Application Brief

Food Testing

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Analysis of Chloramphenicol and Metabolites in Royal Jelly and Honey Using SPE Coupled with LC/MS/MS

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Abstract

This study developed and validated a method for quantitative analysis of chloramphenicol, thiamphenicol, florfenicol and its metabolite florfenicol amine in royal jelly and honey. Sample preparation was done on an Agilent Bond Elut Plexa PCX followed by analysis on an Agilent 6470 LC/MS/MS. The method delivered a reliable solution with good recoveries and reproducibility for chloramphenicol and chloramphenicol-related compounds.

Experimental

Target analytes

Four target analytes in this application included chloramphenicol, thiamphenicol, florfenicol, and its metabolite florfenicol amine.

Instrument method

The samples were run on an Agilent 1260 Infinity II LC system coupled to an Agilent 6470 triple quadrupole LC/MS system equipped with an Agilent Jet Stream Electrospray ion source. Agilent MassHunter workstation software was used for data acquisition and analysis.

HPLC conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 100 × 3.00 mm, 2.7 µm (p/n 695975-302)
Flow Rate	0.4 mL/min
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phase	A) Water + 0.1% formic acid B) ACN
Gradient	Solvent B increased from 10% to 100% in 7 minutes

MS conditions

Parameter	Value
Gas Temperature	250 °C
Flow Rate	0.4 mL/min
Gas Flow	7 L/min
Nebulizer	40 psi
Sheath Gas Flow	12 L/min

Table 1. Target analytes MRM conditions.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	CE (V)	Ionization Mode
Chloramphenicol	321.0	257.0 152.0	100	10 15	Neg
Thiamphenicol	356.0	335.9 184.9	100	8 15	Neg
Florfenicol	353.9	289.9 184.9	120	10 15	Neg
Florfenicol Amine	248.1	230.1 130.1	70	4 20	Pos

Sample extraction

Accurately weigh 2 g of royal jelly sample in a 50 mL centrifuge tube. Add 0.25 g of trypsin and 2 mL of water, and shake for 2 hours at 40 °C in the dark. Add 8 mL of acetonitrile with 2% ammonium hydroxide for extraction, vortex for 1 minute, then centrifuge for 10 minutes at 4 °C. The supernatant is dried with N_2 at 40 °C until only water is left. Compensate the volume to 5 mL with pure water for Bond Elut Plexa PCX, 500 mg, 6 mL (p/n 12258506) cleanup.

For honey sample, 5 g of honey mixed with 20 mL of pure water is vortexed and centrifuged for 10 minutes at 4 °C. The supernatant is then ready for Bond Elut Plexa PCX, 200 mg, 6 mL (p/n 12108206) cleanup. The SPE procedure is shown in Figure 1.

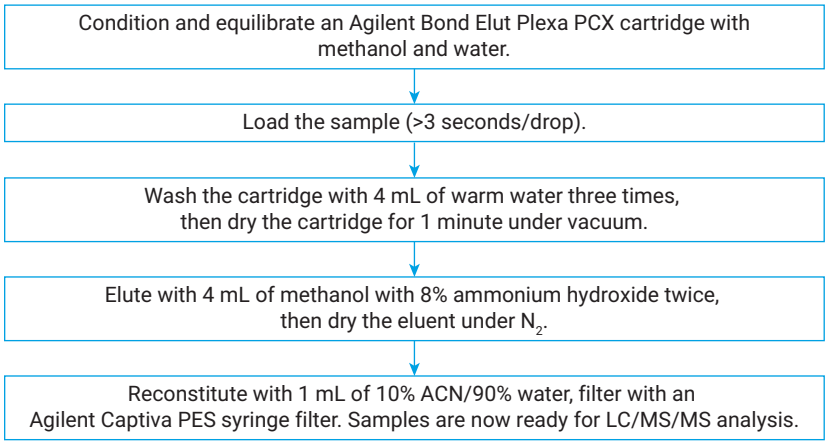

Figure 1. SPE cleanup workflow chart.

Preliminary results and discussion

Table 2. Method recovery and RSDs.

Matrix	Analyte	1 ng/g Spiking (2 ng/g for Florfenicol Amine)		2 ng/g Spiking (4 ng/g for Florfenicol Amine)		5 ng/g Spiking (10 ng/g for Florfenicol Amine)	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Royal Jelly	Chloramphenicol	100.5	1.0	115.0	4.3	116.9	0.6
	Thiamphenicol	85.0	3.0	72.0	5.7	78.7	3.7
	Florfenicol	93.7	4.9	96.7	6.4	105.9	6.9
	Florfenicol Amine	87.7	1.8	83.0	4.8	80.2	4.1
Honey	Chloramphenicol	87.5	3.5	97.8	2.1	106.4	1.8
	Thiamphenicol	99.4	1.3	96.2	1.4	105.6	0.4
	Florfenicol	96.4	3.1	93.6	5.3	96.6	3.7
	Florfenicol Amine	79.0	0.6	86.3	1.1	92.4	2.8

Conclusion

A method has been developed with Bond Elut Plexa PCX SPE cartridges; a polymeric cation exchanger mixed with hydrophobic interaction product. This sample preparation, coupled with LC/MS/MS delivers excellent recoveries and reproducibility for the analysis of chloramphenicol, thiamphenicol, florfenicol, and its metabolite florfenicol amine in royal jelly and honey. The method delivered high sensitivity for the targets in both positive and negative modes for mass spectrometry analysis.

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