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Dual wavelength spectrophotometric method for simultaneous estimation of torsemide and amiloride hydrochloride in their combined dosage form

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective dual wavelength spectrophotometric method for the simultaneous determination of Torsemide (TOR) and Amiloride HCl (AMI) in combined tablet dosage form. The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The principle for dual wavelength method is —the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest. The method was based on determination of Torsemide at the absorbance difference between 258.20 nm and 297.40nm and Amiloride HCl at the absorbance difference between 276.80 nm and 300 nm. The linearity was obtained in the concentration range of 5-25 µg/ml for Torsemide and 5-25 µg/ml for Amiloride HCl. The accuracy and precision of the method was determined and validated statically. The method showed good reproducibility and recovery with % RSD less than 2. Method was found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Torsemide and Amiloride HCl in bulk, and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Key words: Torsemide, Dual wavelength, Amiloride HCl, Recovery.

INTRODUCTION

Torsemide is a pyridine-sulfonyl urea. It is loop diuretic mainly used in the management of edema associated with congestive heart failure and to reduce the swelling and fluid retention caused by various medical problems, including heart or liver disease.[(1)] Its chemical name is. 1-{4-[(3-methylphenyl)amino]pyridine-3-sulfonyl}-3-(propan-2-yl)urea.[(2)]The structure of Torsemide is shown in Fig 1.

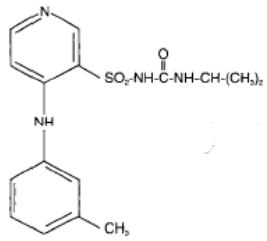

Fig 1. Structure of Torsemide

Amiloride HCl is an antikaliuretic-diuretic agent, is a pyrazine-carbonyl-guanidine that is unrelated chemically to other known antikaliuretic or diuretic agents. Amiloride is used in conjunction with diuretics to spare potassium loss. It is the salt of a moderately strong base (pKa 8.7).[(3)] It is designated chemically as 3,5-diamino-6-chloro-N-

(diaminomethylene) pyrazine -2-carboxamide monohydrochloride, dehydrate.[4]The structure of Amiloride HCl is shown in Fig 2.

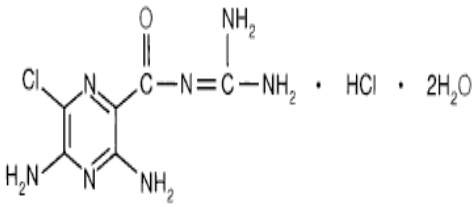

Fig 2. Structure of Amiloride Hcl

Torsemide has been marketed in combination with Amiloride HCl in tablets for the treatment of essential hypertension. Torsemide and Amiloride HCl is used combine in the treatment for management of primary hyper tension and edema in congestive heart failure.Literature survey revealed that, Torsemide is official in United States Pharmacopoeia [5]; Amiloride HCl is official in Indian Pharmacopoeia and United States Pharmacopoeia[6,7]. However, the combination is not official in any pharmacopoeia. On detailed literature survey, it was found that through individually these drugs have been analyzed by many methods, but there is no method available for the estimation of Torsemide and Amiloride HCl drugs in the combine dosage form. Therefore, it was thought worthwhile to develop simple, precise, accurateUVspectrophotometric method for simultaneous determination of Torsemide and Amiloride HCl in tablets. The proposed method was applied to the determination of analyts in pharmaceutical preparations, with satisfactory result. Validation was done with respect to various parameters, as required under ICH guideline.

MATERIALS AND METHODS

Instrumentation

A UV-visible spectrophotometer, model UV 1800 (Shimadzu) was used to measure absorbance of the resulting solutions using UV-Probe software version 2.31. A Digital analytical balance (Wenstar DA13-220) and ultrasonic sonicator (Equitron) were used in the study.

Reagents and materials

Pure Torsemide (TOR) & Amiloride HCl (AMI) kindly gifted as a gift sample by Intas (astron) pharma, Ahemdabad, India and Vapi care pharma, Vapi, India respectively. TORSINEX-A Tablet formulation procured from local market. All analytical grade chemicals and solvents were obtained from Merck (India). Methanol used as solvent in the study. Borosil volumetric flasks of 10, 50,100 ml capacity and pipettes – 1ml, 5ml,10ml, beakers, measuring cylinders etc.

Preparation of solutions

Preparation of Standard Stock Solutions

Torsemide (1000 µg/ml)

Accurately weighed TOR (10 mg) was transferred to a 10 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (1000 µg/ml).

Amiloride HCl (1000 µg/ml)

Accurately weighed AMI (10 mg) was transferred to a 10 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (1000 µg/ml).

Preparation of Working Standard Solutions

Torsemide (100 µg/ml)

Standard Stock solution (5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with methanol.

Amiloride Hcl (100 µg/ml)

Standard Stock solution (5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with methanol.

Preparation of calibration curve

Calibration curve for Torsemide

Aliquots of working standard solution of TOR (100 µg/ml) 0.5, 1, 1.5, 2 and 2.5ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get concentrations 5, 10, 15 20 and 25 µg/ml. Absorbance difference of each solution was measured at 258.20nm and 297.40nm using methanol as a

blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.

Calibration curve for Amiloride HCl

Aliquots of working standard solution of AMI (100 µg/ml) 0.5, 1, 1.5, 2 and 2.5ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get concentrations 5, 10, 15 20 and 25 µg/ml. Absorbance difference of each solution was measured at 276.80nm and 300nm using methanol as a blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.

Method

The working standard solutions of TOR and AMI were prepared separately in methanol having concentration of 20 µg/ml and 10 µg/ml respectively. They were scanned in the wavelength range of 200-400 nm. From the overlain spectra, four wavelengths 258.20 nm, 297.40 nm, 276.80 nm and 300 nm were selected for quantitation of the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of TOR is carried out by measuring the absorbance difference value at between 258.20 nm and 297.40 nm where AMI have same absorbance at both the wavelength. The difference between 258.20 nm and 297.40 is directly proportional toconcentration of TOR in the mixture. Thequantitative determination of AMI is carried out by measuring the absorbance difference value at 276.80 nm and 300 nm where TOR has same absorbance at both the wavelength. The difference between 276.80 nm and 300 nm is directly proportional to concentration of AMI in the mixture.

Preparation of sample solution

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 10 mg TOR and 5 mg AMI was accurately weighed. The powder was transferred to 100 ml volumetric flask and shaken vigorously with methanol for 15 min and the solution was sonicated for 15 minutes and filtered through Whatman filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 20µg/ml of TOR and 10µg/ml of AMI respectively. The absorbance difference values were read at 258.20 nm and 297.40 for TOR and 276.80 nm and 300 nm for AMI by thus concentration was obtained by solving the regression line equations.

Method Validation[8]

The developed method was validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantitation (LOQ)in accordance with the ICH guideline.

Linearity and Range

Linearity was studied by preparing standard solutions at 5 different concentrations. The linearity range for Torsemide and Amiloride HCl were found to be 5-25 µg/ml and 5- 25 µg/ml respectively. Linearity was assessed in the terms of slope, intercept and correlation coefficient for both the drugs.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate (intraday) precision and reproducibility (interday precision).

1) Intraday Precision

Solutions containing 10, 15, 20 µg/ml of TOR and 10, 15, 20 µg/ml of AMI were analysedthree times on the same day and %R.S.D was calculated.

2) Interday Precision

Solutions containing 10, 15, 20 µg/ml of TOR and 10, 15, 20 µg/ml of AMI were analyzed on three different successive days and %R.S.D was calculated.

3) Repeatability

Method precision of experiment was performed by preparing the standard solution of TOR (15 µg/ml) and AMI (15 µg/ml) for six times and analysed as per the proposed method. Coefficient of variation (%CV) was not more than 2%.

Accuracy

Accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100%, 120%) taking into consideration percentage

recovery of added bulk drug samples. The experiment was repeated three times by spiking previously analysed samples of tablet with three different concentrations of standards.

Limit of Detection (LOD)
Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 x (N / S)

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification (LOQ)
Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 x (N / S)

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

Selection of wavelength for simultaneous estimation of TOR and AMI
To determine the wavelength for measurement, TOR (20 µg/ml) and AMI (10 µg/ml) solutions were scanned between 400-200 nm. Absorbance difference were obtained at their λmax 258.20 nm and 297.40 nm for TOR and 276.80nm and 300nm for AMI respectively.

METHOD VALIDATION
Linearity & Range
The linearity of TOR and AMI was found to be in the range of 5-25 µg/ml and 5-25 µg/ml respectively. Calibration curve of TOR and AMI are shown in Figure 6 and 7 respectively. Linear regression analysis data and summary of validation for TOR and AMI are shown in Table 1.

Precision
1. Intraday Precision
The data for Intraday precision for TOR and AMI is in range of % RSD was found to be0.25-1.01% for TOR at 258.20nm -297.40nm and 0.61-1.29% for AMI at 276.80nm- 300nm.
2. Interday Precision
The data for Intraday precision for TOR and AMI is in range of % RSD was found to be 0.48-1.19% for TOR at 258.20nm -297.40nm and0.30-0.93% for AMI at 276.80nm- 300nm.
3. Repeatability
The data for repeatability for TOR and AMI was found to be 1.07% for TOR at 258.20nm -297.40nm and 0.91% for AMI at 276.80nm- 300nm.

Accuracy
Accuracy of the method was confirmed by recovery study from marketed formulation at three levels (80%, 100%, and 120%) of standard addition. Percentage recovery for TOR and AMI were found to be in the range of 98.05 – 101.66% and 99.88 - 101.00%.Data indicating recovery studies of Torsemide and Amiloride HCl are shown in Table 2.

LOD and LOQ
LOD values for TOR and AMI were found to be 0.83µg/ml and 0.23µg/ml respectively. LOQ values for TOR and AMI were found to 2.53µg/ml and 0.71µg/ml respectively.

Analysis of Marketed formulation
Applicability of the proposed method was tested by analyzing the commercially available tablet formulation. (TORSINEX-A). It is shown in Table: 3.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

Parameters	TOR	AMI
Wavelength (nm)	258.20- 297.40	276.80-300
Beer's Law Limit (µg/ml)	5-25	5-25
Regression equation (y = a + bc)	y = 0.0155x + 0.0171	y = 0.0399x + 0.0106
Slope (b)	0.0155	0.0399
Intercept (a)	0.0171	0.0106
Correlation Coefficient (r2)	0.9997	0.9992
Repeatability (% RSD, n=6)	1.07	0.91
Interday (n=3) (% RSD)	0.48-1.19	0.30-0.93
Intraday(n=3) (% RSD)	0.25-1.01	0.61-1.29
LOD(µg/ml)	0.83	0.23
LOQ (µg/ml)	2.53	0.71

Table :2 Data indicating recovery studies of Torsemide and Amiloride HCl

%Level of recovery	Amount of drug taken (µg/ml)		Amount of drug added (µg/ml)		Total amount found (µg/ml) ± S.D. (n=3)		% Recovery	
	TOR	AMI	TOR	AMI	TOR	AMI	TOR	AMI
80%	10	5	8	8	18.30±0.0008	8.99±0.0008	101.66	99.97
100%	10	5	10	10	19.60±0.0021	10.10±0.0004	98.05	101.25
120%	10	5	12	12	21.80±0.0020	11.01±0.0008	99.09	100.02

Table: 3 Analysis of marketed formulation

Tablet	Drug	Label claim (mg)	Amount found (mg)	% label claim
Toesinex-A	Torsemide	10	9.95 ±0.0004	99.5
	Amiloride HCl	5	4.95 ±0.0061	99.01

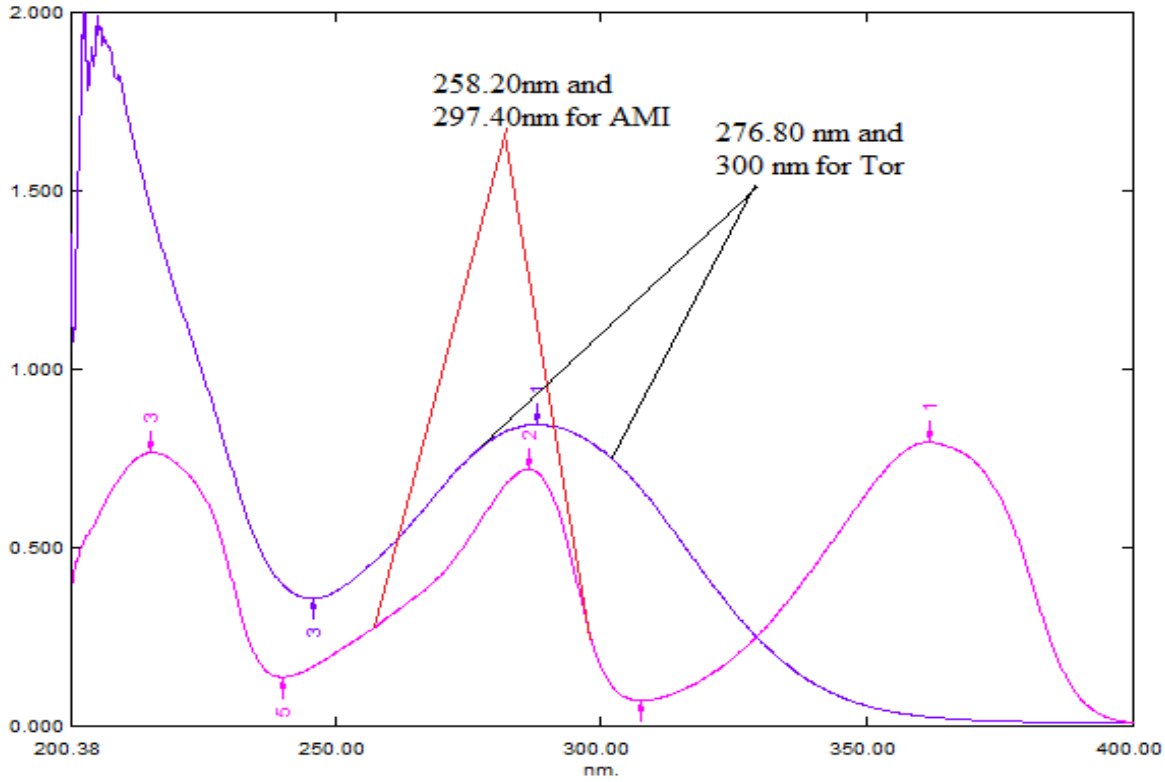

Figure 3: Overlain Spectra of TOR (20µg/ml) and AMI (10 µg/ml)

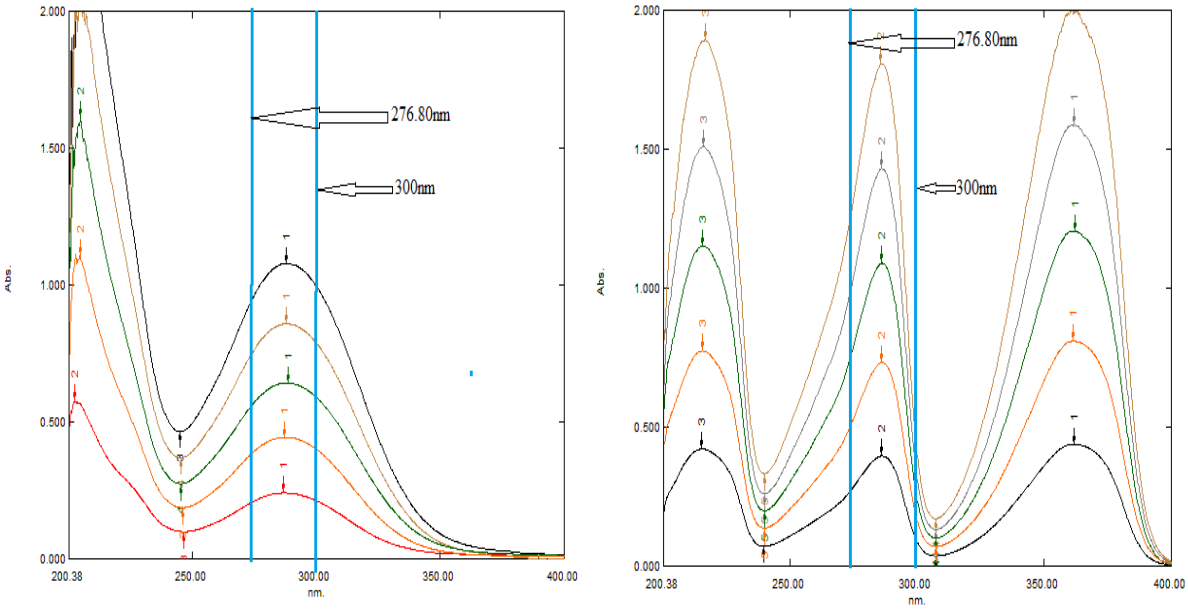

Figure 4: Spectra of Torsemide and Amiloride HCl for different conc. At 276.80 nm and 300 nm where TOR has same absorbance and AMI has different absorbance

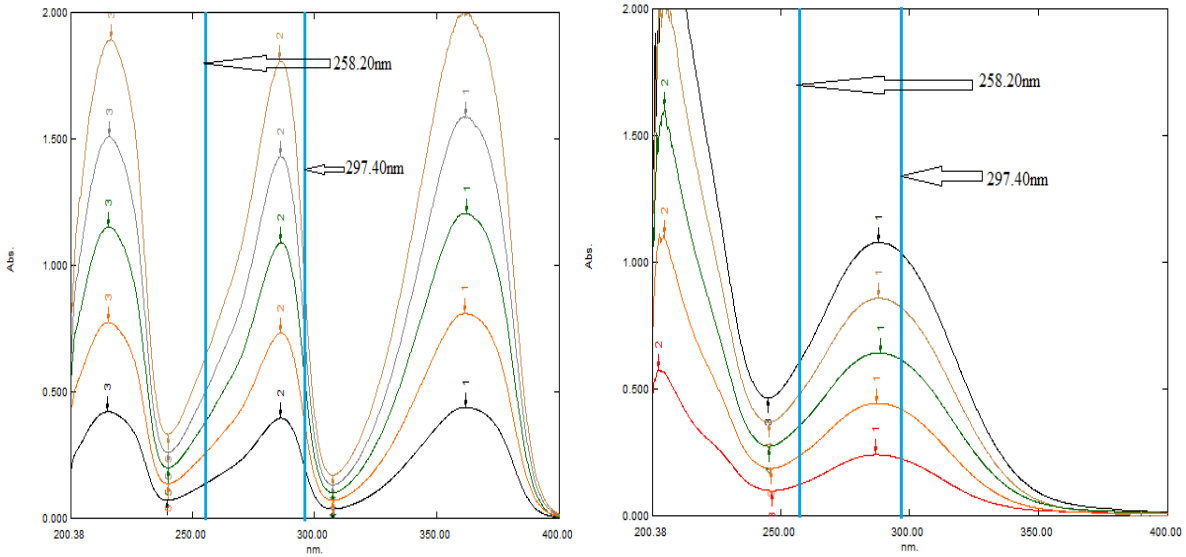

Figure 5: Spectra of Amiloride HCl and Torsemide for different conc. At 258.20nm and 297.40nm where AMI has same absorbance and TOR has different absorbance

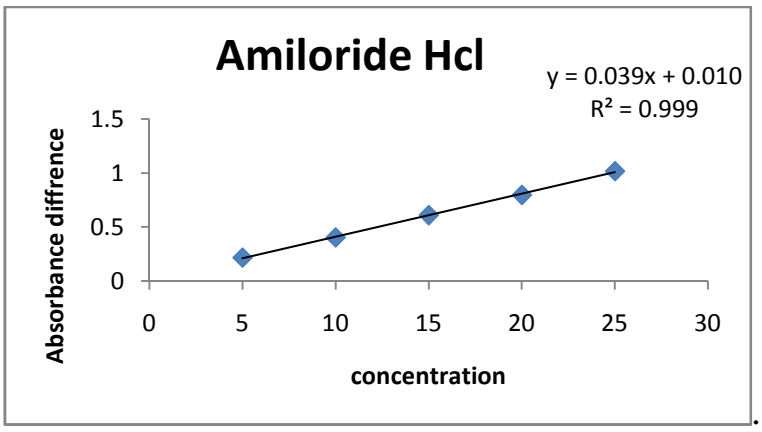

Figure 6: calibrationcurve for Amiloride HCl

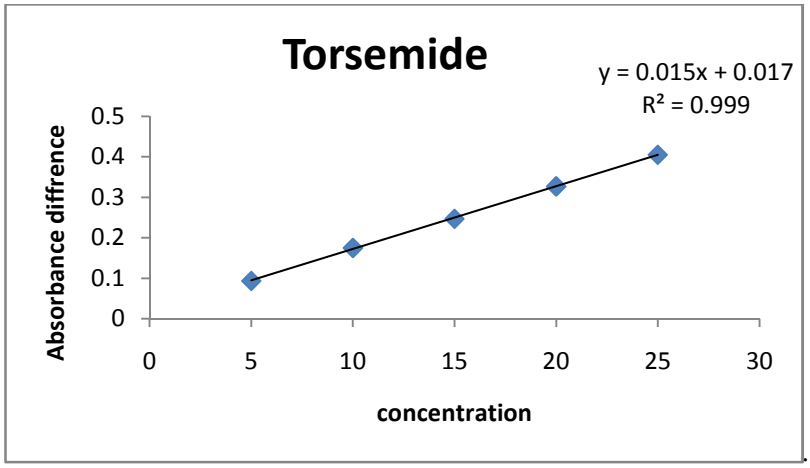

Figure 7: Calibration curve for Torsemide

CONCLUSION

A simple, rapid, accurate and precise spectrophotometric method has been developed and validated for the routine analysis of torsemide and amiloride HCl in API and tablet dosage forms. The spectrophotometric method is suitable for the simultaneous determination of Torsemide and Amiloride HCl in multi-component formulations without interference of each other. The dual wavelength method is rapid, simple and sensitive. The developed method is recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations.

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