Veterinary Drug Residues Survey in Meat: An HPLC Method with a Matrix Solid Phase Dispersion Extraction

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A multiresidue technique for the isolation and liquid chromatography determination of 14 veterinary drug residues in meat is described (10 antibiotics, 2 anthelmintics, 1 coccidiostat, and 1 other chemotherapeutic drug). Matrix solid phase dispersion (MSPD) was chosen as the extraction technique for this screening method. Two fractions were collected by elution with methylene chloride and ethyl acetate. No additional purification was needed. A reversed-phase C18 column was used for the high performance liquid chromatography (HPLC) to separate the analytes with gradients of ammonium acetate buffer−acetonitrile−methanol as mobile phase. Photodiode array and fluorimetric detectors were used for this analysis. Validation data are presented. The limits of detection for each analyte are shown and are under the regulatory tolerances. The percentage recoveries and the linearity of the method were evaluated comparing spiked samples and standard solutions. Meat naturally contaminated with sulfamethazine was used for the evaluation of the repeatability of the method, which was satisfactory.

Keywords: *Matrix solid phase dispersion; HPLC; sulfonamides; benzimidazoles; nicarbazin; chloramphenicol; furazolidone*

INTRODUCTION

Antibacterial, anthelmintic, and coccidiostat compounds are commonly used for prevention and/or treatment of diseases in animal productions. As a feed additive in subtherapeutic doses, some of them like sulfamethazine and virginiamycin contribute to the maintenance of optimal health and promote growth of animals. Such compounds have become an integral part of the livestock-producing industry.

However, their use may induce the presence of residues in food products, especially if proper withdrawal times for treated animals have not been respected. To prevent any health problems with consumers, the authorities have regulated the use of veterinary drugs by fixing maximum residue limits (MRLs) or by prohibiting the use of many substances. Food industries also took some measures to protect consumers and guarantee the quality of their products by checking their suppliers.

Because of the need to control numerous residues and most residue methods deal with a single molecule or small families of compounds, analysis of all residues remain prohibitively expensive and time consuming. Consequently, there is a high need for multiresidue methods which permit routine screening of many samples for many residues. Some authors have investigated such multiresidue methods for meat products by HPLC (Malisch et al., 1992) for the sulfonamides, chloramphenicol, and nicarbazin, and by GC-FID (Mineo et al., 1992) for 23 antibiotics and 13 other drugs.

However, the extraction−purification of these methods involves numerous and varying analytical steps which are labor intensive and time consuming and do not permit work on a large number of samples. For this reason we chose the matrix solid phase dispersion extraction (MSPD) first developed by Barker (Barker et al., 1989), for its easy use, its possible automation, and its multiresidue potential. For meat survey, this technique has been used successfully for the extraction of 5 benzimidazoles (Long et al., 1990), furazolidone (Soliman et al., 1990; Long et al. 1991), nicarbazine (Schenck et al., 1992), cefquinome (Barker et al., 1993), chloramphenicol (Macho et al., 1996), sulfamethazine (Renson et al., 1993; Shearan et al., 1994), sulfaguanidine (Macho et al., 1996), and other sulfonamides (Tamura et al., 1994). The development of the extraction method has been inspired from those publications and adapted to a multiresidue application. The aim was to prepare extracts from a single sample that permits screening of several compounds representing diverse classes of drugs, reducing the time and the cost of the analyses.

For the identification step, enzyme, radioimmuno (Boyd et al., 1994; Rosen et al., 1994; Renson et al., 1993; Le Boulaire et al., 1996), and receptor or microbial assays (Brunner et al., 1993; Langeloh and Petz, 1993; Charriere et al., 1996; Zomer et al., 1996) have been developed for rapid detection. However, the more complex are the matrices, the greater is the percentage of false results, due to interferences and cross reactions. These identification methods are not always specific or sufficiently accurate (Correge et al., 1994). On the contrary, HPLC combined with a diode array system proved to be able to detect a wide range of molecules and ensure their identification. Numerous authors cited for the use of MSPD extraction on meat employed this combination system. Indeed, the retention time and spectrum provide strong evidence of their identity and more specificity than the previous referenced methods. Quantification is also possible and can be essential when MRLs exist.

In this study, statistical data from the method validation are given, and a comparison was made between the MSPD technique with solvent or buffer extraction and liquid/liquid transfer usually practiced in residue analysis.

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Table 1. Liquid Chromatography Conditions

F1[a]		F2[b]	
time, min	% of B in A	time, min	% of B in A
0−5	14	0−5	44
5−22	14 to 22	5−15	44 to 64
22−30	22 to 54	15−25	64
30−35	14	25−30	44

[a] Flow rate 1 mL/min. [b] Flow rate 0.5 mL/min.

MATERIALS AND METHODS

Solvents and Reagents. The solvents used were of LC quality, available from commercial sources. Water for HPLC analysis was double-distilled water. Standards compounds (sulfathiazole, STH; sulfamerazine, SMR; sulfachloropyridazine, SCP; sulfamethazine, SMT; sulfamethoxypyridazine, SMO; sulfamethoxazole, SMA; sulfaquinoxaline, SQX; sulfadimethoxine, SDX; thiabendazole; mebendazole; nicarbazin; chloramphenicol, and furazolidone) were purchased from Sigma (St. Louis, MO). Virginiamycin was a gift from Smith-Kline Beecham (Louvain, Belgium). Working solutions were prepared in methanol at desired concentrations and stored at 4 °C.

Extraction Materials. Bulk C18 (40 μm; 12% load, end capped, octadecylsilyl-derivatized silica) was supplied by J. T. Baker (Noisy Le Sec, France). The bulk phase (20 g) was washed twice with hexane, methylene chloride, and methanol and dried at 37 °C before use. Syringe barrels (10 mL), which were used to prepare elution columns for samples, were thoroughly washed with hot soapy water, rinsed several times with distilled water, and dried overnight in an incubator at 37°C. Filter paper discs 16 mm in diameter were made in our laboratory by stamping them out. Porcelain mortars with pestles, an extraction system (Visiprep DL, Supelco, St Quentin Fallavier, France), and a rotary evaporator (Büchi) were needed for the extraction.

Extraction Procedure. Blank and spiked meat samples (30 g) were available from a local butcher, or pork positive samples came from Nestlé Research Centre. The samples were cut into pieces and blended, and an aliquot (0.5 g) was then weighed in a mortar. For the spiked material, 20 μL of a standard solution at the proper concentration was added to the meat subsample in the mortar and stored at 4 °C overnight. The prewashed C18 material (2 g) was gently ground with the meat using a pestle to obtain a homogeneous material. The resultant C18/tissue matrix mixture was transferred to a 10-mL syringe barrel previously plugged with a filter disc. A paper disc was put on the top. The mixture was then compressed with the syringe plunger to a final volume of 4.5 mL and placed on an SPE extraction system. This preparation was first washed with 8 mL of hexane. Flow was controlled at approximately 50 drops/min. The first fraction (F1) was then eluted with 8 mL of methylene chloride. Elution with another 8 mL of ethyl acetate gave a second fraction (F2). Both fractions collected were dried with a rotary evaporator at 40 °C. The dry extracts were dissolved in 250 μL of the mobile phase:ammonium acetate buffer (0.01 M; pH 5.2): acetonitrile:methanol, respectively 86:10:4; v/v/v for F1 and 56:31:13; v/v/v for F2.

HPLC Analysis. The analysis of standards and samples was conducted by a Hewlett-Packard HP1090M liquid chromatography apparatus equipped with a photodiode array and fluorescence detectors using Chemstation software. The chromatographic column was a reversed-phase octadecylsilyl (ODS) derivatized silica column (Spherisorb C18 ODS II; 250 mm length; 4.6 mm i.d.; 5 μm particle size). Analysis was performed at 35 °C. Two chromatographic systems were used for the different fractions with the same basic phases: an ammonium acetate buffer 0.01 M, pH 5.2, filtered under reduced pressure through a 0.45 μm pore-size filter for phase A and a mixture of acetonitrile:methanol (70/30; v/v) for phase B. The starting mobile phase composition for F1 was 14% of B in A held for 5 min, increasing to 20% B in A from 5 to 22 min, increasing to 54% B in A from 22 to 30 min and finally reequilibrated to 14% B in A for 5 min before the next injection. The flow rate was fixed at 1 mL/min. For F2, the starting mobile phase composition was 44% of B in A held 5 min, increasing to 64% B in A, from 5 to 15 min, held from 15 to 25 min 64% B in A and finally in the same way reequilibrated to 44% B in A for 5 min. The flow rate was fixed during at 0.5 mL/min. The injection volumes were 100 μL.

Diode array detector wavelengths were 270 and 365 nm for F1, while they were 254, 291, and 348 nm for F2. Fluorometric conditions were applied for F2: the wavelength of excitation was fixed at 308 nm and the emission at 365 nm. The chromatographic conditions are summarized in Table 1.

RESULTS

A detection limit determination is requested to characterize an analytical screening or confirmatory method. This limit allows deduction of the presence of the analyte with reasonable statistical certainty. According to the Commission's decision 93/256/EEC and in agreement with the CCMAS 1993 (Codex Committee for Methods of Analyses and Sampling), its establishment is equal to the mean of the measured apparent content of blank samples plus three times the standard deviation of the mean, or alternatively in the case of spectrometric determinations, three times the peak-to-peak noise.

The detection limits obtained using these two models are presented in Table 2. The results were measured

Table 2. Limits of Detection in μg kg^{-1} (ppb) in Meats and MRLs Fixed by the EU and the FAO (20 Pork Meat Samples and 10 Veal Meat Samples Tested)

		detection limit					
		mean + 3 × SD[b]		3 × mean		MRL, ppb	
	detection wavelength, nm	pork	veal	pork	veal	EEC	FAO
sulfathiazole	270	10.5	2.5	7.0	3.5	100	100
sulfamerazine	270	5.5	5.5	7.5	7.5	100	100
sulfachloropyridazine	270	9.0	3.5	7.0	3.5	100	100
sulfamethazine	270	2.5	5.0	3.0	5.0	100	100
sulfamethoxypyridazine	270	42.5	23.5	66.5	35	100	100
sulfamethoxazole	270	3.5	3.5	4.5	4.0	100	100
sulfaquinoxaline	365	3.0	6.0	6.0	6.5	100	100
sulfadimethoxine	270	2.5	4.0	5.0	6.5	100	100
furazolidone	365	2.5	2.0	3.5	3.5	5	not allocated
chloramphenicol	290	6.5	5.5	7.0	5.0	0	0
thiabendazole	fluo	0.4	ND[a]	0.8	ND[a]	100	not allocated
thiabendazole	290	9.0	9.5	8.0	15.5	100	not allocated
mebendazole	254	1.0	2.0	1.0	1.5	100	not allocated
virginiamycin	254	6.5	2.0	7.5	2.5	not allocated	100 (USA)
nicarbazin	348	0.7	0.4	1.0	0.5	not allocated	not allocated

[a] ND: not determined. [b] SD: standard deviation.

Table 3. Percentage Recoveries of Spiked Samples in Meat

fortified level	*n*	concentration[a] measured	recovery, %	fortified level	*n*	concentration[a] measured	recovery, %
			Sulfathiazole				
50 ppb	2	19.6	39	200 ppb	2	90.3	45
75 ppb	2	32.8	66	250 ppb	2	134.5	54
100 ppb	10	43.6 ± 12.5	44	150 ppm	2	85.5	57
150 ppb	2	70.4	47	250 ppm	2	125.0	50
			Sulfamerazine				
50 ppb	2	36.2	72	150 ppb	2	110.8	74
75 ppb	2	52.4	70	200 ppb	2	128.6	64
100 ppb	15	79.6 ± 11.6	80	250 ppb	2	203.6	81
			Sulfamethoxypyridazine				
50 ppb	2	30.1	60	150 ppb	2	101.6	68
75 ppb	2	45.1	60	200 ppb	2	117.9	59
100 ppb	2	64.9	65	250 ppb	2	191.4	77
			Sulfamethazine				
50 ppb	2	34.9 ± 2.7	70	150 ppb	2	109.8	73
75 ppb	2	50.3 ± 12.6	67	200 ppb	2	127.2	64
100 ppb	5	77.0 ± 1.6	77	250 ppb	2	199.6	80
			Sulfachlorpyridazine				
50 ppb	2	42.4	85	150 ppb	2	114.5	76
75 ppb	2	63.6	85	200 ppb	2	150.3	75
100 ppb	13	76.1 ± 10.2	76	250 ppb	2	218	87
			Sulfamethoxazole				
50 ppb	2	39.7	79	150 ppb	2	118.7	79
75 ppb	2	55.1	74	200 ppb	2	133.6	67
100 ppb	13	85.6 ± 18.5	86	250 ppb	2	210.1	84
			Sulfaquinoxaline				
50 ppb	2	32.3	65	200 ppb	2	108.6	54
75 ppb	2	43.6	58	250 ppb	2	163.0	65
100 ppb	13	73.2 ± 8.0	73	150 ppm	2	107.0	71
150 ppb	2	90.5	60	250 ppm	2	179.8	72
			Sulfadimethoxine				
50 ppb	2	38.6	88	150 ppb	2	127.9	85
75 ppb	2	58.4	78	200 ppb	2	146.2	73
100 ppb	10	79.6 ± 9.7	80	250 ppb	2	223.7	90
			Furazolidone				
5 ppb	2	2.6	51	50 ppb	2	21.7	43
10 ppb	2	4.4	44	100 ppb	6	87.1 ± 13	87
20 ppb	2	6.0	30	150 ppb	2	112.2	75
			Thiabendazole				
100 ppb	8	93.6 ± 22.8	94	4 ppm	2	2.74	69
2 ppm	2	2.0	100	8 ppm	2	7.52	94
			Mebendazole				
100 ppb	8	96.8 ± 21.1	97	4 ppm	2	3.3	83
2 ppm	2	1.9	97	8 ppm	2	8.2	102
			Virginiamycin				
50 ppb	2	37.5	75	100 ppb	4	78.1 ± 7.7	78
			Nicarbazine				
20 ppb	2	10.4	52	100 ppb	8	55.1 ± 8.1	55
			Chloramphenicol				
50 ppb	4	36.0 ± 5.2	72	100 ppb	2	75.3	75

[a] Mean of *n* replicates ± SD.

for 20 different blank samples of pork meat and 10 of veal meat. Their distribution was gaussian (Shapiro's test at 98%). The wavelengths and the MRLs required by the EU and the FAO are also mentioned in this table for each compound to permit assessment if its suitability.

Relative recoveries were calculated as the ratio between the response of the analyte in the MSPD-extracted fortified samples and the response of the standard solutions (not in matrix). Spiked samples with different analytes at different levels were assayed. All the results are presented in Table 3, with the calculated recovery percentages. For the sulfonamides at the level of 100 ppb, the data were obtained on measurements at different time intervals.

The method had linear responses from 50 to 250 ppb, for both standards and extracted spiked samples. Quantification of each sample content was calculated from the calibration curves based on the peak height of duplicate standards. Correlation coefficients were 0.998 for all calibration curves.

The repeatability is the closeness of agreement between mutually independent test results obtained under repeatable conditions with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time. It gives an idea of the accuracy of the entire method. This value was estimated using real sulfamethazine-contaminated meat samples in order to work under realistic conditions. Table 4 shows the standard deviation of four different samples of pork meat.

Chromatograms corresponding to a standard (A), a blank tissue sample (B), and two incurred samples of

Table 4. Interassay Variation for the Determination of Sulfamethazine in Meat by MSPD−HPLC

sample identification no.	n^a	method of calculation[b]	measured content, ppb, mean ± SD	CV, %
1	6	h	36.1 ± 2.9	8
2	5	h	197.8 ± 23.6	12
2	2	a	213.5 ± 21.4	10
3	3	h	164.9 ± 6.6	4
3	3	a	169.8 ± 12	7
4	2	h	58.8 ± 5.7	

[a] Number of duplicates. [b] Calculated by the height (h) or the area (a).

pork meat (C and D), respectively, containing 36 and 165 ppb, are presented in Figure 1. Peaks in C and D having the same retention time as sulfamethazine in standard A have been compared. Their spectra are identical as shown in Figure 2.

DISCUSSION

Extraction Technique. A critical aspect of drug residue analysis is the sample extraction and purification steps required to isolate the residue from biological matrix components. In most of the methods for the analysis of drug residues, extraction of the analyte is first performed by a buffer or a solvent extraction using a homogeneizer or a blender. These procedures need large solvent volumes, followed by a liquid/liquid purification and are often the limiting step for a multiresidue method. Indeed, for each family of compounds, some optimal conditions of pH, solvent composition and volume for such extractions exist. As the two liquids must not be miscible, the variety of the extracting solvents is limited. Solubility of the analyte in one of the liquids is the only property that can be used in the liquid/liquid extraction principle. Others disadvantages of this technique are the tendency of samples to form

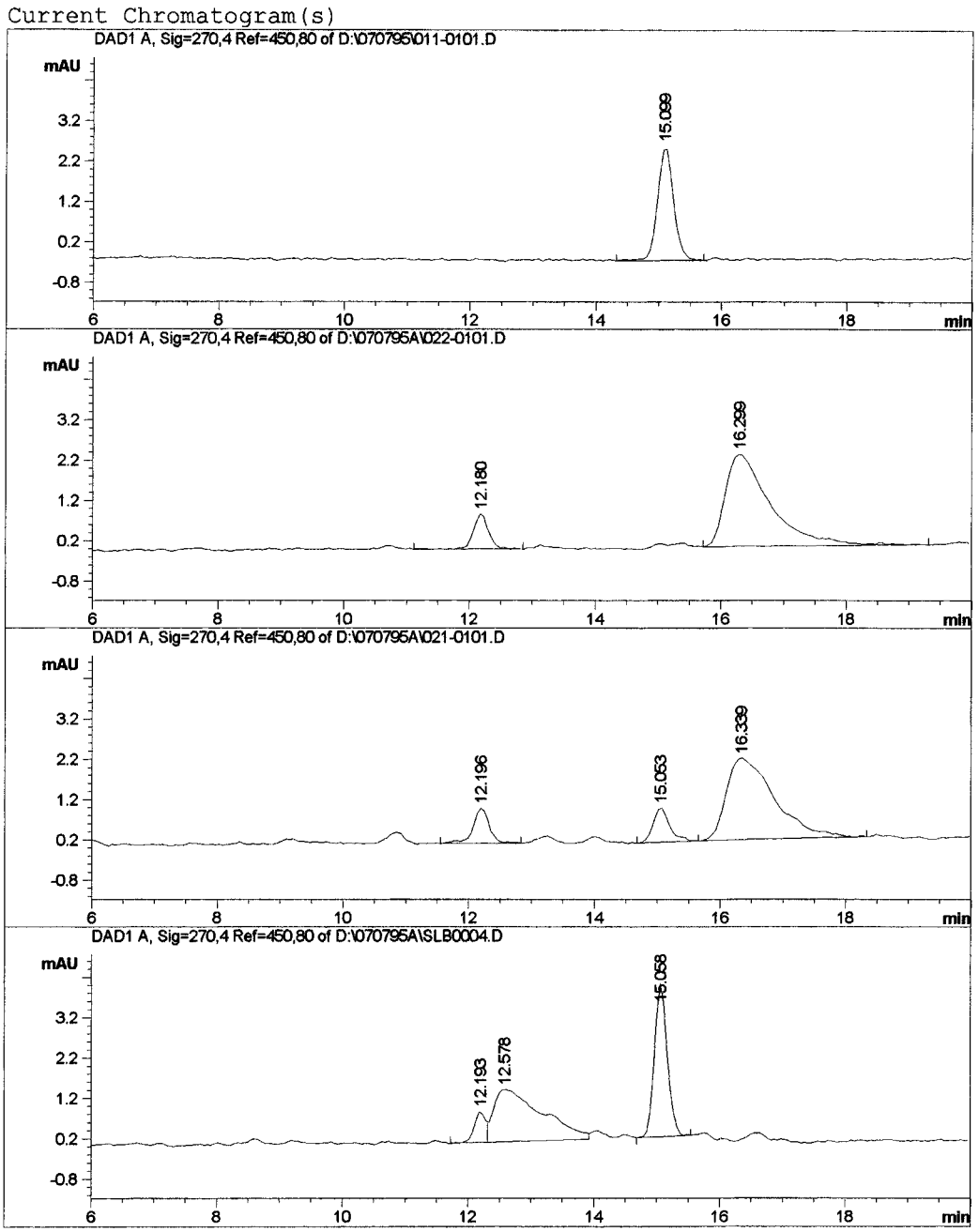

Figure 1. LC chromatograms (UV at 270 nm) of (A) sulfamethazine standard solution (100 ppb) and the dichloromethane extracts of (B) control blank sample and (C, D) incurred samples (respectively, 36 and 165 ppb concentrations).

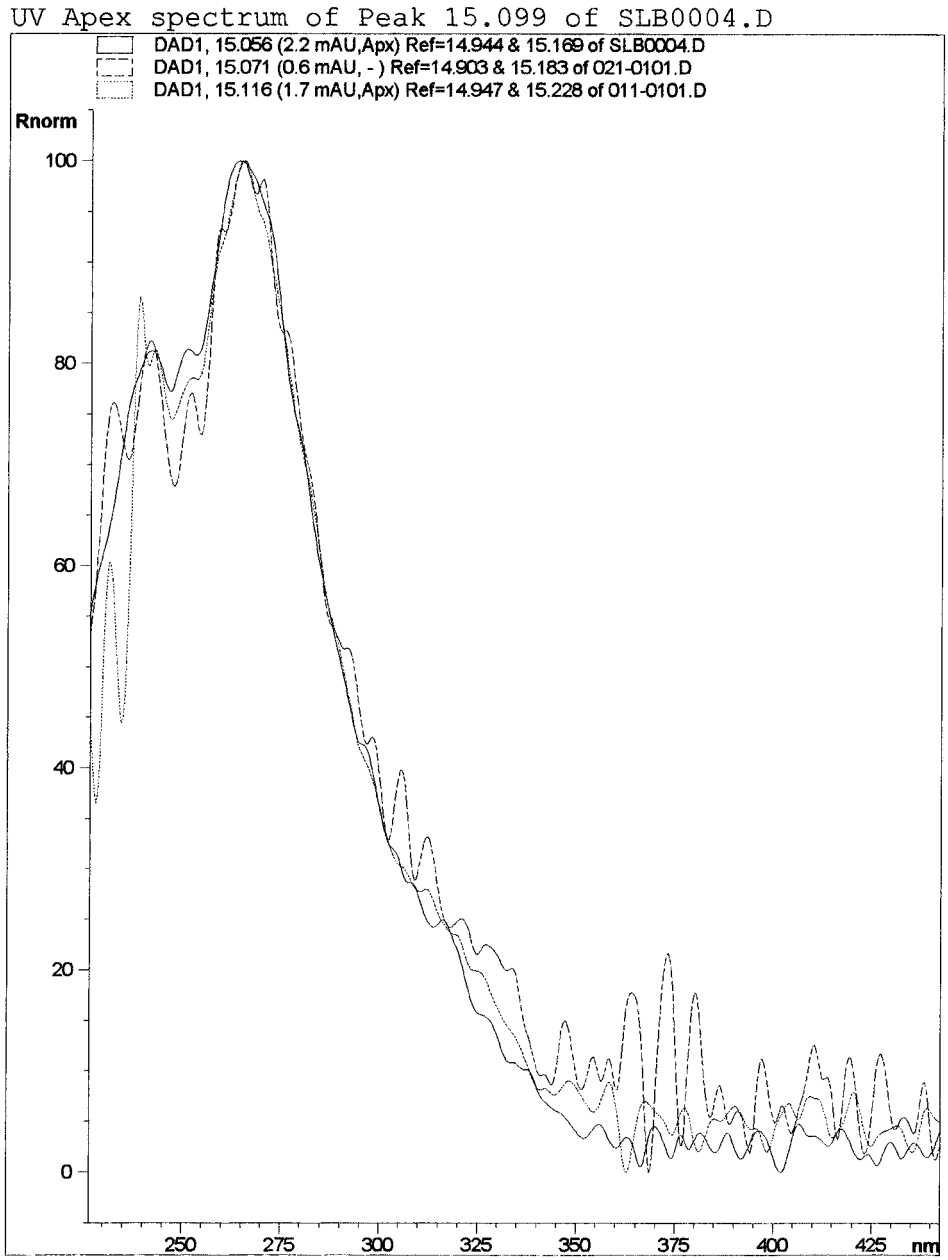

Figure 2. Overlayed normalized UV spectra (200-450 nm) of A, C, and D peak chromatograms at 15 min.

intractable emulsions after shaking, extensive solvent use, and the expense of glassware and washing. This led us to avoid this type of extraction for a multiresidue method, when speed and automation perspectives were requested.

On the past few years, solid phase extraction (SPE) has began to replace liquid/liquid purification. The compounds to be isolated can be retained using the solid surface or bonded phase as the extracting technique. The SPE method is more selective and repeatable and can be easily automated by a laboratory robot. Usually applied for liquid samples, this approach can also be considered for the first extraction step of a nonliquid sample. The matrix solid phase dispersion technique consists of a matrix homogenization with a solid silica phase fashioned into a column as in SPE. Analytes and matrix interferences are retained on the mixed solid phase material. Specific elutions allow recuperation of the analyte after the elimination of matrix compounds by a wash steps.

The MSPD mechanisms appear to encompass sample homogenization, cellular disruption, extraction, fractionation, and purification in a single process.

It has been demonstrated that mixing biological samples with silica-bonded supports provokes a disruption of the sample structure by the mechanical blending, while the octadecylsilane phase induces a lot of chemical interactions within the matrix components. Such evidence has been given by Barker (Barker et al., 1993) in conducting the MSPD fractionation on Mycobacterium, one of the most difficult bacteria to disrupt. The blend analyzed by scanning electron microscopy shows lysis and fractionation of the cells. The examination of a homogenized C18-liver by the same way supports the idea that complete cellular lysis and disruption is occurring. These observations underline that the MSPD extraction can efficiently replace the mechanical forces obtained by a cellular disrupter, a stirring rod (Ultra-Turrax), or a tissue homogenizer (Waring Blendor) often used in tissue extractions or the detergents used in chemical procedures. Furthermore chemical interac-

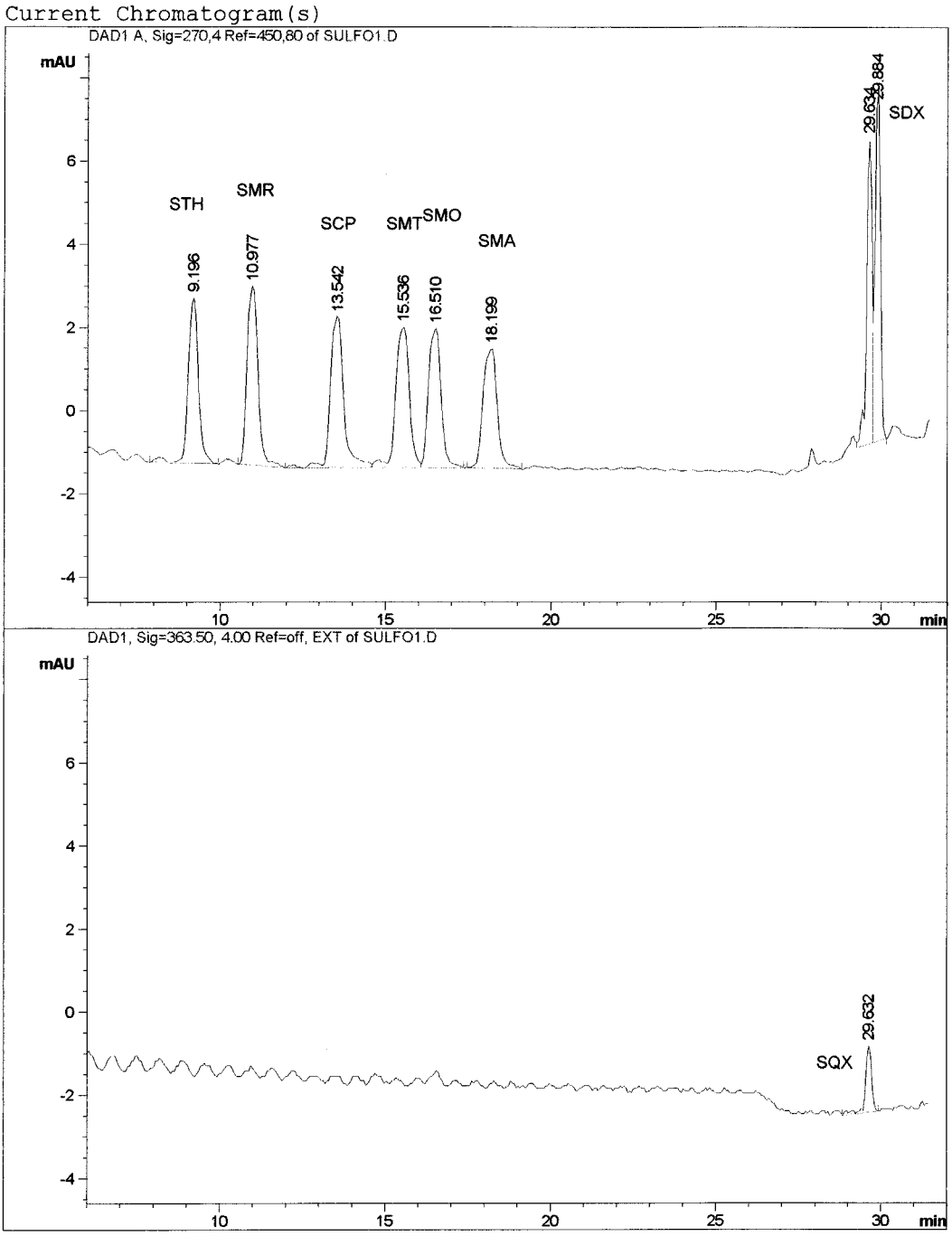

Figure 3. Representative LC chromatograms of sulfonamides (20 ng) obtained from the UV detector analysis at 270 nm (A) and 365 nm (B).

tions between the matrix and the C18 allow specific solvent elution of the interest molecules.

The advantages of this process are numerous. Considerable *savings of time* is reached. We noticed that extraction of 12 samples can be achieved in one-half day instead of 1 day and one-half day using classical multiresidue methods tested.

The elution can be managed *automatically* in the same way as an SPE column. This automation avoids the need to attend the sample, and the extraction can be performed overnight, the results being available during the next morning. Rosen (Rosen et al., 1994) established that up to 30 MSPD columns per day can be prepared by one person and processed overnight by automation. This time-savings reduces the cost of the analysis when existing methods tend to be highly time-, labor-, and material-consuming and are subsequently prohibitively expensive, having a low sample throughput capacity. In addition, the automation of the method improved precision, reproducibility, and recovery for the SPE (Jordan, 1993) and may give a more rugged and reliable method in our application.

There is also a gain brought about in *solvent consumption*. The volumes of organic solvents are small, for example 89% reduction was obtained compared to Malisch's method (Malisch et al., 1992). Moreover, the exposure of laboratory workers to these solvents is also minimized.

MSPD also eliminates many steps and problems associated with classical isolation techniques (emulsion), reduces transfers, and consequently increases extraction

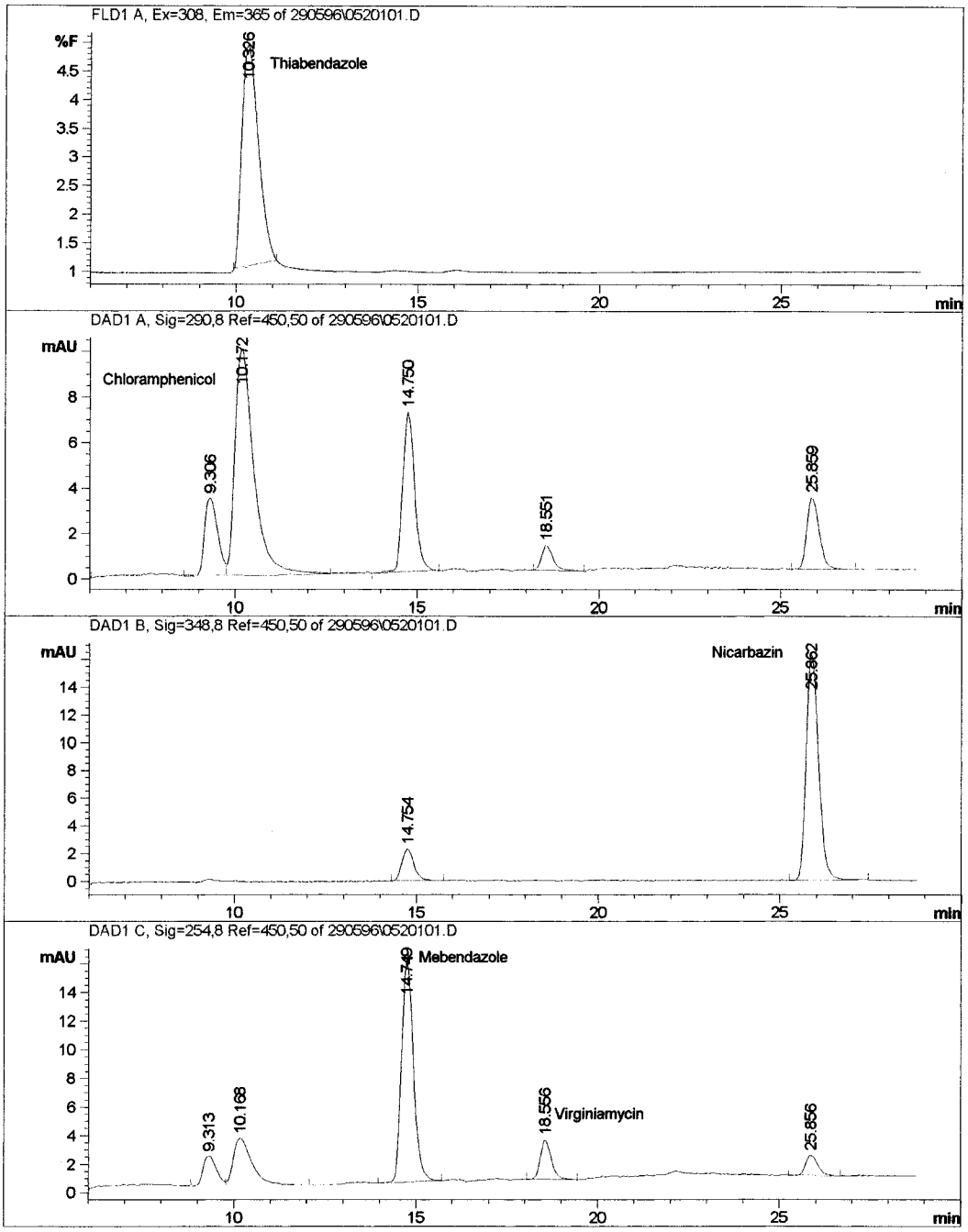

Figure 4. Representative LC chromatograms of thiabendazole, mebendazole, chloramphenicol, nicarbazin, and virginiamycin (20 ng) obtained from the fluorometric (A) and UV detector analysis at 348 nm (B), 290 nm (C) and 254 nm (D).

yields. This technique also reduces laboratory equipment investments.

Identification. The HPLC/UV diode array and fluorescence methods chosen allow the separation of the analytes and identification of them by their retention time and their spectrum and makes possible their quantification.

The *solvent system* studied was a mixture of acetonitrile:methanol and an ammonium acetate buffer (0.01 M). It was found to be the most adequate for our application. Actually, the concentration gradient permits the separation of the compounds in F1, and the same eluents were employed for the second MSPD-fraction F2. Consequently, the same HPLC apparatus and the same mobile phase composition were required using various chromatographic systems. Before the analysis of the F2 group, a reequilibration of the system was just needed.

In order to reach maximum sensitivity, each analyte's spectrum was studied under these HPLC conditions and the *maximum absorbencies* were chosen (Table 2): 270 nm was selected for the sulfonamides, except for sulfaquinoxaline (SQX) for which 365 nm was preferred. Sulfadimethoxine and sulfaquinoxaline tend to be coeluted after many injections on the same column, but they can be easily distinguished by the absorbency of SQX at 365 nm. A chromatogram of those compounds is shown in Figure 3 (experiments reported in Figure 1 and 3 have not been carried out with the same HPLC column, leading to a slightly different retention time). A furazolidone was also detected at this wavelength. In the F2 fractions, no coelution was noted. 290 nm was chosen for chloramphenicol and thiabendazole while 254

nm was selected for virginiamycin and mebendazole. At 348 nm, the nicarbazin was detected and the presence of mebendazole can be confirmed. The thiabendazole tends to have a large peak width and to induce tailing. A fluorimetric detector was used to detect and quantify thiabendazole residues. Excitation and emission wavelengths were also improved, respectively, fixed at 308 nm and 365 nm. A chromatogram is presented in Figure 4.

The *interferences* are much higher at lower wavelengths. In the range of 350−450 nm, no matrix compounds absorb. Therefore, furazolidone, nicarbazin, and sulfaquinoxaline have a very sensitive detection. On the other hand, at 270 nm in the sulfonamide chromatogram, one major peak is eluted between sulfachloropyridazine (SMP) and sulfamethoxazole. This may explain the poor detection limit for SMP. This major peak seems to be a natural component of meat present both in veal and pork. However no confusion can be made, confirmation of the presence of the molecule was validated against standards, blank, and spiked material. Actually, the unknown peak spectra are normalized and overlaid with the standard spectra to confirm the suspicion, allowing one to differentiate, with great certainty, the residue examined from a matrix contaminant.

The quantification by calibration curves and the possible use of an internal standard and correction factors for the extraction, permit us to determine if the MRL has been exceeded. It is worthy to note that this methodology is applicable to various matrices.

Performance of the Method. The *limits of detection* were sufficient for most of the drugs. Indeed, they were below the tolerance whatever the mode of calculation. As shown in the Figure 1, the matrix noise produced is quite low and allows for good sensitivity. The detection limit remains far below the tolerance levels for the 100 ppb MRL. Chloramphenicol, recently banned, could not be detected in a 5 ppb spiked material. Another more sensitive method, such as GC-MS, would be necessary to reach a detection limit under the 1 ppb level.

The *recoveries* were calculated at different concentrations. While they were satisfactory for most of the sulfonamides, sulfathiazole gives recoveries between 40 and 60% depending on the concentration. Malisch (Malisch et al., 1992) found the same results with another extraction method. He suggested the relatively low recoveries could be explained by the formation of an Amadori compound. For furazolidone, recoveries at concentrations under 100 ppb are lower but are compensated by the sensitivity of detection. Nicarbazin was also in the same situation. On the other hand, benzimidazoles, virginiamycin, and chloramphenicol were well recovered.

The *linearity* of the method tested on the sulfonamides seems to be acceptable. In the range of 50 to 250 ppb a linear response was observed for MSPD extracts from muscle.

The *repeatability* calculated on identical test material, in the same laboratory, by the same operator, using the same equipment was also satisfactory. The method gives coefficients of variation under 12% for the fourth material analyzed. It can be noticed that the quantification by peak height seems to give lower results on the same sample.

CONCLUSION

To prevent the consumption of drug residues in food of animal origin and to conform to legislation, industries have to check their raw materials. This implies a large number of samples and a wide range of compounds to analyze. Consequently, there is a need to develop a new methodology with multiresidue extraction of drugs and their metabolites from biological matrices combined with a detection system that can unambiguously identify and accurately quantify the residues of concern.

The multiresidue capability of MSPD makes it attractive. The mechanisms of such a technique appear to encompass sample homogenization, cellular disruption, extraction, fractionation, and purification in a single process. In addition, this technique is easy to perform, does not need expensive or special equipment, and can be automated. The statistical criteria fixed by the European system for a screening method are respected. Regarding recoveries, the extraction seems to be sufficient to detect a number of violative residue levels, except for prohibited residues of chloramphenicol which need a more sensitive detection method such as GC-MS for example.

The present method can be extended to other molecules, for example the family of ivermectin (Schenck et al., 1992), acetylgestagens (Rosen et al., 1994), tetracyclines (Long et al., 1990), clorsulon (Schenck et al., 1991) or β-agonists (Boyd et al., 1994) which have been extracted by MSPD techniques. A survey of compounds in a sample can be possible for other compounds not included yet, by identifying new peaks in the chromatograms or by eluting them with other solvents. Furthermore, other matrices can be treated by this way, like liver (Boyd et al., 1994), (Schenck et al., 1992), kidney (Rosen et al., 1994), milk (Long et al., 1990), catfish tissue (Long et al., 1990; Walker et Barker, 1994), or salmon (Reimet et Suarez, 1992).

This rapid screening procedure can be also associated with several detection modes such as immuno or receptor assays in a short time or HPLC for the identification and the quantification of target residues. The MSPD technique represents a new approach in the field of biological matrices extraction and provides great possibilities for the analysis of a wide range of compounds.

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