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Original Article

A RAPID AND COST EFFECTIVE UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF INDINAVIR SULPHATE IN CAPSULES

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ABSTRACT

Objective: To develop a cheap and rapid UV spectrophotometric method for the quantitative estimation of Indinavir sulphate (400mg) in capsules and validate as per ICH guidelines.

Methods: The optimized method uses a diluent 100% potassium dihydrogen ortho phosphate buffer (pH 3.0) for the estimation of assay of Indinavir sulphate whose λmax is 259 nm.

Results: The developed method resulted in Indinavir sulphate exhibiting linearity in the range 20-80 µg/ml. The precision is exemplified by relative standard deviation of 1.49%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 5 µg/ml, and 16.27 µg/ml respectively.

Conclusion: A cheap and rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Indinavir sulphate in capsules as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: UV, Indinavir sulphate, Method development, Validation.

INTRODUCTION

Indinavir sulphate (**Figure 1**) is a human immunodeficiency virus (HIV) protease inhibitor used for treating acquired immune deficiency syndrome (AIDS). Indinavir sulphate is usually prescribed in combination with other protease inhibitors, nucleoside analogues or reverse transcriptase inhibitors[1-3]. IUPAC name of Indinavir sulphate is [1(1*S*,2*R*),5(*S*)]-2,3,5-trideoxy-N-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)-5-[2-[[(1,1-dimethylethyl)amino] carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-phenyl methyl)-D-*erythro*-pento namide sulphate (1:1) salt.

The drug has a molar mass of 613.88 g/mol for the free base and 711.88 g/mol for the sulphate salt and is commercially available as capsules (trade name: INDIVAN) containing the equivalent of 400 mg of indinavir free base.

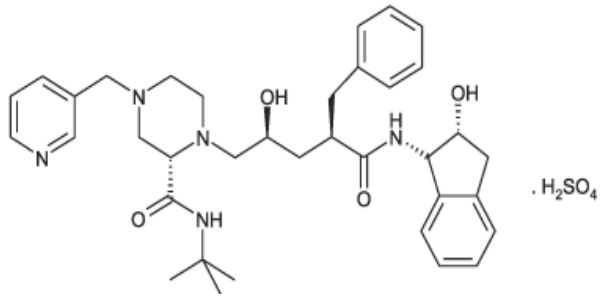

Fig. 1: Structure of Indinavir sulphate

A detailed literature survey reveals LC methods for the analysis of Indinavir sulphate individually and in various combinations in biological matrices[4-10], capillary zone electrophoresis method for the analysis of indinavir sulphate raw material[11], few RP-HPLC methods for the determination of assay of Indinavir in bulk and in capsule dosage forms[12-13]. There exists only one UV spectrophotometric method reported for the determination of assay of Indinavir sulphate in capsule dosage forms using bromocresol purple and bromothymol blue[14]. Hence we here report a new, cheap and rapid UV spectrophotometric method for the quantitative estimation of Indinavir sulphate in INDIVAN capsules.

MATERIALS AND METHODS

Materials

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101) and a sonicator (sonica, model 2200 MH) were used in this study.

Chemicals and Reagents

Analytically pure sample of Indinavir sulphate with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [INDIVAN] was procured from MEDPLUS, Hyderabad, India with labelled amount 400mg of Indinavir sulphate. Potassium dihydrogen ortho phosphate (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India). 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Method

Solvent: Solvent used is prepared by adding 2.72 grams of potassium dihydrogenortho phosphate to 1000 ml of distilled water and later pH was adjusted to 3.0 using 30% v/v of ortho phosphoric acid in water. Solvent was then filtered through 0.45 µm nylon membrane filter.

Selection of suitable detection wavelength

Suitable wavelength for the total experiment was determined by recording UV spectrum in the range of 200-400 nm for Indinavir sulphate and suitable wavelength selected was 259 nm (Figure 2).

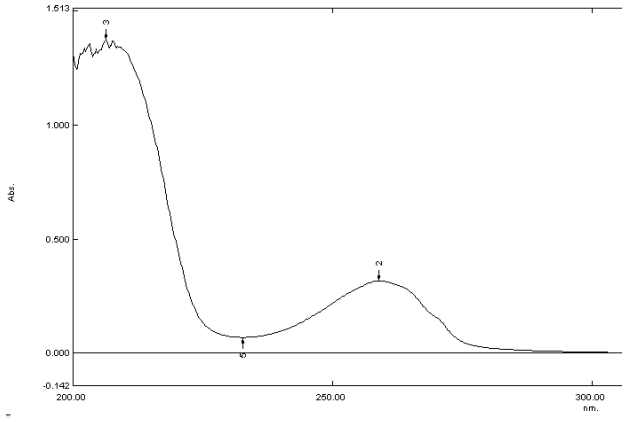

Fig. 2: UV spectrum of Indinavir sulphate

Preparation of stock and working standard solution

10mg of Indinavir sulphate was accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 4ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 40µg/ml, treated as working standard, 100% target concentration.

Preparation of stock and working sample solution

Ten tablets were weighed separately and the average weight was determined. The average weight was weighed from the ten tablets grinded in a pestle and mortar, transferred to a 100 ml volumetric flask containing 100ml diluent and then stirred for 10 minutes, followed by filtration through 0.45µ nylon membrane filter to get sample stock solution of 4mg/ml. 1 ml of the above stock solution was pipetted out and made up to 100 ml to get working sample solution equivalent to a concentration of working standard of 40 µg/ml.

RESULTS AND DISCUSSION

Method Development

Various solvents were explored, including Potassium dihydrogen orthophosphate, triethylammonium phosphate and ammonium acetate buffers varying pH in the ranges of 2-7. Indinavir sulphate was found to be soluble and stable for minimum of 1 hour at room temperature using pH 3.0 Potassium dihydrogen orthophosphate buffer and hence this buffer was initiated for the determination of suitable detection wavelength and working concentration of standard. In order to test the applicability of the developed method to a commercial formulation, INDIVAN was studied at working concentration.

Absorbance and assay for working concentration of sample at 259 nm was in acceptance limits (98-102%) with the standard working concentration during extraction of drug in the sample using the solvent for 10 minutes. The protocol affords reproducible quantification of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control. Hence the method is optimized.

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines [15] for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ).

Precision

System precision

Six replicate recording of absorbance at 259nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning absorbance for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in **Table 1**.

Method precision

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Table 1: System precision results of Indinavir sulphate.

n	Absorbance
1	0.332
2	0.330
3	0.341
4	0.335
5	0.343
6	0.338
Average	**0.336**
SD	**0.005**
% RSD	**1.48**

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 259nm of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 2).

Table 2: Intra day precision results of Indinavir sulphate

n	% Assay
1	98.6
2	98.1
3	101.2
4	99.5
5	101.8
6	100.3
Average	**99.91**
S.D.	**1.49**
% RSD	**1.49**

Intermediate Precision (Inter day precision / Ruggedness)

Six consecutive recording of absorbance at 259nm of the sample solution from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (Table 3).

Linearity

Standard solutions of Indinavir sulphate at different concentrations level (50%, 75%, 100%, 125%, 150%, 175% and 200%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding absorbance at 259nm. The results show an excellent correlation between absorbance and concentration level of drug within the concentration range (20-80 µg/ml) for the drug and the results are given in **Table 4.** The correlation coefficients were greater than 0.995, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 20-80 µg/ml.

Table 3: Inter day precision results.

n	Day 1	Day 2	Day 3
1	98.6	100.68	99.45
2	98.1	101.1	99.32
3	101.2	101.3	98.3
4	99.5	100.6	100.1
5	101.8	100.2	100.2
6	100.3	99.8	99.43
Average	**99.91**	**100.61**	**99.46**
SD	**1.49**	**0.555**	**0.681**
% RSD	**1.49**	**0.55**	**0.68**

Table 4: Calibration data for indinavir sulphate

% Level	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3
50	20	0.182	0.160	0.168
75	30	0.244	0.228	0.221
100	40	0.335	0.320	0.313
125	50	0.426	0.408	0.396
150	60	0.513	0.484	0.499
175	70	0.596	0.582	0.591
200	80	0.689	0.673	0.680
Regression equation		y = 0.0085x-0.0026	y = 0.0086x-0.0226	y = 0.0087x-0.0299
Regression coefficient		0.9983	0.9984	0.995

Table 5: Results of accuracy studies for indinavir sulphate

Concentration Level (%)	*%Mean Recovery
50	99.5
100	100.3
150	99.26

*Mean of three replicates

Table 6: Robustness results of indinavir sulphate sample

Variation parameter	Variation	%RSD
pH(± 0.2)	3.2	0.796
	3	1.702
	2.8	0.813
Wave length (± 2nm)	261	1.003
	259	0.45
	257	1.298

Table 7: Optical characteristics and validation parameters of Indinavir sulphate

Parameters	Results
Detection wavelength (nm)	259
Beer's Law limits (µg/ml)	20 - 80
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.009
Regression equation (y = mx+c)	y = 0.0086-0.0183
Correlation coefficient (r^2)	0.9972
Slope (m)	0.0086
Intercept (c)	-0.0183
% Relative Standard Deviation (% RSD) System precision	1.48
(% RSD) Intra-day precision	1.49
(% RSD) Inter-day precision	≤ 2
Accuracy (% Mean Recovery)	
50 % Level	99.5
100 % Level	100.3
150 % Level	99.26
LOD (µg/ml)	5
LOQ (µg/ml)	16.27
Robustness	
pH(± 0.2) (% RSD)	≤ 2
Wavelength (± 2nm) (% RSD)	≤ 2

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in **Table 5.** The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the drug concerning % assay despite deliberate variations done concerning pH ± 0.2 and detection wavelength ± 2nm (Table 6).

Sensitivity

The sensitivity of measurement of Indinavir sulphate by use of the proposed method was estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD) and Sandell's sensitivity. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 5 µg/ml, and 16.27 µg/ml respectively. Optical characteristics and validation parameters results are summarized in Table 7.

CONCLUSION

A cheap and rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Indinavir sulphate in capsules as per ICH guidelines. The developed method resulted in Indinavir sulphate exhibiting linearity in the range 20-80 µg/ml. The precision is exemplified by relative standard deviation of 1.49%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 5 µg/ml, and 16.27 µg/ml respectively. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of Indinavir sulphate in tablets in various pharmaceutical industries.

CONFLICT OF INTERESTS

Declared None

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REFERENCES

1. Vacca JP, Dorsey BD, Schleif WA, Levin RB, McDaniel SL, Darke PL, Zugay J, Quintero JC, Blahy OM, Roth E, Sardana VV, Schlbach AJ, Graham PI, Condra JH, Gotlib L, Holloway MK, Lin J, Chen IW, Vastag K, Ostovic D, Anderson PS, Emini EA, Huff JF. L-735,524:an orally bioavailable human immunodeficiency virus type 1 protease inhibitor. Proc Natl Acad Sci USA 1994;91(9):4096-4100.
2. Yeh KC, Deustsch PJ, Haddix H, Hesney M, Hoagland V, Ju WD, Justice SJ, Osbournem B, Sterrett AT, Stone JA, Woolf E, Waldman S. Single-Dose Pharmacokinetics of Indinavir and the Effect of Food. Antimicrob Agents Chemother 1998;42(2):332-338.
3. Patrick AK, Potts KE. Protease Inhibitors as Antiviral Agents. Clin Microbiol Rev 1998;11(4):614-627
4. Woolf E, Haddix HM, Matuszewski B. Determination of an in vivo metabolite of a human immunodeficiency virus protease inhibitor in human plasma by high-performance liquid chromatography with tandem mass spectrometry. J Chromatogr A 1997;762(1-2):311-319.
5. Burger DM, de Graaff M, Wuis EW, Koopmans P, Hekster YA. Determination of Indinavir, an HIV-protease inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;703:235-241.
6. Dailly E, Thomas L, Kergueris MF, Joillet P, Bourin M. High-performance liquid chromatographic assay to determine the plasma levels of HIV-protease inhibitors (amprenavir, indinavir, nelfinavir, ritonavir and saquinavir) and the non-nucleoside reverse transcriptase inhibitor (nevirapine) after liquid–liquid extraction. J Chromatogr B Biomed Sci Appl 2001;758:129-135.
7. Foisy ML, Sommadossi JP. Rapid quantification of indinavir in human plasma by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl 1999;721(2);239-247.
8. Jayewardene AL, Zhu F, Aweeka FT, Gambertoglio JG. Simple high-performance liquid chromatographic determination of the protease inhibitor Indinavir in human plasma. J Chromatogr B Biomed Sci Appl 1998;707(1-2):203-211.
9. Jayewardene A, Kearney B, Stone JA, Gambertoglio JG, Aweeka FT. An LC-MS-MS method for the determination of Indinavir, an HIV-1 protease inhibitor, in human plasma. J Pharm Biomed Anal 2001;25(2):309-317.
10. 10.Rose MJ, Merschman SA, Eisenhandler R, Woolf EJ, Yeh KC, Lin L, Fang W, Hsieh J, Braun MP, Gatto GJ, Matuszewski BK. High-throughput simultaneous determination of the HIV protease inhibitors Indinavir and L-756423 in human plasma using semi-automated 96-well solid phase extraction and LC-MS/MS. J Pharm Biomed Anal 2000;24(2):291-305.
11. Elisabete AP, Gustavo AM, Marina FMT. Development and validation of a capillary electrophoresis method for the determination of sulphate in Indinavir sulphate raw material. Journal of the Brazilian Chemical society 2006;17(2);251-256.
12. Johnson BD, Howard A, Varsolona R, McCauley J, Ellison DK. Indinavir. Anal. Prof. Drug Substances 1999;26:319-357.
13. United States Pharmacopeial Convention;Pharmacopeial Forum, Rockville, MD 2000;26:1638.
14. Erk N. Spectrophotometric determination of Indinavir in bulk and pharmaceutical formulations using bromocresol purple and bromothymol blue. Pharmazie 2004;59(3):183-186.
15. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures:Text and Methodology ICH Q2 (R1). 2005.