Metrohm

Application Bulletin 141/5 e

Analysis of edible oils and fats

The seven most important parameters for quality control

Branch

General analytical chemistry; food; pharmaceutical

Keywords

Titration; potentiometric titration; Karl Fischer titration; KFT; polarography; Rancimat; automation; DIS-Cover; oxidation stability; oxidative stability; iodine number; iodine value; peroxide number; peroxide value; saponification number; saponification value; acid number; acid value; free fatty acids; FFA; hydroxyl number; hydroxyl value; nickel traces; Ni; edible oil; edible fat; branch 1; branch 7; EN ISO 660; EN ISO 3657; EN ISO 3960; EN ISO 3961; EN ISO 6886; EN ISO 8534; EN ISO 27107; AOAC 920.159; AOAC 920.160; AOAC 984.20; AOAC 993.20; ASTM D5554; ASTM D5558; ASTM E1899; AOCS Cd 1d-92; AOCS Cd 12b-92; GB/T 26626; GB/T 21121; USP<401>; Ph.Eur. 2.5.1; Ph.Eur. 2.5.4; Ph.Eur. 2.5.5; Ph.Eur. 2.5.6; JOCS 2.5.1.2

Summary

As the determination of the exact content of individual glycerides in fats and oils is difficult and time-consuming, several fat sum parameters or fat indices are used for the characterization and quality control of fats and oils.

Fats and oils are not only essential for cooking, they are also an important ingredient in pharmaceuticals and personal care products, such as ointments and creams. Consequently, several norms and standards describe the determination of the most important quality control parameters.

This Application Bulletin describes eight important analytical methods for the following fat parameters in edible oils and fats:

- Water content according to
  - EN ISO 8534,
  - GB/T 26626,
  - AOAC 984.20
- Oxidation stability according to
  - AOCS Cd 12b-92,
  - EN ISO 6886,
  - GB/T 21121,
  - JOCS 2.5.1.2
- Iodine value based on
  - EN ISO 3961,
  - ASTM D5554,
  - AOAC 920.159,
  - AOAC 993.20,
  - USP<401> Method II,
  - Ph.Eur. 2.5.4 Method B,
  - AOCS Cd 1d-92
- Peroxide value according to
  - EN ISO 27107,
  - EN ISO 3960,
  - AOAC 965.33,
  - Ph.Eur. 2.5.5,
  - USP<401>
- Saponification value according to:
  - EN ISO 3657,
  - ASTM D5558,
  - AOAC 920.160,
  - USP<401>,
  - Ph.Eur. 2.5.6
- Acid value, free fatty acids (FFA) according to
  - EN ISO 660,
  - USP<401> Method I,
  - Ph.Eur. 2.5.1
- Hydroxyl value according to
  - ASTM E1899
- Nickel traces using polarography

Special care is taken to avoid chlorinated solvents in these methods. Also, as many of the mentioned methods as possible are automated.

Water content

Summary

Coulometric Karl Fischer is preferred method for this analysis because of the low water contents of pure oils and fats. For butter and margarines, which exhibit relatively high water contents, volumetric Karl Fischer titration is recommended.

Instruments

- Coulometric KF titrator

  or
- Volumetric KF titrator

Electrodes

Coulometric

Double Pt-wire electrode for coulometry	6.0341.100
Generator electrode with diaphragm	6.0344.100

Volumetric

Double Pt-wire electrode for volumetry	6.0338.100

Reagents

Coulometric

- Hydranal Coulomat Oil or equivalent
- Hydranal Coulomat CG or equivalent

Volumetric

- Hydranal Composite 2 or Hydranal Composite 1 or equivalent
- Methanol, dry, p.a.
- 1-Decanol, p.a.

Solutions

Solvent mixture	Methanol / 1-decanol, Φ(MeOH) = 66% (v/v)

Standard

- Standard with a water content of 10 mg/g

Sample preparation

Hard fats should be melted before adding them into the titration vessel.

Butter and margarine should first be homogenized as the water is inhomogeneously distributed in them. They should not be heated over 25 °C, otherwise phase separation may occur.

Analysis

Sample (Coulometric)

Approximately 100 mL of coulometric reagent is added into the titration cell and conditioned until a constant drift is achieved (< 10 µg/min is typical). A syringe is rinsed 3 times with the sample (approx. 1 mL) and this sample is then discarded. The syringe is filled with sample, and an appropriate amount of sample (see table below) is added to the titration cell and titrated to the end point.

Table 1: Sample sizes dependent on the expected water content according to EN ISO 8534

Expected water content / %	Minimal sample amount / g
0.0001	10
0.001	10
0.01	5
0.1	2
1	0.2

Titer (Volumetric)

Approximately 30 mL of solvent mixture is added into the titration cell and conditioned until a constant drift of approximately 10–20 µL/min is reached. The water standard is filled into a dry syringe and approx. 1 g of water standard is added into the titration cell and immediately titrated to the end point.

Sample (Volumetric)

Approximately 30 mL of solvent mixture is added into the titration cell and conditioned until a constant drift of approximately 10–20 µL/min is reached. The sample is filled into a dry syringe (without needle). An appropriate amount of sample (see table below) is added to the titration cell and titrated to the end point.

Table 2: Sample sizes dependent on the expected water content according to EN ISO 8534

Expected water content / %	Minimal sample amount / g
0.01*	20
0.1*	5
1	1
5	0.2
10	0.1
20	0.05

* For these water contents Metrohm recommends the coulometric Karl Fischer titration.

Parameters

Coulometric

Mode	KFC
Start drift	20 µg/min
EP at	50 mV
Dynamics	70 mV
Min. rate	15 µg/min
Stop criterion	Rel. drift
Rel. stop drift	5 µg/min
Extraction time	0 s

Volumetric (titer and sample)

Mode	KFT Ipol
Start drift	20 µL/min
EP at	250.0 mV
Dynamics	100 mV
Stop criterion	Drift
Stop drift	20 µL/min
Extraction time	0 s

Calculations

Coulometric

$$w_{H2O} = \frac{EP}{m_S \times 10000}$$

w_{H2O}: Water content in the sample in %
EP: Water found up to the end point in µg
m_S: Mass of sample in g
10000: Conversion factor for %

Volumetric

Titer

$$Titer = \frac{m_{Std} \times w_{Std}}{V_{EP}}$$

Titer: Titer of the selected titrant in mg/mL
m_{Std}: Mass of standard in g
w_{Std}: Certified water content of standard in mg/g
V_{EP}: Titrant consumption up to the end point in mL

Sample

$$w_{H2O} = \frac{V_{EP} \times Titer \times 100}{m_S \times 1000}$$

w_{H2O}: Water content in the sample in g / 100 g
Titer: Titer of the selected titrant in mg/mL
V_{EP}: Titrant consumption up to the end point in mL
100: Conversion factor g to 100 g
m_S: Mass of sample in g
1000: Conversion factor mg to g

Comments

- EN ISO 8534 recommends volumetric Karl Fischer titration for samples with a water content of 1 to 100 mg of water. Coulometric Karl Fischer titration is recommend for samples with a water content of 10 µg to 10 mg of water.
- For fats and oils with a water content of 1% or lower, coulometric Karl Fischer titration is recommended as a greater precision can be achieved.
- The method differs from EN ISO 8534 in the following way:
  - A mixture of methanol and 1-decanol is used as solvent instead of a mixture of methanol and chloroform.
- For the preparation of the syringe for the titer determination, see Metrohm Application Bulletin AB-424
- The added sample weight is determined by weighing the full syringe before the addition of the sample and then again after the addition of the sample into the titration cell. The difference is determined and used as a sample weight.

References

- Application Bulletin AB-424
  Titer determination in volumetric Karl Fischer titration
- EN ISO 8534
  Animal and vegetable fats and oils – determination of water content – Karl Fischer method (pyridine free)
- GB/T 26626
  Animal and vegetable fats and oils – Determination of water content – Karl Fischer method
- AOAC 984.20
  Moisture in oils and fats. Karl Fischer method

Oxidation stability

Summary

The Rancimat method is an accelerated aging test. Air is passing through the sample in the reaction vessel at a constant elevated temperature. In this process, fatty acids are oxidized. At the end of the test, volatile secondary reaction products are formed, which are transported into the measuring vessel by the air stream and absorbed in the measuring solution (deionized water). This results in a sudden increase of the electrical conductivity, which is continuously recorded. The time until secondary reaction products are formed and detected is called induction time. It characterizes the oxidation stability of oils and fats.

Instruments

- Rancimat
- Equipment for determining the temperature correction

Reagents

- Deionized water

Sample preparation

No sample preparation is required for this method.

Liquid oils can be weighed in directly. In case of problems, weighing solid fat into the bottom part of the reaction vessel, the sample can be previously melted in a water bath. Care has to be taken that the temperature of the water bath is not considerably higher than the melting point of the sample. Otherwise deterioration of the sample can be expected.

Analysis

Before the determination can be started, the temperature of the heating block has to be stable. Fill each measuring vessel with 60 mL deionized water and place it on the Rancimat together with the measuring vessel cover with the integrated conductivity cell. Use a new and clean reaction vessel. Weigh in 3 g of sample into the bottom part and close it with the reaction vessel cover with the air inlet tube attached. Connect the two tubing for the air supply, place the reaction vessel in the heating block, and start data recording immediately.

Parameters

Sample size	3 g
Measuring solution	60 mL deionized water
Temperature	80 – 160 °C
Gas flow	20 L/h
Evaluation	Induction time

Example determination

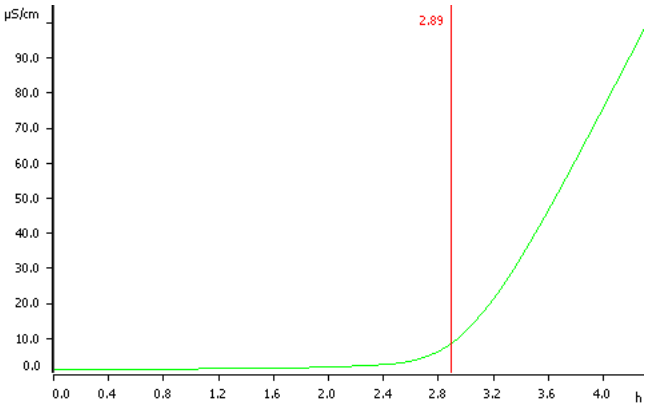

Fig. 1: Determination of oxidation stability of sunflower oil at a temperature of 120 °C, induction time 2.89 h.

Typical results

Sample	Temperature/°C	Induction time/h
Corn oil	120	approx. 5
Hazelnut fat	120	10 – 12
Hazelnut oil	120	7 – 11
Lard	100	1 – 3
Linseed oil	110	0.5 – 2
Margarine	120	2 –. 6
Olive oil	120	6 – 11
Palm oil	120	7 – 12
Peanut fat	120	9 – 10
Peanut oil	120	3 – 15
Pumpkin seed oil	120	approx. 7
Rapeseed oil	120	3 – 5
Safflower oil	120	1 – 2
Sesame oil	120	approx. 5
Soybean oil	120	1 – 7
Sunflower oil	120	1 – 4
Tallow	120	3 – 8

Comments

- Temperature is the most critical parameter in this application. Therefore, a temperature correction has to be included in the method settings to compensate for the cooling due to the gas flow. Tabled values are available for different temperatures and gas flow rates in the manual of the instrument software. For optimum reproducibility of results, it is recommended to determine the temperature correction using the optional equipment for determining the temperature correction. For more information see the instructions for use of the instrument.
- The induction time is usually determined at 120 °C. However, the temperature can be set in a way that the induction time lies within 4 to 10 hours. As a rule of thumb, the induction time decreases by a factor of 2 when the temperature is increased by 10 °C and vice versa.
- It is recommended to use a new reaction vessel for each determination to avoid side reactions due to contamination. To remove particles (e.g., from the cardboard box) the reaction vessel is air-cleaned inside and outside by a sharp stream of nitrogen before the sample is weighed in.

References

- AOCS Cd 12b-92
  Sampling and analysis of commercial fats and oils – Oil Stability Index (OSI)
- EN ISO 6886
  Animal and vegetable fats and oils – determination of oxidative stability (accelerated oxidation test)
- GB/T 21121
  Animal and vegetable fats and oils – Determination of oxidation stability
- JOCS 2.5.1.2
  Oxidation stability - CDM (Conductometric Determination Method)
- Metrohm Application Bulletin 204
  Oxidation stability of oils and fats – Rancimat method

Iodine value

Summary

The iodine number is used for the characterization of fats and oils and as quality control parameter. As a sum parameter, it allows quantification of the amount of unsaturated fatty acids present in fats and oils. The more unsaturated fatty acids, the more iodine reacts with the double bonds, leading to a higher iodine value.

Usually, after the addition of the reaction solution (Wijs solution), the samples have to be placed in the dark for up to 2 hours. Here, we describe a modified analysis based on EN ISO 3961, ASTM D5554, AOAC 920.159, AOAC 993.20, and AOCS Cd 1d-92. For this modified analysis, magnesium acetate is added as catalyst, reducing the reaction time from up to 2 h to 5 min.

Instruments

- Sample changer with Swing Head and DIS-Cover
- Titrator with DET mode
- 2x Burette 20 mL (Glacial acetic acid, $Mg(CH_3COO)_2$)
- 4x Burette 50 mL (H_2SO_4, ICl, KI, $Na_2S_2O_3$)
- Propeller Stirrer

Electrodes

iPt Titrode	6.0471.300

Reagents

- Sulfuric acid, $c(H_2SO_4)$ = 0.5 mol/L, volumetric solution
- Potassium iodate, KIO_3, p.a.
- Potassium iodide, KI, p.a.
- Sodium thiosulfate, $c(Na_2S_2O_3)$ = 0.1 mol/L, volumetric solution
- Magnesium acetate, $Mg(CH_3COO)_2$, purum
- Glacial acetic acid, p.a.
- Iodine chloride, Wijs-solution, c(ICl) = 0.1 mol/L, volumetric solution

Solutions

Titrant	$c(Na_2S_2O_3)$ = 0.1 mol/L If possible this solution should be bought from a supplier.
Potassium iodide solution	β(KI) = 100 g/L 50 g potassium iodide is weighed into a 500 mL volumetric flask and filled up with distilled water.
Magnesium acetate solution	$w(Mg(CH_3COO)_2)$ = 3% 15 g magnesium acetate is weighed into a 500 mL volumetric flask and filled up with glacial acetic acid.
Reaction solution	c(ICl) = 0.1 mol/L in glacial acetic acid If possible this solution should be bought from a supplier.

Standard

Iodate standard	Potassium iodate is dried in a drying oven for 2 h at 110 °C and allowed to cool down in a desiccator for at least 1 h.

Sample preparation

No sample preparation required.

Analysis

Titer

Approximately 70 mg potassium iodate is weighed into a 250 mL beaker and 80 mL distilled water is added to dissolve it. Afterwards 10 mL β(KI) = 100 g/L as well as 25 mL $c(H_2SO_4)$ = 0.5 mol/L are given to the solution. The solution becomes dark brown and the originated iodine is titrated with $c(Na_2S_2O_3)$ = 0.1 mol/L up to the first end point.

Blank

20 mL glacial acetic acid, 25 mL c(ICl) = 0.1 mol/L and 10 mL $w(Mg(CH_3COO)_2)$ = 3% are given into a 250 mL brown glass beaker. The beaker is closed with a lid and left standing for five minutes. 15 mL β(KI) = 100 g/L is given to the solution and the originated iodine is titrated with $c(Na_2S_2O_3)$ = 0.1 mol/L until the first end point.

Sample

An appropriate amount of sample is weighed into a 250 mL brown glass beaker (see table below) and placed onto the sample rack. 20 to 25 mL of glacial acetic acid (see below),

25 mL c(ICl) = 0.1 mol/L and 10 mL w($Mg(CH_3COO)_2$) = 3% are then added. Afterwards the beaker is closed with a lid and left standing for five minutes. 15 mL β(KI) = 100 g/L is given to the solution and the originated iodine is titrated with c($Na_2S_2O_3$) = 0.1 mol/L until the first end point.

Table 3: Sample sizes dependent on the expected iodine value

Expected IV / g / 100 g	Sample amount / g	Solvent volume / mL
< 1.5	15.00	25
1.5 – 2.5	10.00	25
2.5 – 5	3.00	20
5 – 20	1.00	20
20 – 50	0.40	20
50 – 100	0.20	20
100 – 150	0.13	20
150 – 200	0.10	20

Parameters

Titer

Mode	DET U
Pause	20 s
Signal drift	20 mV/min
Max. waiting time	38 s
Meas. point density	4
Min. increment	50 µL
Max. increment	off
EP criterion	5
EP recognition	greatest

Blank/Sample

Mode	DET U
Signal drift	20 mV/min
Max. waiting time	38 s
Meas. point density	4
Min. increment	10 µL
Max. increment	off
EP criterion	5
EP recognition	all

Calculations

Titer

$$\text{Titer} = \frac{m_s \times 6}{V_{EP1} \times c(Na_2S_2O_3) \times M_A}$$

Titer:	Titer of the selected titrant
m_s:	Mass of standard in mg
6:	Stoichiometric factor
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c($Na_2S_2O_3$):	Concentration of the selected titrant in mol/L; here c($Na_2S_2O_3$) = 0.1 mol/L
M_A:	Molecular weight of the analyte; here 214.00 g/mol

Sample

$$IV = \frac{(V_{blank} - V_{EP1}) \times f \times c(Na_2S_2O_3) \times M_A}{10 \times m_S}$$

IV:	Iodine value of the sample in g iodine / 100 g
V_{blank}:	Blank value consumption for the used quantity of solvent in mL
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c($Na_2S_2O_3$):	Concentration of the selected titrant in mol/L; here c($Na_2S_2O_3$) = 0.1 mol/L
f:	Correction factor («titer») without unit
M_A:	Molecular weight of the analyte; here 126.90 g/mol
m_s:	Sample size in g
10:	Conversion factor

Example determination

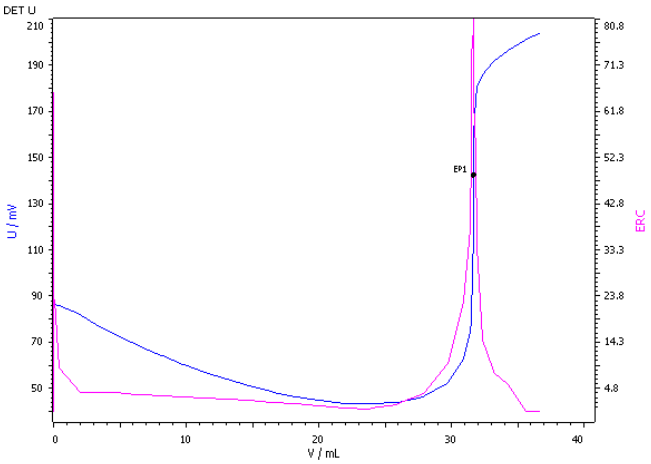

Fig. 2: Determination of the iodine value (blue = titration curve, pink = ERC)

Comments

- The method for determining the iodine value is adapted from EN ISO 3961, ASTM D5554, AOAC 993.20, AOCS Cd 1d-92, USP<401> Method II, and Ph.Eur. 2.5.4 Method B. The following changes are made:
    - Magnesium acetate is used as catalyst shortening the reaction time from 1 – 2 h to 5 minutes.
    - Glacial acetic acid is used as solvent instead of a mixture of cyclohexane and glacial acetic acid.
- The method differs from AOAC 920.159 in the following points:
    - Magnesium acetate is used as catalyst shortening the reaction time from 0.5 – 1 h to 5 minutes.
    - Glacial acetic acid is used as solvent instead of tetrachloromethane.

References

- EN ISO 3961
  Animal and vegetable fats and oils – determination of iodine value
- ASTM D5554
  Standard test method for determination of the iodine value of fats and oils
- AOAC 920.159
  Iodine Absorption Number of Oils and Fats
- AOAC 993.20
  Iodine Value of Fats and Oils
- AOCS Cd 1d-92
  Sampling and analysis of commercial fats and oils: iodine value of fats and oils cyclohexane-acetic acid method
- USP<401>
  Fats and fixed oils
- Ph.Eur. 2.5.4
  Iodine value

Peroxide value

Summary

The peroxide number provides information about the number of peroxide compounds in the oil and hence can be used as an indicator of the age and quality of the edible oil at hand. The lower the peroxide numbers the better and/or more fresh the oil.

Instruments

- Sample changer with Swing Head and DIS-Cover
- Titrator with DET mode
- 1x Burette 5 mL
- 1x Burette 10 mL
- 3x Burette 20 mL
- 2x Burette 50 mL
- Propeller Stirrer

Electrodes

iPt Titrode	6.0471.300

Reagents

- Sulfuric acid, c(H_2SO_4) = 0.5 mol/L, volumetric solution
- Potassium iodate, KIO_3, p.a.
- Potassium iodide, KI, p.a.
- Sodium thiosulfate, c($Na_2S_2O_3$) = 0. 1 mol/L, volumetric solution
- Glacial acetic acid, p.a.
- 1-Decanol, p.a.

Solutions

Titrant	c($Na_2S_2O_3$) = 0.01 mol/L or 0.001 mol/L Prepared by dilution of the c($Na_2S_2O_3$) = 0.1 mol/L with distilled water.
Auxiliary solution	Saturated solution of KI.
Potassium iodide solution	w(KI) = 10% 50 g potassium iodide is weighed into a 500 mL volumetric flask and filled up with distilled water.
Solvent mixture	Glacial acetic acid / 1-decanol with approximately 20 mg I_2 / L Φ(1-decanol) = 40% (v/v)

Standard solution

Iodate standard	Potassium iodate is dried in a drying oven for 2 h at 110 °C and allowed to cool down in a desiccator for at least 1 h. Approximately 0.65 g is weighed into a 1 L volumetric flask and filled up to the mark with dist. water.

Sample preparation

No sample preparation is required.

Analysis

Titer

0.75 to 1.25 mL potassium iodate standard solution is dosed into a 250 mL beaker. 80 mL distilled water, 10 mL w(KI) = 10% as well as 25 mL c(H_2SO_4) = 0.5 mol/L are added to the solution. The solution becomes dark brown and the originated iodine is titrated with c($Na_2S_2O_3$) = 0.01 mol/L or c($Na_2S_2O_3$) = 0.001 mol/L up to the first end point.

Blank

50 mL solvent mixture and 0.5 mL auxiliary solution are dosed into a 250 mL brown glass beaker and closed with the DIS-cover. After one minute 80 mL distilled water is added and the solution is titrated with c($Na_2S_2O_3$) = 0.01 mol/L or c($Na_2S_2O_3$) = 0.001 mol/L until the first end point.

Sample

5 or 10 g of sample (depending on the expected value) is weighed into a 250 mL brown glass beaker and placed onto the sample rack. 50 mL solvent mixture and 0.5 mL auxiliary solution are added and the beaker is closed with the lid of the OMNIS DIS-cover system. After one minute 80 mL distilled water is added and the solution is titrated with c($Na_2S_2O_3$) = 0.01 mol/L or c($Na_2S_2O_3$) = 0.001 mol/L until the first end point.

Parameters

Titer

Mode	DET U
Pause	20 s
Signal drift	20 mV/min
Max. waiting time	38 s
Meas. point density	4
Min. increment	50 µL
Max. increment	500 µL
EP criterion	5

EP recognition	all

Blank/Sample

Mode	DET U
Signal drift	5 mV/min
Min. waiting time	10 s
Max. waiting time	72 s
Meas. point density	4
Min. increment	10 µL
Max. increment	200 µL
EP criterion	20
EP recognition	greatest

Calculations

Titer

$$\text{Titer} = \frac{\beta(KIO_3) \times m_s \times 6}{V_{EP1} \times c(Na_2S_2O_3) \times M_A}$$

Titer:	Titer of the selected titrant
$\beta(KIO_3)$:	Exact mass concentration of the standard solution in mg/L
m_s:	Volume of the added standard solution in L
6:	Stoichiometric factor
V_{EP1}:	Titrant consumption until the first equivalence point in mL
$c(Na_2S_2O_3)$:	Concentration of the selected titrant in mol/L; here $c(Na_2S_2O_3)$ = 0.001 mol/L
M_A:	Molecular weight of the analyte; here 214.00 g/mol

Sample

$$PV = \frac{k \times (V_{EP1} - V_{blank}) \times f}{m_S}$$

PV:	Peroxide value of the sample in meq O_2 / kg
V_{EP1}:	Titrant consumption until the first equivalence point in mL
V_{blank}:	Blank value consumption for the used quantity of solvent in mL
f:	Correction factor («titer») without unit
m_s:	Sample size in g
k:	Conversion factor, 1 for $c(Na_2S_2O_3)$ = 0.001 mol/L, 10 for $c(Na_2S_2O_3)$ = 0.01 mol/L

Example determination

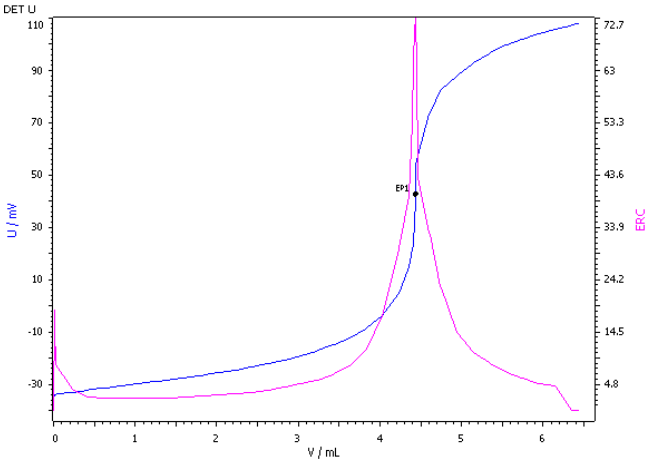

Fig. 3: Determination of the peroxide value (blue = titration curve, pink = ERC)

Comments

- The stirrer has to be set to a higher speed (14) for dissolving of the oil than for the titration (10). Otherwise results may not be reproducible.
- A small quantity of iodine is added to the solvent mixture to enable the determination of reliable blank value. A condition for this is, however, that exactly the same amount (10.0 mL) of solvent mixture be used for each determination of both the blank and the sample.
- Before each determination series, all dosing units, especially the one for the solvent mixture, have to be prepared. This ensures that the amount of iodine added together with the solvent mixture is always the same during a series.
- As the peroxide value depends on the sample size, it was decided by ISO/TC 34/SC 11 to keep the sample size to fixed to 5 g for PV greater than 1, and to 10 g for PV less than or equal to 1.
- The method for determining the peroxide value is adapted from EN ISO 27107 and EN ISO 3960. The following changes are made:
  - H_2SO_4 instead of HCl is used in the titer determination.
  - A mixture of 1-decanol and glacial acetic acid is used as solvent instead of a mixture of isooctane and glacial acetic acid.

- The method differs from AOAC 965.33, USP<401> and Ph.Eur. 2.5.5 Method A in the following way:
    - A mixture of 1-decanol and glacial acetic acid is used as solvent instead of a mixture of chloroform and glacial acetic acid.
    - 50 mL of solvent is used instead of 30 mL.
- Samples with peroxide numbers below 1 are titrated with c($Na_2S_2O_3$) = 0.001 mol/L as a higher consumption of titrant results in better reproducibility.

References

- EN ISO 27107
  Animal and vegetable fats and oils – determination of peroxide value – potentiometric end-point determination
- EN ISO 3960
  Animal and vegetable fats and oils -- Determination of peroxide value -- Iodometric (visual) endpoint determination
- AOAC 965.33
  Peroxide Value of Oils and Fats
- USP<401>
  Fats and fixed oils
- Ph.Eur. 2.5.5
  Peroxide value

Saponification value

Summary

The saponification number is used for the characterization of fats and oils and as a quality control parameter. The saponification number contains the information on the average molecular weight of all fatty acids present in the sample. The lower the molecular weight of all fatty acids, the higher is the saponification number and vice versa.

Instruments

- Titrator with DET mode
- Burette 50 mL
- Stirrer
- Reflux condenser
- Heating device

Electrodes

Solvotrode easyClean	6.0229.020

Reagents

- Hydrochloric acid, c(HCl) = 0.5 mol/L, volumetric solution
- Potassium hydroxide, p.a.
- Ethanol, p.a.
- TRIS, p.a.

Solutions

Titrant	c(HCl) = 0.5 mol/L If possible this solution should be bought from a supplier.
Ethanolic potassium hydroxide solution	c(KOH) = 0.5 mol/L in ethanol If possible this solution should be bought from a supplier. The solution should be colorless or straw yellow. For the preparation of a stable colorless solution see paragraph 5.1 of ISO 3657.
Electrolyte	c(TEABR) = 0.4 mol/L in ethylene glycol Metrohm No. 6.2320.000

Standard

TRIS	TRIS is dried over night in a drying oven at 105 °C and allowed to cool down in a desiccator for at least 1 h.

Sample preparation

Weigh out an appropriate amount of sample (see table below) in a round-bottomed flask. Add 25 mL of ethanolic c(KOH) = 0.5 mol/L and a magnetic stirring bar. Attach the reflux cooler, heat up and boil gently for 60 minutes, tilting the flask back and forth now and then.

Table 4: Sample sizes dependent on the expected saponification value

Expected SV / mg KOH / g	Sample amount / g
150 – 200	2.2 – 1.8
200 – 250	1.7 – 1.4
250 – 300	1.3 – 1.2
> 300	1.1 – 1.0

Analysis

Titer

About 420 mg TRIS is weighed into a titration vessel. 20 mL deionized water and 50 mL ethanol are added. After a pause of 20 s the solution is titrated with c(HCl) = 0.5 mol/L to the first equivalence point. In between measurements, the electrode membrane is rehydrated for 1 min in deionized water.

Blank

Perform a sample preparation without sample for the blank test. After cooling, dilute the flask contents sufficiently with ethanol, insert the electrode and the burette tip, then back-titrate the KOH excess with c(HCl) = 0.5 mol/L to the first equivalence point. Between measurements, rehydrate the electrode membrane for 1 min in deionized water.

Sample

After cooling, dilute the flask contents sufficiently with ethanol, insert the electrode and the burette tip, then back-titrate the KOH excess with c(HCl) = 0.5 mol/L to the first equivalence point. Between measurements, rehydrate the electrode membrane for 1 min in deionized water.

Parameters

Titer

Mode	DET U
Pause	20 s
Signal drift	20 mV/min
Max. waiting time	38 s
Meas. point density	4
Min. increment	50 µL
Max. increment	off
EP criterion	5
EP recognition	greatest

Blank/Sample

Mode	DET U
Pause	20 s
Signal drift	20 mV/min
Max. waiting time	38 s
Meas. point density	4
Min. increment	10 µL
Max. increment	off
EP criterion	5
EP recognition	greatest

Calculations

Titer

$$\text{Titer} = \frac{m_s}{V_{EP1} \times c(HCl) \times M_A}$$

Titer:	Titer of the selected titrant
m_s:	Mass of standard in mg
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c(HCl):	Concentration of the selected titrant in mol/L; here c(HCl) = 0.5 mol/L
M_A:	Molecular weight of the analyte; here 121.14 g/mol

Sample

$$SV = \frac{(V_{blank} - V_{EP1}) \times f \times c(HCl) \times M_A}{m_S}$$

SV:	Saponification value of the sample in mg KOH / g
V_{EP1}:	Titrant consumption until the first equivalence point in mL
V_{blank}:	Blank value consumption for the used quantity of solvent in mL

c(HCl):	Concentration of the selected titrant in mol/L; here c(HCl) = 0.5 mol/L
f:	Correction factor («titer») without unit
M_A:	Molecular weight of KOH; 56.1056 g/mol
m_s:	Sample size in g

Example determination

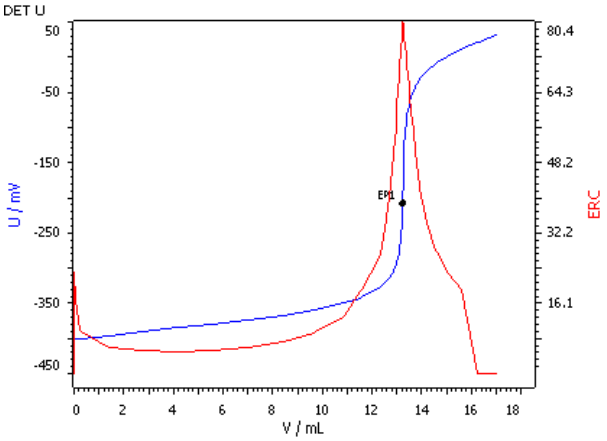

Fig. 4: Determination of the saponification value (blue = titration curve, red = ERC)

Comments

- Samples difficult to saponify should be boiled for 2 h.
- The potassium hydroxide solution should be colorless or straw yellow. A description for the preparation of a stable colorless solution can be found in the norm ISO 3657.
- For further information concerning the handling of the Solvotrode easyClean, please study the instructions sent with the electrode.
- ASTM D5558 and AOAC 920.160 differ from the method described here, in that they use 50 mL of KOH solution and thus also a higher sample size of 4 to 5 g.
- Ph.Eur. 2.5.6 differs from the method described here in that they use different sample sizes depending on the expected saponification value.

References

- EN ISO 3657
  Animal and vegetable fats and oils – determination of saponification value
- AOAC 920.160
  Saponification Number (Koettstorfer Number) of Oils and Fats
- ASTM D5558
  Standard Test Method for Determination of the Saponification Value of Fats and Oils
- USP<401>
  Fats and fixed oils
- Ph.Eur. 2.5.6
  Saponification value

Acid value, free fatty acids

Summary

The acid number or acid value and the free fatty acid content are used for the characterization of fats and oils and as quality control parameter. The higher the acid number and free fatty acid content the lower the quality of the oil. The acid number additionally increases with the age of an oil as triglycerides are converted into fatty acids and glycerol as an effect of time.

Instruments

- Sample changer
- Titrator with DET mode
- Burette 20 mL
- Stirrer

Electrodes

Solvotrode easyClean	6.0229.020

Reagents

- Ethanol, p.a.
- Diethyl ether, peroxide-free, p.a.
- Phenolphthalein, p.a.

Solutions

Titrant	c(KOH) = 0.1 mol/L in ethanol or methanol If possible this solution should be bought from a supplier.
Solvent mixture	Ethanol / diethyl ether, Φ(EtOH) = 50% (v/v) Neutralized, just before use, with KOH in presence of 0.3 mL phenolphthalein solution per 100 mL solvent mixture.
Phenolphthalein solution	Phenolphthalein in ethanol, β(phenolphthalein) = 1 g / 100 mL.

Standard

Benzoic acid	Benzoic acid is dried in a desiccator over night.

Sample preparation

No sample preparation required.

Analysis

Titer

100 – 120 mg benzoic acid is weighed into the titration vessel and dissolved in 50 mL ethanol. The solution is then titrated using c(KOH) = 0.1 mol/L until after the first equivalence point.

Sample

An appropriate sample amount is weighed into a 150 mL beaker (see table below). 50 to 100 mL solvent mixture is added and the sample dissolved. After a pause of 30 s the solution is titrated until the first equivalence point using alcoholic c(KOH) = 0.1 mol/L.

Table 5: Sample sizes dependent on the expected acid value

Expected AV / mg KOH / g	Sample amount / g	Accuracy / g
0 – 1	20	0.05
1 – 4	10	0.02
4 – 15	2.5	0.01
15 - 75	0.5	0.001
> 75	0.2	0.001

Parameters

Titer

Mode	DET U
Signal drift	50 mV/min
Max. waiting time	26 s
Meas. point density	4
Min. increment	10 µL
Max. increment	off
EP criterion	5
EP recognition	all

Sample

Mode	DET U
Signal drift	20 mV/min
Max. waiting time	38 s
Meas. point density	4
Min. increment	10 µL
Max. increment	off
EP criterion	5
EP recognition	all

Calculations

Titer

$$\text{Titer} = \frac{m_s}{V_{EP1} \times c(KOH) \times M_A}$$

Titer:	Titer of the selected titrant
m_s:	Mass of standard in mg
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c(KOH):	Concentration of the selected titrant in mol/L; here c(KOH) = 0.1 mol/L
M_A:	Molecular weight of the analyte; here 122.12 g/mol

Acid value

$$AV = \frac{V_{EP1} \times f \times c(KOH) \times M_A}{m_S}$$

AV:	Acid value of the sample in mg KOH / g
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c(KOH):	Concentration of the selected titrant in mol/L; here c(KOH) = 0.1 mol/L
f:	Correction factor («titer») without unit
M_A:	Molecular weight of KOH; 56.1056 g/mol
m_s:	Sample size in g

Free fatty acids

$$FFA = \frac{V_{EP1} \times f \times c(KOH) \times M_A}{10 \times m_S}$$

FFA:	Acid value of the sample in %
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c(KOH):	Concentration of the selected titrant in mol/L; here c(KOH) = 0.1 mol/L
f:	Correction factor («titer») without unit
M_A:	Molecular weight of the acid chosen for the expression of the result in g/mol according to the fat type (see table below)
m_s:	Sample size in g

Table 6: Choice of fatty acids for the free fatty acid content

Type of fat	Expressed as	Molar mass / g/mol
Coconut oil, Palm kernel oil and similar oils	Lauric acid	200
Palm oil	Palmitic acid	256
Oils from certain cruciferae	Erucic acid	338
All other fats	Oleic acid	282

Example determination

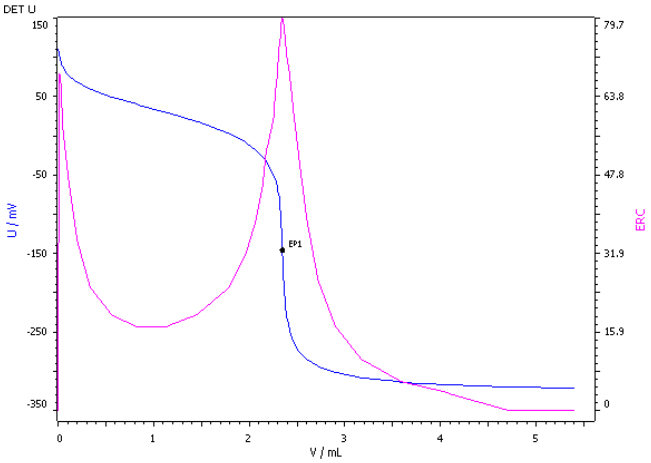

Fig. 5: Determination of the acid value (blue = titration curve, pink = ERC)

Comments

- For hard or animal fats, a solvent mixture of one volume ethanol and three volumes *tert*-butyl methyl ether or toluene is recommended.

  This mixture should also be neutralized.
- In the case of rapeseed oil, which has a maximum of erucic acid content of 5%, the acidity shall be given as oleic acid.
- If the results of the free fatty acids are simply reported as acidity, without further definition, this is by convention, expressed as oleic acid. If the sample contains mineral acids, these are, by convention, determined as fatty acids.
- For the determination of the free fatty acids with 859 Titrotherm, see Application Bulletin 315
- For further information concerning the handling of the Solvotrode easyClean please study the leaflet sent with the electrode.
- Ph.Eur 2.5.1 differs from the here described method in that petrol ether 100-120 °C is used in the solvent mixture instead of diethyl ether.

References

- EN ISO 660
  Animal and vegetable fats and oils – determination of acid value and acidity
- USP<401>
  Fats and fixed oils
- Ph.Eur. 2.5.1
  Acid value

- Application Bulletin AB-315
  Determination of free fatty acids (FFA) in edible oils with 859 Titrotherm

Hydroxyl value

Summary

Most standards for the determination of the hydroxyl value need long refluxing times and/or toxic pyridine for the determination. The herein described determination according to ASTM E1899 has the advantage that neither toxic pyridine nor long refluxing times are required.

Instruments

- Sample changer
- Titrator with DET mode
- 1x Burette 50 mL (acetonitrile)
- 2x Burette 20 mL (reaction solution, titrant)
- 1x Burette 2 mL (distilled water)
- Magnetic stirrer for sample changer
- DIS-Cover

Electrodes

Solvotrode easyClean	6.0229.010

Reagents

- Acetonitrile, HPLC quality
- Toluene-4-sulfonyl-isocyanate, purum (TSI)
- Ethanol, purity >99.8%
- Potassium hydrogen phthalate, KHP, p.a.

Solutions

Titrant	Tetrabutyl ammonium hydroxide, c(TBAOH) = 0.1 mol/L in isopropanol/methanol, Φ(MeOH) = 50% (v/v) If possible, this solution should be bought from a supplier.
TSI solution	The solution reacts vigorous with water, it is therefore recommended to work in a fume hood and under protective gas. Approximately 250 mL acetonitrile is given into a 500 mL volumetric flask and 20 mL TSI is added. The flask is filled up to the mark with acetonitrile and mixed. The reaction solution is stable for approximately 1 month.

Standard

KHP	KHP is dried in a drying oven for 2 h at 120 °C and allowed to cool down in a desiccator for at least 1 h.

Sample preparation

No sample preparation required.

Analysis

Titer

60 mL distilled water is added to approximately 180 mg KHP and the suspension stirred for about one minute in order to dissolve the KHP. The solution is then titrated until the first equivalence point using c(TBAOH) = 0.1 mol/L.

Sample

An appropriate amount of sample (see calculation below) is weighed into the titration vessel and dissolved in 10 mL acetonitrile. The beakers are covered and the solution is stirred for 30 s (stirring rate 8). 10.0 mL TSI solution are added and the sample is covered again and the mixture stirred (stirring rate 4). After 5 minutes 0.5 mL distilled water is added, the lid is again closed and the solution stirred for another 60 s (stirring rate 4). 40 mL acetonitrile is added and the solution is titrated until after the second end point with c(TBAOH) = 0.1 mol/L.

After each titration, the burette and vessel are rinsed first with ethanol, then with distilled water and the electrode is then conditioned for 1 min in distilled water.

$$m_S = \frac{40}{OHV_{expected}}$$

m_S: Sample amount in g

$OHV_{expected}$: Expected hydroxyl value

Parameters

Titer

Mode	DET U
Pause	30 s
Signal drift	50 mV/min
Max. waiting time	26 s
Meas. point density	4
Min. increment	10 µL
Max. increment	off
EP criterion	5
EP recognition	greatest

Sample

Mode	DET U
Pause	30 s
Signal drift	50 mV/min
Max. waiting time	26 s
Meas. point density	4
Min. increment	10 µL
Max. increment	off
EP criterion	5
EP recognition	all

Calculations

Titer

$$Titer = \frac{m_S}{V_{EP1} \times c(TBAOH) \times M_A}$$

Titer: Titer of the selected titrant

m_S: Mass of standard in mg

V_{EP1}: Titrant consumption until the first equivalence point in mL

c(TBAOH): Concentration of the selected titrant in mol/L; here c(TBAOH) = 0.1 mol/L

M_A: Molecular weight of the analyte; here 204.22 g/mol

Sample

$$OHV = \frac{(V_{EP2} - V_{EP1}) \times f \times c(TBAOH) \times M_A}{m_S}$$

OHV: Hydroxyl value of the sample in mg / g KOH

V_{EP1}: Titrant consumption until the first equivalence point in mL

V_{EP2}: Titrant consumption until the second equivalence point in mL

c(TBAOH): Concentration of the selected titrant in mol/L; here c(TBAOH) = 0.1 mol/L

f: Correction factor («titer») without unit

M_A: Molecular weight of the analyte; here 56.1 g/mol

m_S: Sample size in g

Example determination

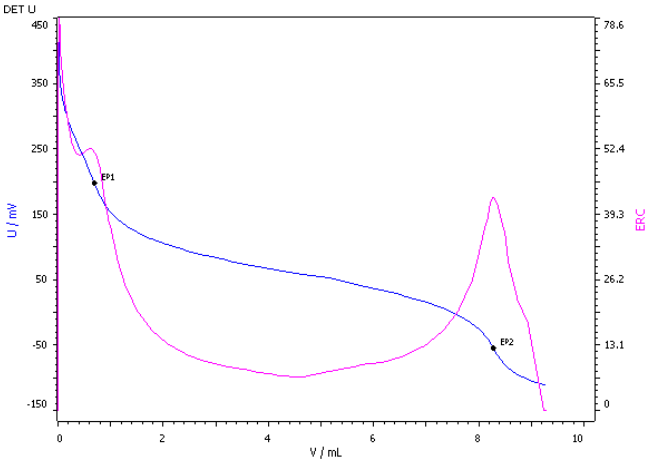

Fig. 6: Determination of the hydroxyl value (blue = titration curve, pink = ERC)

Comments

- The ASTM method is presented here, as it is faster (12 min) than method EN ISO 4629-2 (40 min). For information about the automated determination of the hydroxyl value according to the EN ISO method, see Metrohm Application Bulletin No. 322.
- For further information concerning the handling of the Solvotrode easyClean please study the leaflet sent with the electrode.

References

- ASTM E1899-08
  Standard test method for hydroxyl groups using reaction with p-toluene sulfonyl isocyante (TSI) and potentiometric titration with tetrabutyl ammonium hydroxide
- Application Bulletin AB-322
  Fully automated potentiometric determination of the hydroxyl number (HN) according to ASTM E1899 and EN ISO 4629-2

Nickel traces

Summary

The production of margarine often involves the hardening of liquid oils by a catalytic hydrogenation of the fatty acids. A catalyst used for this process is nickel. Polarography can be used to determine traces of nickel impurities in the final product.

Instruments

- VA instrument
  capable of operating a mercury electrode and supporting DP mode

Electrodes

WE	Multi-Mode Electrode pro (MME pro)	6.1246.120
	Mercury drop capillary	6.1226.030
AE	Separate Pt rod electrode	6.0343.000
RE	Ag/AgCl reference electrode c(KCl) = 3 mol/L	6.0728.020
	Electrolyte vessel filled with c(KCl) = 3 mol/L	6.1245.010

Reagents

- Hydrochloric acid, w(HCl) = 30%, for trace analysis*, CAS 7647-01-0
- Ammonium hydroxide solution, w(NH_3) = 25 %, for trace analysis*, CAS 1336-21-6
- Dimethylglyoxim disodium salt octahydrate, Na_2DMG, for analysis, CAS 75006-64-3
- Ni standard stock solution, β(Cu^{2+}) = 1 g/L, commercially available
- Nitric acid, w(HNO_3) = 65 %, for trace analysis* CAS 7697-37-2
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

* e.g. Merck suprapur®, Sigma-Aldrich TraceSelect® or equivalent

Solutions

DMG solution	c(Na_2DMG) = 0.1 mol/L Dissolve 0.304 g Na_2DMG in 10 mL ultrapure water. This solution needs to be prepared daily.

Standard solution

Ni standard	$\beta(Ni^{2+})$ = 10 mg/L 0.5 mL Ni standard stock solution (1 g/L) and 0.05 mL nitric acid (65%) are made up to 50 mL with ultrapure water.

Sample preparation

Weigh out accurately 2.5 g sample in a round-bottomed flask. Add 2.5 mL w(HCl) = 30%, attach a reflux condenser, heat up the solution and keep boiling for 15 minutes. Rinse out the warm solution with a small quantity of ultrapure water into a separating funnel. Separate and collect the aqueous phase. Extract the round-bottomed flask and the fatty phase another three times with hot ultrapure water. Filter the combined aqueous extracts through a paper filter (e.g «White Ribbon Filter» grade 589/2) into a 100 mL volumetric flask, add 5 mL $w(NH_3)$ = 25% and make up to the mark with ultrapure water.

Analysis

Measuring solution

20 mL sample extract (after sample preparation)

0.1 mL DMG solution

Pipette 20 mL of the prepared sample solution (corresponding to a 0.5 g portion of the original sample) into the polarography vessel and add 0.1 mL DMG solution. The pH of the measuring solution has to be 9.5 ± 0.1.

The concentration of Ni is quantified by two additions of Ni standard solution $\beta(Ni^{2+})$ = 10 mg/L.

Parameters

Volumes

Sample amount	0.5 g
Cell volume	20.1 mL

Voltammetric

Electrode	DME
Mode	DP – Differential pulse
Initial purge time	300 s
Hydrodynamic measurement	No
Sweep	
Start potential	-0.8 V
End potential	-1.4 V
Pulse amplitude	0.05 V
Pulse time	0.04 s
Voltage step	0.006 V
Voltage step time	0.6 s
Sweep rate	0.01 V/s

Substance and calibration

Name	Nickel
Peak potential	-1.0 V
Tolerance	0.05 V
Calibration method	Standard addition

Example determination

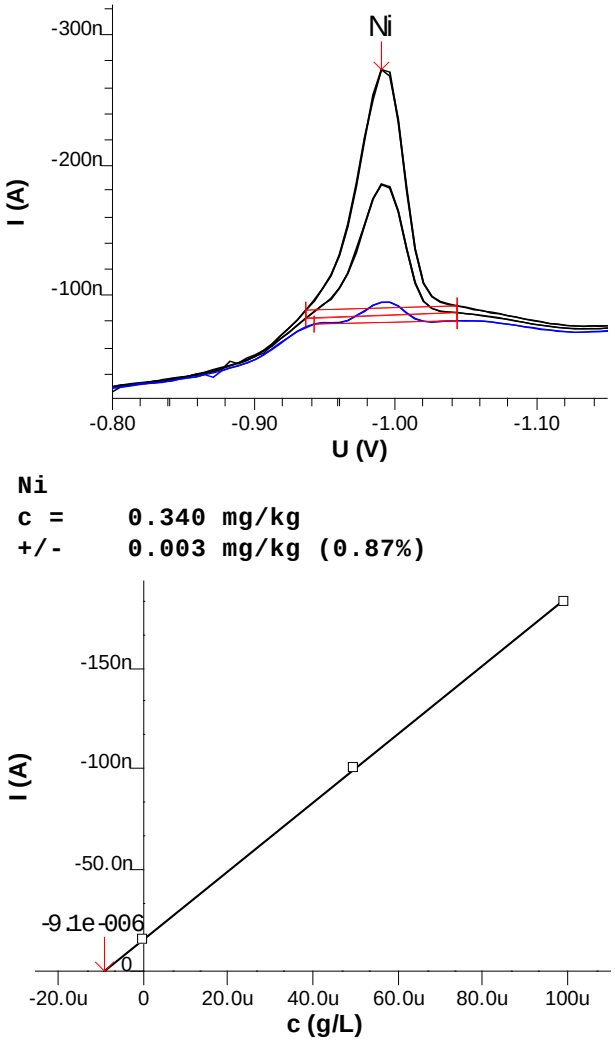

Fig. 7: Voltammogram and calibration curve of a determination of Ni in margarine (2.7 g sample extracted into 100 mL)

Comments

- Combustion as decomposition is unsuitable because volatile nickel carbonyl is lost in process.
- To determine the reagent blank the sample preparation procedure is carried like described just without the sample. The blank concentration is determined with the same parameters as for the sample. The blank concentration is then subtracted from the sample concentration.

Date

May 2019

Author

Competence Center Titration

Competence Center Voltammetry, CVS and Stability

Metrohm International Headquarters