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Simultaneous Extraction of Metformin, Linagliptin and Empagliflozin From Human Plasma Using Mixed-Mode SPE with Oasis (WCX) and Analysis by LC-MS/MS

PMN Rajesh and Dr. Padmakar Wagh
India Technology Centre, Waters India pvt. Ltd, Bangalore

INTRODUCTION

The combination of Metformin, Linagliptin, and Empagliflozin is available as tablets formulation for oral use in diabetes. Metformin introduced in 1950 as glucose-lowering agents to treat non-insulin-dependent diabetes mellitus. It reduces elevated blood glucose concentration in diabetic patients, but it does not increase insulin secretion. Empagliflozin is an oral , potent, and selective inhibitors of sodium glucose contranspoter 2, inhibition of which reduces renal glucose cotransporter 2, inhibition of which reduces renal glucose reabsorption, and results in increased urinary reabsorption and increased urinary glucose extraction. Linagliptin is an oral inhibitor of dipeptidyl peptidase-4 approved for treatment of type 2 diabetes Metformin, Linagliptin, and Empagliflozin. These drugs are generally prescribed in multi-component dosage forms, which are available in the market. In view of this, a simple, precise, and accurate LC-MS/MS method for the simultaneous estimation in pharmaceutical dosage forms by reverse phase high performance liquid chromatography with mass spectrometry has been developed.

EXPERIMENTAL DETAILS

System: ACQUITY® UPLC® I-Class with Xevo® TQ-S micro Mass Spectrometer

Column: ACQUITY UPLC HSS Cyano (CN) 1.8 µm, 2.1 mm x 50 mm

Mobile phase A: 2 mM Ammonium acetate

Mobile phase B: Acetonitrile

Gradient:

Time (min)	%A	%B
0.0	90	10
0.4	90	10
1.5	5	95
2.5	5	95
3.0	50	50
4.0	50	50
4.5	90	10

Flow rate: 0.4 mL/min

Column temp.: 40 °C

Injection volume: 2 µL

Ionization mode: ESI+

Table 1. MRM transitions for metformin, linagliptin, and empagliflozin.

	Precursor ion	Product ion
Metformin	130.006	60.047
Linagliptin	473.265	420.176
Empagliflozin	451.176	71.031

Sample Preparation: For the extraction of Metformin, Linagliptin, and Empagliflozin from plasma, a mixed-mode solid phase extraction (SPE) clean-up strategy with Oasis® WCX Cartridges was employed. Spiked plasma samples (200 µL) were precipitated with diluted ammonia solution (400 µL) and the sample supernatant (500 µL) was loaded onto a pre-conditioned Oasis WCX Cartridge using the SPE extraction protocol shown below.

Table 2. SPE protocol for sample extraction.

WCX 1 CC, 30 mg cartridges
↓
Condition with 1 mL of MeOH
↓
Equilibrate with 1 mL of 4% ammonia in water
↓
Load the sample
↓
Wash with 1 mL of 4% ammonia in water
↓
Wash with 1 mL of water
↓
Elute with 2% formic acid in ACN (1 mL x 2)
↓
Evaporate the sample at 50 °C for 20 min
↓
Reconstitute the dried sample with 0.2 mL of 0.2% acetic acid in 50:50 acetonitrile and water

Table 3. Sample extraction recovery.

Compound name	% SPE recovery
Metformin	95
Linagliptin	80
Empagliflozin	52

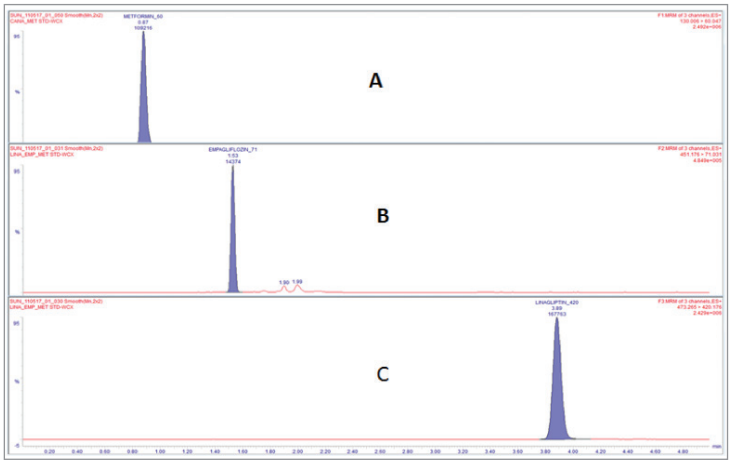

Figure 1. Extracted chromatograms of Metformin (A), Linagliptin (B) and Empagliflozin (C).

RESULT

Successful simultaneous analysis of Metformin, Linagliptin, and Empagliflozin from plasma was achieved using a single SPE extraction method with Oasis mixed-mode WCX Cartridges and subsequent LC-MS/MS analysis.

ORDERING INFORMATION

Description	P/N
Oasis WCX 3 cc 30 mg Cartridge	186002494
ACQUITY UPLC HSS CN, 1.8 µm, 2.1 x 50 mm Column	186005986
12 x 32 mm glass screw neckvial, Quick Thread, Lectrabond cap, preslit PTFE/Silicone septa, Total Recovery	18600385c

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Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990