ISCO3/LAB/00/03 Physico-chemical characterization of ozonized oil. Iodine Value

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Title

ISCO3/LAB/00/03 Iodine Values in Ozonized Oils

1.1. Brief background

The iodine value (IV) is a measure of the total number of double bonds present in the sample. It represents the quantity of iodine (in grams) that will react with the double bonds in 100 g of sample.[1] In other words, IV is the number that express in grams the quantity of halogen, calculated as iodine, that can be fixed in the prescribed conditions by 100 g of the substance.[2] The IV is one of the quality indicator to take into consideration during the quality analysis of an ozonized oil. During ozonization process, the IV decrease, compared to the values of the original non ozonized oil. E.g. Sesame Oil 113.65 ± 1.50 g/100 g (non ozonized), ozonized during 120 min 13.90 ± 0.50 g/100 g.[1]

1.2. Purpose

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

1.3. Scope

This procedure specifies the analytical procedure, chemical and devises to assay the iodine value using a titrimetric method (visual endpoint). The method is basically the procedure describes in the *European Pharmacopoeia* (7th ed), Method A.[2]

1.4. Acronyms, abbreviations and definitions

IV	Iodine Value: is the number that express in grams the quantity of halogen, calculated as iodine, that can be fixed in the prescribed conditions by 100 g of the substance
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

Iodine value method is reproducible only if the exact conditions of the test are carefully followed. Any changes in strength of reagent, sample size or reaction time may produce varying results.

3.2 Principle of Detection

The exact specified weight of the sample is accurately weighed into a glass stoppered iodine flask, and dissolved in chloroform. The measured volume of Hanus reagent is accurately added and after thorough mixing, is placed in the dark for exactly 30 min. A corresponding reagent blank is simultaneously prepared.

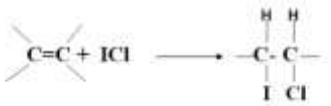
At the end of the specified time, the reaction is stopped by adding potassium iodide and diluting with water to prevent loss of the free iodine. The amount of iodine present is determined by titrating with sodium thiosulfate using starch indicator. The difference between a reagent blank titration and the titration of the test sample represents the amount of iodine absorbed by the sample. The iodine value is calculated as grams of iodine per 100 g of sample.

Hanus reagent is a solution of iodobromide in concentrated acetic acid. The iodine combines with double bonds slowly under these conditions. A large excess of the halogens must be present to complete the reaction. At the end of the reaction the unconsumed iodine should be greater than 60 % of the total. If the sample titration is less than 60 % of the blank titration, take a smaller sample and repeat the analysis.

3.3 Reaction Scheme

The reaction scheme is as follow:

Fatty acids react with a halogen [iodine] resulting in the addition of the halogen at the C=C double bond site. In this reaction, iodine monochloride reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine.



After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product.

$$ICl + KI \rightarrow KCl + I_2$$

This causes the remaining unreacted ICl to form molecular iodine. The liberated I_2 is then titrated with a standard solution of 0.1N sodium thiosulfate.

Titration step: I_2 (purple) + 2 $Na_2S_2O_3$ → $Na_2S_4O_6$ + 2NaI (colorless)

Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is an oil. Starch is used as the indicator for this reaction so that the liberated iodine will react with starch to give purple colored product and thus the endpoint can be observed.

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.
Class A volumetric 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.
Starch p. a. (e. g. soluble acc. to Zulkowsky)
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)
Hanus Solution, A.O.A.C.
Formic acid. p.a.

4.2.1 Potassium iodide 10 % solution

Dissolve 10 g of potassium iodide in 100 mL g of freshly boiled water at room temperature. The solution has to be kept protected from light (use amber glassware).

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.

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 Iodine values shall be taken first and the Iodine 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.

Iodine monochloride is caustic. So handle the reagent with gloves.

5.2 Sample quantity according to the IV

For more accuracy the quantity of sample will depend of the expected IV (see table for details). Accurately weigh the sample into a tared 250 mL iodine flask. Determine the sample size from the following table. Accurately record weight to the nearest mg.

Expected IV	Mass of substance to be examined (g)
< 20	1.0 ± 0.1
20 - 60	(0.5 - 0.25) ± 0.1
60 - 100	(0.25 - 0.15) ± 0.1
> 100	(0.15 - 0.10) ± 0.1

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. 0.15 g (0.1 - 0.2) g of the sample, accurately weighed, into a 200 mL Erlenmeyer flask with glass stopper (Erlenmeyer flak will be previously dried or rinsed with glacial acetic acid). Add 5 mL of the solvent: chloroform. Add very slowly 15 mL of Hanus Reagent (iodine monobromide, dissolved in glacial acetic acid). Close the flask and keep in the dark for 30 min, shaking frequently. Add 10 mL of a 100 g/L solution of potassium iodide and 100 mL of water. Titrate with 0.1 mol/L sodium thiosulfate solution, shaking vigorously until the yellow colour is almost discharged. Add 1 mL starch solution and continued the titration adding the 0.1 M sodium thiosulfate dropwise until the color is discharged (V1 mL of 0.1 M sodium thiosulphate). Carry out a blank titration under the same conditions (V2 mL of 0.1 M sodium thiosulphate).

6.2. Calculation of Iodine Value

IV) = [(V2– V1)·C· 12,69)]/m

IV = Iodine value g/100 g
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 (0.1 M)
m = weighed portion of substance in grams

6.2.1 Unit

IV is expressed in gram per 100 g of sample.

6.3 Calibration and Standardization

Test should be done.

6.4 Interlaboratory test

Inter laboratory test in terms of precision of the method is expected of approximately 10 % of reproducibility in term of relative standard deviation.

6.5 Repeatability

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 %.

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 %.

7. Data Reporting

The test report should specify:

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

1. Zanardi I, Travagli V, Gabbrielli A, Chiasserini L, Bocci V. Physico-chemical characterization of sesame oil derivatives. Lipids. 2008 Sep;43(9):877-86.
2. Europe. C. European Pharmacopoeia 7th Edition, Druckerei C. H. Beck, ISBN 978-92-871-9700-2, Nördlingen, Germany. Method: 2.5.5. Iodine Value. European Pharmacopoeia, 2010:137-8.

9. Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
ISCO3/LAB/00/03	12/07/2016	Draft under review	First version
	03/10/2016	Final version. Minor edition corrections	Version 1

10. Document Records

	Name	**Title**	**Signature**	**Date**
Author	Gregorio Martínez-Sánchez. Ph.D. E.mail: gregorcuba@yahoo.it	Member ISCO3		12/07/2016
Reviewers				
Authoriser / Approved	ISCO3 Board and members 2015-2020	All members		03/10/2016