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

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ABSTRACT
A simple, accurate, sensitive and validated RP-HPLC method for simultaneous determination of Drotaverine hydrochloride and Aceclofenac in combined tablet dosage form has been developed. Separation was carried out on Jasco HPLC system equipped with Hypersil GOLD C_{18} column (250 x 4.6 mm i.d.) and UV/VIS detector using Methanol: 10 mM potassium dihydrogen phosphate buffer in ratio of (80:20, v/v) as mobile phase and detection was carried out at 231 nm. Ambient temperature conditions were maintained. Results were linear in the range of 4-20 µg/ml for Drotaverine hydrochloride and 4-24 µg/ml for Aceclofenac. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Keywords: RP-HPLC, Drotaverine hydrochloride, Aceclofenac, Tablet dosage form.

INTRODUCTION

Drotaverine hydrochloride (DRO) chemically 1-[(3, 4-[diethoxyphenyl) methylene]-6,7-Diethoxy-1, 2,3,4-tetrahydroisoquinolene is an papaver analogue mainly used as an antispasmodic and smooth-muscle relaxant in pain associated with gastrointestinal colic[1]. Aceclofenac (ACE) chemically 2-[(2, 6-dichlorophenyl) amino] phenylacetoxyacectic acid is a phenylacetic acid derivative with potent analgesic and anti-inflammatory properties[2].

Literature survey reveals several high performance liquid chromatographic (HPLC) [3-6] methods for determination of DRO in human plasma and in pharmaceutical formulations either as single and in combination with other drugs. Spectrophotometric methods for simultaneous estimation of DRO with other drugs have also been reported [7-9]. HPLC methods have been reported for the determination of ACE either in single or in combination with other drugs[10-13]. HPTLC methods have been reported for determination of ACE in single or in combination with other drugs[14,15]. Spectrophotometric methods for simultaneous estimation of ACE with other drugs also reported[16,17].

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 quantification 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[18].

MATERIALS AND METHODS

Chemicals and reagents:

Working standards of pharmaceutical grade DRO and ACE were obtained as generous gifts from Alkem Drugs & Pharmaceuticals Ltd. (Haridwar, India) and was used as such without further purification. The pharmaceutical dosage form used in this study was Canefo-D tablets (Medopharm, Chennai, 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) purchased from Merck specialties Pvt. Ltd. (Mumbai, India) and double distilled water were used in analysis.

Instrumentation and chromatographic conditions:

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump and UV-2075 plus UV/VIS detector and JASCO Borwin 1.50 version software was used for analysis. Separation was carried out on Hypersil GOLD C_{18} (250 x 4.6 mm i.d.) column using Methanol: 10mM Potassium dihydrogen phosphate buffer in ratio of (80:20, v/v) as mobile phase at flow rate of 1 ml/min. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 231 nm. All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of standard solutions:

Standard stock solutions of pure drugs were prepared separately by dissolving 10 mg of each drug in 100 ml of mobile phase to get concentration of 100 µg/ml for both DRO and ACE.

Preparation of sample solution:

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of ACE (8 mg of DRO) was transferred to 10 ml volumetric flask containing 7 ml of mobile phase and ultrasonicated for 5

min. The volume was made up to the mark with the mobile phase and filtered through Whatman paper No. 41. 0.1 ml of filtrate was further diluted to 10 ml with mobile phase to get solution of concentration 10 µg/ml of ACE (8 µg/ml of DRO). After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

System suitability:

The system suitability was assessed by six replicate injections of the mixture containing 10 µg/ml of both the drugs. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table 1: System suitability parameters for RP-HPLC method

Parameters	Values	
	ACE	DRO
Theoretical plates	3103	4283
Asymmetry Factor	1.18	1.36
HETP (cm)	0.00805	0.00583
Resolution*	-	3.03

*With respect to previous peak

Method validation:

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines[18].

Linearity:

Aliquots 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml of working standard solutions of ACE (100 µg/ml) and 0.4, 0.8, 1.2, 1.6 and 2 ml of DRO (100 µg/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.

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 were calculated, results of which are represented in Table 2.

Table 2: Recovery studies of DRO & ACE

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Total amount found (µg/ml)	% Recovery*	% RSD*
ACE	10	8	17.87	99.27	1.477
	10	10	19.86	99.31	0.641
	10	12	22.15	100.70	0.711
DRO	8	6.4	14.27	99.11	0.464
	8	8	15.92	99.55	1.557
	8	9.6	17.42	99.01	0.459

* Average of three determinations

Limit of detection and Limit of quantitation:

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

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 three factors were selected for change: flow rate of the mobile phase (1.0 ± 0.02 ml/min), a wavelength at which the drugs were recorded (231 ± 1 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: 10 mM Potassium dihydrogen phosphate buffer in ratio of (80:20, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 3.240 **±** 0.0619 and 4.313 **±** 0.0525min (Mean ± S.D.) for ACE and

DRO respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

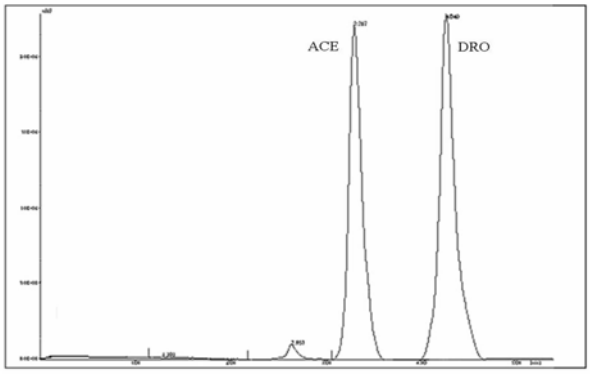

Figure 1: Representative chromatogram obtained for standard mixture of ACE (10 μg/ml, 3.240 ± 0.0619 min), DRO (10 μg/ml, 4.313 ± 0.0525 min)

Results were found to be linear in the concentration range of 4-24 μg/ml for ACE and 4-20 μg/ml for DRO. The correlation coefficients for the plots were 0.9991 for ACE and 0.9989 for DRO. The proposed method was also evaluated by the assay of commercially available tablets containing ACE and DRO. The % assay was found to be 98.81 ± 1.182 for ACE and 102.37 ± 0.752 for DRO (mean ± S.D., n = 6). The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method (data not shown), 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 3.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Parameters	ACE	DRO
Linearity range (μg/ml)	4-24	4-20
Correlation co-efficient	0.9991	0.9989
Slope (m)	17824	22187
Intercept (c)	5518	13004
Limit of detection (μg/ml)	0.354	0.896
Limit of quantitation (μg/ml)	1.170	2.957
Accuracy (% Recovery)	99.27 - 100.70	99.01 – 99.55
Precision (% RSD)		
Intraday (n = 3)	1.033	0.822
Inter day (n = 3)	0.751	0.491

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.

Acknowledgement: The authors express their gratitude to Alkem Drugs & Pharmaceuticals Ltd. (Haridwar, India) for the gift sample of pure Drotaverine hydrochloride and Aceclofenac. Thanks also extended to Dr. A. R. Madgulkar, Principal, AISSMS College of Pharmacy for providing necessary facilities and her constant support.

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