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QUANTITATIVE DETERMINATION OF AMBROXOL HYDROCHLORIDE IN SYRUPS BY RP-HPLC AND UV SPECTROSCOPY

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ABSTRACT

RP-HPLC and UV Spectroscopy methods for the quantitation of ambroxol hydrochloride in syrups have been developed. Both methods were precise and accurate. A number of inactive ingredients present in the synthetic syrup did not interfere with the assay procedures. Although the methods had also good recovery for commercial syrups, but it was difficult to determine which method was more accurate than the other.

INTRODUCTION

Ambroxol [trans-4-(2-amino-3,5-dibromobenzyl) - amino - cyclohexanol], as the hydrochloride, is used as a

bronchosecretolytic and expectorant drug. Ambroxol hydrochloride is found in pharmaceutical preparations mainly as tablets and syrups in Indonesia [1]. No official method of analysis is described for this drug in the USP XXII [2], BP 88 [3] and DAB IX [4]. A RP-HPLC method for determination of ambroxol hydrochloride in pharmaceutical solution preparations [5] and in biological fluids [6] were reported. A GC-MS method for analysis of ambroxol in horse's urine was also reported [7]. The authors have developed a RP-HPLC method for determination of ambroxol hydrochloride in tablets [8]. The present report describes a RP-HPLC method and an UV spectroscopic method for determination of ambroxol hydrochloride in syrups.

EXPERIMENTAL

Chemicals and reagents

All the chemicals and reagents were HPLC and analytical grade and used as received. Ambroxol hydrochloride was supplied by Helm AG Pharmaceutical grade. All the excipients for synthetic syrup were pharmaceutical grade. The commercial syrups (A,B,C) were purchased locally.

Equipment

A HPLC (LC-6A,Shimadzu) equipped with SCL-6A system controller, SPD-6A UV-VIS detector, a Rheodyne 7125

injector fitted with a 20 Ul loop and an integrator (C-R3A, Shimadzu). A RP 18 column (Lichrospher 100, 10 Um, 244 x 4.0 mm i.d, E.Merck) was used.
A Hitachi U-2000 spectrophotometer with 10-mm quartz cells was used.

Chromatographic Condition

The mobile phase consisted of acetonitrile-ammonium acetate(10 mM), triethylamine(10 mM) adjusted to pH 4.0 with acetic acid (20:80,v/v) for the synthetic syrups and (25:75,v/v) with 0.01M di-n-butylamine addition for all commercial syrups. Flow rate was 2.0 ml min^{-1}. The column effluent was monitored at 245 nm and the temperature was ambient.

Preparation of standard solutions

Standard solution for chromatography was prepared daily by dissolving ambroxol hydrochloride in mobile phase to achieve concentration 0.048 mg/ml.
For UV spectroscopy, the calibration curve was prepared daily in concentration range 0.02 to 0.10 mg/ml in 0.1 N hydrochloric acid.

Pharmaceutical dosage forms

The synthetic syrups were prepared at 3 concentration levels (12;15 and 18 mg/5 ml). Three commercial brands of syrups containing 15 mg/5 ml according to the label

were used. The three brands were designated as A,B and C respectively.

Sample preparation

For chromatography, 1.0 ml syrup was transferred to a 25 ml volumetric flask and completed to volume with mobile phase. Dilute 10.0 ml of this solution with mobile phase to 25.0 ml, filter prior to injection.
For UV spectroscopy, 1.0 ml syrup was transferred to a 50 ml volumetric flask and diluted with 0.1 N hydrochloric acid to volume. Determine the absorbances at maximum wavelength (307.4 nm).

Validation

The RP-HPLC and UV spectroscopy methods were validated for the linearity, LOD (limit of detection), LOQ (limit of quantitation), precision and recovery. Linearity was performed by analysing standard solution of ambroxol hydrochloride. LOD, LOQ for RP-HPLC were calculated according to Carr and Wahlich [9], whilst for UV-spectroscopy according to Randez-gil et al[10]. Precision was performed by analysing ten different aliquots from synthetic syrups (15 mg/5 ml). A three point recovery study was performed for synthethic syrups and standard addition method [11] for all commercial syrups.

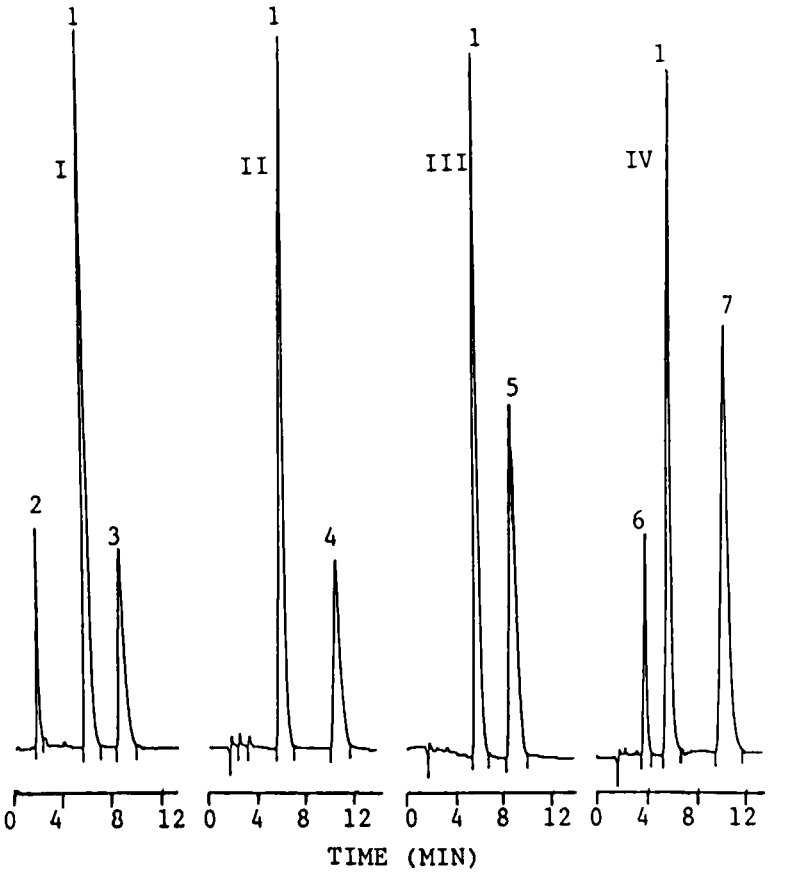

FIGURE 1

HPLC chromatograms of Ambroxol Hydrochloride (1) and other ingredients (2-7) in various preparations : I synthetic syrup, II commercial syrup A,III commercial syrup B, IV commercial syrup C

RESULTS AND DISCUSSION

A characteristic chromatogram of ambroxol hydro - chloride in synthetic syrup was presented in figure 1. Ambroxol hydrochloride was eluted as a single band with a retention time of 5.72 min. It was well resolved from

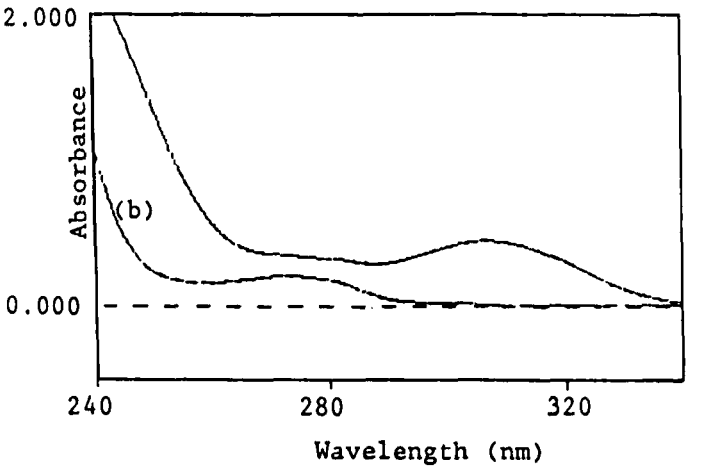

FIGURE 2

UV Spectra of Ambroxol Hydrochloride in synthetic syrup and matrix of synthetic syrup (b)

the other components in the matrix that eluted at 1.8 (citric acid and EDTA) and 8.5 min (benzoic acid). The separation condition for synthetic syrup is the same as for tablet [8]. Under this condition, a linear correlation between peak area and concentration extended from 2.10 - 98.00 Ug/ml (r=0.99992,n=6). Limit of detection was 0.18 Ug/ml and limit of quantitation was 0.60 Ug/ml. The precision, expressed as the relative standard deviation (RSD), of ten replicate analysis was 0.65%. For commercial syrups, the mobile phase need to be modified by adding 0.01 M di-n-butylamine to achieve good resolution between ambroxol hydrochloride and the other component

TABLE 1

Assay results of ambroxol HCl in synthetic syrup

Content (mg/ml)	Mean recovery * % ± SD RP-HPLC	UV Spectroscopy
2.4	99.72 ± 0.57	101.14 ± 0.73
3.0	100.26 ± 0.13	101.07 ± 0.32
3.6	102.55 ± 0.54	101.97 ± 0.36

* average of five determination

TABLE 2

Assay Results Of Ambroxol HCl In Commercial Syrups

Method	Formula	% of the label claim* Original	Added	Theory	Found	% Recovery
I	A	94.90	20.00	114.90	114.62	99.76
			40.00	134.90	131.94	97.80
	B	100.54	20.00	120.54	119.77	99.36
			40.00	140.54	140.44	99.93
	C	93.60	20.00	113.60	113.06	99.52
			40.00	133.60	132.64	99.28
II	A	98.28	16.67	114.95	112.82	98.15
			33.33	131.61	131.27	99.74
	B	98.12	16.67	114.79	114.97	100.16
			33.33	131.45	132.55	100.84
	C	103.13	16.67	119.80	120.02	100.18
			33.33	136.46	137.38	100.67

* average of five determination

I RP-HPLC

II UV-Spectroscopy

in the matrix. The characteristic chromatograms for ambroxol hydrochloride in product A,B and C were presented in figure 1.

A typical UV spectra of ambroxol hydrochloride in synthetic syrup was presented in figure 2. Spectra of the matrix was also included as comparison. The absorbances were determined at 307.4 nm, at which the interference ofthe other components in the matrix, was relative small. Under this condition, a linear correlation between absorbance and concentration extended from 16.0 to 160.0 Ug/ml (r=0.99996, n=7). Limit of detection was 0.28 Ug/ml and limit of quantitation was 0.95 Ug/ml. The precision, expressed as the relative standard deviation (RSD%), of ten replicate analysis was 0.47 %. The results of recovery studies of ambroxol hydrochloride in synthethic and commercial syrups by RP-HPLC and UV spectroscopy were summarized in table 1 and 2.

This experiment showed that both methods were suitable for routine analysis in quality control laboratory. Although the methods also showed good recovery for commercial syrups, but it was difficult to determine which method was more accurate than the other, because the original concentrations obtained were relatively different, whilst the exact compositions were unknown.

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