General methods for tests and analysis of food products

Solutions for regulated Instrumental Analysis Methods with AAS, GC, HPLC, ICP, KF and TLC

2016-1

EMD Millipore Corp. is a subsidiary of Merck KGaA, Darmstadt Germany

Content

Application Index

Matrix/sample	Parameter	Technique	Norm	
pH measurement in meat and meat products				
Meat (pork loin)	pH	pH meter/non destructive	ISO 2917:1999	
Meat (sausage)	pH	pH meter / destructive	ISO 2917:1999	
pH determination in fruit and vegetables				
Apple juice	pH	pH meter	ISO 1842:1991	
Orange juice	pH	pH meter	ISO 1842:1991	
Apricot jam	pH	pH meter	ISO 1842:1991	
Carrot jam	pH	pH meter	ISO 1842:1991	
Chloride content in meat and meat products				
Meat & meat products	Chloride	Titration	ISO 1841-1:1996	
Fatty acid methyl esters (FAME) in oil samples				
Sunflower oil	FAME*	GC with FID	EN ISO 12966-2:2011 EN ISO 12966-4:2015	* Fatty acid methyl Esters (FAME)
Determination of lead and cadmium in fish				
Fish	Cadmium, Lead	AAS/GFAAS	EN 14084:2003	
Determination of deoxynivalenol (DON)				
Wheat	DON**	HPLC	EN 15791:2009	**Deoxynivalenol
Salt content in meat and meat products with different methods				
Cold cut	Sodium	AAS	EN 15505:2008	
Cold cut	chloride	titration	ISO 1841-1:1996	
Salami	Sodium	AAS	EN 15505:2008	
Salami	chloride	titration	ISO 1841-1:1996	
Water content determination in oil samples				
Oil	Water	KF titration / coulometry	DIN EN ISO 8534:2009	
Determination of trace elements in foodstuff				
Meat (Beef)	trace elements***	ICP-OES	EN 13805:2002	***Fe, Mg, Ca, Na, K
Meat (Chicken)	trace elements***	ICP-OES	EN 13805:2002	***Fe, Mg, Ca, Na, K
Milk	trace elements***	ICP-OES	EN 13805:2002	***Fe, Mg, Ca, Na, K
Determination of pesticides				
Tomato	pesticides	LC-MS/MS	EN 15662:2009	

Introduction

This compilation discusses regulated food analysis. It presents complete solutions for regulated instrumental analysis with focus on general methods of tests and analysis of foodstuff.
All highlighted methods follow international norms:

ISO (International Organization for Standardization)
http://www.iso.org/iso/home.html

DIN (Deutsches Institut für Normung)
http://www.din.de/en

CEN (European standards maintained by the European Committee for Standardization)
https://www.cen.eu/

To discuss regulated food analysis, an appropriate starting point is to understand the term food; which is any substance(s) being consumed to provide nutritional support. Food contains essential nutrients, such as fats, proteins, vitamins, or minerals.

Food is typically of plant or animal origin, but also certain fungi's are edible. In the preparation of fermented and pickled foods (leavened bread, cheese, etc.) fungi's and ambient bacteria are also used. Inorganic substances such as salt and baking soda are used to preserve or chemically alter an ingredient, i.e. used as food additives.

Beverages are liquid(s) for drinking, usually excluding water, and this may include tea, coffee, liquor, beer, juice, or soft drinks. The most important types of soft drinks are; ready-to-drink essence flavored beverages (practically always carbonated), ready-to-drink beverages containing fruit or fruit juice, and beverages intended for drinking after dilution.

Food can thus be of both solid (fruit, meat, vegetables, and even frozen liquid or in other words ice-cream) and liquid (beverages) form.

- Are energy drinks foods or dietary supplements?
- What is the difference between a beverage and a dietary supplement?
- When is the ice-cream becoming a beverage and at what stage is it food?

A fair question is thus to ask why we differentiate between food and beverages but at the same time use the abbreviation F&B to define a sector/industry that specializes in the delivery of food.

Milk

Milk is a liquid produced by the mammary glands of mammals. Milk can be adjusted by separating part of the milkfat therefrom, or by adding thereto cream, concentrated milk, dry whole milk, skim milk, concentrated skim milk, or nonfat dry milk. Milk may be homogenized.

The Codex Alimentarius provide the following definitions:

1. Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing.

2. Milk product is a product obtained by any processing of milk, which may contain food additives, and other ingredients functionally necessary for the processing.

3. Composite milk product is a product of which the milk, milk products or milk constituents are an essential part in terms of quantity in the final product, as consumed provided that the constituents not derived from milk are not intended to take the place in part or in whole of any milk constituent.

4. A reconstituted milk product is a product resulting from the addition of water to the dried or concentrated form of the product in the amount necessary to re-establish the appropriate water to solids ratio.

5. A recombined milk product is a product resulting from the combining of milkfat and milk-solids-non-fat in their preserved forms with or without the addition of water to achieve the appropriate milk product composition.

6. Dairy terms means names, designations, symbols, pictorial or other devices which refer to or are suggestive, directly or indirectly, of milk or milk products.

Only a food complying with the definition in definition 1. may be named “milk”. If such a food is offered for sale as such it shall be named “raw milk” or other such appropriate term as would not mislead or confuse the consumer. Milk which is modified in composition by the addition and/or withdrawal of milk constituents may be identified with a name using the term “milk”, provided that a clear description of the modification to which the milk has been subjected is given in close proximity to the name.

India is the world's largest producer of milk, and New Zealand, the European Union's member states, Australia, and the United States are the largest exporters of milk/milk products.
China and Russia are the world's largest importers of milk and milk products.

Meat

Meat is defined as the edible flesh of an animal, typically a mammal or bird.
The flesh of domestic fowls is sometimes distinguished as poultry.
Meat is also the edible part, as of a piece of fruit or a nut.

In gastronomy, red meat is red when raw and not pale in color when cooked, and white meat is pale in color before and after cooking.

In science red meat is defined as meat that contains more myoglobin than a white meat, white meat being defined as non dark meat from chicken (excluding leg or thigh), or fish.

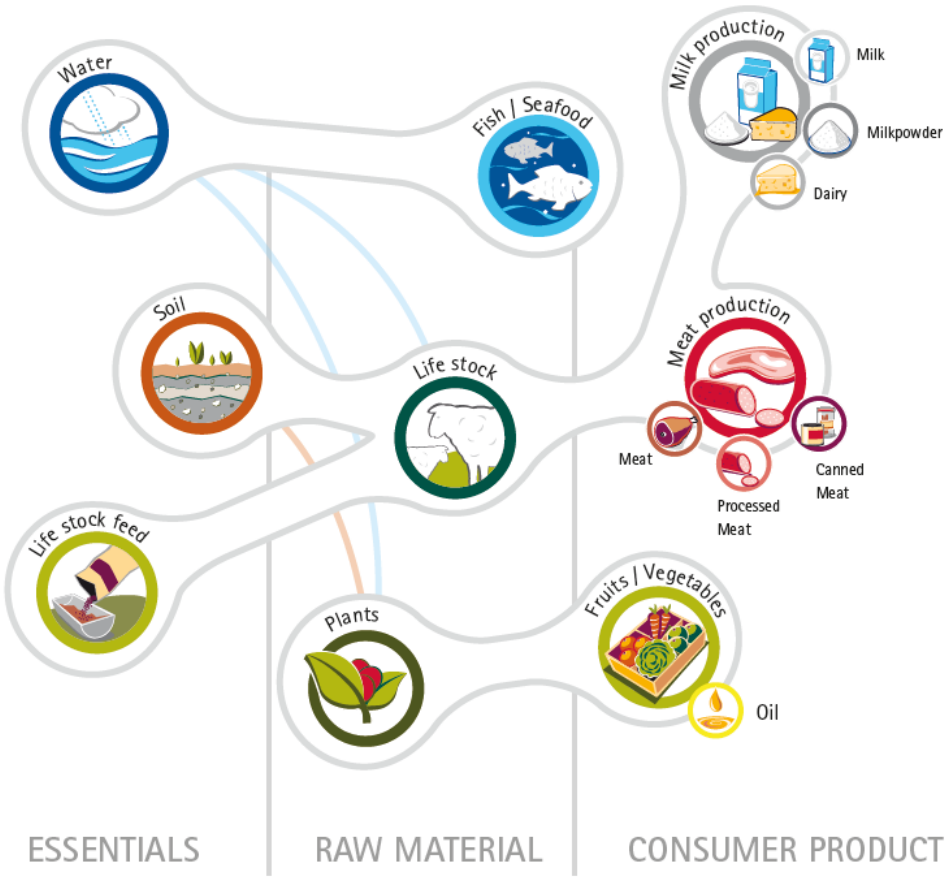
Pork for example, are red meats using the nutritional definition and white meats using the gastronomy definition. *This can cause confusion.*

Food Testing

Instrumental analysis is the main focus in this compilation but on the next page you can find an overview that illustrate the wider perspective of foodstuff testing, with reference to biomonitoring and rapid testing. We thus encourage you to consider the whole process. Practically this means that a commercial product of processed meat (for example a sausage) has been affected not only in its manufacturing procees but already from the soil growing the cattle feed. Food related testing is thus more than only instrumental analysis of finalized products. One could for instance argue that environmental testing (i.,e soil and water quality, air monitoring, etc) is relevant.

On the following pages you will find HPLC and UHPLC methods presented for toxin and pesticide screening (MS-detection); Karl Fischer (KF) methods for water determination, Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma (ICP) methods for metal content determination, GC methods for fatty acid methyl esters (FAME) and different examples of classical titration analysis. Separate sections are dedicated to introduce the different analytical techniques used and an update on regulatory demands and different food legislations.

Food Testing Overview



	Water	Soil	Feed	Cattle/ vegetable	Production process	Final product
Analysis type:						
Rapid tests	Waste Water Analysis: COD N in different forms	Nitrate, Phosphorous, Sulfur		Ascorbic Acid, Sugars, Disinfection control, Nitrate, Sulfur	Ascorbic Acid; Sugars, Disinfection control	Lactic Acid in Milk, pH in meat, Hydroxymethylfurfural in honey
Instrumental	pH, Trace elements, Pesticides	Trace elements, Pesticides	antibiotics	Pesticides, Toxins		Heavy metals Pesticides Antibiotics Toxins Water content Salt content
Microbiological	For drinking water and food process water only: E. Coli and Coliforms		Pathogen and spoilage testing e.g.: Salmonella, Listeria pathogenic E. coli, Clostridia, Enterobacteriaceae, YeastsEt Moulds, Aspergillus		Environmental Monitoring: Air Sampling, Surface monitoring using contact plates and slides, ATP tests	Pathogen and spoilage testing: e.g.: Salmonella, Listeria pathogenic E. coli, Clostridia, Enterobacteriaceae, Yeasts Et Moulds, Aspergillus

*The table list some typical examples

pH measurement in meat and meat products

ISO 2917:1999

The method utilise the measured potential difference between a glass electrode and a reference electrode, which are placed in a sample extract of the meat or meat product.

Two procedures are used because of different sample type:
1. non-destructive method for measurement in pork loin
2. destructive method for measurement in a fermented sausage

Reagents *(only use recognized analytical grades, unless otherwise specified. Water should comply with at least grade 3 in accordance with ISO 3696)*

In the norm, it is described how you prepare buffer solutions but we can offer ready-solutions: The following Certipur® solutions can also be offered with 20°C specifications.

Buffer solutions:
1. Citric acid - sodium hydroxide - hydrogen chloride
   traceable to SRM from NIST and PTB pH 4.00 (25°C) Certipur® (1.09445)
2. Potassium dihydrogen phosphate – disodium hydrogen phosphate
   traceable to SRM from NIST and PTB pH 7.00 (25°C) Certipur® (1.09407)
3. Boric acid - potassium chloride -sodium hydroxide
   (traceable to SRM from NIST and PTB pH 9.00 (25°C) Certipur® (1.09408)

Sodium hydroxide solution
c(NaOH) = 1 mol/l (1 N) Titripur® Reag. Ph Eur Reag. USP (1.09137)

Cleaning:
Ethanol; 96% EMSURE® Reag. Ph Eur (1.59010)
Diethyl ether; EMSURE® ACS,ISO,Reag. Ph Eur (1.00921)

Apparatus
- High-speed rotational cutter, capable to homogenizing the laboratory sample (not exceeding 4.0 mm in diameter)
- pH-meter, accurate to the nearest 0.01 pH
- Combined electrode (in which the indicator and reference electrode are joined in one shaft)
- Shaft homogenizer (able to operate at a rotational frequency of 20000 min^{-1}
- Magnetic stirrer

pH measurement in meat and meat products

ISO 2917:1999

Sampling is not part of this norm but a recommended method is given in ISO 3100-1. It is important to have representative samples and it is suggested to start with at least 200 gram.

Calibration of pH meter
Calibrate the pH-meter according to the manufacturers instruction using at least two buffer solutions, while stirring with the magnetic stirrer. If the pH-meter does not include a temperature correction system, the temperature of the buffer solution shall be within the range 20±2 °C.

Non-destructive method
Pierce a hole in the sample with a knife or a sharp pin and insert the electrode. If the pH-meter does not include a temperature correction system, the temperature of the sample shall be within the range 20±2 °C.

For non-destructive measurement use a spear tip pH electrode. Select a representative point of the sample. If it is considered useful to know the differences between the pH measured at several points in the sample, repeat the measurements at various points of the sample.

Destructive method
Homogenize the laboratory sample. Makes sure the temperature of the sample does not rise above 25 °C. Homogenize a certain mass of the prepared test sample in excess potassium chloride solution (10 times more) by means of shaft homogenizer. Introduce the electrodes into the sample extract and set the temperature correction system of the pH meter to the temperature of the extract.
If the pH-meter does not include a temperature correction system, the temperature of the sample shall be within the range 20±2 °C. While stirring with the magnetic stirrer, measure the pH.
When a constant value has been reached, read the pH directly from the instrument.

Tips:
Fill a suitable airtight container with the homogenized sample. It is recommended to analyze the sample as soon as possible, but always within 24 h after homogenization. The exact time of pH measurement should be noted. With fresh meat, which generally is kept at temperatures from 0-5°C, it is necessary to use a temperature correction system.

Results (report the result to the nearest 0.05 pH unit).
Pork loin: 5.80; 5.75; 5.90
Fermented sausage 4.95; 5.00; 4.95

pH determination in fruit and vegetables

ISO 1842:1991

The method utilise the measured potential difference between two electrodes dipped in the liquid to be tested.

Reagents *(only use recognized analytical grades, unless otherwise specified. Water should comply with at least grade 3 in accordance with ISO 3696)*

In the norm, it is described how you prepare buffer solutions but we can offer ready-solutions: The following Certipur® solutions can also be offered with 20°C specifications.

Buffer solutions:

1. Citric acid - sodium hydroxide - hydrogen chloride
   traceable to SRM from NIST and PTB pH 4.00 (25°C) Certipur® (p/n 1.09445)

2. Potassium dihydrogen phosphate – disodium hydrogen phosphate
   traceable to SRM from NIST and PTB pH 7.00 (25°C) Certipur® (p/n 1.09407)

3. Boric acid - potassium chloride -sodium hydroxide
   (traceable to SRM from NIST and PTB pH 9.00 (25°C) Certipur® (p/n 1.09408)

Apparatus

- pH-meter, accurate to the nearest 0.01 pH
- Combined electrode (in which the indicator and reference electrode are joined in one shaft)

pH measurement in fruit and vegetables

ISO 1842:1991

Calibration of pH meter

Calibrate the pH-meter according to the manufacturers instruction using at least two buffer solutions, while stirring with the magnetic stirrer. If the pH-meter does not include a temperature correction system, the temperature of the buffer solution shall be within the range 20±2 °C.

Sample preparation

- Liquid products: Mix the laboratory sample carefully until it is homogeneous.
- Thick or semi-thick products: Mix a part of the laboratory sample and grind it, if necessary with a blender or mortar. If the product obtained still too thick, add an equal mass of distilled water and mix well.

Test portion

Use as a test portion a volume of the prepared sample sufficient for immersion of the electrodes, according to the apparatus used.

Determination

Introduce the electrodes into the test portion and set the temperature correction system of the pH meter to the temperature of measurement.

Carry out the determination using the procedure appropriate to the pH meter used.
When constant value has been reached, read the pH directly, to at least 0.05 pH unit.
Carry out two determinations on two separate test portions.

Expression of results

Take as the result the arithmetic mean of the results of the two determinations. Report the results to at least 0.05 pH unit.

pH values of the analyzed samples:

Apple juice: 3.70 and 3.70	Mean: 3.70
Orange juice: 3.99 and 3.99	Mean: 3.99
Apricot jam: 3.46 and 3.47	Mean: 3.47
Carrot jam: 4.60 and 4.59	Mean: 4.60

Chloride content in meat and meat products

ISO 1841-1:1996

The method utilise the extraction of a test portion with hot water and precipitation of proteins. After filtration and acidification, excess of silver nitrate solution is added to the extract, and titration is carried out of the excess with potassium thiocyanate solution.

Reagents (*only use recognized analytical grades, unless otherwise specified. Water should comply with at least grade 3 in accordance with ISO 3696)*

Water, distilled and halogen free (for chromatography; LiChrosolv® (1.15333)
Nitrobenzene, ACS reagent, ≥99.0% (8.06770)
Nitric acid; Suprapur® c(HNO3) = 4 mol/L (1.00441)

Carrez Clarification Kit reagent kit for sample prep in food analysis, 5-fold concentrate (1.10537)
(Ready-to-use Carrez clarification kit can accelerate the analyses)

Silver nitrate solution; Reag. Ph Eur,Reag. USP; c($AgNO_3$) = 0.1 mol/L (0.1 N) Titripur® (1.09081)
Potassium thiocyanate, EMPLURA® (1.05124)
Ammonium iron(III) sulfate , EMSURE® ACS,ISO,Reag. Ph Eur (1.03776)

Dissolve in water about 9.7 g of potassium thiocyanate.
Transfer quantitatively to a 1000 mL one-mark volumetric flask and dilute to the mark with water.
Standardize the solution to the nearest 0.0001 mol/L against the silver nitrate solution using the ammonium iron(III) sulfate solution as indicator.

Apparatus:
Analytical balance
Homogenizing equipment
One-mark volumetric flask, of capacity of 200mL
Conical flask of capacity 250 mL
Burette of capacity 50 mL
Boiling water bath

Chloride content in meat and meat products

ISO 1841-1:1996

Procedure:
Weigh about 10 g of the sample (to the nearest 0.001 g) and transfer it quantitatively to a conical flask (referred to as test portion)

Deproteination (elimination of protein from the sample)

1. Add 100 ml of hot water to the test portion.
2. Heat the flask and its contents for 15 minutes in the boiling water bath.
3. Every 3-5 minutes shake the contents of the flask.
4. Allow the flask and its contents to cool to room temperature and add 2 mL of Carrez I and 2 mL of Carrez II solution. Mix thoroughly after each addition.

Allow the flask to stand for 30 minutes at room temperature.
Transfer the contents quantitatively to a 200 mL volumetric flask and dilute to the mark with water. Mix contents and filter through a fluted filter paper.

Determination:
Transfer 20 ml of the filtrate to a conical flask and add 5 mL of the diluted nitric acid and 1 mL of ammonium iron(III) sulfate solution as indicator. Transfer 20 mL of the silver nitrate solution to the conical flask, then add 3 mL of the nitrobenzene and mix thoroughly. Shake vigorously to coagulate the precipitate. Titrate the content of the conical flask with potassium thiocyanate until the appearance of a persistent pink coloration. Record the volume of the potassium thiocyanate solution required, to the nearest 0.05 mL.

Blank test: Carry out a blank test using the same volume of silver nitrate solution.

Calculation: Chloride content=58.44 x (V2-V1)/m x C

V1:is the volume, in milliliters of the potassium thiocyanate solution used in the determination
V2: is the volume, in milliliters of the potassium thiocyanate solution used in the blank test
m: is the mass, in grams of the test portion
C: is the concentration of the potassium thiocyanate solution in moles per liters

Calculation in case of analyzed sample (2015/34018)
V1= 16.75, V2= 19.90. m= 10.112 and c=0.1

Chloride content=1.82 %

(Tips: To calculate the salt content: chloride x 1.65) Salt Content = 3.00%

Fatty acid methyl esters (FAME) in oil samples

EN ISO 12966-2:2011 and EN ISO 12966-4:2015

The method utilise the fact that methyl esters are formed by transmethylation with methanolic potassium hydroxide. Using capillary gas chromatography, fatty acid methyl esters (FAMEs) are separated on a highly polar stationary phase with respect to their chain length, degree of (un)saturation, and geometry and position of the double bonds.

In the highlighted norms, it is described how you work and below you can find relevant products:

Reagents (*only use recognized analytical grades, unless otherwise specified. Water should comply with at least grade 3 in accordance with ISO 3696)*

Water, distilled and halogen free (for chromatography; LiChrosolv® (1.15333)
Methanol for gas chromatography ECD and FID SupraSolv® (1.06011)
Sodium hydrogen sulfate monohydrate; for analysis EMSURE® (1.06352)
Isooctane for gas chromatography ECD and FID SupraSolv® (1.15440)
Potassium hydroxide; pellets for analysis EMSURE® (1.05033)

FAME standards

Apparatus:
Screw-top test tubes, 10 mL
Glass sample vials
One-mark volumetric flask, capacities 50 and 100 mL

Gas-chromatograph with FID equipped with a:
Capillary GC column: SLB®-IL60 Capillary GC Column; L×I.D. 60 m×0.32 mm, df 0.26 µm

Experimental conditions:
Carrier gas: helium, 2.2 ml/min; flame gases: hydrogen and air; make-up gas: nitrogen
Injector temperature: 250 °C
Detector (FID) temperature: 300 °C
Oven temperature: See table
Injection volume: 1 µL

Time (min)	Temperature (°C)
0-2	80
2-152	80-230 (1°C/min)
152-162	230

Fatty acid methyl esters (FAME) in oil samples

EN ISO 12966-2:2011 and EN ISO 12966-4:2015

Procedure: (Sample: Sunflower oil-high oleic acid content)

1. Preparation of fatty acid methyl esters

Pipette 60 µL of the test sample into a 10 ml screw-top test tube.
Add 5 mL of isooctane, and vortex.
Add 400 µL of 2 mol/L potassium hydroxide solution, and immediately put on the cap, tighten, and shake vigorously for 1 minute. The solution become clear and shortly afterwards becomes cloudy again as glycerol separates. Allow to stand for approximately 2 minutes.
Add approximately 1 g of sodium hydrogen sulfate and shake briefly. Draw off the isooctane layer and transfer to a sample vial. The isooctane solution is suitable for analyses using GC.

2.Calculations

The individual FAMEs are identified by their retention times and in comparison with FAME reference standards. Peaks of unknown identity should not be included in the summation of peak areas when calculating fatty acid composition, unless they have been confirmed to be fatty acids. It is also possible to summarize unknown peaks as such.

AFFAME = AFAME/ΣA x 100 where:

AFFAME: the area fraction of individual fatty acid methyl esters
AFAME: is the area of the individual fatty acid methyl ester
ΣA: is the sum of areas under all peaks of all individual fatty acid methyl esters

Results:

FAME	Retention Time (min)	%
Methyl palmitate	68.4	4.10
Methyl palmitoleate	70.8	0.15
Methyl octadecanoate	82.7	2.94
cis-9-oleic acid methyl ester	84.6	83.5
Methyl Linoleate	88.2	5.95
gamma-Linolenic acid methyl ester	92.8	0.11
Methyl cis-11-eicosenoate	95.6	0.42
Methyl Linolenate	97.3	0.30
cis-11,14,17-Eicosatrienoic acid methyl	107.8	0.97
Methyl cis-5,8,11,14,17-Eicosapentaenoate	119.1	0.33
Unidentified		1.21
Sum		100.00

Determination of lead and cadmium in fish

EN 14084:2003

The method analyze samples that are digested in closed vessels, using nitric acid and a microwave oven. The resulting solution is diluted with water, and the lead and cadmium contents are determined by graphite furnace atomic absorption spectrometry (GFAAS) with matrix modifier.

Reagents (*only use recognized analytical grades, unless otherwise specified.*
Water should comply with at least grade 3 in accordance with ISO 3696)
Thus use only reagents/water, with an element level low enough not to affect results.

Water, LiChrosolv® (1.15333)
Nitric acid 65% Suprapur® (1.00441)
Magnesium nitrate hexahydrate 99.99 Suprapur® (1.05855)

Lead (1000 mg/l Pb in HNO_3 0.5 mol/L) traceable to SRM from NIST. CertiPUR® (1.19776)
Cadmium (1000 mg/l Cd in HNO_3 0.5 mol/L) traceable to SRM from NIST CertiPUR® (1.19777)

Apparatus:
Laboratory mill (e.g. knife mill)
Laboratory microwave oven
Atomic absorption spectrometer
Graphite tubes
Element specific lamps

Determination of lead and cadmium in fish

EN 14084:2003

Procedure:

1. Homogenize the sample with a laboratory mill.

Suggestion: in some cases, the drying of the sample is needed in a way that does not affect the element contents, e.g. by freeze drying.

2. Sample preparation

- Weigh 0.5-1.5 g of sample in a vessel.
- Add 5.0 mL of nitric acid. After 30 minutes add 5.0 mL of distilled water and mix it gently.
- Allow samples to predigest by standing open for a minimum of 15 minutes before sealing vessels and proceeding to heating program.

Typically an oven programme includes a stage at low power with increasing temperature for a few minutes followed by one or more stages at higher power settings. A gradual increase between the selected stages is recommended in order to prevent sudden pressure peaks to occur inside the pressure vessels.

Suggestion: samples with high carbon content (e.g. sugar, fat) may cause sudden pressure peaks during the process. Allow these samples to predigest by standing an overnight.

3. Microwave heating program

Step	Time (min)	Temperature (°C)
1	20	Up to 200 °C
2	20-30	200 °C
3	From 30 and onwards	Cooling down

Suggestion: when digesting unknown samples, take care since a too large sample amount may rupture the safety membrane of the digestion vessel. In particular, samples with high carbon content (e.g. sugar and fat) may cause sudden pressure peaks during the process. In all cases, the sample intake should be in strict compliance with the manufacturers recommendation.

Determination of lead and cadmium in fish

EN 14084:2003

4. Measurement with graphite furnace technique

Graphite furnace technique is required for determination of lead and cadmium. Use pyrolytically coated tubes with platforms. Program the autosampler to deliver sample volume to the graphite furnace, which gives a background absorbance of not more than about 0.5 absorbance units. Instrumental parameters with an injection volume of 20 µL.

Element	Wavelength (nm)	Background Correction	Parameter	Step 1	Step 2	Step 3	Step 4
Cd	228.8	Zeeman	Temp (°C)	110	450	1000	2500
			Ramp (°C/sec)	10	150	0	0
			Hold (sec)	30	20	3	3
Pb	217	Zeeman	Temp (°C)	120	800	1200	2500
			Ramp (°C/sec)	10	150	0	0
			Hold (sec)	10	20	3	3

5. Calculation

Construct a standard curve and read the concentration of the metal from the curve. Calculate the content (c), as mass fraction of the element to de determined in µg/kg of sample:

$c = ((a-b) \times V)/m$

a: is the concentration in the sample solution in µg/L
b: is the mean concentration in the reagent blank solution in µg/L
V: is the volume of sample solution in mL
m: is the sample mass in gram

Result:
(in case of sample 2015/33632
- Patagonotothen spp - fish)

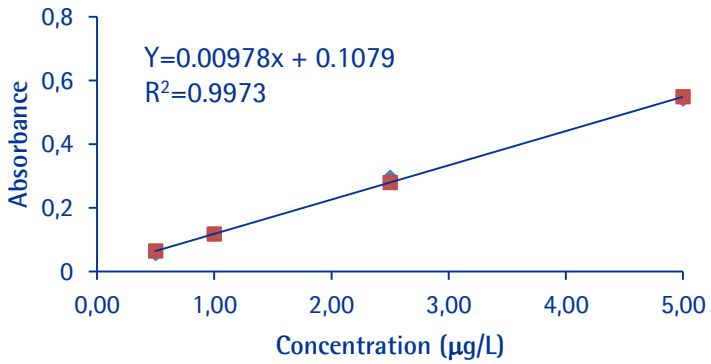
	Lead	Cadmium
m (g)	1.0115	1.0015
a	0.8493	0.3025
b	-	-
V (mL)	100	100
C (µg/kg)	84	29.9

Determination of lead and cadmium in fish

EN 14084:2003

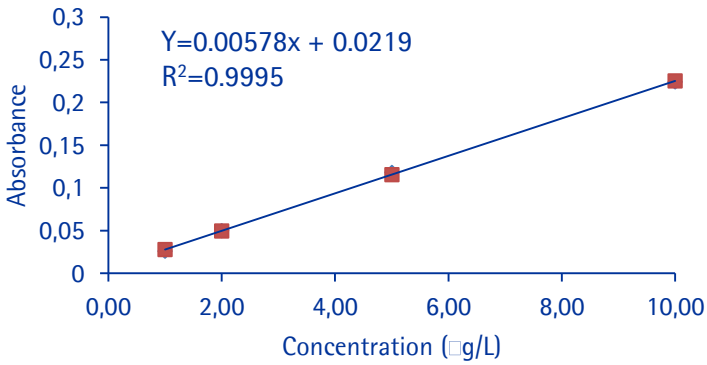
6. Calibration data – Cadmium (Cd)

Sample ID	"SignalAbs (Height)"	"Conc (µg/L)
Cd Blank	0,002	0,0000
Cd standard 1	0,056	0,5000
Cd standard 2	0,116	1,0000
Cd standard 3	0,296	2,5000
Cd standard 4	0,542	5,0000
Cd Blank	0,002	0,0005
Cd Blank	0,002	0,0065
Cd 33632	0,034	0,3025
Cd Ha94	0,191	1,6413
Cd 2.5 ppb std	0,296	2,4974
Cd blank	0,002	0,0005



7. Calibration data – Lead (Pb)

Sample ID	"SignalAbs (Height)"	"Conc (µg/L)
Pb Blank	0,003	0,0000
Pb standard 1	0,026	1,0000
Pb standard 2	0,050	2,0000
Pb standard 3	0,118	5,0000
Pb standard 4	0,224	10,0000
Pb Blank	0,003	0,0022
Pb Blank	0,002	-0,0218
Pb 33632	0,022	0,8493
Pb Ha94	0,036	1,4433
Cd 25.0 ppb std	0,122	5,2115
Pb Blank	0,002	-0,0524



Pb Ha94 and Cd Ha94 are reference materials

Determination of deoxynivalenol (DON)

EN 15791:2009

Deoxynivalenol (DON) is extracted from the sample using water. The aqueous extract is cleaned up with an immunoaffinity column to remove impurities from the sample. Subsequently DON is quantitatively determined by HPLC with UV detection.

The EN norm describe the complete procedure and below you can find relevant products:
Reagents (*only use recognized analytical grades, unless otherwise specified.)*

Methanol gradient grade; LiChrosolv® Reag. Ph Eur (1.06007)
Water, LiChrosolv® (1.15333)

Deoxynivalenol (DON) analytical standard
DON calibration solution: 0.025; 0.05; 0.1; 0,2; 0,5; 1,0; and 2,5 µg/mL

Apparatus:
Analytical balance
Homogeniser
Laboratory shaker
Vortex mixer
Screw cap flasks, with volumes of 500 mL
Funnels
Filter papers
Volumetric flasks
Evaporator with a stream of air or nitrogen

DON immunoaffinity clean-up columns

HPLC system equipped with:

Analytical column: Purospher® STAR RP-18 endcapped (5 µm), Hibar®, 125x4 mm (1.50036)
Equipped with Purospher® STAR RP-18 endcapped (5 µm) LiChroCART® 4-4 guards (1.50250)

Determination of deoxynivalenol (DON)

EN 15791:2009

1.Sample preparation:

- the samples should be finely ground and thoroughly mixed using a mill.

We have used wheat (id 2015/B/4586) as sample matrix

2.Sample extraction

Weigh a 25.0 g test portion into a 500 mL screw cap flask.
Add 200 mL of deionized water, cap and shake for 1 hour with a shaker.
Prepare a funnel with filter paper.
Filtrate the extracted sample into a clean 500 mL screw cap flask.

Tips: Sometimes the filtration takes a long time. It can be accelerated with the usage of two different pore size filter paper

3. Immunoaffinity Column Clean-up

Attach a reservoir to an immunoaffinity column. Transfer 2.0 mL of the filtered extract into the reservoir. Allow the solution to pass slowly through the column by gravity at a rate of 1-2 drop/s. When all the extract has passed completely through the immunoaffinity column, pass 5 mL of deionized water through the column. Remove residual liquid by passing nitrogen or air through the column for about 5 seconds. Discard all the eluate from this stage of the clean-up procedure.

Tips: The capacity of the used immunoaffinity column has to be checked regularly.
The column shall have a capacity of not less than 2 500 ng.

Finally, place a HPLC autosampler vial under the column and pass 0.5 mL of methanol through the column and by gravity collect the eluate. After the last drops of methanol have passed through the column allow the methanol to remain on the column for approximately 1 minute. Then add another 1.0 mL of methanol and collect the eluate.
Carefully pass nitrogen or air through the column in order to collect any residual eluate.

4.Preparation of the test solution for HPLC analysis

Place the vial with the eluate in the evaporator and carefully evaporate to dryness under nitrogen or air at ca. 50°C. Immediately afterwards cool the HPLC vial to ambient temperature and reconstitute the residue with 1.0 mL of HPLC mobile phase.
Mix well with vortex mixer for at least 30 seconds to ensure the residue is completely re-dissolved. In case of turbidity filtrate the test solution through a syringe filter unit.

Determination of deoxynivalenol (DON)

EN 15791:2009

5. Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5 µm), 125x4 mm, Hibar® (1.50036)
Injection:	100 µL
Detection:	UV detection, 220 nm
Flow Rate:	0,7 mL/min
Mobile Phase:	Methanol and water 20:80 (v/v)
Temperature:	25 °C
Diluent:	Mobile phase
Sample:	Wheat
Pressure Drop:	120 Bar

6. Calculation

$w(DON)=c(DON) \times (V(3))/(V(2)) \times (V(1))/(m(s))$ where

c(DON):	is the mass concentration of DON as determined through calibration
V(1):	is the total volume of extraction solvent (200 mL)
V(2):	is the volume of the aliquot of extract used for clean-up
V(3):	is the total volume of test solution
m(s):	is the mass of the extracted test portion

Calculation in case of sample 2015/B/4586

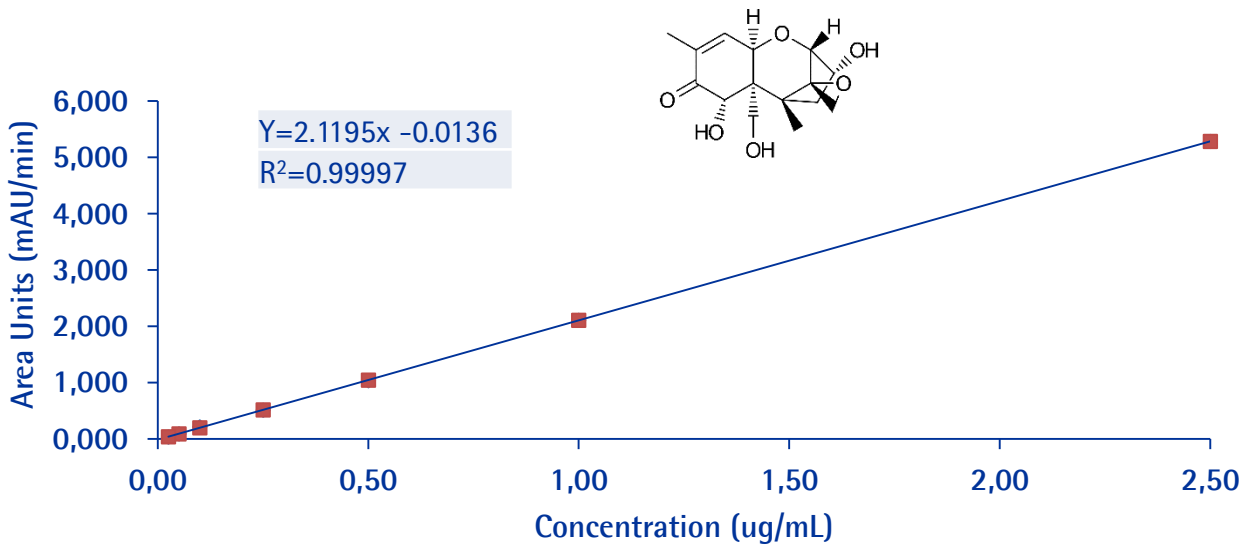
c(DON):	1.221
V(1):	200 mL
V(2):	2 mL
V(3):	1 mL
m(s):	25 g

w(DON): 4.9 mg/kg

Thus in the analyzed sample 4.9 mg/kg Deoxynivalenol (DON) could be found

Determination of deoxynivalenol (DON)

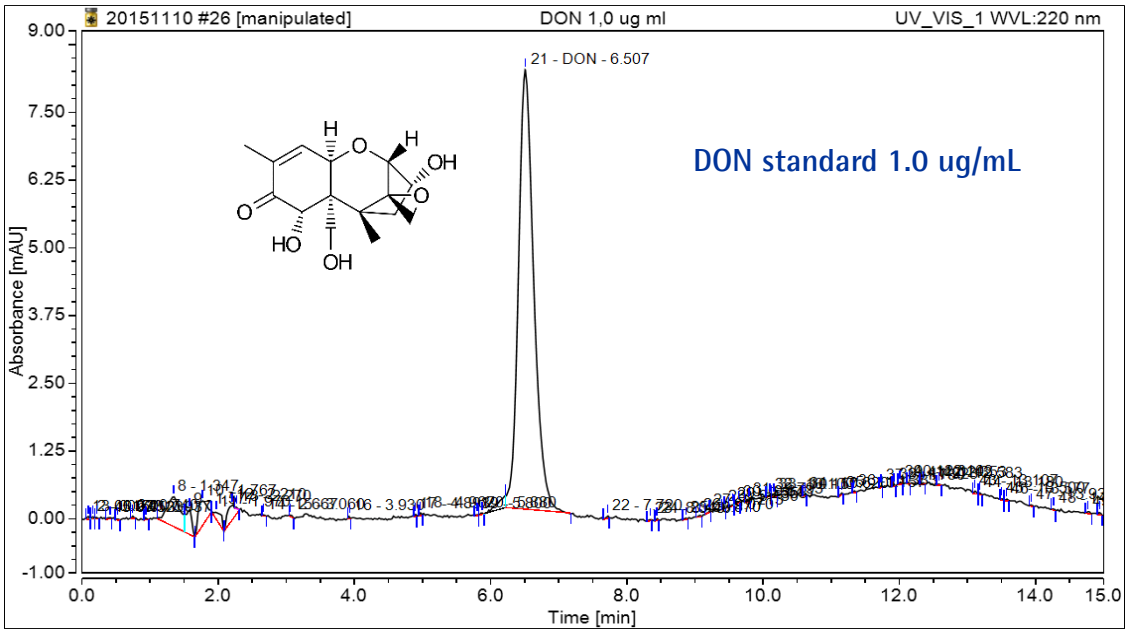
EN 15791:2009

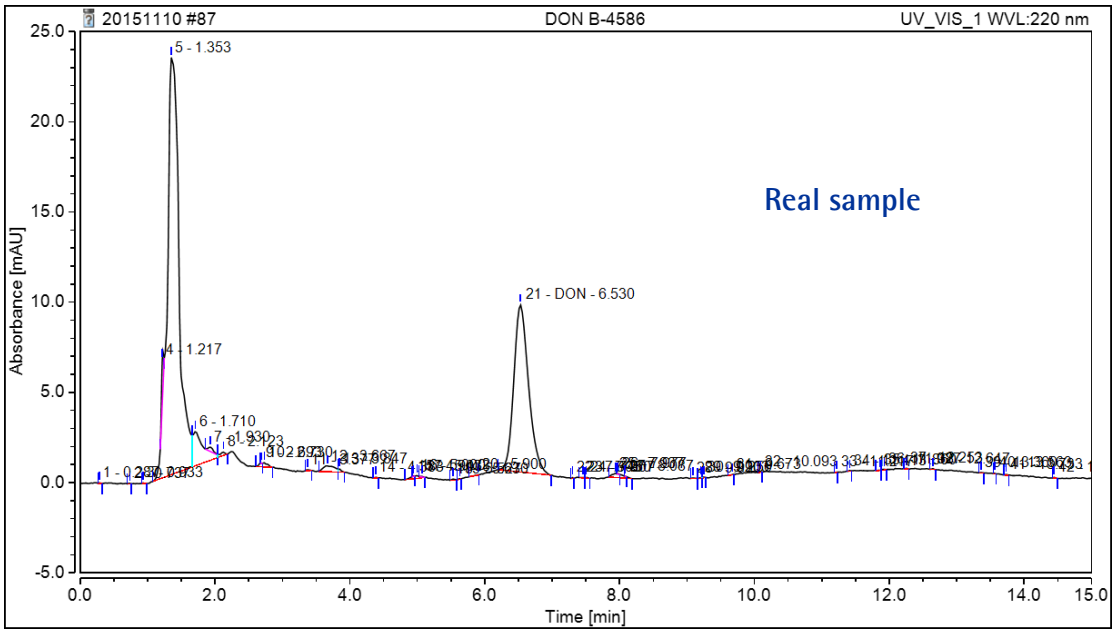


Calibration Results				
ID	level	Concentration (ug/mL)	Area (mAU/min)	Height (mAU)
DON 2.5 ug/mL	04	2.5000	5.286	18.75
DON 1.0 ug/mL	05	1.0000	2.109	8.107
DON 0.5 ug/mL	01	0.5000	1.034	4.127
DON 0.25 ug/mL	02	0.2500	0.509	1.777
DON 0.1 ug/mL	03	0.1000	0.219	0.961
DON 0.05 ug/mL	06	0.0500	0.092	0.398
DON 0.025 ug/mL	07	0.0250	0.034	0.176

Determination of deoxynivalenol (DON)

EN 15791:2009





		RT (min)	Width 50% (min)	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	DON	6.5	0.23	5.1	1.2	4285

Same sample – different methods

On the following pages we have included examples of salt content analysis from same samples (one type of Italian cold cut and one salami) but with different analytical techniques. The sodium content is determined with atomic absorption spectroscopy and the chloride content is determined via titration.

In case of nutrition value determination, according to EU Regulation No. 1169/2011, the salt content has to be written on the product label (previously only the sodium was written on it). In this regulation, it is prescribed that the salt content can be calculated only from sodium (salt content=sodium x 2.5).

There are other regulations where the limits are given in sodium-chloride.
It is not given what kind of measurement (chloride based or sodium based) has to be applied.

The advantage of a chloride based method is speed (much faster) and there is no need for big capital instrumental investments. Usually all the internal labs in the meat industry use the chloride based method. However, a titration method is less exact, with higher uncertainty.

In case of sodium determination, you need an instrument, but this provide you with a more precise method, and you have to use this procedure in case of nutrition value determination.

The experience from industry is, that especially in the meat industry, several food additives are used, which have sodium content beside the salt (e.g. sodium nitrite). In that case the salt content based on sodium are usually higher than the salt content based on chloride. The latter statement is verified in this example; where a difference of 0.22 and 0.14% in salt content was determined for the Italian cold cut and the salami sample, respectively.

Chloride content in meat and meat products

ISO 1841-1:1996

The method utilise the extraction of a test portion with hot water and precipitation of proteins. After filtration and acidification, excess of silver nitrate solution is added to the extract, and titration is carried out of the excess with potassium thiocyanate solution.

Reagents (*only use recognized analytical grades, unless otherwise specified)*

Water, distilled and halogen free (for chromatography; LiChrosolv® (1.15333)
Water should comply with at least grade 3 in accordance with ISO 3696)

Nitrobenzene, ≥99.0% (8.06770)
Nitric acid; Suprapur® c(HNO3) = 4 mol/L (1.00441)

Carrez Clarification Kit reagent kit for sample prep in food analysis, 5-fold concentrate(1.10537)
(Ready-to-use Carrez clarification kit can accelerate the analyses)

Silver nitrate solution; Reag. Ph Eur,Reag. USP; c($AgNO_3$) = 0.1 mol/L (0.1 N) Titripur® (1.09081)
Ammonium iron(III) sulfate , EMSURE® ACS,ISO,Reag. Ph Eur (1.03776)
Potassium thiocyanate, EMPLURA® (1.05124)

Dissolve in water about 9.7 g of potassium thiocyanate.
Transfer quantitatively to a 1000 mL one-mark volumetric flask and dilute to the mark with water.
Standardize the solution to the nearest 0.0001 mol/L against the silver nitrate solution using the ammonium iron(III) sulfate solution as indicator.

Apparatus:
Analytical balance
Homogenizing equipment
One-mark volumetric flask, of capacity of 200mL
Conical flask of capacity 250 mL
Burette of capacity 50 mL
Boiling water bath

Chloride content in meat and meat products

ISO 1841-1:1996

Procedure:
Weigh about 10 g of the sample (to the nearest 0.001 g) and transfer it quantitatively to a conical flask (referred to as test portion)

Deproteination (elimination of protein from the sample)

1. Add 100 ml of hot water to the test portion.
2. Heat the flask and its contents for 15 minutes in the boiling water bath.
3. Every 3-5 minutes shake the contents of the flask.
4. Allow the flask and its contents to cool to room temperature and add 2 ml of Carrez I and 2 ml of Carrez II solution. Mix thoroughly after each addition.

Allow the flask to stand for 30 minutes at room temperature.
Transfer the contents quantitatively to a 200 ml volumetric flask and dilute to the mark with water. Mix contents and filter through a fluted filter paper.

Determination:
Transfer 20 ml of the filtrate to a conical flask and add 5 ml of the diluted nitric acid and 1 ml of ammonium iron(III) sulfate solution as indicator.

Transfer 20 ml of the silver nitrate solution to the conical flask, then add 3 ml of the nitrobenzene and mix thoroughly. Shake vigorously to coagulate the precipitate.

Titrate the content of the conical flask with potassium thiocyanate until the appearance of a persistent pink coloration. Record the volume of the potassium thiocyanate solution required, to the nearest 0.05 ml.

Blank test: Carry out a blank test using the same volume of silver nitrate solution.

Calculation:

Chloride content=58,44 x (V2-V1)/m x C

V1:is the volume, in milliliters of the potassium thiocyanate solution used in the determination
V2: is the volume, in milliliters of the potassium thiocyanate solution used in the blank test
m: is the mass, in grams of the test portion
C: is the concentration of the potassium thiocyanate solution in moles per liter (L).

Chloride content in meat and meat products

ISO 1841-1:1996

Samples:
sample ID 2015/P/20298 (Italian cold cut)
Sample ID 2015/36814 (salami)

Results:

Chloride content=58,44 x (V2-V1)/m x C
Salt content = Chloride content x 1.65

	sample 2015/P/20298 (cold cut)	sample 2015/36814 (salami)
V1	18.4	15.7
V2	19.9	19.9
m	10.0514	10.0597
C	0.1	0.1
Chloride content (%)	0.87	2.43
Salt content (%)	1.43	4.01

Determination of Sodium content in meat

EN 15505:2008

The sample is digested in closed vessels in a microwave oven in nitric acid. The resulting solution is diluted with water, and the sodium content is determined by flame-AAS using matrix modifier.

Reagents (*only use recognized analytical grades, unless otherwise specified.)*

Thus use only reagents/water, with an element level low enough not to affect results.

Water, LiChrosolv® (p/n 1.15333)
Water should comply with at least grade 3 in accordance with ISO 3696)

Nitric acid 65% Suprapur® (1.00441)
Sodium (1000 mg/l Na in HNO_3 0.5 mol/l) traceable to SRM from NIST; CertiPUR® (1.70238)
Cesium chloride 99.995 Suprapur® (1.02039)

Apparatus:
(All glassware and plastic ware should be carefully cleaned and rinsed to Avoid cross contamination. The exact method of cleaning is described in the EN 13804 standard.)

Laboratory mill (e.g. knife mill)
Laboratory microwave oven
Atomic absorption spectrometer

Element specific lamps:
For sodium element specific lamps with wavelength of 589,0 nm is applied.
(Tips: Sodium can be measured with AAS in emission mode.)

Acetylene with appropriate quality.
Air

Determination of Sodium content in meat

EN 15505:2008

Procedure:

1. Homogenize the sample with a laboratory mill.

Suggestion: in some cases, the drying of the sample is needed in a way that does not affect the element contents, e.g. by freeze drying.

2. Sample preparation

- Weigh 1.0-2.5 g of sample in a vessel.
- Add 5.0 ml of nitric acid. After 30 minutes add 5.0 ml of distilled water and mix it gently.
- Allow samples to predigest by standing open for a minimum of 15 minutes before sealing vessels and proceeding to heating program.

Tips:In case of samples with high fat content, reduce the test portion 0.5-1.0 g.
In case of samples with high water content, the test portion can be increased up to 2.0-3.0 g.
Samples with high carbon content (e.g. sugar, fat) may cause sudden pressure peaks during the process. Allow these samples to predigest by standing an overnight.

3. Microwave heating program

Step	Time (min)	Temperature (°C)
1	0-15	Up to 190 °C
2	15-35	200 °C
3	From 35 and onwards	Cooling down

4. Dilution

Pipette a suitable volume of the sample solution, add 1 ml of Cs-solution and dilute (practically to 500 ml) this volume with 2,7 % nitric acid so that the final concentration of Na is within the range of measurement of the element.

5. Atomic absorption spectrometry

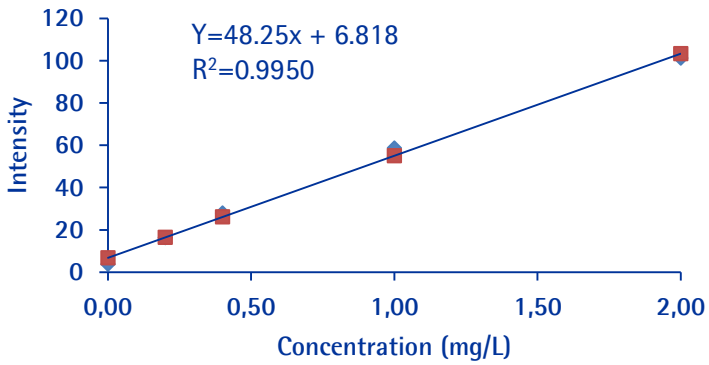
Before every determination, adjust the instrument as specified in the manufacturer's operating manual. The exact settings of our instrument is attached in a separate file.

Determination of Sodium content in meat

EN 15505:2008

6. Calibration - Sodium

Sample ID	"Intensity" (Height)"	"Conc (mg/L)
Blank	3.7	0.0000
Std1	16.3	0.2000
Std2	28	0.4000
Std3	58.6	1.0000
Std4	101.2	2.0000
P-20298	50.4	08246
36814	55.1	0.9231



7. Calculation

c=(a x V x F)/m where

c: is the mass fraction of sodium in milligram per kilogram
a:is the content of the element, in mg/l
V:is the volume of the digestion solution, in ml
F: is the dilution factor of the test solution
m:is the initial sample, in grams

	sample 2015/P/20298 (cold cut)	sample 2015/36814 (salami)
a (mg/L)	0.8246	0.9231
V (mL)	500	500
F (dilution)	25	50
m (g)	1.5713	1.3890
Sodium content (%)	0.66	1.66
Salt content (%)	1.65	4.15

Water content determination in oil samples

DIN EN ISO 8534:2009

Dissolved fat is titrated against an iodine solution and sulfur dioxide (SO_2) is oxidized by iodine in the presence of water. The alcohol reacts with SO_2 and a nitrogeneous (RN) base to form an intermediate alkylsulfite salt, which is then oxidized by iodine to an alkylsulfate salt. This oxidation reaction consumes water contained in the sample.

Reagents for Karl Fischer analysis:
only use recognized analytical grades, unless otherwise specified.)

CombiCoulomat frit (1.09255.0500)
One-component reagents: contain all the reactant in the titrant solution

Water standard (1.88052.0010, 1%)

Apparatus: Cou-Lo Aquamax for example

Determination:
1. check the apparatus by measuring a standard (inject 0.5 mL)
2. if the result comes back within ±10%, it's usable
3. inject the sample into the titration vessel (0.5 mL sample)
4. record the mass
5. enter the sample mass in the instrument
6. the instrument will calculate the final result
7. measure the sample two times, then take the average
8. the difference must be within 10% between the two measurements

Results:

	Standard	Sample 1	Sample 2
Total weight (g)	3.3128	2.9408	2.9561
Sample weight (g)	0.4561	0.4657	0.4786
Result (mg/kg)	9767.38	292.53	292.17

Determination of trace elements

EN 13805:2002

This is a method for the determination of iron, magnesium, potassium, sodium and calcium in foodstuffs. The sample is mineralized through pressurized digestion with nitric acid. In the resulting digestion solution, iron, magnesium, potassium, sodium and calcium are quantified by inductively coupled plasma optical emission spectrometry (ICP-OES).

Reagents:
(only use recognized analytical grades, unless otherwise specified. The concentration of iron, magnesium, potassium, sodium and calcium in the reagents and water used shall be low enough not to affect the results of the determination)

Water, LiChrosolv® (1.15333)
Nitric acid 65% Suprapur® (1.00441)
Multi-element stock solution-Certipur® ICP multi-element standard solution IV (1.11355.0100)
ρ(Fe) = 1000 mg/L
ρ(Mg) = 1000 mg/L
ρ(K) = 1000 mg/L
ρ(Na) = 1000 mg/L
ρ(Ca) = 1000 mg/L

Multi-element standard and calibration solutions
The standard and calibration solutions are prepared from the stock solution by dilution in glass volumetric flasks. For calibration, prepare at least five (5) calibration solutions of different concentrations. The acid concentration shall correspond to the concentration in the measurement solution. The preparation of the solutions below is given as an example.

Calibration solutions of ρ(Fe, Mg, K, Na, Ca) = 0.5, 1.0, 2.5, 5.0, and 10.0 mg/L for ICP-OES.

Fill five 100 mL volumetric flasks with 10-20 mL of water, add 10 mL of nitric acid and mix. Cool the solutions to ambient temperature, and pipette exactly 0.05, 0.1, 0.25, 0.5, 1.0 mL of multi-element stock solution for the respective calibration solutions of mass concentrations 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, 5.0 mg/L, 10.0 mg/L into the five different 100 mL volumetric flasks.
Mix the solutions and dilute to volume with water.

The calibration solutions described here shall be understood as examples. The concentrations prepared shall be in the linear range of the measuring device. The acid concentration of the calibration solutions shall be matched to the acid concentration in the sample solution.

Determination of trace elements

EN 13805:2002

Blank solution

The blank solution contains water, nitric acid in amounts that correspond to the concentrations in the measurement solution, for example 10 mL of nitric acid in 100 mL of water.

Apparatus

Microwave Reaction System
ICP- OES (Axial viewing).

Digestion of the sample

Mineralize the sample in pressurized digestion in accordance with EN 13805:2002. The digestion requirements are based on the specifications of the instrument manufacturer, the reactivity of the sample, the maximum pressure stability of the digestion vessel and the attainable temperature.

Precisely weigh 0.8-0.9 g of sample in digestion vessel and mix it 5 mL nitric acid and 1 mL water. The digestion solution that results from the pressurized digestion according to the norm can be used directly or can be diluted for the subsequent quantification of iron, magnesium, potassium, sodium and calcium.

Inductively coupled plasma optical emission spectrometry (ICP – OES)

Start the instrument and let it stabilize, then optimize it according to the manufacturer's specifications and begin measurements. Use the blank solution to zero the instrument. Use the calibration solutions and measure the emission of the element to be determined.

Evaluations

Relevant analytical lines and the limits of quantification (LOQ) using ICP–OES.

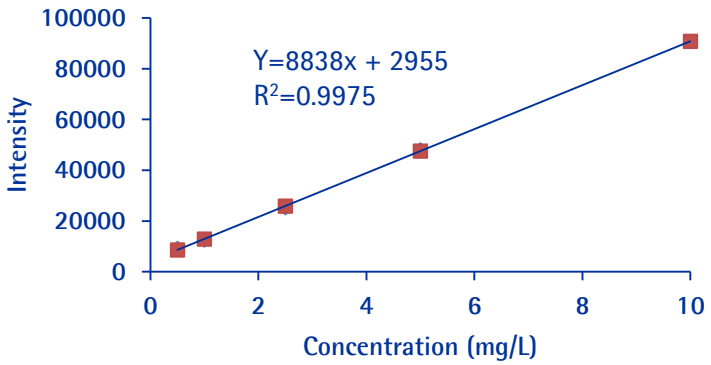
Element	Wavelength of emission (nm)	LOQ (mg/kg)
Fe	239.562	2.0
Mg	280.271	1.0
Ca	315.887	3.0
Na	589.592	3.0
K	766.490	1.5

Determination of trace elements

EN 13805:2002

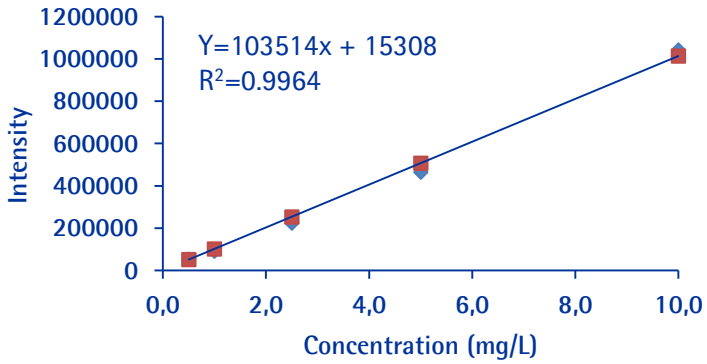
Calibration Data – Calcium (Ca):

Sample ID	"Intensity" (Height)"	"Conc (mg/L)
1	9208	0.5
2	12475	1.0
3	25234	2.5
4	48070	5.0
5	90670	10.0



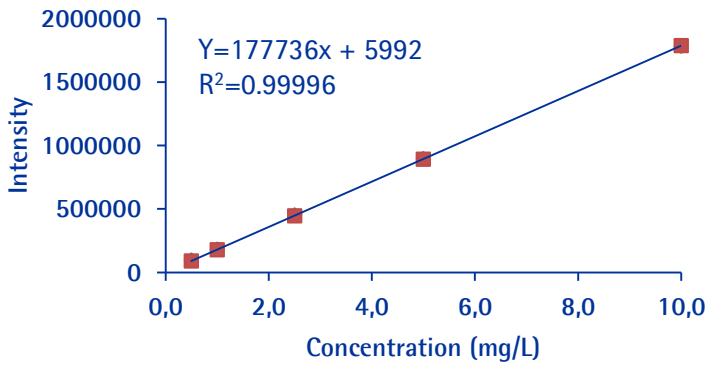
Calibration Data – Sodium (Na)

Sample ID	"Intensity" (Height)"	"Conc (mg/L)
1	52753	0.5
2	91055	1.0
3	224058	2.5
4	464668	5.0
5	1042380	10.0



Calibration Data – Magnesium (Mg)

Sample ID	"Intensity" (Height)"	"Conc (mg/L)
1	98003	0.5
2	184064	1.0
3	453123	2.5
4	895895	5.0
5	1781856	10.0

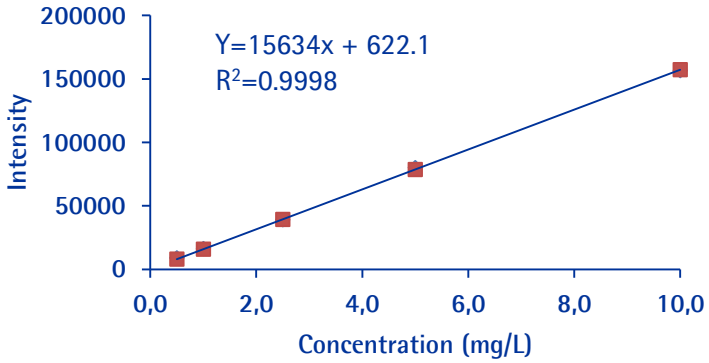


Determination of trace elements

EN 13805:2002

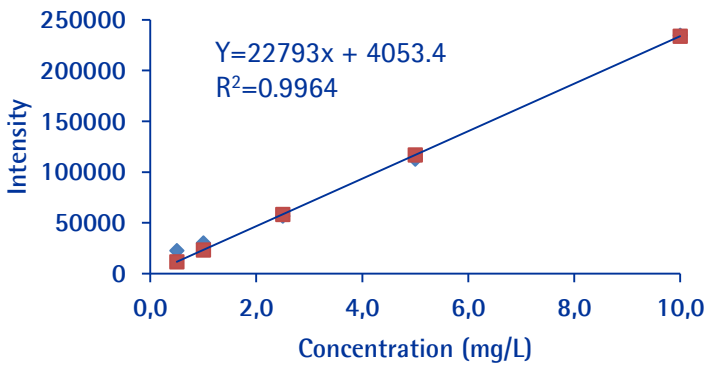
Calibration Data – Iron (Fe):

Sample ID	"Intensity" (Height)"	"Conc (mg/L)
1	9052	0.5
2	16226	1.0
3	38950	2.5
4	80062	5.0
5	156491	10.0



Calibration Data –Potassium (K)

Sample ID	"Intensity" (Height)"	"Conc (mg/L)
1	22605	0.5
2	30335	1.0
3	56651	2.5
4	112827	5.0
5	234966	10.0



Results: The table below present mean values of Iron (Fe), Magnesium (Mg), Calcium (Ca), Sodium (Na) and Potassium (K) found in the three different type of samples.

Sample	Fe	Mg	Ca	Na	K
	(mg/Kg)				
Beef	31.04	246.3	69.34	921.8	2692.5
Chicken	4.996	364.8	38.91	499.9	2946.3
Milk	1.329	123.5	402.2	402.2	1284.3

Residues

Definition

Pesticides

1. The designation pesticide applies to any substance or mixture of substances intended to prevent, destroy, or control any unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of pure articles. The designation includes substances intended for use as growth regulators, defoliants, or desiccants, and any substance applied to crops before or after harvest to protect the product from deterioration during storage and transport.
2. Pesticides are commonly used in agriculture. Pesticides may stay in small amounts (called residues) in or on fruits, vegetables, grains, and other foods. To make sure the food is safe for consumption, official bodies like the United States Environmental Protection Agency (EPA) regulates the amount of each pesticide that may remain in and on foods.
3. Pesticides are categorized into four main substituent chemicals: herbicides; fungicides; insecticides and bactericides.

Antibiotics

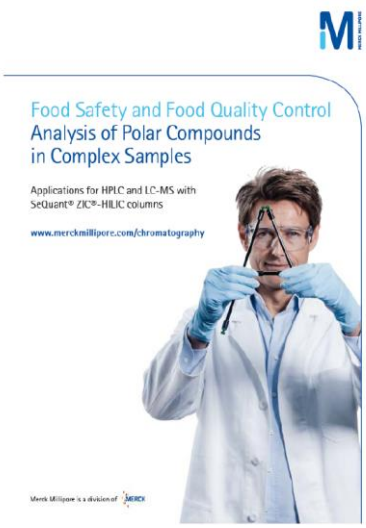
1. During their lifetime animals may have to be treated with different medicines for prevention or cure of diseases. In food producing animals such as cattle, pigs, poultry and fish this may lead to residues of the substances used for the treatment in the food products derived from these animals (e.g. meat, milk, eggs). The residues should however not be harmful to the consumer. To guarantee a high level of consumer protection, legislation requires that the toxicity of potential residues is evaluated before the use of a medicinal substance in food producing animals is authorized. If considered necessary, maximum residue limits (MRLs) are established and in some cases the use of the relevant substance is prohibited.

Further reading on pesticide and antibiotic residues:
http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Pesticides/default.htm
http://www.epa.gov/pesticides/index.htm
http://www.agf.gov.bc.ca/pesticides/
http://ec.europa.eu/food/food/index_en.htm
http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm
http://ec.europa.eu/sanco_pesticides/public/index.cfm
http://en.wikipedia.org/wiki/Pesticide

Pesticides

Pesticides are biological (such as a virus, bacterium, antimicrobial or disinfectant) or chemical substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Target pests can include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, spread disease or are vectors for disease.

There are currently 507 pesticides that are listed with maximum residue limits by the European Union. Many of these are difficult to analyze using traditional methods. For example chlormequat and mepiquat are two very hydrophilic pesticides, they are widely used as plant growth regulators. They act by inhibition of vegetative growth and promotion of flowering in a wide range of fruits, vegetables, cereals and cotton. They are eliminated in soil through microbiological processes and the end-product is carbon dioxide, but can accumulate in plants, animals and humans. The United States Environmental Protection Agency (US-EPA) has listed the compounds and hence it requires to be measured.



In previous application compilations you can find additional methods for residue screening.

Determination of Pesticides

EN 15662:2009

This is a method for the analysis of pesticide residues in foods of plant origin, such as fruits (including dried fruits), vegetables, cereals and processed products thereof. The method can analyze 300 components at the same time, using acetonitrile extraction/partitioning and SPE – QuEChERS method for clean-up followed by GC-MS and/or LC-MS/MS.
In this example, only 10 components were determined though.

Reagents:
(only use recognized analytical grades, unless otherwise specified)

Water (1.15333)
Acetonitrile (1.00029)
Methanol, LC-MS grade
Ammonium formate, LC-MS grade
Formic acid (1.00264.1000)
Magnesium sulfate anhydrous (1.06067)
Sodium chloride (1.06404)
Bondesil PSA, 40um, 100 g
Carbon SPE Bulk Sorbent, 25g bottle

Pesticide Standards
Azoxystrobin
Buprofezin
Fenpyroximate
Hexythiazox
Myclobutanil
Penconazole
Tetraconazole
Tolylfluanid
Trifloxystrobin
Triflumizole

Determination of Pesticides

EN 15662:2009

Sample preparation (tomatoes)

1. Take a representative sample (10 g) a place in a suitable container
2. Add 10 ml of formic acid: acetonitrile (1:1 volume/volume-%) and homogenize

3. Add buffer-salt mixture, homogenize
   - Magnesium sulfate (4 g)
   - Sodium chloride (1 g)
   - Centrifuge 4200 rpm, 2.5 min

4. Take as much as possible from the upper phase (4 mL)
5. Add sorption mixture salt to this phase, homogenize
   - Carbon SPE Bulk Sorbent (35.0 mg)
   - Bondesil-PSA (113.0 mg)
   - Magnesium sulfate (652.0 mg)
   - Centrifuge 4200 rpm, 2.5 min

6. Fill 1 ml from the upper phase into a vial

LC-MS/MS analysis (use an appropriate UHPLC system)
MS-MS detector: Q Trap MS/MS system or similar

HPLC column: Fused core C18 (10 cm x 3,0 mm, 2,7µm)
Guard Column: Fused core C18 (0.5 cm x 3,0 mm, 2,7µm)
Eluent system: A: 1 mmol/l ammonium formate with 0.1% formic acid in water
B: Methanol

Flow rate: 500 µL/min
Injection: 20 µL
Temperature: 40 C°
Gradient profile:

Time (min)	A (%)	B (%)
0	95	5
2.0	65	35
8.5	5	95
15.0	5	95
16.0	95	5
20.0	95	5

Determination of Pesticides

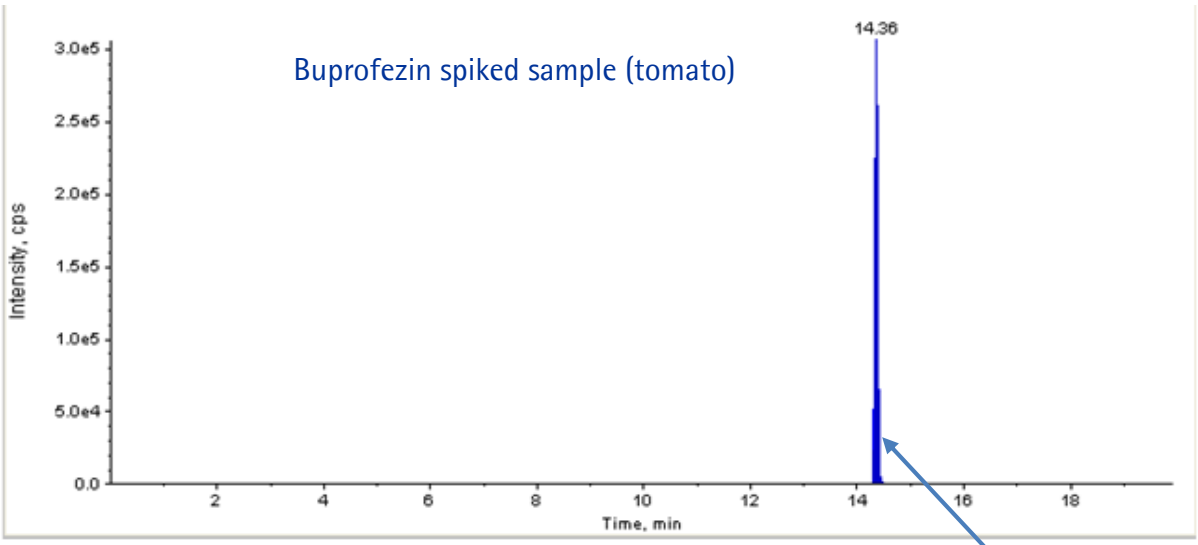
EN 15662:2009

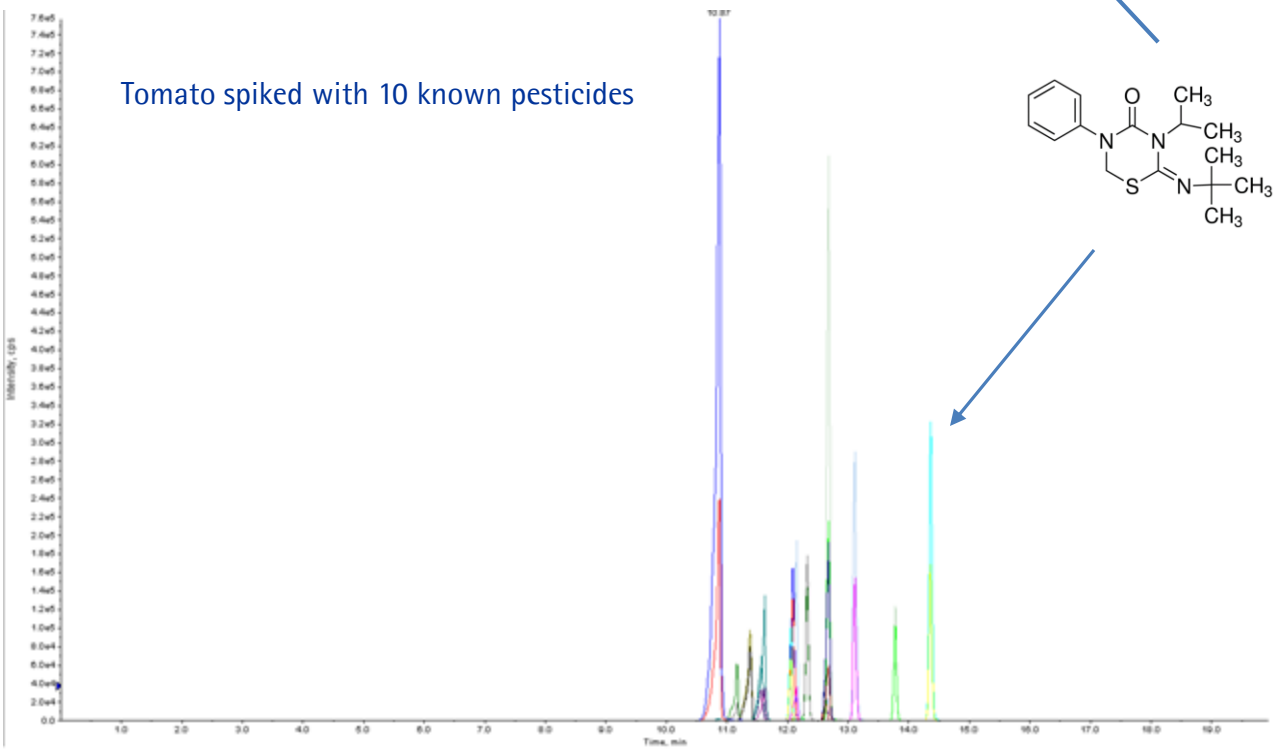
MS-MS parameters	
Polarity	+
CUR	40 psi
CAD	High
IS	5500 V
Temp	400 °C
GS1	35 psi
GS2	45 psi

	Q1	Q3	DP-(V)	EP- (V)	CE- (V)	CXP-(V)	RT (min)
Azoxystrobin 1	404.1	372.3	31	6.5	19	6	11.04
Azoxystrobin 2	404.1	344.2	31	6.5	25	6	11.04
Buprofezin 1	306.1	201.3	50	4	17	4	13.34
Buprofezin 2	306.1	106.0	50	4	31	4	13.34
Fenpyroximate 1	422.3	366.3	46	6	21	4	14.62
Fenpyroximate 2	422.3	135.2	46	6	45	4	14.62
Hexythiazox 1	353.3	228.0	41	6.5	19	4	14.02
Hexythiazox 2	353.3	168.3	41	6.5	31	4	14.02
Myclobutanil 1	289.1	70.2	41	5.5	31	4	11.56
Myclobutanil 2	289.1	125.1	41	5.5	39	4	11.56
Penconazole 1	284.2	159.1	36	5.5	35	4	12.28
Penconazole 2	284.2	70.2	36	5.5	29	4	12.28
Tetraconazole 1	372.1	159.0	46	4	39	4	11.79
Tetraconazole 2	372.1	70.1	46	4	39	4	11.79
Tolylfluanid 1	347.2	137.2	41	5.5	37	4	12.23
Tolylfluanid 2	347.2	238.0	41	5.5	15	4	12.23
Trifloxystrobin 1	409.3	186.1	41	4	23	4	12.85
Trifloxystrobin 2	409.3	206.3	41	4	19	4	12.85
Triflumizole 1	346.2	278.3	36	4	15	4	12.89
Triflumizole 2	346.2	73.1	36	4	23	4	12.89

Determination of Pesticides

EN 15662:2009





Instrumental Techniques

in norm methods

In this compilation, we discuss several parameters, which have to be checked by the food industry. It brings you examples using norm methods, defined by global organizations or local authorities, for the determination of different parameters. But how have these methods been selected, and is there any other alternative?

The norms follow the development of the analytical techniques, but by their nature, they do this slowly. A new technique has to be tested and probed for its robustness and usability.

Another important factor is that the list of critical parameters and also the concentration ranges of some components are changing in the norms over time. The number of molecules which prove to be harmful is increasing. The increase comes from new technologies, new human-made materials, but also from the continuous development of our knowledge about diseases. This means, that we have to develop measuring techniques for new materials, but also develop new techniques for new matrices or for very low concentration ranges.

Before we start with a quantitative determination, we have to make sure that the target molecule or element is brought into a measurable form, that neither physical nor chemical bounds will effect our result, and that the measured result comes only from the target compound indeed. In the case of food, this may often be quite a troublesome process. This is why, whenever possible, methods that are less sensitive for contaminants and methods needing a simple sample preparation are preferred in the food industry.

In the following chapters, you will find an overview and a short introduction to the techniques mentioned in the compilation. We give you a coarse-grained description, with some hints to the above mentioned aspects.

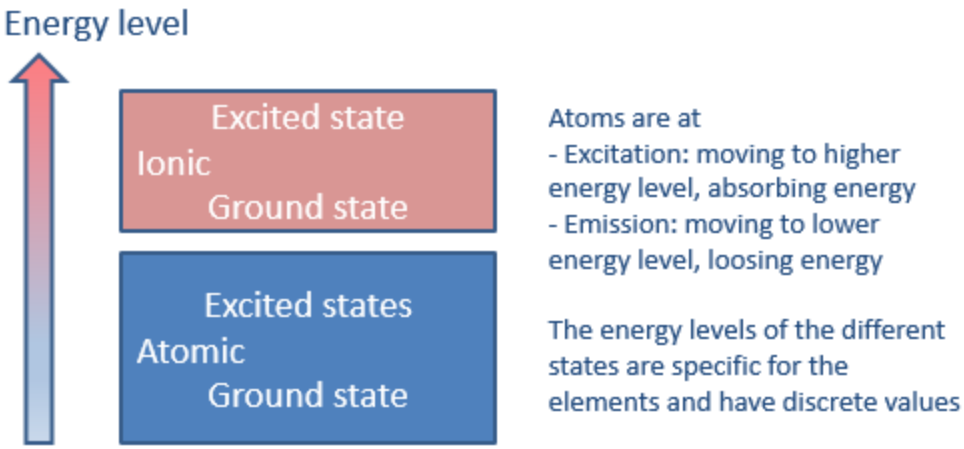
Instrumental Techniques

Atomic spectroscopy

In atomic spectroscopy we can perform qualitative and quantitative determination of elements based on one of their characteristics on atomic level. It can be:

- Absorption: AAS
- Emission: ICP

In both cases we have to transform the targeted element into it´s atomic form to perform the measurement.



Optical spectroscopy started with Newton in the 17th century, but it was a long way until the lines of emission and absorption spectra were perceived to be of atomic origin and their use to an analytical measurement technique was developed.

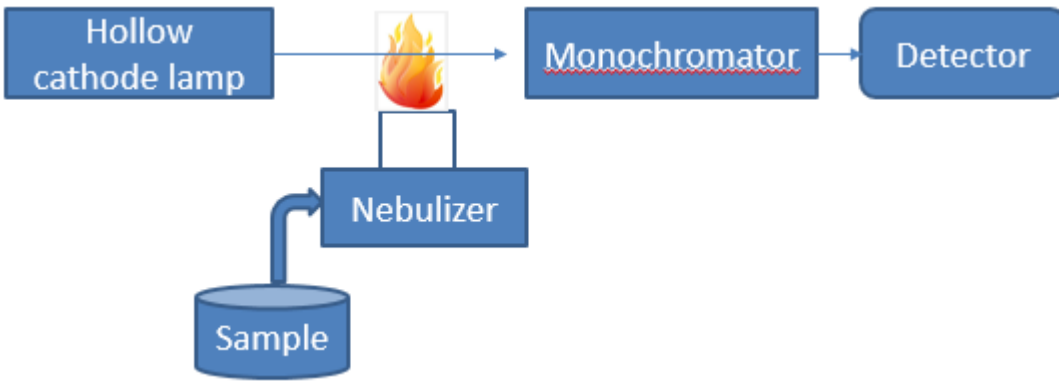
First steps were made in atomic emission spectroscopy, allowing the discovery of several new elements, like cesium, rubidium, thallium, indium or gallium.

Today in the majority of cases AAS or ICP are used for the determination of element compositions.

Instrumental Techniques

Atomic absorption spectroscopy (AAS)

Atomic absorption quantifies the absorption of ground state atoms in gaseous state.
To make a transition to higher energy level the atoms absorb light from visible or UV range.
The concentration can be determined based on the absorption using a calibration curve.



We differentiate between single and double beam instruments. In double beam instruments the light from the lamp is split into "sample" and "reference" beam. The light source normally used is a hollow cathode lamp (HCL) or an electrodeless discharge lamp (EDL).

Atomization is the separation of particles, in this cases to atomic level. This is done by exposing the analyte to high temperatures. We differentiate between systems with:

- Flame
- Graphite tube
- Vapor hydride generator

These atomizers aspirate the sample into the light path where it is illuminated by the lamp.
The lamp is specific for each element and emits at it´s characteristic wavelength.
For the quantitative analysis a blank and a calibration curve is needed.

The method is suitable for about 70 elements. Limitation is the wavelength: generally we can work at a wavelength above 200 nm , but hydrogen and some other elements have their resonance line below 200 nm.

Instrumental Techniques

Atomic absorption spectroscopy (AAS)

- **Flame atomizer**

We need a mix of oxidant and fuel gas for the flame (like air-acetylene, with a typical temperature around 2200°C or N_2O-acetylene with ca. 2700°C).
In the average 5-15% of the nebulized sample will reach the flame.
The sample volume has to be around 0.5-1.0 ml.

- **Graphite furnace**

A graphite coated furnace is used to vaporize by heating the tube with the sample using a high current power supply. No need of sample preparation, small sample size and direct analysis of solids is possible. The system is able to atomize the entire sample and retain the atomized sample in the light path for an extended period of time, enhancing the sensitivity of the technique.
The determination of about 40 elements only is possible, but with microliter sample volumes and with detection limits typically 100-1000 times better than those of flame atomizer systems.

- **Vapor generator**

To separate the analyte from the sample matrix we can use the chemical vapor generation method, where a gaseous species is generated as a result of a chemical reaction.
We differentiate between:

1. Cold vapor method (CVAAS) for the determination of Mercury (Hg) and
2. Hydride generation method (HGAAS) for elements forming gaseous covalent hydrides (As, Bi, Ge, In, Pb, Sb, Se, Sn or Te).

In the applications interferences can occur, like ionization, matrix, chemical or background interference, which can increase or decrease the size of our signal. As they can significantly influence our result, they have to be considered at the method development.

A good example is Cd (228.802 nm) and As (228.812 nm). In such cases the wavelength has to be changed or one of the alternate elements has to be removed.

Instrumental Techniques

Inductively coupled plasma (ICP)

Plasma (Greek, "anything formed") is one of the four fundamental states of matter, the others being solid, liquid, and gas. A plasma has properties unlike those of the other states.
A plasma can be created by heating a gas or subjecting it to a strong electromagnetic field.

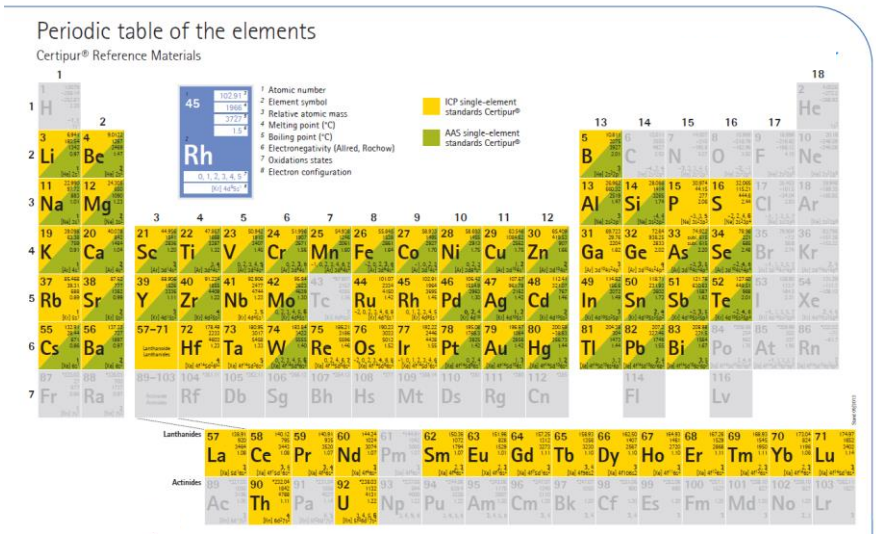
An inductively coupled plasma (ICP) is a type of plasma source in which the energy is supplied by electric currents which are produced by electromagnetic induction, that is, by time-varying magnetic fields.*

**[A. Montaser and D. W. Golightly, eds. (1992). Inductively Coupled Plasmas in Analytical Atomic Spectrometry. VCH Publishers, Inc., New York]*

Inductively Coupled Plasma Emission Spectrometry (ICP-ES) is based on the characteristic energy emission of the atoms and ions of the elements in the sample getting from excited state to ground state.

For this process a high temperature plasma is used (10 000 K can be reached in an argon plasma). For the detection of the emitted light a photomultiplier tube or tubes are physically positioned to detect specific wavelengths or, in more modern units, detection is done by semiconductor photodetectors (charge coupled devices , CCDs).

The measured intensities are compared to the intensities of standards of known concentration to calculate the elemental concentrations of the unknown sample. Also in this technique special combination of elements can lead to interferences. In most cases this can be corrected by special instrument software.



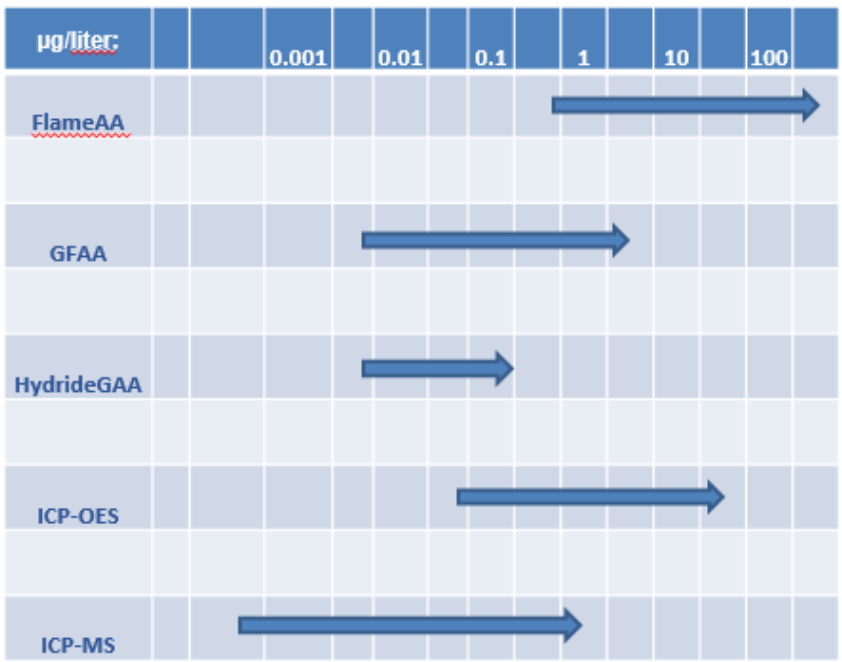
Instrumental Techniques

Inductively coupled plasma with mass detection (ICP-MS)

A special form of ICP is to combine with mass detection (ICP-MS), where a high-temperature ICP source is combined with a mass spectrometer. The elements in the sample are converted by the ICP to ions, and where they are separated and detected by the mass spectrometer. This offers several advantages:

- simple and complex matrices can be handled with low level matrix interferences due to the high-temperature of the ICP source
- detection limits are usually equal to or better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)
- very good detection capability compared to ICP-AES with the same sample throughput
- a higher throughput than GFAAS can be obtained
- isotopic information is possible

In the table you can see the typical detection ranges of the different atomic spectroscopy techniques:



Instrumental Techniques

pH

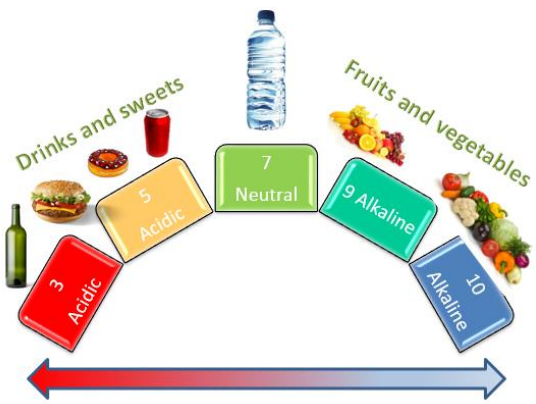
pH is a fast measurable parameter to obtain first information about the quality of different types of raw food or processed food.

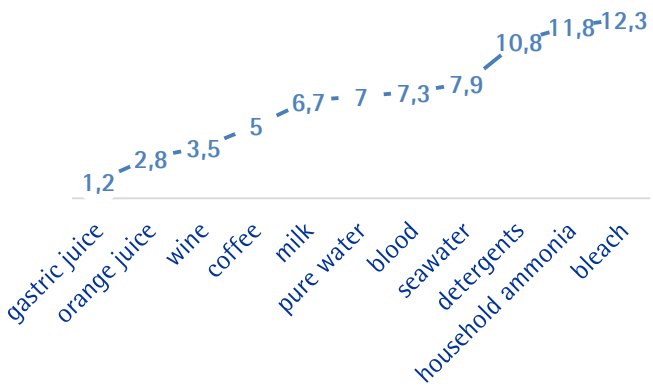
If we take milk as an example, the pH of milk is around 6.8, and it is tested both upon collection and at the end point of delivery. In various processes such as sterilization, the pH is regularly checked since a lower value helps to speed up the process. However, as an example, the lowered pH levels can also indicate that the cattle carry leukocyte infections.

Another example is meat. The pH of carcasses constitutes an important initial test to determine the condition of the animal prior to slaughter, the quality of the breeding and the signs of stress during slaughter. The typical pH value, ranging from 5.4 to 7.0, can also provide an indication of whether the fresh meat was properly stored, as the pH varies in different parts of the animal based on the muscular mass (as an example, loin has a lower pH value).

Too high pH values indicate a loss of aroma and a visibly darker meat resulting in a lower market value. In addition to raw meat, the ingredients used in the production of ham and sausages are often refrigerated. By simply checking the pH at the liquefier's intake and drainage points, one can determine if any ammonia has leaked out.

Some typical examples for pH values in food:





Instrumental Techniques

pH

The concept of pH was first introduced by the Danish chemist Søren Peder Lauritz Sørensen at the Carlsberg Laboratory in 1909, and revised to the modern pH in 1924. The exact meaning of the "p" in "pH" is disputed. According to the Carlsberg Foundation, pH stands for "power of hydrogen". Another suggestion is that the "p" stands for the Latin terms pondus hydrogenii (Engl.: quantity of hydrogen), potentia hydrogenii (Engl.: capacity of hydrogen), or potential hydrogen.

The current use in chemistry is that p stands for "decimal cologarithm of", as also in the term pKa, which is used for acid dissociation constants. By definition, pH is a measure of the acidity or alkalinity of a water solution. The acidity or alkalinity of a water solution is determined by the relative number of hydrogen ions (H^+) or hydroxyl ions (OH^-) present.

Usually pH is assumed to be the negative logarithm of the hydrogen ion concentration:
$pH = - \log10 [H^+]$.

H^+ concentration (mol/L)	OH^- concentration (mol/L)	pH
1	0.00000000000001	0
0.1	0.0000000000001	1
0.01	0.000000000001	2
0.001	0.00000000001	3
0.0001	0.0000000001	4
0.00001	0.000000001	5
0.000001	0.00000001	6
0.0000001	0.0000001	7
0.00000001	0.000001	8
0.000000001	0.00001	9
0.0000000001	0.0001	10
0.00000000001	0.001	11
0.000000000001	0.01	12
0.0000000000001	0.1	13
0.00000000000001	1	14

This way a simple scale of 0 – 14 has been created: the pH scale.
The pH scale is logarithmic, and therefore pH is a dimensionless quantity.

Instrumental Techniques

pH

Actually more precisely we are talking about the hydrogen (H+) activity.
A pH measurement determines only the concentration of active hydrogen ions in a solution, and not the total concentration of hydrogen ions. Only in dilute solutions are all anions and all cations so far apart that the H+ ion concentration and the H+ ion activity are identical.

This is the reason for the observed pH change in pure water with temperature.
If the temperature rises in pure water, the dissociation of hydrogen and hydroxyl ions increases. Since pH is related to the concentration of dissociated hydrogen ions alone, the pH value actually decreases although the water is still neutral.

According to the Nernst equation:

$$E = E^0 + \frac{RT}{F}\ln(a_{H^+}) = E^0 - \frac{2.303RT}{F}\mathrm{pH}$$

E is a measured potential,
E^0 is the standard electrode potential,
R is the gas constant,
T is the temperature in kelvin,
F is the Faraday constant.

For H+ the number of electrons transferred is equal to one.

This means that the electrode potential is proportional to pH when pH is defined in terms of activity and the pH of a given sample is changing with the temperature of the sample.

pH is usually determined by indicator strips or measured with instruments, using a pH sensitive glass electrode, a reference electrode, and a temperature sensor.
They can be built in one, as a combined glass electrode.

The pH electrode uses a specially formulated, pH sensitive glass in contact with the solution, which develops a potential (i.e. voltage) proportional to the pH of the solution.
The reference electrode is designed to maintain a constant potential at any given temperature.

Instrumental Techniques

pH

In the measurement process, the calibration of the electrode is very important: the potential is proportional to the pH, but to know the absolute value we have to use calibration solutions, in an optimal case in the range or close to the pH-range of our sample.

Most instruments offer an automated calibration curve calculation, based on a 2 or a 3 point pH buffer calibration.

pH seems to be easy to measure, but there are several potential error factors:

- Quality of the calibration buffer
- Any problem with the calibration process
- Correct re-calibration time
- Errors coming from the function of the electrode at high or low pH (alkaline error, acid error)
- Correctness of the temperature compensation
- Aging of the electrode

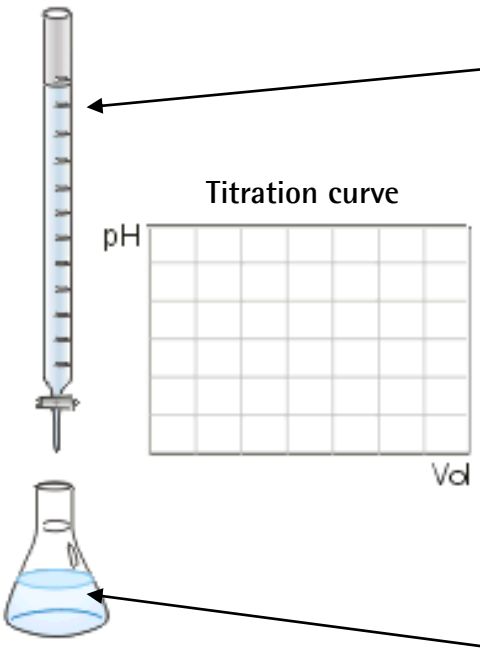
Instrumental Techniques

Titration

For food samples, titration is a very widely used method for the determination of different ingredients.

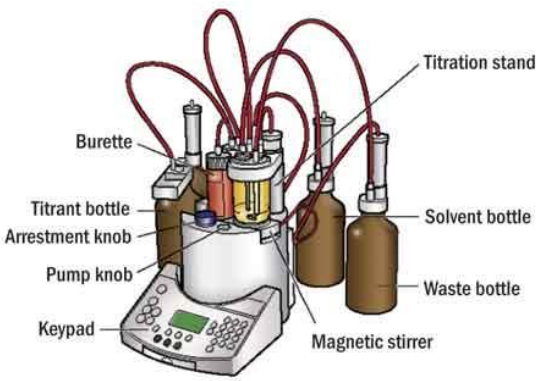
Titration can be performed manually or with instruments.
It can be easily adjusted to the need of the company, depending on the number and diversity of samples, on the time, the budget, and the personal capacity available for the lab.

Principle:
Titration is an analytical technique which allows the quantitative determination of a specific substance (analyte) dissolved in a sample.



Volumetric solution in the burette

Consumption of volume solution in ml
≅ quantity of sample in mg



Sample or (volumetric standards)
+ indicator in water

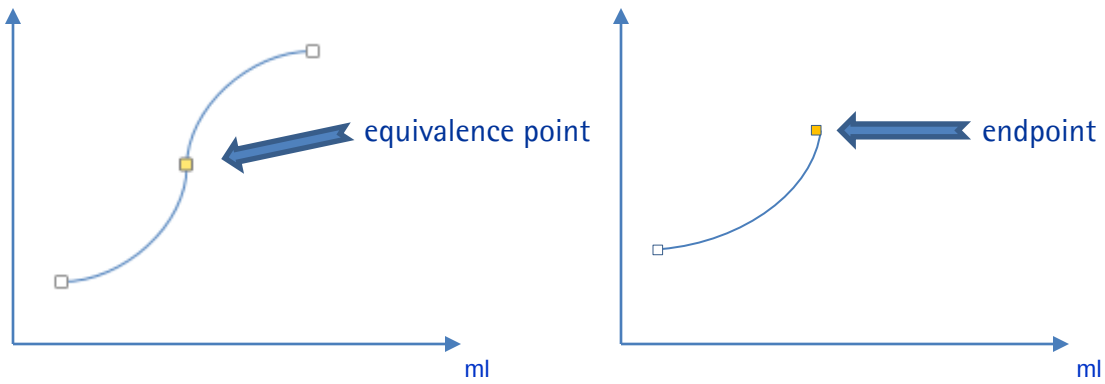
End point is indicated mostly through color change or potential jump with a titrator

Volumetric titration is based on a complete chemical reaction between the analyte and a reagent (titrant) of known concentration which is added to the sample.

Instrumental Techniques

Titration

We can differentiate titrations based on the reaction type, based on the detection method, or based on what titration curve we aim for (equivalence point or endpoint).



Classical titrations were performed using an indicator with color change (see examples for acid/base titration in the table).

Nowadays it is more common to use an electrode for the detection also in the manual titrations.

Indicator	Color on acidic side	Range of Color change	Color on basic side
Methyl violet	Yellow	0.0-1.6	Violet
Bromophenol blue	Yellow	3.0-4.6	Blue
Methyl orange	Red	3.1-4.4	Yellow
Methyl red	Red	4.4-6.3	Yellow
Litmus	Red	5.0-8.0	Blue
Bromothymol blue	Yellow	6.0-7.6	Blue
Phenolphtalein	Colorless	8.3-10.0	Pink
Alizarin yellow	Yellow	10.1-12.0	Red

Instrumental Techniques

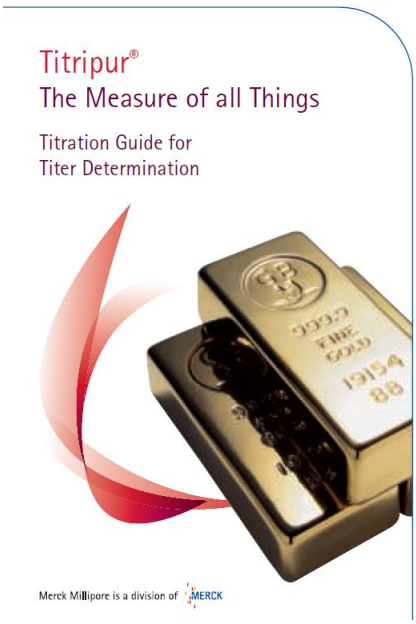
Titration

Some examples for typical titrations in food samples are:

- Acid/Base — saponification value, using a pH glass electrode
- Redox — vitamin C content, using a platinum redox electrode
- Complexometric — calcium content, using photometric or ion-selective sensor
- Thermometric — salt as sodium content, using a temperature sensor
- Precipitation — salt as chloride content, using silver ring electrode

Titrant:
We will understand the importance of the exact concentration of the titrant (the so-called titer), if we consider that our result is calculated from it!

The stability of a titrant over time has to be taken into account case by case.
There can be different factors leading to a titer deviation:



- Inaccurate preparation of the titrant
- Purity of the used titrant
- Changes due to instability

An example for the latter are basic titrants, such as hydroxides.
They take up carbon dioxide from the air over time and their molarity is changing.
Another example is the well-known Karl Fischer titration, where it is basically unavoidable, that moisture reaches the titrant from the environment.

The factor for the effective concentration is determined by means of a titer determination, i.e. the titration of a substance of exactly known concentration, usually a primary standard.

Instrumental Techniques

Traceability of Volumetric Solutions and Volumetric Standards to Standard Reference Material (SRM) from NIST

Volumetric method	Standard Reference Material (SRM)	Volumetric Solution	Volumetric standards Product offering
Acidimetry	Tris(hydroxymethyl)aminomethane (NIST)	HCl	Tris(hydroxymethyl)aminomethane volumetric standard, secondary reference material for acidimetry, traceable to NIST Standard Reference Material (SRM) Certipur® Reag. USP 1.02408.0080
		HCl	Sodium carbonate volumetric standard, secondary reference material for acidimetry, traceable to NIST SRM Certipur® Reag. Ph Eur1.02405.0080
Alkalimetry	Potassium hydrogenphtalate (NIST)	NaOH	Sodium carbonate volumetric standard, secondary reference material for acidimetry, traceable to NIST SRM Certipur® Reag. Ph Eur 1.02405.0080
	Benzoic acid (NIST)	NaOH	Potassium hydrogen phthalate Volumetric standard, secondary reference material for alkalimetry, traceable to NIST Standard Reference Material (SRM) Certipur® Reag. Ph Eur,Reag. USP 1.02400.0080
Argentometry	Potassium chloride (NIST)	AgNO3 solution	Benzoic acid volumetric standard, secondary reference material for alkalimetry, traceable to NIST Standard Reference Material (SRM) Certipur® Reag. Ph Eur,Reag. USP 1.02401.0060
Reductometry	Potassium dichlorate (NIST)	Sodium thiosulfate solution	Sodium chloride volumetric standard, secondary reference material for argentometry, traceable to NIST SRM Certipur® 1.02406.0080
		Sodium thiosulfate solution	Potassium dichromate volumetric standard, secondary reference material for redox titration, traceable to NIST SRM Certipur® Reag. USP 1.02403.0100
Oxidometry	di-Sodium oxalate (NÍST)	Potassium permanganate solution	Iron(II) ethylenediammonium sulfate Volumetric standard, secondary reference material for redox titration, traceable to NIST Standard Reference Material (SRM) Certipur® 1.02402.0080
		Potassium permanganate solution	di-Sodium oxalate volumetric standard, secondary reference material for redox titration, traceable to NIST Standard Reference Material (SRM) Certipur® Reag. USP 1.02407.0060
Complexometry	Zinc (NIST)	EDTA (Titriplex III solution)	Zinc volumetric standard, secondary reference material for complexometry, traceable to NIST SRM Certipur® Reag. Ph Eur 1.02409.0100
		EDTA (Titriplex III solution)	Calcium carbonate volumetric standard, secondary reference material for complexometry, traceable to NIST Standard Reference Material (SRM) Certipur® Reag. USP 1.02410.0100

Instrumental Techniques

Titration

Further potential error factors:

Sensor:
- by performing a titration with pH endpoint detection, you have to take care about all the factors mentioned in the pH chapter
- Generally you have to care about the maintenance of your electrode to have the required level of sensitivity and response time

Temperature:
- by performing a titration with pH endpoint detection, you have to take care about the factors mentioned in the pH chapter

- Generally any volumetric titration is influenced by the change of density with temperature and the thus related concentration change (as an example in the summer, if you perform a titer determination in the morning at 22°C and the temperature in the lab will increase to 32°C in the afternoon, you will have a significant titer change)

Conditioning of the tools/equipment:
You have to make sure you use the appropriate glassware and you don`t forget the regular maintenance

Sample handling:
To make sure that your results are reliable, you have to evaluate the whole titration workflow, including sampling, handling/storage of the sample, weighing (including the evaluation of the balance).

Instrumental Techniques

Karl Fischer titration

A special type of titration is the so-called Karl Fischer titration for the determination of the water content.

The water content has an important role in the quality and the shelf life of most food products. But how can we determine specifically the water content, in all its forms, but without any other decomposition products? This can either be done with big and expensive instrumentation (for example GC, NMR, etc) – or by the water specific KF titration.

The reaction:
$H_2O + I_2 + SO_2 + CH_3OH + 3RN \rightarrow [RNH]SO_4CH_3 + 2[RNH]I$

Water and iodine are consumed in equimolar amounts in the reaction – it means that, if you know the amount of I_2 consumed, you know the amount of water that was present in the sample

The end point is detected most commonly by a bipotentiometric method.

We differentiate between the volumetric and the coulometric method.

	Titrimetry	Coloumetry
Suitable for samples with:	High moisture content	Water contents in the ppm range
Titration	Bipotentiometric	Bipotentiometric
Iodine Generation	Included with the reagents	Generated at an electrode

Rule-of-thumb:
Samples with > 1% water = volumetric method
Samples with < 1% water = coulometric method

Instrumental Techniques

Karl Fischer titration

In most cases the needed sample preparation is only a homogenization.
You can do this using a homogenizer directly in your titration vessel.

In general, in the KF titration you have to take care about various side reactions with aldehydes and ketones, but in food samples usually the concentration of these compounds is very low, and the result concerning the water content will not be significantly influenced.

At the titration you should keep in mind some important aspects:

- there can be water in different forms (surface, inner holes, complexes) in your sample so make sure you know what you measure
- choose the right method fitting to your water content
- check if your titer is still correct
- pH and temperature can influence your result

Instrumental Techniques

Chromatography

Chromatography is a collective term for different techniques to separate mixtures.
A carrier/vehicle, which is called the mobile phase, take the sample mixture through a permanent structure with/without a chemically modified material called the stationary phase. The various constituents of the sample mixture travel at different speeds and interact indifferently with the stationary phase, causing them to separate. The separation process is based on differential partitioning between the mobile and stationary phases.

We have differences in the choice of:
- stationary phase carrier or base material
- composition of the mobile phase
- physical parameters (such as temperature, pressure, different sizes in length, diameter)

We can differentiate between the techniques based on the mobile and the stationary phase.

- Gas Chromatography (GC):	gas + column
- Liquid Chromatography (LC):	liquid + column
- Thin layer Chromatography (TLC):	liquid + plate

If you have a big number of samples with a complex matrix and wish to use minimum sample preparation, you can apply thin layer chromatography. If you are interested in volatile compounds, gas chromatography.is the preferred technique. If you wish to check contaminants in low concentration, ultra/high performance liquid chromatography (HPLC/UHPLC) is a viable alternative as it provide very high separation efficiency and will elute the molecules of interest in sharp peaks to allow sensitive detection.

Following the chromatographic step, there has to be a tool for the detection and quantitative determination of the compounds. Different sort of molecules and different concentration ranges require different detection techniques.

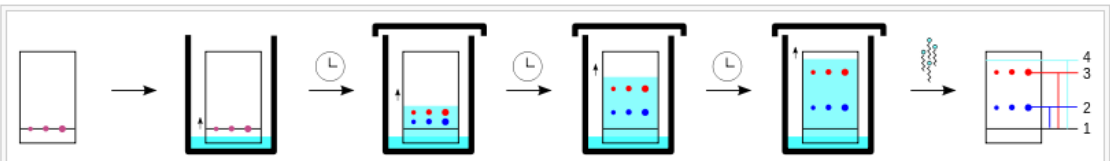
To select the most appropriate detection mode, four important parameters should be taken into consideration; chemical nature of the analytes, potential interferences, limit of detection (LOD) and limit of quantitation (LOQ) required, linearity range, availability and/or cost of detector.

Instrumental Techniques

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a technique in which compounds are separated on a thin layer of adsorbent material, typically a coating of silica gel on a glass, aluminum foil, or plastic sheet.

TLC is a straightforward and extremely cost-efficient technique allowing sample preparation and chromatographic separation in one step due to its high sample matrix tolerance.



Development of a TLC plate, a purple spot separates into a red and blue spot

A small spot of solution containing the sample is applied to a plate. A small amount of an appropriate solvent (eluent) is poured into a separation chamber. The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate.

Compounds are separated by the differences in their attraction to the stationary phase and because of the differences in solubility in the solvent. In some cases, if you have colored components you can visually detect the different compounds on the plate.
More frequently used options are:

1. using a special solution, which gives a color reaction with the analyte (such as iodine)

2. UV light (366nm to see some fluorescent organic compounds or 254 nm using a plate with an adsorbent layer fluorescent at this wavelength, on which the spots of the analyte quench this fluorescence)

3. after elution of the spot, any other known analysis technique can be used to identify the compound (like MS)

TLC is often used as a manual technique, but there are different possibilities to automatize the application, the detection, or the evaluation and documentation.

Instrumental Techniques

Thin layer chromatography with mass detection (TLC-MS)

Over the last few years, several coupling techniques for mass spectrometry have emerged to broaden the scope of TLC-MS analysis enabling unequivocal substance identification.

The most widely used technique for coupling TLC to MS is the elution-based sampling.
It is a semi-automatic system to extract analytes from the TLC plate and transfer them online into the mass spectrometer. It is suitable for all thin layer materials and every eluent that is LC-MS compatible. The interface can be easily connected to any kind of LC-coupled mass spectrometer. Therefore the fields of application are quite broad.

Perhaps the strongest feature of elution-based coupling is its complete independence from the mass spectrometer – any MS system can be used, in principal. Plus, offline extraction into vials is possible, if additional analysis (with NMR, for example) is needed. In online TLC-MS mode, the interface sits between the HPLC pump and the MS system, and is pretty much "plug and play". It's fair to say that such systems offer the lowest entry barriers into TLC-MS, and can be used with almost any kind of plate.

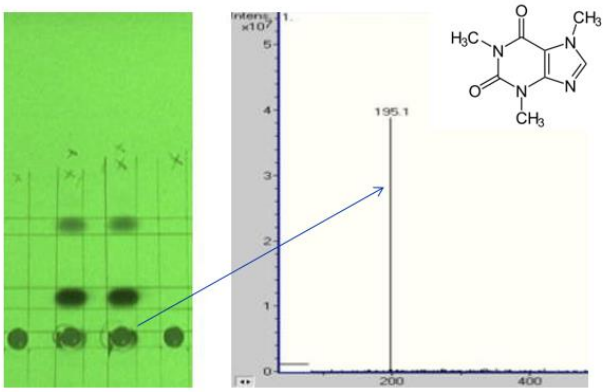


plate after extraction

MS identification of caffeine

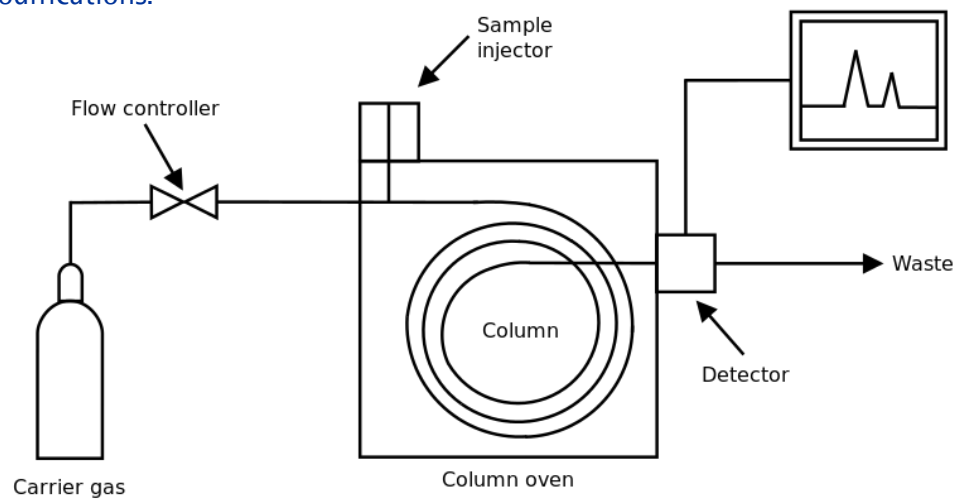
Instrumental Techniques

Gas Chromatography (GC)

Gas chromatography (GC) is a technique for the separation of volatile, thermally stable compounds. It is simple, easy to use, and can be highly automated.

In gas chromatography, a gas is used to carry a mixture across a bed of material, using pressure as force. Therefore you have as part of the system a source for gas (tank or gas generator) including regulators to control the gas flow. The most often used carrier gases are Helium, Hydrogen, Nitrogen.

You also need an oven, an injector for sample input, the column and a detector. The injection has to be done under pressure. The stationary phase is a capillary column, with different inside surface modifications.



The most commonly used detectors are the flame ionization detector (FID) and the thermal conductivity detector (TCD).

TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons.

In recent years mass spectrometers (MS) are getting more common in connection with GC. They are highly effective and sensitive, even in a small quantity of sample.

Instrumental Techniques

Liquid Chromatography (LC)

High Performance Liquid chromatography (HPLC) is a separation technique where you select the most suitable mode of separation, depending on sample solubility and how the analytes of interest differ from other compounds or matrix in sample. In reversed phase (RP) mode the mobile phase is polar and the stationary phase is non-polar. The major distinction between analytes is their hydrophobicity where samples should be soluble in water or a polar organic solvent.

In normal phase (NP), the mobile phase is non-polar while the stationary phase is more polar, and where the major distinction between analytes is not their hydrophobicity, and where the samples should be soluble in a hydrophobic solvent like hexane and the mobile phase is a weak to moderate solvent for the sample.

In HILIC mode, the mobile phases are the same as for reversed phase, but with the opposite elution strength. The major distinction between analytes is their hydrophilicity and the sample should be soluble in a polar organic solvent or organic solvent - water mixtures. The traditional approach for polar and hydrophilic compounds utilized reversed phase separation with ion-pairing mechanism and were applied for analytes being ionic or potentially ionic. In this situation the mobile phase contains a buffer, an ion-pair reagent and a polar organic solvent. Typical ion-pair reagents are; alkyl sulfonates (heptane sulfonic acid, octane sulfonic acid) and used for bases; and where quaternary amines (tetrabutylammonium chloride) are used for acids.

Choose the right HPLC Column

Chromatographic resolution is mainly affected by the selectivity (α). Changing the mobile phase composition or the stationary phase, is the most powerful way of optimizing selectivity whereas the particle size, pore size, length of the column, temperature, mobile phase strength have much less effect. Therefore, if satisfactorily results are not met, or no retention is achieved, it is better to change to another selectivity using a different column type and/or a different mobile phase.

Resolution (Rs or R) can be expressed in terms of three parameters (k, α, and N) which are directly related to experimental conditions. k is the average retention factor for the two bands, N is the column plate number and α is the separation factor (or selectivity factor). The parameters k and α is determined by the experimental conditions (composition of the mobile phase; stationary phase chemistry and temperature), and where N is affected by column length, particle size and pore size.

Instrumental Techniques

Liquid Chromatography (LC)

Choosing the Right Column Format

Find the best column configuration for minimum analysis time, with high efficiency and resolution. Match up method goals to make sure that the chosen format has the ability to produce resolution that fits the purpose of the application, e.g. choose right column length, column inner diameter, particle size and pore size. If you need high mass loadability, a larger column (both in length and diameter) is recommended as it can accommodate more mass. If you work with traditional detectors like UV, RI, FL etc 4.6 or 3.0 mm i.d. columns are suitable but for MS generally a 2 mm or smaller i.d. column is recommended.

If you have sufficient resolution, it is possible to speed up the separation by increasing the flow rate or shorten the column length. Silica-based materials are physically strong and will not shrink or swell, being compatible with a broad range of polar and non-polar solvents, and therefore often the initial choice. Most silica based columns are stable from pH 2-7.5, and historically, polymeric packing materials provided better column stability under pH extremes. A polymer-based packing material is compressible and may shrink or swell with certain solvents. Therefore care must be taken if a polymeric column is used, and upper backpressure limit is lower than corresponding silica based stationary phases. Newer high-purity silica based phases, like Purospher STAR, are stable at pH 1.5-10.5, with the surface functional groups bound to the base silica particle at multiple attachment points via polymeric modification.

Particle Size

Smaller particle sizes provide higher separation efficiency and higher chromatographic resolution than larger particle sizes. However, larger particle sizes offer faster flow rates at lower column back-pressure, and are less prone to clogging, and of these reasons more tolerant to matrix effects. Typical particle sizes range from 3-20 µm, but new 2 µm particle sizes are available to maximize resolution on short Purospher STAR columns. A 5 µm particle size represents the best compromise between efficiency and back-pressure for most non-high through put applications.

Carbon Load

For silica-based reversed-phase packing materials, carbon load indicates the amount of functional bonded phase attached to the base material. Phases with lower carbon loads are more weakly hydrophobic, which may significantly reduce retention times over phases with higher carbon loads. However, a higher carbon load will give higher capacity and often greater resolution, especially for compounds of similar hydrophobicity.
Carbon load is not a relevant parameter for columns used in normal phase or HILIC mode.

Instrumental Techniques

Liquid Chromatography (LC)

Pore Size

Choose a pore large enough to completely enclose your target molecule. If your molecule is larger than the pore, size exclusion effects will be seen and it will be difficult or impossible to retain. In general, packing materials with a smaller pore size have higher surface areas and higher capacities than packing materials with larger pore sizes. A larger surface area typically indicates a greater number of pores, and therefore a higher overall capacity. Smaller surface areas equilibrate faster, which is important for gradient elution analyses. Larger pores are better for interaction with large compounds, such as proteins.

Endcapping

Silica-based reversed-phase packing materials have free silanol groups that will interact with polar compounds. Endcapping the bonded phase minimizes these secondary interactions. Choose endcapped phases if you do not want interactions with polar compounds. Choose non-endcapped phases if you want enhanced polar selectivity, for stronger retention of polar organic compounds.

Mobile phase selection – solvents and buffers

The mobile phase solvent strength is a measure of its ability to elute analytes of the column. It is generally controlled by the concentration of the solvent with the highest strength; for example, in reverse phase HPLC with aqueous mobile phases, the strong solvent would be the organic modifier; in normal phase and HILIC, it would be the most polar one. Worth pointing out is that cyano-bonded phases are easier to work with than plain silica for normal phase separations.
The aim is to find the correct concentration of the strong solvent. With many samples, there will be a range of solvent strengths that can be used within the aforesaid capacity limits.
Other factors (such as pH) may also affect the overall retention of analytes.

Detection

Fluorescence, electrochemical or mass detectors can be used for trace analysis.
UV detectors are most commonly used as it is a robust, inexpensive and versatile detection technique. Most compounds absorb light, especially at low UV wavelengths. It is possible to use a Diode Array Detector (DAD) and allow monitoring at multiple wavelengths simultaneously.
The downside is that UV detectors are not analyte specific and requires that the analyte absorb more light than sample matrix at the set wavelength

Further information can be found in the Chrombook.

Instrumental Techniques

Mass Spectrometry (MS)

Mass spectrometry is today regarded as an established routine detection technique. MS detectors can be coupled to various separation techniques such as liquid chromatography (LC), thin layer chromatography (TLC), or gas chromatography (GC) where the hyphenation with LC is by far the most frequent setup. In contrast to more simple detectors, i.e. UV, RI, FL etc, MS generates data about molecular masses and detailed structural parameters and thereby offers the possibility to discriminate between co-eluting peaks in selected ion monitoring mode. The latter reduces the requirement for chromatographic retention and resolution before detection, yet it is always better to have retained and completely resolved peaks to prevent ion suppression or ion enhancement effects.

Mass analyzers can be quadrupole, magnetic sector, time-of-flight, ion trap or ion cyclotron resonance type. A quadrupole mass analyser consists of four parallel rods that have fixed DC and alternating RF potentials applied to them. The HPLC system handles dissolved analytes under ambient pressure (760 Torr) and delivers the sample to the MS, where the detection of the gaseous, ionized samples is performed under high vacuum conditions (10-5-10-6 Torr). The transfer of the analyte solution from the LC to the MS is accomplished via an interface. The interface stepwise converts the sample to an aerosol, ionizes it and removes the solvent. Ions are then focussed and passed along the middle of the quadrupoles. Their movement will depend on the electric fields so that only ions of a particular mass to charge ratio (m/z) will have a stable path to the detector. The RF is varied to bring ions of different m/z into focus on the detector and thus build up a mass spectrum.

Depending on the physical properties and the molecular mass of the molecules different types of interfaces are used, where they vary among each other in how they ionize the molecules and the pressure applied during this process. At present all the common ionization techniques operate under ambient pressure; i.e. electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), matrix assisted laser desorption/ionization (MALDI) and the less prominent atmospheric pressure photo Ionization (APPI). ESI and APCI are by far the most widely used in LC-MS hyphenation. The more esoteric techniques, electron ionization (EI) and chemical ionization (CI) work under high vacuum conditions with the advantage of being suitable for GC-MS hyphenation. Quadrupole mass spectrometers commonly have two configurations when used with liquid-chromatography, either as a simple single quadrupole system or placed in tandem. The latter principle, the triple quadrupole mass spectrometer, enables ion fragmentation studies (tandem mass spectrometry or MS/MS) to be performed.

Instrumental Techniques

Mass Spectrometry (MS)

Electrospray Ionization (ESI)
In ESI mode liquid solutions of charged or polar substances, delivered with a HPLC system, are sprayed utilizing a metal capillary ("spray needle") and a nebulizer gas (nitrogen) in the MS. Resulting droplets are dried (desolvatization) and volatilized isolated analyte ions are transferred to the detector. Thermal stress is very low hence the analyte molecules do not decompose. ESI is almost unlimited regarding molecule size and suitable for medium to strong polar molecules, e.g., amines, carboxylic acids, heteroaromatics, and sulfonic acids. ESI is applied when fragmentations are unwanted and molecular masses of biomolecules have to be determined. ESI-MS is well suited for hyphenation with LC, and as long as flow rates do not exceed maximum 1-2 mL/min attainable sensitivity is very high, however more common is flow rates between 1-500 µL/min.

In liquid solution molecules are either already ionized or becomes protonated or deprotonated by additives in the sample solution and the mobile phase. To achieve best sensitivity, mobile phases used should be set at a pH where analytes are ionized, and a rule of thumb is to use neutral to basic pH (7-9) for acids whereas more acidic pH (3-4) is advisable for basic compounds. If the analytes of interest have multiple pKa values and may change their ionisation state, other pH values may be more beneficial both in terms of ionisation of the analyte and behaviour in the column. Thus depending on the choice of solvent and additives either positive and/or negative ESI mode can be used. Typically, positive mode is applied in combination with more basic molecules, while acid compounds are analyzed in negative mode. 0.1% formic acid is commonly added to the mobile phase in positive ESI mode to provide a low pH (□3) to protonate the analytes(s). Acidic analytes will be neutralised under such conditions wherefore negative ESI mode is preferred and higher mobile phase pH is recommended. Volatile buffers like ammonium acetate or ammonium formate are used in the pH range 4.5-7 to deprotonate the analyte(s), and for high pH it possible to use either ammonium carbonate or ammonium hydroxide (aqueous ammonia).

For both negative and positive ESI it is a prerequisite that all solvents and additives are volatile in order to avoid contamination of the mass spectrometer and that the total mobile phase ionic strength is adequate (generally 2-25 mM) to prevent unnecessary down-time for cleaning of detector. Strong acids like hydrochloric acid or nitric acid are unsuitable for two reasons: They form ion pairs with analyte molecules (analyte signal suppression) and display strong oxidizing properties. Trifluoroacetic acid (TFA) is a special case: It is widely used as an ion pairing reagent to improve the liquid chromatographic separation of peptides or proteins. On the other hand, TFA can cause strong ion suppression in mass spectrometry (mainly in negative ESI mode) and also contaminates the LC-MS system.

Instrumental Techniques

Mass Spectrometry (MS)

Unfortunately both a quantitative estimation of these effects as well as general recommendations is not possible, as their strength strongly depend on the MS system used. Triethylamine as an alternative additive behaves in a similar manner. If the use of TFA is unavoidable, a weak acid (such as propanoic acid) or isopropanol can be added to the eluent to decrease a signal suppression effect.

Buffers do not only adjust the pH of the eluent and lead to ionization of a target molecule; they can also form adducts with the analyte. Adducts [M+buffer], e.g. with ammonium, alkali, halogens, formate or acetate, will lead to the detection of an additional peak in the MS spectrum; even a complete suppression of the analyte signal is possible when the vapour pressure of the resulting adduct (mainly alkali) is decreased significantly. As a reason of this and in order to keep the ESI source clean, volatile buffers are recommended. Non-volatile salts like phosphates, borates, sulphates or citrates will precipitate in the MS source, block it and cause tedious cleaning procedures!

Atmospheric pressure chemical ionization (APCI)
This technique is complementary to ESI and also useful for LC-MS hyphenation. It does not require a mobile phase with conducting properties wherefore acetone or acetic acid esters can be used as solvents and thus allows for a coupling of APCI with normal phase chromatography. In APCI mode, the analyte solution is vaporized prior to the ionization. Subsequently solvent molecules (aqueous-organic, e.g. methanol, propanol, acetonitrile, acetone etc., combined with 2 – 20 mM of a volatile organic buffer such as formic or acetic acid, ammonium acetate, ammonium formate or triethylamine) become ionized with a corona needle where their charge is then transferred to the analyte molecules via proton transfer or abstraction. APCI is suitable for the analysis of less polar, weakly ionizable substances with small or medium molecular weight (analytes without acidic or basic functional groups, e.g. hydrocarbons, alcohols, aldehydes, ketones, esters) and therefore complementary to ESI, as long as the sample is thermally stable and vaporizable. Fragmentations are generally observed with APCI. Highest sensitivity is achieved with acetonitrile, methanol or water as solvents, and where the degree of analyte ionization can be optimized via eluent pH. As for ESI flow rates up to maximum 1-2 mL/min can be tolerated.

Regulations in Food Testing

Chemical analysis of food is a pre-requisite for safeguarding correct labeling of food and protection of consumers against adulteration and misbranding of food. Of course, such tasks can only be achieved together with suitable food legislation, increased controls by food authorities, continuous studies by food safety agencies and universities to improve knowledge about food (processing), and by enhanced responsibility of the food industry. The latter is supported by food quality management systems like HACCP to prevent and / or control chemical, microbiological and physical hazards within the food supply chain. Such quality assurance procedures require chemical analysis throughout the whole food processing chain, starting from the raw materials and up to the final, labeled food product [1]. For ensuring that food complies to a certain minimum standard very often obligatory quality standards are applied in defining the ingredients and what the food must contain at minimum, including the nutritional composition [2, 3, 4]. Sometimes those standards also include the analytical procedures to be used. Examples for such encompassing quality standards are the US standards of identity or the Codex Alimentarius standards [2, 3].

Nutritional data on packaged food are necessary for helping consumers to choose food in accordance to their individual dietary needs and for reducing diet-related diseases [6, 7, 8, 34]. Accordingly, labeling regulations describe in detail the requirements for nutritional labeling (nutrients, amounts and caloric values) on food packages [5, 6]. To ensure a consistent nutrient declaration, the food manufacturer needs to perform additional testing for nutrients like sugars, organic acids, sugar alcohols, fat and fatty acids, protein and sodium as well as for vitamins and minerals [5].

All of these above described measures for ensuring food safety, provision of wholesome food, and consumer protection against adulteration and misbranding require reliable data obtained by chemical analysis of food. Reliable analytical results are also essential to facilitate international food trade.

Use of Quality Management Systems in Laboratories and Lab Accreditation

The reliability of chemical data depends significantly on how these data have been measured. The implementation of a quality management system for laboratories is an approved measure for safeguarding that the technical equipment and analytical methods are fit for purpose and reproducible [9] and that the staff is suitably qualified and experienced for their tasks.

Consequently, lab accreditation according to an international recognized system is a pre-condition for elaboration of reliable and internationally accepted analytical data [9].

The most important standard for laboratories is the international DIN ISO standard 17025 'General requirements for the competence of testing and calibration laboratories' [9, 10] which is also one of the most important standards for the worldwide globalization of trade. It addresses the technical competence of laboratories to carry out specific tests and is used worldwide by laboratory accreditation bodies as core requirement [9, 10]. The DIN ISO 17025 Standard comprises five elements that are 'Scope', 'Normative References', 'Terms and Definitions', 'Management Requirements' and 'Technical Requirements' [9]. The section 'Technical requirements' includes factors which determines the correctness and reliability of the tests and calibrations performed in laboratory like human factors, accommodation and environmental conditions, test and calibration methods, method validation, equipment, measurement traceability, sampling the handling of test and calibration items [9].

EU – Regulation 882/2004 - Consequences for food testing lab Accreditation

Within the European Union the official laboratories (i.e. those laboratories carrying out the analysis of samples taken during official controls by the competent food authorities of each member state) must be accredited according to DIN ISO 17025 as requested by EU Regulation 882/2004 [11]. The accreditation, however, may relate to individual tests or a group of tests.

Contract or third party laboratories need to be accredited according to this standard only if the results obtained by those laboratories are legally defensible data i.e that these data are used or are intended to be used in the event of a dispute between the competent food authorities and a food operator / manufacturer. However, as an indirect consequence of this regulation the European food market no longer accepts contract labs for food testing which are not accredited [10].

Accreditation in the USA as requested by the Food Safety Modernization Act

In 2011, the USA enacted the Food Safety Modernization Act (FSMA) [12]. It is an enormous reform program to improve food safety and to avoid intentional as well as non-intentional food adulteration within the whole supply chain also involving food testing labs. The FSMA is divided in four main parts. Part 2 of the FSMA ('Improving capacity to detect food safety problems') deals with the detection of food safety problems throughout the food supply chain. Section 202 includes rules to ensure that food testing laboratories (encompassing independent private laboratories, foreign laboratories, and laboratories operated by Federal, State, and local government agencies) meet prescribed standards for quality.

The FDA must establish criteria for laboratory accreditation and develop standards that laboratories must meet to be accredited. FDA missed the statutory deadline in 2013, but FDA officials have indicated a working group is developing a draft proposal and a proposed rule should be published by early 2016.

Correspondingly, accreditation bodies for lab accreditation need to established by the FDA [12]. Publicly available registry of accreditation bodies shall also be built up by the FDA [12]. According to Section 202 of the FSMA the FDA standards that must be met by accredited labs will include among others that

- appropriate sampling techniques are used,
- analytical procedures must be fit for purpose,
- reports of analyses must be certified,
- an internal quality system must be established and maintained,
- complaint evaluation and response procedures must exist, and
- technicians are qualified by training and experience.

These requirements are more or less the requests described by the DIN ISO Standard 17025 for lab accreditation and therefore already quite common for many labs throughout the world. It is planned that the FDA-accredited labs must be used by food manufacturers/ operators at least for

- any specific legal or regulatory testing requirement when addressing an identified or suspected food safety problem;
- any testing required by FDA to address an identified or suspected food safety problem;
- any testing to support admission of an imported food; and
- any testing under an import alert that requires successful consecutive tests.

A key requirement of section 202 is that accredited labs must provide the results of such tests directly to the FDA [12]. Reasons for this requirement may be that due to the other provisions of the FSMA, the number of necessary food samples will increase significantly. On the other hand the FDA does not have the necessary resources to deal with such a sudden rise of food samples to be tested. The FDA, therefore, makes the food manufacturer/operator responsible that the samples are tested and 'outsourced' to third parties, such as contract testing laboratories [14].

The use of official methods in food analysis

In food analysis, it is especially the complexity of the food matrix which has the largest impact on the performance and reliability of the analytical methods and procedures used [5]. The food matrix consists mainly of chemical compounds like protein, carbohydrate and lipids affecting significantly the performance of analytical methods. For example, high-fat or high-sugar foods can cause different types of interferences compared to low-fat or low-sugar food [5].
The application of extraction steps and digestion procedures, although, being a pre-condition for getting accurate analytical results, is often not only time-consuming, but sometimes also bears the risk of artifact formation [15]. Therefore, analytical methods for food analysis always need to take into account the characteristics and composition of the specific food matrix.

Several non-profit (scientific) organizations, such as the Association of Analytical Communities (AOAC), develop, standardize, and endorse official methods for food analysis. Such official methods play an important role in the analysis of foodstuffs, to ensure that food meets the legal requirements. As a consequence there are e.g. in the USA legal provisions requesting the use of a specific analytical method [16]. Furthermore, such official methods allow for comparability of results between different laboratories that follow the same procedure and for evaluating results obtained using new or more rapid procedures [5].

Official methods by WHO/FAO and Codex Alimentarius

The Codex Alimentarius Commission established in 1962 by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) develops international standards and safety practice for foods and agricultural products (the so-called Codex Alimentarius).

The Codex Alimentarius includes general requirements, code of practices and standards [17]. The Codex Commodity standards include methods for analysis of the respective commodity. Codex Volume 13 includes a list of official methods for analysis and sampling. The methods are sorted according to the specific commodity for which they can be applied (e.g. cereals, fats and oils, infant formulas etc.). There are also some methods which can be used for all kind of food exemplified by the method for detecting and quantification of the sweetener Cyclamate [18]. Codex recommends the use of the listed methods in application of DIN ISO 17025 [18].

Codex methods are elaborated by international organizations occupying themselves with a food or a group of foods and selected by the Codex Commission for Analysis [19]. Within this selection preference is given to those test methods that meet the criteria by the Codex Commission for Analysis for accuracy, precision, selectivity, limit of detection, sensitivity etc. The selection of methods also takes into account practicability and applicability under normal laboratory conditions. Consequently, preference will also be given to methods which have applicability for routine use and which are applicable uniformly to various groups of commodities. [19]. It should be mentioned that the majority of the analytical methods cited in Codex standards are those of the AOAC [22]. This is due to the fact that AOAC has had official observer status in Codex Alimentarius since its foundation and has given input on the development of Codex standards [22].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) sets standards for purity of food additives. It provides a compendium of food additive specifications comprised of four volumes [20]. The first three volumes are the food additive specifications themselves in alphabetical order while the last volume includes the revised and updated analytical methods, test procedures and laboratory solutions used by and referenced in the specifications. JECFA specifications include guidance on the analytical methods that should be used for testing according to the JECFA specification. Wherever possible, this is done by reference to the fourth volume of the compendium which includes the revised and updated analytical methods [20]. Otherwise details of the test procedures are described in the individual specifications monographs.

As JECFA specifications have been elaborated for a worldwide use, the referenced methods require the use of apparatus and equipment that is available in most laboratories [20]. According to JECFA, methods involving more recently developed techniques or equipment will not normally be quoted until such techniques are accepted internationally and are generally available at reasonable cost. Taking the advances in analytical chemistry in consideration JECFA is reviewing the analytical methods from time to time. In principle, it is possible to deviate from the JECFA methods, however, provided that the use of such other method or the modification to a JECFA method gives results of equivalent accuracy and specificity to those referenced in the respective JECFA specification [20].

Official methods for food analysis in the US

The FDA has established food definitions and standards which are published in 21 CFR 100-169 [2, 21] including standards of identity and quality. The standards of identity have been set for a wide variety of food products establishing specifically the ingredients a food must contain at minimum levels for expensive ingredients as well as at maximum levels for inexpensive ingredients (like e.g. water). Those standards mostly specify official analytical methods which are to be used for analysis. These methods have been elaborated by the international scientific organizations like the AOAC or by US organizations like e.g. the AACC (American Association of Cereal Chemists) or the AOCS (American Oil Chemists Society).

The Compendium Official Methods of Analysis of the AOAC International contains over 3000 methods adopted by the AOAC appropriate for a wide variety of food products and other materials [2, 22]. 21 CFR Section 2.19 defines the AOAC methods as "official methods" which are to be utilized by the FDA in case there is no analytical method described in a regulation [22, 23]. Accordingly, the FDA, and the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) are using the AOAC methods to check if the food complies to the specific legal requirements, like nutritional labeling information on foods, presence or absence of undesirable residues or residue levels [21]. The AOAC reviews, selects and also develops methods. Once a method is selected by the AOAC it undergoes single lab validation and a full collaborative study involving 8–10 laboratories [22]. After a successful completion and approval by the AOAC Official Methods Board, the AOAC method will be published in AOAC Compendium and Journal [22].

Food chemicals codex (FCC) is a compendium containing standards for identification and purity for known food additives and chemicals (including provision of the respective analytical methods) used in food products either in the United States or internationally. Although the FCC standards have been developed in cooperation with the FDA and industry in the United States and elsewhere, the FCC does not provide information about the regulatory status of a food additives and chemicals [24]. However, some countries other than the US (e.g. Australia, Canada, New Zealand) recognize the FCC standards as legal requirements for food additives [24]. The FCC monographs include general information about the use of the particular food additive, chemical data, minimum standards for identity, purity, and quality of food additive and validated testing methods for verifying the purity and quality of the quoted food additive.

Official methods for food analysis in the EU

Existing official methods within the EU can be found e.g. in the German official collection of food analysis methods according § 64 to the German Food and Feed Act (so called Official Collection of § 64 LFGB Methods) including more than 1300 analytical procedures for food analysis [25, 26]. Experts from food control, science and the food industry develop analytical procedures and assess performance, reliability, and comparability of such methods. They decide for which field of application the methods are to be used. Before a method is included in the Official Collection, the methods are statistically tested by a number of labs in course of a inter-laboratory comparison test and are standardized [26].

In Germany, the Official Collection of § 64 LFGB Methods can be applied without any further justification [25, 26]. However, if a lab wants to use a different method instead, such deviation needs to be justified.

In EU legislation, official methods are often specified in case of product specific regulations to ensure that a certain standard of food quality can be maintained. An example is 'Regulation 2548/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis' which contains a number of official methods [27].

In EU food legislation, however, the traditional approach for food analyses which foresees the use of such official or routine methods is more and more pushed aside by the so called performance criteria approach.

The Performance Criteria approach within the EU

As a result of advances in analytical chemistry the concept of routine methods and reference methods is more and more superseded by the so-called Performance Criteria approach, in which performance criteria for the analytical method and procedures for the validation of screening and confirmatory methods are established [11, 28].

Such change of paradigms is considered in EU Regulation 882/2004 [28]. This Regulation describes the principle requirements for sampling and analytical methods as conducted by the official laboratories of the EU member states. Laboratories involved in the analysis of official samples need to work in accordance with internationally approved procedures (e.g. DIN, CEN, ISO, IUPAC, Codex Alimentarius) or criteria-based performance standards and use methods of analysis that have been validated in accordance with e.g. IUPAC Harmonised Guidelines [11].

This has already been reflected in the EU Regulations 401/2006 [29], 333/2007 [30], 1882/2006 [31] for sampling and analytical methods for contaminants like nitrate, heavy metals, benz(a)pyrene and 3-MCPD. While these regulations describe in detail the sampling procedures (requirements for the personnel, number of samples per lot, precautions to be taken etc.), the regulation does not include (strong) recommendation for analytical methods, but specific requirements for the performance criteria like precision, recovery rate, measurement uncertainty etc. are defined and must be fulfilled by the used validated analytical methods. Also requirements regarding the laboratory quality management system to be applied are included.

For pharmacologically-active residues of authorized and non-authorized veterinarian medicinal products, e.g. certain antibiotics (like chloramphenicol) or substances having an anabolic effect (e.g. Clenbuterol), analytical methods can be selected by the respective lab but also need to be validated and to fulfill the performance criteria as given by EU Decision 2002/657 [32, 33]. The lab itself must comply with the quality norms as requested by EU Regulation 882/2004. However, the National Reference Laboratories develop and validate suitable analytical methods which can be used for analysis of veterinary drugs residues in food as described by the relevant EU legislation [33].

The above described measures aim to ensure the reliability of analytical results for food analysis. In the following compilation, analytical applications for food analysis will be described which may contribute to the reliability of analytical data by simplifying the sample preparation procedures.

References and useful links

Food legislation is a very dynamic legislation. Therefore, food regulations change frequently or are often amended. The given links to food legislation may not in all cases provide the most up-to-date version of the regulation. The most up-to-date legislation is to be found on the respective government websites.

[1] "Regulation (EU) No 852/2004 of the European Parliament and of the Council on hygiene of foodstuffs", Official Journal of the European Communities, L139/1, 2004 and subsequent amendments. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32004R0852:EN:NOT

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[3] Standards of the Codex Alimentarius, www.codexalimentarius.org

[4] Deutsches Lebensmittelbuch,

[5] "Introduction to food analysis" Nielsen, S.S. in: "Food Analysis, Springer US, USA, 4th Edition, 2010, 5-13.

[6] "Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers", Official Journal of the European Communities, L304/18, 2011. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF

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[17] „Understanding the Codex Alimentarius, Secretariat of the Joint FAO/WHO Food Standards Programme, FAO, Rome, 3rd edition, 2006.

[18] "Recommended methods of sampling and analysis, part A and B", Codex Standard 234-1999, Codex Alimentarius, Secretariat of the Joint FAO/WHO Food Standards Programme, FAO, Rome, 2011.

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[20] "Combined compendium of food additives", Joint FAO/WHO Expert Committee on Food Additives, FAO, Rome 2006.

[21] ""Food labelling", Curtis P. and Dunlap, W. in "Guide to food laws and regulations" Blackwell Publishing, USA, 2005, 85-111.

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European Union:
Eurlex:
http://eur-lex.europa.eu/de/index.htm.
This website allows you to search for the Official Journal of the EU and to get access to the text of the regulations (also accessible as consolidated versions)
European Commission:
http://ec.europa.eu/food/food/index_en.htm
European Food safety Agency
http://ec.europa.eu/food/efsa_en.htm

USA:
FDA (Food) homepage
http://www.fda.gov/Food/default.htm
US-DA homepage. The US-DA is responsible for safety of food derived from agriculture
http://www.usda.gov/wps/portal/usda/usdahome?navid=FOOD_SAFETY