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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Montelukast Sodium and Bambuterol Hydrochloride in Bulk and Tablet Dosage Formulation.

Varun Pawar[1]✉, Sanjay Pai[1] and Roa G.K.[1]

[1]Al-Ameen Collage of Pharmacy, Near Lalbagh Main Gate, Bangalore-560027, India.

ABSTRACT

Montelukast is leukotrine receptor blocker and bambuterol is β_2 receptor agonist, and a combination of these drugs is used in the management of asthma. A simple, precise, rapid and selective simultaneous equation by corrective absorbance method has been developed and validated for the simultaneous determination of montelukast and bambuterol in bulk and marketed tablet formulation. The method involves solving the simultaneous equation by calculating the concentration of one analyte in the sample and the concentration of the second absorbing component of interest which was then calculated from the corrected absorbance (total absorbance minus absorbance of the interfering substances) in the usual way. Methanol was used as a solvent, the wavelength maxima of montelukast and bambuterol was found to be 283 nm and 265 nm, respectively. The absorptivity of montelukast at 283 and 265 nm was calculated and found to be 383.15 and 285.67 $g^{-1}L\ cm^{-1}$ respectively, for bambuterol at 265 nm it was 10.68 $g^{-1}L\ cm^{-1}$, while at 283 nm, bambuterol has very negligible absorptivity. An equation was derived to calculate the concentration of montelukast and bambuterol in combined tablet formulation simultaneously. Montelukast and bambuterol were found to be linear in the concentration range of 10-80 μgmL^{-1} and 40 – 240 μgmL^{-1}, respectively. The method was found sensitive, accurate and precise for simultaneous estimation of montelukast and bambuterol in combined tablet dosage forms.

Keywords: Montelukast, Bambuterol, Spectrophotometry, Absorptivity.

INTRODUCTION

Montelukast is leukotrine receptor blocker, administered orally as tablet in the dose of 5-10 mg per day. Chemically it is represented as (R-(E))-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl) -3-(2-(1-hydroxy-1-methylethyl) phenyl) propyl)thio) methyl) cyclopropaneacetic acid, monosodium salt with molecular formula $C_{35}H_{35}ClNNaO_3S$ (Fig. 1). It is not an official drug in any pharmacopoeia.

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✉ Email:varunpawar@yahoo.com.in

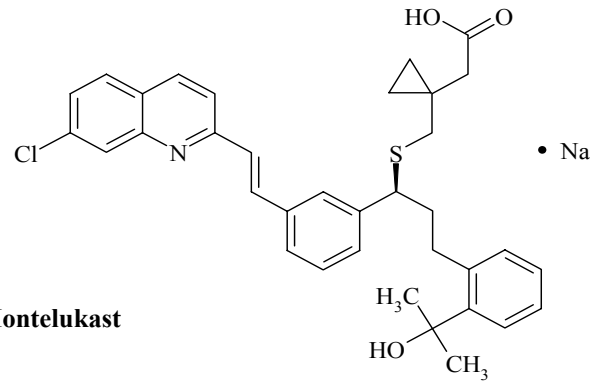

Fig. 1: Structural formula of Montelukast

Bambuterol is a β_2 receptor agonist, administered orally as tablet in the dose of 5-10 mg per day. Chemically it is represented as [3(dimethylcarbamoyloxy)-5-[1-hydroxy-2-(tert-butylamino) ethyl]phenyl] N,N-dimethylcarbamate with the molecular formula $C_{18}H_{29}N_3O_5$. It is an official drug in British pharmacopoeia.

The review of literature revealed that several methods are available for the determination of montelukast and bambuterol individually or in combination with other drugs. But no method mentioned in the literature explained the simultaneous estimation of montelukast and bambuterol in bulk, dosage forms and in biological fluids. The present study describes a simple, rapid, accurate, reproducible and economical method for simultaneous estimation of montelukast and bambuterol using corrective absorbance method.

EXPERIMENTAL

Instruments and Materials

Pure Montelukast sodium and bambuterol hydrochloride were obtained as gift samples from Sun Pharmaceuticals Pvt. Ltd. India. The spectrophotometer used was Shimadzu UV-Visible spectrophometer 1601 model with spectral bandwidth of 3 nm and wavelength accuracy (with automatic wavelength correction) of 0.5 nm. Analytical grade methanol was purchased from Qualigens Fine Chemicals, Mumbai, India. All the apparatus and instruments were calibrated and validated as per calibration and validation protocol specified before starting the experimental work.

Selection and standardization of the solvent

Drugs should have adequate absorbance in the same solvent for the simultaneous determination. In water bambuterol absorbs below 230 nm, possibility of water as solvent was ruled-out. In methanol, bambuterol absorbs below 275 nm and montelukast has a very good absorptivity in entire UV range, hence methanol was selected as a possible solvent for analysis.

Determination of wavelength maxima and molar absorptivity

Standard solution consisted of 50 μgmL^{-1} of montelukast and bambuterol was prepared separately in 10 mL volumetric flasks. Standard solutions of both drugs were scanned in the UV range 220 to 400 nm, using methanol as blank **(Fig 2)**. The wavelength maxima of montelukast and bambuterol were found to be 283 nm and 265 nm, respectively. The absorbances of both drugs were recorded at 265 and 283 nm; and molar absorptivity **(є)** for drugs was calculated from the formula:

Є = A/C.....Eqn.1,

Where, A: absorbance, C: concentration of analyte in $g100mL^{-1}$.

The absorptivity of montelukast at 283 nm and 265 nm was found to be **383.15** and **285.67,** respectively. The absorptivity of bambuterol at 265 nm was found to be **10.68;** while at 283 nm it was negligible

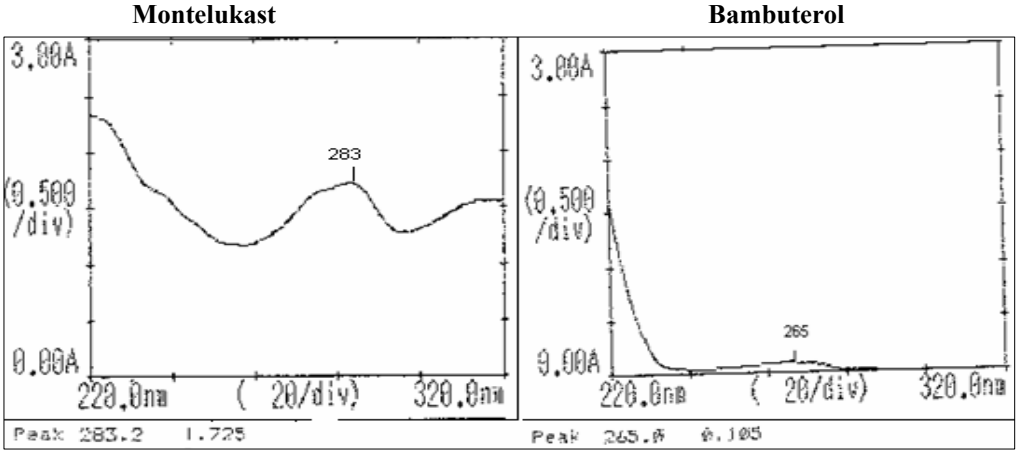

Fig. 2 The UV-Spectra indicating wavelength maxima

Development of Simultaneous Equation

The equation for simultaneous estimation of montelukast and bambuterol in combination was developed by 'Corrective Absorbance Method.[1]

$C_{MON} = A_1 / \epsilon_{x1}$ **……...Eqn.2**,

$C_{BAM} = [A_2 – (C_{MON} X \epsilon_{x2})]/\epsilon_{y2}$ **…….Eqn.3**.

Where, A_1 and A_2: absorbance of the sample at λ_1 and λ_2, respectively

ϵ_{x1} and ϵ_{x2}: absorptivity of Montelukast at λ_1 and λ_2, respectively

ϵ_{y2}: absorptivity of Bambuterol at λ_2,

C_{MON} and C_{BAM}: concentrations of montelukast and bambuterol in $g100mL^{-1}$

λ_1: 283 nm

λ_2: 265 nm

The equations 2 and 3 were modified, for the purpose of calculating the concentration of montelukast and bambuterol in the sample, to be as the following;

$C_{MON} = A_1 / 383.15$**…….. Eqn. 4**,

$C_{BAM} = [A_2 – (C_{MON} X 285.67)]/10.68$**..... Eqn. 5**.

Equation (4) and (5) are the modified forms of the equation for the simultaneous estimation of montelukast and bambuterol in combination.

Preparation of standard mixture solution

Accurately weighed 10 mg of each of the standard drugs montelukast and bambuterol was transferred into clean, dry 10 mL volumetric flask and dissolved into a sufficient volume of methanol. The volume was made up to 10 mL with methanol to get the concentration of 1000 μgmL^{-1}. Aliquots of this solution were transferred into separate 10 mL volumetric flasks and the volume was then adjusted with methanol to get the concentrations in the range between 1- 100 μgmL^{-1}.

Validation of Analytical method

Validation is the process of establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

1. Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOD and LOQ were calculated statistically from formula[3]:

LOD = 3.3 (SD / S), LOQ = 10 (SD / S)

Where, **SD**: Standard Deviation of y- intercepts of regression lines,

S: Slope of the calibration curve.

The LOD and LOQ of montelukast and bambuterol by statistical and visualization methods were mentioned in Table (1).

2. Linearity

The linearity of montelukast and bambuterol were found to be **1 – 80** and **40 – 240 μgmL^{-1}**, respectively.

The values of the percentage curve fittings were in the limits of acceptance criteria (Table 1).

3. Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision is usually expressed as the standard deviation or relative standard deviation. In the present study, developed method was validated for method, system, inter-day and intra-day precision.

As the values of %RSD of all precision study were within the acceptable limits (less than 2%), both the method as well as the system provides good precision and reproducibility (Table 1).

4. Sensitivity

Absorbance of standard solutions of montelukast and bambuterol was taken at 283 and 265 nm. Sandell's sensitivity **(Π)** for drugs was calculated from the following formula, at both wavelengths.[1]

$$\Pi\ (\mu g/cm^{-3}\ AU) = \frac{\text{Conc. of drug } (\mu g 100mL^{-1})}{\text{Absorbance}} \times 0.001$$

The Sandell's sensitivity for montelukast at 283 and 265 nm was found to be 0.00026 and 0.00035 $\mu gcm^{-3}AU^{1}$, respectively; and for bambuterol at 265 nm it reached 0.009441 $\mu gcm^{-3}AU^{-1}$.

5. Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the preanalyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. **(Table 1)**.

Table 1. Validation Parameters

SI. No.	Parameters		Montelukast	Bambuterol
1	LOD	μgmL^{-1}	2.53	5.54
	LOQ	μgmL^{-1}	7.66	16.79
2	Linearity	μgmL^{-1}	10 - 80	40 - 240
		Regression eq^{n}	0.0389x - 0.0129	0.001x + 0.004
		R^2	0.9996	0.999
3.	Precision	Method (SD) %RSD	0.0488 0.101	0.8255 1.7046
		System (SD) %RSD	0.0018171 0.101	0.001169 0.0816
		Inter-day (SD) %RSD	0.0727 0.15	0.9095 1.933
		Intra-day (SD) %RSD	0.1112 0.2301	0.96 1.9952
4.	Sensitivity $\mu g/cm^{-3}$ AU	265 nm	0.00035	0.009441
		283 nm	0.00026	-
5.	Accuracy	80%	98.3 %	98.35 %
		100%	92.34 %	92.64 %
		120%	98.7 %	99.86 %

Procedure for the analysis of tablet formulation

Sample: Montelukast sodium and Bambuterol hydrochloride Tablet

Brand name: Montek Plus (10mg of Montelukast + 10 mg of Bambuterol).

Manufacturer: SUN Pharmaceutical Ind. Ltd.

Preparation of sample stock solution

193 mg of tablet powder equivalent to 10 mg each of montelukast and bambuterol was weighed. The powder was dissolved in a sufficient volume of methanol and filtered through a Whatmann filter paper. Filtrate was made up to 100 mL with methanol, 5 mL of filtrate was transferred to 10 mL volumetric flasks, and then the volume was made up with methanol. Absorbance of this solution at 283 nm and 265 nm was recorded as A_1 and A_2. Using equation 4 and 5, concentration of montelukast and bambuterol was calculated in tablets.

The amount of montelukast and bambuterol in marketed tablet formulation conformed to the label claim (montelukast sodium 10 mg and bambuterol hydrochloride 10 mg) **(Table 2).**

RESULTS AND DISCUSSION

The combination of montelukast and bambuterol tablets is administered for the management of asthma. In the present study, a new, reliable, reproducible, simple UV method for the simultaneous determination of montelukast and bambuterol in combined dosage forms was developed and evaluated. The method was developed by corrective absorbance, using methanol as solvent. The wavelength maxima for montelukast and bambuterol were 283 nm and 265 nm, respectively. The absorptivity of montelukast at 283 and 265 nm was 383.15 and 285.67, respectively. The absorptivity of bambuterol at 265 nm was 10.68. An equation was developed to calculate concentration of montelukast and bambuterol in combined tablet formulation simultaneously. The methods were validated based on ICH; analytical method validation guidelines. The values of R^2 for linearity, %RSD for precision and recovery study for accuracy were within the limits of acceptance criteria of ICH; and this indicates repeatability, reproducibility and accuracy of the method.

The proposed method for simultaneous estimation of montelukast and bambuterol was found to be simple, accurate, precise, sensitive, economical and rapid. The method can be employed to perform the routine analysis conducted in quality control laboratories.

Table 2: Analysis of tablet formulation

Stock vol.	A_1*	A_2*	Conc. (μgmL^{-1})		% Label Claim	
			Montelukast	Bambuterol	Montelukast	Bambuterol
5 mL	1.784	1.38	46.56	46.70	93.12	93.40

***Average of three determinations**

CONCLUSION

The UV method has been developed for the simultaneous estimation of montelukast sodium and bambuterol hydrochloride in bulk and tablet formulation. The methods were validated based on ICH analytical method validation guidelines. The values of R^2 for linearity, %RSD for precision, and recovery study for accuracy were within the limits of acceptance criteria of ICH; and this indicates repeatability reproducibility,and accuracy of the method. The proposed method for simultaneous estimation of monelukast and bambuterol was found to be simple, accurate, precise, sensitive, economical and rapid. The method can be employed when performing the routine analysis in quality control laboratories.

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تطوير وتقييم طريقة التحليل الطيفي باستخدام الأشعة فوق البنفسجية للتقدير المتزامن لمادتي مونتيلوكاست صوديوم و بامبيوترول هايدروكلورايد في المواد الخام والمضغوطات

فارون باور[1] وسانجيه بيه[1] و روا ج.ك[1].

[1] كلية الأمين للصيدلة، جامعة نيرلالباجن، بانغالور، الهند

ملخـص

إن دواء المونتيلوكاست هو عبارة عن حاجب لمستقبلات اللوكوتريين.أما البامبيوترول فهو محفز لمستقبلات بيتا-2. والخليط من هذين الدوائين يستعمل عادة في علاج الربو. تم تطوير وتقييم معادلة بسيطة ودقيقة وسريعة وانتقائية للتحديد المتزامن للمونتيلوكاست البامبيوترول في المادة الخام والحبوب بواسطة طريقة الامتصاص المعدل.إن هذه الطريقة تتضمن حساب تركيز احد المواد التي يراد تحليلها بالعينة ومن ثم حساب تركيز المادة الممتصة الثانية عن طريق الامتصاص المعدل وهو عبارة عن (الامتصاص الكامل-امتصاص المواد المتداخلة) وتم استعمال الميثانول كمذيب. وجد أن الحد الأعلى للطول الموجي للمونتيلوكاست البامبيوترول هو 283 و 265 نانومتر على التوالي.
كما تم حساب معامل الامتصاص للمونتيلوكاست عند الطول الموجي 283 و265 نانومتر ووجد انه يساوي 383.51 و 285.76 $g^{-1}Lcm^{-1}$ على التوالي. أما بالنسبة للبامبيوترول فان معامل الامتصاص عند الطول الموجي 265 نانومتر كان 10.86 وعند الطول الموجي 283 نانومتر كانت قيمته ضئيلة جدا ويمكن إهمالها. تم اشتقاق معادلة لحساب تركيز المونتيلوكاست و البامبيوترول في مضغوطة تحوي المادتين بشكل سريع وكانت العلاقة بينهما خطية عند التراكيز (10-80) و(40-240) μgml^{-1} على التوالي. لقد وجد أن هذه الطريقة دقيقة لحساب تركيز كل من المونتيلوكاست و البامبيوترول في مضغوطة تحوي كلاهما.

الكلمـات الدالـة: مونتلوكاست، بامبيوتورل، التحليل الطيفي، معامل الامتصاص.

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