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MBTH (3-Methyl-2-Benzothiazolinone Hydrazone Hydrochloride) as a Chromogenic Reagent for Estimation of Faropenem

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ABSTRACT

A visible spectrophotometric method which is simple, precise and economical has been developed and validated for the estimation of Faropenem in pharmaceutical bulk and tablet dosage form. Identification was carried out using a UV-Visible double beam spectrophotometer with working wavelength at 639.5 nm. It was based on the formation of a colored complex between Faropenem and MBTH (3-Methyl-2-Benzothiazolinone hydrazone hydrochloride) reagent in presence of Ferric chloride. The method was validated with respect to its specificity, linearity range, accuracy, and precision in analytical media. Regression analysis showed good correlation in the concentration range 25-300 µg/ml and the % relative standard deviation as 0.1262. Statistical treatment of data reflects that the proposed method is accurate and easily applicable for determination of Faropenem in bulk and pharmaceutical preparation. The different experimental parameters effecting the development and stability were studied carefully and optimized. A result of analysis for the method was validated statistically and recovery studies were also performed.

Keywords: Faropenem, MBTH (3-Methyl-2-Benzothiazolinone hydrazone hydrochloride) reagent, Ferric chloride, Ultraviolet-Visible double beam- spectrophotometer.

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INTRODUCTION

Faropenem is a novel β-lactam antimicrobial with a penem (furanem) structure that is being developed for use as an oral therapy for community-acquired respiratory tract infections. Although recent studies have highlighted the broad-spectrum antibacterial activity of faropenem, new attention has focused on its activity against the respiratory pathogens. Faropenem is chemically 6-(1-hydroxyethyl)-2-[(2R)-tetrahydrofuran-2-yl]-2,3-didehydropenam -3-carboxylic acid. It exhibits structural similarities with both the penicillins and cephalosporins. The primary mode of action of faropenem is consistent with that of other β-lactam antibiotics, namely binding to penicillin-binding proteins. Faropenem has been shown to demonstrate high stability to a number of β- lactamases. It is official in Martindale (The extra pharmacopoeia). Literature survey reveals that the drug was determined by HPLC, there is no method reported for Faropenem by visible spectrophotometry. Therefore the need for fast, low cost and selective method is obvious especially for routine quality control analysis of pharmaceutical formulation. The structure of Faropenem is shown in Figure 1.

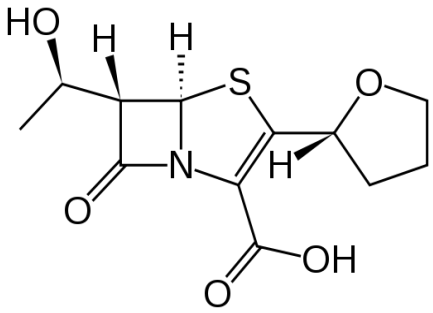

Figure 1: Structure of Faropenem

MATERIALS AND METHODS:

Instrument:

ELICO double beam Ultra Violet –Visible Spectrophotometer SL-244 with 1cm matched quartz cells were used for all spectral measurements.

Reagents:

All chemicals used were of analytical reagent grade

- 0.2% w/v MBTH: 0.2 g of MBTH dissolved in 100 ml of distilled water.
- Preparation of Ferric chloride (0.7% w/v): Dissolve 0.7 g of anhydrous Ferric chloride in 100 ml of 0.5N Hydrochloric acid.
- Preparation of Hydrochloric acid (0.5N): 8.5 ml of Hydrochloric acid is taken and made up to 100 ml with water.

Standard Stock Solution:

A standard stock solution containing 1 mg/ml was prepared by dissolving 100 mg of Faropenem in 100 ml of distilled water. From this, a working standard solution containing 100 µg/ml was prepared with distilled water.

Assay:

Aliquots of standard drug solution of Faropenem containing 0.25-3 ml (100 µg/ml) are taken and transferred into series of graduated test tubes. To each test tube 1.5 ml of MBTH solution and 2 ml of 0.7% w/v Ferric chloride were added. The contents are shaken thoroughly. Reaction mixtures were allowed to stand for a period of 20 minutes. During this waiting period the solutions develop green color. Then, the final volume is made upto 10 ml with distilled water. Now the absorbance was measured at 639.5 nm against reagent blank. The absorption maxima of Faropenem against the corresponding reagent blank is shown in Figure 2. Calibration curve was prepared from absorbance values so obtained. The linearity values are mentioned in Table 1 and the plot is as shown in Figure 3.

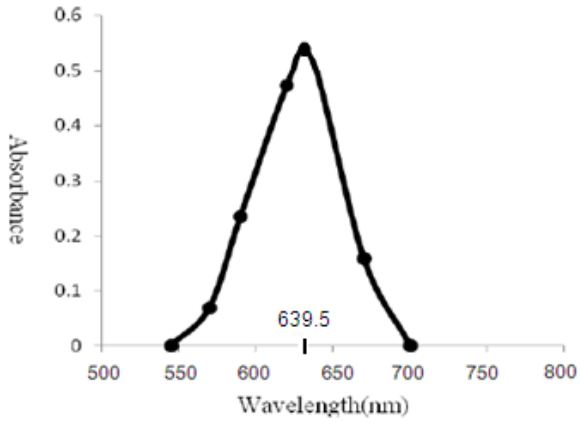

Figure 2: Absorption spectra of Faropenem with MBTH

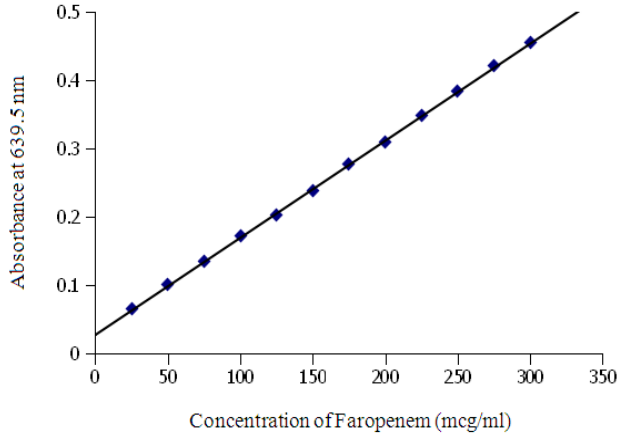

Figure 3: Linearity curve for Faropenem

Preparation of sample solution:

Tablets containing Faropenem (Faronem 200 mg, marketed by Ranbaxy laboratories limited) were successfully analyzed by the proposed methods. Twenty tablets of Faropenem (Faronem 200 mg, marketed by Ranbaxy laboratories limited) were accurately weighed and powdered. Tablet powder equivalent to 100 mg of Faropenem was dissolved in 100 ml of distilled water. The concentration of the resultant solution is 1 mg/ml. This solution was filtered. Now the filtrate was suitably diluted and analyzed as given under the assay procedure for bulk samples. The results are represented in Table 3. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Faropenem, by the proposed method.

Optimization of conditions and absorption spectrum of the reaction product:

Condition under which reaction of Faropenem with MBTH reagent fulfills the essential requirements was investigated. All the conditions studied were optimized at room temperature ($32\pm 2\ ^{0}C$).

Effect of order of addition of reactants:

After fixing all other experimental variables, a few further experiments were performed to ascertain the influence of order of addition of reactants on the color development and it was found that the addition of MBTH reagent followed by Ferric chloride to the aliquots of drug solution gave maximum intensity of the color.

Chemistry of the colored species:

In this mechanism, the drug reacts with MBTH in the presence of Ferric chloride to give a colored product. This is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH forms an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the green colored product. The colored chromogen shows the maximum absorbance at 639.5 nm.

Recovery Studies:

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug 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.

RESULTS AND DISCUSSIONS

In the present work a method has been developed for the estimation of Faropenem from tablet

formulation. The developed method was based on formation of a colored complex between drug and the MBTH reagent. The condition required for formation of colored complex was optimized. Statistical analysis was carried out and the results were satisfactory. Relative standard deviation values were low that indicates the reproducibility of the proposed methods. Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. The optical characteristics such as Absorption maxima, Beer's law limit, Molar absorptivity and Sandell's sensitivity are represented in Table 2. The regression analysis using the method of least square was made for slope (m), intercept (c) and correlation obtained from different concentrations and the results are also summarized. In conclusion, the proposed method is simple, economical, sensitive, precise, reliable and reproducible for the routine estimation of Faropenem in bulk as well as in tablet formulation.

Table 1: Linearity Values

Concentration (µg/ml)	Absorbance
25	0.0646
50	0.1011
75	0.1350
100	0.1712
125	0.2028
150	0.2386
175	0.2773
200	0.3090
225	0.3482
250	0.3829
275	0.4213
300	0.4551

Table 2: Optical Characteristics and Precision Data

Parameters	Proposed method
λmax (nm)	639.5
Beers law limit (µg/ml)	25 – 300
Molar absorptivity(L/mole/cm)	4.9772×10^2
Sandell's sensitivity(µg/cm^2/0.001 Absorbance unit)	0.5740
Regression equation	
Slope (m)	0.001
Intercept (c)	0.0281
Correlation coefficient (r)	0.9949
Precision (% Relative standard deviation)	0.1262
Standard error of estimate	0.002
LOD (µg/ml)	1.287
LOQ (µg/ml)	3.9

***y = mx +c, where 'x' is the concentration in micrograms/ml and 'y' is absorbance unit**

Table 3: Assay of Faropenem in Tablet Formulations

Tablet Formulation	Labeled amount (mg)	Amount obtained (mg) by proposed method*	% Recovery by the proposed method **
01	200	199.88 ± 0.17	99.15 ± 0.11
02	200	199.51 ± 0.23	99.45 ± 0.19
03	200	201.22 ± 0.12	99.79 ± 0.24

*Average of three determinations **After spiking the sample

CONCLUSION

A novel, cost effective, simple and sensitive visible spectrophotometric method, using MBTH (3-Methyl-2-Benzothiazolinone hydrazone hydrochloride) reagent was well established by the assay of Faropenem in pure form and in pharmaceutical preparations. The developed method was also validated and the procedure does not involve any critical reaction conditions or tedious sample preparation. From the statistical data, it was found that the proposed method was accurate, precise, reproducible and can be successfully applied for the analysis of Faropenem without any interference from additives and excipients.

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