Your Essential Guide to Sugar Analysis with Liquid Chromatography

Waters™

CONTENTS

Food and Beverage Industry Trends

There are many trends that drive innovation and reformulations in the food and beverage industry. Most recently the introduction of sugar taxes in several geographies and the consumer focus on health and well-being has led to the reduction of sugar in products, as well as new low- or no-sugar products being released. Alternative beverage products to carbonated soft drinks also continue to grow with product types including energy drinks, sports drinks, fortified waters, and functional dairy products. Lactose-free versions of popular food and beverage products are also increasing in availability.

Testing Sugars and Sugar Substitutes

Laboratories play an important role in product reformulation and development, supporting product optimization by confirming the concentration and ratios of sugars. Laboratory testing also supports product quality and label claims, ensuring product consistency, the correct values on labeling, and protecting brand reputation.

Methods of Testing

There are several test methods used for the determination of sugar in food and beverages. These can be split into non-chromatographic and chromatographic methods. Some examples of the non-chromatographic methods include enzymatic analysis and refractometry (%Brix).

Chromatographic approaches typically employed include partitioning/HILIC-based, ligand exchange-based, and anion exchange-based separations. The use of LC allows for the separation of individual sugars including mono-, di-, tri-, and oligosaccharides in food and beverages, providing information on the types of sugar present (profiling) and amounts in samples (quantitation).

This eBook provides a short overview of important aspects of the analysis of sugars by liquid chromatography, focusing on of sample preparation and sample analysis. We hope you find the information useful.

Please contact us if you need further details.

The Waters Arc™ HPLC System.

Certified laboratory filters for any sample type.

Filtration of Samples

For some samples, such as beverages, sample preparation may involve a simple dilution and filtration only. The main goal of these steps is to dilute the sugars to an appropriate concentration, and adjust the sample solution with a diluent suitable for injection onto the LC column.

Filtration of samples is a preventative procedure, which reduces the risk of blocking instrument components or LC columns. Preventative steps can help laboratories save time and money by reducing the amount of unscheduled downtime and prolonging column lifetime.

When choosing the filter that best meets your application needs some things to consider include:

- Sample type
- Extraction solvent composition
- Volume
- Pore size

View our Laboratory Filters.

Solid-Phase Extraction

More complex food and beverage samples, which contain proteins, and lipids such as fats, will require additional sample preparation steps beyond dilution and filtration. Proteins and lipids can contaminate the column and LC system used for analysis, which can result in unscheduled downtime. The use of protein precipitation and centrifugation, clarification reagents and/or solid-phase extraction (SPE) can be effective for removing proteins and lipids.

Reversed-phase SPE can offer a simple pass-through SPE step to remove hydrophobic matrix co-extractives such as fats. Sep-Pak™ C_{18} is available in different cartridge and plate formats to meet laboratory throughput needs.

Sep-Pak silica-based SPE cartridges and plates.

View our Sep-Pak Sample Extraction (SPE) Products.

Maximize efficiency with Waters Standard and Certified Sample Vials.

Vials

Vials are an important part of your LC workflow. Key components of sample vials include the:

- Glass
- Cap
- Septa

Each of these components can have a potential impact on routine analysis and should be considered when selecting a vial. Some method-related risks associated with the quality of vials to consider when selecting a sample vial for your testing method include:

- Analyte absorption
- Unexpected 'ghost' peaks
- Analyte photosensitivity (stability)
- Sample size
- Mechanical effects (autosampler needle damage/blocking)

Selecting the correct vial for your test method can have an impact on the quality, reliability, and consistency of results.

Visit our Vial Selector Online Tool.

Liquid Handling: Work Smarter

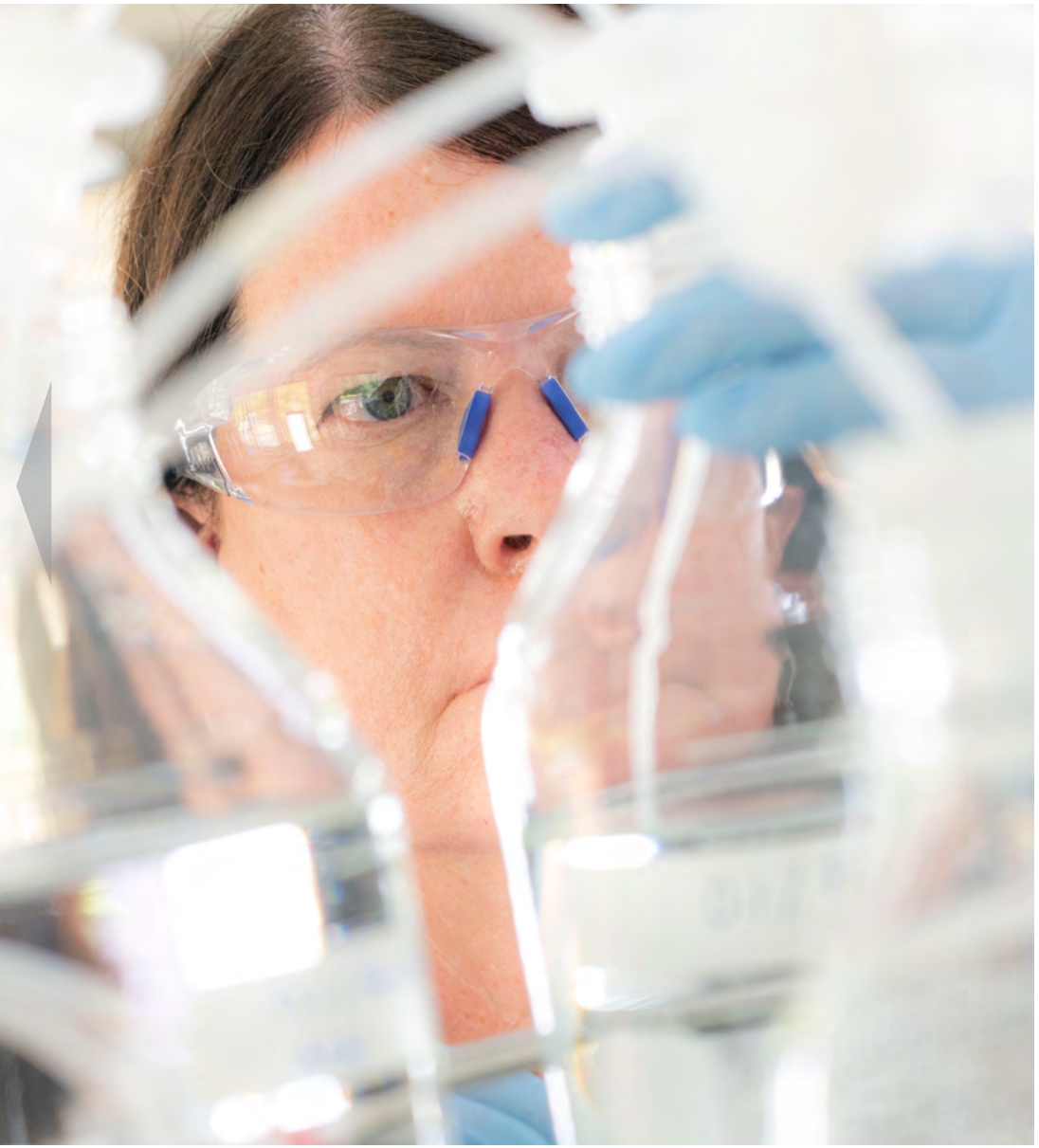
A common and significant step in routine testing workflows is the preparation of samples and standards. The accuracy, repeatability, and traceability of this step are essential for the reliable quantitation of sugars.

Automation of routine liquid handling steps this can provide several benefits, including:

- Reduced risk of pipetting errors
- Reduced waste
- Increased traceability
- Reduced risk of repetitive strain injury
- Freeing analysts to be deployed for other high-value tasks

Read this case study on Liquid Handling Automation System Streamlines Sample Preparation for Nutritional Analysis.

The Andrew+™ Robot and OneLab™ automated liquid handling platform.

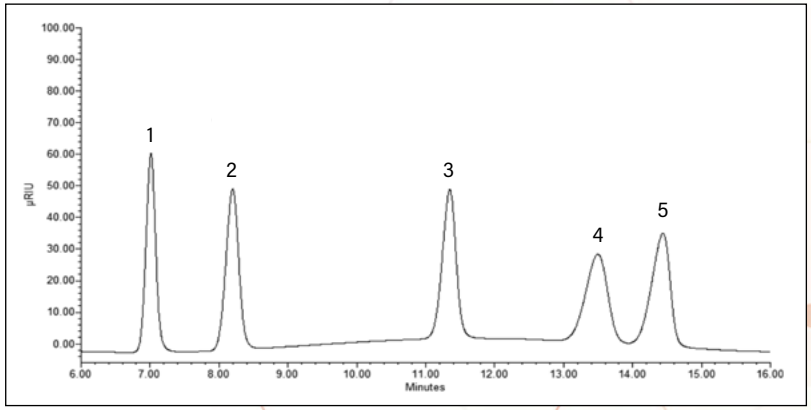


Separation and Retention

Sugars such as glucose, sucrose, and lactose do not retain on reversed-phase chromatography columns, such as C_{18}. Alternative stationary phases for the retention and separation of sugars include ion-exchange (IEX), ligand exchange, and hydrophilic interaction liquid chromatography (HILIC). These different separation mechanisms each have advantages and disadvantages.

Some important aspects to consider when implementing an LC method for the analysis for sugars and sweeteners in beverages include:

- Retention of target analytes
- Separation of target analytes from background interference
- Sample turnaround time
- Cost per analysis
- Solvent consumption

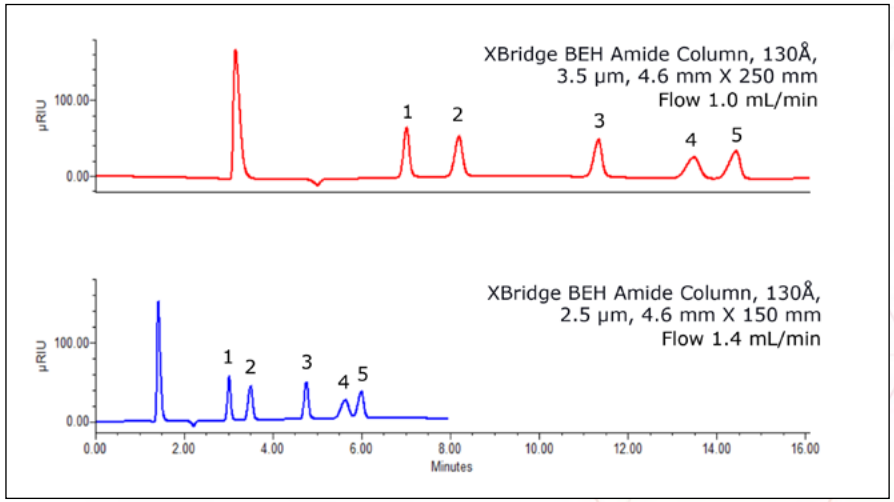


Chromatogram of sugar standards (5 mg/mL) using an XBridge™ BEH Amide Column, 3.5 µm, 4.6 mm X 250 mm on an Arc HPLC System with RI detection. The peak elution order is 1. fructose, 2. glucose, 3. sucrose, 4. maltose, and 5. lactose.

Analysis Time

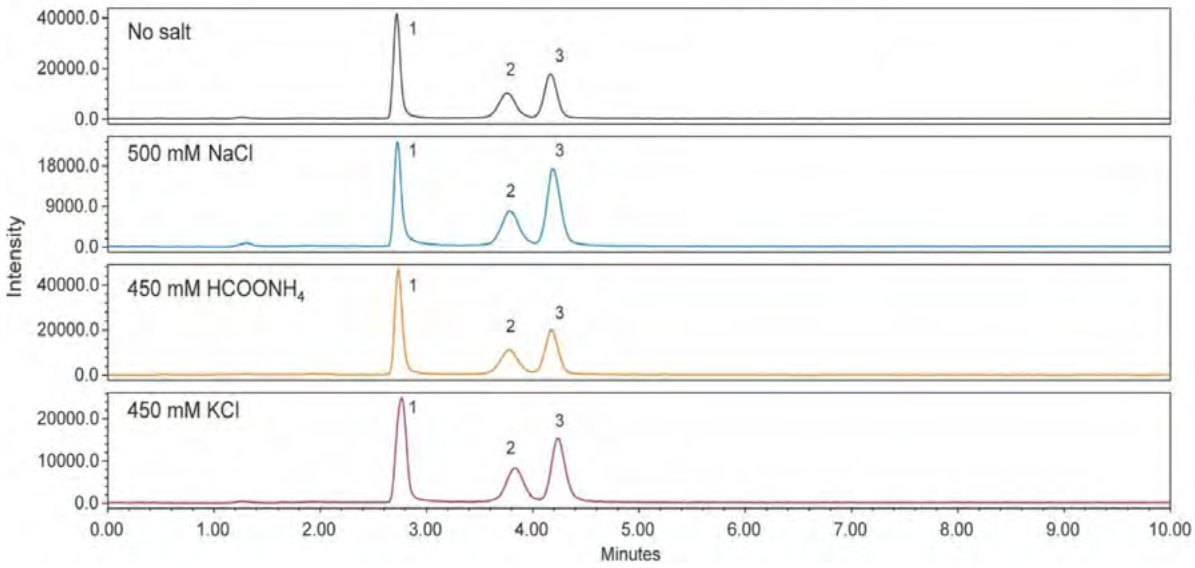
Lengthy testing procedures have an impact on laboratory productivity as well as the time it takes to make decisions, which can impact product development and the release of manufactured product. The ability to reduce analytical run times can deliver both savings in time and cost per analysis. While the speed of analysis is a crucial factor, it should not impact the separation of our target analytes such that the accuracy of quantitative data is compromised.

Smaller particle sizes and solid-core particle technologies provide greater chromatographic efficiency. To take advantage of smaller particle sizes, the LC systems with low extra-column dispersion should be considered. Utilizing smaller particles allows the use of shorter columns and/or narrower bore columns, resulting in reduced analysis time and lesser mobile phase consumption, while achieving the desired chromatographic resolution.



Comparison of sugar standards (5 mg/mL) chromatograms from the two XBridge BEH Amide Columns on an Arc HPLC System with RI detection. The peak elution order is 1. fructose, 2. glucose, 3. sucrose, 4. maltose, and 5. lactose.

View this eBook on Selecting a Liquid Chromatography Solution for Sugars and Sugar Substitute Testing.



Comparison of SIR chromatograms run on an ACQUITY Arc System with an ACQUITY QDa Mass Detector and XBridge BEH Amide XP Column. The peak elution order is 1. fructose, 2. galactose, and 3. glucose.

Read this application note on Quantification of Mono and Disaccharides in Foods Using the ACQUITY QDa Mass Detector.

Salt Interferences

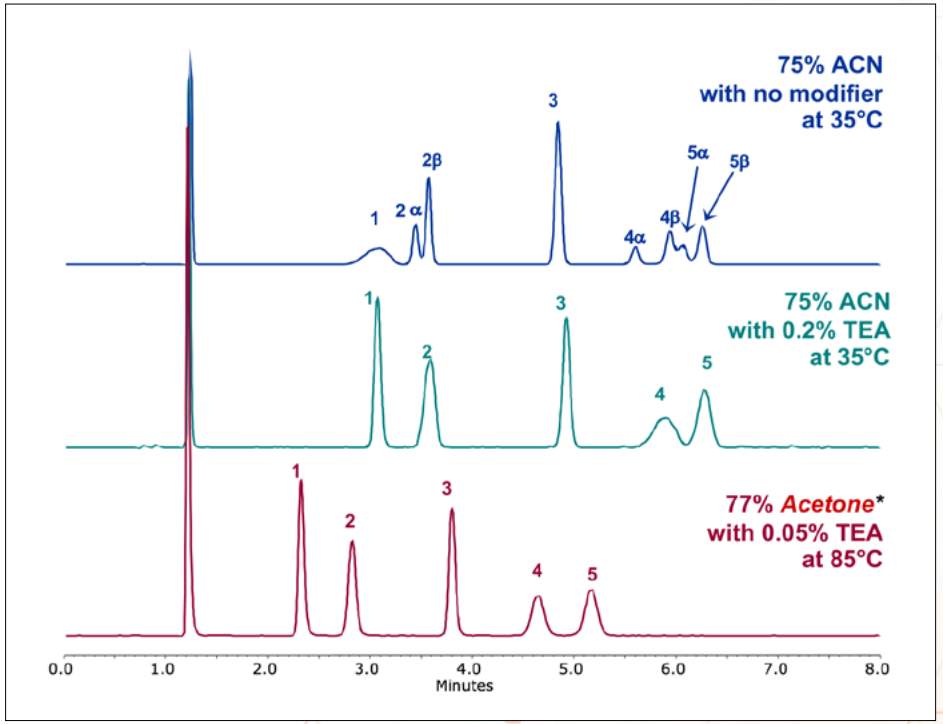
Many food and beverage samples contain other ingredients or components that can interfere with the analysis of sugars. The salt concentration of a sample can impact the quality of the chromatographic separation on several column stationary phases used for sugar analysis. The presence of salts can result in unwanted ionic interference or retention and co-elution with the target sugar peaks.

The chromatograms on the left shows the separation of three early eluting sugars (fructose, galactose, and glucose), with and without the presence of a high concentration of different salts. No impact of salts is observed on the resolution or shape of these early eluting peaks. An ACQUITY™ QDa™ Mass Detector has been used in this example for detection. Such orthogonal detection provides scientists with information rich mass spectral data ensuring no co-elution go unnoticed and chromatography is optimized.

Mutarotation

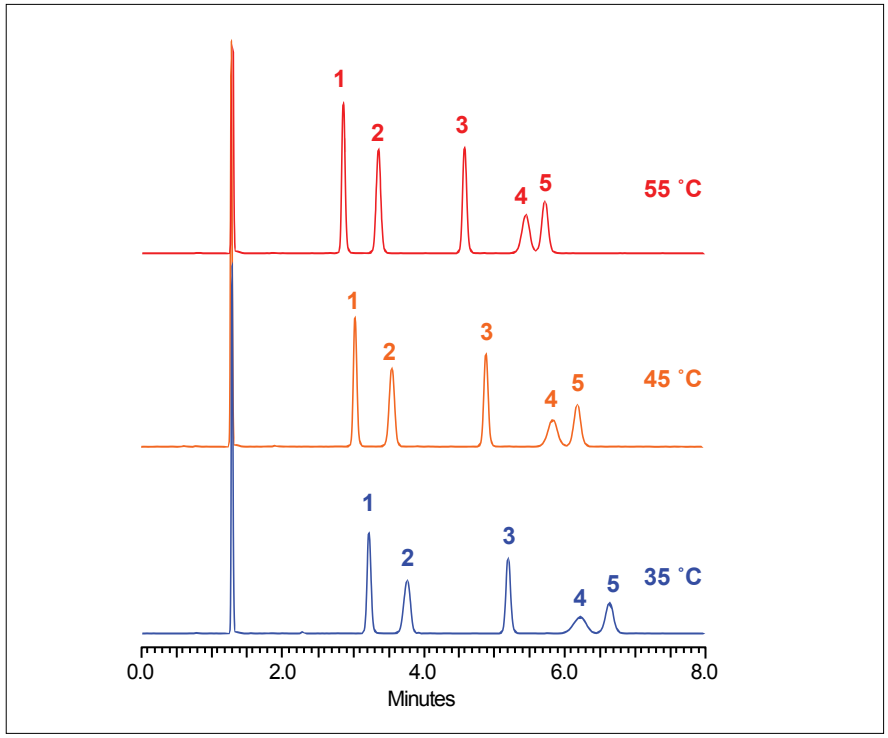
In solution, sugars such as glucose, maltose, and lactose undergo mutarotation, which is the interconversion between alpha (α) and beta (β) anomers. The interconversion between the two forms can be slow in contrast to the mass transfer kinetics of a chromatographic separation. This can result in split peaks for some sugars due to the separation of the two anomeric forms.

Routine LC methods for the analysis of sugars typically employ high pH and/or elevated temperature to collapse reducing sugars to a single peak. The high pH conditions can either be from the addition of a mobile phase additive or the result of a local alkaline environment on the column stationary phase. The chromatograms to the right demonstrate the effects of pH and temperature on the peak shape of common sugars using the BEH Amide Chemistry.



Effect of pH and temperature on peak shape of sugar standards (1 mg/mL) using an ACQUITY UPLC™ BEH Amide Column, 1.7 µm, 2.1 mm x 150 mm. The peak elution order is 1. fructose, 2. glucose, 3. sucrose, 4. maltose, and 5. lactose.

**Requires use of appropriate LC solvent compatibility kit.*



Chromatograms of sugar standards (1 mg/mL) at varied column temperatures using an ACQUITY UPLC BEH Amide Column, 1.7 µm, 2.1 mm x 150 mm on an ACQUITY UPLC System. The peak elution order is 1. fructose, 2. glucose; 3. sucrose, 4. maltose, and 5. lactose.

Schiff Base Formation

In some instances, sugars can chemically bond with amine groups on certain column stationary phases and form Schiff bases. This formation occurs as a result of the reducing sugar structure opening, which happens during the interconversion between the anomeric forms. This can lead to in shortened column lifetimes as the number of available chromatographic sites decrease over time. This reaction is dependent on temperature and in extreme cases, as column temperature is elevated there can be a loss in the recovery of reducing sugars as they are stuck on the stationary phase due to the formation of imine.

Columns with an amide ligand, such as the BEH Amide, do not form Schiff bases. The chromatograms to the left shows the performance of a BEH Amide Column as column temperature is increased.

Detection of Sugars

Sugars typically contain low or no chromophore and cannot be detected by UV detector. Some alternative detectors for sugar analysis include:

- Refractive Index (RI)
- Evaporative Light Scattering (ELS)
- Mass Spectrometry (MS)

When selecting the detector(s) for testing sugars some factors to consider include:

- Sample complexity
- Sensitivity requirements
- Laboratory infrastructure (gas supply and extraction)
- Ease of implementation

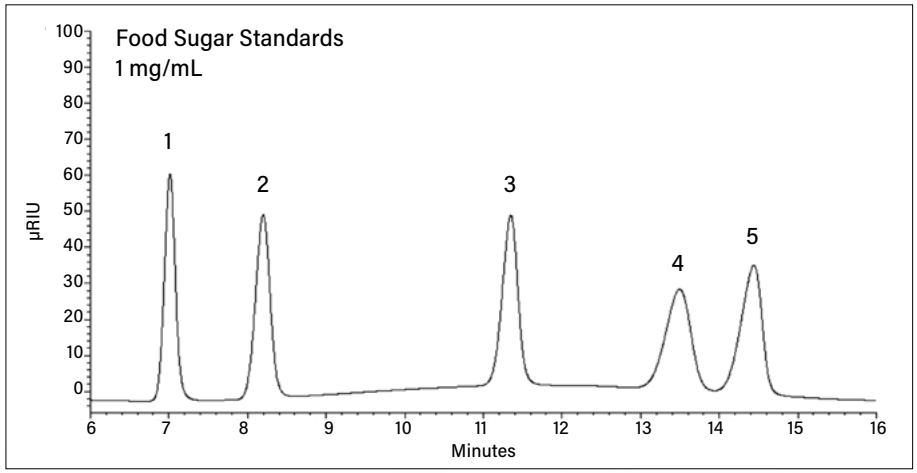
Read this white paper on Liquid Chromatography Detectors for the Analysis of Compounds With a Weak or No Chromophore for Food QC and Composition Testing.

ACQUITY UPLC H-Class PLUS System with ACQUITY QDa Mass Detector and multiple optical detectors.

Refractive Index Detection

Refractive Index (RI) detectors, such as the Waters 2414, are routinely used for the analysis of sugars or sweeteners that do not contain strong chromophores. Methods that employ RI detection are often easy to implement as they utilize a simple isocratic mobile phase and do not require the derivatization of compounds. The use of isocratic methods also removes the need for equilibration steps between injections.

The main limitation of RI detectors is that they are not compatible with gradient elution methods. This limits the ability to use programmed changes in mobile phase composition, to better separate some sugars or sweeteners from each other, or from other closely eluting ingredients. RI has a lower sensitivity when compared to other detectors, however advances in LC technology and RI detectors have improved the performance of LC-RI methods.

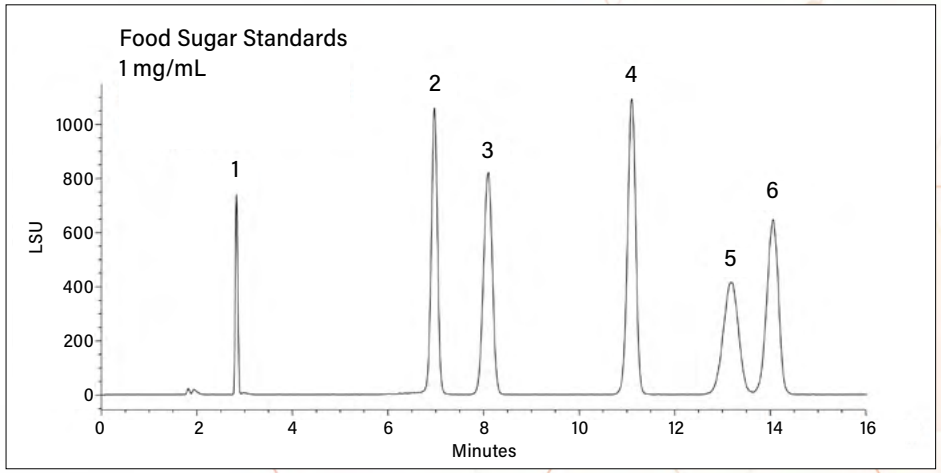


Food sugar standards using an XBridge BEH Amide Column and RI detection. The peak elution order is 1. fructose, 2. glucose, 3. sucrose, 4. maltose, and 5. lactose.

Evaporative Light Scattering Detection

Evaporative Light Scattering (ELS) detectors, such as the Waters 2424, offer near-universal detection of non-volatile and semi-volatile sample components. ELS detection is a widely used technique for the analysis of sugars. The detector has advantages in terms of sensitivity and compatibility with gradient elution methods, when compared to RI.

Non-volatile buffers are not compatible with ELS detectors, so some legacy sugar methods that employ non-volatile buffers as the mobile phase would not be suitable for use. ELS detectors often show non-linear responses, requiring the use of calibration curves with quadratic or log-linear fits for accurate quantification.



Food sugar standards using an XBridge BEH Amide Column and ELS detection. The peak elution order is 1. p-toluamide, 2. fructose, 3. glucose, 4. sucrose, 5. maltose, and 6. lactose.

ACQUITY QDa Mass Detector.

Mass Detection

Mass Spectrometry (MS) detectors, such as the ACQUITY QDa Mass Detector, provide a selective tool for the determination of sweeteners and sugars with a weak or no chromophore. The increased selectivity achieved through routine mass detection provides greater confidence in compound identification by significantly improving the ability to discriminate between different analytes.

The use of MS detection allows for lower detection limits to be achieved due to enhanced specificity compared to traditional LC-UV techniques. Information rich mass spectral data provides more information from the same separation while reducing complexity and increased confidence when profiling sugars and sugar alcohols.

Like the ELS detector, non-volatile buffers are not compatible with mass detection, which means that some legacy methods for sugars will not be compatible with this detector.

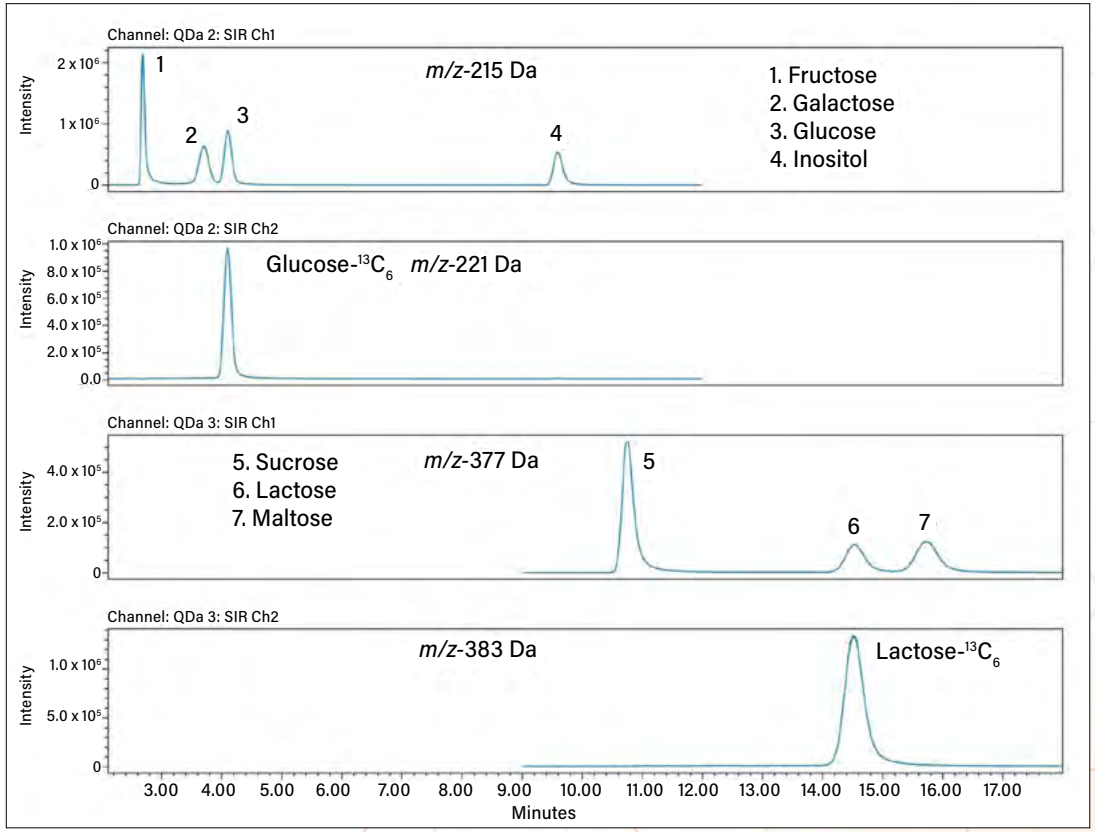
MS Scanning Modes

The ACQUITY QDa Mass Detector can operate in either full scan mode, which is a scanning experiment, or selected ion recording (SIR), which is a static experiment focusing on a specified mass or a series of masses.

When MS scan mode is used, a programmed range of masses are scanned allowing the collection of data across a range of *m/z* values, this is most typically used for qualitative analysis. SIR mode fixes the *m/z* value for a defined period (dwell time). Use of SIR mode allows for increased specificity and sensitivity and is most often used for targeted, quantitative experiments. Multiple masses can be acquired in SIR mode in a single acquisition method.

Learn more about our ACQUITY QDa Mass Detector.

Read this application note on Quantification of Mono and Disaccharides in Foods Using the ACQUITY QDa Mass Detector.



SIR [M+Cl]- chromatograms of sugar standards mix solution and internal reference standards.

Comparison of Detectors

Detector	Strengths	Considerations
2414 RI Detector 	■ Excellent linearity and precision ■ Simple isocratic methods with no need for re-equilibration between injections ■ Ease of use, robust, and low maintenance	■ Not compatible with gradient methods ■ Limited selectivity and sensitivity
2424 ELS Detector 	■ Compatible with gradient methods ■ Near-universal detection of non-volatile and semi-volatile sample components ■ Response independent of analyte's optical properties	■ Requires clean nitrogen supply and removal ■ May exhibit a non-linear response ■ Not compatible with non-volatile buffers
ACQUITY QDa Mass Detector 	■ Superior selectivity and sensitivity relative to optical detection ■ Compatible with HPLC and UHPLC enabling faster analysis time ■ Mass spectral data ■ Co-elutions identified due to enhanced selectivity	■ Requires clean nitrogen supply and removal ■ Not compatible with non-volatile buffers ■ May require use of stable isotope labeled standards for quantification methods

Strengths and considerations for each of the detectors discussed in this eBook for sugar testing.

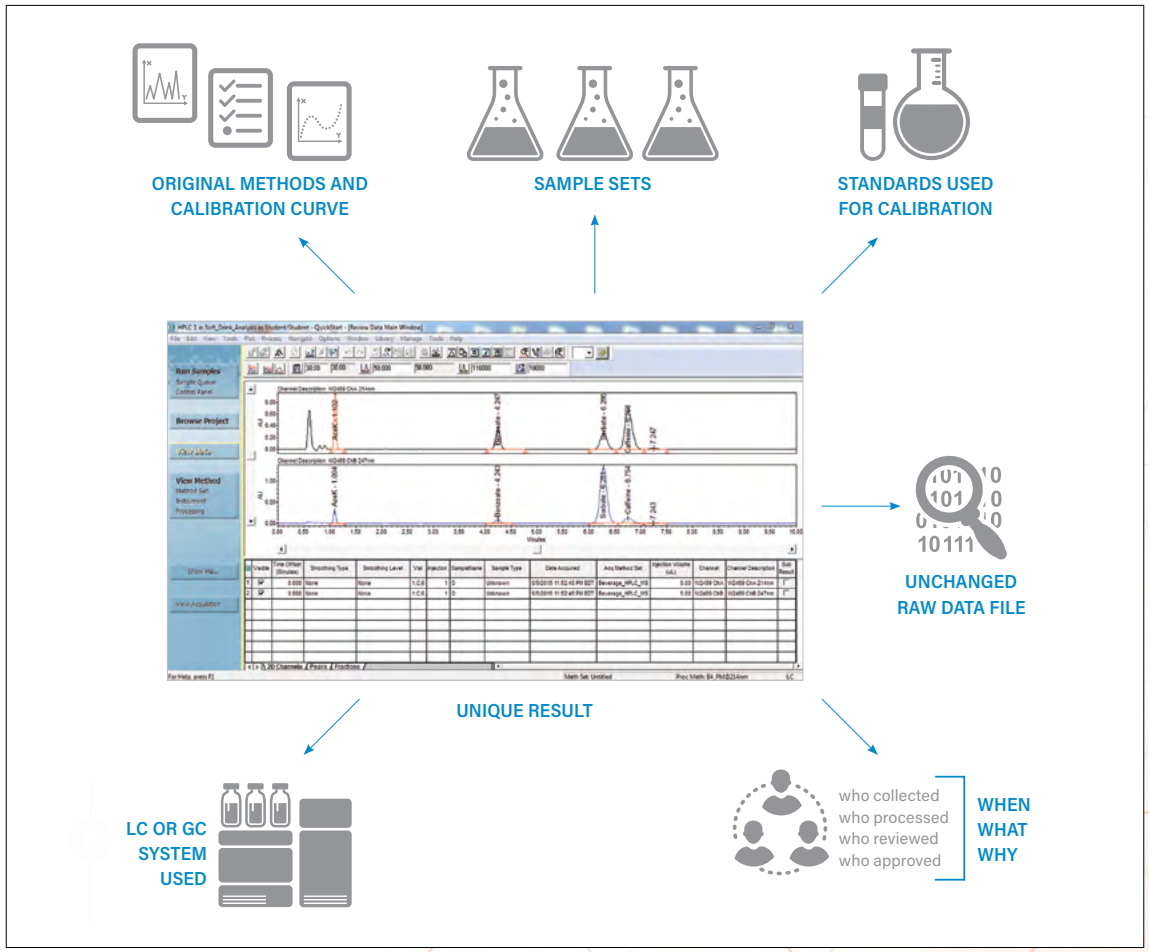
Chromatography Data Systems

Chromatography Data Systems (CDS) are an important component of routine LC food testing systems and are critical for:

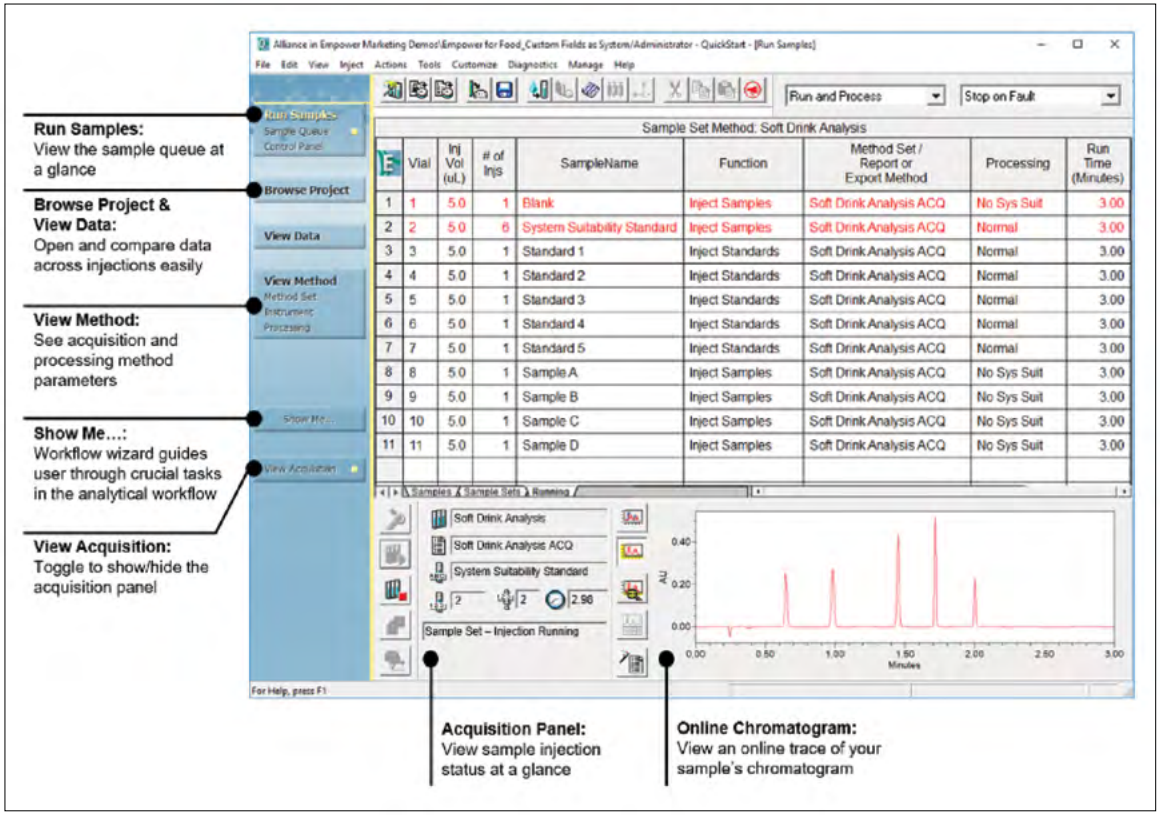
- Instrument control
- Data collection
- Data processing
- Data reporting
- Data archiving

Alongside LC, food and beverage testing laboratories may employ other separation techniques such as gas chromatography (GC) or ion chromatography (IC). The ability for a CDS software to provide comprehensive control of LC, IC, and GC systems allows a laboratory to standardize on a single CDS platform, reducing staff training needs and improving laboratory efficiency and data integrity.

A wide variety of data is captured in a CDS, including calibration curves, sample results, and reports. An important function of any CDS software is the ability to easily find information relating to a sample analysis.



All aspects of the chromatographic analysis are automatically captured within Empower™ Chromatography Data System (CDS), within an embedded relational database, allowing scientist to easily find their data.



The Empower QuickStart interface has a navigation bar on the left, guiding users systematically through the process of running samples, reviewing data, and reporting results. All tasks are performed in a single window.

Simplified Workflows

A key consideration when selecting a CDS software is usability. A streamlined user interface allows for an efficient workflow from sample login through to final report.

The CDS interface should allow users a single window to easily:

- Set-up and manage methods
- Browse projects
- Run samples
- View injection status
- View data
- Process data
- Create reports

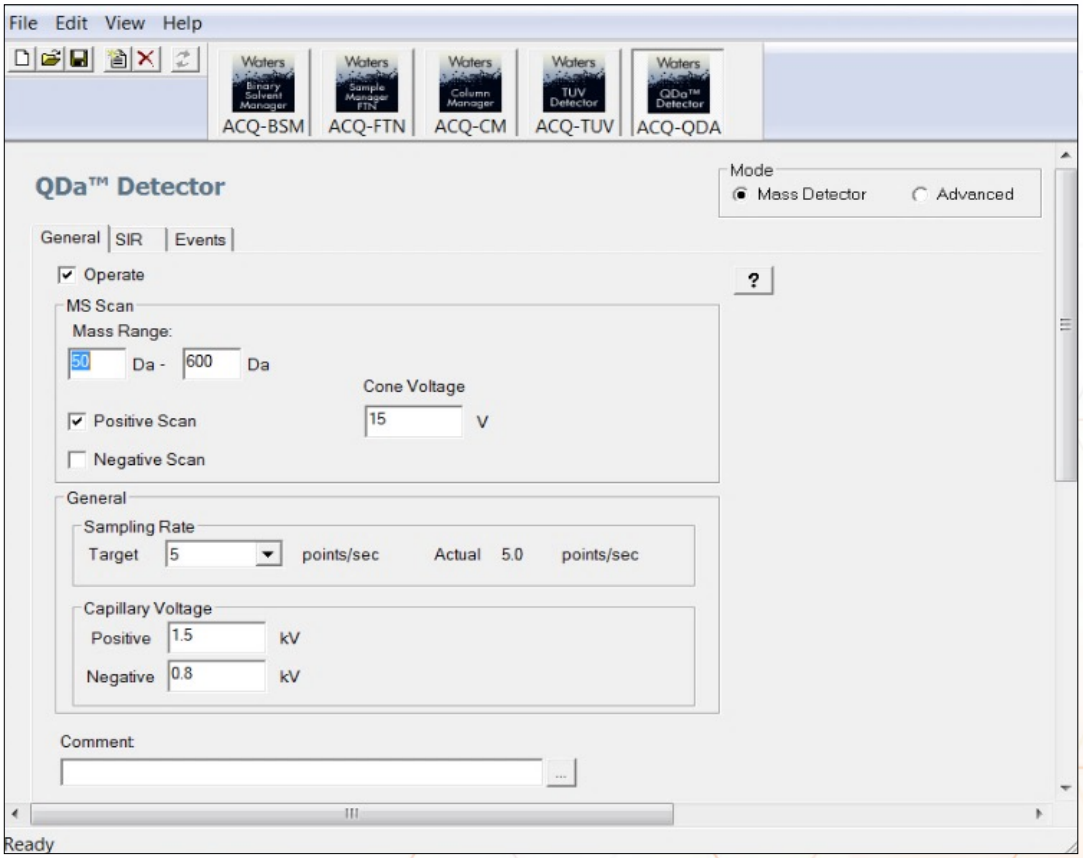
To simplify workflows and eliminate transcription errors, utilizing custom calculation fields in a CDS can remove the need to perform calculations manually or in another software. Calculations can be carried out automatically when raw data is processed, increasing laboratory efficiency and reducing the risks of transcription errors.

Empower™

Advanced Detection Techniques

Empower CDS allows the use of advanced detection techniques, such as mass detection, enabling a more selective determination of sugars in complex matrices, and providing spectral information. This helps with peak identification and troubleshooting, enabling greater confidence in results and enhanced productivity.

In the image to the right you can see how easy it is to set up a method for the ACQUITY QDa Mass Detector in Empower Software.



Method settings for the ACQUITY QDa Mass Detector within Empower CDS. The ACQUITY QDa Mass Detector is specifically designed for use by chromatographers with limited experience in mass detection.

Read this infographic on how to Improve Operational Effectiveness for Routine Food Testing.

Enabling Technologies and Services from Waters

Food and beverage manufacturers rely on analytical data every day to make important decisions around product quality, safety, and manufacturing process optimization. Analytical data is also vital to support new product development and existing product reformulation, providing important information on ingredients, shelf life, and sensory evaluation. Laboratories constantly need to adapt their analytical capabilities to meet the different testing requirements that new products and reformulation of products may bring.

Waters is uniquely positioned to support your analytical testing needs, helping you to improve internal efficiencies and to deliver a safe and consistent product:

- Benefit from flexible configurations and simple, reliable, and cost-effective workflows
- Explore the advantages of routine mass detection under Empower Software control
- Trust the quality and consistency of Waters columns and consumables
- Simplify workflows with custom calculations in Empower Software
- Rely on the technical expertise of our chemists for applications support
- Automate liquid handling to ensure reproducible and fully traceable experiments

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Waters™

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