# CHROMATOGRAPHY

Think about **food**
Care about **safety**

Vol. 8

## Additives

MACHEREY-NAGEL
www.mn-net.com

ADIS INTERNATIONAL

MN Since 1911

# Focus on additives

The global production and trading of food and feed products makes monitoring and control difficult.

Only analysis of food and raw materials for the production of food can ensure safe and edible food. Food safety is an essential part of our quality of life.

**MN shows examples for food analysis that will help you with your work and make our food safer!**

## Vol. 8: **Food additives**

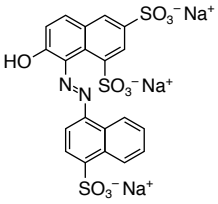
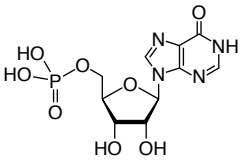
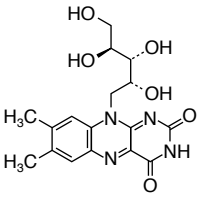
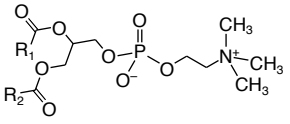
Food stuffs are frequently supplemented with additives in order to regulate or stabilize flavor, color, shelf life, nutritional value and texture. Food additives also ensure a smooth production process [FDA – http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm094211.htm].

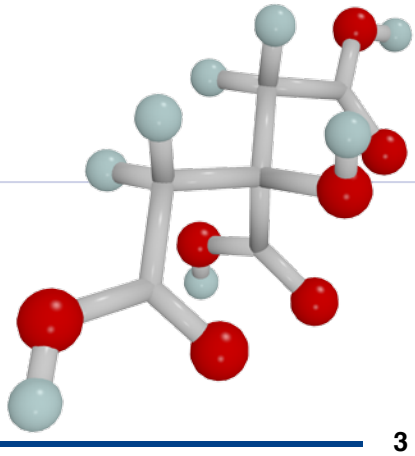
Within the EU food additives are regulated by the European Food Safety Authority (EFSA).

Outside the EU a Joint Expert Committee on Food Additives (JECFA) has been established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [FAO – http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/].

# Compounds of interest

## Overview of selected food additives according to FDA

| **Types of ingredients**<br>Purpose / function | Examples of uses | Examples | Typical molecules |
|---|---|---|---|
| **Color additives** | | | |
| Offset color loss due to exposure to light, air, temperature extremes, moisture and storage conditions<br>Correct natural variations in color<br>Enhance colors that occur naturally<br>Provide color to colorless and "fun" foods | Many processed foods, e.g., candies, snack foods, margarine, cheese, soft drinks, jams / jellies, gelatins, pudding and pie fillings | FD&C Blue Nos. 1 and 2, FD&C Yellow Nos. 5 and 6, Orange B, Ponceau 4R, Beta-carotene<br>(Note: Exempt color additives are not required to be declared by name on labels but may be declared simply as colorings or color added) |  |
| **Flavor enhancers** | | | |
| Enhance flavors already present in foods (without providing their own separate flavor) | Many processed foods | Monosodium glutamate (MSG), hydrolyzed soy protein, autolyzed yeast extract, disodium guanylate disodium inosinate |  |
| **Nutrients** | | | |
| Replace vitamins and minerals lost in processing (enrichment)<br>Add nutrients that may be lacking in the diet (fortification) | Flour, breads, cereals, rice, macaroni, margarine, salt, milk, fruit beverages, energy bars, instant breakfast drinks | Thiamine hydrochloride, riboflavin (vitamin $B_2$), niacin, niacinamide, folate or folic acid, beta carotene, alpha tocopherols, ascorbic acid, vitamin D,<br>Amino acids (*L*-tryptophan, *L*-lysine, *L*-leucine, *L*-methionine) |  |
| **Emulsifiers** | | | |
| Allow smooth mixing of ingredients, prevent separation<br>Keep emulsified products stable<br>Reduce stickiness<br>Control crystallization<br>Keep ingredients dispersed<br>Help products dissolve more easily | Salad dressings, peanut butter, chocolate, margarine, frozen desserts | Soy lecithin, mono- and diglycerides, egg yolks, polysorbates, sorbitan monostearate |  |
| **pH control agents and acidulants** | | | |
| Control acidity and alkalinity<br>Prevent spoilage | Beverages, frozen desserts, chocolate, baking powder | Lactic acid, citric acid, ammonium hydroxide, sodium carbonate | |



# Typical samples, preparation

## 5'-Mononucleotides in infant formula and adult / pediatric nutritional formula

### Sample pretreatment

- Accurately weigh approximately 1 g powder, or 10 mL liquid milk or ready-to-feed infant / nutritional formula, into a 50 mL centrifuge tube.
- Dissolve in 30 mL extraction solution (NaCl, 1 mol/L – EDTA, 5 mmol/L).
- Add 1.0 mL TMP intermediate standard (~80 µg/mL).
- Cap the tube and vortex mix.
- Allow sample to stand for 10 min to ensure complete hydration.
- Dilute to a final volume of 50 mL with water.
- Cap the tube and vortex mix.

### Solid phase extraction (SPE)

***MN Appl. No. 305960***

B.D. Gill et al., Journal of AOAC International Vol. 95, No. 3, 2012, 1–4

*Column type:*
CHROMABOND® SB, 6 mL, 1000 mg
REF 730323

*Note:*
Throughout the extraction procedure, do not let the column run dry but drain to the top of the column bed only. When draining the column the flow rate should be < 2 mL/min.

*Column conditioning:*
4 mL methanol, then 2 x 5 mL water

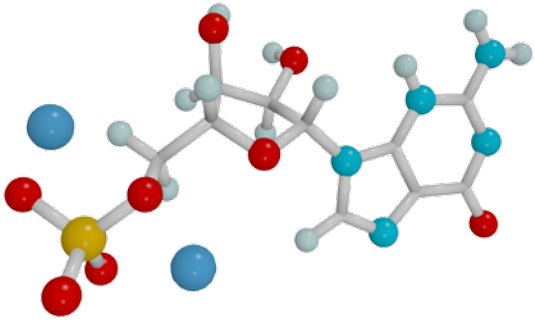
*Sample aspiration:*
4 mL sample solution

*Washing:*
4 mL KBr (0.3 mol/L)

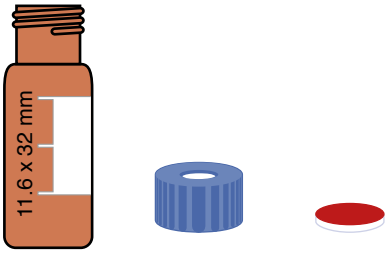
*Elution:*
4 mL $KH_2PO_4$ (0.5 mol/L, pH 3.0)

*Compounds investigated:*
uridine 5′-monophosphate (UMP); inosine 5′-monophosphate (IMP); adenosine 5′-monophosphate (AMP); guanosine 5′-monophosphate (GMP); cytidine 5′-phosphate (CMP); thymidine 5′-monophosphate (TMP, internal standard)

**CHROMABOND® vacuum manifold for 12 columns**
REF 730150



Transfer into a sample vial, e.g., 1.5 mL screw neck vials N 9, amber, flat bottom, label and scale, wide opening, REF 702284 and ready assembled screw closures N 9, blue, center hole with septum silicone / PTFE, REF 702287.1




It is recommended to filter the prepared sample through disposable filters **CHROMAFIL® Xtra PET-45/25**
REF 729220

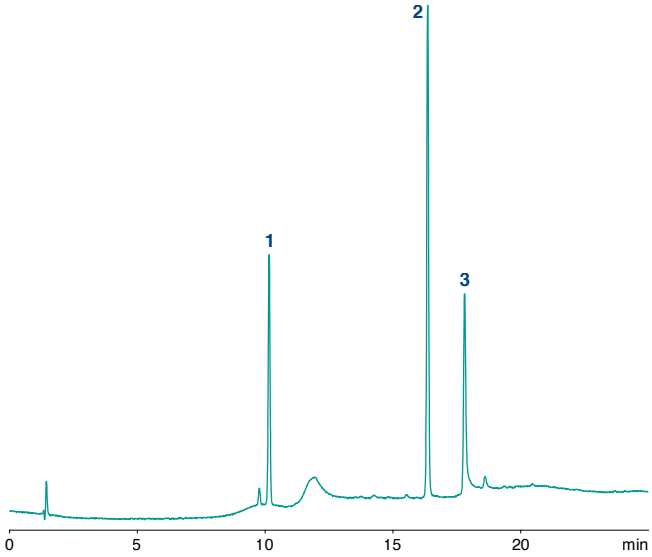
**For other applications see *www.mn-net.com/apps***

## Subsequent analysis: HPLC

### Synthetic antioxidants

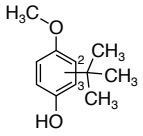
*MN Appl. No. 126980*

*Column:* EC 100/4 NUCLEOSHELL® RP 18plus, 2.7 µm
REF 763234.40

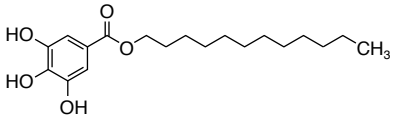



**Peaks**

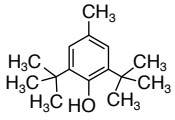
1. Butylhydroxyanisole



2. Dodecyl gallate



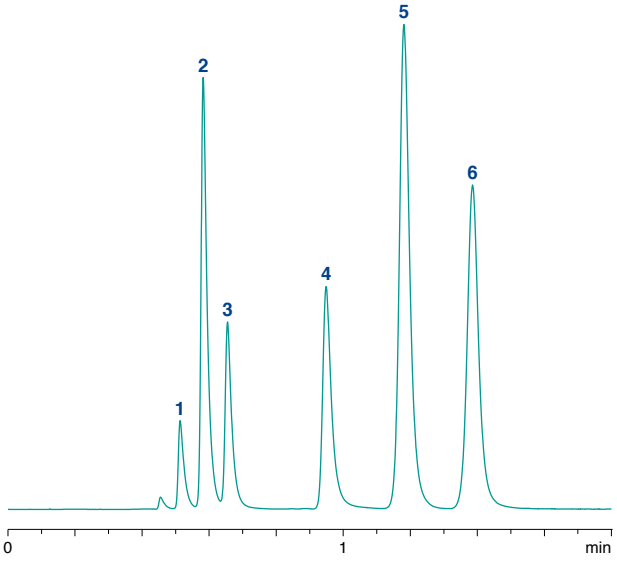
3. Butylhydroxytoluene



### Energy drink components

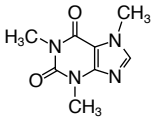
*MN Appl. No. 125010*

*Column:* EC 100/4 NUCLEOSHELL® HILIC, 2.7 µm
REF 763334.40

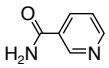



**Peaks**

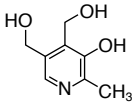
1. Caffeine



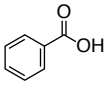
2. Niacinamide



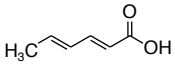
3. Pyridoxine



4. Benzoic acid



5. Sorbic acid



6. Riboflavin see page 3

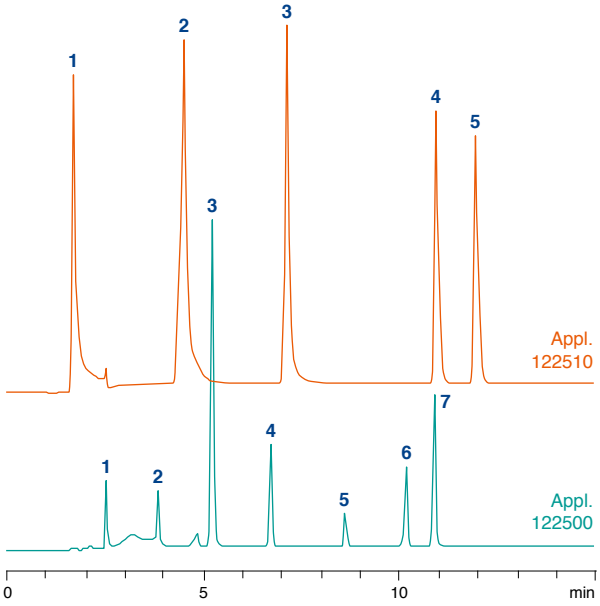
**Detailed conditions are available online at *www.mn-net.com/apps***

## Subsequent analysis: HPLC

### Food dyes (red and yellow dye mixtures)

***MN Appl. No. 122500/122510***

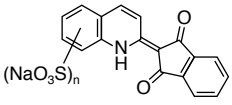
*Column:* EC 250/4 NUCLEODUR® $C_{18}$ Gravity, 5 µm
REF 760101.40




**Peaks**

Red dye mixture
1. Ponceau 6R (E126)
2. Ponceau 4R (E124)
3. Azorubine (E122)
4. Erythrosine (E127)
5. Fast red E

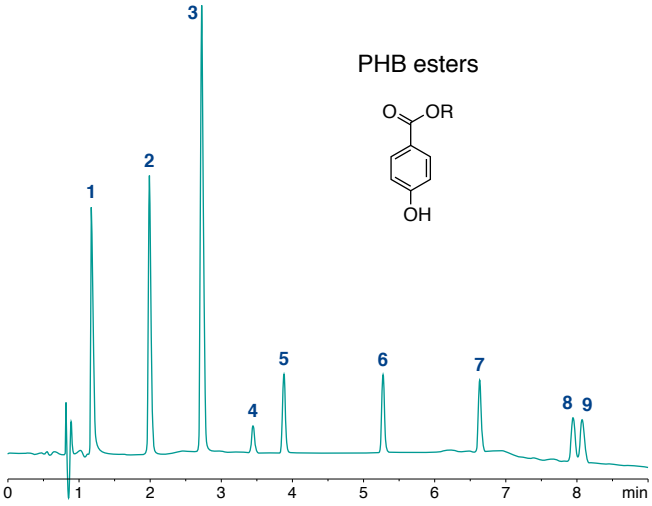
Yellow dye mixture
1., 2. Tartrazine (E 126)
3. Fast yellow
4.-6. Quinoline yellow (E 104)
7. Yellow orange S (sunset yellow CFC, E 110)




### Ultra-fast separation of preservatives

***MN Appl. No. 123180***

*Column:* EC 50/4 NUCLEODUR® $C_8$ Gravity, 1.8 µm
REF 760755.40, coupled with
EC 50/4 NUCLEODUR® $C_{18}$ Gravity, 1.8 µm
REF 760079.40




**Peaks**

1. Salicylic acid (0.056 mg/mL)
2. Benzoic acid (0.052 mg/mL)
3. Sorbic acid (0.049 mg/mL)
4. PHB phenoxy ester (0.105 mg/mL)
5. PHB methyl ester (0.025 mg/mL)
6. PHB ethyl ester (0.026 mg/mL)
7. PHB propyl ester (0.027 mg/mL)
8. PHB iso-butyl ester (0.025 mg/mL)
9. PHB *n*-butyl ester (0.025 mg/mL)

## Detailed conditions are available online at ***www.mn-net.com/apps***

MN suggests using guard columns to protect your main column and significantly increase column life.

The **Column Protection System** (REF 718966) is suitable for all analytical HPLC columns with 1/16" fittings!

# Product information

## Ordering information

| Step | Product | Pack of | REF |
|---|---|---|---|
| **SPE** | CHROMABOND® SB, 6 mL, 1000 mg | 30 | 730323 |
| | CHROMABOND® vacuum manifold for 12 columns | 1 | 730150 |
| **Filtration** | Disposable filters CHROMAFIL® Xtra PET-45/25 | 100 | 729220 |
| **Vials and caps** | 1.5 mL screw neck vials N 9, amber, label and scale | 100 | 702284 |
| | Ready assembled screw closures N 9, blue, center hole with septum Silicone white / PTFE red | 100 | 702287.1 |
| **HPLC 1** | | | |
| Column | EC 100/4 NUCLEOSHELL® RP 18plus, 2.7 µm | 1 | 763234.40 |
| Guard column | EC 4/3 NUCLEOSHELL® RP 18plus, 2.7 µm | 3 | 763238.30 |
| Guard column holder | Column Protection System | 1 | 718966 |
| **HPLC 2** | | | |
| Column | EC 100/4 NUCLEOSHELL® HILIC, 2.7 µm | 1 | 763334.40 |
| Guard column | EC 4/3 NUCLEOSHELL® HILIC, 2.7 µm | 2 | 763338.30 |
| Guard column holder | Column Protection System | 1 | 718966 |
| **HPLC 3** | | | |
| Column | EC 250/4 NUCLEODUR® $C_{18}$ Gravity, 5 µm | 1 | 760101.40 |
| Guard column | EC 4/3 NUCLEODUR® $C_{18}$ Gravity, 5 µm | 3 | 761903.30 |
| Guard column holder | Column Protection System | 1 | 718966 |
| **HPLC 4** | | | |
| Column | EC 50/4 NUCLEODUR® $C_8$ Gravity, 1.8 µm | 1 | 760755.40 |
| | EC 50/4 NUCLEODUR® $C_{18}$ Gravity, 1.8 µm | 1 | 760079.40 |
| Guard column | EC 4/3 NUCLEODUR® $C_{18}$ Gravity, 1.8 µm | 3 | 761901.30 |
| Guard column holder | Column Protection System | 1 | 718966 |

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