Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1067 (2005) 93–100
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Simple confirmatory assay for analyzing residues of aminoglycoside antibiotics in bovine milk: hot water extraction followed by liquid chromatography–tandem mass spectrometry

Sara Bogialli, Roberta Curini, Antonio Di Corcia*, Aldo Laganà, Monica Mele, Manuela Nazzari

Dipartimento di Chimica, Università "La Sapienza", Piazza Aldo Moro 5, 00185 Roma, Italy

Available online 11 November 2004

Abstract

A simple, selective and sensitive procedure for determining nine widely used aminoglycoside antibiotics (AGs) in bovine whole milk is presented. It is based on matrix solid-phase dispersion with heated water, at 70 °C, as extractant followed by liquid chromatography (LC)–tandem mass spectrometry (MS) using an electrospray ion source. After acidification and filtration, 0.2 ml of the aqueous extract was injected into the LC column. MS data acquisition was performed in the multi reaction monitoring mode, selecting two (three, when possible) precursor ion > product ion transitions for each target compound. Analyte recoveries ranged between 70 and 92%. Using aminosidine (an AG not used in veterinary medicine) as surrogate internal standard, the accuracy of the method at three spike levels varied between 80 and 107% with R.S.D. not larger than 11%. The limits of quantification were between 2 ng/ml (apramycin) and 13 ng/ml (streptomycin). They are well below the tolerance levels set by both the European Union and the U.S. Food and Drug Administration.

Keywords: Aminoglycoside antibiotics; Bovine; Milk; Matrix solid-phase dispersion; Heated water; LC–MS/MS

1. Introduction

The aminoglycosides (AGs) are broad-spectrum antibiotics that have bactericidal activity against some Gram-positive and many Gram-negative organisms (Fig. 1). Their clinical use has been limited by side effects of nephrotoxicity and ototoxicity [1,2]. In veterinary medicine and animal husbandry, AGs are widely used in the treatment of bacterial infections, e.g. bacterial enteritis and mastitis, and have been added to feeds for prophylaxis and to promote growth [3,4]. No AGs are currently permitted for use as growth promoters in the European Union. Those most commonly used as therapeutic agents are gentamicins C, neomycin B, dihydrostreptomycin and streptomycin. Others may include apramycin and spectinomycin. To ensure the safety of food for consumers, both the European Union (EU) Commission [5] and the U.S. Food and Drug Administration (FDA) [6] have laid down maximum residue limits (MRLs) of several AGs in milk (Table 1).

Sensitive and reliable methods are necessary for the analysis of veterinary drug residues, including AGs, in food products of animal origin. They are used by statutory agencies responsible for ensuring food safety and for enforcing the regulations governing the use of drugs in food-producing animals. Microbiological assays are by far the most commonly used methods for antibiotic residue analysis in food because they can cheaply detect all of the different classes of antibiotics. However, it is well recognized these methods need to be supported by highly selective and sufficiently sensitive chemical methods. When liquid chromatography (LC) is coupled with mass spectrometry (MS), it becomes the most powerful technique for detecting antibiotic residues in food. LC–MS satisfies the important requirement set by the Commission Decision 2002/657/EC [7] stating "Methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods".

* Corresponding author. Tel.: +39 06 4958251; fax: +39 06 490631.
E-mail address: antonio.dicorcia@uniroma1.it (A. Di Corcia).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.10.033

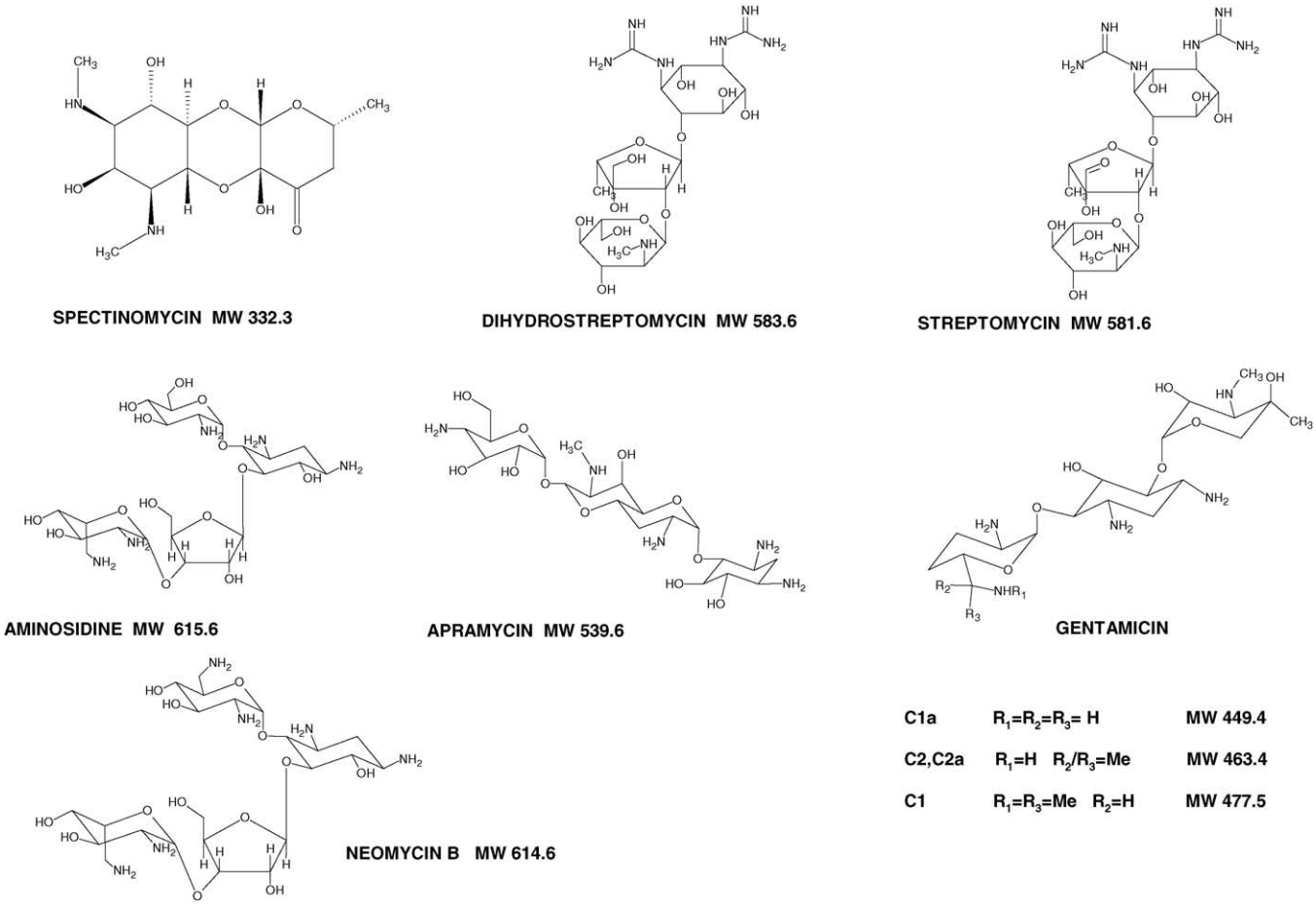

Fig. 1. Chemical structures and molecular weights of selected aminoglycoside antibiotics.

Despite their large use in veterinary medicine, only two confirmatory methods based on LC–MS and aimed at detecting one [8] or two [9] AGs in milk are quoted in the literature. Carson and Heller [8] described a procedure for confirming residues of spectinomycin in milk based on ion-pair reversed-phase solid-phase extraction and LC–MS with a quadrupole ion trap. However, the authors concluded that additional refinement was necessary for correct quantification of the spectinomycin in bovine raw milk. Two years later, Heller et al. [9] modified the above procedure for developing a confirmatory method aimed at quantifying gentamicin and neomycin in milk. Even in this case, the authors concluded that their method could not be amenable to a quantitative regulatory procedure without further refinement.

Table 1
Maximum residue limits (ng/ml) set by the European Union (EU) and U.S. Food and Drug Administration (FDA) for selected aminoglycoside antibiotics in bovine milk

	EU[a]	FDA[b]
Spectinomycin	200	–
Dihydrostreptomycin	200	125
Streptomycin	200	125
Aminosidine	–	–
Apramycin	NP[c]	–
Gentamicin C[d]	100	–
Neomycin B	1500	150

[a] From [5].
[b] From [6].
[c] NP, not permitted in lactating cattle.
[d] As sum of four components of the gentamicin complex.

Recently, we have developed two simple, rapid and sensitive confirmatory methods for determining sulfonamide antibacterials [10] and the two amphoteric penicillins [11], i.e. amoxicillin and ampicillin, in bovine milk. These procedures are based on extraction by the matrix solid-phase dispersion (MSPD) technique and LC–MS equipped with a single quadrupole [10] or triple quadrupole [11] for final extract analysis. The uniqueness of these methods is that the analytes are extracted with heated water. Over conventional extraction with organic solvents, the use of heated water as extractant offers distinct advantages in that no use of toxic and expensive solvents is made and selective extraction can be achieved by suitably adjusting the extraction temperature. Selective extraction reflects in that many endogenous compounds are not co-extracted with the analytes so that little manipulation of the extract needs before LC–MS final analysis.

The aim of this work has been that of developing a confirmatory method for determining nine AGs in bovine raw milk

at the EU and FDA regulatory levels by suitably modifying the procedure mentioned above.

2. Experimental

2.1. Materials

Spectinomycin (SPC), dihydrostreptomycin (DHS), streptomycin (STR), apramycin (APR), gentamicin (GC) C1, C1a, C2, C2a, neomycin B (NEO) and the surrogate internal standard (IS), aminosidine (AMN), were obtained from Sigma–Aldrich (Milwaukee, WI). We prepared 1 mg/ml stock solutions of each aminoglicoside by dissolving 10 mg of the pure analytical standards in 10 ml water/methanol (50:50, v/v) solution. For recovery studies, the same composition was used to obtain a final concentration of 12 μg/ml of a single working composite standard solution, and a 24 μg/ml solution of the IS from the stock solution. When unused, all the above solutions were stored at 4 °C.

Ethylenediaminotetracetic acid sodium salt (Na_2EDTA) was purchased from Fluka AG, Buchs, Switzerland. Heptafluorobutyric acid (HFBA) was from Sigma–Aldrich. Methanol "Plus" of gradient grade was obtained from Carlo Erba, Milan, Italy. Sand (Crystobalite, 40–200 mesh size) was from Fluka. Before use, metal impurities on the surface sand were blocked by treatment with EDTA. In particular, 60 g of sand was packed in a plastic tube (2.6 mm i.d.) and 120 ml of 0.2 mol/l EDTA were passed through the tube at flow-gravity. Partial drying of the sand was carried out by vacuum with a water pump. Thereafter, sand was completely dried in an oven at 100 °C.

2.2. Samples

Whole pasteurized bovine milk samples used for this study were collected from local markets. The samples used for recovery and sensitivity studies were previously determined to be free of the antibiotics considered.

2.3. Extraction apparatus

The design of the homemade extraction apparatus used in this work was very similar to that shown in a previous paper [12], with the exception that nitrogen was bubbled in water to eliminate any trace of dissolved oxygen and the analyte-containing water leaving the extraction cell was collected in a calibrated glass tube instead of a sorbent cartridge. A 16 cm × 8.3 mm i.d. stainless-steel column was used as extraction cell.

2.4. Sample preparation and extraction

For recovery studies, milk samples were spiked with known variable amounts of AGs. Under continuous agitation, 15 min were allowed for equilibration at room temperature. Twelve grams of EDTA-treated sand were taken and poured in a porcelain mortar containing 3 ml of milk and the mixture was blended with the pestle for less than 15 min, until an apparently dry material was obtained. This material was then packed into an extraction cell, taking care to tap the tube to avoid loose packing of the particles. Any void space remaining after packing the solid material was filled with sand. A stainless steel (2 μm pore size) and a polyethylene (20 μm pore size) frits were located respectively above and below the packing. The tube was then put into the oven and heated at 70 °C for 5 min. Four millilitres of water was then passed through the cell at 1 ml/min flow rate to extract the analytes and, if present, the surrogate internal standard. The choice of the parameters mentioned above for extracting the analytes resulted from preliminary experiments (see below) showing that this situation offered maximum recovery of the analytes and a restricted number of co-extractives. When experiments were performed to assess the extraction yield by heated water, 600 ng of the IS was added to the extract. To make aqueous extract similar at the LC mobile phase composition, the pH of the extract was adjusted to 4.1 with 1 mol/l HFBA and then filtered through a glass filter (pore size 1.2 μm, 25 mm d, Carlo Erba, Milano, Italy). After filtration, a completely uncolored and transparent solution was obtained. By following the procedure described above, the guard column was replaced with a new one after about 150 injections of milk extracts. Finally, 0.2 ml of the milk extract was injected into the LC column.

2.5. Instrumental analysis

The liquid chromatograph consisted of a Waters pump (Model 1525 μ, Milford, USA), a 200 μl-injection loop, and Alltima HP HL 5-μm C-18 guard (7.5 mm × 4.6 mm i.d.) and analytical (250 mm × 4.6 mm i.d.) columns (Alltech) thermostated at 35 °C and was interfaced to a benchtop triple-quadrupole mass spectrometer (Model Micromass 4 MICRO API, Waters). Mobile phase component A was 1 mmol/l HFBA in methanol and component B was aqueous 1 mmol/l HFBA. At 1.0 ml/min, the mobile phase gradient profile was as follows (t in min): t_0, A = 20%; t_{16}, A = 60%; t_{17}, A = 100%; t_{20}, A = 100%; t_{21}, A = 20%; t_{30}, A = 20%. Analytes retention times varied ≤0.5% over 2 weeks. A diverter valve led the effluent into the electrospray ion source (ESI), operating in the PI mode, with a flow of 200 μl/min only between 6 and 17 min of the chromatographic run. High-purity nitrogen was used as drying and curtain gases; high-purity argon was used as collision gas. Desolvatation gas was set at 650 l/h while the cone gas was set at 50 l/h; the probe and desolvatation temperature was maintained respectively at 100 and 350 °C. The gas pressure in the collision cell was set at 3 mbar. Capillary voltage was 3000 V, extractor voltage was 0.2 V. Cone voltage, collision energy and others transmission parameters were optimized for each analyte (data are reported in Table 2). Mass axis calibration of each mass-resolving quadrupole Q_1 and Q_3 was performed by infusion of a sodium

Table 2
Time-scheduled multi reaction monitoring conditions for detecting aminoglycoside antibiotics and limits of quantification of the method

Compound	MRM transition (*m/z*)	Cone voltage (V)	Collision energy (eV)	LOQ (ng/ml)
Spectinomycin[a]	*351*[b,c] > 315 *351* > 333	32	20	5
Dihydrostreptomycin[a]	**293**[d] > 176 **293** > 409	20	12	3
Streptomycin[a]	**308**[e] > 176 **308** > 263	20	15	13
Aminosidine[f]	**309** > 161 **309** > 455	15	12	–
Apramycin[f]	**271** > 163 **271** > 217	15	12	2
Gentamicin C1a[f]	**226** > 129 **226** > 322	10	6	5
Gentamicin C2, C2a[f]	**233** > 126 **233** > 143 **233** > 322	12	6	7
Gentamicin C1[f]	**240** > 139 **240** > 157 **240** > 322	15	10	6
Neomycin B[f]	**308** > 161 **308** > 455	15	10	4

[a] Time window, 0–11.1 min; dwell time, 100 ms.
[b] Single-charge precursors in italics.
[c] *m/z* 351 originates from formation of $[M + H_2O + H]^+$.
[d] Double-charge precursors in bold face.
[e] *m/z* 308 originates from formation of $[M + CH_3OH + 2H]^{2+}$.
[f] Time window, 13.2-17 min; dwell time, 50 ms.

and cesium iodide solution at 10 μl/min. Unit mass resolution was established and maintained in each mass-resolving quadrupole by keeping a full width at half maximum of approximately 0.7 amu. All the source and instrument parameters for monitoring aminoglycosides were optimized by standard solutions of 5 μg/ml infused at 10 μl/min by a syringe pump. The multi reaction-monitoring (MRM) mode was used for quantitation by selecting at least two fragmentation reactions for each analyte (Table 2).

3. Results and discussion

3.1. Critical analytical step

Initial extraction experiments gave poor recovery of those AGs bearing several amino groups (Table 3). We supposed this negative result could be due to the presence on the surface of the siliceous material (sand) supporting the biological matrix of metal impurities able to strongly bind compounds bearing amino groups, such as AGs. Protonation of the amino groups by extracting with heated water acidified to pH 1.5 increased recovery of the analytes (data not shown here), but they were still unsatisfactory. Attempts to extract AGs by further decreasing the pH to 1 failed probably because of analyte decomposition. Treatment of the sand with 0.2 mol/l Na_2EDTA reached the goal of achieving good recovery of all the AGs considered (Table 3). The action of the Na_2EDTA should be that of deactivating metal impurities present on the sand surface by formation of stable complexes. This hypothesis, however, was not supported by the observation that any benefit obtained by the Na_2EDTA treatment disappeared if sand was washed exhaustively by water to eliminate any trace of unreacted Na_2EDTA.

Table 3
Effect of sand treatment with 0.2 mol/l Na_2EDTA on recovery (%) of 10 aminoglycosides in milk

Compound	Untreated sand[a]	Na_2EDTA-treated sand and washed[b]	Na_2EDTA-treated sand[c]
Spectinomycin	77	76	90
Dihydrostreptomycin	74	78	101
Streptomycin	50	55	90
Aminosidine	45	30	83
Apramycin	35	32	72
Gentamicin C1a	22	17	68
Gentamicin C2 + C2a	22	18	76
Gentamicin C1	21	16	81
Neomycin B	22	17	78

Spike level: 200 ng/ml. Extractant: water heated at 70 °C.
[a] R.S.D. ranges (%; $n = 4$) 9–12.
[b] R.S.D. ranges (%; $n = 4$) 8–10.
[c] R.S.D. ranges (%; $n = 4$) 7–13.

3.2. Optimization of the chromatographic conditions

Because of their highly hydrophilic nature, ion-pair reversed-phase LC is normally used to chromatograph AGs on a C-18 column. To make the mobile phase compatible with use of the ESI source, Heller et al. [9] proposed the addition to the LC mobile-phase of the volatile heptafluorobutyric acid (HFBA) ion-pair agent at 10 mmol/l concentration. Under this condition, however, we noted a remarkable shortening of the column life that was accompanied by gradual deterioration of the column performance and a weak response of the ESI/MS detector. Hydrolysis of the chemical groups bonded to the silica surface by the excessively acidic mobile phase was considered responsible for degradation of the life and performance of the column. Therefore, we performed experiments aimed at finding the minimum HFBA concentration able to produce good peak shape for the analytes and to improve the sensitivity of the method as well. Fig. 2 shows the variation of the signal-to-noise ratio (S/N) of selected AGs considered by varying the HFBA concentration in the mobile phase. As can be seen, decreasing the HFBA concentration down to 1 mmol/l resulted in a great enhancement of the S/N. With the exception of SPC, a decrease of the S/N was again observed by further decreasing the HFBA concentration. We noted that, by passing from 10 to 1 mmol/l HFBA, the increase of the S/N was mainly due to a decreased noise. Differently, broadening of the chromatographic bands for the AGs, except for SPC was responsible for the decrease of the S/N, when further decreasing the HFBA concentration to 0.5 mmol/l. With the purpose of developing a method able to determine simultaneously the AGs under study, we selected a MeOH/water solution acidified with 1 mmol/l HFBA as mobile phase. Noticeably, under this condition, the chromatographic characteristics of the LC column did not change significantly over 3 months of work.

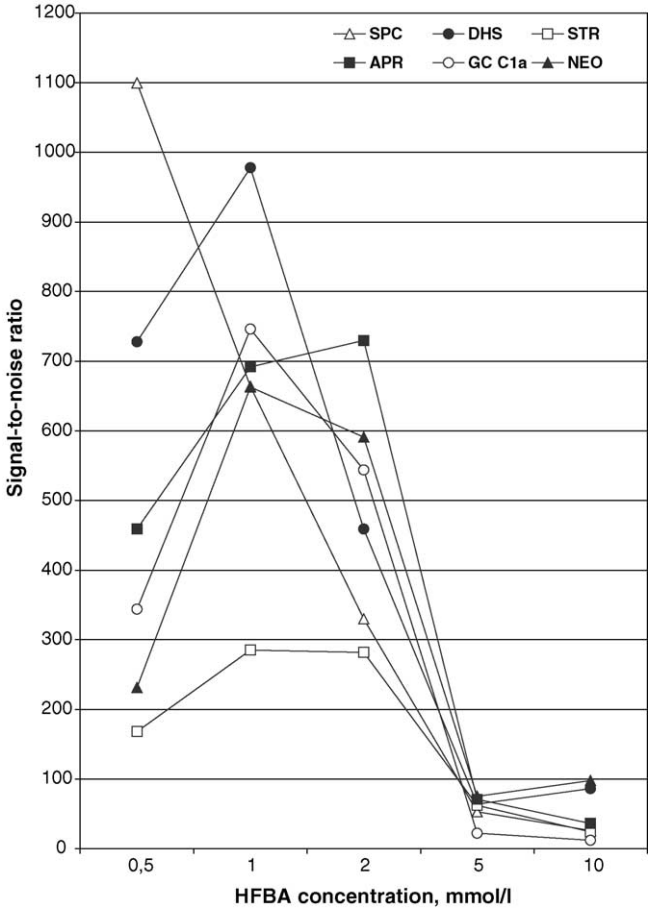

Fig. 2. Variation of the signal-to-noise ratio for selected aminoglycoside antibiotics by varying the heptafluorobutyric acid concentration in the LC mobile phase. Spectinomycin (SPC), dihydrostreptomycin (DHS), streptomycin (STR), apramycin (APR), gentamicin (GC), neomycin B (NEO).

3.3. Effect of the temperature on analyte recoveries

We evaluated the temperature effect on recoveries of AGs by performing extractions at various temperatures. The aim of this study was also that of finding the minimum extraction temperature able to give good recovery of the analytes and the lowest amount of matrix components that could contaminate the ion source and/or interfere with the rest of the analysis. For this study, a sample of milk was spiked with the analytes and the surrogate internal standard at 200 ng/ml and 5 ml of water were passed through the extraction cell at 1 ml/min flow-rate. At each temperature, three extractions were carried out and results are reported in Fig. 3.

Raising the temperature of the extractant from 40 to 70 °C had the effect of improving the extraction yield of all the analytes. When trying to further increase the recovery of the analytes by increasing the water temperature to 90 °C, severe loss of most of the analytes was experienced. Maybe, this loss was due to hydrolytic attack of the oxygen bridge linking the sugar moieties. Thus, an extraction temperature of 70 °C was used for subsequent experiments.

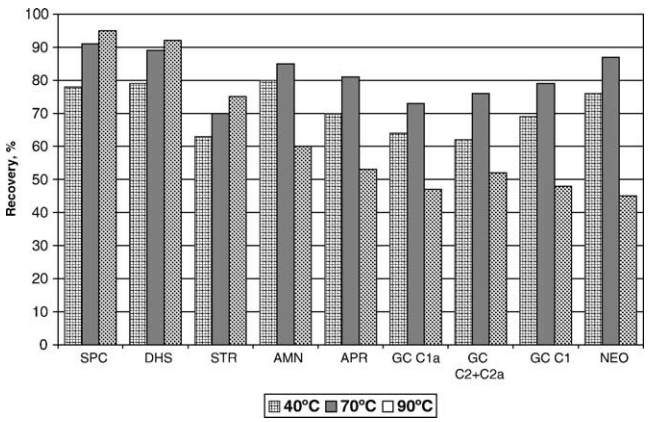

Fig. 3. Effect of the water temperature on recovery of aminoglycoside antibiotics added to bovine milk at 200 ng/ml level. Spectinomycin (SPC), dihydrostreptomycin (DHS), streptomycin (STR), apramycin (APR), gentamicin (GC), neomycin B (NEO).

3.4. The effect of the extractant volume on analyte recoveries

Besides affecting the extraction yield of the target compounds, the water volume passing through the extraction cell can influence the sensitivity of the method, as this method does not include any concentration step of the extract. For the purpose of finding the minimum volume of water able to extract efficiently the analytes, experiments were performed in triplicate by spiking a milk sample with the analytes and the surrogate internal standard at 200 ng/ml level and extracting with increasing water volumes. A volume larger than 4 ml of water did not increase significantly analyte recovery. Thus, the best compromise between method sensitivity and extraction yield was that of passing through the extraction cell 4 ml of water heated at 70 °C.

3.5. Matrix effect

Initially, recovery of the analytes in milk was estimated by comparing their peak areas with those obtained from injection of a standard solution containing the same nominal concentrations of the analytes. Under this condition, apparently low recovery of early eluting AGs was obtained, while recovery of late-eluting AGs were well higher than 100%. We found that, when added *post-extraction* to a milk extract, the response of early eluting AGs decreased with respect to pure standards, while the contrary occurred for the late-eluting AGs. These anomalous matrix effects occurring for AGs when injected from a milk extract were already observed by Heller et al. [9]. To obviate matrix effects on the ion signals of target compounds in biological matrices, many authors have proposed the adoption of analyte-fortified control tissue extracts as reference standards [9,13–19]. On developing an assay for determining a certain drug in human plasma, Matuszewski et al. [20] utilized plasma samples from five different subjects. To evaluate eventual differences in matrix effects from subject to subject, both the analyte and the internal standard were added *post-extraction* to the five plasma extracts. In this situation, the measurement of the analyte concentration was fairly accurate but not precise (R.S.D. = 14–22%). On this basis, the authors concluded that the extent of the matrix effect provoking ion signal weakening for the analyte and/or the internal standard can change by changing the source. It is conceivable that these variations can also occur when analyzing contaminants in other biological matrices. In this case, using a generic analyte-fortified control milk extract as reference standard will affect analyte quantitation in incurred samples. For all the AGs considered here, including aminosidine candidate for use as internal standard, we conducted a study aimed at assessing variations of the matrix effect (if present) by varying the source of the milk. For this purpose, six commercial milk samples from different producers were extracted as reported in Section 2. Prior to LC–MS analysis, the extracts were spiked with the analytes and the surrogate IS at the 200 ng/ml level. Quantification of the AGs in milk extracts was performed by comparing their absolute peak areas to those of the same compounds injected from a standard solution. Results of these experiments are presented in Table 4. As can be read, the matrix effect influenced remarkably the ion signal intensities of all the AGs considered. Anyway, the extent of the matrix effect for protonated AGs appeared to be only slight dependent on the particular milk extract analyzed, as R.S.D. were in all cases not higher than 15%. This means that, when using LC-ESI/MS, an analyte-fortified control milk extract could be used as reference standard to improve the accuracy of the analysis of AGs in incurred milk samples.

Table 4
Accuracy[a] and precision[b] data of aminoglycoside antibiotics directly added to milk extracts from six different sources (spike level = 200 ng/ml).

Compound	Accuracy (%)	R.S.D. (%)
Spectinomycin	−24	11
Dihydrostreptomycin	−20	12
Streptomycin	−19	14
Aminosidine	+4	13
Apramycin	+27	15
Gentamicin C1a	+71	15
Gentamicin C2 + C2a	+111	11
Gentamicin C1	+132	12
Neomycin B	+90	14

[a] Calculated as [(mean calculated concentration − spiked concentration)/spiked concentration] × 100. The concentration of each aminoglycoside (included aminosidine candidate for use as internal standard) in every milk extract was calculated by comparing its absolute peak area to that of the same aminoglycoside injected from a standard solution.
[b] Expressed as relative standard deviation (R.S.D., %).

3.6. Accuracy and precision

Following criteria reported in the EU guidelines [7], this method was validated at three different concentrations corresponding to one half of the maximum residue limit (MRL) (Table 1), the MRL and 1.5 times the MRL. At each analyte concentration, five measurements were performed with the criterion of adding the surrogate internal standard (aminosidine) *before* analyte extraction. Measurement of the various concentrations of AGs in milk was done by using milk extracts spiked with the analytes and the surrogate as reference standards. Results are reported in Table 5, while a typical MRM LC–MS/MS chromatogram of milk spiked with AGs each one at one half of the tolerance level set by EU is visualized in Fig. 4. The accuracy data varied between 80 and 107% with standard deviations not higher than 11%. Thus, this method meets requirements reported in the EU guidelines [7] indicating that a method can be considered accurate and precise when accuracy data are comprised between 80 and 110% with relative standard deviations not higher than 20%.

3.7. Linear dynamic range

Under the instrumental conditions reported in Section 2, the linear dynamic range of the ESI/MS/MS detector was es-

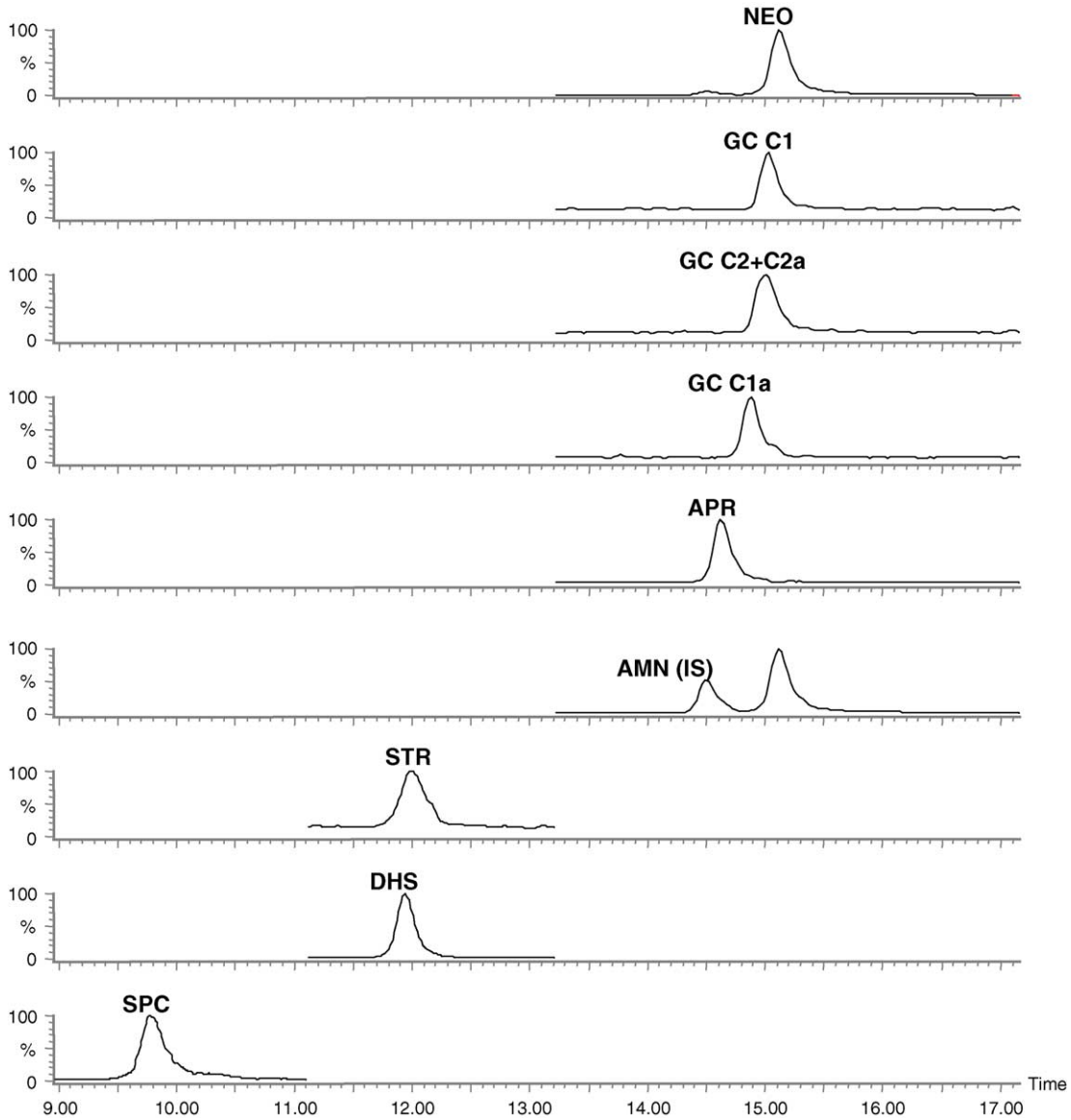

Fig. 4. MRM LC-ESI/MS/MS from analysis of a bovine milk sample spiked with aminoglycoside antibiotics at one half of the tolerance level set by the European Union. Spectinomycin (100 ng/ml) (SPC), 100 ng/ml dihydrostreptomycin (DHS), 100 ng/ml streptomycin (STR), aminosidine (AMN) is the surrogate internal standard added to milk at 100 ng/ml level, 50 ng/ml of each gentamicin (GC), 750 ng/ml neomycin B (NEO). Apramycin (APR) is not permitted for veterinary use in European Union and it was added arbitrarily at the 50 ng/ml level.

timated for all the analytes. Amounts of each analyte varying from 0.2 to 600 ng and a constant amount of 30 ng of the internal standard were injected from suitably prepared standard solutions into the LC column. At each analyte amount, three replicate measurements were made. Signal against amount-injected curves were then constructed by averaging the peak area resulting from the sum of the signals for parent and fragment ions of each analyte and relating this area to that of the internal standard. Results showed that ion signals of the nine aminoglycosides were linearly correlated with injected amounts up to 400 ng, with R^2 ranging between 0.9969 and 0.9999.

3.8. Limits of detection (LODs) and quantification (LOQs)

LOQs of the method were estimated from a MRM LC–MS/MS chromatogram (not shown here) resulting from analysis of a milk extract spiked with AGs at 20 ng/ml level. After extracting the sum of the ion currents of the transitions selected for each analyte, the resulting trace was smoothed twice by applying the mean smoothing method (Mass Lab software, Waters). Thereafter, the peak height-to-averaged background noise ratio was measured. The background noise estimate was based on the peak-to-peak baseline near the an-

Table 5
Accuracy (%) and precision data on analyzing aminoglycoside antibiotics in milk at concentrations close to MRLs set by EU

Compound	MRL/2[a,b]	MRL[c]	1.5 MRL[d]
Spectinomycin	107	98	102
Dihydrostreptomycin	107	104	102
Streptomycin	97	88	95
Apramycin	101	96	94
Gentamicin C1a	93	88	86
Gentamicin C2 + C2a	86	91	88
Gentamicin C1	85	80	80
Neomycin B	95	99	93

[a] MRLs reported in Table 1. For apramycin, whose use is not permitted by the EU an arbitrary concentration of 100 ng/ml was chosen as MRL.
[b] R.S.D. ranges (%; $n = 5$) 6–10.
[c] R.S.D. ranges (%; $n = 5$) 7–11.
[d] R.S.D. ranges (%; $n = 5$) 9–11.

alyte peak. LOQs were then calculated on the basis of a minimal accepted value of the signal-to-noise ratio (S/N) of 10 (see again Table 2). LOQs of the method are below tolerance levels set by EU and FDA for residues of the aminoglycoside antibiotics considered in bovine milk (Table 1). LODs of the method ranged between 1 ng/ml (dihydrostreptomycin and apramycin) and 6 ng/ml (streptomycin). When performing detection with a MS/MS arrangement, the most important condition to be satisfied for ascertaining the presence of a targeted compound is that at least two precursor ion > product ion transitions give signals distinguishable from the background ion current. Accordingly, a definition of LOD (S/N 3) of each analyte was adopted, considering in each case the ion giving the worst S/N.

4. Conclusions

This work has again shown that the environmentally friendly and inexpensive water, besides to be an effective extractant for polar and medium-polar contaminants in biological matrices, produces sufficiently clean extracts requiring little manipulation before final analysis by LC–MS. Besides to be highly specific, the ESI/MS/MS detector provides sensitivity for analyzing AGs in milk at levels well below EU and FDA tolerance levels.

References

[1] T. Koeda, K. Umemura, M. Yokota, in: H. Umezawa, I.R. Hooper (Eds.), Aminoglycoside Antibiotics, Springer-Verlag, Berlin, 1982, p. 293.
[2] C.A. Hammett-Stabler, T. Johns, Clin. Chem. 44 (1998) 1129.
[3] B. Shaikh, E.H. Aleen, J. Assoc. Off. Anal. Chem. 68 (1985) 1007.
[4] K.N. Woodward, G. Shearer, in: H. Oka, H. Nakazawa, K. Harada, J.D. MacNeil (Eds.), Chemical Analysis for Antibiotics Used in Agriculture, AOAC International, Arlington, VA, 1995 (Chapter 3).
[5] Commission Regulation (EC) N° 1931/1999 of 9 September 1999, in Off. J. Eur. Communities; N° L240 of 10 September 1999, Commission Regulation (EC) N° 868/2002 of 24 May 2002, in Off. J. Eur. Communities; N° L137 of 25 May 2002, Commission Regulation (EC) N° 1181/2002 of 1 July 2002, in Off. J. Eur. Communities N° L172, of 2 July 2002 and Commission Regulation (EC) N° 1530/2002 of 27 August 2002, in Off. J. Eur. Communities N° L230, of 28 August 2002.
[6] Code of Federal Regulation, Title 21 (Food and Drugs), vol. 6, Part 556 (Revised 1 January 2003).
[7] Commission Decision 2002/657/EC of 12 August 2002, Implementing Council Directive (EC) 96/23 concerning the performance of analytical methods and the interpretation of the results (text with EEA relevance), Off. J. Eur. Commun. L221 (2002) 8.
[8] M.C. Carson, D.N. Heller, J. Chromatogr. B 718 (1998) 95.
[9] D.N. Heller, S.B. Clark, H.F. Righter, J. Mass Spectrom. 35 (2000) 39.
[10] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, M.L. Polci, J. Agric. Food Chem. 51 (2003) 4225.
[11] S. Bogialli, V. Capitolino, R. Curini, A. Di Corcia, M. Nazzari, M. Sergi, J. Agric. Food Chem. 52 (2004) 3286.
[12] C. Crescenzi, G. D'Ascenzo, A. Di Corcia, M. Nazzari, S. Marchese, R. Samperi, Anal. Chem. 71 (1999) 2157.
[13] L.G. McLaughlin, J.D. Henion, P.J. Kijak, Biol. Mass Spectrom. 23 (1994) 417.
[14] S. Riediker, R.H. Stadler, Anal. Chem. 73 (2001) 1614.
[15] D.K. Matabudul, I.D. Lumley, J.S. Points, Analyst 127 (2002) 760.
[16] S. De Baere, M. Cherlet, K. Baert, P. De Backer, Anal. Chem. 74 (2002) 1393.
[17] J. Rosén, Analyst 126 (2001) 1990.
[18] S. Riediker, J.-M. Diserens, R.H. Stadler, J. Agric. Food Chem. 49 (2001) 4171.
[19] E. Daeseleire, H. De Ruyck, R. Van Renterghem, Rapid Commun. Mass Spectrom. 14 (2000) 1404.
[20] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.