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Enhancement of Plasma Extraction Recovery of Empagliflozin and Metformin Combination using Liquid-Liquid Extraction and Vacuum Evaporation Techniques

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ABSTRACT

Two different approaches were optimized for extraction of empagliflozin and metformin from spiked plasma samples. Liquid-liquid extraction technique was applied using diethyl ether. Good results were obtained for empagliflozin but liquid-liquid extraction failed to simultaneously extract metformin and empagliflozin from human plasma that may be attributed to the high hydrophilicity of metformin with relatively low Log P that diminishes its ability to migrate to the organic layer. Plasma sample was divided into two parts in order to optimize the extraction procedure for each drug using the most suitable method followed by combining them together again as one sample showing high recovery outcomes. Plasma sample preparation involved direct precipitation of plasma proteins to obtain supernatant contains metformin that was concentrated using vacuum evaporation and then it was added to the reconstituted other plasma part that contains empagliflozin after its liquid-liquid extraction and vacuum evaporation till dryness.

Keywords: Empagliflozin; Metformin; Sample preparation; Vacuum Evaporation; Liquid-Liquid Extraction.

INTRODUCTION

Empagliflozin (figure 1) is a sodium glucose linked co-transporter 2 (SGLT-2) inhibitor. Preclinical studies have demonstrated safety, tolerability, and efficacy in terms of glycemic control and HbA1c level in type 2 diabetes mellitus patients in comparison to other anti-diabetic drugs. Empagliflozin has showed safety in type 2 diabetics with renal impairment [1]. It can be used as a single treatment or in combination with metformin (figure 2).

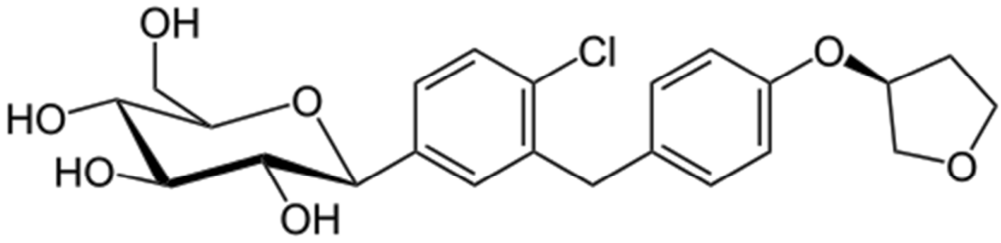

Figure 1: Chemical structure of empagliflozin

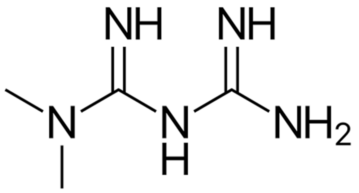

Figure 2: Chemical structure of metformin

Literature review of empagliflozin analysis and its common combinations

Although many analytical procedures were reported for the analysis or bio-analysis of the common recently approved anti-diabetic combinations [2-3], and some analytical methods were reported for determination of empagliflozin alone [4-5] or linagliptin alone [6-13] or binary mixture of empagliflozin and linagliptin [14], or binary mixture of linagliptin and metformin [15-24], only one chromatographic method [25] and one spectrophotometric method [26] were developed for simultaneous determination of empagliflozin and metformin without need for derivatization reaction to enhance the maximum absorption that was required with similar gliptin, saxagliptin, with low lambda max value at 205 nm [27]. In addition, many pharmacokinetic studies were reported regarding safety and tolerability of empagliflozin [28-44] but details about plasma extraction techniques were not previously described in full details.

Therefore, the novelty of the present work was achieved as the first described sample preparation with full details for enhanced plasma extraction of empagliflozin and metformin. Good results were obtained for empagliflozin using liquid-liquid extraction but it failed to simultaneously extract metformin and empagliflozin from human plasma that may be attributed to the high hydrophilicity of metformin with relatively low Log P that diminishes its ability to migrate to the organic layer. Modification of the method was adopted by dividing the plasma sample into two parts in order to optimize the extraction procedure for each drug using the most suitable extraction method followed by combining them again after the reconstitution step. Plasma sample preparation involved direct precipitation of plasma proteins to obtain supernatant contains metformin that was concentrated using vacuum evaporation and then added to the reconstituted other plasma part after its liquid-liquid extraction and vacuum evaporation till dryness.

Instrumentation, reference samples and working solutions

Vacuum evaporator Christ® (S/N 37399708, Germany), Vacuum pump Vacwbrand® (DVP2C-TYR012, Germany), Vortex Scientifica® (S/N 265349, Europe), Centrifuge Hettich® (S/N 012444807, Germany), Waters Acquity® UPLC Xevo TQD system (USA) interfaced with a triple quadrupole mass spectrometer, equipped with electrospray ionization and Mass Lynx software version 4.1 were used.

Empagliflozin (99.81 %), metformin (100.65 %) were supplied from Boehringer Ingelheim pharmaceutical company (Germany). Formic acid, diethylether, HPLC grade acetonitrile were purchased from Sigma Aldrich (Germany). Working solution contains both 50 µg/mL of empagliflozin and 50 µg/mL of metforminin was prepared in 50% acetonitrile/water.

Optimized sample preparation

For determination of empagliflozin and metformin in plasma, 0.5 mL plasma was spiked with 10 µl of the working solution (50 µg/mL of both drugs), vortexed for 1 minute, and separated into two parts. The first part (0.25 mL) was vigorously mixed with 1 mL of acetonitrile. Two mL of diethyl ether was added followed by vortex for 1 minute and centrifugation for 15 minutes at 6000 RPM. Then 1.5 mL of the upper layer was separated, vacuum evaporated at 40˚ C - 1400 RPM till complete dryness of the sample. The sample was reconstituted with 250 µl of 50% acetonitrile/water, centrifuged for 5 minutes at 6000 RPM to ensure complete reconstitution. The other 0.25 mL of the plasma sample was extracted by direct precipitation technique using 1.5 mL of acetonitrile, centrifugation for 5 minutes at 6000 RPM, then the supernatant was vacuum evaporated till obtaining concentrated 250 µl that was added to the other reconstituted 250 µl and the resultant 0.5 mL was transferred to the vials as one sample after vortex for 3 min.

Ten µl was injected into the UPLC-MS/MS system using BEH C_{18} column (50 mm × 2.1 mm, 1.7 µm), isocratic elution based on (0.1% formic acid: acetonitrile, 50:50, *v/v*) as a mobile phase, column temperature at 55˚C and flow rate at 0.2 $mLmin^{-1}$. The mass detector was operated in MRM mode using ESI monitoring the transition pairs of (m/z 451.13 to m/z 71.1) for empagliflozin and (m/z 130 to m/z 71.2) for metformin in the positive mode (figure 3). The optimum values for mass detection parameters like ion source temperature, cone voltage and collision energy were set at 120˚C, 20 V and 25 eV for both drugs. The optimized method was proved to be sensitive for extraction of the drugs from human plasma with extraction recovery of 72 % for empagliflozin and 71 % for metformin.

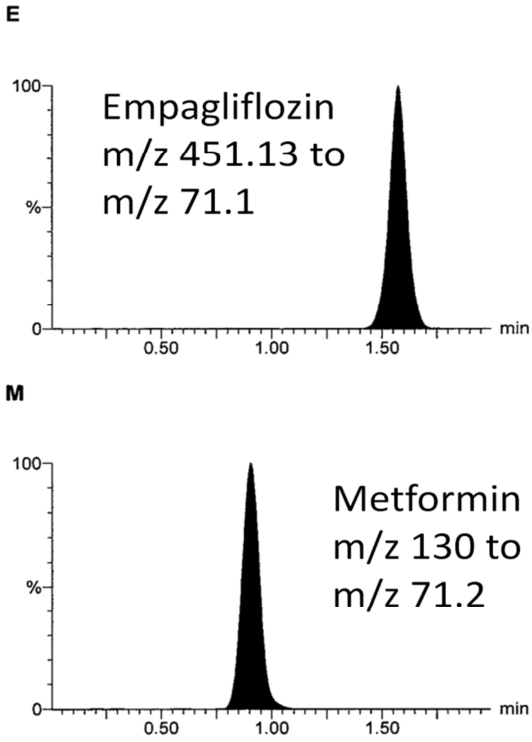

Figure 3: Chromatogram of spiked plasma sample contains empagliflozin (1 µg/mL) and metformin (1 µg/mL) after sample preparation

CONCLUSION

The developed procedure was applied successfully for extraction of empagliflozin and metformin from spiked plasma samples. Only 0.5 mL plasma was needed for the proposed extraction technique that will be suitable for human volunteers' studies. The method is applicable to pharmacological, clinical and bioequivalence studies after developing a UPLC-MS/MS study using internal standards, validation according to ICH guidelines and application to a human based pharmacokinetic study that will be considered as a future work by the author.

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