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Extractive Spectrophotometric Methods for the Determination of Racecadotril in Pure and Pharmaceutical Formulations

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ABSTRACT

Two sensitive extractive spectrophotometric methods have been developed and validated for the estimation of Racecadotril in pure and pharmaceutical dosage forms. The developed methods are based on the formation of colored ion-association complex of drug with Tropaeolin ooo[TPooo] and Alizarin red-s [ARS] extractable in chloroform. The optimum conditions such as effect of concentration of dyes, pH etc on color development have been established for these methods The extracted complexes showed absorbance maxima at 482 and 420nm for these developed methods. Beer's law is obeyed in the concentration ranges between 8-40µg/ml for the two methods respectively. These newly developed methods can be applied for the determination of other drugs in commercial capsules and results of analysis were validated statistically in accordance with ICH guidelines.

Keywords: Racecadotril, Spectrophotometric methods, Tpooo,ARS, Chloroform.

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INTRODUCTION

Racecadotril **(**Figure.1)[1,2] is chemically Benzyl N – [3-(acetyl thio)-2 benzyl propanoyl]glycinateis used as an anti diarrheal drug to treat diarrhoea in infants, young children and adults.

Very few analytical methods[3-8] have been reported in the literature for the determination of this drug in dosage formulations. This prompted the author to develop two simple and rapid extractive spectrophotometric methods [TPooo & ARS] with a one-step extraction procedure for determination of Racecadotril in pharmaceutical dosage forms.

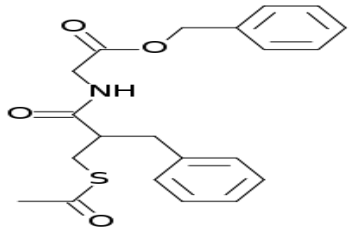

Figure.1 Structure of Racecadotril

MATERIALS AND METHODS

Apparatus:

Spectral and absorbance measurements were carried out by using ELICO UV– Visible Double beam spectrophotometer[Model:SL-159] equipped with 1.0cm thickness matched quartz cells. Systronics digital pH meter was used to adjust (pH) of the buffer solution. Pharmaceutical grade Racecadotril was received as gift sample from Cipra Lab Ltd, Hyderabad, India.(99.8% pure) was used.

Preparation of Standard drug solution:

A stock standard solution containing 1.0mg.mL^{-1}racecadotril was prepared by dissolving accurately weighed (100mg) of pure drug with double distilled water in 100mL calibrated flask. This stock solution is further diluted appropriately with double distilled water to get a working standard concentration of 200µg.mL^{-1} for the given below proposed methods

Reagents and Solutions:

All chemicals and reagents were used of analytical grade and solutions were prepared in double distilled water.

TPooo solution (Fluka; 0.2%):

Prepared by dissolving 200mg of tropaeoline ooo in 100ml of distilled water.

ARS solution (Fluka; 0.2%):

Prepared by dissolving 200mg of Alizerine Red-S in 100ml of distilled water.

Hydrochloric acid (0.1M,Sd-Fine chemicals, India):

Prepared by diluting 8.5mL of concentrated acid to 1 Litre of double distilled water.

Proposed Procedure for TPooo & ARS[9]:

Different aliquots of drug solution(05-2.5ml;200µg.mL^{-1}) were transferred into a series of 100ml separating funnels. To this add 5.0ml of HCL Buffer (i.e. HCL and Sodium acetate), 5.0ml of various dye solutions (TPooo or ARS) were added and total volume was made upto 15ml with double distilled water. To this 10ml of chloroform was added and the contents were shaken for 5 minutes. The organic layer was separated and the absorbance of colored solution is measured spectrophotometrically (482nm for TPooo and 420nm for ARS against blank similarly prepared) which is stable for 24hrs. for the two proposed methods, standard calibration plots were prepared by plotting the absorbance versus drug concentration, and the concentration of the unknown was read from the plotted calibration graphs, computed from the respective regression equations derived using the absorbance concentration data.

Assay procedure for Capsules:

Twenty capsules were weighed and their shells were removed. A quantity of capsule powder equivalent to 100mg of racecadotril were taken in volumetric flask (100mL) and was shaken with methanol (10.0mL) for 10min and the volume was made upto the mark with distilled water. This solution was then filtered through whatman filter paper and the aliquot portion of the filtrate was diluted to 100.0mL with distilled water to get sample solution and analyzed as given in the above proposed assay procedures.

RESULTS AND DISCUSSION

Method Development:

The optimization studies for the color development for the proposed methods for the assay of racecadotril were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species and were found to be same as described as shown in Table 1&2. The optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of racecadotril were taken and colored complexes were developed separately by following the above proposed procedures. The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank shown in (Figure.2&3). These spectra's showed a single well-defined peak with characteristics absorption maxima which were stable for more than 90min, where as the blank in each method has low or no

absorption in this region. These wavelengths (absorption maxima) for each proposed methods were used for the visible spectrophotometric analysis of racecadotril in pure and in capsule formulations respectively.

Table.1: Optimum conditions established in method TPooo for Racecadotril

Parameter	Optimum Range	Conditions in procedure	Remarks
λ_{max}(nm) Tpooo	470-500	482	-----
Effect of acid or buffer on color development	0.08-0.12 HCL for TPooo	0.1M HCL for TPooo	Variation of concentration or pH of acid beyond the upper and lower limits resulted in low absorbance values.
Choice of organic solvent for extraction of the colored complex	Chloroform for TPooo	Chloroform for TPooo	The water immiscible solvents tested for the extraction of the colored complex into organic phase, which include (chlorobenzene, carbon tetrachloride, benzene, n-butanol and chloroform). Chloroform was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.
Effect of shaking time on extraction.	1-5 min	2 min	Constant absorbance values were obtained for shaking periods between 1-5 min
Effect of temperature on the colored species.	Laboratory Temperature (28 to 30^0C)	Laboratory Temperature	At low Temperature (<20^0C) the extraction of colored species was found to be improper. At high Temperature (>35^0C) the stability of the colored species was found to be less.
Stability of the colored species in organic solvent	1-60 min	----	

Table.2: Optimum conditions established in method ARS for Racecadotril

Parameter	Optimum Range	Conditions in procedure	Remarks
λ_{max}(nm) ARS	405-440	420	----------
Effect of acid or buffer on color development	0.08-0.12 HCL for ARS	0.1M HCL for ARS	Variation of concentration of acid beyond the upper and lower limits resulted in low absorbance values.
Choice of organic solvent for extraction of the colored complex	Chloroform for ARS	Chloroform for ARS	The water immiscible solvents tested for the extraction of the colored complex into organic phase, which include (chlorobenzene, carbon tetrachloride, benzene, n-butanol and chloroform). Chloroform was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.
Effect of shaking time on extraction.	1-5 min	2 min	Constant absorbance values were obtained for shaking periods between 1-5 min

Effect of temperature on the colored species.	Laboratory Temperature (28 to 30^0C)	Laboratory Temperature	At low Temperature (<20^0C) the extraction of colored species was found to be improper. At high Temperature (>35^0C) the stability of the colored species was found to be less.
Stability of the colored species in organic solvent	1-60 min	-----	-----

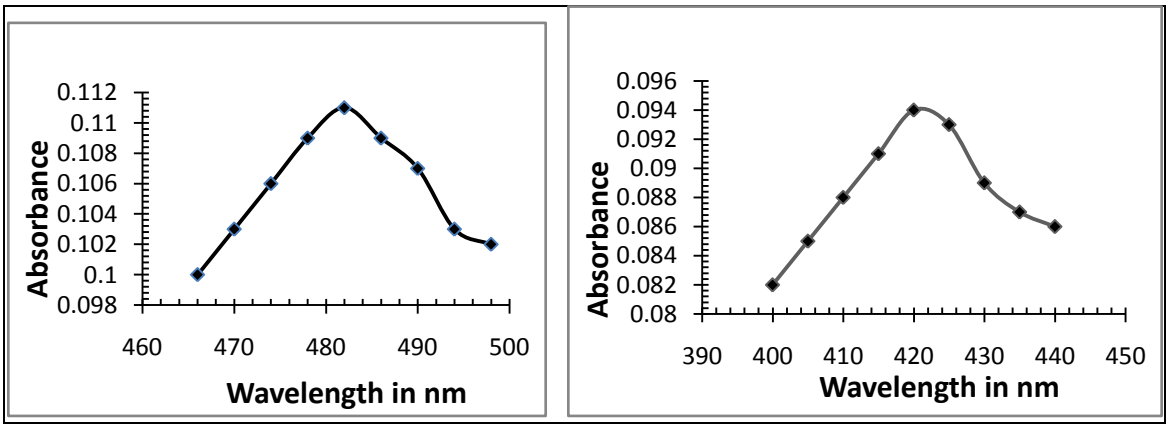

Figure .2 Spectrum of Racecadotril with TPoooFigure.3 Spectrum of Racecadotril with ARS

METHOD VALIDATION:

Linearity Range and Analytical data:

Linearity ranges for each proposed spectrophotometric method for quantitative analysis of racecadotril were made by plotting calibration curves over the concentration ranges cited The statistical parameters (optical characteristics) such as Beer's law limits, Correlation coefficient, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from six replicate samples containing 3/4th of the amount of the upper beer's law limits) were calculated for all the proposed methods and the results are summarized in Table.3.

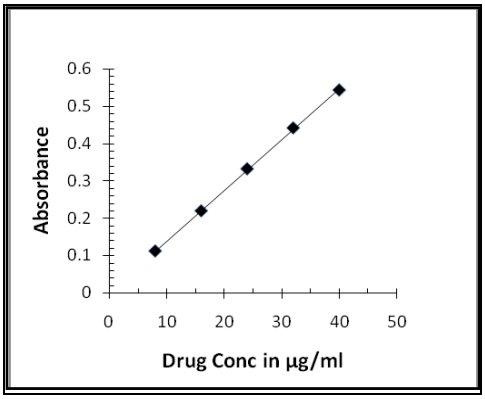

Figure.4 plot of Racecadotril with TPooo

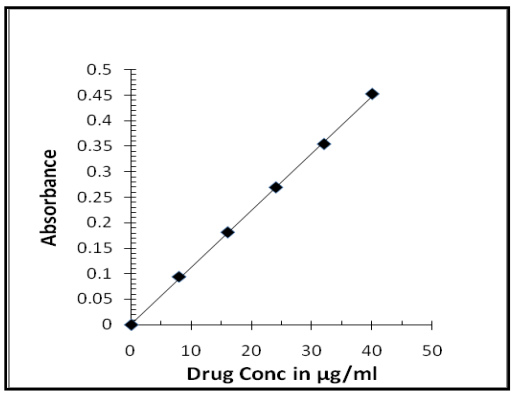

Figure.5 plot of Racecadotril with ARS

Precision:

The precision of each proposal methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of racecadotril for the proposed methods. The percentage relative standard deviation and percentage of error (at 0.05and0.01confidence limits) were calculated for the proposed methods (Table.3).

Accuracy:

To determine the accuracy of each proposed method, different amounts of bulk samples of racecadotril within the Beer's law limits were taken any analyzed by the proposed method. The results were recorded inTable.3.

Table.3: Optical and regression characteristics, precision and accuracy of the proposed methods for Racecadotril

Parameter		TPooo	ARS
λ_{max} (nm)		482	420
Beers law limits(µg/ml)		8.0 - 40.0	8.0 - 40.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)		8.978 x 10^3	9.416 x 10^3
Sandell's Sensitivity (µg cm^{-2}/0.001 absorbance unit)		0.0325	0.03100
Optimum photometric range (µg/ml)		10.0-36.0	9.5-35.0
Regression equation (Y=a+bc); Slope (b)		0.0131	0.0111
Standard Deviation on slope (S_b)		0.0001313	0.000154
Intercept (a)		0.0029	0.0033
Standard Deviation on intercept (S_a)		0.00348	0.004102
Standard error on estimation (S_e)		0.00332	0.003911
Correlation coefficient (r)		0.9998	0.9997
% Relative Standard deviation*		0.8116	0.5708
% Range of error(Confidence Limits)	0.05 Level	0.678	0.4773
0.01 Level		1.03	0.7060

* Standard deviation of six determinations

Application to formulations:

In order to evaluate the analytical applicability of the proposed methods the results obtained by the proposed methods in label claim compared statistically with those obtained by a literature UV- spectrophotometric method[12] by applying student's t-test for accuracy and F-test for precision. Table.4 gives the results of the assay and reveals that there is close agreement between the results obtained by the proposed methods (label claim) the reference method[12] with respect to accuracy and precision for racecadotril. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t =2.36 and F = 4.88), suggesting that the proposed methods are as accurate and precise as the literature method.

Table.4: Determination of Racecadotril in dosage forms by the proposed methods

Capsule Formations	Amount Taken (mg)	Amount found by proposed Methods*		Reference Method[9]	Percentage recovery by proposed methods	
		TPooo	ARS		Tpooo	ARS
Rodotril(Dr Reddy's Pharmaceuticals Ltd)	100 mg	99.95±0.12 F=4.94 T=1.77	99.92±0.11 F=0.641 T=1.77	99.96±0.16	99.89	99.59

* Standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit,F= 2.36, t =4.88.

Nature of colored species:

As racecadotril possesses a secondary amino group, it forms an ion association complex with two acid dyes [Tpooo & ARS] which is extractable into chloroform from aqueous phase. The protonated nitrogen (positive charge) of racecadotril is expected to attract the appositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction [Figure.6].

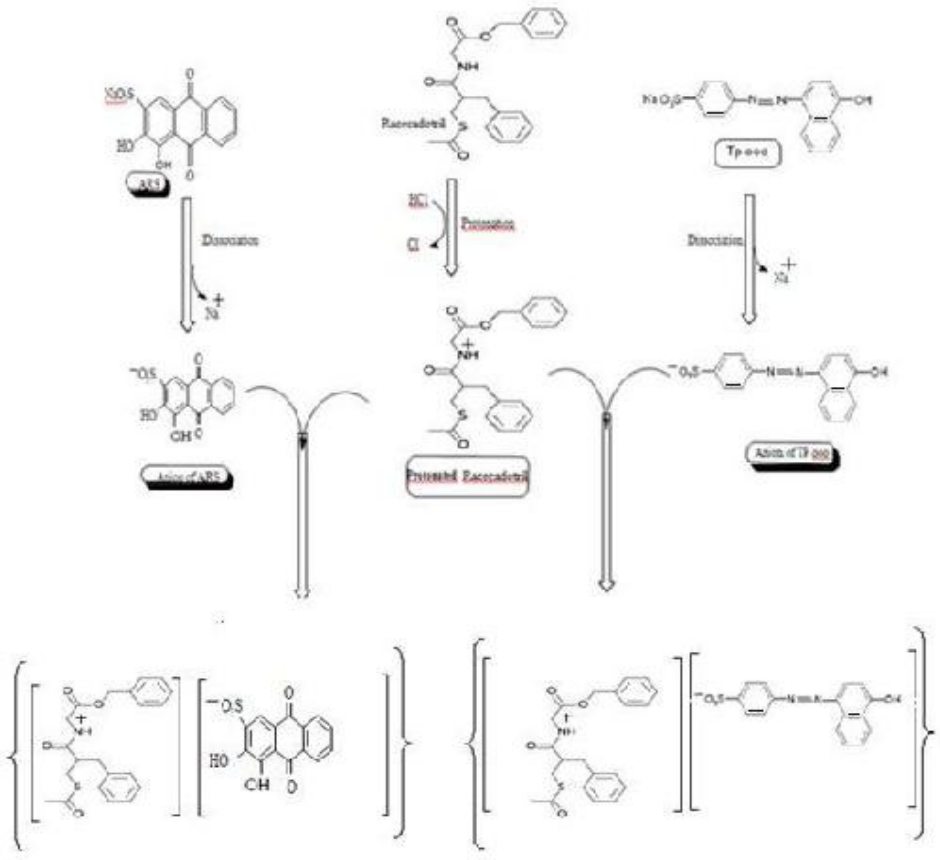

Figure.6: Reaction mechanism of racecadotril with TPooo and ARS

CONCLUSION

The Present research work demonstrated the feasibility of the use of visible spectroscopy and ion complexation reaction for the determination of racecadotril in pure and its dosage formulations. The simplicity, sensitivity and selectivity make these proposed methods a suitable alternative to the HPLC methods. The developed two sensitive spectrophotometric methods has been optimized and validated suggesting that the procedures developed can be adopted as routine laboratory methods in quality control laboratories where modern instruments are not available.

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REFERENCES

1. Cezard JP, Salazar-Lindo E. Racecadotril in acute diarrhea. Indian Pediatr.2005;42:502-3.
2. Schwartz JC. Racecadotril A new approach to the treatment of diarrhea. J Antimicrob Agent. 2000;14:75-9.
3. Reddy KM, Babu JM, Sudhakar P, Sharma MS, Reddy GS, Vyas K. LC-MS method for the quantification of racecadotril, determination of racecadotril in human plasma by LC-MS/MS.Pharmazie. 2006; 61(12):994-998.
4. Prabu SL, Singh T, Joseph A, Dinesh C. Determination of Racecadotril by HPLC in capsules. Indian J.Pharm Sci, 2007;69(6):819-821.
5. Srinivasa RaoP, NappinnaiM. UV and RP-HPLC estimation of Racecadotril. Asian J.Chem. 2007;19(5): 3697-702.
6. Ventrichelvan.T, Prabakaran.S. New spectrophotometric methods for the determination of racecadotril in bulk drug and capsular. Indian J.Pharm.Sci.2007; 69(2):307-9.
7. Venumadhav E, Amreen Nishat, Neeha T, BhargaviP, A.Swetha A, Devala Rao G. New analytical methods for the estimation of Racecadotril in bulk and pharmaceutical formulations. Rasayan J Chem.2010;1:348-351.
8. Raju BH, Narasimha Ch, Delhiraj N, Devala Rao G. Spectrophotometric Methods for the Determination of Racecadotril in Bulk and Pharmaceutical Dosage Forms.Asian Journal of Research in Chemistry.2011,4(4):.548- 550.
9. Raghubabu K, Jagannadharao V, Ramu B Kalyana.Extractive visible spectrophotometric

determination of voriconazole in tablet dosage form using ion-association methods. Asian Journal of Research in Chemistry. 2012; 5(5):595-599.