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Method Development and Validation for the Simultaneous Quantification of Betamethasone and Loratadine by Spectrophotometry

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Abstract

Two simple yet accurate spectrophotometric techniques for simultaneously determining betamethasone and loratadine from formulation are described in the text. Dual wavelength approach is the foundation of the first method. The chosen wavelengths for the estimation of betamethasone and loratadine were 239.2 nm (λ1), 254.6 nm (λ2), 218 nm (λ3), and 263.8 nm (λ4), where the difference in absorbance measured at λ1 and λ2 used to determine begtamethasone and difference in absorbance measured at λ3 and λ4 used to determine Loratadine. First-order derivative spectrophotometry is used in the second technique. The wavelengths used were 247.2 nm (ZCP of Loratadine) for Betamethasone analysis free from sample matrix interference and 239.2 nm (ZCP of Betamethasone) for Loratadine estimation. Based on ICH Q2 (R2) guidelines, both approaches have been validated for a number of criteria. The ranges of 1-6 µg/ml for betamethasone and 5-30 µg/ml for loratadine were found to exhibit linearity of both the approaches. The techniques are found to be straightforward and useful for routinely assessing the formulations of betamethasone and loratadine for quality control.

Keywords:- Spectrophotometry, Betamethasone, Loratadine, Dual wavelength method, First order derivative method.

Introduction:

Betamethasone (BET) Fig. 1, Glucocorticoid with immunocompromised(Mehta AB, 2016), metabolic and anti-inflammatory activities19 Hence, Betamethasone is mainly used as anti-allergic and anti-asthamatic10. Betamethasone is listed in Indian Pharmacopoeia (IP, 2018), British Pharmacopeia (BP, 2020), United state pharmacopoeia (USP, 2020), European pharmacopeia (EP, 2008) and Japanese Pharmacopeia (JP, 2006). An examination of the literature reveals that numerous analytical techniques have been created involving spectrophotometric (Ravikumar, 2013), HPLC (Chandra nath saha, 2009), HPTLC (Lingjun, 2020), and LC-MS (Mayank, 2015) for the quantification of BET in various formulations. Loratadine (LOR) Fig. 2, Piperidine histamine with H1 receptor antagonist mechanism. Hence, Loratadine is commonly used as non-sedating antihistaminic with Antiallergic (Bhoomi, 2023), Antipruritic activity (Bhoomi, 2024). Loratadine is official in British Pharmacopeia (BP, 2020), United state pharmacopoeia (USP, 2020) and European Pharmacopeia (EP, 2008). An examination of the literature reveals that numerous analytical techniques have been created involving spectrophotometric (Ganokar, 2011), Capillary zone electrophoresis (Gabriel, 2014), HPLC (Sujatha, 2014), Titrimetry (Qian, 2020), LC-MS (Niranjani, 2018) and GC-MS (Martens, 1995) for the quantification of LOR in many of formulations. The present manuscript describes novel and cost-effective method for the simultaneous estimation of BET and LOR in combined dose. The described method is validated in respect of ICH guideline Q2R2.22

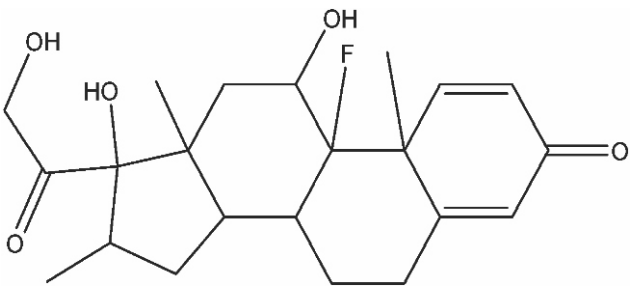

Figure 1. Structure of Betamethasone

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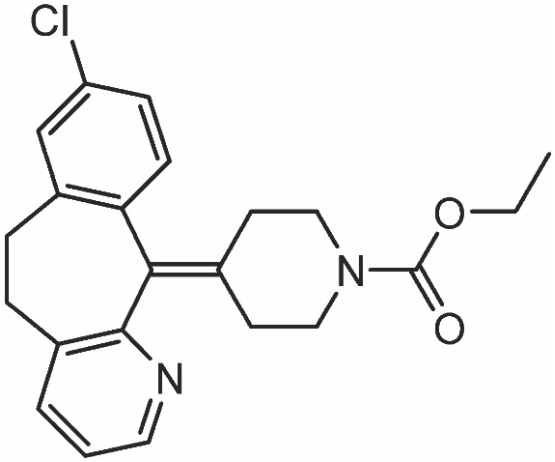

Figure 2. Structure of Loratadine

Experimental:

Apparatus:

The absorbance and spectrum were measured using a Shimadzu UV-1700 (Japan) UV/Vis spectrophotometer with a 2 mm spectral breadth and 0.5 mm wavelength precision. 10 mm matched quartz cells with UV-probe software were used to create the spectra. The Frontline FS4 ultrasonic bath and the Sartorius CP2245 analytical balance (Germany) were used in the experiment.

Materials and Reagents:

Vadish Pharma Pvt. Ltd. gave me a free sample of BET, a pure analytical standard with a 99.97% w/w purity, whereas Cadila Pharma Pvt. Ltd. gave me a gift sample of LOR, which has a 99.93% w/w purity. We bought AR-grade methanol from Bombay's SD Fines Chemicals.

Preparation of solutions:

Preparations of standard solutions and working standard solutions:

In separate 100 ml volumetric flasks, the standard API of BET and LOR (100 mg) were carefully weighed and dissolved in methanol to yield the solutions 1 mg/ml. 10 ml of the stock solutions were carefully transferred to 100 ml volumetric flasks to make working solutions for each having concentrations 100 µg/ml for both the molecules.

Preparation of sample solution:

To measure the amount of BET and LOR, 20 tablets were chosen at random, weighed, and the average weight was computed and pulverized into powder. Accurately weighed 15 mg of standard BET powder was mixed with tablet powder because the dose ratio is high, so to quantify the exact amount of BET in sample; standard addition method was used. Sample mixture containing approximate 10 mg BET and 50 mg LOR, dissolved with 25 ml methanol, and keep in a sonicator for half an hour. After the solution was filtered, the residue was washed completely. To make the final solution; the filter and rinse were mixed in a flask and the required volume of methanol was added with solvent; which approximately contained BET (100 µg/ml) and LOR (500 µg/ml). Transfer 3 ml of above working sample solution to 100 ml volumetric flask to prepare final sample solution.

Method Development:

Dual Wavelngth Method:

Different BET and LOR solutions were prepared in methanol and analyzed in the 200–400 nm spectral range. The overlapped spectra of the two materials show the presence of four distinct wavelengths. (I) BET; response difference (A239.2– A254.6) was noticed at wavelengths 1 and 2, while LOR responses were the same at these two wavelengths (239.2 & 254.6 nm). (II) LOR; response difference (A218 – A263.8) was noticed at wavelengths 3 and 4, although BET response was the same at these two wavelengths (218 & 263.8 nm) (Patel, 2017). In conclusion, these four wavelengths were used to calculate the concentrations of BET and LOR (Figure 3). The capacity of a dual wavelength method to measure unknown concentrations of substances of interest in a solution containing an interfering component is one of its most useful features.

First Derivative Method:

The zero order spectra were calculated using software to get the first derivative spectrum. These two wavelengths were selected for additional examination because the second drug had a reasonable response at the point of zero crossing of the first drug, and the first drug had a reasonable response at the point of zero crossing of the second drug (Bhoomi, 2023). The overlay derivatized spectra show that BET and LOR exhibit zero-crossing points at 239.2 nm and 247.2 nm, respectively. These two points were utilized to calculate BET and LOR. The derivatized overlay spectra are shown in Figure 4.

Validation of Method:

The procedure was validated in accordance with ICH Q2(R2) requirements (ICH, 2023).

Linearity:

The calibration graphs were created throughout the respective ranges of 1-6 µg/ml and 5-30 µg/ml for both BET and LOR. BET (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml) and LOR (0.5, 1.0, 1.5, 2.0, 2.5, & 3.0 ml) were pipetted out, and the working solutions were precisely put into different batches of 10 ml volumetric flasks. solvent was added to the volumes to achieve the desired result.

Precision (Repeatability and Reproducibility):

Method precision

The spectrophotometer's repeatability was assessed by continuously monitoring and recording the absorbance of mentioned solutions (n = 6) for BET (4 µg/ml) and LOR (20 µg/ml) without altering the parameters of the planned method, which was used to confirm the accuracy

of the device. The results were given as a percentage RSD.

Intermediate precision

The relevant findings for diverse concentrations of BET and LOR solutions (3, 4 and 5 µg/ml and 15, 20, and 25 µg/ml) were evaluated three times on the same day and on three different consecutive days. in order to determine the intended strategy. The results are given as %RSD.

Limits of quantification (LOQ) and detection (LOD):

The formulas below were solved to determine the LOD and LOQ of the suggested approach.

3.3 X σ/S is the LOD.

10 X σ/S is the LOQ.

where S is the calibration chart's slope and σ is the responses' standard deviation.

Accuracy (Recovery study)

The % recovery study was assessed by computing the recovered amount for BET and LOR using the conventional addition approach. To the pre-quantified sample solution, known quantities of BET and LOR working solutions were recovered at 50%, 100%, and 150% levels.

Results and Discussion:

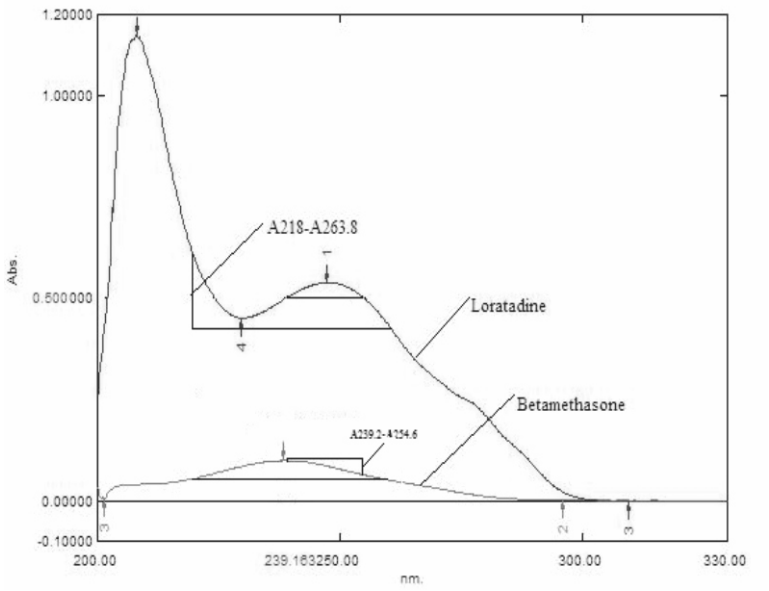

Figure 3. UV spectra of BET and LOR

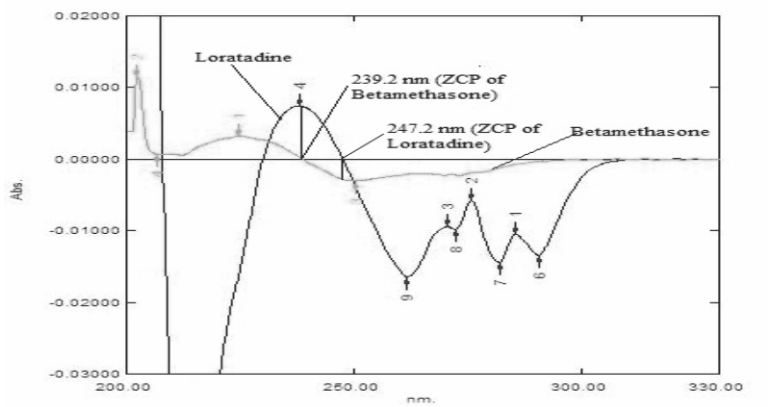

Figure 4. First order derivative spectra of BET and LOR

Linearity:

Working standards were recorded at 6 different BET and LOR concentrations, falling between 1-6 µg/ml and 5-30 µg/ml, for BET and LOR accordingly. Absorbance versus concentration was graphed to create the calibration charts shown in Fig 5,6,7 and8, which were then subjected to linear regression analysis. The outcomes show a significant correlation between the reaction falling under the specified range and the drug concentration in Table 1. BET and LOR had linear responses in the first technique. The second technique demonstrated a linear response for both drugs within the previously specified concentration range. It was therefore capable of being quantified.

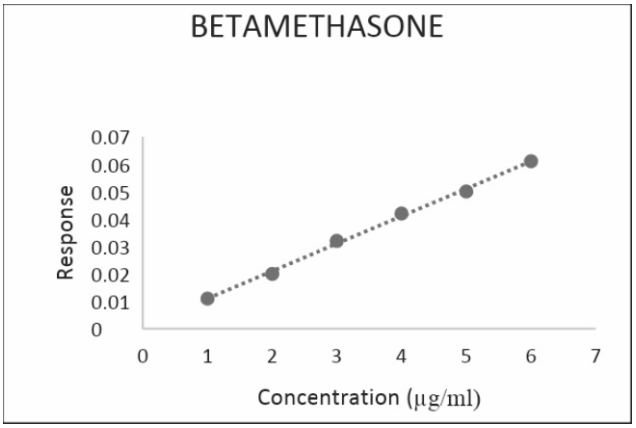

Figure 5. Calibration chart of BET by Dual wavelength method

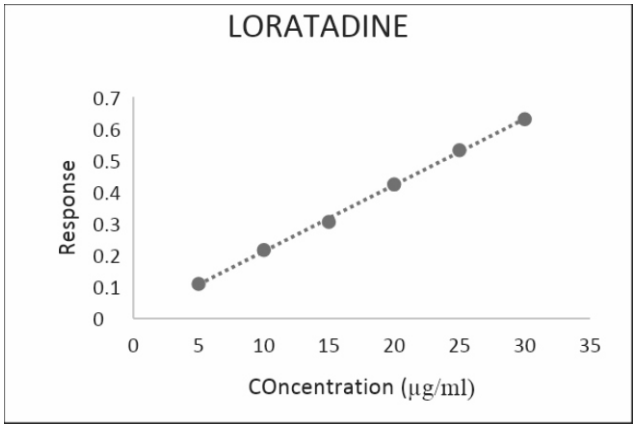

Figure 6. Calibration chart of LOR by Dual wavelength method

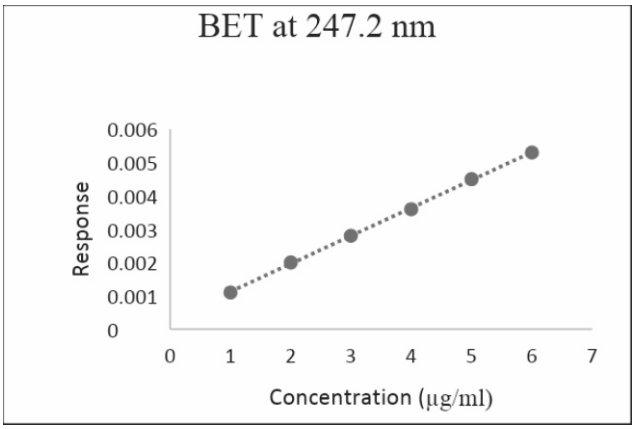

Figure 7. Calibration chart of BET at 247.2 nm by First derivative method

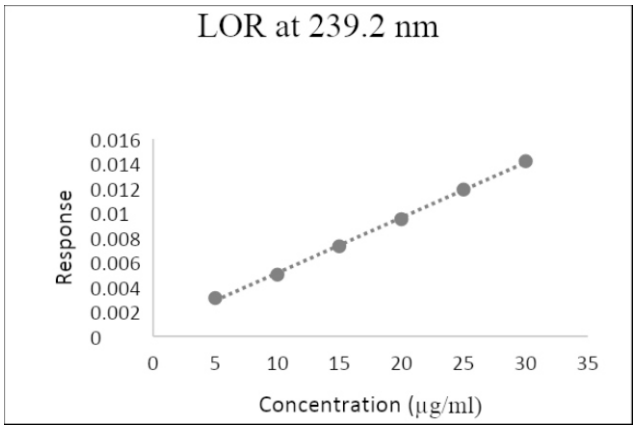

Figure 8. Calibration chart of LOR at 239.2 nm by First derivative method

Precision:

Table 2 presents the findings from the study of repeatability. By repeatedly scanning 4 µg/ml and 20 µg/ml BET and LOR solutions, the accuracy of the method was examined. The repeatability study's % RSD values were sufficiently low, indicating that the suggested methodology was accurate in both methods. A accuracy level is revealed by evaluating intermediate variations. The percentage RSD was calculated by multiple examination of 3 working solutions (15, 20, and 25 µg/ml for LOR and 3, 4, and 5 µg/ml for BET). The BET and LOR percentage RSD values for intra-day and inter-day, respectively. It was discovered that the procedures provided are accurate, with respectable percentage RSD values for research with intermediate precision.

Accuracy:

Three replications of an accuracy study were conducted, and the RSD of the percent recovery was used to calculate the percentage recovery. The information is shown in Table 2. Good recovery of the spiked drugs was obtained at each higher concentration, indicating the accuracy of both procedures.

LOD and LOQ:

Table 3 displays the Detection and Quantification limit for LOR and BET. The LOD and LOQ for BET and LOR were calculated at their respective wavelengths using the ICH guideline formulae described above. The methods are sensitive because of the low LOD and LOQ.

Analysis of marketed formulation:

In tablet dose form, these methods were utilized to estimate BET and LOR concurrently. The formulation analysis was more in line with the label claim, and the methods can be used for regular BET and LOR evaluation in the formulation of pharmaceuticals [Table 4].

All summarized validated parameters are presented in Table 5.

Conclusion:

The procedures for estimating BET and LOR in formulations that are described have been created and

approved in compliance with ICH guidelines. It can be concluded from the results of the formulation analysis using the suggested approach that the approach has a linear response for both BET and LOR, with 1-6 µg/ml and 5-30 µg/ml of range, respectively. The results of the recovery investigation indicate that the suggested method has a low standard deviation and is accurate. The low percentage RSD value of intermediate precision demonstrates the precision of the chosen approach. The sensitivity of the procedure was shown by the LOD and LOQs.

The developed methods that are discussed are quick, easy to use, economical, accurate, exact in terms of repeatability, and have an intermediate level of sensitivity. The creation of a precise, quick method with increased sensitivity was given first priority. Therefore, for routine BET and LOR in their combined pharmaceutical formulation, the recommended approaches can be implemented.

Table 1: Linearity and Correlation coefficient

Parameter	Dual wavelength		First order derivative	
	BET	LOR	BET	LOR
Regression equation	Y=0.01X-0.001	Y=0.0209X-0.0025	Y= 0.0008X + 0.0003	Y = 0.0004x + 0.0007
Linearity µg/ml	1 - 6	5 - 30	1 – 6	5 - 30
Correlation coefficient	0.9977	0.9990	0.9997	0.9990

Table 2: Precision and Accuracy study

Parameter	Dual wavelength		First order derivative	
	BET	LOR	BET	LOR
%RSD (n=6); Repeatability	0.4718	0.6878	0.9764	0.7145
Intraday Precision, % RSD (n=3)	0.91-1.64	0.37-0.50	1.03-1.64	0.68-1.29
Interday Precision, % RSD (n=3)	1.11-1.96	0.85-1.83	1.22-1.77	1.70-1.80
% Recovery ± SD (n=3)	101.0 ± 1.36	99.67 ± 1.22	99.96 ± 1.14	102.0 ± 0.80

Table 3: LOD and LOQ study

Parameter	Dual wavelength		First order derivative	
	BET	LOR	BET	LOR
Limit of Detection (µg/ml)	0.0675	0.0869	0.22	0.57
Limit of Quantification (µg/ml)	0.2047	0.2635	0.69	1.74

Table 4: Analysis of Formulation

Parameter	Dual wavelength		First order derivative	
	BET	LOR	BET	LOR
Assay ± SD (n=6)	102.9 ± 1.14	102.0 ± 0.80	99.97 ± 0.58	101.3 ± 1.57

Table 5: Summary of Validation parameters

Parameter	Dual wavelength		First order derivative	
	BET	LOR	BET	LOR
Wavelength (nm)	239.2-254.6	218-263.8	247.2	239.2
Linear range (µg/ml)	1-6	5-30	1-6	5-30
Regression equation Y=mX+C	Y=0.01X-0.001	Y=0.0209X-0.0025	Y= 0.0008X + 0.0003	Y = 0.0004x + 0.0007
Regression coefficient (r2)	0.9977	0.9990	0.9997	0.9990
Method Precision %RSD (n=6)	0.4718	0.6878	0.9764	0.7145
Intraday Precision %RSD (n=3)	0.91-1.64	0.37-0.50	1.03-1.64	0.68-1.29
Interday Precision %RSD (n=3)	1.11-1.96	0.85-1.83	1.22-1.77	1.70-1.80
LOD (µg/ml)	0.0675	0.0869	0.22	0.57
LOQ (µg/ml)	0.2047	0.2635	0.69	1.74
Accuracy Mean ± SD (n=3)	101.0 ± 1.36	99.67 ± 1.22	99.96 ± 1.14	102.0 ± 0.80
Assay Mean ± SD (n=6)	102.9 ± 1.14	102.0 ± 0.80	99.97 ± 0.58	101.3 ± 1.57

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Conflict of interest

There are no conflicts of interest for the authors in relation to this study.

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