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Development of a harmonised method for the profiling of amphetamines III. Development of the gas chromatographic method

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Abstract

This study focused on gas chromatographic analysis of target compounds found in illicit amphetamine synthesised by the Leuckart reaction, reductive amination of benzyl methyl ketone, and the nitrostyrene route. The analytical method was investigated and optimised with respect to introduction of amphetamine samples into the gas chromatograph and separation and detection of the target substances. Sample introduction using split and splitless injection was tested at different injector temperatures, and their ability to transfer the target compounds to the GC column was evaluated using cold on column injection as a reference. Taking the results from both techniques into consideration a temperature of 250 °C was considered to be the best compromise. The most efficient separation was achieved with a DB-35MS capillary column (35% diphenyl 65% dimethyl silicone; 30 m × 0.25 mm, d_f 0.25 μm) and an oven temperature program that started at 90 °C (1 min) and was increased by 8 °C/min to 300 °C (10 min). Reproducibility, repeatability, linearity, and limits of determination for the flame ionisation detector (FID), nitrogen phosphorous detector (NPD), and mass spectrometry (MS) in scan mode and selected ion monitoring (SIM) mode were evaluated. In addition, selectivity was studied applying FID and MS in both scan and SIM mode. It was found that reproducibility, repeatability, and limits of determination were similar for FID, NPD, and MS in scan mode. Moreover, the linearity was better when applying FID or NPD whereas the selectivity was better when utilising the MS. Finally, the introduction of target compounds to the GC column when applying injection volumes of 0.2 μl, 1 μl, 2 μl, and 4 μl with splitless injection respectively 1 μl with split injection (split ratio, 1:40) were compared. It was demonstrated that splitless injections of 1 μl, 2 μl, and 4 μl could be employed in the developed method, while split injection and splitless injections of 0.2 μl should be avoided.

Keywords: Amphetamine; Impurities; Profiling; Optimisation; Separation; Gas chromatography–mass spectrometry

1. Introduction

It is well known that amphetamine profiling can be useful in comparison of illicit drug samples. Normally, such profiling analysis is performed by gas chromatography (GC) with a flame ionisation detector (FID) [1–6], although GC in combination with a electron capture detector (ECD) [2], nitrogen phosphorous detector (NPD) [6], and mass spectrometry (MS) [3] have also been used for this purpose. As early as 1973, Strömberg [2] used a packed OV-17 column (3%

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diphenyl, 97% dimethyl silicone) to separate target compounds in Leuckart amphetamine in 26 min, and detection was accomplished simultaneously with a FID and a ECD. Lambrecht and Rasmussen [3] employed a SE-30 capillary column (100% dimethyl silicone) to determine the presence of typical by-products in amphetamine synthesised by the Leuckart route; the GC analysis was performed in 42 min, and detection was achieved using MS and FID on two separate GC instruments. Alm et al. [1] used a slightly more polar GC column (HP-UPC, 5% diphenyl and 95% dimethyl silicone) and separated target compounds in Leuckart amphetamine in only 27 min; detection was performed with a FID. King et al. [4] accomplished separation of target compounds in street amphetamine on a BP 5 column (5% diphenyl, 95% dimethyl silicone) in 25 min, and detection was done with a FID. Based on the work of Alm et al. [1], Kärkkäinen et al. [5] developed a new method to analyse target compounds in street amphetamine, which entailed the use of a 50 m HP Ultra 2 column (5% diphenyl, 95% dimethyl silicone) for separation and a FID for detection; the analysis was performed in 57 min, with hydrogen as carrier gas. Kongshauh et al. [6] separated amphetamine target compounds on an SPB 1 column (100% dimethyl silicone) in 26.5 min, and used a NPD for detection. Methods other than gas chromatography have also been used to analyse target compounds. In 1984, Lambrecht and Rasmussen [7] applied high performance liquid chromatography (HPLC) to accomplish separation and quantification of by-products in street amphetamine. Also, Lambrecht later published two additional papers [8,9] describing the use of HPLC for this purpose.

Many different GC methods have been used to separate and quantify impurities in street amphetamine. However, most of the techniques that have been developed have focused on analysis of amphetamine synthesised by the Leuckart route, and, to our knowledge, there are no scientific reports describing optimisation of separation and quantification of impurities in amphetamine manufactured by any other routes. This is the third in a series of six articles describing the development of a harmonised method for the profiling of amphetamine. The objective in this investigation was to develop a gas chromatographic technique to quantify identified target compounds found in street amphetamine synthesised by three different routes: the Leuckart reaction, reductive amination of benzyl methyl ketone, and the nitrostyrene method. GC was chosen because it offers superior resolving power and a high degree of stability, and it is user friendly and has traditionally been employed for amphetamine profiling. Selection and optimisation of the following operating parameters for the GC system were made: (1) sample introduction technique; (2) stationary phase and temperature program of the column; (3) the detector; (4) volume of sample injected.

2. Materials and methods

2.1. Chemicals

All chemicals used were at least of analytical reagent grade. Syntheses of the reference compounds have been described previously [10].

2.2. Sample preparation

2.2.1. Amphetamine samples

A portion of amphetamine (600 ± 2 mg) was weighed in a test tube (12 ml), after which 6 ml of phosphate buffer (0.063 M, pH 7.00) was added, and the system was subjected to horizontal or rotatory shaking for 30 min. If necessary, the pH of the buffer was subsequently readjusted to 7.00 ± 0.02 using 0.1 M sodium hydroxide or 0.1 M hydrochloric acid. Thereafter, 600 μl of isooctane containing tetracosane at a concentration of 10 μg/ml as internal standard, was added, and the mixture was shaken for 30 min and then centrifuged for 3 min at 3000 rpm to facilitate phase separation. A portion of the organic phase was then removed and placed in a GC vial and analysed.

2.2.2. Control sample

A stock solution containing 16 compounds each at a concentration of 1 mg/ml (Table 1) was prepared in isooctane. The mixture was diluted 100 times to give a concentration of 10 μg/ml, and this sample, referred to as the modified Grob extract, was used in subsequent studies as a control for the conditions of the GC system. The following parameters were monitored for each of the target compounds: peak symmetry, resolution, width of peak at half the peak height, and retention time in relation to one of the alkanes in the sample (different alkanes were used in different studies). Initially, the sample was analysed 10 days in a row, and the mean value and standard deviation (S.D.) were calculated for each parameter. In subsequent analyses, values that were larger or smaller than the calculated means ± three times the S.D. were considered to indicate that the gas chromatographic system needed to be inspected, and, if necessary, that equipment should be cleaned or replaced.

2.3. GC–FID and GC–MS analyses

Quantitative analysis was performed on a HP 6890 gas chromatograph (Agilent Technologies) equipped with a FID. Qualitative analysis was carried out on the same kind of gas chromatograph but detection was done with a HP 5973 mass spectrometer (MS; Agilent Technologies). Detection was conducted either simultaneously on a dual column system or using two separate GC instruments. Separation of analytes was performed on a 100% methyl silicone column (HP Ultra 1; 25 m × 0.20 mm, d_f 0.33 μm) connected to a pre-column (i.e., retention gap; 2.5 m × 0.25 mm [i.d.]). One of three different instrumental set-ups were employed to accomplish simultaneous GC–FID and GC–MS detection. Either a y-splitter (Agilent Technologies) was used to connect two columns to the same pre-column, or a divider (Gerstel) or a two-hole ferrule (Agilent Technologies) was employed to install two columns in the same injector. The oven temperature program was 60 °C for 1 min followed by an increase of 10 °C/min to a final temperature of 300 °C, which was held for 10 min. Samples were introduced by either split or splitless injection. The former involved injection of 1 μl of sample per column at 260 °C, with a total gas flow of 37.4 ml/min and a split ratio of 1:20 (gas saver 20 ml/min after

Table 1
Compounds in the control sample

n-Decane
n-Dodecane
n-Tridecane
2,6-Dimethylaniline
n-Tetradecane
Dicyclohexylamine
Dodecanoic acid methyl ester
n-Heptadecane
n-Octanol
2,6-Dimethylphenol
2-Ethylhexanoic acid
Decanoic acid methyl ester
Undecanoic acid methyl ester
n-Pentadecane
n-Hexadecane
n-Octadecane

1.5 min). For splitless injection, 1 μl of sample was introduced per column at 260 °C, with a splitless time of 1 min and a total gas flow of 60 ml/min (gas saver 20 ml/min after 1.5 min). Helium was used as carrier gas in the constant flow mode at 25 cm/s. A liner (vol. 990 μl) packed with glass wool and an empty deactivated glass liner (vol. 250 μl), both from Agilent Technologies, were used for split and splitless injection, respectively. The FID was utilised at a temperature of 305 °C and with gas flows as follows: hydrogen 40 ml/min, air 450 ml/min, and helium 30 ml/min (used as make up gas). The mass spectrometer was operated with a solvent delay of 3.5–6 min, depending on the type of injection. The scanning range was 30–550 amu, with a sampling rate of 2.83 scans/s. The transfer line, the ion source and the quadrupole of the MS were used at temperatures of 305 °C, 230 °C and 150 °C, respectively.

2.4. Optimisation of sample introduction

Four samples of amphetamine were used in this study; two were synthesised by the Leuckart reaction, and one each by reductive amination route, and the nitrostyrene route. Extraction of each amphetamine sample was performed in triplicate, and the portions were combined to obtain four homogeneous extracts (one per sample). Each extract was divided into several portions, which were stored in separate GC vials. Thereafter, each extract was analysed three times by the GC method specified above, employing split and splitless injection at temperatures of 220 °C, 240 °C, 260 °C, and 280 °C. In addition, the extracts were analysed three times using COC injection. The COC inlet was maintained at a temperature 3 °C higher than that of the GC oven.

2.5. Optimising the separation of target compounds

Four samples of amphetamine were used to optimise the gas chromatographic separation of the target compounds. These four samples were synthesised with the same synthetic routes as the samples used in the optimisation of sample introduction study, but they were not the same samples. Moreover, the preparation of impurity extracts of these amphetamine samples was made in the same way as in this previous study. Some target compounds that were missing in the synthesised amphetamine, and for which standards were available [12], were added in appropriate amounts to the sample extracts in question. The prepared extracts were divided into several different portions, which were stored in separate GC vials. Three replicates of each extract were analysed on the following GC columns (length, i.d., and d_f given within parentheses): HP Ultra 1 (25 m, 0.20 mm, and 0.33 μm), HP Ultra 2 (25 m, 0.20 mm, and 0.33 μm), and HP-50+ (25 m, 0.20 mm, and 0.31 μm). In addition, the reductive amination sample was analysed on a HP-1701 column (25 m, 0.20 mm, and 0.20 μm). Each of the indicated columns was tested using the same selection of six different oven temperature programs to separate the analytes (three replicates of the prepared extracts were analysed with each temperature program): all six started at 60 °C (1 min), but they were, respectively, increased by 2 °C/min, 4 °C/min, 6 °C/min, 8 °C/min, 10 °C/min, or 12 °C/min until reaching 300 °C (maintained for 10 min). The final temperature of the HP-1701 was 280 °C, since higher temperatures for this column were not recommended by the manufacturer. Separation of analytes was performed under the GC conditions described in Section 2.3, but injections were done only in the split mode at 250 °C.

2.6. Selection of the detection technique and optimisation of detector operating conditions

A control sample was prepared in toluene and used in optimisation of the detection technique. The compounds included in this sample were the same as those in the control sample used to monitor the performance of the analytical system (Table 1), with the following exceptions: *n*-decane, *n*-octanol, 2-ethylhexanoic acid, and *n*-tetradecane were not included, and *n*-nonadecane, and *n*-tetracosane were added. Furthermore, to enable evaluation of the NPD, the following nitrogen-containing compounds were included: trimipramine, ketamine, *N*-methyl-diphenethylamine, and 4-methyl-5-phenylpyrimidine. The prepared samples were analysed by GC with four different detection techniques: FID, NPD, and MS in both scan mode and selected ion monitoring (SIM) modes. The analytes were separated on a DB-35MS column connected to a fused silica pre-column (2.5 m (*L*) and 0.25 mm (i.d.)) or a DB-35MS pre-column (2.5 m (*L*), 0.25 mm (i.d.) and d_f 0.10 μm). The temperature program started at 90 °C (1 min) and was then increased with a gradient of 8 °C/min until 300 °C, which was held for 10 min. Splitless injection, 1 μl per column, was performed at an injector temperature of 250 °C, and with a single tapered liner packed with glass wool (Agilent Technologies). The other GC conditions used were as described in Section 2.3, except that the MS recorded ions between 40 amu and 300 amu with a scan rate of 2.83 scan/s, and an ultra ion source, an ultra draw out plate, and an ultra repeller (all three from Agilent Technologies) were employed in the GC–MS analyses. The target compounds in the MS chromatograms were quantified with one specific ion for each substance. The NPD analyses were done with a detector temperature of 310 °C, a TID-4 bead (Detector Engineering & Technology) operating at 2.8 V, an extended jet (Agilent Technologies), and with gas flows as follows: hydrogen 4 ml/min, air 55 ml/min, and nitrogen (as make-up gas) 10 ml/min. The hardware function *adjust offset* of the gas chromatograph was not used in the analyses.

2.7. Optimisation of the injection volume

The content of by-products in illicit amphetamine varies between different seizures, and accordingly the intensity of recorded chromatograms will vary between seizures. For less intense impurity profiles, it would be useful to have the option to increase the sample volume injected on the GC to improve the quality of the generated data. In analogy, it would also be convenient to have the option to decrease the injection volume when analysing more concentrated impurity extracts that otherwise would exceed the linear range of the detector. An alternative to changing the injection volumes could be to dilute the sample prior to injection, or to repeat the sample preparation step using less amphetamine. However, these two alternatives are more laborious and were considered less attractive for routine analysis.

In the present study, a control sample was prepared in toluene at five different concentrations, i.e. 40 μg/ml, 5 μg/ml, 1 μg/ml, 0.5 μg/ml and 0.25 μg/ml. The compounds of the control sample were according to Table 1, with the following exceptions: *n*-decane, *n*-octanol, 2-ethylhexanoic acid, and *n*-tetradecane were not included, and *n*-nonadecane and *n*-tetracosane were added. The samples were subjected to analysis in six replicates using GC–MS and different injection volumes such that the same amount of sample (1 μg) theoretically should be introduced on the GC column (Table 2). The GC–MS analyses were performed using the same conditions as used when evaluating the performance of the different gas chromatographic detectors.

3. Results and discussion

3.1. Optimisation of sample introduction

The two vaporisation techniques, split and splitless injection (performed at different injection temperatures), were compared to cool-on-column injection (COC) to evaluate their efficiency in introducing a number of identified target compounds in the prepared organic extracts to the GC column. The target substances had previously been found to be stable when stored

Table 2
Experimental design showing the injection conditions for the different control sample extracts

Sample concentration (μg/ml)	Injection volume (μl)	Injection mode
40	1	Split (split ratio 1:40)
5	0.2	Splitless (splitless time 0.2 min)
1	1	Splitless (splitless time 1 min)
1	1	Splitless (splitless time 0.5 min)
0.5	2	Splitless (splitless time 1 min)
0.25	4	Splitless (splitless time 1 min)

in isooctane and toluene [11]. The COC injection can be regarded as an ''absolute'' introduction technique, since the sample is not heated in the injection block, and the entire sample is delivered directly to the column. Introduction of samples under different vaporisation conditions was assessed with respect to random (u_{ran}), systematic (u_{sys}), and total (u_{tot}) errors.

The random error (Eq. (1)) was estimated by calculating the mean of the relative standard deviations (R.S.D.; $n = 3$) of all target compounds (Table 3) with respect to their relative response factors (RRFs; i.e., the peak area of a certain impurity per peak area of an internal standard).

$$u_{ran}\,(\%) = \frac{\sum_i^n \text{R.S.D.}}{n}, \quad n = \text{number of target compounds} \tag{1}$$

The systematic error (u_{sys}) was determined in a three-step process. First, the relative deviation was determined for each target compound when a sample was injected using the investigated vaporisation techniques and the reference method COC injection (Eq. (2)). Secondly, the absolute values of the relative deviations was determined for each target compound (Eq. (3)). Finally, the systematic error (u_{sys}, Eq. (4)) was obtained by calculating the mean of the absolute values.

$$\text{relative deviation}\,(\%) = \frac{\text{RRF}\,(\text{vaporisation technique}) - \text{RRF}\,(\text{COC})}{\text{RRF}\,(\text{COC})} \times 100 \tag{2}$$

$$\text{absolute value} = |\text{relative deviation}| \tag{3}$$

Table 3
Random errors (%) for target compounds in samples injected at different temperatures

Target compound	On-column	Splitless				Split			
		220 °C	240 °C	260 °C	280 °C	220 °C	240 °C	260 °C	280 °C
Benzyl methyl ketoxime, isomer one	2.86	1.35	4.98	11.00	0.82	2.12	6.37	2.36	2.77
Benzyl methyl ketoxime, isomer two	2.97	2.47	2.89	8.57	0.51	10.20	1.10	1.57	3.05
N-Formylamphetamine	2.38	2.15	7.29	7.66	3.38	3.48	3.20	0.43	21.78
N-Acetylamphetamine	2.67	4.26	22.55	16.61	17.59	9.50	10.29	6.25	1.82
N-(β-Phenylisopropyl)benzaldimine	3.09	4.53	24.03	7.73	9.42	2.06	1.85	2.26	0.35
Average (nitrostyrene extract)	2.79	2.95	12.35	10.31	6.34	5.47	4.56	2.57	5.95
N-(β-Phenylisopropyl)benzaldimine	2.59	2.83	0.99	4.99	4.58	3.56	1.48	1.02	0.96
N-Benzylamphetamine	1.30	1.60	2.19	2.17	0.88	1.02	0.39	1.40	1.40
N,*N*-Di-(β-phenylisopropyl)amine, isomer one	1.05	1.89	1.33	1.80	0.40	0.94	0.47	1.55	1.54
N,*N*-Di-(β-phenylisoropyl)amine, isomer two	1.34	1.33	0.84	1.75	0.80	0.88	0.64	1.59	1.58
N-Benzoylamphetamine	0.69	1.88	1.95	4.21	1.09	1.43	0.77	2.68	2.65
Average (reductive amination extract)	1.39	1.91	1.46	2.98	1.55	1.57	0.75	1.65	1.63
4-Methyl-5-phenylpyrimidine	0.16	5.25	1.72	6.09	6.10	1.47	0.86	1.45	0.55
4-Benzylpyrimidine	0.20	4.99	1.97	6.37	6.49	4.05	2.81	2.21	3.12
N-(β-Phenylisopropyl)-benzaldimine	1.96	5.27	2.38	6.31	2.79	6.89	3.51	5.21	10.88
N-Benzylamphetamine	0.12	3.42	1.45	3.90	3.76	1.45	0.17	2.67	1.31
N,*N*-Di-(β-phenylisopropyl)amine, isomer one	2.00	1.23	1.63	4.29	2.92	0.82	0.71	0.93	0.39
N,*N*-Di-(β-phenylisopropyl)amine, isomer two	3.96	7.62	2.40	5.61	3.59	0.44	0.51	1.12	0.54
N,*N*-Di-(β-phenylisopropyl)methylamine	0.41	2.25	2.37	4.34	2.70	4.59	5.97	1.14	2.50
N,*N*-Di-(β-phenylisopropyl)formamide, isomer one	0.05	0.46	0.97	1.80	1.38	1.56	3.85	3.38	0.94
N,*N*-Di-(β-phenylisopropyl)formamide, isomer two	0.41	0.22	1.10	1.68	1.43	2.15	1.21	1.69	3.57
Average (Leuckart extract 1)	1.03	3.41	1.78	4.49	3.46	2.60	2.18	2.20	2.64
4-Methyl-5-phenylpyrimidine	0.35	2.04	0.93	1.27	1.11	0.45	0.29	0.91	1.24
N-Formylamphetamine	0.69	2.11	0.96	1.22	0.61	0.90	0.18	0.26	1.95
4-Benzylpyrimidine	0.27	1.86	1.03	1.34	0.93	0.69	0.24	0.49	1.40
N-(β-Phenylisopropyl)-benzaldimine	1.15	4.89	1.45	0.92	3.37	0.10	0.23	1.14	4.81
N-Benzylamphetamine	0.47	5.13	1.19	1.45	0.51	2.31	3.45	0.56	5.06
N,*N*-Di-(β-phenylisopropyl)amine, isomer one	1.52	1.64	2.02	1.06	0.73	0.38	0.20	0.78	0.42
N,*N*-Di-(β-phenylisopropyl)amine, isomer two	1.37	1.33	0.99	1.06	1.16	0.30	0.25	0.54	0.29
N,*N*-Di-(β-phenylisopropyl)methylamine	0.33	1.30	1.05	0.64	0.36	0.37	0.31	0.64	0.39
N-Benzoylamphetamine	2.92	1.67	1.06	0.72	1.64	0.49	0.31	0.78	1.14
2,4-Dimethyl-3,5-diphenylpyridine	4.14	3.63	26.86	24.79	32.66	0.31	0.60	0.89	1.44
N,*N*-Di-(β-phenylisopropyl)formamide, isomer one	0.54	0.55	0.36	0.24	0.18	0.15	0.15	0.37	0.28
N,*N*-Di-(β-phenylisopropyl)formamide, isomer two	0.64	0.00	0.36	0.20	0.21	0.12	0.07	0.33	0.29
Average (Leuckart extract 2)	1.20	2.18	3.19	2.91	3.62	0.55	0.52	0.64	1.56
Average (all extracts)	1.44	2.62	3.98	4.57	3.68	2.10	1.69	1.57	2.59
Average (nitrostyrene extract excluded)		2.55	2.37	3.47	3.17	1.45	1.14	1.37	1.95

$$u_{\text{sys}}\,(\%) = \frac{\sum_i^n \text{absolute value}}{n}, \quad (4)$$

n = number of target compounds

Total error (u_{tot}) was estimated with the formula often used for calculating combined measurement uncertainty:

$$u_{\text{tot}} = \sqrt{(u_{\text{sys}})^2 + (u_{\text{ran}})^2} \quad (5)$$

The random, systematic, and total errors for the different injection conditions are given in Tables 3–5.

With the exception of the nitrostyrene extract, the random error (repeatability) was less than 5%. Substantial deviation for compounds in this extract was due to instrumental problems. The random error of the split injections were lower than for the splitless injections. The split and splitless injections had their lowest random errors at 220 °C and 260 °C, respectively, but differences between temperatures were in general small (Table 3).

Systematic errors in GC injection primarily arise due to problems with unsuccessful transfer of target compounds onto the column when using vaporising inlets, and these values are all fairly high compared to the random errors. To allow comparison of the systematic errors for the split and splitless techniques, and to eliminate the discrimination caused by the split flow, RRFs of target compounds obtained with split injection were multiplied by a factor equal to the split ratio (i.e., 20). The results obtained when analysing the nitrostyrene extract exhibited pronounced deviations, which most likely was due to problems with the instrumentation. Excluding the

Table 4
Systematic errors for target compounds in samples injected at different temperatures

Target compound	Splitless injection				Split injection			
	220 °C	240 °C	260 °C	280 °C	220 °C	240 °C	260 °C	280 °C
Benzyl methyl ketoxime, isomer one	20.72	9.64	0.25	1.04	62.04	65.48	65.65	66.31
Benzyl methyl ketoxime, isomer two	62.92	57.80	53.20	54.19	4.82	6.15	6.08	9.13
N-Formylamphetamine	20.58	12.73	2.85	2.80	70.08	70.27	67.75	50.87
N-Acetylamphetamine	81.29	78.56	136.12	146.32	17.62	4.09	11.23	11.02
N-(β-Phenylisopropyl)benzaldimine	43.12	48.33	39.13	17.56	251.52	235.80	236.76	231.04
Average (nitrostyrene extract)	45.73	41.41	46.31	44.38	81.22	76.36	77.49	73.67
N-(β-Phenylisopropyl)benzaldimine	68.11	80.91	108.36	126.22	56.79	51.65	49.98	59.58
N-Benzylamphetamine	1.75	4.32	3.07	2.63	15.25	6.92	3.71	3.60
N,*N*-Di-(β-phenylisopropyl)amine, isomer one	10.12	4.08	4.78	4.36	14.25	5.73	4.50	3.90
N,*N*-Di-(β-phenylisopropyl)amine, isomer two	8.37	2.01	3.12	3.27	19.04	9.94	0.04	0.17
N-Benzoylamphetamine	15.62	10.23	7.03	0.57	12.86	8.45	2.51	3.87
Average (reductive amination extract)	20.79	20.31	25.27	27.41	23.64	16.54	12.15	14.22
4-Methyl-5-phenylpyrimidine	22.76	21.02	25.03	21.36	12.47	11.42	9.29	13.23
4-Benzylpyrimidine	21.41	21.02	25.14	21.36	14.97	12.34	13.37	13.09
N-(β-Phenylisopropyl)benzaldimine	15.18	14.35	23.59	36.97	9.87	0.76	0.51	3.50
N-Benzylamphetamine	7.14	5.34	7.27	5.41	5.36	1.49	0.44	1.32
N,*N*-Di-(β-phenylisopropyl)amine, isomer one	0.58	1.38	3.62	2.97	34.82	36.41	37.30	36.95
N,*N*-Di-(β-phenylisopropyl)amine, isomer two	23.77	15.08	15.32	13.64	92.89	87.62	84.85	86.02
N,*N*-Di-(β-phenylisopropyl)methylamine	3.17	4.21	3.00	1.03	3.15	1.58	5.27	3.27
N,*N*-Di-(β-phenylisopropyl)formamide, isomer one	2.68	1.38	0.20	0.71	5.57	6.41	1.96	4.88
N,*N*-Di-(β-phenylisopropyl)formamide, isomer two	3.64	1.86	0.20	0.28	5.43	8.96	1.67	3.44
Average (Leuckart extract 1)	11.15	9.52	11.49	11.53	20.50	18.55	17.18	18.41
4-Methyl-5-phenylpyrimidine	26.93	14.23	6.02	4.87	45.69	34.02	25.29	16.16
N-Formylamphetamine	9.28	0.89	7.73	7.72	19.82	10.77	3.70	0.77
4-Benzylpyrimidine	19.92	9.27	2.11	1.41	43.54	31.98	22.99	13.89
N-(β-Phenylisopropyl)benzaldimine	13.09	22.82	26.75	23.43	12.01	7.01	1.04	7.11
N-Benzylamphetamine	6.19	17.66	25.83	26.21	4.99	0.58	6.85	12.18
N,*N*-Di-(β-phenylisopropyl)amine, isomer one	16.78	5.06	5.14	6.49	16.11	9.89	4.09	0.13
N,*N*-Di-(β-phenylisopropyl)amine, isomer two	13.83	1.05	8.05	10.43	20.06	13.60	7.51	3.48
N,*N*-Di-(β-phenylisopropyl)methylamine	6.91	3.52	12.65	15.08	4.47	0.86	3.22	6.47
N-Benzoylamphetamine	3.35	11.58	16.54	17.79	37.99	33.61	27.61	23.37
2,4-Dimethyl-3,5-diphenylpyridine	0.44	18.19	30.53	40.65	3.77	6.37	11.15	13.00
N,*N*-Di-(β-phenylisopropyl)formamide, isomer one	0.44	2.85	7.77	9.94	10.97	13.00	15.89	18.26
N,*N*-Di-(β-phenylisopropyl)formamide, isomer two	3.86	6.08	9.93	11.73	13.33	15.13	17.82	20.14
Average (Leuckart extract 2)	10.09	9.43	13.25	14.65	19.40	14.74	12.26	11.25
Average (nitrostyrene extract excluded)	12.5	11.6	15.0	16.0	20.6	16.4	13.9	14.3

Table 5
Total errors (%) for target compounds in samples injected at different temperatures

	Splitless injection				Split injection			
	220 °C	240 °C	260 °C	280 °C	220 °C	240 °C	260 °C	280 °C
Random error, all	2.62	3.98	4.57	3.68	2.10	1.69	1.57	2.59
Systematic error (nitrostyrene results excluded)	12.51	11.55	14.95	16.02	20.60	16.40	13.94	14.30
Total error	12.77	12.26	15.68	16.42	20.71	16.49	13.99	14.53

nitrostyrene results, the smallest systematic errors were obtained at 240 °C and 260 °C for splitless and split injections, respectively (Table 4).

Splitless injection at 240 °C led to the smallest total error and should, therefore, offer good performance, especially when analysing samples containing low concentrations of target compounds. Split injection at a higher temperature (260 °C) was almost equally successful (Table 5). Cool-on-column injection did not seem to be robust enough for amphetamine profiling in routine and was, therefore, considered inappropriate. More specifically, amphetamine samples can contain very high concentrations of impurities, and introducing them directly into the column can cause problems such as excessive contamination of the GC system. Consequently, a choice needed to be made between the two vaporising techniques.

For the current method, the authors chose 250 °C as the inlet temperature, a compromise of both injection techniques. Based on the generated data it was also decided that samples should be introduced using splitless injection. Split injection could be advantageous for samples of high concentration and might, therefore, be an alternative for such samples in the future, but at this stage the split injection mode was not included as a part of the method.

3.2. Optimising the separation of target compounds

The performance of the tested columns was assessed by measuring the separation power and inertness of the column, and resolution of target compounds. The overall separation power was estimated on the basis of the number of peaks that could be integrated in the chromatogram. The sensitivity of the integration was calibrated separately for the chromatograms of the individual samples relative to the peak areas of the internal standard. The concept of graphical resolution was applied to investigate the resolving power of the columns. Inertness was estimated on the basis of peak asymmetry, which was calculated by dividing the first half-peak width

Table 6
Impurities identified in amphetamine synthesised by the Leuckart, reductive amination, and nitrostyrene methods

No.	Compound	Type of identification
1	1,3-Diphenyl-2-propylamine	Synthesised reference material
2	*N*,*N*-Di-(β-phenylisopropyl)methylamine, isomer 1	Synthesised reference material
3	*N*,*N*-Di-(β-phenylisopropyl)methylamine, isomer 2	Synthesised reference material
4	1-Benzyl-3-methylnaphthalene	Reference material
5	1,3-Dimethyl-2-phenylnaphthalene	Reference material
6	2,6-Dimethyl-3,5-diphenylpyridine	Reference material
7	2,4-Dimethyl-3,5-diphenylpyridine	Synthesised reference material
8	2,6-Diphenyl-3,4-dimethylpyridine	Reference material
9	*N*,*N*-Di-(β-phenylisopropyl)formamide, isomer 1	Synthesised reference material
10	*N*,*N*-Di-(β-phenylisopropyl)formamide, isomer 2	Synthesised reference material
11	2-Benzyl-2-methyl-5-phenyl-2,3-dihydropyrid-4-one	Reference material
12	Pyridine 14[a]	Mass spectra
13	Pyridine 7[a]	Mass spectra
14	Pyridine X[a]	Mass spectra
15	Phenyl-2-propanol	Synthesised reference material
16	Acetylamphetamine	Synthesised reference material
17	*N*-(β-Phenylisopropyl)benzaldimine	Synthesised reference material
18	Benzylamphetamine	Synthesised reference material
19	1-Oxo-1-phenyl-2-(β-phenylisopropylimino)propane	Synthesised reference material
20	Benzoylamphetamine	Synthesised reference material
21	2-Oxo-1-phenyl-(β-phenylisopropylamine)ethane	Synthesised reference material
22	2-Methyl-3-phenylaziridine	Synthesised reference material
23	Dimethyl-3-phenylaziridine	Mass spectra
24	2-Phenylmethylaziridine	Mass spectra
25	Phenyl-2-propanoxime, isomer one	Synthesised reference material
26	Phenyl-2-propanoxime, isomer two	Synthesised reference material

[a] Had almost identical mass spectra as identified pyridines and were, therefore, considered as such.

at 10% of the peak height by the second half-peak width at 10% peak height. Symmetry values smaller than 1.0 were inverted. Consequently, an inertness value of 1.0 describes ideal peak shape. Both the resolution and asymmetry of peaks were calculated using the peak performance functions of the macro tools in the Agilent GC Chemstation (Rev. A06.03) software. The temperature program was optimised to separate the target compounds that had been found to be stable in isooctane and toluene in an earlier study [11], and also some compounds whose identities were verified in subsequent work based on their mass spectra or available reference material. Table 6 presents a list of the target compounds investigated in this study, and Table 7 summarises the resolution and peak symmetries of the compounds and the separation power of the columns under the different separation conditions.

For all of the tested columns, the non-polar columns Ultra 1 and Ultra 2 offered the greatest separation power (Table 7); the superior efficiency of these columns is already well known. However, for the current application, other features were more interesting. Resolution of the target compounds was considered to be the most significant selection criterion, and column inertness was nearly as important, because it illustrates the ability of the column to separate compounds with different chemical characteristics. As highlighted in Table 7, the HP-50+ column provided the best resolution of the target peaks, and it also displayed the best symmetries of the target compounds. The final choice of separation conditions was based upon visual inspection of the chromatograms generated using the HP-50+ column and temperature gradients of 8 °C/min, 10 °C/min, and 12 °C/min; slower gradients were considered unrealistic for the method due to their longer run times. Based on this visual evaluation a temperature program of 8 °C/min was chosen for the method. The HP-50+ column was successfully employed in a subsequent study aimed at optimising the extraction of target compounds [12].

However, it was later found that this column had an unacceptable noise level caused by excessive bleeding

Table 7
Performance of the GC columns when using different temperature programmes

Column	Temperature program (°C/min)	Separation power (no. of integrated peaks)	Percent of target peaks with resolution ≥ 1	Column inertness (mean value of target peak asymmetry)
Ultra 1	2	125	74	2.74
	4	128	72	2.49
	6	125	65	2.57
	8	113	62	2.54
	10	112	66	2.23
	12	107	65	2.29
Ultra 2	2	141	75	2.98
	4	140	66	2.43
	6	129	74	2.51
	8	126	72	2.62
	10	126	75	2.19
	12	114	79	2.11
HP-50+	2	130	89	2.36
	4	117	85	2.22
	6	100	88	2.00
	8	102	85	1.84
	10	103	85	1.76
	12	99	82	1.76
HP-1701[a]	2	75	Not evaluated (n.e.)	n.e
	4	75	n.e.	n.e.
	6	68	n.e.	n.e.
	8	64	n.e.	n.e.
	10	63	n.e.	n.e.
	12	62	n.e.	n.e.
HP-35	8	n.e.	87	1.91
	10	n.e.	87	1.43
	12	n.e.	89	1.53
DB-35MS	8	n.e.	87	1.28
	10	n.e.	87	1.42
	12	n.e.	72	1.33
DB-17MS	8	n.e.	88	1.78
	10	n.e.	84	1.43
	12	n.e.	83	1.50

[a] The column was not further investigated due to its inferior separation power.

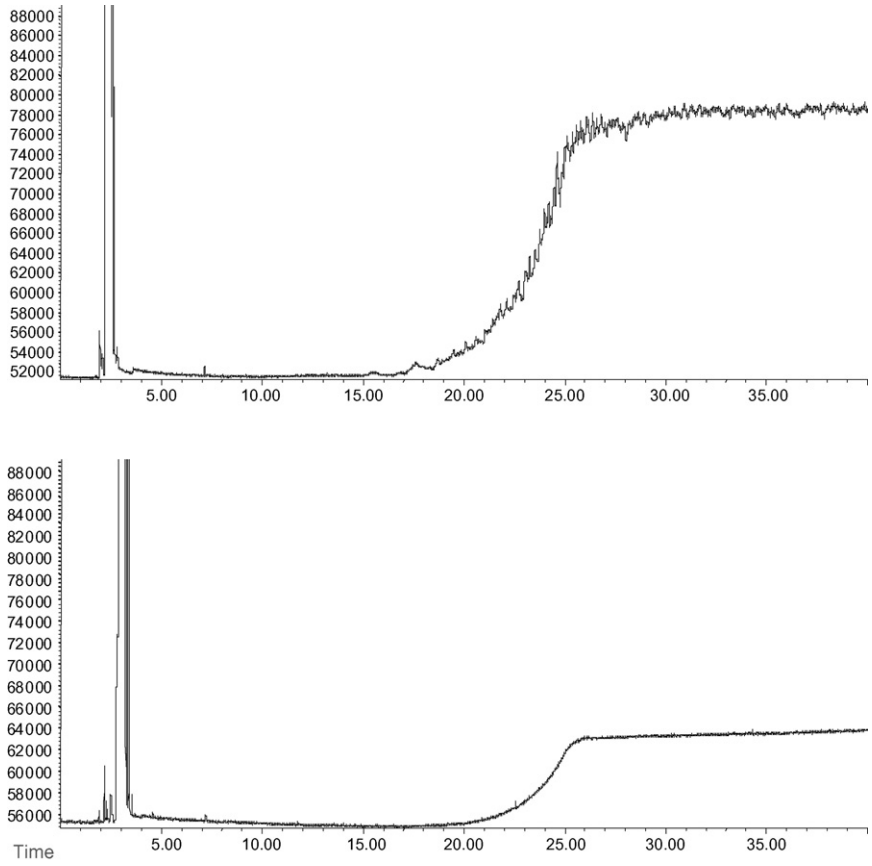

Fig. 1. Bleeding exhibited by the HP50+ column (above) and the HP Ultra 2 column (below). The temperature program was 60 °C (hold 1 min), followed by 8 °C/min to 300 °C hold for 10 min, and detection was achieved with a FID.

(Fig. 1). Although this problem did seem to vary from column to column this was considered unacceptable and the column had to be replaced. Therefore, the following three columns were also evaluated: HP-35 (25 m (*L*), 0.20 mm (i.d.), d_f 0.33 μm; Agilent Technologies), DB-35MS (30 m (*L*), 0.25 mm (i.d.), d_f 0.25 μm; Agilent Technologies), and DB-17MS (30 m (*L*), 0.25 mm (i.d.), d_f 0.25 μm; Agilent Technologies). The separation power of these columns was not evaluated, since resolution of target compounds and column inertness were the most important criteria for the choice of column. These three columns were subjected to the same type of experiments that had been used to evaluate the other four columns with regard to resolving power and inertness. The data were recorded only for the temperature gradients that required an analysis time of 30 min or less (i.e., 8 °C/min, 10 °C/min and 12 °C/min). The performance of each of the tested columns is summarised in the bottom part of Table 7.

All of these three columns provided good separation of the target compounds. The DB-35MS had a superior inertness (Table 7) and exhibited significantly less bleeding in comparison to the other columns. Thus, comprising to satisfy all the requirements, DB-35MS with a temperature gradient of 8 °C/min was chosen for the current profiling method. The performance of the columns mentioned above has been illustrated in Fig. 2.

3.3. Selection of the detection technique and optimisation of detector operating conditions

Detection by NPD, FID, and MS in the scan and SIM modes were evaluated and compared with respect to repeatability (within-day stability), reproducibility (between-day stability), sensitivity, selectivity, and linearity.

3.3.1. Repeatability (within-day stability)

To evaluate the different detectors with regard to repeatability, the modified Grob control sample used in this study was injected 20 times in 1 day at each of three different laboratories, and R.S.D. values of the RRFs were calculated for all target compounds. The repeatability results for the four detection techniques that were assessed are presented in Table 8.

The results show that the different detectors were rather similar with regard to repeatability: FID 2%, MS in the scan mode 2–5%, MS in the SIM mode 1–4%, and NPD 2%.

3.3.2. Reproducibility (between-day repeatability)

To evaluate the different detectors with regard to reproducibility of the results, the modified Grob control sample was injected 20 times once every day for 20 consecutive days at three different laboratories. Reproducibility calculations were performed as for repeatability, and the results for the four evaluated detection techniques are summarised in Table 9.

The FID apparently offered the best reproducibility (R.S.D. 2–3%). By comparison, somewhat greater between-day variation was noted for the other detectors: NPD 6%, MS in the scan mode 5%, and MS in the SIM mode 3–6%.

3.3.3. Limit of determination

Different concentrations of the modified Grob control sample used in this study (three replicates of each; concentrations given in Table 10) were injected at two different laboratories to study the limit of determination (LOD) for the investigated detectors. The lowest concentration with acceptable repeatability was considered to be the LOD. Obviously, this concentration is difficult to define; hence between-day repeatability values were used as a reference and, based on these values, it was decided that R.S.D. values less than 10% could be considered acceptable. The results are presented in Table 10 as the average R.S.D. values of the RRF values of each target compound at various concentrations.

The lowest concentrations at which FID, NPD, and MS in the scan and SIM modes offered sufficient repeatability were 0.05 μg/ml, 0.05 μg/ml, 0.05–0.1 μg/ml, and 0.01–0.05 μg/ml, respectively. To summarise, the results provided by the three various detectors were comparable, except that MS in the SIM mode had 5–10 times lower LOD.

3.3.4. Selectivity

The better selectivity of the mass spectrometer compared to the FID is demonstrated in Fig. 3, where chromatograms of the modified Grob extract obtained by utilising FID, and mass spectrometer in full scan and SIM mode are visualised. Data recorded with full scan mode of the MS can be greatly improved by extracting the signal of one specific ion for a target compound when co-elution with unknown compounds occur. Thus the results clearly illustrate the outstanding selectivity of MS compared to FID.

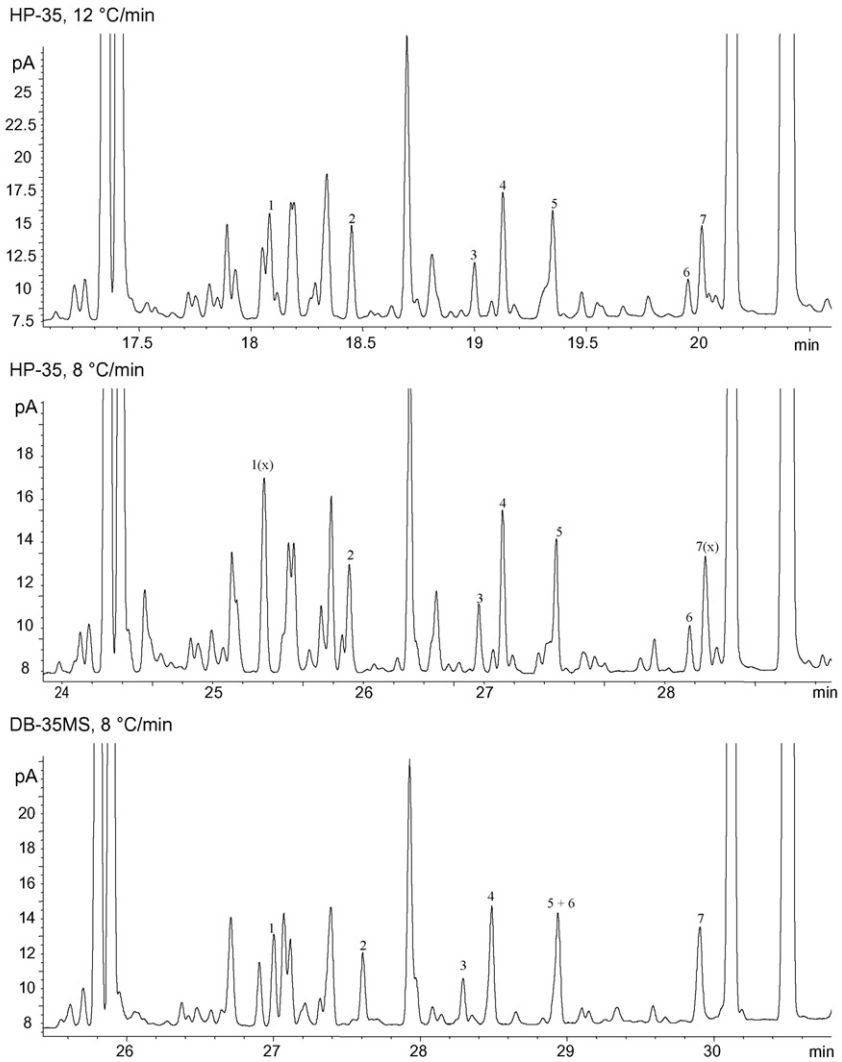

Fig. 2. Parts of chromatograms illustrating separation of Leuckart amphetamine impurities on different columns utilizing oven temperature gradients of 8 °C/min and 12 °C/min. Identification of peaks: (1) 1-benzyl-3-methylnaphthalene; (2) 1,3-dimethyl-2-phenylnaphthalene; (3) 2,6-dimethyl-3,5-diphenylpyridine; (4) 2,4-dimethyl-3,5-diphenylpyridine; (5) pyridine 7; (6) pyridine 14; (7) 2,6-diphenyl-3,4-dimethylpyridine; (X) unknown co-eluting compound(s).

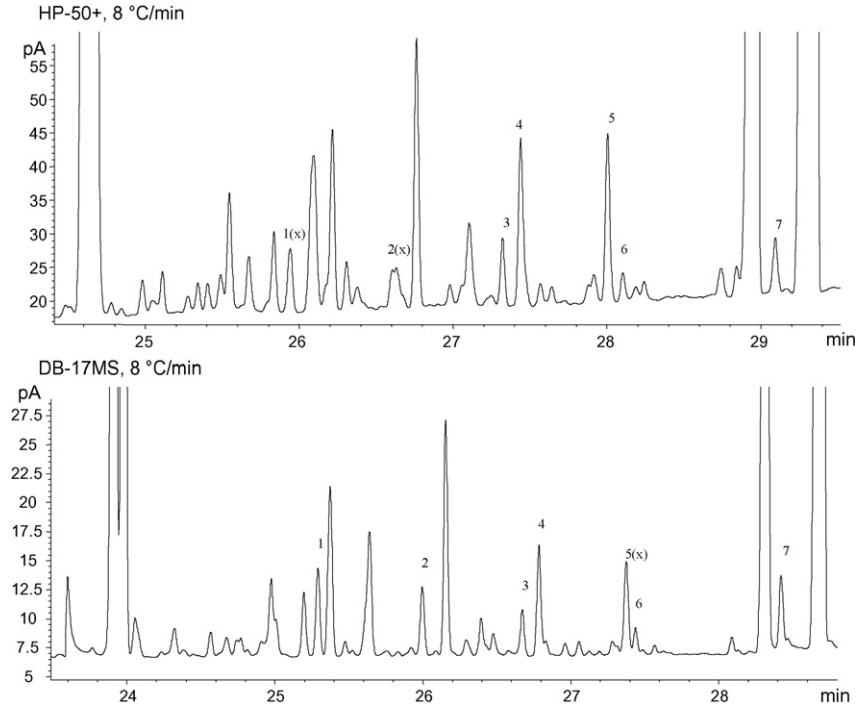

Fig. 2. (*Continued*).

3.3.5. Linearity

The linearity of the different detectors was investigated by analysing the modified Grob extract at three different laboratories, utilising samples with a concentration of target compounds in the range of 0.01–100 μg/ml, 0.05–500 μg/ml, and 0.05–500 μg/ml, respectively, when considering MS in the scan and SIM modes. The same concentrations were used to investigate the linear range of the FID and NPD, with the exception of 0.05–10,000 μg/ml used in one of the laboratories. The linearity of calibration curves was determined by measuring the Pearson product–moment coefficient of correlation (r^2) and the coefficient of determination (R^2) [13]. The

Table 8
Repeatability (%) of different detection techniques

Compound	Laboratory 1			Laboratory 2				Laboratory 3		
	FID	SCAN	SIM	FID	SCAN	SIM	NPD	FID	SCAN	SIM
n-Octanol	3	5	4	–	–	–	–	–	–	–
n-Dodecane	2	5	4	0	1	0	–	2	2	1
2,6-Dimethylphenol	3	4	4	1	1	1	–	2	1	1
n-Tridecane	2	4	4	0	1	1	–	1	1	1
2,6-Dimethyl aniline	3	4	4	i.s.	i.s.	i.s.	i.s.	1	1	1
Decanoic acid methyl ester	2	4	4	0	1	0	–	1	1	0
Undecanoic acid methyl ester[a]	5	3	4	1	1	0	–	2	6	2
Dicyclohexylamine	5	6	5	3	5	44[b]	3	15	15	21
Dodecanoic acid methyl ester[c]	2	3	4	1	1	0	–	1	2	3
n-Heptadecane	1	4	4	1	1	1	–	1	2	0
n-Octadecane	1	5	4	1	1	0	–	1	2	1
n-Nonadecane	1	4	4	1	1	0	–	0	2	1
N-Methyl-diphenethylamine	–	–	–	1	2	2	1	3	2	2
Ketamine	–	–	–	1	4	5	1	4	2	1
Tetracosane	2	17[d]	7	2	2	2	–	1	4	2
Trimipramine	–	–	–	16[e]	3	3	2	2	3	3
Average	2	5	4	2	2	1	2	2	3	3

[a] Undecanoic acid methyl ester and pentadecane co-eluted on the FID column and were not completely separated on the MS column. Only undecanoic acid methyl ester was measured in the SIM mode.
[b] Dicyclohexylamine started to elute before the specific ions were recorded.
[c] Dodecanoic acid methyl ester and hexadecane were not completely separated. Only dodecanoic acid methyl ester was measured in the SIM mode.
[d] High R.S.D. value at laboratory 1 was due to use of the standard ion source.
[e] The compound co-eluted with alkanes originating from the GC vial septum; this value was excluded as an outlier from the average.

Table 9
Reproducibility (%) of different detection techniques measured as between-day repeatability

Compound	Laboratory 1			Laboratory 2				Laboratory 3		
	FID	SCAN	SIM	FID	SCAN	SIM	NPD	FID	SCAN	SIM
Octanol	2	6	7	–	–	–	–	–	–	–
Dodecane (C12)	3	6	7	0	2	2	–	1	3	2
2,6-Dimethylphenol	2	5	7	1	4	5	–	2	2	2
Tridecane (C13)	2	5	6	2	3	2	–	2	3	2
2,6-Dimethyl aniline	2	5	7	i.s.	i.s.	i.s.	–	1	3	1
Decanoic acid methyl ester	6	5	6	1	5	7	–	1	3	2
Undecanoic acid methyl ester[a]	1	5	6	1	4	7	–	2	10	8
Dicyclohexylamine	*16*[b]	*15*[b]	*18*[b]	9	8	*170*[b,c]	9	7	8	10
Dodecanoic acid methyl ester[d]	2	5	6	2	5	10	–	1	8	5
Heptadecane (C17)	1	5	5	2	6	4	–	1	3	2
Octadecane (C18)	1	4	5	2	7	4	–	1	3	2
Nonadecane (C19)	1	4	5	3	9	6	–	1	3	2
N-Methyl-diphenethylamine	–	–	–	2	*15*[b]	*18*[b]	2	2	3	3
Ketamine	–	–	–	9	*31*[b]	*28*[b]	8	7	4	4
Tetracosane (C24)	2	7	7	3	*27*[b]	*19*[b]	–	3	5	5
Trimipramine[e]	–	–	–	*23*[b]	*21*[b]	*24*[b]	4	2	3	–
Average	3	6	7	4	11[f]	22[f]	6	2	5	3
Average (outliers excluded)	2	5	6	3	5	5	–	–	–	

[a] Undecanoic acid methyl ester and pentadecane co-eluted on the FID column and were only partly separated on the MS column. Only undecanoic acid methyl ester was measured in the SIM mode.
[b] Values in italic were excluded as outliers.
[c] Dicyclohexylamine started to elute before the specific ions were recorded.
[d] Dodecanoic acid methyl ester and hexadecane were partly separated. Dodecanoic acid methyl ester and hexadecane and were only partly separated. Only undecanoic acid methyl ester was measured in the SIM mode.
[e] The compound co-eluted with alkanes originating from the GC vial septum.
[f] High R.S.D. values were caused by use of the standard ion source.

calculated correlation coefficients in the investigated concentration ranges were for most compounds, when utilising the MS in both SIM and full scan mode, in the region of 0.999 and the same results were obtained for calibration curves when utilising the FID and NPD. As expected, the linear range was greater for FID and NPD.

Taking repeatability, reproducibility, and linearity into account, it is obvious that the FID performed equally well or better than the MS. However, mass spectrometry in the scan and SIM modes provided outstanding selectivity, and MS in the SCAN mode had the ability to identify the target compounds as full mass spectra. These aspects of the MS make it a prominent candidate as the detector of choice for the final profiling method. Since both the FID and MS performed very well it was decided to study the between laboratory reproducibility before the final choice of detection technique [14].

3.4. Optimisation of injection volume

The response of the target compounds obtained in the experiments as outlined in Table 2 was compared to assess whether the same sample amount was introduced on the column at the different injection settings. The standard injection method (1 μl splitless, splitless time 1 min) was chosen as the reference. Each target compound was normalised to the peak area sum of all target compounds in the same chromatogram. First, an *F*-test was used to compare whether the different injection alternatives had the same standard deviation or not, and thereafter a *T*-test (p 0.05) was applied to evaluate whether the injection alternatives were comparable. The results verified (data not shown) that splitless injections of 1 μl, 2 μl, and 4 μl gave the same response, while 0.2 μl splitless injections were less successful. Hence, the operator can freely choose injection

Table 10
Results of the LOD studies

	Laboratory 1 (R.S.D. %)				Laboratory 2 (R.S.D. %)				
	0.005 μg/ml	0.01 μg/ml	0.05 μg/ml	0.1 μg/ml	0.005 μg/ml	0.01 μg/ml	0.05 μg/ml	0.1 μg/ml	0.5 μg/ml
FID									
Dodecane (C12)	25	15	4	0.1	n.d.[a]	n.d.	1.5	1.8	1.4
2,6-Dimethylphenol	22	5	6	0.0	n.d.	n.d.	3.8	2.2	1.8
Tridecane (C13)	14	11	15	0.0	n.d.	n.d.	4.7	5.8	1.0
2,6-Dimethyl aniline	52	20	6	0.0	n.d.	n.d.	8.2	6.3	1.4
Decanoic acid methyl ester	9	19	19	0.3	n.d.	n.d.	4.4	4.4	1.3
Undecanoic acid methyl ester	7	8	7	0.0	n.d.	n.d.	6.9	2.2	0.74
Dicyclohexylamine	44	53	16	0.1	n.d.	n.d.	12	15	3.6

Table 10 (*Continued*)

	Laboratory 1 (R.S.D. %)				Laboratory 2 (R.S.D. %)				
	0.005 μg/ml	0.01 μg/ml	0.05 μg/ml	0.1 μg/ml	0.005 μg/ml	0.01 μg/ml	0.05 μg/ml	0.1 μg/ml	0.5 μg/ml
Dodecanoic acid methyl ester	52	64	*52*[b]	0.1	n.d.	n.d.	25	3.3	1.2
Heptadecane (C17)	28	25	9	0.0	n.d.	n.d.	10	6.3	1.4
Octadecane (C18)	14	24	*39*[b]	0.0	n.d.	n.d.	6.7	5.3	1.1
Nonadecane (C19)	26	5	4	0.0	n.d.	n.d.	3.6	4.6	0.58
Tetracosane (C24)	8	7	11	0.1	–	–	–	–	–
Trimipramine	–	–	–	–	n.d.	n.d.	1.5	15	3.3
Ketamine	–	–	–	–	n.d.	n.d.	n.d.	20	5,1
N-Methyl-diphenethylamine	–	–	–	–	n.d.	n.d.	10	5.9	1.9
4-Methyl-5-phenylpyrimidine	–	–	–	–	n.d.	n.d.	17	5.4	4.7
Average	25	21	9.7	0.1	–	–	8.2	6.9	2.0
Average of alkanes	19	15	8.6	0.1	–	–	5.3	4.8	1.1
SCAN									
Dodecane (C12)	n.d.	12	13	5	n.d.	n.d.	5.5	2.2	2.3
2,6-Dimethylphenol	n.d.	16	12	6	n.d.	n.d.	5.3	2.0	3.3
Tridecane (C13)	n.d.	10	22	4	n.d.	n.d.	3.4	4.3	3.3
2,6-Dimethyl aniline	n.d.	11	16	7	n.d.	n.d.	1.7	*79*[b]	0.34
Decanoic acid methyl ester	n.d.	7	14	6	n.d.	n.d.	2.1	0.84	0.76
Undecanoic acid methyl ester	n.d.	7	20	6	n.d.	n.d.	5.7	2.7	0.75
Dicyclohexylamine	n.d.	43	30	14	n.d.	n.d.	*54*[b]	*79*[b]	7.2
Dodecanoic acid methyl ester	n.d.	27	18	7	n.d.	n.d.	7.3	3.8	2.4
Heptadecane (C17)	n.d.	24	18	9	n.d.	n.d.	7.0	2.3	1.9
Octadecane (C18)	n.d.	27	17	5	n.d.	n.d.	4.6	0.90	2.3
Nonadecane (C19)	n.d.	32	27	3	n.d.	n.d.	0.66	2.2	2.6
Tetracosane (C24)	n.d.	30	12	4	–	–	–	–	–
Trimipramine	–	–	–	–	n.d.	n.d.	n.d.	1.0	4.4
Ketamine	–	–	–	–	n.d.	n.d.	n.d.	15	3.6
N-Methyl-diphenethylamine	–	–	–	–	n.d.	n.d.	14	4.1	2.8
4-Methyl-5-phenylpyrimidine	–	–	–	–	n.d.	n.d.	9.9	3.3	0.49
Average		20	18	6.3	–	–	5.6	3.4	2.6
Average of alkanes		22	18	5.0	–	–	3.5	2.0	2.5
SIM									
Dodecane (C12)	10	3	3	2	45	10	3.5	2.0	0.40
2,6-Dimethylphenol	6	2	2	3	113	93	*82*[b]	26	17
Tridecane (C13)	15	6	3	2	27	12	6.2	6.9	1.6
2,6-Dimethyl aniline	5	1	2	4	78	80	5.4	1.6	0.01
Decanoic acid methyl ester	4	1	2	3	41	9.0	4.9	1.1	0.06
Undecanoic acid methyl ester	6	2	2	3	43	10	3.9	0.80	1.9
Dicyclohexylamine	26	25	8	5	–	–	–	–	–
Dodecanoic acid methyl ester	4	5	1	2	51	16	5.4	1.4	1.2
Heptadecane (C17)	11	7	1	1	86	20	4.7	2.9	0.67
Octadecane (C18)	15	9	2	1	41	25	8.3	1.4	1.3
Nonadecane (C19)	11	8	1	1	52	38	21	4.6	4.9
Tetracosane (C24)	14	10	1	1	–	–	–	–	–
Trimipramine	–	–	–	–	41	107	25	16	10
Ketamine	–	–	–	–	42	7.3	5.5	10	8.4
N-Methyl-diphenethylamine	–	–	–	–	68	74	24	9.8	2.3
4-Methyl-5-phenylpyrimidine	–	–	–	–	40	34	23	7.4	5.4
Average	10.6	6.6	2.3	2.3	55	38	10.8	6.6	4.0
Average of alkanes	12.7	7.2	1.8	1.3	50	21	8.7	3.0	1.5
NPD									
Trimipramine	–	–	–	–	n.d.	n.d.	10	2.2	2.3
Ketamine	–	–	–	–	n.d.	n.d.	20	–	–
N-Methyl-diphenethylamine	–	–	–	–	n.d.	n.d.	4.7	2.4	1.4
4-Methyl-5-phenylpyrimidine	–	–	–	–	n.d.	n.d.	6.5	2.7	0.72
Dicyclohexylamine	–	–	–	–	n.d.	n.d.	16	4.1	3.8
Average					–	–	9.3	6.3	2.3

[a] Abbreviations: n.d., not detected; –: not determined.

[b] Values in italic were excluded as outliers.

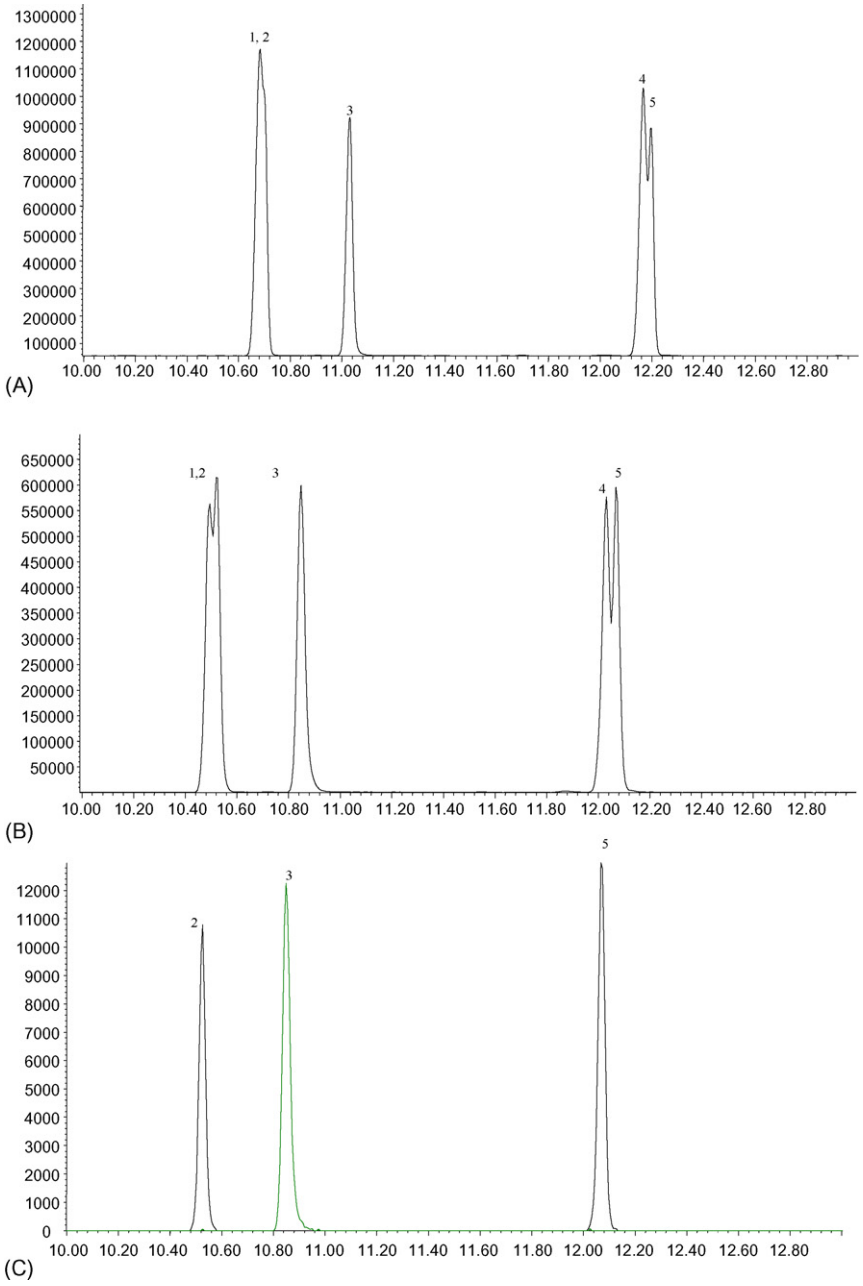

Fig. 3. Chromatograms of the modified Grob mixture recorded using FID (A) and MS in the full scan mode (B). The analytes can be selectively detected by extracting specific ions from the full scan or SIM runs, as illustrated in C. Identification of compounds: (1) pentadecane; (2) undecanoic acid methyl ester; (3) dicyclohexylamine; (4) hexadecane; (5) dodecanoic acid methyl ester.

volumes in the range of 1–4 μl depending on the nature of the sample, while injection of 0.2 μl should be avoided.

Moreover, for 1 μl injections it was found that a splitless time of 0.5 min provided better peak shapes of early eluting compounds than a splitless time of 1.0 min did. Therefore, it was decided to use a splitless time of 0.5 min for this injection volume in the developed GC method. However, it should be noted that, despite this finding, a splitless time of 1.0 min has been used for 1 μl injections in other experiments performed within this pan-European project [14,15]. This situation is a result of the order in which experiments were carried out.

4. General conclusions

The GC method developed in this study performed extremely well. Indeed, sample introduction, which is normally the most critical step in gas chromatography, did not pose any problem since the results were nearly the same under different conditions. However, the cool-on-column injection was superior to the vaporisation techniques, but it was rejected for practical reasons.

Due to the complexity of the samples, chromatographic separation proved to be a great challenge. Nonetheless, highly satisfactory operation was achieved within acceptable analysis time.

Analyses of amphetamine samples can be performed with splitless injection varying the injection volume between 1 μl and 4 μl. This is a very practical advantage since samples with low concentrations can be repeatedly analysed by just increasing the volume of sample injected into the gas chromatograph.

The choice of final detection technique could not be decided upon in this study, but the MS showed advantages, which are due to the unique selectivity of the mass spectrometric detection. Before a final choice of detection technique could be reached, it was decided to also study the between laboratory reproducibility of the MS and FID [14].

The optimised GC method can be summarised as follows:

- *GC*: HP 6890 gas chromatograph;
- *Column*: 35% phenyl methyl silicone column 30 m (L) × 0.25 mm (i.d.), d_f 0.25 μm (DB-35MS, Agilent Technologies), attached to a 35% phenyl methyl silicone pre-column, approximately 2.5 m (L) × 0.25 mm (i.d.), d_f 0.10 μm (DB-35MS, the thin-film column can be ordered as a custom-made product from Agilent Technologies)
- *Column connector*: undeactivated press-fit connector used to couple the pre-column to the analytical column;
- *Carrier gas*: Helium, approximately 41 cm/s at 90 °C, constant flow (see retention time locking);
- *Injection volume*: 1 μl splitless, 60 ml/min total flow after 0.5 min or 2 μl or 4 μl splitless, 60 ml/min total flow after 1 min (gas saver 20 ml/min after 1.5 min), single tapered liner packed with glass wool;
- *Temperatures*: injector, 250 °C; oven *T*-programs, 90 °C (1 min); 8 °C/min; 300 °C (10 min);
- *Retention time locking*: retention time of the internal standard (nonadecane) locked at 15.00 min.

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