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Derivative Spectrophotometry Method for Simultaneous Estimation of Rupatadine and Montelukast in their Combined Dosage Form

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ABSTRACT

This study describes the development and validation for the simultaneous estimation of rupatadine and montelukast by the first-order derivative UV spectroscopy method. The quantification was achieved by the first-order derivative spectroscopy method at 273.46 nm and 297.27 nm over the concentration range of 5-25 µg/ml for estimation of rupatadine and montelukast in a combined tablet formulation. Procedure does not require prior separation of components from the sample. Rupatadine and montelukast were determined at 15 µg/ml with a mean recovery of 99.59 + 0.225 and 99.21 + 0.76, respectively. Calibration curves were linear with a correlation coefficient of 0.9994 and 0.9992 for rupatadine and montelukast, respectively. The relative standard deviation was found to be <2.0%. The present result shows that the proposed method can be successfully used for simultaneous determination of the drug content in marketed formulations.

Key words: Combination tablet, first-order derivative spectroscopy, montelukast, rupatadine

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INTRODUCTION

Rupatadine is a non-sedating H1-antihistamine (second generation) and platelet-activating factor receptor with a chemical name 8-chloro-6,11-dihydro-11-(1-((5-methyl-3-pyridyl)methyl)-4-piperidylidene)-5*H*-benzo(5,6) cyclohepta(1,2-b)pyridine. It is potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria.[1-2]

Montelukast is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series with the chemical name 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl]-3[2-(1-hydroxy-1-methylethyl) phenyl] propylsulfanylmethyl] cyclo-propyl] acetic acid sodium salt. It is developed as a therapeutic agent for the treatment of bronchial asthma by Merck and Co.[3]

Rupatadine and montelukast are available in a single dosage form but not in combination therapy in the market. Fixed dose combination therapy of rupatadine and montelukast is indicated for the treatment of asthma, allergic rhinitis, and urticaria. Rupatadine also enhances the effect of montelukast in asthma.

Some analytical methods for the quantitative determination of rupatadine in pharmaceutical formulations are described in the literature like stability indicating the high-performance liquid chromatography (HPLC) method.[4] Method available for the determination of montelukast

sodium include HPLC,[5] protein precipitation,[6] liquid chromatography/tandem mass spectrometry(LC-MS/MS),[7] liquid-liquid extraction using HPLC with the fluorescence detector,[8] its *S*-enantiomer in human plasma by stereoselective high performance liquid chromatography (HPLC) using column-switching[9] and its determination in human plasma by the column-switching HPLC method,[10] derivative spectroscopy, HPLC,[11] microwave-assisted extraction (MAE) technique,[12] method for the application of pressurized liquid extraction technology for pharmaceutical solid dosage form[13] Residual acetate analysis in bulk drug.[14]

No spectrophotometric method or the HPLC method has been reported in the literature for the simultaneous determination of montelukast and rupatadine in their commercial formulations. It would therefore be beneficial to provide accurate, precise, and reliable methods for simultaneous determination of montelukast and rupatadine. The present work describes two analytical procedures for the quantitation of montelukast in co-formulation with rupatadine using reversed phase HPLC and first-derivative spectrophotometry.

MATERIALS AND METHODS

Chemicals

Rupatadine and montelukast were obtained as a gift sample from Zydus Cadila Healthcare Limited (Ahemdabad, India) and Vent Unimark Remedies with the percentage purity of 99.10% and 98.04%, respectively. Tablets containing 10 mg rupatadine and 10 mg montelukast also provided by Zydus Cadila Healthcare Limited (Ahemdabad, India). HPLC grade methanol was obtained from Spectrochem and E-Merck Limited. HPLC grade distill water was procured from Zydus Cadila Healthcare Limited, prepared by triple glass distillation and filtered through a nylon 0.45 μm-47 mm membrane filter (Gelman Laboratory, Mumbai, India).

Instrumentation

Spectrophotometric analysis was performed on Perkin_ Elmer lambda 19 (Perkin-Elmer, Norwalk, CT, USA) a computer-controlled double-beam UV_Visible spectrophotometer using 10 mm quartz cells with a slit width of 1 nm and a scan speed of 60 nm/min.

Solutions

Preparation of standard stock solutions of rupatadine and montelukast.

Stock solutions of rupatadine and montelukast were prepared by accurate weighing of 50 mg in each case and dissolving in a methanol up to 100 ml in volumetric flasks. For simultaneous quantitative studies of both drugs, a series of standard working solutions containing both the drugs were prepared by an appropriate dilution of a mixture of stock solutions. All the volumetric flasks were wrapped with a aluminum foil.

Preparation of standard working solution for assay

The standard working concentration for the determination of the assay of both the drugs was 15 μg/ml achieved by appropriate dilution from the standard stock solutions. All the volumetric flasks containing rupatadine and montelukast were wrapped with aluminum foil and stored in the dark.

Conditions

Measurement of derivative spectra

First-derivative UV spectra for the solutions of rupatadine and montelukast were recorded in a 10 mm cell over the range 250-375 nm using methanol in the reference cell. Each spectrum was recorded in triplicate. The zero-crossing point for rupatadine and montelukast were recorded. Characteristic wavelengths (ZCPs) for rupatadine and montelukast were confirmed by varying the concentration of both drugs. For each replicate measurement, the cell was refilled with fresh solution.

RESULTS AND DISCUSSION

Derivative spectrophotometric method development

The zero-order absorption spectra of rupatadine and montelukast solutions in methanol are shown in Figure 1. The spectra display overlapping in the region of 200-300 nm. This makes the determination of rupatadine in the presence of montelukast by conventional UV spectrophotometry difficult, but the determination of montelukast from 310 to 375 nm might be possible without the interference from rupatadine. The derivative spectrophotometry technique was, however, chosen for the determination of both the drugs since it could remove broadband contributions from excipients and might also overcome the interference from peak overlapping. Derivative spectra of different orders were studied for both rupatadine and montelukast individually and simultaneously. The experiments showed that the first-derivative spectra of rupatadine and montelukast were simple and gave results with suitable precision at a Dl value of 4 nm. In these first-derivative spectra, the signals at 273.4

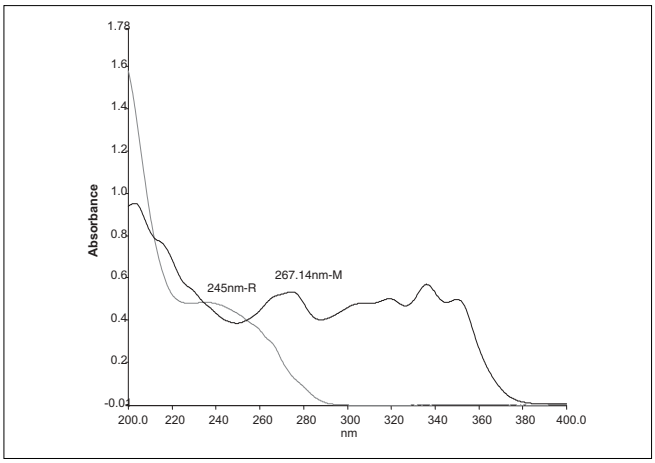

Figure 1: Overlain spectrum of rupatadine and montelukast (15 µg/ml) in methanol

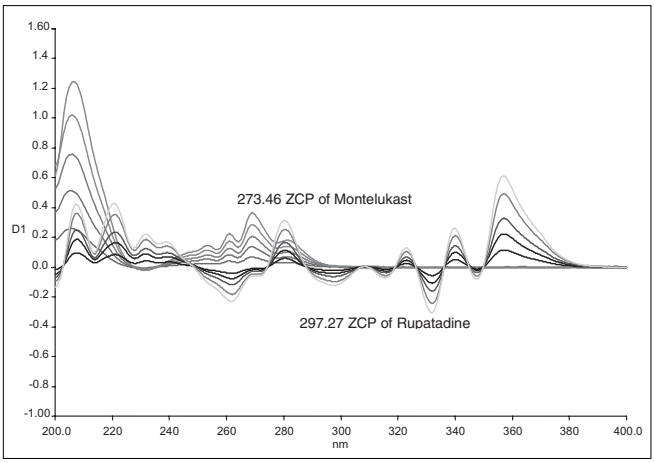

Figure 2: Overlain first-order derivative spectrum of rupatadine and montelukast in methanol

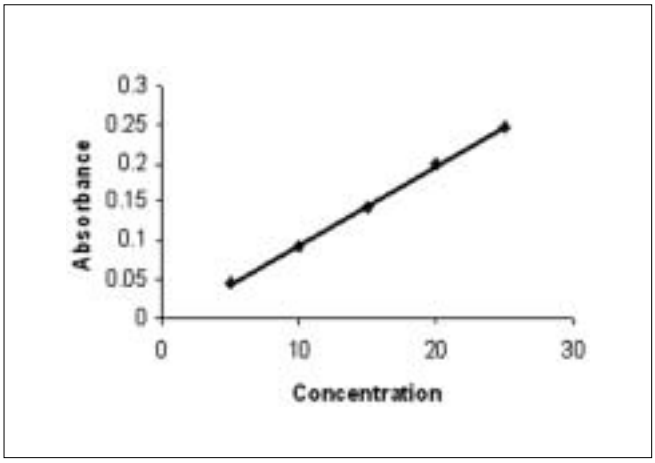

Figure 3: Calibration curve for rupatadine at 273.4 nm

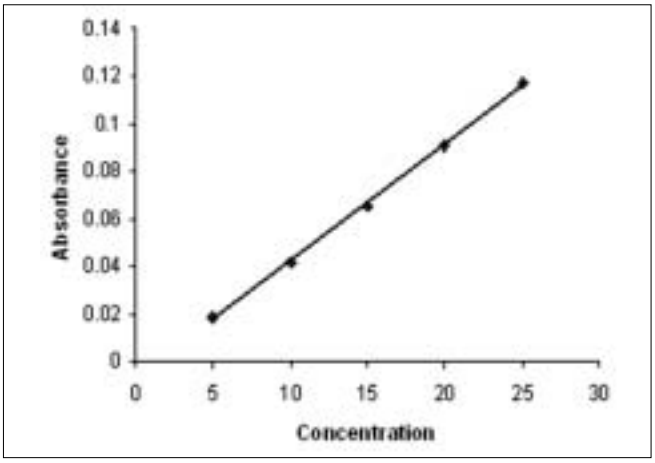

Figure 4: Calibration curve for montelukast at 297.27 nm

nm (zero-crossing point of montelukast) are proportional to the rupatadine concentration and the signals at 297.2 nm (rupatadine reads zero) are proportional to the montelukast concentration [Figure 2].

Method validation

The developed method was method validated for the simultaneous assay determination of rupatadine and montelukast using following parameters.

Calibration graph (linearity of derivative method)

To obtain calibration curves for both rupatadine and montelukast, the first-derivative spectra of standard drug solutions in combination were recorded at five varied concentrations of rupatadine (5-25 µg/ml) and montelukast (5-25 µg/ml) which are shown in Figures 3 and 4. The spectrum was measured three times for each concentration. The correlation coefficients of calibration

Table 1: Regression analysis of the calibration curves for rupatadine and montelukast for the proposed spectrometry

Parameter	Absorbance (273.46)	Absorbance (297.27)
Linear range (µg/ml)	5-25 µg/ml	5-25 µg/ml
Slope	0.0102	0.0049
Intercept	0.0072	0.0065
Standard deviation of slope	0.000147	0.000040
Standard deviation of intercept	0.000155	0.000098

Table 2: Summary of validation parameters of derivative spectroscopy method

Parameters	Rupatadine	Montelukast
Recovery %	98.5±0.73	98.08±1.08
Repeatability (RSD, n=6)	1.92	0.73
Precision (CV)		
Intra-day (n=3)	0.87	0.90
Inter-day (n=3)	1.13	1.33
Limit of detection (µg/ml)	0.76 µg/ml	0.36 µg/ml
Limit of quantitation (µg/ml)	2.30 µg/ml	1.09 µg/ml
Solvent suitability	Suitable for 24 hrs	Suitable for 24 h

Table 3: Assay Results of marketed formulation

Formulation	Actual concentration (µg/ml)		Rupatadine % ± SD (n = 3)	Montelukast % ± SD (n = 3)
	Rupatadine	Montelukast		
Tablet	15	15	100.22% ±1.10	99.74% ± 1.4

plots for rupatadine and montelukast were 0.9994 and 0.9992 indicating good linearity in both cases that are presented in Table 1.

Accuracy

Accuracy of the first-derivative method for simultaneous determination of rupatadine and montelukast was checked at independently prepared concentration levels at 7.5,15, and 22.5 µg/ml (*n*=3). The mean recovery data for each level (at 95% confidence limits) and its percentage recoveries are shown in Table 2.

Precision

Intra-day precision of the method was evaluated for mixtures of rupatadine and montelukast at three different independent concentrations i.e. 10, 15, and 20 µg/ml (*n*= 3) by determining their assay. The R.S.D. values ranged from 0.82 to 1.13% and from 1.0 to 1.33% for rupatadine and montelukast, respectively [Table 2]. Inter-day precision of the method was tested for 3 days at the same concentration levels. Solutions for calibration curves were prepared fresh every day. The R.S.D. values ranged from 0.46 to 0.87 and from 0.73 to 0.9% for rupatadine and montelukast, respectively [Table 2].

Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Assay determination for rupatadine and montelukast from formulations

Sample stock solution preparation

Weigh accurately tablets powdered equivalent to about 10 mg of rupatadine and montelukast in a 100-ml volumetric flask and make up the volume with methanol. The suspension was sonicated for 30 min to dissolve, and the final volume was made up to mark with methanol. Filter it through 0.22 µm-47 Millipore HVLP nylon filters. All the volumetric flasks containing rupatadine and montelukast were wrapped with an aluminum foil and stored in the dark.

Simultaneous quantification of rupatadine and montelukast

Simultaneously quantifications of rupatadine and montelukast were done according to Radhakrishana *et al*.[13] The proposed validated method was successfully applied to determine rupatadine and montelukast in their combined tablet dosage form. The working concentration for the determination of assay of both the drugs was 15 µg/ml achieved by appropriate dilution from the sample stock solution. All the volumetric flasks containing rupatadine and montelukast were wrapped with the aluminum foil and stored in the dark. The results obtained for rupatadine and montelukast were comparable with the corresponding labeled amounts that are presented in Table 3.

CONCLUSION

The present results provide clear evidence that the proposed method can be successfully used for simultaneous determination of drug content in marketed formulations

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