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# Automated screening procedure using gas chromatography–mass spectrometry for identification of drugs after their extraction from biological samples

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ABSTRACT

A novel analytical screening procedure has been developed, using computer-controlled gas chromatography–mass spectrometry (GC–MS), to detect 120 drugs of interest to road safety. This paper describes GC–MS methodology suitable for use on extracts of biological origin, while extraction procedures will be the subject of a future communication. The method was devised to identify drugs in extracts of blood samples, as part of an investigation into the involvement of drugs, other than alcohol, in road accidents. The method could be adapted to screen for other substances. The method depends on a "macro" program which was written to automate the search of GC–MS data for target drugs. The strategy used was to initially search for each drug in the database by monitoring for a single characteristic ion at the expected retention time. If a peak is found in this first mass chromatogram, a peak for a second characteristic ion is sought within 0.02 min of the first and, if found, the ratio of peak areas calculated. Probable drug identification is based on the simultaneous appearance of peaks for both characteristic ions at the expected retention time and in the correct ratio. If the ratio is outside acceptable limits, a suspected drug (requiring further investigation) is reported. The search macro can use either full mass spectra or, for enhanced sensitivity, data from selected ion monitoring (which requires switching between groups of ions during data acquisition). Quantitative data can be obtained in the usual way by the addition of internal standards.

## INTRODUCTION

While the relationship between alcohol and road accidents has been well documented, little is known of the contribution of other drugs to road safety. A major difficulty has been the need to analyse a large number of blood samples, taken from road users involved in accidents, for the presence of drugs which could impair driving performance. A great variety of chemically dissimilar substances are used as medicines and social drugs and have the capacity to impair driving performance and the potential, therefore, to contribute to road accidents. This presents a considerable analytical challenge, which has been managed in previous studies by restricting the analytical screen to a relatively small number of drugs which are considered most likely to be involved in road accidents. Even so, batteries of analytical methods (radioimmunoassay, thin-layer chromatography, gas

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chromatography, high-performance liquid chromatography) have been used to screen for drugs in blood samples taken in road accident studies [1–4], as in many toxicological screens. These methods are often time-consuming and generally lack specificity, although drug findings can be confirmed by subsequent gas chromatography–mass spectrometry (GC–MS) [1,4].

Since many drugs contain nitrogen, GC with nitrogen–phosphorus detection (GC–NPD) can be used for initial screening [5], an approach taken in some previous road accident studies [6–8] and in other drug screening procedures [9–11]. GC–NPD provides a single analytical procedure which is sufficiently sensitive and selective to detect a large number of drugs. However, as drug detection is based only on retention time, searching complex chromatograms is laborious, and possible drug findings need to be confirmed by an additional analysis, such as GC–MS. Another problem, which we found in a previous study [8], was that even GC–NPD chromatograms of extracts of blood samples contained large numbers of extraneous peaks which eluted close to the retention times of drugs: these all required subsequent analysis by GC–MS although most proved negative. In addition, important non-nitrogenous drugs, especially tetrahydrocannabinol and its metabolites and anti-inflammatory agents, cannot be detected by NPD.

This report describes a GC–MS method of screening for 120 drugs of possible concern to road safety. Compared with previous methods, it has better specificity and sensitivity, and is faster in searching for the targeted drugs. In cases where the identification of a drug is critical, such as in forensic work and in analysis by selected ion monitoring (SIM), it may be necessary to confirm the drug finding by appropriate re-analysis to increase the number of ions used. Sample extraction procedures have not been investigated in this study, which instead focuses on the problem of the efficient identification of drugs in complex chromatograms, where there are many possible drugs present. The biomedical application of this method will be the subject of a separate paper.

## EXPERIMENTAL

### *Materials*

Water was purified by the Millipore-Q decontamination system (Millipore, Sydney, Australia). Organic solvent were HPLC grade (Waters Assoc., Division of Millipore, Sydney, Australia) except chloroform (nanograde, Mallinckrodt, Melbourne, Australia). All were found to be free from contaminants by GC analysis of a thousand-fold concentrated sample. Glassware was washed using Extran 300 detergent (BDH Chemicals, Melbourne, Australia), followed by a Milli-Q water wash and a methanol wash. After drying, all glassware was rinsed in dichloromethane prior to use. A list of drugs of interest to road safety was prepared, based on the drugs reported most frequently in road accident studies in Australia and other commonly used drugs with the potential to impair driving performance. Most reference drug standards were obtained from pharmaceutical

companies (see Acknowledgements). Benzoylecognine, 3,4-methylenedioxymethylamphetamine (MDMA) and 9-carboxy-11-nor-$\Delta^9$-tetrahydrocannabinol were obtained from Alltech Assoc. (Deerfield, IL, U.S.A.) and tetrahydrocannabinol (THC) was a gift of the Research Triangle Institute (Research Triangle Park, NC, U.S.A.). *n*-Decane and *n*-triacontane were obtained from Sigma (St. Louis, MO, U.S.A.). Other chemicals were of analytical reagent grade.

*Equipment*

Samples for GC analysis (usually 1 ml) were placed in 1.5-ml crimp-top glass autosampler vials (Sun Brokers, Wilmington, NC, U.S.A.). Small volumes (less than 200 $\mu$l) were contained in smaller conical glass inserts (Microsun Insert, Sun Brokers). GC–MS analysis was performed on a Hewlett-Packard 5890 gas chromatograph and 5970 series mass-selective detector (Hewlett-Packard Australia, Melbourne, Australia). Automated injections were made with a Hewlett-Packard 7673A autosampler. Programming and data processing were carried out using a Hewlett-Packard 59970A workstation and Version 3.1.1 Pascal software. The injector was fitted with a wide-bore (4 mm) quartz liner, which contained a small plug of quartz wool in the centre. Free drugs and methylated derivatives were analysed on a 22 m × 0.32 mm I.D. fused-silica capillary column, coated with 0.52-$\mu$m cross-linked 5% phenyl methyl silicone gum (HP-5, Hewlett-Packard). Samples which had been treated with N-methyl-bis(trifluoroacetamide) (MBTFA; Pierce, Rockford, IL, U.S.A.) as a derivatising agent caused a loss of column performance when underivatised samples were analysed. Therefore, a separate column was used exclusively for analysis of samples treated with trifluoroacetylating reagent: this was a 12 m × 0.32 mm I.D. fused-silica column coated with 0.25-$\mu$m SE-30 gum (Econo-Cap, Alltech Assoc.). Each column was protected by a 20 cm length of the same type of column which was fitted as a pre-column, and changed when there was evidence of column activation. Separate analyses were performed for underivatised, methylated and trifluoroacetylated drugs. This reduced the total number of ions being monitored each time to a manageable size, as well as being consistent with conventional extraction procedures.

The following GC–MS conditions were used: injector temperature, 260°C; open-split interface temperature, 290°C; oven program, 40°C for 1 min, then increasing at 10°C/min to 290°C; carrier gas, helium, column head pressure, 105 kPa (22-m column) or 70 kPa (12-m column); column flow-rate at 40°C, 2.5 ml/min; sample size, 5$\mu$l; split ratio, 10:1.

*Reference samples*

To obtain reference GC and MS data, drug solutions (concentration 100 ng/$\mu$l) were prepared in chloroform and 1 ml was placed in a crimp-top autosampler vial. Hydrocarbon standards in chloroform (decane, 54 $\mu$g in 50 $\mu$l; triacontane, 26 $\mu$g in 100 $\mu$l) were added to the 1-ml sample to enable standardisation of GC retention times. Drugs which were obtained as salts were first dissolved in water

(1–2 mg in 1 ml), then the pH of the solution was adjusted with either 2 ml of 0.05 *M* sodium borate, pH 9.2 (for basic drugs) or 1 ml of 15% hydrochloric acid, pH 3 (for acidic drugs). The free drug was extracted with 1 ml of a mixture of dichloromethane–hexane–ethyl acetate (6:3:1) by vortex-mixing for 5 min and, after separation by centrifugation, the organic phase was transferred to a sample vial and concentrated to about 50 μl under nitrogen, then made to 1 ml with chloroform. An aliquot (usually 100 μl) was placed in a GC vial and made to 1 ml with chloroform, giving a final concentration of about 100 ng/μl. Drug solutions were stored at 4°C until analysed.

*Derivatisation*

Trifluoroacetyl derivatives were made of drugs with free hydroxyl, primary or secondary amino groups. This improved their chromatographic properties and enabled better resolution of peaks. The 1-ml drug solution in chloroform in the GC autosampler vial was concentrated to about 20 μl, 50 μl of MBTFA (Pierce) were added, and the vial was heated at 60°C for 30 min. Care was taken to keep the solvent dry and free from protic solvents to prevent cleavage of the trifluoroacetate derivatives. Then the sample was concentrated to about 20 μl under nitrogen and made to 1 ml with chloroform. Acidic drugs were similarly methylated with freshly prepared ethereal diazomethane (200 μl), except that the reaction was carried out at room temperature.

*Extracts from tissue*

Forensic samples were obtained from the Government Analyst (Hobart, Australia) as extracts of blood, urine or liver (TOXI-LAB Analytical Systems, Kansas City, MO, U.S.A.). This is similar to the extraction method described for reference samples. Extracts of biological samples were concentrated to 20–50 μl and placed in the small-volume inserts in the autosampler vials before GC–MS analysis. Blank plasma samples were similarly extracted to check on extraneous peaks and possible false positives.

*GC–MS analysis*

*Database.* Mass spectra and retention times of drugs were generated from the reference drug samples, which were analysed either individually or in simple mixtures. The "standard" retention times for decane (TC10 = 6.06 min) and triacontane (TC30 = 27.65 min) were chosen arbitrarily from an initial GC–MS run. Retention times from subsequent GC–MS analyses of reference drugs were corrected (TD, Table I), where necessary, by a transformation of the retention times of the two hydrocarbons to the standard times.

*GC–MS.* Full mass spectra were acquired by scanning over the mass range *m/z* 500 to 40 approximately once per second. For maximum sensitivity in analysing unknown samples, however, SIM is required. Because only twenty ions could be monitored at one time while there were 120 drugs to be searched for, the follow-

TABLE I

GC–MS DATA USED BY THE MACRO TO SCREEN FOR EACH DRUG

| Compound[a] | Retention time TD (min) | ION1[b] *(m/z)* | ION2 *(m/z)* | Peak ratio (ION1/ION2) |
|---|---|---|---|---|
| *Free drugs* | | | | |
| Benzaldehyde[c] (d)[d] | 5.42 | 105.05 | 77.05 | 1:1 |
| C10 | 6.07 | 71.05 | – | – |
| Valproic acid | 8.15 | 73.10 | 102.05 | 2:1 |
| Amphetamine | 8.17 | 91.05[e] | 65.05 | 2:1 |
| Methylamphetamine | 9.08 | 58.05 | 91.05 | 15:1 |
| Tranylcypromine | 9.58 | 132.10 | 115.05 | 5:3 |
| Ethosuximide | 9.88 | 55.05 | 113.05 | 1:1 |
| Ephedrine | 12.12 | 58.05 | 77.05 | 10:1 |
| MDMA | 14.00 | 58.05 | 135.05 | 20:1 |
| Clofibrate | 14.25 | 128.00 | 169.05 | 5:1 |
| Metronidazole | 15.55 | 81.05 | 124.05 | 5:4 |
| Tolbutamide (d) | 15.61 | 91.05 | 171.00 | 5:2 |
| Carbimazole | 15.79 | 186.05 | 114.05 | 2:1 |
| Paracetamol | 16.15 | 109.05 | 151.05 | 5:2 |
| Methyl phenidate | 16.58 | 84.10 | 91.05 | 10:1 |
| Pethidine | 16.82 | 172.10[f] | 247.15 | 1:1 |
| Pheniramine | 17.60 | 169.10 | 58.05 | 5:3 |
| Caffeine | 17.70 | 194.10 | 109.05 | 7:5 |
| Alprenolol | 17.78 | 72.10 | 249.15 | 20:1 |
| Ketamine | 18.00 | 180.05 | 209.10 | 5:1 |
| Methylphenobarbitone | 18.58 | 218.10 | 246.10 | 15:1 |
| Captopril | 18.82 | 70.05 | 198.10 | 10:1 |
| Phenyltoloxamine | 19.02 | 58.05 | 255.15 | 20:1 |
| Phenobarbitone | 19.21 | 204.10 | 232.10 | 10:1 |
| Theophylline | 19.23 | 180.05 | 95.00 | 2:1 |
| Fenoprofen | 19.30 | 242.10 | 197.10 | 1:1 |
| Dexchlorpheniramine | 19.68 | 203.05 | 58.05 | 1:1 |
| Clonidine | 20.52 | 229.00 | 171.95 | 2:1 |
| Ranitidine | 20.65 | 137.10 | 94.05 | 5:4 |
| Diphenylpyraline | 20.70 | 99.05 | 114.10 | 2:1 |
| Diclofenac | 20.90 | 214.05 | 242.05 | 5:4 |
| Dextromethorphan | 21.03 | 59.05 | 271.20 | 1:1 |
| Methadone | 21.10 | 72.10 | 294.20 | 50:1 |
| Dextropropoxyphene | 21.24 | 58.05 | 208.10 | 5:1 |
| Procyclidine | 21.41 | 84.10 | 204.15 | 20:1 |
| Amitriptyline | 21.59 | 58.05 | 275.20 | 50:1 |
| Hyoscyamine | 21.68 | 124.10 | 289.20 | 4:1 |
| Cocaine | 21.69 | 82.05 | 182.10 | 3:2 |
| Mianserin | 21.72 | 193.10 | 264.15 | 5:4 |
| Procainamide | 21.73 | 86.10 | 120.05 | 5:1 |
| Nortriptyline | 21.81 | 44.00 | 202.10 | 10:1 |
| Trimipramine | 21.82 | 58.05 | 249.15 | 5:1 |
| Imipramine | 21.88 | 234.15 | 280.20 | 5:2 |

*(Continued on p. 212)*

TABLE I (*continued*)

| Compound[a] | Retention time TD (min) | ION1[b] (*m/z*) | ION2 (*m/z*) | Peak ratio (ION1/ION2) |
|---|---|---|---|---|
| Doxepin | 21.92 | 58.05 | 277.15 | 50:1 |
| Primidone | 22.00 | 190.10 | 146.05 | 5:4 |
| Desipramine | 22.10 | 234.15 | 195.10 | 1:1 |
| Benzhexol | 22.13 | 98.10 | 218.15 | 20:1 |
| Triprolidene | 22.20 | 208.10 | 278.20 | 10:3 |
| Promethazine | 22.41 | 72.05 | 284.15 | 20:1 |
| Trimeprazine | 22.62 | 58.05 | 298.15 | 10:3 |
| Benztropine | 22.81 | 83.05 | 140.10 | 5:4 |
| Carbamazepine | 22.85 | 193.10 | 236.10 | 5:2 |
| Phenytoin | 22.86 | 180.05 | 252.10 | 5:3 |
| Oxazepam | 22.95 | 268.05 | 239.05 | 10:7 |
| Hyoscine | 23.09 | 94.05 | 138.10 | 3:1 |
| Cyproheptadine | 23.14 | 287.15 | 215.10 | 5:3 |
| Pizotifen | 23.23 | 295.15 | 96.00 | 5:4 |
| Azatidine | 23.36 | 246.15 | 290.20 | 6:5 |
| Dothiepin | 23.39 | 58.05 | 202.10 | 50:1 |
| Codeine | 23.45 | 299.15 | 162.10 | 2:1 |
| Sulphamethoxazole | 23.64 | 92.05 | 253.05 | 10:1 |
| Dihydrocodeine | 23.65 | 301.20 | 244.10 | 7:1 |
| Lorazepam | 23.69 | 239.05 | 274.00 | 1:1 |
| Morphine | 23.88 | 285.15 | 162.10 | 3:1 |
| Diazepam | 23.89 | 256.10 | 283.05 | 1:1 |
| $\Delta^9$-THC | 23.95 | 299.20 | 314.20 | 5:4 |
| Methdilazine | 24.19 | 296.15 | 199.05 | 1:1 |
| Disopyramide | 24.31 | 195.05 | 212.05 | 5:3 |
| Chlorpromazine | 24.38 | 58.05 | 318.10 | 5:1 |
| Desmethyldiazepam | 24.47 | 242.05 | 269.05 | 1:1 |
| Chlordiazepoxide | 24.61 | 282.10 | 247.10 | 5:1 |
| Oxycodone | 24.93 | 315.15 | 230.10 | 5:2 |
| Trimethoprim | 25.00 | 290.15 | 259.10 | 5:2 |
| Chloroquine | 25.11 | 86.10 | 319.20 | 10:1 |
| Haloperidol | 25.29 | 224.10 | 237.10 | 5:4 |
| Flunitrazepam | 25.42 | 285.10 | 312.10 | 1:1 |
| Metoclopramide | 25.43 | 86.10 | 184.00 | 10:1 |
| Trifluoperazine | 25.49 | 407.15 | 267.05 | 1:1 |
| Diamorphine | 25.56 | 327.15 | 369.15 | 3:2 |
| Nifedipine | 25.82 | 329.10 | 284.15 | 1:1 |
| Hydrochlorothiazide | 25.94 | 268.95 | 228.00 | 4:1 |
| Temazepam | 26.00 | 271.05 | 300.05 | 10:1 |
| Fentanyl | 26.05 | 245.15 | 146.05 | 3:1 |
| Nitrazepam | 26.70 | 280.05 | 253.10 | 5:4 |
| Sulindac | 27.17 | 296.05 | 239.05 | 5:3 |
| Quinine | 27.20 | 136.10 | 189.10 | 13:1 |
| Clonazepam | 27.34 | 314.05 | 280.05 | 1:1 |
| C30 | 27.65 | 71.05 | – | – |
| Clomiphene | 27.82 | 86.10 | 405.20 | 50:1 |

TABLE I (*continued*)

| Compound[a] | Retention time TD (min) | ION1[b] *(m/z)* | ION2 *(m/z)* | Peak ratio (ION1/ION2) |
|---|---|---|---|---|
| Dextromoramide | 27.97 | 100.10 | 265.15 | 5:4 |
| Miconazole | 28.23 | 159.00 | 334.95 | 5:1 |
| Diltiazem | 28.26 | 58.05 | 121.10 | 20:1 |
| Prochlorperazine | 28.38 | 373.15 | 272.05 | 3:1 |
| Thioridazine | 29.95 | 98.10 | 378.15 | 10:3 |
| Verapamil | 30.46 | 303.20 | 151.10 | 10:1 |
| Pholcodine | 30.92 | 114.10 | 100.10 | 10:7 |
| Glibenclamide | 35.17 | 169.05 | 287.10 | 5:1 |
| *Methylated drugs* | | | | |
| Valproic acid | 6.65 | 87.10 | 116.10 | 10:3 |
| Salicylic acid | 9.98 | 120.05 | 92.05 | 10:7 |
| Allopurinol (1)[g] | 11.39 | 164.05 | 80.05 | 1:1 |
| Ibuprofen | 14.07 | 161.15 | 220.15 | 4:1 |
| Allopurinol (2)[g] | 15.06 | 164.05 | 136.05 | 10:1 |
| Tolbutamide (d) | 15.74 | 91.05 | 185.00 | 2:1 |
| Captopril | 16.86 | 70.05 | 231.10 | 10:1 |
| Captopril ($Me_2$) | 17.80 | 70.05 | 128.05 | 5:3 |
| Diflunisal | 17.97 | 232.05 | 264.05 | 2:1 |
| Methylphenobarbitone | 18.00 | 232.10 | 175.05 | 10:1 |
| Phenobarbitone ($Me_2$) | 18.00 | 232.10 | 175.05 | 10:1 |
| Fenoprofen | 18.36 | 197.10 | 256.10 | 5:4 |
| Phenobarbitone | 18.58 | 218.10 | 246.10 | 20:1 |
| Naproxen | 19.56 | 185.05 | 244.10 | 2:1 |
| Chlorpropamide | 19.73 | 111.00 | 175.00 | 1:1 |
| Mefenamic acid | 20.43 | 223.10 | 255.15 | 10:9 |
| Tolbutamide (d) | 20.51 | 91.05 | 155.00 | 5:4 |
| Ketoprofen | 20.74 | 209.10 | 105.05 | 5:4 |
| Probenecid | 21.16 | 270.10 | 135.05 | 5:3 |
| Diclofenac | 21.55 | 214.05 | 242.05 | 2:1 |
| Benzoylecgonine | 21.69 | 82.05 | 182.10 | 5:4 |
| Phenytoin | 21.87 | 180.05 | 266.10 | 10:9 |
| Tolbutamide | 22.09 | 91.05 | 129.00 | 10:9 |
| Nitrazepam | 24.32 | 294.10 | 248.10 | 5:4 |
| Bendrofluazide ($Me_2$) | 24.86 | 254.00 | 347.00 | 10:7 |
| Clonazepam | 25.11 | 329.05 | 294.10 | 10:7 |
| Warfarin | 25.14 | 279.10 | 322.10 | 5:1 |
| Bendrofluazide | 25.31 | 240.00 | 333.00 | 2:1 |
| Enalaprilat | 25.35 | 220.15 | 317.15 | 10:1 |
| Enalaprilat ($Me_2$) | 25.73 | 234.10 | 331.15 | 10:1 |
| Nitrazepam ($Me_2$) | 25.98 | 267.10 | 294.10 | 10:9 |
| Chlorothiazide ($Me_2$) | 26.47 | 323.95 | 245.00 | 3:2 |
| Clonazepam | 26.67 | 328.05 | 294.10 | 1:1 |
| Frusemide ($Me_2$) | 27.13 | 81.05 | 358.05 | 5:2 |
| Chlorothiazide ($Me_3$) | 27.13 | 308.95 | 337.00 | 5:4 |
| $\Delta^9$-THC acid metabolite | 27.21 | 343.20 | 299.20 | 5:4 |
| Indomethacin | 27.40 | 139.00 | 371.10 | 5:2 |

*(Continued on p. 214)*

TABLE I (*continued*)

| Compound[a] | Retention time TD (min) | ION1[b] (*m/z*) | ION2 (*m/z*) | Peak ratio (ION1/ION2) |
|---|---|---|---|---|
| Bumetanide ($Me_2$) | 28.54 | 392.15 | 349.10 | 1:1 |
| Bumetanide | 29.23 | 318.10 | 378.15 | 1:1 |
| Sulindac | 30.70 | 233.05 | 354.10 | 5:3 |
| *Trifluoroacetylated drugs* | | | | |
| Amphetamine | 7.54 | 140.05 | 118.05 | 5:4 |
| Methylamphetamine | 8.93 | 154.05 | 110.05 | 2:1 |
| Tranylcypromine | 9.48 | 116.05 | 69.00 | 10:3 |
| Ephedrine | 9.61 | 154.04 | 110.05 | 5:1 |
| Metronidazole | 10.81 | 141.00 | 221.05 | 5:4 |
| Methoxyphenamine | 11.00 | 154.05 | 148.05 | 2:1 |
| Paracetamol | 11.04 | 108.00 | 205.05 | 5:3 |
| MDMA | 12.97 | 154.10 | 289.10 | 5:3 |
| Alprenolol ($TFA_2$) | 14.50 | 266.05 | 308.05 | 10:7 |
| Methylphenidate | 14.65 | 180.05 | 150.05 | 10:1 |
| Hydralazine ($TFA_2$ or $TFA_3$) | 14.84 | 281.00 | 295.00 | 5:1 |
| Oxprenolol ($TFA_2$) | 15.25 | 266.05 | 308.05 | 10:7 |
| Clonidine | 15.50 | 290.05 | 199.00 | 5:1 |
| Hyoscyamine | 16.20 | 124.10 | 271.20 | 5:2 |
| Metoprolol ($TFA_2$) | 16.39 | 266.05 | 308.05 | 5:3 |
| Propranolol ($TFA_2$) | 17.24 | 266.05 | 308.05 | 10:7 |
| $\Delta^9$-THC | 17.41 | 410.20 | 339.10 | 1:1 |
| Hyoscine | 17.52 | 94.05 | 399.15 | 5:1 |
| Morphine ($TFA_2$) | 18.29 | 364.10 | 477.10 | 5:2 |
| Codeine | 18.68 | 282.15 | 395.15 | 2:1 |
| Metoclopramide | 19.42 | 86.10 | 280.00 | 15:1 |
| Nortriptyline | 19.47 | 232.15 | 290.15 | 20:1 |
| Desipramine | 19.78 | 208.10 | 362.10 | 10:3 |
| Dihydrocodeine | 22.61 | 397.15 | 284.15 | 2:1 |
| Pholcodine | 24.35 | 100.10 | 114.10 | 10:9 |
| Terfenadine ($TFA_1$ or $TFA_2$) | 27.85 | 262.15 | 433.20 | 10:1 |

[a] Abbreviations: MDMA = 3,4-Methylenedioxymethylamphetamine; $\Delta^9$-THC = $\Delta^9$-tetrahydrocannabinol; $\Delta^9$-THC acid metabolite = 9-carboxy-11-nor-$\Delta^9$-tetrahydrocannabinol; $Me_2$ = dimethylated; $Me_3$ = trimethylated; $TFA_2$ = di(trifluoroacetylated); $TFA_3$ = tri(trifluoroacetylated).

[b] ION1 was the base peak, unless otherwise specified.

[c] Benzaldehyde was a decomposition product of ephedrine.

[d] (d) indicates a drug decomposition product.

[e] Base peak of amphetamine was *m/z* 44.

[f] Base peak of pethidine was *m/z* 71.

[g] Different positional isomers.

ing approach was taken. Two diagnostic ions (usually including the base peak) were chosen for each drug. An acquisition method file was created in which up to twenty ions were monitored during each of ten time periods (the maximum available with this instrument). Groups of ions were monitored during appropriate

A

```
NAME DRUGS
WRITELN 701, #10,#10,#10,#10
TAB HEADER, PRINTER:
WRITELN 701,"*****************************************************************"
WRITELN 701, #10,#10,#10,#10
CH 4:8,71
GETS
A=X
IF Y<50000
    WRITELN701,"*****************************************************************"
    WRITELN 701, "C10 AREA VERY LOW, CHECK THAT IDENTITY IS CORRECT"
    WRITELN 701,"*****************************************************************"
    WRITELN 701, #10,#10,#10,#10
ENDIF
CH 25:30,71
GETS
B=X
IF Y<50000
    WRITELN 701,"*****************************************************************"
    WRITELN 701, "C30 AREA VERY LOW, CHECK THAT IDENTITY IS CORRECT"
    WRITELN 701,"*****************************************************************"
    WRITELN 701, #10,#10,#10,#10
ENDIF
TC10=6.06
TC30=27.65
P=1
.....(START OF REPEATING SECTION OF MACRO)
TD=DRUGTIME
TDCOR=(A+((TD-TC10)/(TC30-TC10)) * (B-A))
CH TDCOR-0.2:TDCOR+0.2, ION1
THRESH THRESH1, TDCOR-1
INT
THRESH THRESH1, -(TDCOR-1)
N=NPEAKS
IF NPEAKS>0
    CH TDCOR-0.2: TDCOR+0.2, ION2
    THRESH THRESH2, TDCOR-1
    INT
    THRESH THRESH2, -(TDCOR-1)
    NB=NPEAKS
    IF NPEAKS>0
        EX
        Z=1
        WHILE N>0
            PE Z
            RO -1
            P1=PEAK_AREA
            RT=RET_TIME
            EX
            Y=1
            M=NB
            WHILE M>0
                PE Y
                RO -1
                IF RT-RET_TIME<0.02
                    IF RT-RET_TIME>-0.02
                        P2=PEAK_AREA
                        ARATIO=P1/P2
                        IF ARATIO < MAXRATIO
                            IF ARATIO > MINRATIO
                                WRITELN 701, "PROBABLE DRUGNAME FOUND"
                                WRITELN 701, #10,#10,#10,#10
                                N=0
                                P=2
                            ENDIF
                        ENDIF
                        IF P=1
                            WRITELN 701, "*****************************************************"
                            WRITELN 701, "IONS FOR DRUGNAME FOUND, RATIO SUSPECT"
                            WRITELN 701, "*****************************************************"
                            WRITELN 701, #10,#10,#10,#10
                        ENDIF
                        MERGE
                        CLEAR 3
                        CLEAR Y
                        CLEAR Z
                        DR 3,X
                        SCR
                        WRITELN 701, #10,#10
                        IF P=2
                            TAB RESULTS, PRINTER:
                            WRITELN 701, #10,#10,#10
                            WRITELN 701, "*****************************************************"
                            WRITELN 701, #10,#10,#10,#10
                            P=1
                        ENDIF
                    ENDIF
                    M=0
                ELSE
                    Y=Y+1
                    M=M-1
                ENDIF
            ENDWHILE
            Z=Z+1
            N=N-1
            EX
        ENDWHILE
    ENDIF
ENDIF
.....(END OF REPEATING SECTION OF MACRO)
QUIT
```

Fig. 1.

(*Continued on p. 216*)

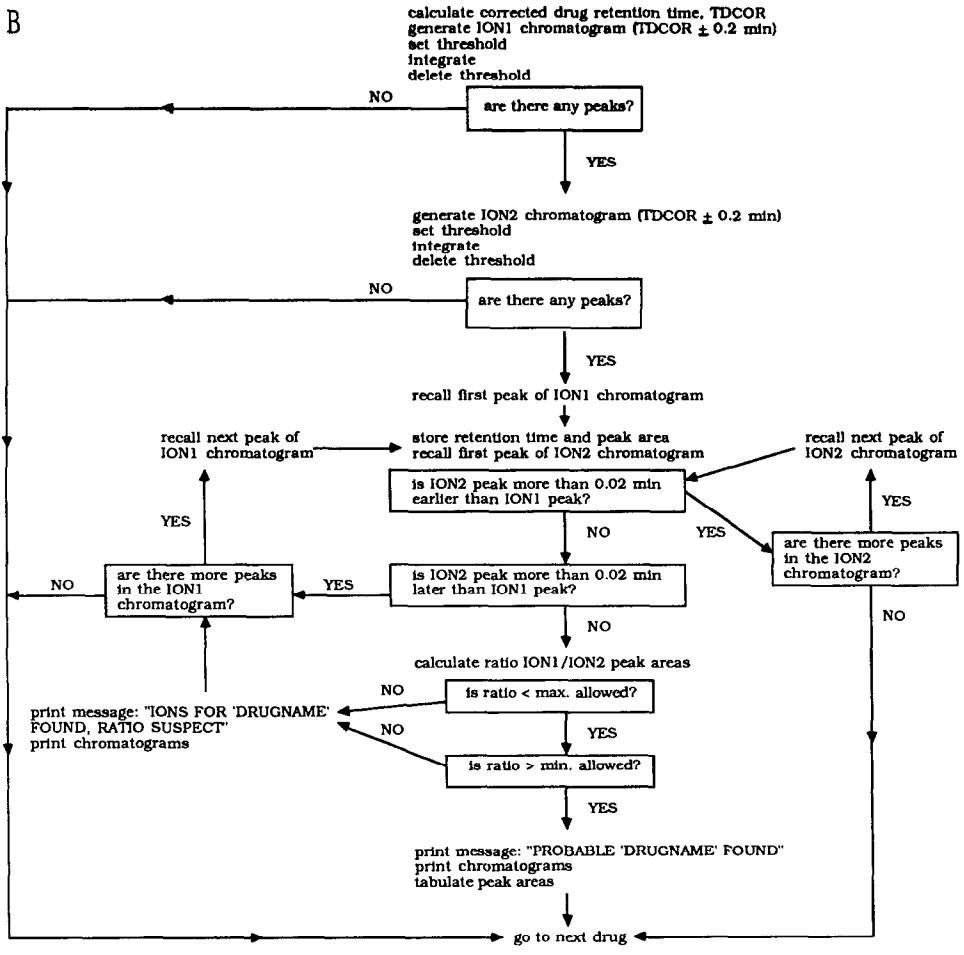


Fig. 1. (A) Listing of the macro program used to search the GC–MS acquisition file for drugs in the data base. The section between the two markers is repeated for each drug with appropriate names and values for the following variables: **DRUGTIME**, the standard drug retention time, based on retention times of 6.06 min for decane and 27.65 min for triacontane; **ION1**, the *m/z* value of the principal diagnostic ion; **THRESH1**, the integration threshold to use for ION1; **ION2**, the *m/z* value of the second diagnostic ion; **THRESH2**, the integration threshold to use for ION2; **MAXRATIO**, the maximum allowable ratio for the peak areas of ION1/ION2; **MINRATIO**, the minimum allowable ratio for the peak areas of ION1/ION2; **DRUGNAME**, the name of the target drug. (B) Flow diagram of the repeating section of the macro program.

time windows, based on the retention times of drugs of interest (Table I). This procedure enabled the maximum possible number of drugs to be included in the analytical screening procedure. Drugs which eluted close to the time when a different group of ions was monitored had to be included in both groups, to allow for changes in retention time. On the other hand, some ions were common to more than one drug, which improved efficiency (eleven drugs were monitored in one group).

Dwell time per ion was 30 ms. Masses of diagnostic ions were calculated to the nearest 0.05 mass units, based on ion structures. Where the empirical formula of the ion was not obvious, an estimate to the nearest 0.05 mass units was made. This level of accuracy was to enable interference from endogenous substances to be kept to a minimum, provided the maximum resolution of the mass spectrometer was used. For example, halogenated ions from drug molecules could be partially resolved from ions derived from lipids which had the same nominal mass, because of the lower mass defect of the halogenated ions.

*Macro.* Editing was automated by means of a "macro" built into the data editing portion of the sequencing software (Fig. 1A and B). This operated by checking a small time window (± 0.2 min) about the expected retention time of each drug for the principal diagnostic ion (ION1) and then, if successful, for the second ion (ION2). The macro program calculated the expected retention time (TDCOR) for each drug in the individual GC run based on a correction for any change in the retention times of decane and triacontane from the standard times. If both the primary (ION1) and secondary (ION2) ions for a drug were detected at the same time (within 0.02 min), the ratio of their peak areas (ION1/ION2) was compared with the expected ratio. Generous limits were allowed, because of the possible variability in relative ion abundances and the potential for interference from other substances in biological samples which could produce interfering ions at the same retention time. Thus if the expected ratio was 2, the limits could be > 1 to <4, although this must be decided for each drug and biological matrix depending on interferences.

In those cases when more than one peak was detected at the mass of ION1 or ION2 within the defined 0.4-min time window about the expected drug retention time, the macro checked all peaks until either a drug was found or all possibilities had been examined. Thus the retention time of the first possible ION1 peak was noted and the ION2 channel examined for the occurrence of a peak within 0.02 min of the retention time of the ION1 peak. If none was found, the next possible ION1 peak was considered and the ION2 channel again examined for a peak within 0.02 min of this second ION1 peak. This process continued until either a suitable pair of peaks was found or all options were exhausted. Results were only reported if there was a probable drug finding.

The macro consists principally of a repeating unit containing information on each drug: name, retention time, the two diagnostic ions with their thresholds for integration and the permissable range of ion ratios (Fig. 1A and B). The software does not allow this to be written as a single subroutine in which variables are substituted. The full macro for a hundred or so drugs is therefore quite long (160 KBytes), although each new drug can be readily added to it from a template of the repeating unit in which only the variables need to be changed.

Separate SIM data acquisition methods and macros were created for underivatised drugs, methylated drugs and trifluoroacetylated drugs. In practice, each biological sample would need to be split and subjected to three different

work-ups (for underivatised, methylated and trifluoroacetylated drugs), and these would then be analysed separately.

## RESULTS AND DISCUSSION

The value of the macro is in the relatively rapid, automated searching of the acquisition file for a large number of possible drugs: while this can be done manually, it would be very time-consuming. The macro took about 5 s to check for each drug, provided that it found no peaks within the time window for the

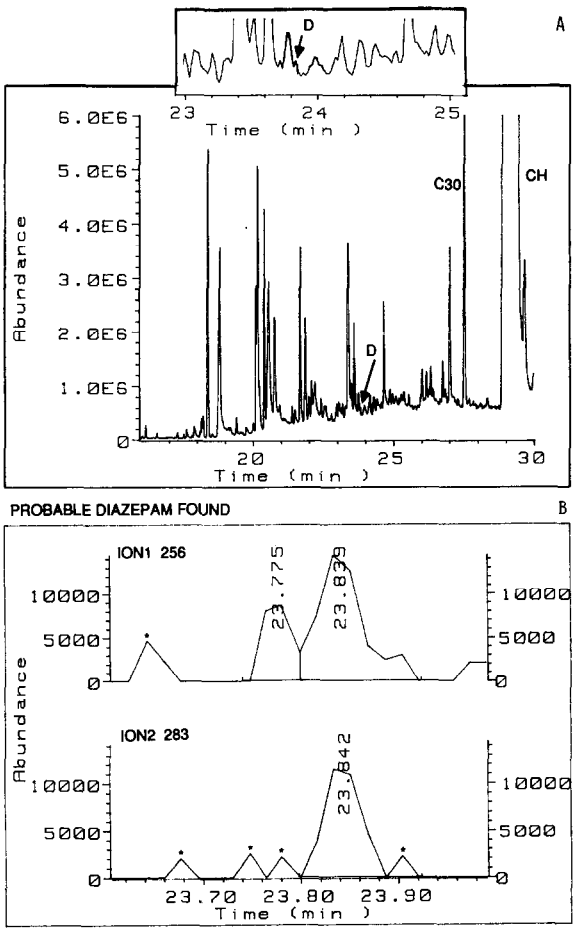


Fig. 2. (A) Portion of TIC chromatogram of an extract of a forensic blood sample. Arrow marks the position of diazepam peak, found in Fig. 2B. Inset: expansion of region from 23 to 25 min. CH is cholesterol. (B) Text and mass chromatograms for diagnostic ions for diazepam generated by the macro from the full scan acquisition data in (A), as part of a report of a probable diazepam finding (see text for details). The macro also tabulated retention times and peak areas for all integrated peaks. * = subthreshold peaks (not integrated).

principal diagnostic ion. Complex chromatograms with peaks for both ions present within the time window could take considerably longer. Automated editing time for biological samples could be up to 30 min per run, depending on the nature of the sample and the integration threshold levels used.

Fig. 2 illustrates the use of the macro in searching a complex chromatogram for target drugs. Fig. 2A shows a portion of a total ion current (TIC) chromatogram obtained by GC–MS analysis of a basic (pH 9) TOXI-LAB solvent extract of a forensic blood sample. Diazepam was present, but at a level giving only a very small peak, even when that region of the chromatogram was expanded

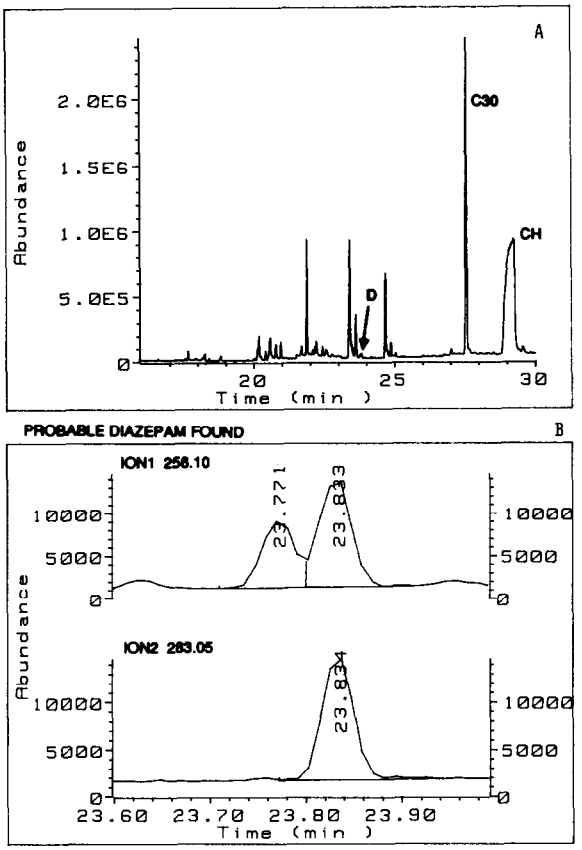


Fig. 3. (A) Portion of TIC chromatogram obtained by SIM re-analysis of the sample used in Fig. 2. The groups of ions monitored at different times are given in Table I. Arrow marks the position of diazepam peak, which is quite small even in SIM mode. (B) Text and mass fragmentograms generated by the macro from the SIM acquisition data in (A). Note that more accurate mass values were used for monitoring the diagnostic ions compared to full scan acquisition (Fig. 2B), and that there was less noise and smoother peak shapes.

(Fig. 2A insert). Fig. 2B shows the mass chromatograms generated by the macro from the full scan data in Fig. 2A. To search for diazepam, the macro first calculated the expected retention time of diazepam (TDCOR = 23.80 min in this run) by correcting for any changes in the retention times of C10 and C30, then examined the chromatogram of the first diagnostic ion for diazepam (ION1 = *m/z* 256) over the time interval within 0.2 min of TDCOR (*i.e.* 23.60–24.00 min). The first ION1 peak was below threshold, but the second (at 23.775 min) was not and this initiated a search in the ION2 (*m/z* 283) chromatogram for a peak occurring within 0.02 min of 23.775 min. No match was found, the second ION1 peak (23.839 min) was located, and this time paired with the ION2 peak at 23.842 min. The ratio of peak areas (ION1/ION2) was 1.39, within the set limits (0.5–2.0), and a report of a probable diazepam finding was printed, with the ION1 and ION2 chromatograms and a table of the areas of all integrated peaks. In this case, confirmation was obtained from a recognizable full spectrum of diazepam at the appropriate retention time, after careful background subtraction.

Fig. 3 shows the results from analysis of the same sample by SIM mode GC–MS. The TIC chromatogram (Fig. 3A) was produced by monitoring the ions listed in Table I for free drugs, sequentially in groups of up to twenty ions at a time. The diazepam peak was still quite small. Fig. 3B shows the mass fragmentograms generated by the macro as in Fig. 2B, except that in this case more accurate values for ION1 (*m/z* 256.10) and ION2 (*m/z* 283.05) were monitored during the SIM acquisition. Due to the inherently longer dwell times of SIM, and the more rapid sampling, there was less noise and smoother peak shapes than when the same nominal masses were acquired from full scans (Fig. 2B).

False positive findings could often be rejected by visual inspection of the mass chromatograms, as illustrated in Fig. 4. The SIM chromatogram of a basic extract (TOXI-LAB) of liver (a forensic sample) shows peaks near the retention times of diazepam and nordiazepam (Fig. 4A), and these drug findings were confirmed by the macro report. A probable finding of haloperidol was also reported by the macro, but inspection of the mass fragmentograms in Fig. 4C shows this to have been an error due to the threshold values having been set too low. The macro integrated an ION1 peak at 25.084 min and paired it with an ION2 peak at 25.075 min (peak ratio 0.54, limits 0.5–2.0), but inspection of the chromatogram (Fig. 4C) shows that neither was a real peak (compare Fig. 4B). The only possible ION1 and ION2 peaks in Fig. 4C did not occur within 0.02 min of each other and would not have led to a report of haloperidol.

It was more important to avoid false negative findings, which would result in drugs being missed altogether, than false positives, which could be detected by examination of the macro report or an additional GC–MS analysis. Interferences in one or both ion channels could cause the ION1/ION2 ratio to be outside the set limits, and this was reported as "IONS FOR **DRUGNAME** FOUND, RATIO SUSPECT", indicating the need for further investigation. This could be readily done for drugs which chromatographed in both free and derivatised forms

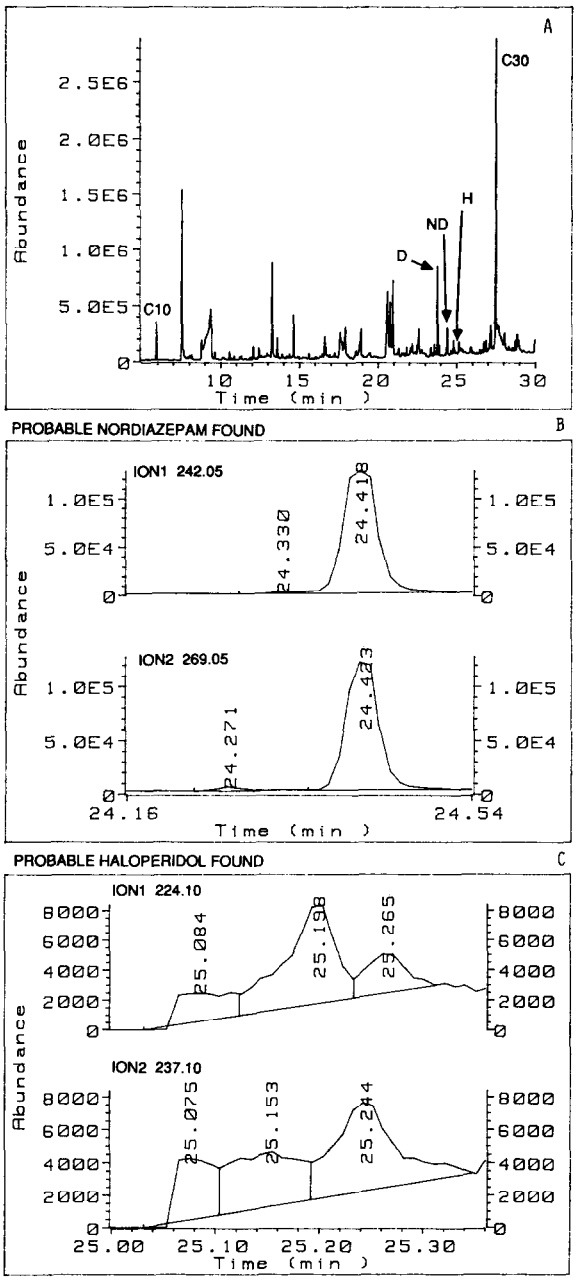


Fig. 4. (A) Portion of a SIM chromatogram of a chloroform extract of liver found to contain diazepam (D) and nordiazepam (ND). H indicates the retention time of haloperidol (see C). (B) Text and mass fragmentograms generated by the macro from the SIM data in (A), showing the identification of probable nordiazepam. (C) Text and mass fragmentogram generated by the macro from the SIM data in (A), showing identification of probable haloperidol. Visual inspection shows this to have been an error, due to integration of noise caused by thresholds being set too low. See text for discussion.

(Table I) by comparison with the macro report on the other analysis. Otherwise re-analysis was required, using specific acquisition methods to identify the particular drug.

Drugs could also be undetected because of deterioration of the chromatographic system, and this was avoided by routine monitoring of GC performance with a mixture of some of the drugs in the search database which were very sensitive to column activity (methylamphetamine, oxazepam and oxycodone). If peak shape indicated activity, the injection port accessories (quartz insert and quartz wool packing, septum and O-ring) were changed. If necessary, a new precolumn was fitted.

Some variability was found even in the corrected drug retention times. Note that the actual retention time of nordiazepam in Fig. 4B (24.42 min) was slightly later than the expected time (TDCOR = 24.35 min, at the centre of the mass chromatogram). The longer retention time was presumably due to the presence of biological molecules which modify the polarity of the liquid phase. This shows the need for the relatively wide limits (TDCOR ± 0.2 min) which were allowed on the search window used by the macro. For the same reason, drugs which eluted near the beginning or (more particularly) the end of a SIM ion group were also included in the adjacent group's acquisition file.

Warnings were also given if the areas of either of the hydrocarbon peaks were low (Fig. 1). This was to avoid the possibility of the wrong peak being assigned as the reference hydrocarbon. In addition, a small C10 peak could indicate a general loss of volatile substances, perhaps during a concentration step.

Caffeine was the only drug reported in blank plasma extracts since, although the SIM chromatogram contained many peaks (as in Fig. 3A), the other criteria in the macro prevented false drug identifications. The method has not yet been evaluated on plasma samples, but the simple solvent extraction procedure described in Experimental was successfully tested on a group of eighteen drugs added to blank plasma. The drugs found in spiked plasma samples included amphetamines, opiates, benzodiazepines, an antihistamine (azatedine), $\Delta^9$-tetrahydrocannabinol and anticonvulsants. The concentrations added to plasma were relatively high (10 μg/ml) as the intention was to test the capacity of the macro to search complex chromatograms rather than the extraction procedure. Success in screening for drugs in biological samples is critically dependent on the efficiency of extraction procedures, and several methods have been described [1–12]. The signal-to-noise ratio is also of decisive importance in analysis of biological samples and becomes more of a problem with the low drug concentrations likely to be seen in practice. The measurement of low levels of drugs in biological samples may require changes to thresholds and even selection of different diagnostic ions, and this will be the subject of future work.

We were unable to analyse some drugs by GC, even as derivatives. Importantly, these included some of the β-blocking drugs: atenolol, labetalol, pindolol and timolol. However, alprenolol, metroprolol, oxprenolol and propranolol were

chromatographed as trifluoroacetyl derivatives (Table I). Leloux *et al.* [13] successfully analysed β-blockers by GC with on-column injection after double derivatisation (trimethylsilylation and trifluoroacetylation), but this was not used in our study.

This method was developed to provide a preliminary screen to detect drugs which could contribute to road accidents, using extracts of plasma suitable for GC analysis. In many cases the evidence of two diagnostic ions, in the correct ratio and occurring at the expected retention time of the drug, would be sufficient evidence for drug identification. Positive drug findings can be confirmed, when necessary, by additional analyses with specific acquisition methods designed to identify the particular drug. In SIM mode, up to twenty ions can be monitored per drug. The macro could be written to use a larger number of co-eluting characteristic ions for fewer target substances, which would increase certainty of identifications.

The Hewlett-Packard GC–MSD system is a widely used instrument, and the screening method described here could be readily adopted by other MSD users. It was not practical to use the quantitative report software supplied with the MSD for drug screening, because it gives full reports on peaks not found. This results in a very lengthy report when screening for over a hundred drugs. The method described in this article only reports on drug findings and is suitable for screening when most samples contain few, if any, of the target drugs. The macro could be applied to screening for other substances, such as metabolites used in clinical diagnosis [14,15], anabolic steroids and other doping agents [16,17] and possibly for pesticides or environmental pollutants.

Copies of the macro containing data on the drugs listed in Table I are available from the authors.

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REFERENCES

1 D. J. Crouch, M. A. Peat, D. M. Chin and D. S. Finkle, *J. Forensic. Sci.*, 28 (1983) 945.
2 A. P. Mason and A. J. McBay, *J. Forensic. Sci.*, 29 (1984) 987.
3 P. Holmgren, E. Loch and J. Schuberth, *Forensic. Sci. Int.*, 27 (1985) 57.
4 S. H. Cosbey, *Forensic. Sci. Int.*, 32 (1986) 245.
5 W. O. Pierce, T. C. Lamoreaux, F. M. Urry, L. Kopjak and B. S. Finkle, *J. Anal. Toxicol.*, 2 (1978) 26.
6 A. W. Missen, *Chem. N.Z.*, 45 (1981) 61.
7 J. Vine and T. R. Watson, *Med. J. Aust.*, 1 (1983) 612.
8 S. McLean, R. S. Parsons, R. B. Chesterman, R. Dineen, M. G. Johnson and N. W. Davies, *Med. J. Aust.*, 147 (1987) 6.
9 B. J. Perrigo, H. W. Peel and D. J. Ballantyne, *J. Chromatogr.*, 341 (1985) 81.
10 A. Turcant, A. Premel-Cabic, A. Cailleux and P. Allain, *Clin. Chem.*, 34 (1988) 1492.
11 R. A. Cox, J. A. Crifasi, R. E. Dickey, S. C. Ketzler and G. L. Pschak, *J. Anal. Toxicol.*, 13 (1989) 224.
12 B. Ford, J. Vine and T. R. Watson, *J. Anal. Toxicol.*, 7 (1983) 116.
13 M. S. Leloux, E. G. DeJong and R. A. A. Maes, *J. Chromatogr.*, 488 (1989) 357.
14 E. Jellum, O. Thoresen, L. Horn, R. Seip, E. Nilsen, E. A. Kvittingen and O. Stokke, *J. Chromatogr.*, 468 (1989) 43.
15 A. G. Antoshechkin, A. B. Golovkin, L. A. Maximova and V. A. Bakharev, *J. Chromatogr.*, 489 (1989) 353.
16 A. K. Singh, M. Ashraf, K. Granley, U. Mishra, M. Madhusudana Rao and B. Gordon, *J. Chromatogr.*, 473 (1989) 215.
17 R. Massé, C. Ayotte and R. Dugal, *J. Chromatogr.*, 489 (1989) 23.