Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 8, pp. 781–784, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Short Communication

Quantitative determination of ambroxol hydrochloride in tablets

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Keywords: *Ambroxol hydrochloride; reversed-phase high-performance liquid chromatography; gas–liquid chromatography; UV spectrophotometry; tablet determination.*

Introduction

Ambroxol [*trans*-4-(2-amino-3,5-dibromobenzylamino)cyclohexanol], as the hydrochloride, is used as a mucolytic and expectorant drug. Ambroxol hydrochloride is marketed in Indonesia as tablets and syrup [1]. There are no analytical specifications for ambroxol hydrochloride in the USP XXII [2], the BP 1988 [3] or the DAB IX [4]. Brizzi and Pasetti [5] have reported a method for the determination of ambroxol hydrochloride in pharmaceutical solutions using reversed-phase high-performance liquid chromatography (RP-HPLC).

The aim of the present work was to develop simple chromatographic and UV spectrometric methods for the routine analysis of ambroxol hydrochloride particularly in tablets.

Experimental

Materials and reagents

Acetonitrile was HPLC grade (J.T. Baker); ammonium acetate and triethylamine (TEA) were analytical grade reagents (E. Merck); chloroform, hydrochloric acid, and ammonium hydroxide were analytical grade reagents (J.T. Baker); cyproheptadine hydrochloride was Egist pharmaceutical grade; ambroxol hydrochloride was obtained from Sigma (as reference standard) and Helm AG (for laboratory-made tablets); excipients for laboratory-made tablets (Avicel PH 101, Emcompres, Primojel, magnesium stearate and talc) were pharmaceutical grade; commercial tablets (A, B, C) were purchased from a local pharmacy at Surabaya.

Chromatography

HPLC. The HPLC analysis was performed on a Shimadzu LC-6A chromatograph with an SCL-6A system controller, SPD-6A UV–vis spectrophotometric detector, a CR3A integrator and a 20 U1 Rheodyne 7125. The separation was carried out on a Lichrosopher 100 RP 18 244 × 4.0 mm i.d. column packed with 10-μm (E. Merck). The mobile phase was acetonitrile–ammonium acetate (10 mM) with triethylamine (10 mM) adjusted to pH 4.0 with acetic acid (25:75, v/v) and the flow rate was 2.0 ml min^{-1}. The column eluent was monitored at 245 nm.

GLC. The GLC analysis was performed on a Shimadzu GC-9A chromatograph with a CR3A integrator, equipped with a glass column (3000 × 3 mm i.d.) packed with 5% OV-101 on 60–80 mesh Chromosorb W (Shimadzu). The column was heated isothermally at 260°C, the temperature of the injector and the flame ionization detector was 300°C. The flow rate of the carrier gas, nitrogen, was 60 ml min^{-1}.

Spectroscopy

A Hitachi U-2000 spectrophotometer with 10-mm quartz cells was used.

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Sample preparation

Twenty laboratory-made/commercial tablets were weighed and the mean weight of tablets was determined. Then the tablets were finely powdered.

For HPLC. A weight equivalent to 2.5 mg of ambroxol hydrochloride was transferred into a 50-ml volumetric flask containing about 40 ml of the mobile phase, ultrasonicated for about 5 min and then diluted to 50 ml with the mobile phase. The solution was filtered through Whatman 40 and 0.5-μm Millex SR filters (Millipore) prior to injection.

For GLC. A weight equivalent to 5 mg of ambroxol hydrochloride was transferred into a glass tube; 2.5 ml of cyproheptadine hydrochloride solution (2.0 mg ml^{-1}) as the internal standard was added and the pH was adjusted to pH 12 with ammonium hydroxide (10%). This mixture was mixed in a vortex mixer with 5.0 ml of chloroform for about 5 min. Subsequently the chloroform layer was separated and filtered through a Whatman 1PS filter prior to injection. Aliquots (5.0 μl) were injected for analysis.

For UV-spectrophotometry. A weight equivalent to 6 mg of ambroxol hydrochloride was transferred into a 100-ml volumetric flask containing about 90 ml of 0.1 M hydrochloric acid; the solution was ultrasonicated for about 15 min, diluted to volume with 0.1 M hydrochloric acid and filtered through a Whatman 40 filter. A 5.0 ml volume of this solution was transferred into a 25-ml volumetric flask, diluted to 25 ml with 0.1 M hydrochloric acid and the absorbance was determined at 244 nm, the wavelength of maximum absorbance of ambroxol.

Validation of methods

The HPLC, GLC and spectrophotometric methods were validated for their linearity, LOD (limit of detection), LOQ (limit of quantitation), precision and recovery. Validation of linearity was performed using dilutions of a standard solution. LOD, LOQ for HPLC and GLC were calculated according to the method of Carr and Wahlich [6]; for UV-spectrophotometry the method of Randez-Gil *et al.* [7] was used. Precision was evaluated by analysing 10 different aliquots from laboratory-made tablets (30 mg $tablet^{-1}$). A three-point

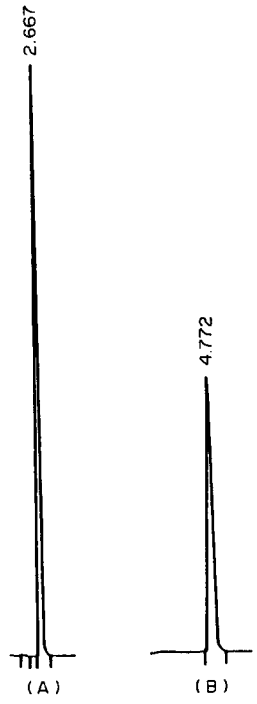

Figure 1
HPLC chromatogram of a standard solution of ambroxol hydrochloride (0.05 mg ml^{-1}) in acetonitrile–10 mM ammonium acetate with 10 mM triethylamine (pH 4.0) (A) 80:20, v/v; (B) 25:75, v/v. Vertical axis: UV detector response (245 nm); horizontal axis: retention time (min).

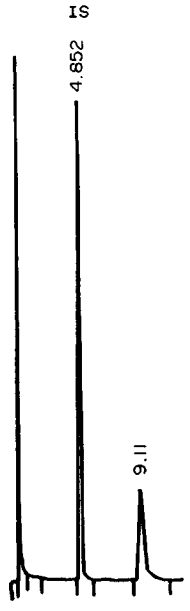

Figure 2
GLC chromatogram of a standard solution of ambroxol hydrochloride (1.01 mg ml^{-1}) and internal standard (IS, 1.00 mg ml^{-1}). Vertical axis: detector response; horizontal axis: retention time (min).

Table 1
Recovery of ambroxol hydrochloride from laboratory-made tablets

Content (mg tablet^{-1})	Group mean recovery (%; n = 5) ± SD		
	HPLC	GLC	UV-spectrophotometric method
24	97.48 ± 1.12	95.52 ± 2.37	100.26 ± 0.65
30	98.14 ± 0.04	101.04 ± 0.64	99.38 ± 0.16
37.5	99.09 ± 0.24	98.04 ± 1.58	98.53 ± 0.37
Grand mean recovery ± SD (%; n = 15)	98.24 ± 1.08	98.20 ± 1.59	99.39 ± 0.84

Table 2
Recovery of ambroxol hydrochloride from fortified commercial tablets

Tablets	HPLC		GLC		UV-spectrophotometric method	
	Added mg tablet^{-1}	Recovery (%)*	Added mg tablet^{-1}	Recovery (%)*	Added mg tablet^{-1}	Recovery (%)*
A	6.0	98.15 ± 0.90	6.0	102.79 ± 0.50	10.0	100.64 ± 0.36
	18.0	99.45 ± 0.47	18.0	101.71 ± 2.90	30.2	100.27 ± 0.24
B	6.0	97.67 ± 0.33	6.0	103.70 ± 0.52	10.4	98.37 ± 0.23
	18.0	100.21 ± 0.36	18.0	106.51 ± 0.85	31.0	98.89 ± 0.48
C	6.0	97.64 ± 0.72	6.0	103.46 ± 0.92	10.4	101.41 ± 1.66
	18.0	102.32 ± 0.36			31.2	100.05 ± 0.09

*Group mean recovery ± SD (n = 5).

recovery study was performed for laboratory-made tablets and the standard addition method [8] was used for commercial tablets.

Results and Discussion

The composition of the mobile phase used for HPLC was a modification of the mobile phase reported previously [5]. A standard solution of ambroxol hydrochloride was analysed using a mobile phase with a lower acetonitrile concentration (25%, v/v). Typical chromatograms of ambroxol hydrochloride using the modified and original solvents are shown in Fig. 1. With the modified solvent, linearity was achieved for 2.10–98.00 μg ml^{-1} (r = 0.99992, n = 6); LOD = 0.18 μg ml^{-1}; LOQ = 0.60 μg ml^{-1}; and the RSD of 10 replicate analyses was 0.65%.

In GLC analysis, the peak of internal standard (cyproheptadine hydrochloride) was adequately separated from the peak of ambroxol hydrochloride, as illustrated in Fig. 2. A linear correlation between peak-area ratio (ambroxol hydrochloride internal standard) and concentration was observed for 0.10–12.00 mg ml^{-1} (r = 0.99958, n = 10); LOD = 2.05 μg ml^{-1}; LOQ = 6.83 μg ml^{-1}; and the RSD of 10 replicate analyses was 1.14%.

A typical UV spectrum of ambroxol hydrochloride from a laboratory-made tablet is shown in Fig. 3. No interference from excipients was observed. A linear correlation between absorbance (at 244 nm) and concentration was observed for 6.00–50.20 μg ml^{-1} (r = 0.99996, n = 7); LOD = 0.08 μg ml^{-1}; LOQ = 0.26 μg ml^{-1}; and the RSD of 10 replicate analyses was 1.13%.

The recoveries of the drug from laboratory-made tablet formulations are presented in Table 1; the grand mean recoveries were 98.24% (HPLC), 98.20% (GLC) and 99.39% (UV-spectrometry). The relatively poor recovery for tablets each containing 24 mg by GLC may be due to extraction error. The accuracy of the procedures were also evaluated on three types of commercial tablets fortified with ambroxol hydrochloride. The recovery

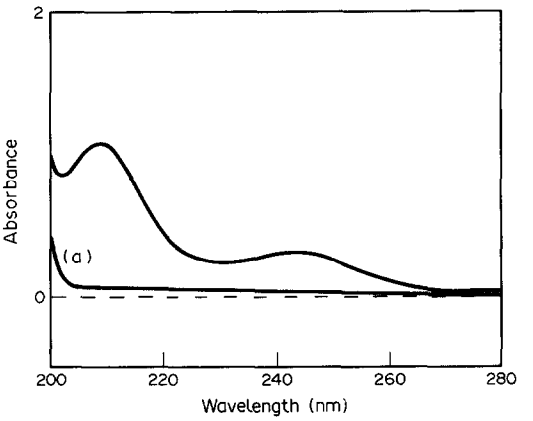

Figure 3
Ultraviolet spectra of ambroxol hydrochloride (0.012 mg ml^{-1}) and excipients of laboratory-made tablet (a).

data are presented in Table 2. A slightly high recovery for tablet B by GLC may be due to the contamination of the ambroxol hydrochloride peak by excipients, which could not be detected by the flame ionization detector.

In conclusion, simple and rapid chromatographic (HPLC, GLC) and UV spectrophotometric methods have been presented for the quantitative determination of ambroxol hydrochloride in tablets. These methods have been found suitable for the routine analysis of tablets in the pharmaceutical industry.

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[Received for review 25 June 1992; revised manuscript received 5 January 1993]