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Screening and confirmation of chloramphenicol in shrimp tissue using ELISA in combination with GC–MS^2 and LC–MS^2

Sandra Impens [a,*], Wim Reybroeck [b], Jan Vercammen [c], Dirk Courtheyn [c], Sigrid Ooghe [b], Katia De Wasch [a], Walter Smedts [d], Hubert De Brabander [a]

[a] *Laboratory of Chemical Analysis, Department of Veterinary Food Inspection, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke B-9820, Belgium*

[b] *DVK-CLO, Department Animal Product Quality, Agricultural Research Centre, Ghent, Brusselsesteenweg 370, Melle B-9090, Belgium*

[c] *State Laboratory (ROLG), Braemkasteelstraat 59, Gentbrugge B-9050, Belgium*

[d] *Federal Agency for Food Safety, Institute for Veterinary Inspection, Wetstraat 56, Brussel B-1000, Belgium*

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Abstract

According to the European Commission Decision 2001/699/EC and 2001/705/EC certain fishery and aquaculture products, imported from China, Vietnam or Indonesia and intended for human consumption, must be subjected to a test in order to ensure the absence of chloramphenicol residues. For that reason an analytical method has been developed and validated based on ELISA for screening and gas chromatography–tandem mass spectrometry (GC–MS^2) or liquid chromatography–tandem mass spectrometry (LC–MS^2) for confirmation. The chloramphenicol ELISA was carried out directly on an aqueous extract of the shrimps or after an extraction with ethyl acetate. Confirmation of suspect samples was performed after extraction with ethyl acetate and defatting with *n*-hexane. The clean-up was based on solid phase extraction using C_{18} cartridges or reversed phase HPLC. After derivatisation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), the final extracts were analysed by GC–MS^2 in the negative ion chemical ionisation mode. Confirmation of chloramphenicol was also possible with LC–MS^2 after the same clean-up. Both selective techniques made it possible to detect chloramphenicol residues at the 0.1 $\mu g\,kg^{-1}$ level starting from 20 g of matrix for enzyme-linked immunosorbent assay (ELISA) with organic solvent extraction, or from 5 g of matrix for ELISA with aqueous extraction.

Keywords: Chloramphenicol; Shrimps; Enzyme-linked immunosorbent assay; Gas chromatography–tandem mass spectrometry; Liquid chromatography–tandem mass spectrometry

1. Introduction

Chloramphenicol (CAP) is an antimicrobial substance produced by the growth of certain strains of the soil bacterium *Streptomyces venezuelae*, but now mainly prepared synthetically. It is a broad spectrum antibiotic which is effective against both Gram-positive and Gram-negative organisms, rickettsiae, chlamydiae, and mycoplasmas. Although its use in human medicine is limited by its toxicity, it has been used particularly in typhoid and other

* Corresponding author. Fax: +32-9-264-74-92.
E-mail address: sandra.impens@rug.ac.be (S. Impens).

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salmonella infections, in the treatment of bacterial meningitis, as an alternative to other agents in various serious infections including brain abscess, epiglottitis, pneumonia, melioidosis and rickettsioses. CAP has many side effects, e.g. it may produce severe or fatal bone marrow depression and aplastic anaemia, and a syndrome of cyanosis and cardiovascular collapse known as the "grey syndrome" may occur, particularly in neonates. Peripheral and optic neuropathies, hypersensitivity reactions, and gastro-intestinal disturbances have also been reported [1]. In veterinary medicine the widespread use and misuse of antibiotics to control diseases in aquaculture species is worldwide and will probably increase as aquaculture product cultivators move towards more intensive animal husbandry, rearing techniques and stocking densities [2]. Because of the well-known risk of anaemia and carcinogenic properties of CAP, the presence of CAP in food has been illegal and unacceptable in the European Community (EC) since 1994 [3].

Therefore, and according to the European Commission Decision 2001/699/EC [4], 2001/705/EC [5], 2002/249/EC [6], 2002/250/EC [7] and 2002/251/EC [8] certain fishery and aquaculture products, imported from China, Vietnam, Indonesia, Thailand and Myanmar and intended for human consumption, must be subjected to a test in order to ensure the absence of CAP residues. Although analytical methods for determination of CAP in various matrices [9–11], and even in shrimp tissue [12–14], have already been published, a detection limit of 0.1 μg CAP kg^{-1} of matrix could not be reached by most of the mentioned analytical approaches.

In this study, an analytical method for screening and confirmation of CAP residues in shrimp tissue was developed. It was tried to incorporate different techniques such as enzyme-linked immunosorbent assay (ELISA), and gas and liquid chromatography in combination with tandem mass spectrometry (GC–MS^2 and LC–MS^2), in order to have a multi-dimensional method to meet unequivocally the EC quality criteria [15]. The ELISA was carried out to screen shrimp tissue samples, GC–MS^2 and LC–MS^2 were applied to confirm suspect samples. All three techniques allowed both qualitative and quantitative measurement of CAP in shrimp tissue.

2. Experimental

2.1. Chemicals

2.1.1. Standards

Chloramphenicol (CAP) was obtained from Sigma–Aldrich Corp. (St. Louis, MO, USA). Florfenicol (FF) was obtained from Schering-Plough Animal Health (OM, USA). Chloramphenicol-d5 (CAP-d5) (ARC Laboratories B.V., Amsterdam, The Netherlands) was used as internal reference standard; thiamphenicol (THAP) was used as GC–MS derivatisation reference standard. Stock solutions at a concentration of 200 ng analyte μl^{-1} in absolute ethanol were prepared and stored at 4 °C. Suitable working solutions were also prepared in order to achieve one standard mixture solution containing 0.02 ng μl^{-1} CAP and 1 ng μl^{-1} CAP-d5. Another standard mixture solution containing 0.06 ng μl^{-1} CAP and 1 ng μl^{-1} CAP-d5 was also prepared.

2.1.2. Reagents for the enzyme-linked immunosorbent assay (ELISA)

All reagents and solvents used were of analytical grade quality and provided by Merck (Darmstadt, Germany). The sample extraction buffer, a phosphate buffer (PBST) at pH 6 ± 0.2, was prepared from 0.96 g of $Na_2HPO_4 \cdot 2H_2O$, 0.17 g of KH_2PO_4, 9 g of NaCl and ultra pure water to a total volume of 1000 ml.

2.1.3. Reagents for extraction and clean-up prior to GC–MS^2

All reagents and solvents used were of analytical grade quality and provided by Merck. The derivatisation reagent, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), needed to obtain suitable extracts for GC–MS^n analysis, was obtained from Macherey-Nagel (Düren, Germany). Decafluorobenzophenone (1 pg μl^{-1}) (ThermoFinnigan, Austin, TX, USA) was used as GC–MS^2 reference standard to check the optimal status of the analytical instrument.

2.1.4. Reagents for extraction and clean-up prior to LC–MS^2

All reagents and solvents used were of analytical quality and provided by Merck. Water and methanol were of HPLC quality and provided by BDH (BDH Laboratory Supplies, Poole, Dorset, UK BH15 1TD).

The sample extraction buffer used was the same as for the ELISA.

2.2. Samples

Blank shrimp tissue was provided by the National Reference Laboratory (WIV-LP, Brussels, Belgium). This blank test material was used during the validation procedure of the ELISA besides a mixture of previous negative tested shrimp samples. Afterwards, for the development and validation procedure of the GC–MS^n and LC–MS^n methods, samples tested as blank by ELISA were also used.

2.3. Apparatus and materials needed for extraction and clean-up of samples

2.3.1. ELISA

The CAP enzyme immunoassay (EIA) kit (type 5091CAP1p) was provided by Euro-Diagnostica B.V. (Arnhem, The Netherlands) and contained all reagents and solvents needed to perform the ELISA. The CAP EIA utilises a specific antibody raised in rabbits against protein conjugated CAP. A test tube shaker, an orbital shaker, a centrifuge and a nitrogen evaporator were used for extraction of the sample prior to performing ELISA.

2.3.2. Materials needed for extraction and clean-up prior to GC–MS^2

A series of devices was used: a balance, a test tube shaker, a centrifuge, a rotary vacuum evaporator, a water bath, a vacuum manifold, an orbital shaker, and a nitrogen (N_2) evaporator or other types of evaporator. Recipients were selectively chosen to be suitable in each step of the procedure. Extraction and subsequent wash step were carried out with Nunc tubes. Further clean-up was performed using solid phase extraction (SPE) techniques with SPE columns (Sep-Pak® Cartridges C_{18} sorbent—6 cc/500 mg, Waters, Milford, MA, USA). Evaporation of the extracts was carried out in suitable glassware. Amber 0.7 ml autosampler vials were used for the derivatisation process to obtain GC–MS injectable extracts.

2.3.3. Materials needed for extraction and clean-up prior to LC–MS^2

The following devices were used: a balance, a centrifuge, a rotary vacuum evaporator and a test tube shaker. Recipients were chosen to be suitable in each step of the procedure. Clean-up was performed using Chem-Elut cartridges (Varian, Walnut Creek, CA, USA).

2.4. Analytical instruments and conditions

2.4.1. ELISA

A microplate reader Titertek Multiskan MCC/340 was used to measure the optical densities at 450 nm.

2.4.2. GC–MS^2

To obtain gas chromatographic and coupled mass spectrometric information, a low-resolution mass spectrometric (LR-MS) GCQ plus (ThermoFinnigan) consisting of a Finnigan gas chromatograph coupled to GCQ ion trap mass spectrometer was used. A Finnigan MAT A200S autosampler was used to inject the samples. In Table 1, an overview of the GC parameters is given. Analyses were performed using a non-polar 5% phenyl-polysilphenylene-siloxane SGE BPX-5 GC-column (30 m × 0.25 mm i.d., 0.25 μm) (SGE Inc., Austin, TX, USA).

MS^2 measurements were performed in negative chemical ionisation mode (NCI) (Table 2). Xcalibur™ software (ThermoFinnigan) version 1.2 was used to perform the interpretation of the analytical results.

2.4.3. LC–MS^2 conditions

The LC–MS system consisted of an HP 1100 series pump, autosampler, vacuum degasser and column compartment, coupled to a LCQ Deca Ion Trap Mass

Table 1
Gas chromatographic parameters for CAP analysis by GC–MS^2

Parameter	Finnigan GC
Split/splitless injector (splitless mode)	
Temperature	260 °C
Split flow	60 ml min^{-1}
Split valve closed at	−0.10 min
Split valve open at	1.00 min
GC temperature program	
Initial temperature	90 °C (hold 1 min)
Segment 1	280 °C (20 °C min^{-1})
Segment 2 (isotherm)	280 °C (hold 15 min)
GC carrier gas	Helium
Column flow	0.91 ml min^{-1}

Table 2
Mass spectrometric parameters for CAP analysis by GC–MS^2

Parameters	GCQ plus
Ion source temperature	200 °C
Transfer line temperature	275 °C
Collision gas (helium) supply pressure	3 bar
Reagent gas (negative chemical ionisation)	Methane
Reagent gas flow	0.3 ml min^{-1}
Electron ionisation energy	70 eV
Segment 1	
Microscans	3
Precursor ion (CAP)	466
Collision energy	0.66 V
Product ion range	280–468 amu
Microscans	3
Precursor ion (CAP-d5)	471
Collision energy	0.74 V
Product ion range	280–475 amu
Segment 2	
Microscans	3
Precursor ion (THAP)	337
Collision energy	0.94 V
Product ion range	180–340 amu

Analyser (ThermoFinnigan) with an electrospray ionisation (ESI) interface. Separation was performed on a Genesis C_{18} 120 Å column (3 μm, 150 mm × 2.1 mm, Jones Chromatography Ltd., Hengoed, UK). An isocratic mixture of 1% acetic acid in methanol/water (55/45; v/v) was used at a flow rate of 0.25 ml min^{-1}. The analytes were detected in full scan MS^2 in negative ion mode. Xcalibur™ software version 1.1 was used to perform the interpretation of the analytical results.

2.5. Methods

2.5.1. Screening with ELISA

Shrimps could be tested on the presence of CAP with an ELISA after a simple sample pre-treatment (extraction with an aqueous extraction buffer). However, better sensitivity could be obtained by performing an extraction with an organic solvent.

2.5.1.1. Sample pre-treatment using an aqueous extraction. Unknown shrimp tissue of 5.0 ± 0.1 g was cut into small pieces and weighed into a Nunc tube. Along with the unknown sample, four blank shrimp tissue samples of which two were fortified with 0.25 μg of CAP kg^{-1} matrix were analysed as well. A 10 ml PBST was added to each sample and the contents were shaken individually for 2 min using a test tube shaker. After 30 min extraction, for which an orbital shaker was used, centrifugation at 4000 rpm for 10 min at room temperature was carried out. Subsequently, the ELISA was performed on the supernatant, according to the procedure instructions delivered along with the test kit.

2.5.1.2. Sample preparation including an organic solvent extraction. Here, the analysis was started with 20.0 ± 0.1 g of unknown shrimp tissue and, next to two blank samples, two other blank materials being fortified with 0.1 μg of CAP kg^{-1} matrix were analysed. Instead of PBST, 10 ml of ethyl acetate was added to each sample. After shaking and centrifugation (under the same conditions as mentioned above), 4 ml of the top layer was evaporated until dry using N_2, redissolved and washed in 2 ml of *n*-hexane. After addition of 1 ml of dilution buffer, mixing and centrifugation, 1 ml of *n*-hexane was added to the bottom layer to repeat the wash step. The resulting sample extract (bottom layer) was used in the ELISA according to the procedure provided with the test kit.

2.5.1.3. ELISA procedure. In one incubation step, rabbit anti-CAP, enzyme-labelled CAP and sample were added to the pre-coated wells. While the specific antibodies were bound by the immobilised antibodies, free CAP (present in the sample) and enzyme conjugated CAP competed for the CAP antibody binding sites. After 1 h incubation, the non-bound enzyme-labelled reagent was removed, and the CAP enzyme conjugate amount was visualised by the addition of a colourless chromogen substrate (tetramethylbenzidine) that was transformed to a coloured product by the bound enzyme conjugate. The substrate reaction was stopped by addition of sulphuric acid and the absorbance was measured at 450 nm. The absorbance was inversely proportional to the CAP concentration in the sample.

2.5.2. Confirmation with GC–MS^2

In Fig. 1, an overview of the procedure for analysis of CAP in shrimp tissue is shown.

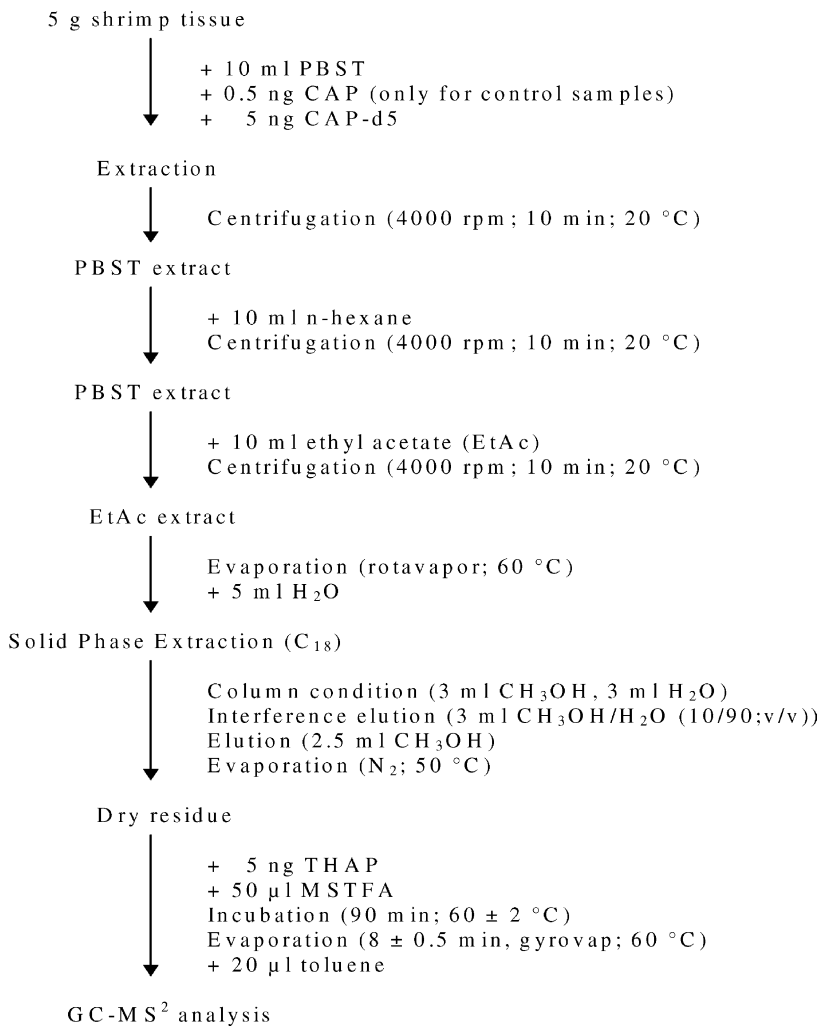

Fig. 1. Overall scheme for the extraction and clean-up procedure for CAP in shrimp tissue using GC–MS^2.

Shrimp tissue was homogenised and weighed into a Nunc tube. Two blank shrimp tissue samples of which one was fortified with 0.1 μg of CAP kg^{-1} of matrix, were analysed as well as control samples. After addition of 10 ml of PBST and 1 μg of CAP-d5 kg^{-1} of matrix to each sample, extraction (30 min while shaking) and centrifugation, the supernatant was washed twice with 10 ml of *n*-hexane in order to remove the remaining lipid material. Subsequently, 10 ml of ethyl acetate was added for a second extraction step, and after centrifugation, extraction was carried out once more. The resulting ethyl acetate upper layer extracts were evaporated until dry, redissolved in 5 ml of ultra pure water and quantitatively passed to the top of the conditioned C_{18} column, using a sample preparation unit coupled to a vacuum source. After interference elution, CAP was eluted with 2.5 ml of methanol. The eluate was evaporated to dryness under N_2, redissolved in 0.5 ml of ethanol and derivatised to enol-trimethylsilyl ether derivatives with MSTFA after addition of 1 μg of THAP kg^{-1} of matrix as a control substance for derivatisation.

A standard solution, of which the amount was equivalent to 0.02 or 0.06 ng of CAP and 1 ng μl^{-1} CAP-d5

on column, was derivatised along with the samples to verify the applicability of the GC–MS device. After incubation and evaporation, the residues were resolved in 20 μl of toluene, and finally, 1 μl was injected into the gas chromatograph.

2.5.3. Confirmation with LC–MS^2

A 30 ml of PBST and 1 μg of THAP kg^{-1} of matrix were added to 10.0 ± 0.1 g of homogenised shrimp tissue. After mixing (15 min) and centrifugation (3500 rpm; 15 min), 8 ml of the supernatant was passed to a 10 ml Chem-Elut column. After 10 min rest, elution was performed with 3 × 12 ml of *t*-butyl methyl ether. The eluate was evaporated (50 °C under vacuum) and the residue was redissolved in 0.3 ml of dilution buffer (from the EIA kit) followed by an addition of 0.3 ml of isooctane/trichloromethane (2/3, v/v). After protein precipitation (water bath, 90 °C) and centrifugation (4000 rpm, 15 min), the aqueous phase was injected into the LC–MS system.

3. Results and discussion

3.1. Screening

The CAP EIA kit is a microtitre-based competitive enzyme immunoassay for screening and quantitative analysis for CAP. Besides shrimp tissue, it can also be used for determination of CAP in urine, milk, egg and tissue samples.

3.1.1. Qualitative screening (initial validation)

In order to comply with the quality criteria for the proper identification according to the European Commission Decision 2002/657/EC [16], detection capability (CCβ), selectivity/specificity, and applicability/ruggedness/stability must be determined. Although applicability, ruggedness and stability already were determined by the producer of the ELISA kit, these parameters were tested within the lab to include its own equipment and experience by integrating little changes in the procedure (kits and solvents with different production numbers, different shrimp tissue types, changes in extraction time and shaking manner, etc.). In order to confirm selectivity and specificity shrimp tissue was fortified with thiamphenicol, florfenicol, streptomycin, sulfamethazine, trimethoprim, penicillin G, ceftiofur, enrofloxacin, erythromycin, oxytetracycline or lincomycin at five times meat maximum residue limit (MRL) concentration level. No inferences could be observed, and furthermore, no signal suppression could be observed when spiked samples contained both mentioned products and CAP (at that time 0.25 μg kg^{-1}).

Although in the EC, CAP is a forbidden substance so quantitative analysis is not necessary, calibration graphs were used to illustrate the test kit's workability in the assumed concentration range. Because at first, Belgian veterinary inspection services had imposed a national minimum required performance limit (National MRPL) level for CAP at 0.3 μg kg^{-1}, initial validation was performed based on this National MRPL value. First, a calibration graph by which two series of blank shrimp tissue samples were fortified with 0.25, 0.5 (twice), 1 and 2 μg of CAP kg^{-1} of matrix, was prepared and analysed. The results are presented in Table 3. It could be concluded that the CAP EIA test kit was suitable to detect CAP in the 0.25–2.0 μg kg^{-1} range. Subsequently, two series of five blank shrimp tissue samples, two series of samples fortified with 0.25 μg of CAP kg^{-1} of matrix and two series of samples fortified with 0.5 μg of CAP kg^{-1} of matrix were analysed. The results of these experiments are illustrated in Table 4. As could be seen, the blanks' mean absorbance was higher than that for the fortified samples.

According to Commission Decision 2002/657/EC [16] and based upon the results of the validation experiments, the decision limit (CCα) and detection capacity (CCβ for the absorbance were 0.13 μg kg^{-1} (absorbance = 1.76) and 0.22 μg kg^{-1} (absorbance = 1.675), respectively.

Table 3
Calibration results for CAP in shrimp tissue using ELISA with an aqueous extraction

Absorbance		Concentration CAP (μg kg^{-1})	B_a/B_0	
Series 1	Series 2		Series 1	Series 2
1.996	1.896	Blank	0.95	0.91
1.679	1.568	0.25	0.80	0.75
1.506	1.457	0.50	0.72	0.69
1.484	1.462	0.50	0.71	0.69
1.280	1.237	1.00	0.61	0.58
1.058	0.976	2.00	0.50	0.46

B_0 = 2.089; B_a = 0.038.

Table 4
Resulting absorbance values (Abs) for the validation experiments

Blank	Abs	0.25 μg kg^{-1} CAP	Abs	0.5 μg kg^{-1} CAP	Abs
A	1.832	F	1.631	A	1.488
B	1.945	G	1.682	B	1.450
C	1.875	H	1.632	C	1.451
D	1.912	I	1.751	D	1.500
E	1.870	J	1.735	E	1.404
A	1.848	F	1.585	A	1.424
B	1.810	G	1.627	B	1.298
C	1.945	H	1.564	C	1.351
D	1.965	I	1.585	D	1.372
E	1.852	J	1.610	E	1.409
Average	1.89	Average	1.64	Average	1.42
S.D.	0.053	S.D.	0.063	S.D.	0.062
CV (%)	2.83	CV (%)	3.86	CV (%)	4.41

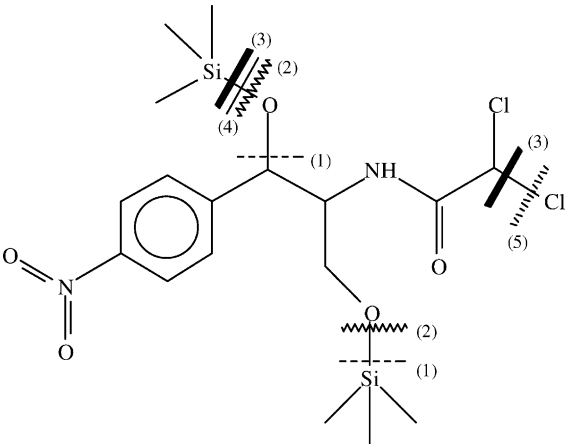

Fig. 2. Principal fragments of chloramphenicol-TMS ether in negative chemical ionisation mode: (1) *m/z* 304; (2) *m/z* 322; (3) *m/z* 358; (4) *m/z* 394; and (5) *m/z* 430.

It could be concluded that the CAP EIA kit can be useful for qualitative screening of CAP in shrimp tissue. A CCβ $\leq$ 0.25 μg kg^{-1} could be reached, meaning that, when a series of shrimps has been contaminated at that level, there is a 95% chance that the shrimps will be considered as being 'suspect'. For lower contamination levels the chance for detection will be smaller and a chemical extraction is advisable. Further experiments, including the more complex ethyl acetate extraction, and a subsequent validation study (three batches of samples on three different days; calculation of CCα and CCβ according to Commission Decision 2002/657/EC) [16]) proved that the linearity range already starts at 0.1 μg of CAP kg^{-1} and a CCβ $\leq$ 0.1 μg kg^{-1} was reached.

3.2. Confirmation

3.2.1. Initial study

Beside detection with MS in electron impact (EI) mode [17], CAP could also be determined using soft ionisation techniques, i.e. negative ion chemical ionisation (NCI), because of the Cl atoms in the molecular structure, resulting in a lower limit of detection than when using EI. After derivatisation with MSTFA to a chloramphenicol-trimethylsilyl ether (CAP-TMS) and redissolving in toluene, detection could be performed at a detection limit of 0.005 ng μl^{-1} taking into account a minimum signal/noise (S/N) ratio of 3. The principal fragments of CAP-TMS, explaining the specific mass spectrum, are shown in Fig. 2.

Although the derivatisation duration time was not very critical, optimal results were obtained after 90 min. It was important to analyse the samples within 24 h after derivatisation, as otherwise the response was much lower. As the TMS ethers are quite volatile, evaporation of MSTFA after derivatisation was very critical and must not take longer than 8 ± 0.5 min. In order to prove specificity and selectivity CAP, CAP-d5, THAP and FF, a fluorinated derivative of THAP and also used in veterinary medicine, were analysed and identified based upon absolute (or relative) retention time and diagnostic transition product ions with specific mass/charge (*m/z*) ratio. When analysing in full scan GC–MS^2 mode, *m/z* 304, 322, 358, 394 and 430 were specific for CAP (precursor ion *m/z* 466); *m/z* 308, 327, 363, 398 and 435 belonged to CAP-d5 (precursor ion *m/z* 471); THAP was identified based upon *m/z* 183, 247, 322 and 337 (precursor ion *m/z* 337); *m/z* 183, 204, 224 and 252 seemed to be specific for FF (precursor ion *m/z* 267) (Fig. 3).

Taking into account the sample analysis recovery (50%), a calibration graph using 6 CAP concentration levels −0, 6.25, 12.5, 18.75, 25 and 37.5 pg μl^{-1} was prepared and analysed in an appropriate way [18]. A good linear relation (R^2 = 0.9936 and

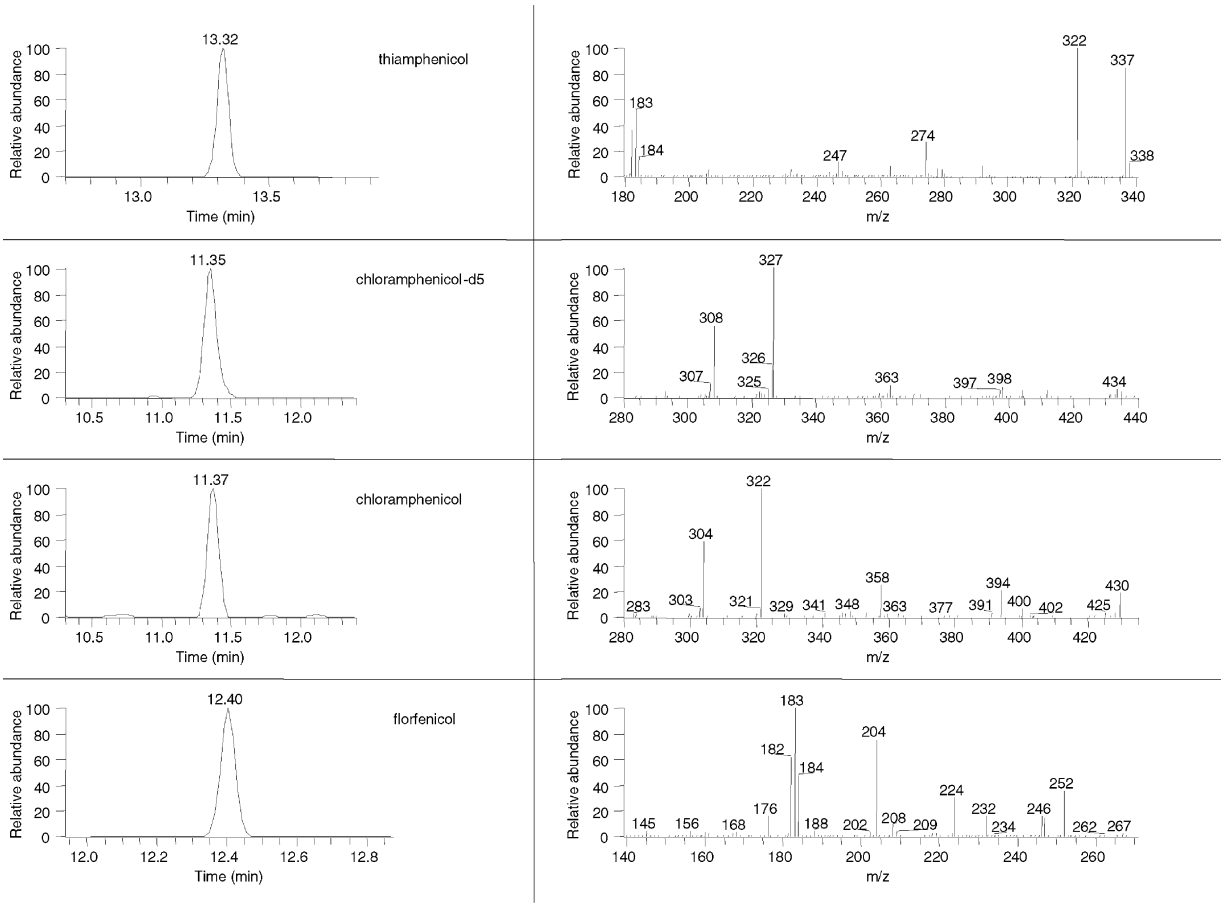

Fig. 3. Identification of CAP, CAP-d5, THAP and FF.

$y = 0.0085x + 0.0066$) between the different concentration levels (measured by area ratio between CAP and CAP-d5) could be observed indicating the applicability of quantitative approach.

3.2.2. Shrimp tissue samples

As Bunch et al. described earlier [14], homogenisation of the sample matrix was important. Concerning SPE, C_{18} columns were chosen in preference to Extrelut and Silicium (Si) columns as for Extrelut quite some losses of analytes could be observed; because of health reasons Si columns were rejected as analyte elution was carried out with a mixture of acetone/toluene instead of elution with methanol when using C_{18} SPE columns. To avoid interfering ions (*m*/*z* 286 and 376) in the CAP mass spectrum especially when the CAP concentration levels were reaching lower limits, methanol gradient grade for liquid chromatography should be used for condition and elution of the C_{18} SPE column.

After optimising the extraction and clean-up procedure, two unknown shrimp tissue samples, provided by the National Reference Laboratory, were analysed using the newly developed method. Results showed that the method was successful and could be used for qualitative routine analysis (Fig. 4).

After method development, 40 blank samples, of which 20 were fortified with 0.1 μg of CAP kg^{-1} were analysed to determine the validation parameters related to qualitative confirmation methods (CCα and CCβ), resulting in a CCα ≤ 0.018 μg CAP kg^{-1} (based upon the S/N ratio for blank and fortified

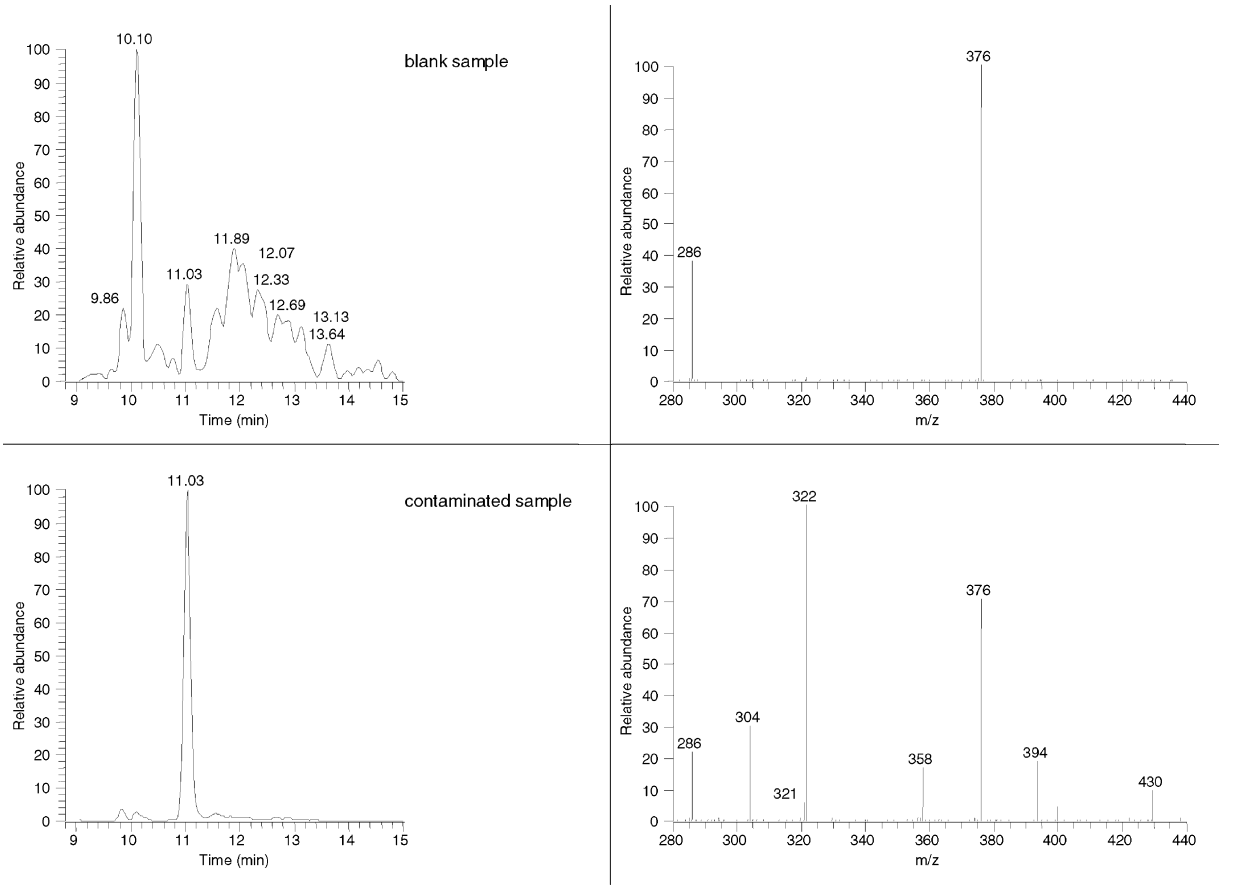

Fig. 4. Analysis of a blank and a contaminated sample.

samples) and a CCβ $\leq$ 0.1 μg CAP kg^{-1}, meaning that there is a 95% chance that a contaminated shrimp tissue sample will be found non-compliant at that concentration level. Of course, sufficient identification points (ion ratios between permitted relative intensity ranges and acceptable relative retention times according to Commission Decision 2002/657/EC [16]) must be achieved.

As some customers claim that confirmation must result in a concentration level on the report sheet (instead of 'positive' or 'negative'), quantification was achieved by analysis of a shrimp tissue sample calibration plot at various CAP concentrations (0, 0.05, 0.1, 0.15, 0.2 and 0.3 μg CAP kg^{-1}), resulting in a regression line $y = 0.4488x - 0.005$ with a correlation coefficient (R^2) of 0.9846. In routine conditions, a calibration graph should be prepared with every batch of samples analysed.

As with different analysis techniques more identification points (IPs) according to Commission Decision 2002/657/EC [16] can be earned, an extraction method suitable for LC–MS^2 analysis of suspected shrimp tissue samples was also developed (Fig. 5). Again, quality criteria could be fulfilled easily and a CCβ $\leq$ 0.028 μg kg^{-1}, CCα $\leq$ 0.011 μg kg^{-1} could be reached.

All ELISA suspected common shrimp tissue could be confirmed with chromatographic mass spectrometric detection. When analysing prepared shrimps, e.g. coated with breadcrumbs, some of the positive ELISA results were not confirmed using MS, indicating false positive results for ELISA with deviating matrices.

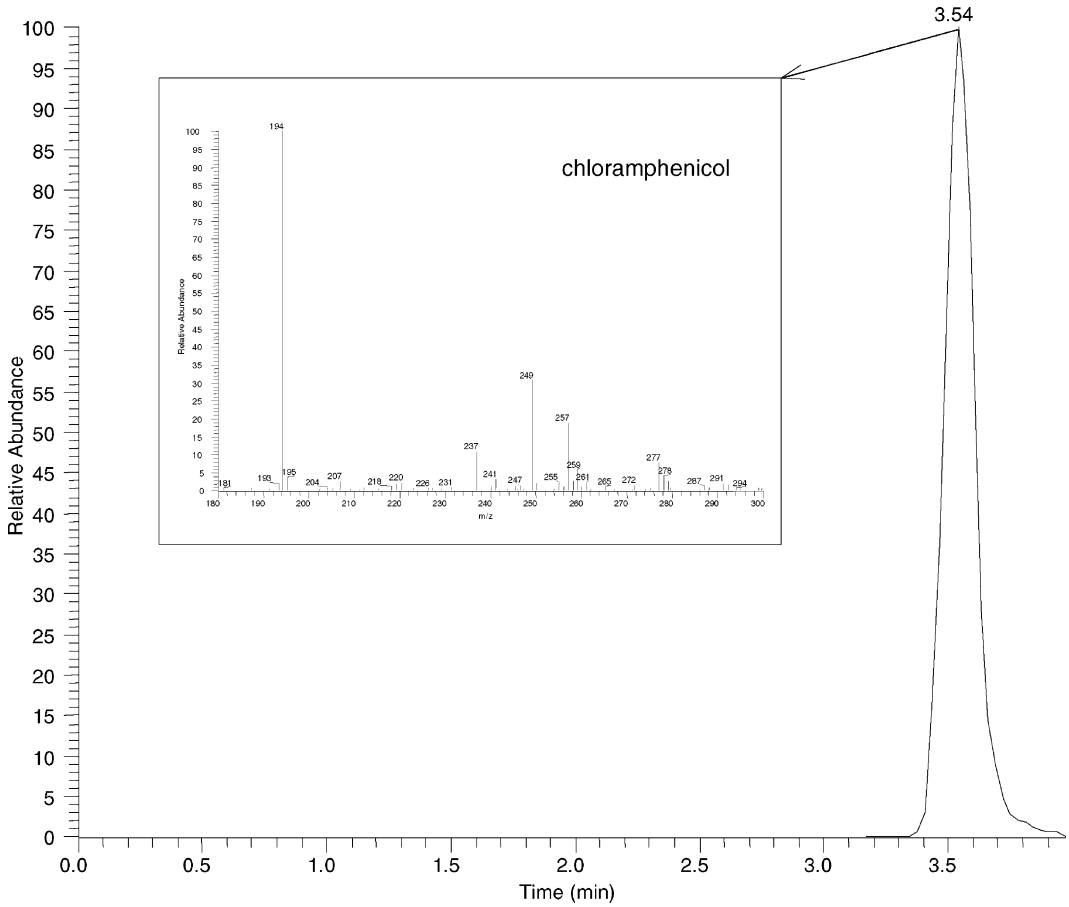

Fig. 5. LC–MS^2 results for shrimp tissue contaminated with 0.1 μg CAP kg^{-1} (ESI full MS^2; precursor ion 322; voltage energy 23.00 V; scan range 180.00–300.00).

4. Conclusion

The multi-technique assay described in this investigation was developed for determination of chloramphenicol in shrimp tissue at the Belgian national MRPL (0.1 μg CAP kg^{-1}). ELISA was chosen for screening, while GC–MS^2 and LC–MS^2 were used to confirm ELISA suspected samples. The developed methods were fast and thus very suitable for routine analysis for which time is always a restricting factor. When quantification was carried out with the external standard method (towards THAP instead of CAP-d5), confirmation analysis could even be performed out of the ELISA extract.

Acknowledgements

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