RESEARCH ARTICLE

Stability Indicating Related Substances Method Development and Validation of Eplerenone and Torsemide Using Reverse Phase High Performance Liquid Chromatography

Challa S. Reddy*, Bubathula T. Rao

Department of Chemistry, Vikas PG College, Vissannapeta, Andhra Pradesh, India

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ABSTRACT

A new simple related substances method was developed and separated its degradation products of Eplerenone and Torsemide using asymmetry C_{18} column with a flow of 1 mL/min on reverse phase high performance liquid chromatography (RP-HPLC). Using the movable phase of acetonitrile 54 and 0.1% Orth phosphoric acid in gradient mode, Eplerenone, Torsemide drugs, and their impurities were separated. The photo diode array detector was monitored at 261 nm. According to the ICH guidelines, the parameters like system precision, linearity, accuracy, method precision, ruggedness, robustness, stability, Limit of Detection (LOD), Limit of Quantification (LOQ), and the degradation studies were validated and found to be acceptable.

Keywords: Eplerenone, reverse phase high performance liquid chromatography (RP-HPLC), RS-Method, Torsemide, Validation.

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INTRODUCTION

Eplerenone, sold under the name inspires maybe a steroidal[1] antimineralocorticoid[2] of the spirolactone group that is used as an adjunct to managing chronic coronary failure[3] and high vital sign[4,5] particularly for patients with resistant hypertension thanks to elevated aldosterone.[6,7] Classed as a selective aldosterone receptor antagonist (SARA),[8] is almost like the diuretic spironolactone.[9,10] Though it is far more selective for the mineral corticoid receptor as compared (i.e., does not possess any anti-androgen,[11] progestogens, glucocorticoid[12] or estrogenic effects) and is specifically marketed for reducing cardiovascular risk[13] in patients following myocardial infarct.[14,15] Eplerenone may be potassium sparing diuretic,[16] which helps the body get obviate water but still keep potassium.

Torasemide, also referred to as torsemide, maybe a medication will not to treat fluid overload thanks to coronary failure, renal disorder[17] and disease,[18] and high vital sign. It is a less preferred treatment for a top vital signs. It's taken orally or by injection into a vein. Common side effects include headache, increased urination, diarrhea,[19] cough,[20] and dizziness.[21] Other side effects may include deafness and low blood potassium.[22] Torasemide may be a sulphonamide and loop diuretic.[23] Its use is not recommended in pregnancy or breastfeeding. It works by decreasing the reabsorption of sodium by the kidneys.

EXPERIMENTAL

Chemicals and Materials

Acetonitrile (HPLC grade), Water (Milli Q), Roth phosphoric acid were purchased from Merck (India) Ltd, Worli, Mumbai, India. All API's of Eplerenone and Torsemide as reference standards were procured from Zydus Cadila, Ahmedabad, India.

Equipment

Waters Alliance e2695 model of HPLC with PDA (photo diode array) detector and the chromatographic software Empower 2.0 was used to separate Eplerenone, Torsemide, and their impurities.

Chromatographic Condition

A symmetry C18 (150 x 4.6 mm, 3.5 µ) column was used for chromatographic separation in an isocratic model at ambient temperature. As a mobile phase, we used a gradient of acetonitrile and 0.1 percent orthophosphoric acid in a 1 mL/min flow rate. The injection volume was 10 µL the experiment lasted 17 minutes.

Table 1: Gives the gradient programme of the method.

Time (minutes)	*Acetonitrile*	*0.1% OPA*
0	20	30
5	50	50
10	80	20
12	50	50
17	50	50

*Author for Correspondence: sudheer192020@gmail.com

Preparation of Standard and Sample Solutions

Making a Standard Solution

Working standards: 25 mg Eplerenone, 20 mg Torsemide, accurately weighed. These standards were put in a 100 mL volumetric flask with 70 mL of diluents and sonicated for 10 minutes to dissolve them. They were then diluted to the required amount with the same diluents and again brought up to the required standard. Add the diluents to 5 mL of the solution mentioned above and mix well.

The Process of Preparing a Test Stock Solution

To determine the concentrations of Eplerenone and Torsemide, place 104 mg of the sample (which contains both 25 and 20 mg) into a 100 mL volumetric flask. Add approximately 70 mL of diluents, sonicate for 30 minutes to completely dissolve the contents, and then dilute to the desired concentration with diluents. Further, Use a 0.45 µ syringe filter to clean the solution.

Prepare a Stock Solution of Impurities

Ten mg of all impurities (Eplerenone imp-1, Eplerenone-2, Torsemidi imp-A, and Torsemidi imp-B) into a volumetric flask of 1000 mL should be accurately weighed before being used. Add 900 mL of diluents, sonicate for 30 minutes to dissolve, and top off with diluents as needed.

Spiked-solution Preparation

Then add 40 mL of diluents to the volumetric flask, followed by the sample and impurity stock solutions. Sonicate the mixture for 15 minutes to dissolve the impurities, and then make the volumetric flask up to the desired concentration with the diluents.

Method Validation

System Precision

Parameters of the system's suitability were analyzed to confirm the system's overall performance. Percent RSD and USP plate count were calculated and found to be within acceptable limits.

Specificity

For an analyte test to be specific, it must distinguish between analytes that have different chemical properties (impurities, degradation products, or exciepents) and those that are present in both the test sample and reference solution. As an added precaution, the chromatograms of blank and impurity-spiked samples had been examined.

Accuracy

This tactic's test results have a high degree of accuracy because of this. The recovery studies looked at it at three different concentrations and came to the same conclusion. Minimum three injections were obtained in each level, with drug concentrations, recovery percentages, and variances all calculated.

Precision

The degree of agreement between individual test results determines the precision of the analytical method. Multiple homogeneous sampling analyses of a homogeneous sample were used to conduct the research. The repeatability, intraday, and interday variations were all considered when evaluating this method's precision. Various time intervals of an equivalent day as well as different days had been analyzed to verify this.

Linearity

An analytical method's ability to produce linear results with the analyte's concentration in a sample over a specified range is known as linearity. In order to determine the linearity range, six different series of ordinary solutions were examined. Regression equations were calculated based on the calibration curve plotted using peak area versus concentration for the quality solution. The slope, intercept, and the correlation coefficient was calculated using the smallest number of squares method.

Stress degradation

The chromatogram of forced degradation preparations should not show any interference from stress degradation peaks. The ICH Q1A stress degradation guidelines were followed when conducting the research (R2). Therefore, the resolution between peaks should be at least 1.0 to keep degradation from mixing up with the principle peak purity. Various types of stress conditions were used in forced degradation studies to achieve degradation of about 20%.

Robustness

To determine an analytical procedure's robustness, look at how well it holds up under normal use when subjected to small but deliberate changes in the method's parameters. It was found that injecting a standard solution into the HPLC system and then altering chromatographic conditions like flow rate (0.2 mL/s), and organic content in the mobile phase (10%) increased robustness. In order to calculate the separation factor, retention time, and peak asymmetry, we looked at how the parameters had changed.

RESULTS AND DISCUSSION

Validation of the Proposed Method

According to ICH guidelines, the method was validated for parameters like system precision, specificity, linearity, LoD, LoQ, accuracy, robustness, and ruggedness.

System Suitability

For a stable baseline, the HPLC system was stabilized for 60 minutes. The following table shows the results of six separate injections of standard solutions. Suitability results were shown in Table 2 and the standard chromatogram was shown in Figure 1.

Table 2: Results of system suitability

System suitability parameter	*Acceptance criteria*	*Drug name*	
		Eplerenone	*Torsemide*
%RSD	NMT 2.0	0.56	0.72
USP Tailing	NMT 2.0	1.01	1.08
USP plate count	NLT 2000	9189	21367

Specificity

The chromatograms of the blank and standard, sample, and placebo have no interaction during the entire runtime. As a result, it establishes the method's uniqueness.

Linearity

Y = 295269.43x + 237412.69 was found to be linear in the eplerenone concentration range of 2.5–37.5 µg/mL, and the regression equation was Y = 295269.43x + 0.9994. Y = 500339.87x + 1214.51 is the regression equation, and the correlation coefficient is 0.9998. Impurity-1 concentrations range from 0.1 to 1.5 µg/mL. Y = 606392.28x + 7428.07 is the regression equation, and the correlation coefficient was 0.9996. The concentration of impurity-2 ranges from 0.1 to 1.5 µg/mL.

This study found linearity between torsemide concentrations of 2–30 µg/mL and the regression equation is Y = 338370.14x + 88137.13, with an R2 value of 0.9997. Regression equation: Y = 429107.81x + 422.01; correlation coefficient: 0.9992; impurity-A concentration range: 0.1–1.5 µg/mL. Impurity-B concentration range from 0.1–1.5 µg/mL, the regression equation is Y = 478151.84x + 2368.22 and the correlation coefficient was found to be 0.9994. Calibration plots of Eplerenone, Torsemide and their impurities were shown in Figures 2-7.

Accuracy

Testing for impurities such as eplerenone and torsemide in the stock solution ensures that the impurity concentration in the sample is 0.5 percent of the test concentration, as required by the test method. Samples injected in triplicate at concentrations of 50%, 100%, and 150% of the target were analyzed. NLT 95.0 percent and NMT 105.0 percent should be the recovery rates. Results of accuracy was given in Table 3.

Method Precision

A test method's precision can be determined by injecting test preparation and then testing the results throughout the entire analytical procedure. Impurities' percent RSD was calculated, and their repeatability was assessed using at least 6 measurements. The RSD values are within the permissible range. Table 4 and 5 gives the method precision results of Eplerenone and Torsemide, sample chromatogram was shown in Figure 8.

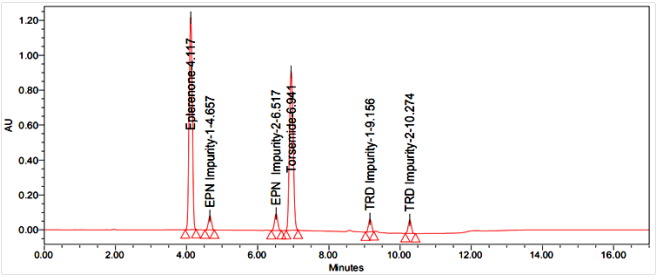

Figure 1: Chromatogram of standard

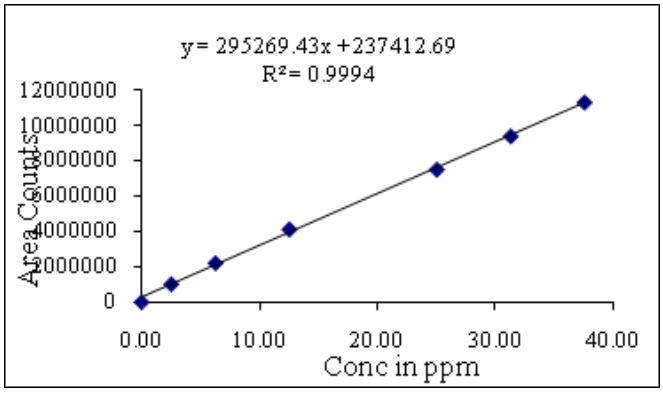

Figure 2: Linearity plot of eplerenone

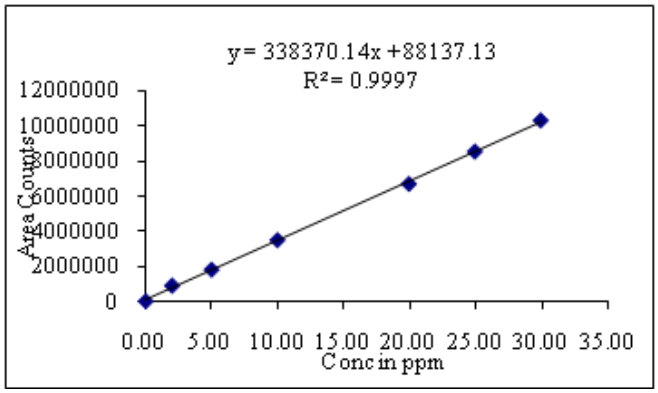

Figure 3: Linearity plot of torsemide

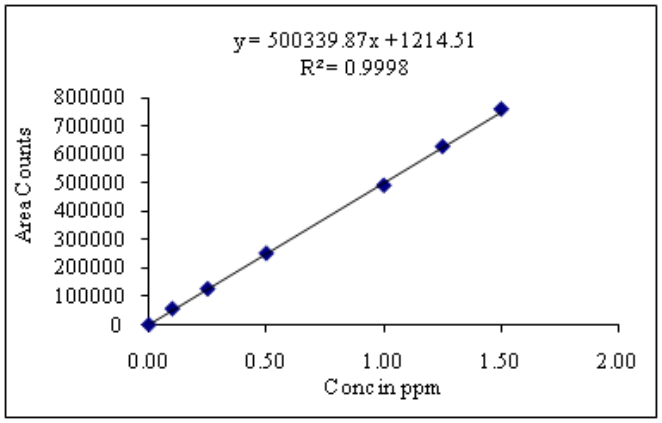

Figure 4: Linearity plot of Eplerenone imp-1

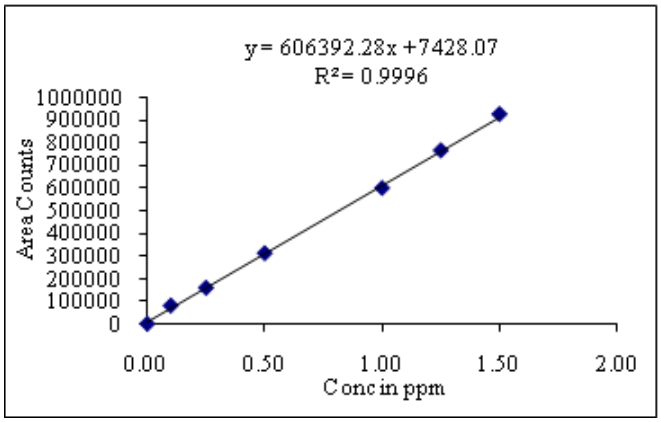

Figure 5: Linearity plot of Eplerenone imp-2

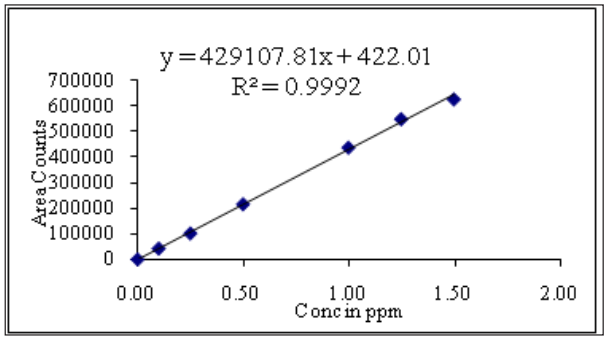

Figure 6: Linearity plot of torsemide imp-A

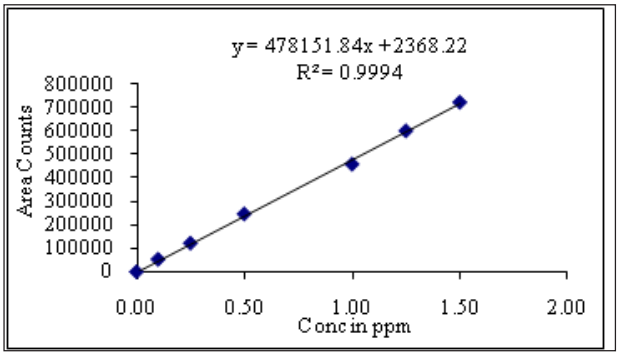

Figure 7: Linearity plot of torsemide imp-B

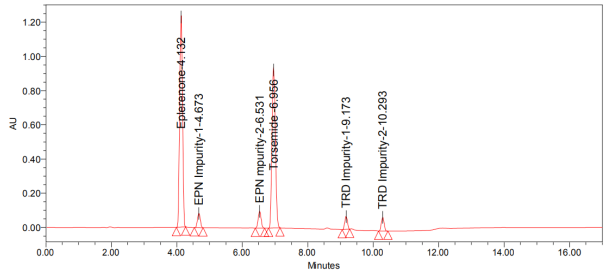

Figure 8: Chromatogram of sample

Table 3: Accuracy results

S. No.	*% Level*	*%Recovery*	
		Eplerenone	*Torsemide*
1	50	100.5	99.8
2	100	100.2	100.6
3	150	99.9	100.1

Table 4: Precision results of eplerenone

	% of related substances		
Sample No.	*Spiked impurities*	*Total impurities*	*%Purity (100-Total impurities)*
1	1.19	0.63	99.37
2	1.21	0.68	99.32
3	1.17	0.65	99.35
4	1.18	0.64	99.36
5	1.19	0.66	99.34
6	1.20	0.62	99.38
Average	1.19	0.65	99.35
% RSD	1.19	3.34	0.02

Table 5: Precision results of torsemide

	% of related substances		
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-Total impurities)*
1	1.01	0.51	99.49
2	1.01	0.55	99.45
3	1.05	0.53	99.47
4	1.03	0.52	99.48
5	1.04	0.57	99.43
6	1.02	0.54	99.46
Average	1.03	0.54	99.46
% RSD	1.59	4.03	0.02

Table 6: Intermediate precision results of eplerenone

	% of related substances		
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-Total impurities)*
1	1.23	0.69	99.31
2	1.28	0.68	99.32
3	1.29	0.66	99.34
4	1.25	0.67	99.33
5	1.27	0.65	99.35
6	1.24	0.64	99.36
Average	1.26	0.67	99.34
% RSD	1.88	2.81	0.02

Table 7: Intermediate precision results of torsemide

	% of related substances		
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-Total impurities)*
1	1.05	0.58	99.42
2	1.09	0.56	99.44
3	1.07	0.57	99.43
4	1.06	0.59	99.41
5	1.08	0.55	99.45
6	1.04	0.52	99.48
Average	1.07	0.56	99.44
% RSD	1.76	4.42	0.02

Table 8: Robustness results

	%RSD for purity	
Parameter name	*Eplerenone*	*Torsemide*
Flow (0.8 mL/min)	1.03	0.81
Flow (1.2 mL/min)	1.11	1.26
Organic solvent (-10%)	0.58	0.33
Organic solvent (+10%)	0.79	0.67

Intermediate Precision

Different days, different analysts, and different instruments analyzed six replicates of a sample solution. Based on these peak areas, mean and standard deviation percent values were calculated. The findings are summarised in the table. Intermediate precision results of Eplerenone and Torsemide were given in the Table 6 and 7.

LOD and LOQ

The calibration curve method was used to determine LOD and LOQ, separately. The compound's LOD and LOQ were determined using a newly developed RP-HPLC method that injected progressively lower concentrations of ordinary solutions. There are LOD concentrations of 0.025, 0.001, 0.001 µg/mL for Eplerenone and impurities 1 and 2 along with their s/n values of 7, 3, 3, and 0.02 µg/mL for Torsemide and its impurities A and B along with their s/n values of 6, 3, 3.

Eplerenone and its impurities 1 and 2 have LOQ concentrations of 0.25, 0.01, and 0.01 µg/mL and s/n values of 27, 24, and 24, respectively. Torsemide and its impurities A and B have LOQ concentrations of 0.2, 0.01, 0.01 µg/mL and a s/n of 26, 24, 24.

Robustness

The robustness of the tactic was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow (±0.2 mL) and organic solvent content (±10%). The alterations caused a significant change in peak area RSD (%), USP tailing factor, and retention times. Robustness results were shown in Table 8.

Stability

At room temperature, sample solution stability was used to determine the stability of Eplerenone and Torsemide initially for up to 24 hours. There is not much of a difference between the two. Results of stability were given in Table 9.

Forced Degradation

In order to partially degrade the drug, the Eplerenone and Torsemide sample was subjected to various degradation conditions. Studies on forced degradation were carried out to show that the method is suitable for degradation products of all types. The studies also give insight into the conditions when drug becomes unstable so that precautions can be taken during formulation to prevent this in the first place. Forced degradation results were shown in Table 10.

Acid Degradation

For each 1 mL of sample, add 1 mL of 1N HCl and leave for 15 minutes in a volumetric flask, followed by 15 minutes of adding 1 mL of 1N NaOH and diluents up to the mark.

Alkali Degradation

Using the 10 mL volumetric flask, add 1 mL of 1N NaOH to the sample and let it sit for 15 minutes before performing the assay. For the next 15 minutes, mix in 1 mL of 1N HCl before adding diluents to reach the desired concentration.

Peroxide Degradation

As much as 1 mL of the sample is placed into each 10 mL volumetric flask, 30 percent hydrogen peroxide is added, and the volume is diluted to the required level.

Reduction Degradation

One millilitre of the sample was transferred into a 10-milliliter volumetric flask, along with one millilitre of a 30% sodium bi sulphate solution, and the solution was diluted to the desired concentration using diluents.

Thermal Degradation

For six hours, a sample solution was baked at 105°C. It was decided to inject the final solution into an HPLC system.

Table 9: Results of stability

Stability	*% purity of Eplerenone*	*% Deviation*	*% purity of Torsemide*	*% Deviation*
Initial	99.77	0.00	99.53	0.00
6 Hrs	99.73	0.03	99.49	0.05
12 Hrs	99.69	0.07	99.43	0.11
18 Hrs	99.65	0.11	99.37	0.17
24 Hrs	99.59	0.17	99.31	0.23

Table 10: Results of forced degradation

Degradation condition	*Eplerenone*			*Torsemide*		
	% Purity	*Purity Threshold*	*Purity Angle*	*% Purity*	*Purity Threshold*	*Purity Angle*
Control	100.02	20.63	4.98	100.15	18.55	5.37
Acid degradation	75.33	24.99	5.04	75.18	20.02	4.86
Alkali degradation	74.29	23.98	5.14	74.89	18.19	4.52
Peroxide degradation	72.68	24.23	5.11	73.19	20.38	5.01
Reduction degradation	71.89	23.68	5.09	72.68	19.87	4.98
Thermal degradation	70.36	24.75	5.16	73.56	20.32	5.02
Hydrolysis degradation	72.53	25.36	4.05	72.98	21.16	4.98

Hydrolysis Degradation

1-mL of sample is placed in a 10 mL volumetric flask, to which 1 mL of water is added and the volume is diluted to the required level.

CONCLUSION

The new method worked well with Eplerenone, Torsemide, and their four impurities, taking 17 minutes to complete, was highly efficient, and met USP's modified SST specifications. As a result of the present study's use of a C18 column, the analytes were eluted better, had better resolution, and improved plate count and tailing. Eplerenone and Torsemide studies using the ICH Q 3A (R2) guidelines have shown that C18 columns can achieve high specificity in a shorter period. Eplerenone and Torsemide were determined and quantified using the proposed method, which was simple, precise, accurate, linear, robust, and fast. In line with their respective label claims, the sample recovery indicated no interference with the estimation. As a result, the technique is frequently used for routine analyses of Eplerenone and Torsemide in combined dosage form because it is simple and convenient.

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