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Micellar Liquid Chromatographic Determination of Lamivudine, Indinavir and Ketoconazole in Dosage Forms and Biological Fluids

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Abstract

A simple, reversed phase high performance liquid chromatographic method has been developed for the determination of lamivudine, indinavir and ketokonazole in pharmaceutical preparations, human plasma and urine. The method was conducted using A Shim-pack VP-ODS (150×4.6 mm i.d) stainless steel column at ambient temperature with ultra violet detection at 225 nm. Micellar mobile phase consisted of 0.07 M sodium dodecyl sulphate , 10% n-propanol, 0.3% triethylamine in 0.02 M phosphoric acid (pH 4.5) was used and pumped at a flow rate of 1.2 mL/ min . The calibration curve was rectilinear over the concentration range of (0.05-1.0) µg/mL and (0.2-5.0) and (0.3-5.0) µg/mL lamivudine , indinavir and ketokonazole respectively. The proposed method was successfully applied to the analysis of these drugs in some dosage forms. The method was extended to the *in-vitro*,

Keywords: HPLC; Micellar mobile phase; Biological fluids

Introduction

The aim of this study was to develop a micellar liquid chromatographic method for the analysis of some coadministered drugs used for the treatment of human immunodeficiency disease (HIV). The studied drugs were lamivudine, ketokonazole and indinavir. The studied compounds were separated and determined in dosage forms and biological fluids. *In vivo* estimation of these drugs was investigated in patient plasma.

Mobile Phase

Micellar liquid chromatography (MLC) offering additional advantages over conventional liquid chromatographic methods. It has proved to be a useful technique in the determination of diverse groups of compounds in several matrices including food and biological samples. MLC allows complex matrices to be analysed without the aid of extraction and with direct injection of the physiological samples. Micelles tend to bind proteins competitively, thereby releasing protein-bound drugs and proteins, rather than precipitating into the column. Proteins compounds are solubilized and washed harmlessly away, eluting with the solvent front. MLC utilizes small amounts of organic modifier and generates less amount of toxic waste in comparison to aqueous-organic solvents, so that they are less toxic, non-flammable, biodegradable and relatively inexpensive (green chemistry) [1,2]. Micelles provide hydrophobic and electrostatic (for ionic surfactants) sites of interaction. In the micelles, three sites of solubilisation can be identified: the core (hydrophobic), the surface (hydrophilic) and the palisade layer (the region between the surfactant head groups and the core). Solutes associated to micelles experience a microenvironment that is different from that of bulk solvent. This is reflected by micelle-induced perturbations in solute physicochemical properties, including changes in solubility, acidity, photophysical properties, and reaction rates [3]. Also, the capability of simultaneous separation of hydrophobic and hydrophilic analytes in the same run without a gradient elution [4]. Unfortunately, some drawbacks were reported concerning the chromtographic efficiency and weak elution strength of pure micellar solution [5,6]. Many articles has been reported for determination of drugs in pharmaceutical preparations and biological fluids [7-9].

The proposed mobile phase was composed of a mixture of 0.07 M sodium dodecyl sulphate (SDS), 10% n-propanol, 0.02 M phosphoric acid and 0.3% triethylamine. The pH was adjusted at pH 4.5 by using phosphoric acid or triethylamine. The mixture was then sonicated for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µm membrane filter. The proposed method is investigated for the determination of some coadministered drugs for treatment of HIV. Ketoconazole is coadministered with antiretroviral drugs in order to increase their duration as it suppress the liver microsomal enzymes [10]. It can be used for therapeutic dose monitoring, bioavailablity and pharmacokinetic studies of these drugs.

Lamivudine

Lamivudine (Figure 1) is (2R-cis)-4-Amino-1-[2-(hydroxyl-methyl) 1,3-Oxathiolan-5-yl]-2(1H)-pyrimidinone [11]. It is used as antiviral against HIV and hepatitis B. It has less cytotoxicity and greater antiviral activity than other antiviral drugs [12]. The United States Pharmacopoeia (USP) determine lamivudine by HPLC method [13]. The USP mobile phase consists of mixture (95:5) of ammonium Buffer and methanol (pH 3.8) conducted through C18 column. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 35°C. Other HPLC methods were reported [14-22].

Ketokonazole

Ketokonazole (Figure1) is 1-[4-(4-{[(2*R*,4*S*)-2-(2,4-Dichlorophe-nyl) -2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl) piperazin-1-yl]ethan-1-one [11]. Ketoconazole is a synthetic, imid-azole antifungal medication used primarily to treat fungal infections. It interferes with the fungal synthesis of ergosterol, a constituent of fungal cell membranes, ketoconazole works principally by inhibiting

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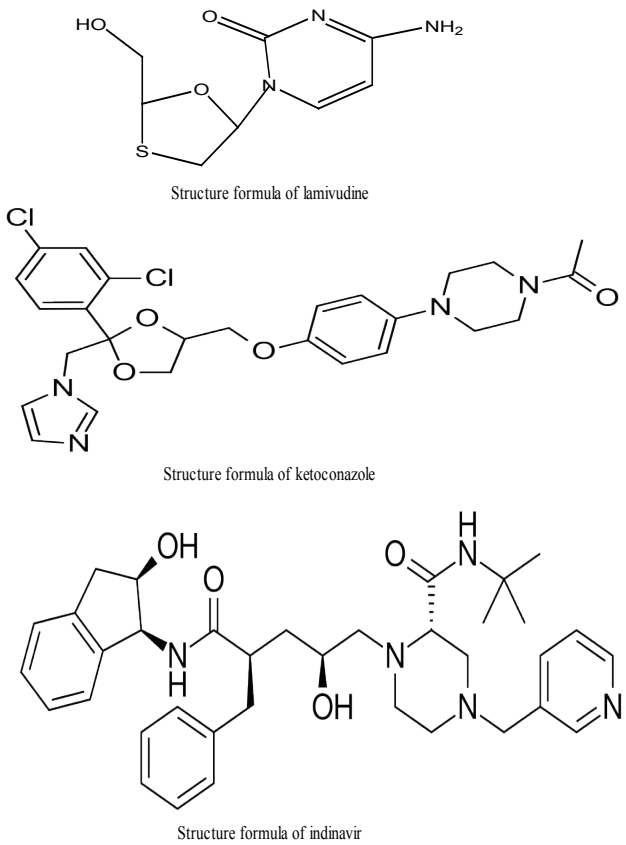

Figure 1: Structural formula of the studied drugs.

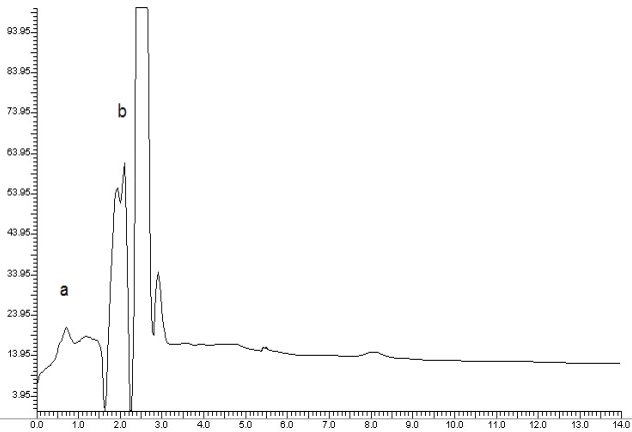

Figure 2: Chromatogram of plain plasma.
a: solvent front
b: plasma peak

the enzyme cytochrome P450 [12]. Ketoconzole has been titrated potentiometrically using perchloric acid as titrant [5]. Several different articles have been published for the determination of ketoconazole spectrophotometrically [23-25] Voltametrically [26], by HPTLC [27], by HPLC [23,28,29] and by LC-MS [30]. The USP [13] Use mobile phase consists of diisopropylamine in methanol (7:3) conducted through C18 column.

Indinavir

Indinavir is 2*S*)-1-[(2*S*,4*R*)-4-benzyl-2-hydroxy-4-{[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]carbamoyl}butyl]-*N*-*tert*-butyl-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide [11]. It is a protease inhibitor used as a component of highly active antiretroviral therapy to treat HIV infection and AIDS [12]. Several HPLC [31-33] and LC-MS [34] methods have been reported for the determination of indinavir. The comparison method [31] uses an analytical column of a Phenomenex Luna® C8 10 mm and a mobile phase was a mixture of di-sodium hydrogen phosphate (0.01 M)+ammonium chloride (0.4%) with pH adjusted to 4.5 (solvent A) and acetonitrile(solvent B), 68:32, v/v, pumped at a flow rate of 1.8 mL/min through the column.

Experimental

Reagents

All chemicals used were of Analytical Reagents grade, and the

- solvents were of HPLC grade.
- Orhtophosphoric acid (Prolabo , Paris, France).
- Methanol (Hipersolv , Merck).
- Sodium dodecyl sulphate (Park Scientific limted, Northompthon, UK), 0.1 M aquous solution was prepared.
- n-Propanol, Ethanol and Triethylamine (Riedel-deHaen, Sleeze, Germany).
- n-Butanol (Honi limited, London, UK).

Materials

Lamivudine was kindly supplied by Eva Pharmaceuticals**,** Cairo**,** Egypt**.** Ketoconazole and indinavir were purchased from Sigma Aldrich. Lamidine® tablets each labeled to contain 150 mg of lamivudine, Batch No. 1183498. Product of Eva pharmaceuticals and were obtained from the local pharmacy. Nezoral® tablets each labled to contain 200 mg of ketoconazole, Batch No.KN364. Product of Jansen pharmaceuticals and were obtained from the local pharmacy. Crixivan® tablets each labeled to contain 200mg of indinavir, Batch No. 58692. Product of Merck pharmaceuticals and were obtained from the local pharmacy.

Instrument

The analysis was performed using Shimadzu™ LC-20A Series Chromatograph equipped with a Rheodyne injector valve with a 20µL loop and a SPD-20A UV detector operated at 225 nm. LC Workstation (Nishinokyo-Kuwabaracho, Nakagyo-Ku, Kyoto 604-8511, Japan).

Column and mobile phase

A Shim-pack VP-ODS column (5 µm) (150 mm × 4.6 mm) was used. The micellar mobile phase consisted of a solution containing a mixture of 0.07 M sodium dodecyl sulphate (SDS),10% n-propanol, 0.02 M phosphoric acid and 0.3% triethylamine. The pH was adjusted at pH 4.5 by using phosphoric acid or triethylamine. The mixture was then sonicated for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Ireland). The micellar mobile phase was freshly prepared. Column stabilization was done by running the mobile phase for 1 hr. in order to obtain stable baseline. The column hold up value was the first deviation of the base line obtained.

Standard solutions

Stock solutions of lamivudine, ketoconazole and indinavir were prepared by dissolving 10.0 mg of the drug in methanol and complete to 25.0 mL in volumetric flask with same solvent. These stock solutions were further diluted with the mobile phase to obtain the working concentration range 0.05-1.0 µg /mL and 0.3-5.0 and 0. 2-5.0 for lamivudine, ketoconzole and indinavir respectively. The stock solutions were found to be stable for at least one week when kept in the refrigerator .The stability of the stock solution was checked through elution of each drug alone. There is no indication of any decomposition of the drugs in the samples.

General Procedures

Construction of calibration graph

Working solutions containing concentration ranges of (0.05-1.0 µg/mL and (0.3-5.0) for lamivudine, ketoconzole respectively were prepared by serial dilution of the stock solutions together with an aliquot of internal standard solution containing 0.1 µg/mL indinavir. 20 µL aliquots were injected (triplicate) and eluted with the mobile phase under the previous chromatographic conditions . A plot of the peak area ratio between the drug and internal standard *versus* concentration of the drug is made to obtain the standard calibration curve. Alternatively, the regression equation was derived. For constructing a calibration curve for indinavir, 0.1 µg/mL lamivudine was used as internal standard and complete the procedure as mentioned above.

Analysis Lamidine, Nezoral and Crixivan tablets and capsules

Ten tablets or the contents of ten capsules were accurately weighed, finely pulverized and mixed well. Aliquot quantities of the powder equivalent to 150 mg, 200 mg and 200 mg of lamivudine, ketoconalzole and indinavir were transferred to a 100 mL volumetric flask. The drugs were extract with 80 mL of methanol, the flasks were sonicated for 20 min. Filter into 100 mL volumetric flask and complete to the volume with methanol. Aliquots of this solution were diluted with the mobile phase and the internal standard (0.1 µg/mL indinavir) was added to lamidine and nezoral extracts. 20 µl were injected (triplicate) and eluted with the mobile phase. The nominal concentration was obtained from the corresponding regression equation. For the determination of indinavir in tablets, the previous procedure was conducted with using 0.1 µg/mL lamivudine as internal standard.

Analysis of lamivudine, ketoconalzole and indinavir in spiked plasma and urine

0.5 mL aliquots of sample plasma was transferred into series of centrifuge tubes, spiked with the working concentrations of the drugs, 0.1 µg/mL indinavir (internal standard), 1.0 mL of acetonitrile was added, then centrifuged at 4000 rpm for 20 min at room temperature. The supernatant solutions were aspirated and filtered using a microfilter paper. 20 µl were injected (triplicate) and eluted with the mobile phase. The nominal concentration was obtained from the corresponding regression equation. For the determination of indinavir in plasma, the previous procedure was conducted with using 0.1 µg/mL lamivudine as internal standard.

Analysis of lamivudine, ketoconalzole and indinavir in patient samples

Lamidine 15 mg and nezoral 200 mg were administered to a healthy volunteer (males 31 years old) in different days after 8 hours of fasting. A blood sample was withdrawn in the morning before administration of drug as blank. 10 mL Blood samples were collected after several time intervals; 30 min., 1 hr, 2 hrs, 3 hrs, 4 and 5 hrs after drug administration. The samples were drawn into test tubes containing sodium citrate as anticoagulant and centrifuged at 2000 rpm for 20 min. The supernatant plasma was transferred into test tubes, 0.1 µg/mL indinavir (internal standard) was added. The procedure of spiked plasma was performed. Crixivan® 200 mg was determined in patient plasma by conducting the previous procedure using 0.1 µg/mL lamivudine as internal standard. The plasma samples were analyzed directly after withdrawing and treatment without the need of freeze-thaw cycles. The study was done on the available volunteer and it can be extended for further applications.

Results and Discussion

The micellar mobile phase was selected due to its previously mentioned advantages [1-4] especially the possibility of direct injection of physiological samples. The proposed method is the only one developed for the simultaneous separation and determination of the studied drugs in biological fluids. In addition of being more sensitive than the other methods.

The drug peaks are eluted at retension times of 3.8, 6.1 and 6.9 for lamivudine, ketoconazole and indinavir respectively. The peaks are separated from plasma peak and are well resolved with resolution factors of 3.83 and 1.33 for lamivudine, ketoconazole and ketoconazole, indinavir respectively (Figures 2 and 3). The other resolution factors were calculated between the peak of the drug and the preceding peak.

Ultra Violet (UV) detection

Several wavelengths were investigated and the wavelength 225 nm was used as it provides high sensitivity for the determination of ketconazole and good sensitivities for the other drugs.

Study of the experimental parameters

The experimental parameters affecting the chromatographic separation of the drugs were studied and optimized. These variables including mobile phase, flow rate and linearity range. Variables were optimized by changing each in turn, while keeping all others constant to obtain the highest number of theoretical plates and good resolution. The optimum condition was using of 0.07 M sodium dodecyl sulphate

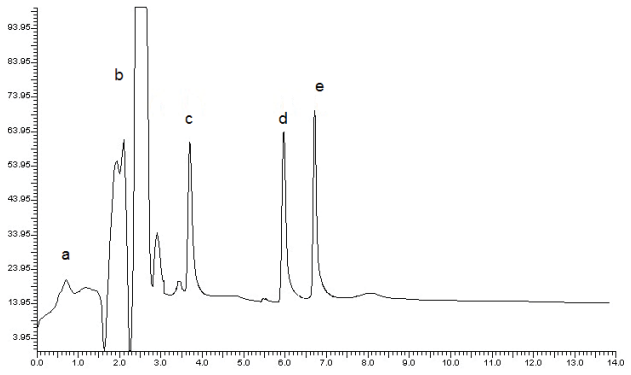

Figure 3: Chromatogram of the studied drugs in patient plasma after 2 hours.
a: solvent front
b: plasma peak
c : Lamivudine
d: ketoconazole
e: indinavir

, 10% n-propanol, 0.3% triethylamine in 0.02 M phosphoric acid (pH 4.5), the mobile phase was pumped at a flow rate of 1.2 mL/ min (Table 1).

Analytical validation

Linearity of the method: After optimizing the conditions, it was found that the relation between the peak area ratios and the final concentrations of the drugs were linear over the ranges (0.03-1.0) µg/mL and (0.2-5.0) and (0.3-5.0) µg/mL lamivudine, indinavir and ketokonazole respectively. Linear regression of analysis of results gave the following equation:

Peak area ratio=0.01+7.4 C,

r=0.9999 for lamivudine

Peak area ratio=0.03+12.7 C

r=0.9999 for ketokonazole

Peak area ratio=0.03 + 9.6 C

r=0.9999 for indinavir

where,

C=concentration in µg/mL

r=correlation coefficient

The results of the statistical analysis of regression data for pure samples, such as standard deviation of the slope (S_b) and standard deviation of intercept (S_a) and the standard deviation of residuals (Sy/x) were calculated and were found to be 0.2, 0.012, 0.016 for lamivudine, 0.3, 0.03, 0.04 for ketoconazole 0.22, 0.06, 0.07 for indinavir. The small values of the figures point out to low scattering of the points around the calibration.

Accuracy and precision

The proposed method was evaluated by studying the accuracy as percent relative error (% Er) and precision as percent relative standard deviation (% RSD) [35]. The results of intraday and interday accuracy and precision for the method are summarized in Table 2. The results show low percent relative error (% Er) and percent relative standard deviation (% RSD) indicating high accuracy and precision. The accuracy of the proposed method was in good agreement with the official and comparison methods [5,23] (Table 3).

Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ) were calculated form the calibration curve according to the formula LOD=3.3 S_a/b and LOQ=10 S_a/b[35] respectively. where S_a is the standard deviation of the intercept of the regression line, b is the slope

Experimental parameter		Number of theoretical plates			Capacity factor K`		
		LAM	KCZ	IDV	LAM	KCZ	IDV
pH	3.0	2360	2880	4310	4.40	7.76	9.0
	4.0	2410	3150	4470	4.41	7.75	9.0
	4.5	2470	3240	4580	4.43	7.71	8.94
	5.0	2435	3210	4450	4.38	7.60	8.80
	6	2360	2910	4360	4.38	7.60	8.90
	7.0	2310	2650	4280	4.40	7.58	8.90
SDS Conc.	0.05	2340	3170	4450	5.0	8.3	9.7
	0.07	2470	3240	4580	4.43	7.71	8.94
	0.1	2440	3120	4420	3.85	7.3	8.3
	0.12	2350	2920	4320	3.0	6.4	7.6
	0.15	2240	2840	4110	2.6	6.0	7.0
Type of organic modifier	Methanol	1180	1850	2280	4.6	8.0	9.5
	Ethanol	2250	2650	3110	4.5	7.9	9.2
	Propanol	2470	3240	4580	4.43	7.71	8.94
	Butanol	2340	2830	3240	4.40	7.4	8.7
Propanol Conc.	5%	1980	2410	3840	5.5	8.2	9.6
	8%	2330	3140	4150	4.8	8.0	9.3
	10%	2470	3240	4580	4.43	7.71	8.94
	12%	2410	3190	4260	3.9	7.3	8.6
	15%	2220	2550	3570	3.5	6.7	8.2
Flow rate (mL/min.)	0.8	1340	1180	2330	6.7	11.5	13.4
	1.0	2110	2660	3880	5.3	9.3	10.7
	1.2	2470	3240	4580	4.43	7.71	8.94
	1.5	2310	2740	3940	3.54	6.2	7.2
	1.8	2010	2110	2140	2.9	5.2	6.0
Experimental parameter		**Selectivity factor (α)**					

		LAM	KCZ	KCZ	IDV
pH	3.0	1.75		1.17	
	4.0	1.74		1.17	
	4.5	1.74		1.16	
	5.0	1.74		1.14	
	6	1.75		1.15	
	7.0	1.75		1.15	
SDS Conc.	0.05	1.66		1.17	
	0.07	1.74		1.16	
	0.1	1.90		1.14	
	0.12	2.13		1.19	
	0.15	2.31		1.17	
Type of organic modifier	Methanol	1.74		1.19	
	Ethanol	1.76		1.16	
	Propanol	1.74		1.16	
	Butanol	1.70		1.18	
Propanol Conc.	5%	1.50		1.17	
	8%	1.70		1.16	
	10%	1.74		1.16	
	12%	1.90		1.18	
	15%	1.91		1.22	
Flow rate (mL/min.)	0.8	1.72		1.17	
	1.0	1.75		1.15	
	1.2	1.74		1.16	
	1.5	1.75		1.16	
	1.8	1.80		1.15	
Experimental parameter		**Resolution factor (Rs)**			
		LAM	**KCZ**	**KCZ**	**IDV**
pH	3.0	3.80		1.30	
	4.0	3.81		1.30	
	4.5	3.83		1.33	
	5.0	3.80		1.32	
	6	3.81		1.30	
	7.0	3.81		1.30	
SDS Conc.	0.05	4.5		2.1	
	0.07	3.83		1.33	
	0.1	3.1		1.26	
	0.12	2.5		1.16	
	0.15	2.0		1.0	
Type of organic modifier	Methanol	2.8		1.11	
	Ethanol	3.2		1.20	
	Propanol	3.83		1.33	
	Butanol	3.9		1.40	
Propanol Conc.	5%	5.1		1.8	
	8%	4.2		1.50	
	10%	3.83		1.33	
	12%	3.1		1.28	
	15%	2.66		1.21	
Flow rate (mL/min.)	0.8	5.7		2.0	
	1.0	4.60		1.60	
	1.2	3.83		1.33	
	1.5	3.10		1.1	
	1.8	2.55		0.9	

Table 1: Effect of different experimental parameters on the column efficiency.
LAM: Lamivudine
KCZ: Ketoconazole
IDV: Indinavir

of the calibration curve . The limit of detections were 0.005, 0.008, 0.021, for lamivudine, ketoconazole and indinavir, respectively. The limit of quantification values were 0.016, 0.024, 0.062, for lamivudine , ketoconazole and indinavir, respectively.

Robustness

The robustness of the method was studied according to the United State Pharmacopoeia [13] and no significant effect on assay data performance was observed with change of pH (4-5), flow rate (1-1.5mL) and mobile phase composition (0.05-0.1 M SDS), (8%-12%propanol) (Table 4).

System suitability

System suitability parameters were measured using three injections of reference solutions of the studied drugs to verify the system performance. The % RSD values were calculated for the number of theoretical plates, capacity factor, tailing factor and retention time Table 5.

Application to dosage forms

The proposed method was successfully applied for the determination of the studied drugs in tablets and capsules. The results shown in Table 3 are in good agreement with those obtained by the official USP method [13] for the determination of lamivudine, ketoconazole comparison methods [31] for the determination of indinavir. The proposed method is fairly sensitive compared with the official and comparison methods.

Content uniformity testing

Due to the high precision of the method, and its ability to rapidly estimate the concentration of the drug in a single tablet and capsule extract with sufficient accuracy, the method is suited for content uniformity testing which is a time consuming process when using conventional assay technique. The steps of the test were adopted according to the United States Pharmacopoeia procedure. The acceptance value AV was calculated for each of the commercially available tablets and it was found to be smaller than the maximum allowed acceptance value L1.The results demonstrated reasonable drug uniformity as shown in Table 6.

Application to spiked biological fluids

The high sensitivity attained by the proposed method allows determination of the drugs in biological fluids with high percentage of recovery Table 7.

Application to patient samples

The plasma samples obtained from the volunteer were investigated using the regression equation of spiked plasma. The maximum plasma level reached after 1 hr for lamivudine, 2 hr for indinavir, and ketoconazole. Hence, the proposed method allows monitoring of the therapeutic drug levels in plasma, bioavailability and pharmacokinetic studies (Figures 2 and 3) (Table 8). The obtained results are with good agreement with the reported plasma concentrations [10,12,31].

Stability

The stability of the methanolic and aqueous sample solutions at room temperature (25°C) for 24 hour after preparation, was verified by reassaying them. There is no indication of any decomposition of the drugs in the samples.

LAMIVUDINE				KETOCONAZOLE				INDIMAVIR			
Conc. added µg/mL	% Found	% RSD	% Er	Conc. added µg/mL	% Found	% RSD	% Er	Conc. added µg/mL	% Found	% RSD	% Er
Intraday				**Intraday**				**Intraday**			
0.03	99.90 ± 0.22	0.22	0.3	0.3	99.50 ± 0.32	0.32	0.18	0.18	99.80 ± 0.23	0.23	0.13
0.5	100.10 ± 0.26	0.26	0.8	0.8	100.20 ± 0.40	0.40	0.23	0.23	100.20 ± 0.3	0.27	0.16
1.00	99.88 ± 00.21	0.21	5.00	5.00	99.90 ± 0.41	0.41	0.24	0.24	99.68 ± 0.26	0.26	0.15
Interday				**Interday**				**Interday**			
0.03	100.20 ± 0.24	0.24	0.14	0.3	100.31 ± 0.32	0.32	0.18	0.3	100.23 ± 0.3	0.25	0.14
0.5	100.13 ± 0.20	0.20	0.12	0.8	99.62 ± 0.33	0.33	0.19	0.8	100.15 ± 0.2	0.23	0.13
1.00	99.5 ± 0.27	0.27	0.16	5.00	99.62 ± 0.3	0.42	0.24	5.00	100.15 ± 0	0.27	0.16

Table 2: Intraday and inter day precision for the proposed method for the determination of the studied drugs. Each result is the average of three separate determinations.

LAMIDINE 150 MG				NEZORAL 200 MG				CRIXIVAN 200 MG			
Proposed method		Official method[13]		Proposed method		Official method[13]		Proposed method		Comparison method[31]	
Amont taken, µg/mL	% Found	Amont taken, µg/mL	% Found	Amont taken, µg/mL	% Found	Amont taken, µg/mL	% Found	Amont taken, µg/mL	% Found	Amont taken, µg/mL	% Found
0.03	100.30	1.00	99.90	0.3	100.30	1.0	99.50	0.3	99.30	0.5	99.82
0.08	99.60	4.00	99.80	0.8	99.30	2.0	99.80	0.8	99.52	0.8	99.20
0.5	99.83	8.00	100.41	2.00	99.82	3.0	100.25	2.00	99.91	1.00	100.50
1.00	99.77	10.0	99.42	5.00	99.71	4.0	100.15	5.00	100.10		100.13
mean ± SD	99.91 ± 0.3		99.90 ± 0.35	mean ± SD	99.80 ± 0.33		99.90 ± 0.38	mean ± SD	99.70 ± 0.41		99.90 ± 0.50
t-test	(2.45)*	(0.26)		t-test	(2.45)*	(1.4)		test	(2.45)*	(1.35)	
F-test	(9.27)*	(1.31)		F-test	(9.27)*	(1.563)		F-test	(9.27)*	(1. 5)	

Table 3: Application of the proposed and comparison method to determination of the studied drugs in dosage forms.

PARAMETERS	LAMIVUDINE			KETOCONAZOLE			INDINAVIR		
	Retention time	Theoritical plate	Capacity factor	Retention time	Theoritical plate	Capacity factor	Retention time	Theoritical plate	Capacity factor
0.05 MSDS	4.2	2340	3.5	6.50	3170	8.3	7.50	4450	9.70
0.07 MSDS	3.8	2470	4.43	6.1	3240	7.71	6.9	4580	8.94
0.1 MSDS	3.4	2440	1.5	5.80	3120	7.30	6.5	4420	8.3
8% propanol	4.1	2330	4.8	6.3	3140	8.0	7.2	4150	9.3
10% propanol	3.8	2470	4.43	6.1	3240	7.71	6.9	4580	8.94
12% propanol	3.4	2410	3.9	5.8	3190	7.30	6.7	4260	8.6
pH 4	3.82	2410	4.45	6.2	3150	7.75	7.0	4470	9.0
pH 4.5	3.8	2470	4.43	6.1	3240	7.71	6.9	4580	8.94
pH 5.0	3.7	2435	4.38	6.0	3210	7.60	6.8	4450	8.8
1.0 mL/min	4.4	2110	5.3	7.2	2260	9.3	5.50	3880	10.7
1.2 mL/min	3.8	2470	4.43	6.1	3240	7.71	6.9	4580	8.94
1.5 mL/min	3.2	2310	3.54	5.0	2740	6.2	5.7	3940	7.2

Table 4: Results of the robustness of the proposed method.

PARAMETER	NUMBER OF THEORETICAL PLATES*	CAPACITY* FACTOR	TAILING FACTOR*	RETENTION TIME*
Lamivudine	0.16	0.14	0.22	0.12
Ketoconazole	0.18	0.15	0.24	0.15
Indinavir	0.2	0.13	0 .27	0.16

Table 5: Results of system suitability study.
* % RSD (n=3)

PARAMETER	PERCENTAGE OF THE LABLE CLAIM		
	Lamidine tablets	Nezoral tablets	Crixivan capsules
Data	99.45	101.10	101.22
	99.50	100.90	100.24
	100.33	99.40	99.31
	100.20	99.53	100.44
	100.63	100.30	99.75
	99.82	99.55	100.32
	99.60	101.21	99.60
	99.12	99.23	99.32
	100.15	99.65	99.35
	100.30	99.21	99.50
Mean	99.91	100.01	99.90
SD	0.53	0.80	0.50
% Error	0.17	0.30	0.15
Acceptance	1.25	1.85	1.10
value(AV)			
Maximum allowed value(L1)	15		

Table 6: Content uniformity testing of Lamivudine, ketoconazole and indinavir in their dosage forms using the proposed method.

LAMIVUDINE		KETOCONAZOLE		INDIMAVIR	
Conc.added µg/mL	% Found	Conc.added µg/mL	% Found	Conc.added µg/mL	% Found
Plasma		**Plasma**		**Plasma**	
0.03	98.90	0.3	101.31	0.3	98.70
0.5	101.10	0.8	99.20	0.8	101.10
1.00	99.30	5.00	98.80	5.00	99.50
mean ± SD	99.76 ± 1.17	mean ± SD	99.77 ± 1.35	mean ± SD	99.77 ± 1.22
Urine		**Urine**		**Urine**	
0.03	100.10	0.3	99.10	0.3	100.42
0.5	100.50	0.8	101.20	0.8	101.15
1.00	99.11	5.00	98.90	5.00	99.21
mean ± SD	99.9 ± 0.72	mean ± SD	99.73 ± 1.27	mean ± SD	100.3 ± 0.9

Table 7: Application of the proposed method for the determination of the studied drugs in spiked plasma.

TABLET/TIME	0.5 HR	1 HR	2 HR	3 HR	4 HR	5 HR
lamidine® 150 mg tablets	0.90	1.61	1.51	1.1	0.83	0.41
Nezoral® 200 mg	1.8	3.1	3.4	2.8	1.9	1.5
Crixivan® 200 mg capsules	0.6	1.0	1.5	0.8	0.52	0.11

Table 8: Determination of the studied drugs in µg/mL in patient plasma.

Conclusion

The proposed method provides simultaneous determination of lamivudine, ketoconazol and indinavir offering additional advantages over comparison methods. The present investigation can be utilized for monitoring of the concentration of the studied drugs in patient plasma. In addition it can be extended for pharmacokinetic studies.

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