JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation OF RP-HPLCMethod for the Simultaneous Estimation of Eplerenone and Torsemide in Pharmaceutical Dosage Form

Krupali S. Patel[1], Atul Bendale[2], Shailesh V. Luhar[3], Sachin B. Narkhede[4]

1. M.PHARM Student, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Gujarat, India.
2. Assistant Professor of Department of Pharmaceutical Analysis, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Gujarat, India.
3. Head of department of Quality Assurance. Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Gujarat, India.
4. Principal of Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Gujarat, India.

ABSTRACT:

A simple, precise, accurate, rapid RP-HPLC Method and UV Spectrophotometric Methods were developed and validated for simultaneous estimation of Eplerenone and Torsemide in pharmaceutical dosage form.RP- HPLC was carried out by using Sheisedo C18 (250 * 4.6 mm, 5µm) column and Acetonitrile: Methanol: water (30:50:20 % v/v/v) as mobile phase, at 1.0 ml/min flow rate. Detection was carried out at 268 nm. Retention time was found to be 2.53 min and 3.27min for Torsemide and Eplerenone, respectively.RP-HPLC method was found to be linear over the range of 40-240µg/ml for Torsemide and 100-600µg/ml for Eplerenone.

KEY WORDS: Eplerenone, Torsemide, RP-HPLC Method,Validation

Article history:

Received 01 April 2016
Revised 14 April 2016
Accepted 16 April 2016
Available online 20 April 2016

Citation:

Patel K. S., Bendale A., Luhar S. V., Narkhede S. B. Development and Validation OF RP-HPLCMethod for the Simultaneous Estimation of Eplerenone and Torsemide in Pharmaceutical Dosage Form. **J Pharm SciBioscientific Res**. 2016, 6(3): *283-290*

For Correspondence:
Krupali S. Patel

M.PHARM Student, Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Gujarat, India.

(www.jpsbr.org)

1. INTRODUCTION[9 – 12]

TORSEMIDE

Torsemide is a loop diuretic drug, chemically it is 3-Pyridinesulfonamide, N-[[(1 methylethyl) amino] carbonyl]-4-[(3-methylphenyl) amino]-1-Isopropyl-3-[(4-m-toluidino-3-pyridyl) sulfonyl] urea .

It is useful in the treatment of hypertension or edema associated with congestive heart failure, renal disease and hepatic disease.Recently a formulation of Torsemide has been launched in market. In this formulation, Torsemide shows a synergistic effect with other combination. Torsemide was determined by several methods for the analysis including gaschromatography (GC), liquid chromatography with UV detection (LC–UV), HPTLC derivative spectrophotometric. Torsemide was determined with or without combination of several drugs by HPLC, spectrophotometry and HPTLC but literature survey revealed that no HPLC method has been reported yet for single estimation for Torsemide. The present study was aimed to develop a simple, rapid, precise, accurate, and selective chromatographic method for estimation of Torsemide oxalate in bulk and dosage forms with the use of buffer in the mobile phase in short duration.

EPLERENONE

Eplerenone has the chemical name Pregn-4-ene-7, 21-dicarboxylic acid, 9, 11-epoxy-17-hydroxy-3-oxo, γ-lactone, methyl ester (7α, 11α, 17α). It is off-

white, crystalline powder with a molecular formula of $C_{24}H_{30}O_6$ and a molecular weight of 414.50gm/mol.

Eplerenone is an aldosterone antagonist used as an adjunct in the management of chronic heart failure. It is clinically used as antihypertensive and diuretic. Literature survey indicated that LC-MS Methods have been reported for its estimation from human plasma and urine, two spectrophotometric methods have been reported for the estimation of Eplerenone in bulk and pharmaceutical dosage forms. The present study describes simple, sensitive and economical spectrophotometric methods for the estimation of Eplerenone in tablet dosage forms.

2. MATERIALS AND METHODS

2.1 RP-HPLC Method Development

2.1.1APPARATUS AND INSTRUMENTS:

- HPLC instrument : Shimadzu LC 2010
  Detector : UV-Visible Detector
  Column : Sheisedo-C_{18} (250mm x 4.6mm, 5 µm)
  Auto Injector : Capacity Loop of 10 µL
  Software : LC Solution
- Digital pH meter
- Digital weighing balance
- Ultra sonicator
- Volumetric flask of 10, 50, 100 ml
- Beaker of 50, 100, 250, 500 ml
- Measuring cylinder of 10, 50, 100 ml
  - Pipettes of 1, 2, 10 ml Capacity

2.1.2 REAGENTS AND MATERIALS

- Eplerenone (Gift Sample by Lupin Ltd)
- Torsemide (Gift Sample by Lupin Ltd)
- Acetonitrile – HPLC Grade (Rankem Lab)
- Methanol – HPLC Grade (Rankem Lab)
- Ortho Phosphoric acid – Analytical Grade (Rankem Lab)
  - Double distilled Water

2.1.3 CHROMATOGRAPHIC CONDITION

- Stationary phase: Sheisedo C_{18} (250mm x 4.6mm, 5 µm) was used at ambient temperature.
- Mobile Phase: Acetonitrile: Methanol : Water (pH-3.4) (30:50:20 % v/v/v)
- Flow rate: 1.0 ml/min
- Injection volume: 10 µl
- Detection: At 268nm with UV Visible detector.

2.1.4 PREPARATION OF SOLUTIONS FOR RP-HPLC

Eplerenone Standard stock solution (1000 µg/ml): 100 mg of Eplerenone was weighed and transferred to a 100 ml volumetric flask and dissolved in Methanol and sonicated for about 10 min. Volume was made up to the mark with Methanol to give a solution containing 1000 µg/ml Eplerenone.

Torsemide Standard stock solution (1000 µg/ml): 100 mg of Torsemide was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in Methanol and sonicated for about 10 min. Volume was made up to the mark with Methanol to give a solution containing 1000 µg/ml Methanol.

Mixed working standard solution (Eplerenone 100 µg/ml and Torsemide 40 µg/ml): 1 ml of Eplerenone and 0.4 ml of Torsemide standard stock solutions were transferred to a 10 ml of volumetric flask and volume was made up to the mark with Methanol to give a solution containing 100 µg/ml Eplerenone and 40 µg/ml Torsemide.

Preparation of mixture of Eplerenone and Torsemide : Accurately weighed 100 mg Eplerenone and 100 mg of Torsemide were transferred to 100 ml volumetric flask. It was dissolved with sufficient Methanol and diluted up to mark with Methanol to give concentration of 1000 µg/ml of Eplerenone and 1000 µg/ml of Torsemide. 1 ml of this solution was further diluted to 10 ml with Methanol to get 100 µg/ml of Eplerenone and 40 µg/ml of Torsemide. Standard Stock solution was diluted further to get the concentration range of 100, 200, 300, 400, 500, 600 µg/ml of Eplerenone and 40, 80, 120, 160, 200, 240 µg/ml of Torsemide.

Mobile phase preparation:

Measure 300ml of Acetonitrile, 500ml of Methanol and 200ml of Double Distilled water and mix together, adjust the pH to 3.4 with orthophosphoric acid. Sonicate the mobile phase for 15min.

2.1.5 Selection of detection wavelength:

The standard solution of Eplerenone (15 µg/ml) and Torsemide(6 µg/ml)in methanol was individually

scanned over the range of 200nm-400nm. Its overlay graph showed that both the drug absorb at 268 nm. So, the wavelength selected for the determination of Eplerenone and Torsemide was 268nm.

2.1.6 Calibration Curve for Both Drugs

Calibration curves were prepared by taking appropriate aliquots (1, 2, 3, 4, 5, 6 ml) of standard Eplerenone and (0.4, 0.8, 1.2, 1.6, 2, 2.4 ml) of Torsemide stock solutions in different 10 ml volumetric flask from Standard stock solution of Eplerenone and Torsemide and diluted up to the mark with Methanol to obtain final concentrations of 100, 200, 300, 400, 500, 600 µg/ml of Eplerenone and 40, 80, 120, 160, 200, 240 µg/ml of Torsemide.

Standard solutions were injected through 10 µl loop system and chromatograms were obtained using 1.0 ml/min. flow rate. The effluent was monitored at 268 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

2.1.7 Assay of Eplerenone and Torsemide in Marketed Tablet Formulation

To determine the content of Eplerenone and Torsemide simultaneously in conventional tablet (PLANEP T 10, label claim 25 mg Eplerenone and 10 mg Torsemide); twenty tablets were accurately weighed, average weight was determined and grounded to fine powder. Tablet was taken and weight equivalent to 25 mg of Eplerenone and 10 mg of Torsemide was transferred into 100 ml volumetric flask. About 10 ml of Methanol was added and sonicated for 10 minutes. The solution was cooled to the room temperature and made up to volume with Methanol.

The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). This Test solution was injected and chromatogram was recorded for the same. The amount of drugs was calculated and the results are given.

2.2 METHOD VALIDATION[1 – 7]

2.2.1 System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter. The test includes Parameters like Number of Theoretical Plates, Resolution, Retention time and tailing factor and recorded in

2.2.2 Linearity and Range.

The linearity was determined at Three levels over the range of 100 - 600 µg/ml Eplerenone and 40 - 240 µg/ml Torsemide. Peak area of above linearity solution preparations were taken at each concentration three times. Mean Peak Area at each concentration was calculated and Graph of Mean Peak Area (y-axis) versus Concentration (x-axis) was plotted.

2.2.3 Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are Calculated.

2.2.4 Precision

A. Repeatability Study:

Standard solutions of 200, 300, 400 µg/ml Eplerenone and 80, 120, 160 µg/ml Torsemide were prepared and chromatograms were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

B. Intra-day precision

Mixed solutions containing 200, 300, 400 µg/ml Eplerenone and 80, 120, 160 µg/ml Torsemide were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Mixed solutions containing 200, 300, 400 µg/ml Eplerenone and 80, 120, 160 µg/ml Torsemide were analyzed on three different days and % R.S.D was calculated.

2.2.5 Limit of Detection and Limits of Quantitation

Limit of Detection (LOD)

From the linearity curve equation, the standard deviation

(SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3× Intercept / Slope

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

LOQ = 10 **×** Intercept / Slope

2.2.6 Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters

a) pH of mobile phase: ± 0.2
b) Flow rate : ± 0.2 ml/min
c) Change in the ratio of component in the mobile phase: ± 2%.

3. RESULTS AND DISCUSSION

3.1. Linearity:

The calibration curve showed (Fig.1 and 2) good linearity in the range of 40-240µg/ml for Torsemide and 100-600µg/ml for Eplerenone with correlation coefficient (r^2) of 0.996 for both the drugs. A typical calibration curve has the regression equation of y = 37.57x + 122.4 for Torsemide and y = 22.43x + 131.78 for Eplerenone. Results are given in Table 6 & 7.

3.2. Precision:

Intraday precision was carried out using test samples prepared and analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The low % RSD values below 2 indicate that the method is precise. The results are given in table 9 to 12.

3.3. Recovery:

At each concentration, sample was injected thrice to check repeatability and from the RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.12% to 101.5% for Torsemide and 98.96% to 100.6% for Eplerenone.

3.4. Robustness:

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (± 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in favor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Torsemide and Eplerenone. The results are given in table 14 & 15**.**

3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD was found to be10.7µg/ml and the LOQ 32.25 µg/ml for Torsemide and the LOD was found to be 19.3µg/ml and the LOQ 58.16 µg/ml for Eplerenoneestimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration.

3.6 DISCUSSION

RP-HPLC method was found to be linear over the range of 40-240 µg/ml for Torsemide and 100-600 µg/ml for Eplerenone. The method has been validated for linearity, accuracy and precision, LOD, LOQ and system suitability according to ICH guideline.

4. CONCLUSION:

A simple, economic, accurate and robust RP-HPLC method have been developed and validated for the estimation of Torsemide and Eplerenone in pharmaceutical dosage form. There was no interference from any excipients in the determination of drugs in dosage form which indicates the method is specific.

The reverse phase liquid chromatography was performed using Sheisedo-C_{18} (250mm x 4.6mm, 5 µm) column and Acetonitrile : Methanol :Water (pH-3.4) (30:50:20 % v/v/v) as mobile phase with flow rate 1 ml/min. The detection was carried out at 268nm. The retention time were found be 2.53 ± 0.01 min. and 3.27 ± 0.01 min. for Torsemide and Eplerenone, respectively. The concentration range of 40-240µg/ml with R^2 = 0.996 for Torsemide and in the concentration range of 100-600µg/ml with R^2 = 0.996 for Eplerenone. Assay of Torsemide found to 100.57 % and Eplerenone found to

100.50%.

All method validation parameters within its acceptance criteria as per ICH guideline so we can conclude that methods are simple, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Torsemide and Eplerenone in pharmaceutical dosage forms.

5. REFERENCES:

1. Analytical Procedures and Methods Validation for Drugs and Biologics, Guidance for Industry, U.S. Department of Health and Human Services, FDA, CDER, CBER, July 2015, pp 3-7

2. Chatwal G R. ,Anand S. K. , Instrumental Method of Chemical Analysis ; 5th Edn. ; Himalaya Publishing House, 2008, pp 2.149-2.184

3. Kalsi P. S. ,Spectroscopy of Organic Compounds; 6th Edn.; New Age International [P] Limited Publishers,2004, pp 9-55

5. Beckett A.H., and Stenlake J.B., Practical Pharmaceutical Chemistry; part- II, 4th Edn.; CBS publishers and distributors, New Delhi, 2004, pp 275-337

6. Dr. KastureA. V., Dr. MahadikK. R., Dr. WagodkarS.G., Dr.More H. N., A Text Book of Pharmaceutical Analysis ; 14th Edn. ; NiraliPrakashan, 2006, pp 48-57

7. ICH Harmonized TripartileGuideline (Nov. 2015), Validation of Analytical Procedures; Text and Methodology Q2[R2], International Conference on Harmonization , Geneva, Switzerland

8. The Merck Index, An Encyclopedia of Chemicals, Drugs And Biologicals; 14th Edn. ; Published by Merck Research Laboratories, 2006, pp 3625

9. Tripathi K.D. , Essential of Medical Pharmacology ; 6th Edn. ; Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2008, pp 561-562

10. Roger Walker, Cate Whittlesea, Clinical Pharmacy and Therapeutic; 4th Edn.; Churchill Livingstone, 2007, pp 265-279

11. " Drug Bank : Eplerenone " http://www.drugbank.ca/drugs/DB00700

12. " Drug Bank : Torsemide "http://www.drugbank.ca/drugs/DB00214

13. British Pharmacopoeia [BP]; The Stationery Office. London, U.K. , Volume – II , 2009 , pp 2170

14. ThulasammaP. and VenkateswarluP. , "Development and validation of RP-HPLC method for the quantitative estimation of Torsemide in Pharmaceutical dosage forms and Human Serum.'', *International Journal of ChemTech Research*, 2014, 6(2), 1103-1109

15. KhianI. J., LoyaP. and SarafM.N., "A Simplified HPLC Method for Quantification of Torsemide from Human Plasma and its Application to a Bioequivalence Study.'', *Indian Journal of Pharmaceutical Science*, 2008, 70(4) , 519-522

16. Amol. Y.G., Bhagwat N.P., and Mohini B.S., "Analytical Method Development and Validation for Quantitative Estimation of Torsemide in Bulk and Pharmaceutical Dosage Form by RP-HPLC.'' *InternationalJournal of Pharmaceutical Chemistry & Analysis*, 2014, 1(1), 6-13

17. M. Naresh CR, and Dr.Chandra K.B., "Development and Validation of Gradient RP-HPLC for Estimation of Impurities in Eplerenone Tablet Dosage Form.'' *International Research Journalof Pharmaceutical & Applied Science,* 2012, 2(3), 58-75

18. JyothirmaiB., Syama SB., Satyadev T.N.V.S.S. and Tata S, "A validated RP-HPLC method for the determination of Eplerenone in human plasma.'' *Pelagia Research Library*,2015, 6(2), 61-68

19. Paraag G, Sandeep S, and Abhishek C, "Development and validation of RP-HPLC method for estimation of Eplerenone in spiked human plasma.'' *Journal of Pharmaceutical Analysis*, 2012, 2(5), 390-393

20. Rujuta D.P. and Dr. Dilip G. Maheshwari, "RP-HPLC Method for the estimation of Torsemide and AmilorideHCl in their Combined Dosage Form.'' *Indo American Journal of Pharmaceutical Research*, 2014, 4(4), 2079-2086

21. Krutika J.B., Darshan M, and Shital F, "Analytical Method Development and Validation for Simultaneous Estimation of Amiloride and Torsemide

in their Combined Pharmaceutical Dosage Form by RP-HPLC Method.''*PharmaTutor*,2014, 2(7), 135-141

Selection of detection wavelength

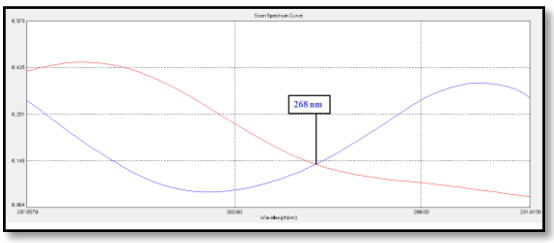

Figure 1 Selection of detection wavelength for HPLC

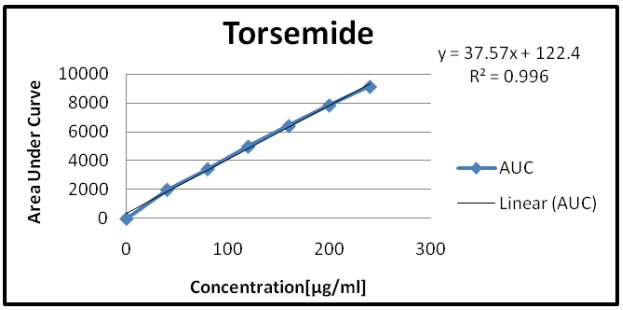

Figure 2Calibration curve of Torsemide

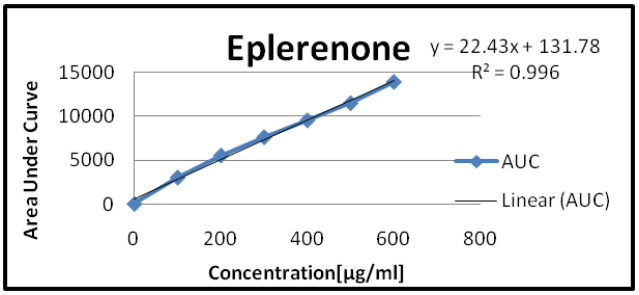

Figure 3 Calibration Curve of Eplerenone

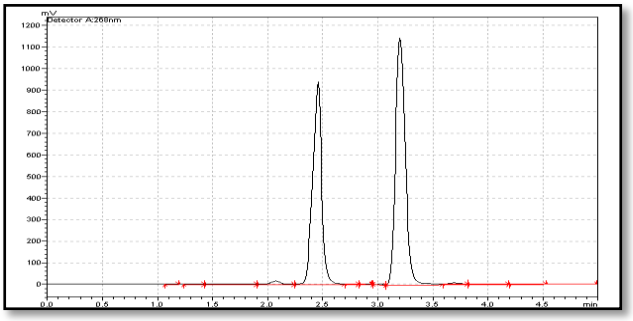

Figure 4 Chromatogram of Tablet Formulation of Eplerenone and Torsemide, Flow Rate 1ml/min, 268nm

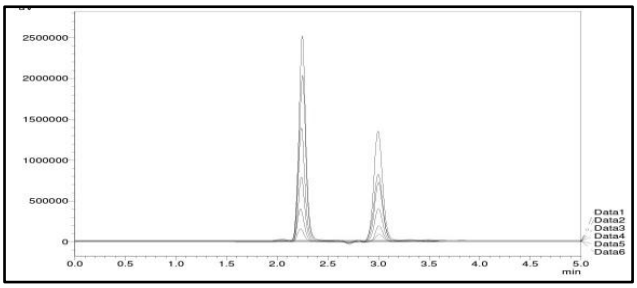

Figure 5 Chromatogram of different concentration of mixture of Eplerenone and Torsemide

Finalized Chromatographic conditions:

Table 1 Finalized Chromatographic conditions Calibration curve for both drugs

Parameters	Specifications
Column	Sheisedo C_{18} (250mm*4.6mm, 5µm)
Mobile phase	Acetonitrile : Methanol : Water (pH-3.4) (30:50:20 % v/v/v)
Flow rate	1 ml/min
Run time	10 min
Detection wavelength	268 nm
Retention time	2.53 min for Torsemide and 3.27 min for Eplerenone

Table 2 Calibration Curve for Eplerenone and Torsemide

Torsemide		Eplerenone	
Conc. (µg/ml)	**Area**	**Conc. (µg/ml)**	**Area**
0	0	0	0
40	2001.462	100	3015.982
80	3430.581	200	5531.050
120	4988.708	300	7627.029
160	6421.579	400	9547.137
200	7844.208	500	11505.233
240	9137.260	600	13945.158

Table 3 Statistical Data of Torsemide and Eplerenone Statistical Data of Torsemide and Eplerenone

Parameters	Result	
	Torsemide	**Eplerenone**
Linearity Range (µg/ml)	40 – 240 µg/ml	100 - 600 µg/ml
Slope	37.57	22.43
Intercept	122.4	131.78
Retention Time (min)	2.53	3.27
Correlation Coefficient (R^2)	0.996	0.996

Table 4 Assay of Eplerenone and Torsemide in Marketed Tablet Formulation

Drug	Actual conc. of Drug (µg/ml)	Con. of Drug Found (µg/ml)	% of Drug found	Avg. of % Drug found	SD	%RSD
Eplerenone	100	99.88	99.88	100.5033	0.693277	0.006898
	100	101.25	101.25			
	100	100.38	100.38			
Torsemide	40	40.79	101.97	100.5733	1.211872	0.01205
	40	39.92	99.8			
	40	39.98	99.95			

Table 5 System Suitability Test Parameters for Torsemide and Eplerenone

Sr. No.	System suitability parameter	Torsemide	Eplerenone	Specification as per IP 2010 and USP 34 NF 29
1	Retention time (min)	2.53	3.27	-
2	Resolution (R)	2.570		>1.5
3	Theoretical plate number (N)	3870.953	4882.381	Not less than 2000
4	Tailing factor (T)	1.077	1.044	Not greater than 2.0

Table 6 Linearity for Torsemide

Conc. (µg/ml)	Area. Mean ± S.D	% RSD
0	0	0
40	2001.895 ± 3.083	0.1540
80	3429.633 ± 3.627	0.1057
120	4988.543 ± 2.135	0.0428
160	6421.877 ± 1.867	0.0290
200	7844.475 ± 1.871	0.0238
240	9137.058 ± 2.957	0.0323

Linearity for Eplerenone

Table 7 Linearity for Eplerenone Accuracy

Conc. (µg/ml)	Area. Mean ± S.D	% RSD
0	0	0
100	3015.156 ± 3.015	0.1000
200	5530.989 ± 2.081	0.0376
300	7627.726 ± 2.102	0.0275
400	9545.984 ± 3.418	0.0358
500	11504.87 ± 2.675	0.0232
600	13945.76 ± 3.376	0.0242

Table 8 Accuracy for Eplerenone and Torsemide

% Recovery	Target Conc., (µg/ml)	Spiked conc., (µg/ml)	Final Conc., (µg/ml)	Conc., Obtained		% of Assay	
				Torsemide	**Eplerenone**	**Torsemide**	**Eplerenone**
80%	80+200	64+160	144+360	144.42	360.42	100.30	100.11
	80+200	64+160	144+360	143.12	359.12	99.38	99.75
	80+200	64+160	144+360	145.76	361.76	101.22	100.48
100%	80+200	80+200	160+400	158.92	398.92	99.32	99.73
	80+200	80+200	160+400	161.99	401.99	101.24	100.49
	80+200	80+200	160+400	159.12	396.12	99.45	99.03
120%	80+200	96+240	176+440	174.46	435.46	99.12	98.96
	80+200	96+240	176+440	173.42	437.42	98.53	99.41
	80+200	96+240	176+440	178.67	442.67	101.51	100.60

Table 9Repetability for Torsemide

Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
80	3433.299 ± 3.607	0.1050
120	4991.876 ± 2.846	0.0570
160	6422.877 ± 3.112	0.0484

Table 10 Repetability for Eplerenone

Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
200	5535.322 ± 4.042	0.0730
300	7626.392 ± 3.609	0.0473
400	9547.650 ± 3.922	0.0410

b) Intra-day and Inter-day precision

Intra-day and Inter-day precision for Torsemide

Table 11Intra-day and Inter-day precision for Torsemide

Intra-day and Inter-day precision for Eplerenone

Concentration (µg/ml)	Intraday Peak Area ± S.D (n=3)	% RSD	Inter-day Peak Area ± S.D (n=3)	% RSD
80	3433.633 ± 5.199	0.1514	3439.633 ± 8.518	0.2476
120	4991.21 ± 6.020	0.1206	4985.876 ± 10.899	0.2186
160	6423.877 ± 6932	0.1079	6428.544 ± 9.211	0.1432

Table 12Intra-day and Inter-day precision forEplerenone

Limit of Detection and Limits of Quantitation

Results of LOD and LOQ

Concentration (µg/ml)	Intraday Peak Area ± S.D (n=3)	% RSD	Inter-day Peak Area ± S.D (n=3)	% RSD
200	5527.989 ± 6.506	0.1177	5526.656 ± 9.538	0.1725
300	7630.059 ± 7.501	0.0983	7630.393 ± 8.920	0.1169
400	9545.317 ± 6.458	0.0676	9547.651 ± 7.755	0.0812

Table 13Results of LOD and LOQ ForEplerenone and Torsemide

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Torsemide	10.7	32.25
Eplerenone	19.3	58.16

Table 14 Result of Robustness for Torsemide

Result of Robustness for Eplerenone

Sr. no.	Torsemide (120 µg/ml)					
	pH		**Flow rate**		**Mobile phase**	
	+ 0.2 units	**-0.2 units**	**+0.2 units**	**-0.2 units**	**+2 %**	**- 2 %**
1	4990.161	4976.123	4979.345	4988.456	4981.457	4991.746
2	4980.345	4987.735	4992.432	4987.274	4990.635	4978.453
3	4988.347	4989.974	4995.346	4998.563	4997.748	4981.674
Mean	4986.284	4984.610	4989.040	4991.431	4989.946	4983.957
S.D	5.222	7.435	8.522	6.204	8.167	6.934
% R.S.D	0.104	0.149	0.170	0.124	0.163	0.139

Table 15 Result of Robustness for Eplerenone

Summary of Validation Parameters for Torsemide and Eplerenone

Sr. no.	Eplerenone (300 µg/ml)					
	pH		**Flow rate**		**Mobile phase**	
	+ 0.2 units	**-0.2 units**	**+0.2 units**	**-0.2 units**	**+2 %**	**-2 %**
1	7629.862	7625.658	7627.658	7625.758	7626.209	7631.732
2	7623.758	7634.879	7632.987	7626.875	7621.743	7626.832
3	7633.635	7639.801	7629.983	7639.534	7631.633	7639.732
Mean	7629.085	7633.446	7630.209	7630.722	7626.528	7632.765
S.D	4.9841	7.1795	2.6716	7.6515	4.9527	6.5117
% R.S.D	0.0653	0.0940	0.0350	0.1002	0.0694	0.0853

Table 16Summary of Validation Parameters for Torsemide and Eplerenone

PARAMETER	Torsemide	Eplerenone
Equation (y = mx + c)	y = 37.57x + 122.4	y = 22.43x + 131.78
Correlation coefficient	0.996	0.996
LOD(µg/ml)	10.7	19.3
LOQ(µg/ml)	32.25	58.16
Repetability (%RSD)	0.0484 – 0.1050	0.0410 – 0.0730
Intraday precision (%RSD)	0.1432 – 0.2476	0.0812 – 0.1725
Inter-day precision (%RSD)	0.1079 – 0.1514	0.0676 – 0.1177
Robustness	0.104 – 0.170	0.0350 – 0.1002
% Recovery	99.12 – 101.5	98.96 – 100.6
Assay	100.57%	100.50%