ISCO3/LAB/00/04 Physico-chemical characterization of ozonized oil. Peroxide Value

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Title

ISCO3/LAB/00/04 Peroxide Values in Ozonized Oils

1.1. Brief background

Over a period of many years, various methods have been developed for the determination of peroxides in fats and oils. The general principle of most of the methods is the liberation of iodine from potassium iodide in an acid medium. The method according to Wheeler was standardized more than 50 years ago by different standardization bodies, and it is widely used to control commodities by producers, receivers and official laboratories. The peroxide value is a measure of the amount of oxygen chemically bound to an oil or fat as peroxides, particularly hydroperoxides.

1.2. Purpose

The purpose of this SOP (Standard Operation Procedure) is to describe the procedure to assay the content of peroxide in samples of ozonized oils.

1.3. Scope

This procedure specifies the analytical procedure, chemical and devises to assay the content of peroxide using a titrimetric method (visual endpoint). The concentration range will be adjusted from 0 to high values (e.g. 4000 mEq O_2 / kg). The method is basically the procedure describes in the *European Pharmacopoeia* (7^{th} ed), modified according the suggestion of Zanardi *et al.* (2008), to increase the detection limit (only by modifying both the temperature and the reaction time with respect to the monograph conditions).

1.4. Acronyms, abbreviations and definitions

PV	Peroxide Value: Quantity of those substances in the sample, expressed in terms of active oxygen, that oxidize potassium iodide under the conditions specified in this SOP.
MW	Molecular weigh
mEq	Milliequivalent
SOP	Standard Operation Procedure

2. Responsibility

Chemical analyst	Guide technical implementation Adjust the method according the characteristics of the sample Issue the final results report

Technical analyst	Preparation of the reagents Perform the technical procedure Notification of possible complications Record and file the original data Perform calculations and give a report of the results

3. Summary of Method

3.1 Background

The chemical reactions of ozone when bubbled into an oil are very complex. The peroxidic species are one of the most important products formed. This group includes hydroperoxides, hydrogen peroxide, polymeric peroxides, ozonides and other organic peroxides. The yield of ozonated derivatives from unsaturated oils depends on reaction conditions (e.g., temperature, time, ozone generator, reactor type, stirring conditions, applied ozone dose). For determining the quality of ozonated products, analytical methods such as peroxide, acidity, and iodine values are usually carried out (ISCO3/QAU/01/03). The peroxide value (PV) represents the quantity of peroxide expressing in milliequivalents of active oxygen contained in 1 kg of the sample.

3.2 Principle of Detection

The sample is treated in solution with a mixture of acetic acid and a suitable organic solvent and then with a solution of potassium iodide. The liberated iodine is titrated with a standard solution of sodium thiosulfate. The endpoint of the tritation based on the iodine liberated by the peroxide is determined iodometricaly (visually) with a starch indicator.

Peroxides and similar products which oxidize potassium iodide under the conditions of the test will contribute to the peroxide value. Variations in procedure may affect the results. Peroxide values are expressed in milliequivalents of peroxide by kg of sample.

3.3 Reaction Scheme

The reaction scheme for hydroperoxides determination in the presence of acetic acid is as follow:

Generation of hydroperoxides, Reaction I: $R\text{-}H + O_3/O_2 \rightarrow ROOH$

Generation of iodine, Reaction II: $KI + CH_3\text{-}COOH + \Delta \rightarrow HI + CH3COO^-K^+$

Reaction II: $ROOH + 2KI + \Delta \rightarrow ROH + H_2O + I_2$ + Starch indicator

Titration step, Reaction IV: I_2 (purple) + $2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$ (colorless)

The reaction of peroxides of the structures R-O-O-R' and R-COH-COH-R' follows an analogous pathway, whilst cyclic peroxides do not react quantitatively under the conditions described here. Alternatively, the ion reaction is of more of general applicability (IFRA2011):

Reaction II $O_2^{2-} + 2I^- + 4H^+ + \Delta \rightarrow 2H_2O + I_2$
Reaction IV $I_2 + 2\ S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$

4. Apparatus and Reagents

4.1 Apparatus

Analytical Balance, ± 1 mg
Stop watch ± 1 s
Magnetic stirrer, with magnetic stirrer rod (of 2.5 cm) and a heating plate
Burette of 10 mL or 25 mL capacity, graduated in at least 0.05 mL, preferably with automatic zero adjustment (pellet titration) or automatic titrimeter with 20 mL of capacity, with a resolution of at least 10 µL and an accuracy of ±0.15% (e.g. a piston burette).

Glassware:
Iodine flask , Erlenmeyer flask, 250 mL with glass stopper.
Pipettes, of 0.5 mL, 1 mL, 10 mL and 100 mL capacity (or automatic pipettes)
Measuring cylinders, of 50 mL and 100 mL capacity
Volumetric flask of 250 mL, 500 mL and 1 L capacity.
Amber stained bottles (for solutions) or amber glassware.

4.2 Reagents

Chloroform p. a.
Glacial acetic acid p. a.
Methanol (reagent grade)
Starch p. a. (e. g. soluble acc. to Zulkowsky)
Formic acid conc.
Sodium thiosulfate solution, 0.01 mol/l (0.01 N) or 0.1 mol/l (0.1 N)
Potassium iodide p. a. (free from iodine and iodates)
Water (demineralized, boiled and cooled down to 20 °C)

4.2.1 Saturated potassium iodide solution

Dissolve approximately 14 g of potassium iodide in approximately 8 g of freshly boiled water at room temperature. The solution must remain saturated (undissolved crystals must be present). The solution has to be kept protected from light (use amber glassware). Test the solutions as follows: add two drops of starch solution to 0.5 mL of the potassium iodide in 30 mL of Glacial acetic acid: chloroform (3:2). If a blue color is formed and if more than one drop of solution thiosulfate standard solution is needed to removed it, discard the potassium iodide solution and prepare a new solution.

4.2.2 Starch Solution 1 %

Soluble starch, 1.0 g is dissolved in deionised water, 100 mL with heating. Formic acid, 0.3 mL is added to stabilize the solution. *Starch solution*. 1085103 *Eur Pharmacopoeia*

4.2.3 Sodium thiosulfate solution

The solution has to be kept well sealed and in the dark. Determine the titer every week or prepare freshly. Use only freshly boiled water for the preparation of this solution, possible purged with nitrogen. Store in an amber-stained bottle.

4.2.4 Solvent Mixture

Glacial acetic acid: chloroform (3:2)

5. Sample Collection, Preservation, Shipment, and Storage

The sample has to be protected from the air, stored in a cool and dark place and should not be opened before the determination is commenced. Sample should not be damaged of changed during transport or storage. The sample should be kept at 5 ± 3 ^{o}C during storage.

Solid fats may not be melted before the determination. A sample of fat is taken from the centre of the sample, and attention must be paid to the fact that no sample is taken from the surface. The sample is transferred into an Erlenmeyer flask previously weighted and closed immediately with a glass stopper.

5.1. Preparation of test sample

The test sample for the determination of peroxide values shall be taken first and the peroxide values shall be determined immediately.

Homogenize the sample, preferably without heating and without aeration. Avoid direct solar radiation. Heat solid samples carefully to 10 °C above their melting point. Samples with visible impurities shall be filtered, the filtration shall be noted in the test report.

5.2 Sample quantity according to the PV

For more accuracy the quantity of sample will depend of the expected PV (see table for details).

Expected PV	Mass of substance to be examined (g)	Recommended Sodium tiosulfate titration solution concentration
< 100	2 - 5 g use the Pharmacopoeia Method	0.01 M
100 - 500	0.5 - 1.2	0.1 M or 0.01 M
500 - 900	0.3 - 0.5	0.1 M or 0.01 M
> 1000	0.2 - 0. 3	0.1 M

6. Procedure

Carry out all steps in a laboratory with diffuse daylight or in artificial light. Avoid direct exposure to sunlight. Observe that all vessels should be free from oxidizing or reducing compounds.

6.1 Test portion added by weighing

Transfer approx. 0.25 g (0.2 - 0.3) g of the sample, accurately weighed, into a 150 mL Erlenmeyer flask with glass stopper. Add 10 mL of the solvent mixture (glacial acetic acid : chloroform 3:2) and saturated potassium iodide solution, 1 mL, freshly prepared and allow to react for 60 s ± 1 s and shaking thoroughly during this period. Place the mixture under magnetic stirring at reflux (60 ° C) for 30 min (Zanardi *et al*. 2008). After that, cool the solution at room temperature add 25 mL of distilled water and shake. Titrate with 0.1 mol/L sodium thiosulfate solution, using 1 mL starch solution. The indicator should be added towards the end of the titration but while the pale straw color is still present. During titration shake until the blue color disappears. Carry out a blank titration under the same conditions.

For dark coloured products the determination of the peroxide value should be performed by potentiometric titration.

6.2. Calculation of peroxide values

$$PV = \frac{1000 \cdot (V_1 - V_0) \cdot c}{m}$$

PV = peroxide value mEq/kg
V_1 = consumption of 0.01 mol/l or 0.1 mol/l sodium thiosulfate solution in the main test
V_0 = consumption of 0.01 mol/l or 0.1 mol/l sodium thiosulfate solution in the blank test
c = molar concentration (molarity) of the sodium thiosulfate solution
m = weighed portion of substance in grams

6.2.1 Conversion of unit

The peroxide values is usually expressed in milliequivalents (meq) of active oxygen per kilogram of oil, but it may also be expressed (in SI units) as millimoles (mmol) of active oxygen per kilogram of oil. The values expressed in millimoles of active oxygen per kilogram is half that expressed in milliequivalents of active oxygen per kilogram. Multiplication of the peroxide values (meq of active oxygen per kg) by the equivalent mass of oxygen (equivalent 8) gives the milligrams of active oxygen per kilogram of oil.

From meq/kg to mmol/L
mmol/L = meq/kg · mmol/2 meq · 1 kg/1000 g · g/L (density) · 1000 mL/L

mmol/L = (meq/kg / 2) / density

Conversion from mmol/L to %
grams peroxide/L = mmol/L · 1 mol/1000 mmol · g/mol (MW of peroxide group 32 g/mol)

% = grams peroxide/L · L/1000 mL · mL/g (inverse density) = grams peroxide/grams product · 100

$\% = (mmol/L \cdot MW) / (density \cdot 10^4)$

6.3 Calibration and Standardization

Accuracy is difficult to evaluate and reproduce, because an adequate standard substance is hard to obtain due to:

- legal restrictions in many countries
- lack of reliable purity information for peroxides
- lack of stability of peroxides

In the International Standard ISO 3960 (2007), third edition, the international collaborative tests with some fatty oils have provided information on the repeatability.
At a level of 2 mEq/kg to 20 mEq/kg (4 to 40 mmol/kg), the relative standard deviation ranges from 4.08% to 11.53%. For high level of peroxide (e.g. ~3000 mEq/kg) the variation expected will be between (210-360) mEq/kg, or a relative standard deviation ranges from ~7 % to 12 %.

6.4 Interlaboratory test

Inter laboratory test in terms of precision of the method is expected of approximately 14% of reproducibility in term of relative standard deviation. This is according the data described in ISO-Standard 3960 (2007).

6.5Repeatability

The absolute differences between two independent single test results, obtained with this same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will be lower of 5%. This is according the data described in ISO-Standard 3960 (2007).

6.6 Reproducibility

The absolute difference between two single results, obtained with this same method on identical test material in different laboratories by different operator using deferent equipment, will be lower than 5 %. This is according the data described in ISO-Standard 3960 (2007).

7. Data Reporting

The test report should specified:

a) all information necessary for the complete identification of the sample
b) the sampling method used, if known
c) the test method used, with reference to this SOP
d) all operating details not specified in this SOP or regarded as optional, together with details of any incidences that may have influenced the test result(s)
e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.
f) where or not user has chosen a smaller samples mass

As the sample mass influence the result, this shall be reported together with the result.

8. References

8.1 SOP References

ISCO3/QAU/01/03. Madrid Declaration on Ozone Therapy 2015-2020 Eng. Schwartz-Tapia A, Martínez-Sánchez G, Sabah F, Alvarado-Guémez F, Bazzano-Mastrelli N, Bikina O, Borroto-Rodrígez V, Cakir R, Clavo B, González-Sánchez E, Grechkanev G, Najm Dawood A H, Izzo A, Konrad H, Masini M, Peretiagyn S, Pereyra, V R, Ruiz Reyes D, Shallenberger F, Vongay V, Xirezhati A, Quintero-Marino, R. **Madrid Declaration on Ozone Therapy**. 2th ed. Madrid: ISCO3; ISBN 978-84-606-8312-4; 2015. 50 p.

8.2 Other References

Council of Europe (2007).European Pharmacopoeia. European Directorate for the Quality of Medicines & Health Care. Technical guide for the elaboration of Monographs on fatty oils and derivatives and some polymers and solvents.67075 Strasbourg Cedex, France.

Council of Europe.(2010). *European Pharmacopoeia 7th Edition*, Druckerei C. H. Beck, ISBN 978-92-871-9700-2, Nördlingen, Germany. Method: 2.5.5. Peroxide Value.

IFRA (International Fragrance Association). Analytical Method: Determination of the Peroxide Value (October 17th, 2011). https://www.google.it/search?q=iodometric+titration+peroxide+value&ie=utf-8&oe=utf-8&gws_rd=cr&ei=mpifVsyKLYSMPpD5pvAJ

ISO-Standard 3960 third edition 2007. Animal and vegetable fats and oils — Determination of peroxide value — Iodometric (visual) endpoint determination.

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9. Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
ISCO3/LAB/00/04	02/02/2016	Draft under review	First version
	15/03/2016	Final version	Version 1

10. Document Records

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