B-TRAXIM 2C

pancosma

Chemical structure of B-TRAXIM 2C in aqueous solution

KEY FINDINGS

B-TRAXIM 2C in aqueous solution

- ***Was always chelated with a glycine bound to the metal atom***
- ***Existed as $[M(Gly)(SO_4)]_X(H_2O)_n$***
- ***Had at least two different chemical forms***

INTRODUCTION AND OBJECTIVE

B-TRAXIM 2C are crystalline chelates of glycine that are more bioavailable than metal sulphates.

The identification of the chemical structure of B-TRAXIM 2C is the starting point to trace these compounds in the digestive system, in the tissues and in blood. In other words, it enables to determine where and how the chelates are absorbed and utilized by the animal, and under which form they exist before and after the absorption.

For regulatory reasons, it is also necessary to have a method to trace B-TRAXIM 2C in premixes and in feed. As no method exists nowadays, it is necessary to develop and validate new analytical methods to trace B-TRAXIM 2C in all these matrixes.

So the first step is to determine the chemical structure of B- B-TRAXIM 2C in water.

MATERIALS AND METHOD

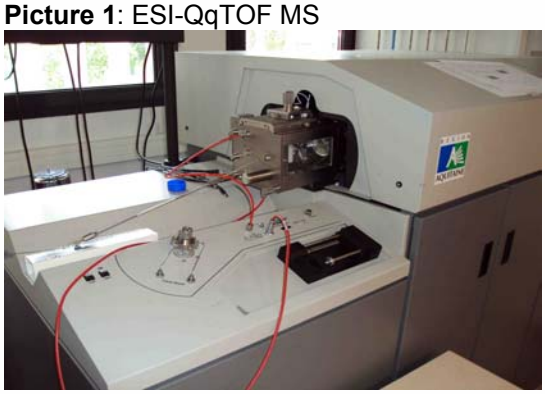
The study was performed at the Laboratory of Bio-Inorganic and Environmental Analytical Chemistry of the CNRS in Pau (France).

The study was performed by high resolution mass spectrometry.

Apparatus:

Electrospray QqTOF mass spectrometer (ESI-QqTOF MS) QSTAR XL from Applied Biosystems (picture 1)

Picture 1: ESI-QqTOF MS



Reagents and solvents:

All the solvents and reagents were of analytical grade.

Samples:

- B-Traxim 2C Zn $[Zn(C_2H_5NO_2)(H_2O)_2(SO_4)]_n$
- B-Traxim 2C Cu $Cu(C_2H_5NO_2)(H_2O)_2(SO_4)]_n$
- B-Traxim 2C Mn $[Mn(SO_4)(C_2H_5NO_2)]_n$
- B-Traxim 2C Fe $\{[Fe(C_2H_5NO_2)(H_2O)_2(SO_4)_2][Fe(C_2H_5NO_2)(H_2O)_4]\}_n$

Method of analysis

Each sample was dissolved at 100mg/L in an aqueous solution at pH 7.4 with an ammonium acetate buffer. The solution was then infused in the mass spectrometer.

The mass accuracy of the mass spectrometer was calibrated with a reserpin standard $(C_{33}H_{40}N_2O_9)$:$[M+H]^+$ 609.28121 amu (atomic mass unit).

The optimum settings were:

- ion spray voltage: 4850V
- curtain gaz: 25V
- GS1: 17V
- GS2: 0V
- collision gas : 15 to 30eV with N_2 depending on the compound.

The mass spectra were recorded across the range m/z 70-2000amu.

First the complete mass spectrum was recorded to identify the fragments which could contain a metal atom. Then the most interesting ions were refragmented (MS/MS) to identify their chemical structure.

The oxidation of Fe(II) in Fe(III) is followed by precipitation of $Fe(OH)_3$ which rendered impossible the characterisation of Fe-glycinate complexes. To prevent this, all the solutions with B-Traxim 2C Fe were degassed by nitrogen bubbling just before analysis. N_2 was bubbled in the solution at the rate of 1min per mL of solution. The solid standard of B-Traxim 2C Fe was dissolved in the degassed solution just before analysis.

Technical Bulletin

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RESULTS AND CONCLUSION

B-TRAXIM 2C Zn in solution

The presence of zinc in the mass spectrum of B-TRAXIM 2C Zn was suspected in 2 parts of the spectrum, the first around 250amu (figure 1) and the second around 475amu (figure 2). The window in the top right of the figures illustrates the theoretical mass spectrum corresponding to the suspected formula.

Figure 1: Mass spectrum of B-TRAXIM 2C Zn from 250 to 260amu.

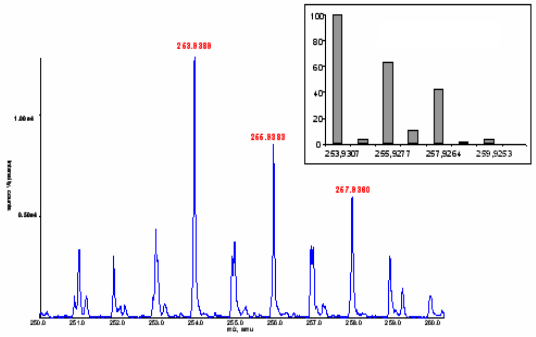
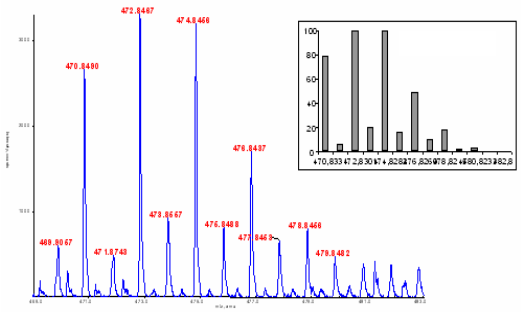


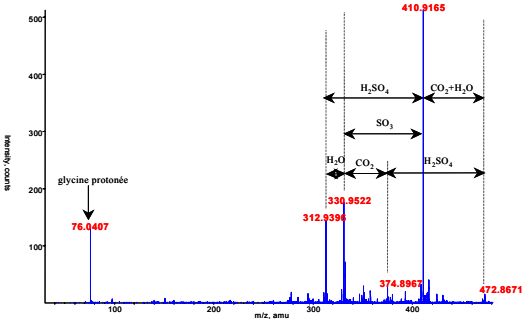
Figure 2: Mass spectrum of B-TRAXIM 2C Zn from 469 to 483amu.



These 2 spectra corresponded to complexes with the formula $Zn(C_2H_5NO_2)(H_2O)(SO_4)$ and $[Zn(C_2H_5NO_2)(SO_4)]_2$ respectively (table 1). The ions at 254 and 473amu were refragmented to verify these structures (figure 3).

The MS/MS spectrum permitted to identify glycine, while losses of sulphate, CO_2 and H_2O fragments were registered, confirming that the mass spectra observed was due to the presence of two zinc glycine complexes.

Figure 3: MS/MS spectrum of the 473amu ion for B-Traxim 2C Zn.



Other B-TRAXIM 2C in solution

The same operation with B-TRAXIM 2C Fe, Cu and Mn led to the identification of 2 different iron glycine complexes, 3 copper glycine complexes and 4 manganese glycine complexes (figure 4 table 1).

Figure 4: Mass spectra of B-TRAXIM 2C Fe

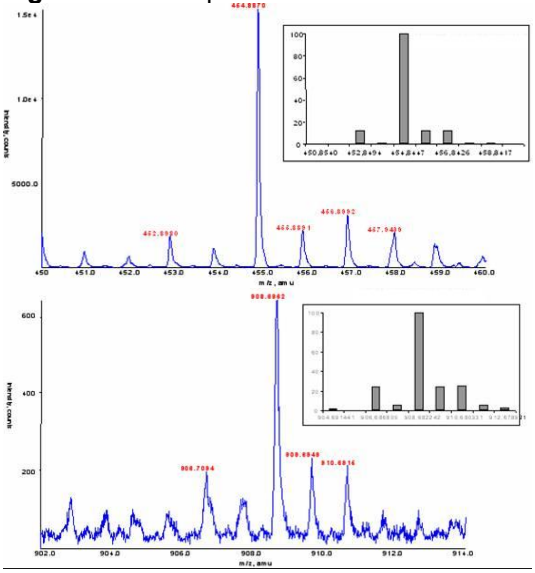


Table 1: Chemical structure of the B-TRAXIM 2C complexes in aqueous solution

	B-TRAXIM 2C Zn	B-TRAXIM 2C Cu	B-TRAXIM 2C Fe	B-TRAXIM 2C Mn
1	$Zn(Gly)(H_2O)(SO_4)$	$Cu(Gly)(H_2O)(SO_4)$	$[Fe(Gly)(SO_4)_2][Fe(Gly)]$	$Mn(Gly)(SO_4)$
2	$[Zn(Gly)(SO_4)]_2$	$[Cu(Gly)(SO_4)]_2(H_2O)$	$\{[Fe(Gly)(SO_4)_2][Fe(Gly)]\}_2$	$[Mn(Gly)(SO_4)]_2$
3		$[Cu(Gly)(SO_4)]_3$		$[Mn(Gly)(SO_4)]_3$
4				$[Mn(Gly)(SO_4)]_4$

For all B-Traxim 2C, glycine complexes were detected in aqueous solution. Except for B-Traxim Mn which was totally anhydrous, the complexes identified in solid state were not found in aqueous solution. For Zn, Fe and Cu the recovered complexes corresponded to the partially or completely dehydrated complexes.

This could be explained either by a fragmentation in the ionisation source, where water would be very quickly fragmented, or by a rearrangement of the complex in solution.

B-Traxim 2C in aqueous solution were always detected as glycine complexes, glycine was always bound to the metal. Their general formula was: $[M(Gly)(SO_4)]_x(H_2O)_n$.