

SPECTROPHOTOMETRIC DETERMINATION OF DROTAVERINE AND ACECLOFENAC IN COMBINED TABLET DOSAGE FORM BY RATIO DERIVATIVE SPECTROSCOPY AND AREA UNDER CURVE (AUC) SPECTROPHOTOMETRIC METHODS

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ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of Drotaverine (DRT) and Aceclofenac (ACE) in combined dosage form has been developed. The first method is Ratio Derivative spectroscopy method (Method A) in which ratio derivative amplitudes were measured at selected wavelengths. Second method is Area under Curve Spectrophotometry (Method B). The amplitudes at 330.13 nm and 228.06 nm in the Ratio derivative spectra were selected to determine DRT and ACE, respectively and wavelength ranges 299-305 nm and 270-277 nm were selected to determine DRT and ACE, respectively by AUC method in methanol-distilled water. Beer's law is obeyed in the concentration ranges of 4-20 $\mu\text{g mL}^{-1}$ and 5-25 $\mu\text{g mL}^{-1}$ for DRT and ACE, respectively in method A while 4-24 $\mu\text{g mL}^{-1}$ and 5-30 $\mu\text{g mL}^{-1}$ by DRT and ACE, respectively in method B. The % assay for commercial formulation was found to be in the range 99.48 – 100.01 % for DRT and 99.52 – 99.89 % for ACE by the proposed methods. Recovery was found in the range 99.18-100.10 for DRT and 99.52 – 101.09% for ACE by ratio derivative spectroscopic method and 99.82 – 101.12% for DRT and 99.73-101.09% for ACE by AUC method for both the Formulations. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and of the proposed methods which were carried out by following ICH guidelines.

Keywords: Drotaverine, Aceclofenac, Ratio Derivative Spectroscopy, Area under Curve.

INTRODUCTION

Drotaverine hydrochloride [DRT], 1-[(3, 4-diethoxy phenyl) methylene]-6, 7-diethoxy-1, 2, 3, 4-tetra hydro isoquinoline is an analogue of papaverine¹. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain². Drotaverine hydrochloride is official in Polish Pharmacopoeia³. A few UV spectrophotometric⁴⁻⁶ and HPLC⁷⁻⁹ methods have been reported individually or in combination with other drugs for estimation of Drotaverine hydrochloride. Aceclofenac [ACE] is chemically designated as 2-(2, 6-Dichloroanilino) phenyl acetoxy acetic acid. It is a NSAID, used in the management of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis^{1, 10}. Aceclofenac is official in Indian pharmacopoeia¹¹. Various UV and HPLC methods for aceclofenac have been reported for its estimation individually or in combination with other drugs¹²⁻¹⁷.

As per our knowledge Absorbance ratio method, Simultaneous equation method, and First order derivative method are available in the literature for the simultaneous estimation of DRT and ACE in combined dosage form¹⁸. Present manuscript describes Ratio Derivative and AUC spectroscopic methods for the determination of DRT and ACE in tablet dosage form. The proposed methods were validated as per the International Conference on Harmonization (ICH) guidelines¹⁹.

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. Electronic balance (Model Shimadzu AUW-220D) was used for weighing.

Reagents and chemicals

Tablets used for analysis were ESNIL manufactured by Dewcare, (Ahmadabad, India) were used for analysis containing DRT 80 mg and ACE 100 mg per tablet. Pure drug sample of DRT (98.5%) and ACE (99.8%) were obtained as a gift sample from Alkem Laboratories (Mumbai, India) and Curex pharmaceuticals (Jalgaon, India). Spectroscopic grade Methanol was used for preparation of stock solution. Double distilled water prepared at lab scale was used throughout the study.

Preparation of Standard Stock Solutions and calibration Curve

Standard stock solution of pure drug containing 1000 $\mu\text{g mL}^{-1}$ of DRT and 1000 $\mu\text{g mL}^{-1}$ of ACE were prepared separately in the methanol. The working standard solutions of DRT (4-20 $\mu\text{g mL}^{-1}$), ACE (5-25 $\mu\text{g mL}^{-1}$) for method A and of DRT (4-24 $\mu\text{g mL}^{-1}$), ACE (5-30 $\mu\text{g mL}^{-1}$) for method B were prepared by dilution of the respective stock solution with Distilled water. Derivative amplitudes of ratio spectra and concentrations were used to construct calibration curves for method A. For method B integrated area under curve was used to construct two simultaneous

equations and these equations were solved and used to calculate amount of analyte in sample solutions.

Preparation of Sample Stock Solution and Formulation analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 80 mg of DRT (100 mg of ACE) was weighed and dissolved in the 80 mL of methanol with the aid of ultrasonication for 5 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with same solvent, adding washings to the volumetric flask and volume was made up to the mark with the solvent. The solution was suitably diluted further with distilled water to get required final concentration of DRT 12 ($\mu\text{g mL}^{-1}$) and ACE ($15 \mu\text{g mL}^{-1}$).

Method A: Ratio Derivative Spectroscopy

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is dependent of concentration of analyte used as a divisor. Using

appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio spectra of different DRT standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of ACE ($15 \mu\text{g mL}^{-1}$) and the ratio derivative of these spectra traced, illustrated in Fig 1. Wavelength 330.13 nm was selected for the quantification of DRT in ACE + DRT mixture. The ratio and ratio derivative spectra of the solutions of ACE at different concentrations were obtained by dividing each with the stored standard spectrum of the DRT ($12 \mu\text{g mL}^{-1}$) (Fig. 2). Wavelength 228.06 nm was selected for the quantification of ACE in ACE + DRT mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions. The amount of ACE (C_{ACE}) and DRT (C_{DRT}) in tablet was calculated by using equations 1 and 2, respectively.

$$C_{\text{ACE}} = [\text{Derivative amplitude at } 228.06 - (0.103)] / (0.4994) \dots (1)$$

$$C_{\text{DRT}} = [\text{Derivative amplitude at } 330.13 - (0.2487)] / (0.4937) \dots (2)$$

Figure 1: First Derivative of Ratio Spectra of DRT when $15 \mu\text{g mL}^{-1}$ of ACE is used as divisor

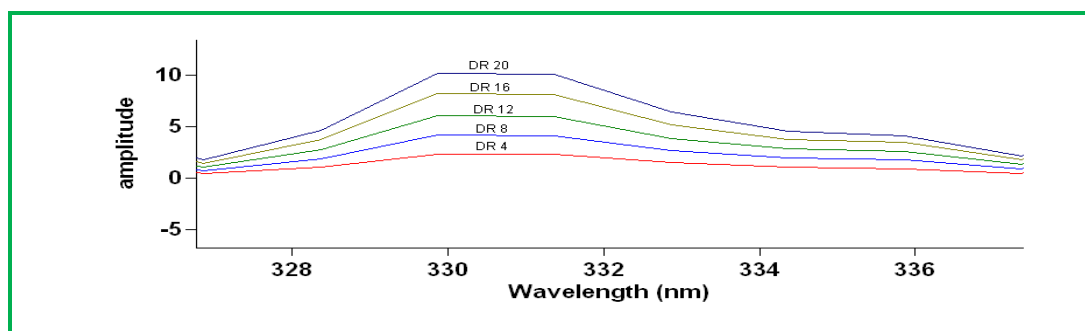


Figure 2: First Derivative of Ratio Spectra of ACE when $12 \mu\text{g mL}^{-1}$ of DRT is used as divisor

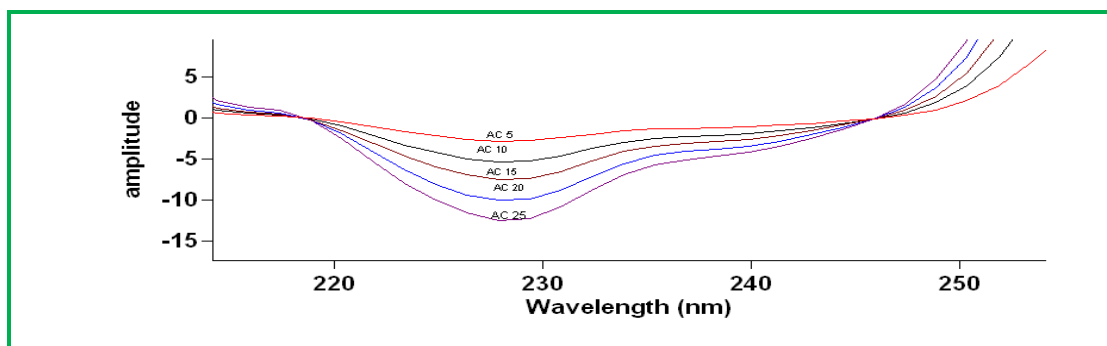


Figure 3: Overlay spectrum of DRT and ACE in Methanol-Distilled water. DRT ($4\text{-}24 \mu\text{g mL}^{-1}$) and ACE ($5\text{-}30 \mu\text{g mL}^{-1}$) for AUC method

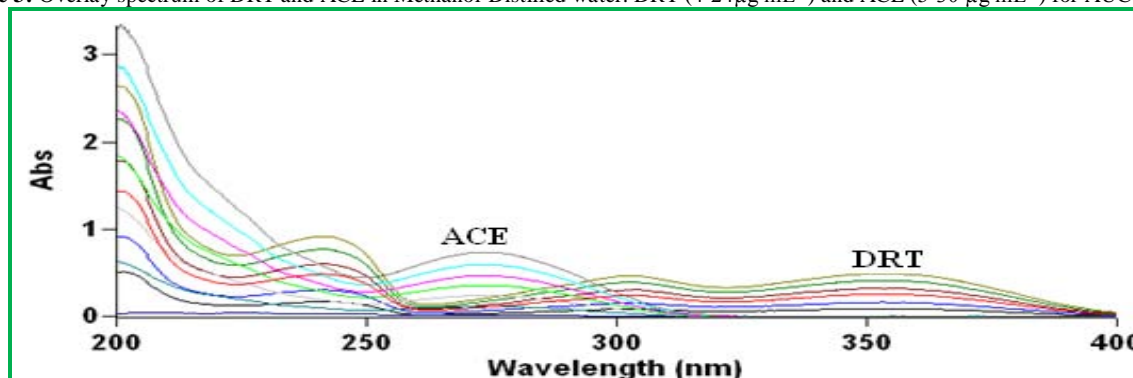


Table 1: Optical characteristics of the proposed methods

Parameter		DROTAVERINE		ACECLOFENAC	
		Method A	Method B	Method A	Method B
λ (nm)		330.13	299-305	228.06	270-277
Beer's law limit ($\mu\text{g mL}^{-1}$)		4-20	4-24	5-25	5-30
Regression Equation ($y = mx + c$)	Slope (m)	0.4937	0.1142	0.4994	0.1612
	Intercept (c)	0.2487	0.0253	0.103	0.0295
Correlation coefficient		0.9996	0.9993	0.999	0.9998
Precision (%RSD.)	Repeatability (n=5)	0.54	0.66	1.15	0.69
	Intra-day (3×3 times)	0.46	0.72	1.15	0.88
	Inter-day(3×5 days)	0.37	0.49	1.10	0.60
	Analyst	0.51	0.73	1.06	0.72
Formulation Analysis (% Assay, %RSD) n=6		99.48, 0.81	100.01, 1.01	99.89, 1.06	99.52, 1.04

Table 2: Result of Recovery studies.

Formulation used	Recovery Level	Recovery of	% Mean Recovery, % RSD by using	
			Method A	Method B
Tablet	50%	Drotaverine	99.30, 0.69	99.82, 0.33
		Aceclofenac	99.52, 0.38	100.32, 0.93
	100%	Drotaverine	99.18, 0.42	101.12, 1.44
		Aceclofenac	101.09, 1.04	101.09, 0.96
	150%	Drotaverine	100.10, 0.49	99.92, 0.99
		Aceclofenac	99.87, 0.73	99.73, 0.29

Method B: Area under Curve

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 $\mu\text{g/ml}$) of DRT and ACE were prepared separately in Distilled water. The solutions of drugs were scanned in the range of 200-420 nm (Fig.3). For Area Under Curve method sampling wavelength ranges selected for estimation of DRT and ACE were 299-305 nm (λ_1 - λ_2) and 270-277 nm (λ_3 - λ_4) and area were integrated between these selected wavelength ranges for both drugs, which showed linear response with increasing concentration hence the same wavelength range were used for preparation of calibration curve was plotted and estimation of tablet formulation. By using integrated areas two simultaneous equations (eq.3 & 4) were constructed and solved to determine concentrations of analytes. Concentration of analytes in mixed standard and the sample solution were calculated using equation (5) and (6).

$$A_1 = 1186.75 C_{\text{DRT}} + 435.8 C_{\text{ACE}} \dots\dots 299-305 \text{ nm} \quad (3)$$

$$A_2 = 618 C_{\text{DRT}} + 1654.6 C_{\text{ACE}} \dots\dots 270-277 \text{ nm} \quad (4)$$

$$C_{\text{DRT}} = A_2 \times a_{X2} - A_1 \times a_{Y2} / a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2} \dots\dots (5)$$

$$C_{\text{ACE}} = A_2 - a_{X2} \times C_{\text{DRT}} / a_{Y2} \dots\dots\dots (6)$$

Where,

a_{X1} (1186.75) and a_{X2} (618) are absorptivities of DRT at (λ_1 - λ_2) and (λ_3 - λ_4) respectively.

a_{Y1} (435.8) and a_{Y2} (1654.6) are absorptivities of ACE at (λ_1 - λ_2) and (λ_3 - λ_4) respectively.

A_1 and A_2 are Absorbances of mixed standard at (λ_1 - λ_2) and (λ_3 - λ_4) respectively. C_{DRT} and C_{ACE} is the conc. of DRT & ACE, respectively in g/100mL.²⁰

Precision of the Method

Precision of repeatability was studied by repeating the procedure five times. To study intraday precision, method was repeated 5 times in a day and the average % RSD was calculated by method A and B, respectively. Similarly the method was repeated on five different days and average % RSD was calculated (Table 1). Precision of analyst was evaluated by repeating the procedure by another analyst working in the same lab. These values confirm the intra and inter day precision.

Recovery studies

The accuracy of the proposed methods were checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs (Table 2). The basic concentration level of sample solution selected for spiking of the drugs standard solution was 6 $\mu\text{g mL}^{-1}$ of DRT and 7.5 $\mu\text{g mL}^{-1}$ of ACE.

RESULTS AND DISCUSSION

The proposed methods for estimation of DRT and ACE in combined dosage form were found to be accurate, simple, precise and rapid. Area under curve method involves formation and solving of simultaneous equation. Once the equations are formed, then only measurement of the area of sample solution at two wavelength ranges and simple calculations are required. Practically no interference from tablet excipients was observed in these methods. Both the methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economical as per ICH guidelines. The values of % RSD and correlation of coefficient for Ratio Derivative spectra were found to be in limit (% RSD 0.37-0.73 and 0.60-1.15) and correlation coefficient was 0.999 for DRT and ACE. The result of recovery studies for Tablet indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis.

CONCLUSION

The proposed methods are simple, precise, accurate, economical and rapid for the determination of DRT and ACE in combined tablet dosage forms. Analysis of authentic samples containing DRT and ACE showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in commercial tablets. It can be easily and conveniently adopted for routine quality control analysis.

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REFERENCES

1. The Merck Index – An Encyclopedia of chemicals, Drugs, and biologicals, 13th edn, Merck Research Laboratories, Whitehouse station. New Jersey: 2001; pg no.6, 609-10.
2. Sethi SD. Textbook of Pharmacology. Elsevier, New Delhi: 2004; pg no.831-40.
3. Dahivelkar PP, Bari SB. Simultaneous derivative and multi-component spectrophotometric determination of Drotaverine hydrochloride and Mefenamic acid in tablets. *Indian J. Pharm. Sci.* 2007; 69: 812-4.
4. Dahivelkar PP, Surana SJ. Spectrophotometric method for simultaneous estimation of Drotaverine hydrochloride and Mefenamic acid in bulk and tablet formulation. *Indian Drugs* 2006; 43: 896-900.
5. Metwally FH, Abdelkawy M. Determination of Nifuroxazide and Drotaverine hydrochloride in pharmaceutical preparation by three independent analytical methods. *J. AOAC Int.* 2006; 89: 78-81.
6. Abdellatif HE, Ayad MM. A comparative study on various spectrometries with thin layer chromatography for simultaneous analysis of Drotaverine and Nifuroxazide in capsules. *Chem. Pharm. Bull. Tokyo.* 2006; 54: 807-13.
7. Panigrahi D, Sharma R. Development and validation of an RP-HPLC method for simultaneous analysis of Drotaverine and Omeprazole in a tablet dosage form. *Journal of Acta Chromatographica.* 2008; 20: 439-50.
8. Mezei J, Kuttel S. A new method for high-performance liquid chromatographic determination of drotaverine in plasma. *J. Pharm. Sci.* 1984; 73: 1489-91.
9. Dahivelkar PP, Bari SB, Bhoir S, Bhagwat AM. High Performance Liquid Chromatographic Estimation of Drotaverine Hydrochloride and Mefenamic Acid in Human Plasma. *Iranian J. Pharm. Research.* 2009; 8: 209-15.
10. Martindale - The Complete Drug Reference, 36th edn. RPS Publishing. Great Britain. 2009; pg no. 14-5.
11. Indian Pharmacopoeia. Indian Pharmacopoeia Commission. Ghaziabad: 2007; 2: pg no- 63-4.
12. Nikam AD, Sampada SP, Gandhi SV. Estimation of paracetamol and Aceclofenac in tablet formulation by ratio spectra derivative spectroscopy. *Indian J. pharm. Sci.* 2008; 70: 635-7.
13. Shah R, Magdum Patil SK, Chougule DK and Naikwade N, Validated Spectroscopic Method for Estimation of Aceclofenac from Tablet formulation. *Research J. Pharm. and Tech.* 2008; 1: 430-2.
14. Rajmane VS, Gandhi SV, Patil UP, Sengar MR. Simultaneous Determination of Drotaverine Hydrochloride and Aceclofenac in Tablet Dosage Form by Spectrophotometry. *Eurasian J. Anal. Chem.* 2009; 4: 184-90.
15. Momin MY, Yeole PG, Puranik MP, Wadher SJ. Reverse phase HPLC method for determination of Aceclofenac and Paracetamol in tablet dosage form. *Indian J. Pharm. Sci.* 2006; 68: 387-9.
16. Musmade P, Subramanian G., Srinivasan KK. High-performance liquid chromatography and pharmacokinetics of aceclofenac in rats. *Analytica Chimica Acta.* 2007; 585: 103-9.
17. Hasan NY, Abdel EM, Elzeany BE, Wagieh NE. Stability indicating methods for the determination of aceclofenac. *Il Farmaco.* 2003; 58: 91-9.
18. Rajmane VS, Gandhi SV, Patil UP, Sengar MR. Simultaneous determination of Drotaverine Hydrochloride and Aceclofenac in Tablet dosage form by spectroscopic method. *Eurasian J. Anal. Chem.* 2009; 4:184-90.
19. ICH-Q2B Validation of analytical procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 1996.
