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Short communication

Simultaneous determination of roxithromycin and ambroxol hydrochloride in a new tablet formulation by liquid chromatography

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Abstract

A rapid and accurate liquid chromatographic method is described for the simultaneous determination of roxithromycin and ambroxol hydrochloride in a new tablet formulation. Chromatographic separation of the two drugs was achieved on a Diamonsil™ C_{18} column (200 mm × 4.6 mm, 5 μm). The mobile phase consisting of a mixture of acetonitrile, methanol and 0.5% ammonium acetate (39:11:50 (v/v), pH 5.5) was delivered at a flow rate of 1.0 ml/min. Detection was performed at 220 nm. Linearity, accuracy and precision were found to be acceptable over the concentration range of 201.2–2012.0 μg/ml for roxithromycin and 42.7–427.0 μg/ml for ambroxol hydrochloride, respectively. Separation was complete in less than 10 min. The proposed method can be used for the quality control of formulation products.

Keywords: Roxithromycin; Ambroxol hydrochloride; Liquid chromatography

1. Introduction

Roxithromycin is a semi-synthetic 14-membered-ring macrolide antibiotic derived from erythromycin. It is more stable than erythromycin under acidic conditions and thus exhibits improved pharmacokinetic properties [1]. Roxithromycin is as effective as or more effective than other macrolides for a wide range of infections and has been clinically used for the treatment of respiratory infections caused by Gram-positive and Gram-negative cocci, Gram-positive bacilli and some Gram-negative bacilli. Ambroxol hydrochloride reduces bronchial hyper-reactivity, stimulates cellular surfactant production, increases the amount of antibiotic penetration and thus reduces daily dose of them and exhibits anti-inflammatory properties as well [2]. An increased interest by pharmaceutical companies has been shown to develop a formulation containing both drugs in order to achieve more favourable effects in clinical trials, for it has been

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verified that ambroxol hydrochloride could increase the concentration of roxithromycin in human lung tissues [3]. In the process of the development, a fast and reliable analytical method is required for the simultaneous determination of both drugs in this compound formulation.

Some methods can be found for the individual determination of roxithromycin and ambroxol hydrochloride. Fluorimetry [4] and liquid chromatography (LC) with different detections, such as amperometric detection [5,6], spectrophotometric detection [7], fluorescence detection [8] and mass spectrometric detection [9–11] have been used for the determination of roxithromycin in biological samples. Methods available for the determination of ambroxol hydrochloride include capillary electrophoresis [12–14], spectrometry [15], gas chromatography [16,17] and LC with potentiometric detection [18], MS detection [19] and UV detection [18,20–23]. However, no references have been found for simultaneous determination of roxithromycin and ambroxol hydrochloride in pharmaceutical preparations. LC with UV detection is often preferred in ordinary laboratories because of its wide suitability and availability. The reported LC methods for the individual determination of the drugs cannot be easily applied for the simultaneous determination of both drugs in the formulation owing to their large differences in physical and chemical properties such as polarity and solubility.

The present paper describes a rapid and accurate LC method for the simultaneous determination of roxithromycin and ambroxol hydrochloride in the new tablet formulation. The performance of the developed method was validated in terms of selectivity, linearity, precision and accuracy.

2. Experimental

2.1. Chemicals and reagents

Roxithromycin reference standard was from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Ambroxol hydrochloride reference standard was from Beijing Taiyang Pharmaceutical Company Ltd. (Beijing, China). Roxithromycin and ambroxol hydrochloride tablets were from Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). Each tablet contains 150 mg roxithromycin and 30 mg ambroxol hydrochloride. HPLC-grade methanol and acetonitrile were from Tedia Company (Fairfield, USA). Ammonium acetate of analytical grade was from Shengyang Chemical Reagents Factory (Shenyang, China). Distilled water was prepared by Milli-Q system (Millipore, USA). All other chemicals and reagents used were of analytical grade unless indicated otherwise.

2.2. Apparatus and chromatographic conditions

Liquid chromatography was performed on a Hewlett-Packard (HP) series 1100 liquid chromatographic system equipped with a G1310A Iso Pump, an HP variable UV-Vis detector, a G1328A Manual Injector with 20 μl loop (Agilent, USA). EChrom 98 chromatography workstation was employed for data collecting and processing (Elite, China). A Shimadzu UV-2201 UV-Vis double-beam spectrophotometer (Shimadzu, Japan) was used for scanning and selecting the detection wavelength.

A Diamonsil^{TM} C_{18} column (200 mm × 4.6 mm, 5 μm) from Dikma Technologies (Beijing, China) was used for the separation. This column is end-capped and possesses specific surface area of 440 m^2/g, porosity of 100 A and carbon content of 17%. The mobile phase consisting of a mixture of acetonitrile, methanol and 0.5% ammonium acetate (39:11:50 (v/v), pH 5.5) was delivered at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 μm membrane filter from Shuangji Chromatograph Company (Tianjin, China) and degassed for 30 min in an ultrasonic bath. Analysis was performed at ambient temperature and detection was performed at 220 nm. The injection volume was 20 μl.

2.3. Preparation of standard solutions

A stock solution with roxithromycin at about 4.02 mg/ml and ambroxol hydrochloride at about 0.85 mg/ml was prepared with the mobile phase. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solutions containing roxithromycin and ambroxol hydrochloride in the concentration ranges of 201.2–2012.0 μg/ml

and 42.7–427.0 μg/ml, respectively. The prepared standard solutions were stored at 4 °C.

2.4. Preparation of sample solution

Ten tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder equivalent to 75 mg roxithromycin was transferred to a 50 ml volumetric flask. After about 10 ml of mobile phase was added, the mixture was shaken well and brought to volume with mobile phase, and filtered. The first 10 ml of the filtrate was rejected, and the subsequent filtrate was used as the sample solution.

3. Results and discussion

3.1. Method development

The primary target in developing this LC method is to achieve simultaneous determination of roxithromycin and ambroxol hydrochloride in the compound formulation under common conditions that are applicable for the routine quality control of this product in ordinary laboratories. Taken into account the instability of roxithromycin in strong acidic and basic media, a mobile phase with weakly acidic or neutral pH value is preferred. The optimal pH value was found to be 5.5 after several tests with the pH ranging from 4.0 to 7.0. Since in ultraviolet region roxithromycin has no significant maximum absorption but end absorption, detection was performed at the wavelength of 220 nm where ambroxol hydrochloride also exhibits significant absorption.

Mobile phase was selected in terms of its components and proportions. This work began with a binary mixture of methanol and 0.5% ammonium acetate in the ratio of 50:50 (v/v, pH 5.5). After several trials, it was found that the incorporation of acetonitrile into the mobile phase favoured the separation and elution of the two drugs. With the increase of acetonitrile, the retention times of both drugs can be shortened significantly. When the ratio of mobile phase of a mixture of acetonitrile, methanol and 0.5% ammonium acetate was gradually changed from 30:20:50 to 45:10:45, the retention time decreased from more than 15–5 min for roxithromycin and from 5 to 2 min for ambroxol hydrochloride. At the same time, the peak shapes of the two drugs, especially that of roxithromycin, were improved. Finally a mobile phase consisting of a mixture of acetonitrile, methanol and 0.5% ammonium acetate in the ratio of 39:11:50 was adopted, which produces good resolution, reasonable retention and acceptable peak shape for both drugs. The tablet matrix was also determined to see if any interference from them existed. No significant peaks from matrix were observed in chromatograms, indicating no interference from the formulation matrix. A typical LC chromatogram for a tablet sample solution is shown in Fig. 1. The retention time is 3.3 min for ambroxol hydrochloride and

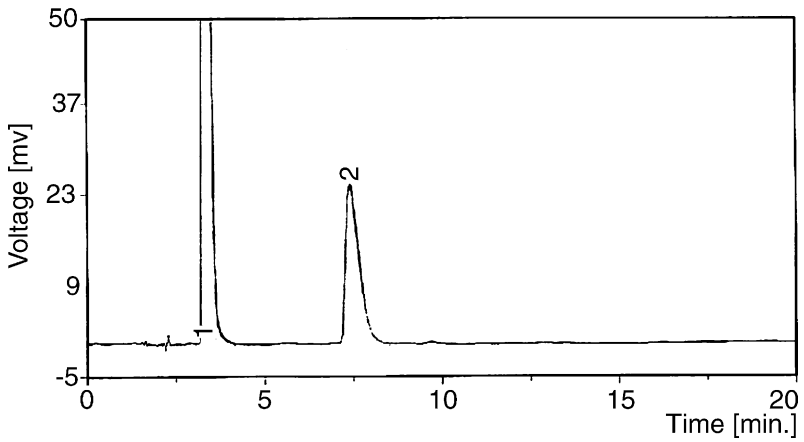

Fig. 1. A typical chromatogram of a tablet sample solution containing 300 μg/ml of ambroxol hydrochloride and 1500 μg/ml of roxithromycin. Peaks: (1) Ambroxol hydrochloride (3.3 min); (2) Roxithromycin (7.4 min).

7.4 min for roxithromycin, respectively. The run time is less than 10 min.

3.2. Linearity

The linearity of the responses of the two drugs was verified at five concentration levels ranging from 201.2 to 2012.0 μg/ml for roxithromycin and 42.7–427.0 μg/ml for ambroxol hydrochloride, respectively. The calibration curve was constructed by plotting mean area response A against concentration C of each drug. The regression equations obtained for the two drugs were $A = -3.32 + 3.97C$ ($r = 0.9995$, $n = 5$) for roxithromycin and $A = -127.7 + 214.4C$ ($r = 0.9999$, $n = 5$) for ambroxol hydrochloride, respectively. The results show that an excellent correlation existed between peak area and concentration of each drug within the concentration range tested.

3.3. Limit of quantitation

The limit of quantitation (LOQ) was defined as the lowest concentration that can be determined with acceptable accuracy and precision, which can be established at a signal-to-noise ratio of 10. LOQ of each drug was experimentally verified by six injections of each drug at its LOQ concentration. The LOQ of roxithromycin and ambroxol hydrochloride were found to be 39.7 and 0.57 μg/ml, respectively.

3.4. Precision

The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The obtained relative standard deviation (R.S.D.) for roxithromycin and ambroxol hydrochloride was 1.5 and 0.28%, respectively.

The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. The R.S.D. of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision. The obtained R.S.D. values were 1.8% for roxithromycin and 1.7% for ambroxol hydrochloride. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days, which was found to be 1.9 and 1.7% for roxithromycin and ambroxol hydrochloride, respectively. The results indicated the good precision of the developed method.

Table 1
Assay results for roxithromycin (150 mg per tablet) and ambroxol hydrochloride (30 mg per tablet) in the formulation product (mean ± S.D., %)

Batch no.	Roxithromycin	Ambroxol hydrochloride
1	103.8 ± 1.4	103.0 ± 1.3
2	98.4 ± 1.6	102.0 ± 1.1
3	100.2 ± 1.5	101.0 ± 1.4

3.5. Accuracy

Accuracy was determined by applying the developed method to synthetic mixtures of excipients to which known amounts of each drug corresponding to 80, 100 and 120% of label claim had been added. The accuracy was then calculated as the percentage of analyte recovered from the formulation matrix. Mean recoveries (mean ± S.D.) for roxithromycin and ambroxol hydrochloride from the formulation are 100.0 ± 1.2% and 99.9 ± 1.4%, respectively. The inter-day accuracy was also determined by assaying the tablets in triplicate per day for consecutive 3 days. Mean recoveries for the inter-day accuracy are 100.3 ± 1.6% for roxithromycin and 100.0 ± 1.7% for ambroxol hydrochloride. The obtained results suggested the accuracy of the developed method for the simultaneous determination of the two drugs in the formulation.

3.6. Assay of the tablets

The validated LC method was applied to the determination of roxithromycin and ambroxol hydrochloride tablets. Three batches of the tablets were assayed and the results are shown in Table 1, indicating that the amount of each drug in the tablet samples met with the requirements (90–110% of the label claim).

4. Conclusion

The proposed LC method is rapid and accurate for the simultaneous determination of roxithromycin and ambroxol hydrochloride in the new tablet formulation.

It can be used for the quality control of the formulation product in ordinary laboratories.

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