Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 867–872
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Quantitative determination of ambroxol in tablets by derivative UV spectrophotometric method and HPLC

Zafer Dinçer, Hasan Basan, Nilgün Günden Göger *

Faculty of Pharmacy, Department of Analytical Chemistry, Gazi University, Etiler, Ankara 06330, Turkey

Received 6 June 2002; received in revised form 30 October 2002; accepted 30 October 2002

Abstract

A derivative UV spectrophotometric method for the determination of ambroxol in tablets was developed. Determination of ambroxol in tablets was conducted by using first-order derivative UV spectrophotometric method at 255 nm ($n = 5$). Standards for the calibration graph ranging from 5.0 to 35.0 μg/ml were prepared from stock solution. The proposed method was accurate with $98.6 \pm 0.4\%$ recovery value and precise with coefficient of variation (CV) of 1.22. These results were compared with those obtained by reference methods, zero-order UV spectrophotometric method and reversed-phase high-performance liquid chromatography (HPLC) method. A reversed-phase C_{18} column with aqueous phosphate (0.01 M)–acetonitrile–glacial acetic acid (59:40:1, v/v/v) (pH 3.12) mobile phase was used and UV detector was set to 252 nm. Calibration solutions used in HPLC were ranging from 5.0 to 20.0 μg/ml. Results obtained by derivative UV spectrophotometric method was comparable to those obtained by reference methods, zero-order UV spectrophotometric method and HPLC, as far as ANOVA test, $F_{\text{calculated}} = 0.762$ and $F_{\text{theoretical}} = 3.89$, was concerned.

Keywords: Ambroxol; Derivative UV spectrophotometry; Reversed-phase high-performance liquid chromatography

1. Introduction

Ambroxol, *trans*-4-(2-amino-3,5-dibromobenzyl)-amino-cyclohexanol, as hydrochloride, is used as a bronchosecretolytic and expectorant drug. It stimulates the transportation of the viscous secretion in the respiratory organs and reduces the standstillness of the secretion. It is administered as hydrochloric salt in daily doses of 30–120 mg using mostly oral formulations like tablets and syrups.

Several spectrophotometric methods have been used for the qualitative and quantitative determination of ambroxol. These are simple UV spectrophotometry [1–3] and flow injection spectrophotometry [4]. In another study, the spectrophotometric determination of ambroxol was carried out by liquid–liquid extraction using bromothymol blue with a flow injection system [5]. Capillary electrophoresis was also applied for the determination of ambroxol in body fluids [8]. Ambroxol was also determined in human plasma,

* Corresponding author. Fax: +90-3122235018.
E-mail address: ngoger@gazi.edu.tr (N.G. Göger).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(02)00664-7

urine and pharmaceutical formulations using gas chromatography [6,7] and high-performance liquid chromatographic methods [9–12].

In this study, a first-order derivative UV spectrophotometric method at 255 nm ($n = 5$) was used for the determination of ambroxol in tablets. There was no derivative UV spectrophotometric study about the determination of ambroxol in tablets in literature. For this reason, it was considered that derivative UV spectrophotometric method would be applicable in routine analysis since it did not require any pretreatment procedure.

Furthermore, quantitative determination of ambroxol in tablets was also performed using a reversed-phase high-performance liquid chromatographic method and zero-order spectrophotometric method (as reference methods). And then results obtained by the proposed method were compared with those obtained by the reference methods.

2. Experimental

2.1. First-order derivative UV spectrophotometric method

2.1.1. Apparatus

A Shimadzu UV-160A double-beam UV–visible spectrophotometer with data processing capacity was used. UV spectra of reference and test solutions were recorded by using 1 cm quartz cells at 200–400 nm range. The first-order derivative spectra were also obtained over the 200–400 nm range ($n = 5$). Spectral band width was 2 nm, and scan speed (slow mode) was set to 480 nm/min. Juan MR 18.22 type centrifuge and a Bondelin Sonorex RK 100H type sonicator were used throughout this study.

2.1.2. Reagents and solutions

Ambroxol hydrochloride was obtained from Bilim Drug Company in Turkey. Encapsulated 30 mg Sekrol® tablets were purchased from the market. For the preparation of standard ambroxol stock solution, 50 mg ambroxol was accurately weighed and dissolved in distilled water in a 50 ml volumetric flask and then adjusted to 50 ml with distilled water. Standard solutions in the range 5.0–35.0 μg/ml were prepared by appropriate dilutions of the stock solution.

2.1.3. Procedure

2.1.3.1. Analysis of tablets. Average mass of 10 tablets was determined and were powdered. A definite amount of powder was transferred to a 50 ml volumetric flask and the volume was then adjusted to the mark with distilled water. The solution was sonicated in an ultrasonic sonicator for 15 min and a portion of the solution was centrifuged at 3000 rpm for 10 min. As necessary, portion of clear centrifugate was diluted to 10 ml with distilled water prior to the analysis. Ambroxol content of the tablet was calculated by referring to calibration curves obtained by using standard solutions of ambroxol ranging from 5.0 to 35 μg/ml for both zero-order at 245 nm and first-order derivative UV spectrophotometric method at 255 nm ($n = 5$).

2.2. HPLC method

2.2.1. Apparatus

The HPLC system consisted of a model HP series 1050 solvent delivery system with a UV–visible dedector set to 252 nm. An HP ODS hypersil column (10 cm × 4.6 mm i.d., 5 μm particle size) and an HP 3396 series II integrator was used. The pH measurement was performed by using a Orion 720 A model equipped with combined pH electrode. Mobile phase filtration was performed with Erich Wiegand GmbH type N 022 AN 18 vacuum pump with All tech. 47 mm, 0.45 μm filter paper. And also Jouan MR 18.22 type centrifuge was used. As a degasser, Bondelin Sonorex RK 100 H was used. Typical operating conditions include flow rate, 1.5 ml/min; operating temperature, room temperature and injection volume, 20 μl.

2.2.2. Reagents and solutions

The mobile phase used in HPLC was aqueous phosphate (0.01 M)–acetonitrile–glacial acetic acid (59:40:1, v/v/v) (pH 3.1). After mixing, the

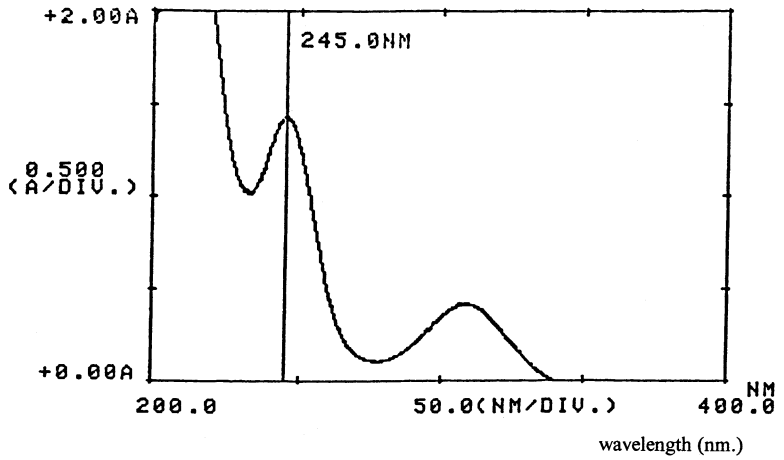

Fig. 1. Zero-order spectrum of ambroxol in distilled water.

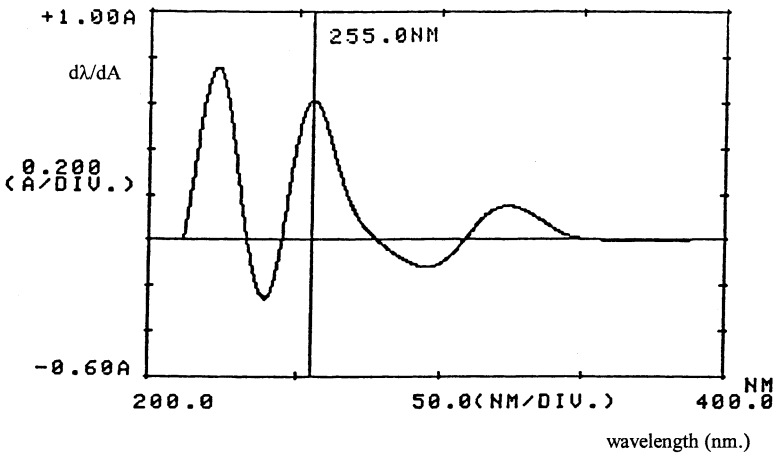

Fig. 2. First-order derivative spectrum of ambroxol in distilled water.

mobile phase was degassed. Aqueous phosphate solution was prepared by dissolving 1.38 g KH_2PO_4 in 1000 ml water. In order to prepare ambroxol stock solution, 50 mg ambroxol was accurately weighed, dissolved in mobile phase and diluted to 50 ml with the mobile phase. Standard solutions ranging from 5 to 20 μg/ml were prepared with the mobile phase. All solutions were prepared with bidistilled water. Acetonitrile and glacial acetic acid were HPLC grade, Riedel-de Haen.

2.2.3. Analysis of tablets

Average mass of 10 tablets was determined. Tablets were powdered and accurately weighed. A definite amount of powdered tablet was transferred to a 50 ml volumetric flask and the volume was then adjusted to the mark with mobile phase. The solution was sonicated in an ultrasonic sonicator for 20 min and the solution was centrifuged at 3000 rpm for 10 min. A clear portion of the centrifugate was diluted to 10 ml with the mobile phase, prior to the analysis. In order to

Table 1
Statistical analysis for the calibration curve of ambroxol in tablets

	Analytical wavelength (nm)	Linearity range (μg/ml)	Regression equation	Correlation coefficient
First-order ($n=5$)	255	5–35	$y=0.0105C+0.0006$	$r=0.9999$
Zero-order	245	5–35	$y=0.0244C-0.0196$	$r=0.9996$
HPLC	252	5–20	$y=28909C+39846$	$r=0.9996$

C is the concentration of analyte (μg/ml).

Table 2
Results obtained in the determination of ambroxol in tablets

Method	Amount labelled (mg/tablet)	Amount found* (mg/tablet)	S.D.	CV
First-order ($n=5$)	30	29.43	0.36	1.22
Zero-order	30	29.89	0.43	1.44
HPLC	30	29.77	0.77	2.59

* Average of five experiments.

Table 3
Recovery analysis of ambroxol in tablets

	Amount labelled (mg/tablet)	Amount added (mg)	Amount recovered (mg)*	Recovery (%)	S.D.
First-order ($n=5$)	30	10	9.86	98.6	0.04
Zero-order	30	10	10.21	102.1	0.05
HPLC	30	10	9.90	99.0	0.07

* Mean of three experiments.

determine ambroxol content of the tablet, ambroxol standard solutions were injected and the calibration curve was obtained as absorbance peak height versus concentration. Tablet solution, 20 μl, was injected, and the detection was at 252 nm. By using calibration curve, amount of ambroxol in tablet was determined.

3. Results and discussion

In this study, quantitative determination of ambroxol in tablets was performed by first-order derivative UV spectrophotometric method and the results were compared with those obtained by the two reference methods, HPLC and zero-order UV spectrophotometric method. Since derivative UV spectrophotometry eliminates the possible scattering effects of colloidal particles and the turbidity problems, it was decided to investigate whether it was applicable or not in routine analysis. It was simple, rapid and sensitive and also it did not require any pretreatment procedure. Zero-order and first-order derivative spectra of ambroxol were shown in Figs. 1 and 2, respectively.

Regression analysis for the first-order derivative UV spectrophotometric method was carried out (Table 1) and the linearity of the calibration graph and adherence of the method to Beer's law were validated by high value of the correlation coefficient (r), 0.9999. Quantitative determination of ambroxol in tablets using derivative UV spectrophotometric method was performed and the results were in good agreement with the labelled amount of ambroxol (Table 2). In addition, coefficient of variation (CV) for the determination

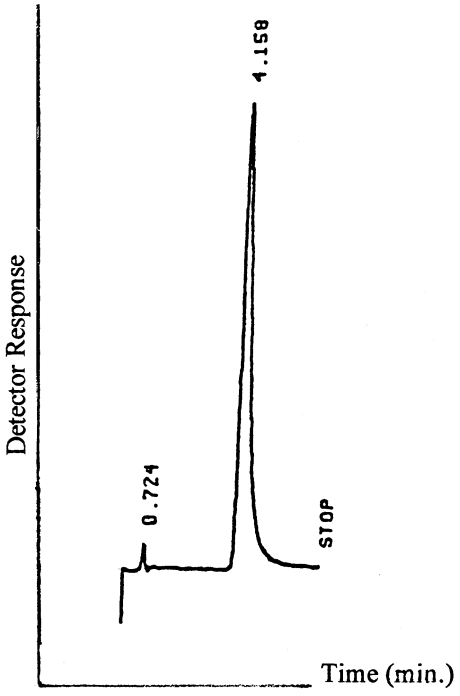

Fig. 3. HPLC chromatogram of ambroxol in mobile phase, consisting of aqueous phosphate (0.01 M)–acetonitrile–glacial acetic acid (59:40:1) (pH 3.12) at 252 nm, flow rate of 1.5 ml/min.

of ambroxol was 1.22. Closeness of the amount found to the labelled amount and the low coefficient of variation value showed that the proposed method was accurate and precise.

Recovery study conducted by the derivative UV spectrophotometric method was performed by spiking the powdered tablets with appropriate amounts of stock solution. The results of the recovery analysis were also represented in Table 3. High recovery, $98.6 \pm 0.04\%$ and low standard deviation confirmed the suitability of the proposed method for the determination of ambroxol in different pharmaceutical formulations.

Determination of ambroxol was also conducted by a modified reversed-phase high-performance liquid chromatographic method. Fig. 3 represents typical chromatogram obtained from the analysis of standard ambroxol. As shown in this figure, standard ambroxol solution was eluted, forming well-shaped, symmetrical single peak, and well separated from the solvent front. Therefore, no additional extractions or separations were required. High correlation coefficient value (Table 1) and low standard deviation (Table 2) proved that HPLC method was precise and accurate. In addition, relatively high recovery value, $99.0 \pm 0.77\%$ (Table 3), was obtained. Furthermore, it was seen that the results obtained with HPLC were in good agreement with those obtained by first-order derivative UV spectrophotometric method.

Table 4
Comparison of the three methods for the determination of ambroxol in tablets, ANOVA test

Amount labelled (30 mg/tablet)	First-order	Zero-order	HPLC
Amount found*	29.43	29.83	29.77
CV	1.22	1.44	2.59

* Average of five experiments. ANOVA, $P = 0.05$; $F_{calculated} = 0.762$; $F_{theoretical} = 3.89$.

Lastly, results obtained by these three methods for the determination of ambroxol in tablets were compared by analysis of variance (ANOVA) test (Table 4) and there was no significant difference ($P = 0.05$) between the results.

Derivative spectrophotometric method was simple, rapid and accurate. Such features render it suitable for the routine analysis in quality control laboratories. The proposed derivative UV spectrophotometric method can also be applied for the determination of ambroxol present in a mixture together with another active substance that absorbs at the same wavelength range with the ambroxol or metabolites of ambroxol.

It can be concluded that the proposed first-order derivative UV spectrophotometric method is suitable for the analysis of ambroxol in commercial tablets.

References

[1] J. Kracmar, J. Kracmarova, Cesk Farm. 29 (1–2) (1980) 19–31.
[2] G. Indrayanta, R. Handayani, J. Pharm. Biomed. Anal. 11 (8) (1993) 781–784.
[3] G. Indrayanto, R. Handajani, Drug Dev. Ind. Pharm. 20 (9) (1994) 1639–1647.

[4] M. Eisman, M. Gallego, M. Valcarcel, J. Pharm. Biomed. Anal. 11 (1993) 301–305.
[5] T. Perez-Ruiz, C. Martinaz-Lozano, A. Sanz, M.T. San Miguel, Talanta 43 (7) (1996) 1029–1034.
[6] L. Colombo, F. Marcucci, M.G. Marini, P. Pierfederici, E. Mussini, J. Chromatogr. 530 (1) (1990) 141–147.
[7] J. Schmid, J. Chromatogr. 414 (1987) 65–75.
[8] T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz, J. Chromatogr. B 742 (1) (2000) 205–210.
[9] J.E. Koundourellis, E.T. Malliou, T.A. Broussali, J. Pharm. Biomed. Anal. 23 (2000) 469–475.
[10] M. Nieder, H. Jaeger, J. High Resolut. Chromatogr. 9 (10) (1986) 561–565.
[11] F.J. Flores-M, C. Hoyo-V, E. Hong, H.G. Castadena, J. Chromatogr. 490 (2) (1989) 464–469.
[12] A. Zarzuelo, M.L. Sayalero, F.G. Lopez, J. Liq. Chromatogr. RT24 (7) (2001) 1007–1014.