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A Procedure for the Sensory Analysis of Gas Chromatographic Effluents

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ABSTRACT

A method is presented for determining those volatile components of foods that have flavour significance. The method leads to quantitative flavour specifications called charm that can be displayed graphically. The procedure is based upon the relative odour detection thresholds of volatile compounds of known gas chromatographic retention indices.

INTRODUCTION

One of the persistent problems in flavour chemistry has been the identification of odour-active components among the many odourless chemicals present in foods. Gas chromatography combined with mass spectroscopy allows chemists to propose the identity of chemical constituents without the necessity of producing pure isolates and without any opportunity to make sensory observations about these components. Thus, the long lists of chemicals detected in apples, grapes and other fruits (Masuda, 1976; van Straten, 1977) have created the illusion that we understand their flavour chemistry much better than we do. An immediate problem is to identify those chemicals that contribute significantly to flavour. That is, we need to arrange the list of constituents into an order of decreasing flavour significance. This requires the application of sensory techniques capable of associating flavour descriptors with chemical constituents.

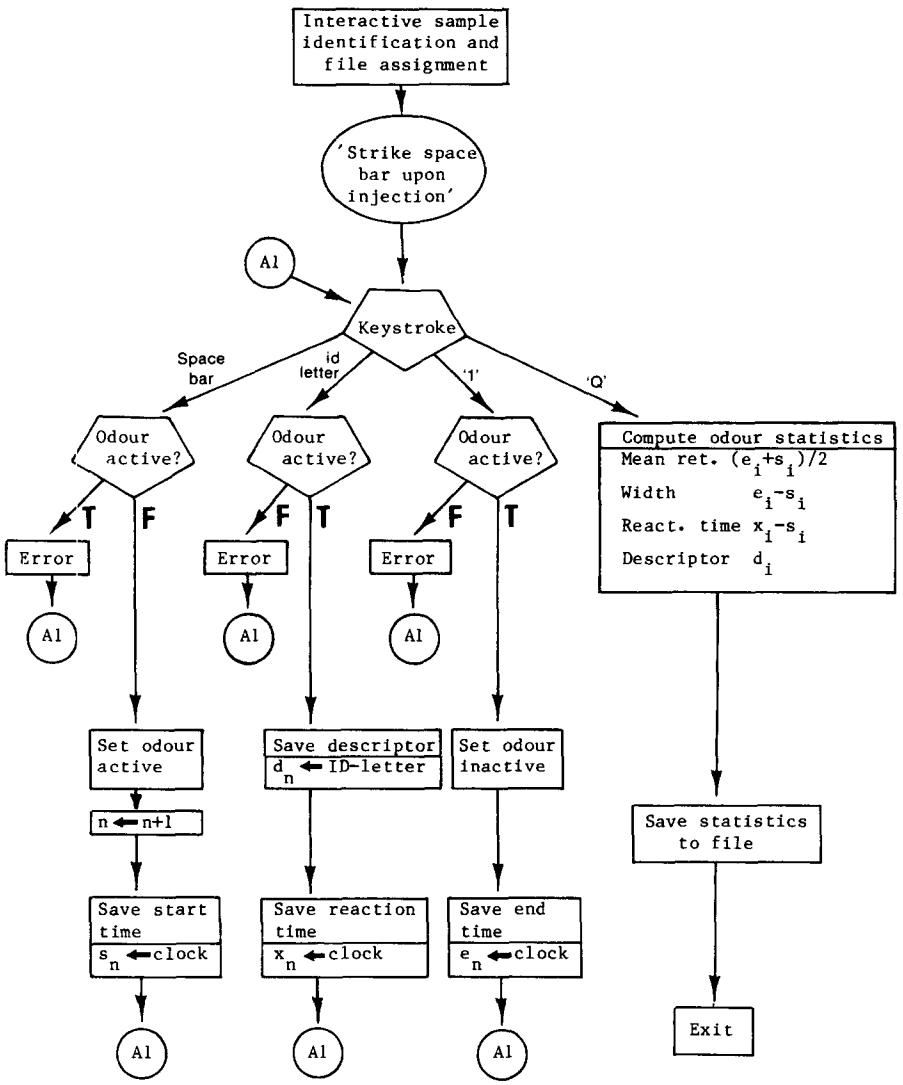
An obvious approach combines sensory analysis with gas chromatography (Fuller *et al.*, 1964). However, the flow of effluent from modern capillary gas chromatographic columns is only about 2 ml/min while the linear velocity of this effluent is very high (30 cm/s or more). Successive puffs of odour-active chemicals, nearly Gaussian in distribution and only 2 to 5 s in duration, must be detected in this stream of carrier gas. Therefore, some type of interface must be constructed to bring these puffs to the nose without disturbing their separation. The interface described by Dravnieks & O'Donnell (1971) used humidified air mixed with the gas chromatographic effluents in order to minimise their desiccating effect on the nasal membrane. Newer designs mix the effluents with larger volumes of humidified air at higher linear velocities to preserve the resolution produced by narrow bore columns (Acree *et al.*, 1976; Drawert and Christoph, 1984).

Given a precise method to transfer chromatographic effluents to human subjects, it is necessary to have a system for recording behaviour, which is both reproducible and responsive to the dynamics of the chromatographic process (Acree, 1984). Methods based on cross-modal matching (Selke *et al.*, 1972) and magnitude estimation (Casimir & Whitfield, 1978) have been described but demand an estimation of odour intensity by the subject while simultaneously sniffing the chromatographic effluent. This is neither easy nor practical while sniffing high resolution gas chromatographic effluents that have band widths of only 2 to 5 s.

A new procedure for the quantitative and qualitative analysis of gas chromatographic effluents is now described. It combines high resolution gas chromatography with the use of *n*-paraffin standards, and uses computerised data collection and reduction, and a sensory procedure based on odour-detection thresholds rather than psychological estimations of stimulus intensity. The procedure produces a dimensionless measure of odour intensity that we call *charm*.

EXPERIMENTAL TECHNIQUE

The procedure provides the subjects with a video-terminal to record their observations. The terminal is connected to a processor containing the program outlined in Fig. 1. The object of the procedure is to determine



CHARM	Collects sensory respose tables.
ADJ	Standardises tables to retention index basis.
BYV	Converts dilution series tables to charm response data.
KWAD	Calculates charm integrals from charm response data.
RK	Produces charm chromatograms from charm response data.

Fig. 1. CHARM, the program used to record sensory responses to gas chromatographic effluents, and summary of ancillary software needed to standardise, reduce, integrate, and produce plots. Hardware independent versions are presently being developed.

the precise retention index, relative to normal paraffins, of the odour-active constituents in a chromatographic effluent, and to apply intensive and/or nominal sensory descriptors to each of them.

To achieve this, a subject sits at the video-terminal while simultaneously sniffing the effluent of a gas chromatograph. At the instant an odour is detected, the subject strikes the space bar on the terminal and the time is recorded on the internal clock of the processor. The subject is then required to strike a key coded for the perceived odour quality. This code can either be previously assigned or developed by the subject during sniffing. The moment the odour is no longer detectable, the subject strikes a character on the keyboard and time is recorded once again. This data is then manipulated to produce a record of the average time each sensory event occurred, its duration, and qualitative descriptor.

Table 1 shows a typical sensory response table produced from apple juice extract. Each row contains the mean time of an odour response, its duration, a descriptor code, and the reaction time. The reaction time is the

TABLE 1
Sensory Response Table

No.	*Descriptor code*	*Mean retention time (s)*	*Response duration (s)*	*Reaction time ($s \times 10^{-1}$)*
1	T	368	3	14
2	T	465	2	14
3	T	509	2	13
4	M	513	5	18
5	T	540	3	44
6	H	625	2	41
7	M	668	6	11
8	T	735	2	14
9	M	1 277	3	20
10	M	1 298	5	16
11	M	1 391	3	13
12	M	1 667	3	3
13	M	1 673	3	23

Data produced from Charm analysis of Freon 113 extracts of apple juice (*Malus domestica* Borkh. cv. Rome Beauty). The apples were crushed in the presence of methanol to prevent enzymatic browning. The extracts were concentrated 20-fold and chromatographed on a 25 m × 0·35 mm fused silica column, coated with methyl silicone (cross-linked bonded phase OV101 0·5 mm film). In this case, T = fruity, H = herbaceous, and M = other.

time the subject took to choose a descriptor after the initial odour detection.

A solution of *n*-paraffin standards is then chromatographed under identical conditions and their retention times used to convert the times in the sensory response table to retention indices. A linear interpolation model is used for temperature-programmed chromatography and a logarithmic model is used for isothermal runs. Table 2 shows the results of linear transformations on the data in Table 1 using a series of *n*-paraffins as standards.

The importance of this part of the methodology cannot be overemphasised since retention indexing associates the sensory response with a reproducible chemical property. Use of retention indices in a rigorous Kovats formulation (Jennings & Shibamoto, 1980) is not essential; an approximation, such as linear modelling of temperature programmed runs, yields equally reproducible results. Fundamental to this procedure is the combining of several different response tables to produce a sensory-developed chromatogram. This requires retention scales which are stable across several chromatographic runs. Retention indexing will assure the required stability.

TABLE 2
Standardised Response Table

No.	*Descriptor code*	*Mean retention index*	*Response duration (index units)*
1	T	788	2
2	T	845	1
3	T	871	1
4	M	873	3
5	T	889	2
6	H	939	1
7	M	964	4
8	T	1 004	1
9	M	1 354	2
10	M	1 369	4
11	M	1 437	2
12	M	1 657	3
13	M	1 662	3

The data in Table 1 were transformed into retention indices using linear interpolation on *n*-paraffin standards.

EXPERIMENTAL PROCEDURE

Coincident response chromatogram

Two experimental procedures each producing different types of sensory developed chromatograms are used. The simplest is to repeat the chromatography several times with one subject, or different subjects, and add the resulting response tables to produce what we call a coincident response chromatogram. A unit experiment comprises a single chromatographic run. The unit experiment generates values of 1 or 0, depending on whether or not the subject detected an odour at a given retention index. Repeated runs of the unit experiment are summed such that there is a count of coincident detections at any index over the complete experiment.

Figure 2 shows the combination of individual experiments to produce a coincident response gas chromatogram. The areas of the peaks in this chromatogram are measures of the relative frequency that an odour was detected in a particular retention region. These frequencies can be used to investigate the properties of the sample (e.g. the occurrence of an odour at a particular retention index) or the behaviour of the subject (e.g. the subject's ability to detect a known compound).

The information contained in coincident response chromatograms is limited by the narrow range in which it provides quantitative results. For instance, odours well above the threshold produce a response every time

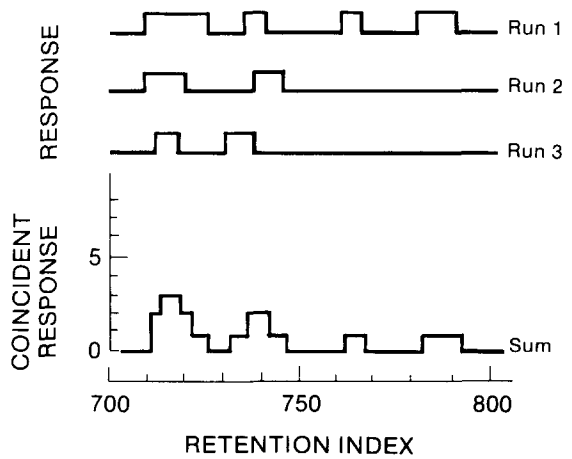


Fig. 2. A stylised coincident response chromatogram developed from simple addition of three sensory experiments.

and those well below the threshold never produce a response. Quantitative results are not produced in either case. Attempts to extend the range of the analysis by changing the concentration of the samples are confused by problems of comparison and the need for unrealistic repetition. These difficulties are eliminated when an approach based on serial dilution is used.

Charm response chromatogram

A more easily interpreted chromatogram is produced by considering the ratio of the total amount of odour-active compounds eluting at a particular index to the threshold amount for that same mixture of compounds. This is equivalent to the concept of Rothe's 'aroma values' (Rothe & Thomas, 1963), Guadagni's 'odour-units' (Guadagni *et al.*, 1966), and Mulders' 'odour values' (Mulders, 1973). The validity as well as the usefulness of these ideas were discussed by Frijters (1979).

The ratio can be estimated by performing repetitions of the analysis with the same sample but at successive dilutions. If the dilution process is continued until no odour is detected, and no response made, then an upper bound for the ratio, c, is a simple function of the dilution factor, d, and the number of coincident responses, n:

$$c = d^{n-1}$$

For a given retention index, c is equal to the ratio a_1/a_n, where a_1 is the amount of odour-active compound eluting from the most concentrated sample and a_n is that amount eluting from the most dilute sample producing odour response. This is clarified by the relationships

$$c = a_1/a_n = (a_1/a_2)(a_2/a_3)\ldots(a_{n-1}/a_n) = d^{n-1}$$

and for any value of n,

$$d = a_{n-1}/a_n$$

A charm response chromatogram is made by plotting c against retention index. The resulting peak areas are relative measures of the odour intensities of the substances eluting from the gas chromatograph in a particular region. The scale of measurement of these areas are referred to as charm and the scale used as the ordinate in a chromatogram is merely instantaneous charm at a given retention index. The name charm is taken from its common meaning '. . . a feature in something or someone

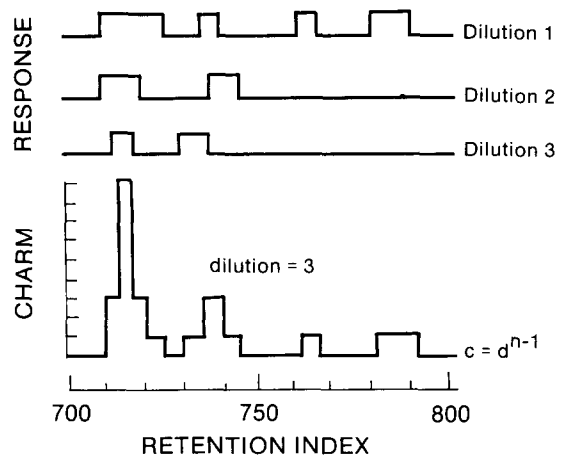


Fig. 3. A stylised charm response chromatogram produced from the relationship $c = d^{n-1}$, where d is the dilution constant and n is the number of coincident responses at any given index.

that attracts or delights people' (Webster's New World Dictionary, 1980). Figure 3 shows graphically how the procedure produces a charm response chromatogram. Listed at the bottom of Fig. 1 are definitions of the programmes used to collect, standardise, reduce, integrate, and produce plots of this data.

The charm algorithm yields a measure of sensory intensity free from the complexities caused by the psychological estimation of stimulus intensity as described by Moskowitz (1976). These intensities are determined independently as they are free of any interaction with components at other retention indices. Inhibition and synergism have been eliminated to the extent that the chromatographic column has separated the components of the mixture. Furthermore, the gas chromatographic process delivers each chemical completely volatised. Thus the areas of these peaks are a monotonic function of the gas phase odour detection thresholds of their constituents.

EXAMPLES

The use of charm is illustrated by first considering the problem of determining the relative odour impact of two isomers of methyl jasmonate. Commercial preparations of methyl jasmonate contain the

two epimers shown in Fig. 4, presumably near their thermodynamic equilibrium of 6% methyl epijasmonate. The problems of preparing pure samples of these epimers for use in sensory analysis was not trivial and required several chromatographic steps (Nishida, 1983. However, their gas chromatographic resolution is simple with a retention index separation of nearly 30 units on methyl silicone (OV101). Also shown in Fig. 4 is the charm response chromatogram produced from six successive three-fold dilutions of commercial methyl jasmonate in Freon 113 separated on OV101. Clearly, the epi-isomer produces most of the odour of the mixture even though it is only 5% of the total mass.

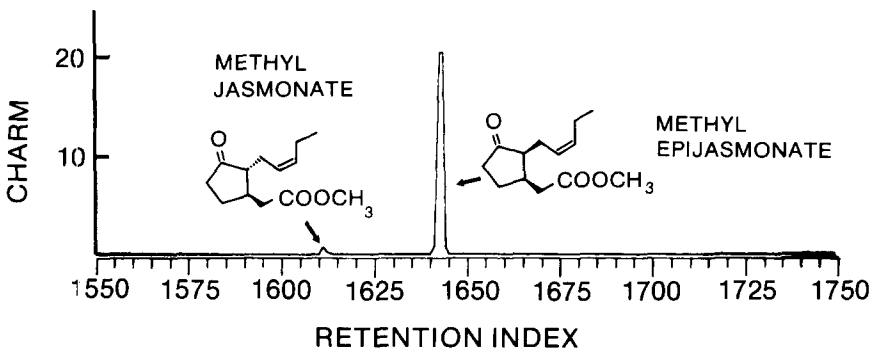


Fig. 4. A charm response chromatogram produced from six successive three-fold dilutions of commercial methyl jasmonate (30 μg/ml in Freon 133). Flame ionisation data indicated 6% methyl epijasmonate isomer.

Given that instantaneous charm is the ratio of the largest amount to the lowest detectable amount eluting at a given instant, and that composition of the peak remains homogeneous in odour-activity over the limits of integration, then the denominator of the ratio is a constant equal to the gas phase threshold amount. Note that homogeneity should not be confused with chemical purity since the peak can be composed of any number of components. However, the sensory nature of the peak must not change between the limits. Thus, integration simplifies to the following:

$$\int c \, \mathrm{d}i = \int a_1/a_n \, \mathrm{d}i = A_I/a_n = C_I$$

where A_I is the total amount of the odour-active components eluting at the mean retention index I. Dividing by the threshold amount, a_n, reduces A_I to the dimensionless odour-units, C_I, that we call charm.

The relative areas produced from the integration of the peaks in Fig. 4 are 3% for methyl jasmonate and 97% for methyl epijasmonate indicating that the latter has 33 times more charm than the former. Dividing the ratio of flame ionisation responses for the two isomers, which is equivalent to A_I/A_J, by the ratio of their charms, C_I/C_J, estimates the ratio of their gas phase detection thresholds, a_n/a_m, as shown in the following:

$$(A_I/A_J)/(C_I/C_J) = (A_I/A_J)/(A_I a_m/A_J a_n) = a_n/a_m$$

To understand these relations it is essential to grasp the notion that even though the number of detectable dilutions changes across a chromatographic peak, the minimum amount detected does not change. The ratio of the detection thresholds provided by the data in Fig. 4 for methyl epijasmonate to methyl jasmonate is 590. This compares well with

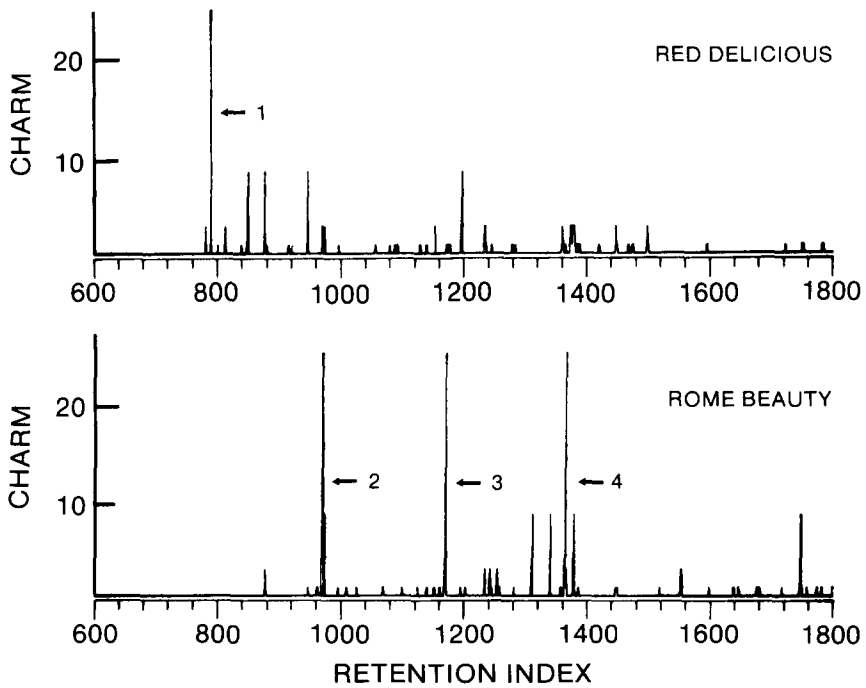


Fig. 5. Charm response chromatograms generated from Freon 113 extracts of Red Delicious and Rome apple juices under the conditions described in Table 1. Peak 1 can be associated with ethyl butanoate (index 789), peak 2 is unknown, peak 3 is due to hexyl butanoate (1168), and peak 4 is due to damascenone (1363).

the value of 440 determined for these compounds in an aqueous headspace using data from a sensory panel.

Another useful application of this methodology is as a bioassay in natural products chemistry. Consider the problem of determining which, among the hundreds of extractable components in apple juice, are the dominant odour contributors. Figure 5 shows charm response chromatograms produced from Freon 113 extracts of Red Delicious and Rome apple juices. It appears that there is one dominant region in Red Delicious and that it is located at an index of 789. This corresponds to ethyl butanoate, a component that has been identified in Red Delicious by Flath *et al.* (1967). In contrast, Rome shows no response in this region but shows response in three others. Red Delicious and Rome are in this sense, complementary in their charm responses.

Areas of charm can be organised into a priority list. Known volatiles

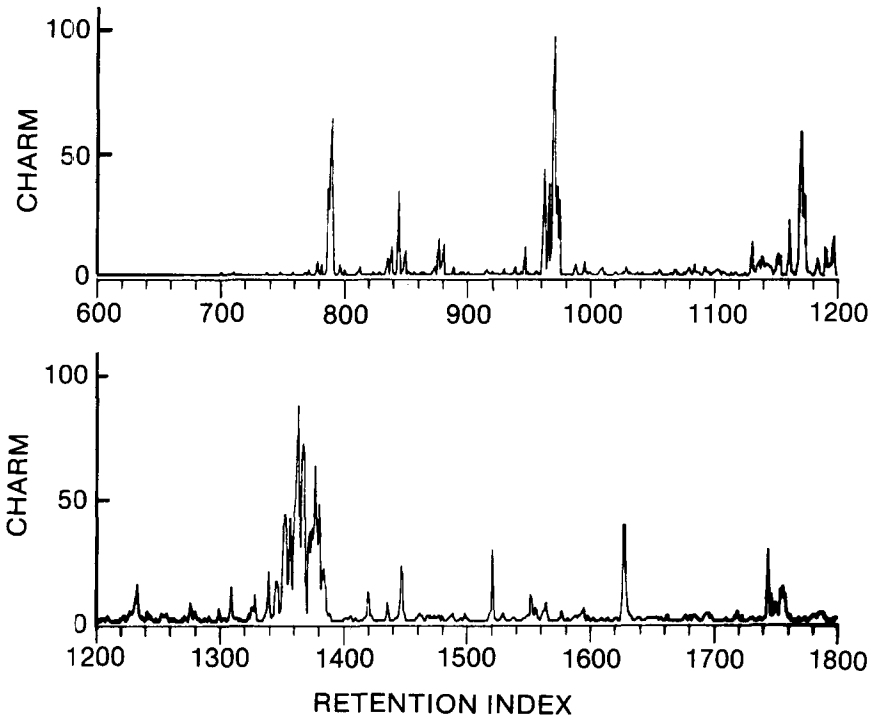


Fig. 6. A composite charm response chromatogram of the extractables in the ten most frequently marketed apple cultivars from New York State. The extracts were produced as described in Table 1.

can be associated with this list and confirmed by appropriate analytical procedures. There remain unknown odour components which will require elucidation. An example of this approach, shown in Fig. 6, is a composite charm response chromatogram from the extracts of ten different apple cultivars. Remarkably, only 50 % of the 20 most 'charming' regions of this chromatogram could be associated with known apple volatiles (Cunningham, 1983). Present studies are directed at the remaining unidentified components.

DISCUSSION

The use of charm in the methodology of sensory analysis formalises the process of sniffing gas chromatographic effluents. The concept of charm was systematically constructed from the idea of odour values. When applied to the identification of natural products it summarises biological activity by means of compact odour profiles and quantitative results.

Charm response to an extract of food will differ from sensory response to the food itself for several reasons. Foremost is that the volatility of each component in a gas chromatographic effluent is 100 % whereas in a food matrix the compounds often have entirely different volatilities. Examining headspace volatiles can, in some instances, avoid these problems. For example, the analysis of headspace volatiles is used to produce a priority list of the chromatographic regions with the most charm. Although this method of volatile collection seldom produces enough isolate for facile identification, extraction and other appropriate isolation schemes could then be used to characterise the components with the most charm.

Designed as a bioassay procedure for the characterisation of odour-active components in gas chromatographic effluents, charm is founded in the measurement of the relative gas phase detection thresholds of individual chemicals. Thus, it provides results which are free from the complexities of a combined sensory response whether the interactions which produce these complexities occur at the receptor level or higher. Perhaps an understanding of how the perceptual system integrates simultaneously the responses of many receptors into a single impression will come from the rationalisation of charm analysis with classical sensory analysis.

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