Application Brief

Food Testing

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Analysis of Flumequine and Oxolinic Acid in Porcine Tissues Using SPE Coupled with HPLC and Fluorescence Detection

Authors

Meicheng Su, Man Jia, and Gang Chen
Institute of Quality Standard and Testing Technology for Agro-Products of CAAS, Beijing, China

Dan Li
China Institute of Veterinary Drugs Control, Beijing, China

Cuiling Wu, Yao Xiao, Congcong Zhang, and Xia Yang
Agilent Technologies, Inc.

Abstract

This study developed and validated a method for quantitative analysis of flumequine and oxolinic acid in pork, porcine liver, and porcine fat. The method uses Agilent Bond Elut C18 SPE coupled with HPLC/FLD analysis. The method provides a reliable solution with good recoveries and acceptable producibility. The method works for residue analysis of flumequine and oxolinic acid in variety of porcine tissues.

Experimental

Target analytes

Two target analytes in this application include flumequine and oxolinic acid.

Instrument method

The samples were run on an Agilent 1260 Infinity II LC system coupled to an Agilent Fluorescence detector (FLD) system. Agilent ChemStation software was used for data acquisition and analysis.

HPLC conditions

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 250 × 4.6 mm, 5 µm (p/n 959990-902)
Flow Rate	0.8 mL/min
Column Temperature	30 °C
Injection Volume	20 µL
Mobile Phase	0.02 mol/L Phosphate/ACN/THF (68/16/16)
Detection Wavelength	Ex = 325 nm, Em = 369 nm

Sample extraction

Figure 1 shows the procedure.

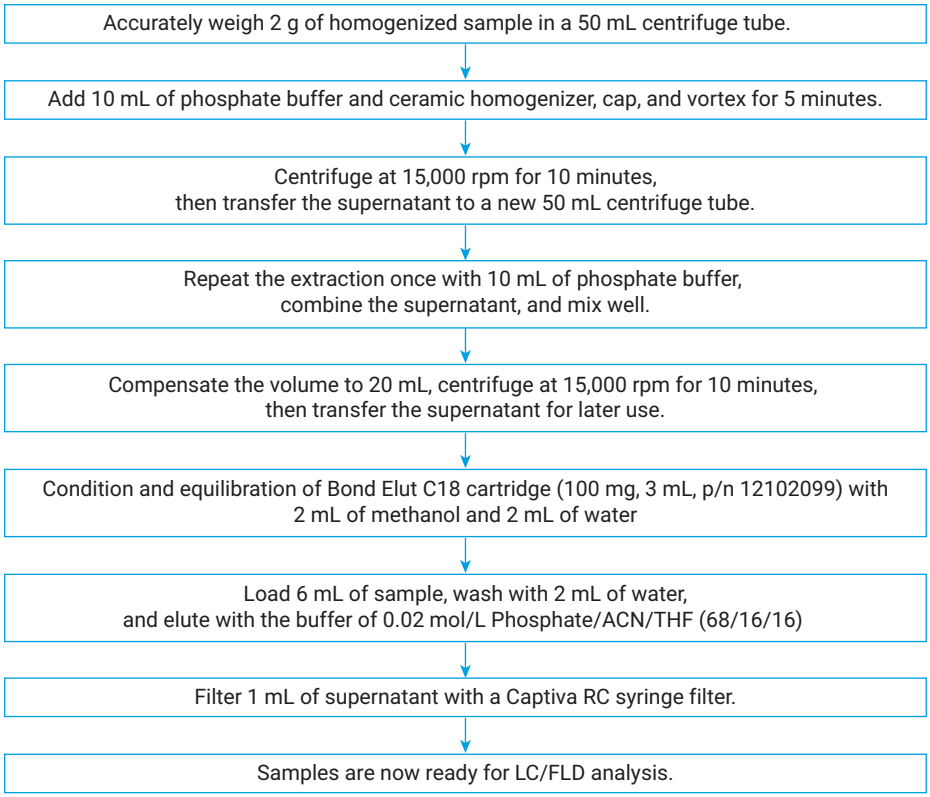

Figure 1. Sample preparation workflow chart.

Results and discussion

Table 1. Method recovery and RSDs for pork.

Analytes	Spiking Level (ng/g)	Batch	Recovery (%)			Intra RSD	Inter RSD	Average Recovery (%)
Flumequine	20	1	76.27	79.25	81.28	3.19	6.27	78.93
		2	78.70	79.58	78.04	0.98		78.77
		3	86.99	91.59	84.61	4.04		87.73
	100	1	94.39	89.27	85.17	5.15	5.08	89.61
		2	92.13	93.65	87.88	3.28		91.22
		3	95.90	99.68	99.89	2.28		98.49
	300	1	98.85	98.09	96.66	1.14	1.82	97.87
		2	95.57	98.45	98.07	1.61		97.36
		3	90.21	99.72	93.91	5.07		94.61
Oxolinic Acid	20	1	79.09	80.76	84.31	3.28	7.94	81.39
		2	85.22	77.79	80.34	4.65		81.12
		3	89.44	93.74	95.71	3.45		92.96
	100	1	86.59	86.76	87.58	0.61	8.51	86.98
		2	87.20	85.26	85.88	1.15		86.11
		3	99.76	100.13	99.94	0.19		99.94
	300	1	100.00	99.55	92.00	4.62	1.08	97.18
		2	99.91	97.84	98.82	1.05		98.86
		3	99.51	99.57	98.34	0.70		99.14

Table 2. Method recovery and RSDs for porcine liver.

Analytes	Spiking Level (ng/g)	Batch	Recovery (%)			Intra RSD	Inter RSD	Average Recovery (%)
Flumequine	20	1	91.49	98.59	93.68	3.88	2.67	94.59
		2	99.99	99.92	98.82	0.66		99.58
		3	97.90	99.84	97.32	1.36		98.35
	100	1	77.98	83.65	71.20	8.75	9.2	77.61
		2	72.46	72.66	70.36	1.81		71.83
		3	85.27	83.09	90.20	4.04		86.19
	300	1	91.09	83.90	92.28	4.91	11.17	89.09
		2	92.19	98.57	97.94	3.59		96.23
		3	79.93	74.32	76.53	3.69		76.93
Oxolinic Acid	20	1	79.50	80.71	77.10	2.38	9.11	79.10
		2	79.57	84.21	79.96	3.22		81.25
		3	98.35	95.59	86.22	7.37		93.39
	100	1	75.62	81.38	73.42	5.60	12.34	76.81
		2	76.29	75.88	75.44	0.56		75.87
		3	95.59	87.71	98.38	5.62		93.89
	300	1	83.01	86.59	81.93	2.98	9.68	83.84
		2	80.34	82.11	76.59	3.68		79.68
		3	96.14	92.81	98.47	2.89		95.81

Table 3. Method recovery and RSDs for porcine fat.

Analytes	Spiking Level (ng/g)	Batch	Recovery (%)			Intra RSD	Inter RSD	Average Recovery (%)
Flumequine	20	1	80.39	80.31	83.78	2.43	6.63	81.49
		2	92.10	99.82	86.09	7.43		92.67
		3	82.82	86.13	85.76	2.14		84.90
	100	1	78.65	79.61	83.99	3.53	1.51	80.75
		2	80.18	77.92	81.24	2.13		79.78
		3	81.55	81.14	83.92	1.83		82.20
	300	1	81.40	79.95	77.49	2.48	2.3	79.61
		2	80.67	78.98	76.24	2.84		78.63
		3	82.69	83.04	80.86	1.42		82.20
Oxolinic Acid	20	1	77.57	82.61	72.51	6.51	4.37	77.56
		2	90.30	75.42	76.80	10.17		80.84
		3	86.45	80.87	86.61	3.86		84.64
	100	1	78.44	83.49	81.24	3.12	2.49	81.06
		2	79.60	81.16	81.06	1.08		80.61
		3	87.11	80.80	85.14	3.83		84.35
	300	1	78.55	80.58	80.90	1.59	1.75	80.01
		2	80.03	84.91	82.03	2.98		82.32
		3	82.94	81.47	83.42	1.23		82.61

Conclusion

This method uses Agilent Bond Elut C18 coupled with fluorescence detection for analysis of flumequine and oxolinic acid in variety of porcine tissues. The method has excellent recoveries and linearity between 5 to 500 ng/mL. High efficiency cleanup by Bond Elut C18 shows no interference on the target peaks. The average recoveries are in the range of 71.8 to 99.5% and 75.8 to 99.9% respectively with acceptable reproducibility (RSD<15%). The limit of quantitation for both targets is 20 ng/g, and the limit of detection is 5 ng/g.

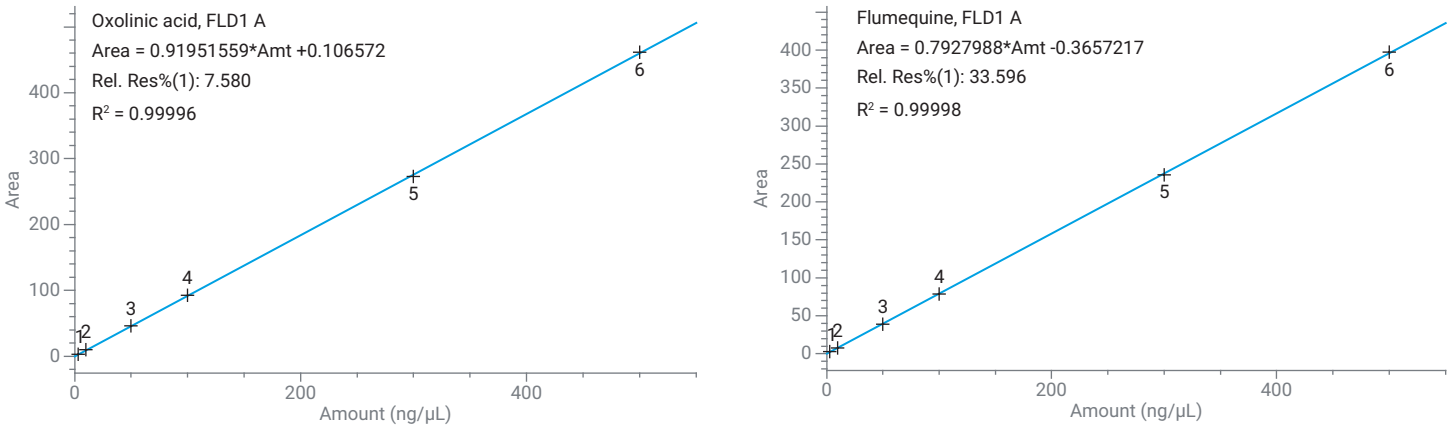

Figure 2. Calibration curves for flumequine and oxolinic acid.

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