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Method Development and Validation of Metadoxine and Atazanavir in Solid Dosage Form by RP-HPLC

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ABSTRACT

The proposed method was found to be simple, precise, accurate and rapid for determination of Metadoxine and Atazanavir from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Metadoxine and Atazanavir in pure form and its dosage forms and can be used for dissolution or similar studies. From the optical characteristics of the proposed method, it was found that Metadoxine obeys linearity within the concentration range of 0.5-70 g/ml. From the results shown, it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy table, it was found that the percentage recovery values of pure drug from the pre-analyzed solution of formulation were in between 97.8 – 99.7, which indicates that the proposed method is accurate and reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

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INTRODUCTION

Pharmaceutical chemistry deals with the chemistry of substances used as a therapeutic agent in medicine. It embraces the main branches of chemistry, radiochemistry analytical, physical, and organic chemistry. Its scope expands with development in medicinal and allied studies and the emphasis shifts as knowledge advance. On the other hand, it is more narrowly concerned with isolation, determination of structure and synthesis of compounds (mainly organic), which may be used in medicine. It also involves the study of metabolism, mechanism of action of drugs and relationship between structure and biological activity.

The pharmaceutical analysis comprises those procedures necessary to determine the "identity, strength, quality and purity" of the drug. Again, it may be defined as the application of analytical procedures used to determine the purity, safety, and quality of drugs and chemicals. It also deals the analysis of raw materials and intermediates in the manufacture of drugs. The pharmaceutical analyst must, therefore, have a firm background in basic organic analysis and in addition, he should have special skills in the quality evaluation of drug products.

Pharmaceutical analysis includes both qualitative and quantitative analysis of drugs and pharmaceutical substances which start from bulk drugs (starting materials to the finished dosage forms) which are applied for identifying or quantifying constituents in a sample.

MATERIALS AND METHODS

LIST OF INSTRUMENTS USED

Table 1: List of Instruments Used

Sr. No.	Instruments/Equipment's/Apparatus
1.	A SHIMADZU HPLC with Class-VP version 6.12 SP1 software, UV-Visible Detector (SPD-10A), PUMP (LC-10AT) and (LC-10ATvp).
2.	UV-Visible double beam Spectrophotometer (ELICO).
3.	UV-Visible double beam Spectrophotometer(THERMO)
4.	Electronic Balance (AFCOSET)
5.	Ultra Sonicator (ENERTECH)
6.	LiChroCART-Lichrospher® 100 (C_{18}) RP Column (250 mm x 4mm x 5 µm.)
7.	P^H Analyzer (ELICO)
8.	Triple Quartz Distillation Unit (BOROSIL)
9.	HPLC Injecting Syringe (25 µl) (HAMILTON)

LIST OF CHEMICALS, REAGENTS, AND STANDARDS

Table 2: List of Chemicals, Reagents, and Standards

Sr.No.	Chemicals / Reagents / Standards	Grade	Batch No	Specification
1	Tetra butyl ammonium hydrogen sulfate	AR	DL8S48123	99.5%
2	Methanol	HPLC	R191L04	99.7%
3	Acetonitrile	HPLC	R054B03	99.9%
4	Triple distilled water	NA	NA	NA
5	Metadoxine sample	NA	GO4532	99.81 %
6	Doxophylline sample	NA	NA	99.98 %
7	Atazanavir sample	NA	NA	99.94 (w/v)
8	Clopidogrel sample	NA	NA	99.99 (w/v)
9	Sodium Hydroxide	NA	NA	NA
10	Hydrochloric Acid	NA	NA	NA

Optimized chromatographic conditions:

Table 3: Optimized Chromatographic Conditions

Parameters	Conditions
Stationary phase (column)	C_{18} RP Column (250 mm x 4.6mm x 5 µm
Mobile Phase	Methanol: 5mM TBHS(50:50%v/v)
Flow rate (ml/min)	1.0 ml
Run time (minutes)	10(Metadoxine), 8(Atazanavir)
Column temperature (°C)	Ambient
Volume of injection loop (□ l)	20
Detection wavelength (nm)	274(Metadoxine), 249(Atazanavir)
Internal standards	Doxophylline and Clopidogrel

METHOD FOR METADOXINE

Preparation of mobile phase:

Methanol and 5mM Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) were properly mixed in the ratio of 50:50.

Preparation of Tetra Butyl Ammonium Hydrogen Sulphate (TBHS): 0.84885gm of Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) was added to 500ml of double distilled water to make 5mM solution of TBHS.

Preparation of standard drug solution:

A stock solution of Metadoxine (1mg/ml) was prepared by dissolving 25 mg of Metadoxine in 25 ml of the volumetric flask containing 10 ml of mobile phase. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase. Working standard solutions of Metadoxine was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly, stock solution of internal standard was prepared by dissolving 25 mg of Doxophylline in 10 ml of the mobile phase, sonicated for 20 min. then made up to the volume with mobile phase. Working standard solutions of Metadoxine were prepared by taking suitable aliquots of drug solution from the standard stock solution 1000µg/ml, spiked with an internal standard solution (0.1ml) and the volume was made up to 10 ml with mobile phase.

Preparation of sample solution

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of Metadoxine was extracted with mobile phase in a 25ml volumetric flask using ultra sonicator. This solution was filtered through 0.45µm filter paper. The solution obtained was diluted with the mobile phase to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in triplicate.

Procedure for calibration curve:

The contents of the mobile phase were filtered before use through 0.45µm filter paper and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to

injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. Then, 20µl of each of standard and sample solutions were injected into the HPLC system for six times to get the chromatograms. The retention time, average peak areas and peak area ratios of drug to the internal standard were recorded. Taking cons. plotted a graph on X-axis and peak area ratios on Y-axis. The linearity range was found to be in between 1-120 µg/ml for Metadoxine. The linearity range and linearity graphs were shown in table.no…..

Analysis of formulations:

The amount of drug present in the pharmaceutical formulation was calculated through peak area ratio of drug to that of the internal standard by using the standard calibration curve, (concentration in µg/ml was taken on X-axis and peak area ratio on Y-axis). A typical chromatogram of Metadoxine in the formulation and internal standard was shown in Fig.

METHOD FOR ATAZANAVIR

Preparation of mobile phase:

Methanol and 5mM Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) were properly mixed in the ratio of 50:50.

Preparation of Tetra Butyl Ammonium Hydrogen Sulphate (TBHS): 0.84885gm of Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) was added to 500ml of double distilled water to make 5mM solution of TBHS.

Preparation of standard drug and internal standard solution:

A stock solution of Atazanavir (1mg/ml) was prepared by dissolving 25 mg of Atazanavir in 25 ml of the volumetric flask containing 10 ml of Methanol and 10 ml of 5mM Tetra Butyl Ammonium Hydrogen Sulphate. The solution was sonicated for about 10 min and then made up to volume with mobile phase. Working standard solutions of Atazanavir was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly, stock solution of internal standard was prepared by dissolving 25mg of Clopidogrel in 25 ml of the volumetric flask containing 10 ml of Methanol and 10ml of 5mM Tetra Butyl Ammonium Hydrogen Sulphate, sonicated for 10min, then made up to the volume with mobile phase. Working standard solutions of Atazanavir were prepared by taking suitable aliquots of drug

solution from the standard stock solution of 1000µg/ml, spiked with an internal standard solution (0.1ml from 1000µg/ml) and the volume was made up to 10 ml with mobile phase.

RESULTS

Method development of Metadoxine:

Linearity:

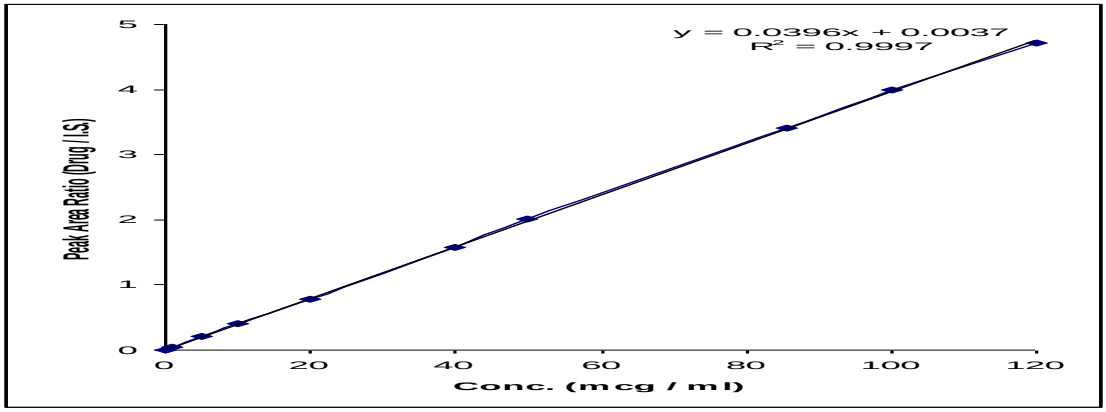

Fig 1: Linearity curve of Metadoxine

Concentration	Peak Area Ratio
1	0.04
5	0.206
10	0.406
20	0.776
40	1.573
50	2.012
85.6	3.41
100	3.995
120	4.721

Table 4: Linearity Table

Table: 5: The amount of Metadoxine present in Tablets:

Formulation	Labeled amount (mg)	HPLC method*		
		Mean ±S.D (amount mg recovered)	%Drug recovered	% RSD
VIBOLIV(tablet)	500	497.96±0.2163	99.592	0.0434

Sr. No	Name of the Peaks	Retention time (min)
1.	Metadoxine	2.2
2.	Doxophylline	3.825

Chromatogram of Metadoxine(20µg/ml) and Doxophylline in pure drug:

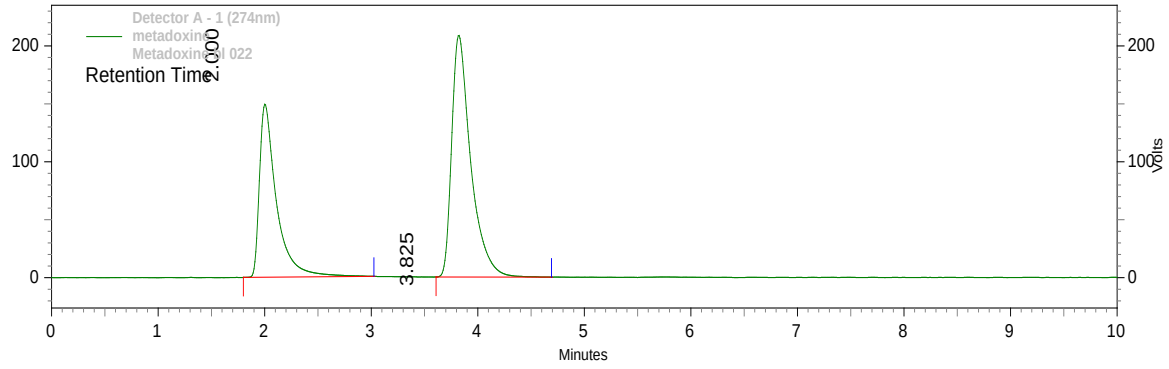

Fig 2: A Typical Chromatogram of Metadoxine (20µg/ml) and Doxophylline in pure drug

Chromatogram of Metadoxine (10 µg/ml) and Doxophylline in Formulation:

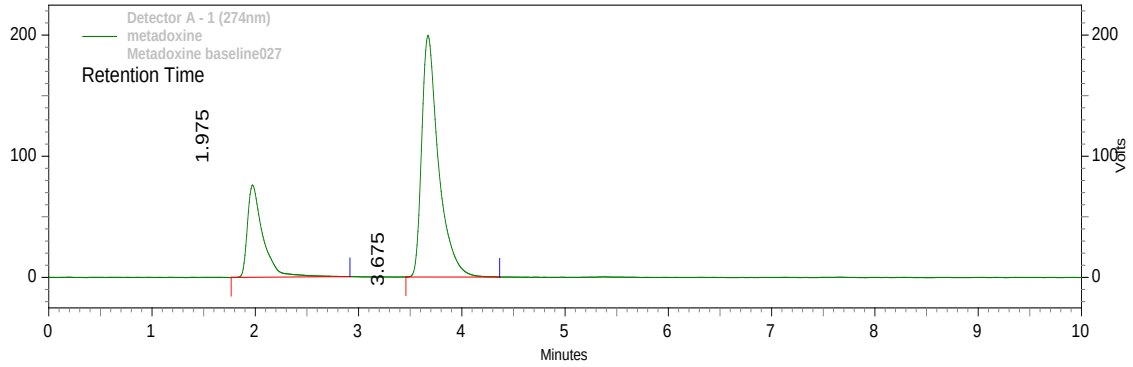

Fig 3: A Typical Chromatogram of Metadoxine (10 µg/ml) and Doxophylline in Formulation

S.NO	Name of the Peaks	Retention time (min)
1.	Metadoxine	1.975
2.	Doxophylline	3.675

Linearity Atazanavir:

Table 6: Linearity table of Atazanavir

Concentration(mcg/ml)	Peak area ratio (drug/I.S.)
0.5	0.0704
1	0.163
5	0.718
10	1.438
20	2.839
50	7.110
100	14.588

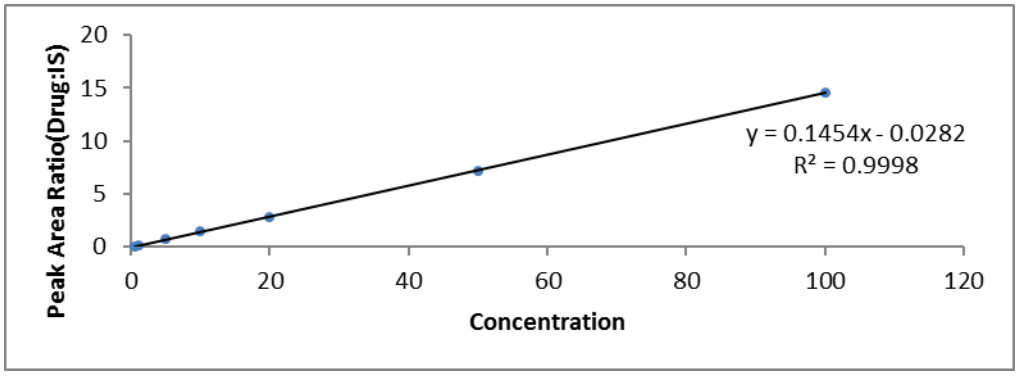

Fig 4: Linearity graph of Atazanavir

Table 7: Amount of Atazanavir present in Formulation

Formulation	Labeled amount (mg)	HPLC method*		
		Mean amount found(mg)	%Recovery by proposed method	% RSD
Atazor	100	100.97 ± 0.9013	100.9741 ± 0.9013	0.8926

Sr. No.	Name of the Peaks	Retention time (min)
1.	Atazanavir	3.217
2.	Clopidogrel	6.183

Chromatogram of Atazanavir (50µg/ml) and Clopidogrel (10µg/ml) in pure form:

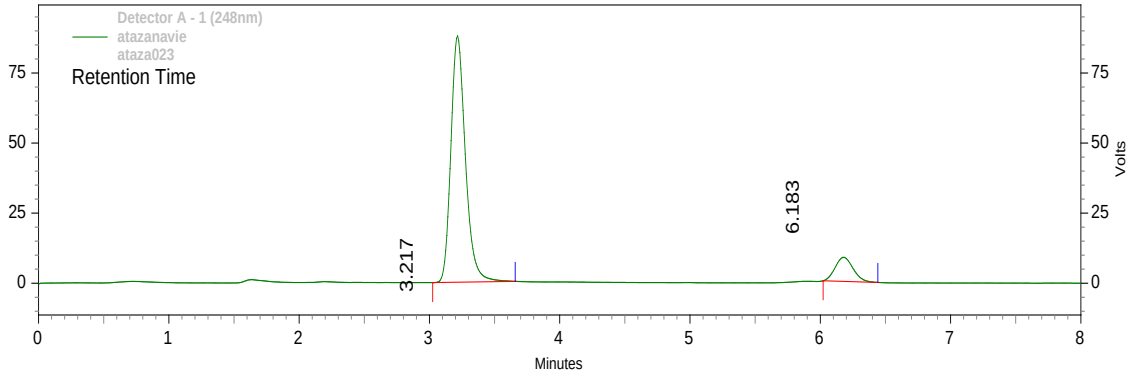

Fig 5: A Typical Chromatogram of Atazanavir (50µg/ml) and Clopidogrel (10µg/ml) in pure form

Chromatogram of Atazanavir (100µg/ml) (formulation) and Clopidogrel (10µg/ml):

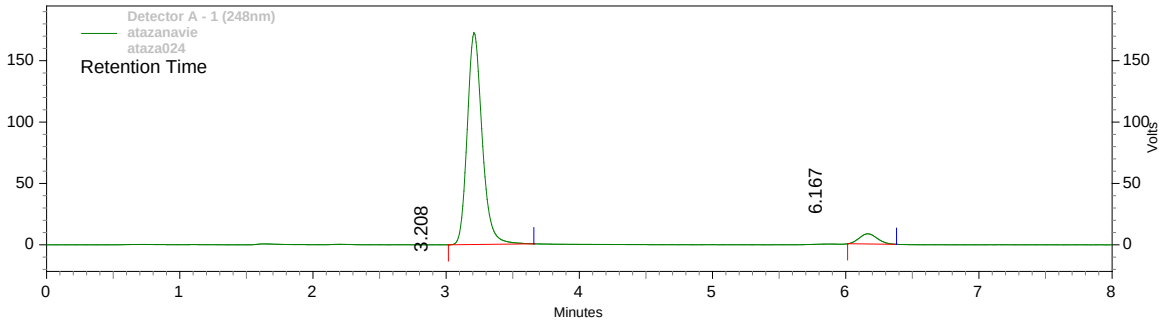

Fig 6: A Typical Chromatogram of Atazanavir (100µg/ml) (formulation) and Clopidogrel (10µg/ml)

Sr. No.	Name of the Peaks	Retention time (min)
1.	Atazanavir	3.208
2.	Clopidogrel	6.167

VALIDATION OF METADOXINE

Precision:

Table 8: Precision Readings of Metadoxine

Sr. No.	Concentration (g/ml)	Ratio(Drug/I.S)	Statistical analysis
1	10	1.426	Mean=1.426 SD=0.014 %RSD=1.007
2.	10	1.438	
3.	10	1.424	
4.	10	1.432	
5.	10	1.438	
6.	10	1.399	

Accuracy:

Table 9: Accuracy Readings of Metadoxine

Sample ID	Concentration (□ g/ml)		%Recovery of pure drug	Statistical Analysis
	Pure drug	Formulation		
S1: 80 %	8	10	98.274	Mean=97.973
S2 : 80 %	8	10	97.314	SD=0.572
S3: 80 %	8	10	98.333	% RSD=0.584
S4: 100 %	10	10	100.445	Mean=100.666
S5: 100 %	10	10	99.694	SD=1.099
S6: 100 %	10	10	101.858	% RSD=1.091
S7: 120 %	12	10	101.511	Mean=100.602
S8 : 120 %	12	10	99.743	SD=0.885
S9: 120 %	12	10	100.551	% RSD=0.879

System suitability:

Table 10: System suitability parameters of Metadoxine

Sr. No.	Parameters	Obtained Values
1.	Theoretical plates (N)	3755.22
2.	Resolution (R) between drug and I.S.	1.35
3.	Tailing factor (T)	1.32
4.	LOD	0.3731
5.	LOQ	1.1206

VALIDATION OF ATAZANAVIR

Precision:

Table 11: Precision readings of Atazanavir

Concentrations (□ g/ml)	Absorbance	Statistical Analysis
20	0.597	Mean = 0.5981 SD = 0.001356 %RSD = 0.2267
20	0.597	
20	0.598	
20	0.599	
20	0.601	
20	0.598	
20	0.597	
20	0.598	

Accuracy:

Table 12: Accuracy Readings of Atazanavir

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis	
	Pure drug	Formulation			
S_1: 80 %	8	10	97.8	Mean	98.133
S_2: 80 %	8	10	98.3	SD	0.2886
S_3: 80 %	8	10	98.3	% RSD	0.2940
S_4: 100 %	10	10	99.7	Mean	99.5
S_5: 100 %	10	10	99.1	SD	0.3641
S_6: 100 %	10	10	99.7	% RSD	0.3481
S_7: 120 %	12	10	99.0	Mean	98.834
S_8: 120 %	12	10	98.75	SD	0.14433
S_9: 120 %	12	10	98.75	% RSD	0.146

Linearity:

Table 13: Linearity table of Atazanavir

Concentration(mcg/ml)	Peak area ratio (drug/I.S.)
0.5	0.0704
1	0.163
5	0.718
10	1.438
20	2.839
50	7.110
100	14.588

DISCUSSION

METADOXINE

From the optical characteristics of the proposed method, it was found that Metadoxine obeys linearity within the concentration range of 0.5-70 µg/ml. From the results shown, it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy table, it was found that the percentage recovery values of pure drug from the pre-analyzed solution of formulation were in between 97.8 – 99.7, which indicates that the proposed method is accurate and reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

ATAZANAVIR

From the linearity table, it was found that the drug obeys linearity within the concentration range of 0.5-100 µg/ml for Atazanavir. From the results shown in the precision table, it was found that RSD is less than 2%; which indicates that the proposed method has good reproducibility. From the results shown in accuracy table, it was found that the percentage recovery values of the pure drug from the pre-analyzed solutions of formulations were in between 97.97-100.66, which indicates that the method was accurate and reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not

interfering the proposed method. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Metadoxine and Atazanavir from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Metadoxine and Atazanavir in pure form and its dosage forms and can be used for dissolution or similar studies.

HPLC is a versatile tool for the qualitative and quantitative analysis of drugs and pharmaceuticals, chemicals and biological materials and has become indispensable in pharmacokinetics studies. The development of highly efficient micro particulate bonded phase has increased the versatility of the technique and has greatly improved the analysis of multi-component mixtures. The systems used are often described as belonging to one or more among four mechanistic type, adsorption, partition, ion exchange and size exclusion. Adsorption and partition systems can be the normal phase (stationary phase more polar than eluent) or reversed phase (stationary phase less polar than eluent).

There is a wide scope for the development of new analytical methods for the assay of, "Metadoxine and Atazanavir" by exploiting their characteristic physical and chemicals properties (dependent on basic moieties and functional groups present in each drug).

ACKNOWLEDGEMENTS

I humbly present this work to the eternal almighty. Indeed my final work is done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

I wish to extend my sincere thanks, **Sri. V. Prasad Rao**, Chairman, Vikas College of Pharmacy, Jangaon for his encouragement that made me finish this work.

ABBREVIATIONS:

HPLC	High-performance Liquid Chromatography
NMT	Not More Than
%	Percent
AUC	Area Under Curve
LC	Liquid Chromatography
PDA	Photodiode Array
ICH	International Conference for Harmonization
GR	General reagent
C18	Octadecyl
UV	Ultraviolet
ml	Milliliter
Min	Minute
MeOH	Methanol
µl	Micro Liter
µ	Micron
µg	Micro gram
N	Normality
M	Molar
ppm	Parts per million
nm	Nanometer
RSD	Relative Standard Deviation
Fig	Figure
cv	Coefficient of variation
HIV	Human Immunodeficiency Virus

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