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DETERMINATION OF PHOSPHORUS IN OILS AND FATS BY DIRECT GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

Results of a collaborative study and the standardized method

Prepared for publication by

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Determination of phosphorus in oils and fats by direct graphite furnace atomic absorption spectrometry: results of a collaborative study and the standardized method

Abstract - A description is given of the development by collaborative study of a standardized method for the determination of phosphorus in crude and refined oils and fats by direct graphite furnace atomic absorption spectrometry. The procedure is rapid and allows determination at levels of 1 - 100 mg/kg.

INTRODUCTION

The determination of phosphorus in edible oils and fats is considered an important parameter for the desliming of crude edible oils and fats. The commonly used methods found in many compilations of standard methods are based on colorimetric determination of phospho(vanado)molybdic complexes (1).

In the oils and fat industry a rapid, accurate and standardized method for the determination of phosphorus is very important for quality control. The classical methods are rather time consuming due to the ashing procedures involved and therefore not very suitable for monitoring the refining process. Graphite furnace atomic absorption spectrometry (GFAAS) using a phosphorus electrodeless discharge lamp has been developed and tested by means of a collaborative study.

METHOD OF ANALYSIS

The method studied in the collaborative study reported is based on earlier work (2). With reference to this work on the determination of phosphorus in oils and fats, an organic matrix modifier is used to enhance the phosphorus signal and to minimize differences in sensitivity between the various phosphatides present in the edible oil.

Comparable results have been found using an electrodeless discharge lamp and a hollow cathode lamp, although in the latter case the precision is not as good. Both atomization 'off the wall' and 'off the platform' have been used. The mode of atomization has no influence on the results of the GFAAS determination.

COLLABORATIVE STUDY

In order to check the validity of the method as an international standard method for IUPAC and eventually for ISO[1], the method has been subjected to an international collaborative study by laboratories worldwide. The method studied took into account that various types of equipment of graphite furnaces can be used either with or without platform.

Materials provided for the study were edible oils (sunflower oil) containing phosphorus at three concentration levels (high, medium and low). Each concentration level was represented by two batches. Each sample was provided in duplicate (blind coded) so that participants received in total 12 samples. Participants were asked to analyze each sample in duplicate and to report both values obtained. A statistical evaluation of the data was made for each level and for each type of sample separately in accordance to ISO 5725 - 1986 using a method published earlier (3).

RESULTS

Data screening. From 18 laboratories data have been received for evaluation. The data from these laboratories have been subjected to tests for outliers according to Cochran and Dixon.

[1] International Organisation for Standardization

Cochran tests As the 12 samples analyzed were in fact 6 pairs of corresponding samples, the differences between these blind (hidden) duplicates have been tested for stragglers and outliers according to Cochran's procedure.

Dixon tests After elimination of the significant outliers (i.e. significant at the 1% level) according to Cochran's test, the six batch averages per laboratory have been tested for stragglers and outliers according to Dixon's procedure.

The data from one laboratory displayed so many deviations that it has been decided to omit the data from this laboratory from further evaluation. All other data have been maintained in the evaluation after discarding the outliers. Hence, for the final calculations of repeatability and reproducibility there remained a total of 17 laboratories.

Precision

In Table 1 the batch averages at each concentration level in liquid oil have been listed. Averages per concentration level have been calculated because the results of two batches at one concentration level are close enough to each other to be representative of the same concentration.

When the values for r (repeatability) and R (reproducibility) as given in table 1 are expressed as functions of their corresponding mean concentration values (m), the following equations are obtained:

Phosphorus in edible oil: $r = 0.23\ m$ and $R = 0.33\ m$

Table 1 Concentration levels, average recoveries (mg/kg) and precision parameters [17 accepted laboratories]

Concentration level	Actual values	Averages		Repeatability			Reproducibility		
	Batch	Batch	Level	s_r	r	CV_r(%)	s_R	R	CV_R(%)
High	30 27	30.42 28.63	29.53	2.30	6.45	7.8	3.51	9.82	11.9
Medium	20 18	20.71 19.29	20.00	1.68	4.71	8.4	2.33	6.53	11.7
Low	8 10	8.45 11.90	10.18	0.91	2.56	9.0	1.24	3.48	12.2

s_r: repeatability standard deviation; r: repeatability limit; CV_r: repeatability coefficient of variation; s_R: reproducibility standard deviation; R: reproducibility limit; CV_R: reproducibility coefficient of variation

DISCUSSION

From the equations $r = 0.19\ m$ and $R = 0.30\ m$ describing the precision of the phosphorus concentration as linear functions it can be concluded that the straight lines corresponding with these equations pass through the origin.

The relative repeatability (the coefficient of variation) does not depend on the level, but the relative reproducibility decreases more or less linearly with the phosphorus concentration. According to Horwitz (4) for an analytical method to be acceptable, the relative reproducibility (CV_R) should be 11.3 % at the 10 mg/kg level, 10.2 % at the 20 mg/kg level and 9.9 % at the 30 mg/kg level. The range of this criterion is reasonably well met by the results in this study.

CONCLUSION

Direct graphite furnace atomic absorption spectrometry is a rapid and sensitive method which allows reliable determination of the total concentration of traces of phosphorus in edible oils and fats.

After an extensive collaborative study it was concluded that the method meets the criterion for an analytical method to determine trace amounts of analyte as stated by Horwitz in 1982.

Based on the repeatability and reproducibility of the results obtained in the collaborative study the Commission has decided to adopt the method. The text of the standardized procedure is given on the following pages.

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2.423 DETERMINATION OF PHOSPHORUS BY DIRECT GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of trace amounts (mg/kg) of phosphorus in all types of crude or refined edible oils and fats.

2. PRINCIPLE

Vaporisation of the oil or fat in a suitable graphite furnace with or without a platform connected to an atomic absorption spectrometer, previously calibrated using standard solutions of soya lecithin and the measurement of the phosphorus content from the observed absorption at a wavelength of 213.5 nm.

3. APPARATUS

3.1 Test tubes - 10 ml.
3.2 Micropipettor - 20 µl.
3.3 Pipettor tips.
3.4 Electric oven - Regulated at 60 ± 2 °C.
3.5 Atomic absorption spectrometer. Equipped with either "peak height" mode and printer, or "continuous" mode and pen recorder (full scale response in 0.2 sec.) together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector (or Zeeman atomic absorption spectrometer).
3.6 Graphite furnace atomizer - Placed in the atomic absorption spectrometer (3.5), equipped with a control unit for temperature programming.
3.7 Graphite tube - Normal (uncoated).
3.8 Platform - Pyrolytic, in combination with uncoated or pyrolytically coated graphite tube (see Note 2).

4. REAGENTS

4.1 Cyclohexane - Analytical grade.
4.2 Lanthanum organometallic standard. A suitable standard (Conostan, 5000 mg/kg) is available from Continental Oil Company, Ponca City, Oklahoma, U.S.A.
4.3 Matrix modifier (note 1) - 0.05 % (m/v) Lanthanum solution is prepared by dissolving 5 g lanthanum standard (4.2) in 50 ml cyclohexane (4.1).
4.4 Blank oil - Refined liquid edible oil with a phosphorus content not greater than 1 mg/kg. (note 3)
4.5 Lecithin - A well-defined lecithin containing 2% phosphorus, e.g. soya lecithin. (note 3)
4.6 Standard stock solution - A stock solution of 400 mg P/kg is prepared by dissolving 1 g of lecithin (4.5) in 4 g cyclohexane (4.1) and 45 g of blank oil (4.4).
4.7 Standard working solutions - 10 mg P/kg, 20 mg P/kg and 40 mg P/kg are prepared by diluting the 400 mg/kg stock solution (4.6) with blank oil (4.4).
4.8 Argon, purity 99.99% minimum.

5. PROCEDURE

5.1 Treatment of samples, blank and standards

5.1.1 Place all samples, blank oil and standard working solutions in the oven (3.4), regulated at 60 ± 2 °C.
5.1.2 Shake samples vigorously.
5.1.3 Weigh 1.00 g sample in a 10 ml test tube together with 1.00 g matrix modifier (4.3) and mix thoroughly. (note 4)
5.1.4 Carry out instructions 5.1.2 - 5.1.3 also for the three standard working solutions (4.6) and the blank oil (4.4).

5.2 Preparation of apparatus

5.2.1 Switch on the atomic absorption spectrometer and the background correction (Deuterium Zeeman).

5.2.2 In accordance with the manufacturer's instructions supplied with the spectrometer, adjust: lamp current, slit, wavelength and amplification. The required wavelength is 213.5 nm.

5.2.3 Optimize the position of the graphite furnace atomizer (3.6) in the atomic absorption spectrometer (3.5) and set the required programme on the control unit of the furnace. If available, place platform in graphite tube.

5.2.4 Pretreat before each injection, the pipettor tip (3.3) by pipetting and then discarding 20 µl cyclohexane.

5.2.5 Inject 20 µl of the standard working solution with a content of 40 mg P/kg (4.7) with the micropipettor (3.2) into the graphite furnace, initiate the temperature programme and record the absorption.

5.2.6 Repeat instruction 5.2.5 until the absorption is constant. (note 5)

Programme for the graphite furnace atomizer (see note 6):

Step	Temp. (° C)	Ramptime (s)	Holdtime (s)	Int. Gasflow (ml/min)
1	600	40	20	300
2	1600	50	40	300
3	2800	0	5	0
4	2800	1	3	50

5.3 Determination

5.3.1 Measurement of the graphite tube blank - Record the absorption, if any, of the graphite tube as such and autozero this absorption.

5.3.2 Measurement of the blank - Inject 20 µl of the blank solution prepared according to 5.1.4 into the graphite furnace, initiate the temperature programme and record the absorption.

5.3.3 Measurement of the working standards - Inject 20 µl of the three standard solutions prepared according to 5.1.4 into the graphite furnace and record the absorptions.

5.3.4 Measurement of sample solutions - Inject 20 µl of the sample solution prepared according to 5.1.3 into the graphite furnace, initiate the temperature programme and record the absorption.

6. CALCULATION AND EXPRESSION OF RESULTS

6.1 Calculation

6.1.1 Measure the peak height on the recorder-chart or take the reading of the display or printer.

6.1.2 Draw a calibration curve by plotting the absorption of the three standards (5.3.2), corrected for the blank (5.3.1), against their respective phosphorus content (note 3).

6.1.3 Measure the absorption of the sample and correct for the blank.

6.1.4 Read the phosphorus content of the sample from the calibration curve.

6.2 Expression of results

Express the results as mg/kg (to two significant figures)

7. QUALITY ASSURANCE

7.1 For general principles of analytical quality control see the section on Quality Assurance in the introductory part of the Compendium of the Standard Methods

7.2 For specific applications of analytical quality control see the Annexe to this standard method.

8. NOTES

1. The amount of phosphorus found depends on the types of phosphatide present in the oil. The addition of lanthanum proved to be essential to find the total amount of phosphorus. Instead of lanthanum calcium also may be used.

2. Both atomization off the wall and atomization off the platform can be used. It is not necessary to change the temperature programme for the graphite furnace.

3. The concentration of phosphorus in the blank oil and in the lecithin is determined with method 2.421 (1).

4. If the expected or found concentration of phosphorus is higher than 40 mg/kg, dilute the sample with blank oil (4.4). In that case multiply the observed concentration (5.3.4) with the dilution factor

5. With a new graphite tube three to four 'determinations' with one and the same working standard have to be carried out in order to obtain an acceptable 'state of equilibrium'.

6. For those having a Varian apparatus the following temperature programme should be used:

Step No.	Temp. (°C)	Time (s)	Gasflow (l/min)
1	120	20	3.0
2	120	20	3.0
3	500	30	3.0
4	500	10	3.0
5	1600	50	3.0
6	1600	20	3.0
7	1600	2	0.0
8	2800	1	0.0
9	2800	2	0.0
10	2800	5	3.0
11	40	20	3.0

APPENDIX

1. Repeatability limit

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than the repeatability limit (r) as calculated from the formulae in Table 1.

Table 1 repeatability (r) and reproducibility limits (R)

P in edible oil	$r = 0.19\ m$	$R = 0.30\ m$

Key: m = corresponding mean concentration value.

2. Reproducibility limit

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit (R) as calculated from the formulae in Table 1.

3. **Trueness (bias).** The bias of the method was demonstrated in the collaborative study of the method (see table of statistical data below) to be negligible when used for the determination of concentration levels of phosphorus in the range 10 - 30 mg/kg.

4. The sensitivity of the method is demonstrated by the low values for r and R at the low absorption levels studied (see table of statistical data below), the limit of detection is 0.1 mg/kg, the limit of determination is 1 mg/kg.

Interference by other elements is not to be expected provided the measurements are carried out at the wave length specific for phosphorus (213.5 nm).

5. Statistical and other data derived from the results of the interlaboratory test

The interlaboratory test carried out at the international level in 1989 by the IUPAC Commission on Oils, Fats and Derivatives, in which 21 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with ISO 5725-1986) summarized in the following table:

Sample		Sunflower oil	
Batch	A (high)	B (medium)	C (low)
Number of laboratories retained after eliminating outliers	17	17	17
Number of outliers (laboratories)	2	1	1
Number of accepted results	32	34	34
Mean value (mg/kg sample)	29.55	20.00	10.18
True or accepted value (mg/kg)	28.50	19.00	9.00
Repeatability standard deviation (S_r in mg/kg)	2.30	1.68	0.91
Repeatability relative standard deviation (CV_r in %)	7.8	8.4	9.0
Repeatability limit (r) [2.8 x S_r]	6.45	4.71	2.56
Reproducibility standard deviation (S_R in mg/kg)	3.51	2.33	1.24
Reproducibility relative standard deviation (CV_R in %)	11.9	11.7	12.2
Reproducibility limit (R) [2.8 x S_R]	9.82	6.63	3.48

S_r: repeatability standard deviation; r: repeatability limit; CV_r: repeatability coefficient of variation; S_R: reproducibility standard deviation; R: reproducibility limit; CV_R: reproducibility coefficient of variation