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## DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF RUPATADINE FUMARATE AND MONTELUKAST SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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### ABSTRACT

The method for the simultaneous estimation of Rupatadine Fumarate (RUPA) and Montelukast Sodium (MONT) in tablet dosage form has been developed, based on simultaneous equation method at two selected wavelengths 213.0 nm and 283.0 nm respectively in Methanol, and also multicomponent method at two selected wavelengths 213.0 nm and 243.0 nm. The linearity ranges for RUPA and MONT was 5–30 µg/ml. The percentage drug estimated in marketed preparation, in simultaneous equation method was 100.12% and 99.83% for RUPA and MONT respectively and in multicomponent method was 100.09% and 99.99% for RUPA and MONT respectively. The method was validated for various parameters as per ICH guidelines and USP requirements. The accuracy of the methods was assessed by recovery studies and was found to be 100.02–100.08% for RUPA and 99.83–100.06% for MONT in simultaneous equation method and 100.0–100.16% for RUPA and 99.83–100.03% for MONT in multicomponent method. These methods are simple, accurate, sensitive, cost effective and specific.

**Key Words:** Rupatadine Fumarate, Montelukast Sodium, Simultaneous equation method, Multicomponent method.

### INTRODUCTION

Rupatadine is a non-sedating H<sub>1</sub>-antihistamine (second generation) and platelet-activating factor inhibitor. Chemically, it is 8-Chloro-6,11-dihydro-11-{1-[(5-methyl-3-pyridinyl)methyl]-4-piperidylidene}-5H-benzo[5,6]cyclohepta[1,2-b] pyridine fumarate. It is potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. (Choudekar R *et al.*, 2012) Montelukast is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series. Chemically, it is Sodium 1-[(R)-m[(E)-2-(7-chloro-2-quinolyl)-vinyl]-α-[o-(1-hydroxy-1-methylethyl)phenethyl]

benzyl }thio)methyl]cyclopropane acetate. (Laskhmana R *et al.*, 2012). It is developed as a therapeutic agent for the treatment of bronchial asthma. The chemical structure of Rupatadine and Montelukast are shown in Figure 1 and 2 respectively. Both drugs are marketed as combined dosage formulation in the ratio of 1:1; RUPA: MONT. The literature review reveals only one analytical method for the simultaneous estimation of Rupatadine fumarate and Montelukast sodium by UV-visible Spectrophotometry (Choudekar R, *et al.*, 2012). Literature review reveals few analytical techniques like Spectrophotometry (Patel DJ *et al.*, 2010; Choudhari V *et al.*, 2010), RP-HPLC (Subasini U *et al.*, 2012; Anirudha RC *et al.*, 2012; Pameela Rani A *et al.*, 2010; Patel DJ *et al.*, 2010; Giriraj P *et al.*, 2012) and HPTLC (Suparna S *et al.*, 2012; Hitesh V *et al.*, 2012) estimations of either Montelukast sodium or Rupatadine fumarate. The combination is not official in any Pharmacopoeia. In view of the need for a suitable method

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for routine analysis in combined formulations, attempts are being made to develop simple, precise accurate and reproducible analytical methods for simultaneous estimation of RUPA and MONT and extend it for their determination in formulation.

## MATERIALS AND METHODS

### Instrumentation

Double Beam UV-Visible Spectrophotometer (Shimadzu UV-Visible spectrophotometer 1601) with 10 mm matched quartz cuvettes were used for all absorbance measurements. Electronic Weighing Balance (Citizen Analytical Balance) was used having sensitivity 0.1 mg. In addition micropipette and sonicator were used in this study.

### Reagents and chemicals

Rupatadine fumarate and Montelukast sodium was generous gift sample from FDC Ltd, (Raigad, India) and EROS MICRO Labs Ltd, (Bangalore, India). A commercial tablet formulation Smarti-M from German Remedies, a division of Cadila Healthcare Ltd, (Mumbai, India) containing 10mg of RUPA and 10mg of MONT was purchased from local market and used within their shelf life period. All other chemicals were used of analytical grade.

### Preparation of Standard Stock Solution

Standard stock solutions of Rupatadine fumarate and Montelukast sodium were prepared by dissolving 25mg of drug in 25 ml of methanol to get standard stock solution of 1000µg/ml. This solution was further diluted to get standard solution of concentration 100µg/ml of RUPA and MONT.

### Selection of $\lambda_{max}$

The stock solution were diluted to get final concentration 10 µg/ml for doth MONT and RUPA. These solutions were scanned in wavelength range of 200 nm to 400 nm to determine the  $\lambda_{max}$ . RUPA shows  $\lambda_{max}$  at 213 nm while MONT at 283 nm respectively (Figure 3).

## METHOD I: SIMULTANEOUS EQUATION METHOD

### Preparation of calibration curves

Appropriate dilutions of the standard stock solution were done separately to get 5, 10, 15, 20, 25 and 30µg/ml of RUPA and MONT respectively. The absorbances were measured at 213.0 nm ( $\lambda_{max}$  of RUPA) and 283.0 nm ( $\lambda_{max}$  of MONT). Beer's Lambert range for RUPA and MONT were selected and working calibration curves for both the drugs were plotted separately. The calibration curve was constructed by plotting absorbance vs concentration at each wavelength and regression coefficients were calculated (Figure 4-5).

### Quantitative determination of RUPA and MONT in tablet

20 tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Weighed tablet powder equivalent to 10 mg of RUPA and 10 mg of MONT was transferred to 50 ml volumetric flask. Methanol was added, ultra-sonicated for 15min. and volume made up to mark with methanol. The solution was filtered through Whatman filter paper No.41. The filtrate was further diluted with methanol to obtain concentration 10µg/ml of RUPA and MONT. The concentration of both RUPA and MONT were determined by measuring the absorbances of sample at both wavelengths 213.0 nm and 283.0 nm.

The concentrations of RUPA and MONT were calculated by solving these simultaneous equations.

$$C_x = (A_1 a_{y2} - A_2 a_{y1}) / (a_{x1} a_{y2} - a_{x2} a_{y1}) \dots \dots \dots (1)$$

$$C_y = (a_{x1} A_2 - a_{x2} A_1) / (a_{x1} a_{y2} - a_{x2} a_{y1}) \dots \dots \dots (2)$$

Where,  $a_{x1}$  = Absorptivity of **MONT** at 213.0 nm

$a_{x2}$  = Absorptivity of **MONT** at 283.0 nm

$a_{y1}$  = Absorptivity of **RUPA** at 213.0 nm

$a_{y2}$  = Absorptivity of **RUPA** at 283.0 nm

$C_x$  and  $C_y$  are concentration of **MONT** and **RUPA** respectively in the sample solution.

$A_1$  and  $A_2$  are the absorbances of the mixture at 213.0 nm and 283.0 nm respectively. The result of the analysis of tablet formulation by simultaneous equation method is shown in Table 1.

## METHOD II: MULTICOMPONENT METHOD

### Preparation of calibration curves

Appropriate dilutions of the standard stock solution were done separately to get 5, 10, 15, 20, 25 and 30µg/ml of RUPA and MONT respectively. The absorbances were measured at 213.0 nm ( $\lambda_{max}$  of RUPA) and 283.0 nm ( $\lambda_{max}$  of MONT). Beer's Lambert range for RUPA and MONT were selected and working calibration curves for both the drugs were plotted separately. The calibration curve was constructed by plotting absorbance vs concentration at each wavelength and regression coefficients were calculated (Figure 6).

### Quantitative determination of RUPA and MONT in tablet

The sample was prepared as per the procedure given in simultaneous equation method and different concentration samples were prepared in ratio 5, 10, 15, 20, 25 and 30 µg/ml of RUPA and MONT respectively. The standard solutions and sample solutions were scanned in multicomponent analysis mode of UVspectrophotometer and found out the concentration of sample solutions. The results are given in Table 1.

### Validation of Developed Methods

The proposed methods have been statistically validated in terms of linearity, accuracy, precision,

repeatability and reproducibility, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2A guidelines (ICH, 2005).

#### a) Linearity

The linearity of the measurement was evaluated by analyzing different concentrations of the standard solution of RUPA and MONT. For both methods, the Beer-Lambert's concentration range was found to be from 5-30µg/ml for both RUPA and MONT.

#### b) Accuracy

The study of accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). A known amount of drug was added to preanalyzed tablet powder and percentage recovery were calculated. The results of recovery studies were presented in Table.2

#### c) Precision

The reproducibility of proposed method was determined by performing tablet assay on the same day at different time interval (intraday precision), on three different days (interday precision) and by different analysts. Result of intraday, interday and different analyst precision is expressed in % RSD.

#### d) Specificity

Specificity study was performed by keeping the sample under various stressed conditions at 60°C and 50°C by adding 1 mL of 0.1N HCl, 0.1N NaOH and 3% H<sub>2</sub>O<sub>2</sub> solutions.

#### e) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where,  $\sigma$  = Standard deviation of the response

S = Slope of the calibration curve

### RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of RUPA and MONT. In simultaneous equation method, wavelengths selected for analysis were 213.0 nm ( $\lambda_{\text{max}}$  of Rupatadine) and 283.0 nm ( $\lambda_{\text{max}}$  of Montelukast). The absorbances of the standard solutions were measured at 213.0 nm and 283.0 nm respectively. The linearity concentration range was 5-30µg/ml for both RUPA and MONT.

**Table 1. Results of assay of tablets**

Methods	Label claim (mg/tab)		Amount found (%)*		Standard deviation(±)	
	RUPA	MONT	RUPA	MONT	RUPA	MONT
Simultaneous equation	10	10	100.12	99.83	0.2091	0.3677
Multicomponent	10	10	100.09	99.99	0.1809	0.1662

\*Average of five determination

**Table 2. Results of Recovery Studies**

Methods	Level of % Recovery	Mean % recovery		± SD		% RSD	
		RUPA	MONT	RUPA	MONT	RUPA	MONT
Simultaneous equation	80%	100.03	99.83	0.3818	0.4413	0.3816	0.4420
	100%	100.02	100.06	0.1569	0.2516	0.1568	0.2514
	120%	100.08	99.91	0.2981	0.3036	0.2978	0.3038
Multicomponent	80%	100.16	99.83	0.2610	0.2594	0.2605	0.2598
	100%	100.00	100.03	0.2645	0.2081	0.2645	0.2080
	120%	100.04	99.91	0.1877	0.1650	0.1876	0.1651

Mean of three estimations, SD is Standard Deviation of n=3 observations, RSD is Relative Standard Deviation

**Table 3. Results Specificity studies**

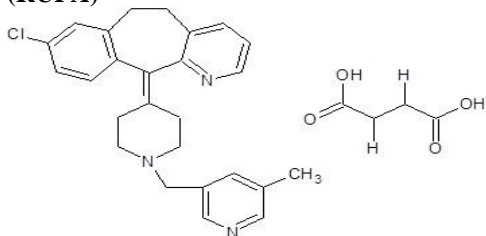
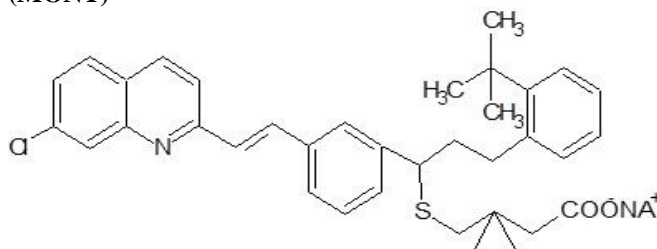
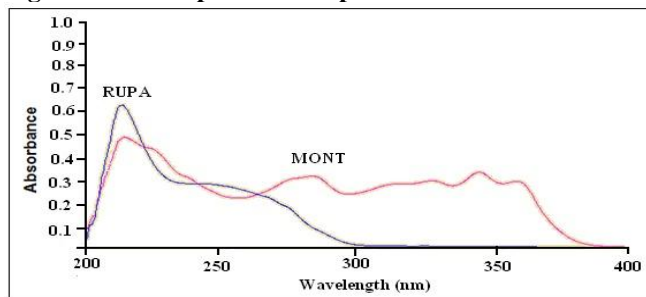
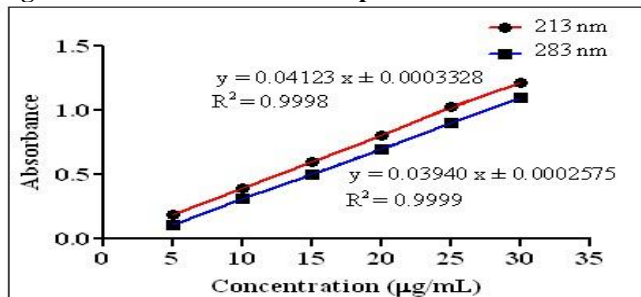
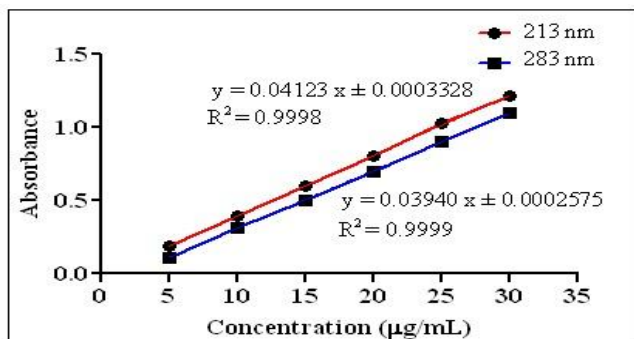
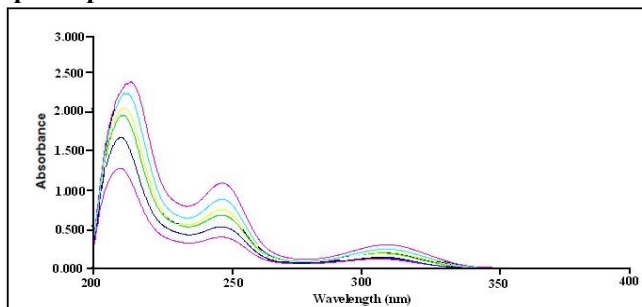
Sr. No.	Conditions	Simultaneous equation		Multicomponent	
		% Labeled Claim of RUPA	% Labeled Claim of MONT	% Labeled Claim of RUPA	% Labeled Claim of MONT
1	Normal	100.90	99.96	100.50	99.95
2	Acid	99.04	99.68	99.03	99.87
3	Alkali	99.01	98.96	99.01	98.84
4	Oxide	98.73	98.34	98.69	98.45
5	Heat	98.61	98.05	98.36	98.02

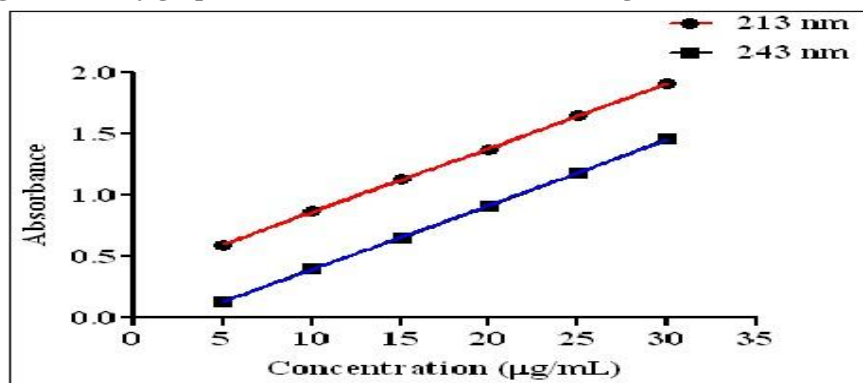
Mean of three estimations

**Table 4. Results of validation Parameters**

Parameters	Simultaneous equation method		Multicomponent method	
	RUPA	MONT	RUPA	MONT
Linearity Range ( $\mu\text{g/ml}$ )	5 – 30	5 – 30	5 – 30	5 – 30
Regression Equation ( $y = mx \pm c$ )	$y = 0.05291x \pm 0.3361$	$y = 0.05317x \pm 0.1302$	$y = 0.05258x \pm 0.3338$	$y = 0.05290x \pm 0.1365$
Correlation Coefficient ( $r^2$ )	0.9999	0.9998	0.9998	0.9997
LOD ( $\mu\text{g/ml}$ )	0.2820	0.2792	0.2806	0.2789
LOQ ( $\mu\text{g/ml}$ )	0.9400	0.9309	0.9355	0.9298
Analysis of tablets (% Assay)	100.12	99.83	100.09	99.99
% Recovery	100.02 – 100.08	99.83 – 100.06	100.00 – 100.16	99.83 – 100.03
Specificity	98.61 – 100.90	98.05 – 99.96	98.36 – 100.50	98.02 – 99.95
Repeatability (% RSD)	0.2091	0.3677	0.1809	0.1662
Intra Day Precision (% RSD)	0.1357	0.1680	0.1069	0.1258
Inter Day Precision (% RSD)	0.1955	0.1814	0.1123	0.1201
Different Analyst (% RSD)	0.1721	0.2709	0.1209	0.1734

RUPA is Rupatadine, MONT is Montelukast,  $y = mx + c$  where  $y$  is absorbance,  $m$  is slope,  $c$  is intercept, LOD is Limit of Detection, LOQ is Limit of Quantitation, RSD is Relative Standard Deviation

**Fig 1. Chemical structure of Rupatadine Fumarate (RUPA)****Fig 2. Chemical structure of Montelukast Sodium (MONT)****Fig 3. Overlain Spectra of Rupatadine and Montelukast****Fig 4. Calibration Curve for Rupatadine****Fig 5. Calibration Curve for Montelukast****Fig 6. Spectra of mixtures containing RUPA and MONT in 1:1 ratio in Multicomponent mode of spectrophotometer**

**Fig 7. Linearity graph of mixture at two selected wavelengths (213 nm and 243 nm)**

The 575.3 and 89.2 are the absorptivity of RUPA and 395.1 and 315.2 are absorptivity of MONT at 213.0 and 283.0 nm respectively. The percent label claim for RUPA and MONT in tablet analysis, by simultaneous equation method was found to be 100.12% and 99.83% respectively. By multicomponent method the percent label claim for RUPA and MONT in tablet was 100.09% and 99.99% respectively. Standard deviation for the six determination of the tablet sample for simultaneous equation and multicomponent method was found to be less than  $\pm 2.0$  indicating precision of the method. Accuracy of the proposed method was ascertained by recovery studies and the results are expressed as % recovery. Percentage recovery by simultaneous equation method was found to be 100.02 – 100.08% and 99.83 – 100.06% and by multicomponent method 100.0 – 100.16% and 99.83-100.03%, for the RUPA and MONT respectively. Standard deviation and coefficient of variance was satisfactorily low, indicating the accuracy of method. By simultaneous equation method, LOD was found to be 0.2820 µg/ml for RUPA and 0.2792 µg/ml for MONT and LOQ was found to be 0.9400 µg/ml for RUPA and 0.9309 µg/ml for MONT respectively. By multicomponent method LOD was found to be 0.2806 µg/ml for RUPA and 0.2789 µg/ml for MONT LOQ was found to be 0.9355 µg/ml for RUPA and 0.9298 µg/ml for MONT respectively. Intra-day, inter-day

and different analysts precision studies were carried out by analyzing tablet powder at different time interval on same day, on three different days and by different analysts respectively.

## CONCLUSION

The experimental results demonstrate that the proposed UV-Spectrophotometric methods using simultaneous equation method and multicomponent method was simple, rapid, sensitive, accurate, precise and economical. Thus these methods can be used for the determination of Rupatadine and Montelukast either in bulk or in the combined solid oral dosage form. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Rupatadine and Montelukast. The developed methods can be successfully used for routine quality control of Rupatadine and Montelukast in their combined dosage form.

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