

Analysis of Beer Using a High Speed U-HPLC Coupled to a Linear Ion Trap Hybrid Mass Spectrometer

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Key Words

- LTQ Orbitrap™
- Differential Analysis
- SIEVE™
- Ultra High Pressure LC

Overview

Ultra high pressure LC coupled to the LTQ Orbitrap™ mass spectrometer for:

- process monitoring and quality control measurements
- differential analysis/profiling of complex mixtures
- structural elucidation of constituents in complex mixtures using multiple levels of fragmentation MSⁿ

We coupled a high resolution/accurate mass instrument to an ultra high pressure LC. The optimized LC method decreased the total analysis time from 130 to 25 minutes, bringing about considerable time and cost savings for routine process monitoring and quality control applications. Reproducible chromatography combined with reliable highly accurate mass measurements on the LTQ Orbitrap enable fast profiling of complex mixtures for their comparison (typing). Moreover, unknown components of the mixtures can be identified and their chemical structures confidently assigned using the MSⁿ fragmentation capabilities of the LTQ Orbitrap.

Introduction

The analysis of beer represents a significant analytical challenge in several respects. For one, beer is a very complex sample containing a wide range of components including vitamins, amino acids, proteins and bitter acids, all imparting peculiar organoleptic properties to the drink. The presence and quantity of certain compounds are monitored to ensure a consistent product. In typical settings for process and quality control of beer production, the analysis of low molecular weight components would benefit from high resolution chromatographic separation, driving the laboratory throughput up and costs down.

Bitter acids are of particular interest in beer analysis due to their considerable impact on the taste of the final product. Being a large group of closely related compounds, bitter acids differ in the type and location of a side chain substituent. Acquisition of accurate mass data in both full scan and MSⁿ mode should enable detailed and confident structural characterization.

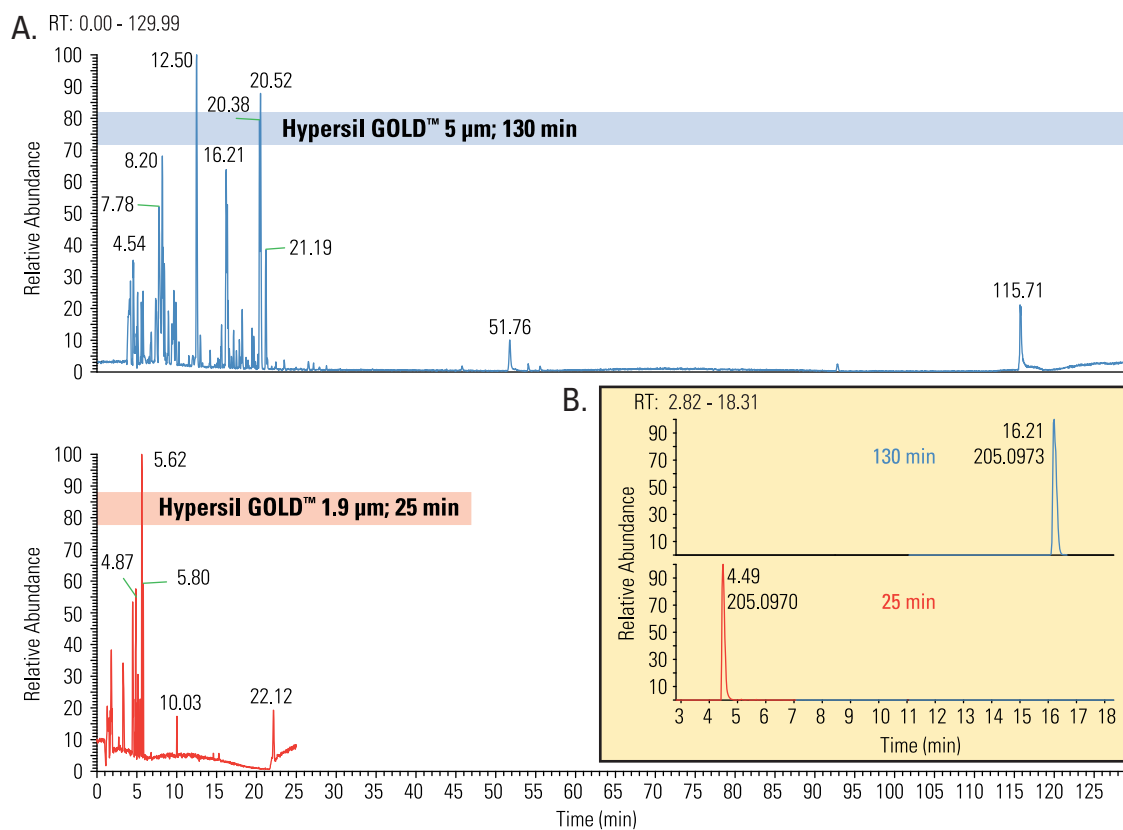


Figure 1: A: LC-MS base peak trace for a Dutch lager sample contrasting the total run time using 5 µm (top) vs 1.9 µm (bottom) particle column material. B. Good chromatography is maintained for the fast separation as exemplified by tracing the elution profile of tryptophan.

Identifying statistically significant differences among multiple LC-MS traces is enhanced considerably by having high mass accuracy and reproducible retention times. For example, one could envision such ‘fingerprinting’ as a routine part of the production monitoring process. For this type of application, an extremely robust LC-MS system is absolutely critical. The system must cope with the potentially deleterious effect of build up of material in the ion source region. We tested this aspect of routine operation by running repetitive beer analyses over a 24-hour period.

Methods

Two different types of beer (Dutch lager and English ale) were obtained from a local supermarket. The beer was degassed for 10 minutes in the ultrasonic bath, and 20 μ L of beer was injected using the Thermo Scientific Accela™ autosampler for each analysis without any other pre-treatment.

Chromatography was performed using an Accela U-HPLC equipped with a reversed phase column. The Accela system enables analysis in a ‘normal’ mode using standard column media (250 x 4.6 mm, 5 μ m) as well as in an ultra-high pressure mode (Hypersil GOLD 50 x 2.1 mm, 1.9 μ m particles).

The column was maintained at 40 °C for the analyses. A linear gradient of 0-95% acetonitrile in 0.1% aqueous formic acid was used. The total run time for the ‘normal’ and U-HPLC run was 130 and 25 min, respectively.

Data Dependent™ analysis was performed on a Thermo Scientific LTQ Orbitrap with full scan data acquired at a resolving power 30,000 (~0.6 sec per scan), and MSⁿ data acquired at a resolving power 7,500 (~0.3 sec per scan), one microscan each. Measurements were done with either external or internal calibration (mass of n-isobutyl-benzenesulfonamide m/z 214.08962 used as a lock mass).

Mass Frontier™ 5.0 software was used to assign the structures and predict the fragmentation pathways.

Both beer types were analyzed in 2-5 replicate injections, and the obtained LC-MS traces were processed using SIEVE 1.1.0 software. The SIEVE parameters were: mass range 150-380 Da, retention time 2-20 minutes, frame m/z width 0.02 Da, frame time width 2.5 min, threshold 500,000.

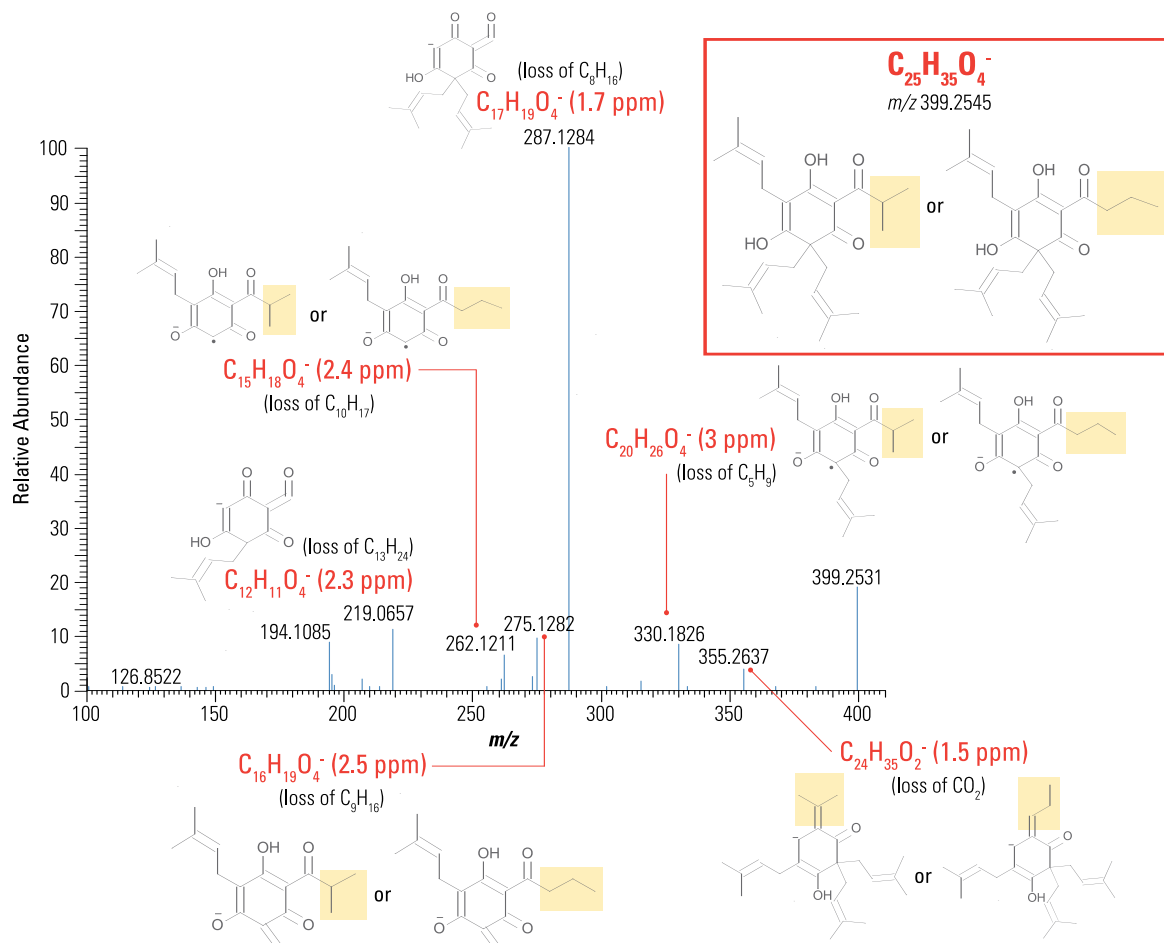


Figure 2: The inset shows a suggested structure for an unknown species of colupulone analysed in negative ion mode. Accurately measured masses (external calibration) for both parent and fragment ions enabled the assignment of elemental composition. Mass Frontier software provided insight into fragmentation pathways of the compound.

Results

The analysis focused on detecting and quantifying the low MW components of the beer samples, including tryptophan, riboflavin and its metabolites, catechins, ferulic acid, and bitter acids.

Using U-HPLC and a 1.9 μm particle column we shortened the total analysis time from 130 min to 25 min while maintaining very high chromatographic resolution (Figure 1A). A closer look at the elution profile of tryptophan shows the peak width at half maximum to be 8.4 s and 4.2 s when using 5 μm and 1.9 μm particle size columns, respectively (Figure 1B).

Spectra acquired with very high mass accuracy (observed mass deviation less than 3 ppm for data with external calibration, respectively) were used for determining elemental composition and identification of compounds (Figure 2). The fast cycle time of the LTQ Orbitrap mass spectrometer enabled us to perform multiple levels of fragmentation at high resolution. Mass Frontier 5.0 software was used to predict the structure and fragmentation pathway for the unknown colupulone (Figure 2).

We employed SIEVE differential analysis software for identification of compositional variations between the two types of beer. The first step of data analysis involves chromatogram alignment (Figure 3). The SIEVE program then highlights statistically significant differences between the analyzed data sets. Information on individual compounds of interest is presented in a concise user friendly tabular or graphical form.

The relative abundancies of several typical compounds present in English ale and Dutch lager samples are shown in Figure 4. The SIEVE results clearly point to a remarkable difference in the content of ferulic acid, the English ale having a much higher concentration.

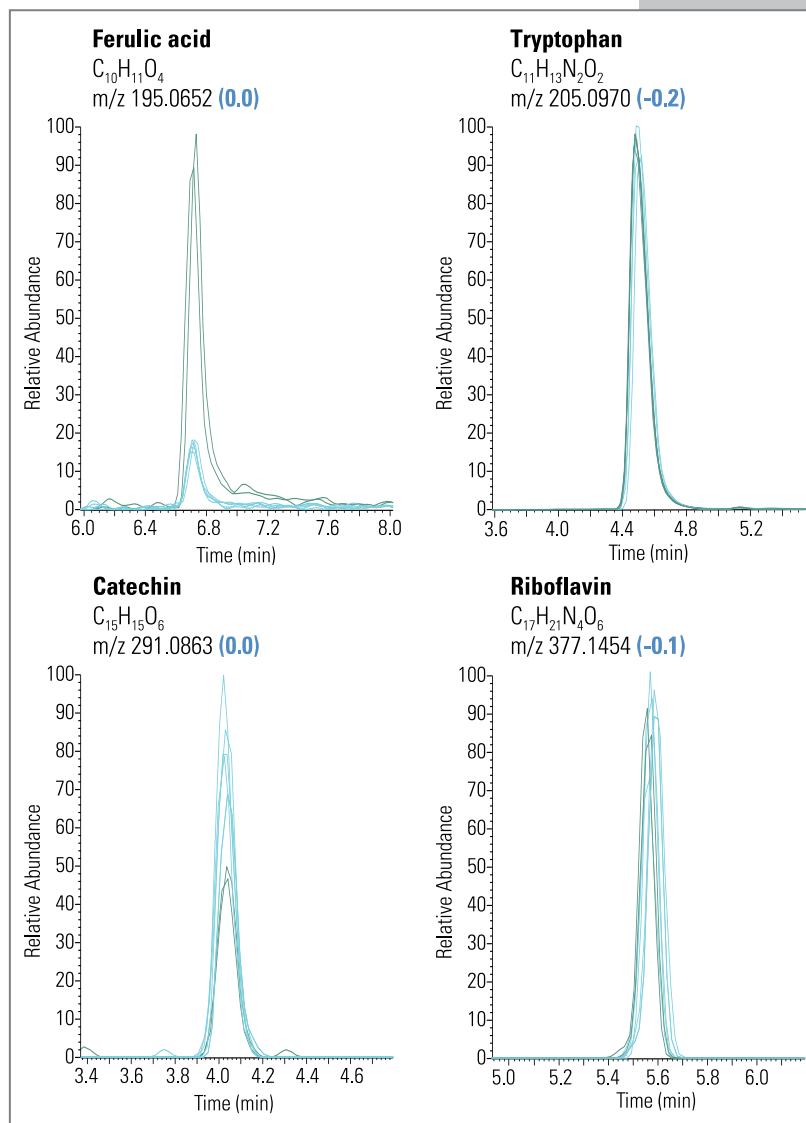


Figure 4: The peak intensities of several compounds typically monitored in beer are compared for the analysed samples of English ale (green) and Dutch lager (blue). The relative abundancies, their molecular formula (M^+H), observed m/z , and mass deviation (mmu) are detailed.

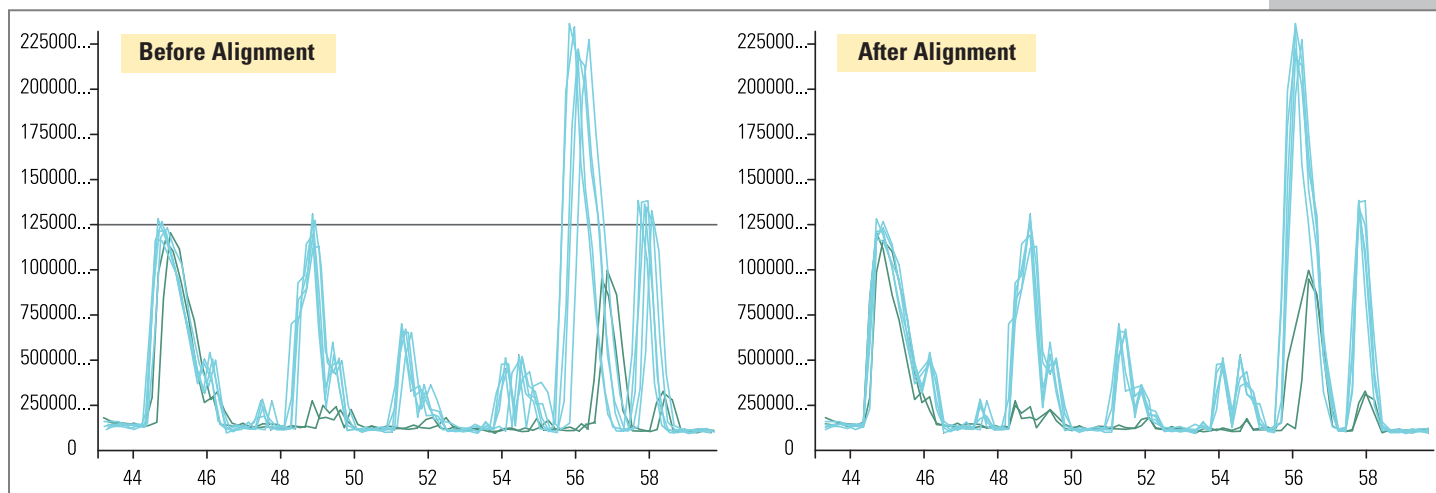


Figure 3: The identification of compositional variations between Dutch lager (blue, five replicate analyses) and English ale (green, two replicates) starts with the alignment of the LC traces using the SIEVE software.

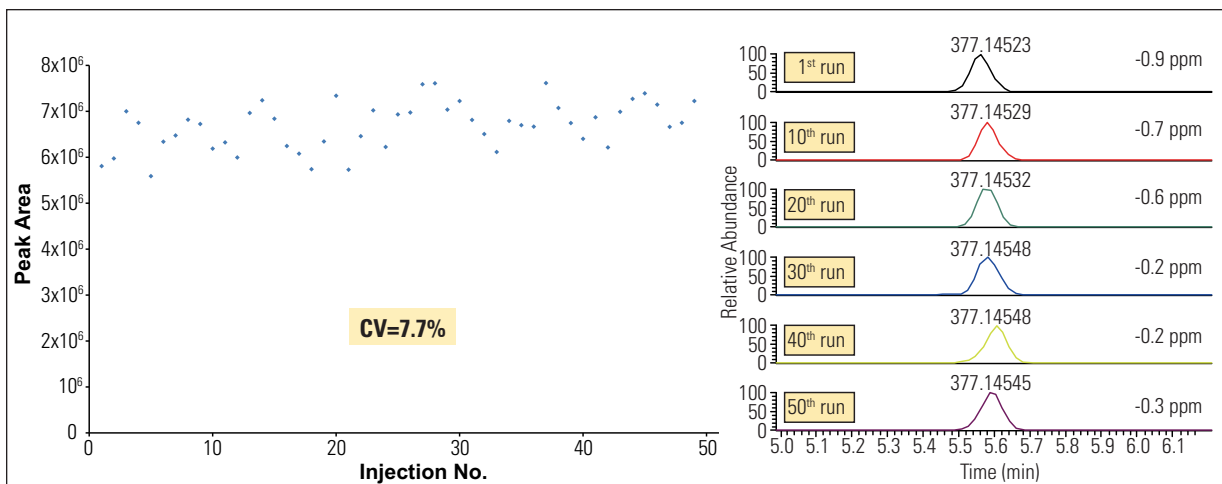


Figure 5: Robustness study monitored the peak area and the mass accuracy over 24-hour period while analyzing 50 repeated injections of Dutch lager. Presented data are for the compound riboflavin (m/z 377.14556) with internal calibration.

The presence of saccharides and proteins in beer makes it a challenging mixture to analyse. The robustness of the system must be assessed. We performed a fully automated analysis of 50 beer samples, corresponding to 24 hours of continuous analysis.

Even under such extreme conditions, the instrument demonstrated remarkable ruggedness (Figure 5). A considerable amount of caramelized deposit was noted at the heated inlet into the mass spectrometer, but this did not interfere with the sensitivity or mass accuracy of the measurements (Figure 6).

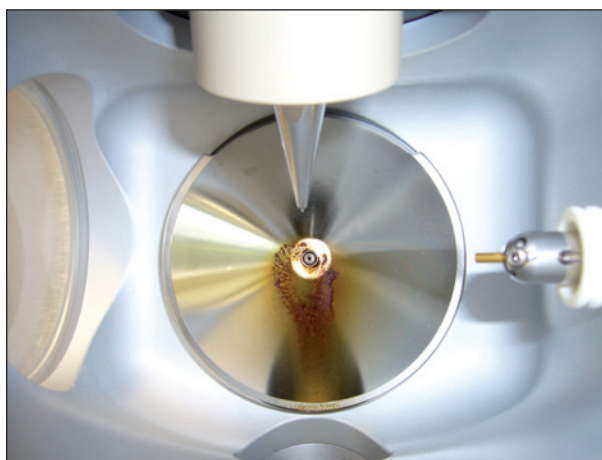


Figure 6: Caramelized deposit accumulated in the ion source after 25 injections of crude beer sample.

Conclusions

The LTQ Orbitrap mass spectrometer coupled to the Accela U-HPLC system enables sample analysis at both normal and ultra-high pressure.

- U-HPLC and small particle columns afford fast analysis times while maintaining very high chromatographic resolution (peak width 4 seconds at half height). These columns also proved to be extremely robust, allowing for an uninterrupted analysis of 50 untreated beer samples (continued analysis over a 24-hour period).
- The mass deviation of the LTQ Orbitrap measurements was always better than 1 ppm using internal calibration, and remained stable for considerable time (over 24 hours).
- Accurate mass measurements greatly increase the confidence in elemental composition assignment. The structure of unknown colupulone was derived using Mass Frontier software relying on accurately measured parent and fragment ions.
- Accurate mass measurements also significantly improve the precision of quantitation by eliminating nearly isobaric interferences. A dramatic enhancement in the performance and accuracy of the quantitation software is observed as well.
- SIEVE differential expression software enables large-scale statistical evaluation of multiple complex LC-MS analyses. Such fast and powerful 'fingerprinting' analysis can be applied in process and/or quality control monitoring.

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