The Automated SPE Assay of Sodium Benzoate and Ascorbic Acid from Orange Juice.

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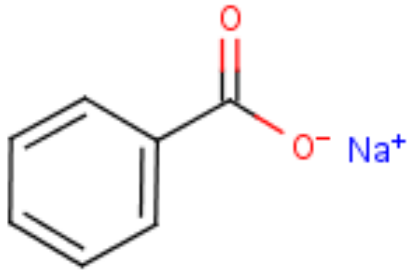
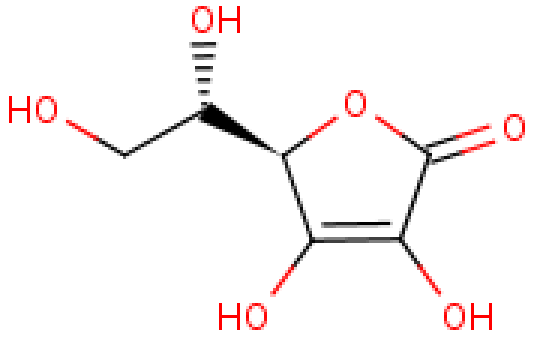
Abstract

Sodium benzoate aka E211, a preservative used in carbonated drinks, can form carcinogen benzene when mixed with vitamin C (ascorbic acid). Four brands have already been removed from the market in 2006. The FDA recently tested 84 soft drink products and found that 54 of them had some detectable benzene. And some had levels as high as 79.2 ppb. Federal rules specify less than 5 ppb in drinking water. This application automates the extraction of Sodium benzoate and vitamin C from orange juice and fruit drinks for analytical processing.

Sodium Benzoate and Ascorbic Acid





Bee Pollen Samples

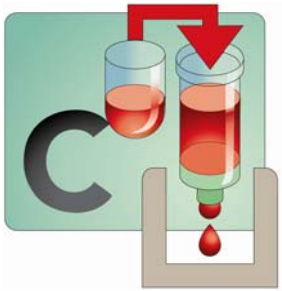
- Orange juice and juice drink samples were prepared
  - 5 ml of juice was combined with with 100 ml of water. Mixture was shaken
  - Orange juice samples were strained with a 0.2 micron filter
  - Samples were analyzed directly for ascorbic acid content.
  - Samples were cleaned by SPE for sodium benzoate analysis

Instrumentation Utilized

- GX-271 Aspec
  - SPE extraction
  - IST C8/anion mixed mode SPE
  - Sample injection
- 305 and 306 Pumps
- 811C Mixer
- 155 UV Detector
- Waters Alantis 4.6 x 150 mm C18 column

SPE Method Optimization

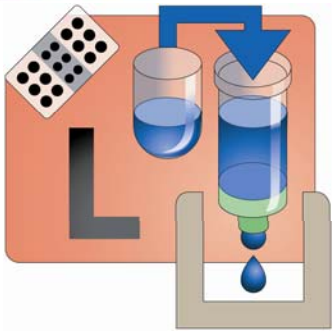
- Optimization is key to precise and accurate analytical measurements
- Sample preparation should be the most scrutinized step in any analyte quantification
- Poor sample preparation can lead to inaccuracies and imprecise measurements.
- Automation can simplify optimization procedures by testing many different sample preparation treatments and optimize the treatment that provides the most accurate and precise results



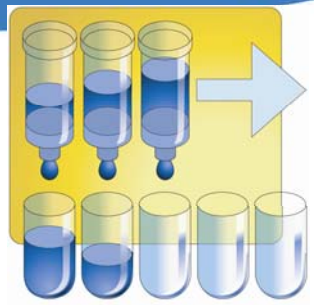
SPE Optimization
Condition

- Condition SPE Cartridge
  - Cartridges are set up with several differing volumes of conditioning agents
  - Several conditioning agents are tested
    - Methanol
    - Water
    - Ethanol
    - Acetonitrile
  - Each volume and agent is tested by Conditioning the column with different volumes and different agents then loading them with analyte. The matrix that elutes from the column is then tested for benzoic acid by injecting onto the HPLC

Conditioning an SPE cartridge via automation is accomplished with software that allows the user to program several variables to optimize the method

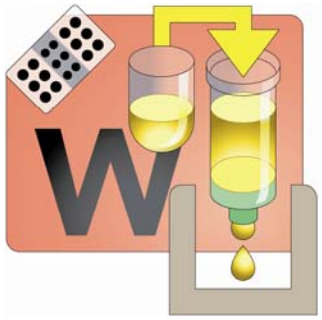


SPE Optimization
Load Sample



- Load sample on cartridge
- Differing volumes of sample are injected onto SPE cartridges
- Differing concentrations of samples are injected each onto SPE cartridges
- Differing matrices are injected each onto SPE cartridges
- Each eluent from each cartridge sample load is tested for benzoic acid by injection onto HPLC.

Loading conditions can be controlled easily with automation software. Each variable can be manipulated for the optimization process.

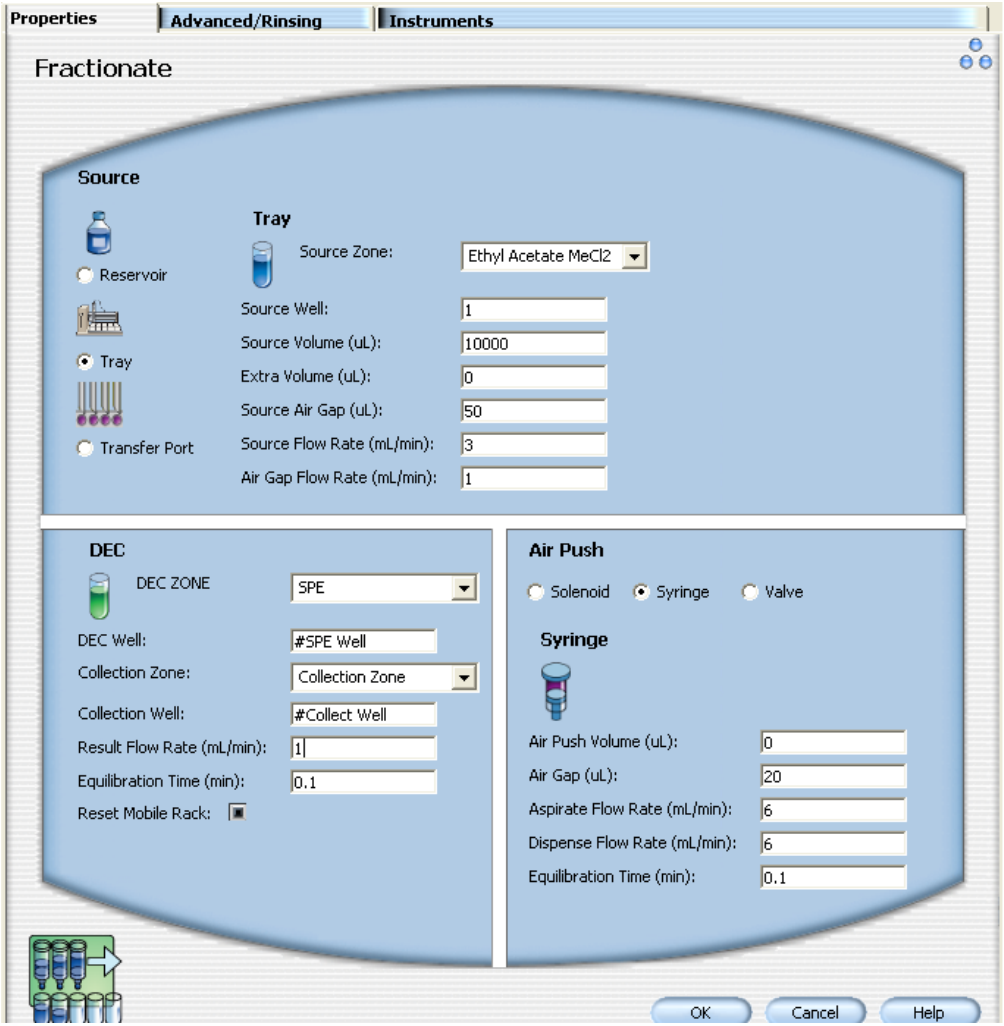


SPE Optimization

Wash Sample

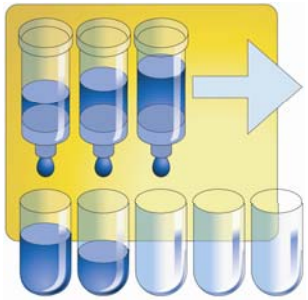
- Once the sample load and condition have been optimized the wash step is then optimized
- Optimized sample is injected onto each cartridge
- Different washes are passed through different cartridges
- Each eluent of the wash is checked for breakthrough of the acaricides.
- Each eluent is checked for interfering compounds that have been washed off.
- Those washes that elute the smallest concentration of benzoic acid and the greatest concentration of interfering compounds are kept and tested with the elution solvents in the next optimization

Optimization of Washing and Eluting can be accomplished within a fractionation task. The SPE cartridge is drawn across a set of test tubes and an allotted amount of wash or eluent is drained into each tube. The contents can then be analyzed for breakthrough of the analyte or elution of any interfering peaks.



SPE Optimization
Elute Benzoic Acid

- After the wash step/steps have been optimized the Elution is then optimized
- Each SPE cartridge has sample loaded and washed
- Each SPE cartridge is then tested with differing elution solvents
- As the solvent is added the eluent is collected in a set of tubes each with the same amount of eluent (this procedure can be used to check washes and loads also)
- Each eluent is checked for breakthrough and concentration of acaricides
- The eluent and wash that delivers the greatest percent of analyte eluted and the least amount of interfering compounds is chosen



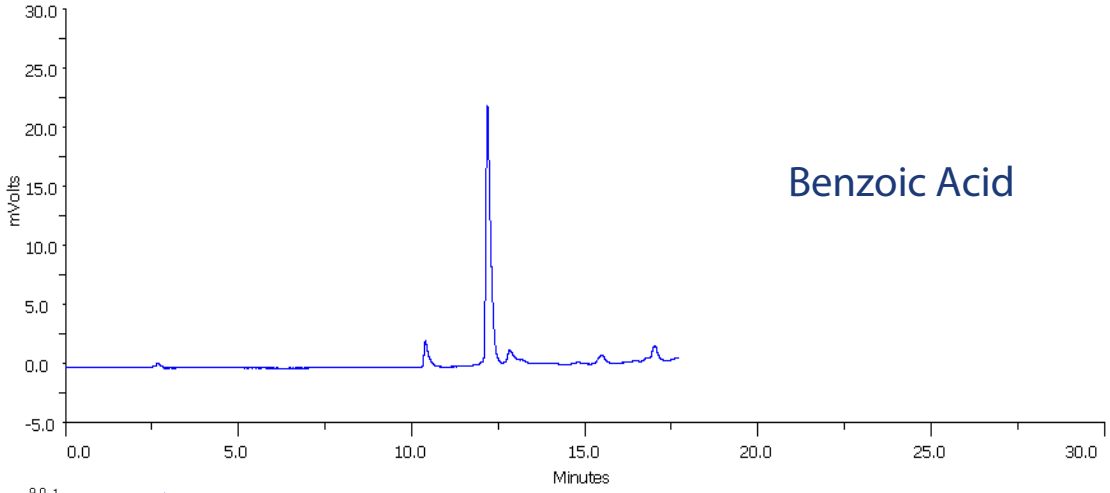
Extraction Parameters

- 5 ml methanol followed by 5 ml water to condition a 3 ml IST C8/anion mixed mode column
- 5 ml water followed by 1 ml water:ethanol:acetic acid 80:20:0.1 as a wash
- Columns were dried
- Elution with 4 ml ethyl methanol 1% ammonium hydroxide
- Dry down with nitrogen and bring back up in 1 ml 50% ACN 50% water solution

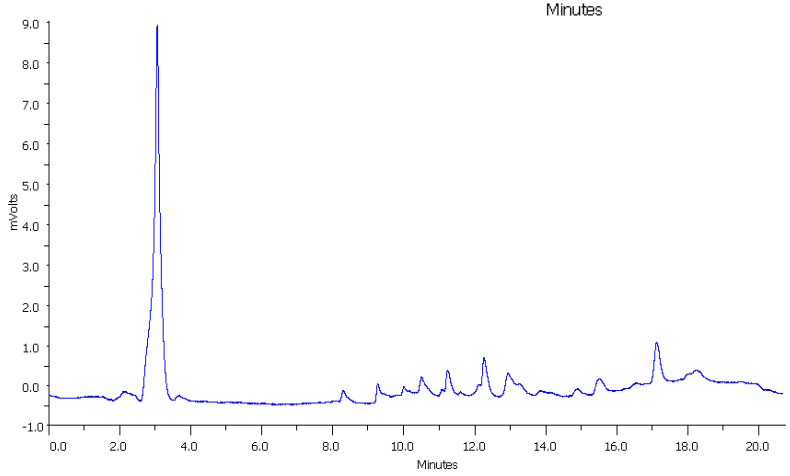
HPLC Separation

- Gradient mobile phase acetonitrile:water/0.1% formic acid 30% ACN for 23 minutes then ramped to 95% over 10 minutes. 95% for 3 minutes then 30% for 5 minutes
- 50 ul injections
- Atlantis C18 column 4.6 x 150 mm
- Mobile phase at 1.0 ml/min
- Peak retention times
  - Ascorbic Acid 2.2 minutes
  - Benzoic acid 12 minutes

HPLC Separation



Benzoic Acid



Ascorbic Acid