Food and Beverage Quality Control and Compositional Testing

Application Notebook



Þ

Vitamins



Introduction

EFFICIENT ANALYTICAL SOLUTIONS TO SUPPORT A SAFE, NUTRITIOUS, AUTHENTIC AND SUSTAINABLE FOOD SUPPLY CHAIN

Welcome to Waters' food quality control and compositional testing application notebook. Alongside insights in gaining workflow efficiencies through the automation of liquid handling, inside this eBook you will find a compilation of our scientists' latest application notes, supporting your development and implementation of new testing methods and technologies for the reliable analysis of:

- Sugar Substitutes
- Sugars
- Vitamins
- Organic acids
- Amino acids

The role of food testing laboratories has never been more critical. Safety, authenticity, sustainability and quality are of major concern to consumers, governments, and food producers. Trust in Waters to provide scalable application procedures and technologies to help you adapt quickly to the challenges, brought in by reformulations and alternative products. Improve internal efficiencies and realize cost and time benefits of less re-analysis, ensuring product quality and workflow optimization. By partnering with us you gain access to unmatched levels of application support and award-winning service which all aim to have your labs running effectively and consistently day-in-day-out.

CONTENTS

| S | ugar Substitutes | 4 |
|---|---|------|
| | Analysis of Soft Drink Additives with No Interference from Aspartame Degradants Using Arc HPLC System | |
| | with PDA Detection | 5 |
| | Analysis of Sugar Alcohols and Allulose Using an | |
| | Arc HPLC System with Refractive Index Detection | 6 |
| | Analysis of Sucralose in Beverages | 7 |
| S | ugars | 8 |
| | Quantification of Mono and Disaccharides in Foods | 9 |
| | Determination of Low Level Lactose in Dairy Products Using UHPLC-MS | 10 |
| v | itamins | . 11 |
| | Simultaneous Analysis of Fat-Soluble Vitamins A, D, and E in Food Using ACQUITY Arc Two-Dimensional Liquid Chromatography | 12 |
| | Determination of Vitamin D and Previtamin D in Food Products | |
| | Differentiation of Natural Vitamin E from Synthetic Vitamin E for Food and Dietary Supplement Nutrition Labeling | 14 |
| | Enhancing the LC-MS/MS Analysis of B-group Vitamins with MaxPeak High Performance Surfaces Technology | 15 |
| | Analysis of Water-Soluble Vitamins and Caffeine in Beverage and Multivitamin Products by Arc HPLC | |
| | System With PDA Detection | 16 |

Organic Acids...

Analysis of Organic Acids using a Mixed-Mode LC Column and an ACQUITY QDa Mass Detector

Analysis of Fourteen Organic Acids in Various Beverages Using the ACQUITY UPLC H-Class PLUS and ACQUITY QDa Mass Detector......

Fast, Accurate and Flexible LC-PDA Method for the Determination of Citric Acid In Beverages.....

Amino Acids.

Instrument Considerations for Successful Adaptation of Amino Acid Analysis Methods Which Utilize Pre-Column Derivatization From an ACQUITY UPLC to an ACQUITY UPLC H-Class PLUS Binary System



.20

.22

Sugar Substitutes

.....

\$,0

Q

d

0-

° 60 0

NN.

Щ ф

T

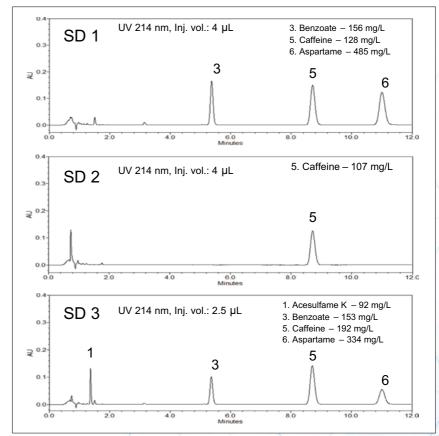
 \bigcirc

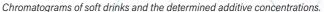
Analysis of Soft Drink Additives with No Interference from Aspartame Degradants

Soft drinks may contain caffeine, benzoate, sorbate, acesulfame K, saccharin, and aspartame as additives. Conformance of the concentrations of these additives in soft drink products to target levels is an essential part of quality control in beverage manufacturing plants. One potential issue in the analysis of soft drinks is aspartame degradation, because its degradants may co-elute and interfere with the quantification of the target additives. This study describes the investigation of the degradation of aspartame and the optimization of the method to eliminate any chromatographic interferences from its degradants. The injection linearity and accuracy of the Arc[™] HPLC System was also investigated, and its performance was compared to volumetric pipettes. This optimized beverage analysis method provides a fast, simple, and accurate HPLC method which can improve the overall productivity in a soft drink manufacturing environment.

APPLICATION BENEFITS

- Analysis of soft drink additives in a 12-minute isocratic run
- No interference from aspartame degradation products
- Inject smaller sample volumes for high additive content samples, minimizing manual sample dilution operation and solvent waste
- Pre-formulated mobile phase, wash solvent, and standards for easy setup and preparation
- Ethanol-based mobile phase and wash solvent for low hazardous waste disposal costs





E

Analysis of Sugar Alcohols and Allulose in Sugar-Free or Reduced-Sugar Products

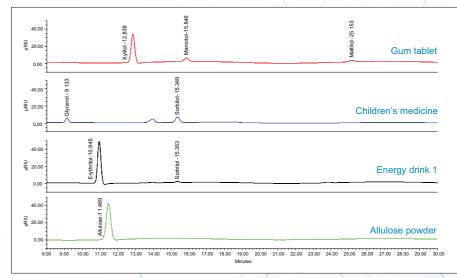
Overconsumption of sugar (sucrose) can lead to many health issues such as obesity, diabetes, dental diseases, and ADHD (attentiondeficit/hyperactivity disorder). To support the reduction of these problems, many food and beverage manufacturers have reformulated or developed new products with sugar substitutes such as sugar alcohols (polyols) or allulose, an epimer of the monosaccharide sugar fructose, which has a lower glycemic index and calories than sugar when digested. There is no fixed daily intake, but it is important to know the value of sugar alcohols and allulose present in food and pharmaceutical products to prevent overconsumption, due to the possible laxative effects of some sugar alcohols. An analytical method has been developed to detect and quantify sugar alcohols and allulose in beverages and other low-calorie products. An Arc HPLC System with refractive index detection (RID) was combined with an Atlantis™ Premier BEH[™] Z-HILIC analytical column, which provides an increase in polar analyte retention and offers a different selectivity when compared to other hydrophilic interaction liquid chromatography (HILIC) chemistries. The proposed analytical workflow may be suitable for supporting manufacturers and contract testing laboratories in standardizing analyses for sugar alcohols and allulose in sugar-free or reduced-sugar products.



Read the Full Application Note

APPLICATION BENEFITS

- Retention and separation of 7 sugar alcohols and allulose using the Atlantis Premier BEH Z-HILIC Column, which is efficient for products using blends of sweeteners
- Simple isocratic liquid chromatography (LC) method, allows ease of method setup for routine product quality control
- Arc HPLC System cycle injector valve reduces downtime and method risks due to possible precipitation of samples
- Detection of components without UV chromophores
- Excellent reproducibility, accuracy, and precision of Arc HPLC-RID with the Atlantis Premier BEH Z-HILIC Column



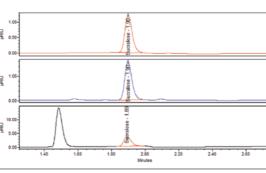
Chromatograms from the analysis of sugar alcohol and allulose containing products.

7

Analysis of Sucralose in Beverages

Sucralose is a non-nutritive sweetener that can be found in a variety of foods and soft drinks. Sucralose is derived from sucrose through the selective replacement of three hydroxyl groups which are substituted with three chlorine atoms. A common detection method for sweeteners is the use of High-Performance Liquid Chromatography (HPLC) coupled with Ultraviolet/ Visible light (UV/Vis) detection, however, sucralose requires alternative detection due to a lack of chromophore. Alternative detectors for the analysis of sucralose include refractive index (RI), evaporative light scattering (ELS), or mass spectrometry (MS). This application note highlights a method for the analysis of sucralose using an ACQUITY Ultra Performance LC[™] H-Class Plus coupled with an ACQUITY[™] Refractive Index Detector (RID). Combined with a CORTECS[™] T3 analytical column and Empower[™] 3 Chromatography Data System, this set-up provides a simple isocratic method for the determination of sucralose in beverages, such as soft drinks and energy drinks.

| Samples | Retention time | Amount (ppm) |
|-----------|----------------|--------------|
| | n=6 (%RSD) | n=6 (%RSD) |
| Sample G1 | 1.90 (0.1) | 57.3 (1.1) |
| Sample P2 | 1.90 (0.1) | 73.0 (0.6) |
| Sample 5H | 1.89 (0.1) | 1423 (1.6) |



Representative chromatograms of sucralose in beverages.

APPLICATION BENEFITS

- Simple isocratic LC method with ease of method setup for routine product quality control
- The ACQUITY RI Detector has a low internal volume which delivers low dispersion and a stable baseline performance for reliable quantitative results
- The thermally isolated optics bench of the ACQUITY RI detector and highly efficient temperature equilibration of the incoming eluent, minimizes baseline drift.
- Excellent method reproducibility, accuracy, and precision



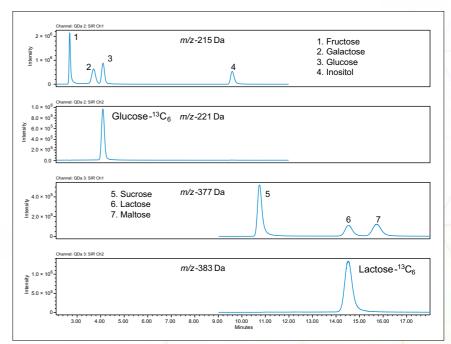
Quantification of Mono and Disaccharides in Foods

Monosaccharides such as fructose, galactose, and glucose, and disaccharides including sucrose, lactose, and maltose are common sugar ingredients in foods. With the increasing concerns of obesity and diabetes in many countries, the need to monitor sugar intake has grown in recent years. Consequently, now there are requirements to provide accurate information about added sugar content on food product labels in order to comply with current FDA food labeling regulations.

High performance liquid chromatography (HPLC) is the method of choice for the analysis of sugars. However, the HPLC analysis of sugars is not a simple task. The main concern is the co-eluting compounds that may interfere with sugar quantification. For example, galactose often co-elutes with glucose. In this work, we improved an existing method and applied it to a wide range of foods, including a chicken feed sample.

APPLICATION BENEFITS

- Separation of galactose and glucose
- Clean chromatogram with minimal interference from complex sample matrix
- Accurate analysis of sugars with 25 minute per injection cycle

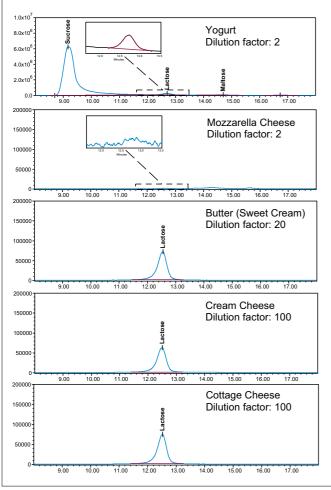




Read the Full Application Note

9

Amino Acids



SIR (377 m/z) chromatograms of lactose in dairy products.



Read the Full Application Note

Determination of Low Level Lactose in Dairy Products

Lactose is found primarily in dairy products and can cause discomfort in people who are lactose intolerant. Lactose intolerance is a consequence of lactase deficiency in the small intestine, resulting in a reduced ability to break lactose down into glucose and galactose. Rates of lactose intolerance vary between regions, from less than 10% in Northern Europe to as high as 95% in parts of Asia and Africa. While low lactose and lactose-free products are available on the market, it is critical to ensure the label claim of these dairy based food products. Some countries have established the threshold levels for the terms of "lactosefree" and "low lactose". These threshold values vary from country to country, but the common threshold levels for lactose-free and low lactose are 10 mg lactose and 1 g lactose per 100 g of final products, respectively. The main challenges in the analysis of lactose at low levels are the detection sensitivity and the matrix interference.

In this application note we demonstrate an analysis method for lactose in dairy products using an ACQUITY Arc[™] System, an ACQUITY QDa Mass Detector, and an XBridge[™] BEH Amide XP Column (2.5 µm, 3.0 × 150 mm). The method limit of quantitation (LOQ) is estimated at 0.0025 g per 100 g of final products with minimal interference from the matrix. The total run time of the chromatography analysis is 25 min.

APPLICATION BENEFITS

- Accurate measurement of low levels of lactose in foods
- Minimal matrix interference using MS detection
- Suitable for analysis of lactose-free and low lactose food products

°-(\$)-°

ð

~% ____

TT

Q

00

a b b b

Simultaneous Analysis of Fat-Soluble Vitamins A, D, and E in Food

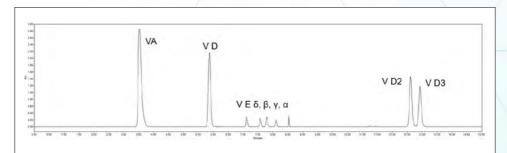
Vitamin A, also known as retinol, plays an important role in promoting body growth, including maintaining the integrity of the epidermis. Vitamin D includes two major forms, i.e. vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol), which promote calcium and phosphorus metabolism and bone formation in mammals. Vitamin E includes tocopherols and tocotrienols. There are 8 active forms of vitamin E due to the variation of methyl substitution on the parent tocopherol and tocotrienol ring, including α -, β -, γ -, and δ -tocopherols. Among them, α -tocopherol is usually singled out in food science as it has the highest activity and antioxidative and anti-aging properties. Infant formula and adult nutritional products and animal feeds are three important types of fat-soluble vitamin fortified products. In actual samples, vitamin A and vitamin E can be quantified directly because their content levels are relatively high and matrix interference is negligible; however, vitamin D is generally added in a small amount, has relatively low UV absorption, and suffers severe interference from the matrix.

Read the Full Application Note

Using the ACQUITY Arc (UHPLC) 2D technology under reversed-phase conditions, the separation of vitamin A, α , β , γ , δ -tocopherol, vitamin D2, and vitamin D3 can be completed simultaneously with one sample injection, and the entire assay only takes 15 min.

APPLICATION BENEFITS

- The two-dimensional column switching ultraviolet detection method can separate vitamins A, D, and α/β/γ/δ-E in a single sample injection, greatly increasing the efficiency of analysis
- With the heart-cutting technique, vitamin D is cleaned-up on the first dimension column, and then separated into vitamin D2 and D3 peaks. This approach helps to eliminate co-elution, which reduces the interference from complex matrix and thus allows for the determination of vitamin D2 and D3.



Chromatogram for the standard of vitamin A, four isoforms of vitamin E, vitamin D2, and vitamin D3.

Vitamins

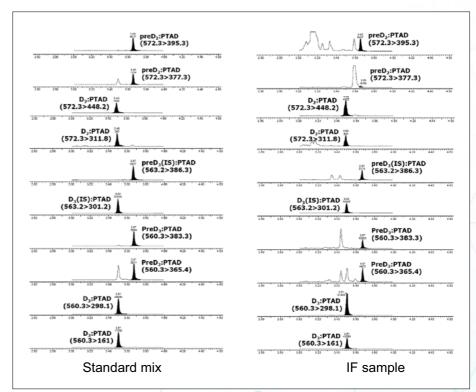
Determination of Vitamin D and Pre-vitamin D in Food Products

Existing standard methods for vitamin D analysis involve saponification, liquid-liquid extraction (LLE), sample clean-up, and liquid chromatography (LC)-UV determination. The most challenging aspect in vitamin D analysis is the diverse interferences from sample matrix. A large number of lipid-like compounds are co-extracted with the vitamin D, and even after extensive sample clean-up, there are still numerous interferences that co-elute and interfere with vitamin D quantitation. Recently, to simplify the sample preparation and to improve the analysis, a derivatization reaction with 4-Phenyl-1,2,4triazoline-3,5-dione (PTAD) and mass spectrometry (MS) was adopted in a new AOAC standard method. This new method has provided much better analytical performance for vitamin D analysis. However, pre-vitamin D is not measured in this new standard.

It is known that vitamin D can thermally isomerize to pre-vitamin D. This transformation is reversible, and both forms are biologically active. It has been reported that the relative content of pre-vitamin D could be up to 22% of the total vitamin D at 80 °C. Therefore, it is prudent to individually determine pre-vitamin D and vitamin D contents in the analysis of vitamin D in foods. This application note demonstrates the determination of total vitamin D by individually measuring the vitamin D and pre-vitamin D in food products.

APPLICATION BENEFITS

- Determination of both pre-vitamin D and vitamin D.
- More accurate and precise determination of total vitamin D analysis.



Typical MRM chromatograms of vitamin D and pre-vitamin D in standard mix solutions (left) and infant formula samples (right).

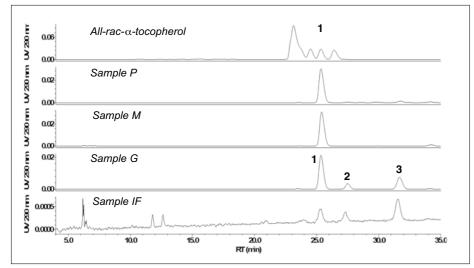
E

Sugar Substitutes

Sugars

Vitamins

Amino Acids



Chromatograms of all-rac-tocopherol, dietary supplements, and infant formula powder. Peak ID: 1) RRR-a-tocopherol; 2) d-tocopherol; 3) g-tocopherol



Differentiation of Natural Vitamin E from Synthetic Vitamin E

To ensure accurate nutritional label claims for vitamin E, the source of vitamin E (natural or synthetic) needs to be verified. This application brief demonstrates the capability of Waters ACQUITY UPC²TM System and TrefoilTM Columns for the differentiation between natural and synthetic vitamin E by separating the stereoisomers of α -tocopherol and its acetate into two or more peaks. Sample preparation for these analyses is simple with no derivatization required and the chromatographic analysis run time is 35 min for the α -tocopherols, and 15 min for the α -tocopheryl acetate, respectively. For the first time, the differentiation between natural and synthetic vitamin E can be run routinely for food, dietary supplements, infant formula and other related products.

APPLICATION BENEFITS

- Reliable differentiation between natural and synthetic Vitamin E
- Simple and fast solution
- Enables true nutritional label claims
- Methodology designed for routine QC environments

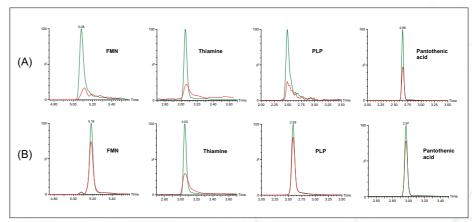
Enhancing the LC-MS/MS Analysis of B-group Vitamins

Waters MaxPeak[™] High Performance Surfaces (HPS) provide an effective solution to mitigate interactions between analytes and metal surfaces in a liquid chromatography flow path. This application note investigates the effects of the MaxPeak HPS on the LC-MS/ MS analysis of B-group vitamins and demonstrates the key benefits for sample analysis, such as energy drinks and vitamin dietary supplements.

Greater sensitivity (3 to 10 times) was observed for riboflavin, thiamine, nicotinamide, flavin mononucleotide, pyridoxal 5'-phosphate, and 5-methyltetrahydrofolate using the Waters ACQUITY Premier Solution. The analytical performance (accuracy and repeatability) of the simultaneous LC-MS/MS analysis of B-group vitamins in energy drinks and vitamin B complex dietary supplements are also presented. The ACQUITY Premier Solution (system and column) showed clear advantages, such as improved sensitivity, less peak tailing, better calibration linearity, and no carry-over issue, over the conventional liquid chromatography solution for the analysis of B vitamins.

APPLICATION BENEFITS

- Waters ACQUITY Premier Solution improves LC-MS/MS analysis of B vitamins
- 3 to 10 times better sensitivity were observed for six B vitamins using the MaxPeak HPS than the conventional stainless-steel surfaces
- Higher response, less peak tailing, and less carry-over are observed with Waters ACQUITY Premier Solution



Comparison of LC-MS chromatograms of FMN, Thiamine, PLP, and Pantothenic acid obtained on the ACQUITY Premier solution (green traces) and the conventional system setup (red traces). (A) Observed during the initial injections of the same standard mix on fresh LC systems. (B) Observed in the B vitamin analysis of the same dietary supplement sample on LC systems that have been extensively used.

Vitamins

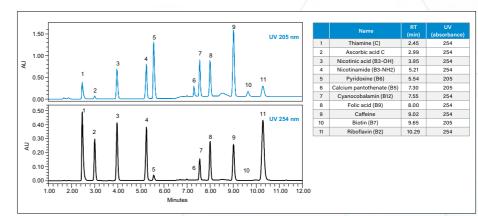
Analysis of Water-Soluble Vitamins and Caffeine in Beverage and Multivitamin Products

The Food and Drug Administration (FDA) has established recommended vitamin intake levels and requires the accurate labeling of products to provide consumers with important information to prevent overdose or overconsumption. Food and beverage manufacturers are required to clearly indicate vitamin compounds, colorings, and other additives that have been included in their products. Manufacturers must comply with strict regulatory requirements, such as European Regulation (EC) No.1925/2006 regarding the addition of vitamins and minerals to foods or Title 21 of the U.S. Code of Federal Regulations (CFR) Part 101 – Food Labeling2 and Part 104 – Nutritional Quality Guidelines for Foods. Once a product has been formulated, food and beverage manufacturers require rapid, reliable, and cost-effective methods to analyze the nutritional content of their products to ensure that their label claims can be substantiated.

This application note outlines a robust analytical method that has been developed to detect and quantify 10 water-soluble vitamins and caffeine in multivitamin tablets and vitamin enhanced beverages. The separation was performed using an Arc HPLC-PDA system combined with an XSelect[™] HSS T3 XP Analytical Column.

APPLICATION BENEFITS

- Efficient test method for multivitamin tablets and vitamin beverages in 16-minutes
- Excellent reproducibility, accuracy, precision of Arc HPLC-PDA with the XSelect HSS T3 XP Column



Separation of 10 vitamins and caffeine at 205 nm and 254 nm.

 \exists

Organic Acids

0-(\$-0

()

Ó

0

\$% ____

Q

0

Sugar Substitutes

Sugars

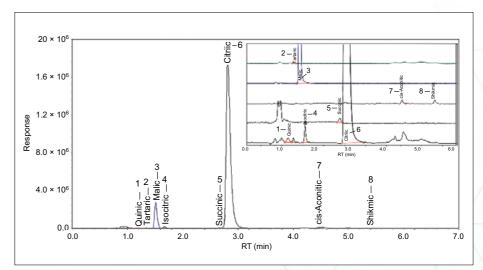
Vitamins

Analysis of Organic Acids Using a Mixed-Mode LC Column

Organic acids (OA) are an important group of compounds for many industries including food and beverages, animal feeds, and human health. The separation of fifteen organic acids on a mixed-mode LC column, the Atlantis Premier BEH C₁₈ AX Column, has been studied using an ACQUITY UPLC H-Class System coupled with an ACQUITY QDa Mass Detector. The effects of the chromatographic conditions, such as organic solvent content, ionic strength and pH of the mobile phase, on the retention and selectivity of organic acids were studied. An analytical method for organic acids has been developed and applied to fruit juices. The performance characteristics of the analytical method, including the limit of quantitation (LOQ), the relationship between the chromatographic peak area and concentration, the precision and the accuracy have been evaluated. This analytical approach has good retention and resolution of OA, the run time is short, and the detection is sensitive and selective. This solution is suitable for the determination of organic acids in fruit juices and beverages as well as other application areas.

APPLICATION BENEFITS

- Excellent retention with improved chromatographic resolution
- Highly sensitive detection that can benefit authenticity testing of fruit juices and beverages
- Highly selective detection that is less prone to interference from co-eluting compounds in the sample matrix
- Fast analysis with run time, less than 8 minutes



SIR chromatogram overlay of a pomegranate juice. The insert shows the detected OA chromatograms at enlarged scale with baseline offset.

Analysis of Fourteen Organic Acids in Various Beverages

Organic acid profiles vary between different beverages, and they contribute to the sensory properties of food and beverages, adding to aroma and taste. It is useful to monitor organic acids in beverages for quality control checks, to ensure ingredients are within product specifications, for consistent taste as well as for the evaluation of fruit juice authenticity and purity.

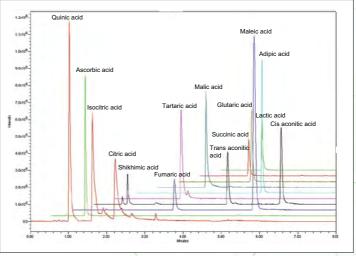
The use of single quadrupole mass detectors such as the ACQUITY QDa, offers several advantages over optical detectors, such as UV-Vis. The advantages include increased selectivity, lower detection limits, and the ability to collect mass spectral information on components of a sample. In this application note, we highlight a method for the separation of fourteen organic acids using the ACQUITY Premier CSH Phenyl-Hexyl Column. The analysis was carried out on an ACQUITY UPLC H-Class PLUS coupled with an ACQUITY QDa Mass Detector. The use of the ACQUITY QDa Mass Detector improves the selectivity of the method, resulting in less impact on the analysis from sample matrix co-elutions, allowing for easier integration of the target organic acids and more accurate analysis.



Read the Full Application Note

APPLICATION BENEFITS

- A single chromatography method for the analysis of fourteen organic acids without the need for sample derivatization, ion-pairing reagents or buffered mobile phase
- Greater selectivity and sensitivity are achieved using the ACQUITY QDa Mass Detector in Single Ion Recording (SIR) acquisition mode, reducing the chromatographic interferences from co-eluting matrix compounds
- A single method for multiple types of beverage samples such as fruit juices, wines, energy drinks, and sparkling water
- Efficient sample analysis with a run time less than 11 minutes per sample



Organic acids detected in a red wine sample.

Fast, Accurate and Flexible Method for the Determination of Citric Acid In Beverages

Citric acid is used as an additive or acidulant in many beverages to improve taste and flavour. An analytical method for citric acid has been developed using the ACQUITY Premier CSH Phenyl-Hexyl Column, with the ACQUITY UPLC H-Class System coupled with an ACQUITY UPLC PDA Detector. The performance of the method was evaluated by assessing parameters such as peak shape, linearity, and precision. The developed method was applied to energy and sports drinks samples. The quantification of citric acid in the samples was reported using Empower 3 Software. The combination of the ACQUITY Premier CSH Phenyl-Hexyl Column, with the ACQUITY UPLC H-Class, coupled with the ACQUITY UPLC PDA method and Empower 3 Software enables the quantification of citric acid in various beverages.

| BBRIN SampleName Inj Channel Name RT Area Height Amount Units USP Tallir 0800 0800 1 SD 10X 1 PDA Ch1 210nm@4.8nm Ctric acid 1.831 192588 7658 1688 ppm 1.70 0800 0800 3 SD 10X 2 PDA Ch1 210nm@4.8nm Ctric acid 1.835 194545 7034 170 ppm 1.71 0800 3 SD 10X 3 PDA Ch1 210nm@4.8nm Ctric acid 1.831 192588 76687 1705 ppm 1.71 0800 SD 10X 4 PDA Ch1 210nm@4.8nm Ctric acid 1.831 192582 76087 1705 ppm 1.72 0800 SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.831 192582 7110 170 ppm 1.72 0800 SD 10X 6 PDA Ch1 210nm@4.8nm Ctric acid 1.841 195096 7110 170 pm | 0.000 | 1 | | | | Component Summary Table Name: Citric acid | | | | | | | |
|---|--------|--------|-------|------------|-----|--|-------------|-------|----------|--------|--------|-------|------------|
| 2 SD 10X 2 PDA Ch1 210nm@4.8nm Ctric acid 1.83 194575 76687 1705 ppm 1.71 3 SD 10X 3 PDA Ch1 210nm@4.8nm Ctric acid 1.835 194575 76687 1705 ppm 1.71 3 SD 10X 3 PDA Ch1 210nm@4.8nm Ctric acid 1.831 194545 77034 1705 ppm 1.71 4 SD 10X 4 PDA Ch1 210nm@4.8nm Ctric acid 1.831 194545 77034 1705 ppm 1.71 9809 5 SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.831 194252 77106 1703 ppm 1.72 6 SD 10X 6 PDA Ch1 210nm@4.8nm Ctric acid 1.844 195096 77110 1710 ppm 1.74 9809 2010X 7 PDA Ch1 210nm@4.8nm Ctric acid 1.838 195932 77426 1718 ppm 1.74 9809 1 | 0.070- | 4 | 2 | SampleName | Inj | Channel | Name | RT | Area | Height | Amount | Units | USP Tailin |
| 3 SD 10X 3 PDA Ch1 210nm@4.8nm Ctric acid 1.837 194545 77034 1705 ppm 1.71 4 SD 10X 4 PDA Ch1 210nm@4.8nm Ctric acid 1.837 194545 77034 1705 ppm 1.72 9880- 9880- 9880- 9880- 9880- 9880- 9880- 9880- 9880- SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.837 194545 77034 1705 ppm 1.72 5 SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.838 194262 77106 1703 ppm 1.72 6 SD 10X 6 PDA Ch1 210nm@4.8nm Ctric acid 1.844 195096 77110 1710 ppm 1.74 7 SD 10X 7 PDA Ch1 210nm@4.8nm Ctric acid 1.831 194565.5 1706.2 1714 Mean 1 1.8 194665.5 1706.2 1714 1744 | 0.060- | | 1 | SD 10X | 1 | PDA Ch1 210nm@4.8nm | Citric acid | 1.831 | 192588 | 76558 | 1688 | ppm | 1.70 |
| 3 SD 10X 3 PDA Ch1 210nm@4.8nm Ctric acid 1.837 194545 77034 1705 ppm 1.71 4 SD 10X 4 PDA Ch1 210nm@4.8nm Ctric acid 1.840 195668 76997 1715 ppm 1.72 5 SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.840 195668 76997 1715 ppm 1.72 5 SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.844 195056 7110 ppm 1.72 6 SD 10X 6 PDA Ch1 210nm@4.8nm Ctric acid 1.844 195056 7110 ppm 1.74 0809 SD 10X 7 PDA Ch1 210nm@4.8nm Ctric acid 1.88 195932 7426 1718 ppm 1.74 0809 Mean 1.8 194666.5 11706.2 | 0.050- | Citric | 2 | SD 10X | 2 | PDA Ch1 210nm@4.8nm | Citric acid | 1.835 | 194575 | 76687 | 1705 | ppm | 1.71 |
| 4 SD 10X 4 PDA Ch1 210nm@4.8nm Ctric acid 1.8d 195668 76997 17.15 ppm 1.72 8809 5 SD 10X 5 PDA Ch1 210nm@4.8nm Ctric acid 1.830 194262 77106 1703 ppm 1.72 6 SD 10X 6 PDA Ch1 210nm@4.8nm Ctric acid 1.844 195096 77110 1710 ppm 1.72 6 SD 10X 6 PDA Ch1 210nm@4.8nm Ctric acid 1.844 195096 77110 1710 ppm 1.74 7 SD 10X 7 PDA Ch1 210nm@4.8nm Ctric acid 1.88 195932 77426 1718 ppm 1.74 Mean 1.8 194686.5 1070.2 | | | 3 | SD 10X | 3 | PDA Ch1 210nm@4.8nm | Citric acid | 1.837 | 194545 | 77034 | 1705 | ppm | 1.71 |
| 3 30 100 3 100 | 0.040- | | 4 | SD 10X | 4 | PDA Ch1 210nm@4.8nm | Citric acid | 1.840 | 195668 | 76997 | 1715 | ppm | 1.72 |
| 7 SD 10X 7 PDA Ch1 210nm@4.8nm Ctric acid 1.838 195932 77426 1718 ppm 1.74 Mean 1.8 194666.5 1708.2 | 0.000 | | 5 | SD 10X | 5 | PDA Ch1 210nm@4.8nm | Citric acid | 1.839 | 194262 | 77106 | 1703 | ppm | 1.72 |
| Mean 1.8 194666.5 1706.2 | 0.020- | | 6 | SD 10X | 6 | PDA Ch1 210nm@4.8nm | Citric acid | 1.844 | 195096 | 77110 | 1710 | ppm | 1.74 |
| Mean 1.8 194666.5 1706.2 | 0000 | | 7 | SD 10X | 7 | PDA Ch1 210nm@4.8nm | Citric acid | 1.838 | 195932 | 77426 | 1718 | ppm | 1.74 |
| 800 02 06 04 0.6 | | | Mean | | | | | 1.8 | 194666.5 | | 1706.2 | | |
| | 0.000 | | % RSD | | | | | 0.2 | 0.6 | 0.4 | 0.6 | | |

An overlay chromatogram of seven injections of sports drink containing citric acid. The Empower summary table reports peak area, retention time, and calculated amount. The mean values, %RSD, and standard deviation are reported here

APPLICATION BENEFITS

- Simple, accurate, and sensitive UPLC-PDA method for citric acid analysis
- Utilization of an ACQUITY Premier CSH Phenyl-Hexyl Column to improve the peak shape and sensitivity of citric acid for the analysis of sports and energy drinks for quality control indicators and to meet product specifications to simplify this
- Data collection and processing with the Empower 3 Quick Start Interface, supporting a streamlined user experience

Amino Acids

0-05-00

ТТ \$

0-0

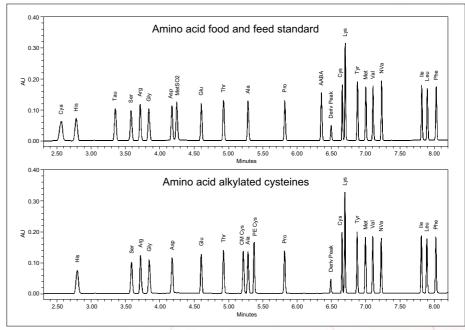
0000

Successful Adaptation of Amino Acid Analysis Methods From an ACQUITY UPLC to an ACQUITY UPLC H-Class PLUS Binary System

In the following study, the methods used for amino acid analysis using AccQ•Tag[™] derivatization will be migrated from the ACQUITY UPLC to the ACQUITY UPLC H-Class PLUS Binary System. It is critical to understand how instrument design differences can impact this process, while preserving all critical performance characteristics, which can include peak shape, resolution, linearity, limits of detection/ quantification, and intra/interday precision to name a few. In this application note, we will show successful method adaptation for multiple amino acid application areas, including those amino acids found in protein hydrolysate, cell culture, food and feed, and alkylated cysteines samples, from the ACQUITY UPLC to the ACQUITY UPLC H-Class PLUS Binary System. The critical performance characteristics have been maintained after method adaptation. Additionally, quantitative analysis of taurine in multiple energy drinks yielded nearly identical results, further proof that the method adaptation has been successful.

APPLICATION BENEFITS

- AccQ-Tag Ultra Chemistry Kit which includes the column, standards and reagents, and eluents for fast, reliable, and reproducible amino acid derivatization, separation, and quantification
- Increased lab productivity and flexibility demonstrated by adaptation of established methods to newer technology
- All critical performance characteristics are maintained, including peak shape, resolution, linearity, limit of quantification, inter/intraday precision after method adaptation

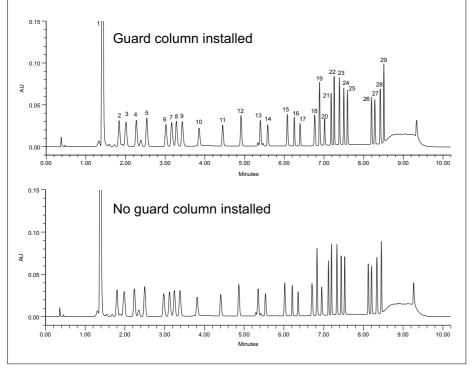


Representative chromatograms for the amino acid analysis food and feed standard and cell culture standard.

Read the Full Application Note

22

Vitamins



Analysis of the cell culture media standard on the AccQ-Tag Ultra Column with (top) and without (bottom) guard column installed

Comparable Chromatographic Performance for Amino Acid Analysis Using the AccQ-Tag Ultra VanGuard Pre-Column

The analysis of complex matrices can cause column performance issues when performed routinely. In plasma, cell culture media, or food samples, the presence of endogenous compounds like lipids or proteins can cause the inlet frit of the column to become blocked over time, leading to increased column pressure and decreased column lifetime. One way to mitigate this is to use guard columns, which can be routinely changed to protect the analytical column from fouling. The work shown here compares the chromatographic performance of the AccQ-Tag Ultra Column with and without an AccQ-Tag Ultra guard for the analysis of neat cell culture media standard.

APPLICATION BENEFITS

- AccQ-Tag VanGuard[™] Pre-Column may increase analytical column lifetime
- Comparable chromatographic performance obtained with and without pre-column



Read the Full Application Note

Food and Beverage Quality Control and Compositional Testing | Application Notebook

Amino Acid Analysis using Andrew+ Automated Preparation

Amino acids are the most basic components that make up proteins, thus making them essential components of cell culture media and food stuffs. Monitoring and optimizing the amino acid components of bioreactor media is essential for ensuring the best growing conditions for the cells. Likewise, it is necessary to confirm that food products meet specified requirements. Therefore, the analysis of amino acids is a critical routine process.

The preparation and analysis of samples is a time-consuming process that can dominate an analyst's time in the laboratory. Automated laboratory preparation systems provide the flexibility of freeing

| | | Andrew + | | Manual | | | | |
|---------|-------|----------|--------|--------|--------|--------|--|--|
| Analyte | 10 µM | 200 µM | 400 µM | 10 µM | 200 µM | 400 µM | | |
| Суа | 0.6 | 0.9 | 2.3 | 2.1 | 2.5 | 1.6 | | |
| His | 0.2 | 1.0 | 2.4 | 0.4 | 2.8 | 1.5 | | |
| Tau | 0.9 | 1.0 | 2.5 | 1.4 | 2,8 | 1.5 | | |
| Ser | 0.7 | 0.8 | 1.6 | 2.0 | 2.8 | 1.5 | | |
| Arg | 0.5 | 1.0 | 2.3 | 0.6 | 2.9 | 1.7 | | |
| Gly | 1.8 | 1.0 | 2.1 | 1.1 | 2.8 | 1.6 | | |
| Asp | 0.5 | 1.2 | 1.2 | 1.0 | 2.7 | 1.7 | | |
| MetSO2 | 0.9 | 0.8 | 2.1 | 1.1 | 2.9 | 1.5 | | |
| Glu | 0.3 | 1.0 | 0.9 | 0.6 | 2.8 | 1.6 | | |
| Thr | 0.6 | 0.8 | 1.5 | 1.4 | 2.9 | 1.5 | | |
| Ala | 0.4 | 0.9 | 0.9 | 0.9 | 2.8 | 1.6 | | |
| Pro | 0.5 | 0.8 | 1.2 | 1.3 | 2.8 | 1.5 | | |
| AABA | 0.3 | 0.8 | 0.9 | 0.6 | 2.8 | 1.5 | | |
| Cys | 0.4 | 1.0 | 2.3 | 0.7 | 2,8 | 1.5 | | |
| Lys | 0.4 | 1.2 | 1.4 | 0.7 | 2,8 | 1.7 | | |
| Tyr | 0.6 | 1.0 | 2.6 | 0.8 | 2.9 | 1.5 | | |
| Met | 0.3 | 0.9 | 1.9 | 0.8 | 2.8 | 1.7 | | |
| Val | 1.5 | 0.8 | 1.1 | 2.2 | 2.9 | 1.5 | | |
| lle | 0.3 | 0.8 | 1.1 | 0.9 | 2.9 | 1.5 | | |
| Leu | 0.4 | 0.8 | 1.2 | 0.8 | 2.8 | 1.6 | | |
| Phe | 0.5 | 1.0 | 2.6 | 0.8 | 2.8 | 1.5 | | |

Food and feed %CV for Andrew+ and manual preparation across 10 μ M, 200 μ M, and 400 μ M solvent panels.

analysts time for other tasks, resulting in a more efficient way of time management. The objective of this application note is to demonstrate the equivalency and robustness of manual preparations of AccQ•Tag labelled amino acids to those prepared using the Andrew+[™] liquid handling robot with amino acid standard kits.

APPLICATION BENEFITS

- The Andrew+ robot provides efficiency without compromising accuracy and precision with calibration line preparation and sample preparation performed in under an hour
- The automation protocol developed requires no manual intervention during the run, taking advantage of features like the Bluetooth configured pipettes which switch between volumes and the gripper device to transfer labware, thus allowing the analyst time to perform other laboratory tasks
- The OneLab cloud-based software allows the user to monitor the run from any internet connected computer or tablet they have available
- The use of automation removes analyst-to-analyst variation allowing laboratories and companies to standardize analysis methods and facilitate method transfer between multiple sites

Read

Read the Full Application Note

Food and Beverage Quality Control and Compositional Testing | Application Notebook

| Sugar Substitutes | Sugars | Vitamins | Organic Acids | Amino Acids |
|-------------------|--------|----------|---------------|-------------|

Links to other Useful Materials

- Selecting a liquid chromatography solution for testing sugars and sweeteners in beverages
- Manufacturer Reduces Vitamin QC Analysis Times by Up to 90%
- Automate Standard Preparations for Food Analyses – A Real-World Evaluation
- Liquid Handling Automation System Streamlines Sample Preparation for Nutritional Analysis
- Improving Method Reproducibility and Efficiency in Food Testing: How Can Liquid Handling Automation Help?





Waters, The Science of What's Possible, Arc, Atlantis, BEH, ACQUITY Ultra Performance LC, ACQUITY, CORTECS, Empower, ACQUITY Arc, XBridge, ACQUITY UPC², Trefoil, MaxPeak, XSelect, AccQ Tag, VanGuard, and Andrew+ are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2022 Waters Corporation. Produced in the U.S.A. August 2022 720007689EN GJ-PDF

 Waters Corporation

 34 Maple Street

 Milford, MA 01757 U.S.A.

 T: 1 508 478 2000

 F: 1 508 872 1990

 waters.com