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**Research Article**

# Extractive spectrophotometric method for determination of rufinamide in bulk and its pharmaceutical dosage form

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## ABSTRACT

A new economic, industrially acceptable and readily adaptable method has been developed following a complexation (acid-dye method) between antiepileptic drugs and Bromocresol green (BCG) dye and validated for determination of this drug in bulk and its pharmaceutical dosage form. Drug used during analysis i.e. Rufinamide was reacted with BCG in presence of hydrochloric acid buffer pH 1.2. The colored complex formed was extracted with chloroform and the absorbance of the solutions were noted which followed a beer's law in concentration range of 10-50 µg/ml for Drug-BCG complex with correlation coefficient close to 0.991. The method was validated as per ICH guideline for accuracy, precision, limit of detection and limit of quantification. The developed Spectrophotometric method has the advantages of speed, simplicity, sensitivity and more affordable instrumentation and could find application as a rapid and sensitive analytical method for Rufinamide.

**Keywords:** Extractive Spectrophotometry, Acid-dye method, Rufinamide, Bromo cresol Green

## INTRODUCTION

Rufinamide is a newer category of drug acting on central nervous system used for the treatment of seizures associated with Lennox-gastaut syndrome [1-3]. The drug is not official in any of the pharmacopeia. Rufinamide is triazole derivative structurally unrelated to any other anti-epileptic drug. Rufinamide is chemically 1-(2, 6-Difluorobenzyl)-1H-1, 2, 3-triazole-4-carboxamide. Principle mechanism of action of Rufinamide is modulation of the activity of sodium channels and in particular, prolongation of the inactive state of channel [4-5]. Rufinamide significantly slowed sodium channel recovery from inactivation after a prolonged prepulse in cultured cortical neurons, and limited sustained repetitive firing of sodium dependent action potentials. Several analytical methods like UV-spectrophotometry [6-7], HPLC [8-10], LC-MS and bioassay [11] have been reported for determination of the drug in bulk, its Pharmaceutical dosage forms and in biological fluids. All these methods require sophisticated instruments and complex sample preparation steps. Moreover due to absence of any conjugated double bonds in structure of Rufinamide, it does not absorb in UV region increasing the difficulties in its estimation by usual detectors. Thus it was thought of interest to develop new, simple, cost effective, accurate, precise and industrially applicable extractive spectroscopic methods for determination of this drug in bulk and its Pharmaceutical dosage form.

## MATERIALS AND METHODS

**Materials:** Rufinamide was supplied by Torrent Research Centre, Ahmadabad, India. All chemicals and reagents used were of analytical grade and purchased from SD Fine Chemicals, India.

**Preparation of standard stock solutions:** Accurately weighed Rufinamide (5 mg) was transferred into 50 ml volumetric flasks, dissolved in and diluted up to the mark with methanol (100 µg/ml).

**Preparation of Dye solutions:** Accurately weighed Bromocresol green dye (50 mg) was transferred into 50 ml volumetric flasks. Distilled water (30 ml) was added, sonicated for 10 min and diluted with distilled water up to the mark (1mg/ml).

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**Preparation of buffer solutions:** All the buffer solutions (pH 1.2, 2.2, 3.3, 4.2, 5.2 6.2 and 7) prepared were as per the formulas listed in IP 2007**.**

**Optimization of solvent for extraction of Drug-Dye Complex and selection of wavelength:** Standard stock solution of Rufinamide (1ml) was transferred into a series of 25ml glass stoppered test-tubes. In each test-tube, BCG dye (1 ml) was added along with 3 ml of 0.1N HCl. The solutions were mixed well and 10 ml of various organic solvents (chloroform, toluene, acetone, diethyl ether and petroleum ether) were added to different tubes. The tubes were vortexed on cyclomixer for 2 min and were kept aside for layers to separate. From each test-tube, the organic layer was collected through separating funnel and anhydrous sodium sulphate was added to remove traces of water and was scanned in the range of 400-800 nm for determination of wavelength maximum and its absorbance.

**Optimization of pH for Drug-Dye complex formation:** Standard stock solution of Rufinamide (1ml) was transferred into a series of 25ml glass stoppered test-tubes. In each test-tube, 1ml of BCG dye solution was added along with 3 ml of different buffers (buffers of varying pH like 1.2, 2.2, 3.2, 4.2, 5.2, 6.2 and 7). The solutions were mixed well and chloroform (5 ml) was added in each tube. The tubes were vortexed on cyclomixer for 2 min and were kept aside for layers to separate. The organic layers were collected through separating funnel and anhydrous sodium sulphate was added to remove traces of water and the absorbances were measured at 418 nm.

**Stochiometric determination of Drug-Dye Complex (JOB'S Curve Method):** Equimolar solution of drug and dye ($1.0 \times 10^{-3}$) were prepared in methanol and distilled water respectively. From this drug solution (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 ml) were transferred into a series of 25 ml glass stoppered test tubes. To these tubes varying volume of dye solution (1.8, 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4 and 0.2 ml respectively) were added in such a way that molar concentration of drug and dye remains constant while their mole fractions vary. To each test-tube buffer pH 1.2 (3 ml) and chloroform (5 ml) were added. The tubes were vortexed on cyclomixer for 2 min and were kept aside for layers to separate. The organic layers were collected through separating funnel and anhydrous sodium sulphate was added to remove traces of water and the absorbances were measured at wavelength maximum (418 nm). Absorbance that is proportional to complex formation was plotted against the mole fractions of the drug component.

**Stability of formed Drug-Dye complex:** Stability of formed drug-dye complex was ascertained by continuously monitoring the absorbance values of the colored complex using UV-spectrophotometer. Absorbance values were noted at an interval of five minutes till there was deflection of more than 2% absorbance value than the initial (up to 90 min). This time interval was termed as stability of the colored drug-dye complex.

## VALIDATION STUDIES [12]

**Calibration Curve (Linearity):** Stock solution of standard Rufinamide (1.0, 2.0, 3.0, 4.0 and 5.0 ml) were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with methanol (10, 20, 30, 40 and 50 µg/ml). Each solution (1 ml) was transferred into five different 25 ml glass stoppered test-tubes. BCG dye (1ml), hydrochloric acid buffer pH 1.2 (3ml), Chloroform (5 ml) were added in each test-tube and vortexed for 2 min. The tubes were kept aside for layers to separate and the organic layer was collected and anhydrous sodium sulphate was added to remove traces of water. UV visible spectrum of each solution was obtained and absorbance of colored ion pair complex was measured at 418 nm. The methodology was adopted in triplicates. A plot of mean absorbance vs. concentration of drug was plotted and linear regression equation was computed.

**Intraday and inter day precision:** Intraday precision was determined at three different concentration levels of calibration curve (10, 30, 50 µg/ml) for three times in the same day. Inter day precision was determined by analyzing above standard over a period of three different days.

**Limit of detection and quantification:** LOD and LOQ were determined using following equation as per ICH guideline:

**LOD= 3.3 σ / S**

**LOQ = 10 σ / S**

Where σ = S.D of y-intercept of calibration curves

S = Mean of slope of calibration curve

**Accuracy:** Accuracy is a measure of the exactness of the analytical method. Accuracy was determined by spiking the known amount of standard solution into the pre-analyzed marketed formulation sample at three different levels (80, 100 and 120%). The % recoveries were determined for drug-dye complexes.

**ASSAY OF MARKETED FORMULATION** (**BANZEL-200 mg Rufinamide**): Twenty tablets were accurately weighed and finely powdered. Powder equivalent to 200 mg Rufinamide was

transferred into 100 ml volumetric flask. Methanol (50 ml) was added and the flask was sonicated for 15 min and diluted up to the mark with methanol. The solution was filtered through whatman filter paper no.41. Aliquot (1 ml) was transferred into a 10 ml volumetric flask and diluted with methanol up to the mark. The solution (1 ml) was transferred into a 25 ml glass stoppered test-tube and analysed as described under 3.1.

## RESULTS AND DISCUSSION

**Chemistry of the Reaction:** The drug in presence of acidic pH (hydrochloric acid buffer pH 1.2) undergoes ionization and form a quaternary amine in solution whereas the dyes at this pH form an anion. Thus a complex is formed between cationic drug and anionic dye which could be easily extracted in organic solvent. The drug and dye forms a complex of a peculiar stochiometric ratio and excess of the components remain ionized in aqueous phase and could not be extracted. The reaction mechanism is depicted in Figure 1.

**Optimization of solvent for extraction of Drug-Dye Complex:** An organic solvent must be selected before the start of analysis in order to ensure complete extraction of Drug-Dye ionic complex from aqueous phase. Amongst various organic solvents tried, Chloroform and Diethylether gave almost similar results with slight higher absorbance values in case of chloroform (Figure 2). Moreover, diethyl ether is more volatile than chloroform. Thus chloroform was selected for extraction of drug-dye complex throughout the analysis.

**Selection of optimum wavelength:** The extracted drug-dye complex was scanned over a range of 400-800 nm for determination of wavelength maximum. It was found to be 418 nm. Figure 3 displays the spectrum of extracted drug-dye complex.

**Optimization of pH for Drug-Dye complex formation:** pH of the solution plays a definite role in formation of Drug-Dye ionic complex. The results obtained clearly indicated that hydrochloric acid buffer pH 1.2 provided optimum environment for formation of Drug-Dye complex and hence was utilized throughout the analysis (Figure 4).

**Stochiometric determination of Drug-Dye Complex (JOB'S Curve Method):** The results indicated that 1:1 (drug: dye) ion-pair is formed through the electrostatic attraction between the positive protonated drug and the anion of dye. The graph of the results obtained gave a maximum at a molar ratio of Xmax ~ 0.5 which indicated the formation of a 1:1 Rufinamide-BCG complex (Figure 5).

**Stability of formed drug-dye complex:** Formed Drug –Dye complex was remained stable up to 70 minutes.

**Validation Studies:** The linearity was established in range of 10-50 µg/ml for Rufinamide-BCG complex. Intra and Inter day precision results clearly indicated that the % RSD values were less than 2% and the method was precise. Limit of detection and quantification were calculated as per ICH guideline. Accuracy study was carried out at three different levels (80, 100 and 120%) by standard addition method. The results of validation studies are summarized in Table 1.

**Assay of Marketed Formulations:** The results of assay of marketed formulations using developed method are depicted in Table 2.

## CONCLUSION

The proposed spectrophotometric method is the first described method for the analysis of Rufinamide by acid-dye complexation methodology. The method was applied for the analysis of Rufinamide in the tablet dosage form. The results were in good agreements with labeled claim. There was no interference from routine excipients and solvents encountered. Hence the proposed method is selective, specific, sensitive, accurate, precise, and cost effective. Therefore it can be used for routine analysis of Rufinamide in bulk and its formulations.

**Table 1** Summary of Validation Parameters

| Sr. No. | Parameter | BCG DYE |
|---|---|---|
| | | **Rufinamide** |
| **1** | Linearity (µg/mL) | 10-50 |
| **2** | Correlation Coefficient ($r^2$) | 0.991 |
| **3** | Intraday Precision (% RSD) (n=3) | 0.92-1.87 ± 0.003 |
| **4** | Interday Precision (% RSD) (n=3) | 0.64-1.17± 0.007 |
| **5** | Accuracy (% Recovery) | 98.15 |

| | | |
|---|---|---|
| **6** | LOD (µg/mL) | 0.60 |
| **7** | LOQ (µg/mL) | 1.82 |

**Table 2** % Assay of Marketed Formulation (n=3)

| **Drug Formulation** | **Labeled Claim** | **% Assay** |
|---|---|---|
| | | **BCG** |
| Tablet | Rufinamide - 200 mg | 98.84 ± 0.016 % |

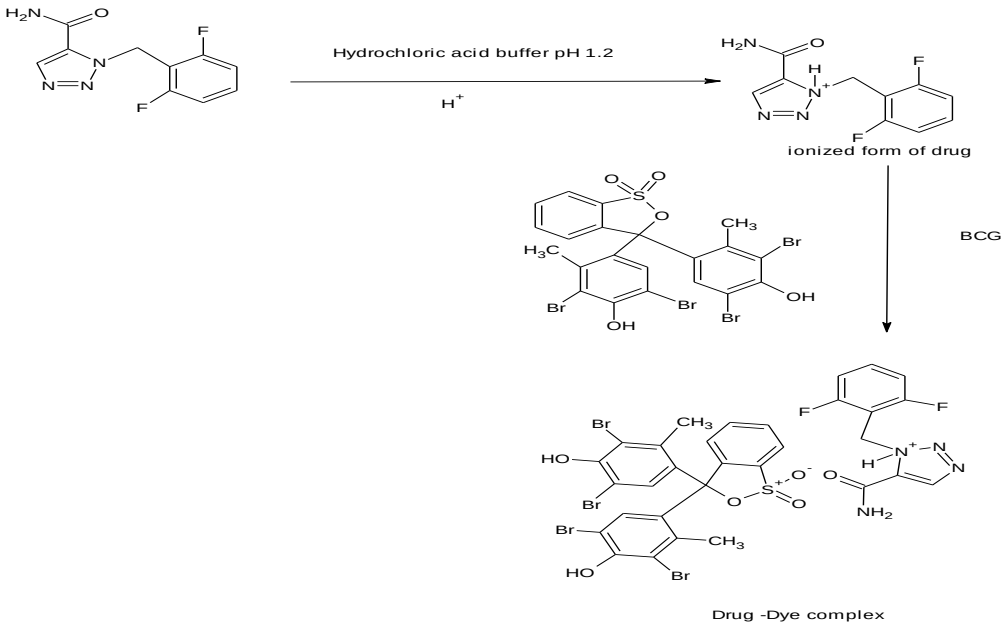


**Figure 1: Reaction mechanism of Drug- Dye Complex**

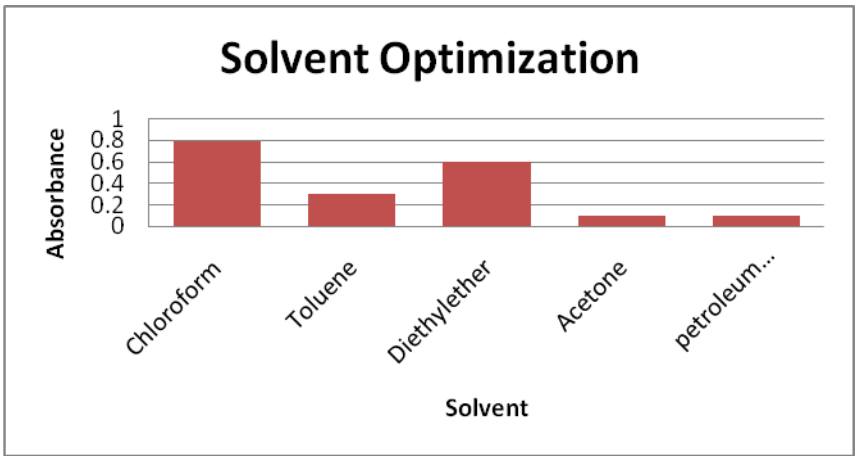


**Figure 2: Optimization of solvent for extraction of drug-dye complex**

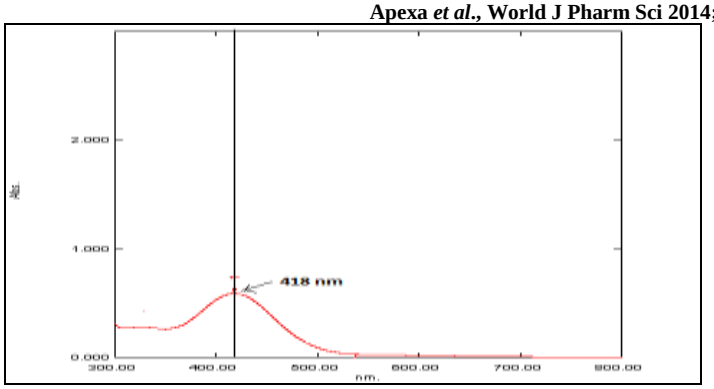


**Figure 3: Spectrum of extracted drug-dye complex-optimization of wavelength**

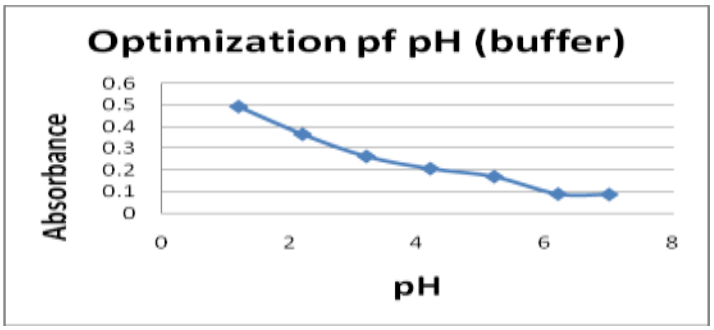


**Figure 4: Optimization of pH**

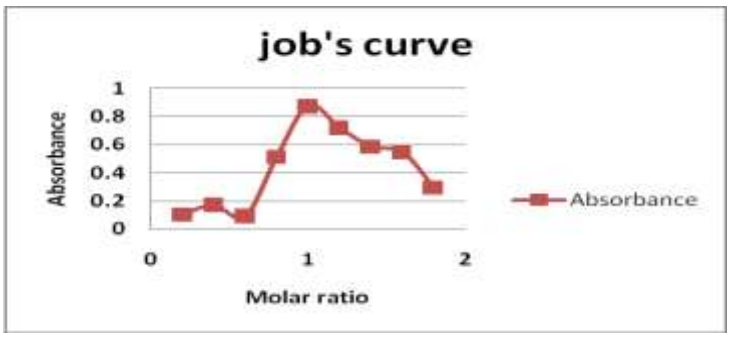


**Figure 5: Job's Curve**

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