Modern Analysis Methodologies: Use and Abuse

By Daniel Joulain, Research Laboratories, Robertet SA, Grasse, France

Perfumes and flavors are complex mixtures made up of raw materials which, in the case of essential oils and solvent extracts, are in themselves highly complex.

For many years, analysts have tried to improve their qualitative and quantitative knowledge of the composition of such mixtures, for a variety of reasons. Modern separation methods, in particular recent so-called *hyphenated* techniques which rely on a powerful data-processing capacity, have enabled us to come closer to this goal.

However, although these techniques may be sophisticated, they have inherent limits which are not always fully understood by their users. The considerable "user-friendly" nature of the analytical tools may gradually dull the awareness and critical capacities of analysts, sometimes leading them to produce erroneous or unrealistic results.

With the help of examples, this article discusses the wide possibilities of certain techniques, and also their limits.

Brief Recent History

Up to 1955, separation techniques were essentially based on chemical fractionation, distillation and crystallization. Identification methods rested most of the time on the measure and comparison of the melting point of characteristic crystallized derivatives and elemental analyses. All this necessitated the use of a large sample, as the analytic methods' resolutions were only mediocre and the detection techniques low in sensitivity.

Starting in the early 1940s, first ultraviolet spectroscopy and then infrared (IR) spectrophotometry were introduced in analytical laboratories. These techniques for the first time provided direct access to molecular information. The decisive step in the analysis of volatile natural mixtures followed the discovery of gas chromatography in 1952.[1] Until the mid-70s, most GC systems were operated with packed, large diameter, short columns, resulting in a poor resolution. Then the use of metal, followed by "open" glass, capillary columns began to spread.

The simultaneous commercialization of microcomputer-driven chromatographs, along with the introduction in 1979 of a multitude of technological improvements in the elaboration of capillary columns with the use of fused-silica tubing, led to the broad use of high-resolution GC today.

A survey of the papers presented since the 7th Congress in Kyoto in 1977 reveals a significant increase of capillary GC determinations. Expressed as a ratio of the total number of reported GC experiments, this peaked at 85% in 1986 at the Washington, DC, Congress.

This spectacular development was accompanied by the systematic use of mass spectrometry to detect and identify, or rather recognize, the successively eluted substances, helped by the increased performance of data processing computer systems and appropriate software. In parallel, high-performance distillation and preparative gas chromatography have progressively disappeared from laboratories during recent years. The abandonment of these techniques is certainly exaggerated and unjustified, as discussed later.

Uses of Analysis

The manufacturing of fragrant and aromatic compositions consists mainly in mixing together somewhat determined ingredients, natural or synthetic, depending on the perfumers' and flavorists' creative imagination with a view toward obtaining a specific result.

Some basic ingredients may be highly complex mixtures: the final composition is, therefore, always very complex, often consisting of several hundreds—if not thousands—of components in proportions varying from a few percent to some parts per million, or even smaller.

In the flavor and fragrance industry, technicians have always searched for a better knowledge of the chemical composition of the ingredients they use, as well as of the finished products available elsewhere.

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The technical analysts' activities are motivated by the following three requirements: natural product knowledge, quality control and market control.

Natural product knowledge: As analytical tools have become available, they have been used by the industry's analytical chemists to penetrate the secrets of natural products in the attempt to identify, after separation, the characteristic and determining substances of the product's organoleptic performance (fragrance, flavor). The analytical studies realized during the 1960s on Bulgarian rose essential oil[2,3] and coffee flavor[4] are two typical examples.

The analysis of rose essential oil led to the discovery of damascenone, and to the opening of a new field in synthetic chemistry, which has brought precious elements—including damascones—for the creation of numerous modern perfumes.

The studies on coffee flavor have demonstrated the outstanding contribution of heteroxyclic molecules to this type of flavor. These studies were to be confirmed later in many analytical reports on the so-called "reaction" flavors, as results of thermal processes (such as cooking and roasting). Here again, the analytical discoveries led to the development of a specific organic chemistry which, otherwise, might not have existed.

While noting that these two examples, rose and coffee, were both studied by a large European firm, one cannot underestimate the research conducted by other laboratories on similar topics, even though they did not always result in detailed publications in the scientific press.

As a matter of fact, it would be unjust to omit the considerable studies which have been accomplished on products for the perfume industry, namely sandalwood, patchouli, musk, galbanum, oakmoss, and others.

During the last 30 years, not one natural product used in fragrance or flavor products has been left unexplored. The compiled analytic studies on essential oils and related extracts, plant products including spices, fruits, vegetables, meats, fish and seafood flavors, as well as the "reaction" flavors, are now available as handbooks in our libraries. The many works regularly published in scientific literature are proof of the incessant activity of this community's research body. The volume and the quality of the acquired knowledge is considerable.

Quality control: The complexity of the products of natural origin goes hand in hand with the possible variables of analytical and organoleptic criteria. This unavoidable variability, however, must be controlled and kept within acceptable limits. The criteria for accepting a natural raw material are the basis for commercial transactions between producers and users. For many natural products, it is difficult to determine such criteria: this is why groups of specialists are working with national and international organizations towards establishing norms; their studies are based on acquired knowledge, as well as that contributed by analytical techniques of the most appropriate kind in order to reach this objective.

Market control: Knowing the constitution of finished products available in the marketplace enables producers to better satisfy requests for duplications and, when needed, to be technologically vigilant for effective defense of patents. Although the analytical methods used for market control are the same as those used for natural product knowledge and quality control, a discussion of market control is beyond the scope of this article.

Two Types of Analytical Methods

Two types of analytical methods can be used, depending on whether the final goal is separation of an unknown substance or measurement of a known substance.

Separation of unknown substances: Analytical methods of this type are aimed at separating a not-yet described substance from a mixture in order to fully identify it. This type is the least frequent in practice and is only used by a relatively small number of analytical chemists. It requires time-consuming separation techniques, as well as a sometimes large quantity of sample. The method always consists of enriching a fraction of the sample with the desired substance, to finally isolating it by means of a preparative or micro-preparative technique.

The methods of enrichment have not evolved much in the course of the last decades, whether it be the technique

of fractional distillation with high number of theoretical plates, molecular distillation, or column chromatography (partition and adsorption). The techniques of enrichment by chemical functions are often beneficial when implemented in parallel with the methods of physical fractionation.

In the course of recent years, supercritical fluid extraction (SFE) and chromatography (SFC), as well as the various techniques of counter-current chromatography (CCC),[5] have led to applications in the field of natural products, particularly with regard to the separation of either highly polar temperature sensitive substances, or highly polar high molecular weight compounds.

In some plant raw materials, such substances are biological or technological precursors of organoleptically important substances which are present in essential oils and industrial extracts. Important efforts have been undertaken recently to identify these precursors to better monitor the production of substances of interest from plant material.

The final aim of these analytical processes always is to obtain a sufficient quantity of the desired substance, of an acceptable purity, in order to realize an NMR study as complete as possible, from which it is practically always possible today to establish a chemical structure.

The determination of the absolute configuration sometimes presents considerable difficulty and requires sophisticated techniques. However, it often constitutes a crucial step towards the complete identification of a natural aromatic molecule, because of the close relationship between chirality and organoleptic properties. It becomes extremely useful to be able to determine the absolute configuration of such a substance, by direct chirospecific chromatographic analysis of a mixture containing that substance.

Recognition of formerly known substances: Analytical methods of this type are today the most widespread in the flavor and fragrance industry. The possibility of direct coupling of a fast scanning quadripolar mass spectrometer with a capillary gas chromatograph (GC/MS) has, during the 1970s, been a determining step towards the rapid identification of volatile constituents in natural mixtures. During the 1980s this technique spread through nearly all laboratories, with the marketing of efficient and low cost GC/MS systems. The techniques discussed in the remainder of this article are primarily used for "the recognition of formerly known substances."

GC/FTIR Technique

The interfacing of a Fourier transform infrared spectrophotometer (FTIR) with a gas chromatograph (conventional GC/FTIR) has been in existence for more than 25 years. However, the first commercial devices were introduced to the market only at the end of the 1970s. They are mostly based on the "light-pipe" technology whose main advantage is that measurement takes place in the carrier gas and interferograms are continuously collected by means of the detector and transformed into IR-spectra *in real time*

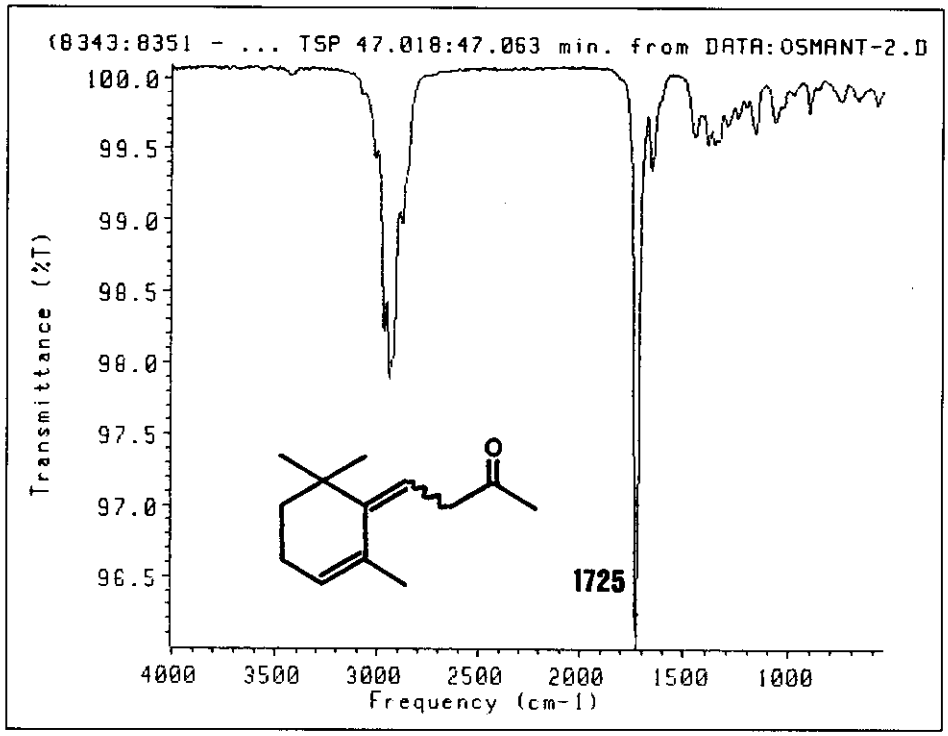

Figure 1. Gas phase FTIR spectrum of a possible retro-α-ionone isomer of osmanthus flowers headspace

mode. The main drawback is a lesser sensitivity compared with a mass detector: 1 to 10 mg for strong IR absorbers and up to 100 mg for weak IR absorbers. The production of spectra in gas phase, significantly different in condensed phase spectra, must not be considered as a drawback because libraries have to be compiled anyway for specific applications. Some are, in fact, available commercially.

The other techniques of matrix isolation and cryodeposition did not present the specific advantage of data acquisition in real time, until the "real-time direct deposition" interface (GC/DD-FTIR) appeared on the market. These techniques are characterized by a significantly higher sensitivity, similar to that of a mass detector, which is a considerable advantage for the identification of minor constituents.

The GC/FTIR data alone can be sufficient for the automatic identification of a constituent in a mixture, subject to the following three conditions:

- Sufficient amount of the constituent must be present.
- Presence of co-eluted impurity, if any, below a certain level.
- The IR spectrum of the compound is already recorded in the reference library.

In this case the GC/FTIR system provides the same service as a conventional GC/MS for which the three conditions mentioned above also prevail. According to our experience, the difference between the two techniques can sometimes be in favor of the GC/FTIR when the molecular ion is not apparent in the mass spectrum as in the case of acetals or certain esters or when isomeric problems arise as sometimes exists with aromatic and heterocyclic molecules. The IR library search usually gives a precise answer, whereas search in standard MS libraries can sometimes lead to ambiguous identifications within the series of homologous compounds; this confusion nevertheless can be eliminated when examining the chromatographic retention indices. If the substance to be identified is not recorded in MS or IR libraries, usually immediate identification is not possible, but IR library search provides information concerning the chemical functions that are present (or absent) within the so-far unidentified molecule, an advantageous feature which is not as readily available using the similar GC/MS operation.

We were recently faced with this problem, during an analysis of the headspace of the silver-white osmanthus flowers. An olfactorily important constituent was not identifiable when exclusively examining its mass spectrum, which however suggested an ionone isomer. On the other hand, the IR spectrum (Figure 1) shows the presence of a non-conjugated carbonyl group and the lack of an exocyclic methylene group, thus suggesting a retro-α-ionone. However, in order to determine the exact chemical structure, it will be compulsory, as we have seen earlier on, to have an authentic reference sample for an efficient comparison, or else it will be necessary to separate a sufficient quantity of the substance to carry out an NMR study.

LC Techniques

Thin layer chromatography (TLC) has been used for many years for the analysis of essential oils. In spite of its indisputable rapidity, this technique is now obsolete, due to its very low resolution, even when using the most sophisticated recent layer technologies.

High Pressure Liquid Chromatography (HPLC) and, to a lesser extent, Size Exclusion Chromatography (SEC) have been extensively tested, but their lower peak capacity (number of resolvable peaks) makes them inferior as compared to capillary GC. Today, the main applications of HPLC for the direct analysis of essential oils are, for example, the control of the genuineness of citrus oils, or the specific determination of certain hazardous components (such as furocoumarins). On the other hand, HPLC is a technique of choice for analyzing non-volatile components in plant extracts (concretes and absolutes), which cannot be safely eluted from any GC column, even in using the most recent developments for the manufacture of short low-diameter columns coated with stationary phases with very high thermal stability.

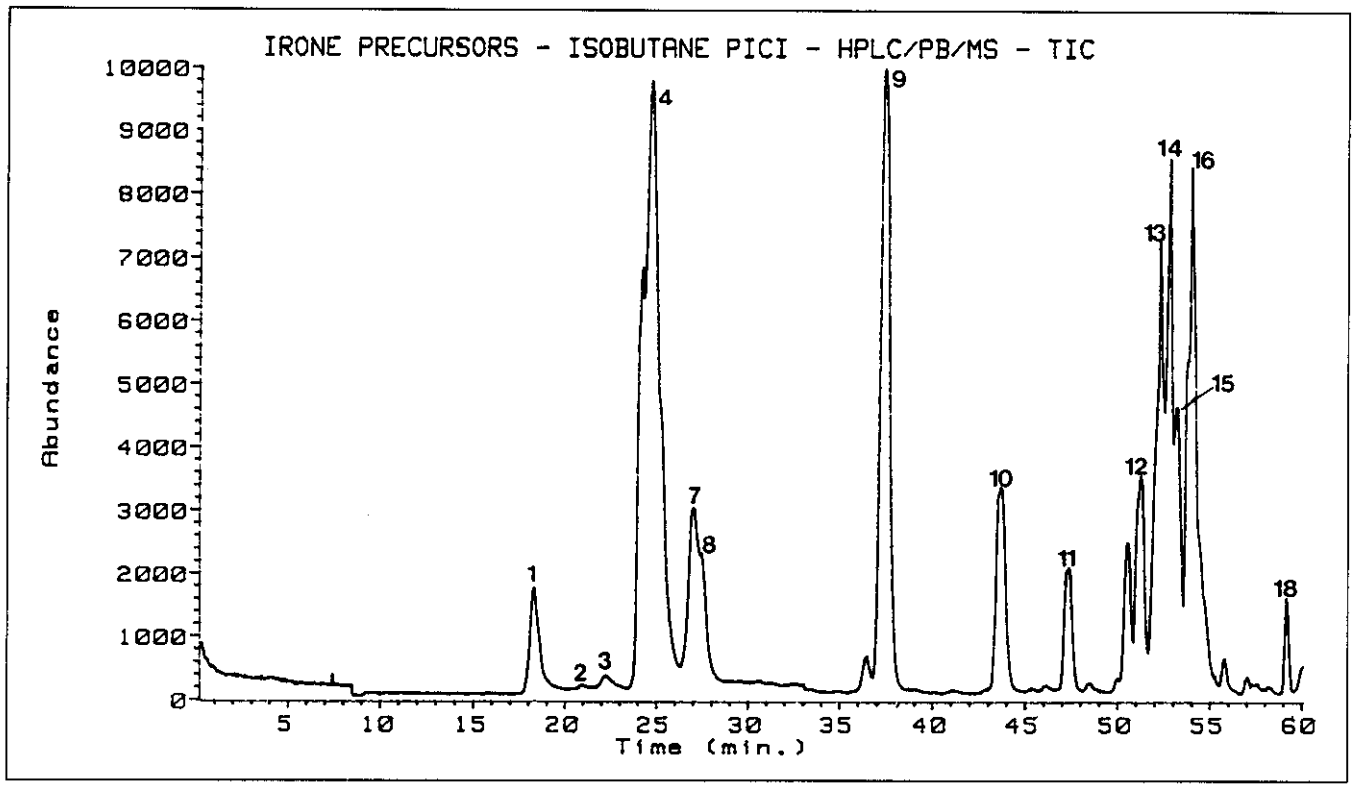

Figure 2. HPLC/MS of *Iris pallida* extract using particle beam positive chemical ionization (CH4) (Portion of total ion current chromatogram); 4: irigenin; 9: iso-iridogermanal; 10: MW 474; 11: MW 528; 14: iriflorental; 15: MW 486; 16: iripallidal

The coupling of HPLC with a mass spectrometer is not a recent technique, but the marketing of new interfacing/ionization technologies is now offering interesting possibilities for the analysis of natural extracts. In 1990 we were able to carry out the direct analysis of an extract of fresh orris rhizomes (*Iris pallida* L.) by particle-beam LC-MS with electron impact as well as positive and negative chemical ionization (Figure 2). We could thus separate and identify a number of triterpenoids, some of which are known irone precursors (such as irripallidal and irriflorental), and we were also able to locate other analogous compounds, which are so far not described and remain to be fully identified.

Liquid phase (HPLC, SEC) as well as supercritical fluid (SFC) chromatographic techniques are very convenient when prefractionation of a mixture is sought, either by polarity (normal or reverse phase conventional HPLC) or by molecular size (SEC) or other criteria (SFC). Adding one (or more) prefractionation steps(s) prior to a capillary GC analysis usually results in a dramatic increase in the overall resolution of the analysis. Efficient off-line and on-line LC-GC experiments have been described.[6]

Multidimensional Chromatographic Techniques

GC-GC: This set of techniques is mainly represented today by bi-dimensional capillary gas chromatography (GC-GC). This technique involves the coupling of two capillary columns, each one having a different function (such as polarity or enantiospecificity), and the use of a variety of switching devices operating within one- or two-column ovens. It is noteworthy that this methodology was applied to essential oil analysis and reported as early as 1977 at the Kyoto Congress.[7]

Such developments are mainly motivated today by a vastly increasing need to evaluate the authenticity of essential oils and aromatic natural extracts, in response to a strong demand from the flavor market. Of particular interest is the direct analysis of a chiral constituent in a natural mixture by GC-GC, using a set of two columns in which the first column carries out a prefractionation of the substance of interest (so-called "heart-cutting") based on either polarity or volatility, and the second chirospecific column performs the chiral separation of the enantiomers.[8] According to our experience, using a conventional GC flame ionization detector (FID) may not always be sufficient to evaluate an enantiomeric purity, since impurities can still co-elute with one of the enantiomers. It is therefore highly preferable to check the identity of the different chromatographic peaks by either MS or IR detection, a precaution that is unfortunately not always taken by many analysts.

GC/IRMS: An even more powerful method for detection of adulteration with added racemic compounds consists in utilizing as the GC detector an isotope ratio mass spectrometer (IRMS) capable of measuring the ratios of stable carbon isotopes 13C/12C for each chromatographic peak. This method is particularly suitable for determining the precise origin of enantiomeric pairs, after proper sepa-

Table I. Carbon isotope ratios† of some cornmint oil components

	δ ^{13}C (parts per thousand, PDB standard)
(–)-limonene	- 32.6*
(+)-3-octanol	- 30.5*
(Z)-3-hexen-1-ol	- 33.4 ± 0.3**
1-hexanol	- 37.3 ± 0.8**
(–)-menthol	- 29.0*

†The $^{13}C/^{12}C$ ratio of a sample is expressed as a δ ^{13}C value, i.e., deviation of ratio in parts per thousand from that of a universal standard which, for carbon, is the calcium carbonate (called PDB) in a fossil from the PeeDee Formation in South Carolina. Thus:

$$\delta^{13}C = [(^{13}C/^{12}C \text{ sample} / {}^{13}C/^{12}C_{PDB})-1] \times 10^3$$

* off-line measurement

** on-line measurement

Table II. Differentiation of natural from synthetic (R)(–)-carvone

		synthetic (R)(–)-carvone	natural (R)(–)-carvone
optical rotation	[α]D/20	- 58°	- 58°
carbon isotope ratio*	δ ^{13}C	- 27.7	- 28.4
hydrogen isotope ratio**	δ ^{2}H	- 170	- 245

* $\delta^{13}C$ is based on parts per thousand of both the sample and the PDB standard, as described in Table I.

** $\delta^{2}H$ is based on parts per thousand of both the sample and the standard, which for stable hydrogen is standard mean ocean water (SMOW). A calculation similar to the one in Table I is used.

ration using the aforementioned chirospecific bi-dimensional GC method.[9,10] This "on-line" GC/IRMS system appears today as one of the most sophisticated instruments for the appraisal of the genuineness of a natural mixture, for the make-up of the isotopic ratios of the different constituents, terpenic or not, results in a fingerprint of the mixture, due to the variety of the biosynthetic pathways of the constituents. A typical example is provided by cornmint *(Mentha arvensis)* oil, in which a number of components are generated through distinct biosynthetic routes, and thus show significantly different 13C/12C ratios (Table I).

In spite of its power, this tool also has limitations. Or, it can raise problems. For example, in performing bi-dimensional GC/IRMS, the "heart-cutting" operation can induce isotopic fractionation and thus generate "drifted" isotope ratio measurements.

In addition, GC/IRMS is useless for the direct determination of the origin of certain substances, such as (R)(–)-carvone that occurs in spearmint essential oil, that cannot be distinguished by this method from synthetic (R)(–)-carvone prepared from (+)-limonene, since in both cases not only the optical rotations but also the carbon isotope ratios are similar (Table II).

We have found that the measurement of the hydrogen isotope ratio could solve this problem. Unfortunately no commercial "on-line" GC/IRMS instrument is able today to perform such a determination. The "off-line" analysis requires a separation of at least 10 mg of pure sample using a micropreparative technique.

The same disadvantage exists with site-specific deuterium NMR, which usually requires hundreds of milligrams of pure substance. In return, in spite of its very high cost, this recent technique is able to characterize very accurately natural molecules of different origins, as in the typical cases of linalool and linalyl acetate[11] or benzaldehyde.[12]

Recently Developed Analytical Techniques

A detailed discussion of all the recently developed analytical techniques would be tedious and is outside the realm of this presentation. Nevertheless some of them are worth mentioning since they undoubtedly have great potential in this specific domain of essential oils and natural extracts.

Among those, one can cite micro-chromatographic techniques, for example involving shorter GC columns (5-10 m) with lower diameter (down to 0.1 mm) that can elute triglycerides with excellent resolution in 15 minutes, or carry out an efficient analysis of mint essential oil, for example, in less than five minutes.[13]

Likewise, increasing the performance of LC/MS technology by using fused silica open tubular columns (FSOT) with lower diameters (50-300 μm), with efficiencies reaching 100,000 theoretical plates per meter, could promote this technique as a serious competitor with GC/MS for solving specific problems.

Simultaneous multiple GC detection using the recently marketed atomic emission detector (AED) can generate with very high sensitivity "heteroatom-specific chromatograms" in a single run, thus allowing the detection, or sometimes the identification, of some trace elements containing nitrogen, sulfur, and chlorine in mixtures of natural origin.

As far as chirospecific GC is concerned, differently derivatized cyclodextrins have been demonstrated to be reliable chiral stationary phases for capillary GC separation of a number of volatile racemates with very different structures and chemical functions. A careful compromise between column length (down to 10 m), optimal elution parameters and highly sophisticated cyclodextrin modifications enables most separations to be carried out[14,15] using a single column, eliminating costly column switchings. Such an advantage is expected to place this methodology at a routine use level in the very near future. Such developments are also compatible with preparative column technology, enabling a pure enantiomer to be separated from a natural mixture in order to carry out further studies, such as sensory evaluations.

Today *hyphenated technique* has become a buzz word used to define a wide variety of combinations of analytical techniques which can also be called hybridized techniques. Successful commercial hybrid instruments have resulted from combining chromatographic methods, spectrometric methods, thermal methods, and kinetic methods with one another. Among examples already cited, GC/IR and GC/MS are the most popular. More complex hybrids such as GC/FTIR/MS, GC-GC/MS-MS, GC/FTIR/MS/AED/FID[16] and others have been developed recently, and some of them have even been tested with essential oils and related mixtures with some success. However attractive their performance may be, it is drastically attenuated by the high cost of these instruments. Moreover, the main feature of this methodology is claimed to be a saving of time since it is supposed to provide a great deal of information within a single analytical run. But this advantage is not so easily fulfilled due to the very laborious and tedious calibration of these instruments.

Qualitative Abuses

These modern analytical techniques would be almost ineffective without the wide use of operating/computing systems. Today the services rendered by data systems—including the very handy GC computing integrators—are outstanding.

But many scientists—as well as non-scientists—seem to have forgotten the meaning of figures, particularly since pocket calculators have come into everyday use! Scientists are thus able to enjoy the comfort (speedy processing) provided by this equipment, and this may develop in them a tendency to neglect to question the meaning of all the data that are fed out to them.

Misuse, and in some cases abuse, of modern instrumentation is often observed when identification of components in a mixture is carried out, for example, by GC and GC/MS. In many cases, analytical chemists rely on chromatographic retention data, using a set of two columns of different polarities, which they compare more or less automatically with previously published data or data which they have compiled in "in house" libraries.[17] More frequently, the GC retention data will be supplemented with mass spectrometer data from automatic searches of commercial or specific mass spectra libraries.

Such methodology is now routinely used in many laboratories. However, in spite of its speed and ease of use, it should be used with great care, as has been pointed out by the Working Group on Methods of Analysis of the International Office of the Flavor Industry (IOFI) in a recent publication on this subject.[18] In their final conclusion, these experts declared "... neither gas chromatographic nor mass spectral evidence on its own can be a satisfactory basis for the conclusive identification. Even a combination of the two may still require an additional form of analysis, such as

Table III. Attempted gas chromatographic quantitation of a 3-component mixture

Injection	Column	Detector	Integrator	Relative percentages as recorded by the integrator		
				2-Phenylethanol	Eugenol	2-Phenylethyl benzoate
hot "on column"	0.5 mm x 25 m methylsilicone	TCD	Shimadzu C-R3A	42.6	36.4	20.9
hot "on column"	0.5 mm x 25 m carbowax 20M	TCD	Varian DS 654	40.3	41.4	18.2
hot "on column"	0.5 mm x 25 m methylsilicone	TCD	Varian DS 654	30.0	39.5	30.4
split mode	0.32 mm x 25 m methylsilicone	TCD	Hewlett-Packard HP 3396	29.0	33.2	37.8
split mode	0.32 mm x 25 m methylsilicone	FID	Hewlett-Packard HP 3396	39.5	32.6	27.0
split mode	0.32 mm x 50 m methylsilicone	MS	Finnigan Data System	33.0	31.7	35.3
split mode with autosampling	0.32 mm x 50 m carbowax 20M	FID	Hewlett-Packard HP 5880A	35.4	29.7	34.8
split mode with autosampling	0.32 mm x 25 m carbowax 20M	FID	Hewlett-Packard HP 5880A	35.5	30.2	34.2
split mode with autosampling	0.32 mm x 25 m methylsilicone	FID	Hewlett-Packard HP 5880A	35.1	30.6	34.2
splitless mode	0.32 mm x 50 m methylsilicone	FID	Shimadzu ICR-1B	40.0	33.4	29.6

NMR or IR spectroscopy in order to ensure absolute certainty."

This caution obviously applies to certain types of compounds which do not display a sufficiently specific mass spectral pattern, or which are highly polar, thus generating unreliable chromatographic retention data, a situation becoming worse with aging columns. However, few analysts remember that no computerized library search has any power of decision! Actually the final decision belongs to the analyst.

Typical abuse of GC/MS semi-automatic or automatic data processing is illustrated in the case of the sesquiterpenoids group. Premature and mistaken identifications may be easily reported, since it is extremely difficult to make safe use of published data concerning this type of natural products that occur very frequently in essential oils. Indeed, such GC and MS data may have been recorded under a variety of conditions, using different instruments and parameter settings.

The particular case of sesquiterpene hydrocarbons is noteworthy: so far, more than 230 naturally occurring sesquiterpenes with molecular weight 204 have been reported. Given one can expect them to be eluted in GC on both polar and non-polar columns within a range of about 300 Kovats units, and one can expect a large number of them to display similar mass spectra (with common fragment ions 105, 119, 133, 161 ...), it is therefore quite understandable that GC/MS identification alone can be hazardous.

Recent compilations of published GC retention data of sesquiterpenoids are thus deplorable, since they may worsen this risk.[19] Conversely, publications of complete and reliable original descriptions (MS, GC, FTIR, NMR) of closely related compounds such as the eight stereoisomers of eudesm-11-en-4-ol are most useful and should be encouraged.[20]

In summary, when investigating mixtures of natural origin, either essential oils or extracts, the identification of a *non-isolated* constituent can be considered as feasible when the following conditions are met:

- The mass spectrum and GC retention indices are strictly identical to those of the reference sample present in the databases. Additional use of infrared data is preferred.
- The databases should be developed by the analyst himself/herself from genuine samples.
- Genuine samples can either be prepared by the analyst (isolation, synthesis) or identified in other natural mixtures previously investigated by others or himself/herself, **using the same reliable methodology.**

It is the analyst's duty to check that any published data used are relevant, and discard any unconfirmed information.

Misuse of instrumentation-generated data can also be a consequence of overlooking the common knowledge of phytochemistry or plant chemotaxonomy. This may lead to wrong interpretation—even from correct data. For instance, in the investigation of a natural mixture, the analyst might incorrectly declare as naturally occurring some products which simply *cannot* be present in the original plant material. Most frequent causes of such misinterpretations are contaminants from foreign plant material, as well as non-plant products, during collection, transportation and industrial processing. Another frequent source of contaminants is careless manipulations during analysis, involving even contamination from the analyst's own skin![21]

Being aware of these sometimes unavoidable drawbacks would prevent any analyst from making thorough carbon NMR studies of di-isooctyl phthalate isolated from a plant extract, or discussing a new contribution of BHT in green tea flavor! Exogeneous contaminations must be distinguished from artifacts, concerning products which are **generated** during processing or analysis. Chemically generated artifacts from isomerization—including cyclizations and racemizations, hydrolysis, eliminations and interesterifications—are well documented and should never be minimized when performing natural products' analyses.

Quantitative Abuses

"Things are seldom what they seem," according to a lyric from Gilbert and Sullivan's *Pirates of Penzance*. This somewhat disenchanted statement applies well to what has just been briefly discussed concerning some *qualitative* aspects of abuses of modern analytical methodologies.

The situation becomes even more tricky when *quantitative* aspects are involved. In the domain of essential oils and related extracts, apart from classical physical determinations (of characteristics such as specific gravity, refractive index and optical rotation), most quantitative measurements are carried out after proper separation using most often GC or HPLC. Detection schemes vary, but data reduction and, ultimately, analyte quantitation are performed in many cases by a computer-based data system or *integrator*. Actually, very few analysts are aware of a number of questions that should be addressed when using data reported by chromatographic detectors and integrators—questions about factors such as repeatability, accuracy and ruggedness.

It has been reported that when four GC integrators were tested using simulated data, they produced not only significantly discrepant results between instruments but also, for a given instrument, widely different effects of noise level and tailing on peaks having different heights and widths.[22]

Differences between results are caused by many other factors, including the analysts themselves, or pressure/flow perturbations inside the injector, or in some other part of the sample path or in the data processing. Standard samples do not necessarily produce standard chromatograms.

This is confirmed by the following experiment which we performed: a three-component model mixture, made of equal parts of 2-phenylethanol, eugenol and 2-phenylethyl benzoate, was submitted to GC analysis, using ten different instruments (GC apparatus and integrators), fitted with different types of columns (narrow and wide bore, polar and non-polar capillary columns) and operating under different modes of injection (split, splitless, on-column) and detection (thermal conductivity, FID, MS). Results are shown in Table III, and do not deserve any comments.

This confirms that in the absence of prior standardization, the apparent high precision, illustrated by the number of decimal places (up to five!), given by modern integrators is illusive or meaningless.

Moreover, the excessive confidence that many analytical

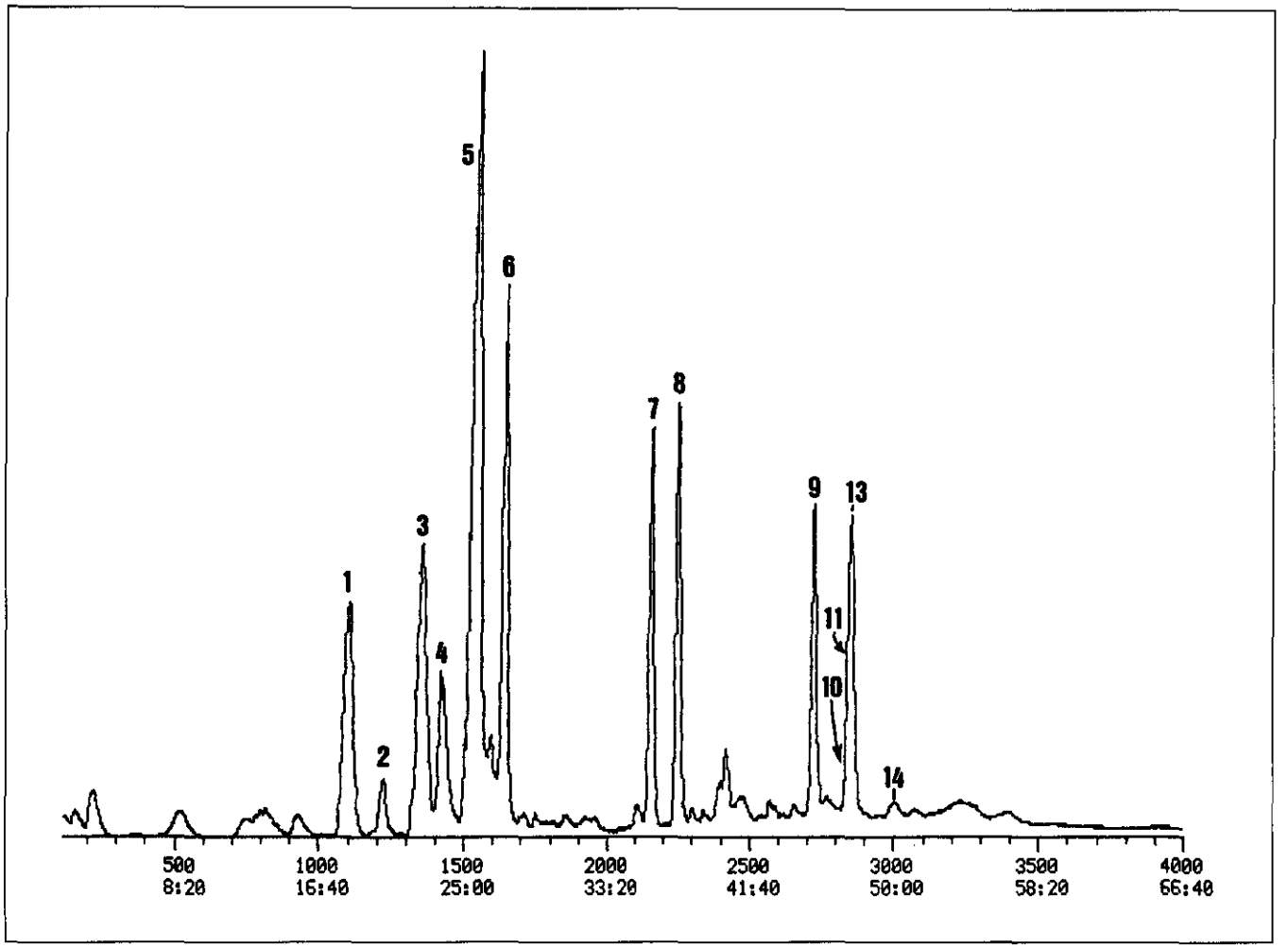

Figure 3. Cold on-column injection of jasmin absolute (column: 0.32 x 6 m fused silica column coated with SE30; detection: MS); 5: phytol; 6: phytyl acetate; 7: squalene; 8: squalene epoxide; 9: phytyl palmitate; 10: phytyl linoleate; 11: phytyl oleate; 13: phytyl linolenate

chemists place in such instrumentation may lead them to conclude that only those components which are detected are present in the mixture! This is practically never the case when plant extracts such as absolutes are analyzed by conventional GC. For example, one may consider that more than 90% of jasmine absolute from hexane extracts of *Jasminum grandiflorum* flowers are made of 15 constituents only.[23] However, while using either standardization of these compounds or while operating under appropriate GC conditions so that squalene, 2,3-oxidosqualene and higher phytyl esters are eluted, one can demonstrate that, actually, these 15 constituents represent less than 60% of the absolute (Figure 3).

Constant awareness of such basic notions as accuracy, precision, repeatability, reproducibility, and bias recognition is required when performing consistent quantitative measurements. In this respect, it is worth mentioning the recent interlaboratory study on the determination of residual benzene in moss extracts.[23]

Finally, in this particular field of natural product analysis, the use of modern instrumentation can sometimes lead to the abuse in which an undeniably accurate result is put forward as a general rule, even though it has only been observed a small number of times. Among many recent examples, one can mention hastily published evaluations of enantiomeric excesses of chiral compounds such as α-ionone or the α-irones,[8] as well as certain rose oil constituents.[24]

Conclusion

Analytical methodologies have reached a high degree of sophistication, and are now so sensitive that tiny amounts of virtually any substance can be detected. Further developments of analytical techniques with increased resolution and specificity are expected to find new applications in this particular essential oil and complex mixtures domain of natural product chemistry. One can also expect these techniques to adapt themselves to new requirements resulting from the foreseeable introduction and development of new industrial processing technologies such as membrane separation techniques, microwave extraction or industrial supercritical fluid chromatography.

Increased analytical skills should be beneficial since, as before, so-far unexpected additional natural products of

interest might be discovered. Likewise, a better knowledge of natural raw materials always results in a better appraisal of their safe use limits. In this respect, our industry now undoubtedly possesses the capacity to define the most reasonable policies.

Analytical chemists should take the lead in responsibly interpreting analytical information. This would prevent this community's research body, and consequently regulatory organizations, from generating any kind of *authoritative misinformation*, which we know can be detrimental to the activity of this industry. To achieve this goal, international and national analytical committees with broad representation from private institutions and official organizations are urged to pursue their work, to define accurate protocols and to build large and reliable databases.

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Address correspondence to Daniel Joulain, Research Laboratories, Robertet SA, BP 100, F-06333 Grasse, France.

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