Application brief | 002201

HPLC chromatography

Improvements in reversed phase chromatography of bisphosphonates with the use of ion pairing reagents

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Introduction

Bisphosphonates are a class of drugs that prevent bone density loss. Due to the ionic nature of these drugs, they are difficult to analyze with traditional reversed phase (RP) liquid chromatography. When using RP chromatography, the ionic nature of the phosphates does not retain. When HILIC is used, separation of the phosphate species is possible, but the peak shape is broad. Therefore, the use of RP chromatography with ion pairing reagents was utilized.

There are two types of ion pairing reagents, either for pairing anionic species (like triethylamine acetate, TEAA) or pairing cationic species (like heptafluorobutyric acid, HFBA). The ion pairing reagents that we will discuss aid in chromatographic separation, compared to ionization enhancers such as hexafluoro-2-propanol (HFIP) or acetic acid for the purposes of MS analysis. With the addition of an ion-pairing reagent, TEAA, the negatively charged bisphosphonates can be retained alongside a significant improvement in peak shape compared to RP chromatography without an ion pairing reagent or HILIC HPLC analysis. One of the negative impacts of an ion pairing reagent is a decrease in MS sensitivity, but improved peak shape reduces the impact of the decreased sensitivity.

Important notes

- TEAA is a useful ion pairing reagent for anionic species.

Materials required

- UHPLC-MS instrument such as a Thermo Scientific™ Vanquish™ Flex system with a Thermo Scientific™ Q Exactive™ mass spectrometer
- Thermo Scientific™ Hypersil GOLD™ column, 1.9 µm, 50 × 2.1 mm (P/N 25002-052130)
- Thermo Scientific™ Triethylamine acetate, TEAA, 2M (P/N 400613)
- Water, UHPLC-MS grade
- Acetonitrile, UHPLC-MS grade
- Thermo Scientific™ SureSTART™ specification certified screw vial and cap kit, level 2: 2 mL volume vial kit, 9 mm opening, AVCS™ (Advanced Vial Closure System) cap silicone/PTFE septum (P/N 6AK80W)

Protocol

1. Make bisphosphonates standards to 100 ng/µL in water.
2. Make mobile phases.
3. Inject and run samples.

Conditions

Mobile phase A	0.1 M TEAA in water		
Mobile phase B	0.1 M TEAA in acetonitrile		
Flow rate	0.4 mL/min		
Gradient program	Time (min)	%A	%B
	0.0	98	2
	3.0	2	98
	4.0	98	2
	8.0	98	2
Column temperature	35 °C, with active pre-heating and still air		
Injection volume	5 µL		
Detection	HESI HRAM (Negative 100–500 *m/z*)		

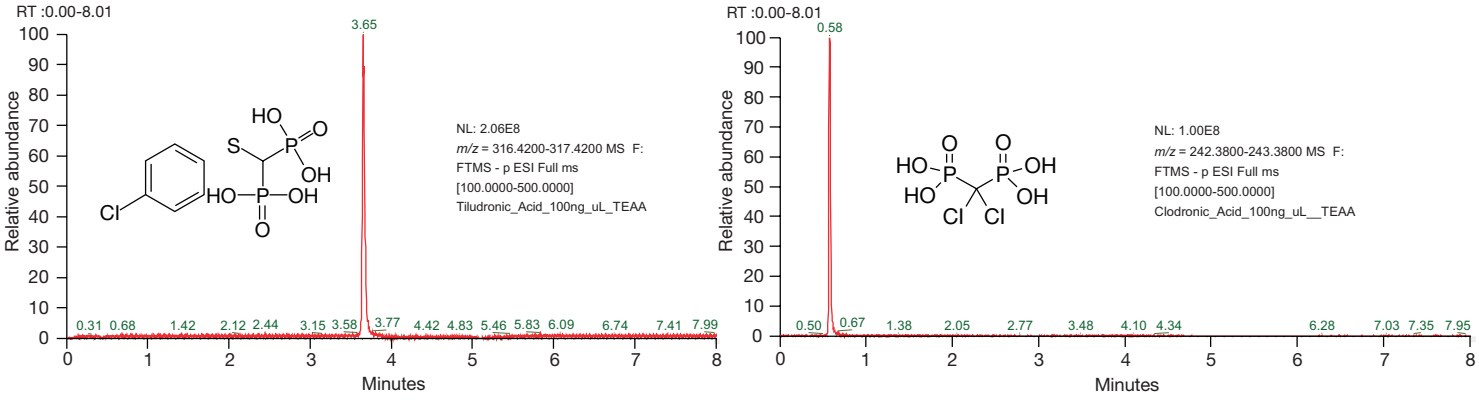

Figure 1. Tiludronate and clodronate

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