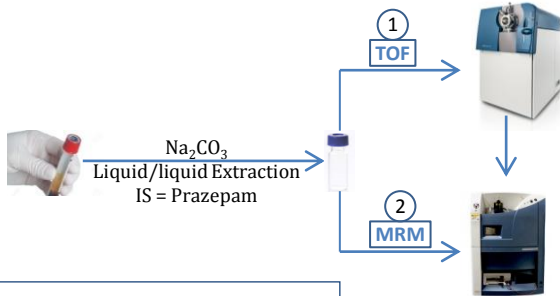


INTRODUCTION

A patient came for the third time at the emergency department for ear-nose-throat symptoms, and finally died of a virulent group A *Streptococcus pyogenes*. As chemsex was evoked by the family, a screening for new psychoactive substances was undertaken.

MATERIAL AND METHODS



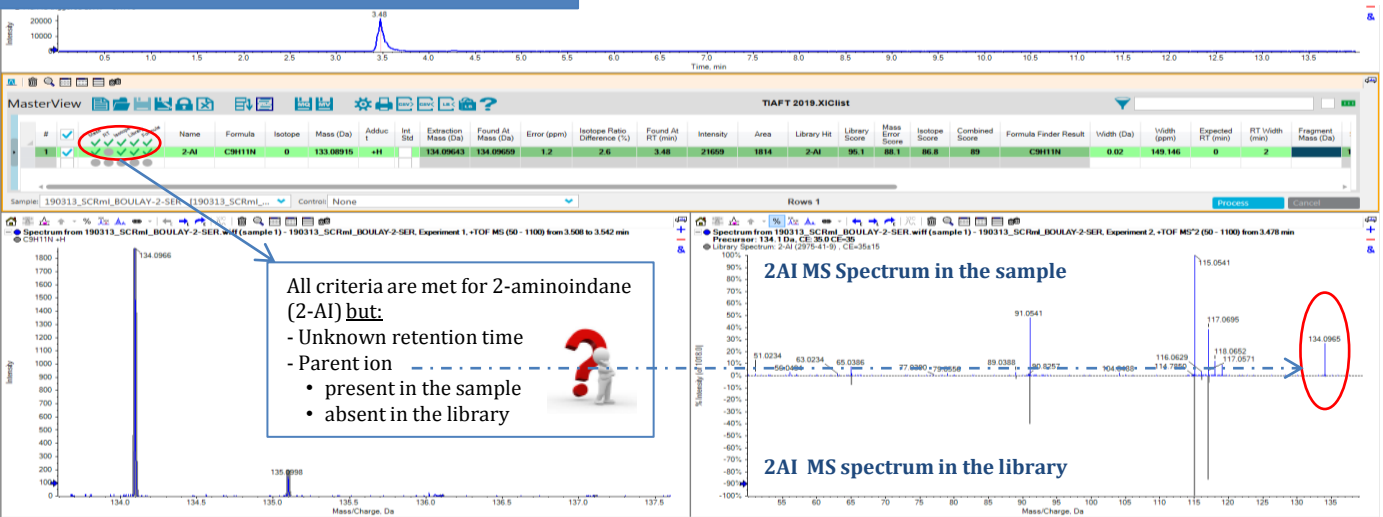
- ① Without reference standard
- ② After reception of reference standard

UHPLC-TOF-MS (Sciex)
 Eksigent LC 100 XL + TripleTOF 4600
Column: Kinetex 2.6 C18, 100 Å, 50 x 3.00 mm (Phenomenex)
Mobile Phase A: 10 mM NH₄ formate
Mobile Phase B: ACN/MeOH (50/50)
Gradient mode: 15.5 min runtime

UHPLC-MS-MS (Waters)
 UPLC Acquity + Quattro Premier
Column: BEH C18, 1.7 µm, 50 X 2.1 mm (Waters)
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in methanol
Gradient mode: 15 min runtime

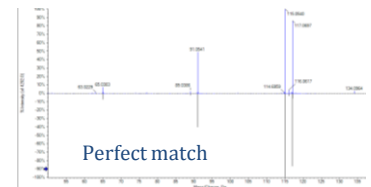
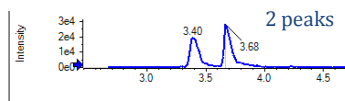
RESULTS

UHPLC-TOF-MS: before reception of reference standard



UHPLC-TOF-MS: after reception of reference standard

Neat standard vs extracted serum:
 → 0.3 min time shift
 → Matrix effect?
 → Sample was spiked with 2-AI



UHPLC-MS-MS: after reception of reference standard



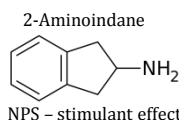
Neat standard vs extracted serum:
 → 0.1 min time shift

BUT

Ion Ratio 133.9 > 116.9 / 133.9 > 115.1
 Standard : 6.62 ≠ Sample : 2.37

DISCUSSION

- ✓ Knowledge of the retention time is useful to identify a compound but reference standard is essential to do so
- ✓ Delivery time are sometimes long → potential stability issue if a case is open
- ✓ Some matrix effects can generate a shift in the retention time
- ✓ LC-MSMS method developed to be as fast as possible: retention time shift even smaller → risk of false positive result if ions ratios aren't checked
- ✓ Same interference in 6 out of 900 other samples but origin still unknown



Conclusion

Even if MRM mode is often preferred in order to increase the sensitivity, identification criteria - including retention time and two transitions - are sometimes insufficient to be 100% sure of the compound identification, especially if the runtime is short. Calculating ion ratios is indispensable and not optional if identification is to be based on MRM data.



Keep in mind