Veterinary Drug Analysis with Supercritical Fluid Chromatography and Triple Quadrupole LC/MS

The Agilent 1260 Infinity Analytical SFC Solution and Agilent 6490 Triple Quadrupole LC/MS

Application Note

Food Testing and Agriculture

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Abstract

This Application Note describes the combination of the Agilent 1260 Infinity Analytical SFC Solution and an Agilent 6490 Triple Quadrupole LC/MS for the measurement of veterinary drugs. The connection of the SFC to the triple quadrupole MS was made through the Agilent Jet Stream Technology electrospray ionization source. The configuration of the system is described, including a split from the SFC to the MS and a separate make-up flow for improved ionization, together with the method parameters. The data show limits of quantitation (LOQs) and limits of detection (LODs) in the lower ppb range for all measured veterinary drugs.

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Introduction

Modern supercritical fluid chromatography (SFC) instruments offer performance advantages compared to classical HPLC instruments. Compared to HPLC mobile phases, SFC's mobile CO_2 phase has lower viscosity, increased diffusion, and better mass transfer capabilities. This enables higher speed separations at lower backpressure. 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 combining it with other detectors, especially mass spectrometers such as triple quadrupole MS instruments, for quantification. Due to its small amount of solvent load, excellent resolution, and narrow peak width, SFC is an excellent front-end for mass spectrometry. Effects such as expansion cooling from decompressed CO_2, the need for splitting, and for a make-up flow to introduce modifiers for improved ionization in electrospray, makes it not as straightforward to use compared to standard LC/MS.

This work describes a robust instrument configuration to connect SFC instruments to modern MS instruments. The introduction of a make-up flow is included in the instrument configuration, to separate 18 veterinary drugs in 3 minutes. Data on LOQs, LODs, linearity, retention time, and area RSDs for the individual compounds are given.

Experimental

Instruments

Agilent 1260 Infinity Analytical SFC Solution (G4309A), with:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High Performance Degasser
- Agilent 1260 Infinity SFC Standard Autosampler
- Agilent 1290 Infinity TCC
- Agilent 1260 Infinity DAD with high pressure SFC flow cell
- Agilent 1260 Infinity Isocratic Pump (G1310B)
- Agilent 6490 Triple Quadrupole LC/MS (G6490A)

Instrument set-up

The configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6490 Triple Quadrupole LC/MS is shown in Figure 1. The column was directly connected to splitter 1. At this first splitter, the make-up flow coming from an isocratic pump was introduced into the flow path. Splitter 1 was connected to a second splitter by a short 0.12-mm id capillary. Here, the flow was split in two, with one part going to the MS and the other part going to the backpressure regulator (BPR) of the SFC module. The connection to the MS was made from a 50-µm id stainless steel capillary, 1-m long (p/n 5067-5939). The split ratio depends on the backpressure generated by this restriction capillary and the pressure set by the BPR. The complete splitter kit can be ordered under p/n G4309-68715

Column

Agilent ZORBAX Eclipse Plus, Phenyl-Hexyl, 2.1 × 150 mm, 1.8 µm (p/n 959759-912)

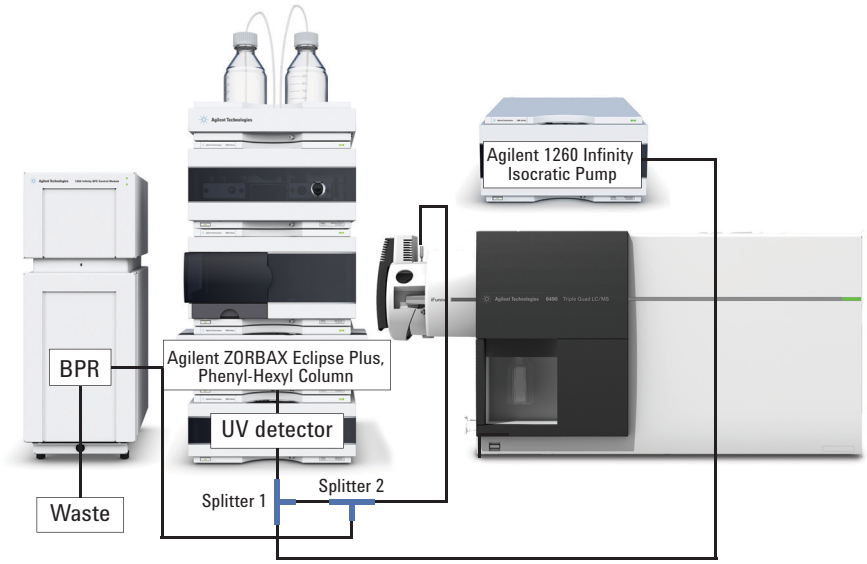

Figure 1. Configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6490 Triple Quadrupole LC/MS. The column was directly connected to splitter 1 (BPR = backpressure regulator, UV detector not used, splitter kit (p/n G4309-68715).

Software

- Agilent MassHunter Data Acquisition Software for triple quadruple mass spectrometer, Version 06.00. including SFC software add-on
- Agilent MassHunter Optimizer Software, Version 06.00
- Agilent MassHunter Qualitative Software, Version 06.00
- Agilent MassHunter Quantitative Software, Version 06.00

MS/MS conditions are shown in Instrument conditions.

Standards

Agilent Veterinary Drug Comprehensive Test Mix (p/n 5190-0554), sub-mix 2 (p/n 5190-0570); concentration 100 µg/mL for each compound

Instrument conditions

SFC conditions	
Flow rate	1 mL/min
Gradient	0 minutes - 5 % B to 3 minutes – 20 % B
Stop time	3 minutes
Post run	1 min at 5 % B
Modifier B	Methanol
Make-up flow	0.2 mL/min
Make-up composition	Acetonitrile + 0.1 % formic acid
Backpressure regulator (BPR) temperature	60 °C
Backpressure regulator pressure	290 bar
Column temperature	40 °C
Injection volume	1 µL, partial loop fill
MS conditions	
Ionization mode	Positive
Capillary voltage	5,000 V
Nozzle voltage	300 V
Gas flow	14 L/min
Gas temperature	150 °C
Sheath gas flow	12 L/min
Sheath gas temperature	400 °C
Nebulizer pressure	60 psi

Table 1. MRM transitions and conditions, showing the identified optimum fragmentor voltage and collision energy values for individual compounds as well as for the quantifier and qualifier ions.

Compound name	Precursor ion (*m/z*)	Product ion 1 (quantifier)	Product ion 2 (qualifier)	Dwell time (ms)	Fragmentor (V)	Collision energy (eV) Product ion 1 (quantifier)	Collision energy (eV) Product ion 2 (qualifier)	Cell accelerator (V)
Brombuterol	365.0	347.0	290.9	5	380	8	16	4
Mapenterol (methylmabuterol)	325.1	237.0	217.0	5	380	12	24	4
Chlorbrombuterol (Bromoclenbuterol)	321.0	247.0	168.1	5	380	12	28	4
Clencyclohexerol	319.1	301.1	203.0	5	380	8	16	4
Mabuterol	311.1	293.1	237.0	5	380	8	12	4
Fenoterol (Th 1165a)	304.2	135.1	107.1	5	380	16	36	4
Isoxsuprine (Isolait)	302.2	284.2	107.1	5	380	8	32	4
Ractopamine	302.2	284.2	107.1	5	380	8	24	4
Clenbuterolhydroxymethyl	293.1	275.1	205.0	5	380	4	12	4
Clenpenterol	291.1	203.0	132.1	5	380	12	28	4
Clenbuterol	277.1	259.1	203.0	5	380	4	12	4
Clenproperol	263.1	245.1	132.1	5	380	4	28	4
Zilpaterol	262.2	244.1	185.1	5	380	8	20	4
Salbutamol (albuterol)	240.2	222.2	148.1	5	380	4	16	4
Cimbuterol	234.2	160.1	143.1	5	380	8	24	4
Tulobuterol	228.1	154.0	119.1	5	380	12	32	4
Terbutaline	226.1	170.1	152.1	5	380	8	12	4
Cimaterol	220.1	202.1	160.1	5	380	4	12	4

Chemicals

All solvents were LC/MS grade. Acetonitrile and methanol were purchased from J. T. Baker, Germany. Fresh, ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

Results and Discussion

For the development of the SFC/MS method, the Agilent Veterinary Drug Comprehensive Test Mix (sub-mix 2) was diluted to 10 ng/mL in methanol. With the final SFC/MS method, the 18 veterinary drugs in this solution were separated within a 3-minute run time, whereas the separation took place within 1 minute. One minute was used for the regeneration of the column, which led to a total run time of 4 minutes. In this separation, the maximum number of overlaying peak maxima was three, which appeared between 1.35 and 1.40 minutes (Figure 2). For the MS detection and final quantification, the two most intense MRM transitions were used (Table 1). Source conditions were optimized as a good compromise for all targeted compounds. The chromatograms shown in Figure 2 represent the quantifier transitions of all veterinary drugs under investigation.

For the creation of the individual calibration curves, a series of dilutions was prepared, starting at 10 ng/mL (10, 5, 1, 0.5, and 0.1 ng/mL). The linearity of all individual calibration curves was typically better than 0.9990 (Table 2). Figure 3 shows two typical examples, where mapenterol was one of the early eluting and higher abundance compounds, eluting without any other coeluting compound. Chlorobrombuterol, in contrast, eluted in the middle of the chromatogram at lower abundance with coelution of several other compounds. Both calibration curves showed excellent linearity.

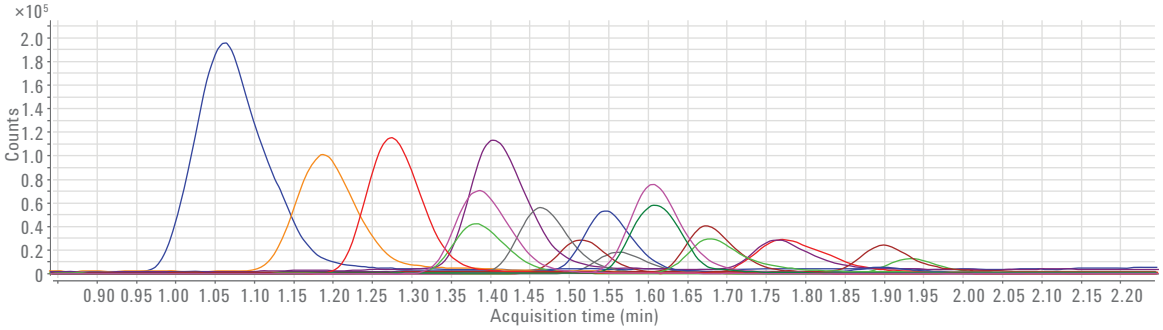

Figure 2. Separation of 18 compounds in the Agilent Veterinary Drug Comprehensive Test Mix (sub-mix 2) at 10 ng/mL in methanol. The complete run time was 3 minutes, and the separation was completed in 1 minute. The compounds and their retention times are given in Table 2.

Table 2. Summary of the measurement of 18 veterinary drugs by SFC/MS.

Compound	RT (min)	RT RSD (%)	Area RSD (%)	R^2	LOQ (pg/mL)	LOD (pg/mL)
Mapenterol (methylmabuterol)	1.059	0.87	5.40	0.9995	76.9	23.1
Mabuterol	1.184	0.91	12.45	0.9987	58.8	17.6
Tulobuterol	1.269	0.70	6.16	0.9998	90.9	27.3
Clenbuterolhydroxymethyl	1.375	2.51	16.69	0.9991	54.9	16.5
Clenpenterol	1.380	0.51	4.58	0.9991	161.3	48.4
Isoxsuprine (Isolait)	1.405	0.59	8.57	0.9985	142.5	42.9
Clenbuterol	1.458	0.57	7.58	0.9998	128.2	38.5
Chlorbrombuterol (bromoclenbuterol)	1.512	0.40	6.58	0.9992	151.5	45.5
Clenproperol	1.540	0.42	9.41	0.9992	277.7	83.4
Brombuterol	1.558	0.41	6.48	0.9995	147.7	44.1
Cimbuterol	1.603	0.47	10.64	0.9997	95.2	28.6
Terbutaline	1.614	0.17	7.32	0.9994	97.1	29.2
Cimaterol	1.665	0.37	10.83	0.9995	210.5	63.0
Salbutamol (albuterol)	1.669	2.39	16.66	0.9993	149.2	44.8
Ractopamine	1.760	0.70	7.89	0.9990	166.5	50.0
Fenoterol (Th 1165a)	1.770	0.29	9.96	0.9997	500.0	150.1
Zilpaterol	1.895	0.46	15.34	0.9995	454.5	136.5
Clencyclohexerol	1.934	1.20	12.89	0.9998	285.5	85.8

For the determination of LOQ and LOD, the concentrations at signal-to-noise (S/N) levels of 10 and 3 were calculated, respectively. The LOQs were calculated from the lowest individual calibration point, which had a S/N > 10. LOQs for all compounds were typically below 200 pg/mL, and one third of the compounds had a LOQ below 100 pg/mL. With regard to the LODs, the majority of compounds had an LOD below 60 pg/mL. These results are in a good accordance with results shown for a LC/triple quadrupole MS multimethod for the screening and quantitation of more than 200 veterinary drug compounds[1]. This method determined approximately 50 % of the compounds targeted in this Application Note with a LOQ below 200 pg/mL (in organic solvent).

For a statistical evaluation, 10 replicate injections of 1 ng/mL were done. The relative standard deviation (RSD) of the retention time was typically below 0.7 %, and the peak area RSD typically between 5 and 10 %, allowing proper quantification (Table 2).

Conclusions

This Application Note describes the combination of the Agilent 1260 Infinity Analytical SFC Solution with a high-end Agilent 6490 Triple Quadrupole LC/MS for the quantitative determination of veterinary drugs. The drugs were separated in a run time of 3 minutes and detected down to a typical LOQ of 200 pg/mL. The linearity of the individual calibration curves was excellent, and the area RSDs were between 5 and 10 %, which is excellent for MS quantification.

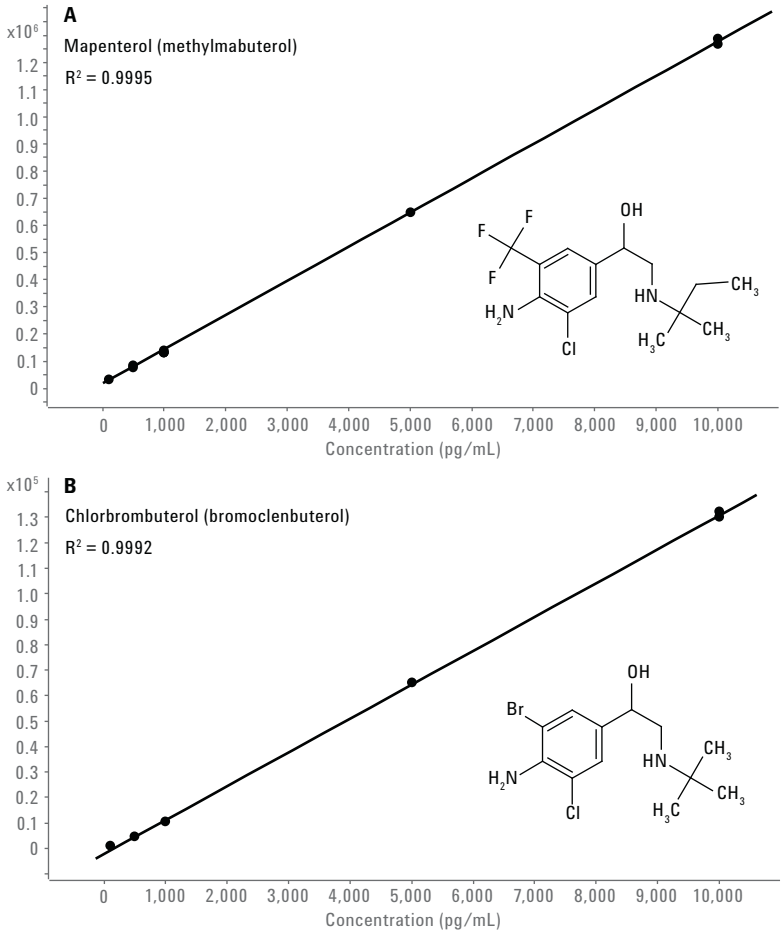

Figure 3. Calibration curves of the veterinary drugs mapenterol and chlorbrompenterol acquired by SFC/MS from 100 pg/mL to 10 ng/mL.

Reference

1. Kempe, G; Glauner, T; Spitzbarth, F. Multi-residue screening and confirmation of veterinary drugs in tissue samples by LC-MS/MS with new triggered MRM acquisition. *61st Annual ASMS Conference on Mass Spectrometry and Allied Topics*, Code: WP20. June 9-13, **2013**, Minneapolis, MN.

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