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Original Research Article

Facile Colorimetric Determination of Duloxetine in Formulations Using Methyl Orange as Ion-Pairing Agent

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Abstract

Purpose: To develop a new and fully validated ion-pair spectrophotometric method for the determination of duloxetine hydrochloride (DX).**Methods:** Ion-pair spectrophotometric method was employed for the determination of duloxetine hydrochloride (DX) in bulk and pharmaceutical formulations using acidic dye methyl orange (MO) as ion-pairing agent at pH 4 (phthalate buffer). The yellow ion-pair complex was extracted with chloroform and spectrophotometrically estimated at 420 nm. The developed method was validated according to ICH and USP guidelines.**Results:** The ion-pair complex of DX and MO obeyed Beer's law in the range of 2 - 20 $\mu\text{g mL}^{-1}$ of DX with a correlation coefficient of 0.998. Recovery was good, with a relative standard deviation (%RSD) of 0.88 - 1.02; precision (inter-day, 0.878 and intra-day, 0.921) was also within validation limits. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.25 and 4 $\mu\text{g mL}^{-1}$, respectively. The method developed was successfully applied to determine DX in a formulation.**Conclusion:** The developed method is accurate, precise, rugged, robust and reproducible. It is also sensitive and specific for the determination of DX in bulk and formulation.**Keywords:** Duloxetine, Methyl orange, Ion-pair, Validation, Spectrophotometry

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INTRODUCTION

Duloxetine hydrochloride (DX) is chemically ((+)-(S)-N-methyl-3-(1-naphthalenyloxy)-2-thiophene propanamine) hydrochloride (Fig 1.). Recently, DX was approved by United States Food and Drug Administration (US FDA) as a balanced selective serotonin and nor-epinephrine re-uptake inhibitor and is used in the treatment of major depressive disorders and diabetic peripheral neuropathic pain [1-3].

The literature on analytical methods for DX revealed that it has been determined by high

performance liquid chromatography (HPLC) [4-6], high performance thin layer chromatography

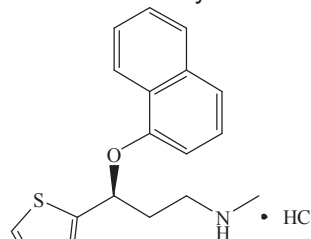


Figure 1: Structure of DX

(HPTLC) [7,8], liquid chromatography-tandem mass spectroscopy (LC-MSMS) [9], ultraviolet (UV) spectrophotometric [10] and colorimetric

methods [11,12]. Recently extractive colorimetric methods using bromocresol green, bromothymol blue and bromophenol blue as ion-pairing chromogens was studied but only partially validated [11,12]. In general, extractive colorimetric procedures are popular for their sensitivity and selectivity towards the active component of interest [13,14]. Due to the ease of this technique, it can be considered for the quantitative determination of many pharmaceuticals.

To the best of our knowledge, there are no previous reports on the determination of DX using methyl orange (MO) as ion-pairing agent. Therefore, in the present study, an ion-pair extractive spectrophotometric quantitative analysis of DX using MO was undertaken. The main aim was to develop a simple, accurate, precise extractive colorimetric method for the determination of DX and validate its suitability for assaying the DX content of formulations according to the requirements of United States Pharmacopeia (USP) and International Conference on Harmonization (ICH) guidelines for method validation [15,16].

EXPERIMENTAL

Chemicals and reagents

All chemicals were of analytical reagent grade procured from Daejung Chemicals & Metals, Gyeonggi-do, South Korea. Doubly distilled water was used to prepare all solutions. Freshly prepared solutions were used for method development and validation. Potassium hydrogen phthalate buffer solution (pH 4) was prepared by standard procedure. Methyl orange (MO, 0.1 %w/v) was prepared in hot water. Standard duloxetine hydrochloride (DX) was obtained from Sigma Aldrich and tablets containing 60 mg duloxetine (Cymbalta, Eli Lilly and Company) were purchased from a retail pharmacy.

Instrumentation

A Shimadzu UV mini-1240 UV-Visible spectrophotometer with 1 cm quartz cells was used for all spectral measurements with Shimadzu UV Probe system software (version 2.1). pH measurements were carried out using a calibrated digital pH meter (Neomet pH-200L, Seoul, South Korea).

Standard solution of the drug

A stock solution of 1 mg mL⁻¹ was prepared by dissolving DX in alcohol (99 %). Working

standards were prepared by suitable dilution of the standard stock solution.

Sample preparation

From the 100 µg mL⁻¹ working standard solution, aliquots were transferred to a series of 100 ml separating funnels and 1 mL of buffer (pH 4) and 1 mL of 0.1 %w/v MO was added to each separating funnel and shaken well followed by the addition of 10 mL of chloroform. The contents were shaken well and kept aside to allow for separation. The chloroform layer was separated and passed through previously dried anhydrous sodium sulphate to remove the water in the organic layer.

Determination of maximum absorbance (λ_{max}) and linearity

Absorption spectrum of the yellow DX-MO ion-pair complex was obtained by scanning the chromogen extracted using chloroform from 350 - 600 nm. To determine Beer's law limit, a calibration curve was constructed by plotting absorbance against concentration.

Validation of the method

For routine use of the method, optimization was carried out for rapid and quantitative formation of colored ion-pair complexes by a number of preliminary experiments. USP [15] and ICH [16] guidelines were followed for method validation.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) of the method was established using the formula: LOD or LOQ = κ SD a/b , where κ = 3 for LOD and 10 for LOQ, while SD is standard deviation with intercept (a) and slope (b) of the standard graph.

Precision

Intra-day precision was calculated by estimating the %RSD of DX solution (12 µg mL⁻¹) at various time intervals (5 times) on the same day, while the inter-day precision was calculated by estimating the %RSD of DX solution (12 µg mL⁻¹) on five consecutive days

Accuracy

Accuracy expresses the agreement between the established value and the true value. This was achieved by recovery studies, i.e., spiking a known quantity of pure drug to a pre-analyzed sample and the proposed analysis procedure was followed.

Application of the developed method to formulations

Twenty commercial DX tablets were weighed and the mean tablet weight was calculated before they were ground to fine powder. A known quantity of the powder was accurately weighed and transferred into a 50 mL volumetric flask. The volume was made up to mark with alcohol, shaken well and filtered through Whatman filter paper no. 40. Suitable aliquots of this solution were taken for the assay of DX.

Interference and placebo study

Studies on interference by common excipients that might be used in formulations were carried out by mixing a known amount of DX (60 mg) with specified amounts of the common excipients such as lactose, starch and talc in their recommended proportions [15]. The analysis of these mixtures was carried out using the above procedure and the recovery values for DX were determined. Likewise, the mixture of above excipients was prepared but without the drug, and the same procedure was used for analysis.

Robustness and ruggedness

Robustness (effect of deliberate change in analytical parameters) was studied by determining the amount of DX in the tablets by slightly varying the wavelength of determination and the dye's (MO) concentration. Ruggedness was established by determining DX in the tablet formulation using two different spectrophotometer Shimadzu UV mini-1240 (system I) and SCINCO, Neosys-2000 DRS-UV provided with liquid sample analysis port (system II) and two different analysts (I and II). The results obtained were within the recommended % RSD limit (< 2%).

Study of bench top stability of chromogen

To study the stability of chromogen, a specified quantity of stock solution (1 mg mL^{-1}) was mixed with the above standardized quantity of buffer and MO, kept aside (10 min) for reaction and extracted with chloroform.

RESULTS

Solvent and buffer

From the trials, potassium hydrogen phthalate buffer was found to be suitable and chloroform was chosen as the most suitable solvent for extraction among those tested which included carbon tetrachloride, dichloromethane and

diethyl ether. The suitability of chloroform for extraction of ion-pair has also been reported by other researchers [11-14]. A volume of 1 mL of MO (0.1 %w/v) was found to be optimal for complete complexation.

Maximum absorbance (λ_{max})

The full scan spectra of DX-MO complex showed maximum absorbance at 420 nm (Fig 2a). Figure 2b indicates that the formed complex obeyed the Beer's law in the range of 2 - 20 $\mu\text{g mL}^{-1}$. The regression equation for the results was $y = 0.0515 x - 0.1052$ ($r = 0.9987$). The molar absorptivity (ϵ) was $1.3967 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity $0.02390 \mu\text{g cm}^2/0.001 \text{ abs unit}$.

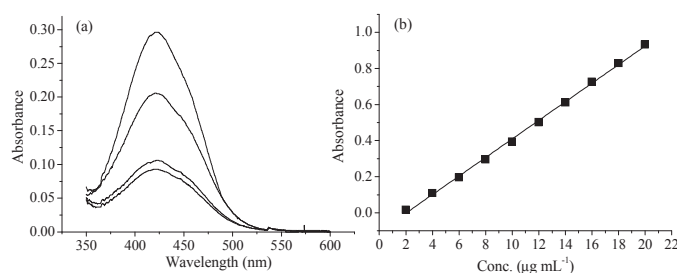


Figure 2: (a) Overlay spectra of DX-MO ion-pair complex (b) Linearity plot

Bench top stability of chromogen

The absorbance of the chromogen from the time of extraction (considered as 0 min) to various time intervals was determined and the results plotted as absorbance against time (plot not shown). The plot shows that the chromogen was stable up to 3.5 h.

Stoichiometry of the reaction

The drug-dye (DX-MO) stoichiometric ratio was calculated by Job's continuous variation method and it was found that DX and MO formed a 1:1 complex [17].

LOD and LOQ

LOD and LOQ were 0.25 and $4 \mu\text{g mL}^{-1}$, respectively. The low values indicate the high sensitivity of the proposed method.

Assay

The assay results for the marketed formulation (tablets) by the proposed and UV (standard) methods [10] were 99.95 and 99.98 %, respectively, of the label claim. No significant differences were found between the calculated and theoretical values, based on t - and F -tests at

Table 1: Assay data

Label claim (mg/tab)	% Amount ^a		% RSD ^a		Confidence	
	Proposed	Reported	Proposed	Reported	t-test ^b	F-test ^b
60	99.95	99.98	0.18	0.20	1.4	2.9

^a Mean of five determinations; ^b The tabulated values of t and F at 95% confidence limit are 2.67 and 6.02 respectively

Table 2: Precision and accuracy

Conc. ($\mu\text{g mL}^{-1}$)	Precision		Accuracy		
	Inter-day	Intra-day	% Spike level	% Recovery ^a	%RSD ^a
	% RSD ^a	% RSD ^a	75	100.1	1.02
12	0.878	0.921	100	99.83	0.88
			125	99.93	0.97

^a Mean of five determinations

95% confidence level, which proves that the proposed method is comparable with that of reference (standard) method (Table 1).

Precision and accuracy of the method

The results obtained for precision of the method are given in Table 2. The relative standard deviation (%RSD) was low, being 0.878 and 0.921 % for inter- and intra-day precision, respectively, and thus indicates high precision (repeatability) of the method. Recovery data are also presented in Table 2. Mean %RSD determined at three levels was in the range 0.88 - 1.02 %, indicating good accuracy of the proposed method.

Effect of MO concentration and volume

The effect of concentration of MO was studied by measuring the absorbance of solutions containing DX ($12 \mu\text{g mL}^{-1}$), 1 mL of buffer and 1 mL of MO solution at various concentration (0.05 - 0.175 %w/v). The results are depicted in Fig 3a. MO concentration of 0.1 %w/v gave maximum absorbance and it was therefore chosen as most suitable for complexation.

The effect of the volume of MO was studied by varying the volume of the added dye (0.5 - 2 mL) while maintaining dye concentration at 0.1% w/v and drug concentration at $12 \mu\text{g mL}^{-1}$ (Fig 3b). The results show that 1 mL of 0.1 %w/v MO was enough to form complex as it produced maximum absorbance. Larger volumes of reagent had no marked effect on ion-pair complex formation.

Effect of pH

The effect of pH was studied by analyzing a solution containing DX ($12 \mu\text{g mL}^{-1}$), 1 mL of 0.1

%w/v MO and 1 mL of buffer of varying pH of 3.8 - 4.2. The results showed that 1 mL of buffer with pH 4 was suitable for the determination of DX.

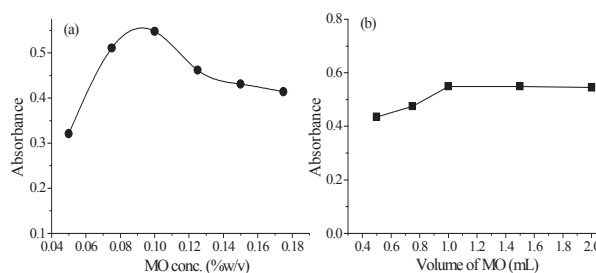


Figure 3: Plot of absorbance vs. (a) MO concentration and (b) MO volume

Interference and placebo influence

The effect of excipient (lactose, starch and talc) interference with the proposed method showed that recovery of the drug was 100.09 ± 0.78 , 99.95 ± 0.60 and 99.58 ± 0.50 , respectively. Since recovery of the drug was high, it therefore means that the added excipients had no significant effect on the assay. Absence of color in the extract obtained from the placebo indicates the selectivity of the method.

Robustness and ruggedness

As Table 3 shows, the results obtained were within the recommended limits for %RSD (< 2%).

DISCUSSION

Anionic MO forms an ion-association complex with the positively charged quaternary amine of DX. The DX-MO complex is a pair of two oppositely charged ions of single unit held together by an electrostatic force of attraction.

Table 3: Robustness and ruggedness of the developed method

Wavelength (nm)	Robustness			Analyst	Ruggedness		
	%RSD ^a	MO conc. (%w/v)	%RSD ^a		%RSD ^a	System	%RSD ^a
419	0.342	0.09	0.546	I	0.125	I	0.156
421	0.421	0.11	0.798	II	0.752	II	0.963

^a Mean of five determinations

The formed yellow ion-pair complex showed maximum absorbance at 420 nm and obeyed.

Beer's law in the concentration range of 2 - 20 µg mL⁻¹ with a very good correlation co-efficient of 0.9987. The developed method was validated for its accuracy, precision, stability, ruggedness and robustness. All the studied parameters were within limits for validation of analytical methods, as prescribed in USP and ICH guidelines with respect to %RSD. Furthermore, the interference study showed that the method was not affected by common excipients used in the study. The proposed method, therefore, is in good agreement with previously reported methods.

CONCLUSION

The developed ion-pair extractive colorimetric method for the determination of DX in bulk and in tablet formulation is sensitive, specific and rapid. The method also showed good accuracy and was selective for the drug. Since the developed method utilizes simple reagents, it should easily be affordable by most analytical laboratories. The developed method is thus recommended for routine determination of DX in tablet formulations validation.

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