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Research Article

Analytical Method Development and Validation for Simultaneous Estimation of Amiloride and Torsemide in their Combined Pharmaceutical Dosage form by RP-HPLC Method

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ABSTRACT

RP-HPLC method was developed for the simultaneous estimation of Amiloride and Torsemide in pharmaceutical dosage form. The separation was achieved by BDS C18 (150 × 4.6 mm, 5 µm) column with Methanol:Phosphate buffer pH 3.6 (10:90 %v/v). Flow rate was maintained at 1.0 ml/ min and UV detection was carried at 288 nm. Retention time for Amiloride and Torsemide was found to be 1.944 min and 8.903 min respectively. The method has been validated for linearity, accuracy and precision. Linearity for Amiloride and Torsemide were in the range of 3-7 µg/ml and 6-14 µg/ml respectively. The percentage recoveries obtained for Amiloride and Torsemide were found to be in range of 99.87- 100.80 and 100.1-101.3 respectively. The developed method was validated as per ICH guidelines. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Amiloride and Torsemide in pharmaceutical dosage form.

Keywords: Amiloride, Torsemide, Methanol, Phosphate buffer pH 3.6, RP-HPLC method

INTRODUCTION

Amiloride and Torsemide are diuretic drugs. Amiloride is a potassium-sparing diuretic, chemically known as, 3,5-diamino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide.[1] It is used in the management of hypertension and congestive heart failure. It inhibits renal epithelial Na^+ Channel.[2] Torsemide is a pyridine-sulfonyl urea type loop diuretic, chemically known as, 1-{4[(3methylphenyl)amino]pyridine-3-sulfonyl}-3(propan-2-yl)urea.[3] It is used in the management of edema associated with congestive heart failure. It inhibit the $Na^+/K^+/2Cl^-$ carrier system It is an anti hypertensive drug and is used in treatment of angina[2]. Amiloride is official in IP, BP, USP[1,3,4] whereas Torsemide is official in USP [3].The chemical structures of Amiloride & torsemide are shown in Fig. 1.Combination drug product of Amiloride and Torsemide (Torsinex-A) is marketed and used in the treatment of Edema associated with congestive heart failure & hypertension. Several analytical methods like HPLC, HPTLC, UPLC have been reported for estimation of Amiloride & Torsemide by single drug and also by combining with other drugs.[5,6,7,8,9] But there was no any method has been reported till date for the simultaneous estimation of Amiloride & Torsemide using the RP-HPLC method. The present paper describes the development and validation of two analytical methods for simultaneous estimation of Amiloride & Torsemide by RP-HPLC method in tablet dosage form. The proposed method are validated as per the ICH guidelines[10,11]

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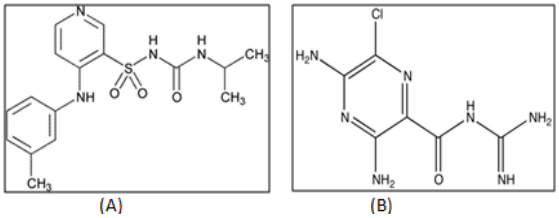

(A) (B)

Fig. 1 Chemical structures of the analytes (A) Amiloride & (B) Torsemide

MATERIALS AND METHODS

Instrumentation

RP-HPLC (LC-2010 C Shimadzu) having variable UV detector and an auto injector. Hypersil BDS C18 (150mm x 4.6 mm, 5 µm) column was used. Column LC solution software was used. An analytical balance (ME5 sartorious) was used in study.

Material and reagent

Amiloride (AML) was obtained from Wockhardtltd.L1chikalthana, Maharastra and Torsemide (TSM) bulk powder was kindly gifted by IPCA Labs ltd., Ahmedabad. Methanol HPLC grade was from Rankem Chemical Ltd., Potassium dihydrogen ortho phosphate was from Sulab reagent, Suvidhinath Labs.

Preparation of Combined Standard Stock solution of AML and TSM:

25 mg of standard Amiloride and 50 mg of standard Torsemide were weighed and transferred to 50 ml volumetric flask and shake to dissolve in methanol. After that the volume was made up to the mark with methanol to obtain 500 µg/ml of Amiloride and 1000 µg/ml of Torsemide. The solution was labeled as 'Stock solution A'.

Preparation of combined Working Standard Solution of AML and TSM:

From the 'Stock solution A' 1 ml of aliquot was pipette out in 10 ml volumetric flask and shake to dissolve in methanol. After that the volume was made up to the mark with methanol to obtain 50 µg/ml of Amiloride and 100 µg/ml of Torsemide. The solution was labeled as 'Working stock solution A'.

Preparation of calibration curve:

From the 'Working stock solution A' (50 µg/ml of Amiloride and 100 µg/ml of Torsemide) 0.6, 0.8, 1.0, 1.2 and 1.4 ml of aliquot was pipette out in 10 ml volumetric flask and made the volume up to the mark with mobile phase to get 3-7 µg/ml of Amiloride and 6-14 µg/ml of Torsemide. The chromatogram was recorded under the finalized chromatographic conditions as described above after getting a stable baseline. Peak areas were recorded for all the peaks. Calibration curves of Amiloride and Torsemide were constructed by plotting the peak area of Amiloride *vs.* Amiloride concentration and peak area of Torsemide *vs.* Torsemide concentration respectively.

VALIDATION PARAMETERS

Validation of developed method was carried out as per ICH guideline.[10] Parameters such as Linearity and range, Accuracy, Precision, LOD and LOQ, specificity, system suitability were taken up as tests for analytical method validation.

Linearity and Range:

Linearity of the proposed method was verified by analyzing five combined different concentrations in the range of 3-7 µg/ml and 6-14 µg/ml for Amiloride and Torsemide respectively. Each concentration was made three times. The calibration curve of Peak area vs. respective concentration was plotted and regression line equation for Amiloride and Torsemide was calculated.

Precision

Precision of the method was determined in the terms of Repeatability, Intraday and Interday precision. Repeatability (% RSD) was assessed by analyzing test drug solution within the calibration range, six times on the same day.

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Intraday variation (% RSD) was determined by analysis of this solution three times on the same day. Interday precision (%RSD) was determined by analysis of this solution on three different days.

Limit of detection (LOD) and limit of quantitation (LOQ)

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y-intercept) and S, is the mean of the slope of calibration plot.

Recovery Studies:

The accuracy of the method was performed by conducting the recovery studies (80,100 and 120%) of pure drugs from marketed formulation, by standard addition method. The amount of Amiloride and Torsemide was calculated at each level and % recoveries were calculated.

Specificity:

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre weighed quantity of drugs and then peak area was measured and calculation was done to determine the quantity of the drugs.

System suitability

Combined standard solutions of Amiloride (5 µg/ml) and Torsemide (10 µg/ml) were prepared and analyzed six times. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they complies with the recommended limit or not.

Application of Proposed Method to dosage form:

To determine the content of Amiloride and Torsemide in their combined dosage form (tablet), 20 tablets were weighed & finely powdered. A quantity of powder equivalent to 50 mg of Amiloride and 100 mg of Torsemide were weighed accurately and transferred to 100 ml volumetric flask and the volume was made up with the methanol and then filtered through whatman filter paper no. 42. From the above prepared solution, further dilutions were prepared in the linearity range using mobile phase. The chromatogram was taken at selected wavelengths and peak areas were calculated. The analysis was done six times.

RESULTS AND DISCUSSION

Method Validation:

The linearity range for AML and TSM were 3-7 µg/mL and 6-14 µg/mL respectively. The results of the recovery studies are found to be satisfactory for AML and TSM and shown in Table 1 and 2 respectively. The result of assay procedure obtained was showed in Table 3. Summary of Other validation parameters including Repeatability, Intraday, Interday, LOD and LOQ, system suitability were found to be satisfactory and are shown in Table 5. Specificity is proven by comparing the chromatogram of Diluent, standard solution and test preparation solution to show that there was no any interference of excipients with the peak of Amiloride and Torsemide, as shown in figure 4, 5, 6, and 7.

CONCLUSION

RP-HPLC method was developed and validated for the determination of Amiloride and Torsemide in their combined pharmaceutical dosage form. The present RP-HPLC method can be considered simple and rapid to apply. This method was validated as per ICH guideline and fulfill the criteria of validation.

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FIGURE AND TABLES

Figure-2: Chromatogram of AML and TSM in Methanol : Phosphate buffer pH 3.6 (10:90)

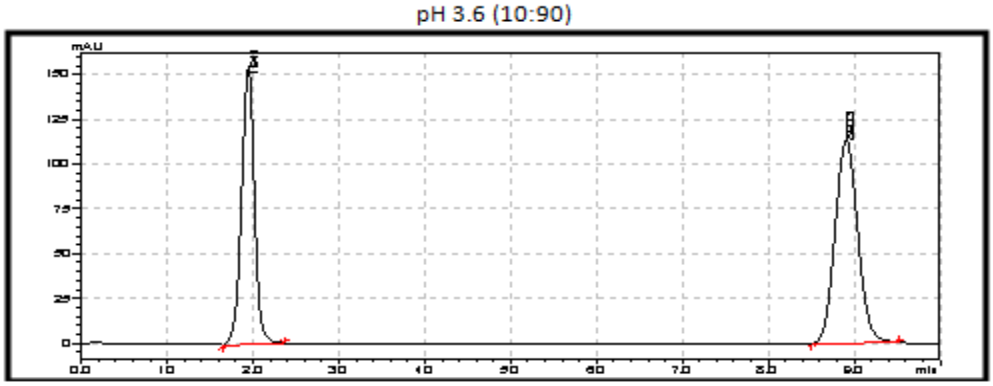

Figure-3: linearity of Amiloride and Torsemide

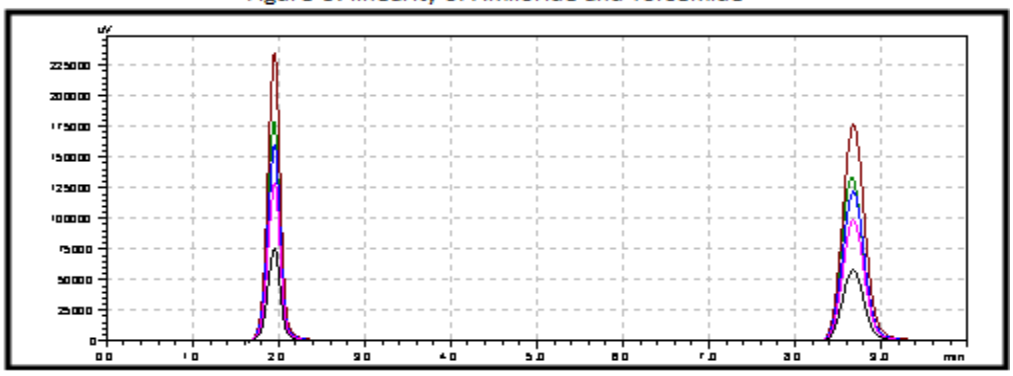

Figure 4: Chromatogram peak of Diluent

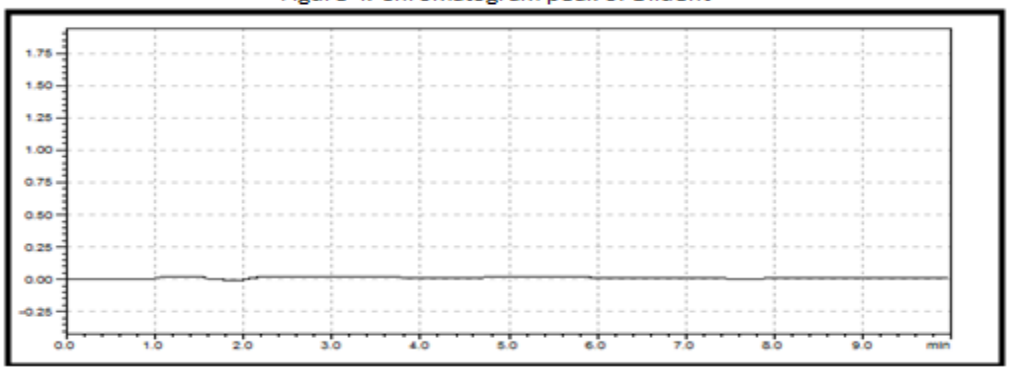

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Figure-5: Chromatogram peak of Standard solution of Amiloride

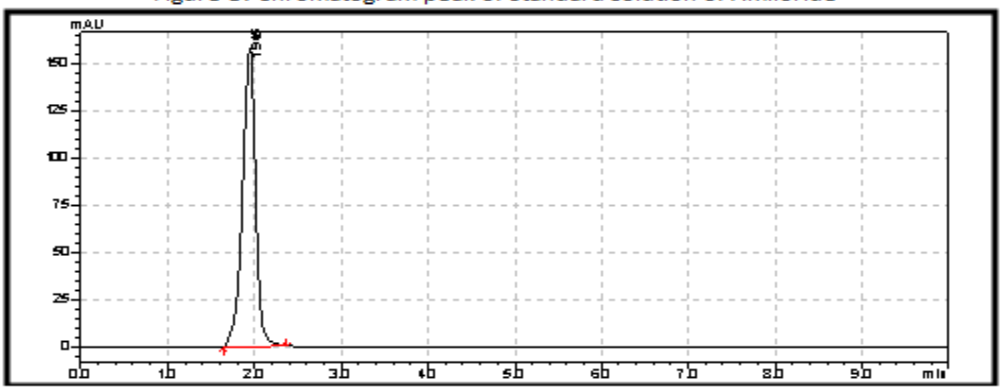

Figure-6: Chromatogram peak of Standard solution of Torsemide

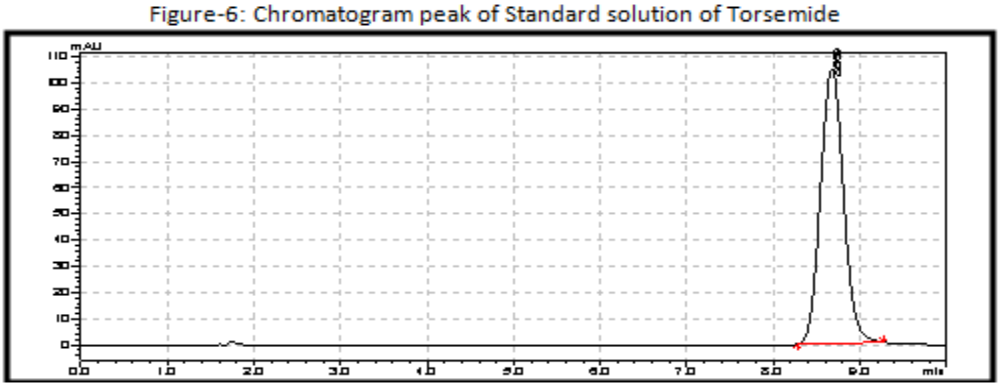

Figure 7: Chromatogram peak of sample solution

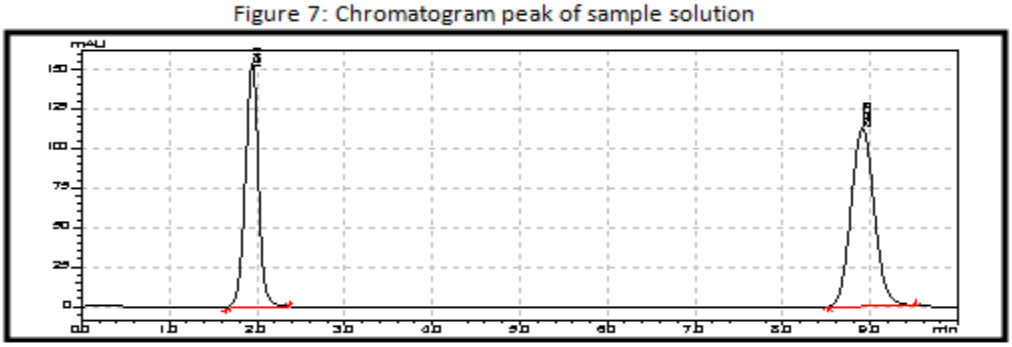

Table-1 Result of Recovery Studies for AML in dosage form:

Amount of AML in mixture (µg/ml)	Amount of Std AML added (µg/ml)	Total amount of AML (µg/ml)	Total amount of AML found (µg/ml) Mean* ± SD	%Recovery
5	0	5	05.09 ± 0.0400	101.80
5	4	9	09.05 ± 0.1058	100.55
5	5	10	9.99 ± 0.5928	99.87
5	6	11	11.02 ± 0.1629	100.15

[*=mean value of 3 determination]

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Table-2 Result of Recovery Studies for TSM in dosage form:

Amount of TSM in Mixture(µg/ml)	Amount of Std TSM added (µg/ml)	Total amount of TSM (µg/ml)	Total amount of TSM found (µg/ml) Mean* ± SD	%Recovery
10	0	10	10.13 ± 0.0953	101.3
10	8	18	18.04 ± 0.0945	100.1
10	10	20	20.08 ± 0.2205	100.4
10	12	22	22.05 ± 0.2875	100.2

[*=mean value of 3 determination]

Table-3: Analysis of AML and TSM in dosage form:

Tablet dosage form	Label claim(mg)		%Recovery ± SD (% of label claim*)	
	AML	TSM	AML	TSM
	5 mg	10 mg	100.06 ± 0.9947	100.09 ± 1.0530

[*=mean value of 5 determination]

Table-4: Regression Characteristics:

Characteristics	AML	TSM
Linearity (µg/ml)	3-7	6-14
Regression Equation	Y= 18985X + 71952	Y= 23931X – 20317
Slope	18985	23931
r^2	0.998	0.996
Intercept	71952	20317
S.D. of Intercept	237.28	303.65

TABLE-5: VALIDATION PARAMETERS:

Parameters	AML	TSM
Repeatability(%RSD) (n=6)	1.065	1.850
Precision (%RSD)		
Intra-day (n=3)	0.2448- 0.6197	0.1990-0.2340
Inter-day (n=3)	0.5641-2.0000	0.2304-0.6796
LOD (µg/ml)	0.0412	0.0418
LOQ (µg/ml)	0.1249	0.1268
% Recovery (n=3)	99.87%-101.80%	100.1%-101.3%
Assay (mean ± S.D.) (n=5)	100.06 ± 0.9947	100.09 ± 1.0530
System suitability		
Theoritical plates	793.64	5349.99
Tailing factor	0.969	1.091
Resolution	-	18.24
Retention time	1.944	8.903

LOD: Limit of Detection, LOQ: Limit of Quantitation, R.S.D.: Relative standard deviation, S.D.: Standard deviation

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