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Ion-pair solid phase extraction for the simultaneous separation and quantitation of metformin and canagliflozin in human plasma by LC-MS/MS

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ABSTRACT

A high-throughput and sensitive liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been developed for the simultaneous determination of metformin and canagliflozin in human plasma. The optimized ion-pair solid phase extraction of the analytes was carried out using 100 µL plasma, followed by reversed-phase chromatography on COSMOSIL 5CN-MS (150 × 4.6 mm, 5 µm) column. An isocratic mobile phase consisting of methanol-20 mM ammonium formate in water, pH 5 (75:25, v/v) was used for separation of the analytes with a resolution factor of 2.18. Mass spectrometer was operated in the multiple reaction monitoring mode at unit mass resolution. Electro spray ionization was used for mass transition of *m/z* 130.1/60.1 and *m/z* 462.1/267.1 for metformin and canagliflozin, respectively. The method was successfully validated for the concentration range of 2.50–2500 ng/mL for both the analytes. The intra- and inter-batch precision determinations for metformin and canagliflozin, expressed as coefficient of variation were lower than 5.5% at all the test concentrations. Recovery of both the drugs was in the range of 78–90% and was reproducible in the quality control samples. No significant matrix effects were observed for the analytes as evident from the IS-normalized matrix factors. Stability of the drugs in whole blood and plasma samples was ascertained under different conditions. The assay showed acceptable performance when applied to clinical samples generated from healthy Indian subjects.

1. Introduction

Monotherapy with metformin (MET) is recommended as a first line therapy for patients with type 2 diabetes mellitus (T2DM) as it provides better glycemic control by decreasing hepatic glucose production, increasing insulin sensitivity and improvement in body weight. However, it has been found in many patients that MET monotherapy is inadequate to control effectively T2DM at some stage and eventually requires add-on treatment with other antihyperglycemic agents [1–3]. Combination or fixed dose combination (FDC) therapy can greatly simplify treatment regimens by minimizing pill burden, better compliance and improved satisfaction especially for patients on multiple medications [2]. Many recent developments in T2DM therapeutics have helped in better management of the disease with drugs that include α-glucosidase inhibitors, dipeptidyl peptidase inhibitors and sodium-glucose cotransporter (SGLT) inhibitors [3]. SGLT-2 inhibitors are recommended by American Association for Clinical Endocrinologists as the first add-on agent for those patients who are unable to achieve glycemic goals with MET monotherapy and have glycosylated haemoglobin (HbA1c) ≥ 7.5% [1,4]. SGLT-2 is a high capacity and low affinity protein mainly expressed in the initial proximal renal tubules and plays a significant role in reabsorbing 80–90% glucose [5]. Canagliflozin (CANA), a SGLT-2 inhibitor has an insulin independent mechanism of action which is complimentary to MET. It lowers renal threshold plasma glucose levels in patients with T2DM and increases urinary glucose leading to mild osmotic diuresis and a net caloric loss. At higher doses (≥ 300 mg per day), CANA can also inhibit SGLT1, found in the intestine and delay postprandial glucose absorption [6]. The FDC of CANA and MET, Invokamet® was first approved by US FDA in 2014 and is currently available in four different dose strengths (50/500 mg, 50/1000 mg, 150/500 mg and 150/1000 mg). The recommended dose is twice daily with food [7]. Following oral administration, CANA reaches peak plasma concentrations within 1–2 h, and the steady state levels are attained in 4–5 days. It has an oral bioavailability of 65% and is 99% protein bound, mainly to albumin. The terminal half-life of CANA for 100 mg and 300 mg dose is 10.6 and 13.1 h, respectively. It is metabolized predominantly by *O*-glucuronidation *via* uridine diphosphate glucuronosyltransferase 1A9 and uridine diphosphate glucuronosyl transferase B4 (UGT2B4) to inactive metabolites, which are excreted renally, while MET, is eliminated

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Table 1
Comparative assessment of methods developed for determination of metformin and canagliflozin in human plasma.

Sr. no.	Technique; internal standard; linear range	Extraction method; plasma volume; mean recovery (%)	Column; mobile phase; elution mode	Retention time (MET/CANA); run time	Application	Ref.
1[a]	CANA LC-MS/MS; $^{13}C_6$-CANA; 5.0–5000 ng/mL MET LC-MS/MS; $^{2}H_6$-MET; 5.0–2500 ng/mL	Liquid-liquid extraction with methyl *tert*-butyl ether; –; –	Waters XBridge™C18 (50 × 2.1 mm, 3.5 μm); methanol-0.01 M ammonium acetate- (70:30, v/v); isocratic	–; –	Bioequivalence study with various immediate release FDC of CANA/MET in 56 healthy fed subjects	[24]
		Protein precipitation with acidic acetonitrile; –; –	Waters XBridge™ Shield RP 18 (50 × 2.1 mm, 3.5 μm); 0.02 M hexafluorobutyric acid in 0.01 M ammonium acetate (A) and acetonitrile (B); gradient	–; –		
2[a]	CANA LC-MS/MS; $^{13}C_6$-CANA; 5.0–5000 ng/mL MET LC-MS/MS; $^{2}H_6$-MET; 5.0–2500 ng/mL	Liquid-liquid extraction with methyl *tert*-butyl ether; –; –	Waters XBridge™C18 (50 × 2.1 mm, 3.5 μm); methanol-0.01 M ammonium acetate- (70:30, v/v); isocratic	–; –	Pharmacokinetic study with immediate release FDC of CANA/MET (150/1000) in 24 healthy subjects in fasting and fed conditions	[25]
		Protein precipitation with acidic acetonitrile; –; –	Waters XBridge™ Shield RP 18 (50 × 2.1 mm, 3.5 μm); 0.02 M hexafluorobutyric acid in 0.01 M ammonium acetate (A) and acetonitrile (B); gradient	–; –		
3	LC-MS/MS; MET-d6 & CANA-d4; 2.5–2500 ng/mL for MET & CANA	Ion-pair solid phase extraction with sodium lauryl sulphate; 100 μL; 87.9/80.3 for MET/CANA	COSMOSIL 5CN-MS (150 × 4.6 mm, 5 μm); methanol-20 mM ammonium formate in water, pH 5.0 (75:25, v/v); isocratic	2.83/2.35 min; 3.5 min	Pharmacokinetic study with immediate release FDC of 150/1000 mg CANA/MET in 18 healthy Indian subjects under fed condition	PM

[a] Separate extraction procedures and chromatographic methods for MET and CANA; MET: Metformin; CANA: Canagliflozin; FDC: Fixed-dose combination; PM: present method.

unchanged in urine [8].

Bioanalytical methods for determination of CANA are essentially based on high-performance liquid chromatography (HPLC) with fluorescence [9] and mass spectrometric detection [10–14] in human plasma [9,10,12,13], human urine [10] and rats plasma [11,14]. Although there are several recent methods for the quantitation of MET with other drugs namely gliptins [15–21], biguanides [22] and statins [23], very few methods report determination of MET and CANA together in biological samples [24,25]. In both the methods separate extraction and chromatographic procedures were optimized for the quantitation of MET and CANA in human plasma [24,25]. The calibration range was established from 5.0–5000 ng/mL for both the analytes. The major focus of these methods was to study the pharmacokinetics of MET and CANA in healthy subjects using an immediate release (IR) FDC formulation, with partial information on method development and validation. A detailed comparison of methods developed for the simultaneous determination of MET and CANA is illustrated in Table 1.

Thus, the objective of the present work was to develop and validate a highly sensitive and rapid LC-MS/MS method for simultaneous determination of MET and CANA in human plasma using a single extraction protocol and identical chromatographic conditions. The developed method was validated as per current regulatory guidelines and was applied to study the pharmacokinetics of these drugs in IR FDC formulation in healthy Indian males.

2. Experimental

2.1. Chemicals and materials

Metformin (MET, 98.62%), metformin-d6 (MET-d6, 99.25%), canagliflozin (CANA, 99.96%) and canagliflozin-d4 (CANA-d4, 97.55%) were purchased from Clearsynth Labs Ltd. (Mumbai, India). HPLC grade methanol, acetonitrile, ammonium formate, ammonium acetate and analytical reagent grade formic acid and acetic acid were obtained from Merck Specialties Pvt. Ltd. (Mumbai, India). Analytical reagent grade sodium hexanesulfonate, sodium octanesulfonate and sodium lauryl sulphate (SLS) of purity ≥ 98% was obtained from Sigma Aldrich Chemicals Pvt. Ltd. (Bangalore, India). Solid phase extraction (SPE) cartridges Strata™-X (30 mg/1 mL) were purchased from Phenomenex India (Hyderabad, India). Water used in the entire analysis was prepared from Milli-Q water purification system procured from Millipore (Bangalore, India). Blank human plasma was obtained from Supratech Micropath (Ahmedabad, India) and was stored at −70 °C until use.

2.2. Liquid chromatographic and mass spectrometric conditions

The chromatographic analysis of MET and CANA was performed on Shimadzu LC-VP HPLC (Kyoto, Japan) under reversed-phase conditions. The analytes were separated on COSMOSIL 5CN-MS (150 × 4.6 mm, 5 μm) column using methanol-20 mM ammonium formate, pH 5.0 (75:25, v/v) as the mobile phase. For isocratic elution, the flow rate of the mobile phase was kept at 1.1 mL/min with 70% flow splitting; flow directed to the ion spray interface was equivalent to 330 μL/min. The column oven and autosampler temperature were maintained at 40 °C and 5 °C, respectively. The pressure of the system was 1900 psi and injection volume was kept at 5 μL.

The LC system was connected to a triple quadrupole mass spectrometer MDS SCIEX API-4000 (Toronto, Canada), having turbo ionspray ionization source and operated in positive ion mode. Detection and quantification of analytes (MET and CANA) and ISs (MET-d6 and CANA-d4) was performed in multiple reaction monitoring (MRM) mode, maintaining a dwell time of 200 ms. The optimized compound dependent, source dependent mass parameters and MRM transitions for both analytes and ISs are summarized in Supplementary Table 1. Quadrupole 1 and 3 were maintained at unit mass resolution and analyst classic software version 1.6.2 was used to control all parameters of HPLC and MS.