Systematic Development and Validation of Analytical Techniques for Simultaneous Quantification of Ertugliflozin and Metformin Hydrochloride in Bulk and Pharmaceutical Formulations

Sohail Khan Saleem Khan[1], Dr.Imran Khan Pathan[2], Dr. Rahil Salim Meman[3]

[1]Research Scholar, Maulana Azad University Maulana Azad University, Jodhpur, Village: Bujhawar, Tehsil Luni Jodhpur-342802 (Raj)
[2]Associate Professor, Maulana Azad University Maulana Azad University, Jodhpur, Village: Bujhawar, Tehsil Luni Jodhpur-342802 (Raj)
[3]Associate Professor, HOD of Ismail Mehta College of Pharmacy, Ambad, Jalna (431203)
Email ID: 1995.sohailkhan@gmail.com

Abstract:

To evaluate Simultaneous Quantification of Ertugliflozin and Metformin Hydrochloride in Bulk and Pharmaceutical Formulations, a quick, reliable and consistent method was developed. The drugs were injected in to the inertsil C18 (250 x 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 co-efficient (R2) 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. In all condition's purity threshold was more than purity angle and within the acceptable range. This approach that can be used in industry in a daily quality assurance process became easy and economical.

Keywords: HPLC, ICH guidelines, Validation, Metformin, Ertugliflozin , Method Development.

1. Introduction

Metformin (MET) chemical name is N, N dimethyl imido dicarbonimidic diamide hydrochloride and molecular formula $C_4H_{11}N_5$. HCl shows in Figure no.1. Metformin HCl is oral antihyperglycemic drugs used in the management of type -2 diabetes. [1, 2]

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 figure no.2.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 in to the circulation. Ertugliflozin is indicated to improve the glycemic in adult patient with type -2 diabetes controls. [3, 4, 5]

Combining antihyperglycemic 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. Agents should ideally have mechanisms of actions that are complementary and that improve glycemic control without 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 disorder characterized by the presences of chronic hyperglycemias by greater or lesser impairment in the metabolism of carbohydrate, lipid, 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 the glucose level [6].

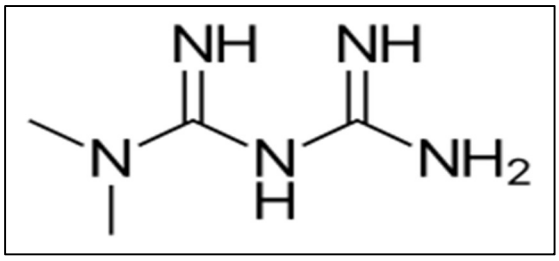


Figure no.1: Metformin HCl

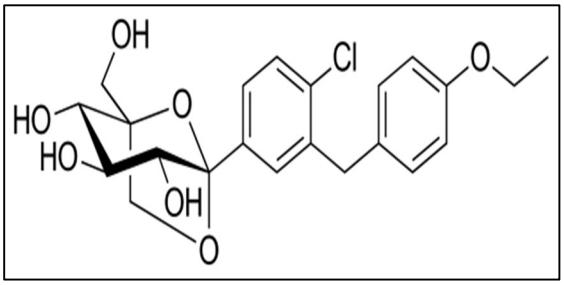


Figure no.2: Ertugliflozin

Literature survey reveals that there were few methods developed for the simultaneous estimation of metformin and ertugliflozin such as RP-HPLC method (Kumari and Bandhakavi, 2020; Sunkara et al., 2021; Harsha Hruditha and Jayachandra Reddy, 2020; Jagadeesh and Annapurna, 2020; China Babu et al., 2019; Nizami et al., 2018). The main objective of the proposed work was to Systematic Development and Validation of Analytical Techniques for Simultaneous Quantification of Ertugliflozin and Metformin Hydrochloride in Bulk and Pharmaceutical Formulations. [8-17]

2. Materials and methods

2.1 Reagents and chemicals

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.

2.2 Instrumentation

Gradient system HPLC equipped with aligned UV detector was used throughout the analysis. The analysis column inertsil C18 250mm x 4.6mm, 5µ 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.

2.3 Experimental work

2.3.1. Buffer preparation: 6.8gm Potassium di-hydrogen phosphate buffer was transferred to 1000mL 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.

2.3.2. MET Standard stock solution (2000 µg/mL): Accurately weighed 200mg of MET and Transferred to 100mL volumetric flask and volume was made up with the Diluents.

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

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

2.3.5. Optimization of Chromatographic Conditions: 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 1.0mL/min. 220nm wavelength, injection volume of 20ul temperature was maintained during the entire process to obtain symmetric peak of MET HCl and ERT.

3. Method of validation [18-19]

3.1 System suitability

System suitability test are a fundamental part of liquid chromatography. It ensures that system is working correctly. Standard solution of ERT and MET HCL was injected in to the chromatographic system and recorded the chromatogram. System suitability parameter such as number of theoretical plates, retention time, and tailing factor were calculated.

3.2 Linearity

Linearity of the method was performed by analyzing a standard solution of MET HCL and ERT to obtain a solution in concentration range is 100 – 300 ug /mL and 1.5-4.5 ug /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.

3.3 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 method of linearity. It may be calculated as

LOD = 3.3 x (SD / Slop)

LOQ = 10 x (SD / Slop)

Where

SD = standard deviation of response (peak area)

Slop = mean of slop of the calibration curve.

3.4 Precision

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

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

3.5 Accuracy

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

3.6 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).

4. Results

4.1 Infrared spectroscopy

A) Ertugliflozin

FT-IR Spectra of Drug standards were obtained by FT-IR Spectrometer. Small quantities of standards were kept directly in the sample compartment of FT-IR and they were scanned in the range of 400-4000 cm^{-1}. FT-IR Spectra of drug was interpreted.

Sr. No.	Functional Group	Frequency
1	C=C	1448
2	C-Cl	742
3	C-H Ar	3082
4	C-O	1072

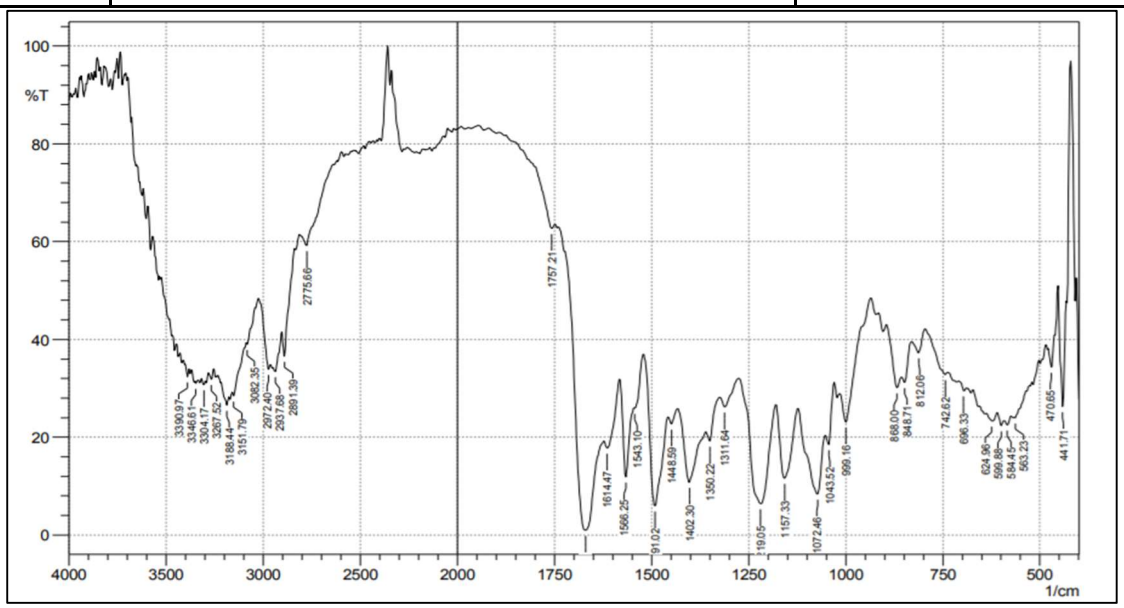


Figure no. 3: IR spectra of ERT

Table no.1 : Interpretation of FT-IR of ERT

b) Metformin HCL

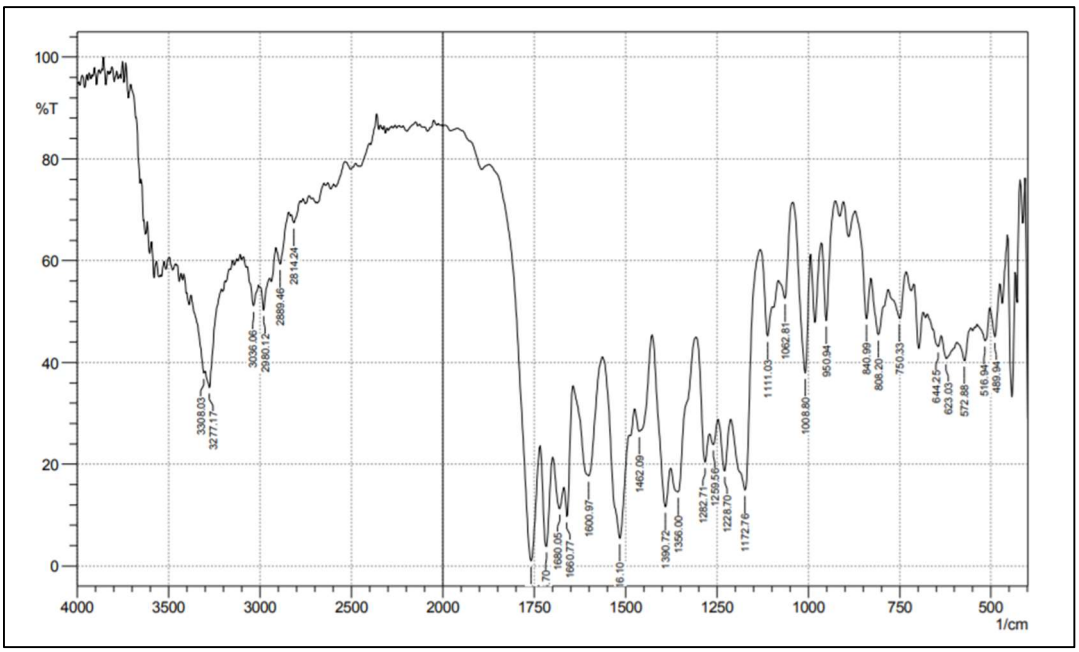


Figure No.4: IR Spectra of MET HCl

Table no.2: Interpretation of IR of MET HCL

Sr. No.	Functional Group	Frequency
1	N-H	3308
2	C-H	3036
3	C=C	1660
4	CH_3	2980

Optimized chromatographic condition for estimation of ERT and MET HCl are finalized shown in below. A representative chromatogram is shown in figure no.5 6 and 7.

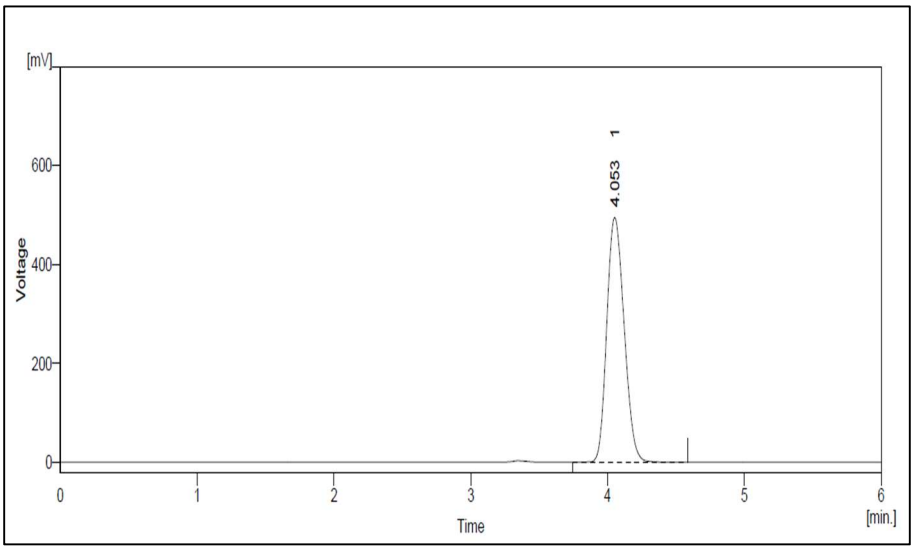


Figure no.5: chromatogram of standard MET HCL

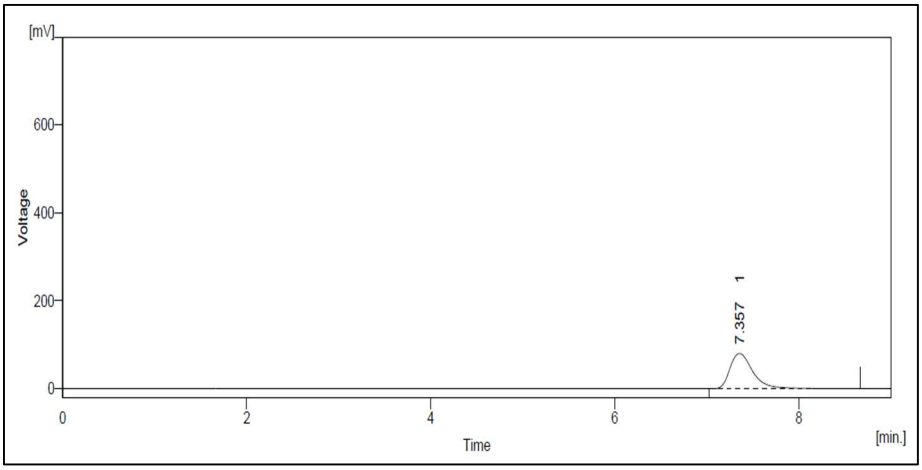


Figure no.6: Chromatogram of Standard ERT

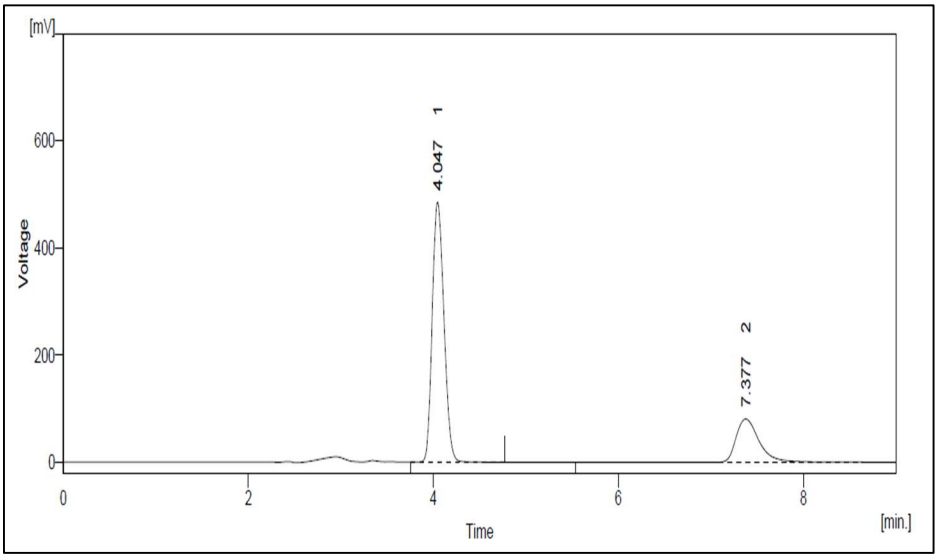


Figure no.7: Chromatogram of Standard MET HCL and ERT

4.2 System suitability

The system suitability parameters like plate count, tailing factor, resolution and % RSD for the five replicates of standard solution were found to be within the acceptance criteria and the results were summarized in Table 3.

Table no.3: 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	-	-

4.3 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 no 4 and figure no. 8(a and b).

Table no. 4: Linearity data for ERT and MET

Metformin HCL			Ertugliflozin	
Sr.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

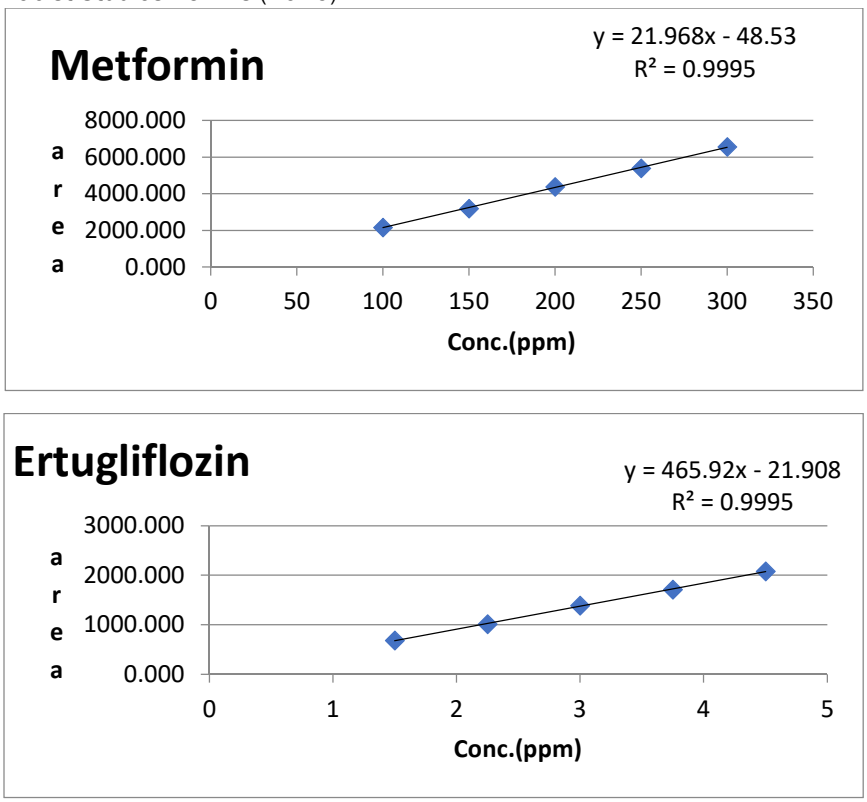


Figure no.8: Graph representing calibration curve (a) - Metformin HCl and b) – Ertugliflozin)

4.4 Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were found to be 1.04 and 9.61µg /mL for MET HCL and 0.0007 and 0.006µg /mL for ERT. THE results are given in table no 5.

Table no. 5: 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

4.5 Precision

- **Method precision / repeatability**

The % RSD value for six replicate injection of a unknown concentration of ERT 3µg /mL and MET HCL 200µg /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 no 6.

Table no. 6: Intraday precision data for estimation of MET And ERT

MET HCl					ERT			
Sr.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

4.6 System precision / intermediate precision

Intermediate precision was determined by measuring the peak area of six replicate was inject into HPLCsystem and was analysed and thewere found within the acceptable limit (%RSD) intermediate precision given in table no 7.

Table no. 7: Interday precision data for estimation of MET and ERT

MET HCl					ERT			
Sr.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

4.7 Accuracy

The percentage recovery was calculated by preparing 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 the table no 8 and 9.

Table no.8: 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	99.889	1.026	1.028
80%	3927.711	80.582	100.728			
80%	3918.322	80.156	100.195			
100%	4334.353	99.047	99.047	99.613	0.549	0.551
100%	4358.51	100.144	100.144			
100%	4347.616	99.649	99.649			
120%	4795.961	120.008	100.006	99.631	0.415	0.417
120%	4774.24	119.021	99.184			
120%	4787.893	119.641	99.701			

*Standard deviation ** % relative standard deviation

Table no.9: 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	100.181	1.065	1.063
80%	1294.611	1.191	99.290			
80%	1306.798	1.216	101.361			
100%	1447.212	1.503	100.182	100.822	0.656	0.651
100%	1456.853	1.522	101.493			
100%	1451.696	1.512	100.792			
120%	1604.435	1.823	101.301	100.814	0.497	0.493
120%	1595.665	1.806	100.307			
120%	1600.309	1.815	100.833			

*Standard deviation ** % relative standard deviation

4.8 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 no 10 and 11.

Table no. 10: Robustness data for MET

Sr.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

Table no. 11: Robustness data for ERT

Sr.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

Figure no.9: Graph representing Rubustness data of Flow rate +2 (1) - Metformin HCl and 2) – Ertugliflozin) at different trail.

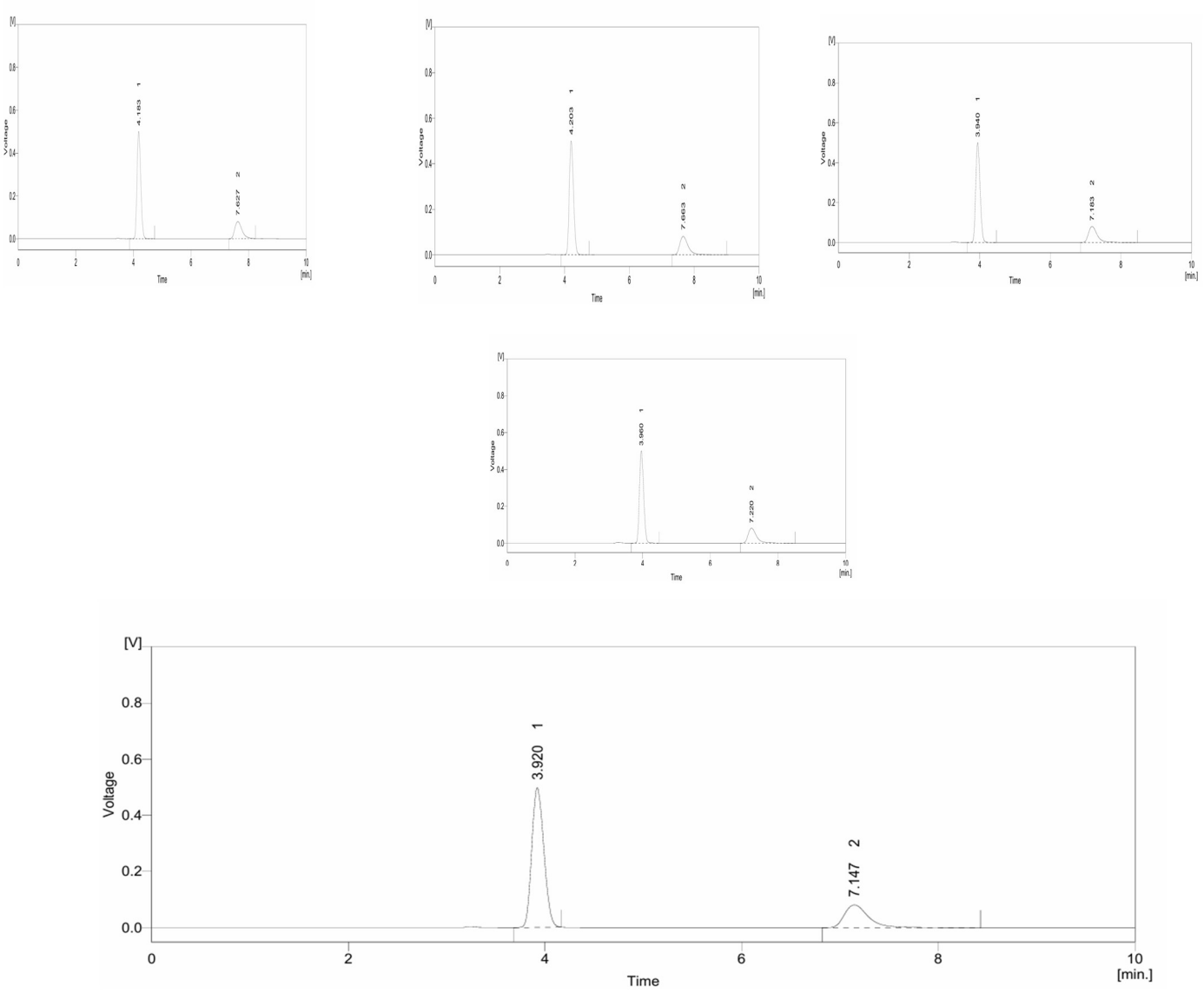


Figure no.10: Graph representing Rubustness data of Flow rate - 2 (1) - Metformin HCl and 2) – Ertugliflozin) at different trail.

4.9 Assay

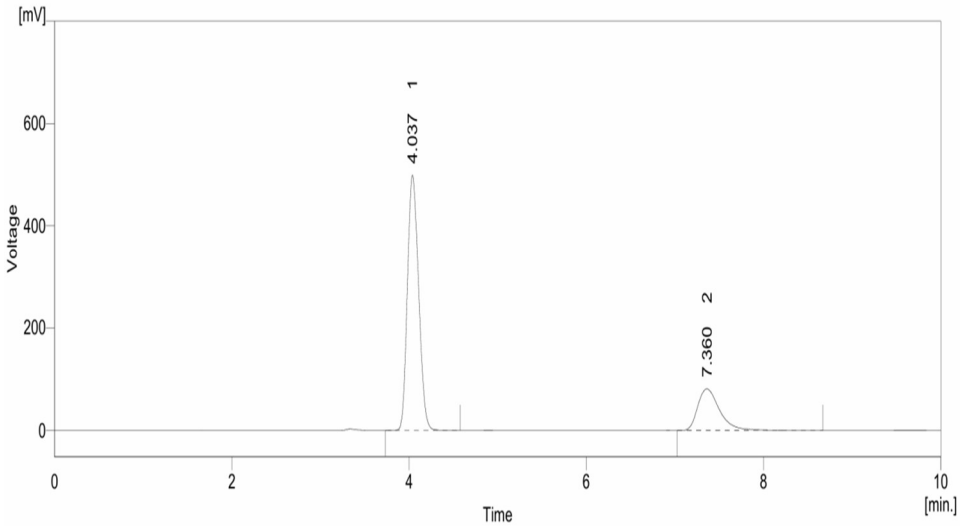
Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out (Table 12 & 13).

Table no. 12: Assay data for MET and ERTU combination marketed formulation

	Serial No	Label Claim (mg)	Result (mg)	% Asaay	Avg % Assay
Metformin	1	325	312.44	96.13	96.75
	2	325	314.97	96.91	
	3	325	315.93	97.20	
Ertugliflozin	1	25	25.05	100.21	99.43
	2	25	24.34	97.37	

	3	25	25.18	100.72	

Figure no.11: Overly spectrum of ERT and MET HCl showing Assay of Standard



Conclusion

A systematic approach was successfully employed for the development and validation of analytical techniques for the simultaneous quantification of Ertugliflozin and Metformin Hydrochloride in bulk drugs and pharmaceutical dosage forms. Metformin Hydrochloride Tablets USP is oral anti hyperglycemic drugs used in the management of type 2 diabetes. The combine dosage form of MET HCL and ERT are used in the treatment of diabetes. 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 was run for appropriate minute with mobile phase buffer (pH): methanol (65:35). Inertsil C18 (250x4.6 mm) column, mobile phase buffer (potassium dihydrogen pH 6.8): methanol (65:35 v/v) with flow rate1.0 ml /min and injection volume 20ul.The calibration curve was linear and regression co-efficient (R2) value was found to be 0.999 and concentration ranging from 50-150ug/ml and 0.5-1.5ug /ml for Ertugliflozin and metformin hydrochloride respectively. The LOD and LOQ of the method were found 1.04ug /ml, 9.61ug /ml and 0.0007ug/ml, 0.006ug/ml for Ertugliflozin and metformin HCl. The detection is carried out at wavelength 220nm. It was found to be simple, precise and accurate. The % RSD also less than 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.

References:

1. Drug profile for Metformin HCl available https://www.drugs.com/pro/metformin.html
2. Drug profile for Metformin HCl available https://www.drugs.com/metformin.html
3. Drug profile for Ertugliflozin available https://www.drugs.com/ppa/ertugliflozin.htmL
4. Juan Pablo Frias. Fixed-dose combination of ertugliflozin and metformin hydrochloride for the treatment of type 2 diabetes. Expert review of endocrinology & metabolism.
5. Drug profile for Ertugliflozin available https://www.drugs.com/mtm/ertugliflozin.htmL
6. Segluromet™ (ertugliflozin and metformin hydrochloride), Prescribing Information. Whitehouse Station, NJ: Merck & Co., Inc.; 2017.
7. Juan Pablo Frias. Fixed-dose combination of Ertugliflozin and metformin hydrochloride for the treatment of type 2 diabetes. Expert Review of Endocrinology & Metabolism.
8. P.Venkateswara Rao, A. Lakshmana Rao, and S.V.U.M Prasad: A New Stability Indicating RP-HPLC Method For Simultaneous Estimation of Ertugliflozin and Sitagliptin In Bulk and Pharmaceutical

Dosage Form Its Validation As Per ICH Guidelines. Indo American Journal of Pharmaceutical Sciences 2018; 05(04): 2616-2627.

9. D. China Babu, C. Madhusudhana Chetty and Sk. Mastanamma: Novel Stress Indicating RP-HPLC method development and validation for the simultaneous estimation of ertugliflozin and sitagliptin in bulk and its formulation. Oriental Journal of Chemistry 2018; 34(5): 2554-2561.
10. Harshalatha P, Kothapalli Bannoth Chandrasekhar and Chandrasekhar Mv: A Novel RP-HPLC Method for Simultaneous Determination of Ertugliflozin and Sitagliptin in Bulk and Tablet Dosage Form. International Journal of Research in Pharmaceutical Sciences 2018; 9(3): 1042-1050.
11. Kavitha D., S. K. Sahoo, Venkateswara Rao P, Nagamani M., and Bhagyalaxmi Ch: Development and Validation of RP-HPLC method for determination of metformin and sitagliptin in bulk and pharmaceutical dosage form. Journal of applied pharmaceutical research 2017; 5(2): 34 – 39
12. P.Shyam Sundar, Ishrath Tabassum, R.Vasanthi1, M. Alagar Raja, K.N.V. Rao, K. Rajeswar Dutt, and Merugu Mahesh: Development and Validation of Stability Indicating RP-HPLC method for the Simultaneous Estimation of Alogliptin and Metformin in Bulk and Pharmaceutical Dosage Form. Indo American Journal of Pharmacy2017; 3 (5): 223-233.
13. W. Abu Dayyih, M. Hamad , E. Mallah, A. Abu Dayyih , R. Awad , Z. Zakaria And T. Arafat: Method Development and Validation of Vildagliptin and Metformin HCl in Pharmaceutical Dosage Form By Reverse Phase–High Performance Liquid Chromatography (Rp-Hplc). International Journal of Pharmaceutical Sciences and Research 2018; 9(7): 2965-2972.
14. Manish D. Patil, Mayank Bapna, Priyanka Shah and Suleman S. Khoja: Development and Validation of Analytical Method for Simultaneous Estimation of Metformin Hydrochloride and Teneligliptin Hydrobromide Hydrate in Pharmaceutical Dosage Form. Journal Pharmaceutical Science Bioscientific Research 2017;7(2):200-208.
15. Ashim Kumar Sen, Denish N. Hinsu, Dhanya B. Sen, Aarti S. Zanwar, Rajesh A. Maheshwari, and Vikas R. Chandrakar: Analytical method development and validation for simultaneous estimation of Teneligliptin Hydrobromide hydrate and Metformin hydrochloride from its pharmaceutical dosage form by three different UV spectrophotometric methods. Journal of Applied Pharmaceutical Science 2016; 6(09): 157-165.
16. Ahmed Gedawy, Hani Al-Salami and Crispin R. Dass: Development and Validation of a New Analytical HPLC Method for Simultaneous Determination of the Antidiabetic Drugs Metformin And Gliclazide. Journal of food and drug analysis. 2019; 2(7): 315-322.
17. Sowjanya P: RP-HPLC Method Development of Metformin in Pharmaceutical Dosage Form. Research &Reviews. Journal of Pharmaceutical Analysis 2015; 4(4): 9-20.
18. ICH. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of analytical procedure: Text and Methodology Q2 (R1); 2005.https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_-Guideline.pdf
19. ICH. Validation of Analytical Procedures: Text and Methodology. Geneva: International Conference on Harmonization, IFPMA; 1996.https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf