



## DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DROTAVERINE HYDROCHLORIDE AND ACECLOFENAC IN THEIR COMBINED TABLET DOSAGE FORM

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### ABSTRACT

A simple, accurate and precise dual wavelength spectrophotometric method was developed for simultaneous determination of Drotaverine hydrochloride and Aceclofenac in combined pharmaceutical dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The wavelengths selected for determination of Drotaverine hydrochloride were 271.5 nm and 280.0 nm, whereas, the wavelengths selected for determination of Aceclofenac were 301.5 nm and 311.0 nm. Methanol was taken as a solvent. Regression analysis of Beer's plots showed good correlation in concentration range of 8-32 µg/ml for Drotaverine hydrochloride and 10-40 µg/ml for Aceclofenac. Accuracy of method was found between 98.33-101.5%. The precision (intra-day, inter-day and repeatability) of method was found within limits. The proposed method was successfully applied to determination of these drugs in commercial tablets.

**Keywords:** Aceclofenac, Drotaverine hydrochloride, Dual wavelength method, UV spectrophotometric method.

### INTRODUCTION

Chemically, Drotaverine (DV) is (1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4 tetra hydro iso quinoline) hydrochloride. It is a benzyloquinoline derivative<sup>1</sup>. It is non-anticholinergic, a highly potent spasmolytic agent and has excellent smooth muscle relaxant properties<sup>2</sup>. It causes smooth muscle relaxation by increasing intracellular levels of cyclic adenosine monophosphate (cAMP) secondary to inhibition of phosphodiesterase. Drotaverine has been shown to inhibit platelet aggregation in a dose dependent manner. Aceclofenac (AF) is 2-[(2,6-Dichlorophenyl)amino]benzene acetic acid carboxymethyl ester<sup>3</sup>. It is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. Literature survey reveals that AF in

bulk and tablet dosage form is official in Indian Pharmacopoeia 2007<sup>4</sup> and British Pharmacopoeia 2008<sup>5</sup>. Several analytical methods have been reported for estimation of DV which include spectrophotometry<sup>5-7</sup>, HPLC<sup>8</sup>, thin layer chromatography<sup>9, 10</sup> and voltammetry<sup>11</sup>. The analytical methods reported for estimation of AF are spectrophotometry<sup>12-14</sup>, HPLC<sup>15-17</sup>, LC-MS<sup>18</sup> and fluorimetry<sup>19</sup>. In the present work, a successful attempt has been made to estimate both these drugs simultaneously using dual wavelength UV spectrophotometric method. This study attempts to develop a simple, accurate and precise analytical spectrophotometric method, which can quantify these drugs simultaneously from a combined tablet dosage form. The developed method was validated as per ICH guidelines and found to comply with the acceptance criteria<sup>20-21</sup>. Structures of both the drugs (DV and AF) are shown in figure 1.



Fig. 1: Chemical structures of the analytes

### MATERIALS AND METHOD

#### Apparatus

Instrument used was an UV-Visible double beam spectrophotometer, make: SHIMADZU (model UV-1800) with a pair of 1 cm matched quartz cells. All weighing was done on Shimadzu analytical balance (Model AU-220). Calibrated glasswares were used throughout the work.

#### Reagents and chemicals

Pure drug samples of DV and AF were obtained as gift samples from Astran Labs, Ahmedabad. Methanol AR was used as solvent.

#### Marketed formulation

The marketed formulation studied was ESNIL tablet manufactured by Cosmas Pharmacls.

Each tablet contains 100 mg Aceclofenac and 80 mg Drotaverine hydrochloride.

#### Preparation of standard stock solution

Accurately weighed quantity of DV (80 mg) and AF (100 mg) was transferred to two separate 100 ml volumetric flasks, dissolved in little amount of methanol and diluted to the mark with methanol (stock solutions: 800 µg/ml of DV and 1000 µg/ml of AF).

### Preparation of working standard solution

100 µg/ml of AF solution was prepared by diluting 10 ml of stock solution to 100 ml with methanol. 80 µg/ml of DV solution was prepared by diluting 10 ml of stock solution to 100 ml using methanol.

### Dual wavelength method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration.

### Study of overlain spectra and selection of wavelength

By appropriate dilutions from the working standard solutions of 80 µg/ml of DV and 100 µg/ml of AF, the solutions of DV (16 µg/ml) and AF (20 µg/ml) were prepared respectively and scanned over the range of 200-450 nm and the overlain spectra were observed for development of suitable method for analysis. The overlain spectra of DV and AF are shown in figure 2. From the overlay spectra two wavelengths 271.5 nm and 280.0 nm were selected as  $\lambda_1$  and  $\lambda_2$  for the estimation of DV. AF shows the same absorbance at these wavelengths. Similarly, wavelengths 301.5 nm and 311.0 nm were selected as  $\lambda_1$  and  $\lambda_2$  for estimation of AF. For calibration curve, from the working standard solutions, appropriate dilutions in the range of 8-32 µg/ml and 10-40 µg/ml for DV and AF respectively were prepared and analyzed. Mixed standards were prepared in the ratio of 4:5, as the formulation contains DV and AF 80 mg and 100 mg respectively.

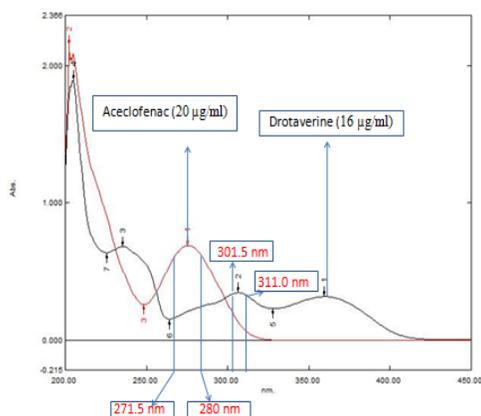


Fig. 2: Overlain zero order spectra of DV and AF in methanol.

### Assay of tablet formulation by dual wavelength spectrophotometry

Ten tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablet powder equivalent to about 80 mg of DV and 100 mg of AF was transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol. The volume was made up to the mark using methanol as solvent. The resulting solution was filtered through Whatman filter paper no.42 and 10 ml of this filtrate was appropriately diluted to get concentration of 80 µg/ml of DV and 100 µg/ml of AF. From the above prepared solution, further

dilutions were prepared to get the concentration of DV (16 µg/ml) and AF (20 µg/ml). The absorbance was measured at the selected wavelengths and concentrations were determined. The analysis was done in triplicate.

### Method validation

#### Linearity and range

Aliquots of standard stock solutions of DV and AF were diluted with methanol to get final concentrations in range of 8-32 µg/ml for DV and 10-40 µg/ml for AF. This calibration range was prepared five times and absorbance was measured at respective wavelengths for each drug separately.

#### Precision

Precision of the methods was determined by performing interday variation, intraday variation and method repeatability studies. In interday variation, the absorbance of standard solutions of DV (8-32 µg/ml) and AF (10-40 µg/ml) were measured on three consecutive days. In intraday variation the absorbances were measured three times in a day. In repeatability study, three concentrations of both the drugs were analysed in triplicate.

#### Recovery studies

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels. Known amount of the two drugs was added to pre-analyzed tablet powder and percentage recoveries were calculated.

#### Ruggedness

The data for ruggedness were obtained from two different analysts.

## RESULTS AND DISCUSSION

### Method development and validation

The overlain spectra of the drugs suggested that a dual wavelength spectrophotometric method was a suitable method for simultaneous determination of Drotaverine hydrochloride and Aceclofenac. Methanol was taken as solvent system, as both the drugs were soluble in this solvent. In dual wavelength method, wavelengths 271.5 nm and 280.0 nm were selected for determination of Drotaverine hydrochloride, whereas 301.5 nm and 311.0 nm were selected for determination of Aceclofenac. Optimized method parameters for dual wavelength spectrophotometry are shown in table 1.

Table 1: Optimized method parameters for Dual Wavelength spectrophotometry

Method parameters	Optimized parameters
Solvent	Methanol
Scanning range	200 nm to 450 nm
Scan speed	Medium
Analytical wavelengths for determination of AF	301.5 nm and 311.0 nm
Analytical wavelengths for determination of DV	271.5 nm and 280.0 nm

#### Linearity

The calibration curves of Drotaverine hydrochloride and Aceclofenac were linear in the range of 8-32 µg/ml and 10-40 µg/ml respectively. The regression equations of calibration curves were

$Y_{DV} = 0.002661 X - 0.000214$ ,  $R^2 = 0.9996$  for Drotaverine hydrochloride and  $Y_{AF} = 0.007893 X + 0.006964$ ,  $R^2 = 0.9989$  for Aceclofenac.

#### Precision

Relative standard deviations (% R.S.D.) for repeatability were found to be 1.3-2.6% and 1.6-2.7% for Drotaverine

hydrochloride and Aceclofenac, respectively. The intraday precision showed % R.S.D. of 1.79-2.33% for Drotaverine hydrochloride and 1.44-2.12% for Aceclofenac. The inter day precision showed % R.S.D. 2.07-3.67% and 1.59-3.73% for Drotaverine hydrochloride and Aceclofenac, respectively. Results of repeatability, intra day and inter day precision of method is illustrated in table 2.

#### Accuracy

The percentage recoveries of drugs from marketed formulation were determined by standard addition of pure drugs at three known concentrations and excellent recoveries were obtained at each level. The percent

recoveries for Drotaverine hydrochloride at three levels were found to be 101.5±0.0006, 98.91±0.0015 and 98.33±0.001.

The percent recoveries for Aceclofenac at three levels were found to be 100.63±0.004, 98.5±0.0015 and 99.11±0.0025. The results of accuracy studies are shown in table 3.

#### Ruggedness

Relative standard deviations (% R.S.D.) for ruggedness were found to be 1.66-2.81% and 0.43-2.28% for Drotaverine hydrochloride and Aceclofenac respectively.

**Table 2: Validation parameters for dual wavelength method**

Parameters	DV	AF
Linearity range	8-32 µg/ml	10-40 µg/ml
Correlation Coefficient	0.9996	0.9989
Precision	% RSD	
Repeatability	1.3-2.6	1.6-2.7
Intraday	1.79-2.33	1.44-2.12
Interday	2.07-3.67	1.59-3.73
% Recovery	98.33-101.5	98.50-100.63
Ruggedness	1.66-2.81	0.43-2.28

\*DV- Drotaverine; AF-Aceclofenac; RSD-Relative Standard Deviation.

**Table 3: Recovery studies**

Name of Drug	Amount of Drug Added (µg/ml)	Dual Wavelength Method	
		%Recovery*	SD
DV	12	101.5	0.0006
AF	15	100.63	0.0040
DV	16	98.91	0.0015
AF	20	98.50	0.0015
DV	20	98.33	0.0010
AF	25	99.11	0.0025

\*Mean of Three estimations DV- Drotaverine; AF-Aceclofenac; SD-Standard Deviation.

#### Application of the method in assay of tablets

The proposed UV method was applied for the determination of Drotaverine hydrochloride and Aceclofenac in their combined pharmaceutical formulation and the results are shown in table 4. The high percentage recovery (98.33-101.5 %) values confirm the suitability of the proposed method for the routine determination of these components in combined formulation.

**Table 4: Results of simultaneous estimation of DV and AF in marketed formulation by Dual Wavelength spectrophotometry method**

Method	mg/tablet%of label claim* (± S.D.)		
	DV AF	DV	AF
Method (DW)	80	100	101.5±0.0006 100.7±0.0012

\*Average of three determinations;

DW-Dual wavelength; DV- Drotaverine; AF-Aceclofenac; SD-Standard Deviation.

#### CONCLUSION

The proposed dual wavelength method gives accurate and precise results for determination of Drotaverine hydrochloride and Aceclofenac in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision and ruggedness. The proposed method was successfully applied to determination of these drugs in commercial tablets.

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#### REFERENCES

- Budavari S. (ed.) The Merck Index 13<sup>th</sup> edition. Merck & Co., Inc., New Jersey, p.3489.
- Sohan SC, Soni R, Sagar BW, Amol AK. Spectrophotometric Methods for Simultaneous Estimation of Nimesulide and Drotaverine. International Journal of ChemTech Research, 2009. CODEN (USA): IJCRGG, ISSN: 0974-4290, Vol.1, No.2:135-8.
- British Pharmacopoeia-2008. Volume- I, London: HMSO Publication, p.44-5.
- Indian Pharmacopoeia-2007. Volume II, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India Ministry of Health and Family Welfare, p.681-2.
- Hisham EA, Magda MA, Suzan MS, Nadia FY. Spectrophotometric and spectrodensitometric determination of Paracetamol and Drotaverine HCl in combination. Spectrochimica Acta PartA 66, 2007; 1147-51.
- Borgmann SH, Parciannelo LM, Arend MZ, Cardoso SG. Direct spectrophotometric determination of Drotaverine in capsules. Pharmazie, 2007; 62:483-5.
- Borgmann HMS, Parciannelo L, Arend MZ, Bajerski L, Cardoso SG. Development and validation of a dissolution method with spectrophotometric analysis for Drotaverine capsules. Sci Pharm. 2008; 76:541-54.
- Giannellini V, Salvatore F, Bartolucci G, Coran SA, Alberti MB. A validated HPLC stability- indicating method for the determination of Drotaverine in bulk drug substance. J of Pharm Biomed Anal, 2005; 39:776-80.
- Ayad MM, Youssef NF, Abdellatif HE, Soliman SM. A

- comparative study on various spectrometries with thin layer chromatography for simultaneous analysis of Drotaverine and Nifuroxazide in capsules. *Chemical and Pharma Bull.* 2006; 54: 6807.
10. Metwally FH, Abdelkawy M, Naguib IA. Determination of Nifuroxazide and Drotaverine hydrochloride in pharmaceutical preparations by three (HPTLC, Spectro, HPLC) independent analytical methods. *J of AOAC Intern*, 2006; 89:78-7.
  11. Ziyatdinova GK, Samigullin AI, Budnikov GK. Voltammetric determination of papaverine and drotaverine. *J of Anal Chem*, 2007; 62:773-6.
  12. Kirti ST, Purushotam KS, Rajesh MJ, Mrinalini CD. Spectrophotometric methods for simultaneous estimation of Diacerein and Aceclofenac. *International Journal of ChemTech Research*, 2009. CODEN (USA): IJCRGG, ISSN : 0974-4290, Vol.1, No.4: 991-5,.
  13. El-Saharty YS, Refaat M, El-Khateeb SZ. Stability indicating spectrophotometric and densitometric methods for determination of Aceclofenac. *Drug development and Industrial pharmacy*, 2002; 28:571-82.
  14. Singhvi I, Goyal A. Visible spectrophotometric estimation of Aceclofenac and Indapamide from tablets using folin-ciocalteu reagent. *Indian J Pharm Sci*, 2007; 69:164-5.
  15. Bhinge JR, Kumar RV, Sinha VR. A simple and sensitive stability indicating RP-HPLC assay method for the determination of Aceclofenac. *J of Chromatogr Sci*, 2008; 46: 440-4.
  16. Shaikh KA, Devkhile AB. Simultaneous determination of Aceclofenac, Paracetamol and Chlorzoxazone by RP-HPLC in pharmaceutical dosage form. *J of Chromatogr Sci.* 2008; 46:649-52.
  17. Hinz B, Auge D, Rau T, Rietbrock S, Brune K, Werner U. Simultaneous determination of Aceclofenac and three of its metabolites in human plasma by high-performance liquid chromatography. *Biomed Chromatogr.* 2003; 17:268-76.
  18. Kang W, Kim EY. Simultaneous determination of Aceclofenac and its three metabolites in plasma using liquid chromatography-tandem mass spectrometry. *J of Pharm and Biomed Anal.* 2008; 46:587-91.
  19. El-Kousy NM. Spectrophotometric and spectrofluorimetric determination of Etodolac and Aceclofenac. *J of Pharm and Biomed Anal.* 1999; 20:185-94.
  20. Robert AN, Alfred HW. *Pharmaceutical Process Validation*. 2005. An international, 3rd ed, Marcel Dekker, New York, Vol.129, p.515-22.
  21. ICH, Q2 (R1): *Validation of Analytical Procedures: Text and Methodology*, Geneva, 2005.