Analysis of edible oils and fats

Branch
General analytical chemistry; food

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

Summary
This Application Bulletin describes the following analytical methods for edible oils and fats:

- Water content according to Karl Fischer
- Oxidation stability – Rancimat method
- Iodine value
- Peroxide value
- Saponification value
- Acid value, free fatty acids (FFA)
- Hydroxyl value
- Nickel traces, using polarography

Special care was taken to avoid chlorinated solvents in these methods. Also as many methods as possible were automated.

Water content

Summary
The coulometric Karl Fischer method is preferred for this analysis because of the low water contents of pure oils and fats. For butter and margarines, which exhibit relatively high water contents, the volumetric Karl Fischer method should be used.

Instruments
- Coulometric KF titrator
  or
- Volumetric KF titrator

Electrodes
- Double Pt-wire electrode for volumetry 6.0338.100
- Double Pt-wire electrode for coulometry 6.0341.100
- Generator electrode with diaphragm 6.0344.100

Reagents
Coulometric
- Hydranal Coulomat Oil or equivalent
- Hydranal Coulomat CG or equivalent

Volumetric
- Hydranal Composite 5 or equivalent
- Methanol, dry, p.a.
- 1-Decanol, p.a.

Solutions
- Solvent mixture Methanol / 1-decanol,
  $\Phi(\text{MeOH}) = 66\% (v/v)$

Sample preparation
Hard fats should be melted before adding them into the titration vessel.
Butter and margarine should first be homogenized as their distribution of water is inhomogeneous. They should not be heated over 25 °C, otherwise phase separation may occur.

Analysis

Sample (Coulometric)
Add approximately 100 mL coulometric reagent to the titration vessel and condition it until a constant drift is achieved (< 10 µg/min is typical). Then fill a syringe 3 times with the sample and discard it. Fill again, and add approx. 0.5 g to 1 g sample to the titration vessel and titrate the water content.

Sample (Volumetric)
Add approximately 30 mL solvent mixture to the titration vessel and condition it until a constant drift of approximately 10 ... 20 µL/min is reached. Fill the sample into a dry syringe (without needle). Add approx. 0.3 g sample to the titration vessel and titrate.

Parameters

Sample (Coulometric)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>KFC</td>
</tr>
<tr>
<td>Start drift</td>
<td>20 µg/min</td>
</tr>
<tr>
<td>EP at</td>
<td>50 mV</td>
</tr>
<tr>
<td>Dynamics</td>
<td>70 mV</td>
</tr>
<tr>
<td>Min. rate</td>
<td>15 µg/min</td>
</tr>
<tr>
<td>Stop criterion</td>
<td>Rel. drift</td>
</tr>
<tr>
<td>Rel. stop drift</td>
<td>5 µg/min</td>
</tr>
<tr>
<td>Extraction time</td>
<td>0 s</td>
</tr>
</tbody>
</table>

Sample (Volumetric)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>KFT Ipol</td>
</tr>
<tr>
<td>Start drift</td>
<td>20 µL/min</td>
</tr>
<tr>
<td>EP at</td>
<td>250.0 mV</td>
</tr>
<tr>
<td>Dynamics</td>
<td>100 mV</td>
</tr>
<tr>
<td>Stop criterion</td>
<td>Drift</td>
</tr>
<tr>
<td>Stop drift</td>
<td>20 µL/min</td>
</tr>
<tr>
<td>Extraction time</td>
<td>0 s</td>
</tr>
</tbody>
</table>

References

- DIN EN ISO 8534
  Animal and vegetable fats and oils – determination of water content – Karl Fischer method (pyridine free)
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). The continuously recorded electrical conductivity of the measuring solution is increasing due to the absorption of the reaction products. Thus their appearance can be detected. The time until secondary reaction products are 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 required.
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 on a water bath. Care has to be taken that the water bath temperature is not far beyond 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 the data recording immediately.

Parameters
<table>
<thead>
<tr>
<th>Sample size</th>
<th>3 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measuring solution</td>
<td>60 mL deionized water</td>
</tr>
<tr>
<td>Temperature</td>
<td>80 … 160 °C</td>
</tr>
<tr>
<td>Gas flow</td>
<td>20 L/h</td>
</tr>
<tr>
<td>Evaluation</td>
<td>Induction time</td>
</tr>
</tbody>
</table>

Example determination

![Graph showing oxidation stability](image)

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

Typical results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature/°C</th>
<th>Induction time/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>120</td>
<td>approx. 5</td>
</tr>
<tr>
<td>Hazelnut fat</td>
<td>120</td>
<td>10 … 12</td>
</tr>
<tr>
<td>Hazelnut oil</td>
<td>120</td>
<td>7 … 11</td>
</tr>
<tr>
<td>Lard</td>
<td>100</td>
<td>1 … 3</td>
</tr>
<tr>
<td>Linseed oil</td>
<td>110</td>
<td>0.5 … 2</td>
</tr>
<tr>
<td>Margarine</td>
<td>120</td>
<td>2 … 6</td>
</tr>
<tr>
<td>Olive oil</td>
<td>120</td>
<td>6 … 11</td>
</tr>
<tr>
<td>Palm oil</td>
<td>120</td>
<td>7 … 12</td>
</tr>
<tr>
<td>Peanut fat</td>
<td>120</td>
<td>9 … 10</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>120</td>
<td>3 … 15</td>
</tr>
<tr>
<td>Pumpkin seed oil</td>
<td>120</td>
<td>approx. 7</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>120</td>
<td>3 … 5</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>120</td>
<td>1 … 2</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>120</td>
<td>approx. 5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>120</td>
<td>1 … 7</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>120</td>
<td>1 … 4</td>
</tr>
<tr>
<td>Tallow</td>
<td>120</td>
<td>3 … 8</td>
</tr>
</tbody>
</table>
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. But for best 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. But the temperature can be chosen 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 every determination to avoid side reactions due to contaminations. 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)
- DIN EN ISO 6886
  Animal and vegetable fats and oils – determination of oxidative stability (accelerated oxidation test)
- Metrohm Application Bulletin 204
  Oxidation stability of oils and fats – Rancimat method
Iodine value

Summary
The determination of the iodine value is based on the addition of iodine to the double bonds of unsaturated fatty acids. The result is given as g I₂ consumed by 100 g sample and is a measure for the unsaturation of an oil.

For the manual determination of the iodine value the beakers have to be placed in the dark after adding the reaction solution, magnesium acetate solution and glacial acetic acid. Before the titration the potassium iodide solution has to be added, all these steps are laborious and time consuming.

The automated determination is done with brown glass beakers and the Robotic DIS-Cover system. This method leads to good and reproducible results.

Instruments
- Sample changer with Swing Head and DIS-Cover
- Titrator with DET mode
- 2x Burette 20 mL (Glacial acetic acid, Mg(CH₃COO)₂)
- 4x Burette 50 mL (H₂SO₄, ICl, KI, Na₂S₂O₃)
- Propeller Stirrer

Electrodes
- iPt Titrode 6.0471.300

Reagents
- Sulfuric acid, c(H₂SO₄) = 0.5 mol/L, volumetric solution
- Potassium iodate, KIO₃, p.a.
- Potassium iodide, KI, p.a.
- Sodium thiosulfate, c(Na₂S₂O₃) = 0.1 mol/L, volumetric solution
- Magnesium acetate, Mg(CH₃COO)₂, purum
- Glacial acetic acid, p.a.
- Iodine chloride, Wijs-solution, c(ICl) = 0.1 mol/L, volumetric solution

Solutions

<table>
<thead>
<tr>
<th>Titrant</th>
<th>c(Na₂S₂O₃) = 0.1 mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>If possible this solution should be bought from a supplier.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Potassium iodide solution</th>
<th>β(KI) = 100 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 g potassium iodide is weighed into a 500 mL volumetric flask and filled up with dist. water.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Magnesium acetate solution</th>
<th>w(Mg(CH₃COO)₂) = 3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 g magnesium acetate is weighed into a 500 mL volumetric flask and filled up with dist. water.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction solution</th>
<th>c(ICl) = 0.1 mol/L in glacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>If possible this solution should be bought from a supplier.</td>
<td></td>
</tr>
</tbody>
</table>

Standard

<table>
<thead>
<tr>
<th>Iodate standard</th>
<th>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.</th>
</tr>
</thead>
</table>

Sample preparation
No sample preparation required.

Analysis

Titer
Approximately 70 mg potassium iodate is weighed into a 250 mL beaker and 80 mL dist. water is added to dissolve it. Afterwards 10 mL β(KI) = 100 g/L as well as 25 mL c(H₂SO₄) = 0.5 mol/L are given to the solution. The solution becomes dark brown and the originated iodine is titrated with c(Na₂S₂O₃) = 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(CH₃COO)₂) = 3% are given into a 250 mL brown glass beaker. The beaker is closed with the 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(Na₂S₂O₃) = 0.1 mol/L until the first end point.

Sample
An appropriate sample amount is weighed into a 250 mL brown glass beaker (see table below) and placed onto the sample rack. 20 to 25 mL glacial acetic acid (see below), 25 mL c(ICl) = 0.1 mol/L and 10 mL w(Mg(CH₃COO)₂) = 3%
are then added. Afterwards the beaker is closed with the 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(Na₂S₂O₃) = 0.1 mol/L until the first end point.

<table>
<thead>
<tr>
<th>Amount of sample and solvent</th>
<th>Expected IV / g / 100 g</th>
<th>Sample amount / g</th>
<th>Solvent volume / mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.5</td>
<td>15.00</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>1.5 – 2.5</td>
<td>10.00</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>2.5 – 5</td>
<td>3.00</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>5 – 20</td>
<td>1.00</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>20 – 50</td>
<td>0.40</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>50 – 100</td>
<td>0.20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>100 – 150</td>
<td>0.13</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>150 – 200</td>
<td>0.10</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

### Calculation

**Titer**

\[
\text{Titer} = \frac{m_s \times 6}{V_{EP1} \times c(\text{Na}_2\text{S}_2\text{O}_3) \times M_A}
\]

- **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(\text{Na}_2\text{S}_2\text{O}_3)**: Concentration of the selected titrant in mol/L; here c(\text{Na}_2\text{S}_2\text{O}_3) = 0.1 mol/L
- **M_A**: Molecular weight of the analyte; here 124.00 g/mol

**Sample**

\[
IV = \frac{(V_{EP1} - V_{\text{blank}}) \times f \times c(\text{Na}_2\text{S}_2\text{O}_3) \times M_A}{10 \times m_s}
\]

- **IV**: Iodine value of the sample in g iodine / 100 g
- **V_{EP1}**: Titrant consumption until the first equivalence point in mL
- **V_{\text{blank}}**: Blank value consumption for the used quantity of solvent in mL
- **c(\text{Na}_2\text{S}_2\text{O}_3)**: Concentration of the selected titrant in mol/L; here c(\text{Na}_2\text{S}_2\text{O}_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 was adapted from the norm DIN EN ISO 3961. The following changes were made:
  - Magnesium acetate was used as catalyst, therefore shortening the reaction time from 1 - 2 h to 5 minutes.
  - Glacial acetic acid was used as solvent instead of a mixture of cyclohexane and glacial acetic acid.

References

- DIN 53241-1
  Determination of the iodine value – part 1: methods using Wijs solution
- DIN EN ISO 3961
  Animal and vegetable fats and oils – determination of iodine value
Peroxide value

Summary
The peroxide number gives information about the number of peroxide compounds in the oil and hence of the age and quality of the edible oil. The lower the peroxide numbers the better and/or newer 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

Reagents
- Sulfuric acid, c(H$_2$SO$_4$) = 0.5 mol/L, volumetric solution
- Potassium iodate, KIO$_3$, p.a.
- Potassium iodide, KI, p.a.
- Sodium thiosulfate, c(Na$_2$S$_2$O$_3$) = 0.1 mol/L, volumetric solution
- Glacial acetic acid, p.a.
- 1-Decanol, p.a.

Solutions

<table>
<thead>
<tr>
<th>Titrant</th>
<th>c(Na$_2$S$_2$O$_3$) = 0.001 mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepared by dilution of the c(Na$_2$S$_2$O$_3$) = 0.1 mol/L with dist. water.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Auxiliary solution</th>
<th>Saturated solution of KI:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Potassium iodide solution</th>
<th>w(KI) = 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 g potassium iodide is weighed into a 500 mL volumetric flask and filled up with dist. water.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solvent mixture</th>
<th>Glacial acetic acid / 1-decanol with approximately 20 mg I$_2$ / L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Φ(1-decanol) = 40% (v/v)</td>
<td></td>
</tr>
</tbody>
</table>

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 required.

Analysis

Titer

0.75 to 1.25 mL potassium iodate standard solution is dosed into a 250 mL beaker. 80 mL dist. water, 10 mL w(KI) = 10% as well as 25 mL c(H$_2$SO$_4$) = 0.5 mol/L are added to the solution. The solution becomes dark brown and the originated iodine is titrated with c(Na$_2$S$_2$O$_3$) = 0.001 mol/L up to the first end point.

Blank

20 mL solvent mixture and 0.2 mL auxiliary solution are dosed into a 250 mL brown glass beaker and closed with the DIS-cover. After one minute 80 mL dist. water is added and the solution is titrated with c(Na$_2$S$_2$O$_3$) = 0.001 mol/L until the first end point.

Sample

5 or 10 g sample (depending on the expected value) is weighed into a 250 mL brown glass beaker and placed onto the sample rack. 20 mL solvent mixture and 0.2 mL auxiliary solution are added and the beaker is closed with the DIS-cover. After one minute 80 mL dist. water is added and the solution is titrated with c(Na$_2$S$_2$O$_3$) = 0.001 mol/L until the first end point.

Parameters

<table>
<thead>
<tr>
<th>Titer</th>
<th>Mode</th>
<th>DET U</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pause</td>
<td>20 s</td>
</tr>
<tr>
<td></td>
<td>Signal drift</td>
<td>20 mV/min</td>
</tr>
<tr>
<td></td>
<td>Max. waiting time</td>
<td>38 s</td>
</tr>
<tr>
<td></td>
<td>Meas. point density</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Min. increment</td>
<td>50 µL</td>
</tr>
<tr>
<td></td>
<td>Max. increment</td>
<td>500 µL</td>
</tr>
<tr>
<td></td>
<td>EP criterion</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>EP recognition</td>
<td>all</td>
</tr>
</tbody>
</table>
### Blank/Sample

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>DET U</td>
</tr>
<tr>
<td>Signal drift</td>
<td>5 mV/min</td>
</tr>
<tr>
<td>Min. waiting time</td>
<td>10 s</td>
</tr>
<tr>
<td>Max. waiting time</td>
<td>72 s</td>
</tr>
<tr>
<td>Meas. point density</td>
<td>4</td>
</tr>
<tr>
<td>Min. increment</td>
<td>10 µL</td>
</tr>
<tr>
<td>Max. increment</td>
<td>200 µL</td>
</tr>
<tr>
<td>EP criterion</td>
<td>20</td>
</tr>
<tr>
<td>EP recognition</td>
<td>greatest</td>
</tr>
</tbody>
</table>

### Calculation

**Titer**  

\[
\text{Titer} = \frac{\beta(\text{KIO}_3) \times m_s \times 6}{V_{\text{EP1}} \times c(\text{Na}_2\text{S}_2\text{O}_3) \times M_A}
\]

- **Titer:** Titer of the selected titrant
- **β(\text{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_{\text{EP1}}:** Titrant consumption until the first equivalence point in mL
- **c(\text{Na}_2\text{S}_2\text{O}_3):** Concentration of the selected titrant in mol/L; here \( c(\text{Na}_2\text{S}_2\text{O}_3) = 0.001 \text{ mol/L} \)
- **M_A:** Molecular weight of the analyte; here 214.00 g/mol

### Sample

\[
\text{PV} = \frac{k \times (V_{\text{EP1}} - V_{\text{blank}}) \times f}{m_s}
\]

- **PV:** Peroxide value of the sample in meq O_2 / kg
- **V_{\text{EP1}}:** Titrant consumption until the first equivalence point in mL
- **V_{\text{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(\text{Na}_2\text{S}_2\text{O}_3) = 0.001 \text{ mol/L}, 10 for c(\text{Na}_2\text{S}_2\text{O}_3) = 0.01 \text{ mol/L}

### Example determination

![Graph showing determination of peroxide value](image)

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

### Comments
- The stirrer has to be set to a higher level (14) for the dissolving of the oil, than for the titration (10). Otherwise irreproducible results can occur.
- Before each determination series a preparation of all dosing units, especially of the solvent mixture has to be done. The solvent mixture contains iodine and the amount of iodine and therefore of the solvent mixture has to be the same during a series.
- As the peroxide value depends on the sample size the ISO/TC 34/SC 11 has decided to fix the sample size to 5 g for PV greater than 1, and at 10 g for PV less than or equal to 1.
- The method for determining the peroxide value was adapted from the norm DIN EN ISO 27107. The following changes were made:
  - H_2SO_4 was used in the titer determination instead of hydrochloric acid.
  - A mixture of 1-decanol and glacial acetic acid was used as solvent instead of a mixture of isooctane and glacial acetic acid.

### References
- DIN EN ISO 27107
  Animal and vegetable fats and oils – determination of peroxide value – potentiometric end-point determination
Saponification value

Summary
The saponification value is expressed as the amount of potassium hydroxide in milligrams required to saponify 1 g of fat under the conditions specified. It contains the information of the average molecular weight of all fatty acids present.

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

<table>
<thead>
<tr>
<th>Titrant</th>
<th>c(HCl) = 0.5 mol/L</th>
</tr>
</thead>
</table>
| If possible this solution should be bought from a supplier.

| Ethanoic potassium                | c(KOH) = 0.5 mol/L in ethanol |
|-----------------------------------| If possible this solution should be bought from a supplier.  
| hydroxide solution                | 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 the sample (see table below) in a round-bottomed flask. Add 25 mL 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.

Amount of sample

<table>
<thead>
<tr>
<th>Expected SV / mg KOH / g</th>
<th>Sample amount / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 – 200</td>
<td>2.2 – 1.8</td>
</tr>
<tr>
<td>200 – 250</td>
<td>1.7 – 1.4</td>
</tr>
<tr>
<td>150 – 300</td>
<td>1.3 – 1.2</td>
</tr>
<tr>
<td>&gt; 300</td>
<td>1.1 – 1.0</td>
</tr>
</tbody>
</table>

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 until 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 electrode and burette tip, then back-titrate the KOH excess with c(HCl) = 0.5 mol/L until the first equivalence point. In between measurements the electrode membrane is rehydrated for 1 min in deionized water.

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

Titer

<table>
<thead>
<tr>
<th>Mode</th>
<th>DET U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pause</td>
<td>20 s</td>
</tr>
<tr>
<td>Signal drift</td>
<td>20 mV/min</td>
</tr>
<tr>
<td>Max. waiting time</td>
<td>38 s</td>
</tr>
<tr>
<td>Meas. point density</td>
<td>4</td>
</tr>
<tr>
<td>Min. increment</td>
<td>50 µL</td>
</tr>
<tr>
<td>Max. increment</td>
<td>off</td>
</tr>
<tr>
<td>EP criterion</td>
<td>5</td>
</tr>
<tr>
<td>EP recognition</td>
<td>greatest</td>
</tr>
</tbody>
</table>

Blank/Sample

<table>
<thead>
<tr>
<th>Mode</th>
<th>DET U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pause</td>
<td>20 s</td>
</tr>
<tr>
<td>Signal drift</td>
<td>20 mV/min</td>
</tr>
<tr>
<td>Max. waiting time</td>
<td>38 s</td>
</tr>
<tr>
<td>Meas. point density</td>
<td>4</td>
</tr>
<tr>
<td>Min. increment</td>
<td>10 µL</td>
</tr>
<tr>
<td>Max. increment</td>
<td>off</td>
</tr>
<tr>
<td>EP criterion</td>
<td>5</td>
</tr>
<tr>
<td>EP recognition</td>
<td>greatest</td>
</tr>
</tbody>
</table>

Calculation

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

\[
SV = \frac{(V_{\text{blank}} - V_{EP1}) \times f \times c(HCl) \times M_A}{m_s}
\]

SV: Saponification value of the sample in mg KOH / g

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

\(V_{\text{blank}}\): Blank value consumption for the used quantity of solvent in mL

Example determination

![Graph showing titration curve and ERC](image)

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 colourless or straw yellow. A description for the preparation of a stable colourless solution can be found in the norm ISO 3657.
- For further information concerning the handling of the Solvotrode easyClean please study the leaflet sent with the electrode.

References

- DIN EN ISO 3657
  Animal and vegetable fats and oils – determination of saponification value
Acid value, free fatty acids

Summary
The acid value corresponds to the amount of carboxylic acid groups in fatty acids and is given in mg KOH per g sample. The older an oil is the higher the acid value as triglycerides are converted into fatty acids and glycerol upon aging.

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.

Amount of sample

<table>
<thead>
<tr>
<th>Expected AV / mg KOH / g</th>
<th>Sample amount / g</th>
<th>Accuracy / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>20</td>
<td>0.05</td>
</tr>
<tr>
<td>1 – 4</td>
<td>10</td>
<td>0.02</td>
</tr>
<tr>
<td>4 – 15</td>
<td>2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>15 - 75</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt; 75</td>
<td>0.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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
Calculation

**Titer**

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

- **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(\text{KOH})**: Concentration of the selected titrant in mol/L; here c(\text{KOH}) = 0.1 mol/L
- **M_A**: Molecular weight of the analyte; here 112.12 g/mol

**Acid value**

\[
\text{AV} = \frac{V_{EP1} \times f \times c(\text{KOH}) \times M_A}{m_s}
\]

- **AV**: Acid value of the sample in mg KOH / g
- **V_{EP1}**: Titrant consumption until the first equivalence point in mL
- **c(\text{KOH})**: Concentration of the selected titrant in mol/L; here c(\text{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**

\[
\text{FFA} = \frac{V_{EP1} \times f \times c(\text{KOH}) \times M_A}{10 \times m_s}
\]

- **FFA**: Acid value of the sample in %
- **V_{EP1}**: Titrant consumption until the first equivalence point in mL
- **c(\text{KOH})**: Concentration of the selected titrant in mol/L; here c(\text{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

**Choice of fatty acids for the free fatty acid content**

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

**Example determination**

![Graph](image)

**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 having a maximum of erucic acid content of 5%, the acidity shall be expressed 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 Titrotherm see Application Bulletin 315
- For further information concerning the handling of the Solvotrode easyClean please study the leaflet sent with the electrode.

**References**

- DIN EN ISO 660
  Animal and vegetable fats and oils – determination of acid value and acidity
- Application Bulletin 315
  Determination of free fatty acids (FFA) in edible oils with 859 Titrotherm
Hydroxyl value (ASTM E1899-08)

Summary
The hydroxyl value is given in mg KOH per g sample and gives information about the degree of esterification within the sample.

Instruments
- Sample changer
- Titrator with DET mode
- 1x Burette 50 mL (acetonitrile)
- 2x Burette 20 mL (reaction solution, titrant)
- 1x Burette 2 mL (dist. H₂O)
- 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

<table>
<thead>
<tr>
<th>Titrant</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSI solution</td>
<td>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.</td>
</tr>
</tbody>
</table>

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**
To approximately 180 mg KHP 60 mL dist. H₂O is added and the suspension stirred for about a 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 dist. H₂O 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 dist. H₂O and the electrode is then conditioned for 1 min in dist. H₂O.

\[
m_s = \frac{40}{\text{OHV}_\text{expected}}
\]

mₙ: Sample amount in g

**Parameters**

**Titer**

<table>
<thead>
<tr>
<th>Mode</th>
<th>DET U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pause</td>
<td>30 s</td>
</tr>
<tr>
<td>Signal drift</td>
<td>50 mV/min</td>
</tr>
<tr>
<td>Max. waiting time</td>
<td>26 s</td>
</tr>
<tr>
<td>Meas. point density</td>
<td>4</td>
</tr>
<tr>
<td>Min. increment</td>
<td>10 µL</td>
</tr>
<tr>
<td>Max. increment</td>
<td>off</td>
</tr>
<tr>
<td>EP criterion</td>
<td>5</td>
</tr>
<tr>
<td>EP recognition</td>
<td>greatest</td>
</tr>
</tbody>
</table>
**Sample**

<table>
<thead>
<tr>
<th>Mode</th>
<th>DET U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pause</td>
<td>30 s</td>
</tr>
<tr>
<td>Signal drift</td>
<td>50 mV/min</td>
</tr>
<tr>
<td>Max. waiting time</td>
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</tr>
<tr>
<td>Meas. point density</td>
<td>4</td>
</tr>
<tr>
<td>Min. increment</td>
<td>10 µL</td>
</tr>
<tr>
<td>Max. increment</td>
<td>off</td>
</tr>
<tr>
<td>EP criterion</td>
<td>5</td>
</tr>
<tr>
<td>EP recognition</td>
<td>all</td>
</tr>
</tbody>
</table>

**Calculation**

**Titer**

\[
\text{Titer} = \frac{m_s}{V_{EP1} \times c(TBAOH) \times M_A}
\]

- **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**

\[
\text{OHV} = \frac{(V_{EP2} - V_{EP1}) \times f \times c(TBAOH) \times M_A}{m_s}
\]

- **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**

[Graph showing titration curve and ERC]

**Comments**

- The ASTM method is presented here, as it is faster (12 min) than the DIN 53240-2 method (40 min). For information about the automated determination of the hydroxyl value according to the DIN 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 isocyanate (TSI) and potentiometric titration with tetrabutyl ammonium hydroxide
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 |
| RE | Ag/AgCl reference electrode | 6.0728.020 |
| RE | 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(NH₃) = 25%, for trace analysis*, CAS 1336-21-6
- Dimethylglyoxim disodium salt octahydrate, Na₂DMG, for analysis, CAS 75006-64-3
- Ni standard stock solution, β(Cu²⁺) = 1 g/L, commercially available
- Nitric acid, w(HNO₃) = 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(Na₂DMG) = 0.1 mol/L |

Standard solution

| Ni standard | β(Ni²⁺) = 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(NH₃) = 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 β(Ni²⁺) = 10 mg/L.

Parameters

Volumes
| Sample amount | 0.5 g |
| Cell volume | 20.1 mL |

Voltammetric
<p>| 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 |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse amplitude</td>
<td>0.05 V</td>
</tr>
<tr>
<td>Pulse time</td>
<td>0.04 s</td>
</tr>
<tr>
<td>Voltage step</td>
<td>0.006 V</td>
</tr>
<tr>
<td>Voltage step time</td>
<td>0.6 s</td>
</tr>
<tr>
<td>Sweep rate</td>
<td>0.01 V/s</td>
</tr>
</tbody>
</table>

**Substance and calibration**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nickel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak potential</td>
<td>-1.0 V</td>
</tr>
<tr>
<td>Tolerance</td>
<td>0.05 V</td>
</tr>
<tr>
<td>Calibration method</td>
<td>Standard addition</td>
</tr>
</tbody>
</table>

**Example determination**

![Voltammogram and calibration curve](image)

- **Ni**
  - $c = 0.340 \text{ mg/kg}$
  - $+/\text{-} 0.003 \text{ mg/kg (0.87\%)}$

**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.

**Author**

Competence Center Titration
Competence Center Voltammetry, CVS and Stability
Metrohm International Headquarters