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Determination of Etidronate (Didronel) in Human and Dog Plasma and urine by LC/MS/MS

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Introduction

Etidronate disodium (Didronel) tablets are indicated for the treatment of symptomatic Paget's disease of bone and in the prevention and treatment of heterotopic ossification following total hip replacement or due to spinal cord injury.

Purpose

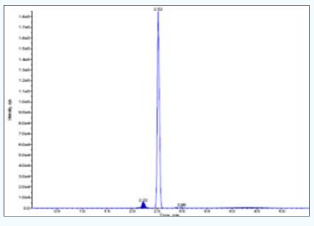
Etidronate disodium acts primarily on bone. It can inhibit the formation, growth, and dissolution of hydroxyapatite crystals and their amorphous precursors by chemisorption to calcium phosphate surfaces. Inhibition of crystal resorption occurs at lower doses than are required to inhibit crystal growth. Both effects increase as the dose increases. The amount of drug absorbed after an oral dose is approximately 3%. In normal subjects, plasma half-life (t1/2) of Etidronate, based on non-compartmental pharmacokinetics is 1 to 6 hours. Within 24 hours, approximately half the absorbed dose is excreted in urine; the remainder is distributed to bone compartments from which it is slowly eliminated. Animal studies have yielded bone clearance estimates up to 165 days. In humans, the residence time on bone may vary due to such factors as specific metabolic condition and bone type. Unabsorbed drug is excreted intact in the feces. Preclinical studies indicate Etidronate disodium does not cross the blood-brain barrier. (Drugs.Com, 2010, Etidronate, http://www.drugs.com/pro/etidronate.html).

Etidronate is not metabolized. The drug is either excreted through urine or absorbed in the bones. The bioavailability in plasma is limited. Therefore, a sensitive and robust method was developed for quantitation of Etidronate levels in plasma and urine. The method can detect Etidronate at levels low enough to be used for therapeutic drug monitoring of the patients taking the drug.

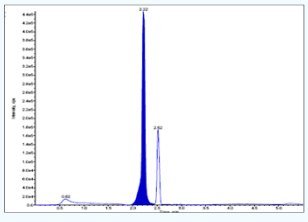
Method

A sensitive LC-MS/MS method was developed for the determination of Etidronate in dog and human plasma and urine. The samples were prepared through ultrafiltration with ^{13}C $^{2}H_3$ Etidronate disodium as the internal standards. The final supernatant was analyzed on the API 5000 LC-MS/MS system by electrospray ionization (ESI) mass spectrometry with multiple reaction monitoring (MRM) of negative ions. The ions monitored were 205→63 for Etidronate and 209→63 for ^{13}C $^{2}H_3$ Etidronate disodium. The ratio of analyte product ion peak area to that of the internal standard were the responses used for quantitation.

Chromatographs



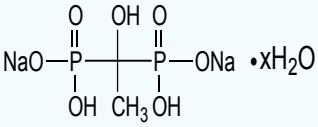
50 ng/mL of Etidronate



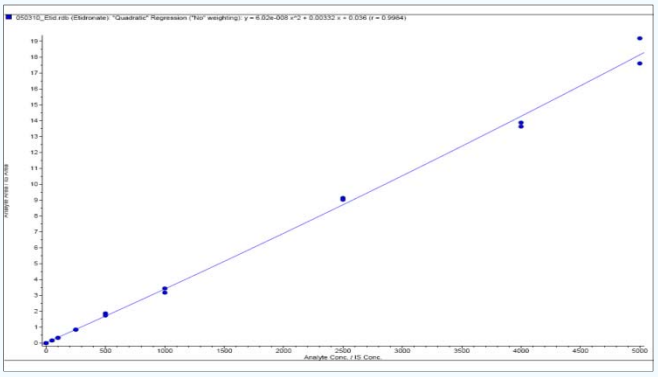
5000 ng/mL of Etidronate

Results and Discussion

The validation showed that the method has quadratic regression (r2 = 0.99) over the concentration range of 50 ng/mL to 5000 ng/mL. No significant interference was observed in blank plasma and urine.
The accuracy of the plasma and urine standards for Etidronate was within 13.54% from the nominal concentration. The precision of the plasma and urine standards for Etidronate did not exceed 13.63% at the LLOQ and was within 13.34% at all other levels.
The intra-day and inter-day accuracy for the determination of Etidronate in plasma and urine samples did not exceed 15.77% for low QC and 18.80% for other QCs. The intra-day and inter-day precision for the determination of Etidronate in plasma and urine samples did not exceed 12.88% CV for low QC and 15.74% for other QCs. Etidronate extracted from plasma and urine was stable at 4ºC (the temperature of the cooled auto-sampler) for at least 24 hours. Etidronate was also stable in plasma and urine after 3 freeze-thaw cycles. The selectivity, sensitivity, linearity, accuracy, precision and robustness of the method are sufficient for analysis of Etidronate in dog and human plasma and urine samples.



Etidronate disodium hydrate



Typical Calibration curve

Conclusion

LC/MS/MS offers a specific and sensitive platform for determination of Etidronate for clinical diagnosis as well research and development which can include Safety, Efficacy, Toxicokinetics as well as Pharmacokinetics in pre-clinical and clinical studies.

Contact

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