Original article

Validated liquid chromatographic method for quantitative determination of Rufinamide active pharmaceutical ingredient form and its impurities

Vikas Parashar, Mangesh Todkar, Fahad Shaikh, Swamy Reddy, Vinny Mehra, Sukumar Sinha[*]

Analytical Research Department, Glenmark Generics Limited, Glenmark Research Centre, Navi Mumbai, Maharashtra 400709, India

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ABSTRACT

Aim: A new reversed-phase liquid chromatography method has been developed for the determination of Rufinamide in active pharmaceutical ingredient form.
Methods: The chromatographic column used is Inertsil ODS 3V, GL Sciences Inc. C18, dimensions (250 mm × 4.6 mm, 5 mm), on Waters 2487 HPLC system. The mobile phase is used in a gradient programme where mobile phase A is 0.1% o-phosphoric acidin water and mobile phase B is a premixed solution of methanol, acetonitrile and tetrahydrofuran in the ratio of 900:70:30 v/v/v. The flow rate applied for the method is 1.0 ml/min and detection wavelength employed is 220 nm. The retention time of Rufinamide API Main peak was found to be 21.3 min. The linearity has been tested for impurities and API over concentration range of Limit of quantitation (LOQ), 0.75 mg/ml to 2.25 mg/ml for Impurity A and LOQ, 0.25 mg/ml to 0.75 mg/ml for Impurity B and LOQ, 0.125 mg/ml to 0.375 mg/ml for both Rufinamide and Impurity C, and the Resulting correlation coefficient were found to be greater than 0.99. The percentage recoveries were found to lie within 80%–120% for LOQ level and 90%–110% for other levels. The method has been validated in accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.
Conclusion: The proposed validated method has been applied for the quantitative analysis of Rufinamide in API form and its impurities, which will help to improve quality Control.

1. Introduction

Rufinamide[1] is a broad-spectrum anticonvulsant approved in the E.U. in 2007 as adjunctive therapy for the oral treatment (tablets) of seizures associated with Lennox–Gastaut Syndrome (LGS) in children 4 years and older and partial-onset seizures in adult and adolescent patients and for the treatment of epilepsy.[2] Molecular Mechanism: this antiepileptic triazole derivative decreases firing by neurons at sodium channels.[3,4] The drug has received orphan drug designation in the U.S., the E.U. and Japan for the treatment of LGS. The Monograph of Rufinamide API, in USP36,[5] details out Related compound-A and Related compound-B. On analysis in the related substances method[6] as per USP36 by HPLC, in-house impurity C, a degradation[7] impurity is eluted in void volume. This impurity C, 1-(2,6-difluorobenzyl)-1*H*-

1,2,3-triazole-4-carboxilic acid is formed as a base degradant and thus cannot be ignored. It has been proven to be formed (to the extent of 7.48% by area normalization) by Base-degradation study carried out by treating the sample with 5 N NaOH and keeping it undisturbed for 12 h without heating and then analyzing the sample. In the proposed Method, both the reported impurities in USP36 Related compound-A and Related compound-B elute well within the gradient programme used. Besides that, Impurity C is also well separated which shall not be possible in the USP Method of analysis (Fig. 1).

2. Materials and methods

Pharmaceutical grade Rufinamide WS, certified to contain 99.7% qualified against USP Standard, HPLC grade Methanol and Acetonitrile, tetrahydrofuran and orthophosphoric acid were purchased from Merck Specialties Chemicals Private Limited (Mumbai, India). HPLC grade water was sourced from Milli Q water purification system, make: TKA Germany.

* Corresponding author. Glenmark Generics Limited, Glenmark Research Centre, Plot No. A-607, T.T.C Industrial Area, MIDC, Mahape, Navi Mumbai 400709, India. Tel.: +91 2267720000, +91 09920290143; fax: +91 2227782525.
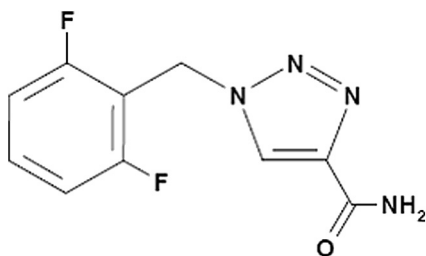E-mail address: sukumars@glenmark-generics.com (S. Sinha).

Fig. 1. Structure of Rufinamide API.

2.1. Instruments

The HPLC system consisted of a Waters 2487 Liquid Chromatograph and used Inertsil ODS 3V, GL Sciences Inc. C18 column (250 mm × 4.6 mm, 5 μm). The system was equipped with a dual wavelength UV-detector and an autosampler. An Elma Sonic S300 H ultrasonic processor model was used for sonication and degassing of the mobile phases. In addition, an electronic balance (Sartorius CPA 225D), a pH meter (Lab India) were used in this study. Shimadzu LC Liquid Chromatograph LC2010A HT with dual wavelength absorbance was used for intermediate precision.

2.2. Chromatographic conditions

Chromatographic separation was achieved on Inertsil ODS 3V, GL Sciences Inc. C18 column (250 mm × 4.6 mm, 5 μm) with UV detection at 220 nm. Aqueous phase was prepared by dissolving 1 ml of ortho phosphoric acid in 1000 ml of water. Organic phase is a mixture of solvents: Methanol, Acetonitrile (of HPLC-grade) and tetrahydrofuran (of GR-grade) in a ratio of 900:70:30 v/v/v respectively. Both the mobile phases are carefully filtered using a vacuum assembly through a 0.45 Nylon membrane filter. The mobile phases are then sonicated and degassed for at least 10 min before being used for analysis. The flow rate applied for the method is 1 mL/min employing a gradient programme mentioned below in Table 1. The column temperature used is 30 °C and the injection volume is 50 μL.

2.3. Preparation of stock solution

Stock Solution A: Accurately weigh and transfer 30.0 mg of Impurity A, 10.0 mg of Impurity B standard and 5 mg of each Impurity C standard and Rufinamide reference standard into 100 ml volumetric flask. Add 50 ml of diluent and sonicate to dissolve. Make up to the mark with diluent and mix.

Stock Solution B: Pipette out 5.0 ml of stock solution A in 100 ml volumetric flask. Dilute and make up to the mark with diluent and mix.

Stock Solution B has been used to prepare aliquots at several concentrations covering the range of linearity for impurities and API.

2.4. Calibration curve (linearity)

Dilutions of stock solution B were done to prepare linearity solutions covering a range of over LOQ, 50%–150% of the

specification limit, which turns out to be 0.75 μg/ml–2.25 μg/ml for Impurity A, 0.25 μg/ml–0.75 μg/ml for Impurity B, 0.125 μg/ml–0.375 μg/ml for Impurity C and Rufinamide. These solutions are prepared and each concentration is injected on the same day. The data generated is analyzed by linear regression analysis to calculate slope, intercept and correlation coefficient.

2.5. Method validation

The method of analysis was validated as per the recommendations of ICH[8] and USP for the parameters like detection limit, quantitation, precision, linearity, accuracy and robustness.

2.5.1. Specificity and selectivity
Selectivity is the ability to measure quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix. It ensures that there is no interference from diluent and/or degradation products and/or impurities or the analyte with each other.

2.5.2. Linearity
Linearity is a measure of the method's ability to obtain results, which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range. The range for linearity study is generally selected on the type of experiment.

2.5.3. Accuracy and precision
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

2.5.4. Limit of detection (LOD)
The limit of detection is the lowest concentration of analyte in a sample that can be detected but not necessary quantified as an exact value.

$$LOD = 3.3 \times \sigma/S$$

where σ and S are standard deviation of response and slope of the calibration line, respectively.

2.5.5. Limit of quantitation (LOQ)
The lowest concentration or amount of analyte in a sample that can be determined quantitatively with an acceptable level of repeatability precision and accuracy.

$$LOQ = 10.0 \times \sigma/S$$

2.5.6. Ruggedness
An investigation of intermediate precision allows to test the ability of the method when subjected to small changes in the environment and/or operating conditions. Typical variations to be studied include days, analysts, equipments etc.

2.5.7. Robustness
A measure of the capacity of the analytical procedure to remain unaffected by small but deliberate variations in method –

Table 1
Gradient programme.

Time (minutes)	% mobile phase A	% mobile phase B
0.01	75	25
30	65	35
65	35	65
75	35	65
80	75	25
90	75	25

Table 2
Specificity.

Selectivity	Retention time
Impurity A	21.20 min
Impurity B	35.52 min
Impurity C	24.23 min
Rufinamide	20.03 min

performance parameters, which provides an indication of its reliability during normal usage.

3. Results and discussion

3.1. Specificity

The specificity of the method has been investigated by subjecting the control sample of Rufinamide to solid state and liquid state forced degradation. Also, the specified impurities were injected individually as well spiked in the API Sample. From the experimental data [Table 2], there are no interfering peaks at retention time of Rufinamide from the chromatogram [Figs. 2 and 3 in Appendix] thereby confirming that neither the specified impurities, nor the degradation products have interfered with the main peak.

3.2. Solution stability

The stability of solution has been checked by injecting the control sample at several intervals upto 72 h and none of the specified impurities A, B, C have been found to increase with respect to the initial level. It has also been checked that there is no significant change in the level of unspecified impurities.

3.3. Limit of detection

The limit of detection is determined from the linearity experiment wherein a low concentration of each of the impurity A, B, C and Rufinamide is analyzed. The LOD concentration for Impurity C and Rufinamide is found to be 0.003% of test concentration and for Impurity A and B, the LOD is found to be 0.01%w/w of test concentration.

3.4. Limit of quantitation

The limit of quantitation is determined from the linearity experiment wherein a low concentration of each of the impurity A, B, C and API Rufinamide is analyzed. The LOQ concentration for Impurity C and Rufinamide is found to be 0.01% of test concentration and for Impurity A and B, the LOQ is found to be 0.02%w/w of test concentration.

3.5. Precision

3.5.1. System precision
The relative standard deviation for six replicate injections of reference solution (a) is found to be 3.04% for Rufinamide.

3.5.2. Method precision
The method precision is performed by estimating the % content of impurities in three control sample and six spiked samples. The relative standard deviation for known impurities, impurity A, impurity B and impurity C, any other unknown individual impurity and total impurities from six spiked samples was calculated and was found to be well within the desired limits.

3.5.3. Intermediate precision/ruggedness
The experimental approach employed for checking the ruggedness of the method is by analysis of nine test preparations of the same lot of Rufinamide API viz., three control sample and six spiked samples by a different analyst, using a different lot of the column, with same dimensions and brand, on a different HPLC instrument on a different day. The mean and percent RSD values for % impurity content were calculated for each set of six sample solutions (spiked samples) and percent cumulative RSD for all twelve sample preparations (six for method precision and six for intermediate precision) and was found to be well within the desired limits.

3.6. Linearity

For establishing the linearity for Rufinamide, Impurity A, Impurity B and Impurity C, a series of standard solutions of Rufinamide, Impurity A, Impurity B and Impurity C were prepared to cover a range of 50%–150% of the specified limits. The specification limit for Bosentan is 0.05% of the test concentration, i.e., 0.25 ppm, the

Table 3
Linearity of related substances.

Sr. No	Conc. (%)	Rufinamide		Impurity A		Impurity B		Impurity C	
		Area	Mean	Area	Mean	Area	Mean	Area	Mean
1	LOQ	8339	8564	9133	8902	10,662	9911	10,516	10,881
		7786		9144		10,836		10,596	
		7540		7798		9582		9761	
		7399		8574		9219		11,292	
		8772		9553		9868		12,495	
		8525		9208		9301		10,627	
2	50	20,406	20,292	148,214	148,095	25,696	28,768	19,804	19,468
		20,263		147,619		30,264		18,721	
		20,206		148,453		30,345		19,879	
3	75	31,186	30,919	203,090	203,236	47,300	48,230	30,308	30,031
		30,219		202,617		48,908		30,130	
		31,352		204,000		48,481		29,656	
4	100	40,346	40,532	251,458	251,611	66,353	65,943	41,832	41,038
		40,446		250,486		65,844		40,748	
		40,803		252,890		65,633		40,533	
5	125	49,053	49,551	301,082	301,278	85,352	85,554	49,836	49,643
		50,501		301,691		85,334		49,800	
		49,098		301,061		85,975		49,292	
6	150	59,171	59,337	368,132	371,574	99,081	99,436	59,004	59,389
		59,839		367,951		99,796		59,269	
		59,000		378,638		99,432		59,893	

specification limit of Impurity A is 0.30% of the test concentration, i.e., 1.5 ppm, the specification limit of Impurity B is 0.10% of the test concentration, i.e., 0.5 ppm, the specification limit of Impurity C is 0.05% of the test concentration, i.e., 0.25 ppm. The data generated is analyzed by linear regression analysis to calculate the slope, intercept and the correlation coefficient [Table 3]. Linearity graphs are plotted [Figs. 4–7 in Appendix]. The method follows linear range over LOQ, 0.75 µg/ml to 2.25 µg/ml (i.e. 50%–150%) for Impurity A, LOQ, 0.25 µg/ml to 0.75 µg/ml (i.e. 50%–150%) for Impurity B and LOQ, 0.125 µg/ml to 0.375 µg/ml (i.e. 50%–150%) for Impurity C & Rufinamide with a correlation coefficient greater than 0.99.

3.7. Accuracy

The accuracy of the method is assessed by using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure) i.e., by spiking the known impurities LOQ, 50%, 100% and 150% w/w of the specified limits. The recovery of Impurity A, Impurity B & Impurity C is within the prescribed range of 80–120% for LOQ level & 90%–110% for other levels.

3.8. Robustness

The evaluation of robustness shows the reliability of analysis with respect to deliberate variations in method parameters. Changes in the column temperature, flow rate, detection wavelength and buffer concentration were made. As a consequence of the evaluation of robustness, a series of system suitability parameters (e.g., resolution test) have been established which ensure that the validity of the analytical procedure is maintained whenever used.

4. Conclusion

From the data of the validation studies performed, it is confidently concluded that the proposed analytical procedure is precise, accurate and robust.

Conflicts of interest

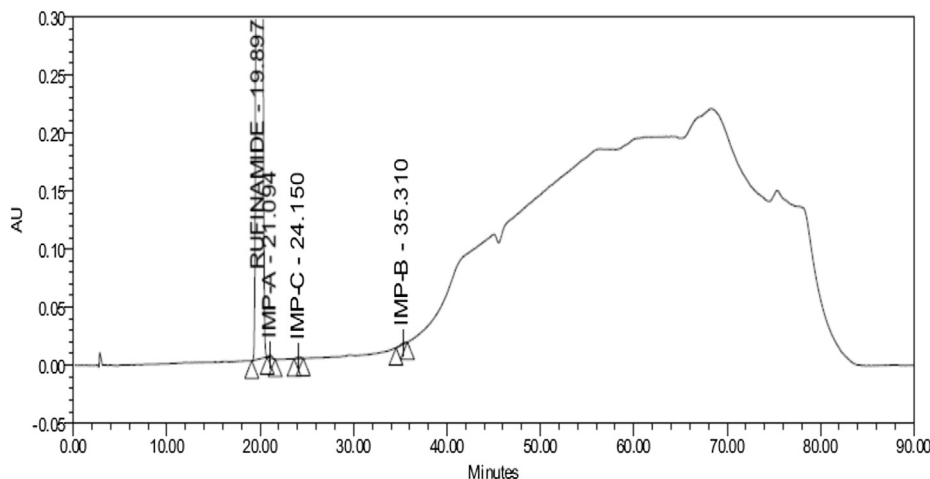All authors have none to declare.

Acknowledgment

Appendix

Fig. 2. Typical chromatogram of Rufinamide.
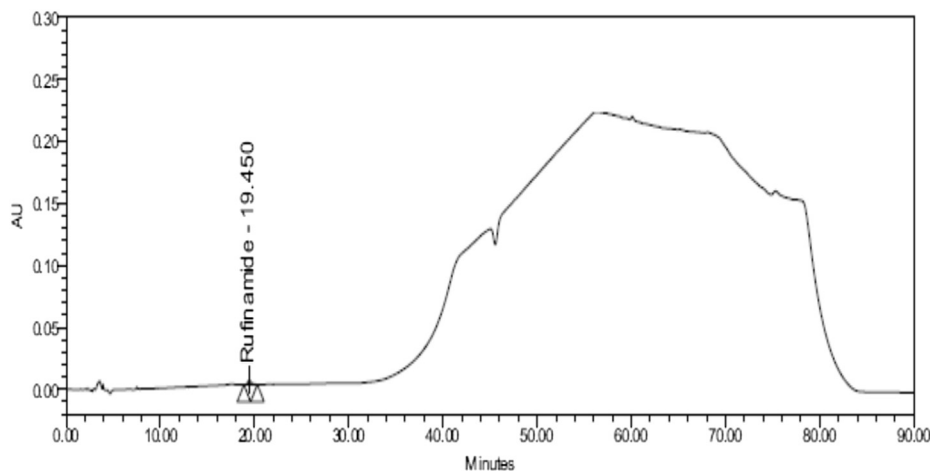
Fig. 3. Typical chromatogram of a reference solution.
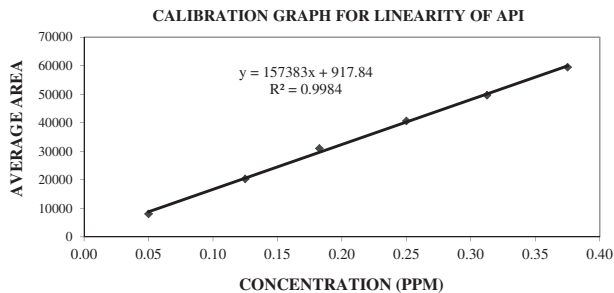
CALIBRATION GRAPH FOR LINEARITY OF API

$y = 157383x + 917.84$
$R^2 = 0.9984$

Fig. 4. Linearity of API.

CALIBRATION GRAPH FOR LINEARITY OF IMPURITY B

$y = 141773x - 4901.6$
$R^2 = 0.9982$

Fig. 6. Linearity of Impurity B.

CALIBRATION GRAPH FOR LINEARITY OF IMPURITY A

$y = 163556x + 9326$
$R^2 = 0.9894$

Fig. 5. Linearity of Impurity A.

CALIBRATION GRAPH FOR LINEARITY OF IMPURITY C

$y = 153152x + 2195.8$
$R^2 = 0.9971$
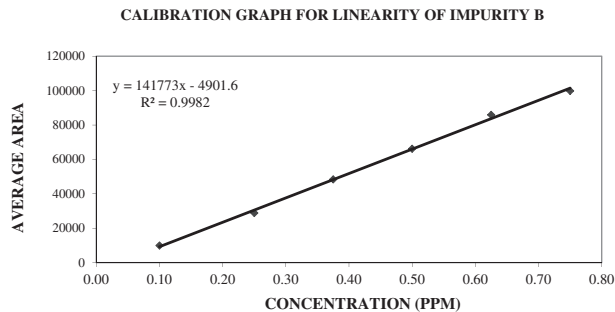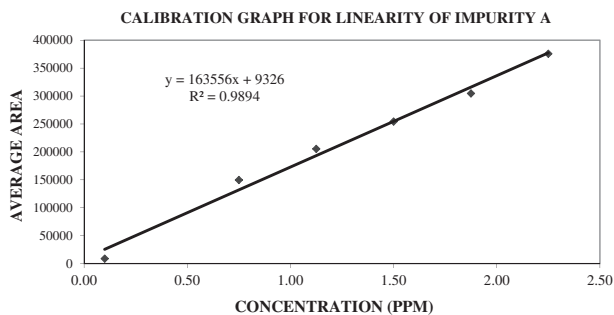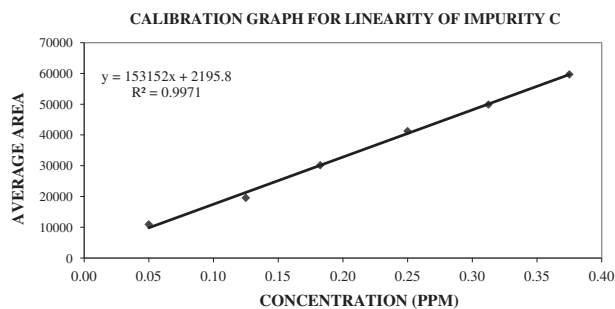
Fig. 7. Linearity of Impurity C.

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Abbreviations

API: active pharmaceutical ingredient
N: normality
w/w: weight by weight
μ: micron
HPLC: high performance liquid chromatography
RS Method: related substances method
ppm: parts per million
v/v: volume/volume
mL/min: milliliter per minute
nm: nanometer
μL: microliter
RSD: relative standard deviation
LOD: limit of detection
LOQ: limit of quantitation
mg/mL: milligram per milliliter
ICH: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use