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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANIOUS ESTIMATION OF EPERELONE AND TORSIMIDE USING RP HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of torsemide and eplerenone in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 55 volumes of Mixed Phosphate Buffer and 45 volumes of Acetonitrile with detection of 261 nm. Linearity was observed in the range 5-15 µg/ml for torsemide (r^2 =0.999) and 12.5-37.5µg /ml for eplerenone (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

KEY WORDS: Torsaemide, eplerenone, HPLC method

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INTRODUCTION

Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at Different rates they become separated in time, like runners in a marathon. Ideally, each component has a Characteristic time of passage through the system. This is called its "retention time." A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it. A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the

identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species (1, 2).

Method validation is the process by which it is established, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended purpose. It is a part of the overall validation process that also includes software validation, instrument qualification and system suitability. Typical analytical characteristics used in method validation are highlighted below. Although all analytical procedures or methods used in a regulated laboratory must be validated, this chart focuses specifically on liquid chromatography.

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. Validation in itself does not improve processes but confirms that the processes have been properly developed and are under control. Method validation is defined as the process of proving (through scientific studies) that an analytical method is acceptable for its intended use. To ensure compliance with quality and safety standards, the United States, Europe, Japan, and other countries have published compendia, or pharmacopeias, that describe official test methods for many marketed drug products. For example, analytical methods found in UnitedStates Pharmacopeia (USP) are legally recognized analytical procedures under section 501 (b) of the Federal Food, Drug, and Cosmetic Act. For these compendia methods, USP provides regulatory guidance for method validation. In addition, validation of analytical methods is covered by the United States Code of Federal Regulations (CFR). A great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe, and Japan. As part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation. The recent FDA methods validation draft guidance documents as well as U.S. both refer to ICH guidelines (3-5).

Eplerenone

(INN) is a steroidal antimineralocorticoid of the spirolactone group that is used as an adjunct in the management of chronic heart failure. It is similar to the diuretic spironolactone, though it is much more selective for the mineralocorticoid receptor in comparison (i.e., does not possess any antiandrogen, progestogen, glucocorticoid, or estrogenic effects), and is specifically marketed for reducing cardiovascular risk in patients following myocardial infarction. Eplerenone is a potassium-sparing diuretic, meaning that it helps the body get rid of water but still keep potassium.

Torasemide (rINN) or torsemide (USAN) is a pyridine-sulfonylurea type 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. It is marketed under the brand name Demadex.

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-potassium exchange 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. Torasemide (INN) or torsemide (USAN) is a novel loop diuretic belonging to pridine sulphonyl urea. It differs from other thiazide diuretics in that a double ring system is

incorporated into its structure. Like thiazides, loop diuretics must be secreted into the tubular fluid by proximal tubule cells. In the thick ascending loop Na+ and Cl- reabsorption is accomplished by a $Na^+/K^+/2Cl^-$symporter. The thick ascending limb has a high reabsorptive capacity and is responsible for reabsorbing 25% of the filtered load of Na^+. The loop diuretics act by blocking this symporter. Because of the large absorptive capacity and the amount of Na^+ delivered to the ascending limb, loop diuretics have a profound diuretic action. In addition, more distal nephron segments do not have the reabsorptive capacity to compensate for this increased load. The osmotic gradient for water reabsorption is also reduced resulting in an increase in the amount of water excreted (6).

Aim is to develop new RP HPLC method for the simultaneous estimation of torsemide and eplerenone pharmaceutical dosage form.

MATERIALS AND METHODS (7-10)

Torsemide

It is sparingly soluble in water, soluble in ethanol, and freely soluble in methanol

Eplerenone

Soluble in dilute ammonia, or sodium hydroxide; also soluble in methanol, ethanol, acetone. Freely soluble in sodium hydroxide solution, in n-butylamine and in dimethylformamide; sparingly soluble in methanol

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of torsemide

10 mg of torsemide was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of eplerenone

10 mg of eplerenone was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard solution

weigh accurately 10mg of torsemide and 10 mg of eprelenone in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of torsemide and 25µg/ml of eprelenone is prepared by diluting 0.875 ml to 10ml with mobile phase. The **Optimized chromatographic conditions** are given in table-1.

Table-1 Optimized chromatographic conditions

Mobile phase	Mixed Phosphate Buffer:CAN
pH	4.0
Column	Inertsil ODS 3V column,C18 (150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	261
Injection volume	20 µl
Run time	6 min
Retention time	About 2.707 min for torsemide and 3.953 min for eplerenone.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The isobestic point was found to be 261 nm for the combination.

The amount of torsemide and eplerenone present in the taken dosage form was found to be 99.83 % and 99.84% respectively (table-2).

Table-2 Assay Results

	Torsemide		Eplerenone	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1136.114	1120.050	2576.974	2541.448
Injection-2	1112.446	1121.051	2535.582	2551.500
Injection-3	1115.176	1123.043	2549.337	2545.160
Injection-4	1116.202	1118.121	2538.795	2551.600
Injection-5	1124.282	1112.446	2544.742	2535.582
Average Area	1120.844	1118.942	2549.086	2545.058
Standard deviatuion	3.615683		6.83985	
%RSD	0.323134		0.26875	
Assay(%purity)	99.83032		99.84198	

The % RSD for the retention times and peak area of torsemide and Eprelenone were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (table-3 and 4).

Table-3 Results for system suitability of torsemide

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.700	1136.114	2877	1.441
2	2.700	1112.446	2966	1.343
3	2.697	1115.176	2961	1.455
4	2.707	1116.202	2976	1.485
5	2.703	1124.282	2971	1.485
Mean	2.7014	1120.844	-	-
SD	0.003782	9.607	-	-
%RSD	0.139984	0.8574	-	-

Table-4 Results for system suitability of eprelenone

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.947	2576.974	2476	1.500
2	3.937	2535.582	2554	1.477
3	3.933	2549.337	2550	1.512
4	3.953	2538.795	2576	1.477
5	3.947	2544.742	2567	1.512
Mean	3.9434	2549.086	-	-
SD	0.008173	16.46919	-	-
%RSD	0.207261	0.646082	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of torsemide and eprelenone is 0.999 and 0.998. The relationship between the concentration of torsemide and eprelenone and area of torsemide and eprelenone is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (table-5 and 6).

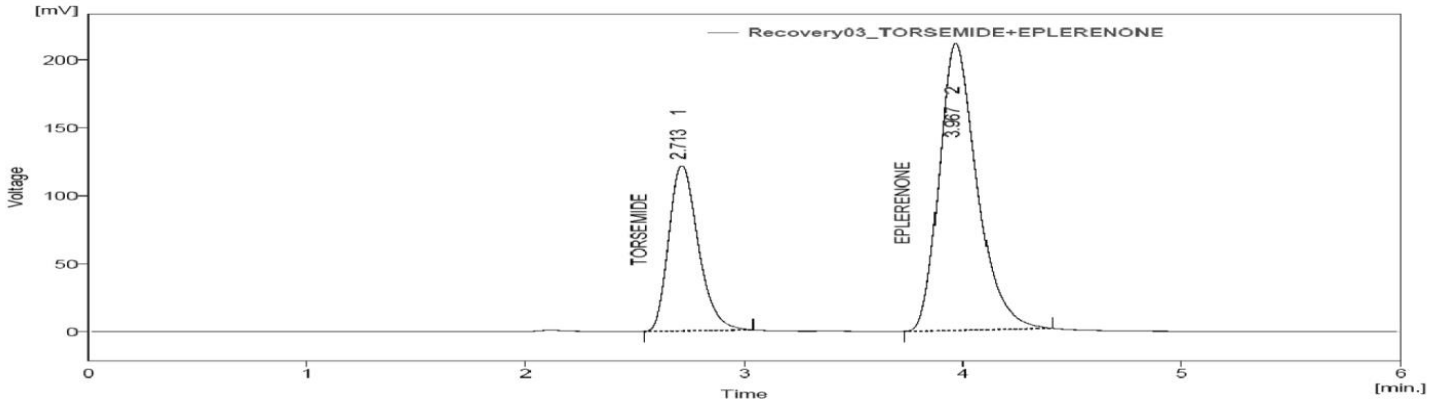
Table-5 linearity of torsemide

S.No.	Conc.(µg/ml)	Area
1	5	495.227
2	7.5	745.541
3	10	995.117
4	12.5	1250.46
5	15	1470.799

Table-6 linearity of eprelenone

S.No.	Conc.(µg/ml)	Area
1	12.5	1122.124
2	18.75	1687.304
3	25	2215.072
4	31.25	2729.514
5	37.5	3254.098

The percentage mean recovery of TORSEMIDE and EPRELENONE is 101.54% and 102.81% respectively (Fig-1, 2 and 3)



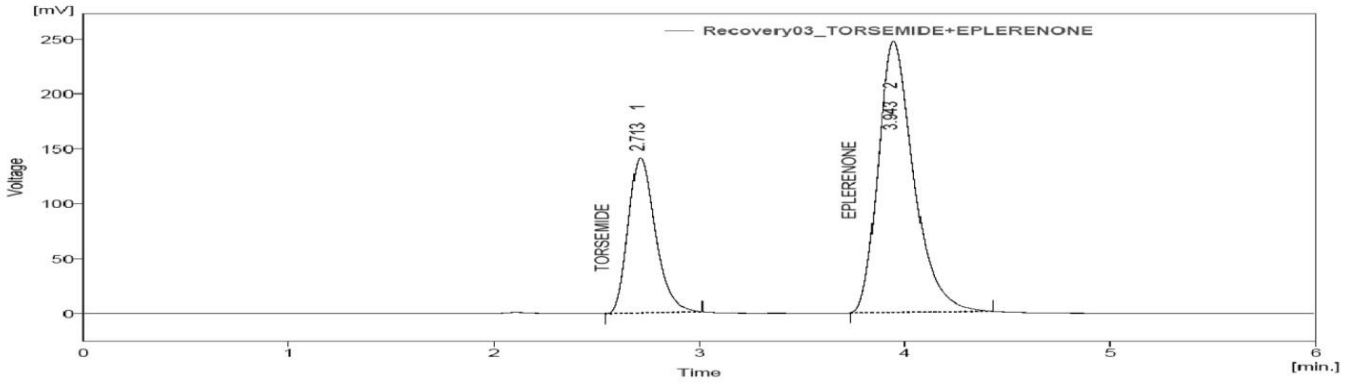
Result Table (Uncal - Recovery03_TORSEMIDE+EPLERENONE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.713	1131.636	121.470	30.68
2	3.967	2556.911	211.267	69.32
	Total	3688.546	332.737	100.00

Column Performance Table (From 50% - Recovery03_TORSEMIDE+EPLERENONE

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.713	0.143	1.515	1985	19853	-
2	3.967	0.187	1.500	2502	25017	4.470

Figure-1 Chromatogram of 50% recovery (injection 1)



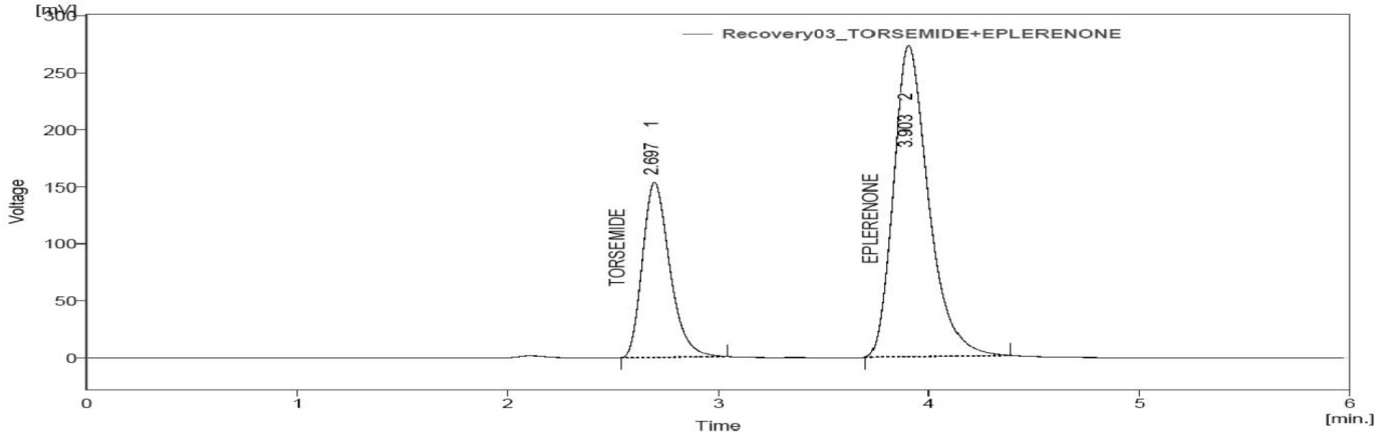
Result Table (Uncal - Recovery03_TORSEMIDE+EPLERENONE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.713	1290.945	141.222	30.26
2	3.943	2975.781	246.913	69.74
	Total	4266.725	388.134	100.00

Column Performance Table (From 50% - Recovery03_TORSEMIDE+EPLERENONE

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.713	0.143	1.412	1985	19853	-
2	3.943	0.183	1.558	2563	25630	4.431

Figure-2 Chromatogram of 100% recovery (injection 2)



Result Table (Uncal - Recovery03_TORSEMIDE+EPLERENONE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.697	1400.738	153.306	30.49
2	3.903	3192.726	272.552	69.51
	Total	4593.464	425.858	100.00

Column Performance Table (From 50% - Recovery03_TORSEMIDE+EPLERENONE

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.697	0.140	1.455	2055	20555	-
2	3.903	0.180	1.524	2605	26052	4.438

Figure-3 Chromatogram of 150% recovery (injection 3)

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged

CONCLUSION

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

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