Using LC/MS/MS 6410 for Analysis of Chloramphenicol, Thiamphenicol, and Florfenicol in Fish Samples

Application Brief

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Chloramphenicol is a banned compound in the EU. It is a zero tolerance compound, and some methods have already been developed for this analysis. The LC/MS/MS is often used for its greater sensitivity and higher selectivity.

In many cases, not only chloramphenicol but also thiamphenicol and florfenicol are also found. This method uses a simple method for detecting all three compounds within less than 6 minutes. Furthermore, the results gave the good results, showing the performance at the negative mode.

Experimental

LC Conditions

Column	Agilent ZORBAX Eclipse Plus, 2.1 mm × 50 mm, 1.8 µm
Mobile phase	A: Water B: Methanol
Flow rate	0.4 mL/min
Gradient	0–2 min/B 10% to 90%; 2–3 min/B 90%; 3.01/B 10%
Stop time	6 min
Column compartment temperature	45 °C
Injection	5 µL
MSD Condition	
Instrument	Agilent 6410A triple quadrupole LC/MS system
Source	ESI –

Highlights

- Using a simple RRLC method can separate the compounds well within 6 minutes
- Quite high sensitivity in negative mode
- The ISTD method can remove the matrix effect and minimumize the sample preparation interference

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Sample Preparation

All SPE cartridges are conditioned with 2 mL of water before use.

1. **Honey**, 1 g sample is diluted to 5 mL with water and 25 µL 10 ppb IS is added. The solution is loaded onto the SPE cartridge and allowed to stand for 5 min. Elution is performed with 10 mL ethyl acetate. The eluate is collected and the solvent is evaporated under a nitrogen stream at 40 °C. The residue is redissolved in 1 mL methanol and put in an ultrasonic bath for 1 min. The solution is filtered, using a syringe filter, before injection. No additional cleanup of the sample solution is performed.
2. **Shrimp**, 1 g of shrimp is defrosted and mixed in a blender. To the 1 g of the mixed shrimp, 3 mL of water and 25 µL 10 ppb IS are added. The portion is centrifuged for 5 min (8,000 rpm). The supernatant is loaded on the cartridge and allowed to stand for 5 min. Elution is performed with 5 mL ethyl acetate. The eluate is collected and the solvent evaporated under a nitrogen stream at 40 °C. The residue is redissolved in 1 mL methanol and put in an ultrasonic bath for 1 min; the solution is filtered before injection.
3. **Chicken**, 1 g of chicken is defrosted and mixed in a blender. To the 1 g of the mixed chicken, 3 mL of water and 25 µL 10 ppb IS are added. The portion is centrifuged for 5 min (8,000 rpm). The supernatant is loaded on the cartridge and allowed to stand for 5 min. Elution is performed with 5 mL ethyl acetate. The eluate is collected and the solvent evaporated under a nitrogen stream at 40 °C. The residue is redissolved in 1 mL methanol and put in an ultrasonic bath for 1 min; the solution is filtered before injection.

MRM Setting

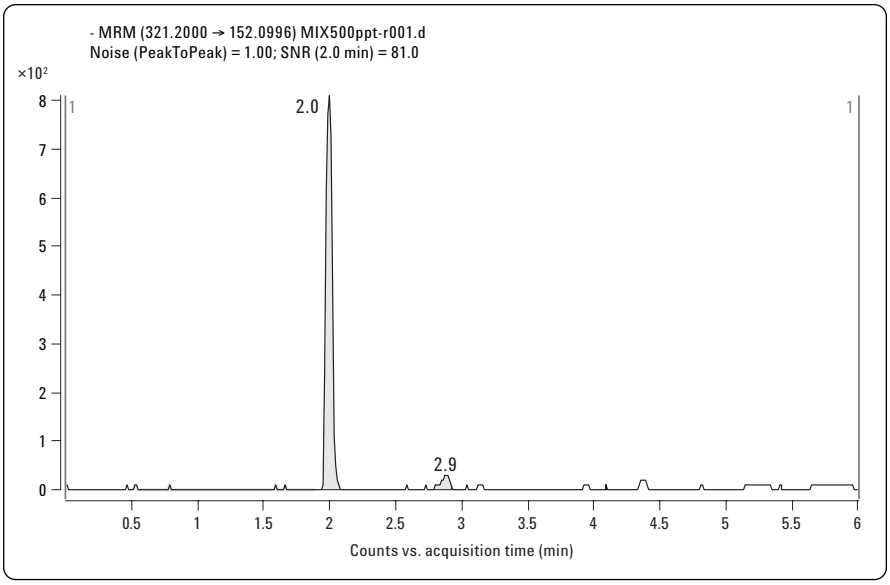
Name	Precursor ion	Product ion	Frag (V)	CE (V)	Dwell (ms)
TAP	354.1	185.1*	120	20	60
	354.1	289.9	120	10	60
FF	356	185.1*	120	20	60
	356	335.8	120	5	60
CAP	321.2	152.1*	120	10	60
	321.2	257.1	120	5	60
D5-CAP (ISTD)	326.2	157.2	130	15	60

Results

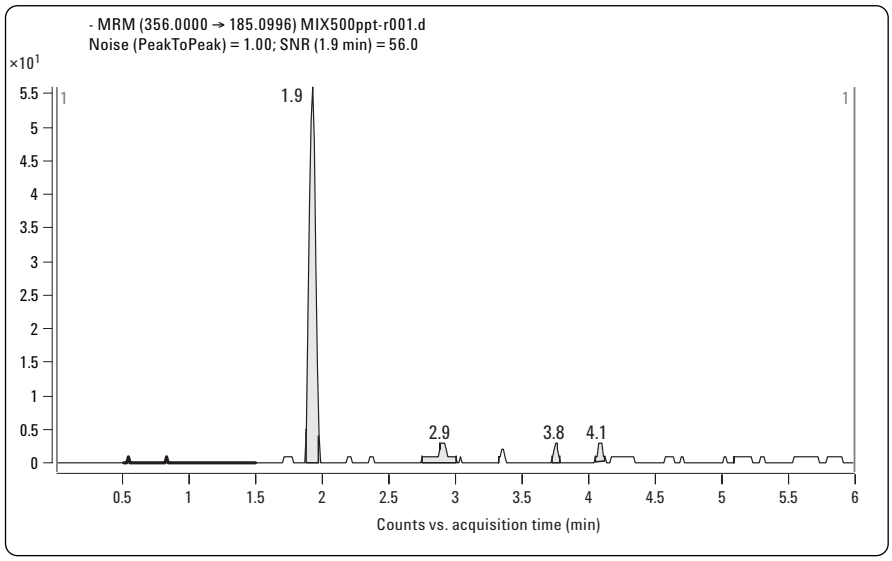
Name	**Linearity** (0.5–20 ppb)
TAP	0.994
FF	0.992
CAP	0.994

Sensitivity

1. CAP: 0.5 ppb S/N = 81



2. FF: 0.5 ppb S/N = 56



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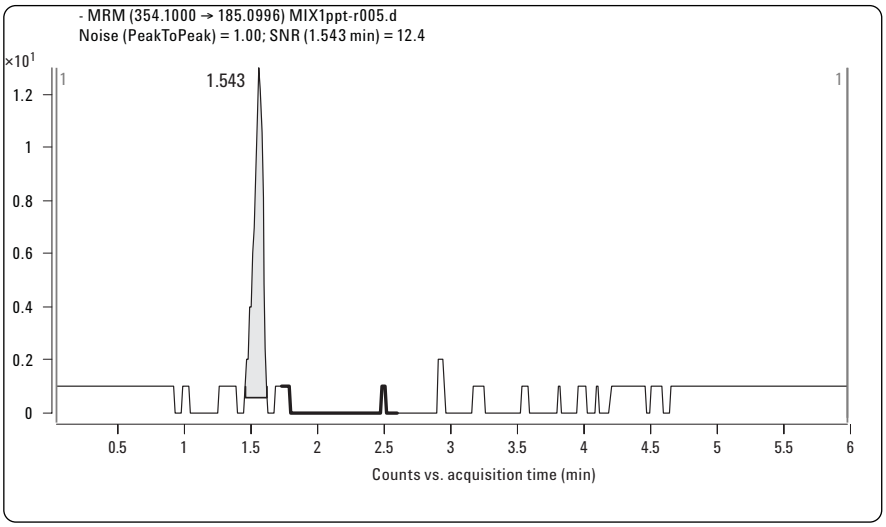
3. TAP 1 ppb, S/N = 12



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