Journal of Chromatography, 647 (1993) 107–119
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2793

Liquid chromatography–mass spectrometry for the identification of minor components in benzothiazole derivatives

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ABSTRACT

Various mass spectrometric techniques were explored for their ability to detect and identify minor components in benzothiazole-derived compounds, namely gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry using a moving-belt, a thermospray and a particle-beam interface and liquid chromatography–tandem mass spectrometry in combination with a thermospray interface. The necessary changes in the liquid chromatographic solvent systems were accomplished by translation of gradient runs into a series of isocratic runs, and a UV photodiode-array detector was used to trace the peaks. The methodology developed and the advantages and limitations of the different techniques employed are discussed.

INTRODUCTION

The use of high-performance liquid chromatography (HPLC) has become invaluable in defining the composition of benzothiazole derivatives, which are used as vulcanization accelerators [1]. The identification of minor components in these products by comparison of the retention times with those of known compounds has always been questionable, as it assumes that compounds with different structures will have different retention times under a particular set of chromatographic conditions, an assumption that has been found to be misleading in our laboratories, especially when the mixtures are complex.

During the last decade, new techniques have become available that can remove the uncertainty when using this method of peak identification, and their application to benzothiazole derivatives has been explored. In this study, various interfaces for combined liquid chromatography–mass spectrometry (LC–MS) [2] were evaluated for

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0021-9673/93/$06.00

their potential in the identification of minor components found in laboratory samples of 2(3*H*)-benzothiazolethione and its derived products. The procedure followed can be summarized as follows: the HPLC gradient elution programmes, initially developed for the analysis of the products using UV detection, were translated into a series of isocratic runs; a UV photodiode-array (PDA) detector was used to correlate the various peaks in the chromatogram. LC–MS was then performed under isocratic conditions using various interfaces, *i.e.*, the moving-belt interface (MBI) in both electron impact (EI) and positive-ion chemical ionization (CI) modes [2,3], the thermospray (TSP) interface in both buffer ionization and discharge-on modes [2,4] and the particle beam interface (PBI) in the EI mode [2,5]. For further structure elucidation, tandem mass spectrometry (MS–MS) was performed in combination with the TSP interface and GC–MS data were also acquired.

In this paper the procedures used and some of the results are outlined and discussed with some typical examples. Lastly, the abilities of the different LC–MS interfaces to characterize successfully the benzothiazole derivatives are compared.

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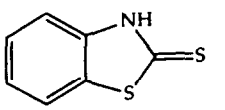

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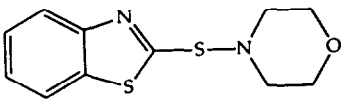

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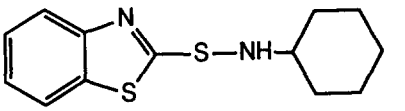

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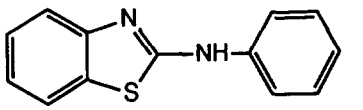

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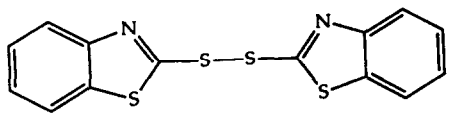

Fig. 1. Structures of the major components studied. **1** = 2(3*H*)-benzothiazolethione; **2** = 4-(2-benzothiazolylthio)morpholine; **3** = N-cyclohexyl-2-benzothiazolesulphenamide; **4** = N-phenyl-2-benzothiazolamine; **10** = 2,2′-dithiobisbenzothiazole.

EXPERIMENTAL

Materials

Acetonitrile was of HPLC quality (Rathburn Chemicals, Walkerburn, UK). Water was purified with a Milli-Q water purification system (Millipore). All other chemicals were of analytical-reagent grade.

Sample solutions containing *ca.* 5 mg/ml were prepared in either acetonitrile or dioxane. Generally, the solutions were centrifuged before use. Samples A and B contained 2(3*H*)-benzothiazolethione (**1** in Fig. 1) as its major constituent, sample C primarily consisted of 4-(2-benzothiazolylthio)morpholine (**2** in Fig. 1) and sample D of N-cyclohexyl-2-benzothiazolesulphenamide (**3** in Fig. 1).

Liquid chromatography

Gradient elution was performed using a Beckman (Anaheim, CA, USA) System Gold injection and solvent-delivery system, a 250 mm × 4.6 mm I.D. Beckman Ultrasphere ODS column and a Hewlett-Packard (Palo Alto, CA, USA) Type 1040-A photodiode-array detector, equipped with a Hewlett-Packard Model 85B computer and operated in the range 200–400 nm.

Three different gradient programmes were applied, using mixtures of solvent A [20% (v/v) acetonitrile in water] and solvent B (acetonitrile). For samples A and B a linear gradient programme was used from 16% to 94% solvent B in 30 min, followed by washing the column with solvent B for 20 min. The flow-rate was 1.6 ml/min. For sample C a gradient programme was applied, in which both the solvent composition and the flow-rate were changed with time (see Fig. 2). For sample D a linear gradient programme was used from 5% to 94% solvent B in 33 min, starting 10 min after injection and followed by washing the column with solvent B

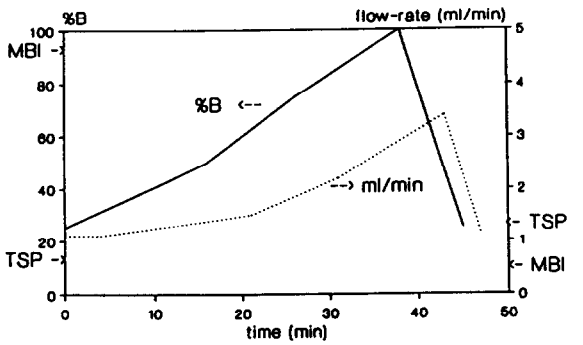

Fig. 2. Gradient elution and flow-rate programme used for the analysis of sample C. Optimum flow-rate and solvent composition conditions for the moving-belt interface (MBI) and the thermospray (TSP) interface are indicated on the abscissae.

for 12 min. The flow-rate was kept at 1.6 ml/min.

For the translation of the gradient runs to a set of isocratic runs, the same instrumentation was used. In an isocratic run either pure solvent B or a fixed percentage of solvent B (*e.g.*, 75%, 50% or 25%) was added to solvent A. The mobile phases used in the LC–MS experiments were made in the same way. The flow-rate used was 1.0 ml/min with mobile phases containing ≥50% of solvent B and 0.5 ml/min with mobile phases containing <50% of solvent B. The column was washed with pure acetonitrile after each run.

Gas chromatography–mass spectrometry

GC–MS was performed on a combination of a Chrompack Packard (Middelburg, Netherlands) Model 438A gas chromatograph and a Finnigan MAT (San José, CA, USA) Model 700 ion trap detector. A 10 m × 0.25 mm I.D. Chrompack CP-Sil-5 capillary column was coupled to the mass spectrometer by means of an open split coupling. A temperature-programmed GC separation was performed with a linear temperature ramp of 15°C/min from 70 to 280°C.

LC–MS using a moving-belt interface

LC–MS experiments with a Finnigan MAT moving-belt interface were performed with a Finnigan MAT HSQ-30 hybrid MS–MS instrument. It was operated in the EI or CI mode (ammonia as reagent gas). The moving-belt interface was operated with a solvent evaporator temperature of 200–220°C, a belt speed of 35 mm/s and the vaporizer at position 7–8.

The LC system consisted of an LKB (Bromma, Sweden) Model 2150 high-pressure pump, a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 20-μl loop, a Beckman 250 mm × 4.6 mm I.D. Ultrasphere ODS column and a Kratos (Manchester, UK) Model 757 variable-wavelength detector, operated at either 254 or 275 nm.

LC–MS experiments with a thermospray interface

LC–MS was performed with a Finnigan MAT thermospray interface on a Finnigan MAT TSQ-70 triple quadrupole mass spectrometer. The thermospray interface was operated with a vaporizer temperature of 100–120°C, a block temperature of 200°C and a repeller potential of 0–50 V. LC–thermospray MS was performed either in the thermospray buffer ionization mode, by using 0.1 mol/l ammonium acetate in water in solvent A instead of water, or in the discharge-on mode (discharge potential 1 kV) with the regular solvent system. No influence on the chromatographic retention time was observed as a result of the addition of ammonium acetate to the solvent system.

The LC system consisted of an LKB Model 2150 high-pressure pump, a Rheodyne Model 7125 injection valve with a 20-μl loop, a Beckman 250 mm × 4.6 mm I.D. Ultrasphere ODS column and a Waters (Rochester, MN, USA) Model 440 fixed-wavelength detector, operated at 254 nm.

LC–MS with a particle-beam interface

LC–MS was performed with a Hewlett-Packard Model 59980A particle-beam interface, fitted on a Finnigan MAT TSQ-70 triple quadrupole mass spectrometer [6]. The mass spectrometer was operated in the EI mode.

The LC system was similar to that used with the thermospray interface. The column (150 mm × 4.6 mm I.D.) was laboratory-packed with 10-μm C_{18} material. A mobile phase of acetonitrile–water (75:25) was used at a flow-rate of 0.5 ml/min.

MS–MS experiments

MS–MS experiments were performed on a Finnigan MAT TSQ-70 triple quadrupole mass spectrometer equipped with a Finnigan MAT thermospray interface. Samples subjected to collision-induced dissociation (CID) were introduced either in the column by-pass or in the LC mode. The collision pressure and energy were optimized for a particular application, and were typically set at 0.05–0.15 Pa of air and 10–50 eV, respectively.

RESULTS AND DISCUSSION

General strategy

The objectives of this study were the assessment of the potential of current LC–MS techniques for the unequivocal characterization of minor components in benzothiazole compounds.

The samples of interest generally have a purity exceeding 95% for the major component, with the remainder consisting of a number (usually up to 10) minor components. The low concentration and number of the minor components practically exclude fraction collection and off-line mass spectrometric analysis. Further, in some fraction collection experiments, unwanted modification of the components in a fraction was observed, with the UV chromatogram of the fraction differing considerably from the original part of the chromatogram. For these reasons, on-line LC–MS was considered to be the method of choice. At first, the MBI was selected because of its ability to be used with both EI and CI, which is most favourable for structure elucidation. For reasons outlined below, the MBI was replaced with the TSP interface; preliminary studies were also performed with a PBI when it became available in our laboratory.

Unfortunately, the LC conditions that were developed for optimum resolution of the minor components and UV detection were not compatible with direct on-line LC–MS analysis with the MBI or TSP interface (*cf.*, Fig. 2), principally because of the gradient elution conditions employed. Considering the facts that the moving belt interface with a direct contact deposition device, as used in this project, does not perform well under gradient elution conditions [2,3], and that the ionization conditions in LC–TSP-MS depend strongly on the mobile phase composition [2,4], it was decided to perform the LC–MS experiments with isocratic elution. Further, the applied flow-rates are generally too high for the MBI, while the range of flow-rate programming is not compatible with the TSP interface (*cf.*,

TABLE I

PEAK CORRELATION FOR 2(3*H*)-BENZOTHIAZOLETHIONE, SAMPLE A

Gradient (min)		UV max. (nm)	100%B	75% B	50% B
254 nm[a]	PDA				
	4.88	243,282		2.77	3.42
7.27	6.87[b]	321			
8.33	–				
	14.16	324			8.24
	15.88	328			10.83
17.10	16.20	237,303			11.35
23.15	22.38	264,301,330		9.02	
26.24	25.33	270		13.70	
33.94	33.00	260–280	6.75		
	33.70	306	8.12		
35.22	35.94	289,370	11.41		

[a] The data for 254 nm were obtained using a different apparatus with a fixed-wavelength UV detector and a different column.
[b] Major component.

Fig. 2). Therefore, it was decided to translate each gradient run into a series of isocratic runs. For the MBI, an additional advantage of this procedure is that the analysis is performed at the highest possible acetonitrile content of the mobile phase. In order to ensure the correlation between the peaks in the chromatograms obtained under gradient conditions and those under isocratic conditions, a PDA detector was used. Following this, the LC–MS analysis was performed under isocratic conditions.

UV photodiode-array detection; peak correlation

The samples of interest were first analysed with the appropriate gradient programme using a PDA detector. The peak purity was checked by comparing UV spectra at the front end, the back end and the top of the chromatographic peak. In this way, a collection of UV spectra and retention time data was obtained for each sample. As an example, these data are summarized for sample A in Table I and for sample C in Table II.

Subsequently, the samples were analysed in a series of isocratic runs using the PDA detector. The peaks detected were checked for peak purity and the UV spectra obtained were compared with the UV spectra obtained under gradient conditions. Corresponding peaks in gradient and isocratic runs could be found in this way (see Tables I and II for the results with samples A and C, respectively). Careful comparison of the UV spectra is obligatory; a conclusion cannot be based on comparing the wavelengths of absorbance maxima only. Although this procedure was cumbersome with the old version of the PDA detector and accompanying software that was available, it worked fairly well provided that the samples were not too complex. It can be considered as a general method of keeping track of UV-absorbing chromatographic peaks when the mobile phase composition must be changed, *e.g.*, for on-line LC–MS analysis. Further, the use of the PDA detector allows the detection of components that show low absorbance at the one fixed wavelength selected with a conventional UV detector for LC (see, for instance, the peaks at retention times 14.16 and 15.88 in Table I).

The availability of UV spectra of the various components in the samples also allowed the generation of cross-correlation tables, based on retention times and UV spectra. For instance, the peaks at 6.87 and 25.33 in the gradient run with sample A correspond to the peaks at 7.12 and 27.90, respectively, in the gradient run with sample C (using a different gradient programme); the second peak is also found in the 75% isocratic runs with both samples, *i.e.*, at 13.70 for sample A and at 13.94 for sample C. By studying the data from a wider variety of

TABLE II

PEAK CORRELATION FOR 4-(2-BENZOTHIAZOLYLTHIO)-MORPHOLINE, SAMPLE A

Gradient (min)		UV max. (nm)	100%B	75% B	50% B	37.5% B
275 nm[a]	PDA					
6.66	5.87	239,278	3.08	3.02	3.78	9.61
	7.12	321			4.07	
10.94	9.33	273			5.34	15.33
	10.91	238,276			5.62	18.11
16.03	14.44[b]	272				
	20.16	279				
	25.54	276–315		10.23		
27.29	26.13	290		10.90		
28.51	27.90	270	5.68	13.94		

[a] The data for 275 nm were obtained using a different apparatus with a fixed-wavelength UV detector and a different column.
[b] Major component.

samples, more extensive cross-correlation tables could be made, which substantially helped in the identification of the compounds in the various samples.

In some instances, the PDA was also helpful in discriminating between isomers. In samples A and sample C a component with a molecular mass of 300 was observed. From the respective UV spectra, shown in Fig. 3, it could be concluded that these components were not identical, but in fact isomers (**8** and **24**). The identity of one of the isomers (**24**) was checked with a standard, while literature data on the UV spectrum of the other (**8**) were available [7].

Obviously, the isocratic conditions that were needed to detect certain components in the mixture with the PDA detector do not necessarily correspond to the conditions required in LC–MS.

LC–MS using a moving-belt interface

The primary reason for selecting the MBI was its ability to generate EI mass spectra that would be easily interpretable from the broad knowledge of EI fragmentation and with the use of on-line library searching. Some typical EI mass spectra obtained in on-line LC–MS with the MBI are given in Fig. 4. An additional feature of the MBI-MS system was the ability to perform exact mass determination via high-resolution measurements.

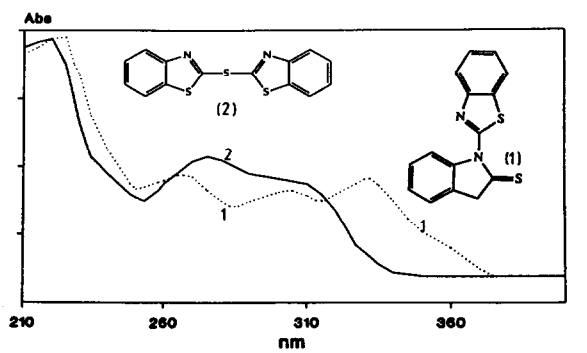

Fig. 3. UV spectra of the two components with molecular mass 300. 1 = [2,3′(2′*H*)-bibenzothiazole]-2′-thione (**8**); 2 = 2,2′-thiobisbenzothiazole (**24**).

In practice, some severe problems were experienced, which seriously limited the potential of the MBI. Although it was possible to obtain EI spectra for some of the minor components (see, *e.g.*, Fig. 4a), the combination of peak tailing in the isocratic runs and the common belt memory effects [2,3,8] led to the inability to obtain clean EI spectra for those components at very low levels, or for ones eluting close to the major compound (see, *e.g.*, Fig. 4b). As a result, it was impossible to obtain interpretable EI spectra in many cases. Generally, the mass region below *m/z* 170 was hardly useful with respect to spectral interpretation. With the use of reconstructed mass chromatograms in the region of a chromatographic peak it can be decided which peaks in a spectrum were actually due to the compound, and which were due to the interferences from belt memory effects. For the peak the spectrum of which is given in Fig. 4b, the peaks at *m/z* 167 and 86 are fragment peaks due to the major component of the sample. Various background subtraction procedures failed to produce relatively reproducible spectra, as a result of which it remains unclear which peaks should be considered during the interpretation. Attempts to solve these problems by using isocratic runs with mobile phases with a higher water content were discontinued because, for some components, thermal degradation was observed as a result of the higher solvent evaporator temperature needed with higher water contents of the mobile phase.

Another difficulty that was experienced is related to the amount of structural information that could be derived from the mass spectra. In the spectrum of N-phenyl-2-benzothiazolamine (**4**), a known constituent of sample A, in Fig. 4c, a loss of 45 u is observed, which basically can only be explained by a loss of CSH, resulting from a major rearrangement of the ion.

The two spectra in Fig. 4d and e are due to components that surprisingly do not show up in the UV chromatograms. The tentative structures assigned to the probable molecular ions at *m/z* 524 and 391 resulted from the following considerations. High-resolution peak matching on *m/z* 391 results in an elemental composition of $C_{20}H_{13}N_3S_3$. Loss of 33 u (SH) results in *m/z*

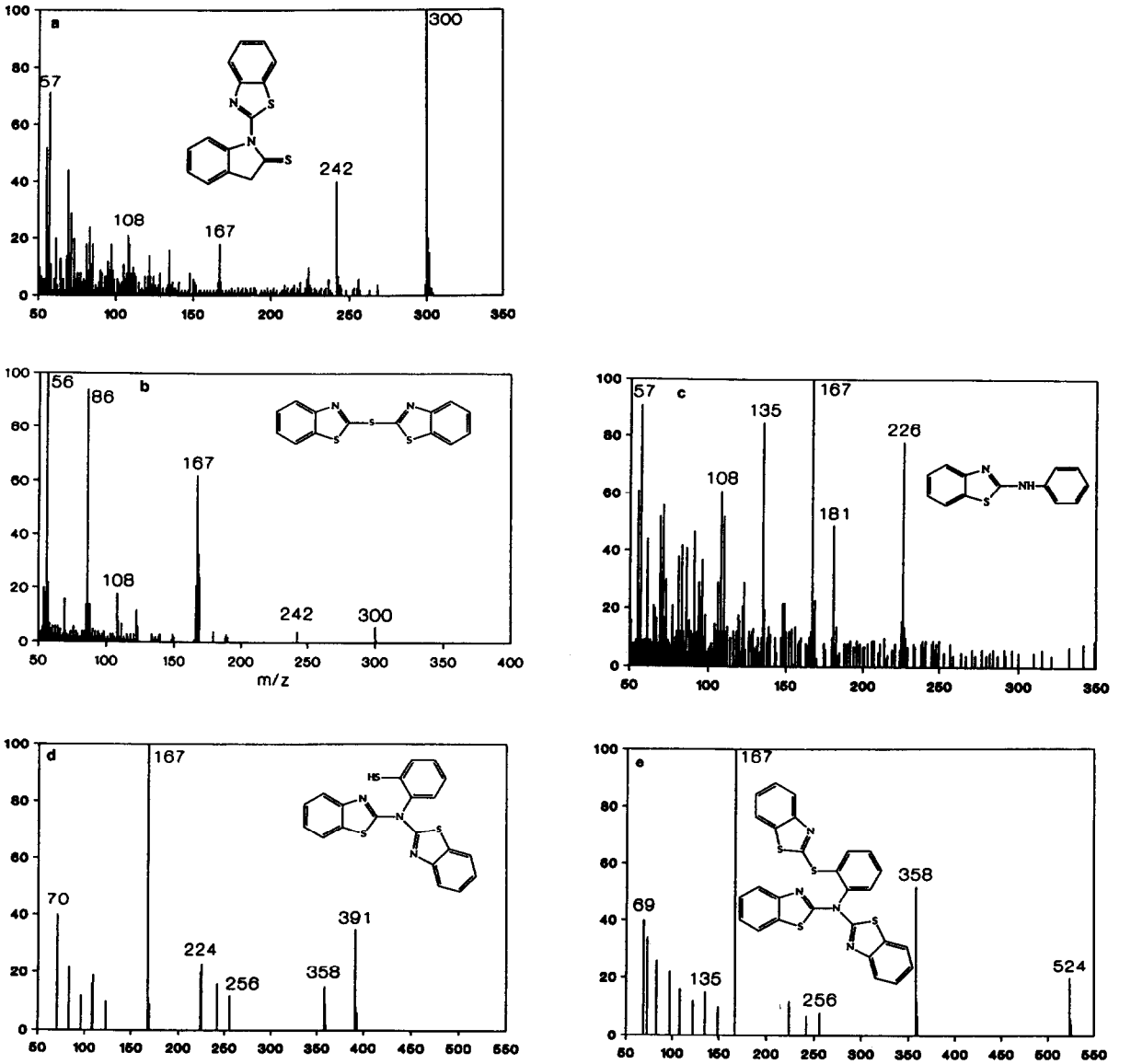

Fig. 4. EI mass spectra for some minor components in sample A or C obtained with LC–MS using a moving-belt interface. (a) Spectrum of [2,3′(2′*H*)-bibenzothiazole]-2′-thione (**8**) (gradient 22.38 min) obtained from sample A with a 75% B in A isocratic run. (b) Spectrum of 2,2′-thiobisbenzothiazole (**24**) (gradient 25.54 min) obtained from sample C with a 100% B isocratic run. (c) Spectrum of N-phenyl-2-benzothiazolamine (**4**) (gradient 16.20 min) obtained from sample A with a 50% B in A isocratic run. (d) Spectrum of **9** (not observed in gradient) obtained from sample A with a 100% B isocratic run. (e) Spectrum of **11** (not observed in gradient) obtained from sample A with a 100% B isocratic run.

358, which fragments to *m/z* 256, 242 and 224. The latter can be explained by the loss of a benzothiazole group. Loss of 166 u (a mercaptobenzothiazole group) from the *m/z* 524 molecular ion also resulted in *m/z* 358, with a similar fragmentation pattern. Other isomers are also possible.

In Tables IV and VI a summary is given of the

compounds that could be identified in the samples A and C, respectively, using the MBI system. The compounds identified are indicated by a number, referring to Table III, in which the names of all compounds found are collected.

LC–MS with a thermospray interface

Considering the difficulties in obtaining sufficient information with the MBI, it was decided to investigate the potential of the TSP interface, which was also available in our laboratory, for this identification problem. Some feasibility tests showed that generally identical spectra are obtained with buffer ionization and discharge-on modes (with only some differences with respect to the observed solvent adduct ions), and that generally no fragmentation is observed. Obviously, some compounds are not amenable to the type of ionization obtained with the TSP interface, *e.g.*, sulphur (S_8) is not detected in TSP whereas it is detected by EI. Further, the response is highly compound dependent, as is demonstrated, *e.g.*, by the fact that the major component in sample A, 2(3*H*)-benzothiazolethione (**1**), yields a signal in buffer ionization that is only three times higher than that of minor component N-phenyl-2-benzothiazolamine (**4**). This may lead to difficulties in detecting and identifying the less polar compounds, an effect that is further enhanced by the fact that these components elute in the final part of the chromatograms.

Another problem met in the experiments with the TSP interface was the difficulty of detecting the peaks of minor compounds in the total ion chromatogram (TIC), as instability of the TSP vaporization process sometimes yielded unstable TIC traces. By systematic searching through the data files using mass chromatography, it was possible to extract the various peaks from the data, but in searching for unknowns such a procedure can be cumbersome.

Nevertheless, with the TSP interface a significant number of peaks in the various samples could be detected and the molecular mass could be determined. In some instances the molecular mass confirmed the presence of expected compounds. This is the case, for instance, with the sulphinamide **20** and the sulphonamide **22** found in sample C, which could be identified from their molecular masses and chromatographic retention times, as standards were available.

However, as fragmentation was lacking, other means must be used for structure elucidation and identification. Some experiments applying repeller-induced fragmentation [9,10] gave some results, especially when the peak intensity was sufficiently large. In many instances, however, a significant decrease in signal intensity was observed, which is partly due to the fragmentation, but which restricted the ability to detect the peak and to produce the accompanying spectrum. Therefore, the potential of tandem mass spectrometry was investigated.

TABLE III

IDENTIFICATION OF THE COMPOUNDS STUDIED

1	2(3*H*)-Benzothiazolethione
2	4-(2-Benzothiazolylthio)morpholine
3	N-Cyclohexyl-2-benzothiazolesulphenamide
4	N-Phenyl-2-benzothiazolamine
5	2(3*H*)-Benzothiazolone
6	Benzothiazole
7-2	2-(Methylthio)benzothiazole
7-3	3-Methyl-2(3*H*)-benzothiazolethione
7-6	6-Methyl-2(3*H*)-benzothiazolethione
8	[2,3′(2′*H*)-Bibenzothiazole]-2′-thione
9	N,N-Di(2-benzothiazolyl)-2-aminobenzenethiol
10	2,2′-Dithiobisbenzothiazole
11	N,N-Di(2-benzothiazolyl)-S-(2-benzothiazolyl)-2-aminobenzenethiol
12	Sulphur
13	Benzenamine
14	N,N′-Diphenylthiourea
15	N-Phenylbenzenamine
16	2-(Phenylthio)benzothiazole
17	10*H*-Phenothiazine
18	2-Phenylbenzothiazole
19	2,2′-Bisbenzothiazole
20	4-(2-Benzothiazolylsulphinyl)morpholine
21	2-(4-Morpholinyl)benzothiazole
22	4-(2-Benzothiazolylsulphonyl)morpholine
23	2-(4-Morpholinyldithio)benzothiazole
24	2,2′-Thiobisbenzothiazole
25	N-Cyclohexyl-2-benzothiazolesulphinamide
26	N-Cyclohexyl-2-benzothiazolesulphonamide
27	N-Cyclohexyl-2-benzothiazolamine

LC–MS–MS with a thermospray interface

MS–MS was applied to this problem in a number of ways, using either product ion or precursor ion scans. In the product ion mode, an ion with a particular m/z value is selected in the first mass analyser and transferred to a collision chamber. The fragment ions generated on collision are mass analysed in the second mass analyser. In the precursor ion mode, the procedure is reversed: an ion with a particular m/z value is selected in the second mass analyser. A signal is only detected when the ions mass analysed in the first mass analyser generate that particular ion on collision. The m/z value of the precursor ion leading to the product ion detected is recorded [11]. Product ion spectra were obtained for selected chromatographic peaks during LC–TSP-MS operation. In this way a considerable number of unknowns could be identified; for others identification was not possible based on the information obtained from the MS–MS data. However, considering the type of products and expected minor components involved, precursor ion scans can be used to trace existing minor components. Precursor ion scans that were applied included the product ions of m/z 134, 166, 168, 87 (for the morpholine derivatives) and 99 (for the cyclohexyl derivatives). Precursor ion scans were performed in combination with LC and also with column-bypass injection of the samples. Subsequently, product ion spectra were acquired from all new precursor ions detected in this way. A number of unknowns could be identified in this way. Unfortunately, a considerable number of product ion spectra contained little information on the identity of the compound. In one of the samples three unknown precursors were detected in a precursor m/z 168 experiment, *i.e.*, at m/z 254, 238 and 252. The product ion spectra of the precursor contained product ions at m/z 168, 87, 71 and 43 for the precursor ion at m/z 254, and at m/z 168, 85 and 57 for the precursor ions at m/z 238 and 252. No identification of the groups attached to mercaptobenzothiazole is possible from these data.

The compounds identified in samples A, B, C and D using LC–MS and LC–MS–MS with the TSP interface are indicated in the Tables IV, V, VI and VII, respectively. It can be concluded that most of the components of these four samples could be identified.

Intermolecular reactivity in thermospray

An interesting aspect of the TSP experiments is the observation of considerable intermolecular

TABLE IV

PEAK IDENTIFICATIONS IN 2(3*H*)-BENZOTHIAZOLETHIONE, SAMPLE A[a]

Gradient (min)	UV max. (nm)	M_r	Identification[b]	MBI	TSP	GC–MS
4.88	243,282	151	**5**	–	×	–
6.87	321	167	**1 + 6**	×	×	×
14.16	324	181	**7**	–	×	×
15.88	328	181	**7**	–	×	×
16.20	237,303	226	**4**	×	×	–
22.38	264,301,330	300	**8**	×	×	×
		391	**9**	×	×	–
25.33	270	332	**10**	×	×	×
		524	**11**	×	×	–
33.00	260–280	256	**12**	×	–	×
33.70	306	?	–	–	–	–
35.94	289,370	?	–	–	–	–

[a] × = –Identified; – = not detected.

[b] See Table III.

TABLE V

PEAK IDENTIFICATIONS IN 2(3*H*)-BENZOTHIAZOLETHIONE, SAMPLE B[a]

Gradient (min)	UV max. (nm)	M_r	Identification[b]	TSP	BPI	GC–MS
	241	93	**13**	×	–	×
7.02	216,248	135	**6**	×	–	×
7.02	321	167	**1**	×	×	×
10.34	272	228	**14**	×	–	×
12.32	324	181	**7-3**	×	×	×
14.56	275	181	**7-2**	×	×	×
17.20	237,303	226	**4**	×	×	×
		169	**15**	×	–	×
		243	**16**	×	×	–
17.69	328	181	**7-6**	×	–	–
18.31	250	199	**17**	×	–	×
20.78	217,295	211	**18**	×	×	×
21.17	250,285	–	–	–	–	–
23.67	300	–	–	–	–	–
		300	**8**	×	–	×
24.24	242,343	268	**19**	×	×	×
34.35	260–280	256	**12**	–	–	×

[a,b] See Table IV.

reactivity. As the samples contained over 95% of the major component, large amounts of this material were generally introduced into the ion source. Spectra acquired during the elution of the major components indicate intermolecular reactions in the ion source. Although these reactions were not studied in detail, some typical results are briefly discussed here.

In the TSP mass spectrum of 2(3*H*)-benzothiazolethione shown in Fig. 5a, a strong

TABLE VI

PEAK IDENTIFICATIONS IN 4-(2-BENZOTHIAZOLETHIO)MORPHOLINE, SAMPLE C[a]

Gradient (min)	UV max. (nm)	M_r	Identification[b]	MBI	TSP	GC–MS
5.87	239,278	268	**20**	–	×	–
7.12	321	167	**1**	–	×	–
9.33	273	220	**21**	×	×	×
10.91	238,276	284	**22**	–	×	–
14.44	272	252	**2**	×	×	×
20.16	279	284	**23**	–	×	–
25.54	276–315	300	**24**	×	×	×
26.13	290	?	–	–	–	–
27.90	270	332	**10**	×	×	×

[a,b] See Table IV.

TABLE VII

PEAK IDENTIFICATIONS IN N-CYCLOHEXYL-2-BENZOTHIAZOLESULPHENAMIDE, SAMPLE D[a]

Gradient (min)	UV max. (nm)	M_r	Identification[b]	TSP	GC–MS
4.77	321	167	**1**	×	×
5.17	250–281	135	**6**	×	×
8.66	277	280	**25**	×	–
11.61	273	296	**26**	×	–
		232	**27**[c]	×	?
19.64	278	264	**3**	×	–
22.82	270	332	**10**	×	×
30.67	297	?	–	–	–

[a,b] See Table IV.
[c] Not detected in LC–UV.

protonated molecule is observed at m/z 168. In addition, peaks are observed at m/z 209, which is an acetonitrile adduct of the protonated molecule, m/z 248 and 262. The latter two peaks, resulting from the addition of 80 and 94 μ, respectively, to the protonated molecule could not be identified by MS–MS. The product ion spectra of m/z 248 and 262 show m/z 168 in both spectra and fragments at m/z 81 and 95, respectively. The same peaks are present in the TSP mass spectrum of 2,2′-dithiobisbenzothiazole shown in Fig. 5b, in which considerable fragmentation is observed. Addition of 80 and 94 u to the major constituent is frequently observed, *e.g.*, also in the analysis of 4-(2-benzothiazolethio)morpholine, resulting in ions at m/z 333 and 347. To prevent the reactions and to avoid severe source contamination in many of the experiments the major component was directed to waste.

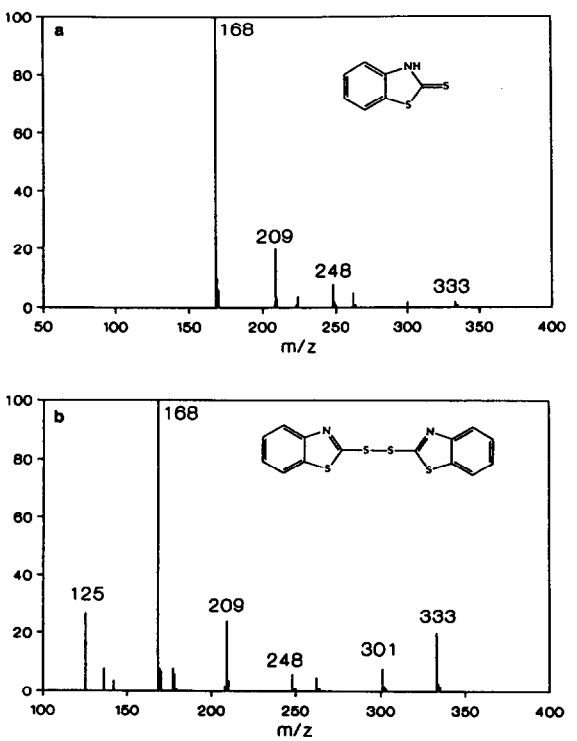

Fig. 5. Thermospray mass spectra obtained with high concentrations of (a) 2(3*H*)-benzothiazolethione (**1**) and (b) 2,2′-dithiobisbenzothiazole (**10**).

GC–MS

In samples A and B, a variety of low-molecular-mass and low-polarity compounds appears to be present. Some of these compounds might not be easily protonated in the TSP ionization applied in LC–MS. Therefore, additional information on the compounds present in the samples may be obtained from GC–MS. Care must be taken with the results as some compounds, such as those in samples C and D, may lack sufficient thermal stability for GC–MS analysis. Further, direct correlation of the identified compounds with the LC retention times is impossible. In

practice, GC–MS helped in and/or confirmed the identification of some of the compounds in samples A and B. The components in samples A–D detected and identified by GC–MS are indicated in Tables IV–VII.

LC–MS with a particle-beam interface

In the final stage of this project, a PBI system became available in our laboratory. Considering the initial strategy developed, in which the MBI was used for its ability to obtain EI spectra, and the results from GC–MS experiments, it was decided to analyse sample B with the PBI. The total ion chromatogram obtained is shown in Fig. 6. The components in sample B that were identified using the PBI are indicated in Table V. These preliminary data confirmed the usefulness of our initial strategy. However, the use of PBI appears to be more appropriate than that of MBI. Some warning must be given here. From both GC–MS and LC–PBI-MS data, it became clear that several components present in the investigated samples do not give clearly interpretable EI spectra. The spectrum of 2,2′-bisbenzothiazole (**19**), shown in Fig. 7, may serve as an example of this. Apart form a strong molecular ion, very few informative fragment peaks are present that would allow the identification of this compound. In this particular instance, the spectrum is available in the NBS library, but with other similar compounds identification will be difficult.

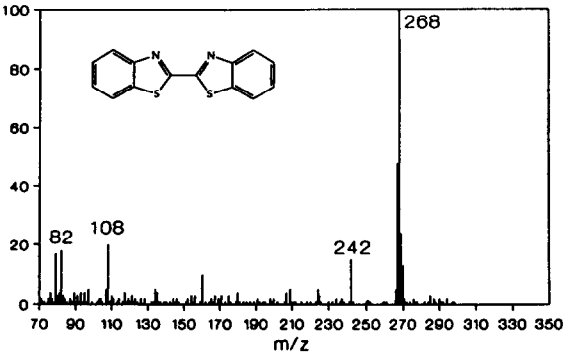

Fig. 7. EI mass spectrum of 2,2′-bisbenzothiazole (**19**).

CONCLUSIONS

The methodology developed, especially TSP in combination with LC–MS and LC–MS–MS and PBI, is capable of characterizing most of the minor components in the benzothiazole-derived compounds. However, greater difficulties would be experienced with components that are below a concentration of 0.1%.

The use of various LC–MS interfaces for these types of compounds allows a comparison of their potential. For identification purposes the availability of EI spectra is most helpful. Therefore, MBI and PBI are the first choice. However, the MBI is a mechanical device that is not easy to operate routinely and suffers from severe memory problems. PBI is simple and reliable, results in useful total ion chromatograms and is capable of EI and solvent-independent CI. It is considered to be the interface of choice for the identifi-

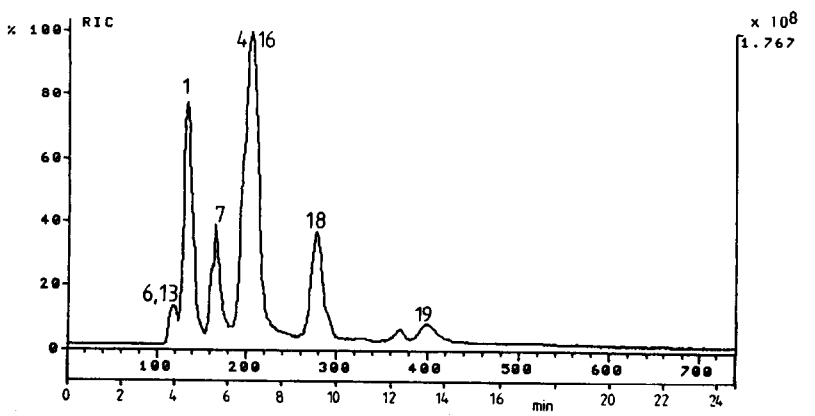

Fig. 6. Total ion current chromatogram of an LC–particle-beam-MS analysis of sample B. For conditions, see Experimental.

TABLE VIII

COMPARISON OF TECHNIQUES USED[a]

Technique	1 (polar stable)	22 (polar labile)	12 (non-polar stable)	10 (non-polar labile)
Moving-belt	+	−	+	±
Particle-beam	+	−	+	±
Thermospray	+	+	−[b]	+
GC–MS	+	−	+	+

[a] + = Good signal; ± = resonable; − = poor or no signal.
[b] Sulphur is not sensitive to TSP ionization owing to insufficient proton affinity.

cation problems with these relatively non-polar and thermally stable compounds. The TSP interface used in combination with MS–MS is also capable of producing useful spectra for the identification of the components in the mixtures. The techniques used in this study are compared in Table VIII. The ability of the various techniques to achieve molecular mass information and structure information from fragmentation is compared for four compounds differing in polarity and stability. It can be concluded that more than one MS technique should be used in identification problems such as this.

ACKNOWLEDGEMENTS

Several workers in our groups were involved in this project and we express our appreciation to them for their practical assistance. H.J.E.M. Reeuwijk (LACDR) is thanked for her extensive work on gradient and isocratic HPLC and the photodiode-array UV spectra, and her help in constructing the peak correlation tables, E.R. Verheij (LACDR and TNO) and G.F. LaVos (TNO) for their experiments with the moving belt, C.E.M. Heeremans (LACDR) for her thermospray work and R.A.M. van der Hoeven (LACDR) for his many hours working with the thermospray, particle-beam and GC–MS systems.

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