

Original Article

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF INDINAVIR SULPHATE IN CAPSULES

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ABSTRACT

**Objective:** To develop a simple and cheap 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 4.0) for the estimation of assay of Indinavir sulphate whose  $\lambda_{max}$  is 259 nm.

**Results:** The developed method resulted in Indinavir sulphate exhibiting linearity in the range 20-80  $\mu\text{g/ml}$ . The precision is exemplified by relative standard deviation of 1.44%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 14.42  $\mu\text{g/ml}$  and 43.69  $\mu\text{g/ml}$  respectively.

**Conclusion:** A simple and a cheap 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(1S,2R),5(S)]-2,3,5-trideoxy-N-2,3-dihydro-2-hydroxy-1H-inden-1-yl)-5-[2-[[[(1,1-dimethylethyl)amino] carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-phenylmethyl]-D-erythro-pentonamide 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.

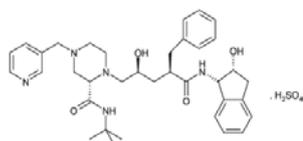


Fig. 1: Structure of Indinavir sulphate

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). 0.45 $\mu\text{m}$  Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Methods

Solvent

Solvent used is prepared by adding 2.72 grams of potassium dihydrogen ortho phosphate to 1000 ml of distilled water and later pH was adjusted to 4.0 using 30% v/v of ortho phosphoric acid in water. Solvent was then filtered through 0.45  $\mu\text{m}$  nylon membrane filter.

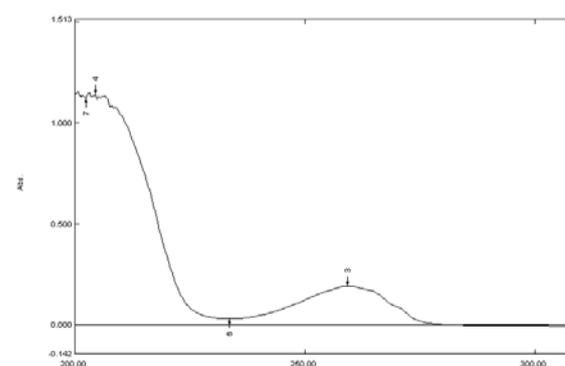


Fig. 2: UV spectrum of Indinavir sulphate

### 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).

### 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 4.0 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 quantitation (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	0.264
2	0.263
3	0.263
4	0.264
5	0.264
6	0.262
<b>Average</b>	<b>0.263</b>
<b>SD</b>	<b>0.0008</b>
<b>% RSD</b>	<b>0.304</b>

**Table 2: Intra day precision results of Indinavir sulphate**

n	% Assay
1	100.2
2	102.5
3	102.5
4	103.5
5	104.0
6	104.4
<b>Average</b>	<b>102.8</b>
<b>S.D.</b>	<b>1.48</b>
<b>% RSD</b>	<b>1.44</b>

**Table 3: Inter day precision results.**

n	Day 1	Day 2	Day 3
1	100.2	100.44	99.48
2	102.5	101.1	99.32
3	102.5	100.3	98.3
4	103.5	100.6	100.3
5	104.0	100.2	100.4
6	104.4	99.8	99.55
<b>Average</b>	<b>102.8</b>	<b>100.4</b>	<b>99.55</b>
<b>SD</b>	<b>1.48</b>	<b>0.555</b>	<b>0.681</b>
<b>% RSD</b>	<b>1.44</b>	<b>0.433</b>	<b>0.7622</b>

### 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).

### 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.

### 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  $\pm$  0.2 and detection wavelength  $\pm$  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 quantitation (LOQ) were found to be 14.42  $\mu$ g/ml and 43.69  $\mu$ g/ml respectively. Optical characteristics and validation parameters results are summarized in Table 7.

**Table 4: Calibration data for Indinavir sulphate**

% Level	Concentration ( $\mu$ g/ml)	Absorbance 1	Absorbance 2	Absorbance 3
50	20	0.146	0.15	0.152
75	30	0.20	0.213	0.211
100	40	0.260	0.258	0.280
125	50	0.324	0.328	0.341
150	60	0.369	0.381	0.375
175	70	0.452	0.433	0.447
200	80	0.502	0.51	0.506
<b>Regression equation</b>		$y=0.0061(x)+0.0207$	$y=0.0058(x)+0.0313$	$y=0.0058(x)+0.039$
<b>Regression coefficient</b>		0.998	0.997	0.996

**Table 5: Results of Accuracy studies for Indinavir sulphate**

Concentration Level (%)	*%Mean Recovery
50	99.5
100	101.8
150	100.3

\*Mean of three replicates

**Table 6: Robustness results of Indinavir sulphate sample**

Variation Parameter	Variation	%RSD
pH( $\pm$ 0.2)	4.2	1.22
	4	1.44
	3.8	1.3
Wave length ( $\pm$ 2nm)	261	0.35
	259	1.44
	257	0.54

**Table 7: Optical characteristics and validation parameters of Indinavir sulphate**

Parameters	Results
Detection wavelength (nm)	259
Beer's Law limits ( $\mu$ g/ml)	20 - 80
Sandell's sensitivity ( $\mu$ g/cm <sup>2</sup> /0.001 absorbance unit)	0.3
Regression equation ( $y = mx+c$ )	$y=0.0059+0.0303$
Correlation coefficient ( $r^2$ )	0.997
Slope (m)	0.0059
Intercept (c)	0.0303
% Relative Standard Deviation (% RSD) System precision	0.304
(% RSD) Intra-day precision	1.44
(% RSD) Inter-day precision	$\leq$ 2
Accuracy (% Mean Recovery)	
50 % Level	99.5
100 % Level	101.8
150 % Level	100.3
LOD ( $\mu$ g/ml)	14.42
LOQ ( $\mu$ g/ml)	43.69
Robustness	
pH( $\pm$ 0.2) (% RSD)	$\leq$ 2
Wavelength ( $\pm$ 2nm) (% RSD)	$\leq$ 2

### CONCLUSION

A cheap and a 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  $\mu$ g/ml. The precision is exemplified by relative standard deviation of 1.44%. Percentage Mean recovery was found to be in the range of 99.5-101.8, during accuracy studies. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 14.42  $\mu$ g/ml and 43.69  $\mu$ g/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.

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