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Determination of casopitant and its three major metabolites in dog and rat plasma by positive ion liquid chromatography/tandem mass spectrometry

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ABSTRACT

A sensitive, selective and quantitative method for the simultaneous determination of casopitant, a potent and selective antagonist of the human Neurokinin 1 (NK-1) receptor, and its three major metabolites M12, M13 and M31 was developed and validated in dog and rat plasma. Acetonitrile containing stable labeled internal standards for the four analytes was used to precipitate proteins in plasma. Chromatographic separation was obtained using a reversed phase column with multiple reaction monitoring turboionspray positive ion detection. The lower and upper limits of quantification for casopitant and its metabolites were 15 and 15,000 ng/mL, using a 50 μL of dog or rat plasma aliquot, respectively. The inter-day precision (relative standard deviation) and accuracy (relative error) in dog plasma, derived from the analysis of validation samples at 5 concentrations, ranged from 4.1% to 10.0% and −10.8% to 8.7%, respectively, for casopitant and its 3 major metabolites. The intra-day precision (relative standard deviation) and accuracy (relative error) in rat plasma, derived from the analysis of validation samples at 5 concentrations, ranged from 3.9% to 6.6% and −9.6% to 8.3%, respectively, for casopitant and its three metabolites. All analytes were found to be stable in analytical solutions for at least 43 days at 4 °C, in dog and rat plasma at room temperature for at least 24 h, at the storage temperature of −20 °C for at least 6 months, and following the action of three freeze–thaw cycles from −20 °C to room temperature. All analytes were also found to be stable in processed extracts at 4 °C for at least 72 h. This assay proved to be accurate, precise, fast and was used to support long-term toxicology studies in dog and rat.

1. Introduction

Casopitant, also known as GW679769 (Fig. 1), is a piperidine derivative that has been shown to be a potent and selective antagonist of the human Neurokinin subtype-1(NK-1) receptor, the primary receptor of substance P (SP), both in vitro and in vivo with good brain penetration properties [1]. NK-1 receptors are widely distributed in the peripheral and central nervous system including areas thought to be involved in the regulation of affective behavior and neurochemical response to stress [2–4]. NK-1 receptors are also found in non-neural tissues such as endothelial and inflammatory cells as well as gastrointestinal, respiratory, and genitourinary tissues. Blocking NK-1 neurotransmitter receptors has been demonstrated to be effective for the treatment of major depressive disorder, one or more anxiety disorders [5,6] and to prevent chemotherapy-induced and post-operative nausea and vomiting [7,8]. Based on this mode of action it has been evaluated for the prevention of chemotherapy-induced and post-operative nausea and vomiting [9,10]. In addition, casopitant has been investigated in a number of chronic dosing indications where the NK-1 receptor is believed to play a role, such as anxiety, depression, insomnia, and over-active bladder.

The nonclinical safety package of casopitant included investigations appropriate for both acute and chronic indications [11]. Following a single oral administration in rats and dogs, casopitant has been shown to be extensively metabolized, widely distributed with quite long retention time in tissues and slow rate of elimination mainly in dog [12]. Of the many metabolites which were found circulating in humans [13], three were considered as major: M12 (coded as GSK631832), M13 (coded as GSK525060) and M31 (coded as GSK517142). Their structures are shown in Fig. 1.

In order to provide safety cover data in the clinical phase, casopitant and its major metabolites M12, M13 and M31 had to be quantified in long-term toxicity studies. For this purpose, a precise, accurate and high-throughput method for the simultaneous quantification of these four analytes had to be developed and validated. The target was to develop a multi-analyte assay with a similar

Abbreviations: LC–MS/MS, liquid chromatography–tandem mass spectrometry; HPLC, high performance liquid chromatography; MRM, multiple reaction monitoring; DMF, dimethylformamide; SIL, stable isotope labeled; IS, internal standard; QC, quality control; LIMS, laboratory information management system; MF, matrix factor; NMF, normalized matrix factor.

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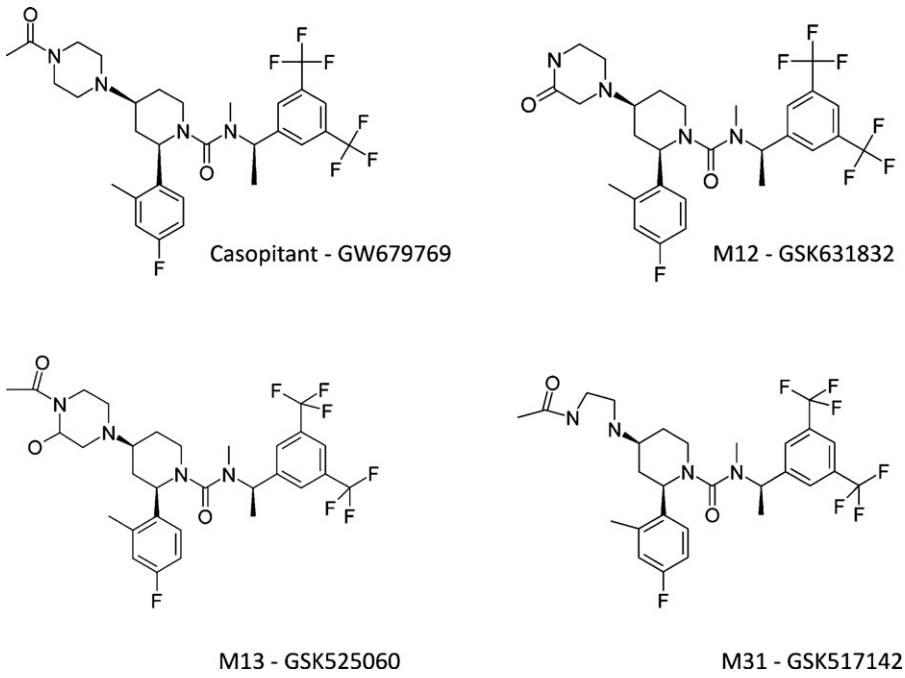

Fig. 1. Chemical structures of casopitant (coded as GW679769) and its metabolites M12 (coded as GSK631832), M13 (coded as GSK525060) and M31 (coded as GSK517142).

productivity, selectivity, precision and accuracy of the previous validated bioanalytical methods which allowed quantification of casopitant, only.

2. Experimental

2.1. Chemicals and materials

Casopitant and its metabolites GSK631832, GSK525060 and GSK517142 were obtained from Chemical Development at GlaxoSmithKline (Tonbridge, UK); stable labeled internal standards (SIL) [$^2H_3{}^{13}C$]-GW679769, [$^2H_3{}^{13}C$]-GSK631832, [$^2H_3{}^{13}C$]-GSK525060 and [$^2H_3{}^{13}C$]-GSK517142 (Fig. 2) were obtained from Isotope Chemistry at GlaxoSmithKline (Stevenage, UK). Dog and rat plasma were obtained from Laboratory Animal Sciences (GSK Verona) and were derived by ethical approved procedures. HPLC grade acetonitrile was obtained from J.T. Baker (Deventer, The Netherlands). Analytical grade ammonium acetate was obtained from Sigma–Aldrich (Steinhein, Germany) and water was from a Millipore Milli-Q system (Billerica, MA).

2.2. Equipment

A Heraeus MultiFUGE (Milan, Italy) centrifuge with a rotor capacity for four 96-well plates and a Mettler AT261 balance (Hightown, NJ, USA) were used. A Tecan Genesis150RSP liquid handler (Zurich, CH) was used for plasma transfer. The HPLC system consisted of an Agilent 1100 G1312A binary pump equipped with an Agilent 1100 G1322A degasser (Waldbronn, Germany). The autosampler was a CTC Analytics HTS PAL (Zwingen, CH). The chromatographic system consisted of a Thermo Hypersil Gold column 3.0 mm × 50 mm, 5 μm (Milan, Italy). Mass spectrometric detection was performed on an Applied Biosystems/MDS Sciex API4000 triple quadrupole (Concord, Ontario, Canada) operating in positive turboionspray mode controlled by Analyst software (version 1.1).

2.3. LC–MS/MS conditions

An isocratic HPLC method was employed for separation. The mobile phase consisted of aqueous 5 mM ammonium acetate/acetonitrile (35:65, v/v). The flow rate was set at 0.7 mL/min. The autosampler was programmed to inject 2.5 μL sample aliquots every 1.5 min.

The API4000 triple quadrupole turboionspray source of the mass spectrometer was operated in positive ion mode, with the curtain gas (nitrogen), ion source 1 and ion source 2 gasses (purified air) set at 25, 50 and 45 psi, respectively. The IonSpray voltage was set at 3500 V, the source temperature was maintained at 600 °C and the source parameters were optimized for casopitant, M12, M13, M31 and their internal standards (IS) in multiple reaction monitoring (MRM) mode. In MRM mode, casopitant was monitored at the transition *m/z* 617–167 and its internal standard [$^2H_3{}^{13}C$]-GW679769 was monitored at the transition *m/z* 621–171. For metabolites of casopitant, the MRM transitions monitored were *m/z* 589–453 for M12 and *m/z* 593–493 for its IS [$^2H_3{}^{13}C$]-GSK631832, *m/z* 633–479 for M13 and *m/z* 637–483 for its IS [$^2H_3{}^{13}C$]-GSK525060, *m/z* 591–184 for M31 and *m/z* 595–184 for its IS [$^2H_3{}^{13}C$]-GSK517142. The product ions were generated with collision energy of 28, 35, 25 and 45 eV for casopitant, M12, M13 and M31, respectively. The collision gas thickness was set at the instrument value of 6. The Declustering Potential (DP), Entrance Potential (EP) and Collision Cell Exit Potential (CXP) were set for all compounds at 65, 10 and 10 V, respectively. A dwell time of 100 and 50 ms was used for the transitions of analytes and internal standards, respectively. The pause time was 5 ms.

2.4. Preparation of standards and quality control samples

Stock solutions for both casopitant and its metabolites were prepared in DMF, while working solutions were prepared in acetonitrile/water (50:50, v/v). Stock solutions of casopitant, M12, M13 and M31 (separate weighing for calibration standards and QC's) were prepared at a concentration of 5 mg/mL. Stock solutions of their stable labeled internal standards were prepared in DMF at a concentration of 1 mg/mL, which were combined and diluted to a unique working SIL solution at 200 ng/mL in acetonitrile. Stock solutions of casopitant and its metabolites were combined and further diluted to obtain working solutions containing all analytes at the concentration of 750, 225, 75, 22.5, 7.5, 2.25 and 0.75 μg/mL.