Research Article Available online through
www.ijrap.net
IJRAP

ESTIMATION OF AMBROXOL HYDROCHLORIDE AND GUIAPHENSIN IN TABLET DOSAGE FORM BY SIMULTANEOUS EQUATION METHOD

Prasanthi N. L.*, Mohan Ch. Krishna, Manikiran S.S., Rao N. Rama

Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur- 522034, Andhra Pradesh, India

Received: 24-07-2010; Revised: 19-08-2010; Accepted: 16-09-2010

ABSTRACT

A simple, precise and reproducible UV spectrophotometric method for simultaneous estimation of ambroxol hydrochloride and guiaphensin by simultaneous equation method has been developed and validated. In this method methanol was utilized as solvent. The method was based on the measurement of absorbance of ambroxol hydrochloride and guiaphensin at 242 and 272 nm respectively. This method obeyed Beer's law in the concentration range of 5-50 mcg/mL for ambroxol and 10-80 mcg/mL for guiaphensin. The results of the analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. This method was successfully applied to the determination of these drugs in pharmaceutical dosage forms.

KEYWORDS: Ambroxol hydrochloride, guiaphensin, simultaneous equation method, spectrophotometry, dosage forms, accuracy.

***Corresponding Author**
N.L. Prasanthi
Department of pharmaceutics
Chalapathi Institute of pharmaceutical Sciences
Lam, Guntur – 522034.
Email ID: prasanthi_pharm@yahoo.com

INTRODUCTION

Ambroxol hydrochloride (AMH) is semisynthetic derivative of vasicine obtained from Indian shrub *Athatoda vasica*. It is the metabolic product of bormhexine. It is official in Martindale the Extra Pharmacopoeia[1]. Chemically it is trans-4-(2-amino-3,5dibromobenzylamino) cyclohexanol hydrochloride. It acts as branchosecretolytic and expectorant drug. It stimulates the transportation of viscous secretions in the respiratory organs and reduces the accumulation of the secretions[2]. Guiaphensin (GUF) is, 3-(2-Methoxyphenoxy)-1,2-propanediol;is reported to increase the volume and reduce the viscosity of tenacious sputum and is used as expectorant for productive cough[3].

Literature survey reveals several spectrophotometric methods have been reported for the qualitative and quantitative determination of AMH from pharmaceutical formulations[4-7]. Various HPLC[8-11], GLC[12, 13], LC-MS[14] and capillary electrophoretic[15] methods are also reported for its determination from biological fluids. GUF including HPLC[16--21], GC[22-24], capillary electrophoresis mass spectrometry[25], X-ray diffraction[26], voltammetry [27]. As combination of ambroxol hydrochloride and guaphenisin is available in market and no spectrophotometric method is reported for their simultaneous estimation. Hence in the present investigation we proposed to develop a simple and accurate method for the determination of AMH and GUF that permits their analysis in dosage forms without interference from excipients and other co formulated drugs

EXPERIMENTAL

Instrumentation and materials

An UV-Visible double beam spectrophotometer (Elico SL-157) with 1 cm matched quartz cells was used for spectrophotometric measurements. All weighing were done on electronic balance (Model Shimadzu AUW-220D). Methanol used was of analytical grade purchased from Loba Chemie Pvt. Ltd. Ambroxol hydrochloride and Guiaphensin were obtained as gift samples from Dr. Reddy's laboratory, Hyderabad.

Preparation of Stock Solutions and Sample solution

Accurately weighed quantities of AMH and GUF were dissolved separately in 20 mL of methanol and volumes were made up to 100 mL with methanol (100 mcg / mL). These solutions of AMH and GUF were used as working standards. Aliquot portions of stock solutions of AMH and GUF were diluted appropriately with methanol to obtain concentration of 20 mcg / mL of AMH and GUF. The working standard solutions were scanned from 200-400 nm to select the wavelengths for estimation. Two wavelengths selected for each formulation for the use of simultaneous equation were 242 and 272 nm for AMH and GUF respectively as shown in Figure 1. For calibration, series of solutions were prepared containing AMH 10, 15, 20, 25, 30 mcg/mL; GUF 10, 20, 30, 40, 50 mcg/mL by diluting the stock standard solution with methanol in standard volumetric flasks (10mL). Є ($A_{1\%,1cm}$) was calculated for each standard drug by measuring the absorbance of 1% solution at 1 cm path length. Similarly, mixed standard solutions were used for UV-spectrophotometric analysis by simultaneous equation method.

Vierordt's simultaneous equation method

Twenty tablets were weighed, powdered and weighed accurately equivalent to 30 mg of AMH and 100 mg of GUF was transferred to a 100ml volumetric flask, dissolve in 50 ml of methanol by ultrasonication for 20 min. The solutions were diluted with the same solvent and filtered through Whatmann no.41 filter paper. The filtrate was diluted with methanol to get final dilution 12 mcg/mL AMH and 40 mcg/mL GUF. Absorbance of these solutions was measured at selected wavelengths as A_1, A_2 and concentrations of the two drugs in each sample were calculated by using the following equations. The method employed solving of simultaneous equation using Cramer's rule and matrices. The simultaneous equations AMH and GUF analysis were,

$$C x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \longrightarrow (1)$$

$$Cy = \frac{A_1 ax_2 - A_2 ax_1}{ay_1 ax_2 - ay_2 ax_1} \longrightarrow (2)$$

where A1, A2 are absorbance of mixture at 242 nm (λ_1) and 272 nm (λ_1) respectively ax1 and ax2 are absorptivities of AMH at λ_1 and λ_2 respectively, ay1 and ay2 are absorptivities of GUF at λ_1 and λ_2 respectively and C_x and C_y are concentration of AMH and GUF respectively.

Recovery Study

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. The precision of an analytical method is expressed as SD or RSD of a series of measurements. It was ascertained by replicate estimation of drug by the proposed method. Test for ruggedness was carried out by repeating the procedure under different days, at different time and by different analyst. Linearity and range study was done by preparing concentration in the range of 80-120% of the test concentration and absorbance values were recorded at 242 and 272 nm. The plot of linearity of AMH and GUF was shown in Figure 2 and 3 respectively.

RESULTS AND DISCUSSION

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of AMH and GUF in their combined dosage form. UV absorption spectrum exhibited maximum absorbance for AMH and GUF at 242 and 272 nm respectively. In this method, AMH and GUF obey Beer's law in the concentration range of 5-50 mcg/mL and 10-80 mcg/mL respectively. Standard 1% solutions were prepared and measured absorbance at both the wavelengths of each respective content of formulations. For AMH, molar absorptivity was found to be 9742.34±0.894 and 1015.68±0.707 at 242 and 272 nm respectively, for GUF 741.39±0.769 and 2132.87±0.852 at 242 and 272 nm respectively. Optical characteristics data of drugs are shown in (**Table 1**). The results of percent estimation of drugs were reported in (**Table 2**). Recovery studies carried out for the method by spiking standard drug as per the ICH guidelines. The results of the recovery study were found to be with in the limit of 98.5-102%, providing the accuracy and showing that the method is free from interference from excipients. The results are reported in (**Table 3**). For ruggedness, proposed method was repeated under different conditions like at different time, on different days and by different analysts. The results are shown in (**Table 4**). From the study of validation parameters namely accuracy, precision, ruggedness (inter day, intraday and different analyst), specificity, linearity and range, it was observed that the method can employed for routine quantitative analysis of tablet dosage form containing AMH and GUF.

ACKNOWLEDGEMENT

The authors are thankful to Chalapathi Educational Society, Guntur for providing the necessary facilities.

REFERENCES

1. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J PharmSci 2006; 68:1-6
2. Reynolds JE., Martindale: The extra Pharmacopoeia. 31st ed., London, published by director of council of Royal pharmaceutical society of Great Britain; 1996: pp 1062.
3. Budavari S., The merck index: An encyclopedia of drugs, chemicals and biological, 12th ed., New Jersy, Published by Merck Reserch laboratories; 1996: pp 62.
4. Khathleen P., Martindale: *the Complete Drug Reference*, 32nd edition, (PhP) Pharmaceutical Press, London; 1999.
5. Kuchekar BS, Shinde GS, Naikwadi IT, Todkar KJ. Spectrophotometric estimation of Ambroxol hydrochloride in tablets. Indian J Pharm Sci. 2003; 30: 193-5.
6. Reddy MN, Rao KV, Swapna M, Sankar DG. Spectrophotometric determination of Ambroxol. East Pharmacist 1998; 125-26.
7. Zafer D, Hasan B, Nilgun FF. Quantitative Determination of Ambroxol in Tablets by Derivative UV Spectrophotometric Method and HPLC. J Pharm Biomed Anal 2003; 31: 867-72.
8. Gunawan I, Ratna H. Quantitative Determination of Ambroxol Hydrochloride in Tablets. J Pharm Biomed Anal 1993; 11: 781-4.
9. Maarit H, Coral B. Validation of an HPLC Method for the Syrup as Pharmaceutical form Stress Test For Stability Evaluation. J Pharm Biomed Anal 2003; 24:1005-10.
10. Botterbolm MHA. Rapid and Sensitive Determination of Ambroxol in Human Plasma and Urine by High-Performance Liquid Chromatography. J Chrom B 1987; 421:211-5.
11. Flores-Murrieta FJ, Hoyo-Vadillo C, Hong E, Castaneda-Hernandex G. Assay of Ambroxol in Human Plasma by High-Performance Liquid Chromatography with Amperometric Detection. J Chrom B 1989; 490:464-9.
12. Koundroullis JE, Elefthria TM, Thepdora AB. High Performance Liquid Chromatographic Determination of Ambroxol in the presence of Different Preservatives in Pharmaceutical Formulations. J Pharm Biomed Ana**.** 2000; 23:469-75.
13. Colombo L, Marcucci F, Marini MG, Pierfederici P, Mussini E. Determination of Ambroxol in Biological Material by Gas Chromatography with Electron Capture Detection. J Chrom B 1990; 530:141-7.
14. Schmid J. Assay of Ambroxol in Biological Fluids in Capillary Gas Liquid Chromatography. J Chrom B 1987; 414: 65-75.
15. Hohyun K, Jeong-Yeon Y, Sang BH, Hee JL, Kyung RL. Determination of Ambroxol in Human Plasma by LC-MS/MS. J Pharm Biomed Anal 2003; 32:209-16.
16. Popisilova M, Polasek M, Jokl V. Determination of Ambroxol and Bromhexine in pharmaceuticals by capillary isotachophoresis. J Pharm Biomed Anal 2001; 24: 421-8.
17. Amer SM, Abbas SS, Shehata MA, Ali NM. Simultaneous determination of phenylephrine hydrochloride, guaifenesin, and chlorpheniramine maleate in cough syrup by gradient liquid chromatography. Journal of AOAC International 2008; 91(2): 276–284.
18. Vasudevan M, Ravisankar S, George M, Ravi J. Simultaneous estimation of terbutaline, bromhexine and guaiphenesin in soft gelatin capsules by HPLC method. Indian Drugs 2000; 37(10): 489–492.
19. Galli V, Barbas C. High-performance liquid chromatographic analysis of dextromethorphan, guaifenesin and benzoate in a cough syrup for stability testing. Journal of Chromatography A 2004; 1048(2): 207–211.
20. Wilcox ML, Stewart JT. HPLC determination of guaifenesin with selected medications on underivatized silica with an aqueous-organic mobile phase. J Pharm Biomed Anal 2000; 23(5): 909–916.

21. Shervington LA. A quantitative simultaneous high performance liquid chromatographic determination of pseudoephedrine HCl, guaifenesin and dextromethorphan HBr. Analytical Letters 1997; 30(5): 927–944.
22. Wilson TD, Jump WG, Neumann WC, SanMartin T. Validation of improved methods for high-performance liquid chromatographic determination of phenylpropanolamine, dextromethorphan, guaifenesin and sodium benzoate in a cough-cold formulation. Journal of Chromatography 1993; 641(2): 241–248.
23. Stavchansky S, Demirbas S, Reyderman L, Chari CK. Simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection. J Pharm Biomed Anal 1995; 13(7): 919–925.
24. Harsono T, Yuwono M, Indrayanto G. Simultaneous determination of some active ingredients in cough and cold preparations by gas chromatography and method validation. Journal of AOAC International 2005; 88(4): 1093–1098.
25. Sharaf MHM, Stiff DD. Determination of guaphenesin in human serum by capillary gas chromatography and electron capture detection. J Pharm Biomed Anal 2004; 35(4): 801–806.
26. Tanaka Y, Kishimoto Y, Otsuka K, Terabe S. Strategy for selecting separation solutions in capillary electrophoresismass spectrometry. Journal of Chromatography A 1998; 817(1): 49–57.
27. Grygar T, Frybort O, Bezdicka P, Pekarek T. Quantitative analysis of antipyretics and analgesics in solid dosage forms by powder X-ray diffraction. Chemia Analityczna 2008; 53(2): 187–200.
28. Tapsoba I, Belgaied JE, Boujlel K. Voltametric assay of Guaifenesin in pharmaceutical formulation. J Pharm Biomed Anal 2005; l (38): 162–165.

Table 1: Optical Characteristics Data af Ambroxol and Guiaphensin

Parameters	**AMH**	**GUF**
Absorption maxima (nm)	242	272
Beer's law limit (mcg/ml)	5-50	10-80
Absorptivity A (1%, 1cm)	235.3	107.6
Molar absorptivity (lt $mole^{-1}$ cm^{-1})	9742.36	2132.87
Regression equation	Y = 0.0228 X - 0.0063	Y = 0.01 X + 0.0063
Correlation coefficient	0.9984	0.9993
Intercept	0.0063	0.0063
Slope	0.0228	0.01

Table 2: results of analysis of tablet formulation and Statistical data

Drug	Labeled claim (mg)	Amount found (mg)*	% labeled claim	SD	RSD	SE
AMH	30	29.97	99.96	0.177	0.0017	0.0794
GUF	100	99.77	99.77	0.513	0.0051	0.2294

**Average of six estimation of tablet formulation*

Table 3: Results of the Recovery Study

Drug	Labeled claim (mg)	Amount Added		% recovery*	SD	RSD	SE
		%	mg/ml				
		80	24	99.97	0.2243	0.0022	0.0915
AMH	30	100	30	99.85	0.2441	0.0024	0.0996
		120	36	99.91	0.2814	0.0028	0.1147
		80	80	100.05	0.1703	0.0017	0.0695
GUF	100	100	100	100.01	0.1935	0.0019	0.0789
		120	120	99.92	0.2497	0.0024	0.1019

**Average of six estimation of tablet formulation*

Table 4: Results of Ruggedness Study

Condition	Amount found (mg)*		% labeled claim		SD		RSD		SE	
	AMH	GUF	AMH	GUF	AMH	GUF	AMH	GUF	AMH	GUF
Intra day	29.90	100.01	99.68	100.01	0.2902	0.1856	0.0029	0.0018	0.1185	0.0757
Inter day	30.04	99.98	100.15	99.98	0.1375	0.2284	0.0013	0.0024	0.0561	0.0932
Different analyst	29.94	99.94	99.84	99.94	0.1596	0.2048	0.0015	0.002	0.0651	0.0836

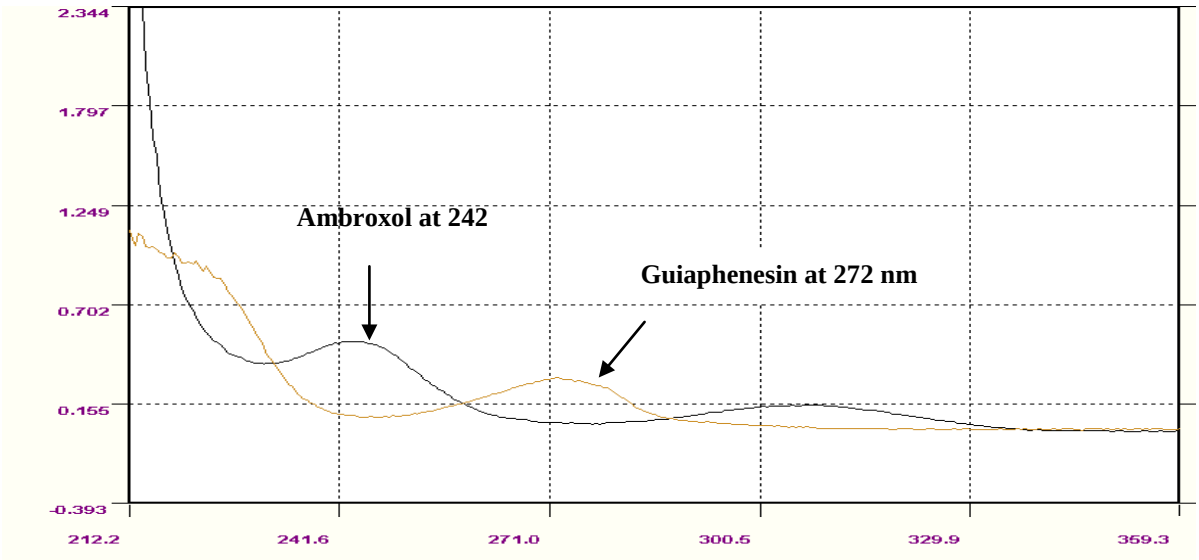

Figure 1: Overlain spectra of Ambroxol hydrochloride and Guiaphensin

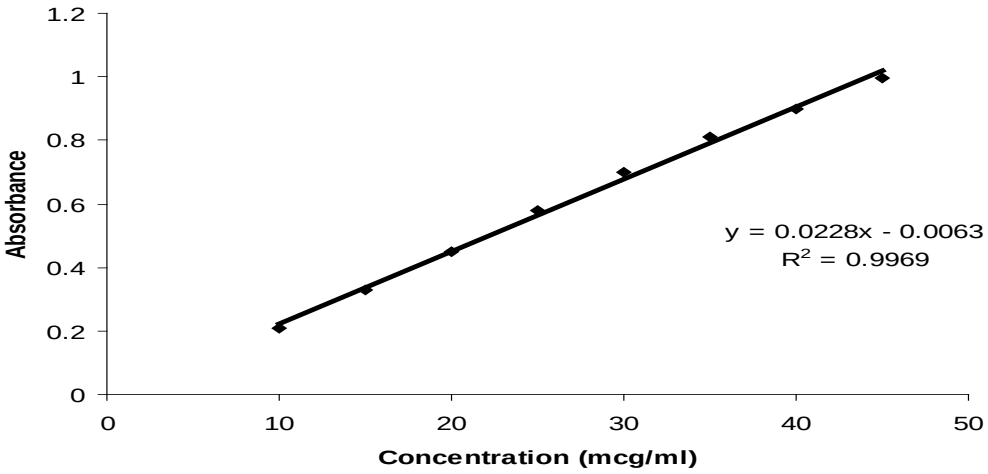

Figure 2: Linearity of Ambroxol hydrochloride at 242 nm

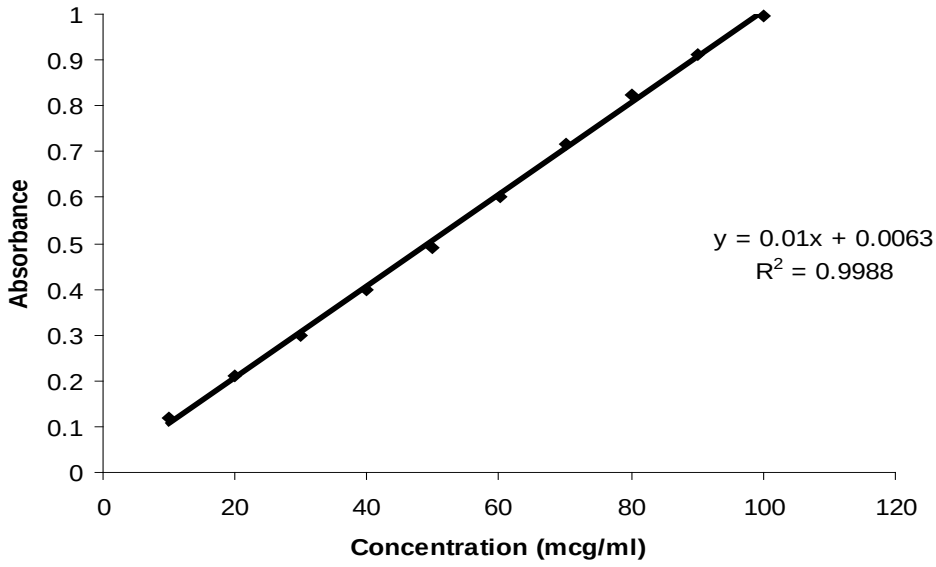

Figure 3: Linearity of Guiaphensin at 272 nm

Source of support: Nil, Conflict of interest: None Declared