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Thermal desorption cold trap-injection in high-resolution gas chromatography: multivariate optimization of experimental conditions

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ABSTRACT

In studies of low concentrations of volatile compounds in air, the method of adsorption on porous polymers and determination by thermal desorption cold trap-injection high-resolution gas chromatography is finding increasing application Factors considered important for injection and chromatographic separation of volatile compounds by this method were investigated with the use of multivariate techniques For the amount injected on to the chromatographic column, the factors of main importance were found to be the temperature of the injection block, the thickness of the internal coating of the cold trap and the flow-rate Strong interaction effects were noted For the sharpness of the chromatographic peaks, the flow-rate was the most important factor

INTRODUCTION

Thermal desorption cold trap-injection high-resolution gas chromatography is an effective method for the determination of low concentrations of volatile compounds in air Some studies have been reported concerning the important factors controlling desorption [1,2], but the injection step has not yet been well examined As many factors presumably exert a joint action both on the injection and on the chromatographic performance, this study was performed using multivariate methods, which take possible interaction effects into account Such interaction effects are common in chemistry, and a traditional approach, *i e* , considering the factors one at a time, is bound to fail if interaction effects are present [3]

Microorganisms such as various species of moulds and bacteria are often found to be the source of contamination of water-damaged buildings, often giving rise to health problems for the inhabitants The aim of this study was to optimize analytical conditions for the determination of volatile metabolites produced by such microorganisms in affected buildings as well as in laboratory studies These studies were performed on a test mixture consisting of eight different compounds, selected as being representative of compounds arising from culture media and of some compounds expected to be produced by microorganisms These compounds also differ sufficiently in polarity and volatility to make these studies of general interest

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OPTIMIZATION

The various experimental factors must be assumed to have a joint action, and it is therefore necessary to approach the problem by multivariate methods The overall strategy was (a) first to identify the most important experimental factors by a screening experiment and (b) then to adjust these factors by a response surface technique to an optimum chromatographic performance For evaluation of the results, we considered it necessary to consider both the amounts of injected material and the chromatographic separation

Attempts at using various chromatographic response functions (CRFs) [4,5,6] which compress the multi-dimensional response into a single criterion were considered unsuitable Such single-criterion response functions over-emphasize short retention times and assume that the eluted peaks are fairly evenly distributed over the whole chromatogram and occur to some extent close to each other The composition of our test mixture did not fulfil this requirement A minimum retention time was not considered necessary because, in this study, the chromatographic separation is not the most time-consuming part of the whole procedure Moreover, in the application of the procedure to real samples, it could not be expected that samples will contain volatile components fulfilling the above-mentioned criteria Instead, in order to achieve a more general optimization, our objective was to obtain a maximum of injected desorbed material and acceptable peak shapes over the whole chromatogram

EXPERIMENTAL

Chemicals and adsorbent

The chemicals used in the test mixture were *n*-hexane (FSA Laboratory Supplies, HPLC grade), dimethyl disulphide (Janssen, p a), 3-methyl-2-pentanone (Aldrich, 99%), benzaldehyde (Kebo, puriss), n-decanal (Aldrich, 98%), *n*-tetradecane (Fluka, puriss) and geosmin, synthesized according to Hansson and co-workers [7,8] Tenax TA (60–80 mesh) (Chrompack, 90 mg per sampling tube) was used as adsorbent in the experiments The sampling tubes were made of glass (159 mm × 6 mm O D × 3 mm I D)

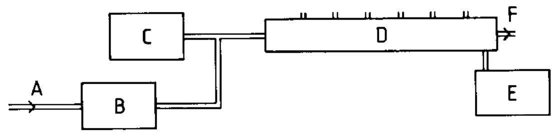

Fig 1 Generation of samples A = compressed air cleaned through oil filters and molecular sieve, B = moisturizing outfit consisting of three water-filled dispersion bottles in a thermostated water bath, C = microinjection pump for continuous injection of the test mixture, D = sampling chamber (Teflon, 900 × 80 × 60 mm^3) with six outlets for sampling, E = Relative humidity meter, F = air outlet

Generation of samples

A sampling atmosphere of the test mixture in low concentration (see Table I) was dynamically generated according to Fig 1 The test mixture was slowly injected (25 nl/min) into a stream of air by means of a microinjection pump (Carnegie Medicin CMA/100) A 50 μl gas-tight syringe (SGE) was used for the injection The air flow-rate was maintained at 40 l/min and the relative humidity of the air was adjusted to 60% Samples were sorbed on Tenax by pumping the sampling atmosphere through the tubes at 100 ml/min for 5 min Three generations were made, and six analyses were run from each generation to check the repeatability and standard deviation before further use of the spiked tubes in the optimization experiments

Injection and chromatographic separation

The experiments were run on a commercial thermal desorption injector (Chrompack 16400 purge and trap injector, modified for thermal desorption injection according to the Chrompack modification manual M-16420-85-2)

The sample, adsorbed on Tenax, was desorbed by heating the sampling tube in the desorption oven A flow of helium transferred the desorbed substances to a cold trap (Fig 2), a fused-silica capillary coated with a 5% phenyl and 95% methyl polysiloxane phase (Chrompack CP-TM-Sil-8CB) Sub-ambient trap temperatures were created by passing a stream of nitrogen cooled by liquid nitrogen through the trap compartment The cold trap was then rapidly heated (15°C/s) in order to inject the sample onto the chromatographic column

Desorption was performed at 220°C for 15 min, using a desorption gas flow-rate of 20 ml/min The measurements were carried out on an HP 5890 gas

TABLE I

TEST MIXTURE SUBSTANCES AND CONCENTRATIONS IN TEST ATMOSPHERE

No	Substance	Concentration (ng l^{-1})	B p (°C)[a]	R S D (%)[b]
1	Hexane	70	68	7
2	Dimethyl disulphide	70	109	6
3	3-Methyl-2-pentanone	82	118	4
4	Toluene	80	111	6
5	Benzaldehyde	72	178	9
6	Decanal	73	208–209	15
7	Tetradecane	68	254	9
8	Geosmin	68	[c]	11

[a] B p = Boiling point at atmospheric pressure
[b] R S D = Relative standard deviation, due to exposure, sample generation, sampling and analysis, based on 18 runs
[c] Data not available

chromatograph with a fused-silica column (HP Ultra 2, 50 m × 0 2 mm I D , coated with cross-linked 5% phenylmethylsilicone, 0 33 μm) and a flame ionization detector The detector temperature was 200°C The starting temperature of the chromatographic separation was 30°C and the final temperature was 200°C An HP 3392A integrator was used as a recorder

Experimental factors and responses

Many factors may influence the results Some factors were known *a priori* to be important and the task was to determine the trend and magnitude of their influence Other factors presumably exert an influence, but their roles remained to be ascertained

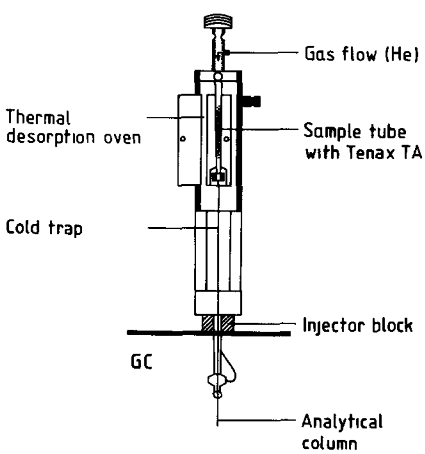

Fig 2 Thermal desorption cold trap (TCT) injector

The following nomenclature will be used x_i denotes the coded setting of factor i The response models described below are expressed in the coded variables The following factors were studied

(1) final temperature of the cold trap (see Fig 2), this setting thus defines the temperature of the sample when transferred to the injection block,

(2) initial temperature of the cold trap,

(3) temperature of the injection block,

(4) thickness of the internal coating of the cold trap,

(5) duration of injection,

(6) additional time during which the chromatographic column was maintained at its starting temperature value,

(7) temperature rise during the chromatographic separation,

(8) flow-rate, this defines the flow-rate both through the cold trap during injection and through the chromatographic column at its starting temperature,

(9) temperature setting of the cold trap after the injection was completed

The range of variations of the experimental factors is specified in Table II Using coded normalized factor settings instead of their natural value has the advantage that the relative importance of each variable can be evaluated directly from the model [3]

The measured results of the chromatographic procedure are called responses For each constitu-

TABLE II

RANGE OF VARIATIONS OF EXPERIMENTAL FACTORS

Factor No	Low level (−)	Medium level (0)	High level(+)
1	+130°C	+160°C	+200°C
2	−100°C	−125°C	−150°C
3	+150°C	+200°C	+250°C
4	A[a]	A[a b]	B[a]
5	1 min	3 min	5 min
6	1 t_0	1 5 t_0	2 t_0
7	3°C/min	6 5°C/min	10°C/min
8	10 cm/s	22 cm/s	34 cm/s
9	<0°C	Room temperature[b]	Room temperature

[a] A = CP-TM-SIL-8CB, d_f = 1 2 μm, I D = 0 32 mm B = CP-TM-SIL-8CB, d_f = 5 0 μm, I D = 0 5 mm

[b] Only two levels tested for these factors

ent (k = 1–8, Table I), two characteristics were measured A_k, the area of the chromatographic peak, and W_k, the width of the peak at half its maximum height

Experimental design

The overall strategy for the study presented in this paper was as follows

(A) run a pilot experiment to validate the experimental domain,

(B) vary the experimental factors considered to be important in a screening experiment with a view to identifying the most important factors,

(C) perform additional experiments to find optimum performance

The pilot experiment consisted of two experimental runs, one in which all experimental factors were set at their upper value, and another in which all experimental factors were set at their lower value This was done to ensure that the responses show a significant variation within the domain and that none of the extreme points yields non-useful responses

To ensure an orthogonal variation of the nine experimental factors, a replicated two-level fractional design 2^{9-5} was used in the screening (entries 1–32, Table III) These experiments were employed to fit a response surface model, containing linear terms $b_i x_i$ and cross-produced terms $b_{ij} x_{ij}$ The coefficients of the cross-product terms are aliased two-factor interactions [9], see Appendix Results are given in the next section

Based on the results from the screening additional experiments were carried out with a view to further optimization of the experimental conditions One significant factor, 4 (thickness of the internal coating of the cold trap), was set at its upper level The following factors were maintained at their average setting throughout the experimental study 3 (temperature of the injection block), 6 (additional time during which the chromatographic column was maintained at its starting temperature) and 7 (temperature rise during the chromatographic separation) One variable, 9 (temperature setting of the cold trap after the injection was completed), was not further varied as its variation was found insignificant in the screening For the remaining factors (1, 2, 5 and 8), experiments were run to complete a variation of these factors on three levels (−1, 0, +1), entries 33–50 This also permits square terms for these factors to be included in the model to describe non-linear effects, *i e*, curvature of the response surface

Mathematical methods

Principles of response surface technique It is reasonable to assume that the variation of the observed responses y (y = A_k and W_k) is functionally related to the detailed settings of the experimental factors However, as the responses are experimentally determined, there will always be an experimental error component (e) We therefore write the functional relationship between the observed response and the experimental factors as

$$y = f(x_1 \quad x_9) + e$$

TABLE III

EXPERIMENTAL DESIGN FOR SCREENING AND OPTIMIZATION

1–9 are the experimental factors, and their settings (+, − or 0) corresponds to the values specified in Table II

Entry	1	2	3	4	5	6	7	8	9	Entry	1	2	3	4	5	6	7	8	9
1	−	−	−	−	−	−	−	−	+	28	+	+	−	+	−	+	−	−	−
2	+	−	−	−	+	+	+	−	−	29	−	−	+	+	+	+	−	−	+
3	−	+	−	−	+	+	−	+	−	30	+	−	+	+	−	−	+	−	−
4	+	+	−	−	−	−	+	+	+	31	−	+	+	+	−	−	−	+	−
5	−	−	+	−	+	−	+	+	−	32	+	+	+	+	+	+	+	+	+
6	+	−	+	−	−	+	−	+	+	33	0	0	0	+	0	0	0	0	+
7	−	+	+	−	−	+	+	−	+	34	+	0	0	+	0	0	0	0	+
8	+	+	+	−	+	−	−	−	−	35	−	0	0	+	0	0	0	0	+
9	−	−	−	+	−	+	+	+	−	36	0	+	0	+	0	0	0	0	+
10	+	−	−	+	+	−	−	+	+	37	0	−	0	+	0	0	0	0	+
11	−	+	−	+	+	−	+	−	+	38	0	0	0	+	+	0	0	0	+
12	+	+	−	+	−	+	−	−	−	39	0	0	0	+	−	0	0	0	+
13	−	−	+	+	+	+	−	−	+	40	0	0	0	+	0	0	0	+	+
14	+	−	+	+	−	−	+	−	−	41	0	0	0	+	0	0	0	−	+
15	−	+	+	+	−	−	−	+	−	42	0	0	0	+	0	0	0	0	+
16	+	+	+	+	+	+	+	+	+	43	+	0	0	+	0	0	0	0	+
17	−	−	−	−	−	−	−	−	+	44	−	0	0	+	0	0	0	0	+
18	+	−	−	−	+	+	+	−	−	45	0	+	0	+	0	0	0	0	+
19	−	+	−	−	+	+	−	+	−	46	0	−	0	+	0	0	0	0	+
20	+	+	−	−	−	−	+	+	+	47	0	0	0	+	+	0	0	0	+
21	−	−	+	−	+	−	+	+	−	48	0	0	0	+	−	0	0	0	+
22	+	−	+	−	−	+	−	+	+	49	0	0	0	+	0	0	0	+	+
23	−	+	+	−	−	+	+	−	+	50	0	0	0	+	0	0	0	−	+
24	+	+	+	−	+	−	−	−	−	51	−	0	+	+	0	0	+	+	0
25	−	−	−	+	−	+	+	+	−	52	−	0	+	+	0	0	+	+	0
26	+	−	−	+	+	−	−	+	+	53	−	0	+	+	0	0	+	+	0
27	−	+	−	+	+	−	+	−	+	54	−	0	+	+	0	0	+	+	0

It is not possible to derive an analytical expression for f from purely theoretical considerations It is reasonable to assume, however, that f can be approximated by a Taylor expansion when the range of variation in the independent factors x_1–x_9 is limited A Taylor expansion will take the form of a polynomial in the independent factors

$$y = b_0 + \Sigma b_1 x_1 + \Sigma\Sigma b_{ij} x_i x_j + \Sigma\Sigma\Sigma b_{ijk} x_i x_j x_k + \quad + e$$

A sufficiently good approximation can often be obtained if the Taylor expansion is truncated after the second degree terms The polynomial coefficients (model parameters) can be estimated by least squares multiple regression of the polynomial to the observed responses

The systematic variation induced by changing the experimental conditions is thus described by the coefficients of the polynomial model In order to be considered significant, an experimental factor must produce a variation in the response above the noise level e, caused by the experimental error The error variation can be assumed to be normally and independently distributed Hence, significant variables can be identified by plotting the corresponding coefficients on normal probability paper A normally distributed random error variation will be depicted by a straight line Significant model coefficients will appear as outliers To the right of the line in the upper right quadrant, or to the left in the lower left quadrant, such effects are either too small or too large to be error variations For details of this technique, see ref 10

Principal component (PC) analysis To analyse the systematic variation of the responses over the entire set of the responses the response matrices were subjected to principal component decomposition There are two kinds of responses A PC model was established for each of them separately Detailed accounts of PC analysis have been given elsewhere [11–13] Here, it is sufficient to say that principal components partition the response matrix into two parts *scores* and *loadings* The scores describe the systematic between-objects variation over the entire set Hence the score vector can be used as a response vector for the entire set of experiments The score value is a linear combination of the original response variables, and thus the error will also have an approximately normal distribution Significant experimental factors can therefore be discerned by a normal probability plot of estimated model parameters obtained by fitting the response model to the score vector A thorough discussion of this technique in screening experiments is given in ref 14, and its application to response surface modelling has been described by Bratchell [15] To avoid overfitting, the principal component models were established through cross-validations [16] Prior to computing the principal components, the original response variables were scaled to unit variance In this way, an equal importance of each response is assumed and blow-up of the variance because of differences in magnitude in the recorded responses is avoided For a discussion on scaling in principal component analysis, see ref 13 The loadings describe how the response variables take part in this systematic variation

RESULTS AND DISCUSSION

The experimental design is shown in Table III The observed responses in these experiments are summarized in Tables IV and V

Analysis of the residuals after fitting a second-order interaction model to the screening design (entries 1–32) indicated a systematic lack of fit Plotting the residuals against the response value predicted by the model showed a U-shaped scatter plot This indicated that an improved model fit was likely to be expected if square terms were also included in the model

Principal component analysis and response model fitting to the score vectors

Peak surfaces Principal component analysis of the data in Table IV afforded one highly significant component and two additional components of borderline significance (72 + 12 + 6% explained variance) Taking into account the fact that the measured peak areas differ in magnitude, this result was to be expected Because of this and because peak areas cannot be negative, a logarithmic transformation of the original data could be expected to yield an improved principal component model fit Principal component analysis of the logarithmically transformed peak areas afforded one significant component which accounted for 70% of the total variance The corresponding score values are summarized in Table IV (t-values) The loadings (p-values) are given as the bottom line in the table

The following response model was obtained by least squares fitting to the score values given in Table IV

$$t_1(\text{log area}) = -4\ 04 + 0\ 004x_1 - 0\ 18x_2 + 1\ 16x_3 + 2\ 02x_4 - 0\ 01x_5 + 0\ 50x_6 + 0\ 26x_7 + 0\ 22x_8 - 0\ 14x_9 + 0\ 02x_1x_2 + 0\ 17x_1x_3 - 0\ 08x_1x_4 - 0\ 27x_2x_3 + 0\ 27x_2x_4 + 0\ 86x_3x_4 + 1\ 32x_1^2 - 0\ 41x_2^2 + 0\ 31x_5^2 + 2\ 37x_8^2$$

The estimated cross-product coefficients represent aliased two-factor interactions, see Appendix A normal probability plot of the estimated coefficients is shown in Fig 3 A plot of the residuals against the estimated score value is shown in Fig 4 The plot does not indicate a lack of fit [17,18] Fig 5–7 show three-dimensional plots of the response surface

Peak widths A one-component PC model was significant according to cross-validation and accounted for 90% of the total variance of the responses in Table V The following response model was determined from the score vector

$$t_1(\text{peak width}) = -1\ 56 - 0\ 06x_1 + 0\ 03x_2 + 0\ 11x_3 - 0\ 05x_4 + 0\ 16x_5 + 0\ 08x_6 - 0\ 58x_7 - 2\ 90x_8 + 0\ 06x_9 - 0\ 14x_1x_2 + 0\ 03x_1x_3 - 0\ 01x_1x_4 + 0\ 08x_2x_3 - 0\ 05x_2x_4 - 0\ 03x_3x_4 + 0\ 23x_1^2 + 0\ 15x_2^2 + 0\ 15x_5^2 + 2\ 19x_8^2$$

A normal probability plot of the estimated coefficients is shown in Fig 8

Evaluation of each response separately

When each response variable was fitted separately with the response functions given above, the results were almost identical to those obtained from the score vectors

TABLE IV

OBSERVED RESPONSES, PEAK AREAS (INTEGRATOR COUNTS)

Entry	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	t_1
1	13 592	4 482	12 917	19 630	8 427	3 844	9 948	7 485	−4 12
2	13 559	4 607	13 945	19 334	11 849	6 922	12 752	10 381	−1 79
3	15 381	5 067	15 208	22 185	12 495	5 039	10 843	8 561	−2 17
4	14 129	3 540	13 558	28 952	13 116	5 422	11 436	8 890	−2 20
5	15 413	5 623	17 807	24 935	13 986	4 967	12 816	10 284	−0 82
6	13 339	4 933	14 987	21 778	11 229	4 438	11 648	9 013	−2 44
7	16 808	5 297	16 011	22 075	11 710	4 230	10 930	8 500	−2 27
8	13 790	4 695	14 010	22 115	10 416	4 236	9 646	7 104	−3 49
9	21 902	6 518	19 217	27 540	13 089	7 568	15 033	12 221	0 90
10	15 919	5 616	16 765	25 260	13 616	8 597	12 875	10 129	−0 28
11	16 733	5 302	15 682	23 122	14 860	7 053	13 288	10 638	−0 53
12	19 294	5 706	16 673	24 584	10 191	5 436	10 497	8 240	−1 87
13	19 367	10 147	30 002	46 446	15 729	7 305	13 426	10 507	2 69
14	19 518	11 376	33 282	43 578	23 107	6 898	14 143	11 044	3 59
15	23 641	7 723	23 590	35 052	18 124	7 386	13 867	11 005	1 98
16	25 281	8 249	25 085	38 009	23 454	10 524	19 763	16 183	4 45
17	14 053	4 600	13 480	19 450	9 082	4 557	10 647	8 068	−3 47
18	–	–	–	–	–	–	–	–	–
19	15 518	5 240	15 893	24 493	12 233	5 337	10 337	7 973	−2 07
20	15 886	4 721	14 366	22 948	11 266	4 287	9 652	7 597	−4 47
21	16 069	5 599	16 967	24 771	12 437	4 030	10 367	7 859	−2 16
22	15 652	6 451	19 078	29 427	16 252	5 739	13 050	10 148	0 14
23	20 424	5 545	16 149	23 914	8 820	3 803	8 786	6 317	−3 39
24	12 232	4 368	13 156	19 135	11 679	4 154	12 096	9 364	−2 96
25	21 156	6 392	18 583	27 056	11 708	7 656	13 378	10 755	0 19
26	14 842	4 994	15 042	21 782	11 082	4 757	10 450	8 352	−2 61
27	17 808	5 573	16 551	24 439	15 320	6 166	13 247	10 157	−0 48
28	20 545	6 456	18 991	28 121	14 424	8 366	14 701	12 142	1 06
29	19 867	11 161	33 232	49 767	22 167	9 795	18 069	15 087	5 12
30	19 117	10 441	30 765	50 515	21 619	7 044	14 756	11 387	3 61
31	23 017	7 729	23 443	34 746	19 408	8 870	16 515	14 086	3 01
32	27 282	8 363	25 444	40 163	21 582	9 806	19 025	14 880	4 21
33	18 876	6 717	19 947	30 280	13 331	8 068	15 788	12 072	1 21
34	18 501	6 619	19 126	26 977	12 294	8 412	15 087	12 418	0 76
35	20 496	7 311	21 355	32 270	14 078	8 021	14 940	12 268	1 59
36	18 339	6 717	19 817	31 283	13 790	7 339	14 647	11 063	0 88
37	19 098	6 872	18 330	29 199	13 367	–	10 587	9 089	−0 46
38	16 853	6 322	17 935	27 160	10 949	7 039	10 115	8 786	−1 09
39	20 079	7 220	21 112	35 534	17 127	8 147	14 720	11 333	1 82
40	22 667	7 414	22 270	38 325	19 736	10 851	16 873	12 924	3 16
41	24 007	6 907	20 827	32 459	15 047	8 050	14 041	10 726	1 34
42	15 107	5 942	16 732	25 363	11 366	6 206	12 696	9 390	−1 03
43	18 596	7 219	20 516	31 667	13 563	7 596	15 293	12 279	1 34
44	20 048	7 294	20 872	32 380	15 308	8 311	15 585	12 654	1 84
45	18 261	6 087	19 066	29 063	12 541	8 562	11 338	9 944	−0 04
46	17 576	6 231	17 318	27 658	9 581	6 390	8 968	7 603	−1 89
47	18 305	6 596	18 401	28 514	11 425	7 515	9 653	8 351	−0 89
48	18 230	7 241	20 924	31 270	19 402	7 985	14 138	10 824	1 55
49	–	–	–	–	–	–	–	–	–
50	23 114	9 640	28 681	41 745	15 081	6 887	13 548	11 249	2 51
51	16 974	5 841	18 496	27 673	12 747	6 839	12 956	9 939	–
52	20 474	7 008	22 463	34 328	15 559	7 951	14 260	11 173	–
53	22 291	6 484	20 647	33 335	17 065	9 061	14 729	12 114	–
54	18 827	6 412	20 260	29 463	13 358	7 484	12 798	10 285	–
p	0 2491	0 3676	0 3788	0 3726	0 3665	0 3473	0 3606	0 3681	

TABLE V

OBSERVED RESPONSES, PEAK WIDTHS (MM)

Entry	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	t_1
1	8 0	11 5	10 25	11 25	13 25	12 5	13 5	17 75	4 33
2	10 5	9 0	8 5	8 75	12 75	11 5	12 75	17 5	3 67
3	2 75	5 25	5 5	5 5	5 5	5 25	5 75	6 0	−0 91
4	2 25	2 75	2 75	–	3 0	2 75	3 0	3 5	−2 97
5	3 25	3 0	3 25	3 0	3 0	2 75	3 25	3 5	−2 44
6	3 5	4 5	4 75	4 75	5 75	5 0	5 75	5 75	−1 09
7	11 0	9 0	8 5	9 0	13 5	12 0	13 25	18 5	3 97
8	9 5	12 5	11 0	11 75	13 5	12 75	14 0	18 5	4 91
9	3 25	3 0	3 0	–	3 0	2 75	3 0	3 75	−2 47
10	3 0	4 75	5 0	5 0	5 75	5 0	5 5	5 75	−1 08
11	7 25	9 0	8 75	9 5	15 5	11 75	13 0	18 0	3 69
12	10 5	11 5	11 75	11 5	13 5	12 75	13 25	17 5	3 98
13	10 5	12 5	12 0	12 25	13 25	11 75	13 0	17 5	4 95
14	6 75	9 5	9 0	9 25	11 75	11 75	12 75	17 0	3 28
15	3 0	4 0	4 5	4 5	5 75	5 5	5 75	6 0	−1 20
16	3 25	3 75	4 0	3 75	3 5	2 75	3 25	3 5	−2 14
17	8 0	11 5	10 25	11 0	13 25	12 25	13 25	17 5	4 25
18	–	–	–	–	–	–	–	–	–
19	–	–	–	–	–	–	–	–	–
20	2 25	2 75	3 0	3 0	3 0	2 75	3 0	3 25	−2 67
21	–	–	–	–	–	–	–	–	–
22	–	–	–	–	–	–	–	–	–
23	–	–	–	–	–	–	–	–	–
24	–	–	–	–	–	–	–	–	–
25	3 0	2 75	3 0	3 0	3 0	3 0	3 0	3 5	−2 54
26	3 25	5 0	5 0	5 25	5 25	5 5	5 5	6 0	−0 98
27	–	–	–	–	–	–	–	–	–
28	–	–	–	–	–	–	–	–	–
29	–	–	–	–	–	–	–	–	–
30	–	–	–	–	–	–	–	–	–
31	–	–	–	–	–	–	–	–	–
32	3 0	3 75	4 0	3 75	3 5	2 75	3 25	3 75	−2 16
33	4 25	4 5	4 0	4 25	4 5	4 25	4 5	5 5	−1 44
34	4 0	4 25	4 0	4 25	4 5	4 25	4 5	5 5	−1 50
35	4 0	4 5	4 0	4 0	4 25	4 25	4 5	5 75	−1 50
36	4 0	4 25	4 0	4 25	4 5	4 0	4 25	5 5	−1 54
37	4 0	4 25	4 0	4 0	4 25	4 25	4 5	5 5	−1 54
38	4 0	4 5	4 0	4 25	4 5	4 0	4 5	5 5	−1 49
39	3 5	4 25	3 75	4 0	4 5	4 0	4 5	5 5	−1 64
40	3 0	3 5	3 25	3 5	3 75	3 5	3 5	3 75	−2 20
41	9 5	9 75	8 75	–	12 0	12 75	13 0	18 0	2 91
42	4 0	4 5	4 0	4 25	4 5	4 0	4 5	5 5	−1 49
43	4 25	4 25	4 0	4 25	4 5	4 25	4 5	5 5	−1 47
44	4 0	4 5	4 25	4 5	4 5	4 25	4 5	5 5	−1 41
45	4 0	4 25	4 0	4 25	4 5	4 25	4 5	5 5	−1 50
46	4 0	4 25	4 0	4 0	4 5	4 0	4 5	5 75	−1 53
47	–	–	–	–	–	–	–	–	–
48	–	–	–	–	–	–	–	–	–
49	–	–	–	–	–	–	–	–	–
50	9 5	–	–	9 0	11 25	11 5	12 75	17 25	3 69
51	–	–	–	–	–	–	–	–	–
52	–	–	–	–	–	–	–	–	–
53	3 5	3 0	3 25	–	3 0	2 75	3 25	3 25	–
54	3 25	3 25	3 0	3 0	3 0	2 75	3 25	3 5	–
p	0 3487	0 3671	0 3656	0 3160	0 3633	0 3338	0 3657	0 3647	

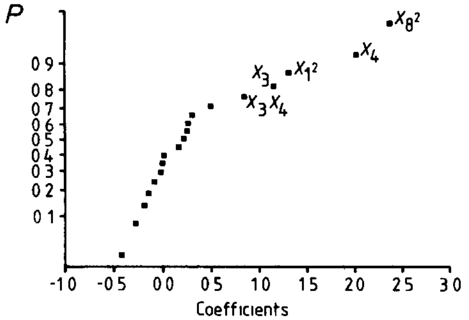

Fig 3 Normal probability plot of estimated coefficients, log peak areas

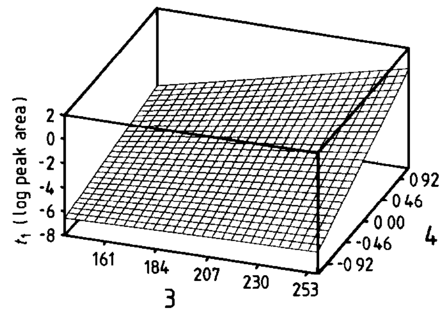

Fig 5 Response surface of log peak areas against temperature of injection block against thickness of internal coating of cold trap

Interpretations

From the results presented above, we conclude that the following experimental factors exert a significant influence on chromatographic performance

Peak areas a weak influence of factor 1 (final temperature of the cold trap), a strong linear influence of 3 (temperature of the injection block) and 4 (inner coating of the cold trap) and a strong interaction effect between 3 and 4, and a strong significant non-linear influence of the flow-rate (8)

Peak widths as expected, the most important factor is 8 (flow-rate), an influence (however weak) of 7 (temperature rise) is also found

Preferred settings of the experimental factors

For obtaining the desired result, maximum injected sample (maximum peak area) and sharp peaks, the following settings of the experimental factors can be inferred from the results above

(1) the final temperature of the cold trap should be at its lower setting,

(2) the initial temperature of the cold trap could be set at any value in the explored range of variation,

(3) because of the strong interaction effect with factor 4, the temperature of the injection block should be set at its upper value,

(4) a cold trap with a thick-layered inner coating should be used (see 3),

(5,6) the duration of the injection and the additional time during which the chromatographic column is maintained at its starting temperature can be set at any value in the explored domain,

(7) the temperature rise during chromatography should be at its higher value to ensure sharp peaks,

(8) the flow-rate should be set at its higher level,

(9) the temperature of the cold trap after injection is completed has no significant influence within the experimental domain

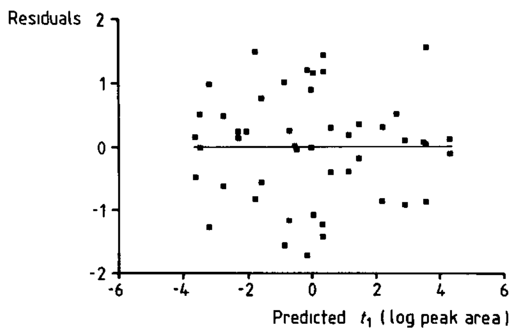

Fig 4 Residuals against estimated score value, log peak areas

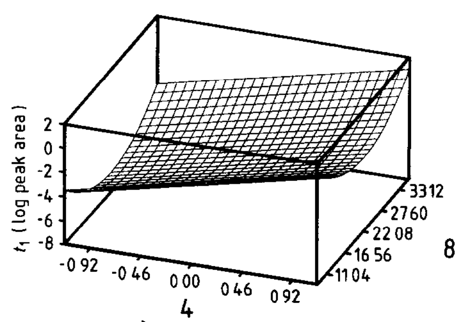

Fig 6 Response surface of log peak areas against thickness of internal coating of cold trap against flow-rate

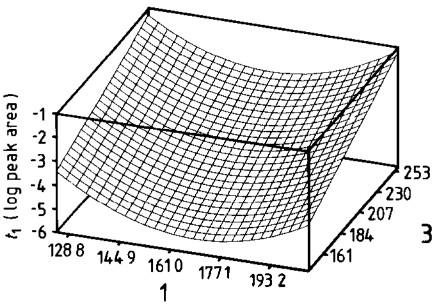

Fig 7 Response surface of log peak areas against final temperature of cold trap against temperature of injection block

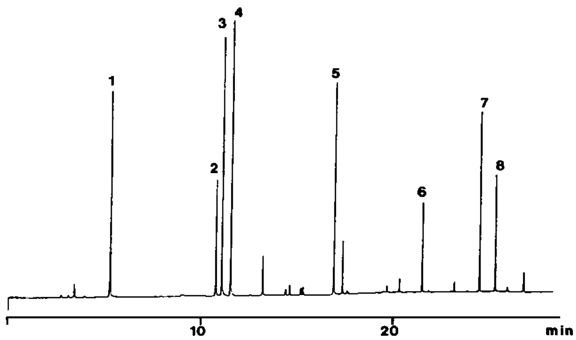

Fig 9 Chromatogram of the test mixture after optimization Substances 1–8 according to Table I Chromatographic conditions are given under Experimental

Replicated experiments carried out under the conditions indicated above are shown in Table III, entries 51–54 The corresponding results are shown in Tables IV and V These results confirm the conclusions with regard to the sharpness of the chromatographic peaks For the peak areas, the results are good but are not at their possible maximum value as predicted by the model An unexpected observation is that the integrated peak areas show a minimum along the flow-rate variation This observation was made for all compounds in the test mixture and is not an artifact The reason for this is not yet fully understood The best results were found at the extremes of the flow-rate variation The flow-rate variation was chosen in order to cover the minimum of the Van Deemter relationships, as determined for geosmin and 3-methyl-2-pentanone

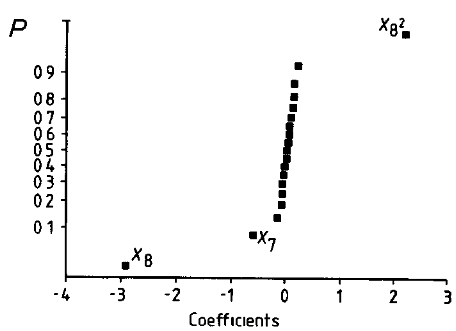

Fig 8 Normal probability plot of estimated coefficients, peak widths

CONCLUSIONS

The results obtained provided the information required for establishing the optimum conditions for the thermal desorption injection and chromatography The advantages of a multivariate strategy are evidenced by the finding of strong interaction effects between the experimental factors We note that a traditional approach, *i e*, considering the factors one at a time, would have failed because of the presence of interaction effects [3]

To simplify the problem of multiple responses, that is, characteristics of all peaks in the chromatograms, we used principal component modelling to obtain a description of the *systematic* variation over the set of experiments This variation is described by the score vectors In all instances the different characteristics were described by one significant component, which thus served as a single criterion Contrary to different chromatographic response functions, the multivariate information is not lost in the principal component model It is always possible to go back to the original responses

One important consequence of the optimized procedure is that the total time of analysis can be kept conveniently short without loss of quality of the eluted peaks (see Fig 9)

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APPENDIX

The 2^{9-5} fractional design used in the screening experiment was constructed from a complete two-level, four-factor factorial design The independent generators [3,9] of the fractional design were 5 = 123, 6 = 124, 7 = 134, 8 = 234, 9 = 1234

The confounding pattern of the aliased two-factor interactions will thus be

12 = 35 = 46 = 78
13 = 25 = 47 = 68
14 = 26 = 58
23 = 15 = 48
24 = 16 = 38 = 57
34 = 28 = 17 = 56

The response model used in the screening experiment thus contained the following terms

$$y = b_0 + \Sigma b_i x_i + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$$

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