ELSEVIER

Journal of Chromatography A, 738 (1996) 57–72

JOURNAL OF CHROMATOGRAPHY A

Direct on-column derivatisation in gas chromatography III. On-column benzylation reagents and the development of 3,5-bis(trifluoromethyl)benzyl-dimethylphenylammonium fluoride, an efficient new on-column derivatisation reagent

Masooma Amijee, Julie Cheung, Robert J. Wells*

Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, NSW 2073, Australia

Received 21 November 1995; revised 19 January 1996; accepted 19 January 1996

Abstract

Direct on-column methylation using methylammonium or sulfonium salts is well established. It has now been demonstrated that benzylammonium salts are effective derivatisation reagents for direct on-column benzylations of acids and phenols. They are particularly useful for the derivatisation of lower aliphatic acids where methyl derivatives are too volatile for convenient GC determination and for simple phenols were reagent by-products from on-column methylation interfere with determination of cresols. 3,5-Bis(trifluoromethyl)benzyldimethylphenylammonium fluoride is a readily prepared and highly efficient new reagent for direct on-column derivatisation in GC and GC–MS. It produces derivatives with phenols which are not only easily detected by GC with electron-capture detection but which also give a total ion current 1.5–3-times higher than the corresponding methyl derivatives in the GC–MS of phenols and acids.

Keywords: Derivatization, GC; Benzylation; Phenols; Bis(trifluoromethyl)benzyldimethylphenylammonium fluoride; Carboxylic acids; Chlorophenols; Nitrophenols

1. Introduction

On-column alkylation using either tetraalkylammonium salts [1–5] or trialkylsulfonium salts [6,7] as an alkylation reagent is a commonly used method for derivatising acidic substances for gas chromatographic 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 chromatograph 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.

The predominant alkylation target for direct on-column methods is methylation but higher alkyl derivatives have been successfully prepared as evidenced by the determination of thiocyanate [8] and alkylsulfates [9] as the butyl esters and theophylline as its ethyl derivative [10] by use of the appropriate symmetrically substituted tetraalkylammonium or trialkylsulfonium salt, respectively.

In Parts I and II of this series of studies, we compared the methylation efficiency and selectivity of a number of different phenyltrimethylammonium, trimethylsulfonium and trimethyloxysulfonium salts

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00088-X

in the direct on-column methylation of a series of acids and phenols, as well as some sulfonamides, benzimidazoles and thiouracils used in animal production [11,12]. In those studies it was found that phenyltrimethylammonium salts were generally more efficient on-column methylating reagents than trimethylsulfonium and trimethyloxysulfonium salts, particularly for many weakly acidic substances. Thus, methylation of the strongly acidic pentachlorophenol was easily achieved with a moderate molar excess of most on-column derivatising agents tested, whereas phenol and the cresols, which are more weakly acidic, were only derivatised completely by phenyltrimethylammonium hydroxide and fluoride. On-column methylation of multifunctional substances, such as sulfonamides and benzimidazoles, with aggressive methylation reagents like phenyltrimethylammonium hydroxide or fluoride resulted in the formation of a complex mixture of products. However, change of the anion of the methylation reagent to either cyanide or acetate furnished alternative derivatisation reagents which were less aggresive, resulting in greater methylation selectivity while retaining excellent efficiency.

Although phenyltrimethylammonium salts have proved efficient on-column derivatisation reagents, they have serious drawbacks in certain applications. Thus, although on-column methylations of phenols with phenyltrimethylammonium salts [12] were efficient, they also resulted in the formation of dimethylaniline as a by-product of the methylation process which caused interference with the quantitation of simple phenyl methyl ethers at low levels. Trimethylsulfonium salts yielded, somewhat unexpectedly, a different interfering by-product, identified as $CH_3SCH_2CH_2SCH_3$, which also eluted close to the combined methylated cresols. Another area of analysis where derivatisation with phenyltrimethylammonium salts is not practicable is in the analysis of volatile fatty acids which would yield, after methylation, even more volatile methyl esters unsuitable for quantitation by routinely employed methods of GC analysis. In this case, a derivative is required which has good chromatographic properties within the readily accessible temperature range of the gas chromatograph.

We report here the development of the new direct on-column derivatisation reagents which yield benzyl and substituted derivatives which have proved complementary and, in some cases, distinctly superior to methyl derivatives for the GC determination of some classes of analytes.

2. Experimental

2.1. Reagents

3,5-Bis(trifluoromethyl)benzyl bromide, 3,5-bis-(trifluoromethyl)benzyl chloride, pentafluoromethylbenzyl bromide, benzyl chloride, triethylamine, dimethylamine hydrochloride and dimethylaniline were purchased from Aldrich (Milwaukee, WI, USA).

Ion-exchange resins were chromatographic 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 sodium fluoride solution followed by 100 ml of deionised water and 100 ml of methanol. The resin was stored in an amber bottle in methanol.

2.1.1. *3,5-Bis(trifluoromethyl)benzyldimethylphenylammonium bromide*

A mixture of 3,5-bis(trifluoromethyl)benzyl bromide (0.75 g) and freshly distilled dimethylaniline (0.6 g) was allowed to stand at 30°C for 24 h, the resulting crystalline mass was triturated with diethyl ether, filtered under vacuum and the pale buff crystals washed on the filter with a further 10 ml ether and dried to yield 1.08 g product. This was recrystallised by dissolution in 6 ml acetonitrile at 60°C, addition of boiling ethyl acetate (20 ml) and cooling to room temperature. Filtration under vacuum followed by washing with a little ethyl acetate and drying yielded 0.96 g colourless crystals m.p. 166–167°C. Prolonged heating of 3,5-bis(trifluoromethyl)benzyl bromide and dimethylaniline during the preparation of the quaternary ammonium salt or heating solutions of the salt in a polar solvent at or above 60°C resulted in the gradual formation of deep purple by-products and a significant lowering of product yield and purity.

2.1.2. 3,5-Bis(trifluoromethyl)benzyldimethylphenylammonium chloride

A mixture 3,5-bis(trifluoromethyl)benzyl chloride (2.0 g) and freshly distilled dimethylaniline (1.6 g) was allowed to stand at 30°C for 10 days and the resulting crystalline mass worked up in the same manner as for the bromide above to yield 0.46 g colourless crystals m.p. 167–169°C.

2.1.3. Pentafluorobenzyldimethylphenylammonium bromide

A mixture of pentafluorobenzyl bromide (2.0 g) and freshly distilled dimethylaniline (1.6 g) was allowed to stand at 30°C for 24 h, the resulting crystalline mass was worked up in the same manner as for the bromide above to yield 0.46 g colourless crystals m.p. 152–154°C. Heating of pentafluorobenzyl bromide and dimethylaniline during the preparation of the quaternary ammonium salt or heating solutions of the salt in a polar solvent at or above 60°C resulted in the rapid formation of deep purple by-products and a drastic lowering of product yield and purity.

2.1.4. Benzyldimethylphenylammonium chloride

A mixture of benzyl chloride (5 g) freshly distilled dimethylaniline (4 g) was heated at 70°C for 24 h, the resulting crystalline mass was worked up in the same manner to other substances described above, except that boiling acetonitrile was employed for recrystallisation which yielded 4.6 g colourless crystals m.p. 87–88°C.

2.1.5. 3,5-Bis(trifluoromethyl)benzyltrimethylammonium bromide

A mixture of 3,5-bis(trifluoromethyl)benzyl bromide (2 g) in 10 ml of a 10% methanolic solution of trimethylamine (prepared by addition of the theoretical amount of sodium methoxide in methanol to a solution of trimethylamine hydrochloride in methanol, cooling to −18°C and filtering from precipitated sodium chloride) was allowed to stand at room temperature overnight, diluted with diethyl ether (50 ml) and the product filtered under vacuum. Recrystallisation from acetonitrile–ethyl acetate (1:2) gave the purified product as colourless crystals (1.6 g) m.p. 208–210°C.

2.1.6. Pentafluorobenzyltrimethylammonium bromide

Prepared from pentafluorobenzyl bromide (1 g) and 5 ml of 10% trimethylamine in methanol by the same method as the 3,5-bis(trifluoromethyl)benzyl derivative above. Yield 0.78 g, m.p. 230–234°C.

2.1.7. 3,5-bis(trifluoromethyl)benzylltriethylammonium bromide

This salt was prepared 3,5-bis(trifluoromethyl)benzyl bromide (2 g) and 10% solution of triethylamine in methanol in a similar manner to the trimethylammonium salt. Recrystallisation from acetonitrile–ethyl acetate (1:1) gave the purified product as colourless crystals (1.8 g) m.p. 130–132°C.

2.1.8. Pentafluorobenzyltriethylammonium bromide

Prepared from pentafluorobenzyl bromide (1 g) and 5 ml of 10% triethylamine in methanol by the same method as the 3,5-bis(trifluoromethyl)benzyl derivative above. Yield 1.0 g, m.p. 67–69°C.

2.1.9. 3,5-Bis(trifluoromethyl)benzyldimethylphenylammonium fluoride

A chromatographic column was prepared from 5 ml of AGMP anion-exchange resin (63–150 μm, fluoride form) in methanol. The column was then washed with methanol (2 ml) and a solution of 3,5-bis(trifluoromethyl)benzyldimethylphenylammonium bromide (0.84 g in 4 ml methanol, 2 m*M* 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 calcium chloride. When all the quaternary derivative had been eluted, enough methanol was added to the combined fractions to bring the total volume to 10 ml (0.2 *M*). This stock solution was used for on-column derivatisation experiments which were usually conducted with the derivatisation reagent at a concentration of 10 m*M*.

2.1.10. Other quaternary ammonium fluorides

The following reagents were prepared by the same method using the appropriate quaternary ammonium bromide or chloride: Benzyldimethylphenylammonium fluoride, 3,5-bis(trifluoromethyl)benzyltriethylammonium fluoride, 3,5-bis(trifluoromethyl)-

benzyltrimethylammonium fluoride, pentafluorobenzyldimethylphenylammonium fluoride, pentafluorobenzyltriethylammonium fluoride and pentafluorobenzyltrimethylammonium fluoride.

2.2. Standards

Phenols and carboxylic acids were the purest grade available from Aldrich.

2.3. On-column derivatisation studies

Unless otherwise stated, on-column derivatisations were investigated by injection of a mixture of a solution of the analyte (70 or 100 mg/kg) 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 10:1.

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 either 20:1. 5:1 or 4:1.

3. Results and discussion

3.1. Derivatisation of phenols

3.1.1. Development of appropriate on-column benzylation reagents

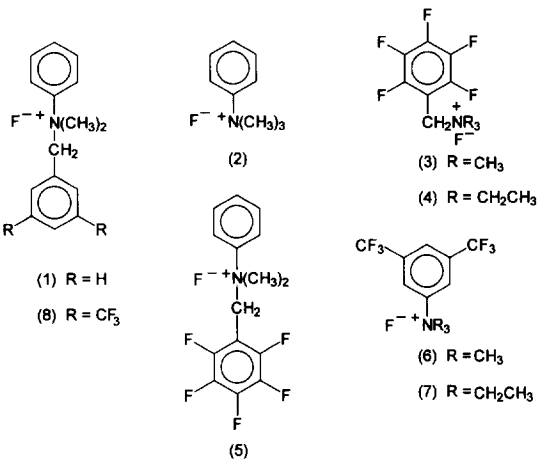
We previously demonstrated that phenyltrimethylammonium fluoride was an efficient non-corrosive alternative to the corresponding hydroxide (Meth-Elute) for direct on-column methylations of acids and phenols [11,12]. Furthermore, the change of quaternary ammonium counter-ion from fluoride to acetate or cyanide imparted methylation selectivity during the derivatisation of multifunctional compounds with little loss in methylation efficiency.

We have now investigated the feasibility of developing new on-column derivatisation reagents in which selectivity of alkyl transfer to an acidic host

could be obtained from a quaternary ammonium salt containing two or more different alkyl substituents.

It was anticipated that the far greater stability of a benzyl carbonium ion over methyl or phenyl carbonium ions would result in the preferential transfer of a benzyl group rather that a methyl or phenyl group during the thermal decomposition of a quaternary ammonium salt in which these groups were present. Therefore, the use of a quaternary ammonium salt such as benzyldimethylphenylammonium fluoride as a direct on-column derivatisation reagent for acids and phenols should result in the formation of benzyl esters and ethers in preference to methylated derivatives.

Table 1 shows that this expectation was fully realised. Indeed, benzyldimethylphenylammonium fluoride (BDMA-F, **1** in Scheme 1) exclusively and efficiently produced benzyl ethers when used for the on-column derivatisation of phenols. The benzyl derivatives of phenols were particularly interesting in that they produced a greater total ion current in the GC–MS than the corresponding methyl derivatives. A further advantage was that the derivatives of 3- and 4-cresol were completely separated on a non-polar capillary column which was unable to resolve either the parent 3- and 4-cresols or their methyl ethers. Comparison between alkylation of phenols



Scheme. 1.

with (BDMA-F) to that with phenyltrimethylammonium fluoride (PTMA-F, **2** in Scheme 1), a neutral on-column methylation reagent shown previously [11] to be as efficient as MethElute, is shown in Table 1. Whereas methylation of 9 phenols (total concentration=3.9 m*M*) with PTMA-F (10 m*M*) gave apparently complete conversion to methyl ethers (no parent phenols detectable by GC–MS), some parent phenols could still be detected when 10 m*M* of BDMA-F was used and alkylation was

Table 1
Comparison of the derivatisation of nine phenols (total concentration=3.9 m*M*) with four on-column derivatisation reagents (concentration=10 m*M*)

Analyte	On-column derivatisation reagent				
	None TIC (cps×10^6)	PTMA-F	BDMA-F	BTBDMA-F	BTBTA-F
		(Ratio of the TIC of derivative peak/TIC of parent compound peak)			
Phenol	44	1.33	2.7[a]	5.25	3.0
2-Cresol	63	1.62	1.32[a]	3.12	1.71
3-Cresol	155[b]	1.23[b]	0.92[a,c]	2.3[c]	1.4[c]
4-Cresol			1.17[a,c]	2.5[c]	1.7[c]
4-Nitrophenol	28	1.54	4.6	4.64	4.1
2,4-Dinitrophenol	6.4	4.53	9.38	10.94	
Chlorophenol	54	1.60	2.81	3.70	
Trichlorophenol	64	1.8	2.30	2.66	
Pentachlorophenol	50	1.97	1.84	3.4	

[a] Some underivatised parent phenol also detected.
[b] 3- and 4-Cresols and their methyl ethers not separated under chomatograghic conditions used.
[c] Benzyl derivatives of cresols well separated. Response factors of derivatives relative to underivatised cresol calculated assuming that the TICs for 3- and 4-cresol were equal and that the TICs for their derivatives were also equal.

incomplete for the less acidic phenols such as phenol and the cresols. However, the total ion current values for the benzyl derivative of each phenol were significantly greater than those of the corresponding methyl derivatives.

The concept of selective benzyl transfer was extended to a search for a derivatisation reagent that produced derivatives which retained the good GC separation characteristics of simple benzyl compounds coupled with enhanced sensitively to both electron-capture detection (ECD) and mass spectrometric (MS) detection and quantitation. Several quaternary ammonium salts were prepared and investigated as on-column derivatisation reagents in this work. These contained either the pentafluorobenzyl group or the 3,5-bis(trifluoromethyl)benzyl group as quaternary derivatives of dimethylanilne, trimethylamine and triethylamine, respectively.

The pentafluorobenzyl group was particularly attractive as an on-column derivatising reagent because of the great sensitivity of pentafluorobenzyl derivatives to ECD coupled with the formation of the pentafluorobenzyl ion in the MS at m/z 181. However, the use of pentafluorobenzyltrimethylammonium fluoride (**3** in Scheme 1), pentafluorobenzyltriethylammonium fluoride (**4** in Scheme 1) and dimethylpentafluorobenzylphenyl-ammonium fluoride (**5** in Scheme 1) as direct on-column derivatising reagents resulted in the formation of a plethora of reagent by-products together with the low yield formation of the required benzyl derivatives.

By contrast, investigation of the corresponding 3,5-bis(trifluoromethyl)benzyl quaternary ammonium fluorides produced two reagents of distinct promise as efficient on-column derivatising reagents.

3.1.2. Relative merits of different 3,5-bis(trifluoromethyl)benzyl quaternary ammonium fluorides as on-column derivatising reagents for phenols

The relative effectiveness of 3,5-bis(trifluoromethyl)benzyltrimethylammonium fluoride (**6** in Scheme 1), 3,5-bis-(trifluoromethyl)benzyltriethylammonium fluoride (**7** in Scheme 1) and 3,5-bis(trifluoromethyl)benzyldimethylphenyl - ammonium fluoride (**8** in Scheme 1) as on-column derivatisation reagents were compared.

Although 3,5-bis(trifluoromethyl)benzyl bromide reacted smoothly and rapidly with trimethylamine and triethylamine to form highly crystalline and readily purified quaternary bromide salts, reaction with dimethylaniline in polar solvents tended to give coloured products. This tendency was particularly evident at temperatures above 60°C when deep purple by-products were gradually formed with a concomitant lowering of the yield and difficulty in purification of the required quaternary bromide. A high yield of the bromide was obtained, however, by storage of a mixture of 3,5-bis(trifluoromethyl)benzyl bromide and a small molar excess of dimethylaniline at about 30°C for 24 h followed by recrystallisation of the resultant crystalline mass from acetonitrile–ether.

Table 2
Total ion current monitoring of a mixed phenols standard after on-column derivatisation with 10 m*M* BTBDMA-F, and 10 m*M* and 20 m*M* BTBTA-F

Analyte	t_R (min)	Total ion current (cps/10^6)		
		BTBDMA-F (10 m*M*)	BTBTA-F (10 m*M*)	BTBTA-F (20 m*M*)
Phenol	9.54	89.7	23.3	55.1
Chlorophenol	10.47	126.8	62.9	92.8
o-Cresol	9.89	66.0	13.8	36.2
m-Cresol	9.99	76.3	22.9	49.4
p-Cresol	10.06	113.2	31.2	67.7
Trichlorophenol	11.5	142.6	103.1	109.7
4-Nitrophenol	12.14	100.0	64.3	67.7
Pentachlorophenol	13.22	82.0	56.5	61.0
2,4-Dinitrophenol	13.4	44.0	21.9	23.7

3,5-Bis-(trifluoromethyl)benzyltriethylammonium fluoride was an inefficient benzylating agent. While moderate to good conversions of phenols to their 3,5-bis(trifluoromethyl)benzyl ethers were obtained using relatively high concentrations (50–100 m*M*) of the fluoride in on-column derivatisation studies, the major reaction pathway was the thermal decomposition of the reagent to 3,5-bis-(trifluoromethyl)-benzyldiethylamine and, presumably, ethylene. This product was identified by its mass spectrum which showed a molecular ion at *m/z* 300 and a very strong parent ion at *m/z* 227.

In contrast, 3,5-bis(trifluoromethyl)benzyl-trimethylammonium fluoride (BTBTA-F) proved to be an effective and efficient on-column derivatisation reagent. Table 1 compares the on-column derivatisation of nine phenols with four different on-column alkylation reagents. BTBTA-F produced good yields of the corresponding benzyl ethers and none of the underivatised parent phenol could be detected. By comparison, phenyltrimethylammonium fluoride (PTMA-F), when used at the same concentration also showed no detectable parent phenols but the methyl derivatives formed gave total ion current values which were significantly less for each phenol than those obtained with BTBTA-F. However, comparison of the derivatisation efficiency of BTBTA-F with that of 3,5-bis(trifluoromethyl)benzyldimethylphenyl-ammonium fluoride (BTBDMA-F) showed that the latter reagent was even more efficient as an on-column derivatisation reagent and gave total ion current values 1.5–2-times higher than those obtained with BTBTA-F.

Data shown in Table 2 demonstrates that BTBDMA-F is a more efficient derivatising agent than the more readily prepared BTBTA-F. Thus every phenol of a 100 mg/kg standard phenol mixture is more efficiently alkylated with 10 m*M* of BTBDMA-F than with either 10 or 20 m*M* of BTBTA-F. However, BTBTA-F is an excellent on-column derivatising reagent for routine GC–MS use, at least equalling MethElute in alkylation efficiency, and gives fewer potential reagent interferences than BTBDMA-F in some applications. The GC–MS total ion current (TIC) profiles of products from on-column derivatisation of phenols at 10 mg/kg with 10 m*M* BTBDMA-F and BTBTA-F are shown in Fig. 1 and Fig. 2. A reagent impurity is present in the BTBDMA-F profile (Fig. 1) which, in this case, does not interfere with the overall analysis.

3.1.3. Efficiency of alkylation with 3,5-bis(trifluoromethyl)benzylammonium salts

GC–MS analysis of 3,5-bis(trifluoromethyl)benzyl ethers of phenols (Fig. 1), formed on-column with BTBDMA-F, produced total ion current values for each of the separate alkylated product which were considerably higher than those produced by analogous methyl ether formed from PTMA-F run under identical conditions (Fig. 3). Furthermore, much of this ion current was accounted for by a dominant *m/z* 227 fragment ion, thus making these derivatives much more sensitive to detection by SIM monitoring than their methyl counterparts. Fig. 1 and Fig. 3 and Fig. 4 compare the GC–MS traces of a series of phenols run with BTBDMA-F as on-column benzylating agent, run with PTMA-F as on-column methylating agent and run without derivatisation, respectively. The total phenol concentration was the same in all cases. These figures clearly demonstrate that derivatisation of phenols allows lower detection limits, particularly for acidic phenols and that the 3,5-bis(trifluoromethyl)benzyl ethers of phenols are superior to the corresponding methyl ethers for this purpose. Fig. 5 and Fig. 6 show the mass spectra of the 3,5-bis(trifluoromethyl)-benzyl ethers of pentachlorophenol and 2,4-dinitrophenol, respectively.

The ability of BTBDMA-F to efficiently alkylate a 7-phenol mixture was investigated using approximate concentrations of 10, 35 and 70 mg/kg, respectively, for each phenol in the mixture and derivatisation reagent concentrations of 0.2, 1, 2, 4 and 10 m*M*. The results of this study are shown in Fig. 7 Fig. 8 Fig. 9.

From this data the following trends are apparent:

- The more acidic phenols such as pentachlorophenol, trichlorophenol and 2,4-dinitrophenol are akylated in preference to less acidic phenols.
- On-column derivatisation with BTBDMA-F in the presence of a molar excess of the phenol mixture results in 80–85% utilisation of the theoretical amount of BTBDMA-F in the alkylation of mixed

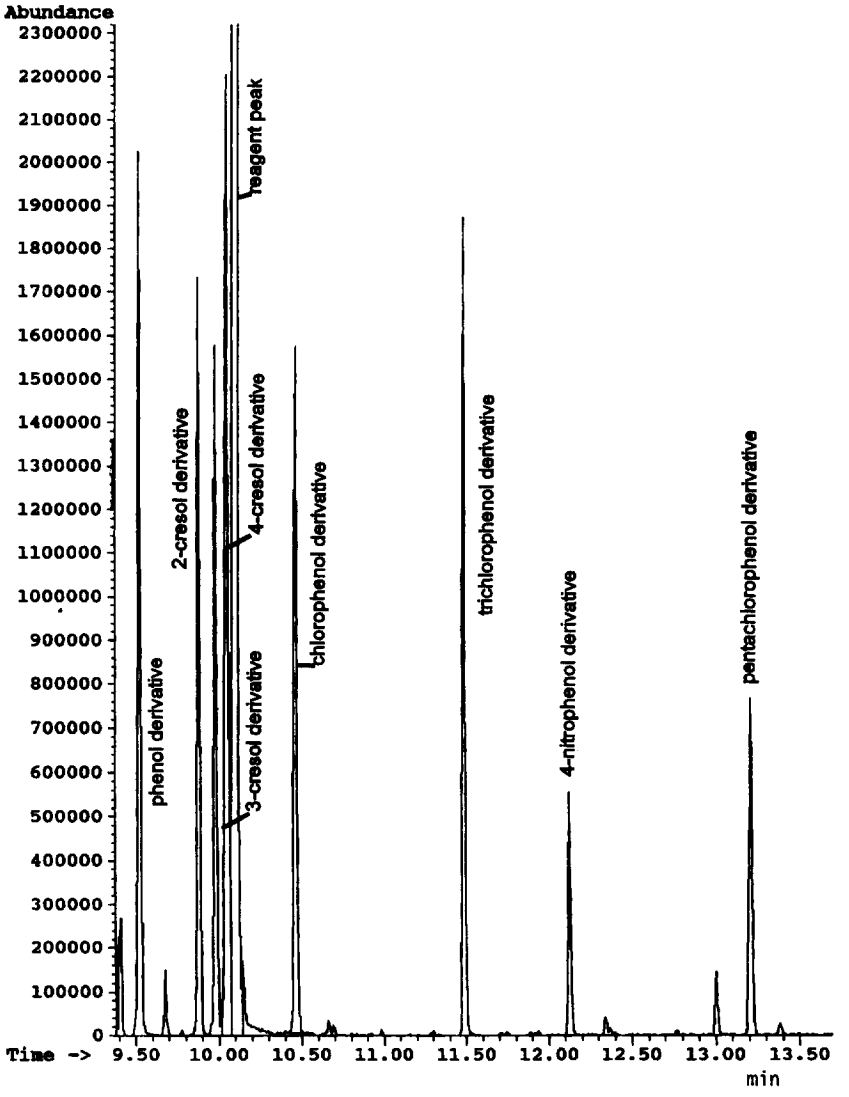


Fig. 1. GC–MS of eight phenols derivatised on-column with 10 mM BTBDMA-F in methanol.

phenols (e.g., alkylation of 3.9 m*M* combined phenols with 1 and 2 m*M* reagent, respectively, shown in Fig. 7).

- Complete alkylation of the 7-phenol mixture occurs with only a 3–6-fold theoretical excess of reagent (concentration dependent).
- Over 90% alkylation occurs with double the theoretical amount of reagent.

BTBDMA-F appears, therefore, to have the selectivity of a typical ion-exchanger for certain analytes. We reported earlier on the selectivity and methylation efficiency of phenyltrimethylammonium salts and obtained evidence that the quaternary salt counter ion had a profound effect on both the selectivity and efficiency of methylation [12]. The selectivity of strong cationic ion-exchange resins for various anions increases in the order OH<OAc<Cl~CN<Br< phenate<benzenesulfonate. In a mixture of phenols, those phenols, such as pentachlorophenol, which

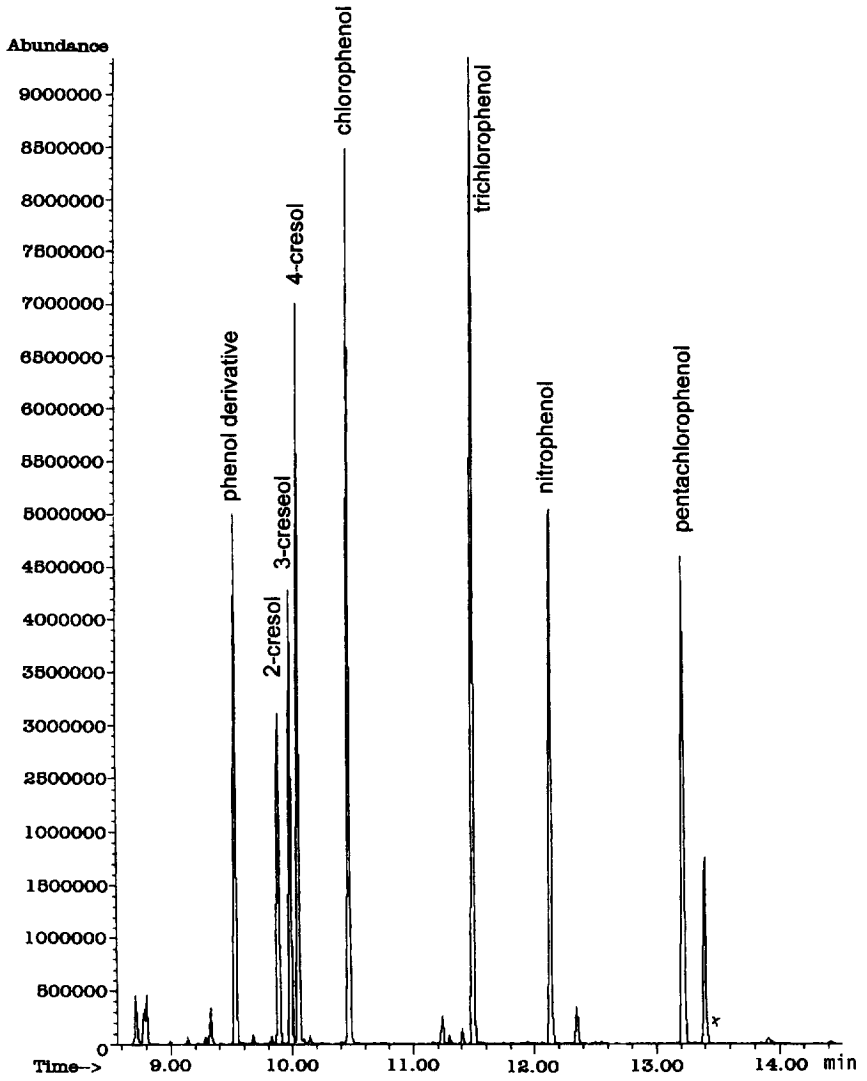


Fig. 2. GC–MS of eight phenols derivatised on-column with 10 mM BTBTA-F in methanol.

form a more strongly bound ion pair with the reagent were preferentially akylated by both BTBDMA-F and PTMA-F [12]. Furthermore, the phenols which form the most stable ion pairs with the reagent are completely alkylated by a very modest excess of BTBDMA-F, whereas less strongly bound phenols require a larger excess of BTBDMA-F to achieve complete on-column alkylation.

The selectivity of alkylation by BTBDMA salts could also be adjusted by choice of the salt counter-ion. Thus, the derivatisaton of a phenol mixture under otherwise identical conditions using 10 m*M* BTBDMA-Cl and BTBDMA-Br are shown in Table 3. Only the fluoride gave complete alkylation of the whole range of phenols in the standard mixture. The chloride was far less effective while pentachlorophenol alone was inefficiently alkylated when the bromide was used.

The modest theoretical excess of reagent required to achieve complete phenol alkylation suggests a that

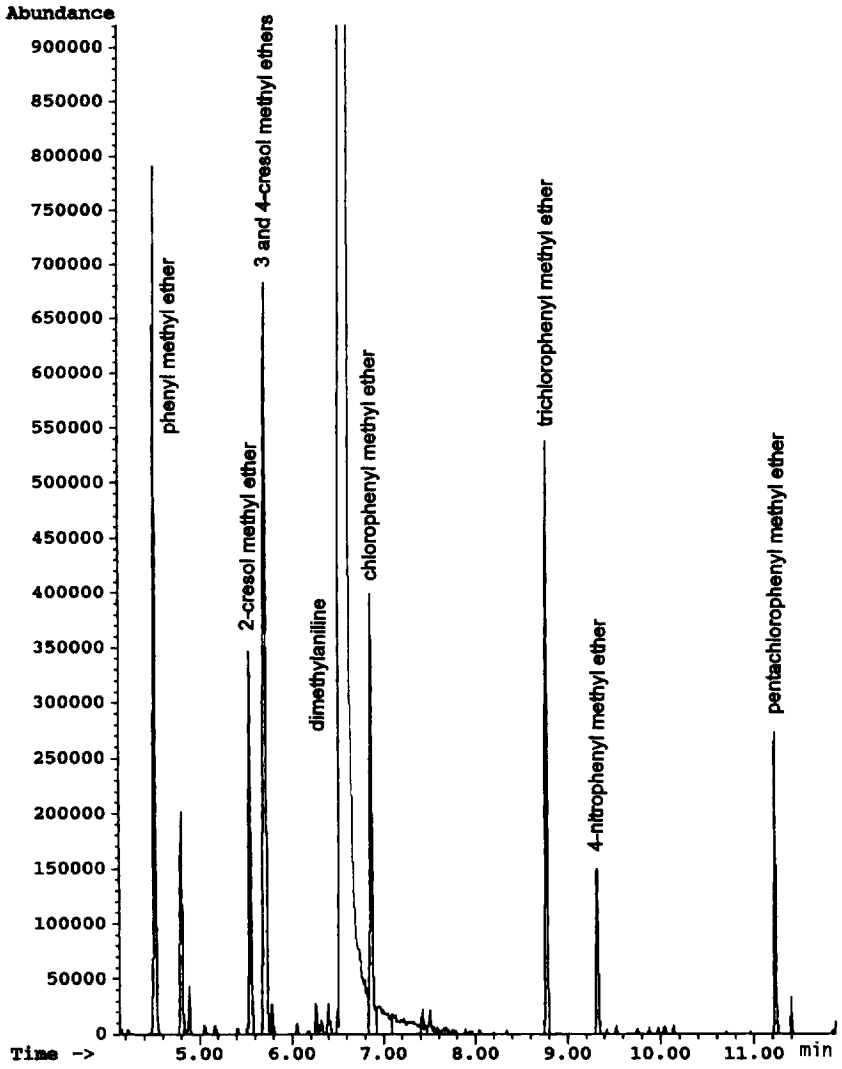


Fig. 3. GC–MS of eight phenols derivatised on-column with 10 mM PTMA-F in methanol.

the on-column thermal derivatisation is a unimolecular decomposition of a phenol-reagent ion pair which has significant stability in methanol solution.

3.2. Derivatisation of carboxylic acids

3.2.1. Higher fatty acids

As a guide to the relative efficiency of on-column methylation with that of 3,5-bis(trifluoromethyl)benzylation using BTBDMA-F and BTBTA-F, the on-column derivatisation of a standard mixture of fatty acids was investigated using methyl heptadecanoate as an internal standard. The results, shown in Table 4, indicate that there is little difference between the derivatisation efficiency of PTMA-F, BTBDMA-F or BTBTA-F. This contrasts with the experience with phenol derivatisation but supported the proposed mode of action of these on-column reagents, discussed earlier, in that fatty acids have much lower affinity than phenols for ion-exchange resins and, therefore, less selectivity would be expected between the use of different reagents. Thus not only does 3,5-bis(trifluoromethyl)benzylation offer no real advantages over methylation, either in

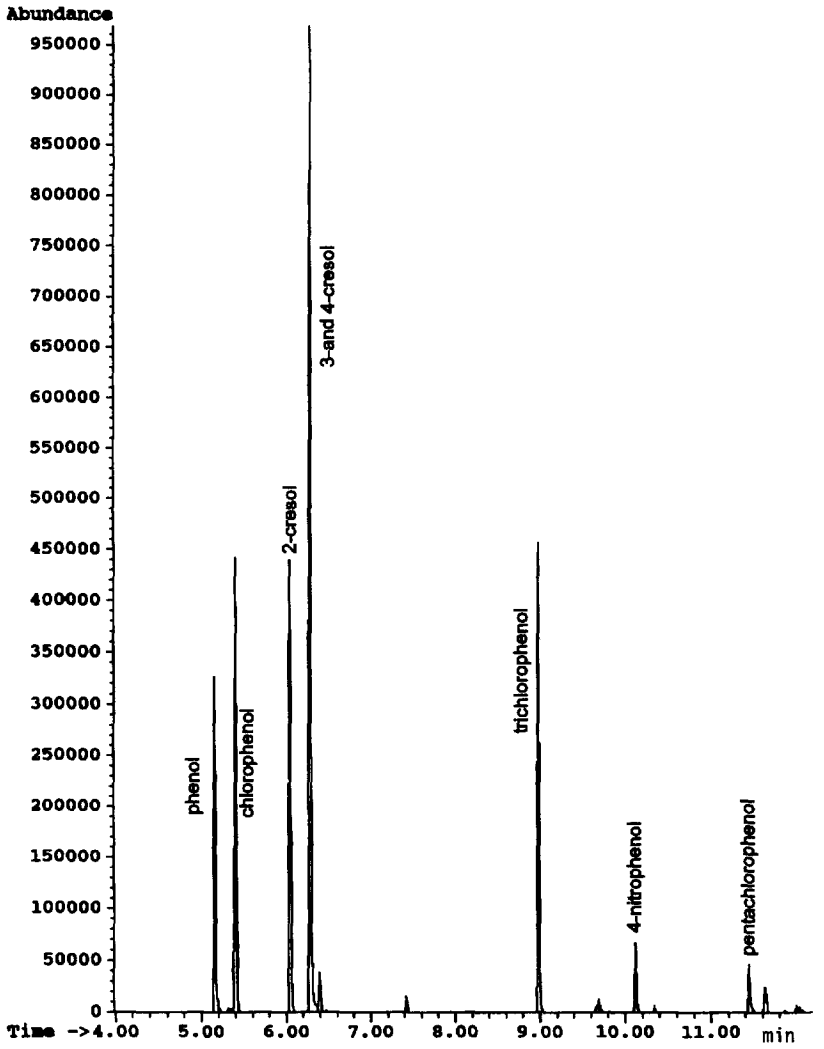


Fig. 4. GC–MS of eight phenols without derivatisation.

the total ion current or selective ion monitoring mode, for fatty acid derivatisation but BTBDMA-F proved no more effective than BTBTA-F as an on-column derivatisation reagent.

3.2.2. Volatile fatty acids

The methyl esters of volatile organic acids are entirely unsuitable for routine GC determination because of their volatility. However, 3,5-bis(trifluoromethyl)benzylation has proved useful for on-column derivatisation of these acids. Table 5 shows the results obtained on three volatile fatty acids using BTBDMA-F or BTBTA-F as on-column derivatisation reagents. Although the derivatisation of acetic, propionic and butyric acids proceeded satisfactorily with both reagents, BTBDMA-F was more than twice as efficient as BTBTA-F for the derivatisation of acetic acid. With propionic and butyric acids BTBDMA-F was also more effective than BTBTA-F, but the advantage was not as great.

The linearity and sensitivity of volatile acid derivatisation with BTBDMA-F was also investigated. Results shown in Table 6 demonstrate that ready detection of volatile acids as their 3,5-bis(trifluoro-

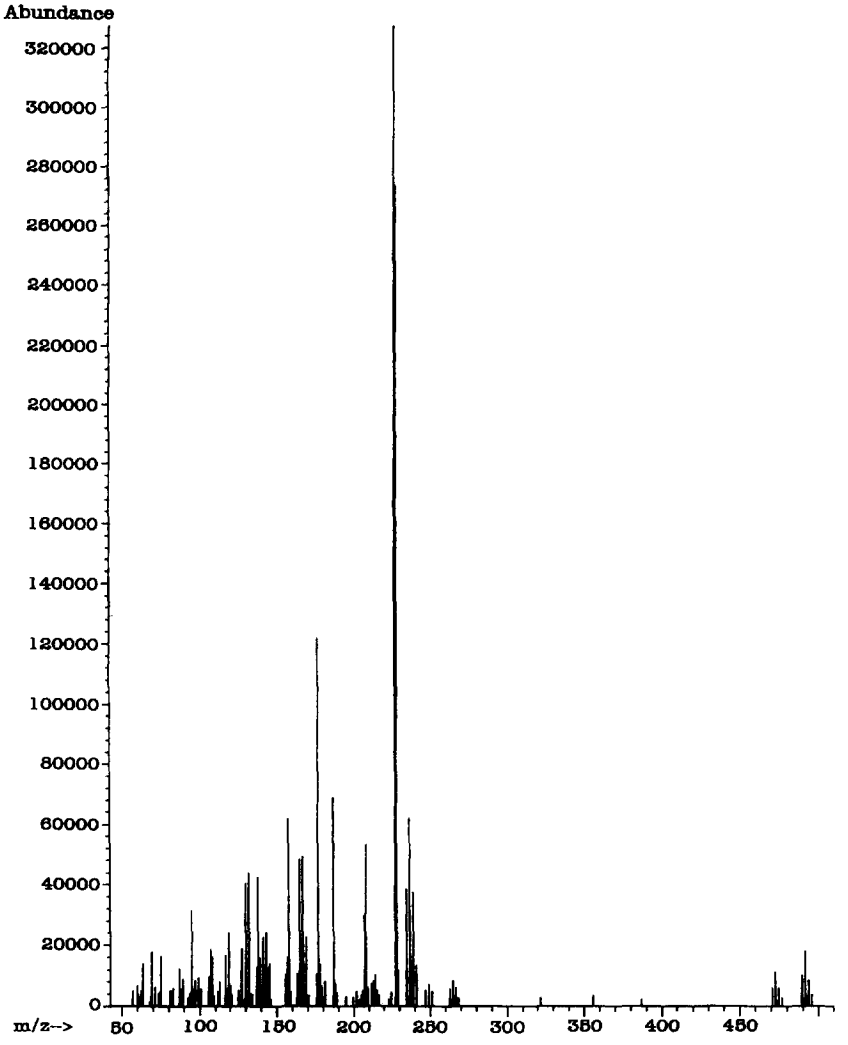


Fig. 5. Mass spectrum of bis(trifluoromethyl)benzyl pentachlorophenyl ether.

methyl)benzyl esters well below the 1 mg/kg level was attainable. Moreover, derivatisation of the acids was essentially linear with acid concentrations between 0.5 and 5 mg/kg.

3.2.3. *Miscellaneous organic carboxylic acids*

The derivatisation of a selection of organic acids with BTBDMA-F yielded 3,5-bis(trifluoromethyl)benzyl esters with high efficiency and with good chromatographic behaviour characteristics in GC and GC–MS. Table 4 shows data for chloroacetic, benzoic, sorbic, 2-(4-isobutylphenyl)propionic and salicylic acid. The conversion of all acids to their 3,5-bis(trifluoromethyl)benzyl esters with 10 m*M* BTBDMA is linear with acid concentrations up to 100 mg/kg with the exception of salicylic acid which can form both mono- and a disubstituted 3,5-bis(trifluoromethyl)benzyl derivatives. At higher salicylic acid concentrations of 50 and 95 mg/kg, a mixture of both mono- and di-3,5-bis(trifluoromethyl)benzyl derivatives were formed whereas at salicylic acid concentrations of 10 mg/kg and lower in the mixed acid standard, salicylic acid formed exclusively a di-substituted derivative and

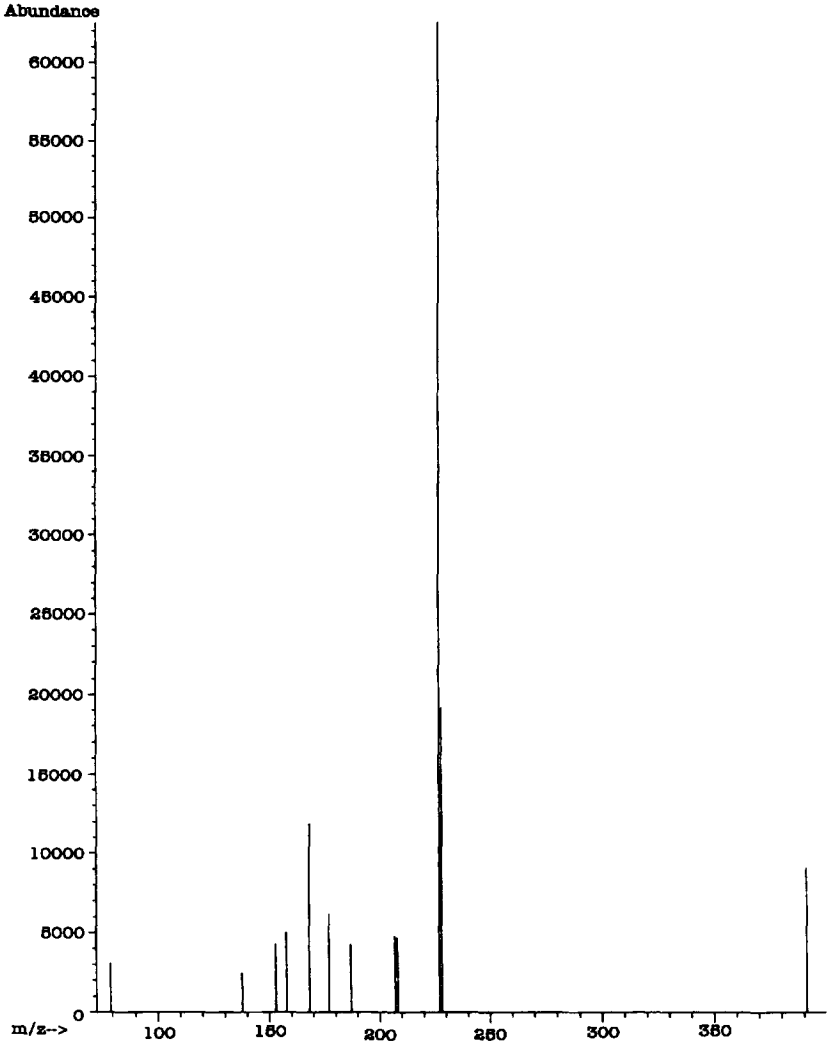


Fig. 6. Mass spectrum of bis(trifluoromethyl)benzyl-2,4-dimitrophenyl ether.

the detector response for the formation of this derivative was linear with salicylic acid concentrations between 0.5 and 10 mg/kg. Detector response for all acids except sorbic acid was linear between 1 and 10 mg/kg

4. Conclusions

It has been demonstrated that thermal decomposition of benzylmethylammonium salts in the presence of acids results in the exclusive transfer of the benzyl group with high yield formation of the benzyl derivative of the acid. This observation has led to the development of a new series of on-column benzylation reagents of which 3,5-bis(trifluoromethyl)benzyldimethylphenylammonium fluoride (BTBDMA-F) and 3,5-bis(trifluoromethyl)benzyltrimethylammonium fluoride (BTBTA-F) proved to be the most effective. BTBDMA-F has been shown to be the reagent of choice because it gives high conversion to benzylated derivatives when used in only moderate theoretical excess. Furthermore, the

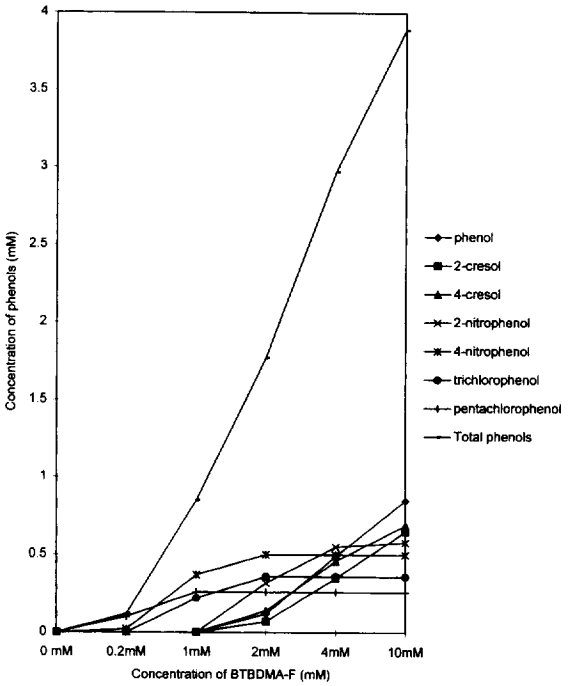


Fig. 7. On-column derivatisation of a mixture of seven phenols (total concentration 3.89 m*M*) with different concentrations of BTBDMA-F.

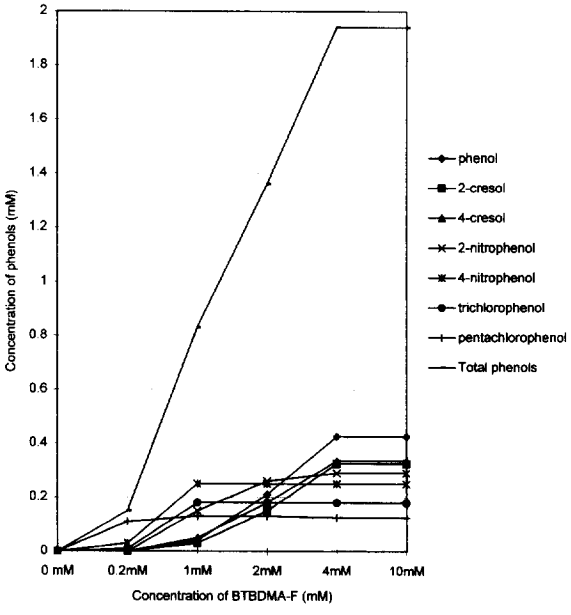


Fig. 8. On-column derivatisation of a mixture of seven phenols (total concentration 1.94 m*M*) with different concentrations of BTBDMA-F.

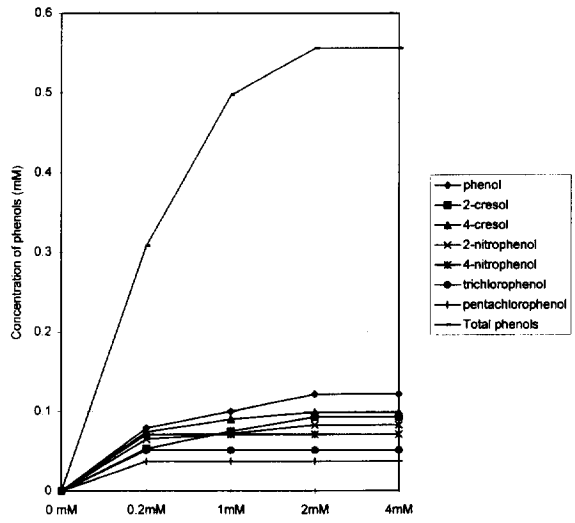


Fig. 9. On-column derivatisation of a mixture of seven phenols (total concentration 0.56 m*M*) with different concentrations of BTBDMA-F.

reagent is non-caustic and has no deleterious effect on column performance with extended use. BTBDMA-F is particularly efficient for derivatisation of phenols, especially chlorinated phenols, and has also been shown to be very useful for the derivatisation of volatile and simple aromatic organic acids.

BTBDMA-F has been demonstrated to be more efficient than MethElute (phenyltrimethylammonium hydroxide) for phenol derivatisation. A reaction mechanism which supports this observation is the formation of a particularly stable ion-pair between the acidic substance and the BTBDMA cation which undergoes efficient unimolecular thermal rearrangement in the injection port of the GC resulting in the high yield formation of the benzyl derivative of the acid.

Acknowledgments

We thank Dr G. Trout for negative ion GC–MS and the Australian Government Analyst for permission to publish is work.

Table 3
Total ion current monitoring after on-column derivatisation of 20 mg/kg each of a mixed phenols standard with 10 m*M* BTBDMA-Cl and 10 m*M* BTBDMA-Br

Phenol	Retention time (min)		Total ion current (cps/1000)			
	t_R(phenol)	t_R(deriv)[a]	BTBDMA-Cl		BTBDMA-Br	
			phenol	deriv	phenol	deriv
Phenol	4.53	5.88	1439	nd	1473	nd
Chlorophenol	4.73	10.23	1749	nd	1726	nd
2-Cresol	5.36	9.27	1641	nd	1778	nd
3-Cresols	5.58	9.69	4793	nd	nd	nd
4-Cresol	5.58	9.76	[b]	nd	nd	nd
Trichlorophenol	8.34	11.37	814	666	954	nd
4-Nitrophenol	9.4	11.81	nd	255	nd	nd
Pentachlorophenol	10.92	13.15	nd	1038	nd	665

Split ratio 20:1, Shimadzu MS. nd, not detected.
[a] t_R(deriv)=retention time of 3,5-bis(trifluoromethyl)benzyl derivative.
[b] 3- and 4-cresol not separated.

Table 4
Total ion current and SIM monitoring of a mixed fatty acid standard (30 mg/kg each acid) after on-column derivatisation with 10 m*M* PTMA-F, BTBDMA-F and 10 m*M* BTBTA-F

Analyte	Retention time (min)		Ion current (cps/1000)					
	t_R (Me)	t_R (Bz)	PTMAF		BTBDMA-F		BTBTA-F	
			TIC	*m/z* 74	TIC	*m/z* 227	TIC	*m/z* 227
C_{12}COOH	4.53	7.01	21 288	7430	27 820	5317	26 343	6077
C_{14}COOH	5.72	7.93	20 649	6450	22 986	4290	22 919	5308
C_{16}COOH	6.82	8.8	24 298	6997	23 710	4895	25 374	5037
C_{18}COOH	7.84	9.7	22 333	5870	21 110	4183	21 088	4053
C_{17}COOMe	7.34		14 914	4119	14 069		12 209	

Table 5
Total ion current (TIC) and selected ion monitoring (SIM) of a mixed volatile acid standard (100 mg/kg of each acid) after on-column derivatisation with 10 m*M* BTBDMA-F and BTBTA-F

Acid	t_R[a] (min)	Ion current (cps/1000)			
		BTBDMA-F		BTBTA-F	
		TIC	*m/z* 227 ion	TIC	*m/z* 227 ion
Acetic	6.41	116 957	18 880	49 450	9613
Propionic	7.22	153 715	38 201	85 837	19 197
Butyric	7.91	110 903	38 153	68 361	20 401

Split ratio 20:1, HP-MSD.
[a] t_R=retention time of 3,5-bis(trifluoromethyl)benzyl derivative.

Table 6
Total ion current (monitoring of different concentrations a mixed volatile acid standard (0.1–10 mg/kg of each acid) after on-column derivatisation with 10 m*M* BTBDMA-F

Acid	t_R^a (min)	Total ion current (cps/1000)					
		Concentration of acid (mg/kg)					
		10	5	2.5	1	0.5	0.1
Formic	5.51	4254	1721	1152	514	461	350
Acetic	6.18	16 024	6542	4051	2061	2206	1834
Propionic	7.01	14 237	5061	2998	1257	729	310
n-Butyric	7.72	20 058	6898	3819	1686	907	300

Split ratio 4:1, Shimadzu MS.
[a] t_R=retention time of 3,5-bis(trifluoromethyl)benzyl derivative.

Table 7
Total ion current monitoring of different concentrations of a standard mixture of acids after on-column derivatisation with 10 m*M* BTBDMA-F in methanol

Acid	t_R^a (min)	Total ion current (cps/1000)					
		Concentration of acid (mg/kg)					
		95[b]	50[b]	10[c]	5[c]	1[c]	0.5[c]
Chloroacetic	8.02	5431	2784	2006	929	164	0
Sorbic	9.7	7634	4461	5105	3004	477	377
Benzoic	10.15	14 796	8414	10 029	5094	1034	960
Ibuprofen[d]	11.91	6169	3254	3473	1715	379	417
Salicylic acid[e]	12.93	9798	7813	8803	4503	1434	2085
Salicylic[f]	10.71	3262	639				

[a] t_R=retention time of 3,5-bis(trifluoromethyl)benzyl derivative.
[b] Split ratio 20:1, Shimadzu MS.
[c] Split ratio 5:1, Shimadzu MS.
[d] Ibuprofen=2-(4′-isobutylphenyl)propionic acid.
[e] Salicylic acid formed only a di-3,5-bis(trifluoromethyl)benzyl derivative at lower acid concentrations.
[f] At higher acid concentrations salicylic acid formed a mixture of mono- and di-3,5- bis(trifluoromethyl)benzyl derivatives.

References

[1] D.R. Knapp, Handbook of Analytical Derivatization Reactions, Wiley, New York, NY, 1979
[2] R.J. Perchalski and B.J. Wilder, in C.E. Pippenger, J.K. Penry and H. Kutt (Editors), Antileptic Drugs: Quantitative Analysis and Interpretation, Raven Press, New York, NY, 1978.
[3] V. Gambario, R. Mariani and E.J. Marozzi, J. Anal. Toxicol., 6 (1982) 321.
[4] I. Brondz and I. Olsen, J. Chromatogr., 598 (1992) 309.
[5] R.L. Crackel, D.J. Buckley, A. Asghar, J.I. Grayn and A.M. Booren, J. Food Sci., 53 (1988) 1220.
[6] W. Butte, J. Chromatogr., 261 (1983) 142.
[7] H. Farber and H.F. Scholer, J. Agric. Food Chem., 41 (1993) 217.
[8] P. Jacob III, C. Savanaprindi, L. Yu, M. Wilson, A.P. Shulgin, N.L. Benowitz, B.A. Elias-Baker, S.M. Hall, R.L. Herning, R.T. Jones and D.P.L. Sachs, Anal. Chem., 56 (1984) 1692.
[9] J.A. Field, D.J. Miller, T.M. Field, S.B. Hawthorne and W. Giger, Anal. Chem., 64 (1992) 3161.
[10] B.L. Lee, P. Jacob III and N.L. Benowitz, J. Chromatogr., 494 (1989) 109.
[11] M. Amijee and R.J. Wells, J. Chromatogr. A, 662 (1994) 123.
[12] M. Amijee, J. Cheung and R.J. Wells, J. Chromatogr. A, 738 (1996) 43.