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Direct on-column derivatisation in gas chromatography II. Comparison of various on-column methylation reagents and the development of a new selective methylation reagent

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Abstract

It has been demonstrated that judicious choice of the derivatisation reagent used for direct on-column methylations can have a profound effect of the products produced, often with little effect on the overall methylation efficiency of the process. Trimethylsulfonium acetate, trimethylsulfonium cyanide and phenyltrimethylammonium acetate are three new selective methylation reagents for direct on-column derivatisation in gas chromatography. Phenyltrimethylammonium acetate is a particularly stable, selective and efficient new direct on-column methylation reagent. This work demonstrates that, for routine on-column methylation where no selectivity is required, phenyltrimethylammonium fluoride (PTMA-F) is as efficient as phenyltrimethylammonium hydroxide. PTMA-F is the preferred reagent because it is neutral and produces less column deterioration than the hydroxide. Where methylation selectivity is required, both phenyltrimethylammonium acetate has very similar efficiency and selectivity as the previously reported cyanide salt. However, the acetate is the more stable and readily utilised than the cyanide and is the best of a number of selective on-column methylation reagents surveyed in this work.

Keywords: Derivatization, GC; Methylation; Benzimidazoles; Phenyltrimethylammonium salts; Trimethylsulfonium salts; Sulfonamides; Phenols; Fatty acids; Chlorophenols; Nitrophenols

1. Introduction

On-column alkylation using either tetraalkylammonium salts [1–5] or trialkylsulfonium hydroxides [6,7] as an alkylation reagent, is a commonly used method for derivatising acidic substances for gas chomatographic analysis. The usual procedure is to inject a solution of analyte and the on-column derivatisation reagent into the hot injection port of the gas chomatograph which results in the decomposition of the alkylation reagent into an amine or sulfide accompanied by the concomitant alkylation of the analyte in high yield. Although previously reported on-column methylation reagents are effective and efficient, they are all highly caustic and therefore likely to cause rapid column deterioration. Furthermore, they demonstrate little selectivity in the methylation of multifunctional substances.

We recently reported the comparison of methylation efficiency and selectivity of three phenyltrimethylammonium salts in the direct on-column methylation of a series of sulfonamides, benzimidazoles and thiouracils used in animal production [8]. In this study it was found that phenyltrimethylammonium hydroxide (PTMA-OH), sold commercially under the trade name of MethElute, is an efficient

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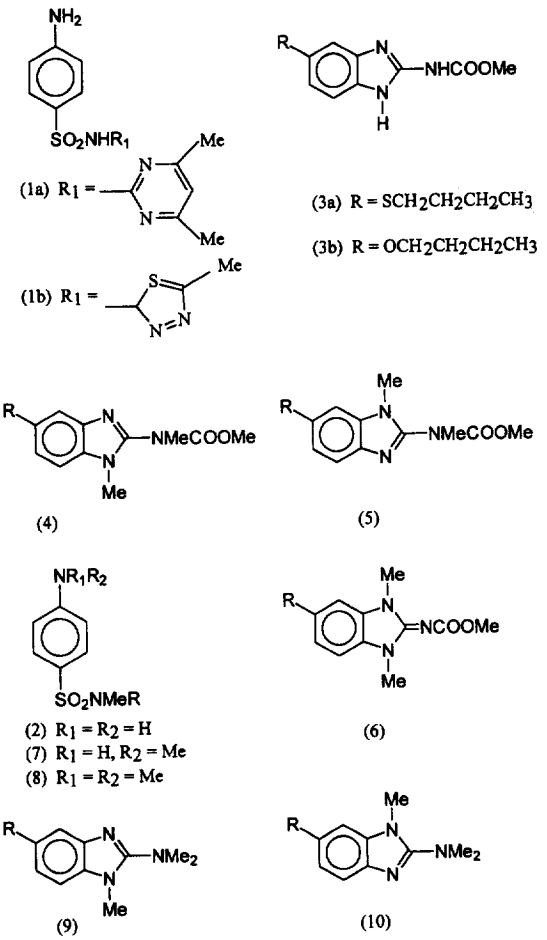
but unselective on-column methylating reagent for multifunctional substances, resulting in the formation of a complex mixture of products when used for methylation of either sulfonamides or benzimidazoles. Phenyltrimethylammonium fluoride (PTMA-F) was found to be somewhat more selective but also resulted in the production of several products. PTMA-F did, however, offer a significant advantage over PTMA-OH because it was neutral and did not have the potential to cause the rapid column degradation that the strongly alkaline PTMA-OH had demonstrated in our hands.

By contrast, phenyltrimethylammonium cyanide (PTMA-CN) proved to be far the most selective of the three reagents surveyed while retaining excellent methylation efficiency. For example, sulfonamides (**1** in Scheme 1) gave over 95% of the monomethylated product (**2** in Scheme 1) after on-column derivatisation with PTMA-CN while benzimidazoles (**3** in Scheme 1) gave two dimethyl derivatives (**4** and **5** in Scheme 1) together with the formation of minor amounts of the isomeric dimethyl derivative (**6** in Scheme 1).

Although phenyltrimethylammonium cyanide [8] has proved a selective and efficient on-column methylation reagent, the utilisation of cyanide counter ion has not met with universal approval in our operational areas because of both the potential toxicity of the salt and the fact that methanolic solutions are strongly alkaline and not particularly stable. Stock solutions are oxidised by atmospheric oxygen and discolour over a 1-week period at ambient temperature but can be kept for up to five weeks in a refrigerator.

In the present study we have extended this initial work with two primary objectives: (1) To survey a range of direct on-column methylation reagents in order to determine which was the preferred reagent for routine use. (2) To determine which particular salt of a particular reagent gave the greatest methylation selectivity while retaining high methylation efficiency.

We now report that phenyltrimethylammonium salts are, in general, the on-column methylation reagents of choice and phenyltrimethylammonium acetate (PTMA-OAc) is an equally selective on-column derivatisation reagent as the corresponding cyanide whilst retaining excellent methylation efficiency for many substances. Significant differences between the methylation products obtained for sulfonamides and benzimidazoles by the use of PTMA-OH or trimethylsulfonium hydroxide (TMSu-OH) are also discussed.



Scheme. 1.

2. Experimental

2.1. Reagents

Phenyltrimethylammonium iodide, cyanide and fluoride were prepared as detailed previously [1].

Silver acetate was prepared by adding a 20% aqueous solution of sodium acetate to an equimolar quantity of a 30% aqueous solution of silver nitrate. The mixture was filtered under vacuum, washed with ice-cold water followed by methanol and dried at 60°C overnight and stored in an amber bottle. Tetramethylammonium fluoride tetrahydrate (TMA-F), trimethylsulfonium iodide and trimethyloxysulfonium iodide were purchased from Aldrich (Milwaukee, WI, USA). MethElute (phenyltrimethylammonium hydroxide, PTMA-OH) and Methprep-II (3-trifluoromethylphenyl)-trimethylammonium hydroxide, (TTMA-OH) were commercial analytical reagents obtained from Pierce (Rockford, IL, USA) and Alltech (Deerfield, IL, USA), respectively.

Ion-exchange resins were chomatographic grades (63–150 μm) available from Bio-Rad Laboratories (Sydney, Australia). The fluoride form of the resin in methanol was prepared by percolation of an aqueous slurry of 50 ml of the commercially available chloride form contained in a chromatographic column with 500 ml of a 2 *M* aqueous solution of sodium fluoride followed by 100 ml of deionised water and 100 ml of methanol. The resin was stored in an amber bottle under methanol. The hydroxide, acetate and other anionic forms of the resin were prepared by the same method with substitution of the appropriate sodium salt for sodium fluoride. The acetate form of the resin was stable to storage at room temperature while the cyanide could be stored in an air-tight bottle in a refrigerator for several weeks. The hydroxide form was best prepared freshly before use.

2.1.1. Phenyltrimethylammonium acetate (PTMA-OAc) and trimethylsulfonium acetate (TMSu-OAc)

These salts were prepared as a 0.2 *M* solution in methanol by stirring a mixture of either phenyltrimethylammonium iodide (5.3 g) or trimethylsulfonium acetate (4.1 g) in methanol (80 ml) with silver acetate (3.32 g) for 1 h. The solution was filtered, the residual silver iodide washed with methanol (10 ml) and the combined solutions adjusted to 100 ml with methanol. Alternatively they could be prepared by the same ion-exchange procedure employed for the preparation of trimethylsulfonium cyanide (below) using sodium acetate in place of sodium cyanide.

2.1.2. Trimethylsulfonium cyanide (TMSu-CN)

A chromatographic column containing 20 ml of AG 1-X8 anion-exchange resin (63–150 μm) was percolated with a 0.1 *M* solution of sodium cyanide. The column was then washed with water (100 ml) and methanol (200 ml). A 0.3 *M* solution of the trimethylsulfonium iodide in methanol (20 mmol total) was added to the column and eluted with methanol. The emergence of the quaternary derivative was monitored by frequent testing of small aliquots of column eluent with aqueous silver nitrate. When all the quaternary derivative had been eluted, enough methanol was added to the combined fractions to bring the concentration to 0.2 *M*. This stock solution was diluted for use in on-column derivatisation experiments.

2.1.3. Trimethylsulfonium hydroxide (TMSu-OH)

This salt was prepared as a 0.2 *M* solution in methanol by stirring a suspension of trimethylsulfonium iodide (4.1 g) in methanol (80 ml) with silver oxide [freshly prepared from silver nitrate (3.4 g) and well washed with water and methanol] until the solids changed colour from brown to pale yellow. The solution was filtered, the residual silver iodide washed with methanol (10 ml) and the combined solutions adjusted to 100 ml with methanol. Alternatively it could be prepared by the same ion-exchange procedure employed for the preparation of the cyanide (above) using sodium hydroxide in place of sodium cyanide.

2.1.4. Trimethylsulfonium fluoride (TMSu-F)

A 0.2 *M* solution in methanol was prepared either by neutralisation of the hydroxide to pH 7 (tested by pH paper using a drop of reagent solution diluted with 5 drops of water) with 40% hydrofluoric acid or by ion-exchange replacement of iodide by fluoride as detailed above for the preparation of PTMA-CN. The ion-exchange procedure yielded a purer product than that from neutralisation which contained minor impurities in some derivatisations which, however, did not interfere with GC–MS results.

2.1.5. Trimethyloxysulfonium hydroxide (TMSuO-OH)

This salt was prepared as a 0.2 *M* solution in methanol by stirring a suspension of finely ground

trimethyloxysulfonium iodide (4.3 g) in methanol (80 ml) with silver oxide (freshly prepared from silver nitrate (3.4 g) and well washed with water and methanol) until the solids changed colour from brown to pale yellow (3 h). The solution was filtered, the residual silver iodide washed with methanol (10 ml) and the combined solutions adjusted to 100 ml with methanol. Preparation of solutions above 10 m*M* by ion-exchange of the iodide was not possible because of the limited solubility of trimethyloxysulfonium iodide in methanol.

2.1.6. Trimethyloxysulfonium fluoride (TMSuO-F) and acetate (TMSuO-OAc)

These salts were prepared by the neutralisation of a 0.2 *M* solution of trimethyloxysulfonium hydroxide in methanol to pH 7 with 40% aqueous HF or to pH 8.5 with glacial acetic acid respectively (tested by pH paper using a drop of reagent solution diluted with 5 drops of water).

2.2. Standards

Sulfonamide standards were obtained from Sigma (St. Louis, MO, USA) and benzimidazole and thiouracil standards were provided by the Curator of Standards, Australian Government Analytical Laboratories, (Pymble, NSW, Australia). Phenols and carboxylic acids were the purest grade available from Aldrich.

2.3. On-column derivatisation studies

Unless otherwise stated, on-column methylations were investigated by injection of a mixture of a solution of the analyte (70–100 mg/kg each) in a 10 m*M* methanolic solution of the requisite derivatisation reagent.

2.4. Equipment operation

2.4.1. Gas chromatography–mass spectrometry

GC analyses were conducted on one of two alternative instruments:

(1) Hewlett-Packard 5890 gas chromatograph, operating in the split injection mode, equipped with a Hewlett-Packard 7673A autosampler and a Hewlett-Packard mass-selective detector (Model 5971A). The column was a Hewlett-Packard HP-1 12 m×0.22 mm fused-silica capillary column with a film thickness of 33 μm (Hewlett-Packard, Palo Alto, CA, USA). Helium was used as the carrier gas. The data were analysed using the software supplied with the 5971A instrument.

(2) Shimadzu GC-17A gas chromatograph, operating in the split injection mode, equipped with a Shimadzu AOC-17 autoinjector and a Shimadzu mass spectrometer (Model GP 5000). The column was a Hewlett-Packard HP-1 30 m×0.25 mm fused-silica capillary column with a film thickness of 25 μm. Helium was used as the carrier gas. The data were analysed using the software supplied with the instrument.

Injector inserts were cleaned and prepared by washing with methanol.

2.5. Acquisition of GC data

GC analyses and on-column derivatisation studies were conducted employing the following standard conditions:

2.5.1. Hewlett-Packard mass-selective detector

GC: injection temperature 250°C, detector temperature 280°C, injection volume 2 μl (with 3 washes between injections), oven equilibration time between runs 0.5 min, oven program: initial temperature 140°C (0.5 min), then 20°C/min to 300°C and hold at 300°C for 3 min. MS: solvent delay 3.5 min, scan parameters *m/z* 50–450, threshold 1500. The split ratio was 20:1.

2.5.2. Shimadzu mass spectrometer

GC: injection temperature 250°C, detector temperature 280°C, injection volume 1 μl (with 3 washes between injections), oven equilibration time between runs 0.5 min, oven program: initial temperature 60°C (1 min) then 15°C/min to 140°C, then 25 C°/min to 280°C and hold at 280°C for 4 min. MS: solvent delay 3.5 min, scan parameters *m/z* 50–450, threshold 1500. The split ratio was 5:1.

3. Results and discussion

3.1. Comparison of the selectivities and efficiencies of on-column methylation reagents for sulfonamides and benzimidazoles

3.1.1. Sulfonamides

We previously reported that on-column derivatisation of four different sulfonamides (**1** in Scheme 1) with PTMA-OH produced a mixture of three derivatives, the structures of which were established as the monomethylated (**2** in Scheme 1) dimethylated (**7** in Scheme 1) and trimethylated (**8** in Scheme 1) from mass spectral data [8]. PTMA-F was less aggressive but also gave the same three products in comparable overall yield. However, PTMA-CN produced over 90% of the monomethylated (**2**). The study has now been expanded to compare the efficiency and selectivity of a number of tetraalkylammonium, trimethylsulfonium and trimethyloxysulfonium salts in the on-column derivatisation of two representative sulfomamides, sulfadimidine (1a) and sulfamethiazole (**1b** in Scheme 1). Results are shown in Table 1 and Table 2. In these studies diphenylsulfone and pentachlorophenol were added as surrogates. Diphenylsulfone was not derivatised and was used to monitor the GC–MS performance. Pentachlorophenol (PCP) has been found to be completely methylated with only a moderate molar excess of an efficient on-column derivatisation reagent and was therefore a convenient additive to estimate the maximum methylation yield to be expected from use of any particular reagent. Both surrogates were used, since it could not be assumed that PCP would be completely methylated with all reagents and therefore false results would be obtained by simply using the total derivative/PCP ratios. Generally inefficient methylation by trimethyloxysulfonium salts is readily demonstrated in this manner.

In addition to the three phenyltrimethylammonium salts previously studied [1], the acetate salt (PTMA-OAc) was also examined in this work. This on-column derivatisation reagent gave results which were very similar to those obtained with PTMA-CN, both in efficiency and in selectivity of methylation. Thus, on-column derivatisation of sulfadimidine with PTMA-CN or PTMA-OAc gave almost identical methylation efficiency and selectivity (Table 1) and sulfamethiazole gave slightly more dimethylated product with PTMA-OAc than with PTMA-CN (Table 2).

In comparing the methylation efficiency of trimethylsulfonium salts as on-column derivatisation reagents, the highly alkaline TMSu-OH was not included because, in our hands, this reagent was found to cause rapid column deterioration. On-column derivatisation of sulfadimidine and sulfamethiazole with TMSu-F gave a similar mixture of

Table 1
Methylation of 100 mg/kg sulfadimidine (**1a**) with 10 m*M* of different on-column derivatisation reagents

Methylation reagent	Total ion current×10^6			% of total methylated sulfonamide			PCP/DPS ratio[b]	Tot. deriv/DPS ratio[c]
	DPS	Methyl-PCP	Total derivatives[a]	Monomethyl	Dimethyl	Trimethyl		
Methprep	6.7	8.5	30	64.67	26.00	9.33	1.27	4.48
PTMA-OH	5.9	10.4	25.6	42.19	26.17	31.64	1.76	4.34
PTMA-F	7.6	19	26	38.85	26.15	35.00	2.50	3.42
PTMA-CN	7.2	12.6	31.8	93.08	6.92	0.00	1.75	4.42
PTMA-OAc	7.2	12.1	33.9	93.81	6.19	0.00	1.68	4.71
TMSu-F	7.6	18.3	28.7	62.72	18.47	18.82	2.41	3.78
TMSu-CN	6.2	11.7	24	88.75	11.25	0.00	1.89	3.87
TMSu-OAc	6.4	13.3	27.2	87.87	12.13	0.00	2.08	4.25
TMSuO-OH	6.5	6.5	20.2	92.08	7.92	0.00	1.00	3.11
TMSuO-F	5.3	8.6	17.9	82.68	17.32	0.00	1.62	3.38
TMSuO-OAc	7.6	19.5	27.4	82.85	17.15	0.00	2.57	3.61
TMA-F	7.6	1.8	5	50.00	28.00	22.00	0.24	0.66

[a] Total derivatives=sum of ion currents for the mono-, di- and trimethylated sulfonamide.
[b] Ratio of the total ion current for pentachlorophenol methyl ether to that of diphenylsulfone.
[c] Ratio of the total ion current for the combined methylated sulfonamides to that of diphenylsulfone.

Table 2
Methylation of 100 mg/kg sulfamethiazole (**2b**) with 10 m*M* of different on-column derivatisation reagents

Methylation reagent	Total ion current×10^6			% of total methylated sulfonamide			PCP/DPS ratio[b]	Total deriv/DPS ratio[c]
	DPS	Methyl-PCP	Total derivatives[a]	Monomethyl	Dimethyl	Trimethyl		
Methprep	7.7	12.9	16.3	83.44	16.56	0.00	1.68	2.12
PTMA-OH	7.1	20	13.6	37.50	46.32	16.18	2.82	1.92
PTMA-F	8.5	27.5	10.2	41.18	58.82	0.00	3.24	1.20
PTMA-CN	7.5	12.6	19.7	88.58	11.42	0	1.68	2.63
PTMA-OAc	7.4	16.9	17.3	90.17	9.83	0.00	2.28	2.34
TMSu-F	7.6	18.3	28.7	62.72	18.47	18.82	2.41	3.78
TMSu-CN	6.9	15	14.2	78.87	21.13	0.00	2.17	2.06
TMSu-OAc	7.3	14.1	16.2	81.48	18.52	0.00	1.93	2.22
TMSuO-OH	7.1	11.6	9.6	58.33	15.63	26.04	1.63	1.35
TMSuO-F	6.8	11.8	9.5	58.95	15.79	25.26	1.74	1.40
TMSuO-OAc	8.3	22.7	15.2	49.34	39.47	11.18	2.73	1.83
TMA-F	7.6	2	1.6	43.75	56.25	0.00	0.26	0.21

[a] Total derivatives=sum of ion currents for the mono-, di- and trimethylated sulfonamide.
[b] Ratio of the total ion current for pentachlorophenol methyl ether to that of diphenylsulfone.
[c] Ratio of the total ion current for the combined methylated sulfonamides to that of diphenylsulfone.

products to those obtained with PTMA-F and methylation efficiencies of PTMA-F and TMSu-F were comparable (Table 1 and Table 2). On-column methylation of the same two sulfonamides with TMSu-OAc or TMSu-CN gave almost the same efficiency and selectivity as that obtained by use of PTMA-OAc or PTMA-CN (Table 1 and Table 2). Thus, for this use, trimethylsulfonium acetate is an attractive alternative to the use of phenyltrimethylammonium acetate. The routine use of either of the cyanide salts (TMSu-CN or PTMA-CN) was not as attractive as the acetates because neither was stable to prolonged storage, even at 40°C and both were susceptible to rapid atmospheric oxidation in methanolic solution.

Table 1 and Table 2 also illustrate that, with the exception of the acetate, trimethyloxysulfonium salts investigated in this work were unsuitable reagents for on-column derivatisation.

3.1.2. Benzimidazoles

As previously reported [1], methylations of benzimidazoles with PTMA-OH, are complex and produce up to five products. Thus, albendazole (**3a** in Scheme 1) gives a mixture of the three dimethylated derivatives (**4a**, **5a** and **6a**) together with two additional trimethylated products which elute earlier than the dimethyl derivatives. The structures **9a** and **10a** have been assigned to these products [1].

Methylation of oxibendazole with TMSu-OH gave the identical set of products to those obtained with PTMA-OH; however, the trimethylated products (**9a**) and (**10a**) were produced in higher yield with TMSu-OH than with PTMA-OH. This suggested that, for benzimidazoles, TMSu-OH behaved as a stronger base than PTMA-OH which resulted in a greater degree of cleavage of the carbamate grouping in benzimidazoles during the direct on-column methylation with TMSu-OH than was caused by methylation with PTMA-OH (Table 4).

Table 3 and Table 4 shows the comparison between the methylation of albendazole and oxibendazole with a variety of on-column derivatisation reagents. Table 3 gives results obtained on a Hewlett-Packard mass-selective detector whereas Table 4 shows results derived from a Shimadzu instrument under different operating conditions. These tables demonstrate that the array of methylation products obtained with multifunction compounds varies and this has been found to be dependent on the injector insert.

With these two benzimidazoles the greatest methylation selectivity is obtained with PTMA-OAc. This reagent produces less rearranged dimethylated product (**6** in Scheme 1) than does the corresponding

Table 3
Methylation of 95 mg/kg of albendazole (**3a**) and oxibendazole (**3b**) with 10 m*M* of different on-column derivatisation reagents (Hewlett-Packard GC–MS system)

Methylation reagent	Total ion current×10^6			% of total methylated benzimidazole			PCP/DPS ratio[b]	Total deriv/PCP ratio[c]
	DPS	Methyl-PCP	Total derivatives[a]	Dimethyl (**4** and **5**)	Dimethyl (**6**)	Trimethyl (**9** and **10**)		
3a								
Methprep	7	20.3	28.80	36.81	29.17	34.03	2.90	1.42
PTMA-OH	7.6	21.7	21.90	19.63	35.62	44.75	2.86	1.01
PTMA-F	7.6	20	17.50	50.86	17.14	32.00	2.63	0.88
PTMA-CN	7.7	20.3	22.50	59.11	40.89	0.00	2.64	1.11
PTMA-OAc	8	17.7	18.60	80.65	19.35	0.00	2.21	1.05
TMSu-CN	6.5	16.9	23.50	69.36	30.64	0.00	2.60	1.39
TMSu-OAc	6.6	18.3	10.20	58.82	41.18	0.00	2.77	0.56
TMSuO-OH	7.5	11.3	9.90	79.80	20.20	0.00	1.51	0.88
TMSuO-F	7	7	8.90	82.02	17.98	0.00	1.00	1.27
TMSuO-OAc	7.2	18.9	21.90	87.21	12.79	0.00	2.63	1.16
3b								
PTMA-F	7.6	20	17.50	50.86	17.14	32.00	2.63	0.88
PTMA-CN	7.7	20.3	22.50	59.11	40.89	0.00	2.64	1.11
PTMA-OAc	8	17.7	18.60	80.65	19.35	0.00	2.21	1.05
TMSu-CN	6.5	16.9	23.50	69.36	30.64	0.00	2.60	1.39
TMSu-OAc	6.6	18.3	10.20	58.82	41.18	0.00	2.77	0.56

[a] Total derivatives=sum of ion currents for all methylated benzimidazoles.
[b] Ratio of the total ion current for pentachlorophenol methyl ether to that of diphenylsulfone.
[c] Ratio of the total ion current for the combined methylated benzimidazoles to that of diphenylsulfone.

Table 4
Methylation of 100 mg/kg of albendazole (**3a**) and oxibendazole (**3b**) with 10 m*M* of four different on-column derivatisation reagents (Shimadzu GC–MS system)

Methylation reagent	Total ion current×10^7			% of total methylated benzimidazole			PCP/DPS ratio[b]	Total deriv/DPS ratio[c]
	DPS	Methyl-PCP	Total derivatives[a]	Dimethyl (**4** and **5**)	Dimethyl (**6**)	Trimethyl (**9** and **10**)		
3a								
PTMA-OH	15.26	15.26	47.71	21.21	20.58	58.21	1.59	3.13
PTMA-F	11.71	17.06	41.05	72.18	21.49	6.33	1.46	3.51
TMSu-F	13.38	23.42	44.42	69.14	18.91	11.95	1.75	3.32
TMA-F	11.31	2.57	25.01	22.27	2.32	75.41	0.23	2.21
3b								
PTMA-OH	14.52	20.43	37.85	17.52	2.51	26.00	1.41	2.61
PTMA-F	12.07	20.89	27.06	62.75	16.19	21.06	1.73	2.24
TMSu-OH	12.77	25.40	29.84	12.33	33.33	54.33	2.01	1.17
TMSu-F	12.67	25.50	14.84	73.25	13.07	13.68	2.01	1.17
TMA-F	12.14	2.50	8.04	27.36	0.00	72.64	2.21	0.66

[a] Total derivatives=sum of ion currents for all methylated benzimidazoles.
[b] Ratio of the total ion current for pentachlorophenol methyl ether to that of diphenylsulfone.
[c] Ratio of the total ion current for the combined methylated benzimidazoles to that of diphenylsulfone.

cyanide which is possibly due to the lower basicity of the reagent.

3.2. Methylation of phenols

3.2.1. Phenol methyl ethers from tetraalkylammonium salts

Although PCP can be analysed by GC–MS under carefully defined conditions, up to a 3–10-fold greater sensitivity may obtained using the methyl ether, depending on the condition of the column and insert used. Fig. 1 shows a typical GC–MS run for free phenols. The most efficient on-column derivatision method to convert a range of environmentally important phenols to their methyl ethers was therefore investigated. Phenyltrimethylammonium hydroxide (MethElute, PTMA-OH) and phenyl-trimethylammonium fluoride, acetate and cyanide gave equally efficient methylation of phenols but they also produced dimethylaniline as a by-product of the methylation process which caused interference with the quantitation of simple phenyl methyl ethers at low levels (see Fig. 2). Therefore, other on-column derivatisation reagents were investigated.

TMA-F is a neutral salt which is commercially available as the solid tetrahydrate. We found that sulfonamides were as efficiently methylated by TMA-F as by MethElute under the same conditions. However, whereas MethElute completely methylated phenols and cresols to methyl ethers, TMA-F in

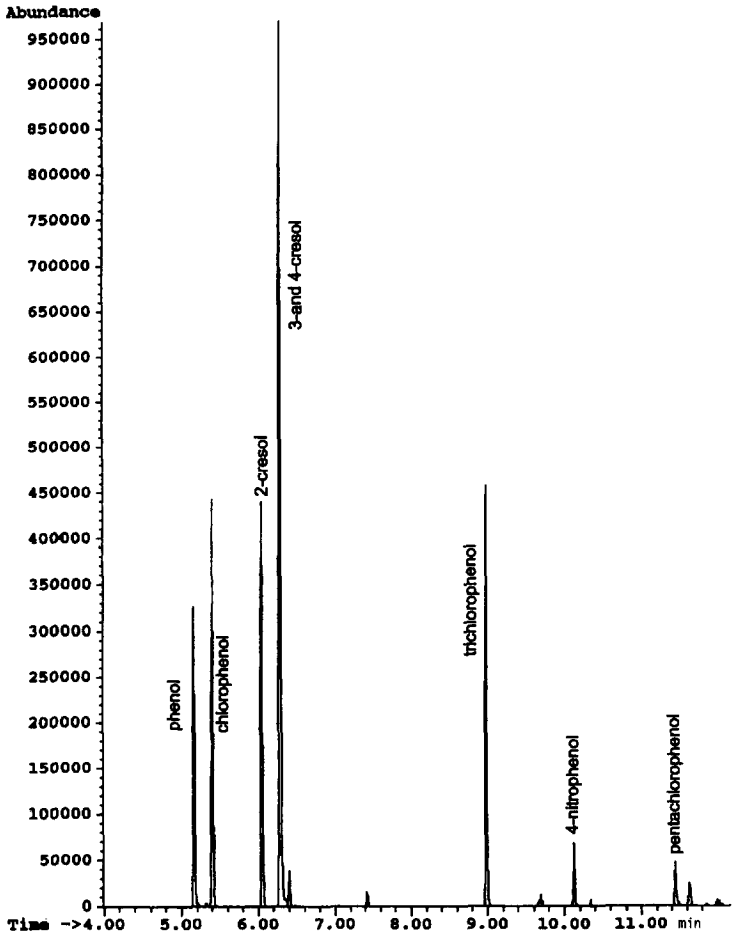


Fig. 1. GC–MS (TIC) of underivatised phenols.

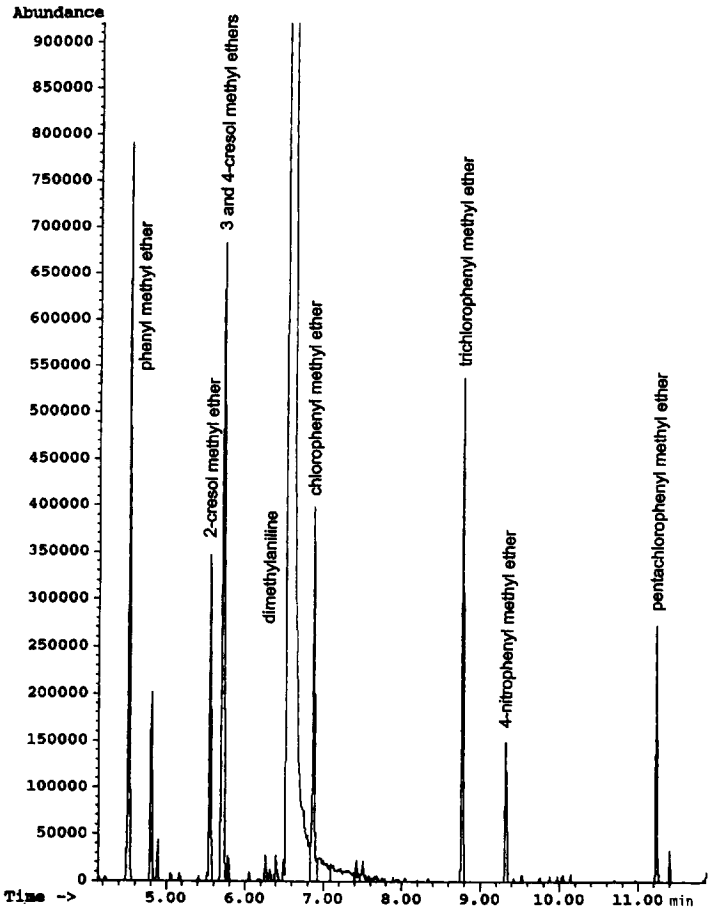


Fig. 2. GC–MS (TIC) of phenols (100 mg/kg) derivatised on-column with PTMA-F (10 m*M*).

Table 5
Methylation efficiencies of a mixture of phenols (70 mg/kg each) with 10 m*M* of different on-column derivatisation reagents

Analyte	TIC (cps×10^6)	TIC of derivative peak/TIC of parent compound peak			
	No on-column derivatisation agent	PTMA-F	TMSu-OH	TMSu-F	TMSuO-F
Phenol	44	1.33	1.23[a]	1.27[a]	0.05[a]
2-Cresol	63	1.62	1.00[a]	0.90[a]	ND
3- and 4-cresol[b]	155	1.23	0.80[a]	0.75[a]	ND
4-Nitrophenol	28	1.54	1.43	1.4	1.03
2,4-Dinitrophenol	6.4	4.53	ND	ND	ND
Chlorophenol	54	1.60	1.24	1.26	0.15[a]
Trichlorophenol	64	1.8	1.04	1.06	0.75
Pentachlorophenol	50	3.97	1.42	1.40	0.92

[a] Some underivatised parent phenol also detected. ND, not detected.
[b] 3- and 4-cresols and their methyl ethers not separated under chromatograghic conditions used.

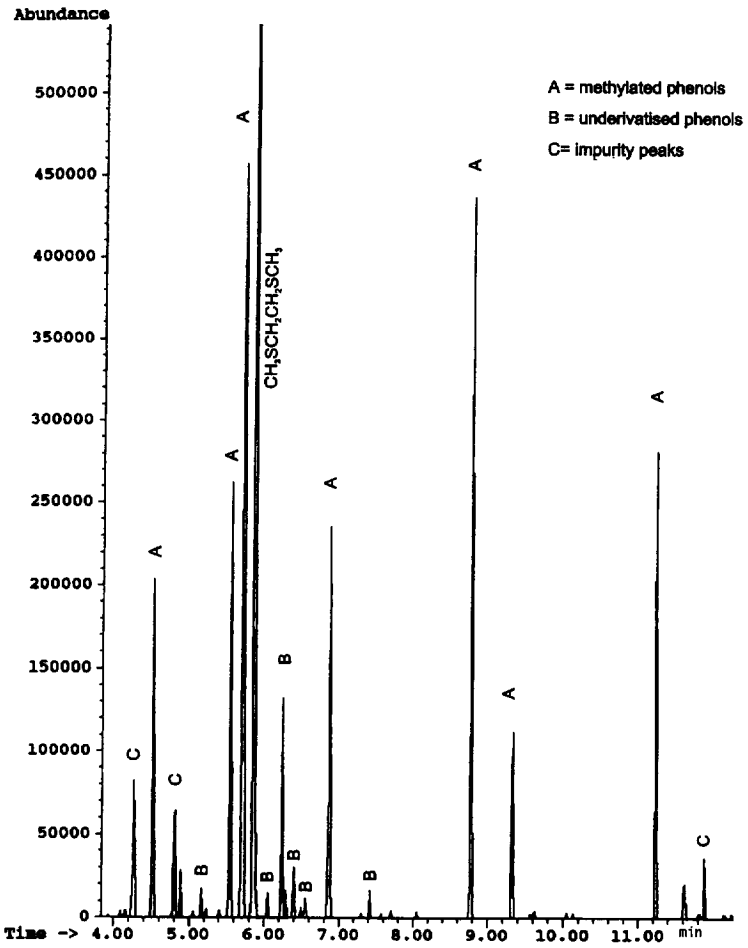


Fig. 3. GC–MS (TIC) of phenols (100 mg/kg) derivatised on-column with TMSu-F (10 m*M*).

methanol or dichloromethane–methanol cleanly produced phenol and cresol derivatives with a molecular mass 32 above the parent. The fragmentation patterns of these derivatives in the mass spectrum was parallel to those obtained for simple methylation products. This observation may be explained by O methylation being accompanied by a concurrent Friedel–Crafts type C methylation of the activated aromatic ring in the GC injection port by this reagent during on-column derivatisation.

Nevertheless, TMA-F appears to be a promising reagent for routine on-column derivatisations under conditions which do not adversely affect column lifetime.

3.2.2. Phenyl methyl ethers from trimethylsulfonium and trimethyloxysulfonium salts

The expected by-product of trimethylsulfonium salts, dimethyl sulfide, is extremely volatile and should cause no chromatographic interference in phenol methylation. The methylation efficiency of trimethylsulfonium salts was therefore investigated. The hydroxide, cyanide, fluoride and acetate were investigated as potential methylation reagents in this work. It was found that all four salts gave equally satisfactory methylation efficiency with the acetate and cyanide being slightly less efficient than the fluoride and hydroxide in the methylation of phenol and cresols.

The selectivity of various trimethylsulfonium salts was similar to that of the analogous PTMA salts. However, at the same reagent concentration trimethylsulfonium salts gave only half the methylation efficiency of their PTMA counterparts. Furthermore, whereas methylation of phenol and cresols is complete with PTMA salts, as exemplified by PTMA-F in Table 5, methylation proceeds to about 50% completion with trimethylsulfonium salts.

Not only was methylation efficiency lower with both these salts but a major by-product was formed which eluted in the same region as the combined methylated cresols at low concentrations (See Fig. 3). This by-product was identified as

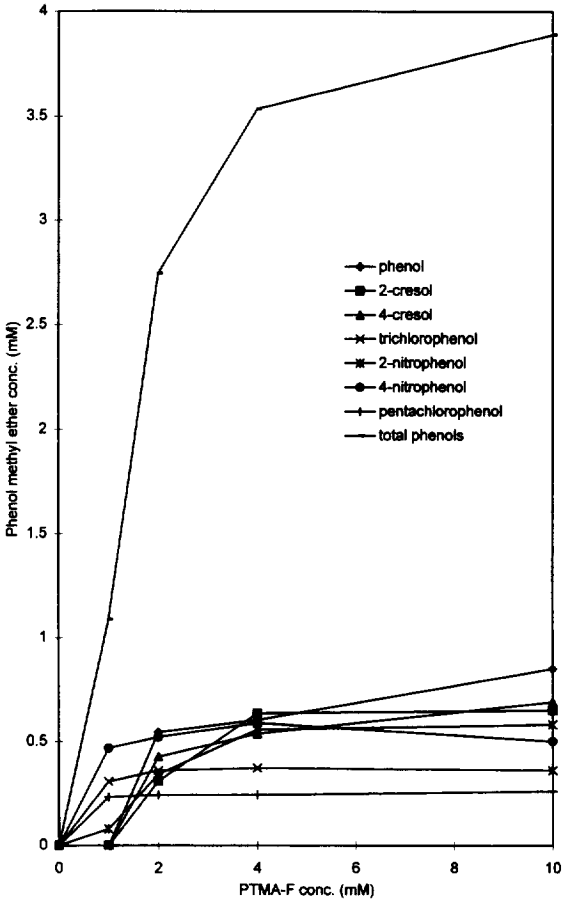


Fig. 4. On-column derivatisation of a mixture of seven phenols (total concentration 3.89 m*M*) with different concentrations of PTMA-F in methanol.

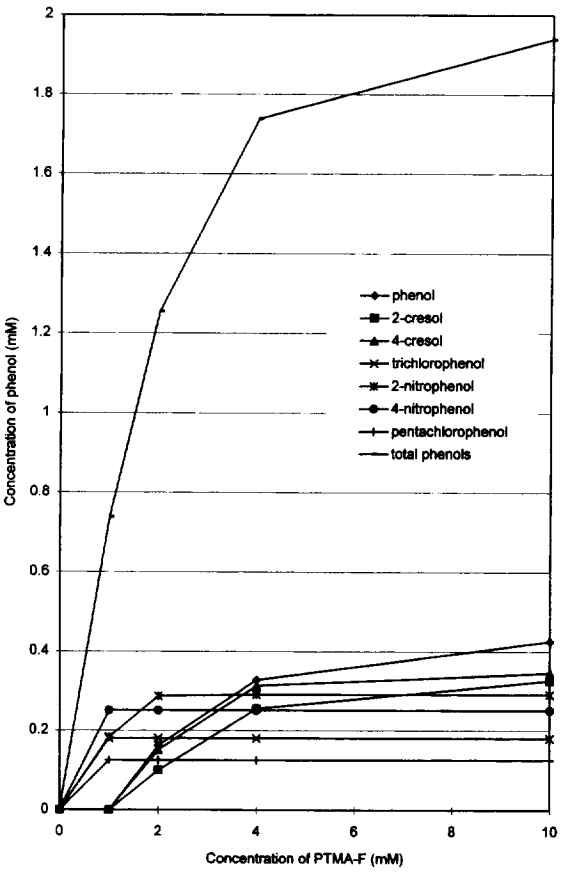


Fig. 5. On-column derivatisation of a mixture of seven phenols (total concentration 1.94 m*M*) with different concentrations of PTMA-F in methanol.

$CH_3SCH_2CH_2SCH_3$, formed by pyrolytic rearrangement of the on-column derivatisation reagents. In addition to $CH_3SCH_2CH_2SCH_3$, on-column methylation with TMSu-OAc gave a further by-product which was tentatively assigned the structure $CH_3SCH_2CH_2SCOCH_3$ on the basis of its mass spectrum. Unfortunately both these methylation by-products appeared in the centre of the GC trace of the methyl derivatives of the lower phenols making TMSu salts even less satisfactory than PTMA salts for interference free methylation of phenols. However it should be noted that all the PTMA salts or TMSu salts investigated in this study gave excellent derivatisation of tri- and pentachlorophenols and allowed detection of these analytes by GC at levels

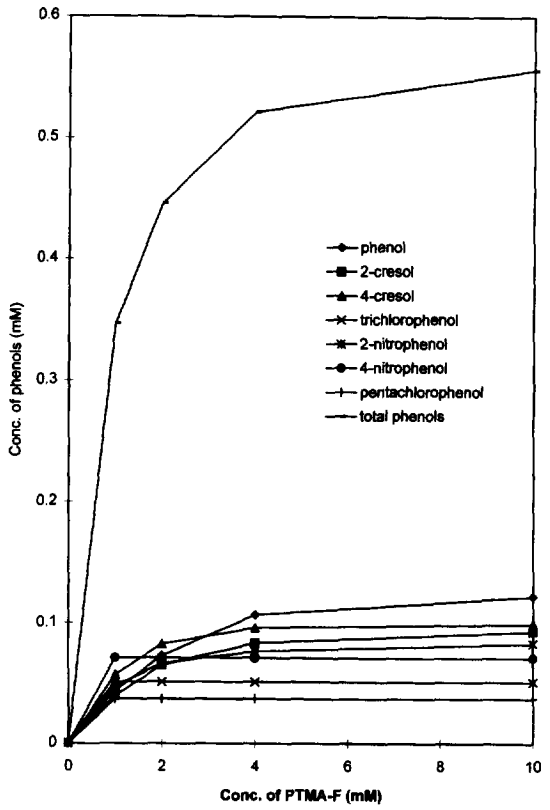


Fig. 6. On-column derivatisation of a mixture of seven phenols (total concentration 0.56 m*M*) with different concentrations of PTMA-F in methanol.

lower than could be attained with the free chlorophenols.

Alkylation of phenols with TMSuO-F proceeded with very low efficiency.

3.3. Differences in methylation efficiency for different phenols

The distinct differences between PTMA and TMSu salts in the methylation of phenols deserves mention. Methylation of less acidic phenols (phenol and cresols) is not complete even at high concentrations of TMSu salts whereas only a moderate molar excess of PTMA salts are required (Table 5). Fig. 4 Fig. 5 Fig. 6 show the methylation of three different concentrations of a standard phenol mixture with PTMA-F which show that: (1) Highly acidic phenols electronegative such as PCP and 4-nitrophenol are more efficiently methylated than less acidic phenols. (2) At high phenol and reagent concentrations only a 2.5-fold molar excess of reagent is required to secure complete methylation of both acidic and less acidic phenols. At lower phenol concentrations a higher molar excess of reagent is required.

The data presented in Figs. 4–6 may be explained by the relative strengths of ion-pair formation between the phenol and the cationic derivatising agent which approximate the relative affinity of the same phenols for ion-exchange resins. Those phenols such as PCP which form very strong ion pairs undergo essentially unimolecular decomposition in the injector port to form methyl ethers and little excess of on-column derivarisation reagent is required. As ion-pair stability decreases, more derivatisation reagent is needed to secure complete methylation.

It would appear that TMSu salts form weaker ion pairs with phenols than PTMA salts.

Table 6
Methylation efficiencies of a mixture of fatty acids (100 mg/kg each) with 10 m*M* of different on-column derivatisation reagents

Methylation reagent	Fatty acid (% yield)				
	C_{12}	C_{14}	C_{16}	C_{18}	Av.
MethElute (PTMA-OH)	77	92	98	89	89
Methprep-II	69	78	82	72	75
PTMA-F	86	102	110	102	100
PTMA-CN	77	84	97	89	87
TMSu-OH	57	68	75	64	66
TMSu-CN	84	97	105	95	95
TMSu-F	76	91	99	94	90
TMSuO-OH	47	60	65	62	59
TMA-F	69	82	89	84	81

All yields are calculated as a percentage using $C_{17}H_{35}COOCH_3$ as internal standard.

Simple aliphatic acids have less affinity for ion-exchange resin than do phenols and therefore different methylation reagents would be expected to show less differences in reagent efficiency for carboxylic acids. Data in Table 6 demonstrates the validity of this prediction. We have also shown that a series of new on-column benzylation reagents have similar concentration-dependent efficiency differences between various analyte types [9].

3.4. Methylation of fatty acids

As a guide to the relative methylation efficiencies of reagents discussed above, the on-column methylation of a mixture of fatty acids was investigated under standard conditions. The results, shown in Table 6, indicate that PTMA or TMSu salts are suitable with PTMA-F being the reagent of choice. However, overall there is less difference in methylation efficiency between PTMA and TMSu salts with fatty acids than with phenols, with all reagents tested giving greater than 50% the yield of methyl esters.

3.5. Column stability with the use of various on-column derivatisation reagents

During this study a steady decrease in column efficiency was noted and it was found that it was the use of TMSu-OH which caused the greatest deterioration although PTMA-OH also had a deleterious effect on column stability. In subsequent work with a new column it was found that phenyltrimethylammonium cyanide, fluoride and acetate or trimethylsulfonium cyanide, fluoride and acetate had no observable detrimental effects on column performance over a period of five weeks of constant use.

4. Conclusions

MethElute and Methprep are perfectly satisfactory in the derivatisation of mono-functional compounds but produce mixtures, often very complex, when used for methylation of multi-functional substances. Also these reagents are highly basic and lead to degradation of capillary GC columns.

It has been demonstrated that judicious choice of the derivatisation reagent used for direct on-column methylations can have a profound effect on the products produced, often with little effect on the overall methylation efficiency of the process. A survey of various reagents has suggested that, for general routine use, PTMA-F is the best choice. It is as efficient as MethElute and Methprep but without causing loss of column efficiency with prolonged use.

When methylation selectivity is required, PTMA-OAc provides a stable, efficient and preferable alternative to the previously reported PTMA-CN.

Acknowledgments

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