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NEW ANALYTICAL METHODS FOR THE ESTIMATION OF RACECADOTRIL IN BULK AND PHARMACEUTICAL FORMULATIONS.

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ABSTRACT

Three simple and sensitive visible spectrophotometric methods (A-C) for the determination of Racecadotril in bulk and pharmaceutical formulations are described. They are based on the formation of colored species by treating with Folin-Ciocalteau (F.C) reagent under alkaline conditions (Method A, $\lambda_{max: 742}$ nm) or 1, 10-phenanthroline (PTL) in the presence of ferric chloride (Method B, $\lambda_{max: 510}$ nm) or 2, 2' -Bipyridine (BPN) in the presence of ferric chloride (Method C, λ_{max} : 520 nm). These methods were extended to the analysis of pharmaceutical preparations and the results are compared with the reference method (USP).

KEY WORDS

Spectrophotometry, Racecadotril, Pharmaceutical preparations.

INTRODUCTION

Racecadotril[1] (RAC) is chemically N-{(R, S)-3-acetylmercapto-2-benzyl}-glycine, benzyl ester. It is an oral enkephalinase inhibitor for use in the treatment of acute diarrhea. A survey of literature revealed only a few reported methods include UV[2], HPLC[3-8], NMR[9] and LC-MS[10] methods for the estimation of Racecadotril in bulk drug and pharmaceutical dosage forms. The analytically important groups of RAC were not exploited for designing suitable spectrophotometric methods. Hence an attempt has been to develop simple and sensitive visible spectrophotometric methods for RAC determination for routine quality control analysis of RAC in formulations. The developed methods are based on the formation of colored species by treating with Folin-Ciocalteau reagent (Redox reaction), 1, 10-phenanthroline (Oxidation followed by complexation reaction), 2, 2' - Bipyridine in presence of Ferric chloride (Oxidation followed by Complexation reaction).

MATERIALS AND METHODS

Instrument:

A Systronics Model 2201 UV-Vis Spectrophotometer with 1 cm matched quartz cells
was used for absorbance measurements.

Reagents:

All the chemicals used were of analytical grade. The commercially available FC reagent (2N) was taken and diluted suitably with distilled water. Aqueous solutions of 1, 10-Phenanthroline (0.198%w/v in 0.1N HCl), Ferric Chloride (0.003M), 2, 2'-

Bipyridine (0.156%w/v in 0.1N HCl), Ortho-phosphoric acid solution (1.3 ml in 100 ml distilled water) were prepared.

Standard Drug solution:

The working standard solution (1mg/ml) of RAC was prepared by dissolving 100 mg of RAC in 100ml of methanol (Method A & C), acetonitrile (Method B).

Procedures

Method A:

Into a series of 10 ml volumetric flasks, standard solution (1000 µg/ml) of RAC in the concentration range of 200- 1400 µg (0.2-1.4 ml) was transferred. Then 1.0 ml of Na_2CO_3 solution, 1.0 ml of FC reagent were successively added and kept aside for 5 min. The volume was made up to 10 ml with water. The absorbance was measured at 742 nm against reagent blank. The amount of RAC was deduced from its Beer-Lambert's plot.

Method B:

Aliquots of standard RAC solution (1000 µg/ml) containing 250 to 1000 µg were transferred into a series of 10 ml volumetric flasks and the volume in all volumetric flasks were equalized with acetonitrile. Then 1.0 ml ferric chloride was added to each flask. Then 1.0 ml of PTL solution was added to all flasks. The contents were gently boiled for 30 min. The flasks were cooled to room temperature and 2.0 ml of Ortho- phosphoric acid was added to all and final volume of all volumetric flasks was brought to 10 ml with water. The absorbance was measured at 510 nm against reagent blank. The amount of RAC in sample was estimated from corresponding calibration graph.

Method C:

Aliquots of standard RAC solution (1000 µg/ml) containing 200 to 1200 µg (0.2-1.2 ml) were transferred into a series of 10 ml volumetric flasks and the volume in all volumetric flasks were equalized with methanol. Then 1.0 ml ferric chloride was added to each flask followed by 1.0 ml of BPN solution was added to all flasks. The contents were gently boiled for 40 min. The flasks were cooled to room temperature and 2.0 ml of Ortho- phosphoric acid was added to all and final volume of all volumetric flasks was brought to 10 ml with water. The absorbance was measured at 520 nm against reagent blank. The amount of RAC in sample was estimated from corresponding calibration graph.

Analysis of pharmaceutical formulations

For Tablets:

About twenty tablets were transferred to a mortar. The tablets were ground in a mortar. From this tablet powder equivalent to 10 mg of RAC was taken and extracted into 100 ml of methanol. Then it was appropriately diluted with the same solvent and used for methods (A&C) and the solvent used for method B was acetonitrile.

For Sachets:

About ten sachets were transferred to a mortar. The sachet powder was ground in a mortar. From this, powder equivalent to 10 mg of RAC was taken and extracted into 100 ml of methanol. . Then it was appropriately diluted with the same solvent and used for methods (A&C) and the solvent used for method B was acetonitrile.

Recovery studies

To study the accuracy, reproducibility and precision of the proposed methods, recovery experiments were carried out. The recovery of the added standard was studied at 3 different levels. Each level was repeated six times. A plot of amount of drug found by proposed method (Y-axis) against standard added (X-axis) was drawn. The intercept on Y-axis indicates the amount of drug present per formulation.

RESULTS AND DISCUSSION

The optimum conditions for each method were established by varying one parameter at a time and keeping the others fixed and observing the effect of product on the absorbance of colored species and incorporated in the procedure. The optical characteristics are given in **Table-1,** together with regression equation for calibration plots.

The precision and accuracy were found by analyzing six replicate samples containing known amount of drugs and the results were summarized in **Table-1, Table-2** shows that the values of recovery is between 98 to 102% and the values of coefficient of variation are sufficiently low indicating that the proposed methods are free of interferences from any excipients like starch, talc, etc and the results are reproducible. Thus these developed methods can be employed for the routine determination of RAC in pure and in pharmaceutical preparations.

Table-1

Optical Characteristics, Regression Data, Precision, and Accuracy of the Proposed Methods for RAC.

Parameter	Method A	Method B	Method C
λ_{max}(nm)	742	510	520
Beer's law limit(µg/ml)	20-140	25-100	20-120
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	2.1×10^{3}	2.675×10^{3}	2.399×10^{3}
Detection limits(µg/ml)	1.965	2.846	0.408
sandell's sensitivity ($\mu g/cm^2$/0.001 abs. unit)	0.183486	0.1441	0.16064
Optimum Photometric range(µg/ml)	1.5-4.5	2.5-11.5	55-245
Regression equation(Y=a+bc) Slope(b)	0.0054	0.0075	0.0063
Standard Deviation of Slope (S_b)	5.32×10^{-5}	9.92×10^{-5}	1.274×10^{-5}
Intercept(a)	0.0024	0.001	0.002
Standard deviation of intercept(S_a)	3.2×10^{-3}	6.1×10^{-3}	7.7×10^{-4}
Standard error of estimation(S_e)	3.57×10^{-3}	8.11×10^{-5}	2.68×10^{-3}
Correlation coefficient(r)	0.9996	0.9994	0.9998
%Relative standard deviation*	0.7264	0.7014	0.5939
%Range of Error (Confidence limits)* 0.05 level 0.01 level	0.762 1.195	0.736 1.154	0.6234 0.977
%Error in bulk samples**	0.33	-0.51	0.25

*Average of six determinations. ** Average of three determinations.

Table-2
Assay and Recovery of RAC in Dosage Forms

Method	Sample	Pharmaceutical Formulation	Labeled Amount (mg)	Proposed Method			Reference method	% Recovery ± R.S.D
				Amount found*(mg)± S.D	t(value)	F(value)		
A	Brand 1	Tablets 1	10	10.02±0.023	0.78	2.06	10.05±0.016	100.2±0.229
		Tablets 2	30	29.05±0.036	1.35	2.67	29.05±0.022	99.5±0.358
		Sachet 1	10	10.01±0.028	0.92	2.17	10.02±0.019	100.1±0.279
		Sachet 2	30	30.03±0.031	0.64	1.22	30.01±0.048	100.3±0.309
	Brand 2	Tablet 1	10	9.95±0.018	0.88	1.96	9.85±0.016	99.8±0.229
		Tablet 2	30	29.05±0.036	1.15	2.23	30.09±0.028	98.9±0.225
		Sachet 1	10	10.06±0.041	0.78	2.56	10.02±0.032	100.08±0.239
		Sachet 2	30	30.05±0.036	1.35	2.79	30.07±0.014	100.6±0.257

*Average ± standard deviation of six determinations, the t and F- values refer to comparison of the proposed with reference method.
Theoretical values at 95% confidence limits t = 2.365 and F = 4.88

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