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New, simple and validated UV-spectrophotometric methods for the estimation of drotaverine hydrochloride in bulk and formulations

Khaggeswar. B*, Harishankar. P, Raghunandan. N

Central Analytical Laboratory, Balaji Institute of Pharmaceutical Sciences, Warangal, India

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ABSTRACT

New, simple and cost effective, accurate and reproducible UV-spectrophotometric methods were developed for the estimation of drotaverine hydrochloride (DHC) in bulk and pharmaceutical formulations. The drug was estimated at 242 nm in 100 mM hydrochloric acid (pH 1.2), 242 nm in methanol: 100 mM phosphate buffer pH 7.4 (25:75), and 243 nm in ethanol: 100 mM phosphate buffer pH 7.4 (25:75). Linearity range was found to be 2-18 µg mL^{-1} (regression equation: absorbance = 0.052 × concentration in µg mL^{-1} + 0.0163; r^2 = 0.9999) in the hydrochloric acid medium (pH 1.2), 5-25 µg mL^{-1} (regression equation: absorbance = 0.0347× concentraion in µg mL^{-1} + 0.0058; r^2 = 0.9999) in methanol: 100 mM phosphate buffer, pH 7.4 (25:75) and 7-25 µg mL^{-1} (regression equation: absorbance = 0.0435 × concentraion in µg mL^{-1} + 0.0002; r^2 = 0.9998) in ethanol: 100 mM phosphate buffer, pH 7.4 (25:75). The apparent molar absorptivity was found to be 2.22 × 10^4 l mol^{-1} cm^{-1}, 1.48 ×10^4 l mol^{-1} cm^{-1} and 1.88 × 10^4 l mol^{-1} cm^{-1}. The quantitation limits were found to be 0.23, 0.31 and 3.56 µg mL^{-1} in the respective media. These methods were tested and validated for various parameters according to ICH guidelines and USP.

Key words: Drotaverine hydrochloride, spectrophotometry, method validation, uv-method, ICH

INTRODUCTION

Drotaverine Hydrochloride (DHC) [1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroiso-quinoline] Figure 1, a hydrated derivative of papaverine, is an effective spasmolytic agent [1].

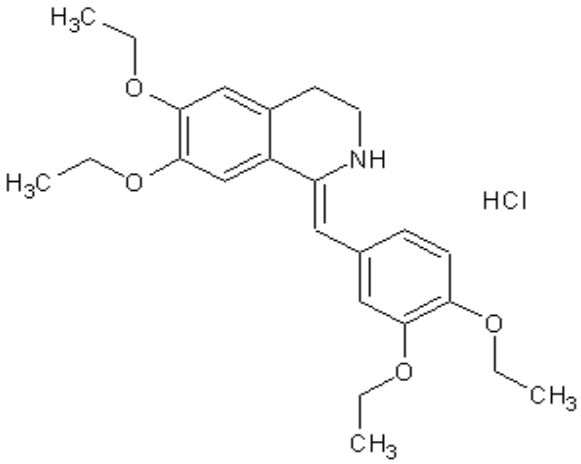

Figure 1. Structure of Drotaverine Hydrochloride

A survey of literature has not revealed any simple UV-spectrophotometric method for estimation of DHC in bulk and formulations. They include HPLC [2-4] and TLC [5]. Other alternative include spectrophotometry [6], differential spectrophotometry [7-8], computer-aided spectrophotometry [9] potentiometry [10-11] square-wave polarography [12] and spectrophotometric method determination using chromophore[14].

But, chromatographic techniques are time consuming, costly and require expertise. A simple and accurate UV-spectrophotometric method can be highly useful for routine analysis of bulk, formulations and dissolution samples and this analysis doesn't require chromophore. The present work aims to present a simple, rapid and sensitive method for the determination of DHC in pure form and in their pharmaceutical preparations and can be used for the quality control and assurance of these drugs in industry.

The objective of the present study was to develop simple, precise, accurate and economic analytical methods with the better detection range for estimation of DHC in bulk, pharmaceutical formulations, and in vitro dissolution studies of oral formulations.

Three analytical methods have been developed in different media for estimation of DHC Media used are 100mM (0.1N HCl), methanol: 100 mM phosphate buffer, pH 7.4 (25:75), ethanol: 100 mM phosphate buffer, pH 7.4 (25:75). No extraction step was involved in the proposed methods, thereby decreasing time and the error in quantitation. The developed methods were validated as per ICH guidelines and USP requirements [14-15] and with suitable statistical tests were performed on validation data [16-17].

MATERIALS AND METHODS

2. Experimental procedures

2.1. Instruments

A double-beam Analytical Technologies Limited, model T60 UV-Visible spectrophotometer connected to computer loaded with UV Win 5.0 software. The instrument has an automatic wavelength accuracy of 1 nm and matched quartz cells of 10 mm path length.

2.2. Materials

DHC was obtained as gift samples from Rantus Pharmaceuticals Pvt. Ltd., India. Tablet Formulation A, labeled to contain 40 mg of DHC per tablet, Tablet Formulation B, labeled to contain 80 mg of DHC per tablet, Injection Formulation C, labeled to contain DHC 20 mg mL^{-1} were collected from local Indian market. All other chemicals and reagents used were of analytical grade.

2.3. Analytical method development

Different media were investigated to develop a suitable UV-spectrophotometric method for the analysis of DHC in formulations. For selection of media the criteria employed were sensitivity of the method, ease of sample preparation, solubility of the drug, and cost of solvents and applicability of method to various purposes. Absorbance of DHC in the selected medium at respective wavelength was determined and apparent molar absorptivity was calculated according to the standard formulae (Table 2).

Table 1. Calibration data of the developed methods (each value is result of nine separate determinations)

100 mM Hydrochloric acid medium (pH 1.2)		
Drug Conc. (µg mL^{-1})	Absorbance at 242 nm (± S.D.[a])	% R.S.D.[b]
02	0.0885±0.0014	1.58
06	0.2958±0.0007	0.24
10	0.4994±0.0041	0.82
14	0.7164±0.0043	0.60
18	0.9182±0.0021	0.23
Methanol: 100 mM phosphate buffer, pH 7.4 (25:75)		
Drug Conc. (µg mL^{-1})	Absorbance at 242 nm (± S.D.[a])	% R.S.D.[b]
05	0.1634±0.0022	1.37
10	0.3411±0.0027	0.81
15	0.5149±0.0038	0.74
20	0.6829±0.0053	0.77
25	0.8546±0.0082	0.97
Ethanol: 100 mM phosphate buffer, pH 7.4 (25:75)		
Drug Conc. (µg mL^{-1})	Absorbance at 243 nm (± S.D.[a])	% R.S.D.[b]
07	0.3083±0.0082	2.65
10	0.4374±0.0061	1.39
15	0.6476±0.0087	1.34
20	0.8690±0.0094	1.08
25	1.0943±0.0076	0.69

a Standard deviation.
b Relative standard deviation.

2.4. Calibration standards

Three different stock solutions of 100 µg mL^{-1} of DHC were prepared in 100 mM hydrochloric acid (pH 1.2) (medium A), methanol: 100 mM phosphate buffer, pH 7.4 (25:75) (medium B) and in ethanol: 100 mM phosphate buffer pH 7.4 (25:75) (medium C) by dissolving 10 mg of

DHC in 100 mL of each media. For preparation of different concentrations, aliquots of stock solutions were transferred into a series of 10 mL standard flasks and volumes were made with respective media. Five different concentrations were prepared in the range of 2-8 µg mL^{-1}, 5-25 µg mL^{-1} and 7-25 µg mL^{-1} of DHC in respective media. DHC was estimated at 242 nm, 242 nm and 243 nm in three media, respectively. The calibration data are presented in Table1.

2.5. Analytical validation

2.5.1. Specificity and selectivity

DHC solutions (10 µg mL^{-1}) were prepared in three selected media along with and without common excipients. (methyl cellulose, hydroxyl propyl methyl cellulose, dextrose, iron oxide yellow, titanium oxide, lactose, starch, microcrystallinecellulose, magnesium stearate, talc and benzalkonium chloride) separately. All the solutions were scanned from 400 to 200 nm and checked for change in the absorbance at respective wavelengths. In a separate study, drug concentration of 10 µg mL^{-1} was prepared independently from pure drug stock and commercial sample stock in selected media and analysed (N = 5). Paired t-test at 95% level of significance was performed to compare the means of absorbance (Table 2).

Table 2. Optical characteristics, statistical data of the regression equations and validation parameters for DHC (each value is result of nine separate determinations)

Parameter	Medium A	Medium B	Medium C
Optical characteristics			
Apparent molar absorptivity (l mol^{-1} cm^{-1})	2.22×10^{4}	1.48×10^{4}	1.88×10^{4}
Regression analysis			
Slope (S.E.[a])	$0.0520(1.0\times10^{-4})$	$0.0347(1.4\times10^{-4})$	$0.0435(4.0\times10^{-4})$
95% confidence limits of slope	0.0510; 0.0529	0.0338; 0.0351	0.0435; 0.0441
Intercept (S.E.[a])	$-0.0163(1.1\times10^{-3})$	$-0.0058(2.1\times10^{-3})$	$-0.0002(6.6\times10^{-3})$
95% confidence limits of intercept	-0.0273; -0.0053	0.0338; 0.0049	-0.0187; 0.0191
Standard error of estimate	2.94×10^{-3}	2.5×10^{-3}	1.3×10^{-2}
Regression coefficient (r^2)	0.9999	0.9999	0.9998
Calculated F-value (critical F-value)[b]	1.0122(2.5787)	1.1521(2.5787)	1.2801(2.5787)
Validation parameters			
Specificity and selectivity - tCal (tCrit)[c]	0.97(2.31)	1.28(2.31)	1.64(2.31)
Linearity (µg mL^{-1})	2-18	5-25	7-25
DL (µg mL^{-1})	0.0762	0.1023	1.1766
QL (µg mL^{-1})	0.2309	0.3100	3.5655
Robustness (mean % recovery ± S.D.)	100.11±1.524	100.81±1.135	100.96±1.729

a Standard error of mean.
b Theoretical value of F(4,45) based on one-way ANOVA test at P = 0.05 level of significance.
c tCal is calculated value and tCrit is theoretical value (at 8 d.f.) based on paired t-test at P = 0.05 level of significance.

2.5.2. Accuracy

To determine the accuracy of the proposed methods, different levels of drug concentrations (LQC, MQC and HQC in respective media) were prepared from independent stock solution and analyzed (N = 9). Accuracy was assessed as the percentage relative error and mean percentage

recovery (Table 3). Standard addition method was done to give additional support to accuracy. In this study, same concentrations of pure drug 5 µg mL^{-1} in the three media were added to a known preanalysed formulation sample and the total concentration was determined using the proposed methods (N = 3). The percent recovery of the added pure drug was calculated as, % Recovery = [(Cv – Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition; Cu, drug concentration in the formulation; Ca, drug concentration added to formulation (Table 4).

2.5.3. Precision

Repeatability was determined by using different levels of drug concentrations prepared from independent stock solution and analyzed (N = 9) (Table 3). Inter-day and intra-day variation and instrument variation were taken to determine intermediate precision of the proposed methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation.

The relative standard deviation (in %) of the predicted concentrations from the regression equation was taken as precision indicating inter-day variation (N = 27) (Table 5).

2.5.4. Linearity

To establish linearity of the proposed method, nine separate series of solutions of the drug (2-18 µg mL^{-1} in medium A, 5-25 µg mL^{-1} in medium B and 7-25 µg mL^{-1} in medium C) were prepared from the stock solutions and analyzed (Figure 2, 3 and 4). Least square regression analysis was done for the obtained data. ANOVA test (one-way) was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions (Table 2).

Table 3. Accuracy and precision data for the developed methods (each value is result of nine separate determinations)

Level	Predicted conc. (µg mL^{-1}) [a]			Mean % recovery (± S.D.)	Accuracy (%) [b]
	Range	Mean (± S.D.)	% R.S.D.		
Medium A					
LQC	3.89-4.07	3.99±0.05	1.47	99.77±1.38	0.22
MQC	9.96-10.05	10.00±0.03	0.31	100.16±0.31	-0.06
HQC	16.92-17.05	17.01±0.11	0.64	100.42±0.59	0.64
Medium B					
LQC	4.95-5.04	5.00±0.04	0.80	99.34±1.57	-0.08
MQC	14.87-15.05	14.97±0.06	0.40	99.84±0.92	0.19
HQC	22.91-23.12	23.01±0.07	0.31	100.27±0.31	-0.07
Medium C					
LQC	7.96-08.12	8.01±0.06	0.77	100.15±0.72	-0.15
MQC	15.85-16.09	15.97±0.07	0.46	99.84±0.46	-0.17
HQC	23.88-24.16	23.98±0.12	0.50	99.91±0.34	0.08

a Predicted concentration of DHC was calculated by linear regression equation.
b Accuracy is given in % relative error (= 100 × [(predicted concentration – nominal concentration)/nominal concentration)]

Table 4. Results of standard addition method (each value is result of three separate determinations)

Method	Conc. of drug in formulation (μg mL^{-1})	Conc. of pure drug added	Total conc. of drug found	% Analytical Recovery (± S.D.)
Medium A	5	2	7.04	100.57±1.76
	5	5	9.98	100.02±0.83
	5	10	15.10	99.33±1.05
Medium B	5	1	6.05	100.21±0.47
	5	5	9.93	99.61±0.55
	5	7	12.14	101.17±1.12
Medium C	5	3	7.96	99.28±1.46
	5	11	16.22	100.31±0.55
	5	19	24.27	100.83±1.17

2.5.5. Detection limit (DL) and quantitation limit (QL)

The DL and QL of DHC by the proposed methods were determined using calibration standards. DL and QL were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation [7] (Table 2).

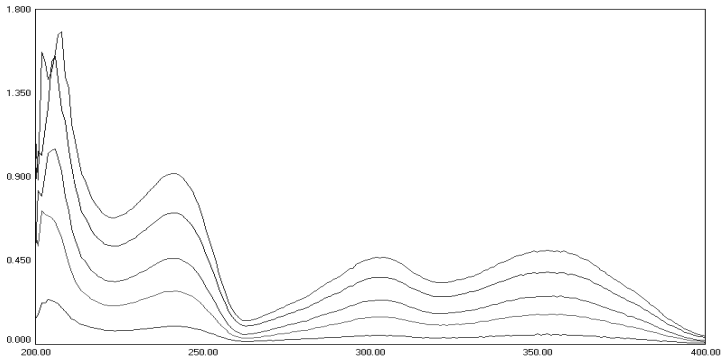

Figure 2. Overlay spectrum of DHC in medium A

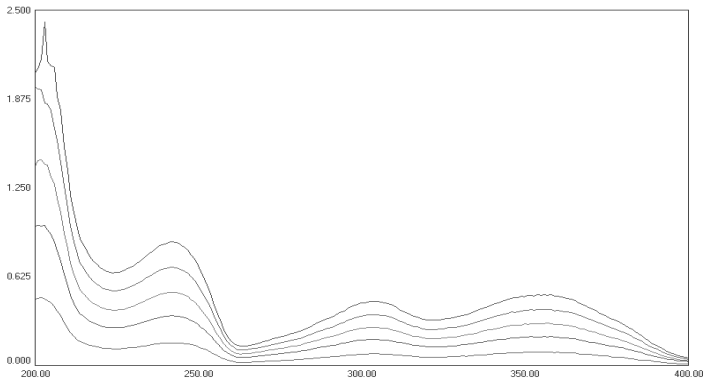

Figure 3. Overlay spectrum of DHC in medium B

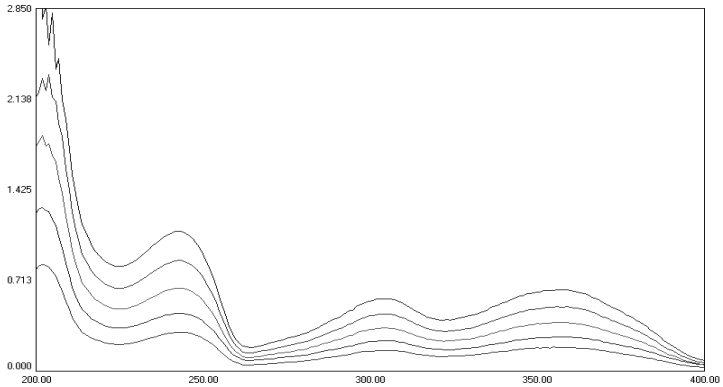

Figure 4. Overlay spectrum of DHC in medium C

Table 5. Results of intermediate precision study

Conc. (µg mL^{-1})	Intra-day repeatability % R.S.D. [a] (*N* = 9)			Inter-day repeatability R.S.D.[a]
	Day 1	Day 2	Day 3	(*N* = 27)
Medium A				
4	0.1925	0.1007	0.0031	1.54
10	0.5042	0.4034	0.3836	0.87
17	0.8674	0.8569	0.8398	0.59
Medium B				
5	0.1653	0.1512	0.1403	1.08
15	0.5112	0.5059	0.4967	0.72
23	0.7918	0.7831	0.7762	0.94
Medium C				
8	0.3503	0.3416	0.3290	0.52
16	0.6965	0.6781	0.6548	0.66
24	1.0483	0.9395	0.9206	1.13

a Percentage relative standard deviation

Table 6. Application of the proposed spectrophotometric methods to the determination of DHC in dosage forms (each value is the average of five separate determinations)

Market Product	Medium A	Medium B	Medium C
	% Assay	% Assay	% Assay
Tablet Formulation A(40 mg)			
Mean ± S.D. (mg)	98.42±1.24	100.12±1.17	100.29±0.85
F[a]	1.82(3.84)		
Tablet Formulation B(80 mg)			
Mean ± S.D. (mg)	101.06±0.73	99.94±1.40	101.17±1.60
F[a]	2.09(3.84)		
Injection Formulation C (20 mg mL^{-1})			
Mean ± S.D. (mg)	98.46±1.83	99.12±1.55	100.26±0.51
F[a]	2.71(3.84)		

[a] *The values in parenthesis are the tabulated values of F at P = 0.05(at 4 d.f.).*

2.5.6. Robustness

Robustness of the proposed method was determined by (a) changing pH of the media by ± 0.1 units and (b) stability of the DHC in the both selected medium at room temperature for 8 h. Three different concentrations (LQC, MQC and HQC) were prepared in both media with different pH. Mean percentage recovery was determined (Table 2).

2.6. Estimation from formulations

2.6.1. Tablets

Twenty tablets were weighed and pulverized. Amount of the powder equivalent to 10 mg of DHC was taken and extracted with three media separately for 30 min. These solutions were diluted suitably to prepare a 100 µg mL^{-1} concentration in respective media. Finally solutions were filtered through Whatman filter paper number 40 and the filtrate was suitably diluted to prepare a 10 µg mL^{-1}, 15 µg mL^{-1} and 20 µg mL^{-1} (Figure 5, 6 and 7) in the following media A, B and C were analyzed using proposed methods (Table 6).

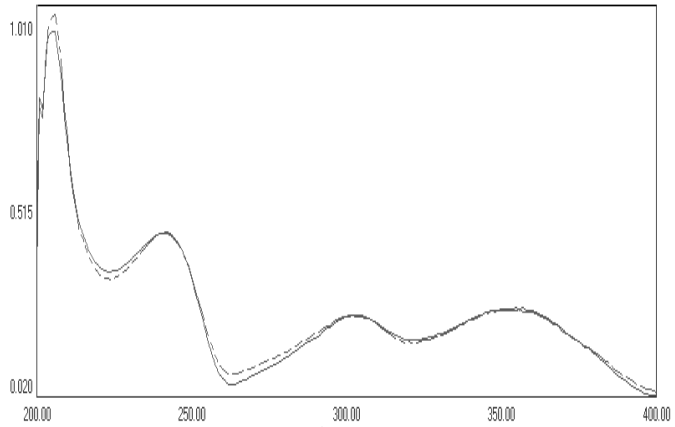

Figure 5. Overlay spectrum of DHC 10 µg mL^{-1} and marketed product (dotted line) in medium A.

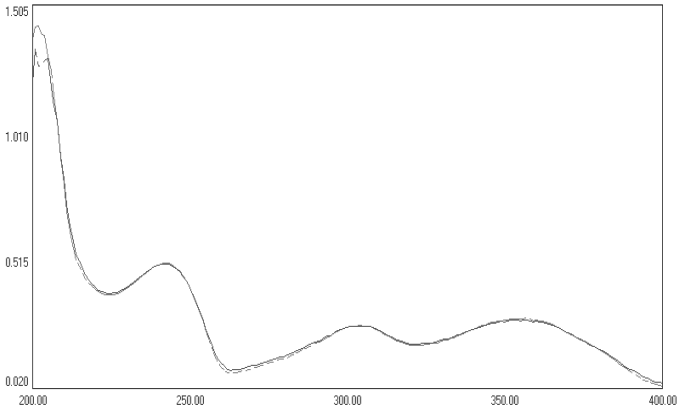

Figure 6. Overlay spectrum of DHC 15 µg mL^{-1} and marketed product (dotted line) in medium B.

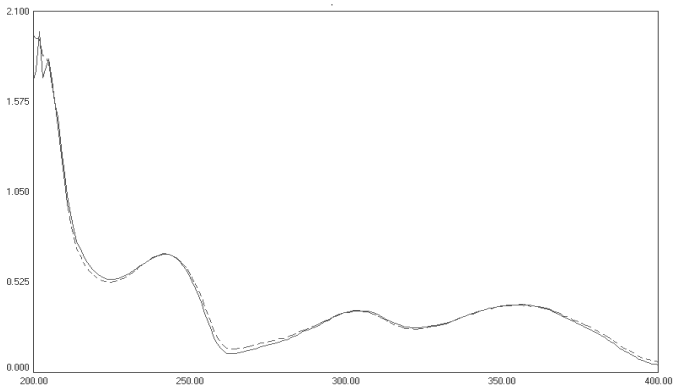

Figure 7. Overlay spectrum of DHC 20 µg mL^{-1} and marketed product (dotted line) in medium C.

2.6.2. Injection

Equivalent aliquots of DHC injection was taken and diluted with three media separately to get 10 µg mL^{-1} concentration and the samples were analysed (Table 6).

RESULTS AND DISCUSSION

For media optimisation various aqueous media like 100 mM hydrochloric acid medium, acetate buffers (pH 3.6–5.8), phosphate buffers (pH 5.8–8.0) and 100 mM sodium hydroxide were investigated. The final decision of using 100 mM hydrochloric acid (pH 1.2), methanol: 100 mM phosphate buffer pH 7.4 (25:75), and ethanol: 100 mM phosphate buffer pH 7.4 (25:75) as a media was based on criteria like: sensitivity of the method, cost, ease of preparation and applicability of the method to dissolution studies. The spectra of DHC in the respective media are shown in Figure 2, 3 and 4. The λmax of DHC in medium A, B and C were found to be 242, 242 and 243 nm, respectively.

3.1. Calibration curve

In medium A, the linear regression equation obtained was at 242 nm, absorbance = 0.052 × concentration in µg mL^{-1} + 0.0163 with r^2 = 0.9999, in medium B at 242 nm, absorbance = 0.0347 × concentraion in µg mL^{-1} + 0.0058 with r^2 = 0.9999 and in medium C at 243 nm, 0.0435 × concentraion in µg mL^{-1} + 0.0002 with r^2 = 0.9998 (Table 2).

3.2. Analytical validation

3.2.1. Specificity and selectivity

The UV-spectrum of DHC was not changed in the presence of common excipients in both the selected media. Absorption spectrum of pure drug sample was matching with the marketed formulation sample in both the selected media (Fig. 2). The calculated t-values were found to be less than that of the critical t-value, indicating that statistically there was no significant difference between mean absorbance of solutions prepared from pure drug sample and marketed formulation sample (Table 2). Therefore proposed methods are specific and selective for the drug.

3.2.2. Accuracy

Accuracy ranged from -0.06% to 0.64%, -0.07 to 0.19% and -0.15% to 0.08% in three media, respectively (Table 3). The excellent mean % recovery values (nearly 100%) and their low standard deviation values represent accuracy. The validity and reliability of the proposed methods was evaluated by recovery studies of standard addition method (Table 4).

3.2.3. Precision

Precision determined by studying repeatability and intermediate precision. Repeatability (% R.S.D.) ranged from 0.31% to 1.47%, 0.31% to 0.80% and 0.46% to 0.77% in the respective media, at all three levels of concentrations (Table 3). In intermediate precision study, lower R.S.D. values indicating that these methods have excellent repeatability and intermediate precision (Table 5)

3.2.4. Linearity

The linearity range was found to be 2–18 µg mL^{-1} at 242 nm in medium A, 5-25 µg mL^{-1} in medium B and 7-25 µg mL^{-1} in medium C. Lower values of parameters like standard error of slope and intercept indicated high precision of the proposed methods (Table 2). The mean slope and intercept values are within the 95% confidence interval. Goodness of fit of regression equations was supported by high regression coefficient values and less calculated *F*-values (Table 2).

3.2.5. DL and QL

DL and QL values are found to be 0.0762 µg mL^{-1} and 0.2309 µg mL^{-1} in medium A, 0.1023 µg mL^{-1} and 0.31 µg mL^{-1} in medium B, and 1.1766 µg mL^{-1} and 3.5655 µg mL^{-1} in medium C, respectively (Table 2).

3.2.6. Robustness

Variation of pH of the selected media by ±0.1 did not have any significant effect on absorbance. The mean % recovery±S.D were found to be 100.11±1.524, 100.8±1.656 and 100.96±1.729 in the three media (Table 2).

3.3. Estimation of formulations

Assay values of formulations were same as mentioned in the label claim; this indicated that the interference of tablet excipient matrix is insignificant in estimation of DHC by proposed methods. The estimated drug content with low values of standard deviation established the precision of the proposed methods. The results obtained from the three methods were compared statistically (Table 6). The F-values did not exceed the tabulated values (for four degrees of freedom) indicating no significant difference between the methods, as far as accuracy and precision are concerned.

CONCLUSION

In summary, the proposed methods were simple, rapid, accurate, precise and inexpensive and can be used for routine analysis of DHC in bulk, pharmaceutical formulations and for dissolution studies of tablets and injection formulations.

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