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Method Development And Validation For Simulataneous Determination Of Torsemdie And Spironolactone In Tablet Dosage Form By RP-HPLC.

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ABSTRACT

Torsemide (TOR) is a novel loop diuretic of pyridine sulfonyl urea group and spironolactone (SPI) is potassium- sparing diuretic which in combination used to treat congestive heart failure. Diuretics are the agents which will increase the rate of urine flow. A simple, accurate, precise, and sensitive validated reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Torsemide and Spironolactone and validated according to International Council of Harmonization guidelines (Q2B). Separation was carried out on Agilent 1220 LC system with EZChromeAlite software equipped with Agilent C18 column (4.6×250mm, 5µm) and UV detector using 10mM potassium dihydrogen Ortho phosphate and acetonitrile as mobile phase in the ratio of 20:80 v/v and detection was carried out at 235nm. The orticle plate count was >2000, % RSD was found to be less than 2%. Results were linear in the range of 10-22 µg mL^{-1} for TOR and 100-220 µg mL^{-1} for SPI. The method was successfully employed for pharmaceutical formulation. The developed method was found to be simple, precise, and sensitive which is validated statistically.
Keywords: RP-HPLC, ICH(Q2B), EZChromeAlite., Torsemide, Spironolactone, tablet dosage form.

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INTRODUCTION

Torsemide is aniline pyridine sulfonyl urea derivative which is used as high ceiling diuretic and cardiovascular agent by inhibiting Na^+/K^+ ions. Spironolactone is a steroidal derivative which act as potassium-sparing diuretic. Diuretics are the agents which will increase the rate of urination and sodium excretion and are used to adjust the volume and / or composition of body fluids in variety of clinical situations including hypertension, heart failure, renal failure, nephrotic syndrome and cirrhosis.

Literature survey revealed that several analytical methods were developed for the determination of different class of diuretics. But there is no official compendial method for the simultaneous determination of Torsemide and Spironolactone. The present study deals with simultaneous estimation of Torsemide and spironolactone by RP-HPLC. All the validation parameters related to this study were evaluated according to ICH guidelines Q2B.

MATERILAS AND METHODS

DRUGS AND CHEMICALS

Acetonitrile, methanol, and water were purchased from Merck (Mumbai, India). All other reagents used were of HPLC grade. Standard bulk drug samples of Torsemide and Spironolactone were provided by Lupin labs Ltd., India. DYTOR PLUS 5 tablets (CIPLA LTD., TOR 5mg + SPI 50 mg) were purchased from APPOLLO pharmacy.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS:

Agilent 1220 LC HPLC system, EZChrome Elite software with UV/VIS detector. Separation was carried out by using mobile phase consisting 20:80 (v/v) 10mM potassium dihydrogen Ortho phosphate and acetonitrile with 1.0 ml/min flow rate. All weights were taken using SHIMADZU balance of model ATX224.

Specifications	
Software	Ezchrom Elite
Column	Agilent C18 Column (4.6×250mm, 5µm)
Pump	LC20AT
Detector	UV-2489
Injector	Rheodyne
Temperature	Ambient

PREPARATION OF TORSEMIDE STOCK SOLUTION:

Standard stock solution of TOR was prepared separately by dissolving 10 mg of the drug in 10 mL of methanol to get a concentration of 1000µg mL^{-1}. One milliliter of this solution was diluted to 10mL to get a concentration of 100µg mL^{-1}.

PREPARATION OF SPIRONLACTONE STANDARD STOCK SOLUTION:

Standard stock solution of SPI was prepared by dissolving 10mg of Spironolactone in 10mL of methanol to get a concentration of 1000µg mL^{-1}. One milliliter of this solution was further diluted to 10 mL with mobile phase to get a concentration of 100 µg mL^{-1}.

PROCEDURE FOR SAMPLE PREPARATION OF FORMULATION

DETAILS OF FORMULATION:

Brand Name	Manufactured By	Concentration of Torsemide (mg)	Concentration of Spironolactone (mg)
DYTOR PLUS 5	CIPLA LTD.	5	50

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Twenty tablets were weighed and powdered. A quantity of tablet powder equivalent to 10 mg of SPL was weighed and transferred to 10 mL volumetric flask containing 8 mL of mobile phase and ultra-sonicated for 20 min, and the volume was made up to the mark with mobile phase. The solution was filtered through 0.41 µ filter paper. One milliliter of this solution was further diluted to 10 mL with mobile phase to get 10 + 100 µg mL^{-1} concentrated TOR & SPI solution. The chromatographic conditions were set before injecting the samples into the system. After stabilizing the system, the tablet sample solution was injected into the system, a chromatogram was obtained peak areas and retention time was recorded. The process was repeated for six injections and the amount of sample was estimated from the respective calibration curves.

SYSTEM SUITABILITY

The system suitability was confirmed by six replicate injections of the mixture containing 16 + 160 µg mL^{-1} of TOR and SPI drugs. The resolution, HETP, number of theoretical plates, peak asymmetry, were calculated from obtained chromatogram. The standard deviation was found to be 0.42 for TOR and 0.84 for SPI respectively. The representation chromatogram for standard solution of mixture is shown in *Fig.1.*

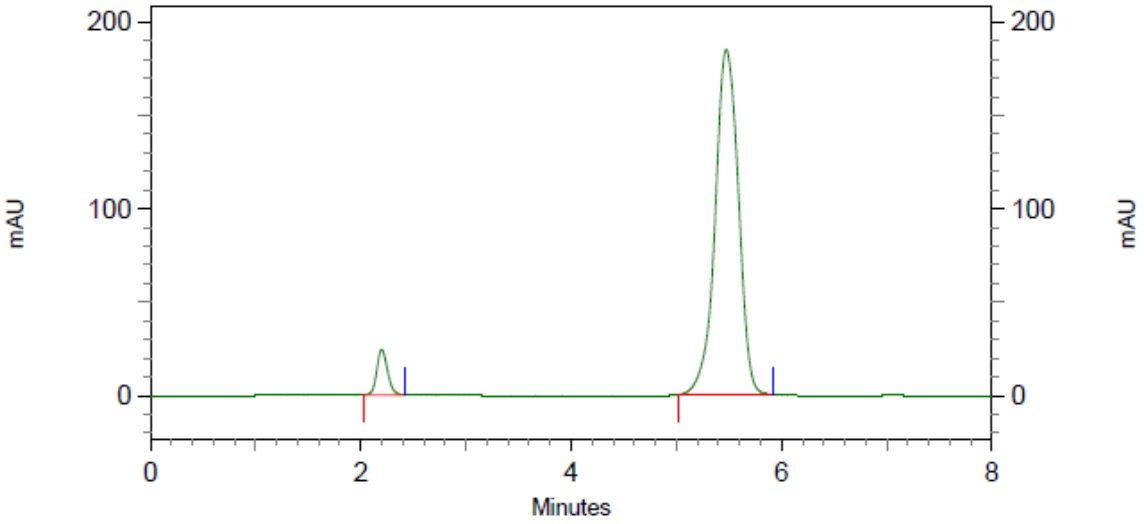

Fig 1: **Representation chromatogram obtained for standard mixture of TOR (16 µg mL^{-1}) SPIR (160 µg mL^{-1}).**

S.NO	System Suitability Parameters	Observed value	
		Torsemide	Spironolactone
1	The Tailing factor for Torsemide and Spironolactone in standard solution	1.19	0.97
2	Theoretical plates for Torsemide and Spironolactone in standard solution	2227	3036
3	Asymmetry factor	1.15	0.97
4	Retention time	2.203	5.490

METHOD VALIDATION

The developed method was validated for its linearity, intra and inter day precision, accuracy and robustness in accordance with ICH guidelines.

SPECIFICITY

The specificity of the method was confirmed by comparing the standard drug and sample by their retention time. It was observed that excipients present in the formulation did not interfere with the peaks of TOR and SPI.

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LINEARITY

A series of standard solutions (not less than 5 is recommended) were prepared in the range of 10-22 µg/ mL^{-1}containing Torsemide and 100-220 µg/ mL^{-1} Spironolactone standards were injected. A plot of average peak area versus the concentration in µg/ mL^{-1} was plotted and the correlation coefficient, y-intercept (const. of regression) and slope (coefficient of regression) of the regression line were calculated.

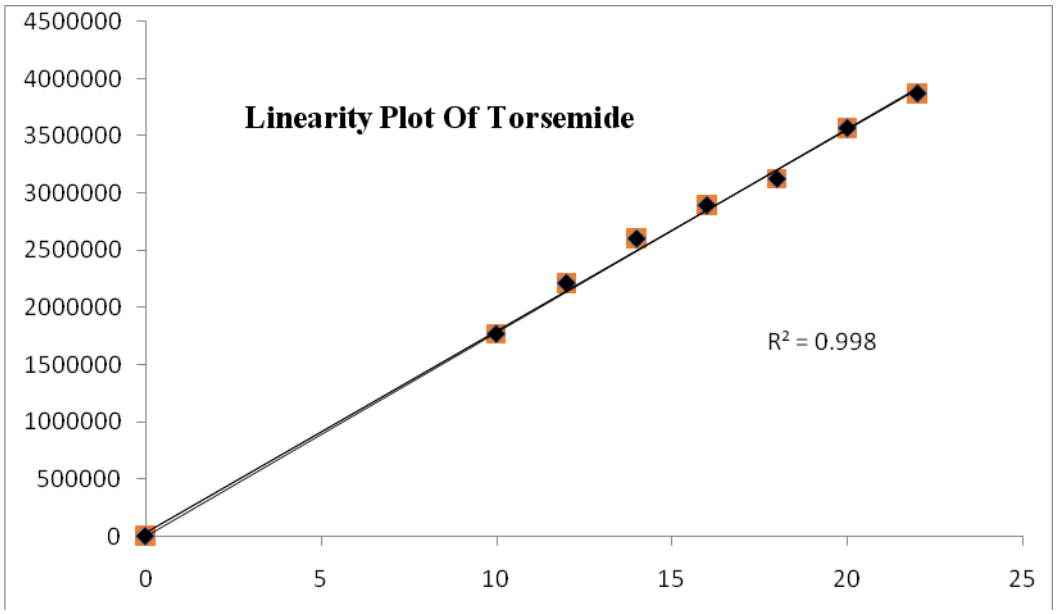

Fig 2: Linearity graph for Torsemide

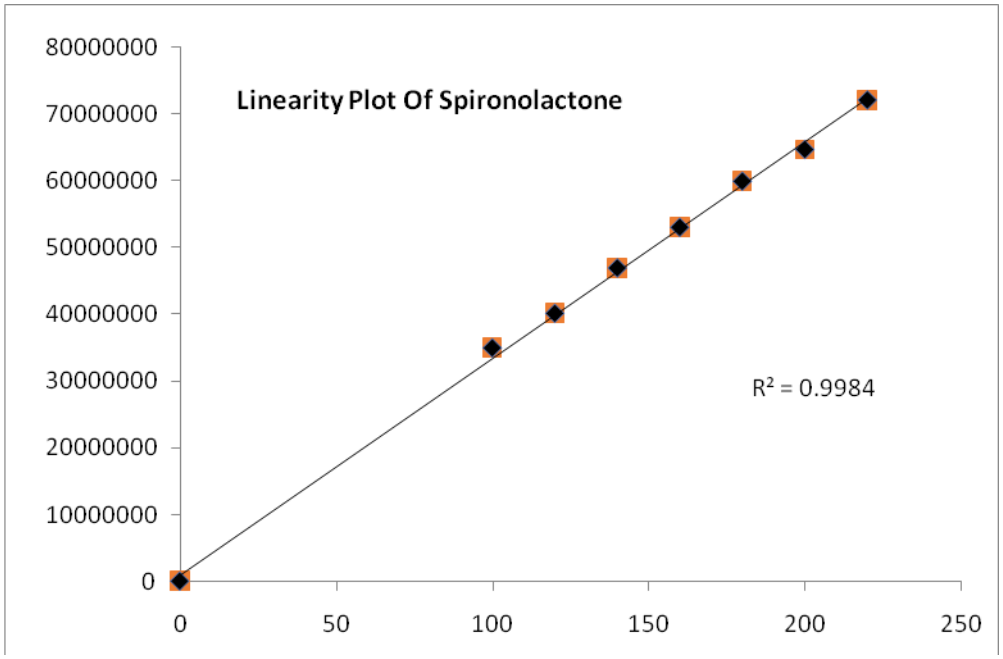

Fig 3: Linearity graph for Spironolactone

PRECISION

The precision of the test procedure was evaluated by injecting the six replicates of standard solutions. The Relative Standard Deviation of six injections was calculated for both intraday and inter day precision.

ACCURACY

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To check the accuracy of the method, recovery studies were carried out by the addition of standard drug solution to a pre-analyzed sample solution at three different levels, 90%, 100%, and 110%. The percentages of recoveries were calculated, the results of which are represented in Table II.

% Recovery	concentration of Torsemide (µg/mL^{-1})	concentration of Spironolactone (µg/mL^{-1})	Amount found in Torsemide	% Recovery of Torsemide	Amount found in Spironolactone	% Recovery of Spironolactone
90	18	180	18.14	100.77	180.16	100.08
	18	180	17.93	99.61	179.76	99.86
	18	180	18.07	100.38	180.40	100.22
100	20	200	20.12	100.6	200.64	100.32
	20	200	20.04	100.2	199.61	99.80
	20	200	20.20	101.0	200.07	100.03
110	22	220	21.86	99.36	220.14	100.06
	22	220	22.15	100.68	220.48	100.21
	22	220	21.91	100.34	219.81	99.91

LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

The Limit of detection of an individual analytical procedure where the lowest amount of analyte in a sample and can be detected but not necessarily quantitated.

$$LOD = \frac{3.3\,\sigma}{S}$$

The Limit of quantification is a parameter the lowest amount of analyte in a sample and can be quantitatively determined with suitable precision and accuracy.

$$LOQ = \frac{10\,\sigma}{S}$$

ROBUSTNESS

In the robustness of the study the influence of deliberate variations of the analytical parameters on retention time of the drugs were examined. The parameters such as wavelength at which drugs were recorded (235±5 nm), flow rate of the mobile phase (1 ±0.2 ml/min), pH of the mobile phase (2 ±0.2), mobile phase composition (80:20 ±5%) were selected and performed. One factor was changed at a time to estimate the effect. One solution of 10 µg mL^{-1} of TOR and SPI were applied for this parameter. It was observed that there is no remarkable change in chromatograms which confirms that the developed method is robust.

RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 10 -22 µg mL^{-1} for TOR and 100-220µg mL^{-1}for SPI, respectively. The correlation coefficients for the plots were 0.9980 for TOR and 0.9984 for SPI. The proposed method was also evaluated by the assay of commercially available tablets containing TOR and SPI. The % assay was found to be 99.7± 1.0 for TOR and 98.01± 1.01 for SPI (mean ± SD, *n* = 6). The method was found to be accurate and precise, as indicated by recovery studies and %RSD not more than 2. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters, showed no marked changes in the chromatograms (RSD < 2), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of the proposed HPLC method is given in *Table III.*

Table III: **Summary of validation parameters of the proposed RP-HPLC method**

S.NO	PARAMETERS	TOR	SPI
1	Linearity Range (µg mL$^{-1)}$	10-22	100-220
2	Correlation coefficient	0.9980	0.9984
3	Accuracy (% Recovery)	99.36-100.77	99.80-100.32

4	Precision (%RSD)		
	Intraday (n=6)	0.66	0.77
	Interday (n=6)	1.22	0.86
5	Specificity	Specific	Specific
6	LOD (µg mL^{-1})	0.35 µg/ml	3.7 µg/ml
7	LOQ (µg mL^{-1})	1.07 µg/ml	11.23 µg/ml

LOD = Limit of Detection; LOQ = Limit of Quantitation; RSD = Relative Standard Deviation; *n* = Number of Determination

CONCLUSION

The developed and validated method by RP-HPLC is proved as simple, accurate, precise and robust so that it can be applied for routine analysis of TOR and SPI in combined tablet dosage form.

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