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A rapid stability indicating LC method for zonisamide using RR-LC

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ABSTRACT

A simple, sensitive isocratic RR-LC method has been developed for the quantitative determination of Zonisamide Related compounds in bulk drug, used as Anticonvulsant. The developed method is also applicable for the Assay determination. Efficient chromatographic separation was achieved on a C18 stationary phase with simple mobile phase combination delivered in a isocratic mode and quantification was carried out using ultraviolet detection at 240 nm at a flow rate of 1.5 mL min^{-1}. In the developed RRLC method the resolution between Zonisamide and its two potential impurities was found to be greater than 4.0. Regression analysis shows an r value (correlation coefficient) of greater than 0.999 for Zonisamide and it's all the two impurities. This method was capable to detect all two impurities of Zonisamide at a level of 0.01 % with respect to test concentration of 1.0 mg mL^{-1} for a 5µL injection volume. The inter and intraday precision values for all two impurities and for Zonisamide was found to be within 2.0 % RSD at its specification level. The method has shown good and consistent recoveries for Zonisamide two impurities (98.2-102.3%). The test solution was found to be stable in diluent for 48 h. The drug was subjected to stress conditions of exposure to acid hydrolysis and base hydrolysis, oxidataion, Humidity, Photolysis and thermal degradation. Considerable degradation was found to occur in acid hydrolysis stress conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.9 %. The developed RR-LC method was validated with respect to linearity, accuracy, precision and robustness.

KEYWORDS

Column liquid chromatography;
Zonisamide;
Forced degradation;
Validation.

INTRODUCTION

Zonisamide 1,2-benzisoxazole-3-methane sulfonamide (Figure 1) is an anticonvulsant. The generic name of Zonisamide is Zonegran, it is prescribed for the treatment certain types of seizures (partial seizures) in patients with epilepsy. It is used in along with other medicines.

Few analytical methods were reported in literature for the quantification of Zonisamide in human plasma [1-2]. One method[3] was reported for stability-indicating LC method for determination of related compounds and for quantitative estimation of Zonisamide.with one impurity and on traditional LC . In this paper we described validation of fast Liquid Chromatograph related compounds method for accurate quantification of Zonisamide two impurities in bulk drug samples along with method validation as per ICH norms. Intensive

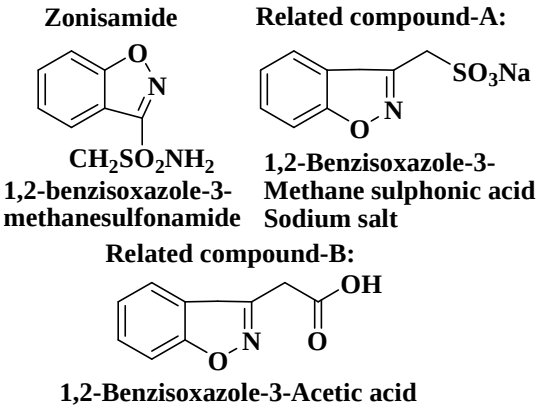

Figure 1: Chemical Structures and labels of Zonisamide and its impurities

stress studies were carried out on Zonisamide accordingly a stability-indicating method was developed, which could separate various degradation products.

The present drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH)[4] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to separation of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Accordingly, the aim of present study was to establish inherent stability of Zonisamide through stress studies under a variety of ICH recommended test conditions and to develop a stability-indicating assay method.

EXPERIMENTAL

Chemicals

Samples of Zonisamide and its related impurities were received from Hetero Laboratories Ltd, Hyderabad, India (Figure 1). HPLC grade acetonitrile and was purchased from Merck, Darmstadt, Germany. Analytical reagent grade Mono basic ammonium phosphate and sodium hydroxide were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurities used in this study were of greater than 99.8% purity.

Equipment

The LC system, used for method development, forced degradation studies and method validation was Agilent 1200 RRLC. The output signal was monitored and processed using Chemistation software on Pentium computer (Digital equipment Co).

Chromatographic conditions

The chromatographic column used was XDB-C18, 50X4.6 with 1.8μm particles. The mobile phase contains a mixture of buffer and Solvent mixture in the ratio of 77:23 (v/v). Buffer consists of 2.8g of Mono basic ammonium phosphate dissolve in 1000 mL of water and adjusted to pH 3.0 using ortho phosphoric acid solution, solvent mixture consists Acetonitrile and methanol in the ratio 1:1.

The flow rate of the mobile phase was 1.5mL min^1. The column temperature was maintained at 45^0C and the detection was monitored at a wavelength of 240 nm. The injection volume was 5μL. Mobile phase was used as diluent.

Preparation of solutions

Preparation of standard solutions

A stock solution of Zonisamide (1.00mg mL^{-1}) was prepared by dissolving appropriate amount in the diluent. Working solutions of 1000 and 100μg mL^{-1} were prepared from above stock solution for related compounds determination and assay determination, respectively. A stock solution of impurities (mixture of related compound-A and B) at a concentration of 1.0 mg mL^{-1} was also prepared in diluent.

Analytical method validation

The developed chromatographic method was validated for selectivity, linearity, range, precision, accuracy, sensitivity, robustness and system suitability[5-12].

Selectivity

Selectivity of the developed method was assessed by performing forced degradation studies[5-12]. The terms selectivity and specificity are often used interchangeably. Selectivity is the ability of the method to measure the analyte response in the presence of its potential impurities. According to ICH[4] stress testing of the drug substance can help the intrinsic stability of the molecule

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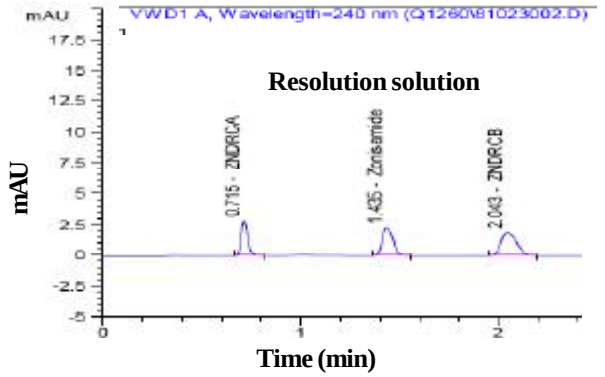

Figure 2: Typical chromatogram of Zonisamide spiked with Impurities

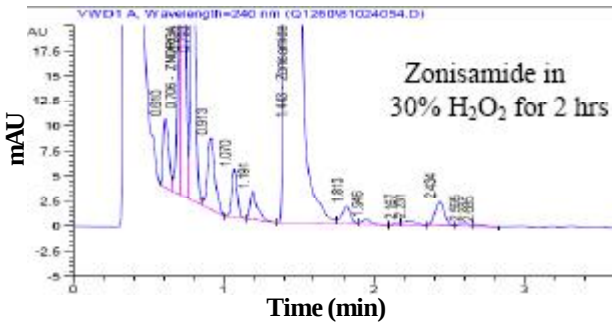

Figure 3: Typical chromatograms of stressed Zonisamide samples

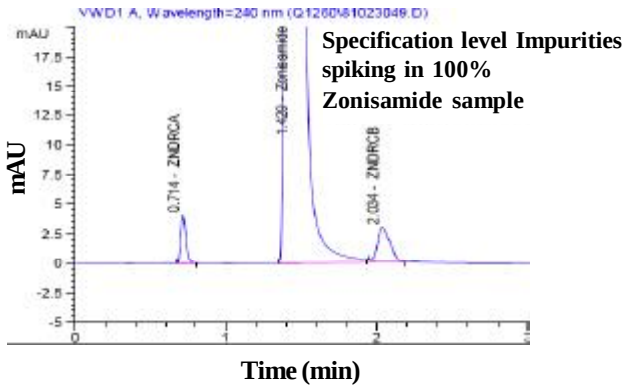

Figure 4: Specification level Impurities spiking in 100% Zonisamide sample

and validate the stability indicating power of the analytical procedure used. Photo stability testing should be an integral part of stress testing. The standard conditions for photo stability testing are described in ICH Q1B[6]. The specificity of the developed LC method for Zonisamide was determined in the presence of its related compounds namely ZNDRCA, ZNDRCB and degradation products. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (100^0C), acid hydrolysis (1N HCl). For heat and light studies, study period was 48 hrs where as for acid and oxidation it was 48 hrs.

Assay studies were carried out for stress samples against qualified reference standard and the mass balance (% assay + % of impurities + % of degradation products) was calculated. Assay was also calculated for bulk samples by spiking all two impurities (related compound-A and B) at the specification level(i.e. 0.10% of analyte concentration which is 1000µg mL^{-1}).

Analytical method validation

Precision

Precision was determined through repeatability (intra-day) and intermediate (inter-day) precision. The precision of the related compounds method was checked by injecting six individual preparations of (1000µg mL^{-1}) Zonisamide Anhydrous. The % RSD for percentage of each impurity was calculated.

Linearity and range

To establish linearity of the method, calibration solutions were prepared from stock solution at six concentration levels for chromatographic purity method-concentration levels ranging from LOQ to 150% (with respect to test concentration of 1000µg mL^{-1}, LOQ, 50, 80,100,120 and 150%) were prepared by diluting the impurity stock solution to the required concentrations. Average peak area at each concentration level was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses. The % y-intercept for chromatographic purity method was calculated. Analytical range of the method was established from the analysis of sensitivity curves. Upper and lower levels of range were also established.

Sensitivity

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantification (LOQ) Related compound-A and B estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a

series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of related compound-A and B and calculated the % RSD for the areas of each impurity.

Accuracy

For determination of accuracy, recovery study was carried out by spiking analysis. A known amount of the impurity stock solutions were spiked to the previously analysed samples at LOQ,50,100 and 150% of the analyte concentration (1000μg mL^{-1}).

The percentage of recoveries related compound-A and B were calculated. Each concentration level was prepared for three times.

Robustness

Robustness study was conducted by making small but deliberate changes in the optimized method parameters. Critical sources of variability in operating procedure such as percent organic strength, buffer strength, temperature of the column were identified. By deliberate change in experimental conditions the resolution between Zonisamide, related compound-A and B was evaluated. The flow rate of the mobile phase was 1.5 mL min^{-1}. To study the effect of flow rate on the resolution, 0.2 units changed i.e. 1.3 and 1.7 mL min^{-1}. The effect of column temperature on resolution was studied at 45^0C and 45^0C instead of 40^0C. In the all above varied conditions, the components of the mobile phase were held constant.

Solution stability and mobile phase stability

The solution stability of Zonisamide in the assay method was carried out by leaving the test solutions of sample in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed 6 h interval up to the study period against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions 6 h interval up to 48 h. Prepared mobile phase was kept constant during the study period.

The solution stability of Zonisamide and its impurities in the related compounds method was carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content of related compound-A and B were determined every 6 h interval up to the study period. Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Content of related compound-A and B was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

Method development and optimization

All the impurities and Zonisamide solutions were prepared in diluent at a concentration of 100 ppm and scanned in UV-visible spectrometer; all the 2 impurities and Zonisamide were having UV maxima at around 240 nm. Hence detection at 240nm was selected for method development purpose.

The reported method for the determination of Zonisamide, is on Traditonal Liquid chromatograph with one impurity. keeping these disadvantages in view, a rapid resolution LC method was developed with in 5 min for the quantification of Zonisamide and its related compounds.

The primary target of this work was to develop a stability indicating chromatographic method for the determination of Zonisamide and its impurities Imp-A and Imp-B. To get separation of Zonisamide from its impurities, and degradation products chromatographic method was developed using different stationary phases like C18, C8 and Cyano; different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-8) and using organic modifiers like acetonitrile and methanol in the mobile phase.

The chromatographic separation was achieved on XDB-C18, 50X4.6 with 1.8μm particles). To decrease the interactions of Zonisamide with stationary phase column (due to hydrophobicity) mobile phase was selected with higher percentage of acetonitrile. Different ratios were tried to optimize the retention time of Zonisamide and resolution between the impurities. Satisfactory results (retention time of Zonisamide is ~1.435 min and the resolution between all the impurities is >4) were obtained with optimized conditions

In the optimized conditions Zonisamide anhydrous, related compound-A and B were well separated with a resolution of greater than 4 and the typical retention

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TABLE 1: System suitability report

Compound	USP Resolution (R_S)	USP tailing factor	No of theoretical plates USP tangent method (N)
Related compound-A	--	1.287	2187
Zonisamide	8.694	1.237	3014
Related compound-B	4.805	1.279	3022

TABLE 2: Batch analysis (As per new developed method)

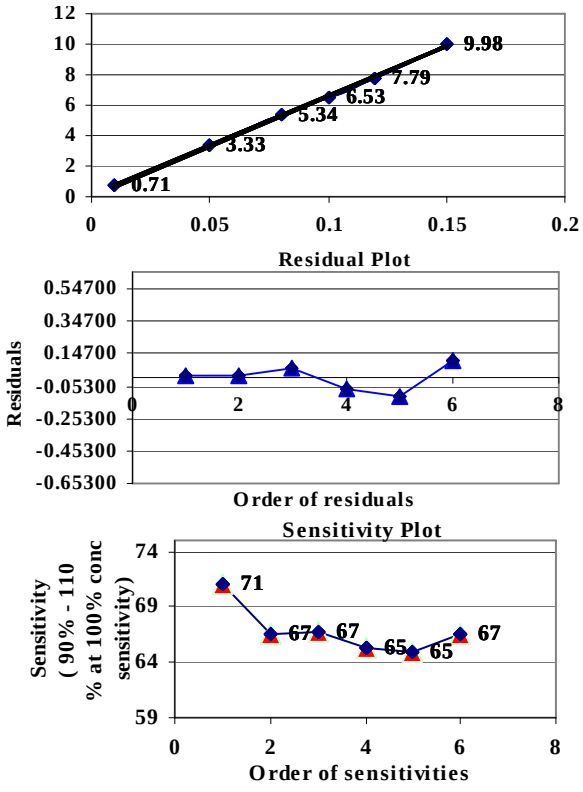
Batch no.:	Related compound-A	Related compound-B	Maximum single unknown impurity	Total Impurities	Assay by HPLC
ZA017	ND	ND	0.01	0.02	99.9
ZA016	ND	ND	0.01	0.01	99.8
ZA015	ND	ND	0.01	0.02	99.7

Where ND = Not Detected

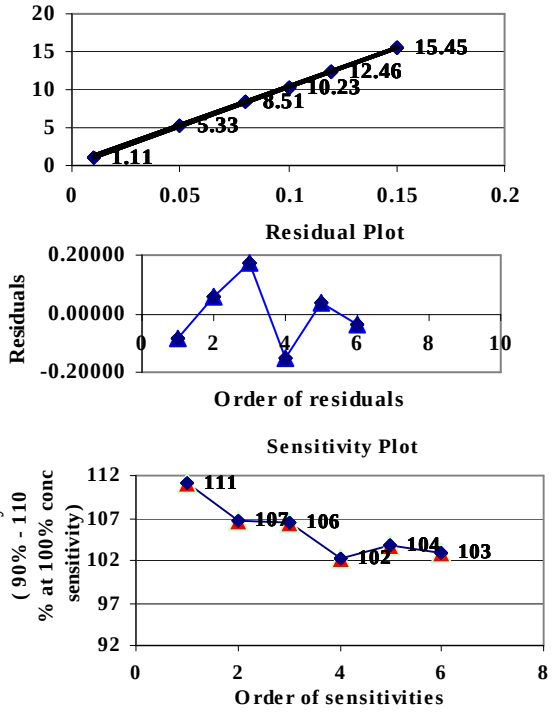
TABLE 3: Linearity results for related compounds estimation

	Related compound-A	Related compound-B	Zonisamide
Trend line equation	Y=65.52722x +0.045	Y=102.09296x +0.171	Y=88.6919x +0.074
Range	0.01-0.10%	0.01-0.10%	0.01-0.10%
Regression coefficient	0.99970	0.99975	0.99971
Slope	65.52722	102.09296	88.6919
Intercept	0.045	0.171	0.074
%Intercept	0.68	1.67	0.82
Residual sum of Squares	0.0328	0.0660	0.0548

Linearity graphs for Related compound-A



Linearity graphs for related compound-B



times of related compound-A and B and Zonisamide were about 0.715, 1.435 and 2.043 min respectively. The system suitability results were given in TABLE 1.

Analysis was performed for different batches of bulk drug samples (n=3) Results were given in TABLE 2.

Method validation

Precision

The %RSD of area of related compound-A and B in precision study were within 2.0 %. Confirming the good precision of the developed analytical method.

Sensitivity

The limit of detection of related compound-A and B was 0.003 and 0.003% (of analyte concentration, i.e.1000 µg mL^{-1}) respectively for 5 µL injection volume. The limit of quantification of related compound-A and B was 0.01 and 0.01% (of analyte concentration, i.e.1000 µg mL^{-1}) respectively for 5 µL injection volume. The % RSD for area of related compound-A and B were below 2.0% for precision at LOQ level.

Linearity

Calibration curve obtained by the least square re-

TABLE 4: Results of accuracy study for related compounds

Added (µg/mL) (n= 3)	% Recovery of related compound-A	% Recovery of related compound-B
0.1	102.30	99.3
0.5	101.6	98.7
1.0	101.3	98.9
1.5	98.2	98.6

n =3, Number of determinations

TABLE 5: Results of robustness study

S. no.	Parameter	Variation	Resolution (R_s) between Zonisamide and ZNDRCB
1	Temperature(± 5^{0}C of set temperature)	(a) At 40°C	4.966
		(b) At 50°C	4.199
2	Flow rate(± 10% of the set flow)	(a) At 1.3mL min^{-1}	4.579
		(b) At 1.7mL min^{-1}	4.380

TABLE 6: Summary of forced degradation results

Stress condition	Time	% Assay of active substance	Mass balance (%Assay + % impurities + % Degradation products)
Oxidation (30% H_2O_2 at 100°C)	2hrs	96.3	99.5
Acid hydrolysis(1N HCl at 80° for 2hrs)	2hrs	99.4	99.4
Base hydrolysis(1N NaOH at 80° for 2hrs)	2hrs	99.5	99.6
Humidity(100%RH)	48 hrs	99.6	99.6
Thermal (100°C)	48hrs	99.5	99.5
Light (photolytic degradation)	1200K LUX	99.2	99.3

gression analysis between average peak area and concentration showed linear relationship with a regression coefficient of 0.999 over the calibration ranges tested.

The results of linearity and range obtained for the two potential impurities were tabulated in the TABLE 3. Linear calibration plot for related compounds method was obtained over the calibration ranges tested, i.e. LOQ to 0.15% for related compound-A and B. The correlation coefficient obtained was greater than 0.999 for all two impurities.

Accuracy

The percentage recovery of related compound-A and B in bulk drug samples ranged from 98.2 to 102.3. HPLC chromatogram of spiked sample with all two impurities in Zonisamide bulk drug sample is shown in figure.

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate and column temperature) revealed that the resolution between closely eluting impurities, namely Zonisamide and related compound-B was greater than 4.0, illustrating the robustness of the method (TABLE 5).

Solution stability and mobile phase stability

The %RSD of assay of Zonisamide during solution stability and mobile phase stability experiments was within 1.0. No significant changes were observed in the content of related compound-A and B during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related substance determination were stable up to the study period of 48 h.

Results of forced degradation studies

Degradation behavior

Stress studies on Zonisamide under different stress conditions suggested the following degradation behavior.

Degradation in acidic solution

The drug was stable to the effect of 1 N HCl. When the drug was exposed to 1 N HCl at 100^{0}C temperature for 2 h, no degradation was observed.

Degradation in basic solution

The drug was stable to the effect of 1 N NaOH. When the drug was exposed to 1 N NaOH at 100^{0}C temperature for 2 h, no degradation was observed.

Degradation in oxidative condition

Zonisamide is sensitive to oxidative condition and was degraded into unknown impurities by oxidation in 30% H_2O_2. The drug was exposed to 30% H_2O_2 at 100^{0}C temperature for 2 h. Zonisamide has shown significant sensitivity towards oxidative treatment . The drug gradually undergone degradation with time and degraded into unknown (~3.2%).

Photolytic conditions

The drug was stable to the effect of photolysis. When the drug powder was exposed to light for an overall

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illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200-watt hours/square meter (w/mhr) (in photo stability chamber), no degradation was observed.

Thermal degradation

The drug was stable to the effect of temperature. When the drug powder exposed to dry heat at 100^0C for 2 days, no degradation was observed.

CONCLUSION

The isocratic RR-LC method developed for quantitative and related compounds determination of Zonisamide in bulk drug is precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Zonisamide samples.

ACKNOWLEDGMENTS

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