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Determination of nitrofuran residues in avian eggs by liquid chromatography–UV photodiode array detection and confirmation by liquid chromatography–ionspray mass spectrometry

Rosa Draisci[a,*], Luigi Giannetti[a], Luca Lucentini[a], Luca Palleschi[a], Gianfranco Brambilla[a], Luigi Serpe[b], Pasquale Gallo[b]

[a]*Istituto Superiore di Sanità, V. le Regina Elena 299, 00161 Rome, Italy*

[b]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Napoli, Italy*

Abstract

A high-performance liquid chromatographic–UV–Vis photodiode-array detection (HPLC–DAD) method for the determination of nitrofuran residues, nitrofurazone, furazolidone and furaltadone, in chicken eggs is described. Confirmation of the identity of nitrofurazone, furazolidone and furaltadone was performed by high-performance liquid chromatography–mass spectrometry (HPLC–MS) using an atmospheric pressure ionization (API) source and an ionspray interface. The nitrofuran residues were extracted from eggs with acetonitrile and the extracts purified by liquid–liquid partitioning. Analytes were chromatographed isocratically with an octadecylsilyl (ODS) column and an UV–Vis detector set at 362 nm and identified by comparing the retention times and UV–Vis spectra of the sample peaks with the reference compounds. The HPLC–DAD limit of detection based on a signal-to-noise ratio (S/N) of 3, was estimated to be 2.5 $\mu g\,kg^{-1}$ for nitrofurazone and furazolidone and 5.0 $\mu g\,kg^{-1}$ for furaltadone. The ionspray HPLC–MS was carried out on the purified extracts. The HPLC–MS method involved the separation of analytes on a C_{18} column with acetonitrile–water (50:50, v/v), containing 1 m*M* ammonium acetate and 0.025% acetic acid, with selected-ion monitoring (SIM) of only protonated molecules, $[M+H]^+$ of the analytes. The overall average recovery in nitrofuran fortified eggs was 85.3±3.8% for nitrofurazone, 88.1±3.9% for furazolidone and 87.1±3.7% for furaltadone. The HPLC–MS limit of detection, based on a S/N of 3, was estimated to be 3.2, 1.6 and 1.0 $\mu g\,kg^{-1}$ for nitrofurazone, furazolidone and furaltadone, respectively. HPLC–MS has shown itself to be a sensitive, selective and rapid method and was successfully used for the confirmation analysis of nitrofurazone, furazolidone and furaltadone in avian eggs for regulatory purposes. © 1997 Elsevier Science B.V.

Keywords: Eggs; Food analysis; Nitrofurans; Furazolidone; Nitrofurazone; Furaltadone

1. Introduction

Nitrofurazone {2-[(5-nitro-2-furanyl)methylene]-hydrazinecarboxamide}, furazolidone [3-(5-nitrofurfurylideneamino)-2-oxazolidinone] and furaltadone [5-morpholinomethyl-3-(5-nitrofurfurylideneamino)-2- oxazolidinone] belong to the class of nitrofurans, with a molecular structure characterized by the presence of a 5-nitro group. This 5-nitro group has a broad antimicrobial activity [1] and for this reason nitrofurans are widely used as veterinary drugs. Nitrofurans are mainly administered to food-producing animals as feed additives and are usually given to prevent and control several bacterial and protozoan infections, such as fowl cholera, coccidiosis blackheads and swine enteritis in poultry and pigs [1]

*Corresponding author.

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and mastitis in dairy cattle [1–4]. However, the use of nitrofurans can leave drug residues in foods of animal origin. Concerning the toxicological properties of nitrofurans, mutagenic activity has been observed in yeasts and fungi and in bacterial and sub-mammalian systems [5–7]. Moreover, nitrofurans have been shown to be tumorigenic in rats and mice [7] and cytotoxic to mammalian cells in culture [8]. The effects of nitrofurans seem to be related to the compound itself, and also to metabolite formation [9]. Owing to the rapid metabolism in the gastrointestinal tract [10], furazolidone administration to pigs and poultry results in very low residual concentrations of the drug in muscle and liver [11]. Studies undertaken on pigs have demonstrated that metabolites of furazolidone covalently bind to cellular protein in vivo [12]. The potential toxicological risk to the consumer by protein-bound metabolites of furazolidone has been shown [13]. Higher furazolidone levels have been found in eggs from treated hens since no further metabolism occurs during the time of egg development [14].

The use of nitrofurans, including furazolidone, in veterinary practice has became questionable in the European Union (EU). A provisional maximum residue limit (MRL) of 5 $\mu g\ kg^{-1}$ was established by the EU for furazolidone [15] and the total amount of nitrofuran residues in animal derived foodstuffs should not exceed this level. However, at the time of writing, this provisional limit has expired and nitrofurans are considered as drugs of Annex IV of EEC Reg. 2377/90 [16,17], i.e. they cannot be used in food-producing farm animals and, thus, no MRL values can be set for them. From 1997, drugs in Annex IV will no longer be registered for use in any farm animals in the EU [17].

Several methods have been reported for the detection of nitrofurazone, and/or furazolidone and/or furaltadone in feeds and biological matrices; the analysis being performed by colorimetry [18], thin-layer chromatography (TLC) [19] and gas chromatography (GC) [20,21]. Reversed-phase (RP) high-performance liquid chromatography (HPLC) with UV–Vis detection has been the most frequently employed technique to determine nitrofurans [4,19,22–26]. However, because of the complexity of the matrices examined and the presence of dissociation products [19,27], HPLC methods using retention time and fixed wavelength data, might not be sufficiently specific to perform certain identification of the analytes. In order to confirm nitrofuran residue identity, UV–Vis photodiode array detection (DAD) has been carried out by comparing the spectra of the standards with those of the analytes [27,28].

Confirmation methods for residues of veterinary drugs in foods should quantitate and confirm the structure of a veterinary drug residue and mass spectrometry (MS) is sufficiently specific in itself to provide unambiguous identification and quantitation of a compound and is indicated as the technique of choice in confirmation analysis of veterinary drug residues in foods [29]. MS coupled with HPLC (HPLC–MS) has great potential for performing drug residue identification in complex biological matrices and recently, identification of furazolidone in swine serum and avian eggs through atmospheric pressure chemical ionization (APCI) flow injection analysis (FIA)–MS [26] and determination of furazolidone in porcine tissues by thermospray LC–MS has been reported [30].

Ionspray [31] is a recently introduced atmospheric pressure ionization (API) method for combined HPLC–MS and is highly sensitive to polar compounds that are ionised in solution [31]. The ionspray HPLC–MS technique performs a mild ionization process, which can generate the intact protonated molecules $[M+H]^+$ of the analytes, thus providing the molecular mass information which is considered the most important criterion for identification of the analyte. Of the new technologies, ionspray HPLC–MS is becoming a common analytical tool due to the recent availability of relatively inexpensive instruments.

In this work, we investigated the possibility of using HPLC–MS coupled via an ionspray interface, to confirm nitrofurazone, furazolidone and furaltadone in eggs, which had previously been analysed by the HPLC–DAD method.

2. Experimental

2.1. Reagents

All the solvents were LC reagent grade, unless

otherwise stated. Acetonitrile, dichloromethane, methanol, *n*-hexane were purchased from Carlo Erba (Milan, Italy), sodium chloride, anhydrous sodium sulfate, sodium acetate, glacial acetic acid (RPE quality) were also from Carlo Erba. Water was purified in a Milli-Q system (Millipore, Bedford, MA, USA). Nitrofurazone, furazolidone and furaltadone were provided by Sigma (St. Louis, MO, USA). Individual standard stock solutions (1000 $\mu g\,ml^{-1}$) of nitrofurazone, furazolidone and furaltadone were obtained by dilution of the pure compounds with methanol. Standard curve solutions in the range 0.04–2.50 $\mu g\,ml^{-1}$ and standard fortification solution (5.00 $\mu g\,ml^{-1}$) were prepared by pooling aliquots of nitrofuran individual standard stock solutions and diluting with methanol. All the above procedures were carried out under shaded light, due to the light sensitivity of nitrofurans. Solutions were stored in the dark at −35°C and were stable for at least one month. Nitrofuran concentrations in unknown samples were determined by interpolation from the standard calibration curve.

2.2. Samples

In order to evaluate drug recoveries, avian egg, chicken liver and muscle blank control samples were obtained from nitrofuran free animals from a farm under study. Standard fortification solution was added to the blank control samples to obtain fortified control samples containing 5.0 μg, 20.0 μg and 50.0 μg of nitrofurazone, furazolidone and furaltadone per kilogram of sample. Recovery was determined by the comparison of the determined amount of each analyte extracted from biological samples with that of the standard curve solution.

Samples of eggs destined for human consumption were collected at different farms whereas chicken tissue samples were collected from different farms and slaughter-houses as part of the National Program of Residue Control [32].

2.3. Sample preparation

Nitrofurazone, furazolidone and furaltadone are light sensitive, therefore sample extraction and clean-up procedures were performed under shaded light. Sample preparation was performed using the Petz procedure [22] with slight modifications. Firstly, 30 ml of acetonitrile was added to 10.0 g of homogenized shelled eggs, chicken liver or muscle, in a polyethylene centrifuge tube and the samples were blended by Ultraturrax at low speed (2 min). The extract was centrifuged at 1000 *g* for 5 min, and the supernatant was transferred to a separatory funnel. Then, 10 ml of sodium chloride (10%, w/v) and 50 ml of dichloromethane were added and the separatory funnel was shaken for a few minutes. After phase separation, the lower organic phase was transferred to a round-bottomed flask by filtration on 5 g of anhydrous sodium sulphate and evaporated to dryness using a rotary vacuum evaporator with a temperature controlled bath (45°C). Finally, the residue was redissolved in 1 ml of a mixture of methanol–20 m*M* sodium acetate (pH 4.6)–acetonitrile (50:40:10, v/v/v), extracted three times with 1 ml of *n*-hexane to remove lipid components, and injected into the HPLC system.

2.4. HPLC–DAD analysis

Liquid chromatography was performed by a Waters Model 600E instrument, equipped with a Mod. 991 photodiode array detector, and a 745 Data Module (Waters Associates, Milford, MA, USA). The chromatography was carried out on a Spherisorb ODS2 S5 (5 μm particle size, 150×4.6 mm I.D.) stainless steel column (Phase Sep, Queensferry, UK) and fitted with a μBondapak C_{18} (10×4.6 mm I.D.) guard column (Lab-Service Analytica, Bologna Italy). Separations of nitrofurazone, furazolidone and furaltadone were performed at 25°C with a 20 m*M* sodium acetate (pH 4.6)–acetonitrile (79: 21, v/v) mobile phase at 1 $ml\,min^{-1}$ flow-rate. A Rheodyne 7125 (Cotati, CA, USA) injection valve equipped with a 50-μl sample loop was used for sample injection. HPLC–DAD analysis was performed by fixing the detector wavelength at 362 nm and the peak spectra were collected in the range between 220 and 550 nm.

The HPLC–DAD calibration curves were obtained by injecting solutions at 0.10, 0.25, 0.50, 1.00 and 2.50 $\mu g\,ml^{-1}$ into the HPLC standard curve. The measurements were repeated five times and averaged, and calibration curve parameters calculated by ordinary linear regression. Nitrofuran quantitative

determination for all samples was worked out by interpolating chromatographic peak areas.

2.5. HPLC–MS analysis

The analyses were performed on a Varian 9010 pump (Palo Alto, CA, USA) liquid chromatograph. A Rheodyne 7125 injection valve equipped with a 20-μl sample loop was used. Separation of nitrofurazone, furazolidone and furaltadone was carried out on a column packed with Supelcosil L C_{18}-DB (Bellefonte, PA, USA) (5 μm particle size, 250×4.6 mm I.D.) operated at 25°C, under isocratic conditions with a mobile phase of acetonitrile–water (50:50, v/v) containing 1 m*M* ammonium acetate and 0.025% acetic acid at a flow-rate of 0.6 ml min^{-1}. The column effluent was split to obtain a flow-rate of 30 μl min^{-1} in MS.

Mass spectral analysis was performed on a PE-SCIEX API I single-quadrupole (PE-Sciex, Thornhill, Canada). The mass spectrometer was equipped with an atmospheric pressure ionization (API) source and an ionspray interface set at a voltage of 5500 V; the orifice potential voltage (OR) was set at 60 V. Full-scan mass spectra were acquired in positive-ion mode over the mass range *m/z* 50–400. For targeted analysis and maximum sensitivity, the selected-ion monitoring (SIM) mode on the $[M+H]^+$ was implemented at *m/z* 199, at *m/z* 226 and at *m/z* 325, for nitrofurazone, furazolidone and furaltadone, respectively. Standard curve solutions were obtained by the SIM HPLC–MS analyses of standard curve solutions containing 0.04, 0.10, 0.25, 0.50 and 1.00 μg ml^{-1} of each nitrofuran. The *m/z* values indicated both in text and in figures are in all cases the truncated values of the more accurate experimental values.

3. Results and discussion

A characteristic HPLC–DAD chromatogram of three nitrofuran standards monitored at 362 nm, and their associated UV–Vis spectra is shown in Fig. 1.

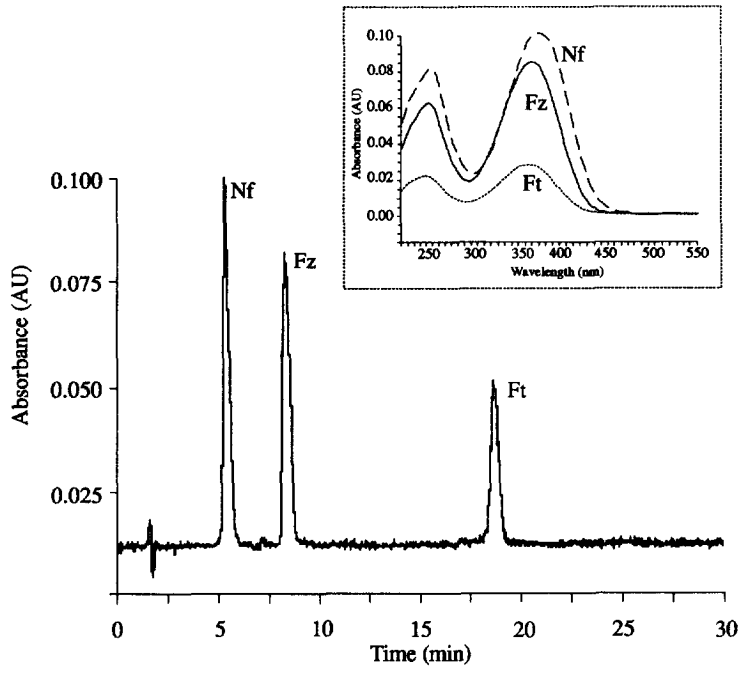

Fig. 1. HPLC–DAD chromatogram of a standard mixture (1.0 μg ml^{-1}) of nitrofurans, with their respective UV–Vis spectra. Peaks: Nf=nitrofurazone; Fz=furazolidone; Ft=furaltadone. Conditions: Spherisorb ODS 2 S5 column (150×4.6 mm, 5 μm); mobile phase: 20 m*M* sodium acetate (pH 4.6)–acetonitrile (79:21, v/v); flow-rate 1 ml min^{-1}; injection volume 50 μl; detection at 362 nm.

The analytes were well separated in 20 min as sharp and symmetrical peaks. Retention times were 5.3, 8.2 and 18.5 min, for nitrofurazone, furazolidone and furaltadone, respectively, and the repeatability of the retention times was less than 1% for 5 injections.

The HPLC–DAD limit of detection was estimated to be 2.5 μg kg^{-1} for nitrofurazone and furazolidone and 5.0 μg kg^{-1} for furaltadone. Linearity of the photometric detector response to nitrofurazone, furazolidone and furaltadone in standard solutions was verified in the range 0.10–2.50 μg ml^{-1} (i.e. 5–125 ng injected) and correlation coefficients ranged from 0.9969 to 0.9981. Representative chromatograms of a blank control and a fortified (20.0 μg kg^{-1}) control egg sample are reported in Fig. 2. Blank control samples did not elute peaks at the

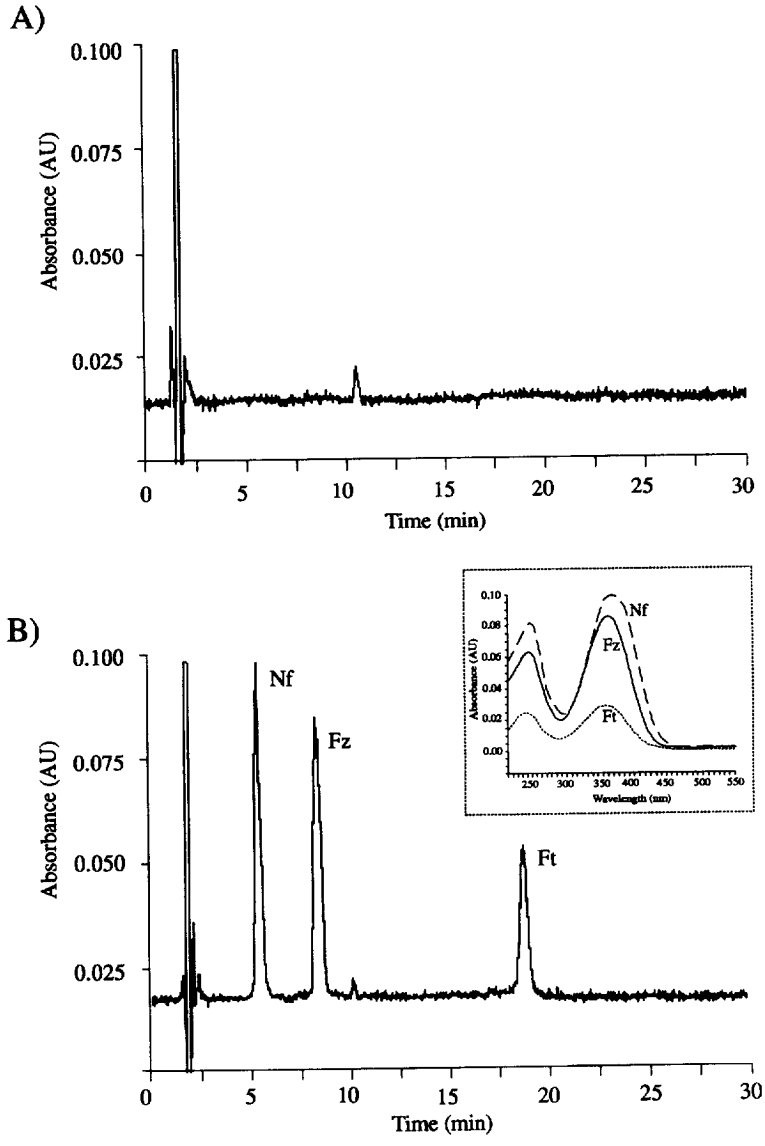

Fig. 2. HPLC–DAD chromatograms of (A) blank and (B) fortified (20 μg kg^{-1}) egg control samples and related UV–Vis spectra. Peaks: Nf=nitrofurazone; Fz=furazolidone; Ft=furaltadone. Conditions as described in Fig. 1.

retention times of nitrofurazone, furazolidone and furaltadone. Identities of nitrofuran peaks were verified on the fortified control samples on the basis of the retention times and the UV–Vis spectra, which were compared with those of authentic standards. Recovery and precision data for the HPLC–DAD method were generated each day for 2 days from the analysis of triplicated blank control egg samples and fortified control egg samples, containing 20 μg and 50 μg of nitrofurazone, furazolidone and furaltadone per gram of sample. The overall recovery (means±S.D.) was 82.8±4.4, 85.3±5.4 and 84.1±6.6 for nitrofurazone, furazolidone and furaltadone, respectively. The precision of the measures depended on the content of drug in the samples. Using data from the separate extractions of the same sample, a relative standard deviation (R.S.D.) of 6.91% for nitrofurazone, 7.72% for furazolidone and 10.12% for furaltadone were found for 20 μg of nitrofuran per kilogram of egg sample. The R.S.D. values for the egg samples fortified at 50 $\mu g\,kg^{-1}$ were 4.71%, 6.31% and 5.96%, for nitrofurazone, furazolidone and furaltadone, respectively.

Although the HPLC–DAD method was shown to be a rapid procedure to verify the presence of nitrofuran residues in a large number of egg samples, deviations in the UV–Vis spectrum of the furazolidone sample peak, as compared with the standard spectrum, were observed in some samples. Fig. 3 shows the HPLC–DAD chromatogram of an egg sample exhibiting a peak at the retention time of furazolidone and its associated UV–Vis spectrum. This spectrum was not congruent in the UV region with the furazolidone standard spectrum, thus indicating the probable presence of coeluting substances at the furazolidone retention time. Interference due to the matrix and/or to dissociation products from furazolidone has been noticed by other researchers [19,27]. Our study demonstrated that the interference can compromise the effectiveness of the confirmation DAD assay.

HPLC–MS was therefore tried in order to achieve the unambiguous identification of nitrofurans. Although HPLC–MS methods with the ionspray interface have not been previously considered for the determination of nitrofurans, we thought that its high sensitivity to polar compounds which are ionised in solution [31] would probably make it suitable for this task.

FIA–MS experiments were performed in order to determine suitable ionspray parameters for absolute sensitivity and S/N ratio, as well as to select the appropriate ions for SIM HPLC–MS experiments. Fig. 4 shows the ionspray mass spectra (mass range m/z=150–400) of nitrofurazone, furazolidone and furaltadone as obtained by FIA in positive ion mode.

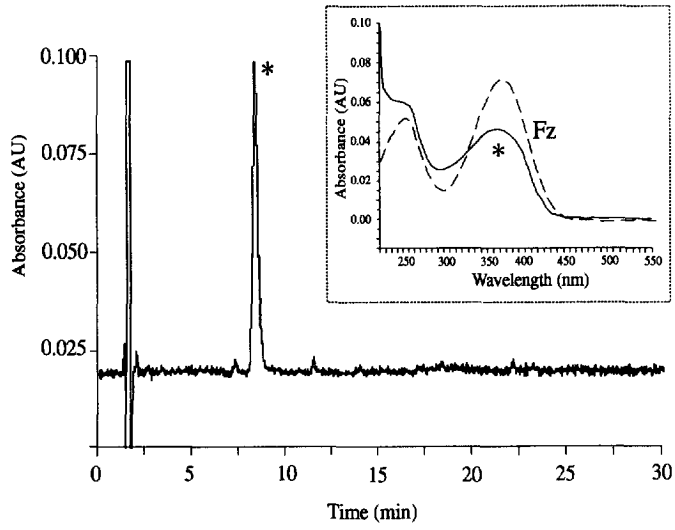

Fig. 3. HPLC–DAD chromatogram of an egg sample and UV–Vis spectrum of the impure LC peak (*) compared with UV spectrum of a furazolidone standard (Fz). Conditions as described in Fig. 1.

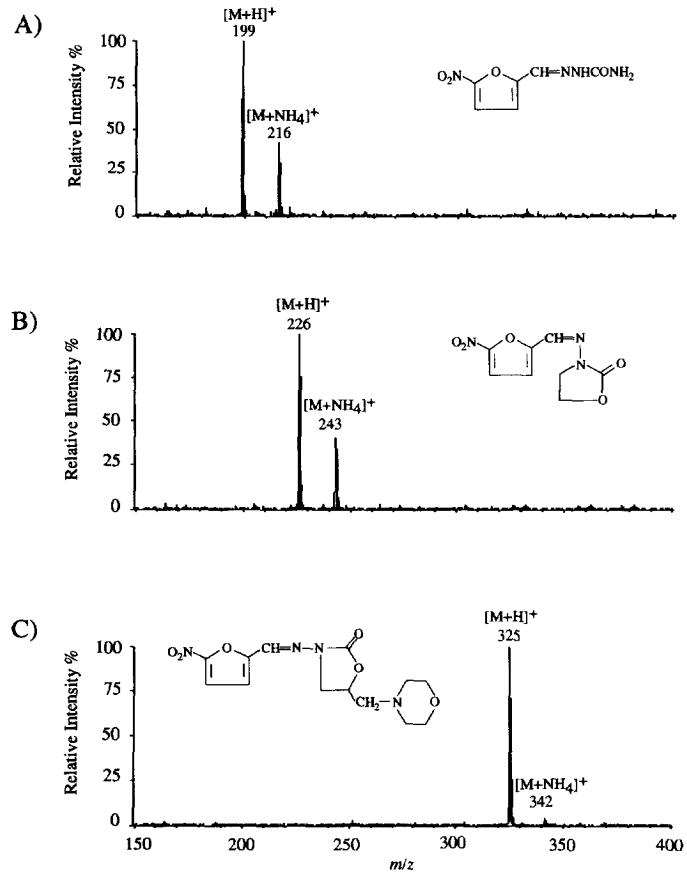

Fig. 4. Full-scan (m/z 150–400) positive ion mass spectra of individual standard solutions (1.0 μg ml^{-1}) of nitrofurans: (A) nitrofurazone; (B) furazolidone; (C) furaltadone. Conditions: flow injection analysis (FIA) mobile phase: acetonitrile–water (50:50, v/v) containing 1 m*M* ammonium acetate and 0.025% acetic acid; flow- rate 30 μl min^{-1}; injection volume 20 μl.

These spectra are simple, exhibiting an abundant peak due to the protonated molecules at m/z 199, at m/z 226 and at m/z 325, for nitrofurazone, furazolidone and furaltadone, respectively. The ammonium adducts, $[M+NH_4]^+$, at m/z 216, at m/z 243 and at m/z 342 for nitrofurazone, furazolidone and furaltadone, respectively, were also observed. The intensity of both nitrofurazone and furazolidone adduct ions $[M+NH_4]^+$ was about 40% of the respective base peak, whereas the relative intensity of the furaltadone adduct ion was below 10%.

The final coupling of HPLC to the MS system was then carried out. Separations were obtained on a C_{18} column at a flow-rate of 0.6 ml min^{-1} and with a mobile phase consisting of acetonitrile–water, (50:50, v/v) containing 1 m*M* ammonium acetate and 0.025% acetic acid. Column effluent was split to achieve a flow-rate of 30 μl min^{-1} to the MS system. Under these conditions an excellent signal and separation were obtained for nitrofurazone, furazolidone and furaltadone, which were eluted at 5.2, 6.3 and 7.7 min, respectively (Fig. 5). The HPLC–MS method was linear over the concentration range 0.04–1.00 μg ml^{-1} with correlation coefficients greater than 0.9999 for all the analytes.

Recovery and precision data for the HPLC–MS method were generated each day for two days from the analysis of triplicate blank control and fortified control egg samples containing 5.0 μg and 20.0 μg of nitrofurazone, furazolidone and furaltadone per kilogram of sample. Representative chromatograms of blank control and fortified control egg samples are

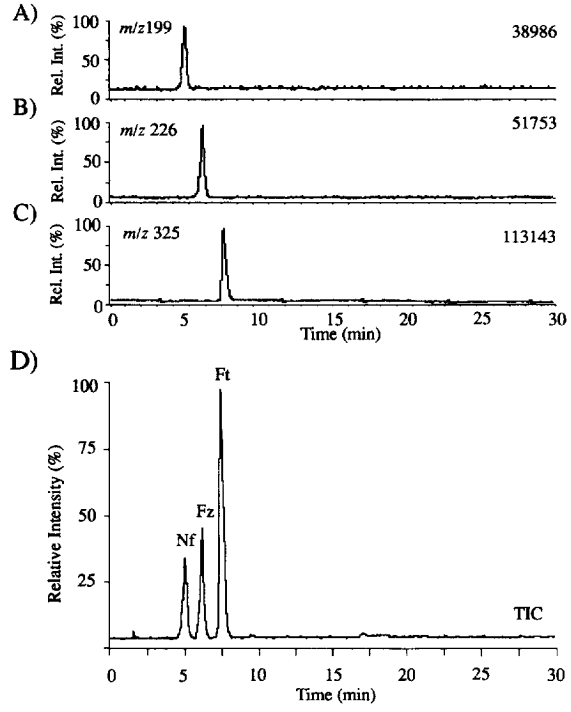

Fig. 5. (A to C) Extracted ion current profiles and (D) total ion current (TIC) profile of an ionspray HPLC–MS analysis of a standard solution (0.5 μg ml^{-1}) of nitrofurans. Peaks: Nf= nitrofurazone ($[M+H]^+$=*m/z* 199); Fz=furazolidone ($[M+H]^+$= *m/z* 226); Ft=furaltadone ($[M+H]^+$=*m/z* 325). Conditions: Supelcosil LC_{18} DB column (250×4.6 mm, 5 μm); mobile phase: acetonitrile–water (50:50, v/v) containing 1 m*M* ammonium acetate and 0.025% acetic acid; flow-rate 0.6 ml min^{-1} (30 μl min^{-1} split to MS); injection volume 20 μl; SIM on the ions *m/z* 199, *m/z* 226 and *m/z* 325.

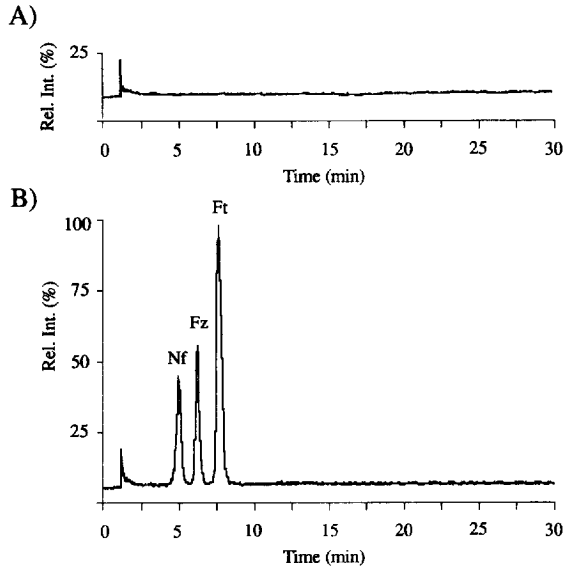

Fig. 6. HPLC–MS chromatograms of (A) blank and (B) fortified (20 μg kg^{-1}) egg control samples. Peaks: Nf=nitrofurazone; Fz= furazolidone; Ft=furaltadone. Conditions as described in Fig. 5.

reported in Fig. 6. No interference peaks were observed around the retention time of nitrofurans in the blank control samples. The average recoveries and precision data for the HPLC–MS method are reported in Table 1. Averages of recovery for the three analytes ranged from 85.0–88.9%. The precision was also satisfactory at these levels with R.S.D. values ranging from 3.97–6.24. The same procedure was used to validate HPLC–MS method for the determination of nitrofuran residues in chicken tissues; the results are reported in Table 1. Averages of recovery at the tested fortified levels were above 83.2% for liver and 83.7% for muscle tissue. The precision was also satisfactory in the tested matrices, with R.S.D. values less than 6.83% and 7.48 for liver and muscle tissue, respectively.

The lowest level validated by HPLC–MS for the three analytes was 5.0 μg kg^{-1}; however, the limit of detection, based on a S/N of 3, was estimated to be 3.2, 1.6 and 1.0 μg kg^{-1} for nitrofurazone, furazolidone and furaltadone, respectively.

The HPLC–MS method was then used for confirmation analyses of nitrofurazone, furazolidone and furaltadone in egg samples [32], which had previously been analysed by the HPLC–DAD method. Identification of the analytes in every HPLC–DAD positive sample was confirmed by HPLC–MS. The HPLC–MS was used to identify furazolidone in the egg samples for which the HPLC–DAD analyses had shown a peak at the retention time of furazolidone with an associated UV–Vis spectrum that was not congruent in the UV region with the furazolidone standard spectrum. The HPLC–MS analysis of these samples were first performed in full-scan (*m/z* 50–400) positive ion mode and the resulting total ion current profiles showed interesting peaks only at *m/z* 226, which was the signal for the $[M+H]^+$, of furazolidone (data not shown). Fig. 7 shows the SIM HPLC–MS chromatogram of an egg sample for

Table 1
Accuracy and precision of the HPLC–MS method in fortified egg, liver and muscle control samples

Sample	Spike level ($\mu g\ kg^{-1}$)	Nitrofurazone			Furazolidone			Furaltadone		
		Measured concentration[a] (mean±S.D.) ($\mu g\ kg^{-1}$)	Recovery[a] (%)	R.S.D. (%)	Measured concentration[a] (mean±S.D.) ($\mu g\ kg^{-1}$)	Recovery[a] (%)	R.S.D. (%)	Measured concentration[a] (mean±S.D.) ($\mu g\ kg^{-1}$)	Recovery[a] (%)	R.S.D. (%)
Eggs	5	4.2±0.3	85.0	6.24	4.4±0.2	87.3	4.97	4.9±0.2	86.4	4.94
	20	17.1±0.7	85.7	3.97	17.8±0.6	88.9	3.40	17.6±0.6	87.8	3.63
Liver	5	4.2±0.3	83.2	5.93	4.2±0.2	84.5	5.37	4.3±0.3	85.1	6.83
	20	17.4±0.7	86.9	4.09	17.5±0.9	87.5	5.11	17.3±0.7	86.7	4.12
Muscle	5	4.2±0.3	83.7	7.48	4.2±0.2	84.0	5.35	4.3±0.2	86.5	5.58
	20	16.8±0.9	84.1	5.61	17.4±1.0	86.9	5.93	17.1±0.8	85.6	4.85

[a] Each value is the average of 6 extractions (3/day for 2 days).

which the unambiguous identification of furazolidone by HPLC–DAD was hampered by coeluting interference that altered the UV–Vis spectrum of furazolidone. The detection of a peak at the same retention time of furazolidone standard in the SIM HPLC–MS trace at m/z 226, corresponding to the $[M+H]^+$ of the analyte, unequivocally confirmed furazolidone residue (87.6 $\mu g\,kg^{-1}$) in the egg sample. Nitrofurazone and furaltadone were not detected in this sample.

HPLC–MS was also used to analyse chicken tissue samples collected as part of the National Plan of Residue Control. In no cases were nitrofuran positive samples detected. This is consistent with the negligible residue of the nitrofuran parent drug found in animal tissues [11] due to the extensive metabolism of the drug [7,10]

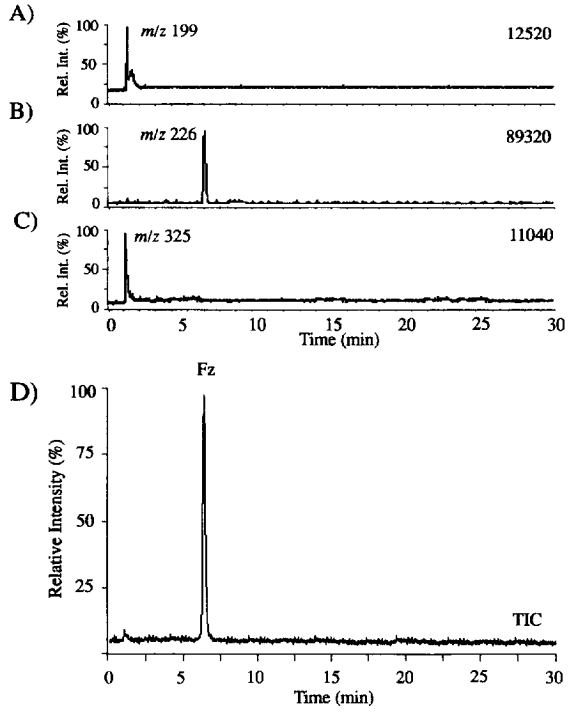

Fig. 7. (A to C) Extracted ion current profiles and (D) total ion current (TIC) profile of an ionspray HPLC–MS analysis of an extract of egg sample, Fz=furazolidone (87.6 $\mu g\,kg^{-1}$). Conditions as described in Fig. 5.

4. Conclusions

The aim of this research was to develop a confirmation method in order to unambiguously identify and quantitate nitrofurans in eggs, due to the uncertain results achieved by the conventional HPLC method.

Ionspray HPLC–MS was used here for the first time for the determination of nitrofurans in eggs. Our study showed that this method is sensitive, selective and rapid and is an excellent form of confirmation analysis in cases of false negatives by HPLC–DAD. Although the latter technique is fast and relatively economic, it is in some cases affected by matrix interference and/or dissociation products from the parent drug, which can lead to false negatives.

This study also proved the suitability of the ionspray HPLC–MS method for the determination of nitrofurans in chicken tissues. The use of HPLC–MS to analyse a number of samples collected under the National Plan of Residue Control resulted in no nitrofuran positive samples. These results suggest that in order to monitor nitrofuran administration, the metabolites of the drug should be identified in animal tissues rather than the parent drug itself.

Future work will focus on the application of the ionspray HPLC–MS method to other veterinary drug residues and other biological matrices, as well as the identification of biotransformation products of nitrofurans in chicken tissues, by HPLC with mass spectrometric and tandem mass spectrometric detection.

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