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Screening of sulphonamides in egg using gas chromatography–mass-selective detection and liquid chromatography–mass spectrometry

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Abstract

A rapid extraction and clean-up procedure for sulphonamide antibiotics in eggs suitable for both GC–MSD and LC–MS end determinations has been developed. The drugs were extracted using acetonitrile, acidified using acetic acid and cleaned-up using cation- and anion-exchange. For determination by GC–MSD, extracts were derivatised with diazomethane followed by pentafluoropropionic acid anhydride. For LC–MS extracts were taken up in water and used directly. The methodology developed was validated at the 100 and 25 $\mu g\ kg^{-1}$ levels, equivalent to the MRL and one quarter of the MRL. Results for the GC–MS procedure were quantitated against deuterated sulphadiazine with relative recoveries ranging from 54% for sulphachloropyridazine to 135.5% for sulphamethazine. Recoveries for the LC–MS procedure ranged from 33% for sulphaguanidine to 92% for sulphamethazine and sulphadimethoxine. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Sulphonamides

1. Introduction

The sulphonamide class of antibiotics are a diverse class of chemically related compounds (Fig. 1). A number have found widespread use in animal husbandry, in particular sulphamethazine, sulphadiazine, sulphaquinoxaline and sulphamethoxypyridazine.

There is a vast literature on methodology for the determination of sulphonamides in animal tissues and fluids. A number of methods have used mass spectrometry (MS) for the final determination, after both gas chromatography (GC) and liquid chromatography (LC) separations. Methods for GC–MS tend to be time-consuming due to the clean-up and derivatisation required prior to GC. For example, Carignan and Carrier [1] used liquid–liquid extraction followed by high-performance liquid chromatography (HPLC) clean-up and derivatisation with diazomethane prior to capillary GC–MS for the determination of sulphamethazine in pig tissues. Liquid–liquid extractions have also been used by other authors prior to GC–MS [2,3]. Mooser [4] used liquid–liquid chromatography followed by silica gel chromatography for clean-up prior to GC–MS for the determination of 13 sulphonamides in animal tissues. One disadvantage of this procedure was that to cover the range of sulphonamides, three different derivatisations were required. Takatsuki and Kikuchi [5] used silica solid-phase extraction (SPE) prior to derivatisation with further clean-up of the derivative

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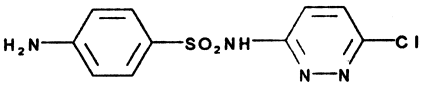

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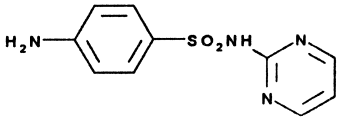

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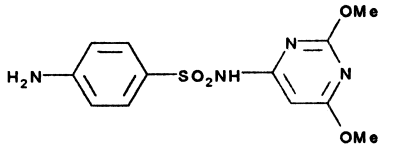

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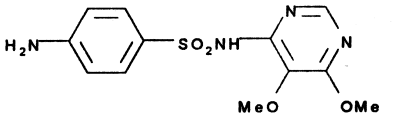

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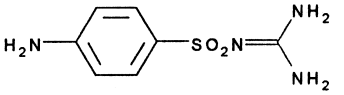

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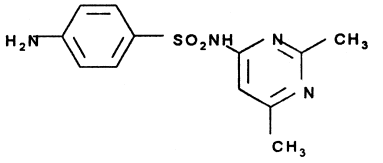

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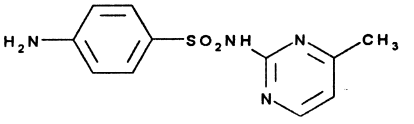

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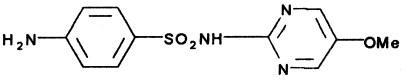

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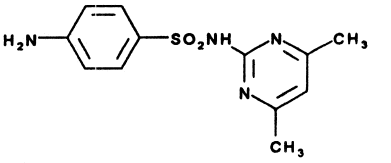

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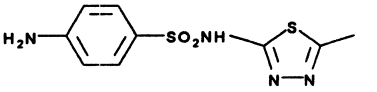

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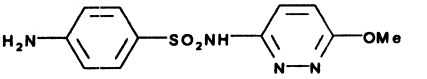

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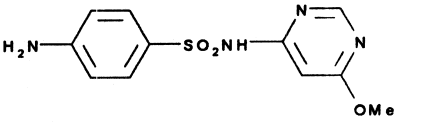

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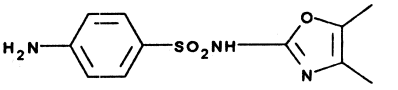

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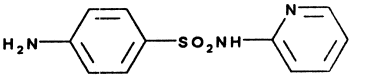

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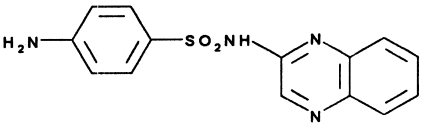

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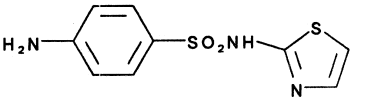

Sulphathiazole (STZ)

Fig. 1. Structures of sulphonamide antibiotics.

using silica gel chromatography for the determination of 6 sulphonamides in tissues and egg. Van Pouke and Van Peteghem [6] used high-performance thin-layer chromatography (HPTLC) following silica solid-phase extraction (SPE) prior to capillary GC–MS.

The most common interface that has been used in LC–MS of sulphonamides is thermospray. Abián et al. [7] used a relatively straightforward clean-up for the determination of 10 sulphonamides in milk. Silica SPE was used as clean-up by Balizs et al. [8] for the determination of four sulphonamides and their N^4-acetyl metabolites in pig muscle.

This laboratory had previously developed a procedure for the determination of six sulphonamides in egg using GC–mass-selective detection (MSD) as the determinative procedure [9]. This paper reports the development of a rapid SPE-based procedure which provides sufficient clean-up for extracts to be screened by either GC–MSD (after derivatisation) or LC–MS determination.

2. Experimental

2.1. Principle

Sulphonamides are extracted from homogenised whole egg using acetonitrile. The sulphonamides are cleaned-up following acidification with acetic acid and separated into two fractions by cation-exchange SPE followed by anion-exchange SPE. Determination of the basic sulphonamide SGN in fraction 1 is by LC–MS. Determination of the remaining amphoteric sulphonamides in fraction 2 is by either LC–MS or GC–MSD following derivatisation with diazomethane and pentafluoropropionic anhydride.

2.2. Reagents

Glacial acetic acid, 0.88 ammonia and potassium hydroxide pellets were obtained from BDH (Poole, UK). Diazald was obtained from Aldrich (Gillingham, UK). Absolute alcohol was obtained from Hayman (Witham, UK). HPLC grade acetone, acetonitrile, dichloromethane, diethyl ether, hexane and methanol were obtained from Rathburn Chemicals (Walkerburn, UK). Pentafluoropropionic acid anhydride (PFPA) was obtained from Pierce Warriner (Chester, UK). Bond-Elut strong anion-exchange (SAX) and strong cation-exchange (SCX) cartridges were obtained from Varian (Walton-on-Thames, UK).

Sulphachloropyridazine (SCP), sulphadiazine (SDZ), sulphamonomethoxine (SMM), sulphamethoxypyridazine (SMP), sulphamerazine (SMR), sulphamethizole (SMT), sulphamethazine (SMZ), sulphapyridine (SPN), sulphaquinoxaline (SQX) and sulphathiazole (STZ) were obtained from Sigma (Poole, UK). Sulphadoxine (SDX), sulphaguanidine (SGN), sulphisomidine (SIM), sulphameter (SME) and sulphamoxole (SMO) were obtained from Riedel-de-Haën (Philip Harris Scientific, Lichfield, UK). Sulphadimethoxine (SDM) was obtained from Fluka (Gillingham, UK). d^4-Sulphadiazine and d^4-sulphamethazine were obtained from Lancaster Synthesis (Morecambe, UK).

2.2.1. Diazomethane preparation

Diazomethane was prepared using the Aldrich kit for the preparation of 100mmol quantities. The glassware used had no ground glass joints. Absolute alcohol (6 ml) was added to a solution of potassium hydroxide (1.25 g) in water (2 ml) in a round-bottomed flask fitted with a separating funnel. The flask containing the alkali solution was heated in a water bath to approximately 65°C. A solution of Diazald (5.35 g) in diethyl ether (50 ml) was added dropwise through the separating funnel. The rate of addition was approximately equal to the rate of distillation. The collecting flask was cooled in an acetone–dry ice bath. When the separating funnel was empty a further 10 ml of diethyl ether was added and the distillation continued. The ethereal diazomethane solution was stored in screw-cap vials at −20°C and could be kept for at least six weeks.

2.3. Apparatus

A homogeniser (Ultra-Turrax, Janke & Kunkel), centrifuge (Jouan CR4.22), Vac-Elut (Varian, obtained from Jones Chromatography), Anatop 10 0.2 μm filters (Whatman), nitrogen blowdown and hot-block (Pierce), vortex-mixer (Fisons) and ultrasonic bath (L&R) were used.

2.4. Extraction

Prehomogenised egg (5 g) and acetonitrile (50 ml) were homogenised for 1 min and then centrifuged for 5 min at 4500rpm (4200 *g*) and 10°C. The supernatant was decanted and glacial acetic acid (5 ml) added.

2.5. Clean-up

Bond-Elut SCX cartridges were prepared by passing 5% acetic acid in acetonitrile (5 ml) through them. The acidified supernatant was then passed through the cartridge and the cartridge washed with methanol (5 ml) followed by acetonitrile (5 ml). The cartridge was then eluted with 5% gr. 0.88 ammonia in acetonitrile (10 ml).

Bond-Elut SAX cartridges were prepared by passing 5% 0.88 ammonia in acetonitrile (5 ml) through them. The SCX eluate was then passed through the cartridge and the eluate collected (fraction 1). A further 2.5 ml either acetonitrile or 5% 0.88 ammonia in acetonitrile was passed through the cartridge and collected. This wash was combined with fraction 1. The SAX cartridge was then washed with methanol (5 ml) followed by acetonitrile (5 ml) and eluted with 5% acetic acid in acetonitrile (10 ml) (fraction 2).

Fractions 1 and 2 were then evaporated to dryness under a stream of nitrogen 45–50°C.

For determination by LC–MS, the residue was redissolved in water (1 ml) by vortex-mixing for 15 s and ultrasonicating for 3 min.

2.6. Further sample treatment prior to GC–MS

The residue after evaporation from fraction 2 was dissolved in dichloromethane (1 ml) by vortex-mixing for 15 s and ultrasonicating for 3 min and filtered through an Anatop 10 0.2 μm filter. The filtrate was evaporated to dryness under a stream of nitrogen at 45–50°C. The residue was redissolved in 1 ml diazomethane in diethyl ether and left for 15 min. The solution was evaporated to dryness under a stream of nitrogen at 45–50°C, the residue dissolved in 5% PFPA in hexane (1 ml) and left for 15 min. The solution was evaporated to dryness under a stream of nitrogen at 45–50°C and redissolved in toluene (50 μl).

2.7. LC–MS

Samples (50 μl) were injected (Gilson 231XL) onto a Bondclone 10 C_{18} 10 μm, 300×3.2 mm column. Mobile phase was 0.05 *M* ammonium acetate (adjusted to pH 4 with acetic acid)–acetonitrile (80:20, v/v). Mobile phase flow-rate was 0.5 ml min^{-1} maintained at 40°C. Detection was by MS using a Micromass Platform operated in the positive ion atmospheric pressure chemical ionisation (APCI) mode. The nominal source tuning parameters were as follows: corona 3.00 kV, high voltage lens 0.10 kV, cone 10 V offset 5 V, source temperature 120°C and APCI probe temperature 500°C. The ions monitored are listed in Table 1. The linearity of response was checked over the ranges 5–50 ppb tissue equivalent (1.25–12.5 ng on-column) and 25–200 ppb (6.25–50 ng on-column).

Table 1
Fragmentation ions monitored for GC–MSD and LC–MS determination of sulphonamides[a]

Sulphonamide	GC–MSD *m/z*	LC–MS *m/z*
d^4-Sulphadiazine (SDZ)	348	269
d^4-Sulphamethazine (SMZ)		283
d^4-Sulphamerazine (SMR)		269
Sulphachlorpyridazine (SCP)	379	285
Sulphadimethoxine (SDM)	405	311
Sulphadoxine (SDX)	405	311
Sulphadiazine (SDZ)	345	251
Sulphaguanidine (SGN)		215
Sulphisomidine (SIM)	373	279
Sulphameter (SME)	375	
Sulphamonomethoxine (SMM)	375	281
Sulphamoxole (SMO)	427	268
Sulphamethoxypyridazine (SMP)	375	281
Sulphamerazine (SMR)	359	265
Sulphamethizole (SMT)	430	271
Sulphamethazine (SMZ)	373	279
Sulphapyridine (SPN)	344	250
Sulphaquinoxaline (SQX)	395	301
Sulphathiazole (STZ)	350	256

[a] For GC–MSD, sulphamethizole and sulphamoxole ions correspond to $[M]^+$ for the derivatised compounds, the remaining ions correspond to $[M-HSO_2]^+$ [3]. For LC–MS, ions correspond to $[M+H]^+$.

2.8. GC–MSD

Analysis was carried out using a Hewlett-Packard 5980 series II gas chromatograph with a 5971 mass selective detector. The column used was a Hewlett-Packard Ultra 1 (crosslinked Me silicone) (12 m×0.2 mm, 0.33 μm). Carrier gas was helium. The system was maintained in constant flow mode with a pressure of 20 kPa at 200°C, giving a linear velocity of 28.6 cm s^{-1}. Injection volume was 0.5 μl in splitless mode. The injector temperature was 250°C and the transfer line to the MSD system was set at 300°C. The initial oven temperature was 140°C and was held at this temperature for 3 min after injection. The oven was programmed at 5°C/min to a temperature of 275°C. The total run-time was 30 min. Detection was in the selected ion mode. The ions monitored are listed in Table 1. Quantification was by ratioing peak areas against the internal standard (d^4-SDZ) peak area and quantifying against a tissue standard at the equivalent spike level. The linearity of response was checked over the range 25–200 ppb tissue equivalent (1.25–10 ng on-column).

2.9. Protocol

For method validation on both GC–MSD and LC–MS, samples were analysed in batches of eight, consisting of one blank sample, one blank sample to be used as a tissue standard and six spikes.

For GC–MSD, samples (except the blank to be used as a tissue standard) were spiked with d^4-SDZ at an appropriate level prior to extraction. The sample to be used as a tissue standard was spiked with a mixed sulphonamide solution at an appropriate level immediately prior to derivatisation.

For LC–MS, samples (except the blank to be used as a tissue standard) at the final dissolution stage were made up in water containing either d^4-SMR or d^4-SMZ at an appropriate level. The sample to be used as a tissue standard was made up in water containing all the sulphonamides to be analysed for at an appropriate level.

The time of preparation for a batch of eight samples was approximately 4 h for HPLC analysis and approximately 6 h for GC–MSD analysis.

3. Results and discussion

The procedure previously developed in this laboratory [9] used silica-based cation-exchange for clean-up following extraction with acidic ethyl acetate. Whilst this clean-up was sufficient for screening purposes by HPTLC, a further series of liquid–liquid extractions were necessary before derivatisation for determination by GC–MSD. The whole procedure gave good recoveries for the six sulphonamides measured, but was time-consuming, a batch of eight taking approximately 1.5 days to prepare. The aim of this work was therefore to improve the extraction and clean-up such that a batch could be prepared within the time-scale of a single day. Additional aims were to broaden the scope of the analysis to more sulphonamides and to be able to apply the protocol to the preparation of samples for both GC– and LC–MS.

Initial work using solvent spikes demonstrated the efficacy of ion-exchange for the retention and elution of sulphonamides. The amphoteric nature of the majority of the common sulphonamides indicated both cation- and anion-exchange might be used. Using silica-based SCX columns a wide range of sulphonamides were retained on the cartridge when loaded in 5% acetic acid in acetonitrile or ethyl acetate. The sulphonamides remained on the cartridge when washed with methanol and could be eluted in high yield using ammonia in either methanol or acetonitrile. On silica-based anion exchangers (SAX), the majority of the sulphonamides investigated were retained when loaded in 5% ammonia in methanol or acetonitrile. The exception to this was sulphaguanidine (SGN) which was not retained at all on the SAX cartridge. The cartridge could then be washed with methanol and eluted with 5% acetic acid in acetonitrile. From these results it was concluded that the sulphonamides could be conveniently fractionated into two groups by use of the SAX column, one containing the basic sulphaguanidine and the second containing the other amphoteric sulphonamides.

Having established the principle of the clean-up protocol, a full extraction and clean-up procedure was then developed. Preliminary work indicated that acetonitrile (without the addition of acid or base

modifier) was effective in extracting the sulphonamides and gave cleaner extracts from egg than ethyl acetate. SCX and SAX columns were then examined individually and also in sequential mode. It rapidly became clear that egg extracts (following the addition of ammonia) could not be loaded directly onto an SAX cartridge. Overloading of the cartridge was thought to be the problem. Good recoveries were obtained from the SCX SPE column. However, the extracts were still not clean enough to be used for GC–MSD. Vastly improved clean-up was achieved when the eluate from the SCX column was passed directly through an SAX column. Collection of the loading fraction from the SAX cartridge enabled determination of the basic sulphonamide whilst elution of the cartridge with acetic acid in acetonitrile enabled the determination of the amphoteric sulphonamides. Inclusion of methanol washes on both cartridges also improved the clean-up. An acetonitrile wash was included prior to elution of the SAX cartridge as it was found that going straight from a methanol wash to elution increased the amount of co-eluting material. This was thought to be due to the presence of residual methanol in the SPE cartridge.

Following blow-down of the SAX eluates, take-up in water was found to be suitable for determination by LC–MS. For GC–MSD, initially methanol was used to dissolve the residue prior to derivatisation. However, some polar material appeared to be transferred using this solvent, which had an adverse effect on the column performance. For this reason dichloromethane was used to dissolve the residue. It was also found that on using an SPE box with metal transfer tubes, sufficient metal was dissolved in the eluates to catalyse the decomposition of diazomethane, resulting in poor derivatisation. Subsequently a box containing no metal was used.

Appropriate conditions for GC–MSD determination had previously been developed. It had been established that the best derivatisation in terms of efficiency of reaction, chromatographic behaviour and fragmentation for MSD determination was a two-stage derivatisation, using diazomethane to methylate followed by pentafluoropropionic anhydride to acylate. It was not possible to derivatise sulphaguanidine using this combination of reagents. The formation of isomeric compounds has been noted in the literature [10] as a potential problem when using diazomethane. It was found that some compounds (for example sulphisomidine, sulphathiazole and sulphamethoxypyridazine) did give two peaks although one peak usually gave a far higher response. Preliminary work with tissue extracts indicated that the presence of tissue residues could have a profound effect on the course of derivatisations and subsequent work was undertaken using tissue standards to counterbalance this effect.

Table 2
Recoveries of sulphonamides relative to d^4-sulphadiazine (internal standard) from egg using GC–MSD determination

		SMO	SPN	STZ	SIM	SDZ	SMR	SMZ	SMM	SMP	SCP	SDX	SDM	SME	SQX	SMT
100 μg kg^{-1}																
Batch 1	Mean	91.9	82.9	80.8	117.1	98.5	100.2	98.0	111.4	83.3	57.5	107.4	103.1	95.3	92.3	61.5
n=4	SD	5.8	7.3	8.7	13.6	1.1	3.6	4.8	19.7	19.2	13.3	11.8	3.5	6.1	6.8	15.8
	RSD	6.3	8.8	10.8	11.6	1.1	3.6	4.9	17.7	23.0	23.1	11.0	3.4	6.4	7.3	25.7
Batch 2	Mean	82.5	99.1	82.1	98.0	100.8	101.9	100.1	93.5	113.7	51.0	108.5	114.4	109.3	85.5	65.3
n=6	SD	6.3	5.3	3.9	4.1	3.5	4.1	4.7	4.2	16.8	8.2	6.0	25.1	4.7	6.5	7.8
	RSD	7.6	5.4	4.7	4.1	3.5	4.0	4.7	4.5	14.8	16.2	5.5	21.9	4.3	7.6	11.9
Overall	Mean	86.3	92.6	81.6	105.6	99.9	101.2	99.2	100.7	101.5	53.6	108.1	109.9	103.7	88.2	63.8
n=10	SD	7.5	10.2	5.8	13.0	2.9	3.8	4.6	14.9	22.9	10.4	8.2	19.7	8.8	7.1	11.0
	RSD	8.7	11.0	7.2	12.3	2.9	3.7	4.6	14.8	22.6	19.4	7.5	17.9	8.5	8.1	17.2
25 μg kg^{-1}																
Batch 1	Mean	99.1	118.1	95.9	101.8	108.8	122.7	135.5	109.1	131.1	77.6	117.9	130.3	129.7	118.5	71.4
n=6	SD	5.9	10.2	20.3	8.8	2.1	7.8	12.8	4.9	8.8	15.6	7.8	10.1	6.4	12.2	14.0
	RSD	6.0	8.6	21.1	8.7	2.0	6.3	9.5	4.5	6.7	20.0	6.6	7.7	4.9	10.3	19.6

Separation of the N^1-methyl-N^4-pentafluoropropionyl derivatives of sulphonamides was achieved on a Hewlett-Packard Ultra 1 (12 m×0.2 mm, 0.33 μm) column.

The GC–MSD based procedure was validated in egg for 15 sulphonamides. d^4-Sulphadiazine was also spiked in prior to extraction for use as an internal standard. The ions monitored are summarised in Table 1. Validation data was obtained at 100 and 25 μg kg^{-1} levels. This is equivalent to the maximum residue limit and one quarter of the MRL. Data is summarised in Table 2.

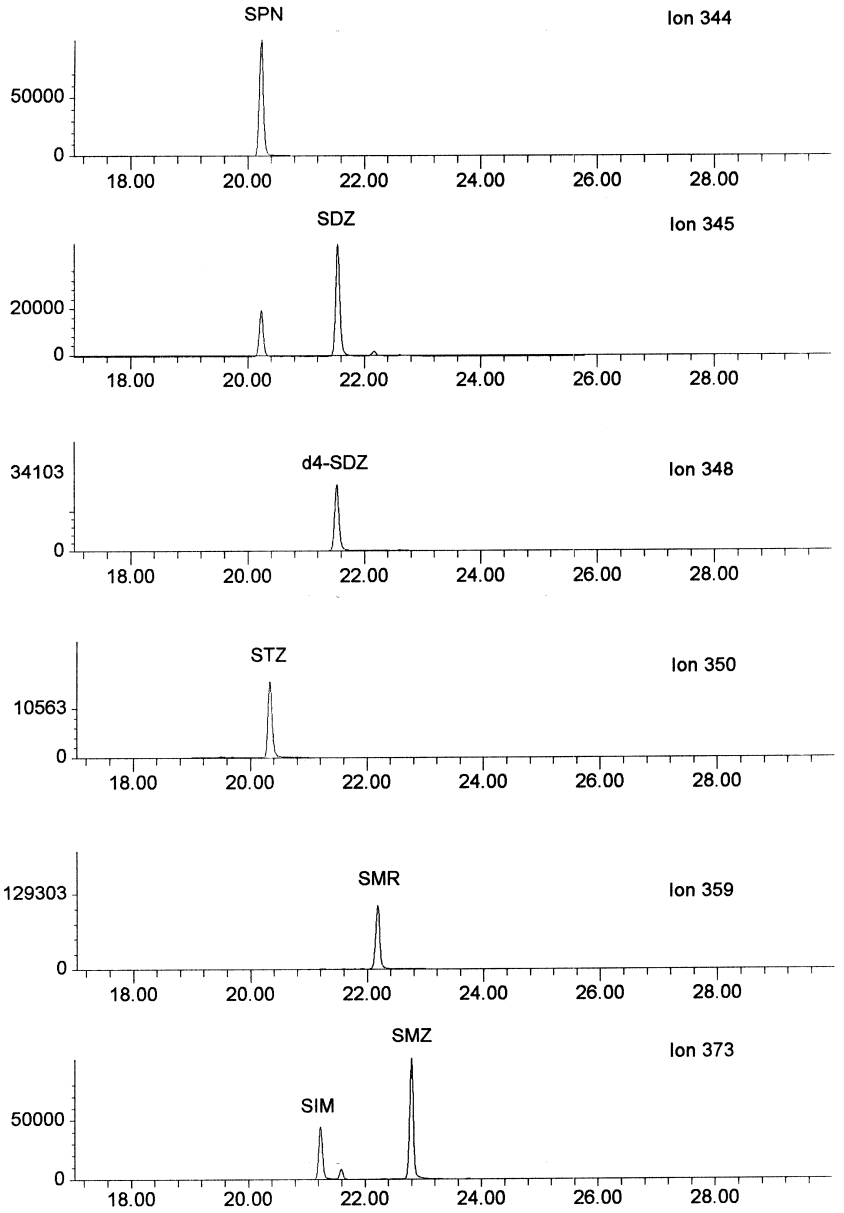

Fig. 2. Illustrative GC–MSD ion traces for a blank egg extract spiked at 100 μg kg^{-1} with 15 sulphonamides and internal standard.

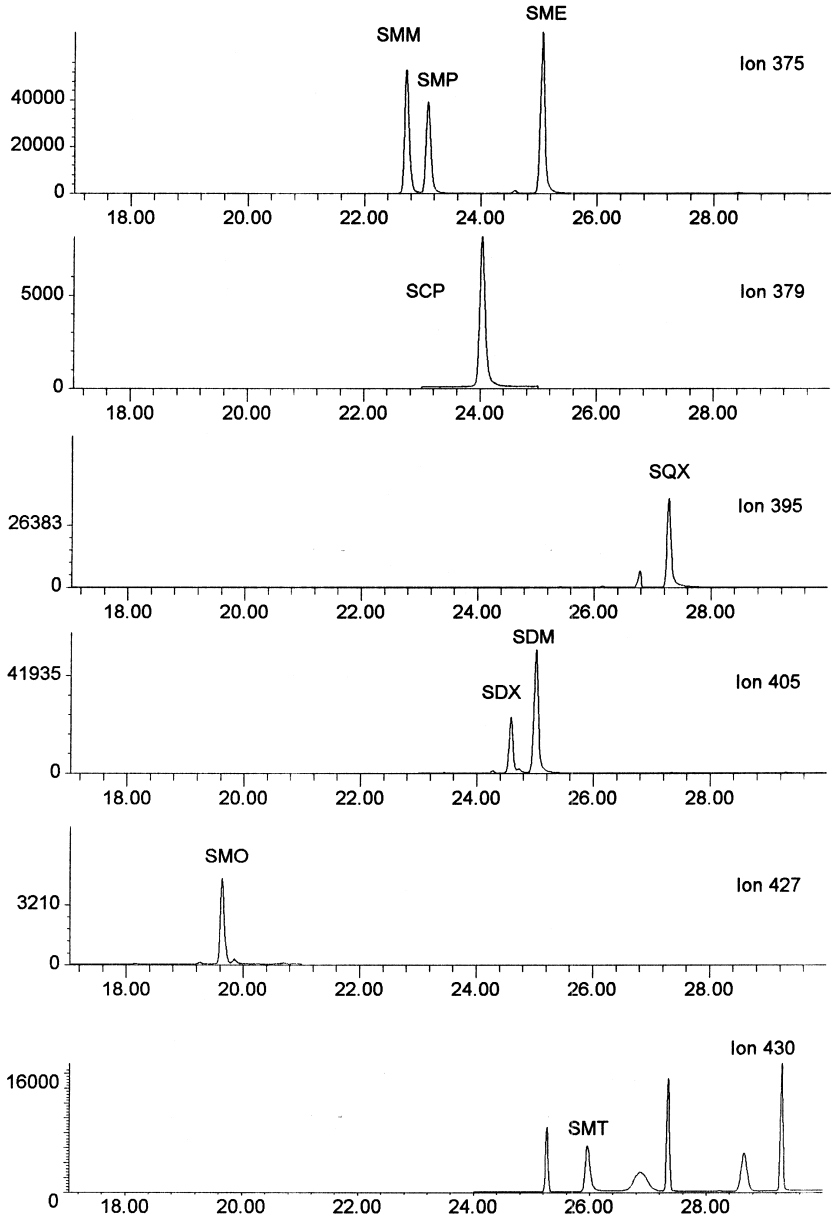

Fig. 2. (*continued*)

Overall recoveries at the 100 μg kg^{-1} level relative to the internal; standard ranged from 54% for sulphachloropyridazine to 110% for sulphadimethoxine. With the exception of sulphamethoxypyridazine, RSDs were below 20%. At the 25 μg kg^{-1} level, relative recoveries ranged from 71% for sulphamethizole up to 135% for sulphamethazine, with RSDs below 20% except for sulphathiazole and sulphachloropyridazine. Some of the variation seen, particularly with sulphamethoxypyridazine, may be accounted for by considering the effect of the presence of tissue residues on the catalysis of the derivatisation reactions and the formation of isomeric compounds. Despite this variation, the method is

sufficiently sensitive to give an indication of both presence and level of the sulphonamides present for screening purposes. Illustrative ion traces for an egg extract spiked at 100 μg kg^{-1} are shown in Fig. 2.

The LC–MS procedure was validated in egg for 15 sulphonamides. Either d^4-sulphamerazine or d^4-sulphamethazine was added to the final extract to act as an injection standard. The ions monitored are summarised in Table 1. Validation data was again obtained at 100 and 25 μg kg^{-1}. Results are summarised in Table 3. Overall recoveries at the 100 μg kg^{-1} level ranged from 43.9% for sulphaguanidine to 92.3% for sulphamethazine. RSDs were below 15% for all analytes except sulphachloropyridazine and sulphamoxole. The very high RSD for sulphamoxole (29.7%) appeared to be the

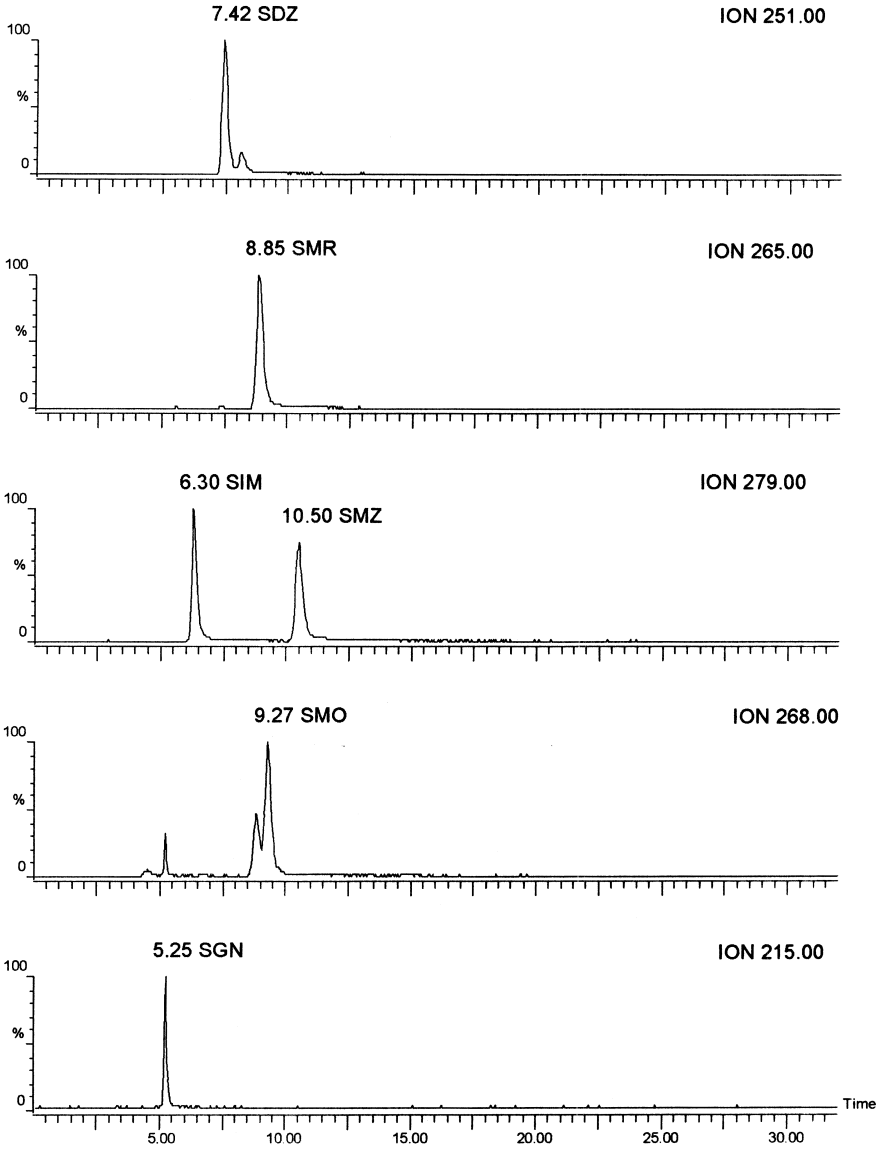

Fig. 3. Illustrative LC–MS ion traces for a blank egg extract spiked at 100 μg kg^{-1} with 15 sulphonamides.

result of one batch giving considerably lower recoveries than the other two. This particular batch had been stored for 14 days at +4°C prior to final analysis. There is some evidence that sulphamoxole breaks down in aqueous solution when stored for long periods of time and this may account for the low and variable recovery obtained in this batch. Overall recovery at the 25 μg kg^{-1} level ranged from 32.7% for sulphaguanidine up to 141.2% for sulphamoxole. The high recovery for sulphamoxole at this level is due to interference from low levels of d^3-sulphamerazine present in the internal standard. Illustrative ion traces for an egg extract spiked at 100 μg kg^{-1} are shown in Fig. 3.

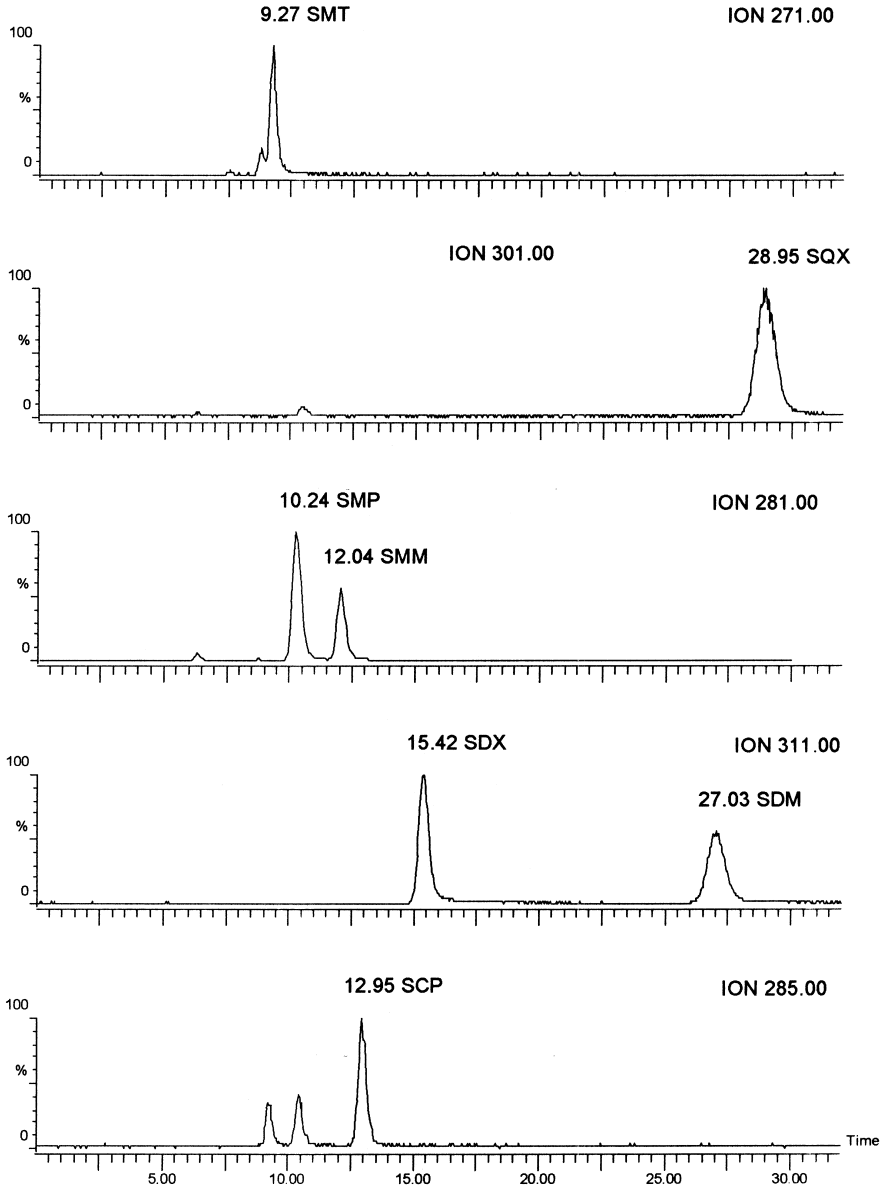

Fig. 3. (*continued*)

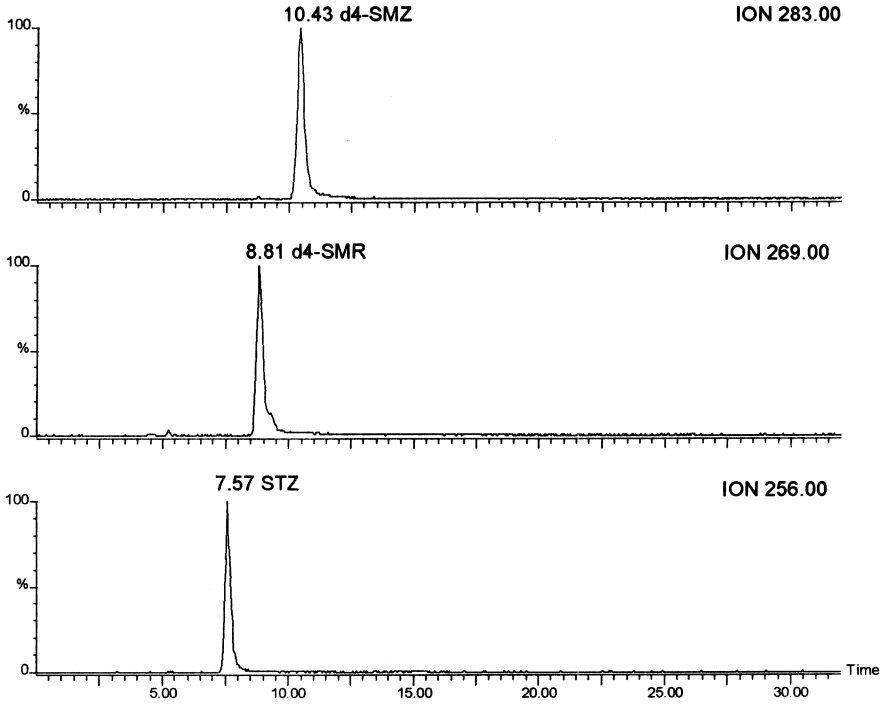

Fig. 3. (*continued*)

Table 3
Recoveries of sulphonamides from egg using LC–MS determination

		SGN	SPN	SDZ	STZ	SMR	SMO	SMT	SIM	SMZ	SMP	SMM	SCP	SQX	SDX	SDM
100 μg kg^{-1}																
Batch 1	Mean	47.0	81.0	72.2	68.4	79.8	59.6	80.3	77.0	91.6	88.5	88.8	61.4	85.4	97.8	102.4
n=6	SD	7.5	4.5	0.7	2.9	2.7	17.7	2.6	2.6	3.6	1.5	2.5	6.1	5.6	4.3	4.9
	RSD	16.0	5.6	0.9	4.2	3.3	29.7	3.3	3.4	4.0	1.7	2.9	9.9	6.6	4.4	4.8
Batch 2	Mean	45.1	83.5	88.3	81.0	92.2	103.0	61.2	90.1	91.5	89.6	83.4	51.9		87.1	84.5
n=6	SD	2.3	4.2	2.4	6.7	2.4	8.5	4.6	3.3	2.7	2.3	2.9	7.4		2.7	2.5
	RSD	5.2	5.0	2.7	8.3	2.6	8.3	7.5	3.7	2.9	2.6	3.5	14.3		3.1	2.9
Batch 3	Mean	37.6	83.3	85.6	76.3	91.9	108.7	61.6	87.2	94.6	89.3		45.6	76.5	88.1	88.6
n=4	SD	3.2	8.3	4.8	6.7	3.1	12.1	6.8	7.4	3.5	3.5		17.7	4.8	1.4	3.6
	RSD	8.5	10.0	5.6	8.7	3.3	11.1	11.1	8.5	3.7	4.0		38.9	6.3	1.6	4.1
Overall	Mean	43.9	82.5	81.6	75.1	87.5	88.2	68.5	84.5	92.3	89.1	86.1	53.9	81.8	91.3	92.2
n=16	SD	6.1	5.3	8.0	7.7	6.6	26.2	10.4	7.3	3.3	2.3	3.8	11.7	6.8	6.0	9.0
	RSD	14.0	6.4	9.8	10.2	7.6	29.7	15.2	8.7	3.6	2.6	4.5	21.6	8.3	6.6	9.8
25 μg kg^{-1}																
Batch 1	Mean	32.7	75.0	84.6	78.8	89.0	141.2	70.0	88.9	89.6	87.9		58.1	66.3	73.2	73.9
n=6	SD	8.4	2.7	4.3	7.0	2.1	4.3	4.2	2.5	5.5	3.3		12.3	8.9	6.6	9.0
	RSD	25.6	3.6	5.0	8.8	2.3	3.0	6.0	2.8	6.2	3.8		21.3	13.4	9.0	12.1

4. Conclusion

A simple and rapid extraction and clean-up procedure has been developed which fractionates the basic sulphonamides from the amphoteric sulphonamides and can be used for screening by either GC–MSD or LC–MS. Recoveries for a range of sulphonamides were good with good RSDs.

Acknowledgements

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