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Der Pharmacia Lettre, 2014, 6 (3):265-270
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ISSN 0975-5071
USA CODEN: DPLEB4

UV spectrophotometric estimation of rupatadine fumarate by first order derivative and area under curve methods in bulk and pharmaceutical dosage form

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ABSTRACT

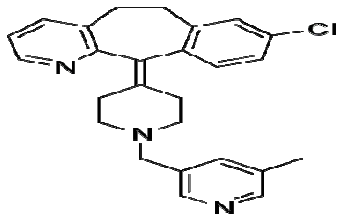
Simple and precise UV spectrophotometric methods by first order derivative and area under curve [AUC] - have been developed and validated for the estimation of rupatadine fumarate in bulk and its tablet formulation. The standard and sample solutions of rupatadine fumarate were prepared in absolute alcohol. Rupatadine fumarate was estimated at 214 nm for the first order derivative UV-spectrophotometric method (A), while in area under curve (AUC) method (B) the zero order spectrum of rupatadine fumarate was measured in between 245 nm to 255 nm. Beer's law was obeyed in the concentration range of 1 to 30 µg / ml with coefficient of correlation value 0.9998 for first order derivative method. Similarly in AUC method, Beer's law was obeyed in the concentration range of 1 to 30 µg / ml with coefficient of correlation value 0.9988. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation were of 2.949 % and 1.9267 % for the above two methods respectively. The proposed methods were successfully applied for the determination of rupatadine fumarate in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Rupatadine fumarate, UV spectroscopy, Derivative spectroscopy, Area under curve method.

INTRODUCTION

Its chemical name is 8-chloro-6,11-dihydro-11-[1-[(5-methyl-3-pyridinyl)methyl]-4-piperidinylidene]-5H-benzo[5,6]cyclohepta[1,2 b]pyridine Fumarate. Rupatadine fumarate is a second generation of antihistamine and platelet activity factor (PAF) antagonist used to treat allergies. Rupatadine possesses anti-allergic properties such as the inhibition of the de-granulation of mast cells induced by immunological and non-immunological stimuli and inhibition of the release cytokines, particularly of the TNF in human mast cell [1]. Literature survey reveals the Spectrophotometric [2-4] titration [5, 6], HPLC [7-11] methods for the estimation of rupatadine fumarate. Simple, rapid and reliable UV spectrophotometric methods are developed for the determination of rupatadine fumarate. These methods can be used for the routine analysis. In the proposed methods optimization and validation of this method are reported.

Structure of rupatadine



MATERIAL AND METHODS

Shimadzu UV-1800 was used with 10 mm matched quartz cell to measure absorbance of solution.

A Shimadzu analytical balance with 0.01 mg was used.

CHEMICAL AND REAGENTS

Reference standard of rupatadine fumarate was obtained from reputed firm with certificate analysis. All spectral absorbance measurements were made on Shimadzu UV-1800 with 10 mm matched cell.

PREPARATION OF STANDARD SOLUTION

About 10 mg of standard rupatadine fumarate was weighed accurately and transferred in 100 ml of volumetric flask. About 30 ml of absolute alcohol was added and sonicated for 15 minutes. The volume was adjusted up to the mark with absolute alcohol to give concentration as 100 µg /ml.

Estimation from tablets

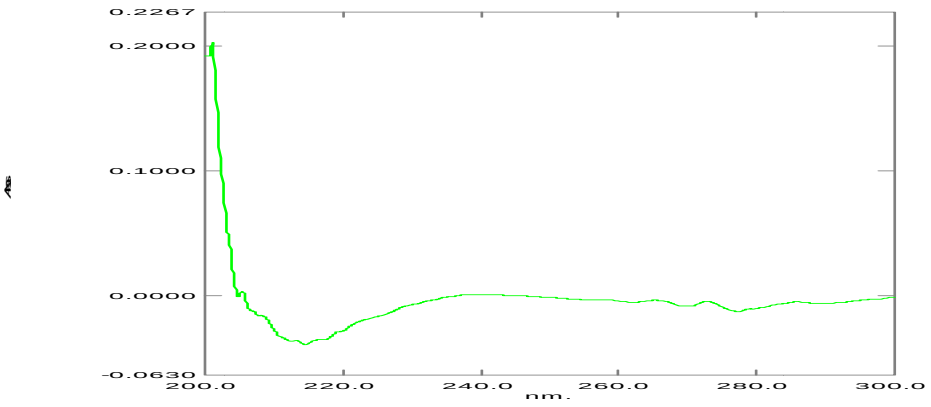
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of rupatadine fumarate was weighed and transferred in 100 ml of volumetric flask. A 30 ml of absolute alcohol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with absolute alcohol to give concentration as 100 µg /ml. Such solution was used for analysis.

Experimental

Method A: First order derivative method

For the selection of analytical wavelength, 10 µg /ml solution of rupatadine fumarate was scanned in the spectrum mode from 300 nm to 200 nm by using absolute alcohol as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured between 210 to 220 nm (Fig. 2).

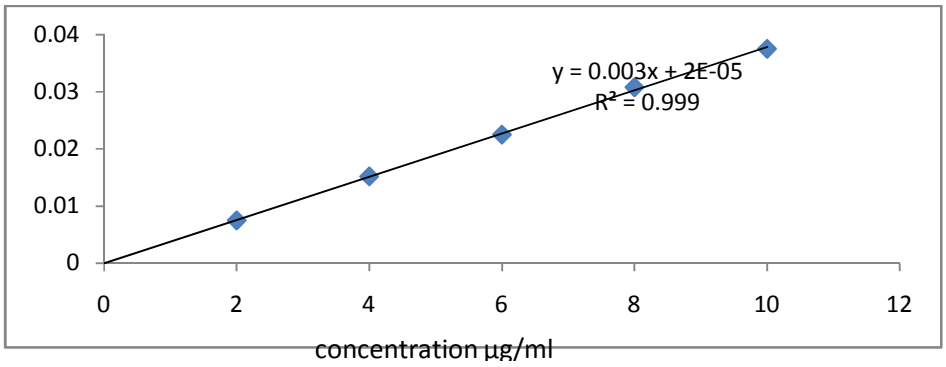
Fig. 2. First order derivative spectrum of rupatadine fumarate (10 µg/ml) showing absorbance at 214 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of rupatadine fumarate was pipette out and volume was adjusted with absolute alcohol as solvent. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectra were obtained by using derivative mode. Amplitudes of the

resulting solutions were measured at between 220 nm to 210 nm by using absolute alcohol as blank. The calibration curve was prepared in the concentration range of 1 to 30 µg/ml. (Fig. 3)

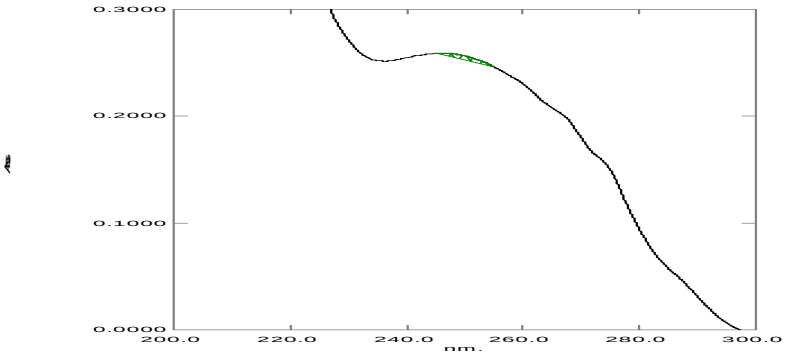
Fig. 3. Calibration curve for rupatadine fumarate at 214 nm by first order derivative Spectroscopy



Method B: Area under curve (AUC) method

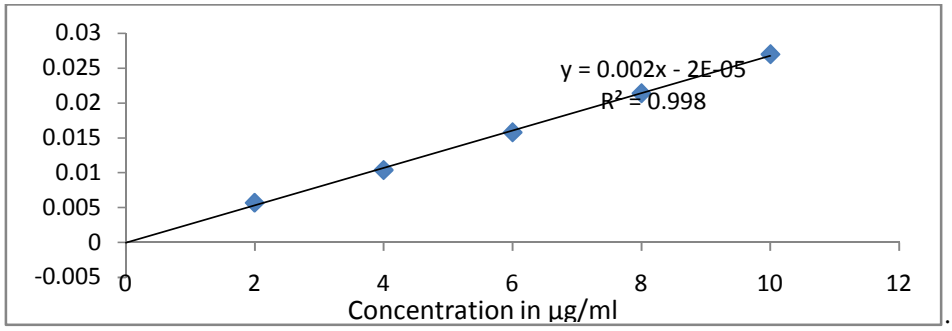
Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 were calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of rupatadine fumarate was scanned in the spectrum mode from 300 nm to 200 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 245 nm to 255 nm (Fig. 4).

Fig. 4. Area under curve spectrum of rupatadine fumarate (10 µg/ml) showing area from 245 nm to 255 nm.



Into series of 10 ml graduated flask, varying amount of standard solutions of rupatadine fumarate were pipette out and volume was adjusted with absolute alcohol. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The AUC calculations were done and the calibration curve for rupatadine fumarate was plotted in the concentration range of 1 to 30 µg/ml (Fig. 5).

Fig. 5. Calibration curve for rupatadine fumarate by area under curve spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve (AUC) method
Detection Wavelength (nm)	214	245-255
Beer Law Limits (µg/ml)	1-30	1-30
Correlation coefficient(r^2)	0.9992	0.9988
Regression equation (y=b+ac)		
Slope (a)	0.0038	0.0027
Intercept (b)	0.00002	0.00002

Validation

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2, 3).

Table 2: Results of recovery of rupatadine fumarate for first order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	1.965	98.25	0.1007	5.125
2	2	4.063	101.57	0.1300	3.364
2	4	5.957	99.283	0.1009	1.694
2	6	8.040	100.50	0.1290	1.613
				Mean =0.1151	Mean =2.949

Table 3: Results of recovery of rupatadine fumarate for area under curve (AUC) method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	2.047	102.35	0.05106	2.494
2	2	3.920	98.00	0.06599	1.683
2	4	5.967	99.450	0.1143	1.916
2	6	7.962	99.525	0.1285	1.614
				Mean =0.0899	Mean =1.9267

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 4.

Table 4: Precision- method precision

Experiment no.	Weight of rupatadine fumarate taken in mg	Content in mg. of rupatadine fumarate	
		First order derivative method	Area under curve method
1	10	10.015	10.026
2	10	10.008	10.015
3	10	10.017	9.976
4	10	9.997	9.992
5	10	9.987	10.027
6	10	10.021	10.031
	Standard deviation	0.01311	0.04392
	%RSD	0.1311	0.4381

Inter-day and intra-day precision
An accurately weighed quantity of tablets powder equivalent to 10 mg of rupatadine fumarate was transferred to 100 ml of volumetric flask. A 30 ml of absolute alcohol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with absolute alcohol to give concentration as 100 µg /ml. Such solution was used for analysis.

For first order derivative method
Solution was scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at between 220 nm to 210 nm by using absolute alcohol as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell 214 nm for first order derivative (method A). Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of rupatadine fumarate was estimated by comparison with standard at 214 nm for first order derivative, table 5.

For area under curve method
Solution was scanned between 300 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 245 nm to 255 nm by using absolute alcohol as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 245 nm to 255 nm (method B). Similarly area under curve of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of rupatadine fumarate was estimated by comparison with standard at 245 nm to 255 nm, table 5.

Table 5: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	First order derivative method	Area under curve (AUC) method
(A)	Intra-day precision (n=3) Amount found ± % RSD	99.60 % 0.2485	99.45% 0.03446
(B)	Inter-day precision (n=3) Amount found ± % RSD	98.484% 0.1366	98.765% 0.00765
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	0.06789	0.00823

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of LOD and LOQ

parameters	First order derivative method	Area under curve (AUC) method
Limit of Detection (µg/ml)	0.5999	0.1686
Limit of Quantification (µg/ml)	1.815	0.5111

Ruggedness
The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of rupatadine fumarate sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of rupatadine fumarate was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The first order derivative and area under curve UV-spectroscopic methods are useful for routine analysis of rupatadine fumarate in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Rupatadine fumarate has the absorbance maxima at 214 nm (method A) and in the AUC spectrum method areas were measured between 245 nm to 255 nm (method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 30 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of rupatadine fumarate in pharmaceutical formulation.

Acknowledgment
Authors express sincere thanks to the Principal, Dr. Tushar M. Desai of D. G. Ruparel college.

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