Research Article

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Simultaneous Estimation of Drotaverine Hydrochloride & Paracetamol in a Tablet Dosage Form by Reverse Phase High-Performance Liquid Chromatographic Method

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Abstract: A simple, rapid and precise reverse phase high-performance liquid chromatographic method has been developed and validated for the simultaneous estimation of drotaverine hydrochloride (DROTA) and paracetamol (PARA) in a tablet formulation. Chromatography was carried out at ambient temperature on a Eurosphere C_{18} column (250 × 4.6 mm) with the isocratic mobile phase Methanol: Water (25: 75 v/v, pH 5.9 adjusted with acetic acid) at a flow rate of 1.2 mL/min. The UV detection was carried out at 274 nm. DROTA and PARA were separated in less than 10 min with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines and the criteria for accuracy, precision, linearity and system suitability were acceptable in all cases.

Keywords: HPLC, Paracetamol, Drotaverine hydrochloride, Method validation

INTRODUCTION:

Multidrug administration is often associated with clinically significant interaction, especially of narrow therapeutic index drugs, either at pre-absorption or post-absorption stage [1,2]. This can limit the desired therapeutic effect of either of drug molecules. Drotaverine hydrochloride (DROTA) is chemically1-[(3,4-diethoxyphenyl)-methylene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, which is used as antispasmodic [3]. Paracetamol (PARA) is chemically N-(4-hydroxyphenyl)-acetamide, and is used as an analgesic and antipyretic [3]. Tablet formulation containing 80 mg of DROTA and 500 mg PARA, is available in the market. Extensive literature survey reveals that several methods such as spectrophotometric [4-6], and HPLC [7-8], were reported for the determination of DROTA. While spectrophometry [9-13], HPLC [14-17], and capillary electrophoresis [18], methods were reported for determination of PARA alone or in combination with other drugs.

The present study was aimed to develop simple, rapid and precise reverse phase high-performance liquid chromatographic method for simultaneous estimation of drotaverine hydrochloride and paracetamol in a tablet dosage form.

METHODS & MATERIALS:

High Performance Liquid Chromatography system Chemito LC 6600 equipped with universal injector with injection volume 20mL, Ultra-Visible (UV-Vis) detector. A Eurosphere C_{18} (KNAVER, Berlin, Germany) column (250 × 4.6 mm) with particle size 5µm forms the stationary phase.

The bulk drugs of drotaverine hydrochloride (DROTA) and Paracetamol (PARA) were obtained as gift samples from from Zellifac Chem, Hydrabad, India and IPCA Laboratories Ltd., Mumbai, India, respectively. All the solvents and reagents used were of HPLC and analytical grade respectively. HPLC grade methanol and water were obtained from Merck Chemicals, India. Tablets of brand name (DROPAR, Accent Pharma) containing Paracetamol (500 mg) and Drotaverine hydrochloride (80 mg) was procured from local pharmacy.

Mobile phase: Methanol: Water (25: 75 v/v, pH 5.9), Methanol (25 mL) was added in water (75 mL) and then pH was adjusted to 5.9 with acetic acid. The mobile phase was ultrasonicated for 30 min and then filtered through 0.45 µm membrane filter. The flow rate was l.2 mL/min and the detector was set at 274 nm. All analyses were made at 25^{o}C and the volume of solution injected was 20 µL.

Standard stock solutions:

DROTA and PARA standard stock solutions: Reference standard of DROTA (10mg) was transferred to 10 mL volumetric flask and dissolved in methanol. The flask was shaken for 30 min and the volume was made up to the mark with mobile phase to obtain standard stock solution of DROTA (1000 µg/mL). Stock solution was filtered through a 0.2 µm membrane filter. The working standard solution of DROTA was prepared from suitable aliquots of stock solution.

Reference standard of PARA (10mg) was transferred to 10 mL volumetric flask and dissolved in methanol. The flask was shaken for 30 min and the volume was made up to the mark with mobile phase to obtain standard stock solution of Paracetamol (1000 µg/ml). Stock solution was filtered through a 0.2 µm membrane filter. The working standard solution of PARA was prepared from suitable aliquots of stock solution.

Working standard solution: The combined working standard solution containing DROTA (8µg/mL) and PARA (50 µg/mL) was prepared in mobile phase.

Determination from formulation: Twenty tablets containing Drotaverine Hydrochloride and Paracetamol (DROPAR*) were weighed accurately to determine average weight, tablets were crushed to fine powder. The tablet powder equivalent to DROTA (80 mg) and PARA (500 mg) was weighed, transferred to a 100 mL volumetric flask and dissolved in mobile phase, shaken for 30 min and the volume was made up to the mark with mobile phase. The content was ultrasonicated for 20 min., the solution was filtered through a 0.2 µm membrane filter paper. This tablet solution was further diluted with mobile phase to obtain mixed sample solutions having concentration DROTA (8µg/mL) and PARA (50 µg/mL).

Method Validation: The method was validated according to the ICH guidelines.[19] The following validation characteristics were addressed: linearity, accuracy, precision, limits of detection and quantization and robustness.

System suitability testing (SST): Standard solutions which contained DROTA (8 µg/mL) and PARA (50 µg/mL) were prepared by appropriately diluting and mixing the corresponding stock standard solutions. System suitability was determined from six replicate injections of the system suitability standard before sample analysis.

Linearity and range: Standard calibration curves were prepared with seven calibrators over a concentration range of 2–10 µg/mL for DROTA and 12.4–60.20 µg/mL for PARA. The data of peak area versus concentration were treated by linear least square regression analysis.

Accuracy: To study the reliability and suitability of the developed method, recovery experiments were carried out. Placebo samples were spiked with different amount of DROTA and PARA at 80, 100 and 120% in duplicate for each one (n = 6) over the theoretical values. Measured values were compared with the theoretical concentration. Recovery for pharmaceutical formulations should be within the range 99.86-100%. The R.S.D. percent of individual measurements was also determined. The results must be less than 5%.

Precision: The precision of the developed method was assessed in terms of repeatability, intra-day and inter-day precision by analyzing six replicate standard samples. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision.

Limits of detection and quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) for the procedure were performed on samples containing very low concentrations of analytes according to the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQs were considered as the lowest concentration of analytes in standards that can be reproducibly measured with acceptable accuracy and precision.

Robustness: The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±1), organic solvent content (±1) and pH of mobile phase (±1).

RESULTS AND DISCUSSION:

Method development and optimization: Typically, method development focuses on identifying buffer type, strength and pH, organic solvent and implementing small changes to optimize selectivity and enhance resolution. Initially, different stationary phases such as C-8 and C-18, with mobile phases containing buffers like phosphate, at different pH and temperature, and organic solvents, different mobile phases like Methanol : Phosphate buffer, Methanol : Acetonitrile : Water and Methanol : Water were tried in order to find the optimum conditions for the separation of Drotaverine hydrochloride and Paracetamol. It was found that mobile phase containing methanol and water (pH 5.9) and stationary phase C-18, give satisfactory results with sharp well defined and resolved peaks with minimum tailing as compared to other mobile phases.

Method validation: When a method has been optimized it must be validated before practical use. By following the ICH guidelines for analytical method validation, Q2 (R1), the SST was performed and the validation characteristics were addressed.

System suitability: The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. All critical parameters tested met the acceptance criteria on all days

Linearity and range: For the construction of calibration curves, five calibration standard solutions were prepared over the concentration range of 2-10 µg/mL for DROTA and 12.4-60.20 µg/mL for PARA. The results, summarized in **Table 1**, show a good correlation between analytes peak area and concentration with, r^2 is 0.9991 and r^2 is 0.9996 for DROTA and PARA, respectively.

Accuracy and precision: Accuracy and precision were established across the analytical range for DROTA and PARA. The intra- and inter-day accuracy and precision were calculated from the QC samples **Table 2 and 3**. Repeatability (intra-day precision) of the analytical method was found to be reliable based on %R.S.D. (<2%) corresponding to the peak areas and retention times. Intermediate precision (inter-day accuracy) was demonstrated on different days and evaluating the peak area data at three QC standards that cover the assay method range. The %R.S.D. values were less than 5% and illustrated the good precision for the analytical method. Results of statistical validation given in **Table 4.**

Sensitivity: The limit of detection and limit of quantitation decide about the sensitivity of the method. Tests for the procedure were performed on samples containing very low concentrations of analytes based on the visual evaluation method. In this method, LOD is determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected. Accordingly, the LOQ is determined by the analysis of samples with known concentration of analytes and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision (R.S.D. <2%). The LOD and LOQ values were found to be 0.0506 and 0.1534 µg/mL for DROTA and 0.2587 and 0.7841 µg/mL for PARA.

Robustness: To ensure the insensitivity of the developed HPLC method to minor changes in the experimental conditions, it is important to demonstrate its robustness. None of the alterations caused a significant change in resolution between DROTA and PARA, peak area, R.S.D., USP tailing factor and theoretical plates (Table 5 and 6).

Analysis of the marketed product: The validated HPLC method was used for the simultaneous determination of DROTA and PARA in their combined dosage form. Five samples of brand (DROPAR) was weighed separately and analyzed. Representative chromatogram is shown in Figure 1. The results, expressed as percentage drug recovery related to label claim, are informed in Table 7. These indicate that the amounts of each drug in the tablets of are within the USP requirements of 90–110% of the corresponding label claims.

CONCLUSION:

A simple and efficient HPLC method has been developed, optimized and validated for the isocratic separation and simultaneous determination of drotavarine hydrochloride and paracetamol in

their combined tablet dosage form. The method, suitable for routine quality control, has been successfully applied to the determination of both analytes in commercial brand of tablet containing this pharmacological association.

Table 1. Results of linearity and range

Drug	Linearity range (µg/mL)	Slope ± S. D	Intercept ± S.D	Correlation Coefficient (r^2) ± S.D
DROTA	2 - 10	6.4810 ± 0.14	0.1 ± 0.1	0.9991±0.0001
PARA	12.4 – 60.20	11.4769 ± 0.10	0.9 ± 0.1	0.9996±0.0001

Table 2. Results of inter-day precision data

Drug	% Mean*	S.D	% R.S.D.	S.E.
DROTA	100.12	0.040	0.039	0.016
PARA	100.18	0.161	0.160	0.065

***Average of six determinations**

Table 3. Results of intra-day precision data

Drug	% Mean*	S.D	% R.S.D.	S.E.
DROTA	99.95	0.454	0.454	0.181
PARA	99.68	0.515	0.514	0.210

***Average of six determinations**

Table 4. Statistical validation of recovery studies

Level of % Recovery	Drug	% Recovery	Standard Deviation*	% Co-efficient of Variation	Standard Error*
80	DROTA	99.86	0.070	0.070	0.040
	PARA	100	0.034	0.034	0.020
100	DROTA	100.09	0.047	0.046	0.027
	PARA	100	0.010	0.01	0.005
120	DROTA	99.80	0.023	0.023	0.013
	PARA	100.02	0.010	0.010	0.005

***Average of three determinations.**

Table 5. Results of the robustness of the method

Factor		**Rt**		**Number of Theoretical plates**	
FlowRate(ml/min)		**DROTA**	**PARA**	**DROTA**	**PARA**
1.1	- 1	2.760	4.967	6520	7215
1.2	0	2.713	4.453	6361	7108
1.3	+ 1	2.690	4.440	6310	7098
Mean ±S.D		2.721± 0.035	4.620 ± 0.300	6397 ± 109.5	7140 ± 64.85
% of Methanol in the Mobile Phase (v/v)		**DROTA**	**PARA**	**DROTA**	**PARA**
24	- 1	2.727	4.460	6389	7226
25	0	2.713	4.453	6331	7108
26	+ 1	2.680	4.372	6298	7099
Mean ±S.D		2.706 ± 0.024	4.428 ± 0.048	6339 ± 46.06	7162 ± 70.86
pH		**DROTA**	**PARA**	**DROTA**	**PARA**
5.8	- 1	2.743	4.480	6388	7135
5.9	0	2.713	4.453	6361	7108
6.0	+ 1	2.689	4.399	6318	7089
Mean ±S.D		2.715 ± 0.027	4.444 ± 0.041	6355 ± 35.30	7110 ± 23.11

Table 6. Results of the robustness of the method

Factor		**Area**		**% Content**	
Flow Rate (ml/min)		**DROTA**	**PARA**	**DROTA**	**PARA**
1.1	-1	52.8996	501.9174	100.4	100.01
1.2	0	52.6371	501.8089	100.0	100.
1.3	+1	52.3790	500.9987	99.50	99.83
Mean ± S.D		52.6385 ± 0.260	501.575 ± 0.502	99.96 ± 0.450	99.94 ± 0.101
% of Methanol in the Mobile Phase (v/v)		**DROTA**	**PARA**	**DROTA**	**PARA**
24	-1	52.7560	502.0015	100.2	100.03

25	0	52.6371	501.8089	100.0	100.0
26	+1	52.4980	501.6894	99.73	99.97
Mean ± S.D		52.6303 ± 0.129	501.8332 ± 0.157	99.97 ± 0.235	100.0 ± 0.030
pH		**DROTA**	**PARA**	**DROTA**	**PARA**
5	-1	52.8678	501.9909	100.4	100.03
5.9	0	52.6821	501.8089	100.0	100.0
6.1	+1	52.3134	501.7463	99.38	99.98
Mean ± S.D		52.6211 ± 0.282	501.8487 ± 0.127	99.92 ± 0.513	100.0 ± 0.025

Table 7. Assay of drotaverine hydrochloride and paracetamol in their combined tablet formulations

Sr. No.	**Amount present (mg/tab)**		**Amount found (mg/tab)**		**Percentage of drug Found**	
	Drota	**Para**	**Drota**	**Para**	**Drota**	**Para**
1	80	500	80.03	500.00	100.26	100.22
2	80	500	80.09	500.00	100.63	100.34
3	80	500	79.86	500.01	99.10	100.41
4	80	500	79.84	500.00	98.95	99.78
5	80	500	80.00	500.00	100.06	100.05
6	80	500	79.96	500.01	99.78	100.29
Mean					99.80	100.18
SD					0.66	0.23

Table 9. Summary of the results of the method validation assays

Parameters	**Drotaverine hydrochloride**	**Paracetamol**
Linearity Range (µg/mL)	2 – 10	12.04 – 60.20
Slope	6.4810	11.4769
Intercept	0.1	0.9
Correlation Coefficient	0.9991	0.9996
Limit of Detection (µg/mL)	0.0506	0.2587
Limit of Quantitation (µg/mL)	0.1534	0.7841
Retention Time (min.)	2.73	4.47
Resolution Factor	-	3.168

Precision (%R.S.D)		
Inter-day	0.039	0.160
Intra-day	0.454	0.514
Mean % Recovery	99.86	100.0
System Suitability tests		
Retention Time (t_R)	2.716	4.453
Capacity Factor (k')	1.71	3.45
Theoretical plate Number (N)	6361	7108
Resolution Factor (R)	-	3.168
Robustness-drug recovery (%± SD)		
Variation of Flow Rate (mL/min)	99.96 ± 0.450	99.94 ± 0.101
Variation of Methanol in the Mobile Phase (v/v)	99.97 ± 0.235	100.0 ± 0.030
Variation of pH	99.92 ± 0.513	100.0 ± 0.025

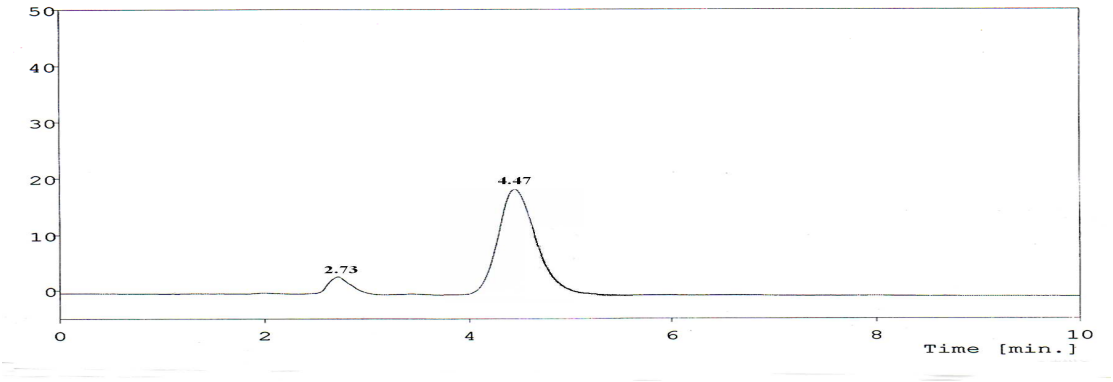

Figure 1. Chromatogram of Drotaverine hydrochloride and Paracetamol in tablet formulation. (Tablet Brand Name DROPAR); Eurosphere C_{18} column; mobile phase Methanol: Water (25: 75 v/v, pH 5.9 adjusted with acetic acid); Flow rate: 1.2 mL/min; detection: UV (0.0-10.0 min; 274 nm); drotaverine hydrochloride, Peak 1: 2.73min; paracetamol, Peak 2: 4.47 min.

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