Research Article

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NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF REFAXIMIN IN FORMULATIONS.

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Abstract:

Three new spectrophotometric methods were proposed for the Quantative Estimation of Rifaximin. Method A(Ferri cyanide method) is oxidation of Rifaximin by excess of ferric salt in Ferricyanide and ferric chloride , Method B is diazotization of Para nitro Aniline (PNA) with sodium nitrate fallowed by coupling with drug in alkaline medium (PNA Method), in Method C (MBTH Method) MBTH was oxidized by the ferric chloride in acidic medium followed by its coupling with the drug. Linearity ranges and RSD will be 6-20ppm and 1.47 for Method A and 2-14 ppm and 0.45 for Method B and 5-30ppm and 0.904 for method C. All these method are Accurate, precise and very effective even at low concentrations and used for the quantitive estimation of Rifaximin in commercial formulations.

Key Words:

Rifaximin, Spectrophotometry, PNA, MBTH, Ferri cyanide.

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Introduction:

Rifaximin is a semisynthetic, rifamycin-based non-systemic antibiotic, meaning that very little of the drug will pass the gastrointestinal wall into the circulation as is common for other types of orally administered antibiotics. It is used in the treatment of traveler's diarrhea and hepatic encephalopathy, for

which it received orphan drug status from the U.S Food and Drug Administration in 1998.

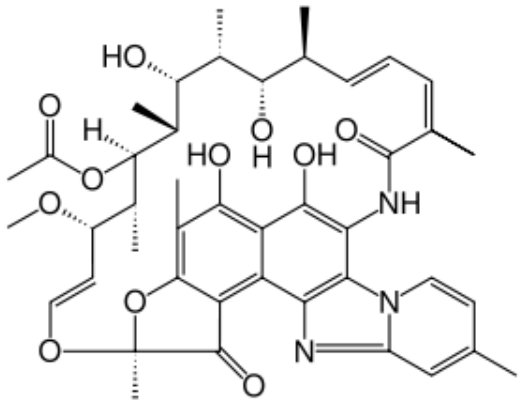

Figure 1: Stricture of Rifaximin.

Rifaximin is licensed by the U.S. Food and Drug Administration to treat traveler's diarrhea caused by E. coli1 Clinical trials have shown that rifaximin is highly effective at preventing and treating traveler's diarrhea among travelers to Mexico, with few side effects and low risk of developing antibiotic resistance2. It is not effective against Campylobacter jejuni, and there is no evidence of efficacy against Shigella or Salmonella species. It may be efficacious in relieving chronic functional symptoms of bloating and flatulence that are common in irritable bowel syndrome3 There was recently a pilot-study done on the efficacy of rifaximin as a means of treatment for rosacea, according to the study, induced by the co-presence of small intestinal bacterial overgrowth4. In the United States, rifaximin has orphan drug status for the treatment of hepatic encephalopathy5. Although high-quality evidence is still lacking, rifaximin appears to be as effective as or more effective than other available treatments for hepatic encephalopathy (such as lactulose), is better tolerated, and may work faster. The drawbacks to rifaximin are increased cost and lack of robust clinical trials for HE without combination lactulose therapy. A recent study suggests that treatment with rifaximin relieves symptoms for some sufferers of irritable bowel syndrome.

Usual Adult Dose for Traveler's Diarrhea is 200 mg orally 3 times a day for 3 days , for Hepatic Encephalopathy is 550 mg orally twice a day and Usual Pediatric Dose for Traveler's Diarrhea for 12 years or older is 200 mg orally 3 times a day for 3 days. Clostridium difficile associated diarrhea (CDAD) has been reported with almost all antibiotics and may potentially be life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea following rifaximin therapy. Mild cases generally improve with discontinuation of the drug, while severe cases may require supportive therapy and treatment with an antimicrobial agent effective against C difficile. Hypertoxin producing strains of C difficile cause increased morbidity and mortality; these infections can be resistant to antimicrobial treatment and may necessitate colectomy. Safety and effectiveness for travelers' diarrhea have not been established in pediatric patients less than 12 years of age. Safety and effectiveness for hepatic encephalopathy have

not been established in pediatric patients less than 18 years of age.

Experimental Procedure:

Reagents and Materials

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Double beam VV-Visible Spectrophotometer is used for measuring the absorbances of the color formed during the analysis.

Preparation of reagents:

MBTH solution: accurately 500 mg of MBTH was weighed and was taken in a 100 ml graduated volumetric flask. It was dissolved in distilled water and made up to the mark.

Potassium ferricyanide solution: accurately 100 mg of Potassium ferricyanide was weighed and taken in a 100 ml graduated volumetric flask. It was dissolved in Double distilled water and made up to the mark.

PNA solution: accurately 100 mg of PNA was weighed and was taken in a 100 ml graduated volumetric flask. It was dissolved in 0.2 M HCl solution and made up to the mark.

Fe (III) solution: accurately 250 mg of anhydrous ferric chloride was weighed and was taken in a100 ml graduated volumetric flask. It was dissolved in distilled water and made up to the mark.

HCl solution (1N): Prepared by diluting 86 ml of conc. HCl to 1000 ml with distilled water and standardized.

$NaNO_2$ solution: accurately 100 mg of $NaNO_2$ was weighed and was taken in a 100 ml graduated volumetric flask. It was dissolved in distilled water and made up to the mark.

NaOH solution (4 %, 1M): accurately 4g of NaOH was weighed and was taken in a 100ml graduated volumetric flask. It is dissolved in distilled water and made up to the mark.

Preparation of working standard drug solution:

The standard Rifaximin (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg /ml (stock solution I). 10 ml of stock solution I was diluted to 100 ml with Methanol (Stock solution II, 100 µg/ml) and the resulting solution was used as working standard solution.

Methods:

Ferricyanide method:-

In a 10mL of calibrated tubes, aliquots of standard drug is transferred and 1 ml of Fe(III) solution is added. The tubes were Stoppard immediately and shaken well for 5 min. Then 0.5ml of potassium Ferricyanide solution was added into each tube and was closed with lids immediately. After 5 min, 1

ml of 1N HCl was added and the final volume was made up to 10 ml with distil water solution attain pale red colour. The maximum absorbance was measured against a reagent blank (colourless).

PNA method:-

In a 10 ml graduated test tubes 1.0 ml of PNA solution and 1.0 ml of $NaNO_2$ solution were successively added and allowed to stand for 2 min. Later, standard drug of elected concentration is delivered into the test tube. Then 1.5 ml of NaOH solution was added and the volume in each tube was made up to 10 ml distil water. Solution attains green colour. The maximum absorbance was measured against a reagent blank (colourless).

MBTH method:-

From the standard stock solution II of Rifaximin, appropriate concentration is pipetted out in to a 10 ml volumetric flasks add 1 ml of water, 0.5 ml of 0.5% MBTH and 0.5 ml of 0.1 N NaOH were added. The contents were heated for 10 min in a water bath at 100 ^{0}C and cooled for 5 min in a water bath at 15 ^{0}C. Then 0.5 ml of 1N HCl and 2 ml of Fe (III) solutions were added successively the final volume was made up to 10 ml with distil water and kept side for 1 hr. Maximum absorbance was against a reagent blank prepared in a similar way

Assay Procedure for Formulations

An amount of finely ground capsule powder equivalent to 100 mg of Rifaximin (XIFAXAN _ 550mg) was accurately weighed into a 100 ml calibrated flask, 60 ml of water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well, and filtered using a Whattman No 42 filter paper. First 10 ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 µg mL-1 Rifaximin) was diluted appropriately to get suitable concentrations for analysis by proposed methods.

Method Validation:

Selection of analytical concentration ranges: (linearity test)

Linearity test was evaluated by measuring the absorbance values of standard solutions. The standard stock solution of Rifaximin, appropriate aliquots were pipetted out in to a seven series of 10 ml volumetric flasks and make up to 10ml mark with the solutions required for each individual method. After color formation absorbance of each concentration was measured at wavelength found for the proposed method. Results were shown in Table:1 and Standard graphs of linearity for proposed methods were shown in Graph-A,B,C respectively.

Accuracy and Precision:

To evaluate the accuracy and precision of the methods, pure drug solution (Within the working limits) was analyzed and being repeated six times. The relative error (%) and relative standard deviation (%) were less than

2.0 and indicate the high accuracy and precision for the proposed methods (Table 2).

Recovery Studies:

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The Percentage recoveries thus obtained were given in Table 3.

Application to Analysis of Commercial Sample:-

In order to check the validity of the proposed methods, Rifaximin was determined in commercial formulation. The results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. These results indicating that there was no significant difference between the proposed methods and the reference methods in respect to accuracy and precision.

Result and Discussion:-

PNA method involves the diazotization of PNA with sodium nitrate fallowed by coupling with drug in alkaline medium. The formed PNA- DRUG complex develop green color, the developed color can be estimated by using spectrophotometer at a wavelength 430 nm. MBTH was oxidized by the ferric chloride in acidic medium followed by its coupling with the drug to form green colored complex having λ max at 620nm.Actually, this is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling agent. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The Ferricyanide method based on the oxidation of Rifaximin by excess of ferric salt (Fe3+) and the reduced state of Fe3+ was utilized besides the un reacted Fe3+. The Fe2+ has tendency to give colored complex on treatment with potassium ferricyanide the developed color can be estimated by using spectrophotometer at a wavelength 790 nm.

The linearity ranges of Rifaximine are found for Ferricyanide method (6-20ppm), PNA Method (2-14ppm), and MBTH method (5-30ppm) respectively. A linear correlation was found between absorbance and concentration of Rifaximin. The graphs showed negligible intercept and are described by the equation: Y = a + bX (where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in ug mL-1 max) .Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient(r) for each system according to ICH guidelines.

The accuracy of the proposed methods were further ascertained by performing

Accuracy studies. The Relative standard deviation of results for three proposed were Ferricyanide method (1.47), PNA Method (0.45), and MBTH method (0.687) respectively and these values are within the range below 2. It indicates that the high accuracy and precision for the proposed methods. The Recovery results were very close to the actual range and it revealed that co-formulated substances did not interfere in the determination.

Conclusions:

Three useful micro methods for the determination of Rifaximine have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. These spectrophotometric methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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Tables:

Parameter	Ferricyanide method	PNA method	MBTH method
Wavelength Max	430	690	790
Correlation coefficient	0.99910.0137	0.9995	0.9995
Slope	0.060796	0.0997	0.0326
Intercept	0.024	0.0084	0.0137
RSD of Precision	1.47	0.45	0.90
RSD of Recovery	0.698	0.772s	0.38s
%of Assay for formulation	99.87	99.90	99.95

Table 1: summery of the proposed methods for Rifaximin

S.No	Ferricyanide method Abs at 12ppm	PNA method Abs at 4 ppm	MBTH method Abs at 10 ppm
1	0.76	0.417	0.352
2	0.78	0.415	0.359
3	0.76	0.416	0.354
4	0.75	0.418	0.357
5	0.77	0.413	0.352
6	0.78	0.414	0.351
RSD	1.47	0.45	0.904

Table 2:Precision test results.

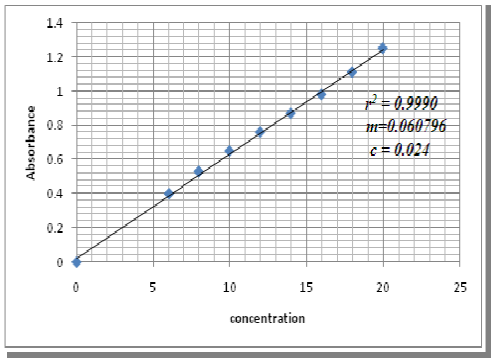
Method	Recovery	Conc. In ppm	Amount of Rifaximine recovered(ppm)	% Recovery	Mean recovery
Ferricyanide method	50%	6	5.98	99.67	99.84
	100%	12	11.98	99.25	
	150%	18	18.11	100.61	
PNA method	50%	2	1.98	99.00	99.67
	100%	4	4.02	100.50	
	150%	6	5.97	99.50	
MBTH method	50%	5	4.97	99.40	99.92s
	100%	10	10.09	100.9	
	150%		14.92	99.47	

Table 3: Result of Recovery studies.

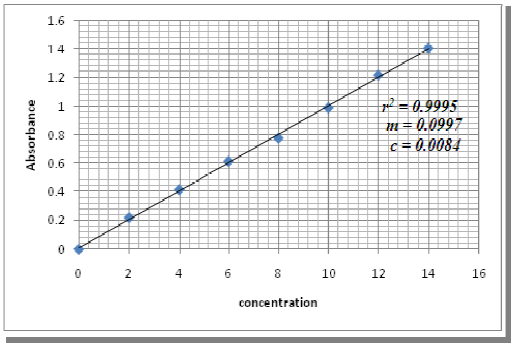
Tablet formulation	Labeled Amount mg	Amount obtaned by Ferricyanide method	Amount obtaned by PNA method	Amount obtaned by MBTH method	% of recovery by Ferricyanide method	% of recovery by PNA method	% of recovery by MBTH method
XIFAXAN	550mg	11.98ppm	3.96ppm	11.94ppm	99.833	99.0	99.5

Table 4: formulation study results.

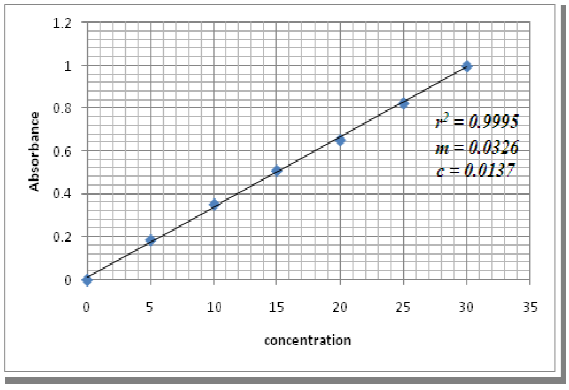
Graphs:



Graph A: A Ferriciande Method



Graph B: PNA Method



Graph C: MBTH Method.