

## THE IDENTIFICATION OF URINARY ACIDS BY COUPLED GAS CHROMATOGRAPHY-MASS SPECTROMETRY

O. A. MAMER, J. C. CRAWHALL AND (MISS) SIOE SAN TJOA

*Department of Clinical Biochemistry, Royal Victoria Hospital, Montreal (Canada)*

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## SUMMARY

A tabulation of the important and characteristic  $m/e$  values in the mass spectra of the trimethylsilyl ether, ester and amide derivatives of acids and related compounds frequently found in urine or other biological fluids is presented as an aid in identifying gas-chromatographically separated components of extracts.

A quick method for routinely extracting millilitre quantities of urine is described along with a silylation technique that is facilitated by the use of easily made and inexpensive reaction vessels.

## INTRODUCTION

In a continuing study of genetically inherited metabolic diseases we have developed a methodology of isolation, silylation and characterization of the ether-extractable urinary acids applicable on millilitre urine samples. The characterization of components in an extract so small as to allow only one gas chromatographic injection is easily made certain by direct comparison of the mass spectrum of each peak with a library of spectra obtained from authentic compounds. Such direct comparison also is valuable in estimating the relative proportions of two or more components eluting simultaneously from the gas chromatograph.

In an effort to reduce the work of those investigators having access to a coupled gas chromatograph-mass spectrometer system and faced with accumulating a library of known spectra, we include in this report a summary of the important and characteristic  $m/e$  values and intensities (relative to the base peak intensity = 100%) of some of the compounds in our library. The compounds we have chosen are trimethylsilyl (TMS) ethers, esters and amides of authentic heterocyclic, aliphatic, aromatic and phenolic acids and related compounds that frequently occur in urine or other biological fluids.

Deciding which  $m/e$  values are important and characteristic in each spectrum has been a somewhat subjective and arbitrary process, but in general, the following considerations were observed. A given  $m/e$  value and its intensity will be listed:

1. if it is the molecular ion and can be detected,
2. or if the intensity is greater than 0.5% of the base peak and it has an  $m/e$  value greater than 50% of the  $m/e$  value of the molecular ion.
3. or if the intensity is greater than 2% of the base peak and it has an  $m/e$  value less than 50% of the  $m/e$  value of the molecular ion but greater than 73.

This approach provides for the listing of relatively weak but highly characteristic heavy fragment ions while ignoring for the sake of brevity the weaker of the usually more intense light fragments that are less important in the identification of an unknown. Considerable flexibility has been allowed however to differentiate between spectra of isomers that are often similar.

Peaks below  $m/e$  73 (trimethylsiliconium ion) are not listed because the time required to scan in excess of the decade  $m/e$  60–600 (approx. 5 sec) would allow a large change in concentration of the eluting derivative in the ion source. The response characteristics of the recording oscillograph limit the use of faster scan rates. The scans were started near the top of the eluting peak on the ascending side so that by the time the molecular ion was recorded, the source pressure was near its maximum value enhancing slightly the intensity of this frequently weak but very important peak (Fig. 1). Some compounds yield more than one TMS derivative if a hindered amine or slowly enolizable carbonyl group is present. In these cases, a spectrum is presented for each derivative.

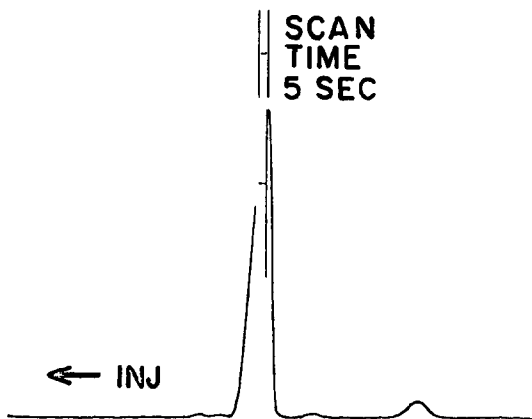


Fig. 1. Typical gas chromatographic peak showing blanking of pen movement during scan of mass spectrum. The scan is begun at a time such that the source pressure is a maximum while the molecular ion intensity is being recorded.

The data provided here can be used as a basis for a card file. Each card could be assigned an  $m/e$  value and would have listed on it all silylated compounds having that  $m/e$  value as a characteristic peak. For example, card # 179 would list *p*-hydroxyphenyl acetic acid, mandelic acid, homovanillic acid, and others. In attempting to identify an unknown, those cards having numbers corresponding to the observed intense  $m/e$  values in the unknown spectrum would be pulled from the file, and at a glance, one would know which compounds are the most likely candidates for direct comparison.

## MATERIALS AND APPARATUS

Most of the spectra were derived from compounds purchased from Sigma Chemical Company of St. Louis, Mo., Nutritional Biochemicals Corporation of Cleveland, Ohio, British Drug Houses Limited of Poole England, and Fisher Scientific Company of Fair Lawn, N.J. The silylation reagent (Tri-Sil/BSA) was purchased from Pierce Chemical Company of Rockford Ill. The gas chromatography-mass spectrometry was done on an LKB 9000. The extractions were done in unstoppered test tubes altered as shown in Fig. 2 which allows very efficient mixing on a "Vortex-Genie" (Fisher Scientific) but prevents the liquid from climbing the tube walls and spilling. Glass-stoppered centrifuge tubes of appropriate size could be used but have the disadvantage of having to prevent the introduction of artifacts by repeated stopper handling.

The silylations were carried out in reaction vessels made with a minimum of skill from Pyrex glass tubing and stoppered with rubber serum caps (Fisher Scientific) in such a way as to expose as little as possible of the rubber surface to the reacting mixture (Fig. 3). Before use, the caps were leached over night in acetone at room temperature to remove extractable potential contaminants. These silylation tubes are more convenient than the commercially available screw cap vials as they are so easily and inexpensively made that they can be discarded after use.

The glassware used in all stages of work-up should be freed of grease or other

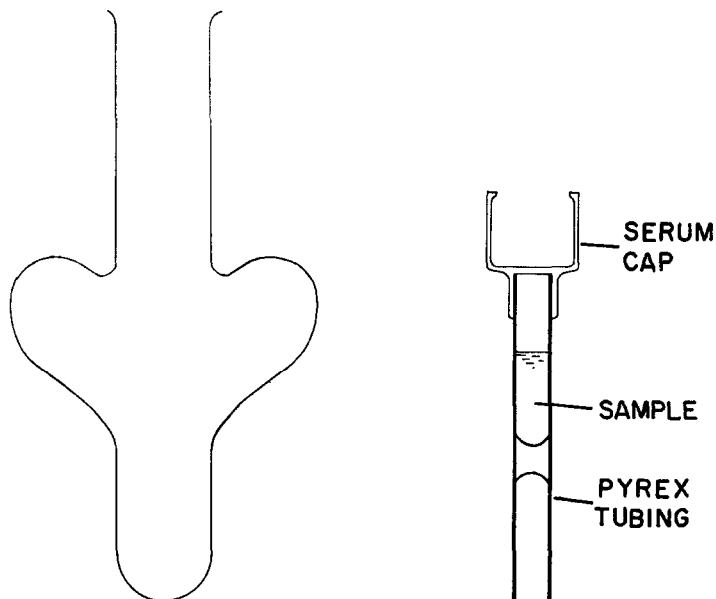


Fig. 2. Cross-section through a test-tube (18 × 150 mm) altered with a barrier that prevents spillage during agitation.

Fig. 3. Silylation reaction tube made by collapsing a short length of Pyrex tubing near the centre. One chamber is closed with a serum cap as shown and is used as the reaction volume. With tubing 3 mm O.D., silylations of microgram samples in 5 microlitre volumes can be easily done and sample recovery by syringe is very simple. The lower half of the tube can be used for support in the water bath or to hold a label.

organic films by rinsing in dilute warm hydrofluoric acid (1%, 30–50°) until the glass surface is uniformly hydrophilic (approx. 5 sec). A final rinse in distilled water is recommended.

#### METHODS

The urine (1–5 ml) is saturated with sodium chloride at room temperature and is made basic (pH 12 approximately) with concentrated sodium hydroxide. The solution is extracted three times with volumes of ether (microanalytical quality) equal to the urine volume. These ether extracts contain the neutral and basic solutes in the urine and for the purposes of the study at hand are discarded. Concentrated hydrochloric acid is added to the aqueous phase until it is strongly acidic (pH 1 approximately). The urine is again extracted three times with volumes of ether (microanalytical grade) equal to the urine volume. The ether extracts are dried over anhydrous sodium sulphate and evaporated to dryness in a silylation tube swept with dry nitrogen. The tube is capped and a volume of Tri-Sil/BSA equal to 2% of the original volume of urine is added by syringe through the serum cap. The tube is stood upright in a water bath (50–60°, 30 min) leaving the cap free of the water surface. Aliquots were withdrawn by syringe through the serum cap and injected on column.

The instrument parameters found suitable for these analyses are as follows: injection sample: 0.1–5  $\mu$ l (depending on sample concentration, 1  $\mu$ l usually); flash heater: 275°; column conditions: 6'  $\times$  1/4", 3% SE 30 Ultraphase on Chromosorb W (HP) 80/100 mesh (Pierce Chemical) temperature programmed from 80° to 280° at 4°/min with no delay; flow rate: approx. 30 ml helium per min; separator 285°; ion source temperature: 290°; ionizing energy and current: 70 eV, 120  $\mu$ A; scan time 4 sec between m/e 60 and 600; acceleration potential: 3.5 kV.

The mass spectra of the authentic compounds listed at the end of this article were obtained by introducing a few crystals (10–100  $\mu$ g) of the authentic compound (as received from the supplier) into a silylation tube along with approximately 0.10 ml of silylating agent (Tri-Sil/BSA). After heating for 30 min in a water bath (50–60°), 1–2  $\mu$ l aliquots were withdrawn by syringe and injected on column. Instrument parameters were the same for the authentic compounds as for the urine extracts.

In the case of compounds having only carboxyl, phenolic and alkyl hydroxyl substitution, silylation is apparently complete after the above reaction time has elapsed and only one peak of any import is noted in the GC of the silylate mixture.

The degree of separation on gas chromatography of the silylates of the authentic compounds is illustrated in Fig. 4. Peaks c and d in Fig. 4 are the *bis*-trimethylsilyl derivatives of 2-hydroxycaproic acid and succinic acid respectively. No trace of the other compound can be detected in the mass spectrum of each when the spectra are taken near the tops of the peaks. Since the chromatography record is a plot of ion beam current *versus* time on the LKB 9000, it is a fairly faithful indication of the partial pressure of the organo-silicon compound in the source at any given time. Return to baseline values of the ion current is assurance that there is indeed little memory of past sample peaks and little anticipation of samples to come. Hence, the spectra reported in the library are of the TMS derivatives of the authentic compounds uncontaminated by silylation agents and solvents. The polysiloxanes which have characteristic mass spectra and appear to be by-products of the silylation reaction

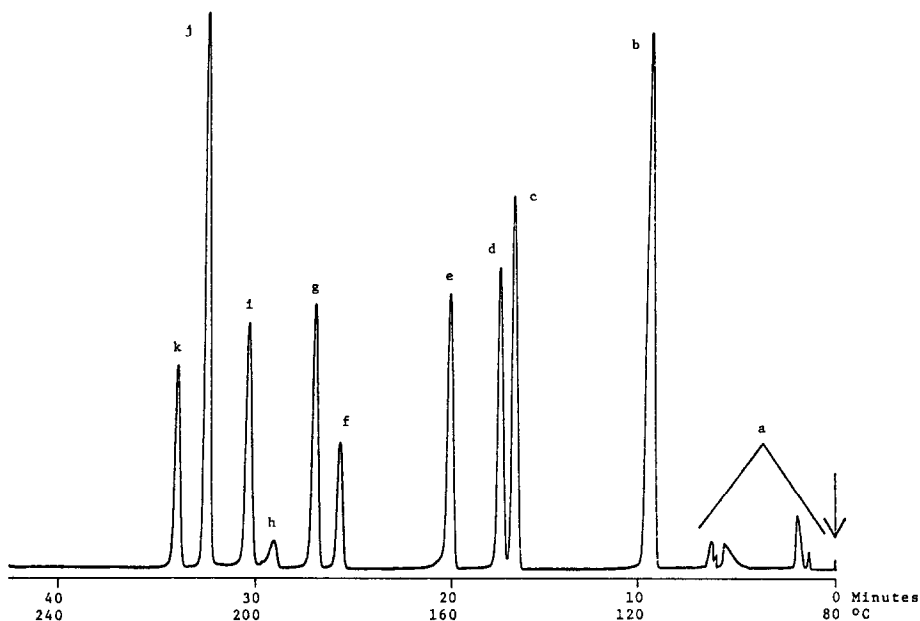
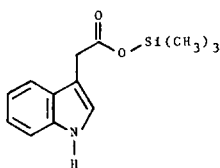


Fig. 4. Gas chromatogram of the TMS derivatives of a mixture of authentic acids frequently found in urine. By their mass spectra, the peaks are identified as: a, silylating reagents and solvents; b, lactic acid; c,  $\alpha$ -hydroxy-caproic acid; d, succinic acid; e, *O*-lactyllactic acid ( $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2\text{CH}(\text{CH}_3)\text{COOH}$ ); f, phenyllactic; g, *p*-hydroxyphenylacetic acid; h, an unknown contaminant of the lactic acid; i, homovanillic and vanillic acid 2:1; j, citric acid; k, *p*-hydroxyphenyllactic acid. The simultaneous elution of vanillic and homovanillic acid TMS derivatives can be detected by an examination of the mass spectrum of peak i as explained in the text.

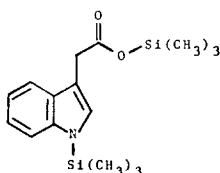
elute much later and at terminal program temperatures and do not contribute in any detectable way to these spectra. Experience has shown that the intensity of the polysiloxane GC peaks increases with sample age and exposure to atmospheric humidity. For this reason the silylates should be analyzed as soon as possible after derivation.

Some compounds having slowly enolizable carbonyl groups or hindered non-tertiary amines will often show more than one significant peak on gas chromatography. The indole acids are typical of this behaviour.

3-Indoleacetic acid, for example, yields two well resolved significant GC peaks. The first peak is unsymmetrical and has a molecular ion of  $m/e$  247. The second peak is symmetrical and has a molecular ion of  $m/e$  319. Further heating of the silylate mixture causes an increase in the size of the second peak at the expense of the first.



I



II

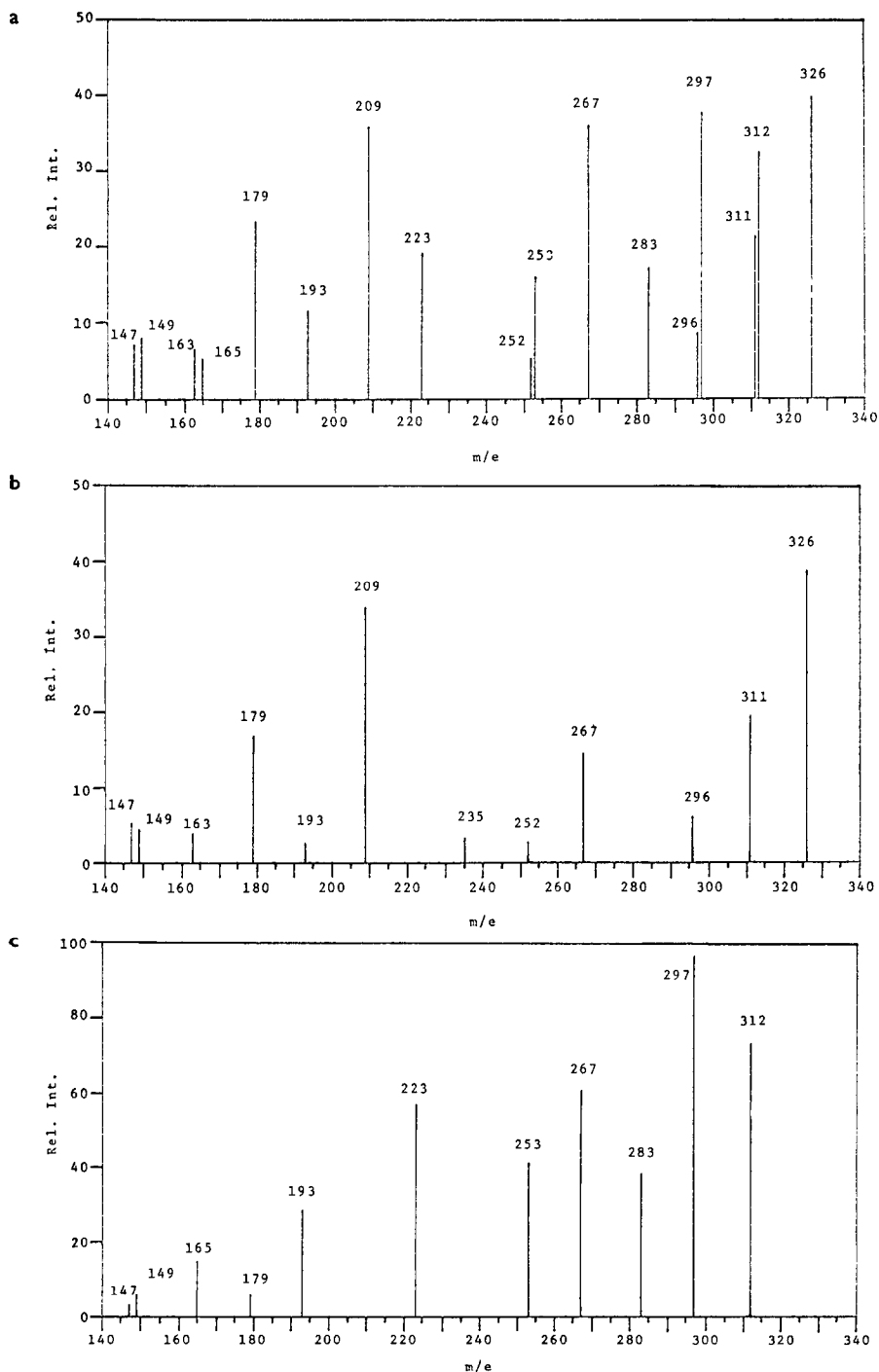


Fig. 5. The partial mass spectrum of (a) peak i in figure 4; (b) authentic homovanillic acid and (c) authentic vanillic acid. The three spectra are normalized with respect to m/e 73 being the 100% base peak. The presence of the spectra of both vanillic and homovanillic acid TMS derivatives is clearly evident in the spectrum of peak i of Fig. 4.

The chromatography and spectrum of the first peak is consistent with it being the trimethylsilyl ester of 3-indoleacetic acid (I), and similarly the second peak is *N,O*-bis-(trimethylsilyl)-3-indoleacetic acid (II).

## RESULTS

Fig. 4 shows the gas chromatogram of a synthetic mixture of the TMS derivatives of some authentic acids frequently found in urine. As previously explained, although peaks c and d elute closely together there is no reasonable trace of one in the other that can be detected in the mass spectra of the two peaks. A different situation occurs however in the case of peak i, the spectrum of which is represented in Fig. 5 (a). Close inspection of peak i would hardly betray the fact that it represents the simultaneous elution of two compounds. Examination of the spectrum of peak i indicates the presence of two molecular ions,  $m/e$  326 and 312, corresponding to homovanillic and vanillic acids respectively. The respective spectra of these two single acids are reproduced in Fig. 5 (b) and (c) and it can be seen that the spectrum of peak i is a contribution of about 1 part vanillic acid to 2 parts homovanillic acid (ignoring relative ion sensitivities). *p*-Hydroxyphenylacetic acid and *p*-hydroxybenzoic acid similarly elute simultaneously with the conditions of chromatography used here and with the aid of a card file embodying these spectra this fact may be detected.

These spectra are useful in identifying components of a derivatized mixture of

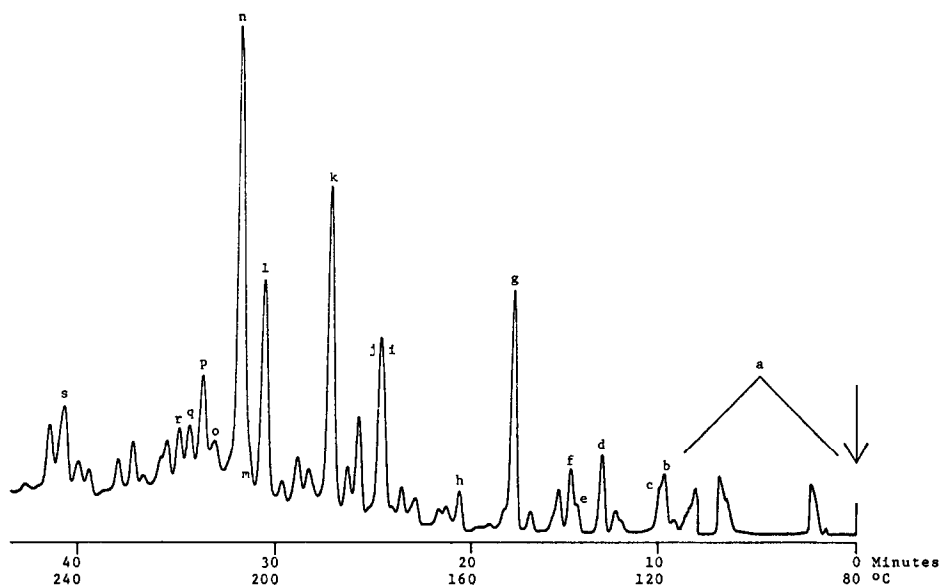


Fig. 6. Gas chromatogram of the acidic substances extracted from the urine of a 3-year-old apparently normal male. The peaks are identified as: a, silylating reagents and solvents; b, lactic acid; c,  $\alpha$ -hydroxyisobutyric acid; d,  $\gamma$ -hydroxybutyric acid; e,  $\alpha$ -methyl- $\beta$ -hydroxybutyric acid; f,  $\beta$ -hydroxy- $\beta$ -methyl butyric acid; g, succinic acid; h, glutaric acid; i and j, see text; k, *p*-hydroxyphenylacetic acid and *p*-hydroxybenzoic acid, approximately 8:1; l, vanillic acid and homovanillic acid approximately 1:3; m (shoulder), *p*-hydroxymandelic acid; n, hippuric acid; o, hydroxyphenylhydracrylic acid (*m*-isomer possibly); p, vanillylmandelic acid; q, *p*-hydroxyphenylacetic acid; r, 3-indoleacetic acid; s, 5-hydroxyindoleacetic acid.

acids extracted from urine. Fig. 6 is the gas chromatogram of the TMS derivatives of the acidic compounds extracted by the method described here from the urine of K.M., the 3-year-old son of one of the authors. The complexity of the mixture is obvious, yet the major peaks and many of the minor ones are readily identifiable using the library in the form of a card file. Unresolved GC peaks are readily identified. For example b and c are lactic and 2-hydroxyisobutyric acids, m (shoulder) and n are *p*-hydroxymandelic and hippuric acids respectively and peak l is the mixture of vanillic and homovanillic acids in approximately the ratio 1:3 respectively.

By comparison with the library, unknown peaks may be partially characterized. For example, i and j (spectra reproduced in Fig. 7) are two compounds eluting nearly simultaneously. Spectrum i (Fig. 7 (a)) was obtained on the ascending side of the peak while spectrum j (Fig. 7 (b)) was obtained on the descending side of the peak. The molecular ion in i appears to be  $m/e$  304 with a stronger ion at 303 representing the loss of a hydrogen atom from 304. This behaviour is typical of hydroxy-substituted fatty

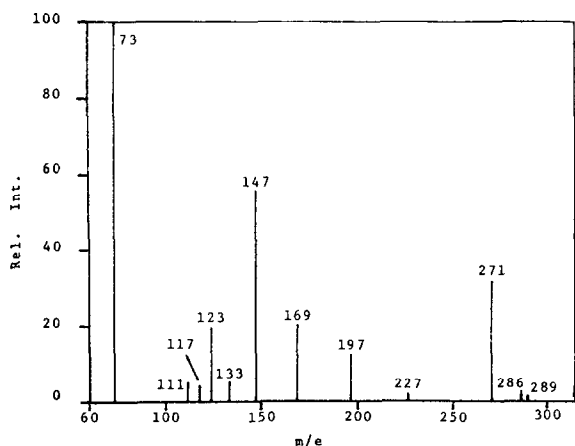
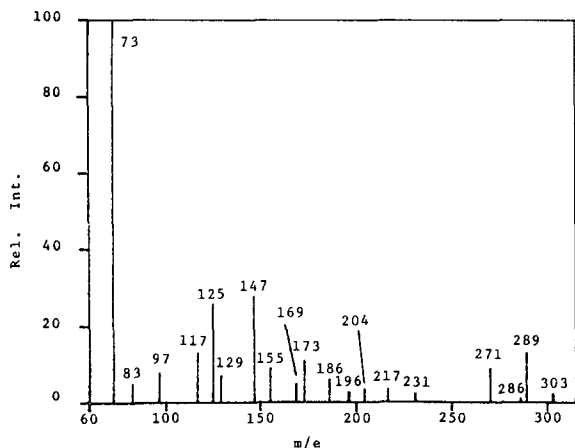


Fig. 7. Spectra taken on the ascending side (a) and descending side (b) of peak labelled i and j in Fig. 6, clearly showing the near simultaneous elution of two unknown compounds under a single envelope. See text for a partial identification of these unknowns.



|                                       |        |        |        |        |       |        |
|---------------------------------------|--------|--------|--------|--------|-------|--------|
| Lactic Acid -ITMS                     | 156    | 01.19  | 198    | 01.19  | 149   | 01.05  |
| 234                                   | 196    | 00.67  | 197    | 00.67  | 127   | 25.75  |
| 213                                   | 147    | 02.62  | 147    | 02.62  | 91    | 03.50  |
| 206                                   | 106    | 100.00 | 106    | 100.00 | 89    | 02.37  |
| 225                                   | 79     | 06.63  | 79     | 06.63  | 75    | 21.88  |
| 191                                   | 77     | 11.06  | 77     | 11.06  | 73    | 100.00 |
| 197                                   | 75     | 17.94  | 75     | 17.94  |       |        |
| 195                                   | 73     | 22.78  | 73     | 22.78  |       |        |
| Lactic Acid -2TMS                     |        |        |        |        |       |        |
| 234                                   | 147    | 00.87  | 147    | 00.87  |       |        |
| 213                                   | 121    | 100.00 | 121    | 100.00 |       |        |
| 206                                   | 91     | 05.56  | 91     | 05.56  |       |        |
| 225                                   | 89     | 07.13  | 89     | 07.13  |       |        |
| 191                                   | 89     | 14.20  | 89     | 14.20  |       |        |
| 197                                   | 77     | 15.27  | 77     | 15.27  |       |        |
| 195                                   | 75     | 08.78  | 75     | 08.78  |       |        |
| 137                                   | 73     | 58.32  | 73     | 58.32  |       |        |
| Phenylacetic Acid -ITMS 208           |        |        |        |        |       |        |
| 208                                   | 02.89  | 115    | 00.47  | 116    | 03.44 |        |
| 193                                   | 04.24  | 117    | 05.32  | 103    | 06.40 |        |
| 164                                   | 30.80  | 137    | 04.96  | 102    | 04.68 |        |
| 137                                   | 04.96  | 137    | 05.73  | 101    | 01.72 |        |
| 117                                   | 04.96  | 137    | 05.73  | 79     | 03.44 |        |
| 91                                    | 28.78  | 117    | 05.32  | 77     | 08.86 |        |
| 75                                    | 68.51  | 121    | 02.20  | 75     | 19.70 |        |
| 73                                    | 100.00 | 121    | 02.20  | 73     | 61.08 |        |
| 73                                    | 100.00 |        |        |        |       |        |
| Urea -2TMS 204                        |        |        |        |        |       |        |
| 204                                   | 04.94  | 220    | 29.73  |        |       |        |
| 189                                   | 60.73  | 207    | 07.05  |        |       |        |
| 179                                   | 04.13  | 206    | 17.80  |        |       |        |
| 171                                   | 10.69  | 205    | 100.00 |        |       |        |
| 147                                   | 01.13  | 173    | 03.71  |        |       |        |
| 157                                   | 100.00 | 172    | 09.09  |        |       |        |
| 133                                   | 03.89  | 171    | 54.17  |        |       |        |
| 132                                   | 05.38  | 145    | 13.71  |        |       |        |
| 131                                   | 05.16  | 135    | 14.85  |        |       |        |
| 130                                   | 05.16  | 131    | 89.02  |        |       |        |
| 100                                   | 08.55  | 103    | 57.58  |        |       |        |
| 99                                    | 05.96  | 79     | 34.24  |        |       |        |
| 87                                    | 07.75  | 77     | 42.99  |        |       |        |
| 79                                    | 11.02  | 75     | 71.97  |        |       |        |
| 75                                    | 18.66  | 73     | 27.77  |        |       |        |
| 74                                    | 15.64  |        |        |        |       |        |
| 73                                    | 38.47  |        |        |        |       |        |
| Benzoic Acid -ITMS 194                |        |        |        |        |       |        |
| 194                                   | 04.71  |        |        |        |       |        |
| 179                                   | 71.21  |        |        |        |       |        |
| 135                                   | 50.00  |        |        |        |       |        |
| 105                                   | 97.14  |        |        |        |       |        |
| 77                                    | 100.00 |        |        |        |       |        |
| 75                                    | 09.07  |        |        |        |       |        |
| 73                                    | 03.64  |        |        |        |       |        |
| g-Methoxyphenyl-acetic Acid -ITMS 238 |        |        |        |        |       |        |
| 238                                   | 00.18  |        |        |        |       |        |
| 223                                   | 02.81  |        |        |        |       |        |
| 195                                   | 02.63  |        |        |        |       |        |
| 193                                   | 01.71  |        |        |        |       |        |
| 179                                   | 16.89  |        |        |        |       |        |
| 165                                   | 01.45  |        |        |        |       |        |
| 147                                   | 00.87  |        |        |        |       |        |
| 121                                   | 100.00 |        |        |        |       |        |
| 105                                   | 05.56  |        |        |        |       |        |
| 91                                    | 07.13  |        |        |        |       |        |
| 89                                    | 14.20  |        |        |        |       |        |
| 77                                    | 15.27  |        |        |        |       |        |
| 75                                    | 08.78  |        |        |        |       |        |
| 73                                    | 58.32  |        |        |        |       |        |
| m-Methoxyphenyl-acetic Acid -ITMS 238 |        |        |        |        |       |        |
| 238                                   | 03.63  |        |        |        |       |        |
| 237                                   | 08.65  |        |        |        |       |        |
| 207                                   | 00.32  |        |        |        |       |        |
| 194                                   | 02.55  |        |        |        |       |        |
| 179                                   | 02.19  |        |        |        |       |        |
| 167                                   | 00.64  |        |        |        |       |        |
| 145                                   | 00.53  |        |        |        |       |        |
| 148                                   | 00.89  |        |        |        |       |        |
| 147                                   | 00.77  |        |        |        |       |        |
| 117                                   | 04.02  |        |        |        |       |        |
| 91                                    | 05.42  |        |        |        |       |        |
| 85                                    | 03.48  |        |        |        |       |        |
| 75                                    | 24.17  |        |        |        |       |        |
| 73                                    | 100.00 |        |        |        |       |        |
| p-Methoxyphenyl-acetic Acid -ITMS 238 |        |        |        |        |       |        |
| 238                                   | 13.05  |        |        |        |       |        |
| 223                                   | 11.28  |        |        |        |       |        |
| 208                                   | 00.37  |        |        |        |       |        |
| 195                                   | 03.28  |        |        |        |       |        |
| 179                                   | 35.38  |        |        |        |       |        |
| 167                                   | 02.26  |        |        |        |       |        |
| β-Hydroxybutyric Acid -2TMS 248       |        |        |        |        |       |        |
| 248                                   | 00.19  |        |        |        |       |        |
| 207                                   | 00.24  |        |        |        |       |        |
| 205                                   | 16.11  |        |        |        |       |        |
| 204                                   | 02.78  |        |        |        |       |        |
| 203                                   | 03.24  |        |        |        |       |        |
| 191                                   | 32.43  |        |        |        |       |        |
| 187                                   | 100.00 |        |        |        |       |        |
| 183                                   | 06.76  |        |        |        |       |        |
| 181                                   | 04.65  |        |        |        |       |        |
| 130                                   | 10.00  |        |        |        |       |        |
| 117                                   | 27.57  |        |        |        |       |        |
| 88                                    | 21.62  |        |        |        |       |        |
| 79                                    | 08.38  |        |        |        |       |        |
| 75                                    | 29.73  |        |        |        |       |        |
| 73                                    | 94.53  |        |        |        |       |        |
| 5-Hydroxyindole -ITMS (ether) 205     |        |        |        |        |       |        |
| 205                                   | 92.27  |        |        |        |       |        |
| 190                                   | 100.00 |        |        |        |       |        |
| 163                                   | 07.83  |        |        |        |       |        |
| β-Phenylglycine -ITMS (ester) 223     |        |        |        |        |       |        |
| 223                                   | 12.19  |        |        |        |       |        |
| 208                                   | 04.16  |        |        |        |       |        |

|                     |       |                    |        |                     |        |                 |               |                   |        |                    |        |       |
|---------------------|-------|--------------------|--------|---------------------|--------|-----------------|---------------|-------------------|--------|--------------------|--------|-------|
| Maleonic Acid -2TMS | 248   | 01.77              | 105    | 100.63              | 219    | 08.03           | Methylmalonic | 261               | 05.12  | 219                | 00.74  |       |
| 249                 | 01.19 | 77                 | 46.54  | 190                 | 03.28  | 262             | 00.50         | 247               | 00.26  | 190                | 03.23  |       |
| 253                 | 00.49 | 75                 | 20.44  | 145                 | 56.39  | 262             | 06.91         | 232               | 15.12  | 171                | 02.00  |       |
| 204                 | 00.35 | 73                 | 66.04  | 133                 | 06.39  | 231             | 00.23         | 217               | 02.67  | 159                | 100.00 |       |
| Fumaric Acid -2TMS  | 189   | 00.27              | 131    | 02.46               | 129    | 01.49           | 218           | 05.94             | 191    | 03.02              | 147    | 50.77 |
| 260                 | 00.00 | 129                | 01.49  | 119                 | 00.51  | 157             | 01.05         | 186               | 01.40  | 133                | 16.38  |       |
| 245                 | 01.08 | 117                | 01.97  | 147                 | 100.00 | 147             | 100.00        | 171               | 01.45  | 103                | 13.69  |       |
| 217                 | 01.08 | 115                | 00.95  | 133                 | 03.78  | 133             | 03.78         | 160               | 01.74  | 79                 | 02.77  |       |
| 201                 | 00.48 | 113                | 00.44  | 129                 | 01.30  | 131             | 02.73         | 147               | 33.72  | 75                 | 16.92  |       |
| 131                 | 01.21 | 102                | 01.06  | 117                 | 01.78  | 117             | 01.78         | 131               | 01.74  | 73                 | 96.92  |       |
| 157                 | 01.81 | 79                 | 03.44  | 116                 | 01.96  | 116             | 01.96         | 129               | 02.44  | α-Hydroxy-iso-     |        |       |
| 155                 | 02.54 | 75                 | 14.95  | 115                 | 01.10  | 115             | 01.10         | 117               | 100.00 | caproic Acid -2TMS |        |       |
| 147                 | 34.38 | 73                 | 99.02  | 94                  | 00.94  | 94              | 00.94         | 75                | 19.77  | 276                |        |       |
| 143                 | 15.35 | 262                | 00.17  | 86                  | 00.92  | 86              | 01.92         | 73                | 88.37  | 276                |        |       |
| 133                 | 05.72 | 261                | 00.33  | 79                  | 03.25  | 79              | 03.25         | 101               | 00.35  | 276                |        |       |
| 115                 | 06.54 | 247                | 02.92  | 73                  | 17.85  | 73              | 17.85         | 262               | 100.00 | 276                |        |       |
| 84                  | 03.50 | 231                | 02.92  | 262                 | 00.76  | 262             | 00.76         | 249               | 01.43  | 155                | 03.78  |       |
| 83                  | 05.13 | 218                | 16.33  | 204                 | 02.92  | 204             | 02.92         | 248               | 03.33  | 147                | 37.19  |       |
| 75                  | 27.05 | 217                | 34.58  | 157                 | 34.58  | 157             | 34.58         | 247               | 15.87  | 131                | 09.95  |       |
| 73                  | 57.94 | 147                | 70.83  | 147                 | 70.83  | 147             | 70.83         | 218               | 19.50  | 113                | 54.37  |       |
| Maleic Acid -2TMS   | 260   | 00.15              | 245    | 24.96               | 260    | 02.92           | 260           | 02.92             | 145    | 04.65              | 101    | 02.27 |
| 260                 | 00.15 | 215                | 01.03  | 170                 | 02.28  | 170             | 02.28         | 172               | 03.57  | 97                 | 02.06  |       |
| 208                 | 05.44 | 147                | 100.00 | 143                 | 04.49  | 143             | 04.49         | 116               | 02.77  | 79                 | 02.49  |       |
| 207                 | 23.58 | 143                | 04.49  | 133                 | 04.83  | 133             | 04.83         | 116               | 02.77  | 73                 | 100.00 |       |
| 206                 | 66.61 | 126                | 02.45  | 115                 | 05.58  | 115             | 05.58         | 101               | 03.02  | -5-Hydroxyindole   |        |       |
| 190                 | 03.07 | 88                 | 01.32  | 262                 | 00.07  | 262             | 00.07         | 97                | 02.49  | -2TMS 277          |        |       |
| 177                 | 02.22 | 83                 | 04.29  | 261                 | 00.17  | 261             | 00.17         | 129               | 04.29  | 277                | 40.00  |       |
| 163                 | 01.51 | 79                 | 02.08  | 247                 | 12.53  | 247             | 12.53         | 116               | 01.51  | 247                | 02.81  |       |
| 161                 | 00.60 | 75                 | 16.09  | 231                 | 00.74  | 231             | 00.74         | 75                | 48.27  | 262                | 02.50  |       |
| 149                 | 00.65 | 74                 | 07.96  | 218                 | 19.75  | 218             | 19.75         | 73                | 100.00 | 235                | 04.49  |       |
| 125                 | 01.93 | 73                 | 85.03  | 209                 | 1.1.60 | 209             | 1.1.60        | 276               | 00.05  | 234                | 02.52  |       |
| 124                 | 02.44 | α-Hydroxy-iso-     |        | 191                 | 1.1.73 | 191             | 1.1.73        | 275               | 00.05  | 233                | 02.52  |       |
| 118                 | 02.20 | valeric Acid -2TMS |        | 157                 | 02.96  | 157             | 02.96         | 261               | 06.62  | 218                | 03.01  |       |
| 117                 | 02.55 | 262                |        | 147                 | 70.37  | 147             | 70.37         | 245               | 00.35  | 205                | 02.43  |       |
| 116                 | 02.39 | 262                |        | 117                 | 100.00 | 117             | 100.00        | 233               | 06.00  |                    |        |       |
| 107                 | 00.65 | 247                | 00.05  | 75                  | 20.99  | 75              | 20.99         |                   |        |                    |        |       |
| 106                 | 08.49 | 247                | 05.90  | 73                  | 95.06  | 73              | 95.06         |                   |        |                    |        |       |
| Glutaric Acid -2TMS | 276   |                    |        | Succinic Acid -2TMS | 262    |                 |               | α-Hydroxy-caproic | 276    |                    |        |       |
| 276                 | 00.47 | 262                | 00.76  | 262                 | 00.76  | 262             | 00.76         | Acid -2TMS 276    | 276    | 00.05              |        |       |
| 254                 | 03.05 | 249                | 01.43  | 249                 | 01.43  | 249             | 01.43         |                   | 275    | 00.05              |        |       |
| 254                 | 03.05 | 248                | 03.33  | 248                 | 03.33  | 248             | 03.33         |                   | 245    | 00.35              |        |       |
| 254                 | 03.05 | 247                | 15.87  | 247                 | 15.87  | 247             | 15.87         |                   | 233    | 06.00              |        |       |
| 254                 | 03.05 | 218                | 01.67  | 218                 | 01.67  | 218             | 01.67         |                   |        |                    |        |       |
| 254                 | 03.05 | 172                | 03.57  | 172                 | 03.57  | 172             | 03.57         |                   |        |                    |        |       |
| 254                 | 03.05 | 145                | 04.65  | 145                 | 04.65  | 145             | 04.65         |                   |        |                    |        |       |
| 254                 | 03.05 | 116                | 02.77  | 116                 | 02.77  | 116             | 02.77         |                   |        |                    |        |       |
| 254                 | 03.05 | 116                | 02.77  | 133                 | 03.02  | 133             | 03.02         |                   |        |                    |        |       |
| 254                 | 03.05 | 131                | 01.67  | 131                 | 01.67  | 131             | 01.67         |                   |        |                    |        |       |
| 254                 | 03.05 | 129                | 04.29  | 129                 | 04.29  | 129             | 04.29         |                   |        |                    |        |       |
| 254                 | 03.05 | 116                | 01.51  | 116                 | 01.51  | 116             | 01.51         |                   |        |                    |        |       |
| 254                 | 03.05 | 75                 | 48.27  | 75                  | 48.27  | 75              | 48.27         |                   |        |                    |        |       |
| 254                 | 03.05 | 73                 | 100.00 | 73                  | 100.00 | 73              | 100.00        |                   |        |                    |        |       |
| α-Hydroxy-caproic   |       | α,α-Dimethyl-β-    |        | α,α-Dimethyl-β-     |        | α,α-Dimethyl-β- |               | α-Hydroxy-caproic |        |                    |        |       |
| Acid -2TMS 276      |       | hydroxybutyric     |        | hydroxybutyric      |        | hydroxybutyric  |               | Acid -2TMS 276    |        |                    |        |       |
|                     |       | Acid -2TMS 276     |        | Acid -2TMS 276      |        | Acid -2TMS 276  |               |                   |        |                    |        |       |
|                     |       | 276                | 00.02  | 276                 | 00.02  | 276             | 00.02         |                   |        |                    |        |       |
|                     |       | 275                | 00.06  | 275                 | 00.06  | 275             | 00.06         |                   |        |                    |        |       |

|     |       |  |       |   |        |   |        |                                       |        |        |
|-----|-------|--|-------|---|--------|---|--------|---------------------------------------|--------|--------|
| 190 | 02.02 | 147  | 09.54 | 175   | 01.42  | 252   | 05.42  | 220                                   | 10.84  |        |
| 188 | 02.25 | 134  | 09.54 | 151   | 02.35  | 175   | 100.00 | 219                                   | 05.31  |        |
| 174 | 02.72 | 118  | 05.59 | 149   | 06.30  | 163   | 02.94  | 205                                   | 03.79  |        |
| 172 | 02.54 | 92   | 03.58 | 147   | 04.07  | 147   | 22.16  | 195                                   | 03.00  |        |
| 147 | 02.70 | 91   | 03.77 | 135   | 10.34  | 256   | 00.88  | 194                                   | 05.15  |        |
| 142 | 01.87 | 75   | 13.38 | 91  | 07.86  | 252   | 02.84  | 193                                   | 57.31  |        |
| 133 | 01.86 | 73   | 84.57 | 73  | 100.00 | 237   | 01.23  | 179                                   | 00.21  |        |
| 130 | 03.80 |  |       |   |        | 225   | 02.11  | 178                                   | 00.46  |        |
| 79  | 07.27 | <b>N-Phenylglycine</b>                     |       |   | 209    | 00.93   | 118    | 01.29                                 | 177    | 00.93  |
| 74  | 06.23 | <b>-2TMS 282</b>                           |       |   | 177    | 02.05   | 117    | 00.94                                 | 163    | 01.11  |
| 73  | 71.74 | <b>-2TMS 295</b>                           |       |   | 295    | 05.07   | 106    | 02.27                                 | 161    | 01.51  |
|     |       | 282  | 45.00 | 280   | 01.80  | 164   | 00.90  | 147                                   | 43.27  |        |
|     |       | 268  | 16.74 | 252   | 02.30  | 149   | 08.46  | 135                                   | 01.37  |        |
|     |       | 267  | 24.63 | 223   | 02.16  | 104   | 00.61  | 133                                   | 05.19  |        |
|     |       | 225  | 08.00 | 208   | 01.05  | 91  | 01.58  | 131                                   | 04.03  |        |
|     |       | 224  | 07.11 | 207   | 00.71  | 90  | 02.73  | 121                                   | 00.74  |        |
|     |       | 223  | 30.74 | 206   | 00.42  | 89  | 02.14  | 119                                   | 00.84  |        |
|     |       | 209  | 01.10 | 178   | 100.00 | 75  | 01.86  | 117                                   | 00.16  |        |
|     |       | 207  | 02.00 | 162   | 00.82  | 73  | 86.03  | 115                                   | 00.64  |        |
|     |       | 195  | 03.03 | 147   | 10.14  | <b>p-Hydroxyphenyl-<br/>acetic Acid -2TMS</b> |        |                                       |        |        |
|     |       | 194  | 07.45 | 106   | 23.78  | 296   | 09.98  | 104                                   | 02.98  |        |
|     |       | 193  | 41.32 | 79  | 05.78  | 296   | 20.23  | 103                                   | 02.82  |        |
|     |       | 181  | 20.65 | 77  | 05.78  | 281   | 08.12  | 91                                    | 06.87  |        |
|     |       | 179  | 01.35 | 75  | 11.28  | 252   | 10.53  | 75                                    | 11.14  |        |
|     |       | 177  | 03.93 | 73  | 84.28  | 237   | 00.59  | 74                                    | 08.73  |        |
|     |       | 165  | 05.37 | <b>m-Hydroxyphenyl-<br/>acetic Acid -2TMS</b> |        |   | 190    | 05.58                                 | 73     | 100.00 |
|     |       | 163  | 01.49 | 296   | 04.03  | 191   | 02.56  | <b>Homovanillyl<br/>Alcohol -2TMS</b> |        |        |
|     |       | 149  | 12.94 | 281   | 03.56  | 181   | 12.79  | 312                                   |        |        |
|     |       | 147  | 10.51 | 252   | 02.60  | 179   | 69.76  | 312                                   | 29.00  |        |
|     |       | 126  | 14.93 | 237   | 00.44  | 163   | 03.72  | 297                                   | 11.00  |        |
|     |       | 91   | 09.12 | 181   | 01.51  | 135   | 02.32  | 287                                   | 04.23  |        |
|     |       | 79   | 06.63 | 179   | 01.15  | 133   | 01.27  | 262                                   | 00.90  |        |
|     |       | 75   | 12.13 | 164   | 11.68  | 118   | 03.48  | 223                                   | 03.85  |        |
|     |       | 73   | 64.12 | 149   | 04.17  | 75  | 13.72  | 209                                   | 100.00 |        |
|     |       | <b>0-Hydroxybenzoic<br/>Acid -2TMS 282</b> |       |   | 179    | 01.92   | 74     | 09.07                                 | 211    | 05.75  |
|     |       | 282  | 00.10 | 177   | 01.15  | 135   | 00.92  | 193                                   | 03.90  |        |
|     |       | 267  | 46.35 | 164   | 11.68  | 117   | 01.22  | 192                                   | 04.20  |        |
|     |       | 249  | 01.24 | 149   | 03.36  | 105   | 00.95  | 179                                   | 17.25  |        |
|     |       | 233  | 00.98 | 135   | 00.80  | 75  | 14.41  | 149                                   | 03.35  |        |
|     |       | 209  | 03.72 | 133   | 01.51  | 73  | 100.00 | 147                                   | 01.58  |        |
|     |       | 193  | 04.69 | 117   | 00.76  | <b>β-Phenylacetic Acid</b>                    |        |                                       |        |        |
|     |       | 181  | 00.97 | 105   | 02.93  | <b>-2TMS 310</b>                              |        |                                       |        |        |
|     |       | 179  | 01.72 | 75  | 26.43  | 295   | 04.72  | 149                                   | 03.55  |        |
|     |       | 177  | 00.66 | 73  | 100.00 | 268   | 01.19  | 141                                   | 03.55  |        |
|     |       |  |       |   |        | 267   | 05.00  | 103                                   | 08.00  |        |
|     |       |  |       |   |        | 222   | 00.61  | 89                                    | 02.75  |        |
|     |       |  |       |   |        | 221   | 02.41  | 75                                    | 05.50  |        |
|     |       |  |       |   |        | <b>Mandelic Acid</b>                          |        |                                       |        |        |
|     |       |  |       |   |        | <b>-2TMS 296</b>                              |        |                                       |        |        |
|     |       |  |       |   |        | 296   | 00.05  |                                       |        |        |
|     |       |  |       |   |        | 281   | 01.84  |                                       |        |        |





acid derivatives. A large  $m/e$  147 suggests that the unknown is a di-silyl derivative. By examining the intensity ratios of  $m/e$  290 and 291 to 289, it would appear that mass 289 has an empirical formula of  $C_{13}H_{29}O_3Si_2$  in keeping with the usual loss of  $CH_3$  radical from the molecular ion of 304. Therefore  $m/e$  304 is probably the molecular ion of  $C_{14}H_{32}O_3Si_2$ , which is the *bis*-trimethylsilyl derivative of  $C_8H_{16}O_3$  which may be an unknown hydroxyoctanoic acid isomer. Peak j appears to have a molecular ion of  $m/e$  286 and apparently is a *bis*-trimethylsilyl derivative of  $C_7H_{10}O_3$  which may be an unknown keto heptenoic acid isomer. There is some evidence of cross-contamination between i and j (*e.g.*  $m/e$  271 and 286 in i and  $m/e$  289 in j)

Below are listed the important and characteristic  $m/e$  values and their corresponding intensities (as % of the base peak) in the mass spectra of some of the silylated compounds in our library. The headings of each spectrum summary include the name of the compound, the number of trimethylsilyl groups in the molecule and the  $m/e$  value of the molecular ion. For example, laevulic acid silylates once and the silylated acid has a molecular ion of  $m/e$  188. The summaries are arranged in order of increasing molecular ion values.

The summaries are printed out by means of a Hewlett-Packard 9100A calculator and although the intensity values appear to be stated to two decimal places, such precision is of course not warranted nor often required. Adequate description of a spectrum for qualitative purposes rarely requires more than two significant digits in the peak intensities and it is only to allow the inclusion of very weak molecular ions (*i.e.* much less than 1% base) that the printer limits were set to two decimal places. In our experience, the intensities of the ions reported are representative and may be expected to vary somewhat with instrument parameters such as ion source temperature and efficiency, ion extraction, focussing and acceleration potentials and not least of all make and model of instrument.

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