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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DROTAVERINE HYDROCHLORIDE AND ACECLOFENAC IN BULK AND FORMULATION BY RP-HPLC

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ABSTRACT

A reserve phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Drotaverine hydrochloride and Aceclofenac in bulk and its pharmaceutical formulation. Separation was achieved with an Inertsil Cyano (CN) column, 250mm x 4.6mm (particles with 5µm). A mixture of methanol and 0.1% formic acid and 0.1ml of 0.1% TEA (70:30) as mobile phase at a flow rate of 1 ml/min and the column temperature was maintained at 25˚c. Dual wavelength detector was performed at 231 nm and sample temperature was maintained at 5˚c with a run time of 10 minutes. The method was rapid, simple and sensitive. The described method of drotaverine hydrochloride and aceclofenac is linear in the range of 10-50µg/ml with correlation coefficient of 0.999 respectively for both Drotaverine hydrochloride and Aceclofenac. The method enables accurate, precise and rapid analysis of Drotaverine hydrochloride and Aceclofenac. It can be conveniently adopted for routine quality control analysis of bulk and pharmaceutical formulation.

KEYWORDS: HPLC, Drotaverine ydrochloride.

INTRODUCTION

Drotaverine hydrochloride (DRO) chemically 1-[(3, 4- [diethoxyphenyl) methylene]-6,7-Diethoxy-1,2,3,4- tetrahydro isoquinolene is an papaver analogue mainly used as an antispasmodic and smooth-muscle relaxant in pain associated with gastrointestinal colic. Aceclofenac (ACE) chemically 2-[(2, 6-dichlorophenyl) amino] phenylacetoxyacetic acid is a phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties. Literature survey reveals that several reverse phase high performance liquid chromatographic (RP-HPLC) methods for determination of DRO and ACE in pharmaceutical formulations either as single and in combination with other drugs. Spectrophotometric methods for simultaneous estimation of DRO and ACE with other drugs have also been reported. To best of our knowledge no reports were found for the simultaneous estimation of the DRO and ACE in combined tablet dosage form by RP-HPLC method. This paper describes a simple, accurate, sensitive and validated RP-HPLC method for simultaneous estimation of these compounds as the bulk drug and in combined tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

The pharmaceutical dosage form used in this study was Alcinac - Spas tablets (Leeford Healthcare Ltd, Mumbai, India) labeled to contain 80 mg of DRO and 100 mg of ACE were procured from the local market. Methanol (HPLC grade), Potassium dihydrogen phosphate (AR grade), Formic Acid and triethyl amine were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

METHODOLOGY

Agilent HPLC system with Ezchrome elite software and with a UV detector. Separation was carried out on Inertsil CN Column (250 x 4.6 mm i.d.) using Methanol: 0.1% Formic acid and 0.1ml of 0.1% TEA in ratio of (70:30) as mobile phase at flow rate of 1 ml/min. Samples were injected using Rheodyne injector with 20µL loop and detection was carried out at 231 nm. All Weighing were done on Shimadzu balance (Model AY-120).

PREPARATION OF STANDARD SOLUTIONS

10 mg of Drotaverine Hydrochloride and Aceclofenac working standards was taken and

separately transferred into 10 ml volumetric flask and both was made up the volume with methanol (1000µg/ml). Equal amounts of both the solutions were pipetted into a 10ml volumetric flask and were made up the volume with mobile phase (10µg/ml). Filtered through 0.45µm membrane filter.

PREPARATION OF SAMPLE SOLUTIONS

180 mg of Drotaverine Hydrochloride and Aceclofenac Tablet powder was accurately weighed to this some amount of mobile phase solution was added and dissolved by shaking and sonicated and diluted to volume with mobile phase solution and then mix and homogenize. From this solution 1 ml was pipetted into 10 ml volumetric flask and then diluted with mobile phase. Filtered through 0.45 µm membrane filter. The solution was scanned in the range of 400-200 nm in 1 cm cell against blank and maximum absorbance was observed at 231 nm.

METHOD VALIDATION

The method was validated for linearity, accuracy, intraday and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity

Aliquots 0.1, 0.2, 0.3, 0.4 and 0.5 ml of working standard solutions of ACE (1000µg/ml) and 0.1, 0.2, 0.3, 0.4 and 0.5 ml of DRO (1000µg/ml) were transferred in a series of 10 ml volumetric flasks and the volume was made up to the mark with the mobile phase. Five replicates per concentration were injected and chromatograms were recorded. The peak areas were recorded and calibration curve was plotted of peak area against concentration of drug. (Fig. No: 1 & 2).

System suitability

The system suitability was assessed by six replicate injections of the mixture containing 10µg/ml of both the drugs. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Precision

One set of three different concentrations of mixed standard solutions of ACE and DRO were prepared. All the solutions were analyzed thrice, in order to record any intra day variations in

the results. For Inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and relative standard deviation (RSD) was calculated.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100% and 120%. The percentage of recoveries was calculated.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following factors were selected for change: flow rate of the mobile phase (1.0 ± 0.1 ml/min), a wavelength at which the drugs were recorded (231 ± 2 nm). One factor at the time was changed to estimate the effect. A number of replicate analyses (n = 3) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Methanol: 0.1% Formic acid & 0.1 ml of 0.1% TEA in ratio of (70:30 v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 4.047 and 7.763 min (Mean ± S.D.) for DRO and ACE respectively.(Fig.No: 3 & 4).

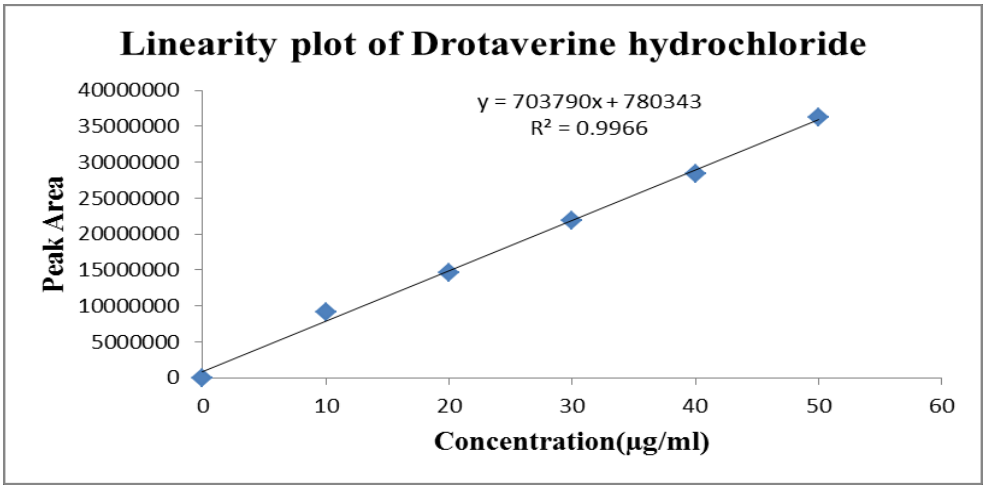

Fig. No.: 1 Linearity plot of Drotaverine Hydrochloride.

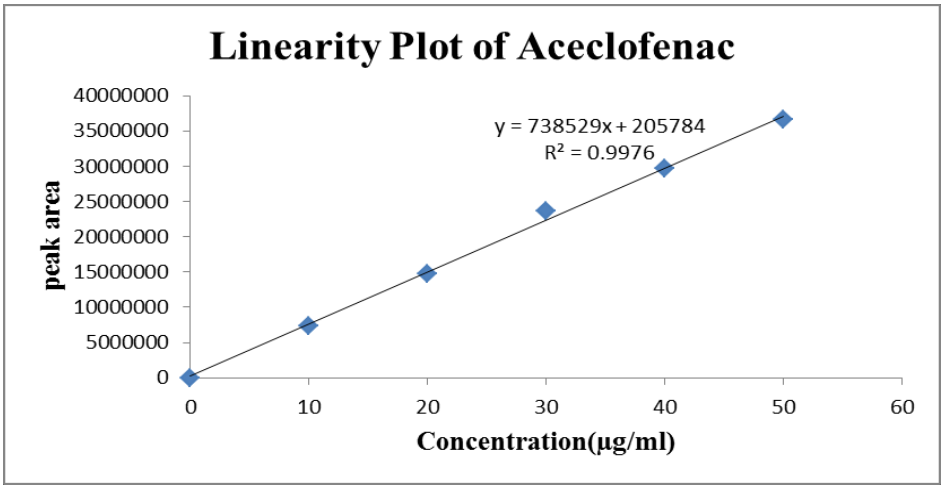

Fig. No.: 2 Linearity plot of Aceclofenac.

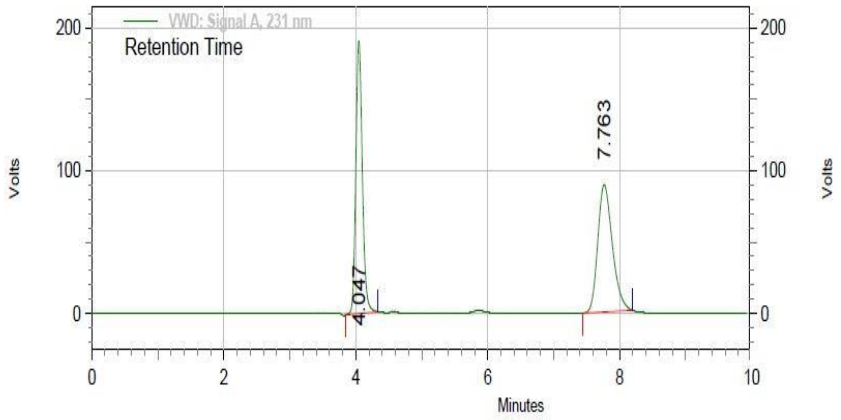

Fig. No: 3 Chromatogram of Standard.

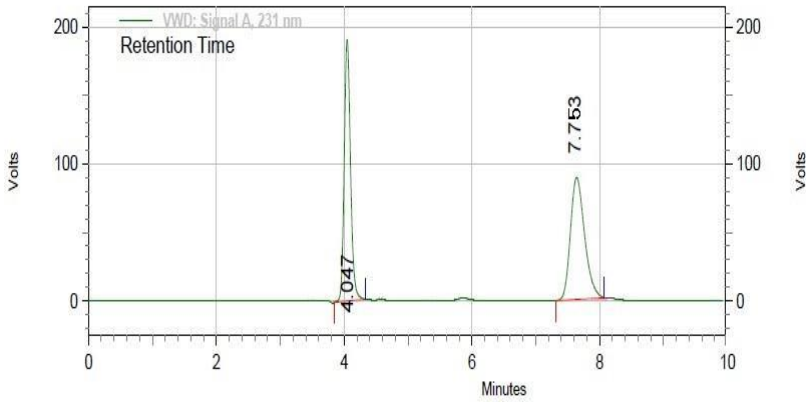

Fig. No.: 4 Chromatogram of Sample.

Results were found to be linear in the concentration range of 10-50□g/ml for DRO and ACE. The correlation coefficients for the plots were 0.996 for DRO and 0.997 for ACE. The proposed method was also evaluated by the assay of commercially available tablets

containing ACE and DRO. The % assay was found to be 98.08 for DRO and 98.3 for ACE. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD < 2), which demonstrated that the RP-HPLC method developed is robust.

The summary of validation parameters of proposed HPLC method is given in Table 1.

VALIDATION ARAMETERS	**ACCEPTANCE CRITERIA**	**RESULTS**	
System Suitability	%RSD of Standard solution should be not more than 2.0.	%RSD of Standard solutions Drot was 0.27 & for Ace was 0.16	
	The tailing factor [Asymmetry] shall not be more than 2.	The tailing factor of standard solution of Drot was 1.2 & for Ace was 0.9	
	Theoretical plate count shall be not less than 2000.	Theoretical plate count is more than 2000 i.e. 8278 for Drot & 5672 for Ace.	
Specificity	Peaks from the diluting solvent, the main peak and should not interfere with one other.	No interference was found in any peaks.	
	%RSD of standard solution should be not more than 2.0.	%RSD of standard solution for both the drugs was within the limits.	
Precision	%RSD of the areas obtained from six replicate injections of Standard solution should be not more than 2.0	RSD % for the area's obtained from six replicate injections of Standard solution is 0.59 for Drot & 0.43 for Ace.	
Linearity	The correlation coefficient should be not less than 0.99.	Correlation coefficient for Drot was 0.996 and for Ace was 0.997	
Accuracy/Recovery	The recovery of both the drugs at different levels should be between 90.0% and 115%	The accuracy of Drot was 98.59% and for Ace was 99.33%.	
Assay	The % assay of drug should be not less than 90 % and not more than 115 %	The % assay for Drotaverine Hydrochloride was 98.08 % and for Aceclofenac was 98.3%	
Validation parameters	**Acceptance criteria**	**Results**	
		DRO	ACE
Robustness	**PARAMETERS**	%RSD of Drot peak area	%RSD of Ace peak area
	Actual Wavelength (231nm)		
	Set Wavelength 229nm	0.772	0.711
	Set Wavelength 233nm	0.862	0.709
	Actual flow rate 1 ml		
	Set flow rate 0.9ml	0.886	0.465
	Set flow rate 1.1ml	0.881	0.702

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise

and robust, thus can be used for routine analysis of DRO and ACE in combined tablet dosage form.

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