Application Brief

Food Testing and Agriculture

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Analysis of Acetyl Progesterones in Pork, Eggs, and Milk Using the Agilent Captiva EMR—Lipid by LC/MS/MS

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Abstract

This study developed and validated a method for the quantitative analysis of four man-made acetyl progesterones in pork, eggs, and milk. The method used the Agilent QuEChERS extraction kit followed with Agilent Captiva EMR—Lipid cleanup by LC/MS/MS analysis. The method provided a reliable solution with acceptable recoveries and reproducibility for the emergent application testing.

Experimental

Target analytes

The four target analytes in this application included flurogestone acetate (FGA), megestrol acetate (MA), melengestrol acetate (MGA), and chlormadinone acetate (CMA).

Instrument method

The samples were run on an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II binary pump (G7120A), an Agilent 1290 Infinity II high performance autosampler (G7167B), and an Agilent 1290 Infinity II thermostatted column compartment (G7116B). The UHPLC system was coupled to an Agilent G6470 triple quadrupole LC/MS system equipped with a Jet Stream electrospray ion source. Agilent MassHunter workstation software was used for data acquisition and analysis.

HPLC conditions

Parameters	Value
Column	Agilent InfinityLab Poroshell 120 SB-C18, 3.0 × 100 mm, 2.7 µm (p/n 685975-302)
Flow Rate	0.4 mL/min
Column Temperature	40 °C
Injection Volume	10 µL
Mobile Phase	A) Water with 2 mM ammonium acetate and 0.1% formic acid B) ACN with 0.1% formic acid
Gradient	Time (min) / %B / Flow rate (mL/min) 0 / 60 / 0.4 4.0 / 90 / 0.4 6.0 / 100 / 0.4
Post Time	2.0 minutes

MS conditions

Parameters	Value
Gas Temperature	250 °C
Gas Flow	7 L/min
Nebulizer	35 psi
Sheath Gas Heater	325 °C
Sheath Gas Flow	11 L/min
Capillary	3,500 V (POS) 0 V (NEG)
Data Acquisition	MRM as shown in Table 1.

Table 1. Target analytes MRM conditions.

Analyte	Polarity	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	CE (V)
CMA	POS	405.5	345	130	10
			309.1	130	15
			301	130	20
MGA	POS	397.4	337.2	138	13
			279.1	138	20
MA	POS	385.5	267.1	138	20
			224.1	138	30
FGA	POS	407.4	285.1	138	25
			267	138	25
			225.1	138	30

Sample extraction

The following products were used for sample preparation.

- Agilent Captiva EMR—Lipid, 3 mL cartridge, 300 mg (p/n 5190-1003)
- Agilent Vac Elut 20 Manifold with tall glass basin and collection rack for 16 × 150 mm test tubes (p/n 12234104)
- Agilent Bond Elut QuEChERS extraction kit, veterinary drugs, nonbuffered (p/n 5982-0032)
- Ceramic homogenizers for 50 mL tubes (p/n 5982-9313)
- Agilent Captiva Nylon syringe filter, 0.2 µm, 13 mm (p/n 5190-5133)

Figure 1 shows the procedure.

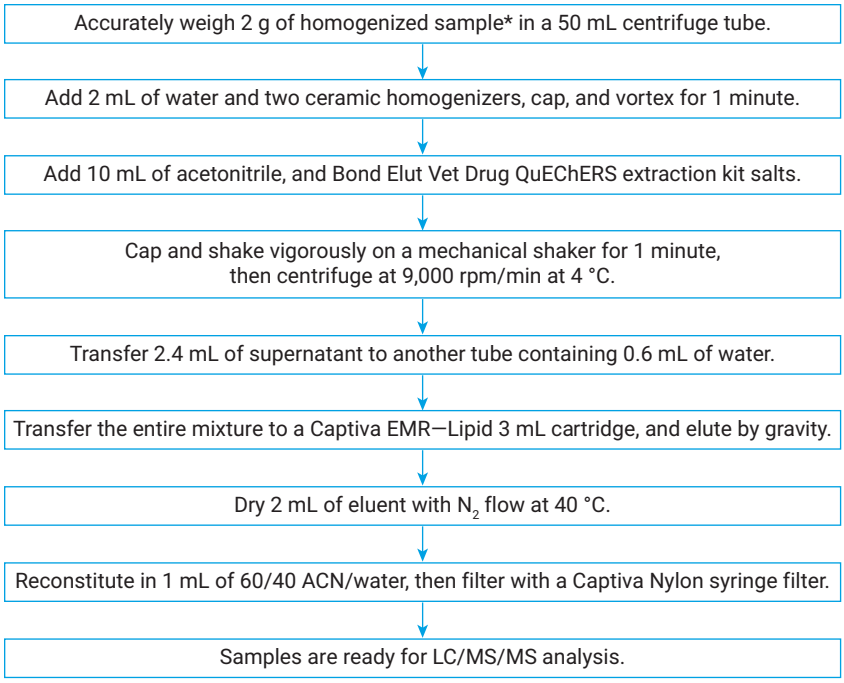

Figure 1. Sample preparation workflow chart. *For pork and egg matrices, 2 g of homogenized sample and 2 mL of water were used for extraction. For milk, 10 mL of milk was used for extraction. No additional water was added to the milk sample.

Results and discussion

Table 2. Method recovery and RSDs.

Analytes	Spiking Level (ng/g)	Pork		Porcine Liver		Porcine Kidney		Egg		Milk	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
FGA	1	114.2	4.7	90.2	2.5	93.9	5.9	93.7	2.7	87.1	0.6
	2	103.6	4.7	84.3	4.8	96.0	4.9	101.7	4.3	92.6	7.8
	10	99.7	8.2	95.1	3.5	112.8	1.2	107.4	9.3	92.1	2.7
CMA	1	104.0	6.3	97.1	9.9	88.6	4.3	94.4	3.3	98.7	2.8
	2	108.7	5.6	100.7	4.8	97.8	5.4	94.4	4.8	99.6	4.1
	10	103.0	2.3	98.5	2.3	102.0	9.8	99.8	6.3	92.9	2.4
MA	1	95.7	3.3	80.1	2.1	91.1	9.6	87.1	2.8	103.1	1.1
	2	112.2	3.5	91.6	3.6	105.7	9.4	98.8	8.2	102.7	1.3
	10	111.6	5.3	101.3	5.5	102.4	9.8	98.7	5.0	80.8	0.4
MGA	1	93.3	7.3	96.7	5.8	96.0	4.0	90.1	7.6	88.1	8.5
	2	109.0	4.5	97.7	9.3	104.2	3.3	99.1	6.6	85.2	4.3
	10	110.6	3.3	108.3	8.8	103.7	8.2	99.1	5.2	92.6	2.2

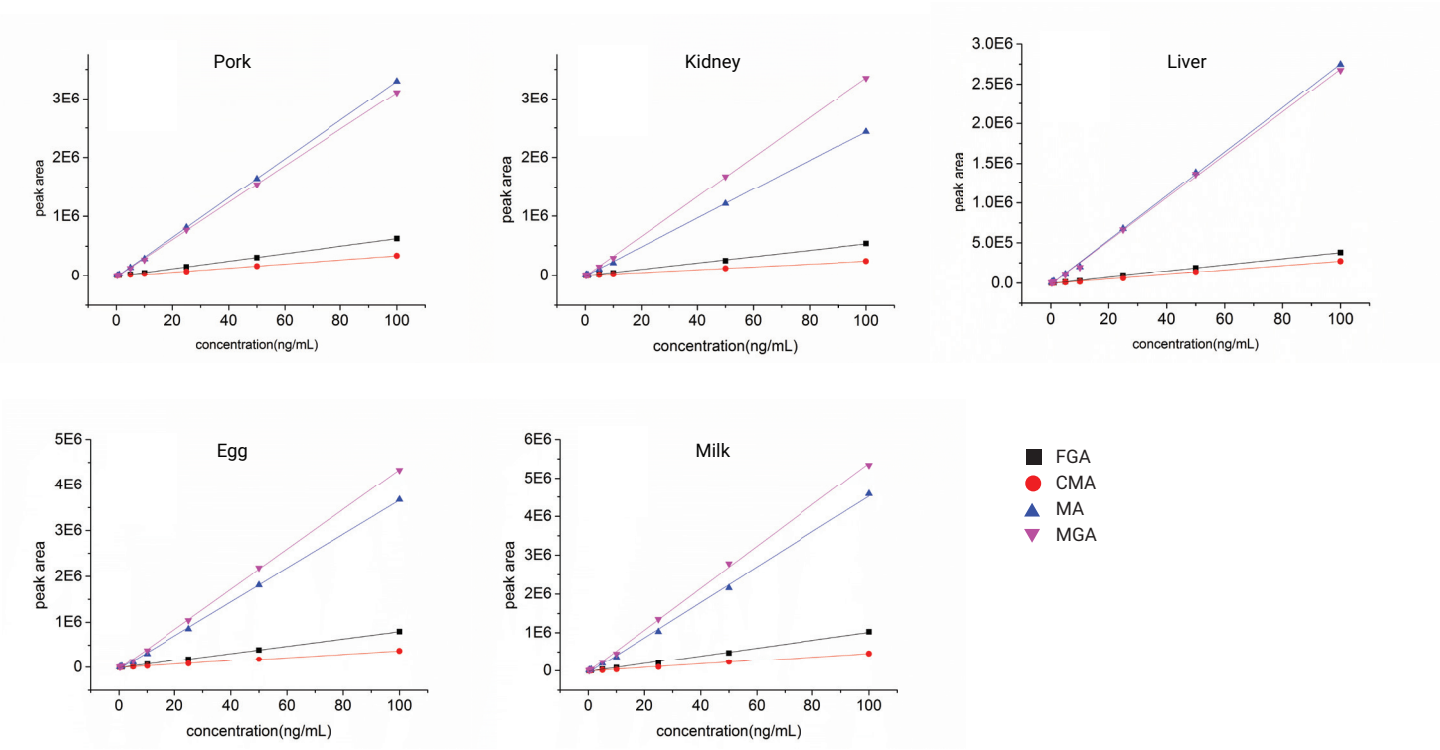

Figure 2. Calibration curves in sample matrices.

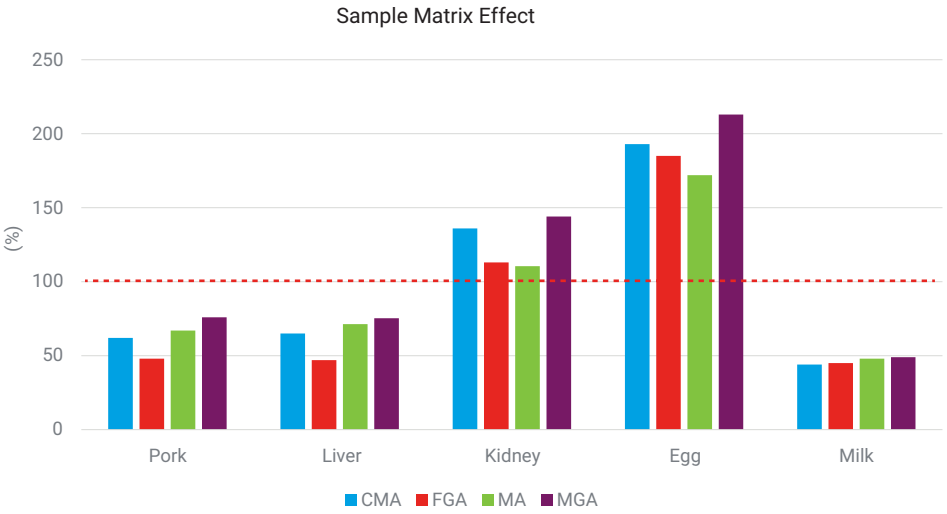

Figure 3. Matrix effect in sample matrices.

Conclusion

A new method using an Agilent QuEChERS extraction kit followed by Agilent Captiva EMR—Lipid cleanup is established for the fast and reliable analysis of four acetyl progesterone compounds in meat, eggs, and milk using LC/MS/MS. The method provided excellent analyte recovery and reproducibility, efficient matrix removal, and a simplified workflow.

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