Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1155-1163 | ISSN:2251-6727

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Empagliflozin and Metformin Hydrochloride in Bulk Drug and Tablet Dosage Form.

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(Received: 14 April 2024 Revised: 1 May 2024 Accepted: 18 June 2024)

KEYWORDS

Empagliflozin, Metformin hydrochloride, RP-HPLC, Methanol, ICH guideline, Validation.

ABSTRACT:

Background

Type 2 diabetes mellitus is a chronic condition that affects millions of people worldwide. The combination of Empagliflozin and Metformin hydrochloride in tablet form is commonly used for the treatment of this condition. Accurate and precise determination of these drugs in their combined dosage form is crucial for quality control and effective therapeutic outcomes.

Objective

To develop and validate a selective, accurate, and precise reverse phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of Empagliflozin and Metformin hydrochloride in combined tablet dosage form.

Method

Chromatographic separation was achieved using a Cosmosil C18 column (250mm x 4.6ID, Particle size: 5 micron) with a mobile phase consisting of Methanol:KH_2PO_4 Buffer (60:40). The retention times (RT) for Empagliflozin and Metformin hydrochloride were 6.7 and 4.1 minutes, respectively. The flow rate was set at 0.8 ml/min, and detection was performed at an ultraviolet wavelength of 227 nm. The method was validated according to ICH guidelines.

Result

The linearity ranges for Empagliflozin and Metformin hydrochloride were 1-5 µg/mL and 40-200 µg/mL, respectively, with regression coefficients (R^2) of 0.9966 and 0.9971. Precision studies indicated that the % R.S.D. was less than 2%. The % assay of Empagliflozin and Metformin hydrochloride were 99.94% and 99.80%, respectively.

Conclusion

The developed RP-HPLC method is selective, accurate, and precise for the simultaneous determination of Empagliflozin and Metformin hydrochloride in combined tablet dosage form. This validated method complies with ICH guidelines and is suitable for routine quality control analysis in pharmaceutical settings.

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Introduction

Diabetes is a long-term metabolous condition marked by elevated blood sugar levels. Type 2 diabetes is the most prevalent and typically affects adults, which occurs when the body became resistance to insulin or doesn't make sufficient insulin [1-2]. Empagliflozin is a medication belonging to the gliflozin class, which inhibits the sodium-glucose cotransporter-2 mechanism, with chemical name 1-chloro-4-[b-Dglucopyranos-1-yl]-2-[4-([S]- tetrahydrofuran-3-yl-oxy) benzyl, it assists in preventing sodium glucose transport protein from being reabsorbed into the blood after the kidneys have filtered it out. Its chemical formula is C23H27ClO7 & its molecular weight is 450.91 g/mol [3].

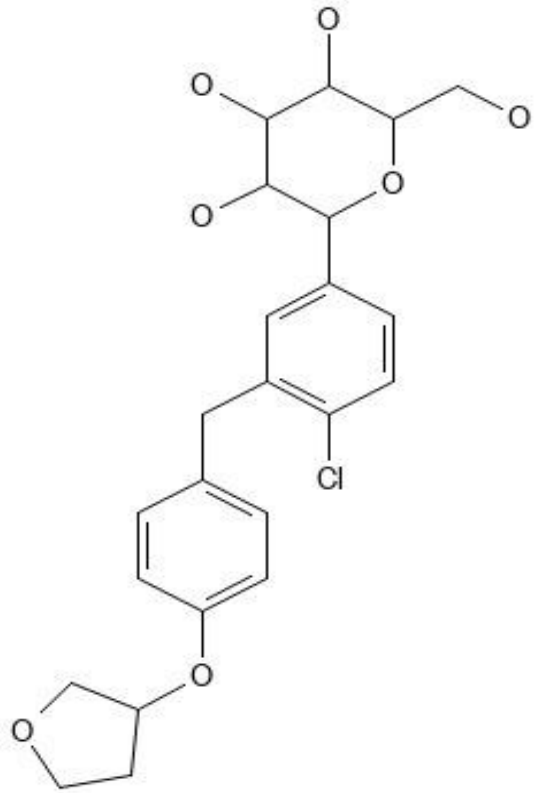

Figure 1: Chemical structure of Empagliflozin

When treating type 2 diabetes, metformin is typically recommended as the first line pharmacologic therapy. Metformin hydrochloride, also known as 1,1-dimethylimido dicarbonimidic diamide hydrochloride, is a medication in the biguanide class, it functions by enhancing the body's tissue's sensitivity to insulin and decreasing glucose production by the liver. Its molecular formula is $C_4H_{12}ClN_5$ and its molecular weight is 165.62 g/mol [4].

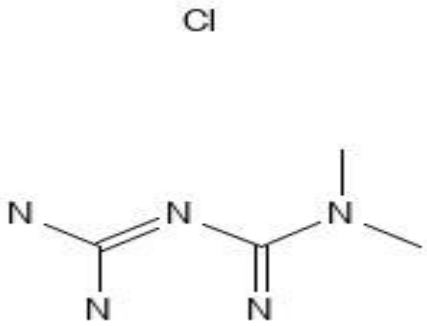

Figure 2: Chemical structure of Metformin hydrochloride

Literature survey showed that there are various techniques for estimation of both the drugs in combination as well as in alone using various analytical techniques like UV spectrophotometer [5-7], LC-MS/MS [8], RP-HPLC [9-16], UPLC [17-18].

Materials and Methods

Equipment

The chromatographic separation was carried out on Cosmosil C18 column (250mm x 4.6ID, Particle size: 5 micron), with UV detector with HPLC workstation software. Double beam UV-Visible spectrophotometer for spectroscopic determinations and Wenser high precision balance was used in the study for weighing.

Chemicals and reagents

Empagliflozin & Metformin hydrochloride pure drugs (API), Combination of Empagliflozin and Metformin hydrochloride tablet (Jardiance Met), Water were purchased from Qualigens, Methanol was procured from Merck Specialties Private Limited and Potassium dihydrogen phosphate buffer from Hexon laboratories, Ortho-phosphoric acid.

Chromatographic conditions

Mobile Phase consists Methanol:10mM KH2PO4 Buffer (60:40) were used. The flow rate was 0.8ml/min with injection volume is 20µl. Ultra violet detection wavelength was obtained at 227nm & at room temperature; the separation was accomplished.

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Preparations of solution

Standard stock solution (Preparation)

Accurately weighed (10mg of each of) Empagliflozin and Metformin hydrochloride were dissolved separately in 10ml of solvent in volumetric flasks A and B. The drug was sonicated for 5 min and finally made up to the mark gives 1000 ppm solution.

Sample stock solution (Preparation)

Accurately weighed 20 tablets & average weight of each tablet was calculated & were ground to obtain fine powder. The average weight of 20 tablet was found to be 603.95 mg. In 603.95 mg contains API dose as 500 mg. So, to get 10mg of API will have to take 12.079 mg, and it was weighed and dissolved it into 10ml to get 1000 ppm of solution.

Result and discussion

Method validation

The method was validated in accordance with ICH criteria, and the parameters, including Linearity, Precision, Accuracy, LOD, LOQ, robustness and system suitability parameters was evaluated.

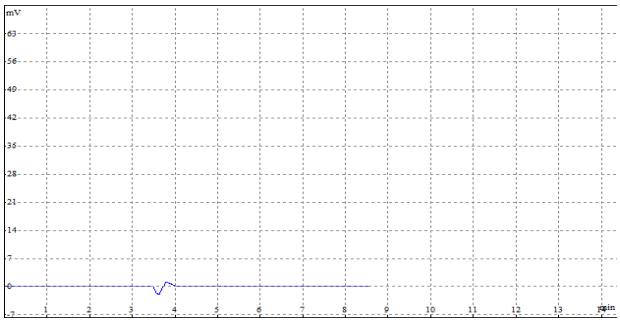

Figure 3: Chromatogram of Blank

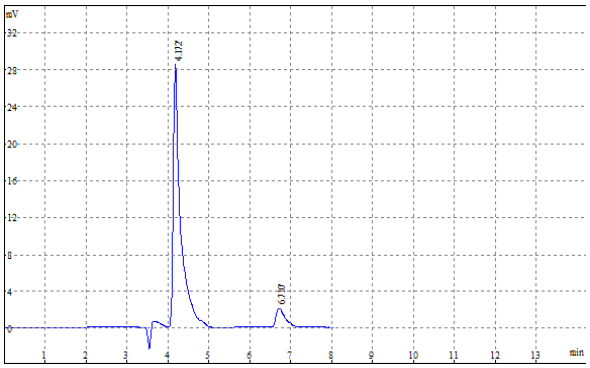

Figure 4: Optimized chromatogram of metformin HCL and Empagliflozin.

Linearity

Linearity was studied with calibration curve by analyzing different concentration of range 1-5 µg/mL for Empagliflozin & 40-200 µg/mL for Metformin hydrochloride. The correlation coefficient (R^2) for given drug was found to be 0.9966 and 0.9971 respectively. The calibration curve for given drug were shown in figure 5 and 6.

Sr. No.	Concentration	Area
1	1	70977
2	2	127135
3	3	193610
4	4	278742
5	5	341802

Table 1: Linearity data of Empagliflozin

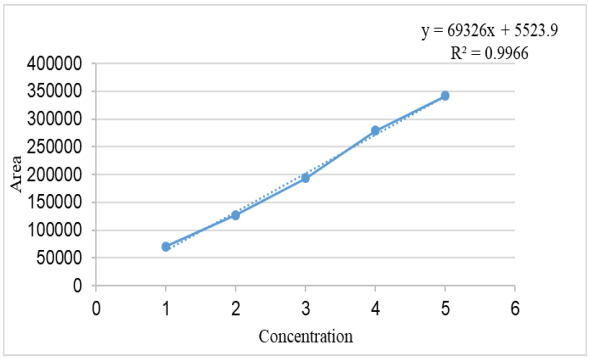

Figure 5: Calibration curve for Empagliflozin

Sr. No.	Concentration	Area
1	40	503302
2	80	1210095
3	120	1984545
4	160	2959474
5	200	3734875

Table 2: Linearity data of Metformin hydrochloride

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JCHR (2024) 14(4), 1155-1163 | ISSN:2251-6727

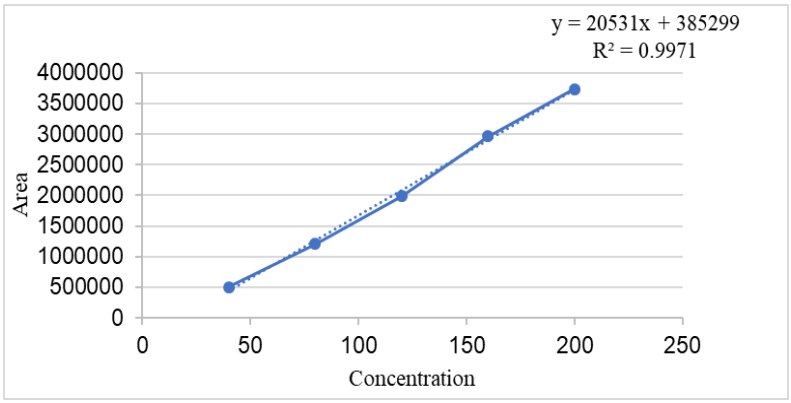

Figure 6: Calibration curve for Metformin hydrochloride

Precision

In order to measure repeatability (intra-day precision), drug solutions were analyzed on the same day and Intermediate precision dealt with differences within the laboratory, such as various days, different equipment, different analysts, etc.

Precision studies of Empagliflozin

			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	%SD	%RSD
1	3 3 3	193610 192586 195486	193894	1470.71139	0.7585131	0.26111922
2	3 3 3	137597 138656 138290	138181	537.848492	0.3892348	

Table 3: Inter-day study of Empagliflozin

			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	%SD	%RSD
1	3 3 3	193610 192586 195486	193894	1470.7114	0.7585131	0.1865489

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2	3 3 3	218724 216571 217688	217661	1076.7539	0.4946931	

Table 4: Intraday study of Empagliflozin

Precision studies of Metformin hydrochloride

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	120 120 120	1984545 1974609 1951216	1970123.3	17111.296	0.8685394	0.3505241
2	120 120 120	1908774 1921951 1910619	1913781.3	7135.026	0.3728235	

Table 5: Inter-day study of Metformin Hydrochloride

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	120 120 120	1984545 1974609 1951216	1970123.333	17111.2964	0.8685394	0.52610726
2	120 120 120	1985443 1987826 1982883	1985384	2472.02811	0.1245113	

Table 6: Intraday study of Metformin Hydrochloride

Accuracy (% Recovery)

The capacity of a procedure to recover a known amount that has been spiked at varying quantities to the assessed sample. Recovery studies were performed at three distinct levels 50%,100%,150%. The solutions were analyzed for % Recovery.

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Sr. No	% Composition	Area of standard (Area Units)	Area of sample (Area Units)	% Recovery (%)	Conc. Taken (ppm)	Conc. Found (ppm)
1	50% Recovery	193610	193463	99.92407417	3	2.997722225
2	100% Recovery	278742	277382	99.51209362	4	3.980483745
3	150% Recovery	341802	338538	99.04506118	5	4.952253059

Table 7: Recovery study of Empagliflozin

Sr. No	% Composition	Area of standard (Area Units)	Area of sample (Area Units)	% Recovery (%)	Conc. Taken (ppm)	Conc. Found (ppm)
1	50% Recovery	1984545	1983370	99.94079247	120	119.928951
2	100% Recovery	2959474	2958044	99.9516806	160	159.922689
3	150% Recovery	3734875	3732725	99.94243449	200	199.884869

Table 8: Recovery study of Metformin hydrochloride

Limit of detection and limit of quantitation

The assay method's detection/quantitation limits are the lowest concentrations that can be detected & quantitated. The following formulas were used to get the LOD and LOQ values; LOD = 3.3 σ/S and LOQ = 10 σ/S, respectively. In this method, σ represents the standard deviation of the responses, while S is the mean of the calibration curve slopes.

Sr. No.	Drug	SD	Slope	LOD	LOQ
1	Empagliflozin	431.87	6932.6	0.205575253	0.6229553
2	Metformin HCL	764.748	82125	0.0307296	0.09312

Table 9: LOD& LOQ of drugs

Robustness

The performance of a successful analytical technique is measured by how well it stands up to small but deliberate variations. The robustness was performed by change in wavelength, change in pH.

Sr. No.	Change in parameter	Concentration	Area	Mean	SD	% RSD
1	Change in wavelength	2 2 2	127135 126086 127856	127026	890.051	0.7006858

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2	Change in pH	2 2 2	127135 127424 126166	126908	658.919	0.5192087

Table 10: Robustness study of Empagliflozin

Sr. No.	Change in parameter	Concentration	Area	Mean	SD	% RSD
1	Change in wavelength	80 80 80	1210095 1223752 1213307	1215718	7140.6	0.5873562
2	Change in pH	80 80 80	1210095 1206072 1218825	1211664	6519.67	0.5380756

Table 11: Robustness study of Metformin hydrochloride

Assay

The assay performed by the marketed formulations (Jardiance met 12.5mg/500mg of EMP & MET.HCL). The prepared sample and standard solution were injected into HPLC and peak areas were recorded. Final percentage amount of drug was calculated.

Sr. No	Drug	Concentration	Area of standard	Area of sample	% Assay
1	Empagliflozin	3 ppm	193610	193495	99.9406022
2	Metformin HCL	120 ppm	1984545	1980581	99.8002565

Table 12: Assay of samples

Conclusion

For the determination of Empagliflozin and Metformin HCL in mixed dosage form, a simple, precise and exact approach was established. All results have %RSD of less than 2%, indicating a high degree. Hence the suggested method was simple to use, affordable, efficient and suitable for routine examination of combination dose form of Empagliflozin and Metformin HCL. The approach was successfully employed for the simultaneous estimation of EMP & MET.HCL in solid dosage form and was verified in accordance with ICH guidelines.

Abbreviations

RP-HPLC: Reverse Phase High-Performance Liquid Chromatography

SGLT2: Sodium- glucose co- transporter

UV: Ultraviolet

LOD: Limit of detection

LOQ: Limit of quantitation

PPM: Parts per million

NLT: Not less than

RSD: Relative standard deviation

NMT: Not more than

R.T: Retention time

µg/ml: Microgram per milliliter

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JCHR (2024) 14(4), 1155-1163 | ISSN:2251-6727

Acknowledgement

The authors are grateful to the Management and Principal, Dr. Laxmikant B. Borse of Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Nashik for providing necessary facilities to carry out the research work.

Conflict of Interest

There is no conflict of interest.

Funding Source

No

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