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RECENT ADVANCES IN DOPING ANALYSIS (6)

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Chiral Derivatisation
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CHIRAL DERIVATISATION

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Introduction

Routine screening of stimulants within IOC accredited laboratories is a two part process. An initial screen using a simple basic extraction into a solvent such as ether or t-butylmethylether is analysed by gas chromatography with NPD detection. Inspection of this chromatogram allows identification of many volatile basic drugs. Once a potential violation of the IOC code is suspected a confirmation of the identity of the substance is required by GCMS before the sample can be reported as containing a banned substance.

The confirmation of many of the amphetamine related substances has been performed by preparation of a suitable derivative which gives unique retention time and mass spectrum. The mass spectrum of many of this class of compound without derivatisation is characterised by the presence of one major low mass ion, no molecular ion and very low intensity confirming ions which renders the use of this spectrum as unsuitable for confirmation. A derivatisation technique commonly used is the "double derivatisation" developed by Donike where sequential addition of MSTFA and MBTFA gives a mixed TMS/TFA derivative. This procedure while giving an excellent derivative has the undesirable consequence of requiring a dedicated GC column, as the reagent, which is not removed before analysis, changes the characteristics of the column phase, making it useless for any other analysis especially if the sample is not derivatised. Since equipment is expensive and needs to be as versatile as possible it was important to develop a derivatisation process which gives:

- A derivative with a useful characteristic spectrum
- Does not change the character of the GC column and thus allow the instrument to have a multifunctional role.

- Gives rapid high yielding reaction with as few manipulations as possible.
- Is amenable to use of a chiral derivatisation reagent.

Pentafluorobenzoyl chloride has been often used as a derivatisation reagent for amines. This was used as the model reagent for an extractive acylation reaction directly on the urine. Conditions were found which gave suitable pentafluorobenzoyl (PFB) derivatives for ephedrine and pseudoephedrine and giving sufficient separation of these isomers to be used for their confirmation.

The chiral reagent Mosher's acid chloride (methoxytrifluoromethylphenylacetyl chloride to give MTP derivatives) was chosen as the chiral reagent to distinguish optical isomers of the amphetamines. The Cologne Laboratory using a "double derivatisation" technique with MSTFA and Mosher's acid chloride had presented previous use of this reagent at the Cologne Workshop for the detection of metabolites of seleginine. Use of the procedure above for MTP derivatives gave very good results.

MATERIALS AND METHODS

The reference compounds were obtained from Sigma (Saint Louis, USA) or the Curator of Standards, Australian Government Analytical Laboratories, Sydney. Pentafluorobenzoyl chloride was obtained from Sigma and the optical active reagents S(+)- and R(-)- Mosher's acid chlorides from Fluka.

The derivatisation reaction for the PFB derivatives of ephedrine is shown in FIG 1 and the general sample derivatisation to give PFB and MTP derivatives is shown in FIG 2.

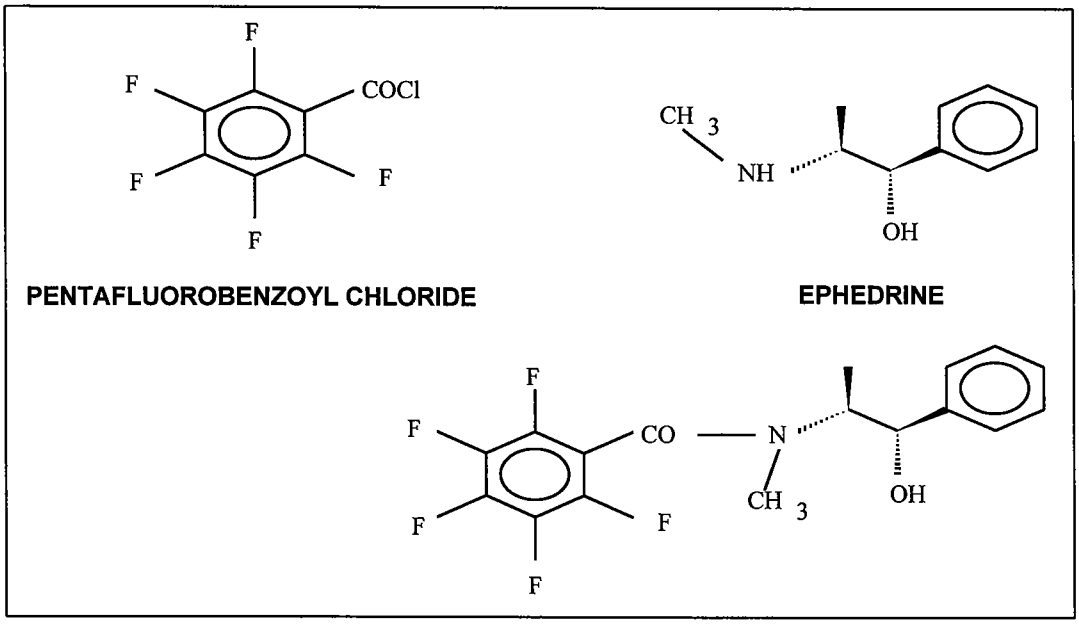


FIG 1

- 1ml URINE
- 5ml HEXANE
- 30ul 2% REAGENT IN HEXANE
- 60ul 6M SODIUM HYDROXIDE
- SHAKE 15mins
- REMOVE ORGANIC SOLVENT AND EVAPORATE
- RECONSTITUE IN 100ul ETHYL ACETATE
- GCMS FULL SCAN HP5890/5970
- GC 17m HP ULTRA 2, 0.2mm ID, 0.1u FILM. 130C THEN RAMP 10C/min TO 300C, SPLIT 10:1

FIG 2

The reaction scheme for the derivatisation of amphetamine using S(+)-Mosher's acid chloride is shown in FIG 3.

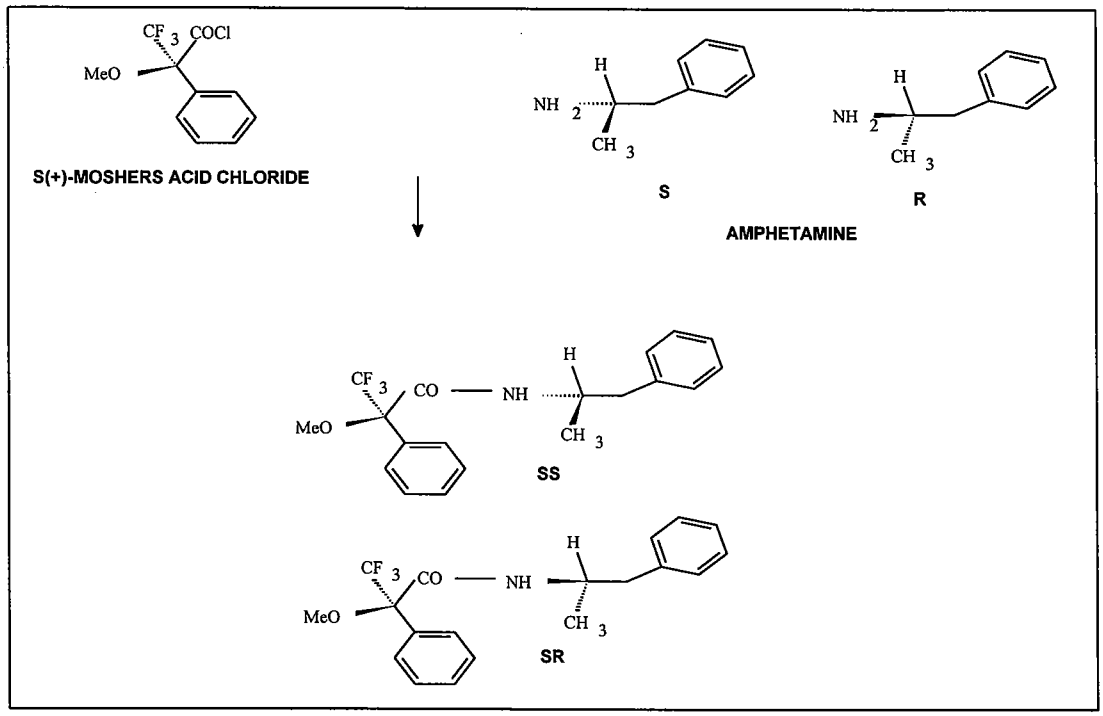


FIG 3

RESULTS

The derivatives were formed in high yield and gave good separation for the compounds tested.

The ephedrine and pseudoephedrine PFB derivatives had a relative retention time of 1.027 and 1.036 respectively to the internal standard methoxyphenamine. This separation is shown in FIG 4. The mass spectra of the two derivatives were identical and the spectrum of ephedrine is shown in FIG 5.

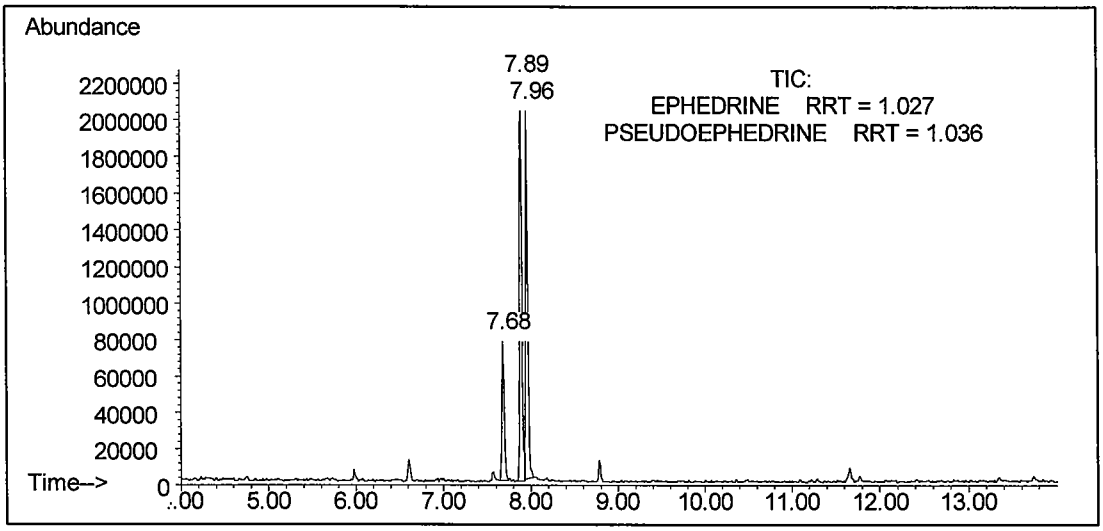


FIG 4

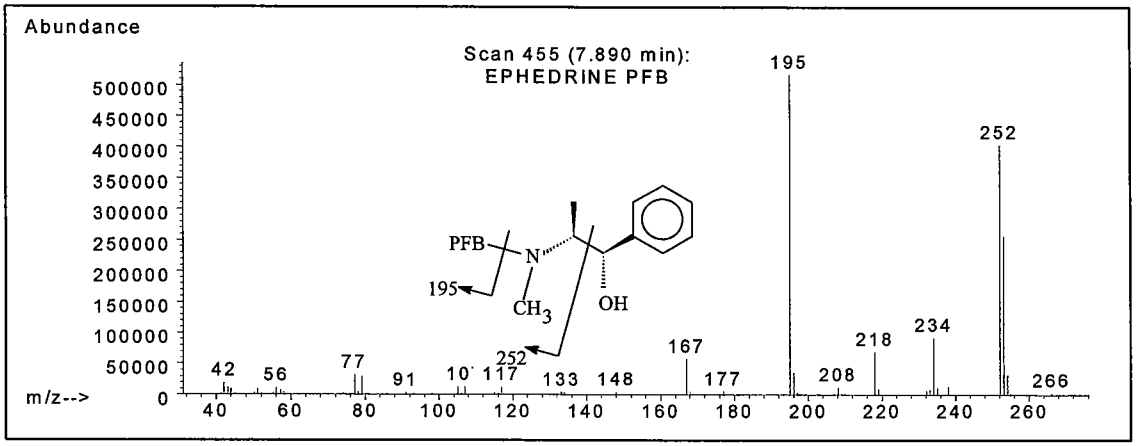


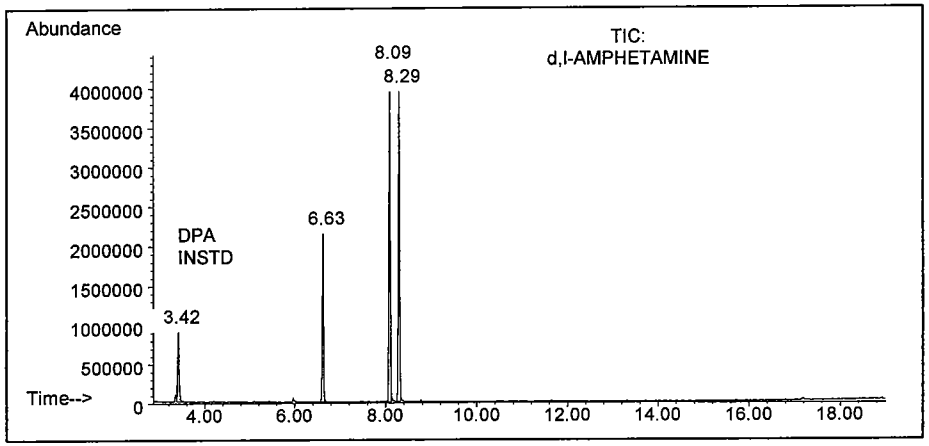
FIG 5

Chiral separation of the amphetamine derivatives is shown in TABLE 1.

COMPOUND MTP DERIVATIVES	RETENTION TIME	RELATIVE RETENTION TIME DIPHENYLAMINE INSTD
d-Amphetamine	8.09	2.365
l-Amphetamine	8.29	2.424
d-Methamphetamine	8.92	2.608
l-Methamphetamine	9.01	2.635
d-Ethylamphetamine	9.13	2.685
l-Ethylamphetamine	9.23	2.715
d-Methylendioxyamphetamine	10.95	3.192
l-Methylenedioxyamphetamine	11.23	3.274
d-Methylendioxymethamphetamine	11.80	3.471
l-Methylendioxymethamphetamine	11.92	3.506
Phentermine	8.181	2.41

TABLE 1

The corresponding TIC and the mass spectrum of one of the chiral isomers is shown in FIGS 6-11. Phentermine, which does not have a chiral centre, is included as it needs to be distinguished from methylamphetamine. It has a retention time between the isomers of amphetamine so it is well separated from methylamphetamine and has a significant ion at m/z 133 which is absent in the spectrum of methylamphetamine.



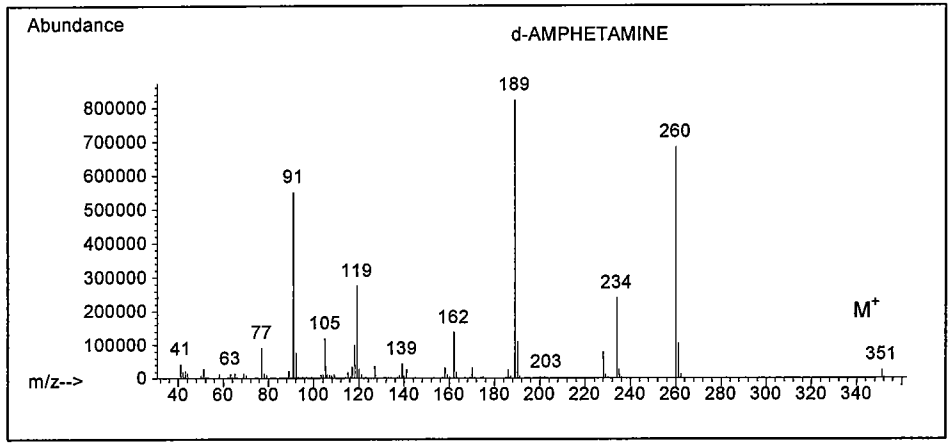
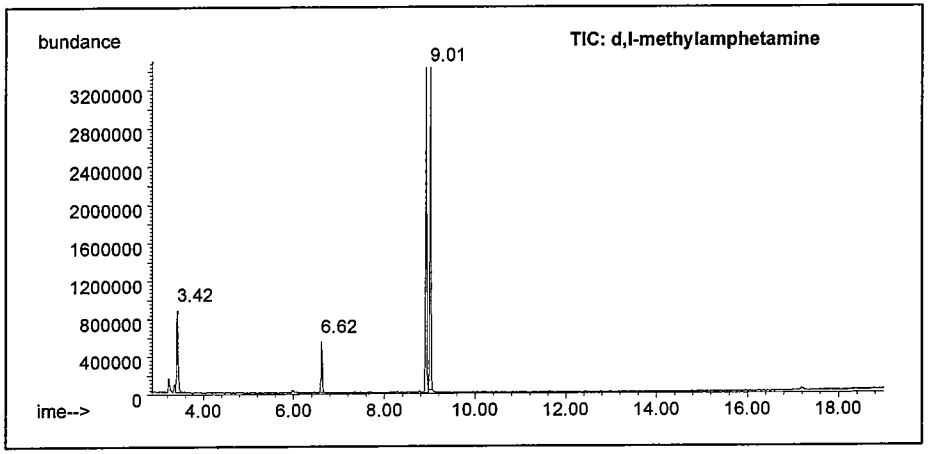


FIG 6



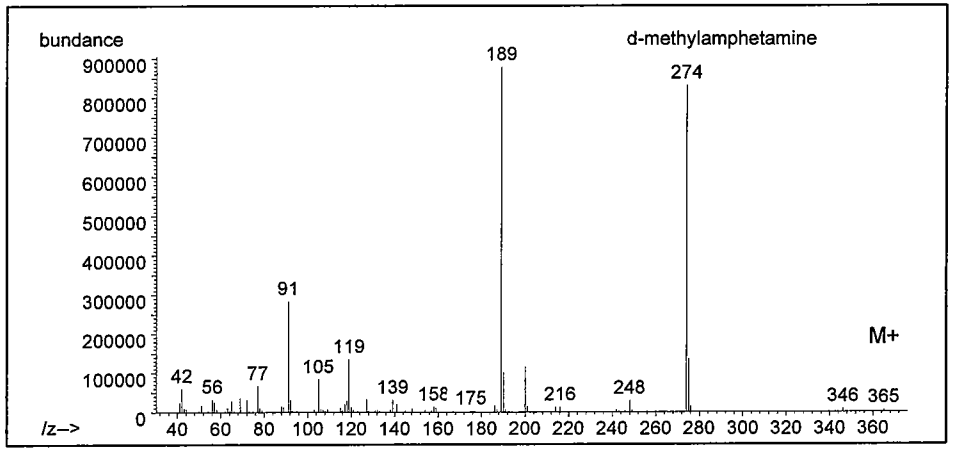


FIG 7

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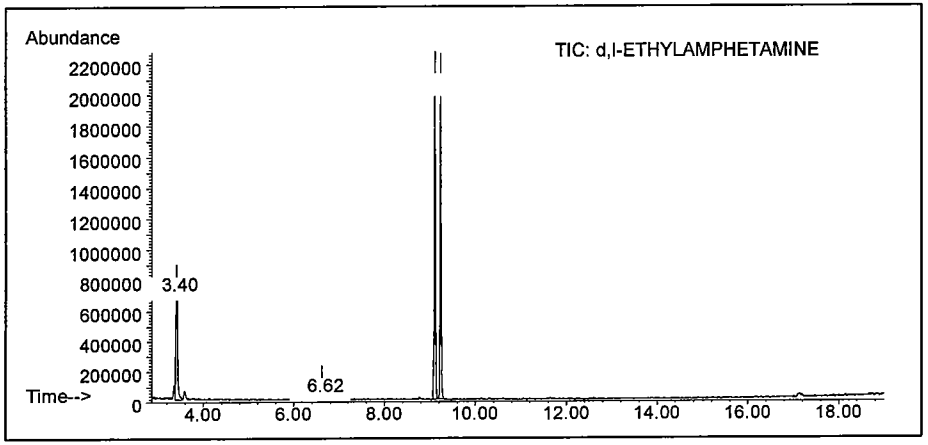

Abundance
TIC: d,l-ETHYLAMPHETAMINE
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
3.40
6.62
Time-->
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00

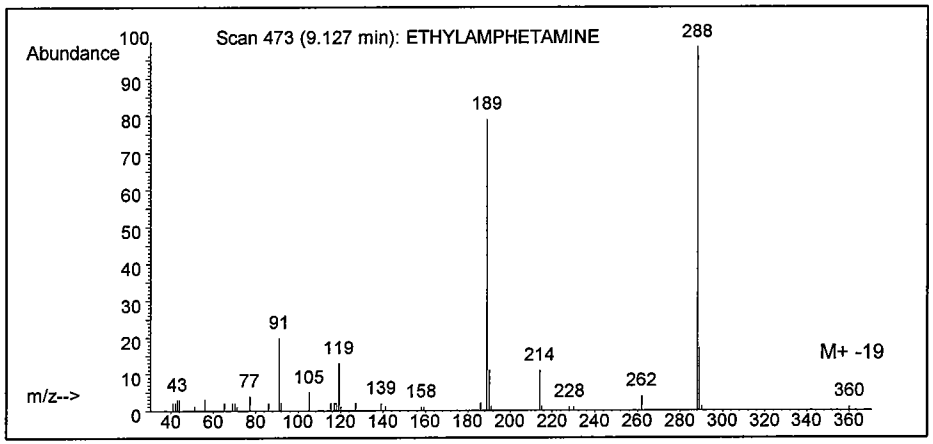
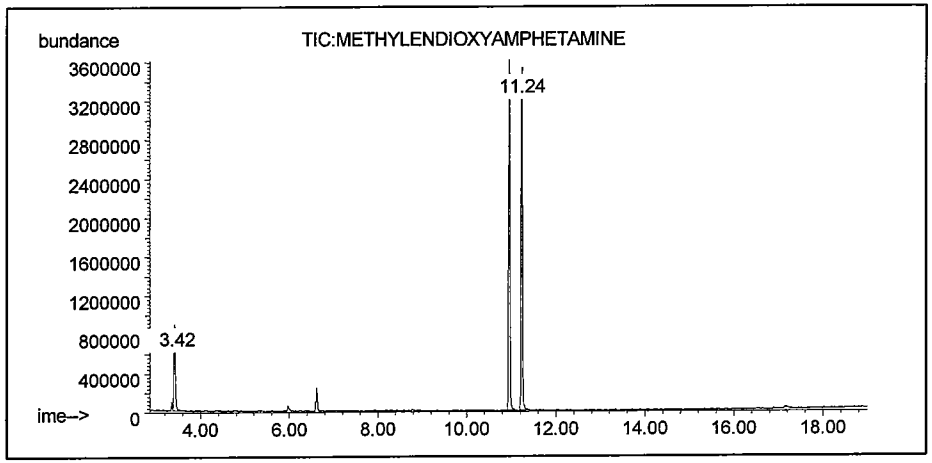

Abundance
100
Scan 473 (9.127 min): ETHYLAMPHETAMINE
288
189
91
119
214
M+ -19
43
77
105
139
158
228
262
360
m/z-->
90
80
70
60
50
40
30
20
10
0
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360

FIG 8


bundance
TIC:METHYLENDIOXYAMPHETAMINE
3600000
3200000
2800000
2400000
2000000
1600000
1200000
800000
400000
0
11.24
3.42
ime-->
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00

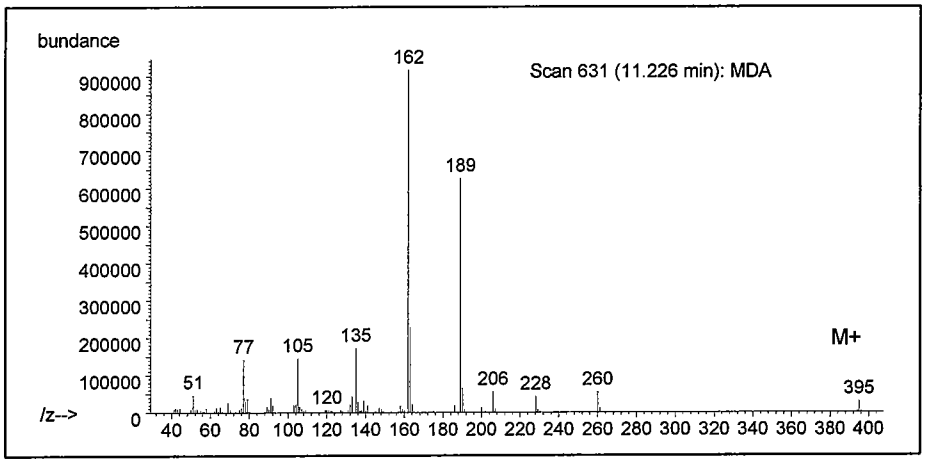
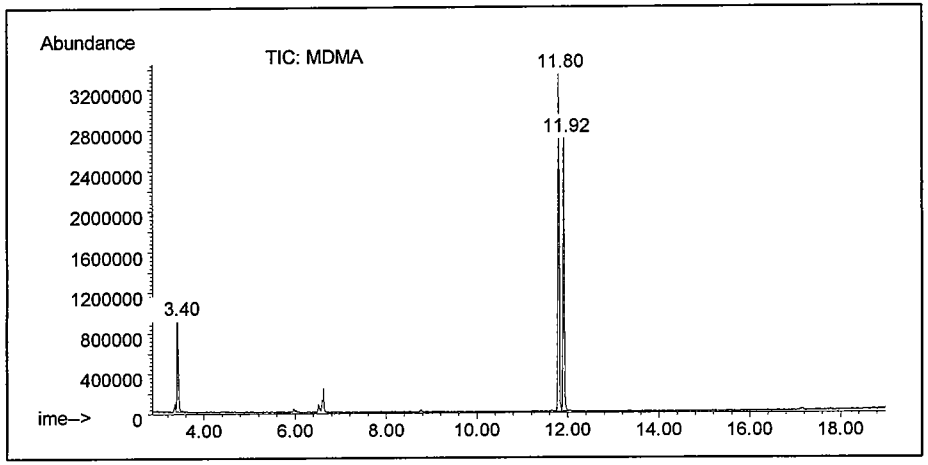

bundance
162
Scan 631 (11.226 min): MDA
189
M+
77
105
135
120
51
206
228
260
395
/z-->
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400

FIG 9



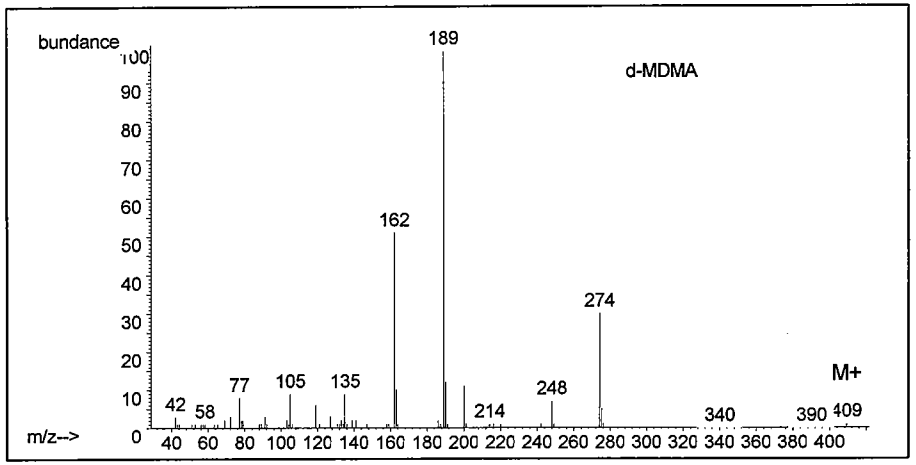
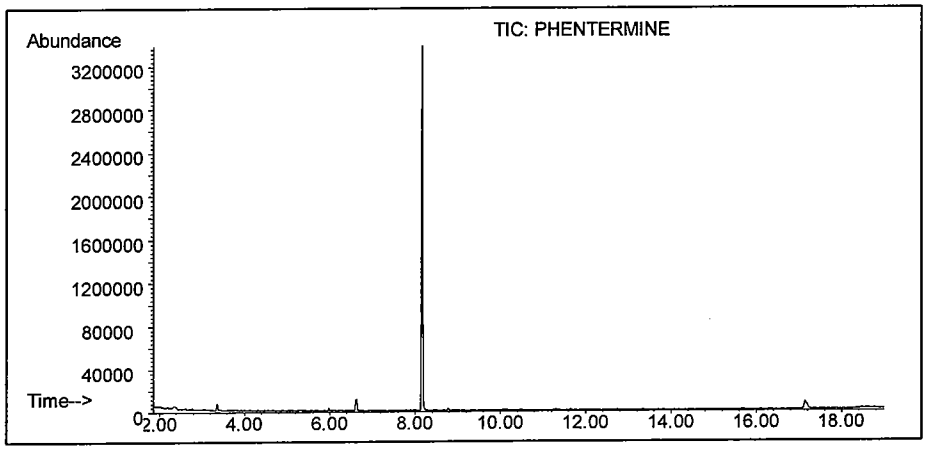


FIG 10



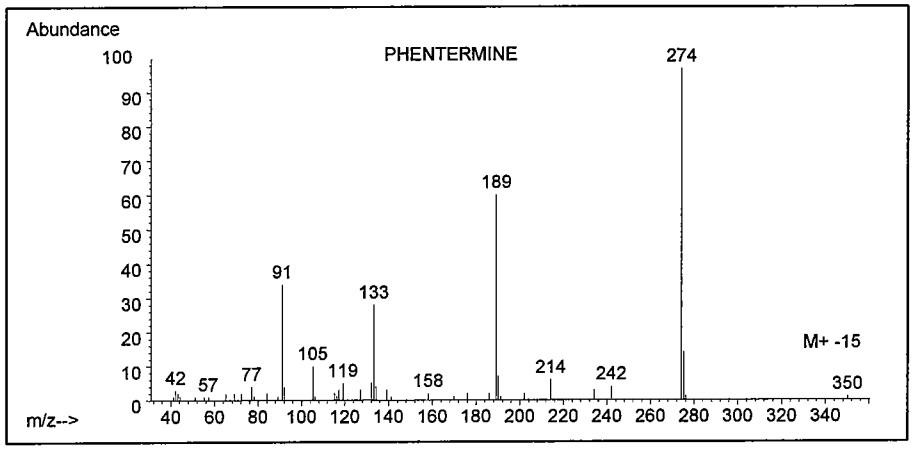


FIG 11

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The separation of a mixture of amphetamines at concentrations of 0.5ug/ml and 0.05ug/ml is shown in FIG 12 and 13 and the full scan comparison of the MDMA spectra is shown in FIG 14.

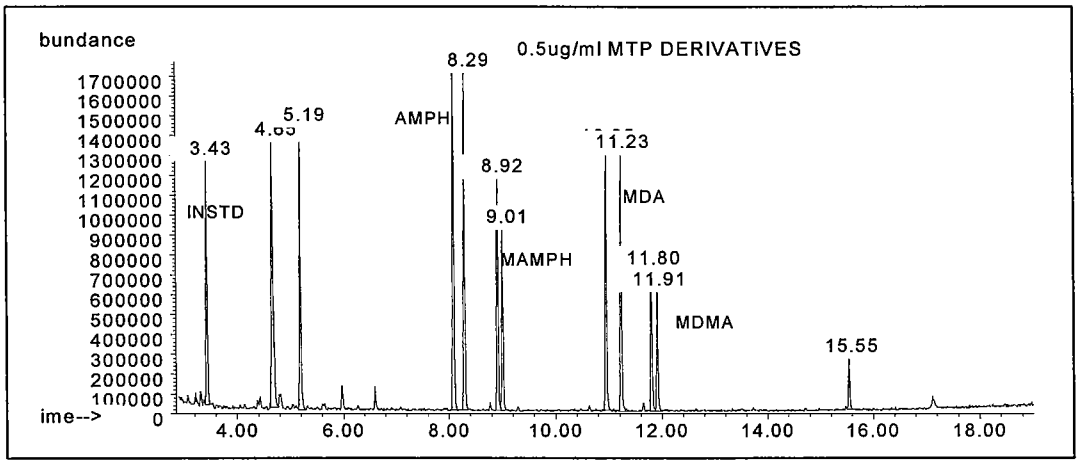

FIG 12

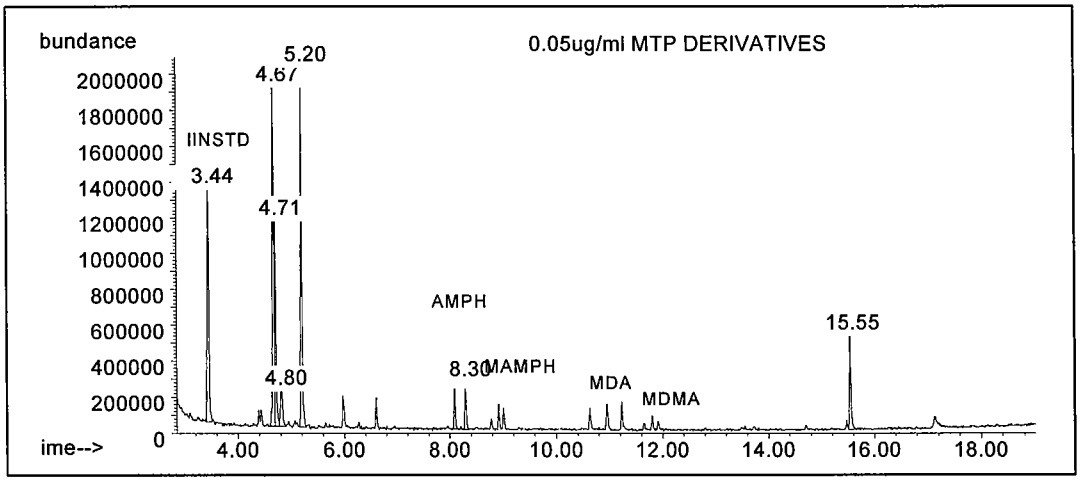

FIG 13

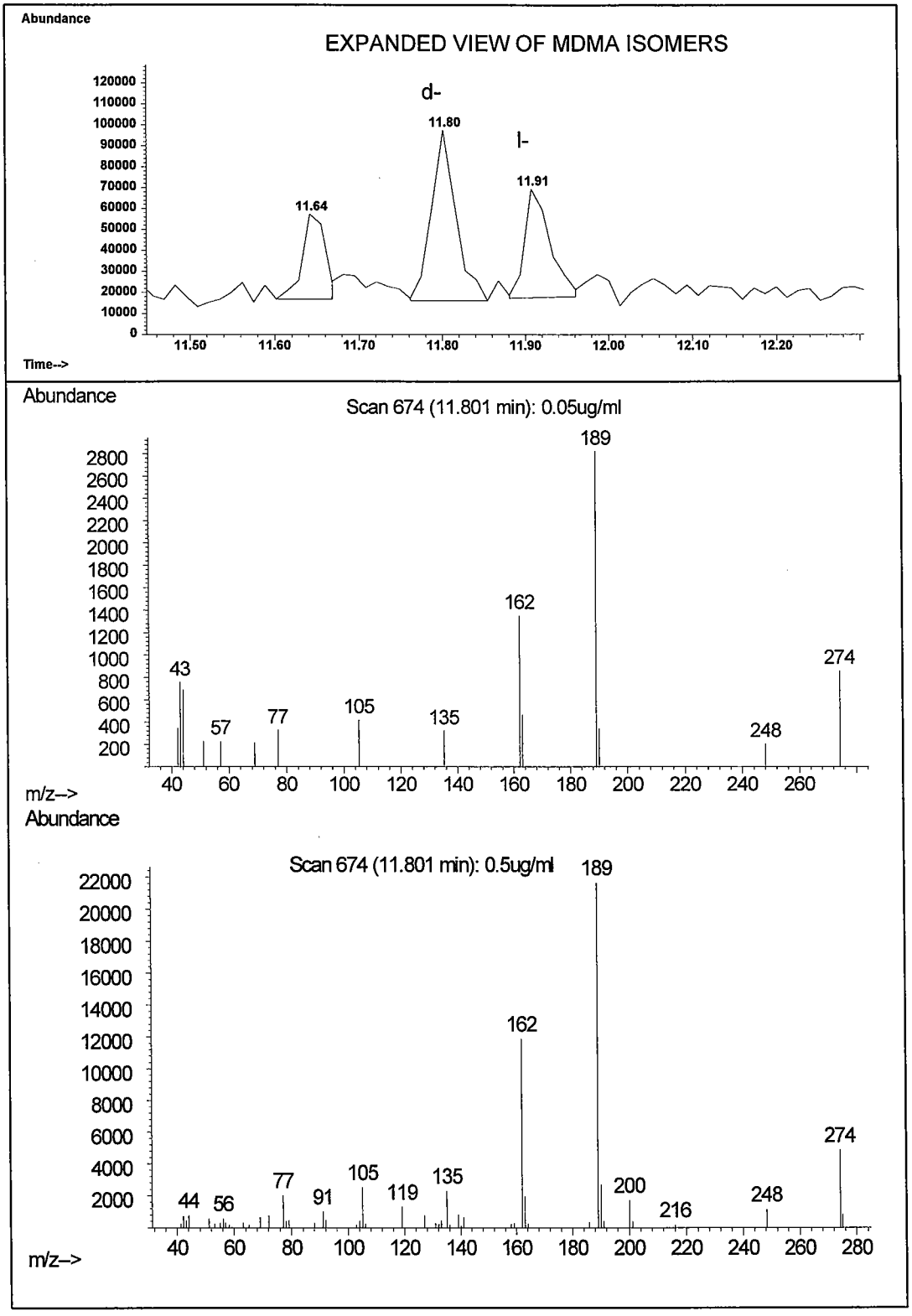
Abundance
EXPANDED VIEW OF MDMA ISOMERS
d-
l-
11.64
11.80
11.91
Time-->
Abundance
Scan 674 (11.801 min): 0.05ug/ml
189
162
43
57
77
105
135
248
274
m/z-->
Abundance
Scan 674 (11.801 min): 0.5ug/ml
189
162
44
56
77
91
105
119
135
200
216
248
274
m/z-->

FIG 15

The detection of 0.05ug/ml of MDMA is very good and is close to the detection limit by full scan. Comparison of the full scan spectra in FIG 15 shows agreement, which can be used to confirm the presence of these two isomers.

CONCLUSION

The derivatisation of amphetamine class can be easily carried out by extractive acylation giving a very rapid confirmation procedure that can resolve the optical isomers. Detection to a level well below 100ug/ml is routinely possible. The utility of this procedure for quantitation of these substance has yet to be determined.

The use of pentafluorobenzoyl derivatives of ephedrine and pseudoephedrine allow them to be confirmed and distinguished. Extractive acylation with Mosher's acid chloride to give MTP derivatives of the ephedrines has to date given variable recovery and appears to be unsuitable using the current procedure.