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Application of Methyl Orange in the Spectrophotometric Determination of Citalopram and Dapotextine in Pharmaceutical Formulations

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method has been proposed for the assay of citalopram (CIT) and dapotextine (DPT) in bulk and pharmaceutical formulations. The method based on the reaction of the selected drugs with methyl orange (MO) in buffered aqueous solution at pH = 5.0 and pH = 3.0 for CIT and DPT, respectively. The formed yellow ion-pair complexes were extracted with dichloromethane and their absorbance was measured at 422 nm. The best conditions of the reaction were studied and optimized. The extracts are intensely colored and very stable at room temperature. The calibration graphs were linear over the concentration range of 1.2 – 4.8 µg/ml for CIT and 0.4 – 4.0 µg/ml for DPT. The drug–dye stoichiometric ratio as determined by the Job's method was found to be 1:1. The proposed method was successfully applied for determination of the drugs in tablets with good accuracy and precision. Excipients used as additive in commercial formulations did not interfere in the analysis. The proposed method can be recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical technique are of great importance.

INTRODUCTION

Citalopram, (CIT, Fig. 1a) 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1, 3-dihydroisobezofuran-5-carbonitrile (Budavari, 2007), 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 (Brunton *et al*., 2008; Sweetman, 2005). 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 (Olesen and Linnet, 1996; Duverneuil *et al*., 2003; Frahnert *et al*., 2003), HPLC with fluorescence detectors (Matsui *et al*., 1995; Rochat *et al*., 1995; Meng and Gauthier, 2005), HLPC/mass spectrometry (Juan *et al*., 2005; Gutteck and Rensch, 2003; Kollroser and Schober, 2003), gas chromatography (Gergov *et al*., 2000; Sane *et al*., 2002), electrophoretic methods (Flores *et al*., 2004; Buzinkaiova and Polonsky, 2000; Bjorhovde *et al*., 2003) and spectrophotometric (Asad, 2006; Asad *et al*., 2008; Badiadka and Kunnummel, 2010) methods. However, few spectrofluorimetric (Vasantharaju *et al*., 2008; Satana *et al*., 2007; El-Sherbiny, 2006) methods have been reported in the literature and most reported methods involve multistep procedures and have poor selectivity's and sensitivities. Dapoxetine HCl (DPT) 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) (Dresser *et al*., 2006). SSRI's are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders. 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 (McMahon *et al*., 2011).

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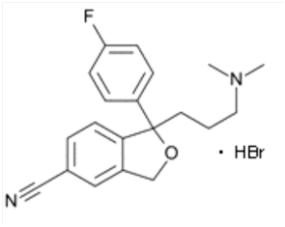
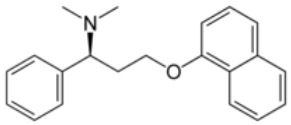
Its structural formula is given in the Fig. 1b. The literature study reveals that several UV spectrometric and chromatographic methods available for dapoxetine in combined tablet formulation (Kim *et al*., 2013; Abirami *et al*., 2012; Giri *et al*., 2012; Banik 2014; Kolsure and Hiremath, 2015; Mehta *et al*., 2011; Nataraj *et al*., 2011; Rohith and Ananda, 2012).

The aim of this study was to develop a sensitive and accurate spectrophotometric method for determination of CIT and DPT in raw material and pharmaceutical dosage forms through ion-pair complex formation between the drugs and methyl orange. The reaction conditions and the application of the method are presented. The constructed calibration curves were utilized in determining the concentration of these drugs in different pharmaceutical preparations available.



A-CIT



B-DPT

Fig. **1**: Chemical structure of the studied drugs.

MATERIAL AND METHODS

Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Visible spectro-photometer (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. Double distilled water was used to prepare all solutions.

i. Stock solutions of pure CIT and DPT were prepared separately by dissolving 20 mg in a 100 ml calibrated flask. Working solutions of lower
ii. A 1×10^{-3} M of methyl orange was prepared by dissolving the accurate weighed amount of 32.73 mg in 100 ml water.
iii. Commercial dosage forms of CIT (cipramax 40mg, copad egypt for trade and pharmaceutical industries) and DPT (joypox 60 mg, 10^{th} of Ramadan, Egypt).
iv. 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.

Construction of Calibration Curves

Into a series of separating funnels, accurately measured aliquots of CIT or DPT in the concentration range shown in (Table 1) were pitted out. Then, 1.0 ml of 1×10^{-3} M of MO, 2.0 ml of buffer solution of pH as recorded in Table 1 was added. The solution was diluted to 10 ml with water. The mixture was extracted with 10 ml dichloromethane by shaking for 2.0 min and then allowed to stand for clear separation of the two phases and organic layer was passed through anhydrous sodium sulphate. The absorbance of yellow colored ion – pair complexes was measured at 422 nm against reagent blank prepared in the same manner except addition of drugs. All measurements were made at room temperature (25±2 ◦C) and calibration plots were drawn to calculate the amount of drugs in unknown analyte samples.

Procedure for Tablets

At least ten tablets of the drugs were weight into a small dish, powdered and mixed well. A portion equivalent to 20 mg of CIT or DPT 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 analysis.

RESULTS AND DISCUSSION

Extractive spectrophotometric procedures due to their sensitivity are widely used in the assay of drugs and hence, ion-pair extractive spectrophotometry has received a considerable attention for the quantitative determination of many pharmaceutical compounds (El-Didamony *et al*., 2015; El-Didamony and Shehata, 2014; Swamy and Basavaiah, 2013). CIT and DTP react with MO in acidic buffer to give dichloromethane soluble ion-pair complexes, which exhibit absorption maxima at 422 nm (Fig. 2). Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for the quantitative determination of CIT and DPT.

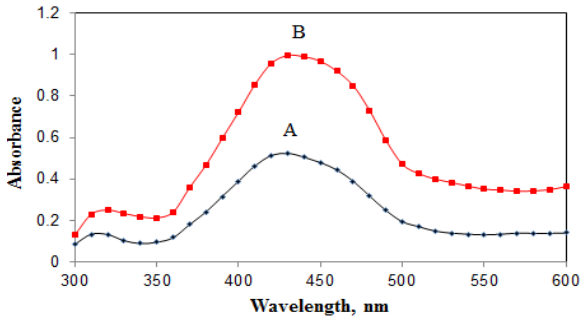


Fig. **2**: Absorption spectra of ion-pair complexes of A-(2.4 µg/ml) CIT-MO and B- (3.2 µg/ml) DPT-MO.

Optimization of Reaction Conditions

The optimization of the method was carefully performed to achieve complete ion-pair complex formation, quantitative extraction of the ion-pair complex and maximum sensitivity. For the ion-pair complex formation found by preliminary experiments, reaction conditions such as pH, type of buffer and organic solvent, volume of the dye, and shaking time were optimized.

Effect of 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 3.4-5.6). It was noticed that the maximum absorbances and reproducible results were observed in NaOAc-HCl of pH 5.0 and pH 3.0 for CIT and DPT, respectively, (Fig. 3). The volume of buffer solution added was studied and adding 2.0 ml buffer solution attained complete color development. For the highest color intensity and maximum absorbance, the buffer solution should be added after mixing the drug–dye solution at neutral pH.

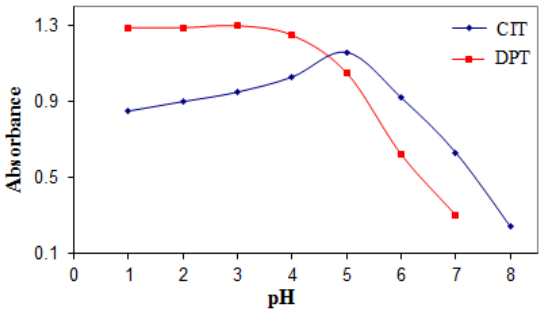


Fig. 3: Effect of pH of buffer solution on ion-pair complex formation between MO, CIT and DPT.

Choice of Organic Solvent

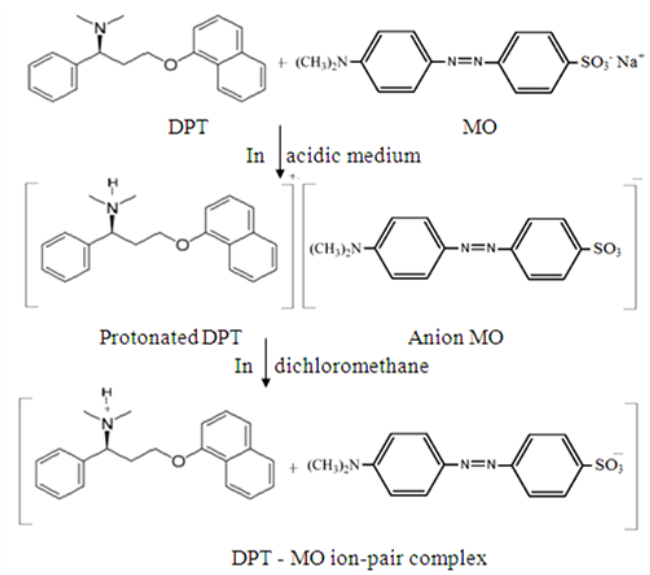
Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained highest absorbance with dichloromethane. It was also observed that only one extraction was adequate to achieve a quantitative recovery of the complexes and the shortest time to reach the equilibrium between both phases. Shaking time of 0.5 – 5 min provided constant absorbance and hence, 2.0 min was selected as the optimum shaking time.

Effect of Dye Concentration

The effect of the dye concentration on the intensity of the color developed at the selected wavelength and constant drugs concentration was tested using different volumes of methyl orange (0.5-3 ml). It was observed that 1.0 ml of 1×10^{-3} MO was necessary for maximum color development of the ion-pair complexes. After this volume, the absorbance remains constant by increasing the volume of the reagent.

Composition of the Ion-pair Complexes

The composition of ion-pair complexes was established by applying Job's method of continuous variations (Job, 1928). The method is simple and widely used for elucidating the composition of complexes and is based on the variation of both the drug and the reagent (MO) of equal molar concentrations, keeping the total volume of the drug and the reagent constant. The plot reached a maximum value at a mole fraction of 0.5 (Fig. 4), which indicated that a 1: 1 (drug: MO) ion-pairs are formed through the electrostatic attraction between positive protonated drugs and methyl orange anions. The suggested mechanism for the reaction product of DPT – MO ion-pair complex formation for example, is given in Scheme 1.



Scheme 1: Suggested mechanism of DPT-MO ion-pair complex formation.

Stability of the Ion-pair Complexes

The stability of the ion-pair complexes formed between the studied drugs and MO was evaluated. Although the ion-pairs were obtained instantaneously, constant absorbance readings were obtained after not less than 20 min of standing at room temperature (25±2 °C). Ion-pairs were stable for at least 6 h without any change in color intensity or in λ_{max}.

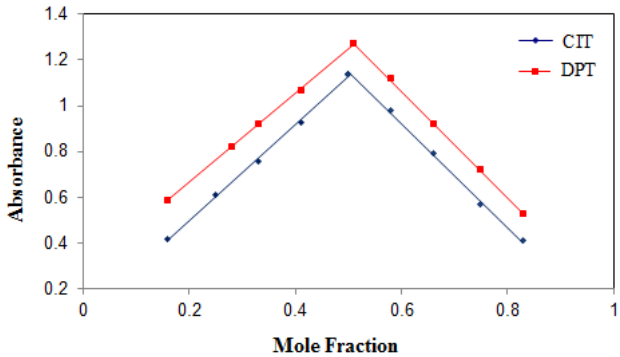


Fig. 4: Job's method of continuous variation graph for the reaction of CIT and DPT with MO, (drug) = (dye) = 5×10^{-4} M.

Effect of Interferences

In order to evaluate the selectivity of the proposed method for the analysis pharmaceutical formulations, the effects of the presence of excipients and additives, which can occur in real samples, were investigated. It was found that the presence of the common excipients of tablets such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate did not interfere with the determination of the studied drugs at the levels normally found in dosage forms.

Linearity and Range

The Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, slope, intercept and correlation coefficient determined for each drug are given in Table 1. A linear relationship was found between the absorbance and the concentration of each drug in the range of 1.2-4.8 µg/ml for CIT and 0.4-4.0 µg/ml for DPT. The correlation coefficients were 0.9988 and 0.9997 for CIT and DPT, respectively, indicating good linearity (Fig. 5).

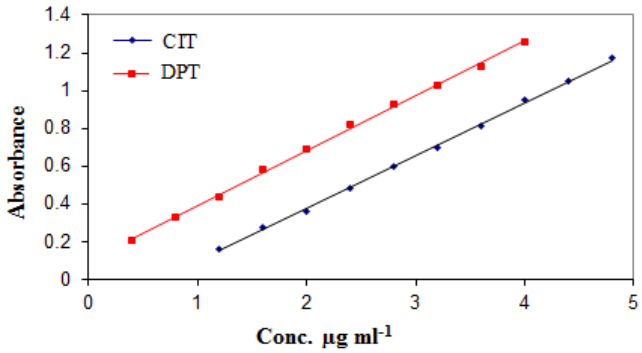


Fig. 5: Calibration curve of ion-pair complexes of CIT and DPT with MO against blank.

Detection and Quantification Limits

The detection and quantification limits as defined by IUPAC (Winefordner and Long, 1983), LOD $=3S_b/m$ and LOQ $=10S_b/m$ (where S_b is the standard deviation of the blank and m is the slope of the calibration graph) were found to be 0.0723 and 0.2416 µg/ml for CIT, 0.0362 and 0.1207 µg/ml for DPT (Table 1). The slope of the calibration graph (m) is the calibration sensitivity according to IUPAC definition.

Table 1: Statistical data of the regression equations for determination of CIT and DPT with MO.

Parameters	CIT	DPT
pH	5.0	3.0
Beer's law limit (µg/ml)	1.2-4.8	0.4-4.0
Molar absorptivity(l mol-1cm-1)	8.04×10^4	10.77×10^4
Sandell's sensitivity (ng/cm2)	4.033	3.174
Correlation coefficient (r)	0.9988	0.9997
Linear regression equation		
Sy/x	0.0109	6.16×10^{-3}
Intercept (a)	-0.164	0.0847
Slope (b)	0.2701	0.3049
S.D of slope (Sb)	6.51×10^{-3}	3.68×10^{-3}
S.D. of intercept (Sa)	0.015	5.73×10^{-3}
Detection Limits (µg/ml)	0.0723	0.0362
Quantitation Limits (µg/ml)	0.2416	0.1207

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

Accuracy and Precision

The validity of the method for the analysis of selected drugs in its pure form and in its pharmaceutical formulations was examined by analyzing the sample using the proposed method. In order to determine the accuracy and precision of the proposed method, solution containing three different concentrations of the studied drugs were prepared and analyzed in six replicates. The analytical results obtained for this investigation are summarized in Table 2. The low values of percent relative standard deviation (RSD, %) indicate good precision and reproducibility of the proposed method.

Table 2: Evaluation of accuracy and precision of the proposed method.

Drugs	Drug Taken µg/ml	Drug Found µg/ml	Recovery[a], %	RE[b]	RSD[c], %	SE
CIT	1.6	1.599	99.94	-0.062	3.851	5.7×10^{-3}
	3.2	3.198	99.96	-0.041	1.552	5.4×10^{-3}
	4.8	4.799	99.98	-0.021	2.562	0.150
DPT	0.8	0.799	99.96	-0.050	1.428	0.016
	2.0	1.999	99.96	-0.041	2.517	0.027
	3.2	3.199	99.98	-0.022	3.145	0.016

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

Tablets Analysis

The proposed method was successfully applied to the determination of CIT and DPT in their commercially tablets. The results were reproducible with low RSD values. The average percent recoveries obtained were quantitative (99.94-99.96), indicating good accuracy of the method (Table 3). The results of analysis of the commercial tablets and the recovery study of drugs suggested that there is no interference from any excipients (such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate), which are present in tablets.

Table 3: Analysis of CIT and DPT in tablets using the proposed method.

Drug formulations	Drug Taken µg ml^{-1}	Drug Found µg ml^{-1}	Recovery[c], %	RE[d]	RSD[e], %
CIT	1.6	1.5993	99.96	-0.043	3.585
(Cipramax, 40 mg)[a]	3.2	3.198	99.94	-0.062	3.928
	4.8	4.798	99.96	-0.042	2.798
DPT	0.8	0.799	99.96	-0.050	2.878
(Joypox, 60 mg)[b]	2.0	1.999	99.96	-0.040	1.878
	3.2	3.198	99.96	-0.041	2.618

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

CONCLUSION

The proposed method is simple, precise, accurate and convenient. Therefore, it can be useful for routine analyses and quality control assay of the examined drugs in raw material and in tablets without fear of interference caused by the excipients expected to be present in tablets. This is for the first time that visible spectrophotometric method is being reported for the assay of DPT. The spectrophotometric method can be applied routinely because it does not require high cost reagents and equipment when it is compared with HPLC analysis.

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