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Beer Analysis Applications Notebook

Solutions for the Complete Brewing Process

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Beer Analysis

Complete Brewing Solutions

Beer is the most widely consumed alcoholic beverage in the world and the third most popular drink after water and tea. Beer is typically brewed from four basic ingredients: water, a starch source (e.g., malted barley) brewer's yeast, and a flavoring agent such as hops. Many varieties of beer result from differences in these ingredients, the additives used, and the brewing process.

Once a manufacturer has developed a popular product they need to ensure that they can reproduce it consistently, and in sufficient quantity, in compliance with any local & national regulations. It also needs to remain stable over the shelf-life of the beer.

Brewers have to ensure that raw ingredients are consistent from batch to batch and methodology is available to detect minute differences in the raw ingredients as it can affect taste, color and the shelf life of the product. Raw ingredient contamination can also result in a food safety issue. Results need to be available quickly so there are no unforeseen delays in the brewing process as these can be costly to the business. Rapid analysis methods are also required during the brewing process to ensure the process is going according to set standards. Failure to monitor various critical parameters during the brewing process can lead to product recalls, vast wastage of the product or down time to the brewing process. The final beer is tested to ensure it meets all quality and labelling criteria to comply with local and national regulations.

Thermo Scientific™ chromatography systems and photometric analyzers offer distinct benefits throughout the beer brewing analysis process. Using these systems and our highly sensitive and selective detectors, state-of-the-art column technologies, along with the proven analytical methods presented here, will help you to:

- Reduce analysis time without compromising resolution, retention, or reproducibility
- Monitor isomerization and decomposition progress during and after brewing to determine flavor and stability
- Maintain and preserve essential flavoring components such as bitterness and smoothness
- Use metabolomic approaches to characterize your beer sample, monitor the fermentation process and study product stability

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Automated Discrete Photometry

Fast and effective quality monitoring for breweries

Over 40 years experience in designing and manufacturing automated analyzers established the ability to measure wine and water and recently progressed to testing beer, wort and malt. Discrete photometric analyzers provide excellent analytical performance for colorimetric and enzymatic testing. Their automated operating systems allow laboratories to simultaneously measure multiple analytes while reducing total analysis time and increasing efficiency. Thermo Scientific™ Gallery™ Plus Beermaster, launched in 2011, is a new generation analyzer available in a bench-top compact system that is reliable and user-friendly. In addition to over 30 beer, wort and malt tests, the analyzer can measure water and several other parameters, like enzyme activity, measurements often required by manufactures. Gallery Plus Beermaster can analyze bitterness, SO_2, beta-glucan, free amino nitrogen, beer color, sugars, pH, acetaldehyde and acids quickly and accurately. This analysis requires no extra sample preparation with the exception of degassing for carbonated samples. With a maximum capacity of 350 photometric tests per hour, the analyzer is very straightforward to use requiring only minimal training to run routine analysis and provide maintenance. Use of this automated system increases efficiency in quality control, reduces costs, and improves productivity in breweries.

- Multiple colorimetric tests can be run simultaneously
- Low reagent volumes guarantee cost efficient testing
- Unique automated pretreatment and measurement for bitterness
- Fast and precise malt analysis of important brewing indicators
- Easy and eco-friendly automation for controlling the brewing process

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Learn More at www.thermoscientific.com/beermaster

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- Automated Bitterness Analysis
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- Beer Color Measurement

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Discrete Photometry for Beer Analysis

Automated Bitterness Analysis

Bitterness is an important quality parameter in beer, for taste, quality of foam, and stability. During the wort boil an isomerization reaction occurs that produces iso-alpha acids. The bitterness test used with the Gallery Plus Beermaster is based on binding iso-alfa acids onto the surface of a solid phase extraction column which is integrated into the photometric analyzer. Samples are first acidified, and then passed through the extraction column which binds bittering substances. The sample matrix is washed out and bittering substances are eluted from the column. Bitterness is measured at 275 nm, a single measurement takes about 7 minutes, and bitterness units are automatically calculated from absorbance results. This example summarizes bitterness analysis results for ten different beer samples. The method correlates fairly well with the iso-octane extraction method and has the added benefit of being more environmental friendly.

Measured			Thermo Scientific Method					Reference Method					
Analysis	**Sample**				**Mean**	**Std Dev**	**SE Mean**			**Mean**	**Std Dev**	**SE Mean**	***P-Value***
Bitterness (BU)	CB44	15.90	18.33	17.66	17.99	0.474	0.330	15.63	16.03	15.83	0.283	0.200	*0.114*
	BA		22.11	21.86	21.98	0.177	0.130	17.50	17.90	17.70	0.283	0.200	*0.035*
	BB		15.91	15.51	15.71	0.283	0.200	13.14	3.58	13.38	0.283	0.200	*0.014*
	BC		20.28	18.47	19.83	1.280	0.910	23.40	24.20	23.80	0.566	0.400	*0.140*
	BD		25.47	24.01	24.74	1.032	0.730	20.25	20.28	20.26	0.021	0.015	*0.103*
	BE		15.44	14.83	15.13	0.431	0.300	12.38	12.45	12.42	0.049	0.035	*0.072*
	BF		26.76	24.56	25.66	1.556	1.100	24.20	24.45	24.33	0.177	0.130	*0.441*
	BG		20.19	22.33	21.26	1.512	1.100	19.85	19.62	19.74	0.163	0.110	*0.391*
	BH		23.18	23.14	23.16	0.028	0.020	26.80	26.70	26.75	0.071	0.050	*0.010*
	BI		17.33	16.92	17.13	0.289	0.200	12.70	17.30	17.25	0.071	0.050	*0.660*
	BJ		26.14	26.01	26.08	0.092	0.065	22.25	22.30	22.28	0.035	0.025	*0.012*

Table 1. QC sample of a commercially available lager. The value is the mean of readings taken at reference method between February 2013 and July 2013.

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Download Poster Note: Automated Wort and Beer Quality Control Analysis

Discrete Photometry for Beer Analysis

Rapid Determination of Beta-glucan

Beta-glucans are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds present in the cell walls of cereals and are capable of clogging process filters. Excessive amounts of beta-glucan may cause haze in the end product and thus impair the taste of beer. For this reason it is important to determine the concentration of beta-glucan, in particular the portion of the beta-glucan polymer with a molecular size of about 10,000 Da or more. This rapid two reagent method was developed using a blank buffer to eliminate sample color interference. A photometric reaction was measured using a wavelength of 405 nm with a side wavelength of 600 nm and method linearity determined to range from 15-500 mg/L. The beer and wort samples tested showed excellent repeatability and reproducibility with a typical variation of 2 % or less. Analysis time for nine samples with ten replicates was less than 40 minutes. Results were compared against the Calcofluor method by flow injection analysis (FIA) using fluorescence dye as recommended by the European Brewing Commission (EBC) 8.13.2, 4.16.2, 3.10.21 and American Society of Brewing Chemists (ASBC) Wort-182s.

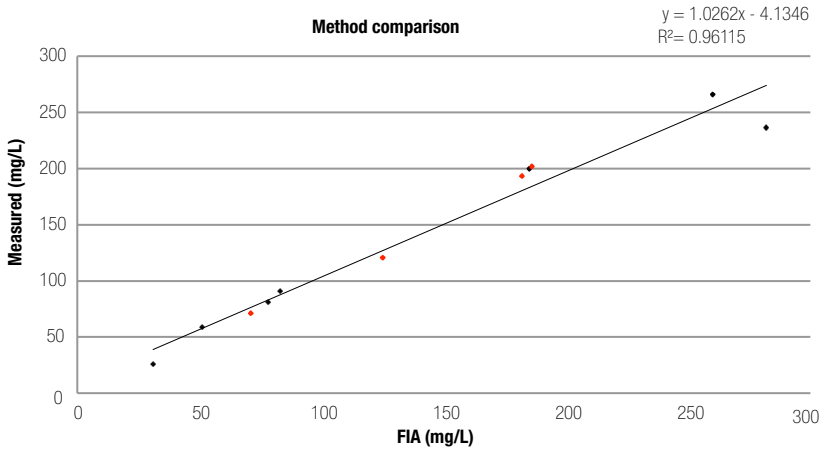

Figure 1. Method comparison showing wort samples (red) and beer samples (black).

Download Poster Note: Rapid Determination of High Molecular Weight 1,3/1,4-Beta-D-Glucan by a Novel Photometric Method

Discrete Photometry for Beer Analysis

Free Amino Nitrogen (FAN) Analysis using NOPA method

In the fermentation process that produces beer, yeast (usually Saccharomyces) converts sugars to ethanol and carbon dioxide. This yeast synthesizes the proteins required for healthy growth from amino acids, created by yeast from ammonia or by removal of the amino group from other alpha amino acids. The alpha amino acids available to the yeast in fermentation are known as FAN. The ninhydrin method measures the content of ammonia in addition to the FAN equaling the total assimilable nitrogen. A rapid 2-reagent NOPA (alpha-amino nitrogen by OPA) method was developed for the automated discrete analyzer using a blank buffer to eliminate sample color interference. Total analysis time for six samples and 60 test requests was approximately 45 minutes. The NOPA method showed excellent repeatability between 2.2 and 3.2 %. In this example the correlation between beer and wort samples is measured according to the EBC FAN protocol and the NOPA method.

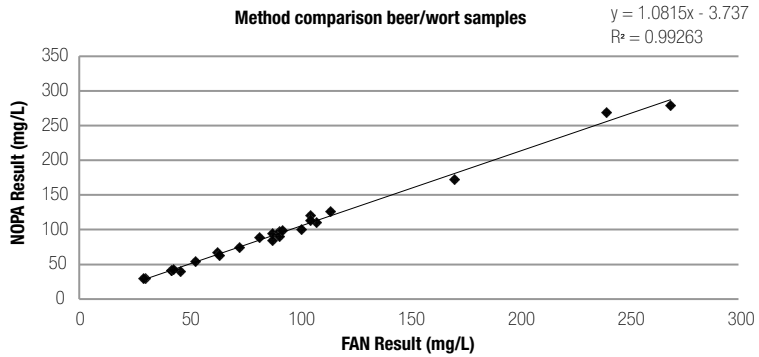

Figure 2. Method comparison. The two wort samples are the ones with highest NOPA concentration.

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Download Poster Note: Beer Reference Sample Correlation between Free Amino Nitrogen (FAN) and NOPA (Nitrogen by OPA)

Discrete Photometry for Beer Analysis

Automated Total SO_2 Method

Sulfur dioxide (SO_2) in beer originates primarily from yeast metabolism. SO_2 reacts with carbonyl compounds to form hydroxysulfonates which increase the flavor threshold of carbonyl compounds responsible for a stale flavor. SO_2 also plays an important role as an antioxidant and is known to provide antimicrobial properties at high concentrations. The SO_2 level is controlled at the end of beer production for the dual purposes of human health and beer quality. SO_2 in beer is typically measured by EBC Method 9.25.3 (or by the similar ASBC Beer-21) para-rosaniline method. In the example below the correlation of beer samples measured by the p-rosaniline method is compared to the total SO_2 method based on a DTNB (5,5´-dinitrobenzoic acid) measurement at 405 nm. This rapid 2-reagent method was done without sample pretreatment prior to analysis. The method is linear from 2 to 50 mg/L, shows good precision for all samples, and has a CV % of less than 1.5 % for most samples.

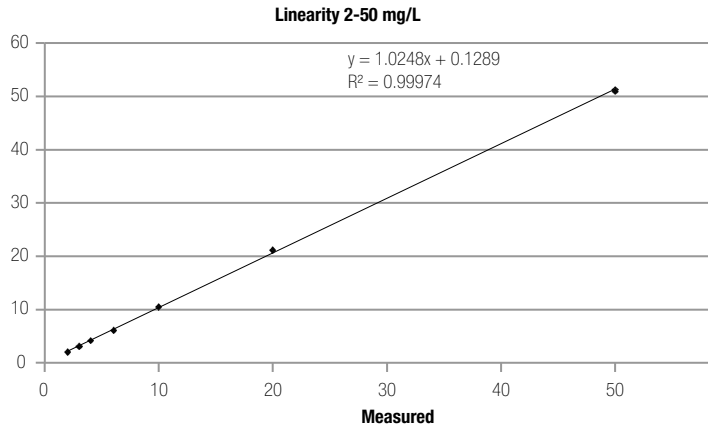

Figure 3. Method linearity.

Sample	SO_2 Total (mg/L)	Ref. value (mg/L)	Bias	CV% (n=10)
Beer 1	9.1	10	-0.9	1.4
Beer 2	4.8	5	-0.2	0.8
Beer 3	7.9	8	-0.1	0.9
Beer 4	3.6	2	1.6	2.5
Beer 5	2.4	2	0.4	10.2
Beer 6	2.8	2.3	0.5	3.4
Beer 7	2.9	1.9	1.0	3.5
Beer 8	5.3	4.4	0.9	1.4
Beer 9	3.5	2.7	0.8	1.1
Beer 10	5.1	3.8	1.3	1.3
Beer 11	1.2	0.8	0.4	1.4
Beer 12	5.4	5.7	-0.3	0.9
Cider 1	54.7	57	-2.3	3.3
Cider 2	76.3	80	-3.7	2.5

Table 2. Value correlation between the Gallery analyzer system reagent SO_2 Total and reference values measured by p-Rosaniline method.

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Download Poster Note: Correlation of Automated Total SO_2 DTNB Method to EBC/ASBC Para-rosalinine Methods

Discrete Photometry for Beer Analysis

Automated Total Polyphenols Method

Total polyphenols are analyzed to prevent haze formation, primarily resulting from protein-polyphenol interactions within the product. Polyphenols, along with proteins, are the two chemical entities which control the colloidal stability of beer. In this example, the EBC / MEBAK Total Polyphenol method was adapted for the automated discrete analyzer. Results are reported in mg/ L to the nearest whole number as a Gallic acid equivalent. The photometric method used correlates well with the EBC reference method. Recovery rates varied form from 94% to 106% and total precision (n=50) was 2.4 % or less for all samples. No significant run-to-run variation was noticed. Method linearity was determined in a range of 79 — 199 mg/L but can be extended with automated dilutions. No sample pretreatment was needed for the beer samples. For a Total polyphenol measurement the analysis turnaround time is approximately 10 minutes for a single request and 55 minutes for 100 requests with very little hands-on time.

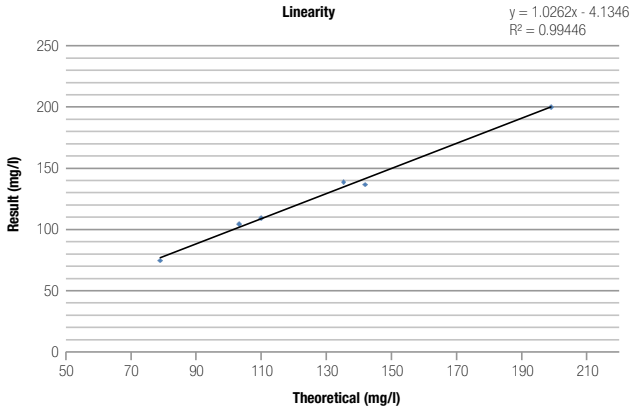

Figure 4. Linearity.

Sample	Assigned Value mg/L	Measured Value (mg/L)	Recovery %
Lager 1	199	198	99
Lager 2	75	74	94
Lager 3	110	108	98
Lager 4	135	136	101
Lager 5	103	101	98
Bitter 1	195	207	106
Bitter 2	142	133	94

Table 3. Method comparison.

Download Poster Note: Evaluation of a Gallery Total Polyphenol Method Performance in Beer (and Wort) using the EBC/MEBAK Protocol

Discrete Photometry for Beer Analysis

Beer Color Measurement

Beer color originates mainly from malt, but other factors can also have an effect. Naturally dark beers are usually brewed using a pale malt or lager malt base and a small proportion of darker malt is added to achieve the desired shade. Very dark beers, such as stout, use dark or patent malts that have been roasted longer. Most common beer color is pale amber (EBC unit ≤ 12). Other factors affecting beer color are increased pH, a Maillard reaction, the type of yeast selected, the use of filtration or the impact of oxidation. In addition, the fermentation process can deposit proteins in beer creating a change in color. In this example, beer (and wort) color measurement is performed with an automated discrete analyzer using a 430 nm filter. Results were compared to those measured with a manual spectrophotometer. The measuring range for the beer color test was determined as 5 – 50 EBC units without dilution and up to 200 EBC with automated pre-dilution. CV % of all measured samples was < 0.15 %.

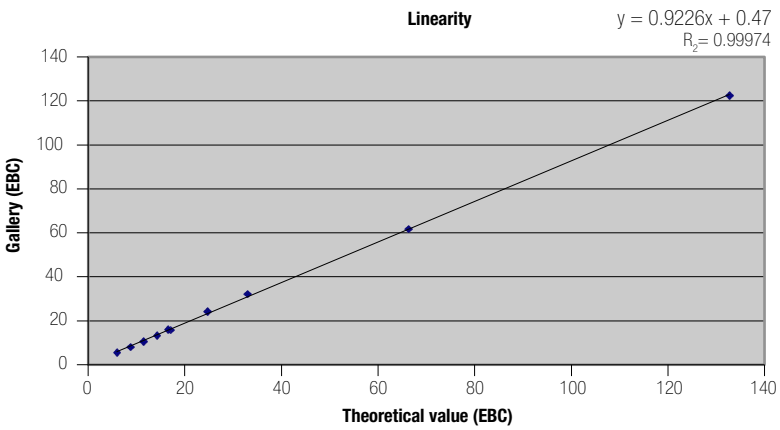

Figure 5. Method linearity.

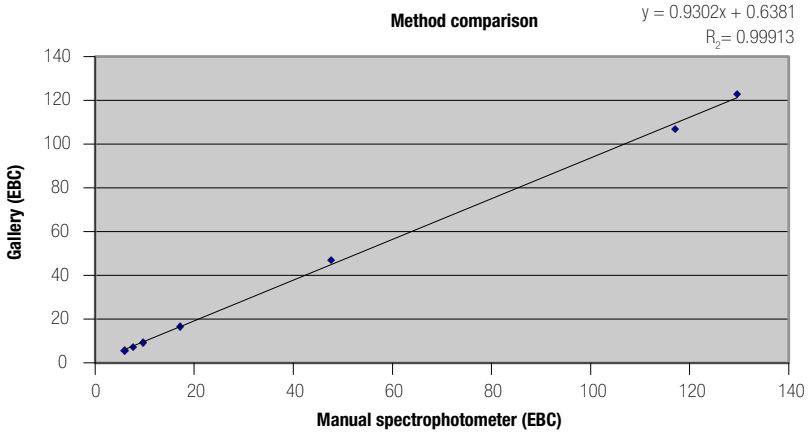

Figure 6. Method comparison.

Download Poster Note: Gallery Method Performance of Beer (and Wort) Color Measurement using the EBC protocol

Unmatched Performance, Reliability, and Value

Meet your stringent requirements for performance, reliability, and value with Thermo Scientific gas chromatograph (GC) and gas chromatography-mass spectrometer (GC-MS) systems. Combine powerful GC-MS instruments with productivity-enhancing software to create complete solutions to the most challenging analytical problems.

GC instruments from Thermo Scientific provide outstanding performance for routine analyses, advanced capabilities, and the flexibility to increase sample throughput. Instant connect injectors and detectors enable you to change modules in minutes to reconfigure the instrument for new workflows, develop new methods and eliminate maintenance downtime. Current developments in GC-MS triple quadrupole technology deliver high sensitivity and selectivity in the small molecule mass range and allow the detection of compounds at low concentrations, even in complex matrix samples. A simple and standard approach using electron impact ionization (EI) enables a very straightforward method for low-level analysis, such as that of dioxins.

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GC and GC-MS for Beer Analysis

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Gas Chromatography for Beer Analysis

Vicinal Diketones

Diketones, such as diacetyl (2,3-pentanedione) or 2,3-butanedione, are naturally occurring products in some alcoholic beverages and foods. They are important ingredients in beer aroma and are characterized by their buttery flavor. In lager beer they are considered off-flavors, hence the importance of carefully monitoring their content in these beverages. In 1999, the European Brewery Convention issued a method for the determination of 2,3-butanedione and 2,3-pentanedione in beers via headspace gas chromatography. One of the critical points of this method is the need to incubate the samples at 35 °C. Most sampling systems cannot achieve this low temperature without employing cryogenic systems. In this study, analysis is performed without a cryogenic system, using a Thermo Scientific™ TriPlus™ 300 Headspace Autosampler and a Thermo Scientific™ TRACE™ 1310 GC system, equipped with an Instant Connect Electron Capture Detector (ECD) module controlled by Chromeleon CDS software.

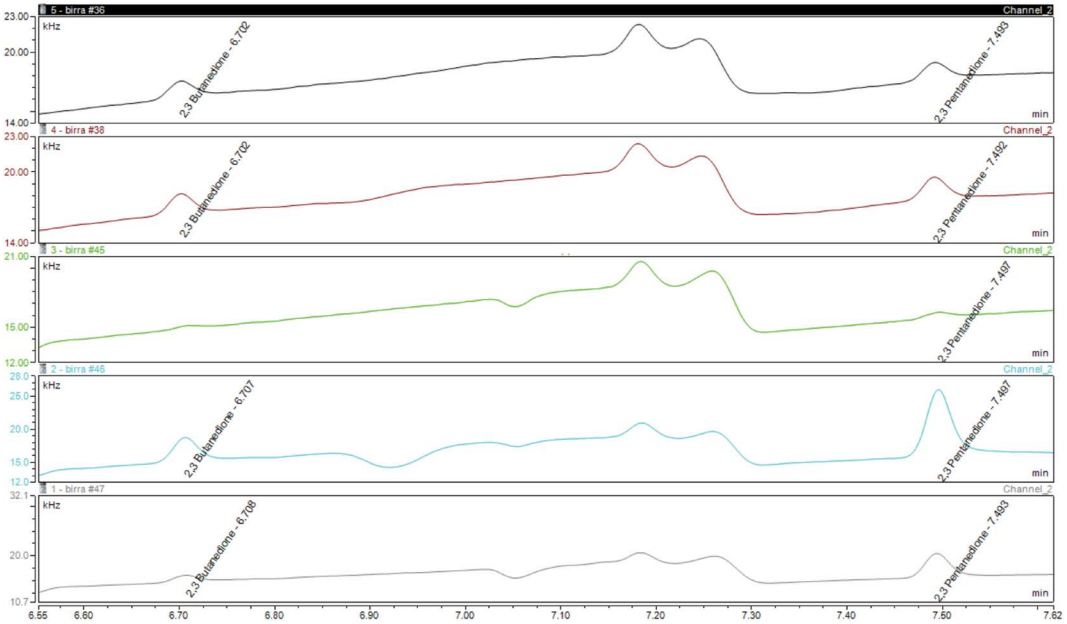

Figure 7. Chromatograms of different brands of beer.

Recommended Conditions	
TriPlus 300 Headspace Autosampler	
Oven Temperature:	35 °C
Manifold Temperature:	40 °C
Transfer Line Temperature:	60 °C
Equilibration time:	40 min, shaking on high
Pressurization Mode:	Pressure, 1 bar
Pressure Equilibration Time:	0.3 min
Loop Filling Mode:	Pressure, 0.5 bar, equilibration time, 0.2 min
Loop Size:	1 mL
Injection Mode:	Standard, injection time, 0.2 min
Purge:	After injection for 1 min at 100 mL/min
Vial Venting:	On
TRACE 1310 GC	
Liner:	Dedicated headspace liner (P/N 453A1335)
Carrier Gas:	Helium, constant flow, 3 mL/min
Column Type:	TR-WAX 60 m x 0.25 mm x 0. 5µm (P/N 260W235P)
Column Oven:	Initial 60 °C, hold 1 min. Ramp at 15 °C/min up to 150 °C. Hold 2 min
Instant Connect SSL Injector	
Inlet temperature:	160 °C
Mode:	Split flow, 60 mL/min, split ratio, 20:1.
Instant Connect ECD	
Temperature:	180 °C
Makeup Gas:	Nitrogen, 15 mL/min

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Download Application Note 10418: Vincinal Diketones in Beer Using Valve-and-Loop Headspace Analysis

Gas Chromatography-Mass Spectrometry for Beer Analysis

Analysis of Nitrosamines

Nitrosamines, also called N-nitrosodiaklyamines, comprise a class of compounds with a variety of alkyl moieties. These compounds are toxic and highly carcinogenic in humans and animals, with higher doses leading to severe liver damage and internal bleeding. Nitrosamines are produced in beer brewing operations during the process of drying germinated malt. Today's GC-MS triple quadrupole technology delivers the sensitivity and selectivity to detect nitrosamines at very low concentrations, even in complex matrix samples like beer. This example describes a turnkey GC-MS/MS method for routine detection and quantitation of food-borne nitrosamine compounds.

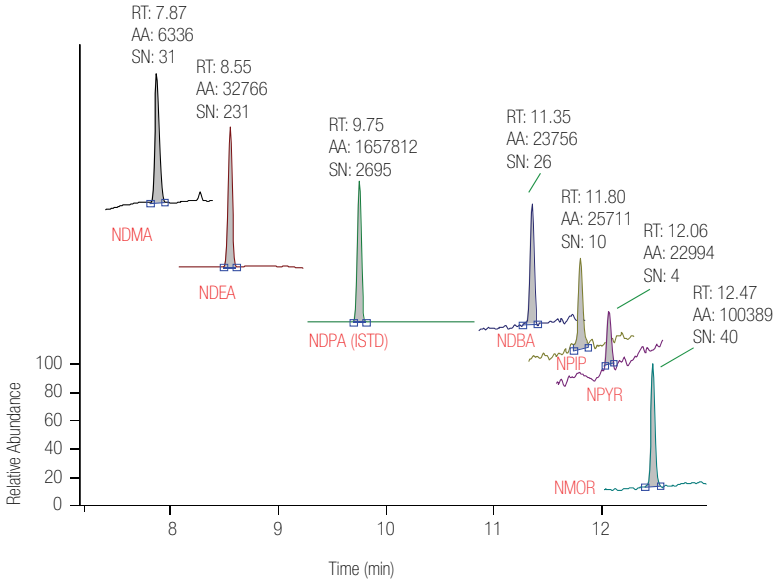

Figure 8. Chromatogram of nitrosamine compound standard solution at 1 ppb.

Experimental Conditions GC-MS/MS Instrument

TRACE 1310 GC	
iC Injector Module	Split/Splitless Injector
Injector Temperature	250 °C
Injection Mode	Splitless
Surge Mode	300 KPa
Splitless Time	1.0 min
Analytical Column	TG-WAX MS, 30m×0.25mm×0.5µm
Carrier Gas	He (99.999% purity)
Flow Rate	1.0 mL/min, constant flow
Oven Program	45 °C for 3 min, 25 °C/min to 130 °C, 12 °C/min to 230 °C, 1 min hold
Transfer Line Temperature	250 °C
Total Analysis Time	14.7 min
Total Cycle Time	18.4 min
TriPlus RSH Autosampler	
Injection Volume	1 µL
Solvent	Dichloromethane
Standard Runs	3 replicate of injections each
Dilution of Standard Mix	1 ppb, 5 ppb, 10 ppb, 25 ppb, 100 ppb, 250 ppb, 500ppb
Internal Standard	NDPA added to each calibration level at 50 ppb
Thermo Scientific™ TSQ™ 8000 Triple Quadrupole GC-MS/MS system	
Ionization Mode	EI
Mass Resolution Setting	Normal
Source Temperature	220 °C
Scan Mode	MRM, retention time-based SRM mode

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Download Application Note 10315: High Sensitivity Analysis of Nitrosamines Using GC-MS/MS

Innovative Ion Chromatography Solutions

Thermo Scientific™ Dionex™ IC systems have led the analytical instrument industry for over 30 years with solutions that represent state-of-the-art technological advancements and patented technologies.

Our High-Pressure™ Ion Chromatography (HPIC™) systems include the Thermo Scientific Dionex ICS-5000+ HPIC system, which is optimized for flexibility, modularity, and ease-of-use, combining the highest chromatographic resolution with convenience. In addition, the Thermo Scientific Dionex ICS-4000 Capillary HPIC system is the world's first commercially available dedicated capillary high-pressure Reagent-Free™ (RFIC™) IC system. The Dionex ICS-4000 system is always ready for the next analysis, delivering high-pressure IC on demand.

Reagent-Free IC systems eliminate daily tasks of eluent and regenerant preparation in turn saving time, preventing errors, and increasing convenience. RFIC-EG systems use electrolytic technologies to generate eluent on demand from deionized water, and to suppress the eluent back to pure water to deliver unmatched sensitivity. RFIC-ER systems are designed to use carbonate, carbonate/ bicarbonate, or MSA eluents for isocratic separations.

At the heart of our ion chromatography portfolio is a unique set of column chemistries that provide high selectivities and efficiencies with excellent peak shape and resolution. Thermo Scientific™ Dionex™ IonPac™ chromatography columns address a variety of chromatographic separation modes including ion-exchange, ion-exclusion, reversed-phase ion pairing, and ion suppression. Our column chemistries are designed to solve specific application challenges, and we offer a variety of selectivities and capacities for simple and complex samples. Additionally, our Dionex IonPac column line is available in standard bore, microbore, and capillary formats for the ultimate application flexibility. We also offer the Thermo Scientific™ Dionex™ CarboPac™ column family, which provides high-resolution separations of a variety of carbohydrates including glycoproteins, oligosaccharides, and complex carbohydrates from dietary fiber.

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Ion Chromatography for Beer Analysis

- Carbohydrates, Alcohols, Organic Acids, Inorganic Anions, and Cations
- Determination of Organic Acids
- Biogenic Amines in Alcoholic Beverages
- Water Quality and Characteristics

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Carbohydrates, Alcohols, Organic Acids, Inorganic Anions, and Cations

Ion chromatography is an efficient technique for the quantification of ions in solution. The compounds of interest for the beer industry range from inorganic ions, organic acids, and hop bittering principles that contribute to the overall taste and bitterness of the beverage to proteins, carbohydrates, and alcohols that are monitored to determine the extent of fermentation. The finished beer product may be analyzed to determine the concentration of added preservatives and colorants, in addition to ensuring manufacturing authenticity. The examples below show the separation of malto-oligosaccharides in beer (Figure 9) and the separation of fermentable sugars in wort (Figure 10) by ion exchange chromatography. Ion-exchange or ion-exclusion chromatography can be used for the determination of many classes of compounds of interest to the brewing industry, including carbohydrates, alcohols, organic acids, inorganic anions, and inorganic cations.

Conditions for Figure 10

Column:	Dionex CarboPac PA1			
Eluent 1:	Deionized water			
Eluent 2:	500 mM Sodium hydroxide			
Gradient:	**Time**	**E1**	**E2**	**Comments**
	Initial	99	1	Reequilibrate
	5.00	99	1	Inject
	6.00	99	1	Back to Load
	20.00	91	9	
	45.00	0	100	
	50.00	0	100	
Flow Rate:	1.0 mL/min			
Inj. Volume:	10 µL			
Detection:	Pulsed amperometry, gold electrode			

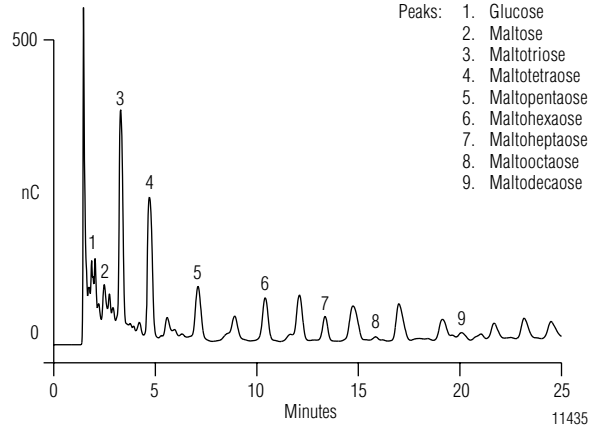

Figure 9. Separation of malto-oligosaccharides in an American beer by ion-exchange chromatography with pulsed amperometric detection.

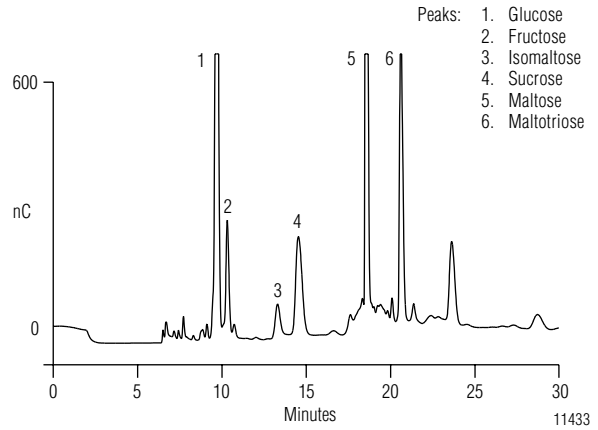

Figure 10. Separation of fermentable sugars in wort by ion-exchange chromatography with pulsed amperometric detection.

Ion Chromatography for Beer Analysis

Determination of Organic Acids

Organic acids are end products of yeast fermentation critical to the flavor of beer, but are also products of bacterial fermentation that introduce a sour flavor, either purposely or unintentionally due to spoilage. The 4 µm resin particle Dionex IonPac AS11-HC-4 µm anion-exchange column is a high resolution, high-capacity column optimized for organic acids in complex matrices, ideal for analysis of beer samples. At standard flow rates, this 4 µm resin particle column operates above 3000 psi, which necessitates the use of a high-pressure-capable system such as the Dionex ICS-5000⁺ HPIC system. if the advantages of eluent generation are to be realized. In the example below, three U.S. domestic lager beers were analyzed on a Dionex HPIC system, using a 4 µm particle-size column.

Column:	Dionex IonPac AS11-HC-4µm with guard, 4 × 250 mm
Eluent Source:	Dionex EGC 500 KOH cartridge
Gradient:	1 mM KOH (-5–8 min), 1–15 mM KOH (8–18 min) 15–30 mM KOH (18–28 min), 30–60 mM KOH (28–38 min), 60 mM KOH (38–45 min)
Flow Rate:	1.5 mL/min
Injection Volume:	10 µL
Temperature:	30 °C
Detection:	Thermo Scientific™ Dionex™ ASRS™ 300 suppressor, 4mm, AutoSuppression, recycle mode
Beer Samples:	A: Lager, B: Lager 2, C: Light Lager
Sample Prep:	Degas, 5-fold dilution

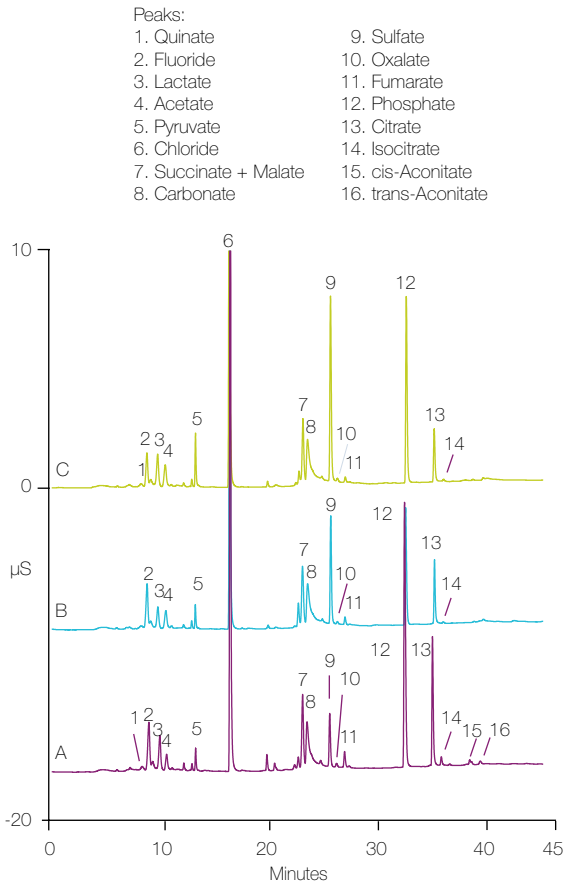

Figure 11. Analysis of beer samples.

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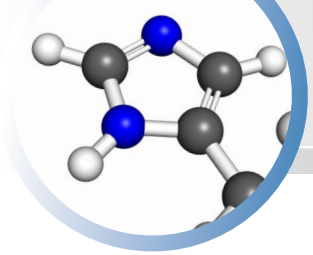
Download Technical Note 126: Determination of Organic Acids in Beer Samples Using an HPIC System



Biogenic Amines in Alcoholic Beverages

Biogenic amines are common in plants and animals, where they have important metabolic and physiological roles. In foods and beverages, biogenic amines can be formed by the decarboxylation of amino acids from microbial activity. Their presence in food is not only important from a toxicological view, but can also be used as an indicator of spoilage. The consumption of an excess amount of these amines, can induce severe toxicological effects and produce various physiological symptoms, such as nausea, respiratory distress, headache, sweating, heart palpitations, and hyper- or hypotension. Putrescine, agmatine, spermidine, and spermine are considered natural beer constituents that primarily originate from malt. The presence of tyramine, cadaverine, and histamine, however has been associated with the activities of contaminating lactic acid bacteria during the brewing process. The described method demonstrates the use of the Dionex IonPac CS18 column for the separation of several target biogenic amines in alcoholic beverages. The combination of three detection configurations, suppressed conductivity detection, integrated pulsed amperometric detection, and UV detection, demonstrates the versatility and potential of cation-exchange chromatography for determining hydrophobic amines in complex matrices.

Column:	Dionex IonPac CG18, CS18, 2 mm
Eluent:	Methanesulfonic acid: 3 mM from 0–6 min, 3–10 mM from 6–10 min,10–15 mM from 10–22 min, 15 mM from 22–28 min, 15–30 mM from 28–35 min, 30–45 mM from 35.1–42 min
Eluent Source:	EGC II MSA
Temperature:	40 °C
Flow Rate:	0.30 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex CSRS ULTRA II, 2 mm, AutoSuppression external water mode

Peaks:		
	1. Putrescine	6.6 mg/L (ppm)
	2. Cadaverine	0.67
	3. Histamine	0.60
	4. Agmatine	7.70
	5. Spermidine	1.2
	6. Spermine	0.73

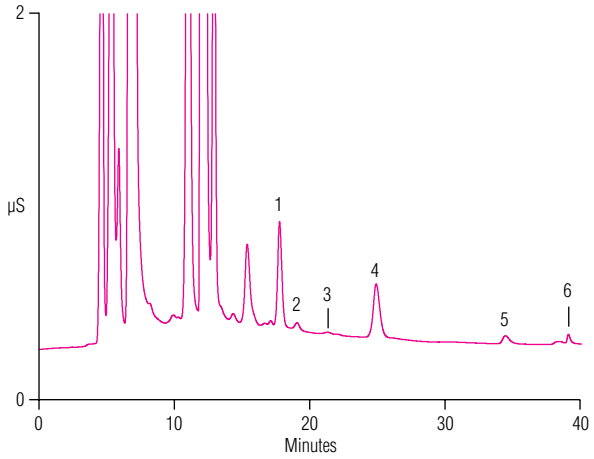


Figure 12. Determination of biogenic amines in wheat beer using suppressed conductivity detection.

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Download Application Note 182: Determination of Biogenic Amines in Alcoholic Beverages by Ion Chromatography with Suppressed Conductivity Detection and Integrated Pulsed Amperometric Detection

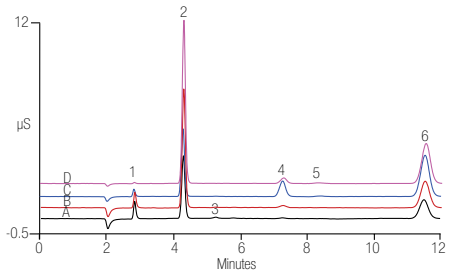
Ion Chromatography for Beer Analysis

Water Quality and Characteristics

Water is a major component in beer and beer production. Therefore water characteristics and quality are important to beer flavor and processing. It is well known that the flavor of many famous beers have been influenced by the regional water. For example, the stout beer, Guinness, originated in a hard water location (Dublin), whereas Pilsner Urquell originated from a soft water location, Pilsen. Burton, England has water with high gypsum (calcium sulfate mineral) which is now associated with the pale ale flavor. Soft water regions desiring the same pale ale flavor add gypsum to simulate the Burton water, known as Burtonisation. Additionally, salt content can affect the yeast, wort, pH, and precipitation. Water quality and the subsequent effect on beer processing and quality are assured by water testing. Here we show examples of water quality testing performed by municipalities for regulatory reasons. However, these requirements are the same as those needed to maintain optimum brewery operations and beer quality.

Column: Dionex IonPac AG22, 4 × 50 mm,
Dionex IonPac AS22, 4 × 250 mm
Eluent: 4.5 mM Sodium carbonate/1.4 mM Sodium bicarbonate
Flow Rate: 1.2 mL/min
Injection: 5 µL
Detection: Suppressed conductivity, Dionex AERS 500 suppressor, 31 mA, recycle mode
Samples: A: City of Berkeley, CA
B: City of Sunnyvale, CA
C: City of Palm Desert, CA
D: City of San Jose, CA

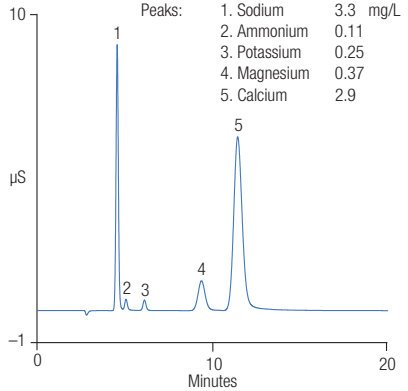
Peaks:	A	B	C	D	
1. Fluoride	0.84	0.79	0.61	0.20	mg/L
2. Chloride	5.9	11.6	10.6	37.7	
3. Nitrite	0.60	—	—		
4. Nitrate	—	0.73	9.1	5.05	
5. Carbonate	—	—	—	—	
6. Sulfate	6.7	11.5	25.1	37.3	



Caption: Figure 13. Municipal drinking water samples.

Column: Dionex IonPac CG12A, CS12A, Capillary, 0.4 mm
Eluent: MSA (RFIC-EG)
Gradient: 6–65 mM from 0 to 30 min
Flow Rate: 10 µL/min
Inj. Volume: 0.4 µL
Col. Temp.: 40 °C
Detection: Suppressed conductivity, Thermo Scientific Dionex CCES™ Cation Capillary Electrolytic Suppressor
Sample: Municipal drinking water undiluted

Peaks:		
1. Sodium	3.3	mg/L
2. Ammonium	0.11	
3. Potassium	0.25	
4. Magnesium	0.37	
5. Calcium	2.9	



Caption: Figure 14. Cations in municipal drinking water on the Dionex IonPac CS12A capillary column.

Download Application Update 196: Anion Determinations in Municipal Drinking Water Samples Using EPA Method 300.1 (A) on an Integrated IC System

Download Application Brief 133: Cost-Effective Determination of Inorganic Anions and Cations in Municipal Drinking Water Using Capillary Ion Chromatography

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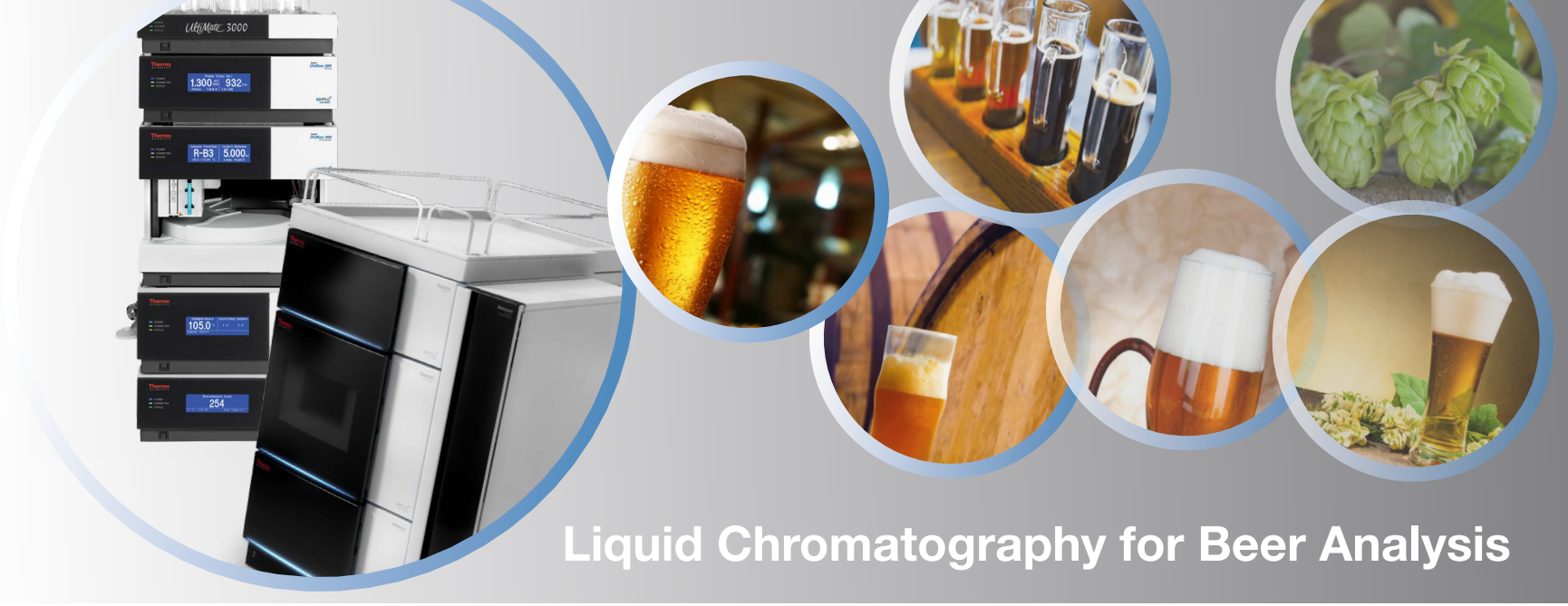

Liquid Chromatography for Beer Analysis

- Targeted Analysis of Polyphenols
- Targeted Analysis of Bitter Acids
- Metabolomic Analysis of Beer
- Ultrafast Analysis of Isohumulones
- Isohumulones, Humulones, and Lupulones
- Analysis of Isohumulones and Reduced Isohumulones

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Liquid Chromatography for Beer Analysis

Targeted Analysis of Polyphenols

Beer contains a complex mixture of phenolic compounds extracted from the starch source and hops. The hop-derived xanthohumol and the iso-alpha acids formed are primarily responsible for the perceived bitterness. Many of these secondary metabolites are not only purported to offer health benefits but are essential to the flavor and stability of the beer itself. Conversely, some secondary metabolites contribute to the degradation of beer during storage with the formation of haze (e.g., catechins and their polymers the proanthocyanidins). In the chromatograms below, two beer samples with differing amounts of hops were measured for a targeted analysis of polyphenols. This example presents multi-channel electrochemical array chromatograms for two different beer samples. Electrochemical array detection reveals the presence of many more polyphenols in a high hops beer (Figure 15A) compared to a regular domestic beer (15B).

Pump:	Thermo Scientific Dionex UltiMate LPG-3400BM with SR-3000 solvent rack
Autosampler:	Thermo Scientific Dionex UltiMate WPS-3000TBSL
UV Detector:	Thermo Scientific Dionex UltiMate DAD-3000RS diode-array detector
	Channel 1: 218 nm Channel 2: 240 nm
	Channel 3: 254 nm Channel 4: 275 nm
EC Detector:	Thermo Scientific™ Dionex™ CoulArray™ detector with thermal organizer
EC Parameters:	16 channel array from 0 to +900 mV in +60 mV increments
Column:	Thermo Scientific™ Acclaim™ 120, C18 (3.0 × 150 mm, 3 µm particle size)
Flow Rate:	0.65 mL/min
Injection Volume:	10 or 20 µL
Mobile Phase A:	20 mM monobasic sodium phosphate, 3% acetonitrile, 0.2% tetrahydrofuran, pH 3.35
Mobile Phase B:	20 mM monobasic sodium phosphate, 50% acetonitrile, 10% tetrahydrofuran, pH 3.45
Mobile Phase C:	90% methanol
Gradient:	0-2 min: 2%B/3%C, 30 min: 97%B/3%C, Curve 7 (concave), 45 min: 97%B/3%C

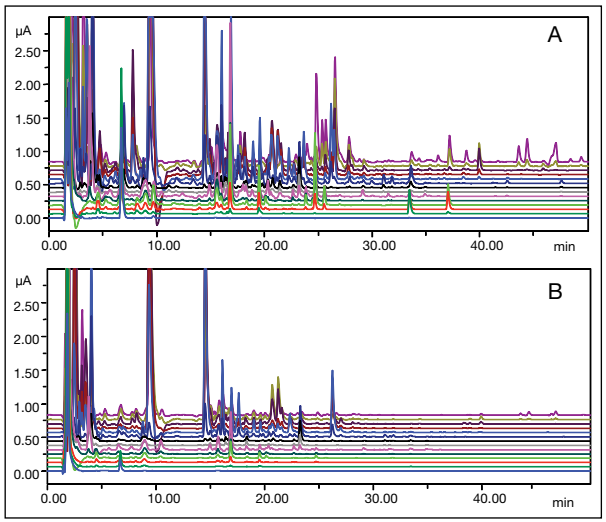

Figure 15. Polyphenol method chromatograms of (A) high-hops beer and B) regular domestic beer.

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Download Application Note 1020: Chalconoids and Bitter Acids in Beer by HPLC with UV and Electrochemical Detection

Targeted Analysis of Bitter Acids

Hops contain a number of important phytochemicals including xanthohumol (a prenylated chalconoid), and alpha- and beta- acids. As part of the beer brewing process, hops or hop extracts are added during the boiling of the wort. The α-acids (humulone, cohumulone and adhumulone) are slowly isomerized into the more soluble iso-acids, the main bittering substances in beer. Unfortunately, α-acids can react with riboflavin and light to produce compounds that give beer an off or skunky taste and smell. β–acids (lupulone, colupulone and adlupulone) do not isomerize during boiling and do not impart bitterness initially. However, during fermentation and storage, -acids slowly create bitterness through oxidation, affecting the long-term character of aged beers. In the example below, a bitter acids standard mixture and an Ultra IPA beer sample were analyzed for targeted analysis of bitter acids.

Column:	Acclaim 120, C18 (3.0 × 150 mm, 3 µm particle size)
Temperature:	35 °C
Flow Rate:	0.65 mL/min
Injection Volume:	20 µL
Mobile Phase A:	25 mM sodium perchlorate, 50% acetonitrile, 2.5 mM perchloric acid
Mobile Phase B:	25 mM sodium perchlorate, 90% acetonitrile, 2.5 mM perchloric acid
Mobile Phase C:	90% methanol
Gradient:	0-3 min: 0%B/3%C, 30 min: 40%B/3%C, 40 min: 97%B/3%C, 45 min: 97%B/3%C
EC Parameters:	Thermo Scientific Dionex UltiMate model 5011A dual channel coulometric electrochemical cell
E1 (blue trace):	+550 mV
E2 (green trace):	+850 mV

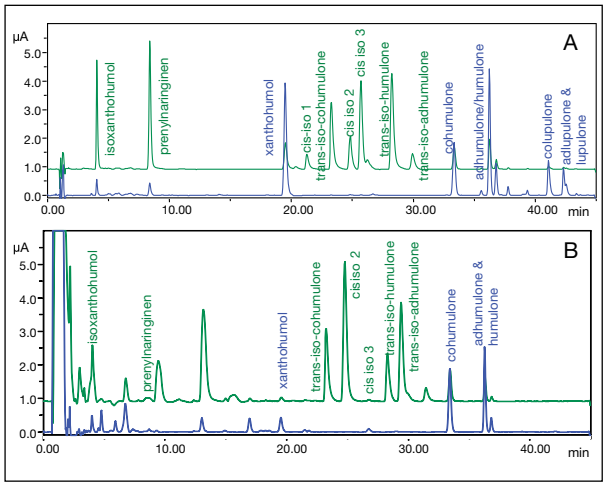

Figure 16. Chromatograms of (A) bitter acids standard mixture; (B) Ultra IPA beer sample.

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Download Application Note 1020: Chalconoids and Bitter Acids in Beer by HPLC with UV and Electrochemical Detection

Metabolomic Analysis of Beer

Untargeted (metabolomic approaches) can be used to differentiate beer samples, study the brewing process, monitor product stability, identify possible adulteration and to authenticate the sample. In this example, a simple metabolomics experiment was conducted to evaluate whether a spectro-electro array platform could be used to differentiate between different beer types, including: matched regular and light American beers, a variety of American microbrews, a European beer from Belgium, an Irish stout, and an American high-hops ultra IPA. Metabolomic profiles containing several hundred analytes—including both known and unknown compounds—were measured in each sample. Principal component analysis (PCA) was then used to differentiate samples for both EC data (Figure 17, Plot A) and UV data (Figure 17, Plot B).

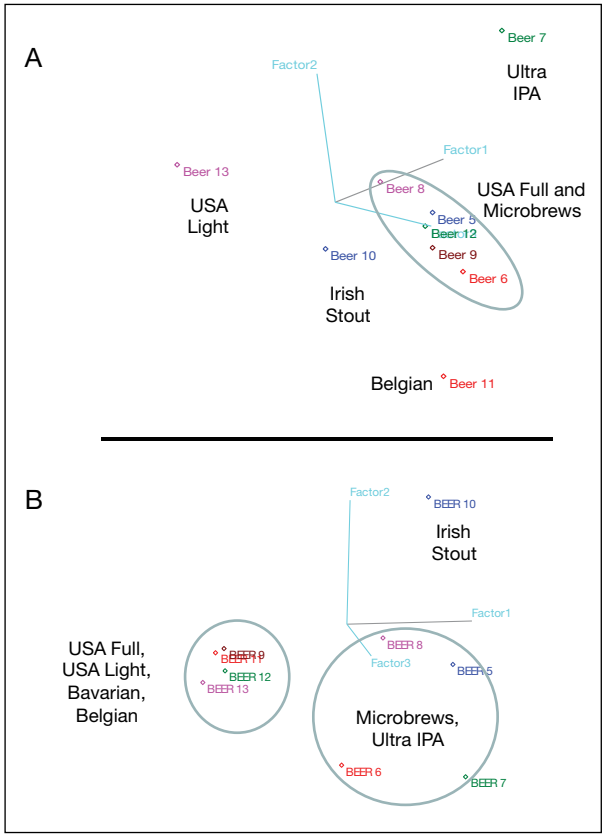

Figure 17. Principal component plots for (A) EC and (B) UV data (USA = American).

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Download Application Note 1065: Gradient HPLC Method for Analysis of Beer Polyphenols, Proanthocyanidins, and Bitter Acids Using a Novel Spectro-Electro Array Platform

Ultrafast Analysis of Isohumulones

Isohumulones (iso-α-acids) account for approximately 80 percent of the typical bitterness of beer. Their antimicrobial effect leads to a sterile beverage, their tensioactive character stabilizes the foam, and they have a major influence on the general flavor, aroma, and smoothness of beer. The three major iso-α-acid variants present in beer differ only in their acyl side chains and comprise iso-n-humulone, iso-cohumulone, and iso-adhumulone. Due to the stereochemistry of iso-α-acids, all of them occur as cis- and trans-isomers. The application shown here provides specific determination and quantitation of each cis- and trans-isomer of the iso-α-acids within nine minutes, including automated beer sample cleanup.

System:	UltiMate 3000 System with On-Line SPE RS configuration
Mobile Phase A:	Water with 1% formic acid and 100 mg/L ethylenediaminetetraacetic acid disodium salt dihydrate
Mobile Phase B:	Acetonitrile
Pressure:	720 bar (max.)
Temperature:	35 °C
Injection Volume:	5 µL beer or 5 µL isohumulone standard
Analytical Flow Path Parameters	
Column:	Thermo Scientific™ Hypersil GOLD™ column, 1.9 µm, 100 × 2.1 mm
Isocratic:	50% B
Flow Rate:	650 µL/min
Detection:	UltiMate VWD-3400RS Variable Wavelength Detector, 2.5 µL flow cell, 270 nm
Automated On-Line SPE Parameters	
Column:	Hypersil GOLD C8 column, 5 µm, 20 × 2.1 mm
Gradient:	0-2 min 25% B at 2000 µL/min, 2-4 min 100% B at 2000 µL/min, 4-7 min 25% B at 200 µL/min, 7-9 min 25% B at 2000 µL/min
Valve Position:	0 min 6_1, 1.5 min 1_2, 2 min 6_1

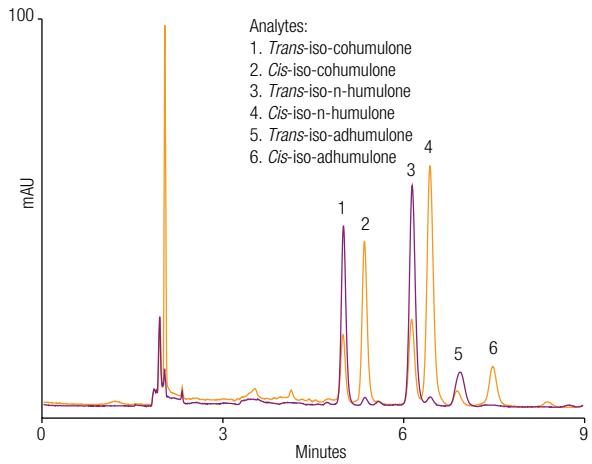


Figure 18. Chromatograms of isohumulone in beer and isohumulones standard.

Isohumulones, Humulones, and Lupulones

Monitoring the isomerization progress as well as the general content of hop α-acids into iso-α-acids in beer during and after the brewing process is mandatory in order to control important beer properties: each iso-α-acid variant provides different contributions to beer bitterness, taste, and foam stability— and they exhibit different lifetimes. Because the degradation products of iso-α-acids also influence the beer attributes mentioned above, the avoidance of hop types containing higher amounts of the less stable α-acid variants is beneficial in terms of the stability of the beer quality characteristics. The chromatogram below shows the separation of hop bitter acids and iso-α-acids in less than 10 minutes, including automated beer sample cleanup.

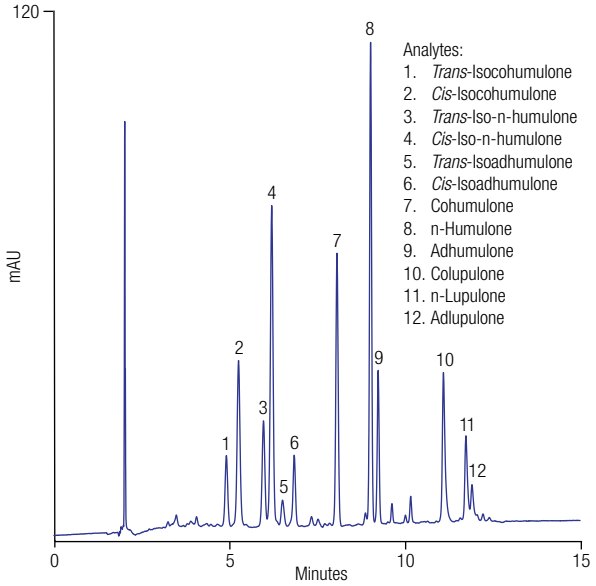


Figure 19. Chromatogram of hop bitter acids and iso-α-acids in beer.

System:	UltiMate 3000 System with On-Line SPE RS configuration
Mobile Phase:	A. Water with 1% formic acid and 100 mg/L ethylenediaminetetraacetic acid disodium salt dihydrate B. Acetonitrile
Pressure:	750 bar (max.)
Temperature:	35 °C
Injection Volume:	15 µL
Analytical Flow Path Parameters	
Column:	Hypersil GOLD, 1.9 µm, 100 × 2.1 mm
Gradient:	0–4 min 50% B, 4–6 min 50–60% B, 6–7 min 60% B; 7–8 min 70% B, 8–11.5 min 70–80% B; 11.5–15 min 50% B
Flow Rate:	650 µL/min
Detection:	VWD-3400RS, 2.5 µL flow cell, 270 nm
Automated On-Line SPE Parameters	
Column:	Hypersil Gold C8, 5 µm, 20 × 2.1 mm
Gradient:	0–2.5 min 25% B at 2000 µL/min, 2.5-5 min 100% B at 2000 µL/min, 5–14 min 25% B at 200 µL/min, 14–15 min 25% B at 2000 µL/min
Valve Position:	0 min 6_1, 1.5 min 1_2, 2 min 6_1

Analysis of Isohumulones and Reduced Isohumulones

Isohumulones decompose to 3-methyl-2-butene-1-thiol (MBT) when exposed to sunlight. MBT is detectable by odor and taste as a skunky off-flavor of light-struck beer at the extremely low threshold of 0.05 ppb. Chemically reduced isohumulones do not decompose with sunlight and are, therefore, often used for beers filled into clear or green bottles. Nevertheless, the German purity law (Reinheitsgebot) does not allow synthetically modified ingredients in beer. The chromatogram below shows the separation of iso-α-acids and tetrahydro-iso-α-acids in less than 10 minutes, including automated sample cleanup.

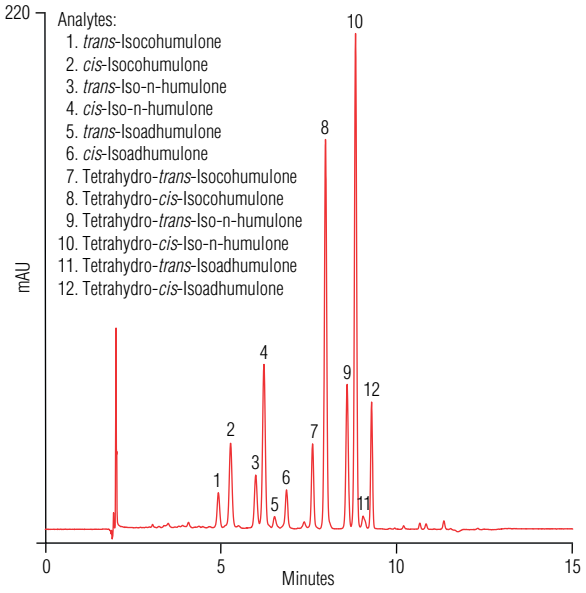


Figure 20. Chromatogram of iso-α-acids and tetrahydro-iso-α-acids in beer.

System:	UltiMate 3000 System with On-Line SPE RS configuration
Mobile Phase:	A. Water with 1% formic acid and 100 mg/L ethylenediaminetetraacetic acid disodium salt dihydrate B. Acetonitrile
Pressure:	750 bar (max.)
Temperature:	35 °C
Injection Volume:	15 µL
Analytical Flow Path Parameters	
Column:	Hypersil GOLD, 1.9 µm, 100 × 2.1 mm
Gradient:	0–4 min 50% B, 4–6 min 50–60% B, 6–7 min 60% B; 7–8 min 70% B, 8–11.5 min 70–80% B; 11.5–15 min 50% B
Flow Rate:	650 µL/min
Detection:	VWD-3400RS, 2.5 µL flow cell, 270 nm
Automated On-Line SPE Parameters	
Column:	Hypersil Gold C8, 5 µm, 20 × 2.1 mm
Gradient:	0–2.5 min 25% B at 2000 µL/min, 2.5-5 min 100% B at 2000 µL/min, 5–14 min 25% B at 200 µL/min, 14–15 min 25% B at 2000 µL/min
Valve Position:	0 min 6_1, 1.5 min 1_2, 2 min 6_1

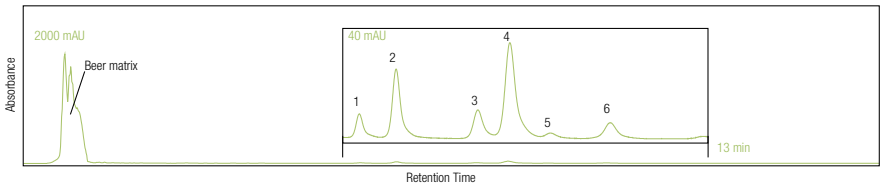
Complete Solutions for Isohumulones in Beer

In general, the content of isohumulones (or iso-α-acids) in beer is expressed as a value of bitterness units (BU). This value, a numerical sum parameter derived from a nonspecific, empirical, and spectrophotometric method, provides no information about the individual contents of each iso-α-acid variant. Furthermore, non-bitter components are also detected and adulterate that BU value. Hence, only the measurement of the pure concentration of iso-α-acids offers precise and comparable information about genuine beer bitterness.

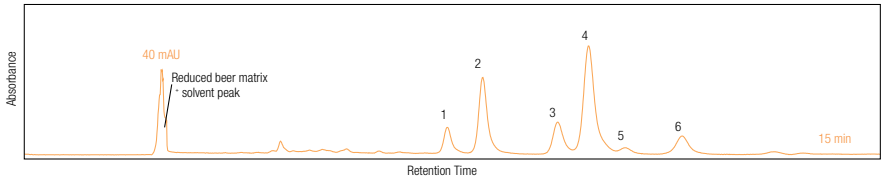
High performance liquid chromatography (HPLC) is the only analytical method that enables specific quantitation of iso-α-acids in beer. Thermo Scientific BeerNHops Solutions for isohumulones in beer provide specific determination and quantitation of all major cis- and trans-isomer variants of the iso-α-acids present in beer.

Each Complete Solution for Isohumulones in Beer consists of an UltiMate 3000 system package and its related isohumulones starter kit. Both are ordered separately. System packages include Chromeleon 7.2 CDS software, as well as the UltiMate 3000 hardware parts needed to set up the entire UHPLC+-focused system. Add the appropriate Thermo Scientific Isohumulones Starter Kit, providing all of the necessary consumables, and the analysis of isohumulones in beer can be started immediately.

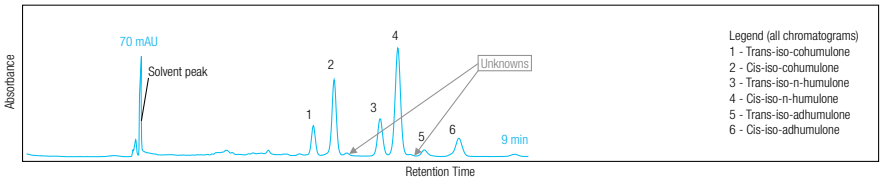
Dedicated Chromeleon 7 CDS eWorkflow™ templates are provided as part of each starter kit. An eWorkflow creates a sequence, starts the run, and ensures that data are processed and reported correctly.



The BeerNHop Solution for Occasional Beer Analyses



The BeerNHop Solution for Direct Injection of Untreated Beer Samples



The BeerNHop Solution for High Throughput

Recommended Resources

Identification and determination of phenolic constituents in natural beverages and plant extracts by means of a coulometric electrode array system.	Achilli, G.; Cellerino, G.; Gamache, P.; Melzi d'Eril, G.	J. Chromatogr., 632, 111-117.	1993
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Determination of Free Phenolic Acids in Wort and Beer by Coulometric Array Detection.	Floridi, S.; Montanari, L.; Marconi, O; Fantozzi, P.	J. Agric. Food Chem., 51, 1548-1554.	2003
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Determination of proanthocyanidins and catechins in beer and barley by high-performance liquid chromatography with dual-electrode electrochemical detection.	Madigan, D.; McMurrough, I; Smyth, M.	Analyst, 119, 863-867.	1994
Phenolic acids from beer are absorbed and extensively metabolized in humans.	Nardini, M.; Natella, F.; Scaccini, C.; Ghiselli, A.	J. Nutr. Biochem. 17 (1), 14–22.	2006
Optimization of gradient HPLC analysis of phenolic compounds and flavonoids in beer using a CoulArray detector.	Rehová, L.; Skeríková, V.; Jandera, P.	J. Sep. Sci., 27, 1345-1359.	2004
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