White Paper

Application of GC-MS/MS in the Analysis of Taints and Off Flavours

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Introduction

Taint and off-flavour compounds can be present in food and drinks causing an atypical smell or taste. Extreme examples can influence the consumer’s perception of a product and can lead to product recalls. These compounds are often present at very low levels that traditional gas chromatography-mass spectrometry (GC-MS) based methods can struggle to detect and identify.

Fortunately developments in mass spectrometry technology have meant that instrument sensitivity can be significantly increased. This technology has been assessed to determine whether it can play a role in supporting the food and drinks industry in the investigation of taints and off flavours.

We assess GC-MS/MS as a tool to identify a number of the common tainting compounds found in the food and drinks and compare this to traditional GC-MS, to determine whether an increase in sensitivity can be seen, and in turn address an old age old problem

MS/MS is used to fragment specific ions in the compound of interest, generating a product ion which should be free of interferences form the matrix and thereby increasing sensitivity.

Taints and Off Flavours

Taint compounds are contaminants which are not intended constituents of the product and can originate from a variety of sources, which include chemicals that are transferred from packaging, storage bags, wooden pallets, shipping containers, storage facilities and corks of bottles. Moreover the use of cleaning products near food production can also be a source. Typical taints cause musky, earthy or even medicinal smells. Compounds from a number of chemical classes, most commonly halophenols, and haloanisoles are responsible. In addition, pyrazines, geosmin and 2-methyl-isoborneol are well documented taint compounds in food.

On the other hand off flavour compounds are present in products as a result of chemical alteration of the product, which include oxidative, biological and enzymatic degradation of ingredients such as fats and oils. Typical off flavours cause a rancid smell of the product.

Halophenols

Halophenols such as chlorophenols, bromophenols and iodophenols, are compounds that are used as chemical intermediates or as finished products in industry. These compounds have low sensory thresholds and may be present as taints in foods and drinks at concentrations in the ppb (parts per billion; µg per kg) or even ppt (parts per trillion; ng per kg) range. At this level traditional GC-Ms techniques struggle to pick up these compounds even though they can be obvious during sensory evaluation. Products that have been contaminated with halophenols have an aroma that is described as disinfectant or medicinal.

Chlorophenols consist of many different compounds with different degrees of chlorination. They are used as fungicides, pesticides, herbicides and disinfectants. W well-known chlorophenol is pentachlorophenol, which has been used extensively for wood preservation. Chlorophenols in drinking water can also result from chlorination of natural phenolic compounds that are present in the treated water, whereas iodophenols can be formed during the use of iodine as a drinking water disinfectant. Bromophenols and their derivatives are widely used as fire-retardants, but also for the preservation of wood and wood products, leather, textiles, paint, plastic, paper and pulp.

An additional important source of halophenols and especially chlorophenols in drinking water is diffusion of organic additives from high density polyethylene water pipes. These additives consist of impurities or by-products of typical phenolic additives that are used as antioxidants in the production of the pipes.

Haloanisoles

Chloro-, bromo- and iodoanisoles are formed by methylation of the hydroxide group of halophenols by moulds and fungi under humid and warm conditions. This methylation is a biological mechanism to detoxify the halophenols. Haloanisoles are easily transferred when moulds grow in the vicinity of a product and contaminated products exhibit musty and mouldly odours. These compounds can be detected on aroma when present at extremely low concentrations (as low as 0.02 ppt or ng/kg) and are responsible for the cork taint in wine.
Taint and Off Flavour Investigation

Typical Taint Analysis Workflow

The first objective in a taint and off flavour investigation is to identify the compound or compounds that are the cause of the atypical smell or taste. When possible and safe to do so, an informal sensory analysis is performed on the affected product, which will help to decide what analytical strategy will be pursued. The headspace of the samples will be analysed if the odour is very strong or a compound is suspected to be highly volatile (e.g., by solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC_MS)). If this does not provide the required information or sensory suspect a less volatile compound, the same is extracted (e.g., using Liken-Nickerson extraction) and the extract will be concentrated (e.g., using a Kuderna Danish apparatus) to increase the relative amount of the tainting compound in the extract.

If a taint or off-odour compound has been identified, the next stage in the investigation is aimed to find the root cause of the problem. Therefore a targeted analytical approach is required to analyse affected samples, raw materials, storage materials, etc. Sample amounts are often limited. Therefore, in addition to concentrating extracts of samples, the most sensitive analytical techniques are required to analyse for these compounds. Confirmatory data may also be needed to prove the origin of the contamination, for example, for legal or insurance purposes.

Most taint and off flavour compounds are potent odour compounds that can be detected by humans in much lower concentrations than any analytical instrument is capable of. Therefore, in addition to concentrating the samples, the most sensitive analytical techniques are required to analyse for these compounds. A very powerful and sensitive technique is tandem mass spectrometry.

Tandem Mass Spectrometry (MS/MS)

In order to be able to analyse complex mixtures of volatile compounds, gas chromatography is coupled to mass spectrometry (GC-MS). The chromatography separates the components then a mass spectrometer operated in scanning mode produces mass spectral data for each component of the chromatographic analysis. This means that detected compounds can be identified from their mass spectra. These spectra can be compared to recorded spectra from commercially available libraries. Analysis time has to be dedicated to scan and record a signal for each mass. Whilst this provides a large amount of data, the sensitivity is lower and this can also be time consuming to generate and analyse.

Compounds can be monitored and quantified by selecting a single mass and ignoring all the other masses, constructing an extracted-ion chromatogram. Sensitivity can be greatly improved by only scanning for selected masses, since analysis time is spend on recording signals for a reduced number of masses. Although this technique of selected-ion monitoring (SIM) is very sensitive, there is still a potential that compounds with similar masses cause interference with the masses of the compounds of interest. Moreover this relies on the expertise of the analyst to select the correct ions to search for. In each of these instances there is the chance that the tainting compound will be missed.

Selectively and therefore sensitivity can be improved by using single-reaction monitoring (SRM) using a triple-quadrupole mass spectrometer. Rather than monitoring a specific ion (as in SIM), selected precursor ions are fragmented and a product ions are monitored. This is highly selective, because for many compounds, it is unlikely that a compound elutes very closely to the compound of interest that can be characterised by the same precursor/product ion (SRM transition). In many gas chromatography-tandem mass spectrometry (GC-MS/MS) applications matrix interference is virtually eliminated. In many instances, this will remove or reduce the need for sample clean-up, which can simplify the analytical procedure and increase efficiency.
In concentrated extracts, the detection limits of GC-MS/MS are lower than the odour thresholds form the majority of taint and off-flavour compounds without the need to extract and concentrate excessive quantities of sample. This is especially important, since the amount of tainted sample is often limited, especially in the case of customer complaints.

Furthermore, GC-MS/MS is a well-established and widely accepted analytical technique. This means that GC-MS/MS data can be used for legal cases or to aid insurance claims.

**Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) Analysis**

Samples are analysed for a large suite of taint and off-flavour compounds using a Thermo Scientific GC that is coupled to a TSQ 8000 triple quadrupole MS. The gas chromatograph is equipped with a TG-WASMX column and samples are injected into a split-splitless injector operated in splitless mode. An oven temperature programme is used to separate the compounds that are injected onto the column. The mass spectrometer is operated in positive electron impact MS/MS mode at 70 eV. SRM transitions of the taint and off-flavour compounds were determined with the aid of the automatic algorithms of the Thermo Xcalibur 2.2 software using commercial standards.

**Example of the Analysis of a Taint Compound**

To illustrate the analysis of a taint compound by GC-MS/MS, a food sample was analysed by both GC-MS and GC-MS/MS looking for 2, 4-dichlorophenol (Figure 1). At a concentration of 0.05 ppb (ng/ml) in the sample, the compound could not be detected from the total-ion current chromatogram (TIC). However, when the molecular ion (m/z 162) was selected in selective ion monitoring (SIM), a signal could be observed, but was difficult to distinguish from the baseline noise. When the same sample was analysed by GC-MS/MS, a significant increase in the signal to noise ratio of the SRM chromatogram (m/z 162 to 98) was observed. The taste threshold in water for 2,4-dichlorophenol is reported to be 0.3 ppb so this clearly demonstrates the benefits of the increase in selectivity (and therefore sensitivity) at a level significantly below the taste threshold.

**Figure 1**

**Top**
Partial chromatograms (from top to bottom: TIC, SIM and SRM)

**Bottom**
Spectrum of 2, 4-dichlorophenol

**Conclusions**

The use of GC-MS/MS for the analysis of taint and off-flavour compounds in food and potable water samples provides a very sensitive and effective analysis technique. Experience demonstrates that food and drinks manufacturers face challenges in retrieving affected samples from the consumer for analysis. Therefore for the majority of occasions, the amount of tainted product that can be analysed is limited to relatively small sample amounts (<100 g). Sensitivity is therefore essential.

GC-MS/MS has demonstrated that as a tool for taint and off-flavour analysis it provides very sensitive, reliable quantitative and confirmatory data, which is needed to identify the root cause of the contamination of a product.

**References**

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Donna has recently joined RSSL with 30 years of experience in a variety of analytical techniques on varied matrices. Her experience includes method development, validation and special analysis on tobacco and related products; pesticide analysis on teas and various crops, and on pesticides for regulatory purposes. She was also a Forensic Scientist for 16 years in the field of drugs and toxicology.

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