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Research Article

Development and Validation of Gradient RP-HPLC for Estimation of Impurities in Eplerenone Tablet dosage form

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ABSTRACT:
A simple reverse phase gradient HPLC method used for simultaneous identification of Eplerenone impurities in market tablet dosage form. Poroshell ECC18 (50mmx4.6mmx2.7µm)column, mobile phase(gradient)A&B(70:30)it's contain potassium dihydrogen orthophosphate diluent, acetonitrile and methanol mixture diluent (90:10) to adjust the ph3.2±0.05with ortho phosphoric acid. Flow rate 1ml/min and injection volume 2µl chromatograms observed at 240nm. System suitability parameters are passed so system is suitable for analysis. Validation parameters specificity, linearity, lod, loq, precision, accuracy, robustness and also Acid and Diene impurities all are within the limit method should be validated its use full for stability studies and other pharmaceutical analysis.

Keywords: Eplerenone, RP-HPLC (Gradient), PH 3.2±0.05, Poroshell EC C18 column

INTRODUCTION

Eplerenone , a selective aldosterone blocker is chemically (Pregn-4-ene7, 21-dicarboxylic acid, 9, 11-epoxy-17- hydroxy-3-oxo, γ-lactone, methyl ester. (Fig: Eplerenone). [1-2] Eplerenone binds to the mineralocorticoid receptor and blocks the binding of aldosterone, a component of rennin-angiotensin-aldosterone system [3-4].

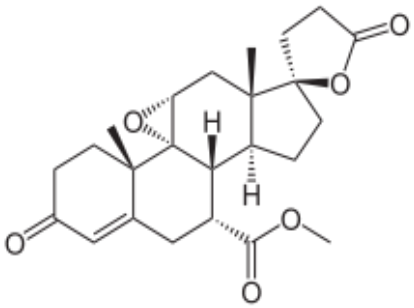


Fig: Eplerenone

This drug is not official in any Pharmacopoeia. So far a few methods have been reported for its estimation of Eplerenone from different pharmaceutical bulk and dosage forms, human plasma and urine. It has been reported for the determination of eplerenone in biological fluids by LC-MS 5. In tablet formulation by UV-Spectroscopy[6], TLC/Densitometry [7] and RP-HPLC [8].

However, to our knowledge, no information related to the RP-HPLC method for estimation of impurities in eplerenone tablet dosage forms has ever been mentioned in literature. Therefore the aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of impurities in eplerenone tablet dosage form 25 mg and 50 mg as per ICH guidelines.[9-10]

MATERIAL AND METHODS

Instrument, Reagents and chemicals

Waters separation module e2695, detector PDA2998 with empower software 2, Eplerenone pure sample is given by DR.REDDYS, Hyderabad, . HPLC grade acetonitrile and methanol were purchased in E. Merck, Mumbai. Potassium di hydrogen ortho phosphate, Ortho phosphoric acid were purchased from Rankem (RFCL LTD, New Delhi) and HPLC grade water was used.

Chromatographic Conditions:

Poroshell EC C18 (50 mm x 4.6 mm, 2.7µm) Column to be used at ambient temperature, wave length 240 nm, flow 1 ml/min, and injection volume 2µl, run time10minutes, elution gradient, mobile phase diluents A&B (70:30).

Mobile phase – A: Weigh accurately and transfer 1.36g of potassium dihydrogen orthophosphate into a 1000 ml beaker containing 700 ml water, dissolve and add 300 ml of water and adjust to pH 3.2 ± 0.05 with dilute orthophosphoric acid and filter through 0.22 micron filter and degas.

Mobile phase – B: Mix Acetonitrile and methanol in the ratio of 90:10

Gradient Program:

Time (Min)	Mobile phase – A (%)	Mobile phase – B (%)
0	70	30
6	30	70
7	30	70
8	70	30
10	70	30

System suitability stock solution: Weigh accurately 5mg of each individual Acid impurity and Diene impurity into a 50ml volumetric flask, add 30ml of diluent and sonicate to dissolve. Make up the volume with diluent.

System suitability solution: Weigh accurately 20mg of Eplerenone working standard into 20ml volumetric flask, add 10ml diluent and sonicate to dissolve. Further add 1ml of System suitability stock solution and make up the volume with diluents and mix.

Standard preparation: Weigh accurately 25 mg of Eplerenone working standard into a 20 ml volumetric flask, add 10 ml of diluent and sonicate to dissolve. Make up to volume with diluent and mix. Further dilute 1 ml to 250 ml with diluent.

25 mg Sample Preparation: Weigh and transfer 10 tablets into 250 ml volumetric flask. Add 120 ml of diluent and sonicate for 15 min and dilute to volume with diluent and mix. Filter the solution using 0.22 micron filter.

50 mg Sample Preparation: Weigh and transfer 10 tablets into 500 ml volumetric Flask. Add 250 ml of diluent and sonicate for 15 min and dilute to volume with diluent and mix. Filter the solution using 0.22 micron filter.

25 mg Placebo Preparation: Weigh the amount of placebo present in equivalent to 10 tablets and transfer into 250ml volumetric flask, add 120ml of diluent and sonicate for 15min and dilute to volume with diluent. Further filtrate the solution through 0.22

50 mg Placebo Preparation: Weigh the amount of placebo present in equivalent to 10 tablets and transfer into 500ml volumetric flask, add 250ml of diluent and sonicate for 15min and dilute to volume with diluent. Further filtrate the solution through 0.22 filter.

Procedure:

Inject about 2µL of blank, placebo preparation, six times of standard preparation, System suitability and sample preparation into the chromatography.

System suitability Requirements:

From SST:

Resolution between Acid impurity and Eplerenone: NLT 2.0

Theoretical plates: NLT 2500

Asymmetry: NMT 2.0

From Standard solution:

RSD for standard replicates: NMT 5.0%

25 mg Calculation for Known & Unknown impurities:

$$\% \text{ of Impurity} = \frac{\text{Impurity Area}}{\text{Avg.std.Area}} \times \frac{\text{Std.Wt}}{50} \times \frac{1}{250} \times \frac{250}{10} \times \frac{1}{\text{L.A}} \times \text{Std. Potency}$$

50 mg Calculation for Known & Unknown impurities:

$$\% \text{ of Impurity} = \frac{\text{Impurity Area}}{\text{Avg.std.Area}} \times \frac{\text{Std.Wt}}{20} \times \frac{1}{250} \times \frac{500}{10} \times \frac{1}{\text{L.A}} \times \text{Std. Potency}$$

Total impurities = Sum of % known impurities + % of unknown impurities

Eplerenone concentration is as follows (in ppm):

Test	Concentration (ppm)			
	Standard		Test	
	Initial	Final	Initial	Final
25 mg Tablets	1250	5	-	1000
50 mg Tablets	1250	5	-	1000

VALIDATION PARAMETERS

System suitability Solution:
Resolution between Acid impurity and Eplerenone: NLT 2.0
Theoretical plates : NLT 2500
Asymmetry : NMT 2.0
From Standard solution:
RSD for standard replicates : NMT 5.0%

Specificity:
The peaks due to diluent, placebo and impurities shall not interfere with the analyte peak and with all-individual known impurities.
All-individual known impurities should be separated from the principal peak.
The Peak purity of principal peak and all-individual impurities shall not be less than 0.99

Limit of Quantitation:
The signal to noise ratio should be 9.5 to 10.5.
In LOQ Precision % RSD of impurity areas from six sample preparations should not be more than 10.0%.
The mean recovery of % impurity about Limit of Quantitation level should not be less than 90.0% and not more than 110.0%.
Limit of Detection:
The signal to noise ratio should be 2.0 to 3.0.

Linearity :
The correlation coefficient of all individual known impurities and Eplerenone is NLT 0.998 at different concentrations levels of LOQ, 25.0%, 50.0%, 100.0%, 150.0% &200.0%.
Should evaluate Y-intercept, Slope of regression, Residual sum squares and Residual standard deviation

Establishment of RR T's &RF Values

PRECISION:
System Precision:
% RSD for six replicates of the standard is NMT 5.0.
Method Precision(Repeatability):
The RSD of % impurities from six spiked sample preparations should not be more than 10.0%.

Accuracy in terms of recovery:
The % mean recovery of all individual known impurities at LOQ, 50%, 100%, 150% and 200% level should not be less than 90.0% and not more than 110.0%.

Intermediate Precision:
The RSD of % impurities from six spiked sample preparations should not be more than 10.0% by different instruments, analysts, Columns and days.
The RSD of % impurities from Twelve spiked sample preparations should not be more than 15.0% by different instruments, analysts, Columns and days.

Range:
Linearity:
The correlation coefficient of known impurities and Eplerenone is NLT 0.998 at different concentrations levels of LOQ, 25.0%, 50.0%, 100.0%, 150.0% &200.0%
Precision:
The RSD of impurity areas from six sample preparations at LOQ and 200% level should not be more than 10.0%
Accuracy:

The mean recovery of known impurities at LOQ and 200% level should not be less than 90.0% and not more than 110.0%
Robustness: Solution stability and mobile phase stability Extraction time of analyte Filter variation Change in mobile phase pH to 3.2 ± 0.2 units Change in column temperature to 25 ± 5 °C Change in flow rate to 1.0 ± 10% **Acceptance criteria:** 1) Difference between % of individual impurities should not be more than 0.04% and total impurities should not be more than 0.1% from related substances of normal condition 2) System should meet the System suitability criteria.

Analytical method validation of related substances by HPLC of Eplerenone tablets 25/50 mg shall be performed by carrying out the following typical analytical parameters.

Details of Validation Parameters System Suitability

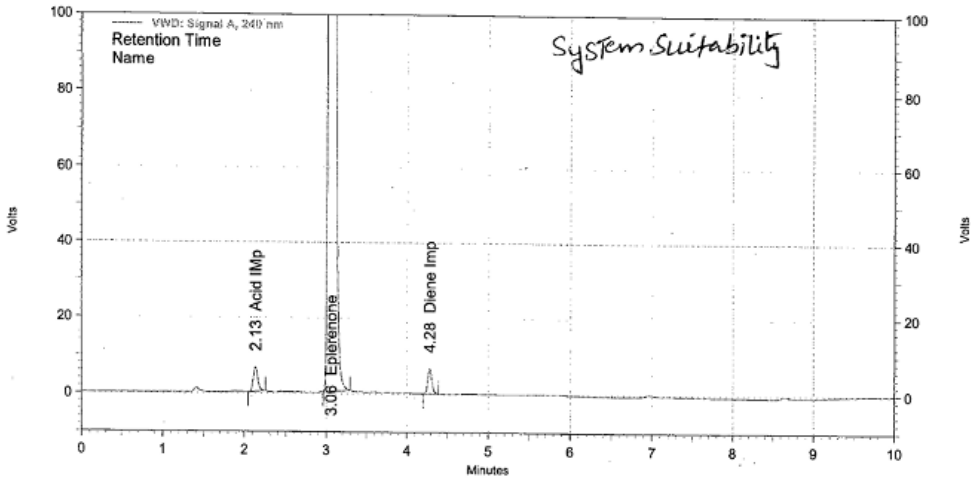
System suitability was demonstrated by injecting standard solution six times as per test method.
The observations are tabulated below-

RESULTS AND CONCLUSION

System suitability Parameters & Acceptance criteria	Resolution (NLT 2.0)	Theoretical plates (NLT 2500)	Asymmetry (NMT 2.0)	% RSD (NMT 5.0)
	9.69	18177	1.3	1.09

The results obtained meets the system suitability requirements, which indicates that the System is suitable for analysis.

Figure 1



SPECIFICITY

Placebo and impurities interference:

Interference from placebo and impurities was carried out by preparing the following specificity samples. Performed related substances on Placebo equivalent to the amount present in test preparation and inject into the chromatography.

By preparing and inject impurities at 1.0 % of test concentration. By preparing active sample as per test concentration. By spiking the active sample with individual known impurities at 1.0 % of test concentration. The above samples were injected and observed for any interference from blank and placebo at the retention time of analyte and known impurity peaks. This was further demonstrated by determining the peak purity of analyte and known impurity peaks.

The results of the peak purity values of analyte and known impurities are tabulated as below.

S. No.	Compound Name	Peak Purity
1	Eplerenone	1.00000
2	Acid impurity	1.00000
3	Diene impurity	1.00000

Acceptance criteria:

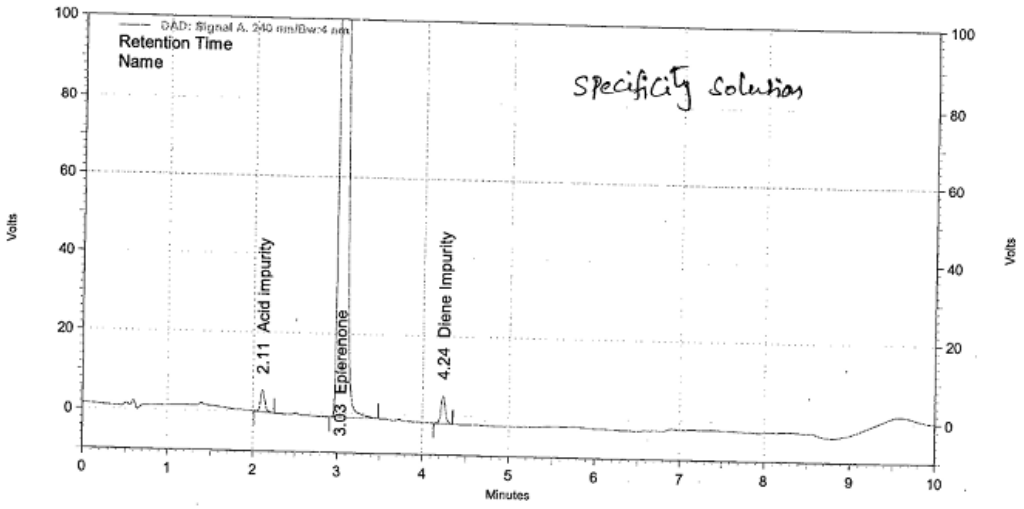
1. The peaks due to blank and placebo solution shall not interfere with the principal and impurities peak.
2. All-individual known impurities should be separated from the principal peak
3. Analyte and known impurities peak purity should be NLT 0.99.

Since no interference of blank, placebo and known impurities was observed at the retention time of analyte. Individual impurity peaks are separated from the analyte peak.

Peak purity of analyte peak and known impurity peaks are greater than 0.99, So the method is specific for Eplerenone 25/50 mg tablets.

RESULTS AND CONCLUSION

Figure 2



Limit of Quantitation

Preparation of stock solutions:

Eplerenone stock solution:

Weighed 9.54 mg of Eplerenone standard and transferred into a 100 ml volumetric flask, added 60 ml Of diluent, sonicated to dissolve and further diluted to volume with diluent.

Acid impurity stock solution:

Weighed 10.52 mg of Acid impurity into 100 ml volumetric flask, added 60 ml of diluent and sonicated to dissolve and further diluted to volume with diluent.

Diene impurity stock solution:

Weighed 9.58 mg of Diene impurity into 100 ml volumetric flask, added 60 ml of diluent and sonicated to dissolve and further diluted to volume with diluent.

Preparation of LOQ level solution: Diluted to 48 µL of Eplerenone stock solution, 42 µL of Acid impurity stock solution and 42 µL of Diene impurity stock solution into 50 ml and make up to volume with diluent.

LOQ Results

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration
1	Eplerenone	9.70	0.0092
2	Acid impurity	10.24	0.0088
3	Diene impurity	10.11	0.0080

Acceptance criteria:
Signal to noise(S/N) Ratio should be 9.5 to 10.5

Results and Conclusion:

LOQ values of Eplerenone and Eplerenone impurities was found within the acceptance limit of Signal to Noise ratio 9.5 to 10.5.

Figure 3

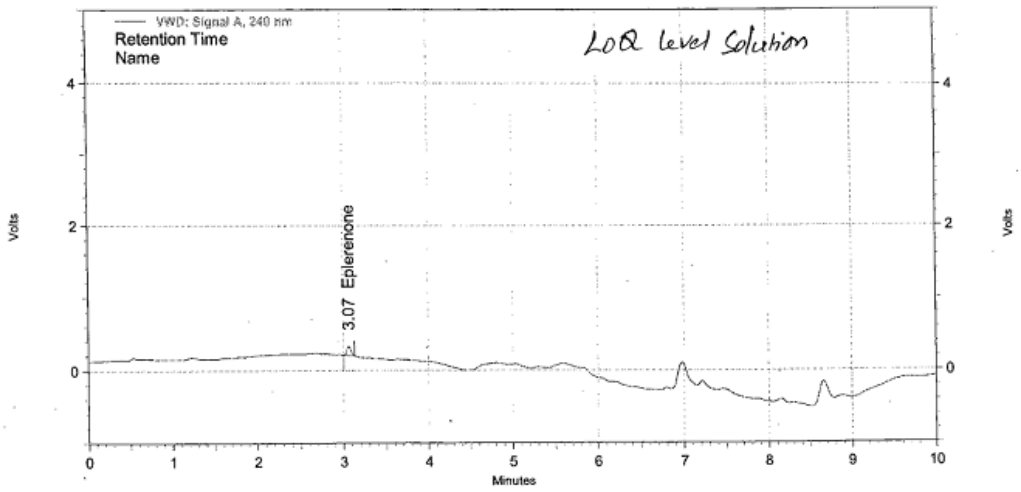


Figure 4

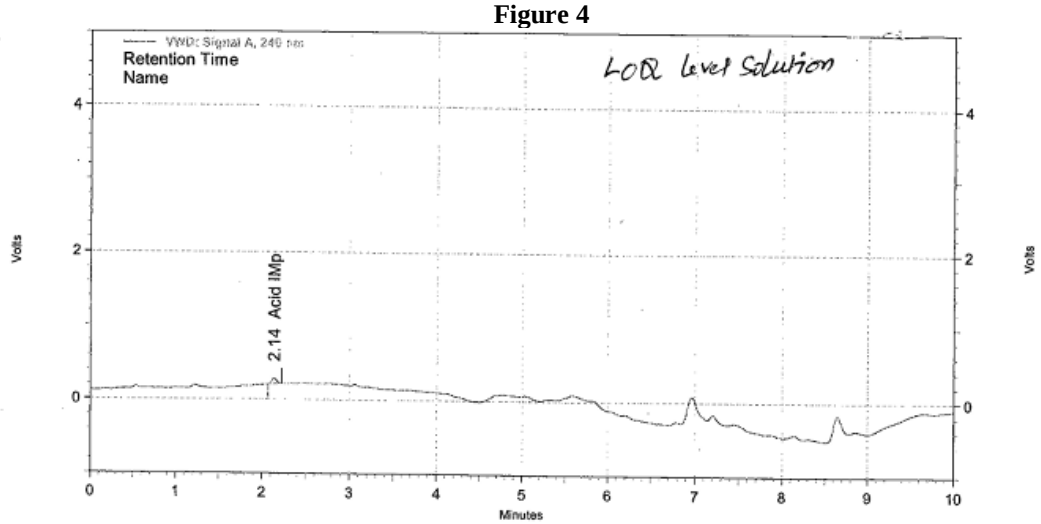
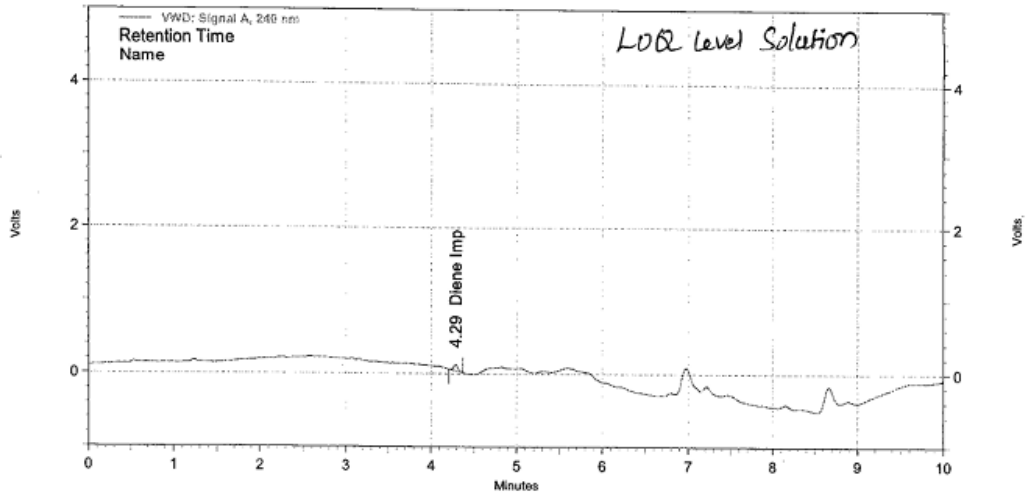


Figure 5



LIMIT OF DETECTION

Preparation of LOD level solution:
Diluted 3ml of the LOQ level solution to 10 ml with diluent.
LOD Results:

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration
1	Eplerenone	2.60	0.0027
2	Acid impurity	2.82	0.0027
3	Diene impurity	2.57	0.0024

Acceptance criteria:
The signal to noise ratio should be 2.0 to 3.0.
Results and Conclusion:
LOD values of Eplerenone and Eplerenone impurities was found within the acceptance limit of Signal to Noise ratio at about 2.0 to 3.0.

PRECISION AT LIMIT OF QUANTITATION LEVEL:
Acceptance criteria:
The RSD of Eplerenone and Individual impurity areas from six preparations at LOQ precision Level should not be more than 10.0%
Conclusion: The % RSD of individual impurity areas from six sample preparations at LOQ precision level is within the acceptance limit.

LINEARITY:
Continued from LOQ level Establishment
Preparation at 25% to 200% Level:

Linear solutions (%)	Stock solution taken in (ml)	Diluted to volume (ml) with diluent
25%	0.625	50

Continued from LOQ level Establishment.
Preparation of LOQ level precision solution: Diluted to 48 µL of Eplerenone stock solution, 42 µL of Acid impurity stock solution and 42 µL of Diene impurity stock solution into 50 ml and make up to volume with diluent. Repeated same procedure for remaining five preparations.

% RSD for the Eplerenone and impurities areas is tabulated as below.

S. No	Eplerenone	Acid impurity	Diene impurity
1	8150	4805	5928
2	6828	5244	5966
3	6992	5281	5832
4	7024	5320	5930
5	7764	5667	6331
6	7174	5627	6323
Avg:	**7322**	**5324**	**6052**
SD:	**518.6**	**312.1**	**217.5**
% RSD:	**7.08**	**5.86**	**3.59**

The Linearity of detector response for impurities was demonstrated by prepared solutions of Eplerenone and Eplerenone impurities over the range of LOQ to 200% level of the target Concentration. (0.5 % of test concentration)
Preparation of LOQ level Linearity solution:
Diluted to 48 µL of Eplerenone stock solution, 42 µL of Acid impurity stock solution and 42 µL of Diene impurity stock solution into 50 ml and make up to volume with diluent.

50%	1.25	50
100%	2.50	50
150%	3.75	50
200%	5.00	50

Results:
Linearity of Eplerenone:

S. No	Linearity Level	Concentration (ppm)	Average area of Eplerenone
1	LOQ	0.092	7307
2	25.0%	1.19	98203
3	50.0%	2.39	196443
4	100.0%	4.77	412543
5	150.0%	7.16	610131
6	200.0%	9.54	815835

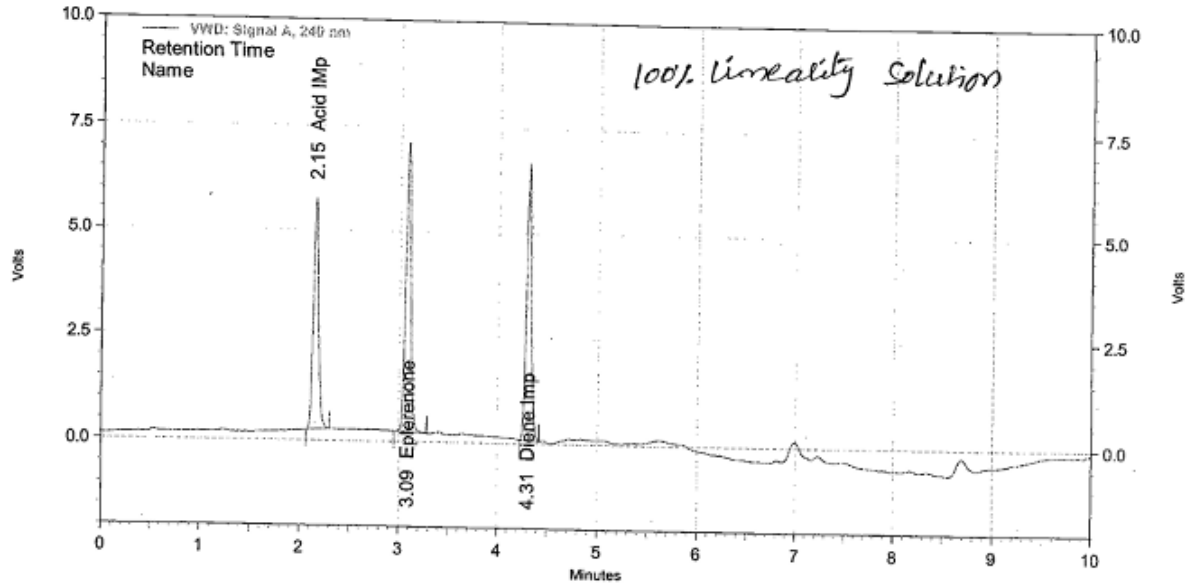
Linearity of acid impurity:

S. No	Linearity Level	Concentration (ppm)	Average area of Acid impurity
1	LOQ	0.088	5432
2	25.0%	1.32	80798
3	50.0%	2.63	164621
4	100.0%	5.26	328952
5	150.0%	7.89	503191
6	200.0%	10.52	667637

Linearity of dine impurity:

S. No	Linearity Level	Concentration (ppm)	Average area of Diene impurity
1	LOQ	0.080	5800
2	25.0%	1.20	95169
3	50.0%	2.40	193859
4	100.0%	4.79	390114
5	150.0%	7.19	585916
6	200.0%	9.58	782394

Figure 6



Acceptance Criteria

The correlation coefficient shall not be less than 0.998

Results:

	Eplerenone	Acid impurity	Diene impurity
Correlation coefficient	0.9999	1.0000	1.0000
Slope	85869	63709	81832
Y-Intercept	3077.14	2438.19	2031.1
Residual sum square	7.8709 x 107	2.8754 x 107	3.3380 x 106
Residual standard deviation	4431	2681	914

Results:
The plot was found to be linear with a correlation co-efficient of more than 0.998

Conclusion:
The study proves that the response of Eplerenone Acid impurity and Diene impurities are linear over the range of LOQ level to 200% level of targeted concentration.

ESTABLISHMENT OF RR T's & RF VALUES FOR IMPURITIES

Note: RRT's & RF Values are calculated from the Linearity levels of 50%, 100% and 200% (i.e., 0.50%, 1.0% and 2.0% of test concentration)

Acid impurity (RRF) =

Acid impurity area Eplerenone wt Eplerenone purity

---------- x ---------- x ----------
Avgarea of Eplerenone Acid impurity wt Acid impurity purity

Diene impurity (RRF) =

Diene impurity area Eplerenone wt Eplerenone purity
---------- x ----------x ----------
Avg area of Eplerenone Diene impurity wt Diene impurity purity

RRF Results:

Impurity areas at 0.25% of Test Concentration	
Acid impurity	Diene impurity
164527	193353
164715	194365
RRF Values	
Acid impurity	Diene impurity
0.783	1.004
0.782	1.009

Impurity areas at 0.5% of Test Concentration	
Acid impurity	Diene impurity
329248	390537
328656	389690
RRF Values	
Acid impurity	Diene impurity
0.746	0.966
0.745	0.964

Impurity areas at 1.0% of Test Concentration	
Acid impurity	Diene impurity
663277	778201
671996	786586
RRF Values	
Acid impurity	Diene impurity
0.760	0.973
0.762	0.983

S. No	**Concentration (%)**	**RRF Values**	
		Acid impurity	Diene impurity
1	0.25%	0.783	1.007
2	0.50%	0.746	0.965
3	1.00%	0.761	0.978
Avg RRF:		**0.763**	**0.983**

Avg RRF:

RF Results:

$$\text{Response Factor (RF)} = \frac{1}{\text{RRF}}$$

RF Values	
Acid impurity	Diene impurity
1.31	1.02

RR T's:

$$\text{RRT} = \frac{\text{Impurity RT}}{\text{Eplerenone RT}}$$

PRECISION: (Repeatability)

Precision was demonstrated by prepared six sample preparations as per the test method representing a single batch. Impurity areas of these samples was determined and the precision of the method was evaluated by computing the percentage-relative standard deviation of the impurity Areas. The results of the precision study are tabulated as below.

Eplerenone Tablets 25 mg Tablets:

S. No	Acid impurity	Diene impurity
1	0.51	0.52
2	0.50	0.51
3	0.50	0.51
4	0.51	0.51
5	0.51	0.52
6	0.51	0.52
Avg:	**0.51**	**0.51**
SD:	**0.01**	**0.00**
% RSD:	**1.08**	**0.72**

Eplerenone Tablets 50 mg Tablets:

S. No	Acid impurity	Diene impurity
1	0.50	0.51
2	0.50	0.51
3	0.50	0.51
4	0.50	0.52
5	0.51	0.52
6	0.50	0.51
Avg:	**0.50**	**0.51**
SD:	**0.00**	**0.00**
% RSD:	**0.69**	**0.75**

Acceptance criteria:

% RSD of individual % impurity from six Spiked sample preparations should not be more than 10.0

Conclusion

The low % RSD observed for Six Spiked sample preparations, which indicate the method is precise.

INTERMEDIATE PRECISION:

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, columns, instruments and days. The results of the intermediate precision study are tabulated as below.

Eplerenone Tablets 25 mg Tablets:

S. No	Acid impurity	Diene impurity
1	0.50	0.51
2	0.52	0.52
3	0.52	0.50
4	0.48	0.50
5	0.48	0.52

6	0.50	0.51
Avg:	**0.50**	**0.51**
SD:	**0.02**	**0.01**
% RSD:	**3.58**	**01/01/80**

Eplerenone Tablets 50 mg Tablets:

S. No	Acid impurity	Diene impurity
1	0.49	0.50
2	0.49	0.50
3	0.48	0.50
4	0.51	0.50
5	0.51	0.49
6	0.51	0.51
Avg:	**0.50**	**0.50**
SD:	**0.01**	**0.00**
% RSD:	**2.50**	**0.88**

The mean % RSD for both method precision and intermediate precision are tabulated below
Note: For Analyst - 1, Column -1 and System -1 results refer Repeatability

Eplerenone Tablets 25 mg Tablets:

S. No	Acid impurity	Diene impurity
1	0.51	0.52
2	0.50	0.51
3	0.50	0.51
4	0.51	0.51
5	0.51	0.52
6	0.51	0.52
7	0.50	0.51
8	0.52	0.52
9	0.52	0.50
10	0.48	0.50
11	0.48	0.52
12	0.50	0.51
Avg:	**0.50**	**0.51**
SD:	**0.01**	**0.01**
% RSD:	**2.59**	**1.47**

Eplerenone Tablets 50 mg Tablets:

S. No	Acid impurity	Diene impurity
1	0.50	0.51
2	0.50	0.51
3	0.50	0.51
4	0.50	0.52
5	0.51	0.52
6	0.50	0.51
7	0.49	0.50
8	0.49	0.50
9	0.48	0.50
10	0.51	0.50
11	0.51	0.49
12	0.51	0.51
Avg:	**0.50**	**0.51**
SD:	**0.01**	**0.01**
% RSD:	**1.91**	**1.75**

Acceptance criteria:

1) The RSD of % impurities from six spiked sample preparations should not be more than 10.0% by different instruments, analysts, columns and days.

2) The RSD of % impurities from Twelve spiked sample preparations should not be more than 15.0% by different instruments, analysts, columns and days.

Conclusion: As the % RSD results obtained on different instruments, analysts, columns and days. Different analysts were found to be within the acceptable limit, which shows that the test method is rugged.

ACCURACY:

The accuracy of the test method was demonstrated by preparing recovery samples (i.e.test Sample with known quantities of known impurities) at the level of LOQ, 50%, 100%,150% and 200% of target concentration (i.e 0.5% of test concentration).

Preparation of stock solutions:

Acid impurity stock solution:

Weighed 99.61 mg of Acid impurity into 100 ml volumetric flask, added 60 ml of diluent and sonicated to dissolve and further diluted to volume with diluent.

Diene impurity stock solution:

Weighed 102.51 mg of Diene impurity into 100 ml volumetric flask, added 60 ml of diluent and sonicated to dissolve and further diluted to volume with diluent. The recovery samples were prepared at each level. The samples at different level were injected and the percentage recovery for the amount added was estimated. The precision of recovery at LOQ level and 200% level was determined by computing the relative standard deviation of Recovery results. The observations are tabulated as below.

Eplerenone tablets 25/50 mg Tablets

Recovery of Acid impurity:

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery	%RSD
LOQ level	0.084	0.08	101.10	99.65	1.29
LOQ level	0.084	0.08	99.24		
LOQ level	0.084	0.08	98.62		
50%	2.49	2.45	98.36	97.97	0.72
50%	2.49	2.43	97.59		
50%	2.49	2.41	96.94		
100%	4.98	4.91	98.67	97.81	2.01
100%	4.98	4.83	96.95		
100%	4.98	5.02	100.88		
150%	7.47	7.32	97.94	98.05	0.97

150%	7.47	7.33	98.16		
150%	7.47	7.45	99.69		
200%	9.96	10.06	101.01		
200%	9.96	9.93	99.66	100.33	1.33
200%	9.96	9.79	98.33		

Recovery of Diene impurity:

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery	%RSD
LOQ level	0.086	0.09	102.47		
LOQ level	0.086	0.09	101.45	103.23	2.17
LOQ level	0.086	0.09	105.75		
50%	2.56	2.53	98.65		
50%	2.56	2.52	98.26	98.45	0.38
50%	2.56	2.54	99.00		
100%	5.13	5.08	99.06		
100%	5.13	5.15	100.48	99.77	1.17
100%	5.13	5.20	101.39		
150%	7.69	7.58	98.60		
150%	7.69	7.71	100.28	99.44	0.93
150%	7.69	7.70	100.10		
200%	10.25	10.40	101.50		
200%	10.25	10.29	100.42	100.96	1.14
200%	10.25	10.17	99.19		

Acceptance criteria:

The recovery at each level shall not be less than 90.0 % and not more than 110.0 %.

Conclusion:

As the recovery results are found between 90.0% to 110.0%, the study proves that the Method is accurate for the estimation of impurities in Eplerenone 25/50 mg tablets over the range of LOQ to 200% level of target concentration.

Range Linearity Results:

S. No	Impurity Name	Correlation coefficient (NLT 0.998)
1	Eplerenone	0.9999
2	Acid impurity	1.0000
3	Diene impurity	1.0000

Precision at LOQ & 200% level:
Results:
Eplerenone tablets 25/50 mg Tablets

S. No	Acid impurity	
	Impurity at LOQ level	**Impurity at 200% level**
1	5665	673807
2	5561	664828
3	5526	655948
4	5571	668031
5	5596	660717
6	5392	663013
Avg:	**5552**	**664391**
SD:	**91.0**	**6144.6**
% RSD (NMT 10.0)	**1.64**	**0.92**

S. No	Diene impurity	
	Impurity at LOQ level	**Impurity at 200% level**
1	6727	793184
2	6660	784816
3	6942	775166
4	6744	789602
5	6809	779692
6	6930	784176
Avg:	**6802**	**784439**
SD:	**114.2**	**6509.5**
% RSD (NMT 10.0)	**1.68**	**0.83**

Accuracy at LOQ & 200% level :

S. No	% Recovery at LOQ level &200% level (Limit: 90.0% to 110.0%)	
	Acid impurity	Diene impurity
LOQ level	99.65	103.23
200.0%	100.96	100.33

Conclusion:
The study proves that method is linear, precise and accurate over the range of LOQ level to 200% level of targeted concentration.

ROBUSTNESS:

Solution stability and mobile phase stability:
Standard and sample solutions was prepared as per test method The % difference of impurities Determined and the observations are tabulated as below.

Bench top solution stability Results:

Time (hours)	% of Acid imp	% of difference	% of Diene imp	% of difference	% of Total impurities	% of difference
Initial	0.02	NA	0.01	NA	0.11	NA
After 24 hours	0.02	Nil	0.01	Nil	0.10	0.1
After 48 hours	0.02	Nil	0.01	Nil	0.11	Nil

Refrigerator solution stability Results:

Time (hours)	% of Acid imp	% of difference	% of Diene imp	% of difference	% of Total impurities	% of difference
Initial	0.02	NA	0.01	NA	0.11	NA
After 24 hours	0.02	Nil	0.01	Nil	0.10	0.01
After 48 hours	0.02	Nil	0.01	Nil	0.11	Nil

Mobile phase stability Results :

Time (hours)	% of Acid imp	% of difference	% of Diene imp	% of difference	% of Total impurities	% of difference
Initial	0.02	NA	0.01	NA	0.11	NA
After 24 hours	0.02	Nil	0.01	Nil	0.10	0.01
After 48 hours	0.02	Nil	0.01	Nil	0.11	Nil

NA= Not applicable

Acceptance criteria:
The difference between initial and bench top and Refrigerator stability samples for % of individual impurities should not be more than 0.04% and % of Total impurities should not be more than 0.1%

Conclusion:
The difference between initial and bench top and Refrigerator stability samples for % of individual impurities **and % of total impurities were found with in the limit of 0.04% and 0.1%,** which indicates that the solution is stable up to 48 hours.

Filter variation:
The filter variation study established by injecting the test solution filtered through 0.22 µm nylon filter, 0.22 µm PVDF filter and Centrifuged as per method. The observations are tabulated as below.

	Centrifuged	Nylon Filter	PVDF Filter
% of Acid impurity	0.02	0.02	0.02
% Difference	NA	Nil	Nil
% of Diene impurity	0.01	0.01	0.01
% Difference	NA	Nil	Nil
% of Total impurities	0.11	0.11	0.11
% Difference	NA	Nil	Nil

Acceptance Criteria:
The difference between filtered portions of % individual impurities should not be more than 0.04% and % of Total impurities should not be more than 0.1%

Conclusion:
The difference between Centrifuged filtered portions of % individual impurities and % of Total impurities were found within the limit of 0.04% and 0.1%

Effect of Extraction time, Column temperature, Buffer pH and Flow rate:

Robustness of test method was demonstrated by injecting system suitability and test preparation under normal condition (i.e as such condition) and each of the altered conditions mentioned below:

Conditions:

- Extraction time
- Change in Column Temperature to 25 ± 5°C
- Change in flow rate to 1.0 ml ± 10%
- Change in PH to 3.2 ± 0.2

The % difference of impurity results obtained under normal condition (i.e as such condition) and each of the changed conditions are tabulated below-

Condition	Resolution (NLT 2.0)	Theoretical plates (NLT 2500)	Asymmetry (NMT 2.0)	% RSD (NMT 5.0)
Normal Condition (i.e as such condition)	9.69	18177	1.30	1.09
Flow changed to 0.9 ml/min	9.75	19826	1.22	2.96
Flow changed to 1.1ml/min	9.62	16280	1.21	3.25
Column Temperature changed to 20°C	9.47	18199	1.35	4.10
Column Temperature changed to 30°C	9.86	16883	1.35	1.90
pH changed to 3.0	8.12	13038	1.39	3.23
pH changed to 3.4	7.69	11545	1.49	2.70

Extraction time:

	15 min	**10 min**	**20 min**
% of Acid impurity	0.02	0.02	0.02
% Difference	NA	Nil	Nil
% of Diene impurity	0.01	0.01	0.01
% Difference	NA	0.01	0.01
% of Total impurities	0.11	0.11	0.11
% Difference	NA	Nil	Nil

Note: Initial data compiled from precision section

Acceptance criteria:

1) The difference between changed conditions of % individual impurities should not be more than 0.04% and % Total impurities should not be more than 0.1% from normal condition.

2) System should meet the System suitability criteria.

Results and Conclusion:

Since system suitability requirements are met for all the above-mentioned changed conditions, % of individual and total impurities observed that not differ by more than 0.04% and 0.1% from normal condition, it proves that the method is robust.

CONCLUSION:

The selected method successfully used for the impurities in Eplerenone tablet dosage form.

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