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Research Article

APPLICATION OF RP-HPLC FOR THE SIMULTANEOUS ESTIMATION OF DROTAVERINE AND ACECLOFENAC IN COMBINED IN SOLID DOSAGE FORM

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Abstract

A new simple, precise, accurate, rapid and reproducible reverse phase high performance liquid chromatographic method has been developed for simultaneous determinationofDrotaverine and Aceclofenac in tablet formulations.The mobile phase used was a combination of ammonium acetate buffer (pH 3.2): acetonitrile(50:50). The detection of the tablet dosage form was carried out at 258 nm and a flow rate employed was 1 ml/min**.** All the parameters were validated according to the ICH guidelines and found to be within limits. Limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal-to-noise ratio. Linearity was obtained in the concentration range of 48-112 µg/ml of Drotaverine and 60-140 µg/ml Aceclofenac with a correlation coefficient of 0.995 and 0.996 respectively. The results of the analysis were validated statistically and recovery studies confirmed the accuracy of the proposed method. The developed method has been successfully used for the simultaneous estimation of both drugs in commercial formulation.**Keywords:** Drotaverine, Aceclofenac, RP-HPLC, Validation, Simultaneous estimation.

Introduction

Drotaverine[1-2]chemically it is a1-[(3, 4-diethoxy phenyl) methylene]-6,7-diethoxy-1,2,3,4-tetra hydro isoquinolene (**Figure 1a**) 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.

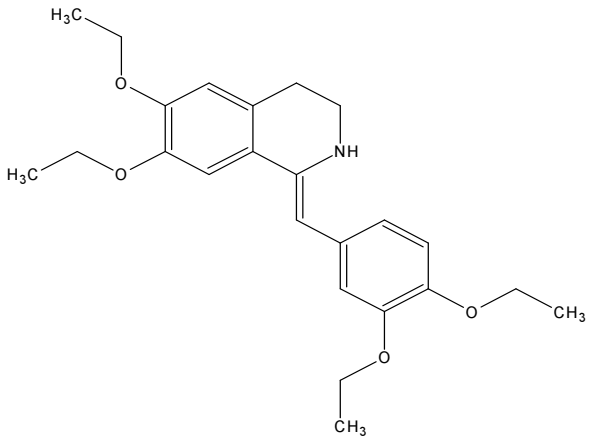

Fig.1a

Aceclofenac is a phenylacetic acid derivative NSAID with a chemical designation of [2-[(2,6-dichlorophenyl)amino]-pheny-lacetoxyacetic acid]. The chemical structure is shown in (**Figure 1b**)[3]. In vitro studies have shown that aceclofenac inhibits mediators of inflammatory activity, including prostaglandin (PG)E2, IL-1β, IL-6 and TNF[4]. Interference with the expressionof cell adhesion molecules has also been observed in humanneutrophils[5].Aceclofenac has also shown stimulatory effects on glycosaminoglycan synthesis in human osteoarthritic cartilage[6].

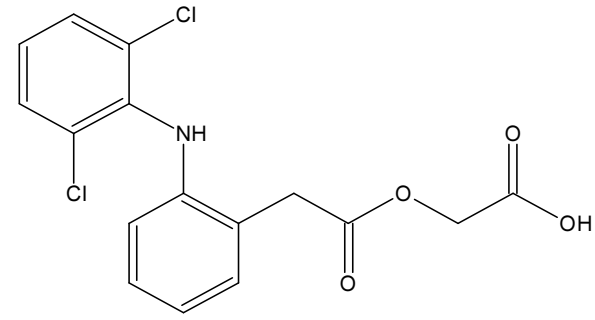

Fig. 1 b

Drotaverine hydrochloride and Aceclofenacthe combination was significantly superior to monotherapy the combination which is lead in patients with primary dysmenorrhoea[7].

Few techniques are reported for the estimation of Drotaverine and Aceclofenacsuch as HPLC[8-10], Stability indicating[11],spectroscopic[12-13] and HPTLC[14]methodsare available. The above fact indicates there is need to develop a sensitive, stable and accurate method, the novelty of the present method involves the use a chief, simple solvent and well separated drug under study in presence of different degrading products. So the present RP-HPLC method for the simultaneous determination of Drotaverine and Aceclofenac in bulk and tablet dosage form can be used in the quality control laboratory for routine analysis.

Materials and Methods

Instrumentation

An isocratic HPLC Cyber lab (Salo Terrace, Millbury, USA) with UV detector equipped with C_{18} RP Hypersil column (250 mm x 4.6mm x 5 µm) was employed for the study.

Reagent and Solution: Acetonitrile HPLC grade, Methanol HPLC grade, water HPLC grade were purchased from Merck chemicals, India.

Chromatographic conditions: For chromatographic analysis, C_{18} RP Hypersil column (250 mm x 4.6mm x 5 µm) was used. Separation was carried out by isocratic elution. The solvent system was a mixture of Ammonium acetate buffer (pH 4.5): Acetonitrile (50:50). It was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 15 min before passing through the instrument. The injection volume was 20 µl and the flow rate was 1ml/min. UV detection was carried out at 258 nm.

Preparation of Mobile Phase: 1000ml of mobile phase was prepared by mixing 500 ml Ammonium acetate buffer (pH 4.5), 500 ml of Acetonitrile and. pH adjusted to 4.5.

Filtration of Mobile Phase: The degassed mobile phase was filtered through 0.45µn filter to avoid the column clogging due to smaller particles.

Selection of mobile phase: Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this Ammonium acetate buffer (pH 4.5): Acetonitrile (50:50) was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions. The detection wavelength was measured by scanning the 10 mg/ml solution of Drotaverine and Aceclofenac in mobile phase, in UV spectrophotometry, overlaid spectra and the wavelength of maximum absorption was selected as 258 nm.

Preparation of standard stock solution

Drotaverine standard stock solution

Stock solution of standard drugs was prepared by weighing accurately 20mg of drotaverine was taken in a 50mL standard flask and make up with mobile phase, it consists of 400µg/mL of Drotaverine.

Aceclofenac standard stock solution

Stock solution of standard drugs was prepared by weighing accurately 25mg of aceclofenac was taken in a 50mL standard flask and make up with mobile phase. it consists of 500µg/mL of Aceclofenac. From this take 5mL and make up to 50mL with mobile phase and sonicated for 5 minutes to dissolve the drugs.

Quantification of Drotaverine and Aceclofenac

Twenty Tablets were weighed and powdered.Tablet powder having weight equivalent to 20mg of drotaverine and 25mg of aceclofenac was weighed accurately and taken in a 50 mL volumetric flask and dissolved in mobile phase. The solution was shaken well and allowed to stand for 15min with intermittent sonication to ensure complete solubility of drug. The contents were made up to the mark with Mobile Phase and filtered through a 0.45µ membrane filter. From the filtrate, dilution was made in a 10mL volumetric flask to get 20µg/mL of Drotaverineand 25µg/mL of Aceclofenac respectively with diluents. The peak area measurements were done by injecting each sample three times and theamount of Drotaverine and Aceclofenac were calculated.

Method Validation

As per the International Conference on Harmonization (ICH) guidelines. The method validation parameters such as specificity, linearity, precision, accuracy, limit of detection/quantization and robustness were optimized.

Linearity

To establish linearity, the stock solutions were prepared (1000µg/mL of both Drotaverine and Aceclofenac) using Mobile Phase as the solvent; again from the stock solution further dilutions were made to yield solutions in the concentration range of 48-112µg/mL of Drotaverineand 60-140µg/mL of Aceclofenac. 20µl of each solution was injected and records the chromatogram at 258nm.

The chromatogram optimized given in (Figure-2) and their system suitability parameters were given in (Table -1), the calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation coefficient was found to be above 0.995 and 0.996 Drotaverine and Aceclofenac.The optical characteristics of Atorvastatin calcium and Fenofibrate shown in (Table-2).

Figure-2: Optimized Chromatogram for Drotaverine and Aceclofenac

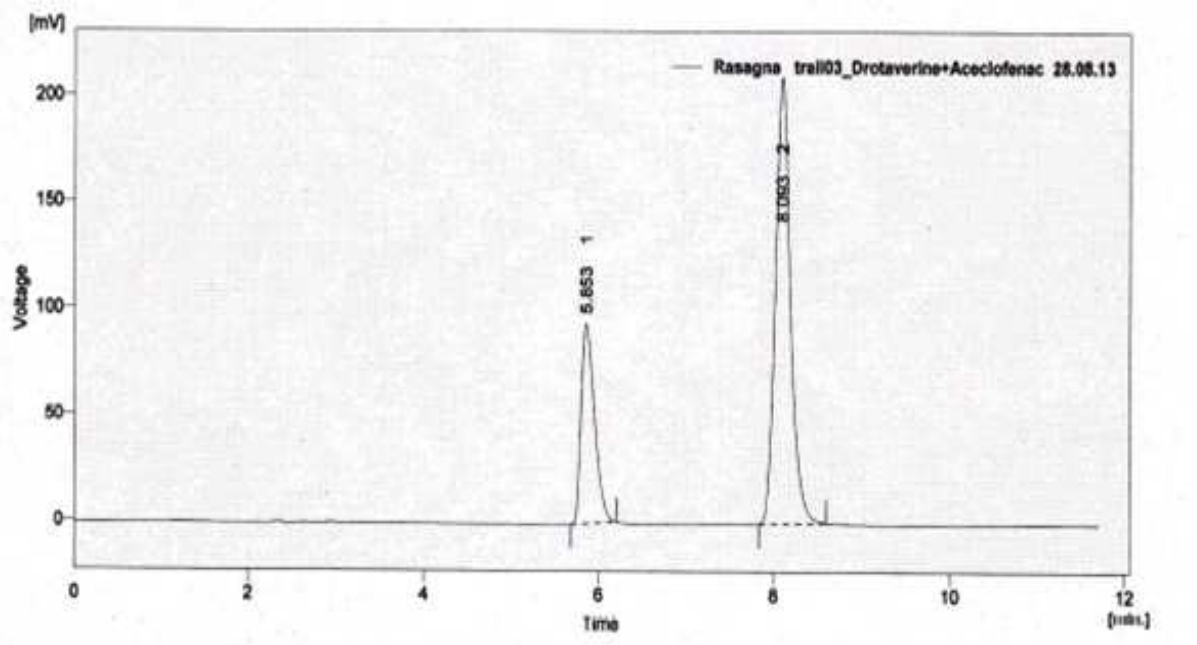

Table-1: System Suitability Parameters for the Optimized Chromatogram by RP-HPLC.

Parameters	Drotaverine	Aceclofenac
Retention time	5.853	8.093
Peak area	1008	2462
Tailing factor	1.34	2.0
Theoretical plates	6318	11627
Resolution	Between Drotaverine and Aceclofenac	7.532

Table-2: Optical Characteristics.

Parameters	Drotaverine	Aceclofenac
Detection Wavelength(nm)	229	281
Beers law limit (µg mL^{-1})	48-112	60-140
Regression equation(y=mx+c)	y = 24.42x-156.5	y = 50.95x-399.4

Correlation coefficient (r^2)	0.995	0.996
Slope (m)	24.42	50.95
Intercept (c)	156.5	399.4
LOD (μg mL^{-1})	3.42	2.05
LOQ (μg mL^{-1})	10.36	6.21

Precision

Precision was determined by analysing standard preparations of Drotaverine(20 μg/mL), Aceclofenac(25μg/mL), for six times. The chromatograms were recorded and the results were summarized in (Table 3).

Table -3: Quantification of tablet formulation (Zerodolol-spas) BY RP-HPLC.

Injection	**Drotaverine**		**Aceclofenac**	
	RT	**Area**	**RT**	**Area**
1	2.130	1773.318	4.787	4694.238
2	2.143	1757.981	4.797	4671.269
3	2.147	1770.881	4.803	4672.796
4	2.147	1774.701	4.807	4714.121
5	2.120	1768.959	4.697	4727.748
6	2.140	1753.751	4.710	4733.290
Average	2.1378	1766.599	4.767	4705.577
SD	0.0108	8.650	0.050	23.671
%RSD	0.50	0.49	1.04	0.50

Accuracy

The accuracy of the method was performed by recovery studies to the pre analyzed formulation, a known quantity of Drotaverine and Aceclofenac of working standard solutions were added at different levels, 80% (64µg/mL for Drotaverine; 80µg/mL for Aceclofenac), 100% (80µg/mL for Drotaverine; 100µg/mL for Aceclofenac), and 120% (96 µg/mL for Drotaverine 120µg/mL for Aceclofenac), (Three replicates each) of the theoretical concentrations were injected the solutions and the chromatograms were recorded.

The percentage of recovery for Drotaverine and Aceclofenac was found to be 98.92%, 100.29% respectively.

The results of recoveryin (Table 4) revealed that no interference was produced due to the excipients used in formulation. Therefore developed method was found to be accurate.

Table-4: Recovery data of Drotaverine and Aceclofenac.

Recovery Level (%)	Drug	Conc. of drug (µg/ml)		% Recovery	%Mean Recovery
		Drug Taken	Std. drug added		
80	Drotaverine	12	64	98.29	98.52
100		12	80	98.36	
120		12	96	98.92	
80	Aceclofenac	30	80	100.16	99.65
100		30	100	98.50	
120		30	120	100.29	

Robustness

The robustness of the method was determined as per USP guidelines under different conditions including change in flow rate and wavelength.The chromatograms were recorded and the results were summarized in (Table 5).

Table-5: Results of robustness by variations in flow rate and wavelength.

Parameter	Value	Drotaverine		Aceclofenac	
		RT	TF	RT	TF
Flow rate	0.8 mL/min	2.823	1.423	6.163	1.267
	1.0 mL/min	5.853	1.34	8.093	2.0
	1.2 mL/min	1.703	1.333	3.723	1.200
Wavelength	256nm	2.133	1.381	4.647	1.257
	258 nm	5.853	1.34	8.093	2.0
	260 nm	2.110	1.429	4.617	1.194

Conclusion

The present method was a sensitive, precise, and accurate HPLC method for the analysis of Drotaverine and Aceclofenac.To optimize the mobile phase, various combinations of buffer and organic solvents were used onC_{18} RP Hypersilcolumn.Then the mobile phase containing a mixture ofammonium acetate buffer (pH 3.2) and acetonitrile mixture was used as mobile phase in the ratio 50:50 v/v was selected at a flow rate of 1.0mL/min for developing the method and the peaks with good shape and resolution were found resulting in short retention time, baseline stability and minimum noise.Retention times of Drotaverine and Aceclofenac were found to be 5.85min, and 8.09min with a good tailing factor and theoretical plates which are within the limits.The LOD value was found to be 3.42µg/mL for Drotaverine 2.05µg/mL for Aceclofenac,which indicates the sensitivity of the method.The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations didn't interfere with the estimation of the drugs by the proposed HPLC method.

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