Analysis of β-Lactam Antibiotics in Incurred Raw Milk by Rapid Test Methods and Liquid Chromatography Coupled with Electrospray Ionization Tandem Mass Spectrometry

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A recently developed confirmatory LC-MS method has been applied to the quantification of five major β-lactam antibiotics in suspect raw bovine milk samples that gave a positive response with receptor-based (BetaStar) and rapid microbial inhibitory screen tests (Delvotest SP). In total, 18 presumptive positive raw milk samples were reanalyzed; 16 samples showed traces of antibiotic residues that could be identified and quantified by the LC-MS method, ranging from the limits of confirmation up to 38 μg/kg. Of the positive samples, only five (∼30%) were found to be violative of EU maximum residue limits. The most frequently detected antibiotic residues were cloxacillin and penicillin G, the former often in combination with amoxicillin or ampicillin. This study compares the results obtained by the three methods on identical samples and addresses how these relate to certain criteria such as sensitivity and selectivity. Furthermore, the limitations of the LC-MS method and the potential impact of the presence of frequently more than one residue in the same milk sample on the response of the rapid test methods are discussed.

Keywords: *Food analysis; raw milk; β-lactam antibiotics; stability; field tests; rapid screening test; LC-MS*

INTRODUCTION

Antibiotics are administered to treat bacterial infections or employed prophylactically to augment growth and yield in livestock production and fish farming. The extensive use of antimicrobial agents in human medicine and agriculture poses a potential risk for public health because of allergic reactions of individuals to antibiotics and/or their metabolites and the increasing incidence of microbial resistance against these compounds (*1*). The most frequent use of antibiotics in the dairy industry is to combat mastitis-causing pathogens, a disease which inflicts significant economic losses estimated in the United Kingdom alone to lie in the region of £80 million per annum (*2*). Thus, to avoid having antibiotics enter the food chain at unacceptable levels, stringent control at primary production is imperative to protect the public health. Therefore, maximum residue limits (MRLs) have been set by the European Union for veterinary drug residues in different food commodities, for example, in the case of milk 4 μg/kg for penicillin G (PEN G), ampicillin (AMPI), and amoxicillin (AMOX) and 30 μg/kg for the semisynthetic isoxazolyl β-lactams cloxacillin (CLOX) and oxacillin (OXA) (*3*).

Numerous commercial test kits have been developed to check the compliance of residue levels of antibiotics in milk at legislative levels. These rapid tests are based either on the inhibition of growth of microbial test organisms (e.g., Delvotest SP), ligand assays using biological receptor (e.g., Charm Test II), or antibodies configured in an enzyme-linked immunoassay (e.g., SNAP). The major advantage is that the majority of the tests can be conducted rapidly, providing an accept/reject decision at the farm level. Furthermore, such tests vary in selectivity and may give evidence for representatives of one or several groups of antibiotics present in the milk sample (*4*−*6*).

There may be a number of reasons for reanalyzing suspect milk samples that give a positive response in a rapid test by a truly confirmatory method, for example, to (1) determine the potential interference by natural inhibitors such as high somatic cell counts, bovine lactoferrin, or lysozyme (*7*−*9*); (2) confirm results in the case of contradictory responses obtained with two independent rapid test methods; and (3) identify individual residues and their concentrations in contaminated milk. Clearly, the goal here is not to revalidate commercial rapid tests as ample data is available in the literature (*6*, *10*, *11*). However, there are only very limited data available on the analysis of β-lactam antibiotics in truly incurred raw milks (*10*, *12*) and practically no information on analyte identification in suspect milks that have been rejected "in the field" on the basis of nonspecific rapid tests.

In this study, the qualitative and quantitative data procured on incurred milk samples by the different analytical approaches are compared and discussed, particularly in terms of sensitivity, potential synergistic effects of antimicrobials resulting in false violation, and predictability of antibiotic formulations used in cow therapy. Finally, the benefits and limitations of LC-MS as a confirmatory tool in suspect samples are addressed and how developments in trace analytics may in the future have an impact on drug screening in general.

EXPERIMENTAL PROCEDURES

Chemicals and Materials. Amoxicillin (13.7% H_2O), ampicillin (sodium salt), penicillin G (sodium salt), cloxacillin (sodium monohydrate salt), and penicillinase were purchased from Sigma (Buchs, Switzerland). Oxacillin (sodium monohy-

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drate salt) was obtained from Riedel-de Haën GmbH and Co (Seelze, Germany). Potassium benzyl(d_7-phenyl) penicillate (chemical purity > 95%, d_7-PEN) that was used as internal standard (IS) was custom synthesized by Toronto Research Chemicals Inc. (North York, ON, Canada). The antibiotics were stored in a dried atmosphere at 4 °C (AMOX, AMPI, CLOX, and OXA), at ambient temperature (PEN G) or at −20 °C (IS). Stock solutions (0.1 mg/mL) were prepared with a mixture of ethanol, acetonitrile, and water (1:1:2, v/v) and stored at −20 °C for a maximum of 1 month as recommended by Tyczkowska et al. (*13*). *Caution: Penicillins are harmful and may cause sensitization by inhalation and skin contact. For this reason, they have to be handled with corresponding precautionary measures.*

Formic acid, *n*-hexane, ethanol, sodium chloride, and disodium hydrogen phosphate dihydrate were of p.a. grade (Merck, Darmstadt, Germany). Water was either purified in-house using a Büchi Fontavapor 260 (Flawil, Switzerland) or purchased from Merck (HPLC grade, LiChrosolv). Methanol (LiChrosolv, Merck) and acetonitrile (J. T. Baker, Phillipsburg NJ) were of HPLC grade.

Bakerbond C_{18} solid-phase extraction cartridges (3 mL, 500 mg, SPE) were obtained from J. T. Baker and cutoff filter devices (Microcon-10, nominal molecular weight limit of 10000 Da) from Millipore Corp. (Bedford, MA). Test kit Delvo SP (ampule format) was obtained from DSM Food Specialties (Delft, The Netherlands), BetaStar from UCB-Bioproducts SA (Braine-L'Alleud, Belgium), SNAP test from IDEXX Laboratories Ltd. (Westbrook, ME), and Charm Rosa from Charm Science Inc. (Malden, MA).

Milk Samples. Raw milk samples (bovine milk, bulk tanker deliveries) with incurred residues (positive response with the β-lactam selective BetaStar and/or SNAP test and nonselective Delvo SP screen test) were obtained from various milk collection centers in the United Kingdom. They were transported in a frozen state to the Nestlé Research Center in Switzerland, where the samples were stored at −30 °C until further analysis.

Rapid Screening Tests. All raw milk samples were examined in the milk collection centers using the commercial BetaStar or SNAP and Delvo tests. The milk samples were retested upon arrival at the Nestlé Research Center by performing the Delvo SP and BetaStar. The presence of β-lactam antibiotics was confirmed by conducting the Delvotest SP in the presence of penicillinase (Sigma P-0389), 1.67 units/100 μL of milk, and incubation for 3 h at 64 °C [the penicillase test is not effective for CLOX and OXA (*14*)]. All tests were conducted with the initial raw milk samples according to the manufacturer's protocol.

Milk Sample Preparation for LC-ESI/MS/MS Analysis. The milk samples were prepared according to the procedure illustrated in Figure 1 and according to ref *15*. In essence, the method entails fortification of the milk sample with an internal standard (d_7-PEN) and removal of milk fat by centrifugation followed by solvent extraction and a solid-phase cleanup step. The column effluent is evaporated under a gentle stream of nitrogen in a heater block (45−50 °C). After the pH has been adjusted to ∼7 by the addition of formic acid, the extract volume is supplemented to 1 mL with distilled water. Finally, the extract is filtered through a cutoff filter device using a tabletop microcentrifuge. Each milk sample was prepared in duplicate and injected at least three times in arbitrary order within an analytical series.

LC-ESI/MS/MS. Measurements were conducted by employing an Alliance 2690 HPLC (Waters, Rupperswil, Switzerland) coupled with a Quattro LC tandem mass spectrometer (Micromass, Manchester, U.K.). Separation was accomplished with a YMC ODS-AQ column (50 × 2 mm i.d., particle size = 3 μm, 120 Å) by running a linear gradient from 100% solvent A (0.1% formic acid solution) to 100% solvent B (35% water in acetonitrile with a total of 0.1% formic acid) in 13 min at a flow rate of 0.3 mL/min. After compound separation, the column was flushed with 100% B for 2 min, increasing the flow rate to 0.4 mL/min, then changing to 100% A within 1 min and simultaneously increasing the flow rate to 0.5 mL/min for column equilibration for 4 min. The column and autosampler temperatures were 35 and 5 °C, respectively. A volume of 10 or 15 μL was injected.

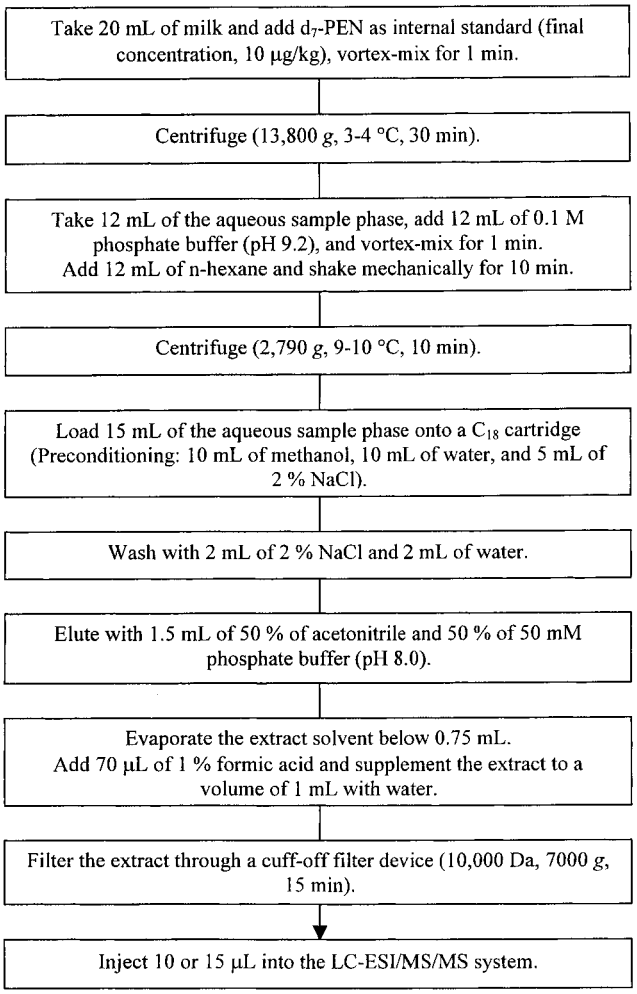

Figure 1. Flowchart of the extraction/cleanup procedure for the analysis of β-lactam antibiotics in bovine milk.

The analytes were detected using electrospray ionization in positive ion mode. The needle voltage was typically set to 3.1 kV and the RF lens voltage to 0.2 V. Source block and desolvation temperatures were 145 and 350 °C, respectively. Nitrogen gas was used for nebulization and desolvation at flow rates of 95 and 700 L/h. The ion energies of the first and second quadrupoles were 0.8 and 1.0 V. The collision gas was argon at a vacuum pressure of 1.73 mTorr. Two or three different fragmentation reactions (selected reaction monitoring, SRM) were observed for each analyte (Figure 2). The settings for cone voltages and collision energies were optimized for each SRM trace and ranged from 18 to 20 V and from −10 to −24 eV, respectively.

Data Evaluation. For quantitation of AMOX, AMPI, PEN G, and CLOX in the incurred milk samples matrix-matched calibration curves were established using blank milk samples fortified at five different concentration levels, resulting in working ranges from 0.3 to 52 μg/kg for CLOX, 0.4−12 μg/kg for AMOX, 1.1−12 μg/kg for AMPI, and 0.8−16 μg/kg for PEN G. Area ratios of the SRM transition showing maximum signal response versus IS were plotted against their respective amount ratios. All statistical calculations were done using robust statistics (*16*).

RESULTS

Analytical Method. The high selectivity and sensitivity of LC-ESI/MS/MS analysis is clearly illustrated for all five β-lactam antibiotics in this study (Figure 2).

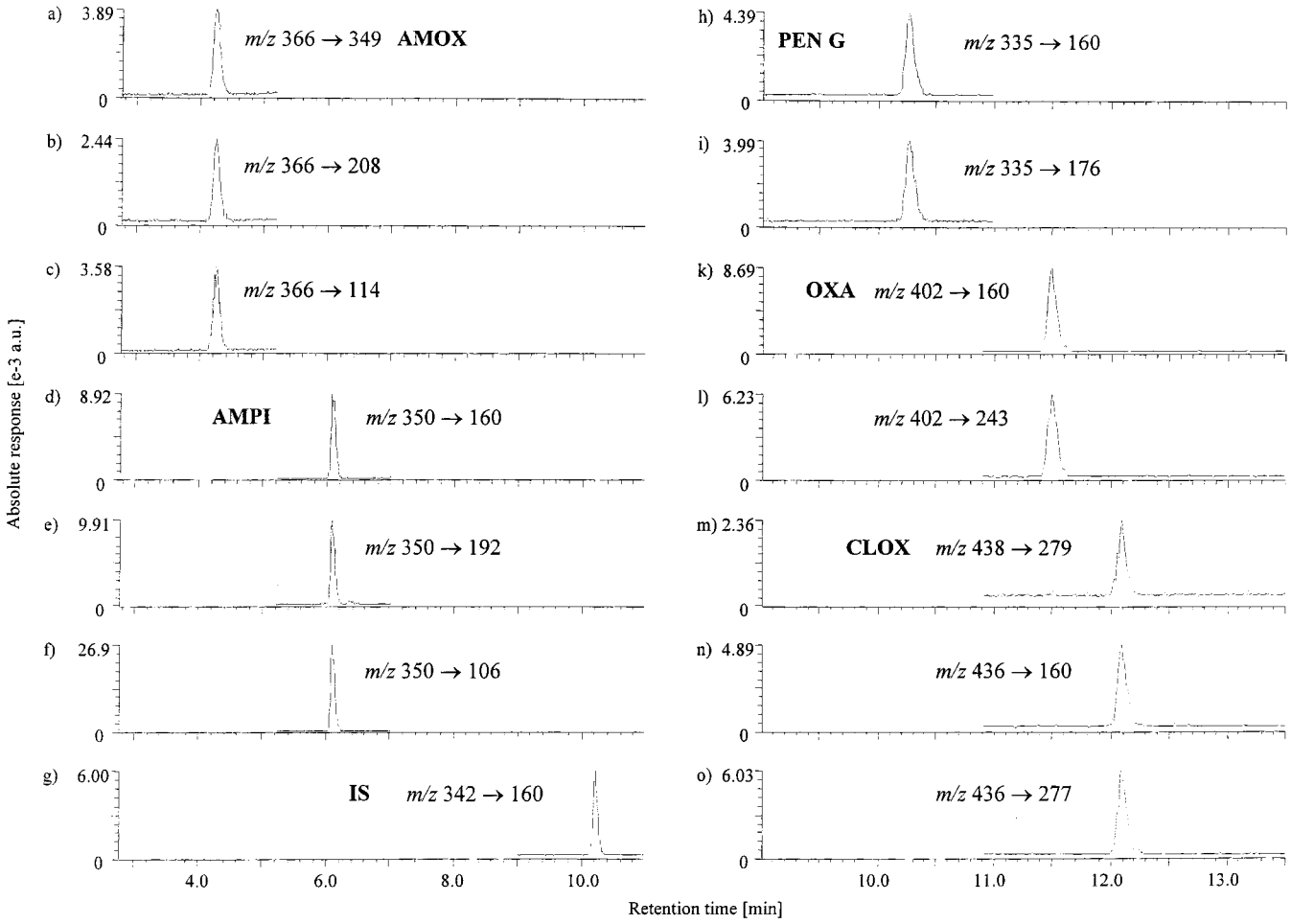

Figure 2. Selected reaction monitoring traces obtained from a blank milk sample fortified with amoxicillin (AMOX, a−c), ampicillin (AMPI, d−f), internal standard (IS, g), penicillin G (PEN G, h, i), oxacillin (OXA, k, l), and cloxacillin (CLOX, m−o) at a level of each 10 μg/kg. The fragmentation transitions for each SRM trace are depicted in the corresponding chromatograms.

The monitoring of two or three compound-specific fragmentation reactions of the protonated molecule provides added confidence in the identification of the analyte; thus, the method complies with the MS confirmation criteria as recommended by 1999/333/EG (*17*) and the Commission Decision 93/256/ECC (*18*). The performance of the complete method encompassing sample preparation and LC-ESI/MS/MS analysis has been reported in detail elsewhere (*15*), and thus only the most salient analytical parameters—evaluated for the five target antibiotics—are summarized here. Typically, the recovery of all analytes in milk ranged from 76 to 94% at an individual analyte concentration level of 4 μg/kg. The coefficients of inter- and intra-assay variation were 4.8−10.8 and 3.2−6.2%, respectively, estimated by analyzing milk samples spiked at a level of 10 μg/kg of each analyte. The limits of confirmation (estimated by evaluating the SRM transitions with the lowest response) are listed in Table 1, the limits of quantitation (LOQ) ranging between 0.1 and 1.14 μg/kg (injection volume = 10 μL). The low levels of analyte confirmation enable identification of β-lactams well below the MRLs required and, as expected, below the detection limits of the rapid tests (Table 1).

Comparative Tests with Incurred Raw Milk Samples. In the normal practice of milk quality control at the raw milk collection stage, samples that give a positive response with a rapid test at procurement are postscreened for confirmation using the BetaStar or Delvo SP test. Eighteen samples of raw milk that failed this control procedure were collected and reanalyzed at our Center, using the BetaStar, Delvo SP, and LC-ESI/MS/MS methods for unambiguous identification and quantitation of five β-lactam residues. Reinvestigation of the samples at our Center gave results that overall corresponded to those produced at the milk collection site. Moreover, of the 18 milk samples collected, 17 gave clearly positive responses with the Delvo SP test conducted at the milk collection center, whereas the response was ambiguous for milk sample 10, which may be attributable to decomposition of the residues during storage and/or transport.

Table 1. EU Maximum Residue Limits (MRLs), Detection Limits of the Screening Tests Delvo SP, BetaStar, and Charm Rosa (CR), and Confirmation Limits of the LC-ESI/MS/MS Method for All Five β-Lactam Antibiotics and Cefalonium

		detection limits (μg/kg)			confirmation limits (μg/kg)
compound	MRL	Delvo SP[a]	BetaStar[a]	CR[a]	LC-ESI/MS/MS
AMOX	4	3−5	2−4	3−4	0.42
AMPI	4	3−5	2−5	3−4	0.52
CLOX	30	20−25	5−10	20−35	1.10
OXA	30	10	5−10	25−40	0.40
PEN G	4	2.5	2−4	2−3	0.53
cefalonium	10[b]	15−25	7.5−15	nd[c]	nd[c]

[a] Manufacturer's information. [b] Expires on July 1, 2001. [c] Not determined.

Table 2. Comparison of Results Obtained from Incurred Raw Milk Samples Analyzed by Screening Tests Delvo SP and BetaStar and by LC-ESI/MS/MS (All Measurements Were Performed in the Same Laboratory)

			LC-ESI/MS/MS				
sample	Delvo SP + or −[a]	BetaStar + or −	+ or −	AMOX (μg/kg)	AMPI (μg/kg)	CLOX (μg/kg)	PEN G (μg/kg)
1	+	+	+	2.94			
2	−	+	+		1.01	4.85	
3	+	+	+			38.2	
4	+	+	+			29.1	
5	+	+	+		1.33	3.67	1.76
6	−	+	−				
7	+	±[b]	+				1.06
8	−	−	+				0.87
9	−	+	+	1.25		8.40	
10	−	+	−				
11	±	−	+				1.15
12	+	+	+				1.33
13	+	+	+				8.70
14	+	+	+		0.55	5.66	
15	−	+	+	0.36		3.12	
16	+	+	+	9.94			
17	+	+	+				1.82
18	+	+	+				4.24

[a] + or −, positive or negative response. [b] ±, positive response with caution.

The β-lactam antibiotics AMOX, AMPI, CLOX, and PEN G were identified and quantified in 16 of the 18 milk samples by using LC-ESI/MS/MS, and as shown (Table 2) either one or a number (up to three) of different residues were found in the same milk sample. The concentration of the individual analytes ranged between 0.9 and 8.7 μg/kg for PEN G (eight samples), between 3.1 and 38.2 μg/kg for CLOX (seven samples), between 0.4 and 9.9 μg/kg for AMOX (four samples), and between 0.55 and 1.33 μg/kg for AMPI (three samples). Typical total ion chromatograms obtained from a blank milk and a contaminated raw milk sample are illustrated in Figure 3. In this particular sample, both CLOX and AMPI are visible, the latter exhibiting a clear response at only spurious amounts (∼1 μg/kg), which is still within the LOQ (injection volume = 15 μL: 0.34 μg/kg), demonstrating the performance of the LC-MS method in terms of specificity and sensitivity.

The presence of β-lactam antibiotics in milk samples 6 and 10 was confirmed by BetaStar but not by Delvo SP and LC-ESI/MS/MS. A Charm Rosa test selective for β-lactams was also performed in these cases and afforded a positive response. This apparent discrepancy (potential false-positives) could be due to the presence of a cephalosporin antibiotic with a higher LOD for Delvo SP than for BetaStar or Charm Rosa (e.g., cefalonium, see Table 1). In addition, this result points to a possible limitation of the current LC-ESI/MS/MS method, as only a few contaminants, that is, five, can be monitored simultaneously during the same analytical run due to the restriction in the number of MS/MS acquisition channels. Moreover, the veterinary drugs applied "in the field" for animal treatment may contain a broad spectrum of antimicrobial agents including also other groups such as aminoglycosides (e.g., streptomycin and gentamicin), macrolides (e.g., erythromycin and tylosin), tetracyclines and sulfonamides, present either as single active components or as antibiotic cocktails.

Even though all samples were rejected on the basis of the β-lactam selective immunoassay and microbial inhibitory test, only four milk samples actually showed levels of individual antibiotics in violation of EU MRLs, that is, AMOX (sample 16), CLOX (sample 3), and PEN G (samples 13 and 18). Apparently contradictory results, that is, positive responses of milk samples 2, 9, and 15 with BetaStar versus negative results with Delvo SP, were clarified by LC-MS identifying in all samples CLOX as the major contaminant, with co-occurrence of AMOX or AMPI. The failure of the Delvo SP to respond to these residues can be explained by the higher detection limit of this commercial test for CLOX (Table 1).

Surprisingly, two raw milk samples that had initially been rejected at milk collection passed the BetaStar and Delvo SP tests when tested in our laboratory. Both samples, that is, numbers 8 and 11, showed trace amounts of PEN G, that is, 0.87 and 1.15 μg/kg, respectively. As correctly claimed by the manufacturers, the LOD for PEN G is in the range of 2−4 μg/kg, that is, not adequate for detection and thus explaining the lack of response. The initial positive result at milk collection may be due to higher levels initially and certain degradation/metabolism of the analytes during storage/transport. This emphasizes strict adherence to correct storage conditions and the avoidance of thawing of the samples during transport. In this context, separate investigations were conducted in our laboratory to determine the impact of temperature/time and matrix on the stability of β-lactam antibiotics in fresh milk (unpublished results), initial results showing that temperatures of −20 °C must be maintained throughout storage to avoid analyte degradation.

DISCUSSION

The LC-ESI/MS/MS method employed in this study provides unambiguous identification of five β-lactam antibiotics and quantitation of individual compounds even well below the LODs of the nonchemical screening and selective immunoassay-based tests. However, only a limited number of contaminants can be determined in this selective multiresidue method because of the restricted number of MS acquisition channels. Metabolites or other antimicrobial agents can be monitored only if they undergo the same fragmentation reactions in the MS collision cell as the target compounds. Thus, LC-ESI/MS/MS is a powerful tool for confirmation of field test results and, in the case of ambiguity, identification and quantification of the individual analytes. In this study, the power of the LC-MS technique could be demonstrated in that residues were identified in 16 of 18 suspect raw milk samples collected "in the field" and indicates the formulation(s)/cocktail used during cow therapy.

The major β-lactams identified in the incurred milks were penicillin G and cloxacillin, which in part reflects the usage of these compounds in mastitis treatments. In fact, >170 formulations are on the market today (taking into account CH-GB-F-USA-I), and of these >50% contain either PEN G or CLOX, with roughly equal distribution of the two antibiotics in the preparations. In the United Kingdom, the intramammary drugs Kloxerate Plus DC and Bovaclox DC are commonly used in dry cow therapy (*2*, *12*), both containing CLOX and AMPI as active ingredients, and in this survey three of the positive milk samples indeed showed the co-occurrence of both compounds. However, because testing was done at tanker level, extrapolation of the individual analytes detected in the contaminated milk to the drugs administered must be done with due caution and can thus be only indicative.

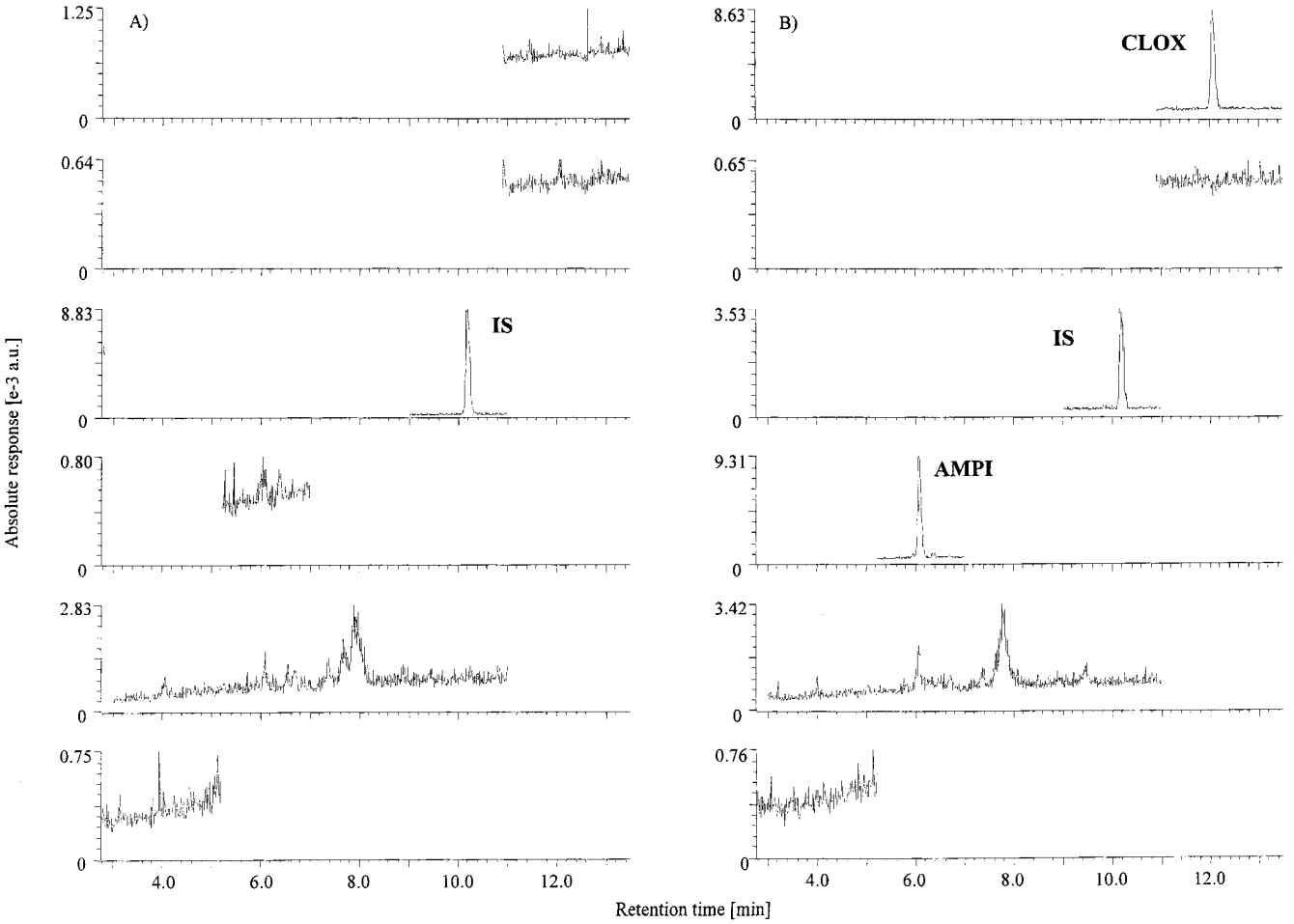

Figure 3. Total ion chromatograms acquired from (A) a blank milk sample and (B) incurred milk sample 2 from Table 2. Both milk samples were spiked with 10 μg/kg internal standard (IS) (injection volume = 15 μL). The concentrations of the analytes found in the contaminated raw milk samples were 1.01 μg/kg ± 8.1% ampicillin (AMPI) and 4.85 μg/kg ± 8.3% cloxacillin (CLOX).

Rapid test kits are designed to respond to residues at or above MRLs, which gives a high degree of certainty (probability) that violative samples will be detected before entering the food chain. However, the test may give positive results below violative levels, leading to so-called false violatives (*19*). This is illustrated in the sensitive response of the BetaStar to CLOX. The frequent usage of CLOX alone or in combinations with other β-lactams in mastitis medication and its detection in this study in >40% of the suspect raw milk samples question—albeit only for this particular antibiotic—the validity of the BetaStar technique as a rapid field test, as the test cannot differentiate "pass/fail" at the EU MRL of CLOX (Tables 1 and 2). Thus, from an economic point of view, there is a great risk of rejecting milk that is still within legislative limits, which necessitates the performance within a short period of time a second test, for example, the Delvo test with an LOD closer to the MRL of CLOX. However, the Delvo test may also provide a false violative in some cases if more than one antibiotic residue is present in the milk due to synergistic effects (*20*). Thus, as shown by the comparative LC-MS data, individual residues may be within legislative limits but due to the synergistic effect will lead to failure in the test.

The advancement of analytical techniques that enable quantification of chemical food contaminants at trace levels in "selective multiresidue" methods will provide a greater degree of knowledge pertaining to the spectrum of antibiotics used in animal-derived foods.

In this context, the frequent occurrence of often two or in one case three residues in the same milk—albeit at levels below MRLs for the individual compounds—raises the question of establishing MRLs for "total antibiotics" in food. Of course, as confirmatory analytical methods—particularly those based on mass spectrometer detection—continuously improve, these may soon not only enable the simultaneous detection of a few compounds of the same class as illustrated here but also be expanded to include other pertinent residues used in formulations (e.g., aminoglycosides, macrolides, and tetracyclines) for a more targeted assessment. This approach would allow more precise contaminant data for better risk assessment and may help to identify potential problem areas. Method development will be continued to incorporate additional antibiotics in the current LC-MS method, that is, to accommodate a greater spectrum of pertinent antibiotics commonly used in mastitis treatment.

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LITERATURE CITED

(1) Schwarz, S.; Noble, W. C. Aspect of bacterial resistance to antimicrobials used in veterinary dermatological practice. *Vet. Dermatol.* **1999**, *10*, 163−176.

(2) Clement, M. Bovine Mastitis: Products and Markets. *Animal Pharm Reports*; SR 173; Surrey, U.K., 1998.
(3) Council Regulation (EEC) 2377/90 of June 26, 1990. *Off. J. Eur. Communities* **1990**, *L 224* (Aug 18).
(4) Nouws, J. F. M.; van Egmond, H.; Loeffen, G.; Schouten, J.; Keukens, H.; Smulders, I.; Stegeman, H. Suitability of the Charm HVS and a microbiological multiplate system for detection of residues in raw milk at EU maximum residue levels. *Vet. Q.* **1999**, *21* (1), 21−27.
(5) Nouws, J. F. M.; van Egmond, H.; Smulders, I.; Loeffen, G.; Schouten, J.; Stegman, H. A microbiological assay system for assessment of raw milk exceeding EU maximum residue levels. *Int. Dairy J.* **1999**, *9*, 85−90.
(6) Suhren, G.; Beukers, R. Delvotest SP for detection of cloxacillin and sulfamethoxazole in milk: IDF interlaboratory study. *J. AOAC Int.* **1998**, *81*, 978−990.
(7) Angelidis, A. S.; Farver, T. B.; Cullor, J. S. Evaluation of the Delvo-X-Press Assay for detecting antibiotic residues in milk samples from individual cows. *J. Food Prot.* **1999**, *62*, 1183−1190.
(8) Carlsson, A.; Björck, L. The effect of some indigenous antibacterial factors in milk on the growth of *Bacillus stearothermophilus var. calidolactis. Milchwissenschaften* **1987**, *42* (5), 282−285.
(9) Van Eenennaam, A. L.; Cullor, J. S.; Perani, L.; Gardner, I. A.; Smith, W. L.; Dellinger, J.; Guterbock, W. M.; Jensen, L. Evaluation of milk antibiotic residue screening tests in cattle with naturally occurring clinical mastitis. *J. Dairy Sci.* **1993**, *76*, 3041−3053.
(10) Ang, C. Y. W.; Luo, W.; Call, V. L.; Righter, H. F. Comparison of liquid chromatography with microbial inhibition assay for determination of incurred amoxicillin and ampicillin residues in milk. *J. Agric. Food Chem.* **1997**, *45*, 4351−4356.
(11) Moats, W. A.; Romanowski, R. D.; Medina, M. B. Identification of β-lactam antibiotics in tissue samples containing unknown microbial inhibitors. *J. AOAC Int.* **1998**, *81*, 1135−1140.
(12) Hillerton, J. E.; Halley, B. I.; Neaves, P.; Rose, M. D. Detection of antimicrobial substances in individual cow and quarter milk samples using Delvotest microbial inhibitor tests. *J. Dairy Sci.* **1999**, *82*, 704−711.
(13) Tyczkowska, K. L.; Voyksner, R. D.; Aronson, A. L. Solvent degradation of cloxacillin *in vitro*: Tentative identification of degradation products using thermospray liquid chromatography−mass spectrometry. *J. Chromatogr.* **1992**, *594*, 195−201.
(14) Fletouris, D. J.; Psomas, J. E.; Mantis, A. J. Determination of some monobasic penicillins in milk by ion-pair liquid chromatography. *J. Agric. Food Chem.* **1992**, *40*, 617−621.
(15) Riediker, S.; Stadler, R. H. Simultaneous determination of five beta-lactam antibiotics in bovine milk using liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Anal. Chem.* **2001**, *73*, 1614−1621.
(16) Danzer, K. Robuste Statistik in der analytischen Chemie. *Fresenius' Z. Anal. Chem.* **1989**, *335*, 869−875.
(17) Commission Recommendation 1999/333/EG of March 3, 1999. *Off. J. Eur. Communities* **1999**, *L 128* (May 21).
(18) Commission Decision 93/256/EEC of April 14, 1993. *Off. J. Eur. Communities* **1993**, *L 118* (May 14).
(19) O'Rangers, J. J.; Condon, R. J. Promise and pitfalls of rapid contaminant analysis. *Conference Proceeding. Nondestructive Testing, Pasteurization Requirements and Antibiotics in Dairy Products*; Lisboa Symposium: Lisboa, Portugal, 1997.
(20) Luitz, M.; Suhren, G.; Heeschen, W. Interactions of antimicrobials in milk—Consequences for the detection by a microbial inhibitor test. *Milchwissenschaften* **1996**, *51* (7), 390−392.

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