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Development and Validation of RP-HPLC method for the quantitative estimation of Torsemide in Pharmaceutical dosage forms and Human Serum

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Abstract: A simple and rapid high performance liquid chromatographic method for the determination and quantification of torsemide has been developed. The chromatographic system consists of shimazu LC-10-AT VP pump, SPD-10 AV VP UV/visible detector. Separation was achieved on the reversed phase Hypersil BDS C_{18} column along with mobile phase consisting of buffer and acetonitrile (55:45 v/v) at pH of 3.8. The sample was introduced through injector valve with a 40 µl sample loop. Therapeutic concentrations of torsemide in serum were measured by reversed – phase liquid chromatography, with detection by ultraviolet absorbance at 261 nm. The results obtained showed a good agreement with the declared content. Recovery values of torsemide in tablet were from 99.94% to 100.08% and in serum 99.92% to 99.96%. The proposed method is rapid, accurate and selective; it may be used for the quantitative analysis of torsemide from pharmaceutical formulations and in human serum.
Keywords: Torsemide tablets, Serum, RP-HPLC, Hypersil BDS C_{18} column.

1. INTRODUCTION

Torsemide [1, 2] chemically 3-[4-[(3-methyl phenyl) amino] pyridine – 3-yl] sulfonyl -1- propan -2- yl urea (Fig.1), is a loop diuretic mainly used in the management of edema associated with congestive heart failure. It is also used at low doses for the management of hypertension [3, 4]. Torsemide acts by inhibiting the $Na^+/K^+/2Cl^-$ carrier system [5] in the lumen of the thick ascending portion of the loop of Henle, resulting in the decrease in reabsorption of sodium and chloride [6-9]. Literature survey revealed several analytical methods for the determination of diuretics in formulation, which employ techniques such as High performance liquid chromatography (HPLC) in human plasma and Urine [10] and few spectroscopic methods[11] for estimation in formulation. Reported methods involve complicated time consuming multi step liquid – liquid extraction techniques.

To the best of my knowledge, no RP-HPLC method has been described for the estimation of torsemide in pharmaceutical formulations and in human serum. Therefore, it was thought-worthwhile to develop simple, precise, accurate RP-HPLC method for determination of torsemide in tablet formulations and human serum.

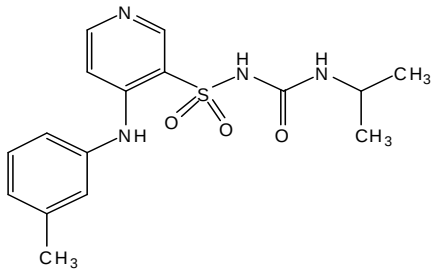

Fig.1: Chemical structure of Torsemide

2. EXPERIMENTAL:

Chemicals:

Acetonitrile and methanol of HPLC grade were purchased from qualigens fine chemicals (Mumbai, India). Water HPLC grade was obtained from a milli – Q - water purification system. Whattman filter paper (No.1) was obtained from Merck and Potassium dihydrogenorthophosphate of AR grade was obtained from S.D fine chemicals (Mumbai, India)

Instrumentation and analytical conditions:

The HPLC method was performed on a Shimadzu (Japan) SPD -10 A VP system comprising an LC – 10AT VP pump, an auto sampler, and an SPD -10 A VP detector. Data processing was by Shimadzu Class – VP software on a Hewlett – Packard computer. Compounds were separated on a 250 mm x 4.6 mm, 5µm particle, Hypersil BDS C_{18} column. Torsemide was eluted with a flow rate of 1.0 ml. min^{-1} using a mobile phase consisting of 0.01M phosphate buffer (pH 3.8) and acetonitrile (55:45 v/v). The wavelength of UV detector was set to 261 nm.

Preparation of stock solution of torsemide:

10 mg of torsemide was dissolved in methanol in 100 ml volumetric flask and made up the volume with the same solvent (Stock solution of 100 µg ml^{-1}). Aliquots were appropriately diluted.

Preparation of standard and working solution:

Clinical study:

Five hypertensive patients participated in this study. The patients were administered a single dose (2x10mg) of torsemide slow release tablets. An indwelling venous cannula was inserted into the antebrachial vein and blood samples were drawn at 1, 2, 3, 4, 5, and 6 h after drug administration. The samples were frozen at -70^{0}C pending analysis. The sampling was carried out under a sodium lamp or extremely subdued light and all tubes and syringes were wrapped in aluminum foil because of the photolability of torsemide.

Sample extraction procedure:

500 µl spiked plasma calibration curve standards and drug samples (2-14µg/ml) were transfer to pre – labeled polypropylene tubes containing 50 µl of mobile phase. The tubes were vortexed for ten seconds. Each of the tubes was added 2.5 ml of extraction solvent (Ethyl acetate). The tubes were vortexed for 10 min at 2000 rpm on a vibramax unit and then were centrifuged at 4000 rpm for 5 minutes in a refrigerated centrifuge at 10^{0}C temperature. From the centrifuged poly propylene tubes approximately 2.0 ml of supernatant extracted solvent was transferred to a new set of pre-labeled poly propylene tubes. The contents of the tubes were evaporated in steam of nitrogen at 40^{0}C for 10 minutes and the residues of the dried tubes were reconstituted with 0.2 ml mobile phase. The contents of the tubes were vortexed and transferred into auto sampler vials and then analyzed with HPLC unit by injecting 40 µl of sample volume.

3. RESULTS AND DISCUSSION:

This work was designed to develop an isocratic method based on RP-HPLC separation for torsemide assay in pharmaceutical dosage formulations as well as in human serum. The goal of this study was to develop a rapid, more accurate, precise, reliable, least time consuming HPLC method for the torsemide drug. This analytical method was developed taking in account the therapeutic and overdose concentration range, has been validated and holds well for the determination of drug in dosage formulations and especially in human serum.

To optimize the operating conditions for isocratic RP-HPLC detection of analyte, a number of parameters such as the mobile phase composition, pH and the flow rate. The ratio of phosphate buffer: acetonitrile was tested as starting solvent for system suitability study. The mobile phase leads to considerable in the chromatographic parameters, like peak symmetry, capacity factor and retention time. The pH effect showed that optimized conditions are reached when the pH value is 3.8, producing well resolved and sharp peaks for drug assayed. Hence forth, in the present method pH adjusted to 3.8 using wavelength 261 nm. The retention time of torsemide was 3.91 min at flow rate 1.0 ml/minute. The chromatograms have been shown in Fig.3.

Torsemide is practically insoluble in water, soluble in methanol; calibration solutions were prepared in methanol. Solubility increases at pH>3. During the HPLC method developments following considerations were made.

Serum deproteination for the determination of drugs is commonly accepted as the simplest method of sample preparation. The developed HPLC procedure for the determination of torsemide was based on extraction from serum sample and involved the injection of the serum samples after precipitation of protein with mobile phase (Phosphate buffer acetonitrile). Peak plasma levels reported for torsemide was 2-20 µg ml^{-1} (Fig.2).

The results of the present study demonstrate that the determination of torsemide was very beneficial for pharmaceutical companies, it also benefits in the studies of drug interactions. Symmetrical peak was observed for torsemide without internal standard shown in Fig.3 the method is accurate, very low LOD and LOQ. The method is very useful because it is less time consuming.

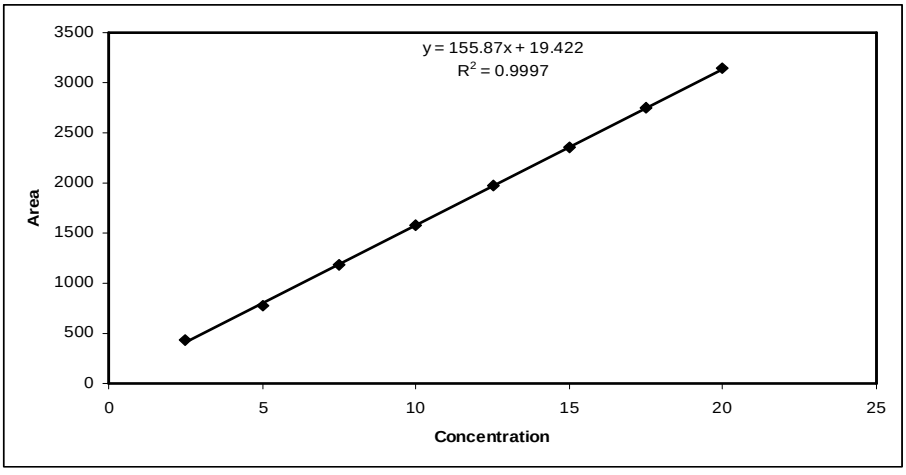

Fig.2: Calibration plot of Torsemide (HPLC)

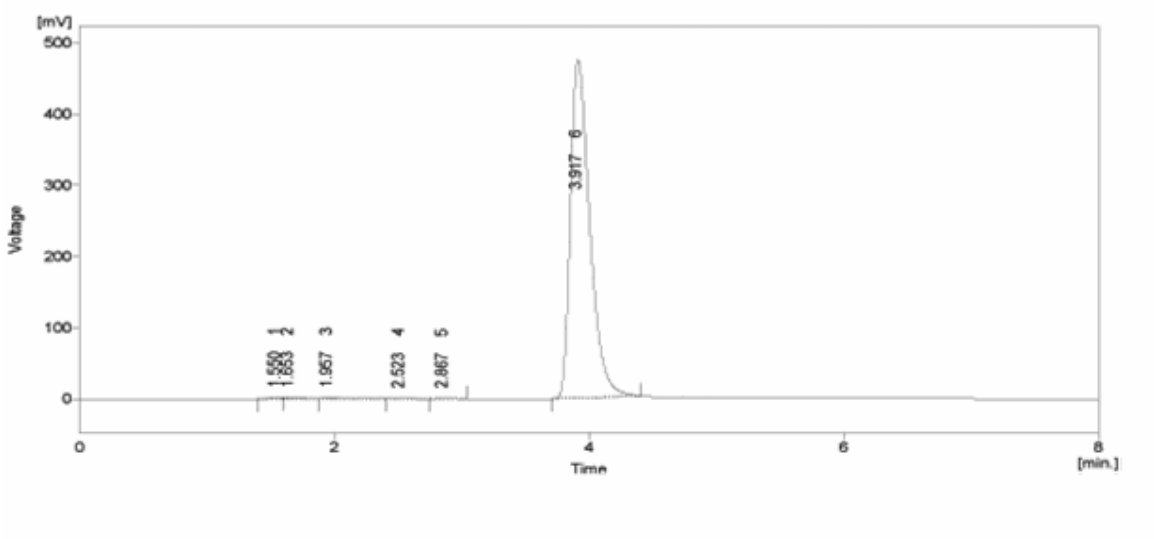

Fig.3: Chromatogram of a diluted solution of tablet formulation containing Torsemide

Method validation:

The method was validated according to International conference on Harmonization Q2B guidelines [12] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for analyte [13, 14].

Linearity:

Six point calibration curves were generated with appropriate volumes of working standard solutions for HPLC method. The calibration range was 2.0 -20.0 µg/ml in the HPLC method of analysis for the drug. The linearity was evaluated by the least square regression method using a weighed data presented in Table.1.

Precision and accuracy:

Inter and Intra day precision and accuracy of the method was evaluated at different independent concentrations by adding known quantities of analyte to the drug product. The results of accuracy revealed that the method was accurate for all above purposes. The method passed the test for repeatability as determined by %RSD of the area of the peaks of six replicate injections at 100^0C test concentration. Results are cited in Table.2.

Specificity:

The method specificity was assessed by comparing the chromatogram (HPLC) obtained from the drug and the most commonly used excipinets mixture with those obtained from blank, (excipients solution in water without drug). The excipients chosen are the ones used commonly in tablet formulation, which included lactose, starch, and cellulose and magnesium sterate. The drug to excipient ratio used was similar in the commercial formulations.

Detection and quantification limit [14]:

The LOD and LOQ for pharmaceutical preparation and in serum were determined and are presented in table.1. The calculated LOD and LOQ values confirmed that method were sufficiently sensitive.

Recovery:

Different concentration of human serum samples and pure samples of drug were linear, accurate, precise and selective by running three replicates of each concentration measured for five days. The mean recoveries are summarized in Table.3.

Table.1: Validation parameters of the HPLC method of Torsemide

Parameters	**values**
Retention time, R_t (min)	3.917
HETP (h, mm)	0.0582
Capacity factor(k^1)	0
Theoretical Plates (N)	4293
Tailing Factor(T)	1.733
Resolution (R)	0.00
LOD (Bulk material) µg ml^{-1}	0.028
LOD (Serum) µg ml^{-1}	0.04
LOQ (Bulk material) µg ml^{-1}	0.073
LOQ (Serum) µg ml^{-1}	0.0121
Slope	155.87
Intercept	19.422
Correlation coefficient	0.9997

Table.2: Inter - day and Intra - day precision and recovery of torsemide in formulations and human serum sammples

S.NO	**Torsemide taken µg/ml**	**Intra - day**			**Inter - day**		
		Found µg/ml	**Recovery ± RSD%**	**RE%**	**Found µg/ml**	**Recovery ± RSD%**	**RE%**
Formulations							
1	2.0	2.001	100.08±0.24	0.08	1.999	99.96±0.081	-0.04
2	4.0	3.99	99.82±0.097	-0.18	3.996	99.92±0.124	-0.08
Human Serum samples							
1	2.0	1.998	99.94 ±0.129	-0.06	1.999	99.95±0.145	-0.05
2	3.0	2.996	99.89±0.064	-0.106	2.997	99.92 ±0.112	-0.08

Table.3: Method accuracy from recovery assay for the studied analyte.

S.No	**Added µgml^{-1}**	**Found µgml^{-1}**	**Recovery ± SD**
Serum (5%)			
1	2.0	1.9996	99.98 ±0.097
2	4.0	3.999	99.97±0.084
3	6.0	5.9958	99.93±0.024
Pharmaceutical formulations			
1	0.4	0.400	100.05±0.273
2	0.8	0.801	100.15±0.223
3	2.0	1.9988	99.94±0.129

APPLICATIONS

Analysis of tablets

Ten commercial tablets and the contents of ten – tablet ingredients were separately weighed and powdered in different mortars. A portion of the powder equivalent to about one tablet and the content of one tablet was weighed accurately, transferred to a 100 ml calibrated flask and suspended in mobile phase for HPLC method. The flasks were completed to volume with same solvent. The samples were filtered through a 0.45µm membrane filter, and then further diluted to suit the calibration graph. Results are shown in Table. 3.

Determination of torsemide in spiked human serum Samples:

The proposed HPLC method was applied successfully to the determination of torsemide in spiked human serum. In order to evaluate the validity of the proposed method, recovery studies were carried out on samples to which known amounts of torsemide was added, with recoveries from 99.82 to 100.08%. Results are shown in Table.3.

4. CONCLUSION:

Some analytical problems of the detection and identification of diuretics and their metabolites are due to the wide variety of their chemical structures and functional groups, wide differences in pka values, low volatility and lack of metabolite studies in several cases. On the other hand, RP-HPLC has emerged as one of the most accepted and widely employed techniques for determination of torsemide in biological fluids (serum) owing to high efficiency, reliability, versatility and being able to be combined with other tools, mass spectrophotometry.

The developed RP-HPLC method is able to determine torsemide from dosage form and also applicable to human serum samples. The results and its validation data show the satisfactory performance of the developed method in monitoring drug alone or along with human serum. The precision, reliability, rapidness, simplicity, sensitivity and economical nature of this HPLC method makes it superior to the other reported HPLC for the assay of torsemide.

The proposed HPLC method provides simple, accurate and reproducible. Quantitative analysis for determination of torsemide in tablets and in human serum without any interference from the excipients. It is a simple analytical procedure and short retention time allows the analysis of a large number or samples in a short period of time. The proposed HPLC method for determination of torsemide has advantages over other analytical methods due to selectivity's and better sensitivity. The retention time observed (3.91 min) enables rapid determination of the drug, which is important for routine analysis. The linearity range, limits of detection and quantification, precision and accuracy were determined to asses the suitability of the method and satisfactory results were obtained.

The developed methods was validated and applied for the determination of drugs in pharmaceutical formulations and in human serum. The high recovery percentage and low RSD reflect the high accuracy and precision of the proposed methods. Moreover the methods are easy, applicable to a wide range of concentrations, besides being less time consuming and depending on simple available reagents thus offering economic and acceptable methods for the routine determination of drug in pharmaceutical formulation and in human serum. In the proposed method the analysis time is quite short and a simple mobile phase is used, for this reason consumption of organic solvent is very slow. The method is rapid, specific, reliable, and cost effective and can be recommended for routine analysis and quality control.

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