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Spectrophotometric and HPLC Methods for Simultaneous Estimation of Roxithromycin and Ambroxol from Tablets

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A simple, accurate, economical and reproducible UV spectrophotometric and a HPLC method for simultaneous estimation of two-component drug mixture of roxithromycin and ambroxol in combined tablet dosage form have been developed. The first developed method employs multiwavelength spectroscopy using six mixed standards and 267 nm and 245 nm as two wavelengths for estimation. Linearity was observed in concentration range of 50-300 μg/mL of roxithromycin and 10-60 μg/mL of ambroxol. Developed HPLC method is reverse-phase chromatographic method using Hypersil C_{18} column and acetonitrile:water in the ratio of 35:65 v/v pH 5.0 as mobile phase. For HPLC method, linearity was observed in concentration range of 100 -500 μg/mL of roxithromycin and 20-100 μg/mL of ambroxol. Results of analysis were validated statistically and by recovery studies.

Key Words: Spectrophotometry, HPLC, Roxithromycin, Ambroxol.

INTRODUCTION

Roxithromycin, chemically 9-[O-[(2-methoxy ethoxy) methyl]oxime] oxacyclotetradecane erythromycin derivative is a macrolide antibiotic used in the treatment of respiratory tract and urinary tract infections[1]. Roxithromycin is official in British Pharmacopoeia[2] and European Pharmacopoeia[3]. Ambroxol, chemically 4{[2-amino-(3,5-dibromophenyl)-methyl]amino}-cyclohexanol is a mucolytic used in the treatment of acute and chronic respiratory tract disorders[4]. Literature survey indicated spectrophotometric[5-10], HPLC[11-20] have been developed for the estimation of roxithromycin and ambroxol, individually and in combination with other drugs. But no method has been established so far, for the simultaneous estimation of these drugs in combined dosage form. The present investigation is an attempt to develop highly sensitive, precise and rapid analytical methods for the simultaneous estimation of roxithromycin and ambroxol from tablet formulations.

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EXPERIMENTAL

Standard bulk drug samples of roxithromycin and ambroxol were provided by Madras Pharmaceuticals, Chennai. Tablets of combined dosage form were procured from the local market. All other reagents used were of analytical grade for spectrophotometric method and of HPLC grade for HPLC method. Shimadzu UV/Vis spectrophotometer (model 1601) with 1 cm matched quartz cell was used for spectrophotometric method. Spectra were recorded using specific program of apparatus, having specifications as follows: spectral bandwidth 3 nm, wavelength accuracy ± 0.5 nm, wavelength readability 0.1 nm increments. For HPLC method, Shimadzu delivery module LC-10AD with UV SPD-10A detector was used.

Method-I: Multiwavelength spectroscopy

Using the overlain spectra of roxithromycin and ambroxol in methanol, the wavelength maxima of both drugs, *i.e.*, 267 and 245 nm, were selected as two sampling wavelengths for this method. Six mixed standards of two drugs in methanol were prepared so as to contain 50-300 μg/mL of roxithromycin and 10-60 μg/mL of ambroxol. All mixed standard solutions were scanned over the range of 400 to 200 nm in multicomponent mode of spectrophotometer using 267 and 245 nm as two sampling wavelengths. The spectral data from these scan were used to determine the concentration of two drugs in the sample solution.

Analysis of commercial formulation: 20 Tablets were accurately weighed and average weight per tablet was determined. Tablets were ground to fine powder and weighed tablet powder equivalent to 250 mg of roxithromycin was transferred to 100 mL volumetric flask. The powder was dissolved in methanol by intermittent shaking and the volume was made upto the mark with methanol. The solution was then filtered through Whatmann filter paper no. 1. Aliquot of this solution was diluted to get a final concentration 250 and 50 μg/mL of roxithromycin and ambroxol, respectively. The sample solution was scanned over the range of 400 to 200 nm in multicomponent mode and concentration of each component was estimated by analysis of spectral data of sample solution with respect to that of mixed standards by the instrument. Results of analysis are reported in Table-1.

Method-II: High Performance liquid chromatographic method

HPLC method was developed using Hypersil C_{18} ODS (5 μ) 250 × 4.6 mm column. Mobile phase selected for this method contained 65 parts of water and 35 parts of acetonitrile adjusted to pH 5.0 with phosphoric acid that was filtered through 0.45 m membrane filter. Flow rate employed was 1.5 mL/min. Detection of eluent was carried out at 220 nm.

Standard stock solution: Standard stock solutions of pure drugs were made separately in mobile phase containing 1000 μg/mL of roxithromycin and ambroxol and filtered through a 0.45 μ membrane filter.

Preparation of calibration curve: For preparation of the drug solutions for the calibration curves in a series of 10 mL volumetric flasks 1, 2, 3, 4 and 5 mL of the pure drug standard stock solutions of roxithromycin and 0.2, 0.4, 0.6, 0.8 and 1 mL of the pure drug standard stock solutions of ambroxol were transferred. The volume in each flask was made up to the mark with the mobile phase. Each solution was injected and a chromatogram was recorded. Mean retention time for roxithromycin was found to be 3.474 min and for ambroxol 8.312 min. The peak area of roxithromycin and ambroxol were calculated and respective calibration curves were plotted against concentration of drug and peak area of drug.

Procedure for analysis of formulations: 20 Tablets of the formulation were weighed and average weight per tablet was calculated. 20 Tablets were crushed and ground to a fine powder. Powder equivalent to 125 mg of roxithromycin was weighed and transferred to a 100 mL volumetric flask containing 50 mL mobile phase. The powder mixture was dissolved in the mobile phase with the aid of ultrasonication. The solution was filtered through Whatmann filter paper no. 41 into another 100 mL volumetric flask. The filter paper was washed with mobile phase and washings were added to filtrate. Volume of filtrate was made up to the mark with the mobile phase. To another 10 mL volumetric flask, 1mL of this solution was transferred and the volume was made up to the mark with the mobile phase. This solution was filtered through a 0.45 μ membrane filter. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 μL fixed sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated five times and the peak areas were recorded. The peak areas of each of the drugs were calculated and the amount of each drug present per tablet was estimated from the calibration curves. The results of analysis are presented in Table-1.

TABLE-1
RESULTS OF ANALYSIS OF COMMERCIAL FORMULATION

Method	Label claim (mg/tablet)		% Label claim estimated*		Standard deviation	
	RO	AM	RO	AM	RO	AM
Method 1	150	30	100.18	100.10	0.1332	0.1329
Method 2	150	30	100.06	100.05	0.7033	0.3620

RO = Roxithromycin; AM = Ambroxol; *Average of five determinations.

Recovery studies: To study the accuracy, reproducibility and precision of the above methods, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at three different levels. Results of recovery studies were found to be satisfactory and are reported in Table-2.

TABLE-2
RESULTS OF RECOVERY STUDIES

Method	Concentration added (mcg/mL)		% Concentration recovered*	
	RO	AM	RO	AM
Method 1	50	10	100.02	99.40
	100	20	100.05	100.06
	150	30	100.08	100.57
Method 2	100	20	102.60	98.12
	200	40	99.20	98.08
	300	60	98.07	99.02

*Average of three determinations; RO = Roxithromycin; AM = Ambroxol.

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