Introduction

Modern Supercritical Fluid Chromatography (SFC) systems offer huge performance advantages compared to classical HPLC instruments. Compared to HPLC mobile phases, SFC mobile CO2 phase has a lower viscosity, an increased diffusion and better mass transfer capabilities. This enables a faster speed of separation at lower back pressure by means of columns with smaller inner diameter and small particle size packing material. Both methodologies, SFC and HPLC, are comparable in terms of sensitivity and stability but provide orthogonal selectivity of the separation. This makes SFC a valuable complementary technique to classical HPLC as well as modern UHPLC.

The application range of SFC can be widened by coupling with other detectors especially ELSD and mass spectrometers like triple quadrupole MS instruments for quantification and QTOF MS instruments for high resolution mass spectrometry. Due to its small amount of solvent load, excellent resolution and narrow peak width SFC is an excellent front end for mass spectrometry. But, effects like expansion cooling from decompressed CO2, the need of splitting and a make-up flow to introduce ionizing agents makes it not that straightforward.

This work shows an instrument configuration to combine SFC instruments to ELSD and modern MS instruments. The effect of CO2 expansion cooling and pre-heating of the column effluent on the peak performance will be shown and discussed. The introduction of a make-up flow will be included in the instrument configuration. Finally, the separation of an environmental and food relevant sample of 18 veterinary drugs in three minutes with MS detection will be shown (Figure 1). Data about the LOD, LOQ, linearity, retention time and area RSDs for the individual compounds will be shown.

Experimental

SFC-BLD method

Solvent: A: CO2
Modifier: B: Methanol
SFC Flow: 3 mL/min
Gradient: 0 min - 5% B, 10 min - 25%
B, Stop time: 10 min. Post time: 2 min.
Make-up Flow: 0.2 mL/min, methanol
Back pressure regulator (BPR) temperature: 60°C
BPR pressure: 250 bar
Column temperature: 40°C
Injection volume: 5 µL, 3 x loop over fill.
Needle wash in vac with methanol
DAD: 254 nm band width 4 nm Ref. 360 nm band with 100 nm Data rate: 20Hz.
ELSD:
Evaporator temperature: 40°C
Nebulizer Temperature: 55°C
Gas Flow Rate: 1.15 SLM
Data rate: 10 Hz
Smoothing: 2 sec
PMT Gain: 5

SFC-QQQ MS method

Solvent: A: CO2
Modifier: B: Methanol
SFC Flow: 1 mL/min
Gradient: 0 min - 5% B to 3 min - 20% B
Stop time: 3 minutes, Post time: 1 min.
Make-up flow: 0.2 mL/min
Make-up composition: Acetone + 0.1% formic acid
BPR temperature: 60°C
BPR pressure: 250 bar
Column temperature: 40°C
Injection volume: 1 µL, partial loop fill.
MS:
Ionization mode: positive
capillary voltage: 5000 V
Nezzle voltage: 300 V
Gas flow: 14 L/min
Gas temp.: 150°C
Sheath gas flow: 12 L/min
Sheath gas temp.: 40°C
Nebulizer pressure: 60 psi
MS conditions see table 1.

Results and Discussion

Considering the retained signal-to-noise at a pre-heating temperature of 30°C and the minimized signal-to-noise RSD at that point the connection from the SFC to the ELSD found its optimum the values of 30°C for pre-heating with 0.2 mL/min make-up flow (Figure 5).

Figure 5 A) Peak height and peak height RSD (%), vs. make-up flow rate at 30°C temperature of split SFC effluent. The peak heights decreased during the addition of a make-up flow but the peak height RSD achieved a minimum at about 4-5% for a make-up flow rate of 0.2 mL/min. B) Signal-to-Noise ratio and S/N RSD (%) vs. make-up flow rate at 30°C temperature of split SFC effluent. The signal-to-noise ratios kept nearly constant during the addition of a make-up flow of 0.2 mL/min and started to decrease for higher make-up flow rates. The signal-to-noise ratio RSD achieved a minimum at about 10-15% for a make-up flow rate of 0.2 mL/min.

SFC-QQQ MS

With the developed SFC MS method, 10 veterinary drugs comprised in the test were separated within three minutes run time, whereas the separation took place within one minute. The maximum number of overlying peak maxima was three, which appeared between 1.35 and 1.40 minutes (Figure 6). For the creation of the individual calibration curves a series of dilutions starting at 10 ng/mL were prepared (3, 5, 10, 0.1 ng/mL). The linearity of all individual calibration curves is typically better than 0.9990 (Table 2). LOD for all compounds are typically below 200 pg/mL and one third of the compounds had an LOD below 100 pg/mL. The majority of compounds had a LOD which is below 60 pg/mL. The relative standard deviation (RSD) of the retention time was typically below 0.1% and the peak area RSDs typically between 5 and 10% allowing proper quantification (Table 2).

Figure 6 Separation of 18 Compounds which are part of the Agilent Veterinary Drug Comprehensive Test Mix (Sub-mix 2) at a concentration of 10 ng/mL in methanol. The complete run time was 3 minutes and the separation was completed in one compound. The compounds and their retention time is given below.

Table 2 Summary of the measurement of 18 Veterinary Drugs by SFC-MS

Conclusions

- This work describes the combination of a Supercritical Fluid Chromatography (SFC) instrument with a high end triple quadrupole mass spectrometer for the quantitative determination of veterinary drugs.
- The veterinary drugs were separated in a run time of three minutes and detected down to a typical LOD of 200 pg/mL.
- The achieved linearity of the individual calibration curves was excellent and the area RSDs were between 5 and 10% which is essential for MS quantification.
- The effects of pre-heating the SFC effluent after the split are described in combination with an ELSD to find an optimum pre-heating temperature getting the best performance about peak height, peak area, signal-to-noise and their relative standard deviation values.
- The introduction of a make-up flow at the optimized temperature and the dependence of the mentioned parameters are described.
- Finally, an optimized pre-heating temperature and make-up flow rate is concluded from the data.