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**Application Summary Compendium**

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# Ion Chromatography: A Versatile Technique for the Analysis of Beer

Thermo Fisher Scientific

## Overview:

This application note describes the use of ion-exchange or ion-exclusion chromatography for the determination of five classes of compounds of interest to the brewing industry, including: carbohydrates, alcohols, organic acids, inorganic anions, and inorganic cations. One of two forms of electrochemical detection is used, pulsed amperometry or conductivity detection.

## Method:

Analyses were carried out using a Dionex chromatographic system consisting of a gradient pump, electrochemical detector with pulsed amperometry and conductivity modes.

Separation using a range of ion exchange and ion exclusion columns are shown.

Equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-5000<sup>+</sup> system and Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software.

## Conclusion:

Although many of these constituents can be determined individually using unrelated analytical techniques, reduced analysis time and equipment costs can be realized by using one instrument with multi-species capability. Ion chromatography is a versatile technique that meets many of the analytical requirements of the beer making process.

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# An Enzymatic Method for Acetaldehyde Testing of Alcoholic Beverages

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## Overview:

Acetaldehyde (ethanal,  $\text{CH}_3\text{CHO}$ ) is found in alcoholic beverages and many other foods, like yogurts, that are produced by fermentation processes. Yeasts and bacteria produce acetaldehyde as their metabolites. It is also naturally present in fruits like apples. Acetaldehyde is produced when the human body breaks down ethanol. Aldehyde dehydrogenase (ALDH2) is the major enzyme responsible for oxidizing acetaldehyde into acetic acid. In 2009, the International Agency for Research on Cancer (IARC) concluded that consuming acetaldehyde with alcohol is carcinogenic to humans.

The objective of this study is to develop and validate a rapid enzymatic method based on photometric UV determination for acetaldehyde and compare it with a liquid chromatographic method.

## Method:

A Thermo Scientific™ Arena™ 20XT analyzer was used for the automated photometric determination. The Thermo Scientific™ Gallery™ and Gallery™ Plus analyzers can also be used for this test. The Thermo Scientific Acetaldehyde system kit was used for enzymatic analysis of acetaldehyde.

20 samples were analyzed during the method validation phase including wine, spirits, beer, and cider.

## Conclusion:

The enzymatic method correlated very well with the liquid chromatography method as shown in this study. Enzymatic determination of acetaldehyde provides a rapid, user-friendly way of analyzing acetaldehyde from alcoholic beverages.

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# FT-NIR Analysis of Czech Republic Beer: A Qualitative and Quantitative Approach

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## Overview:

Beers from the Czech Republic are renowned for their refreshing taste and quality. Czech beer is often classified as 10°, 11°, or 12°. This notation refers to the amount of sugars present in the liquid extract (wort), prior to fermentation that may later be transformed into alcohol by yeast. Analysis of beer is required for accurate, reproducible production and labeling.

This method looks at Fourier transform near-infrared (FT-NIR) spectroscopy, which is a rapid and robust technique, demonstrating its capacity to rapidly and accurately measure and predict multiple components simultaneously.

## Method:

A Thermo Scientific™ Antaris™ II FT-NIR analyzer was used to generate spectra for 86 degassed beer samples. Spectra were analyzed quantitatively for four components; these were alcohol, original gravity (density), real extract and apparent extract, using a Partial Least Squares (PLS) method.

## Conclusion:

Antaris FT-NIR Analyzer successfully predicted both qualitative as well as quantitative parameters from a single data collection taking 20 seconds.

The four components: alcohol content, original gravity, real extract, and apparent extract were correctly predicted with correlation coefficients better than 0.990 and low root mean square errors indicating the model is appropriate and robust.

The method is rapid and without the need for sample preparation.

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# Rapid Determination of High Molecular Weight Beta-Glucan Using a Photometric Method

Liisa Otama, Sari Tikanoja, Sari Hartikainen and Annu Suoniemi-Kähärä  
Thermo Fisher Scientific, Vantaa, Finland

## Overview:

In the malting and brewing process used for beer production, one important analyte is beta-glucan ( $\beta$ -glucan). It is important to determine the concentration of  $\beta$ -glucan, as an excess may cause haze in the end product and thus impact appearance of the beer, as well as clogging process filters during production. The application note describes an automated novel method for analyzing  $\beta$ -glucans from wort and beer samples.

## Method:

The  $\beta$ -glucan method was validated using a Thermo Scientific™ Gallery™ discrete photometric analyzer. Similar results were obtained using the Thermo Scientific™ Gallery™ Plus, Thermo Scientific™ Arena™ and Thermo Scientific™ Gallery™ Plus Beermaster analyzers. A Beta-Glucan (High MW) test kit from Thermo Fisher Scientific was used and provides ready-to-use non-hazardous reagents for up to 350 tests.

## Conclusion:

Beer and wort samples showed excellent repeatability and reproducibility with a typical variation of 2% or less. Total analysis time for nine samples with ten replicates, a total of 90 results, was less than 40 minutes. In preliminary analysis of the beer and wort samples, this method correlates well with results obtained by fluorometry using Calcofluor fluorescence dye as recommended in EBC 8.13.2, 4.16.2, 3.10.2 and ASBC Wort-18.

An improvement over the existing fluorometric methods, the open on-board stability of these novel non-hazardous reagents was determined to be at least 30 days. This study demonstrates that the novel  $\beta$ -glucan method is precise and a suitable alternative method for routine use.

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# Correlation of the Free Amino Nitrogen and Nitrogen by O-Phthaldialdehyde Methods in the Assay of Beer

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## Overview:

In normal fermentation, yeast converts sugar to ethanol and carbon dioxide. Yeast synthesizes the proteins required from amino acids that may be created from ammonia or by removal of the amino group from other alpha amino acids. The alpha amino acids available to yeast during fermentation are known as Free Amino Nitrogen (FAN).

Levels of FAN in wort influence the formation of higher alcohols, specifically ethanol, which contributes to the flavor of beer.

The ninhydrin method describes FAN measurement and is used by the leading brewing governing bodies. The correlation between the Brewing Analytes Proficiency Testing Scheme (BAPS) beer samples measured according to the EBC FAN protocol and the Thermo Scientific™ Gallery™ system alpha-amino nitrogen by o-Phthaldialdehyde (NOPA) method are examined.

## Method:

The NOPA method was demonstrated using a Thermo Scientific™ Gallery™ discrete photometric analyzer. Similar results were obtained with the Thermo Scientific™ Gallery™ Plus, Arena™, and Gallery™ Plus Beermaster analyzers. A NOPA test kit from Thermo Fisher Scientific was used.

## Conclusion:

All samples tested by the Thermo Scientific NOPA method demonstrate result levels similar to samples tested according to the EBC FAN protocol for beer. The Gallery analyzer method is an easy to use, robust method for the measurement of FAN in beer and wort. The advantage of using an automated analyzer is its ability to measure multiple analytes like beta-glucan and SO<sub>2</sub> in addition to performing the NOPA measurement on the same sample.

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# Isohumulones - Robust Iso- $\alpha$ -Acids Assaying in Beer within Ten Minutes

Michael Heidorn, Thermo Fisher Scientific, Germering, Germany

## Overview:

Isohumulones (iso- $\alpha$ -acids), derived by humulones ( $\alpha$ -acids), are essential constituents of hop resins, forming approximately 80% of the typical bitterness of beer. Their antimicrobial effect leads to a sterile beverage, their tensioactive character stabilizes foam and they have a major influence on the general flavor, smell, and smoothness of beer.

This application note describes the robust and reproducible determination of beer bitterness, by precisely measuring the contents of isohumulones in untreated beer.

## Method:

Commercial beers were analysed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 System with On-Line SPE RS Configuration and an Isohumulones Starter Kit. Chromatographic separation of isohumulones was performed using a Thermo Scientific™ Hypersil GOLD™ 1.9  $\mu$ m 100  $\times$  2.1 mm column. Detection was carried out using a Thermo Scientific™ Dionex™ UltiMate™ VWD-3400RS Variable Wavelength Detector.

Column Part Number	Description
25002-102130	Hypersil GOLD 1.9 $\mu$ m 100 $\times$ 2.1mm

## Conclusion:

The application provides a fast specific determination and quantitation of each *cis*- and *trans*-isomer of the isohumulones (iso- $\alpha$ -acids), with a total run time of 10 minutes from sampling to result. By using online solid phase extraction (SPE), untreated beer samples can be directly injected as all SPE steps are performed automatically.

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# Correlation of an Automated Discrete Analysis Sulfur Dioxide Method to Standardized Para-rosaniline Methods in the Analysis of Beer

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Thermo Fisher Scientific, Vantaa, Finland

## Overview:

Sulfur dioxide ( $\text{SO}_2$ ) in beer, originates from yeast metabolism. It reacts with carbonyl compounds to form hydroxysulfonates, which can produce a stale unwanted flavour. Sulfur dioxide is measured by the European Brewery Commission (EBC) Method 9.25.3 or the American Society of Brewing Chemists (ASBC) Beer-21 para-rosaniline ( $\rho$ -rosaniline) method.

$\text{SO}_2$  levels (usually present as the bisulfate ion free form,  $\text{HSO}_3^-$ ) help brewers monitor beverage quality. This method evaluates total  $\text{SO}_2$  using an automated discrete analyser, to standardized  $\rho$ -rosaniline methods for beer and cider analysis.

## Method:

Commercial beer and cider samples were analyzed by the Thermo Scientific™ Gallery™ discrete photometric analyser using a total sulfur dioxide test that does not require sample pre-treatment. A fully automated table top photometric analyzer enables simultaneous analysis of multiple parameters from the same sample. For total  $\text{SO}_2$ , the Thermo Scientific method is based on a reaction between  $\text{SO}_2$  and 5, 5'-dinitrobenzoic acid (DTNB) in basic conditions and is performed at 37 °C, using a 405 nm filter for detection.

## Conclusion:

All samples tested by the  $\text{SO}_2$  Total method showed results similar to the EBC (9.25.3)  $\rho$ -rosaniline method. The Gallery method was easy to use and provided a robust method for  $\text{SO}_2$  Total measurement for both cider and beer samples.

Ready-to-use Thermo Scientific liquid reagents eliminated reagent preparation, which saved time, and the volume-optimized methods minimized reagent waste.

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# High Sensitivity Analysis of Nitrosamines Using GC-MS/MS

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<sup>1</sup>Alpha Analytical Pte. Ltd., Singapore, <sup>2</sup>Thermo Fisher Scientific, Singapore

<sup>3</sup>Health Sciences Authority, HSA Singapore

## Overview:

Nitrosamines is the common term used for compounds of the class of N-nitrosodialkylamines. Nitrosamines in food are mainly produced from nitrites, added to food as preservatives. Another source of nitrosamines is during the drying process of germinated malt in beer production. Nitrosamine levels in malt and beer have been significantly reduced in the brewing process, due to regular control, requiring high analytical performance.

This application note describes a comprehensive GC-MS/MS method for the high sensitivity detection of nitrosamine compounds at low levels.

## Method:

Nitrosamines were isolated from beer samples using an adapted method from AOAC Official Method (2000), 982.11. Chromatographic analysis was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler and a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with a Thermo Scientific™ TraceGOLD™ TG-Wax MS column 30m x 0.25mm x 0.5µm. All samples were analyzed on a Thermo Scientific™ TSQ 8000™ Triple Quadrupole GC-MS/MS system with the unique AutoSRM analysis software. Data processing was carried out with Thermo Scientific™ TraceFinder™ software, for quantitation and targeted screening workflows.

Part Number	Description
26088-2230	TraceGOLD TG-WaxMS 30m x 0.25mm x 0.5µm
31303233-BP	BTO Septa 11mm in Blister Packaging
365D0291	TriPlus RSH Syringe 10µl FN 57mm 26s Gauge

## Conclusion:

The TSQ 8000 GC-MS/MS can successfully quantify the concentration of nitrosamine components in real samples without any uncertainty, and can serve as a turnkey method for routine use in beer and food safety control. The presented method is fast, allows high sample throughput and provides results with very high sensitivity.

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# Chalconoids and Bitter Acids in Beer by HPLC with UV and Electrochemical Detection

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Thermo Fisher Scientific, Chelmsford, MA

## Overview:

In this study, two targeted assays were developed for the measurement of either polyphenols (including catechins and proanthocyanidins) or xanthohumols and bitter acids.

The bitter acid method was used to study beer stability. A metabolomic approach was also developed where patterns of both known and unknown analytes were used to study differences between beer samples—an approach that is relevant to quality control.

## Method:

A Thermo Scientific™ Dionex™ UltiMate™ 3000 System with a Thermo Scientific™ Acclaim™ 120, C18 3µm 3.0 x 150mm column. Detection was carried out using UV (Thermo Scientific™ Dionex™ DAD-3000RS diode-array) and EC (Thermo Scientific™ Dionex™ CoulArray detector with thermal organizer). Data was analyzed using the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System version 6.8 and Thermo Scientific™ Dionex™ CoulArray™ software 3.1. EC-array data were transferred to Pirouette® software for chemometric analysis.

Column Part Number	Description
063691	Acclaim 120 C18 3µm 3.0 x 150mm

## Conclusion:

The polyphenol method employs a targeted approach to accurately and sensitively measure various phenols, phenolic acids and polyphenols in beer, not possible by UV alone. Metabolomic approaches using the patterns of numerous known and unknown analytes can be used to differentiate between different samples. Such an approach can be used to study fermentation, product stability, and authenticity.

Use of EC detection eliminated the need for solid phase extraction procedures for sample pre-concentration commonly used in UV detection methods.

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# Multi-mycotoxin Screening and Quantitation Using UHPLC, High Resolution and Accurate Mass

Milena Zachariášová, Jana Hajšlová, Institute of Chemical Technology, Prague, Czech Republic  
Michal Godula, Thermo Fisher Scientific, Prague, Czech Republic

## Overview:

Mycotoxins are a group of naturally occurring chemicals produced by microscopic filamentary fungi. They can grow on a variety of different cereals, including barley. Reliable analytical methods for fast and effective monitoring of mycotoxins during the beer production chain are essential.

The aim of this study was to introduce a method for analysis of 32 mycotoxins in beer, based on very simple sample preparation and ultra high performance liquid chromatography (UHPLC) coupled with full spectral Mass Spectrometry (MS) detection.

## Method:

Mycotoxin standards, including Fusarium toxins and aflatoxins were used. Beer samples were treated to precipitate out contaminants. A Thermo Scientific™ Accela™ UHPLC system with a Thermo Scientific™ Hypersil GOLD™ aQ, 1.9µm 100 x 2.1mm column, was used for the separation of target analytes. Detection was carried out using a Thermo Scientific™ Orbitrap™ LC-MS system.

Column Part Number	Description
25302-102130	Hypersil GOLD aQ 1.9µm 100 x 2.1mm

## Conclusion:

The method enables rapid determination of trace levels of multiple mycotoxins occurring in complex beer samples.

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# Gradient HPLC Method for Analysis of Beer Polyphenols, Proanthocyanidins, and Bitter Acids Using a Novel Spectro-Electro Array Platform

Paul A. Ullucci, Ian N. Acworth, Marc Plante, Bruce A. Bailey  
and Christopher Crafts, Thermo Fisher Scientific, Chelmsford, MA, USA

## Overview:

Recent trends from microbreweries in the U.S. is the development of a wide range of extremely bitter beers created by the addition of extra hops during the brewing process. 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 also are essential to the flavor and stability of the beer itself.

This application note describes a method to develop gradient high-performance liquid chromatography (HPLC) methods to measure specific analytes in beer samples and, in a metabolomic approach, to distinguish between different beer samples and study beer stability.

## Method:

The Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system fitted with a Thermo Scientific™ Acclaim™ 120, C18 3µm 3.0 × 150 mm column and DAD-3000RS UltiMate 3000 Diode Array Detector was used along with Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector.

Data was analyzed using the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software with CoulArray software version 3.1.



Column Part Number	Description
063691	Acclaim 120 C18 3µm 3.0 x 150mm

## Conclusion:

The polyphenol method employs a targeted approach to accurately and sensitively measure various phenols, phenolic acids and polyphenols in beer, not possible by UV alone. Metabolomic approaches can be used to study fermentation, product stability and authenticity. The bitter acid method enabled the sensitive targeted measurement of beer stability over a two week period.

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# Analysis of Elemental Contaminants in Beverages using the Thermo Scientific iCAP 7200 ICP-OES

Patrícia Coelho, Applications Chemist,  
Thermo Fisher Scientific, Cambridge, UK

## Overview:

The use of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) for analysing elemental contaminants in beer and other different types of beverages is described.

The elements commonly analysed in beverages, both contaminants and major constituent elements are:

Major nutrients	Contaminants		
Ca	Al	Fe	Zn
Mg	As	Mn	
K	Cd	Pb	
Na	Cu	Sn	

## Method:

The Thermo Scientific™ iCAP™ 7200 ICP-OES was used for the direct analysis of beer. The Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS) simplifies method development and provides an option of immediate analysis. The Food Safety template was used which contains all of the required method parameters and standard concentrations.

A British ale was used, one drop of a silicone anti-foaming agent was added to reduce the influence of the dissolved CO<sub>2</sub> gas on nebulization and transport.

## Conclusion:

This method makes the analysis of beverages rapid and analyst friendly allowing both experienced and inexperienced users alike to vastly reduce the method development time for these types of samples, resulting in cost effective analyses. In addition to the time saving on method development, removal of the digestion stage and the use of internal standards produces an easy to use, versatile method capable of analyzing beer and also a wide variety of food and beverage samples.

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