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Food Additives

An overview on analytical
methods

Food colors Directive EC/94/36

Sweeteners Directive EC/96/83

Miscellaneous Directive EC/95/2

According to Regulation 882/2006/EC all
Official Laboratories involved in food
analysis in EC member states should be
accredited according to ISO 17025

The latter foresees that all used methods
should be validated

FLEP criteria for analytical methods
(international accepted) :

FLEP = Food Low Enforcement
Practitioners :

i.e. for a concentration of an analyte of 10
mg/kg till 100 mg/kg :

max acceptable RSD_r = 7.5 %,

acceptable %Recovery = 80-110%

No analytical result quantifying a FA with a defined maximum limit should be delivered as long as the used method is not validated!!!!

If not validated methods are used, results are useful only to be registered in laboratory's archive.

Analysis of FA in Food

Authorized and non authorized
additives :

Different approach

Special case : Sudan Red

Non authorized colors in spices :

Simultaneous analysis of Sudan Red, **E160b**
(Annatto, bixin, norbixin, non authorized
in spices) and Rodhamine B possible

Minimum LOD required

Identification of an hypothetical color :
mandatory!!

Use of HPLC /DAD adequate

Commission Decision 2005/402/EC

E100 Curcumin

E102 Tartrazine

E104 Quinoline Yellow

E110 Sunset Yellow FCF,
Orange Yellow S

**E120 Cochineal, Carminic
acid, Carmines**

E122 Azorubine,
Carmoisine

E123 Amaranth

E124 Ponceau 4R,
Cochineal Red A

E127 Erythrosine

E128 Red 2G non authorized

E129 Allura Red AC

E131 Patent Blue V

E132 Indigotine, Indigo
carmine

E133 Brilliant Blue FCF

E142 Green S

E154 Brown FK

E155 Brown HT

Simultaneous analysis HPLC/UV or DAD

HPLC /DAD

E160b

Annatto, bixin, norbixin

matrix specific

*Carotenoids **simoultaneous:***

E160a Carotenes

1.)Mixed carotenes

2.)Beta-carotene

E160d Lycopene

E160e Beta-apo-8-carotenal E160f

Ethyl ester of beta-apo-8-carotenic acid

E161b Lutein

E161g Canthaxanthin

*Caramel : estimation as group (**5-HMF indicator**)*

AAS or ICP

E171 Titanium dioxide in chick peas , chewing gums e.t.c.

E172 Iron oxides and hydroxides

(natural occurrence should be taken into account)

E173 Aluminium (health concerns)

may be restricted

General problems related with the analysis of food colors

FLEP CRITERIA OF METHODS OF ANALYSIS NEED TO BE MET. New max. limits may be proposed.

Recoveries for the analysis of artificial colours are matrix specific and need to be calculated and ev. improved. A lot of work needs to be done. Only in water based drinks methods are validated and accredited.

Analysis of E100 and E120 easy going

Analysis of E160b under constant development

Other Natural colors :
Not quantification but identification the main issue.
Interference of other natural colors contained in food.
Possible quantification of lycopene should be necessary in the future.
Differentiation between caramel colors, may be necessary in the future.

International accepted Data base of natural occurrence of Fe necessary i.e. honey

Sweeteners

POLYOLS example:
simultaneous analysis
and identification (?)
with HPLC/RI or other
detectors.

Columns expensive and
sensitive.

E420 Sorbitol

1.) Sorbitol

2.) Sorbitol syrup

E421 Mannitol

E965 Maltitol

1.) Maltitol

2.) Maltitol syrup

E966 Lactitol

E967 Xylitol

E950 **Acesulfame-K** E951 **Aspartame**

E954 **Saccharin**

: simultaneous analysis with HPLC/DAD.

E952 Cyclamic acid (Specific method for cyclamic acid)

(HPLC/DAD).

Identification is necessary

Improvement of extraction of aspartame from some matrices is important.

If phosphate Buffer is used for the mobile phase :

Caution some analytical systems are very sensitive.

Maintenance of analytical column mandatory.

Miscellaneous additives

Methods used :

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Enzymatic analysis

liquid chemistry

(accompanied with spectrophotometry)

HPLC/DAD, UV, FLD

Ion Chromatography

GC/FID/MS

Enzymatic analysis

Alternative : HPLC/UV or DAD

E325 Sodium lactate

E326 Potassium lactate

E327 Calcium lactate

E330 Citric acid

E331 Sodium citrates

1.) Monosodium citrate

2.) Disodium citrate

3.) Trisodium citrate

E332 Potassium citrates

1.) Monopotassium citrate

2.) Tripotassium citrate

E333 Calcium citrates

1.) Monocalcium citrate

2.) Dicalcium citrate

3.) Tricalcium citrate

E334 Tartaric acid (L(+)-)

E335 Sodium tartrates

1.) Monosodium tartrate

2.) Disodium tartrate

E336 Potassium tartrates

1.) Monopotassium tartrate

2.) Dipotassium tartrate

E337 Sodium potassium
tartrate

Enzymatic analysis

alternative HPLC/UV analysis of amino acids

E620 Glutamic acid

E621 Monosodium glutamate

E622 Monopotassium glutamate

E623 Calcium diglutamate

E624 Monoammonium glutamate

E625 Magnesium diglutamate

Enzymatic analysis

- Matrix specific trueness
- Every new matrix should be tested (participation in interlaboratory studies necessary)
- Acids, Flavor enhancers i.e. glutamates
- Attention to natural occurrence of glutamic acid and some organic acids
- Internationally accepted data base with reference to glutamates is needed

Liquid Chemistry as a first choice :

Legislative Limit of occurrence

Titration reagents should be checked constantly alternative : Ion Chromatography –check for stability of sulfites

E220 Sulphur dioxide

E221 Sodium sulphite

E222 Sodium hydrogen sulphite

E223 Sodium metabisulphite

E224 Potassium metabisulphite

E226 Calcium sulphite

E227 Calcium hydrogen sulphite

E228 Potassium hydrogen sulphite

Liquid Chemistry i.e. sulfites

Adequate for a wide range of matrices, repeatability
sufficient at low levels, satisfactory at high levels.

LOQ may reach the legislative limit of 10 mg/kg

Dried fruits : homogenization of samples is required,
use of water to mix with samples is suggested.

Interference : honey (HMF) and volatile reductants

Combination with a spectrometric determination of
sulfites in distillate with a SO_2 specific reagent is
recommended. Repeatability here should be
checked

Liquid chemistry :

Nitrites, nitrates, phosphates

Internationally accepted data base in blank
foodstuffs is needed (natural occurrence)

Nitrites, phosphates method adequate

Liquid Chemistry and spectrophotometry:

Legislation limit in a very limited range of products according to the recent amendment of Directive EC/95/2 (Directive EC/2006/52)
alternative : Ion Chromatography

E249 Potassium nitrite

E250 Sodium nitrite

E251 Sodium nitrate

E252 Potassium nitrate

Alternative for liquid chemistry :

Ion chromatography

Participation in ring tests is strongly recommended.

Liquid chemistry : phosphates

expressed as P_2O_5

E338 Phosphoric acid

E339 Sodium phosphates

- 1.) Monosodium phosphate
- 2.) Disodium phosphate
- 3.) Trisodium phosphate

E340 Potassium phosphates

- 1.) Monopotassium phosphate
- 2.) Dipotassium phosphate
- 3.) Tripotassium phosphate

E341 Calcium phosphates

- 1.) Monocalcium phosphate
- 2.) Dicalcium phosphate
- 3.) Tricalcium phosphate

E343 Magnesium phosphates

- 1.) Monomagnesium phosphate
- 2.) Dimagnesium phosphate

E450 Diphosphates

- 1.) Disodium diphosphate
- 2.) Trisodium diphosphate
- 3.) Tetrasodium diphosphate
- 4.) Tetrapotassium diphosphate
- 5.) Dicalcium diphosphate
- 6.) Calcium dihydrogen diphosphate

E451 Triphosphates

- 1.) Pentasodium triphosphate
- 2.) Pentapotassium triphosphate

E452 Polyphosphates

- 1.) Sodium polyphosphate
- 2.) Potassium polyphosphate
- 3.) Sodium calcium polyphosphate
- 4.) Calcium polyphosphate

E280 Propionic acid

E281 Sodium propionate

E282 Calcium propionate

E283 Potassium propionate

E260 Acetic acid

E261 Potassium acetate

E262 Sodium acetates

1.) Sodium acetate

2.) Sodium hydrogen acetate (sodium diacetate)

E263 Calcium acetate

GC/ FID

Propionic acid/ acetic acid:

Steam distillation : Repeatability and recovery should be tested and eventually improved. Internal Standard is very useful, simultaneous analysis of sorbates.

Caution natural occurrence of acetates and propionates possible

GC/MS (wide range of use with identification capacity, only for volatile additives, volatilization has an impact on repeatability and recovery)

CO (as Methane, non authorized), **E284 Boric acid**
E285 Sodium tetraborate (borax) (as BF₃)
Head space system usually necessary

HPLC / FLD

Normal phase Tocopherol analysis

E306 Tocopherol-rich extract

E307 Alpha-tocopherol

E308 Gamma-tocopherol

E309 Delta-tocopherol

Separation of tocotrienoles mandatory

H P L C /DAD/UV/Vis Very wide range of use : main instrument for the analysis of FA

Specific analysis of single additives :

E234 Nisin

E385 Calcium disodium ethylene diamine
tetraacetate (Calcium disodium EDTA)

(ion pair analysis, recoveries in diverse
matrices need to be improved)

H P L C /DAD/UV reversed phase

E300 Ascorbic acid

E301 Sodium ascorbate

E302 Calcium ascorbate

**Analyte is light sensitive, possible impact on
repeatability**

H P L C /DAD/UV reversed phase

: **simultaneous analysis**, natural occurrence of benzoates possible

E200 Sorbic acid

E202 Potassium sorbate

E203 Calcium sorbate

E210 Benzoic acid

E211 Sodium benzoate

E212 Potassium benzoate

E213 Calcium benzoate

E214 Ethyl-p-hydroxybenzoate

E215 Sodium ethyl-p-hydroxybenzoate

E216 Propyl-p-hydroxybenzoate

E217 Sodium propyl-p-hydroxybenzoate

E218 Methyl-p-hydroxybenzoate

E219 Sodium methyl-p-hydroxybenzoate

H P L C /DAD/UV reversed phase

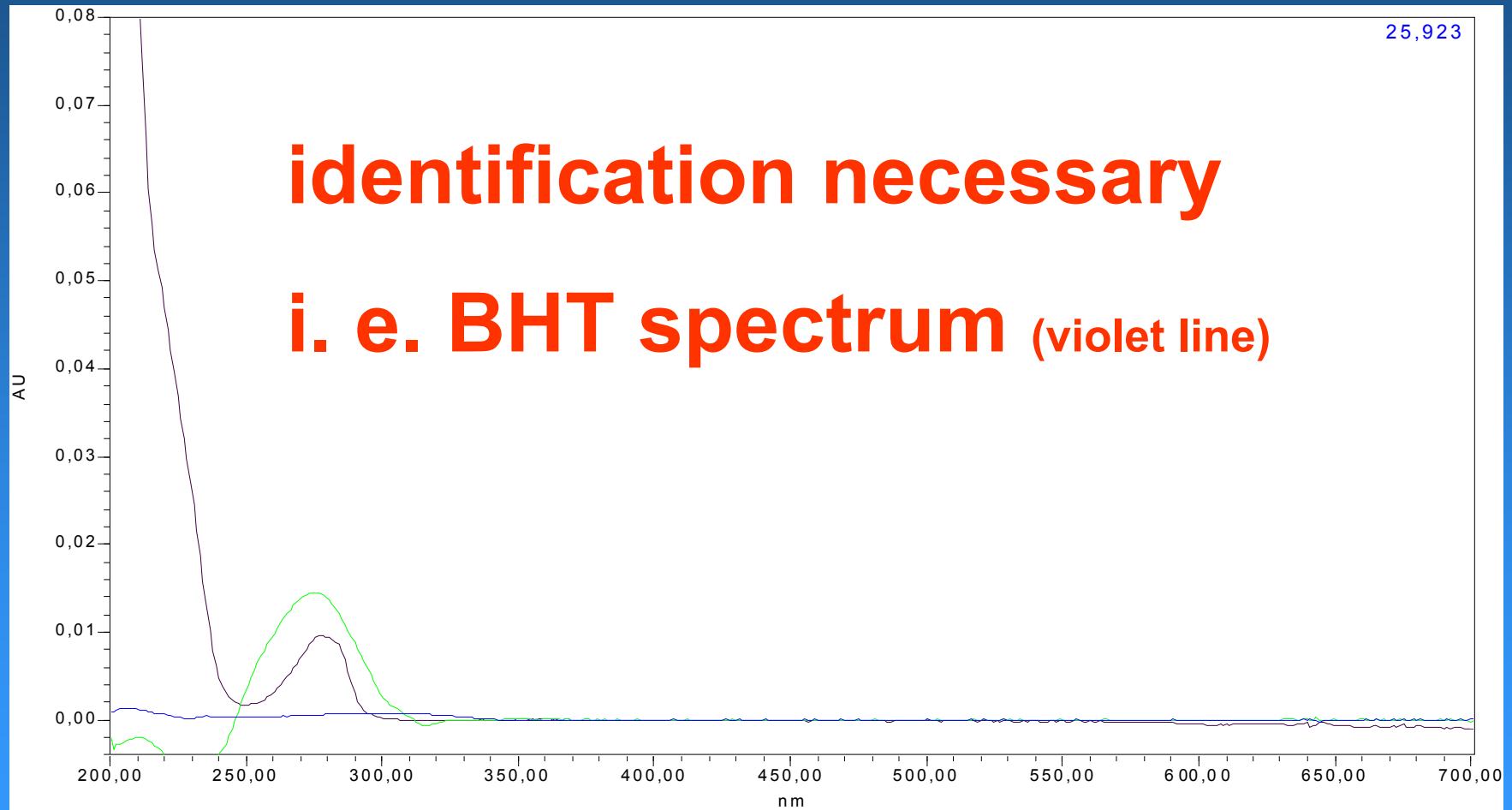
Antioxidants : **simultaneous** analysis

E320 Butylated HydroxyAnisole (BHA)

E321 Butylated HydroxyToluene (BHT)

E 319 Tert-Butylated-HydroQuinone (TBHQ)

H P L C /DAD/UV reversed phase



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Thank you for your attention !