Preservative Analysis by Liquid and Gas Chromatography

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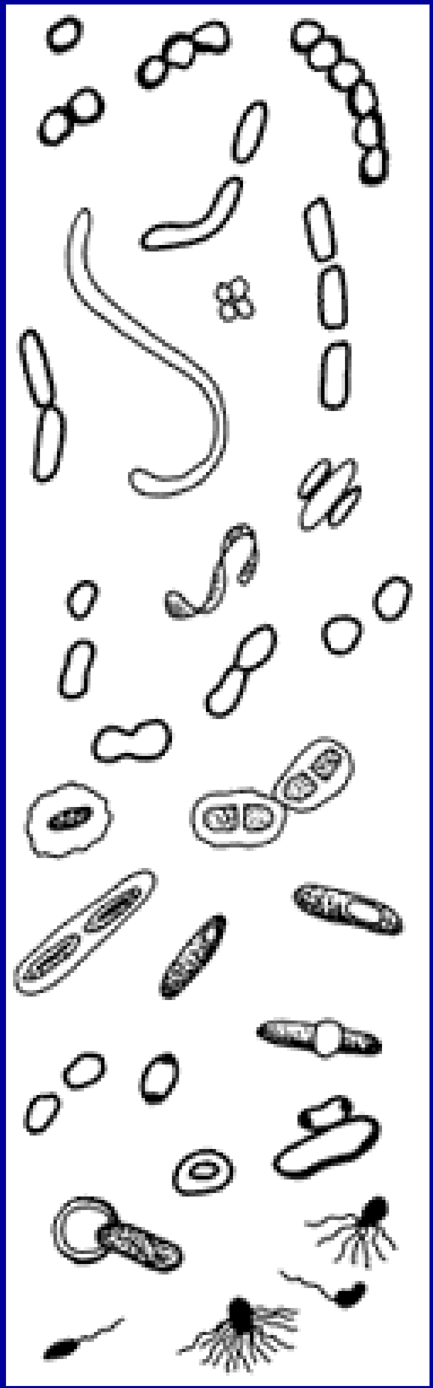
Food Preservatives
Chemical Antimicrobial Agents

Bacteria need to have certain conditions (water content, pH, temperature) in order to flourish…

…chemical preservatives can be used to kill or prevent the growth of molds, yeasts, and bacteria.

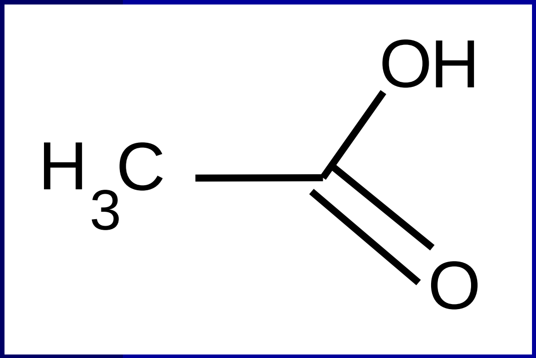
The type of preservative used depends on the food.



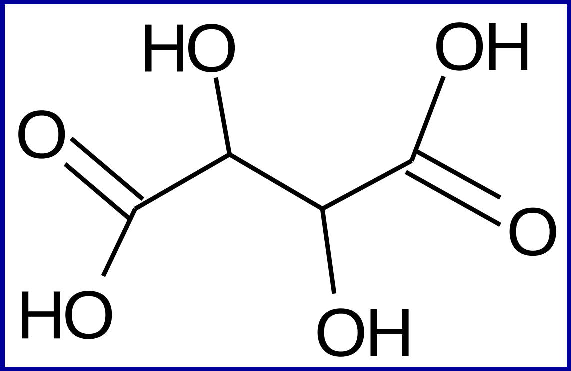
Food Preservatives

Food Organic Acids for pH Adjustment

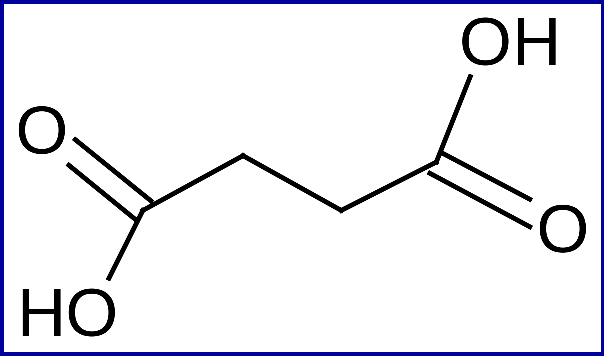
1. Acetic acid



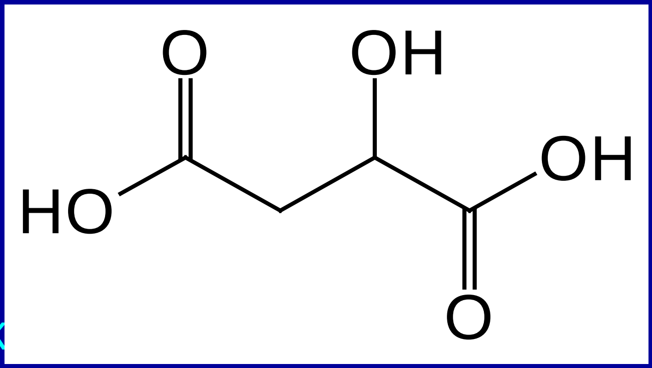
2. Tartaric acid



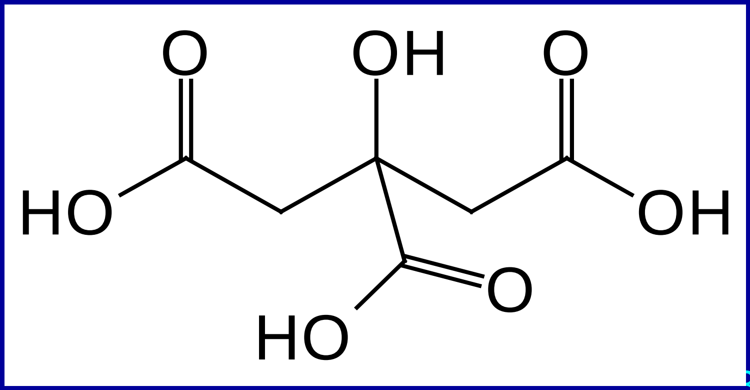
3. Succinic acid



4. Malic acid



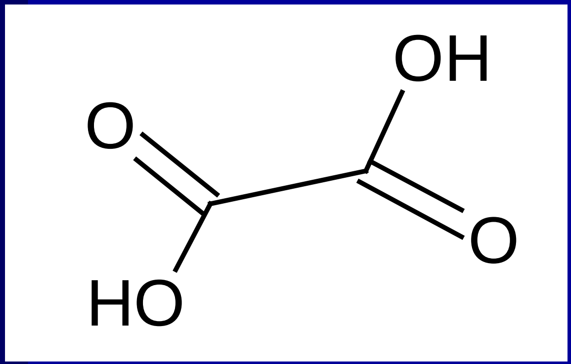
5. Citric acid



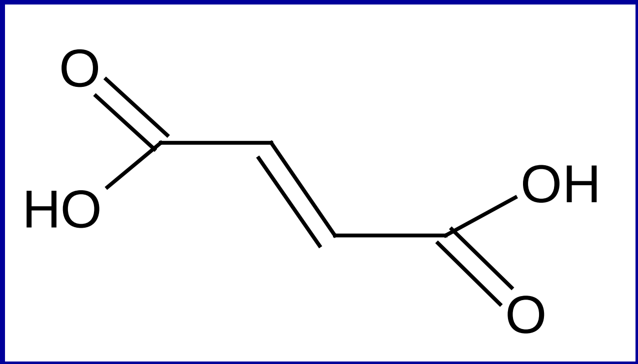
Food Preservatives

Food Organic Acids for pH Adjustment

6. Oxalic Acid



7. Fumaric Acid



Sources of Common Organic Acids:

- Malic acids – fruit
- Citric acid - fruits
- Oxalic acid – spinach
- Tartaric acid – grapes
- Fumaric acid - candies

Food Preservatives

Food Buffers for pH Stability

Acetic Acid / Sodium Acetate Buffer System

$$CH_3COOH \rightarrow CH_3COO^- + H^+$$

$$CH_3COONa + H^+ \rightarrow CH_3COOH + Na^+$$

Compounds that can act as food buffers: organic acids and their salts, proteins, phosphates

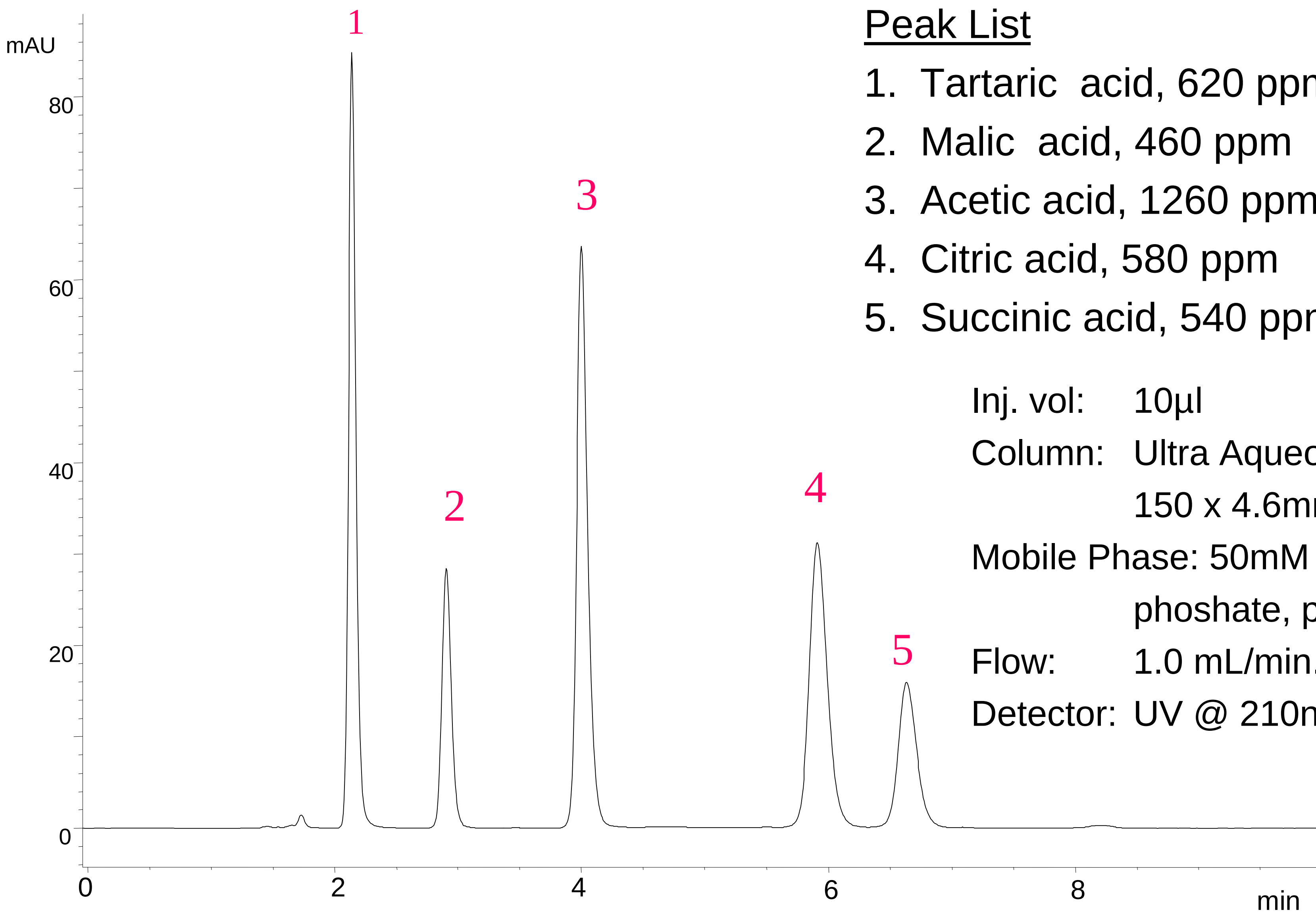
Food Preservatives

Analysis of Organic Acids by HPLC

- Modes of Separation
  - Reversed phase
  - Ion exclusion (separates electrolytes)
- Mobile Phases
  - High aqueous
  - Low pH
- Sample Preparation
  - Aqueous extraction
  - SPE clean-up

Food Preservatives

Analysis of Organic Acids by HPLC



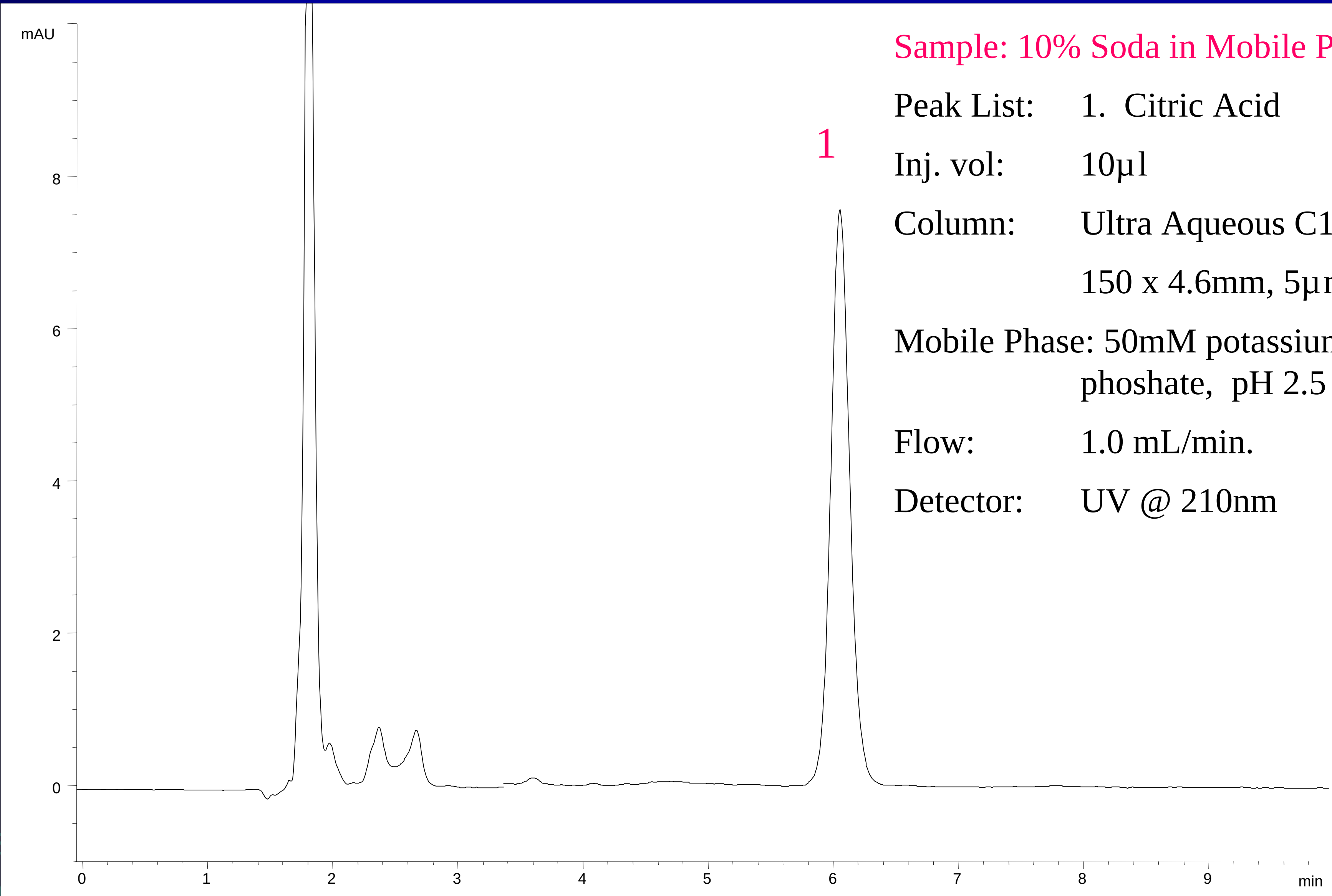
Peak List

1. Tartaric acid, 620 ppm
2. Malic acid, 460 ppm
3. Acetic acid, 1260 ppm
4. Citric acid, 580 ppm
5. Succinic acid, 540 ppm

Inj. vol: 10µl
Column: Ultra Aqueous C18
150 x 4.6mm, 5µm
Mobile Phase: 50mM potassium phoshate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm

Food Preservatives

Analysis of Lemon-Lime Soft Drink



Sample: 10% Soda in Mobile Phase

Peak List: 1. Citric Acid

Inj. vol: 10µl

Column: Ultra Aqueous C18
150 x 4.6mm, 5µm

Mobile Phase: 50mM potassium phoshate, pH 2.5

Flow: 1.0 mL/min.

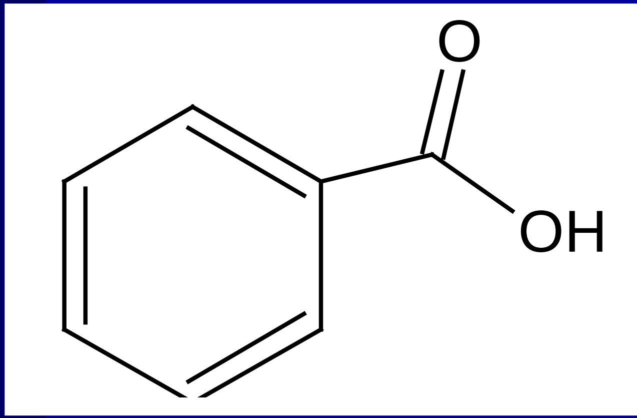
Detector: UV @ 210nm

Chemical Antimicrobial Agents

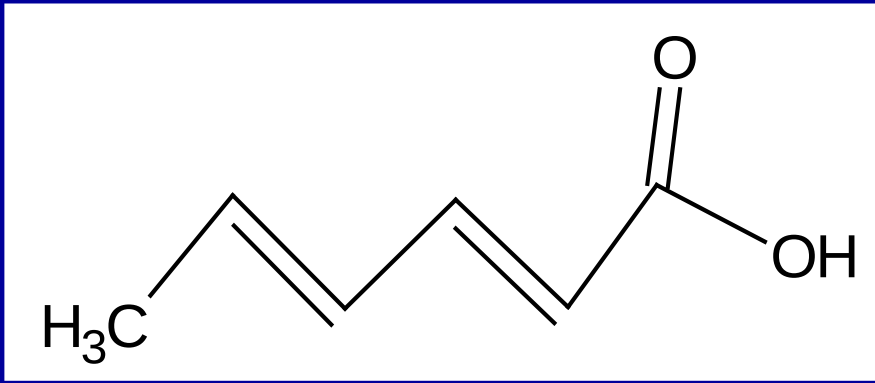
- Sulfites
  - Inhibit bacteria, not yeast
  - Used in wines
  - Some people are sulfite sensitive
- Nitrites
  - Inhibit botulism (bacterial spores) in meat
  - Can form nitrosamines
- Sorbates & Benzoates
  - Specific inhibitors of bacteria
- Propionates
  - Act on molds & rope bacteria, not yeasts
- Parabens
  - Act on yeasts & molds (higher cost)
  - Also used as antioxidants

Microbial Inhibitors

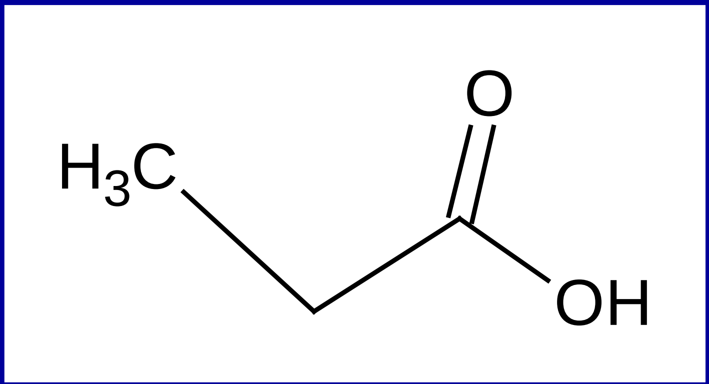
Chemical Antimicrobial Agents



Benzoic Acid



Sorbic Acid



Propionic Acid

Food Preservatives

Analysis of Benzoic and Sorbic Acid

- Reversed Phase
  - Phenyl stationary phases – selectivity
- Acidic Mobile Phases
  - Suppress ionization
  - Increasing methanol content reduces the retention
- Detection - UV
  - 228nm more sensitive for benzoic
  - 259nm more sensitive for sorbic

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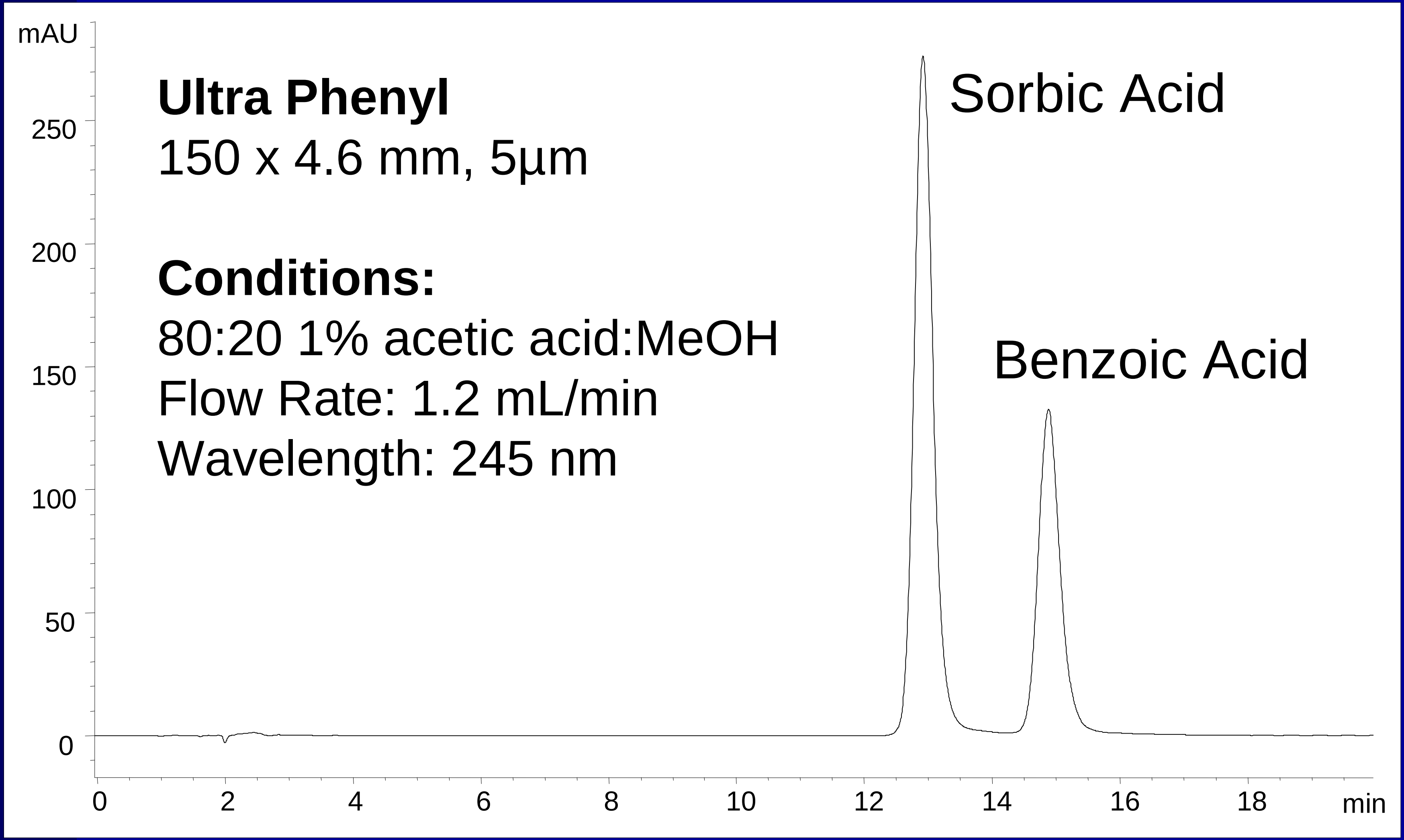
Analysis of Benzoic and Sorbic Acid



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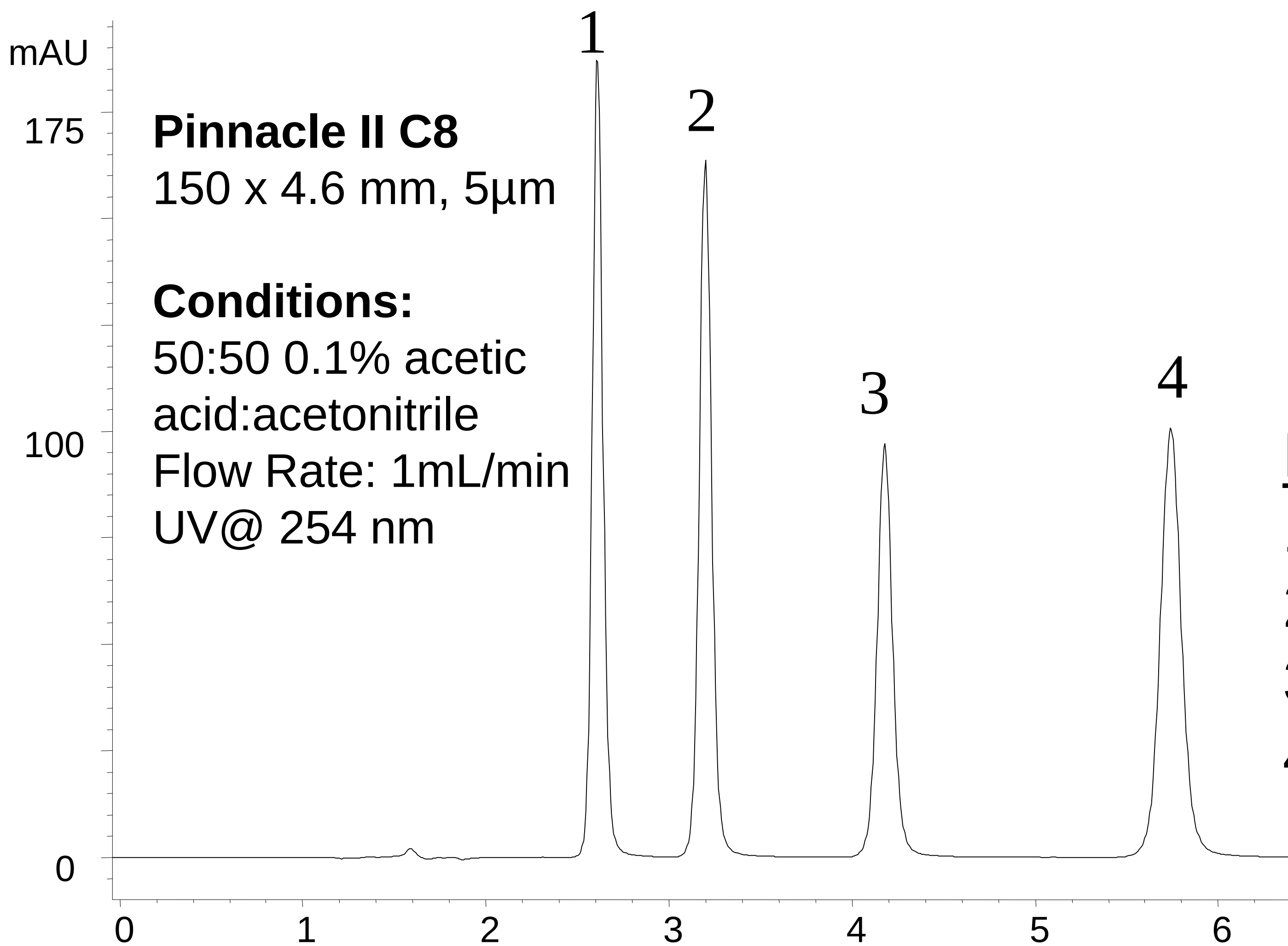
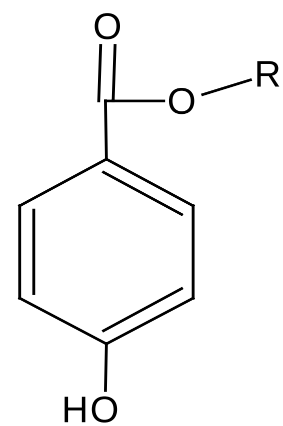
Analysis of Parabens by HPLC





Pinnacle II C8
150 x 4.6 mm, 5µm

Conditions:
50:50 0.1% acetic acid:acetonitrile
Flow Rate: 1mL/min
UV@ 254 nm

Parabens:

1. methyl
2. ethyl
3. propyl
4. butyl

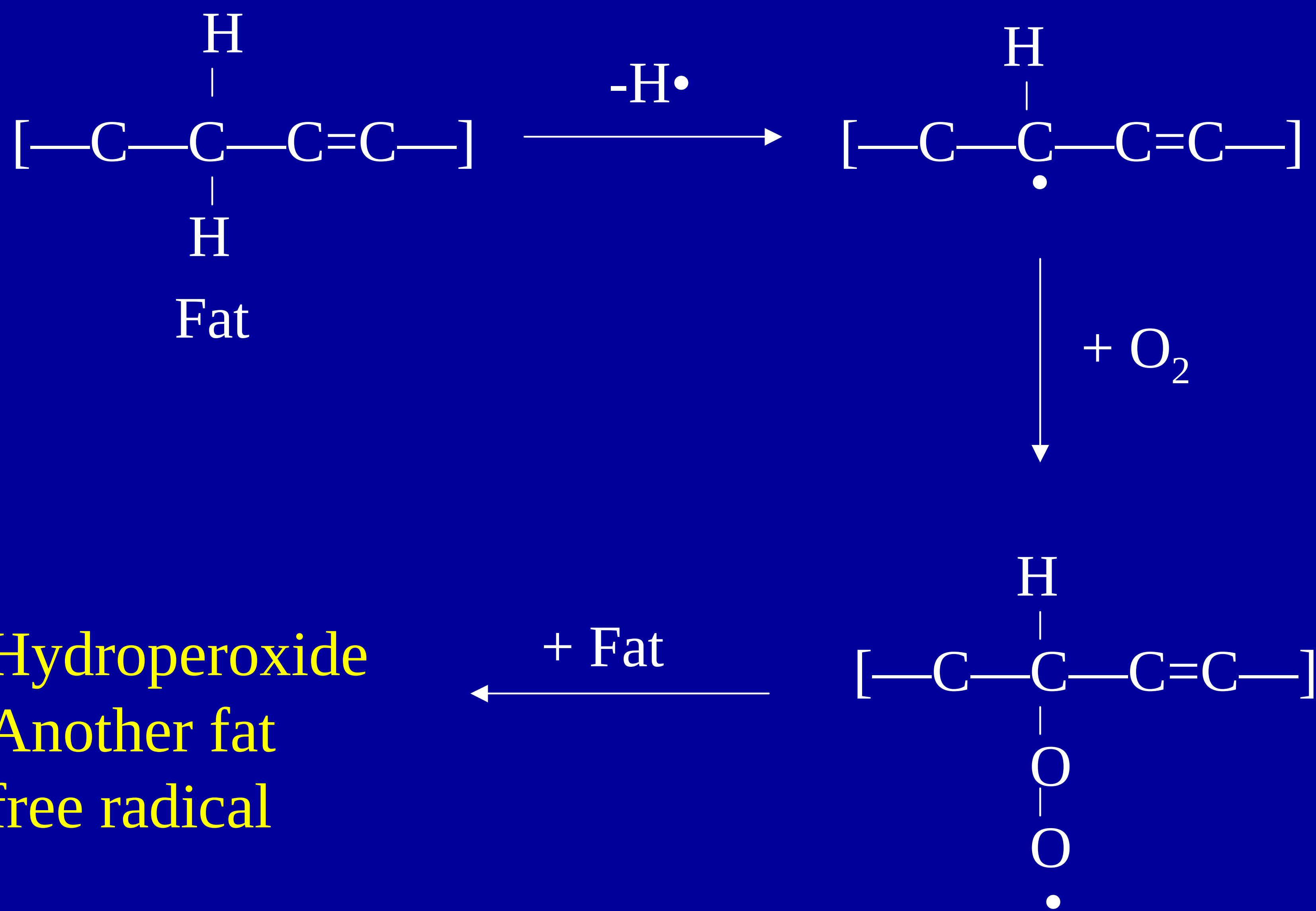
Lipid Oxidation in Food Systems

- A Leading Cause of Food Spoilage
- Free-Radical Reaction:
  - Light & free-radical producing compounds initiate the reaction
  - Hydroperoxides (ROOH)
    - Form first
  - Further reactions of ROOH
    - Produce hydrocarbons, aldehydes, alcohols, acids, and epoxides

Off-Flavors from Lipid Oxidation

- Chemical Changes
  - Sensory Evaluation
    - Stale, cardboard, metallic, fishy
  - Chemical Evaluation
    - Monitor intermediate & end products
- Antioxidants Used as Preservatives
  - Protect flavor, color
  - Extend shelf life

Free Radical Oxidation Reaction



- Hydroperoxide
- Another fat free radical

Edible Oil Processing

Crude Oil

↓

Degumming → Removes water, protein, phospholipids (lecithin), carbohydrates

↓

Refining → Caustic added to remove FFA (soap stock)

↓

Bleaching → Heat to 85C, act. carbon or diatomaceous earth

↓

Deodorization → Distillate consists of: FFA, tocopherols, phytosterols in soy

↓

Edible Oil

Typical Fatty Acid Compositions

Fatty Acid	Corn Oil	Soy Oil	Olive Oil	Peanut	Lard
C14:0			Trace	Trace	3
C14:1					Trace
C16:0	13	11	14	6	29
C16:1			2	Trace	3
C18:0	4	4	2	5	14
C18:1	29	25	64	61	38
C18:2	54	51	16	22	11
C18:3		9			Trace
C20:0	Trace	Trace	Trace	2	Trace
C20:1					Trace
C20:2					Trace
C22:0	Trace	Trace		3	

Marine/Fish Oil Fatty Acids

Fatty Acid	Herring	Cod	Trout
C14:0	6.4	1.4	2.7
C16:0	12.7	19.6	20.9
C16:1	8.8	3.5	3.9
C18:0	0.9	3.8	8.3
C18:1	12.7	13.8	18.4
C18:2ω6	1.1	0.7	7.3
C18:3ω3	0.6	0.1	1.6
C18:4ω3	1.7	0.4	3.2
C20:1	14.1	3.0	ND
C20:4ω6	0.3	2.5	1.7
C20:5ω3	8.4	17.0	5.8
C22:1	20.8	1.0	ND
C22:5ω3	0.8	1.3	Trace
C22:6ω3	4.9	29.8	7.0

From: deMan, Principles of Food Chemistry

Food Preservatives

Lipid Oxidation Inhibitors

Rancidity can be Slowed Down by Disrupting the Free Radical Reaction

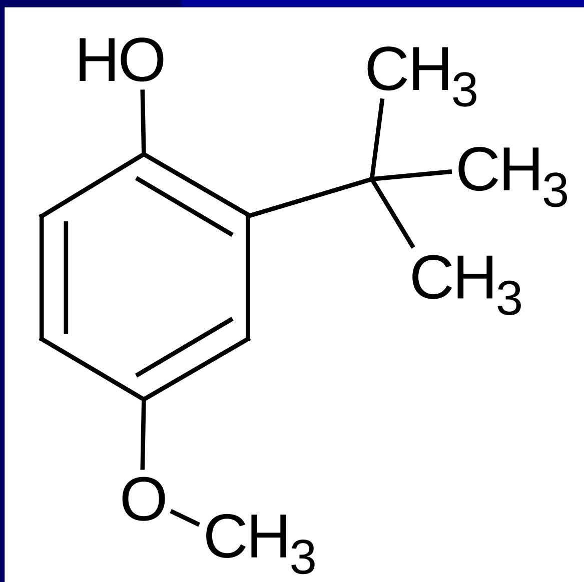
$^1O_2$ Quenchers include: β-Carotene
Tocopherols
BHA/BHT

To monitor oxidative rancidity:

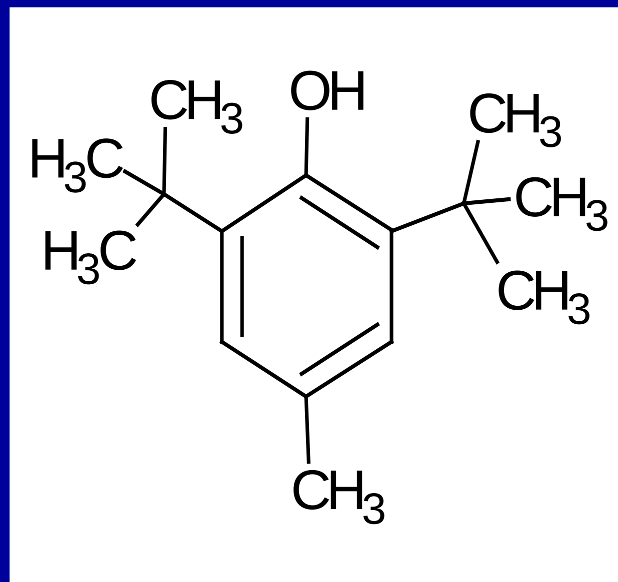
- Peroxide value monitors the initial products
- Hexanal is one of the rancidity products that can be monitored by headspace GC

Food Preservatives

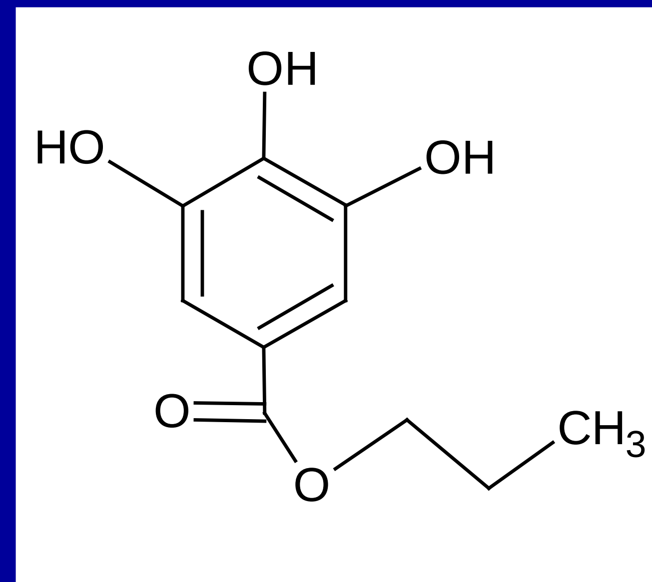
Lipid Oxidation Inhibitors



BHA



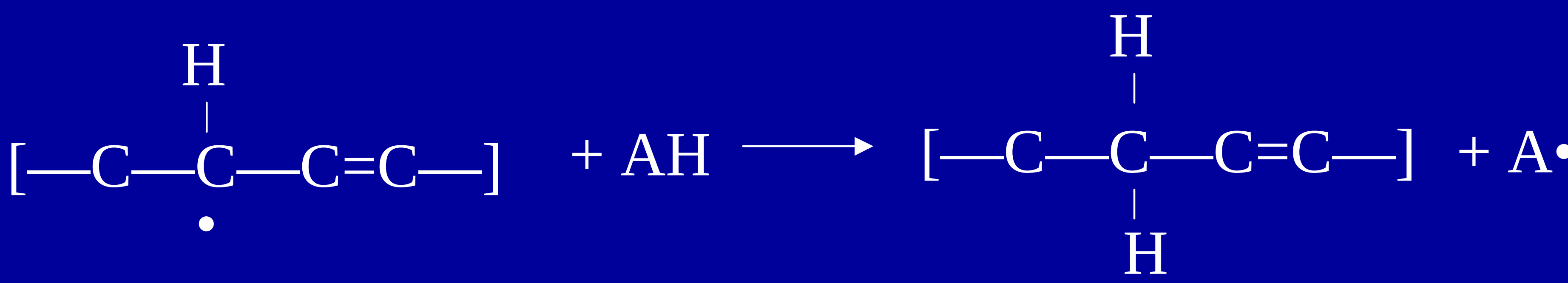
BHT



Propyl Gallate (PG)

Monohydric and polyhydric phenols can be used in food systems as lipid oxidation inhibitors

Antioxidants: How They Work



Where AH is an antioxidant such as BHT

The antioxidant can protonate:

- The fat free radical
- The peroxy free radical

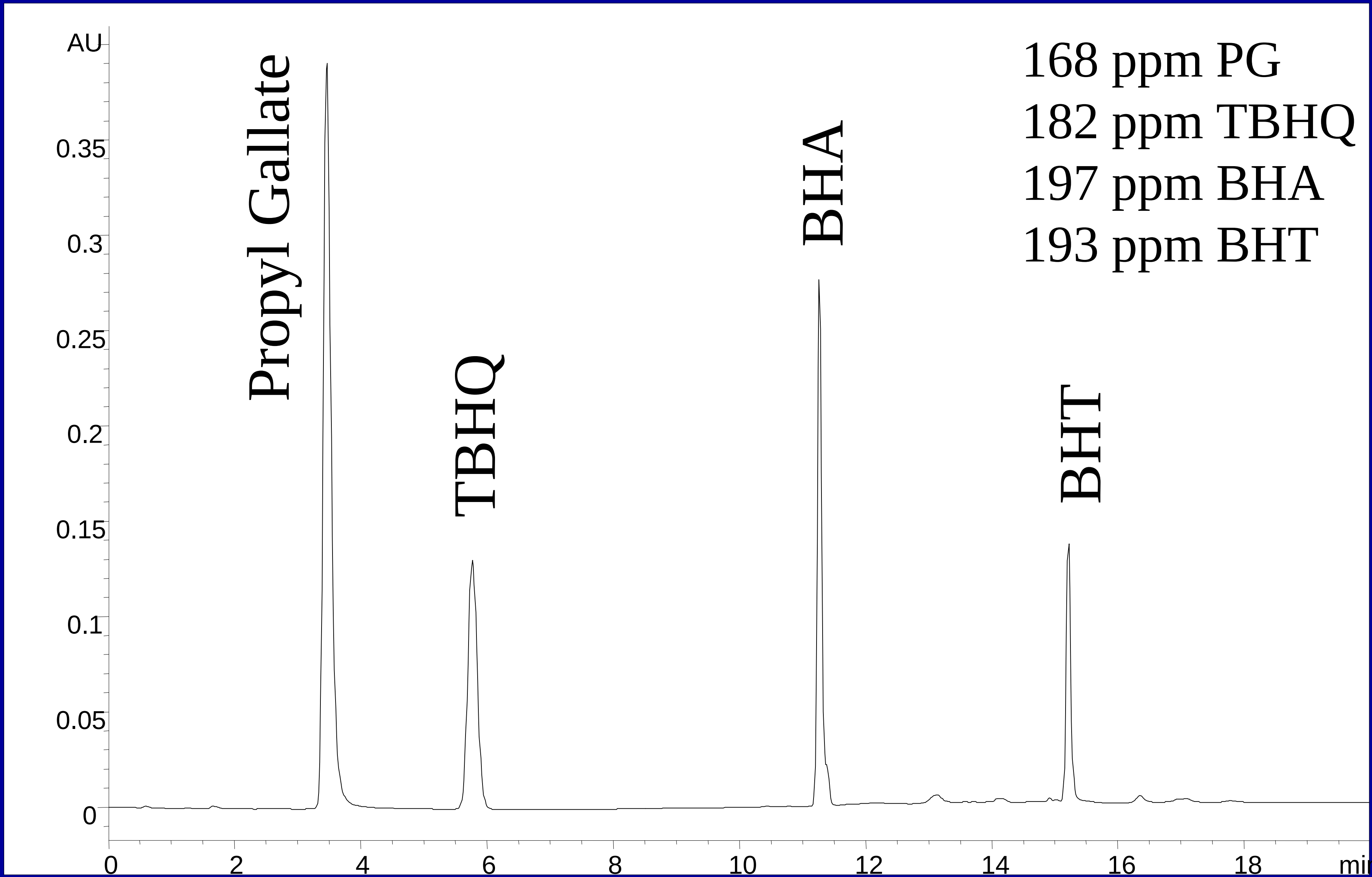
⇒The antioxidant free radical has much greater stability because of its resonance forms

Food Preservatives- Antioxidants

- Oxidation is a Free-Radical Reaction
  - Antioxidants significantly slow oxidation
  - *cis* oxidizes faster than *trans*
  - Conjugated fatty acids oxidize faster than non-conjugated
- Phenolic Antioxidants
  - Monohydric/polyhydric phenols
  - Regulated at <0.02% by weight (fat)
- Natural Antioxidants
  - Typically less regulated

Antioxidants

Analysis of Phenolics by HPLC



Analysis of Phenolics by HPLC

Column: Pinnacle II C18, 150 mm x 4.6 mm, 5 µm

Mobile Phase A: 1% acetic acid in purified water

Mobile Phase B: Methanol

Time (minutes)	% B
0	50
4	50
10	90
25	90
26	50

Flow: 1.0 mL/min, Temp. @ 30°C

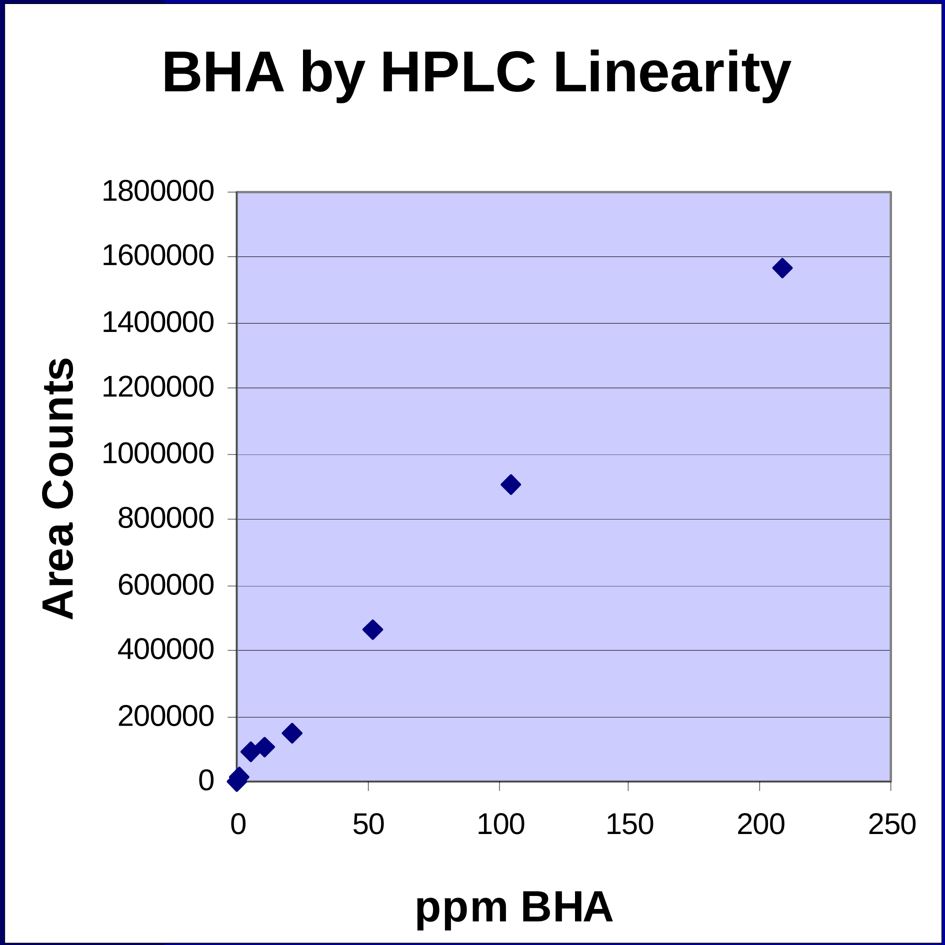
Detector: UV @ 280 nm

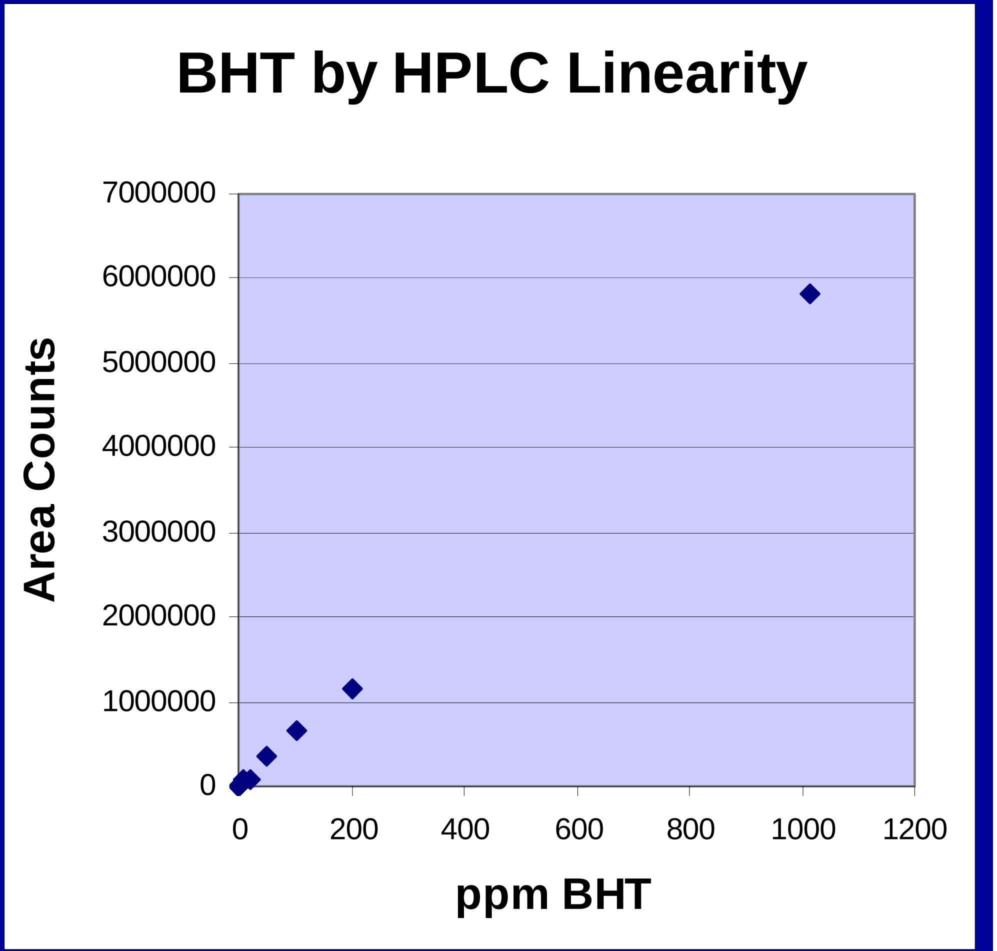
Linearity Study for BHA & BHT by HPLC

- Linear Range Study
  - HPLC procedure
  - Comparison to the GC procedure
  - Standard range: 0.1 – 10,000 ppm

- Results
  - BHA: 10,000 ppm and 1000 ppm out of range
  - BHT: 10,000 ppm standard was out of range
  - Both compounds were detected at 0.1 ppm
  - 0.5ppm would be a reasonable LOD
  - For a typical sample dilution of 1:10, LOD of 5 ppm

Linearity Study for BHA & BHT by HPLC

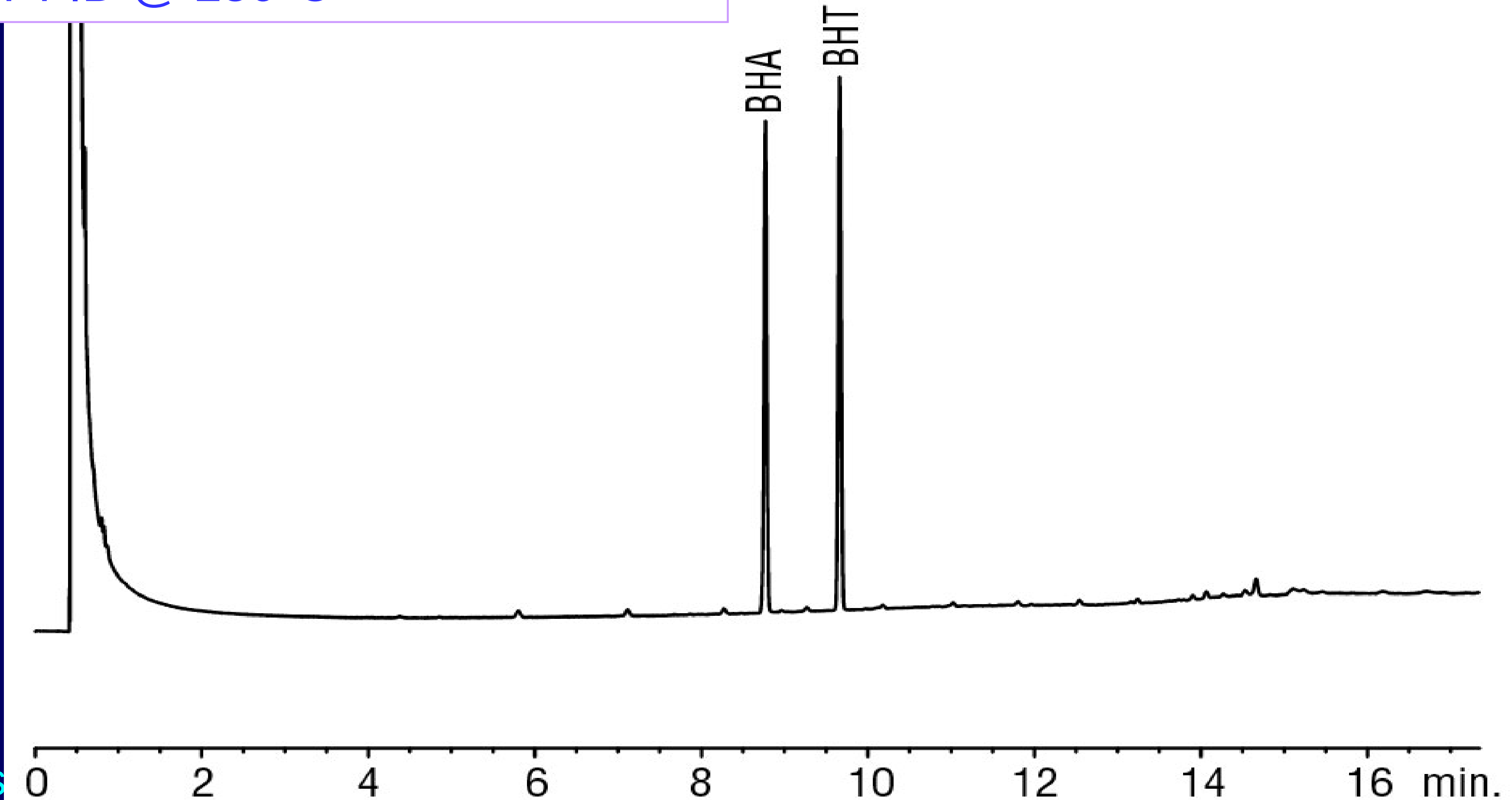




R2 = 0.9932 (BHA) and 0.9997 (BHT)

Analysis of BHA & BHT by GC

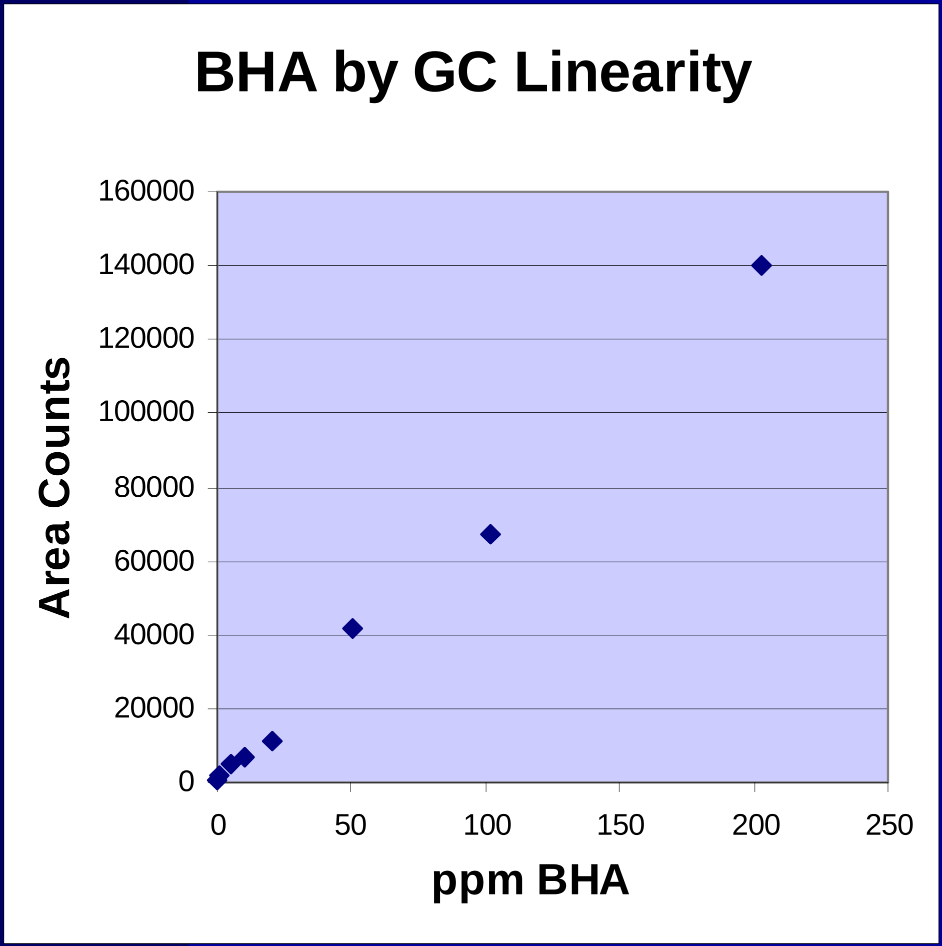
Column: Rtx®-50, 30mx0.53mm, 0.5µm
Injector: 250°C, direct injection
Carrier: Helium at 20 cm/sec.
Oven: 50°C to 200°C at 10°C/min.
Detector: FID @ 250°C

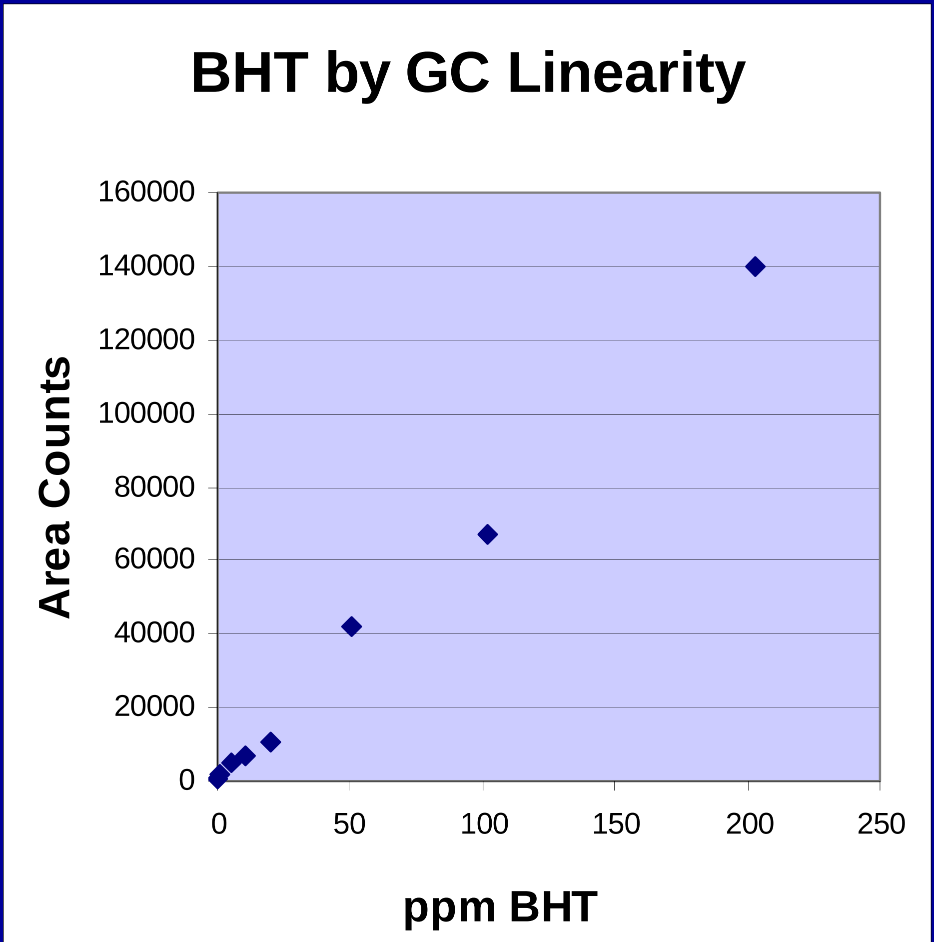


Linearity Study for BHA & BHT by GC

- Linear Range Study
  - GC procedure
  - Comparison to the HPLC procedure
  - Standard range: 0.1 – 10,000 ppm
- Results
  - BHA & BHT: 10,000 & 1000 ppm out of range
  - Both compounds were detected at 0.1ppm
  - BHA detected at 0.01 ppm
  - LOD of <1 ppm

Linearity Study for BHA & BHT by GC

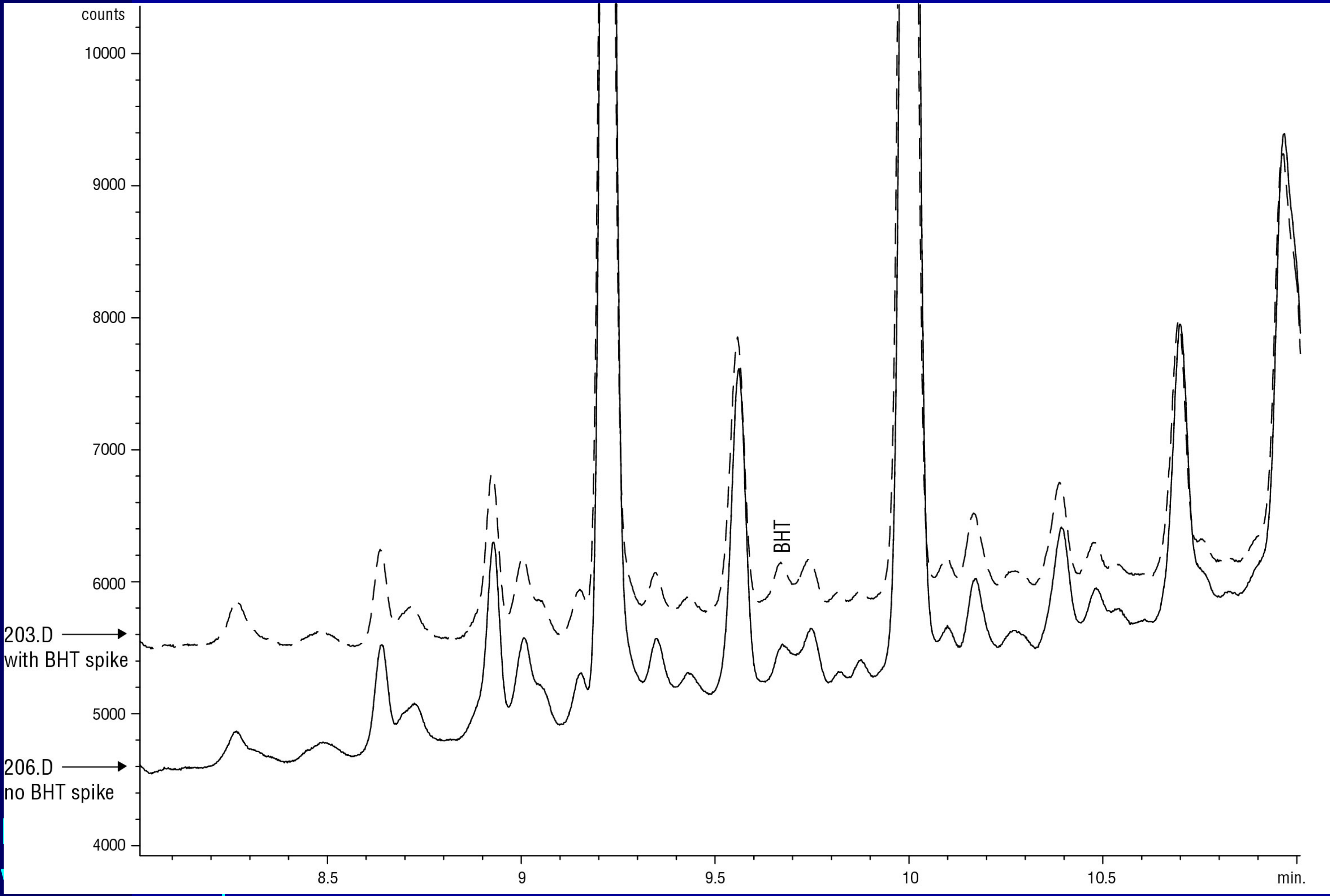




Concentration range: 0.1-200 ppm

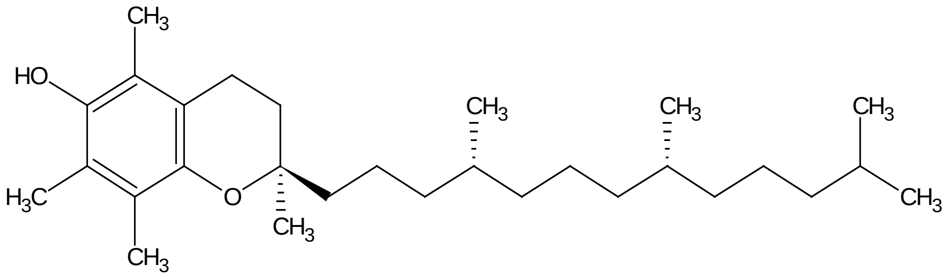
R2 = 0.9956 (BHA) and 0.9981 (BHT)

Analysis of BHT in Marine Oil by GC

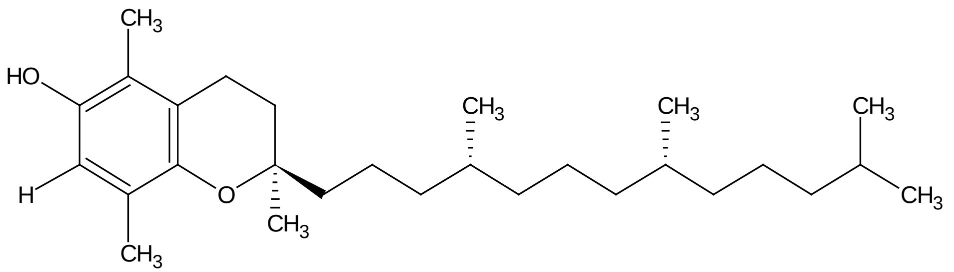


Food Preservatives

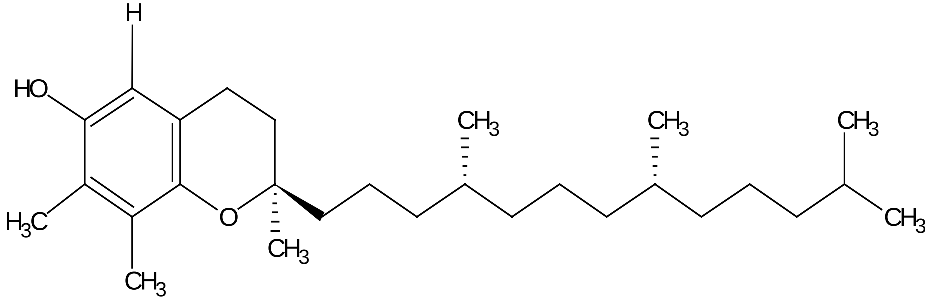
Analysis of Tocopherols – ‘Natural’ Additives



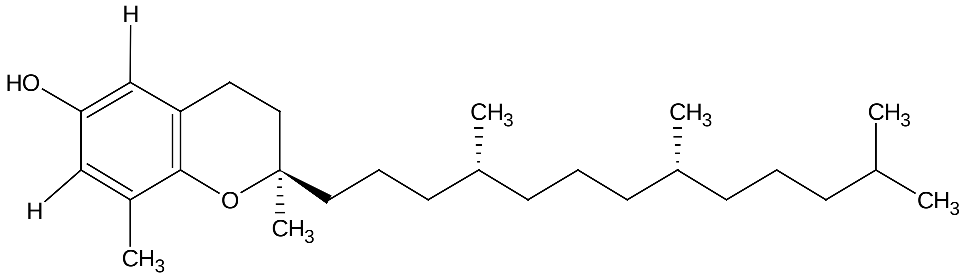
α-Tocopherol (12%)



β-Tocopherol (1%)



γ-Tocopherol (62%)



δ-Tocopherol (25%)

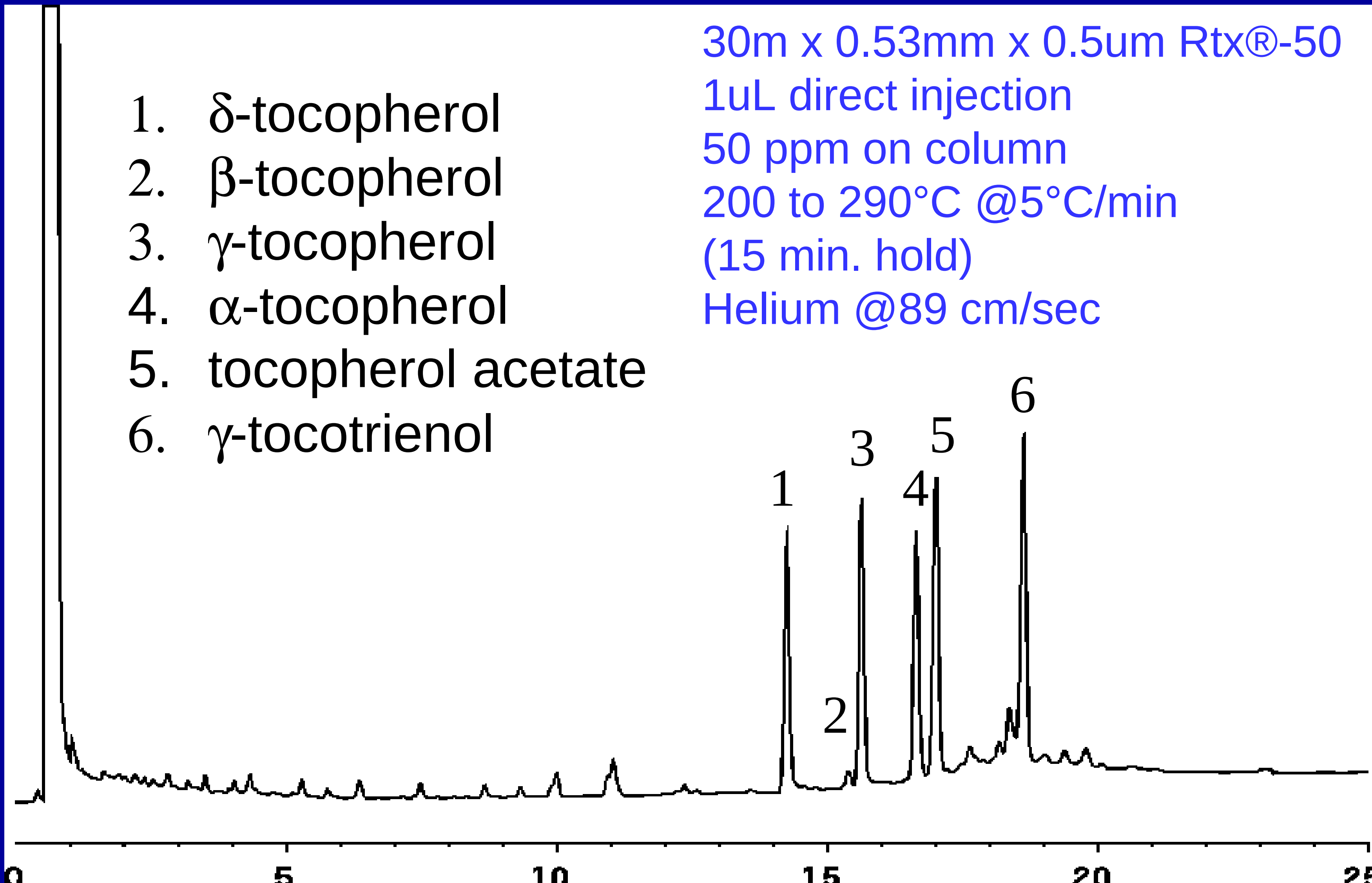
Food Preservatives

Analysis of Tocopherols by GC & HPLC

- GC
  - Medium polarity column
  - Direct injection
  - Analytes can be light, oxygen sensitive
- HPLC
  - Normal phase (e.g. bare silica)
  - Seed oils can be diluted in hexane
  - Extraction necessary for products
  - UV (295nm) or fluorescence (295/330)

Food Preservatives

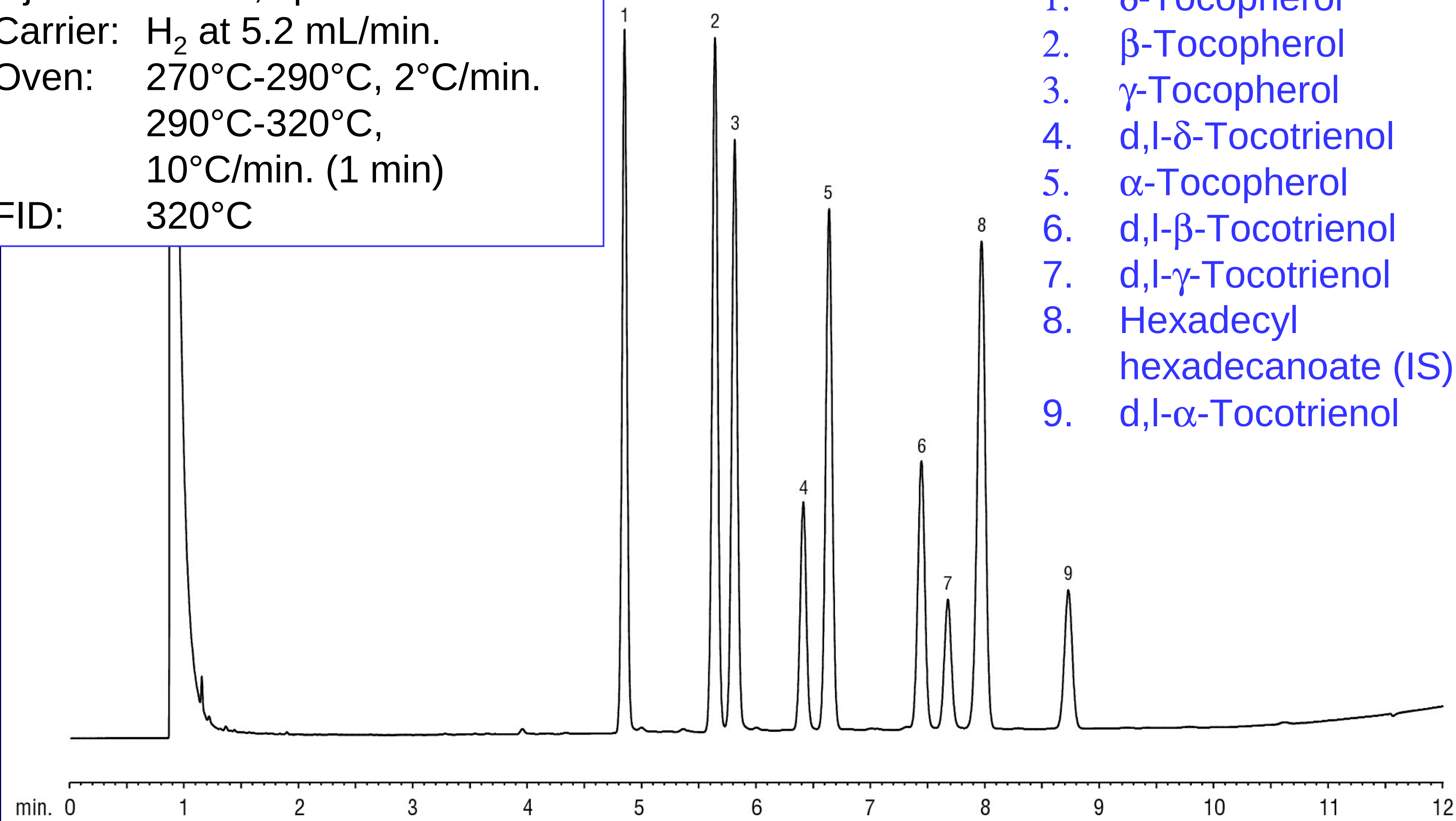
Analysis of Tocopherols by GC



Analysis of Tocopherols by GC

Rtx®-20, 30m x 0.53mm x 0.5mm

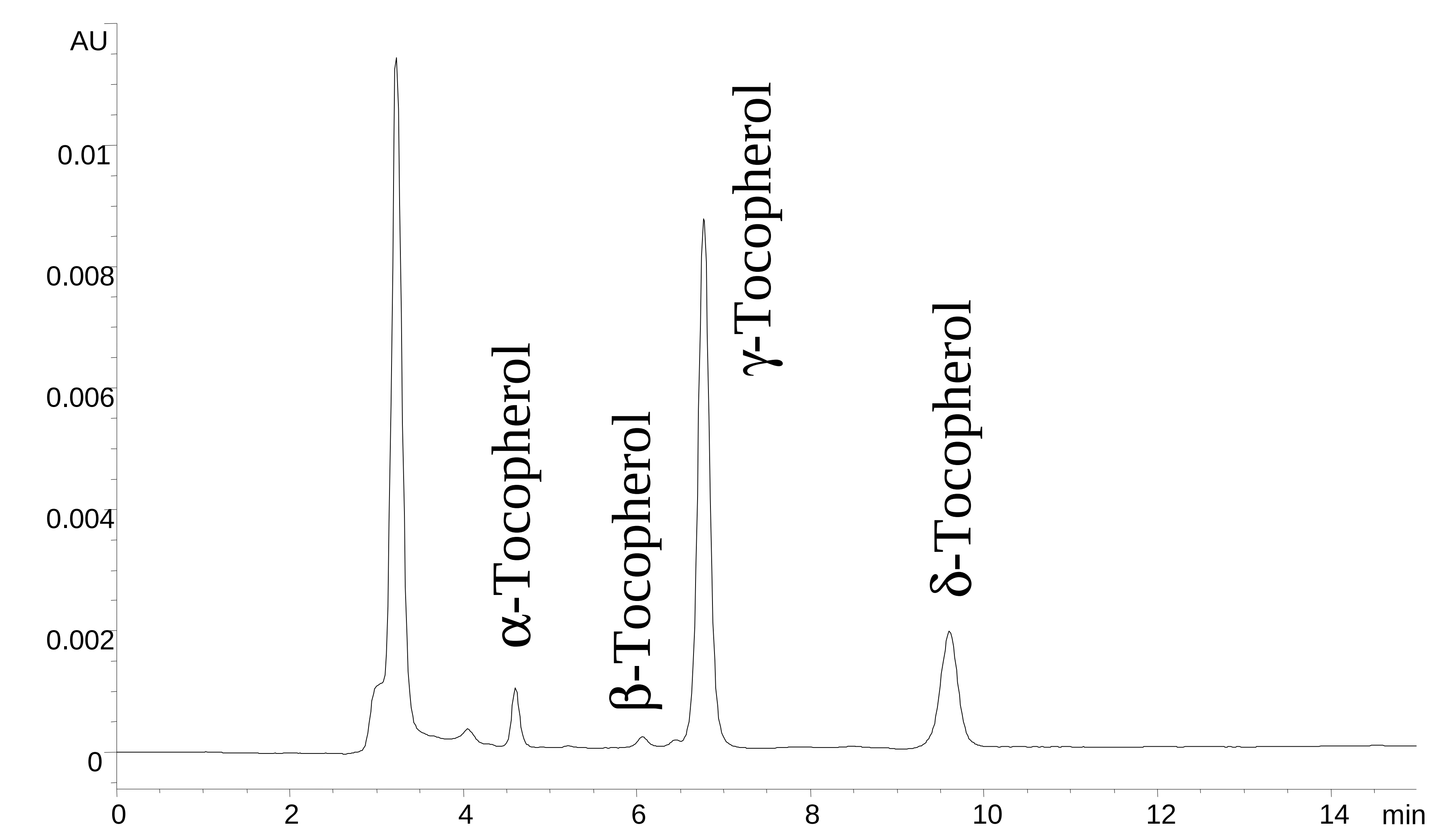
Injector: 320°C, split 20:1
Carrier: H_2 at 5.2 mL/min.
Oven: 270°C-290°C, 2°C/min.
290°C-320°C,
10°C/min. (1 min)
FID: 320°C



1. δ-Tocopherol
2. β-Tocopherol
3. γ-Tocopherol
4. d,l-δ-Tocotrienol
5. α-Tocopherol
6. d,l-β-Tocotrienol
7. d,l-γ-Tocotrienol
8. Hexadecyl hexadecanoate (IS)
9. d,l-α-Tocotrienol

Food Preservatives

Tocopherols by HPLC: 2.5% Soy Oil in Hexane



Pinnacle II Silica, 150 mm x 4.6 mm x 5 µm. Mobile phase: 0.5% IPA in hexane @ 0.6 mL/min, UV detection @ 295 nm

Summary of Preservative Analysis

- Function of Preservatives
  - Control microbial growth
  - Limit lipid oxidation
- Chromatographic Analysis – HPLC vs. GC?
  - HPLC
    - Relatively easy sample preparation
    - Samples with high water content
    - Solid samples, especially water soluble
  - GC
    - Can be very sensitive
    - Typically efficient separations – good for complex matrices