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50 Years of Mass Spectrometry at Firmenich: A Continuing Love Story

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Abstract: Mass spectrometry (MS) has been intensively used in the field of flavor and fragrance since its beginning in the 1950s, and it remains an essential technique for current and future research in this field. After a short historical section on the introduction and development of MS at Firmenich, this work reviews the main applications of MS-based techniques published by Firmenich researchers over the past 5 years. It exemplifies the use of gas chromatography (GC)–MS for the discovery of new odorant – hence volatile – molecules in a broad range of natural products, such as fruits, meats, and vegetables. Non-volatile compounds play a major role in taste attributes and are also possible precursors of odorant molecules. Their identification by liquid chromatography (LC)–MS in the context of malodor generation from sweat is a typical example of such a relationship. With their high selectivity and sensitivity, GC–MS and LC–MS instruments are used in the fields of flavor and fragrance not only for identification, but also as unique tools for the accurate quantitation of compounds in complex matrices. This is particularly important for regulatory analyses such as dosage of potential allergens in perfumes and for the development of delivery systems. Finally, because of the rapid response time of MS, the kinetics of processes such as the release of flavors in the mouth during food consumption can be monitored by direct sampling into the mass spectrometer.

Keywords: Direct sampling MS · Flavor · Fragrance · GC–MS · LC–MS

Analysis of natural products has always been a major field of research in the flavor and fragrance industry in general and at Firmenich R&D in particular. Volatile compounds are the essence of perfumes and similarly have a major role in the flavor of foods. Therefore, analysis of volatile compounds has long constituted the main interest of the R&D analytical experts at Firmenich. In this context, any technique that helps identify unknown constituents is of critical importance. Hence, the emergence in the late 1950s of mass spectrometry (MS) for structure elucidation immediately raised the interest of Firmenich scientists. As a result, from a historical perspective, Firmenich played a significant role in the development of MS in Switzerland and beyond, as testified by the pioneering work of Klaus Biemann[1] from the Massachusetts Institute of Technology. His work was supported by Firmenich, which subsidized the purchase of Biemann's first mass spectrometer in 1958. In the early 1960s, the company was among the first in Switzerland to be equipped with such an instrument, with the first mass spectrum (which was ethyl methyl sulfide) recorded in September 1961 on an Atlas MAT CH4 mass spectrometer (Fig. 1). MS quickly delivered on its promises, allowing, for instance, the identification of Furaneol in 1965,[2] which today is still among the top ingredients sold by Firmenich. Later, in 1981, Dr. B. Willhalm, on behalf of Firmenich, co-signed the founding document for the creation of the Swiss Group for Mass Spectrometry (SGMS).

The next decade was characterized by the introduction of gas chromatography–mass spectrometry (GC–MS) instruments, which brought another dimension to the identification of unknown volatile compounds. During this Golden Age, hundreds of new chemical structures were elucidated, leading to numerous publications, and, most important, to a proprietary library of electron impact (EI) mass spectra containing over 120,000 entries. This library increases by about 4,000 spectra each year, which, in addition to other commercial libraries, constitutes a unique asset for all Firmenich analytical scientists. Indeed, nowadays, single quadrupole GC–MS instruments are still the most common mass spectrometers that colonize our laboratories. They have become routine instruments used daily for aroma analysis of many foods, essential oil composition studies, quality control analyses, and regulatory quantitative analyses. The ionization technique that is most often used is still EI. Chemical ionization (CI) is also used for molecular weight confirmation. Single quadrupole MS instruments are now more and more hyphenated to two-dimensional GCs or are combined with GC–olfactometry, where the human nose is used in parallel with MS to identify the most powerful odorants within a food or a plant extract. Beyond single quadrupoles, new generations of MS instruments appeared in Firmenich R&D laboratories over the last five years: high-resolution time-of-flight (TOF)-MS and triple quadrupole GC–MS. These instruments have found many applications in the elucidation of unknown

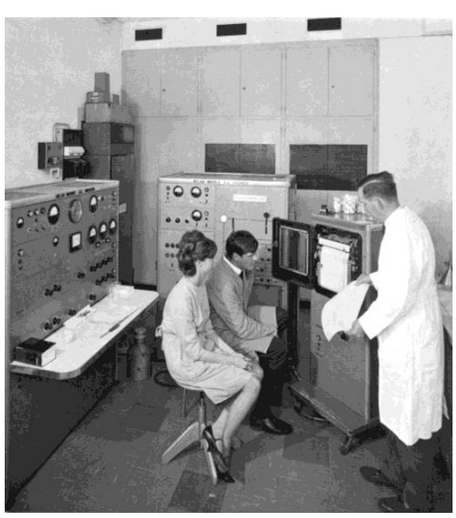

Fig. 1. ATLAS CH4 mass spectrometer on the Firmenich premises, with B. Willhalm standing on the right.

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chemicals or complex and sensitive quantifications.

For the past 20 years, the study of non-volatile products has no longer been overlooked. Non-volatile products give foods their taste. In homecare or body care products, fragrance interacts with many ingredients of the client's product. Perfume precursors or capsules are composed of non-volatile chemicals that need to be analyzed as well. In 1996, Firmenich acquired the recently launched *Thermo LCQ,* a benchtop ion trap MS instrument that 'democratized' access to LC–MS capabilities. Since then, many other LC–MS instruments have been routinely used, such as LC–TOF for structural identification and LC triple quadrupoles for quantification, similarly to volatile analyses. Firmenich's latest acquisition is the *Thermo* Q-Exactive Plus, a quadrupole-orbitrap MS coupled with a next-generation ultra-high-pressure liquid chromatography (UHPLC) instrument. This instrument will be used primarily for natural products analysis. The accurate mass will allow automatic searches in large chemical databases (ChemSpider, PubChem) which will help in the identification of previously known compounds. The structural elucidation of unknown compounds based on high-resolution full scan and MS/MS spectra will be eased. For targeted analyses, the high resolution will enhance the capabilities of quantifying trace products in complex matrices.

Interestingly, for about the last 20 years, MS instruments have no longer been located in a central MS laboratory but are spread across many laboratories. In addition, both GC–MS and LC–MS instruments are often found in the same laboratories, so that researchers can combine the two techniques and analyze both the volatile and the non-volatile parts of the same food or plant as a whole. A striking example of this possibility is the study of the biological origin of an odor (see below).

In the following sections, we describe examples of the uses of GC–MS, LC–MS and direct-sampling MS techniques in our field of flavor and fragrance. We have selected only recent examples, most of which have been published by Firmenich R&D over the past 5 years.

GC–MS

The analysis of plants and food extracts or of essential oils with the aim of discovering new molecules not previously described in a natural product is still an important activity in the flavor and fragrance industry. With most of the major compounds already known, however, new structures are associated with trace constituents. In this case, MS is the main technique that is sensitive enough to provide structural information. The analyst generally gathers all MS information available from a single quadrupole GC–MS such as the molecular ion in EI or in CI and the EI fragments. The use of high-resolution TOF-MS greatly facilitates interpretation, since it can give both the molecular formula of the compound and also that of the EI-MS fragments, thereby facilitating the understanding of fragmentation. For an unknown trace compound, the measurement of reliable chromatographic data, such as linear retention indices on non-polar and polar GC columns, can also be performed with confidence by GC–MS and these data are useful in structural elucidation. When combined with selective chromatographic isolation techniques, GC–MS can be extremely sensitive and selective. Thus, Naef *et al.*[3] were able to isolate a thiol fraction of bell pepper by using an affinity column that was based on mercury benzoate. The analysis of the extract by single quadrupole GC–MS led to the identification of as many as 19 new sulfur compounds, which were subsequently synthesized. Sulfur compounds are among the most powerful odorants in many foods and their identification is important for understanding the aroma. Similarly, Frérot *et al.*[4] identified sulfur compounds in petai, also called stink beans, used in Thai cuisine. The use of high-resolution GC–TOF-MS helped in the identification of many cyclic polysulfides.

In their analysis of finger lime, a rare citrus fruit from Australia, Delort and Jaquier[5] used a traditional GC–MS technique. Since terpenic compounds are known to possess similar mass spectra, particular care was taken in measuring retention times, more exactly linear retention indices, and six new terpenyl esters were identified. Similarly, Perry *et al.*[6] determined the aroma composition of honeydew melon by using classical GC–MS. They developed a GC–MS–MS method to help register a new flavor ingredient, namely, (*E,Z*)-2,6-nonadienyl acetate, which was previously unknown in natural products. As it turned out, CI in the negative mode was the most sensitive technique and the compound could be unambiguously shown to occur in the melon fruit.

When classical GC–MS and high-resolution GC–TOF-MS cannot provide undisputable identification of unknown compounds, the analyst can use his or her skill in synthetic organic chemistry. During the analysis of cooked chicken, Delort *et al.*[7] were faced with an unknown minor compound presenting the same molecular formula, $C_{10}H_{14}O$ (150 Da, four insaturations), and a very similar EI-mass spectrum as 2,4,7-decatrienal but with different GC retention times. A diluted solution of the pure product was obtained by normal-phase HPLC. The solution was submitted to two microreactions followed by GC–MS analysis. Sodium borohydride added 2 Da to the molecular ion, suggesting the presence of a carbonyl group. Subsequent catalytic microhydrogenation showed the presence of a compound having a mass of 156 Da that could be measured only in negative CI–GC–MS. This demonstrated the presence of one ring, and the product could be identified as (*E*)-3-(2-ethylcyclopent-1-en-1-yl) acrylaldehyde, as shown in Fig. 2.

Similarly, Frérot and Bagnoud[8] used a systematic chemical synthesis approach to identify all of the unsaturated γ-lactones in butter oil.[9] GC–MS and GC–TOF-MS

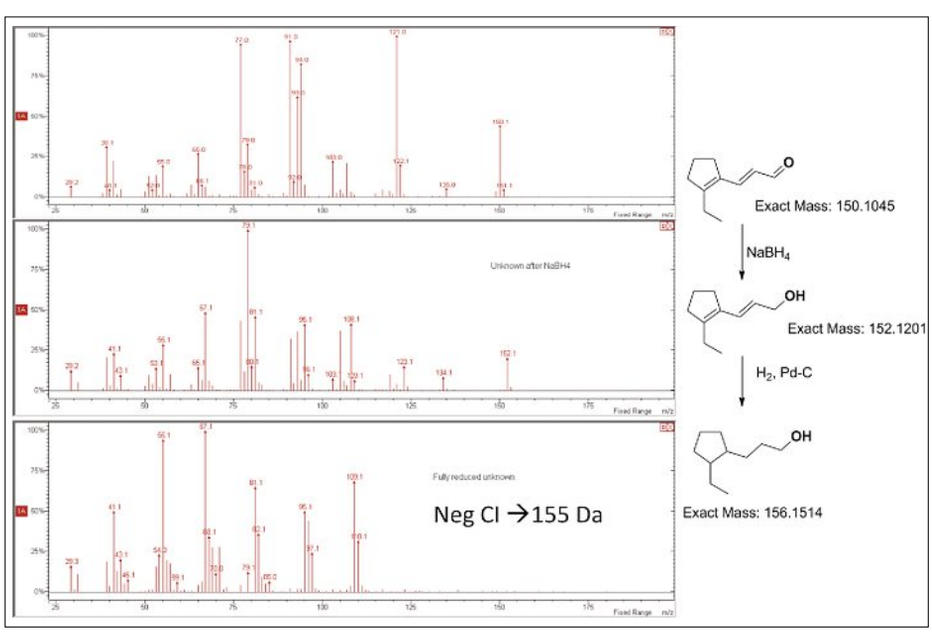

Fig. 2. Identification of (*E*)-3-(2-ethylcyclopent-1-en-1-yl)acrylaldehyde in chicken by microreactions and GC-MS analyses.

were not sufficient to obtain the position of the double bond in a few products.

Rochat *et al.*[10] used a combination of GC–olfactometry and comprehensive two-dimensional GC–MS techniques[11] to identify the key odorants in shrimp. TOF-MS was used as the detector since it is able to scan at the acquisition speed required by GC × GC chromatography.

Other analyses using GC–MS and recently published by Firmenich cover a broad range of products, including beer fermentation with an innovative and home-made automated fast sampling device,[12] latrine malodors,[13] urine,[14] oyster leaves,[15] and human milk (the latter in collaboration with the University of Erlangen).[16]

Protecting the health of consumers and the environment is of major concern for flavor and fragrance companies. GC–MS also plays a central role in regulatory analyses. In fine fragrances, for instance, it is mandatory to determine whether the content of some ingredients that have been identified as potential allergens is below or above 10 ppm. As fragrance oils usually contain more than hundred constituents, the selectivity and sensitivity of GC–MS is required. In a collaborative study, Chaintreau *et al.*[17] proposed a method for the determination of twenty-four suspected allergens. The work showed that the method gave similar results with different GC–MS instruments.

In homecare applications (washing powders, *etc.*) and body care products (soaps, shampoo, *etc.*), most of the perfume ingredients end up in the environment as diluted aqueous solutions upon the use of such products. In this context, their biodegradability, persistence in the environment and bioaccumulation potential must be assessed before they can be used at large scale and monitored accurately. In particular, the recent REACH regulation imposes strict policies, and the rule 'no data; no market' is a decisive incentive for companies producing or importing chemicals in the European Community. GC–MS and LC–MS are unique tools to provide robust data for understanding the fate of perfumery chemicals in the environment. For instance, Begnaud *et al.*[18] have developed a method based on thermodesorption with stable isotope dilution to accurately quantify musks and other typical perfumery ingredients in waters from the aeration tank of sewage plants. The method was fully validated with a limit of detection of 1 to 25 ppb, depending on the compound.

Recently, Frérot *et al.*[19] developed a GC–MS–MS method for the quantification of the malodorous compounds hydrogen sulfide and methyl mercaptan. They used a solid-phase microextraction method with in-fiber derivatization. The concentration factor that they achieved, combined with the sensitivity of the triple quadrupole GC–MS in the multiple reaction monitoring mode, made this method nearly as sensitive as the human nose for these two compounds.

LC–MS

Sulfur compounds are potent odorants with positive attributes, such as in fruits and wines, but are also malodorants, such as in sweat. In particular, Troccaz *et al.*[20] used GC–MS to discover 3-mercapto-3-methylhexan-1-ol (transpirol) as a stinking component of human sweat. Incubation of non-volatile fractions of sterile sweat with underarm bacteria was carried out to identify which products release transpirol. LC–MS analyses led to the elucidation of its precursors, the cysteine conjugates (Fig. 3).[21] Later, Starkenmann *et al.*[22–24] demonstrated the role of thiol conjugates in the oral perception of fruits and vegetables. In particular, they showed that naturally occurring, odorless cysteine-S-conjugates, such as S-(*R/S*)-3-(1-hexanol)-L-cysteine in wine, S-(1-propyl)-L-cysteine in onion, and S-((*R/S*)-2-heptyl)-L-cysteine in bell pepper, are transformed into volatile thiols in the mouth by microflora. This very important piece of work on odorous thiol precursors was greatly eased by the availability of both GC–MS and LC–MS capabilities in the same laboratory.

Frérot and coworkers[8,25] also enjoyed using both instruments during the analysis of butter oil when both the volatile and the non-volatile part could be analyzed to assess their respective role in the perception of creamy and fat nuances.

Metabolomics is another domain in which the combination of both volatile and non-volatile analyses by MS is pertinent. With such an approach, one study carried out in collaboration with Prof. J. L. Wolfender and S. Rudaz[26] at the University of Geneva combined the information provided by GC–FID/MS and UHPLC–TOF-MS to successfully discriminate and classify cold-pressed lemon oils according to their geographic origin and their production processes.

Other studies have been performed with the aim of finding new taste-active ingredients by investigating the non-volatile part of many savory foods using UHPLC–MS instruments (Fig. 4). Taste-active derivatives of glutamic acid such as succinyl-Glu were recently discovered in soy sauce and quantified using a triple quadrupole UHPLC–MS.[27] Strombine was shown to be the sweet, umami principle of dried scallop.[28] A very sulfury-smelling vegetable consumed in China, *Toona chinensis*, was analyzed by UHPLC–MS, and a rare norcysteine derivative was discovered and shown to be the precursor of a volatile sulfur-containing odorant molecule.[29]

In perfumery, which is the realm of volatile molecules, HPLC–MS may seem to be a technique of lesser importance. However, in various homecare or body care products, the perfume needs to be micro-encapsulated in polymers and HPLC–MS finds many applications in this field. Thus HPLC coupled with a quadrupole-linear ion trap instrument (Q-Trap) was used by Jacquemond *et al.*[30] to quantify various residual isocyanate monomers in polyurea capsules slurries. Derivatization of the reactive and unstable isocyanate groups by dibutylamine allowed a sensitive and reliable quantification of the monomers and of the small residual oligomers that failed to fully polymerize. This analysis helped researchers to understand the outcome of the polymerization reaction and to assess the efficiency of the scavenging of unhealthy residual isocyanates by ammonia. In another study, the same instrument was used to quantify encapsulated tea polyphenols.[31]

Direct Sampling MS

The discriminating power of mass spectrometers combined with their extreme sensitivity and rapid response time enables the analysis of mixtures in real

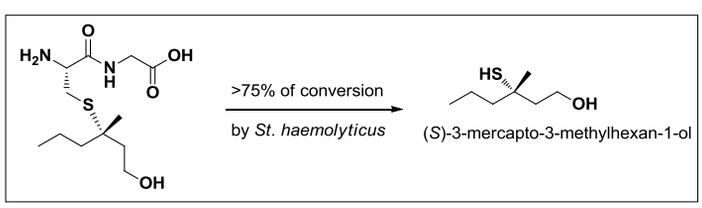

Fig. 3. Generation of sweat sulfury odor by the underarm microflora.

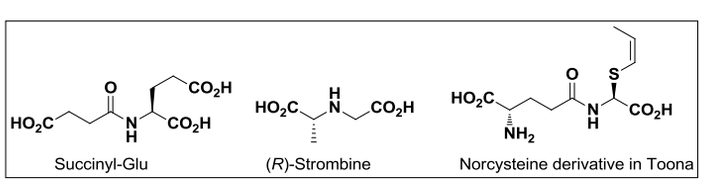

Fig. 4. Taste-active compounds in soy sauce, scallop, and toona.

time. By omitting the chromatographic step, it is possible to achieve a time resolution of less than seconds and hence to monitor the kinetics of processes such as the release of different constituents of a flavor in the mouth during food consumption. With this approach, based on techniques such as atmospheric pressure chemical ionization MS (APCI-MS), proton transfer reaction MS (PTR-MS), or selected ion flow tube MS (SIFT-MS), the analytes are directly transferred from a gaseous sample such as the breath into the spectrometer source with a specially designed interface that is usually directly connected to the nose of a panelist. Initially developed by A. Taylor at Nottingham University, the APCI-MS technique has been implemented, improved, and heavily used at Firmenich for more than a decade to characterize and optimize the delivery of flavors during eating.[32,33] As shown in Fig. 5, the selectivity, fast response time, and high sensitivity offered by the mass spectrometer are critical in monitoring an individual constituent of the flavor mixture, breath by breath, without preconcentration.

Direct analysis is not limited to gaseous samples. Several techniques have emerged during the past decade that allow direct analysis of complex solid or liquid samples, such as DART, DESI, DAPCI, JEDI, ESSI, DESSI, LDI, and ESTASI to name a few by their acronyms. The detailed principle of each technique is beyond the scope of this publication, but the first two are the most established. Direct analysis in real time (DART) was introduced by IonSense, Inc., in 2005. It allows analysis of solid, liquid, or gaseous samples at atmospheric pressure and ground potential by simply placing them between a DART® ion source and a mass spectrometer. The analytes are ionized by a gun that provides a beam of neutral metastable species. In June 2006, we had the opportunity to conduct an evaluation of the IonSense DART® interface. The results obtained[34] prompted us to acquire this interface. After the development of a new home-made probe that improves the reproducibility as well as the sensitivity by a factor of 3 to 4, the technique was used for business-relevant applications, such as measurement of the release kinetics from chewing gums into the saliva of a free versus encapsulated cooling agent.[35]

Future trends of relevance for our needs and applications continue to be in the direction of more selective, more sensitive, and more robust instruments. We are also following with much expectation the developments of miniaturized mass spectrometers that we hope will allow us, without compromising performance, to carry out at-line and field analyses. These new instruments will also improve the sustainability of MS in terms of power consumption, heat generation, noise, and footprint. Finally, data generation and data treatment have not been addressed in this review, but any improvement of their user-friendliness and automation is of prime importance for current and future uses of MS at Firmenich.

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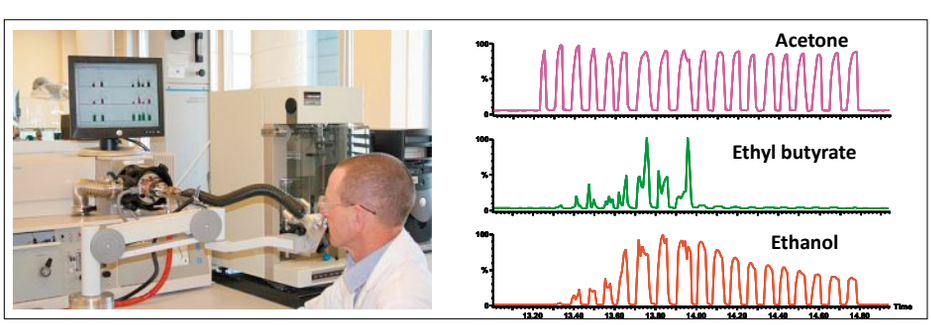

Fig. 5. APCI-MS setup and volatiles in the breath during the consumption of a gelatin gel. Acetone (top) is normally present in the breath as a result of metabolic activity in the liver. Ethyl butyrate (center) is released as the gel melts. After the final swallowing action, the volatile concentration in the breath declines rapidly. Ethanol (bottom) is also released as the gel melts, but persists in the breath because of its solubility in the mucous membranes.

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