ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NEW STABILITY-INDICATING REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND EMPAGLIFLOZIN IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of this study was to develop and validate a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of the metformin and empagliflozin in tablet dosage forms.

Methods: The chromatographic conditions were optimized and it was run through Std. BDS (250 mm × 4.6 mm, 5 m) column with mobile phase consisting of 0.1% orthophosphoric acid buffer: acetonitrile in the ratio of 50:50. The flow rate was 1 ml/min and optimized wavelength was 210 nm. Temperature was maintained at 30°C.

Results: The retention times of metformin and empagliflozin were found to be 2.588 min and 3.679 min and percentage relative standard deviation (RSD) of the metformin and empagliflozin was found to be 0.59 and 1.2, respectively. Percentage recovery was in the range of 100.01-100.65% for metformin and empagliflozin, respectively.

Conclusion: A sensitive, rapid, and specific method has been developed for the simultaneous estimation of metformin and empagliflozin using RP-HPLC in tablet dosage form.

Keywords: Metformin, Empagliflozin, Reverse-phase high-performance liquid chromatography, Validation, Stability indicating.

INTRODUCTION

Metformin is chemically named as N,N-dimethyl imidodicarbonimidic diamide [1], is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus. It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption, and increasing insulin-mediated glucose uptake.

Empagliflozin is chemically named as [2S, 3R, 5R, 6R]-7-{4-[4-Chloro-3-[[3S]-oxolan-3-yloxy]phenyl]methyl}piperidin-6-hydroxymethyl]oxane-3,4,5-triol [2]. Empagliflozin is a sodium glucose co-transporter-2 inhibitor, which lowers blood glucose in people with type 2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting the excretion of excess glucose in the urine (Fig. 1).

According to the literature survey, there are only few reverse-phase high-performance liquid chromatography (RP-HPLC) methods and ultraviolet (UV) methods available for the estimation of metformin and empagliflozin individually and in combination with other drugs [3-8]. Hence, an attempt was made to develop RP-HPLC method for simultaneous estimation of metformin and empagliflozin in tablet formulation to decrease the retention times and run time. It can be adopted in regular quality control test in industries and laboratories.

METHODS

Chemicals and reagents

Acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, potassium dihydrogen phosphate, triethylamine, and orthophosphoric acid procured from Rankem Chemicals. HPLC grade methanol and water were purchased from SD Fine chemicals.

Combination of metformin and empagliflozin tablets were purchased from local market.

Instrumentation

Waters HPLC 2965 system equipped with autoinjector and photodiode array detector with Empower 2 Software was used for method development. UV-visible spectrophotometer PG Instruments T60 with a special bandwidth of 2 mm and 10 mm and matched quartz was used for spectral measurements of metformin and empagliflozin solutions.

Chromatographic conditions

The chromatographic separation and analysis were performed using a Std. BDS column (250×4.6 mm, 5 m particle size) with the mobile phase consisting of buffer and acetonitrile in the ratio of 50:50 at a flow rate of 1 ml/min and detection wavelength was 210 nm. Injection volume was 10 ml and column temperature was maintained at 30°C. Optimized chromatogram was shown in Fig. 2.

Standard preparation

Accurately weighed and transferred 34 mg of metformin and 2 mg of empagliflozin into a 10 ml and 100 ml clean, dry volumetric flasks and 3/4 ml of diluent was added and sonicated for 30 minutes. Final volume was made up with diluent. 1 ml solution was pipetted out from the above two stock solutions and transferred into a 10 ml volumetric flask and made up to final volume with diluent. Typical chromatogram was shown in Fig. 3.

Sample preparation

A total of 20 tablets were weighed and average weight was calculated for each tablet. Then, the tablets were powdered and equivalent weight...
of one tablet was calculated. It was transferred into a 250 ml volumetric flask 200 mL of diluent was added, sonicated for 25 min. Using diluent final volume was made and filtered. 0.2 ml was pipetted out from the filtered solution into a 10 ml volumetric flask and final volume was made with diluent.

**Assay preparation**

Stock solutions of standard preparations were made from the active pharmaceutical ingredient (API) and sample preparations were prepared from formulation using appropriate diluent. Working solutions were prepared from stock solutions and both sample and standards were injected into chromatographic system for the determination of assay.

**Validation of HPLC method[9,10]**

**System suitability**

System suitability was carried out to check the system performance. All the essential characteristics including the percentage RSD, the United States Pharmacopeia (USP) tailing, the USP plate count, and the USP resolution were verified. The results are shown in Table 1.

**Specificity**

The specificity of the method was carried out by injecting blank, placebo, standard, and sample solutions into the chromatographic system. The interference of the analyte peaks with the excipients was analyzed.

**Linearity**

The linearity test was performed to ensure correlation response of concentration range of the analyte and the peak area. The linearity studies were performed using stock solution containing 850 µg/ml and 5 µg/ml metformin and empagliflozin. These solutions were further diluted with diluents to yield the concentration range of 2.215–1275 ppm and 1.25–7.5 ppm of metformin and empagliflozin. The correlation coefficients were calculated. The calibration plots are shown in Figs. 4 and 5.

**Precision**

The precision of the method was performed by intraday and interday variation studies. The intraday and interday studies were performed by injecting six injections of test preparations into the chromatographic system. The percentage RSD and SD were calculated. The results are presented in Table 2.

**Accuracy**

The accuracy was determined by calculating the percentage recoveries of known amounts of each analyte of metformin and empagliflozin claim to the excipients, and the accuracy results were expressed as percentage of analyte recovered. The results are shown in Table 3.

**Limit of detection (LOD) and Limit of quantification (LOQ)**

The limit of detection (LOD) can be defined as the lowest amount of analyte in a sample can be detected and limit of quantification (LOQ) was determined as the lowest amount of analyte that was quantified. These parameters were calculated using the formula,

![Fig. 1: (a) Chemical structure of metformin, (b) chemical structure of empagliflozin](image)

![Fig. 2: Optimized chromatogram of metformin and empagliflozin](image)

![Fig. 3: Typical chromatogram of metformin and empagliflozin](image)

| Table 1: System suitability studies of metformin and empagliflozin |
|---|
| Property | Metformin | Empagliflozin |
| RT | 2.588 min | 3.679 min |
| Theoretical plates (N) | 2886±63.48 | 4616±63.48 |
| Tailing factor (T) | 1.38±0.117 | 1.11±0.117 |

RT: Retention time

![Fig. 4: Calibration plot of metformin](image)

![Fig. 5: Calibration plot of empagliflozin](image)

| Table 2: Intraday precision results for metformin and empagliflozin |
|---|
| S. No. | Metformin (%) | Empagliflozin (%) |
| 1 | 7921030 | 250856 |
| 2 | 8015260 | 245837 |
| 3 | 8015344 | 247309 |
| 4 | 7949328 | 246258 |
| 5 | 8044578 | 241978 |
| 6 | 8012992 | 249282 |
| Mean±SD | 7993089±47205 | 246920±3074.3 |
| %RSD* | 0.59 | 1.2 |

%RSD: Relative standard deviation
Table 3: Accuracy results for metformin and empagliflozin

| Sample     | Amount added (ug/ml) | Recovery (%) | %RSD* |
|------------|----------------------|--------------|-------|
| Metformin  |                      |              |       |
| 425        | 100.64               | 1.41         |       |
| 850        | 101.18               | 1.29         |       |
| 1275       | 101.89               | 0.15         |       |
| Empagliflozin |                    |              |       |
| 2.5        | 101.51               | 0.97         |       |
| 5          | 99.89                | 0.47         |       |
| 7.5        | 100.34               | 0.96         |       |

%RSD: Relative standard deviation

Table 4: Robustness data of metformin and empagliflozin

| S. No | Robustness condition | Metformin %RSD* | Empagliflozin %RSD* |
|-------|----------------------|-----------------|---------------------|
| 1     | Flow minus (0.9 ml)  | 0.1             | 0.3                 |
| 2     | Flow plus (1.1 ml)   | 0.5             | 0.7                 |
| 3     | Mobile phase minus   | 0.3             | 0.5                 |
| 4     | Mobile phase plus    | 0.3             | 0.4                 |
| 5     | Temperature minus (25°C) | 0.1             | 0.1                 |
| 6     | Temperature plus (35°C) | 0.3             | 0.8                 |

%RSD: Relative standard deviation

Table 5: Degradation data of metformin and empagliflozin

| Type of degradation | Metformin | Empagliflozin |
|---------------------|-----------|---------------|
|                     | Area      | % recovery    | % degraded    | Area  | % recovered | % degraded |
| Acid                | 7685632   | 97.45         | 2.55          | 239367 | 98.09       | 1.91       |
| Base                | 7757303   | 96.98         | 3.02          | 236010 | 97.03       | 2.97       |
| Peroxide            | 7899186   | 98.42         | 1.58          | 239152 | 98.02       | 1.98       |
| Thermal             | 7924395   | 99.60         | 0.4           | 243284 | 99.10       | 0.9        |
| UV                  | 7947101   | 99.08         | 0.92          | 243360 | 99.23       | 0.77       |
| Water               | 7964453   | 99.08         | 0.92          | 244395 | 99.41       | 0.59       |

UV: Ultraviolet
CONCLUSION
A simple and efficient method was developed for the method development and validation for simultaneous estimation of the metformin and empagliflozin by RP-HPLC in tablet dosage form. Validation was carried out as per the International Conference on Harmonisation guidelines and method validation data showing satisfactory results. Degradation studies revealed that the method is stability indicating. Hence, the proposed method can be applicable for routine quality control analysis of tablet dosage forms in laboratories.

CONFLICTS OF INTEREST
All authors have none to declare.

AUTHORS’ CONTRIBUTIONS
The research was proposed and designed by G.Rajitha. The experimental work of the validated method development was carried out by Y.Ramya Yadav and A.Geetha Susmita. The manuscript was drafted by A. Geetha Susmita which was further edited by G. Rajitha. Authors read and approved the final manuscript.

REFERENCES
1. Kar M, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Indian J Pharm Sci 2009;71:318-20.
2. Shyamala KN, Mounika J, Nandini B. Validated stability-indicating RP-HPLC method for determination of Empagliflozin. Pharm Lett 2016;8:457-64.
3. Venkata SM, Ram BJ, Rajan DS, Adinarayana G, Ramana MK. Development of a validated HPLC method for the estimation of metformin HCl and propranolol HCl. Br J Pharm Res 2014;4:1909-22.
4. Lakshmi KS, Rajesh T, Sharma S. Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. Int J Pharm Pharm Sci 2009;1:162-6.
5. Pandya RH, Rathod R, Maheswari DG. Bioanalytical method development and validation for simultaneous determination of linagliptin and metformin drugs in human plasma by RP-HPLC method. Pharmacophore 2014;5:202-18.
6. Murthy TG, Geethanjali J. Development of a validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and rosuvastatin calcium in bulk and in-house formulation. J Chromatogr Sep Tech 2014;5:1-7.
7. Kavitha KY, Geetha G, Hariprasad R, Kaviarasu M, Venkatnarayanan R. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form. J Chem Pharm Res 2013;5:230-5.
8. Ramesh J, Kumar NS. Stability indicating RP-HPLC method development and validation for the simultaneous determination of vildagliptin and metformin in pharmaceutical dosage form. Int J Pharm Pharm Sci 2017;9:150-7.
9. Validation of Analytical Procedures. Text and Methodology. Geneva: Q2 (R1); 1996.
10. Validation of Analytical Procedures: Text and Methodology. ICH Q2 (R1) Harmonised Tripartite Guideline; 2006;15-64.