A STUDY ON QUALITY, EVALUATION AND RATIONAL USE OF MEDICINE WITH REGULATORY PERSPECTIVES

A thesis submitted in fulfillment of the requirements for the degree of

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IN

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BY

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TABLE OF CONTENTS

TABLE OF CONTENTS i

DECLARATION iv

CERTIFICATE v

ACKNOWLEDGEMENTS vi

ABSTRACT ix

LIST OF ABBREVIATIONS xii

LIST OF SYMBOLS xv

LIST OF FIGURES xvi

LIST OF TABLES xviii

CHAPTER 1 INTRODUCTION 1

1.1. Background 2

1.2. Poor Quality Medicine 5

1.3. SFFC Drugs: A Pandemic Threat 5

1.4. SFFC or NSQ Drugs in India 6

1.5. Near Infrared Spectroscopy and Chemometric 11

1.6. Medicine Prescribing Preference and Rational Use 16

1.7. Purpose of the Research 17

REFERENCES 20

CHAPTER 2 QUALITY PROFILING OF GENERIC PRODUCTS 31

2.1. QUALITY PROFILING OF AMOXICILLIN TRIHYDRATE GENERIC PRODUCTS… 32

2.1.1. Background 32

2.1.2. Materials and Methods 33

2.1.3. Results and Discussions 35

2.1.4. Conclusions 40

2.2. METHOD DEVELOPMENT AND QUALITY PROFILING OF DICLOFENAC SODIUM GENERIC PRODUCTS 41

2.2.1. Background 41

2.2.2. Materials 43

2.2.3. Product Identification 43

2.2.4. Assay Method Development 44

2.2.5. Assay Method Validation 45

2.2.6. Generic Products Collection 45

2.2.7. Assay of Market Products 46
2.2.8. Results and Discussions 46
2.2.9. Conclusions 54

REFERENCES....56

CHAPTER 3 MEDICINE EVALUATION THROUGH DEVELOPMENT AND VALIDATION OF NEAR INFRARED AND CHEMOMETRIC PROCEDURE....62

3.1. NEAR INFRARED AND CHEMOMETRIC MODEL DEVELOPMENT FOR AMOXICILLIN TRIHYDRATE CAPSULE....63
3.1.1. Background 63
3.1.2. Materials, Instrument and Software 64
3.1.3. Methods....65
3.1.4. Reference Method 67
3.1.5. Chemometric Method Development for Identification 67
3.1.6. Chemometric Method Development for Quantification....68
3.1.7. Chemometric Method Validation for Identification....73
3.1.8. Chemometric Method Validation for Quantification 73
3.1.9. Results and Discussion....73
3.1.10. Model Assessment on Real Samples....78
3.1.11. Conclusions 79
3.2. NEAR INFRARED AND CHEMOMETRIC MODEL DEVELOPMENT FOR DICLOFENAC SODIUM TABLET 80
3.2.1. Background 80
3.2.2. Materials, Instruments and Software....81
3.2.3. Methods....81
3.2.4. Reference Method 85
3.2.5. Results and Discussions 86
3.2.6. Model Assessment on Real Sample 93
3.2.7. Conclusions 94

REFERENCES....96

CHAPTER 4 RATIONAL USE AND PRESCRIBING PREFERENCE OF MEDICINE.....100

4.1. GENERIC MEDICINES AND PRESCRIBING PREFERENCE 101
4.1.1. Study Design 102
4.1.2. Data Analysis 104
4.1.3. Results....104
4.1.4. Discussions....108
4.1.5. Conclusions 110
4.2. TREATMENT NON ADHERENCE : RATIONAL TO IRRATIONAL USE 110
4.2.1. Study Design 111

4.2.2. Survey Respondents 111
4.2.3. Methods 112
4.2.4. Statistical Analysis 113
4.2.5. Results 113
4.2.6. Discussions 117
4.2.7. Conclusions 119

REFERENCES 121

CHAPTER 5 CONCLUSIONS 123

APPENDIX A 126

A.1 Linear regression parameters with p-value and RMSE values of the amoxicillin calibration models for optimization 126

A.2 Linear regression parameters with p-value and RMSE values of the diclofenac calibration models for optimization 130

APPENDIX B 135

B.1 Survey questionnaire for medical practitioners 135

B.2 Survey questionnare for patients 140

LIST OF PUBLICATIONS 141

INTERNATIONAL & NATIONAL PRESS MEDIA RECOGNITION 142

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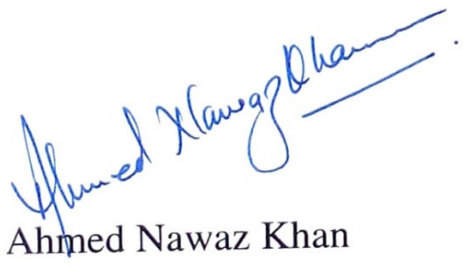
DECLARATION

I hereby declare that the work reported in the Ph.D. thesis entitled **"A Study on Quality, Evaluation and Rational Use of Medicine with Regulatory Perspectives"**, submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work carried out under the supervision of **Dr. Malairaman Udayabanu** and **Prof. (Dr.) Roop K. Khar**. I have not submitted this work elsewhere for any other degree or diploma.



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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"A Study on Quality, Evaluation and Rational Use of Medicine with Regulatory Perspectives"**, submitted by **Ahmed Nawaz Khan (Enroll. No. 106751)** in the fulfillment for the award of degree of **Doctor of Philosophy** in **Pharmacy** at **Jaypee University of Information Technology, Waknaghat, India** is a bonafide record of his original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

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Date: 29/02/2016
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Dedicated To My Parents

Abbi & Mummy

ABSTRACT

Quality of medicine, proper diagnostic, good counseling and rational use of medicine are the foremost attributes in quality healthcare outcomes. Evidently every country is the victim of substandard or spurious drugs, which result in life threatening issues, loss in trust on healthcare system and financial loss of consumer and manufacturer as well. Such poor quality drugs tend to cause unsuccessful treatment. Therefore, the expanding issues over the extent of substandard or spurious medicines remain a challenge, mainly when the large sample sizes in market are not fully monitored. Not only poor qualities of medicines are responsible for the treatment failure but irrational use of medicine by medical practitioners and patients also have a role to play; especially patient's behavior towards treatment adherence and instructions compliance. These are some of the critical issues which call for stringent and predominant regulations in making and following the rules, policies and schemes for better healthcare system by the regulatory bodies. Thus in order to cover these issues though a holistic approach we worked on quality evaluation of medicine available in the market, medical practitioners' perception towards various market medicines, patients' behavior towards treatment adherence, and in developing fast and reliable quality evaluation model for large number of market product.

For quality evaluation 46 amoxicillin trihydrate (250 mg label claim) and 32 diclofenac sodium (50 mg label claim) generic products were collected from open market of North India. Out of 46 amoxicillin products, 28.26% were found to be out of Indian Pharmacopoeia specifications, including 13.04% products which were of substandard quality. Out of 32 diclofenac products; 34.37% were found to be out of Indian Pharmacopoeia specification including 15.62% substandard. Such an extent might cause further morbidity and even resistance. And the circumstances may become worst if the prices are high and quality could not be controlled. This makes the health situation miserable for public and descend their trust, which demands the evidence of medicine safety before approval and thereafter too. Thus Indian drug regulatory bodies need to be entreated to encounter these critical issues strictly.

Monitoring large number of market samples is another issue for the regulatory body. Requirements governing the quality assurance of drug are highly demandable with fast and

efficient techniques. Conventionally used techniques like high performance liquid chromatography and ultra-violet spectroscopy are time and money consuming; and are sample destructive. Therefore, our study describes the method to establish models for non-destructive identification and quantification of 78 amoxicillin trihydarte (250 mg as 100%) and 67 diclofenac sodium (50 mg as 100%) in-house formulations using chemometric tools. Therefore, on diffuse reflectance mode, an identification model based on discriminant analysis was successfully processed with 76 amoxicillin formulations; after two outlier removal. Model was found to be specific using cefadroxil reference standard as negative control. Same samples were also used for quantitative analysis and 96 regression models were processed and finally a best model was selected with partial least square algorithm with four latent variables which resulted in 0.9937 correlation of coefficient followed by 2.17% root mean square error of calibration, 2.38% root mean square error of prediction, 2.43% root mean square error of cross-validation.

For diclofenac sodium, a model from discriminant analysis was designed for identification using 67 formulations. While for quantitative analysis various pre-treatments of the spectra of same formulations were examined and 96 regression models were designed. Six samples were found to be outliers thus removed from calibration set and the model was again calibrated on 41 sample set and as a result a partial least squares regression model with constant pathlength and without smoothing was extracted based on 3.04% root mean square error of calibration, 3.32% root mean square error of prediction and was 4.68% as root mean square error of cross-validation. Identification model was found to be specific for diclofenac sodium using aceclofenac formulation as negative control. Quantitative model was found accurate, precise and robust under 2% relative standard deviation; and linear (r=0.999); that proved to be an alternate to the referenced HPLC method. Therefore, developed near infrared-chemometric models for identification and quantification are intended to be alternatives of the existing analytical tests for its reliability and time saving features. These approaches can solve the problems associated with monitoring large number of market products by conventional methods to save time, money and resources.

As Indian Pharmaceutical market is well known for generic medicines and the government promotes them due to their affordability. These medicines are manufactured by big, medium and small size companies and their quality are generally checked by analytical

methods; though real evaluation of medicines can only be ensure by medical practitioners who prescribe them which is based on therapeutic responses and adverse effects they notice. A survey of 111 medical practitioners was conducted on one to one basis in a form of questioner. Branded generic is preferred over innovator branded generic and generic by 63.1% of medical practitioners; because 64.9% believe that it has good therapeutic response and 68% experienced that it has mild adverse effects while only 0.9% assumed it has high adverse effect. Patients only adhere to their 70-90%, 40-60% and 10-30% instructions according to 33.3%, 45.9% and 16.2% medical practitioners respectively.

Medication non adherence and instructions non compliance are the emerging trends in the deprivation of success in treating illness, particularly in developing countries. To address the prevalence of non adherence and compliance across the Northern India we conducted a cross sectional survey. This was a survey based on about 4161 patients who are or were once under treatment. About 44.1% (N=4151) of the patients stop medication during treatment before its completion and 53.8% (N=4160) of patient generally miss one or more than four doses in between treatment which show high prevalence of non adherence to the treatment. While with respect to non compliance about 73.1% (N=4161) patients do not show full compliance. Non adherence is the major problem tends to poor health care across the nation. Patient non-adherence needs prior attention by implementing patient involvement in treatment decision and educating them. If not confronted today, it may cause big harm to the public health in near future.

LIST OF ABBREVIATIONS

ADR	Adverse Drug Reactions
AE	Adverse Effects
AMOX	Amoxicillin Trihydrate
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
ASTM	American Society for Testing and Materials
BAMS	Bachelor of Ayurvedic Medicine and Surgery
BDS	Bachelor of Dental Surgery
BG	Branded Generic
BUMS	Bachelor of Unani Medicine and Surgery
CDSCO	Central Drugs Standard Control Organization
D & C Act	Drug and Cosmetic Act
DCGI	Drugs Controller General of India
DICLO	Diclofenac Sodium
DM	Doctorate of Medicine
DNB	Diplomate of National Board
DPLS	Discriminant Partial Least Squares
EMA	European Medicine Agency
FDCs	Fixed Drug Combinations
FIP	Pharmaceutical Associations of International Pharmaceutical Federation
FT	Fourier Transform
G	Generic
GR	Guaranteed Reagent
HPLC	High Performance Liquid Chromatography
IBG	Innovator Branded Generic
ICH	International Conference on Harmonization
IMPACT	International Medical Products Anti Counterfeiting Taskforce
InGaAs	Indium Gallium Arsenide
INR/Rs.	Indian Rupee
IP	Indian Pharmacopoeia
IR	Infrared

M	Molar
MBBS	Bachelor of Medicine- Bachelor of Surgery
MCH	Master of Chirurgiae
MD	Doctor of Medicine
MP	Medical Practitioners
MS	Doctor of Surgery
MS	Mass Spectrometry
MSC	Multiplicative Scatter Correction
NIR	Near Infrared
NIRS	Near Infrared Spectroscopy
NLEM	National List of Essential Medicines
NSAID	Nonsteroidal Anti-Inflammatory Drug
NSQ	Not of Standard Quality
PCA	Principal Component Analysis
PCR	Principal Component Regression
PCs	Principal Components
PLS	Partial Least Square
PRESS	Predicted Residual Sum of Squares
PSM	Partnership for Safe Medicines
PTFE	Polytetrafluoroethylene
PvPI	Pharmacovigilance Program of India
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
RMSECV	Root Mean Square Error of Cross Validation
RMSEP	Root Mean Square Error of Prediction
RS	Reference Standard
RSD	Relative Standard Deviation
Schedule H	Class of Prescription Drugs
SEARPharm	South East Asia Region Pharmaceutical Forum
SEC	Standard Error of Calibration
SECV	Standard Error of Cross Validation
SEL	Standard Error of Laboratory

SEP	Standard Error of Prediction
SFFC	Spurious/Falsely-labeled/Falsified/Counterfeit
SIAS	Stability in Analytical Solution
SIMCA	Soft Independent Modeling of Class Analogy
SNV	Standard Normal Variate
TLC	Thin Layer Chromatography

LIST OF SYMBOLS

r	Coefficient of Correlation
r^2	Coefficient of Determination
µm	Micrometer
µl	Microliter
pH	Scale Used to Specify Acidity or Basicity
w/v	Weight by Volume
min	Minutes
ml/min	Milliliter per Minute
mg	Milligram
mg/ml	Milligram per Milliliter
v/v	Volume by Volume
nm	Nanometer
^oC	Celsius
R	R Software Package
χ^2	Chi-square
N	Total Sample Size
₹	Rupee
%	Percent

LIST OF FIGURES

Figure 1.1 Challenging issues in the regulation of medicine 4
Figure 1.2 Decline in substandard and spurious drugs cases from 1995 to 2009.....11
Figure 2.1 Full scale chromatogram of amoxicillin trihydrate reference standard.....36
Figure 2.2 Maximum retail price versus assay plot showing amoxicillin generic products (N=46) quality 39
Figure 2.3 Diclofenac identification in commercial generic products by TLC 47
Figure 2.4 Auto scale chromatogram of DICLO certified reference standard spiked with its known impurity A and E.....47
Figure 2.5 Comparative price versus assay plot showing quality, out of pharmacopoeia specification and substandard DICLO products 53
Figure 3.1 Full range raw NIR mean spectra (a) Amoxicillin API, (b) Microcrystalline Cellulose, (c) Magnesium Stearate, (d) Crosscarmellose Sodium, (e) Placebo, (f) Cefadroxil RS.....66
Figure 3.2 Determination of calibration/training set (blue solid circles) using kennard-stone algorithm for 50 samples out of 78 samples 68
Figure 3.3 Region of interest (a) and (b) in the second-derivative spectra of calibration/training set.....69
Figure 3.4 Outlier detection plot for amoxicillin calibration/training set, high leverage value shows outlier as shown in boxes 70
Figure 3.5 Full range second-derivative overlaid spectra of amoxicillin calibration set with Norris derivative filter after standard normal variate pretreatment.....71
Figure 3.6 Scatter Score plot as variance for PLS1-PLS2-PLS3 obtained for amoxicillin data set 72
Figure 3.7 Scatter Score plot as variance for PLS4-PLS5-PLS6 obtained for amoxicillin data set 72
Figure 3.8 Identification of amoxicillin sample by discriminant analysis based on mahalanobis distance.....74
Figure 3.9 Linearity plot for the calibration (○) and validation (＋) samples of amoxcillin, solid line shows the data fit.....78
Figure 3.10 Full range raw NIR mean spectra (a) Diclofenac API, (b) Placebo, (c) Microcrystalline Cellulose, (d) Magnesium Stearate, (e) Crosscarmellose Sodium.....83
Figure 3.11 Determination of calibration/training set (blue solid circles) using kennard-stone algorithm for 47 samples out of 67 samples 84
Figure 3.12 Identification of diclofenac sample by discriminant analysis based on mahalanobis distance 87
Figure 3.13 Outlier detection plot for diclofenac calibration/training set, high leverage value shows outlier as shown in boxes 89
Figure 3.14 Scatter Score plot as variance for PLS1-PLS2-PLS3 obtained for diclofenac calibration set 89
Figure 3.15 Scatter Score plot as variance for PLS4-PLS5-PLS6 obtained for diclofenac calibration set 90
Figure 3.16 Linearity plot for the calibration (○) and validation (＋) samples diclofenac, solid line shows the data fit.....93
Figure 4.1 Prescribing preference of IBG, BG and G medicines by medical practitioners.....105
Figure 4.2 Therapeutic responses views on IBG, BG and G medicines by medical practitioner.....105
Figure 4.3 Adverse effects views on IBG, BG and G medicines by medical practitioners.....107

Figure 4.4 Price views on IBG, BG and G medicines by medical practitioners.....107
Figure 4.5 Stop medication tendency of patients (N=4151) before completion of treatment 114
Figure 4.6 Missing dose tendency of patients (N=4160) during treatment 115
Figure 4.7 Compliance of patients (N=4161) with respect to follow the instructions of medical practitioners
.....115

LIST OF TABLES

Table 1.1 Reports on substandard and spurious drugs defrauds in India between 2002-2004 10
Table 2.1 Amoxicillin trihydrate generic products assay and price 37
Table 2.2 Price wise distribution of passed and failed AMOX products 40
Table 2.3 Validation of developed HPLC method for diclofenac sodium 49
Table 2.4 Diclofenac generic products assay and price 51
Table 2.5 Price wise distribution of passed and failed DICLO products 53
Table 3.1 Models selection based on good performance out of 96 models 71
Table 3.2 Eigenvalue analysis for identifiaction of amoxcillin 74
Table 3.3 Effect of PLS factors on PRESS and RMSECV for amoxicillin quantitative model 75
Table 3.4 Accuracy evaluation between proposed model and reference method of amoxicillin 76
Table 3.5 Validation parameters for evaluating the proposed NIR amoxicillin model for quantification 77
Table 3.6 Amoxicillin market products evaluation by NIR proposed model 79
Table 3.7 Eigenvalue analysis for identification of diclofenac 86
Table 3.8 Effect of PLS factors on PRESS and RMSECV for diclofenac quantitative model 88
Table 3.9 Validation parameters for evaluating the proposed NIR diclofenac model for quantification 91
Table 3.10 Accuracy evaluation between proposed and reference method 92
Table 3.11 Diclofenac market products evaluation by NIR proposed model 94
Table 4.1 Sample profile of medical practitioners, N=111 103
Table 4.2 Scale for adverse effects of medicine 104
Table 4.3 Sample profile of patients, N= 4161 112
Table 4.4 Non adherence and non compliance question asked during patients' survey 113
Table 4.5 Chi square test for association between different categorical variables 116

CHAPTER 1

INTRODUCTION

1.1. Background

With a population of more than 1.24 billion [1], right to health is a fundamental right in India and has been recognized in the national constitution and statutory laws as well as in international laws [2]. Globally, about 2 billion people, one third of the global population lack access to essential medicines [3]. As medicine are the life saving entities and thus are more essential for the treatment, while they account for 20-60% of care cost and 50-90% of this cost is being paid by the patient, particularly in low and middle income countries [4]. India is a developing country where more than 40% of the population survives on less than US $1 a day [5] and if a patient needs medicines he has to pay more than half of this. As ensuring public health is a national priority, therefore medicines are essential both to a country's economy and to the health of its people [4], however their poor quality expand the burden of healthcare cost and even cause the morbidity and mortality [6], [7]. Moreover, poor quality drugs influence the healthcare system and even drugs which have significant therapeutic effect can also lead to adverse or unwanted effect that may be lead to low or high risk [8]. Therefore, patient safety has become one of the most significant factors in the healthcare and concerns equally to all the stakeholders. Involvement of people as a well active, concerned and informed consumer may help in making their healthcare experience safer and better. In healthcare practice, medicines are the source to treat, cure and mitigate the disease conditions for saving millions of lives. However side by side medicines are inappropriately, ineffectively and economical inefficiently used throughout the global healthcare system; specifically in developing countries [9]. Such irrational use of medicines in terms of prescribing, dispensing, selling and consumption is a worldwide problem [10]. Indian regulatory authorities, industries, medical practitioners, pharmacist, nurses and even patients as well have a significant role in creating safety culture across the country. In terms of pharmaceuticals, ensuring patient safety requires good quality of medicines, less side effects or adverse effects (AE) and rational use of medicines [11]. As shown in Figure 1.1, these situations emerge as challenging issues for the regulatory authorities. Among them first challenge is the quality of medicines, as over the past two decades providing safe and quality products has become a major area of drug regulation and scientific analytical investigation. Prevalence of poor quality drug or substandard product encounters a major stringent issue for the global health system [5] and it cannot be ignored. Day by day

availability and detection of spurious/falsely-labeled/falsified/counterfeit (SFFC) or not of standard quality (NSQ) medicine in the market deteriorate the public credibility in health system. And consumption of these medicines can be responsible for failure of treatment or even death [12]. However, some of the complicating factor which may affect the quality of the drug products are less or more than the label claim, unwanted excipients, impurity and sometimes no active pharmaceutical ingredient (API) [13]. Considering the expansion of the pharmaceutical industry and the degree of potentially mortal diseases, any amount of substandard or spurious medicines is unacceptable because it rises the morbidity and mortality [14]. Even such medicines are the most possible suspect for the antimicrobial resistance in case of antibiotics and treatment failure [15]. And they also induce loss of confidence in health systems and health care takers; economic loss for patients and their families; and business loss to the genuine producers [16].

Second important challenging issue for the regulatory authorities is the evaluation process of large numbers of products available in the market. India has more than 8100 manufacturing units for drug formulations [17] and seven national testing laboratories, while there are 134 approved private testing laboratories [18]. Thus, if on an average each manufacturing unit manufactures 15 products, it means there must be more than 100000 drug products in the market. Quality monitoring of these market products are only done in national testing laboratories and the annual average evaluation capacity of all these seven laboratories is only 15000 samples [19]. Therefore, a challenge concern rises for the evaluation of the rest of the products in the market.

Likely poor quality medicines are one of the possible suspects for the treatment failure; irrational use of medicines by patient is also responsible for such breakdown. Therefore, third challenging issue is the irrational use of medicines. Medicines are inappropriately, ineffectively and economical inefficiently used throughout the global healthcare system; specifically in developing countries [9]. “The overuse, underuse or misuse of medicines results in wastage of scarce resources and widespread health hazards” [10]. Poly-pharmacy, unseemly use of antimicrobials, prescribing medicines not in accordance with clinical guidelines, ill-suited self medication and patients’ non adherence to the treatment are some of the irrational use of medicines [10]. And this non adherence issue is exceedingly common in medical care [20]. Some of the major causes include poor quality of medicines [21]; cost of medicines, side effects and patient behavior [22]–[24]. Indian

government and regulatory authorities have stepped up various program and schemes for the patient care and patient safety. However, there are only limited evidences available on effective interventions in improving adherence in India like fixed dose combinations; recommendation of generic medicines to reduce cost burden on patients and prolonged or sustained release medicines. Moreover these interventions are irrespective of unknown prevalence of non adherence. In India, great emphasis is placed on monitoring the AE under Pharmcovigilance Program of India (PvPI) and less concern is paid on non adherence. And this lacking focus on the use of medicines recognizes that a functional planning is a prerequisite for being able to implement some other interventions.

In healthcare practice, medicines are the source to treat, cure and mitigate the disease conditions for saving millions of lives. However side by side medicines are inappropriately, ineffectively and economical inefficiently used throughout the global healthcare system; specifically in developing countries [9]. Hence, irrational use of medicines in terms of prescribing, dispensing, selling and consumption is a worldwide problem [10].

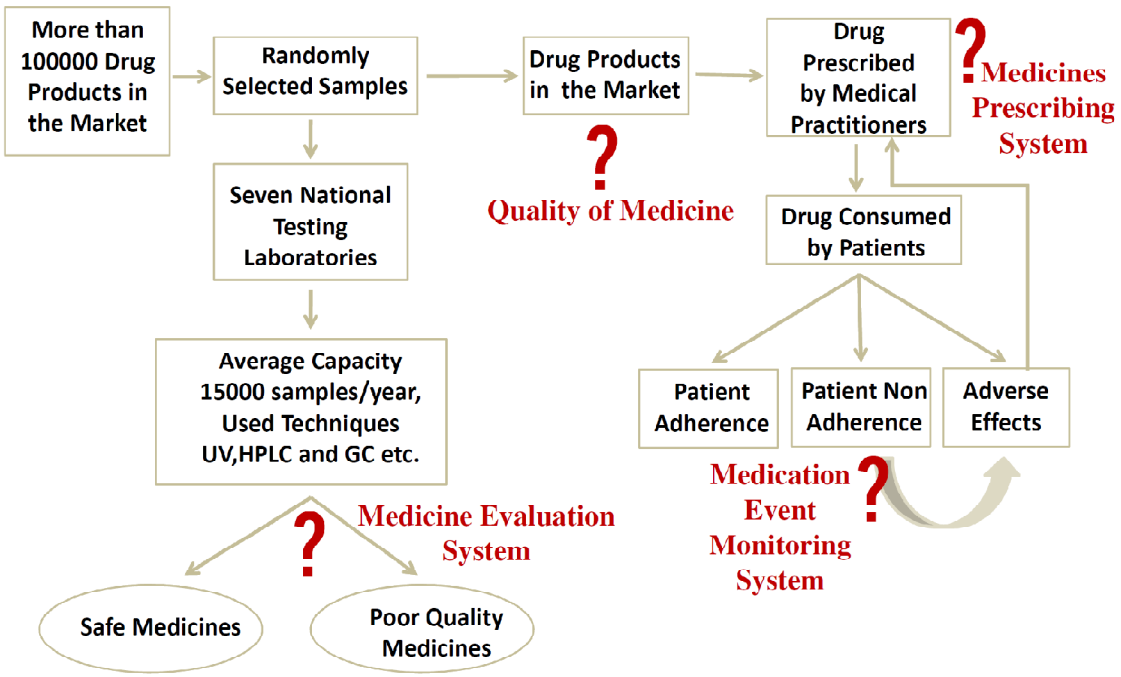

Figure 1.1 Challenging issues in the regulation of medicine

1.2. Poor Quality Medicine

However, due to cheap price, accessibility and availability of counterfeit or substandard products in the market, people accept and buy these products preferably on genuine or branded products [25]. Consumer does not know about the manufacturer or the quality of the product and many time they are unaware of expired, degraded or substandard products which ultimately results in failure of the treatment and with antibiotics cause antimicrobial resistance [26], [27]. Substandard product arises correspondingly due to lack of expertise, unfair manufacturing practices or insubstantial infrastructure; whereas counterfeit is the product of black marketer [27]. The problem of poor quality is already very serious and steadily growing and is likely to cause much more damage in the near future [28]. As such poor quality drug does not bear any universal definition as it may vary from country to country [29]. In general poor quality drug are SFFC drugs that can cause treatment failure or even death [30]. Accordingly, International medical products anti-counterfeiting taskforce (IMPACT) of World Health Organization (WHO) defines SFFC medicines as "medicines which are deliberately and fraudulently mislabelled with respect to identity and/or source, and also which may include products with correct ingredients or with the wrong ingredients, without active ingredients, with insufficient or too much active ingredient, or with fake packaging".

1.3. SFFC Drugs: A Pandemic Threat

Poor quality drug or substandard product encounters a major stringent issue for the global health system [5] and it cannot be ignored. Russia, China, India, Brazil, Mexico, Pakistan, Southeast Asian and Middle Eastern countries are considered as the chief operators in distribution and manufacturing of counterfeit drugs [31]. A decade ago, it was examined by WHO that 10% of the global medicines were counterfeit. However, contrary to its previous communicated data WHO-IMPACT pointed out that data was not much authentic [32]. It means no absolute extent is reported. Now, it is questionable that what are the causes and influences of this problem. In turn, one reason is poverty and other is ignorance and these could contribute to the demand for counterfeit and substandard drugs [5]. Moreover, ignorance of poor quality, unregistered medicines, lenient penalties,

inadequate enforcement of laws are some of the significant causes which provoke the situation [27].

Day by day, consumption by a patient, availability and detection of SFFC or NSQ medicine in the market, may deteriorate the public credibility in health system. Consumption of SFFC medicines can be responsible for failure of treatment or even death [12], [30]. Unbelievably, 0.20 to 0.30 million people die every year in China just because of counterfeit and substandard drugs product [12]. No such data is available in India, yet many patients are dying every year. According to a report revealed by International Policy Network, globally 0.70 million deaths were reported for malaria and tuberculosis because of counterfeit drugs [33]. This data reveals the loop holes in the regulatory system and the cautions for avoiding the poor quality medicines.

1.4. SFFC or NSQ Drugs in India

India is the largest manufacturer of generic drugs and probably 12-25% of the medicines supplied globally are contaminated, substandard and counterfeit [33]. Being the world's largest manufacturers of active pharmaceutical ingredients and finished products, it is likely that India along with China could be the major contributors to spurious medications according to Patrick Lukulay, vice president of US Pharmacopoeial Convention's global health programs [34]. In a report, it has been declared by the European Commission that 75% of the global cases of SFFC medicines originate from India [35]. Indian Government officials initiated an investigation to scrutinize the drugs product which are supplying by India to Nigeria when India was accused along with other 29 Asian countries as the main originator of counterfeit drugs [36]. On one side, India extensively interacts with the African countries in providing quality medicine at affordable prices, while on other side predictive blames were imposed on India and China for exporting the fake or substandard quality of anti-malarial, antibiotics and contraceptives drug product to Uganda and Tanzania. In turn, India and China is denying for such blames [37]. At present, Indian drug regulatory authority has taken various steps against the causes and they have put all their efforts to improve the drug regulation in the country.

In India, as per Drug and Cosmetic Act (D & C Act), 1940, under section 17, 17A and 17B poor quality drug comprises of misbranded, spurious and adulterated drugs

respectively [38]. With the 2008 amendment of D & C Act, Indian drug regulatory authority that is Central Drugs Standard Control Organization (CDSCO) has categorised NSQ products in three categories A, B, C that is helpful in categorising the products during quality evaluation [39]. Category A incorporates spurious and adulterated drug products; which conceal the real identity of the product or formulation and be similar to some well-known brand. These products may or may not contain active ingredients and generally manufactured by unlicensed antisocial people or sometimes by licensed manufacturers. Products that consist of adulterant/substituted product or incorporate some filth material are known as adulterated drugs. Category B include grossly sub-standard drugs in which product fails the disintegration or dissolution test and where active ingredient assay get below 70% and 5% of permitted limit for thermolabile and thermostable product respectively for tablets or capsules. In case of parenteral preparation, failing sterility, pyrogen/endotoxin test or inappropriate toxicity, and fungus presence in any liquid preparation hold such products in this substandard category. Category C involved products with minor defects like emulsion cracking, change in formulation colour, small variation in net content, sedimentation in clear liquid preparation, failing of weight variation test, spot or discolouration on product, uneven coating, presence of foreign matter and labelling errors.

India is considered as the main originator and distributor of SFFC drugs. However, no authentic evidences exist against the country according the data provided by the government and non government agencies of India. Many researchers have investigated only individual drugs or narrow range of drug preparations and formulations. Currently, no large randomized studies of drugs quality have been done in India [40]. In the year 2000, it has been stated that around 35.0%, 23.1% and 13.3% global sales of counterfeit medicines come from India, Nigeria and Pakistan, respectively and counterfeiting includes all therapeutic classes of drug and mainly antibiotics [41]. A decade ago, Indian government officials estimated that 9% of the drug products were of substandard quality [42]. Although according to Indian press media, 30-40% of the total marketed drugs are considered as spurious, but this data is without any scientific confirmation [43]. Under laboratory analysis in a survey accomplished in 2007 by South East Asia Region Pharmaceutical (SEARPharm) Forum, a group of Pharmaceutical Associations of International Pharmaceutical Federation (FIP) and WHO, 10743 samples were collected from 234 retail outlets. About 3.1% were estimated as spurious and 0.3% were out of pharmacopoeial standard [43]. In 2007, 294

fixed drug combinations (FDCs) products were unlawfully available in the market since these were not approved by the Drugs Controller General of India (DCGI) [44]. In 2008, out of 183020 chemist shops, 8418 chemist licenses were suspended or cancelled by the State Drugs Control Organizations on behalf of their trade with spurious drugs [45]. According to CDSCO, estimation of the data during 2003-2008 indicates 6.3-7.5% of the samples were of substandard quality and 0.16-0.35% were encountered as spurious [43]. In 2009, CDSCO reported that in 1995-96, 10.64% and 0.30% tested samples out of 32 770 were substandard and spurious respectively, while in 2007-2008 6.42% and 0.16% tested sample out of 42 354 were substandard and spurious respectively [46]. It was good achievement by the drug authority.

Nevertheless, in 2009, 24136 samples of 62 brands of drugs product were collected in a nationwide survey to find those products which are covertly manufactured and thus to explore the extent of spurious drug in India. Samples were drawn from over 100 pharmacy outlets from various regions of India, which were belong to nine therapeutic categories of 30 manufacturers. Survey affirmed that only 11 products (0.046%) were spurious. Supplementary information revealed by the State Drugs Control Departments declared 1146 (4.75%) products were of substandard quality [47]. Hereby, it can be observed from the government data that spurious drugs are at same level while there is a great decline in the number of substandard drugs from 10.64% in 1995-96 to 5.75% in 2008-09 [46], [48] as shown in Figure 1.2. These kinds of inspections and surveys by the government officials are some driving steps for the public safety. However, stringent actions are yet to be taken for the betterment of public health**.** Overlaying the effects of inferior manufacturing standards, deterioration with inactive or toxic fillers, relabeling of time expired drugs and degradation during storage are closely associated with drug quality [49], which must be checked regularly by fast and efficient techniques.

Manufacturing of spurious and substandard quality drug products is a fraudulent activity and their availability in the market is the life threatening issue for the public health. In 2008, a pilot study performed in two major cities of India, Delhi and Chennai to explore the extent of substandard and counterfeit drugs available in market, under which it was estimated that 12% and 5% samples from Delhi and Chennai, respectively, were of substandard quality [50]. In 2007-08 maximum instance were form Maharashtra and in 2008-09 Kerala was the leading manufacturer of the spurious and substandard drugs [48]. In

2007 four deaths were reported in Maharashtra related to spurious drugs [51]. While more serious results came in news when it was reported that 300 infant died in 2012 in Kashmir because of ceftriaxone substandard quality product which was used to treat pneumonia [52].

No absolute and entire data is reported for substandard and spurious drugs after 2010 by CDSCO, non government organizations or any individual research. For last 3 years, Government has noticed several cases of spurious and substandard drugs importation. In 2009, at Chennai sea port, CDSCO officials caught 3 cases of unregistered bulk drugs originating from China [53]. Cases related to the substandard quality drug product importation in India showed 35, 35, 34 cases for 3 consecutive years 2009-2010, 2010-2011 and 2011-2012, respectively [54]. On a surprise inspection by the CDSCO officials, 85 sales outlets out of 130 were trafficking with the banned drugs in Delhi and Bhiwandi city [55]. News from the country reveals numerous incidences as shown in Table 1.1 [56]. It is highly recommended to investigate individually every drug product that is available in the domestic market.

Considering the expansion of the pharmaceutical industry and the degree of potentially mortal diseases, any amount of substandard or spurious medicines is unacceptable because it rises the morbidity and mortality [14], [57]. Only few published data admit the extent of the problem and its influence on the public health [14], [57], [58]. Thus, there is requirement of immediate attention and research by the regulatory authority towards this public safety issue.

Table 1.1 Reports on substandard and spurious drugs defrauds in India between 2002-2004

Year	Region	Report
2002	New Delhi	Two arrested for running fake medicines racket: 1662 kg of the spurious/fake drugs, Avil, Betnesol, Diclowin, Erythrocin, Voveran and Zintec, forgery labelled as the product of Cipla, Ranbaxy, Cadila, Glaxo and Smithkline Beechem, were seized in New Delhi.
2003	Jaipur	Spurious drugs recovered at Sriganganagar, Rajasthan: Drug Control Department, Rajasthan, seized several products.
2003	New Delhi	Delhi police seized 100 kg of spurious version of nimesulide, ranitidine, and betadine drugs made in Agra, Meerut and Ghaziabad.
2003	Mumbai	Maharashtra FDA raided spurious manufacturer in Palghar, and seized spurious and substandard drug amoxicillin, ampicilline and Solutone (used in multivitamins) worth around US $60,000 (INR 30 lakh) worth of spurious drugs
2004	Faridabad	Spurious Domstal tablets recovered at Faridabad: Health Department of Haryana from a licensed drug trader seized 10,000 tablets of spurious Domstal product.

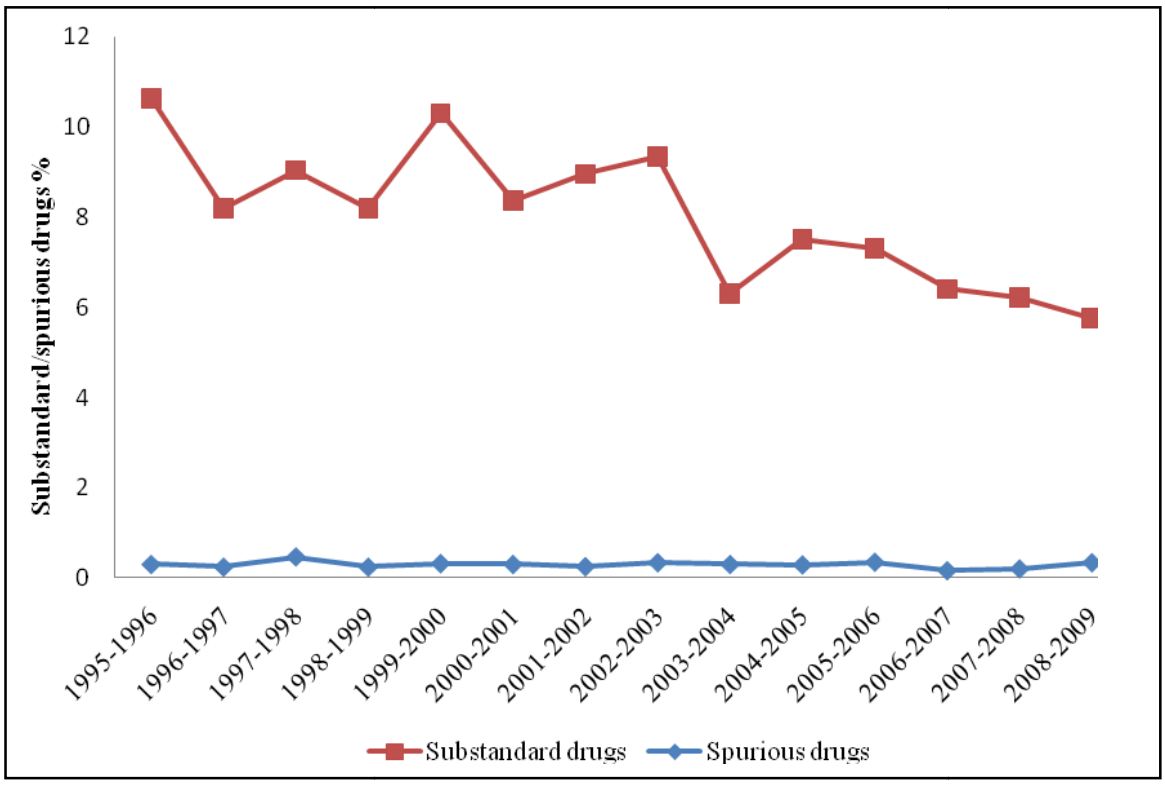

Figure 1.2 Decline in substandard and spurious drugs cases from 1995 to 2009
Substandard drugs (–■–) and spurious drugs (–◆–)

1.5. Near Infrared Spectroscopy and Chemometric

Over the past two decades providing safe and quality products has become a major area of drug regulation and scientific analytical investigation. Poor quality, unbalanced amount or no API at all in the drug products prone to cause severe consequences as mortality and morbidity [59]. Other critical outcomes of such situation cover drug resistance, adverse clinical outcomes such as lack of therapeutic effects and treatment failure, toxicity, side effects and sometimes lethal particularly in developing countries [14], [60]. On such grounds, content assay, toxic impurities and adulterants are the critical factors for monitoring large numbers of commercial pharmaceutical products by conventional techniques. Currently, the most prevailing techniques in the area of evaluation of pharmaceutical products are chromatography and spectrophotometry. More recently, there is a continuing demand for rapid, reliable and economical methods for direct quality measurements of drug products [61]. Therefore, one of the fundamental and remarkable transformations in twentieth century in analytical science happened when the near infrared

spectroscopy (NIRS) expand out the knowledge; which has been now widely accepted as a new concept [62]. This technique as a non-destructive analytical tool has recently become progressively significant in pharmaceutical industries, food technologies, agriculture, biomedical sciences and for exploring substandard and spurious drugs [61], [63]–[67]. Therefore, NIRS has characterized with several advantages for the industrial control and chemical analysis.

It is well known fact that unlike specific chemical peak in mid-infrared (IR) or high performance liquid chromatography (HPLC), visual examination of the spectra cannot interpret any information [68]. In particular, it is feasible to obtain multivariate data from the sample in a affordable, rapid and nondestructive way [69]. Therefore the phase of development of the emerging subject of chemometric appears, and the immense commercial interest in countering such challenges is continuously exploring. This approach has been proven useful also for chemical pattern recognition especially suitable for solving differentiation of main analyte and excipients in complex matrices [64]. Literature presents various reports focused on the determining drug stability, tablet coating, tablet hardness and polymorphic content and particle size of powders [64], [70].

Near infrared bands are typically broad, overlapping and 10-100 times weaker corresponding to their fundamental mid infrared absorption bands, which directly influence the sensitivity. However, productively low absorption coefficient allow high penetration depth and based on this rational; strongly absorbing viscous liquid and highly scattering solid samples may get easily analyzed [68]. Moreover, NIRS permits analysis of low viscosity liquids, high viscosity slurries, solids and smaller particles, webs and pellets and even intact tablets and capsules with little or no sample preparation under the transmission, diffuse transmission, reflectance and diffuse reflectance mode [71]–[73]. Spectroscopic regions of interest in near infrared (NIR) range usually exist between 14493-3333 cm^{-1}(690-3000 nm) and as per international definition of American Society for Testing and Materials (ASTM); it comes between 12821-4000 cm^{-1} (780-2500 nm) [71]. The most apparent absorption band or most active molecular bonds in NIR region are associated to overtones and combinations of fundamental vibrations of –CH, -OH, -NH, -CO functional groups. Frequency and intensity of such NIR absorption bands are the result of anharmonicity and fermi resonance [68], [71].

NIRS is not only to determine the chemical through the analysis of the vibrational molecular bonds in the NIR spectrum, but also to create an optical model that behave like unique pattern recognition for the samples [68]. This declares the possibility of using spectra to determine complex attributes of raw material or drug matrices.

NIR spectroscopy has been examined to assess its suitability to detect API in different types of products such as tablet, capsules and gels etc. [68]. This contributes as an alternative to the other more sophisticated and labor-intensive technology that would verify excessive cost to determine quality of raw material or finished products [64]. On this account NIRS may be a good alternative if it is combined with multivariate calibration methods for determination of a complex mixture in pharmaceuticals for quality evaluation. These approaches can solve the problems associated with the large number of variables compared to the number of samples measured, which is distinct for NIR data. Due to the mathematical simplicity and physical or chemical elucidation these have been widely used in distinct applied fields of chemometric.

Univariate analysis is quite convincing in terms of conventional regulation while multivariate analysis remained a challenge. Hereby NIRS require some sort of data processing that is chemometric calculations which can relate spectral information to sample properties. However, to develop such calibration design requires multiple samples, numerous hours of work and several of computer calculations are required. Therefore, according to European Medicine Agency(EMA), the scope of NIRS as an analytical procedure require chemometric analysis for the purpose of authentication of physicochemical properties, identification, qualification and assay of starting materials, intermediates and finished products [74]. Several chemometric and statistical algorithms can be employed in qualitative and quantitative analysis since these techniques have been proven to be successful in extracting the desired information from untreated NIR spectra. Like for identification and classification purpose; multivariate analytical techniques like principal component analysis (PCA), discriminant partial least squares (DPLS), cluster analysis and soft independent modeling of class analogy (SIMCA) are used [70]. While for calibration and optimization of quantitative procedure principal component regression (PCR) and partial least square (PLS) algorithm are applied on the dataset [70].

Pretreatment of the spectrum are prerequisite for accurate and precise result thus the most common preprocessing approaches such as first and second derivative transformation, Savitzky–Golay filtering, Norris derivative filtering, standard normal variate (SNV), multiplicative scatter correction (MSC), peak ratio or normalization, and sometimes spectral subtraction or a combination are some fundamental necessity [63], [75]. These are excellent for differentiation between samples and spectral differences [76]. However, they require expertise and supervision to avoid unstable calibration model.

European Medicine Agency has published guidelines for the NIRS calibration and validation, otherwise except this no harmonized regulations are established or drafted [74]. However, NIRS has already been incorporated as an established monograph in United State Pharmacopoeia, European Pharmacopoeia and Indian Pharmacopoeia (IP). In spite of its extensive acceptability in pharmaceutical industries, it lacks specific monograph of pharmaceuticals for a multivariate method of quantification [65]. And specifically this technique remains largely unexplored in India notably in drug products quality testing.

Performance of calibration is calculated according to equation 1-3 as standard error of calibration (SEC), standard error for cross validation (SECV) and bias according to the given equation as prescribed by the EMA [74].

$$\mathrm{SEC} = \sqrt{\frac{\sum_{i=1}^{n} (y_{C,i} - Y_{C,i})^2}{n - p}} \qquad (1)$$

where, Y_C is NIRS predicted value of calibration set, y_C is reference method value of calibration set and n is the number of samples and p is the number of coefficients like principle component or factor.

$$\mathrm{SECV} = \sqrt{\frac{\sum_{i=1}^{n} (y_{CV,i} - Y_{CV,i})^2}{n}} \qquad (2)$$

where, Y_{CV} is NIRS predicted value of calibration set, y_{CV} is reference method value of calibration set and n is the number of samples and p is the number of coefficients like principle component or factor.

$$\text{Bias} = \frac{\sum_{i=1}^{n}(y_i - Y_i)}{n} \qquad (3)$$

where, Y is NIRS predicted value, y is reference method value and n is the number of samples.

Collectively, root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) and root mean square error of cross validation (RMSECV) are known as figure of merit [65]. The essential criterion for examination of average accuracy and precision of multivariate model are the variability of difference between the predicted and reference values for a set of independent validation samples and expressed as mentioned in equation 4 and 5 as standard error of prediction (SEP) and standard error of laboratory (SEL) respectively [74] and overall model performance estimated by root mean square error (RMSE) [77] equation 6.

$$\text{SEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_{V,i} - Y_{V,i})^2}{n}} \qquad (4)$$

where, Y_V is NIRS predicted value for independent validation set, y_V is reference method value and n is the number of samples.

$$\text{SEL} = \sqrt{\frac{\sum_{i=1}^{n}(y_{1,i} - y_{2,i})^2}{n}} \qquad (5)$$

where, $Y_{1/2}$ is reference method value , measured at different laboratory conditions and n is the number of samples.

$$RMSE = \frac{1}{n}\sum_{i=1}^{n} e_i^2 \quad (6)$$

where, n is the number of samples of model errors ε calculated as (e_i, $i = 1,2, \ldots ,n$) [77]. Other parameters which are partially required for model authenticity are regression coefficient of correlation (r) and coefficient of determination (r^2) [74], [78], [79].

1.6. Medicine Prescribing Preference and Rational Use

Globally million of people are injured, disabled and even died because of medical error and among them disability is more common than death [80]; and these errors can be diagnostic error, preventive error and treatment error or other error like lack of communication or instrument failure [81]. It indicates that medical practitioners (MP) play a vital role in society in diagnosing patients and treating them with medication. Therefore, well practice and considerable knowledge is of the elemental significance to MP for their professional endeavor. According to World Bank report, India is the lower middle income country having population of 1252 million and out of it about 742 million people live on a daily cost of about 88 Indian Rupee (INR) and among them 296 million live on about 55 INR only [82]. Wealthy people have easy access to the high quality of healthcare benefits while poor and middle class are far away from it [83]–[85], and the patients who adopt health care access in public sector found it to be of poor quality [86]. However, public healthcare is the only option specifically poor population can afford.

Focusing on the accessibility and affordability of the drug products in the country, India excels as the ‘pharmacy of the developing world’ [87]. Indian Government instructed to all Central Government hospitals and Central Government Health Scheme dispensaries to prescribe generic medicines in large extent as possible. Physicians are also instructed by State Government to prescribe generic medicines [88].

According to WHO “The overuse, underuse or misuse of medicines results in wastage of scarce resources and widespread health hazards” [10]. Inability to follow medical practitioners instructions, non cooperation in healthcare programs, delay in seeking care, negligence in appointments; forms different types of non compliant behaviors [89], [90]. Few others include receiving a prescription and not made up it at drug store, taking an

erroneous dosage, incorrect timing of dose administration, missing one or more doses or ceasing the treatment too soon by not taking repeated medicines [91]. Some underlying causes of poor adherence are cost of medicine, clinical side effects of medicine and patient behavior [24]; and poor quality of medicine [21]. For instance, as a consequence of less adherence to anti-inflammatory medication, problem of juvenile idiopathic arthritis and more inflamed joint occurs [92]. Similarly, in case of antiretroviral therapy, children with HIV/AIDS transpire to high viral load [93]. And most harmful consequence linked to the reappearance of tuberculosis due to lower adherence and early suspension of the treatment [94], [95]. In chronic disease report from developed countries suggest 50% adherence only [96], while magnitude of the problem is quite big in developing nations [97]. Today, India does not have much information and evidence on non adherence. In particular, non adherence is a threat to patient health with many devastating consequences like patient safety risk [21], morbidity and mortality [24].

Every year in United States, 125000 cardiovascular disease patients die due to non adherence to the treatment [97], while developing countries like India do not hold any data on such related subject. Among few reported studies; a study of tuberculosis patients (N=538) showed 16% patients non adherence to the treatment [98] and another study of schizophrenia patients (N=115) showed 41.9% patients non adherence to the treatment [99]. In a study on hypertensive patients; only 24.1% (N=473) patients showed adherence; it means 75.9% showed non adherence to the treatment [100]. Sometimes adherence and compliance could be used interchangeably. However more specifically following the instructions of medical practitioner is termed as compliance [101]. Irrespective of disease currently no research has specifically depicts how many patients have the tendency to show non adherence and non compliance.

1.7. Purpose of the Research

According to Partnership for Safe Medicines (PSM) organization; India is the largest manufacturer of generic medicines and probably 12-25% of the medicines supplied globally are contaminated, substandard and counterfeit [102]. The problem of poor quality is already very serious that steadily growing and is likely to cause much more damage in the near future [28]. Only few published data admit the extent of the problem and its influence on

the public health. Thus, surveying the pervasiveness of low quality medications is a fundamental need for ensuring the welfare and benefits for all the associated sections. Recently no study has been done so far in India except CDSCO monitored studies. Therefore, there is requirement of immediate attention and research towards this public safety issue.

One way to solve evaluation process of large number of market samples issue is to increase the number of testing laboratories and other is to use some alternate techniques which are fast and efficient for large number of samples. Evaluation of the drug product are generally done by thin layer chromatography (TLC), IR spectroscopy, HPLC, mass spectrometry (MS), colorimetric methods, dissolution assay and visual inspection; which are considered as well established and pharmacopoeial recognized techniques [103]. However there are certain complications with most of these pharmacopoeial approaches like the invasive nature of analysis, consumption of organic and inorganic solvents, sample preparation, elution of hazardous waste and time consuming. For these reasons efficient analytical approaches with rapid, reliable and non invasive nature are required primarily for routine drug analysis specifically for large number of samples. Hence to overcome such difficulties; Fourier Transform (FT) NIRS has already been proven as a significant analytical technique not only for pharmaceutical product quality estimation but also for detection of counterfeit or spurious drugs at a very low cost with little or no sample preparation [61]. However, it is not a standalone technique thus require some mathematical calculations. Therefore, development of NIRS model requires chemometric analysis in combination with NIRS that defines the scope of NIRS procedure or model used for the intended purpose [74]. Chemometric like PCA, PLS, PCR, DA and SIMCA are some of the widely used methods [61]. However, they require expertise and supervision to avoid unstable calibration model.

Altogether in India, great emphasis is placed on monitoring the AE under PvPI and less concern is paid on non adherence and this lacking focus on the use of medicines recognizes that a functional planning is prerequisite for being able to uncover necessary interventions. However, there are only limited evidences available on effective interventions in improving adherence in India like fixed dose combinations and prolonged or sustained release medicines, and these interventions are irrespective of unknown prevalence of non

adherence. Despite the overwhelming benefit of fixed dose combination, non-adherence remains in a manner that poses a major warning to patient safety.

Even exploring the quality of large number of market products is a big challenge; thus under either MP or patient monitoring events, we can also monitor the poor quality medicines as the actual quality in terms of therapeutic action and AE can only be observed by the MP who prescribe them and the patients who use them. Observations from such study will help in making better policies and interventions to improve the public health. Therefore a holistic attempt must be initiated to work on these issues for protecting and promoting the public health.

To study these issues we worked on the following objectives:

Objective 1: To evaluate quality of amoxicillin trihydrate and diclofenac sodium generic products available in the Indian market

Objective 2: To develop and validate identification and quantification models using near infrared spectroscopy and chemometric analysis for:

(a) amoxicillin trihydrate capsule formulations; and

(b) diclofenac sodium tablet formulations

Objective 3: To conduct two cross sectional surveys:

(a) to demonstrate medicine prescribing preference by medical practitioners and their perspective for medicine therapeutic response and AE with respect to quality of medicines; and additionally their perspective towards patient compliance;

(b) to demonstrate the prevalence of treatment adherence and compliance by patients; and explore the most imprudent age, gender and monthly income groups of patients with respect to rational use of medicines.

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CHAPTER 2

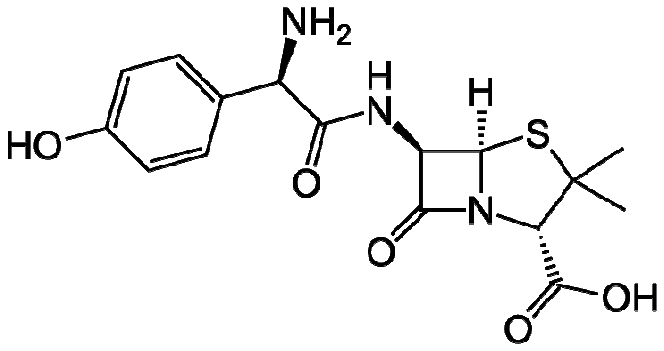
QUALITY PROFILING OF GENERIC PRODUCTS

2.1. QUALITY PROFILING OF AMOXICILLIN TRIHYDRATE GENERIC PRODUCTS

2.1.1. Background

"The dose makes the poison" as stipulated by Paracelsus. Consequently, the presence of active ingredient and its compliance to the pharmacopeia specifications considered as the most obvious paradigm for certifying the quality of drugs formulations [1]. From the majority of the developing countries; there are several reports of failing treatments, development of antimicrobial resistance and serious adverse drug reactions (ADR) including death [2], [3]. In all probability, this is because of meager medications like SFFC medicines [2]. According to World Health Organization, "SFFC medicines include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient or too much active ingredient, or with fake packaging" [4]. Broadly poor quality medicines are divided into two categories viz. substandard and counterfeit [5]. From India, 12-25% of supplied medicines globally may be contaminated, substandard and counterfeit [6]. Without any scientific evidence Indian media consider 30-40% availability of the poor quality drug in domestic market [7]. When a product is manufactured it is being utilized by the patients; and availability of good quality medicines strengthen the chances of better treatment for the individual patient which advances improved results for general well being by and large [8]. In developing countries this poor quality situation stresses the patients without the known extent [5]. Likely in developing nations; medicines account for 25-70% of total heath care expenses [9]. Thereupon in the case of India where 58.01% and 21.2% of the population has daily living cost of less than Rs. 144 and Rs. 88.6 respectively [10] then how could people afford the high price of medicines in addition to survival. Therefore Indian Government always emphasizes and promotes the affordable generic medicines for prescription and utilization [11]. The futile increase in the price of medicines is being wrangled on in the financial health care and public health domain, as it usually results in a reduced access of essential drugs [12]. Therefore to counteract the issue of spurious and substandard quality medicine in India there is an urgent demand for more research or routine analysis to document the magnitude of the problem.

Antibiotics are the life-saving medicines [13], thus, an ideal antibiotic therapy is a prerequisite to treat the systemic spread of the infection and to prevent their complications [14]. Prescribing antibiotics irrationally is a general trend in India [15]. However, antibiotic medicines are considered as the most poor quality class or counterfeit worldwide and observed as the major threat for patients especially children [16]. Penicillin antibiotics like amoxicillin is largely prescribed drugs [17] and it is used as broad spectrum antibiotics which is considered as susceptible to the β lactamase-negative strains and species like *Streptococcus pneumonia, Streptococcus species* (α-and β-hemolytic strains only) and *Haemophilus influenza* etc. [18]. In recent years no study has been done overall or for any individual drug to explore the situation leaving behind no clear documentation on the current extent of the problem. Hence, the purpose of this pilot study was to explore quality and affordability of amoxicillin products; and scope in creating awareness to public and attention to regulatory bodies to evaluate such products.



Amoxicillin Structure

2.1.2. Materials and Methods

2.1.2.1. Chemicals and reagents

Amoxicillin, trihydrate reference standard, was directly purchased from Sigma-Aldrich. HPLC grade acetonitrile (Lichrosolv), potassium dihydrogen phosphate (LiChropur), potassium hydroxide GR grade were procured from Merck (India). The polytetrafluoroethylene (PTFE) filter of 0.45 µm and nylon filter of 0.20 µm pore size from Millipore system (Millipore Inc., USA) was used throughout the evaluation.

2.1.2.2. Instrument

A HPLC system (Waters, Milford, MA, USA) equipped with Alliance 2695 separations module with photodiode array detector was used in this study. A reverse phase octadecylsilane bonded C-18 (250 mm × 46 mm, 5 μm) column (Waters) was employed throughout the analysis. All samples for evaluation were weighed on high sensitive analytical balance TB-215D (Denver Instrument, Germany). Chromatograms were recorded and processed using Empower Pro Software (Waters).

2.1.2.3. Generic product collection

All amoxicillin generic products used in this study were purchased without prescription from the open market from different location of Northern India. In total 46 generic products were collected which comprise 43 different products of amoxicillin trihydrate (AMOX) capsules and two tablets, having 250 mg dose. Among them, one capsule product was collected in two batches thus in total 46 products were collected.

2.1.2.4. Sample preparation

Standard and sample preparation for the identification and quantitative evaluation of amoxicillin in the finished pharmaceutical product, HPLC being highly sensitive and pharmacopeial established method was preferred. Thus in accordance to IP [19], 0.02M monobasic potassium phosphate buffer was prepared as a solvent mixture and adjusted to pH 5.0±0.05 using 4.5% potassium hydroxide (w/v) and finally filtered through 0.20 μm membrane nylon filter and degassed in an ultrasonic bath. An isocratic mobile phase of acetonitrile and solvent mixture in 4:96 v/v was used with a flow rate of 1.5 ml/min. Each analytical run was carried for 10 min with 10 μl injection volume, and data was acquired at 230 nm and processed. The solvent mixture was used as diluent in the preparation of analytical sample solutions. AMOX working reference standard (RS) solution of 1.2 mg/ml concentration was used as system suitability solution.

Test samples were prepared by mixing 250 mg equivalent to amoxicillin form content of ten capsules or tablets in diluent to prepare 1.2 mg/ml concentration. Samples were sonicated for 10 min and filtered using 0.45 μm PTFE. Each sample was prepared in triplicate and also injected in triplicate to ensure the precise assay result. Against the mean area of five injections of AMOX RS; assay of amoxicillin in the test sample was determined

on percent label claim of the API present in individual capsule or tablet. According to IP for ten capsules the assay requires to be within 89.5–110.6% of the label claim and for ten tablets assay requires to be within 89.4-110.8% of the label claim [20]. While according to the CDSCO a product is said to be substandard quality only if assay found 5% below the IP limit [21]. To calculate the assay, simple formulas were used

Assay (mg/capsule) =

$$\frac{\text{Mean area of sample} \times \text{Concentration of standard} \times \text{Potency of standard} \times \text{Molecular weight of drug} \times \text{Average capsule content weight}}{\text{Mean area of standard} \times \text{Concentration of sample} \times \text{Molecular weight of drug salt} \times 100}$$

Assay % =

$$\frac{\text{Mean area of sample} \times \text{Concentration of standard} \times \text{Potency of standard} \times \text{Molecular weight of drug} \times \text{Average capsule content weight} \times 100}{\text{Mean are of standard} \times \text{Concentration of sample} \times \text{Molecular weight of drug salt} \times \text{Label claim} \times 100}$$

2.1.3. Results and Discussions

Our aim was not to expose any particular product or defame any company. Hence, product identity is not revealed. However the challenges involved in the collection and evaluation of substandard drugs in the market cannot be neglected; as all products were procured from the open market without prescription.

In evaluating the generic product, firstly weight variation test was done and each product passed the test. Then it was necessary to face up the interaction of complex matrix of the product. Thus, an assumed placebo was prepared by blending microcrystalline cellulose, magnesium stearate, croscarmellose, sodium and colloidal silicon dioxide. And this placebo was spiked with the reference standard to confirm the interference. System suitability was demonstrated to be appropriate for routine analysis purpose, thus, AMOX RS peak was confirmed by theoretical plate count, tailing factor and maximum absorption at 230 nm wavelength. Peak purity was also checked with the higher purity threshold than purity angle. The observed retention time and peak area with system suitability are shown in Figure 2.1.

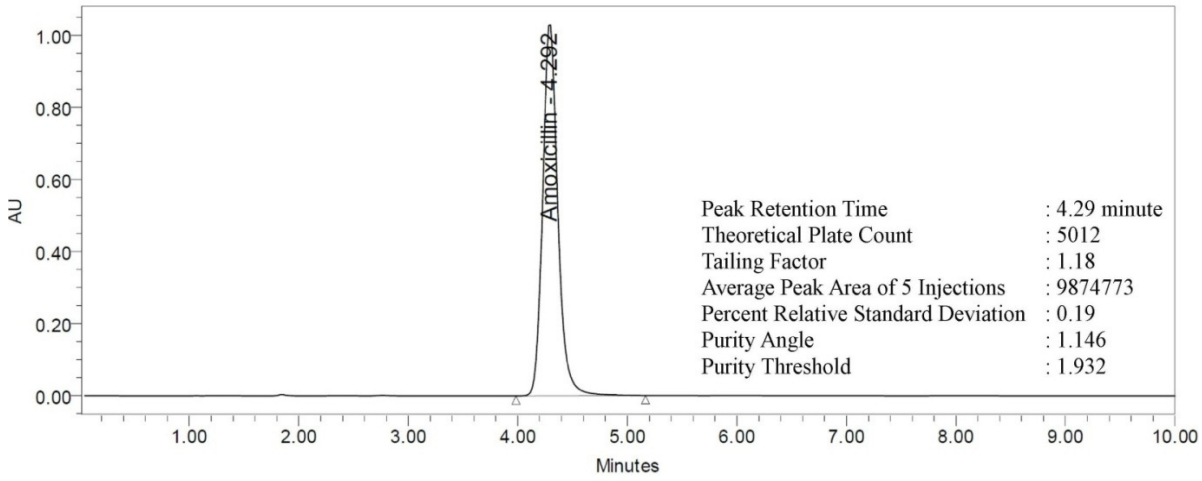


Figure 2.1 Full scale chromatogram of amoxicillin trihydrate reference standard

Due to listed in National List of Essential Medicines (NLEM) [22], its extensive production [23] and extensive utilization [24]; AMOX was selected. The analytical techniques chosen need to be specific to every product; thus, amoxicillin capsule monograph was selected from IP 2010. However, the presence of excipients and another evaluation test like impurity profiling can determine the overall quality of the product. In order to compare the quality of AMOX capsules or tablets, we identified its presence and calculated the amoxicillin quantity with respect to label claim. Out of 46 products the percentage content of 13 products were not matched with the label claim. As shown in Table 2.1, 13 products were failed to follow the IP specification. And among them, six products were failed according to the specified limit by CDSCO. Thus, 28.26% products were out of IP specification, and 13.04% products were of substandard quality, high demand for antibiotics which are often prescribed in developing countries and is a very profitable market for the manufacturers who deliberately manufactured such products.

Table 2.1 Amoxicillin trihydrate generic products assay and price

Sample Code	Assay I (%) (Mean of triplicate injections)	Assay II (%) (Mean of triplicate injections)	Assay III (%) (Mean of triplicate injections)	Mean Assay (%)	Standard Deviation (%)	Relative Standard Deviation (%)	Maximum Retail Price per Capsule or Tablet (Rs.)
AMOX-01A	98.80	99.37	101.36	99.84	1.34	1.34	5.90
AMOX-01B	99.69	101.30	100.82	100.60	0.83	0.83	2.90
AMOX-02	90.49	89.58	89.21	89.76	0.66	0.74	4.80
AMOX-03	95.75	93.59	93.26	94.20	1.35	1.43	5.07
AMOX-04	92.55	93.56	94.05	93.39	0.76	0.81	3.50
AMOX-05	85.54	86.66	86.22	86.14	0.56	0.65	5.75
AMOX-06	85.66	85.32	85.30	85.43	0.20	0.23	3.25
AMOX-07	89.06	90.39	89.47	89.64	0.68	0.76	2.90
AMOX-08	95.72	95.00	94.63	95.12	0.55	0.58	3.96
AMOX-09	88.95	90.11	89.17	89.41	0.62	0.69	5.50
AMOX-10	100.22	100.53	100.71	100.49	0.25	0.25	3.00
AMOX-11	89.86	91.48	90.96	90.77	0.83	0.91	3.60
AMOX-12	90.57	91.31	91.30	91.06	0.42	0.46	3.40
AMOX-13	93.05	89.72	90.98	91.25	1.68	1.84	1.50
AMOX-14	81.92	83.72	83.47	83.04	0.98	1.18	6.50
AMOX-15	22.41	22.40	22.17	22.33	0.14	0.63	4.50
AMOX-16	92.23	92.34	92.10	92.22	0.12	0.13	2.90
AMOX-17	97.89	99.18	99.08	98.72	0.72	0.73	4.70
AMOX-18	99.08	99.65	99.79	99.51	0.38	0.38	3.90
AMOX-19	90.12	91.48	90.53	90.71	0.70	0.77	3.80
AMOX-20	100.21	99.88	101.08	100.39	0.62	0.62	4.70
AMOX-21	87.08	89.12	88.77	88.32	1.09	1.23	5.50
AMOX-22	89.13	90.83	90.58	90.18	0.92	1.02	4.10
AMOX-23	94.52	95.89	95.16	95.19	0.69	0.72	4.10
AMOX-24	93.52	94.27	94.39	94.06	0.47	0.50	5.75
AMOX-25	90.47	90.72	91.20	90.80	0.37	0.41	3.20
AMOX-26	95.39	95.62	96.15	95.72	0.39	0.41	5.25
AMOX-27	96.14	96.00	95.86	96.00	0.14	0.15	1.40
AMOX-28	91.23	91.98	90.00	91.07	1.00	1.10	5.63
AMOX-29	91.09	91.52	91.22	91.28	0.22	0.24	6.50
AMOX-30	83.21	84.46	83.71	83.79	0.63	0.75	3.95
AMOX-31	91.88	91.01	91.94	91.61	0.52	0.57	3.60
AMOX-32	92.24	93.67	93.64	93.18	0.82	0.88	3.83
AMOX-33	86.89	86.60	86.76	86.75	0.15	0.17	3.25
AMOX-34	91.52	92.92	92.82	92.42	0.78	0.84	4.07
AMOX-35	83.26	85.36	84.09	84.24	1.06	1.26	3.50
AMOX-36	89.77	90.23	90.44	90.15	0.34	0.38	6.20
AMOX-37	87.26	88.99	88.87	88.37	0.97	1.10	5.00

AMOX-38	91.20	89.28	90.39	90.29	0.96	1.06	3.40
AMOX-39	27.94	27.61	27.54	27.70	0.21	0.76	3.20
AMOX-40*	105.92	106.12	105.76	105.93	0.18	0.17	4.00
AMOX-41*	86.95	84.85	85.55	85.78	1.07	1.25	5.43
AMOX-42	90.75	93.28	91.82	91.95	1.27	1.38	5.87
AMOX-43	79.95	81.31	81.24	80.83	0.77	0.95	2.90
AMOX-44	90.87	91.80	91.46	91.38	0.47	0.51	2.50
AMOX-45	98.80	98.45	98.55	98.60	0.18	0.18	3.97

**Tablets, Total number of samples N=46*

India has to ensure the continuity of the generic competition in order to respect, protect and fulfill the right to health of its people [25]. Thus additionally, we raise quibble over the consideration of price to be one of the controlling parameters influencing the access of medicines in India. Under article 21 of the Indian constitution; the right to health includes availability and accessibility to affordable drugs. While substandard product such as Amox-14; and out of pharmacopeia specification products like Amox-05, Amox-21, Amox-09 and Amox-41 were available in the market at high prices, range between Rs. 5.43-6.50 as shown in Figure 2.2. In addition, such quality also has a big contribution in antimicrobial resistance and unfortunately for one decade there is no antibiotic in the pipeline of the invention.

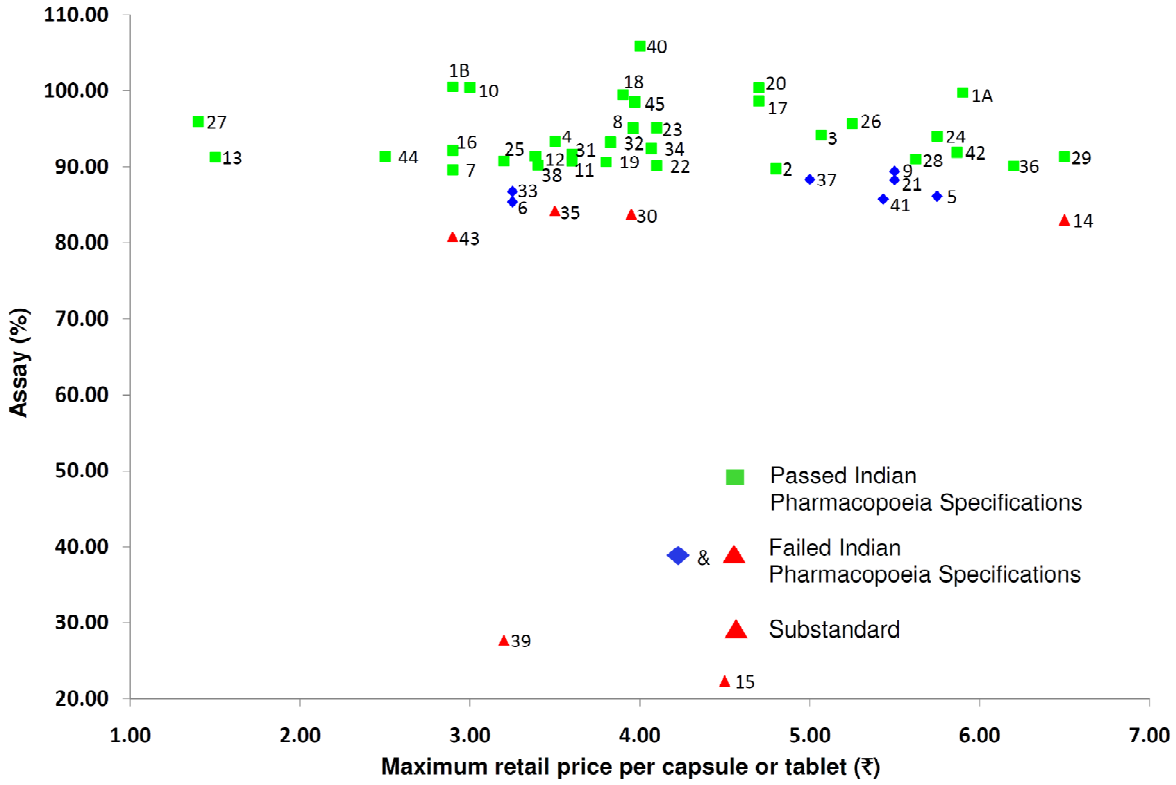


Figure 2.2 Maximum retail price versus assay plot showing amoxicillin generic products (N=46) quality

Patient demand affordable and quality generic products like Amox-13 and Amox-27, which are manufactured by the companies who may have no motive to more profit but are more patient-centric. Raising competition with expanding the drug production ultimately lowers the price, improve the access and verify public benefits.

Fisher's exact test with two-tailed p-value 0.87 showed no significant difference between assay and price of the evaluated products. Based on Indian pharmaceutical generic scope and affordability; Indian Government promotes the generic medicines and recommend to prescribe them by the medical practitioners; but the practitioners must be aware of the sources, quality and all possible benefits and risk of the prescribed medication so as to educate the patients. Moreover, pharmacist's involvement in switching the branded medicines to generic medicines may be a good intention only if pharmacists know the quality of medicines besides the trade brokerage.

Another concern is selling the 'Schedule H' drug without prescription. As products were purchased directly from the retailer and wholesalers, and they didn't ask for any prescription or the buyer identity. Maybe they consider medicines as a commodity. Thus, in

short D & C Act 1940 and Rules 1945 are improperly followed. Therefore good pharmacy practice guidelines [26] has to be followed for the safety of patients. Inversely due to patient's lack of knowledge, unawareness of AE and seller ignorance during trade may increase morbidity and mortality.

It can be concluded that several products available in the market are of substandard quality. The high extremity of this situation, as shown by the results is not significantly related to low cost only as show in Table 1.2. Precisely there is no single step to restrain the issue of quality medicine, however; the interventions adopted by regulatory authorities must be more practicable and durable to uproot the cause of the problem. As a part of interventions, education and providing information to the public may enhance awareness. Other feasible strategies have been increasing the number of testing laboratories, and use a fast and efficient method like NIRS or capillary electrophoresis for monitoring the poor quality medicines trade.

Table 1.2 Price wise distribution of passed and failed AMOX products

Maximum retail price per capsule/tablet (Rs.)	No. of products failed	No. of products passed
1-2	-	2
>2-3	1	5
>3-4	6	11
>4-5	2	5
>5-6	4	6
>6-7	1	2

2.1.4. Conclusions

When people in developing countries don't have access to quality medicine at an affordable price, improving regulatory system should be the first challenge that has to take care off. As many substandard products are available in the market and ready to use by the patients for their treatment. These results would create awareness among the public and drug regulatory authorities about a brief extent of the problem. The patient can compromise with price but patients' health cannot be compromised with poor quality. Therefore, situation demands the evidence of safety before approval and thereafter too. Improved quality of antibiotics and inclusion of additional parameters for their effective supply and strategies may further eliminate the antibiotic resistance. Study determine the requirement

for further investigation into how this substandard product are available in the market and explore other quality compromised medicines in order to protect the public health and promote the health system.

2.2. METHOD DEVELOPMENT AND QUALITY PROFILING OF DICLOFENAC SODIUM GENERIC PRODUCTS

2.2.1. Background

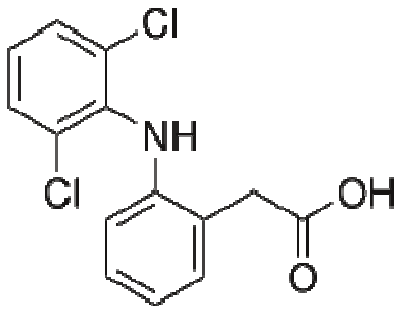
Globally more than 2 billion population is deprived of the essential medicines [9], this may be due to higher cost of the drugs [25] and low grade of medicines, thus considered as the prospective menace for the public health [27], [28]. Markedly for developing nations it may have a compelling impact and lead to clinical loss along with the economical burden [5], [28]. Some of the complicating factors that affects the quality of the drug products are with regard to the quantity of API which mismatches with the label claim or occasionally no API, unwanted excipients and impurity content [29], [30]. Irrespective of the causes, poor quality affects the health in the same way which ultimately insignificant to the patient [31]. Therefore, this global issue of substandard medication needs a comparative consideration as it influences a broad set of population. Drug products from the market undergo evaluation under routine check by the regulatory authorities. Their API may differ noticeably due to complex method of formulation and production but it must be within pharmacopoeia specification. Many studies showed the significant differences in the quality of products [32], [33]. The incompetency in regulation to control the falsified and substandard drugs is leading to a severe impact on the health and economic ramifications in low and middle income countries [34]. Consequently, availability of poor quality medicines diminishes the possibilities of fruitful treatment for individual patients which obstruct improved results all together [8]. Moreover, failing treatments and critical AE including death are some of the major incidences from developing countries [2], [3] and it may be the result of inadequate medications related to SFFC medicines [4]. Assuredly, the complete list of undesirable effects and number of incidences due to substandard or spurious drug is still unknown.

Mainly low and middle-income countries have a weak pharmacovigilance and drug regulatory system [34]. Therefore as general public health disputes, the issue of the proximity of substandard and SFFC medicines; solutions for open utilization ought to draw

watchful consideration primarily for developing nations [35]. A study suggests 12-25% of the medicines distributed globally from India are contaminated, substandard and counterfeit [6]. Evidently such confusion makes the regulatory system miserable for the public health.

Substandard medications are most likely a bigger issue influencing more individuals, and therefore unquestionably need a comparative consideration. No study has been performed in the last few years to cross check the extent and thus real extent of the problem still remains unknown. Moreover, impractical pricing of medicines is further influencing to the crisis in the public health domain and may undermine efforts to improve healthcare [3]. Worldwide awareness has been growing on the increasing incidence of substandard and spurious drug, whereas India is still lacking on the issue. Thus, to counteract the issue of spurious and substandard quality medicine in India there is an urgent need for additional research or routine analytical evidences to explain the magnitude of the problem.

Most often used medicines like amoxicillin, azithromycin, metformin [36] and diclofenac [37] etc. should be evaluated on priority. Thus we have selected diclofenac sodium (DICLO) tablet generic products. Diclofenac (2-[(2,6-dichlorophenyl)amino] benzeneacetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) belongs to phenylacetic acid class. Irrespective of its 40-60% bioavailability and fatal gastrointestinal AE like stomach or intestinal bleeding, ulceration, inflammation and perforation of stomach etc.; [38], [39] it is widely used for the symptomatic relief of pain and inflammation; and has favorable therapeutic effect in arthritis, musculoskeletal disorder, toothache and dysmenorrhea [40], [41]. It is a ‘Scheduled H’ drug under D & C Act and Rules [42] and also included in NLEM of India and globally the most widely prescribed NSAID [37]. Based on the widely prescribed and some reported substandard quality of diclofenac [43] we aimed this pilot study to explore the quality of diclofenac generic products and to observe how the prices are associated with corresponding quality.



Diclofenac

2.2.2. Materials

2.2.2.1. Chemical and reagent

Diclofenac sodium API and excipients for placebo were provided by the Ranbaxy (India). Certified reference material of diclofenac sodium was purchased from Sigma Aldrich. High performance liquid chromatography grade methanol (Lichrosolv), phosphoric acid (EMSURE), monobasic sodium phosphate GR grade were procured from Merck (India). Water used during analysis was purified through a Millipore Milli-Q (Waltham, MA, USA) water system.

2.2.2.2. Instruments

A HPLC (Waters, Milford, MA, USA) equipped with Alliance 2695 separations module and 2996 photodiode array detector was employed throughout the analysis using octadecylsilane bonded C-18 (250 mm × 46 mm, 5µm) column (Waters). All samples were weighed using TB-215D (Denver Instrument, Germany) analytical balance. And chromatograms were processed using Empower Pro software (Waters).

2.2.3. Product Identification

For identification, TLC was used in accordance to IP [44]. For this, a precoated silica gel 60 F254 plate was spotted with 1 µl of 10 mg/ml concentration of DICLO RS and all market products. After air drying the plate was sprayed with a 0.5 percent w/v solution

of potassium dichromate in dilute sulfuric acid. Spots were visualized and identified corresponding to the retention factor value of DICLO-RS.

2.2.4. Assay Method Development

A new HPLC method was developed using reference of Diclofenac Sodium tablet monograph mentioned in United State Pharmacopoeia [45]. DICLO-RS was used as a control. A blend of microcrystalline cellulose, talc, croscarmellose sodium, magnesium stearate and colloidal silicon dioxide was prepared and assumed as placebo.

In accordance with developed method, 0.01M phosphoric acid and 0.01M monobasic sodium phosphate (1:1) buffer was prepared as solvent mixture and adjusted to pH of 2.5±0.05 using 5% phosphoric acid (v/v) and ultimately filtered through 0.20 µm membrane nylon filter and degassed in ultrasonic bath. Mobile phase comprised of 70 volume of methanol and 30 volume of phosphate buffer, which was degassed through sonication and vacuumed prior to use. Diluent of methanol and water (70:30) was used in the preparation of analytical sample solutions. DICLO-RS solution of 0.2 mg/ml was used as system suitability solution. The analysis was carried out at 1.0 ml per min flow rate under isocratic mode for 15 min run time. The column was held at ambient temperature, the volume of injection was 10 µl. Peak area response was detected by extracting chromatogram at 254 nm. Filter compatibility was done with one-use 0.45 µm nylon filter and PTFE filter while supernatant of the centrifugate was used as control. Analytical stability of standard and sample in solvent at 25^{o}C was done at 0, 1, 3, 6, 12, 18, and 24 hours. A solution of DICLO-RS spiked with two known impurity of diclofenac that is Impurity A (1-(2,6-dichlorophenyl)indolin-2-one and Impurity E (indoline-2-one); were used as resolution solution to ensure specificity. Resolution, R between Impurity A and Impurity E was set at not less than 5 and between Impurity A and diclofenac not less than 2 as a peak resolution criterion. For assuring the correct result percent of relative standard deviation (RSD) of peak area response for replicate samples were posed not to be more than 2. A sample of standard spiked with placebo was also prepared to verify the system suitability with injection run time for 30 minutes.

2.2.5. Assay Method Validation

In accordance with the Q2(R1) International Conference on Harmonization (ICH) guidelines method was validated with recommended parameters which include specificity, linearity, accuracy, precision and robustness [46]. Validation sample set were quantified against mean peak area of six injections. For system suitability criterion, peak tailing factor must be less than 2, peak area %RSD of five injection of standard solution must be less than 2 and theoretical plates should not be less than 3000. The peak purity was determined based on lower purity angle than purity threshold of the main peak.

Specificity of method was shown by spiked samples and no peak was eluted with the main peak of API which further validated with spectra match plot. Method linearity was illustrated by the standard calibration curve of six samples in the range of about 0.14-0.26 mg/ml (that is 70%, 80%, 90%, 100%, 110% and 130% of the 0.2 mg/ml concentration). Accuracy and precision were established by assessing recovery and %RSD values obtained with three test solutions, each at concentration of 0.14, 0.20, and 0.26 mg/ml corresponding to 70%, 100% and 130% of the API concentration. Recovery was estimated by comparing calculated theoretical concentration from the standard curve and the nominal concentration. Method robustness was demonstrated by changing in flow rate, column oven temperature, minor component and extracting wavelength. The robustness was tested with a 0.2 mg/ml standard solution, and explained by the effect of parameter modification on peak theoretical plate count and tailing factor. Overall %RSD for robustness was fixed not to be more than 2 and peak purity must pass.

2.2.6. Generic Products Collection

Simple sampling process was done when one of the authors posed as customer and purchased 32 drug products directly without prescription from storefront wholesaler or retailer of open market located in urban and semi-urban areas of Northern India. There were 31 products of different companies and among them one product was in two batches. Thus in total 32 products of DICLO tablet were procured which were of 50 mg dose except one with 100 mg dose. Once procured all generic products were stored at ambient temperature with low humidity and no sunlight until assay evaluation.

2.2.7. Assay of Market Products

Ten tablet of each product were transferred in to 100 ml amber color volumetric flask. Initially half of the volume was made up with diluent and vigorously shaken mechanically for about 30 minutes till all tablets disintegrate. Some samples were not dissolved mechanically due to coating; therefore they were sonicated for 15 minutes. Thereafter volume was made up and a concentration of about 0.20 mg/ml of DICLO was obtained. Before injection, each sample was filtered with new disposable PTFE filter.

All 32 market products were assayed using aforementioned in house developed HPLC method in duplicate. Six injections of DICLO-RS of 0.20 mg/mL prepared in mobile phase were used to determine to fulfill the system suitability criterion. Against the peak area response of DICLO-RS; assay was determined from the percent label claim of API content in individual product. For calculating the precise assay result; %RSD of two preparations was fixed not to be more than 2. For assay; to pass the pharmacopoeia specification each product must be within 89.3–110.8% of the label claim. While in accordance to the guidelines by Indian pharmaceuticals regulatory authority that is CDSCO; products which fail assay 5% below the pharmacopoeia specification that is below 84.3% are considered to be substandard product and NSQ [21]. This minor change in the range was due to considering ten tablets of a product and to compensate the sampling error as mentioned in IP.

2.2.8. Results and Discussions

Ultra-violet spectroscopy recommended by IP was considered as primitive method and due to use of 100% methanol as diluents mentioned in diclofenac monograph; preliminary results were showed variations in the assay. Therefore, a new method of HPLC was developed and validated for the evaluation of the commercial generic DICLO products. However, for ensuring identity of DICLO, recommended TLC method was followed according to IP. Figure 2.3 showed all products contain the claimed API.

In filter compatibility analysis, 0.45 µm PTFE filter were selected for filtering reference standard and samples. Relative retention time of impurity E, impurity A and diclofenac was about 3.55, 6.62 and 8.38 min as shown in

Figure 2.4, and all peaks passed the resolution.

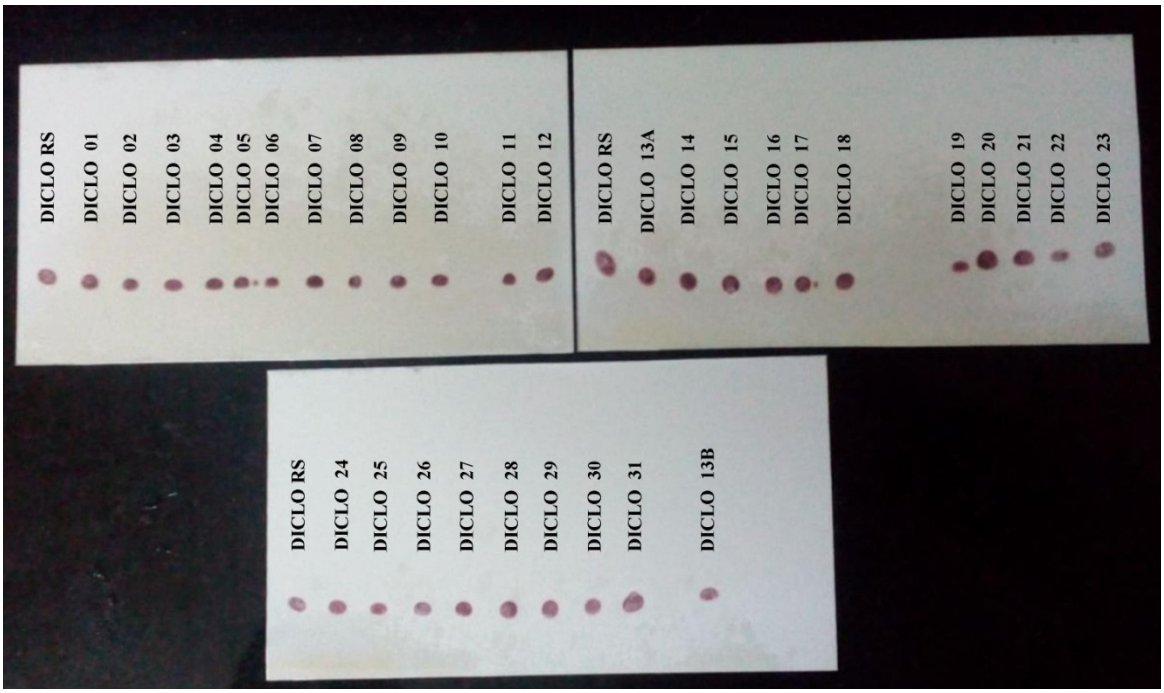


Figure 2.3 Diclofenac identification in commercial generic products by TLC

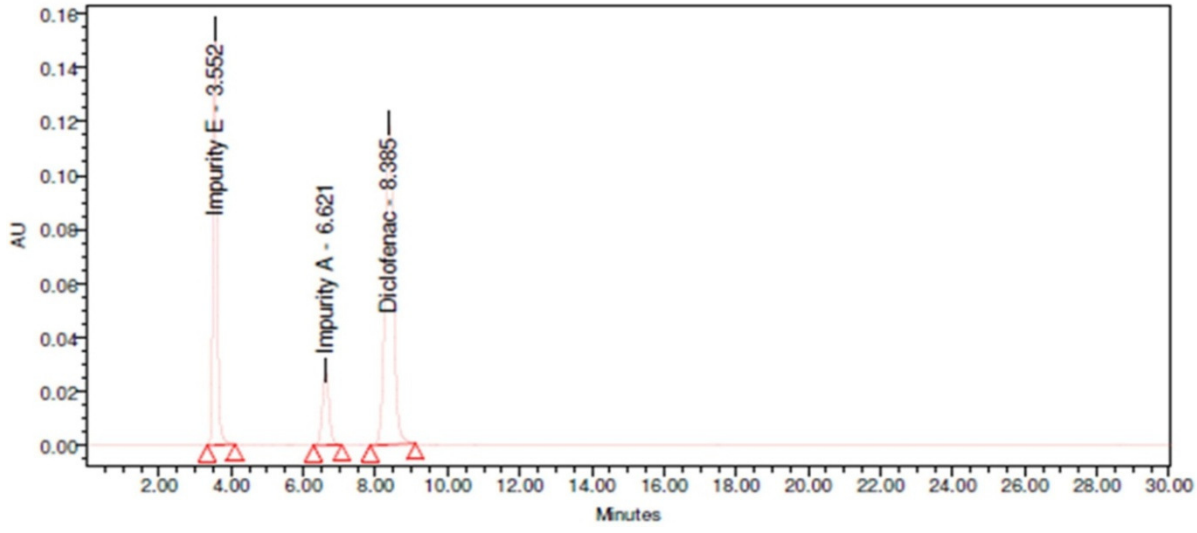


Figure 2.4 Auto scale chromatogram of DICLO certified reference standard spiked with its known impurity A and E

According to the recommended ICH guidelines Q2 (R1) proposed method was validated as shown in Table 2.3. For linearity the sample area count versus concentration was evaluated by linear least square regression. Comparable slopes (9937) and intercepts (−72612) were obtained. Linearity was shown by the good correlation 0.9997, between area count and the drug concentration as shown in Table 2.3. The accuracy results showed recoveries between 99.0-101.7%. The method precision and system precision were determined which showed 0.27 and 0.94 %RSD respectively. The robustness results signify that the peak area was not significantly affected by changing the flow rate by ±10%, column oven temperature by ±5ºC, 10% relative change in minor component of the mobile phase and changing the wavelength by ±5 nm, as shown in Table 2.3. To calculate the assay, simple formulas were used

Assay (mg/tablet) =

$$\frac{\text{Mean area of sample} \times \text{Concentration of standard} \times \text{Potency of standard} \times \text{Molecular weight of drug} \times \text{Average tablet weight}}{\text{Mean area of standard} \times \text{Concentration of sample} \times \text{Molecular weight of drug salt} \times 100}$$

Assay % =

$$\frac{\text{Mean area of sample} \times \text{Concentration of standard} \times \text{Potency of standard} \times \text{Molecular weight of drug} \times \text{Average tablet weight} \times 100}{\text{Mean are of standard} \times \text{Concentration of sample} \times \text{Molecular weight of drug salt} \times \text{Label claim} \times 100}$$

It was an exploratory pilot scale research to investigate the quality of drug products in the Indian market. Out of total 32 products, 34.37% failed to pass the pharmacopoeia specification including 15.62% substandard quality products as shown in Table 2.4. The potential consequence of such under-dose medications is a matter of concern to the regulatory authorities. These differences may affect the therapeutic effectiveness of products and trust on health system.

Table 2.3 Validation of developed HPLC method for diclofenac sodium

Validation Parameters	Value
System Suitability	
Mean Peak Area	1938427
Retention Time	8.3
Tailing Factor	0.94
Capacity Factor	7.42
Theoretical Plates	5542
Specificity	
Sample Spiked with Placebo	purity angle (0.027)< purity threshold (0.257)
Difference % of Control and Spiked Sample(with Impurity)	-1.5
Linearity	
Correlation Coefficient	0.9997
Regression Coefficient	0.9994
Slope	9937
Intercept	-72612
Precision (% RSD)	
System Precision	0.27
Method Precision	0.95
Accuracy (% Recovery)	
At 70% level	99.0-99.43
At 100% level	101.30-101.70
At 130% level	100.38-100.84
Overall % Recovery	100.46
Overall % RSD	0.99
SIAS Standard at 25 °C for 24 hours	
Cumulative % RSD	1.3
SIAS Sample at 25 °C for 24 hours	
Cumulative % RSD	0.88
Assay %	100.3-102.1
Robustness	
For Standard	
System Suitability	
(under modified conditions)	

Theoretical plates	> 4000
Tailing factor	< 2
% RSD(five injections)	< 2
For Sample (% RSD)	
Control	0.23
Flow Minus	0.38
Flow Plus	0.35
Temperature Minus	0.4
Temperature Plus	0.2
Minor Component Minus	0.50
Minor Component Plus	0.26
Wavelength Minus	0.21
Wavelength Plus	0.63

SIAS- Stability in analytical solution, RSD: Relative standard deviation

A possible explanation for existence of substandard medicines in the market may likely because of negligence in manufacturing, non conformance to good manufacturing practice [21] or may be to gain more profit by the manufacturer without knowing the negative consequences of this poor quality like loss of trust on medical practitioners, loss of trust on health system and increased burden on patient. It is noteworthy that not only the different products have different assay result; but two batches of same product can have varied results. For example DICLO-13A found to be substandard while DICLO-13B passed the test as shown in Table 2.4.

Table 2.4 Diclofenac generic products assay and price

Sample Code	Assay I (%)(Mean of replicate injection)	Assay II (%) (Mean of replicate injection)	Mean Assay (%)	Standard Deviation (%)	Relative Standard Deviation (%)	Maximum Retail Price per tablet (Rs.)
DICLO-01	92.35	93.10	92.73	0.53	0.57	4.95
DICLO-02	88.41	88.47	88.44	0.04	0.05	1.24
DICLO-03	105.37	105.40	105.39	0.02	0.02	1.94
DICLO-04	94.07	94.63	94.35	0.40	0.42	3.00
DICLO-05	90.52	89.62	90.07	0.64	0.71	1.70
DICLO-06	43.04	42.57	42.81	0.33	0.78	1.30
DICLO-07	94.91	93.84	94.38	0.76	0.8	1.66
DICLO-08	91.67	91.42	91.55	0.18	0.19	2.40
DICLO-09	93.28	94.10	93.69	0.58	0.62	3.20
DICLO-10	90.39	91.14	90.77	0.53	0.58	1.47
DICLO-11	75.19	74.20	74.70	0.70	0.94	0.12
DICLO-12	106.72	107.14	106.93	0.30	0.28	0.30
DICLO-13A*	61.35	60.80	61.08	0.39	0.64	0.18
DICLO-13B*	99.18	99.71	99.45	0.37	0.38	0.18
DICLO-14	93.38	94.08	93.73	0.49	0.53	0.30
DICLO-15	100.24	100.11	100.18	0.09	0.09	2.60
DICLO-16	93.94	93.88	93.91	0.04	0.05	1.85
DICLO-17	89.41	89.22	89.32	0.13	0.15	0.15
DICLO-18	96.39	97.08	96.74	0.49	0.5	0.12
DICLO-19	45.60	45.64	45.62	0.03	0.06	0.18
DICLO-20	87.51	85.69	86.60	1.29	1.49	2.17
DICLO-21	88.26	88.19	88.23	0.05	0.06	0.19
DICLO-22	89.13	88.71	88.92	0.30	0.33	0.13
DICLO-23	83.84	84.77	84.31	0.66	0.78	1.54
DICLO-24	83.67	83.80	83.74	0.09	0.11	1.06
DICLO-25	85.67	86.11	85.89	0.31	0.36	1.92

DICLO-26	95.89	96.46	96.18	0.40	0.42	1.21
DICLO-27	91.31	92.33	91.82	0.72	0.79	2.50
DICLO-28	89.67	89.89	89.78	0.16	0.17	0.70
DICLO-29	98.90	99.04	98.97	0.10	0.1	0.85
DICLO-30	92.44	92.23	92.34	0.15	0.16	1.80
DICLO-31[#]	97.38	97.89	97.64	0.36	0.37	1.30

[*]*Two batches,* [#] *100 mg dose*

All products were procured without prescription and this calamity signifies that how D & C Act and Rules are misapplying. Medicine seller and buyer both are considering it as a commodity. Medicine should be dispense only as per the rules and guidelines [26]. Medicines are quite complex molecules which may cause morbidity and mortality if use without medical practitioners or pharmacists instructions. Thus good pharmacy practice is highly demanded.

Weight variation test was also done on each sample and no product was found failed in the test. The intended study not only identified and quantified DICLO content in different marketed generic brands and local generics but also their relative significance with the cost. As shown in Table 2.4, DICLO-18 of 0.12 rupee per tablet passed the assay while at the same price another generic product DICLO-11 failed in the test. On the other hand, products labeled with high price like DICLO-06, DICLO-23, DICLO-20 and DICLO-25 were failed in the assay; while DICLO-01, DICLO-04, DICLO-15 and DICLO-16 were passed the evaluation. High severity of underlying situation, as indicated by the results was not significantly related to low cost only as show in Table 2.5.

After demonstrating Fisher's exact test, a two tailed p-value 0.432 showed no significant difference between price and assay value. Therefore, products tagged with high price do not guarantee the good quality and product of low price do not ensure poor quality as shown in Figure 2.5. It indicates poor quality products exist in the market irrespective of the price and these substandard products range from low price to high price. Further investigations on other category of drugs are necessary to address the concern for quality and affordability of medicines in India.

Table 2.5 Price wise distribution of passed and failed DICLO products

Maximum retail price per tablet (Rs)	No. of products failed	No. of products passed
0.1-0.5	5	5
>0.5-1	-	2
>1-2	5	8
>2-3	1	3
>3-4	-	2
>4-5	-	1

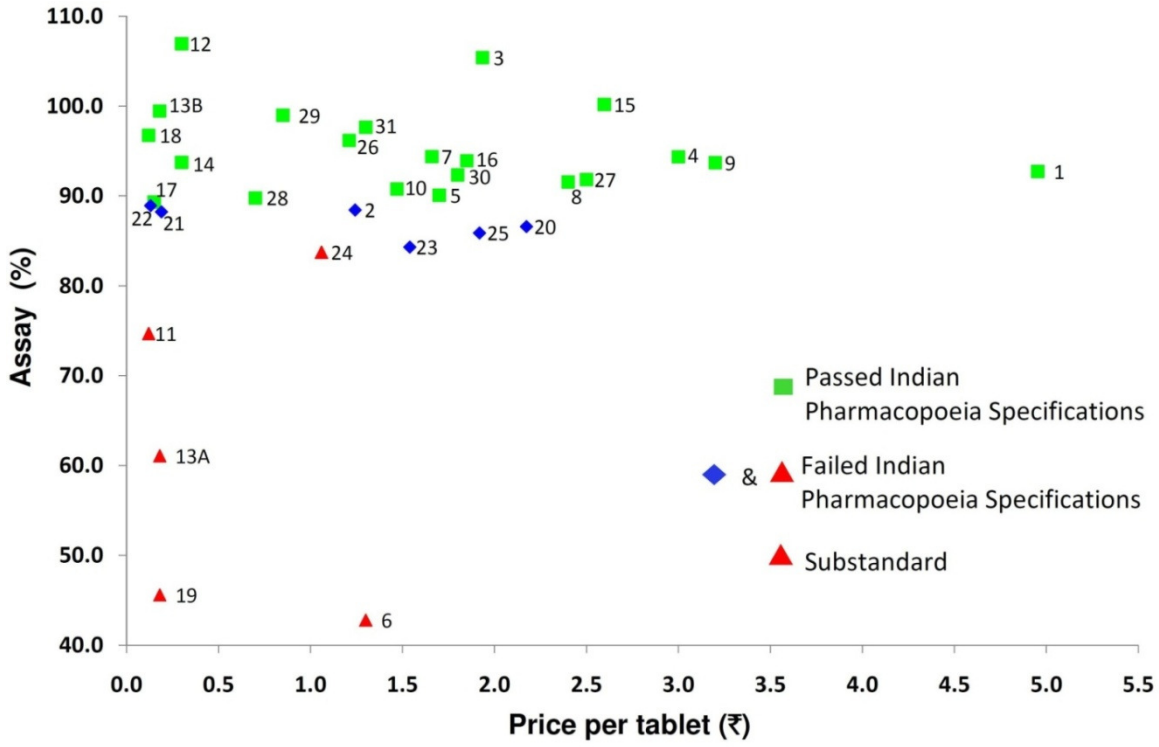


Figure 2.5 Comparative price versus assay plot showing quality, out of pharmacopoeia specification and substandard DICLO products

In general, these outcomes highlight the assorted nature of poor quality medication issues that have critical consequences for public health. Such issue should be undertaken by the pharmaceutical researchers or academicians, concerned regulatory agencies, medical

practitioners and consumer as well. The Indian government, national regulatory authority and state regulatory authorities need to be very stringent in complying with quality assurance and quality control. Authorities has to review and implement the already recommended interventions by Mashelkar Committee Report [47]. Additional efforts are required to enhance the current manufacturing practices along with the process involved in registration of drugs to control the flow of impoverished medicines in the market.

This work additionally accentuates the requirement for productive oversight of pharmaceutical products, with legitimate observing of manufacturers and their distribution systems to bring down the danger for public being exposed to products of low quality, low safety and low efficacy.

2.2.9. Conclusions

An HPLC assay method has been developed and validated for DICLO generic market products. The assay method has been validated to be specific, linear (r = 0.9997), accurate (recovery 99.0–101.7%), precise (method precision %RSD = 1.39 and system precision %RSD = 0.91) and robust. Proposed method can be used for future evaluation of diclofenac sodium tablets.

From the result of this study it is evident that there is a high predominance of low quality DICLO products in northern India and it may be due to non harmonized regulatory system which makes it a challenge to quantify the prevalence of poor quality medicines across the country. Thus there is urgent requirement of large scale study for sufficient data to estimate the true extent of poor quality medicines. Our study aim was not to defame any faulty product or company. It was only directed to publicize maximum awareness to the consumers, pharmacists, medical practitioners and drug regulatory authorities about extent of the problem. This quality assessment of diclofenac products may be regarded as an initiating step for further evaluation of such products or other marketed drug products for the patient safety. Besides affordability and non affordability issue; ambiguous quality of generics tends to cause huge loss to consumers, therefore medicine regulations, policies, practices and research are required to be patient centric. Under urgency it is required to focus on controlling the availability of substandard drugs in the market that are produced as a result of the poor manufacturing and quality-control practices or deliberately falsified drugs. Among the primary challenges; first is to improvise the product quality by

enforcement of good manufacturing practice rules and second is regulatory authorities must harmonized and confront in order to make some feasible interventions for improving this crude situation.

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CHAPTER 3

MEDICINE EVALUATION THROUGH DEVELOPMENT AND VALIDATION OF NEAR INFRARED AND CHEMOMETRIC PROCEDURE

3.1. NEAR INFRARED AND CHEMOMETRIC MODEL DEVELOPMENT FOR AMOXICILLIN TRIHYDRATE CAPSULE

3.1.1. Background

Antibiotics medicines are life saving entities since they cure, treat, prevent and mitigate the infectious condition, thereby they are safeguarding the public health. Antibiotics like ampicillin, amoxicillin, co‑trimoxazole, gentamicin, erythromycin, and ciprofloxacin are the most counterfeited products globally [1]. AMOX is among the most prescribed drugs and produced at large scale worldwide [2]. Surprisingly, WHO listed AMOX on top of 47 antibiotics in 2010 being most counterfeit active ingredient in the world [3]. Poor quality, insignificant amount or no API in drug products tend to cause grievous consequences such as mortality and morbidity [4]. Such situation also accounts for drug resistance and adverse clinical outcomes such as lack of therapeutic effects, treatment failure, toxicity, and side effects [1], [5]. On such grounds, monitoring and quality profiling of large numbers of pharmaceutical products by fast, efficient and inexpensive analytical method are highly demandable for drug regulatory authorities.

Dry formulations can be evaluated noninvasively on reflectance mode [6] while liquid formulations on transmission mode using NIRS [7]. Contrary to transmission mode, reflectance measurement impart the remarkable bulk chemical information with small particles size and also with lambertian (diffuse reflectors) surfaces; it is mainly because it is not predominantly affected by surface scattering or reflectance losses due to the exclusion of major portion of the specular component [8]. Many researchers have been worked on NIR‑chemometric models for the qualification and quantification of AMOX [2], [9]–[11]; however qualitative identification model which can identify AMOX in capsule powder formulations which are analogous to commercial market products; and quantitative analysis of same API in these formulations has not been reported previously in India, so that it could be used for evaluation of market products. Therefore using our developed spectral library, we designed models based on diffuse reflectance that can be used for AMOX identification and quantification in capsule formulations which were very similar to commercial products. Adding new spectra of new AMOX product or batch will update calibration model which will facilitate an easy and direct comparison between different products without use of

reference samples in future. Evaluation with conventional HPLC method requires sample preparation and is expensive, sample destructive, and time consuming [12]. Therefore, a NIR-chemometric method is intended to be an alternative of the reference HPLC method for its reliability and time saving features.

3.1.2. Materials, Instrument and Software

Amoxicillin trihydrate, magnesium stearate, and croscarmellose sodium were provided as gift samples by Ranbaxy Laboratories Ltd., India, while microcrystalline cellulose-avicel and colloidal silicon dioxide-aerosil was provided as gift samples by FMC Biopolymer Brussels, Belgium and Evonik Industries, Germany, respectively. Amoxicillin trihydrate RS and cefadroxil RS were directly procured from Sigma Aldrich. HPLC grade acetonitrile (Lichrosolv), potassium dihydrogen phosphate (LiChropur), potassium hydroxide GR grade were procured from Merck (India). Polytetrafluoroethylene filter of 0.45 μm and nylon filter of 0.20 μm pore size from Millipore system (Millipore Inc., USA) was used throughout the HPLC analysis. All AMOX commercial samples used in this study were purchased over the counter from the open market of Northern India.

A Thermo Scientific Antaris II FT-NIR analyzer equipped with an integrating sphere coupled with indium gallium arsenide (InGaAs) detector was employed to generate diffuse reflectance spectra, and data were collected by means of inbuilt RESULT software. A HPLC (Waters, Milford, MA, USA) equipped with Alliance 2695 separations module and 2996 photodiode array detector was used in reference analysis using octadecylsilane bonded C-18 (250 mm × 4.6 mm, 5 μm) column. All samples during analysis were weighed using TB-215D (Denver Instrument, Germany) analytical balance. All chemometric data analysis and modeling were carried out using Chemometric Software Package TQ Analyst 7.2.0 (Thermo Fisher Scientific Inc., MA, USA), along with the R Version 3.0.3 and Statistical Software MATLAB version 7.6.0 (MathWorks, Natick, USA).

3.1.3. Methods

3.1.3.1. Capsule content formulation

Capsule formulation was comprised of amoxicillin trihydrate equivalent to amoxicillin used as an API in a range of approximately 50–110% of 250 mg AMOX. This range was grounded on to have more formulations and maximum variability. Total content was fixed at 400 mg based on commercial capsule size available in the market for 250 mg AMOX that is one (hard gelatin capsule size). Label claim with 100% of API, in this case, was 250 mg AMOX making this a high‑dose capsule. Four common excipients for AMOX capsule were microcrystalline cellulose (5-90%), magnesium stearate (0.25-5%), croscarmellose sodium (10-25%), and colloidal silicon dioxide (0.1-1%) and their selection was based on a short survey of the United States National Library of Medicine portal where various AMOX capsules belong to different companies including the Indian Companies with their ingredient were mentioned [13]; and their added quantity was derived from available monographs [14]. AMOX and added excipients at different quantity were formulated by quadratic mixture modeling [15] and blended.

A NIR preliminary study was accomplished to determine the optimum number of scans for each sample and number of latent variables. This study proclaimed 32 scans and for maximum variability it entailed six latent variables (LVs). Therefore as per the ASTM guidelines for sample set during method development which define 6× (number of LVs + 1) sample for calibration and 4× (number of LVs) for validation set were opted out [11]. Samples were formulated accurately, providing inconsiderable variability to create a stable calibration model. A total of 78 compositions were prepared by weighing appropriate amount of ingredients on highly sensitive analytical balance stored in 10‑mL scintillation vials and ordered mixing were done manually. Samples were formulated accurately, providing inconsiderable variability to create a stable calibration model. Additionally to provide the robustness, some generic AMOX commercial capsules were evaluated as independent validation samples using the predictive model.

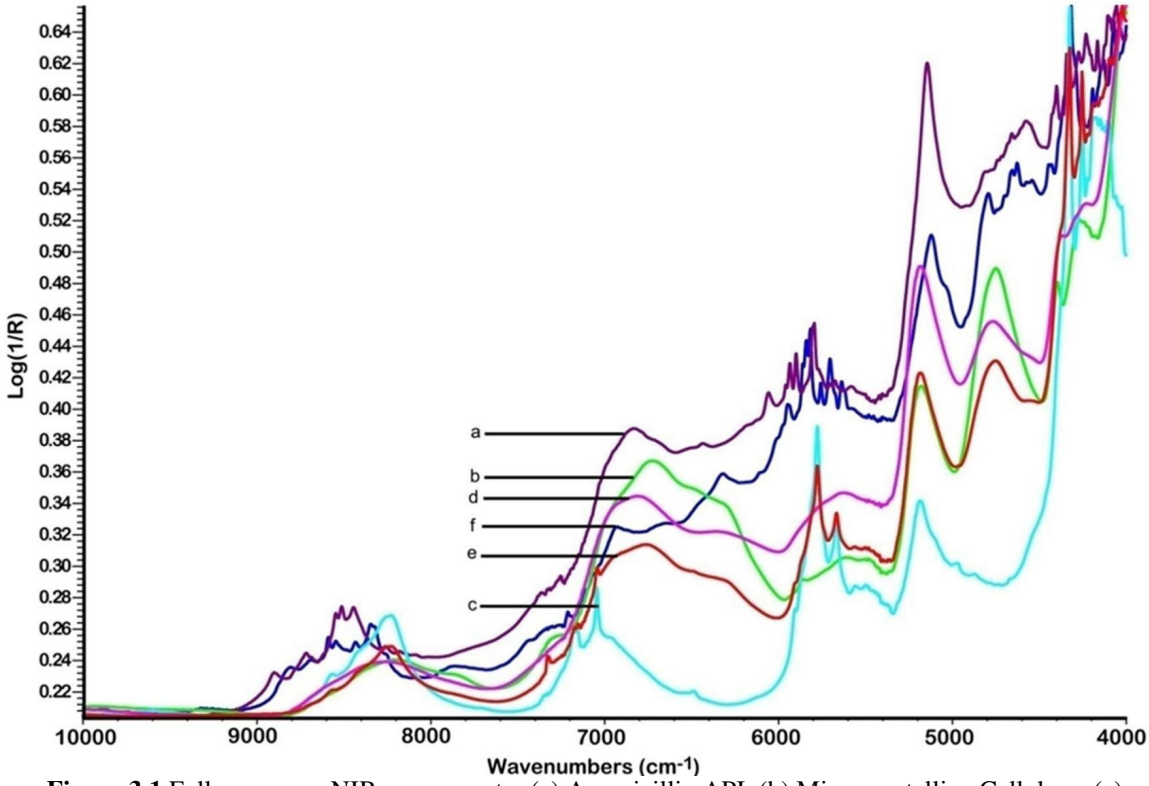

Figure 3.1 Full range raw NIR mean spectra (a) Amoxicillin API, (b) Microcrystalline Cellulose, (c) Magnesium Stearate, (d) Crosscarmellose Sodium, (e) Placebo, (f) Cefadroxil RS

3.1.3.2. Near infrared spectroscopy data acquisition

This study was accomplished in three stages; model development with optimization, validation and model applicability on real samples. Method development with optimization was determined with a set of 50 calibration standards known as a training set and validated with 28 validation standards known as test set containing the same original AMOX range as in calibration samples.

A placebo of all excipients in equal amount was also formulated, and the total content of each formulation was divided into three aliquots. Simultaneously spectra were recorded in triplicate using integrating sphere in diffuse reflectance mode at 8 cm^{-1} resolution over the spectral range of wavenumber 4000–10,000 cm^{-1}. To avoid the error during NIRS, each spectrum was acquired after shaking or whirling of the sample vial for mixture homogeneity as per recommendations [8] with 32 scans corresponding to measurement time on few seconds. Hence, 711 spectra were procured totally on account of 78 formulations including placebo. External validation set spectrum were acquired separately. Figure 3.1 shows the pure NIR raw spectrum of AMOX and a similar molecule

cefadroxil and all excipients except colloidal silicon dioxide. Before developing chemometric model, a standard analysis of variance (ANOVA) between two randomly selected calibration standards was checked for feasibility, and a high value of F-ratio showed sufficient variation between the samples thus allowed us to continue for method development. Every time before scanning, powdered sample in the vial was rotated and not tapped, as tapping can cause particles segmentation which may give greater density at the bottom of the powder sample and hence increase the reflectance.

3.1.4. Reference Method

IP prescribes IR and HPLC for identification and assay respectively for AMOX trihydrate product [16]. Thus being highly sensitive; HPLC was preferred for both identification and assay. The peak homogeneity of each chromatogram was expressed in terms of peak purity values. Resulted assay values were used as reference values in NIR-chemometric model development.

3.1.5. Chemometric Method Development for Identification

On the ground of mahalanobis distance, kennard-stone algorithm [17] was used for selecting 50 calibration standards out of 78 formulations as shown in Figure 3.2 using the prospectr package in R Software (The Comprehensive R Archive Network) [18] For developing AMOX identification model; Discriminant analysis algorithm was applied on 50 standards including a mean spectrum of pure AMOX API and mean of six spectra of placebo. Finally three outliers were observed, and the model was optimized after removing two outliers except placebo, as it was included by choice to make the model more accurate.

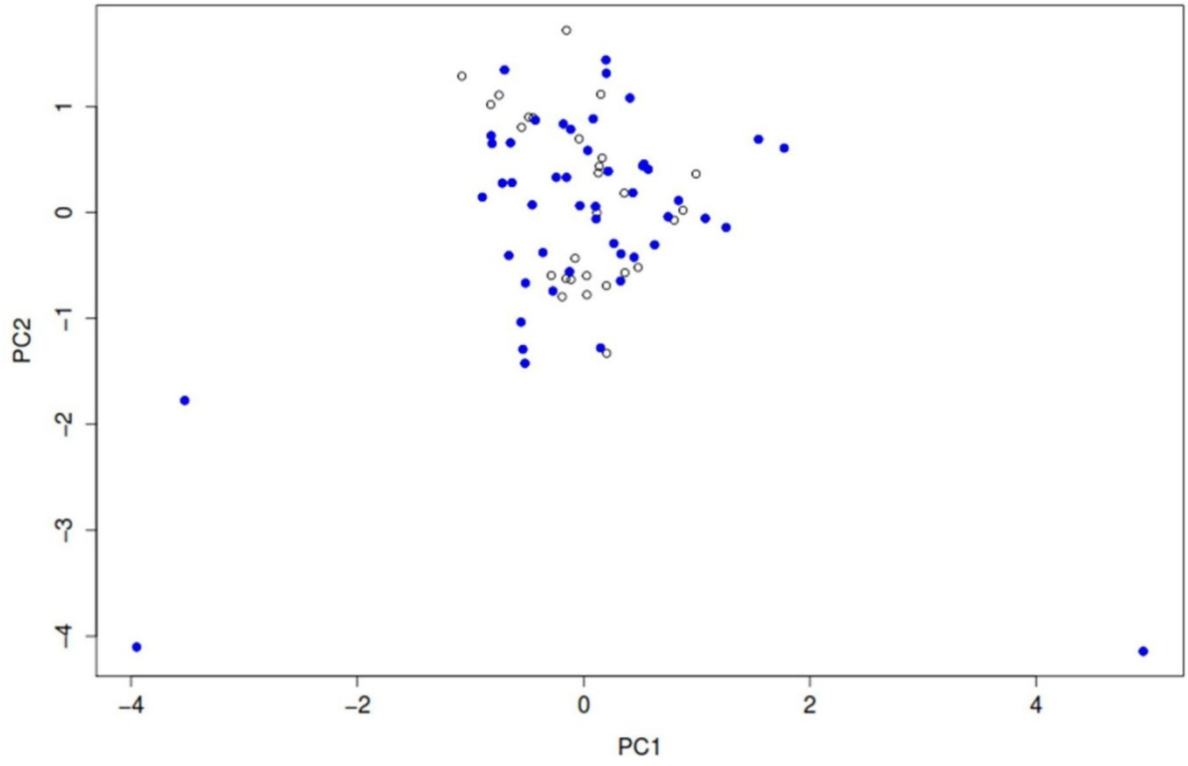

Figure 3.2 Determination of calibration/training set (blue solid circles) using kennard-stone algorithm for 50 samples out of 78 samples

3.1.6. Chemometric Method Development for Quantification

Same 50 calibration standards were utilized for quantitative analysis using two well known factor analysis based multivariate techniques that are PCR and PLS. Each spectrum was pretreated with various data processing techniques. Initially a calibration curve between reference value and NIR predicted values was plotted using six factors and resulted in 0.9861 of correlation coefficient with RMSEC of 3.68% which was not justifiable. Like identification model, there were three spectral leverages found in this evaluation as done by the Chauvenet test [19] as shown in Figure 3.4, where except placebo two other outliers were removed. For applying chemometric on spectral data for creating the calibration; spectral pretreatment methods and wave number range of interest must be selected. Being the multivariate method, the entire range found to be rich in information was considered. However, during optimization of the model, regions of interest were selected accordingly as shown in Figure 3.3.

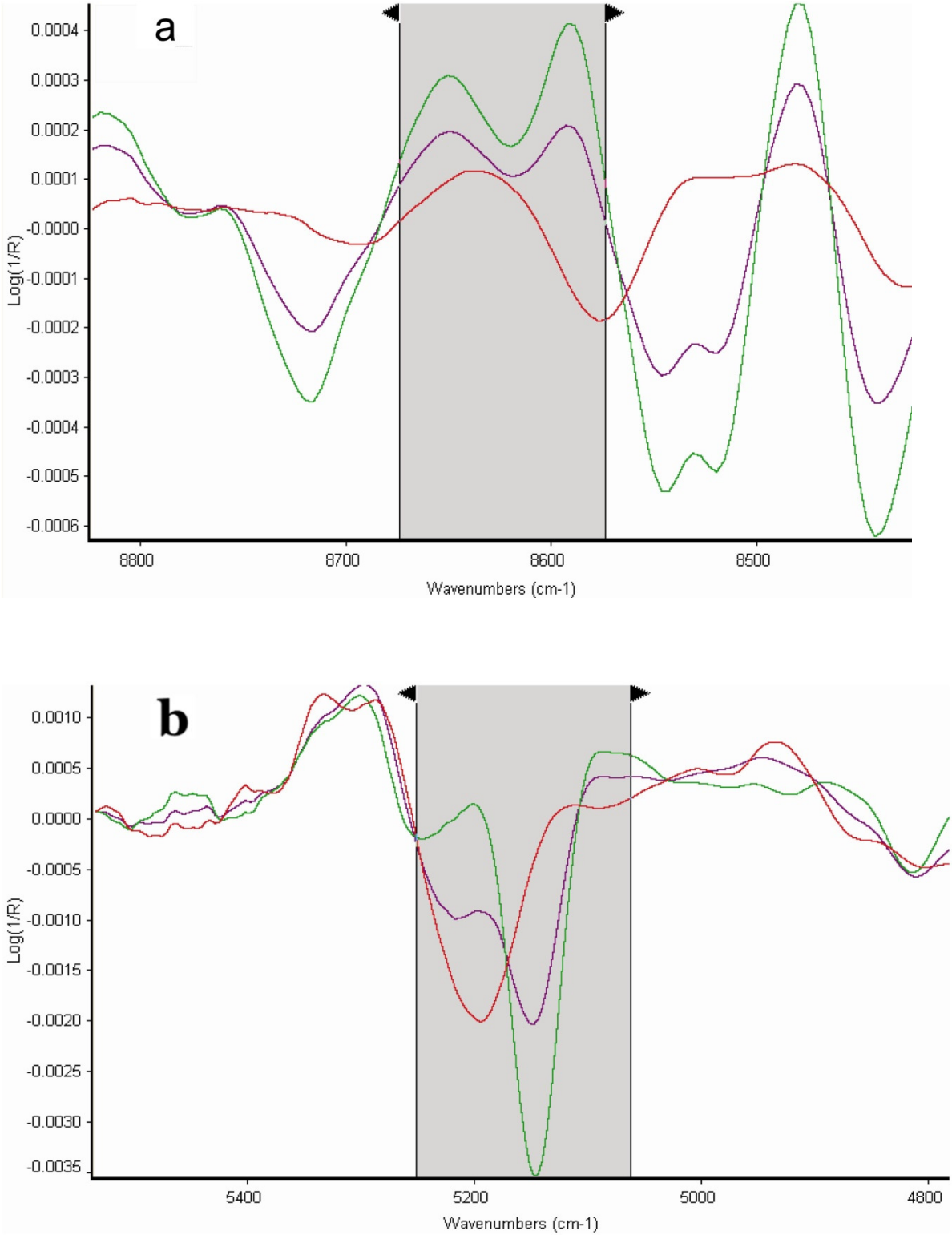

Figure 3.3 Region of interest (a) and (b) in the second-derivative spectra of calibration/training set.

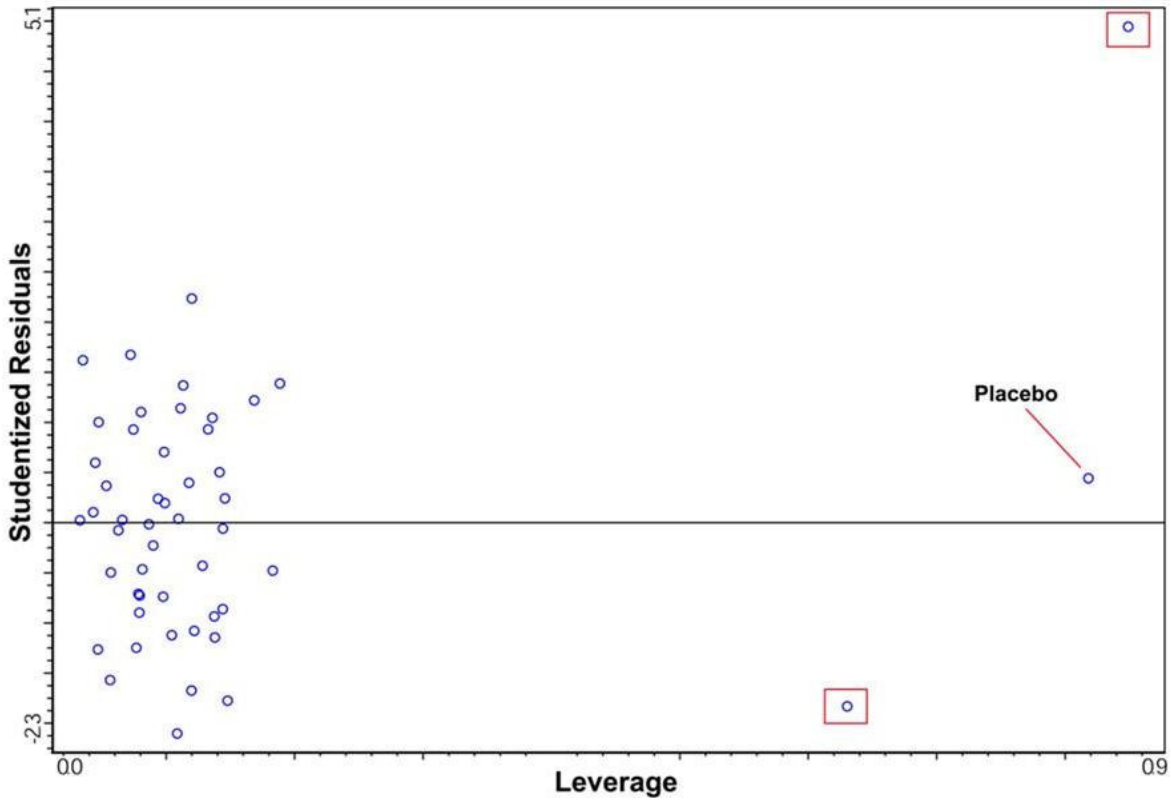

Figure 3.4 Outlier detection plot for amoxicillin calibration/training set, high leverage value shows outlier as shown in boxes

Several chemometric models were developed, as mentioned in Appendix A.1, to demonstrate the ability of NIRS. Model accomplished covering multivariate algorithm such as PLS and PCR followed by leave one out cross validations. There were 96 regression models were devised (see Appendix A.1) and based on the p-value of intercept and slope along with minimum RMSEC and RMSEP, many models were filtered out as shown in Table 3.1 and ultimately a PLS model finally opted based on latent variable, RMSECV value. In this NIR model, data was first pretreated with SNV; and then second derivative with Norris filtering were applied as shown in

Figure 3.5. For this model, factor 6 was optimal for maximum variability as shown in Figure 3.6 and Figure 3.7. It is feasible to identify from second derivative spectra, two regions of high correlation, especially located from 5064 to 5253 cm^{-1} and 8573 to 8674 cm^{-1} as shown in Figure 3.3. The first region corresponds to the fundamental combination band of O-H stretching and C-H bending while the other region corresponds to a second overtone of C‑H bending. Thus, this model was not only based on purely mathematical calculation but was an artifact corresponded to the notable spectral region.

Table 3.1 Models selection based on good performance out of 96 models

	Models			
	PCR[a]	**PCR after outliers removal[a]**	**PLS[b]**	**PLS after outliers removal[b]**
Number of calibration samples	50	48	50	48
Number of validation samples	28	28	28	28
Number of latent variables	10	10	6	4
RMSEC(%)	5.34	1.97	3.16	2.17
RMSEP(%)	5.71	2.37	2.69	2.38
RMSECV(%)	9.62	2.39	6.92	2.43
Model Performance-RMSE(%)	5.35	1.77	2.22	1.35

[a]*With SNV correction, Second derivative, no filtering,* [b]*With SNV correction, Second derivative, Norris filter*

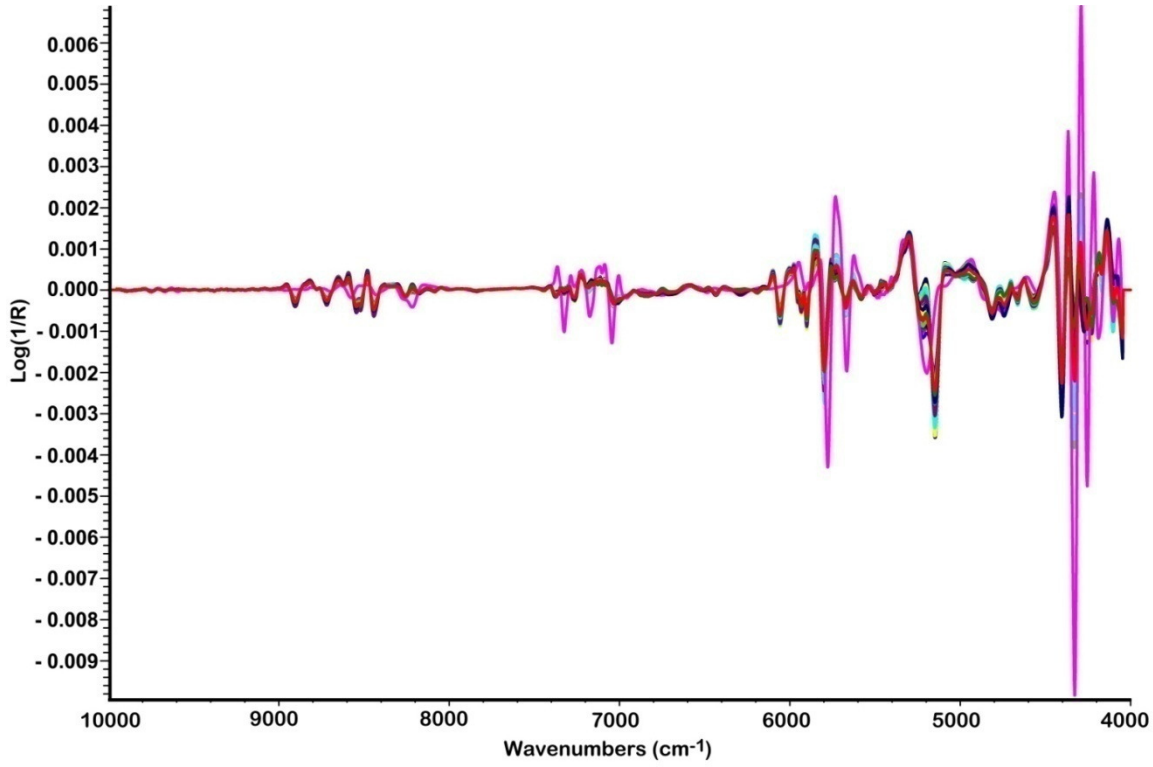

Figure 3.5 Full range second-derivative overlaid spectra of amoxicillin calibration set with Norris derivative filter after standard normal variate pretreatment.

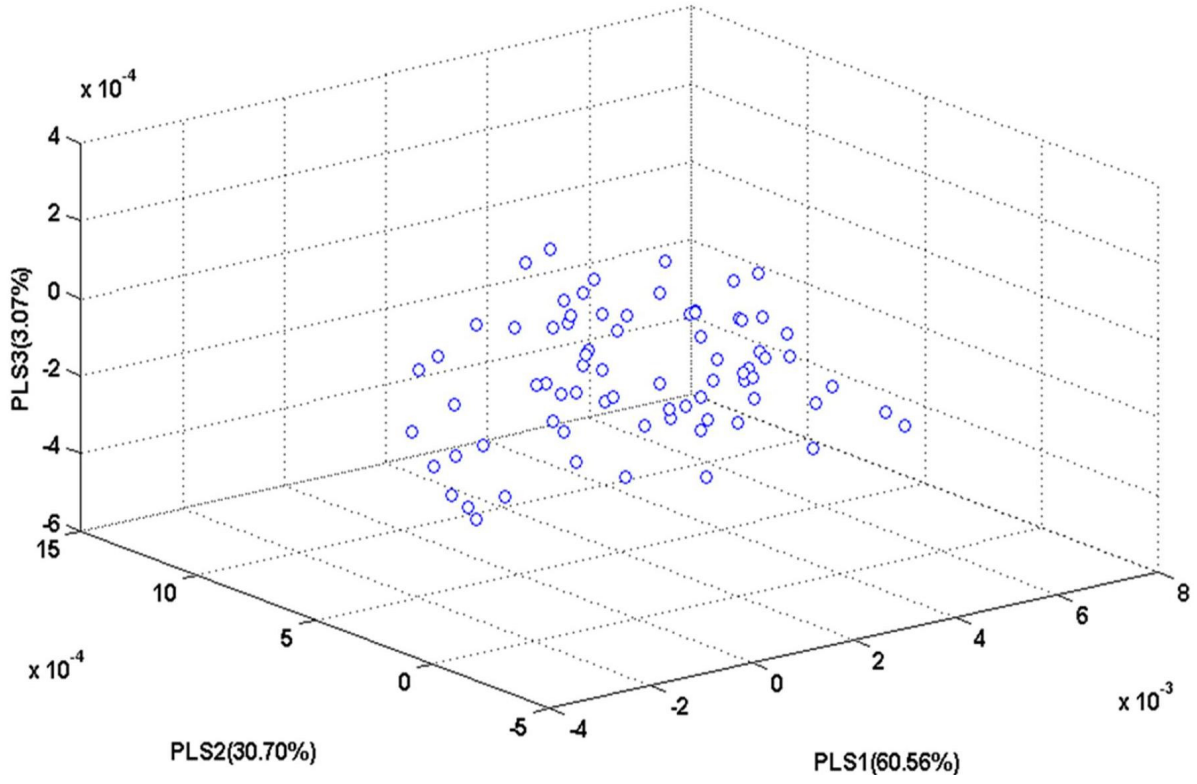

Figure 3.6 Scatter Score plot as variance for PLS1-PLS2-PLS3 obtained for amoxicillin data set

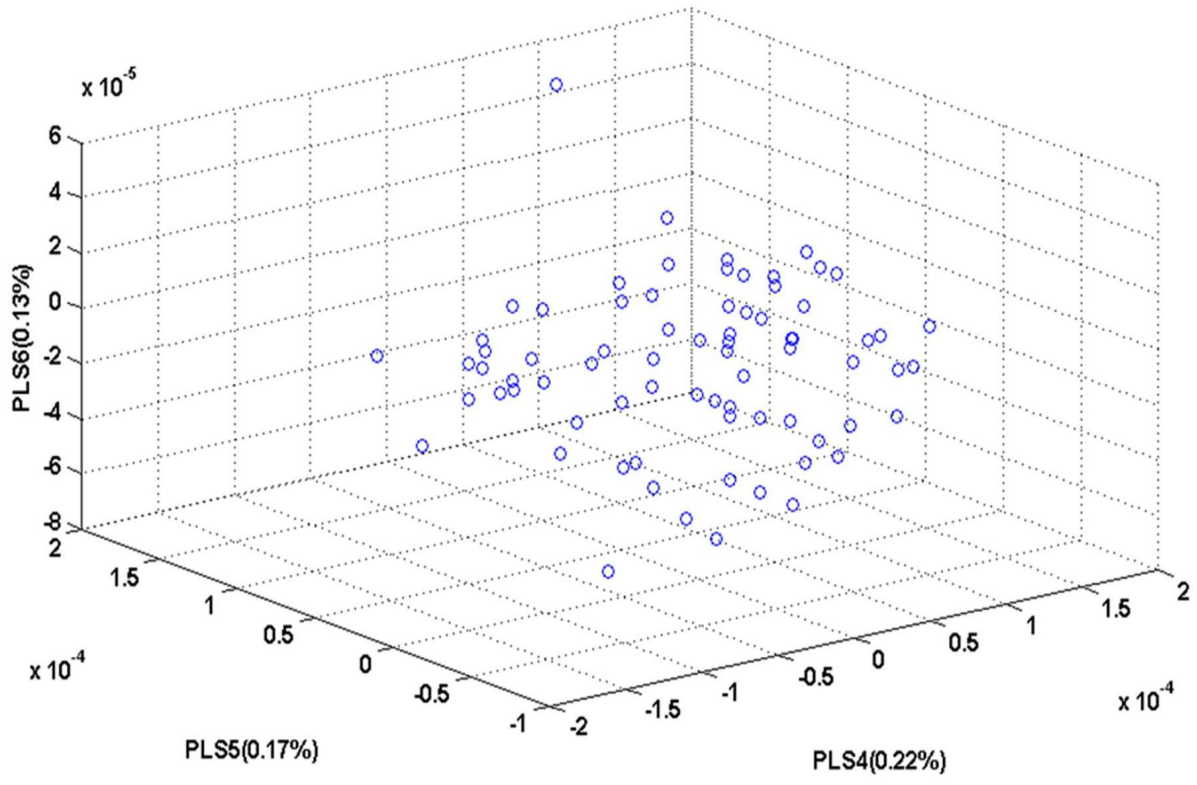

Figure 3.7 Scatter Score plot as variance for PLS4-PLS5-PLS6 obtained for amoxicillin data set

3.1.7. Chemometric Method Validation for Identification

For validation, samples must be independent and cannot be included in the training set during development. Samples used as independent validation set were divided into two sets; internal validation set or test set, and external validation set.

The test set was comprised 28 samples. And since ampicillin classifies as penicillin; and cefadroxil belongs to cephalosporin family which is much similar to penicillin class, therefore, external validation set included the commercial product of each ampicillin and cefadroxil along with three AMOX commercial products. In addition, for the specificity of the model, cefadroxil RS was included in the external validation set as the negative control to design a robust model.

3.1.8. Chemometric Method Validation for Quantification

There were 28 samples used as the internal validation sample. In accordance with the recommended validation parameters by EMA and ICH guidelines like specificity, linearity, accuracy, precision, and robustness; demonstration for quantitative analysis were covered using five independent formulations and three commercial real capsules as external validation set.

3.1.9. Results and Discussion

3.1.9.1. Model development and validation for identification

The detection and the subsequent identification of AMOX relied heavily on the overtone region of the NIRS spectra. Using the DA algorithm mean spectrums of three aliquots for each sample in triplicate were calculated and distribution model generated by estimating the variance at each data point of spectrum in the range of analysis. Identification model was optimized on 48 samples training set. Principle components up to 6 described the 99.9% variability as shown in Table 3.2. Specificity of the model was evaluated with cefadroxil RS as a negative control. The result suggests that the proposed model is well efficient to differentiate AMOX from cefadroxil and ampicillin also as depicted in Figure 3.8.

Table 3.2 Eigenvalue analysis for identifiaction of amoxcillin

Principal Component	Full Spectrum Contribution(%)	Analysis Region Contribution(%)
1	82.1084	74.8052
2	92.927	93.3556
3	96.6501	98.6079
4	99.6079	99.7681
5	99.842	99.9267
6	99.933	99.9547

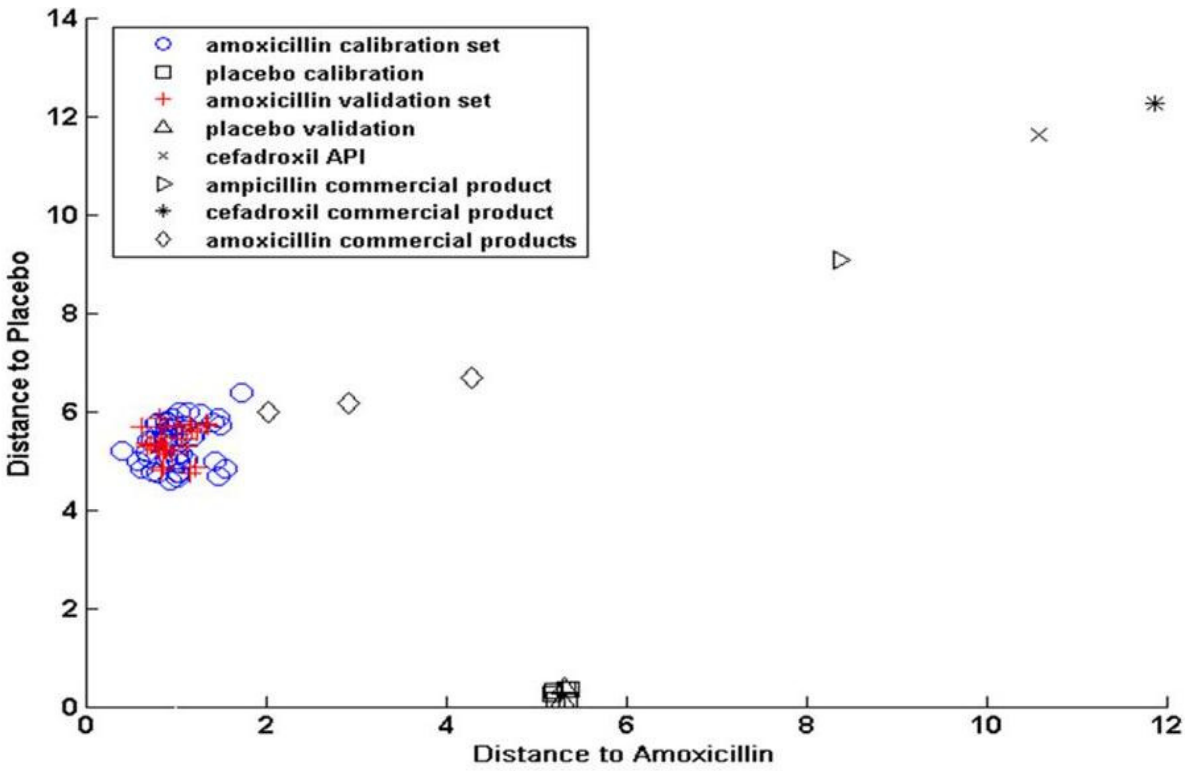

Figure 3.8 Identification of amoxicillin sample by discriminant analysis based on mahalanobis distance

3.1.9.2. Model development and validation for quantification

NIRS quantitative method was established to explore the quantity of amoxicillin in AMOX capsule formulations. A quantitative model was optimized on 48 samples training set using PLS with latent variable up to four which shows maximum spectral information. However, before removal of outliers LVs (factors) were six. As a result, four LVs a good

correlation coefficient was found to be 0.9937 with RMSEC 2.17%. The accepted PLS models were eventually used to predict the AMOX in 28 samples test set. The accuracy of the NIRS prediction equation was then evaluated using linear regression analysis between NIRS-predicted values and those acquired by the reference HPLC method. Therefore, RMSEP of 2.38% was estimated which indicate a high degree of correlation in this method. This correlation between the spectra and reference values was examined using established PLS method and was optimized by cross-validation. With cross-validation, each sample was eliminated one at a time from the training set, then a new calibration was executed, and a predicted score was determined for the removed sample. This mechanism was repeated until every sample had been omitted once using the leave one out method. In this case to determine the model over fitting, minimum predicted residual sum of squares (PRESS) and RMSECV of 2.43% were estimated with factor four as shown in Table 3.3.

Table 3.3 Effect of PLS factors on PRESS and RMSECV for amoxicillin quantitative model

Factor	PRESS Value	RMSECV(%)
0	19118.18	19.95734
1	542.7632	3.36267
2	420.2064	2.95877
3	317.9843	2.57384
4	282.9449	2.4279
5	298.4666	2.4936
6	360.5332	2.74064
7	338.8173	2.65682
8	251.3927	2.28853
9	262.3107	2.33769
10	268.2074	2.36382

The proposed quantitative model was validated using parameters usually recommended such as accuracy, precision, linearity, and robustness in accordance with the ICH and EMA guidelines.

3.1.9.3. Accuracy

Accuracy of the proposed model was obtained by performing a comparison of NIR predicted data with the reference HPLC data that was shown to be accurate conducted at low, medium, and high AMOX amount which is approximately 70%, 90%, and 110%,

respectively, of the label claim. Table 3.4 shows the trueness of the predicted values with a reference method. In paired t-test with eight degrees of freedom, the obtained experimental t-stat value was smaller than the critical t-value; and high p-value than 0.05 alpha value shows that there is no significant difference between the proposed and referenced result with a 95% confidence level. These data show that the percentage difference between the validated NIR method and reference method is insignificant for assay and that there is a good correlation between the two methods with respect to AMOX. As a result, the proposed NIR method can be accounted as a suitable alternative to the reference HPLC method for the evaluation of AMOX capsules. Moreover, there is no official procedure for validating NIR accuracy as such; thus it may be evaluated through RMSEC, RMSEP, and RMSECV which are also known as figure of merit as shown in Table 3.5.

Table 3.4 Accuracy evaluation between proposed model and reference method of amoxicillin

Sample	Pharmacopoeial HPLC Assay (%)*	NIR Predicted Assay (%)*	t-test paired p-value
Low (70%)	69.75±1.04	67.38±0.57	0.2843
Medium (90%)	89.36±0.25	89.92±1.48	0.0994
High (110/%)	110.01±0.74	109.14±0.14	0.0918

**Mean values and standard deviation of each sample in triplicate.*

3.1.9.4. Precision

Repeatability was evaluated as intraday precision that was performed with the same analyst on three independent validation samples and applying the model to the same formulation samples three times on three consecutive days. In this study, the precision was estimated by three determinations I, II, and III at 80%, 90%, and 110%, respectively, in triplicate as mentioned in Table 3.5. Less than 2% relative standard deviation shows that the proposed method may be considered as precise in relation to HPLC method.

Table 3.5 Validation parameters for evaluating the proposed NIR amoxicillin model for quantification

Validation Parameter	Validation Unit	Value
Accuracy (Figure of merit)	RMSEC	2.17%
	RMSEP	2.38%
	RMSECV	2.43%
Precision (Repeatability)	RSD Ist determination	0.65%
	RSD IInd determination	0.44%
	RSD IIIrd determination	1.37%
Linearity	Slope	0.958
	Intercept	4.580
	Correlation coefficient (*r*)	0.996
Robustness	Predicted AMOX amount at	
	T= 22 ^{o}C	100.2±0.75%
	T= 25 ^{o}C	100.9±1.65%
	T= 28 ^{o}C	100.1±1.22%
	Variation between groups, p-value	0.64

3.1.9.5. Linearity

Linearity correlation between the predicted values from selected PLS model and the reference value of three determinations in triplicate as 70%, 90%, and 110% was evaluated. Linear regression analysis was performed between the two applied methods and the compatibility of results was estimated by general equation:

$$y = bx + a$$

where y is the NIR predicted value, x is reference value, a is intercept and b is the slope. For AMOX determination, the regression equation was y = 0.958x + 4.58, being the confidence interval for the slope (0.882; 1.035) and for the intercept (–2.35; 11.51) included 1 and 0, respectively, as mentioned in Table 3.5. Therefore within the AMOX range of the training set the proposed model for AMOX allowed a suitable linearity with r = 0.994 for all sample set when it is compared to the reference method as shown in Figure 3.9.

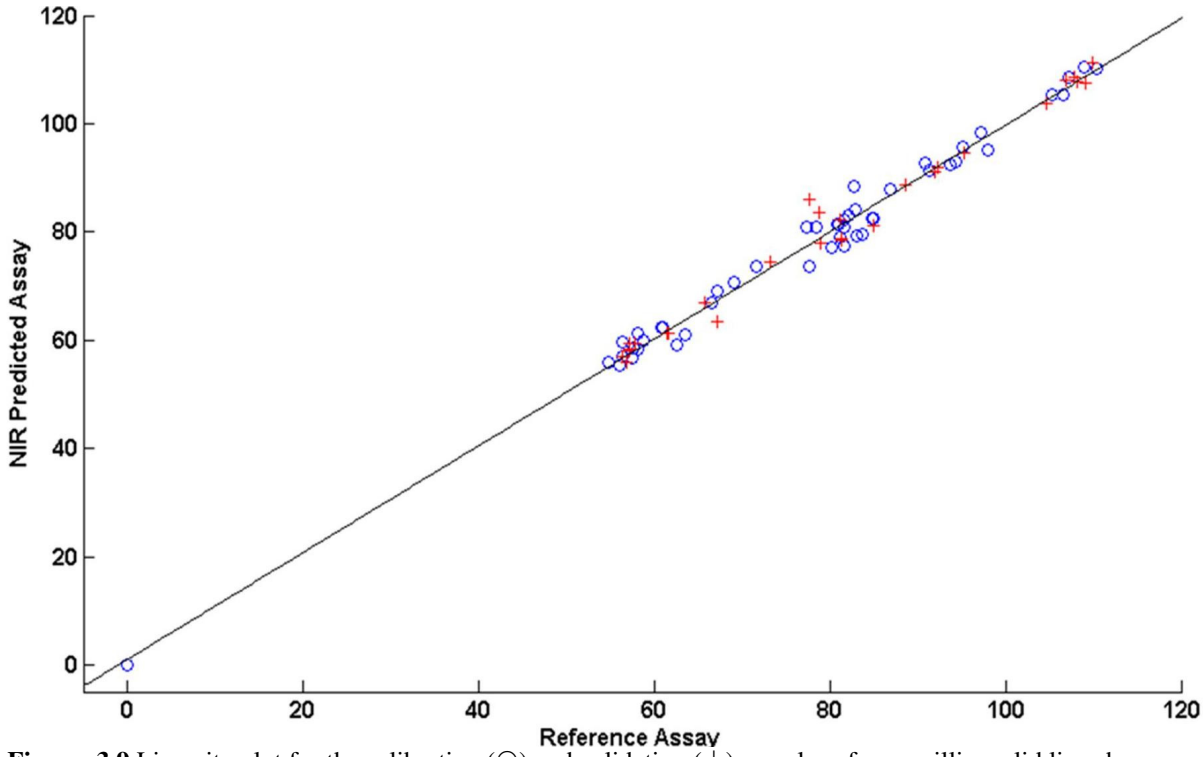

Figure 3.9 Linearity plot for the calibration (○) and validation (+) samples of amoxcillin, solid line shows the data fit

3.1.9.6. Robustness

Robustness of the proposed method was assessed through the results of three determinations in triplicates of a 100% label claim AMOX sample at three different temperatures as shown in Table 3.5. Percent RSD was less than two and result were unaffected by small and deliberate temperature variations and found no significant difference between the predicted values and the reference result, upon paired t-test; p-value greater than 0.05 shows reliability and a good agreement between both analytical methods.

3.1.10. Model Assessment on Real Samples

The identification and determination of the content of three generic samples were carried out by applying the HPLC method reported in AMOX monograph of IP. NIR proposed identification and quantitative model was applied on same commercial products and obtained results were finally compared with the result estimated by the reference method. For identification based on DA; all three AMOX commercial sample found close in distance to AMOX formulated samples while others similar molecules like cefadroxil and

ampicillin commercial products were clearly distinguished as shown in Figure 3.8. In quantitative analysis, content assay estimation from proposed method and reference method was compared by applying paired t-test which shows NIR method to be an alternative and effective method as mentioned in Table 3.6.

3.1.11. Conclusions

The present analysis supports the points raised in several research articles that an NIRS in combination with chemometric analysis can perform equal to or often with very small error than the reference method. Present NIR-chemometric models implied as good correlation with the reference HPLC method. Moreover, validation result verified that developed methods were as accurate as reference analytical technique. Thus, it has been shown a feasible alternative to HPLC for the identification and assay of AMOX capsules. These models emphasize the importance of NIRS and chemometric analysis because it is fast, nondestructive and can be employed to analyze solid sample with minimal or no sample preparation. Pharmaceutical regulatory authorities are expected to provide alternate and quick techniques for routine analysis specifically for a large number of samples and therefore in the rapidly growing area of analytical method development NIRS have been introduced as a challenging field. The proposed model will be used for identifying and assaying a large number of AMOX products quickly and may be utilized for quality profiling of spurious and substandard medicines. It takes less than two minutes to analyze a sample once the calibration model has been set up. We hope this research will stimulate the quality assessment study of other antibiotics and other category of drug products in India and in other countries as well.

Table 3.6 Amoxicillin market products evaluation by NIR proposed model

Market Sample	Average content weight of a capsule (mg)	Product label claim of amoxicillin(mg)	Pharmacopoeial HPLC Assay (%)	NIR Predicted Assay (%)	t-test paired p-value
AMOX-01	293.65	250	90.36±0.37	90.29±0.56	0.4554
AMOX-02	294.86	250	93.57±0.5	93.28±1.77	0.6472
AMOX-03	291.76	250	94.65±0.65	96.37±1.65	0.1154

3.2. NEAR INFRARED AND CHEMOMETRIC MODEL DEVELOPMENT FOR DICLOFENAC SODIUM TABLET

3.2.1. Background

Pharmaceutical market is loaded with large number of samples, thus to extract the quality of product we designed NIRS and multivariate analysis. And based on one of the most prescribing non-steroidal anti-inflammatory drugs (NSAIDs) we picked out DICLO which has verified efficacy in treating variety of acute pain and postoperative pain, gout, inflammation in acute and chronic musculoskeletal disorders and dysmenorrhoea [20], [21]. It is among the best known compound of the aryl acetic acid derivatives and widely available as sodium or potassium salt.

Only few developed model are reported for the diclofenac quantification using transmission mode using NIR spectral acquisition on coated tablet [22] and using artificial neural network multivariate method on powder form [23]. In one report diclofenac API was tested in a formulation with having only one excipients [23]. Therefore, the purpose of this study was to design fast, specific and accurate models to identify and quantify the main analyte that is diclofenac from a complex matrix by applying NIR spectroscopy and multivariate analysis. Thus, rapid identification and estimation of the main analyte of the formulation sample with the NIR method has been demonstrated and validated. And at last they were applied on some commercial market products to assess the scope of theses proposed models.

Development of NIRS model requires chemometric analysis in combination with NIRS spectral acquisition that defines the scope of NIRS procedure used for the intended purpose. European Medicine Agency (EMA) has published a guidelines draft for the NIRS calibration and validation, otherwise except this no harmonized regulation are established or drafted. PCA, SIMCA, DA, cluster analysis and correlation algorithm are used for calibration and optimization of identification or qualitative procedure. On the other hand for quantitative procedures LVs for PLS regression; and principal components (PCs) for PCR are employed [24]–[27].

3.2.2. Materials, Instruments and Software

Diclofenac sodium, magnesium stearate, talc and croscarmellose sodium were gifted by Ranbaxy Laboratories Ltd. India. Microcrystalline cellulose (Avicel) was provided as gift sample by FMC Biopolymer Brussels, Belgium and colloidal silicon dioxide (Aerosil) by Evonik Industries, Germany.

Diclofenac sodium RS was directly procured from Sigma Aldrich. Methanol (Lichrosolv), phosphoric acid (EMSURE), monobasic sodium phosphate GR grade was purchased from Merck (India). Milli-Q water from Millipore (Waltham, MA, USA) was used during HPLC analysis.

A Thermo Scientific Antaris II FT-NIR analyzer equipped with an integrating sphere coupled with InGaAs detector was utilized to induce diffuse reflectance spectra and data was assembled by means of inbuilt RESULT software. All NIR data was further analyzed using chemometric software TQ Analyst 7.2.0 (Thermo Fisher Scientific Inc., MA, USA), along with the R version 3.0.3 and statistical software MATLAB version 7.6.0 (MathWorks, Natick, USA).

High performance liquid chromatography was employed as reference method. Thus, an Alliance 2695 separation module and 2996 photodiode array detector (Waters, Milford, MA, USA) was used with Waters C-18 (250 mm × 46 mm, 5µm) column. And chromatograms were extracted using Empower Pro software (Waters).

3.2.3. Methods

3.2.3.1. Preliminary study

For the development of models, a preliminary study was done to check the required number of formulations. Thus LV and number of optimal scans were estimated after conducting as preliminary analysis and as a result 5 LV for maximum variability and 32 scans were found adequate for superior precision. In accordance with ASTM guidelines, for training or calibration set ample number of sample that is 6 × (number of LVs + 1); and for test or validation set 4 × (number of LVs) sample are recommended for making model [11]. Thus 67 compositions in total were formulated and divided in to calibration (training) and validation (test) set according to ASTM guidelines.

3.2.3.2. Tablet content formulation

Tablet formulation was accomplished using DICLO as API within a range of 70-130% of 50 mg. Formulation with 50 mg of content was considered as 100% and total weight of the each tablet formulation was 100 mg. Also every formulation was composed up to one gram which evolved ten tablets. These tablets were kept in powder form without make them compressed. Often used excipients like microcrystalline cellulose (5-20%), magnesium stearate (0.5-5%), talc (1-30%), croscarmellose sodium (0.5-5%) and colloidal silicon dioxide (0.1-1%) [13], were weighed in range according to recommended specifications [14] and blended with API using quadratic mixture modeling process [15]. Samples were formulated accurately for providing inconsiderable variability to create a stable calibration model. Therefore, samples were prepared by weighing appropriate amount of ingredients on high sensitive analytical balance which stored in 10-mL scintillation vials and ordered mixing were done manually.

3.2.3.3. NIRS data acquisition

Each powder formulation contents was divided in to three aliquots and kept in a quartz flat bottom vial. Using Integrating sphere, diffuse reflectance was measured with in a spectral range of 4000 to 10000 cm^{-1} with 8 cm^{-1} spectral resolution. Equal amount of all excipients were mixed and used as placebo. For each sample, a mean spectra of triplicate spectrum was used for calculation and each spectrum was collected from the average of 32 scans. Figure 3.10 shows the pure NIR raw spectra of diclofenac, placebo and all excipients.

3.2.3.4. Data pretreatment

Due to non chemical information like particle size and water content etc.; spectral data preprocessing is highly essential. Therefore, different pretreatments were applied during model optimization like first and second derivative transformation, savitzky golay filtering, norris derivative filtering, peak normalization, MSC, SNV [28], [29]. However it should be noted that eventually no smoothing was applied to the raw spectra as there was no difference in the spectra before or after this pretreatment. Baseline analysis was also applied

on the raw spectra but there was no change in loading peaks so the raw spectra were analyzed without pretreatment at constant pathlength.

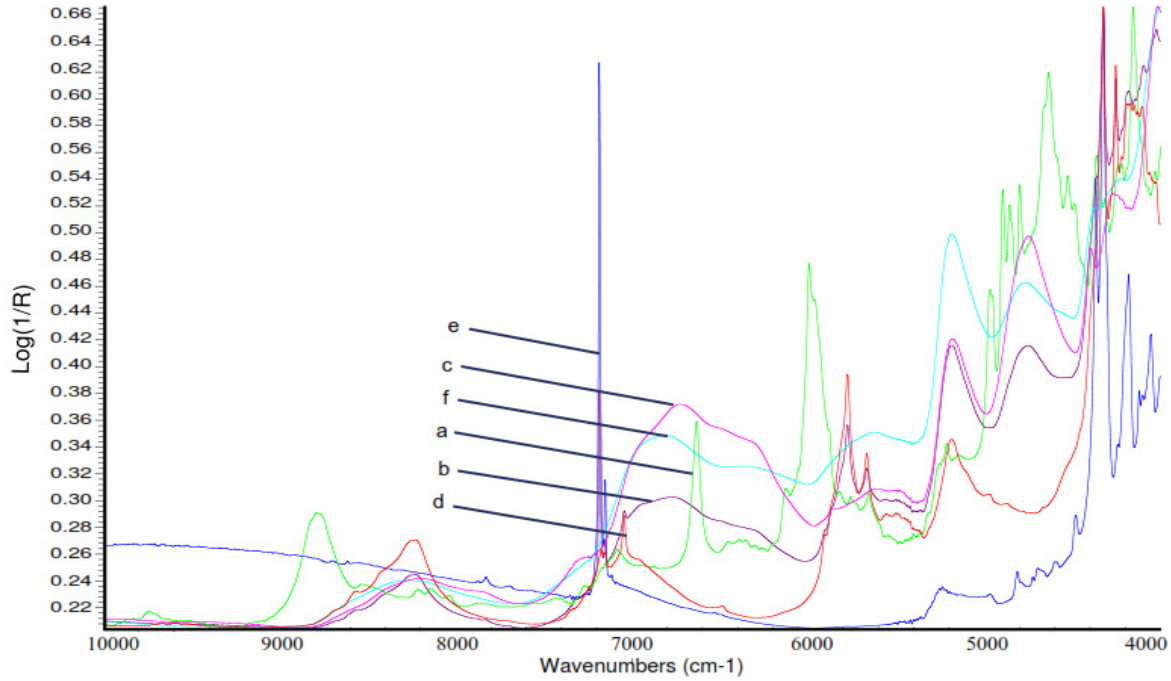

Figure 3.10 Full range raw NIR mean spectra (a) Diclofenac API, (b) Placebo, (c) Microcrystalline Cellulose, (d) Magnesium Stearate, (e) Crosscarmellose Sodium

3.2.3.5. Selection of training and test set

Based on the mahalanobis distance, 47 sample and 20 samples were selected as calibration and validation set respectively using Kennard-stone algorithm [17] in 'prospectr' package R software [18] as depicted in

Figure 3.11. Five independent samples to the training and test set were also prepared as external validation set for quantitative model development.

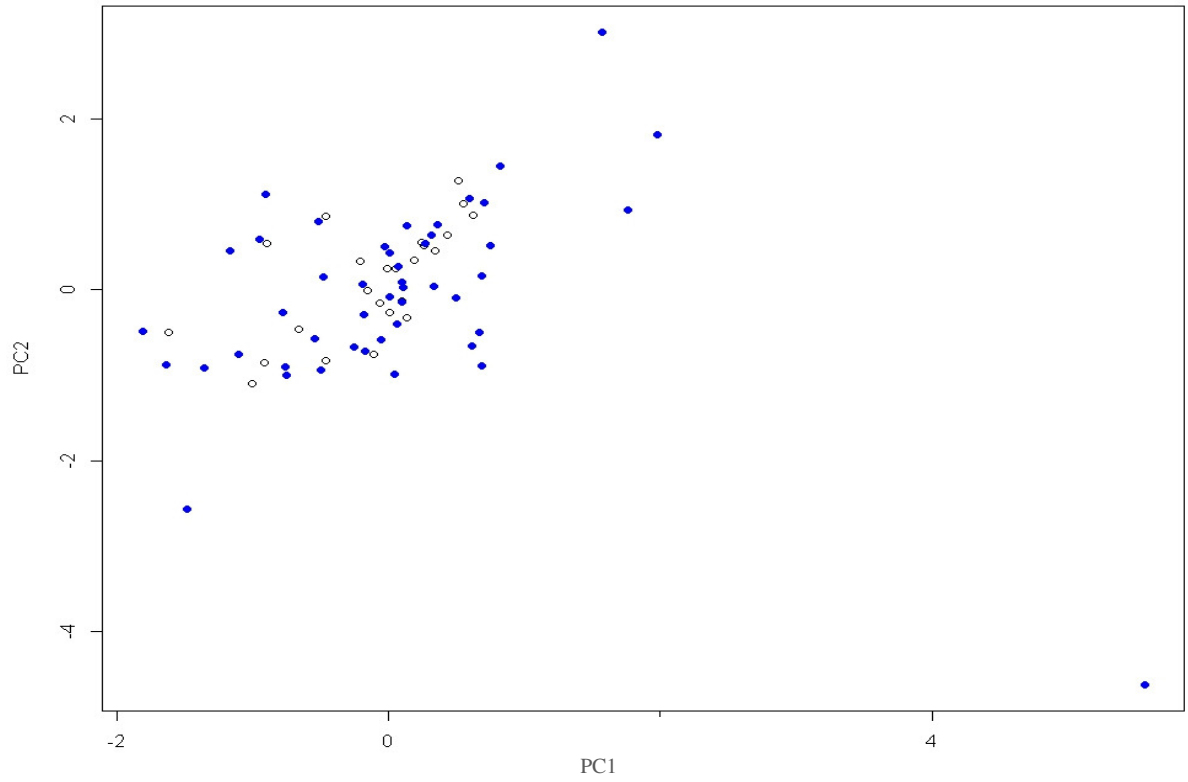

Figure 3.11 Determination of calibration/training set (blue solid circles) using kennard-stone algorithm for 47 samples out of 67 samples

3.2.3.6. Market sample collection

Five commercial sample of DICLO were procured from the open market of Northern India. These samples were used as external validation set for identification and quantitative model assessment.

3.2.3.7. Model development for identification

Using discriminant analysis algorithm, diffuse reflectance spectral library was formed with 47 calibration samples and additionally three samples of placebo.

3.2.3.8. Model development for quantification

For quantitative estimation diffuse reflectance data of the calibration set along with placebo were analyzed using PCR and PLS multivariate factor analysis techniques through

HPLC reference data. There were 96 linear regression models were created (see Appendix A.2).

3.2.3.9. Model validation for identification

Internal validation or test set was constituted of 20 samples and two sample of placebo. Additionally to check the model specificity, a similar commercial product to diclofenac that is aceclofenac was used as a negative control.

3.2.3.10. Model validation for quantification

Model specificity for quantification was covered along with quantitative accuracy, precision, linearity and robustness corresponding to EMA guidelines [30]. Two validations set that is internal validation set which was comprised of 20 samples that were included during model development; and external validation set which was independently formulated, were used to endorse the model validation. These independent validation set were not used in structuring the multivariate model.

3.2.4. Reference Method

UV spectroscopy being a primitive and conventional method was not preferred. Thus to be more authentic; an in-house developed and validated HPLC method was employed to ensure the identity and quantity of DICLO in formulations. In which 0.01M phosphoric acid and 0.01M monobasic sodium phosphate (1:1) buffer of pH of 2.5±0.05 was prepared as solvent mixture which was filtered through 0.20 µm membrane nylon filter and degassed in ultrasonic bath. Methanol and phosphate buffer (70:30) was prepared as mobile phase which further get sonicated and vacuumed for degassing. Methanol and water (70:30) was used as diluents in the preparation of testing sample. For system suitability solution, reference standard of DICLO solution of 0.2 mg/ml was used. Each sample injection volume of 10 µl was analyzed under isocratic mode at 1.5 ml/min for 15 min run time. Obtained chromatograms were processed at 254 nm for identification; and drug peak area was used to calculate the content in the samples. And results were used as reference data for the development of multivariate model with NIR data.

3.2.5. Results and Discussions

The study was accomplished in three phase; model development and optimization, validation and model applicability on commercial samples. Identification and quantification of all samples were processed using in house validated HPLC method against DICLO RS. Peak purity was confirmed by lower purity angle then purity threshold. HPLC assay results were used as reference data for the multivariate model development.

Feasibility of the chemometric model development was checked by ANOVA between two samples of calibration set. And a high F-ratio signified adequate variation thus allowed to start model development. Vial of sample was rotated too and forth before each reading was acquired during NIR data acquisition [8]. Therefore in total 653 spectra were acquired on account of 72 formulations (including five external validation samples) and a placebo.

3.2.5.1. Model development and validation for identification

The diclofenac detection was counted in the overtone region of the spectrum. PC 5 explained the 99.67% variability as shown in Table 3.7 Using commercial samples of one aceclofenac and five DICLO tablets, specificity of the model was determined; and resulted data signified the developed model as a well compatible model to identify DICLO specifically as shown in Figure 3.12.

Table 3.7 Eigenvalue analysis for identification of diclofenac

Principal Component	Full Spectrum Contribution	Analysis Region Contribution
1	86.8	82.9947
2	92.5168	90.5984
3	96.7482	96.1597
4	98.9327	98.5321
5	99.674	99.5651

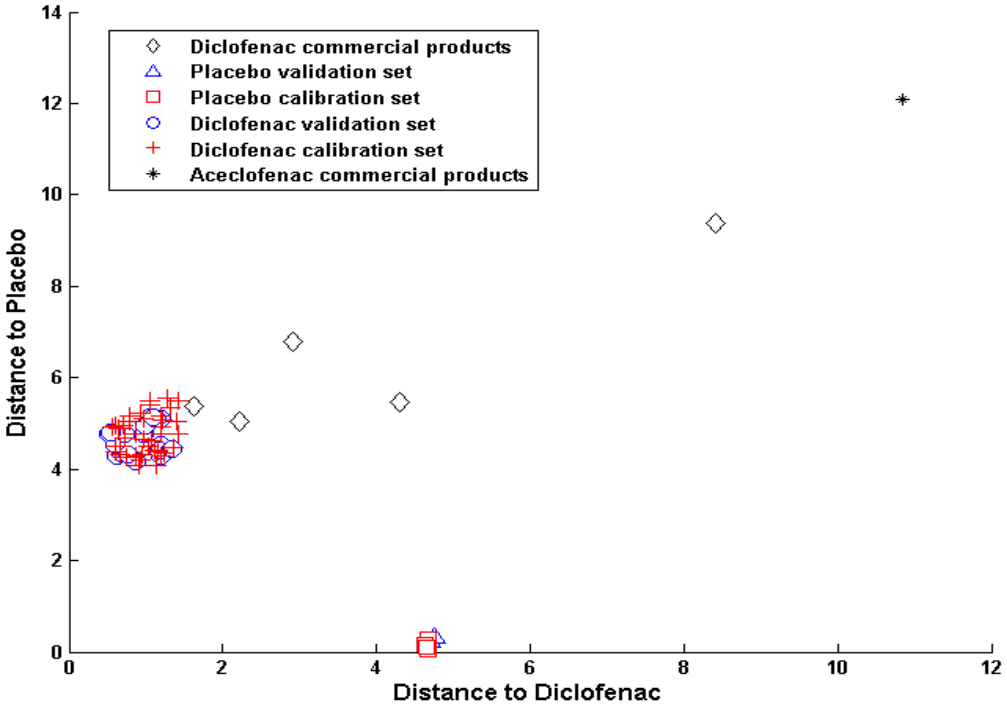

Figure 3.12 Identification of diclofenac sample by discriminant analysis based on mahalanobis distance

3.2.5.2. Model development and validation for quantification

Being multivariate whole range of wavelength was abundant source of information. For illustrating the NIRS ability, different chemometric models were developed, as shown in Appendix A.2.

Based on the p-value of intercept and slope along with minimum RMSEC and RMSEP, among 96 linear regression models with different pretreatment; a model processed under PLS algorithm with constant pathlength and without smoothing pretreatment was opted. This PLS model selection was based on LVs, RMSECV value and overall performance as RMSE. In this case to determine the model over fitting, minimum PRESS and RMSECV of 2.34% were estimated with factor four as shown in

Table 3.8 Effect of PLS factors on PRESS and RMSECV for diclofenac quantitative model

Factor	PRESS Value	RMSECV(%)
0	8455.45996	14.36074
1	5737.55957	11.82964
2	2130.50879	7.20858
3	1481.6217	6.01142
4	1372.60437	5.78603
5	565.42401	2.34291
6	578.36549	2.87654
7	601.65478	2.97142
8	749.65855	3.47857
9	658.47154	3.35871
10	709.52741	3.25436

At first with NIR-chemometric predicted data against reference data selected model have shown a correlation of 0.971 with RMSEC of 2.72 mg (5.44%) which was found to be unjustified for a good model. And that may be because of some leverage. Thus, a plot of leverage against studentized residual [8] was estimated that was identified by the Chauvenet test [19]. Six samples except placebo were considered as outlier and removed from the calibration set due to high leverage value as shown in Figure 3.13. To propose an accurate and specific model placebo was intentionally not considered as outlier, and thus rest of six samples were removed for the calibration set.

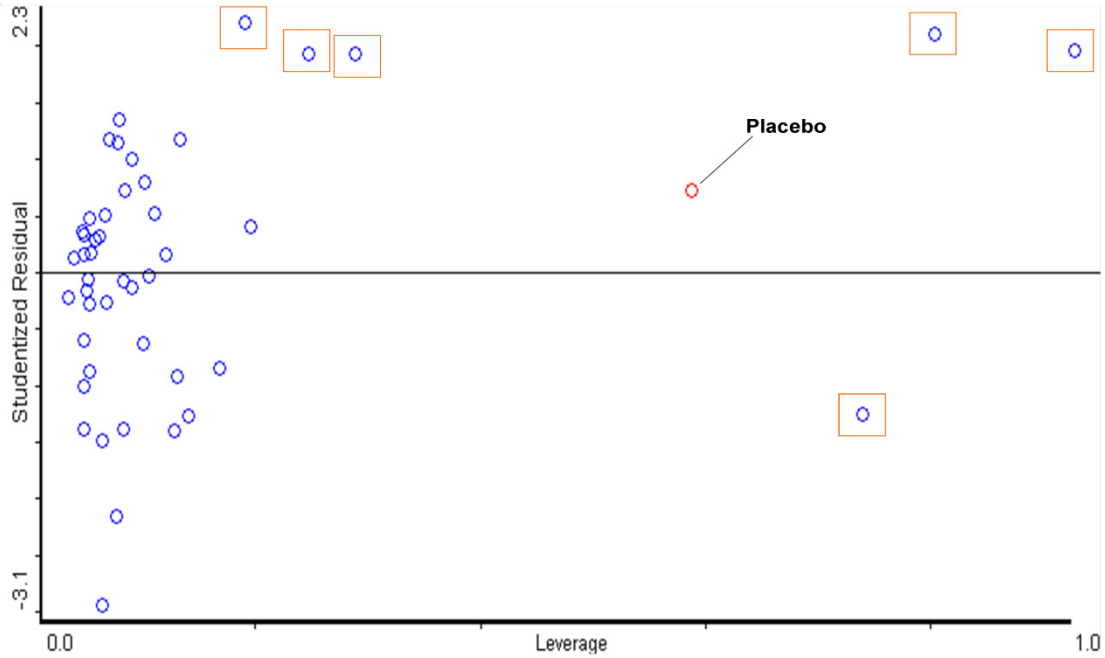

Figure 3.13 Outlier detection plot for diclofenac calibration/training set, high leverage value shows outlier as shown in boxes

Subsequently calibration was done and this time again the latent variables were found to be five to show optimum spectral information as shown in Figure 3.14 and Figure 3.15. Ultimately a good correlation coefficient was established to be 0.991 with RMSEC 1.52 mg (3.04%).

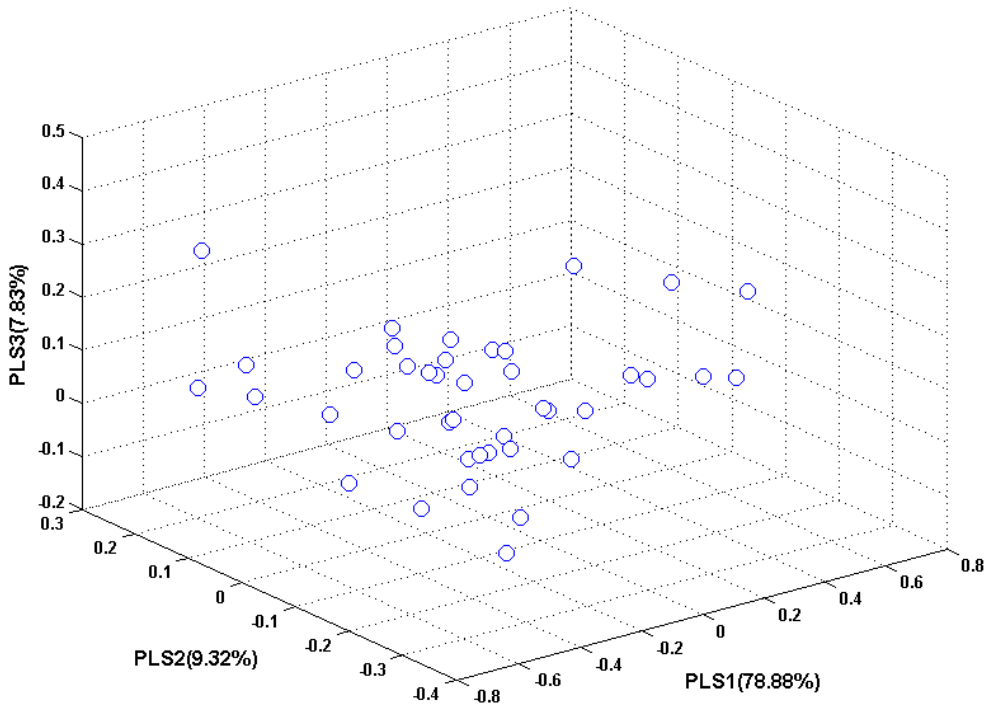

Figure 3.14 Scatter Score plot as variance for PLS1-PLS2-PLS3 obtained for diclofenac calibration set

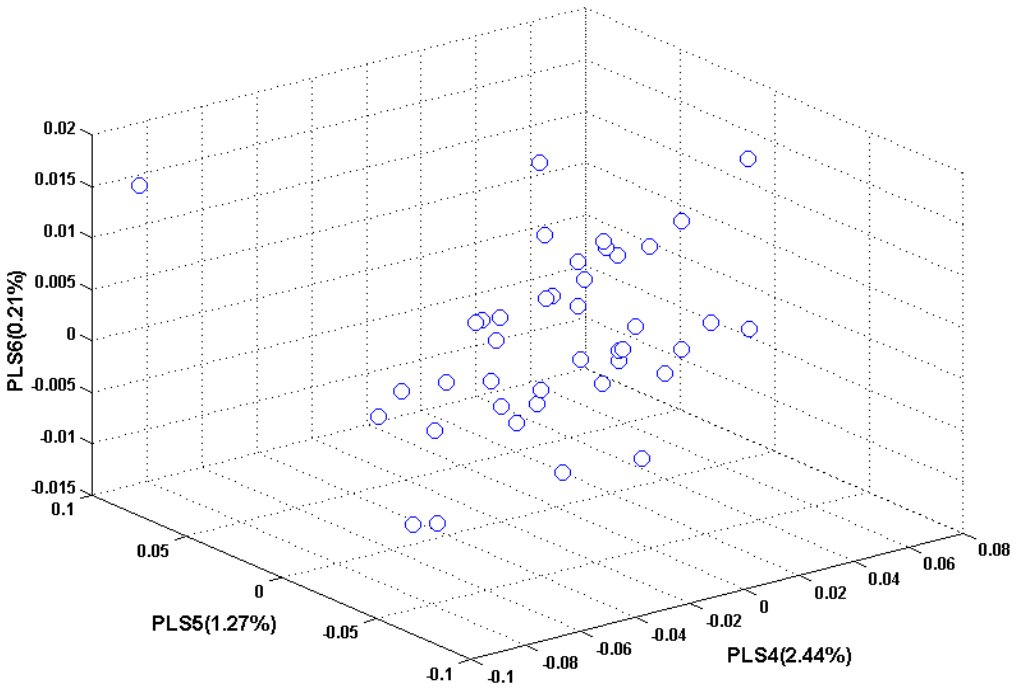

Figure 3.15 Scatter Score plot as variance for PLS4-PLS5-PLS6 obtained for diclofenac calibration set

In accordance to EMA guidelines, internal validation was performed on the developed PLS model to calculate DILCO in the validation set of 20 samples. Linear regression analysis between predicted and reference result were accomplished. Root mean square error of prediction of 1.66 mg (3.32%) signifies high degree of correlation as shown in Table 3.9. The correlation between the spectra and reference values was examined using this established PLS method, and was optimized by cross-validation. With cross-validation, each sample was eliminated one at a time from the calibration set then a new calibration was executed and a predicted score was determined for the removed sample. This mechanism was repeated until every sample has been omitted once using the leave one out method. To overcome the over fitting problem minimum PRESS and RMSECV were estimated. Factor five showed least RMSECV as shown in Table 3.8.

Table 3.9 Validation parameters for evaluating the proposed NIR diclofenac model for quantification

Validation Parameter	Validation Unit	Value
Accuracy (Figure of merit)	RMSEC	1.52 mg (3.04%)
	RMSEP	1.66 mg (3.32%)
	RMSECV	2.34 mg (4.68%)
Precision (Repeatability)	%RSD Ist day	0.62
	%RSD IInd day	0.71
	%RSD IIIrd day	0.65
Linearity	Slope	1.004
	Intercept	-0.329
	Correlation coefficient (*r*)	0.999
Robustness	Predicted DICLO amount (mg) at	
	T= 22 ^{o}C	49.52±0.60
	T= 25 ^{o}C	49.96±0.41
	T= 28 ^{o}C	49.55±0.5
	Variation between groups	
	p-value	0.35

As recommended by the EMA and ICH guidelines the developed model was validated using five independent samples with respect to accuracy, precision, linearity and robustness.

3.2.5.3. Accuracy

Three sample in triplicate containing diclofenac sodium at three different levels that is 70%, 80% and 110% of the label claim, were used to predict the main analyte and compare with the HPLC reference value as shown in Table 3.10. On doing paired t-test, a p-value of more than 0.05 showed no significant difference with 95% confidence between NIR-chemometric predicted and referenced HPLC value. It implied the proposed model to be an alternative to the referenced HPLC method. Another way to show the accuracy of the method is to mention the RMSEC, RMSEP and RMSECV that is also known as figure of merit as shown in Table 3.9.

Table 3.10 Accuracy evaluation between proposed and reference method

Sample	Pharmacopoeial HPLC Assay (mg)*	NIR Predicted Assay (mg)*	t-test Paired p-value
Low (70%)	35.19±0.20	35.46±0.34	0.46
Medium (80%)	39.93±0.07	40.12±0.27	0.24
High (110%)	55.16±0.21	55.30±0.30	0.36

**Mean values and standard deviation of each sample in triplicate*

3.2.5.4. Precision

Using three determination of 100% label claim of diclofenac sodium in intraday precision was estimated by single analyst on three consecutive days as shown in Table 3.9. A %RSD less than of two showed precise description of proposed method with respect to HPLC referenced method.

3.2.5.5. Linearity

From five determinations of 70%, 80%, 90%, 100% and 110% of the label claim, linearity correlation was estimated using NIR-chemometric predicted values from PLS model and HPLC reference values. Regression equation for this linear analysis was found to be y = 1.004x-0.329 Table 3.9. Therefore within the diclofenac sodium range of the calibration set the proposed model for diclofenac allowed a suitable linearity with r= 0.999 for all sample set compared to the reference values as shown in Figure 3.16.

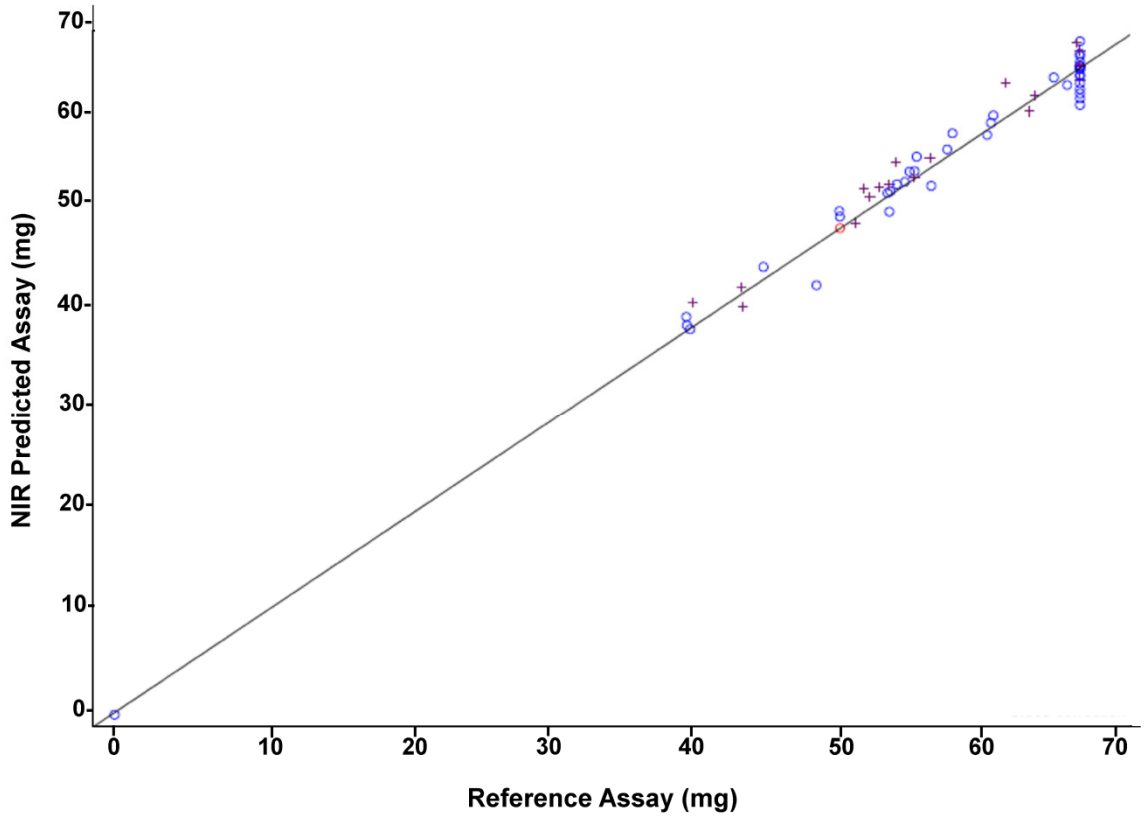

Figure 3.16 Linearity plot for the calibration (○) and validation (+) samples diclofenac, solid line shows the data fit

3.2.5.6. Robustness

Using three determinations of 100% label claim of diclofenac sodium, evaluation in triplicate were done at three different temperatures as shown in Table 3.9. The estimated variation in values were found to be less than two as %RSD, which once more indicated no significant differences compared to the HPLC reference method values. A p-value from the paired t-test showed reliability and good correlation between predicted and referenced values.

3.2.6. Model Assessment on Real Sample

Proposed NIR-multivariate models were demonstrated using five commercially available DICLO tablets. Samples were initially crushed into powder and sieved to remove the coating particles then further used for evaluation. A threshold of mahalanobis distance to diclofenac was set at 4.5 on x-axis to pass the identification test. Therefore, four products

were identified to be similar to the formulated samples while and one product was considered as outlier. This product was the DICLO-3, which was having low content of diclofenac as shown in Table 3.11. This also shows the result of quantitative prediction from proposed PLS model and reference method result. Upon paired t-test, p-value more than 0.05 shows proposed method to be a good alternate method. Only two products were estimated correctly while three were not quantified accurately. This may be because of the different source of API, different excipients contents or particles size.

Table 3.11 Diclofenac market products evaluation by NIR proposed model

Market Product	Product Label Claim of Diclofenac(mg)	In-house HPLC Assay (mg)	NIR Predicted Assay (mg)	t-test Paired p-value
DICLO-01	50	48.80±0.29	37.41±0.15	0.00
DICLO-02	50	47.08±1.35	46.72±0.08	0.63
DICLO-03	50	19.96±0.02	16.39±0.25	0.04
DICLO-04	50	46.11±0.39	46.68±0.19	0.05
DICLO-05	50	43.77±0.28	48.36±0.26	0.03

3.2.7. Conclusions

Evaluation with the conventional methods is time and money consuming. For these reasons efficient analytical approaches with rapid, reliable and non invasive nature are required for routine drug analysis of large number of samples. Therefore, proposed NIR-multivariate models for identification and quantification are intended to be alternatives of the existing analytical tests for its reliability and time saving features. These approaches can solve the problems associated with the large number of variables compared to the number of samples measured, which is distinct for NIR data. Regulatory authorities require fast and efficient method for evaluation of large number of products and thus NIRS along with multivariate analysis show the promising outcome with respect to diclofenac sodium. Proposed models reduced the sample evaluation time from 15 minutes in case of HPLC to two minutes in case of multivariate model which reduces analysis cost as well. The proposed models were found non-destructive and therefore may lend themselves to large numbers of market products for assurance purposes. The models are easy to use; however

require more number of API sources to add in library to make the models more specific, accurate and precise.

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CHAPTER 4

RATIONAL USE AND PRESCRIBING PREFERENCE OF MEDICINE

4.1. GENERIC MEDICINES AND PRESCRIBING PREFERENCE

Indian Pharmaceutical market is well known for generic medicines and the government promotes them due to their affordability. These medicines are manufactured by big, medium and small size companies and their quality are generally checked by analytical methods; though real evaluation of medicines can only be ensure by medical practitioners who prescribe them which is based on the therapeutic responses and adverse effect they notice. High cost of healthcare and medicines affect lives of consumers specifically poor people; as cost of the medicine is highly affected by the market liberalization which proliferate the private sector [1]. In order to provide affordable medicine access to large population, Indian government always efforts to discontinue the unscrupulous practices of big pharmaceutical companies, who encourage the MP to prescribe their branded medicines over generic medicines. On one side government wants to bring down the prices of medicine in term of health care cost while on other side poor quality medicine exist in the market and on account of these, some negative consequences occur daily to the patient [2], thus a question rises; Does survival need medicine quality or its affordability? Thoroughly both are prerequisite simultaneously. Another major issue emerges from the patient side like with increasing chaotic burden of life, the willingness and regularity of treatment schedules are reduced enormously among patients. This concept of treatment non adherence or treatment noncompliance has led to severe consequences of reduction in clinical benefit and increased risk of morbidity and mortality in the patients [3]. Therefore practicing medical profession is considered as the responsibility for overall clinical results so that quality of healthcare may improve in regular practice.

On such grounds a survey of 111 MP was conducted on one to one basis in a form of questionnaire to determine the medicine prescribing preference by the MP and general criterion for their selection among Innovator Branded Generic (IBG), Branded Generic (BG) and Generic (G) products based on therapeutic response, adverse effect and affordability. This study aimed to get the perspectives of MP to explore the challenges for the regulatory authorities or government of India to mitigate healthcare system and improve quality of medicines. Additionally instructions following behavior for treatment that is compliance by the patients was also determined based on the perspective of medical practitioners.

4.1.1. Study Design

This study was accomplished as a cross-sectional study of 111 MP from seven states of Northern India (Uttar Pradesh, Uttarakhand, New Delhi/Delhi, Haryana, Punjab, Himachal Pradesh and Jammu & Kashmir). They had one to more than ten years experience and different qualifications like M.B.B.S, B.D.S., M.D., D.M. etc. as shown in Table 4.1. Medical practitioner having qualification in Unani and Ayurveda were also included in this study as they generally do practice with allopathic medicine; although they are not registered with Indian Medical Council. Selection of MP was random and they were specialized in different field like general physician, dentist, pediatricians, dermatologist and gynecologist etc. The survey instrument was a questionnaire (Appendix B.1) conducted face-to-face consisting of several multiple choice questions about medicine prescribing preference, therapeutic response and adverse effects of three standards in market and their price views based on affordability and non affordability for a person living on a daily cost of INR 88. Study was mainly focused on prescribing preference among three standards which are IBG, BG and G products. IBG are the products by companies who have invented drugs and are highly involved in inventions, research and manufacturing, BG are the product by companies who are highly involved in research and manufacturing; and G are the products by companies who are only involved in manufacturing. Scale used for multiple choice questions were less, moderate and most preferable for prescribing preference; good, moderate, mild and poor for therapeutic response; none, mild, moderate, high and severe for adverse effects (degree of scale shown in Table 4.2) And price view were also asked based on the affordability of IBG, BG and G medicine to the people who live on a cost of Rs. 88 daily An interval scale question was queried about the percent of adherence to their instruction by their all visiting patients. This survey was comprised of many other questions but due to specific research objectives, we have measured only prescribing preference, therapeutic response, adverse effects, affordability of medicines and instructions compliance (adherence).

Table 4.1 Sample profile of medical practitioners, N=111

Demographic Variables		Frequency	Percent
Location			
	Uttar Pradesh	35	31.5
	Uttarakhand	4	3.6
	New Delhi/Delhi	21	18.9
	Haryana	2	1.8
	Punjab	2	1.8
	Himachal Pradesh	27	24.3
	Jammu and Kashmir	20	18.0
Qualification			
	M.B.B.S.	21	18.9
	M.D.	33	29.7
	M.S.	1	.9
	B.D.S.	29	26.1
	B.U.M.S.	9	8.1
	B.A.M.S	11	9.9
	Higher Degree(DM, Mch, DNB etc)	4	3.6
	Others: M.D. in Ayurveda/Unani/Dental	3	2.7
Specialization			
	Dentist	29	26.1
	Dermatologist	8	7.2
	Diabetologist	3	2.7
	ENT Specialist	1	.9
	General Physician	53	47.7
	Gynaecologist	4	3.6
	Neonatologist	1	.9
	Opthalmologist	1	.9
	Orthopedic	1	.9
	Pediatrician	8	7.2
	Preventive Medicine	1	.9
	Radiologist	1	.9
Practicing Experience			
	Up to 1 Year	8	7.2
	Up to 2 Year	20	18.0
	Up to 4 Year	8	7.2
	Up to 6 Year	4	3.6
	Up to 8 Year	10	9.0
	Up to 10 Year	2	1.8
	More than 10 Year	59	53.2

Abbreviations: MP: Medical practitioners, MBBS : Bachelor of Medicine- Bachelor of Surgery, MD: Doctor of Medicine, MS: Doctor of Surgery, BDS: Bachelor of Dental Surgery, BAMS: Bachelor of Ayurvedic Medicine and Surgery, BUMS: Bachelor of Unani Medicine and Surgery, DM: Doctorate of Medicine, MCH: Master of Chirurgiae, DNB: Diplomate of National Board

Table 4.2 Scale for adverse effects of medicine

None: no adverse effect observed
Mild: require only substitution by new medicine product
Moderate: require high attention and addition of new medicines product and/or substitution by new medicines products
High: require immediate treatment
Severe: require immediate hospitalization

4.1.2. Data Analysis

Descriptive statistics were used to examine the perspective of the MP. The Chi-square (χ^2) test was used to examine the associations between the different attributes. Statistical analyses were performed using the SPSS software, version 17.0, and statistical significance was assumed for p-value less than or equal to 0.05.

4.1.3. Results

Out of 111 respondents, 47.7% were general physician and 26.1% were dentist. Among all 53.2% MP were having more than ten years of experience as shown in Table 4.1, the sample profile of the survey respondents. On account of multiple option scale for three commercial standard medicine; IBG, BG and G; some of the respondent were not exclusively answered and they selected same option between two or three standards like some MP choose both IBG and BG as most preferable In terms of most preferable medicines 21.6%, 63.1% and 19.8% MP prescribe the IBG, BG and G respectively, while as less preferable medicines 28.8%, 4.5%, 41.4% MP prescribe IBG, BG and G respectively as shown in Figure 4.1. About 59.5% and 64.9% MP considered IBG and BG respectively as showing good therapeutic response (TR) while G has good TR according to only 15.3% MP as depicted in Figure 4.2. A chi-square test was performed to determine the association between qualification and medicine prescribing preference of IBG, BG and G medicines.

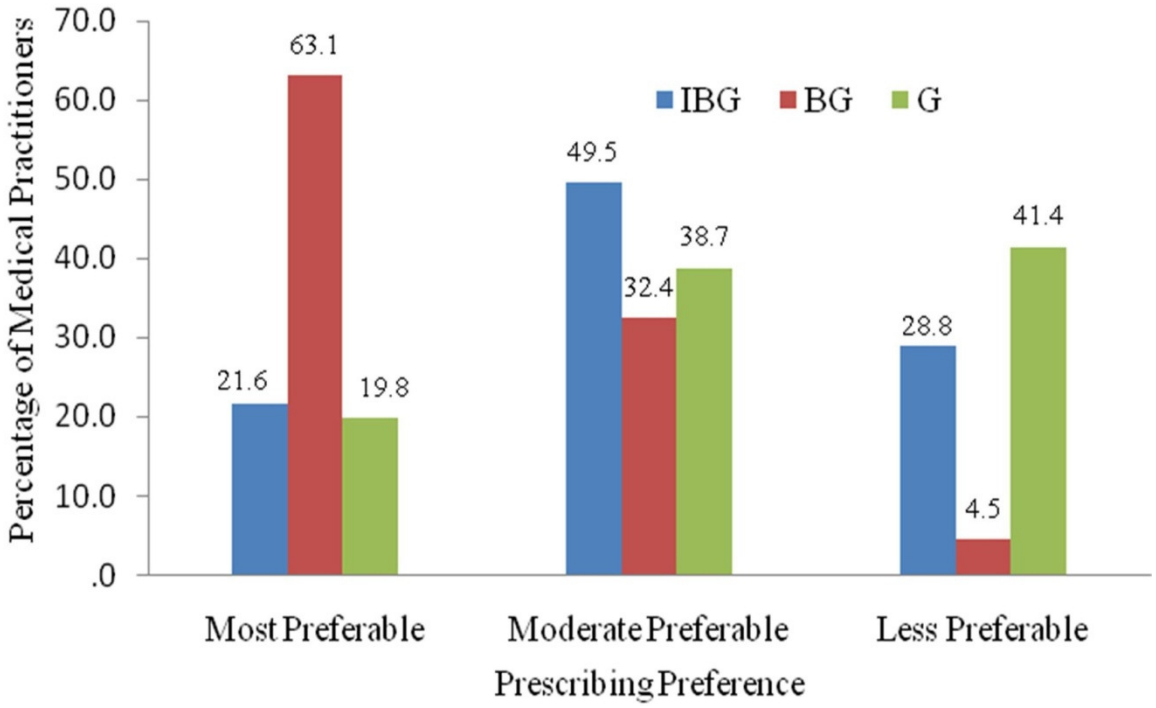

Figure 4.1 Prescribing preference of IBG, BG and G medicines by medical practitioners

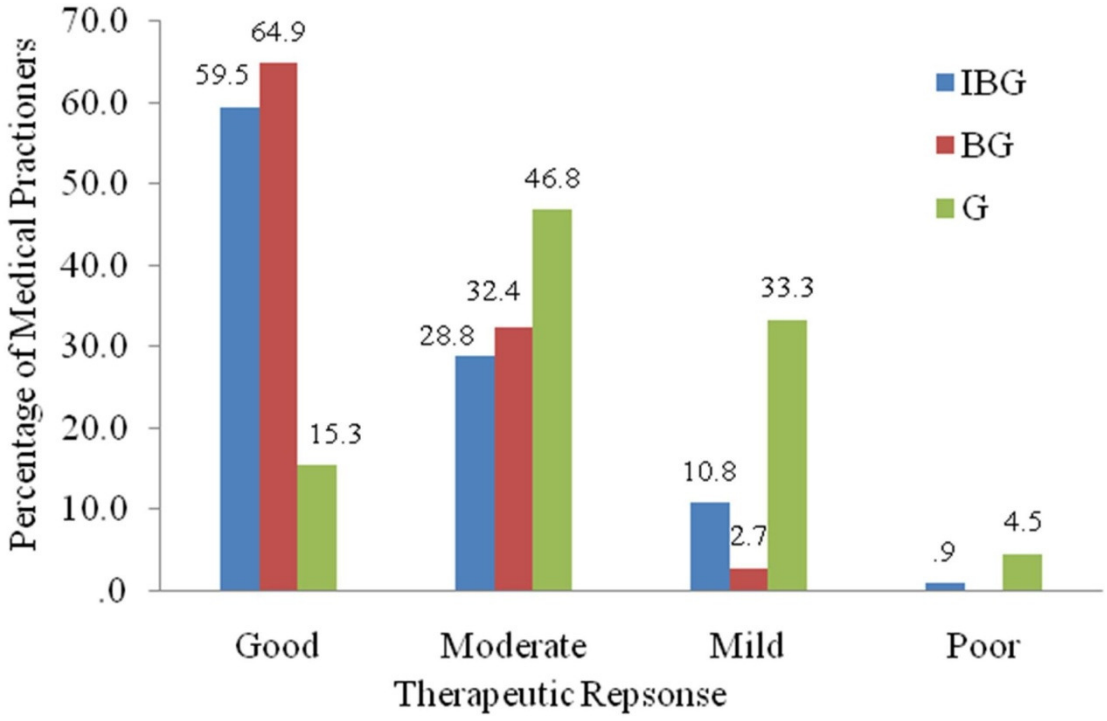

Figure 4.2 Therapeutic responses views on IBG, BG and G medicines by medical practitioner

It was observed that there was significant association between qualification and IBG, BG and G preference as in all cases $p < 0.05$. Out of 21 M.B.B.S., IBG, BG and G standards are most preferred by 19.0%, 47.6% and 23.8% MP, and same three standards are

moderately preferred by 52.4%, 42.9% and 33.3% MP while less preferred by 28.6%, 9.5% and 42.9% MP respectively. Out of 33 M.D., IBG, BG and G standards are most preferred by 48.5%, 69.7% and 15.2% MP, and same three standards are moderately preferred by 42.4%, 30.3% and 21.2% MP whereas less preferred by 9.1%, 0% and 63.6% MP respectively. Out of 29 B.D.S., IBG, BG and G standards are most preferred by 6.9%, 86.2% and 0% MP, and same three standards are moderately preferred by 55.2%, 13.8% and 75.9% MP while less preferred by 37.9%, 0% and 24.1% MP respectively. Out of 9 B.U.M.S. that is Unani practitioners, IBG, BG and G standards are most preferred by 0%, 44.4% and 66.7% MP, and same three standards are moderately preferred by 33.3%, 55.6% and 11.1% MP while they are less preferred by 66.7%, 0% and 22.2% MP respectively. Out of 11 B.A.M.S. that is Ayurvedic practitioners., IBG, BG and G standards are most preferred by 0%, 36.4% and 45.5% MP, and same three standards are moderately preferred by 54.52%, 45.5% and 36.4% MP whereas they are less preferred by 45.5%, 18.2% and 18.2% MP respectively.

Chi-square test between prescribing preference and adverse effect of three tested standards shows no association as $p>0.05$ in all cases. However, significant association found between prescribing preference and therapeutic response of BG χ^2 (4, N=111) = 21.751, $p<0.05$ and G χ^2 (6, N=111) = 19.486, $p<0.05$ but no association in case of IBG χ^2 (6, N=111) = 14.068, $p>0.05$. Related to AE, 69.4%, 65.8% and 55% MP considered IBG, BG and G having mild AE respectively; and 2.7%, 0.9% and 8.1% believed IBG, BG and G having high AE respectively as depicted in Figure 4.3. As there is price concerned results shows that 20.7%, 33.3% and 76.6% MP believe that IBG, BG and G are affordable to the people who live on a daily cost of 88 INR. IBG, BG and G has moderate price for same people according to 27%, 54.1%, 14.4% MP respectively as illustrated in Figure 4.4.

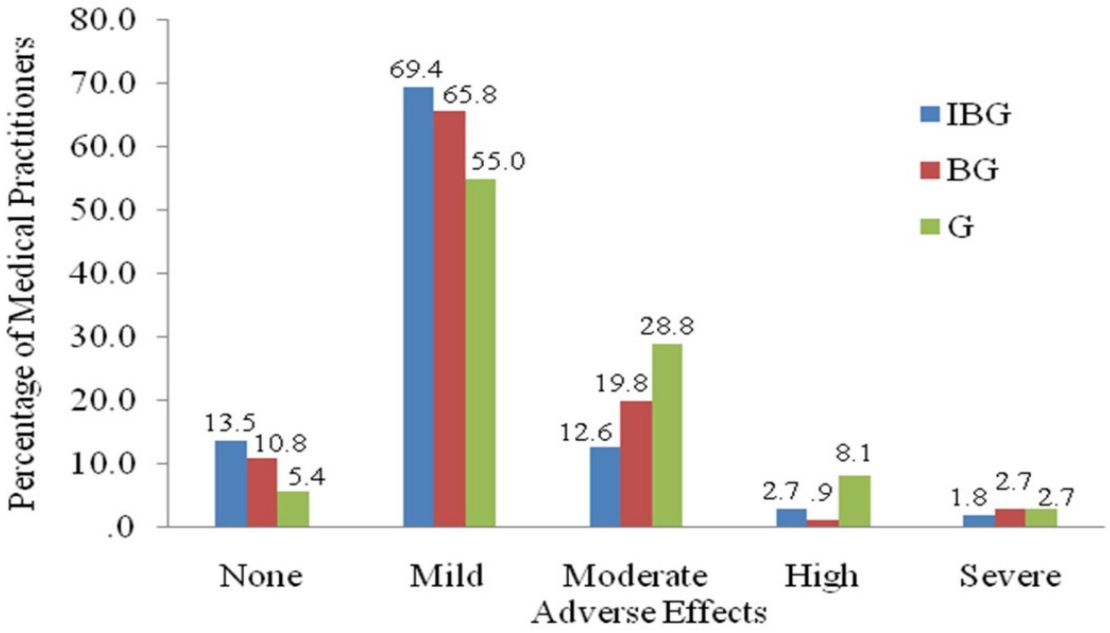

Figure 4.3 Adverse effects views on IBG, BG and G medicines by medical practitioners

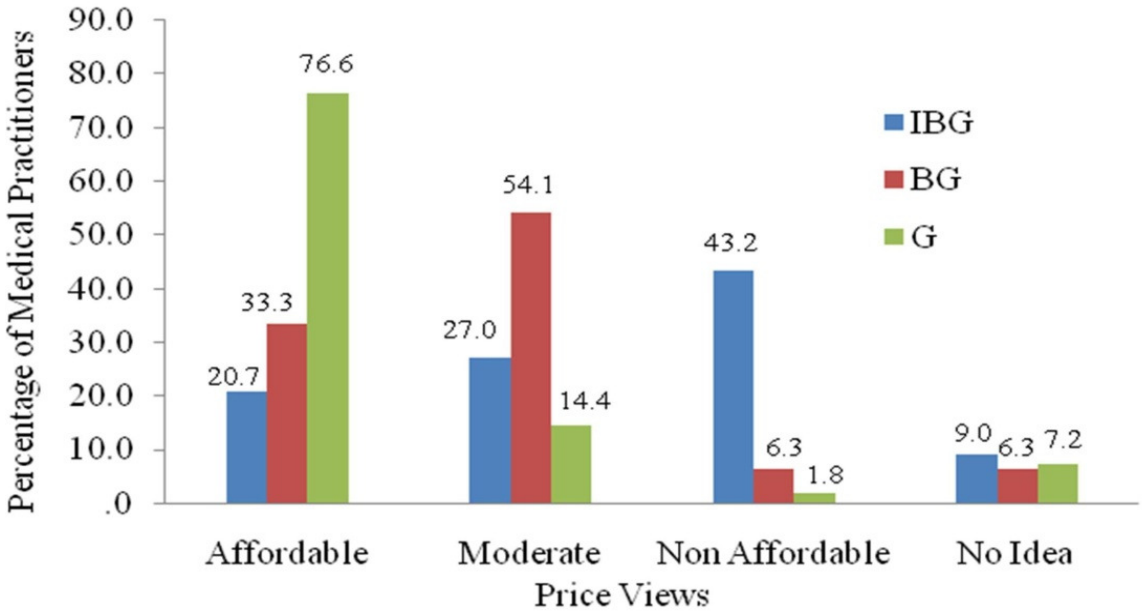

Figure 4.4 Price views on IBG, BG and G medicines by medical practitioners

For determining the patient adherence to MP's instruction, result shows that only one MP said that all his patients follow his 100% instructions; whereas 33.3%, 45.9%, 16.2% said their all patients follow their 70-90%, 40-60% and 10-30% instructions respectively, while 3.6% said their all patient do not follow any instruction.

4.1.4. Discussions

Based on perspectives of MP this study adds to scaling evidence of the need for medication prescribing pattern and required interventions for improving the quality of medicines, and patient adherence and upgrading the knowledge of MP. Over the years India is producing BG and G medicine and some foreign multinational companies also manufacturing the generics known as IBG. In order to afford medication under healthcare cost by majority of Indian population; government promotes the G medicines over BG and IBG. MP prefer BG as most prescribing standard. The reason may be their moderate to good therapeutic response and moderate to mild adverse effect. It shows better therapeutic response than IBG and G and have less adverse effects than IBG and G. One half of MP considered as it has moderate price and one third MP believe it is affordable to more than 59% of Indian population. IBG is the moderately preferred by half of the MP as favorably it has moderate to good TR and mild to none AE; although unfavorably its moderately preference may be because of non affordability by the visiting patient to MP. This study shows majority of the MP considered G medicine as most affordable medicine than BG and IBG. However, majority of MP do not prefer to prescribe G may be due to moderate to poor therapeutic response and moderate to high adverse effect. With comparison to IBG and BG, more MP reported moderate and high adverse effect with G medicine in which high attention and addition and/or substitution of new medicines product is required and sometimes require immediate treatment. Generally G is less preferable by MP and the reason may be their poor therapeutic response or high adverse effect as reported by more MP and where it is moderate preferable it is may be because of the financial status of the visiting patients. More research has to be done to uncover the real cause. Generic medicines competition and low price can significantly be a factor in affordability in low-income countries [4]. In this study MP's perspectives show that G medicines are most affordable to 742 million Indian who live on a daily cost of 88 INR, whereas more MP considered BG as moderately affordable and some believe it as affordable while more MP believe IBG as non-affordable and some think moderately affordable to the same population.

Although G substitution and competitive tendering has made a positive impact on hospital budget but this should be complemented with improved strategies of patient safety that prevents costs of medication errors [2].

As general tendency except poor people, patient do not compromise with quality of medicine against price thus government has to take more stringent action for those generic products which are of low quality and cease them before they enter in to market or unapproved them. Statistically there was significant association between MP's qualification and their prescribing preference for three market standards. Large number of M.B.B.S and M.D. most preferably prescribe BG, moderately prescribe IBG and less preferably prescribe G. While large number of B.D.S most preferably prescribe BG, moderately prescribe G and less preferably prescribe IBG. Majority of B.U.M.S. practitioners most preferably prescribe G, moderately prescribe BG and less preferably prescribe IBG, whereas B.A.M.S. practitioners most preferably prescribe G, moderately prescribe IBG and less preferably prescribe BG.

In order to confront the poor medicine existence, patient non-adherence and improve healthcare services, we found three main areas that need urgent attention and efforts. First, the MP need to render close attention to financial and nonfinancial outlook when making prescribing decisions for individual patients. Their most prescribing preference is for BG, and moderate to less preference to G. As per their perspectives generic bear not equivalent quality as BG and IBG in terms of therapeutic response, so government has to take better steps to improve the quality of G medicines. Only in such a way the vision of better healthcare with affordable price can be accomplish, alternatively it directly or indirectly affects the public health and ultimately reduces the economic growth of India.

Second, high and severe AE of IBG and G are disclosed by some MP, additionally G have high AE responded by somewhat more MP. In general it may be because of poor quality or medication errors which can increase the risk of adverse events, increased length of hospital stay, increased healthcare costs and ultimately increase the morbidity and mortality [5]–[8]. Although an initiative has been taken by the Indian government as PvPI to monitor the ADR and make awareness among the health care professionals [9]; more stringent reporting of AE or ADR should be achieved with the help of regulatory authorities, pharmaceutical companies, healthcare professionals and academician. The only need of the hour is to report every observed AE or ADR to the PvPI.

And third, this study reveals that majority of the patients do not follow the MP's instruction sincerely and there are very few who fully adhered to all instructions. Healthy

discussion of treatment schedules between patient and health care provider is quoted with the term ‘concordance’ and it should be ideally promoted while prescribing. The process of concordance should not be limited just to prescribe medicines but also to gain patient support that will ultimately increase patient compliance [10]. There are numerous reasons for the non-adherence but patients relationship and communication with the health care provider are the most important factor for the same [3].

4.1.5. Conclusions

This study extracted several facts regarding MP’s outlook and awareness of these attributes may help regulatory bodies to step up promising interventions for improving the health policies, drug price control and quality of medicines. Non-adherence to medication is a considerable issue which is concomitant for patients and healthcare system. Strengthening and endorsing patient involvement in treatment decisions and enhances patient education by medical or drug regulatory authorities may improve the adherence and healthcare outcomes. Policies and strategies based on such medical practitioner’s perspectives are necessity for improving and making better healthcare system.

4.2. TREATMENT NON ADHERENCE : RATIONAL TO IRRATIONAL USE

Not only poor qualities of medicines are responsible for the treatment failure but patients also have a role to play. Rational uses of medicine contribute for successful treatment completion while irrational uses provoke uncomfortable situations. Medication non adherence and instructions non compliance are the emerging trends in the deprivation of success in treating illness, particularly in developing countries. Fixed dose combinations and prolonged or sustained release medicines are some adopted approaches by pharmaceutical industries and Indian Government; however such interventions are irrespective of unknown prevalence of non adherence and non compliance. Therefore goal of the present research was to address the prevalence of non adherence and compliance across the Northern India so that further research could be done to know reasons and find the related solutions. Thus, one of the primary steps in estimating non adherence is to identify which patients are at high risk of this problem. Therefore we aimed to identify the

most affected community groups based on age, gender and family monthly income for their non adherence and non compliance practices. However, the complexity of elements that correspond to non-adherence cannot be trivialized but this research tend the regulatory authorities to design interventions for the most affected segment of the community and help policy maker to plan some efficient scheme to solve this problem for the best use of medicines. This was a survey based on about 4161 patients who are or were once under treatment.

4.2.1. Study Design

One way out to assess the adherence encompasses direct behavior observation or therapy surveillance. However, subjective self reports are extensively used concept which is simple, fast, inexpensive, least resource intensive and highly useful in clinic interview and large scale assessment [11], [12]. Questionnaires (Appendix B.2) for the patient create good modus operandi to estimate the adherence level. A subjective means for measuring the adherence require systematize patient administered questionnaires. Therefore, questionnaires which relate to specific medical tendency to determine specific behavior may be the preferable predictors for measuring the adherence. Thus to obtain patients perspectives, we conducted a cross sectional survey; based on self reported questionnaire by a large population from six north Indian states and national capital of India. Questionnaire was in four languages that is English, Hindi, Urdu and Punjabi due to public diversity. Data was collected via face-to-face interviews of several patients near hospital areas, residential locations, colleges, roads and school children to be filled by patient themselves or by their guardians incase of age between 13-16 years. Therefore this research demonstrate the variation in medication adherence, tendency and responsibilities of the respondents who are patient or were be a sufferer in past. Survey was conducted between June 2014 and August 2015. This survey was comprised of many other questions but stick to our research objectives; we have measured only treatment non adherence and non compliance.

4.2.2. Survey Respondents

Patients were belong to different regions of Northern India like Uttar Pradesh, Uttarakhand, Haryana, Punjab (including Chandigarh), Himachal Pradesh, Jammu and

Kashmir and Delhi. More than 30 district and 50 villages of Northern India were covered. Patients were both male and female; and they were having different family monthly income. Their age were lied between below 19 (not less than 13) years and above 65 years. The demographic profile of patients is depicted in Table 4.3.

Table 4.3 Sample profile of patients, N= 4161

Demographic variables		Frequency	Percent
Age (years)			
	Below 19	1008	24.2
	20-24	796	19.1
	25-34	822	19.8
	35-44	694	16.7
	45-54	546	13.1
	55-64	218	5.2
	Above 65	76	1.8
Gender			
	Male	2563	61.6
	Female	1597	38.4
Location			
	Delhi	555	13.3
	Himachal Pradesh	700	16.8
	Haryana	556	13.4
	Jammu & Kashmir	451	10.8
	Punjab	215	5.2
	Uttar Pradesh	889	21.4
	Uttarakhand	795	19.1
Monthly Income (rupees)			
	2000-5000	890	21.4
	5001-10000	1062	25.5
	10001-20000	968	23.3
	20001-30000	601	14.4
	More than 30000	638	15.3

4.2.3. Methods

The primary aim of the survey was to perceive responsibility and treatment adherence with respect to the medication and medical practitioners' instructions. To

measure adherence we created two attributes, first was how many patients stop medication before completion of treatment when getting relief and second was how many patients miss doses during treatment. Third attribute we measured was compliance towards the medical practitioner's instructions. Three questions were asked as mentioned in Table 4.4. In drafting this questioner there was strict adherence to the concept of clarity, simplicity, and ease of questioning.

Table 4.4 Non adherence and non compliance question asked during patients' survey

Variable	Question	Options
Stop Medication Before Treatment Completion	In a 5 days treatment, on improvement, what do you do generally? (Without asking doctor)	Stop medication before one day Stop medication before two day Stop in mid of treatment Always complete the treatment
Missing Dose	How many times you generally miss medicine dose in between your treatment of 5 days (among 10 doses)?	One dose Two doses Three doses Four or more doses Never miss
Compliance	How much percent of instructions do you follow given by your doctor about medicines?	100% 70- 90% 40-60% 10-30% 0%

4.2.4. Statistical Analysis

Data analysis was performed using SPSS version 17.0 (SPSS, Inc.). The alpha value for association was 0.05. The categorical data for assessing proportion of patient's in-agreement with stop medication before completion of treatment and miss the dose during treatment were analyzed using the frequency. Most associated community groups were determined based on Chi-Square test which signifies the association between categorical responses with age, gender and family monthly income.

4.2.5. Results

As shown in Table 4.3, 4161 respondents were from teenagers up to more than 65 years of age; and they were further classified based on monthly income and gender. Patients or respondents were randomly selected from all over Northern India. As shown in Figure 4.5. patients' tendency to stop medication before completion of treatment have shown that

44.1% (N=4151) of the patients do not adhere to the treatment, which means that when these respondents get some relief, they follow a tendency to stop medication before one or two day and sometimes in mid of the treatment without asking medical practitioners. Moreover 53.8% (N=4160) of respondents generally miss one, two, three and even four or more doses during treatment as shown in Figure 4.6. It is clearly indicated that 55.6% (N=4151) patients always complete the treatment and 46% (N=4160) patients never miss the dose, which signifies that about half of the population rationally use the medicine.

Patient tendency towards compliance or following the instructions of medical practitioners during the treatment has been manifested that about 73.8% (N=4161) are non compliant to the instructions while only 26.2% patients show full compliance. Even more than 20% (N=4161) patients follow half or less than half of the instructions as shown in Figure 4.7.

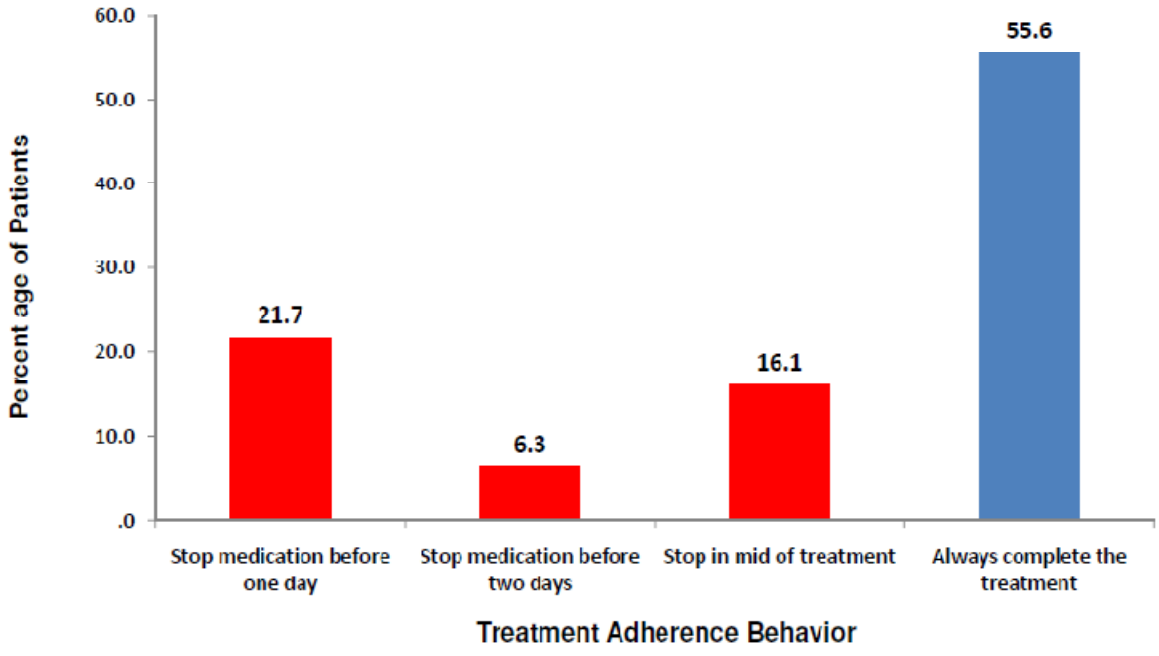

Figure 4.5 Stop medication tendency of patients (N=4151) before completion of treatment

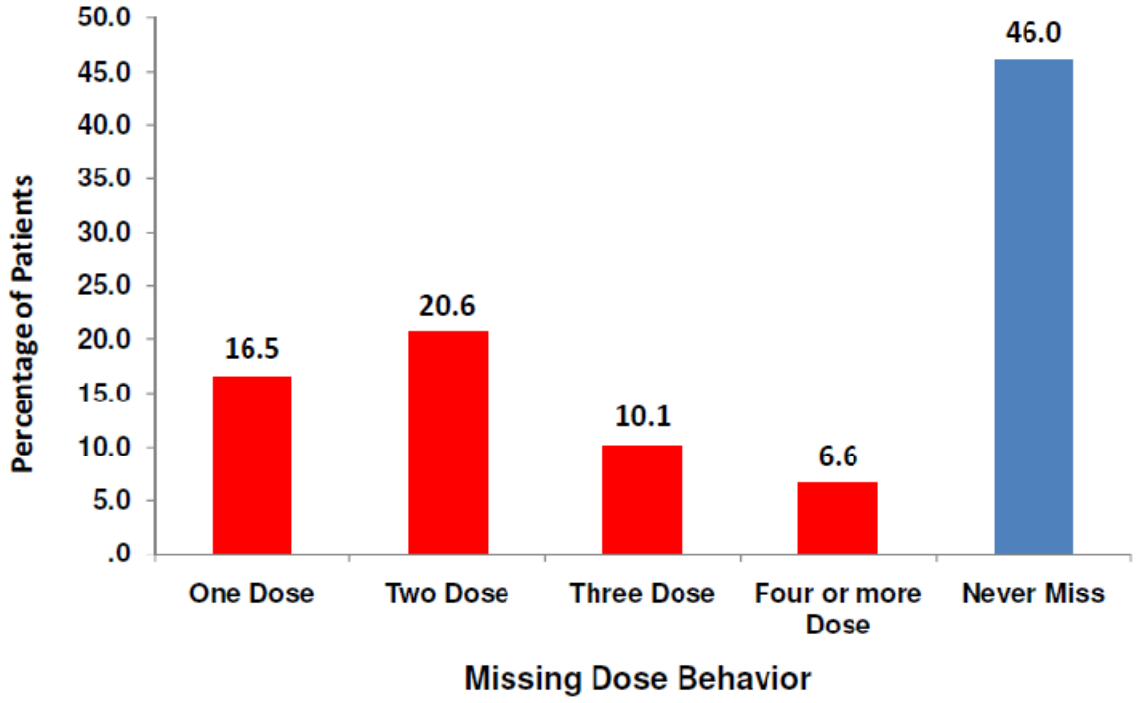

Figure 4.6 Missing dose tendency of patients (N=4160) during treatment

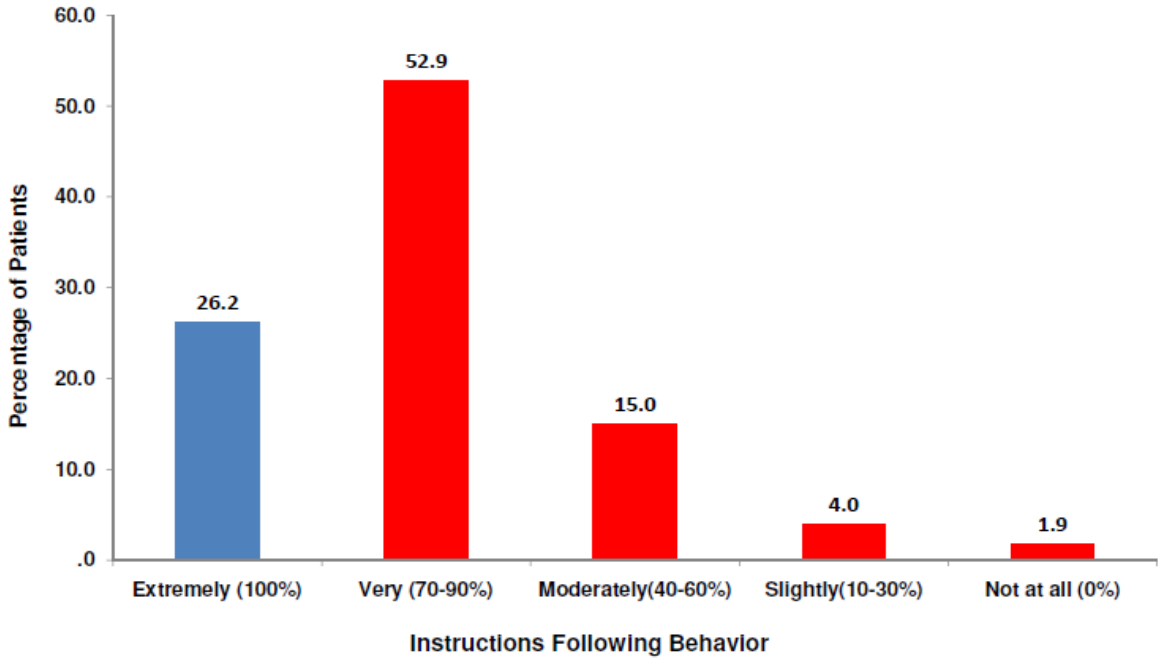

Figure 4.7 Compliance of patients (N=4161) with respect to follow the instructions of medical practitioners

To ensure the relationship between different variables, Chi-square test was applied as shown in Table 4.5. There was no relationship between treatment completion and age. Moreover, as demonstrated by Chi-square test with p-value greater than 0.05, more than

53% (N=1005) patients who were below 19 year of age do not complete the treatment on getting relief without asking the medical practitioners. Maximum non adherence is shown by the age group between 20-24 years, and maximum adherence by the group above 65 years of age.

Table 4.5 Chi square test for association between different categorical variables

Behavior	Age	Gender	Monthly Income
Stop Medication Before Treatment Completion	p=0.063 χ^2=40.273 df=28 N=4157	p=0.004 χ^2=22.343 df=8 N=4157	p=0.000 χ^2=166.110 df=16 N=4155
Missing Dose	p=0.002 χ^2=54.772 df=28 N=4160	p=0.023 χ^2=17.730 df=8 N=4160	p=0.000 χ^2=113.888 df=16 N=4158
Compliance	p=0.000 χ^2=76.193 df=28 N=4161	p=0.480 χ^2=7.536 df=8 N=4161	p=0.000 χ^2=109.148 df=16 N=4159

Note: p= p-value, χ^2= chi square critical value, df= degree of freedom, N= number of patients

With missing dose tendency and age; Chi-square test determined the significant relationship with p-value less than 0.05. In this case 43.3% (N=76) patients above 65 age have missing dose behavior. About 56.3% (N=822) patients between age 25-34 and 51.2% (N=1007) patients below age 19 have the tendency to miss the dose. Thus, with increase in age missing dose behavior also increases but except the patients above 65 years of age.

In the same way Chi-square analysis with contingency table between compliance and age has shown significant relationship with p-value less than 0.05. More than 80% (N=796) of patients between 20-24 age; and more than 77% (N=1008) below 19 years age do not show full compliance.

Patients' gender was significantly associated with this adherence tendency with a p-value less than 0.05. As there were 24.1% (N=2560) males and 18.1% (N=1596) females stop medication one day before the treatment complete; however, 53.5% (N=2560) males and 59.0% (N=1596) females always complete the treatment.

Patients' gender and missing dose tendency has a significant relationship as observed in this study with p-value less than 0.05. About 10.1% (N=2562) males and 6.4% (N= 1597) females have the tendency to miss one dose during treatment. And about 13.5% males and 7.1% females have the tendency to miss two doses during treatment. While about 44.5% males and 48.5% females never miss the dose. However, instructions following behavior have no significant relationship with gender as determined by p-value greater than 0.05.

On demonstrating the Chi-square test, p-value less than 0.05 shows that patients' adherence behavior has significantly associated with family monthly income. About 34% (N=638) patients who have monthly income more than Rs. 30000 do not complete the treatment. More than 52% (N=1061) patients who have monthly income between Rs. 5001-10000 and more than 46% (N=889) patients who have monthly income between Rs. 2000-5000 do not complete the treatment. With the exception of lowest income group; with subside the family monthly income; patient adherence to complete the treatment decreases.

Similarly, missing dose behavior also has significant relationship with the monthly income as manifested by p-value less than 0.05. About 54% (N=4158) respondents of all income group have the tendency to miss the doses. Missing dose behavior is estimated within groups shows patients who have family monthly income of Rs. 5001-10000 have maximum tendency to miss the dose while the group having family monthly income more than Rs. 30000 have minimum missing dose behavior. Second most prone group to miss the dose is the group having family monthly income of Rs. 2000-5000.

Instructions following tendency also has the significant relationship with monthly income since p-value less than 0.05. Lowest and highest income group have the maximum compliance tendency. About 78% (N=968) patients who have family monthly income of Rs. 10001-20000 and about 77.5% (N=1062) patients who have Rs. 5001-10000 do not express full compliance.

4.2.6. Discussions

Perceived necessity is not equivalent as perceived efficacy and thus with the highest contribution from medical practitioners, pharmacists, nurses, medical or drug regulatory bodies; patients and public also contribute a lot in making the healthcare system better. It is

a common trend what we see all around in our community that when people start feeling better, they themselves decide not to complete the treatment without medical practitioners permission. Generally patient do not notice improvement in symptoms or if little improvement is there they do not consider it much, as most of them want fast relief. Consequently they stop to take medication and further ask the physician to modify medication otherwise move to other medical practitioners. And therefore patients' forwardness in demanding high quality care to make it possible only with the right information, choosing treatment appropriately and participates actively in the treatment. But how can we ensure that patients have the information and resources which they need to contribute in the healthcare system? Little consideration has been paid to the patients' thoughts regarding medicines, and such thoughts may have well significance for working out the non-adherence to medication. This research was conducted to review patient adherence to treatment in an attempt to know the patient contribution in respect to relationship with healthcare system.

From the result, tendency of patients to stop medication during treatment before its completion without asking medical practitioners has shown that about 44.1% (N=4151) patients do not adhered completely to the treatment. Possible reason for non adherence may be the negative belief of patient. Mutual positive belief on doctors and medication may benefit the healing function. Moreover, 53.8% (N=4160) of patient generally miss one or more than four doses in between treatment which show high prevalence of non adherence to the treatment. Numerous self-distinguished reasons include poor memory, worries about prescriptions, fear of side effects, lack of knowledge of disease or treatment schedule which contribute to treatment non adherence by the patients [13]. About 73.1% (N=4161) patients do not follow the complete instructions. It is worth noting that our previous study of medical practitioners survey have shown that according to the perspectives of 99% (N=111) medical practitioners, their all patients do not follow theirs all instructions thus show non compliance. Non compliance remains a grave health care issue and thus it demands good quality research focused on investigating compliance improving strategies.

Consequently, there are unlikely to be simple adherence solutions to enhancing patient compliance. Thus the primary concern is how the consumption of medicines by patients should be monitored to address the underlying result of non adherence and non compliance.

Teenagers and young generation up to 24 years are more imprudent group in stopping medication before treatment completion and show non compliance for instructions. However missing dose behavior is increase with increase in age up to 65 years whereas on above 65 years; patients missing dose tendency decrease. Women are less irregular then men towards stop medication before treatment completion as well as in terms of missing doses. Lower income group are more prone in following the non adherence trend and missing dose behavior. However, an interesting result show that lowest and highest income group show more compliance. Strategies to monitor and improve adherence are key components of pharmaceutical care plans, especially for patients with chronic diseases, such as hypertension, diabetes, and atherosclerotic heart disease. However, changing such mindset is difficult process. Therefore the only formula for most part entails quality education, good relationship and communication between patient and physician. Improving doctor patient relationship can be proved a standout amongst the most usually pushed approaches to enhance compliance. Thus far, primary approach to improve the situation; pharmacists are on an ideal position to assess and treat adherence-related problems that can adversely affect patients' health outcomes.

Adherence may increase if patient are provided clear, simple and understandable information about diagnosis, medication, dosage regimen and most important poor consequences of the missing dose, leaving the treatment before completion and noncompliance to instructions. In such a way they are expected to manage their dosing regimen and follow the instructions by practitioners. This study contribute a preliminary insight into the concept by which the tendency to take medicines and compliance may influence adherence. As such, this study assists as a blueprint for action by all medical exponents. And further studies of functional interventions and exploring the reason associated with it are required to intensify adherence as mandatory to improve health outcomes.

4.2.7. Conclusions

Rational use of medicine is followed by half of consumers. Half of them are susceptible towards irrational use. Irrational use as non adherence is the major problem tends to poor health care across the nation. Young generation, low income family and males are most imprudent groups of patients who are more irregular during treatment with respect

to adherence and compliance. Therefore, for improving adherence to treatment we need some practicable initiatives and appropriate predictor which are focused on patient. Upgrading healthcare require a joint effort on the part of medical practitioners, pharmacist, nurse, policy makers and patients.

This research will most importantly benefit the medical practitioners and policy makers in term of encouraging new health system initiative and developing better treatment undertaking by increasing access to data extracted from the treatment refusal, medication non adherence and physician's instructions negligence by the patients. In addition, future patients also get direct potential benefits by knowing current patients degree of non adherence and carelessness towards health care.

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CHAPTER 5

CONCLUSIONS

CHAPTER 5

No study has been performed on the quality of market products in the last many years to put a check on government estimates; and thus real extent of the poor quality or substandard medicines still remains ambiguous due to many high level claim of poor quality by others nation. This study found high prevalence of poor quality of amoxicillin 28.26% (N=46) and diclofenac 34.37% (N=32) generic products in the Indian market. The potential consequence of such under dose medications is a matter of concern to the regulatory authorities. Under urgency it is required to focus on controlling the availability of substandard drugs in the market that are produced as a result of the poor manufacturing and quality-control practices or deliberately falsified drugs. Worldwide awareness has been growing on the increasing incidence of substandard and spurious drug, whereas India is still lagging on the issue. Thus, to counteract the issue of spurious and substandard quality medicine in India there is an urgent need for additional research or routine analytical evidences to explain the magnitude of the problem across the country. This work additionally accentuates the requirement for productive oversight of pharmaceutical products, with legitimate observing of manufacturers and their distribution systems to bring down the danger for public being exposed to products of low quality, low safety and low efficacy.

Evaluation with the conventional methods is time and money consuming. Therefore evaluation of large number of samples of amoxicillin and diclofenac formulations can be sorted out by using the developed identification and quantification near infrared - chemometric models. Owing to reliability and time saving features; NIR-Chemometric procedures were intended to be an alternative tool for the existing analytical techniques.

Generic medicines are highly recommended by the regulatory bodies, however due to poor therapeutic response; most of medical practitioners do not prefer generic medicines. It indicates some quality gaps exist and therefore a medicine monitoring event program must be conducted by the regulatory bodies. Another challenge is how consumption of medicines by patients should be monitored to address the underlying result of non adherence and non compliance. About 73.9% (N=4161) patient do not show full compliance as they reported, while as per 99% medical practitioners; their all patient do not show full compliance. This is a matter of concern for the regulatory authorities and policy makers.

CHAPTER 5

Rational use of medicine must be regularly monitored and followed. Strategies to monitor and improve adherence are key components of pharmaceutical care plans, especially for patients with chronic diseases, such as hypertension, diabetes, and atherosclerotic heart disease. One of the primary steps in estimating non adherence is to identify which patients are at high risk of this problem. Therefore this study addressed the prevalence of non adherence and compliance across the Northern India. This study shows that 44.1% (N=4151) of the patients stop medication during treatment before its completion and 53.8% (N=4160) of patient generally miss one or more than four doses in between treatment which show high prevalence of non adherence to the treatment. Patients who had Rs. 5001-10000 monthly income have more tendency to miss the dose while the group having more than Rs. 30000 monthly income have minimum missing dose behavior. About 80% (N=796) patients between 25-34 years age and 77% (N=1008) below 19 years of age are more imprudent in showing non compliance. Women are somewhat more regular in showing adherence as when get relief during treatment; 18.1% females (N=1596) and 24.1% males (N=2560) stop medication one day before the treatment complete. The complexity of elements that correspond to non-adherence cannot be trivialized but this research tend the regulatory authorities to design interventions for the most affected segment of the community and help policy maker to plan some efficient program to solve this problem for the best use of medicines. And it also tends to initiate further research to explore the reasons and consequences of such irrational use trend.

Clearly wait and watch approach cannot be applied and even there is no quick fix at all. On major part, quality monitoring of medicines and patient non adherence study should be done by the health care contributors and by the pharmaceutical fraternity and regulatory authorities. This exploring research demonstrates that regulatory authorities have to look into these issues for the ways to improve the situation and empower the patients. Therefore, they must confront them as new challenges with respect to patient safety; which ultimately may improve healthcare system, reduce financial burden on patients and enhance the trust on healthcare system.

APPENDIX A

A.1 Linear regression parameters with p-value and RMSE values of the amoxicillin calibration models for optimization

Model	Pathlength Correction	Spectra	Pretreatment	Set	Intercept	Intercept p-Value (%)	Slope	Slope p-value (%)	Correlation coefficient (r)	RMSE (%)
PLS	Constant	Original	Without Smoothing	Calibration Set	3.1080	18%	0.9598	17%	0.9797	3.8945
PLS	Constant	Original	Without Smoothing	Validation Set	0.2565	93%	0.9821	61%	0.9843	3.4742
PLS	Constant	Original	Savitzky-Golay Filter	Calibration Set	4.4752	11%	0.9421	10%	0.9706	4.6727
PLS	Constant	Original	Savitzky-Golay Filter	Validation Set	-0.0890	98%	0.9852	71%	0.9798	3.9320
PLS	Constant	First Derivative	Without Smoothing	Calibration Set	0.9593	46%	0.9876	45%	0.9938	2.1636
PLS	Constant	First Derivative	Without Smoothing	Validation Set	2.1133	39%	0.9652	25%	0.9882	2.9117
PLS	Constant	First Derivative	Savitzky-Golay Filter	Calibration Set	1.2578	40%	0.9837	39%	0.9918	2.4786
PLS	Constant	First Derivative	Savitzky-Golay Filter	Validation Set	1.9394	46%	0.9673	31%	0.9866	3.0953
PLS	Constant	First Derivative	Norris Derivative Filter	Calibration Set	1.7706	32%	0.9771	30%	0.9885	2.9382
PLS	Constant	First Derivative	Norris Derivative Filter	Validation Set	-0.5693	85%	0.9985	97%	0.9841	3.3971
PLS	Constant	Second Derivative	Without Smoothing	Calibration Set	1.1909	41%	0.9846	40%	0.9923	2.4087
PLS	Constant	Second Derivative	Without Smoothing	Validation Set	2.6463	39%	0.9680	39%	0.9819	3.4917
PLS	Constant	Second Derivative	Savitzky-Golay Filter	Calibration Set	0.7288	52%	0.9906	51%	0.9953	1.8837
PLS	Constant	Second Derivative	Savitzky-Golay Filter	Validation Set	3.3208	33%	0.9734	52%	0.9783	4.0115
PLS	Constant	Second Derivative	Norris Derivative Filter	Calibration Set	1.5934	34%	0.9794	33%	0.9896	2.7875
PLS	Constant	Second Derivative	Norris Derivative Filter	Validation Set	1.7478	56%	0.9722	44%	0.9828	3.4469
PLS	MSC	Original	Without Smoothing	Calibration Set	2.6945	22%	0.9651	20%	0.9824	3.6287
PLS	MSC	Original	Without Smoothing	Validation Set	1.8337	51%	0.9651	31%	0.9846	3.3660

PLS	MSC	Original	Savitzky-Golay Filter	Calibration Set	2.9764	19%	0.9615	18%	0.9806	3.8091
PLS	MSC	Original	Savitzky-Golay Filter	Validation Set	0.8582	76%	0.9744	45%	0.9852	3.3851
PLS	MSC	First Derivative	Without Smoothing	Calibration Set	0.9348	47%	0.9879	46%	0.9939	2.1377
PLS	MSC	First Derivative	Without Smoothing	Validation Set	2.1985	28%	0.9752	31%	0.9921	2.3271
PLS	MSC	First Derivative	Savitzky-Golay Filter	Calibration Set	0.9409	47%	0.9878	46%	0.9939	2.1410
PLS	MSC	First Derivative	Savitzky-Golay Filter	Validation Set	2.2495	27%	0.9744	30%	0.9921	2.3315
PLS	MSC	First Derivative	Norris Derivative Filter	Calibration Set	3.9664	13%	0.9487	12%	0.9740	4.3985
PLS	MSC	First Derivative	Norris Derivative Filter	Validation Set	6.1194	4%	0.9053	1%	0.9824	3.9179
PLS	MSC	Second Derivative	Without Smoothing	Calibration Set	3.5822	15%	0.9537	14%	0.9765	4.1815
PLS	MSC	Second Derivative	Without Smoothing	Validation Set	-4.9526	6%	1.0596	6%	0.9892	3.1050
PLS	MSC	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.1359	43%	0.9853	41%	0.9926	2.3542
PLS	MSC	Second Derivative	Savitzky-Golay Filter	Validation Set	1.9789	40%	0.9819	52%	0.9897	2.6967
PLS	MSC	Second Derivative	Norris Derivative Filter	Calibration Set	0.9533	47%	0.9877	45%	0.9938	2.1551
PLS	MSC	Second Derivative	Norris Derivative Filter	Validation Set	2.1969	29%	0.9730	28%	0.9918	2.3734
PLS	SNV	Original	Without Smoothing	Calibration Set	8.3728	3%	0.8916	2%	0.9442	6.3940
PLS	SNV	Original	Without Smoothing	Validation Set	7.6128	11%	0.8675	2%	0.9511	6.4984
PLS	SNV	Original	Savitzky-Golay Filter	Calibration Set	3.1205	18%	0.9596	17%	0.9796	3.9017
PLS	SNV	Original	Savitzky-Golay Filter	Validation Set	1.0119	72%	0.9719	41%	0.9851	3.4115
PLS	SNV	First Derivative	Without Smoothing	Calibration Set	1.0440	45%	0.9865	43%	0.9932	2.2566
PLS	SNV	First Derivative	Without Smoothing	Validation Set	3.6274	12%	0.9549	11%	0.9897	2.6768
PLS	SNV	First Derivative	Savitzky-Golay Filter	Calibration Set	0.8845	48%	0.9886	47%	0.9943	2.0735
PLS	SNV	First Derivative	Savitzky-Golay Filter	Validation Set	2.7080	20%	0.9651	18%	0.9914	2.4459
PLS	SNV	First Derivative	Norris Derivative Filter	Calibration Set	1.2532	40%	0.9838	39%	0.9919	2.4729
PLS	SNV	First Derivative	Norris Derivative Filter	Validation Set	0.2122	93%	0.9975	93%	0.9892	2.7285
PLS	SNV	Second Derivative	Without Smoothing	Calibration Set	0.9970	46%	0.9871	44%	0.9935	2.2068
PLS	SNV	Second Derivative	Without Smoothing	Validation Set	3.0141	22%	0.9657	25%	0.9883	2.8307
PLS	SNV	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.3585	38%	0.9824	37%	0.9912	2.5753
PLS	SNV	Second Derivative	Savitzky-Golay Filter	Validation Set	4.2018	10%	0.9508	11%	0.9875	2.9549

PLS	SNV	Second Derivative	Norris Derivative Filter	Calibration Set	0.9666	46%	0.9875	45%	0.9937	2.1732
PLS	SNV	Second Derivative	Norris Derivative Filter	Validation Set	1.4361	49%	0.9844	54%	0.9917	2.3743
PCR	Constant	Original	Without Smoothing	Calibration Set	6.6794	5%	0.9136	4%	0.9558	5.7108
PCR	Constant	Original	Without Smoothing	Validation Set	-0.7620	84%	0.9866	77%	0.9729	4.7046
PCR	Constant	Original	Savitzky-Golay Filter	Calibration Set	4.6377	10%	0.9400	9%	0.9695	4.7589
PCR	Constant	Original	Savitzky-Golay Filter	Validation Set	-0.1439	97%	0.9855	72%	0.9793	3.9860
PCR	Constant	First Derivative	Without Smoothing	Calibration Set	1.1297	43%	0.9854	41%	0.9926	2.3506
PCR	Constant	First Derivative	Without Smoothing	Validation Set	2.2455	36%	0.9649	24%	0.9883	2.8843
PCR	Constant	First Derivative	Savitzky-Golay Filter	Calibration Set	1.2403	40%	0.9840	39%	0.9919	2.4595
PCR	Constant	First Derivative	Savitzky-Golay Filter	Validation Set	1.7627	48%	0.9712	35%	0.9878	2.9303
PCR	Constant	First Derivative	Norris Derivative Filter	Calibration Set	1.3060	39%	0.9831	38%	0.9915	2.5246
PCR	Constant	First Derivative	Norris Derivative Filter	Validation Set	2.0978	41%	0.9672	29%	0.9872	2.9893
PCR	Constant	Second Derivative	Without Smoothing	Calibration Set	1.2713	40%	0.9836	39%	0.9917	2.4925
PCR	Constant	Second Derivative	Without Smoothing	Validation Set	2.1022	52%	0.9726	49%	0.9794	3.7318
PCR	Constant	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.8256	31%	0.9764	30%	0.9881	2.9867
PCR	Constant	Second Derivative	Savitzky-Golay Filter	Validation Set	0.5992	83%	1.0039	91%	0.9864	3.2156
PCR	Constant	Second Derivative	Norris Derivative Filter	Calibration Set	1.4499	37%	0.9812	35%	0.9906	2.6600
PCR	Constant	Second Derivative	Norris Derivative Filter	Validation Set	2.3473	39%	0.9637	28%	0.9853	3.2090
PCR	MSC	Original	Without Smoothing	Calibration Set	2.2462	26%	0.9709	25%	0.9854	3.3094
PCR	MSC	Original	Without Smoothing	Validation Set	1.7199	51%	0.9706	35%	0.9870	3.0336
PCR	MSC	Original	Savitzky-Golay Filter	Calibration Set	2.8801	20%	0.9627	19%	0.9812	3.7492
PCR	MSC	Original	Savitzky-Golay Filter	Validation Set	1.1388	68%	0.9709	38%	0.9856	3.3466
PCR	MSC	First Derivative	Without Smoothing	Calibration Set	0.8415	49%	0.9891	48%	0.9945	2.0275
PCR	MSC	First Derivative	Without Smoothing	Validation Set	1.3276	53%	0.9833	51%	0.9915	2.3934
PCR	MSC	First Derivative	Savitzky-Golay Filter	Calibration Set	0.8433	49%	0.9891	48%	0.9945	2.0288
PCR	MSC	First Derivative	Savitzky-Golay Filter	Validation Set	1.4040	51%	0.9824	50%	0.9914	2.4140
PCR	MSC	First Derivative	Norris Derivative Filter	Calibration Set	2.8248	21%	0.9634	19%	0.9816	3.7130

PCR	MSC	First Derivative	Norris Derivative Filter	Validation Set	0.9717	73%	0.9774	51%	0.9852	3.2763
PCR	MSC	Second Derivative	Without Smoothing	Calibration Set	1.1877	42%	0.9846	40%	0.9923	2.4096
PCR	MSC	Second Derivative	Without Smoothing	Validation Set	1.5290	54%	0.9905	75%	0.9882	2.9367
PCR	MSC	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.0468	44%	0.9865	43%	0.9932	2.2600
PCR	MSC	Second Derivative	Savitzky-Golay Filter	Validation Set	2.2269	38%	0.9787	48%	0.9881	2.8869
PCR	MSC	Second Derivative	Norris Derivative Filter	Calibration Set	0.9564	46%	0.9876	45%	0.9938	2.1591
PCR	MSC	Second Derivative	Norris Derivative Filter	Validation Set	1.7838	38%	0.9782	38%	0.9921	2.3183
PCR	SNV	Original	Without Smoothing	Calibration Set	3.9455	13%	0.9489	12%	0.9741	4.3870
PCR	SNV	Original	Without Smoothing	Validation Set	0.0809	98%	0.9791	58%	0.9813	3.9070
PCR	SNV	Original	Savitzky-Golay Filter	Calibration Set	3.1573	18%	0.9591	17%	0.9794	3.9253
PCR	SNV	Original	Savitzky-Golay Filter	Validation Set	0.9655	73%	0.9722	41%	0.9850	3.4316
PCR	SNV	First Derivative	Without Smoothing	Calibration Set	0.8012	50%	0.9896	49%	0.9948	1.9731
PCR	SNV	First Derivative	Without Smoothing	Validation Set	1.3524	52%	0.9835	52%	0.9917	2.3691
PCR	SNV	First Derivative	Savitzky-Golay Filter	Calibration Set	0.8059	50%	0.9896	49%	0.9948	1.9803
PCR	SNV	First Derivative	Savitzky-Golay Filter	Validation Set	1.9415	37%	0.9767	38%	0.9909	2.4814
PCR	SNV	First Derivative	Norris Derivative Filter	Calibration Set	0.8380	49%	0.9892	48%	0.9946	2.0238
PCR	SNV	First Derivative	Norris Derivative Filter	Validation Set	1.4900	50%	0.9825	52%	0.9906	2.5313
PCR	SNV	Second Derivative	Without Smoothing	Calibration Set	0.7572	52%	0.9902	50%	0.9951	1.9173
PCR	SNV	Second Derivative	Without Smoothing	Validation Set	2.4377	32%	0.9691	30%	0.9886	2.7830
PCR	SNV	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.1732	42%	0.9848	40%	0.9924	2.3968
PCR	SNV	Second Derivative	Savitzky-Golay Filter	Validation Set	3.7127	12%	0.9582	14%	0.9892	2.7482
PCR	SNV	Second Derivative	Norris Derivative Filter	Calibration Set	0.9600	46%	0.9876	45%	0.9938	2.1642
PCR	SNV	Second Derivative	Norris Derivative Filter	Validation Set	1.6965	42%	0.9798	42%	0.9917	2.3733

Note: PLS: Partial Least Square, PCR: Principle Component Regression, MSC: Multiple Scattering Correction, SNV: Standard Normal Variate, RMSE: Root Mean Square Error (Overall Model Performance Parameter)

A.2 Linear regression parameters with p-value and RMSE values of the diclofenac calibration models for optimization

Model	Pathlength Correction	Spectra	Pretreatment	Set	Intercept	Intercept P-Value	Slope	Slope P-value	R	RMSE(mg)
PLS	Constant	Original	No Smoothing	Calibration Set	0.576	53%	0.989	52%	0.9947	1.52
PLS	Constant	Original	No Smoothing	Validation Set	1.691	44%	0.987	74%	0.9867	1.66
PLS	Constant	Original	Savitzky-Golay Filter	Calibration Set	1.200	37%	0.979	36%	0.9892	1.75
PLS	Constant	Original	Savitzky-Golay Filter	Validation Set	1.350	53%	0.991	82%	0.9876	1.57
PLS	Constant	First Derivative	No Smoothing	Calibration Set	4.713	7%	0.916	6%	0.9571	3.47
PLS	Constant	First Derivative	No Smoothing	Validation Set	0.530	84%	0.991	85%	0.9809	1.63
PLS	Constant	First Derivative	Savitzky-Golay Filter	Calibration Set	4.735	7%	0.915	6%	0.9567	3.51
PLS	Constant	First Derivative	Savitzky-Golay Filter	Validation Set	0.331	90%	0.995	91%	0.9805	1.66
PLS	Constant	First Derivative	Norris Derivative Filter	Calibration Set	1.169	37%	0.979	36%	0.9895	1.72
PLS	Constant	First Derivative	Norris Derivative Filter	Validation Set	4.925	0%	0.921	1%	0.9926	1.26
PLS	Constant	Second Derivative	No Smoothing	Calibration Set	0.604	52%	0.989	51%	0.9946	1.25
PLS	Constant	Second Derivative	No Smoothing	Validation Set	5.252	13%	0.894	9%	0.9653	2.33
PLS	Constant	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.145	38%	0.980	37%	0.9897	1.71
PLS	Constant	Second Derivative	Savitzky-Golay Filter	Validation Set	5.843	0%	0.911	1%	0.9897	1.59
PLS	Constant	Second Derivative	Norris Derivative Filter	Calibration Set	1.241	36%	0.978	35%	0.9889	1.78
PLS	Constant	Second Derivative	Norris Derivative Filter	Validation Set	3.281	5%	0.948	8%	0.9922	1.14
PLS	MSC	Original	No Smoothing	Calibration Set	1.968	24%	0.965	23%	0.9822	2.25
PLS	MSC	Original	No Smoothing	Validation Set	-2.990	4%	1.061	27%	0.9778	2.00
PLS	MSC	Original	Savitzky-Golay Filter	Calibration Set	3.000	15%	0.946	14%	0.9728	2.77

PLS	MSC	Original	Savitzky-Golay Filter	Validation Set	-2.406	20%	1.055	46%	0.9589	2.71
PLS	MSC	First Derivative	No Smoothing	Calibration Set	1.595	29%	0.971	28%	0.9856	2.01
PLS	MSC	First Derivative	No Smoothing	Validation Set	3.829	4%	0.931	4%	0.9901	1.24
PLS	MSC	First Derivative	Savitzky-Golay Filter	Calibration Set	1.641	28%	0.971	27%	0.9852	2.03
PLS	MSC	First Derivative	Savitzky-Golay Filter	Validation Set	3.611	5%	0.935	5%	0.9906	1.20
PLS	MSC	First Derivative	Norris Derivative Filter	Calibration Set	2.662	17%	0.952	16%	0.9758	2.61
PLS	MSC	First Derivative	Norris Derivative Filter	Validation Set	5.247	3%	0.906	3%	0.9832	1.60
PLS	MSC	Second Derivative	No Smoothing	Calibration Set	1.711	27%	0.969	26%	0.9846	2.08
PLS	MSC	Second Derivative	No Smoothing	Validation Set	7.077	0%	0.882	1%	0.9841	1.72
PLS	MSC	Second Derivative	Savitzky-Golay Filter	Calibration Set	2.014	23%	0.964	22%	0.9818	2.25
PLS	MSC	Second Derivative	Savitzky-Golay Filter	Validation Set	4.383	3%	0.928	4%	0.9891	1.35
PLS	MSC	Second Derivative	Norris Derivative Filter	Calibration Set	4.073	9%	0.927	9%	0.9626	3.25
PLS	MSC	Second Derivative	Norris Derivative Filter	Validation Set	10.016	6%	0.822	6%	0.9098	3.45
PLS	SNV	Original	No Smoothing	Calibration Set	1.360	35%	0.976	34%	0.9877	1.92
PLS	SNV	Original	No Smoothing	Validation Set	-2.053	14%	1.052	35%	0.9766	2.15
PLS	SNV	Original	Savitzky-Golay Filter	Calibration Set	9.632	1%	0.828	1%	0.9100	4.96
PLS	SNV	Original	Savitzky-Golay Filter	Validation Set	4.767	22%	0.910	19%	0.9547	2.49
PLS	SNV	First Derivative	No Smoothing	Calibration Set	2.516	19%	0.955	18%	0.9772	2.56
PLS	SNV	First Derivative	No Smoothing	Validation Set	2.545	35%	0.951	32%	0.9785	1.72
PLS	SNV	First Derivative	Savitzky-Golay Filter	Calibration Set	2.692	17%	0.952	16%	0.9755	2.63
PLS	SNV	First Derivative	Savitzky-Golay Filter	Validation Set	1.470	58%	0.968	49%	0.9805	1.66
PLS	SNV	First Derivative	Norris Derivative Filter	Calibration Set	2.766	16%	0.951	15%	0.9750	2.64
PLS	SNV	First Derivative	Norris Derivative Filter	Validation Set	-1.626	12%	1.034	41%	0.9864	1.49
PLS	SNV	Second Derivative	No Smoothing	Calibration Set	1.563	29%	0.972	28%	0.9859	1.99

PLS	SNV	Second Derivative	No Smoothing	Validation Set	7.727	5%	0.851	4%	0.9537	2.66
PLS	SNV	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.423	32%	0.974	31%	0.9871	1.91
PLS	SNV	Second Derivative	Savitzky-Golay Filter	Validation Set	0.592	76%	0.993	85%	0.9892	1.24
PLS	SNV	Second Derivative	Norris Derivative Filter	Calibration Set	2.034	24%	0.963	23%	0.9816	2.28
PLS	SNV	Second Derivative	Norris Derivative Filter	Validation Set	1.644	37%	0.971	36%	0.9907	1.13
PCR	Constant	Original	No Smoothing	Calibration Set	0.684	50%	0.987	48%	0.9937	1.64
PCR	Constant	Original	No Smoothing	Validation Set	3.027	19%	0.965	38%	0.9854	1.76
PCR	Constant	Original	Savitzky-Golay Filter	Calibration Set	1.028	40%	0.982	39%	0.9908	1.63
PCR	Constant	Original	Savitzky-Golay Filter	Validation Set	0.528	83%	1.006	90%	0.9836	1.75
PCR	Constant	First Derivative	No Smoothing	Calibration Set	1.721	28%	0.969	27%	0.9845	2.10
PCR	Constant	First Derivative	No Smoothing	Validation Set	4.144	3%	0.926	3%	0.9902	1.25
PCR	Constant	First Derivative	Savitzky-Golay Filter	Calibration Set	1.747	28%	0.969	27%	0.9842	2.14
PCR	Constant	First Derivative	Savitzky-Golay Filter	Validation Set	3.906	4%	0.930	4%	0.9903	1.23
PCR	Constant	First Derivative	Norris Derivative Filter	Calibration Set	1.273	34%	0.977	33%	0.9886	1.79
PCR	Constant	First Derivative	Norris Derivative Filter	Validation Set	4.702	1%	0.923	1%	0.9919	1.25
PCR	Constant	Second Derivative	No Smoothing	Calibration Set	1.069	39%	0.981	38%	0.9904	1.65
PCR	Constant	Second Derivative	No Smoothing	Validation Set	6.010	1%	0.894	1%	0.9848	1.56
PCR	Constant	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.117	38%	0.980	37%	0.9900	1.69
PCR	Constant	Second Derivative	Savitzky-Golay Filter	Validation Set	4.373	5%	0.935	10%	0.9858	1.63
PCR	Constant	Second Derivative	Norris Derivative Filter	Calibration Set	1.133	38%	0.980	37%	0.9898	1.71
PCR	Constant	Second Derivative	Norris Derivative Filter	Validation Set	3.964	2%	0.939	5%	0.9917	1.28
PCR	MSC	Original	No Smoothing	Calibration Set	1.859	26%	0.967	25%	0.9832	2.18
PCR	MSC	Original	No Smoothing	Validation Set	-2.442	6%	1.048	35%	0.9805	1.80
PCR	MSC	Original	Savitzky-Golay Filter	Calibration Set	3.032	15%	0.946	14%	0.9726	2.78

PCR	MSC	Original	Savitzky-Golay Filter	Validation Set	-2.769	13%	1.061	41%	0.9611	2.65
PCR	MSC	First Derivative	No Smoothing	Calibration Set	1.585	29%	0.972	28%	0.9857	2.00
PCR	MSC	First Derivative	No Smoothing	Validation Set	3.602	7%	0.939	9%	0.9885	1.32
PCR	MSC	First Derivative	Savitzky-Golay Filter	Calibration Set	1.686	28%	0.970	27%	0.9848	2.06
PCR	MSC	First Derivative	Savitzky-Golay Filter	Validation Set	4.073	4%	0.929	4%	0.9890	1.30
PCR	MSC	First Derivative	Norris Derivative Filter	Calibration Set	2.999	15%	0.946	14%	0.9727	2.77
PCR	MSC	First Derivative	Norris Derivative Filter	Validation Set	4.949	6%	0.909	6%	0.9793	1.73
PCR	MSC	Second Derivative	No Smoothing	Calibration Set	2.054	23%	0.963	22%	0.9815	2.27
PCR	MSC	Second Derivative	No Smoothing	Validation Set	4.940	2%	0.917	2%	0.9880	1.41
PCR	MSC	Second Derivative	Savitzky-Golay Filter	Calibration Set	2.245	21%	0.960	20%	0.9797	2.38
PCR	MSC	Second Derivative	Savitzky-Golay Filter	Validation Set	4.632	3%	0.923	4%	0.9878	1.41
PCR	MSC	Second Derivative	Norris Derivative Filter	Calibration Set	2.253	22%	0.959	21%	0.9795	2.42
PCR	MSC	Second Derivative	Norris Derivative Filter	Validation Set	0.206	94%	1.001	99%	0.9779	1.79
PCR	SNV	Original	No Smoothing	Calibration Set	2.172	25%	0.962	24%	0.9808	1.65
PCR	SNV	Original	No Smoothing	Validation Set	0.791	79%	1.002	97%	0.9772	2.02
PCR	SNV	Original	Savitzky-Golay Filter	Calibration Set	3.250	13%	0.942	12%	0.9706	2.88
PCR	SNV	Original	Savitzky-Golay Filter	Validation Set	-3.167	11%	1.068	38%	0.9576	2.80
PCR	SNV	First Derivative	No Smoothing	Calibration Set	3.862	10%	0.931	9%	0.9648	3.12
PCR	SNV	First Derivative	No Smoothing	Validation Set	2.871	32%	0.938	23%	0.9751	1.94
PCR	SNV	First Derivative	Savitzky-Golay Filter	Calibration Set	3.987	9%	0.928	9%	0.9636	3.20
PCR	SNV	First Derivative	Savitzky-Golay Filter	Validation Set	2.770	34%	0.938	23%	0.9755	1.96
PCR	SNV	First Derivative	Norris Derivative Filter	Calibration Set	3.612	11%	0.937	11%	0.9679	2.07
PCR	SNV	First Derivative	Norris Derivative Filter	Validation Set	4.982	2%	0.921	4%	0.9865	1.54

PCR	SNV	Second Derivative	No Smoothing	Calibration Set	3.521	11%	0.937	10%	0.9680	2.98
PCR	SNV	Second Derivative	No Smoothing	Validation Set	7.476	9%	0.848	5%	0.9430	2.94
PCR	SNV	Second Derivative	Savitzky-Golay Filter	Calibration Set	1.659	29%	0.970	27%	0.9850	2.06
PCR	SNV	Second Derivative	Savitzky-Golay Filter	Validation Set	2.363	25%	0.969	39%	0.9882	1.43
PCR	SNV	Second Derivative	Norris Derivative Filter	Calibration Set	1.862	26%	0.966	25%	0.9831	2.20
PCR	SNV	Second Derivative	Norris Derivative Filter	Validation Set	2.523	35%	0.954	34%	0.9803	1.62

Note: PLS: Partial Least Square, PCR: Principle Component Regression, MSC: Multiple Scattering Correction, SNV: Standard Normal Variate, RMSE: Root Mean Square Error (Overall Model Performance Parameter)

APPENDIX B

B.1 Survey questionnaire for medical practitioners

1. Filling Date: (DD/MM/2014)

***2. Your current location:**

- ◯ Uttar Pradesh
- ◯ Uttarakhand
- ◯ New Delhi/Delhi
- ◯ Haryana
- ◯ Punjab
- ◯ Himachal Pradesh
- ◯ Jammu & Kashmir
- ◯ Others

***3. Your Qualification: (You can select more than one option)**

- ☐ M.B.B.S.
- ☐ M.D.
- ☐ M.S.
- ☐ B.D.S.
- ☐ B.U.M.S.
- ☐ B.A.M.S.
- ☐ Higher Degree(DM, Mch, DNB etc.)
- ☐ Others: M.D in Ayurveda/Unani/Dental

***4. Your professional specialization?**
(Note: Like General Physician, Cardiologist, Gynecologist, Neurologist, Orthopedic, Pediatric etc)

5. Your practicing experience in medical profession?

- ◯ Up to 1 Year
- ◯ Up to 2 Years
- ◯ Up to 4 Years
- ◯ Up to 6 Years
- ◯ Up to 8 Years
- ◯ Up to 10 years
- ◯ More than 10 Years

＊6. Which medicine products you preferentially prescribe?
(Note:
#Innovator Branded Generic : Generic products of those companies who are highly involved in inventions, research and manufacturing

#Branded Generic : Generic products of those companies who are highly involved in research and manufacturing

#Generic: Generic products of those companies who are involved in only manufacturing)

	Less Preferable	Moderate Preferable	Most Preferable
Innovator Branded Generic Products (for eg. products of Eli Lilly, Pfizer, GSK, Novartis etc.)	◯	◯	◯
Branded Generic Products (for eg. products of Ranbaxy, Dr. Reddy's, Zydus Cadila, Cipla etc.)	◯	◯	◯
Generic Products (for eg. products of ZenLabs, Alto Healthcare, Ind-swift etc.)	◯	◯	◯

＊7. What are your general views about therapeutic response of:
(Note--
 # Poor : Ineffective & Patient Always Complain

 # Mild : Less Effective & More Complains

 # Moderate : Effective & Few Complains

 # Good : Always Effective & No complain)

	Poor	Mild	Moderate	Good
Innovator Branded Generic Products	◯	◯	◯	◯
Branded Generic Products	◯	◯	◯	◯
Generic Products	◯	◯	◯	◯

***8. What are your general views about price of :**
(Note:
 # No Idea- No Idea about price

 # Non Affordable- For the person whose per day living cost is less than $2.0

 # Moderate- For the person whose per day living cost is less than $2.0

 # Affordable - For the person whose per day living cost is less than $2.0)

	No Idea	Non Affordable	Moderate	Affordable
Innovator Branded Generic Products	◯	◯	◯	◯
Branded Generic Products	◯	◯	◯	◯
Generic Products	◯	◯	◯	◯

***9. What are your general views about adverse effects of :**
(Note :
 # None- No adverse effects observed

 # Mild: Require only substitution by new medicines products

 # Moderate: Require high attention and addition of new medicines products and/or substitution by new medicines products

 # High: Require immediate treatment

 # Severe: Require immediate hospitalization)

	None	Mild	Moderate	High	Severe
Innovator Branded Generic Products	◯	◯	◯	◯	◯
Branded Generic Products	◯	◯	◯	◯	◯
Generic Products	◯	◯	◯	◯	◯

10. From where you get information about medicine uses?
(Note : Rating Scale :
0-No information from this source
1- Minor,
2-Less,
3-Moderate
4-High,
5-Major)

	0	1	2	3	4	5
Medical Council of India	☐	☐	☐	☐	☐	☐
Medical Representative of Pharmaceutical Companies	☐	☐	☐	☐	☐	☐
Conferences, Workshops, Seminars of Pharmaceutical Companies	☐	☐	☐	☐	☐	☐
Conferences, Workshops, Seminars of Medical or Paramedical Professional Organisations	☐	☐	☐	☐	☐	☐
Self study and knowledge	☐	☐	☐	☐	☐	☐

11. How would you rate your knowledge of Drug-Drug Interactions of those drugs which you prescribed?

◯ **Minimal: aware of few side effects/adverse effects/interactions only as informed by the patient themselves**

◯ **Below average: aware of at least two moderate interactions of each among all the prescribed drugs**

◯ **Average: aware of at least five moderate interactions of each among all the prescribed drugs**

◯ **Good: aware of all moderate and major interactions which are life threatening or require hospitalization but not addition of drugs in the prescription, or substitution of prescribed drug**

◯ **Excellent: aware of all moderate and major interaction which are life threatening or require hospitalization or addition of drugs in the prescription, or substitution of prescribed drug**

12. How would you rate your knowledge of Drug-Food Interactions of those drugs which you prescribed?

◯ **Minimal: aware of few side effects/adverse effects/interactions only as informed by the patient themselves**

◯ **Below average: aware of at least two moderate interactions of each among all the prescribed drugs**

◯ **Average: aware of at least five moderate interactions of each among all the prescribed drugs**

◯ **Good: aware of all moderate and major interactions which are life threatening or require hospitalization but not addition of drugs in the prescription, or substitution of prescribed drug**

◯ **Excellent: aware of all moderate and major interaction which are life threatening or require hospitalization or addition of drugs in the prescription, or substitution of prescribed drug**

13. How many new patients consult you everyday?

◯ **1-20**

◯ **21-40**

◯ **41-60**

◯ **61-80**

◯ **81-100**

◯ **More than 100**

14. How much your instructions followed by how many patients?

	100% Instructions Followed By	70-90% Instructions Followed By	40-60% Instructions Followed By	10-30% Instructions Followed By	0% Instruction Followed By
100% Patients	◯	◯	◯	◯	◯
70-90% Patients	◯	◯	◯	◯	◯
40-60% Patients	◯	◯	◯	◯	◯
10-30% Patients	◯	◯	◯	◯	◯
0% Patient	◯	◯	◯	◯	◯

B.2 Survey questionnare for patients

Survey

For Patient/Patient's Guardian

Objective: To know about the patient awareness and experience about medicine, and their participation in healthcare.

Note 1: This survey is being conducted by Dept. of Pharmacy, Jaypee University, Waknaghat, Solan with the permission of Institute Review Board (IRB) , JUIT, Waknaghat, Solan, H.P. India.

Note 2: *All information given by you will be just for academic research only and your personal information will be kept confidential.*

Name :……**Age:**……**Gender:** Male/Female , Address(if possible)……..
……Mobile No. (if possible)…….

Your Location(must):_____(Village/Town/City) Date: ____/____/2014

Q.1. What is your family monthly income?

Rs. 2000- 5,000☐ Rs.5001-10,000☐ Rs. 10,001-20,000☐ Rs. 20,001-30,000☐ More than 30,000☐

Q.2. Which medicine system do you follow?

Allopathic ☐ Unani/ Ayurvedic ☐ Mark **1**—most preferable,
Homeopathic ☐ **2**—preferable,
3—less preferable.

Q.3. Do you get free essential medicines from government hospital under Central or State government scheme?

Not aware about free medicines ☐
Aware and also avail ☐
Aware but do not take ☐

If you are aware but do not take medicine, specify why?..

Because they do not work well ☐ Only few available, not all ☐
Always all unavailable ☐ It takes much time to get ☐

Q.4. If you buy same medicine from two different location and one is you nearby store (your location), what have you observed?

Same response Observed ☐ Near by store medicine have better response than other area store medicine ☐
Nothing Observed ☐ Other area store medicine have better response than my nearby store medicine ☐

Q.5. How much far your medicines are accessible to you?

Up to 2 km ☐ Up to 5 km ☐
Up to 10 km ☐ Up to 20 km or more ☐

Q.6. When you get ill, what you prefer?

Consult to Government Doctor ☐ Mark **1**—most preferable,
Consult to Private Doctor ☐ **2**—preferable,
Consult medical store ☐ **3**—neutral,
Self medication ☐ **4**—less preferable.

Q.7. Do you check on label?

Expiry Date Yes☐ No☐ Maximum Retail Price Yes☐ No☐

Q.8. In a 5 days treatment, on improvement, what do you do generally? (Without asking doctor)

Stop medication before One day ☐ Stop medication before Two day ☐
Stop in mid of treatment ☐ Always complete the treatment ☐

Q.9. How many times you generally miss medicine dose in between your treatment of 5 days (among 10 doses)?

One dose ☐ Two dose ☐
Three dose ☐ Four or more ☐
Never miss ☐

Q.10. How much percent of instruction do you follow given by your doctor about medicines?

100% ☐ 70- 90% ☐
40-60% ☐ 10-30% ☐
0% ☐

Q.11. Do you save your all (old & new) prescriptions written by your doctor?

100% ☐ 70- 90% ☐
40-60% ☐ 10-30 % ☐
0% ☐

LIST OF PUBLICATIONS

[1] **A. N. Khan** and R. K. Khar, "Current scenario of spurious and substandard medicines in India: A systematic review," *Indian Journal of Pharmaceutical Sciences*, vol. 77, no. 1, pp. 2–7, 2015.

[2] **A. N. Khan,** A. Kaur, R. K. Khar, and S. Khanam, "Medicine prescribing preference and patient adherence: perspectives of Indian medical practitioners," *Journal of Young Pharmacist*, vol. 7, no. 4(suppl.), pp. 446-454, 2015.

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