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RP-HPLC method for simultaneous estimation of Drotaverine hydrochloride and Aceclofenac in their combined tablet dosage form

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ABSTRACT

Drotaverine hydrochloride and aceclofenac are prescribed for the treatment of adult patients with muscular pain associated with spasm. This study describes a rapid, simple, precise and accurate RP-HPLC method for simultaneous estimation of drotaverine hydrochloride and aceclofenac in their combined tablet dosage form. The separation was achieved on a Phenomenex-Gemini (150mm x 4.6mm, 5 µm) column with an isocratic mixture of methanol, acetonitrile and water in the ratio of 60:30:10, pH adjusted to 3.0, at a flow rate of 1.0 ml/min and UV detection at 298.5 nm. The retention time for drotaverine hydrochloride and aceclofenac was 1.06 and 4.19 min respectively. The method was linear in the range of 8-40 µg/ml and 10-50 µg/ml for drotaverine hydrochloride and aceclofenac respectively. The correlation coefficients were 0.9997 for drotaverine hydrochloride and 0.9995 for aceclofenac. The method was validated as per ICH guidelines and successfully applied for estimation of drotaverine hydrochloride and aceclofenac in commercially available tablet dosage form.

Key words: Drotaverine hydrochloride, Aceclofenac, RP-HPLC.

INTRODUCTION

Drotaverine (DRV), [(1 - (3, 4 – diethoxybenzylidene) - 6, 7 – diethoxy - 1, 2, 3, 4 tetrahydroisoquinoline) hydrochloride], a benzylisoquinoline derivative [1] is a highly potent spasmolytic agent and has excellent smooth muscle relaxant properties [2,3]. Its antispasmodic activity is due to inhibition of phosphodiesterase enzyme IV. It causes smooth muscle relaxation by increasing intracellular levels of cyclic adenosine monophosphate (cAMP) secondary to inhibition of phosphodiesterase. Aceclofenac (ACF), 2-[(2,6-dichlorophenyl)amino]phenyl]acetyl] oxyacetic acid is used as anti-inflammatory drug. It is official in B.P.[4] and I.P.[5]. The combined tablet formulation of DRV and ACF is available for the treatment of adult patients with muscular pain associated with spasm. Reported methods for estimation of DRV include spectrophotometry [6-8], HPLC [9], thin layer chromatography [10,11] and voltammetry [12]. Analytical methods reported for estimation of ACF are spectrophotometry [13-15], HPLC [16-18], LC-MS [19] and fluorimetry [20]. A stability indicating HPLC method has been reported for simultaneous estimation of DRV and ACF in

tablet dosage form [21]. The present study provides a simple, rapid, precise and accurate RP-HPLC method with run time of only 5 min for simultaneous estimation of DRV and ACF in tablet dosage form and is useful in routine quality control of dosage forms. The method was validated as per ICH guidelines [22].

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade methanol and acetonitrile (S.d. fine chem.ltd.) and triple distilled water were used for analysis. Pure drug samples of DRV and ACF were received as gift samples from Astran Labs, Ahmedabad, Gujarat, India. Tablets of this combination (ESNIL tablets, Cosmas Pharmacls), labeled content 80 mg of DRV and 100 mg of ACF, were procured from the local market.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a HPLC system (Shimadzu, LC-10AT) with UV/Vis detector (SPD-10A) using Rheodyne injector (7725i) with 20 µl fixed loop and data analysis was done using Clarity software. Separation and analysis were carried out on Phenomenex-Gemini (150mm x 4.6mm, 5 µm) column. Mobile phase consisted of a mixture of methanol, acetonitrile and water (60:30:10 v/v/v), adjusted to pH 3.0 with 5% o-phosphoric acid, filtered through 0.45 micron membrane filter and delivered at ambient temperature at flow rate of 1.0 ml/min. Detection wavelength was 298.5 nm.

Standard solutions and calibration curves

Standard stock solutions (1000 µg/ml of DRV and 1250 µg/ml of ACF) were prepared in methanol. Working standard solutions (100 µg/ml of DRV and 125 µg/ml of ACF) were prepared by separately diluting 10 ml of stock solutions to 100 ml with methanol. Working standard solutions were diluted further to get concentration range of 8, 16, 24, 32, 40 µg/ml of DRV and 10, 20, 30, 40, 50 µg/ml of ACF using mobile phase and analyzed by the developed HPLC method. Calibration curves were constructed by plotting peak areas versus concentration and correlation coefficients were computed.

Selection of detection wavelength

An aliquot of 2.4 ml of working standard solution of DRV (100 µg/ml) and ACF (125 µg/ml) each were diluted separately to 10 ml with mobile phase to get solutions containing 24 µg/ml of DRV and 30 µg/ml of ACF. Each solution was scanned between 200-400 nm using a UV visible spectrophotometer. Wavelength for detection was selected from the overlay spectra of DRV and ACF.

Assay of tablet formulation

Twenty tablets were weighed and powdered and a quantity of tablet powder equivalent to 100 mg of DRV or 125 mg of ACF was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatman filter paper and 1 ml of the filtrate was diluted to 10 ml with methanol. From the diluted solution, 2.4 ml was further diluted to 10 ml with mobile phase and analyzed by HPLC.

System suitability

System suitability for the RP-HPLC method was determined by calculating relative standard deviation in retention time of 6 replicate injections, tailing factor and resolution of peaks.

Validation

Linearity

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity of response for DRV and ACF was assessed by analysis of five independent levels of concentrations in range of 8-40 µg/ml for DRV and 10-50 µg/ml for ACF. Graphs of concentration versus area of DRV and ACF peaks were plotted and correlation coefficients were determined.

Precision

Precision of the method was evaluated by determining repeatability, intraday precision and interday precision and expressed in terms of % relative standard deviation (%RSD).

Repeatability

Combined standard solutions at three levels 16, 24 and 32 µg/ml of DRV and 20, 30 and 40 µg/ml of ACF were prepared in triplicate and analyzed. The peak area obtained with each solution was measured and % RSD was calculated.

Intraday precision

Solutions prepared for calibration curve, containing 8-40 µg/ml of DRV and 10-50 µg/ml of ACF were analyzed five times on the same day and % RSD was calculated.

Interday precision

Solutions containing 8-40 µg/ml of DRV and 10-50 µg/ml of ACF were prepared and analyzed on five consecutive days and % RSD was calculated.

Accuracy

Recovery studies were carried out by addition of standard drug solution (0.8, 1.6, 2.4 ml of 100 µg/ml of DRV and 125 µg/ml of ACF) to pre-analyzed sample solution containing 80 µg/ml and 100 µg/ml of DRV and ACF respectively and diluted suitably to get three levels of concentration. Each solution was analyzed and the amount of DRV and ACF was calculated at each level and % recoveries were computed.

LOD and LOQ

They were estimated from the set of five calibration curves. The equations used were LOD = 3.3 × (SD/slope) and LOQ = 10 × (SD/slope), where SD is standard deviation of the Y- intercepts and slope is mean slope of the five calibration curves.

Solution stability

The standard and sample solutions prepared as per the procedure were kept at ambient laboratory conditions and analyzed by HPLC after 12 hours to monitor for change in concentration or presence of additional peaks of degradation products. No change in concentration was observed and no new peaks were found after 12 hours indicating that the solutions are stable.

RESULTS AND DISCUSSION

The simultaneous estimation of DRV and ACF in their combined tablet dosage form was carried out by RP-HPLC using methanol, acetonitrile and water as mobile phase in the ratio of 60:30:10 v/v/v (pH 3.0) and Phenomenex-Gemini C18 column as the stationary phase. This was found to give optimum separation and the optimized chromatographic conditions are shown in Table 1. The results of system suitability parameters such as relative standard deviation in retention times

of replicate injections, tailing factor and resolution are presented in Table 2. The retention time of DRV and ACF was 1.06 and 4.19 min respectively. Chromatogram showing retention times of DRV and ACF is shown in figure 1.

Table 1: Optimized chromatographic conditions

Column	Phenomenex-Gemini C18
Mobile phase	Methanol:acetonitrile:water (60:30:10 v/v/v) (pH 3.0 with o-phosphoric acid)
Flow rate	1.0 ml/min
Detection wavelength (UV)	298.5 nm
Temperature	Ambient
Retention time of DRV	1.06 min
Retention time of ACF	4.19 min

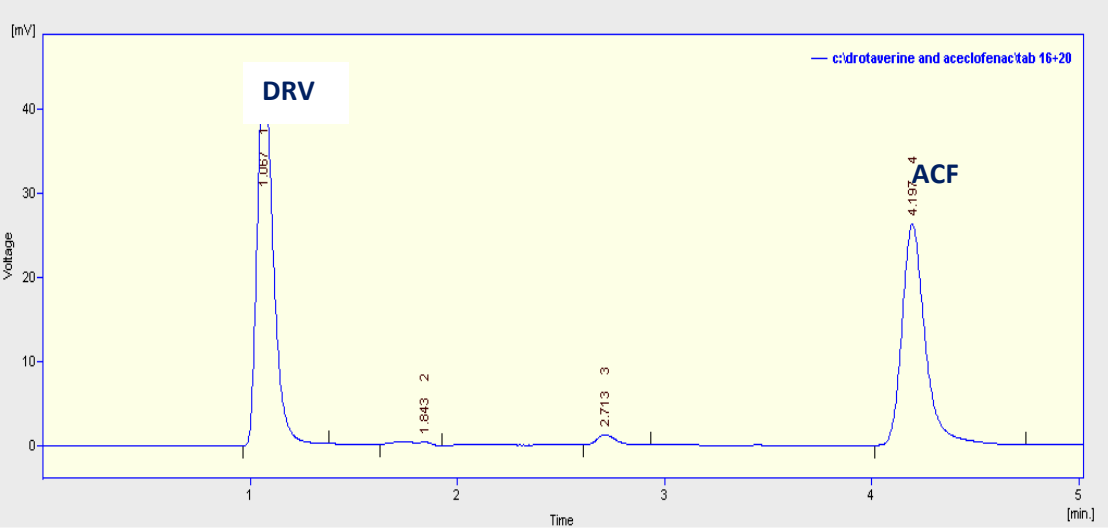

Fig. 1: Chromatogram showing retention times of DRV (24 µg/ml) and ACF (30 µg/ml)

Table 2: System suitability parameters

Parameters	Results	
	DRV	ACF
% RSD in retention time (n=6)	0.26	0.33
Tailing factor	1.42	1.31
Resolution	8.95	

Selection of detection wavelength

Wavelength selected for detection was 298.5 nm where DRV and ACF both showed adequate absorbance (Fig.2).

Validation

Linearity

The linearity was studied in the concentration range 8-40 µg/ml for DRV and 10-50 µg/ml for ACF. The correlation co-efficient for DRV and ACF were found to be 0.9997 and 0.9995 respectively. Calibration curves for the drugs are shown in Fig.3. Overlain chromatograms of five concentrations of binary mixtures of DRV and ACF are shown in Fig.4.

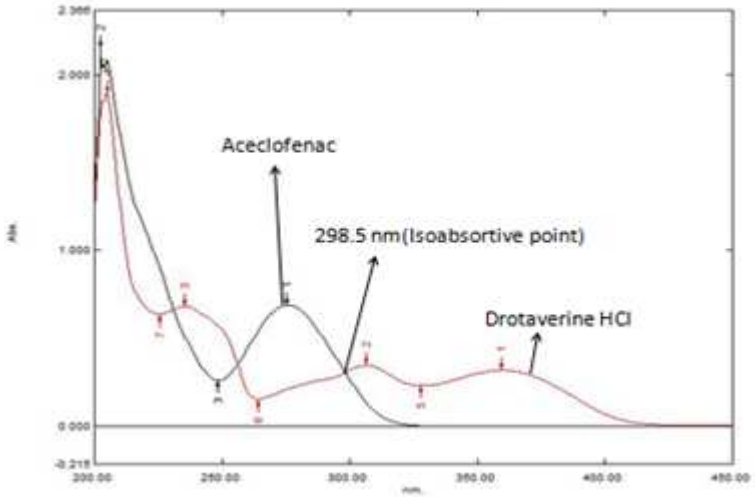

Fig.2: Overlain UV spectra of DRV and ACF showing wavelength for detection

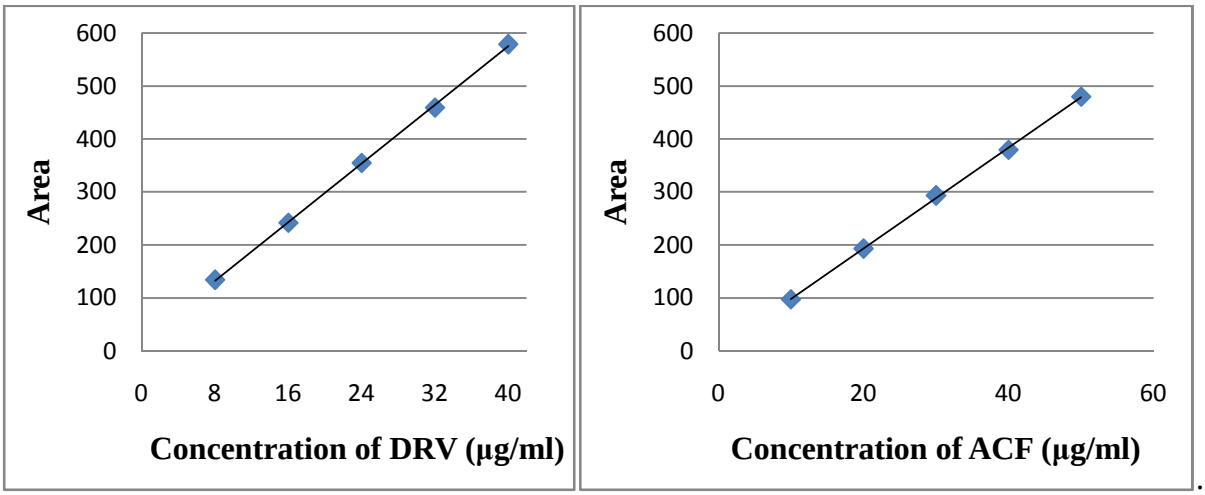

Fig 3: Calibration curves for DRV (8-40 µg/ml) and ACF (10-50 µg/ml)

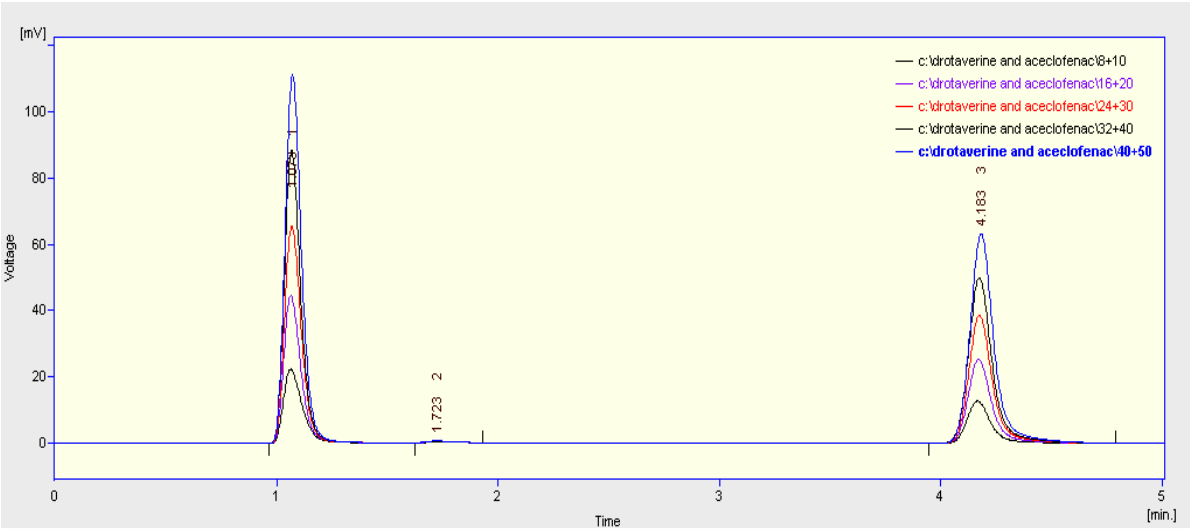

Fig. 4: Overlain chromatogram of mixtures of DRV (8-40 µg/ml) and ACF (10-50 µg/ml)

Precision

Results of repeatability, intra-day and inter-day precision are expressed as %RSD. In repeatability study, dilutions at three levels were analyzed in triplicate and % RSD was found to be 0.57-0.65 for DRV and 0.65-0.87 for ACF. Results for intraday and interday precision are shown in Table 3. Intraday variation ranges from 0.34-0.80% for DRV and 0.50-0.96% for ACF. Interday precision was found to be 1.51-2.45% for DRV and 1.21-1.97% for ACF.

Table 3: Results of precision study

DRV			ACF		
Concentration (µg/ml)	**Intraday % R.S.D. (n=5)**	**Interday % R.S.D. (n=5)**	**Concentration (µg/ml)**	**Intraday % R.S.D. (n=5)**	**Interday % R.S.D. (n=5)**
8	0.80	2.45	10	0.79	1.97
16	0.56	2.26	20	0.96	1.62
24	0.34	1.82	30	0.60	1.21
32	0.47	1.89	40	0.54	1.83
40	0.49	1.51	50	0.50	1.47

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation (ESNIL tablets) at three levels of standard addition in triplicate. The results are shown in Tables 4 and 5 for drotaverine and aceclofenac respectively. Percentage recovery for DRV was 99.29 - 101.75%**,** while for ACF, it was found to be in range of 98.60-99.55%.

Table 4: Recovery data for DRV from tablet formulation

Concentration of DRV in sample (µg)	Amount of standard DRV added (µg)	Total concentration of DRV (µg)	Mean concentration recovered (n=3)(µg)	DRV % Recovery
8	8	16	8.14	101.75
8	16	24	16.09	100.56
8	24	32	23.83	99.29

Table 5: Recovery data for ACF from tablet formulation

Concentration of ACF in sample (µg)	Amount of standard ACF added (µg)	Total concentration of ACF (µg)	Mean concentration recovered (n=3) (µg)	ACF % Recovery
10	10	20	9.86	98.60
10	20	30	19.91	99.55
10	30	40	29.66	98.87

LOD and LOQ

They were estimated from the set of five calibration curves using standard deviation of the Y-intercepts and mean slope of the five calibration curves. LOD and LOQ for DRV were found to be 0.025 µg/ml and 0.229 µg/ml respectively and for ACF were 0.076 µg/ml and 0.695 µg/ml respectively. Results of all validation parameters are summarized in Table 6.

Assay of tablets

The validated method was applied to estimate DRV and ACF in marketed tablets. Results of assay are presented in Table 7. The content of DRV and ACF in tablets was found to be 99.79 and 101.69% of label claim respectively.

Table 6: Summary of method validation parameters

PARAMETERS	DRV	ACF
Linearity range	8-40 µg/ml	10-50 µg/ml
Correlation coefficient	0.9997	0.9995
Precision		**% R.S.D**
Repeatability (n=3)	0.57-0.65	0.65-0.87
Intraday (n=5)	0.34-0.80	0.50-0.96
Interday (n=5)	1.51-2.45	1.21-1.97
% Recovery (n=3)	99.29-101.75	98.60-99.55
LOD (µg/ml)	0.025	0.076
LOQ (µg/ml)	0.229	0.695

Table 7: Assay of market formulation

Tablet	Label claim (mg/tablet)		Assay (% of label claim)	
	DRV	ACF	DRV	ACF
ESNIL	80	100	99.79	101.69

CONCLUSION

The proposed method is a rapid, accurate and precise analytical method for simultaneous determination of DRV and ACF in marketed tablet formulation and is easily applied for routine analysis as the total run time is short. Method validation has been demonstrated by determination of linearity, precision, accuracy, LOD and LOQ.

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