HUMAN HEALTH

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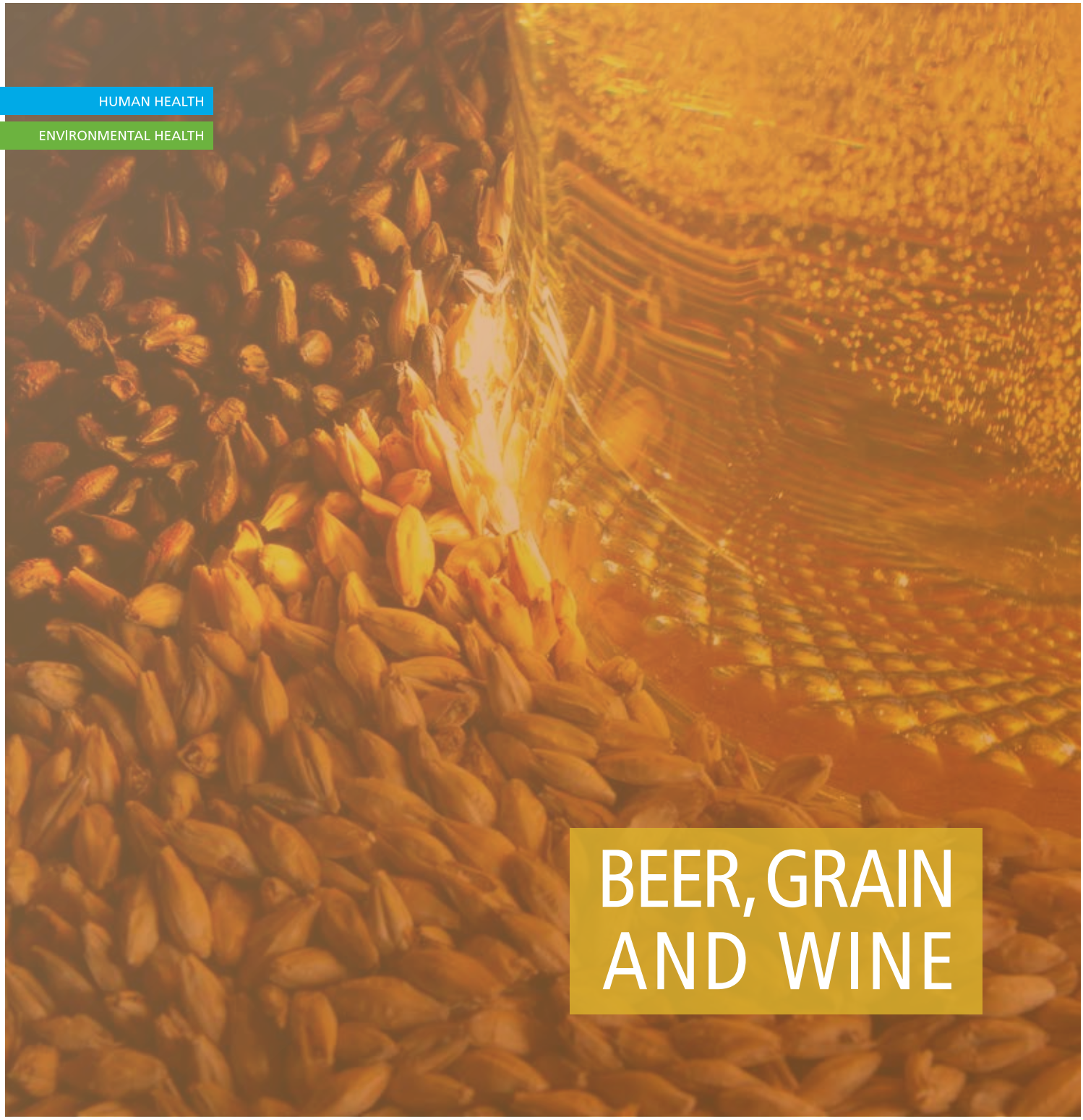

BEER, GRAIN AND WINE

Beer, Grain and Wine Compendium

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APPLICATION NOTE

Gas Chromatography – Mass Spectrometry

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The Determination of Low Levels of Nitrosamines in Beer Using the Clarus 680 GC/MS and a D-Swafer System

Introduction

Nitrosamines are a class of compounds that are often found in food and other organic products. They are highly carcinogenic and many countries apply controls on the acceptable levels of these compounds in food. Nitrosamines are formed as food is heated through the reaction of amines with nitrites, which are sometimes added as a preservative.

Malt and its derivative products are of particular concern and beer represents (along with fried bacon and tobacco) the major source of ingested nitrosamines in humans. Historically, malt was roasted over open fires and the nitrogen oxide gases in the smoke would react with amines in the malt to form nitrosamines. Modern malt production uses indirect fire roasting and the levels of nitrosamines have consequently dropped significantly – by a factor of over 50x from malt produced 20 years ago.

Nitrosamines generated during malt production will pass into beer. Examples of maximum acceptable levels of nitrosamines in beer are 5 µg/kg in the United States, 0.5 µg/kg in Italy, Switzerland and Germany and 2-15 µg/kg in Russia.

The main compound that is monitored in malt and beer is nitrosodimethylamine (NDMA). This compound and its homolog, nitrosodiethylamine (NDEA) are the compounds targeted in this application note.

To determine NDMA and NDEA at low concentrations in beer typically involves a liquid-liquid extraction followed by a multi-step extensive sample clean-up regime and determination by gas chromatography, including a highly specific and selective detector, such as a thermal energy analyzer (TEA) detector.

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In this application note, we present a more efficient and rapid method of analysis. It uses a fast and simple single-step liquid-liquid extraction technique followed by direct injection of the extract into a GC/MS system for separation and quantification.

A Swafer™ heartcutting system is used to selectively transfer timed cuts of the effluent, that contain the analytes, from one GC column into the inlet of a second column with a different stationary phase. This technique eliminates the solvent and bulk of other compounds extracted from the sample matrix from the chromatography in the second column, which provides an extra level of analytical selectivity and reduces the need for the sample clean-up procedures.

A quadrupole MS detector system was used in electron ionization mode to monitor the chromatography on the second column. This approach means that more sample residue is likely to accumulate over time in the injector liner, but liner replacement is a much easier option than a multi-stage sample clean-up regime. To achieve the detection limits sought, data collection used single ion recording (SIR). This provided another degree of selectivity to overcome potential matrix interferences.

A second detector (flame ionization) was used to monitor the chromatography on the precolumn and establish the heartcut and the backflush timings.

Experimental

Instrumentation

Figure 1 gives a schematic diagram of the analytical system, which is from the Swafer Utilities Software that was used to develop this method. Table 1 lists the full operating conditions for the final method.

Table 1. **Analytical Conditions for the Determination of Nitrosamines in Beer.**

Gas Chromatograph	PerkinElmer Clarus 680
Oven Temperature	35 °C for 1 min., then 10 °C/min. to 185 °C (16-minute run)
Injector	Programmable Split/Splitless (PSS)
Injector Temperature	35 °C for 1 min., then 200 °C/min. to 250 °C and hold until the end of the run
Carrier Gas	Helium
Injector Pressure	Initially 27 psig then 2 psig at 12.81 min. Maintained at 2 psig during oven cooldown.
Injector Split Flow Rate	Initially Off, then 25 mL/min. at 3 min.
Detector	a) Flame Ionization (FID) b) Mass Spectrometer (MS)
FID Temperature	250 °C
FID Combustion Gases	Air: 450 mL/min., Hydrogen: 45 mL/min.
FID Range	x1
FID Attenuation	x4
MS Transfer Line Temperature	200 °C
MS Filament Temperature	200 °C
MS Data Collection	a) SIR m/z 74 from 12.00 – 13.50 min. (for NDMA) b) SIR m/z 102 from 15.00 – 16.00 min. (for NDEA) Dwell Time 0.5 sec Interchannel Delay 0.02 sec
Switching/Backflush System	D-Swafer configured in D4 mode
Precolumn	30 m x 0.250 mm x 0.25 µm PerkinElmer Elite™ 1
Analytical Column	30 m x 0.250 mm x 0.25 µm PerkinElmer Elite Wax (connected to MS)
Restrictor Tubing	51 cm x 0.100 µm deactivated fused silica (connected to FID)
(Midpoint) Pressure at D-Swafer	18 psig throughout
Timed Events	PSS Pressure set to 27 psig at -1.50 min. (to raise pressure after oven cooldown) PSS Split Flow set to 0 mL/min. at -1.00 min. (for splitless mode) PSS Split Flow set to 25 mL/min. at 3.00 min. (to vent liner) Switching Valve On at 9.27 min. (to cut NDMA) Switching Valve Off at 9.48 min. Switching Valve On at 12.60 min. (to cut NDEA) Switching Valve Off at 12.80 min. PSS Pressure set to 2 psig at 12.81 min. (to backflush)
Sample Injection	Normal injection of 3.0 µL of sample dichloromethane extract using an autosampler

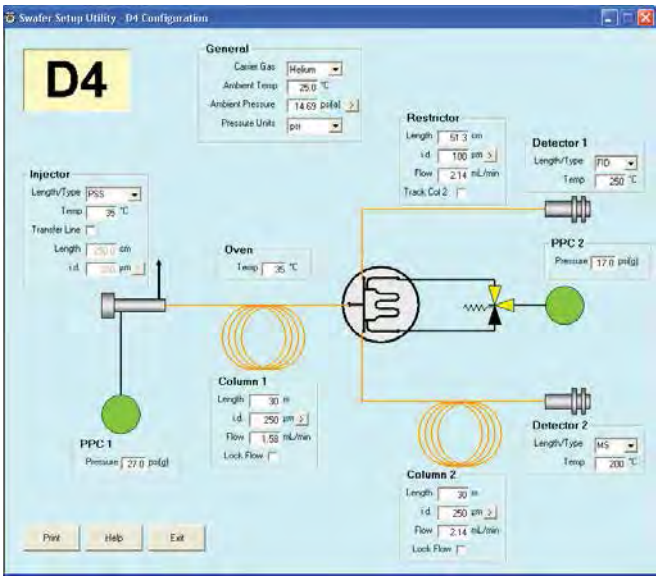

Figure 1. Swafer Utilities Software used for the determination of nitrosamines in beer.

Sample Preparation

Approximately 25 mL of the beer sample was poured into a 50 mL beaker and placed in a cool ultrasonic bath for two minutes to de-gas the sample.

10.0 ±0.5 g of de-gassed beer were weighed to a precision of 0.01 g into a 15-mL polypropylene centrifuge tube.

1-mL of 1.0 M sodium hydroxide solution prepared in de-ionized water and 3.0 ±0.1 g of crystalline sodium chloride were added to the sample and shaken to dissolve the salt.

1-mL of dichloromethane was added using a Grade-A bulb pipette and carefully shaken with a gentle rocking motion with the treated beer sample for 10 minutes. Vigorous shaking was avoided to minimize the formation of emulsions.

The tube was centrifuged at high speed for 20 minutes. In instances where emulsions had formed, the contents of the tube were rocked backwards and forwards a few times and then re-centrifuged (this was usually effective at breaking up the emulsion). An example of a successful extraction is given in Figure 2.

Figure 2. Example of successful beer extraction.

Using a dropper pipette, a sufficient volume of the lower organic layer was transferred directly into an autosampler vial for GC analysis. Note that it was not necessary to transfer the whole extract. Care was taken to ensure that none of the aqueous phase was transferred to the vial along with the extract. The vial was sealed with a suitable cap and refrigerated until analysis.

Method Development

Figure 3 shows a TIC of a concentrated standard mixture containing a series of nitrosamines including NDMA and NDEA. In this chromatogram, the D-Swafer has been set to direct the effluent from the precolumn into the analytical column, which is connected to the mass spectrometer. In practice we will need to see nitrosamines at a concentration below the 1 ppb level in the samples (equivalent to 10 ppb in the extracts). This is more than 1000x less than the concentration shown here.

Another point that should be mentioned is the role of the PSS injector in this analysis. The sample extracts are in dichloromethane. This is a highly volatile solvent that boils at 40 °C (at atmospheric pressure). It is not a good solvent to use for splitless injection as it is difficult to refocus at the column inlet. The injection of large volumes will easily cause injector blow-out and cause peak distortion and loss of injected sample. In this method the PSS is set to a low temperature (35 °C) during injection and then heated to vaporize the rest of the injected sample. This provides a much more controlled vaporization process and the 3 µL injection volume provides symmetrical and well separated peaks as shown in Figure 3. One concern in using the PSS at low temperatures like this is the time needed to cool the injector back to this temperature before the next run. The Clarus® 680 PSS uses an optimized heatsink and a high-speed dedicated cooling fan to achieve this cooling in less than four minutes.

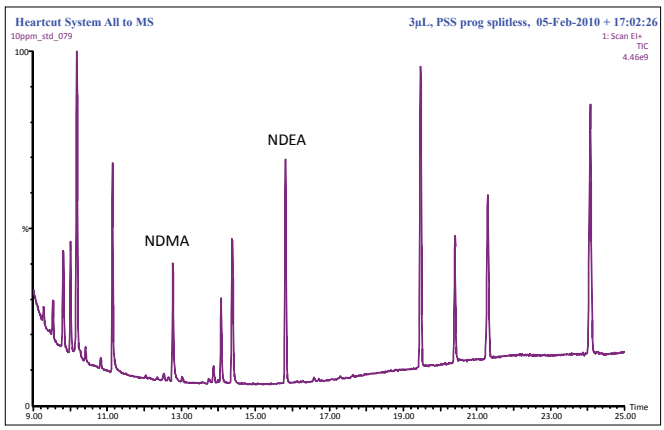

Figure 3. Total ion chromatogram (m/z 35 to 150) from a 3 µL injection of a 10 ppm standard mixture of nitrosamines with the precolumn effluent directed to the second column and the MS detector.

The concern regarding detection limits is illustrated in Figure 4. This shows a TIC obtained from a beer extract run under the same conditions as used for Figure 3. The chromatogram is plotted with an expanded time scale but with a similar response scale. Expected elution times of the two nitrosamines are indicated. We need to see peaks at less than 1000x the size of those shown in Figure 3 without significant interference from co-eluting peaks from the sample matrix. This is clearly a challenge using this type of method as much better sensitivity and much better selectivity are needed.

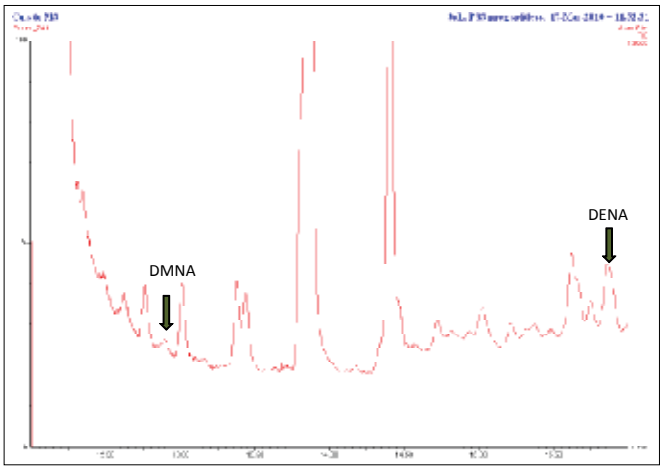

Figure 4. Total ion chromatogram of an extract taken from an American porter ale sample with the precolumn effluent directed to the second column and the MS detector.

Better sensitivity and selectivity are easily obtained on a quadrupole mass spectrometer by operating it in the SIR mode.

Figure 5 and Figure 6 give the structures and mass spectra of the two nitrosamines being monitored. These figures were taken from the NIST® Mass Spectral Search Program v. 2.0 supplied with the Clarus 680 MS system. In each case there is a strong molecular ion at m/z 74 and m/z 102 for NDMA and NDEA respectively. These ions were used in a SIR MS method to collect and process the data.

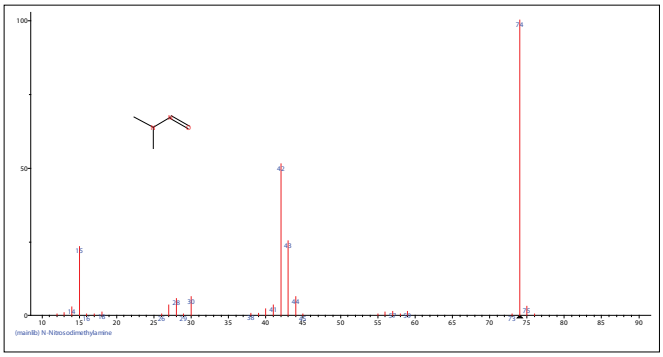

Figure 5. Structure and mass spectrum of nitrosodimethylamine.

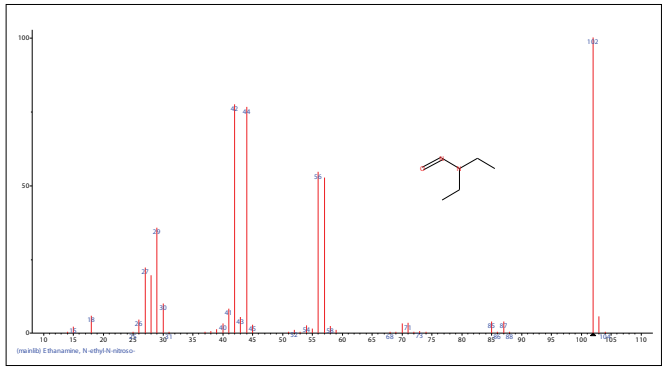

Figure 6. Structure and mass spectrum of nitrosodiethylamine.

Figure 7 shows chromatography of a much more dilute standard mixture of nitrosamines under the same conditions as used for Figure 3 and Figure 4. These peaks would represent nitrosamine concentrations of 1 ppb in the samples, and clearly indicate the potential to detect nitrosamines at levels below 1 ppb in the samples.

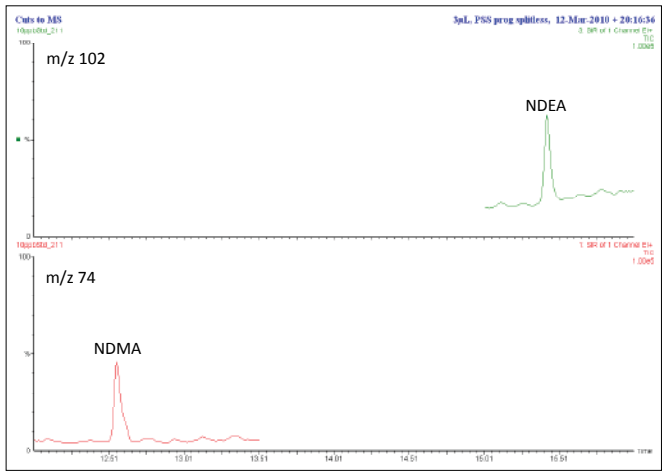

Figure 7. SIR chromatographic traces at m/z 74 and 102 showing expected elution times of NDMA and NDEA respectively from an injection of a 10 ppb stand mixture of nitrosamines (equivalent to 1 ppb in samples).

However, when a beer extract is chromatographed as shown in Figure 8 under the same conditions as used for Figure 7, there are significant interferant peaks in the chromatography that would obscure the nitrosamine peaks at the required levels. This chromatogram has the same scaling as Figure 7. Some further improvements to the selectivity are required.

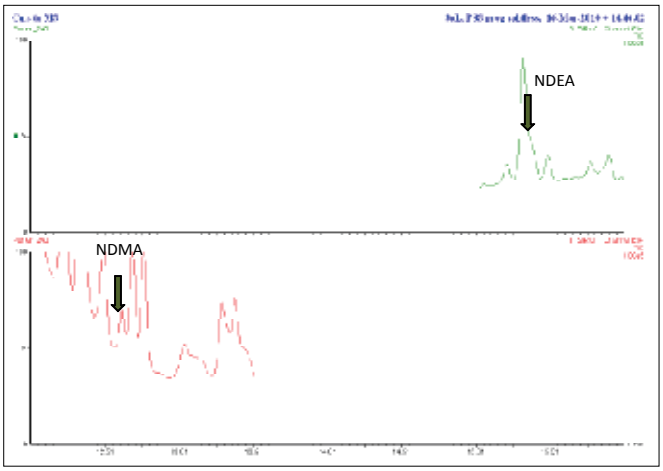

Figure 8. SIR traces at m/z 74 and 102 showing expected elution times of NDMA and NDEA respectively.

The use of the D-Swafer in the D4 heartcutting configuration provides an additional high degree of selectivity by transferring narrow cuts around the elution times of the nitrosamines from the precolumn on to the analytical column. This way, the solvent and the bulk of the extracted sample matrix are eliminated completely from the chromatography being monitored. Because the analytical column stationary phase is very polar, peaks that would co-elute with the nitrosamines on the non-polar precolumn and cut with them to the analytical column, would become separated by the different stationary phase. Figure 9 shows chromatography of the concentrated nitrosamine standard mixture indicating the regions around the eluting nitrosamines that are to be heartcut to the analytical column.

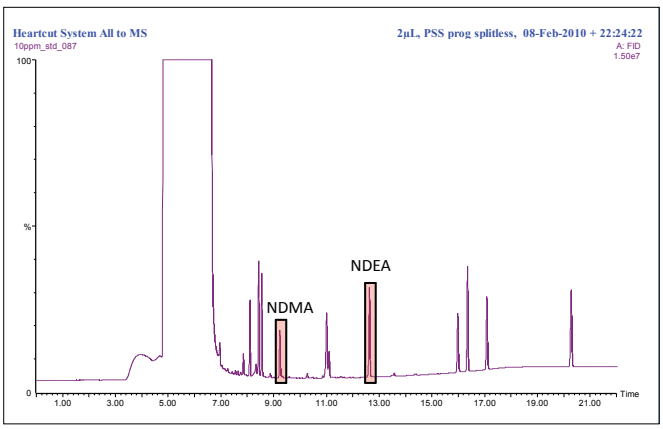

Figure 9. Precolumn chromatogram of 10 ppm nitrosamine standard mixture. This chromatogram was produced with the precolumn effluent switched to the D-Swafer outlet restrictor and the FID. The regions to be heartcut are highlighted.

Figure 10 shows a chromatogram from the same beer extract shown in Figure 4 and Figure 8, which was run under the same conditions used for Figure 9 but with the heartcutting switching applied. The heartcut regions are indicated by the drop in signal from the FID. After the NDEA peak has been heartcut, the pressure at the GC injector is reduced to a low value to initiate backflushing of the precolumn. This is indicated by the absence of chromatography beyond the last heartcut. Backflushing helps keep heavy sample material out of the Swafer and the detector, eliminates the need for extended temperature programming to elute heavy sample material from the system and reduces the analysis time.

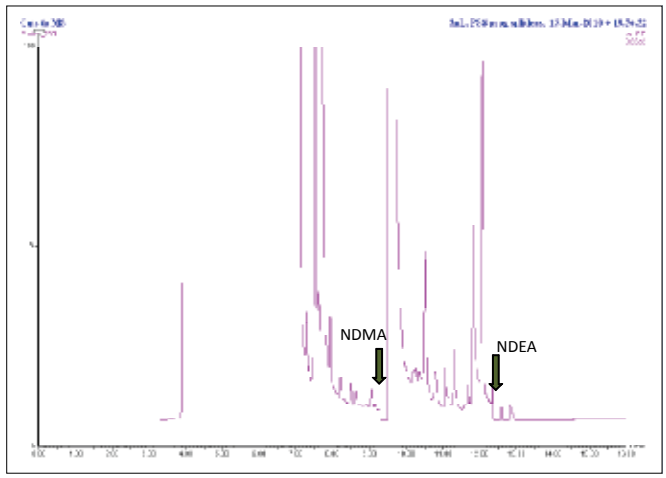

Figure 10. FID precolumn chromatogram of an American porter ale extract showing regions that have been heartcut to the analytical column.

Figure 11 shows the corresponding SIR chromatography of the heartcuts directed to the analytical column illustrated in Figure 10. Now the traces are very clean and the nitrosamine peaks, which are 0.39 ppb and 0.11 ppb for NDMA and NDEA respectively are seen and quantified with confidence. Compare these chromatograms against those in Figure 8 to see the improvements in selectivity brought about by the D-Swafer heartcutting technique.

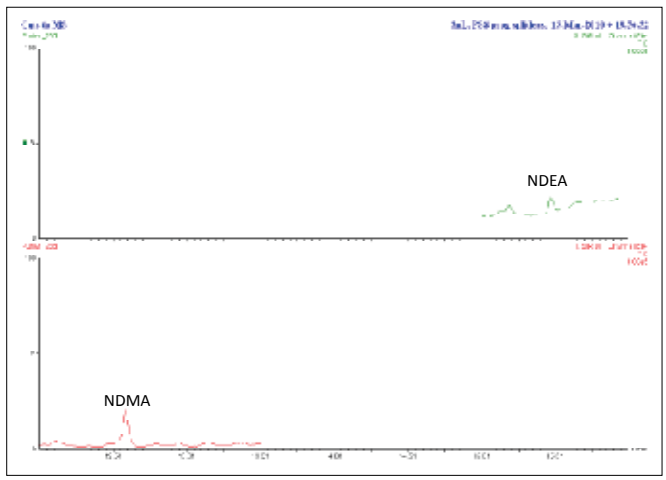

Figure 11. Analytical column SIR chromatography of an American porter ale extract.

Performance

Using these conditions, the performance of the system was checked using standard mixtures of known concentrations. Figure 12 and Figure 13 show the calibrations for NDMA and NDEA respectively over a range of effective sample concentrations from 0.05 ppb or less to 2.0 ppb. Excellent linearity for this method is demonstrated for both analytes.

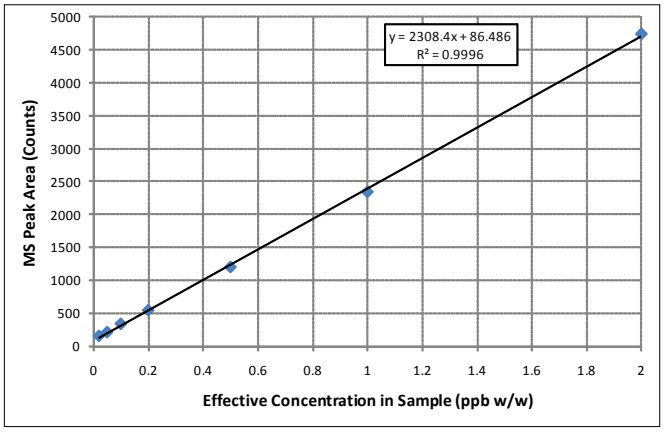

Figure 12. Calibration data for NDMA.

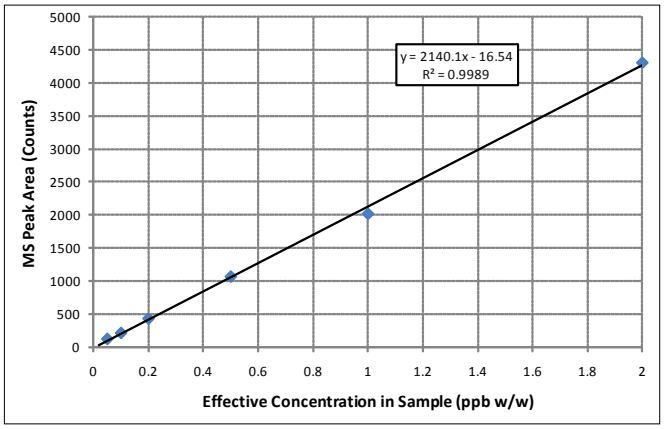

Figure 13. Calibration data for NDEA.

Results from Beer Samples

A variety of commercial beers were purchased from a local store and samples of home brewed beer (all grain recipes) were extracted and chromatographed using this method. The results are given in Table 2. All results are well within the USA FDA guideline of 5.0 ppb (CPG 510.600).

***Table 2.* Results obtained from various beer samples.**

Sample	NDMA (ppb)	NDEA (ppb)
Oktoberfest (Germany)	<0.02	<0.05
IPA (USA)	0.09	<0.05
English Bitter (Home Brew)	0.08	<0.05
Pilsner Lager (Home Brew)	<0.02	<0.05
Imperial Stout (USA)	0.25	<0.05
Coffee Porter (USA)	0.39	0.11
IPA-B (USA)	0.08	<0.05
Pale Ale (Home Brew)	0.16	<0.05
Paler Ale (USA)	0.14	<0.05

Making the Method More Robust

The use of an internal standard would improve the robustness of the method by reducing errors that result from partitioning the solvent into the sample or evaporation of the solvent during sample handling.

A deuterated form of NDMA was obtained and added at the 50 ppb level (equivalent to 5 ppb in the sample) to the dichloromethane extraction solvent. This NDMA-D6 compound had a primary (molecular) ion of m/z 80 in its mass spectrum so the SIR method was adjusted to also monitor the signal at this mass.

This deuterated compound is a perfect internal standard as it is chemically equivalent to the NDMA being determined and is subjected to the same sources of error.

Figure 14 shows chromatography of a standard mixture that included the internal standard.

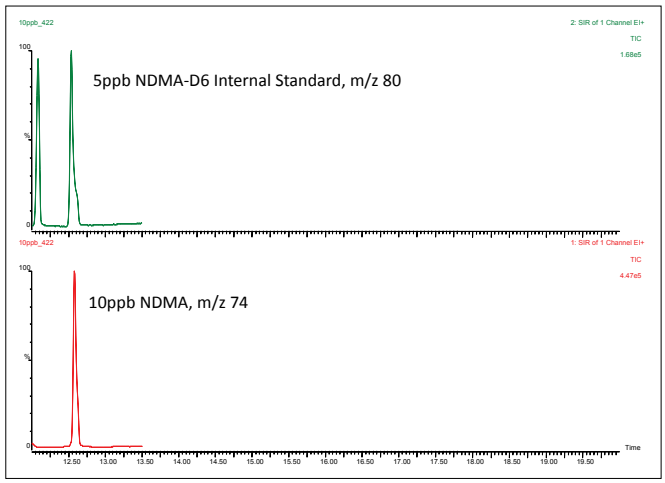

Figure 14. Chromatography of a standard mixture showing peaks for both NDMA and NDMA-D6 internal standard. Concentrations reflect those in the sample.

Figure 15 shows a calibration profile for the response ratio versus the concentration ratio for NDMA versus NDMA-D6. Excellent linearity is demonstrated at levels down to 0.1 ppb.

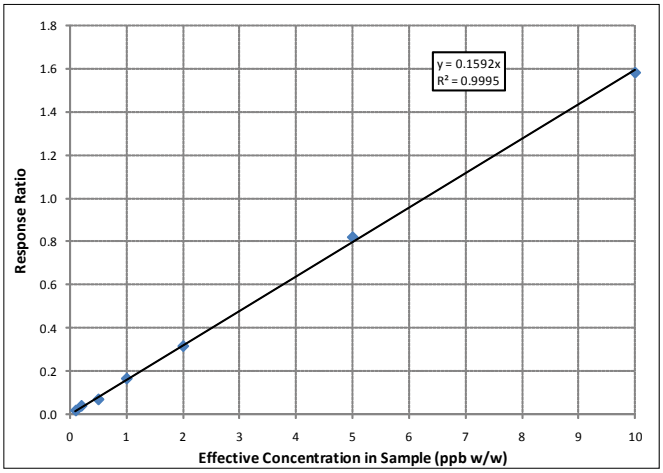

Figure 15. Calibration data for NDMA based on ratio to internal standard response.

Table 3 shows the instrumental precision obtained for multiple injections of the same standard mixture of NDMA and NDMA-D6. A relative standard deviation of 2.5% for the quantitative precision is excellent for this type of analysis – low level analytes in a very dirty sample matrix.

Table 3. **Precision data obtained for NDMA using NDMA-D6 internal standard.**	
n	10
Mean Result (ppb)	2.27
RSD %	2.46

Another set of beer samples was selected for analysis. These were strong beers (apart from the American cream ale) with final alcohol concentrations of 7 to 10 ABV% so significant amounts of malt were used in their production. This is a severe test for the method as interferences from the sample matrix will be higher.

Figure 16 shows chromatography of an extract taken from one of the strong beer samples and Table 4 summarizes the results for all the samples tested.

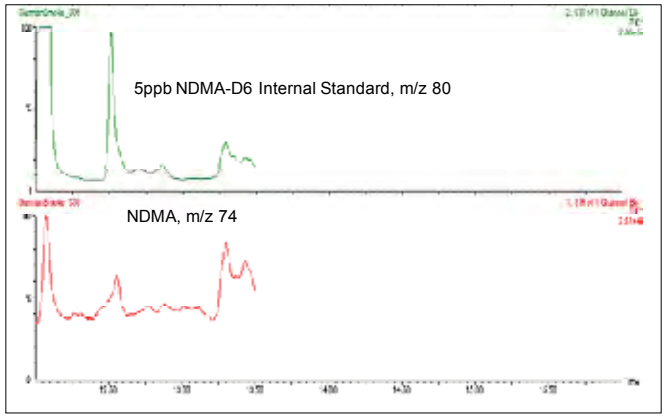

Figure 16. Chromatography of extract taken from Czech dark lager sample using DMA-D6 internal standard.

Table 4. **Results from various beer samples.**	
Beer Sample	**NDMA (ppb)**
Polish Porter	0.43
German Rauchbier	0.32
Russian Porter	0.80
Italian Birra Blonde	0.22
USA Cream Ale	<0.2
Czech Dark Lager	0.76

Extending the Method to Other Nitrosamines

Although the initial work focused on two nitrosamines, this method may be easily adapted to monitor other nitrosamines.

Figure 17 through Figure 19 shows how the method was extended to include nitrosopyrrolidine (NPYR) by simply adding another heartcut and extending the run time.

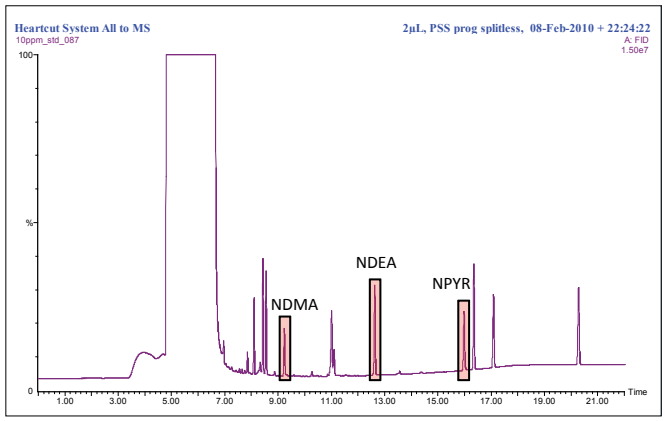

Figure 17. Precolumn FID chromatogram of 10 ppm standard mixture showing elution of NPYR and suggested additional heartcut times.

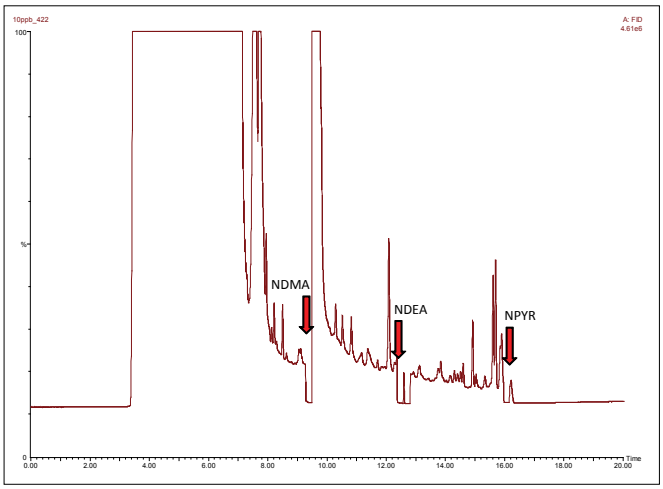

Figure 18. Precolumn FID chromatogram of beer extract showing cuts transferred to analytical column. Temperature program was extended to 225 °C and chromatographic run time was extended to 20 minutes.

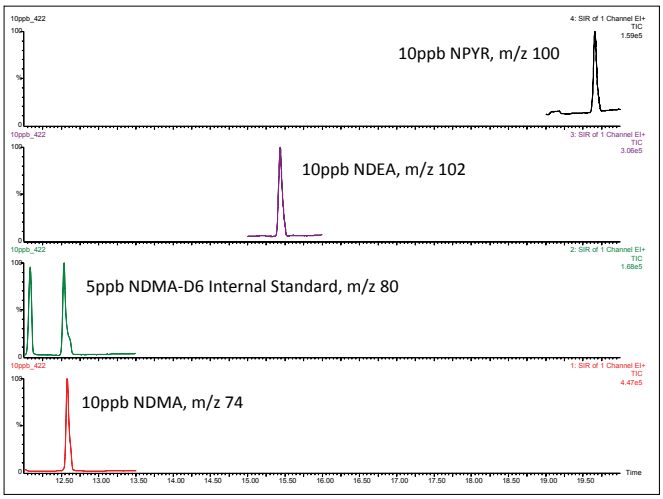

Figure 19. Analytical column SIR chromatography of spiked beer sample showing three analytes and the internal standard.

Conclusion

The need for methods to determine the levels of nitrosamines in beer has been a requirement for many years. Over time, a succession of different methods were developed yet need extensive sample clean-up, concentration procedures and exotic detection systems. The method presented in this application note uses a very simple and straightforward single-step extraction procedure with a minimum of solvent (1 mL). Sample extracts are much 'dirtier' than those produced in other methods but the combination of the heartcutting technique with SIR data collection on a mass spectrometer delivers the necessary selectivity without compromising the detection limits.

The combination of the heartcut technique and the fast cooling oven and PSS injector of the Clarus 680 enables the total chromatographic cycle time to be reduced to less than 20 minutes.

Examples have been shown in which nitrosamines in beer are seen at levels of 0.1 ppb or even lower.

Although this method is targeted towards beer analysis, it can be applied to the analysis of nitrosamines in other sample types.

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APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Characterization of Hop Aroma Using GC/MS, Headspace Trap and Olfactory Port

Introduction

Hops are a critical ingredient in beer. They provide an important balance to the malt in the taste of many beers. They also aid the brewing process in precipitating out proteins, etc. during the boil. Hops also have preservative properties that help keep beer fresh and free from bacteriological attack.

Hops contribute to the taste of beer in three ways:

- Bittering – hops contain compounds such a humulones that are very insoluble in water but isomerize on boiling to form isohumulones, which are partially soluble and impart the bitter flavor to beer.
- Flavoring – compounds such as terpenes and esters provide the fruity, citrus, earthy, resiny flavors to many beers.
- Aroma compounds – these are the volatile organic compounds that migrate into the vapor above the head of beer and gives the beer its characteristic smell. This can be flowery, citrusy, fruity, etc. They form a very important part of the overall flavor of beer.

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There are many types of hops that deliver a very wide range of flavors. Hops need to be stored carefully and be used when fresh since the flavor will degrade as they age. Consequently there is a need to characterize the quality of hops so that the brewer can develop and deliver the required product.

Aroma characterization of hops is complex; there are many compounds in hops that contribute to flavor. Table 1 lists the composition of typical hops and Table 2 lists some of the key aroma compounds. The traditional way to evaluate hop quality is to use an experienced brewer to assess the hops organoleptically by crushing a few of the hops in their fingers and smelling the released aroma. This is effective but not objective and lacks the quantitative information needed to make correct decisions on how to utilize the hops.

Table 1. Composition of typical hops.

Component	%
Vegetative material (cellulose, lignin, etc.)	40
Proteins	15
Total resins (bittering compounds)	15
Water	10
Ash	8
Lipids, wax, pectin	5
Tannins	4
Monosaccharides	2
Essential oils (flavor/aroma compounds)	0.5 to 2

Table 2. Key hop aroma compounds.

Component	Comment
Myrcene	Pungent flavor; normally oxidized during the boil into other flavor compounds such as linalool and geraniol and their oxides
Humulene	Delicate and refined flavor characteristic of noble hops; broken down by boiling into oxidative flavors
Caryophyllene and farnesene	Herbal spicy character -- not well characterized

This application note describes a system that is able to provide both an objective chemical analysis of hop aroma using gas chromatography/mass spectrometry and, at the same time, provide the means for the user to monitor the olfactory character of each component as it elutes from the chromatographic column. Such an approach allows the user to gain a fuller characterization of a particular hop sample.

Analytical System

The analytical system comprises five main components:

HS Trap

Static headspace (HS) sampling is very suited for extracting aroma compounds out of hops. A weighed amount of hops (pellets or leaves) is placed in a glass vial and sealed as shown in Figure 1. This vial is then heated in an oven at a set fixed temperature and for a set fixed time period. A portion of the vapor is then extracted from the vial by the headspace sampling system and introduced into the GC column for separation and analysis.

Figure 1. Hops inside a headspace vial awaiting analysis.

While extremely convenient, static headspace sampling only delivers a very small fraction of the headspace vapor into the GC column and so it is really best suited to high concentrations of compounds. In the analysis of complex samples, it is often found that low levels of some components are critical to the overall aroma of that sample. To increase the amount of sample value introduced into the GC column, a headspace trap system was used.

Using this technology, most or even the entire headspace vapor is passed through an adsorbent trap to collect and focus the VOCs. The trap is then rapidly heated and the desorbed components are transferred to the GC column. In this way, the amount of sample vapor entering the GC column can be increased by a factor of up to 100x. This technique is ideally suited for hop aroma analysis.

Figures 2 to 4 are simplified representations of the HS trap operation – there are other valves and plumbing needed to ensure that sample vapor goes where it should and not anywhere else. Essentially, the principle is very similar to classical static headspace but at the end of the vial equilibration step, after the vapor is pressurized, it is fully vented through an adsorbent trap. This process may be repeated to effectively vent the entire headspace vapor through the adsorbent trap. Once the trap is loaded, it is rapidly heated and the desorbed VOCs are transferred to the GC column.

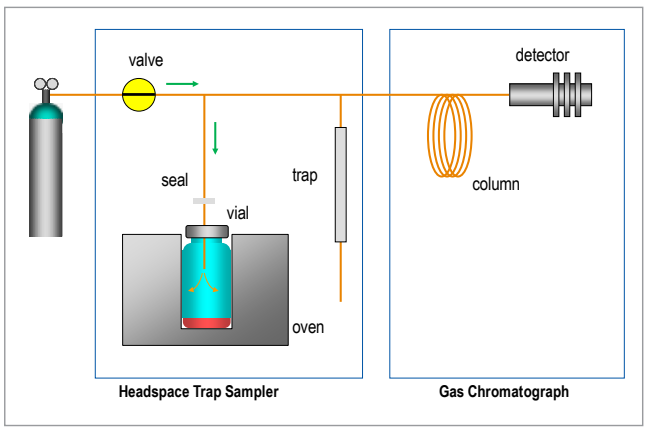

Figure 2. Schematic diagram of the HS trap system showing the equilibrated vial being pressurized with carrier gas.

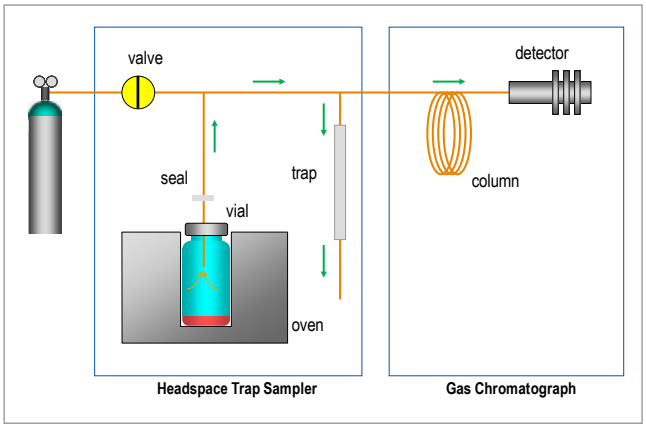

Figure 3. Schematic diagram of the HS trap system showing the pressurized headspace being released from the vial into the adsorbent trap.

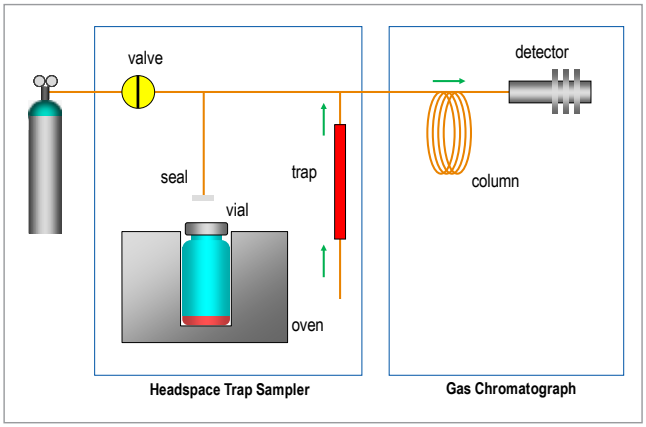

Figure 4. Schematic diagram of the HS trap system showing the VOCs collected in the adsorbent trap being thermally desorbed and introduced into the GC column.

Clarus 680 GC

The workhorse Clarus® 680 GC is an ideal complement to the rest of the system. The chromatography is undemanding so simple methods may be used. For olfactory monitoring, it is important to have sufficient time between adjacent peaks for the user to discern them from each other. It is also beneficial to load the column with as much sample as possible without overload to provide the best opportunity for the user's nose to detect them. For this reason, a long column with a thick stationary phase is used. Because many of the components in hops are highly polar (acids, esters, ketones, etc.) a very polar Carbowax®-type stationary phase is used for the separation.

Figure 5. The Clarus 680 SQ 8 GC/MS system.

S-Swafer System

Because the column effluent needs to supply both the MS and the olfactory port, some form of splitting device is required. This should not affect the integrity of the chromatography in any way and so should be highly inert and have low-volume internal geometry. The use of a make-up gas in the splitter provides additional control and stability of the split flow rates.

S-Swafer™ is an excellent active splitting device and well suited to this purpose. Figure 6 shows the S-Swafer configured to split the column effluent between the MS detector and the SNFR olfactory port. The split ratio between the detector and the olfactory port is defined by the choice of restrictor tubes connected between the Swafer outlets and the MS and SNFR.

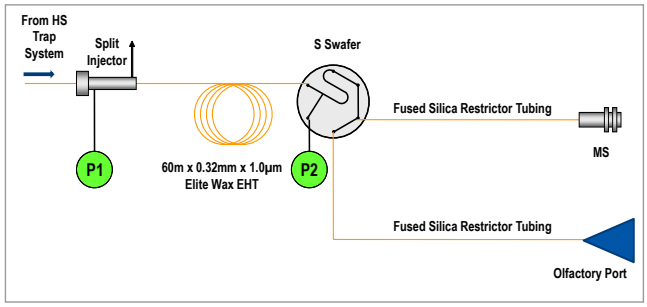

Figure 6. S-Swafer configured for use with the Clarus SQ 8 GC/MS and the SNFR.

The Swafer utility software, which is included with the Swafer system, may be used to calculate this split ratio. Figure 7 shows how this calculator was used to establish the operating conditions for the S-Swafer for this application.

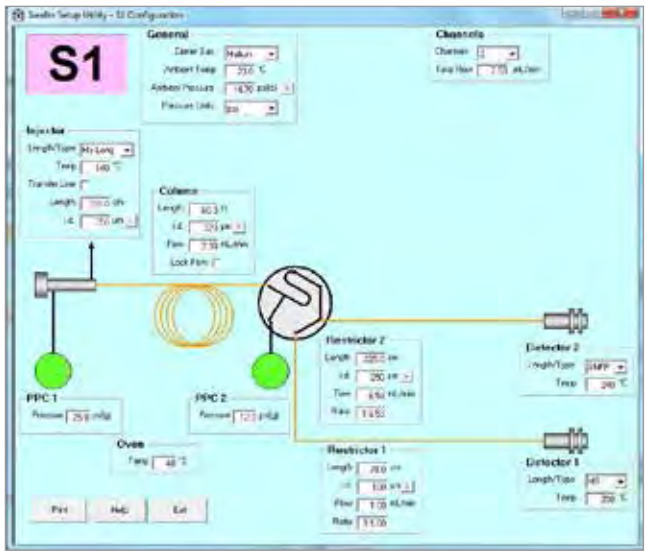

Figure 7. The Swafer utility software showing the settings used for this hop aroma characterization work.

Clarus SQ 8 Mass Spectrometer

A mass spectrometer is an important part of an aroma characterization system. It's important not only to detect and describe the aromas of the various components eluting from the GC column but to also to identify what those components are and possibly what their levels in the hops are.

The Clarus SQ 8 quadrupole mass spectrometer is ideally suited for this purpose and will quickly identify and quantify components using classical spectra in the supplied NIST library. This software is also able to interact with the olfactory information as described later in this document.

GC SNFR Accessory

Figure 8 shows a picture of the SNFR accessory. This is connected to the GC via a flexible heated transfer line. The split column effluent travels to the glass nose-piece through deactivated fused silica tubing.

While monitoring the aroma compounds eluting from the GC column, the user is able to capture vocal narration via a built-in microphone and aroma intensity by adjustment of a joystick.

Figure 8. The SNFR olfactory port accessory.

Analytical Conditions

Table 3. HS Trap conditions.

Headspace system	PerkinElmer® TurboMatrix™ 110 HS Trap
Vial equilibration	80 °C for 15 minutes
Needle	120 °C
Transfer line	140 °C, column connected directly to HS trap
Carrier gas	Helium at 25 psig
Dry purge	5 min
Trap	Air toxics, 30 °C to 300 °C, hold for 5 min
Extraction cycles	1 with 40 psig extraction pressure

Table 4. GC conditions.

Gas Chromatograph/ Mass Spectrometer	PerkinElmer Clarus 680 SQ 8
Column	60 m x 0.32 mm x 1.0µm Elite-5MS connected directly to the HS trap
Oven	40 °C for 2 min, then 4 °C/min to 240 °C for 8 min
Carrier gas	13 psig at Swafer
Injector	PSS at 300 °C, carrier gas off

Table 5. MS conditions.

Scan range	m/z 35 to 350
Scan time	0.8 s
Interscan delay	0.1 s
Source temp	250 °C
Inlet line temp	250 °C

Table 6. Olfactory port conditions.

Olfactory port	PerkinElmer SNFR
Transfer line	225 cm x 0.250 mm at 240 °C
Humidified air	500 mL/min with jar set to 37 °C

Table 7. Swafer conditions.

Swafer	PerkinElmer S-Swafer in the S1 configuration
Settings	Developed using the Swafer utility software – see Figure 7

Table 8. Sample details.

Sample preparation	Hops (leaves or pellets) were ground with a rotary coffee grinder and 1 g was weighed into a sample vial and sealed
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicone septum

Typical Chromatography

Figure 9 shows total ion chromatograms (TIC) of four typical hops from different countries. Part of the German Hallertau is highlighted and is expanded in Figure 10. The power of the MS enables a particular peak to be identified from its mass spectrum (as shown in Figure 11) by searching the NIST spectral library supplied with the Clarus SQ 8 system. The results of this search are given in Figure 12. Results of this search very strongly indicate that the peak eluting at 36.72 minutes is 3,7-dimethyl-1,6-octadien-3-ol, otherwise known as linalool. Linalool is a very important aroma compound and will provide a delicate flowery aroma to the beer. The amount of linalool (or any other compound once identified) may be quantified by calibrating the GC/MS with standard mixtures of this compound.

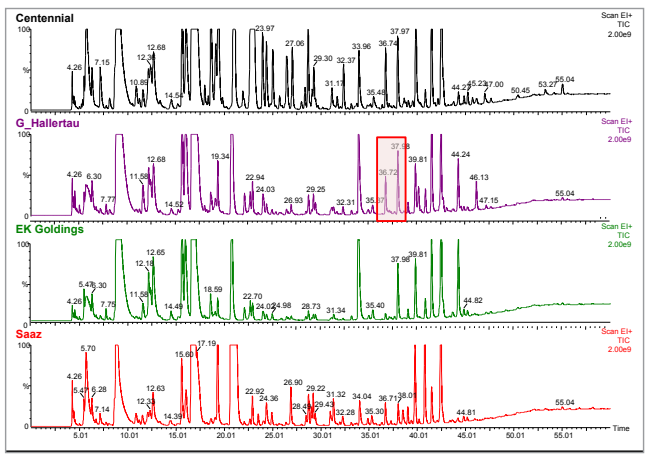

Figure 9. Typical TIC chromatograms of four hop samples.

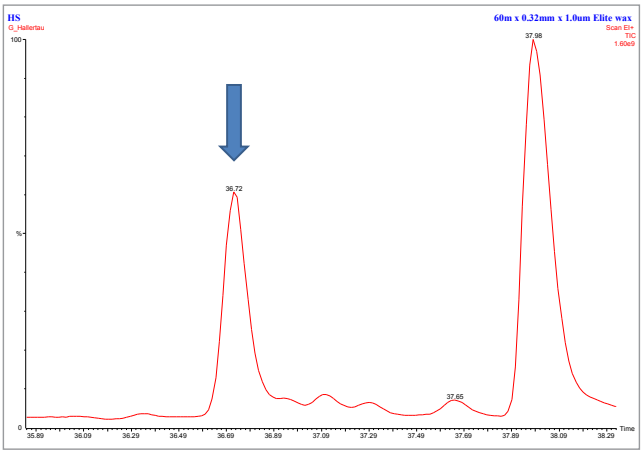

Figure 10. Highlighted detail from Figure 9.

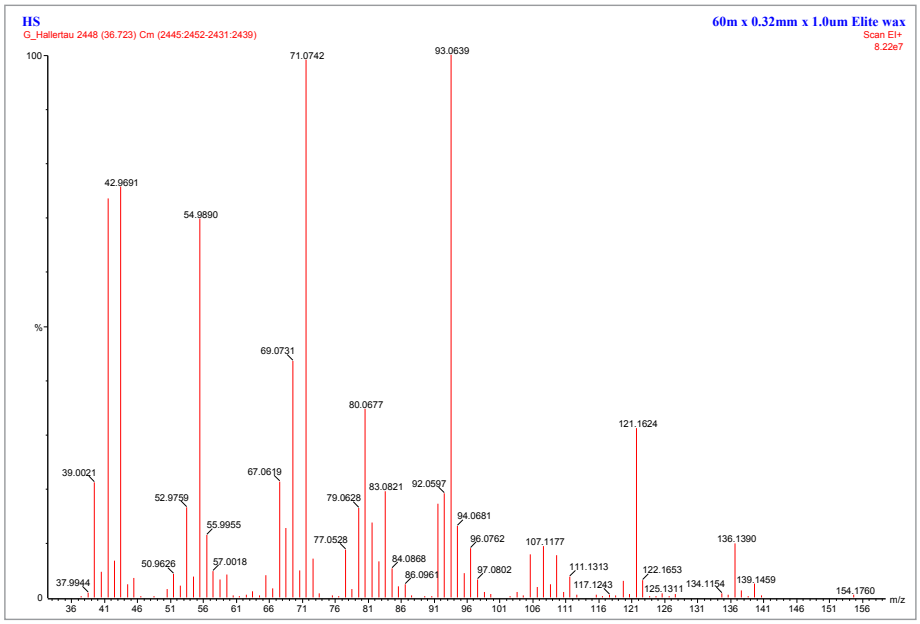

Figure 11. Mass spectrum from peak highlighted in Figure 10.

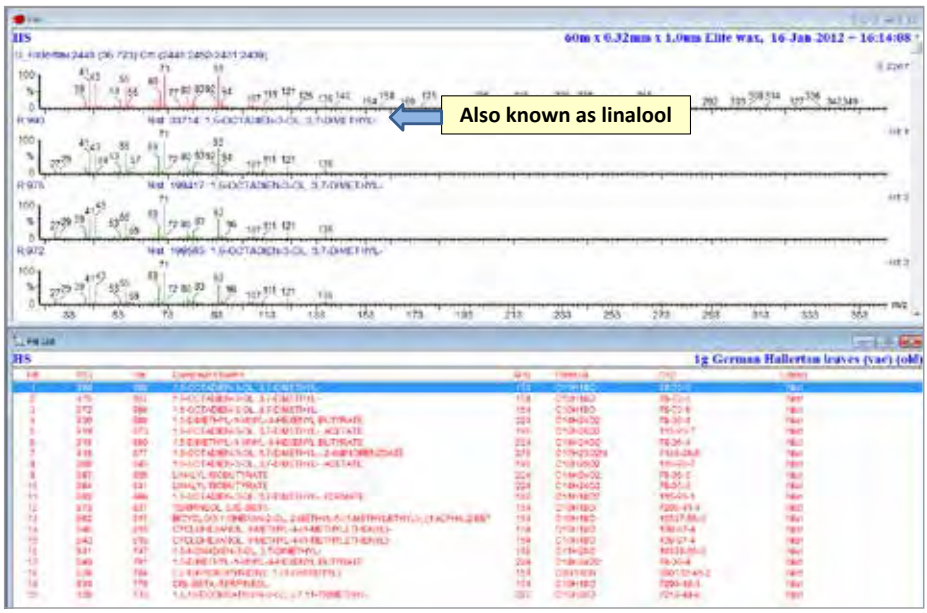

Figure 12. Results from library search on mass spectrum shown in Figure 11.

By performing further identifications of the chromatographic peaks, a profile of the hop character may be established. Figure 13 shows further peaks identified in the German Hallertau chromatogram previously shown in Figure 9. Annotated peaks are mainly aliphatic acids which indicate a degree of oxidation in the hops in this particular sample. The strongly flavored myrcene peak is rather smaller than expected. These observations indicate that this particular sample is rather old (which was true – this was a really old sample that had been poorly stored).

Figure 14 shows chromatography of four additional hop samples.

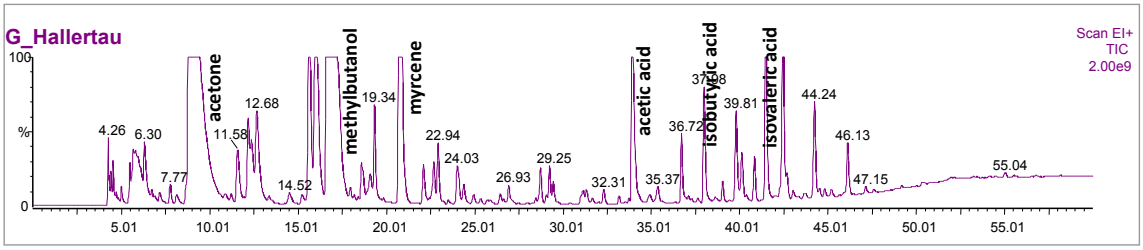

Figure 13. Typical TIC chromatograms of four hop samples.

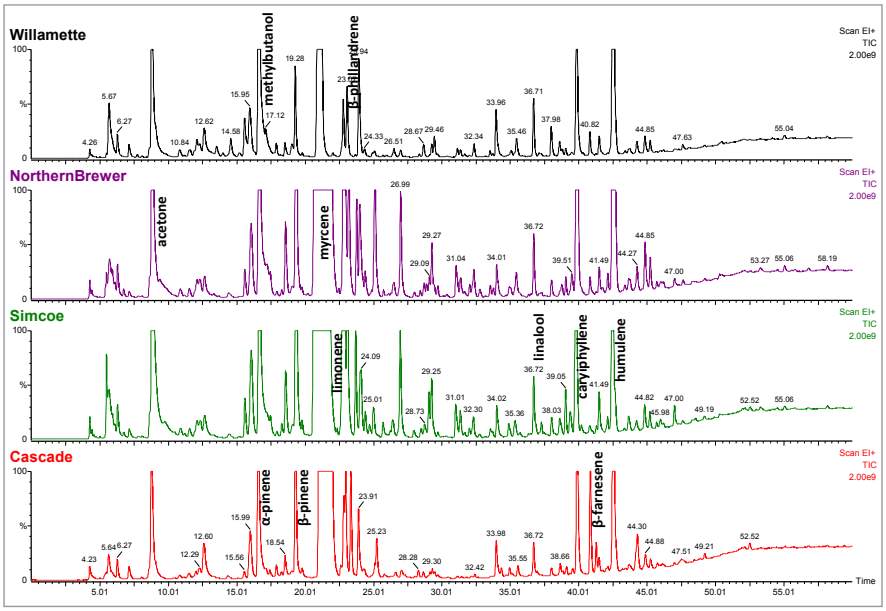

Figure 14. TIC chromatograms of a further four hop sample.

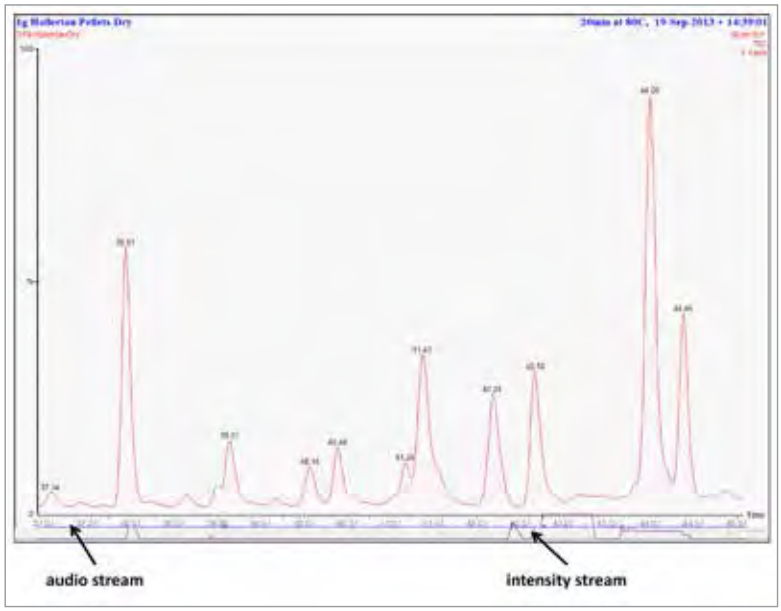

Figure 15. Example of a hop chromatogram being reviewed within the TurboMass™ software with the audio narration and aroma intensity graphically overlaid.

Olfactory Characterization

Figure 15 shows an example of a hop chromatogram with the audio narration and intensity recordings graphically overlaid. Audio narration is stored in a standard WAV file format that may be replayed from this screen to the operator from any point in the displayed chromatogram by means of a simple mouse-click. The narration WAV file may also be played back from most media applications including the Microsoft® Media Player, which is included with the Windows® operating systems. The audio data may be transcribed into text at the time of the recording. The Nuance® Dragon® Naturally Speaking software performs this function. It is included in the SNFR product. Table 9 shows a typical report from a hop analysis showing the user's transcribed narration and the recorded aroma intensity from the joystick. This report is formatted as a comma-separated value (CSV) file suitable for direct importation into Microsoft® Excel® or other application software.

Table 9. Typical output report showing text transcribed from the audio narration and the corresponding aroma intensity data.

Project Name	OKTOBERFEST.PRO
Sample Name	019-HallertauDry

Time Stamp	Spoken text	Intensity
1.05	coming up on a minute	0
2.13	two minutes	0
5.15	a sweet smell	0
5.20	very faint	0
6.07	nothing there	0
6.65	very very faint smell	2
6.88	off order	3
7.12	like sour milk	2
7.25	sour milk	4
7.30	was a very good banana smell	5
7.35	fruity smell	4
8.18	like a sour milk	4
8.23	sour milk	4
9.17	fruit there	2
10.02	nothing there	0
10.10	large peak and I smell nothing	0
11.52	burning smell	2
11.58	almost woody	0
12.00	little sweet	1
12.45	almost a hint of coffee	0
13.22	that's an off smell	3
13.25	a rancid smell	3
13.82	something	3
13.88	almost	0
13.90	medical	0
15.43	medical smell	2
15.47	is almost toffee like	2
15.57	very pleasing	4
16.43	off order	0
17.92	slight sweet	0
18.58	bubblegum	0
19.88	hint of something sweet	0
21.00	off order of skunk	3
21.08	definite skunk	5
22.90	something	3

Start Time	9/19/2013 2:39:02 PM
Duration	60.00

Time Stamp	Spoken text	Intensity
23.02	almost like a match	1
23.07	a sulfur smell	0
25.18	subtle	2
25.22	subtle	0
25.33	not quite sure what that was	0
25.70	nothing there	0
30.70	little off odor	1
33.67	foul smell	2
36.23	smell of cardboard must	0
36.35	bananas	2
36.82	almost mint	2
38.08	that was a nice fruit	3
38.20	very citrus	0
42.47	hot	4
42.50	pepper	2
42.70	again	3
42.82	it's an off odor	6
42.85	are very bad off order	6
43.08	a sweaty socks smell	6
43.72	that's a fruity smell	2
43.73	very pleasing	2
45.78	floral	2
46.30	a burning smell	2
46.37	burning match almost	2
47.02	pepper smell	1
47.95	pepper	1
48.93	sweet	1
49.13	a sweet smell	3
49.88	interesting smell	1
49.92	can't describe it	0
50.32	ah	3
50.35	medical smell again	4
50.40	medicinal	4
54.08	solvent	1

Conclusions

The addition of an olfactory port to a HS GC/MS system extends its application for aroma characterization of samples such as hops. The ability to directly correlate organoleptic perception against hard analytical data provides insights difficult to obtain otherwise.

This system should be of interest to brewers and researchers involved in the following:

- Quality control of raw hops
- Product development
- Trouble shooting of off-flavors
- Storage/aging studies
- Comparison studies
- Aroma analysis of finished beer
- Reverse engineering of competitive products

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APPLICATION BRIEF

ICP - Mass Spectrometry

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The Elemental Analysis of Grains with the NexION 300/350 ICP-MS

Introduction

Trace elemental analysis of grains can provide associations between air pollution sources and soil variables. The elements themselves are distributed unevenly throughout the cereal grain, with the germ and the outer layers having the highest concentrations. Therefore, the elemental analysis requires the ability to measure both trace and high levels.

The elemental capabilities and dynamic range of inductively coupled plasma mass spectrometry (ICP-MS) make it ideally suited for the analysis of food materials. The ultratrace detection limits of ICP-MS permit the determination of low-level contaminants, such as Pb, As, Se, and Hg, while the macro-level nutritional elements, such as Ca, Mg, K, and Na, can be quantified using the extended dynamic range capability of ICP-MS which provides the ability to measure concentrations over nine orders of magnitude. However, there are still a number of challenges to overcome, including complex sample matrices, high levels of dissolved solids, and interferences. With the proper ICP-MS instrumental conditions and design, all of these issues can be overcome, allowing for the successful analysis of food samples, as described elsewhere[1]. This work will focus on the analysis of grains.

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Experimental

Sample Preparation

NIST® 8433 Corn Bran and NIST® 8436 Wheat Flour were used in this work. Approximately 0.5-0.6 g were digested in duplicate with 5 mL of nitric acid (Fisher Scientific™, Optima grade) and 2 mL of hydrogen peroxide (Fisher Scientific™, Optima grade) in pre-cleaned PTFE microwave sample vessels. The digestion program consisted of 30 min of heating and 15 min of cooling, as shown in Table 1. All samples were completely dissolved, resulting in clear solutions that were diluted to a final volume of 50 mL with deionized water. No further sample dilutions were necessary. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Preparation blanks, consisting of the acid mixture, were taken through the same microwave digestion program as the samples.

Table 1. Microwave Digestion Program.

Step	Power (W)	Ramp (min)	Hold (min)
1	500	1	4
2	1000	5	5
3	1400	5	10
4 (cooling)	0	—	15

Instrumental Conditions

All data in this study were generated under normal operating conditions on a PerkinElmer NexION® 300/350X ICP-MS using an autosampler. The instrumental operating conditions are shown in Table 2.

Table 2. ICP-MS Instrumental Operating Conditions for this Application.

Parameter	Value
Nebulizer	Glass concentric
Spray chamber	Glass cyclonic
Cones	Nickel
Plasma gas flow	18.0 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.98 L/min
Sample uptake rate	300 µL/min
RF power	1600 W
Total integration time	0.5 (1.5 seconds for As, Se, Hg)
Replicates per sample	3
Universal Cell Technology™*	Collision mode

*PerkinElmer, Inc.

Calibration

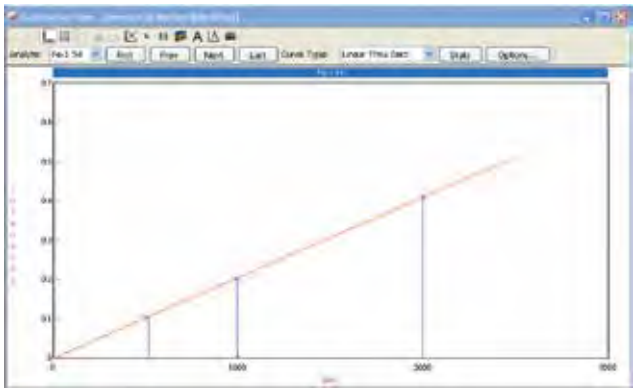
Multielement calibration standards, representing all the analytes in the SRM, were made up from PerkinElmer Pure single and multielement standards and diluted into 10% HNO_3. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Calibration standard ranges were based on whether the analyte was expected to be a high-level nutritional element like potassium (K) or sodium (Na), low/medium-level essential element like manganese (Mn) or iron (Fe), or trace/ultratrace contaminant such as lead (Pb) or mercury (Hg).

Depending on the certified value of the analytes, five different calibration ranges were made up to cover the complete range of elements being determined:

- High-level nutritional analytes: 0-300 ppm
- Medium-level essential analytes: 0-20 ppm
- Low-level essential analytes: 0-2 ppm
- Trace-level contaminants: 0-200 ppb
- Ultratrace-level contaminants: 0-20 ppb

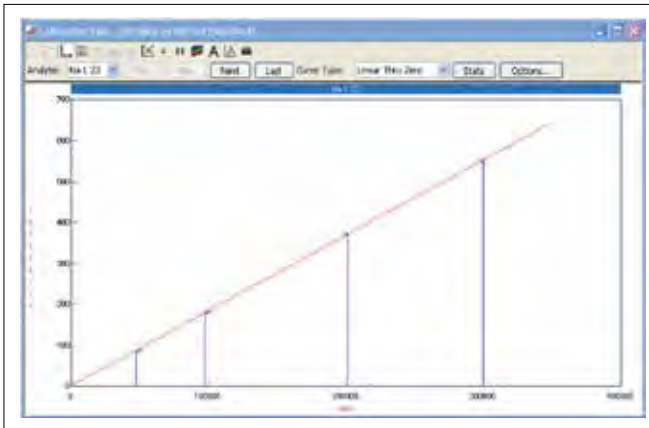
Figures 1 to 5 show representative calibration curves for each range.

In addition to the analyte elements used for the multielement calibration, the standards, blanks, and samples were also spiked on-line using a mixing tee with a solution of ^{6}Li, Sc, Ge, In, and Tb for internal standardization across the full mass range. Acetic acid was added to the internal standard solution to compensate for residual carbon left over from the sample digestion.



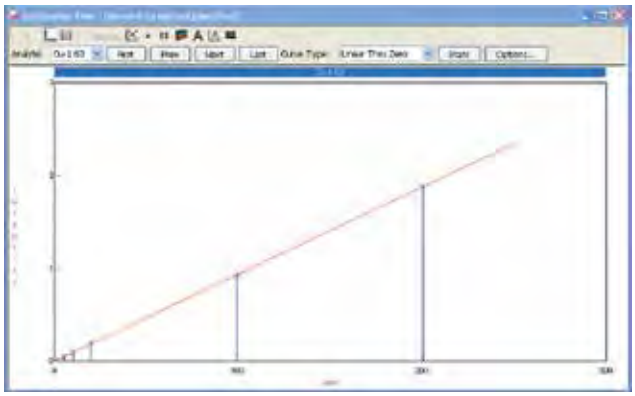
^{54}Fe Correlation Coefficient = 0.99997.

Figure 1. Calibration curves for ^{54}Fe (0-2 ppm).



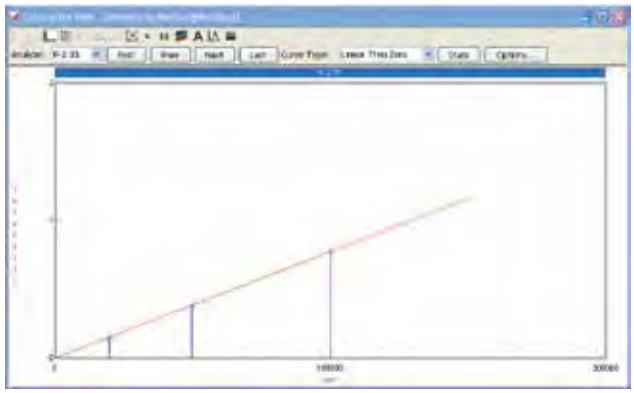
^{23}Na Correlation Coefficient = 0.99996.

Figure 2. Calibration curve for ^{23}Na (0-300 ppm).



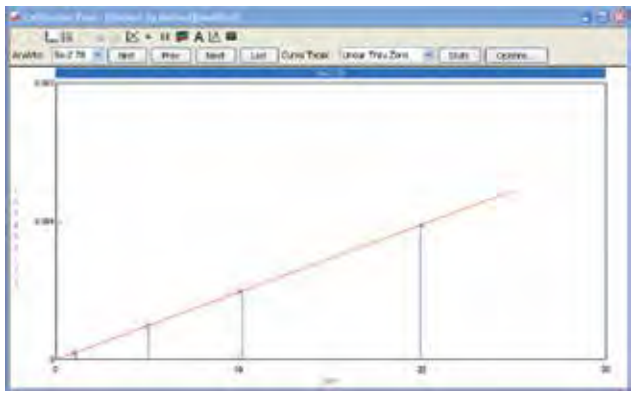
^{63}Cu Correlation Coefficient = 0.99999.

Figure 3. Calibration curve for ^{63}Cu (0-200 ppb).



^{31}P Correlation Coefficient = 0.99999.

Figure 4. Calibration curve for ^{31}P (0-100 ppm).



^{78}Se Correlation Coefficient = 0.99995.

Figure 5. Calibration curve for ^{78}Se (0-20 ppb).

Results

Quantitative results for two sample preparations of the NIST® 8436 Wheat Flour and NIST® 8433 Corn Bran reference materials are shown in Tables 3 and 4. All elements in every sample were determined with Universal Cell operating in Collision mode using helium as the cell gas. Figures in parentheses ( ) in the Reference Value column are not certified values but are included for information purposes only. The data show very good agreement with the certified values, especially for the elements that suffer from known spectral interferences. The elements that are outside the specified limits are mostly the ones that are well recognized as environmental contaminants, which have most likely been impacted by the sample preparation procedure.

Table 3. Analysis of NIST® 8436 Wheat Flour using the NexION 300/350 ICP-MS.

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	–	0.62
Na	23	16.0±6.1	17.0
Mg	26	1070±80	1030
Al	27	11.7±4.7	11.8
P	31	2900±220	2330
S	34	1930±280	1460
K	39	3180±140	2950
Ca	44	278±26	262
V	51	0.021±0.006	0.026
Cr	52	0.023±0.009	0.053
Fe	54	41.5 ±4.0	41.4
Mn	55	16.0±1.0	15.1
Co	59	0.008±0.004	0.007
Ni	60	0.17±0.08	0.17
Cu	63	4.30±0.69	4.18
Zn	66	22.2±1.7	20.6
As	75	(0.03)	0.01
Se	78	1.23±0.09	1.22
Sr	88	1.19±0.09	1.19
Mo	98	0.70±0.12	0.72
Cd	111	0.11±0.05	0.11
Sn	118	–	0.032
Sb	121	–	0.002
Ba	137	2.11±0.47	2.04
Hg	202	0.0004±0.0002	<0.0007
Pb	208	0.023±0.006	0.35
Tl	205	–	<0.0001
Th	232	–	0.001
U	238	–	0.001

Table 4. Analysis of NIST® 8433 Corn Bran using the NexION 300/350 ICP-MS.

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	2.8±1.2	3.2
Na	23	430±31	399
Mg	26	818±59	787
Al	27	1.01±0.55	1.15
P	31	171±11	158
S	34	860±150	738
K	39	566±75	548
Ca	44	420±38	434
V	51	0.005±0.002	0.005
Cr	52	(0.11)	0.08
Fe	54	14.8±1.8	13.7
Mn	55	2.55±0.29	2.53
Co	59	(0.006)	0.005
Ni	60	0.158±0.054	0.143
Cu	63	2.47±0.40	2.54
Zn	66	18.6±2.2	17.0
As	75	(0.002)	<0.006
Se	78	0.045±0.008	0.056
Sr	88	4.62±0.56	4.56
Mo	98	0.252±0.039	0.255
Cd	111	0.012±0.005	0.013
Sn	118	–	0.015
Sb	121	(0.004)	0.003
Ba	137	2.40±0.52	2.26
Hg	202	0.003±0.001	0.005
Pb	208	0.140±0.034	0.122
Tl	205	–	<0.0001
Th	232	–	<0.00008
U	238	–	<0.00002

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION 300/350X ICP-MS to effectively measure macro-level nutritional elements in the same analysis run as lower-level elements, without having to dilute the samples. The agreement between experimental and certified results for NIST® 8436 Wheat Flour and NIST® 8433 Corn Bran demonstrates the accuracy of the analysis. Instrument design characteristics eliminate deposition on the ion optics, leading to long-term stability in high-matrix samples, while permitting trace levels to be accurately measured.

References

1. "The Determination of Toxic, Essential, and Nutritional Elements in Food Matrices Using the NexION 300/350 ICP-MS", PerkinElmer Application Note.

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APPLICATION NOTE

FT-NIR Spectroscopy

The Determination of Protein and Moisture in Samples of Wheat

Summary

NIR spectroscopy has many valuable uses throughout the various stages of the manufacturing process particularly for raw material qualification and quantitation. The technique offers a fast and reliable alternative to traditional quantitative methods which often take many hours to complete. This note describes the use of FT-NIR spectroscopy to determine the protein and moisture content in ground wheat raw materials used in the agricultural industry. We have established the feasibility of determining such properties with an estimated prediction error of less than 0.5%.

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Experimental

All spectra were recorded on a PerkinElmer® FT-NIR Spectrometer fitted with an in-board solid sampling accessory. Seventy different ground wheat samples were supplied and measured with no additional milling or grinding. Spectra were recorded by filling a standard sample cup with the sample and scanning in interleaved mode. This mode of operation alternately takes a background spectrum as well as the ratioed spectrum which minimizes changes in atmospheric effects.

Three replicate measurements of each of the calibration samples were collected, and the mean spectrum used for the generation of the calibration equations. The sample cup was emptied and refilled for the collection of the three replicate spectra to obtain a more representative spectrum of the sample. A rotating sample cup is also available, which removes the need to scan multiple replicates for these types of samples.

To support the validation tests, a random set of sample spectra was collected approximately one week later. Data was collected over the range 10000 to 3800 cm^{-1} at 16 cm^{-1} resolution with approximately one minute scanning time. It may be possible to scan the samples using considerably less scanning time and still achieve the desired accuracy. Data was collected over the whole range of the NIR spectrum since this data set may be used to determine a number of other properties in wheat from these spectra. A typical spectrum representative of the wheat samples is shown in Figure 1.

A partial least squares analysis (PLS) was performed on the data (70 spectra). It is possible to predict values for protein and moisture content in wheat in the independent validation set.

Various mathematical pretreatments were tested and a second derivative function chosen to provide SEP value of 0.28 for protein and 0.49 for moisture using 6 PLS factors and full cross validation. Full cross validation excludes each standard in turn from the calibration set, performs the calibration and then predicts the excluded standard using that calibration. Smaller prediction errors may be obtained using a larger number of PLS factors.

However, it was decided to optimize the calibration for robustness which is better achieved by performing independent validation over time. Figures 2 and 2a are the illustrated plots of Estimated versus Specified values, first for protein and second for moisture. This provides an adequate starting point for the calibration model.

These graphs show that protein has a slightly tighter model than moisture. This may be due to the samples' changing moisture content in storage. It is recommended to store calibration samples in dry conditions, especially if there is a significant time lapse between reference and NIR measurements. The regression model summaries for the full cross validation model are shown in Table 1.

To support validation, a series of samples were run a week later and both the protein and moisture content predicted using the calibrated model. Table 2 shows the results along with the reference values supplied. Additional statistics in terms of the total M-distance and residual ratio give an indication of how well the model covers these samples.

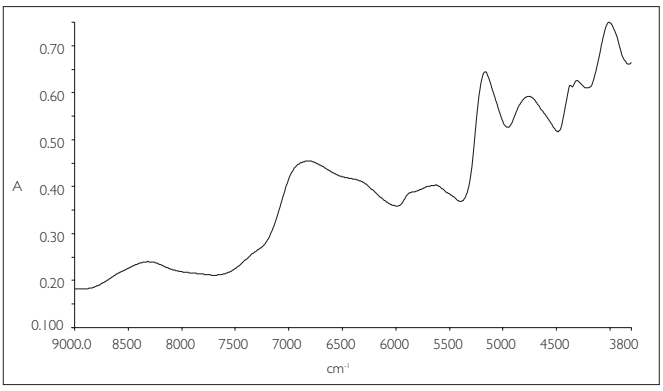

Figure 1. Typical spectrum of ground wheat.

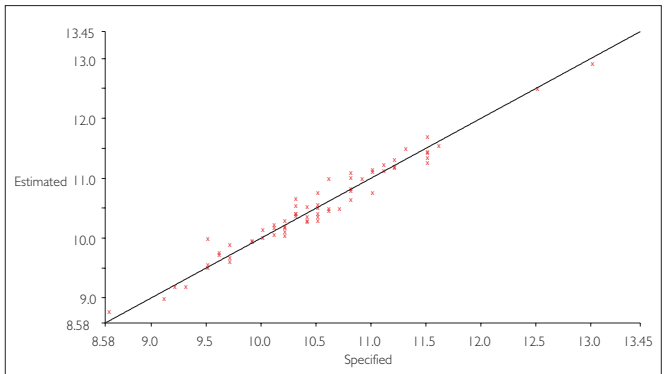

Figure 2a. Estimated vs Specified plot for Protein/Full Cross Validation.

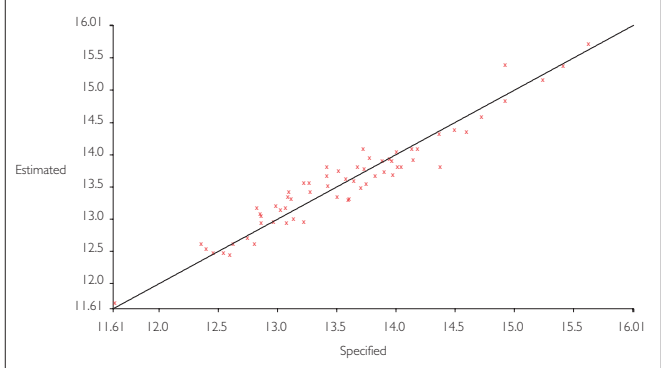

Figure 2b. Estimated vs Specified plot for Moisture/Full Cross Validation.

Table 1. **Summary of Calibration Reports for i) Protein and ii) Moisture in Wheat.**

i) Protein		**Number of LVs used : 6 + intercept**			
LV Number	**Correl. of LV with property**	**Regression Coefficient**	**Std. error of R.C.**	**t-value**	**Sig. Lev.%**
1	0.8298	5.82	0.1961	29.67	0.00
2	0.2590	1.669	0.1646	10.14	0.00
3	0.4893	2.66	0.1637	16.24	0.00
4	0.1555	0.9108	0.1656	5.50	0.00
5	0.2314	1.328	0.1635	8.12	0.00
6	0.1613	0.9859	0.1611	6.12	0.00
Intercept	0.1966	-0.06268	0.0196	-3.19	0.22
Std Error of Prediction: Estimate		**= 0.1659**	**Actual = 0.2824**		
Multiple Correlation		**= 0.9819**			
Mean Property Value		**= 10.46**			
% Variance (R squared)		**= 96.4107**			
Std Error of Estimate (SEE)		**= 0.159**			
F-value		**= 268.6**			
ii) Moisture		**Number of LVs used : 6 + intercept**			
LV Number	**Correl. of LV with property**	**Regression Coefficient**	**Std. error of R.C.**	**t-value**	**Sig. Lev.%**
1	0.5654	3.965	0.2389	16.59	0.00
2	0.5432	3.935	0.2351	16.74	0.00
3	0.2324	2.214	0.2546	8.70	0.00
4	0.2632	1.72	0.2454	7.01	0.00
5	0.3195	2.228	0.2220	10.03	0.00
6	0.0845	0.9262	0.2334	3.97	0.02
Intercept	0.2766	0.08827	0.0279	3.16	0.25
Std Error of Prediction: Estimate		**= 0.2314**	**Actual = 0.4938**		
Multiple Correlation		**= 0.9642**			
Mean Property Value		**= 13.55**			
% Variance (R squared)		**= 92.9637**			
Std Error of Estimate (SEE)		**= 0.2189**			
F-value		**= 123.3**			

Table 2. **Samples 1 and 2.**

QUANT+ V4.00 PREDICTION RESULTS PLS1								
		Sample 1			**Sample 2**			
Sample		V20030 (1 of 2)			V20033 (1 of 2)			
Calc.Name		R01V2030.SP			R01V2033.SP			
Normalization		None			None			
Method		WHEAT.MD Ver: 2 ID: 3294			WHEAT.MD Ver: 2 ID: 3294			
Total M-Distance		0.379			0.611			
Residual Ratio		1.33			1.15			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Protein	**10.13%**	**10.00**	**0.275**	**0.397**	**12.15%**	**12.50**	**0.28**	**0.595**
Total M-Distance		0.368			0.555			
Residual Ratio		1.18			1.33			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Moisture	**12.96%**	**12.34**	**0.378**	**0.387**	**12.57%**	**12.44**	**0.383**	**0.547**
Prediction complete					Prediction complete			

Table 2. **Samples 3 and 4.**

QUANT+ V4.00 PREDICTION RESULTS PLS1								
		Sample 3			**Sample 4**			
Sample		V20073 (1 of 2)			V20077 (1 of 2)			
Calc.Name		R01V2073.SP			R01V2077.SP			
Normalization		None			None			
Method		WHEAT.MD Ver: 2 ID: 3294			WHEAT.MD Ver: 2 ID: 3294			
Date		10-Apr-1997 15:55:02			10-Apr-1997 15:55:05			
RMS Error		1.807e-006			1.612e-006			
Peak to Peak Error		2.126e-005			2.116e-005			
Total M-Distance		0.652			0.573			
Residual Ratio		1.84			1.47			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Protein	**9.463%**	**9.50**	**0.281**	**0.63**	**9%**	**9.10**	**0.279**	**0.563**
RMS Error		1.691e-006			1.654e-006			
Peak to Peak Error		1.932e-005			2.022e-005			
Total M-Distance		1.06			0.508			
Residual Ratio		1.51			1.44			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Moisture	**15.94%**	**15.61**	**0.398**	**0.977**	**13.76%**	**14.03**	**0.382**	**0.507**
Prediction complete					Prediction complete			

Table 2. **Samples 5 and 6.**

QUANT+ V4.00 PREDICTION RESULTS PLS1								
		Sample 5			**Sample 6**			
Sample		V20181 (1 of 2)			V20380 (1 of 2)			
Calc.Name		R01V2181.SP			R01V2380.SP			
Normalization		None			None			
Method		WHEAT.MD Ver: 2 ID: 3294			WHEAT.MD Ver: 2 ID: 3294			
RMS Error		1.318e-006			1.441e-006			
Peak to Peak Error		1.229e-005			1.754e-005			
Total M-Distance		0.111			0.427			
Residual Ratio		0.982			1.17			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Protein	**10.89%**	**11.00**	**0.269**	**0.167**	**10.78%**	**10.50**	**0.276**	**0.438**
RMS Error		1.353e-006			1.579e-006			
Peak to Peak Error		1.423e-005			1.735e-005			
Total M-Distance		0.17			0.358			
Residual Ratio		0.963			1.31			
Property	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**	**Calc.Value**	**(Ref Value)**	**R-Error**	**M-Distance**
Moisture	**14.11%**	**13.40**	**0.372**	**0.217**	**12.52%**	**12.58**	**0.378**	**0.379**
Prediction complete					Prediction complete			

Conclusion

The example detailed here illustrates that it is possible to determine a number of properties present in ground wheat samples with accuracy which is of a similar order to that of the reference method using FT-NIR spectroscopy. Based on the samples supplied, it has been shown that FT-NIR and partial least squares can be used to determine protein and moisture in ground wheat to within 0.5% SEP.

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FIELD APPLICATION REPORT

Gas Chromatography

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Brewing QC Applications Using Headspace Sampling-Gas Chromatography

Introduction

Headspace sampling coupled with gas chromatography (HS-GC) is a widely used technique for the analysis of beer throughout the world. HS-GC is typically used for quality control (QC), to identify problems or changes occurring in the brewing or fermentation process that affect the taste or quality of the final product.

Four of the major HS-GC analyses that are typically performed at breweries are described in Table 1. The first, and most important, is monitoring for vicinal diketones (VDK) in the beer, which include 2,3-butanedione (diacetyl) and 2,3-pentanedione. VDK are considered extremely important since they are known to affect the taste of the beer. These components produce a butter-like flavor and are also considered non-beneficial at high levels. Many heavier beers, such as European beers, have VDK at higher levels than the lighter beers typically produced in the U.S. and they still maintain good flavor. VDK concentrations typically range from 1-50 ppb in lighter beers, but they can reach several hundred ppb in darker beers.

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Table 1. **Four Typical HS-GC Analyses Performed in the Brewing Process.**

1	Vicinal diketones (VDK)	2,3-Butanedione (diacetyl) 2,3-Pentanedione
2	Acetaldehyde	Acetaldehyde
3	Trihalomethanes	Dibromochloromethane Bromoform Chloroform Dichlorobromomethane
4	Sulfur	DMS (dimethyl sulfide) Sulfur dioxide Hydrogen sulfide

A second common HS-GC analysis performed in the brewing process is monitoring acetaldehyde. Acetaldehyde is reduced to ethanol by yeast during secondary fermentation, but oxidation of the finished beer may reverse this process, converting ethanol back to acetaldehyde. Acetaldehyde can also be a product of bacterial spoilage caused by Zymomonas or Acetobacter. In addition, background levels of acetaldehyde can be tasted in beers that use beechwood chips to drop the yeast before it can be reduced to ethanol. Acetaldehyde has the taste and aroma of fresh-cut green apples and has also been compared to grass, green leaves and latex paint. The typical levels of acetaldehyde that are monitored are 1-20 ppm.

A third HS-GC analysis typically performed on beer is monitoring of trihalomethanes. These can be harmful and are usually introduced into the beer through the municipal water supply. Municipal water is often treated with chlorine, resulting in a variety of chlorinated hydrocarbon disinfection byproducts. The QC check for trihalomethanes is typically performed on incoming water, but not always on the large numbers of samples taken from the finished product. Chloroform is usually the most prominent trihalomethane component identified in this analysis.

The fourth HS-GC test commonly performed is for the identification of sulfur compounds in beer. Dimethyl sulfide (DMS), sulfur dioxide (SO_2), and hydrogen sulfide (HS) are monitored by some brewers. DMS has the taste and aroma of sweet corn. This comes either from the malt, as a result of the short or weak boil of the wort, slow wort chilling or bacterial infection. Hydrogen sulfide is an indicator of the performance characteristics of the yeast, since some yeasts can produce significantly different levels of hydrogen sulfide. Sulfur dioxide is often encountered due to its presence as a preservative. When present in beer at low quantities, these sulfur components can be considered acceptable, but above very low ppb levels, they give off an unpleasant taste and smell (e.g., rotten eggs).

Although these four QC tests are usually performed individually, some breweries will combine two tests in the interest of time and sample throughput. For example, the test for VDK may also be used to identify the presence of chloroform, and the test for acetaldehyde may also be used to identify sulfur compounds.

Experimental

All analyses were performed using a PerkinElmer® TurboMatrix™ automated headspace sampler (TurboMatrix HS-40 and HS-110) and a Clarus® gas chromatograph (Figure 1). The Clarus GC was configured with both a flame ionization detector (FID) and an electron capture detector (ECD).

Figure 1. TurboMatrix automated headspace sampler (right) with the Clarus gas chromatograph (left).

Beer samples require degassing prior to headspace analysis. Full degassing of beer is important to prevent dissolved carbon dioxide (CO_2) from influencing vial pressure during the headspace heating process and to minimize GC baseline disturbances from CO_2 eluting during chromatography. Repeated shaking in an oversized container and allowing the foam to settle is one way to degas the samples, but filtration or sonication is easier and more efficient.

Samples were prepared by transferring the beer to a wide mouth beaker and sonicating them briefly (only 5-15 seconds is required). Using a wide-mouth beaker that is at least 10 times the volume of the beer measured is recommended. (Note: sonicating the beer directly in the bottle will cause an instantaneous geyser of beer foam to elevate 10-24 inches in height!) After degassing, 5-10 mL of beer sample was placed into a headspace vial (PerkinElmer Part No. B0104236) and sealed with PTFE/butyl rubber septa (PerkinElmer Part No. B0159356).

Results

Experiment 1 – Vicinal diketones: 2,3-butanedione (diacetyl) and 2,3-pentanedione

The desired 1-50 ppb detection limits are achieved by using an electron capture detector (ECD). The column used for the vicinal diketones analysis is an Elite-5, 60 meter x 0.53 mm x 1.5 µm (PerkinElmer Part No. N9316103). The HS and GC conditions required for the analysis are listed in Tables 2 and 3. A typical chromatogram showing the presence of VDK is displayed in Figure 2. Note: some beer methods use a manual headspace technique,[1] requiring attended analysis and yielding less reproducible results than the automated system demonstrated here.

Table 2. **HS Conditions.**

Sample Temperature:	60 °C
Needle Temperature:	80 °C
Transfer Line Temperature:	100 °C
Equilibration Time:	15 min
Pressurization Time:	1.0 min
Injection Time:	0.1 min
Withdrawal Time:	0.0 min
Carrier Pressure:	35 psi

Table 3. **GC with ECD Conditions.**

Initial Temperature:	45 °C
Hold Time 1:	1.3 min
Rate 1:	40 °C/min
Final Temperature:	150 °C
Hold Time 2:	0.6 min
Injector Temperature:	100 °C
Liner:	Zero Dilution
Split:	25 mL/min
ECD:	150 °C
ECD Attenuation:	1
Makeup Gas (Argon/Methane):	30 mL/min

Experiment 2 – Acetaldehyde

The detection limits required for acetaldehyde determination (1-20 ppm) are achieved using a flame ionization detector (FID). The column used for the acetaldehyde experiment is an Elite BAC-1, 30 meter x 0.32 mm x 1.8 µm (PerkinElmer Part No. N9316579). The conditions required for the headspace sampler and gas chromatograph are listed in Tables 4 and 5. A chromatogram showing the presence of acetaldehyde along with 2-propanol, which is used as the internal standard in this analysis, is displayed in Figure 3. Note that dimethylsulfide is also identified in the chromatogram, confirming the presence of this sulfur-containing compound.

Table 4. **HS Conditions.**

Sample Temperature:	60 °C
Needle Temperature:	80 °C
Transfer Line Temperature:	100 °C
Equilibration Time:	15 min
Pressurization Time:	1.0 min
Injection Time:	0.1 min
Withdrawal Time:	0.0 min
Carrier Pressure:	35 psi

Table 5. **GC with FID Conditions.**

Initial Temperature:	45 °C
Hold Time 1:	1.3 min
Rate 1:	40 °C/min
Final Temperature:	150 °C
Hold Time 2:	0.6 min
Injector Temperature:	100 °C
Liner:	Zero Dilution
Split:	25 mL/min
FID:	150 °C

Experiment 3 – Trihalomethanes (THMs)

The low-ppb detection limits necessary for trihalomethane analysis are achieved using an electron capture detector (ECD). THMs are introduced into the process with the water used to make the beer. The THM test can be run on incoming water, processed water and also the beer itself. To measure all four trihalomethanes (chloroform, dichlorobromomethane, bromoform and dibromochloromethane), a temperature-programmed GC analysis is required. Consequently, this is not considered a high-throughput application. Many brewery QC labs will determine the presence of chloroform (typically the most common THM) during VDK analyses of the finished product, as depicted in Figure 2. However, other labs will perform a separate analysis to determine total THM content.

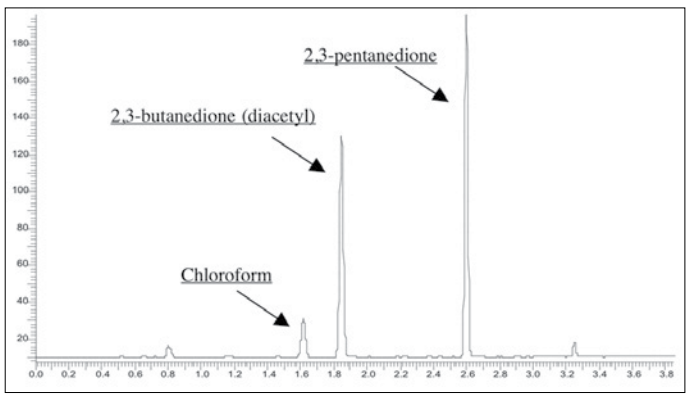

Figure 2. Vicinal diketones determination at 10-ppb concentration (ECD).

Experiment 4 – Sulfur Compounds

Low-ppm detection limits for sulfur-compound analysis can be achieved using a flame ionization detector (FID). Typically, dimethylsulfide is also quantified on the FID during the acetaldehyde determination, so the column and conditions used are those listed under Experiment 2, and Figure 3 displays an example chromatogram. If ppb detection limits are required, a sulfur detector such as a Chemiluminescence or another sulfur-specific detector would be required with your PerkinElmer GC.

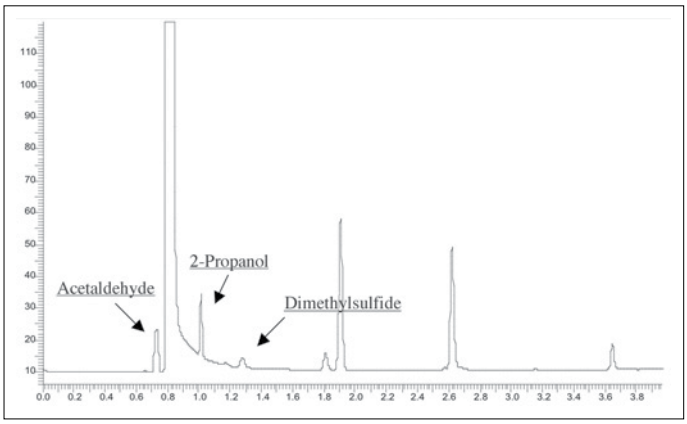

Figure 3. Acetaldehyde determination at 10-ppm concentration (FID).

Conclusions

The PerkinElmer HS-GC system has the capabilities needed to perform the vital QC checks required throughout the beer-making process. Undesirable components introduced into or created by the brewing process can be sampled, separated, identified and quantified using flame ionization or electron capture detection at a ppm or ppb level, respectively. In addition, the integrated system described here provides automated headspace analysis, yielding more reproducible results that can be acquired with unattended operation.

The four experiments described here can be performed separately, but some QC labs will perform two of the experiments simultaneously due to throughput considerations. For example, sulfur compounds (typically DMS) will be determined during the acetaldehyde analysis and trihalomethanes (typically chloroform) will be determined during the VDK analysis. If these screening tests indicate that the targeted components exist at undesirable levels, more specific analyses will typically be performed as part of a follow-up procedure.

It is possible to perform all four analyses simultaneously on the same HS-GC system. This entails splitting the GC column effluent between the FID and ECD detectors, and choosing a GC column and conditions that will separate all the components. The Elite-5 column – 60 meter x 0.53 mm x 1.5 µm (N9316103) – has been successfully used to accomplish this. However, both the oven-temperature ramp and the overall run time have to be increased to successfully separate all the components of interest, so the overall throughput of this analysis method is low.

References

1. Methods of Analysis, The American Society of Brewing Chemists, Eighth Revised Edition St. Paul, MN, 1992 (some sections revised 1996).

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APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Monitoring Volatile Organic Compounds in Beer Production Using the Clarus SQ 8 GC/MS and TurboMatrix Headspace Trap Systems

Introduction

Beer is a popular beverage produced by the fermentation of hopped malt extracted from barley and other grains. Although simple in concept, beer is a highly complex mixture of many compounds including sugars, proteins, alcohols, esters, acids, ketones, acids and terpenes. Flavor is an important quality of any beer and the chemical content of the beer is obviously responsible for that flavor. Aroma is an extremely important part of the flavor and so there is a strong interest by brewers in the volatile organic compounds (VOCs) in beer that affect its aroma.

Some VOCs have a positive effect on aroma (attributes) and some have a negative effect (defects). The ability to characterize these in beer products before, during and after fermentation would be an important tool in process control, quality assurance and product development.

This application note describes a system comprising a headspace trap sampler to extract and concentrate VOCs from a beer sample and deliver them to a gas chromatograph/ mass spectrometer (GC/MS) for separation, identification and quantification.

The purpose of our experiments is to demonstrate that attributes and defects can all be monitored using one detector and from a single injection with mass spectrometry (MS). The associated benefits include a quicker return on investment, enhanced productivity, more information from a single analysis, and less bench space requirements.

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Instrumentation

In this analysis, we utilized a headspace trap system for sample introduction to characterize the flavor of beer. This technique ensures that non-volatile material in beer does not enter the analytical system, which can cause system contamination. The headspace trap extracts the volatile components from a large sample and focuses them onto an inline adsorbent trap. It also facilitates very easy sample preparation – a volume of beer is dispensed into a vial and sealed. The subsequent analysis is then fully automated.

A PerkinElmer® TurboMatrix™ Headspace Trap connected to a PerkinElmer Clarus® SQ 8 GC/MS was used for these experiments. Using a headspace trap instead of the classical headspace technique enables up to 100 times improved detection limits over classical headspace methods.

A slightly-polar 60 m x 0.25 mm x 1.0 µm Elite 5 (5% phenyl-silicone) column was used. This thick-film column provided sufficient retention to separate the early-eluting most volatile components and provided the dynamic range necessary to chromatograph both high level and low level components in the beer.

Experimental

Overview

Several experiments were performed that are key to the brewing industry:

- Quantitation of dimethyl sulfide (DMS), 2,3-butanedione (diacetyl), 2,3-pentandione and t-2-nonenal.
- Characterization of several types of beers
- Fermentation profiling
- Analysis of raw materials
- Aging studies

Analytical Method

The experimental conditions for this analysis are given in Tables 1 to 4.

Table 1. **HS Trap Conditions.**

Headspace System	TurboMatrix (40 or 110) HS Trap
Vial Equilibration	80 °C for 20 min
Needle	120 °C
Transfer Line	140 °C, long, 0.25 mm i.d. fused silica
Carrier Gas	Helium at 31 psig
Dry Purge	7 min
Trap	Air Toxics, 25 °C to 260 °C, hold for 7 min
Extraction Cycles	1 with 40 psig extraction pressure

Table 2. **GC Conditions.**

Gas Chromatograph/ Mass Spectrometer	Clarus SQ 8
Column	60 m x 0.25 mm x 1.0 µm Elite-5MS
Oven	35 °C for 5 min, then 6 °C/min to 245°
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 2.0 mL/min (28.6 psig initial pressure), HS Mode ON

Table 3. **MS Conditions.**

Scan Range	35 to 350 Daltons
Scan Time	0.1 s
Interscan Delay	0.06 s
Source Temp	180 °C
Inlet Line temp	200 °C
Multiplier	1700V

Table 4. **Sample Details.**

Sample preparation	5 mL of each sample was pipetted into a sample vial and sealed
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicone septum

Calibration

A 10-point calibration was prepared for four target 'defect' compounds. The detection limit goal was 5.0 parts per billion (ng/mL). The standards were acquired in simultaneous Full Scan and Single Ion Monitoring acquisitions (SIFI). Examples of the chromatographic peaks and their signal to noise ratios at the 5.0 ppb level are given in Figures 1 to 4.

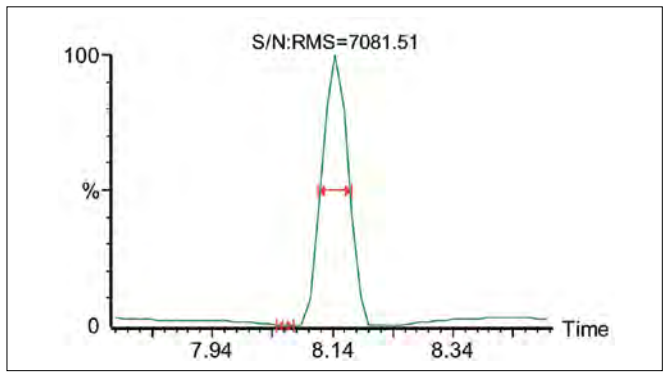

Figure 1. SIM chromatogram of dimethyl sulfide peak at 5.0 ppb.

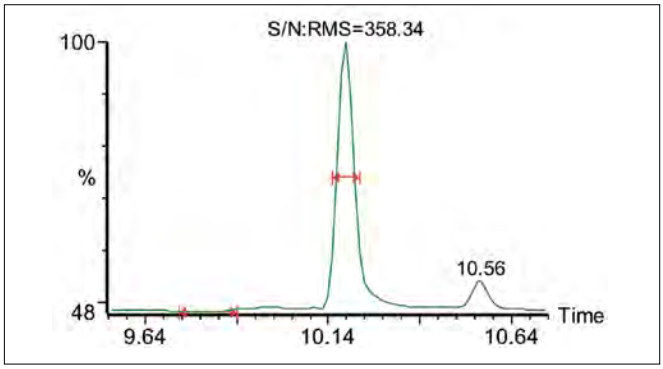

Figure 2. SIM chromatogram of 2,3-butanedione peak at 5.0 ppb.

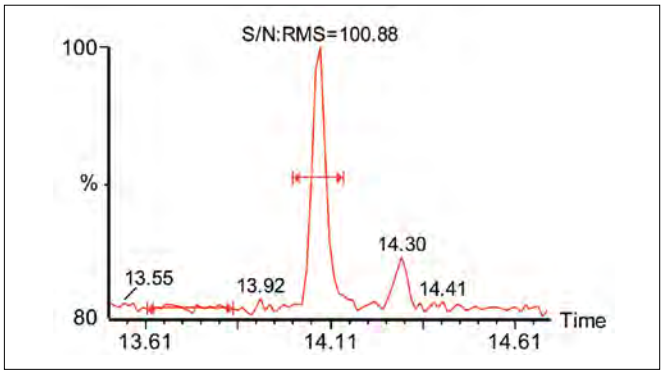

Figure 3. SIM chromatogram of 2,3-pentanedione peak at 5.0 ppb.

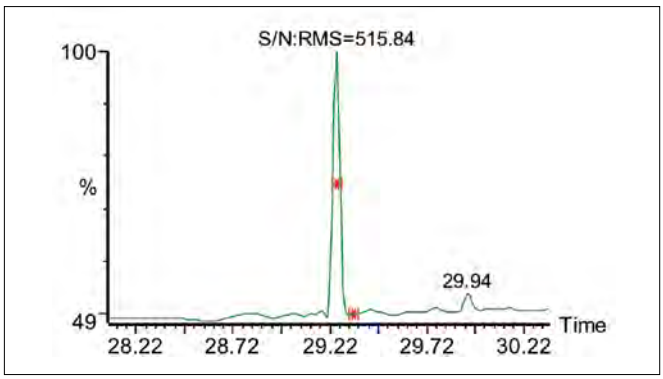

Figure 4. SIM chromatogram of t-2-nonenal peak at 5.0 ppb.

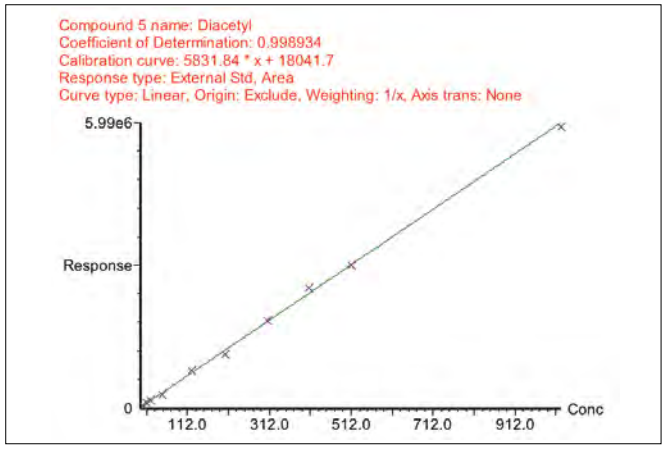

Figure 5. Calibration profile for 2,3-butanedione (diacetyl).

The calibration results are presented in Table 5. An example of one of the calibration plots is given in Figure 5. These data demonstrate a good linear response for these components in at low levels in a highly complex matrix.

Characterization of Beer

The MS detector enables the identification of components in beer. Figure 6 is an example of such characterization that was analyzed in our research center in Shelton, CT. Figure 7 is a comparison of the component identities and responses found in two competitive products.

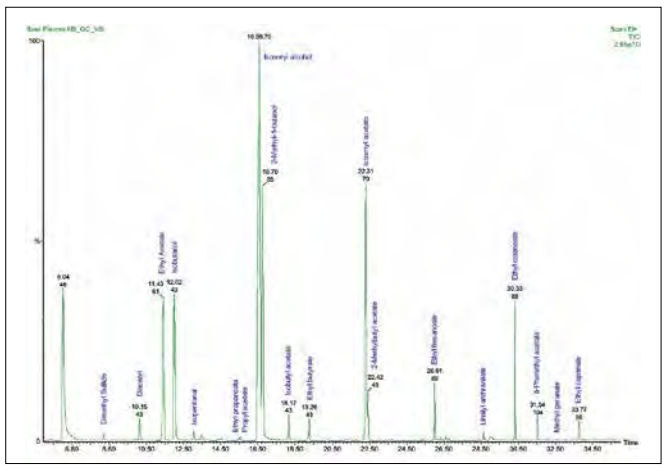

Figure 6. Typical chromatographic profile of volatile flavor compounds in an American pale ale.

***Table 5.* Calibration Data.**

Component Name	Signal to Noise Ratio Ratio at 5 ng/mL	r^2 over range 5 to 1000 ng/mL	Signal to Noise Ratio Ratio at 5 ng/mL	r^2 over range 5 to 1000 ng/mL
Dimethyl Sulfide	821 to 1	0.9934	7081 to 1	0.9945*
2,3-Butanedione	12 to 1	0.9989	358 to 1	0.9943
2,3-Pentanedione	20 to 1	0.9975	470 to 1	0.9983
t-2-Nonenal	19 to 1	0.9958	516 to 1	0.9960

*Reduced range due to overloading.

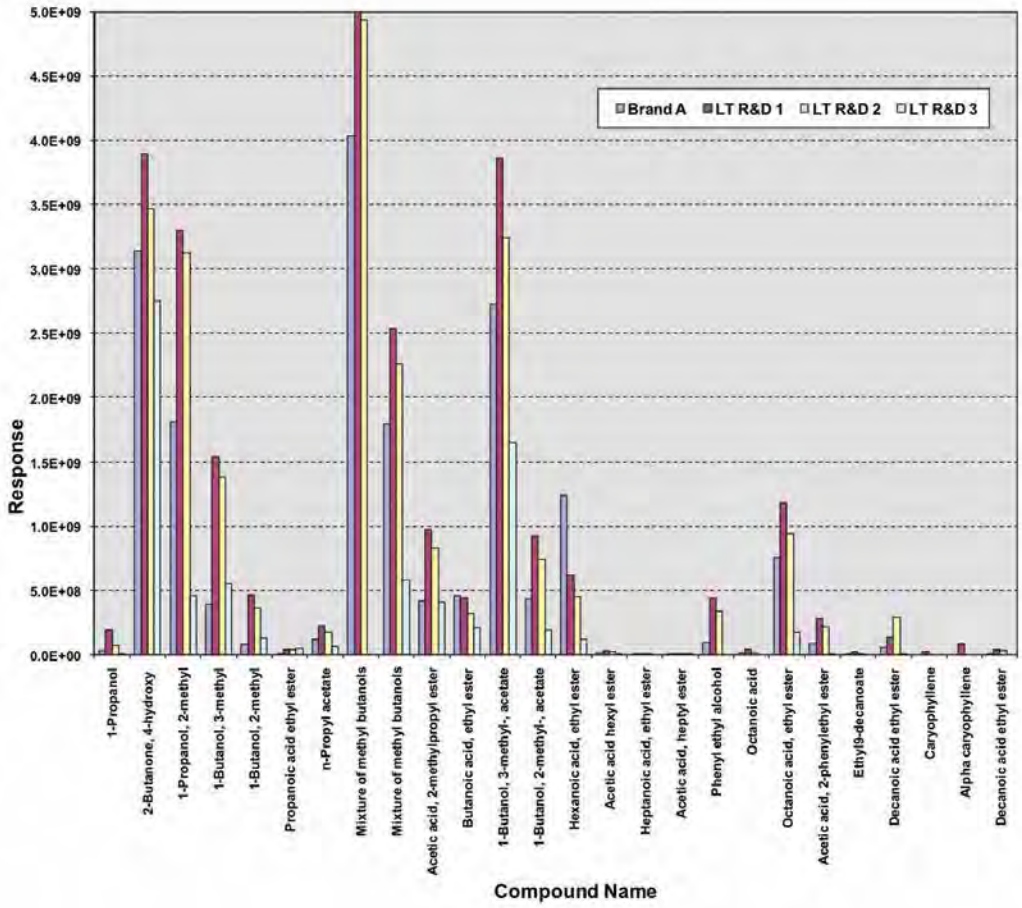

Figure 7. Comparison between two brands of beer (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

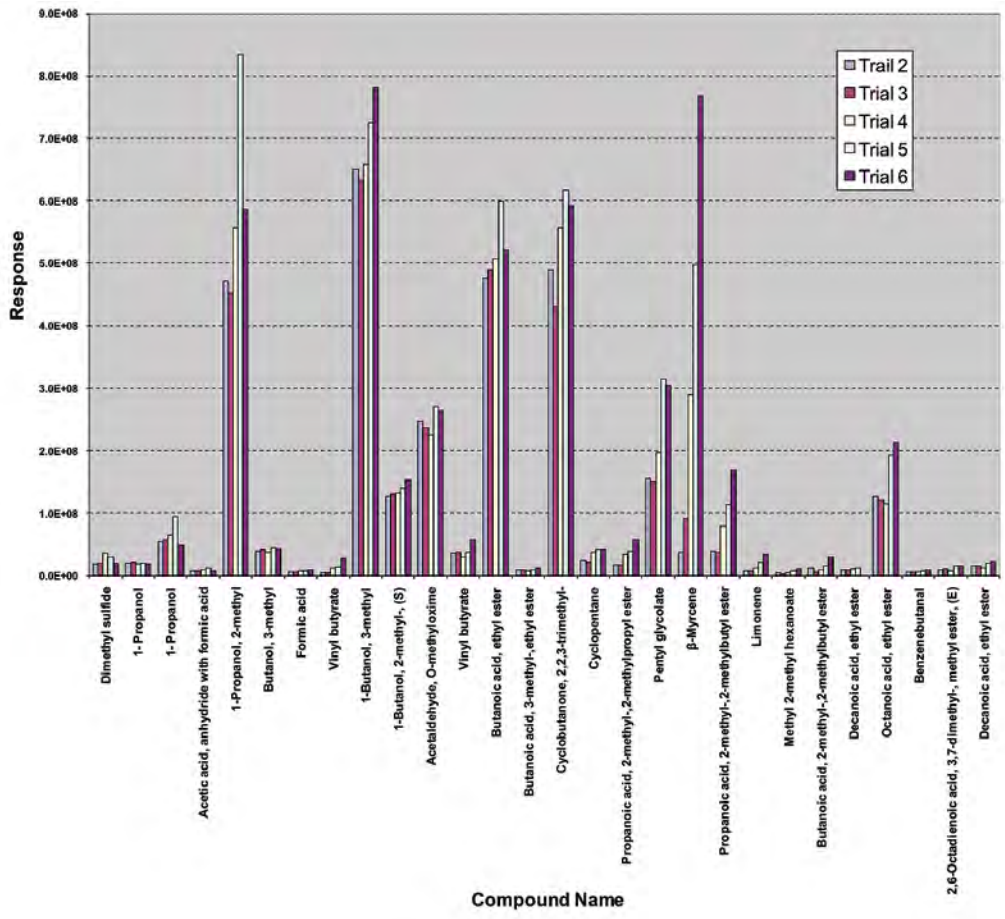

Figure 8. Comparison between different fermentations of the same beer type (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

Figure 8 shows the results of a research study comparing the flavor profiles of a beer from five different fermentations.

Fermentation Process

This analyzer provides the ability to obtain analytical results during the fermentation process.

An experimental batch of American pale ale was brewed and fermentation initiated. A sample was analyzed every eight hours starting with time zero and completing on day eight.

Specific gravity is often used as an indicator of the fermentation progress and is shown for this beer in Figure 9. The final gravity of 1.012 was achieved in about 100 hours.

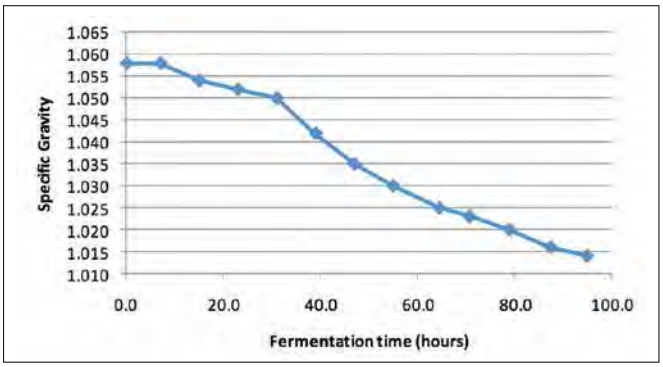

Figure 9. Specific gravity profile for the experimental beer during the fermentation process.

The concentrations of key components in the beer were checked during the fermentation process. The profiles of two key 'defects', 2,3-butanedione and dimethyl sulfide are shown in Figures 10 and 11 respectively. Trans-2-nonenal was not detected.

Analysis of Raw Materials

Figure 12 displays the results of a study comparing the components of different hops in order to understand and to improve the taste of beer.

Some beers use adjuncts to impact special flavors. The same system may be used to characterize these. Figure 13 displays the results of a comparison between orange peel from different suppliers for use in Belgian-style beers.

Aging Studies

Beer is a very complex matrix that ages over time due to chemical and biological activity so storage conditions are critical to its quality.

Exposure to air promotes the formation of aldehydes and other undesirable compounds that can impair the flavor of a good beer. The Clarus system is capable of monitoring such compounds. A compound of major concern is t-2-nonenal ('wet cardboard' flavor), which we monitored during the fermentation studies, yet was undetected.

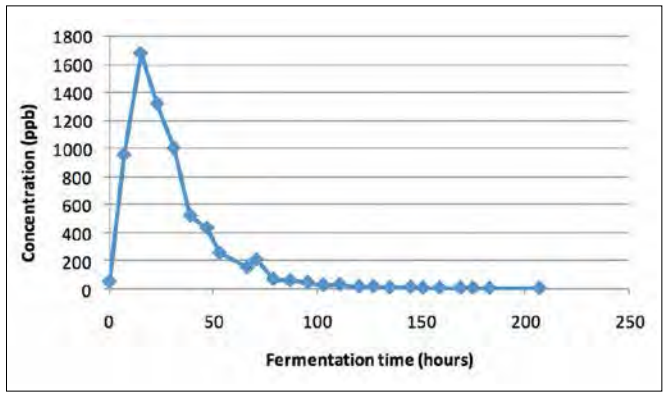

Figure 10. Concentration profile of 2,3-butanedione for the experimental beer during the fermentation process.

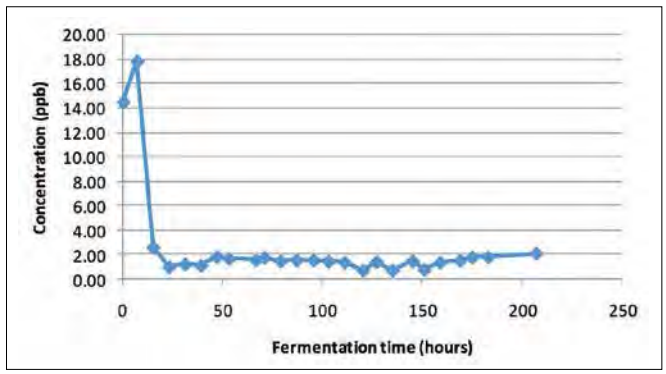

Figure 11. Concentration profile of dimethyl sulfide for the experimental beer during the fermentation process.

Another flavor concern is that bittering components (isohumolones) react to light and produce mercaptans and other volatile sulfur compounds giving a 'skunky' flavor to the beer. Figure 14 shows chromatograms of the same beer kept in the dark and also in bright sunlight. Major differences in the composition of the beer VOCs are apparent. Figure 15 identifies one of the sun-stuck components as an olefinic thiophene

Conclusion

The combination of the TurboMatrix HS Trap extraction technology with the state of the art Clarus SQ 8 GC/MS is a very powerful, yet easy to use tool for investigating many aspects of the beer production process. Virtually anything that is volatile and organic can be monitored in beer using a single column and applied conditions. The system may be deployed for checking raw materials, monitoring fermentation, quality control testing of a final product, product development, aging studies and trouble shooting.

Traditionally, this work would have been performed by skilled tasters, which of course continues to be an important part of any brewing process. The opportunity to compliment taste and olfactory determinations with hard objective analytical data can only enhance the art of making quality beer.

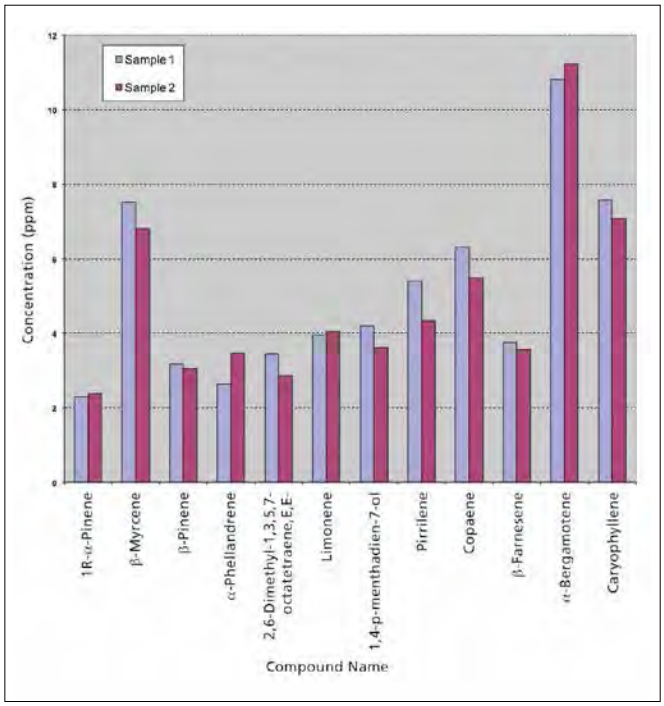

Figure 12. Hop VOC profiles (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

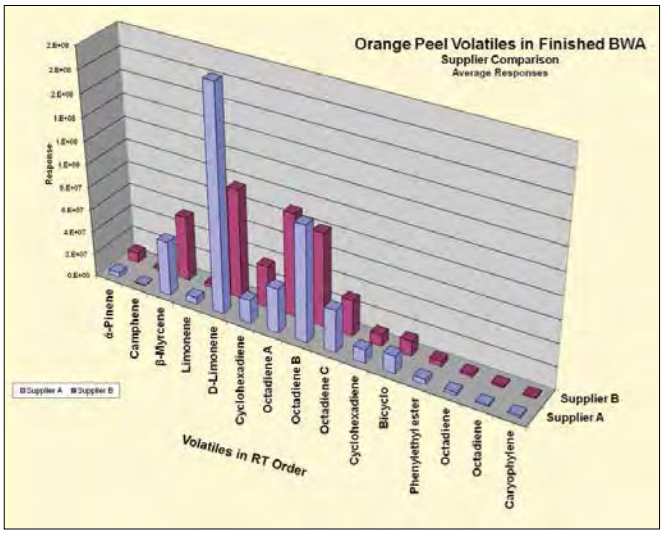

Figure 13. Flavor profiles in orange peel (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

In this note, we have conducted many of the critical analyses relevant to beer brewing. We have shown good performance in determining levels of defects such as diacetyl and dimethyl sulfide. We have identified flavor components in the beer, hops and adjuncts.

All this is possible on a system that simply requires the beer to be sealed in a vial and placed on an autosampler tray. The system does the rest.

Acknowledgement

We thank Bill Yawney, the QC Manager, from Long Trail Brewing Company for allowing us to use some of his data in this application note. In addition, Bill provided us with valuable expert advice on the brewing process and the analysis of beer.

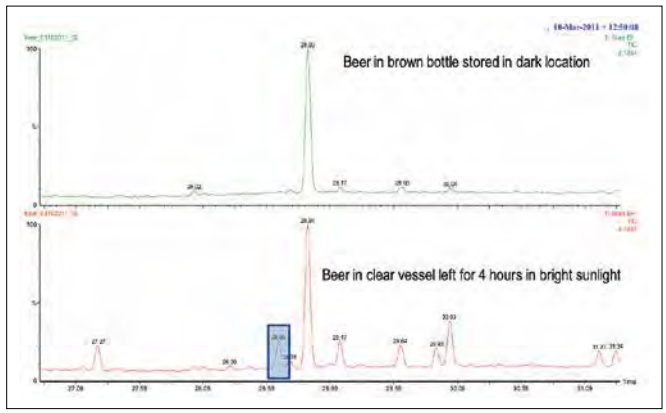

Figure 14. Effect of sunlight on beer volatiles.

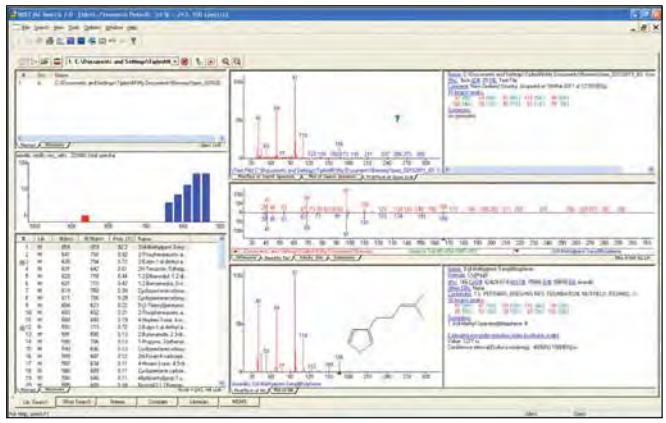

Figure 15. Library search on spectrum obtained from the peak highlighted in Figure 14.

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FIELD APPLICATION REPORT

ICP Optical Emmission

Beer Analysis Using the Optima ICP

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Introduction

Beer is one of the oldest beverages with references dating all the way back to 6000 B.C. The analysis of elements is an important parameter for determining the quality of beer. The analysis of beer is complicated by the presence of alcohol, dissolved solids and carbonation. Some elements affect the taste of beer, including Fe and Cu. These are usually found in very low concentrations, so the instrument's detection limits are important. Because of the low levels found, it is desirable to avoid dilution of the samples. Some elements are found at much higher concentrations, such as K, which can be several hundred mg/L. Inductively coupled plasma optical emission spectroscopy's (ICP-OES) multi-element capabilities, large dynamic range, and low detection limits (using axial viewing), make it ideal for the determination of metals in beer. The extensive linear range of ICP allows the analysis of both the low level elements as well as the major elements, without further dilution.

The direct analysis of beer by ICP can be challenging. The alcohol content requires matrix matching the standards to the samples containing ethanol. Also, the sample introduction system must be optimized for the volatile, organic ethanol component of the matrix. Due to high levels of dissolved solids, the nebulizer and injector must be capable of handling the samples without clogging. The carbonation in the beer samples must be removed to prevent out-gassing during thenebulization process and to eliminate poor reproducibility.

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The American Society of Brewing Chemists, Inc.[1] has undertaken a round robin study to develop a method for the determination of beer using ICP. Most of the parameters used in the latest study were also used in this analysis. Four beer samples were analyzed using PerkinElmer Optima™ ICP optical emission spectrometers. The samples represented different brands and types and they were split between the two labs.

Experimental

Instrumentation

Either the PerkinElmer Optima 5300DV or the PerkinElmer Optima 2100DV ICP model can be used for this analysis. The Optima 5300DV ICP system is a simultaneous ICP system with an echelle polychromator and a Segmented-Array Chargecoupled Detector (SCD). Simultaneous measurement of the background and analyte emission allows for accurate correction of transient background fluctuations. The Optima 2100DV ICP has a high speed, high resolution, double monochromator with a CCD array detector. Dynamic Wavelength Stabilization ensures wavelength accuracy and reliability.

A baffled cyclonic spray chamber with a Burgener Mira Mist® nebulizer and the 1.2 mm quartz injector were used for this analysis to minimize the volatility affect of the ethanol and the presence of high dissolved solids. The hardware and instrument parameters are detailed in Tables 1 and 2, respectively.

Table 1. Hardware

Nebulizer	Burgener Mira Mist® N077-5330
Spray Chamber	Baffled, Glass Cyclonic N077-6053
Injector	1.2 mm i.d. Quartz N077-5226
Injector Support Adapter	1.2 mm i.d. N077-6091
Torch	Quartz, Single Slot Paddle Torch N077-0338

Table 2. Instrument Parameters

RF Power	1400 W
Plasma gas	17 LPM
Aux gas	1.0 LPM
Nebulizer gas	0.5 LPM
Pump	2.0 ml/min
Torch cassette position	-3.0 mm
Replicates	3
Integration Time	5 min. 20 max.
Radial viewing distance	15 mm

Due to the different viscosities and alcohol content of the various beers, internal standards were used. Yttrium and gallium were used as internal standards for both radial and axial viewing. All solutions were spiked with Ga and Y so that the final concentration in solution was 100 mg/L Ga and 20 mg/L Y. The elements, wavelengths, viewing mode, and internal standards used are listed in Table 3.

Table 3. Instrument Parameters

Element	Wavelength nm	Viewing Mode	Internal Standard
K	766.490	Radial	Ga Radial
Na	589.592	Radial	Ga Radial
Mg	279.077	Radial	Y Radial
Ca	317.933	Radial	Y Radial
Fe	238.204	Axial	Y Axial
Cu	324.752	Axial	Ga Axial
Zn	213.857	Axial	Ga Axial
Y	371.031	Radial & Axial	—
Ga	417.206	Radial & Axial	—

Sample Preparation

A portion of each of the beers was taken and allowed to stand for several minutes with mild shaking to release the majority of the carbonation. They were then degassed in an ultrasonic bath for 15 minutes. An aliquot of beer was taken and spiked with the internal standard. The samples were also acidified with trace metal grade nitric acid to 7% (7 ml concentrated HNO_3/100 mL). The standards and blank were made to contain 5% ethanol and 7% HNO_3 to matrix match the beer sample matrix.

Results

As can be seen in Table 4, the concentration values for K can reach very high levels in the beer, while Fe and Cu are present at very low concentrations. Due to the large linear dynamic range of ICP, it is possible to calibrate for K up to as high as 1000 mg/L. This allows the analysis of very high levels of K without the need for further dilution and the need for a second analysis of each sample after dilution. (Figure 1)

Table 4. Concentration, mg/L

Element	Beer A	Beer B	Beer C	Beer D
K	590	203	273	212
Na	42.6	15.8	58.9	13.6
Mg	106	48.5	60.1	52.1
Ca	47.1	29.6	34.0	49.7
Fe	0.053	0.032	0.025	0.044
Cu	0.044	0.002	0.014	0.013
Zn	<0.002	0.152	<0.002	<0.002

The analysis was performed using several wavelengths to check for potential spectral interferences. The agreement between the wavelengths was better than 4% RSD for the major elements and typically less then 10% RSD for elements above 0.05 mg/L. Four separate aliquots of the samples were analyzed and the % RSD was less than 5% for the major elements. Some variations were higher because of potential contamination, especially for Cu and Zn which were near detection limits for some samples.

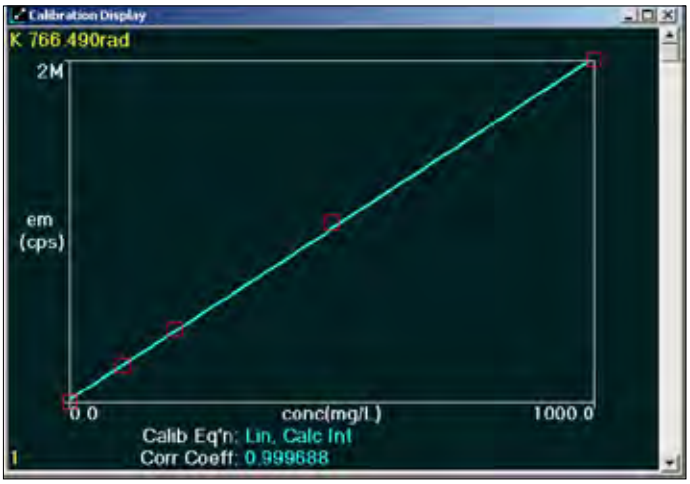

Figure 1. Potassium Calibration Curve.

Conclusion

As a sample, beer presents significant challenges for accurate and precise analysis. ICP can meet the challenge with the use of Dual View optics, optimized sample introduction systems and careful preparation of samples and standards. The samples had no spectral interferences at the chosen wavelengths. Internal standards are necessary for accurate analysis. Using this procedure the analysis of beer can be straight forward.

References

1. American Society of Brewing Chemist, Inc. Report of sub committee, Elemental Analysis of Beer and Wort by Inductively Coupled Plasma – Atomic Emission Spectroscopy. D. Sedin Chairman, Journal 62:190, 2004.

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CASE STUDY

Food Quality

Long Trail Brewing Uses Headspace Sampling to Improve Beer Taste and Production Efficiency

Long Trail Brewing Company is a microbrewery located in Bridgewater Corners, Vermont that produces the second best selling draft beer in the state of Vermont. "Beer production is very simple but it gets complicated when you try to make it the same every time," said Bill Yawney, Quality Assurance Manager for Long Trail. "Our philosophy is that people drink beer because they like the taste so we focus on identifying and ensuring the presence of positive attributes that provide the great taste that keeps our customers coming back for more. A modern headspace trap, gas chromatrograph/mass spectrometer (GC/MS) from PerkinElmer helps us monitor the taste of our beer and ensure that our process is working correctly. We also use these instruments in the product development process to deliver a world class beer flavor."

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Challenge

Andy Pherson founded Long Trail Brewery in the basement of the old Bridgewater Woolen Mill in 1989. The company was named after a hiking trail that runs the length of Vermont. Long Trail's flagship beer is Long Trail Ale, a Dusseldorf-style Altbier. Long Trail Ale is Vermont's best selling craft beer and Long Trail draft is outsold in the state of Vermont only by mega-brewer Anheuser Busch. The Long Trail Visitor Center receives an estimated 72,000 visitors each year, making it one of the state's top tourist attractions. The company now distributes its beer to 13 additional states in the Northeast and Mid Atlantic regions. Founder Pherson retired in 2006 and the company is still privately owned.

"The owner of this company had the vision that analytical instruments could play a critical role in our process control, quality assurance and product development," Yawney said. "He gave me the job of selecting the right instruments and implementing them in our brewery. I interviewed four companies and quickly narrowed the choice down to PerkinElmer and one other. I selected the PerkinElmer TurboMatrix headspace trap sampler and Clarus 500 Gas Chromatograph (GC) with a Clarus 500 mass spectrometer (MS) detector because they offer several significant advantages. The headspace trap repeatedly pressure cycles samples to extract as much vapor as possible, resulting in detection limits up to 100 times lower than standard methods. The GC's programmable pneumatic control makes it possible to control and monitor all injector, detector and auxiliary gases electronically, substantially reducing the time needed to measure and set flows. The mass spectrometer has, not only the ability to quantify compounds, but the ability to identify them, as well."

Solution

Headspace sampling is the state-of-the-art method for sampling the aroma of beer and other food products. The beer sample is placed into a vial and sealed. The vial is heated to release the vapor into the headspace or empty area of the vial. The vapor is then extracted and analyzed using gas chromatography.

At equilibrium the concentration in the headspace phase is proportional to the original concentration in the sample. Determining the concentration of the headspace phase enables the composition of the sample to be established. Polar compounds in beer are more soluble in water than in air so only less than 0.5% of the compound in the sample may pass into the headspace. The headspace trap technique can enhance detection limits by injecting the entire headspace volume into the trap, pausing to allow the headspace to refill with vapor and repeating the injection process several times.

Long Trail uses a number of different detectors with the PerkinElmer Clarus GC, a flame ionization detector (FID) and a mass spectrometer. The FID has certain specific uses, however, the mass spectrometer is the primary detector able to detect beer defects such as acetaldehyde, dimethyl sulfide and vicinal diketones such as diacetyl and 2,3- pentanedione. It is also used to analyze volatile aldehydes, ketones, carbonyl and furfural compounds, some of which are involved in beer staling. The mass spectrometer can measure many beer attributes, as well, such as higher alcohols, esters and hop aroma compounds.

Outcome

Acetaldehyde has the taste and aroma of green apples or grass that, like many other compounds, provides a positive taste at low levels but can cause a negative flavor at higher levels. Acetaldehyde is reduced to ethanol by yeast during secondary fermentation. It is important not to remove the yeast too early from a fermentation to allow the process to complete. Acceptable levels are typically in the range of 1 to 20 parts per million (ppm).

Dimethyl sulfide contributes to the taste of ales at the right level but it's important to avoid excessive amounts because it produces to an unpleasant cooked cabbage aroma. Long Trail typically sets a maximum level of 50 parts per billion (ppb). Analysis results are used to fine-tune the process, particularly the boiling time and the venting system, to meet this specification.

Diacetyl is a naturally occurring compound that provides a buttery flavor that can cause problems at higher levels. Beer naturally produces diacetyl as it ferments, often to excessive levels. As the beer sits in the fermenter, yeast reabsorb the diacetyl, reducing it to acetoin. The human threshold for detection of diacetyl has been reported to be between 50 and 100 ppb. Acceptable levels of diacetyl are dependant on the beer style. In lagers, it is generally considered a defect above the threshold, yet, it may make a positive contribution to the flavor profile of some ales and is vital to some barrel aged syles.

The time it takes for yeast to reduce diacetyl in the conditioning period is called the diacetyl rest and is important information with regards to brewery throughput. "At Long Trail, the ability of the PerkinElmer GC/MS to measure diacetyl reduction over time has allowed us to maximize product throughput with confidence that diacetyl reduction has progressed to our desired targets. Previously, we based this solely on information from beer science journals and taste testing."

Long Trail uses the GC to measure the floral bouquet of hops. A wide assortment of compounds give hops their particular flavor and the GC/MS can identify and quantify many of them. The analytical data is then calibrated against human perception in taste tests. This type of analysis has been particularly valuable in ensuring that the company's two breweries produce beer with exactly the same flavor.

The GC is also used to evaluate other flavors. When Long Trail had to switch suppliers of orange peel it compared the old and new suppliers' products and determined the appropriate use rate for the new supplier's product. Long Trail switched from a coarse to a fine coriander mill and discovered by quantitative analysis that less of the fine coriander needed to be added to its product to deliver the same flavor.

Understanding what makes beer go stale

Long Trail also is engaged in a project to model the compounds that cause beer to go stale. When beer is exposed to oxygen, aldehydes and other carbonyls turn into compounds that produce unpleasant flavors. Modern fillers produce bottled beer with only 30 ppb of oxygen, however, staling compounds can form from a combination of heat and time alone. The key to improving beer shelf-life is to minimize the levels of precursors in the first place. The approach is to heat abuse product and compare it to fresh product in order to determine which compounds are involved in staling. This will hopefully lead to the ability to predict staling by analyzing the levels of precursors in fresh product. Additionally, process changes could be made to minimize the amounts of the precursors, once identified.

Long Trail uses GC results to guide its product development efforts. "In one case, our goal was to make a well designed thin brown ale." Yawney said. "Our in-house tasters did not like our first try so we compared it to our competition with the GC and found that our ester profile was too strong. We made process changes that resulted in a less pronounced ester profile and in-house taste testing rated the revised product as superior to the competition. It is then typical for Long Trail to use the GC/MS to evaluate production size trials of laboratory R&D produced recipes.

"Breweries around the world have been using this type of analytical equipment for years, much of the time to monitor beer defects. What makes Long Trail's approach unique is our focus on beer attributes," Yawney concluded. "With an eye on potential product defects, focusing on the positive organoleptic qualities of this wonderful substance means we are focusing on the enjoyable experience we wish for all our customers and the reason they purchase our products."

View from the Long Trail mezzanine where you can follow a self guided tour of the brewery.

Boiling wort in the brew kettle as viewed through the port for hop additions.

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APPLICATION NOTE

Atomic Absorption

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Milano, Italy

Elemental Analysis of Beer by Flame Atomic Absorption Spectrometry with the PinAAcle 900 AAS

Introduction

Beer is a widely consumed beverage with both organic and inorganic components. The concentrations of the inorganic components may vary depending on raw materials and brewing processes. Knowledge of the type and concentration of inorganic components in beer is of considerable interest from various perspectives, as they may affect taste, appearance, product stability, and health of the consumer[1]. The determination of elements in beer by flame atomic absorption spectrometry (FAAS) is a well-known procedure[2]. For example, the American Society of Brewing Chemists (ASBC) in St. Paul, Minnesota, USA, is proposing the regular determination of calcium (Ca), copper (Cu), iron (Fe), and sodium (Na) in beer by FAAS[3].

FAAS has the benefit of providing precise and accurate measurements at a lower cost per element than more advanced elemental techniques, and also requires less operator training than many other trace elemental techniques. The PinAAcle™ 900 FAAS provides an intuitive, highly efficient system capable of simplifying analyses while maintaining peak performance and unmatched productivity.

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Experimental

Instrumentation

All measurements were performed on a PerkinElmer PinAAcle 900T atomic absorption spectrometer equipped with high sensitivity nebulizer (HSN) and ceramic impact bead. An air-C_2H_2 flame with a 10 cm 3-slot solid titanium burner head was used for the determination of copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn). Aluminum (Al) was determined with N_2O-C_2H_2 flame on a 5 cm solid titanium burner head. A nebulizer spacer was used for calcium (Ca) and sodium (Na) to reduce sensitivity, and for Al to improve N_2O flame stability and minimize interferences. Lumina™ cableless hollow cathode lamps were used for all elements.

Sample Preparation

Several brands of beer were purchased in a local supermarket in Singapore. When available, the same brand was purchased in two different packaging materials: a glass bottle and a metal can. A total of five bottled and six canned beers were analyzed. Sample aliquots for analyses were obtained by pouring the beers in 50 mL polyethylene autosampler tubes with caps. Samples were degassed of CO_2 by ultra-sonication at full power for 30 minutes and then acidified to 2 % (v/v) with HNO_3 (70 % w/v, Clean Room Chemical, Air Products and Chemicals Inc, Allentown, Pennsylvania, USA).

All elements were measured against external calibration curves with linear-through-zero regression, except Na, which used a non-linear through zero regression. Standards were prepared by serial dilutions of 1000 mg/L PerkinElmer Pure single-element standards in 2% HNO_3 (v/v).

Elements usually present at trace levels (Al, Cu, Fe, Mn, and Zn) were determined directly in the undiluted beers. The calibration solutions for these elements were prepared in 5% (v/v) ethanol (99.5% GR grade, Kanto Chemical Co. Inc., Japan) for matrix matching. For Al determination, 0.2% lanthanum (La) (w/v) was added to all samples and standards as an ionization buffer (La_2O_3 99.5% LAB grade, Merck, Germany).

For the determination of Ca and Na, the samples were diluted 30 fold with ≥ 18 MΩ ultrapure water (MilliQ system, Millipore, Billerica, Massachusetts, USA). Calibration standards were prepared in 1% HNO_3 (v/v). No ethanol was added, due to the dilution factor. La 0.2% (w/v) was added as a releasing agent (to avoid phosphate suppression on Al) and as an ionization suppressant for Na and Ca (releasing agents are cations that react preferentially with an interferent). Table 1 shows the instrumental conditions used for this work.

Results and Discussion

Each beer sample was given a number to identify the brand and container type. Samples labeled "G" were from glass bottles, while samples labeled "M" were from metal cans. Results, reported in Table 2, show that Ca and Na are present at high concentrations (mg/L), while other elements are present at µg/L levels, as expected. The data showed good quality for all beers tested, with respect to their elemental contents, based on the current ASBC guidelines. These results indicate that the container material (glass or can) does not significantly contribute to the element content of the beer, with the exception of Mn, which is always a little higher in the bottled beers.

Due to the low level of Al in the beer samples tested, it could not be detected by FAAS in most samples. Instead, a more sensitive technique, such as graphite furnace atomic absorption spectrometry (GFAAS), should be used for Al determination. The PinAAcle 900T (and 900H) can easily be switched between flame and graphite furnace modes, offering the capability to determine the low concentration elements by GFAAS using a single system. For analysis using flame-only atomic absorption, the PinAAcle 900F is also available.

Table 1. Instrument settings for the analysis of beer.

Element	Wavelength (nm)	Slit (nm)	Lamp Current (mA)	Units	Calibration Standards	Air (L/min)	Nitrous Oxide (L/min)	Acetylene (L/min)
Al	309.27	0.7	25	mg/L	2, 5	---	10.0	7.98
Ca	422.67	0.7	10	mg/L	0.5, 0.8, 2.5	8.68	---	2.48
Cu	324.75	0.7	15	µg/L	40, 100, 200	10.0	---	3.16
Fe	248.33	0.2	30	µg/L	100, 250, 500	10.0	---	3.16
Mn	279.48	0.2	20	µg/L	50, 125, 250	10.0	---	3.16
Na	589.00	0.2	8	mg/L	0.5, 0.8, 2.5	8.68	---	2.48
Zn	213.86	0.7	15	µg/L	50, 125, 250	10.0	---	3.16

Table 2. Results for the analysis of multiple beer samples using flame atomic absorption.

Sample	1M	2G	3M	3G	4M	4G	5M	5G	6G	7M	7G
Al (mg/L)	0.14	ND	ND	ND	ND	ND	ND	ND	ND	0.19	ND
Ca (mg/L)	79	104	72	93	139	143	107	143	96	77	76
Cu (µg/L)	35	31	63	62	37	33	45	44	46	36	35
Fe (µg/L)	43	42	45	31	22	30	52	68	54	25	25
Mn (µg/L)	62	144	107	136	87	118	123	153	190	64	72
Na (mg/L)	40	47	35	46	86	82	72	69	207	196	187
Zn (µg/L)	2.4	0.7	6.1	3.6	1.2	0.3	2.5	29	1.2	4.4	5.3

ND = not detected

Quality Control

For beer analysis, there are no certified reference materials (CRMs) available with certified elemental content. For this reason, quality control (QC) procedures were implemented by running selected samples in duplicate and after performing a spike to demonstrate the method's capability for precision and recovery. Due to the spread in concentration levels, spike additions were performed at mid-range of calibration curves, to provide a detectable signal increase (Table 3). Due to limited sample, not all elements were analyzed in all samples.

Analytical results of some samples run in duplicate were utilized to demonstrate analytical precision. Sample duplicates were carried through the full sample preparation process. The obtained results, reported in Table 4, show a good level of repeatability, even when using disposable plastic-ware, which was used in this application, instead of the typical calibrated glassware.

Conclusions

The present work reports the usage of the PinAAcle 900T AAS in flame mode for the determination of several elements relevant to the beer industry. The procedure is simple, fast, and accurate, requires no sample digestion, and can be applied to the quality control of beer manufacturing products when using a customer-validated application. The reported results prove that the PinAAcle 900 FAAS has the capability to determine elements in beer with high accuracy and precision.

Table 3. Spike recovery tests.

Element	Spike Level	% Recovery					
		3M	3G	4M	4G	6G	7G
Ca	1 mg/L		90	105			103
Cu	100 µg/L	93			97	95	
Fe	250 µg/L	98			98	101	
Mn	125 µg/L		100	95			94
Na	1 mg/L		103	110			103
Zn	125 µg/L		99	96			93

References

1. S. Caroli "The Determination of Chemical Elements in Food: Applications for Atomic and Mass Spectrometry", Wiley 2007.
2. PerkinElmer Flame-AAS Cook Book 03030152 rev. 2009.
3. American Society of Brewing Chemists (ASBC), http://www.asbcnet.org, International Check Sample Service, May 2010.

Consumables Used

Component	Part Number
Al Hollow Cathode Lamp	N3050103
Ca Hollow Cathode Lamp	N3050114
Cu Hollow Cathode Lamp	N3050121
Fe Hollow Cathode Lamp	N3050126
Mn Hollow Cathode Lamp	N3050145
Na Hollow Cathode Lamp	N3050148
Zn Hollow Cathode Lamp	N3050191
10 cm 3-slot Titanium Burner Head	N0400103
5 cm 1-slot Titanium Burner Head	N0400101
Al – 1000 mg/L Standard	N9300184 (125 mL) N9300100 (500 mL)
Ca – 1000 mg/L Standard	N9303763 (125 mL) N9300108 (500 mL)
Cu – 1000 mg/L Standard	N9300183 (125 mL) N9300114 (500 mL)
Fe – 1000 mg/L Standard	N9303771 (125 mL) N9300126 (500 mL)
Mn – 1000 mg/L Standard	N9303783 (125 mL) N9300132 (500 mL)
Na – 1000 mg/L Standard	N9303785 (125 mL) N9300152 (500 mL)
Zn – 1000 mg/L Standard	N9300178 (125 mL) N9300168 (500 mL)
Autosampler Tubes	B0193233 (15 mL) B0193234 (50 mL)

Table 4. Duplicate tests.

Sample	3M		3G		4M		4G		6G		7G	
Replicate	1	2	1	2	1	2	1	2	1	2	1	2
Ca (mg/L)			93	93	139	134					76	75
Cu (µg/L)	63	59					33	32	46	43		
Fe (µg/L)	45	42					30	31	54	40		
Mn (µg/L)			136	135	87	89					72	69
Na (mg/L)			46	46	86	85					187	189
Zn (µg/L)			3.6	3.2	1.2	1.2					5.3	4.0

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PRODUCT NOTE

Gas Chromatography/
Mass Spectrometry

Clarus® SQ 8 GC/MS with TurboMatrix Headspace Trap System Application Pack for Monitoring Volatile Organic Compounds in Beer Production

Beer is a popular beverage produced by the fermentation of hopped malt extracted from barley and other grains. Although simple in concept, beer is a highly complex mixture of many compounds including sugars, proteins, alcohols, esters, acids, ketones and terpenes. Flavor is an important quality of any beer and the chemical content of the beer is responsible for that flavor. Aroma is also an extremely important part of the beer's trademark, so there is a strong interest by brewers in the volatile organic compounds (VOCs) in beer.

Some VOCs have a positive effect on aroma (attributes) and some have a negative effect (defects). The ability to characterize these in beer products before, during and after fermentation is an important tool in process control, quality assurance and product development. This application pack contains all the consumables needed to perform your analysis.

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For the Better

Monitoring Volatile Organic Compounds in Beer Production Application Pack Pack includes one of each of the following items (some items may ship separately):	**Part No. N9300908**
Description	**Part No.**
Application note: Monitoring Volatile Organic Compounds in Beer Production Using the Clarus SQ 8 GC/MS and TurboMatrix™ Headspace Trap Systems	
Elite-5 Column Length: 60 m, Inner Diameter: 0.25 mm, Film Thickness: 1.00 µm, Temperature Limits: -60 to 325/350 °C	N9316080
2 mm Split Mode Quartz Liner for Programmable Split/Splitless Injector	N6121004
Thermogreen Low Bleed Injector Port Septa – 6/Pkg.	N6101748
Graphite/Vespel Ferrules, 1/16 in x 0.4 mm – 10/Pkg.	09920104
20 mL PTFE/Silicone Convenience Kit: contains 20 mm PTFE/Silicone assembly (100/pack), 22 mL crimp clear vials (100/pack) with write on patch and 20 mm caps (100/pack)	N9303992
TurboMatrix Air Monitoring Headspace Trap	M0413628
Ferrules for PTFE Cold Trap – 10/Pkg.	L4275110
Marathon Filament for GC/MS	N6470012
Ergonomic Manual 20 mm Hand Crimper	N6621037
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APPLICATION NOTE

FT-NIR Spectroscopy

Verification of Tartrates Used in the Wine Industry

Summary

Tartrates are used extensively in the wine industry to clear the 'muddiness' or sediment before bottling (materials used in this process are called 'finings'). This application example describes the use of NIR to discriminate between two typical samples, based on calcium tartrate and potassium bitartrate.

Why choose NIR for this Application?

There are many different types of finings used in the beer, cider and wine making industries. These range from aluminosilicates to gelatin, egg white, fish glue and polyvinylpolypyrrolidone or polyclar. Since many of the finings are the bi-products of other processes, they are often of extremely variable quality. For example, particle size distribution varied widely from sample to sample. NIR reflectance is an ideal method for finings analysis since no sample preparation is involved (other than transferring a few grams of sample to a glass vial), and both chemical and physical property information is available.

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Experimental

Four samples were provided for analysis. Two of type 1 (calcium tartrate based) and two of type 2 (potassium bitartrate/calcium tartrate mixture). The samples were prepared for analysis by placing a few grams of sample into a glass vial. NIR spectra of the four samples were generated using a PerkinElmer FT-NIR System equipped with in-board Reflection Accessory. Typical scan conditions:– 11000-3800 cm^{-1}, 8 cm^{-1} resolution, 16 scans.

Results

Type 1 sample spectra (based on calcium tartrate) are shown in Figure 1. The spectra are similar; the variation in baseline indicating a difference in particle size distribution between the two samples which can be removed by converting to a second derivative as shown in Figure 2. It is also easier to see the bands due to the calcium tartrate itself in the second derivative rather than the water bands which dominate Figure 1.

Type 2 samples (based on a potassium bitartrate/calcium tartrate mixture) are shown in Figure 3. Note the strong band at around 4700 cm^{-1} indicative of tartrate.

Analysis

It is a simple task to create a short COMPARE™ library and use it to verify the identification of test samples. This was done for the finings samples, see table 1. The results for a test sample of type 1 (batch 5412) and type 2 (batch 5431) indicate that the task of separating type 1 and type 2 samples is easily accomplished. Resolution, intensity, noise and water blanking COMPARE filters were switched on to minimize unwanted spectral and sample interferences.

Table1. Compare library for identification of finings.

Compare – 5412.SP			
File	Correlation	Factor	Comments
type1.sp	0.9980	0.9714	Batch 5412
type1a.sp	0.9615	0.8213	Batch 5396
type2a.sp	0.1151	0.1506	Batch 5430
type2.sp	0.0679	-0.0754	Batch 5430

Compare – 5431.SP			
File	Correlation	Factor	Comments
type2.sp	0.9976	0.9330	Batch 5431
type2a.sp	0.9588	1.0889	Batch 5430
type1a.sp	0.1000	-0.0693	Batch 5396
type1.sp	0.0718	-0.0575	Batch 5412

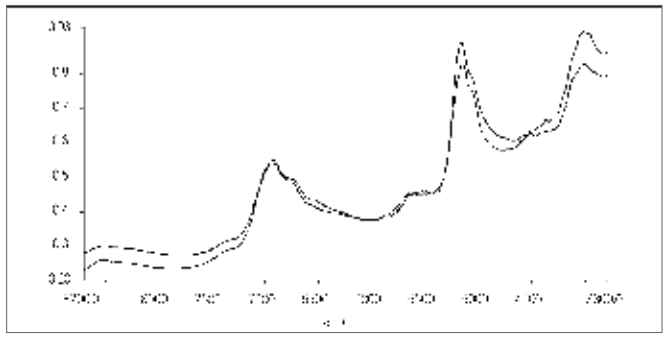

Figure 1. Sample spectra based on calcium tartrate.

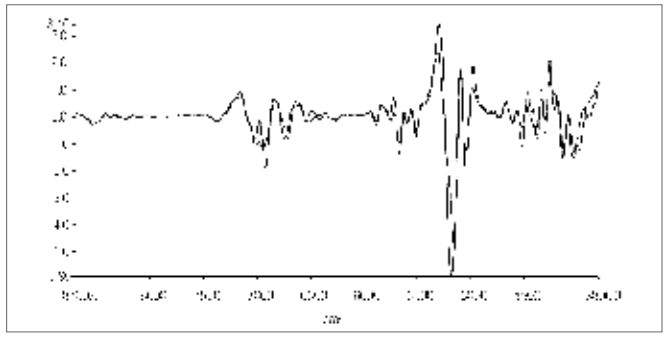

Figure 2. Second derivative of sample spectra based on calcium tartrate.

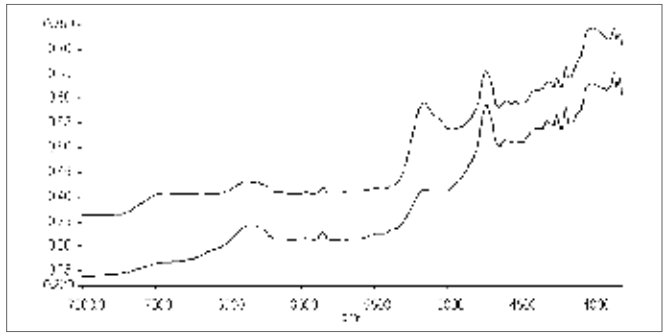

Figure 3. Sample spectra based on potassium bitartrate/calcium tartrate.

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APPLICATION BRIEF

ICP - Mass Spectrometry Analytics

Authors:

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Geographical Differences of Trace Elements in Wines – Analysis with NexION 300X/350X ICP-MS and Visualization with TIBCO Spotfire Software

Introduction

Traceability of the wine origin is important for brand protection. Elemental profiles of wines have been shown to be specific for their geographic origin[1,2], since the levels of trace metals in wines are related to the soil in the grapevine cultivation area.

In this study, a total of 75 Italian red wines from different regions and grape types were analyzed by ICP-MS to determine whether elemental profiles correlate to the region of origin. Results were imported into TIBCO Spotfire® software for statistical calculations and to display geospatial distribution.

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Methods

All wines in this study were red wines produced in different regions of Italy and from various grape types, the majority bottled in 2011 or 2010, with a few older wines. Regions were Lombardy (Lombardia), Abruzzo, Tuscany (Toscana), Trentino-Alto Adige, Apulia (Puglia), Sicily (Sicilia) and Sardinia (Sardegna).

All analyses were performed on a PerkinElmer NexION® 300X ICP-MS in both Standard and Collision modes.

Table 1. Instrumental Conditions

Parameter	Condition
Instrument	NexION 300X ICP-MS
Nebulizer	Glass concentric
Spray chamber	Glass cyclonic
Sample uptake rate	0.25 mL/min
RF power	1500 W
Internal standard	Ge, Rh, Re at 10 ppb
Dwell time	50 ms
Collision mode	He = 4 mL/min

All samples were filtered and diluted four times with 2% (v/v) HNO_3. Internal standards were used to compensate for possible matrix effects during sample introduction. An internal standard mix (Ge, Rh, and Re) was added on-line by merging flows of the sample and internal standard mix.

Results for all samples were compiled into a single table in Microsoft® Excel, with columns of elements, and the ICP-MS values (in cps) for each sample in rows. For most of the samples, information on the region and city of origin, the type of grape, and year of production were available, and added as additional category columns to the table. The table was opened in TIBCO Spotfire software and the data used for various calculations and visualizations. Standard S Plus statistical algorithms such as Principal Component Analysis (PCA) were used. The category columns enable different grouping and sorting options for raw data and the resulting statistical outputs, and for color coding of graphs.

Results

The levels of 39 elements were measured for each sample; these vary widely, from phosphorus at high mg/L levels to rare earth elements at sub µg/L levels. PCA was used to investigate the relationship between the geographic origin of wines and their elemental profiles.

PCA is a data analysis method used to reduce the dimensionality of multivariate data and to derive meaningful patterns from the complex information.

PCA transforms or projects the variables for each sample into a lower dimensional space, while retaining the maximal amount of information about the variables. Resulting principal components for each sample are a combination of the original variables after the transformation. The largest difference in the combined variables between the samples is described by Principle Component 1 (PC1), the next largest by component 2 and so on.

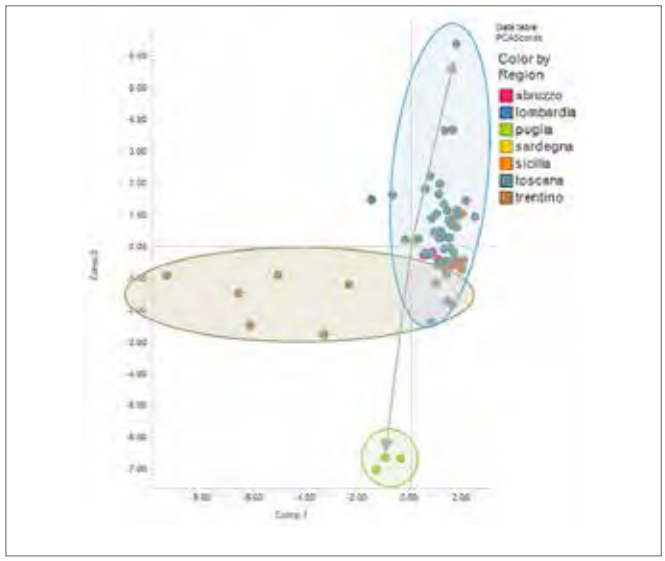

Figure 1. Scores Plot for PC1 vs. PC3 showing separation of the Puglia (Apulia) wines in green from the Toscana (Tuscany) wines in blue and the Trentino wines in brown.

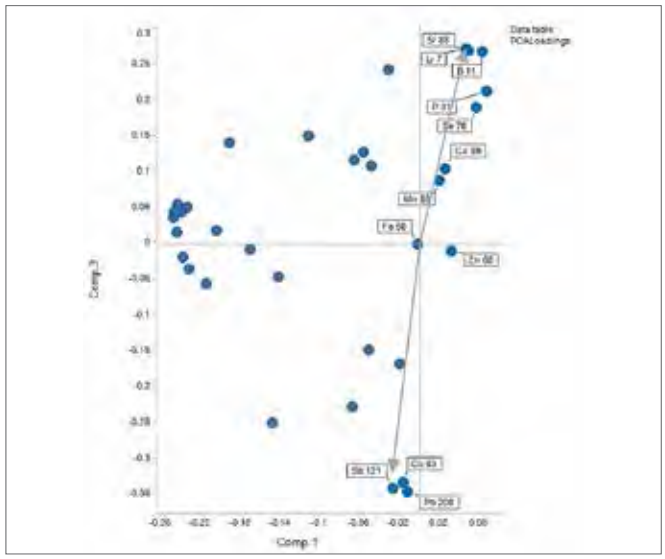

Figure 2. Loadings Plot of PC1 vs. PC3 showing that the strongest contributors to the separation of the Puglia (Apulia) wines are the higher levels of Sb, Cu and Pb, with some Toscana (Tuscany) wines having higher levels of Sr, Li and B.

A Scores Plot summarizes the relationship between the samples, a plot of PC1 vs. PC2, or PC1 vs. PC3 will show the samples grouped according to the larger differences between them; this information is displayed in TIBCO Spotfire software scatter plots. A Loadings Plot of the same components shows the weighting for each variable as a distance from the origin. The plot is a means of interpreting the patterns seen in the Scores Plot.

For these wine analyses, the levels of the 39 different elements from the ICP-MS results are the variables. PCA calculations used the functions within the TIBCO Spotfire Statistics Services, including autoscaling of values in each element column, thus giving the same variance ranges across the samples, independent of concentration and ICP-MS instrument response.

The Scores Plot from the initial autoscaled PCA results show a strong separation of the three Puglia wines from all other wines using PC1 vs. PC3 (Figure 1). The corresponding Loadings Plot (Figure 2) indicates that this separation was most strongly

correlated to the higher levels of Cu, Sb and Pb in these wines. Other wines, particularly those from Tuscany, are partly grouped by having higher levels of Sr, Li and B. Trentino wines correlate to increased levels of a number of elements, which will be described for the PC1 vs. PC2 interpretation that follows.

A bar chart of the levels of Cu and Pb for each sample, sorted by region, confirms the higher levels for the Puglia samples (Figure 3). It is not known whether these relatively high levels are due to the soil type, grape type, or cultivation and production methods for these wines. For example, high levels of Cu may be due to the use of copper compounds as mildewcides and fungicides. Increased levels may also relate to the use of brass equipment during production and bottling.

The various wines from the Trentino region were also partly grouped in a Scores Plot of PC1 vs. PC2; the loadings plot (Figure 4) suggests that these wines have higher levels of a number of rare earth elements. These elements have been reported previously[3, 4], as having variable levels in wines due to the use of bentonite, an absorbent clay, to precipitate proteins from the wine. Thus, these elements are not considered to be reliable indicators of geographic origin.

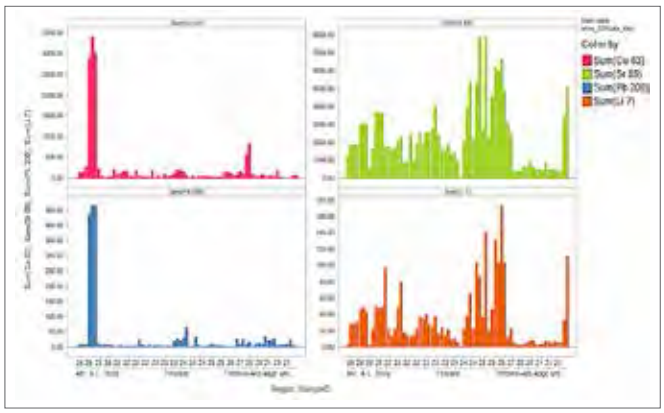

Figure 3. Bar chart of the concentration of Cu (red), Pb (blue), Sr (green), and Li (orange) for each sample, grouped by region, shows that the wines from Puglia (Apulia) have higher levels of Cu and Pb, while several of the wines from Toscana (Tuscany) have higher levels of Sr and Li.

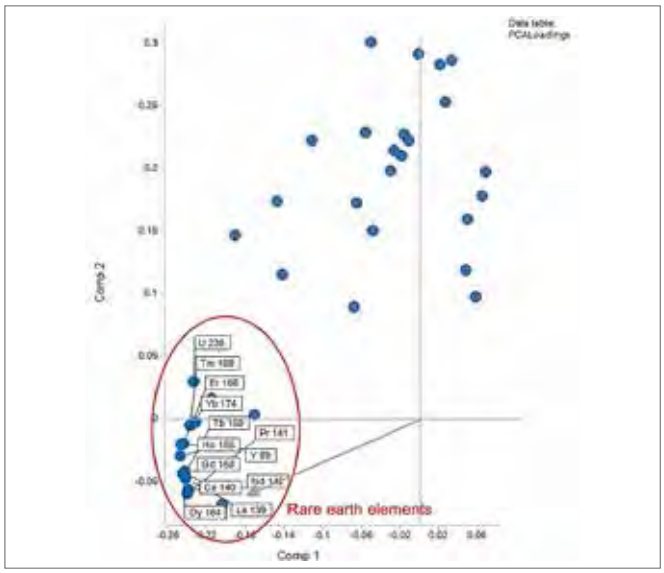

Figure 4. Loadings Plot of PC1 vs. PC2 showing that the increased levels of many rare earth elements differentiate the Trentino wines.

A new PCA analysis of the data was made, after excluding the data columns for the rare earth elements. The new Scores Plot of PC1 vs. PC2 (Figure 5) gave a clearer differentiation of the groupings for the Tuscany and Trentino regions.

The differentiators in the loadings plot (Figure 6) are clustered into groups of elements, which may be classified by geochemistry. This grouping has been reported for other wines[5], and may relate to the local rainfall and climate of the grapevines. The chalcophilic elements (Cu, Zn, Sb, As, Sn) have a lower affinity for oxygen and prefer to bind to sulfur as insoluble sulfides. These elements are at higher levels in Tuscany wines, as are siderophilic elements such as Ni and Co which track with Fe. Lithophilic elements (Li, Ba, Cs, Sr etc.) are highly soluble and associate with the soil; these are at lower levels in Trentino wines.

TIBCO Spotfire software visualizations allow the user to quickly group information in the element data table in different ways.

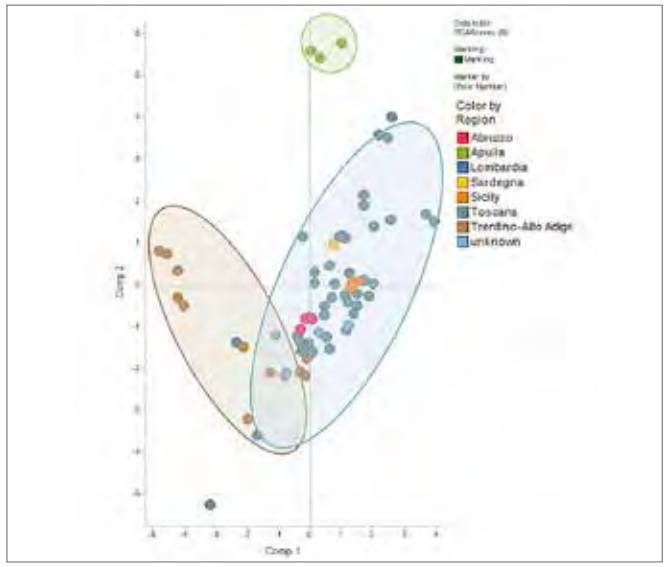

Figure 5. Scores Plot for PC1 vs. PC2 using only the non-rare-earth elements, showing separation of the groups of the Puglia (green), Trentino (brown), and Toscana (blue) wines.

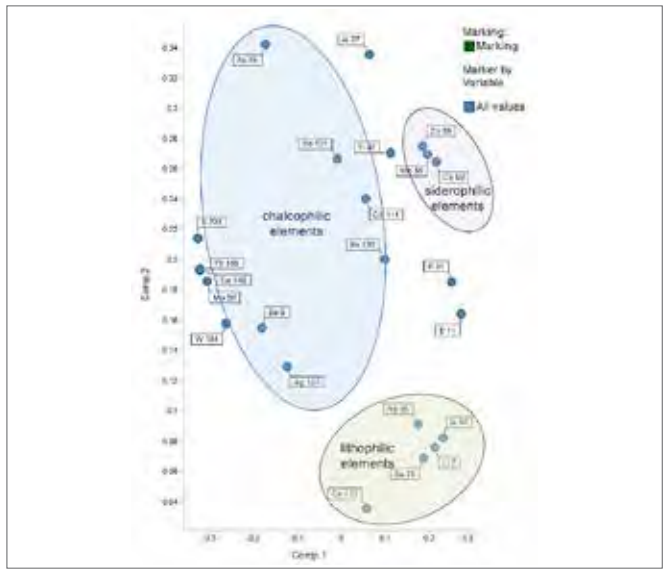

Figure 6. Loadings Plot showing a partial grouping of elements that differentiate the wines into the categories of the Goldschmidt geochemical classification of elements.

For example, a bar chart showing concentrations of Li for each sample (Figure 7), is ordered first by region, and then color-coded by city. This view of the data highlights the difference in Li levels by region. The higher levels in many wines from Florence in Tuscany are clearly visible.

Custom expressions are easily created to show the levels for a combined group of elements. A category for various chalcophilic elements (the sum of concentration for Zn, Sb, As, and Sn but not Cu) gives a clear view of the differences in concentration of this group of elements for all the samples (Figure 8).

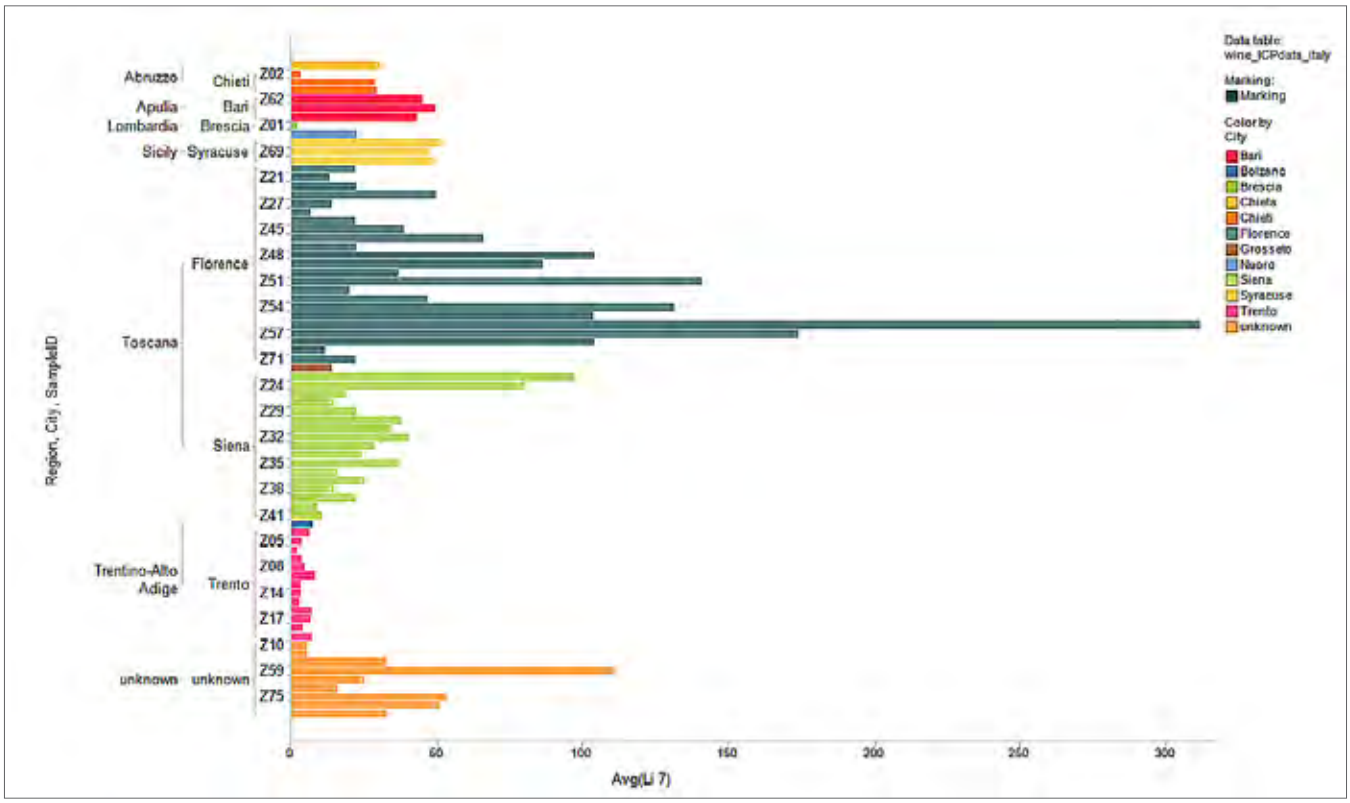

Figure 7. Bar chart showing the Li level for each sample, with samples ordered by region, then grape type and colored by city. Highest levels are for the wines from Florence in Tuscany, shown in blue.

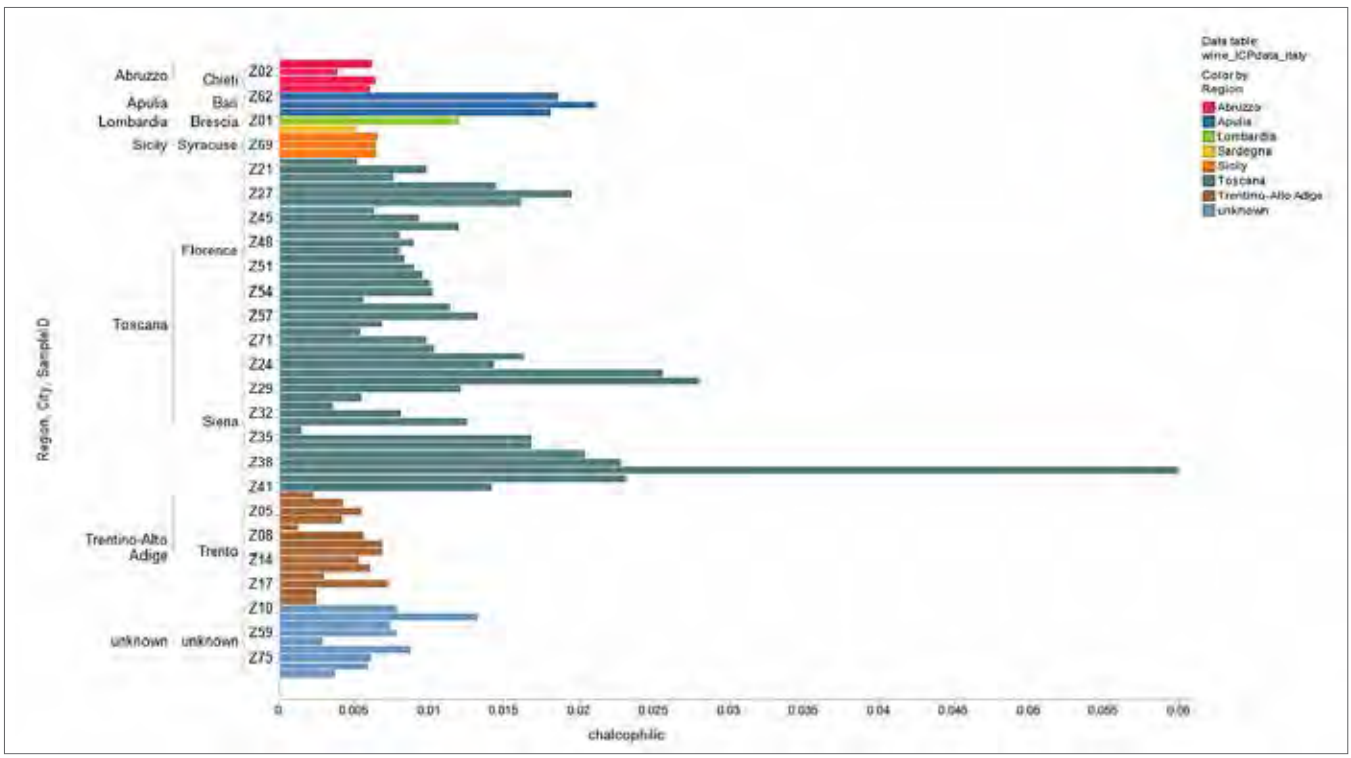

Figure 8. Bar chart for samples ordered by region then city, with bars colored by region, showing that levels for the custom chalcophilic category (sum of Zn, Sb, As, and Sn levels) are higher for the Toscana (Tuscany) wines (blue) than for the Trentino wines (brown).

Previously reported elemental analysis of Italian wines[6] suggested that Sr and Rb levels were correlated to the soils of origin in provinces of Abruzzo in central Italy. With these results, levels of Rb were quite constant for all samples, although Sr levels did vary. The levels of Sr were highest in some of the wines from Florence in Tuscany and Syracuse in Sicily (Figure 9).

A map chart (Figure 10) shows the geospatial distribution of a selected element or customized group of elements. The region names for a shape file of Italy are linked in TIBCO Spotfire software to the region column in the data table, and regions in the map chart are color-coded by intensity for an element. Here, chalcophilic and lithophilic element groups are contrasted with high intensity levels in red and low levels in blue. Wines from the Florence area of Tuscany are relatively low in chalcophilic elements compared with other regions, but higher in lithophilic elements. Wines from Sicily are relatively high in lithophilic elements, but lower in chalcophilic elements.

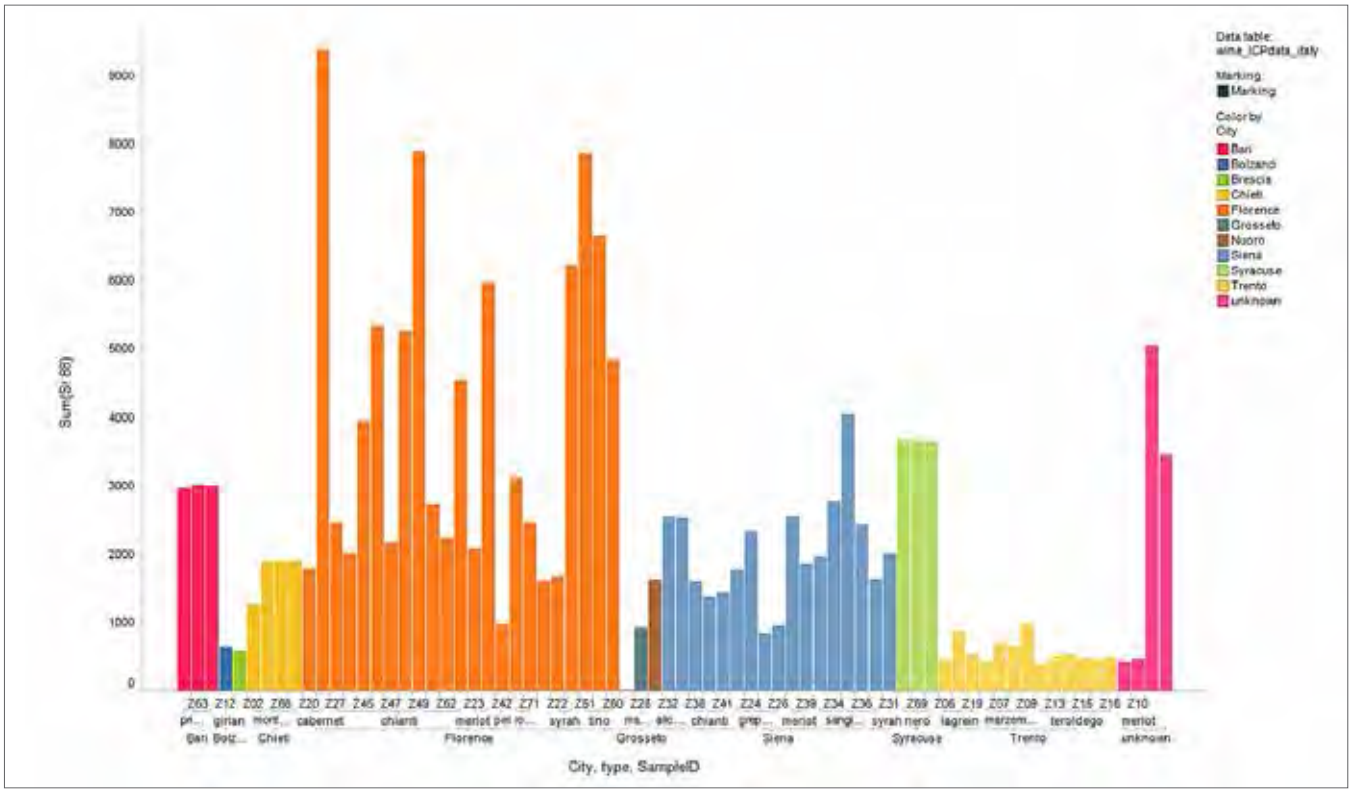

Figure 9. Bar chart showing levels of Sr for each sample, with columns colored by city, and ordered by city and grape type.

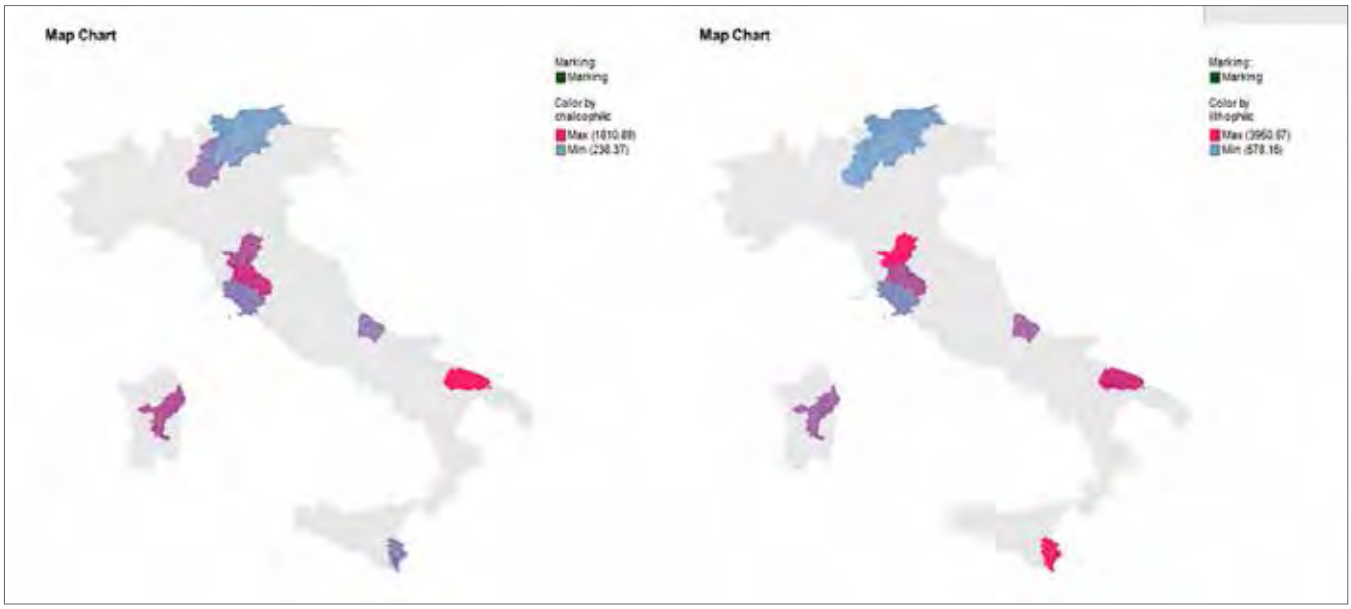

Figure 10. Map charts showing the distribution by region of the chalcophilic elements (Zn, Sb and As) on the left and lithophilic (Li, Ba, Cs, Sr etc.) elements on the right, with red for the highest intensity and blue as the lowest.

Conclusion

The ICP-MS analysis of a large number of wines from Italy provided results which were used for statistical analysis and geospatial mapping with TIBCO Spotfire software. Results indicated significant differences in elemental levels in many of the wines, some of which were linked to specific geographical regions. Further research will be needed to investigate whether these differences relate to soil and rainfall, or are correlated to viniculture production differences.

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APPLICATION BRIEF

Food/Nutraceutical

Microwave Digestion of Malt and Barley

Introduction

At PerkinElmer, we understand that sample preparation is one of the most critical steps in the analytical process. Often accounting for 60% of your analytical timetable, it has a fundamental impact on laboratory throughput and analytical performance. Any errors within the sample preparation process will undermine the quality of your data at all subsequent stages of your analysis. Great results begin with good preparation and our Titan MPS Microwave Sample Preparation System delivers the clean, clear solutions you need for reliable results.

The next page will provide you with the tools you need to quickly and efficiently develop digestion methods for your unique sample preparation needs.

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For the Better

The following describes a guideline method for microwave assisted digestion of the raw materials used in the production of beer or spirits.

To provide the greatest consistency and accuracy it is important that the sample for digestion be representative of the larger raw material batch. Homogenization via grinding or blending is recommended prior to weighing the sample.

Using a few mL of DI H_2O to transfer samples from a weigh-boat into the vessel, will not adversely affect the digestion.

All reagents used during sample digestion should be trace-metal grade or better to prevent baseline contamination.

Equipment

PerkinElmer Titan MPS

Standard 75 mL Digestion Vessel

Reagents

HNO_3 (70%) 10.0 mL

Procedure

Weigh 300 mg of the sample into the digestion vessel. Add 10.0 mL of HNO_3. Gently swirl the mixture and wait approximately 10 min before closing the vessel. This application uses the following temperature program.

Temperature Program

Step	Target Temp [°C]	Pressure Max [bar]	Ramp Time [min]	Hold Time [min]	Power [%]*
1	150	30	8	2	80
2	180	35	2	20	100
3	50	35	1	15	0
4	-	-	-	-	-
5	-	-	-	-	-

Notes: To avoid foaming and splashing wait until the vessels have cooled to room temperature (about 20 min). Carefully open the digestion vessel in a fume hood wearing hand, eye and body protection since a large amount of gas will be produced during the digestion process.

If the digestion is partially successful (the solution contains solids, is opaque or is dark and foamy), increase the hold time in step 2 by 10 minute increments until successful. If a hold time of 40 minutes in step 2 is not successful, raise the temperature in step 2 by increments of 10 °C until successful. Optionally, 2 mL of H_2O_2 can be added to the digestion reagents to aid digestion, which will increase the pressure of the digestion.

Results

Clear solution.

Summary

Malt or Barley is digested in an acid solution with a PerkinElmer Titan MPS.

Notes: This application serves only as a guideline and may need to be optimized for your sample.

**This application is designed for the digestion of 16 samples. Decrease the power at the first step by 5% per sample when using fewer than 16 samples Minimum power is 40% regardless of the number of samples digested.*

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APPLICATION BRIEF

Food/Nutraceutical

Microwave Digestion of Beer

Introduction

At PerkinElmer, we understand that sample preparation is one of the most critical steps in the analytical process. Often accounting for 60% of your analytical timetable, it has a fundamental impact on laboratory throughput and analytical performance. Any errors within the sample preparation process will undermine the quality of your data at all subsequent stages of your analysis. Great results begin with good preparation and our Titan MPS Microwave Sample Preparation System delivers the clean, clear solutions you need for reliable results.

The next page will provide you with the tools you need to quickly and efficiently develop digestion methods for your unique sample preparation needs.

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The following describes a guideline method for microwave assisted digestion of the in-process batch or final product of beer or spirits.

To provide the greatest consistency and accuracy it is important that the sample for digestion be representative of the larger raw material batch. Homogenization via blending is recommended prior to weighing the sample. Carbonated products should be allowed to stand open for 15 minutes and then de-gassed via sonication for an additional 10 minutes.

Using a few mL of DI H_2O to transfer samples from a weigh-boat or cup into the vessel, will not adversely affect the digestion.

All reagents used during sample digestion should be trace-metal grade or better to prevent baseline contamination.

Equipment

PerkinElmer Titan MPS

Standard 75 mL Digestion Vessel

Reagents

HNO_3 (70%) 10 mL

Procedure

Put 5 mL of the sample into the digestion vessel. Add 10 mL of HNO_3. Gently swirl the mixture and wait approximately 10 min before closing the vessel. This application uses the following temperature program.

Temperature Program

Step	Target Temp [°C]	Pressure Max [bar]	Ramp Time [min]	Hold Time [min]	Power [%]*
1	150	30	8	2	80
2	180	35	2	20	100
3	50	35	1	15	0
4	-	-	-	-	-
5	-	-	-	-	-

Notes: To avoid foaming and splashing wait until the vessels have cooled to room temperature (about 20 min). Carefully open the digestion vessel in a fume hood wearing hand, eye and body protection since a large amount of gas will be produced during the digestion process.

If the digestion is partially successful (the solution contains solids, is opaque or is dark and foamy), increase the hold time in step 2 by 10 minute increments until successful. If a hold time of 40 minutes in step 2 is not successful, raise the temperature in step 2 by increments of 10 °C until successful. Optionally, 2 mL of H_2O_2 can be added to the digestion reagents to aid digestion, which will increase the pressure of the digestion.

Results

Clear solution.

Summary

Beer and original wort is digested in an acid solution with a PerkinElmer Titan MPS.

Notes: This application serves only as a guideline and may need to be optimized for your sample.

**This application is designed for the digestion of 16 samples. Decrease the power at the first step by 5% for each sample less than 16. Minimum power is 40% regardless of the number of samples digested.*

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