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Methylation reagents for the direct on-column derivatisation of veterinary residues

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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.

MethElute (PTMA-OH) and MethPrep (TTMA-OH) are perfectly satisfactory in the derivatisation of mono-functional compounds but produce mixtures, often very complex, when used for methylation of multi-functional substances.

Phenyltrimethylammonium cyanide (PTMA-CN) is readily prepared and is a far more selective alternative to MethElute for direct on-column methylation whilst still providing good yields of methylation products. Overall methylation efficiency is also dependent on the GC injector temperature whilst the condition of the injection liner can exert a significant effect on both methylation efficiency and selectivity.

INTRODUCTION

The analyses of many biologically active substances are routinely performed by HPLC because such substances are not suitable for analysis by gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) without prior derivatisation. A wide range of these are acidic and can be converted into methylated derivatives which are more volatile and therefore far more amenable to GC–MS analysis. Analytical results obtained by HPLC are frequently required to be confirmed by the GC–MS analysis of a methylated derivative. Examples of drug groups which require methylation to be satisfactorily detected and quantified by GC or GC–MS are: sulfonamide antimicrobials (**1**, see Fig. 1), benzimidazole anthelmintics (**2**, **3**, see Figs. 2 and 3), thiouracil thyrostatics (**4**, see Fig. 4) and thiazide diuretics.

In addition to these examples may be added acidic herbicides such as 2,4-D and wood preservatives such as pentachlorophenol which are often analysed by GC as their methylated derivative.

Derivatisation may be achieved by a number of methods of which reaction with diazomethane [1], methylation with methyl iodide–potassium carbonate [2] or phase-transfer methylation with methyl iodide–tetrahexylammonium bromide [3–5] are typical examples. Although many of these procedures are simple and give high product yields, they add an extra step and often involve the use of toxic or hazardous reagents.

Phenyltrimethylammonium hydroxide (PTMA-OH) and 3-trifluoromethylphenyltrimethylammonium (TTMA-OH) hydroxide are commercially available analytical reagents which are sold under the trade names of MethElute and MethPrep, respectively. They have been widely used for the on-column derivatisation of acidic substances in GC [6,7]. Trimethylsulfoxonium hydroxide (TMSO-OH) [8,9] has also been used for the same purpose. These sub-

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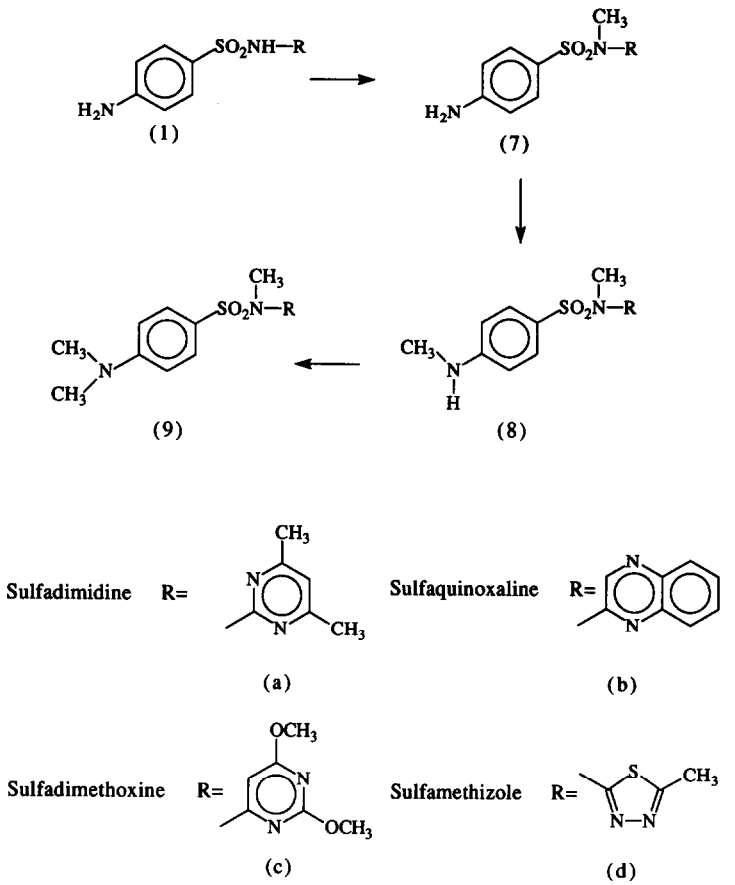


Fig. 1. Methylation products of sulfonamides.

stances all offer the advantage of *in situ* formation of the methylated derivative in the GC injection port but, in our hands, have proved somewhat non-selective in the methylation of some compound classes as illustrated later in this paper.

During studies on methylation under phase transfer conditions or using ion-exchange resins it has been found that the use of tetraalkylammonium salts or resins converted to the fluoride rather than the hydroxide form often offer advantages in terms of yield and selectivity [10]. This work suggested the replacement of the hydroxide anion of PTMA-OH with an alternative counter ion such as fluoride or cyanide could give greater selectivity.

We now report the investigation of phenyltrimethylammonium fluoride (PTMA-F) and phenyltrimethylammonium cyanide (PTMA-CN) as reagents for the direct on-column derivatisation of a series of substances used in veterinary treatments. Emphasis was placed on various sulfonamides (**1**), benzimidazoles (**2**, **3**) and thyrostatic substances (**4**). There are significant differences between the products formed when different methylation reagents are used.

EXPERIMENTAL

Reagents

MethElute (PTMA-OH) and Methprep-II (TTMA-OH) were commercial analytical reagents obtained from Pierce (Rockford, IL, USA) and Alltech (Deerfield, IL, USA), respectively.

Phenyltrimethylammonium iodide was prepared by refluxing a 20% solution of dimethyl-

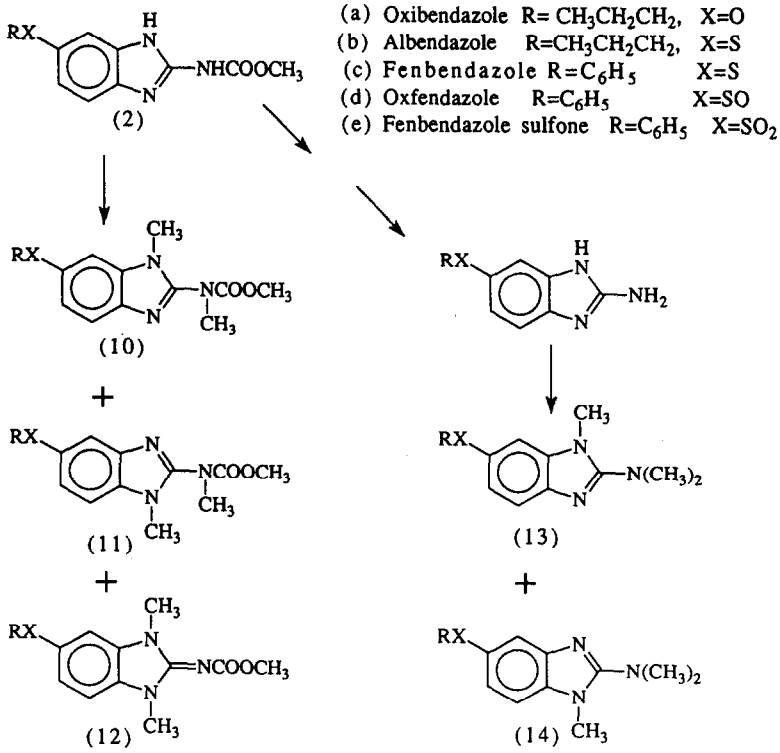


Fig. 2. Methylation products of benzimidazoles.

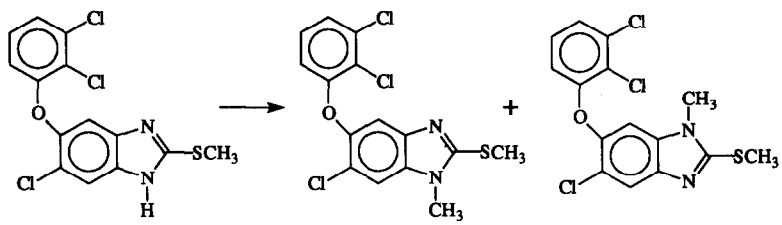
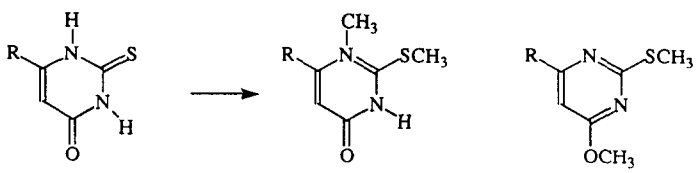


Fig. 3. Methylation products of triclabendazole.



R = H, CH_3, $CH_3CH_2CH_2$, C_6H_5

Fig. 4. Methylation products of thiouracils.

aniline in acetonitrile with methyl iodide, followed by recrystallisation from acetonitrile–ethyl acetate. It was converted to the respective hydroxide, fluoride or cyanide derivatives by the following general procedure:

A chromatographic column containing 500 ml of AG 1-X8 anion-exchange resin [200–400 mesh (38–75 μm wet bead size), Bio-Rad Labs.] was percolated with a 0.1 *M* solution of either sodium hydroxide, sodium fluoride or sodium cyanide. The column was then washed with water (1000 ml) and methanol (2000 ml). A 0.5 *M* solution of the quaternary ammonium salt in methanol (20 m*M* total) was applied 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 used for on-column derivatisation experiments.

Trimethyloxysulfonium hydroxide was prepared as a 0.2 *M* solution in methanol by stirring a suspension of trimethyloxysulfonium iodide (4.4 g) in methanol (800 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 (100 ml) and the combined solutions adjusted to 1000 ml with methanol.

Trimethyloxysulfonium fluoride (0.2 *M* in methanol) was prepared by neutralisation of the hydroxide to pH 7 with 40% hydrofluoric acid.

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, Australia).

On-column derivatisation studies

Unless otherwise stated, on-column methylations were investigated by injection of a 1:1 mixture of a solution of the analyte (100 μg ml^{-1}) and a 0.2 *M* methanolic solution of the requisite quaternary ammonium salt.

Equipment operation

Gas chromatography–mass spectrometry

GC analyses were conducted on a 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 0.33 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 mass-selective detector.

Injector inserts were cleaned and prepared by washing with methanol.

Acquisition of GC data

All GC analyses and on-column methylation studies were conducted employing the following standard conditions for the analyses of sulphonamides and benzamidazoles:

GC. Injection temperature 250°C, detector temperature 280°C, injection volume 2 μl (with three 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.

On-column methylation studies for thiouracils employed the following standard conditions:

GC. Injection temperature 250°C, detector temperature 280°C, injection volume 2 μl (with three washes between injections), oven equilibration time between runs 0.5 min, oven program: initial temperature 60°C (1.0 min) then 15°C/min to 140°C, 25°C/min to 280°C and hold at 280°C for 2 min.

MS. Solvent delay 2 min, scan parameters *m/z* 50–500, threshold 1000.

Mass spectra of methylated analytes

Sulfonamides

N_1-Methylsulfadimidine (**7a**). *m/z* 228 ($M^+ - 64$, 73%), 227 ($M^+ - 65$, 100%), 136 ($C_6H_2N_2Me_2NMe-$, 12%), 121 ($H_2NC_6H_4$-NMe–, 12%), 108 ($C_6H_2N_2Me_2-$, 28%), 107 ($C_4HN_2Me_2-$, 13%), 107 ($C_6H_6N_2-$, 13%), 92 ($H_2NC_6H_4-$, 24%), 67 (10%), 65 (20%).

N_1,N_4-Dimethylsulfadimidine (**8a**). *m/z* 242 ($M^+ - 64$, 100%), 241 ($M^+ - 65$, 93%), 136, ($C_6H_2N_2Me_2NMe-$, 5%), 136 ($H_2NC_6H_4NMe-$, 12%), 108 ($C_6H_2N_2Me_2-$, 28%), 122 ($C_4HN_2Me_2-$, 13%), 107 ($C_6H_6N_2-$, 13%), 92 ($H_2NC_6H_4-$, 24%), 67 (10%), 65 (20%).

N_1,N_4,N_4-Trimethylsulfadimidine (**9a**). *m/z* 256 ($M^+ - 64$, 100%), 255 ($M^+ - 65$, 70%), 242 (5%), 241 (11%), 136 ($C_6H_2N_2Me_2NMe-$, 35%), 135 (12%), 134 (20%), 121 (12%), 120 (17%), 108 ($C_6H_2N_2Me_2-$, 8%), 107 ($C_6H_6N_2-$, 7%), 105 (9%), 104 (13%), 79 (7%), 78 (5%), 77 (11%), 67 (10%).

N_1-Methylsulfadimethoxine (**7c**). *m/z* 260 ($M^+ - 64$, 96%), 259 ($M^+ - 65$, 100%), 140 (29%), 92 (55%), 65 (39%).

N_1,N_4-Dimethylsulfadimethoxine (**8c**). *m/z* 274 ($M^+ - 64$, 100%), 273 ($M^+ - 65$, 71%), 259 (7%), 154 (9%), 140 (21%), 122 ($C_4HN_2Me_2-$, 25%), 121 (14%), 120 (21%), 107 (10%), 106 (28%), 82 (10%), 79 (17%), 78 (14%), 77 (25%), 65 (7%).

N_1,N_4,N_4-Trimethylsulfadimethoxine (**9c**). *m/z* 288 ($M^+ - 64$, 100%), 287 ($M^+ - 65$, 32%), 274 (11%), 273 (11%), 168 (7%), 140 (15%), 136 (31%), 135 (15%), 134 (14%), 122 (6%), 121 (11%), 120 (19%), 108 (5%), 105 (11%), 104 (9%), 83 (5%), 82 (6%), 79 (7%), 78 (5%), 77 (16%), 68 (5%).

N_1-Methylsulfaquinoxaline (**7b**). *m/z* 250 ($M^+ - 64$, 84%), 249 ($M^+ - 65$, 100%), 159 (8%), 158 (8%), 157 (5%), 156 (20%), 140 (7%), 131 (10%), 130 (17%), 129 (11%), 117 (5%), 116 (6%), 108 ($C_6H_2N_2Me_2$–37%), 107 ($C_6H_6N_2-$, 12%), 106 (8%), 102 (11%), 93 (8%), 92 (54%), 91 (8%), 90 (26%), 66 (5%), 65 (25%), 64 (7%).

N_1,N_4-Dimethylsulfaquinoxaline (**8b**). *m/z* 265 (19%), 264 ($M^+ - 64$, 100%), 263 ($M^+ - 65$, 63%), 170 (12%), 158 (5%), 154 (7%), 131 (8%), 130 (8%), 129 (9%), 122 ($C_4HN_2Me_2-$, 66%), 121 (24%), 120 (9%), 107 (6%), 106 (43%), 105 (7%), 104 (8%), 102 (8%), 91 (5%), 90 (17%), 79 (17%), 78 (9%), 77 (19%), 76 (5%), 66 (5%), 65 (9%), 64 (7%), 63 (9%).

N_1,N_4,N_4-Trimethylsulfaquinoxaline (**9b**). *m/z* 279 (21%), 278 ($M^+ - 64$, 100%), 277($M^+ - 65$, 35%), 264 (33%), 263 (19%), 207 (12%), 137 (9%), 136 (76%), 135 (21%), 134 (6%), 131 (9%), 130 (5%), 129 (5%), 122 ($C_4HN_2Me_2-$, 13%), 121 (7%), 120 (45%), 118 (7%), 115 (5%), 106 (12%), 105 (12%), 104 (13%), 103 (6%), 102 (75), 92 (6%), 91 (6%), 90 (20%), 79 (15%), 78 (7%), 77 (23%), 76 (7%), 65 (7%), 64 (5%), 63 (6%).

N_1-Methylsulfamethizole (**7d**). *m/z* 284 (M^+ 83%), 156 (66%), 108 (58%), 92 (100%), 70 (63%), 65 (47%), 51 (35%).

N_1,N_4-Dimethylsulfamethizole (**8d**). *m/z* 299 (15%), 298 (M^+ 100%), 207 (13%), 176 (26%), 170 (21%), 138 (10%), 122 (74%), 117 (13%), 107 (12%), 106 (51%), 90 (16%), 79 (17%), 77 (31%), 69 (12%), 65 (15%).

N_1,N_4,N_4-Trimethylsulfamethizole (**9d**). *m/z* 314 (14%), 313 (M^+ 20%), 312 (100%), 311 (8%), 298 (14%), 207 (11%), 184 (14%), 136 (84%), 120 (50%), 119 (9%), 118 (11%), 105 (14%), 104 (14%), 92 (8%), 79 (11%), 78 (9%), 77 (12%), 70 (11%), 69 (14%), 59 (13%).

Benzimidazoles

Dimethyloxibendazole (**10a**). *m/z* 278 (11%), 277 (M^+ 88%), 235 (10%), 234 (27%), 218 (14%), 178 (19%), 177 (32%), 176 (100%), 175 (7%), 174 (7%), 162 (7%), 148 (11%), 147 (14%), 134 (5%), 119 (5%), 106 (7%), 105 (5%), 90 (5%), 80 (6%), 79 (5%), 72 (64%), 59 (9%).

Dimethyloxibendazole (**11**). *m/z* 278 (8%), 277 (M^+ 85%), 235 (14%), 234 (17%), 219 (5%), 218 (21%), 177 (15%), 176 (100%), 175 (6%), 162 (7%), 161 (7%), 149 (7%), 148 (12%), 147 (10%), 134 (5%), 106 (6%), 92 (7%), 79 (5%), 72 (5%), 66 (7%), 59 (8%).

Dimethyloxibendazole (rearranged) (**12**). *m/z* 278 (10%), 277 (M^+ 71%), 247 (13%), 246 (100%), 234 (15%), 233 (8%), 219 (11%), 205 (5%), 204 (61%), 203 (12%), 189 (9%), 188

(5%), 187 (5%), 177 (15%), 176 (8%), 174 (8%), 161 (6%), 160 (5%).

Trimethyldecarbomethoxyoxibendazole (**13**). *m/z* 234 (9%), 233 (M^+ 48%), 204 (7%), 191 (12%), 190 (100%), 176 (9%), 162 (11%), 148 (9%), 147 (12%).

Trimethyldecarbomethoxyoxibendazole (**14**). *m/z* 234 (14%), 233 (M^+ 100%), 218 (21%), 204 (34%), 191 (13%), 190 (16%), 189 (7%), 176 (44%), 163 (7%), 162 (59%), 161 (12%), 160 (12%), 159 (5%), 149 (6%), 148 (27%), 147 (31%), 145 (5%), 134 (6%), 121 (6%), 106 (7%), 71 (5%).

Dimethylalbendazole (**10**). *m/z* 295 (9%), 294 (16%), 293 (M^+ 100%), 250 (14%), 235 (10%), 234 (36%), 194 (7%), 193 (23%), 192 (30%), 191 (14%), 164 (7%), 163 (8%), 159 (6%), 150 (5%), 149 (5%), 72 (19%), 59 (5%).

Dimethylalbendazole (**11**). *m/z* 294 (18%), 293 (M^+ 100%), 264 (8%), 250 (8%), 234 (28%), 193 (9%), 192 (54%), 191 (15%), 164 (10%), 163 (7%), 106 (5%), 90 (10%), 83 (5%).

Dimethylalbendazole (rearranged) (**12**). *m/z* 294 (14%), 293 (M^+ 63%), 264 (6%), 263 (15%), 262 (100%), 250 (11%), 249 (7%), 235 (10%), 220 (19%), 219 (29%), 218 (6%), 207 (5%), 205 (6%), 204 (12%), 192 (8%), 186 (5%), 59 (6%).

Trimethyldecarbomethoxyalbendazole (**13**). *m/z* 250 (14%), 249 (M^+ 86%), 234 (15%), 220 (16%), 207 (15%), 206 (100%), 205 (9%), 192 (17%), 178 (6%), 177 (7%), 176 (10%), 165 (6%), 164 (18%), 163 (14%), 162 (6%), 131 (6%), 118 (6%), 95 (5%).

Trimethyldecarbomethoxyalbendazole (**14**). *m/z* 250 (15%), 249 (M^+ 100%), 234 (24%), 220 (29%), 207 (8%), 206 (19%), 205 (17%), 192 (21%), 178 (22%), 177 (15%), 176 (13%), 165 (8%), 164 (19%), 163 (23%), 161 (5%), 132 (6%), 131 (5%), 122 (6%), 109 (5%), 91 (5%), 65 (7%).

Dimethylfenbendazole (**10**). *m/z* 328 (19%), 327 (M^+ 100%), 270 (11%), 269 (25%), 268 (88%), 239 (19%), 90 (6%).

Dimethylfenbendazole (**11**). *m/z* 328 (14%), 327 (M^+ 100%), 270 (10%), 269 (19%), 268 (63%), 239 (9%), 207 (11%), 82 (6%), 77 (9%).

Dimethylfenbendazole (rearranged) (**12**). *m/z* 328 (21%), 327 (90%), 297 (22%), 296 (100%), 270 (15%), 269 (10%), 207 (37%), 148 (18%).

Trimethyldecarbomethoxyfenbendazole (**13**). *m/z* 284 (18%), 283 (M^+ 100%), 269 (5%), 268 (10%), 255 (5%), 254 (20%), 253 (9%), 241 (7%), 240 (13%) 239 (35%), 225 (5%), 224 (5%), 159 (5%), 142 (11%), 134 (9%), 131 (7%), 109 (6%), 77 (6%).

Trimethyldecarbomethoxyfenbendazole (**14**). *m/z* 284 (14%), 283 (M^+ 100%), 269 (8%), 268 (48%), 255 (6%), 254 (33%), 253 (7%), 241 (6%), 240 (16%) 239 (47%), 224 (6%), 207 (5%), 184 (5%), 142 (14%), 134 (8%), 132 (5%), 127 (6%), 118 (7%), 109 (5%).

Dimethyloxfenbendazole (**10**). *m/z* 344 (16%), 343 (M^+ 51%), 328 (35%), 327 (100%), 295 (70%), 281 (41%), 270 (16%), 269 (24%), 268 (98%), 267 (21%), 266 (53%), 240 (17%), 239 (20%), 236 (72%), 234 (37%), 225 (12%), 209 (32%), 208 (27%), 207 (73%), 191 (18%), 159 (44%), 158 (12%), 147 (20%), 131 (19%), 118 (19%), 95 (19%), 78 (14%), 72 (47%), 59 (27%).

Dimethyloxfenbendazole (**11**). *m/z* 344 (12%), 343 (M^+ 39%), 328 (15%), 327 (95%), 295 (48%), 282 (18%), 281 (32%), 269 (32%), 268 (88%), 266 (56%), 250 (27%), 240 (11%), 240 (11%), 239 (18%), 236 (45%), 234 (40%), 208 (22%), 207 (100%), 191 (18%), 159 (30%), 147 (15%), 131 (13%), 119 (1%), 118 (23%), 73 (38%).

Dimethyloxfenbendazole (rearranged) (**12**). *m/z* 344 (16%), 343 (M^+ 51%), 328 (35%), 327 (100%), 295 (70%), 281 (41%), 270 (16%), 269 (24%), 268 (98%), 267 (21%), 266 (53%), 240 (17%), 239 (20%), 236 (72%), 234 (37%), 209 (32%), 208 (27%), 207 (73%), 191 (18%), 159 (44%), 158 (12%), 147 (20%), 131 (19%), 118 (19%), 96 (19%), 77 (26%), 272 (47%), 59 (27%).

Trimethyldecarbomethoxyoxfenbendazole (**13**). *m/z* 299 (14%), 284 (22%), 283 (100%), 282 (6%), 269 (9%), 268 (36%), 254 (27%), 253 (12%), 240 (21%), 239 (30%), 222 (35%), 207 (11%), 192 (5%), 191 (6%), 190 ((%), 159 (10%), 142 (9%), 134 (10%), 133 (6%), 132 (8%), 131 (9%0, 118 (9%), 77 (12%), 51 (7%).

Trimethyldecarbomethoxyoxfenbendazole (**14**).

m/z 300 (18%), 299 (95%), 284 (23%), 283 (100%), 281 (11%), 270 (8%), 269 (9%), 268 (46%), 254 (28%), 253 (9%), 251 (14%), 240 (20%), 239 (47%), 236 (17%), 223 (10%), 222 (75%), 221 (11%), 209 (8%), 208 (18%), 207 (48%), 206 (15%), 193 (19%0, 191 (7%), 190 (23%), 163 (13%), 162 (15%), 159 (27%), 147 (12%), 145 (24%), 141 (14%), 134 (12%), 133 (10%), 131 (13%), 130 (11%), 118 (15%), 104 (11%), 77 (22%), 51 (10%).

Monomethyltriclabendazole (**4**). *m/z* 378 (8%), 376 (27%), 375 (20%), 374 (M^+ 100%), 373 (17%), 372 (99%), 370 (7%), 357 (9%), 343 (23%), 342 (15%), 341 (88%), 340 (22%), 339 (93%), 328 (5%), 325 (7%), 302 (6%), 269 (8%), 256 (9%), 229 (9%), 227 (13%), 207 (13%), 198 (6%), 196 (9%), 169 (7%), 168 (9%), 166 (9%), 164 (6%), 154 (8%), 153 (6%), 152 (13%), 151 (14%), 111 (10%), 110 (8%), 109 (22%), 100 (7%), 97 (8%), 85 (8%), 75 (11%), 74 (8%), 73 (10%).

Monomethyltriclabendazole (**5**). *m/z* 377 (10%), 376 (40%), 375 (25%), 374 (M^+ 100%), 373 (21%), 372 (100%), 357 (6%), 343 (15%), 342 (10%), 341 (55%), 340 (10%), 339 (53%), 327 (13%), 326 (7%), 325 (9%), 304 (5%), 303 (14%), 302 (13%), 269 (8%), 256 (9%), 255 (10%), 227 (10%), 208 (10%), 207 (12%), 198 (8%), 196 (8%), 170 (7%), 169 (7%), 168 (14%), 152 (26%), 151 (20%), 109 (17%), 102 (6%), 101 (9%), 76 (12%), 75 (11%), 74 (8%), 73 (6%), 66 (13%), 63 (14%).

RESULTS AND DISCUSSION

Effect of injection temperature

The effect of the injection temperature on methylating power and efficiency was compared for PTMA-OH and PTMA-F using the methylation of sulfadimidine (**1a**) and oxibendazole (**2a**) at different injector block temperatures as a general guide. A 100 μg ml^{-1} solution of oxibendazole and sulfadimidine in 0.2 *M* methanolic PTMA-F was analysed using injector block temperatures between 180 and 280°C. The methylation efficiency was estimated from the combined total ion current produced by GC–MS monitoring of methylated products. The results of this study are shown in Table I.

These results indicate an injection temperature of 240–260°C is optimal for maximum derivatisation efficiency of both oxibendazole and sulfadimidine. This injection temperature is also optimal for both derivatising agents as is the overall methylation efficiency. At lower injection temperatures there is little difference in methylation efficiency of sulfadimidine for either of the two reagents. By contrast, methylation efficiency of oxibendazole is better with PTMA-OH than with PTMA-F at temperatures below 250°C. However, in general, total derivatisation efficiency decreases with decrease in temperature and is very poor below 200°C.

In terms of selectivity, it is clear from Table I that the hydroxide is a more vigorous and non-selective methylation reagent than is the corresponding fluoride. However, with either reagent there is very little variation of methylation selectivity with increasing injection block temperature above a temperature of 200°C although the ratios of various products are subject to some variation as the injection temperature is increased. At 180°C oxibendazole gave only two dimethylated products (**10** and **11**) but this selectivity was associated with a methylation efficiency of only 20% that obtained at 250°C. Above 200°C, the formation of a third rearranged dimethylated product in the methylation of oxibendazole becomes important and constitutes about 40% of the combined derivatives at 250°C.

Methylation efficiency

Effect of injector insert

It was found during this work that following the replacement of an unclean injector liner with a fresh one, a maximum reproducible value for overall methylation efficiency and selectivity was attained only after about 20 on-column methylation injections. Thus the methylation of oxibendazole (**2a**) with PTMA-CN gave 67% of a monomethyl derivative together with a combined yield of 33% of two dimethyl derivatives at 250°C when a fresh injector insert was used. After 20 further injections involving a variety of methylation reagents, the same methylation mix-

TABLE I

EFFECT OF INJECTION TEMPERATURE ON THE METHYLATION EFFICIENCY OF OXIBENDAZOLE AND SULFADIMIDINE BY TMPA-OH AND TMPA-F

The structures of products formed are numbered and are discussed in detail later in the paper. TIC = Total ion current.

Methylation agent	Analyte	Injector temp (°C)	No. of products	Ratio of products %					Combined TIC × 10^3
				13a	**14a**	**10a**	**11a**	**12a**	
PTMA-F	Oxibendazole	180	2			50	50		181
		200	3			37	37	26	270
		220	3			35	30	35	295
		260	3			28	28	44	580
		280	3			30	23	47	380
PTMA-OH	Oxibendazole	180	3			30	30	40	260
		200	3			45	35	20	415
		220	3	18	16	16	16	34	440
		250	5	21	18	14	14	33	550
		280	5	21	20	13	13	33	625

Derivatising agent	Analyte	Injector temp. (°C)	No. of products	Ratio of products %			Combined TIC × 10^3
				7a	**8a**	**9a**	
PTMA-F	Sulfadimidine	180	2	80	20		100
		200	2	80	20		294
		220	2	83	17		380
		250	2	83	17		450
		280	2	83	17		520
PTMA-OH	Sulfadimidine	180	3	48	31	21	86
		200	3	37	27	36	230
		220	3	30	30	40	350
		250	3	27	25	48	408
		280	3	27	25	48	456

ture of oxibendazole and PTMA-CN gave combined yield of 100% of the two dimethyl derivatives. Both the selectivity and overall methylation efficiency was subsequently maintained for the lifetime of the injector insert. Similar behaviour was found for other methylation reagents. For example, the methylation of sulfadimidine (**1a**) with PTMA-OH gave 33% of the trimethylated derivative with a fresh insert. This value rose to and was maintained at 55% as the insert was subsequently used.

Comparison of the efficiencies of on-column and phase transfer methylations

Information on the efficiency of direct on-column methylation was obtained by a direct comparison of the methylation of triclabendazole (**3**) using a phase-transfer procedure [3–5] and on-column methylation with PTMA-F. The phase-transfer methylation reaction has previously been reported to give yields in the range of 70–80% [3–5]. Triclabendazole was the chosen analyte because it methylated in good yield using either method and produced only two products (**5** and **6**) in approximately the same ratio.

In order to assess the comparability of phase transfer and direct methylation a standard solution of triclabendazole in methanol was mixed 1:1 with 2 *M* methanolic PTMA-F for direct injection. A parallel phase-transfer methylation was conducted on the same amount of triclabendazole standard solution. This solution was then evaporated to near dryness, methylated by published procedures [4] and the product made up to the same concentration as the triclabendazole in the direct injection experiment.

TABLE II

COMPARISON OF THE METHYLATION EFFICIENCY OF TRICLABENDAZOLE BY TMPA-F AND PHASE TRANSFER AT DIFFERENT ANALYTE CONCENTRATIONS

The concentration of injection solutions were adjusted to ensure that the concentration of the analytes were equivalent for either derivatisation method. The total ion current (TIC) for each of the two possible monomethylated products **4** and **5** are listed together with the combined TIC for **4** plus **5**

Concentration of analyte (in solution injected) $\mu g\ ml^{-1}$	Phase transfer, TIC $\times 10^3$			PTMA-F, TIC $\times 10^3$		
	Peak 1	Peak 2	Total	Peak 1	Peak 2	Total
110	2366	1173	3539	2744	1650	4394
55	872	401	1273	1108	631	1739
28	260	112	372	243	128	371
5	–	–	–	14	–	14

The results shown in Table II demonstrate that derivatisation using on-column injection with PTMA-F is at least as efficient as phase-transfer methylation at higher analyte concentrations, at an intermediate level both methods give comparable results whilst at 5 $\mu g\ ml^{-1}$ only PTMA-F gives a detectable product using the MS in full scan mode. Thus, not only is direct on-column methylation a very convenient technique but it appears to give methylated derivatives in yields comparable to alternative methods.

Effect of variation of methylation reagent on selectivity

Sulfonamides

Work on the derivatisation of sulfonamides has been reviewed previously [11]. On-column derivatisation of sulfonamides (**1**) with PTMA-OH gave significantly different results than those obtained by use of PTMA-F or PTMA-CN. Thus, with PTMA-OH, all sulfonamides tested gave a mixture of three derivatives consisting of monomethylated (**7**), dimethylated (**8**) and trimethylated (**9**) substances as judged from the mass spectrum of each peak (see Fig. 5). Very little variation of derivatisation pattern was obtained by alteration of injector temperature.

By contrast, PTMA-F gave predominantly mono- and dimethyl derivatives which were identical to those obtained by phase-transfer alkylation [3–5] and PTMA-CN yielded the monomethyl derivative almost exclusively. Phase-transfer methylation of sulfonamides produced the monomethyl derivative, however it was found that the phase-transfer methylation of three out of the four sulfonamides tested gave unsatisfactory yields. The direct on-column methylation efficiencies of PTMA-F and PTMA-CN were comparable to that obtained with the PTMA-OH but PTMA-CN showed far greater methylation selectivity than the corresponding fluoride or hydroxide. Therefore PTMA-CN appears to be the reagent of choice for the on-column derivatisation of sulfonamides. It is interesting to note that methylation using TTMA-OH gave predominantly dimethylated and monomethylated derivatives and therefore possesses a methylation selectivity similar to that of PTMA-F.

Methylation of sulfadimethoxine (**1c**) and sulfamethizole (**1d**) by both TMSO-OH and TMSO-F gave predominantly monomethyl derivatives but methylation efficiency was lower than that obtained for PTMA-F or PTMA-CN.

The variation in product patterns with various methylation reagents is shown in Fig. 6. The products and the apparent relative yields obtained from on-column methylation of a series of different sulfonamides at 250°C with PTMA-OH, PTMA-F, PTMA-CN, TTMA-OH, TMSO-OH and TMSO-F is shown in Table III.

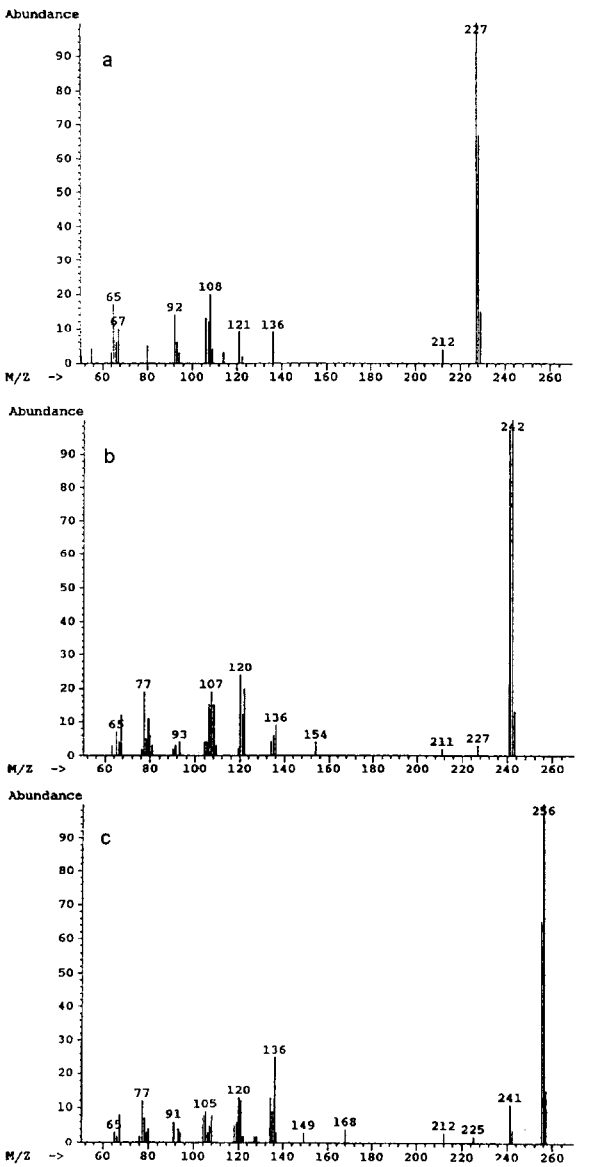


Fig. 5. The mass spectra of methylated sulfadimidines (monomethyl, **7a**, dimethyl, **8a** and trimethyl, **9a**).

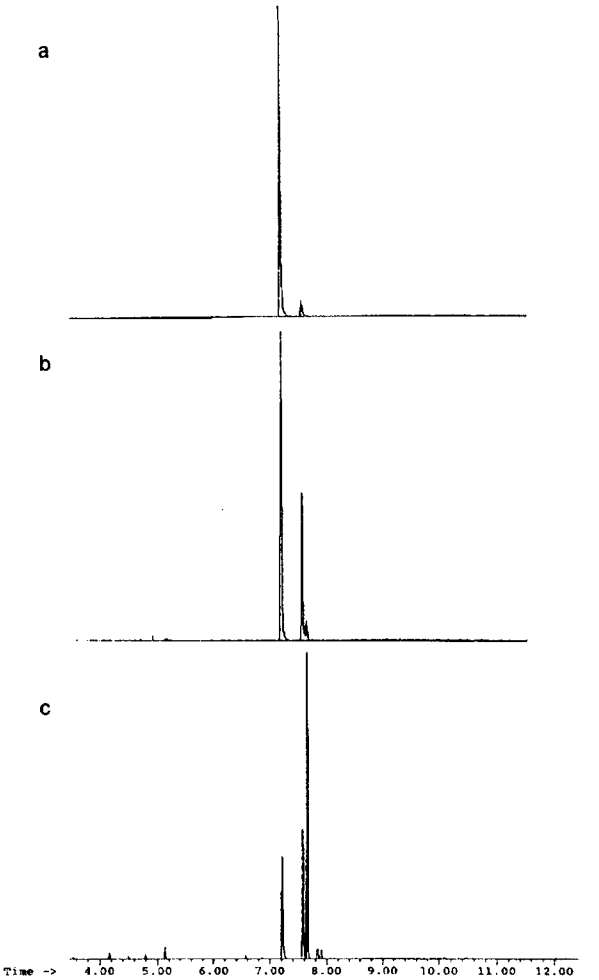


Fig. 6. The variation in product patterns in the methylation of sulfadimidine with three methylation reagents (PTMA-OH, PTMA-F and PTMA-CN). The three products in increasing retention time are **7a**, **8a** and **9a** (mono-, di- and trimethylation products). (a) PTMA-CN, (b) PTMA-F, (c) PTMA-OH. Time scale in min.

Benzimidazoles

In previous work [2] on the confirmation of the HPLC analysis of benzimidazoles by methylation with methyl iodide–potassium carbonate in acetone followed by GC–MS detection, the formation of two dimethyl benzimidazole derivatives was reported. The mass spectra of these derivatives were very similar and, although GC peaks were completely separated, the GC retention times were close and the alternative N,N′-dimethyl structures **10** and **11** were assigned to these substances.

We have studied the direct on-column methylation of a series of typical benzimidazoles with several different methylation reagents including phenyltrimethylammonium hydroxide, fluoride and cyanide salts. The results of this study are summarised in Table IV together with data on

TABLE III

COMPARISON OF THE METHYLATION OF SOME SULFONAMIDES WITH DIFFERENT ON-COLUMN METHYLATION REAGENTS

Sulfonamide	Monomethyl	Dimethyl	Trimethyl
Sulfadimidine (**1a**) (retention time, min)	7.26	7.62	7.70
PTMA-OH (% formed)	27	25	48
TTMA-OH (% formed)	40	32	28
PTMA-F (% formed)	63	29	8
PTMA-CN (% formed)	94	6	
TMSO-OH	100	low yield	
TMSO-F	100	low yield	
Extractive Me (%)	100	(good yield)	
Sulfaquinoxaline (**1b**) (retention time, min)	8.63	8.97	9.01
PTMA-OH (% formed)	36	36	28
TTMA-OH (% formed)	56	24	6
PTMA-F (% formed)	41	59	
PTMA-CN (% formed)	99	1	
TMSO-OH	96	4	(moderate yield)
Phase transfer Me (%)	100	(very poor yield)	
Sulfadimethoxine (**1c**) (retention time, min)	7.93	8.24	8.28
PTMA-OH (% formed)	13	32	55
TTMA-OH (% formed)	38	48	14
PTMA-F (% formed)	41	59	
PTMA-CN (% formed)	98	2	
TMSO-OH	100	25% yield of TMSO-F	
TMSO-F	95	5	(moderate yield)
Phase transfer Me (%)	100	(very poor yield)	
Sulfamethizole (**1d**) (retention time, min)	7.96	8.35	8.47
PTMA-OH (% formed)	13	38	49
TTMA-OH (% formed)	19	51	30
PTMA-F (% formed)	25	53	23
PTMA-CN (% formed)	91	9	
TMSO-OH	100		
TMSO-F	87	17	
Phase transfer Me (%)	100	(very poor yield)	

methylation using TTMA-OH, TMSO-OH and TMSO-F, respectively.

Methylations of benzimidazoles with PTMA-OH, PTMA-F and TTMA-OH are complex and produced up to six products. By contrast, PTMA-CN, TMSO-OH and TMSO-F yielded predominantly two dimethylated products suitable for confirmation purposes. TMSO-OH and TMSO-F are less aggressive methylation reagents than PTMA-OH and TTMA-OH and give similar product mixtures to those given by PTMA-CN, however the methylation efficiencies of the TMSO-derived reagents are less than that of PTMA-CN and therefore the use of TMSO-OH and TMSO-F offers no advantages over PTMA-CN either in methylation efficiency or

TABLE IV

COMPARISON OF THE METHYLATION OF SOME BENZIMIDAZOLES WITH DIFFERENT ON-COLUMN METHYLATION REAGENTS

Benzimidazole	Trimethyldecarbomethoxy-		Dimethyl		Dimethyl (rearranged)
Oxibendazole (retention time, min)	5.24	5.37	6.23	6.31	7.95
PTMA-OH (% formed)	21	18	14	14	33
TTMA-OH (% formed)	16	13	23	19	29
PTMA-F (% formed)	14	13	19	17	37
PTMA-CN (% formed)			48	52	
TMSO-F (% formed)	Monomethyl 5.83 min, m/z 263(M +)		Very poor yield		
Phase transfer Me (%)			49	51	
Albendazole (retention time, min)	6.12	6.29	6.96	7.11	8.11
PTMA-OH (% formed)	27	25	11	10	27
TTMA-OH (% formed)	20	19	20	23	18
PTMA-F (% formed)	19	16	19	18	28
PTMA-CN (% formed)			45	46	9
TMSO-OH (% formed)	Poor yield		45	55	
Phase transfer Me (%)			45	55	
Fenbendazole (retention time, min)	7.85	8.05	8.52	8.75	9.63
PTMA-OH (% formed)	29	32	12	14	13
TTMA-OH (% formed)	16	17	25	30	12
PTMA-F (% formed)	18	17	21	27	17
PTMA-CN (% formed)			45	55	
TMSO-OH (% formed)	Poor yield		45	55	
Phase transfer Me (%)			49	51	
Oxfendazole (retention time, min)	9.03	9.35	9.52	9.95	11.10
PTMA-OH (% formed)	32	28	14	13	10
TTMA-OH (% formed)	14	9	27	31	10
PTMA-F (% formed)	4	4	41	39	12
PTMA-CN (% formed)			48	52	
Phase transfer Me (%)			54	46	
Fenbendazole sulfone (retention time, min)			9.74	10.27	11.30
PTMA-F (% formed)			47	46	7
PTMA-CN (% formed)			70	48	
Phase transfer Me (%)			61	39	
Triclabendazole (retention time, min)			8.94	9.10	
PTMA-OH (% formed)			52	48	
TTMA-OH (% formed)			52	48	
PTMA-F (% formed)			50	50	
PTMA-CN (% formed)			51	49	
TMSO-OH			54	46	
Phase transfer Me (%)					

selectivity although optimum conditions for derivatisation by these reagents were not explored in this work.

Methylation of benzimidazoles with PTMA-CN gave the same two dimethylated products as those obtained by phase transfer methylation. These dimethyl derivatives produced almost identical mass spectra which were consistent with the alternative structures **10** and **11** whilst a third later eluting dimethyl benzimidazole produced during methylation with PTMA-OH, PTMA-F and TTMA-OH was assigned the symmetrical structure **12** on the basis of its mass spectrum. Thus, for oxibendazole, each derivative had predominant ions at m/z 277 (M^+) and 176 ($M^+ - C_3H_6 - COOCH_3$) with more minor fragments at m/z 235 ($M^+ - C_3H_6$), 234 ($M^+ - C_3H_7$) and 218 ($M^+ - COOCH_3$) consistent with structures **10a** and **11a**. By contrast, the third dimethyl derivative had a mass spectrum containing only three ions at m/z 277 (M^+), 246 ($M^+ - OCH_3$) and 204 ($M^+ - OCH_3 - C_3H_6$) which is more consistent with structure **12a** than with **10a** and **11a**.

In addition to the three dimethyl derivatives discussed above, direct on-column methylation of benzimidazoles with either PTMA-OH or TTMA-OH yield two additional products which elute earlier than the dimethyl derivatives discussed above. Thus oxfendazole gave two additional products with molecular ions at m/z 299 and similar mass spectra which corresponded to trimethyldecarbomethoxy derivatives. Both of these substances had the same major fragment ions. On this basis the unsymmetrical structures **13a** and **14a** were assigned to these products.

It can be seen in Table IV that there is a significant difference in methylation pattern obtained from direct on-column derivatisation which is dependent on the methylation reagent employed. Reagent selectivity increases in the order PTMA-OH < TTMA-OH < PTMA-F < PTMA-CN ≥ TMSO-OH(F). However, whilst PTMA-OH, TTMA-OH, PTMA-F and PTMA-CN give the same methylation efficiency, TMSO-OH and TMSO-F give lower methylation yields. Thus in terms of efficiency and selectivity PTMA-CN is the reagent of choice for the on-column methylation of benzimidazoles. The exception is triclabendazole which cannot undergo the rearrangement or degradation reactions of the other benzimidazoles and PTMA-OH, TTMA-OH or PTMA-F are equally satisfactory derivatising reagents for this substance. The results of methylation of albendazole (**2b**) and fenbendazole (**2c**) with three different methylation reagents are shown in Figs. 7 and 8 and the mass spectra of the three classes of methylated derivatives of oxibendazole (**10a**, **12a** and **13a**) are illustrated in Fig. 9.

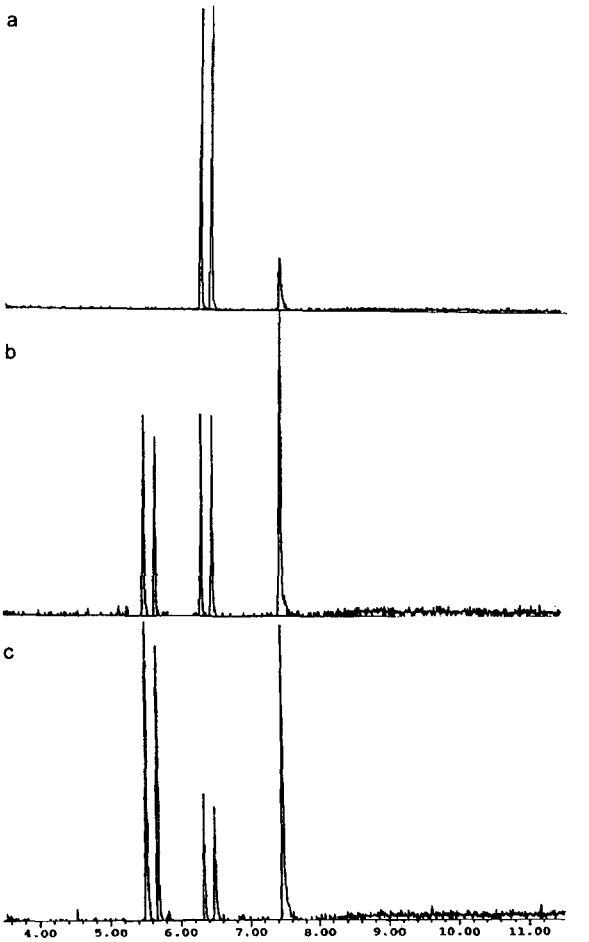


Fig. 7. The variation in product patterns in the methylation of albendazole with three methylation reagents (PTMA-OH, PTMA-F and PTMA-CN). The five products in increasing retention time are **13b**, **14b**, **10b**, **11b** and **12b**. (a) PTMA-CN, (b) PTMA-F, (c) PTMA-OH. Time scale in min.

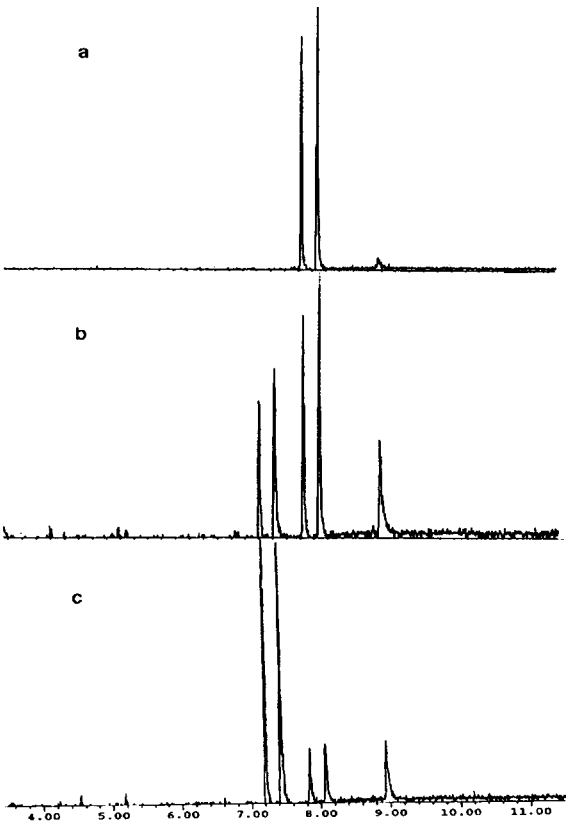


Fig. 8. The variation in product patterns in the methylation of fenbendazole with three methylation reagents (PTMA-OH, PTMA-F and PTMA-CN). The five products in increasing retention time are **13c**, **14c**, **10c**, **11c** and **12c**. (a) PTMA-CN, (b) PTMA-F, (c) PTMA-OH. Time scale in min.

Thiouracils

On-column derivatisation of thiouracils gave two dimethylated derivatives with all reagents whereas phase transfer methylation gave a single product identical to the later eluting dimethyl derivative. The structure **15** has been previously assigned to the phase transfer methylation product [12] but the structure of the second dimethylated derivative formed in on-column methylation is tentatively assigned the structure **16**. The results obtained from on-column methylation of thiouracils are shown in Table V. Although some methylation selectivity can be achieved by variation of the on-column derivatisation reagent, such selectivity is not great and no reagent is clearly superior for *in situ* derivatisation of this class of substances.

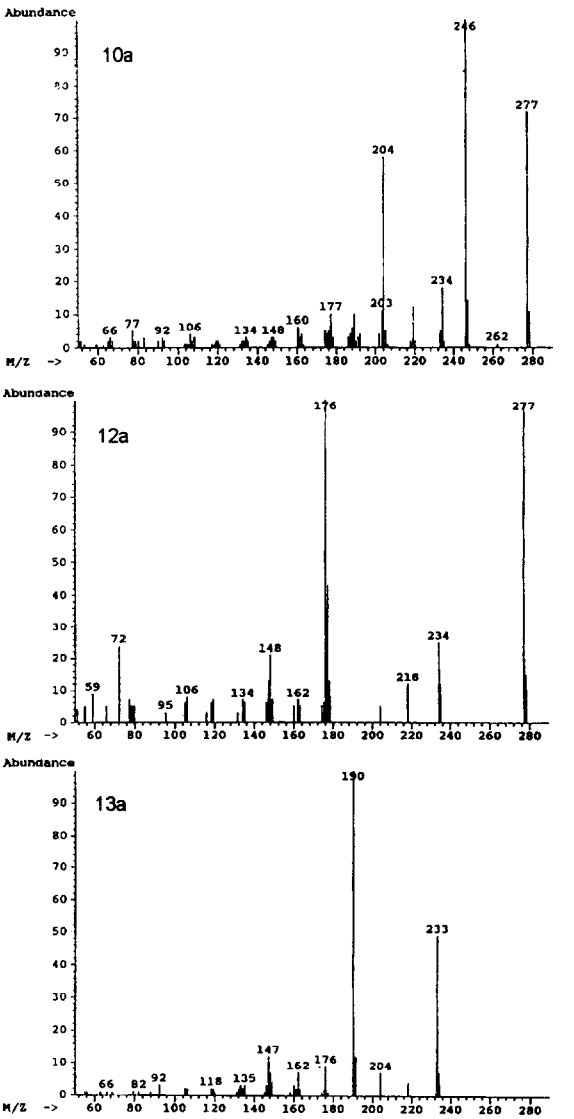


Fig. 9. The mass spectra of dimethylated oxibendazole (**10a**), the isomeric dimethylated oxibendazole (**12a**) and the trimethylated degradation product (**13a**).

CONCLUSIONS

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.

MethElute (PTMA-OH) and MethPrep

TABLE V

COMPARISON OF THE METHYLATION OF SOME THIOURACILS WITH DIFFERENT ON-COLUMN METHYLATION REAGENTS

Derivatising agent	Thiouracil substituent	Product 15		MS Fragmentation	Product 16		MS Fragmentation
		%	Time (min)		%	Time (min)	
TMPA-OH	None	60	4.73	156 (100%), 141 (42),	40	6.18	156 (40%), 141 (5), 123
TMPA-F		45		126 (3), 125 (3), 111	55		(4), 112 (15), 111 (100),
TMPA-CN		40		(10), 110 (47), 95 (18)	60		110 (28), 109 (73)
TTPA-OH		45			55		
Phase transfer					100		
TMPA-OH	5-Methyl		5.80	170 (100%), 155 (37),		6.71	170 (66%), 155 (9), 127
TMPA-F		66		125 (9), 124 (39), 109	34		(9), 126 (22), 125 (100),
TMPA-CN		33		(14)	67		124 (35), 123 (36), 109
TTPA-OH		60			40		(8), 95 (22)
Phase transfer					100		
TMPA-OH	5-Propyl	55	8.42	198 (100%), 183 (18),	45	9.50	198 (47%), 197 (20), 183
TMPA-F		45		170 (12), 169 (50),	55		(30), 169 (100), 153 (16),
TMPA-CN		70		110 (11), 96 (88)	30		88 (15), 83 (25)
TTPA-OH		45			55		
Phase transfer					100		
TMPA-OH	5-Phenyl	80	9.28	232 (100%), 217 (28),	20	10.16	232 (68%), 217 (8), 188
TMPA-F		45		186 (35), 171 (20),	55		(10), 187 (60), 186 (28),
TTPA-OH		67		145 (17), 129 (15),	33		185 (25), 145 (18), 116
Phase transfer				128 (15), 102 (16)	100		(20), 102 (18), 83 (100)

(TTMA-OH) are perfectly satisfactory in the derivatisation of mono-functional compounds but produce mixtures, often very complex, when used for methylation of multi-functional substances.

PTMA-CN is readily prepared and is a far more selective alternative to MethElute for direct on-column methylation whilst still providing good yields of methylation products. Overall methylation efficiency is also dependent on the GC injector temperature whilst the condition of the injection liner can exert a significant effect on both methylation efficiency and selectivity.

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