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Liquid chromatography and tandem mass spectrometry method for the quantitative determination of saxagliptin and its major pharmacologically active 5-monohydroxy metabolite in human plasma: Method validation and overcoming specific and non-specific binding at low concentrations

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ABSTRACT

A liquid chromatography and tandem mass spectrometry (LC–MS/MS) method was developed and validated to simultaneously determine the concentrations of saxagliptin (Onglyza™, BMS-477118) and its major active metabolite, 5-hydroxy saxagliptin to support pharmacokinetic analyses in clinical studies. The dynamic range of the assay was 0.1–50 ng/mL for saxagliptin and 0.2–100 ng/mL for 5-hydroxy saxagliptin. Protein precipitation (PPT) with acetonitrile was used to extract the analytes from plasma matrix before injecting on an Atlantis® dC18 column (50 mm × 2.1 mm, 5 μm) for LC–MS/MS analysis. The sample pre-treatment process was carefully controlled to disrupt DPP4-specific binding and non-specific binding observed at lower concentrations. The recoveries for both analytes were >90%. The assay was selective, rugged and reproducible; storage stability of at least 401 days at −20 °C was demonstrated. Under these chromatographic conditions, the isomers of saxagliptin and 5-hydroxy saxagliptin were chromatographically separated from saxagliptin and 5-hydroxy saxagliptin. The assay has been used to support multiple clinical studies and regulatory approvals.

1. Introduction

Saxagliptin (1*S*,3*S*,5*S*)-2-[(2*S*)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0]hexane-3-carbonitrile, BMS-477118, Onglyza™, Fig. 1 and also in the fixed dose combination of saxagliptin and metformin as Kombiglyze™ is an orally active, selective, long-acting and reversible dipeptidyl-peptidase 4 (DPP4) inhibitor that is marketed in USA, Europe and many other countries for the treatment of type 2 diabetes mellitus [1–6]. DPP4 inhibitors enhance levels of active glucagon-like peptide 1 (GLP-1) and other incretins, and facilitate glucose-dependent insulin secretion. In addition, GLP-1 inhibits glucagon release, slows gastric emptying, reduces appetite, and regulates the growth and differentiation of the insulin producting β cells in pancreatic islets [1].

To support saxagliptin clinical studies, several bioanalytical methods [7,8] were developed and validated using high performance liquid chromatography with tandem mass spectrometry (LC–MS/MS). Structures for saxagliptin and its active, monohydroxylated metabolite, 5-hydroxy saxagliptin, (1*S*,3*S*,5*S*)-2-[(2*S*)-2-amino-2-(3,5-di-hydroxy-1-adamantyl) acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile, are shown in Fig. 1. The plasma elimination half-life values of saxagliptin and 5-hydroxy saxagliptin are approximately 2.5 and 3.5 h [1], respectively, which is in contrast to a plasma DPP4 inhibition half-life of up to 24 h. It was proposed that pharmacologically active concentrations of saxagliptin and 5-hydroxy saxagliptin below the limits of quantitation of the available assays at the time (1 and 2 ng/mL for saxagliptin and 5-hydroxy saxagliptin, respectively)

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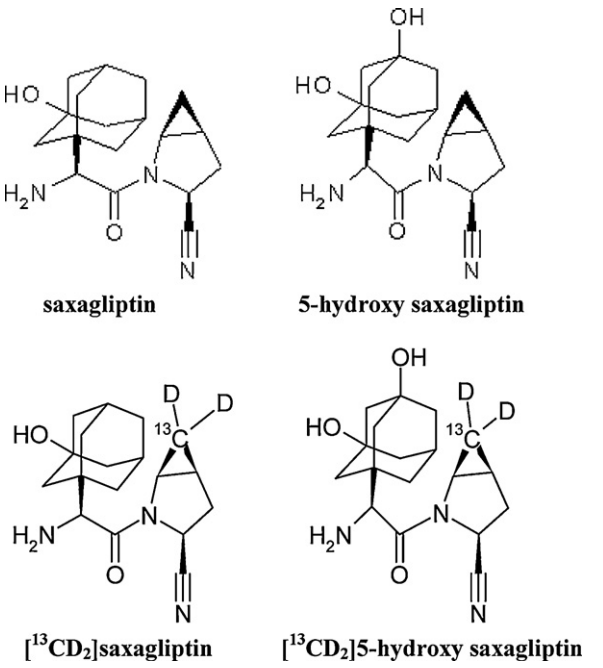

Fig. 1. Structure of saxagliptin (BMS-477118), 5-hydroxy saxagliptin and corresponding stable-isotope labeled internal standards.

were contributing to DPP4 inhibition in a secondary elimination phase that was not characterized with these earlier bioanalytical methods [7,8].

In this article, we describe efforts to develop and validate a simple, high-throughput LC–MS/MS method with a lower limit of quantification (LLOQ) in the sub-ng/mL range for the simultaneous quantification of saxagliptin and 5-hydroxy saxagliptin in human plasma, which could be utilized to better characterize a secondary pharmacokinetic elimination phase. The assay utilizes protein precipitation with CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate) and sonication for sample preparation prior to LC–MS/MS analysis. The CHAPS treatment and sonication steps are critical to improve the recovery at lower concentration levels by disrupting DPP4-specific and non-specific absorption. Results of the assay validation, including assessment of precision and accuracy, QC stability, incurred sample reproducibility, potential interference from concomitant medicines, and cross-validation with an earlier assay are also discussed.

2. Experimental

2.1. Materials

Saxagliptin (Onglyza®, BMS-477118), 5-hydroxy saxagliptin, stable labeled saxagliptin ([$^{13}CD_2$]BMS-477118), stable labeled monohydoxylated metabolite ([$^{13}CD_2$]5-hydroxy saxagliptin) (Fig. 1) and the authentic standards for saxagliptin diastereomers (Fig. 2) were obtained from Bristol-Myers Squibb (Princeton, NJ, USA). After salt correction, the purities of saxagliptin and 5-hydroxy saxagliptin were 93.9% and 83.9%, respectively. HPLC-grade acetonitrile, methanol and ammonium hydroxide were purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Formic acid (minimum purity 96%) was purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA) and ammonium acetate was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate, minimum purity 98%) was purchased from Sigma–Aldrich (St Louis, MO, USA). Water was obtained from an in-house Barnstead

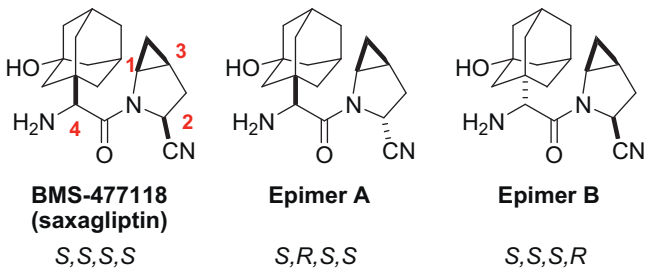

Fig. 2. Structure of saxagliptin and its stereoisomers.

De-ionized Water System (Billerica, MA, USA). Control human plasma (dipotassium EDTA) was purchased from Bioreclamation (NY, USA).

2.2. Instrumentation

The HPLC system consisted of a Shimadzu Autosampler (SIL-5000, set at ambient temperature), Shimadzu System Controller (Model SCL-10A Vp), Binary Pumps (Model LC 10AD Vp) and solvent degasser (Model DGU-14A) (Shimadzu Scientific Instrument, Columbia, MD, USA). A Sciex API 4000 mass spectrometer (Sciex, Toronto, Canada) was used as the detector. A Tomtec robotic liquid-handling system (Hamden, CT, USA) was used for the final transfer step in the sample preparation. Data were collected and processed using Analyst software v. 1.4 (Sciex, Toronto, Canada).

2.3. Chromatographic conditions

Saxagliptin and 5-hydroxy saxagliptin were separated on a 2.1 mm × 50 mm Atlantis® dC18 5 μm column (Waters, Milford, MA, USA). The chromatography was performed at room temperature, under gradient conditions. Mobile phases A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. Needle washes 1 and 2 were methanol:acetonitrile:formic acid (50:50:0.1, v/v/v) and water:acetonitrile:formic acid (70:30:0.1, v/v/v), respectively. Retention times of saxagliptin and 5-hydroxy saxagliptin were 2.3 and 1.4 min, respectively. A typical injection of 10 μL was sufficient to obtain required sensitivity. The typical cycle time is 6.0 min. The steps in Table 1 represent the typical mobile phase gradient program.

2.4. Mass spectrometer conditions

The mass spectrometer was a Sciex API-4000 equipped with a TurboIonSpray® source, operated in positive ionization mode, using selected reaction monitoring (SRM). The mass spectrometer was operated in unit resolution mode, with Q1 and Q3 set at 0.7 Da full width at half maximum (FWHM). Nitrogen was used as the nebulizer, curtain and collision gas. The optimized TurboIonSpray®

Table 1
HPLC gradient for mobile phase (A) and mobile phase (B).

Time from injection (min)	Total flowrate (mL/min)	% A	% B
0.01	0.300	96.0	4.00
3.00	0.300	70.0	30.0
3.50	0.300	5.00	95.0
3.51	0.300	5.00	95.0
3.52	0.600	5.00	95.0
4.50	0.600	5.00	95.0
4.51	0.300	5.00	95.0
4.52	0.300	5.00	95.0
4.53	0.300	96.0	4.00
6.00	0.300	96.0	4.00
6.01	Controller		Stop