

# Application Example

## Method of Quantification

### AAAAA, BBBBB and CCCCC in Pig Blood by LC - Electro-Spray Tandem Mass - Spectrometry.

## Mass-spectrometric (MS) condition.

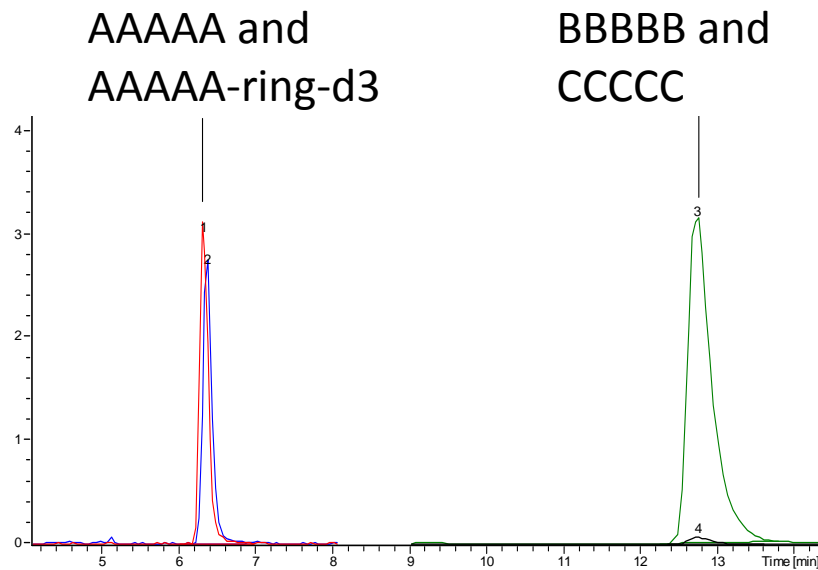
### MS- Parameters in Segments of Injection

<u>Compounds:</u>	AAAAA BBBBB CCCCC
<u>Internal Standard</u>	AAAAA-ring-d3
<u>System:</u>	HPLC Agilent 1100 Series and Auto-sampler G 1329A ALS 1200 Series with Frizzier G1330B FC/ALS Therm. The Auto-sampler operated by temperature 4C.
<u>Column:</u>	Kromasil 250X2.0 5U 100A ( 5µm) Ser. N 286756-6 The column operated by room temperature.
<u>Detector:</u>	Ion Trap MS Esquire 3000 Plus (Bruker Daltonics) equipped with Electro-Spray Source.
<u>Software:</u>	Chem Station for LC 3D. Rev.A.09.03 (1417). Copyright® Agilent Technologies 1990-2002. Method AAAAA.m Bruker Daltonik esquire 5.0. Build 169 Esquire Control Version 5.0 (Built 65). Copyright® Bruker Daltonik GmbH 1998- 2002. AAAAA.ms Bruker Daltonik esquire 5.0. Build 169. Quant Analysis Version 1.4 (Build 49) Copyright® Bruker Daltonik GmbH 1999- 2002. Method QA AAAAA8.ms
<u>Condition:</u>	
Injection Volume:	40 µl
Flow Rate:	0.2 ml / min
Pressure Limits:	300 bar
Stop Time:	25 min
Post Time:	15 min
<u>Solvents:</u>	
A	acetonitrile
B	water + 0.1% Acetic Acid

Parameters	Segment			
	1	2	3	4
	-	AAAAA AAAAAA-ring-d3	BBBBB CCCCC	-
<u>Time of Opening Window</u>	0	4.0	8.75	18.1
<u>Time of Close Window</u>	4.0	6.85	13.95	25
<u>Mode</u>				
Mass Range Mode	Std/ Normal	Std/ Normal	Std/ Normal	Std/ Normal
Ion Polarity	Positive	Positive	Positive	Positive
Ion Source Type	ESI	ESI	ESI	ESI
Alternating Ion Polarity	Off	Off	Off	Off
Divert Valve	Waste	Source	Source	Waste
<u>Tune Source</u>				
Trap Drive	-	52.3	33.4	-
Octopole RF Amplitude, Vpp	-	50.0	108	-
Lens 2, Volt	-	80.8	-60.0	-
Capillary exit, Volt	-	99.2	108.0	-
Skimmer, Volt	-	26.1	40.0	-
Lens 1, Volt	-	- 6.4	-5.0	-
Oct 1 DC, Volt	-	5.94	12.00	-
Oct 2 DC, Volt	-	1.02	1.70	-
Dry Temp (Set), °C	-	365	365	-
Nebulizer (Set), psi	-	40.0	40.0	-
Dry gas (Set), L/min	-	9.0	9.0	-
HV Capillary, V	-	4107	4500	-
HV End Plate Offset, V	-	-500	-500	-
<u>Trap</u>				
Rolling ,Averages, cts	-	2	2	-
Scan Begin, m/z	-	130	50	-
Scan End, m/z	-	210	230	-
Averages, Spectra	-	10	5	-
Max. Accu Time, µs	-	100.00	600.0	-
ICC Target	-	25000	50000	-

# Application Example

The MS Esquire 3000 Plus (Bruker Daltonics) equipped with Electrospray Source was ran in Positive Mode (ES+) with Manual MS in the Segment 2 and with Multiply Reaction Monitoring (MRM) in the Segment 3



## MS2 Parameters in Segments of Injection

Compound	Chromatogram	RT	MS Mode	Amplitude
BBBBB	EIC 181+ MS2 (227)	11.8	MRM	0.82
AAAAA	EIC 180.9; 151.9 +MS2 (200)	6.2	Manual	0.9
CCCCC	EIC 166.1; 195 +MS2 (212)	12.0	MRM	0.9
CCCCC-ring-d3	EIC 182.9; 154 +MS2 (200)	6.1	Manual	0.9