ISSN : 0974-7419 Volume 14 Issue 1

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 14(1) 2014 [11-16]

Simple and specific validated derivative spectrophotometric method for simultaneous quantification of drotaverine HCl and mefenamic acid combination in tablets

Panikumar D.Anumolu[1,2]*, G.Sunitha[2], Y.Venkat raju[2], P.R.Sathesh Babu[2], C.V.S.Subrahmanyam[2]

[1]R & D Center, Jawaharlal Nehru Technological University, Department of Pharmaceutical Sciences, Hyderabad, Andhra Pradesh-500072, (INDIA)

[2]Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Andhra Pradesh-500090, (INDIA)

E-mail : panindrapharma@yahoo.co.in

ABSTRACT

A simple and specific first-order-derivative spectorophotometric method has been developed and validated for simultaneous quantification of drotaverine HCl and mefenamic acid in combined tablet dosage forms without any prior separation of components from the sample. Drotaverine HCl was determined at a wavelength of 253.8 nm (zero-crossing wavelength point of mefenamic acid). Similarly, mefenamic acid was measured at 304 nm (zero-crossing wavelength point of drotaverine hydrochloride) in phosphate buffer, pH 6.8 as solvent. The first derivative amplitude- concentration plots were rectilinear over the range of 4-24 µg/mL for drotaverine HCl and mefenamic acid. Detection and quantitation limit were 0.4 and 1.21 µg/mL for drotaverine HCl and 0.33 and 1.0 µg/mL for mefenamic acid, respectively. The % assay in commercial formulation was found to be in the range 99.00 – 100.00 for drotaverine HCl and 99.72 – 100.20 for mefenamic acid by the proposed method. The method was validated for precision and accuracy as per ICH guidelines. The proposed method can be effectively applied for routine analysis of drotaverine HCl and mefenamic acid in tablets.

KEYWORDS

Drotaverine HCl;
Mefenamic acid;
First-derivative;
Simultaneous;
Validation.

INTRODUCTION

Drotaverine HCl chemically (Figure-1A) 6,7,3',4'-Tetraethoxy-1-benzal-1,2,3,4-tetrahydroisoquinoline hydrochloride, is an analogue of papaverine with smooth muscle relaxant properties. Mefenamic acid (Figure-1B) chemically, 2-[(2, 3-Dimethylphenyl) amino] benzoic acid, is an analgesic, anti-inflammatory, and antipyretic properties by inhibition of cyclooxygenase[1-6]. The combined dosage form of DRT and MEF is therapeutically used for uterine irritability, primary and secondary dysmenorrhoea.

Absorption spectroscopy is simple, common and useful technique for quantification of drugs in various fields, but problem of selectivity are seen in multi-component analysis due to the overlapping of spectra. Derivative spectroscopy provides a superior selectivity and spectral discrimination than common absorption spectroscopy. It is the dominant approach for resolution of one analyte whose peak is concealed by a large over-

Full Paper

lapping peak of another analyte in multi-component analysis. For this reason, diverse procedures for the resolution of overlapping derivative peaks have been applied. It has a wide range of applications in chemical, pharmaceutical, food, clinical and environmental analysis and also in the quantification of many drugs in the presence of their degradation products (or) multi- component mixtures[7-9].

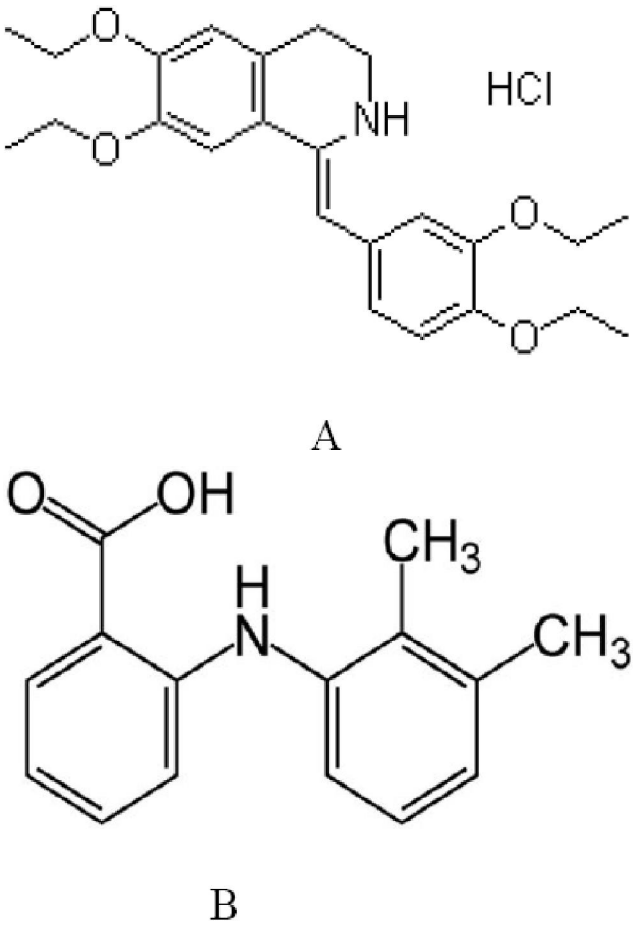

Figure 1 : Chemical structure of drotaverine HCl (A) and mefenamic acid (B)

A detailed literature survey revealed that few simultaneous analytical methods reported for quantification of drotaverine HCl and mefenamic acid by liquid chromatrography, spectrophotometry and derivative spectrophotometric methods[10-13]. To the best of our knowledge, only one method reported on the use of first derivative spectrophotometry and one method on second derivative for the simultaneous quantification of drotaverine HCl and mefenamic acid in methanol as solvent, but methanol is environmental toxic and expensive than aqueous buffers. It is also found that the parallel increase in electronic noise inbuilt in the making of the higher order spectra[14]. Literature data signify the need of simple, economic, eco friendly and specific analytical method for simultaneous quantification of drotaverine HCl and mefenamic acid combination in tablets. Hence an attempt has been made to develop a simple, economic, eco-friendly and specific first derivative analytical method for simultaneous quantification of drotaverine HCl and mefenamic acid bulk drug and combination in tablet dosage form using phosphate buffer, ph 6.8 as solvent and validated as per ICH guidelines[15].

EXPERIMENTAL

Double beam 1800 UV-Visible spectrophotometer (Shimadzu, Japan), analytical balance (Shimadzu AUX 220,Japan), pH meter (Elico, Hyderabad) and ultrasonic cleaner (Sonica) were used for the study. Drotaverine HCl and mefenamic acid were obtained as a gift samples from Aurobindo pharmaceutical and Wan bary Limited, Hyderabad, India. Methanol, potassium dihydrogen orthophosphate and sodium hydroxide were purchased from Sd Fine-Chem Ltd., Mumbai; Double distilled water was used throughout the study. DRT and MEF combination tablet formulations - Doverin-M (Intas Pharmaceuticals Ltd) and DROFEM (FDC Ltd) were purchased from local market.

Preparation of standard stock solutions

Each of standard drotaverin HCL (10 mg) and mefenamic acid (10 mg) were weighed and transferred into two separate 10 mL volumetric flasks and dissolved in methanol. The flasks were shaken and volume was made up to the mark with phosphate buffer, pH 6.8. From this 1 mL solution was diluted to 10 mL with phosphate buffer pH 6.8 to obtain standard solution of drotaverine HCl and mefenamic acid having final concentration of 100 µg /mL of each.

Selection of wavelengths

Standard solution of drotaverine HCl and mefenamic acid were diluted appropriately with phosphate buffer pH 6.8 to obtain a solution containing drotaverine HCl (12 µg /mL) and mefenamic acid (12 µg/ mL). Spectra of these diluted solutions were scanned in the

spectrum mode between 200 to 400 nm using phosphate buffer pH 6.8 as a blank. These zero-order spectra of drotaverine HCl and mefenamic acid were transformed to corresponding first-derivative spectra in the range of 200 to 400 nm.

Derivative conditions

First-order derivative spectra of drotaverine HCl (12 µg/ mL) and mefenamic acid (12 µg/ mL) were overlapped. The zero-crossing point (ZCP) values of mefenamic acid at which the drotaverine HCl showed some derivative response were recorded. The wavelength 253.8 nm was selected for the quantification of drotaverine HCl (where the derivative response for mefenamic acid was zero). Similarly, 304 nm was selected for the quantification of mefenamic acid (where the derivative response for drotaverine HCl was zero). Characteristic wavelengths (zero-crossing points) for drotaverine HCl and mefenamic acid were confirmed by varying the concentrations of both drugs.

Preparation of sample solutions

Twenty tablets of two different brands (Doverin-M and DROFEM), containing 80 mg of drotaverin HCl and 250 mg of mefenamic acid were taken and accurately weighed. Average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 80 mg drotaverin HCl and 250 mg mefenamic acid was transferred to 100 mL volumetric flask. 25 mL methanol was added to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with phosphate buffer pH 6.8. The solution was filtered through whatmann filter paper (No- 41).The filtrate was further diluted to obtained sample solutions of concentrations within linearity range. The derivative absorbance of sample solutions were measured at selected wavelengths used for the quantification of drugs.

METHOD VALIDATION

The selected method was validated for linearity, accuracy, precision, specificity, LOD and LOQ by the following procedures.

Linearity

Appropriate aliquots of standard stock solutions of drotaverine HCl (100 µg/mL) and mefenamic acid (100 µg/mL) were taken in two different sets of 10 mL volumetric flasks and diluted upto the mark with phosphate buffer, pH 6.8 to obtain final concentrations of 4 - 24 µg /mL for both drugs. The first-derivative spectra were recorded using the prepared solutions against phosphate buffer pH 6.8 as blank. The values of first-derivative absorbance were plotted against corresponding concentrations to construct the calibration curves.

Accuracy

The accuracy of the method was determined by calculating recoveries of drotaverine HCl and mefenamic acid by the method of standard additions. Known amounts of drotaverine HCl and mefenamic acid (80%, 100% and 120%) levels were added to pre quantified sample solutions. These solutions were further diluted with phosphate buffer pH 6.8 and analyzed by using phosphate buffer pH 6.8 as blank. The recovery was verified by estimation of drug in triplicate at each specified concentration level and calculated % RSD.

Precision

The intra-day and inter-day precision of the proposed first-derivative spectrophotometric simultaneous method was determined by estimating the corresponding response three times on the same day and three different days for three different concentrations of both drugs (8, 16, 24 µg/ mL). The results are reported in terms of relative standard deviation (% RSD).

Specificity

The specificity of the proposed method was evaluated through the analysis of a placebo solution, which it was prepared with the excipients of the pharmaceutical formulation. Thus, the mixture of component inert was prepared in their usual concentration employed in tablets than the method was applied in order to check if any component of the formulation could generate a response or a read with absorption band similar to the drug.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for the procedure were performed on sample containing very low concentrations of analyte

Full Paper

as per ICH guidelines. From the linearity data the limit of detection and limit of quantification was calculated using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

The limit of quantification (LOQ) may be expressed as:

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response S = slope of the calibration curve of the analyte

RESULTS AND DISCUSSION

The technique of derivative spectroscopy may be used with minimum error for the quantification of one analyte, whose peak is mystified by a large overlapping peak of another analyte. Figure-2 shows overlaid zero-order spectra of standard solution of drotaverin HCl and mefenamic acid at 12 µg/mL and spectra were found to be similar in nature and overlapping. It was observed that drotaverine HCl and mefenamic acid impart significantly at their corresponding λ_{max} value for absorbance. Hence, the derivative graphical method was used to estimate drotaverine HCl and mefenamic acid in presence of each other.

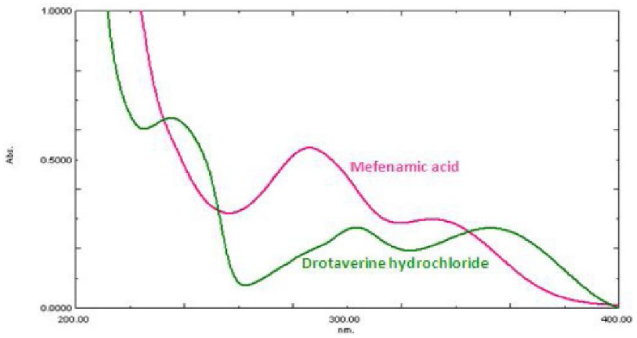

Figure 2 : Zero-order UV overlaid spectrum of drotaverine hydrochloride (12 µg/mL) and mefenamic acid (12 µg/mL) in pH 6.8 phosphate buffer

First-order Derivative overlaid spectra of drotaverine HCl and mefenamic acid was shown in Figure- 3. The first-derivative spectrum of mefenamic acid has zero absorbance at 253.8 nm, where drotaverine HCl gives the significant derivative response; while the

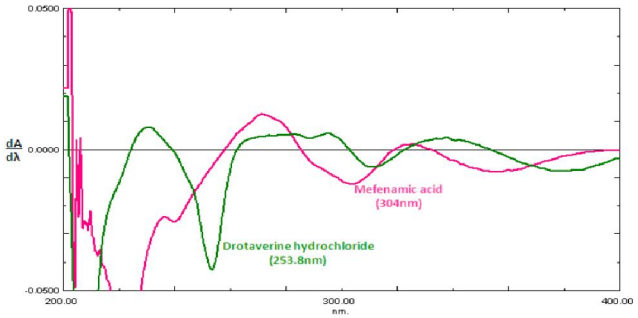

Figure 3 : First-order UV overlaid spectrum of drotaverine hydrochloride (12 µg/mL) and mefenamic acid (12 µg/mL) in pH 6.8 phosphate buffer

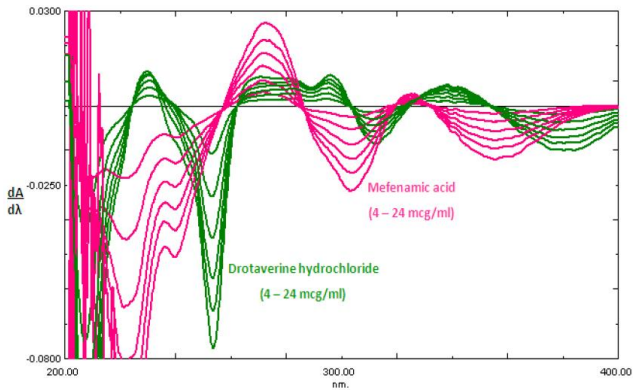

Figure 4 : UV first-derivative linearity range of drotaverine hydrochloride (4-24 µg/mL) and mefenamic acid (4-24µg/mL)

TABLE 1 : Optimized conditions for the proposed method

S.No.	Parameter	Drotaverine	Mefenamic acid
1	Absorption maxima (nm)	253.8	304
2	Beer's Law Limit (µg/ mL)	4 - 24	4 - 24
3	Slope	-0.0033	-0.001
4	Intercept	-0.0014	0.0001
5	Correlation coefficient	0.999	0.999
6	LOD (µg/ mL)	0.4	0.33
7	LOQ (µg/ mL)	1.21	1.00

TABLE 2 : Precision of the method

Conce (µg/mL)	Intra-day precision		Inter-day precision	
	Conc.found (µg /mL) (AM ± SD) (n=3)	% RSD	Conc.found (µg/mL) (AM± SD) (n=3)	% RSD
Drotaverine HCl				
8	8.78 ± 0.115	1.309	8.72 ± 0.129	1.479
16	16.9 ± 0.206	1.218	16.75 ± 0.256	1.528
24	24.3 ± 0.246	1.012	24.33 ± 0.281	1.154
Mefenamic acid				
8	8.92 ± 0.123	1.378	8.88 ± 0.117	1.317
16	16.8 ± 0.272	1.619	16.7 ± 0.215	1.287
24	25.6 ± 0.273	1.066	25.4 ± 0.287	1.129

TABLE 3 : Accuracy of the method (Recovery studies)

Formulation	Recovery level (%)	Recovery of analyte	Theoretical content (mg)	Amount found (AM ± SD) (mg) (n=3)	Recovery (%)	% RSD
Doverin-M	0	DRT	16	16.05 ± 0.207	100.36	1.289
		MEF	50	49.34 ± 0.386	98.68	0.782
	80	DRT	28.8	28.53 ± 0.333	99.06	1.167
		MEF	90	88.80 ± 1.171	98.70	1.318
	100	DRT	32	32.08 ± 0.363	100.2	1.131
		MEF	100	98.21 ± 1.099	98.21	1.119
	120	DRT	35.2	35.35 ± 0.535	100.4	1.513
		MEF	110	111.6 ± 1.650	101.40	1.478
DROFEM	0	DRT	16	16.14 ± 0.160	100.8	0.991
		MEF	50	49.97 ± 0.715	99.94	1.43
	80	DRT	28.8	28.32 ± 0.303	98.33	1.069
		MEF	90	90.49 ± 0.666	100.54	0.735
	100	DRT	32	31.96 ± 0.34	99.87	1.063
		MEF	100	98.87 ± 0.349	98.87	0.352
	120	DRT	35.2	35.19 ± 0.212	99.97	0.602
		MEF	110	110.9 ± 0.825	100.81	0.743

TABLE 4 : Analysis of commercial tablets (assay)

Formulation	Drotaverine HCl			Mefenamic acid		
	Label claim (mg)	Amount found (mg) (AM ± SD) (n=3)	% RSD	Label claim (mg)	Amount found (mg) (AM ± SD) (n=3)	% RSD
Doverin-M	80	79.62 ± 0.754	0.946	250	248.11 ±1.63	0.656
DROFEM	80	79.78 ± 0.971	1.21	250	250.43 ±1.37	0.547

first-derivative spectrum of drotaverine HCl has zero absorbance at 304 nm, where mefenamic acid gives the significant derivative response. Therefore, 253.8 nm was selected for estimation of drotaverine HCl and 304 nm was selected for the estimation of mefenamic acid.

The calibration curves shows that, the developed method was linear in the concentration range of 4-24 µg/mL for both drugs. (Figure 4) Limit of detection and limit of quantification values were indicated that the method shows high sensitivity. The optimized conditions for developed method are shown in TABLE 1. No significant difference between intra-day and inter-day precision, revealed that the method is reproducible (TABLE 2). The % recovery was within the range between 97-101 (TABLE 3) and %RSD for commercial formulation was shown less than 2 (TABLE 4). This indicates that the method is accurate and reliable.

CONCLUSION

Now a day's organic solvents superseded by eco-friendly solvents like hydrotropic agents, water and aqueous buffers while analytical method development for quantification of drugs (or) substance is preferred to defend the environmental less toxic and eco-friendly. Keeping this point into consideration, we made an attempt to develop a simple, eco-friendly, sensitive, specific and economic first derivative spectrophotometric method for simultaneous quantification drotaverine HCl and mefenamic acid in pure form and in tablet dosage forms by using phosphate buffer pH 6.8 as solvent. The assay values were in good concurrence with their respective labeled claim, which suggested no interference of formulation excipients in the estimation and obtained results from validation evidenced the proposed method was scientifically sound. Therefore, the developed method can be readily accepted by pharmaceutical quality control laboratory for routine analysis.

ACKNOWLEDGEMENT

The authors are grateful to the management of

Gokaraju Rangaraju College of pharmacy for providing needful facilities and supporting for this investigation.

REFERENCE

[1] C.Anthony Mottat, M.David Osserton; Brian widdop, Clarke's analysis of drugs and poisons. 3rd Edition. Pharmaceutical press, 208 **(2004)**.

[2] Indian pharmacopoeia. Ghazianad: The Indian pharmacopoeia commission, **1&2**, 477-480 **(2007)**.

[3] British pharmacopoeia. British pharmacopoeia commission, **2**, 1385-1386 **(2008)**.

[4] USP-NF. The official compendia of standards. Asian Edition, USP Convention, INC., Rockville, 1092 **(2007)**.

[5] S.C.Sweetman, Martindale; The Complete Drug Reference. London: Pharmaceutical Press, 165-166 **(1999)**.

[6] K.C.Singh, P.Jain, N.Goel, A.Saxena; Inter J Gyneco Obst., 84 **(2004)**.

[7] N.Swetha Bhavani, S.Hima Bindu, J.Sai Supriya, C.H.Sandhya Rani, Panikumar D.Anumolu; J App Pharm., **4**, 2 **(2013)**.

[8] N.Sirisha, A.Haripriya, N.Swetha Bhavani, R.Bhagirath, M.Satyanarayana, Panikumar D.Anumolu; Der pharmacia letre., **5**, 2 **(2013)**.

[9] A.Haripriya, N.Sirisha, S.Vishal, K.Ramakrishna, Panikumar D.Anumolu; Asian J, Research chem., **5**, 8 **(2012)**.

[10] D.C.Permanand, K.L.Senthilkumar, B.Senthilkumar, M.Saravanakumar, M.Thirumurthy; Der pharma chemical, **5**, 2 **(2010)**.

[11] D.Prasad Prakash, B.Sanjay Baburao, Suvarna Bhoir, B.Ashok Mukund; Iranian journal of pharmaceutical research., **8**, 3 **(2009)**.

[12] J.Dilipbhai Patel, A.Bhavik Patel, P.Bhuvan Raval, M.Vipul Vaghela; Journal of pharmacy research., **3**, 1 **(2010)**.

[13] P.P.Dahivelkar, V.K.Mahajan, S.B.Bari, A.A.Shirkhedkar, R.A.Fursule, S.J.Surana; Indian journal of pharmaceutical sciences., **69**, 6 **(2009)**.

[14] Beckett AH and Stenlake JB; Practical pharmaceutical chemistry; 4th Edition, the athlone press, 269-299 **(2007)**.

[15] International Conference on Harmonization, Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology, Q2 (R1), November **(2005)**.