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# DETERMINATION OF TOTAL AND 'FREE' HEXANE CONTENT IN EXTRACTION MEALS

## Results of a collaborative study and the standardised method

*Prepared for publication by*

A. HAUTFENNE[1], W. D. POCKLINGTON[2] and J. P. WOLFF[3]

[1]Université Catholique de Louvain, Unité des Procédés (PRCD), B-1348 Louvain-la-Neuve, Belgium
[2]Laboratory of the Government Chemist, Waterloo Road, London SE1 8XY, UK
[3]Institut des Corps Gras, 10/A, rue de la Paix, F-75002 Paris, France

*Membership of the Commission during the period of the collaborative study (1983–85) was as follows:

*Chairman*: M. Naudet (France); *Vice-Chairman*: A. Hautfenne (Belgium); *Secretary*: W. D. Pocklington (UK); *Titular Members*: Ö. Levin (Sweden); J. Pokorný (Czechoslovakia); H. Wessels (FRG); *Associate Members*: J. L. Beare-Rogers (Canada); A. Dieffenbacher (Switzerland); E. Fedeli (Italy); J. Gracian Tous (Spain); B. Jacobsberg (Belgium); E. Kurucz (Hungary); A. T. Møller (Denmark); F. H. Koelman (Netherlands); M. Teupel (FRG); A. Visapää (Finland); R. G. Manning (USA); G. Zwerenz (Austria); *National Representatives*: N. G. Bazan (Argentina); A. R. Johnson (Australia); R. Lago (Brazil); D. G. Chobanov (Bulgaria); A. P. Tulloch (Canada); G. Hølmer (Denmark); A. M. Gad (Egypt); R. Linko (Finland); J. P. Wolff (France); G. Ostermann (FRG); V. M. Kapoulas (Greece); N. V. Bringi (India); M. McCarthy (Ireland); A. Dolev (Israel); E. Tiscornia (Italy); T. Hashimoto (Japan); A. S. H. Ong (Malaysia); P. W. Hendrikse (Netherlands); F. B. Shorland (New Zealand); W. Zwierzykowski (Poland); R. Stoica (Romania); G. D. Carr (South Africa); M. Gassiot Matas (Spain); R. Ohlson (Sweden); H. Brüschweiler (Switzerland); R. Perin (Turkey); A. E. Waltking (USA); V. L. Rubajlo (USSR).

# Determination of total and 'free' hexane content in extraction meals: results of a collaborative study and the standardised method

***Abstract*** *- The development, by collaborative study, of a standardised method for the determination of total residual "hexane" content in extraction meals is described. The procedure involves the complete desorption of hydrocarbons by heating the moistened meal in a closed flask. The hydrocarbons content of the headspace is then determined by GLC using a capillary or packed column and expressed in terms of hexane content. If the extraction meal is not moistened, desorption of hexane into the headspace is incomplete. Conventionally the "free" hexane content is the quantity of hexane determined on the sample as such without preliminary moistening.*

## INTRODUCTION

*Hexane used industrially is, in reality, a mixture of volatile hydrocarbons. In oil-extraction plants the determination of this residual hexane in extraction meals is important in order to monitor the manufacturing process. It is obligatory, in many countries, to monitor the conformity of meals with the legislation on transportation, due to potential dangers resulting from the presence in the meals of an inflammable solvent which, with air, may form explosive mixtures.*

*The collaborative study on the determination of residual hexane in extracted meals showed that the re-humidification of the meal increased significantly the hexane released during the heating of the meal.*

*Consequently it is necessary to distinguish between total hexane and the hexane, referred to as "free hexane", releasable from the meal without re-humidification.*

## COLLABORATIVE STUDIES AND RESULTS

### Determination of total hexane content

*The method studied was derived from the H.P. Dupuy procedure (1,2). It involves the complete desorption of hexane by heating at 110°C the moistened meal in a closed flask and the "total residual hexane" is then determined in the headspace by GLC using a capillary or packed column.*

*The technique consists of introducing the sample into a glass vial, with the addition of an amount of water equivalent to 50% by weight of the sample, and crimp-sealing the vial with a septum. After heating the vial at 110°C for 90 min. the headspace is analysed by GLC.*

*Two international collaborative studies have been organised successively, in 1982 and 1983. The study in 1982 involved 12 participants who analysed three samples:*

- *rapeseed meal*
- *soyabean meal*
- *sunflowerseed meal*

*The soyabean and sunflowerseed meals contained less than 500 ppm hexane, the limit usually fixed by legislation on transportation. The rapeseed meal prepared in the experimental workshop of ITERG at Bordeaux-Pessac had a residual hexane content well above this limit. The statistical evaluation of the results from this first study is given in* ***TABLE I.***

*The study in 1983 involved 15 participants and three samples were analysed for their solvent content:*

- *soyabean meal*
- *rapeseed meal and*
- *the same rapeseed meal homogenised in the laboratory*

*The results of this second study are given in* ***TABLE II.*** *The statistical analysis was carried out in accordance with* ***ISO 5725*** *and is given in the* ***TABLE*** *included in the text of the method* ***1.171 Determination of total hexane content in extraction meals.***

*It was found that the homogenisation of the sample in the laboratory does not improve the reproducibility significantly. If the statistical analysis of results for this second collaborative study is limited to 9 laboratories which participated in the collaborative studies in 1982 and 1983, the variation coefficients of reproducibility are as follows:*

- *soyabean.....18%*
- *rapeseed.....20%*

*It can therefore be concluded that the poor reproducibility of the method derives more from the difficulty of procedure than the unavoidable heterogeneity of samples. As the residual hexane amounts required to be determined are low (generally about 0.05 - 0.1%), the reproducibility appeared to be adequate for adoption of the method given below.*

*During the collaborative study in 1983, 5 operators compared the proposed method with the DGF method (3) (determination of hexane by GLC headspace obtained from meal which had not been remoistened.) They obtained the results given as indicated in* ***TABLE III.***

***TABLE I. STATISTICAL ANALYSIS OF RESULTS FOR TOTAL HEXANE - COLLABORATIVE STUDY 1982***

| Sample | Rapeseed residue | Soyabean residue | Sunflower seed residue |
|---|---|---|---|
| Number of laboratories | 12 | 12 | 12 |
| Number of results | 24 | 24 | 24 |
| Number of laboratories used after elimination of outliers | 10 | 11 | 11 |
| Mean value (in mg/kg) | 971 | 400 | 450 |
| Repeatability standard deviation ($S_r$) | 39 | 19 | 22 |
| Repeatability coefficient of variation % | 4.0 | 4.6 | 4.8 |
| Repeatability value r ($S_r$ x 2.83) | 111 | 52 | 62 |
| Reproducibility standard deviation ($S_R$) | 289 | 83 | 125 |
| Reproducibility coefficient of variation % | 30 | 21 | 28 |
| Reproducibility value R ($S_R$ x 2.83) | 817 | 235 | 353 |

***TABLE II. RESULTS FOR TOTAL HEXANE (expressed in mg/kg) - COLLABORATIVE STUDY 1983***

| Sample: Lab. | Soyabean residue | Rapeseed residue | Homogenised rapeseed residue |
|---|---|---|---|
| 01 | 520 - 497 | 389 - 380 | 344 - 395 |
| 03 | 267 - 270 | 138 - 138 | 116 - 115 |
| 04 | 520 - 560 | 430 - 410 | 370 - 360 |
| 05 | 383 - 389 | 363 - 317 | 323 - 317 |
| 06 | 480 - 450 | 320 - 350 | 370 - 320 |
| 07 | 360 - 362 | 265 - 278 | 302 - 290 |
| 08 | 630 - 630 | 500 - 520 | 410 - 420 |
| 09 | 330 - 346 | 340 - 356 | 304 - 306 |
| 10 | 510 - 530 | 490 - 480 | 490 - 420 |
| 11 | 500 - 510 | 450 - 460 | 410 - 440 |
| 12 | 586 - 491 | 371 - 318 | 345 - 254 |
| 13 | 521 - 397* | 438 - 417 | 376 - 349 |
| 14 | 556 - 556 | 648 - 678 | 518 - 588 |
| 15 | 332 - 236 | 212 - 164 | 186 - 163 |
| 16 | 420 - 416 | 359 - 352 | 316 - 308 |
| Mean | 452 | 378 | 341 |

*a third determination gave 540

***TABLE III. RESULTS FOR RESIDUAL HEXANE (expressed in mg/kg) - INFLUENCE OF WATER ADDITION***

| Sample / Lab. | Soyabean residue | | Rapeseed residue | | Homogenised rapeseed residue | |
|---|---|---|---|---|---|---|
| | with water | without water | with water | without water | with water | without water |
| 01 | 520 - 497 | 400 - 380 | 389 - 380 | 290 - 280 | 344 - 395 | 280 - 290 |
| 02 | 380 | 240 | - | - | - | - |
| 03 | 267 - 270 | 252 - 257 | 138 - 138 | 130 - 126 | 116 - 115 | 124 - 134 |
| 08 | 630 - 630 | 220 - 220 | 500 - 520 | 240 - 260 | 410 - 420 | 260 - 260 |
| 12 | 586 - 491 | 365 - 340 | 371 - 318 | 240 - 228 | 345 - 254 | 256 - 246 |
| Mean | 474 | 301 | 346 | 224 | 299 | 231 |

*The comparison of mean values shows that the meal re-humidification increases significantly the content in hexane released during the heating of the meal. It is therefore necessary to distinguish between total hexane (as determined by the method studied in 1982 and 1983 and adopted by the Commission on Oils, Fats and Derivatives) and hexane, referred to as "free" hexane, releasable from meal without re-humidification.*

*Consequently, the Commission decided (in 1984) to extend the study of the total hexane determination to that of the determination of the "free" hexane.*

## Determination of 'free' hexane content

*The method studied was derived from the DGF procedure (3). It involves the desorption of hexane by heating at 80°C in a closed vial, after the addition of an internal standard, and the free" hexane is then determined in the headspace by GLC using a packed or capillary column.*

*The international collaborative study was organised in 1984, the study involving 13 participants who analysed 3 samples:*

- *1. fresh rapeseed meal (moisture: 8.8%)*
- *2. fresh rapeseed meal (sample 1 re-humidified to moisture 12.1%)*
- *3. stored sunflowerseed meal (moisture: 9.0%)*

*The results and the statistical evaluation of this analysis determined in accordance with ISO 5725 are given, respectively, in **TABLE IV** and in the **TABLE** included with the text of the method **1.172 Determination of "free" hexane content in extraction meals** which follows this report.*

*TABLE IV. RESULTS FOR "FREE" HEXANE (expressed in mg/kg) - COLLABORATIVE STUDY 1984*

| Sample | 1 | 2* | 3 |
|---|---|---|---|
| | Rapeseed meal (fresh) | Rapeseed meal (fresh) | Sunflower seed meal (stored) |
| Moisture: | 8.8% | 12.1% | 9.0% |
| Lab. | | | |
| 01 | 740 - 710 | 766 - 674 | 239 - 231 |
| 02 | 780 - 760 | 680 - 720 | 240 - 270 |
| 03 | 570 - 600 | 470 - 490 | 160 - 170 |
| 04 | 510 - 490 | 450 - 500 | 180 - 200 |
| 05 | 410 - 450 | 440 - 480 | 110 - 130 |
| 06 | 43 - 41 | 423 - 517 | 146 - 155 |
| 07 | 710 - 710 | 600 - 600 | 170 - 150 |
| 08 | 626 - 607 | 604 - 605 | 75 - 74 |
| 09 | 631 - 616 | 650 - 640 | 174 - 198 |
| 10 | 460 - 420 | 490 - 500 | 140 - 160 |
| 11 | 814 - 814 | 825 - 813 | 262 - 262 |
| 12 | 650 - 650 | 740 - 690 | 180 - 190 |
| 13 | 436 - 467 | 452 - 456 | - |

*Sample 1 rehumidified to 12.1% moisture

*TABLE V. COMPARISON OF RESULTS FOR TOTAL AND "FREE" HEXANE - COLLABORATIVE STUDY 1984*

| Sample | 1 | | 2* | | 3 | |
|---|---|---|---|---|---|---|
| | Rapeseed meal (fresh) | | Rapeseed meal (fresh) | | Homogenised rapeseed meal (stored) | |
| Moisture: | 8.8% | | 12.1% | | 9.0% | |
| | Total | Free | Total | Free | Total | Free |
| Hexane means in mg/kg | 720 | 624 | 602 | 599 | 381 | 178 |

*Sample 1 rehumidified to 12.1%

*The reproducibility appeared adequate for the adoption of the method. The comparison of mean values given in **TABLE V** shows that "free" hexane is always lower than total hexane. The divergence is more significant as the moisture of the meal is lower. The determination of "free" hexane is only significant for a given percentage of moisture in the sample. This method is, consequently, more a routine method than a reference method.*

## CONCLUSION

*On the basis of the results the Commission decided to adopt the method for "free" hexane. As indicated above, the method for total hexane was adopted by the Commission in 1983. The texts of both standardised procedures are given in the following pages.*

# 1.171 *DETERMINATION OF TOTAL HEXANE CONTENT IN EXTRACTION MEALS*

## 1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of the total amount of volatile hydrocarbons, expressed generally in terms of hexane, remaining in oil-seed residues after extraction of the seed with hydrocarbon based solvents.

## 2. DEFINITION

The total residual hexane content in extraction meals is the quantity of volatile hydrocarbons determined by the present method and expressed in milligrams per kilogram (ppm) of oil-seed residue.

## 3. PRINCIPLE

Desorption of the residual hydrocarbons by heating the sample at 110°C with water in a closed vessel. After calibration, determination of the hydrocarbons contained in the headspace by gas chromatography using capillary or packed columns. Expression of the results as hexane.

## 4. APPARATUS

4.1 Septum vials, 50 - 60 ml capacity, all with the same volume to within 2%.

4.2 Septa suitable for vials (4.1) about 3 mm thickness, inert to hexane. (Note 1).

4.3 Metallic foil, eg. of aluminium.

4.4 Crimping pliers.

4.5 Syringe, 10 µl.

4.6 Gas syringe, graduated, 1 ml, preferably with valve.

4.7 Gas chromatograph with flame ionisation detector and integrator and/or recorder. If a capillary column is used, the apparatus shall be equipped with a 1/100 split injector.

4.8 Glass capillary column suitable for gas chromatograph (4.7), approximately 30 m long and 0.3 mm internal diameter, coated with methylpolysilsoxane (Note 2) (film thickness 0.2 µm) or, failing this, a packed column approximately 1.7 m long packed with 150 - 180 µm diatomaceous support acid-washed (Note 3) and coated with methyl-poly-siloxane (Note 2).

4.9 Electric oven regulated at 110°C.

## 5. REAGENTS

5.1 Technical hexane or a light petroleum with a composition similar to that used in industrial extraction, or failing these, n-hexane.

5.2 Carrier gas: hydrogen, nitrogen or helium, thoroughly dried and containing less than 10 mg/kg of oxygen.

5.3 Auxiliary gases: hydrogen, 99.9% pure, containing no organic impurities.
air containing no organic impurities.

## 6. SAMPLING AND SAMPLE STORAGE

It is essential that loss of solvent from the sample be prevented. The laboratory sample shall be in a completely sealed container (preferably in a crimped metal box) and shall be stored at - 20°C or below, for example in a deep-freezer.
Plastic containers shall not be used.
The determination of residual hydrocarbons shall be carried out as soon as the container - kept at + 4°C for 24 h before opening - has been opened.

## 7. PROCEDURE

### 7.1 Calibration curve

Add 6 ml water to a vial (4.1) (Note 4). Add rapidly a specific quantity of solvent (5.1) (Note 5) measured accurately by means of the syringe (4.5). Seal with a septum (4.2). Cover with a foil (4.3). Crimp with pliers (4.4). Place the vial in the oven (4.9) at 110°C for 15 min.
Warm the gas syringe (4.6) to 60°C.
Remove the vial from the oven and leave to cool for 2 min.
By means of the warmed gas syringe (4.6), take exactly 0.5 ml of the headspace and inject immediately into the chromatograph (4.7), with the injection and detection temperatures of the chromatograph (4.7) set at 120°C, the oven temperature set at 40°C and the carrier gas pressure at 0.3 bar.
Determine the peak areas obtained for the solvent.
Repeat this operation for different quantities of solvents (5.1) (Note 5). Determine the peak areas for the solvent in each case.
Plot the calibration curve expressing the relation between the solvent peak areas and the mass of solvent introduced into the vials (Note 6).

### 7.2 Test portion analysis

Weigh rapidly, to within 0.1 g, 5 g of the laboratory sample into a vial (4.1). Add 2.5 ml of distilled water. Seal the vial with the septum (4.2). Cover with foil (4.3). Crimp with pliers (4.4). Place the vial in the oven (4.9) at 110°C for exactly 90 min. (Note 7).
Warm the gas syringe (4.6) to 60°C.
Remove the vial from the oven. Leave to cool for 2 min.
Agitate by inverting. By means of the heated gas syringe, take exactly 0.5 ml of the headspace and inject immediately into the chromatograph (4.7), with the injection and detection temperatures set at 120°C, the oven temperature set at 40°C and the carrier gas (5.2) pressure at 0.3 bar. Determine the sum of the peak areas of hexane and various hydrocarbons which usually make up the technical solvents (Note 8). Determine from the calibration curve the corresponding quantity, $m_1$, of solvents present in the vial.

### 7.3 Number of determinations

Carry out two determinations in rapid succession.

## 8. EXPRESSION OF RESULTS

The total hexane content expressed in mg/kg (ppm) is given by the formula:

$$\frac{m_1}{m_o}$$

where:

$m_o$ is the mass, in g, of the test portion,

$m_1$ is the mass of solvents, in µg, of solvents present in the flask.

Report as the final result the mean of the results of the two determinations, provided the requirements for repeatability (9.1) are met. If the requirements for repeatability are not met, discard the results and carry out a further two determinations on the test sample.

## 9. PRECISION

The results of one interlaboratory test organized at an international level (for different samples) gave the statistical results shown in the **TABLE** in the **Appendix.**

### 9.1. Repeatability

When the mean of the duplicate determinations lies within the range of the mean values cited in the **TABLE** the difference between the results of the two determinations, carried out in rapid succession by the same operator, using the same apparatus for the analysis

of the same test sample, should not be greater than the **repeatability value (r)**, which can be deduced from the **TABLE**, for the level of hexane in the sample examined.

### 9.2 Reproducibility

When the means of the duplicate determinations, obtained in two different laboratories using this standard method for the analysis of the same laboratory sample, lie within the range of the mean values cited in the **TABLE**, the difference between the mean results obtained by those laboratories should not be greater than the **reproducibility value (R)**, which can be deduced from the **TABLE**, for the level of hexane in the sample examined.

## 10. NOTES

1. A material such as nitrile rubber, for example Perbunan, or butyl rubber with a polytetrafluoroethylene or polychloroprene seal, for example Neoprene, is suitable. These septa often have a very high mechanical resistance. It is therefore preferable to perforate them with a pin before taking the sample from the headspace and using this perforation so that the syringe needle is not damaged on insertion. Re-use of the septa is not recommended.

2. SE 30 is suitable.

3. Chromosorb WAW is suitable.

4. This volume is determined by the fact that the test portion (5 g) hydrated with water (2.5 ml) will occupy a volume of 6 ml.

5. Three additions, for example, with 2, 5 and 10 µl of solvent (5.1) are usually sufficient for constructing the calibration curve. They correspond respectively to 268, 670, 1340 mg/kg of solvent in a test portion of 5 g meal in the case of technical hexane and to 264, 660, 1320 mg/kg in the case of n-hexane.

6. It may be assumed that 1 µl of solvent introduced into the vials has a mass of 670 µg in the case of technical hexane, and 659 µg in the case of n-hexane.

7. It is important to leave the vials in the oven for the same length of time for each sample.

8. Hydrocarbons which usually make up the technical solvents are: 2-methylpentane, 3-methylpentane, methylcyclopentane, cyclohexane, etc., A typical chromatogram of these solvents is represented by Figure 1. Do not include peaks due to oxidation products. Some of these products may be present in significant amounts.

## APPENDIX

### STATISTICAL ANALYSIS OF RESULTS FOR TOTAL HEXANE

(Collaborative study 1983 - duplicate determinations)

| Sample | Rapeseed residue | Soyabean residue | Homogeneised rapeseed residue |
|---|---|---|---|
| Number of laboratories | 15 | 15 | 15 |
| Number of results | 30 | 30 | 30 |
| Number of laboratories after elimination of outliers | 15 | 15 | 15 |
| **Mean value (in mg/kg)** | **452** | **378** | **341** |
| Repeatability standard deviation ($S_r$) | 35 | 19 | 29 |
| Repeatability coefficient of variation % | 7.8 | 5.1 | 8.6 |
| **Repeatability value r** ($S_r$ x 2.83) | **100** | **54** | **83** |
| Reproducibility standard deviation ($S_R$) | 109 | 129 | 108 |
| Reproducibility coefficient of variation % | 24 | 34 | 32 |
| **Reproducibility value R** ($S_R$ x 2.83) | **308** | **365** | **305** |

# 1.172 DETERMINATION OF 'FREE' HEXANE CONTENT IN EXTRACTION MEALS

## 1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of the "free" volatile hydrocarbons, expressed generally in terms of hexane, remaining in oil-seed residues after extraction with hydrocarbons based solvents.

## 2. DEFINITION

The "free" hexane content in extraction meals is the quantity of volatile hydrocarbons determined by the present method and expressed in milligrams per kilogram (ppm) of oil-seed residue.

## 3. PRINCIPLE

Desorption of volatile hydrocarbons by heating at 80°C in a closed vessel after addition of an internal standard. After calibration, determination of these hydrocarbons in the headspace by gas chromatography using packed or capillary columns. Expression of the results as hexane.

## 4. APPARATUS

4.1 Septum vials, 20 ml capacity, all with the same volume to within 2%.

4.2 Septa suitable for vials (4.1) approximately 3 mm thickness, inert to hexane. (Note 1).

4.3 Metallic foil, eg. of aluminium.

4.4 Crimping pliers.

4.5 Syringe, 10 µl.

4.6 Gas syringe, graduated, 1 ml, preferably with valve.

4.7 Gas chromatograph with flame ionisation detector and integrator and/or recorder. If a capillary column is used, the apparatus shall be equipped with a 1/100 split injector system.

4.8 Packed columns suitable for gas chromatograph (4.7), approximately 2 m long, packed with 150-180 µm diatomaceous support, acid washed (Note 2) and coated with squalane (10%) or methylpolysiloxane (Note 3), or failing this, a glass capillary column approximately 30 m long and 0.3 mm internal diameter, coated with methylpolysiloxane (film thickness 0.2 µm) (Note 3).

4.9 Heating bath fitted with a rack to hold the sealed vials. The bath should be thermostatically controlled at a temperature of 80°C. For continuous use, glycerine is recommended as the heating liquid.

## 5. REAGENTS

5.1 Technical hexane or a light petroleum with a composition similar to that used in industrial extraction, or failing these, n-hexane.

5.2 n-Heptane, analytical reagent quality (Note 4).

5.3 Cyclohexane, analytical reagent quality (Note 4).

5.4 Carrier gas: Hydrogen, nitrogen or helium thoroughly dried and containing less than 10 mg/kg of oxygen.

5.5 Auxiliary gases: Hydrogen, 99.9% pure containing no organic impurities.
Air containing no organic impurities.

## 6. SAMPLING AND SAMPLE STORAGE

It is essential that loss of solvent from the sample be prevented. The laboratory sample shall be in a completely sealed container (preferably in a crimped metal box) and shall be stored at + 4°C.
Plastic containers shall not be used.
The determination of hydrocarbons shall be carried out as soon as the container has been opened.

## 7. PROCEDURE

### 7.1 Determination of the calibration factor (Note 5)

Weigh to the nearest 0.1 g, 5 g of a meal containing very low level of extraction solvent (Note 6) into each of 5 septum vials (4.1). Seal each vial with a septum (4.2) and an aluminium foil cap (4.3). Add, using the syringe (4.5), variable quantities of technical hexane (5.1) to 4 of 5 vials, according to the following table (Note 7); do not add solvent to one of the vials.

| Vial no. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| µl technical hexane added: | 1 | 2 | 4 | 7 |

Allow the sample vials to stand at room temperature for 24 hours (Note 8).

Then, add 5 µl of a n-heptane (5.2) or cyclohexane (5.3), as an internal standard, to each sample vial, making the addition with the syringe (4.5) through the septum. At 15 minutes intervals, place one of the sample vials in the heating bath, the heating medium coming up to the neck of each vial, and heat at 80°C for exactly 60 minutes.
Warm the gas-tight syringe (4.6) to 60°C.
Without removing the vial from the heating bath, withdraw about 1 ml of the gaseous phase from the headspace through the septum with the heated gas syringe. Inject the sample immediately into the chromatograph (4.7) with the injection and detection temperatures set at 120°C, the oven temperature set at 40°C, and the carrier gas (5.4) pressure at 0.3 bar (Note 9).

For each of the 4 vials, containing added technical hexane (5.1), a calibration factor, F, may be determined by the formula:

$$F = \frac{C_s \cdot A_i}{C_i \cdot (A_H - A_B - A_i)}$$

where:

$A_B$ is the peak area of the hydrocarbons present in the oil-seed residue, to which technical hexane has not been added, ie. in the oil-seed residue present in the 5th vial less the peak area of the internal standard (Note 10).

$A_H$ is the total peak area of the hydrocarbons including internal standard present in the oil-seed residue of the vial (Note 10).

$A_i$ is the peak area corresponding to the internal standard.

$C_i$ is the concentration of internal standard expressed in milligrams per kilogram of oil-seed residue. For an addition of 5 µg to 5 g sample, $C_i$ = 680 for n-heptane and $C_i$ = 780 for cyclohexane.

$C_s$ is the concentration of technical hexane added to the oil-seed residue present in the vial, expressed in milligrams per 1000 g of meal.
It is given by the following table:

| Vial no. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| concentration of technical hexane added in mg/1000 g: | 134 | 268 | 536 | 938 |

If n-hexane is used, $C_i$ is given by the following table:

| Vial no. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| concentration of technical hexane added in mg/1000 g: | 132 | 264 | 528 | 924 |

Calculate the mean calibration factor from the results obtained for the 4 vials. The mean calibration factor should be 1.0 $\pm$ 0.1 and the differences between the individual results should be small.

### 7.2 Test portion analysis

Weigh to the nearest 0.1 g, 5 g of the test portion into a vial (4.1). Seal it immediately with the septum (4.2). Cover with foil (4.3) and crimp with pliers (4.4). Inject 5 ul of the internal standard (5.2 or 5.3) (Note 4) into the vial through the septum using the syringe (4.5). Immerse the vial up to the neck in the heating bath (4.9) regulated at 80°C for exactly 1 hour.
Warm the gas-tight syringe (4.6) to 60°C.
Without removing the vial from the heating bath, withdraw about 1 ml of the gaseous phase from the headspace through the septum with the heated gas syringe. Inject the sample immediately into the chromatograph (4.7) with the injection and detection temperatures set at 120°C, the oven temperature set at 40°C and the carrier gas (5.4) pressure at 0.3 bar.

Determine the total peak area due to hydrocarbons (including the peak area of the internal standard) and the peak area due to the internal standard.

### 7.3 Number of determinations

Carry out two determinations in rapid succession.

## 8. EXPRESSION OF RESULTS

The "free" hexane content expressed in mg/kg (ppm) is given by the formula:

$$\frac{(A_H - A_i)}{A_i} \cdot F \cdot C_i$$

where:

- $A_H$ is the total peak area due to hydrocarbons (including the peak area of the internal standard). (Note 10).
- $A_i$ is the peak area due to the internal standard.
- $C_i$ is the concentration of the internal standard expressed in milligrams per kilogram of oil-seed residue.
  For an addition of 5 µg to 5 g sample, $C_i$ = 680 for n-heptane and $C_i$ = 780 for cyclohexane.
- F is the calibration factor determined according to 7.1.

Report as the final result the mean of the results of the two determinations, provided the requirements for repeatability (9.1) are met. If the requirements for repeatability are not met, discard the results and carry out a further two determinations on the test sample.

## 9. PRECISION

The results of one interlaboratory test organized at an international level (for different samples) gave the statistical results shown in the **TABLE** in the **Appendix**.

### 9.1. Repeatability

When the mean of the duplicate determinations lies within the range of the mean values cited in the **TABLE** the difference between the results of the two determinations, carried out in rapid succession by the same operator, using the same apparatus for the analysis of the same test sample, should not be greater than the **repeatability value** (r), which can be deduced from the **TABLE**, for the level of hexane in the sample examined.

### 9.2 Reproducibility

When the means of the duplicate determinations, obtained in two different laboratories using this standard method for the analysis of the same laboratory sample, lie within the range of the mean values cited in the **TABLE**, the difference between the mean results obtained by those laboratories should not be greater than the **reproducibility value** (R), which can be deduced from the **TABLE**, for the level of hexane in the sample examined.

## 10. NOTES

1. A material such as nitrile rubber, for example Perbunan, or butyl rubber with a polytetrafluoroethylene or polychloroprene seal, for example Neoprene, is suitable. These septa often have a very high mechanical resistance; in this case it is preferable to perforate them with a pin before taking the vapour from the headspace and using this perforation so that the syringe needle is not damaged on insertion. Re-use of the septa is not recommended.

2. Chromosorb WAW is suitable.

3. SE 30 is suitable.

4. If the hexane used for extraction or calibration contains appreciable amounts of cyclohexane, n-heptane should be used as the internal standard.

5. Commercial oil-seed residues usually have a water content of 12 to 14%. For samples having a different water content, the calibration has to be made with an oil-seed residue that has the same water content as the sample.

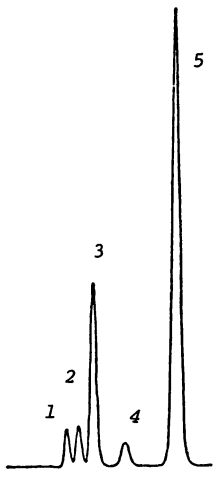
6. The meal used for the calibration should be of a similar nature as the sample. A meal can be used to determine the calibration factor if it contains only small amounts of extraction solvent (less than 0.01%). If it contains a greater amount of solvent, this should be reduced by leaving the meal in thin layers in the open for some hours in air.

7. When a sample to be tested has a high "hexane" content, the quantity of technical hexane added to vials 3 and 4 must be sufficiently high so that the levels of hexane contained in the meal of these vials corresponds to the high level of solvent expected in the sample.

8. In the case of shorter contact times, it cannot be certain that the extraction solvent is absorbed completely by the residue and that the absorption-desorption equilibrium of the extraction solvent between meal and air has been attained.

9. For routine analyses, an automatic sampler may be used.

10. Hydrocarbons which usually make up the technical solvents are: 2-methylpentane, 3-methylpentane, methylcyclopentane, cyclohexane, etc., A typical chromatogram of these solvents is represented by Figure 1. Do not include peaks due to the oxidation products. Some of these products may be present in significant amounts.




1: 2-Methylpentane

2: 3-Methylpentane

3: n-Hexane

4: Methylcyclopentane

5: Cyclohexane (internal standard)
(with other retention times: n-heptane)

**Figure 1** Gas chromatogram of hexane hydrocarbons from extraction meal

## APPENDIX

### STATISTICAL ANALYSIS OF RESULTS FOR "FREE" HEXANE

(Collaborative study 1984 - duplicate determinations)

| Sample | Fresh rapeseed meal | Fresh rapeseed meal | Stored sunflower seed meal |
|---|---|---|---|
| Moisture | 8.8% | 12.1%* | 9% |
| Number of laboratories | 13 | 13 | 12 |
| Number of results | 26 | 26 | 24 |
| Number of laboratories after elimination of outliers | 11 | 11 | 12 |
| **Mean value (in mg/kg)** | **624** | **599** | **178** |
| Repeatability standard deviation ($S_r$) | 17 | 33 | 12 |
| Repeatability coefficient of variation % | 2.7 | 5.5 | 6.7 |
| **Repeatability value r ($S_r$ x 2.83)** | **48** | **94** | **34** |
| Reproducibility standard deviation ($S_R$) | 129 | 125 | 55 |
| Reproducibility coefficient of variation % | 21 | 21 | 31 |
| **Reproducibility value R ($S_R$ x 2.83)** | **364** | **353** | **156** |

*Sample 1 (moisture 8.8%) adjusted to moisture level of 12.1%

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## REFERENCES

1. Dupuy H.P. and Fore S.P., J. Am. Oil Chem. Soc., 1970, 47, 231.

2. Prevot A. and Coustille J.L., Rev. Franc. Corps Gras, 1981, 28, 413

3. Arens M. and Kroll E., Fette-Seifen Austrichmittel, 1983, 85, 307