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Spectrophotometric determination of indinavir in bulk and pharmaceutical formulations using bromocresol purple and bromothymol blue

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A simple, sensitive and selective method for the determination of indinavir (IND) in bulk and in pharmaceutical formulations is described. The method is based on extraction of this drug into chloroform as ion-pair with sulphonphthalein dyes as bromocresol purple (BCP) and bromothymol blue (BTB). The optimum conditions of the reactions were studied and optimized. The absorbance of the yellow products was measured at 427 nm for IND-BCP and 420 nm for IND-BTB. The calibration graphs were linear over the range 4.0–60.2 $\mu g \cdot ml^{-1}$ of drug in chloroform, using the two dyes. The composition of the ion-pairs was established by the molar ratio method. For IND the motar ratio was determined to be 1 : 1 by measurement of first derivative signals at 273 nm. A calibration graph was established for 3.0–70.6 $\mu g \cdot ml^{-1}$ of IND for first derivative spectrophotometry. The developed method was applied successfully for the determination of IND in pharmaceutical formulations. The data obtained were compared the data given by first derivative spectrophotometry. No differences were found.

1. Introduction

The HIV protease inhibitor indinavir (IND) is chemically designated as *N*-[2(*R*)-hydroxy-1(*S*)-indanyl]-5-{[2(*S*)-*tert*-butylaminocarbonyl]-4-(3-pyridyl–methyl) piperazino}-4 (S)-hydroxy-2 (R)-phenyl-methyl-pentanamide (L-735, 524, MK-639, Crixivan®).

No official (pharmacopoeial) method has been found for the assay of IND in its formulations.

In the literature, only HPLC methods have been described for the quantitative determination of indinavir and its metabolites in human plasma (Rentsch 2003; Frerichs et al. 2003; Crommentuyn et al. 2003; Turner et al. 2003; Justesen et al. 2003; Chi et al. 2002; Cociglio et al. 2003; Ray et al. 2002; Villani et al. 2001; Yamada et al. 2001; Marchei et al. 2001; Laussine et al. 2001; Aymard et al. 2000; Langmann et al. 1999; Zhong and Yeh 1999; Li et al. 1999; Van Heeswijk 1998).

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs; for lercanidipine (Erk 2003a), reboxetine (Erk 2003b), sildenafil citrate (Dinesh et al. 2002), enoxacin (Süslü and Tamer 2002), ranitidine (Perez- Ruiz 2001), terfenadine (Amin and Issa 1999), atorvastatin (Erk 2003c) and for nizatidine (El-Yazbi et al. 2003). Derivative spectrophotometry is an analytical technique for extracting quantitative and qualitative information from spectra composed. Derivative UV-Vis spectrophotometry involves calculating and plotting one of the mathematical derivatives of a spectral curve. Although the derivative transformation does not increase the information content of a given spectrum, this method shows good sensitivity and specificity and permits discrimination in the face of the broad band interference arising from turbidity or non-specific matrix absorption.

The present work describes a new spectrophotometric method which is less expensive than HPLC. The method is based on the formation of ion-pair complexes with bromocresol purple (BCP) and bromothymol blue (BTB) in acidic buffer. The data obtained were compared with data obtained by first derivative spectrophotometry.

2. Investigations, results and discussion

In Fig. 1 the first derivative spectra for the three concentrations of IND in methanol are shown. For the determination of IND peak-zero amplitude at 273 nm was measured.

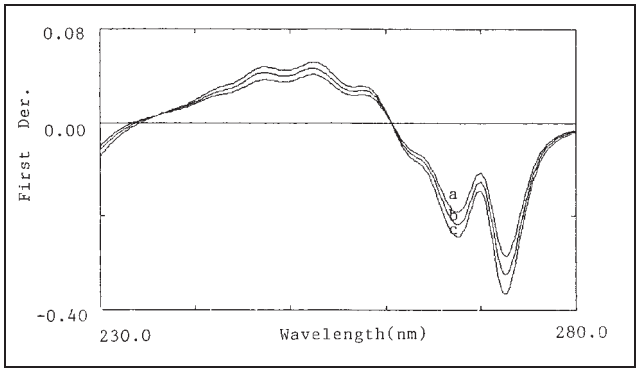

Fig. 1: First derivative spectra of a) 20.2 $\mu m \cdot ml^{-1}$; b) 40.6 $\mu m \cdot ml^{-1}$ IND in methanol

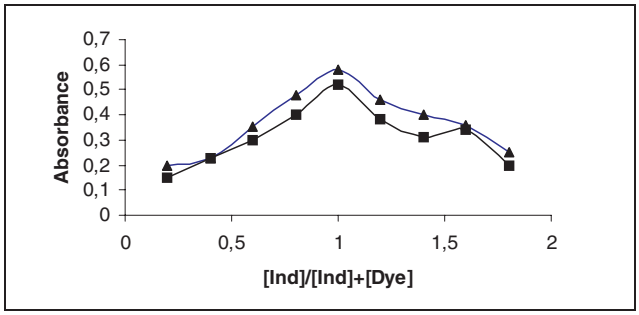

Fig. 2: Continuous variation plots for (a) ▲ IND–BCP; (b) ■ IND–BTB in chloroform (2.0×10^{-3} M)

The ion-pair complexes with BCP and BTB absorb maximally at 427 and 420 nm, respectively. The influence of pH on the formation of the ion-pair complex of IND has been studied. Different pH values (2.0–9.0) were tested. pH 3.8 potassium hydrogen phthalate buffer solution was found to be the optimum solvent for BCP or BTB complexes. The effect of dyestuff was studied by adding different volumes of dyestuff solution to a constant amount of IND. The maximum absorbance, in each case, was found with 3.0 ml of dyestuffs. The effect of the extracting solvent used both on extraction efficiency and color intensity was examined. Chloroform, dichloromethane, benzene, methylene chloride proved useful solvents; chloroform was selected because of its slightly higher efficiency and considerably lower extraction ability for the reagent blank. For both methods, the effect of temperature on the colored complexes was studied. It was found that the colored complexes were stable up to 27 °C. At higher temperature the drug concentration was found to increase due to the volatile nature of chloroform. As a result, the absorbance of the colored complexes increased. However, the resultant complexes were stable for more than 2 h at 24–26 °C. Shaking times ranging from 0.5 to 5.0 min did not change the color intensity, and so a min shaking time was selected. Consequently, the yield of a single extraction with 10 ml of chloroform with an organic: aqueous phase of 1 : 2 was practically 100%. The most favorable sequence was "drug-reagent-buffer-chloroform" for the highest absorbance and stability. Other sequences needed longer time in addition to lower stability.

The effect of additives in formulations was investigated. The interference of common excipients (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide, and magnesium stearate) and other concomitant substances was determined by measuring the absorbance of the solutions containing 40.0 $\mu g \cdot ml^{-1}$ of the drug and various amounts of excipients. The tolerance of each foreign compound was taken as the largest amount yielding an error of less than $\pm$ 1.2% in the analytical signal of IND.

The molar ratio of drug to reagent in the complex formed was investigated by the Job's method of continuous variation. It was found to be 1 : 1 (Fig. 2). The stability constants calculated using Harvey and Manning equation (De Beer et al. 1994) for the IND-BCP or the IND-BTB complexes were found to be 4.4 ± 0.05, or 4.9 ± 0.1, respectively.

Under the conditions mentioned above, the main characteristics of the procedures have been established. Table 1 summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients and relative standard deviation. To test the reproducibility of the proposed methods, 10 replicate determinations of 40.0 $\mu g \cdot ml^{-1}$ of IND were made. The coefficient of variation was found to be less than 1.5% for all the procedures.

The procedures were validated by evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability and recovery. The LOD and LOQ were calculated from the calibration curves as kSD/b where k = 3 for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD were found to be 0.5 $\mu g \cdot ml^{-1}$ for the IND-BCP method, 0.5 $\mu g \cdot ml^{-1}$ for the IND-BTB method, and 0.2 $\mu g \cdot ml^{-1}$

Table 1: Analytical data for indinavir ion-pair complexes

Method	λ_{max}	Conc. range ($\mu g \cdot ml^{-1}$)	Molar absorptivity ($l \cdot mol^{-1} \cdot cm^{-1}$)	Sandell sensitivity ($\mu g \cdot cm^{-2}$)	Linear intercept (a)	Regression slope (b)	Corr.coeff. (r)	RSD* (%)
BCP	427	4.0–60.2	2.27×10^4	0.58	0.054	0.13	0.9992	1.00
BTB	420	4.0–60.2	3.58×10^4	1.24	0.068	0.84	0.9994	0.08
First deriv. spec.	273	3.0–70.6	9.28×10^4	–	0.024	0.063	0.9999	0.06

* Average of ten determinations

Table 2: Spectrophotometric determination of indinavir (IND) in pure drug and in pharmaceutical formulations

Sample	Added ($\mu g \cdot ml^{-1}$)	First deriv. spec.	%Found $\pm$ SD (n = 5) BCP	BTB
IND (Pure drug)	20.0	101.2 ± 0.69	99.2 ± 1.00	101.7 ± 0.98
	40.0	98.2 ± 1.05	99.2 ± 1.12	98.2 ± 1.34
	60.0	99.9 ± 0.92	100.5 ± 0.96	99.9 ± 0.85
Commercial formulations[a]		99.1 ± 0.24	98.9 ± 0.95	101.9 ± 0.99
			t: 0.98 (2.26)[b]	t: 1.12

[a] Labelled to contain 200.0 mg indinavir in one tablet of Crixivan® capsule
[b] Values in parentheses are the theoretical values at p = 0.95. Theoretical values at % 95 confidence limit t = 2.26

for the first derivative spectrophotometric method, while the quantification limits (LOQ) were found to 1.5 $\mu g \cdot ml^{-1}$ for the IND-BCP method, 1.5 $\mu g \cdot ml^{-1}$ for the IND-BTB method, and 0.9 $\mu g \cdot ml^{-1}$ for the first derivative spectrophotometric method. The accuracy, precision, and repeatability of the method was tested by means of the recovery test. Five replicate determinations at three different concentrations were carried out to test of the methods (Table 2). The standard deviations were found to be less than 1.3% indicating good accuracy, precision, and repeatability of the proposed method.

The method has been successfully applied to the determination of IND in commercial capsules. The results obtained are shown in Table 2.

The first derivative method, and was chosen as the analytical reference method. BCP, and BTB methods were evaluated in comparison. The results obtained are summarized in Table 2. No significant differences were found between the methods for the same batch at the 95% confidence level (student's t- test).

The proposed method is simple, sensitive and suitable for the determination of IND in bulk and pharmaceutical dosage forms. Precision and recovery data clearly indicate the reproducibility and accuracy. Analysis of synthetic samples of the drug has shown the non-interference of common excipients and additives. Hence all the developed methods may be recommended for routine and quality control analysis of pharmaceutical preparations.

3. Experimental

3.1. Apparatus

A Shimadzu 1601 double beam spectrophotometer with 1 cm matched cells was used for all absorbance measurements. pH measurements were made with a NEL model 890 pH meter equipped with a combined glass-calomel electrode and an ultrasound generator.

3.2. Reagents

All chemicals were of analytical reagent grade unless otherwise specified. Double distilled deionized water was used to prepare all solutions. Freshly prepared solutions were always employed. Potassium hydrogen phthalate buffers were prepared by dissolving 1.020 g potassium hydrogen phthalate in water and completing to 50 ml with water and pH adjusting by addition of 0.1 M hydrochloric acid. 0.10% (w/v) BCP or 0.5% (w/v) BTB were prepared in water and in respective methods.

Bulk IND drug was kindly supplied by MSD Pharm. Co. (Rahway, NJ, USA) and used without further purification. The IND capsules labelled as containing 200 mg/capsule, were purchased from the local pharmacy.

Stock solutions were prepared by dissolving 10 mg of the drug in 100 ml methanol. Freshly prepared solutions were always employed.

3.3. Procedure for the assay of bulk samples

Into a series of 50 ml separating funnels, 5.0 ml of buffer solutions of pH 3.8 and 3.0 ml of dyestuff solutions were placed. Appropriate 5.0 ml drug solution (4.0–60.2 $\mu g \cdot ml^{-1}$ for BCP or BTB) was added to each funnel and mixed well. The funnels were shaken vigorously with 10 ml chloroform for 2 min, then allowed to stand for clear separation of the two phases. The absorbance of the organic phase at 427, or 420 nm was measured in each case against a reagent blank similarly prepared. All measurements were made at room temperature (24 ± 2 °C). The procedures were repeated for other analyte aliquots and the calibration plots were drawn to calculate the amount of drugs in unknown analyte samples.

3.4. Procedure for pharmaceutical formulations

Crixivan capsules were obtained in a local pharmacy. The amounts of IND declared are 200 mg per capsule. The contents of 10 capsules was completely removed from shells. Accurately weighted quantities of these powders equivalent to 200 mg of IND were transferred to 50 ml volumetric flasks. About 25 ml of methanol were added to dissolve the drug. After sonicating and shaking the mixture for 15 min, it was completed to volume with the same solvent, mixed and passed through a Whatman no 42 filter. Necessary amounts of filtrate were diluted to 100 ml with methanol and the same procedure was applied as described in section 3.3.

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