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Analytical Techniques for Determination of Torsemide and its Combinations: A Review

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ABSTRACT:

Torsemide is chemically 3-pyridine sulfonamide N-[[(1- methylethyl) amino] -carbonyl]-4-[(3-methylphenyl) amino]. Torsemide is a loop diuretic drug used for treatment of high blood pressure (hypertension). It acts by inhibiting the Na+/ K+/ 2Cl– carrier system (via interference of the chloride binding site) in the lumen of the thick ascending portion of the loop of Henle, resulting in the decrease in reabsorption of sodium and chloride. This review focuses on the recent developments in analytical techniques for estimation of Torsemide alone or in combinations with other drugs in various biological media like human plasma and urine. This review will critically examine the (a) sample pretreatment method such as solid phase extraction (SPE), (b) separation methods such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), high performance thin layer chromatography (HPTLC), liquid chromatography coupled to tandem mass spectrometry (LC-MS) and capillary electrophoresis (CE), other methods such as spectrophotometry, diffuse reflectance near infrared spectroscopy and electrochemical methods.

KEY WORDS: Analytical Techniques, Torsemide, Solid phase extraction, HPLC, Spectrophotometry.

1. Introduction

Torsemide is sulfonylurea derivative and chemically known as 3-[4-[(3-methylphenyl) amino] pyridin-3-yl] sulfonyl-1-propan-2-ylurea. Torsemide is a new high-efficiency loop diuretic, acting the same as other pyridine sulfonylurea medications in blocking the $Na^+–K^+–2Cl^-$ carrier to promote excretion of water on the thick ascending limb of the loop of Henle. It has been successfully used to treat oedematous states associated with chronic congestive heart failure , renal disease and hepatic cirrhosis and low-dose torasemide has also been used to control arterial hypertension . Research proves that torasemide is safer and better tolerated than furosemide in chronic heart failure patients and associated with a trend in reducing all-cause mortality. Lower incidences of abnormal serum potassium levels and hypotension were also observed in patients receiving torasemide compared with those receiving other diuretics.

Torasemide inhibits the $Na^+/K^+/2Cl^-$-carrier system (via interference of the chloride binding site) in the lumen of the thick ascending portion of the loop of Henle, resulting in a decrease in reabsorption of sodium and chloride. This results in an increase in the rate of delivery of tubular fluid and electrolytes to the distal sites of hydrogen and potassium ion secretion, while plasma volume contraction increases aldosterone production. The increased delivery and high aldosterone levels promote sodium reabsorption at the distal tubules, and By increasing the delivery of sodium to the distal renal tubule, torasemide indirectly increases potassium excretion via the sodium-potassiumexchange mechanism. Torasemide's effects in other segments of the nephron have not been demonstrated. Thus torasemide increases the urinary

excretion of sodium, chloride, and water, but it does not significantly alter glomerular filtration rate, renal plasma flow, or acid-base balance. Torasemide's effects as a antihypertensive are due to its diuretic actions. By reducing extracellular and plasma fluid volume, blood pressure is reduced temporarily, and cardiac output also decreases[1-4].

2. Physical and Chemical Property

Torsemide is white to yellowish-white, crystalline powder almost odorless. IUPAC name of Torsemide is 1-[4-(3-methylanilino)pyridin-3-yl]sulfonyl-3-propan-2-ylurea (Fig.1.). Chemical formula of Torsemide is $C_{16}H_{20}N_4O_3S$. Molecular weight is 348.421 gm/mol. Soluble in water. [4,5]

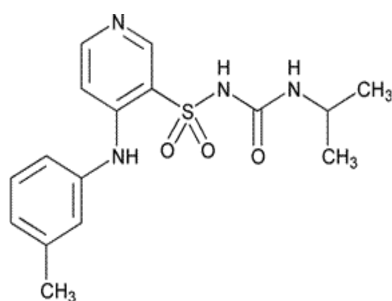

Fig.1. Chemical Structure of Torsemide

3. Analytical Methods

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products. After therapeutic administration of drug formulation, to check therapeutic concentration of drug in human body, there is a need for analytical methods. Analytical Methods are necessary for therapeutic drug monitoring. High Performance Liquid Chromatography (HPLC) together with various types of detection UV (ultraviolet), FD (fluorescence detection) and MS (mass spectrometry) has become the method of choice for analytical method development. Apart from these methods other analytical methods also used which are High performance thin layer chromatography , Ultra performance liquid chromatography, Thin layer chromatography, Voltammetry, Capillary electrophoresis are also widely used.

3.1 Capillary electrophoresis method

Rosa Marıa Alonso *et al* [6] developed a cyclodextrin assisted capillary electrophoresis method for the simultaneous determination of Torsemide and its metabolites. The most critical parameter is the pH of the running buffer. Optimal separation of the four studied compounds was achieved in less than 6.5 min, using an electrolyte of 60 mM borate buffer with no organic modifier and 25 mM methyl-b-cyclodextrin concentration adjusted to pH 8.0 at a potential of 30 kV. Detection wavelength and temperature were 197 nm and 208 ^{0}C respectively. Separation was performed on a fused-silica capillaries were 58.5 cm x 50 μm I.D.x 375 μm O.D. The method was successfully applied to the quantitative determination of these compounds in their corresponding pharmaceutical formulation.

3.2 Electrochemical method / Voltammetric method

Voltametric method was developed by M. Fernandez [7] for Torsemide by using differential-pulse adsorptive stripping voltametry at a hanging mercury drop electrode. The drug in Britton–Robinson buffer (pH 2.5). Under these conditions, the current showed a linear dependence with concentration in the range 2.29×10^{-7}–3.65×10^{-6} mol l^{-1}. The reproducibility, in terms of relative standard deviation, for 10 determinations of 4.89×10^{-7} and 9.83×10^{-7} mol l^{-1} solutions was 1.80 and 0.90%, respectively; the detection limit was 80 ppb (2.29×10^{-7} mol l^{-1}). The method was applied to urine samples spiked with torasemide, to obtain a recovery of 100.6 ± 3.4%.

3.3 Spectrophotometric method

(A) UV Spectroscopic method

K. Damodar Reddy *et al* [8] were developed a Kinetic Spectrophotometric method for determination of drugs Levofloxacin, Moxifloxacin, Pseudoephedrine and Torsemide based on oxidation by alkaline $KmnO_4$. Kinetics of the oxidation reaction is followed spectrophotometrically, as one of the reaction product, Mn(VI), absorbed at 610 nm. Initial rate and fixed time method are used for the construction of calibration curves Beer's law is obeyed in the range 6.25-37.5 µg ml-1 for LEV; 5-30 µg ml-1 for MOX; 6.25-37.5 µg ml-1 for PSE and 2.5-15 µg ml-1 for TOR.

A simple, accurate and precise dual wavelength spectrophotometric method was developed for simultaneous determination of Torsemide (TOR) and Amiloride HCl (AML) in their combined pharmaceutical

dosage form. 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 wavelengths selected for determination of TOR were 299.66nm (λ1) nm and 323.21nm (λ2) nm and the wavelengths selected for determination of AML were 276.11nm (λ3) and 300.0nm (λ4). Methanol was taken as a common solvent. The calibration curve was linear in the concentration range of 4-24 µg/ml for TOR and 4-14 µg/ml for AML. Developed method was successfully applied for the estimation of TOR and AML in their combined dosage form.[9]

A simple and cost effective spectrophotometric method is described for the determination of torsemide in pure form and in pharmaceutical formulations. The method is based on the formation of blue colored chromogen when the drug reacts with Folin-Ciocalteu (F-C) reagent in alkaline medium. The colored species has an absorption maximum at 760 nm and

obeys beer's law in the concentration range 30 – 150 µg mL^{-1}. The absorbance was found to increase linearly with increasing concentration of TSM, which is corroborated by the calculated correlation coefficient value of 0.9999(n=8). The apparent molar absorptivity and sandell sensitivity were 1.896x103 L mol^{-1} cm^{-1} and 0.183 µg cm^{-2}, respectively. The slope and intercept of the equation of the regression line are $5.4x10^{-3}$ and $1.00x10^{-4}$ respectively. The limit of detection was 0.94.The optimum experimental parameters for the reaction have

been studied. The validity of the described procedure was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of TSM in pharmaceutical formulations[10].

Three simple, accurate, and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Torsemide (TOR) and spironolactone (SPI) in combined tablet dosage form. The methods employed were absorbance ratio, I; first order derivative spectroscopy method, II; and area under curve (AUC) method, III. Torsemide showed absorbance maxima at 288 nm and spironolactone showed at 238 nm in methanol as solvent.

Beer's law was obeyed in concentration range of 0-25 mcg ml-1 for both drugs for all proposed three methods. The first developed method makes use absorbance ratio method using 255 nm as isobestic point. The second method is based on first order derivative spectroscopy to overcome spectral interference from other drug, wavelengths 315 nm and 225 nm were selected for the determination of the TOR and SPI respectively. Third method is area under curve method, the sampling wavelengths range selected are 294-290 nm and 240-236 nm with linearity for TOR and SPI respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines [11].

Simple and sensitive validated spectrophotometric methods has been developed for the assay of five drugs *viz.,* Gemifloxacin (GEM), Ondensetron (OND), Alfuzocin (ALF), Dulaxetine (DUL) and Torsemide (TOR) in pure and pharmaceutical formulations. The proposed method was based on the oxidation of drug by Chloramine-T (excess) and subsequent determination of unreacted Chloramine-T using methyl orange as analytical tool which absorbs at 510 nm. Beer's law is obeyed in the concentration of 2-14, 4-28 , 3-24 , 3-21 and 1.5- 8.5 µg mL^{-1} for GEM, OND, ALF, DUL and TOR respectively. Different variables affecting the reaction were studied and optimized. The proposed methods were applied successfully to the determination of the examined drugs in pure and pharmaceutical dosage forms with good accuracy and precision [12].

A simple and cost effective spectrophotometric method is described for the determination of Torsemidein pure form and in pharmaceutical formulations. The method is based on the formation derivative when the drug reacts with picric acid solution. The colored derivative solution has an absorption maximum at 350 nm; also obeys Beer's law in the concentration range 5-25 µg/mL. The absorbance was found to increase linearly with increasing concentration of torsemide, which is corroborated by the calculated correlation coefficient value of 0.997. The slope and intercept of the equation of the regression line are 0.035 and 0.002 respectively. The optimum experimental parameters for the reaction have been studied. The validity of the described procedure was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of Torsemide in pharmaceutical formulations [13].

A method for simultaneous estimation of torsemide (TRS) and spironolactone (SPL) in combined tablet dosage form has been developed. The method employs the application

of multicomponent mode of analysis. This method utilize 50 % v/v methanol in distilled water. TRS show maximum absorbance at a wavelength of 288 nm and SPL at 238 nm. Where the linearity ranges for TRS and SPL were 1-5µg/ml and 5-25 µg/ml respectively. The procedure was successfully applied for the simultaneous determination of both drugs in laboratory prepared mixture and in market available tablet dosage form. The accuracy of the method was assessed by recovery studies and was found to be 98.15±0.64 and 100.27±0.15 for TRS and SPL respectively. Results of the analysis were validated statistically so that it can be used for routine analysis of TRS and SPL in combined tablet dosage form [14].

3.4 Chromatographic method

(A) High performance liquid chromatography with UV spectroscopy

Four sensitive and precise methods for determination of torsemide in presence of its degradation product and in pharmaceutical formulation were developed and validated. Method A is the second derivative spectrophotometry at 262.4 nm with mean percentage recoveries 100.06±0.75. Method B is first derivative of the ratio spectra spectrophotometry, at 232.4, 244.6 nm and at the total peak amplitude from the maximum at 232.4 nm to the minimum at 244.6 nm (1DD232.4+244.6nm). Method C is a TLC-densitometric one, for torsemide separation using acetone : chloroform : ethyl acetate (4:4:2 v/v) as a developing system. Method D is HPLC one, it provides complete separation of torsemide from its degradation product on C8 column with UV detection at 287 nm and recovery 99.98±0.76. The proposed methods have been successfully applied to the analysis of torsemide in pharmaceutical formulations without interference from other additives and the results were statistically compared with the official method [15].

(B) Reverse phase high performance liquid chromatography

The chromatographic separation was performed on a Zorbax SB C_{18} analytical column (250 x 4.6 mm, 5 µm, Agilent) with column temperature set at 25°C. The mobile phase was an aqueous solution of 10 mM ammonium formate, adjusted to pH 2.5 with formic acid (mobile phase A) and acetonitrile (mobile phase B), with gradient elution: 0 min, B 30%; 11.2 min, B 60%; 11.3 min, B 30 %, hold for 10 minutes. The flow rate was 1 mL min^{-1} and the injection volume was 30 µL for LC–UV analysis. Detection was performed at 290 nm. The calibration curves show high linearity with the coefficients of correlation (*r*) greater than 0.9982. The obtained recovery values (95.78–104.92%) and relative standard deviation values (0.12–5.56%) indicate good accuracy and precision. Lower limit of detection (LOD) and limit of quantitation (LOQ) values are obtained with the LC–UV method, indicating higher sensitivity of the proposed method. [16].

Noorulla et.al.[17] developed a simple, precise, accurate, rapid Reverse phase- High performance Liquid chromatographic method for the simultaneous estimation of Torsemide and Eplerenone in tablet dosage form was developed and validated in the present studies. The mobile phase consists of a mixture of mixed phosphate buffer and Acetonitrile in the proportion of (55:45). The retention time was found to be 2.70 and 3.95 min for Torsemide and Eplerenone respectively. HPLC analysis was carried out at a wavelength at 261 nm with a flow rate 1ml/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 and 0.998 for Torsemide and Eplerenone respectively. The method was validated for accuracy, precision, robustness, ruggedness, specificity.

A simple, specific, precise and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Torsemide in Bulk and Pharmaceutical dosage form. The proposed RP-HPLC method was carried out on Zorbax C18 (250x4.6mm), 5µm column with mobile phase phosphate buffer and methanol (50:50) (v/v). The pH of phosphate buffer was adjusted by Ortho- phosphoric acid to 3.5. The flow rate was 1.3 mL/min and the detection wavelength was 288nm. The retention time of torsemide was found at 6.0±0.2min. The method was validated for specificity, precision, accuracy, linearity and robustness. The linearity range was 10-30 µg/mL and correlation coefficient (r^2) was found to be 0.9980. The mean % recovery for Torsemide was found to be 99.80. The developed method could be employed for the routine analysis of Torsemide from different formulations and for the Torsemide calculations as well.[18]

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 x 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 40-240 µg/ml for Torsemide and 100-600µg/ml for Eplerenone.[19]

A simple, rapid and selective method was developed. The method was validated and found to be linear in the range of 100-4000 ng/ml. Chromatographic peaks were separated by means of a 5 µm, C18 silica column using acetonitrile and phosphate buffer (0.05 M) in proportion of 40:60 (pH 4.0) as a mobile phase. The retention time of torsemide was 5.00±0.20 min. The chromatograms showed good resolution and no interference from plasma. The mean recovery from human plasma was found to be above 82%. Both inter-day and intra-day accuracy and precision data showed good reproducibility. This method was applied to a single dose bioequivalence study. Log transformed values were compared by ANOVA followed by classical 90% confidence interval. Confidence limits for C_{max}, AUC_{0-t} and AUC_{0-inf} ranged from 98.6 to 102.8, 101.8 to 105.3 and 102.4 to 105.5 respectively. These results suggested that the analytical method was linear, precise and accurate. Test and reference product were found to be bioequivalent [20].

Torsemide (TSM) is a loop diuretic used in the treatment of edema, and spironolactone (SPL) is a potassium-sparing diuretic used in hypokalemia. Both are potential diuretics used in combination to treat congestive heart failure. This paper describes a simple, sensitive, accurate, and validated reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous quantification of these compounds as the bulk drug and in tablet dosage forms. Separation was carried out on Jasco HPLC system equipped with Neosphere C_8 column (150 × 4.6 mm i.d.) and UV/VIS detector using acetonitrile:10 mM potassium dihydrogen phosphate buffer in ratio of 60:40 (*υ/υ*) as the mobile phase, and detection was carried out at 240 nm. Results were linear in the range of 2–12 µg mL^{-1} for TSM and 5–30 µg mL^{-1} for SPL. The method was successfully applied for the analysis of drugs in pharmaceutical formulation. Results of the analysis were validated statistically and by recovery studies [21].

A high-performance liquid chromatographic method for the determination of Torsemide is described. The assay uses a reversed-phase gradient system and UV-detection. The chromatographic separation was carried out on a µBondapak C18 column with a mobile phase consisting of Acetonitrile:Phosphate Buffer 0.05M (pH 2.4) in ratio of 70:30. The method was validated and found to be linear in the range of 50-100 µg/ml. The retention time of Torsemide was 6.00 ± 0.20 min. The chromatograms showed good resolution and no interference with impurity. The mean recovery of the Torsemide was found to be above 99.9%. Both accuracy and precision data showed good reproducibility. The linearity range was found to be 50-100 µg/ml with coefficient of variation of 0.998 at calibration point. The limit of detection and limit of quantization for Torsemide were found to be 148.1ng and 448.9 ng respectively. These results suggested that the analytical method was linear, precise, and accurate [22].

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 [23].

A simple, highly robust (quality by design (QbD) approach), precise and accurate method using high performance liquid chromatography coupled to mass spectrometry has been established for the simultaneous separation, identification and quantitation of a Torsemide (TOR), spironolactone (SPI) and their degradant impurities. The chromatographic separations of drugs and impurities were achieved on a inertsil ODS3 µm C18, 150 mm × 4.6 mm, while the isocratic elution using a ternary mobile phase mixture of methanol, acetonitrile and water (5:3:2 v/v/v) at a flow rate of 0.2 mL/ min was adopted for achieving optimum separations. The quantitation of torsemide and spironolactone was accomplished by UV detection at 254 nm and identification of the degradants were done by comparing identical mass in mass spectrometer. The recoveries of the torsemide and spironolactone were obtained higher than 98 % with good

validation parameters; linearity ($r^2 > 0.994$), LOD and LOQ was 10 and 33 ng for TOR and 75 and 248 ng for SPI respectively. The quality by design (QbD) approach has been successfully utilized to prove the method is robust even deliberate changes in critical parameters [24].

Validated high performance liquid chromatographic (HPLC) method for estimation of Torsemide (TOR) and Spironolactone (SPI) in tablet dosage form. Isocratic RP-HPLC separation was achieved on Licrosphere C18 column (250 x 4.6mm) using Methanol: Acetonitrile: Phosphate buffer, pH 6.5 (60:20:20 v/v) at flow rate of 1.5 ml/min at 30° C temperature. Quantitation was achieved by UV detection at 252 nm over the conc. range 0-25 µg/ml for both the drugs with mean recoveries of 100.01% + 0.12 and 100.64% + 0.20 for TOR and SPI respectively. This method is simple, precise and sensitive and applicable for the simultaneous estimation of TOR and SPI in tablet pharmaceutical combined dosage form[25].

Simple, accurate and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Spiranolactone and Torsemide in combined tablet dosage form has been developed and validated. Beer's law was obeyed in the concentration range of 5-25 µg/mL for both drugs in methanol. The RP-HPLC method uses a Shimadzu LC 10 AT_{VP} system with a Luna C_{18} column and methanol: acetonitrile : phoasphate buffer, pH 3.5 (60 : 20 : 20 % v/v) as the mobile phase. The detection was carried out using a diode array detector set at 238 nm. The recoveries were found to be in the range of 99.56±0.04 to 100.75±0.15 and 99.56± 0.35 to 100.33 ± 0.56 for Torsemide and Spiranolactone, respectively. Developed method was found to be simple, precise, sensitive and may be used for routine analysis of Torsemide and Spiranolactone in a pharmaceutical formulation. Results of analysis were validated statistically per ICH guidelines [26].

HPLC and spectrophotometirc methods were developed and validated for the quantitative determination of two diuretics, Spiranolactone and Torsemide. The different analytical performance parameters such as linearity, precision, accuracy, specificity and robustness were determined according to ICH Q2 B guidelines. Chromatography was carried out by isocratic technique or a reverse phase C18 Inertsil (250 mmx 4.6 mm), 5µm column with mobile phase composed of methanol: water (80:20). The UV spectrophotometric determinations (dual wavelength) were performed at 210 nm and 268 nm for spiranolactone and 243 and 330.5 nm for torsemide. Both the methods were accurate and precise with recoveries ranging from 98.12 % and 101 % for both drugs and RSD < 2%[27].

One HPLC and one UV spectrophotometric method have been developed for the determination of torsemide (TRS) in tablet dosage form. The first method is based on determination of TRS in tablet dosage form by RP- HPLC method. Chromatgraphy was carried out on a nucleosil C-18,250 x 4.6 mm column using a mixture of phosphate buffer and methanol (50:50 v/v) as the mobile phase at a flow rate of 1.3 ml/min. Run time was 15 min. Detection was done at 288 nm and retention time of the drug was 7.05 min. This method produced linear responses in the concentration range 60-140 µg/ml of torsemide. The accuracy of the method was assessed by recovery studies and was found to be 99.90± 0.41 for torsemide. The second method is based on the estimation of torsemide in tablet dosage form by UV spectrophotometry using 50% v/v methanol in distilled water. Beer's law obeyed over the concentration range 2-26 µg/ml at 288 nm with apparent molar absorptivity of 1.26×10^4. Both developed methods were found to be applicable for routine analysis of drug in tablet dosage form. The result of the analysis were validated statistically.The results were compared obtained from UV spectrophotometry and HPLC [28].

(D) Stability indicating chromatographic method

An isocratic reverse phase liquid chromatography method has been developed for quantitative determination of Torsemide and Spironolactone alongwith their related compounds using a 150 X 4.6mm, 5µ Hypersil BDS C8 column with a mobile phase composition of buffer pH 5.0: methanol in equal quanitites. The flow rate was 1.0mL min^{-1} and wavelength was set at 260 nm. Resolution between torsemide and its impurity, and that due to Spironolactone and its impurity canrenone was more than 2.0 and 3.0 respectively. The method was validated for selectivity, linearity, accuracy, precision, limit of detection and limit of quantitation. Impurities of torsemide and spironolactone gave linear response. For the assay study torsemide and spironolactone showed linear response. The stress studies showed that the method was specific, selective to study torsemide, spironolactone and impurity1, impurity 2, impurity 3 of torsemide and impurity 4 of spironolactone. The peak purity of analyte showed that unknown degradation products formed during stress studies did not interfere with the determination of all the studied analytes.

The mass balance for assay was achieved for torsemide and spironolactone[29].

A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Eplerenone and Torsemide In Their Combined Dosage Form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Eplerenone and Torsemide. In Their Combined Dosage Form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (pH 3.5): Methanol (60:40) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 276 nm. Retention time of Eplerenone and Torsemide were found to be 3.200 min and 5.023 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Eplerenone 6.25-18.75 µg/ml and for Torsemide 2.5-7.5 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Eplerenone and Torsemide In Their Combined Dosage Form. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in alkaline degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form [30].

(E) High performance thin layer chromatographic method

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Torsemide in the presence of its degradation products. The stationary phase used was precoated silica gel 60F254. The mobile phase used was a mixture of ethyl acetate–acetonitrile–water 6.5:3.0:0.5 (v/v). The detection of spots was carried out at 297 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 200 to 800 ng/spot for Torsemide [31].

Validated high performance thin liquid chromatographic (HPTLC) method for estimation of Torsemide (TOR) and Spironolactone (SPI) in tablet dosage form. A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the determination of Torsemide and Spironolactone in dosage form. The stationary phase used was precoated silica gel 50 F254. The mobile phase used was a mixture of ethyl acetate: acetone: acetic acid (10.5: 4: 1.5v/v/v). The detection of spot was carried out at 269.0 nm. Developed method was validated in terms of linearity, accuracy, precision, repeatability and specificity. Limit of detection and limit of quantification of Torsemide and Spironolactone were found to be 120 ng/spot and 178 ng/spot, respectively. The linearity range for Torsemide and Spironolactone was found to be 360-850 ng/spot with correlation coefficient of 0.998.The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of methods[32].

A simple, sensitive, and precise high-performance thin-layer chromatographic method has been developed for the estimation of torsemide and amiloride hydrochloride in the pharmaceutical dosage form. Thin-layer chromatographic (TLC) aluminum plates precoated with silica gel 60 F_{254} were used as the stationary phase, while chloroform-methanol-ammonia (7.5: 3.5: 1, *v/v*) as mobile phase was used. The R_F values observed were 0.46 ± 0.01 and 0.24 ± 0.01 for torsemide and amiloride hydrochloride, respectively. The densitometric analysis was carried out in absorbance mode at 286 nm. The method was linear in the range of 100–600 ng $spot^{-1}$ for torsemide and 50–300 ng $spot^{-1}$ for amiloride hydrochloride. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The limit of detection and limit of quantitation were found to be 19.94 ng $spot^{-1}$ and 100 ng $spot^{-1}$, respectively, for torsemide. The limit of detection and limit of quantitation were found to be 12.86 ng $spot^{-1}$ and 50 ng $spot^{-1}$, respectively, for amiloride. The proposed method was successfully applied to the estimation of torsemide and amiloride hydrochloride in the pharmaceutical dosage form[33].

A new simple High Performance Thin Layer Chromatographic (HPTLC) method for determination of Spironolactone and Torsemide in combined tablet dosage form has been developed and validated. The mobile phase selected was n-Hexane: Ethyl acetate: Methanol: Glacial acetic acid (7: 3: 1.5: 0.5 v/v/v) with UV detection at 263 nm. The retention factor for SPL and TSM were found to be 0.67 ± 0.03 and 0.34 ± 0.03. The method was validated with respect to linearity, accuracy, precision and robustness. Results found to be linear in the concentration range of 100-1000 ng/band for SPL and TSM respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 99. 75 ± 0.134 for SPL and 98.67 ± 0.153 for TSM [34].

(F) High performance liquid chromatography coupled with mass spectrometry

A sensitive and selective method using high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC–ESI–MS) to determine the concentration of torasemide in human plasma samples was developed and validated. Tolbutamide was chosen as the internal standard (IS). The chromatography was performed on a Gl Sciences Inertsil ODS-3 column (100 mm×2.1 mm i.d., 5.0 µm) within 5 min, using methanol with 10 mM ammonium formate (60:40, v/v) as mobile phase at a flow rate of 0.2 mL/min. The targeted compound was detected in negative ionization at *m/z* 347.00 for torasemide and 269.00 for IS. The linearity range of this method was found to be within the concentration range of 1–2500 ng/mL (*r*=0.9984) for torasemide in human plasma. The accuracy of this measurement was between 94.05% and 103.86%. The extracted recovery efficiency was from 84.20% to 86.47% at three concentration levels. This method was also successfully applied in pharmacokinetics and bioequivalence studies in Chinese volunteers.[35]

The chromatographic separation was performed on a Zorbax SB C_{18} analytical column (250 x 4.6 mm, 5 µm, Agilent) with column temperature set at 25°C. The mobile phase was an aqueous solution of 10 mM ammonium formate, adjusted to pH 2.5 with formic acid (mobile phase A) and acetonitrile (mobile phase B), with gradient elution: 0 min, B 30%; 11.2 min, B 60%; 11.3 min, B 30 %, hold for 10 minutes. The flow rate was 1 mL min^{-1} and the injection volume was 10 µL for LC–MS analysis. Detection was performed by using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The calibration curves show high linearity with the coefficients of correlation (*r*) greater than 0.9982. The obtained recovery values (95.78–104.92%) and relative standard deviation values (0.12–5.56%) indicate good accuracy and precision. Lower limit of detection (LOD) and limit of quantitation (LOQ) values are obtained with the LC–MS method, indicating higher sensitivity of the proposed method. [36]

g) High performance liquid chromatography coupled with PDA detector

A sensitive, accurate, precise and rapid HPLC-PDA method was developed and validated for the simultaneous determination of torasemide and spironolactone in human plasma using Design of experiments. Central composite design was used to optimize the method using content of acetonitrile, concentration of buffer and pH of mobile phase as independent variables, while the retention factor of spironolactone, resolution between torasemide and phenobarbitone; and retention time of phenobarbitone were chosen as dependent variables. The chromatographic separation was achieved on Phenomenex C18 column and the mobile phase comprising 20 mM potassium dihydrogen *ortho* phosphate buffer (pH-3.2) and acetonitrile in 82.5:17.5 *v/v* pumped at a flow rate of 1.0 mL min–1. The method was validated according to USFDA guidelines in terms of selectivity, linearity, accuracy, precision, recovery and stability. The limit of quantitation values were 80 and 50 ng mL^{-1} for torasemide and spironolactone respectively. Furthermore, the sensitivity and simplicity of the method suggests the validity of method for routine clinical studies [37].

Table 1: Development Parameters for Chromatographic Methods

Development Parameters for Chromatographic Methods Drugs	Method	Year	Mobile phase(v/v)	Column/ Stationary phase	Flow rate (mL/min)	Wave length (nm)	Ref
Torsemide	HPLC	2016	Phosphate buffer pH 4 : acetonitrile (3:2).	C8 (150 mm × 4.6 mm i.d.), 5 µm	1	287	15
Torsemide	HPTLC	2016	Acetone : chloroform : ethyl acetate (4:4:2)	Silica gel 60 F 254 TLC plate	-	287	15
Torsemide and its impurities	RPHPLC-UV	2012	10 mM ammonium formate, adjusted to pH 2.5 with formic acid (mobile phase A) and acetonitrile (mobile phase B), with gradient	Zorbax SB C_{18} (250 x 4.6 mm, 5 µm,	1	290	16

			elution: 0 min, B 30%; 11.2 min, B 60%; 11.3 min, B 30 %, hold for 10 minutes				
Torsemide and Eplerenone	RPHPLC	2017	Mixture of phosphate buffer and Acetonitrile (55:45)	Inertsil ODS 3V column C_{18}	1	261	17
Torsemide	RPHPLC	2014	Phosphate buffer and methanol (50:50)	Zorbax C18 (250x4.6mm), 5µm	1.3	288	18
Torsemide and Eplerenone	RPHPLC	2016	Acetonitrile: Methanol: water (30:50:20)	C18 (250 x 4.6 mm, 5µm)	1	268	19
Torsemide in human plasma	HPLC	2008	Acetonitrile and phosphate buffer (0.05 M) 40:60 (pH 4.0)	C18 silica			20
Torsemide and spironolactone	RPHPLC	2012	Acetonitrile:10 mM potassium dihydrogen phosphate buffer 60:40	Neosphere C_8 column (150 × 4.6 mm i.d.)			21
Torsemide	RPHPLC	2012	Acetonitrile:Phosphate Buffer 0.05M (pH 2.4) in ratio of 70:30	µBondapak C18			22
Torsemide and Amiloride	RPHPLC	2014	Methanol:Phosphate buffer pH 3.6 (10:90)	BDS C18 (150 × 4.6 mm, 5 µm)	1	288	23
Torsemide, spiranolactone and its degradants	RPHPLC	2018	Mixture of methanol, acetonitrile and water (5:3:2)	Inertsil ODS3 µm C18, 150 mm × 4.6 mm	0.2	254	24
Torsemide and spiranolactone	RPHPLC	2010	Methanol: Acetonitrile: Phosphate buffer, pH 6.5 (60:20:20)	Licrosphere C18 column (250 x 4.6mm)	1.5	252	25
Torsemide and spiranolactone	RPHPLC	2012	Methanol: acetonitrile : phoasphate buffer, pH 3.5 (60 : 20 : 20)	Luna C_{18}			26

Torsemide and spiranolactone	RPHPLC	2010	Methanol: water (80:20)	C18 Inertsil (250 mmx 4.6 mm), 5μm			27
Torsemide	RPHPLC		Phosphate buffer and methanol (50:50)	Nucleosil C-18,250 x 4.6 mm	1.3	288	28
Torsemide and spiranolactone	Stability indicating	2013	buffer pH 5.0: methanol(50:50)	150 x 4.6mm, 5μ Hypersil BDS C8	1	260	29
Torsemide and Eplerenone	Stability indicating	2017	Buffer (pH 3.5): Methanol (60:40)	LC- 20 AT C18 (250mm x 4.6 mm x 2.6 μm)	1	276	30
Torsemide	HPTLC	2011	Ethyl acetate–acetonitrile–water 6.5:3.0:0.5	Silica gel 60F254	-	297	31
Torsemide and spiranolactone	HPTLC	2010	Ethyl acetate: acetone: acetic acid (10.5: 4: 1.5)	Silica gel 60F254	-	269	32
Torsemide and Amiloride	HPTLC	2014	Chloroform-methanol-ammonia (7.5: 3.5: 1)	Silica gel 60F254	-	286	33
Torsemide and spiranolactone	HPTLC	2010	n-Hexane: Ethyl acetate: Methanol: Glacial acetic acid (7: 3: 1.5: 0.5)	Silica gel 60F254	-	263	34
Torsemide	LC-MS	2016	10 mM ammonium formate (60:40)	Inertsil ODS-3 column (100 mm×2.1 mm i.d., 5.0 μm)	0.2	ESI-MS	35
Torsemide	LC-MS	2012	Aqueous solution of 10 mM ammonium formate, adjusted to pH 2.5 with formic acid (mobile phase A) and acetonitrile (mobile phase B), with gradient elution: 0 min, B 30%; 11.2 min, B 60%; 11.3 min, B 30 %, hold for 10 minutes	Zorbax SB C_{18} analytical column (250 x 4.6 mm, 5 μm	1	ESI-MS	36

Torsemide and spiranolactone	HPLC-PDA	2015	20 mM potassium dihydrogen *ortho* phosphate buffer (pH-3.2) : acetonitrile (82.5:17.5)	Phenomenex C_{18} column	1		37

Table 2: Validation Parameters for Chromatographic Methods

Drugs	**Method**	**Year**	**Linearity range**	**LOD**	**LOQ**	**Ref**
Torsemide	HPLC	2016	20-60 µg/mL (TOR)	0.22 µg/mL (TOR)	0.66 µg/mL (TOR)	15
Torsemide	HPTLC	2016	0.25–2.50 mg/mL (TOR)	0.08 (µg/band) (TOR)	0.25(µg/band) (TOR)	15
Torsemide and its impurities	RPHPLC-UV	2012	70–130 µg/ mL (TOR)	0.035 µg/ mL (TOR)	0.100 µg/ mL (TOR)	16
Torsemide and Eplerenone	RPHPLC	2017	5-15µg/ml (TOR) 12.5-37.5 µg/ml (EPL)	-	-	17
Torsemide	RPHPLC	2014	10-30 µg/ml (TOR)	-	-	18
Torsemide and Eplerenone	RPHPLC	2016	40-240 µg/ml (TOR) 100-600 µg/ml (EPL)	10.7 µg/ml (TOR) 19.3 µg/ml (EPL)	32.25 µg/ml (TOR) 58.16 µg/ml (EPL)	19
Torsemide in human plasma	HPLC	2008	100- 4000 ng/mL (TOR)	-	-	20
Torsemide and spironolactone	RPHPLC	2012	2–12 µg/mL (TOR) 5–30 µg/mL (SPI)	-	-	21
Torsemide	RPHPLC	2012	50-100 µg/ml (TOR)	0.1481 µg/ml (TOR)	0.4489 µg/ml (TOR)	22
Torsemide and Amiloride	RPHPLC	2014	6–14 µg/mL (TOR) 3–7 µg/mL (AML)	0.0418 µg/mL (TOR)	0.1268 µg/mL (TOR) 0.1249 µg/mL (AML)	23

				0.0412 µg/mL (AML)		
Torsemide, spiranolactone and its degradants	RPHPLC	2018	2–12 µg/mL (TOR) 10–60 µg/mL (SPI)	10 ng(TOR) 75 ng (SPI)	33 ng(TOR) 248 ng (SPI)	24
Torsemide and spiranolactone	RPHPLC	2010	0-25 µg/mL (both drugs)	1 µg/mL (TOR) 0. 9 µg/mL (SPI)	10 µg/mL (TOR) 1 µg/mL	25
Torsemide and Spiranolactone	RPHPLC	2012	5-45 µg/mL (TOR) 5-25 µg/mL(SPI)	0.01 µg/mL (TOR) 0.004 µg/mL (SPI)	0.03 µg/mL (TOR) 0.010 µg/mL (SPI)	26
Torsemide and Spiranolactone	RPHPLC	2010	10-50 µg/mL (TOR) 25-125 µg/mL(SPI)	-	-	27
Torsemide	RPHPLC	-	2-26 µg/ml (TOR)	-	-	28
Torsemide and Spiranolactone	Stability indicating	2013	1-20 µg/mL (TOR) 5-100 µg/mL(SPI)	0.043 µg/mL (TOR) 0.022 µg/mL (SPI)	0.13 µg/mL (TOR) 0.65 µg/mL (SPI)	29
Torsemide and Eplerenone	Stability indicating	2017	2.5-7.5 µg/ml (TOR) 6.25-18.75 µg/ml (EPL)	0.067 µg/mL (TOR) 0.168 µg/mL (EPL)	0.203 µg/mL (TOR) 0.508 µg/mL (EPL)	30
Torsemide	HPTLC	2011	200-800 ng/band (TOR)	31 ng/band (TOR)	94 ng/band (TOR)	31
Torsemide and spiranolactone	HPTLC	2010	400-1000 ng/spot (TOR) 600-2500 ng/spot (SPI)	240 ng/spot(TOR) 24 ng/spot (SPI)	420 ng/spot(TOR) 70 ng/spot (SPI)	32
Torsemide and Amiloride	HPTLC	2014	100–600 ng/ spot (TOR) & 50–300 ng/ spot (AML)	19.94 ng/ spot (TOR) & 12.86 ng/ spot (AML)	100 ng/ spot (TOR) & 50 ng/ spot (AML)	33
Torsemide and spiranolactone	HPTLC	2010	100-1000 ng/band (Both drugs)	-	-	34
Torsemide	LC-MS	2016	1–2500 ng/mL (TOR)	0.5 ng/mL (TOR)	1.0 ng/mL (TOR)	35

Torsemide	LC-MS	2012	0.7–1.3 µg/mL(TOR)	0.0002 µg/mL (TOR)	0.0006 µg/mL(TOR)	36
Torsemide and spiranolactone	HPLC-PDA	2015	0.3-6.0 µg/mL (TOR) 0.1-2.0 µg/mL(SPI)	25 ng/mL (TOR) 15 ng/mL (SPI)	80 ng/mL (TOR) 50 ng/mL (SPI)	37

3.5. Thermal method

Thermal treatment of torasemide form A resulted in several effects which were divided into five steps. These were investigated and discussed applying TG-MS and TG-FTIR with additional information derived from SEM, hot-stage and FTIR microscopy. The investigated crystal form of torasemide represents a mixed solvate including ethanol and water. Its desolvation, the solid-solid transformation into the anhydrate mod. II and the melting of this anhydrate is elucidated using thermal analysis and microscopic observations (FTIR and hot-stage microscopy). The released and evaporated solvents were determined with coupled techniques. On further heating the structural identification of evolved gases allowed the analysis of the degradation pathway of torasemide up to 340°C[38].

4. Significance of Methods and Limitations

The selection of analytical method is very important task, by imparting main focus on important analytical parameters as selectivity, efficiency, sensitivity and robustness. The sensitivity is the most important method characteristic in the evaluation of the samples with small amount of analyte and these can be evaluated by comparing the values of LOD (Limit of detection) obtained by different methods. The principal advantages of voltammetric method are its rapidity and simplicity. Each voltammetric run takes few seconds. It involves no clean up procedures. Capillary electrophoresis method gives more specificity, and accuracy. Spectrophotometry has always provided analytical techniques characterized by instrumental simplicity, moderate cost and portability. In these methods, fluorescence detection offers superior sensitivity and selectivity compared to that provided by UV detection. On the other hand, the HPLC shows advantages over spectrophotometric methods lies in its separation capability. Through chromatographic separations, the analytes of interest can be detected and quantified in the presence of degradation products and excipients. Additionally, capillary electrophoresis can be an excellent alternative separation technique to HPLC, however its limit of detection is usually about 50 times poorer than that of HPLC. At present, HPLC is the most widely used technique for the analysis of bulk drugs and their formulations. However, HPLC methods show limitations as cost of columns, solvents and a lack of long-term reproducibility due to the proprietary nature of column packings. The choice of proper detection mode in HPLC analysis is crucial to ensure that all the components are detected. With UV detection, the use of this problem could be overcome by using a multiple wavelength scanning program which is capable of monitoring several wavelengths simultaneously. In general, fluorescence detection offers superior sensitivity and selectivity compared to that provided by UV detection. Liquid chromatography combined with mass spectrometry (LC-MS) is considered as one of the most important techniques of the last decade of 20th century. It became the method of choice for analytical support in many stages of quality control and assurance within the pharmaceutical industry .The main advantage of mass spectrometric is its capability for analyzing both chromophoric and non-chromophoric drug compounds with superior selectivity and sensitivity. As the other techniques, this method shows some disadvantages as high cost, few well-defined analytical procedures fit in the current GMP requirements and variability associated with unstable ionization source and significant interference from the formulation excipients.

5. Conclusion

The presented review provides information about the various methods available in the literature for the determination of Torsemide alone and in combination with other drugs and determination of Torsemide in biological samples. The analysis of the published data revealed that the HPLC was exclusively used for determination of Torsemide. Determination of Torsemide in formulation and biological samples, were commend HPLC-MS/MS method, since this method combines the HPLC Separation ability with MS Sensitivity and selectivity, allowing the

unambiguous identification of Torsemide. Sometime, HPLC with UV Detection is applicable because this method provides accurate results and low cost compared to more advanced detection techniques. This review carried out an overview of the current state-of-art analytical methods for determination of Torsemide.

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