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CONFERENCE

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TITLE

Determination of Ibandronate (a Complex Bisphosphonate) in Human Plasma by LC-MS/MS

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ABSTRACT

Novel Aspect: A rugged and reliable assay for the analysis of a bisphosphonate compound via a mild and efficient derivatization approach

Introduction

Ibandronate is a biophosponate that alters the cycle of bone formation and breakdown in the body. It is used to treat or prevent osteoporosis in women after menopause. Biophosphonates are extremely hydrophilic and structurally similar to many endogenous phosphorylated compounds, making their selective analysis in biological samples very challenging in both sample purification and analyte detection. In our current study, we report the method for the determination of ibandronate in human plasma employing solid-phase extraction with derivatization by trimethylsilyl (TMS) diazomethane, a mild and safer methylation agent than diazomethane, and analysis using reversed-phase HPLC with Turbo Ion Spray® MS/MS detection. Our data shows that the method reported here is both rugged and reproducible.

Methods

Samples were spiked with internal standard and processed by weak anion exchange (WAX) solid-phase extraction with derivatization by TMS. The derivatization takes place on the cartridge by methylating the hydroxyl groups on the phosphates of ibandronate and transforms the tertiary amine into a quaternary amine, thus achieving higher sensitivity in electro-spray ionization in positive mode. It also prevents interference from phosphate groups contained in plasma and helps with adequate retention in hydrophilic interaction chromatography (HILIC). The extracted samples of ibandronate and its deuterated internal standard were delivered by Shimadzu pump and CTC autosampler through an LC program utilizing a flow rate of 0.9 mL/minute, and detected on a Sciex API 4000 tandem mass spectrometer in positive ion mode.

Preliminary results

A method that enables sensitive and selective quantification of ibandronate by LC/MS/MS following derivatization with trimethylsilyl diazomethane has been developed. The partially automated sample processing is relatively simple and reproducible as shown by the validation results. The method can be used to analyze samples with concentrations of 500 pg/mL to 100 ng/mL. The intra-assay precision ranged from 3.4% to 6.4%, while the inter-assay precision ranged from 4.1% to 9.2%. The intra-assay accuracy ranged from -6.2% to 3.4% while the inter-assay accuracy ranged from -4.2% to 4.9%. Ibandronate has been shown to be stable in plasma for at least 62 days at -70°C and through 4 freeze-thaw cycles. It is also stable in processed samples for at least 159 hours at 4°C and for 86 days at -20°C in water. In addition, the selectivity, dilution reproducibility, matrix effect and recovery were also demonstrated. The assay was successfully applied to the analysis of over 5000 clinical samples in less than two months. Hence, the assay was demonstrated to be rugged, sensitive, selective, accurate and reproducible.