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Highly sensitive determination of alendronate in human plasma and dialysate using metal-free HPLC-MS/MS

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ABSTRACT

A highly sensitive method was developed for the analysis of alendronate in human plasma and dialysate using MonoSpin™ SAX® extraction and metal-free high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS) following methylation with trimethylsilyldiazomethane. The chromatographic separation of the derivatives for alendronate and alendronate-d_6 was achieved on an *L-column2* ODS metal-free column (50 mm × 2 mm i.d., particle size 3 μm) with a linear gradient elution system composed of 10 mM ammonium acetate (pH 6.8) and acetonitrile at a flow rate of 0.3 ml/min. Quantification was performed by multiple reaction monitoring (MRM) with positive-ion electrospray ionization (ESI). Distinct peaks were observed for alendronate and for the internal standard on each channel within 1 min. The regression equations showed good linearity within the ranges of 2.0–100 ng/0.5 ml for the plasma and 1.0–100 ng/0.5 ml for the dialysate, with the limits of detection at 1.0 ng/0.5 ml for the plasma and 0.5 ng/0.5 ml for the dialysate. Extraction efficiencies for alendronate for the plasma and dialysate were 41.1–51.2% and 63.6–73.4%, respectively. The coefficient of variation (CV) was ≤8.5%. The method was successfully applied to the analyses of real plasma and dialysate samples derived after intravenous administration of alendronate.

1. Introduction

As a bisphosphonate, alendronate is a potent inhibitor of bone resorption and is widely used for the prevention and treatment of primary and secondary osteoporosis [1–7]. Alendronate toxicity to soft tissues such as oral ulceration and acute pancreatitis has been pointed out [8–12]. Alendronate may induce osteonecrosis of the jaw if invasive dental procedures are performed [13,14]. If invasive dental procedures are performed. Hence, the discontinuation of the medication is highly required if the patient needs dental treatments. From the viewpoint of legal medicine, the detection of alendronate in a patient of osteonecrosis of the jaw may suggest the dereliction by the doctor, although such a case has not been reported. For that reason, therapeutic drug monitoring (TDM) has been required to establish optimal therapy regimens for clinical treatment, and drug analysis for forensic diagnosis. Furthermore, because the relationship between the alendronate concentration in blood and dialysate has been unknown for renal failure and dialysis patients, the optimal or recommended dosage for use has not been clarified. However, to the best of our knowledge, no reports have investigated the concentration of alendronate in body fluids after its administration. Currently, blood, urine, tears, dialysate, exudate or stomach contents are used as useful samples in clinical and forensic fields.

The main chemical feature of these drugs is the covalent linking of their two phosphate groups to a carbon atom. Moreover, bisphosphonates such as alendronate, ibandronate, and clodronate do not absorb ultraviolet light and are poorly retained by reverse-phase sorbents due to the strong hydrophilic properties of bisphosphonates and their strong interaction with metal ions. In general, bisphosphonates have been reported to be unsuitable for analysis by high-performance

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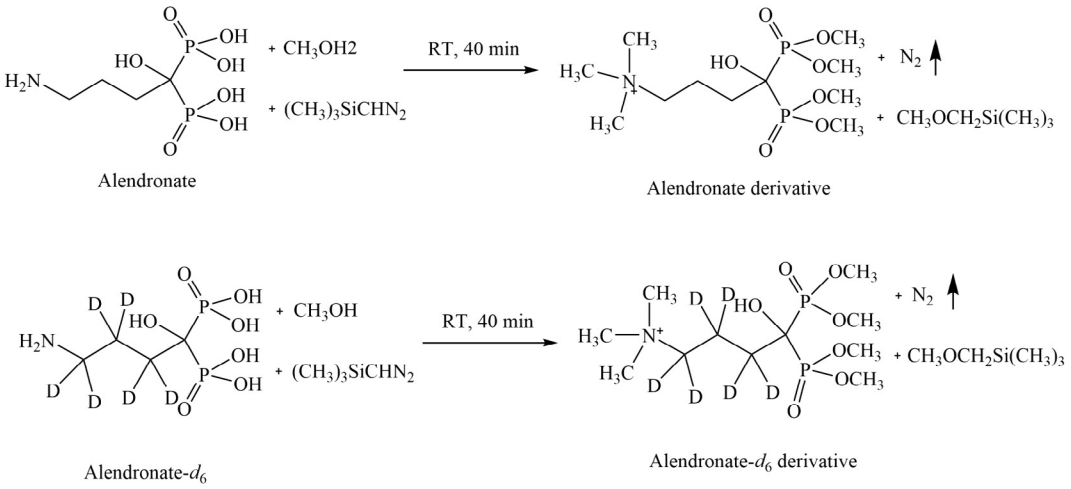

Fig. 1. The derivatization of alendronate and alendronate-d_6 with trimethylsilyldiazomethane.

liquid chromatography (HPLC) or HPLC-tandem mass spectrometry (HPLC-MS/MS) [15,16]. Therefore, determination of these bisphosphonates by HPLC or HPLC-MS/MS requires derivatization to reduce the polarity of the bisphosphonates behind the anion exchange columns during solid-phase extraction (SPE) (Fig. 1). Several methods have been reported for the determination of bisphosphonates using HPLC with fluorescence detection [15,17,18] and HPLC-mass spectrometry (HPLC-MS) [19–22]. Most of these techniques have limitations as a result of problems with carryover or low sensitivity due to the stainless steel parts used, since the stainless steel parts in particular show a high affinity for phosphorylated molecules, including bisphosphonates, which is due to a process known to happen in stainless steel emitters [20,23].

To improve carryover or intensity of bisphosphonates, metal-free hardware, such as the sample needle, injection valve, and column, has been recommended for HPLC-MS analyses [24–26]. In the present study, we have established a new, simple, sensitive, and selective metal-free HPLC-MS/MS method for the analysis of alendronate in human plasma and dialysate samples. To the best of our knowledge, no studies have reported metal-free HPLC-multiple reaction monitoring (MRM) for the simultaneous quantification and confirmation analysis of alendronate. The derived MS/MS spectrum library can be used for the screening of alendronate obtained from clinical samples.

2. Materials and methods

2.1. Materials

Alendronate sodium trihydrate and alendronate-d_6 as an internal standard (IS) were purchased from TCI Co., Ltd., (Tokyo, Japan) and TLC PharmaChem (Vaughan, Canada), respectively. The derivatization reagent, trimethylsilyldiazomethane (2.0M in hexane) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-MS-grade acetonitrile and water were purchased from Wako Pure Chemical Industries, Inc. (Osaka, Japan). Other common chemicals used were of the highest purity commercially available. The ultra-pure water from the Milli-Q ultrapure system (Komatsu Electronics Co., Ltd., Ishikawa, Japan) was used in all experiments. MonoSpin™ SAX® columns were purchased from GL Sciences Inc. (Tokyo, Japan).

2.2. Preparation of standard solutions and quality control

Individual stock standard solutions (1 mg/ml) of alendronate and IS (alendronate-d_6) were prepared separately by dissolving an accurately weighed quantity of each drug in ultra-pure water each month. The solutions were then stored at −30 °C. Working standard solutions of these drugs were prepared by appropriate dilution of the stock standard solutions with the UFLC mobile phase (10 mM ammonium acetate in 50% acetonitrile). All working standard solutions were freshly prepared when required. Calibration standards were prepared by mixing appropriate amounts of the working standard solutions and drug-free plasma or alendronate-free dialysate to achieve different concentrations ranging from 1.0 to 100 ng/0.5 ml for alendronate. Quality control (QC) samples (5–100 ng/0.5 ml) for alendronate were prepared using the same procedure.

2.3. Preparation of plasma and dialysate samples

Drug-free whole blood samples were obtained intravenously, in the presence of heparin sodium as an anticoagulant, from healthy volunteers. Alendronate-free dialysates were obtained from dialysis patients who had not been administered with alendronate. This study was reviewed and approved by the Ethics Committees of Showa University School of Medicine (No. 1249) and the Ethics Committees of Makita General Hospital (UMIN ID. 000027182), respectively. To prepare drug-free plasma samples and alendronate-free dialysate, heparinized whole blood or dialysate was centrifuged at 1700*g* at 4 °C for 10 min, respectively. The plasma or dialysate was then decanted into a clean centrifuge tube and stored at −80 °C until use.

2.4. MonoSpin™ SAX® extraction and derivatization procedure

MonoSpin™ SAX® columns were conditioned with 500 µl each of 750 mM NaF and ultra-pure water, respectively, and then centrifuged at 6000*g* for 30 s. 500 µl of ultra-pure water was added to 500 µl of the plasma or dialysate sample containing 20 µl of the drug mixture (alendronate and IS). The sample solution was applied to the conditioned MonoSpin™ SAX® column, which was centrifuged at 15,000*g* for 1 min. The column was then washed with 500 µl of ultra-pure water at 6000*g* for 30 s. After washing, the analytes were eluted and collected from the column with 500 µl of 2% TFA-water at 10,000*g* for 1 min. The elution was evaporated to dryness using an evaporator, reconstituted in 20 µl of ultra-pure water, and methylated with 200 µl of methanol and 100 µl of trimethylsilyldiazomethane. The solution was incubated at ambient temperature for 40 min. The solvent was then evaporated to dryness under a stream of nitrogen. The residues were reconstituted in 100 µl of the mobile phase, and 10-µl of the sample was sent to the autosampler for injection into the HPLC-MS/MS system.

2.5. Metal-free HPLC-MS/MS system and conditions

A metal-free HPLC-MS/MS system consisting of a Shiseido Nanospace SI-2 series liquid chromatograph (Shiseido, Tokyo, Japan)