Development of a Validated Stability-Indicating HPTLC Method for Rufinamide in Bulk and Its Pharmaceutical Dosage Form

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A sensitive, selective, precise and stability indicating high-performance thin layer chromatographic method for the analysis of rufinamide (RF) in bulk drug and its formulations was developed and validated. The method employed thin layer chromatography aluminum plates precoated with silica gel 60 F254 as the stationary phase. The solvent system consisted of chloroform : methanol : glacial acetic acid (9 : 1 : 0.1 v/v/v). This system was found to give compact spots for RF (RF value of 0.68 ± 0.02). RF was subjected to acid and alkali hydrolysis, oxidation, photodegradation and dry heat treatment. Also, the degraded products were well separated from the pure drug. Densitometric analysis of RF was carried out in the absorbance mode at 210 nm, which is wavelength maxima for the degradant. The linear regression data for the calibration plots showed a good linear relationship with an $r^2$ of 0.9989 in the concentration range of 1,000–3,500 ng. The method was validated for precision, accuracy, ruggedness and recovery. The limits of detection and quantitation were 196.59 and 595.74 ng spot$^{-1}$, respectively. The drug undergoes degradation under acidic and basic conditions. All the peaks of degraded products were resolved from the standard drug with significantly different RF values.

Introduction

Rufinamide (RF), [1-{(2,6-difluorophenyl)methyl}-IH-1,2,3-triazole-4 carboxamide], is an antiepileptic drug approved for the treatment of partial seizures associated with Lennox–Gastaut syndrome in adults and children (up to 4 years) and older. Lennox–Gastaut syndrome consists of a variety of treatment-resistant seizures and is most common among pediatric patients (1–4). In the literature, various spectrophotometric methods and HPLC methods have been reported for quantification of RF in its available market formulations in the presence of impurities and degradation products (5–10).

No article related to the stability-indicating high-performance thin layer chromatographic (HPTLC) determination of RF in pharmaceutical dosage forms has been reported. The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drug substances and products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. A very viable alternative for stability indicating the analysis of RF is HPTLC. The advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. The focus on the present study was to develop an accurate, specific, reproducible and stability, indicating a method for the determination of low levels of RF in the presence of its degradation products, bulk drug and stability of its dosage forms. The proposed method was validated as per the ICH guidelines.

Experimental

Materials

RF was supplied by Torrent Research Centre, Ahmedabad, India. All chemicals and reagents used were of analytical grade and were purchased from SD Fine Chemicals, India. Solvents like chloroform (purity ≥99.8%), methanol (purity ≥99%), glacial acetic acid (purity ≥97.5%) were utilized during analysis. Market sample used during the study was BANZEL 10–35 (200 mg rufinamide tablets) purchased from local pharmacy.

CAUTION: Use of chlorinated organic solvents can cause cancer. Due precautions should be taken for their handling, storage and disposal.

HPTLC instrumentation

The samples were spotted in the form of bands of width 3 mm with a Camag microlitre syringe on a precoated silica gel aluminium plate 60 F254 (E. Merck, Germany) using a Camag Linomat IV (Switzerland). The mobile phase consisted of chloroform : methanol : glacial acetic acid (9 : 1 : 0.1 v/v/v). The plates were prewashed by methanol and activated at 60°C for 5 min prior to chromatography. Samples were applied as bands 3 mm long, at 5 mm intervals under a stream of nitrogen. The slit dimensions were 3 × 0.1 mm. Linear ascending chromatogram development to a distance of 8 cm was performed in twin trough TLC developing chamber (Camag) at room temperature and previously saturated for 30 min with a mobile phase. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on the Camag TLC scanner III in the absorbance mode at 210 nm. The wavelength of 210 nm was selected as it was found to be wavelength maximum for the determination of degradation product. The source of radiation utilized was deuterium lamp.

Calibration curves

A stock standard solution of RF (1 mg mL$^{-1}$) was prepared in methanol. Aliquot (1 mL) was transferred in a 10-mL volumetric flask and was diluted with methanol (100 µg mL$^{-1}$) to prepare working standard solution. From the working standard solution, 10–35 µL were spotted on the TLC plate to obtain concentrations 1,000–3,500 ng of RF, respectively. The data of peak area

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versus drug concentration were treated by linear least square regression analysis and were selected as working range for the assay and recovery. Linearity was also determined over the range of 1,000–3,500 ng mL\(^{-1}\).

**Method validation**

**Linearity**
The linearity of response for Rf was assessed in the range of 1,000–3,500 ng spot\(^{-1}\) for standard drug.

**Precision**
Method precision. The intraday precision of the proposed method was determined by estimating the corresponding responses three times on the same day for three different concentrations of Rf (1,000, 2,000 and 3,500 ng spot\(^{-1}\)) for the method. The results are reported in terms of relative standard deviation (RSD).

Intermediate precision. The interday precision of the proposed method was determined by estimating the corresponding responses on three different days over a period of 1 week for three different concentrations of Rf (1,000, 2,000 and 3,500 ng spot\(^{-1}\)) for the method. The results are reported in terms of RSD.

**Accuracy**
Accuracy of the developed method was accessed by the standard addition technique to preanalyzed sample of market formulation with three concentrations of drugs corresponding to 80, 100 and 120% and determining the recovery of added drug. At each level of the amount, three determinations were performed.

**Limits of detection and quantification**
The limits of detection (LOD) and quantitation (LOQ) of the drugs were calculated using the following equations as per the ICH guidelines.

\[
\text{LOD} = 3.3 \times \sigma/S, \\
\text{LOQ} = 10 \times \sigma/S.
\]

where \(\sigma\) is the standard deviation of the response, and \(S\) is the standard deviation of \(y\)-intercept of regression lines.

**Specificity**
The specificity of the method was ascertained by analyzing standard drug and sample. The spot for Rf in the sample was confirmed by comparing the Rf and spectra of the spot with those of standard. The peak purity of the sample was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot.

**Assay of marketed formulation**
To determine the content of Rf in conventional tablets (label claim: 200 mg per tablet), 10 tablets were powdered and powder equivalent to 200 mg of Rf was weighed. The extraction solvent employed was methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 100 mL. The resulting solution was filtered to obtain final concentration of 2,000 \(\mu\)g mL\(^{-1}\). Aliquot quantity (1 mL) was further transferred in a 10-mL volumetric flask and was diluted with methanol up to the mark. The resultant solution (10 \(\mu\)L) was spotted onto the plate followed by development and scanning. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

**Accelerated degradation of Rf**

**Acid- and base-induced degradation (hydrolysis)**
Accurately weighed Rf (100 mg) was dissolved in 100 mL of methanol. The drug was subjected to accelerated degradation under acidic and basic conditions by refluxing with 1 N HCl and 1 N NaOH, respectively, at 80 °C for a period of 3 h. The accelerated degradation in acidic and basic media was performed in the dark in order to exclude the possible effects of light on the drug. The resultant solutions were appropriately diluted, and chromatograms were run after spotting. The hydrolyzed products were resolved from pure drug using HPTLC.

**Hydrogen peroxide-induced degradation (oxidation)**
Accurately weighed 100 mg of drug was dissolved in 100 mL of methanol. Subsequently, 10 mL of hydrogen peroxide (3% v/v) was added and the solution was heated in boiling water bath for 1 h till the removal of excess hydrogen peroxide. The solution was spotted on TLC plates, and plates were developed and analyzed.

**Photochemical degradation**
The photochemical stability of the drug was studied by exposing the drug solution to direct sunlight for 48 h.

**Dry heat degradation**
Rf (10 mg) was stored at 100 °C for 12 h under dry heat condition. After 12 h, the drug sample was diluted in methanol and spotted on TLC plate, and plates were developed and analyzed.

**Results**

**HPTLC method development**
The TLC procedure was optimized with a view to develop a stability-indicating assay method. Both the pure drug and degraded products were spotted on the TLC plates and run in different solvent systems. The solvent system consisted of chloroform: methanol: glacial acetic acid (9:1:0.1 v/v/v) gave a good resolution, sharp and symmetrical peak. This system was found to give compact spots for Rf (Rf value of 0.68 ± 0.02). It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and presaturation of TLC chamber with a mobile phase for 30 min ensure good reproducibility and peak shape of Rf (Figure 1).

**Validation**
Using the optimized chromatographic conditions, the proposed method was validated in terms of linearity, LOD, LOQ, precision, accuracy and specificity.
Linearity
The linear regression data for the calibration curves ($n = 3$) showed a good linear relationship over the concentration range of 1,000–3,500 ng ($r^2 = 0.998$).

Precision
Method precision and intermediate precision. Percentage RSD of intra- and interday studies were found to be in the range as shown for the HPTLC method (Table I). The low % RSD values of intra- and interday variations reveal that the proposed method is precise.

Accuracy (% recovery)
The recovery experiments were carried out by the standard addition method. The mean percent recovery obtained was 98.92 ± 0.54 (Table II). The low value of % RSD indicates that the method is accurate.

LOD and LOQ
The LOD and the LOQ for Rf were calculated as in the text. LOD and LOQ for Rf were found to be 196.59 and 595.74 ng spot$^{-1}$.

Table I
Summary of Validation Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>1,000–3,500 ng spot$^{-1}$</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.9989</td>
</tr>
<tr>
<td>LOD (ng spot$^{-1}$)</td>
<td>196.59</td>
</tr>
<tr>
<td>LOQ (ng spot$^{-1}$)</td>
<td>595.74</td>
</tr>
<tr>
<td>Accuracy ($n = 3$)</td>
<td>98.92 ± 0.54</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td></td>
</tr>
<tr>
<td>Intraday ($n = 3$)</td>
<td>0.57–0.79</td>
</tr>
<tr>
<td>Interday ($n = 3$)</td>
<td>0.46–1.43</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
</tbody>
</table>

Table II
Recovery Studies

<table>
<thead>
<tr>
<th>Amount of standard added (in ng)</th>
<th>Amount recovered (ng)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>982.5</td>
<td>98.25</td>
<td>1.05</td>
</tr>
<tr>
<td>500</td>
<td>1474.1</td>
<td>99.43</td>
<td>0.86</td>
</tr>
<tr>
<td>1,000</td>
<td>1967.8</td>
<td>99.26</td>
<td>0.88</td>
</tr>
<tr>
<td>1,500</td>
<td>2451.3</td>
<td>98.74</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Figure 1. Chromatogram of standard Rf (0.68 ± 0.02). Mobile phase consisted of chloroform : methanol : glacial acetic acid (9 : 1 : 0.1 v/v/v).

Figure 2. Chromatogram for acid-induced degradation of Rf.

Figure 3. Chromatogram for base-induced degradation of Rf.
respectively. These data show that the method is sensitive for the determination of Rf.

Specificity
The peak purity of Rf was assessed by comparing its respective spectra at the peak start, apex and peak end positions of the spot, i.e., \( r(S, M) = 0.9996 \) and \( r(M, E) = 0.9991 \) for Rf. A good correlation was also obtained between the standard and sample (test formulation) spectra of Rf (0.9996).

Stability in sample solution
Solutions of two different concentrations (1,000 and 2,000 ng) were prepared from sample solution and stored at room temperature for 2, 4, 6, 10, 12, 24 and 48 h. They were then applied on the plate, after development the chromatogram was evaluated for additional spots if any. There was no indication of compound instability in the sample solution.

Assay of marketed formulation
A single spot at Rf of 0.68 was observed in the chromatogram of the drug samples extracted from conventional tablets. The drug content was found to be 98.15 with a % RSD of 1.41.

Accelerated degradation

Acid- and base-induced degradation
The chromatograms of the acid (Figure 2) and base (Figure 3) degraded samples for Rf showed an additional peak at a Rf value of 0.23. The concentration of the drug was found to be changing from the initial concentration, indicating that Rf undergoes degradation under both the conditions. The rate of alkaline degradation was higher when compared with that of acidic condition.

Hydrogen peroxide-induced degradation
The sample did not show any other peak except that of standard Rf. The result indicates that Rf does not degrade under oxidative stress condition (Figure 4).

Photochemical degradation product
The photodegraded sample showed no additional peak. No significant degradation was observed in standard that was left in day light for 48 h (Figure 5).
Dry heat degradation
The dry heat sample showed no additional peak. No significant degradation was observed in standard even after exposure at 100°C for 12 h (Figure 6).

Isolation and characterization of degradation product
TLC analysis of acid and base hydrolyzed accelerated degradation suggested that the degradation product resolved in both the cases is same which was further authenticated by mass, IR and UV analysis. To isolate the degradation product, 1 g of RF was dissolved in 100 mL of methanol and 250 mL of 1 N NaOH was added to it and the resultant solution was refluxed for 6 h. Complete hydrolysis was confirmed by TLC, and the resultant solution was extracted with chloroform (ethyl-acetate and other non-halogenated organic solvents were tried for extraction, but showed lower recovery values and poor solubility for degradant). Chloroform was evaporated to dryness and the residue was crystallized using chloroform : methanol (9 : 1). The degradation product was characterized using spectrometric analysis. Mass spectra showed the molecular ion peak (m/z) at 240 for the degradant (Figure 7), while for standard RF was at 239 (Figure 8). The doublet in IR of standard RF (Figure 9) close to 3,178 cm⁻¹ characteristic of –NH₂ group was found missing in case of IR spectra of degradation product (Figure 10). The NMR spectra for degradant further confirmed its structure (Figure 11). Based on spectral data, the

![Figure 7. Mass spectrum of degradation product of RF.](image1)

![Figure 8. Mass spectrum of standard RF.](image2)
Figure 9. IR spectrum for standard Rf.

Figure 10. IR spectrum for degradation product of Rf.
The structure was identified as 2-(2,6-diflorobenzyl)-2H-1,2,3-triazole-4-carboxylic acid. The structure was supported by appropriate mechanistic explanation (Figure 12).

Discussions

The mobile phase selected for analysis provided optimum resolution and polarity for separation of degradation products from the drug. The method was utilized for the estimation of available tablet dosage form. There was no interference from the excipients commonly present in the conventional tablets. It may, therefore, be inferred that degradation of Rf had not occurred in the marketed formulation that was analyzed by this method. The low % RSD value indicated the suitability of this method for routine analysis of Rf in pharmaceutical dosage forms. Rf was found to be stable in oxidation, photolytic and thermal conditions, while it is susceptible to acid and base hydrolysis. The acid and base degradation products were found to be same. The degradation product was isolated and characterized using various analytical techniques. The same method could be utilized for dissolution testing as well as for studying pharmacokinetic parameters of drugs in biological samples.

Conclusion

The developed HPTLC technique is precise, specific, accurate and stability indicating one. Statistical analysis proves that the method is reproducible and selective for the analysis of Rf in bulk drug and its pharmaceutical formulations. It may be extended to study the degradation kinetics of Rf. As the method could effectively separate the drugs from their degradation products, it can be employed as a stability indicating one.

References

of Pharmaceutical, Biological and Chemical Sciences, (2011); 2: 855–865.


