Analysis of Pesticides in Animal Fluids by LC-MS-MS

Instrumentation: Agilent HPLC system **with** AB Sciex LC/MS/MS System
Column: Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm
Elution Type: Gradient
Needle wash: 1:1:1:1 ACN/MeOH/IPA/H2O w/0.2% FA.
Mobile Phase A: 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H2O
Mobile Phase B: 5 mM ammonium acetate, pH 5.0 in ACN
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	80	20
2	0.5	80	20
3	8.0	0	100
4	10.0	0	100
5	13.0	STOP	STOP

Post run: 4 min
Total cycle time: 17 min
Flow Rate: 0.3 mL/min
Col. Temp: 30 °C
Inj. Vol.: 10 µL
Detection: Tandem Mass Spec (MS-MS) **@ amu (550 °C)** (Ctrl + Click to follow link)
Detector Info: AB Sciex API 4000 (Ctrl + Click to follow link)
MS Conditions
Source: Positive ESI
Nebulizer: 40 psig
Gas flow: 10 L/min
Gas temp: 350 °C
Vcap: 4000 V
MRM: Instrument Acquisition Data Used for the Analysis of 13 Pesticides:

Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)
Acephate	1) 184.0 > 94.9 2) 184.0 > 111.0	60	3 15	2.55
Methamidophos	1) 142.0 > 94.0 2) 142.0 > 124.9	60	8 8	2.54
Pymetrozine	1) 218.1 > 105.0 2) 218.1 > 78.0	115	20 50	2.97
Carbendazim	1) 192.1 > 160.0 2) 192.1 > 105.0	95	18 40	5.07
Imidacloprid	1) 256.1 > 209.1 2) 256.1 > 175.0	60	12 18	5.53
Thiabendazole	1) 202.1 > 175.0 2) 202.1 > 131.0	110	27 38	5.65
Propoxur	1) 210.1 > 111.0 2) 210.1 > 92.9	50	12 15	6.89
Carbaryl	1) 202.0 > 145.0 2) 202.0 > 115.0	50	3 40	7.30
Ethoprophos	1) 243.1 > 130.9 2) 243.1 > 172.9	80	15 15	8.50
Imazalil	1) 297.1 > 158.9 2) 297.1 > 200.9	80	22 15	8.52
Penconazole	1) 284.1 > 158.9 2) 284.1 > 172.9	80	32 32	8.95
Cyprodinil	1) 226.1 > 93.0 2) 226.1 > 108.0	120	35 35	9.23
Kresoxim methyl	1) 314.0 > 222.1 2) 314.0 > 235.0	70	10 10	9.44
TPP (IS)	1) 327.1 > 77.0 2) 327.1 > 151.9	70	45 45	9.49

1) Quantifier transition channel
2) Qualifier transition channel

Sample Preparation:

Samples were extracted and cleaned using dispersive SPE. The tubes were capped tightly and vortexed for 1 min. The tubes were centrifuged. A 200 µL aliquot of extract was transferred into an autosampler vial. An aliquot of 10 µL 1% FA in ACN was added immediately. Then 800 µL of water or appropriate standard solutions (prepared in water) were added. The samples were capped and vortexed thoroughly for LC/MS/MS analysis.

Linearity and limit of quantification (LOQ):

The linear calibration range for all of the pesticides tested was 5–250 ng/g. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 100, 200, and 250 ng/g. Triphenyl phosphate (TPP) was used as an internal standard at 100 ng/g. A 5 ng/g quantification limit LOQ (5 ppb) was established for all of the pesticides.

References:

http://www.interchim.fr/cat/QuEChERs_Spinach-5990-4395EN_interchim.pdf
P. Payá, M. Anastassiades; "Analysis of Pesticide Residues Using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) Pesticide Multiresidue Method in Combination with Gas and Liquid Chromatography and Tandem Mass Spectrometric Detection," Anal Bioanal Chem., 2007, 389, 1697-1714.
L. Zhao, J. Stevens, "Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection. Agilent Technologies publication 5990-4248EN.