ISCO3/LAB/00/02 Physico-chemical characterization of ozonized oil. Acid Values

Index

Title

ISCO3/LAB/00/02 Acid Values in Ozonized Oils

1.1. Brief background

The acid value (AV) the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance.[1]

1.2. Purpose

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

1.3. Scope

This procedure specifies the analytical procedure, chemical and devises to assay the acid value using a titrimetric method (visual endpoint). The method is basically the procedure describes in the *European Pharmacopoeia* (7th ed), Method A.[1]

1.4. Acronyms, abbreviations and definitions

AV	Acid Values: The number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance.
FFA	Free fatty acids
KOH	Potassium hydroxide
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 acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds and it is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. A known amount of sample dissolved in organic solvent, is titrated with a solution of potassium hydroxide (KOH) with known concentration and with phenolphthalein as a colour indicator.

Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid. Edible oil contains > 1%. Pharmaceutical oil must not have any acidity. The values of AV in ozonized oil vary according to the manufacture.

3.2 Principle of Detection

The acid value is determined by directly tritrating oil/fat in alcoholic medium against standard potassium hydroxide.

Acid 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.

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:
Erlenmeyer flask, 50 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 50 mL, 250 mL, 500 mL and 1 L capacity.
Amber stained bottles (for solutions) or amber glassware.

4.2 Reagents

Ethanol 96 % p. a.
Toluene (Methylbenzene) p.a.
Phenolphtalein solution 1 % in ethanol. R1. 1063703 *Eur Pharmacopoeia*
Potassium hydroxide solution 0.1 N (2.805 g of KOH in 500 mL of ethanol)
HCL 1 M solution
Water (demineralized, boiled and cooled down to 20 °C)

4.2.1 Potassium hydrogen solution 0.1 N

Dissolve 2.805 g of potassium hydroxide in 500 mL of ethanol 96% p.a. Standardized the solution as fallow: Take 20 mL add 5 drops of Phenolphtalein solution 1 %, tritrating with HCL 1 M solution. Calculate the correction factor (Cf) as follow:

Cf = Volume of HCL used · Cf of HCl

The solution should be colorless and has to be kept protected from light (use amber glassware).

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 ºC 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 AV shall be taken first and the AV 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.

Handle the reagent with gloves.

5.2 Sample quantity according to the AV

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

Expected AV	Mass of substance to be examined (g)	Accuracy of weighing of test portion (g)
< 1	20	0.05
1 - 4	10	0.02
4 - 15	2.5	0.01
15 - 75	0.5	0.001
> 75	0.1	0.0002

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. 1 g (0.9 - 1.2) g of the sample, accurately weighed (or fit the weight according to table in 5.2), into a 50 mL Erlenmeyer flask with glass stopper (Erlenmeyer flak will be previously dried or rinsed with ethanol). Add 5 mL of the solvent: toluene. Close the flask and shake. Add 5 drops of Phenolphtalein solution 1 %. If necessary, heat to about 90 °C to dissolve the substance to be examined. Titrate with 0.1 N potassium hydroxide in ethanol solution, shaking vigorously until a light pink color appears (end point) at least 15 s. If the used volume of KOH is less than 0.5 mL, the test should be repeated using more quantity of sample. When heating has been applied to aid dissolution, maintain the temperature at about 90 °C during tritation. Test should be repeated at least 3 times.

6.2. Calculation of Acid Value

Acid Values = (mL of KOH · N · 56.1) / weight of sample (g)

% Free Fatty Acid (FFA) = AV · 0.503

AV = Acid value mg of KOH
N = (Normality of KOH, 0.1 N · Cf)
Cf= Correction factor
mL of KOH (= mL consumed of 0.1 N KOH – mL of KOH consumed in the tritration of the blank)

The difference between the replicates shall not be greater than ±1 mg of KOH.

6.2.1 Unit

AV is expressed in mg of KOH.

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. 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/00/02	17/11/2016	Draft under review	Draft
	30/01/2017	Final version. Minor edition corrections	Version 1

10. Document Records

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Author	Gregorio Martínez-Sánchez. Ph.D. E.mail: gregorcuba@yahoo.it	Member ISCO3		17/11/2016
Reviewers				
Authoriser / Approved	ISCO3 Board and members 2015-2020	All members		30/01/2017