

AMPHENICOLS ANALYSIS IN MEAT BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Introduction

Chloramphenicol (CAP), florfenicol (FLOR) and thiamphenicol (TAP) are broad spectrum antibiotics from the family of amphenicols. CAP was first extracted from bacterium *Streptomyces venezuelae* in the year 1947 and was approved by the US Food and Drug Administration in 1949 as the first broad spectrum antibiotic. It was widely used in the treatment of infectious conditions ranging from bronchitis to bacterial meningitis. However, in the 1960s, toxicity of CAP was highlighted in terms of effects on bone marrow. Since 1990s, use of CAP was banned and a zero tolerance policy for food residues was established. FLOR and TAP are structurally related to CAP but do not present such toxicity. FLOR is e.g. proposed for treatment of bovine respiratory disease and TAP for the treatment and control of respiratory and intestinal infections in cattle and poultry. Maximum residue limits are available in European Union and Switzerland for these two substances (e.g. 200 and 50 µg/kg in muscle for FLOR and TAP, respectively). Considering these information, analytical methodologies have to be developed to detect and quantify the presence in food and in particular in meat of these compounds.

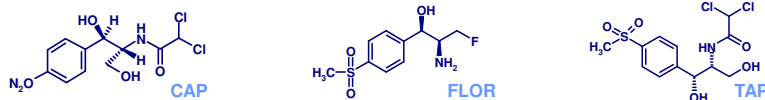


Figure 1: CAP, FLOR and TAP molecules

Results

Sample preparation

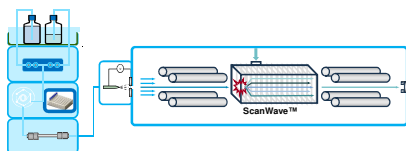
1. Solid-Liquid Extraction

- 5 grammes of meat
- Addition of 10 ml of ethyl acetate
- Shaking
- Centrifugation
- Evaporation until dryness
- Reconstitution in 500 µl of MeOH
- Transfer into a 15 ml polypropylene (PP) tube
- Addition of 2x5 ml of NaCl 4% to the initial tube
- Transfer into the 15 ml PP tube

2. SPE purification

- Conditioning : 2 ml MeOH + 2 ml H₂O
- Loading
- Washing : 3 ml H₂O and 2 ml H₂O/MeOH (50/50)
- Drying : 5 minutes
- Elution : 2 ml MeOH
- Volume adjustment to 5 ml with H₂O

UHPLC-MS/MS



LC : Acquity UPLC (Waters®)
MS-MS : Xevo TQ MS (Waters®)
Column UPLC : Acquity BEH C18, 1.7 µm, 2.1 x 100 mm (Waters®)
Mobile phase : A : H₂O/MeOH (98/2), B : MeOH
Gradient : A/B: 0 min: 95/5 v:v; 0.50 min: 95/5; 1.6 min: 10/90; 1.9 min: 10/90; 2.05 min: 95/5
Flow : 450 µl/min
Injection : 10 µl
Column temperature : 40°C
Sample temperature : 10°C
Interface : ESI Neg

Table 1: MRM transitions for CAP, CAP-D5, FLOR and TAP

MRM transitions (ESI-)	Parent ion [m/z]	Product ion [m/z]	Cone voltage [V]	Collision voltage [V]
CAP	321	152	28	16
	321	257	28	12
CAP-D5	321	194	28	12
	326	157	25	15
FLOR	356	336	22	10
	356	185	22	18
	356	119	22	26
TAP	354	185	30	20
	354	290	30	12
	354	240	30	18

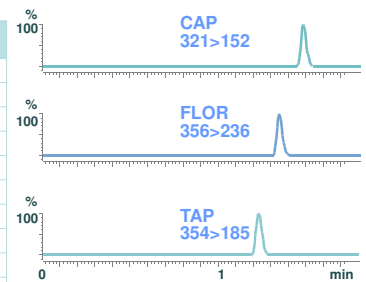


Figure 2: EIC of CAP, FLOR and TAP (QC sample at 1, 200 and 50 µg/kg, respectively)

Quantitative aspects

Matrix effect evaluation

1 µg/kg CAP
50 µg/kg TAP
200 µg/kg FLOR

- Matrix effect was evaluated by comparison between a standard solution and a spiked meat sample at the same concentrations

➔ Strong matrix effects: 40 to 80%!

➔ Need for a correction!

Matrix effect correction

CAP Correction with an isotopically labelled standard

FLOR TAP No isotopically labelled standard available

- Screening analysis without isotopically labelled standard for a quantitative approximation
- Standard addition procedure for suspect samples

Validation

3 days 3 concentration levels 4 repetitions

Table 2: Validation results

	Conc. levels [ng/ml]	Trueness [%]	Repeatability (RSD; [%])	IP (RSD; [%])	CI (t=1.83; [%])
CAP	1	111	13	16	30
	5	108	15	17	30
	10	110	5	10	20
FLOR	150	126	6	8	19
	300	127	5	5	12 <input checked="" type="checkbox"/>
	450	116	4	4	9
TAP	25	71	16	24	30
	50	76	11	14	20
	75	66	9	8	10

IP : intermediate precision; CI : confidence interval