Use of Multispectral Analysis in the Characterization of a Perfume*

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Complex multicomponent samples are encountered in all areas of chemistry, such as the analysis of hazardous waste, the characterization of petroleum feed stocks and processes, the identification of naturally occurring flavors and fragrances, to name a few. Because of the complex nature of perfumes, it is often very difficult to identify and quantitate important compounds in them. The analysis of minor components, for example, is usually hampered by severe chromatographic overlap.

Multispectral Analysis

A new analytical approach is Multispectral Analysis (MSA), which uses gas chromatography/atomic emission detector (GC/AED) to screen for the elements present, gas chromatography/infrared spectrophotometry (GC/IRD) to screen for functional groups, and combined gas chromatography/infrared spectrophotometry/mass spectrometry (GC/IRD/MSD) to identify the components.

This article reports on the use of MSA to characterize the components in a commercially available perfume. The same approach is applicable to any complex essential oil, synthetic blend, or final product. The analytical scheme for component identification can be summarized as follows:

1. Run the sample on GC/AED to produce a series of element-selective chromatograms. These chromatograms will indicate the elements that are present (and those which are absent) in the chromatogram. Note the element content indicated for each peak in the chromatogram. This element screening can be used to prioritize which peak to identify first. If nitrogen compounds are of special interest, they can be found and worked on first.
2. Run the sample on combined GC/IR/MS. The IR data is first plotted as a series of selected wavenumber chromatograms (SWCs) chosen to reflect any functional groups that could be present. These include aliphatic, aromatic, unsaturated, hydroxyl, carbonyl, ether, ester, nitro, amine, and other functionality. This information is used to note the functional group content of each GC peak. At this point, from the AED and IR data, we can now say that a given peak is, for example, an aromatic nitro ether.
3. From the MS data, plot extracted ion chromatograms, for any ions of interest. These can be used to screen for structures like methyl esters, monosubstituted benzenes, terpene alcohols, and others. They can also be used to screen for a particular compound using the molecular ion of that compound. These plots are then used to determine further information about each peak. At this point we now can say that a given peak is, for example, a nitro disubstituted benzene ether with an aliphatic side chain.
4. Lastly, run MS and IR library searches for the peaks of interest. In many cases the compounds will be identified directly from this search process. However, there are many times when library searching fails to provide for the correct identification due, for instance, to spectral overlap, spectral similarities, or the absence of the compound from the library. In these cases the dossier of information obtained by steps 1-3 above can be used to choose between ambiguous library search results, or at least give some data on peaks which are absent from the library. At the very least, for a compound that cannot be identified, we can say that it is, for example, a nitro disubstituted benzene ether with an aliphatic side chain that has a probable molecular weight of 196. The power of this approach is illustrated in the following analysis of a commercial perfume.

Element Screen with AED

For the GC/AED system, the gas chromatograph was set up using a fairly short thin-film column programmed to a high enough temperature for complete elution of the heavier components (see Table I). Details of the design and operation of the atomic emission detector are covered elsewhere.[1,2]

Figure 1 shows four of the seven element chromatograms obtained with the atomic emission detector. These chromatograms indicate that the majority of the perfume components contain C, H and O, but a few contain just C and H. Also apparent is the presence of four nitrogen-containing compounds. Elements screened for but which

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are absent include Br and S. There seem to be three unexpected sub-part-per-million chlorine containing compounds (see Figure 2). The AED is useful in distinguishing minor peaks by heteroatom content. It can be difficult to detect trace components with chlorine or other halogens by GC/MS, especially when they coelute with large amounts of other compounds.

The AED can also confirm elemental presence by looking at the atomic spectra which are collected during the chromatography. Figure 3 shows three of these "snapshots" that were taken during the analysis of the three GC peaks that may contain chlorine. The three atomic emission lines, at the correct wavelengths and with the correct relative intensities, prove that chlorine is present in two of the three peaks, and strongly suggest the presence of chlorine in the third.

Figure 4 shows the IRD Total Response Chromatogram (TRC) and the MSD Total Ion Chromatogram (TIC) for the perfume sample. Comparison with the AED C, H and O chromatograms allows correlation of the retention times. Not surprisingly, most of the components contain oxygen of some functionality.

Table I. Conditions

HP 5890A Gas Chromatograph (for AED system)	
Column:	25 m x 0.32 mm x 0.17 micrometer film of HP-1 (methyl silicone)
Carrier Gas:	Helium at 180 kPa, 2 mL/min
Oven:	40°C (2 min) to 240°C at 3°C/min with 10 min hold
Injection Port:	250°C
Sample Injection:	1.0 µL, split 100 to 1
HP 5921A Atomic Emission Detector **AED parameters**	
Cavity:	350°C
Transfer Line:	300°C
HP 5890A Gas Chromatograph (for IRD/MSD system)	
Column:	50 m x 0.32 mm id x 0.52 micrometer film of HP-1 (methyl silicone)
Carrier Gas:	Helium at 180 kPa, 2.0 mL/min
Oven:	40°C (2 min) to 240°C at 3°C/min with 10 min hold
Injection Port:	250°C
Sample Injection:	1.0 microliter, split 20 to 1
HP 5965B Infrared Detector **IRD parameters**	
Light Pipe:	250°C
Transfer Lines:	260°C
Sweep Gas:	Nitrogen, 35 kPa inlet, 100 kPa outlet
Scan Parameters:	8 cm^{-1} resolution, 2 coads, 3 scans/second stored
Detector:	Wide Band MCT, 550 to 4000 cm^{-1}
HP 5971A Mass Selective Detector **MSD parameters**	
Mass Range:	10 to 310 daltons
Scan Parameters:	2 A/D samples, 1.4 scans/second stored

Selected Wavenumber Chromatograms with IRD

For the GC/IRD/MSD system, the gas chromatograph was set up using a long intermediate film thickness methyl silicone column operated similarly (see Table I). The column effluent was split at the end of the column at a 10 to 1 ratio with the bulk of the flow going to the infrared spectrophotometer and the lesser amount going to the mass spectrometer. The details of this parallel configuration are described elsewhere.[3,4]

In order to further characterize the oxygenates, several selected wavenumber (functional group) chromatograms were extracted looking for carbonyls, ethers and alcohols. As an example of this further refinement, aldehyde carbo-

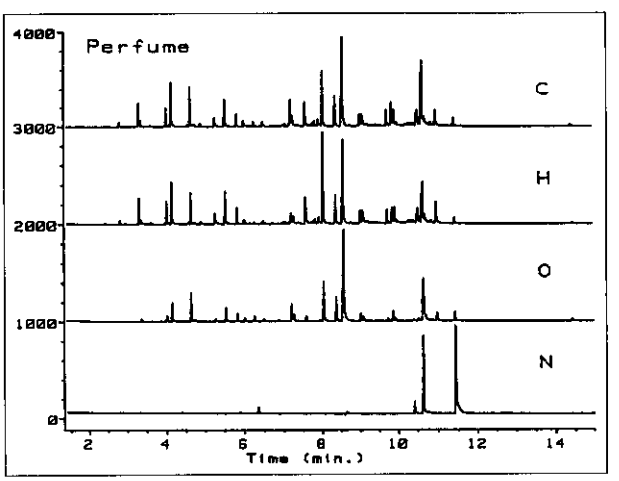

Figure 1. AED element specific chromatograms: carbon, hydrogen, oxygen and nitrogen

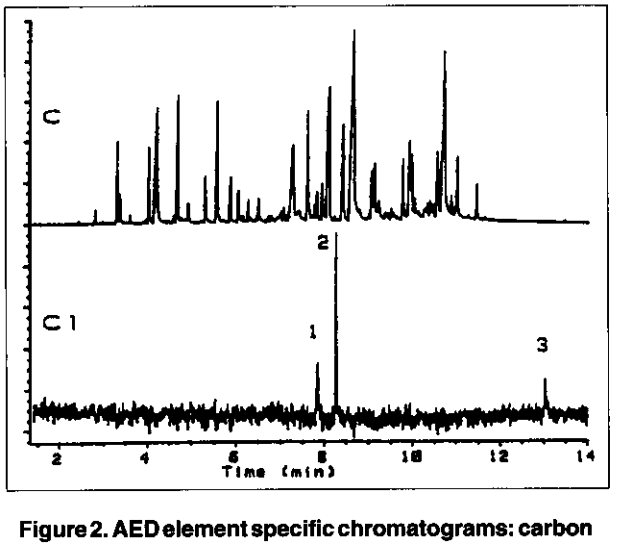

Figure 2. AED element specific chromatograms: carbon and chlorine

nyl and aldehyde carbon-hydrogen were examined as shown in Figure 5. The four starred peaks in the carbonyl chromatogram are ones that have aldehyde carbon-hydrogen. The specific compounds were then identified via library searching. If aldehydes were of interest only, they could thus be quickly found.

Similarly, to find the alcohols, the selected wavenumber chromatograms were generated for the carbon-oxygen ether bond and the oxygen-hydrogen bond. Figure 6 shows these chromatograms with the coincident peaks starred. These compounds were then identified via library searching later. The unidentified alcohols are ones that are not in the Flavor and Fragrance library, but are alcohols, nonetheless.

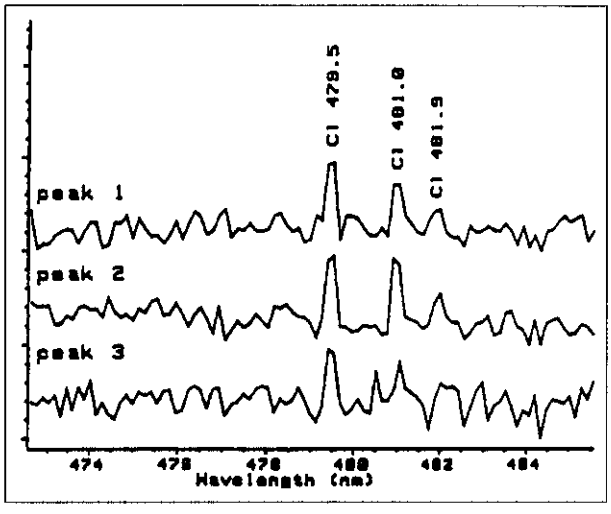

Figure 3. AED atomic spectra "snapshots" of three chlorine-containing peaks

Extracted Ion Screen with MSD

Valuable information can also be found using extracted ion data. Figure 7 shows two of these ion range chromatograms. Many diterpenes have a m/z 136 ion and phthalates have a m/z 149 ion. This information is very useful in classification. In the m/z 149 chromatogram the largest GC peak is diethyl phthalate, a major fixative for perfumes. The other peak with m/z 149 is piperonal (MW 150), which, like many aldehydes, has a major M-1 ion.

The ions at m/z 152 and 154 are indicative of oxygenated terpenes. Many of these compounds are important in perfume, such as linalyl acetate, RT 16.5 minutes. Figure 8 shows these two extracted ion profiles.

Nitrogen-containing Compounds

Some of the most interesting compounds in this perfume sample are the ones containing nitrogen (see Figure 9). Clearly, the nitrogen-containing peak at 10.65 minutes on the AED trace is partially coeluting with another peak containing C, H and O only and with a retention time a few seconds later.

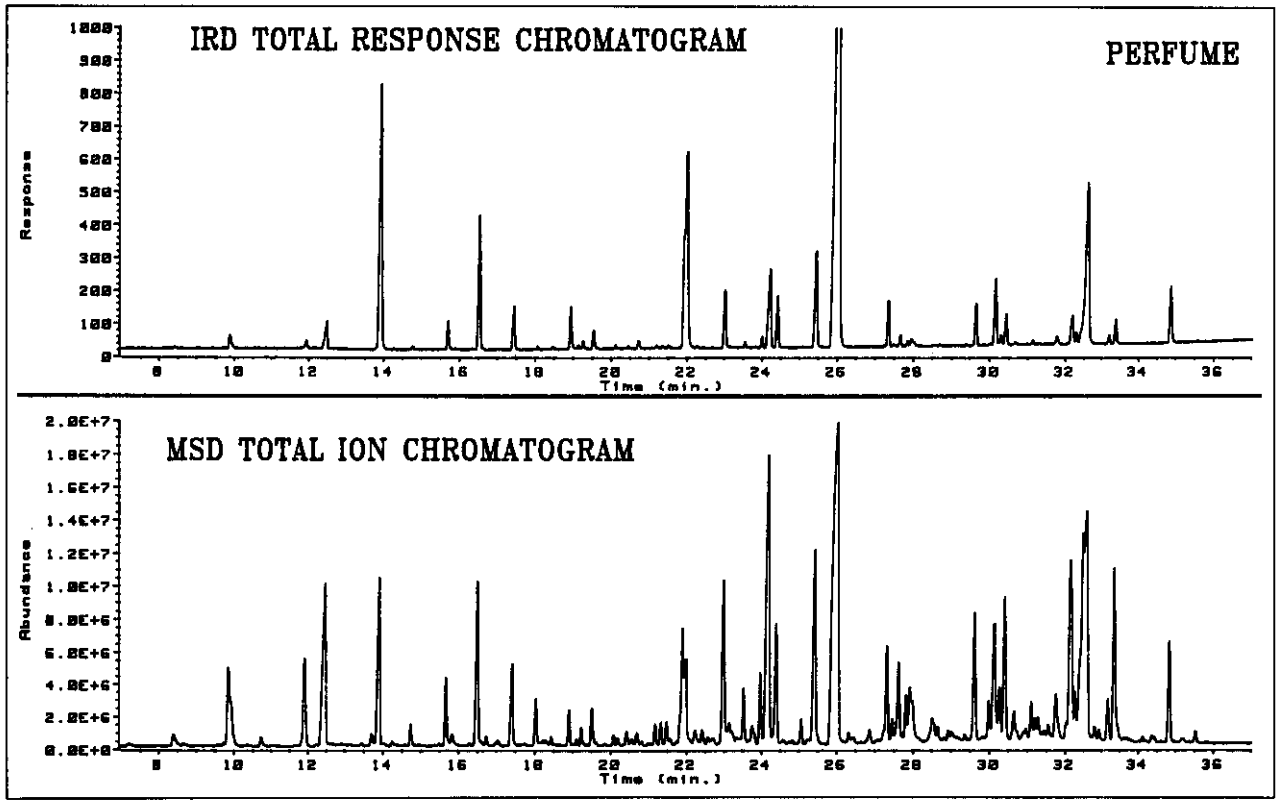

Figure 4. IRD total response chromatogram (TRC) and MSD total ion chromatogram (TIC) for perfume sample

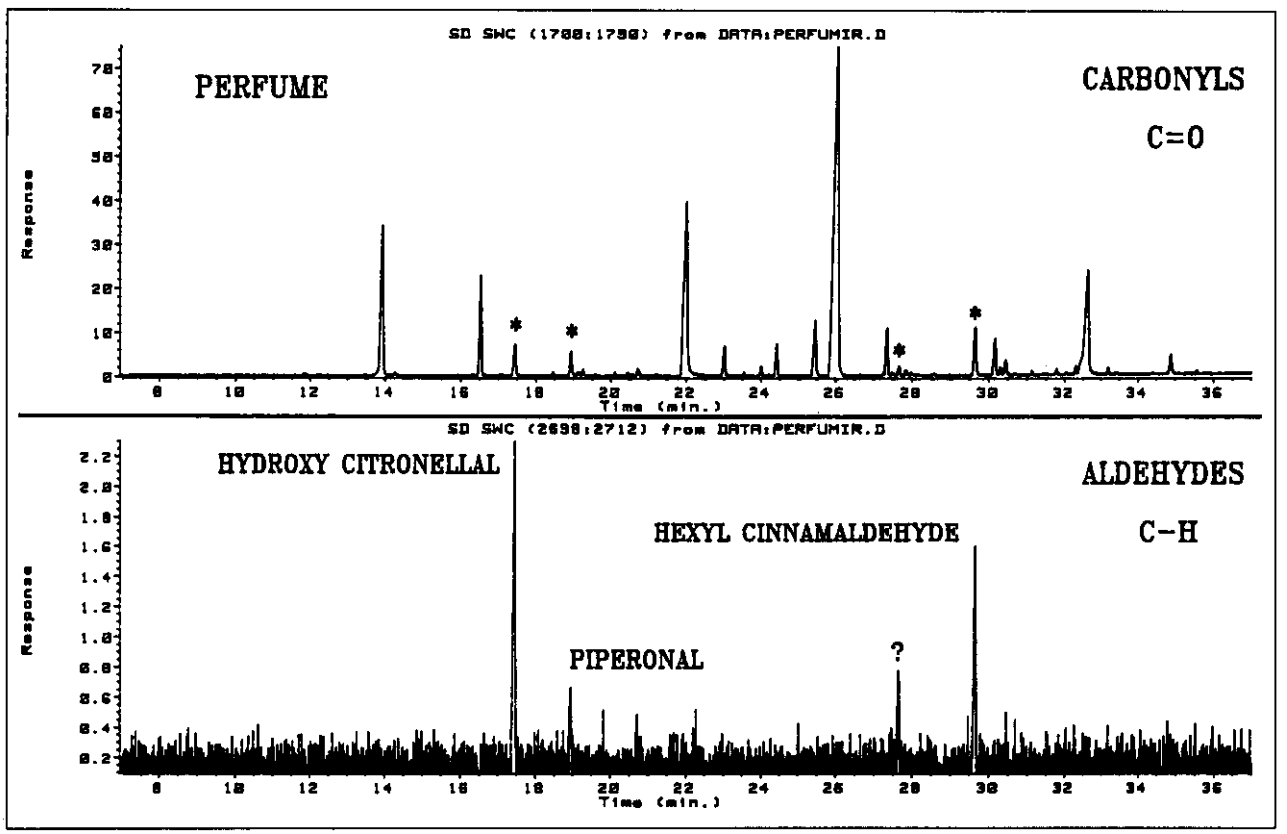

Figure 5. IR selected wavenumber chromatograms for carbonyl (1700:1790 cm^{-1}) and aldehyde C-H (2698:2712 cm^{-1})

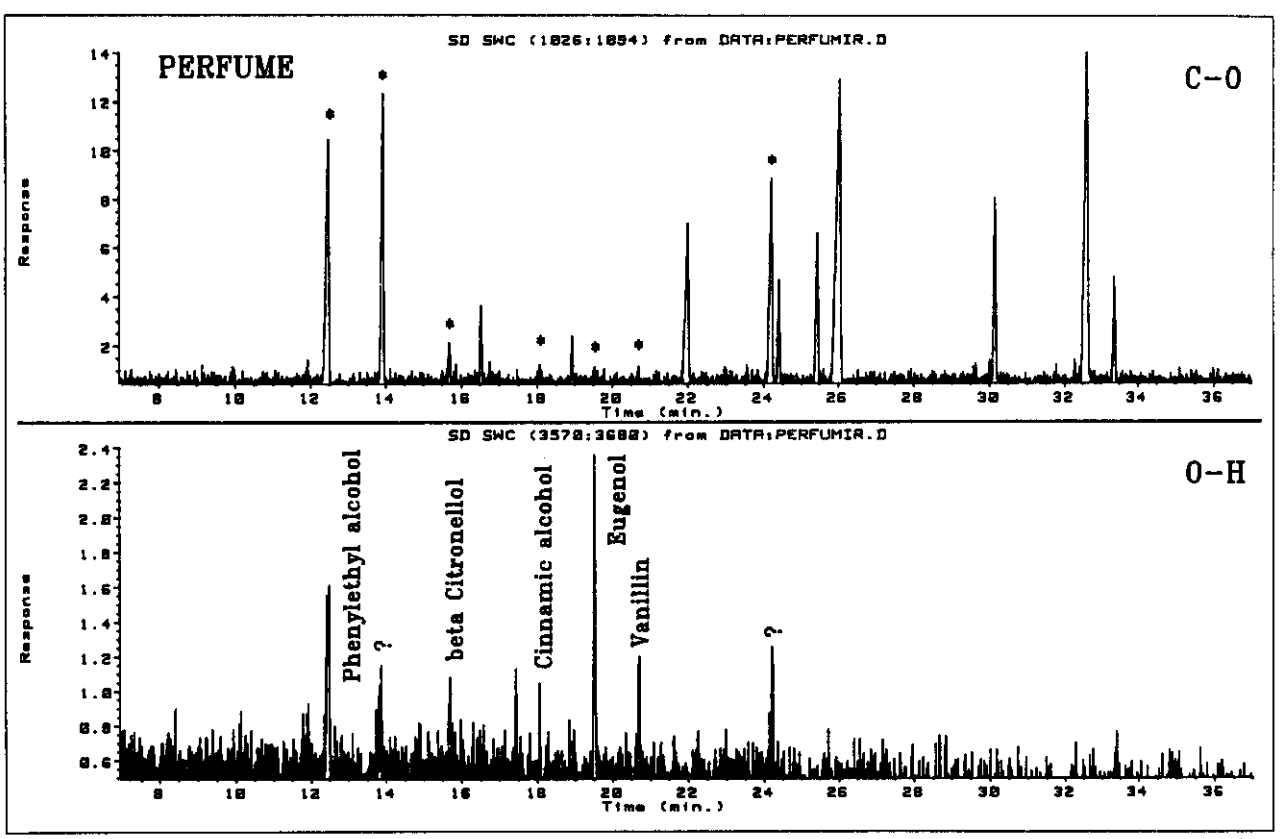

Figure 6. IR selected wavenumber chromatograms for ether C-O (1026:1094 cm^{-1}) and hydroxyl O-H (3570:3680 cm^{-1})

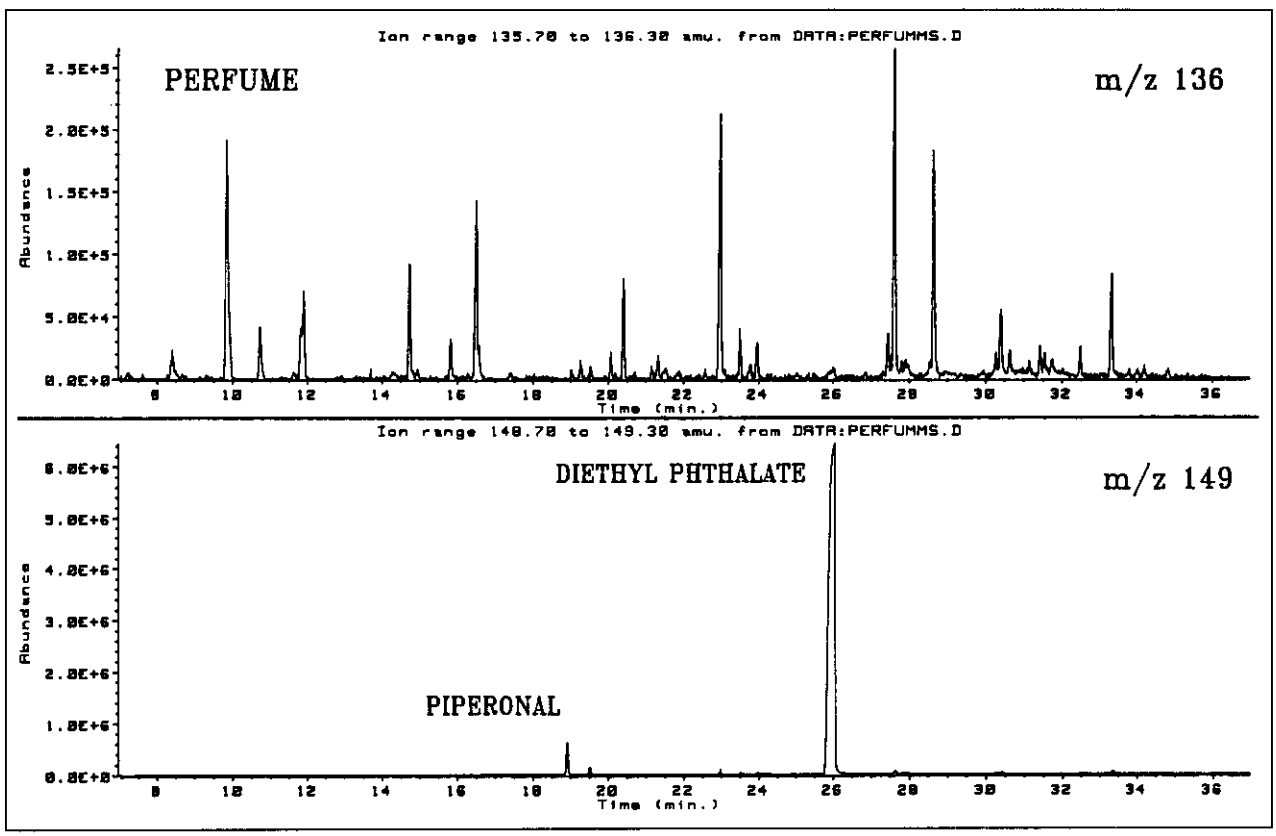

Figure 7. MS extracted ion ranges for terpenes (m/z 136) and phthalates (m/z 149)

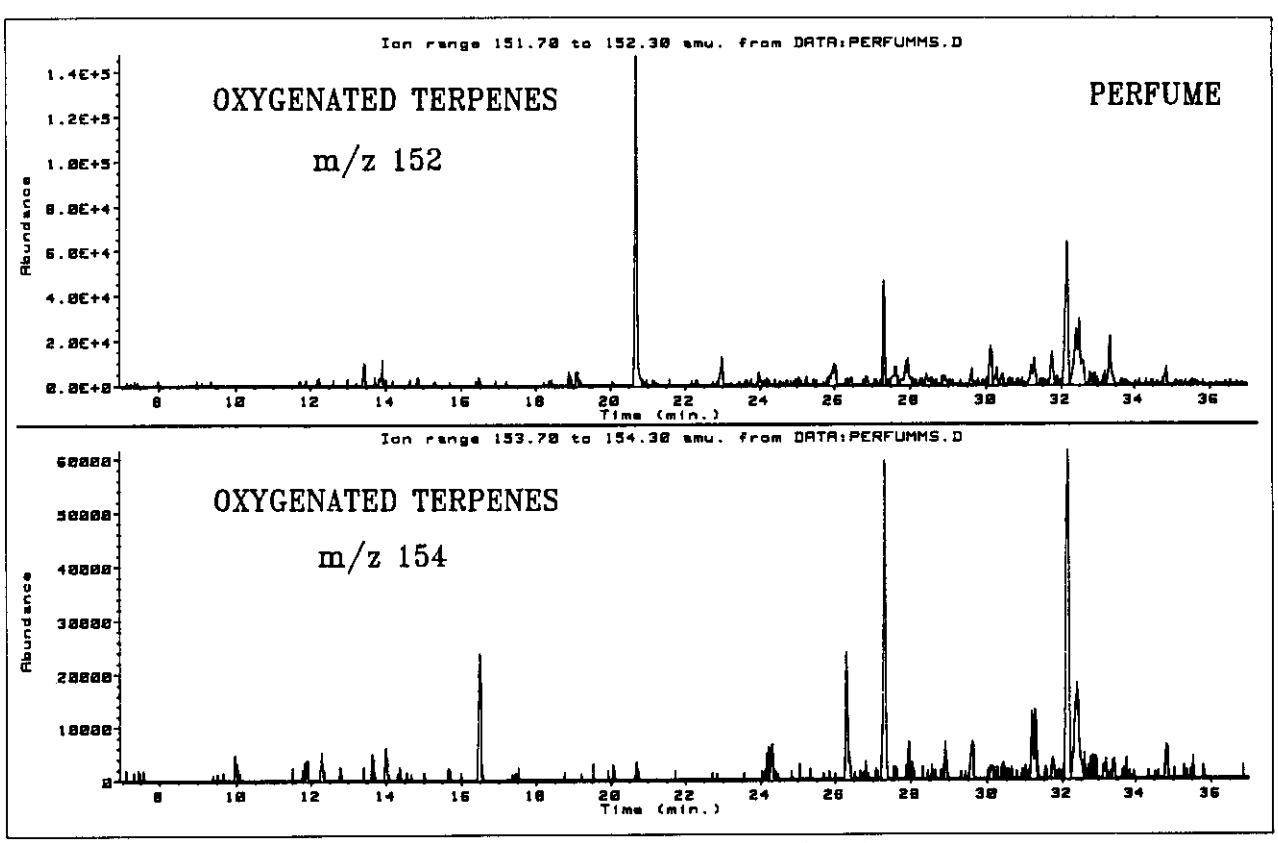

Figure 8. MS extracted ion ranges for oxygenated terpenes (m/z 152 and m/z 154)

This nitrogen-containing peak is at 32.50 minutes on the IRD/MSD chromatograms. They are not amines or nitriles but are nitros as shown in Figure 10. Library searching indicates this peak to be musk xylene (see Figure 11).

To illustrate how all of these techniques of multispectral analysis apply, the peak at 34.9 minutes will be examined in detail. The infrared and mass spectra of this peak are shown in Figure 12. The process of structure elucidation is outlined in Table II. Library searching identified this compound as musk ketone, the structure of which is in agreement with the multispectral data.

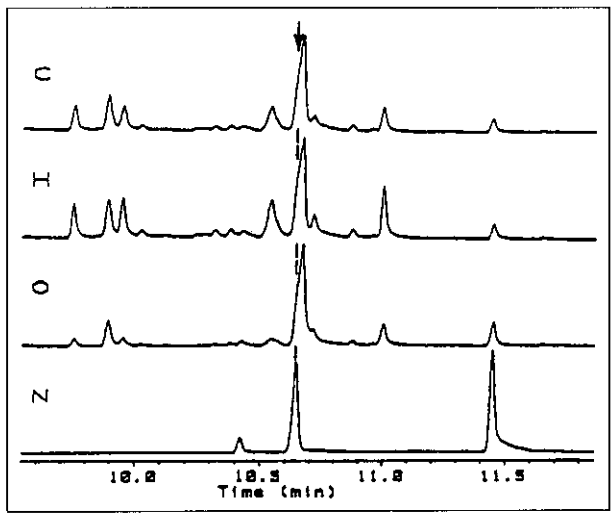

Figure 9. AED partial element specific chromatograms for showing overlapping of nitrogen-containing compound

Library Searches

The library searches were performed using the 130,544-entry Wiley MS library and the 2004-entry H-P Flavor and

Table II. Structure elucidation rationale for peak at 34.9 minutes

AED	C, H, O, N only
IR	Ketone carbonyl at 1724 cm^{-1}
IR	Aromatic C-H at 3037 cm^{-1}
MS	Aromatic ion at m/z 91
IR	Nitro at 1351 cm^{-1} and 1549 cm^{-1}
MS	$CH_3C{=}O$ ion at m/z 43
MS	Labile methyl with M-15
AED	C:N ratio 7:1
MS	Even number of N, $C_{14}N_2$
MS	294:295 ratio, C_{14}
AED	C:O ratio 2.8:1, $C_{14}N_2O_5$
MS	Molecular weight 294, $C_{14}N_2O_5H_{18}$
IR	Library search, excellent results, -> Musk Ketone

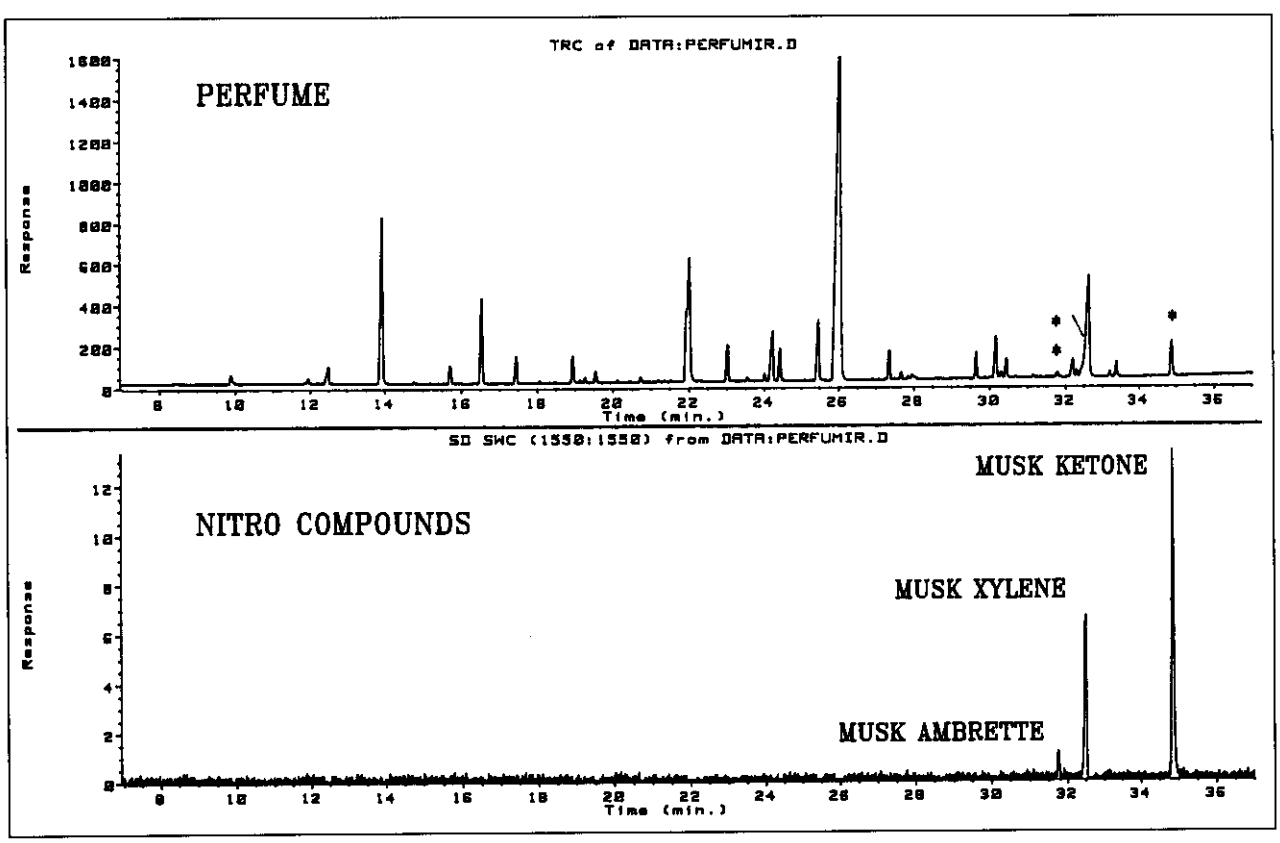

Figure 10. IR total response chromatogram with nitrogen-containing peaks marked and selected wavenumber chromatogram for nitro compounds

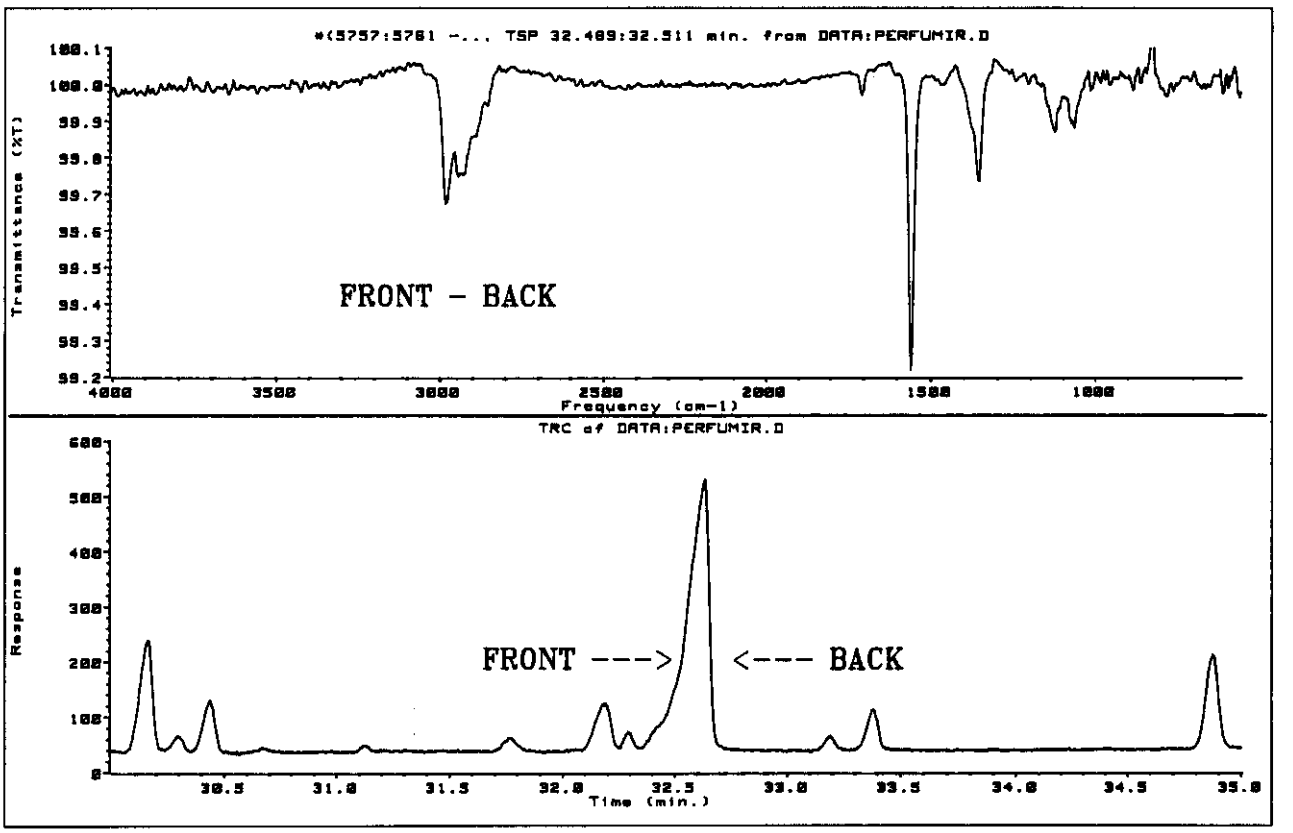

Figure 11. IR spectrum of overlapped nitrogen-containing peak, musk xylene

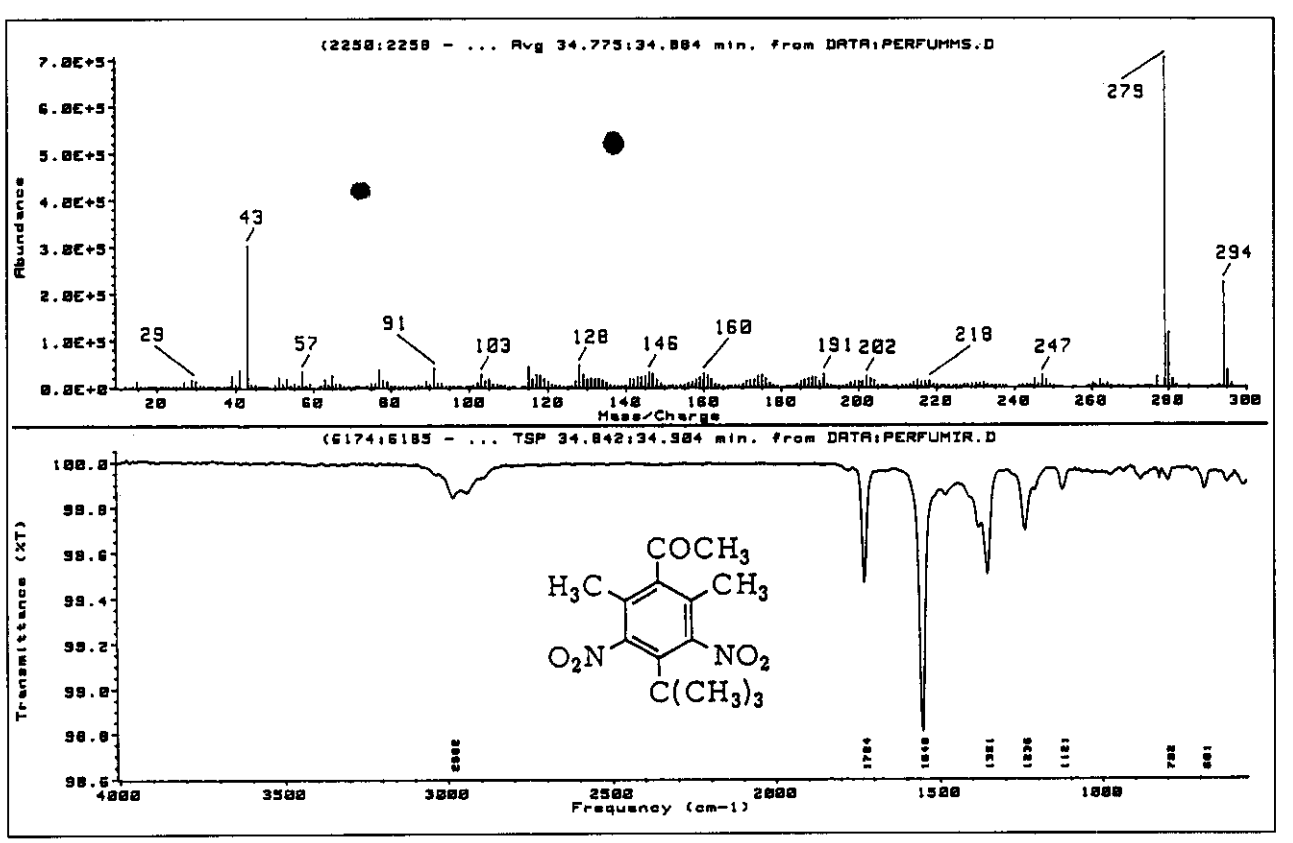

Figure 12. Mass and infrared spectra of peak at 34.9 minutes, musk ketone

Table III. Major components found in perfume

Retention time (min.)	Compound
8.4	β-pinene
9.9	limonene
10.0	benzyl alcohol
11.8	linalool
12.4	phenylethyl alcohol
13.9	phenylmethyl acetate
14.7	1-α-terpineol
15.7	β-citronellol
16.5	linalyl acetate
17.4	hydroxycitronellal
17.9	cinnamic alcohol
18.4	myrcenyl acetate
18.9	piperonal
19.1	methyl anthranilate
19.5	eugenol
20.1	geranyl acetate
20.7	vanillin
21.9	coumarin
23.0	α-isomethyl ionone
24.0	α-methyl ionone
24.4	2-methylbutyl salicylate
25.4	pentyl salicylate
25.9	diethyl phthalate
27.3	hedione
28.0	patchouli alcohol
29.6	hexyl cinnamic aldehyde
31.8	musk ambrette
32.6	musk xylene
34.9	musk ketone

Figure 13. Combined library search results for peak at 34.9 minutes, musk ketone

Comparison of Results from
PBM Search of Library file: **Data: Wiley L**
Avg 34.775:34.884 min. from **Data: Perfumms. O**
and
IR Search of Library file: **Data: Ref. L**
ASP 34.842:34.904 min. from **Data: Perfumir. O**

Class 1 (on both lists)

No common compounds found in separate reports

Class 2 (in only one library)

CAS Number	PBM Qual	IR Qual	MWt	Formula	Name
1. 057207-24-6	42	—	276	C14H9D3O6	4-D3-Halfordin
2. 000224-42-0	36	—	279	C21H13N	Dibenz[a,j]acridine
3. 000224-42-0	36	—	279	C21H13N	Dibenz[a,j]acridine
4.	36	—	279	C21H13N	Dibenzo[c,h]acridine
5.	36	—	279	C21H13N	Dibenzo[a,c]acridine
6.	36	—	279	C21H13N	Dibenzoacroline[a,h]
7. 037984-02-4	—	912	231	C12H9NO25	Sulfide, M-Nitrophenyl Phenyl
8. 007745-93-9	—	911	216	C7H6BrNO2	2-Bromo-4-nitrotoluene, 98%
9. 000620-55-8	—	898	215	C12H9NO3	Ether, M-nitrophenyl phenyl
10. 036438-55-8	—	886	178	C11H14O2	3-Methylphenyl 2-Methylpropionat

Class 3 (in both libraries, but on only one list)

CAS Number	PBM Qual	IR Qual	MWt	Formula	Name
11. 000081-14-1	—	969	294	C14H18N2O5	Musk Ketone
12. 000089-96-3	—	847	140	C8H9Cl	Benzene, 1-chloro-2-ethyl

Fragrance Vapor Phase IR Library. One of the features of the IR software is that of combining library search results. This produces a single hit list merged by common CAS Registry numbers into three classes. Class 1 contains those entries which are on both hit lists. Class 2 contains those entries which fall on only one list because the entry is not present in the other library. Class 3 consists of those entries which are in both libraries, but showed up in only one of the two hit lists. An example of this combined library search is shown in Figure 13 for the peak at 34.9 minutes, Musk Ketone. Note that this compound is in the Wiley library, but the spectrum is of very low quality.

Table III lists the major components found using all of the Multispectral Analysis tools described.

Conclusion

As seen with this perfume sample, Multispectral Analysis—the method of element, functional group, and ion prescreening described here—provides a powerful tool for unknown identification. It saves time by leading the analyst directly to compound types of interest. It provides higher confidence in library search identifications. In those cases of compounds that are not in the libraries, it provides useful information that can be used to greatly expedite spectral interpretation.

References

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