

DEVELOPMENT OF VALIDATED RP-HPLC METHOD FOR ESTIMATION OF EMPAGLIFLOZIN AND METFORMIN IN COMBINED FORMULATION

Abstract

Aim: The aim of the present study include development of validated RP-HPLC method for estimation of Empagliflozin and Metformin in combined dosage form by using LC-MS compatible volatile mobile phase.

Methodology: Appropriate separation of drugs was achieved using C18 column as a stationary phase and **Acetonitrile:** Water (50: 50, v/v) at a flow rate 1mL/min as mobile phase. Detection was done at 230 nm.

Results: The R_t of Metformin and Empagliflozin was found to be 2.20 ± 0.02 min and 3.64 ± 0.02 min respectively. When the marketed formulation was analyzed by the developed method, the % drug contents were found to be 98.57 ± 1.28 and 99.86 ± 1.02 %w /w for EM and MH, respectively. The method was found to be linear in a range of 11.25 – 56.25 $\mu\text{g/mL}$ for Empagliflozin and 85 – 425 $\mu\text{g/mL}$ for Metformin. Detection limit and quantitation limit were found to be 0.30 and 0.92 $\mu\text{g/mL}$ for EM and 1.12 and 3.36 $\mu\text{g/mL}$ for MH, respectively. The accuracy and precision results were found to be near 100 % w/w for both the drugs. The method was also found to be robust and specific.

Conclusion: The developed RP-HPLC method was found to be linear, sensitive, accurate, precise, specific and robust for the analysis of Empagliflozin and Metformin in combined dosage form.

Keywords

Empagliflozin, Metformin, RP-HPLC, Method validation

1. Introduction

Empagliflozin (EM) chemically known as (1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl)-D-glucitol (Fig. 1) is a sodium glucose co-transporter-2 (SGLT-2) inhibitor approved to improve glycemic control in adult patients with type II diabetes. The reabsorption of glucose from the glomerular filtrate takes place by SGLT2. Inhibition of SGLT2 decreases renal glucose reabsorption resulting in enhanced glucose excretion¹. It is not yet official drug in IP, BP and USP.

Metformin HCl (MH) is an anti-hyperglycemic agent, used for the treatment of type II diabetes. Metformin decreases blood glucose levels by increasing insulin sensitivity, increasing glucose uptake and utilization in the peripheral tissue. It also decreases glucose production in liver as well as absorption of glucose by intestine¹. Its chemical name is 1, 1-dimethylbiguanide hydrochloride (Fig. 2). It is official in IP, BP and USP²⁻⁴.

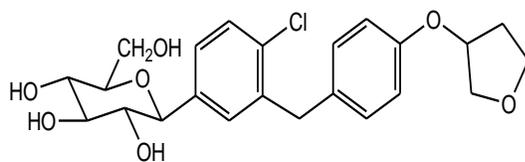


Fig. 1 Empagliflozin

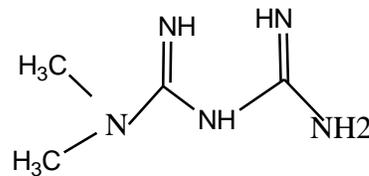


Fig. 2 Metformin

Combination of EM and MH is approved by FDA on 26 Aug 2015 and CDSCO on 17 Oct 2017 as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus⁵⁻⁶.

The thorough literature survey revealed reporting of UV-Visible spectroscopic⁷⁻¹¹, HPLC¹²⁻¹⁸ and UPLC¹⁹⁻²⁰ methods for determination of EM in combination with MH. From above literature, it was clear that, there are number of UV-Visible spectroscopic methods reported for estimation of EM and MH in combined dosage form. The HPLC methods reported for EM and MH used buffers like phosphate as a part of mobile phase. Use of buffers decrease column life as well as it requires thorough washing after analysis which increases analysis time. Therefore, it was decided to develop a RP-HPLC method for estimation of EM and MH in combined tablet formulation by using simple volatile mobile phase.

2. Materials and Methods

Pure standard sample of Empagliflozin (99.33 % w/w) was procured from Manus Aktteva Biopharmaceutical, Gujarat. Pure sample of Metformin (99.7 %, w/w) was obtained as a gratis sample from Nulife Pharmaceuticals, Pune. Tablet Synjardy containing EM and MH was purchased from local market. AR grade chemicals and solvents were procured from Research Lab, Mumbai.

2.1 Instrumentation

- UV-Visible Spectrophotometer (Shimadzu-1800, Japan) with 10 mm matched quartz cells was used for selection of wavelength.
- All weighings were done on an electronic balance (Shimadzu model AUX 220) and Ultra Sonicator was used for sonication.
- HPLC System- Cyberlab HPLC (Model: LC-100B) binary gradient system with DAD detector, 772Si Rheodyne injector (20 μ l), gradient mixer (GM-100), Neosphere C18 column (4.6 X 250 nm, 5 μ m) and DS-100 control data system was used.

2.2 Preparation of standard solutions

Accurately weighed 11.25 mg of EM and 85 mg of MH powder was dissolved in 10 mL of methanol to get stock solution. This stock solution was further diluted by taking appropriate aliquotes to get working standard solution of 11.25 μ g/mL of EM and 85 μ g/mL of MH.

2.3 Selection of wavelength for analysis

The standard drug solutions were scanned in 200-400 nm range in UV spectroscopy. From the UV spectroscopy overlay spectrum of EM and MH it was observed that both the drugs absorb radiations of 230 nm. Therefore analysis was done at 230 nm.

2.4 Optimization of chromatographic conditions

Combination of water and methanol in various ratios was tried as a mobile phase. Tailing of MH was observed. Therefore methanol was replaced by acetonitrile. Combinations of water and acetonitrile in various proportions were tried.

2.5 Analysis of marketed formulation

Average weight of the tablet was determined by accurately weighing twenty tablets. Tablets were triturated to get a fine powder. To the powder equivalent to 85 mg of MH, 50 mL of methanol was added in a 100 mL volumetric flask. It was sonicated for 20 min and volume was made with methanol. This suspension was filtered through Whatman filter paper No. 42. Working sample containing 22.50 µg/mL of EM and 170 µg/mL of MH was prepared by diluting suitable volumes of this stock solution with methanol. Analysis of this formulation solution was done by the developed method and % drug contents were determined.

2.6 Method Validation

The **developed** method was validated as per ICH guidelines Q2(R1)²¹.

2.6.1 Linearity and Range, Limit of detection and Quantitation:

Linearity was checked by preparing a series of dilutions in a range from standard stock solution and verifying the linear relationship between concentration and response.

Detection limit and quantitation limits were determined by formulae,

$$\text{LOD} = 3.3 \sigma / S \quad \text{and} \quad \text{LOQ} = 10 \sigma / S$$

Where, σ is SD of smallest response and S is slope of the calibration curve.

2.6.2 Accuracy

Tablet powder was spiked with pure drugs at 80, 100 and 120 % levels and analysis of samples were carried out by developed method to determine % drug recovery.

2.6.3 Precision

Repeatability and intermediate precision studies were performed to establish precision. In case of repeatability studies, sample containing 100 % of the test concentration was analyzed six times within a short interval of time in a day. Interday analysis was done to confirm the intermediate precision. Values of % RSD were found to be less than 2 indicated method is precise.

2.6.4 Robustness

Robustness was estimated by checking the stability of analytical solutions at RT. The effect of variations like change in detection wavelength (± 2 nm) and the composition of mobile phase (± 2 mL) was also determined.

2.6.5 Specificity

The specificity of the method was determined by peak purity analysis. Purity angle and purity threshold were determined.

3. Result and Discussion

3.1 Optimization of chromatographic conditions

An excellent resolution with good peak shapes was obtained by using Acetonitrile: Water (50: 50, v/v) at a flow rate 1mL/min as mobile phase. MH is highly hydrophilic and polar drug with negative log P value eluted out faster at 2.20 ± 0.02 min. Whereas, EM a lipophilic drug eluted out later with R_t 3.64 ± 0.02 min (Fig. 3). The resolution between the two drugs was 2.78.

The number of theoretical plates for EM and MH were 2900 ± 100 and 2100 ± 100 respectively. The Tailing factor for EM was 1.08 ± 0.02 and for MH was 1.12 ± 0.02 .

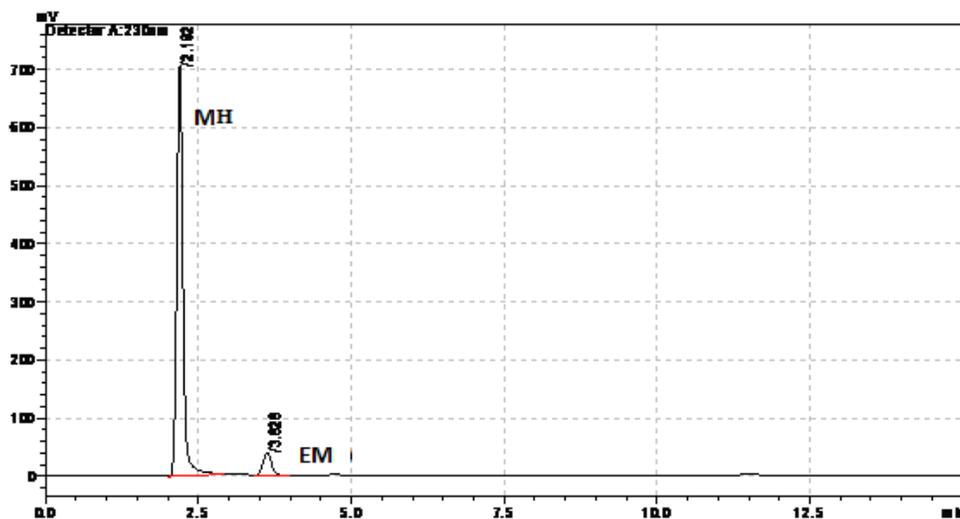


Fig. 3: Typical chromatogram of standard mixture of MH and EM

3.2 Analysis of marketed formulation

Analysis of this formulation solution was done by the developed method (Fig. 4) and % drug contents were determined. The % drug contents were found to be 98.57 ± 1.28 and 99.86 ± 1.02 % w/w for EM and MH, respectively.

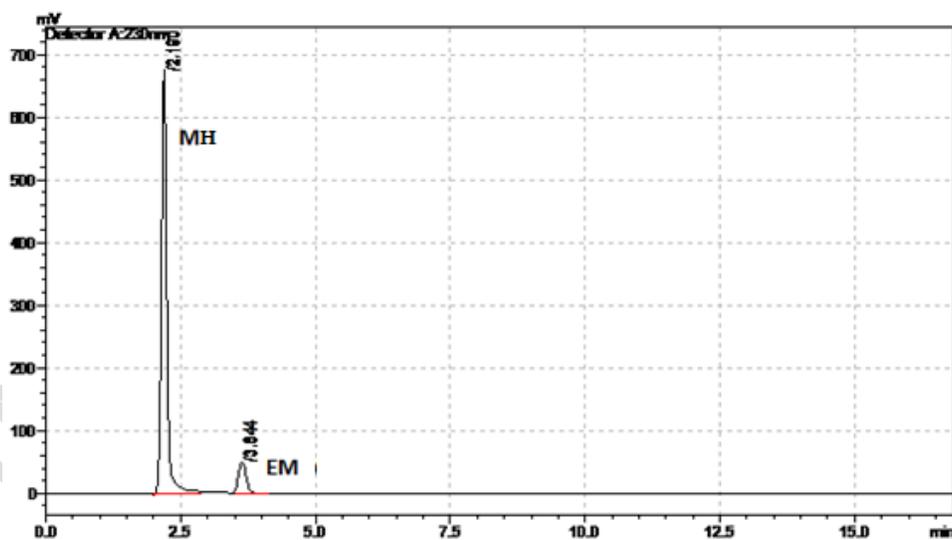


Fig. 4: Typical chromatogram of MH and EM in formulation

3.3 Method Validation

3.3.1 Linearity and Range:

The method was found to be linear in a range of $11.25 - 56.25 \mu\text{g/mL}$ for EM and $85 - 425 \mu\text{g/mL}$ for MH with correlation coefficients 0.9999 and 0.9991 for EM and MH respectively. Values close to 1 indicated good linearity (Fig. 5 and 6).

Detection limit and quantitation limit were found to be 0.30 and 0.92 $\mu\text{g/mL}$ for EM and 1.12 and 3.36 $\mu\text{g/mL}$ for MH, respectively. From such small values of limits, sensitivity of the method was proposed.

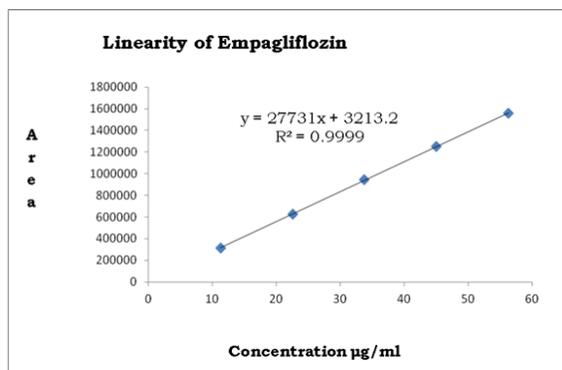


Fig. 5: Linearity plot of EM

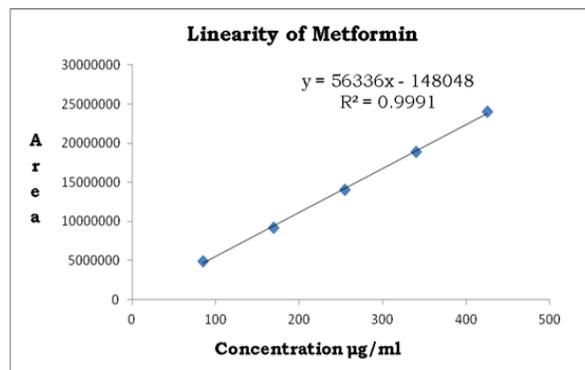


Fig. 6: Linearity plot of MH

3.3.2 Accuracy

The % drug recovery was found to be in a range of 98.86 to 99.08 and 100.59 to 101.27 % w/w for EM and MH, respectively. Values close to 100 confirmed the accuracy of the method.

3.3.3 Precision

The % drug contents were found to be in a range of 99.12- 100.34 % w/w for both the drugs. Values of % RSD were found to be less than 2 indicated method is precise.

3.3.4 Robustness

Robustness was estimated by checking the stability of analytical solutions at RT and solutions were found to be stable for 8 h at RT. The % RSD values of the deliberate variations like change in detection wavelength (± 2 nm) and the composition of mobile phase (± 2 mL) were found to be less than two proved robust nature of the method.

3.3.5 Specificity

The purity angle was found to be less than purity threshold, concluded peak purity.

Table 1 represent results of various method validation parameters.

Table 1: Result of various validation parameters

| Parameter | Empagliflozin | Metformin HCl |
|--|---------------------------------|-------------------------|
| Assay (% w/w \pm SD, % RSD) n=3 | 98.57 \pm 1.28, 1.30 | 99.86 \pm 1.02, 1.02 |
| Linearity range ($\mu\text{g/mL}$) n=3 | 11.25 – 56.25 | 85 – 425 |
| Correlation coefficient (R^2) | 0.9999 | 0.9991 |
| Limit of detection ($\mu\text{g/mL}$) | 0.30 | 1.12 |
| Limit of quantification ($\mu\text{g/mL}$) | 0.92 | 3.36 |
| % Recovery | 80 | 100.59 \pm 1.18, 1.17 |
| (% w/w \pm SD, % RSD) n=3 | 100 | 101.27 \pm 1.09, 1.08 |
| | 120 | 98.86 \pm 1.22, 1.23 |
| Repeatability, n=6 | 99.12 \pm 1.16, 1.17 | 100.91 \pm 1.11, 1.10 |
| (% w/w \pm SD, % RSD) | | |
| intermediate precision, n=9 | 99.56 \pm 1.02, 1.02 | 100.23 \pm 1.00, 1.00 |
| (% w/w \pm SD, % RSD) | | |
| Robustness | Change in composition of mobile | 99.74 \pm 1.03, 1.03 |
| | | 100.23 \pm 1.21, 1.21 |

| | | | |
|-----------------------------------|--|------------------------|-------------------------|
| (% w/w \pm SD, % RSD) n=3 | phase (\pm 2 mL) | | |
| | Change in detection wavelength (\pm 2 nm) | 98.92 \pm 0.87, 0.88 | 100.29 \pm 1.18, 1.18 |
| Purity angle, n=3 | | 0.194 | 0.227 |
| Purity threshold, n=3 | | 0.289 | 0.394 |

4. CONCLUSION

The HPLC methods reported for EM and MH have used buffers like phosphate as a part of mobile phase. Use of buffers decrease column life as well as it requires thorough washing after analysis which increases analysis time. Therefore, a RP-HPLC method using Acetonitrile:Water (50:50, v/v) as a mobile phase was developed and validated for the determination of EM and MH simultaneously in tablet formulation. The assay values and Recovery values were found to be nearer to 100% w/w. Whereas %RSD for precision and robustness studies was found to be less than two. The developed HPLC method was validated and found to be linear, sensitive, accurate, precise, specific and robust.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

5. REFERENCES

1. European Medicines Agency, Assessment Report, Procedure No. EMEA/H/C/003770/0000, 26 March 2015, EMA/238334/2015.
2. Indian Pharmacopoeia, Government of India, Ministry of health and family welfare. Vol-II, Gaziabad, India, Indian Pharmacopoeia Commission; 2014. p. 2186-2187.
3. British Pharmacopoeia, Vol-II, The Stationary Office on behalf of Medicines and Health Care Products Regulatory Agency (MHRA), London, United Kingdom; 2008. p. 1410-1411.
4. United States Pharmacopoeia-34 National Formulary-29, Vol-III, United States Pharmacopoeial Convection, Rockville; 2011. p. 3442.
5. www.accessdata.fda.gov/drugsatfda_docs/nda/2015/206111Orig1s000TOC.cfm (visited on 07/12/2017).
6. www.cdscoonline.gov.in/CDSCO/Drugs (visited on 07/12/2017).
7. Bassam M. A., Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of Empagliflozin and metformin: Applied to recently approved pharmaceutical formulation, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2016**, 168, 118–122.
8. Bassam M. A., Application of spiking technique coupled with derivative spectrophotometry for the analysis of a novel anti-diabetic combination of two coformulated drugs with highly different concentrations, *Der Pharma Chemica*, **2016**, 8(10), 12-14.

9. Padmaja N.; Mulagiri S. B.; Veerabhadram G., Development and validation of UV spectrophotometric method for simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage forms, *Der Pharmacia Lettre*, **2016**, 8(13), 207-213.
10. Kadam A. T.; Zameeruddin M.; Arsul V., Development and validation of analytical methods for the simultaneous estimation of Metformin hydrochloride and Empagliflozine, *Journal of Medical and Pharmaceutical Innovation*, **2017**, 4(21), 21-27.
11. Bassam M. A.; Rola M. E.; Mahmoud M. Y.; et.al., Mean centering method for determination of Empagliflozin and Metformin, *Marmara Pharm J*, **2017**, 21(3), 669-674.
12. Godasu S. K.; Sreenivas S. A., A new validated RP-HPLC method for the determination of Metformin HCl and Empagliflozin in its bulk and pharmaceutical dosage forms, *Int J Pharm Sci Research*, **2017**, 8(5), 2223-2232.
13. Shaik M. N.; Sadath A., RP-HPLC method development and validation for the simultaneous estimation of Metformin and Empagliflozine in tablet dosage form, *International Journal on Engineering Technology and Sciences*, **2015**, 2(11), 2349-3976.
14. Patil S.D.; Amurutkar S.V.; Chatpalliwar V. A., Upasani C. D., Development and validation of RP-HPLC method for Empagliflozin and Metformin HCL, *Journal of Innovations in Pharmaceutical and Biological Sciences*, **2017**, 4(4), 185-189.
15. Hussain B., Karuppasamy C., Suresh Y., Somasekhar G., Jyothsna M., Venkatesh, Method development and validation of Metformin and Empagliflozin in pharmaceutical dosage forms in RP-HPLC, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, **2016**, 4(3), 91-100.
16. Rupasi P.; Raju M. B., Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin in bulk and in a synthetic mixture, *Int J Pharm*, **2016**, 6(4), 138-147.
17. Geetha S. P., Lakshmana R K, Prasad KRS, Suresh B K, Development and validation of stability indicating reversed phase High-Pressure Liquid Chromatography method for simultaneous estimation of Metformin and Empagliflozin in bulk and tablet dosage form, *Asian J Pharm Clin Res*, **2016**, 9(1), 126-135.
18. Madan Mohan Reddy M., Gowri Sankar D., Seshagiri Rao J, Stress degradation studies and development of validated stability indicating assay method by RP-HPLC for simultaneous estimation of Metformin and Empagliflozin in presence of degradation products as per ICH guidelines, *J Sci Res Pharm*, **2017**, 6(2), 20-33.
19. Babu B. S.; Nataraj K.S., Pawar A. K. M., Gnananath K., Method development and validation for the determination of Metformin HCl and Empagliflozin in its bulk and pharmaceutical dosage forms by RP-Ultra Performance Chromatography method, *Int J Pharma Bio Sci*, **2017**, 8(4), 253-258.
20. Padmaja N.; Veerabhadram G, A novel stability indicating RP-UPLC-DAD method for determination of Metformin and Empagliflozin in bulk and tablet dosage form, *Orient. J. Chem.*, **2017**, 33(4), 1949-1958.
21. ICH Harmonised Tripartite Guideline, *Validation of Analytical Procedures: Text And Methodology Q2(R1)*, November 2005.