ISSN : 0974-7419 Volume 14 Issue 11

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 14(11) 2014 [426-429]

Visible spectrophotometric assay of racecadotril in bulk and commercial formulations using 2,4-DNP and nitro methane

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ABSTRACT

A direct, simple and sensitive visible spectrophotometric method for racecadotril determination in bulk and its formulations is described. It is based on the formation of pink colored species by condensation reaction between drug and 2,4-DNP, followed by substitution with nitro methane in non aqueous medium under alkaline conditions and exhibits λ_{max} at 560nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration range (20-60) µg/ml. The proposed method is applied to commercial available formulations and the results are statistically compared with those obtained by the reported UV reference method (methanol as solvent λ_{max} at 231nm) and validated by recovery studies. The results are found satisfactory and reproducible.

KEYWORDS

Anti diarrheal drug;
Statistical analysis;
Beer's law;
Regression equation.

INTRODUCTION

Racecadotril (RCT)[1] (Figure1) is an anti diarrheal drug and oral enkephalinase inhibitor. Chemically it is (RS)-benzyl N-[3-(acetylthio)-2-benzyl propanoyl] glycinate with empirical formula of $C_{21}H_{23}NO_4S$ and representing molecular weight of 385.476. It is a white crystalline powder that is soluble in methanol, ethanol, acetonitrile and dichloromethane. The drug is mainly used for the treatment of acute diarrhea of bacterial and viral aetiology. The drug acts by inhibition of enkephalinase, this produces an increases in the levels of enkepalins that act in the enterocyate, thus inhibits hyper secretion.

In literature, several analytical methods such as HPLC[2-6], HPLC tandem MS[7], HPTLC[8], NMR[9], spectrophotometry and spectrofluorometry[10], UV[11,12] & visibleSpectrometric[13,14] have been reported for the determination of RCT in bulk and formulations. Devala Rao et al reported three visible spectrophotometric methods using FC, 1,10-phenanthroline(PTL)-

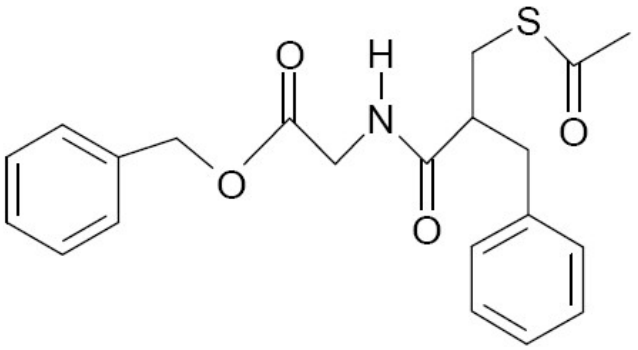

Figure 1: Chemical structure of racecadotril

$FeCl_3$ and 2,2'-Bipyridine(BPN)-$FeCl_3$ reagents for the determination of the drug in bulk and formulations. For routine quality control analysis, cost effective visible spectrophotometric methods are required and preferred. The functional groups present in the drug not fully exploited. So, there is a scope for development of sensitive accurate and flexible visible spectrophotometric methods for the determination of RCT in pharmaceutical preparations. It was noted in the literature[15,16] that ketones (keto steroids also) forms 2, 4-dinitro phenyl hydrazone, and without prior elimination of the excess reagent, development with nitro methane in alkaline medium (Janovsky reaction) is a basis for the colorimetric determination in the present investigation. The method can be extended for the routine quality control analysis of pharmaceutical products containing RCT.

MATERIALS AND METHODS (EXPERIMENTAL)

Apparatus and chemicals

A Milton Roy UV/Visible spectrophotometer model-1201 with 10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-362 was used for pH measurements. All the chemicals used were of analytical grade.

2,4-DNP (Otto,0.05%, $2.52x10^{-2}$M prepared by dissolving 0.025g of 2,4-DNP in a mixture of 10ml of methanol and 0.5ml of Conc. HCl and diluted to 50ml with methanol) solution, Nitro methane(Otto, GR98%), Benzyl tri methyl ammonium hydroxide (BTMAH, Otto,40% solution in methanol), Di methyl formamide(Otto,GR99%) were used.

Preparation of standard stock solution

The standard stock solution (1mg/ml) of RCT was prepared by dissolving 100mg of RCT in 100ml methanol. The working standard solution of RCT (200µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent. The prepared stock solution was stored at 4 ºC protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

Preparation of sample solution

About 20 sachets were weighed to get the average weight and pulverized. The powder equivalent to 100mg of RCT was weighed, dispersed in 25ml of Isopropyl alcohol, sonicated for 15 minutes and filtered through Whatman filter paper No 41.The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Determination of wavelength maximum (λ_{max})

3.0ml of standard RCT solution in methanol in 10ml of calibrated test tube is taken (protected against light), 0.5ml of 2,4-DNP reagent was added and allowed to stand for 20 minutes at room temperature. Later 1.5ml of nitromethane, 1.5ml of dimethyl formamide and 0.3ml of benzyl tri methyl ammonium hydroxide reagent in methanol added. The volume was made up to the mark with methanol. The contents were shaken for 30 seconds. From the absorption spectra (Figure 2), it was concluded that 560nm is the most appropriate wavelength for analyzing RCT with suitable sensitivity.

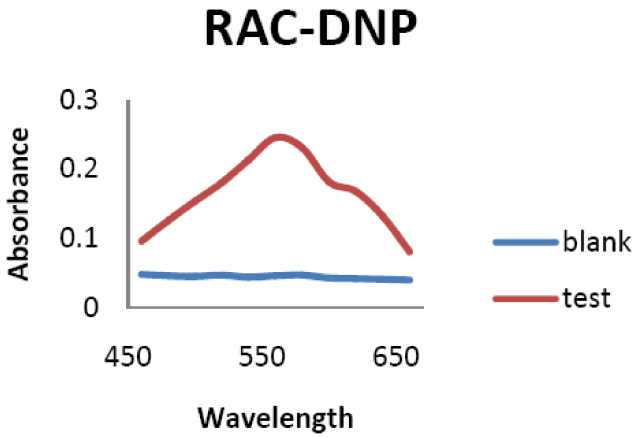

Figure 2 : Absorption spectra of RCT-2,4DNP-NM-BTMAH system

Analytical procedure

Aliquots of standard RCT solution in methanol (1.0-3.0ml, 200µg/ml) were delivered into a series of 10ml of calibrated test tubes (protected against light). 0.5ml of 2,4-DNP reagent was added to each tube and allowed to stand for 20 minutes at room temperature. Later 1.5ml of nitromethane, 1.5ml of dimethyl formamide and 0.3ml of benzyl tri methyl ammonium hydroxide reagent in methanol were successively added

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to each tube. The volume was made up to the mark with methanol. The contents were shaken for 30 seconds and absorbances were read immediately at 560nm against a reagent blank. The amount of RCT in a sample was computed form the calibration graph (Figure 3)

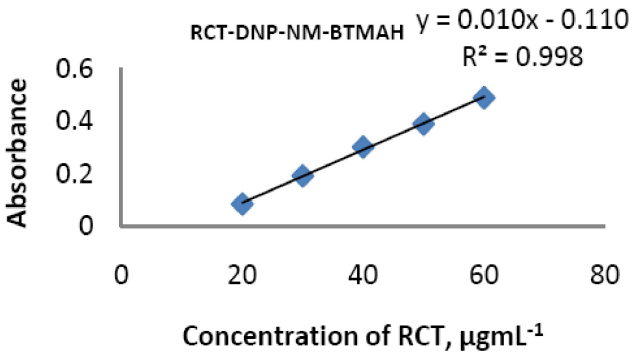

Figure 3 : Calibration graph of RCT-2,4DNP-NM-BTMAH system

TABLE 1 : Optical characteristics, precision and accuracy of proposed method

Parameter	Values
λ_{max} (nm)	560
Beer's law limit(µg/ml)	20-60
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.013245033
Molar absorptivity (Litre/mole/cm)	29103.74
Regression equation (Y)*	
Intercept (a)	-0.110
Slope(b)	0.010
Correlation Coefficient (R^2)	0.998
%RSD	1.85
% Range of errors(95% Confidence limits)	
0.05 significance level	1.94
significance level	3.0

***Y = a+ b x, where Y is the absorbance and x is the concentration of RCT in µg/ml**

TABLE 2 : Assay of racecadotril in pharmaceutical formulations by proposed and reference methods

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Method			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
RCT-2,4-DNP-	Sachet-1	10	9.82 ±0.087	0.67	2.35	9.86 ±0.13	98.2 ±0.88
	Sachet-2	30	29.63 ± 0.29	0.11	2.12	29.63 ± 0.43	98.79 ± 0.99

*** Sachet 1 (10mg) and Sachet 2 (30mg) of Enuff from Hetro HC company; **Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits; t =2.57 and f = 5.05; # Recovery of 10mg added to the pre analyzed sample (average of three determinations); Reference method (reported UV method) using methanol ($2\lambda_{max}$=231nm)**

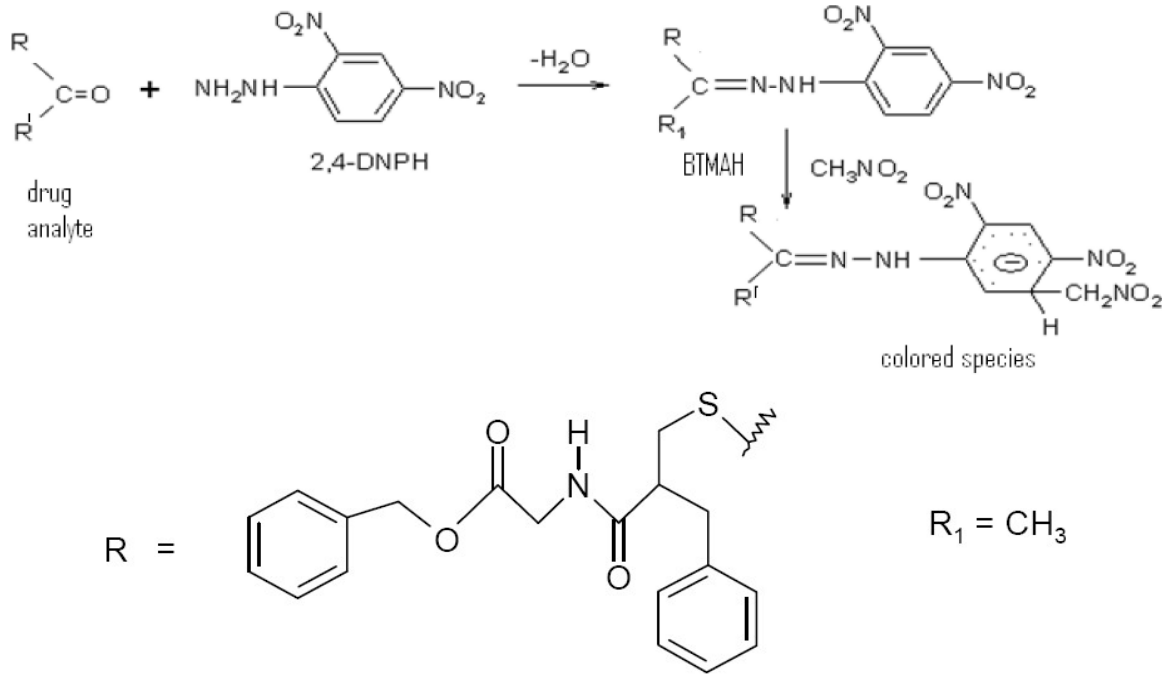

Figure 4 : Probable scheme of the reaction

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, the order of addition of reagents and solvent for final dilution of the colored species were studied. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in TABLE 1.

Chemistry of colored species

In the present investigation, the method involves initially the formation of condensation product from drug with 2,4-DNPH reagent and then followed by substitution with nitro methane in the presence of BTMAH as the drug possess the keto group (Figure 4-Scheme).

CONCLUSION

The reagents utilized in the proposed method are normal cost, readily available in all laboratories and small scale pharmaceutical industries and the procedures do not involve any critical reaction conditions or tedious sample preparation. The proposed method can be used as alternative method to reported ones and provide wide choice for the routine determination of the RCT depending upon the availability of chemical and situation arising due to the presence of concomitants.

ACKNOWLEDGEMENTS

The authors thanks to R&D division Tychy Industries, Hyderabad AP India for providing gift sample of the drug and also thanks to University authorities for providing necessary facilities in this work.

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