Journal of Chromatography B, 941 (2013) 10–16

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of allopurinol and oxypurinol in human plasma and urine by liquid chromatography-tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 11 June 2013
Accepted 19 September 2013
Available online 1 October 2013

Keywords:
Allopurinol
Metabolite
HPLC-MS/MS
Human plasma
Pharmacokinetics

ABSTRACT

Allopurinol is used widely for the treatment of gout, but its pharmacokinetics is complex and some patients show hypersensitivity, necessitating careful monitoring and improved detection methods. In this study, a sensitive and reliable liquid chromatography-tandem mass spectrometry method was developed to determine the concentrations of allopurinol and its active metabolite oxypurinol in human plasma and urine using 2,6-dichloropurine as the internal standard (IS). Analytes and the IS were extracted from 0.5 ml aliquots of plasma or urine using ethyl acetate and separated on an Agilent Eclipse Plus C_{18} column using methanol and ammonium formate–formic acid buffer containing 5 mM ammonium formate and 0.1% formic acid (95:5, v/v) as the mobile phase (A) for allopurinol or methanol plus 5 mM ammonium formate aqueous solution (95:5, v/v) as the mobile phase (B) for oxypurinol. Allopurinol was detected in positive ion mode and the analysis time was about 7 min. The calibration curve was linear from 0.05 to 5 μg/mL allopurinol in plasma and 0.5–30 μg/mL in urine. The lower limit of quantification (LLOQ) was 0.05 μg/mL in plasma and 0.5 μg/mL in urine. The intra- and inter-day precision and relative errors of quality control (QC) samples were ≤11.1% for plasma and ≤ 8.7% for urine. Oxypurinol was detected in negative mode with an analysis time of about 4 min. The calibration curve was linear from 0.05 to 5 μg/mL in plasma (LLOQ, 0.05 μg/mL) and from 1 to 50 μg/mL in urine (LLOQ, 1 μg/mL). The intra- and inter-day precision and relative errors were ≤7.0% for plasma and ≤9.6% for urine. This method was then successfully applied to investigate the pharmacokinetics of allopurinol and oxypurinol in humans.

1. Introduction

The xanthine oxidase inhibitor allopurinol (Fig. 1A) is the most frequently prescribed medication for the treatment of gout and hyperuricaemia [1,2]. Allopurinol is rapidly absorbed from the gastrointestinal tract and metabolized by xanthine oxidase to oxypurinol (Fig. 1B), the main metabolite responsible for the pharmacological activity [3,4]. Oxypurinol is also a potent inhibitor of xanthine oxidase, the enzyme that converts hypoxanthine to xanthine and xanthine to uric acid. Like allopurinol, it is excreted via the kidney. However, oxypurinol exhibits a prolonged serum half-life [5,6], complicating allopurinol dosing and increasing the risk of side effects in some patients. A new allopurinol intravenous preparation is in development, necessitating a reliable and practical analytical method for high-throughput detection in pharmacokinetic and therapeutic drug monitoring studies.

Several methods have been developed for the measurement of allopurinol and oxypurinol in plasma and urine, including HPLC with ultra-violet detection [7–11] or electrochemical detection [12–14]. Brown and Bye [7] described the HPLC method for allopurinol and oxipurinol assay within human plasma and urine with linear calibration curves for allopurinol over the range 0.068–1.36 μg/ml in plasma and 0.68–136 μg/ml in urine and for oxipurinol 0.076–15.2 μg/ml in plasma and 15.2–304 μg/ml in urine. However, this method is based on complex ion-exchange chromatography. Moreover, the selectivity of UV detection is reduced by the interferences of residual protein [8–10], detection of single sample extends as long as 20 min [10], preparation method is time-consuming [11], and sensitivity is still limited [8–11]. Electrochemical detection involves complex sample preparation using enzymatic reactions [13] or solid phase extraction [14]. Other methods such as capillary electrophoresis detection [15,16] and micelle-stabilised room temperature phosphorescence detection [17] require specific modifications for plasma and urine sample detection. HPLC-MS method [18] has been adopted to determine allopurinol and oxypurinol in plasma by protein precipitation and liquid–liquid extraction following the lower limit of quantification

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http://dx.doi.org/10.1016/j.jchromb.2013.09.028

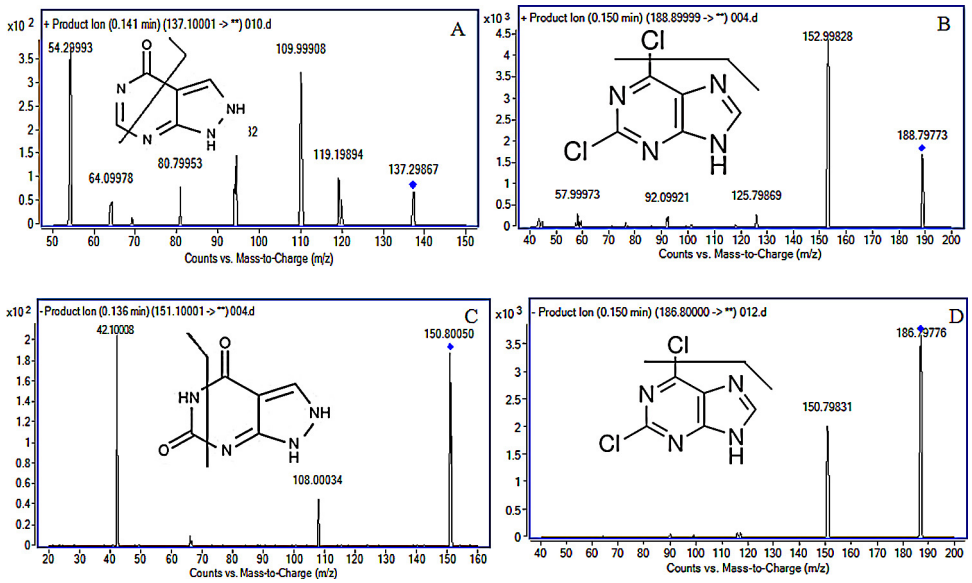

Fig. 1. Chemical structures and mass spectra of (A) allopurinol $(M+H)^+$, (B) 2,6-dichloropurine $(M+H)^+$, (C) oxypurinol $(M-H)^-$, and (D) 2,6-dichloropurine $(M-H)^-$.

of the assay was 50 ng/mL for allopurinol and 100 ng/mL for oxypurinol, yet the paper did not present the concrete assay method. Modern HPLC-MS/MS method [19] for the determination of oxypurinol in urine is suitable for the quantification of oxypurinol in the urine of patients ($n = 34$) participating in a clinical trial to optimize therapy of gout with allopurinol but the selectivity and sensitivity are still not sufficient for general application.

Because allopurinol and its metabolite oxypurinol are main for the treatment of gout and hyperuricaemia so a validated analytical methods for urine matrix to explore relationships with plasma urate concentrations is also needed. As a result, we have developed novel HPLC-MS/MS method to systemically measure allopurinol and oxypurinol in both human urine and plasma for the first time. This simple, sensitive, and practical method was fully validated and then successfully applied to a pharmacokinetic study of an injectable allopurinol formulation in healthy Chinese volunteers.

2. Materials and methods

2.1. Chemicals and reagents

Intravenous Preparations of Allopurinol Sodium (0.5 g, Batch No. 120601) were prepared for a clinical study by Hainan Generalsanyang Pharmaceutical Co., Ltd. (China). Allopurinol standard reference (purity: 99.5%, lot. C10118000) was provided by Dr. Ehrenstorfer GmbH Company. Oxypurinol standard (purity: 99.5%, lot. D454550) was supplied by Toronto Research Chemicals Inc. The internal standard 2,6-dichloropurine (purity: 99.5%, lot. 100603-200401) was provided by Wuhan Ariel Chemical Technology Co., Ltd. (China). Chromatographic pure grade methanol was purchased from Merck, chromatographic pure grade formic acid and ethyl acetate from Dikma Company (USA), mass spectrum pure grade ammonium formate from Sigma (USA), and analytical grade HCl from Guangzhou Chemical Agent Factory (China). Deionized water was produced using a Milli-Q academic reagent grade water purification system (Millipore, USA) and distilled before using.

2.2. Apparatus

The chromatographic analysis system (Agilent 1100 series LC system, Agilent Technologies, Inc., USA) included a quaternary pump, autosampler, column oven, and degasser. The analytes and internal standard were detected using an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Inc., USA) with electrospray ionization. Ultra-high purity (UHP) nitrogen was used as the drying gas. Raw data were processed using the Agilent MassHunter Chemstation (B.01.03). A Salvis Lab vacuum drying apparatus (Vacucenter VC20, CH-6343 Rotkreuz, Switzerland) was used to evaporate the supernatant extracted from plasma or urine samples. Pharmacokinetic parameters were calculated using DAS 2.1.1 software (Mathematical Pharmacology Professional Committee of China).

2.3. LC-ESI-MS/MS

Chromatographic separation was performed at 40 °C on an Agilent Eclipse Plus-C18 column (4.6 mm × 150 mm, 3.5 μm). The mobile phase consisting of methanol −5 mmol/l ammonium formate aqueous solution (95:5, v/v) with or without 0.1% formic acid was employed for allopurinol and oxypurinol, separately. The flow rate for detection of both analytes was 0.5 mL/min.

The mass spectrometer was operated in positive ion multiple reaction monitoring (MRM) mode for allopurinol detection and negative ion MRM mode for oxypurinol detection. The optimized condition consisted of a nebulizer set at 45 psi, drying gas flow of 10 L/min at 350 °C, and a HV capillary set at 4000 V.

Impact energy and split voltage of allopurinol were 30 units and 125 V separately and these were 20 units and 135 V for 2,6-dichloropurine. The dwell time for each transition was 0.2 s and the electron multiplier set at 300 V. The injection volumes were 5 μL plasma and 2 μL urine.

The impact energy of oxypurinol and IS were separately 18 units and 22 units with a split voltage of 115 V and 135 V, separately. The dwell time for each transition was 0.2 s and the electron multiplier set to 400 V. The injection volumes were 5 μL plasma and 1 μL urine.

2.4. Preparation of stock solutions, calibration curves, and quality control (QC) samples

Stock solution of allopurinol in mobile phase A, oxypurinol in mobile phase B, and 2,6-dichloropurine in corresponding mobile phase A or B were prepared individually at a concentration of 1 g/L. Stocks were then serially diluted with the mobile phase