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Original Article

UTILIZATION OF ION-PAIR COMPLEX FORMATION FOR THE SPECTROPHOTOMETRIC DETERMINATION OF SOME ANTIDEPRESSANT DRUGS IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Objective: A new, simple and sensitive spectrophotometric method has been developed for the determination of some antidepressant drugs, namely desvenlafaxine (DSV), dapoxetine (DAP) and citalopram (CIT) in pharmaceutical formulations.

Methods: The proposed method was based on the formation of yellow colored ion-pair complex between the studied drugs and two sulpho phthalein dyes *viz.,* bromophenol blue (BPB), and bromothymol blue (BTB) in acidic medium. The optimizations of the reaction conditions were investigated. Beer's law limits, Sandell's sensitivity, correlation coefficient, detection, and quantification limits were calculated.

Results: The formed complexes showed absorption maxima at 412 nm and 410 nm measured for all the drugs with BPB and BTB, respectively. The Job's method of continuous variations indicated that a single l: l ion-pair complex was formed. Calibration curves were linear over the concentration range of 0.8–11.4, 0.8–6.4 and 0.8–8.0 µg/ml for DSV, DAP and CIT, respectively.

Conclusion: The proposed method has been applied successfully for the analysis of the investigated drugs in pure and in their dosage forms. No interference was observed from common excipients present in pharmaceutical formulations. The proposed method is suitable for quality control applications.

Keywords: Desvenlafaxine, Dapoxetine, Citalopram, Sulphonphthalein dyes, Extraction spectrophotometry, Pharmaceutical formulations

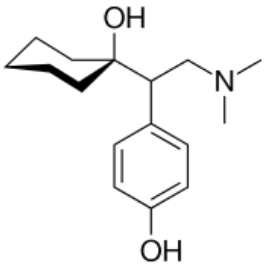
INTRODUCTION

Desvenlafaxine succinate is a newer antidepressant drug, which is chemically 1-[(1RS)-2-(Dimethylamino)-1-(4-hydroxyphenyl) ethyl] cyclohexanol succinate monohydrate (DSV, fig. 1a). Desvenlafaxine succinate is a structurally novel SNRI (serotonin-norepinephrine reuptake inhibitor) useful for the treatment of MDD (major depressive disorder). Desvenlafaxine (O-desmethyl-venlafaxine) is the major active metabolite of the antidepressant venlafaxine, a medication used to treat major depressive, generalized anxiety and panic disorders. Literature survey reveals HPLC coupled to spectrophotometric [1–3], spectrofluorimetric [4–6] or coulometric detection [7], capillary electrophoresis [8–11], adsorptive stripping voltammetric [12], HPLC-ESI/MS [13] and LC-MS/MS [14–17] have been used. Desvenlafaxine succinate is not official in any pharmacopoeia.

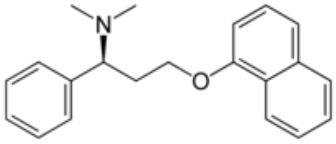
Dapoxetine HCl (DAP, fig. 1b) is designated chemically as (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1 phenylpropan-1-amine. This drug is mainly useful in erectile dysfunction as selective serotonin reuptake inhibitor (SSRI) [18]. SSRI's are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders. They can also sometimes be effective and used in treating premature ejaculation problems, impotence and some cases of insomnia. The drug's mechanism of action is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time [19]. The literature study reveals that several UV spectrometric and chromatographic methods available for dapoxetine in combined tablet formulation [20–27]. No method has been reported for individual dapoxetine HCl using visible spectrophotometric methods.

Citalopram, (CIT, fig. 1c) 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl) -1, 3-dihydroisobezofuran-5-carbonitrile [28], is a second generation antidepressant and one of the recently introduced SSRIs. It is used for managing depression, social anxiety disorder, panic disorder, and obsessive-compulsive disorder [29–31]. Several methods have been devised for the determination of citalopram in pharmaceutical preparations and biological fluids. These include high performance liquid chromatography (HPLC) with UV detectors [32–34], HPLC with fluorescence detectors [35–37], HLPC/mass spectrometry [38–40], gas chromatography [41, 42], electrophoretic methods [43–45] and spectrophotometric [46–48] methods. However, few spectrofluorimetric [49, 50] methods have been reported in the literature, and most reported methods involve multistep procedures and have poor selectivity's and sensitivities.

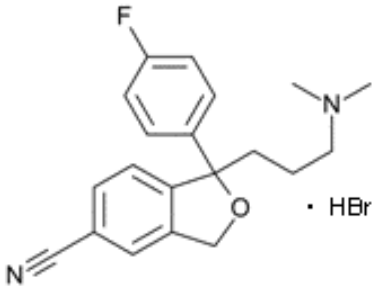
Our present study has been designed to describe a simple, accurate, rapid and precise spectrophotometric methods for the determination of DSV, DAP and CIT in bulk and tablet dosage forms.



a-DSV



b-DAP



c-CIT

Fig. 1: Chemical structure of the investigated drugs

MATERIALS AND METHODS

Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Visible spectrophotometer (Biotech Engineering Ltd. (UK), with wavelength range 190 nm ~ 1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells. The pH values of buffer solutions were measured using Jenway instrument pH-meter (combined electrode).

Reagents and solutions

All of the chemicals used were of analytical or pharmaceutical grade and used without further purification. Distilled water was used to prepare all solutions.

i. A 1.0×10^{-3}M of bromophenol blue and bromothymol blue (Aldrich Co., Ltd., Gillingham-Dorst, Germany), were prepared by dissolving 66.996 mg and 62.438 mg from each dye in 2 ml methanol then, add 20 ml distilled water and diluted to 100 ml in a calibrated flask with distilled water to the mark.

ii. Series of buffer solutions of KCl-HCl (pH 1.0-2.2), NaOAc-HCl (2.2-6.8) and NaOAc-AcOH (3.4-5.6) pH were prepared by standard methods.

iii. A pharmaceutical grade of DSV, DAP and CIT certified to be 99.85% pure was obtained as a gift were kindly supplied from Egyptian International Pharmaceutical Industries Company (EIPICo), Egypt. Stock solutions of pure DSV, DAP and CIT were prepared separately by dissolving accurately weighed 10 mg of each drug in distilled water and finally the volume was made up to 100 ml with distilled water (100 µg/ml).

General recommended procedures

Into a series of separated funnels, accurately measured aliquots of DSV, DAP and CIT, in the concentration range shown in (table 1) were pitted out. A volume of 2.0 ml of 1×10^{-3}M BPB or BTB were added. Then, 2.0 ml of buffer solution of pH = 2 for each system was added, and the volume was completed to 10 ml with distilled water. The ion-pairs were extracted with 10 ml of dichloromethane by shaking for 2.0 min and then, the combined dichloromethane extracts were dried over anhydrous sodium sulphate. The absorbance of colored ion-pair complexes was measured within 30 min of extraction against a reagent blank prepared in the same manner except the addition of drugs. In both the methods, a standard curve was prepared by plotting the increasing absorbance values versus concentrations of the drug. A linear equation for the standard curve was calculated by linear regression.

Procedure for tablets

At least ten tablets of the drugs were weight into a small dish, powdered and mixed well. A portion equivalent to 10 mg of DSV, DAP and CIT were weight and dissolved in distilled water, filtered into a 100 ml calibrated flask and diluted to volume with water. Solutions of working range concentration were prepared by proper dilution of this stock solution with water and followed the above procedure for the calibration curve.

RESULTS AND DISCUSSION

Absorption spectra of the yellow color of the studied drugs with BPB or BTB ion-pair complex with a maximum absorbance (λ_{max}) at 412 and 410 nm, respectively is shown in fig. 2. The measurements were made at these wavelengths for bulk and tablet samples. The studied drugs contain a tertiary amino group which is protonated in the acid medium while the sulphonic acid group is present in BPB and BTB that is the only group undergoing dissociation in the pH range 1.0–5.0. The color of such dyes is due to the opening of lactoid ring and subsequent formation of the quinoid group. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated drug forms ion-pairs with the dyestuffs which are quantitatively extracted into dichloromethane [51, 52]. The suggested reaction pathway for the reaction product of DSV–BPB ion–pair complex formation, for example, is given in Scheme 1.

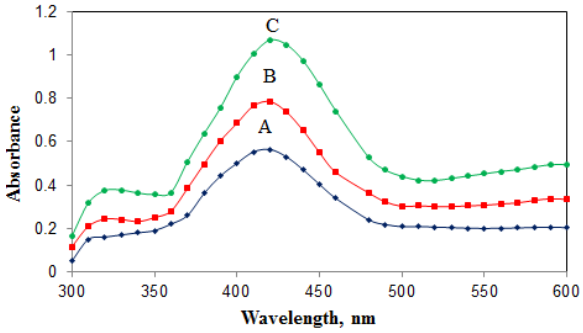


Fig. 2: Absorption spectra of ion-pair complexes of: A-CIT (4 µg/ml), B-DSV (6.4 µg/ml) and C-DPA (5.2 µg/ml) with BPB

Optimization of the reaction conditions

The optimum reaction conditions for determination of the ion-pair complexes were established. Then linearity, accuracy, precision, sensitivity, and stability of proposed methods were described and these developed methods applied to pharmaceutical preparations as tablets and obtained results evaluated statistically.

Effect of buffer type and pH

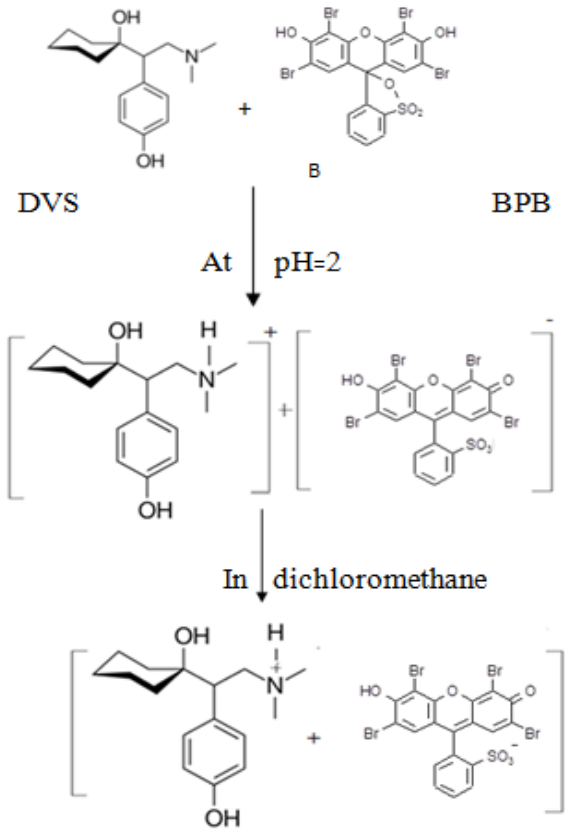
It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl–HCl (pH 1.0–2.2), NaOAc–HCl (pH 2.2–6.8) and NaOAc–AcOH (pH 3.6–5.6). It is evident that the maximum color intensity and maximum absorbance were found in KCl–HCl buffer at pH = 2 for both DSV, DAP and CIT (fig. 3, 4). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5–5.0 ml). The higher absorbance value obtained at using 2.0 ml of buffer solutions.

Effect of reagent concentration

The DSV, DAP and CIT concentrations were kept constant, while the concentrations of BPB or BTB was varied from 0.5–4.0 ml of 1.0×10^{-3}M. The results showed that the absorbance of the extracted ion-pairs increased by increasing the BPB or BTB concentrations till 1.0 ml. After this volume, the absorbance remains constant by increasing the volume of the reagents. So any excess of reagents has no effect on the determination of the drugs.

Choice of organic solvents

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained the highest absorbance with dichloromethane. It was also observed that only one extraction was adequate to achieve a quantitative recovery of the complexes and the shortest time to reach the equilibrium between both phases.



Scheme 1: Suggested mechanism of DSV-BPB ion-pair complex formation

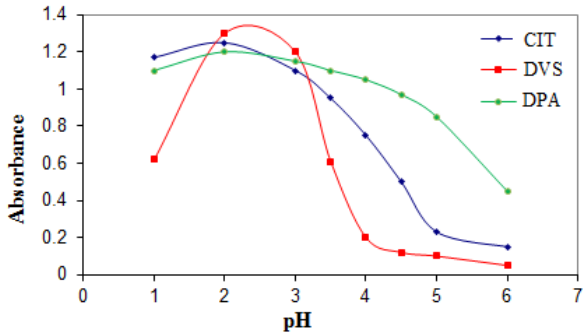


Fig. 3: Effect of pH of buffer solution on the ion-pair complex formation between (11.4 µg/ml) ▪ DSV, (5.2 µg/ml) ● DAP and (8 µg/ml) ♦ CIT with 1.0×10^{-3}M BPB

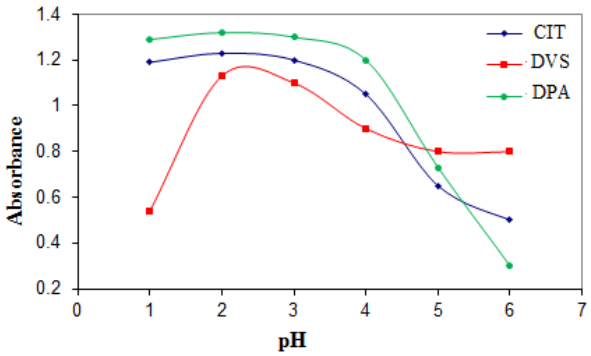


Fig. 4: Effect of pH of buffer solution on the ion-pair complex formation between (8.0 µg/ml) ▪ DSV, (6.4 µg/ml) ● DAP and(8 µg/ml) ♦ CIT with 1.0×10^{-3}M BTB

Effect of temperature on the colored complexes

The effect of temperature on colored complexes was studied over the range 20-50 °C. It was found that the absorbance of the ion-pair complex was constant up to 25 °C. At higher temperatures, the drug concentration was found to increase due to volatile nature of the dichloromethane. Therefore, the temperature chosen was 25 °C as the best temperature for determination of the investigated drugs in pure and pharmaceutical formulations.

Effect of shaking time for extraction

Shaking time ranging from 0.5-4.0 min was tested to ascertain the extraction of the complex. Maximum and constant absorbance value were obtained when extracted after 1.5 min shaking. Therefore, shaking time of 2.0 min was maintained throughout the experiment.

Composition of the ion-pair complexes

In order to establish the molar ratio between DSV, DAP and CIT on one side and BPB or BTB reagent used on the other, Job's method of continuous variation was applied [53]. In this method, 5.0×10^{-4} M solutions of drugs and reagents were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug, [drug]/[drug]+[dyestuff] as shown in (fig. 5, 6). This measurement showed that 1: 1 complex was formed with each dyestuff.

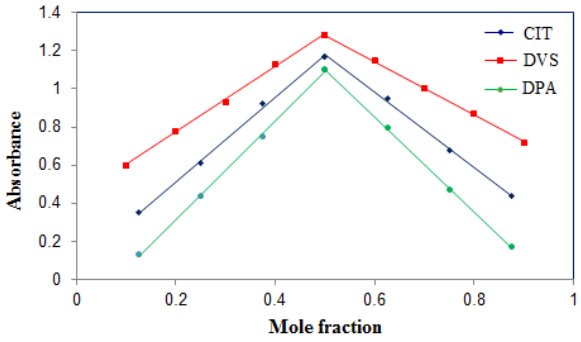


Fig. 5: Job's method of continuous variation graph for the reaction of DSV, DAP and CIT with BPB, [drug] = [dye] = 5×10^{-4} M

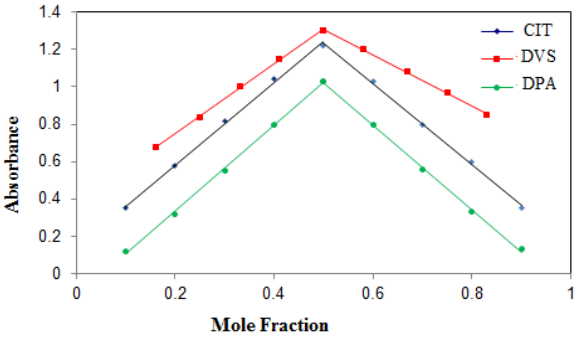


Fig. 6: Job's method of continuous variation graph for the reaction of DSV, DAP and CIT with BTB, [drug] = [dye] = 1.0×10^{-4} M

Quantification

Under the optimum conditions described above, the calibration graphs were constructed for the investigated drugs. The molar absorptivity, Sandells sensitivity, concentration range, regression equation and correlation coefficient for each drug are tabulated in (table 1). A linear relationship was found between the absorbance at λ_{max}. Regression analysis of Beer's law plotted at λ_{max} reveals a good

correlation (fig. 7, 8). The graphs showed a negligible intercept, which was calculated by the least-squares method's regression equation, A = a+bC (where A is the absorbance of 1.0 cm layer, b is the slope, a is the intercept and C is the concentration of the measured solution in µg/ml.

The calculated molar absorptivity of drugs–BTB ion-pair complexes was found to be higher than the drugs–BPB ion-pair complexes. The limit of detection (LOD) and limit of quantitation (LOQ) are calculated according to ICH guidelines [54]. The results are as shown in (table 1).

Table 1: Analytical parameters and optical characteristics of the proposed method

Parameters	BPB			BTB		
	DSV	DAP	CIT	DSV	DAP	CIT
λ_{max} (nm)	412	412	412	410	410	410
Beer's law limit (µg/ml)	1.6–11.4	0.8–5.2	0.8–8.0	0.8–8.0	0.8–6.4	0.8–8.0
Molar absorptivity(l mol^{-1} cm^{-1})	3.03×10^4	8.02×10^4	4.91×10^4	3.99×10^4	8.85×10^4	5.15×10^4
Sandell's sensitivity (ng/cm^2)	8.682	4.262	6.605	6.611	3.862	6.298
Correlation coefficient (r)	0.9994	1.000	0.9989	0.9993	1.000	0.9988
Linear regression equation						
$S_{y/x}$	0.0111	3.21×10^{-3}	0.0105	9.41×10^{-3}	2.19×10^{-3}	0.0115
Intercept (a)	0.0795	-0.1195	0.0880	0.0716	0.1566	0.1234
Slope (b)	0.1105	0.2692	0.1382	0.1506	0.1839	0.1446
SD of slope (S_b)	1.78×10^{-3}	1.92×10^{-3}	3.13×10^{-3}	2.81×10^{-3}	6.56×10^{-3}	3.43×10^{-3}
SD of intercept (S_a)	0.0111	3.70×10^{-3}	9.77×10^{-3}	8.76×10^{-3}	2.04×10^{-3}	0.0107
Detection limits (µg/ml)	0.0483	0.0213	0.0679	0.0559	0.1070	0.0711
Quantitation limits (µg/ml)	0.1611	0.0713	0.2264	0.1865	0.3567	0.2372

A = a+bC, where A is the absorbance and C is the concentration of drug in µg/ml.

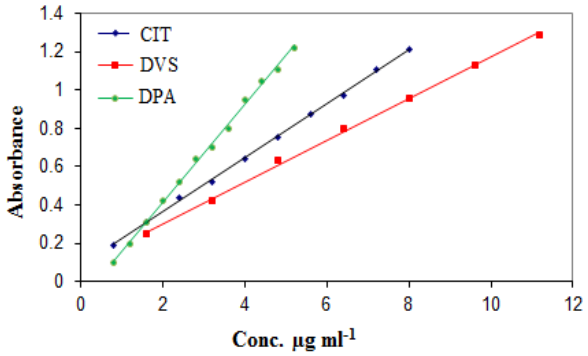


Fig. 7: Calibration curve of ion-pair complexes of DSV, DAP and CIT with BPB against blank

Accuracy and precision

In order to determine the accuracy and precision of the recommended procedure five replicate determinations at three different concentrations of the studied drugs were carried out. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively and indicated that the proposed method is highly accurate and reproducible (tables 2, 3).

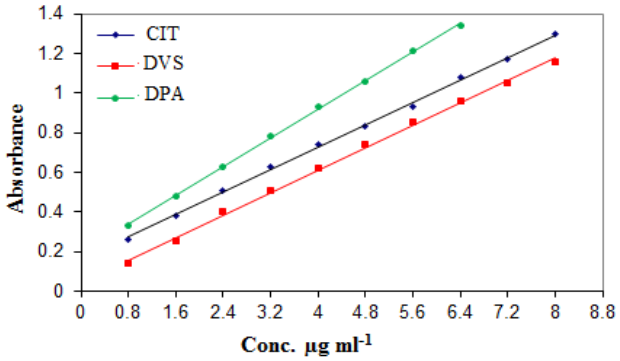


Fig. 8: Calibration curve of ion-pair complexes of DSV, DAP and CIT with BTB against blank

Table 2: Evaluation of accuracy and precision of the proposed method using BPB

Drugs	Drug taken µg/ml	Drug found µg/ml	Recovery[a], %	RE[b]	RSD[c], %	SE
DSV	3.2	3.198	99.96	-0.041	6.471	0.0138
	6.4	6.397	99.96	-0.040	1.926	7.9×10^{-3}
	9.6	9.596	99.96	-0.042	1.876	0.011
DAP	3.2	6.471	99.96	-0.041	3.198	0.0138
	6.4	1.926	99.96	-0.041	6.397	7.9×10^{-3}
	9.6	1.876	99.96	-0.041	9.596	0.011
CIT	1.6	1.599	99.96	-0.043	5.888	0.011
	4	3.997	99.94	-0.061	0.906	2.7×10^{-3}
	6.4	6.397	99.96	-0.041	0.572	2.7×10^{-3}

[a]Mean value of five determinations., [b]Relative error., [c]Relative standard deviation.

Analysis of dosage forms

To evaluate the validity and reproducibility of the methods, known amounts of the DSV, DAP and CIT drugs were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The percent recoveries are given in (Tables 4, 5). Interference studies revealed that the common excipients and other additives such as lactose, starch, gelatin, talc and magnesium trisilicate, that are usually present in the tablet dosage forms did not interfere at their regularly added levels.

Table 3: Evaluation of accuracy and precision of the proposed method using BTB

Drugs	Drug taken µg/ml	Drug found µg/ml	Recovery[a], %	RE[b]	RSD[c], %	SE
DVS	1.6	1.599	99.94	-0.062	4.609	6.5×10^{-3}
	4.8	4.796	99.92	-0.081	2.338	9.1×10^{-3}
	6.4	6.396	99.94	-0.061	1.310	6.5×10^{-3}
DAP	1.6	1.5992	99.95	-0.050	2.469	5.7×10^{-3}
	3.2	3.1992	99.97	-0.025	2.684	0.0118
	4.8	4.7976	99.95	-0.050	1.272	7.2×10^{-3}
CIT	1.6	1.599	99.94	-0.062	3.714	0.011
	4.0	3.998	99.96	-0.041	2.982	0.010
	6.4	6.397	99.96	-0.040	2.101	0.011

[a]Mean value of five determinations., [b]Relative error., [c]Relative standard deviation.

Table 4: Determination of the studied drugs in pharmaceutical preparation using BPB

Drug formulations	Drug taken µg/ml	Drug found µg/ml	Recovery[a], %	RE[b]	RSD[c], %
DVS	1.6	1.599	99.98	-0.025	1.112
(Prismaven, 100 mg)[d]	4.0	3.997	99.94	-0.06	1.963
	6.4	6.396	99.94	-0.061	2.268
DAP	1.6	1.599	99.97	-0.025	2.206
(Joypox 60 mg)[e]	3.2	3.198	99.95	-0.050	2.105
	4.8	4.798	99.97	-0.031	2.416
CIT	3.2	3.197	99.92	-0.081	1.961
(Cipramax, 40 mg)[f]	6.4	6.397	99.96	-0.041	2.152
	9.6	9.594	99.94	-0.060	1.273

[a]Mean value of five determinations, [b]Relative error, [c]Relative standard deviation, [d,e]tength of ramdan for pharmaceutical industries, Egypt, [f]copad egypt for trade and pharmaceutical industries, Egypt

Table 5: Determination of the studied drugs in pharmaceutical preparation using BTB

Drug formulations	Drug taken µg/ml	Drug found µg/ml	Recovery[a], %	RE[b]	RSD[c], %
DVS	1.6	1.598	99.92	-0.081	3.865
(Prismaven, 100 mg)[d]	4.8	4.799	99.98	-0.021	1.152
	6.4	6.397	99.96	-0.040	1.550
DAP	1.6	1.599	99.97	-0.025	1.814
(Joypox, 60 mg)[e]	3.2	3.198	99.95	-0.050	2.521
	4.8	4.797	99.95	-0.050	2.029
CIT	1.6	1.599	99.98	-0.025	1.724
(Cipramax, 40 mg)[f]	4.0	3.998	99.96	-0.040	1.772
	6.4	6.399	99.99	-0.011	1.573

[a]Mean value of five determinations., [b]Relative error., [c]Relative standard deviation., [d,e]tength of ramdan for pharmaceutical industries, Egypt., [f]copad egypt for trade and pharmaceutical industries, Egypt.

CONCLUSION

The proposed method is rapid, simple, accurate and in addition, offer advantages in the wide applicability of the new method for routine quality control is well established by the assay of DSV, DAP and CIT in pure form and in pharmaceutical preparations. Moreover, the method is free from interference by common additives and excipients. The reagents utilized in the proposed method are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation.

CONFLICT OF INTERESTS

Declare none

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