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Research Article

STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DROTAVERINE AND ACECLOFENAC

SUGANTHI AZHLWAR* AND THENGUNGAL KOCHUPAPPY RAVI

Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, 395, Sarojini Naidu Road, Coimbatore-641 044. E-mail: suganlemu@gmail.com

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of drotaverine and aceclofenac, using a RP-18 column and a mobile phase composed of 0.1%trifluoro acetic acid: acetonitrile (45: 55 %v/v), pH 3.4 adjusted with 1%triethyl amine. The retention time of drotaverine and aceclofenac were found to be 5.8 and 10.5 min, respectively. Linearity was established for drotaverine and aceclofenac in the range of 0.8-8 µg/ml and 1-10 µg/ml, respectively. The percentage recoveries of drotaverine and aceclofenac were found to be 99.97±0.3250and 99.25±0.2567%, respectively. Both the drugs were subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, and photolytic degradation. The degradation studies indicated drotaverine to be susceptible to alkali and neutral hydrolysis, H_2O_2, dry heat and direct sun light while aceclofenac showed degradation under acid, alkali and neutral hydrolysis. The degradation products of drotaverine and aceclofenac were well resolved from the pure drugs with significant differences in the retention time values. This method can be successfully employed for simultaneous quantitative analysis of drotaverine and aceclofenac in bulk drugs and formulations.

Keywords: Drotaverine, Aceclofenac, Degradation products, HPLC-PDA.

INTRODUCTION

Drotaverine and aceclofenac fixed dose combination tablet contains 80 mg drotaverine and 100 mg aceclofenac. Drotaverine hydrochloride (DRO) chemically 1-[(3,4-[diethoxyphenyl) methylene]- 6,7-diethoxy-1, 2,3,4-tetrahydroisoquinolene is mainly used as an antispasmodic and smooth-muscle relaxant[1]. Aceclofenac, (ACE) chemically, 2-[(2',6- dichlorophenyl) amino] phenyl-acetoxyacectic acid, is a phenylacetic acid derivative with potent analgesic and anti-inflammatory properties. It is official in *Indian Pharmacopoeia*[2]. Literature survey revealed RP-HPLC simultaneous determination of DRO in presence Nifuroxazide[3] as well as Omeprazole[4] in pharmaceutical samples. Spectro-photometric[5,6] and HPTLC[7,8] methods have been reported for the estimation of DRO in combination with other drugs. Spectrofluorometric[9] measurement of DRO also has been reported. HPLC method has been reported for estimation of ACE in formulations in combination with other drugs[10]. Few spectrophotometric[11,12,13] and HPTLC[14,15] methods are also reported for aceclofenac. Extensive literature survey reveals that no chromatographic method has been reported for simultaneous determination of Drotaverine hydrochloride and Aceclofenac in tablet dosage form.

The International Conference on Harmonization (ICH) guideline entitled 'Stability testing of new drug substances and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances[16]. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine DRO and ACE simultaneously, in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought necessary to study the stability of DRO and ACE under acidic, alkaline, neutral hydrolysis, oxidative, dry heat, and photolytic conditions. This paper reports validated stability-indicating HPLC method for simultaneous estimation of DRO and ACE in presence of their degradation products.

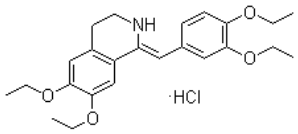


Fig. 1: Structure of drotaverine(DRO)

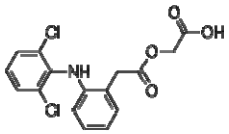


Fig. 2: Structure of aceclofenac (ACE)

MATERIALS AND METHODS

Materials

Pure samples of drotaverine and aceclofenac were obtained as gift samples from AD Pharmaceuticals Ltd, India. Tablet Canfeo-D was purchased from local market. Acetonitrile and water used were of HPLC grade and were purchased from Merck, India. The liquid chromatograph mass spectrometer Shimadzu LCMS-2010EV, which consisted of following components: a binary gradient pump, variable wavelength programmable PDA detector with auto sampler system was employed for the present study.

Instrumentation and chromatographic conditions

The chromatographic analysis was performed using Compaq Intel Core-2 DUO HP W/907 software on a pre-packed RP-18 column (250×4.6 mm, 5 µm particle size). In addition, an electronic balance (Shimadzu. Elec.balance BL-220H), a p^H meter (Eli co L127), a sonicator (Leclasonic ultrasonic cleaner), a hot air oven (Inlab equipments Ltd) were used in the study. Separation was achieved using a mobile phase consisting of 0.1%trifloro acetic acid: acetonitrile (45:55v/v), at a flow rate of 1ml/min and the eluent was monitored using PDA detector at 243 and 275 nm. The column was maintained at ambient temperature and injection volume of 20 µl was used.

Preparation of standard sample solutions

Standard Preparation

Standard stock solutions containing 80 µg /ml of DRO and 100 µg/ml were prepared in separate 100 ml volumetric flasks using acetonitrile. A stock solution containing mixture of DRO and ACE in the ratio of 8:10 was also prepared using acetonitrile. Working solutions were prepared by diluting the stock solutions with mobile

phase to contain 0.8-8 µg/ml for DRO and 1-10 µg/ml for ACE. These solutions were used to obtain the calibration graph by plotting peak area versus concentrations and regression equations were computed for both the drugs. (Table 1)

Sample preparation

Twenty tablets (Canfeo-D, Medopharm, Chennai) each containing 80 mg of DRO and 100 mg of ACE were weghed, and powder equivalent to to 8 mg of DRO was weighed accurately and taken into 100ml volumetric flask. The drugs were extracted into acetonitrile, volume was adjusted to 100ml, vortexed and then filtered through a 0.22µm nylon filter. From this solution, further dilutions were made using mobile phase to get a final concentration of 8 µg/ml of DRO and 10 µg/ml of ACE. Twenty microlitres of solution was injected into HPLC system to obtain chromatogram for standard drug solution (6 replicates) and sample solution (6 replicates). Concentrations of DRO and ACE in the formulation were calculated by comparing the peak area of sample with that of standard.

Forced degradation studies

Forced degradation studies of both the drugs were carried out under conditions of acid/base/neutral hydrolysis, oxidation, dry heat and photolysis. Dry heat and photolytic degradation of drug product were carried out in solid state. For each study, four samples were prepared: the blank solution stored under normal condition, the blank subjected to stress in the same manner as the drug solution, zero time sample containing the drug which was stored under normal conditions and the drug solution subjected to stress treatment.

Forced degradation studies were conducted separately and for the combination for DRO and ACE at a concentration of 80 µg/ml and 100 µg/ml respectively. Then the study was extended for the formulation. Forced degradation with acidic media was performed by heating the drug under reflux with 1M hydrochloric acid for 2 ½ hours. Base hydrolysis included heating the drug solution under reflux with 0.01M NaOH for 1 hour. To study neutral hydrolysis, the drug was dissolved in acetonitrile and heated under reflux with water for 12 hours. Degradation with hydrogen peroxide was performed by treating the drug solution with 10% H_2O_2 (v/v) for 4 hours at room temperature. For thermal degradation, solid drugs were kept in Petri dish in oven at 80°C for 12 hours. The photolytic degradation study was also performed by exposing the drug to sunlight for 5 hours.

The solutions were then left to equilibrate to room temperature and an aliquot of sample was withdrawn and diluted with mobile phase to get the concentration equivalent to 8 µg/ml of DRO and 10 µg/ml of ACE. Then 20 µl solutions was injected into the HPLC system and analyzed under the chromatographic condition described earlier.

RESULTS AND DISCUSSION

Different mobile phases were employed and proposed chromatographic condition was found to be appropriate for the quantitative determination of drotaverine and aceclofencac in presence of their degradation products. The optimum mobile phase consisted of 0.1%triflouro acetic acid : acetonitrile (45: 55, v/v) having pH 3.4 adjusted with 1%triethyl amine, selected because it was found to ideally resolve the peaks of DRO (R_t 5.8 min) and ACE (R_t 10.5min), with adequate separation in presence of their degradants at a flow rate of 1 ml/min. UV detection wavelength at 243 and 275nm, injection volume 20µl, ambient temperature for column and HPLC system was found to best for analysis.

ICH guidelines recommend 10-20% degradation for establishing stability indicating nature of the assay method[16] while Singh and Bakshi, in their article on stress testing suggested a target degradation of 20-80%[17]. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 10-80%, this could not be achieved in some cases even after exposure for prolonged duration (12 hours). Table 1 indicates the extent of DRO and ACE degradation under various stress condition. Fig. 3 and 4 show normal chromatogram of bulk and formulation while fig.5-10 shows the chromatogram of forced degraded samples. The degradation study indicated that DRO was susceptible to alkaline hydrolysis more than neutral hydrolysis. Degradation also occurred when exposed to H_2O_2, and direct sunlight whereas ACE was susceptible to alkaline and neutral hydrolysis more than acid hydrolysis and stable to H_2O_2, thermal and direct sunlight. Specificity of the method for the simultaneous estimation of DRO and ACE in presence of their degradants was demonstrated by the absence of co-eluting peaks with the main peaks.

Table 1: Summary of degradation studies for DRO and ACE

Degradation condition	Time (h/day)	%DRO	%ACE	t_R(min) of DP	
				DRO	ACE
Acid,1M HCl (reflux at 80°c)	2 ½ hrs	94.5	69.97	----	9.8
Base,0.01 M NaOH (reflux at 70°c)	1 hr	64.15	27.10	7.4	9.8
Neutral , water (reflux at 80°c)	12 hrs	84.53	59.97	7.4	9.8
Oxidative, 10%v/v H_2O_2(ambient, in dark)	4 hrs	69.53	96.45	4.4, 7.4	----
Dry heat (80°c)	12 hrs	97.2	95.12	-----	-----
Direct sun light(photolysis)	5 hrs	66.96	91.88	7.4	-----

t_R - retention time, ND - no degradation observed, DP – degradation product

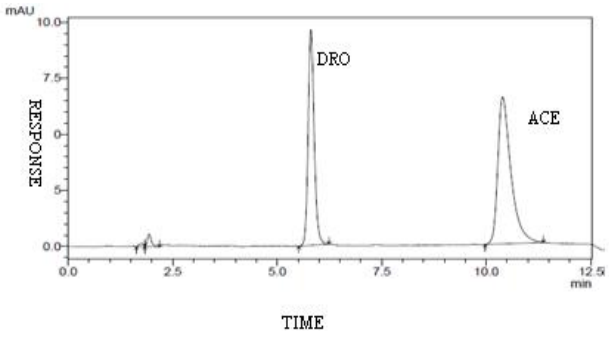


Fig. 3: Chromatogram of mixture of untreated DRO and ACE (bulk)

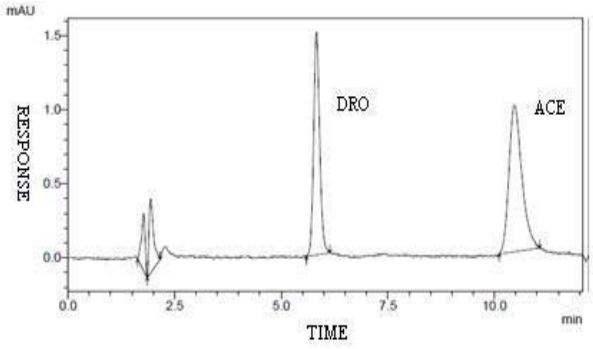


Fig. 4: Chromatogram of untreated marketed formulation of DRO and ACE

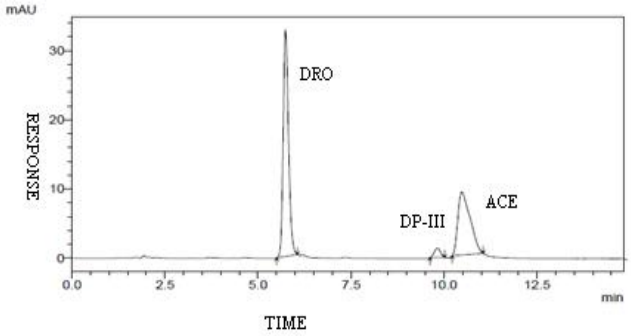
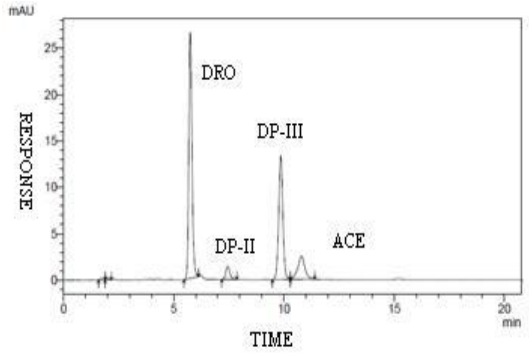


Fig. 5: Chromatogram of mixture of DRO and ACE degradated with 1M hydrochloric acid



lol

Fig. 6: Chromatogram of mixture of DRO and ACE degradated with 0.01M sodium hydroxide

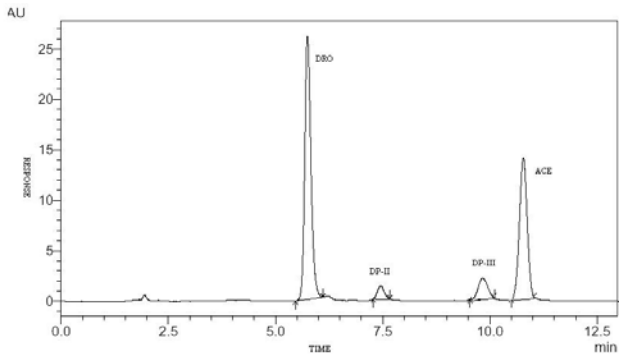


Fig. 7: Chromatogram of mixture of DRO and ACE degradated under neutral hydrolysis

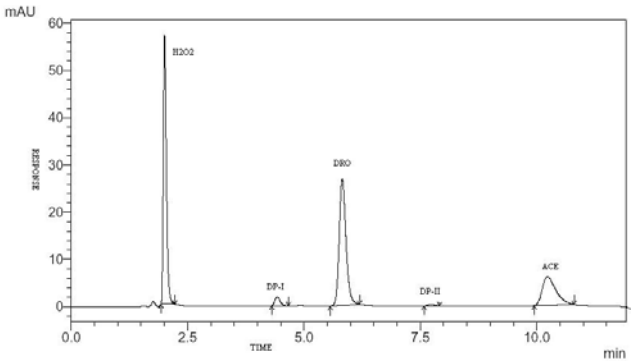


Fig. 8: Chromatogram of mixture of DRO and ACE degradated with 10%v/v hydrogen peroxide

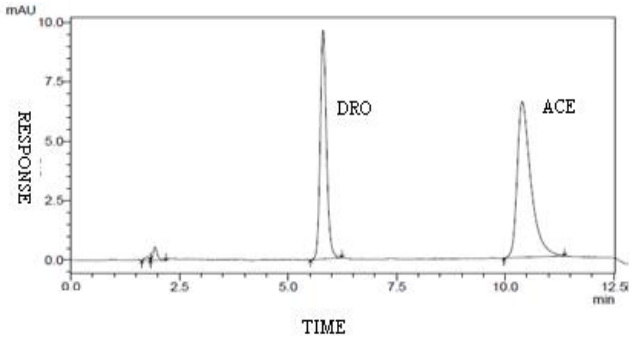


Fig. 9: Chromatogram of mixture of DRO and ACE degradated under dry heat

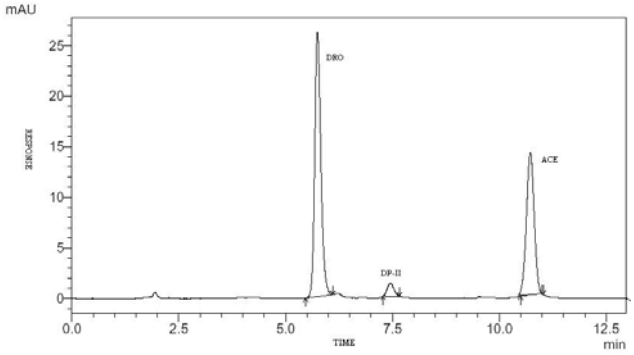


Fig. 10: Chromatogram of mixture of DRO and ACE degradated under direct sunlight

Table 2: Summary of validation and system suitability test parameters

PARAMETER (units)	DRO	ACE
Linearity range (µg/ml)	0.8-8	1-10
Correlation Coefficient(±SD)(n=3)	0.9997±0.00029	0.9877±0.021
LOD (ng/ml)	10	25
LOQ (µg/ml)	0.8	1.0
Recovery (%±RSD)(n=3)	99.97±0.3250	99.25±0.2567
Interday precision (%RSD)	0.0425	0.0296
Intraday Precision (%RSD)	0.0250	0.0755
Robustness	Robust	Robust
Retention Time (min)	5.8	10.5
Theoretical plates	9583	7634
Tailing Factor	1.1	1.3
Resolution	-------	11.6

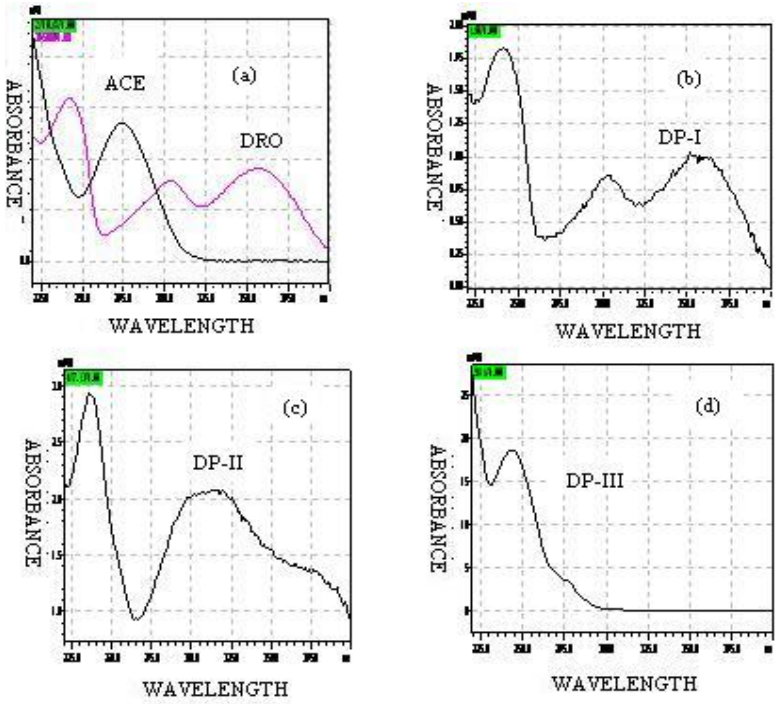


Fig 11. Comparative UV spectra between DRO, ACE and degradation products. (a) overlay spectra of DRO and ACE (b) DP-I (c) DP-II (d) DP-III.

The UV overlay spectra of pure DRO and ACE was compared with the spectrum of the drugs subjected to different forced conditions; Changes in the spectra were observed for all degradants of DRO except acid hydrolysis and dry heat (Fig –to -) while aceclofenac showed spectral changes only on acid, base and neutral hydrolysis (Fig). Purity of all degradation products was confirmed by comparing their spectra with the spectrum of DRO and ACE. Comparison of the spectra of two major degradation products, DP-I and DP-II, with the DRO spectrum (Figs. 3a and b) suggested the degradation of drotaverine to drotaveraldine and perparaldine[18]. The major degradation product of ACE was DP-III, which might be diclofenac[19]. The goal of determining DRO and ACE in presence of degradation products by the proposed stability indicating RP-HPLC-DAD method was successfully achieved and the method can be also used for routine quality control of tablets.

The described method has been validated for Linearity, LOD, LOQ, accuracy, precision, robustness and system suitability. The standard solutions for linearity were prepared at ten different concentration levels. The calibration curve for DRO and ACE was found to be linear over the range of 0.8-8 µg/ml and 1-10 µg/ml, respectively. Accuracy of the method was carried out by recovery studies using standard addition method at three different concentration levels. Summary of validation and system suitability test parameters are given in table 2.

Repeatability of measurements of peak area was carried out using six replicates of concentration (4 and 5 µg/ml of DRO and ACE respectively). The intra- and inter-day variation of the method was carried out for one concentration level. The low % CV values of within a day, day to day variations and analyst to analyst variation for DRO and ACE revealed that the proposed method is precise.

For robustness evaluation of both the drugs, few parameters like flow rate, percentage of methanol in mobile phase and pH of mobile phase were deliberately changed. Table 3 shows the robustness evaluation of the method. Each factor selected was changed at three levels with respect to the optimized parameters. Robustness of the method was done at the concentration levels 4 and 5µg/ml for DRO and ACE, respectively and the method was found to be robust.

Table 3: Robustness evaluation of the method

Factor	Level	Asymmetric factor		Number of theoretical plates		Resolution
		DRO	ACE	DRO	ACE	
Flow Rate (mL min^{-1})						
0.9	-0.1	1.1	1.5	6992	3960	11.6
1	0	1.2	1.1	7680	5290	11.6
1.1	+0.1	1.2	1.1	7688	5312	11.6
%B of mobile phase						
54	-1	1.6	1.4	5010	3578	11.6
55	0	1.2	1.1	7680	5290	11.6
56	+1	1.4	1.3	6851	4269	11.6
pH of mobile phase						
3.3	-0.1	1.3	1.2	4172	6785	11.6
3.4	0	1.2	1.1	7680	5290	11.6
3.5	+0.1	1.2	1.3	7680	4128	11.6

Table 4: Assay of DRO and ACE from tablet dosage form

Drug	Labeled amount (mg)	Amount found (mg)	Assay(%)±SD [a]
DRO	80	79.68	99.65±0.42
ACE	100	100.35	100.98±0.1

[a] n = 6

Assay of DRO and ACE from its tablet dosage form

The assay results of DRO and ACE in tablet dosage forms were comparable with thevalues of labeled claim. The results presented in Table 4 indicate the suitability of the method for routine analysis of DRO and ACE from their combined tablet dosage form.

CONCLUSION

The study presents a simple stability-indicating HPLC method for the simultaneous estimation of DRO and ACE in presence of their degradation products and validated as per ICH guidelines. Statistical analysis proved that the method developed was accurate, precise and repeatable. The method was successfully used for the estimation of drugs in pharmaceutical formulation. Assay results for combined dosage form using proposed method showed 99.65±0.42and 100.98±0.68, for DRO and ACE respectively. There was no interference observed due to excipients or other components present in the tablet dosage form. The results indicated the suitability of the method to study stability of DRO and ACE under various forced degradation condition viz. acid, base, neutral, oxidative, dry heat and photolytic degradation. It can be concluded that the developed method may be employed for analysis of stability samples of DRO and ACE since the method could separate the drugs from their degradation products.

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