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Application of nanoUPLC-QToF MS for screening of veterinary drugs

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Introduction

For small molecule applications in various working fields such as food-, forensic- and doping analysis generic multi-target screening methods using full scan accurate mass spectra are under development. These methods allow screening for an unlimited number of compounds and possibilities for retrospective searches for new emerging compounds. Furthermore, bioactivity-driven hazard identification before confirmation of suspected samples by mass spectrometry is nowadays more often used. For hazard identification but also for forensic applications sample size can be limited. Therefore, it is interesting to scale down UPLC methods to nanoUPLC format. The first step, developing a nanoUPLC QToF MS method for > 100 veterinary drugs is demonstrated.

Analytical Method

Optimisation of the analytical method was done with a standard mixture containing approx 100 different antibiotics. Separation and detection was achieved on respectively a Waters nanoAcquity and Xevo QToF-MS. Trap and chromatographic conditions were optimized for optimal separation and peak shape for most of the veterinary drugs included.

Optimized method

Mobile phase:	Linear gradient Water/acetonitrile 0.1% formic acid
Trap column:	HSS T3 20 × 0.18mm, 5µm
Analytical column:	BEH 130Å C_{18} 150mm × 75µm, 1.7µm
Injection volume:	2µL
Trap:	2 min on 2µL/min
Flow rate:	600nL/min
Run time:	32 min (2 min trap + 30 min separation)
QToF MS:	Operating in ESI^+
Resolution:	FWHM 10 000
Method:	MS^e mode scan from m/z 50 till m/z 1000

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Results

The nanoAcquity is standard installed with a symmetry C18 trap column. With this trap relative broad peaks were observed (**Fig 1a**). To have better focusing of the relative polar compounds a HSS T3 trap column for nanoUPLC was developed. This trap column resulted in an improved peak shape for most antibiotics (**Fig 1b**).

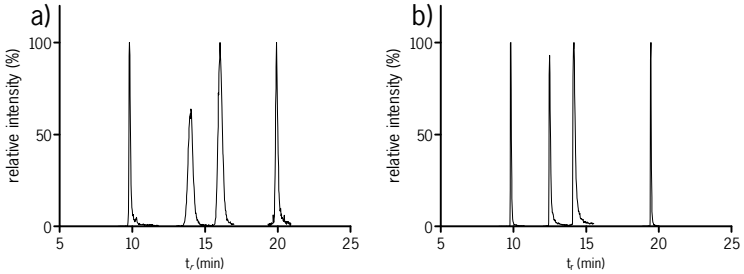

Figure 1. Peak shapes after trap on a) symmetry C_{18} and b) a HSS T3 column

Still a drawback of ToF MS screening methods is the lack of fragmentation data. With the Xevo QToF MS it is possible to do a MS^e experiment. One function contains the MS data and an additional function contains the MS/MS data. This MS^e data was used to for example distinguish between the NSAIDs fenbufen and ketoprofen which both have a m/z 255.1021 (**Fig 2**).

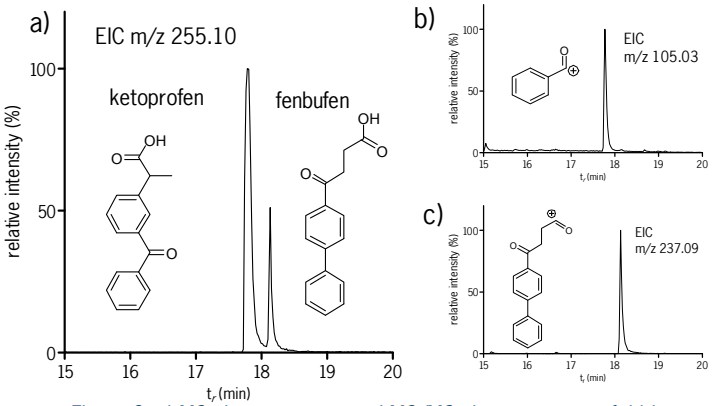

Figure 2. a) MS chromatogram and MS/MS chromatogram of b) ketoprofen and c) fenbufen

Conclusion

- A robust nanoUPLC QToF-MS method has been developed with a good retention time repeatability (RSD < 1% for 16h)
- MS^e is a helpful tool to identify compounds with identical elemental composition