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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DROTAVERINE HYDROCHLORIDE AND PARACETAMOL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, rapid and economic 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 Hypersil (BDS) C_{18} (150mm x 4.6 mm), 5 µm column with the isocratic mode. The mobile phase consisting of Acetate Buffer P^H 3: Acetonitrile (45:55% v/v) at a flow rate of 1 ml/min. The UV detection was carried out at 230 nm. DROTA and PARA were separated in less than 10 min. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The proposed method was optimized and validated as per the ICH guidelines.

KEY WORDS: Paracetamol, Drotaverine hydrochloride, HPLC, Method validation.

1.INTRODUCTION

Drotaverine hydrochloride [DRT], 1-[(3, 4-diethoxy phenyl) methylene]-6, 7-diethoxy-1, 2, 3, 4- tetra hydro isoquinolene is an analogue of papaverine. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain. Paracetamol is chemically known as N-(4-hydroxyphenyl) acetamide. Paracetamol is used as an antipyretic and analgesic drug. Literature Survey reveals that the Paracetamol is Official in I.P, B.P and U.S.P, But Drotaverine Hcl is unofficial in any pharmacopoeia. Literature survey revealed HPLC methods for estimation of paracetamol with different drug combination like Nimesulide, Aceclofenac, and Diclofenac Potassium. And estimation of Drotaverine Hcl with different combinations like Mefenamic acid and Omeprazole in pharmaceutical formulations. But there is no method for estimation of Drotaverine Hcl with Paracetamol Combination by HPLC. So it is worthwhile to develop simple, rapid and accurate method for estimation of Drotaverine Hcl with Paracetamol Combination by HPLC. The present paper describes a simple, accurate and precise method for simultaneous estimation of drotaverine hydrochloride and paracetamol in combined tablet dosage form. The present RP-HPLC method was validated following the ICH guidelines.

2.EXPERIMENTAL

Reagents and chemicals : Acetonitrile, Methanol, and water of HPLC grade were procured from Merck and Qualigens fine Chemicals, respectively (Mumbai, India). The gift sample of drotaverine hydrochloride (DROTA) was obtained from Zellifac Chem, Hyderabad and Paracetamol (PARA) was obtained from IPCA Laboratories Ltd., Mumbai. Tablets of brand name (DROPAR, Accent Pharma) containing Paracetamol (500mg) and Drotaverine hydrochloride (80mg) was procured from local pharmacy.

Instrumentation and Chromatographic Conditions : Chromatography was carried out at ambient temperature on a Hypersil (BDS) C_{18} (150mm x 4.6 mm), 5 µm column with the isocratic mode. The mobile phase consisting of Acetate Buffer P^H 3: Acetonitrile (45:55% v/v) at a flow rate of 1 ml/min. The UV detection was carried out at 230 nm and injection volume was 20 µl.

Preparation of buffer : Dissolve 8.2gms of Sodium acetate in 100 ml of milli Q water and sonicated for 15min, adjust the pH 3.0 with ortho phosphoric acid.

Preparation of mobile phase : The mobile phase was prepared by mixing the buffer and acetonitrile in the ratio 45:55. It was filtered through membrane filter applying vacuum.

Diluent Preparation: Mobile phase is used as diluent.

Drotaverine hydrochloride and Paracetamol Standard Stock Solutions: Weighed accurately equal quantity of about 50.2 mg of paracetamol and 8.1 mg of Drotaverine Hcl working standard to a 25 ml volumetric flask. Added about 10 ml of diluent and sonicated to dissolve. Finally make up volume to the mark with diluent.

Sample Preparation: Weighed accurately 20 tablets and calculate the average weight. Weighed and transferred 70.2 mg tablet powder to a 25 ml volumetric flask added about 10 mL of diluent and sonicate with occasional shaking till the tablets disintegrate completely and continue sonication with occasional shaking for about 30 minutes. Make volume up to the mark with diluent and mix. Dilute 1.0 ml of supernatant solution to 25 ml with diluent and mix. Filter the solution through 0.45 µm Millipore filter. Collect filtrate by discarding first few ml of filtrate.
Assay procedure : 20µl of the standard stock solutions were injected and the retention time and peak area were determined. The sample solutions were also analyzed by injecting 20µl of the solution and the peak area were determined. The amount of Paracetamol and Drotaverine hydrochloride present in commercial tablets was calculated by comparing the peak area of standard and sample.

3.RESULTSANDDISCUSSION

A reverse phase HPLC Method was developed for estimation of DROPAR tablet formulation. The separation was achieved by Hypersil (BDS) (150 mm X 4.6 mm, 5µm) column and acetate buffer and acetonitrile (45:55% v/v) as mobile phase at a flow rate of 1 ml/min, the detection was carried out at 230 nm. The retention time of drug was found to be for paracetamol was 2.85 min and drotaverine was found to be 5.53 min. The method was found to be accurate and precise as indicated by results of recovery studies and %RSD not more than 2%. LOD and LOQ for paracetamol were found to be 0.16µg/mL and 0.48µg/mL respectively and for drotaverine were 0.29µg/mL and 0.87µg/mL respectively. The proposed method was found to be specific as there is no interference from common tablet excipients like lactose, starchetc.
Method Validation : The method was validated according to the ICH guidelines. The following validation characteristics were addressed: linearity, accuracy, precision, recovery studies, LOD & LOQ and robustness.
Accuracy : Prepared solutions in triplicate by spiking Drotaverine Hcl and Paracetamol drug substance at levels from 10% to 60% of target concentration as per the test method and analyzed. The mean % recovery of the Drotaverine Hcl and Paracetamol at each level should be not less than 98.0% and not more than 102.0%.
Precision : Standard solution was prepared as per the test method and injected five times as per the test procedure. Precision study was performed to find out intra-day and inter-day variations. The %RSD for intra-day precision was 0.1731% for drotaverine hydrochloride and 0.1008% for paracetamol and inter-day precision was 0.1926% for drotaverine hydrochloride and 0.1205% for paracetamol. Both the values were well within the limit of 2% as per ICH guidelines.
Linearity: A Series of solutions were prepared using Drotaverine Hcl and Paracetamol working standard at concentration levels from 10% to 50% of target concentration (10%, 20%, 30%, 40% and 50%). Measure the peak area response of the solution at each concentration. The results, summarized in Table show a good correlation between analytes peak area and concentration with, r^2 is 0.9989 and r^2 is 0.9997for DROTA and PARA, respectively.
Limit of detection and limit of Quantitation: The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.
LOD = 3.3 X Standard deviation of y intercept / Slope of calibration curve.

LOD of Drotaverine Hydrochloride & Paracetamol was found to be 0.29µg/mL and 0.16µg/mL respectively.

The LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

LOQ = 10 X Standard deviation of y intercept / Slope of calibration curve

LOQ of Drotaverine Hydrochloride & Paracetamol was found to be 0.87µg/mL and 0.48µg/mL respectively.
Robustness : Robustness of the method was determined by making slight deliberate changes in chromatographic conditions like change in ratio of mobile phase constituents, change in flow rate and change in column temperature. It was observed that there were no marked changes in the chromatogram. It suggests that the developed method is robust.
Ruggedness : Analyst to Analyst variability study was conducted by different analysts under similar conditions at different times. Five samples were prepared and each was analyzed as per the test method. The Overall% RSD was found within the limits. The results are shown in the Table.

4.CONCLUSION

Simple, accurate and efficient HPLC method has been developed and validated for the isocratic separation. Hence the developed HPLC method for the simultaneous determination of paracetamol and drotaverine hydrochloride can be used for routine analysis of both these components in combined dosage form.

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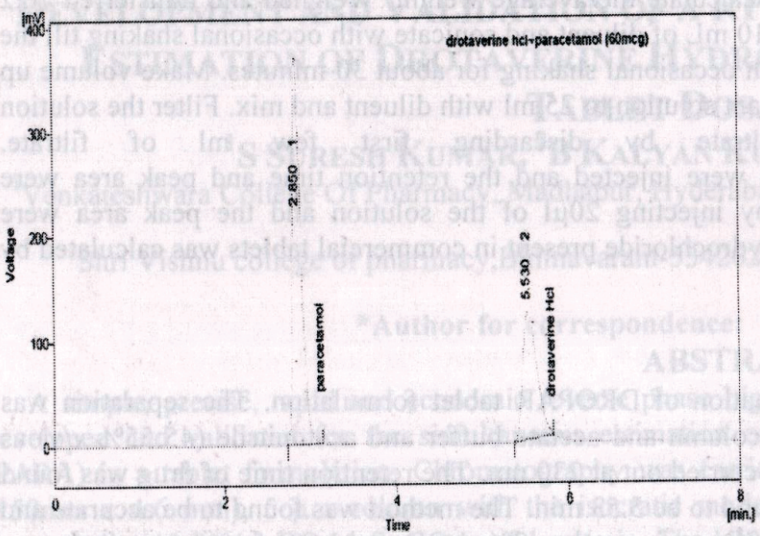

Figure1. Shows a representative chromatogram of drotaverine hydrochloride (rt-5.530) and paracetamol (rt-2.850)

Drug	Level of recovery	%recovered
Drotaverine HCl	40	99.90
	50	99.98
	60	99.96
Paracetamol	40	100.01
	50	99.91
	60	99.913

Table 1. Statistical Validation of Recovery Studies

Drug	Amount present (mg/tab)	Amount found (mg/tab)	%label claim
Drotaverine HCl	80	79.34	99.18
Paracetamol	500	500.50	100.10

Table2:Results of Determination of Intra-day,Inter-day Precission data

Parameters	Drotaverine HCl	Paracetamol
Wavelength	230nm	230nm
Linearityrange(µg/ml)	10-50	10-50
Slope±S.D	23.153	43.92
Intercept±S.D	+2.3818	-2.561
Correlation coefficient(r^2) ±S.D	0.9989	0.9997
L.O.D. (µg/ml)	0.29	0.16
L.O.Q. (µg/ml)	0.87	0.48
Retentiontime(min)	5.53	2.85
%R.S.D.Inter-day	0.1926	0.1205
%R.S.D.Intra-day	0.1731	0.1008
Mean%recovery	99.94	99.944

Table 3.Summary of the Results of the Method Validation Assays

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