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Analysis of sulphonamides using supercritical fluid chromatography and supercritical fluid chromatography–mass spectrometry

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ABSTRACT

Packed-column supercritical fluid chromatography has been used for the separation of mixtures of sulphonamides on silica and amino-bonded stationary phases utilizing carbon dioxide with methanol modifier as the mobile phase. The effect of modifier concentration, column pressure and modifier identity on retention was also studied. Packed-column supercritical fluid chromatography–mass spectrometry (SFC–MS) of these mixtures utilizing both moving-belt and modified thermospray interfaces was also studied. The identification of sulphamethazine in a spiked porcine kidney extract was performed by SFC–MS using the moving-belt interface.

INTRODUCTION

The sulphonamides were initially used in human medicine against a wide variety of microbes, but acquired resistance has limited their application and they are now mainly employed to counter urinary tract infections [1]. Due to their wide spectrum of activity they are administered for numerous purposes in veterinary medicine including the treatment of acute and chronic bacterial infection in swine [2], the protection of honeybees [3] and fish [4] against infection and in combatting infectious diseases of cattle [5]. They have also been added in subtherapeutic doses to animal feeds to promote growth [6,7]. These drugs can be absorbed in animal tissues such that when the half-life of the drug within tissue is sufficiently long then the risk of the presence of residues in tissues prepared for human consumption occurs. Following ingestion the potential for acquired resistance occurs such that should the sulphonamides be

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required for therapy then the agent may be ineffective [8]. Current U.S. regulations specify a maximum residue level of 0.1 ppm in animals, where swine liver is quoted as the animal tissue of prime interest [9,10], illustrating the need for sensitive analytical methods for sulphonamide screening.

Numerous techniques have been employed to screen sulphonamide residues in meat but a major disadvantage is the extensive clean-up required prior to the analytical step. Thin-layer chromatography is prone to interferences and is insufficiently quantitative for residue analysis [11]. Gas chromatographic methods have been shown to be sensitive and specific but the non-labile nature of the sulphonamides means that derivatization to the N_1-methyl or N_1-acyl derivative is required [12]. Many liquid chromatographic procedures exist for the detection and determination of sulphonamides in animal-based matrices but no universal mobile or stationary phase can be applied to these analyses. With the use of relatively non-specific ultraviolet (UV) [5,9,11,13,14] and fluorescence [15] detection confirmation has often proved necessary where interferences have arisen. This has mainly been performed using gas chromatography–mass spectrometry (GC–MS) which then introduces the problems associated with GC analyses [16]. This problem has been countered by using high-performance liquid chromatography (HPLC) coupled with tandem MS which also limits sample clean-up required [17]. MS techniques have included the use of collision-activated dissociation–mass-analysed kinetic energy spectrometry (CAD–MIKES) [10] and tandem MS of the $[M+1]^+$ ion formed using chemical ionization [12] to screen sulphonamides in swine tissue extract, forgoing any chromatographic step, where interference occurred from dibutylphthalates possibly from clean-up contamination [10].

The use of packed-column supercritical fluid chromatography–mass spectrometry (SFC–MS) using a moving-belt interface has been reported previously where a mixture of sulphonamide homologues was chromatographed on a silica stationary phase [18]. A more recent report showed capillary-column SFC where manipulation of the stationary phase and mobile phase characteristics did not provide complete separations of the test compounds [19]. Further chromatographic runs were performed by packed column SFC using ODS2 and cyano stationary phases but again variation of the experimental conditions could not separate the sulphonamides which was consistent with our preliminary findings [19].

We now report the results of experiments which were used to study the separation of sulphonamide standards by packed-column SFC with UV and MS detection together with one example of a pig's kidney extract spiked with sulphamethazine.

EXPERIMENTAL

For SFC and SFC–MS a Hewlett-Packard 1084B high-performance liquid chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) modified for SFC operation [20] was used. The chromatograph was coupled to a VG 7070E forward geometry mass spectrometer (VG Analytical, Wythenshawe, U.K.) equipped with a moving-belt interface for the acquisition of electron-impact (EI) and ammonia chemical ionization (CI) data. Interfacing of the chromatograph with the moving-belt interface was effected using a Finnigan MAT thermospray deposition device (Finnigan MAT, San

Jose, CA, U.S.A.). The spray deposition device was connected in-line via a "T" piece between the UV detector exit and the outlet back-pressure regulator of the chromatograph, thus effecting a split of the eluent. In order that the full density range of the SFC could be used, the end of the stainless-steel tubing used in the spray deposition device was slightly crimped. The spray deposition device was connected to a 6 V/12 V power supply which was adjusted through a Rheostat to prevent freezing of the mobile phase at the tip. This resulted in approximately 50% of the eluent being transferred to the mass spectrometer. For direct-introduction SFC–MS a Finnigan MAT 4500 equipped with a thermospray source in the filament on mode was used. Modification of the source involved replacement of the coiled vapourizer with a straight piece of stainless-steel tubing which was crimped at the end to maintain supercritical conditions. Source heating was performed using a standard thermospray control box. The thermospray source was connected to the SFC using the stainless-steel transfer line and "T" piece already described. Calibration of the thermospray source was performed using poly(propylene glycol) average molecular weight 3000 (Aldrich, Milwaukee, WI, U.S.A.) [21]. Both the Finnigan MAT 4500 and VG 7070E mass spectrometers were controlled using a Finnigan MAT INCOS 2300 data system.

Instrument-grade liquid carbon dioxide supplied in cylinders with a dip tube (BOC, London, U.K.) and glass-redistilled methanol, 2-methoxyethanol, acetonitrile, 2-propanol, dimethylformamide and propylene carbonate were used for mobile phases. The carbon dioxide was introduced directly into the "A" pump of the 1084B and the solvent modifier (usually methanol) was placed in the "B" pump, which was operated in the HPLC mode. The liquid carbon dioxide and the pump heads of the chromatograph were cooled to −25°C using a Neslab RTE-4Z refrigerated bath (Neslab Instruments, NH, U.S.A.).

For SFC, 100 mm × 4.6 mm I.D. columns packed with 5-μm amino-bonded Spherisorb and 5-μm Spherisorb (Phase Separation, Queensferry, U.K.) were used. Detection was by a UV detector.

SFC–MS measurements in the EI mode were performed at 70 eV with a source temperature of 180°C (indicated) and the source housing pressure was in the region $5 \cdot 10^{-6}$ Torr. CI was performed with a source temperature of 100°C (indicated) and a source housing pressure in the region of 0.6 Torr. The belt vapourizer was set at 220°C (indicated). SFC–MS measurements using the thermospray source were all performed in the filament on mode. The source heater temperature was set at 140°C and the vapourizer temperature was set at 140°C.

RESULTS AND DISCUSSION

SFC of sulphonamides

Our objectives in these studies were to evaluate the utility of packed-column SFC for the separation and analysis of a mixture of sulphonamide standards and to investigate the possibility of effecting on-line SFC–MS, using both the moving-belt and thermospray interfaces, for these mixtures. The structures of the sulphonamides that were studied are shown in Fig. 1. In previous reports the problem of chromatographing basic compounds on silica has been discussed with reference to ergot and cinchona alkaloids where subsequent use of an amino-bonded stationary phase provided the chromatographic resolution required [18,22]. When studying the

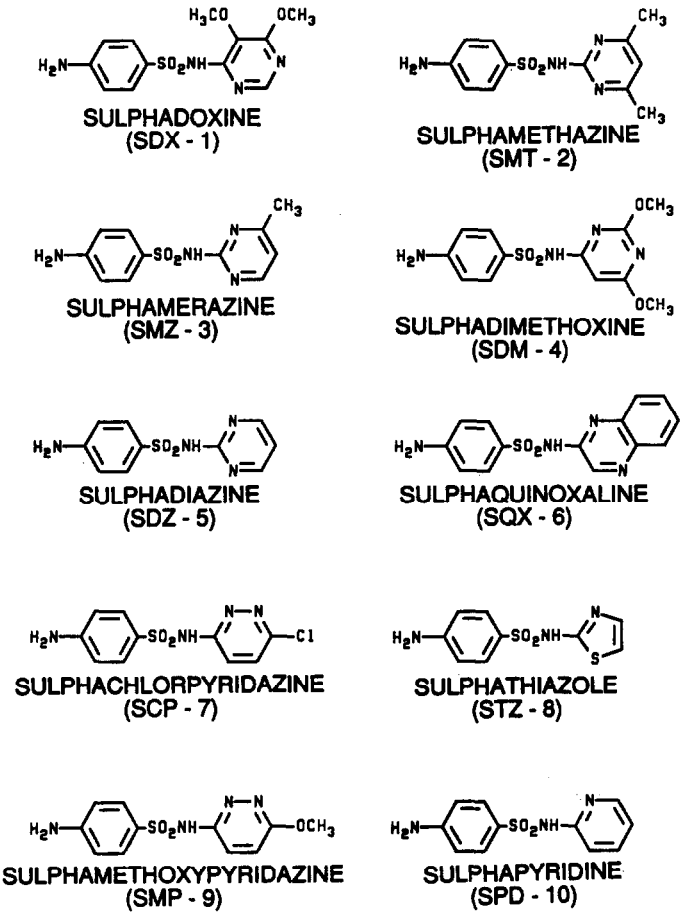

Fig. 1. Structures of the sulphonamides.

sulphonamides, though it was found to be possible to separate mixtures on silica this was not entirely satisfactory because separation could only occur at low pressure. This meant that the chromatographic run time was relatively long and the peaks were broad [18]. It therefore seemed expedient to attempt the same separations using an amino-bonded stationary phase. The separation of sulphonamide homologues sulphamethazine (SMT-2), sulphamerazine (SMZ-3) and sulphadiazine (SDZ-5) proved much superior with the chromatographic run time virtually halved (2.5 min using the amino-bonded stationary phase compared with just over 4 min using silica [18]). The high modifier concentration and column pressure meant that sharper peaks were obtained which in turn would lead to increased sensitivity and better detection limits. The change in selectivity of the phase altered the elution order of the mix of five sulphonamides reported previously [19] such that sulphadimethoxine (SDM-4) and sulphamethoxypyridazine (SMP-9) eluted between sulphamerazine and sulphadiazine and proved difficult to separate.

The minimum detectable quantity using the UV detector was also measured at a signal-to-noise ratio of 2 for these five sulphonamides. Each standard was chromatographed singly on the amino-bonded stationary phase at its own wavelength of maximum absorption. The experimental detection limits ranged from 1.5 ng for sulphamerazine to 5 ng for sulphamethoxypyridazine.

At this point in the experimental work five additional sulphonamides became available. These were sulphadoxine (SDX-1), sulphaquinoxaline (SQX-6), sulphachlorpyridazine (SCP-7), sulphathiazole (STZ-8) and sulphapyridine (SPD-10). The separation of nine of the ten of the sulphonamides on the amino-bonded stationary phase was investigated taking into account the previously observed coelution of sulphadimethoxine and sulphamethoxypyridazine. It was found only to be possible to chromatograph eight of the standards with baseline resolution since sulphamerazine and sulphapyridine coincided (Fig. 2). The mobile phase employed a gradient system changing from 15% methanol in carbon dioxide to 25% methanol after 4 min. The increase in modifier concentration had the effect of eluting the final peaks more quickly and also made them much sharper (if the percentage of methanol had remained constant the peaks due to sulphachlorpyridazine and sulphathiazole would have eluted after 17 and 23 min, respectively). The oven temperature was set at 90°C to ensure that supercritical conditions were maintained which had the additional effect of enhancing the separation of the earlier eluting peaks due to the decrease in mobile phase density. The total run time of approximately 9.5 min could be reduced to under 8 min by using a mobile phase containing 20% methanol in carbon dioxide increasing after 4 min to 25% methanol but this gain in time was partially negated by the loss of resolution between sulphadoxine and sulphamethazine.

The different selectivity of the silica stationary phase was then studied to see if it could be exploited to enhance existing separations. A single 10-cm column proved poor but the use of two columns in series proved extremely helpful. It was possible to

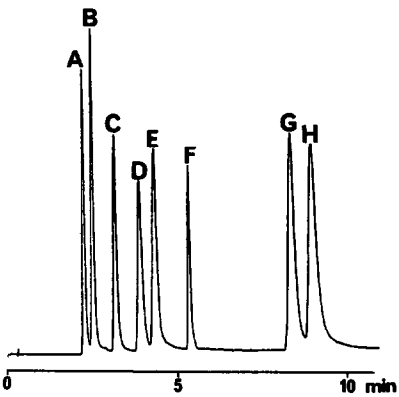

Fig. 2. UV trace (270 nm) obtained from SFC of a mixture of (A) sulphadoxine (1-SDX), (B) sulphamethazine (2-SMT), (C) sulphamerazine (3-SMZ), (D) sulphadimethoxine (4-SDM), (E) sulphadiazine (5-SDZ), (F) sulphaquinoxaline (6-SQX), (G) sulphachlorpyridazine (7-SCP) and (H) sulphathiazole (8-STZ) on a 100 × 4.6 mm I.D. column packed with 5-μm amino-bonded Spherisorb. The mobile phase was initially carbon dioxide modified with 15% methanol at a flow-rate of 4 ml/min. After 4 min the concentration of methanol was increased to 25%. Column pressure, 361 bar; temperature, 90°C.

separate the same mixture of eight sulphonamides though sulphamethazine and sulphaquinoxaline could not be baseline-resolved (Fig. 3). The most interesting facet of these separations was the influence of the stationary phase on the elution order. This is particularly helpful since the instrumental sensitivity was poor for the later eluting sulphonamides. If any single sulphonamide were being screened then the stationary phase could be varied to yield maximum sensitivity. The separation of eight sulphonamides could not be bettered using tandem silica columns since sulphapyridine coincided with sulphadiazine and sulphamerazine coincided with sulphamethoxypyridazine.

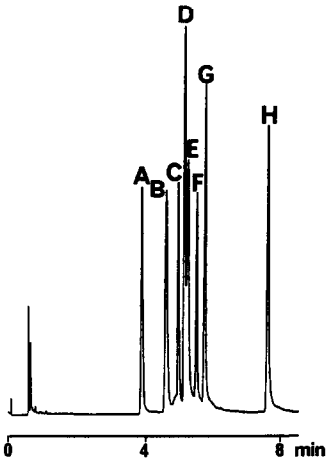

Fig. 3. UV trace (254 nm) obtained from SFC of a mixture of (A) sulphadoxine (1-SDX), (B) sulphadimethoxine (4-SDM), (C) sulphachlorpyridazine (7-SCP), (D) sulphaquinoxaline (6-SQX), (E) sulphamethazine (2-SMT), (F) sulphamerazine (3-SMZ), (G) sulphadiazine (5-SDZ) and (H) sulphathiazole (8-STZ) on two 100 mm × 4.6 mm I.D. columns mounted in series and packed with 5-μm Spherisorb. The mobile phase was initially carbon dioxide modified with 12% methanol at a flow-rate of 4 ml/min. After 5 min the concentration of methanol was increased to 20%. Column pressure, 263 bar; temperature,75°C.

As a further comparison between the amino-bonded and silica stationary phases the effect of change in modifier concentration on retention times was monitored (Figs. 4 and 5). It was apparent that a reduction in the modifier concentration served to enhance the separation and that this proved very much easier on the amino-bonded column. On the tandem silica system marked changes were required to produce a moderately small effect on the separation. The change in retention times with change in column pressure proved less easy to predict, such that when pressure was decreased some peaks merged rather than moved further apart (Figs. 6 and 7). In Fig. 6 it seems that the mobile phase became subcritical somewhere between 200 and 150 bar to radically alter retention times.

In order to monitor the effect of different modifiers on sulphonamide retention times with a view to enhancing existing separations the use of several solvents was reviewed where the chromatographic runs were performed under the same chromatographic conditions. Supercritical conditions for methanol, 2-propanol and acetonitrile were determined by the method already reported [18]. We concluded that the

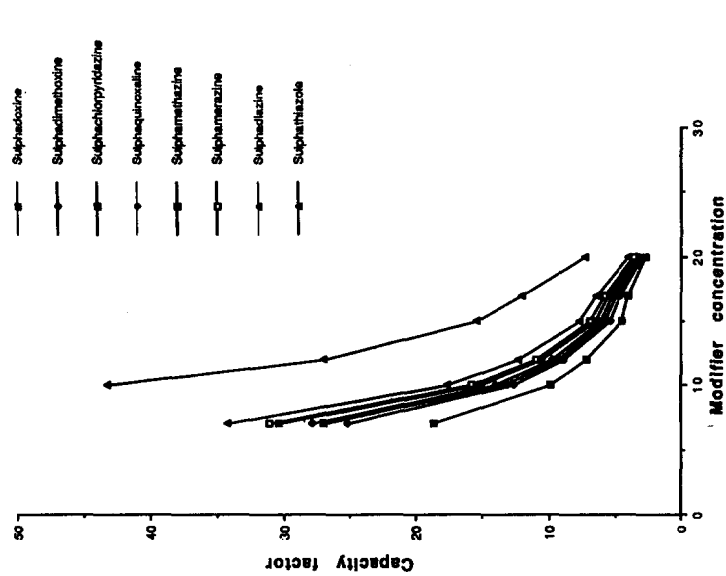

Fig. 4. Effect of modifier concentration on the retention times of the sulphonamides (as in Fig. 2) on a 100 mm × 4.6 mm I.D. column packed with 5-μm amino-bonded Spherisorb. The mobile phase was carbon dioxide modified with varying amounts of methanol at a flow-rate of 4 ml/min. Column pressure, 361 bar; temperature, 90°C.

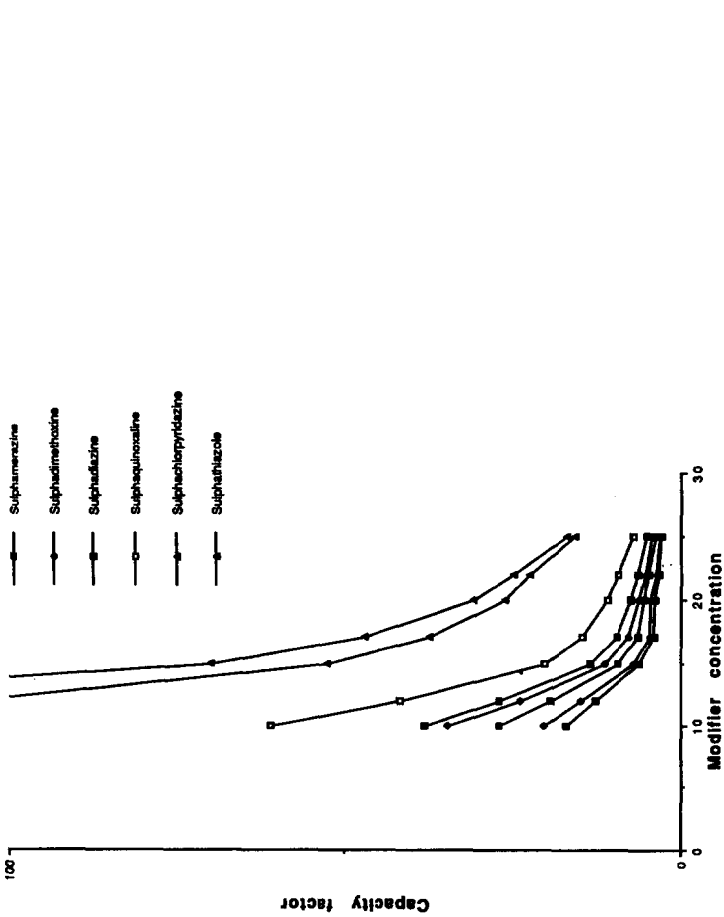

Fig. 5. Effect of modifier concentration on the retention times of the sulphonamides (as in Fig. 3) on two 100 mm × 4.6 mm I.D. columns mounted in series and packed with 5-μm Spherisorb. The mobile phase was carbon dioxide modified with varying concentrations of methanol at a flow-rate of 4 ml/min. Column pressure, 230 bar; temperature, 75°C.

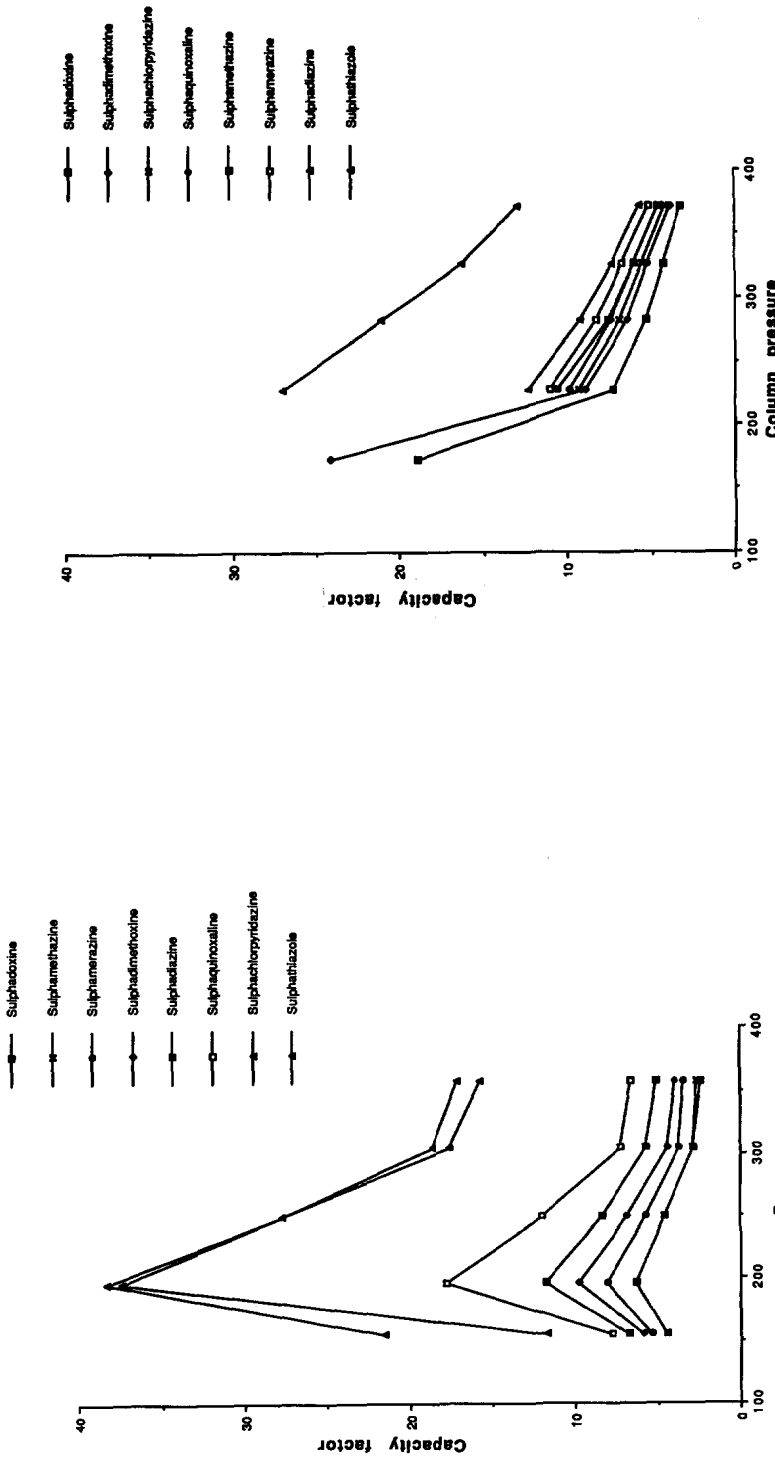

Fig. 6. Effect of column pressure on the retention times of the sulphonamides (as in Fig. 2) on a 100 mm × 4.6 mm I.D. column packed with 5-μm amino-bonded Spherisorb. The mobile phase was carbon dioxide modified with 25% methanol at a flow-rate of 4 ml/min. The column pressure was varied throughout and the temperature was 90°C.

Fig. 7. Effect of column pressure on the retention times of the sulphonamides (as in Fig. 3) on two 100 mm × 4.6 mm I.D. columns mounted in series and packed with 5-μm Spherisorb. The mobile phase was carbon dioxide modified with 12% methanol at a flow-rate of 4 ml/min. Column pressure was varied throughout and the temperature was 90°C.

experimental conditions employed for these solvents were suitable for the other modifiers by extrapolation of the conditions used by Levy and Ritchey [23] in previous modifier studies. All of the modifiers with the exception of dimethylformamide (DMF) produced a greatly increased analysis time compared to methanol (Fig. 8). 2-Methoxyethanol could be used to enhance the separation of the earlier eluting sulphonamides but this was accompanied by unacceptable peak broadening which occurred with all the other modifiers even DMF despite the reduced run time.

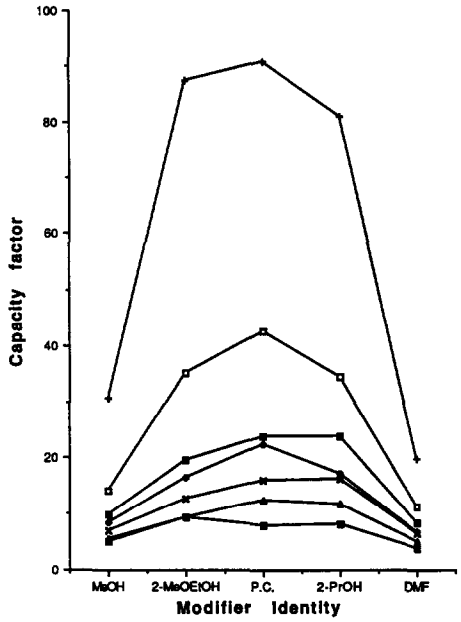

Fig. 8. Effect of modifier identity on the retention times of (⊡) sulphadoxine (1-SDX), (▲) sulphamethazine (2-SMT), (×) sulphamerazine (3-SMZ), (◇) sulphadimethoxine (4-SDM), (■) sulphadiazine (5-SDZ), (□) sulphaquinoxaline (6-SQX) and (+) sulphachlorpyridazine (7-SCP) on a 100 mm × 4.6 mm I.D. column packed with 5-μm amino-bonded Spherisorb. The mobile phase was carbon dioxide modified with 20% of either methanol, 2-methoxyethanol, propylene carbonate, 2-propanol or dimethylformamide at a flow-rate of 4 ml/min. Column pressure, 340 bar; temperature, 80°C.

SFC–MS of sulphonamides

UV detection, though useful, is of limited value when confirmatory data are required, particularly when screening unknown samples. Having established optimum separations for the sulphonamides the logical progression was to chromatograph the same mixture on-line with the mass spectrometer. The reconstructed total ion current trace is shown for the on-line SFC–MS of eight sulphonamides (Fig. 9) run under the same chromatographic conditions as those in Fig. 2. It is clear that the chromatographic integrity has been retained. However the on-line study of this mixture was not entirely routine. The first five sulphonamides were very reproducible but sulphaquinoxaline, sulphachlorpyridazine and sulphathiazole gave variable responses. This seemed to coincide within the increase in modifier concentration during the

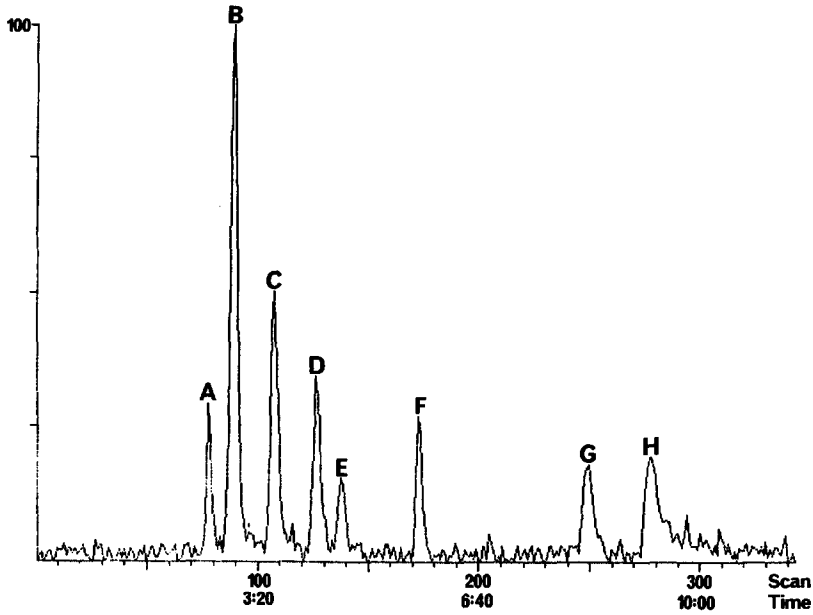

Fig. 9. Computer-reconstructed total ion current trace obtained by SFC–EIMS of the same mixture as in Fig. 2 using a moving-belt interface. SFC conditions as in Fig. 2. For peak identification see Fig. 2. Time in min:s.

chromatographic run. It may be that the spray depositor was not heated enough to maintain supercritical conditions at the high modifier concentration and some precipitation may have occurred at the vapourizer tip. This effect has been noted with other groups of compounds and generally took place after a modifier concentration gradient was employed within a chromatographic run.

The sulphonamides all undergo similar fragmentations under EI conditions and these have been described elsewhere [24]. It was found that the presence of solvent in the source drastically affected spectra where the intensities of diagnostically important ions, resulting from loss of SO_2, increased while those resulting from cleavage of the $S–N_4$ bond diminished to become relatively unimportant. The spectra became very much simpler and slight molecular or protonated molecular ions were also noted. The comparative mass spectra of sulphadimethoxine obtained under EI conditions with no solvent present, under EI conditions with solvent in the source and under ammonia CI are shown in Fig. 10, and the major fragments (relative abundances > 10%) of the sulphonamides together with their relative intensities are listed in Tables I and II.

As a means of evaluating SFC and SFC–MS as a routine analytical tool for the screening of sulphonamides a sample of porcine kidney extract containing approximately 3300 ng/g sulphamethazine was chromatographed on-line with the mass spectrometer. An amino-bonded silica column was used to ensure that the sulphamethazine was retained relative to the endogenous components within the matrix [25]. An injection volume of 8 μl was used corresponding to 26 ng. Since the UV detection limit was 2 ng the peak due to sulphamethazine was readily detected (Fig. 11) and

indeed it could easily be seen at the 13-ng level. For on-line analysis 13 ng of sulphamethazine were passed to the moving-belt interface following the split. Due to the high amount of endogenous material present the peak due to sulphamethazine could not be seen in the reconstructed total ion current trace during the relevant time

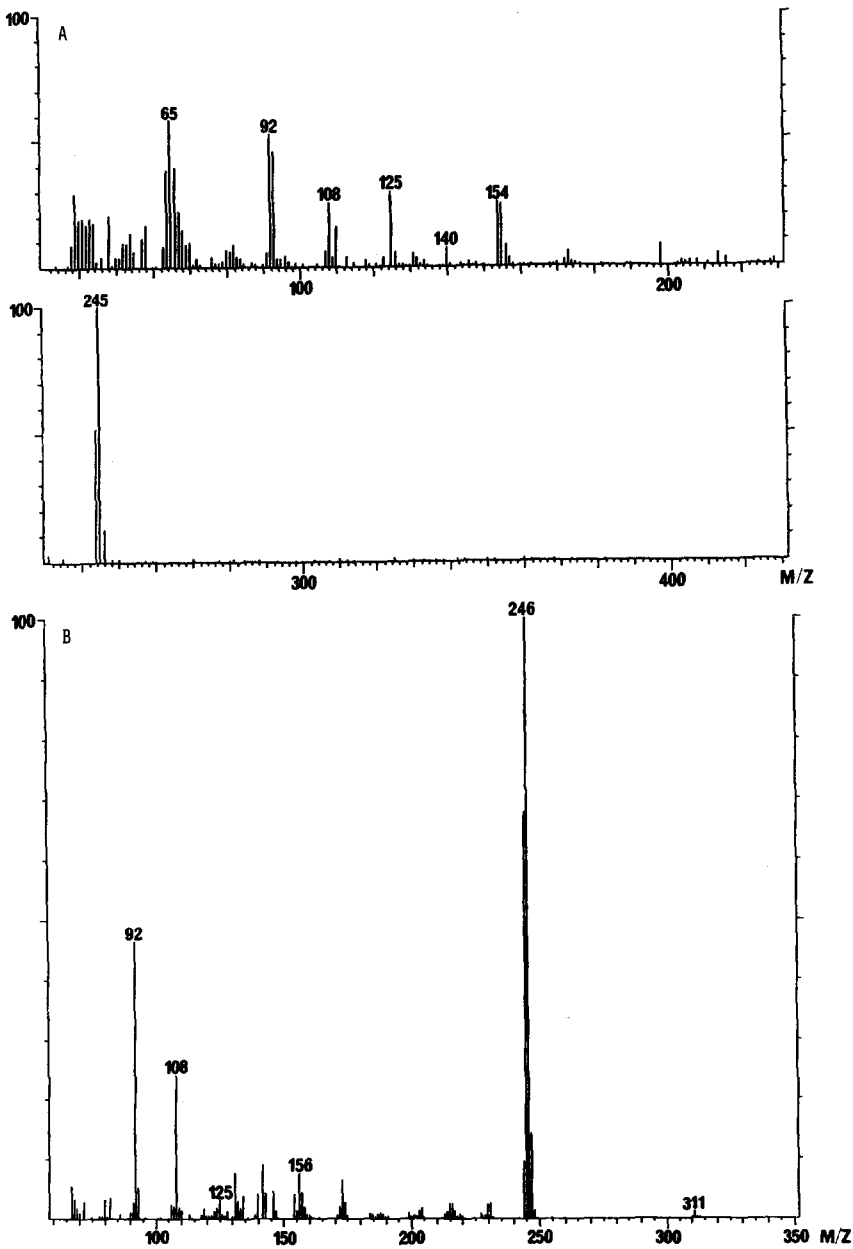

Fig. 10.

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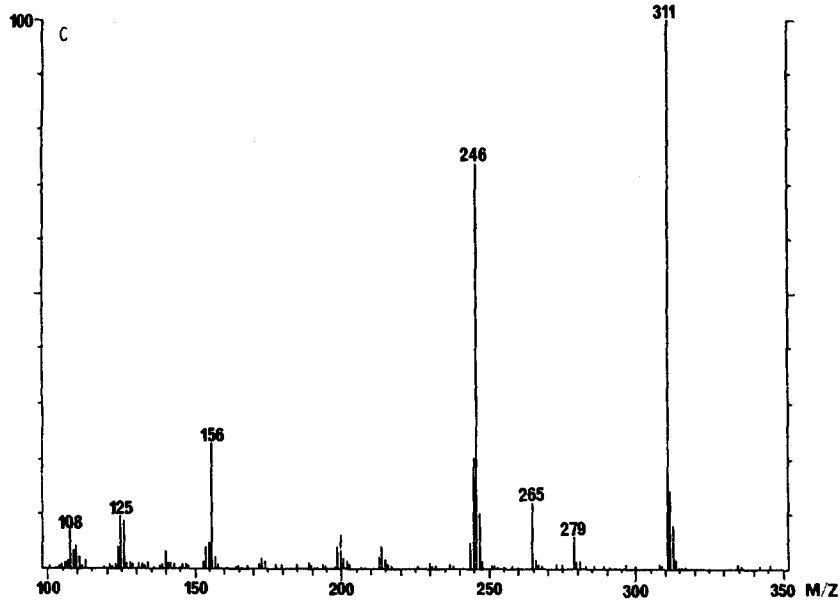

Fig. 10. (A) EI mass spectrum of sulphadimethoxine obtained by SFC–MS using the moving-belt interface. (B) EI mass spectrum of sulphadimethoxine obtained by SFC–MS with solvent present in the source. (C) Ammonia CI spectrum of sulphadimethoxine obtained by SFC–MS using the moving-belt interface.

region. However screening of *m/z* 214 (the base peak for sulphamethazine under EI conditions) showed a peak at scan 79 corresponding to a retention time of just under 3 min (Fig. 12). A comparison of a background-subtracted spectrum from scan 79 and the spectrum obtained for sulphamethazine standard from the belt is shown in Fig. 13. The presence of endogenous material meant that minor diagnostic peaks are not present but the base peak at *m/z* 214 is obvious as is *m/z* 213. Fragments at masses 65, 92, 108 and 123 were also present while *m/z* 156 was difficult to differentiate from the background. The ion at mass 149 was probably due to the presence of dibutyl-phthalates from clean-up contamination. A comparison of retention times and the EI spectrum offer a strong indication that the peak is indeed sulphamethazine. Further sensitivity could be gained from the use of single ion monitoring.

Lack of sensitivity had been noted with the moving-belt interface so the sulphonamides were also chromatographed on-line using a modified thermospray interface. The initial intention was to use the same mixture as had been run on-line using the moving-belt interface. This was shown fairly rapidly to be impractical as the problems noted with use of a gradient became more pronounced such that it became very difficult to see the later eluting sulphonamides. In the thermospray source the response depended on the vapourizer temperature and was very compound-dependent. This had been optimized using sulphamethazine as the test compound. When the mixture was chromatographed at this "optimum" temperature of 140°C, sulphadiazine yielded a very badly tailing peak which may have been due to involatility or precipitation at the vapourizer tip. This had the effect of obscuring the later eluting

TABLE I

IMPORTANT IONS AND THEIR RELATIVE INTENSITIES OBTAINED FOR THE SULPHONAMIDES UNDER ELECTRON IMPACT CONDITIONS WITH AND WITHOUT SOLVENT PRESENT IN THE SOURCE

Peak	Mol.wt.	Electron impact		Solvent-moderated electron impact	
SDX	310	64(100)	65(57)	92(87)	108(46)
		66(62)	92(37)	140(22)	156(15)
		93(97)	108(17)	227(44)	231(35)
		125(47)	244(17)	245(100)	246(67)
SMT	278	64(32)	65(67)	92(40)	108(18)
		66(31)	92(54)	123(8)	212(12)
		93(38)	123(45)	213(82)	214(100)
		213(92)	214(100)		
SMZ	264	64(37)	65(73)	92(62)	108(25)
		66(32)	92(61)	109(10)	198(12)
		93(42)	109(40)	199(100)	200(88)
		199(100)	200(81)		
SDM	310	64(67)	65(80)	92(47)	108(24)
		66(47)	92(70)	156(8)	244(10)
		93(60)	108(32)	245(68)	246(100)
		244(54)	245(100)	247(14)	
SDZ	250	64(65)	65(95)		
		66(47)	92(70)		
		93(61)	108(30)		
		185(100)	186(85)		
SQX	300	64(78)	65(47)		
		91(40)	92(43)		
		93(53)	118(81)		
		145(100)	236(32)		
SCP	284	64(100)	65(73)	92(31)	108(17)
		66(48)	92(60)	156(5)	219(100)
		93(60)	108(32)	220(54)	221(37)
		125(20)	219(35)	222(17)	284(6)
STZ	255	64(91)	65(41)	92(100)	93(23)
		66(43)	92(31)	108(70)	140(20)
		93(91)	100(100)	156(90)	157(11)
		108(16)	191(11)	191(75)	255(15)
SMP	280	64(30)	65(42)	215(100)	216(48)
		80(30)	92(47)	280(6)	
		93(32)	108(26)		
		215(100)	216(38)		
SPD	249			92(47)	93(9)
				108(22)	183(13)
				184(100)	185(71)

TABLE II

IMPORTANT IONS AND THEIR RELATIVE INTENSITIES OBTAINED FOR THE SULPHONAMIDES UNDER AMMONIA CHEMICAL IONIZATION AND THE THERMOSPRAY SOURCE IN THE FILAMENT-ON MODE

Peak	Mol.wt.	Ammonia chemical ionization		"Thermospray"	
SDX	310	156(21)	245(25)	311(100)	312(14)
		246(27)	311(100)		
		312(13)			
SMT	278	213(12)	214(29)	279(100)	280(16)
		279(100)	280(20)		
SMZ	264	199(33)	200(52)	265(100)	266(15)
		265(100)	266(16)		
SDM	310	156(23)	245(20)	311(100)	312(17)
		246(74)	311(100)		
		312(14)			
SDZ	250	92(12)	156(15)	251(100)	252(11)
		185(41)	186(56)		
		251(100)	252(15)		
SQX	300	125(14)	145(21)		
		146(29)	156(14)		
		235(19)	236(62)		
		301(100)	302(17)		
SCP	284	93(52)	125(78)		
		130(62)	156(43)		
		219(84)	220(71)		
		285(100)	287(46)		
STZ	255	92(40)	93(46)		
		101(34)	108(40)		
		125(39)	156(75)		
		191(94)	256(100)		

peaks completely. Increasing the vapourizer temperature to the optimum temperature for sulphadiazine (160°C) greatly reduced this tailing but an additional effect was the reduction of the response of the earlier eluting sulphonamides, notably sulphadoxine and sulphamethazine. As a result a mixture containing only sulphadoxine, sulphamethazine, sulphamerazine and sulphadimethoxine was chromatographed on-line (Fig. 14).

Although this was performed at the "optimum" vapourizer temperature for sulphamethazine it was clearly seen that all of the sulphonamides tailed badly. This may be due to partial precipitation of the compounds within the vapourizer as the solvent density increases or at the vapourizer tip upon solvent expansion into the source. The spectra obtained were extremely simple consisting of protonated molecular ions. The spectrum obtained for sulphadimethoxine is shown in Fig. 15. The peaks occurring at masses 265 and 279 are due to sulphamerazine and sulphamethazine, respectively.

Fig. 11. UV trace (270 nm) obtained from SFC of a porcine kidney extract containing a known amount of sulphamethazine (3300 ng/g) on a 100 mm × 4.6 mm I.D. column packed with 5-μm amino-bonded Spherisorb. The mobile phase was carbon dioxide modified with 15% methanol at a flow-rate of 4 ml/min. Column pressure, 361 bar; temperature, 75°C.

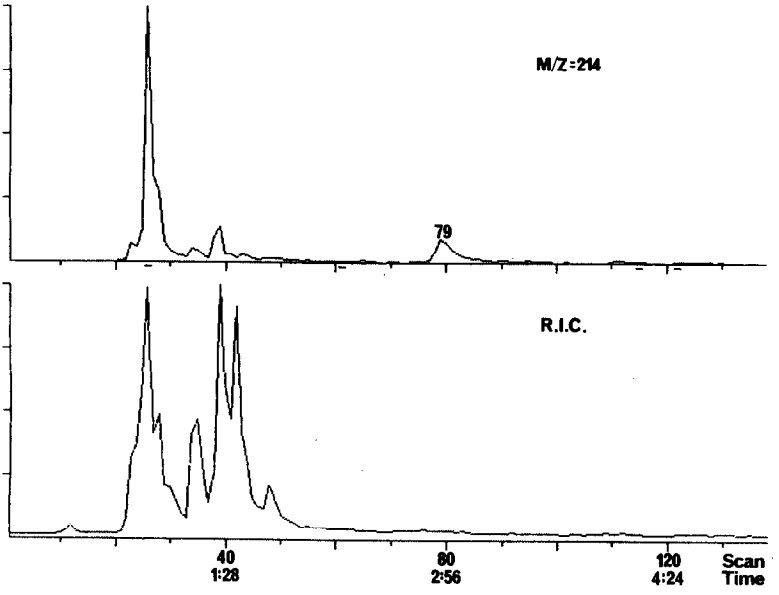

Fig. 12. Computer-reconstructed total ion current and single ion trace obtained by SFC–MS of a porcine kidney extract containing a known amount of sulphamethazine (3300 ng/g) using a moving-belt interface. SFC conditions as in Fig. 11.

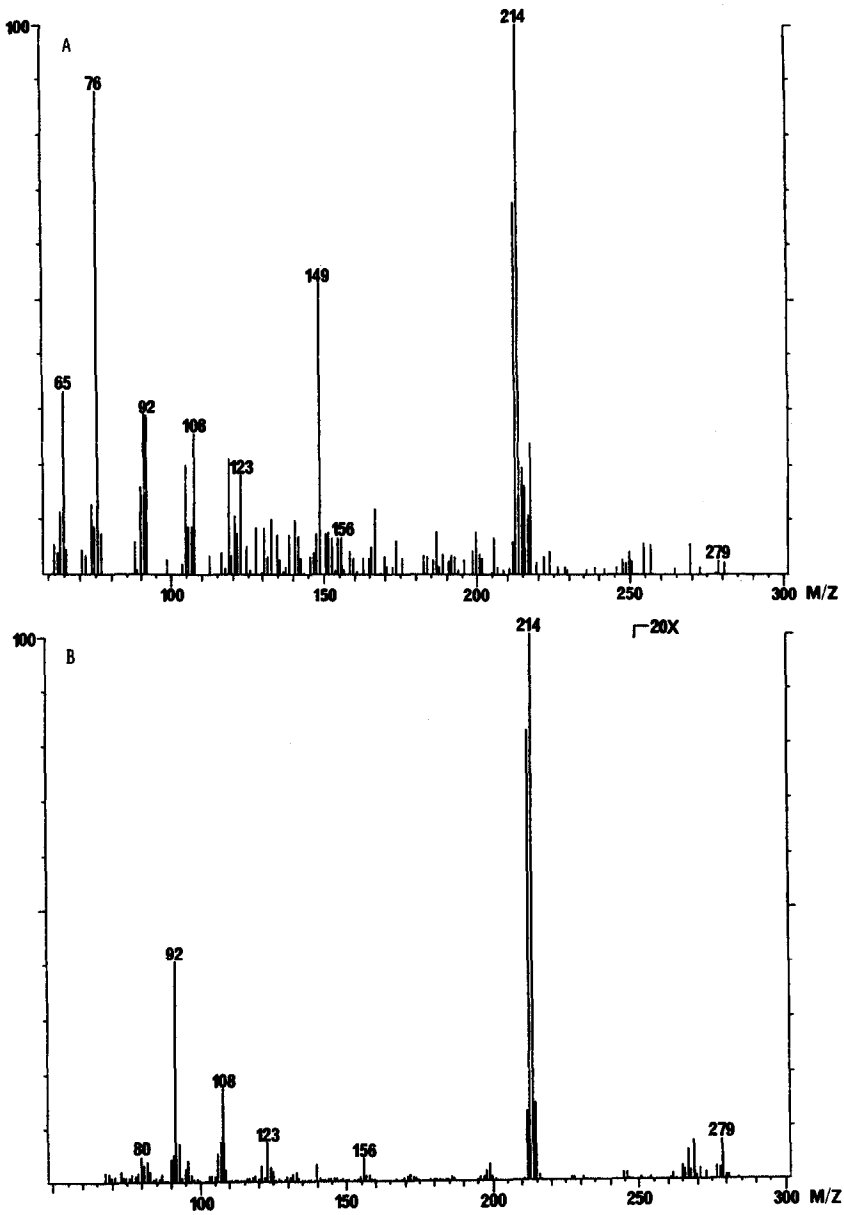

Fig. 13. (A) EI mass spectrum of sulphamethazine from the porcine kidney extract obtained by SFC–MS using a moving-belt interface. (B) EI mass spectrum of sulphamethazine standard obtained by SFC–MS using a moving-belt interface.

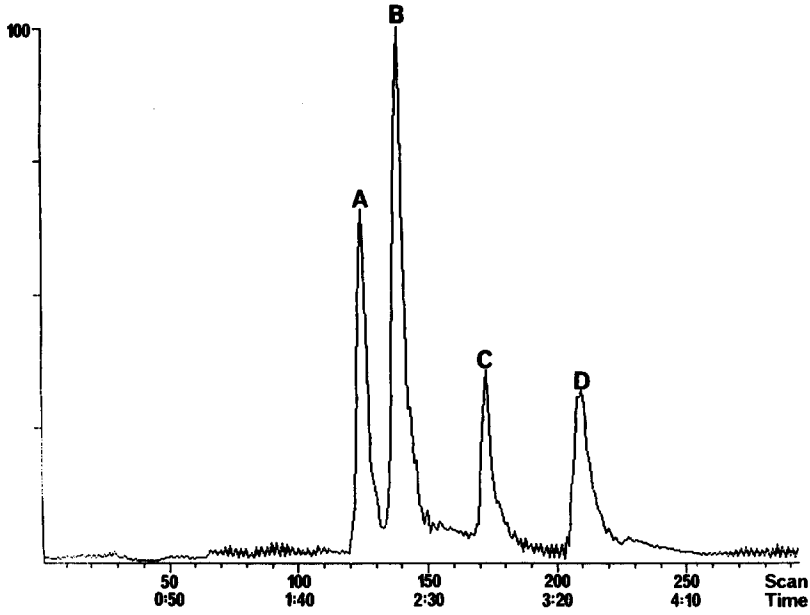

Fig. 14. Computer-reconstructed total ion current trace obtained from SFC–MS of (A) sulphadoxine (1-SDX), (B) sulphamethazine (2-SMT), (C) sulphamerazine (3-SMZ) and (D) sulphadimethoxine (4-SDM) using a modified thermospray source. This was performed on a 100 mm × 4.6 mm I.D. column packed with 5-μm amino-bonded Spherisorb. The mobile phase was carbon dioxide modified with 15% methanol. Column pressure, 356 bar; temperature, 90°C. The source and vapourizer temperatures were both set at 140°C.

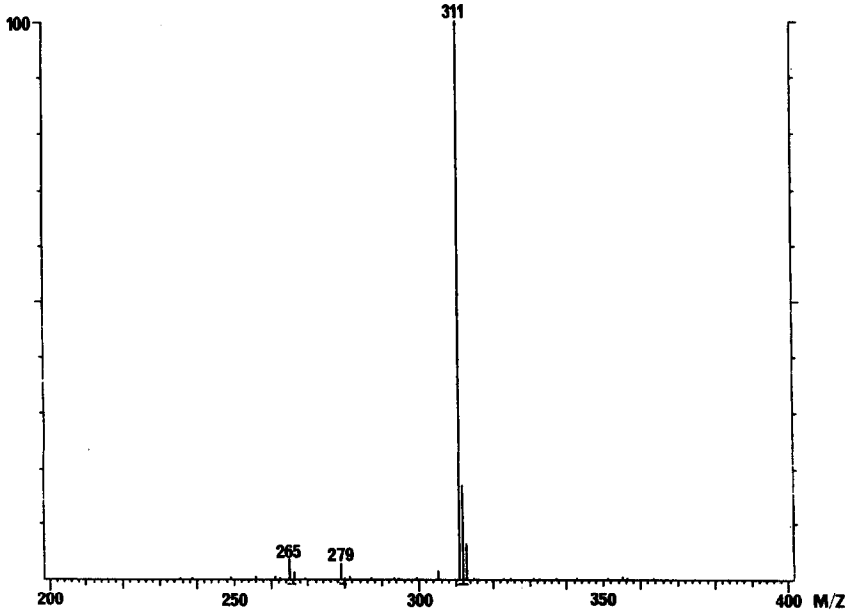

Fig. 15. Filament-on SFC mass spectrum of sulphadimethoxine.

CONCLUSION

Following these studies routine analysis of sulphonamides by SFC using UV detection is now possible. Both amino-bonded and silica stationary phases can be employed and their different selectivities mean that a particular column can be chosen for a specific problem. The mobile phase used throughout the experimental work was extremely simple, being supercritical carbon dioxide containing methanol as the modifier. All other modifiers proved to be of limited value. The separations obtained by this method were quick and extremely reproducible. Detection limits in the low nanogram range have been obtained.

SFC–MS of sulphonamides using a moving-belt interface can provide both EI and CI data. Use of a modifier concentration gradient can cause variable response for sulphonamides which elute after the gradient is implemented. Those sulphonamides which elute prior to the gradient were very reproducible. This method was used to look at a pigs kidney extract spiked with sulphamethazine. SFC–MS using a thermospray interface is also possible. It is more sensitive than the moving belt but peaks due to the sulphonamides showed tailing produced within the interface and the use of a gradient shows a more marked effect than with the moving-belt interface.

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