Spectrophotometric determination of some calcium channel blockers using Sulfochlorophenol-S, Bromopyrogallol red, Eriochromecyanine-R and Pyrocatechoil violet

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ABSTRACT

Four simple, sensitive and accurate spectrophotometric methods have been developed for the determination of some calcium channel blockers: Amlodipine besylate (ADB), Diltiazem hydrochloride (DTZ) and Verapamil hydrochloride (VPM) in pharmaceutical formulations. These methods based on formation ion pair complexes with Sulfochlorophenol-S (SCPS), Bromopyrogallol red (BPR), Eriochromecyanine-R (ECC) and Pyrocatechol violet (PCV) in acidic medium. The colored products are extracted with chloroform and measured spectrophotometrically at 462, 600, 440 and 442 for ECC, SCPS, BPR and PCV, respectively. Beer's low was obeyed in the concentration range, for ECC: 25 - 175 µg·ml^{-1}, 50 - 150 µg·ml^{-1} or 100 - 250 µg·ml^{-1}, for SCPS: 300 - 800 µg·ml^{-1}, 200 - 700 µg·ml^{-1} or 100 - 550 µg·ml^{-1}, for BPR: 50 - 400 µg·ml^{-1}, 200 - 700 µg·ml^{-1} or 200 - 700 µg·ml^{-1} for VPM, DTZ or ADB, respectively and for PCV: 50 - 250 µg·ml^{-1} for VPM or 200 - 500 µg·ml^{-1} for DTZ with molar absorptivity, for ECC: 2.2×10^4, 2.1×10^4, 1.6×10^4 L·mol^{-1}·cm^{-1}, for SCPS: 3.8×10^3, 5.6×10^3, 8.1×10^3 L·mol^{-1}·cm^{-1}, for BPR: 11×10^3, 4.8×10^3, 6.9×10^3 L·mol^{-1}·cm^{-1} for VPM, DTZ or ADB, respectively and for PCV: 19.5×10^3 L·mol^{-1}·cm^{-1} for VPM and 6.6×10^3 L·mol^{-1}·cm^{-1} for DTZ and relative standard deviation, for ECC: 0.76%, 0.86%, 0.46%, for SCPS: 0.94%, 0.96%, 0.86%, for BPR: 0.96%, 0.95%, 0.55% for VPM, DTZ or ADB, respectively and for PCV: 0.81% for VPM and 0.65% for DTZ. These methods have been successfully applied for the assay of drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

1. INTRODUCTION

The calcium channel blockers are divided into three chemical classes, each with different pharmacokinetic properties and clinical indications [1].

1.1. Diphenylamine Derivatives

Verapamil is the only member of this class. Verapamil is the least selective of any calcium channel blocker, and has significant effects on both cardiac and vascular smooth muscle cells.

Verapamil hydrochloride contains not less than 99.0% and not more than the equivalent of 101% of (2RS)-2-(3,4-dimethoxyphenyl) 5-[2-(3,4 dimethoxyphenyl) ethyl] (methyl) amino-2-(1-methyl ethyl) pentanenitrile hydrochloride, calculated with reference to the dried substance.
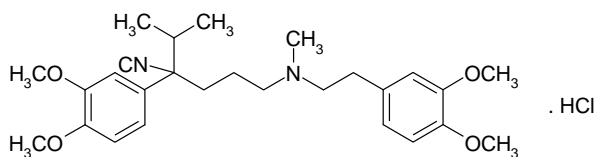
Verapamil hydrochloride (VPM).

1.2. Benzothiazepine Derivatives

Diltiazem is the only member of this class. Like verapamil, diltiazem affects both cardiac and vascular smooth muscle cells; however, it has a less pronounced negative intropic effect on the heart than does verapamil.

Diltiazem hydrochloride contains not less than 98.5% and not more than the equivalent of 101.0% of (2S,3S)-3-acetyloxy-5-[2-(dimethyl-amino) ethyl]-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)-one hydrochloride, calculated with reference to the dried substance.
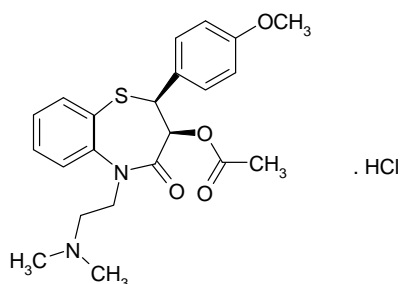

Diltiazem hydrochloride (DTZ).

1.3. 1,4 Dihydropyridine Derivatives

This rapidly expanding class of calcium channel blockers includes the first-generation (nifedipine), second-generation: "amlodipine, felodipine, isradipine" and others. Some of the newer agents, such as amlodipine and nicerdipine, have the advantage that they show little interaction with other cardiovascular drugs, such as digoxin or warfarine that are often used concomitantly with calcium channel blockers.

Amlodipine besylate contains not less than 99.0% and not more than 101.0% of 2-[(2-amino-ethoxy)-methyl]-4-2-chlorophenyl), 1,4-dihydro-6-methyl-3,5-pyridine dicerboxylic acid, 3-ethyl-5-methyl ester besylate.
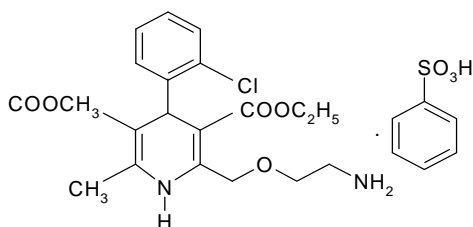
Amlodipine besylate (ADB).

Calcium channel blockers have been determined by a variety of analytical techniques, such as HPLC [2-10], HPTLC [11-16], gas chromatography [17-21], Micellar electrokinetic chromatography [22-25], electroanalytical methods [26-28] and spectrophotometric methods [29-34].

In this communication, four new spectrophotometric methods for the determination of amlodipine, diltiazem and verapamil have been discussed. The methods based on formation of chloroform-extractable ion-pair complexes with Sulfochlorophenol-S (SCPS), Bromopyrogallol red (BPR), Eriochromecyanine-R (ECC) and Pyrocatechol violet (PCV).

2. EXPERIMENTAL

2.1. Apparatus

A spectronic^{TM} Genesys^{TM}, ultraviolet/visible spectrophotometer (Milton Roy Co., Westhaven, USA) was used for measure absorbance.

A Milwakee SM 101 pH meter, (Portugal) was used for pH measurements.

2.2. Chemicals and Reagents

Stock solutions containing 1 mg·ml^{-1} of each of the studied drugs were prepared. 50 mg of verapamil and diltiazem were accurately weighed (Alexandria Co. For pharmaceuticals, Alexandria, Egypt), carefully transferred into 50 ml volumetric flasks, then dissolved in double distilled water, sonicated for about 2 min and completed to volume with the same solvent. For amlodipine 50 mg was accurately weighed, carefully transferred into 50-ml volumetric flasks, then dissolved in 2 ml methanol, sonicated for about 2 min and completed to volume with double distilled water. Working standard solutions containing 50 - 500 µg·ml^{-1} were prepared by a series of dilutions of the stock solutions with water. ECC (Merck, Darmstadt, Germany) 0.2% w/v stock solution was prepared by dissolving 200 mg of the commercial preparation in double distilled water into 100-ml volumetric flask and then diluted to volume with the same solvent. SCPS (Sigma, St. Louis, MO, USA) 0.1% w/v stock solution was prepared by dissolving 100 mg of the commercial preparation in double distilled water into a 100-ml volumetric flask and then diluted to volume with the same solvent. BPR (Sigma, St. Louis, MO, USA) 0.05% w/v stock solution was prepared by dissolving 50 mg of the commercial preparation in double distilled water into a 100-ml volumetric flask and then diluted to volume with the same solvent. PCV (Sigma, St. Louis, MO, USA) 0.1% w/v stock solution was prepared by dissolving 100 mg of the commercial preparation in double distilled water into a 100-ml volumetric flask and then diluted to

volume with the same solvent.

Stock 1 M solutions of each of the following acids; acetic, hydrochloric, nitric, oxalic and sulfuric acids which obtained from El-Nasr Co. (for pharmaceuticals and chemicals, Egypt) were freshly prepared in double distilled water. Stock solutions of citric acid, 0.1 - 5 M were freshly prepared in double distilled water.

2.3. Procedure for Calibration Curve

Into 60-ml separating funnel: for ECC; 1 ml of 3 M citric acid was transferred, followed by 1 ml of working standard solution of either VPM, DTZ or ADB, in the general concentration ranges 25 - 175 $\mu g \cdot ml^{-1}$, 50 - 150 $\mu g \cdot ml^{-1}$ or 100 - 250 $\mu g \cdot ml^{-1}$, respectively. Then suitable aliquot of corresponding dye was added to the resultant solution, 2 ml of 0.2% w/v ECC solution for DTZ, 1.5 ml of 0.2% w/v ECC solution for VPM and ADB. For SCPS; 2 ml of 0.5 M sulfuric acid for VPM, 1.5 ml of 0.3 M sulfuric acid for DTZ and ADB was transferred, followed by 1 ml of working standard solution of VPM, DTZ or ADB, in the general concentration ranges 300 - 800 $\mu g \cdot ml^{-1}$, 200 - 700 $\mu g \cdot ml^{-1}$ or 100 - 550 $\mu g \cdot ml^{-1}$, respectively. Then suitable aliquot of corresponding dye was added to the resultant solution, 1.5 ml of 0.1% w/v SCPS solution. For BPR; 1.5 ml of 1 M sulfuric acid was transferred, followed by 1 ml of working standard solution of VPM, DTZ or ADB, in the general concentration ranges 50 - 400 $\mu g \cdot ml^{-1}$, 200 - 700 $\mu g \cdot ml^{-1}$ or 200 - 700 $\mu g \cdot ml^{-1}$, respectively. Then suitable aliquot of corresponding dye was added to the resultant solution, 1.5 ml of 0.05% w/v BPR solution. For PCV; 0.5 ml of 1 M sulfuric acid was transferred, followed by 1 ml of working standard solution of either VPM or DTZ, in the general concentration ranges 50 - 250 $\mu g \cdot ml^{-1}$ or 200 - 500 $\mu g \cdot ml^{-1}$, respectively. Then suitable aliquots of corresponding dye were added to the resultant solution, 1.5 ml of 0.1% w/v PCV solution. The contents were mixed well and shacked with three 3 ml portions of chloroform. Then the extracts were combined into 10-ml volumetric flask and completed to volume with chloroform. The combined extracts were filtered over 2 gm anhydrous sodium sulfate and first portion of the filtrate was rejected. The absorbance of the chloroform extract was measured at λ_{max} = 462, 600, 440 and 442 nm against a reagent blank treated similarly, respectively.

2.4. Procedure for the Assay of Drug in Dosage Forms

An accurately weight amount equivalent to 100.0 mg of each drug from composite of 20 powdered tablets was carefully transferred into 100 ml volumetric flask, followed by addition of about 50 ml double distilled water, and the mixture was shaken well, sonicated for about 10 min and then completed to volume with the same solvent. The resulting solution was filtered where the first portion of the filtrate was rejected [4]. Appropriate volume was diluted quantitatively with the same solvent to prepare a working standard solution containing 50 - 500 $\mu g \cdot ml^{-1}$ and then was subjected to analysis as explained under general procedure.

3. RESULTS AND DISCUSSION

Upon interaction of the studied drugs with these acidic dyes such as SCPS, ECC, BPR and PCV, highly colored ion pair complexes extractable with chloroform, were formed. Absorption spectra of diltiazem hydrochloride as a presentative example and the formed ion pair complexes with ECC, SCPS, BPR and PCV in chloroform, are shown in **Figure 1**, The reagent blanks prepared under similar conditions showed no absorption.

3.1. Composition and Formation Constant of Ion-Pair Complexes

Using job's method of continuous variation [35], the results revealed a 1:1 ratio between ECC and the studied drugs, **Figure 2**, 1:4 ratios between SCPS and the investigated drugs, **Figure 3**, 1:1 ratio between BPR and the studied drugs, **Figure 4** and 1:1 ratio between PCV and the studied drugs, **Figure 5**. The formation constant (K_f) of the studied drugs in complex with ECC, SCPS, BPR and PCV were calculated also the Gibbs free energy change of the reaction were calculated [36] and the collected data are illustrated in **Table 1**.

3.2. Optimization of the Reaction Conditions

The optimum conditions for quantitative estimation of the drug were established via number of preliminary experiments.

3.2.1. Effect of Extracting Solvent

Preliminary experiments were carried out without adjusting variables. The absorption intensities of the formed complexes were greatly affected by different immiscible solvents, it was observed that the highest absorption intensity was obtained on using chloroform as an extracting solvent; thus it was selected for all the subsequent work.

3.2.2. Effect of Acid Type

This study revealed that the use of acids led to higher absorption intensities than those obtained upon using buffers. Among different acids, citric acid gave the maximum color intensity than others with ECC but sulfuric acid gave the maximum color intensity with SCPS, BPR and PCV.
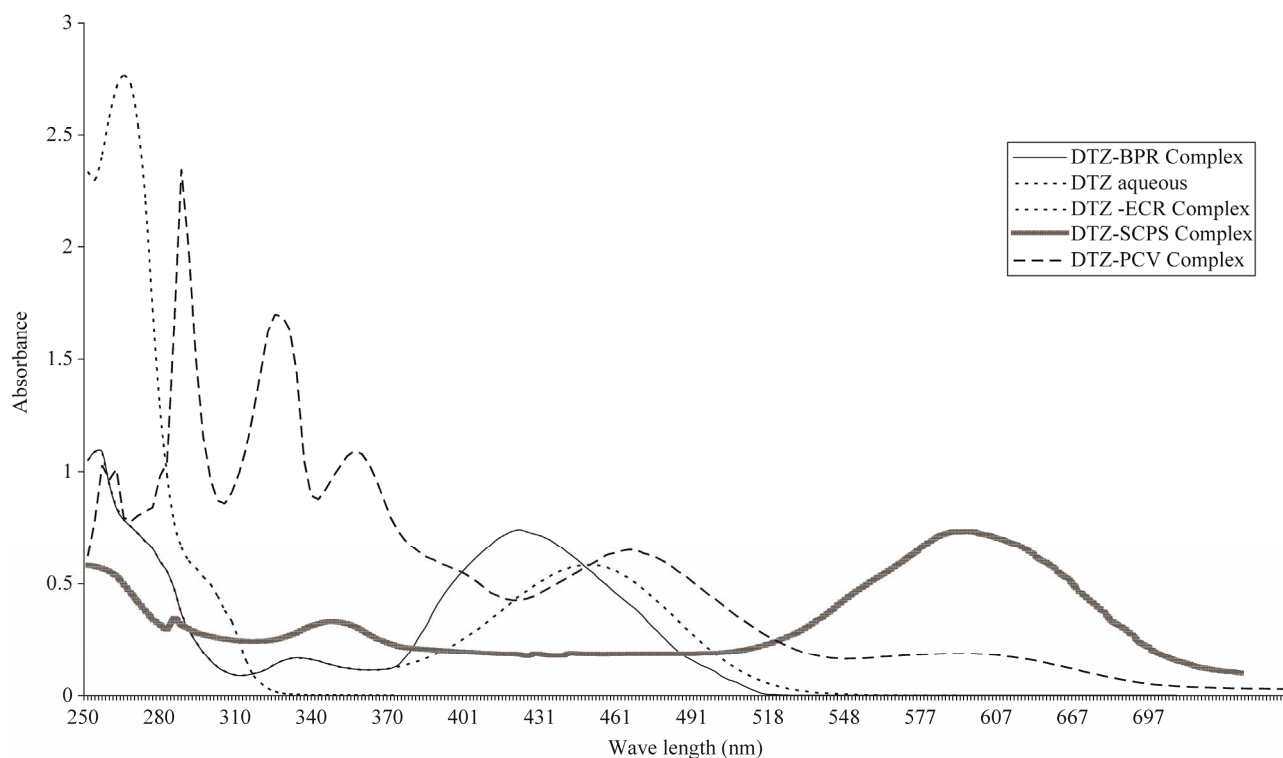
Figure 1. Absorption spectra of aqueous diltiazem hydrochloride 30 µg·ml^{-1}, 15 µg·ml^{-1}, 50 µg·ml^{-1} and 50 µg·ml^{-1} with BPR, ECC, SCPS and PCV dyes respectively, to form diltiazem hydrochloride ion pair complexes with these dyes in chloroform.

Table 1. Calculated stability constants and molar absorptivities of the formed chelates between the studied drugs and the four dyes.

Drug	Dye	Parameter			
		Formation constant (K$_f$)	Log (K$_f$)	ΔG	ε (L^{-1}·mol^{-1}·cm^{-1})
VPM	ECC	8.8×10^4	4.9	-2.8×10^4	2.3×10^4
	SCPS	1.5×10^3	3.2	-1.8×10^4	3.7×10^3
	BPR	5.9×10^3	3.8	-2.1×10^4	1.1×10^4
	PCV	1.3×10^4	4.1	-2.3×10^4	1.8×10^4
DTZ	ECC	1.6×10^5	5.2	-2.9×10^4	2.1×10^4
	SCPS	1.4×10^4	4.1	-2.4×10^4	5.8×10^3
	BPR	6.8×10^3	3.8	-2.2×10^4	5×10^3
	PCV	5.5×10^3	3.7	-2.1×10^4	7.1×10^3
ADB	ECC	1.17×10^8	8.2	-4.6×10^4	1.6×10^4
	SCPS	5.9×10^4	4.8	-2.7×10^4	8×10^3

### 3.2.3. Effect of Acid Concentration and Volume

As shown in **Figures 5-8**, 3 M citric acid gave the maximum color intensity with the highest stability in case of using ECC; 1 M sulfuric acid gave the maximum color intensity with the highest stability in case of using BPR and PCV; 0.5 M sulfuric acid gave the maximum color intensity with the highest stability in case of using SCPS with VPM. 0.3 M sulfuric acid gave the maximum

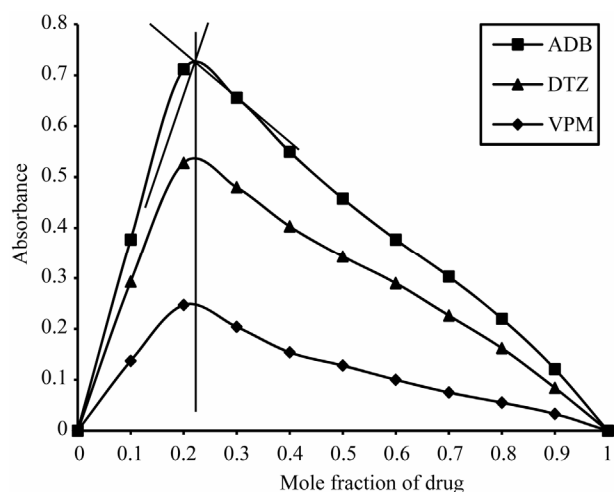color intensity with the highest stability in case of using SCPS with ADB and DTZ.

The optimum volume was also studied for each acid where; in case of using 0.5 - 3 ml of 3 M citric acid; 1.5 ml gave the maximum color intensity with the highest stability as indicated in **Figure 9** For ECC. In case of using 0.5 - 3 ml of 0.3 M sulfuric acid 1.5 ml gave the maximum color intensity with the highest stability in
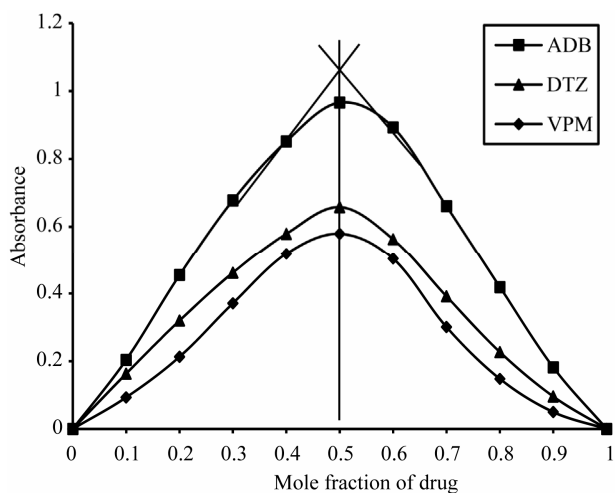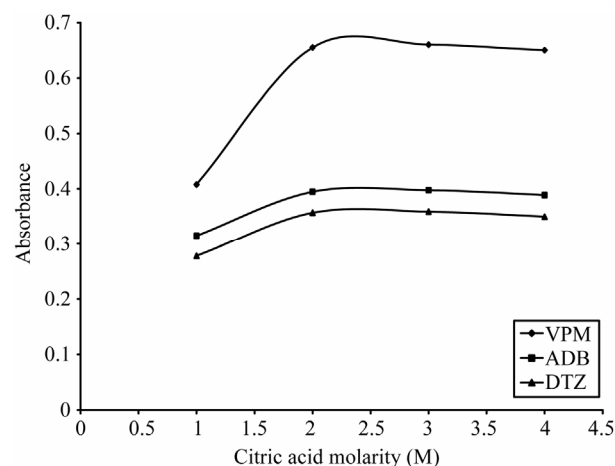

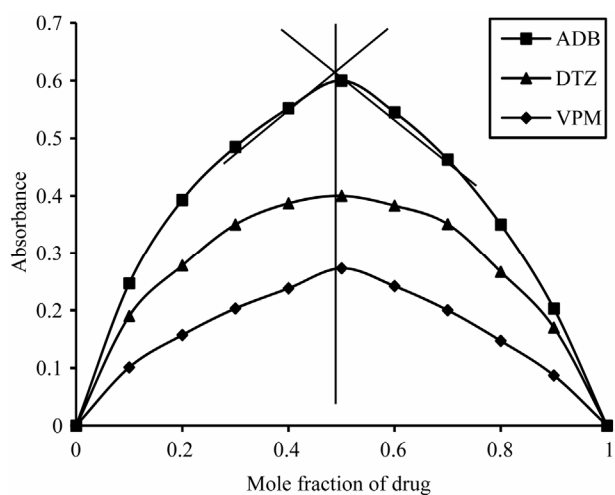**Figure 2.** Job's plots method of continuous variation between the studied drugs and ECC ion pair complex.

Figure 3. Job's plots method of continuous variation between the studied drugs and SCPS ion pair complex.

Figure 4. Job's plots method of continuous variation between the studied drugs and BPR ion pair complex.
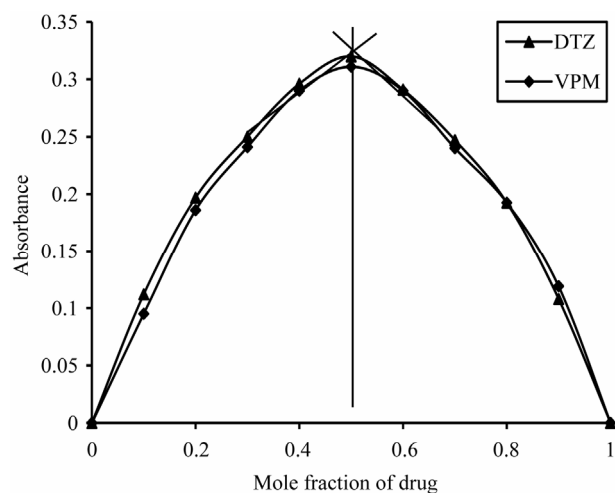
Figure 5. Job's plots method of continuous variation between the studied drugs and PCV ion pair complex.
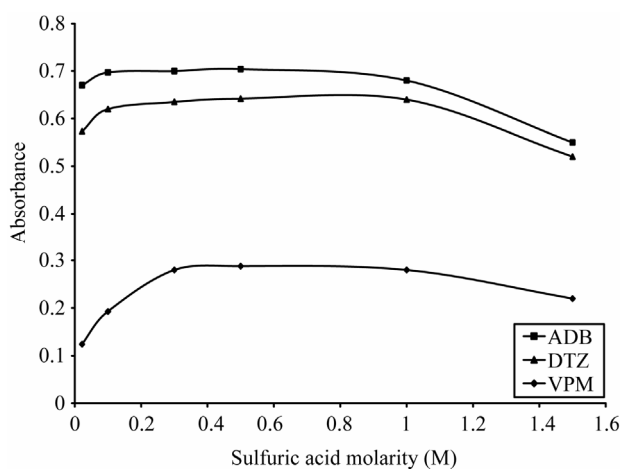

Figure 6. Effect of citric acid morality on the absorption spectra of formed ion pair complex using 15 µg·ml^{-1} from each studied drug and 0.2% w/v ECC.

Figure 7. Effect of sulfuric acid morality on the absorption spectra of formed ion pair complex using 50 µg·ml^{-1} of each studied drug and 0.1% w/v SCPS.
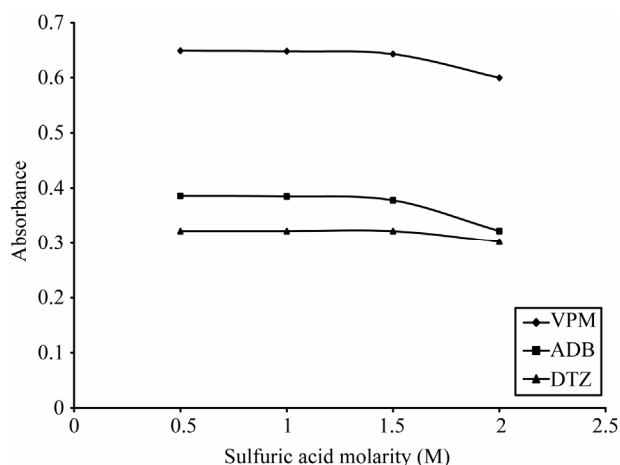
Figure 8. Effect of sulfuric acid morality on the absorption spectra of formed ion pair complex using 30 µg·ml^{-1} of each studied drug and 0.05% w/v BPR complex.

Figure 9. Effect of sulfuric acid morality on the absorption spectra of formed ion pair complex using VPM 20 µg·ml^{-1}, DTZ 50 µg·ml^{-1} and 0.1% w/v PCV.
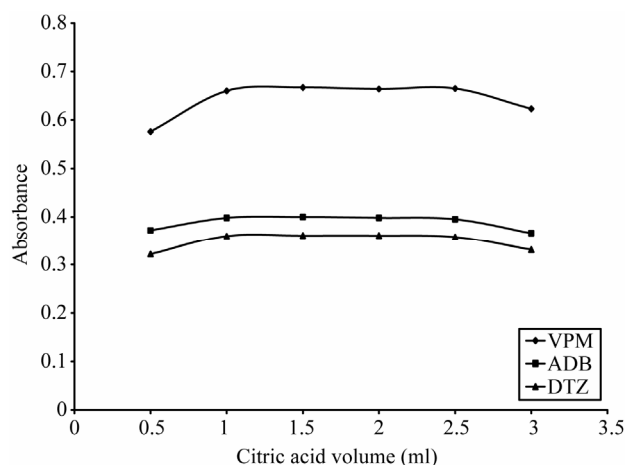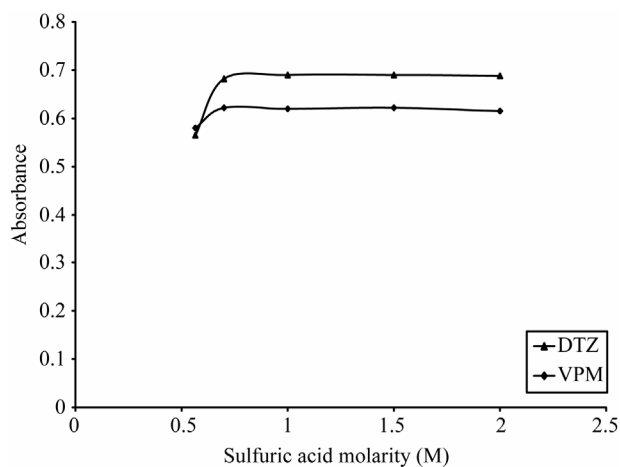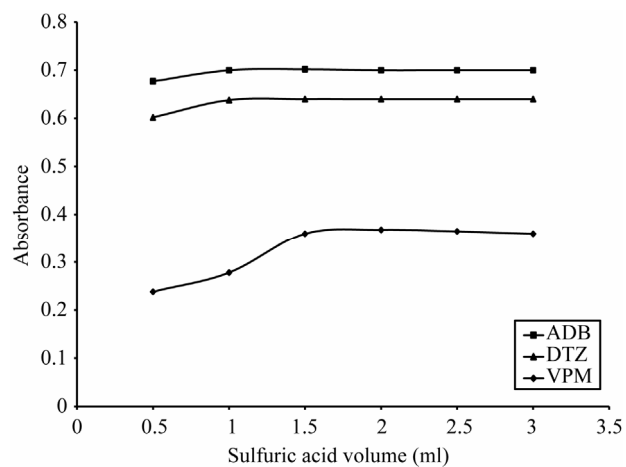

Figure 10. Effect of citric acid volume on the absorption spectra of the formed ion pair complex using 15 µg·ml^{-1} of each studied drug and 0.2% w/v ECC.
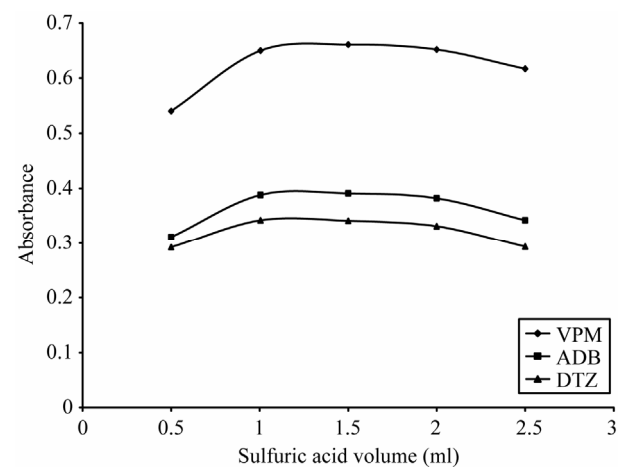

Figure 11. Effect of sulfuric acid volume on the absorption spectra of the formed ion pair complex using 50 µg·ml^{-1} of each studied drug and 0.1% w/v SCPS.

Figure 12. Effect of sulfuric acid volume on the absorption spectra of the formed ion pair complex using 30 µg·ml^{-1} of each studied drug and 0.05% w/v BPR.

case of ADB and DTZ, respectively. 2 ml of 0.5 M sulfuric acid in case of VPM gave the maximum color intensity with the highest stability as indicated in **Figure 10**. For SCPS, in case of using 0.5 - 2.5 ml of 1 M sulfuric acid 1.5 ml gave the maximum color intensity with the highest stability as indicated in **Figure 11**. For BPR, in case of using 0.1 - 1.5 ml of 1 M sulfuric acid 0.5 ml gave the maximum color intensity with the highest stability as indicated in **Figure 12** For PCV.

3.2.4. Effect of Dye Concentration

The absorption intensities of the formed complexes were dependent upon the dye concentration; for ECC dye, the optimum range was 0.1% - 0.3% w/v, thus 0.2% w/v was selected, **Figure 13**. For SCPS dye, the optimum range was 0.05% - 0.2% w/v, thus 0.1% w/v was selected, **Figure 14**. For BPR dye, the optimum range was

Figure 13. Effect of sulfuric acid volume on the absorption spectra of the formed ion pair complex using VPM 20 µg·ml^{-1}, DTZ 50 µg·ml^{-1} and 0.1% w/v PCV.

Figure 14. Effect of eriochrome cyanine concentration on the absorption spectra of the formed ion pair complex using 15 µg·ml^{-1} of each studied drug.

Figure 15. Effect of Sulfochlorophenol-S concentration on the absorption spectra of the formed ion pair complex using 50 µg·ml^{-1} of each studied drug.

Figure 16. Effect of Bromopyrogallol red concentration on the absorption spectra of the formed ion pair complex using 30 µg·ml^{-1} of each studied drug.
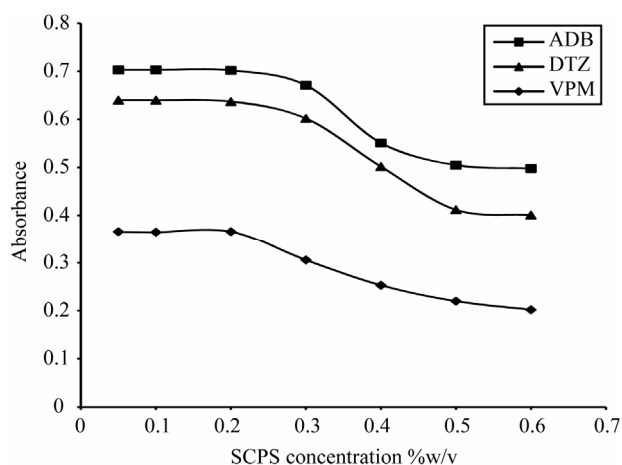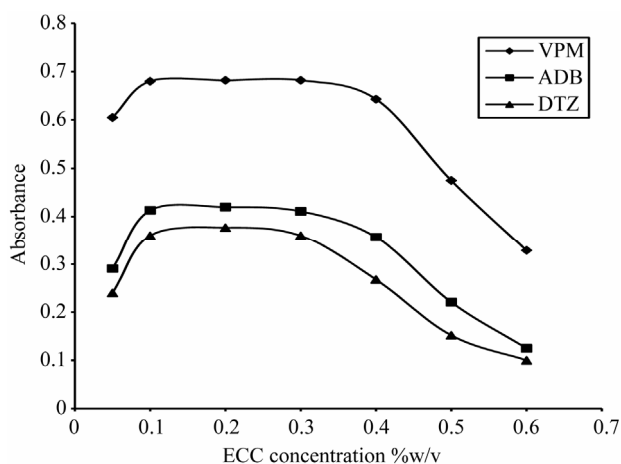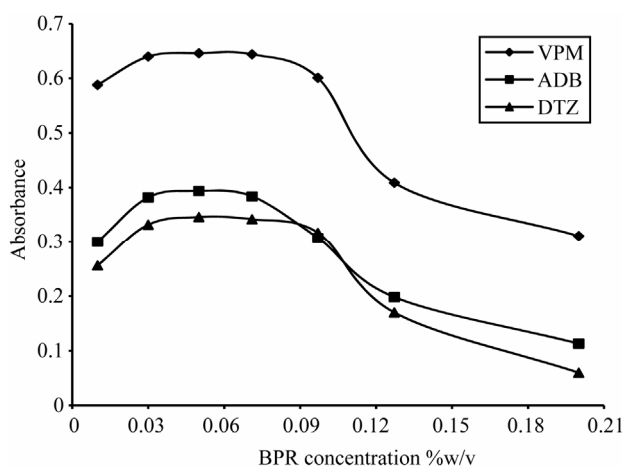
0.03% - 0.07% w/v, thus 0.05% w/v was selected, **Figure 15**. For PCV dye the optimum range was 0.07% - 0.2% w/v, thus 0.1% w/v was selected, **Figure 16**. Then the formed colored complex was extracted using different volumes of dyes. For ECC dye, optimum range was 0.5 - 3 ml, thus 2 ml for DTZ and 1.5 ml for both ADB and VPM was selected, **Figure 17**. For SCPS and BPR dyes, the optimum range were 0.25 - 2 ml and 0.5 - 2 ml respectively, thus 1.5 ml for each drug was selected, **Figures 18** and **19**. For PCV dye, the optimum range was 0.5 - 2.5 ml, thus 1.5 ml in case DTZ and 2 ml in case of VPM was selected, **Figure 20** and **21**.

3.3. Analytical Data

Standard calibration graphs for the studied drugs with each dye were constructed by analyzing a series of concentrations of the drugs. Beer's plots showed linear cor-

Figure 17. Effect of pyrocatechol violet concentration on the absorption spectra of the formed ion pair complex using VPM 20 µg·ml^{-1}, DTZ 50 µg·ml^{-1}.
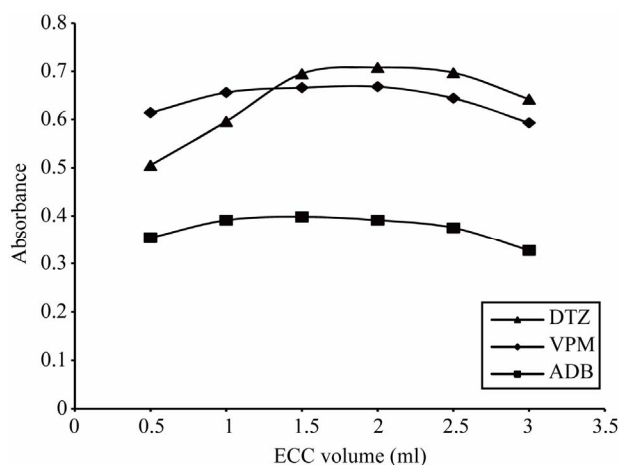
Figure 18. Effect of eriochrome cyanine concentration on the absorption spectra of the formed ion pair complex using 15 µg·ml^{-1} of each studied drug.

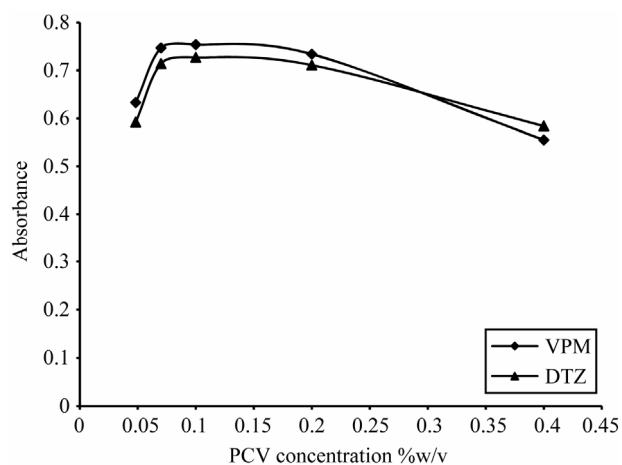**Figure 19.** Effect of 0.1% w/v Sulfochlorophenol-S volume on the absorption spectra of the formed ion pair complex using 50 µg·ml^{-1} of each studied drug.

Figure 20. Effect of 0.05% w/v Bromopyrogallol red volume on the absorption spectra of the formed ion pair complex using 30 µg·ml^{-1} of each studied drug.
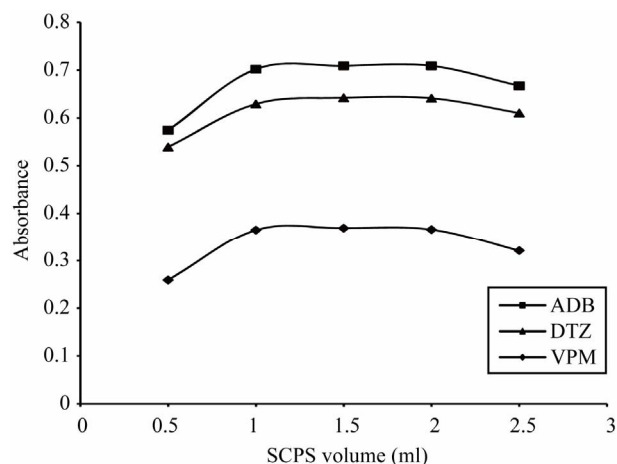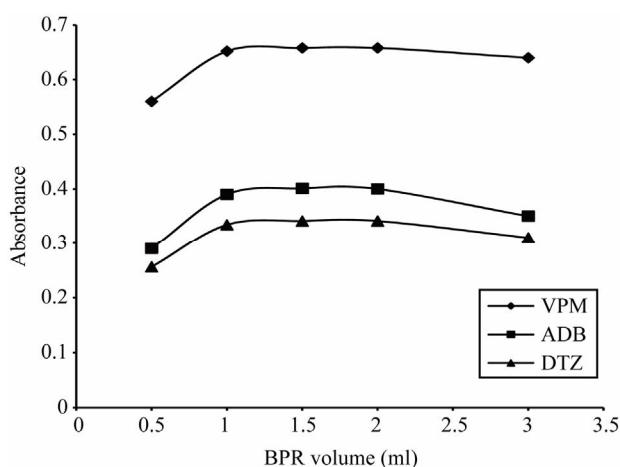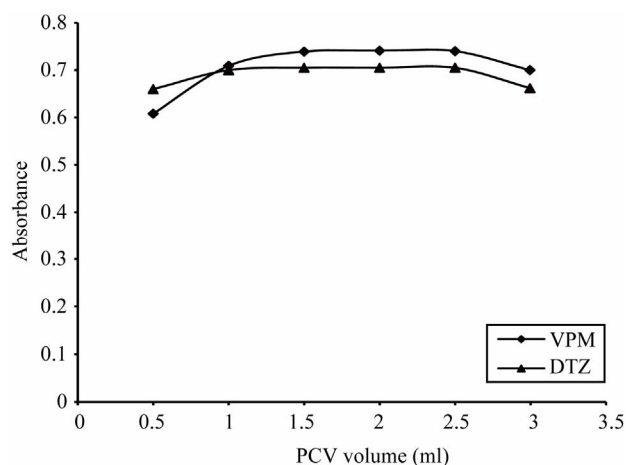

Figure 21. Effect of 0.1% w/v pyrocatechol violet volume on the absorption spectra of the formed ion pair complex using VPM 20 µg·ml^{-1} and DTZ 50 µg·ml^{-1}.

relations over a wide range of drug concentrations with good correlation coefficients. Data of such studies are illustrated in **Table 2**. The slopes (b) of the calibration curves indicated the high sensitivity of the proposed method while the small intercept reflected the low interfering background [37].

The accuracy of the proposed method was demonstrated by recovery experiments, which were carried out by adding a fixed amount of pure drug to pre-analyzed dosage forms. The analytical results obtained were summarized in **Table 3**. The percentage of RSDs can be considered to be very satisfactory.

The performance of the proposed methods was statistically compared with those of reported ones [38-40] using student's t-test and the variance ratio F-test [41], **Table 4**. This comparison did not show any significance differences indicating good accuracy and high sensitivity of the proposed procedure.

The effect of the presence common excipients such as: starch, lactose, sucrose, glucose, magnesium stearate, gum acacia and talc was studied. The excellent recoveries, obtained in **Table 5** for ECC as representative example, indicated that the presence of these excipients did not interfere with the proposed method. This could be explained on the basis that all these additives are natural and almost non extractable by organic solvents as well as very or non basic at all and so they do not contribute in the reaction.

The Precision [42] of the proposed method was checked by replicate analysis of five separate standard solutions of each compound at three concentration levels. The calculated relative standard deviation values were below 2% indicating good repeatability and reliability of the proposed method [37,43]. The results and their statistical analysis were summarized in **Table 6**. for ECC as representative example.

Table 2. Quantitative parameters for proposed method.

Parameter	ECC method			SCPS method			BPR method			PCV method	
	VPM	DTZ	ADB besylate	VPM	DTZ	ADB besylate	VPM	DTZ	ADB besylate	VPM	DTZ
Linear range ($\mu g \cdot ml^{-1}$)	2.5 - 17.5	5 - 15	10 - 25	30 - 80	20 - 70	10 - 55	5 - 40	20 - 70	20 - 70	5 - 25	20−50
Intercept (a)	0.00160	0.0318	−0.0433	−0.00798	0.027	0.0484	−0.0226	0.025	0.0539	−0.0275	−0.063
SE of intercept	0.00652	0.00594	0.00514	0.01047	0.0051	0.01058	0.00547	0.0085	0.0057	0.0038	0.00891
Slope (b)	0.0445	0.0444	0.02981	0.00771	0.012	0.01319	0.0228	0.0144	0.01137	0.04075	0.0187
SE of slope	0.00055	0.00056	0.00029	0.00018	0.0001	0.00028	0.00022	0.00011	0.00012	0.00023	0.0002
Correlation coefficient (r)	0.9997	0.9998	0.9999	0.9989	0.9998	0.9991	0.9997	0.9995	0.9998	0.9999	0.9995
D determination coefficient (r^2)	0.9994	0.9995	0.9997	0.9978	0.9997	0.9982	0.9995	0.9989	0.9996	0.9998	0.9995
$\varepsilon \times 10^3$ ($L \cdot mol^{-1} \cdot cm^{-1}$)	2.2	2.1	1.6	3.8	5.6	8.1	11	4.8	6.9	19.5	6.6
LOD ($\mu g \cdot ml^{-1}$)	0.44	0.4	0.525	4	1.2	2.4	0.7	1.7	1.5	0.28	1.4
LOQ ($\mu g \cdot ml^{-1}$)	1.5	1.3	1.7	13.5	4.3	8	2.4	6	5	0.94	4.8

Table 3. Analysis of pharmaceutical preparations using ECC, SCPS, BPR and PCV methods, applying standard addition technique.

Drug	Pharmaceutical preparation	Claimed (mg/tablet)	Dye	Added ($\mu g \cdot ml^{-1}$)	% Recovery ± SD
VPM	Verapamil® tablet	60	ECC	2.5	100.1 ± 0.82
			SCPS	30	99.8 ± 0.86
			BPR	10	98.2 ± 0.75
			PCV	5	99.8 ± 0.98
DTZ	Altiazem® tablet	5	ECC	2.5	98.5 ± 0.79
			SCPS	20	99.2 ± 0.84
			BPR	10	99.6 ± 0.71
			PCV	10	98.8 ± 0.91
ADB besylate	Amlodipine® tablet	40	ECC	5	99.9 ± 0.74
			SCPS	20	98.1 ± 0.87
			BPR	10	99.5 ± 0.90

Table 4. Statistical comparison between the proposed and reported methods for determination of studied drugs in their pharmaceutical formulations.

	% Recovery ± SD**		
	VPM	DTZ	ADB besylate
Reported method***	100.2 ± 0.600	99.9 ± 0.814	99.96 ± 0.926
ECC method	98.9 ± 1.152 $t^* = 2.3$, $F^* = 3.7$	99.7 ± 0.84 $t^* = 0.267$, $F^* = 1.07$	99.3 ± 0.638 $t^* = 1.35$, $F^* = 2.11$
SCPS method	100.15 ± 0.720 $t^* = 0.66$, $F^* = 1.49$	99.8 ± 1.33 $t^* = 1.43$, $F^* = 2.67$	100.18 ± 0.497 $t^* = 0.47$, $F^* = 3.47$
BPR method	99.48 ± 1.123 $t^* = 1.26$, $F^* = 3.51$	99.9 ± 0.935 $t^* = 0.072$, $F^* = 1.32$	99.58 ± 1.026 $t^* = 0.61$, $F^* = 1.23$
PCV method	100.2 ± 1.206 $t^* = 0.01$, $F^* = 4.04$	100.26 ± 0.903 $t^* = 0.728$, $F^* = 1.23$	----------

Table 5. Assay of the studied drugs in presence of some common excipients using eriochrome cyanine method.

Excipients	Added* (mg)	Taken** ($\mu g \cdot ml^{-1}$)	% Recovery*** ± RSD		
			ADB besylate	DTZ	VPM
Starch	100	5	99.2 ± 0.97	100.6 ± 1.18	99.4 ± 1.0
Lactose	50	5	99.3 ±1.34	99.3 ± 1.33	99.5 ± 1.38
Glucose	50	5	99.7 ± 1.04	98.9 ± 1.24	99.5 ± 0.77
Sucrose	50	5	100.3 ± 0.94	99.7 ± 1.03	99.4 ± 0.85
Mg stearate	10	5	99.5 ± 1.42	99.5 ± 0.84	99.3 ± 1.14
Gum acacia	10	5	100.2 ± 0.96	99.9 ± 1.44	101.2 ± 1.57
Talc	10	5	99.9 ± 0.73	99.7 ± 1.29	99.5 ± 0.96

Table 6. Evaluation of the precision of Eriochromecyanine method.

Sample No.	% Recovery								
	VPM			DTZ			ADB besylate		
	10 $\mu g \cdot ml^{-1}$	12.5 $\mu g \cdot ml^{-1}$	15 $\mu g \cdot ml^{-1}$	5 $\mu g \cdot ml^{-1}$	10 $\mu g \cdot ml^{-1}$	15 $\mu g \cdot ml^{-1}$	10 $\mu g \cdot ml^{-1}$	15 $\mu g \cdot ml^{-1}$	25 $\mu g \cdot ml^{-1}$
1	97.7	98.4	99.3	98.2	99.5	99.2	98.6	98.9	98.8
2	97.3	98.8	98.5	98.2	99.5	99.2	98.6	98.2	98.8
3	97.7	98.0	99.3	98.2	99.5	98.8	98.3	98.9	98.8
4	97.7	98.0	99.3	98.2	99.3	99.2	98.6	98.9	98.3
5	96.0	98.8	99.3	100.1	100.0	98.8	98.3	98.2	98.4
Mean	97.3	98.4	99.1	98.6	99.6	99.0	98.5	98.7	98.6
SD	0.74	0.40	0.36	0.85	0.26	0.22	0.16	0.36	0.25
RSD	0.76	0.41	0.36	0.86	0.26	0.22	0.17	0.37	0.25

4. CONCLUSION

The proposed methods are advantageous in comparison to other existing spectrophotometric methods because the detection limits are low. The molar absorptivities are also comparable with low RSDs. No interference from common excipients was encountered. Thus the proposed methods are simple, sensitive, accurate, precise and easy to use and might be preferred to other published methods for assay of the drug in clinical and quality control laboratories.

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