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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ERTUGLIFLOZIN AND METFORMIN HCl IN BULK AND PHARMACEUTICAL DOSAGE FORM BY HPLC

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ABSTRACT: Analytical method for simultaneous estimation of Ertugliflozin (ERT) and Metformin hydrochloride (MET) was developed and validated by high-performance liquid chromatography (HPLC) as per ICH guidelines. The drugs were injected into the inertsil C18 (250 × 4.6 mm) maintained at room temp and wavelength 220 nm. The mobile phase consists of buffer (potassium dihydrogen pH 4.0) and methanol (65:35 v/v). The flow rate is maintained at 1.0 mL/min. The calibration curve was linear and regression coefficient (R^2) value was found to be 0.999 and concentration ranging from 1.5-4.5 µg/mL and 100-300 µg/mL for Ertugliflozin and Metformin hydrochloride respectively. The LOD and LOQ of the method were found 1.04 µg/mL, 9.61 µg/mL and 0.0007 µg/mL, 0.006 µg/mL for Ertugliflozin and Metformin HCl. The developed method was found to be simple, precise, specific, linear and accurate as validated as per USP and International Conference on Harmonization (ICH) guidelines.

INTRODUCTION: Ertugliflozin (ERT) chemical name is (1S, 2S, 3S, 4R, 5S) – 5 –[4- chloro -3 – [(4-ethoxyphenyl) methyl] -6,8-dioxybicyclo [3, 2, 1] octane -2, 3, 4-triol compound with (2S) - 5oxypyrrolidine – 2-carboxylic acid and molecular formula $C_{22}H_{25}ClO_7$ show in **Fig. 1**. Ertugliflozin is inhibiter of sodium-glucose co-transporter -2 (SGLT-2) is the pro dominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Ertugliflozin is indicated to improve glycemic in adult patients with type -2 diabetes controls [1-3].

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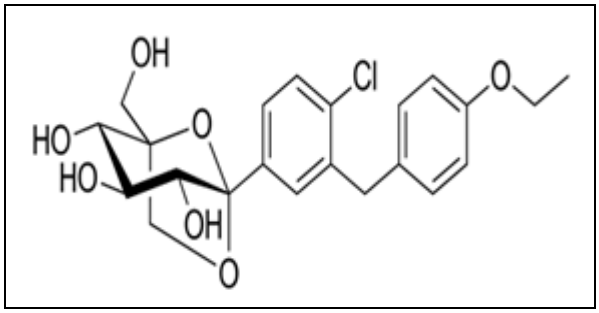


FIG. 1: ERTUGLIFLOZIN

Metformin (MET) chemical name is N, N dimethyl imido dicarboximide diamide hydrochloride and molecular formula $C_4H_{11}N_5$. HCl shows in **Fig. 2**. Metformin HCl is oral antihyperglycemic drugs used in the management of type-2 diabetes [4-5].

Combining anti-hyperglycemic agents in order to rapidly and safely achieve the best possible glycemic control is the standard of care today for the management of type 2 diabetes.

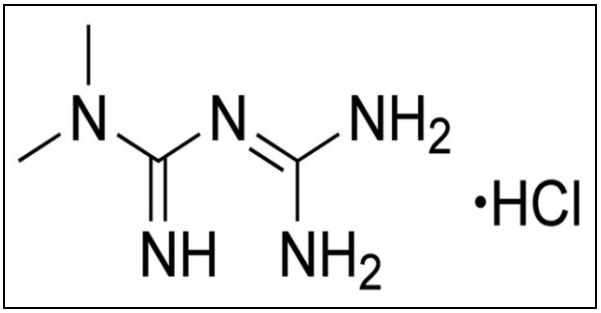


FIG. 2: METFORMIN HCl

Agents should ideally have mechanisms of actions that are complementary and that improve glycemic control without an unacceptable gain in body weight or hypoglycemia. Ertugliflozin and metformin hydrochloride (ertugliflozin/metformin, Segluromet) is a recently approved fixed-dose combination tablet containing the sodium-glucose co-transporter 2(SGLT-2) inhibitor ertugliflozin and metformin. Diabetes is a group of metabolic disorders characterized by the presence of chronic hyperglycemia by greater or lesser impairment in the metabolism of carbohydrate, lipids, and proteins.

The result is a high level of glucose in the blood the two active substances such as ertugliflozin and metformin HCl to lower glucose level [6]. SGLT-2 inhibitors are an important class of antihyperglycemic agents that are efficacious as monotherapy and in combination with other antihyperglycemic agents. Given their favorable effects on glycemic control as well as 'extra-glycemic' parameters such as body weight and blood pressure, they are ideal agents for appropriate patients with type 2 diabetes. The fixed-dose combination of ertugliflozin with metformin is an effective combination that is conveniently administered and may improve medication adherence and persistence [7].

Even though numerous methods are available for the estimation of metformin hydrochloride available individually and in combination with another drug, whereas five methods available for the estimation of ertugliflozin and sitagliptin no method has been reported estimation of ertugliflozin and metformin hydrochloride simultaneously [8-16].

MATERIALS AND METHODS: Standard of ERT and MET HCl were obtained from Merck. Buffer and methanol were obtained from Rankem. All solvent and reagent were of analytical grade.

Instrumentation: Gradient system HPLC equipped with an aligned UV detector was used throughout the analysis. The analysis column inertsil C18 250 mm × 4.6 mm, 5 µ thermo scientific was used as a stationary phase. The instrumental settings were flow of 1.0 mL/min and injection volume 20µL column oven temperature was ambient.

Buffer Preparation: 6.8 gm Potassium dihydrogen phosphate buffer was transferred to 1000 mL beaker, and 800 mL water was added shacked to dissolve, and volume was made up with water, pH 4.0 was adjusted with diluted o-Phosphoric acid.

MET Standard Stock Solution (2000 µg/mL): Accurately weighed 200 mg of MET and Transferred to 100 mL volumetric flask and volume were made up with the Diluents.

ERT Standard Stock Solution (30 µg/mL): Accurately weighed 30 mg of ERT and Transferred to 100 mL volumetric flask and volume were made up with the Diluents, Transfer 1mL of this solution to 10 mL volumetric flask and volume was made up with the Diluents.

Standard Working Solution (MET 200 µg/mL, ERT 3 µg/mL): 1 mL of standard stock solution was transferred to 10 mL volumetric flask and volume was made up with the diluents.

Optimization of Chromatographic Conditions: a various combination of mobile phase was screened with respect to resolution, theoretical plate, capacity factor, and other system suitability parameters. finally the separation was performed with freshly prepared mobile phase consist of buffer (ph 4.0): methanol in the ratio of 65:35 at flow rate of 1.0 mL/min. 220 nm wavelength, injection volume of 20 ul temperature was maintained during the entire process to obtain symmetric peak of MET HCl and ERT.

Method of Validation: [17-18]

System Suitability: System suitability test is a fundamental part of liquid chromatography. It ensures that system is working correctly. The standard solution of ERT and MET HCl was

injected into the chromatographic system and recorded the chromatogram. System suitability parameters such as number of theoretical plates, retention time, and tailing factor were calculated.

Linearity: Linearity of the method was performed by analyzing a standard solution of MET HCL and ERT to obtain a solution in the concentration range is 100-300 µg/mL and 1.5-4.5 µg/mL for MET HCL and ERT respectively. The area of each level was calculated and graph of area versus concentration was plotted. The correlation coefficient was calculated in linearity plot.

LOD (Limit of Detection) and LOQ (Limit of Quantitation) of ERT and MET HCl: LOD and LOQ of ERT and MET HCL were determining by calibration curve used to determine the method of linearity.

It may be calculated as

$$LOD = 3.3 \times (SD / Slope)$$

$$LOQ = 10 \times (SD / Slope)$$

Where; SD= standard deviation of response (peak area), Slope= mean of slop of the calibration curve.

Precision: Precision of the method was determined by injecting six replicate of sample an unknown concentration of ERT 3 µg/mL and MET HCl 200 µg/mL have been analyzed by injecting into an HPLC column on the same day.

The intermediate precision was estimated by injecting samples prepared at the sample concentration on their different days. The % RSD for the ERT and MET HCl was calculated.

Accuracy: The accuracy of this method was determined by three different levels (80%, 100%, and 120%) by adding of unknown amount of standard to sample at each level. Each sample was injected thrice.

Robustness: Robustness is the measure of optimized method capacity to remain unaffected by small but deliberate variation in method parameters such as mobile phase flow rate (+0.2 mL/min), melting point (+0.2) and pH (+ 0.2).

RESULTS AND DISCUSSION:

Optimization of Chromatographic Conditions: Optimized chromatographic conditions for estimation of ERT and MET HCl are finalized shown in below. A representative chromatogram is shown in **Fig. 3, 4** and **5**.

- ✓ **Column:** Inertrsil C18 (250 × 4.6 mm)
- ✓ **Mobile Phase:** Buffer (pH 4.0): Methanol (65:35)
- ✓ **Flow Rate:** 1.0 mL/min
- ✓ **Detection Wavelength:** 220 nm
- ✓ **Runtime:** 6 min
- ✓ **Injection Volume:** 20.0µl

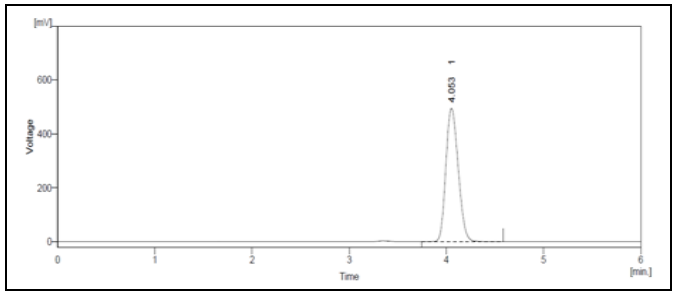


FIG. 3: CHROMATOGRAM OF STANDARD MET HCl

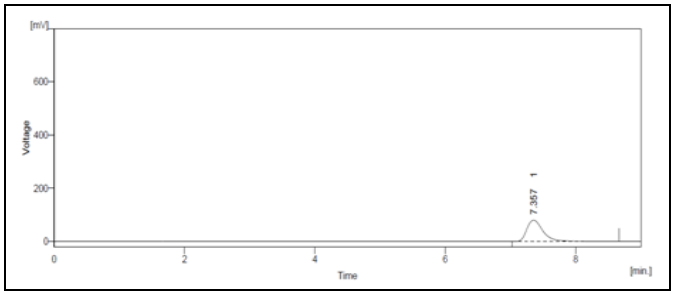
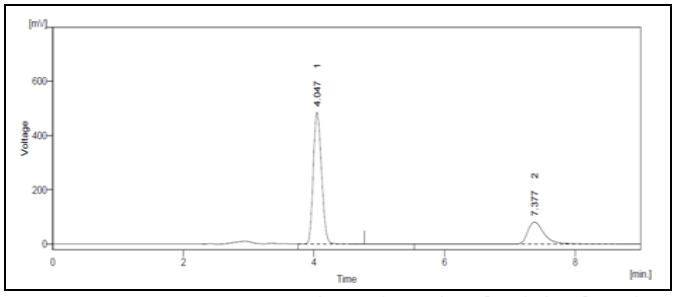


FIG. 4: CHROMATOGRAM OF STANDARD ERT



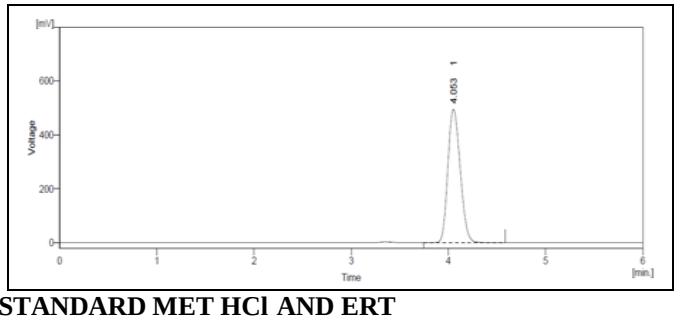


FIG. 5: CHROMATOGRAM OF STANDARD MET HCl AND ERT

TABLE 1: RESULTS FOR SYSTEM SUITABILITY TEST

Parameters	ERT	MET HCl
Theoretical plates per column	4435	4430
Symmetry factor/tailing factor	1.661	1.273
Retention time (min)	7.357	4.053
Resolution	-	-

System Suitability: The system suitability was performed by injecting mix standard solution containing 200 µg/mL MET HCL and 3 µg/mL ERT in six replicates. For two of them, the peak asymmetric was < 1.5, and the theoretical plate number is > 2000and % RSD of ERT, and MET HCl was less than 2. The result indicates that the system suitability parameter is within the acceptable limit. The results are shown in **Table 1**.

Linearity: The linearity of the method was established by determining the constructing calibration graph between tested calibration level and corresponding peak area for ERT and MET HCl in triplicate. Over a range of 1.5-4.5 µg/mL and 100-300 µg/ml, respectively.

The correlation coefficient was > 0.999 for all two drugs. The results are given in **Table 2** and **Fig. 6A** and **6B.**

TABLE 2: LINEARITY DATA FOR ERT AND MET

	Metformin HCl		Ertugliflozin	
S. no.	Concentration µg/mL	Area	Concentration µg/mL	Area
01	100	2171.289	1.5	682.424
02	150	3205.757	2.25	1015.305
03	200	4383.295	3	1388.885
04	250	5397.235	3.75	1710.713
05	300	6567.476	4.5	2081.916

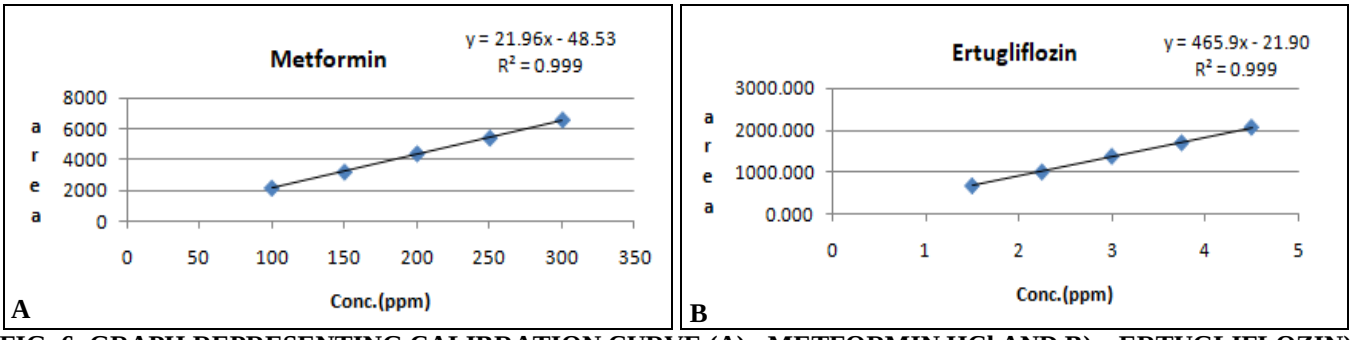


FIG. 6: GRAPH REPRESENTING CALIBRATION CURVE (A) - METFORMIN HCl AND B) – ERTUGLIFLOZIN)

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were found to be 1.04 and 9.61 µg/mL for MET HCL and 0.0007 and 0.006 µg/mL for ERT. THE results are given in **Table 3**.

TABLE 3: LOD AND LOQ

Drug	LOD	LOQ
Metformin HCl	1.04µg /mL	0.0007µg /mL
Ertugliflozin	9.61µg /mL	0.006µg /mL

LOD = limit of detection, LOQ = limit of quantitation

TABLE 4: INTRADAY PRECISION DATA FOR ESTIMATION OF MET AND ERT

	MET HCl				ERT			
S. no.	Conc. µg/mL	Area	SD*	% RSD**	Conc. µg/mL	Area	SD*	% RSD**
1	50	2134.69	25.292	0.58	1.5	677.7	9.623	1.428
	50	2160.4				662.98		
	50	2166.92				681.08		
2	100	4325.68	20.104	0.308	3	1379.2	15.635	1.138
	100	4361.45				1356.24		
	100	4374.55				1386.11		
3	150	6521.85	20.104	0.308	4.5	2023.19	29.888	1.452
	150	6534.7				2071.54		
	150	6495.28				2077.8		

*Standard deviation** % relative standard deviation

Precision:

Method Precision / Repeatability: The % RSD value for six replicate injection of an unknown concentration of ERT 3 µg/mL and MET HCL 200 µg/mL carried out on the same day was found to be < 2% which indicate that the method repeatable. The results for method precision are given in **Table 4**.

System Precision / Intermediate Precision: Intermediate precision was determined by measuring the peak area of six replicate was inject into the HPLC system and was analyzed and they were found within the acceptable limit (% RSD) intermediate precision given in **Table 5**.

TABLE 5: INTERDAY PRECISION DATA FOR ESTIMATION OF MET AND ERT

	MET HCl				ERT			
S. no.	**Conc. µg/mL**	**Area**	**SD***	**% RSD****	**Conc. µg/mL**	**Area**	**SD***	**% RSD****
1	50	2136.837	13.781	0.64022	1.5	678.385	7.078328	1.049754
		2162.571				666.111		
		2158.252				678.357		
2	100	4325.727	21.0758	0.48455	3	1380.585	16.21286	1.18237
		4365.814				1352.496		
		4357.055				1380.57		
3	150	6500.112	23.3342	0.3582	4.5	2069.503	32.81717	1.60041
		6541.25				2012.653		
		6501.597				2069.485		

*Standard deviation, ** RSD, relative standard deviation

Accuracy: The percentage recovery was calculated by preparing a standard concentration of ERT and MET HCL with concentration level of 80%, 100%, and 120%. The percentage recovery obtained was found to be in the range of 99.889% - 99.631% for MET HCl and 100.181% - 100.814% for ERT. The acceptable limits of mean recovery are 100% - 102%. Good recovery of the spiked drugs was obtained at each added concentration the results are given in **Table 6** and **7**.

TABLE 6: ACCURACY DATA FOR MET

% level	**Area of sample spike with std**	**Amount recovered (mcg/mL)**	**% recovery**	**Average**	**SD***	**% RSD****
80%	3892.77	78.996	98.745			
80%	3927.711	80.582	100.728	99.889	1.026	1.028
80%	3918.322	80.156	100.195			
100%	4334.353	99.047	99.047			
100%	4358.51	100.144	100.144	99.613	0.549	0.551
100%	4347.616	99.649	99.649			
120%	4795.961	120.008	100.006			
120%	4774.24	119.021	99.184	99.631	0.415	0.417
120%	4787.893	119.641	99.701			

*Standard deviation, ** % relative standard deviation

TABLE 7: ACCURACY DATA FOR ERT

% level	**Area of sample spike with std**	**Amount recovered (mcg/mL)**	**% recovery**	**Average**	**SD***	**% RSD****
80%	1298.166	1.199	99.894			
80%	1294.611	1.191	99.290	100.181	1.065	1.063
80%	1306.798	1.216	101.361			
100%	1447.212	1.503	100.182			
100%	1456.853	1.522	101.493	100.822	0.656	0.651
100%	1451.696	1.512	100.792			
120%	1604.435	1.823	101.301			
120%	1595.665	1.806	100.307	100.814	0.497	0.493
120%	1600.309	1.815	100.833			

*Standard deviation, ** % relative standard deviation

TABLE 8: ROBUSTNESS DATA FOR MET

S. no.	**Flow rate +2**	**Flow rate -2**	**MP* +2**	**MP. - 2**	**pH +2**	**pH -2**
1	4230.902	4501.112	4218.439	4479.887	4151.541	4439.114
2	4282.374	4545.746	4273.793	4488.280	4194.555	4501.851
3	4308.608	4563.092	4304.293	4445.926	4212.298	4519.246
Avg. area	4273.961	4536.650	4265.508	4471.364	4186.131	4486.737
SD**	39.530	31.976	43.522	22.426	31.242	42.150
%RSD***	0.925	0.705	1.020	0.502	0.746	0.939

* Mobile Phase**Standard deviation, *** % relative standard deviation

Robustness: The method was found to be robust when minor changes were made in optimized chromatographic condition such as mobile phase flow rate (+ 0.2 mL/min), M.P (+ 0.2), and pH (+ 0.2). It was observed that there was no marketing change in the analytical data of the drug which indicate good reliability during normal usage. The results are given in **Table 8** and **9**.

TABLE 9: ROBUSTNESS DATA FOR ERT

S. no.	Flow rate +2	Flow rate -2	MP* +2	MP. - 2	pH +2	pH -2
1	1348.702	1434.725	1345.997	1377.816	1318.788	1417.948
2	1356.931	1395.881	1330.659	1411.576	1302.794	1401.004
3	1365.275	1445.626	1363.905	1430.417	1329.980	1431.849
Avg. area	1356.969	1425.411	1346.854	1406.603	1317.187	1416.934
SD**	8.287	26.148	16.640	26.651	13.664	15.447
%RSD***	0.611	1.834	1.235	1.895	1.037	1.090

* Mobile Phase**Standard deviation, ***% relative standard deviation

CONCLUSION: The combined dosage form of MET HCl and ERT is used in the treatment of diabetes. Various methods are reported in combination with another drug, but no HPLC method is available for the estimation of MET HCL and ERT combination. HPLC method for the estimation of these simultaneously has been developed and validated according to the ICH guideline. All the validation parameter including system suitability, linearity, accuracy, precision, LOD, LOQ, and robustness were within the recommended limits of the ICH.

The optimized chromatogram parameters were established appropriately with mobile phase buffer (pH): methanol (65:35). Inertsil C18 (250 × 4.6 mm) column, mobile phase buffer (potassium dihydrogen pH 6.8): methanol (65:35 v/v) with flow rate 1.0 mL /min and injection volume 20 µL. The detection was carried out at wavelength 220 nm. It was found to be simple, precise and accurate. The % RSD also <2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of MET HCl and ERT combine dosage form.

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CONFLICTS OF INTEREST: Authors does not have a conflict of Interest

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