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Validation of UV Spectrophotometric Method for Determination of Riluzole Pharmaceutical Formulation as Hydrotropic Solubilizing Agents

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Abstract: Ultraviolet absorption spectrophotometric method for the estimation of poorly water soluble drug like Riluzole in pharmaceutical formulations has been developed. Aqueous solubility of this selected model drug was to a great extent (55 to 112 fold) in 5.0 M sodium benzoate. Riluzole shows maximum absorbance at 348 nm. Beer's law was obeyed in the concentration. The value of LOD and LOQ were 0.4365 µg/ml, 0.2654 µg/ml Riluzole. Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide; acetonitrile, hexane, acetone and carbon tetrachloride have been employed for solubilization of poorly water-soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity and error in analysis due to volatility.Results of analysis were validated statistically and by recovery studies.

Key words: Riluzole · Hydrotropic Solubilization · Sodium Benzoate

INTRODUCTION

Riluzole (RZ) is a benzthiazole derivative. Chemically, Riluzole is 6-(trifluoromethoxy)-1, 3- benzothiazol-2-amine. Riluzole (RZ) is an NMDA receptor antagonist used to treat amyotrophic lateral sclerosis. Riluzole (RZ) preferentially blocks TTX sensitive sodium channels, which are associated with damaged neurons [1,2]. This reduces influx of calcium ions and indirectly prevents stimulation of glutamate receptors. Literature survey reveals that few HPLC methods are reported for the determination of RZ in plasma [3,4]. The term "hydrotropy" has been used to designate the increase in aqueous solubility of various poorly water-soluble compounds due to the presence of a large amount of additives. Sodium benzoate, sodium salicylate, niacinamide, sodium hydroxide and urea have been employed to enhance the aqueous solubility of poorly water-soluble drugs [5]. Various organic solvents like methanol, chloroform, ethanol, dimethyl formamide, benzene, hexane, acetone, toluene, carbon tetrachloride, diethyl ether and acetonitrile are widely used in spectrophotometric estimations of poorly water-soluble drugs. Most of these organic solvents are toxic, costlier and sources of pollution. Inaccuracy in spectrophotometric estimations due to volatility of organic solvents is another drawback of these solvents. There was tremendous increase in aqueous solubility of Riluzole in 5M Sodium benzoate solution. Sodium benzoate does not show absorbance above 365 nm. The Beer's law was obeyed in the range of 5 to 45 mcg/ml at 348 nm for Riluzole in presence of Sodium benzoate. In the present investigation Riluzole tablets have been estimated by BP method (Spectrophotometric) which involved use of an organic solvent, methanol and also by the hydrotropic solubilization technique which involved use of Sodium benzoate as hydrotropic solubilizing agent. The results of analysis obtained by the proposed method compared very well with those obtained by the pharmacopoeial method. Recovery studies and low values of standard deviation, % coefficient of variation and standard error validated the proposed method. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form by hydrotropic agents [6-20]. Recovery studies and statistical analysis were used to validate the methods. It is thus concluded, that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully employed in the routine analysis of these drugs in pharmaceutical dosage forms.

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Experimental

Instruments and Chemicals: UV/Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). All chemicals used are of AR grade from S.D.Fine chemicals, Mumbai Commercial tablets 50 mg strength were procured from local pharmacy of commercial brand i.e. Rilutek® (CIPLA) were procured from local market.

Preliminary Solubility Studies of Drugs: In the preliminary studies, it was found that there was considerable enhancement in the aqueous solubility of Riluzole in 5.0 M sodium benzoate, 1 M sodium acetate, 1 M sodium bicarbonate, 1M sodium chloride, 1 M sodium gluconate, 1M thiourea, 1M trisodium citrate and 1 M urea solutions. Since these solutions do not absorb above 370 nm, it was thought to use these agents' hydrotropic agents, to extract out the drugs having λ max above 348 nm, from their corresponding solid dosage forms. Recovery studies and statistical analysis were used to validate the methods. Solubility of Riluzole were determined at 35±1°C. An excess amount of drug was added to screw capped 30 ml glass vials containing different aqueous systems viz. distilled water, buffer of pH 9.4, buffer of 5.0 M Sodium benzoate. The vials were shaken mechanically for 24 hrs at 35±1° in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hrs and then centrifuged for 15 min at 3000 rpm. The supernatant of each vial was filtered through Whatmann filter paper No. 41.

Calibration Curve: In a 100 ml volumetric flask, about 50 mg Riluzole (accurately weighed) was transferred. To this flask 10 ml of 5M Sodium benzoate solution was added and drug was dissolved in it. Distilled water was used to make up the volume up to the mark to give a stock solution (1mg/ml). This stock solution was diluted suitably with distilled water to produce various standard solutions containing 50, 100, 150, 200, 250,300,350 and 400 mcg/ml of drug. Absorbances of these solutions were observed at 348 nm against corresponding reagent blank.

Vierordt's Simultaneous Equation Method [21]: Twenty tablets were weighed and powdered finely. A portion of this powder containing 50 mg Riluzole was accurately weighed and transferred to a 500 ml volumetric flask. Methanol (300 ml) was added and the suspension was heated to 40°C and shaken for 20 min. After cooling, it was diluted to 500 ml with methanol and filtered through a sintered glass funnel. The filtrate was diluted suitably with methanol to produce a solution containing 0.01% w/v of Riluzole. The absorbance of this solution was noted at 348 nm. The drug content was calculated using as the value of A [1%, 1cm]. For selection of analytical wavelength for Simultaneous Equation Method. The wavelength 348 nm was selected. The absorbencies of Riluzole were measured at 348nm. This method of analysis is based on the absorption of drugs X and Y at the wavelength maxima of the other. The quantification analysis of Riluzole mixture were performed by using Eqn-1.

$$CX = A2ay1 - A1ay2 / ax2ay1 - ax1ay2$$

Absorbance Ratio Q-Analysis Method: In absorption ratio method [21,22], absorbances of both the drugs were calculated at two selected wavelengths; among which λ_1 is the wavelength of isoabsorptive point of both drugs and λ_2 is the λ max of either drug among drugs. From the overlain spectra wavelength 309 (isoabsorption point) and 323 (λ max of Riluzole) were selected for study. The absorbencies at 309 nm and 323 nm for Riluzole were obtained. The concentration of the individual components were calculated by using the following equations; Cx = Qm-Qy/Qx-Qy)×A1 /ax1(Eqn.2), Cy = Qm-Qy/Qy-Qx)×A1 /ax 1(Eqn.3) where Qm = A_2 /A_1, A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at ?max of one of the two components, Qx = ax2 /ax1, Qy = ay2 /ay1, ax 1 and ax 2 represent absorptivities of Riluzole.

Analysis of Riluzole in Tablets by the Proposed Method: Tablet powder equivalent to 50 mg Riluzole was transferred to a 100 ml volumetric flask containing 10 ml of 5 M sodium benzoate solution. Flask was shaken for about 10 minutes to solubilize the drug present in tablet powder and volume was made up to the mark with distilled water. After filtration through sintered glass funnel, the filtrate was appropriately diluted with distilled water and absorbance was noted at 348 nm against reagent blank. Using the calibration curve, the drug content was computed. Recovery studies were performed by spiking the preanalyzed tablet powder with Riluzole bulk drug sample at three levels and determining the drug content by the proposed method. Each type of analysis was performed six times.

Table 1: Results of analysis of commercial tablet formulations

S No.	Hydrotropic Solution	TF	LC (mg/tab)	% LC estimated* (mean±S.D.)	Coeff. of variation	S.E.
1	5 M sodium benzoate	I	50	100.3±0.06	0.29	0.18
2	1 M sodium acetate	I	50	99.98±0.1	0.36	0.17
3	1 M sodium bicarbonate	I	50	100.15±0.5	0.75	0.28
4	1M sodium chloride	I	50	101.6±0.67	0.16	0.42
5	1 M sodium gluconate	II	50	99.94±0.21	0.18	0.50
6	1M thiourea	II	50	99.99±0.78	0.44	0.20
7	1M trisodium citrate	II	50	99.93±0.45	0.12	0.19
8	1 M urea	II	50	100.4±0.15	0.88	0.71

TF (I) - Tablet formulation, LC- Label claim, SE- Standard error, *Mean of three determinations, I, II

Table 2: Recovery study for spiked concentration of drugs added to the preanalyzed dosage form

S.No	Hydrotropic Solution	TF	LC (mg/tab)	Drug Added (spiked mg)	% LC estimated* (mean±S.D.)	Coeff of variation	S.E.
1	5 M sodium benzoate	I	50	40	100.04±0.21	0.11	0.25
2	1 M sodium acetate	I	50	40	99.97±0.04	0.13	0.26
3	1M sodium bicarbonate	I	50	40	100.08±0.11	0.05	0.69
4	1M sodium chloride	I	50	40	101.20±0.02	0.16	0.44
5	1 M sodium gluconate	II	50	40	100.3±0.56	0.62	0.76
6	1M thiourea	II	50	40	101.0±0.37	0.22	0.26
7	1M trisodium citrate	II	50	40	101.11±0.04	0.98	0.60
8	1 M urea	II	50	40	100.4±0.21	0.26	0.51

TF- Tablet formulation, AD- Amount of drug, LC- Label claim, SE- Standard error, *Mean of three determinations, I-

Table 3: Analysis Data of Tablet Formulations with Statistical Evaluation

Tablet Formulation	Label Claim (mg/Tablet)	%Label Claim Estimated*(Mean±S.D.)	% Coeff. of Variation	Standard Error
I	50	49.06±0.11	0.14	0.36
II	50	50.06±0.08	0.28	0.09

* Mean (n = 6)

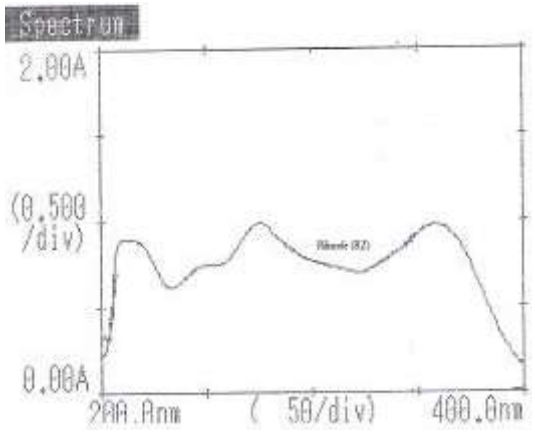

Fig. Overlain spectra of riluzole

Recovery Studies: For recovery studies, tablet powder (formulation I), equivalent to 50 mg drug was taken in a 25 ml volumetric flask. In this flask, 5 mg of pure drug (corresponding spiked drug) was transferred, 20 ml of 5.0 M sodium benzoate solution was added and the flask was shaken for about 10 min. The volume was made up to the mark with distilled water and filtered through Whatman filter paper No. 41. The solution was diluted appropriately with distilled water and analyzed for drug content. A similar procedure was repeated using 1.0 M other hydrotropic solutions, in place of 5.0 M sodium benzoate solution, in all the cases. The results of analysis of recovery studies are presented in Table 2.

Validation of the Developed Methods [23]: The developed methods for simultaneous estimation of Riluzole were validated as per ICH guidelines. Accuracy check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. Precision of the method was verified by repeatability and intermediate precision studies. Repeatability check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet

formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. Intermediate precision of the method was checked by assay the sample solution on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision) This study indicates that the solutions can be analyzed within 48-72 h without having any bad effect on chemical stability of the drug in presence of agent. The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ by using the equations 3.3s/s for LOD and 10s/s for LOQ, where s stands for standard deviation of Y-intercept and S stands for slope of the calibration curve.

RESULTS AND DISCUSSION

The primary objective of the present investigation was to employ hydrotropic solutions to extract the drugs from their dosage forms precluding the use of costlier organic solvents. Formulations I estimated by British Pharmacopoeial method (standard analytical method) were 101.05 and 99.99 respectively. The mean percent label claims estimated by proposed method for tablet formulations I were 99.86 which are very close to 100, indicating the accuracy of the method. The values of the mean percent label claims obtained in case of the proposed method are very comparable with those obtained by use of British Pharmacopoeial method. Validation of the proposed method is further confirmed by the low values of standard deviation, percent coefficient of variation and standard error.Results of solubility studies indicated that, enhancements in aqueous solubilities in 1 M sodium benzoate, 1 M sodium acetate, 1 M sodium bicarbonate, 1M sodium chloride, 1 M sodium gluconate, 1M thiourea, 1M trisodium citrate and 1 M urea solution, as compared to solubility in distilled water, were more than 50 and 112 fold in case of Riluzole study proves that increase in solubilities of these three drugs in hydrotropic solutions are not due to alteration in pH, but are due to hydrotropic phenomenon. Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for Riluzole. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods % COV were not more than 2.0% indicates good repeatability and intermediate precision. The value of LOD and LOQ were 0.4365 µg/ml, 0.2654 µg/ml Riluzole in method II respectively.This indicates that the enhancement in the aqueous solubility of Riluzole in 5.0 M hydrotropic solutions was largely due to hydrotropy. Part A solution of drug was kept at room temperature for 48 hrs. There was no precipitation of drug in Part A solutions within 48 hrs. In addition, drug contents of Part A solutions (after 48 hrs) were same as those of Part B solutions (fresh solutions). This study reveals that the estimations can be done within 48 hrs at least, without having any detrimental effect on drug stability. It is evident that there is good agreement between the amounts estimated and those claimed by the manufacturers. Percent label claims are very close to 100, with low values of standard deviation, % coefficient of variation and standard error. Accuracy, reproducibility and precision of the proposed methods, were further confirmed by percent recovery values, which were close to 100 with low values of standard deviation, % coefficient of variation and standard error (Table 2).The mean percent recovery values ranged from 100.03 to 101.05 and were very close to 100. Also, the values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error were significantly low. Thus, the proposed method of analysis was very well validated. It may be concluded that the proposed method of analysis, using urea as the hydrotropic solubilizing agent is new, simple, cost-effective, environmentally friendly, safe, accurate and reproducible. Urea and the commonly used tablet excipients did not interfere in Spectrophotometric estimation at 348 nm. Decided advantage is that organic solvent (methanol) is precluded but not at the expense of accuracy. The proposed method is worth adopting in pharmacopoeia.

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