Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1067 (2005) 85–91
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Quantitative determination of four nitrofuran metabolites in meat by isotope dilution liquid chromatography–electrospray ionisation–tandem mass spectrometry

Pascal Mottier*, Seu-Ping Khong, Eric Gremaud, Janique Richoz, Thierry Delatour, Till Goldmann, Philippe A. Guy

Department of Quality and Safety Assurance, Nestlé Research Centre, Nestec Ltd., P.O. Box 44, Vers-chez-les-Blanc, 1000 Lausanne 26, Switzerland

Abstract

A confirmatory method based on isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed for the low-level determination of residues of four nitrofuran veterinary drugs in meat, e.g., furazolidone, furaltadone, nitrofurantoin, and nitrofurazone. The procedure entails an acid-catalysed release of protein-bound metabolites, followed by their in situ conversion into the 2-nitrobenzaldehyde (NBA) imine-type derivatives. Liquid–liquid extraction and clean-up on a polymeric solid phase extraction cartridge are then performed before LC–MS/MS analysis by positive electrospray ionisation (ESI) applying multiple reaction monitoring of three transition reactions for each compound. Reliable quantitation is obtained by using one deuterated analogue per analyte (d_4-NBA derivative) as internal standard (IS). Validation of the method in chicken meat was conducted following the European Union (EU) criteria for the analysis of veterinary drug residues in foods. The decision limits (CCα) were 0.11–0.21 μg/kg, and the detection capabilities (CCβ) 0.19–0.36 μg/kg, thus below the minimum required performance limit (MRPL) set at 1 μg/kg by the EU. 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.

Keywords: Nitrofuran antibiotics; Meat; Liquid chromatography mass spectrometry; Semicarbazide; Furazolidone; Furaltadone; Nitrofurantoin; Nitrofurazone

1. Introduction

Nitrofurans are Shiff's base derivatives of nitrofuraldehyde, known to be effective against a variety of pathogenic bacteria. The most common nitrofurans are furazolidone, nitrofurantoin, nitrofurazone and furaltadone (Fig. 1), which have been widely used as feed additives in food-producing animals like cattle, swine, poultry, cultured fish and shrimps, for prophylactic and therapeutic treatment of diseases causally link to bacteria or protozoa. Nitrofuran-type drugs are rapidly metabolised in vivo, leading to a significant decrease in plasma levels of the parent compound [1,2] and a concomitant accumulation of some metabolites in proteins, generating stable adducts that can be detected in tissues over periods up to 30 days [3]. The stability of (tissue-bound) residues of nitrofuran drugs was extensively studied, and it was demonstrated that upon common food preparation techniques like cooking, baking, grilling and microwaving, the residues were stable or at least did not degrade to a significant extent [4]. Following concerns about their carcinogenicity and mutagenicity, these nitrofurans were placed in Annex IV of Regulation 2377/90/EEC (1995), which prohibits the use of certain chemicals in food-producing animals in the European Union (EU), and in products from third countries intended for the EU market. The EU Commission Decision of 13 March 2003 [5] has set up a MRPL at 1 μg/kg (for each nitrofuran metabolite) for any method dealing with the analysis of nitrofurans in poultry meat and aquaculture products. Analysis of these drugs is generally based on the acid-catalysed release

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

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.08.160

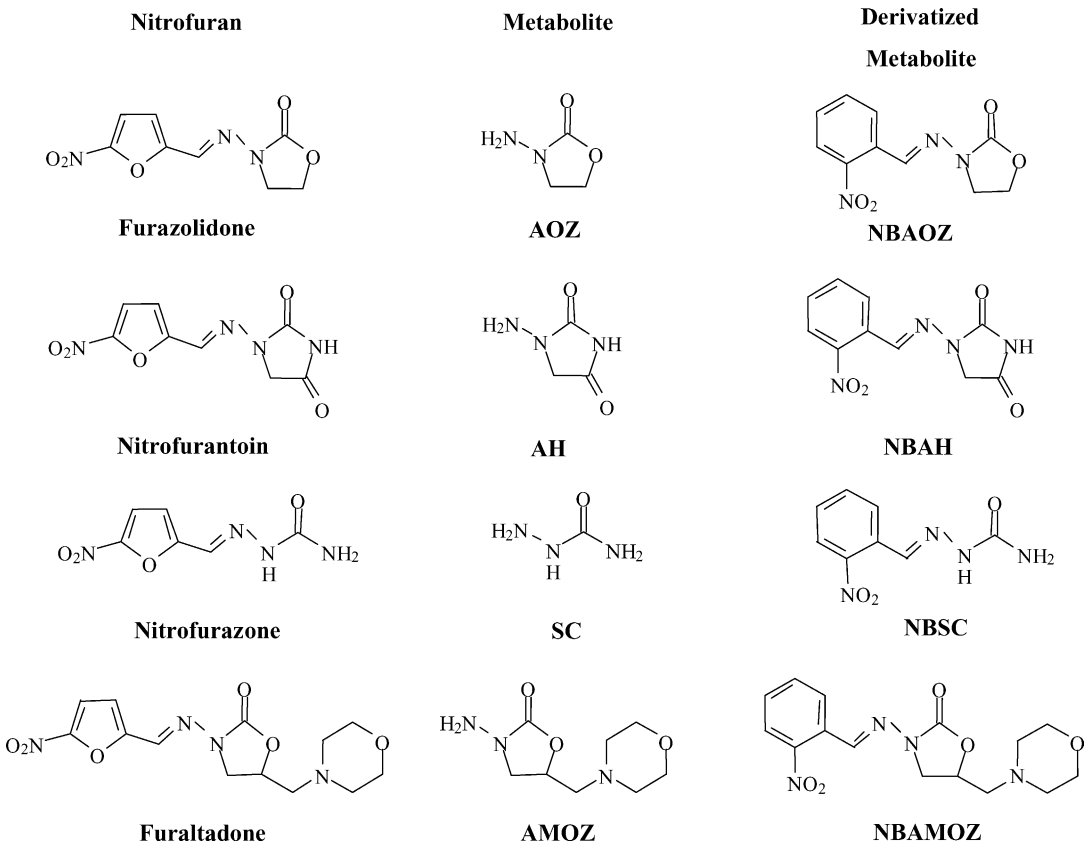

Fig. 1. Chemical structures of nitrofuran parent drug compounds along with their metabolites and NBA derivatives.

of protein-bound metabolites, followed by their conversion into NBA imine-type derivatives (Fig. 1). Due to their higher sensitivity and selectivity, and considering the low MRPL set by the EU, MS techniques and particularly tandem MS are attractive methods to confirm the presence of nitrofuran residue in foods of animal origin. Currently, only few MS-based published methods allow the simultaneous determination of the four nitrofuran metabolites. Leitner et al. [6] describes a liquid chromatography–electrospray ionisation tandem mass spectrometry (LC–ESIMS/MS) procedure able to detect nitrofuran metabolites in muscle tissue at limits of quantitation (LOQ) ranging from 2.5 to 10 μg/kg. Semicarbazide as its 4-nitrobenzaldehyde derivative was used as sole IS. Edder et al. [7] proposed a similar procedure with LOQs ranging from 0.5 to 5 μg/kg in poultry and shrimps. No IS was used during this study, but matrix effects and recoveries of derivatisation and extraction steps were taken into account through quantitation by matrix-matched calibrations. However, considering (a) the commonly observed signal suppression effect in ESI, (b) the large difference in MS response of the four derivatised nitrofuran metabolites [6], and (c) the time-consuming approach of matrix-matched calibration curve when analysing a wide range of foodstuffs, the best approach for a reliable quantitation remains the use of one isotope-labelled IS for each analyte under survey. In a previous paper [8], we described the synthesis of the four stable ^{13}C-isotope-labelled NBA-derivatised nitrofuran metabolites, using $^{13}C_6$-toluene as starting reagent.

The present work describes the full validation of a confirmatory and quantitative method for the determination of four nitrofuran metabolites in chicken meat employing isotope dilution LC–ESIMS/MS (see [9]). The four ISs used were d_4-NBA derivatives of these residues (instead of ^{13}C-NBA derivatives due to the commercial availability of the deuterated NBA).

2. Experimental

2.1. Chemicals and reagents

3-Amino-2-oxazolidinone (AOZ), semicarbazide (SC) hydrochloride, 1-aminohydantoin (AH) hydrochloride and NBA were obtained from Sigma-Aldrich (Buchs, Switzerland). 3-Amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) and NBA derivatives of nitrofuran metabolites (NBAH, NBAMOZ, NBAOZ, and NBSC) were supplied by Witega (Berlin, Germany). 3,4,5,6-d_4-NBA (chemical purity > 99%, isotopic purity > 99%) was from Toronto Research Chemical (North York, Canada). Individual d_4-NBA derivatives of nitrofuran metabolites were obtained by condensation of 3,4,5,6-d_4-NBA with an

excess of AH, AMOZ, AOZ or SC, and were further purified by preparative LC [8]. All other solvents and reagents as well as Lichrolut EN solid phase extraction (SPE) cartridges (200 mg) were obtained from Merck (Darmstadt, Germany).

2.2. Standard solutions

Individual stock standard solutions of NBAH, NBAMOZ and NBAOZ (2 mg/ml) were prepared by dissolution in methanol, and that of NBSC (0.2 mg/ml) in acetonitrile/distilled water (50:50, v/v). d_4-NBX stock solutions were prepared in a similar way. All solutions were stored at −20 °C. Precise concentrations of these solutions were verified by UV spectrophotometry using the molar extinction coefficients [8] after proper dilution with water. Calibration solutions containing both labelled and unlabelled derivatives of nitrofuran metabolites were prepared by successive dilutions of the stock standard solutions in water. Aliquots of calibration solutions were stored at −20 °C until use.

2.3. Food samples

Meats (chicken, pork) were mainly of Asian origin. Samples, typically 200 g, were first minced using a kitchen homogeniser (Moulinex, France), then sub-sampled (20 g) and stored at −20 °C in airtight containers until analysis. For method validation, chicken meat from animals grown under strictly controlled conditions ("bio" label) was used.

2.4. Sample preparation

2.4.1. Extraction

A well homogenised sample (5 g) was weighed into a 50-ml Falcon polypropylene tube (Becton Dickinson, Le Pont-de-Claix, France). Hydrochloric acid (0.125N, 25 ml) was added, and the mixture thoroughly homogenized by means of an Ultra-Turrax. NBA (50 mM solution in DMSO, 250 μl) was added and the slurry incubated in a water bath at 37 °C overnight. After cooling, the mixture was neutralized at pH 7.1 ± 0.2 with both sodium hydroxide (1 M, 2.5 ml) and dipotassium hydrogenphosphate (1 M, 1 ml), and the final pH adjusted with NaOH (1 M). The slurry was further fortified with an aqueous solution containing the four d_4-labelled nitrofuran derivatised metabolites (50 ng/ml each, 500 μl) and allowed to stand for 15 min after mixing. Ethyl acetate (15 ml) was added to the slurry before being thoroughly hand-shaked for 2 min and centrifuged at 3600 × *g* for 10 min at room temperature (centrifuge Mistral 2000, MSE Scientific Instrument, England). The organic phase was collected into a 15-ml Falcon tube, evaporated to dryness under a stream of nitrogen at 40 °C and the dry residue reconstituted with distilled water (3 ml). A further washing step of the aqueous extract with hexane (1 ml) was performed to remove the lipidic components.

2.4.2. Clean-up

A Lichrolut EN SPE cartridge was conditioned successively with ethyl acetate (9 ml), methanol (3 ml), and distilled water (9 ml). The previous aqueous extract was loaded onto the cartridge and after penetration, the column was washed successively with distilled water (3 ml) and hexane (3 ml), and dried by sucking through air (Visiprep vacuum manifold, Supelco, Switzerland) after each solvent addition. The derivatised nitrofuran metabolites were finally eluted with ethyl acetate (9 ml) and collected in a 15-ml Falcon tube. The eluate was evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted with acetonitrile/distilled water (30:70, v/v, 250 μl). The resulting solution was filtered through a 0.2-μm nylon filter (Nalgene, Rochester, NY, USA) directly into an HPLC vial.

2.5. LC–ESIMS/MS

Analyses were performed on a C_{18} SymmetryShield LC 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 LC 200 Micro Pump series system (Norwalk, CT, USA). The mobile phase was as follows: solvent A: water containing acetic acid 0.025% (v/v); solvent B: acetonitrile. The linear gradient program was: 0–0.5 min 10% B; 0.5–3 min from 10 to 35% B; 3–8 min 35% B; 8–9 min from 35 to 100% B; 9–12 min 100% B; 12–13 min from 100 to 10% B; and 13–22 min 10% B at a flow rate of 0.3 ml/min. The injection volume was 50 μl and between injections, the needle was rinsed with a solution of water/acetonitrile 1:1 (v/v). The entire LC flow was directed into the MS detector between 0.5 and 9 min using a VICI diverter (Valco Instrument Co., Houston, TX, USA). MS detection was done in the positive ESI mode on a Sciex API 3000 triple stage quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray ionisation source. Nitrogen was used for the gas nebuliser, TurboIonSpray gas, and curtain gas at a pressure of 15 psi, and a flow rate of 7.5 l/min and 10 ml/min, respectively. The source block temperature was set at 350 °C and the electrospray capillary voltage to 5.5 kV. Nitrogen was used as collision gas at a pressure set of 5 mTorr. Data acquisition was performed using the Sciex Analyst software alternating three transition reactions for each nitrofuran derivatised metabolite and two transition reactions for its corresponding d_4-labeled analogue used as IS. The dwell time for each transition reaction was set at 25 ms. The various transition reactions and their collision energies are shown in Table 1.

2.6. Quantitation

Derivatised nitrofuran metabolites were quantified by means of an external calibration curve (response ratio versus amount ratio) constructed in water at six calibration levels ranging from 0 to 4 ng injected on-column (covering the

Table 1
Transition reactions monitored by LC–ESIMS/MS (collision energies in eV are within brackets), and peak area ratios along with their limit of acceptance according to [9]

Analyte	Transition Reactions (*m/z*) used for:		Peak area ratio ± limit (%)
	Quantitation	Analyte confirmation	
NBAMOZ	335 → 291 (18)	335 → 128 (30)	0.50 ± 20
		335 → 262 (24)	0.28 ± 25
d_4-NBAMOZ	339 → 295 (18)	339 → 266 (24)	0.29 ± 25
NBSC	209 → 166 (15)	209 → 134 (17)	0.32 ± 25
		209 → 192 (17)	0.84 ± 20
d_4-NBSC	213 → 170 (15)	213 → 196 (17)	0.91 ± 20
NBAH	249 → 134 (19)	249 → 104 (29)	0.52 ± 20
		249 → 178 (20)	0.24 ± 25
d_4-NBAH	253 → 138 (19)	253 → 108 (29)	0.60 ± 20
NBAOZ	236 → 134 (18)	236 → 101 (18)	0.12 ± 30
		236 → 149 (20)	0.18 ± 30
d_4-NBAOZ	240 → 138 (18)	240 → 153 (20)	0.12 ± 30

0–4 μg/kg range). The concentration of isotopically labelled ISs was fixed at 5 ng injected. The stability of the calibration solutions was verified by checking the slope of the calibration curves, which should be 1.0 ± 0.2. Calibration standards were injected before and after each analytical series, and both data sets were used to establish the calibration curves. The linearity was checked by calculating the standard deviation of the average of response factors (peak area ratios divided by the corresponding analyte concentration ratios of all standards), which should be <15% to assume a linear response [10]. Final results were expressed as the free nitrofuran metabolites, by multiplying values obtained from the calibration curves by a factor of 0.602, 0.361, 0.464 and 0.434 for AMOZ, SC, AH and AOZ, respectively.

2.7. Confirmation criteria

Nitrofuran metabolites were considered as positively identified in meat samples when: (a) the ratio of the retention time of the analyte to that of the corresponding IS corresponded to that of the calibration solution within a ±2.5% tolerance, and (b) the peak area ratios of the various transition reactions were within the tolerances set by the EU criteria [9] and shown in Table 1.

2.8. Method validation

Recoveries and precisions (within- and between-day) were calculated from the analysis of six blank chicken meats fortified with each derivatised nitrofuran metabolite at three fortification levels (1.0, 1.5 and 2.0 μg/kg) and performed by the same operator on three separate occasions in a two-week period. Fortification was done after the hydrolysis step. The individual fortification levels converted to the free nitrofuran metabolites are given in Table 2. Within-laboratory precision was obtained by following the same protocol but analyses were performed by two different operators on one occasion in a one-month period. Repeatability at the 95% confidence level was deduced from the within-day precision using an expansion factor of 2.77. Similarly, the intermediate reproducibility was calculated from the within-laboratory precision using the same expansion factor.

2.9. Measurement of uncertainties

The estimation of measurement uncertainty was based on the results of in-house testing of spiked samples. Its significant relevance corresponds to the range over which analytical results will fall provided that the analytical system is "under control". The analytical parameters taken into account were precision (repeatability, intermediate reproducibility), trueness and calibration data (standard preparation, linear regression). Each step involved in the sample preparation (i.e., weight of test portion, preparation and dilution of ISs, volumes, injection, etc.) was assigned to a defined uncertainty and summed as a final value (cause and effect diagrams). The final uncertainty was calculated using an expansion coefficient of 2, which represents a confidence interval of 95% [11–13].

3. Results and discussion

3.1. Method development

Our extraction method is essentially that of Leitner et al. [6] with some modifications. The significant difference concerns the quantitation approach, as each derivatised nitrofuran metabolite was quantitated against its own deuterated analogue. Preliminary trials were conducted to assess the stability of the underivatised nitrofuran metabolites in chicken meat samples during the hydrolysis step. Thus, unbound metabolites (e.g., AH, AMOZ, AOZ, and SC) were spiked at

Table 2
Performance data of LC–ESIMS/MS analysis of nitrofuran residues in chicken meat

	AH			AMOZ			AOZ			SC		
	0.46[a]	0.70[a]	0.93[a]	0.60[a]	0.90[a]	1.20[a]	0.43[a]	0.65[a]	0.87[a]	0.36[a]	0.54[a]	0.72[a]
Under repeatability conditions[b]:												
Overall recovery (%) (n = 18)	110	119	115	104	108	104	122	123	117	90	91	85
Within-day precision (%)	6	8	6	4	5	2	4	7	3	13	6	2
Between-day precision (%)	7	8	14	8	8	9	6	6	10	20	12	21
r (μg/kg)[c]	0.09	0.18	0.18	0.07	0.15	0.06	0.06	0.15	0.08	0.12	0.08	0.04
Under intermediate reproducibility conditions[b]:												
Overall recovery (%) (n = 12)	110	121	98	94	101	91	114	118	103	89	93	85
Within-laboratory precision (%)	12	15	12	6	11	5	6	12	5	24	21	35
iR (μg/kg)[d]	0.17	0.36	0.31	0.09	0.27	0.15	0.09	0.27	0.12	0.22	0.28	0.56
$\pm U$ (μg/kg)[e]	0.10	0.18	0.20	0.07	0.19	0.16	0.10	0.21	0.08	0.13	0.14	0.26

[a] Fortification levels (μg/kg).
[b] For details, see Section 2.8.
[c] Repeatability at 95% confidence level.
[d] Intermediate reproducibility at 95% confidence level.
[e] Expanded uncertainty at 95% confidence level.

the 5 μg/kg level in blank chicken meat, and the derivatisation agent was added either immediately or at different times after spiking. Analysis was then conducted as described in Section 2. Results showed that AH and SC were relatively stable in this matrix whereas a large proportion of AOZ and AMOZ (up to 40%) was trapped or destroyed even immediately after the spiking of nitrofuran metabolites (Fig. 2). Leitner et al. [6] reported losses of about 30% for all four nitrofuran metabolites spiked in similar conditions. These data have a major implication on the nature of the ISs to be used. Indeed, for a correct quantitation, both the chemical and the chromatographic behaviours of the IS are expected to be as similar as possible than those of the endogenous compound. Considering the loss of AMOZ and AOZ in chicken meat, the supplementation of deuterated metabolites as ISs is not appropriate. Indeed, the deuterated AOZ and AMOZ will not have the same loss during sample work-up as the protein-bound metabolites and this would falsify the analyte/IS concentration ratio (i.e., the quantitation would be over evaluated). Consequently, the choice of deuterated derivatised metabolites (d_4-NBA-metabolites) was retained. Moreover, these ISs were added after the hydrolysis and neutralisation steps to provide an accurate quantitation of the released metabolites in the extract and also to avoid a possible clip-off of IS molecules, which would have generated false positive results (considering that the deuterium species are located on the NBA part of the molecule). The extraction procedure described here provides absolute yields of ca. 23% for NBAH, 40% for NBAMOZ, 43% for NBAOZ and 45% for NBSC. These yields were calculated from the ratio of the peak area of the IS in the sample to the mean peak area of the IS of the corresponding standard calibration and thus included the loss of analyte during the extraction/purification steps as well as ion suppression effects.

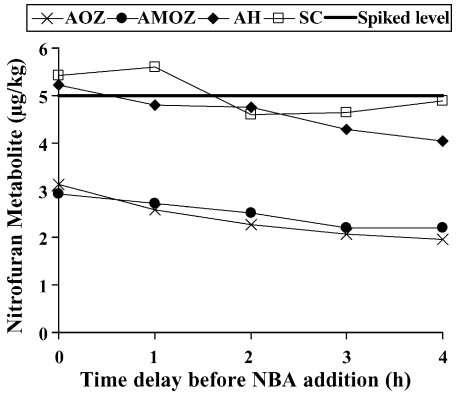

Fig. 2. Stability of nitrofuran metabolites spiked at the 5 μg/kg level in chicken meat prior to NBA addition.

3.2. LC–ESIMS/MS

The best MS sensitivity for the four analytes was obtained using the positive ESI mode. Thus, protonated molecules $(M + H)^+$ were obtained for NBAH, NBAMOZ, NBAOZ and NBSC at m/z 249, 335, 236 and 209, respectively. The collision induced dissociation (CID) fragmentation pathways of the derivatised nitrofuran metabolites have been described previously by Delatour et al. [8] and will not be elaborated further. Chromatograms of an incurred meat are shown in Fig. 3. A good separation of the four compounds was obtained and no significant drift of their retention time was observed over time ($n > 200$). No interfering peak was observed at the transition reactions used to depict the presence of unlabeled analytes in a blank matrix containing only the ISs. Matrix effects were assessed by building matrix-matched calibration curves and comparing their slope with that of solvent-based curves. Differences were observed, with relative slope ratio of 26, 11, 17 and 18% for NBAH, NBAMOZ, NBAOZ and NBSC, respectively, meaning that a complete compensation of the matrix effect by the ISs

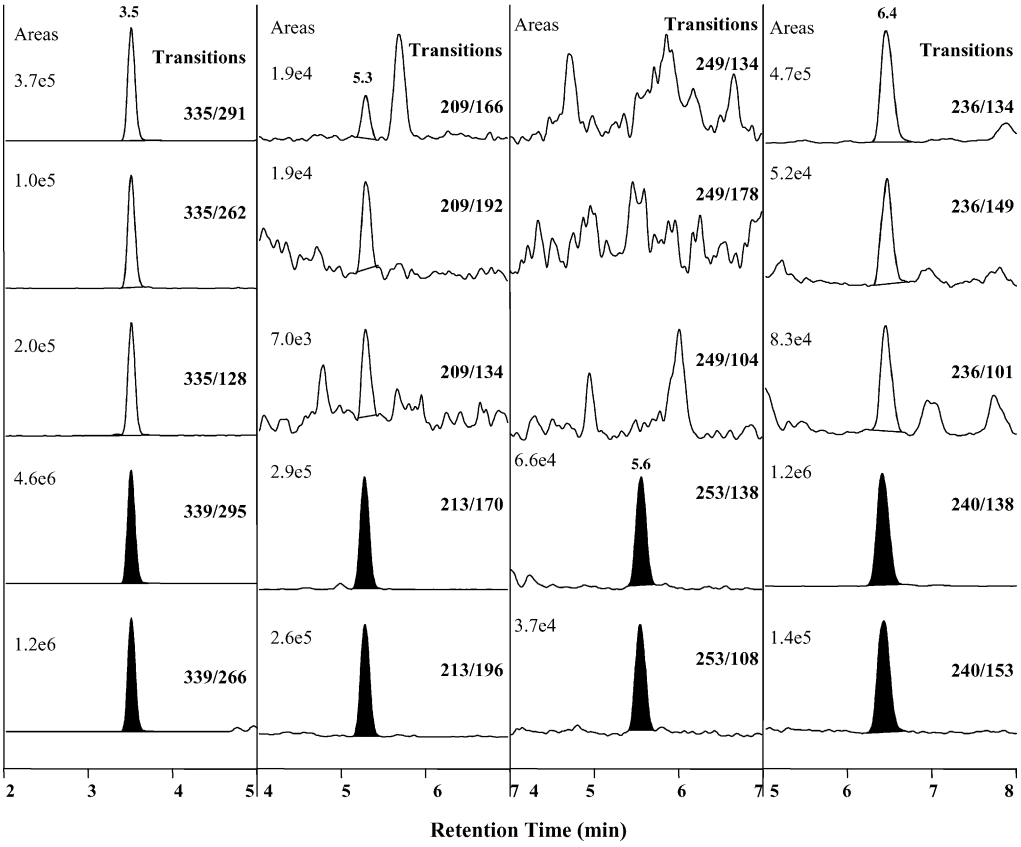

Fig. 3. MRM chromatograms obtained by LC–ESIMS/MS in the positive mode of an incurred chicken extract containing 0.21 μg/kg of AMOZ, 0.09 μg/kg of SC (<CCα) and 0.74 μg/kg of AOZ. From left to right are peaks of NBAMOZ, NBSC, NBAH, and NBAOZ. IS peaks are darkened.

was not achieved. However, final results obtained by both types of calibration curves were not significantly different (data not shown). Consequently, only water-based calibration curves were considered for convenience during this validation.

3.3. Method performance characteristics

The selectivity of this method is warranted by the use of three transitions reactions for each analyte, which count for 5.5 identification points (IPs), as defined by the EU criteria [9]. For nitrofurans, which belong to the Annex IV (banned compounds with no MRL) of Council Regulation 2377/90, the minimum number of IPs to consider for their quantification is four. Consequently, our method fulfils this requirement. Table 2 summarizes the performance data of this procedure (after conversion of values into nitrofuran metabolites concentrations). Within- and between-day precisions were found satisfactory for the four compounds under survey (≤21%). Recoveries ranged between 85 and 122%. As well, within-laboratory precisions were acceptable for AH, AMOZ and AOZ (≤15%) but less satisfactory for SC (≤35%). Intermediate reproducibility (iR) values were systematically higher than the uncertainty data supporting the fact that the iR, in this validation scheme, is a more realistic indicator of the "true" uncertainty than that obtained by calculation.

CCα and CCβ limits were calculated following the calibration curve procedure as explained in the EU guidelines [9]. This procedure was preferred over the signal-to-noise (S/N) ratio method (i.e., analysis of 20 blank materials and measurement of the S/N ratio at the retention time where the analyte is expected), as this method only gives a time-related image of the CCα and CCβ limits. On the other hand, the calibration curve procedure makes use of values obtained by different operators over a one-month period, and thus is a better approach to take into account instrument instability and/or instrument cleanliness. CCα and CCβ limits by the calibration curve procedure were thus 0.21 and 0.36; 0.12 and 0.21; 0.11 and 0.19; 0.20 and 0.34 μg/kg for AH, AMOZ, AOZ and SC, respectively. To check these calculated limits (which still are extrapolated data), further spiking experiments were performed at the CCα values and the results obtained confirmed the relevance of these limits. Consequently, the proposed method enables a selective and confirmatory detection at levels below the MRPL set at 1 μg/kg. Validation of the method in chicken meat was further performed through participation in a ring-test organised by the Swiss Federal Office of Public Health, showing our data with *z*-score values ranging within −1.05 to −0.36.

3.4. Analysis of incurred meats

The usage of this LC–ESIMS/MS method in quality control operations was demonstrated in our laboratory in which >100 meat-based samples were injected without loss of LC column performance or excessive pollution of the MS detector. AH was not detected in these samples, confirming previous findings that the parent drug nitrofurantoin is less frequently used in veterinary medicine [7]. AOZ, AMOZ and SC were respectively detected in 15% (max., 193 μg/kg; median, 0.6 μg/kg), 10% (max., 9 μg/kg; median, 0.5 μg/kg) and 21% (max., 19.6 μg/kg, median, 10.9 μg/kg) of the meat-based products analysed. However, the presence of SC in meat may originate from other sources than from the use of the parent drug nitrofurazone. Pereira et al. [14] demonstrated that some meats coated with flour were contaminated with SC, the origin of which was due to azodicarbonamide used in the cereal industry as a dough maturing and bleaching agent. Furthermore, SC has also been found in foods after treatment with sodium hypochlorite, related to certain food processing methods used for disinfection or bleaching [15]. Thus, considering these recent findings, SC is not an established marker for nitrofurazone administration in animal food production.

4. Conclusion

A quantitative LC–ESIMS–MS method has been developed for the determination of four nitrofuran metabolites in meat-based products and validated following the EU criteria for the analysis of veterinary drug residues. The method clearly demonstrates good accuracy and the ability to quantify the presence of these residues at levels below the MRPL. Our limited survey on different meats shows that furazolidone and furaltadone are the main antibiotics used to treat animals, whereas the presence of SC in meat could have other origins than the illegal use of nitrofurazone. The applicability of this method was also demonstrated during the analysis of other food matrices such as fish and egg. Furthermore, a complete validation of the determination of nitrofuran residues in honey is currently under finalization.

References

[1] G.R. Mir, R.P. Uppal, Indian J. Anim. Sci. 54 (1984) 888.
[2] L.H.M. Vroomen, M.C.J. Berghams, P. van Leeuven, T.B.D. van der Struijs, P.H.U. de Vries, H.A. Kuiper, Food Addit. Contam. 3 (1986) 331.
[3] L.A.P. Hoogenboom, M.C.J. Berghmans, T.H.G. Polman, R. Parker, I.C. Shaw, Food Addit. Contam. 9 (1992) 623.
[4] R.J. McCracken, D.G. Kennedy, J. Chromatogr. B 691 (1997) 87.
[5] Commission Decision 2003/181/EC, 13 March 2003, amending decision 2002/657/EC as regards the setting of minimum performance limits (MRPLs) for certain residues in food animal origin, Off. J. Eur. Communities L71 (2003) 17.
[6] A. Leitner, P. Zöllner, W. Lindner, J. Chromatogr. A 939 (2001) 49.
[7] P. Edder, S. Vargas, D. Ortelli, C. Corvi, Clin. Chem. Lab. Med. 41 (2003) 1608.
[8] T. Delatour, E. Gremaud, P. Mottier, J. Richoz, F. Arce Vera, R.H. Stadler, J. Agric. Food Chem. 51 (2003) 6371.
[9] 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. Communities L221 (2002) 8.
[10] M. Rodriguez, D.B. Orescan, Anal. Chem. 70 (1998) 2710.
[11] Guide to the Expression of Uncertainty, ISO, Geneva, Switzerland, 1st ed., 1993.
[12] Quantifying Uncertainty in Analytical Measurement, Eurachem/CITAC guide, in: S.L.R. Ellison, M. Roesslein, A. Williams (Eds.), 2nd ed., 2000.
[13] Protocol for uncertainty evaluation from validation data. In VAM Project 3.2.1 Development and Harmonisation of Measurement Uncertainty Principles, LGC/VAM, 2000.
[14] A.S. Pereira, J.L. Donato, G. de Nucci, Food Addit. Contam. 21 (2004) 63.
[15] R. Gatermann, P. Hach, L. Hartig, K. Hoenicke, M. Mandix, S. Otte, Poster Presented at the International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 2003.