

Analysis as an Aid to Perfumery Or Who Needs Perfumers Anyway?

By John Mayo, Bush Boake Allen Ltd., London, England

The perfumery industry has existed, in some form or other, since the beginning of recorded history. In relative terms, it is only in recent years that perfumers have ceased to be totally and exclusively dependent upon natural products for their ingredients. Moreover, most of our knowledge of and our ability to measure the chemical composition of these natural products has only been derived in the past twenty-five years or so.

It is worth emphasizing this point because it is necessary to understand how the role of the analyst has changed and is still changing in order that perfumers may be aware and take advantage of these changes.

For many years the role of the analysts was to control the quality of materials purchased for and produced by the fragrance industry. Prior to the late 1950s they were largely trying to control the quality of natural products without knowing

the chemical identities of many and probably most of the constituents and with no ready means of determining the quantities of these constituents. Thus, the tests used were built around measurements of physical constants such as refractive index, specific gravity, melting or boiling point, and by measurements relating to functional groups such as ester value, iodine value, oximation, total alcohols and so on. The same restrictions, of course, applied to the checking of compounded products, i.e., fragrances, since once the mixing had taken place there was no simple way to check, by analysis, what had been added.

The wide variation in availability and costs of natural materials gave rise to heavy adulteration of the more expensive items with those that were cheaper and more freely available. Classic examples were the adulteration of lemon oil with turpentine, the addition of rosin to gums,

balsams, cassia and orange oils and the dilution of citronella oil with mineral oil. Some of this adulteration was, and still is, crude in the extreme but the sophistication of natural products soon became big business and was mainly conducted in a controlled and responsible manner. The supply houses employed analysts and perfumers to produce blended products which would pass their customers' tests both on analysis and odour.

This situation was naturally beneficial to both analysts and perfumers since it increased the size of the job market for both. A great deal has been said about the "fiddling" or sophistication of natural products, but I personally believe that it is a practice that has been, and still is, of considerable benefit to the fragrance industry. Not only has it acted as a spur to the analyst but it has been an important area for the development and learning of the skills associated with successful blending. It has also increased very significantly the availability of natural products at a range of prices which normally are reasonable reflections of the quality and purity. You really have no right to express indignation when your analyst tells you that the Geranium Bourbon you have just purchased at £1.50/kilo is not very pure.

New Analytic Techniques

The traditional role of the analyst began to change very dramatically about twenty-five years ago and this change was initiated by the development of equipment which enabled the commercial exploitation of a whole spectrum of new analytical techniques. I should emphasise that this development was not a fortuitous gift to the analysts but essentially resulted from the efforts of analysts and physical chemists who could see the potential advantage to be gained by finding ways of applying scientific principles to the solution of everyday problems. There is no doubt that today's sophisticated instruments have resulted from a high level of cooperation between the research scientist—which includes analysts—and the instrument manufacturers.

Chromatography

Without doubt pride of place—in terms of advantage to the fragrance industry—must go to chromatography.

The basic principle of chromatographic separation has been known for very many years, certainly from the beginning of the 20th Century. In the days before the invention of the biro every schoolchild was aware that a drop of ink

on a blotting paper gradually spread outwards from the centre and gave rise to a number of concentric rings of different colour. Similarly, a solution of dyestuffs poured into a vertical glass column packed with, say, Fullers Earth, gave rise to distinct coloured bands in the column and these bands represented individual chemical components of the dye. Such a technique could be used to isolate the components for analysis.

In essence, this is the basis of all chromatography. A material or a mixture of materials is absorbed into or adsorbed onto a liquid or a solid, known as the stationary phase and is then eluted by the passage of a gas or liquid stream. The vital factor is that under standard conditions, i.e., the same stationary phase, identical pressure, temperature, column size and gas or liquid flow rate, the time taken for a given chemical to be eluted from the system is constant. Thus, if it were possible to detect individual components as they emerged from a column it would be possible, by measuring the time factor, to determine the identity of the components and, moreover, if the detector system could be made to give an indication of the amount of each component detected then we would have a system capable of both qualitative and quantitative analysis.

The determined efforts to exploit this principle began around the mid 1950s. We do not have time to go into a detailed history of the developments but a few illustrations will give some idea of the tremendous advances that have been made in the past 25 years.

The modern gas chromatograph has its own data station, i.e., computer, and has automatic sampling and multi-ramp temperature programming facilities. It can also operate simultaneously on two different stationary phases. The columns most used today are:

The Modern Gas Chromatograph

metres in length with a diameter of 0.5 mm. This column also has a stationary phase and support material coated in a thin layer on the inside wall.

- **Wall Coated Open Tubular Column (WCOT)**—usually made of fused silica with the stationary phase alone coated on the inside wall. The internal diameter is 0.22 mm at the length of 25 to 50 metres.

Columns Most Used Today

- **Packed Columns**—usually made of glass, 3 metres in length and 4 mm in diameter and with a stationary phase coated on a support material.
- **Support Coated Open Tubular Column (SCOT)**—also made of glass, from 25 to 50

The separation power of these columns is enormous. In the early days we were very excited when we produced columns with a separative power which in distillation terms was equivalent to 40 to 60 theoretical plates and 100 plates was a triumph. Today's columns would equate to around 40,000-50,000 theoretical plates. Figures 3-5 will give some idea of how the efficiencies of columns have improved.

Similarly, there have been tremendous advances in the development of the detector systems used to measure the chemicals as they emerge from the chromatographic column. The detector system most commonly used today is

based on flame ionisation. Other methods used are electron capture and gas density balance systems, the latter being very valuable for the accurate measurement of specific materials such as alcohol. There are also detector systems designed to measure specific elements such as sulphur, nitrogen and halogens.

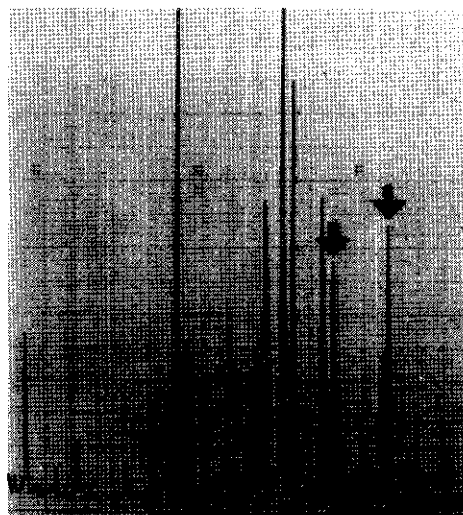
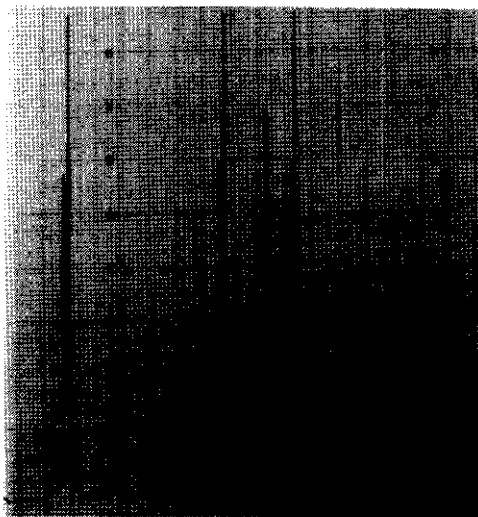
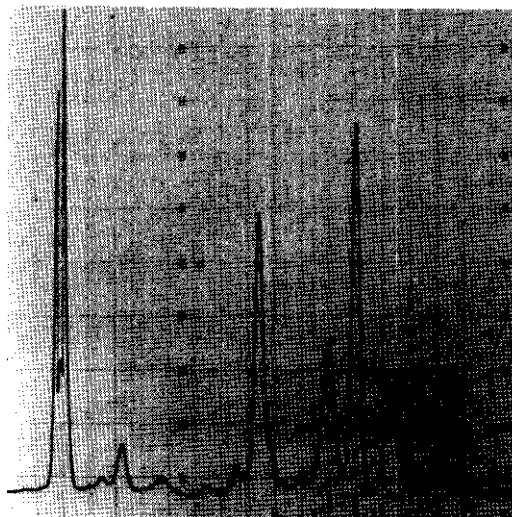
The degree of response of the detector system is related to the quantity of each individual component present as is instanced by the varying sizes of the peaks on the charts. Unfortunately this response factor is not necessarily linear but nevertheless it is possible to build into the data system factors which can correct for such nonlinearity.

The advances made in the sensitivity of chromatographic equipment have made possible the very detailed analysis of minute quantities of materials and for gas chromatography detection limits are as low as 1×10^{-10} gm. This facility to measure extremely low levels has made possible a new technique, known as head space analysis, which is, in effect, the analysis of a sample of air.

Gas chromatography (GC) has one major limitation in that it can normally only be applied to materials of relatively high volatility. However, the basic principle of chromatography can still be applied to molecules of high molecular weight and a technique known as HPLC (High Pressure Liquid Chromatography) has been developed for this purpose. In simple terms this technique involves the elution of the components of the added sample by pumping a liquid, known as the mobile phase, through the column. A typical column will measure 250 mm in length with an internal diameter of about 5 mm. The pressure used to force the mobile phase through the column may be as high as 2-3000 p.s.i. The detector systems most commonly used with HPLC are UV or R.I. measurements.

Spectroscopy

The second field of analysis which is of prime importance is spectroscopy and, at the risk of offending the purist, I will include Mass Spectrometry under this heading. The major developments in this field, in terms of industrial availability and exploitation, followed some 5-10 years after chromatography but, here too, the scientific principles had been long established. Spectroscopy is concerned with the scattering or diffraction of electromagnetic radiation and the relationship of such scattering to molecular structure. As an analytical tool it can only be applied to pure substances. The techniques in common use are:



Comparison of the separative power of 3 different GC columns on a sample of patchouli penang. In each case, the arrowed peaks are α -copalene (right) and α -gurjunene (left). From top: Packed SP2100, SCOT SP2100, and SP2100 WCOT.

- Infrared (IR) spectroscopy which is concerned with absorption by vibrational transitions within the molecule
- Ultraviolet (UV) Spectroscopy which is concerned with absorption by electronic transmissions within the molecule
- Nuclear Magnetic Resonance (NMR) which is concerned with the measurement of absorptions resulting from spin transitions within the molecule
- Mass Spectrometry (MS) which is based on measurement of the masses of individual fragments of a molecule

In brief summary it may be said that IR and UV identify specific functional groups within a molecule, NMR gives information on the spacial arrangement of the molecule and MS identifies and quantifies the fragments of a ruptured molecule. From all of this the chemist derives the pieces of a jigsaw which must be fitted together to give the final answer.

In the early days much of the work carried out by the analyst was involved in the elucidation of structures of varying complexity. In pursuit of this goal it must be said that the Mass Spectrometer was of prime importance. However, once a structure had been determined the spectrum became a highly specific fingerprint for the given chemical. Thus if a spectrum and chromatographic retention time can be readily matched against a known standard then identification is complete and absolute.

At this stage I must point out that modern electronics, computers and data systems have played a vital role in the development of modern analytical techniques. If we had been confined to the manual interpretation of analytical data such as gas chromatographic charts or mass spectra the level of advance in our knowledge over the past twenty-five years would have been only a minute fraction of that which has actually been achieved. Fortunately we are able to collect, store and interpret the mass of information via computerised data systems.

The logical step, once the techniques had been developed, was to link chromatography directly to mass spectrometry and a computer-based data system. In this way we had the facility via the chromatography unit to split complex mixtures, quantitatively, into pure chemicals which could be instantly identified by the mass spectral fingerprint. The output from a modern

gas chromatograph/mass spectrometer system is enormous. For example, a thirty minute or so time span is sufficient to produce a qualitative and quantitative measurement of maybe several hundred components in an essential oil sample. Prior to the 1950s a team of highly competent scientists could, and in some cases did, spend a lifetime's work studying an essential oil and only succeed in isolating and determining the structures of not more than 10% of the components.

Although chromatography can be used to detect materials of extremely low levels, a coupled GC/MS system under normal operating conditions will usually only determine components of a mixture down to levels of 1 part in 10,000. Thus a routine analysis could easily miss trace components which could be of considerable importance in terms of olfactory properties. In order to overcome this problem, use is made of the human nose—still the most sensitive piece of analytical equipment available to us. Quite simply, the gas stream from the chromatograph is split with one part being fed into the mass spectrometer and the other part is released through a funnel to atmosphere and is monitored by a trained nose. In this way, the presence of trace materials can be indicated and the relevant parts of the sample can be prepared in higher concentration for more detailed analysis.

Modern Analysis' Value to the Perfumer?

My own view is that by far the most important gain to the perfumer has been that modern analysis has enabled the organic chemist to synthesise a vast number of materials for the perfumer to use. There can be no doubt that a very large number of the materials that you regard today as standard ingredients for fragrances would not be available, or even known, were it not for chromatography and, though perhaps to a lesser extent, spectroscopy. Without these techniques we might still be struggling to develop economically viable processes for even relatively simple materials such as geraniol, linalol, citral and citronellal and, of course, all their derivatives.

Once these new facilities were available a major effort was directed toward the analysis of essential oils and natural products. For some companies the approach was to establish the structure of, and subsequently synthesise, components with important odour characteristics and use the synthetics to extend the natural supply or to boost certain characteristics of the oil. Other companies were primarily interested in establishing new products for manufacture and sale to the fragrance and flavour industries.

Whatever the motive, the end result was an enormous increase in our knowledge of essential oils and in the number of materials available to the perfumer.

It is true to say that the ability to establish the structure of many important materials is a long way ahead of our ability to synthesise the identical materials. Nevertheless, knowledge of the identity and structure will frequently indicate possible routes to other molecules of similar but not identical structure and this too has been a fruitful field for the organic chemist. Again, there have been many instances where analysis has identified impurities in synthetic processes and these impurities have themselves proved to be valuable products.

The biggest challenge facing the perfumer today is probably the demand for products giving high performance in difficult or aggressive media. This is an obvious area where the perfumer can derive a great deal of assistance from the analyst. Most fragrance ingredients are, in chemical terms, highly reactive and the bases into which they are introduced are becoming more and more complex and can cause severe breakdown with some fragrance ingredients. With all the facilities available today the analyst can monitor the performance of a fragrance in the end product. Not only is it possible to obtain accurate scientific measurements of the stability of the individual components of the fragrance, but the effect of the fragrance on the components of the base can also be measured. There is no point in developing a stable fragrance if the fragrance itself destroys one of the active ingredients in the end product medium.

Once aware of the possibilities of analysis each reader can think of ways in which it may help to solve individual problems. Obvious examples are the measurement of emanation rates, not only of a fragrance but of the individual components from an air freshener or the scientific measurement of the retention of fragrance ingredients on fabric. But do not expect miracles all the time. Remember the analysts are specialists also and it is far better to discuss problems with them at the earliest possible stage so that you can jointly work out the best approach to use your combined skills.

The ever increasing legal and safety restrictions are a problem to everybody, but here too analysts can often help. Not only can they check that your ingredients are suitable for use but they may be able to identify problem components in essential oils or impurities in valuable synthetics. An obvious example is the identification of bergaptenes or furocoumarins as being

the problem materials in bergamot oil.

And, of course, there is the problem of matching or type work which every perfumer has to face from time to time, if not most of the time. The analyst can extract the fragrance from virtually any medium, measure the dose-rate and give a skeletal formulation for the fragrance. Of course, the analysis will determine discreet chemicals only, but the analyst can give further assistance by interpreting the results and indicating which ingredients are present solely as synthetic chemicals or part synthetic and part natural and what essential oils have been used. But, again, I would strongly recommend that you collaborate with the analysts at all stages. Your nose can often be of equal, and sometimes more, assistance to them than their GC and MS.

The Analyst

I have been in this industry for a sufficient time that I can remember what it was like before, during and after the advent of the so-called modern analytical techniques. I have seen some perfumers treat these new facilities with suspicion and even open hostility. Equally, I have seen young, enthusiastic analysts become so cocky about the capabilities of their equipment that they openly suggest we no longer require perfumers.

Fortunately most perfumers and analysts today realise that they need each other and the ever increasing complexity of the problems the perfumer must tackle will ensure that this need will continue and increase. The wise perfumer of today will not merely take the results provided by the analyst but will actively seek help and collaboration in solving the problem as a whole. Equally, the good analyst of today does not merely pass on, via the internal post, a mass of analytical data but actively seeks to discuss the results with the perfumer so that both can pool their knowledge and skills to ensure the best possible results.

And so, I would strongly urge all perfumers to seek out your analyst and cultivate good cooperative relationship. Even the most sceptical will find it rewarding. For some the day may come when the salesman rushes up to you to announce that he has sold one of your fragrances. Your initial glow of satisfaction may be tempered when you learn that your classic haute-couture perfume, undoubtedly a masterpiece, is going into a sanitary chemical and you may be further put out when you discover that you have lost the formula. It is at times like this when you take your problem to him or her that you discover the true value of the analyst.

