

## UTILISATION OF OXIDATIVE COUPLING REACTIONS FOR THE ESTIMATION OF DROTAVARINE

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### ABSTRACT

Three simple spectrophotometric methods (A–C) for the assay of Drotavarine (DRT) in pure state and in formulations have been developed based on the oxidative coupling reaction of DRT, with N,N-dimethylamino-para-phenylenediamine (DMPD) in the presence of chloramine-T (CAT) (Method A), 4-aminophenazone (4-AP) in the presence of  $\text{IO}_4^-$  (Method B) and 3-methyl benzothiazolinone hydrazone (MBTH) in the presence of cerium (IV) (Method C). Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 4.0–20.0  $\mu\text{g/mL}$ , 4.0–20.0  $\mu\text{g/mL}$  and 10.0–50.0  $\mu\text{g/mL}$  for methods A, B and C, respectively. The results of analysis have been validated statistically and by recovery studies.

**Key word :** Drotavarine, Oxidative coupling

### INTRODUCTION

DRT is an isoquinoline antispasmodic agent for oral administration and chemically known as 1- [(3,4-Diethoxyphenyl)methylene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline; 1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline. A few numbers of methods such as HPLC<sup>2–5</sup>, spectrophotometry<sup>6–8</sup> TLC<sup>9</sup>, ion exchange<sup>10</sup> and GC<sup>11,12</sup> were reported for the estimation of DRT. Literature survey revealed that visible spectrophotometric methods are not reported for its quantitative determination in bulk drug and pharmaceutical formulations. Three visible spectrophotometric methods (A, B and C) based on the oxidative coupling reaction of DRT with the reagents such as DMPD-CAT (Method A), 4-AP –  $\text{IO}_4^-$  (Method B) and MBTH-Ce (IV) (Method C) have been developed. All the methods are applicable to the determination of DRT in bulk form and in formulations.

### EXPERIMENTAL

**Instruments :** Spectral and absorbance measurements were made on Systronics UV-Visible Spectrophotometer 117 with 10 mm matched quartz cells. An Elico LI-120 digital pH meter was used for pH measurements.

**Reagents :** All the chemicals used were of analytical grade. All the solutions were prepared fresh in doubly distilled water.

Aqueous solutions of  $2.39 \times 10^{-3}$  M DMPD (Merck) and  $7.11 \times 10^{-4}$  M CAT (Loba) were prepared for method A. Aqueous solutions of  $2.46 \times 10^{-2}$  M 4-AP (Ferack) and  $4.68 \times 10^{-3}$  M  $\text{IO}_4^-$  (BDH) were prepared for method B. Aqueous solutions of  $8.58 \times 10^{-3}$  M MBTH (Loba) and  $1.58 \times 10^{-2}$  M ceric ammonium sulphate in 0.36 N  $\text{H}_2\text{SO}_4$  (BDH) were prepared for method C.

**Preparation of drug solutions :** Twenty-five mg of DRT was boiled with 10 mL of 5N HCl in the flask, cooled and the excess of HCl was removed under vacuum. The residue was dissolved in distilled water and made up to 50 mL with the same solvent to get a stock solution of 500  $\mu\text{g/mL}$  of hydrolyzed DRT (HDRT). Stock solution was diluted stepwise with distilled water to obtain the working standard solutions of concentrations 200  $\mu\text{g/mL}$  for method A–C. Sample solutions for formulations (tablet or injection) were prepared exactly in the same manner as given under the standard solutions with prior filtration before making up to volume and analyzed as described for pure samples.

#### Method A

Aliquots of DRT solution (0.5 – 2.0 mL, 200  $\mu\text{g/mL}$ ) were transferred into a series of 25 mL calibrated tubes. Then 1.0 mL of DMPD solution and 1 mL of CAT solution were added and the tubes were kept aside for 15 min. The solutions in each tube were made up to the mark with methanol. The absorbance was measured at 520 nm against a reagent blank prepared in a similar way. The amount of DRT was computed from its calibration graph.

#### Method B

Aliquots of standard DRT solution (0.5–2.5 mL, 200  $\mu\text{g/mL}$ ) were transferred into a series of 25 mL calibrated tubes. Then 2.0 mL of 4-AP and 5.0 mL of  $\text{NaIO}_4$  solutions were added and kept aside for 5 min. The volume was made up to the mark with distilled water. The absorbance was measured at 530 nm against a similar reagent blank. The amount of DRT was computed from its calibration graph.

#### Method C

Aliquots of standard DRT solution (0.5 – 2.5 mL, 200  $\mu\text{g/mL}$ ) were transferred into a series of 10 mL calibrated tubes. The total volume in each tube was brought to 3.0 mL with distilled water. One mL each of MBTH and ceric ammonium sulfate were added and the tubes were kept aside for 5 min. at room temperature. The solutions in each tube were made up to mark with distilled water and the absorbances were measured after 5 min. at 640 nm. against a reagent blank. The amount of DRT was computed from its calibration graph.



## RESULTS AND DISCUSSION

The optimum conditions for the colour development of method were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table 1. The precision of each method was found by measuring absorbances of six replicate samples containing known amounts of drug and the results obtained are incorporated in Table 1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and are presented in Table 1. The accuracy of each method was ascertained by comparing the results by proposed and reference methods (UV) statistically (Table 2). This comparison shows that there is no significant difference between the results of proposed methods and those of the reference ones. The similarity of the results is obviously evident that during the application of these methods, the additives and excipients that are usually present in tablets do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were performed by adding a fixed amount of the drug to the preanalysed formulations. The amount of drug found and the % recovery was calculated in the usual way.

**Table 1. Optical characteristics, precision and accuracy of the proposed methods for DRT**

Parameters	Method A	Method B	Method C
$\lambda_{\max}$ (nm)	520	530	640
Beer's Law limits ( $\mu\text{g/mL}$ )	0.5 – 2.5	0.5 – 2.5	0.5 – 2.5
Molar absorptivity ( $\text{l mole}^{-1}\text{cm}^{-1}$ )	$1.12 \times 10^4$	$1.24 \times 10^4$	$5.78 \times 10^3$
Sandell's sensitivity ( $\mu\text{g/cm}^2/0.001$ absorbance unit)	0.036	0.032	0.069
Regression Equation $y = a + bc^*$			
Slope (b)	0.029	0.032	0.015
Intercept (a)	0.0001	0.0005	0.0032
Correlation coefficient (r)	0.9999	0.9999	0.9999
Relative Standard Deviation (%)**	0.289	0.322	0.201
% Range of error ** (0.05 level confidence limit)	0.242	0.271	0.168

\* $Y = a + bc$ , where c is the concentration in  $\mu\text{g/mL}$ .

\*\*From six determinations.

**Table 2. Determination of DRT in pharmaceutical formulations**

Sample <sup>Δ</sup> (Tablets)	Labeled method (mg)	Amount obtained (mg)						
		UV* Method	Proposed method			Recovery (%)		
			A	B	C	A	B	C
T <sub>1</sub>	40	39.9	39.8	39.9	39.8	99.7	99.8	99.7
T <sub>2</sub>	40	39.8	39.9	39.9	39.7	99.8	99.8	99.5
T <sub>3</sub>	80	79.7	79.8	79.9	79.8	99.8	99.9	99.8
T <sub>4</sub>	80	79.5	79.7	79.8	79.8	99.6	99.8	99.8

<sup>Δ</sup>Four different batches of tablets from a pharmaceutical company.

The proposed methods are applicable for the assay of drug (DRT) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and  $\lambda_{\text{max}}$  among the proposed methods are C > B > A, respectively. The proposed methods are simple, selective and can be used in the routine determination of DRT in bulk samples and formulations with reasonable precision and accuracy.

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