

Beverage profiling rethought - thanks to SICRIT® "electronic nose" technology

The analysis of beverages is a relevant subdiscipline of food analysis. In addition to classic residue control, ensuring product authenticity is becoming increasingly important. At the same time, increasing digitalization and automation in food production require the inclusion of relevant analytical data to optimize and document processes. Plasmion GmbH's ion source technology can make an important contribution here for the universal and broad use of mass spectrometry in these areas of application.

The analysis of beverages is an important part of food analysis. Chemical analysis covers a wide range of tasks that encompass all aspects of quality assurance. Thus, both the desired ingredients, i.e. minerals, vitamins, or flavors, must be analyzed and the raw materials and end products must be tested for foreign substances and contaminants. An increasingly important issue are the numerous counterfeit products, which make it necessary to determine their authenticity, e.g., on the basis of characteristic chemical markers. For this reason, and in terms of digitalization and customer awareness in the food sector, the requirements for continuous monitoring of the supply and production chains are increasing.

To meet these demanding tasks, food analysis uses a wide variety of instrumental methods such as IR spectroscopy and gas or liquid chromatography. However, when it comes to the taste or odor of food, human-sensory olfactometry is also an important component of quality control or recipe optimization. Instrumental-analytical measurement data can also make an important contribution to ensuring product quality or early detection of product defects and help to significantly increase the reliability of previous measures.

Mass spectrometry in food analysis

In addition to spectroscopic and purely chromatographic methods, mass spectrometry-based detection in standard and special beverage analysis is well established in routine laboratory work and, due to its depth of information, represents the upper end of instrumental laboratory measurement methods. For example, LC/MS methods are used to determine maximum levels of residues such as pesticides or polyaromatic hydrocarbons (PAH) that must be complied with by law. Especially for beverages, aroma profiling and the determination of off-flavor components are tasks that are often realized by GC/MS methods.

The disadvantage of mass spectrometry-based methods, however, in contrast to optical methods, is that a sample must always be taken for an analysis and then fed to the measuring instrument. Samples are therefore usually collected at specific times during the manufacturing process and processed in the laboratory at different times (at-line analysis). This means that questions requiring a prompt analysis result can hardly be answered using standard LC/MS or GC/MS.

The innovative concept of SICRIT® technology

The approach taken by SICRIT® (soft ionization by chemical reaction in transfer) technology, on the other hand, opens up new fields of application for mass spectrometric analysis, especially with regard to rapid analysis and flexible sampling. SICRIT® ion source technology addresses the interface between sampling and measurement in the mass spectrometer: While in both GC/MS and LC/MS the sample measurement is performed methodically and instrumentally in a combination of chromatographic separation of the analytes and mass spectrometric detection, the SICRIT® technology decouples the detection from a given separation technique and thus enables flexible feeding of the sample to the mass spectrometer. This is made possible by the ionization principle used. Ionization takes place directly in the extension of the atmospheric pressure inlet system of LC mass spectrometers, i.e. the gaseous sample is drawn in and ionized on its way into the detector. The ionization itself is based on cold plasma ionization, which can be operated in various carrier gases such as nitrogen or CO₂, but

also in room air. As a result, each LC-MS instrument is thus converted into an “electronic nose” capable of directly ionizing the gas phase in front of the source and analyzing it in real-time. An important feature of the ionization method is its broad and soft ionization spectrum, which usually leads to the formation of the protonated molecular ion and allows identification based on molecular mass. This is particularly advantageous when the ion source is coupled with high-resolution mass spectrometry (HRMS), where the exact mass can be accurately determined. The flow-through principle outlined above not only allows online screening without the need for chromatographic separation, but also flexible coupling with chromatographic separation techniques. This offers the unique possibility of implementing previously separate LC/MS and GC/MS routines on a single instrument, combining the advantages of the high separation performance of GC with the high resolution and sensitivity of modern LC-MS instruments.

Application scenario: Residue control with flexible sample preparation

What the combination of GC and high-resolution mass spectrometry (HMRS) can be used for is shown in Figure 1 a) using the example of saturated hydrocarbons. The detection of hydrocarbon compounds (MOSH/MOAH) is also coming into focus in the field of food safety and represents a complex analytical issue that cannot be fully answered by the established LC-GC-FID approach. The fragment-free ionization of even nonpolar molecules by SICRIT[®] allows the unambiguous assignment of an n-alkane standard mix (chain lengths C8 to C20) based on the exact molecular mass, thus circumventing the problem of similar fragment spectra as encountered with alternative GC-EI-MS analysis.

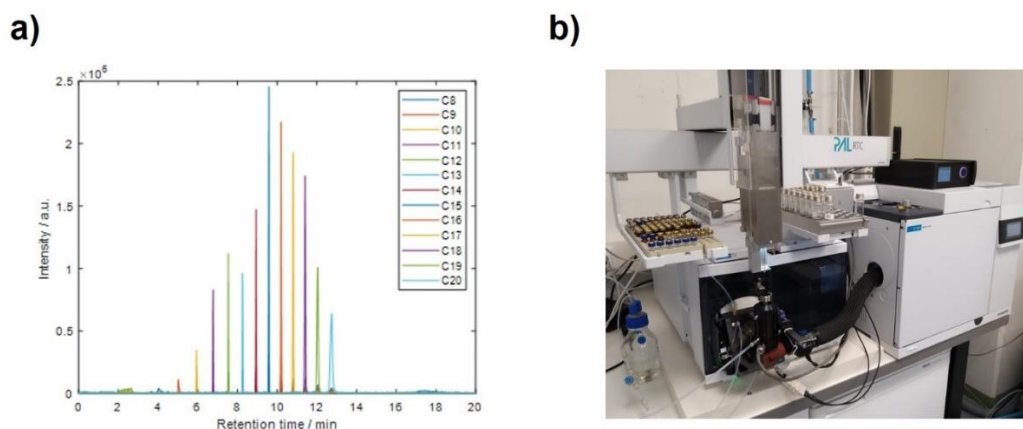


Figure 1:a) Reconstructed EIC-chromatogram of n-Alkanes C8-C20 based on molecular ions in positive mode. 1b) Laboratory setup for sample analysis using SICRIT[®]-MS including autosampler and gas chromatography (CTC PAL RTC, Agilent Ultivo TQ, Agilent 8860 GC).

For many analytical problems, however, the decisive advantage of flow-through ionization lies in the possibility of performing rapid analyses with high sensitivity even without chromatographic separation. In this case there are various sampling methods. In addition to the simplest option of detecting volatile components (VOCs) of a solid or liquid sample by holding it directly in front of the source, small amounts of liquid can be evaporated and analyzed directly via a heated injector module, for example, or commercially available SPME fibers can be heated directly in front of the source and analytes enriched from real samples can be desorbed. Figure 1 b) shows a laboratory setup combining automated sample preparation, a direct injection system and - optionally - GC coupling with a triple quad LC mass spectrometer. In a project together with CTC Analytics AG (Zwingen, CH), the potential of automated sample enrichment using SPME (solid phase microextraction) fibers in combination with SICRIT[®]-MS was investigated at ETH Zurich. In particular, the broad ionization spectrum of the SICRIT[®] ion source proved to be advantageous here, since in addition to pesticides, which could be determined in the ppt range in grape juice, nonpolar PAHs could also be quantitatively detected in the ppb range without chromatographic

separation. [1,2] With regard to residue analysis and the analytical sensitivity required for this, SICRIT®-MS technology provides sufficient instrumental detection limits even without the temporal focusing provided by chromatography. For example, phthalates can be detected in the lower ppb range when injected directly from standard solutions. Such a rapid method, with analysis times well below one minute, would thus be well suited for rapid estimation of plasticizer contamination in beverages, for which EFSA proposes an indicative limit of 50 µg/kg for the definition of medium contamination. It could be used e.g. for pre-screening to reduce the amount of samples that are given to routine GC/MS analysis, thus increasing efficiency and significantly reducing costs.

Application Scenario: Direct Headspace Sampling for Classification

The depth of information that can already be achieved with simple headspace sampling in combination with high-resolution mass spectrometry is shown by the experimental data from a study conducted by Prof. Schmitt-Kopplin's Comprehensive Foodomics group at the Technical University of Munich (TUM) at the Helmholtz Zentrum München. Here, different white wine varieties were analyzed for their characteristic markers, i.e., their molecular fingerprint. Such molecular information can be used both to make assessments about optimal production or aging, and to determine authenticity. In the tests, three different white wines were compared, specifically the grape varieties Pinot Blanc, Sylvaner and Riesling. The measurement data were recorded on a high-resolution FT-ICR-MS (Fourier transform ion cyclotron resonance) mass spectrometer in positive mode and the measurement signals were assigned to molecular formula. In the previous work of the group, the signature of wines and historical champagne finds was analyzed by direct infusion of diluted samples in combination with conventional electrospray ionization (ESI).[3] With this measurement technique, more than 4000 components could be determined, which could be used for the classification of the wines. However, the very volatile components, which are important for the aroma of the wines, are hardly covered. It was precisely these components that the measurement of undiluted wine samples using the SICRIT® ion source was aimed at. By analysis of the sample headspace, the signals could be assigned to the most important aroma substance classes such as esters, phenols, fatty acids and monoterpenes. In total, more than 230 volatile components could be determined from each of the wine samples using this very simple method of sampling, and a good 170 of these were VOCs that could be detected using SICRIT® ionization alone (see Figure 2 a).

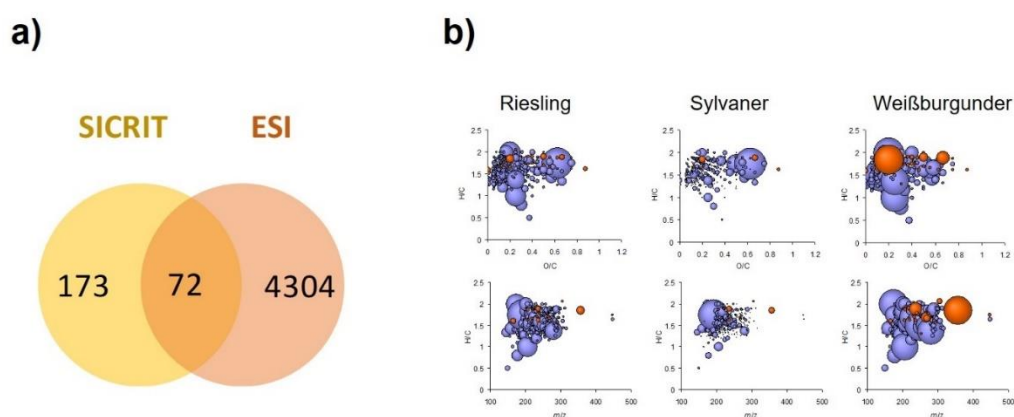


Figure 2: 2 a) – Illustration of the complementary molecular information that can be obtained from the wine samples by SICRIT® headspace measurement. 2 b) van Krevelen (top) and H/C vs. m/z diagrams for Riesling, Sylvaner and Pinot Blanc analyzed with (+) SICRIT® FT-ICR MS. Shown in blue are compounds of the elements CHO, in orange compounds of the elements CHON.

This shows that a broader view of the constituents of complex samples is possible via the complementary approach of headspace analysis. This applies to the odor-forming VOCs, which are most important for the sensory perception of the wine consumer. Figure 2 shows the results of the headspace measurements graphically. Although the volatile signature of the aromas contained in the wines is very similar, even small differences can

be characteristic. For example, Pinot Blanc has some nitrogen-containing compounds that were not detected in the other two varieties and shows more VOCs overall with higher masses. Together with the information resulting from the non-volatile components, a comprehensive profile can thus be established, which can provide information on the aroma diversity, the presence of off-flavors, pesticide residues or even on the geographical origin and the growing conditions.

Application scenario: Simple online monitoring of production processes.

Particularly in the case of beverages that exhibit a high diversity of flavors, the production process is critical and natural differences in the starting materials or small deviations in the production process can have a major impact on the taste of the final product. Fermentation or roasting processes have a decisive influence on the flavor profile and require product quality control. In coffee roasting, for example, test roasting and subsequent tasting is an indispensable part of quality management to optimize roasting profiles and document the flavor profile. However, human sensory quality control, which is often used in food production, requires extensive training of the testers and is difficult to standardize. Therefore, approaches to provide an "electronic nose" for food sensory analysis using instrumental analytical methods have been tested repeatedly in the past. Here, the SICRIT[®] ion source offers the possibility of continuous in-process monitoring based on mass spectrometry. Advantageously, cold plasma ionization does not require special carrier or reactant gases and the flow-through principle allows easy implementation of MS analysis into the process (in-line analysis). Figure 3 shows a laboratory experiment in which the ion source was overflowed with the exhaust air of a coffee roaster and the roasting processes taking place could be monitored in real-time using high-resolution mass spectrometry. The chemical changes during the heating of the beans, which causes them to break open, is clearly visible. This releases flavoring substances, which then undergo Maillard reactions, for example. Such a real-time measurement is also conceivable for use in brewing or distillation processes. Another possible application of direct measurement is the simple realization of (online) migration studies, another relevant area of food quality, in order to detect or prevent the transfer of odor-active or harmful ingredients from packaging into the food.

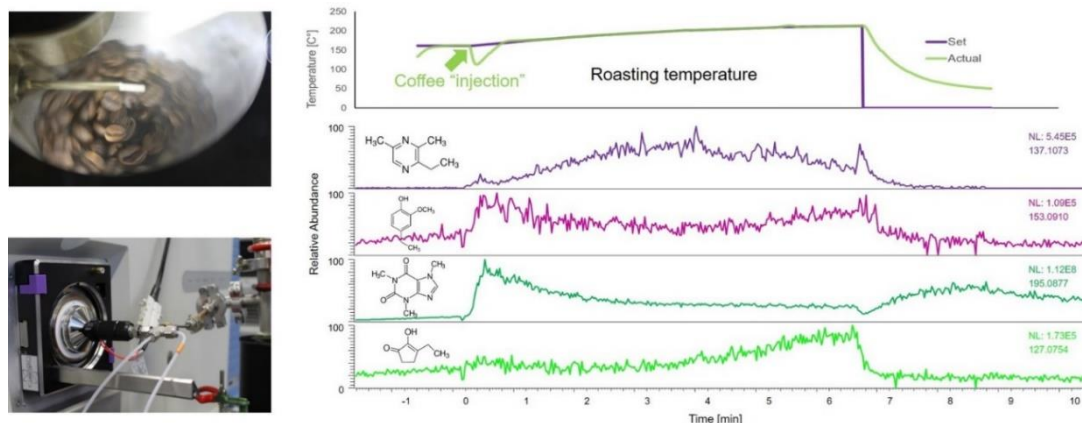


Figure 3: Online monitoring of the roasting process of coffee beans using SICRIT[®]-HRMS. During roasting, aroma substances are released and, in some cases, undergo reactions. The chemical processes can be monitored in real-time.

New applications of mass spectrometry in the food sector

The novel ion source design thus combines advantages that enable a wide range of application scenarios that are impossible or difficult to realize with classical GC/MS and LC/MS approaches. In particular, the ability to perform real-time measurements without the need for chromatographic pre-separation can be used for both rapid qualitative screening and online monitoring for volatile compounds. The concept of flow-through ionization clearly differs from the approach of the DART (Direct Analysis in Real Time) MS, which was also developed for

real-time screening. On the one hand, SICRIT® ionization does not require a noble gas for plasma generation, and on the other hand, ionization takes place at room temperature, which eliminates thermally induced measurement artifacts as with DART. Due to the simple combination with sample preconcentration techniques such as SPME or chromatographic techniques such as GC or LC, SICRIT® additionally offers the possibility to obtain detailed or complementary information from a sample by means of different approaches and thus to answer even complex questions.

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References

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