Analysis of Food Additives in Beverages Using Syringe Filter Filtration and HPLC

Application Note

Food Testing and Agriculture

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Abstract

Agilent Captiva Premium syringe filters were tested thoroughly for sample preparation in the analysis of food additives in beverages. The sample preparation method targeted sample filtration for direct injection or dilute-and-shoot prior to HPLC analysis. Thirteen popular food additives were selected for filter evaluation, covering a wide variety of chemical and physical properties. An HPLC method using an Agilent Poroshell 120 EC-C18 column was used to evaluate the recovery of target analytes after filtration. The results demonstrated that, with appropriate selection of filtration membrane based on sample medium and target analytes, Agilent Captiva Premium syringe filters provided excellent recoveries for a wide variety of food additives. The developed method was then used to identify food additives in sixteen beverage products.

Introduction

Non-nutritive food additives, such as preservatives, sweeteners, colorants, and stimulants, are frequently used in beverage and food products. These additives are generally safe, but could be harmful at certain levels, potentially causing allergy and hyperactivity in children, and so their use might be restricted in some countries. Therefore, the analysis of these compounds in food quality control is important to ensure that the use of additives meets international food quality control criteria.

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Many synthetic or artificial additives are water soluble, making them ideal for analysis by HPLC. Most beverage products are already LC-compatible with less complicated sample matrices, and so these samples are usually suitable for direct injection, or dilution followed by injection onto LC columns. However, particulates in food samples can have negative impacts on columns. As a result, filtration prior to HPLC is usually the major or only sample preparation procedure for the analysis of additives in beverages. In particular, for some samples with a large number of particulates such as juice, filtration using depth filters becomes more necessary. Some drinkable products, such as drinkable yogurt or iced coffee, have milk added, which contains proteins and requires simple protein precipitation by organic solvents prior to filtration.

The major concern when using sample filtration is whether it causes sample loss. The potential sample loss can result from unwanted interaction between the filter membrane and analytes. The target compounds' physical properties, chemical structure, ionization properties and molecular weight, as well as product formulations, could affect the interactions. On the other hand, the membrane property also contributes to the interactions, such as the membrane polymer's chemical structure, hydrophobicity or hydrophilicity, polymer formulation, and purity, among others. When unwanted interactions happen, analytes can be bound nonspecifically onto the membrane during filtration.

Poorer solubility of analytes in sample media can also result in compound loss as semidissolved compound clusters can be blocked by membrane filtration. For relatively less polar compounds, the target's solubility in the sample medium can greatly impact filtration recoveries [1]. Therefore, the sample medium is another factor that can induce sample loss, as it can directly affect compound solubility and membrane wettability.

As a result, the selection of filter membrane depends on the sample medium, and membrane and sample interaction. However, the interaction of membrane and target compounds is usually hard to predict; therefore, filter-membrane selection usually starts from consideration of the sample medium. For aqueous-based samples, hydrophilic-type membranes are preferred, such as cellulose acetate (CA), polyethersulfone (PES) and regenerated cellulose (RC). For organic-solvent-based samples, especially for aggressive solvents, polypropylene (PP) or polytetrafluoroethylene (PTFE) filters should be used. For mixtures of organic/aqueous samples, PTFE, PP, nylon (polyacrylamide, PA), RC, and PES filters can be used according to solvent composition. Preliminary tests on filtration recovery are therefore highly recommended, using selected filters to prevent unwanted interactions. Other factors contributing to filter selection include sample matrix and volume and request from detection instruments,and so forth.

Agilent Captiva Premium syringe filters have been tested thoroughly for cleanliness and are supplied with either HPLC or LC/MS certificates, demonstrating that they are entirely free of detectable extractables under testing conditions [2]. These thorough tests and certification help reduce concerns on potential contamination introduced through filtration.

In this study, different types of syringe filters were evaluated for the analysis of a broad group of food additives in beverages, based on filtration recoveries. The selected appropriate syringe filters were then used to prepare beverage or food samples prior to HPLC analysis.

Experimental

Instrumentation

The HPLC method was developed based on an Agilent Poroshell 120 column method [3] using an Agilent 1200 HPLC system.

Column:	Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695975-302)
Eluent:	A: 20 mM Ammonium acetate, pH 4.8 B: acetonitrile
Injection volume:	3 µL
Flow rate:	0.851 mL/min
Gradient:	Time % B(min) 0.01 14 2.1 52 2.8 52 2.81 100
Total cycle time:	4.5 min with 3 min for sample run and 1.5 min for post equilibrium
Temperature:	30 °C
Detector:	DAD SL with the signal set to 235 and 254 nm

Chemicals and reagents

Pure food additive standards and ammonium acetate were purchased from Sigma-Aldrich Corp. (St Louis, MO, USA). HPLC grade acetonitrile (ACN) and methanol (MeOH) were from Honeywell (Muskegon, MI, USA).

Thirteen popular food additives were selected to assess filter performance, including preservatives, colorants, and sweeteners. Figure 1 shows the chemical structure and pKa values of the food additives.

Solutions and standards

Individual stock solutions (5.0 mg/mL) of each compound were prepared by dissolving compound powder in Milli-Q water or methanol. Solutions were mixed by vigorously vortexing to ensure complete dissolution. A mixed standard solution, containing 50 µg/mL of the 12 compounds and 300 µg/mL of aspartame, was made in water by appropriate dilution of compound stock solutions. This standard mixture was used for HPLC method development and peak identification. For the evaluation of filtration recovery, a combined spiking solution with higher concentration was made by combining 200 µL of each stock solution of the 12 compounds and 600 µL of aspartame stock. This solution was used to spike into beverage samples to make equivalent concentrations of 50 µg/mL (150 µg/mL for aspartame).

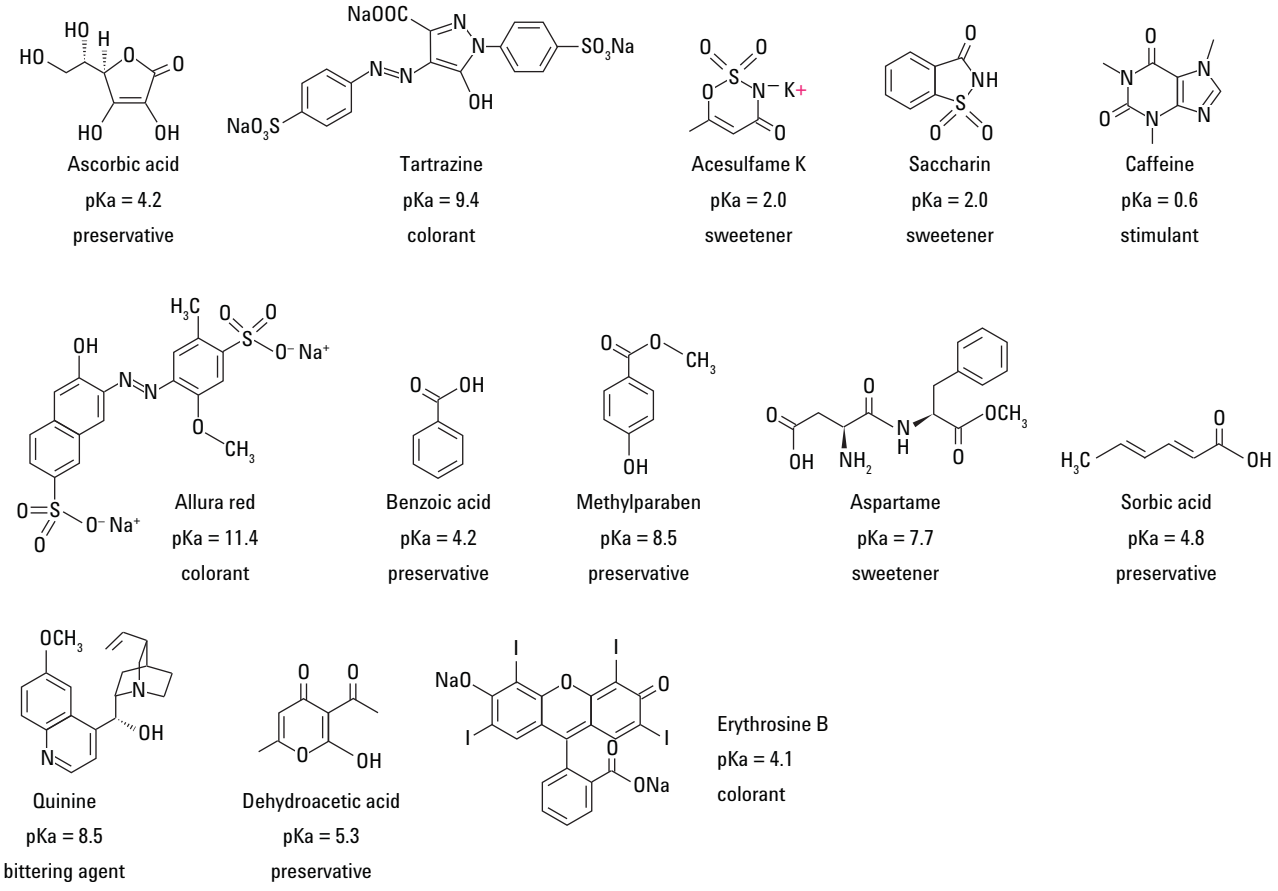

Figure 1. Food additives used in this work.

Sample preparation

Sixteen different beverage products were purchased from a local supermarket. These samples were either directly used or appropriately diluted with water or organic solvent, depending on the abundance of additives and the sample matrix. Organic solvents such as acetonitrile or methanol were added to milk-containing samples to precipitate proteins. The samples were then filtered to remove the particulates and subsequently analyzed by HPLC.

Two samples were selected to be spiked with additive standards to evaluate filtration recovery. A sports drink, Thirst Quencher, was used for aqueous-based sample evaluation. The appropriate volume of standard spiking solution was added to a Thirst Quencher blank to make a final additive concentration of 50/150 µg/mL. Two milliliters of this sample was either centrifuged, or filtered through various Captiva Premium syringe filters prior to HPLC analysis. A dietary drink, Dannon Yogurt, was used for the evaluation of samples in aqueous/organic solvent media. Similarly, the mixed spiking solution was added to the Dannon Yogurt blank to make a final concentration of 50/150 µg/mL. The spiked sample was then diluted with an equal volume of methanol to make the final sample medium as 1:1 MeOH:water. As before, 2 mL of the sample was either centrifuged or filtered by various Captiva Premium syringe filters prior to HPLC analysis.

The following syringe filter types were evaluated for filtration recovery of food additives.

For samples in aqueous media:

- Agilent Captiva Premium PES syringe filter, 25 mm, 0.2 µm (p/n 5190-5098) and 0.45 µm (p/n 5190-5099)
- Agilent Captiva Premium RC syringe filter, 25 mm, 0.2 µm (p/n 5190-5110) and 0.45 µm (p/n 5190-5111)
- Agilent Captiva Premium CA syringe filter, 28 mm, 0.2 µm (p/n 5190-5116) and 0.45 µm (p/n 5190-5117)

For samples in aqueous/organic solvent media:

- Agilent Captiva Premium RC syringe filter, 25 mm, 0.2 µm (p/n 5190-5110) and 0.45 µm (p/n 5190-5111)
- Agilent Captiva Premium PTFE syringe filter, 25 mm, 0.2 µm (p/n 5190-5086) and 0.45 µm (p/n 5190-5087)
- Agilent Captiva Premium glass fiber/PTFE syringe filter, 25 mm, 0.2 µm (p/n 5190-5128) and 0.45 µm (p/n 5190-5129)
- Agilent Captiva Premium nylon syringe filter, 25 mm, 0.2 µm (p/n 5190-5092) and 0.45 µm (p/n 5190-5093)

Results and Discussion

HPLC separation and peak identification

HPLC columns packed with superficially porous particles have been demonstrated to be superior to conventional columns packed with fully porous particles, by providing much higher column efficiency and significantly shorter runtime. The Agilent Poroshell 120 column offers similar efficiency and selectivity to the sub-2 µm column, without high back pressure. This allows the use of Poroshell 120 columns on regular 400 bar HPLC systems, while still achieving high efficiency, shorter run times, and solvent savings.

The HPLC method was based on a previous method using the Poroshell 120 column [3]. Baseline separation was achieved within just 3 minutes. Two wavelengths (235 nm and 254 nm) were used to monitor all of the targets within one run. The LC chromatogram for the 50 µg/mL standard is shown in Figure 2 for peak identification.

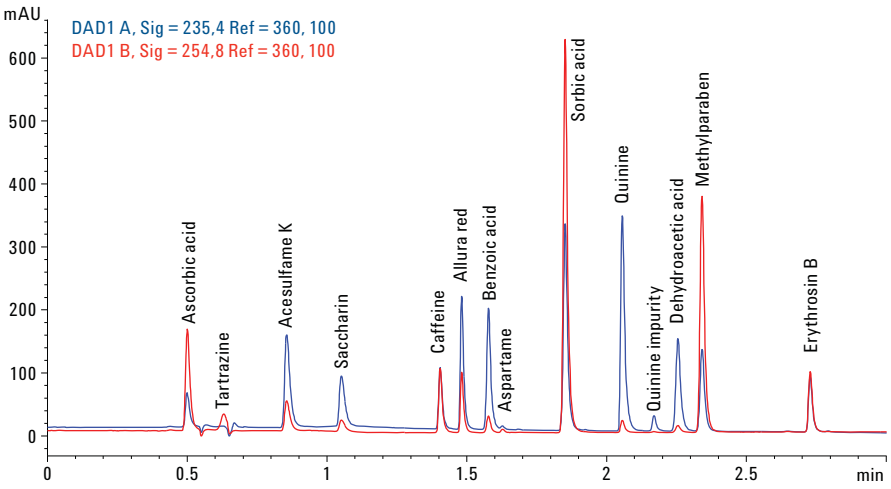

Figure 2. HPLC chromatogram for 50 µg/mL mixed standard (13 food additives).

Filtration recovery evaluation

Since there is no limit on sample size, 25-mm syringe filters were used for evaluation, because these filters could represent the worst adsorption situation due to their largest membrane contact surface area.

The results were evaluated as relative recovery, which is the peak area comparison of analytes in a filtered sample to those in a centrifuged sample. Assuming no analyte loss by centrifugation, the closer the peaks in filtered samples with those in centrifuged samples, the higher filtration recovery. The results are shown in Figures 3 and 4.

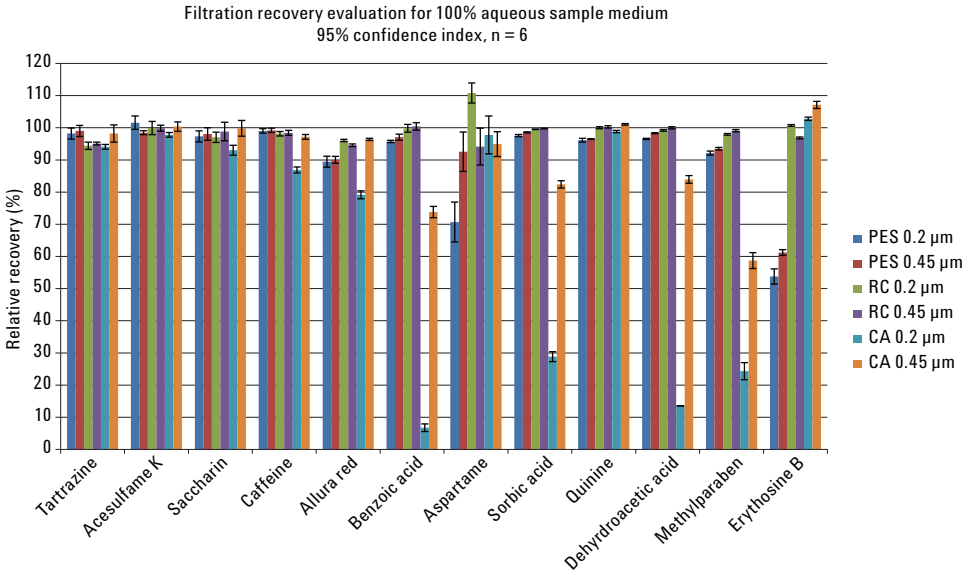

Figure 3. Filtration recovery evaluation results for 100% aqueous sample media, 95% confidence index, n = 6. Thirst Quencher samples directly filtered prior to HPLC injection, relative recovery (%) = normalized peak area ratio for filtered sample compared to centrifuged sample.

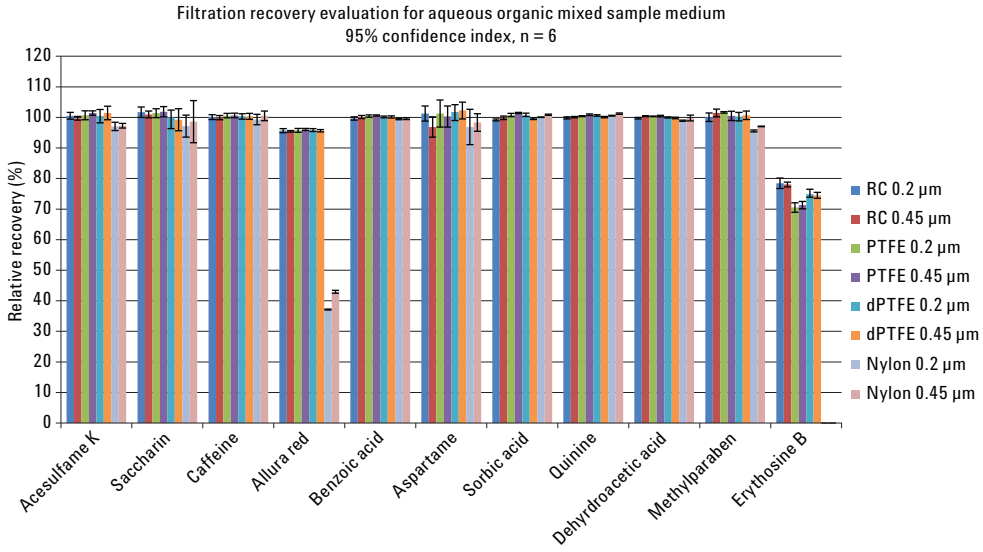

Figure 4. Filtration recovery evaluation results for aqueous/solvent sample media, 95% confidence index, n = 6. Dietary drink samples diluted with MeOH to generate a 1:1 MeOH:water sample medium, vortexed and filtered prior to HPLC injection, relative recovery (%) = normalized peak area ratio for filtered sample compared to centrifuged sample.

For samples in 100% aqueous media, filters with hydrophilic membrane were selected. Regenerated-cellulose (RC) filters provided excellent recovery (>95%) for all of the target analytes. However, PES filters and cellulose acetate (CA) caused loss of some compounds during filtration, especially the CA filters, which led to significant loss of several acids. When the membrane showed adsorption of the target analytes, 0.2-µm membrane filters had more significant adsorption than 0.45-µm membrane filters. This was probably due to the higher density of the 0.2-µm membrane compared to the 0.45-µm membrane. Therefore, when not completely necessary, the 0.45-µm filter should be selected to prevent unwanted membrane adsorption.

For samples in aqueous/organic sample media, filters with hydrophobic membranes were included. Excellent and consistent recoveries were evident for all food additives, except erythrosin B, when using RC, PTFE, and depth PTFE syringe filters. Depth PTFE syringe filters provided easier filtration with less resistance, without suffering more analyte adsorption. For some beverage samples such as juice, the use of depth filters is highly recommended. In general, low recoveries of erythrosin B were seen, probably linked to its relative low solubility in the sample medium. Nylon filters showed significant adsorption (< 50% of recovery) for the food colorants allura red and erythrosin B.

The evaluation results show that RC filters offered the highest recoveries overall in both aqueous and aqueous/organic sample media. Compared to the filtration recoveries by RC filters for aqueous-based samples and aqueous/solvent-based samples, slightly higher recoveries (up to 5%) of most analytes occurred for aqueous/solvent-based sample media. These were linked to the solubility of the compounds in the sample medium. Better solubility helped prevent analyte loss during filtration. Food colorants seemed to be lost easily by filtration. A follow-up study will be conducted to evaluate the filtration impact on food dyes analysis.

It was demonstrated that filtration by 0.45-µm filters prior to LC injection offered acceptable particulate removal to protect the Poroshell 120 column [4]. Since Poroshell 120 on a regular 400-bar HPLC system was used in this study, RC 0.45-µm syringe filters were selected instead of RC 0.2-µm filters for the investigation of 16 beverage samples.

Filtration of beverages

The developed method was used for food additive analysis of 16 beverage products, to further evaluate method suitability in practical applications. As shown in Table 1, different sample preparation procedures were followed for those products. Various food additives were found in 14 products, which were identified based on retention time and UV-spectrum comparison with standards. The chromatogram comparisons are shown in Figure 5, and additive identification and filtration recovery results are in Table 1.

Conclusions

Filtration using syringe filters was shown to be an easy, simple, efficient, and robust sample preparation technique for the analysis of food additives in beverages and drinkable food products. The beverage samples could be either directly filtered or diluted followed by filtration, prior to HPLC analysis. For products containing large amount of particulates such as fruit juice, we recommend using depth filters to prevent the filter membrane being clogged quickly. The selection of filters, including membrane type and pore size, filter dimension, depth or regular, is based on the sample medium and matrix, the properties of target analytes and the needs of instrument analysis. Agilent provides an online selection tool to guide the appropriate filter selection [5]. To prevent unwanted loss during filtration, we suggest conducting a quick-recovery evaluation prior to the use of filters for real-world samples.

In conclusion, Agilent Captiva Premium regenerated-cellulose syringe filters were shown to be an excellent choice for food additive analysis in beverage products, by providing excellent filtration recovery and particulate removal efficiency.

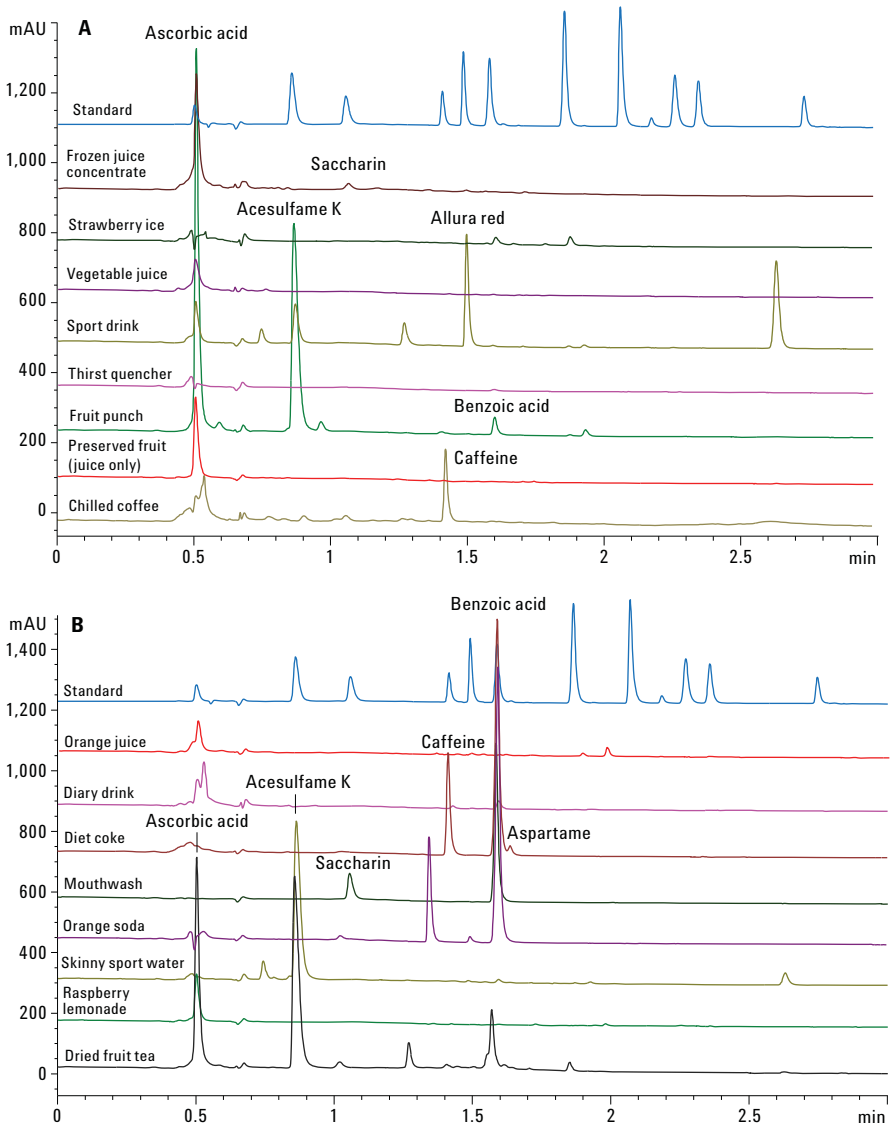

Figure 5. Analysis of food additives in beverage products by HPLC/UV using filtration as the major sample preparation technique. Samples filtered through Agilent Captiva Premium 0.45-µm RC syringe filters. A) Chromatograms comparison for beverage products 1-8; B) Chromatograms comparison for beverage products 9-16.

Table 1. Food additive analysis in beverage products.

Beverage product	Sample preparation	Additives found	Relative filtration recovery (%) mean, n = 3
Frozen juice concentrate	Thaw at room temperature, 10x dilution with water	Ascorbic acid	103.4
		Saccharin	103.1
Strawberry ice	Thaw at room temperature, 2x dilution with MeOH, vortex	N/A	N/A
Vegetable juice	10x dilution with water, vortex	Ascorbic acid	99.4
Sport drink	Direct use	Ascorbic acid	97.0
		Acesulfame K	99.3
		Allura red	94.6
Thirst quencher	Direct use	N/A	N/A
Fruit punch	Direct use	Ascorbic acid	100.8
		Acesulfame K	100.2
		Benzoic acid	100.2
Preserved fruit (only juice analyzed)	10x dilution with water, vortex	Ascorbic acid	98.6
Chilled coffee	3x dilution with acetonitrile, vortex	Caffeine	101.6
Diet coke	Direct use	Caffeine	99.1
		Benzoic acid	99.4
		Aspartame	99.2
Mouthwash	50x dilution with water, vortex	Saccharin	101.4
		Benzoic acid	100.4
Orange soda	Direct use	Allura red	96.2
		Benzoic acid	99.2
Skinny sport water	Direct use	Acesulfame K	99.9
Dairy drink	2x dilution with MeOH, vortex	Benzoic acid	99.9
Raspberry lemonade	5x dilution with water, vortex	Ascorbic acid	100.1
Orange juice	5x dilution with water, vortex	Ascorbic acid	90.9
Dried fruit tea	Dissolve one bag of tea power in 50 mL of water, followed with 10x dilution with water, vortex	Ascorbic acid	100.5
		Acesulfame K	100.0

Relative recovery (%) = normalized peak area ratio for filtered sample to centrifuged sample.

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