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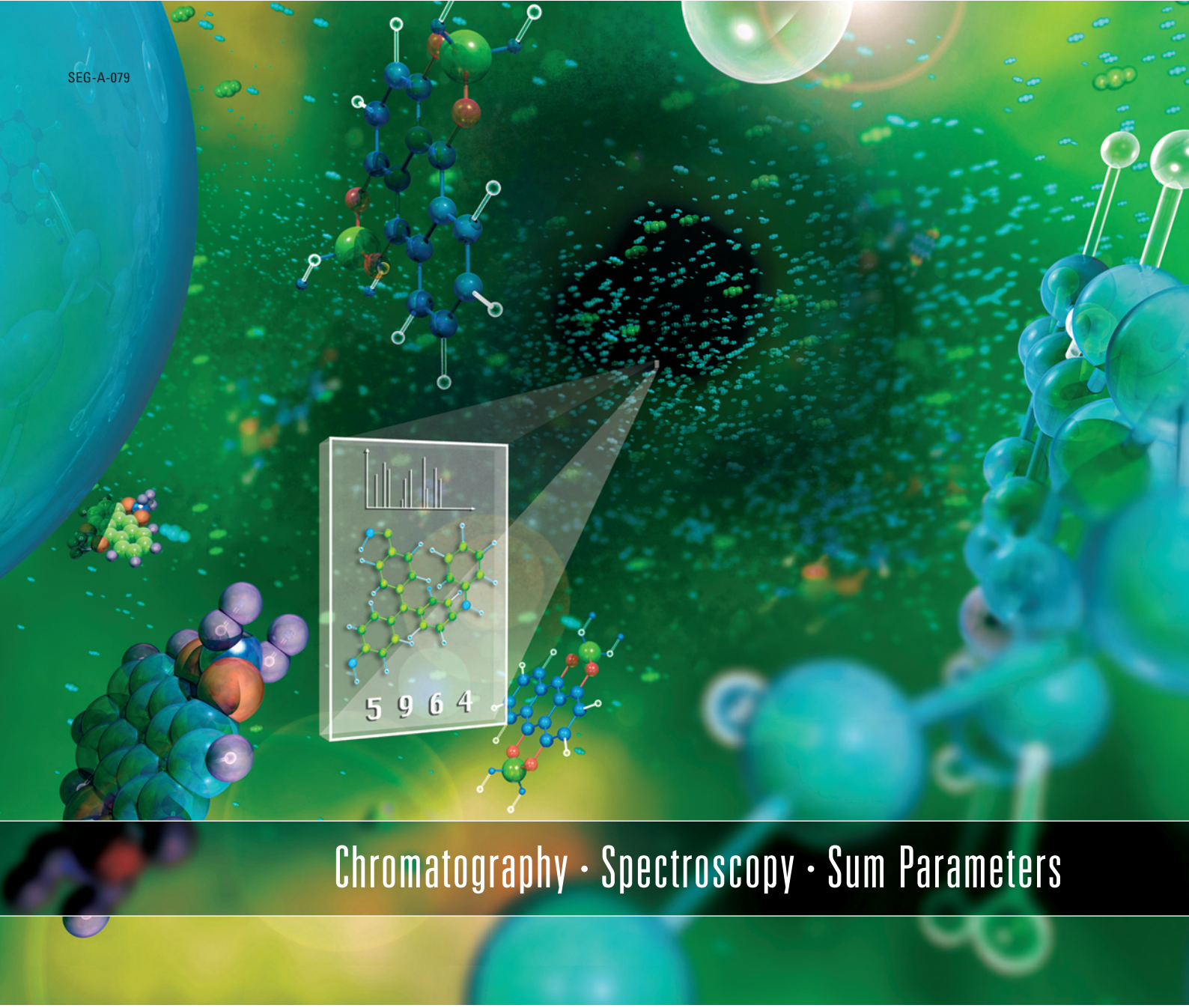

Chromatography · Spectroscopy · Sum Parameters

Application Book Volume 3

Food & Beverage

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Application Book Volume 3

Food & Beverage

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Publisher:
Shimadzu Europa GmbH
Albert-Hahn-Str. 6-10 · 47269 Duisburg, Germany
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Shimadzu thanks Prof. Luigi Mondello,
University of Messina, Italy for his kind support.

Design and Production:
m/e brand communication GmbH GWA , Düsseldorf

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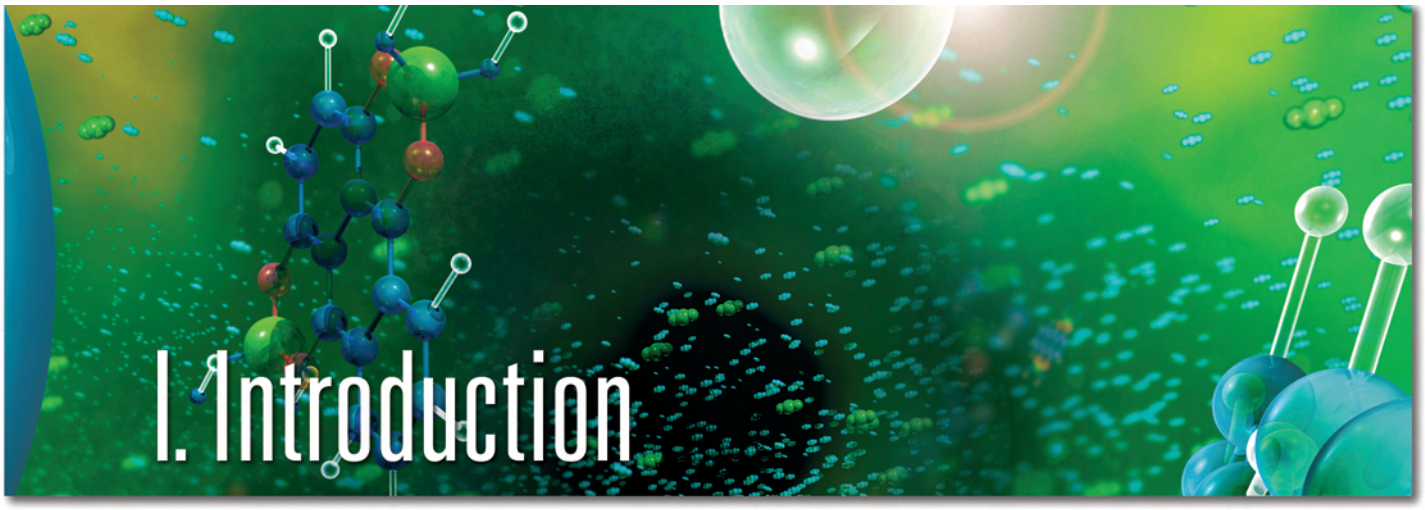

I. Introduction

The analysis of food and beverage products is an increasingly important task world-wide. Many aspects have to be taken into account whenever the analysis of food and beverages is concerned. Quality control is highly demanding in modern food processing in order to assure the required, standardized taste for the consumer. At the same time environmental pollution increases the necessity for thorough control of possible contaminants such as pesticides in food and beverages. Severe hygiene requirements must be fulfilled as well, leading to stringent controls of water not only for beverages but also for food processing.

Food products derive, essentially, from plants, fruits or animals and can consist of many different compounds. The characterization of a food product can therefore be considered as one of the most challenging tasks in Analytical Chemistry. Full elucidation of the qualitative and quantitative profile of a food or beverage requires a wide range of analytical techniques. Although in many cases only specific aspects of a product are investigated, it is obvious that a food analysis laboratory needs to be equipped with many different analytical methods.

Shimadzu, as one of the world-leading manufacturers of analytical instrumentation, offers the range of instruments required in a food laboratory: ranging from liquid and gas chromatography, also coupled to mass spectrometric detection, to spectroscopic techniques (for elemental analysis as well as molecular spectroscopy) and also including TOC (Total Organic Carbon) determination.

This Application Book 'Food and Beverage' provides examples from different fields and for various types of analysis, such as the detection and quantitation of contaminants in food (e.g. pesticides or bisphenol A), the characterization of a product through flavor and fragrance evaluation or quality control process in matrices such as beer or honey. The book provides the reader with valuable details on latest techniques used in food analysis laboratories as well as helpful information on successful method development.

For more information about the Shimadzu product range, as well as applications, please visit

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II. Contaminants

Analysis of NDMA (N-nitrosodimethylamine) in malt for

Barley for malting

Figure 2: Traditional direct fired floor kiln (capacity ~ 20 t)

NDMA sources

Exposure to N-nitrosodimethylamine (NDMA) can come from many directions – the environment, the workplace, the food and drink we consume. Some of the primary sources of exposure are tobacco (smoked and chewed), cured meats (particularly bacon), some cheeses, fish, toiletries and cosmetics (e.g. shampoos and cleansers) and combustion gases such as traffic fumes. Workplace exposure can occur in industries such as pesticides and rubber products manufacturing, fish processing and tanning. NDMA has been found in groundwater samples. It is naturally present in the stomach from digestion of some foods.

Effects of NDMA on human health

Laboratory studies on animals have demonstrated that NDMA is carcinogenic and it is possible that induction of tumours involves interaction with genetic material. As the metabolism of NDMA appears to be similar in humans to that in animals it is considered highly likely that NDMA is also carcinogenic to humans. In common with N-nitroso compounds in general, NDMA is known to be harmful to the livers of animals and humans.

NDMA in beer and whisky

Not too long ago beer and whisky could have been considered as potential primary sources of NDMA but the risk is now minimal because of changes made in the production of one of the key ingredients viz: malt. Following a study in Germany in the 1970s [1] it was established that a significant source of NDMA in beer was from the malt used for brewing and the malting industry was summarily challenged to reduce levels to as low as practically possible in as short a time as possible.

Formation of NDMA

NDMA in malt is formed during the kilning process if oxides of nitrogen (NO_x), principally N_2O_3 and N_2O_4 from fuel combustion, come into contact with the malt in the early stages of drying. Although there are several possible routes to NDMA formation, it appears that nitrosation of hordenine is the principal one [2]. Hordenine, a protein breakdown product formed during the germination stage of malting, is nitrosated by N_2O_3 and N_2O_4 and, as kilning proceeds and the temperature rises, so the nitrosated hordenine breaks down to form dimethylamine (DMA) which is then further nitrosated to form NDMA. A simplified pathway is shown in Figure 1:

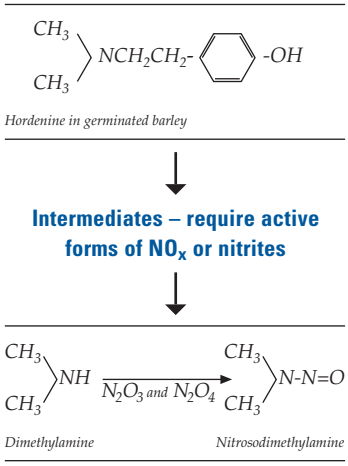


Figure 1

Concentrations of NDMA in malt and beer

Traditionally malt kilns (Figure 2) were direct fired i.e. the products of combustion from the fuel used to heat the air for drying passed directly through the grain bed. NDMA formation was therefore likely to have been a normal part of the process of malting, but its presence was probably not known about and so not a cause for concern.

However, with increasing awareness of potentially harmful substances in the food chain and improvements in analytical equipment and techniques, NDMA in malt was identified as a problem. Although palliative treatments like reducing the pH at the surface of the grain during kilning by burning sulphur in the air stream offered a short term solution, indirect firing was seen as the ultimate answer. In indirect firing the hot air carrying the products of combustion from the heating fuel passes to exhaust through a heat exchanger which heats incoming, clean, ambient air to dry the malt. Major investment in indirect fired kilns (Figure 3) in malting companies around the world was set in train and the ability to obtain NDMA levels in malt of around 1 µg/kg is now commonplace; previously some levels had been recorded in the low hundreds. In the few remaining malting plants where direct fired kilns are still in operation the installation of low NO_x burners and/or palliative sulfur burning in the early stages of kilning provides almost equivalent control of NDMA formation, thus enabling maltsters to continue to meet the very tight specifications set by brewers and distillers.

Control of NDMA

Very low levels of NDMA in malt are now being consistently achieved and these levels are diluted a further 10-fold during the brewing of beer. Nevertheless, there remains a duty of care to all those who drink malt based beverages to ensure that the potentially harmful effects of

beer and whisky production

By Stan Sole, Crisp Malting Group Ltd, Great Ryburgh, Fakenham, Norfolk, UK

Figure 3: Modern indirect fired kiln (capacity ~ 200 t)

NDMA are kept to an absolute minimum. To that end the monitoring of NDMA levels in malt continues to be necessary.

Analysis of NDMA at Crisp Malting Group

Previously a Thermal Energy Analyzer was used to determine levels of NDMA in malt samples, but replacement with GCMS-QP2010 in early 2004 has proved very successful. Since that time hundreds of samples have been routinely analysed without problems and excellent correlation with laboratories using Thermal Energy Analyzers has been achieved. Figure 4 shows the chromatogram of a malt sample with a concentration of 0.75 ppb (µg/kg). Details of the method are given below.

NDMA in malt: method details

Malted barley is blended with water in a homogenizer/blender and subsequently filtered through a Whatman No. 54 filter paper. NDPA internal standard and sodium chloride are added to the filtrate. After liquid/liquid extraction with DCM (dichloromethane) the DCM phase is dried with anhydrous sodium sulphate, filtered and evaporated in a water bath at 55 °C to about 1 mL. The cooled DCM phase is transferred to a GC-MS vial and is ready for injection.

GC-MS conditions:

Column: Stabilwax, 30 metres, 0.25 mm ID, 0.5 µm film thickness

Oven temperature: 50 °C for 1 min, then 20 °C/min to 175 °C holding at 175 °C for 1 min, then 40 °C/min to 250 °C holding at 250 °C for 10 min.

Injection port temperature: 240 °C

Interface temperature: 250 °C

Ion source temperature: 200 °C

References:

[1] Nitrosamines in Malt and Beer; Wainwright, T., J. Inst. Brew., 1986, Vol 92, pp 73- 80

[2] The Chemistry of Nitrosamine Formation: Relevance to Malting and Brewing; Wainwright, T., J. Inst. Brew., 1986, Vol. 92, pp 49-64

- **NDMA is considered to be carcinogenic**
- **The GCMS-QP2010 has proved to be very successful in replacing a Thermal Energy Analyzer for determination of levels of NDMA in malt samples**

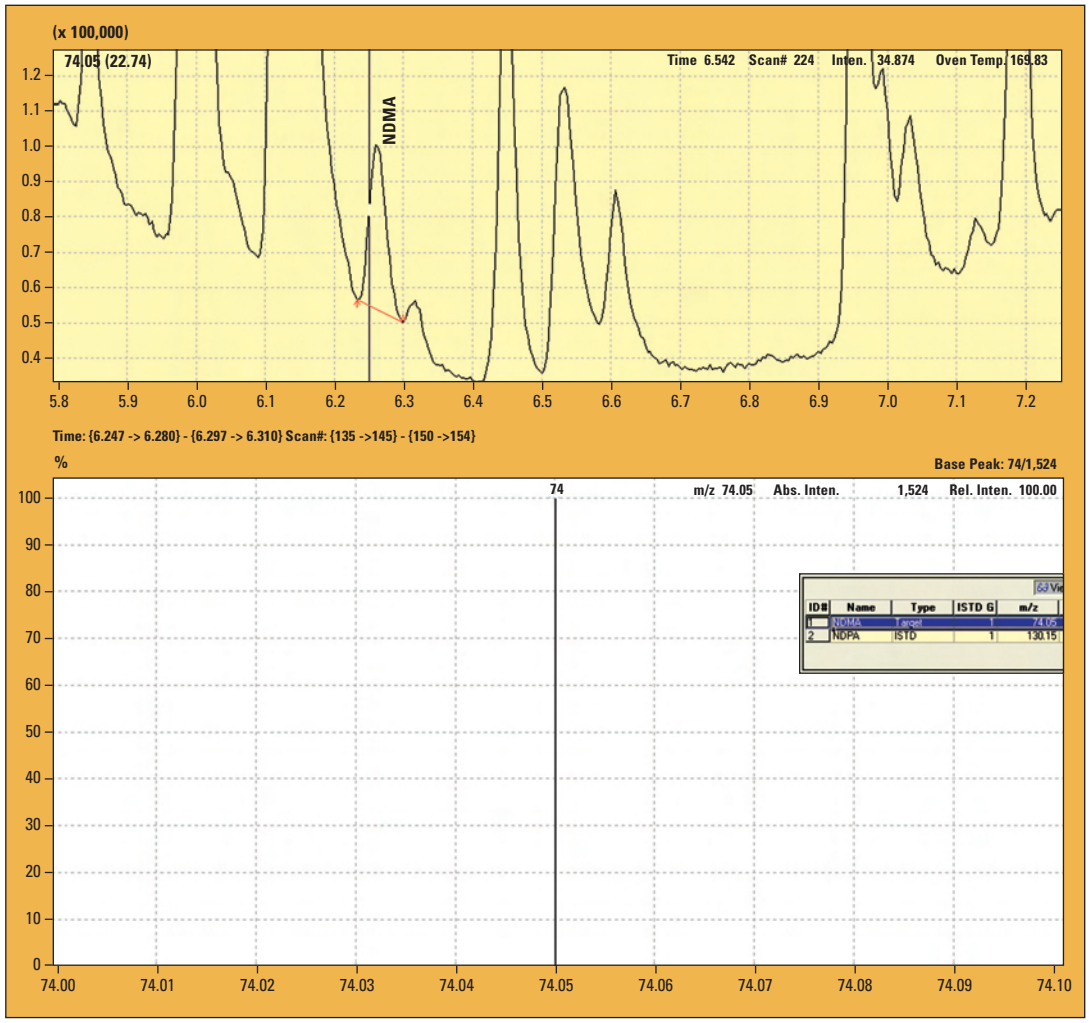


Figure 4: Chromatogram of NDMA

An apple a day keeps the doctor away?!

Mycotoxins are

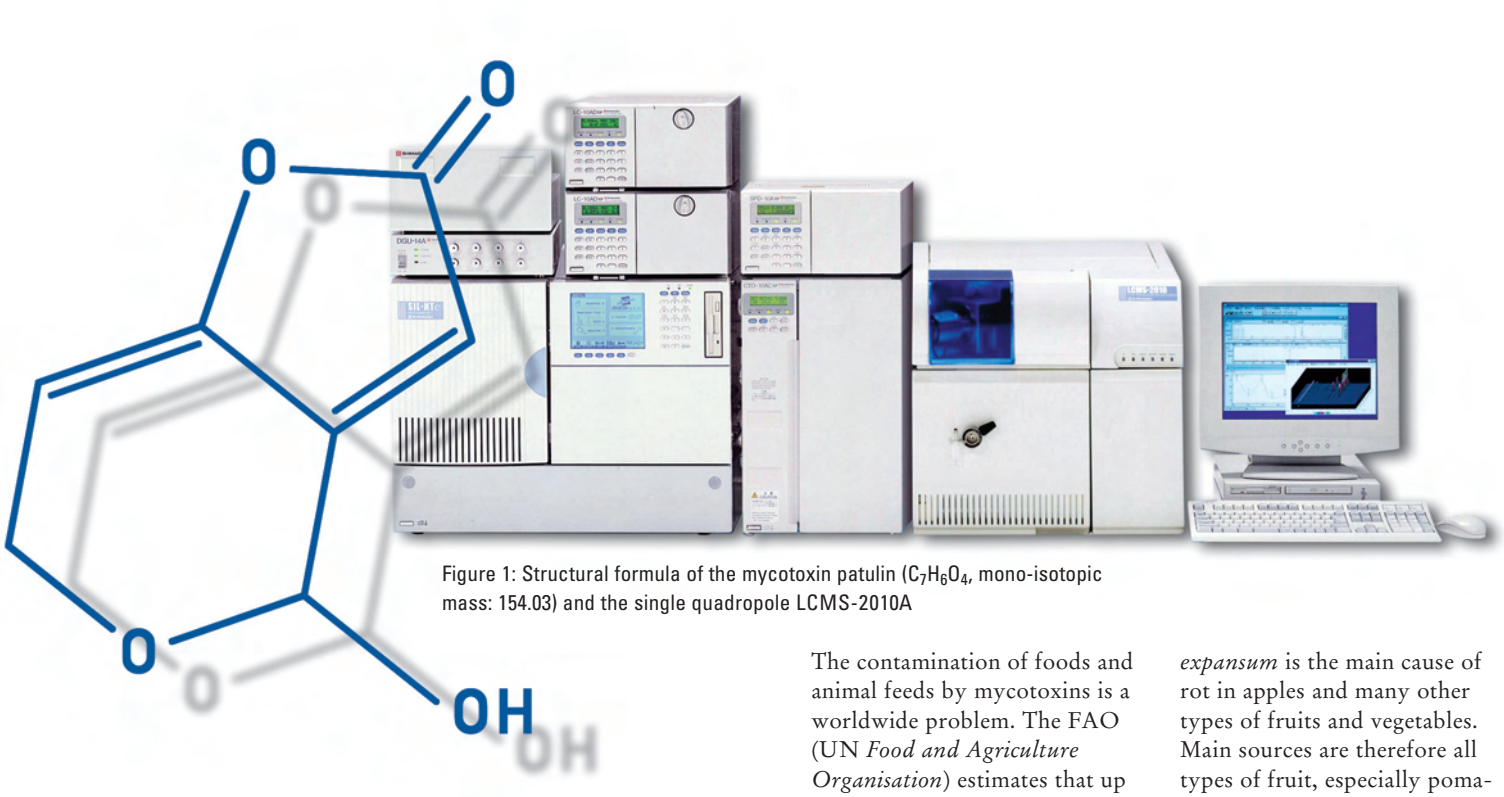


Figure 1: Structural formula of the mycotoxin patulin ($C_7H_6O_4$, mono-isotopic mass: 154.03) and the single quadropole LCMS-2010A

- **Through increased sensitivity in analytical research mycotoxins were discovered as contaminants**
- **HPLC/MS is a new method for identification and quantification of patulin (mycotoxin)**
- **Various toxins can be determined in a single analysis**

Fungal toxins have posed a human health hazard since the early days of food crop cultivation. Already in the Bible, there are reports of a disease that can occur after digestion of ergot. In the Middle Ages hundreds of thousands of people died due to ergot poisoning.

In search of the cause for particular diseases that rule out known triggers such as microorganisms, plant toxins or pesticide residues, mycotoxins were discovered. Over time it was established that foods infected with fungi are responsible for several diseases in livestock.

That the same compounds could also be present in human foods and be the possible causes of disease was only discovered when analytical research methods became more sensitive and a large number of foods and food components could be analyzed.

The contamination of foods and animal feeds by mycotoxins is a worldwide problem. The FAO (UN *Food and Agriculture Organisation*) estimates that up to 25 % of the world food production is affected by mycotoxins.

In approximately 20 % of the cereal crops in the EU, mycotoxins can be detected. To date there are hardly any consolidated findings about the effects of small amounts – especially during lifelong uptake.

The mycotoxin patulin

Patulin is formed by various *Penicillium-*, *Aspergillus-* and *Byssochlamis-species. Penicillium-expansum* is the main cause of rot in apples and many other types of fruits and vegetables. Main sources are therefore all types of fruit, especially pomaceous fruit such as apples and pears, which are often attacked by brown rot (blight) (*Penicillium expansum*).

Mycotoxins can also attack peaches, apricots and cherries. Citrus fruits and plums are less problematic. In approximately 40 % of brown rot in apples, patulin could be detected. The infected areas in apples can contain more than 80 mg/kg patulin. This means that only a small number of mouldy apples are required to cause patulin contaminations in large volumes of apple

Concentration (ppb)	1	2	3	Mean value (1-3)	% RSD
5	1293	1266	1216	1258	3.10
10	2773	2815	3027	2872	4.74
25	6560	6271	7081	5402	7.60
50	14006	14425	14441	14291	1.73
100	28602	29175	28655	28811	1.10
250	69991	75810	74773	73525	4.22
500	165437	161378	156406	161074	2.81
1000	291363	280538	278373	283425	2.46

Table 1: Calibration from 5 ppb up to 1000 ppb

a world-wide problem – Highly sensitive determination of the fungal toxin patulin

juice to patulin concentrations of 50 µg/ kg or even higher.

EU Commission limits the patulin mycotoxin in apple products

Since November 2003, the following maximum concentrations of patulin in apple products have been established in the European Union.

The mycotoxin patulin is a five-ring unsaturated lactone [4-Hydroxy-4H-furo(3.2-c) pyran-2(6H)-on ($C_7H_6O_4$)].

More selective, faster detection using LC-MS

The analysis of patulin in foods is commonly carried out according to DIN ISO 8128-1 using HPLC/PDA. There is, however, a growing trend to use the hyphenated technique HPLC-MS for identification and quantification. In order to attain a higher sensitivity and selectivity for the determination of patulin at the trace level, an HPLC method using mass spectrometric detection was developed as described below. This method uses Shimadzu's single quadrupole LCMS-2010A for mass spectrometric detection.

The chromatographic separation was carried out on a 2 mm reversed-phase column in less than 15 minutes. The toxin is protonated in the mass spectrometer via APCI (atmospheric pressure chemical ionization) (detected at $[M^+H^+]^+$ = 155 m/z). Using this analysis method, it is possible to detect patulin safely down to the sub-ppb range. Figures 2 and 3 show the chromatogram in single ion monitoring (SIM) mode (at m/z = 155) and a calibration from 5 to 1000 ppb with excellent linearity.

Not only patulin can be detected with excellent sensitivity using LC-MS. Other highly sensitive routine LC-MS methods exist for mycotoxins such as aflatoxins, ochratoxin and fusaric toxins such as deoxynivalenol. Due to the high selectivity of MS detection, the simultaneous determination of various toxins in a single analysis is also possible.

The use of such highly sensitive detection methods for mycotoxins in foods enables accurate monitoring of the mycotoxin concentration and forms the basis for further research into health hazards that can arise from mycotoxin contamination in foods.

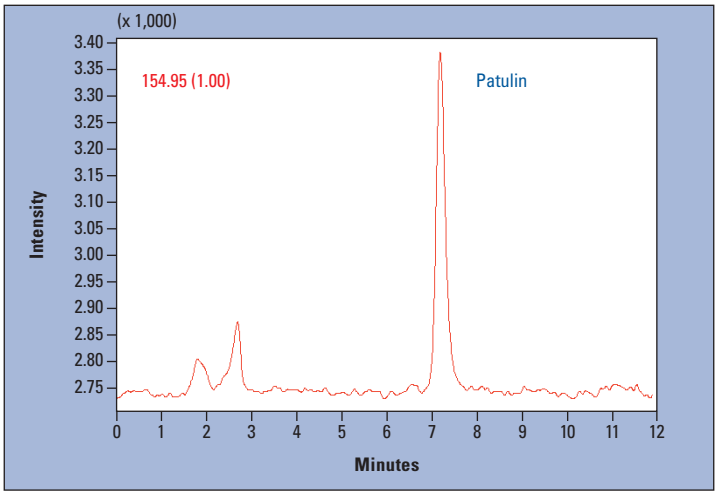


Figure 2: Detection of patulin using APCI in SIM mode

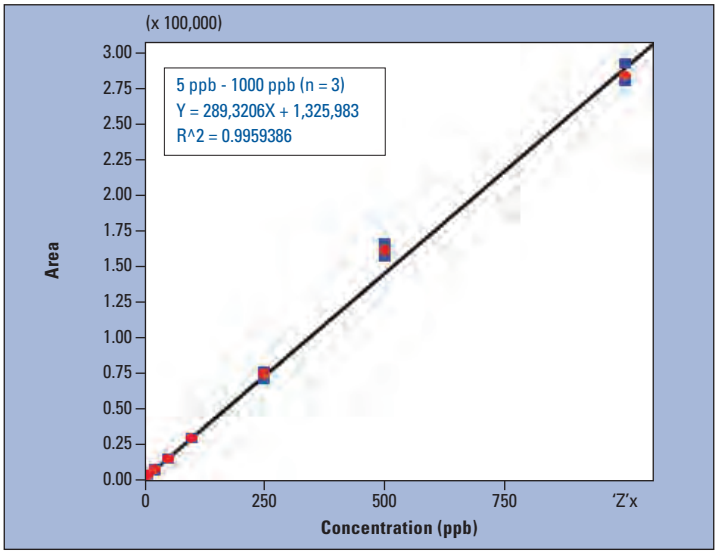
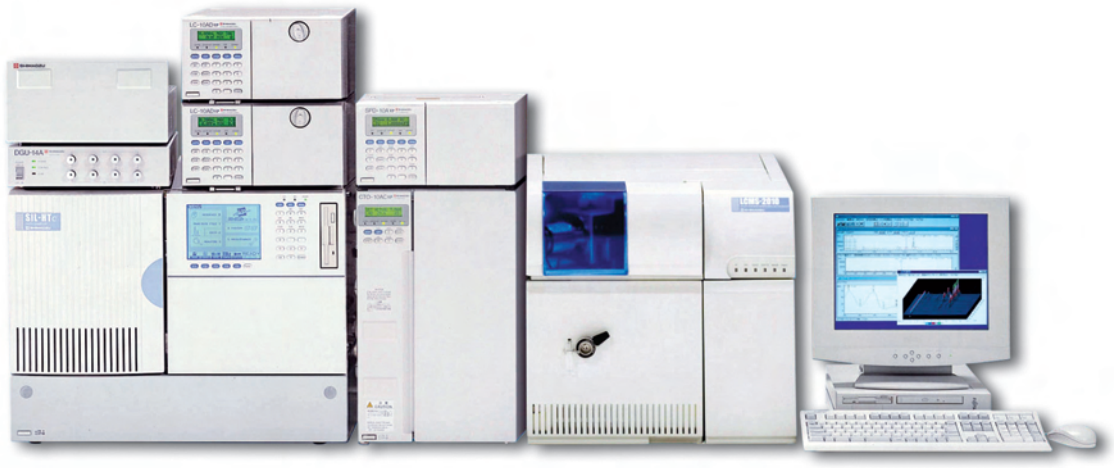


Figure 3: Calibration from 5 ppb up to 1,000 ppb

Product	Maximum concentration (µg/kg)
Fruit juices, especially apple juice, and fruit juice additives in other beverages, fruit nectars and fruit concentrates, spirits, apple cider and other beverages derived from apples or apple juice containing fermented beverages	50
Solid apple products intended for direct consumption, including apple compote and apple sauce	25
Apple juice and solid apple products, including apple compote and apple puree for nursing and infants, which are marked and sold for this purpose. Other supplements for nursing and infants.	10

Table 2: Maximum concentration for Patulin

Make sure you know what you're dealing with! Analysis



The LCMS-2010 covers the complete range of analytical challenges

- **LC-MS method can detect different herbicide species simultaneously with excellent sensitivity**
- **Excellent separation**
- **Development into a fast HPLC-MS method is possible**

In recent years, pesticides have been used with much greater care and more restraint. Increasing scientific evidence as well as improved detection methods have played an important role.

In agriculture, users have changed their general attitude towards herbicides, fungicides and pesticides. Continuous control and analysis of pesticides should be a matter of routine. As knowledge and detectability will further increase in the future, the old saying, "Make sure you know what you're dealing with!" will, as always, remain applicable in the positive sense.

The so-called phenoxy herbicides have been on the market since the 1940s. These, at the time new, types of pesticides proved to be fast-acting and very effective. Even today, phenoxypropionic herbicides (such as fluazifop, fluazifopbutyl and quizalofopethyl) are still being used in agriculture worldwide, as they possess strong herbicidal properties. The mode of action is based on the disruption of the biosynthesis of fatty acids via the inhibition of the acetyl-CoA carboxylase enzyme.

Some types of the phenoxy herbicides received a bad reputation through their use as a military defoliant (Agent Orange), during the Vietnam War. The well documented damaging after-effects on the population did not, however, originate from the herbicide itself. One of the components of Agent Orange was contaminated with a high concentration of dioxin. With each gram of the herbicide mixture sprayed, up to 50 micrograms of the highly toxic dioxin was being applied simultaneously.

Today, the use of modern phenoxy herbicides, such as fluazifop, is allowed in the cultivation of medicinal plants and herbs under very strict conditions for the control of weeds. In addition to their high effectiveness, these herbicides distinguish themselves by an extraordinarily low toxicity to mammals. Poisoning can only occur after exposure to very high levels of these compounds resulting from careless storage or negligent maintenance.

Recently, however, the potential hormonal action of biocides and pesticides is under critical discussion. As fluazifop-P belongs to the biocides that are suspected of hormonal action, an efficient control of the pollution levels of foods by herbicide residues is therefore imperative.

Residue analysis using LC-MS

The phenoxy herbicides are normally used as a salt of the phenoxycarboxylic acid (for instance fluazifop) or an ester (fluazifop-butyl). Residue analysis for quizalofop is routinely carried out using HPLC/LCMS and for fluazifop using GC/GCMS.

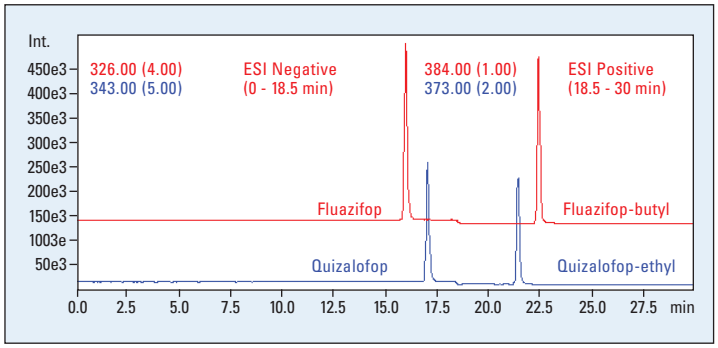


Figure 1: Simple LC-MS analysis: allows the simultaneous determination of several phenoxy herbicides

# of phenoxypropionic herbicides using LC-MS

Figure 1 illustrates a simple LC-MS method using the single quadrupole LCMS-2010 system, which allows the simultaneous determination of fluazifop, fluozifopbutyl and quizalofop and quizalofopethyl. Electrospray ionization (ESI) at atmospheric pressure was used as the ionization method. The carboxylic acid types are ionized in the negative ionization mode, the ester types in the positive ionization mode. In the separation of herbicides using reversed-phase liquid chromatography, the ester types elute much later than the free acids. When the MS detector is switched from the negative to the positive ionization mode, after elution of the acids, all different herbicide species can be detected simultaneously with excellent sensitivity.

Figures 1 and 2 show the chromatograms of four herbicides detected in the SIM mode and the corresponding mass spectra with their characteristic masses. By using a semi-micro C18 HPLC column, the sensitivity of the method could be increased significantly, resulting in a calibration of 0.8 up to 500 ppb with excellent linearity for each compound. This is shown in Figure 3 for fluazifop (m/z 326).

The excellent separation of compounds using this method leaves room for further method optimization in order to reduce the total analysis time. In terms of instrumentation, further development into a fast HPLC/MS method is certainly possible, making use of the fast switching times of the LCMS-2010 in multi-sequence mode and using the faster SIL-HT autosampler (15 s per standard injection).

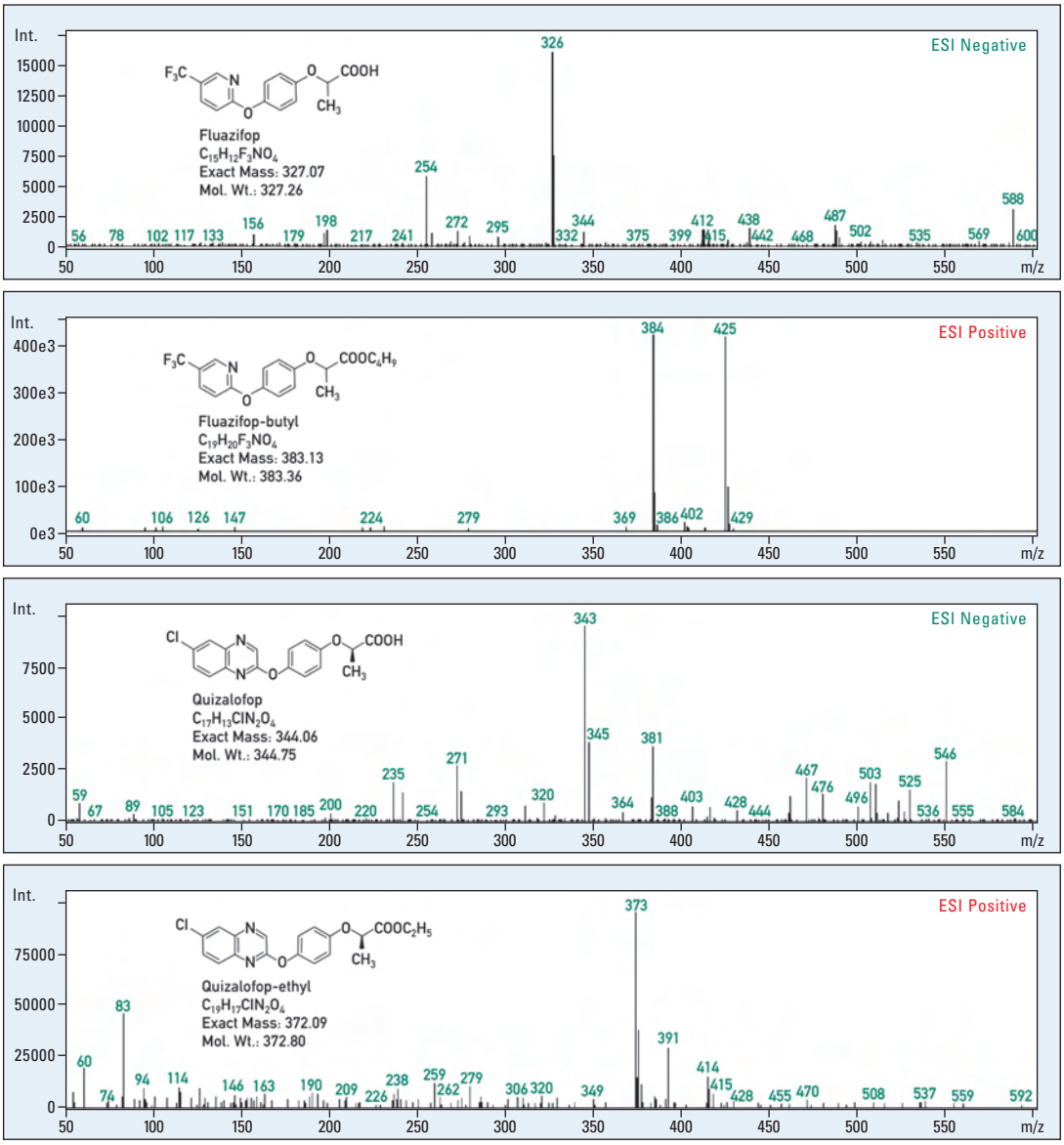


Figure 2: Chromatograms of four herbicides in the SIM-mode with their characteristic masses

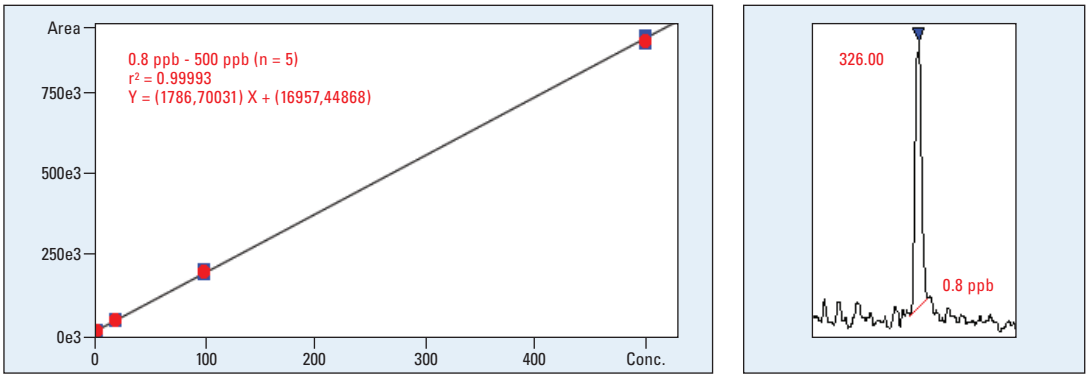


Figure 3: Linearity and sensitivity for the analysis of fluazifop

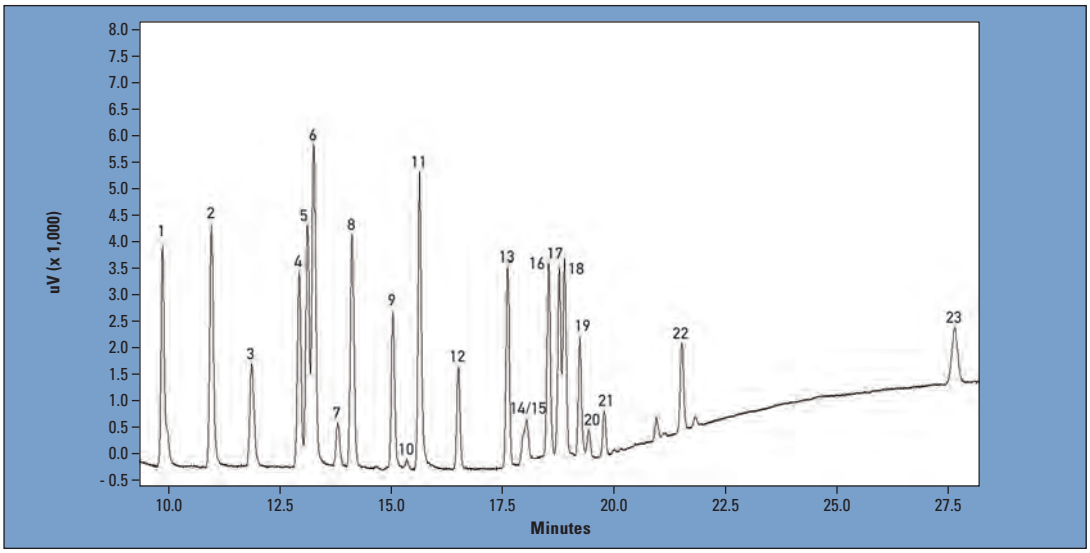


Figure 1: Standard analysis of an OCP standard (23 compounds) using an RTX-5 column 30 m, 0.25 mm ID, 0.25 µm film

Fast GC-ECD analysis of organochlorine pesticides

The analysis of organophosphorus (OPP) and organochlorine (OCP) pesticides in environmental and food matrices is of major importance in routine analysis. The large number of compounds to be detected requires a proper screening method in order to complete the analysis in a reasonable time.

In the search for a method which reduces analysis time while maintaining resolution, the use of narrow bore columns has become significant in routine work [1].

Although many publications exist describing Fast GC using FID, FTD and FPD, this paper describes the use of ECD. As the peak width at half height (FWHM) in a chromatogram recorded with 0.1 mm ID column is expected to be about 0.5 s [2], the detector needs to have low dead volume, selectable filter time constant, and to supply enough data points across the peak [3]. The latter is referred to as the sampling frequency.

With the GC-2010, it is possible to freely select the filter time constant and the sampling frequency down to 4 ms (minimum filter time constant) and up to 250 Hz (maximum acquisition frequency) respectively, for all detectors.

In GC analysis using standard columns of about 30 m length with inner diameter 0.25 mm and 0.25 µm film, the typical run time for an OCP standard containing 23 compounds is about 29 minutes. Figure 1 shows the chromatogram of such a standard (for concentration refer to Table 1).

The retention time of the p,p-DDD (peak 22) is about 21 minutes. The column used was a 5 % phenyl substituted Dimethylsiloxane column and the temperature program was 100 °C, 1 min, 50 °C/min to 170 °C for 1 min, 5 °C/min to 220 °C, 10 °C/min to 260 °C, 20 °C/min to 280 °C for 10 min. Carrier gas was N_2 with a starting pressure of 77 kPa, corresponding to a linear velocity of 23 cm/s. The injection was carried out in splitless mode (1 µL).

This method was then transferred to the Fast GC method using a CPsil 8, 9 m, 0.1 mm, 0.1 µm and H_2 as carrier gas. The result is shown in figure 2. All 23 compounds were better separated and the retention time of p,p DDD (peak 22) was less than 3.6 minutes. The program used was 80 °C, 1 min, 60 °C/min up to 280 °C for 3 min with an initial head pressure of 324 kPa and an average linear velocity of 100 cm/s kept constant during the entire chromatographic run. The filter time constant and the sampling frequency were selected as 20 ms and 63 Hz respectively.

Injection volume was 1 µL with a split ratio of 40:1. The signal-to-noise ratio of alpha-HCH, for example is about 440:1 in this analysis, compared to 220:1 in the splitless standard measure-

- **Proper screening method in a reasonable time**
- **Narrow-bore columns have become significant in routine work**

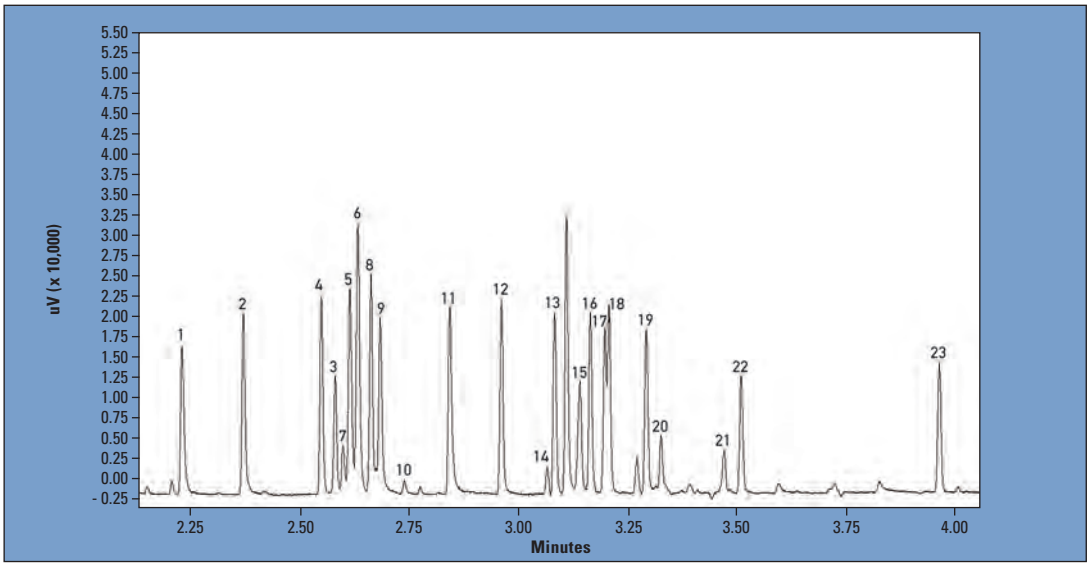


Figure 2: Fast analysis of the OCP standard containing 23 compounds (injection 1 µL, split 40:1, temperature program: 80 °C, 1 min, 60 °C/min to 280 °C 3 min. H_2 linear velocity 100 cm/s, ECD: make up gas: 80 mL/min, acquisition frequency 63 Hz, filtertime constant 20 ms.

ment, indicating the increased sensitivity due to the sharper peaks.

The full width half maximum (FWHM) of alpha-HCH as an example is about 0.5 s which is the typical FWHM observed with this kind of column proving the suitability of the ECD-2010 for fast analysis in the field of organochlorine pesticides beyond any doubt. The limit of detection for alpha-HCH, for example, is about 0.1 ppb with a split ratio of 40 : 1, requiring that the signal-to-noise level be at least 3 : 1.

To apply the splitless injection technique, a high pressure injection in combination with a slightly thicker film on the column has to be used. This is demonstrated in Figure 3.

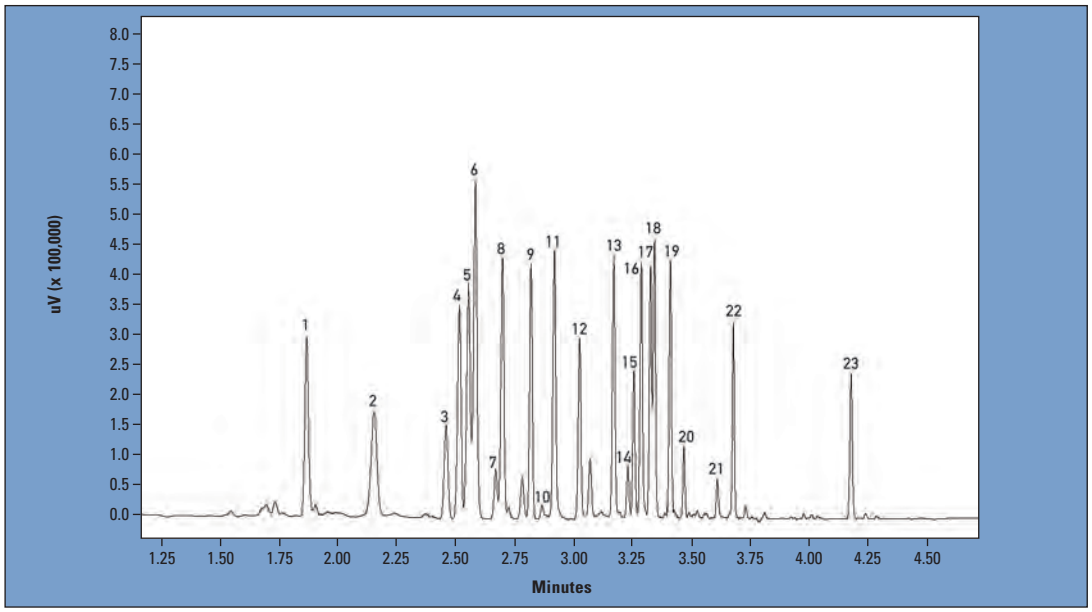


Figure 3: Chromatogram recorded with the OCP standard mix. Injection 1 µL splitless, high pressure pulse 400 kPa. Column RTX-5 10 m, 0.18 mm, 0.4 µm. Temperature program: 100 °C, 1 min, 60 °C/min to 280 °C for 3 min. H_2 120 cm/s.

Here a 10 m, 0.18 mm, 0.4 µm (5 % Phenyl substituted) column was used with 100 °C initial temperature and a constant linear velocity of 120 cm/s. All other parameters were unchanged. Looking again at alpha-HCH, the detection limit calculated from the signal-to-noise ratio (3:1) is about 0.01 ppb in this case.

The analysis of pesticides is regulated by the well known multi-residue method referred to as S 19 in Germany and DIN EN 1528-3, DIN EN 12393-2 in Europe. The method described above was also adapted to measurement of real samples prepared according to this procedure. Figure 4 shows a chromatogram recorded from a grape eluate containing chlorpyrofos and cypermethrin. This was measured using the thin film column (see Figure 2).

The determination of organochlorine pesticides in food matrices can be performed well using Fast GC-ECD. The detection limit of this method is below 0.1 ppb for several compounds using a split of 40:1 and about 0.01 ppb using the splitless technique with a column of increased film thickness in combination with high pressure injection.

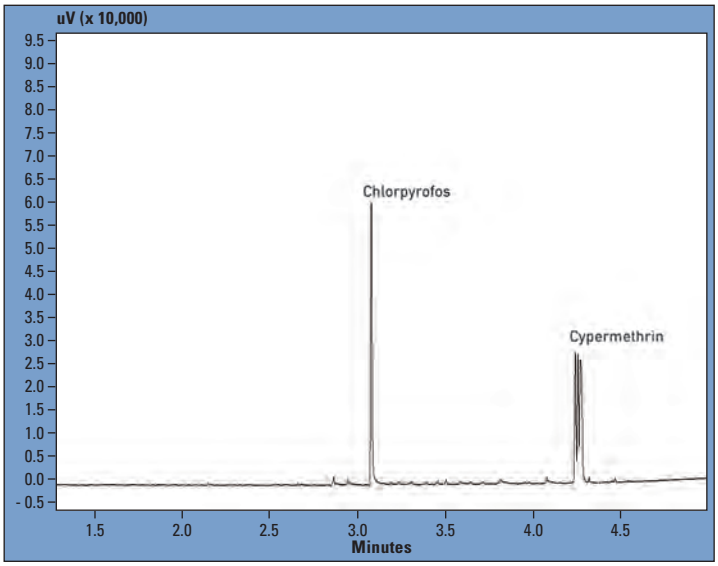


Figure 4: GPC eluate (S 19) of a grape sample measured with Fast GC-ECD: Chlorpyrofos 0.53 ng/mL (corresponds to 0.48 ng/kg grapes) and cypermethrin 0.55 ng/mL (corresponds to 0.5 ng/kg).

References

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[2] Baier, H.-U. and Mondello L.: Die schnelle Gaschromatographie in der Lebensmittelanalytik in Schnellmethoden zur Beurteilung von Lebensmitteln und deren Rohstoffen Kap. 3.2, Behrs Verlag 2004. Im Druck.

[3] Hinshaw. J.: LCGC (2002) vol 15 p. 152

		RT	Conc (ppb)
01 Pentachlorobenzole	Target	2.23	21.3
02 Tecnazene	Target	2.37	22.5
03 Benfluraline	Target	2.58	52.8
04 alpha-HCH	Target	2.548	22.1
05 HCB	Target	2.613	24.1
06 Pentachloroanisole	Target	2.631	20.6
07 beta-HCH	Target	2.598	20.4
08 Lindane	Target	2.662	28.8
09 delta-HCH	Target	2.818	23.2
10 epsilon-HCH	Target	2.683	1
11 Pentachloroaniline	Target	2.842	26
12 Heptachlor	Target	2.96	30.4
13 Aldrine	Target	3.109	21.7
14 Isobenzane	Target	3.065	5
15 Bromophosmethyle	Target	3.139	22.6
16 Isodrine	Target	3.163	22.04
17 cis-Heptachloroepoxide	Target	3.196	25
18 trans-Heptachloroepoxide	Target	3.206	25
19 Bromophosethyle	Target	3.291	50.36
20 trans-Chlordane	Target	3.325	5
21 cis-Chlordane	Target	3.469	5
22 p,p`DDD	Target	3.508	22.4
23 Mirex	Target	3.961	21.84

Table 1: Concentration of the OCP standard

Organophosphorus pesticide determin

Shimadzu's GCMS-QP2010 Plus

- **Qualitative and quantitative determination of pesticides in food**
- **Time matters**
- **Maximum sensitivity**

The analysis of organophosphorus pesticides in environmental and food samples is one of the most challenging tasks in analytical chemistry. The numerous food scandals in recent years demonstrate the immense importance of comprehensive quality control.

Therefore, it is necessary to detect the contaminants of interest at the lowest possible concentration levels in the sample. Analytical instrumental procedures, especially the chromatographic methods offer very reliable tools for the qualitative and quantitative determination of pesticides in food- and environmental samples.

Especially GC-MS is well suited for the analysis of pesticides with its excellent sensitivity.

In the EI mode compounds can be unequivocally identified via their classical EI spectra, acquired in the scan mode. This is performed via comparison with mass spectral libraries. Shimadzu offers a unique pesticides library containing 578 MS spectra obtained in EI mode, and 383 MS spectra recorded in NCI mode. Additionally to the mass spectra the Pesticide library also contains the Linear Retention Indices for all compounds. The use of Linear Retention Indices as search criteria in libraries greatly enhances the reliability of identification. Negative chemical ionization (NCI) is especially suitable for highly sensitive detection of organophosphorus pesticides. Using this ionization method, it is possible to achieve quantitative and accurate determinations down to the fg-range. NCI is a selective analytical technique which enables the detection of compounds that, on the basis of their chemical structure, are able to capture electrons. NCI applications are, for example, the detection of chlorinated pesticides and phosphoric acid ester pesticides (for example lindane, chlorpyrifos).

EI and NCI can be carried out on the GCMS-QP2010 series without hardware modifications using the same ion source (Combi Source). The user simply indicates in the GCMSsolution software which ionization mode is to be applied for the analysis. The system is then optimized automatically via a dedicated tuning procedure for both modes.

Precise Fast GC

Time plays an important role in pesticide analysis. The analysis results must be available as soon as possible so that, in the case of positive results, the necessary measures can be taken immediately. Also in this respect, the GCMS-QP2010 lives up to its outstanding qualities: with the proven GC-2010 system in the

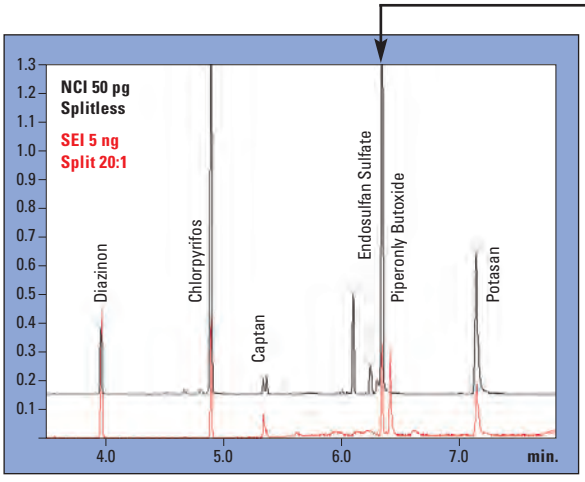

Figure 1a: Fast SEI and NCI-Scan of an organophosphorus pesticide standard

ation with GC-MS in EI and NCI mode

Fast GC mode, results become available almost immediately. Important features are the high scan rate of the MS detector of up to 10,000 amu/s and the high data acquisition rate (up to 50 spectra/s). These features allow, also for peaks with a width of significantly less than 1 second, acquisition of accurate mass spectra that are suitable for matching against an MS library.

Figure 1a shows the chromatogram of a pesticide standard using Fast GC-MS in the EI and NCI scan mode. A 10 m column with an internal diameter of 0.1 mm and 0.4 mm film thickness (RTX-5) was used. The chromatograms were obtained in the constant linear velocity mode using a carrier gas velocity of 50 cm/s. The retention time for the pesticide endosulfan sulfate was less than 6.5 min. Figure 1b shows the mass spectra of endosulfane sulfate in EI and NCI mode.

Endosulfan sulfate and another pesticide, chlorpyrifos, have been detected in a tea sample. Figure 2 shows the NCI data of a mixture prepared with a non-contaminated matrix (0.5 pg of each component was added) and the result of the tea sample (method DFG S19). Unequivocal identification of endosulfan sulfate and chlopyrofos can be obtained down to the low fg range.

The data impressively demonstrate the suitability of fast NCI/GCMS for the determination of organophosphorus pesticides for natural matrices with very high sensitivity and precision. This is an important prerequisite in routine analysis.

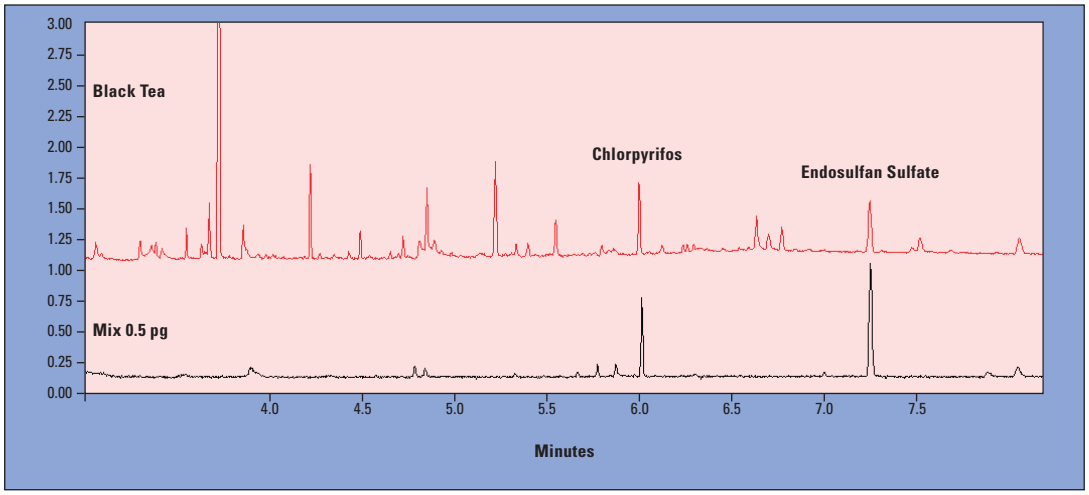

Figure 2: Fast NCI-Scan of a pesticide standard solution and a real tea sample

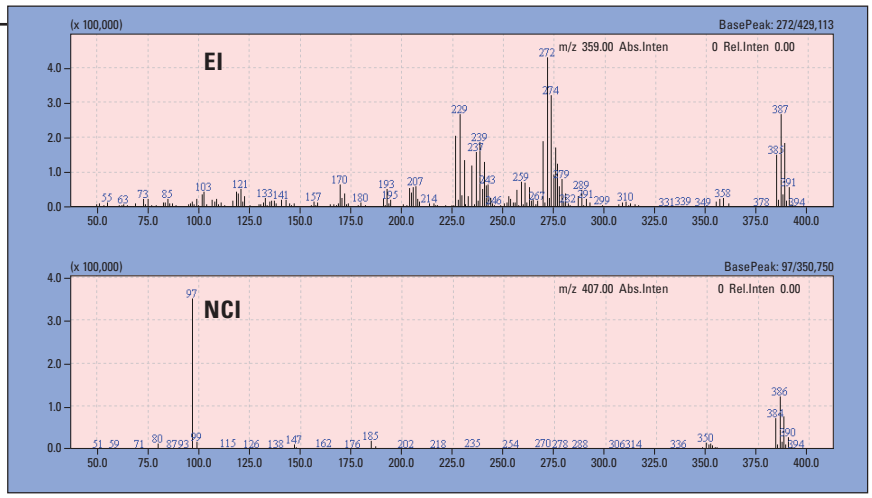

Figure 1b: EI and NCI Spectra of Endosulfane sulfate, respectively

"High" on hemp beer? Unusual study investigates beer consumption and positive THC test results

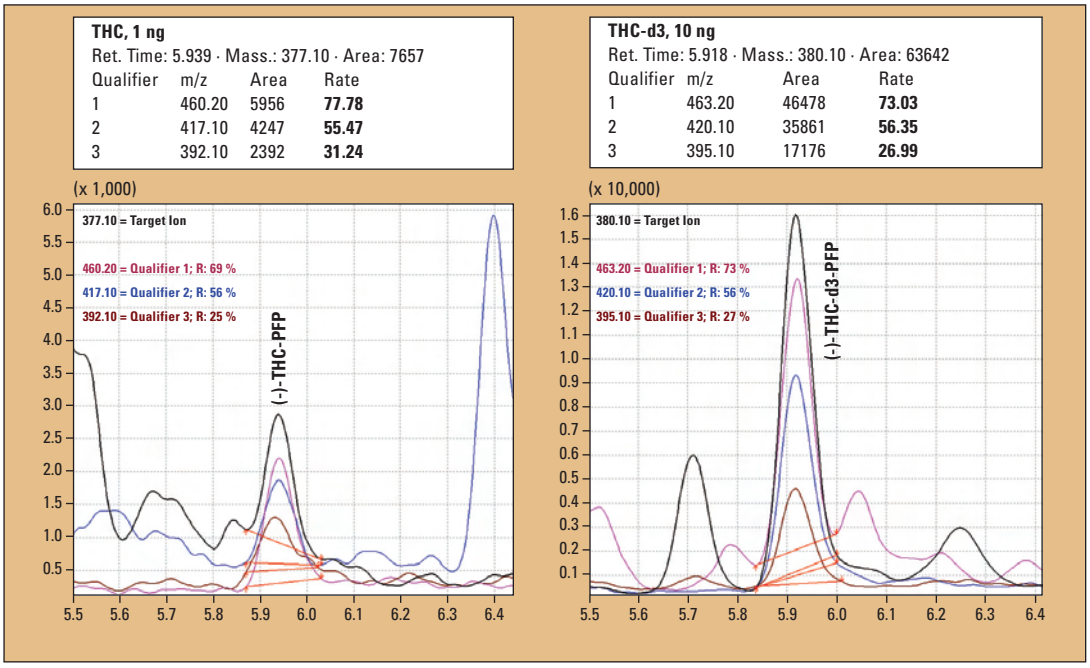

Figure 2: Sample chromatograms from the matrix calibration with spiked 'Beck's' beer: mass traces of THC (1 ng/mL) and the internal standard THC-d3 (10 ng/mL) after solid-phase extraction of 1 mL sample and PFPA derivatization. The insert shows the ratios of the individual qualifier ions to the target ion, determined under optimal conditions.

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- **Method development**
- **Sample pretreatement**
- **Analysis using GCMS-QP2010**

A company which for reasons of safety regularly spot-checks its employees for drug use heard an unlikely story when a 23-year old employee tested positive with considerable traces of THC, indicating use of hashish or marijuana. According to the young man, he had simply been drinking hemp beer on the previous night, although this still amounted to 15 bottles of the German hemp-containing 'Cannabis Club' specialty beer.

A urine sample tested positive for cannabis metabolites. He had been randomly selected for a drug-use screening test. A screening test was carried out on the urine sample via enzyme immunoassay (CEDIA) and tested positive for cannabinoids at a level of > 150 ng/mL, clearly indicating use of tetra-hydrocannabinol (THC) containing drugs such as hashish or marijuana. The result was confirmed by a laboratory in Stockholm, Sweden* and the concentration of THC carboxylic acid, the THC main metabolite, was determined at a comparatively high level of 650 ng/mL.

Figure 1: Two of the authors in 'stressful' experimental conditions

The young man's statement has raised many questions:

- Is the import of hemp beer into Sweden even permitted?
- Does consumption of hemp beer lead to a positive THC test result?
- How much hemp does such a 'specialty beer' contain?
- How much hemp beer does one need to drink in order to reach a urine concentration level of > 150 ng/mL?

A laboratory in Dessau, Germany tackled these questions after receiving a request for assistance from their colleagues in Stockholm. And the German team certainly did not shy away from heroic self-testing!

Hemp in beer

Hemp has played an important role as a crop plant, food and raw material for thousands of years (see separate box on page 24). Since early days, hemp was used in the brewing of beer. But in the 16th century, the 'German (beer) purity law', displaced hemp as a flavoring compound for beer. Meanwhile, hemp is experiencing a renaissance as a food additive and also in the form of 'hemp beer', available from various breweries. Under federal regulations, the hemp content in alco-

holic and non-alcoholic beverages in Germany may not exceed a value of 5 µg/kg ('German Federal Institute for Consumer Health Protection and Veterinary Medicine', known as 'German Federal Institute for Risk Assessment' since 2002).

The study

The laboratory in Dessau was able to order the German beer in question via the Internet (www.hemp-wholesale.com). However, the young man had brought the beer back to Sweden after his holiday in the Netherlands. A request by the Stockholm laboratory for more information from the Swedish Customs Office learned that this beer was well known and was considered to be quite safe – based on the low THC concentration listed on the label and the fact that the THC content was not detectable with the methods available in the Customs Office laboratory. The 'specialty beer' brewed in Bavaria listed the following ingredients: 95 % beer, 5 % spring water flavored with hemp (ethereal hemp oil), 4.9 vol % alcohol with a THC content of < 0.05 mg/kg.

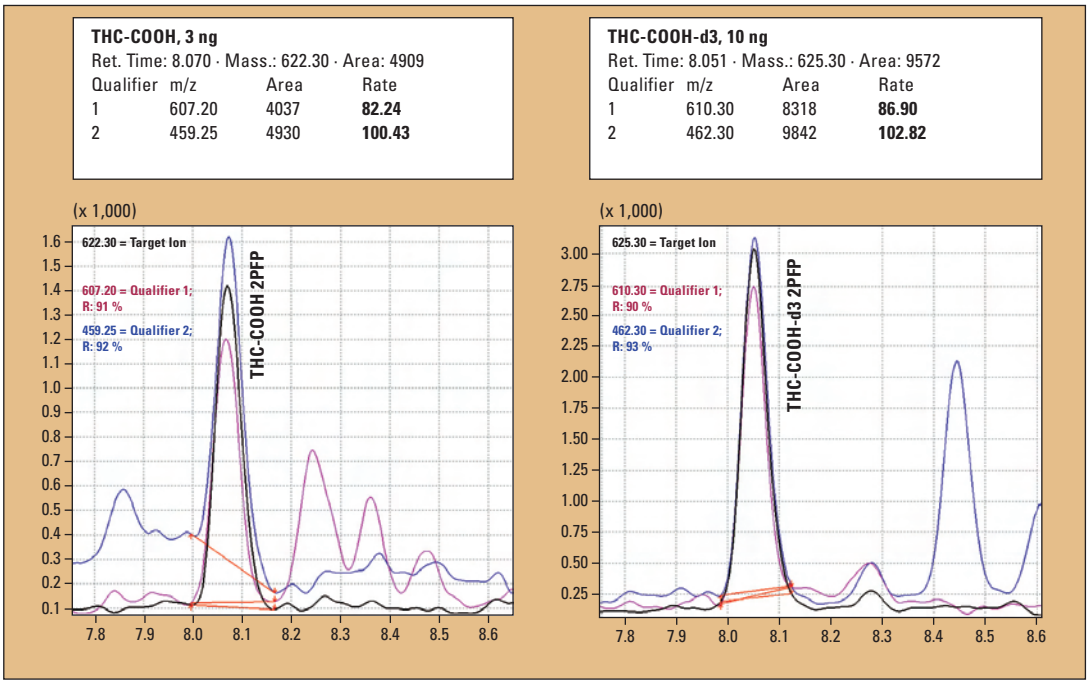


Figure 4: Sample chromatograms from matrix calibration with spiked pooled urine: mass traces of THC carboxylic acid (3 ng/mL) and the deuterated internal standard (10 ng/mL) after solid-phase extraction of 1 mL sample and PFPA/PFPOH derivatization. The insert shows the ratios of the individual qualifier ions to the target ion, determined under optimal conditions.

At first, a method had to be developed that could determine the THC content in beer. According to the 'German purity law', the THC-free 'model beer' 'Beck's' (Beck Brewery, Bremen, Germany) was used as reference. For matrix calibration, 1 mL sample volumes of this beer were mixed with different THC concentrations and with 10 ng THC-d3 (internal standard), respectively. After sample pretreatment using solid-phase extraction and acylation with PFPA (pentafluoropropionic acid anhydride), the samples were analyzed using Shimadzu's GCMS-QP2010 in the SIM mode (Figure 2). Prior to GC-MS analysis, carbon dioxide was removed by heating the beer and applying ultrasound. The detection limit of THC calculated according to DIN 32645 was 0.25 ng/mL (Figure 3).

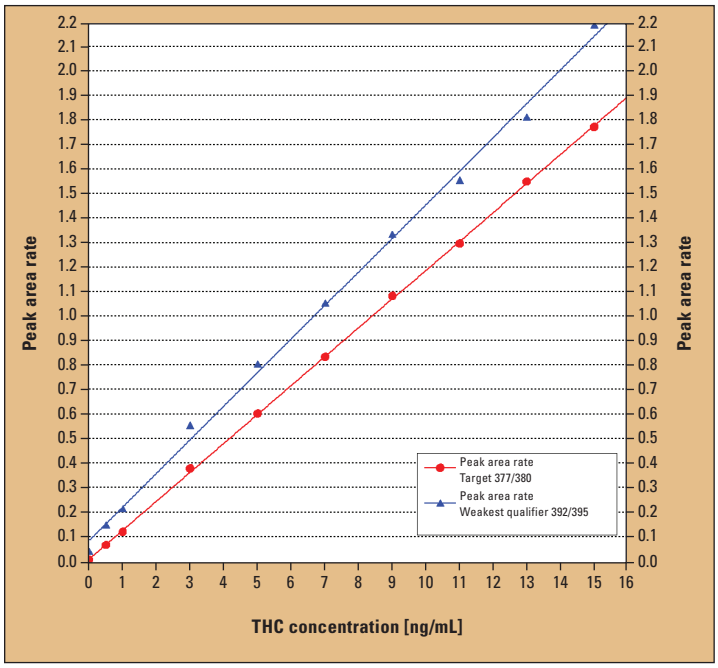


Figure 3: THC matrix calibration in 'Beck's' beer

Suitable initial experiments with the 'Cannabis beer' quickly revealed that a higher sample volume needed to be prepared for accurate quantification. After preparing 5 – 10 mL sample volumes, it was possible to determine a THC content of 3.2 and 2.2 ng/mL in two samples, whereas the THC content determined in 3 samples of a different batch was only 1.0 ng/mL. In all cases, the THC concentration was clearly under the legal limit of 5 ng/mL. This clearly pointed to the fact that the THC metabolite concentrations in the urine sample of the test person could not originate from the consumption of 15 bottles, each containing 0.33 L of beer.

Fun science – the 'self-experiment'

In a fearless self-experiment, three of the authors volunteered to drink 7 bottles of 'specialty beer' each within 3 hours (Figure 1). During this drinking session and also afterwards, urine samples were collected over a

time period of approximately 40 hours and analyzed for THC carboxylic acids using a routine method. For this purpose, 1 mL aliquots of the samples were mixed with 10 ng THC carboxylic acid-d3 (internal standard) and subjected to alkaline hydrolysis and subsequent solid-phase extraction. After derivatization with PFPA/PFPOH (pentaflourpropionic acid anhydride/pentafluoro-propanol), the samples were analyzed using the GCMS-QP2010 in the SIM mode. The detection limit according to DIN 32645 was determined via matrix calibration from the spiked THC carboxylic acid-free pooled urine with 0.54 ng/mL (Figures 4 and 5).

Despite all efforts, THC carboxylic acid could only be detected in a single sample (approximately 2.5 hours after the last bottle of beer was consumed, at a concentration of 0.38 ng/mL). Also for sample volumes of 5 mL, none of the main metabolites was detected in any of the samples.

Lies don't go far

The results clearly disproved the statements of the test person, even when accepting that resorption and metabolism to THC carboxylic acid (elimination kinetics) may proceed at different rates depending on the individual. The 'specialty beer' cannot be held responsible for the high THC carboxylic acid concentration detected in the test person's urine sample. Obviously, he had used other THC-containing substances in addition to drinking the beer.

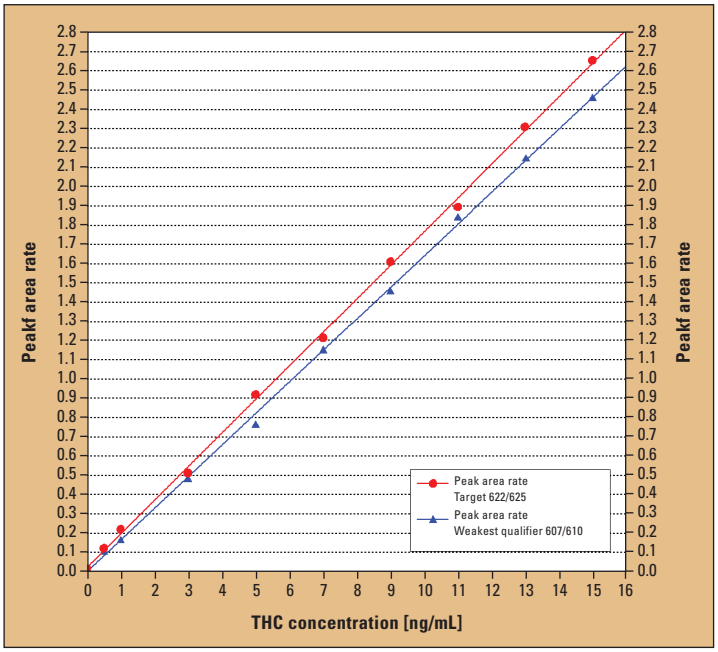


Figure 5: THC carboxylic acid matrix calibration in pooled urine

Hemp – a very old crop plant

Hemp is one of the oldest and most valuable crop and raw material plants, which has also played an important role in our society up to the beginning of the twentieth century.

Already 12,000 years ago, hemp was cultivated as a crop in Persia and China. Hemp seeds were eaten, their fibers were used to make clothing and later in the production of paper. In fact, hemp was the most commonly cultivated economically useful crop.

In the 13th century, hemp was brought to Europe and became the raw material for paper. The hemp plant was also popular for its medicinal powers. However, the popularity of hemp slowly declined in the industrial revolution. Synthetic compounds quickly displaced cannabis for medicinal purposes. Cotton and wood lobbyists banned hemp from the market and the industrial use of hemp became unprofitable. Lastly, hemp became disreputable in the 1930s because of its use as a raw material for drugs such as hashish or marijuana.

Hemp in foods

Hemp belongs to the Cannabaceae family and is related to the hop. Only the female flowers of the dioecious plant form the physiologically nutritional seeds and the strongly smelling and aromatic resin that has provided hemp with its famous-notorious reputation in the form of hashish and marijuana. Depending on the designated use and THC content, various types of hemp can be distinguished. Plants or plant parts with a THC content of more than 0.3 % are designated as drugs according to Appendix 1 of the German Narcotics Act and are therefore prohibited.

Hemp seeds are botanically classified as nuts and are used as additives in various types of foods – either directly as ground hemp nuts or as ethereal oil. Hemp seeds, as well as hemp oils derived from the seed, have a fine 'nutty' and somewhat 'lemony' taste and are also very healthy. They contain vitamin B, B1, E and F as well as essential fatty acids (linoleic and linolenic acids) in relatively high concentrations. Addition of hemp products, therefore, improves the taste and nutritional value of foods.

Seeds and their seed coats, in contrast to the surrounding hulls and resin, do not contain even traces of psychoactive THC. THC can only enter food products when the seeds are not cleaned thoroughly. Therefore, danger of psychoactive effects due to consumption of hemp-containing foods is non-existent.

Bisphenol A – Consumption inevitable

Fast determination of bisphenol A residues in plastic bottles, containers and cans

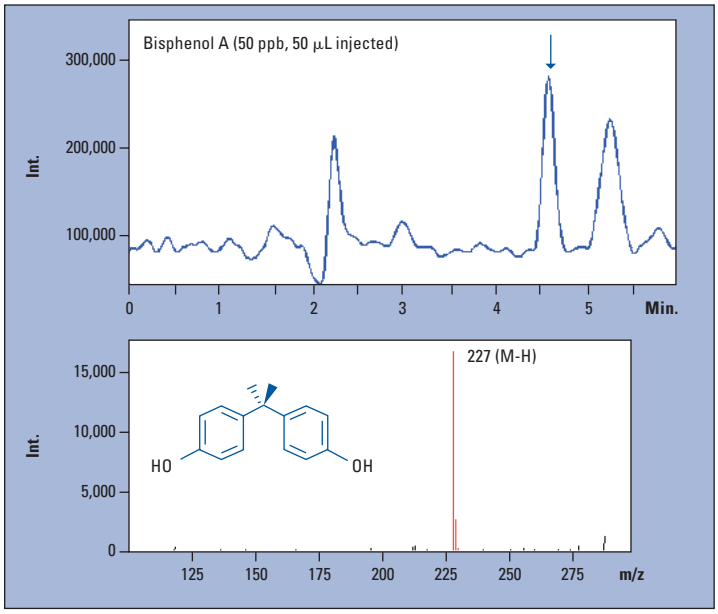


Figure 1: Bisphenol A: structural formula, TIC (scan neg. 100 – 300 m/z) and mass spectrum (M-H)-

Bisphenol A (2,2-Bis(4-hydroxyphenyl) propane) is one of the most significant industrial chemicals worldwide and has been used for 50 years in the manufacture of polycarbonates and epoxy resins for various applications. Polycarbonates are used as packing materials (plastic bottles, airtight containers), as lenses, as data carriers (CDs) and as foils. Epoxy resins are used as adhesive-, lacquer and cast resins for instance for coating of cans (BADGE – bisphenol A diglycidyl ether) and dental fillings. In Germany alone, more than 400,000 tons of bisphenol A are used every year.

Results from the USA

The estrogen-like action of bisphenol A (BPA) has been known since the 1930s. As the release of BPA from plastic materials and resins was at that time believed to be low, the health risk for humans was considered to be negligible. Now that scientists in the United States have found new evidence for the teratogenic effect of BPA, this compound is again the center of attention. Researchers have determined that very low BPA dosages can lead to genetic defects in mice.

Environmental agency advises restriction

Polycarbonates based on BPA used for baby bottles and drinking mugs and epoxy resins used for coatings of cans have been increasingly critically examined. Although toxicologists have not agreed until now on the assessment of the health risks caused by BPA and the use of BPA in baby bottles could not be prohibited, the German Federal Environmental Agency had already requested urgently to restrict the use of BPA in food packaging and especially in baby bottles.

LC-MS offers higher sensitivity

BPA is commonly analyzed via GC-MS or HPLC. In order to attain a higher sensitivity and selectivity for the determination of traces of BPA in food samples, an HPLC method with mass spectrometric detection was developed and is described below. The single quadrupole LCMS-2010A was used as mass spectrometric detector. Using this method, BPA could be detected accurately in the lower ppb range. The chromatographic separation could be carried out in less than 5 minutes using a 2 mm reversed phase column. The BPA is deprotonated during electrospray ionization and shows a mass of 227 m/z in the negative mode.

Using the SCAN mode in the total ion chromatogram (TIC), 50 ppb BPA can be detected. In the corresponding mass spectrum the quasi-molecular ion (M-H)- is detected at 227 m/z (Figure 1).

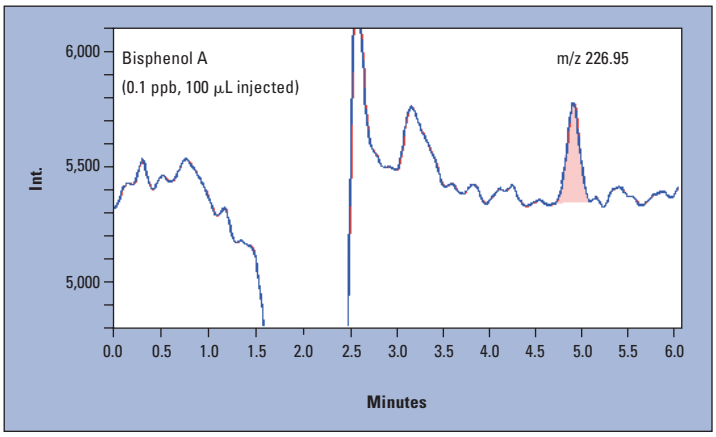


Figure 2: Detection of 0.1 ppb bisphenol A in SIM mode

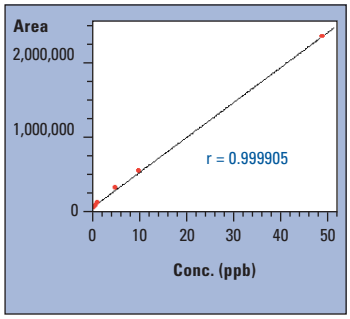


Figure 3: Calibration 0.05 up to 50 ppb

In the single ion-monitoring mode (SIM mode) for mass 227, BPA can be determined at concentration levels as low as 0.05 ppb (Figures 2 and 3) using this method. The use of such highly sensitive detection methods for BPA residues in foods allows for accurate monitoring of released BPA amounts and forms the basis of further investigation into health risks which can result from the use of BPA in packaging materials.

- **HPLC method with mass spectrometric detection**
- **Sensitivity covers lower ppb range**

Colorful, but hazardous: forbidden dyes

Fast LC-MS determination of Sudan Red I-IV, Butter Yellow and Para Red

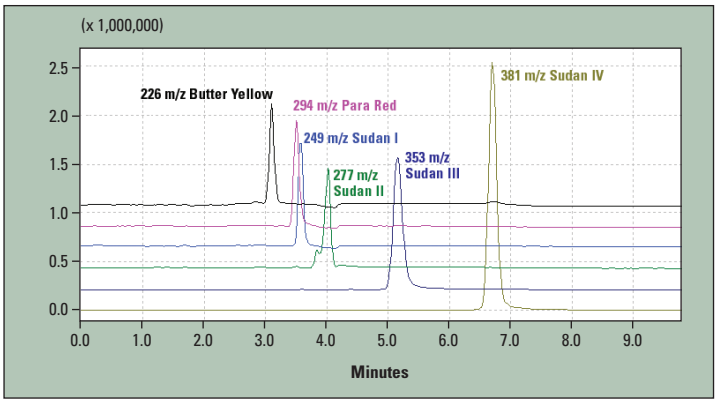


Figure 1: Mass traces of the separation of Butter Yellow, Para Red and Sudan Red I - IV on a Pathfinder® AS Silica 100 column (3.5 µm, 2.1 x 50 mm) in the positive mode

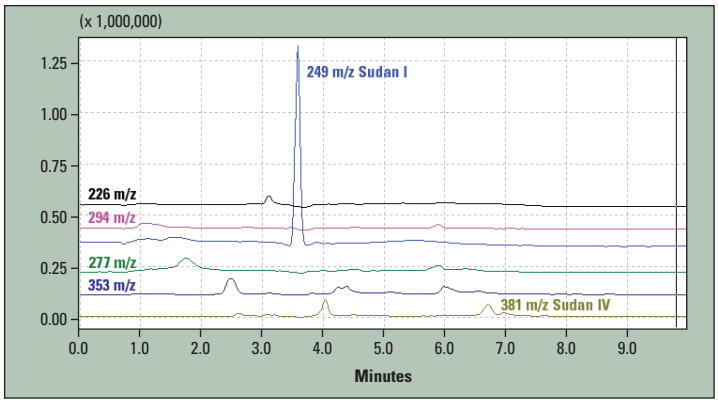


Figure 2: Chromatogram of an extract of a chicken spice mixture containing the prohibited Sudan Red I and IV, dyes, measured in the positive ionization mode

- **Analytical methods with highest sensitivity and reliability needed**
- **Mass spectrometry is the preferred detection mode**
- **HPLC, APCI, LC-MS**

In May 2003, the European early warning system issued a warning from France on the detection of Sudan Red dye in Indian chilli powder. During further investigations, the dyes Sudan Red II - IV had also been detected in various foods such as meat products, colored pastas, paprika powder, spice mixtures, tomato sauces as well as palm oils.

Sudan dyes are synthetic, fat-soluble red and orange-colored azo dyes. They are used as colorings for numerous household products such as floor waxes or shoe polish. However, the use of azo dyes is no longer permitted in foods, since the European Union has banned the use of Sudan-based dyes as food colorings.

In addition to the Sudan dyes, the 'Chemisches Untersuchungsinstitut Bergisches Land' in Wuppertal, Germany has detected Butter Yellow (dimethyl yellow) in curry and nitroanaline red (Para Red) in paprika powder. These dyes are also prohibited as food colorings.

Since January 2004, chillies and chilli products are specifically tested for the presence of Sudan Red I - IV upon import. These spices and foods may be imported into the EU only when the appropriate analysis certificate is present. In addition, food control authorities carry out random checks on products on the market. In the first half of 2004, 125 violations had already been detected.

LC-MS for selectivity and sensitivity

In chilli powders and spice mixtures, the prohibited dyes were usually detected in relatively high concentrations. When these spices were used in the production of meat products, however, the concentration of the prohibited dyes decreased down to several milligrams per kilogram in the end product, for instance in spicy sausages. Since a positive test result for these dyes requires the complete shipments to be destroyed, the applied testing procedures require analytical methods with the highest sensitivity and reliability.

The usual detection methods for Sudan dyes use liquid chromatographic separation methods, usually coupled to diode-array detection. In order to increase the sensitivity as well as the selectivity, mass spectrometry is the preferred detection mode. The above-mentioned dyes can be ionized using common LC-MS ionization modes such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). In the method proposed by the 'Chemisches Untersuchungsamt' of Bielefeld, Germany, the analytes of interest are separated using semi-micro HPLC and are detected via APCI and a single-quadrupole mass spectrometer (Shimadzu LCMS-2010).

Fast detection method

The sample preparation procedure is very straightforward. One gram of sample is extracted with acetonitrile for 30 min. An aliquot is withdrawn and filtered. The sample is subsequently injected directly into the LC-MS system. Depending on the dye

in foods

Dr. Heinz-Dieter Winkeler, Chemisches Untersuchungsamt in Bielefeld, Germany

concentration, a dilution step may be necessary.

The Sudan Red dyes I - IV are extremely lipophilic azo dyes which are strongly retained on reversed-phase columns. Short analysis times can be attained using short chromatographic columns. For the proposed application, a 50 x 2.1 mm column was selected. A water/methanol gradient (65 - 100 %, 200 µL/min) was used to elute the six dyes from a Shimadzu Pathfinder® AS column within seven minutes. Figure 1 shows the chromatogram of mass traces of a separation of the four Sudan Red dyes as well as Butter Yellow and Para Red.

The extrapolated detection limits in a matrix-adjusted calibration setting are, for Sudan Red I - IV and Butter Yellow between 5 and 20 pg total (RMS noise of S/N 5:1) and 20 pg total for Para Red. Using this method with 10 µL injection volumes, concentrations into the lower µg/L range can be detected.

As the concentrations of Sudan Red detected in spices were between 2 and 4 g/kg and in spice mixtures between 40 and 150 mg/kg, the attained detection limits are at present sufficient. Experiments with spiked matrix solutions showed no significant ion suppression for all analytes. Spiked matrix samples were linear in the tested range of 0.1 mg/L up to 2.0 mg/L.

Figure 2 presents the chromatogram of mass traces of an extract of a positively tested chicken spice mixture (1 : 10 dilution, 1 µL injection volume). In this extract, 132 mg/kg Sudan Red and 5 mg/kg Sudan Red IV were detected.

Apart from Butter Yellow, the dyes can also be ionized in the negative mode. Negative ionization can, for instance, be used for the verification of inconclusive analysis results obtained during the positive ionization mode.

Summary

The single quadrupole LC-MS system is a sensitive and selective method and is especially suitable for the determination of Sudan Red I - IV, Butter Yellow and Para Red. The robustness of the instrument allows a simple sample preparation procedure. With a 50 mm long separation column, the dye mixture can be analyzed within seven minutes. The detection limits for the azo dyes using this method are within a range of 5 - 20 µg/kg food sample, and more gain in sensitivity can be attained through variation of the sample preparation procedure or increasing the injection volume.

LC-MS conditions		
Separation column:	Pathfinder® AS 100; 50 x 2.1 mm; 3.5 µm Shimadzu/Shant Laboratories	
Eluents:	A = Water (Milli-Q®), B = methanol (HPLC-grade, Roth)	
Gradient:		
Initial concentration:		65 % B
0 - 1 min	linear up to	95 % B
1 - 3 min	linear up to	100 % B
3 - 10 min	isocratic	100 % B
5 min	equilibrate at	65 % B
Flow rate:	200 µL/min	
Temperature:	55 °C	
MS-parameter positive ionization		
Mode:	Atmospheric Pressure Chemical Ionization (APCI) positive	
Scan range:	100 - 500	
SIM masses:	Sudan I	249 m/z $[MH]^+$
	Sudan II	277 m/z $[MH]^+$
	Sudan III	353 m/z $[MH]^+$
	Sudan IV	381 m/z $[MH]^+$
	Butter Yellow	226 m/z $[MH]^+$
	Para Red	294 m/z $[MH]^+$

Bovine blood in chocolate... Spectrosco

Model	ICPE-9000
Frequency	27.120 [MHz]
Output Power	1.3 [kW]
Cooling gas flow rate	16 [L/min]
Plasma gas flow rate	1.2 [L/min]
Carrier gas flow rate	0.7 [L/min]
Nebulizer	Coaxial nebulizer
Chamber	Cyclone chamber
Plasma torch	Standard torch
Observation	Axial & Radial

Table 1: Instrumental conditions

Modern myths tell not only of the tarantula which crawled out of the Yucca plant and suddenly appeared in the living room, but also of the story of bovine blood and all of the foods to which it is supposedly added – red jelly babies, red wine and even chocolate (including white?). But consumers, parents, children and "sweet teeth" can rest assured: their favorite "melt in the mouth" treats may be enjoyed without any worry.

The General Food Law of the European Union regulates the use of ingredients and additives in food products, and excludes blood as a permitted additive. Bernd Schartman of the Swiss chocolate company Lindt & Sprüngli refers to two possible origins of the "bloody" rumor. In the first, a research project in the former Eastern Germany investigated the use of dried blood in order to enhance the color of chocolate. Whether this research has actually been applied is not known. In any case, no one needs to worry today. The second possible source: a resourceful inventor applied for a patent for a process that could be used to increase the protein content of foods by adding blood. However, the patent was never issued [2].

Food safety strictly monitored

In Germany, the Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)) ensures clarity with respect to food safety. The BLV carries out many tasks in food analysis including publishing a collection of official procedures for sampling and analysis of foods, tobacco products, cosmetic products and consumer goods. The research results are statistically tested and standardized in round robin tests. The BVL operates a national and an EU reference laboratory for pharmacologically active residues and contaminants, which tests for compliance with the maximum allowable values.

Chromatographic and spectroscopic analyses are used to determine threshold values in residue monitoring, as well as for quantitative analysis of contaminants. UV-VIS spectrometers are suitable for qualitative and quantitative analysis of food samples in the absorption, transmission and reflection mode. FTIR spectrometers from near to the far infrared range are used for specific identification of analytes. The fluorescence spectrometry completes the entire bandwidth of molecular spectroscopy for high-sensitivity quantitative analysis.

Quantification of trace and ultratrace elements in aqueous and organic solutions is carried out using atomic absorption and ICP spectrometers at the highest sensitivities and lowest detection limits, as well as with X-ray fluorescence spectrometers which also allow analysis of solid samples without prior sample preparation.

Legal basis for the control of popular beverages

Heavy metals present as natural constituents of the Earth's crust are under continuous investigation as food contaminants. Some heavy metals such as copper, zinc, iron and manganese are essential to organisms in trace concentrations. Other heavy metals, however, do not have any identifiable metabolic function and can be toxic already at very low levels. Lead, cadmium and mercury are considered as dangerous and toxic and have been banned under the "ElektroG" (Electrical and Electronic Equipment Act) since July 2006. This directive governs the sale, return and environmentally sound disposal of electrical and electronic equipment.

Contamination of food products with these heavy metals is primarily caused by industrial activities such as the release of industrial and traffic exhaust gases into the atmosphere where they are subsequently transported via air currents before settling onto the Earth's surface. The heavy metals also enter into the soil through fertilizers such as sludges where they are enriched, or seep into deeper layers down into the groundwater. Through migration, they ultimately end up in the food chain.

Drinking water is the most important source of food and its quality has been assured since 2003 in the revised European Drinking Water Directive (TVO 2001). The European Drinking Water Directive is aimed at the protection of human health from polluted water. The directive covers all types of water, whether used for drinking, cooking, for preparation of meals and beverages as well as for personal hygiene and cleaning of objects that come into contact with foods. The average water consumption in German households is approximately 130 liters per person per day (for international figures, see Figure 1), 4 liters of which is used as drinking water. While the total water consumption has continuously decreased over the past 20 years, the consumption of carbonated mineral water has doubled within the same timeframe. In 1985, a German citizen was still content with 57 liters per day, while the consumption in 2005 was, statistically considered, almost 114 liters. The legal provisions for mineral water and drinking water are different. Although official authori-

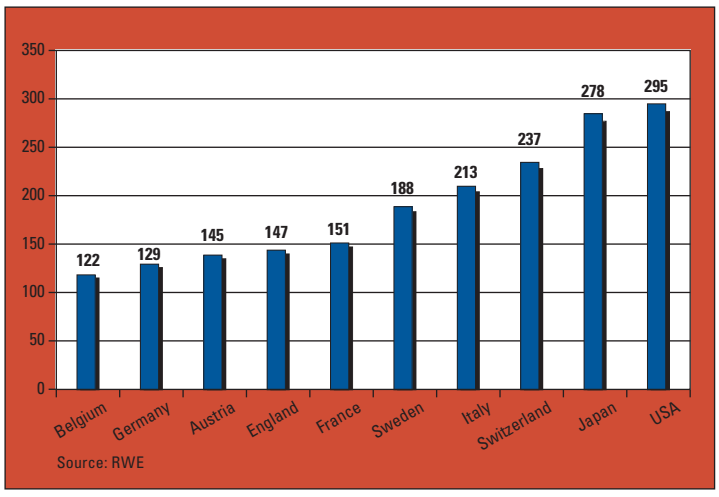


Figure 1: Daily water consumption (liter per capita)

py in food analysis

zation and quality control via chemical analysis is required for the distribution of mineral water, the requirements are not quite as rigorous as for drinking water from the tap. Only ten ingredients must be tested for compliance with threshold values. Mineral water is controlled most frequently by private companies. The analysis results and the inspecting authority carrying out the tests must be identified on the label.

The situation is very different for two other, very popular beverages in Germany: beer and wine. The average beer consumption per person was, at 115.2 liters, slightly above that of mineral water (for European figures, see Figure 2). Beer is, therefore, together with tap water, one of the most popular beverages. Beer brewing and beer quality have been regulated by law in Germany for almost 500 years.

An additional law, the German Order on Wines (Weinverordnung) dating from 22 May, 2002 (Federal Law Gazette Part 1, No. 31) includes the classification of specific regions of wine production (such as for German table wine or Vin de Pays) and provides guidelines on cultivation and processing as well as testing procedures and maximum allowable concentrations of important elements such as aluminum, cadmium, zinc and others.

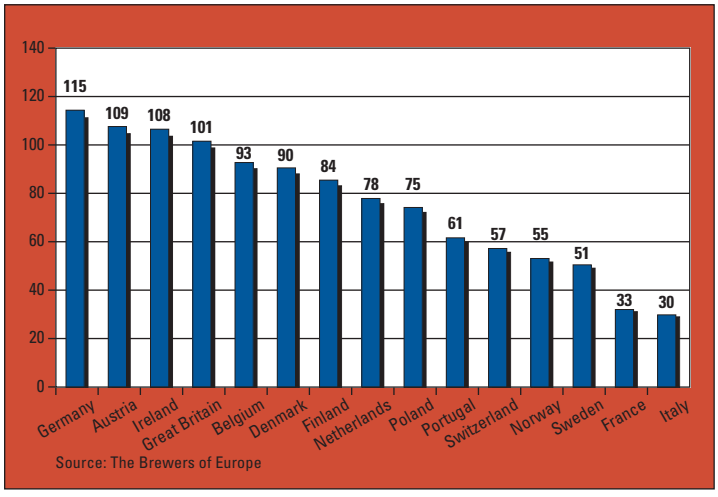


Figure 2: Annual beer consumption (liter per capita)

Multi-element analysis using the ICPE-9000

Shimadzu's ICPE-9000 ICP spectrometer, which excels through high sensitivity, a wide dynamic range and high sample throughput, was used for the quantitative determination of the above metals according to the Wine Ordinance. This simultaneous ICP with CCD detector (charge-coupled device) is equipped with a unique optical system setting new standards with its high performance and speed.

In the ICPE-9000, the liquid samples are vaporized in the plasma torch and the atoms and ions released during vaporization are exited to emit radiation. The optical system processes the emitted radiation, which is subsequently detected using the CCD detector whereby the emission spectra of all elements under investigation are recorded.

The high sensitivity of the detector enables accurate resolution, even of very closely neighboring elemental emission lines such as copper (213.60 nm) and phosphorus (213.62 nm). The intensity of the radiation is proportional to the concentration of the elements present in the sample. The quantitative determination of the elements was carried out against calibration curves from aqueous multi-element standards which, in the case of wine analysis, were adjusted with 15 % ethanol to resemble a wine matrix.

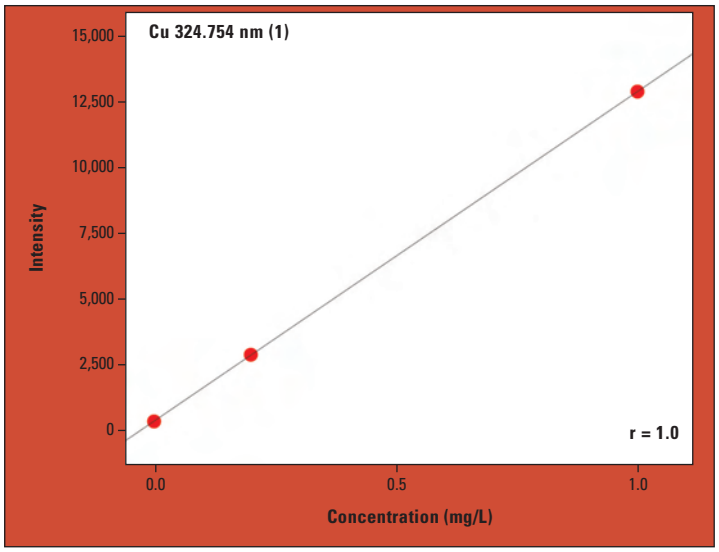


Figure 3: Copper calibration curve

Figure 3 shows the calibration curve of copper in the concentration range up to 1 mg/L. The elements calcium, magnesium, potassium, sodium, iron, titanium and zinc were analyzed according to the same procedure, whereby the axial as well as the radial acquisition mode was applied. The relative standard deviations of the analysis results for five replicate measurements were lower than 1.5 % for these elements. The instrumental parameters are summarized in Table 1.

Summary

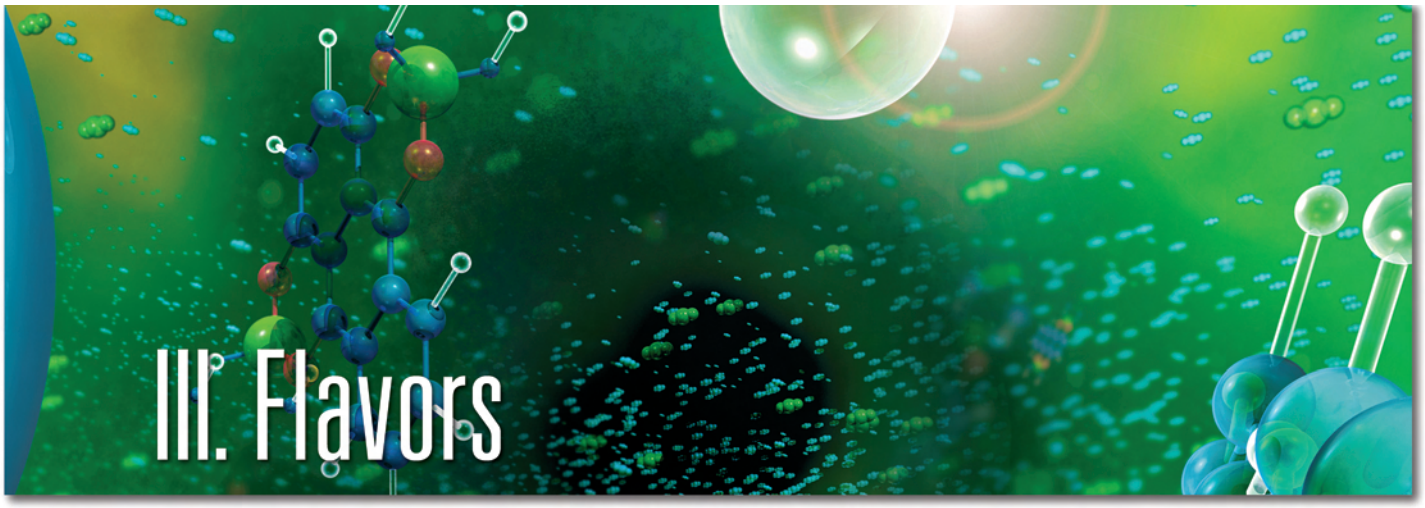
Continuous control of our foods and beverages according to international standards such as the European Drinking Water Directive and the German Order on Wines assures users of the highest possible safety standards together with a constantly high quality. With the simultaneous multi-element ICPE-9000 ICP spectrometer, Shimadzu offers a modern tool for the reliable determination of all relevant parameters in these types of applications.

- **UV-VIS, FTR, ICP**
- **Multi-element-analysis with high sensitivity**

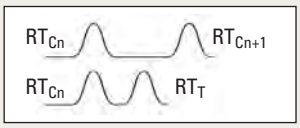
References

[1] Aufgaben des BVL im Bereich der Lebensmittelanalytik, Website: www.bvl.bund.de http://www.bvl.bund.de/cln_007/nn_491400/DE/01_Lebensmittel/04_Lebensmittelanalytik/LebensmittelAnalytik_node.html_nnn=true

[2] Blut in Schokolade und in Nougatcremes Website: www.ammenmaerchen.de http://www.ammenmaerchen.de/overview.php?did=127&title=Blut-in-Schokolade-und-in-Nougatcremes


III. Flavors

Sniff, sniff, take a whiff Accurate determination of flavors and fr



RT : Retention Time
RT_{Cn} : RT for n-alkane with n-carbons
RT_{Cn+1} : RT for n-alkane with n+1-carbons
RT_T : RT for target compound

- **The retention time is calculated using n-alkanes as reference standards**
- **LRI = 100 x n + 100 x (RT_T - RT_{Cn}) / (RT_{Cn+1} - RT_{Cn}) (Temperature Program Condition)**

Figure 1: Calculation algorithm for Linear Retention Index (LRI)

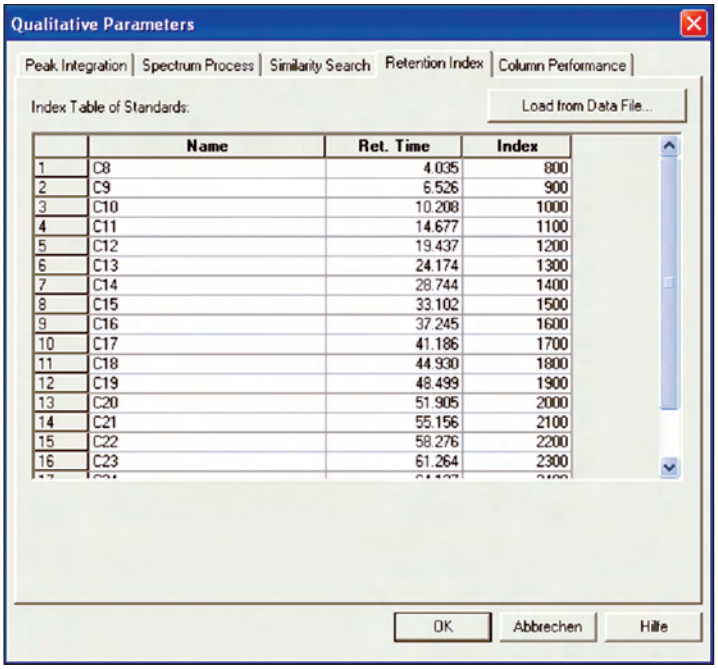
- **The concept of Linear Retention Index**
- **Automatically calculated in GC-MS software**
- **Combined with MS library search**

In the flavor and fragrance industries as well as in basic research of essential oils, analysis of the materials of interest is quite complex even when using modern equipment. A common problem occurs when dealing with groups of compounds having similar structures (e.g. sesquiterpenes in essential oils) due to similarity of fragments generated by the ionization process, thus leading to acquisition of nearly identical spectra for different compounds.

In this case, commercial libraries are not always capable of giving the correct peak assignment. Also, multiple entries of a compound inside one library have often been observed, making identification rather difficult.

Linear Retention Index (LRI)

In order to eliminate false positive or false negative identification, the concept of linear retention index (LRI) can be used. This concept is based on numbers derived from the retention times of alkanes observed when applying a linear temperature ramp (Figure 1). The basic theory is described in the literature [1]. LRI values depend only on the type of stationary phase used (e.g. polymethylsiloxanes, wax etc.) but are independent of the column dimensions. As mentioned before, the retention index relationship is linear if temperature program conditions are used.



	Name	Ret. Time	Index
1	C8	4.035	800
2	C9	6.526	900
3	C10	10.208	1000
4	C11	14.677	1100
5	C12	19.437	1200
6	C13	24.174	1300
7	C14	28.744	1400
8	C15	33.102	1500
9	C16	37.245	1600
10	C17	41.186	1700
11	C18	44.930	1800
12	C19	48.499	1900
13	C20	51.905	2000
14	C21	55.156	2100
15	C22	58.276	2200
16	C23	61.264	2300

Figure 2: n-alkanes used for the calculation of LRIs in an unknown sample

Automatic calculation of LRI values for unknown compounds

In the GCMSsolution software, the Linear Retention Index is calculated automatically. First, a standard sample of n-alkanes is measured. This data file is then linked to the data file to be analyzed (Figure 2) and the Linear Retention Indices of all unknown compounds are calculated automatically.

FFNSC library

Professor Luigi Mondello, University of Messina in Italy, has been working in this field for years [1-3], acquiring much experience in the complexity of the subject. Over this time, he and his research group developed a database known as the Flavour & Fragrance Natural & Synthetic Compounds Library (FFNSC 1.2) for electron impact (EI) analysis. At present, this library contains approx. 1200 mass spectra with retention indices, all of which have been measured with a Shimadzu GCMS-QP2010 instrument.

Library search using Linear Retention Index Filter

For the identification of unknown compounds in addition to the comparison of the mass spectra, the Linear Retention Indices in the new FFNSC Ver. 1.2 are compared with the Linear Retention Indices calculated for the compounds to be analyzed (Figure 3).

Automatic update of Retention Times

At the same time, the retention times in the list of compounds to be quantitated (targets) are also updated using the AART (Auto-

agrances with FFNSC library

matic Adjustment of Retention Times) function button which utilizes the LRI values. The AART function is particularly useful if a column was changed or part of the column was cut due to contamination. With one measurement of n-alkanes the retention times in the ID table of all target compounds are updated. The method parameters (pressure, temperature) are not changed, ensuring that the chromatographic performance is still effective.

Summary

In GC-MS, identification of unknown compounds is usually performed by comparison of the measured mass spectra with mass spectra obtained from a commercially available MS library. However, there are drawbacks to this identification method. In many cases, very similar mass spectra for isomeric compounds e.g. terpenes can be found.

Linear Retention Indices are useful for the identification of unknown compounds in a sample, especially isomers. Although the concept of Retention Indices is well known, it has never before been calculated automatically in a GC-MS software and used in combination with MS library search.

In the GCMSsolution software, the LRI values are calculated automatically from a measurement of n-alkanes using the same column. The LRI can be used as filter criterion in MS library search to enhance the accuracy of identification of compounds with very similar mass spectra, e.g. isomers.

The FFNSC library contains the mass spectra and the LRI values of almost 1200 compounds. All data were acquired from pure standard compounds using the GCMS-QP2010.

The LRI values are also used to update retention times automatically if a column was changed or cut (AART), as the LRI values depend only on the type of column stationary phase and are independent of column dimensions.

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[2] Robert Shellie, Luigi Mondello, Philip Marriott, Giovanni Dugo. Characterisation of lavender essential oils by using gas chromatography-mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography. J. Chromatogr. A, 970, 225 - 234 (2002). ISSN 0021 - 9673 (September 2002, Elsevier, B.V., Amsterdam, The Netherlands).

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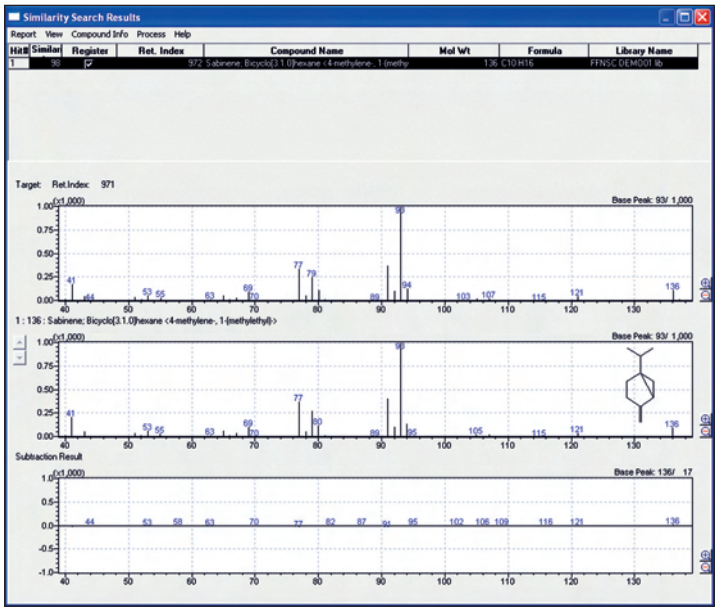
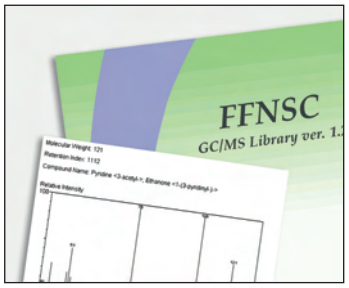


Figure 3: Library search result for Sabinene from FFNSC library using LRI in addition to the mass spectrum



Fruits, not only sweet Comparative analysis of aroma compounds

solid-phase microextraction fibers (SPME) using GC-FID and GC-MS

"African pears" – fruits of the baobab tree (courtesy of Dr. Honoré Tabuna)

- **The right type of fiber is key to analysis**
- **Main components out of 40 compounds identified constituting the aroma**

The so-called African pear (*Dacryodes edulis Burseraceae*) is a well known plant in West Africa, with edible fruits and bark, leaves, stem and roots which have use in local medicine against various diseases. The fruit is usually eaten raw or boiled, and the pulp is also roasted to form a type of butter. Essential oil compositions are known for parts of African pear plants growing in (Democratic Republic of) Congo and Nigeria. No information, however, is available on the composition of the aroma compounds of the fruits.

Following a series of publications on applications of headspace solid phase microextraction (HS-SPME) coupled with gas chromatography and gas chromatography-mass spectrometry (GC-FID and GC-MS) for extraction and identification of aroma compounds of various fruits, flowers and spices (Bonino et al., 2003; Diaz-Maroto et al., 2002; Jelen et al., 2000; Jirovetz et al., 2001; Vercammen et al., 2000), HS-SPME is of increasing importance in the aroma analysis of exotic fruits (Jirovetz et al., 2003; Shang et al., 2002).

For this reason, the combined HS-SPME with GC-FID and GC-MS (GC-14 and GCMS-QP5000 from Shimadzu, both with 2 columns of different polarity) was used for the first time in the pulp aroma compound analysis of African pear fruits from Cameroon.

A decisive point of this study was the fact that a range of different SPME fibers are already commercially available. It is also known that by using different types of SPME fibers, a dramatic change in the composition of the analyzed samples is often observed (e.g. aromatic and medical active plants: Bicchi et al., 2000 or fruit juices: Widder and Eggers, 2001). The aim of this study was, therefore, to find the right type of fiber which can extract qualitatively and quantitatively all aroma-active compounds from *D. edulis* which are responsible for the characteristic and pleasant odor impression.

Sample preparation

Dacryodes edulis fruits were bought at a local market in Ngaoundere (northern Cameroon) in September 2002, immediately after the harvest. The species identity was confirmed by a local botanist, and the control specimen was deposited at the National Herbarium of Yaoundé.

The samples investigated were prepared from a total of 5 fruits which were peeled and the pulp separated from the stone using a commercial stainless steel knife.

The pulp (300 g) was portioned in 5 x 60 g samples and each was placed in a 240 mL flask (Supelco Co., product no. 23231), olfactorally evaluated by professional perfumers (Dragoco Co., Vienna, Austria, now Symrise) and afterwards closed with hole caps (Supelco Co., product no. 23237) with Teflon/silicone septa (Supelco Co., product no. 23245-U).

The pulp samples were heated in a water bath at 40 °C for 1 hour and the volatiles extracted by solid-phase microextraction from the headspace with the following fibers: 50/30 mm DVB/Carboxen/PDMS on a 2 cm Stable-Flex coated glass fiber (Supelco 57348-U), 50/30 mm DVB/Carboxen/PDMS/Stable-Flex fiber (Supelco 57328-U), 70 mm Carbowax/DVB/Stable-Flex fiber (Supelco 57336-U), 65 mm PDMS/DVB/Stable-Flex fiber (Supelco 57326-U) and 85 mm Carboxen/PDMS/Stable-Flex fiber (Supelco 57334-U).

GC-FID and GC-MS analysis

Subsequent desorption of the analytes took place in the hot injector (250 °C) of the GC-14 (FID: 320 °C) or GCMS-QP5000 respectively. For the GC-FID measurements the carrier gas was hydrogen. The temperature program was: 40 °C/5 min to 280 °C/5 min, with a heating rate of 6 °C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 mm (BioRad, Germany) and 60 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 mm (Restek, USA). Quantification was achieved using peak area calculations in %, and compound identification was carried out partly using correlations between retention times (Retention indices according to Adams, 2001; Davies, 1990; Jennings & Shibamoto, 1980; Kondioya & Berdaque, 1996; Tudor, 1997).

For the GC-MS experiments the carrier gas was helium; injector temperature 250 °C; interface

# of African pears via differently coated

Compound	RI	S-1	S-2	S-3	S-4	S-5	Aroma impressions
Dimethyl sulfide	309	0.3	0.1	0.4	0.4	0.2	sharp, Allium-like
Ethanol	503	tr	nd	0.5	0.2	0.1	ethereal, alcohol-like
2-Butanol	590	0.2	0.1	0.7	0.2	0.1	medicinal, ethereal
Hexanal	801	tr	0.1	0.1	0.2	0.2	fatty, grassy, green
2-Methyl butanoic acid	837	0.8	0.7	1.3	0.9	0.6	fruity-fatty, spicy
(Z)-3-Hexen-1-ol	861	tr	tr	0.7	0.5	0.2	green ("leaf alcohol"), fresh
Hexanol	865	0.1	tr	0.6	0.4	0.2	alcoholic, ethereal, medicinal
Heptanal	899	tr	0.1	0.2	0.1	tr	fatty, sweet, woody, nutty, fruity
α-Thujene	925	0.1	0.1	0.3	0.2	tr	herbal, green
α-Pinene	934	59.8	59.1	47.1	55.6	60.5	woody, pine-like
Camphene	946	1.4	1.6	2.1	1.7	1.6	fresh, camphoraceous
Isoamyl propionate	952	0.6	0.5	0.8	0.3	0.2	fruity, pineapple-like
Sabinene	974	1.4	1.6	2.1	1.5	1.5	spicy, warm-woody
β-Pinene	981	8.0	7.9	6.7	7.7	8.2	woody, pine-like
Myrcene	989	14.2	13.9	12.9	14.0	14.8	sweet-balsamic, plastic-side-note
α-Phellandrene	1004	0.2	0.5	0.4	0.3	0.3	minty, herbal, spicy
δ-3-Carene	1011	0.3	0.2	0.1	0.2	0.2	sweet, refined limonene-note
π-Cymene	1027	0.5	0.7	0.5	0.4	0.2	weak citrus-note
Limonene	1031	3.8	4.0	3.4	6.4	4.3	citrus-, lemon- and orange-note
α-Terpinene	1034	0.1	0.2	0.1	0.2	tr	terpene-like
β-Phellandrene	1036	0.1	0.1	tr	0.1	tr	herbal, spicy
1,8-Cineole	1038	tr	0.1	tr	0.6	tr	fresh, eucalyptus-like
(Z)-β-Ocimene	1040	tr	nd	nd	0.1	nd	spicy (estragon- and basil-notes)
(E)-β-Ocimene	1048	tr	nd	nd	0.1	tr	spicy (estragon- and basil-notes)
γ-Terpinene	1061	tr	0.1	0.1	tr	tr	herbal, citrus-note
Terpinolene	1090	tr	0.1	0.2	tr	tr	sweet-piney, slightly sweet-anisic
Linalool	1101	0.1	0.1	1.3	tr	nd	floral, citrus-lemon-orange notes
Nonanal	1104	nd	0.1	0.8	nd	nd	fatty, waxy
2-Phenyl ethyl alcohol	1116	1.9	1.8	2.3	1.7	1.9	floral, rose-note
(Z)-Pinocarveol	1139	1.2	1.2	2.5	1.0	1.0	camphoraceous
Verbenol	1178	1.8	1.7	3.1	1.6	1.9	minty, spicy
Terpinene-4-ol	1183	0.4	0.5	2.4	0.1	0.9	spicy, woody-earthy, liliac-notes
α-Terpineol	1198	0.4	0.3	1.1	0.2	0.8	liliac odor, floral, fruity
Decanal	1204	tr	0.1	0.6	tr	tr	sweet-waxy, floral, citrus-note
Verbenone	1215	tr	0.1	0.4	0.1	tr	minty, spicy
Carvone	1255	tr	nd	0.2	tr	nd	spicy, fresh, herbal
α-Copaene	1391	0.2	0.2	0.1	0.3	tr	woody, spicy
β-Caryophyllene	1437	1.1	1.1	0.7	0.6	0.4	terpene-odor, woody, spicy
Aromadendrene	1459	0.1	0.3	0.1	0.2	tr	woody, spicy
α-Humulene	1472	tr	0.1	0.1	0.1	tr	weak woody
Nerolidol	1565	tr	nd	0.9	tr	0.1	rose-, apple-, citrus-like green
δ-Cadinol	1658	tr	tr	0.6	0.1	nd	spicy
α-Cadinol	1675	tr	0.1	0.5	0.1	nd	spicy
Farnesol	1834	tr	nd	0.3	0.1	nd	floral-oily

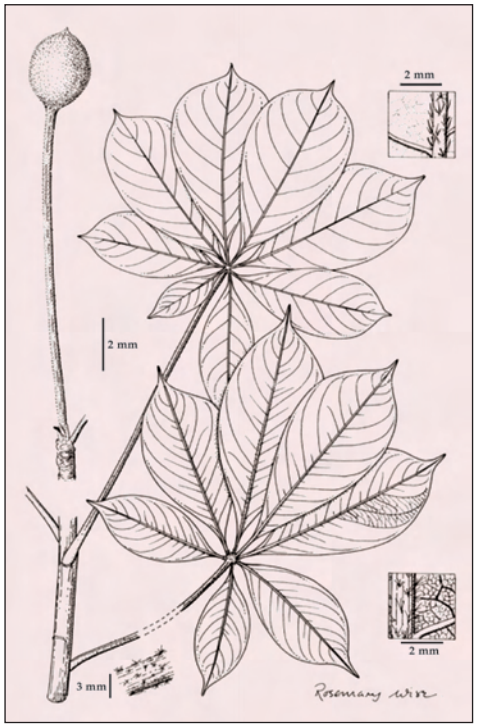
S-1: 50/30 µm DVB/Carboxen/PDMS/2 cm-Stable-Flex – **S-2:** 50/30 µm DVB/Carboxen/PDMS-Stable-Flex
S-3: 70 µm Carbowax/DVB/Stable-Flex – **S-4:** 65 µm PDMS/DVB/Stable-Flex – **S-5:** 85 µm Carboxen/PDMS/Stable-Flex
tr: trace compound (less than 0.1 %) – **nd:** not detected

Table 1: Headspace aroma compounds from the pulp of *Dacryodes edulis* fruits from Cameroon by using differently coated SPME fibers in order of their retention indices (RI) on a carbowax column in percentage (%-peak area, calculated from GC-FID analysis). Aroma impressions of identified headspace SPME pulp constituents from published data elsewhere (Arctander, 1969; Bauer et al., 1997; Fazzalari, 1978; Furia & Bellanca, 1975; Ohloff, 1994; Sigma-Aldrich, 2001).

temperature 300 °C, EI-mode- with 70 eV, and the scan-range was 41 - 450 amu. All other parameters were the same as for the GC-FID analysis. Mass spectra correlations were performed using Wiley, NBS, NIST and our own library as well as published data (Adams, 2001; Jennings & Shibamoto, 1980; Joulain & König, 1998).

Results and discussion

The pulp samples of ripe African pear fruits from Cameroon were olfactorally evaluated by professional perfumers as follows: pleasant warm-woody-balsamic (pinene-like), fresh-fruity (citrus-like), sweet-fruity (direction of ripe plum), weak minty-floral and in the background fatty and spicy aroma.



Leaf and fruit of the baobab tree

About 50 volatiles could be detected and more than 40 of them identified in the pulp headspace of *D. edulis*. Monoterpenes in particular, such as α-pinene (47.1 % - 60.5 %) myrcene (12.9 % - 14.8 %), β-pinene (6.7 % - 8.2 %) and limonene (3.4 % - 6.4 %) were found to be main compounds in the pulp headspace of *D. edulis* fruits (Table 1, page 35).

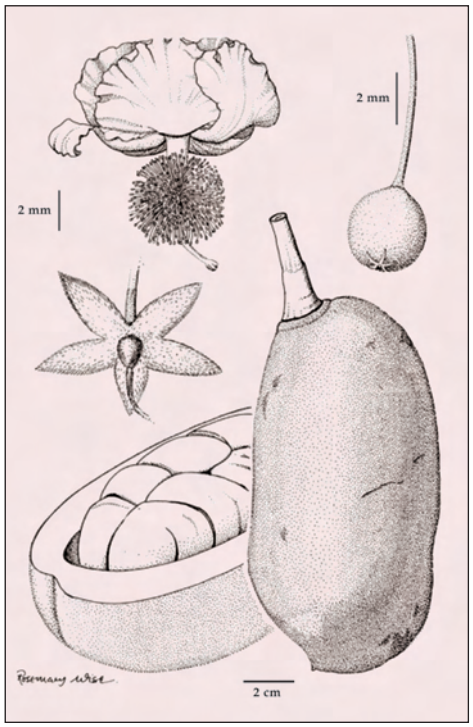
Using correlations of analytical data with odor-notes of identified essential oil compounds published elsewhere (Arctander, 1969; Bauer et al., 1997; Fazzalari, 1978; Furia & Bellanca, 1975; Ohloff, 1994; Sigma-Aldrich, 2001; see Table 1), we can deduce the following:

- dominating sweet-woody-balsamic odor impressions can be attributed to the main compounds α- and β-pinene as well as myrcene
- fresh-fruity (citrus-like) and sweet-fruity (direction of ripe plum) aroma notes are known from limonene, p-cymene, and some terpinene derivatives
- spicy odor is exhibited by some mono-terpenes, such as sabinene, phellandrene and ocimene derivatives, and sesquiterpenes, such as aromadendrene and cadinols
- green and fresh (camphoraceous- and minty-note) odor impressions are characteristic for hexane derivatives (green-grassy), camphene, pinocarveol, verbenol, verbenone (fresh-minty) as well as linalool, terpinen-4-ol and α-terpineol (floral).

Comparison of the analytical results from the extraction with the different SPME fibers clearly shows that by using the DVB/Carboxen/PDMS/Stable-Flex Fibers all aroma active compounds in the headspace of African Pears can be indentified and detected using GC-FID and GC-MS analysis. Thus also all olfactoric impressions of the identified compounds can be correlated with the overall aroma of *D. edulis*. Using the 3 other fibers, some compounds which add a considerable amount to the characteristic flavor of the pulp of African pears could not be detected.

Summary

To summarize the investigation of pulp HS-SPME aroma compounds of *Dacryodes edulis* fruits from Cameroon with GC-FID and GC-MS, we can report that α-pinene, β-pinene, myrcene and limonene are the main components among more than 40 compounds identified and mainly account also for the aroma impression of the African pear. Other Headspace compounds are present in medium or low concentrations and also have some impact on the overall aroma. These main and minor compounds could only be extracted in detectable concentrations from the headspace of the African pear by using the DVB/Carboxen/PDMS/Stable-Flex fibers. This fiber was thus found to be optimal for the aroma compounds analysis of exotic fruits.



We acknowledge the olfactory evaluations and the advisory function for the smell correlations of Wolfgang Höppner and Volker Hausmann, chief perfumers of Dragoco Co., Vienna

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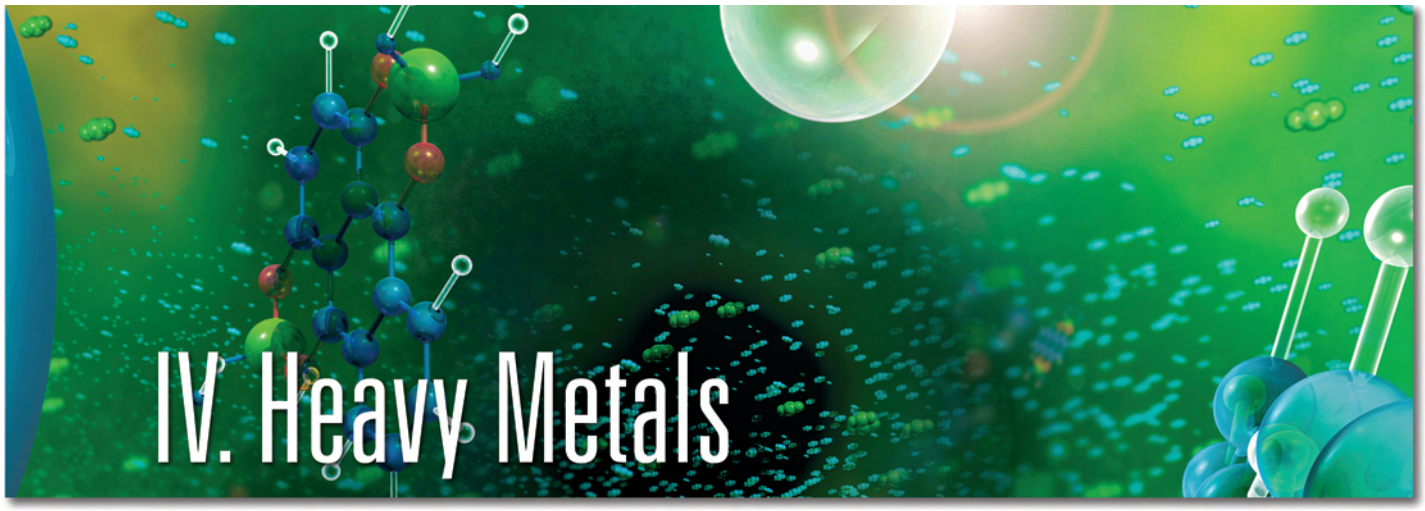

IV. Heavy Metals

Well, cheers! AAS in drinking water analy

Figure 1: The AA-6300 fully automatic atomic absorption spectrometer

- **Quantitative determination of lead, copper and nickel**
- **Complete hardware and software for determination of all relevant parameters**

Since January 1st 2003 the new European Drinking Water Regulation (TVO 2001) has been in effect in Germany. The goal of this regulation is to protect human health from the adverse effects which can result from the consumption of polluted water.

This law applies to all types of water used for drinking, cooking, food and beverage preparation as well as for personal hygiene and cleaning of objects that come into contact with foods. For these applications, water may not contain any chemical compounds in concentrations regarded as hazardous to human health. For many classes of compounds, especially heavy metals, there are well-defined maximum contaminant levels which may not be exceeded and which as a result need regular monitoring.

Atomic absorption spectroscopy (AAS) is an important technique for the quantitative determination of element concentrations.

AAS in the flame- and graphite furnace mode enables accurate determination of extremely low concentrations of metals down to the ultra-trace range. Fully automatic multi-element analysis of sample series for up to 20 elements, as well as the optimization of system parameters is possible using Shimadzu's AA-6300 atomic absorption spectrometer (Fig. 1).

The use of sophisticated background compensation techniques guarantees a high quality of the analytical results even for complex sample matrices and spectral interferences and allows, in this way, secure monitoring during routine analyses.

All of Shimadzu's atomic absorption spectrometers from AA-6200 to AA-6800 model series feature the highly sensitive GFA-EX7i graphite furnace with digital control, and can be used for electrothermal atomization. During fully automated operation, calibration from stock solutions in the desired concentration range is carried out using the ASC-6100 autosampler and the ASK-6100 dilution station.

Programming of the analysis sequence is performed via the WizAArd system software, which already contains all element-specific parameters and functions for recalibration and quality control. Automatic data storage after each measurement, as well as export functions from the system computer into the network or database are integrated as standard.

The determination of lead, copper and nickel in drinking water

Appendix 2 Part II of the drinking water regulation ratified on January 1st 2003 includes lead, copper and nickel under toxicological aspects. The presence of these metals therefore needs to be monitored frequently.

These parameters are now much stricter in comparison with the previous version of the drinking water regulation. The maximum contaminant level for lead has been decreased in the new EC guideline from 0.05 to 0.01 mg/L. The reason for this is the known high toxicity of lead, especially for children and adolescents as well as for pregnant women.

The assessment of copper in drinking water is also stricter under the new regulation. The previous maximum contaminant level of 3 mg/L has been reduced to 2 mg/L. Even though copper is a widely distributed metal and is a trace element for humans, it can, after longer exposure, lead to severe health problems in infants and small children even at concentration levels of 10 mg/L.

Lowering the maximum contaminant level for nickel in the new drinking water regulation from 0.05 mg/L to 0.02 mg/L, should prevent nickel pollution levels of

Element	Pb	Cu	Ni
Wavelength [nm]	283.3	324.8	232.0
Slit width [nm]	0.7	0.7	0.2
Atomization	Graphite furnance	Flame	Graphite furnance
Lamp current D_2 BGC*[mA]	10	6	12
Lamp current SR BGC*[mA]	8/300	10/500	10/400

Table 1: Instrumental parameters for the determination of the elements lead, copper and nickel

sis

drinking water that could lead to further increase of the already frequently occurring nickel allergies in humans.

For the quantitative determination of these metals, the AA-6300 atomic absorption spectrometer was used. This system operates in the wavelength range of 185 - 900 nm and consists of a Czerny-Turner monochromator with a holographic grating (1800 lines/mm). The detector system consists of a photomultiplier for the 185 - 600 nm range and a Si-detector for the 600 - 900 nm range and is therefore extremely powerful for the determination of ultra-trace level concentrations. The double-beam optics ensure excellent stability during long time operation. With this system configuration, concentrations of heavy metals such as lead, copper and nickel can be determined in drinking water according to the new drinking water regulation.

The AA-6300 enables the atomization of these elements in the flame mode (copper) or the electrothermal atomization in combination with the GFA-EX7i graphite furnace (lead and nickel). Table 1 shows the instrumental parameters used.

For the elements copper and nickel, background compensation was carried out using the deuterium technique. For the determination of lead, the high speed self reversal technique was used to compensate for spectral interferences. Calibration of the elements was carried out in the linear range as shown in Figure 2 for the element lead in concentrations of 0.005 up to 0.02 mg/L. Using the ASC-6100 autosampler enables fully automatic multi-element analysis sequences.

Water for human consumption must be free from impurities, be pure and fit for consumption. This requirement will be met when the generally accepted state of the technologies are applied during water treatment-, purification, and distribution and when water for human consumption meets the requirements of paragraphs 5 through 7 of the drinking water regulation. Continuous monitoring according to international standards, for instance the regularly updated drinking water regulation will provide the consumer with the greatest possible assurance.

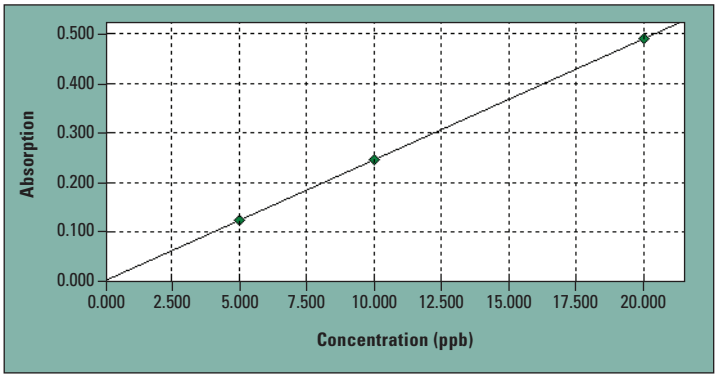


Figure 2: Calibration curve for the element lead

In this application area, Shimadzu offers a complete product range consisting of hardware and software for the accurate determination of all relevant parameters as well as the competence and know-how of a market leader in analytical instrumentation.

Precious, yet dangerous The determination of heavy and precious metals in flour using ETA-GFAAS

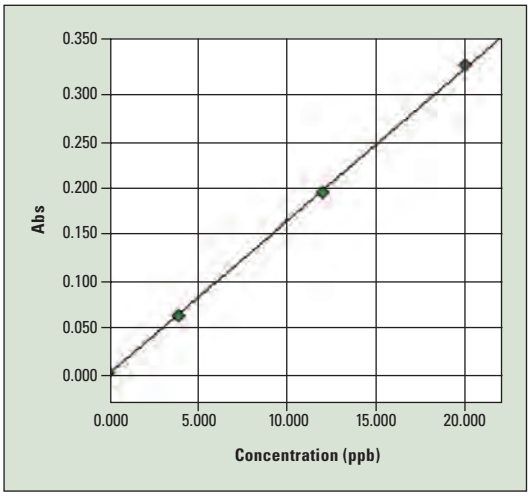


Figure 2: Rh calibration: 20 µL of 0 / 4 / 12 / 20 µg/L

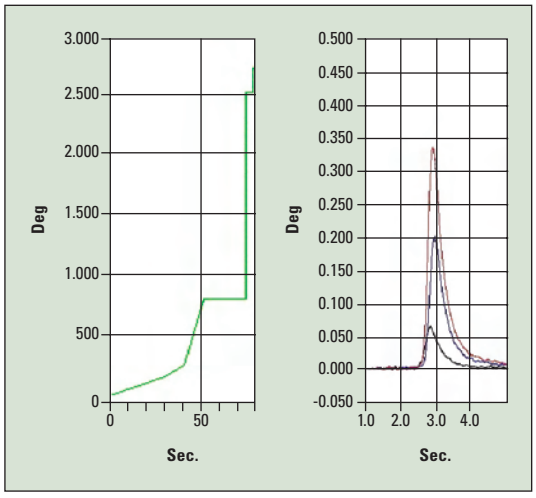


Figure 3: Peak profile at an ashing temperature of 800 °C and an atomization temperature of 2500 °C

Figure 1: AA-6300 with GFA-EX7i for the electrothermal atomization of Pb, Cd, Pt, Pd and Rh

■ Detection of ultra-trace amounts

■ Fully automatic multi-element analysis

The AAS analysis of heavy metals such as lead and cadmium is an established method. Using modern atomic absorption spectrometers in the flame mode, lead concentrations of 0.1 mg/L can be accurately determined. Electrochemical atomization is used for lower concentrations in the range of 4 to 20 µg/L. In addition to these "classical" heavy metals, the analysis of precious metals such as platinum, palladium and rhodium is gaining significance. These elements are released from lead-free gasoline by automotive catalyzers and contribute to environmental pollution.

Growing concern for the environment, as well as increased requirements of the quality and control measures for our daily basic needs such as drinking water, lead to increasingly refined and precise analytical methods. The sensitivity of analytical systems is being improved continuously and the detection limits are decreasing, even to ultra-trace level. Today, environmental- and food analysis are receiving much attention.

In 1999, the Environmental Council of the European Commission drastically reduced the limiting values for heavy metals. The value lead per liter drinking water was lowered from 50 µg to 10 µg/L, as lead can lead to nervous system damage in children and pregnant women.

Systems such as the AA-6300 (Figure 1) with double-beam optics and double-detector technology in the wavelength range of 185 – 900 nm enable fully automatic multi-element analysis. In combination with the GFA-EX7i graphite furnace, even ultra-trace amounts of heavy and precious metals can be detected accurately.

The topic of this investigation is the detection of several precious and rare metals (present in very small amounts at the ppb level in the environment with respect to the total earth mass) in different types of flour samples: wheat flakes, white flour, chickpea flour as well as course meal from biological cultivation.

The following elements were determined:

- Lead (taking into account the pollution that can be traced to lead-containing gasoline, which has already been taken off the market) and cadmium: these analytes are included in the EC regulations n. 466/2001 (maximum amount of pollutants in foods).
- Platinum and rhodium: these analytes are connected mainly with the emissions of relatively new vehicles.

Experimental methods

All experiments were carried out on Shimadzu's model AA-6300 and AA-6800 atomic absorption spectrometers equipped with deuterium and high speed self-reversal method for background compensation. For electrothermal atomization the high sensitivity GFA-EX7i graphite furnace with digital control was used. In the fully automatic mode, calibration from stock solutions was carried out using the ASC-6100 autosampler with the ASK-6100 dilution station.

The experimental results were obtained from standard solutions, diluted sample solutions as well as from certified reference solutions. The instrumental parameters were mostly copied from the system software.

Figure 2 shows the calibration curve for the element rhodium in the measuring range of 4 up to 20 µg/L at an injection volume of 20 µL. Figure 3 shows the peak profile at an ashing temperature of 800 °C and an atomization temperature of 2500 °C.

The samples show concentrations of 5 to approximately 250 µg/L and were accurately determined in the preselected calibration range. Two samples that were outside this calibration range were automatically diluted and remeasured, after calculating the dilution factors in the linear range of the calibration curve.


V. Quality Control — Beverage

Analysis of solvent residues in packaging foils Headspac

Figure 1: HT200H headspace sampler

- **Headspace autosampler HT200H**
- **High-performance, ease-of-use, cost-effective**

Headspace sampling has set new standards in the gas chromatographic analysis of volatile compounds. The first headspace application was the determination of alcohol in blood. Gas-phase sampling has led to lower sample contamination onto the separation column when compared with injection of a liquid blood matrix.

This principle has since been developed further for other applications such as the determination of volatile halogenated hydrocarbons and aromatics in environmental analysis. In addition, there are countless applications in polymer chemistry, pharmaceutical analysis and food technology. In many cases, headspace techniques are used to replace time-consuming and complex sample pretreatment procedures.

Today, many types of headspace autosampler are available. They differ from each other mainly in the way sample handling is carried out. Usually a part of the gas-phase resulting from incubation of a sample is transferred – via a gastight syringe, sample loop or constant pressure valve – from the sample vial to the GC system for separation and analysis. Whichever technique is applied, consistent automation of incubation and sampling is crucial for the reproducibility of the analytical results. Shimadzu's HT200H headspace autosampler (Figure 1) is a prime example of high-performance, ease of use and cost-effectiveness, all combined within a single instrument.

The HT200H can handle up to 40 headspace vials. Both standard volume 10-mL and 20-mL vials can be used. The incubation oven offers six sample positions. In this way, several samples can be incubated simultaneously, also during longer chromatographic runs. This offers great advantages at short incubation times, especially when using the integrated shaker. Transport of the vials takes place via an electric "grab" mechanism. This enables use of simple crimped aluminum vial caps. The HT200H can be controlled as "stand-alone" through a simple internal keypad or via a PC with user-friendly software (Figure 2).

Solvent residues in packaging foils

The following example describes a typical headspace application in the determination of solvent residues in a printed packaging foil*.

The HT200H was used in combination with a GC-2014.

In order to test the reproducibility, four empty headspace vials were first spiked with 1 µL of a solvent mixture. Due to the low dosages, the solvents evaporate completely during incubation. This technique is called "total evaporation".

There is no matrix present which could influence the solvent concentrations in the gas-phase. In this way, it was possible to quantify total evaporations during headspace analysis using the usual calibration methods such as external or internal standards, as long as total evaporation without matrix effect also applies to the samples. Table 1 summarizes the results of the solvent residue analyses. The values indicated in the table represent the absolute masses in the headspace vials.

The excellent reproducibility is also demonstrated in the chromatograms in Figure 3.

The packaging foils investigated in this example contain low amounts of ethanol and ethyl acetate solvent residues. For the quantification, pieces of foil with surface areas of 50 cm^2 were placed in headspace vials and, after incubation times of 15 minutes, aliquots of the gas-phase were sampled, injected into the gas chromatograph and quantified. The presence of the foil leads to a matrix effect, meaning that the solvent residues are not evaporated completely into the gas-

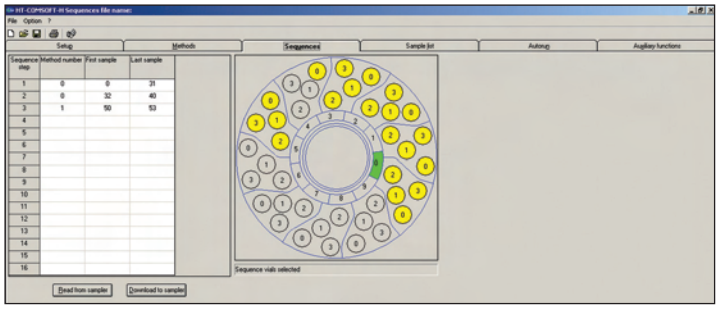


Figure 2: Comsoft-H – sample sequence

e autosampler HT200H

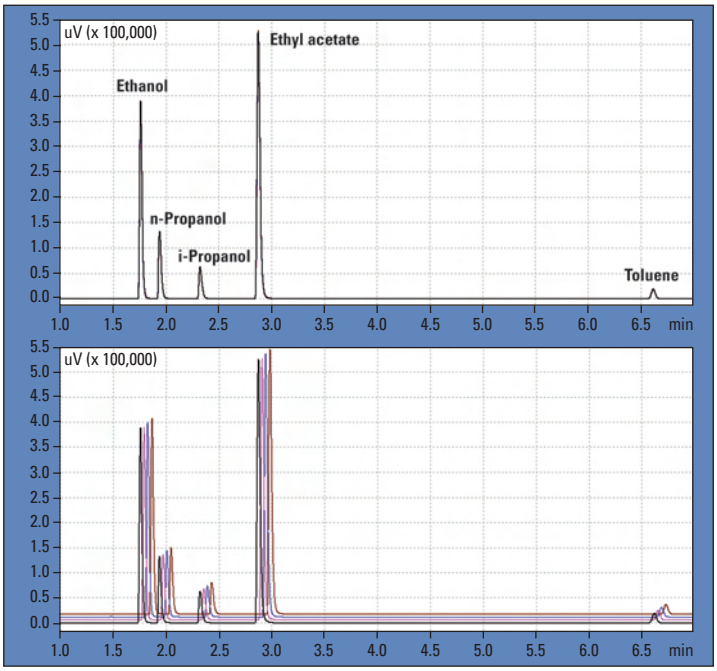


Figure 3: Comparison of chromatograms – overlay and 3D

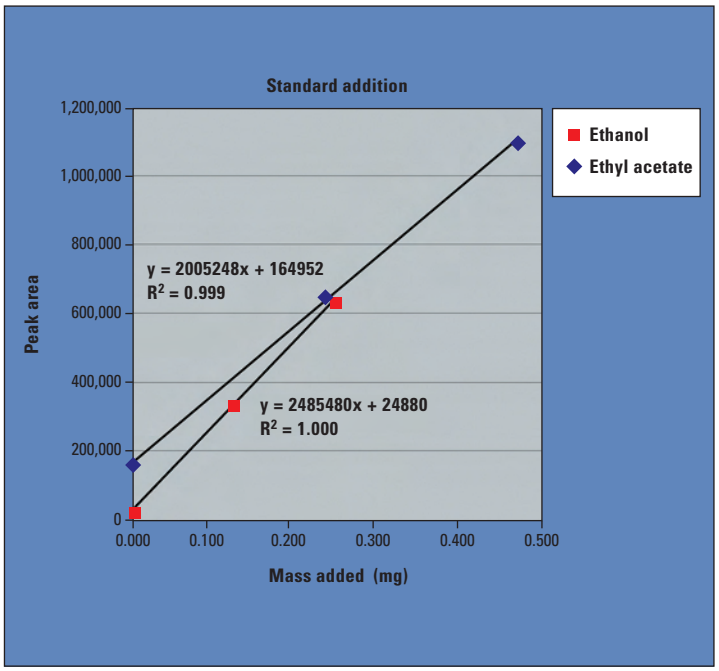


Figure 4: Graphic representation of standard addition

phase. Specifically, a state of equilibrium is established between the gas-phase and the foil constituents. This matrix effect must be taken into account during quantification. A calibration in the presence of the matrix – in this case the foil – is therefore recommended.

Consequently, with reference to the solvent residue, a calibration was carried out in the form of a standard addition of the solvent mixture in Table 1. 0.5 µL and 1.0 µL of the solvent mixture specified above were added to two identical samples (foils in headspace vials) using a micro-liter syringe, and subsequently measured. The results are shown in Table 2 and in the diagram in Figure 4. The solvent residue concentration in the foil can be calculated from the slope and intercept of the calibration line. According to this, 50 cm^2 of the foil sample contains 0.010 mg ethanol as well as 0.082 mg of ethyl acetate.

Significant deviations

When the peak areas (or the areas resulting from spiking with different solvents) obtained from standard addition analyses are compared with the data obtained from complete evaporation, significant deviations are evident. Using standard addition, the peak areas with respect to solvent component are up to 30 % smaller. This is due to the matrix effect, which in this case is relatively small. In practice, the matrix effect can influence the results by several orders of magnitude. It depends on both the sample components being analyzed and the type of matrix.

Ethanol added (mg)	Peak area	Result (mg)
0.000	22,349	0.010
0.124	338,163	
0.247	636,242	

Ethyl acetate added (mg)	Peak area	Result (mg)
0.000	156,937	0.082
0.235	652,248	
0.469	1097,364	

Table 2: Standard addition of ethanol und ethyl acetate

* The support of Huhtamaki Deutschland GmbH & Co KG is gratefully acknowledged.

Measuring value (mg)	Ethanol	n-Propanol	i-Propanol	Ethyl acetate	Toluene
Measuring value 1	0.2485	0.0836	0.0413	0.4725	0.0091
Measuring value 2	0.2443	0.0821	0.0405	0.4637	0.0089
Measuring value 3	0.2470	0.0830	0.0410	0.4691	0.0090
Measuring value 4	0.2486	0.0835	0.0413	0.4712	0.0091
Mean value	0.2471	0.0831	0.0410	0.4691	0.0090
RSD (%)	0.81	0.83	0.92	0.83	1.06

Table 1: Headspace measurements of a solvent mixture

CO_2 determination in beer Fast help with TOC

Stock solution	Dilution factor	Calibration points	Area units
1,000 mg/L C	20	50	205.9
	10	100	407.5
	5	200	803
	2	500	2120

Table 1: IC calibration curve with automatic dilution function

Quality assurance is only one of the attributes for brand name products such as beer. And quality is dependent on the quality of the starting materials, strict production processes as well as specialists whose keen eyes oversee the entire production.

Finally, millions of consumers are testing their favorite brand of beer every day.

An important player in the production process and a condition for quality is invisible: carbon dioxide, formed during the fermenting process. After filling the beer into barrels and bottles, carbon dioxide ensures that the necessary pressure is maintained. This is an important factor for guaranteeing shelf life and fresh taste of the beer. A constant concentration of carbon dioxide also ensures a steady taste and consistent quality of the beer. The concentration of carbon dioxide also plays an important role in other soft drinks. Beer contains 4 – 6 g/L CO_2; soft drinks contain 4 – 10 g/L CO_2.

Quality control during beverage production therefore requires a routine method for fast and accurate determination of CO_2. Traditional procedures such as titration and manometric methods are usually time-consuming, not very selective and difficult to automate. In collaboration with the König brewery in Duisburg, Germany, a new procedure was developed for the determination of CO_2 content using a TOC analyzing system (Figure 1).

Analytical system and measuring method

For this application, a Shimadzu TOC-V_{CPH} with autosampler (ASI-V) was used. A typical method for TOC determination is the differential method where the total carbon content (TC) is determined initially and subsequently the inorganic carbon content (IC). The difference between both parameters represents the organic carbon content.

The IC method was used for CO_2 determination. The sample was injected into a vessel containing a phosphoric acid solution. The phosphoric acid converts all carbonates and hydrogen carbonates to CO_2. Carrier gas is used to transfer the CO_2 from the sample solution to the NDIR detector where it is selectively detected. The peak area of the NDIR analog output signal is then integrated. For evaluation of the correlation between peak area and IC concentration, the TOC system is calibrated using an IC standard solution.

Sample preparation for CO_2 determination

In a vessel containing 180 mL beer, 5 mL of a 32 % sodium hydroxide solution was added in order to convert dissolved carbonic acid into carbonates. After mixing, the solution was transferred to the autosampler. As the CO_2 concentration is relatively high, the TOC-V_{CPH} automatically dilutes the sample by a factor of 5 respectively 10. This also minimizes the influence of the aggressive, alkaline matrix.

Calibration and measurement results

For the IC determination, the TOC-V_{CPH} was calibrated with a carbonate/hydrogen carbonate solution. The automatic dilution function again simplifies the calibration procedure. Only one

Figure 1: TOC-V_{CPH} with autosampler

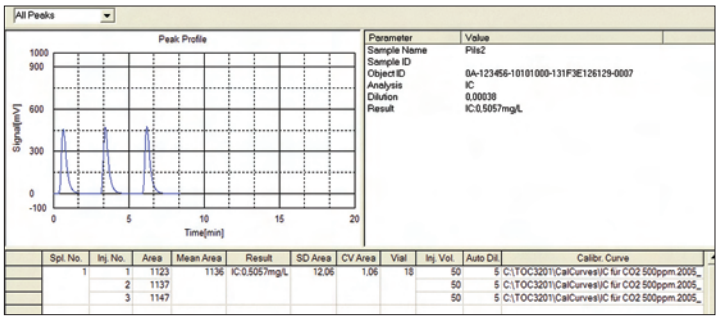


Figure 3: IC determination of a beer sample

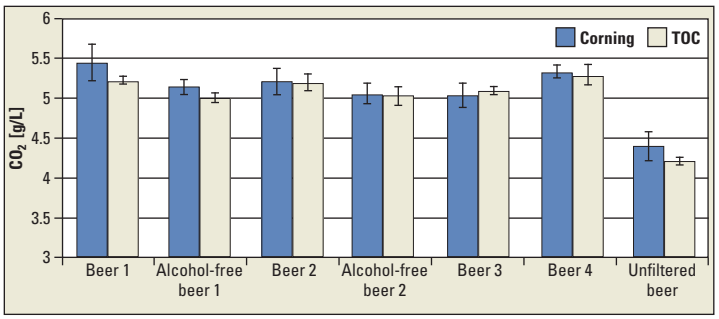


Figure 4: Bar graph

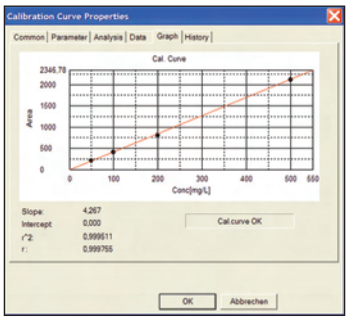


Figure 2: IC calibration curve 500 ppm

standard solution is prepared manually and the instrument subsequently carries out the entire dilution sequence (see Table 1 and Figure 2).

The calibration curve expresses area units in terms of carbon concentration. The values obtained are multiplied by a factor of 0.00038 in order to obtain the CO_2 content in the original solution (beer).

Figure 3 shows an IC determination of a beer sample.

The CO_2 content of various types of beer was determined using this method. In order to check the plausibility of the data, the results were compared with a reference method (Corning method). Figure 4 shows the results. IC determination resulted in less scatter of the data compared with the reference method. In absolute terms, these values were always within the data deviation zone of the reference method.

Summary

IC determination using the TOC analyzer has established itself as a suitable alternative to the classical methods for CO_2 determination. In comparison with the traditional methods, this new method is much more selective, easier to handle and to automate. The excellent reproducibility, the wide measuring range and the fully automatic dilution function of the TOC-V_{CPH} enable its use in routine analyses in an industrial brewery. Furthermore, the TOC system can be used in additional applications in the brewery (for instance for testing of process- and wastewater).

Cheers!

Shimadzu thanks the König brewery in Duisburg for providing their measuring data.

- **IC routine method for accurate determination of CO_2**
- **Much more selective, easy to handle**

Headaches from sulfur? The truth abo

Wine containing a residual sugar content of less than 5 g/L:	
Red wine: all quality levels	160 mg/L
White wine, rosé wine, "Rotling"	210 mg/L
Wine containing a residual sugar content of 5 g/L or more Red wine:	
Table- and "vin de pays", quality wine, "Kabinett"	210 mg/L
White wine, rosé wine, "Rotling":	
All wine types	300 mg/L
"Spätlese" (late harvest)	350 mg/L
"Beerenauslese", "Trockenbeerenauslese", Ice Wine	400 mg/L
Wines that are labelled "suitable for diabetics – consumption only after consultation with a physician" [please refer to § 13 section 3 "Weinverordnung" (251)]	400 mg/L

Table 1: Permitted maximum total sulfurous acid content

Source: Decree (EG) Nr. 1493/1999 of the Council for viticulture products, paragraph V: 17 May 1999 "On the Common Market organisation for wine Appendix V – Threshold values and requirements for certain oenological processes") CONSLEG: 1999R1493 – 01/05/2004

- **Ion chromatography as a simple alternative to classical method**
- **Analytical run within 20 min**

Viticulture and wine making tradition dates back to over 8000 years. In worshipping Dionysus and Bacchus, wine was elevated to god-like status. Wine nurtured humanity, it intoxicated and it provided consolation… However, a good measure also stimulates appetite and the senses. Does truth really lie in the wine, as in the saying "in vino veritas"?

From an analytical view, red and white wines are very different due to their difference in tannin levels. Other constituents of wines are water, alcohol, glycerine, sugar, acids, minerals as well as components at trace-level concentrations.

An important wine constituent since ancient times is sulfur. This natural component prevents wine from turning into vinegar, deters bacterial decay and oxidation and greatly prolongs a wine's shelf life. Without sulfur it would not be possible to taste many a good vintage wine.

Sulfur is applied during wine-making as a preservative and hygienic stabilization measure. Sulfur blocks highly active oxidation enzymes and thereby prevents oxidation. The growth of wild yeasts and microorganisms (such as acetic acid bacteria) is inhibited and the oxygen in air is bound by the sulfur. Sulfur dioxide is a reducing agent and reacts so strongly with oxygen that all other detrimental reactions with other compounds do not take place. In this way, young wines are prevented from turning brown and their shelf life is greatly increased.

In contrast, unsulfured wines often taste stale and dull after storage and darken in color as they age faster, a fact already known in Roman times. Recent experiments attempting to do without sulfur are technically complex and these types of wines often have an unusual taste.

Headaches from sulfur?

Sulfur in small quantities does not cause any problems in the human body. According to the latest scientific research, headaches after wine consumption are not caused by the sulfur content but rather by histamine. This protein building block is formed through bacteria after fermentation.

Persons sensitive to very small amounts of sulfur suffer from indisposition, headaches and nausea. These symptoms can also occur after consumption of unsulfured alcoholic beverages. Sulfur concentrations in wine are much too low to cause problems. Dry red wines contain the lowest amounts of sulfur, about 80 - 100 mg. Only sweet berry or so-called "Trockenbeeren-Auslese" can contain up to 400 mg/L. These are, however, only enjoyed in small amounts due to their sweetness. Other foods such as hamburgers or canned vegetables contain much more sulfur.

Caution should be observed in case of sulfur allergies. Allergic persons can react quite severely, and may even suffer from sulfur-

Sample	Sulfite Content
Red wine 1	267 mg/L
Red wine 2	192 mg/L
Rosé wine 1	50 mg/L
Rosé wine 2	52 mg/L
White wine 1	467 mg/L
White wine 2	129 mg/L

Table 2: Overview of the sulfite content in various wines

ut the wine...

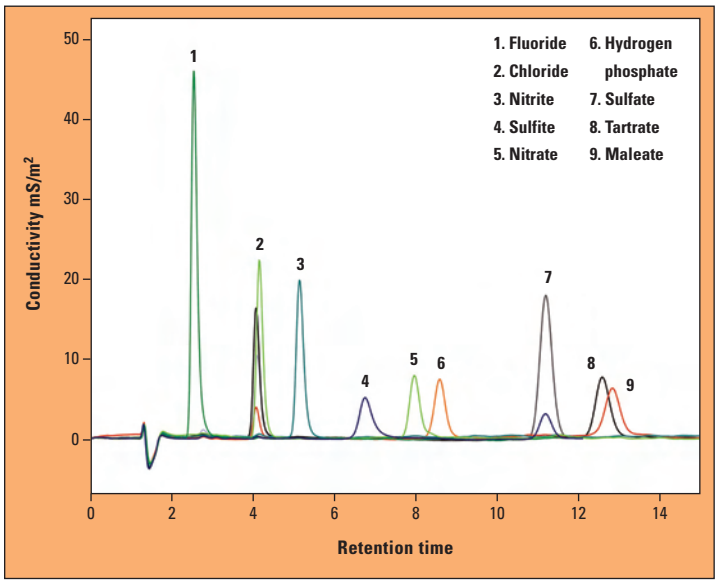


Figure 1: Standards of various anions under analytical conditions

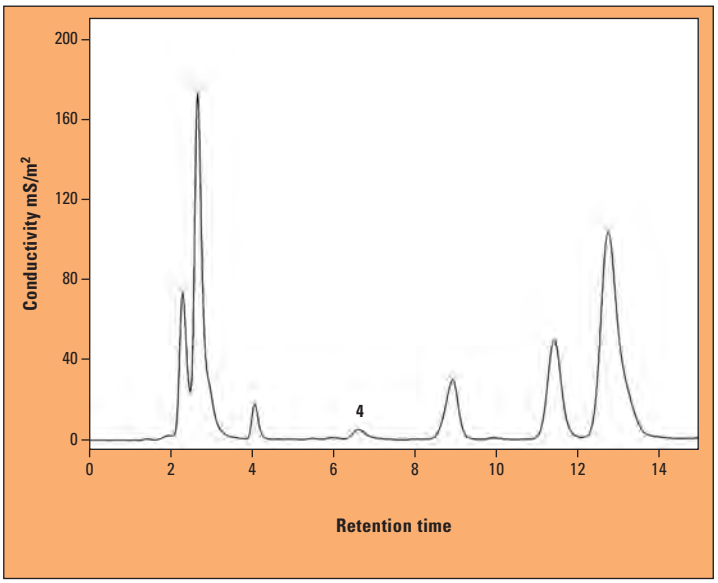


Figure 2: Chromatogram of a red wine sample

induced asthma attacks. Sulfur occurs naturally in wine and concentrations of up to 30 mg/L have been found.

In addition, sulfur interferes with vitamin B1. Therefore, persons with a deficiency of this vitamin should be very careful when consuming sulfur-containing foods and beverages.

Difficult without sulfur

Sulfur-free wines do not exist, as SO_2 is formed during fermentation (15 - 30 mg/L, depending on the type of yeast or the fruit). Sulfur is even used in organic viticulture, although in much lower amounts. The maximum legally allowable sulfur content (total SO_2) is between 160 and 400 mg/L, depending on the type of wine (Tab. 1). These values are so low that they do not affect the taste of the wine and will not have a negative effect on the body.

Today, sulfur is added during wine making in three different forms. It can be added as a powder (potassium pyrosulfite, $K_2S_2O_5$) applied directly on the grapes. During harvesting and crushing of the grapes the powder is thoroughly mixed. Sulfur can also be added to the fermented grape juice, either in the form of liquid SO_2 (easily produced by pressurized sulfur dioxide gas) or in the form of sulfurous acid (H_2SO_3). Potassium pyrosulfite and liquid sulfur dioxide react with water or wine to form sulfurous acid. The addition of sulfuric acid (H_2SO_4) is prohibited.

Analytical procedure

Classical methods for the determination of the sulfite content, for example fast determination of total sulfurous acid content according to Dr. Hans Rebelein, are based on titration. In the illustrated case the sulfite determination was carried out via an ion chromatographic method using a Suppressor IC with a special column guaranteeing well-separated sulfite peaks with respect to other ions.

In addition to sulfite, this method also detects sulfate as well as chloride and a number of additional anions (see Figure 1). The chromatographic run takes 20 minutes including a column rinse to condition the column for the next chromatographic analysis.

All of the wines investigated exhibited sulfite concentrations within the threshold values (see Table 2).

The results show that ion chromatography is a simple alternative to the established methods, especially when considering that food analysis is increasingly significant and chromatographic techniques are being applied in many special analyzes to monitor wine quality.

So when people wake up next morning with a fierce headache, they should not blame this on the wine they enjoyed the night before.

Truth lies in the must Must content as wine quality indicator –

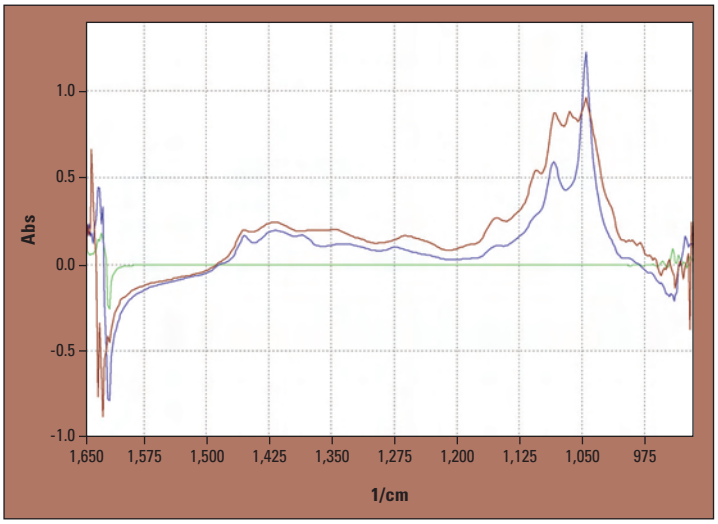


Figure 1: Infrared spectra of liquids after subtraction of the reference (water) where green = water difference, blue = wine and red = must. The fingerprint region is shown.

- **Fast analytical technique for identification and quantification of IR active components**
- **Calibration of must with the aid of IR spectroscopy is possible**

Wine is one of the oldest cultural products in human history. Vines have been cultivated for over 8000 years. The oldest known archaeological evidence of wine-making is an 8000-year old wine- and fruit press found near Damascus. Awareness of the medicinal effects of wine also dates back to this time. Hippocrates (460 – 377 B.C.) recommended wine diluted with water as a remedy against headaches and digestive disorders.

Winemaking is a rather simple process: freshly harvested grapes are crushed and the resulting juice (must) is collected. The must contains fermentable sugars and natural yeasts which, either by themselves or with the help of additional yeast cultures, start the fermentation process in which mainly ethyl alcohol and carbon dioxide are formed. The latter is a gas and escapes.

The fermentation process comes to a halt when all of the sugars are fermented or the alcohol concentration becomes too high and kills off the yeasts. At this point the must has turned into wine.

Spectroscopic methods for quality assurance

A meticulous quality control procedure is essential, and during each stage of the production process spectroscopic methods such as FTIR spectroscopy are applied for quality assurance or for product characterization, for example the determination of must content in grapes. Must is the juice collected from grapes after crushing or pressing, before the process of fermentation has begun.

This application note describes the determination of must content of different types of grapes from the Trieste region in Italy according to the Brix method. The Brix value [% Brix] is measured using a refractometer and is a percentage indication of the general must value or sugar content of the grapes. This value, in turn, indicates the potential alcohol content of the future wine. In Germany this determination is usually measured in degrees Oechsle (°OE) and in other European regions in KMW (Klosterneuburger Mostwaage, where 1 KMW = 5 °OE), Babo, KMN and Baumé. The must weight is an important classification criterion for the quality of a wine.

This article will present FTIR as a suitable analytical technique for very rapid measurement of must content based on acquisition of infrared spectra.

FTIR – fast analysis of IR-active compounds

FTIR spectroscopy is a fast analytical technique for the identification and quantification of IR-active compounds, for instance those present in must and wine. Both samples consist mainly of water, sugar, acids and other ingredients whereby the crucial distinction is the alcohol present in the wine.

In IR spectroscopy it is well known that water present in the samples strongly influences the infrared spectra. Nevertheless, a measurable region was found in the fingerprint where the IR-bands of the must ingredients and of the wine are clearly visible (Figure 1).

As mentioned before, both the must and the wine differ with respect to their sugar (must) and alcohol (wine) content as indicated in the spectral range of 1200 up to 1000 cm^{-1}. This is also the case in other wavelength ranges. As both substances can be identified in the water background, these wavelength ranges can also be used for quantification.

Measurement

Measurement was carried out using a Shimadzu IRPrestige-21 FTIR spectrophotometer in the conventional transmission mode

Standard	Spectrum	Must content (% Bx)
1	most_sample4	32.0
2	most_sample2	27.2
3	most_sample3F	16.9
4	most_sample2_1	26.0
5	most_sample1F	16.5

Table 1: List of the standards used

FTIR spectroscopy

using a flow cell with a layer thickness of 50 µm. The cell is equipped with water-resistant CaF_2 windows. As infrared radiation is also a form of heat radiation, the cell was thermostatted in order to maintain a constant temperature in the sample. Variation of temperature of the analytical system during spectral acquisition can lead to high measuring errors in the quantification of liquid samples and errors as high as 0.0125 Abs per 1 °C have been reported.

The accuracy of the FTIR system with respect to the baseline can be specified to within 0.005 Abs with water serving as reference as well as sample. In comparison, for refractometers an error range of 0.1 up to 0.5 % Bx is reported, depending on the measuring range. The temperature stability of the cell is achieved with an error of ± 1 °C. In this measurement set-up, the water spectrum was used as reference and measured against the sample.

Quantification

In this case, as an indicator of the early stages during wine production, the must content and its determination according to Brix is considered to be a one-component system. Using mathematical quantification models, the Brix parameter can be correlated to the absorption of a spectrum.

Although the "must content" characteristic property is considered here, the conventional calibration method – plotting the concentration of one component against a discrete analytical wavelength – does not lead to a suitable quantification model. When taking into account the characteristics reflected in the must content, a multivariate model spanning the entire spectrum or parts of it will be a more suitable tool for tackling this quantification issue.

The infrared spectrum represents all ingredients and their corresponding characteristics. The PLS (Partial Least Square) method was therefore used for this application. In PLS, a factor set is sought after which represents the standard spectral set and its components. Based on the established factors, the content of a certain component (in this case must) can be determined in a sample.

For this model, 5 must samples (Table 1) were determined in 10-fold using an autosampler. 48 measuring values were acquired in the calibration (Table 2). In order to test the calibration model, a sample was created from must standards 2 and 1. The result is listed in Table 3. The deviation of the Brix mean value of five measurements with 0.0138 % Bx lies below the error that can result from a false reading of 0.2 % Bx on the refractometer scale. Each single measurement lies in turn below the reading error.

Evaluation

This limited standard model illustrated that a calibration of must with the aid of infrared spectroscopy is possible. The quality of calibration models is obviously dependent on the quality of the standards and reference methods used. This model can be further improved by using more standards, enabling in turn more accurate calibration. It is also important to use a reliable reference method, fast processing of the standards – as these are not stable over time – and temperature control as the measurements are strongly influenced by temperature variations.

Report of PLS Calibration	
Algorithm	PLS I
Number of components	1
Number of references	48
Range [1]	2432.60 – 3000.60
Range [2]	1122.59 – 1558.41
Centered data	Yes
Components	Must content
Number of factors	3
Correlation coefficient	0.99905
MSEP	0.00186
SEP	0.04319

Table 2: Calibration model of the must application

No.	Sample	Must content (% Bx)
1	most_sampleF1and2__003	22.3893
2	most_sampleF1and2__002	22.4411
3	most_sampleF1and2__001	22.4413
4	most_sampleF1and2__004	22.3474
5	most_sampleF1and2__005	22.3119
	Mean value	22.3862
	Difference in terms of the mean value	0.0138

Table 3: Testing the model using a sample representing a must content of 22.4 % Bx

We acknowledge with thanks the friendly support and use of the standards provided by the laboratory of Dr. Diust in Corni di Rosazzo and Emanuele Canu of Shimadzu in Milan, Italy.

Drinking water even purer with TOC

A timesaver for large sample numbers: the ASI-V autosampler

Germany has implemented the European drinking water guidelines into its national legislature after the European Commission ratified the "Directive on the quality of water intended for human consumption" on May 28, 2001. After a transition period, this law was adopted on January 1st 2003, in accordance with European law.

- **NPOC method for drinking water analysis**
- **ISP module reduces handling steps**

Indicator parameter

The drinking water directive introduces the category of indicator parameter value specifications. These are not directly linked to health problems but have an indicator function. This list of indicator parameters also includes the TOC value (total organic carbon), for which no limiting value or criterion has been defined, but which can be considered as a cautionary warning for action under unusual circumstances.

Oxidizability

Another indicator parameter included in the list is oxidizability. This is a measure of the sum of all chemically oxidizable organically bound compounds present in water. With reference to drinking water limiting values, this parameter is no cause for direct health concern but can lead to regermination or undesirable disinfection byproducts. Oxidizability is proportional to the sum of organically bound carbons that are determined as DOC (dissolved organic carbon) or TOC. Oxidizability can, therefore, be replaced by the TOC parameter. The frequency of determination of the parameter indicators depends on the volume of water that is produced or released in a water supply area.

Copper

The TOC parameter is also associated with another relevant parameter i.e. copper. In the drinking water directive, the limiting value for copper is reduced to 2 mg/L. This limiting value also requires that the newly issued restrictions for the use of copper materials described in DIN 50930-6 be strictly observed. Copper can be applied without any problems in all types of water at a pH value higher than 7.4. In addition to the pH value, the concentration of dissolved organic carbon (DOC) is also significant. In the pH range 7.0 – 7.4 the TOC seems to influence the solubility of copper. The limiting value of the TOC in this pH range is 1.5 mg/L.

TOC determination in drinking water

When examining carbon compounds in drinking water, it is apparent that the amount of inorganic carbons, such as carbonates and hydrogen carbonates, is much higher than the organic fraction. The organic fraction is only 1 % of the total carbons. A TOC determination via the differential method (TOC = TC - IC) will not be appropriate in this case, as the calculated TOC value is prone to large statistical errors. According to EN 1484 (instructions for the determination of total organic carbon and dissolved organic carbon), the differential method can only be applied when the TIC value (total inorganic carbon) is smaller than the TOC value.

For drinking water analysis the NPOC method (non purgeable organic carbon) is therefore used. The drinking water sample is first acidified to a pH value of 2. In this way the carbonates and hydrogen carbonates are transformed into carbon dioxide. The CO_2 is then removed via sparging with carrier gas. The amount of volatile and therefore spargeable organic carbon can be disregarded in drinking water. What remains is a solution of non-volatile organic carbon compounds. These can be oxidized to CO_2 and detected via NDIR.

TOC-V series

This application can be easily carried out using Shimadzu's TOC-V series. For a large number of samples the ASI-V autosampler, in which the samples are automatically acidified and sparged in the sample vials, is recommended. During TOC measurement of the first sample, the next sample is already being sparged in order to save valuable time. But even when an autosampler is not available, the NPOC method can still be automated. The TOC-V series is equipped with an ISP (integrated sample preparation) module, which consists of an 8-port valve and a syringe with sparging gas connection. In addition to acidification and sparging in the syringe, the ISP also enables automatic dilution. This feature facilitates an extended measuring range, dilution of highly contaminated samples and the preparation of a series of calibration samples from a stock solution. The ISP module can, therefore, considerably reduce time-consuming sample handling steps.

The accompanying TOC-Control software offers, in addition to instrument control and data acquisition, numerous functions that greatly simplify quality assurance in TOC analysis. The quality assurance functions use control charts according to criteria of the German "Allgemeine Qualitäts-Sicherung" (AQS) which regulates quality control. The TOC-Control V software automatically consults these control charts and issues a warning when extraordinary events arise. In addition, simple control samples that initiate various actions (warning message, stopping the analysis runs or reanalysis of the last samples) when limiting values are being exceeded, can be defined in the autosampler.

Regarding luke-warm Cervisia*... Determination of hops in beer –

Belgian brewery uses spectrophotometer

The Romans in Asterix's days called it "luke-warm cervisia". This "grain juice" became known throughout the world as beer. Today there are about 30 different types of beer with, at times, varying ingredients. Scientifically speaking, beer is a beverage produced from starch containing raw materials, boiled and fermented with hops. Whereas German beer drinkers may be the heaviest users, the large European breweries are located in the Netherlands, Denmark, the UK, Ireland, France and Belgium. There are approximately 1200 breweries in Germany, which market 5000 different brands; the Benelux countries brew about 1500 labels in 150 breweries.

In 1999 beer production in Belgium was approximately 15 million hectoliter. One third of this amount is for export; 10 million hectoliter are consumed in Belgium itself. Not only in Belgium but all over Europe, beer is one of the most popular beverages (see Table 1). Northern and middle Europeans prefer beer whereas wine is the preferred beverage in southern Europe.

Country	Annual beer consumption in L per person
Germany	131.1
Ireland	123.7
Denmark	116.7
Austria	113.3
United Kingdom	101.0
Belgium	86.4
The Netherlands	81.0
Finland	80.0
Luxemburg	67.0
Spain	62.2
Portugal	61.7
Sweden	39.0
Greece	39.0
France	37.0
Italy	25.4

Table 1: Annual beer consumption in Europe

»A drop of hops...«

...according to ancient brewer wisdom. Hops have been used as a regular ingredient in beer since the 16th century. In addition to increasing shelf life and stability of the foam, hops are above all responsible for one thing: the incomparable, bitter-aromatic taste. The higher the hops content in beer, the more bitter and distinctive the taste.

The determination of hops content in beer, carried out in the analytical laboratory, assures that the beer is of constant quality. Maximum and minimum concentrations are usually not mandatory – more important is that the quality of a certain type of beer is assured by a constant hops concentration. One Belgian brewery carries out the determination of hops content using a Shimadzu UV-VIS spectrophotometer. The UV-1700 with its built-in system validation function is well suited for quality control. The analytical wavelength is 275 nm. After dilution with iso-octane the total hops concentration can be determined instantly.

In addition, using calculation models of the UV software, an additional differentiation of α-(humulon, cohumulon, adhumulon) und β-(lupulon, colupulon, adlupulon) bitter acids can be carried out.

The sample is then further diluted and subsequently measured at three different wavelengths of 275 nm, 325 nm and 350 nm (see Table 2).

Using the following mathematical equations, the ratio between α- and β- bitter acids can be calculated directly via the UV Probe software (Table 2).

Dilution factor (DIL) = [VA*VB*VC] / [SW*ALQA*ALQB]
Total volume of original sample (L) [VA]
Total volume of first dilution (mL) [VB]
Total volume of third dilution (mL) [VC]
Weight of sample (mg) [SW]
Aliquot A (mL) [ALQA]
Aliquot B (mL) [ALQB]
α-acid (mg/L) = (-51,56*WL1) + (73,79*WL2) – (19,07*WL3)
β-acid (mg/L) = (55,57*WL1) – (47,59*WL2) + (5,10*WL3)
Bkg (mg/L) = (8,34*WL1) – (15,74*WL2) + (37,19*WL3)
% α-acid = α-acid*DIL*100
% β-acid = β-acid*DIL*100
% Bkg = Bkg*DIL*100

Table 2: Ratio of α- and β-bitter acids

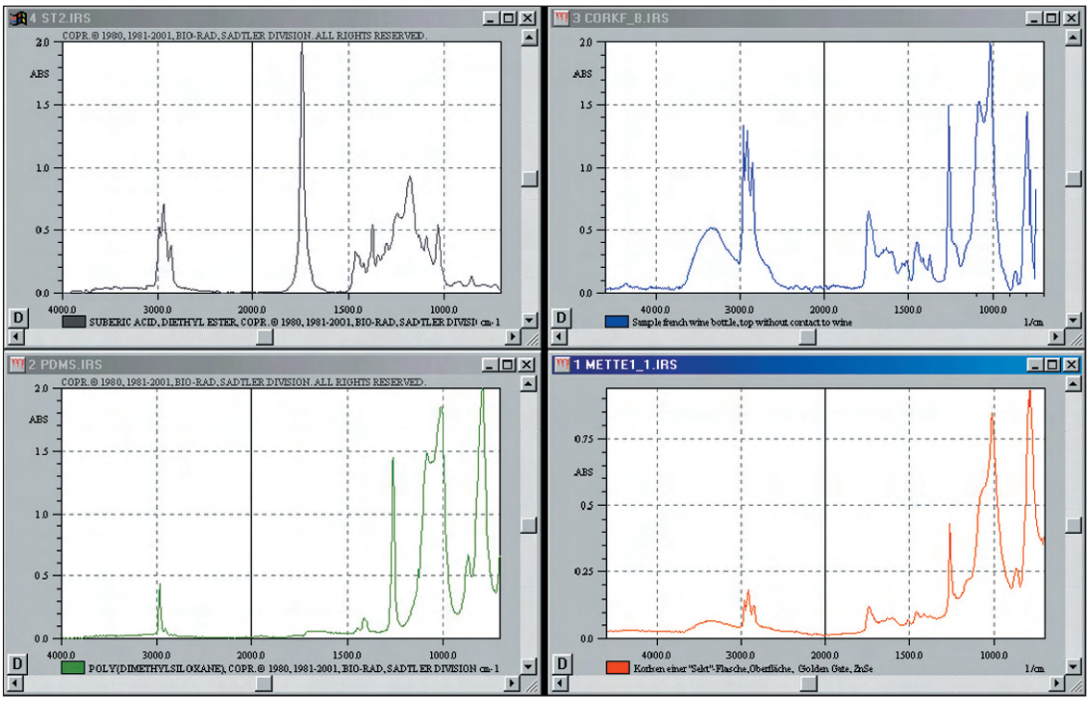
Additional interesting information on the topic beer can be found at the following websites:

1) www.bier.de
general information on beer
2) www.ebc-nl.com
European beer convention for analytical methods
3) www.bierwereld.com
Beer links

*precursor of beer in ancient times

- **Hops assure constant quality of beer**
- **UV-VIS spectrophotometer is perfect for quality control**

What makes a cork pop? FTIR identifies lubricants



IR spectra: Left: top suberine acid diethylester; bottom: polydimethylsiloxane, Right: top: cork of a wine bottle; bottom: cork of a champagne bottle

- **Paraffin and polysiloxane on surface of corks**
- **Product quality control contributes to marketing success**

It is said that the truth is found in wine. This is, however, only partially true, at least when it applies to pricing. Not only the quality of the wine, but other factors contribute to the final price – the bottle for instance, distribution and even the type of cork used. This small piece of nature at the end of the bottleneck gives the wine an emotional added value and underlines the authenticity of wines and champagnes.

For the beverage industry there is a price difference between a one piece cork and a cork pressed from granulate material. What applies to both is, that they should be easily pulled from the bottle. Both paraffin and modern polysiloxane are applied to the cork surface as lubricants. These compounds also contribute to the overal price of the wine.

In times of heavy competition for market share, the beverage industry pays close attention to what their competitors are doing and to their own market research. They have a keen eye for their competitors, their marketing strategies and pricing policies. An important factor is product quality control, which included the cork and the paraffin or polysiloxane lubricants used.

Infrared spectroscopy can be helpful in the task of identifying these compounds. The Shimadzu FTIR-8400 was equipped with a single reflectance unit which allows surface analysis whereby the infrared beam can penetrate the sample surface to a depth of approximately 2 µm. The corks are first cut into slices in order to allow the surface to be measured.

The infrared spectrum of the surface measurement is carried out for polydimethylsiloxane and suberine (suberine acid diethylester). The results have shown that depending on the function of the cork, the polymethylsiloxane layer has a varying thickness. Research on a small sample of European wine bottle corks show that paraffin is used less frequently than siloxanes.

The conclusions that the beverage manufacturers draw from these types of analyses are, of course, proprietary. What is important is that the product pays off. And that is indeed the case, when the consumer is satisfied with respect to quality, price and what the product promises. And vice versa, who does not recognize this: a cork that is stuck in a wine bottle? Or the champagne bottle where the cork does not want to pop?

Cork – its properties

Cork contains suberine, lignine and cellulose. These keep cork afloat. Cork cells consist of a cellulose skeleton which is cross-linked with lignine. Together this forms the backbone for suberine, a natural polymer ester.

The physical properties of cork are also interesting. As a natural raw material, cork has a limited permeability to liquids and gases, is heat- and sound resistant, elastic and chemically neutral. This makes cork a very attractive material for home decorating, finds use in life jackets and seals wine and champagne bottles.


VI. Quality Control — Food

Parallel determination of carbohydrates and organic aci

Figure 1: HPLC system

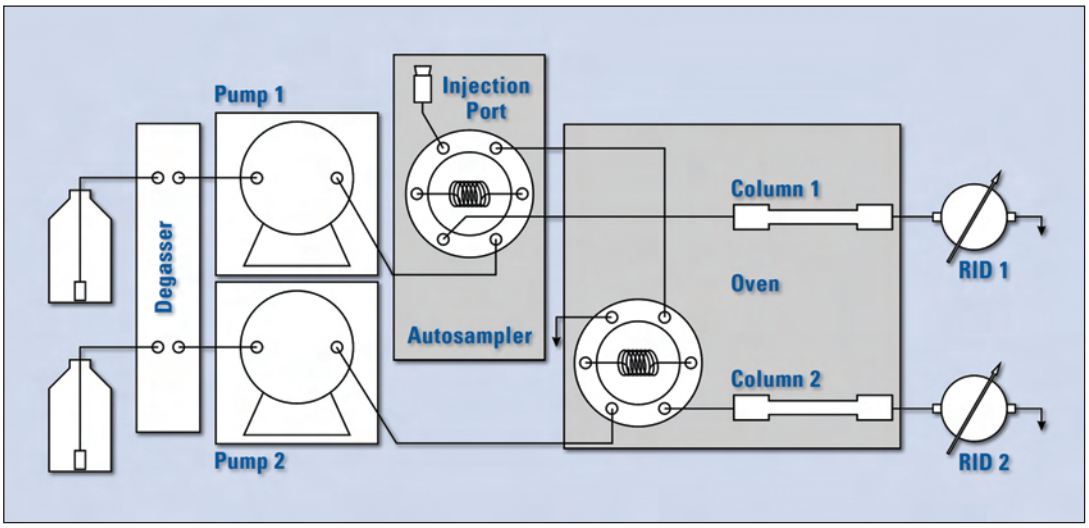


Figure 2: Flow scheme

■ **Parallel LC configuration on two columns with a single injection**

It would be good to solve several analytical problems at once within a single analysis, in order to save time – as long as the chromatographic system will allow sufficient separation of all analytes on the same column under equal separation conditions. This approach, however, quickly fails with widely differing compound classes or when the separation would require several different separation principles or columns. When these analyses are to be carried out simultaneously – as required by the number of samples or the need to acquire information on all sample components – the only option is to purchase a second analytical system.

One compromise would be a "parallel determination" on two columns with a single sample injection. In this case, similar conditions for both separations should be selected with respect to mobile phases, column temperature and run time. The following application example illustrates the possibilities of such a parallel LC configuration.

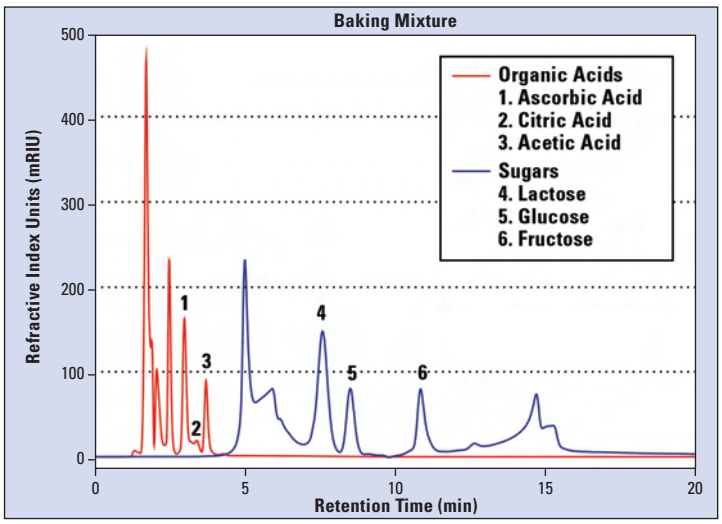


Figure 3: Chromatograms of sugar and organic acids

The determination of sugars and organic acids

The analysis involves a complex mixture containing sugars and organic acids. As the sample is a powder and soluble in water, it made sense to use an aqueous mobile phase in order to prevent separation artefacts at these relatively large injection volumes.

The HPLC system is shown in Figure 1. Each separation system uses a separate pump. The injection system uses a loop injection. The sample is transferred to a sample loop (similar to manual injection) and subsequently injected into the flow line via switching of the injection valve. A switching valve, coupled to the autosampler valve, is installed into the column oven in such a

ds with LC using dual RI detection

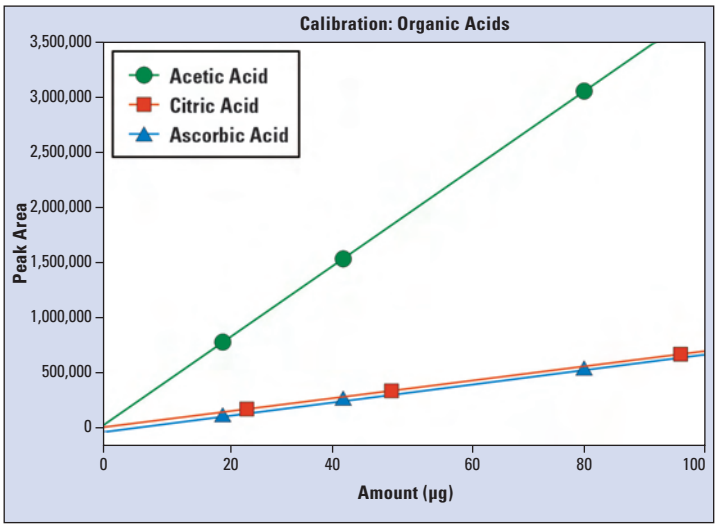


Figure 4a: Calibration curve

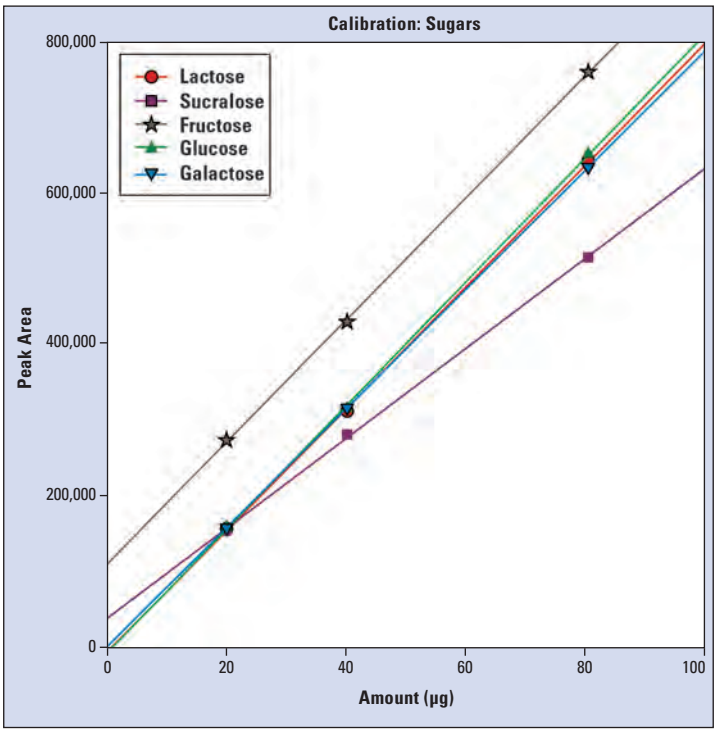


Figure 4b: Calibration curve

way that the sample is present in two sample loops. The flow scheme is shown in Figure 2.

In this way, mixing of both mobile phases is ruled out. Simultaneous switching of the injection valve and the autosampler valve will start the analysis simultaneously on the second system. For the system described here, each separation channel uses a refractive index detector.

As these detectors are relatively insensitive and the analyte concentrations in the sample were low, it was necessary to dissolve a large quantity of sample material. In this way, it was possible to quantitatively detect several sugars and organic acids in the samples. Chromatograms for the determination of sugars and organic acids are presented in Figure 3. Calibration curves, as well as the concentrations determined using these graphs, are shown in Figures 4a and 4b. Figure 5 presents a short overview of the chromatographic method parameters.

Summary

Parallel LC can be employed for many different types of applications, using any type of detector.

Due to the complexity of the system, a step-by-step approach is recommended for method development. In this respect it is best to occasionally test whether a parallel determination is possible and also useful. Depending on the type of sample compounds, it may also be necessary to optimize the sample loop volumes, especially when limited amounts of sample are available.

HPLC Parameters
Temperature: 50 °C Flow: 1.0 mL/min Detector: RID (1 per channel)
Determination of organic acids Column: Shodex RSpak DE-413 Mobile Phase: 10 mm o-phosphoric acid (85 %) in water
Determination of sugars Column: Shodex SUGAR SP0810 Mobile Phase: water

Figure 5: Overview of the method parameters

16 times faster with the GC-2010 Determination of fatty acid of animal and vegetable origins by conventional and Fast GC analysis

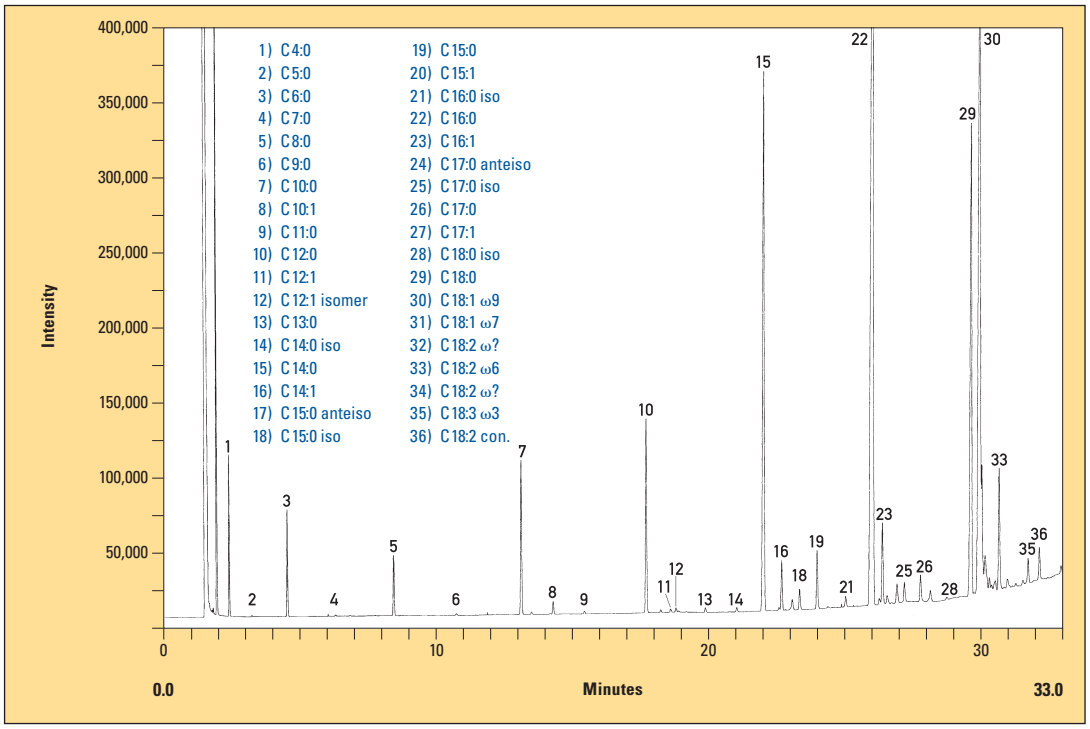


Figure 1: Butter FAMEs "Conventional" Analysis

Column: Rtx Wax 30 m x 0.25 mm i.d.; 0.25 µm film; Inj. Vol.: 1 µL (1:10 in hexane); Split Ratio: 1:50 (250 °C)
T. Progr.: 50 °C to 250 °C at 3.0 °C/min
Carrier gas: H_2; Linear velocity u: 36.2 cm/s (constant)
Detector: FID (250 °C); H_2: 50 mL/min, Air: 400 mL/min, Make-up: 50 mL/min kPa (N_2)
Interval: 40 ms; Filter Time Constant: 200 ms

- **Analysis time reduced by factor 16.5**
- **Fast GC suitable for every kind of sample**
- **High productivity, sensitivity and reproducibility**

The analysis of food products is one of the important tasks in analytical chemistry. In quality control the amount of samples to be analyzed per day can be quite high. Thus analysis time is an important factor for the productivity of a control laboratory. A solution for the achievement of high productivity is the application of the Fast GC method. In Fast GC the retention times can be reduced drastically compared with "conventional" GC methods.

For Fast GC short columns with small inner diameters and thin stationary phase films are used. With these kind of columns retention times can be reduced without loss of resolution.

The very small inner diameter used means that you need high pressures applied to achieve a high linear velocity required for a good separation. This linear velocity, is kept constant (constant linear velocity mode) to ensure optimum resolution for every part of the chromatogram and further reduce the retention time by reducing the elution temperature.

Also very important is the use of high heating rates for the reduction in retention time. With the GC-2010 you can use pressures up to 970 kPa and heating rates up to 140 °C/min. Of course, the cooling speed is also high with 3 min from 300 °C to 50 °C.

To achieve good and reproducible data the detector sampling frequency must match the speed of

methyl esters (FAMES) on fats and oils

the chromatography. Thus the detectors in the GC-2010 have a maximum sampling rate of 250 Hz (4 ms interval) and a selectable filter time constant down to 4 ms (minimum).

The following application was developed at the University of Messina by Professor Luigi Mondello and Professor Giovanni Dugo. It shows the use of the Fast GC method for the analysis of butter FAMES. It is shown that the analysis time can be reduced from more than half an hour to 2 min, i.e. a factor of 16.5!

Figure 1 shows the chromatogram for the "conventional" GC. The column used was a standard column 30 m, 0.25 mm I.D., 0.25 µm film thickness. Linear velocity was constant at 36.2 cm/s. Heating rate was 3 °C/min. Retention time is 33 min.

Fast GC analysis

Figure 2 shows the same sample measured with Fast GC using a 10 m column, with 0.1 mm I.D. and a film thickness of 0.1 µm. Linear velocity was constant at 116 cm/s and heating rate 90 °C/min. Retention time obtained was 2 min.

The method of Fast GC is not restricted to any type of compounds but it can be used for every kind of sample with high advantage in terms of productivity, without loss of sensitivity, reproducibility (< 1 %) or separation.

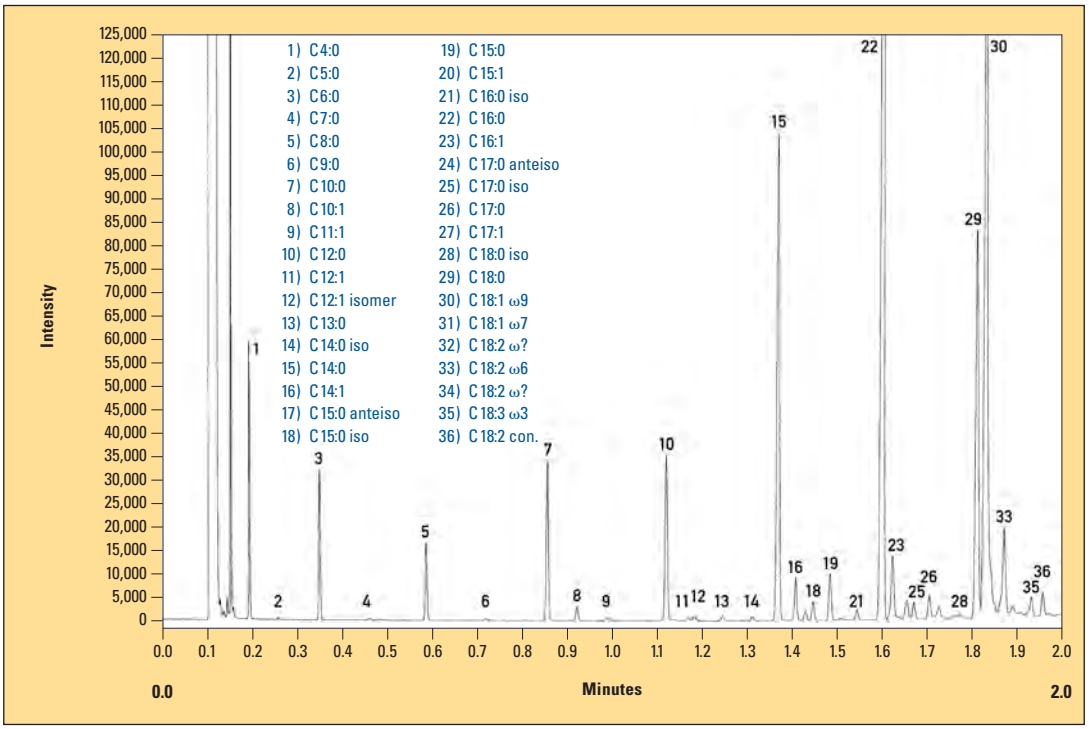


Figure 2: Butter FAMEs Fast GC analysis

Column: Rtx Wax 10 m x 0.10 mm i.d.; 0.10 µm film; Inj. Vol.: 0.2 µL (1:20 in hexane); Split Ratio: 1:200 (250 °C)
Gas save mode: after 2 min reduction of split ratio to 1:10
T. Progr.: 50 °C to 250 °C at 90.0 °C/min
Carrier gas: H_2; Linear velocity u: 116.0 cm/s (constant)
Detector: FID (250 °C); H_2: 50 mL/min, Air: 400 mL/min, Make-up: 50 mL/min kPa (N_2)
Interval: 4 ms; Filter Time Constant: 50 ms

Figure 1: Bee approaching an apple blossom

Figure 2: Phacelia flower with pollen collecting bee

Enjoy without guilt

Honey and GC analysis

Locally produced honey is one of the foods least contaminated with residues of environmental pollutants and pesticides. Although the 1.5 kg per-capita consumption of honey in Germany is negligible in comparison with the amounts of meat, vegetables and fruits that find their way into the shopping basket, honey is nevertheless subject to analytical testing as if it belonged to one of the main foods for human consumption. Since 1988, pesticide residue analysis has been carried out at the Federal Institute for Agriculture at the University of Hohenheim in Germany on several thousand honey samples every year. Quality control is an important issue but the main focus is the identification of factors which may jeopardize the image of locally produced honeys. The results are primarily important for informing beekeepers. The criteria in honey analysis are very close to those of drinking water analysis, with determination limits in the low ppb range.

Why these procedures?

Where no maximum levels have been assigned, a reliable maximum value, usually 10 to 50 µg/kg, is used for plant food sources. As honey was considered as a plant food source (today it is recognized as an animal food source) analytical methods were developed and established that comply with these legal maximum levels. In this way, honey – which as a natural product is regarded as being especially pure – is subject to very stringent quality control using highly sensitive analytical methods.

But even when using very sensitive methods, it is still quite difficult to detect pesticide residues in honey. The problem is that these pesticides are frequently used on cultivated plants in full bloom, which at the same time serve as an important source of nectar and pollen for honeybees and other insects. Although pesticide contamination of nectar in orchard flowers or rapeseed fields can be detected easily, the search for pesticide residues in harvested honey often produces negative results. Why is this so?

Honey in GC analysis

To investigate this contradiction, laboratory, field and semifield studies as well as tent tests were carried out in recent years where the pesticides were traced from the flower up to the ready-for-harvest honey in the beehive. The availability of reliable miniaturized extraction methods and highly sensitive measuring instruments was indispensable in these studies. The objective was to detect the pesticide compounds originally applied to the plants, in the collected nectar of honeybees as transported in their honey sacs.

Analyses were carried out using Shimadzu's GC-17A and GC-2010 gas chromatographs, each equipped with ECD detectors as well as a GCMS-QP5050A.

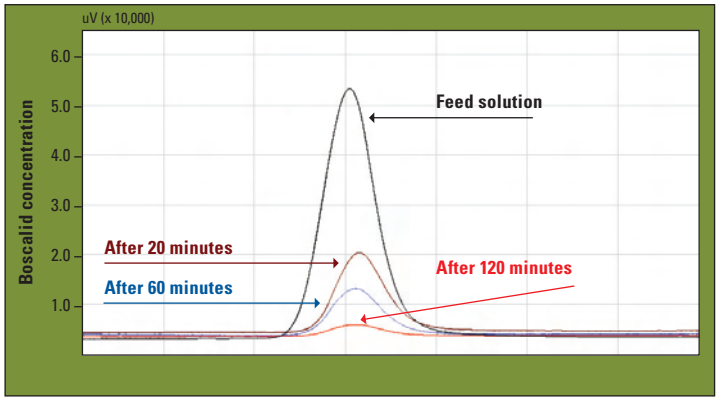


Figure 4: Degradation of the fungicide boscalid in honey sacs, shown via overlaid ECD chromatograms

Figure 3: Dissected honey sac filled with nectar

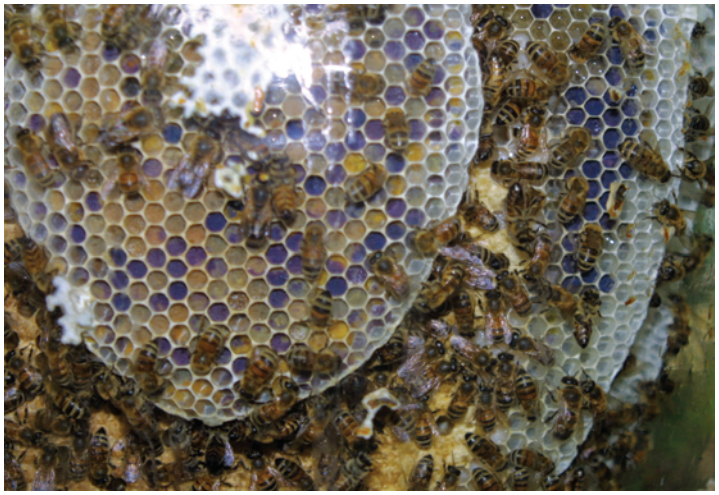


Figure 5: Pollen stored in the honeycomb

Depending on the type of plant and weather, honeybees usually visit between 50 and several hundred flowers before they return to the beehive with a full honey sac. During their nectar gathering flights they select one particular type of flower, meaning that they visit only one type of plant and also safeguard the pollination process (Figures 1 and 2). Under suitable experimental conditions, it is possible to guarantee that all collecting bees actually fly into the experimental plot – a rapeseed field that was sprayed with pesticides. Each bee transports on average approximately 40 µL of nectar. This nectar contains the active compound of the pesticide spray subsequently determined in the laboratory.

After returning from the field, the forager bees must be intercepted at the hive entrance. This is done using a converted automobile vacuum cleaner that instantaneously covers the bees with CO_2 snow. In the laboratory the honey sacs, up to 2000 per experiment, are dissected individually (Figure 3). The active target compounds are then isolated using liquid-liquid extraction methods and subsequently determined using gas chromatography.

The results show that the bees encounter mid ppm range pesticide concentrations in the flowers, which then find their way into the nectar. An interesting observation is that very high fluctuation margins are measured between individual bees in one series, although the bees have all been released into the flowering fields simultaneously. In the honey sacs of some of the nectar collecting bees extremely low pesticide levels – with 0,1 pg/µL being below the quantitation limit – were found. Based on the high number of visited flowers, a more evenly distributed pesticide level was expected in the honey sacs.

Bees under laboratory conditions

In the laboratory, caged groups of bees were fed with sugar solutions containing active pesticides. After a predetermined time frame, the honey sacs of these bee samples were examined in order to determine whether active pesticide levels were being reduced within the honey sacs. Indeed, for some lipophilic compounds a radical decrease in the active pesticide levels could be determined. The active pesticide molecules apparently diffused into the tissue of the honey sac, which in turn suggests that the bees already reduced the pesticide content in the collected nectar during their collecting flights.

As the times that bees spend collecting nectar in the flower fields vary, this may be the cause for the strongly fluctuating measuring values. Bees clearly reduce the pesticide content acquired from the sprayed flowers already during their flight. The nectar delivered when the bees return to their hives already shows significantly reduced pesticide contaminations (Figure 4).

In the beehive, the nectar is processed into honey. The collected nectar is passed on from bee to bee. The worker bees enrich the nectar with endogenous substances and extract water from the honey during honey production. In this way, a self-preserving food substance is produced and stored in the cells of the honeycomb (Figure 5).

Further measuring sequences have shown that additional pesticide reduction processes take place during storage of honey, such as diffusion processes from the collected nectar into the beeswax of the honeycomb cells walls. These processes apply especially to the lipophilic active compounds and take place at the beginning of the honey production process when the water content is still relatively high. The question as to why pesticides rarely present a problem with respect to honey quality, even though beekeepers have always been very critical regarding the use of pesticides in orchards or rapeseed fields, is now close to being answered.

- **Detection of active pesticide components applied to the plants in the collected nectar of honeybees**
- **Determination limits in low ppb range**

First class quality TN_b for quality control

Figure 1: TOC-V_{CSN} and TNM-1

Illustration of a sugar beet (Beta vulgaris subsp. vulgaris var. altissima) – www.wikipedia.de

■ **Chemiluminescence detector enables the determination of total bound nitrogen and makes complex sample preparation redundant**

Sum parameters are frequently used in environmental applications. In these types of analyses, the traditional Kjeldahl method is steadily being replaced by the determination of total nitrogen (TN_b). Shorter analysis times and improved automation are significant advantages of TN_b determination.

The European Standard "EN 12260 – Determination of bound nitrogen (TN_b) following oxidation to nitrogen oxides", describes the determination of TN_b using chemiluminescence detection.

Determination of the sum parameters TOC and TN_b is a straightforward analytical method and is therefore used in many application areas. The following example demonstrates the use of TN_b determination in quality control of sugar beets.

TN_b analysis in sugar beets

In Germany, sugar beets are used as raw material in the production of granulated sugar. Optimal fertilization is crucial for a good sugar beet harvest. A high nitrogen supply increases crop growth but also decreases the sugar content. Within the scope of quality control, the bound nitrogen content is an important parameter for indication of the sugar content of the beets. In this case TN_b determination is a perfect analytical method.

Analysis system and measuring method

Shimadzu's TOC-V_{CXN} in combination with a TNM-1 chemiluminescence detector is a suitable measuring instrument. This module enables the determination of the total bound nitrogen. When required, TN_b determination can also be carried out simultaneously with TOC determination, all within four minutes. The TN_b determination is based on catalytic combustion at 720 °C. The nitrogen oxide generated during combustion is captured using a chemiluminescence detector. In this way, Shimadzu complies with the guidelines of the EN 12260 Standard.

Sample preparation

The sample material is first crushed to a paste. 500 mg of this paste are slurried in one liter of ultrapure water. The resulting suspension is placed into vials and transferred to the TOC-

# of sugar beets

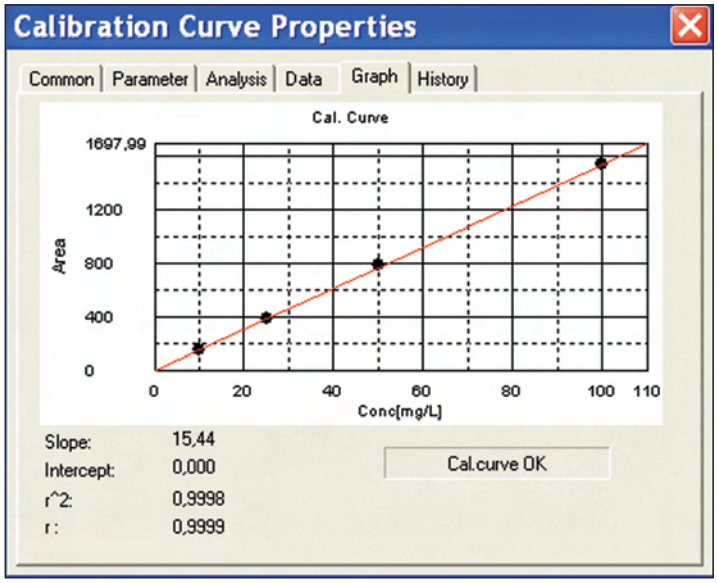


Figure 2: TN calibration curve 10 - 100 mg/L

V_{CXN} system using the ASI-V autosampler. The vials are stirred continuously during analysis. This measuring system has already been proven effective for efficient sample handling of suspensions during TOC analysis.

Calibration

The TNM-1 nitrogen module was calibrated using a KNO_3 standard solution. For the multipoint calibration, the automatic dilution function was used. From a stock solution of 100 mg/L, several dilutions were prepared automatically and measured by the TOC-V_{CPN} system. The calibration curve obtained in this way is shown in Figure 2. After calibration, the prepared suspensions were measured. Since reproducibility is critical in this type of analysis, each sample was injected ten times successively into the TOC system.

Results

Figure 3 shows good reproducibility of successive injections of one sample. In this way, sugar beets are apparently quantifiable with respect to TN_b and do not require any complex sample preparation procedure or any use of a solid sample module.

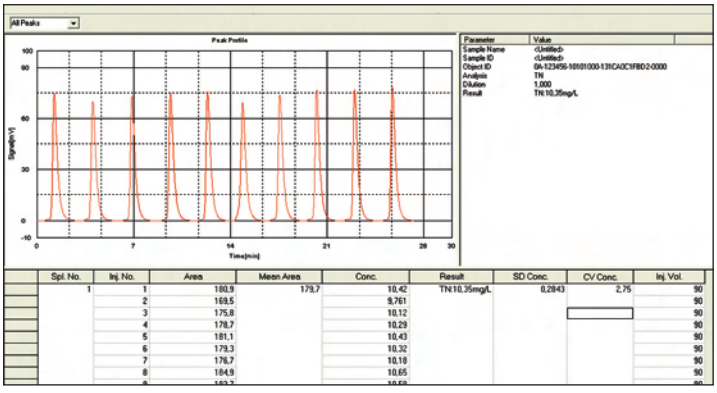


Figure 3: Peak graph of a TN determination

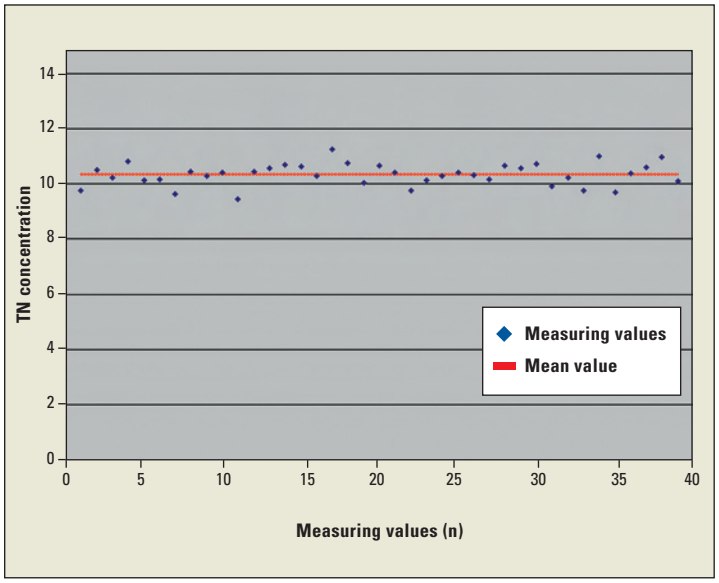


Figure 4: TN results of the sugar beets

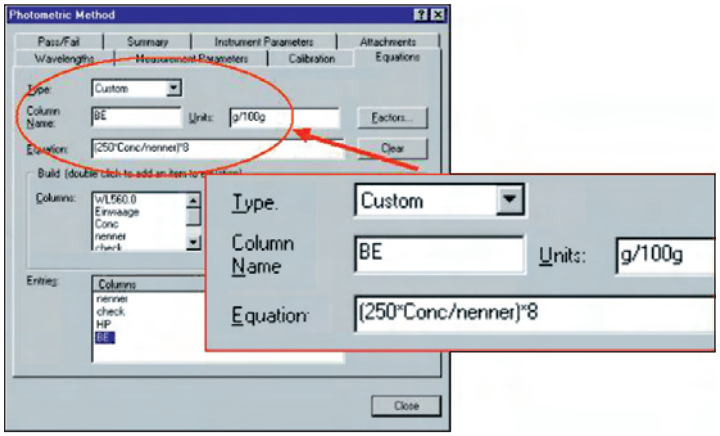
Measuring sequence [n]	TN_b (Suspensions) [mg/L]	TN_b (Sugar beets) [Mass %]	Injections [n]	RSD [%]
1	10.24	2.05	10	3.4
2	10.48	2.10	10	4.6
3	10.35	2.07	10	2.8
4	10.53	2.11	9	4.9*

Table 1: Results of four measuring sequences (*1 outlier was not evaluated)

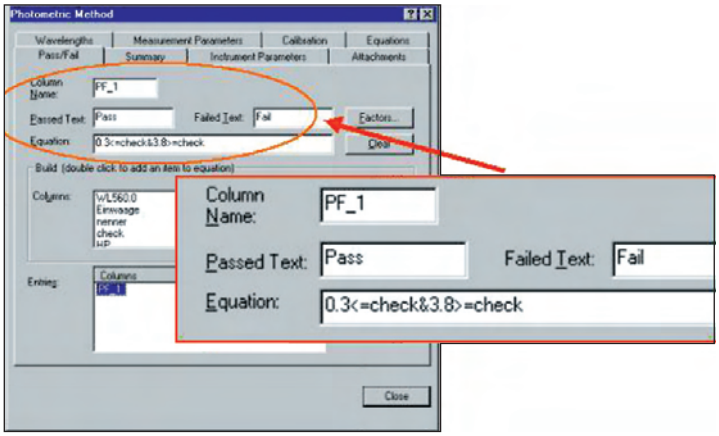
Table 1 summarizes the results of four measuring sequences. In addition, the diagram in Figure 4 shows an overview of the analytical results.

Highlighted piece by piece

Determination of hydroxyproline content in meat and meat products



Customer defined equation



Setting of Pass/Fail criteria

- **UV-VIS spectrometry determines hydroxyproline as a key indicator of meat products**
- **Applicable for determination of nitrite/nitrate and total phosphates**

Common experience shows that meat is the most nutritious of all foods according to Justus von Liebig, one of the best-known chemists in Germany. As a main food item on our plates, meats and meat products must be constantly subjected to stringent quality control.

One of the analytical methods used is UV-VIS spectrophotometry. This article describes the determination of hydroxyproline according to the German standard § 35 LMBG. Hydroxyproline is a parameter for the metabolism of collagen. Via the hydroxyproline content, the collagen content can be calculated, which in turn is an indication of the meat content in meats and meat products.

For the determination of hydroxyproline a double-beam UV-1700 UV-VIS spectrophotometer with UVProbe software is used. In addition to the quantification of hydroxyproline via a calibration curve, it is also possible to use various arithmetic calculation functions and to define pass/fail criteria. A report generator facilitates printing of the results. The photometric determination is carried out via solutions of red 4-dimethyl aminobenzaldehyde at a wavelength of 560 nm. The method can be automated using a peristaltic sipper in combination with a flow cell. The data is evaluated via a 6-point calibration curve.

During measurement it is possible to switch between standard and sample measurements. The standards, therefore, do not have to be determined before the actual samples but can be included in the sample analysis.

With the use of user definable arithmetic calculation functions, it is possible, for instance, to recalculate the hydroxyproline content from units in µg/0.1 mL to g/100 g. In this way, the collagen content can be calculated directly from the hydroxyproline content.

The pass/fail function of the software allows the definition of various control criteria. The sample tables show whether a measured value meets these criteria. The pass/fail function is employed during method development in order to test whether the sample concentration lies within the calibration range. In addition to the hydroxyproline method, the UV-1700 spectrophotometer is suitable for other meat quality control methods: the determination of nitrite/nitrate in cold cuts or the determination of total phosphate in meats and meat products.

				µg/0.1 mL			g/100 g	
	Sample ID	Type	Ex	Conc	WL560.0	HP	BE	PF_1	Einwaage
1	Probe 1_a	Unkno		0.625	0.088	0.110	0.877	Pass	1.426
2	Probe 1_b	Unkno		0.959	0.123	0.168	1.345	Pass	1.426
3	Probe 2_a	Unkno		2.142	0.247	0.327	2.616	Pass	1.638
4	Probe 2_b	Unkno		2.142	0.247	0.327	2.616	Pass	1.638
5	Probe 3_a	Unkno		4.499	0.494	0.924	7.393	Fail	1.217
6	Probe 3_b	Unkno		4.499	0.494	0.924	7.393	Fail	1.217
7	Probe 4_a	Unkno		0.167	0.040	0.020	0.161	Fail	2.078
8	Probe 4_b	Unkno		0.186	0.042	0.022	0.179	Fail	2.078
9									

Fail: Concentration above calibration area
- Probe 3: Conc > 3.8 µg/0.1 mL (highest Standard)
- Probe 4: Conc > 0.3 µg/0.1 mL (lowest Standard)

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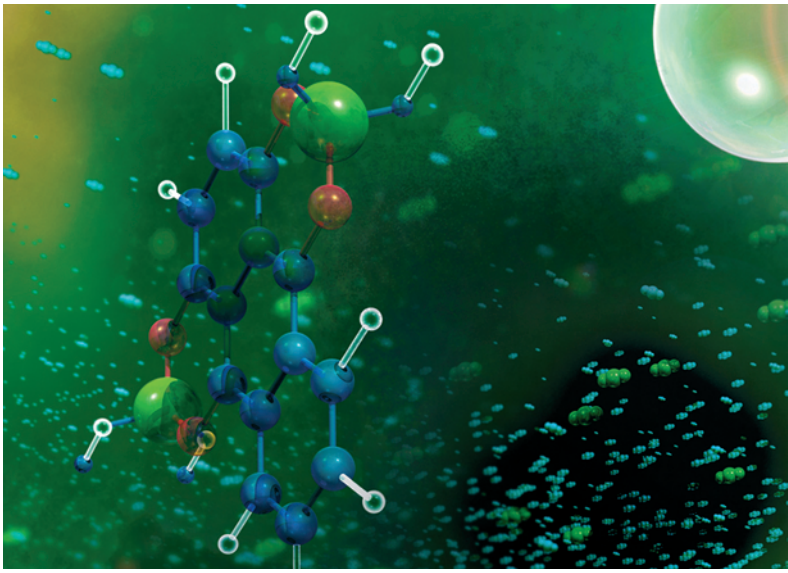

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