Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

ELSEVIER

Journal of Chromatography A, 994 (2003) 75–84

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of the antibiotic chloramphenicol in meat and seafood products by liquid chromatography–electrospray ionization tandem mass spectrometry

Pascal Mottier*, Véronique Parisod, Eric Gremaud, Philippe A. Guy, Richard H. Stadler

Nestlé Research Center, Nestec Ltd, Vers-chez-les-Blanc, 1000 Lausanne 26, Switzerland

Received 30 December 2002; received in revised form 5 March 2003; accepted 11 March 2003

Abstract

A confirmatory method based on isotope dilution liquid chromatography–electrospray ionization tandem mass spectrometry is described for the determination of the antibiotic chloramphenicol (CAP) in foods. The method is quantitative and entails liquid–liquid extraction followed by a clean-up step on a silica gel solid-phase extraction cartridge. Mass spectral acquisition is done in the negative ion mode applying multiple reaction monitoring of two diagnostic transition reactions for CAP (m/z 321→257 and m/z 321→152). In addition, the presence of two chlorine atoms in the CAP molecule provides further analyte certainty by assessing the $^{37}Cl/^{35}Cl$ ratio using the transition reactions m/z 323→257 and m/z 323→152. Validation of the method in chicken meat is conducted according to the latest European Union criteria for the analysis of veterinary drug residues at levels of 0.05, 0.10, and 0.20 μg/kg, employing [$^2H_5$]-chloramphenicol as internal standard. The decision limit and the detection capability were calculated at 0.01 μg/kg and 0.02 μg/kg, respectively. At the lowest fortification level (i.e. 0.05 μg/kg), precision values below 14 and 17% were achieved under repeatability and within-laboratory reproducibility conditions, respectively. The accuracy of the method was within 20, 15, and 5% of the target values at the 0.05, 0.10, and 0.20 μg/kg fortification levels, respectively. The applicability of this procedure was demonstrated by the analysis of other meat (turkey, pork, beef) and seafood (fish, shrimps) products. The method is robust and suitable for routine quality control operations, and more than 200 sample injections were performed without excessive pollution of the mass spectrometer or loss of LC column performance.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Meat; Fish; Food analysis; Antibiotics; Chloramphenicol

1. Introduction

Intensive surveillance has recently been conducted by certain European Union (EU) member states due to the finding of residues of chloramphenicol (CAP) in animal-derived foods (mainly aquaculture products) originating from China and Vietnam [1,2]. CAP is a broad-spectrum antibiotic, and its use in food-producing animals is prohibited in many countries such as the USA, Canada, Australia, and EU member states. Relatively low levels of CAP may give rise to an irreversible type of bone marrow depression, which may lead to aplastic anemia [3]. No maximum residue limit (MRL) has

*Corresponding author. Tel.: +41-21-785-8231; fax: +41-21-785-8553.
E-mail address: pascal.mottier@rdls.nestle.com (P. Mottier).

0021-9673/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0021-9673(03)00484-9

been established for CAP in animal-derived foods as its toxic effects are not dose-dependent, but rather related to the hypersensitivity of certain individuals. However, as reflected in recent EU website Food Safety Alerts [4], the indiscriminate and illegal use of CAP in some countries—potentially to mask poor hygienic conditions and/or to augment growth and yield—may lead to unacceptable exposure of consumers. In the past decades, several analytical methods have been developed and reviewed for the detection and quantitation of CAP in foods and biological fluids [3,5]. For confirmatory purposes at trace levels, however, mass spectrometry (MS) is the generally accepted technique as detection of residues in complex matrices is possible with the additional advantage of analyte confirmation. Thus, gas chromatography–mass spectrometry (GC–MS) methods using either electron impact (EI) or chemical ionization (CI) have been reported, but these procedures still require a tedious derivatization step prior to final analysis [6–8]. On the other hand, liquid chromatography coupled with either thermospray or atmospheric pressure chemical ionization mass spectrometry (LC–MS) techniques have been described without derivatization of the analyte, thus facilitating the analysis [9–12]. To our knowledge, no analytical method using liquid chromatography–tandem mass spectrometry (LC–MS–MS) has been reported to date for the quantitative determination of CAP in different animal-derived foods. However, MS-MS techniques have already shown their impact in enhancing analyte selectivity, which is pivotal when dealing with complex matrices [13].

The purpose of this study was to develop a confirmatory and quantitative method for the determination of CAP at trace levels in a variety of animal-derived foods employing isotope dilution LC–electrospray ionization (ESI)–MS–MS. The method is fully validated using chicken meat spiked with CAP at the ng/kg level, and the applicability of the procedure was further demonstrated for other types of meat and seafood products of different geographical origin. The in-house validation criteria follow the latest EU recommendations for the analysis of veterinary drug residues [14].

2. Experimental

2.1. Chemicals

Chloramphenicol or 2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide was supplied by Fluka (Buchs, Switzerland). Deuterated [$^{2}H_5$]chloramphenicol (d_5-CAP, ring-d_4, benzyl-d_1; chemical purity >98%, isotopic purity 99.8%) was obtained from the Cambridge Isotope Laboratory (Andover, MA, USA). Radiolabeled [^{14}C]CAP (55 mCi/mmol) was purchased from Moravek (Brea, CA, USA). β-Glucuronidase (Type H-2 from *Helix pomatia*, 131 000 units/ml) was from Sigma (Buchs, Switzerland). Silica gel (SiOH) solid-phase extraction cartridges (500 mg, 3 ml) were from J.T. Baker (Phillipsburg, NJ, USA). All other reagents and solvents were of analytical reagent grade and supplied by Merck (Darmstadt, Germany). Deionized and bi-distilled water was obtained from a Milli-Q water purification apparatus (Millipore, Bedford, MA, USA).

2.2. Food samples

Meat (chicken, turkey, pork, beef) and seafood (dab, shrimps, fish in dry powdered form) samples were of Asian origin. The samples, typically 200 g, were first minced using a kitchen homogenizer (Multi moulinette, Moulinex, France), then sub-sampled (20 g) and stored at −20 °C in airtight containers until analysis. For method validation, chicken meat originating from animals grown under strictly controlled conditions (''bio'' label) was used.

2.3. Sample preparation

2.3.1. Extraction

A well homogenized sample (3.00±0.05 g) was weighed into a 50 ml Falcon polypropylene tube (Becton Dickinson, Le Pont de Claie, France) and fortified with 0.5 μg/kg of the internal standard d_5-CAP (150 μl of an aqueous 10 ng/ml solution). A 0.1 *M* sodium acetate buffer solution (adjusted to pH 5 with concentrated acetic acid) (25 ml) was added and the mixture homogenized using an Ultra-Turrax homogenizer at 9000 rpm for 2 min. (When needed,

β-glucuronidase (200 μl, 26 200 units) was pipetted into the slurry, which was then incubated overnight at 37 °C). CAP was extracted with ethyl acetate–diethyl ether (75:25 v/v) (15 ml) by first thoroughly hand shaking the mixture for 2 min, followed by centrifugation at 2200 *g* (centrifuge Mistral 2000, MSE Scientific Instrument, Leicestershire, UK) for 5 min at room temperature (RT). The upper organic phase was carefully collected and the analyte extracted as described below.

2.3.2. Clean-up

A SiOH solid-phase extraction (SPE) cartridge, attached to a 80-ml solvent reservoir, was conditioned with successively methanol (5 ml), acetone (5 ml) and ethyl acetate–hexane (24:9 v/v) (5 ml). The crude extract was poured into the reservoir followed by the addition of hexane (60 ml) and the solvent mixture allowed to elute through the cartridge at a flow-rate of 1–2 drops/s applying a slight vacuum (Visiprep vacuum manifold, Supelco, Buchs, Switzerland). After penetration, excess solvent was removed by increasing the vacuum for a few seconds. CAP and the internal standard were eluted with 5 ml of a 0.05 *M* di-potassium hydrogenphosphate buffer solution (adjusted to pH 10 with 0.1 *N* sodium hydroxide) and the eluate mixed with ethyl acetate (25 ml). After mixing and centrifugation (2200 g for 2 min at RT), the upper organic phase was removed and evaporated under reduced pressure (100 mbar, 45 °C) down to a volume of ca. 1 ml. After transfer to an eppendorf tube, the extract was evaporated to dryness under a stream of nitrogen at RT and reconstituted with water (300 μl). The extract was washed with toluene (600 μl) by shortly vortexing and subsequently centrifuged (Centrifuge Eppendorf 5415C, Hamburg, Germany) at 8400 *g* for 2 min at RT. The aqueous phase was collected and filtered through a 0.2 μm nylon filter (Semadeni, Ostermundingen, Switzerland) directly into an HPLC vial.

2.3.3. Recovery

Recovery experiments were conducted using radiolabeled [^{14}C]CAP spiked in chicken meat at a level of 2.5 μg/kg to optimize the extraction procedure. Radioactivity was measured by liquid scintillation counting with a LKB-Wallac 1219 Rackbeta counter (Perkin-Elmer Life Sciences, Regensdorf, Switzerland). For this purpose, the final extract (300 μl) was thoroughly mixed with Ultima Flo M scintillator (10 ml) (Packard, Meriden, USA) prior to radioactivity measurement.

2.4. HPLC

HPLC analyses were performed on a C_{18} reversed-phase SymmetryShield HPLC column (15 cm×2.1 mm I.D., 3.5 μm particle size) fitted with a SymmetryShield RP_{18} precolumn (1 cm×2.1 mm I.D., 3.5 μm particle size) (Waters, Milford, MA, USA) using a Perkin-Elmer HPLC 200 pump series system (Norwalk, CT, USA). The mobile phase was as follows: solvent A: water; solvent B: acetonitrile. The linear gradient program was: 0–3 min 0% B; 3–10 min 100% B; 10–15 min 100% B; 15–16 min 0% B and 16–21 min 0% B running at a flow-rate of 0.3 ml/min. Using these conditions, the retention time of CAP was observed at 8.2±0.1 min (n=250). The injection volume was 15 μl and between injections, the needle was rinsed with a solution of isopropanol–methanol–water–formic acid (5:3:2:0.1, v/v). The entire HPLC flow was directed into the MS detector between 6 and 12 min using a VICI diverter (Valco Instruments, Houston, TX, USA).

2.5. ESI–MS–MS

MS detection was done on a Sciex API 3000 triple stage quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray ionization source (resolution 0.7 a.m.u. at full width half mass). Nitrogen was used for the gas nebulizer, TurboIonSpray gas and curtain gas at a pressure of 0.87 bar, and flow-rates of 7.5 l/min and 13 ml/min, respectively. The source block temperature was set at 350 °C and the electrospray capillary voltage to 3.5 kV. Nitrogen was used as collision gas with a pressure set at 5 mTorr (1 Torr= 133.322 Pa). The declustering potential and the dwell time for each transition reaction were set at 65 V and 100 ms. Data acquisition was performed using the Sciex Analyst software in negative multiple reaction monitoring (MRM) alternating two transition re-

actions for ^{35}Cl (used for quantitation) and two transition reactions for ^{37}Cl (used for peak confirmation) for both CAP and d_5-CAP. The different transition reactions and collision energies are shown in Table 1.

2.6. Quantitation

CAP was quantified by means of two external calibration curves (response ratio vs. amount ratio) constructed in water at seven calibration levels ranging from 0 to 1 μg/kg. One calibration curve was based on the transition m/z 321→257 and the second curve on m/z 321→152. The concentration of the deuterated internal standard was fixed at 0.5 μg/kg. The lowest quantity injected from a standard solution was 7.5 pg (23 fmol) on-column. Calibration solutions were prepared from successive dilutions of the stock standard solution in water, the precise concentration of which was determined from the extinction coefficient of CAP ($E^{1\%}_{1\text{cm}}=298$, $\lambda=278$ nm [15]). Aliquots of calibration solutions were stored at −20 °C until use. Calibration standards were injected before and after an analytical series, and both data sets were used to establish the calibration curves. Linearity was checked by calculating the average of response factors (peak area ratios divided by the corresponding analyte concentration ratios of all standards), which should be <15% to assume linear response [16].

2.7. Confirmation criteria

CAP was considered as positively identified in meat and seafood products when the following criteria were met: (a) the retention time of the analyte was within ±1% of the retention time of the deuterated internal standard; (b) the presence of a signal at each of the four diagnostic transition reactions for CAP and d_5-CAP; (c) ^{37}Cl/^{35}Cl ratios for m/z 323→257 vs. m/z 321→257, and m/z 323→152 vs. m/z 321→152 were within 0.33±20% and 0.65±15%, respectively (see Results and discussion).

2.8. Method validation

Accuracy and precision (within- and between-day) were calculated from the analysis of six blank chicken meats fortified with CAP at each of the three specified fortification levels (0.05, 0.10, and 0.20 μg/kg) and performed by the same operator on three separate occasions. Within-laboratory precision was obtained by following the same protocol but analyses were performed by three different operators on one occasion. Since no MRL exists for CAP, the decision limit (CCα) and the detection capability (CCβ) were calculated and replace the limit of detection (LOD) and the limit of quantitation (LOQ). CCα is defined as the lowest concentration level from which it can be decided whether the identified substance is present with a statistical certainty of $1-\alpha$. CAP belongs to the Group A substances, listed in Annex I of Council 96/23/EC [17], where $\alpha=1\%$. CCβ is the smallest concentration of the analyte that can be detected, identified and quantified in a sample with an error probability of β, with $\beta\leq5\%$. These limits were determined graphically using the data generated during the determination of the within-laboratory precision. Peak area ratios of CAP/d_5-CAP found experimentally at the three fortification levels (y-

Table 1
Transition reactions and their corresponding collision energies used for the quantification of CAP

	^{35}Cl transitions (m/z) (used for quantitation)	Collision energy (eV)	^{37}Cl transitions (m/z) (used for confirmation)	Collision energy (eV)
CAP	321→257	14	323→257	14
	321→152	23	323→152	23
d_5-CAP	326→262	14	328→262	14
	326→157	23	328→157	23

Peak areas from both ^{37}Cl and ^{35}Cl transition reactions in standard solutions and sample extracts for CAP and d_5-CAP were ratioed and compared to obtain a further confirmatory criterion.

axis) were first plotted against their corresponding nominal concentration (x-axis). The slope, intercept and standard error of the intercept (SE_{int}) from the resulting linear regression line were then calculated using Excel software. CCα is defined as the concentration corresponding to the intercept value$+2.33\times SE_{int}$, whereas CCβ is defined as the concentration corresponding to the intercept value$+3.97\times SE_{int}$.

3. Results and discussion

3.1. Extraction

Our extraction and clean-up procedures were adapted from previous publications [18,19], with the aim to reduce the number of individual steps and to allow a high sample throughput while still achieving detection of the analyte at the ng/kg level. Thus, preliminary studies were first conducted to optimize solvents and solid-phase extraction conditions to recover CAP from foods by using radiolabeled [^{14}C]CAP. Using the described methodology, the overall absolute recovery of [^{14}C]CAP spiked at a concentration of 2.5 μg/kg into a blank chicken meat was calculated at 60±5% (n=4). The principal loss of analyte was shown to occur by partial extraction of CAP into the aqueous buffer. Recovery of CAP could be improved by additional solvent extraction steps with ethyl acetate–diethyl ether, but this would have prolonged the method which already achieves adequate sensitivity.

A potential drawback of the procedure is the clean-up step, which entails the use of a rather large volume of organic solvent (75 ml of the ethyl acetate–diethyl ether–hexane solvent mixture) that needs to pass through the SPE cartridge. This step requires approx. 45 min, and 8–12 samples can be processed simultaneously. To limit solvent usage/waste and expedite the extraction step, we attempted to decrease the volume of hexane (25 ml instead of 60 ml) added before the elution step. However, this resulted in a 20% loss of CAP as determined by measuring the radioactivity in the eluate.

Reports in the literature indicate that enzymatic digestion is necessary to liberate bound residues of CAP from its glucuronide conjugate when extracting the analyte from pig liver and kidney [20]. This step is apparently not necessary when working with muscle tissue of swine [20,21] and trout [22]. As no data are available for poultry meat, we analyzed CAP in two incurred chicken meats with and without incorporation of the enzymatic digestion step. Similar results ($P>0.01$, $n=4$) were obtained, confirming the absence of glucuronidation in chicken muscle and therefore concluding that the digestion step can be omitted for this matrix.

3.2. LC–ESI-MS–MS

CAP and its internal standard were first analyzed by ESI-MS to optimize the MS conditions. The full mass spectra of CAP and its deuterated internal standard display several intense ions at m/z 321.1 and 323.1, and at m/z 326.1 and 328.1, respectively, which correspond to the characteristic isotopic cluster of the two chlorine atoms (Fig. 1). The isotope ratio of the two chlorine ions (^{35}Cl and ^{37}Cl) showed a relative intensity of 66% (m/z 323) and 62% (m/z 328), which is in good agreement with the theoretical values calculated at 65%.

CAP was then analyzed by LC–ESI-MS–MS in negative ionization product ion scan mode by selecting m/z 321.1, the $^{35}Cl_2$ $[M-H]^-$ ion as the precursor ion (Fig. 2A). Negative ionization of CAP was shown to give a better sensitivity [23]. Two main fragment ions were obtained from the collision-induced dissociation (CID) experiment giving rise to m/z 257.1 and m/z 152.1 when the collision energy was set at 20 eV, accompanied by several minor fragment ions at m/z 249.1, m/z 194.1 and m/z 176.6. The fragment ions obtained from the CID spectrum of CAP are in good agreement with previous findings [23]. The fragment ion obtained at m/z 152.1 corresponds to the base peak of the MS–MS spectra acquired under these conditions, generated by homolytic cleavage of the carbon–carbon bond on the alkyl branch. The fragment ion observed at m/z 257.1 represents the loss of hydrogen chloride and methanol from the parent molecule. CID experiments were conducted by selecting m/z 323.1, which corresponds to the $^{35}Cl_1$ $^{37}Cl_1$ $[M-H]^-$ ion of CAP (Fig. 2B). Here, a similar fragmentation pattern was observed with the appearance of m/z 257.1, m/z 250.0,

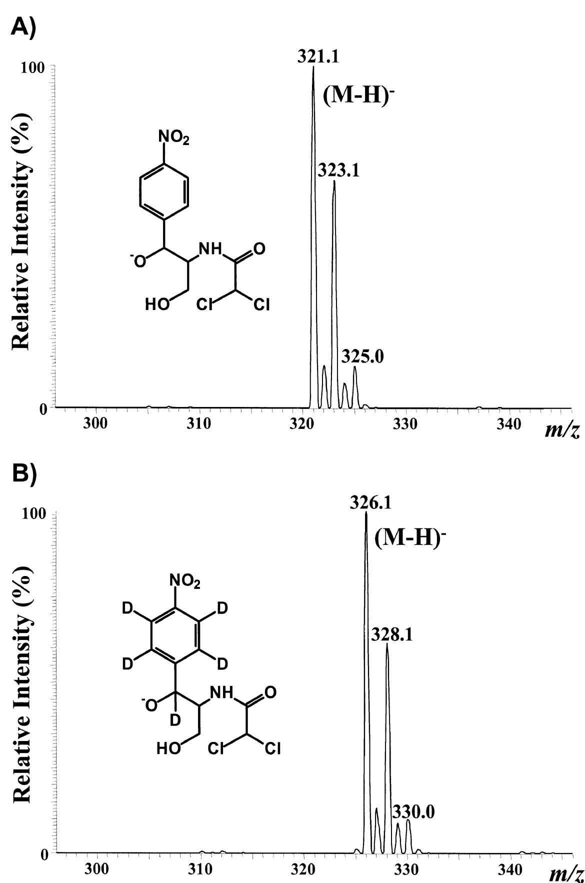

Fig. 1. Full scan electrospray mass spectra of (A) chloramphenicol and (B) d_5-chloramphenicol, depicting also their respective chemical structures.

m/z 194.1 and *m/z* 152.1. However, a new fragment ion was observed at *m/z* 259.1. The two fragment ions *m/z* 257.1 and *m/z* 259.1 show similar relative intensities substantiating the presence of a chlorine atom in this fragment (^{35}Cl and ^{37}Cl isotope, respectively). The CID of the corresponding d_5-CAP [M–H]$^-$ ions produced similar fragmentation as observed for the unlabeled CAP. Thus, the peak areas from the two transition reactions *m/z* 321→257 and *m/z* 321→152 for CAP (and *m/z* 326→262 and *m/z* 326→157 for d_5-CAP) were monitored for quantitation, with the latter transition showing signals more intense by a factor of approx. 1.5 when compared to the former. Moreover, as CAP contains two chlorine atoms, two diagnostic transition reactions at *m/z* 323→257 and *m/z* 323→152 (and *m/z* 328→262 and *m/z* 328→157 for d_5-CAP) were also monitored. Calculation of the chlorine isotope ratio for CAP and d_5-CAP in each corresponding transition provided additional analyte certainty.

Fig. 3 depicts the typical transition reactions obtained from a blank chicken meat and an incurred shrimp sample (calculated at 0.04 μg/kg). Each MS–MS chromatogram was clean with no interferences due to matrix constituents. With regard to the incurred sample of shrimp, signal-to-noise ratios of 16 and 39 were observed for *m/z* 321→257 and *m/z* 321→152, respectively. A calibration curve prepared in blank chicken meat was compared to a standard curve prepared in distilled water, over the same range of concentrations. Both curves showed slope equivalence, thus demonstrating the absence of matrix effect.

Calibration curves were linear over the range of concentrations considered, as checked by the response factor test. However, day-to-day variations of the slope of calibration lines (0.854±0.036 and 0.916±0.034 for *m/z* 321→257 and *m/z* 321→152, respectively, n=22) were observed. This reinforces the need for recording a calibration along with each analytical series to compensate for these variations.

3.3. Method validation

According to EU criteria for the analysis of veterinary drug residues in live animals and animal products [14], a system of identification points (IPs) is used to define the number of ions and their corresponding ratios that should be measured when using confirmatory MS techniques. For the LC–MS–MS analysis of CAP, which is listed as belonging to Group A substances (with no MRL), a minimum of four IPs are required. In our case, measurement of the two transition reactions *m/z* 321→152 and *m/z* 321→257 earns 1.5 IPs each. Measurement of the two transition reactions *m/z* 323→152 and *m/z* 323→257 for the isotopic chlorine ratio calculation earns an additional 1.5 IPs each. Thus, our LC–ESI-MS–MS method accumulates a total of six IPs and therefore meets these criteria.

For additional confirmation of the presence of

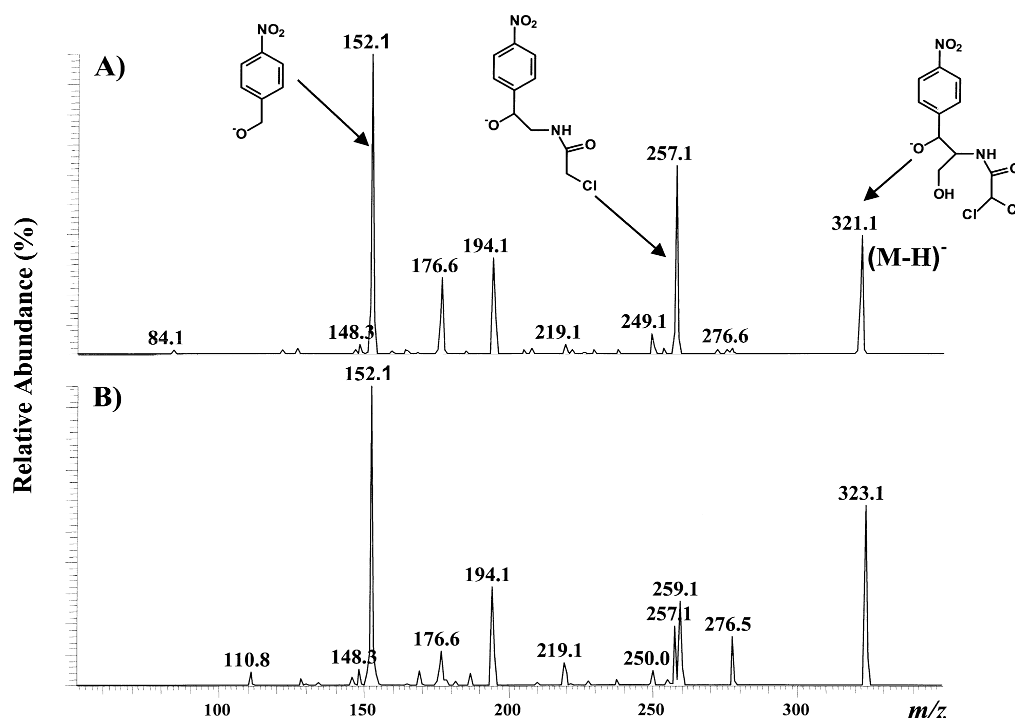

Fig. 2. Full scan product ion spectra of CAP obtained by selecting (A) *m/z* 321 and (B) *m/z* 323 as precursor ion. Nitrogen was used as collision gas with a pressure and collision energy set at 5 mTorr and 20 eV, respectively. The chemical fragmentation structure has been attached to the major fragment ions observed.

CAP in the extracts, the chlorine isotope ratios (^{37}Cl/^{35}Cl) of *m/z* 323→257 vs. *m/z* 321→257 and *m/z* 323→152 vs. *m/z* 321→152 were calculated from the analysis of standard solutions at 0.33 and 0.65, respectively. These two ratios were then compared to those obtained from the extract samples. A standard deviation of 20 and 15%, respectively, was accepted for confirming the presence of CAP. The same comparison of ratios was performed for the d_5-CAP to confirm that the internal standard response was not polluted by interfering chemicals. However, at concentrations below 0.04 μg/kg, the MRM signal observed from the transition reaction *m/z* 323→257 was too weak and the corresponding chlorine ratio deviated from the above specification. In such cases, confirmation of CAP was based only upon the ratio obtained from the transition reactions *m/z* 323→152 vs. *m/z* 321→152, while still achieving the minimum of four IPs.

The performance data of the LC–ESI-MS–MS method is summarized in Table 2 and shows that the two transition reactions used in quantitation provide comparable results. At a fortification level of 0.05 μg/kg, the precision values of repeatability and within-laboratory reproducibility were calculated at below 14 and 17%, respectively ($n=18$). Moreover, these precision values were improved at higher fortification levels (<8 and 10%, respectively at a spiking level of 0.20 μg/kg). Both transition reactions gave similar results for the decision limit (CCα) and the detection capability (CCβ), i.e. 0.01 and 0.02 μg/kg, respectively. Taking into account the initial amount of sample (3 g weighed) and 60% recovery of CAP, then these values correspond to approximately 0.9 and 1.8 pg, respectively (i.e. 2.8 and 5.6 fmol) injected on-column.

Though LOD and LOQ have been replaced by CCα and CCβ, an estimate of these former limits was performed by extrapolating the *S/N* ratio of the peak areas obtained from a chicken meat sample

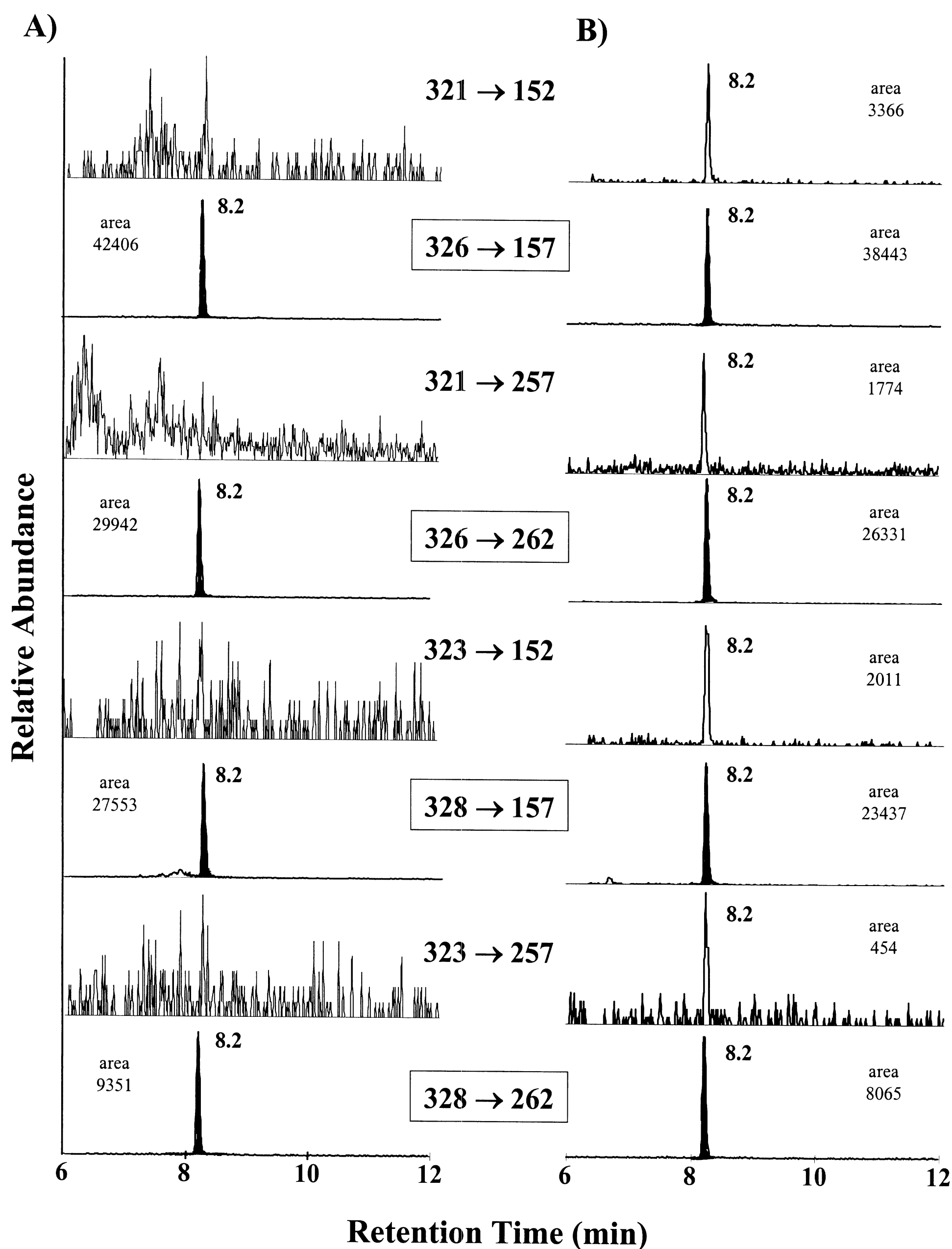

Fig. 3. LC–ESI-MS–MS chromatograms acquired in MRM mode for (A) a CAP free chicken meat and (B) an incurred shrimp sample with a CAP content calculated at 0.04 μg/kg. The different transition reactions of the d_5-CAP internal standard spiked at 0.5 μg/kg are bordered. The first four transition reactions were used for quantitation whereas the other four transition reactions were monitored to check the correct $^{37}Cl/^{35}Cl$ ratio of the two chlorine molecules present in CAP.

Table 2
Performance data of the LC–ESI-MS–MS method for the analysis of CAP in spiked chicken meats

	Fortification levels (μg/kg)					
	0.05		0.10		0.20	
Transition reactions (*m/z*)	321→257	321→152	321→257	321→152	321→257	321→152
Under repeatability conditions[a]						
Overall mean±SD (*n*=18)	0.06±0.01	0.06±0.01	0.11±0.01	0.12±0.01	0.21±0.01	0.21±0.02
Accuracy (%)	129±16	122±11	109±10	117±9	105±8	104±7
Within-day precision (%)	11	9	5	5	7	8
Between-day precision (%)	14	10	8	6	8	8
Under within-laboratory conditions[b]						
Overall mean±SD (*n*=18)	0.06±0.01	0.06±0.01	0.11±0.01	0.11±0.01	0.21±0.02	0.20±0.01
Accuracy (%)	118±18	117±19	111±13	111±11	105±9	101±6
Within-laboratory precision (%)	17	17	13	11	10	7

[a] Six negative chicken meats spiked at each of the three fortification levels and analyzed on three separate occasions by the same operator using the same equipment over a 2-week period.
[b] Six negative chicken meats spiked at each of the three fortification levels and analyzed by three operators using the same equipment over a 1-month period.

fortified at a 0.05 μg/kg level. The S/N ratio observed at this spiking level was calculated at 15±1 and 46±4 ($n=30$) for m/z 321→257 and m/z 321→152, respectively. Thus, the LOD ($S/N=3$) and LOQ ($S/N=10$) were estimated at 0.01 and 0.03 μg/kg for m/z 321→257, respectively; and at 0.003 and 0.01 μg/kg for m/z 321→152, respectively. These values are within the same range as compared to the calculated decision limit (CCα) and the detection capability (CCβ).

Table 3 summarizes the data obtained from different incurred and spiked meat, shrimp and fish samples. Some meat-based commercial products such as fried chicken in breadcrumbs, spring rolls, and petfoods were also analyzed to successfully extend the applicability of the method (data not shown). Valida-

Table 3
CAP content in some incurred and spiked food matrices[a]

	n[b]	Transition *m/z* 321→257	Transition *m/z* 321→152
Incurred samples			
Chicken A	6	0.16±0.01 (10)	0.16±0.01 (8)
Chicken B	6	0.37±0.03 (7)	0.37±0.02 (5)
Fish (dried extract)	3	0.15±0.02 (13)	0.15±0.03 (20)
Shrimps A (dried extract)	3	0.24±0.00 (1)	0.24±0.01 (5)
Shrimps B	4	0.04±0.01 (21)	0.04±0.01 (28)
Spiked samples[c]			
Chicken C	6	0.12±0.01 (9)	0.12±0.01 (7)
Turkey	3	0.11±0.01 (7)	0.11±0.00 (3)
Pork	3	0.12±0.00 (2)	0.11±0.01 (6)
Beef	3	0.11±0.01 (4)	0.11±0.00 (1)
Dab	3	0.14±0.04 (26)	0.13±0.03 (22)
Shrimps B	6	0.16±0.02 (10)	0.15±0.02 (11)

[a] Values are expressed in μg/kg and are mean±SD (% RSD).
[b] Number of replicates.
[c] All samples, but shrimps B, were found negative and further spiked at the 0.10 μg/kg level.

tion of the method in seafood was further performed through participation in a FAPAS® ring test [24]: assigned value in an incurred prawn test material 1.09 μg/kg—our result 1.13 μg/kg, *z*-score 0.1. Moreover, the usage of the LC–ESI-MS–MS method in routine quality control operations was demonstrated in our laboratory in which >200 sample extracts could be injected without loss of LC column performance or excessive pollution of the MS.

4. Conclusion

A quantitative method has been developed for the determination of trace levels of chloramphenicol in different meat and aquaculture products. Tandem mass spectrometry using MRM transitions on analyte-specific fragment ions enable selective and confirmatory detection, without the need of cumbersome chemical derivatization of the parent compound as required in GC-based methods. CAP is quantified with good precision and accuracy at the ng/kg level using stable isotope-labeled CAP as an internal standard. Furthermore, the method has been validated according to EU criteria for the analysis of veterinary drug residues, and successfully applied in routine quality control for the presence of CAP residues in animal-derived foods.

References

[1] Commission Decision of 19 September 2001, Off. J. Eur. Commun. L251 (2001) 11.
[2] Commission Decision of 30 January 2002, Off. J. Eur. Commun. L30 (2002) 50.
[3] J.E. Roybal, in: S.B. Turnipseed, A.R. Long (Eds.), Analytical Procedures For Drug Residues in Food of Animal Origin, Science Technology System, West Sacramento, CA, 1998, p. 227.
[4] http://www.fas.usda.gov
[5] J.J. Allen, J. Assoc. Off. Anal. Chem. 68 (1985) 990.
[6] F.A. Bencsath, S.M. Plakas, A.R. Long, Biol. Mass Spectrom. 23 (1994) 665.
[7] L.A. Van Ginkel, H.J. van Rossum, P.W. Zoontjes, H. van Blitterswijk, G. Ellen, E. van der Heeft, A.P.J.M. de Jong, G. Zomer, Anal. Chim. Acta 237 (1990) 61.
[8] T. Nagata, H. Oka, J. Agric. Food Chem. 44 (1996) 1280.
[9] W.J. Blanchflower, A. Cannavan, R.J. McCracken, S.A. Hewitt, D.G. Kennedy, Residues of veterinary drugs in food, in: N. Haagsma, A. Ruiter, P.B. Czedik-Eysenberg (Eds.), Proceedings Euroresidue II Conference, The Netherlands, Federation of European Chemical Societies, 1993, p. 196.
[10] B. Delépine, P.J. Sanders, J. Chromatogr. 582 (1992) 113.
[11] V. Hormazabal, M.J. Yndestad, J. Liq. Chromatogr. 24 (2001) 2477.
[12] A. Di Corcia, M. Nazzari, J. Chromatogr. A 974 (2002) 53.
[13] R.H. Stadler, New Food 3 (2000) 32.
[14] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Off. J. Eur. Commun. L221/8 (2002).
[15] M.C. Rebstock, H.M. Crooks, J. Controulis, Q.R. Bartz, J. Am. Chem. Soc. 71 (1949) 2458.
[16] M. Rodriguez, D.B. Orescan, Anal. Chem. 70 (1998) 2710.
[17] Council Directive 96/23/EC, Off. J. Eur. Commun. L125 (1996) 10.
[18] J.F.M. Nouws, J. Laurensen, M.M.L. Aerts, Arch. Lebensmittelhyg. 38 (1987) 7.
[19] J.-M. Diserens, C. Renaud-Bezot, M.-C. Savoy-Perroud, Dtsch. Lebensm. Rundsch. 87 (1991) 205.
[20] A.D. Cooper, J.A. Tarbin, W.H.H. Farrington, G. Shearer, Food Addit. Contam. 15 (1998) 637.
[21] H.J. Keukens, W.M.J. Beek, M.M.L. Aert, J. Chromatogr. 352 (1986) 445.
[22] M. Baradat, J. Alary, J.P. Cravedi, Residues of veterinary drugs in food, in: A. Ruiter, P.B. Czedik-Eysenberg (Eds.), Proceedings Euroresidue II Conference, The Netherlands, Federation of European Chemical Societies, 1993, p. 160.
[23] T.L. Li, Y.J. Chung-Wang, Y.C. Shih, J. Food Sci. 67 (2002) 21.
[24] FAPAS Veterinary Drug Residues Report 0241 (2002).