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AUTOMATED ANALYSIS OF VARIOUS COMPOUNDS WITH A WIDE RANGE OF BOILING POINTS BY CAPILLARY GAS CHROMATOGRAPHY BASED ON RETENTION INDICES

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SUMMARY

A simple and reliable automated method has been developed for the analysis of various compounds with a wide range of boiling points by temperature-programmed capillary gas chromatography (GC). It consists of the identification based on corrected retention indices and the quantitation by a multi-internal standard technique. The full automation of the analysis was achieved by the connection of a GC system to a personal computer. Some factors affecting the reproducibility of retention indices and the quantitation are discussed. As an example of the application of the method, the analysis of a commercial cosmetic product was demonstrated.

INTRODUCTION

Gas chromatography (GC) is one of the best methods for the analysis of mixed unknown volatiles. There are many chromatographic techniques for the identification of unknown compounds, involving parameters such as absolute retention time, relative retention time, equivalent chain length (ECL) and retention index (I). Among them, the I system proposed by Kováts[1] in 1958 is considered to be the most suitable technique for routine GC analysis, in terms of reliability and ease of measurement.

Recent progress in fused-silica capillary column technology and the improvement of GC instruments in the 1980s have made it more useful, and many studies on its applications have been reported[2-11]. Anderson and Stafford[6] reported the screening of drugs based on the I system. D'Agostino and Provost[3] also applied it to the analysis of chemical warfare agents in environmental samples. The reproducibility of I in temperature-programmed capillary GC analysis has been investigated in detail by Schepers *et al.*[5] and Knöppel *et al.*[2]. However, these reports describe only the behaviour of compounds with low or medium boiling points, and there has been no study of compounds with high boiling points.

On the other hand, the quantitative analysis of the compounds is also required. This has been scarcely discussed for compounds with a wide range of boiling points analysed by temperature-programmed capillary GC. Unknown samples frequently

contain a variety of compounds with low to high boiling points. Therefore, the development of a versatile method for the analysis of such samples is required.

In the present paper we will discuss the factors affecting the reproducibility of retention indices and quantitation in the simultaneous analysis of compounds with a wide range of boiling points by temperature-programmed capillary GC, and report a reliable automated method.

EXPERIMENTAL

Gas chromatography

All experiments were carried out using an Hewlett-Packard Model 5890A gas chromatograph equipped with a flame ionization detector and a split/splitless injection system. A 12.5 m × 0.20 mm I.D. fused-silica capillary column, Ultra 1, with a film thickness of 0.11 μm of a cross-linked dimethylpolysiloxane was used. The oven temperature was programmed from 120 to 325°C at a rate of 5°C/min with a 5-min hold at 325°C. The injection and detector temperatures were set at 340°C. Helium was used as a carrier gas at a linear velocity of 45 cm/s at 120°C. A split injection technique was used with a splitting rato of 80:1 in all analyses. All samples were automatically injected into the gas chromatograph by an Hewlett-Packard Model 7671A automatic sampler. Peak areas and retention times were obtained by an Hewlett-Packard Model 3392A integrator. These data were transferred to a personal computer, PC-98XA (NEC, Japan) with a 20-Mbyte hard disk, connected to the integrator by RS-232C interfaces, and were then processed by the personal computer.

Reagents and chemicals

Authentic hydrocarbons with even carbon numbers in the range of n-C_{12}–n-C_{44} were used as references for the calculation of retention indices. The n-C_{42} alkane, which is not commercially available, was synthesized from behenic acid by the Kolbe reaction[12]. Other hydrocarbons and all the standard compounds used in the present study were obtained from various suppliers and were used without further purification. All the reagents were of reagent grade.

Preparation of samples

A solution of reference hydrocarbons and all solutions of standard compounds were prepared by mixing 25 mg of each hydrocarbon or standard compound with 100 ml of chloroform. An unknown sample solution was prepared as follows; a sample containing 0.5–10 mg of each compound of interest was placed in a 30-ml beaker, and was dried in an electrical drying oven at 105°C for 2 h. To the sample, 10 ml of chloroform with (for the quantitation) or without (for the identification) a known amount of internal standards were added.

Automated capillary GC analysis

Fig. 1 illustrates an outline of the automated GC analysis. All sample solutions (2 μl) were injected into a gas chromatograph by using an automatic sampler. After the analysis of each sample, the data on retention times and peak areas were transferred to a personal computer. For the reference hydrocarbons, standard retention times were determined and the new retention times used to calculate retention indices in the
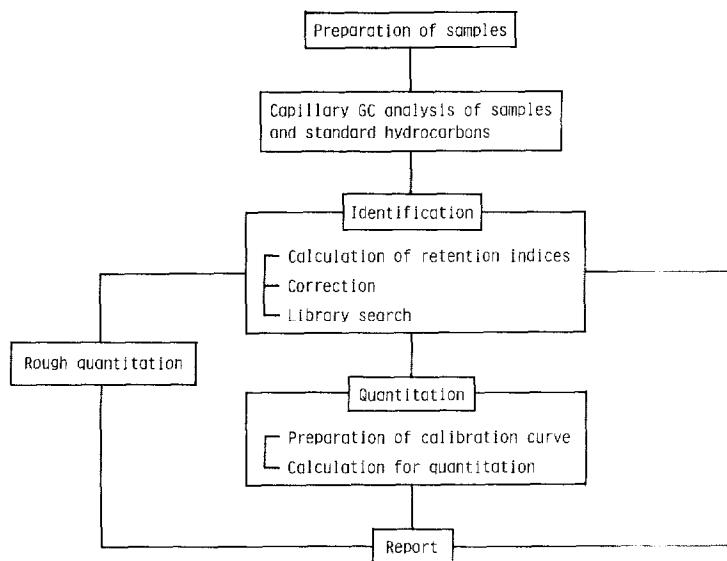
Fig. 1. An outline of the automated analysis system.

subsequent analysis. For an unknown sample, the retention indices, $I_{org.}$, of each peak were calculated using the expression proposed by Van den Dool and Kratz[13]

$$I_{org.} = 100n + 2 \cdot 100 \cdot \frac{T_X - T_{Cn}}{T_{Cn+2} - T_{Cn}} \qquad (1)$$

where X is a compound of interest, C is a standard hydrocarbon, T is the retention time and n is the carbon number of a standard hydrocarbon immediately prior to the compound X. Then, the corrected retention indices, $I_{corr.}$, were calculated according to

$$I_{corr.} = I_{org.} (aX^3 + bX^2 + cX + d + 1) \qquad (2)$$

where X is the peak area of the compound of interest, and a, b, c and d are constants (see Fig. 6). The corrected retention indices were then searched with an in-house retention index data base to identify the compounds. The results were printed out in a suitable format. A series of such processes was carried out automatically. When n-C_{28} alkane was added to the sample as an internal standard, semiquantitative analysis was carried out and the results printed out together with those for the identification. The subsequent quantitative analysis of the compounds identified, including the preparation of calibration graphs, quantitation and printing out of the results, was also carried out automatically.
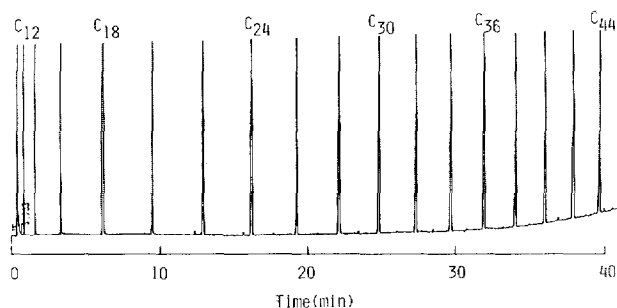
Fig. 2. Gas chromatogram of a mixture of standard hydrocarbons

RESULTS AND DISCUSSION

Optimization of the GC conditions

The GC conditions were investigated with respect to the analysis time, resolving power and thermal stability of the columns. Polar columns with polyethylene glycol as a liquid phase gave unsatisfactory results because of the insufficient long-term stability and the strong retention of compounds with high boiling points. On the other hand, an Ultra 1 column with a thin film (0.11 μm) of cross-linked methyl silicone gave symmetrical peaks and good resolving power for compounds not only with low and medium boiling points but also with high boiling points. A column with a thick film of the same liquid phase afforded unsuitably long retention times for compounds with high boiling points. Therefore, the Ultra 1 column was used in the subsequent studies.

Based on the results described above and the investigation of other factors such as the column temperature, length and inner diameter of the column, the linear velocity of the carrier gas, etc., the optimum GC conditions were established as shown in the Experimental section. Under these conditions, a variety of compounds with *I* values in the range of 1200–4400 were analyzed within 40 min, as shown in Fig. 2. This method
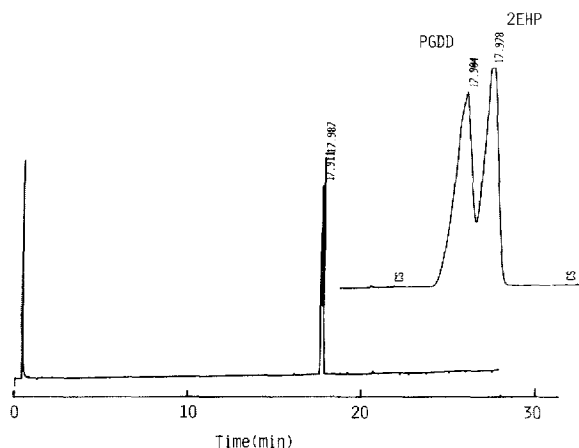

Fig. 3. Resolving power of the proposed method. Peaks: PGDD = propylene glycol didecanoate (*I* = 2482.3); 2EHP = 2-ethylhexyl palmitate (*I* = 2488.3).

gave relatively good resolving power in spite of the use of a short column (15 m), as shown in Fig. 3 which indicates a 20% valley separation of two compounds differing by 6.0 index units in retention indices, PGDD ($I = 2482.3$) and 2EHP ($I = 2488.3$).

Reproducibility of retention indices

The practical usefulness of the method proposed depends strongly on the reproducibility of the retention indices. Therefore, several factors affecting the reproducibility were investigated. Table I shows the effect of the injection methods on the reproducibility of retention indices in the replicate analyses ($n = 10$). A standard sample solution containing five compounds was separately injected, or co-injected together with the reference hydrocarbons. Both injection methods gave satisfactory reproducibilities within 0.5 index units standard deviation for all the compounds. However, slightly poorer reproducibility was obtained for compounds with high boiling points when a separate injection method was applied. In the present study, a separate injection method was chosen because of the convenience of the analysis of practical samples.

In GC analysis of compounds with high boiling points over a long period, the remaining compounds in a column and the use of high temperatures frequently result in thermal decomposition of the liquid phase, which greatly affects the reproducibility of retention indices. Therefore, the long-term reproducibility of retention indices under nominally unchanged chromatographic conditions was investigated using the various types of compounds listed in Table II. As shown in Fig. 4, the variations in the retention indices over a period of 6 months were within 3 index units for almost all the compounds, and no variation in the resolving power and the peak shape was observed during this period. These results indicate that the method proposed permits the relatively reliable identification of compounds with a wide range of boiling points over a long period.

Table III shows the retention indices of several compounds, determined with three columns having different lot numbers. Excellent inter-column reproducibility was obtained for all the compounds. Other factors affecting the reproducibility of retention indices, such as the solvents and the linear velocity of the carrier gas had no

TABLE I

EFFECT OF THE INJECTION METHODS ON THE REPRODUCIBILITY OF RETENTION INDICES IN REPLICATE ANALYSES

Sample	Retention index	Standard deviation	
		Separate injection	Co-injection
Lauryl alcohol	1442.8	0.44	0.05
Methyl stearate	2109.4	0.30	0.09
Propylene glycol didecanoate	2482.5	0.25	0.10
Myristyl myristate	2947.2	0.18	0.20
Octyldodecyl erucate	4129.2	0.42	0.35

TABLE II

COMPOUNDS USED AND THEIR RETENTION INDICES

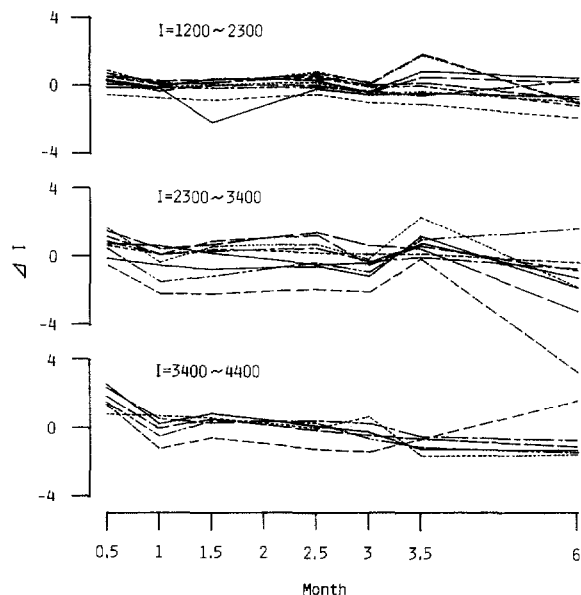| Sample	Retention index
Methyl caprate	1281.5
Laurylamine	1426.2
Lauryl alcohol	1441.7
o-Hydroxybiphenyl	1455.6
2,6-Di-*tert.*-butylhydroxytoluene	1471.2
Cetyl alcohol	1856.4
Methyl palmitate	1904.7
Stearyl alcohol	2060.9
Methyl stearate	2108.2
Ethylene glycol monopalmitate	2200.2
Ethylene glycol monostearate	2403.1
Propylene glycol didecanoate	2481.6
Glycerol tri-2-ethylhexanoate	2602.5
Squalene	2663.1
Myristyl myristate	2946.3
Cholesterol	3003.5
dl-α-Tocopherol acetate	3132.2
Pentaerythritol tetra-2-ethylhexanoate	3267.4
2-Tetradecyloctadecanol	3381.2
Diisostearyl malate	3465.3
Ethylene glycol dipalmitate	3656.0
Distearylmethylamine	3699.9
2-Hexadecyleicosanol	3782.1
Ethylene glycol distearate	4056.8
Octyldodecyl erucate	4126.3

Fig. 4. Long-term reproducibility of retention indices of various compounds.

TABLE III

COMPARISON OF RETENTION INDICES DETERMINED WITH THREE COLUMNS HAVING DIFFERENT LOT NUMBERS

Sample	Retention index		
	Column 1	Column 2	Column 3
Methyl caprate	1281.5	1282.9	1283.8
Laurylamine	1426.2	1427.2	1427.2
Di-*tert.*-butylhydroxytoluene	1471.2	1472.7	1472.8
Stearyl alcohol	2060.9	2062.3	2061.4
Propylene glycol didecanoate	2481.6	2482.5	2482.1
Glycerol tri-2-ethylhexanoate	2602.5	2603.5	2602.5
dl-α-Tocopherol acetate	3132.2	3132.1	3131.4
Ethylene glycol dipalmitate	3656.0	3658.9	3656.8
Distearylmethylamine	3699.9	3700.2	3700.5

influence on the reproducibility. On the basis of these results, the error window of retention indices in the identification of unknown compounds was determined to be 4 index units.

Influence of the concentration of a compound on the retention index

As described above, the method proposed gave excellent reproducibility of the retention indices for various compounds with the same concentration. However, it was found that the retention indices were dependent upon the concentration of the compound of interest, as shown by the dotted line in Fig. 5. The retention indices gradually increased with increasing concentration of the compounds, and a ten-fold increase in the concentration resulted in an increase of 4-8 retention index units. Similar results were obtained for all the compounds listed in Table II. The
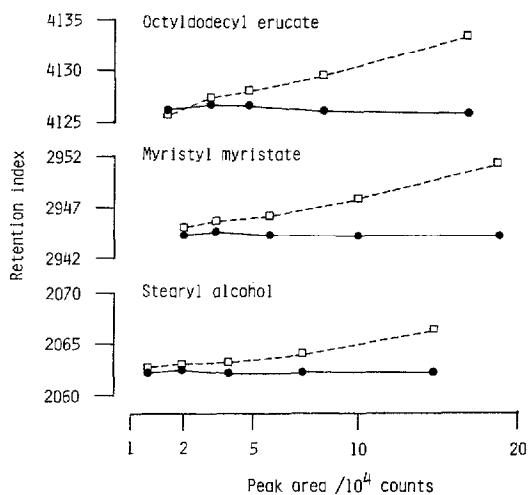

Fig. 5. Relationship between peak areas and retention indices: □---□, before correction; ●--●, after correction.

concentration dependence of retention indices is a significant problem in the practical use of the method proposed. The dependence decreased on a capillary column with a thick film of liquid phase, however the column was not suitable for the present study as described above. This behaviour suggests that the degree of the variation was related to the amounts of compounds injected. Therefore, the relationship between the degree of the variation in retention indices and the peak areas which are proportional to the amounts was examined. As shown in Fig. 6, a definite correlation was found to exist between these two, which could be expressed approximately as the third degree of a function of the peak areas. Therefore, we attempted to correct retention indices on the basis of peak areas. The full line in Fig. 5 shows the relationship between the peak areas and retention indices after this correction. No variation in the corrected retention indices was observed over the whole range of peak areas. Thus, the concentration dependence of the retention indices can be cancelled by the correction based on peak areas.

Quantitation

The on-column injection technique gives superior quantitation and high sensitivity, having been used in quantitative capillary GC analysis. However, this technique is not suitable for routine analysis because of the tendency to column decomposition and the need for special equipment for automatic analysis. This led us to investigate the quantitation by a split injection technique which is most widely used in capillary GC analysis.

Fig. 7 shows the reproducibility of the response factors for eight compounds relative to $n\text{-}C_{28}$ alkane (an internal standard). The single internal standard technique gave good reproducibility within 3% relative for compounds which were eluted in the proximity of the internal standard, however poor reproducibility was observed for other compounds. The reason for this behaviour is considered to be that non-uniformity of splitting of the components vaporized in an injection port takes place
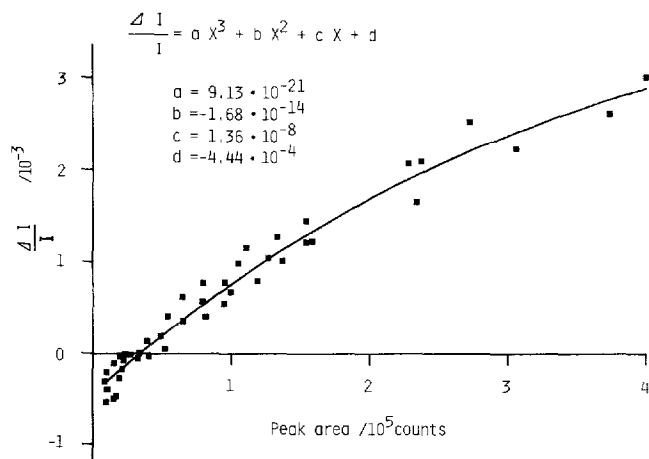

Fig. 6. Relationship between peak areas and the degree of the variation in retention indices, $\Delta I/I = (I_x - I)/I$, where I_x is the retention index of a target compound the peak area of which is X, I is the retention index of the target compound in concentration of 0.025%.
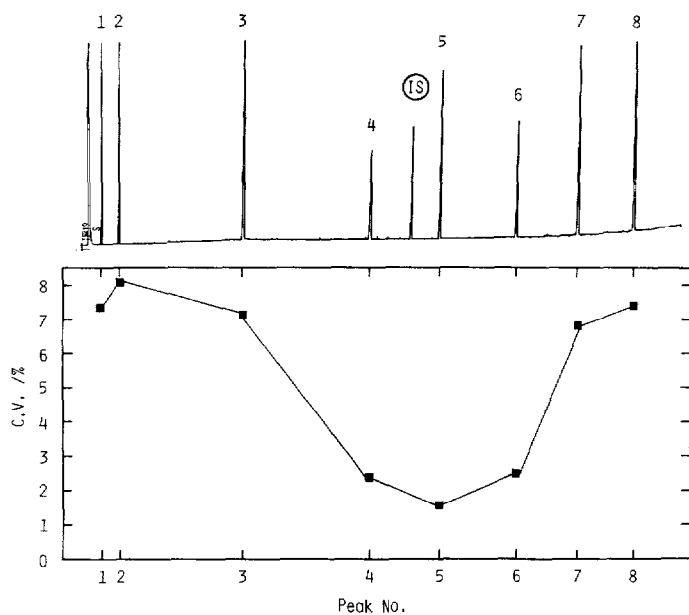
Fig. 7. Reproducibility of relative response factors in replicate analyses by a single internal standard (IS) technique.

depending on the differences in volatility of the components. Therefore, when a compound with a boiling point similar to that of a compound to be quantified is used as an internal standard, relatively good reproducibility can be obtained since these compounds are thought to undergo a similar degree of preferential loss. This suggests that the use of several internal standards with different boiling points would make it possible to quantify simultaneously compounds with a wide range of boiling points. Therefore, the use of a multi-internal standard technique was examined. The chromatogram was divided into three regions (A, B and C in Fig. 8) and an internal standard eluting in the same region as the unknown compounds was used to calculate their relative response factors. As shown in Fig. 8, this technique gave good reproducibility for all the compounds.

The accuracy of the method was tested by adding known amounts of six compounds with a wide range of boiling points to a commercial rinse and lotion. The recoveries were 97–101% as shown in Table IV. Fig. 9 shows the long-term reproducibility of the relative response factors. Satisfactory reproducibility was obtained over a period of 28 days.

The use of more than three internal standards was also investigated, however, similar reproducibilities were observed. Therefore, the number of internal standards employed was three.

These results indicate that the multi-internal standard technique permits the simultaneous quantitation of compounds with a wide range of boiling points in capillary GC analysis by a split injection technique.
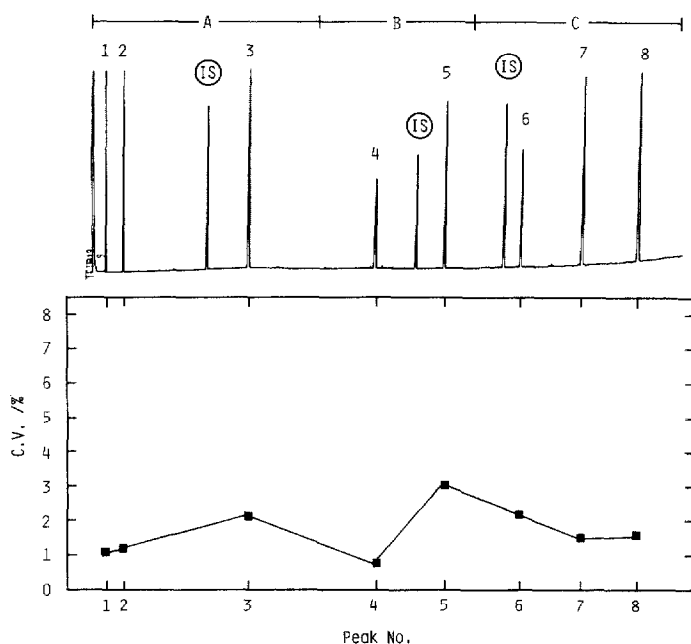
Fig. 8. Reproducibility of relative response factors in replicate analyses by a multi-internal standard technique.

Automation

The method proposed involves several time-consuming and cumbersome processes; the calculation and correction of retention indices, a library search based on corrected retention indices, etc. Therefore, a series of the processes were fully automated by connection of the GC system to a personal computer as shown in the Experimental section. All the analyst has to do is to place the samples in an automatic sampler and then push a button on the integrator attached to the gas chromatograph in

TABLE IV

RECOVERY IN THE ANALYSIS OF A COMMERCIAL RINSE AND LOTION BY THE METHOD PROPOSED

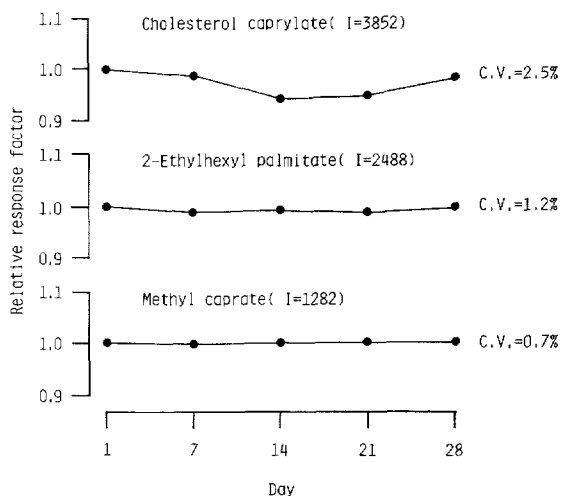| Compound added	Added (%)	Found (%)		Recovery (%)	
		Rinse	Lotion	Rinse	Lotion
Methyl caprate	0.300	0.296	0.291	98.7	97.1
2-Heptylundecanol	0.300	0.302	0.300	100.6	100.0
Eicosanol	0.300	0.295	0.290	98.2	96.6
2-Ethylhexyl palmitate	0.300	0.304	0.297	101.2	99.0
Cholesterol caprylate	0.300	0.295	0.295	98.4	98.4
Pentaerythritol tetra-2-ethylhexanoate	0.300	0.298	0.304	99.4	101.3

Fig. 9. Long-term reproducibility of relative response factors over a period of 28 days.

order to start the analysis. The automation of these processes would make the method more useful.

Application

The method proposed was applied to the analysis of a commercial cosmetic product. Fig. 10 shows the chromatogram of the analysis of a commercial lotion. All the compounds of interest could be identified by the library search based on their corrected retention indices and quantified, as shown in Table V. These results are in good agreement with those obtained by GC–mass spectrometry. This method was successfully applied also to the analysis of other cosmetic products and several kinds of industrial materials.
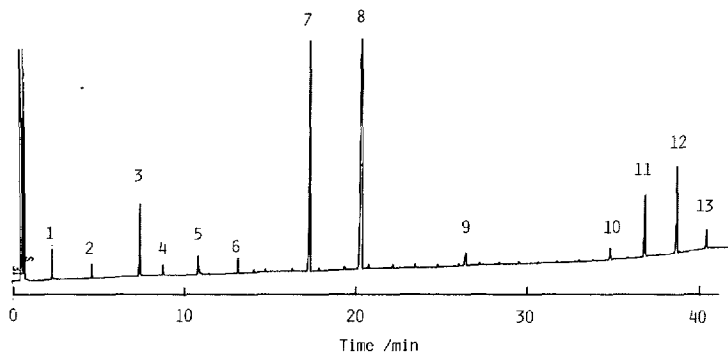

Fig. 10. Analysis of a commercial lotion. See Table V for peak identification.

TABLE V

RESULTS OF THE ANALYSIS OF THE COMMERCIAL LOTION BY THE METHOD PROPOSED

Peak No.	Retention time (min)	Retention index	Difference*	Compound identification	Content (%)
1	2.475	1467.0	2.2	Ethyl *p*-hydroxybenzoate	0.06
2	4.743	1671.1	3.3	*n*-Butyl *p*-hydroxybenzoate	0.08
3	7.565	1856.3	2.3	Cetyl alcohol	0.24
4	8.952	1938.4	2.4	Oxybenzone**	0.07
5	11.041	2060.9	0.7	Stearyl alcohol	0.14
6	13.397	2198.5	3.6	Escalol 507***	0.09
7	17.648	2460.3	0.6	Cetyl isooctanoate	1.68
8	20.628	2660.1	−1.6	Stearyl isooctanoate	0.44
9	26.938	3130.7	1.8	Tocopherol acetate	1.01
10	35.523	3895.4	−0.8	Jojoba oil	0.81
11	37.535	4097.2	2.0	Jojoba oil	0.81
12	39.456	4300.9	2.7	Jojoba oil	0.89
13	41.259	4470.0	0.9	Jojoba oil	0.99

* The difference between the *I* of a target compound and that of the corresponding compound in our data base.

** 2-Hydroxy-4-methoxybenzophenone.

*** 2-Ethylhexyl *p*-dimethylaminobenzoate.

CONCLUSION

A simple and reliable automated method has been developed for the simultaneous analysis of compounds with a wide range of boiling points by using temperature-programmed capillary GC. It is based on the use of a corrected retention index system and a multi-internal standard technique, which permits the reliable identification and precise quantitation of such compounds. The method provides a very useful means for the simultaneous analysis of mixed unknown volatiles.

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