Commission Implementing Regulation (EU) 2016/1240 of 18 May 2016 laying down rules for the application of Regulation (EU) No 1308/2013 of the European Parliament and of the Council with regard to public intervention and aid for private storage (Text with EEA relevance)

ANNEX V

SKIMMED MILK POWDER

[[F1]A1.

This Annex does not apply in relation to public intervention schemes in England, otherwise than in connection with exceptional market conditions which are the subject of a declaration under section 20 of the Agriculture Act 2020.**]**

[[F2]A2. This Annex ceases to apply in relation to public intervention schemes in Scotland, otherwise than in connection with public intervention measures which the appropriate authority takes under Article 219(1) of Regulation (EU) No 1308/2013, for a period of five years beginning on 1 July 2023.**]**

Textual Amendments

F2 Annex 5 para. A2 inserted (S.) (1.7.2023) by The Public Intervention and Private Storage Aid (Amendment and Suspension) (Scotland) Regulations 2023 (S.S.I. 2023/150), regs. 1, **9(15)** (with reg. 10)

PART I

Sampling and analysis of skimmed-milk powder offered for intervention

1. Samples per lot shall be taken in accordance with the procedure laid down in International Standard ISO 707. However, paying agencies may use another method of sampling provided that it complies with the principles of that standard.

2. Number of packages to be selected for taking samples for analysis:

(a) lots containing up to 800 25-kg bags: at least eight;

(b) lots containing more than 800 25-kg bags: at least eight, plus one for each additional 800 bags or fraction thereof.

3. Weight of sample: samples of at least 200 g are to be taken from each package.

4. Grouping of samples: no more than nine samples are to be combined in a global sample.

5. Analysis of samples: each global sample is to undergo an analysis to verify all the quality characteristics laid down in Part II of Annex V to Delegated Regulation (EU) 2016/1238.

6. Where samples show defects:

(a) where a composite sample shows a defect with regard to one parameter, the quantity from which the sample came is rejected;

(b) where a composite sample shows a defect with regard to more than one parameter, the quantity from which the sample came is rejected and samples are taken from the remaining quantities from the same plant; the analysis of those samples shall be decisive. In that case:

— the number of samples laid down in point 2 is doubled,

— where a composite sample shows a defect with regard to one or more parameters, the quantity from which the sample came is rejected.

[F3PART IA

Methods of analysis of skimmed milk powder for public intervention

Parameter	Method
Protein	ISO 8968 part 1
Fat	ISO 1736
Water	ISO 5537
Acidity	ISO 6091
Lactates	ISO 8069
Phosphatase test	ISO 11816 part 1
Insolubility index	ISO 8156
Scorched particles [a]	ADPI
Micro-organisms	ISO 4833-part 1
Buttermilk	Appendix I
Rennet whey [b]	Appendix II and III
Acid whey [c]	ISO 8069 or On-the-spot inspections
Sensory checks [d]	ISO 22935 part 2 and 3

a Scorched particles' analyses may be conducted systematically. However, such analyses shall always be conducted if no sensory checks are performed.

b The method to be applied shall be approved by the paying agency (one or both methods).

c The method to be applied shall be approved by the paying agency.

d Sensory checks shall be performed where deemed necessary after risk based analysis approved by the paying agency.

Textual Amendments

F3 Inserted by Commission Implementing Regulation (EU) 2018/150 of 30 January 2018 amending Implementing Regulation (EU) 2016/1240 as regards methods for the analysis and quality evaluation of milk and milk products eligible for public intervention and aid for private storage.

Appendix I

SKIMMED MILK POWDER: QUANTITATIVE DETERMINATION OF PHOSPHATIDYLSERINE AND PHOSPHATIDYLETHANOLAMINE

Method: reversed-phase HPLC

1. PURPOSE AND FIELD OF APPLICATION

The method describes a procedure for the quantitative determination of phosphatidylserine (PS) and phosphatidylethanolamine (PE) in skimmed milk powder (SMP) and is suitable for detecting buttermilk solids in SMP.

2. DEFINITION

PS + PE content : the mass fraction of substance determined using the procedure here specified. The result is expressed as milligrams of phosphatidylethanolamine dipalmitoyl (PEDP) per 100 g powder.

3. PRINCIPLE OF THE METHOD

Extraction of aminophospholipids by methanol from reconstituted milk powder. Determination of PS and PE as o-phthaldialdehyde (OPA) derivatives by reversed-phase (RP) HPLC and fluorescence detection. Quantification of PS and PE content in the test sample by reference to a standard sample containing a known amount of PEDP.

4. REAGENTS

All reagents shall be of recognised analytical grade. Water shall be distilled or water of at least equivalent purity, unless otherwise specified.

4.1. **Standard material: PEDP, at least 99 % pure**

Note: Standard material shall be stored at – 18 °C.

4.2. **Reagents for standard sample and test sample preparation**

4.2.1. *HPLC-grade methanol*

4.2.2. *HPLC-grade chloroform*

4.2.3. *Tryptamine-monohydrochloride*

4.3. **Reagents for o-phthaldialdehyde derivatisation**

4.3.1. *Sodium hydroxide, 12 M water solution*

4.3.2. *Boric acid, 0,4 M water solution adjusted to pH 10,0 with sodium hydroxide (4.3.1)*

4.3.3. *2-mercaptoethanol*

4.3.4. *o-phthaldialdehyde (OPA)*

4.4. **HPLC elution solvents**

4.4.1. *Elution solvents shall be prepared using HPLC-grade reagents.*

4.4.2. *HPLC-grade water*

4.4.3. *Methanol of tested fluorimetric purity*

4.4.4. *Tetrahydrofuran*

4.4.5. *Sodium dihydrogen phosphate*

4.4.6. *Sodium acetate*

4.4.7. *Acetic acid.*

5. APPARATUS

5.1. **Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg**

5.2. **Beakers, 25 and 100 ml capacity**

5.3. **Pipettes, capable of delivering 1 and 10 ml**

5.4. **Magnetic stirrer**

5.5. **Graduated pipettes, capable of delivering 0,2, 0,5 and 5 ml**

5.6. **Volumetric flasks, 10, 50 and 100 ml capacity**

5.7. **Syringes, 20 and 100 µl capacity**

5.8. **Ultrasonic bath**

5.9. **Centrifuge, capable of operating at 27 000 × g**

5.10. **Glass vials, about 5 ml capacity**

5.11. **Graduated cylinder, 25 ml capacity**

5.12. **pH-meter, accurate to 0,1 pH units**

5.13. **HPLC equipment**

5.13.1. *Gradient pumping system, capable of operating at 1,0 ml/min at 200 bar*

5.13.2. *Autosampler with derivatisation capability*

5.13.3. *Column heater, capable of maintaining the column at 30 °C ± 1 °C*

5.13.4. *Fluorescence detector, capable of operating at 330 nm excitation wavelength and 440 nm emission wavelength*

5.13.5. *Integrator or data processing software capable of peak area measurement*

5.13.6. *A LiChrospher® — 100 column (250 × 4,6 mm) or an equivalent column packed with octadecylsilane (C 18), 5 µm particle size.*

6. SAMPLING

Sampling shall be carried out in accordance with ISO Standard 707.

7. PROCEDURE

7.1. **Preparation of the internal standard solution**

7.1.1. *Weigh 30,0 ± 0,1 mg of tryptamine-monohydrochloride (4.2.3) into a 100 ml volumetric flask (5.6) and make up to the mark with methanol (4.2.1)*

7.1.2. *Pipette 1 ml (5.3) of this solution into a 10 ml volumetric flask (5.6) and make up to the mark with methanol (4.2.1) in order to obtain a 0,15 mM tryptamine concentration*

7.2. **Preparation of the test sample solution**

7.2.1. *Weigh 1,000 ± 0,001 g of the SMP sample into a 25 ml beaker (5.2). Add 10 ml of distilled water at 40 °C ± 1 °C by a pipette (5.3) and stir with a magnetic stirrer (5.4) for 30 minutes in order to dissolve any lumps*

7.2.2. *Pipette 0,2 ml (5.5) of the reconstituted milk into a 10 ml volumetric flask (5.6), add 100 µl of the 0,15 mM tryptamine solution (7.1) using a syringe (5.7) and make up*

to the volume with methanol (4.2.1). Mix carefully by inversion and sonicate (5.8) for 15 min

7.2.3. *Centrifuge (5.9) at 27 000 g × g for 10 minutes and collect the supernatant in a glass vial (5.10)*

Note: Test sample solution should be stored at 4 °C until the HPLC analysis is performed.

7.3. **Preparation of the external standard solution**

7.3.1. *Weigh 55,4 mg PEDP (4.1) into a 50 ml volumetric flask (5.6) and add about 25 ml of chloroform (4.2.2) using a graduated cylinder (5.11). Heat the stoppered flask to 50 °C ± 1 °C and mix carefully till the PEDP dissolves. Cool the flask to 20 °C, make up to the volume with methanol (4.2.1) and mix by inversion*

7.3.2. *Pipette 1 ml (5.3) of this solution into a 100 ml volumetric flask (5.6) and make up to the volume with methanol (4.2.1). Pipette 1 ml (5.3) of this solution into a 10 ml volumetric flask (5.6), add 100 µl (5.7) of 0,15 mM tryptamine solution (7.1) and make up to the volume with methanol (4.2.1). Mix by inversion*

Note: Reference sample solution should be stored at 4 °C until the HPLC analysis is performed.

7.4. **Preparation of the derivatising reagent**

Weigh 25,0 ± 0,1 mg of OPA (4.3.4) into a 10 ml volumetric flask (5.6), add 0,5 ml (5.5) of methanol (4.2.1) and mix carefully to dissolve the OPA. Make up to the mark with boric acid solution (4.3.2) and add 20 µl of 2-mercaptoethanol (4.3.3) by syringe (5.7).

Note: The derivatising reagent should be stored at 4 °C in a brown glass vial and is stable for one week.

7.5. **Determination by HPLC**

7.5.1. *Elution solvents (4.4)*

Solvent A: Solution of 0,3 mM sodium dihydrogen phosphate and 3 mM sodium acetate solution (adjusted to pH 6,5 ± 0,1 with acetic acid): methanol: tetrahydrofuran = 558:440:2 (v/v/v)

Solvent B: methanol

7.5.2. *Suggested eluting gradient:*

Time (min)	Solvent A (%)	Solvent B (%)	Flow rate (ml/min)
Initial	40	60	0
0,1	40	60	0,1
5,0	40	60	0,1
6,0	40	60	1,0
6,5	40	60	1,0
9,0	36	64	1,0
10,0	20	80	1,0
11,5	16	84	1,0
12,0	16	84	1,0

16,0	10	90	1,0
19,0	0	100	1,0
20,0	0	100	1,0
21,0	40	60	1,0
29,0	40	60	1,0
30,0	40	60	0

Note: The eluting gradient may require slight modification in order to achieve the resolution shown in figure 1.

Column temperature: 30 °C.

7.5.3. *Injection volume: 50 µl derivatising reagent and 50 µl sample solution*

7.5.4. *Column equilibration*

Starting up the system on a daily basis, flush the column with 100 % solvent B for 15 minutes, then set at A:B = 40:60 and equilibrate at 1 ml/min for 15 minutes. Perform a blank run by injecting methanol (4.2.1).

Note: Before long-term storage flush the column with methanol: chloroform = 80:20 (v/v) for 30 minutes.

7.5.5. *Determine the PS + PE content in the test sample*

7.5.6. *Perform the sequence of the chromatographic analyses keeping constant the run-to-run time in order to obtain constant retention times. Inject the external standard solution (7.3) every 5-10 test sample solutions in order to calculate the response factor*

Note: The column shall be cleaned by flushing with 100 % solvent B (7.5.1) for at least 30 minutes every 20-25 runs.

7.6. **Integration mode**

7.6.1. *PEDP peak*

PEDP is eluted as a single peak. Determine the peak area by valley-to- valley integration.

7.6.2. *Tryptamine peak*

Tryptamine is eluted as a single peak (Figure 1). Determine the peak area by valley-to-valley integration.

7.6.3. *PS and PE peaks groups*

Under the described conditions (Figure 1), PS elutes as two main partially unresolved peaks preceded by a minor peak. PE elutes as three main partially unresolved peaks. Determine the whole area of each peak cluster setting the baseline as reported in Figure 1.

8. CALCULATION AND EXPRESSION OF RESULTS

PS and PE content in the test sample shall be calculated as follows:

$C = 55{,}36 \times ((A_2)/(A_1)) \times ((T_1)/(T_2))$

where:

C = PS or PE content (mg/100 g powder) in the test sample
A_1 = PEDP peak area of the standard sample solution (7.3)
A_2 = PS or PE peak area of the test sample solution (7.2)
T_1 = Tryptamine peak area of the standard sample solution (7.3)
T_2 = Tryptamine peak area of the test sample solution (7.2).

9. ACCURACY OF THE METHOD

Note: The values for repeatability were calculated according to the IDF International Standard (*).

9.1. **Repeatability**

The relative standard deviation of the repeatability, which expresses the variability of independent analytical results obtained by the same operator using the same apparatus under the same conditions on the same test sample and in a short interval of time, should not exceed 2 % relative. If two determinations are obtained under these conditions, the relative difference between the two results should not be greater than 6 % of the arithmetic mean of the results.

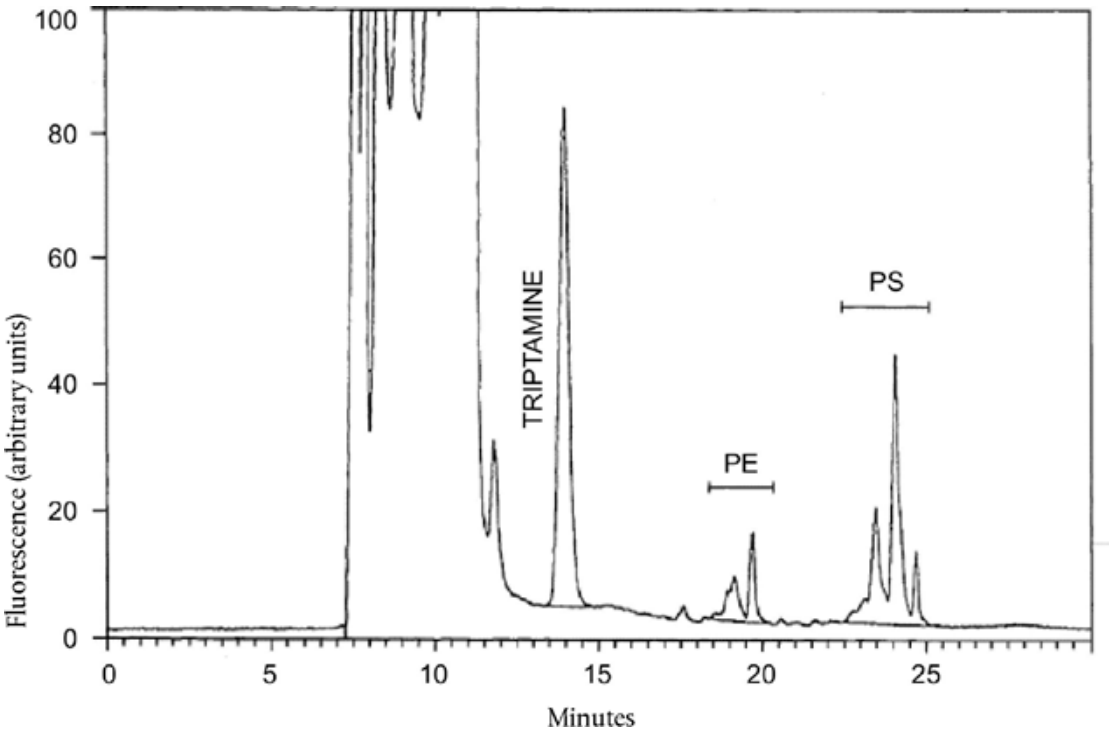
9.2. **Reproducibility**

If two determinations are obtained by operators in different laboratories using different apparatus under different conditions for the analysis on the same test sample, the relative difference between the two results should not be greater than 11 % of the arithmetic mean of the results.

10. REFERENCES

10.1. *Resmini P., Pellegrino L., Hogenboom J.A., Sadini V., Rampilli M., ' Detection of buttermilk solids in skimmilk powder by HPLC quantification of aminophospholipids ' . Sci. Tecn. Latt.-Cas., 39,395 (1988).*

Figure 1 **HPLC pattern of OPA-derivatives of phosphatidylserine (PS) and phosphatidylethanolamine (PE) in methanol extract of reconstituted skim-milk powder. Integration mode for the peaks of PS, PE and tryptamine (internal standard) is reported**



Appendix II

DETECTION OF RENNET WHEY IN SKIMMED MILK POWDER FOR PUBLIC STORAGE BY DETERMINATION OF CASEINOMACROPEPTIDES HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1. SCOPE AND FIELD OF APPLICATION

This method allows detection of rennet whey in skimmed milk powder intended for public storage by determination of the caseinomacropeptides.

2. REFERENCE

International Standard ISO 707 - Milk and Milk Products - Guidance on sampling.

3. DEFINITION

The content of rennet whey solids is defined as the percentage by mass as determined by the caseinomacropeptide content by the procedure described.

4. PRINCIPLE

— Reconstitution of the skimmed milk powder, removal of fat and proteins with trichloroacetic acid, followed by centrifugation or filtration;

— Determination of the quantity of caseinomacropeptides (CMP) in the supernatant by high-performance liquid chromatography (HPLC);

— Evaluation of the result obtained for the samples by reference to standard samples consisting of skimmed milk powder with or without the addition of a known percentage of whey powder.

5. REAGENTS

All reagents shall be of recognised analytical grade. The water used shall be distilled water or water of at least equivalent purity.

5.1. **Trichloroacetic acid solution**

Dissolve 240 g of trichloroacetic acid (CCl_3COOH) in water and make up to 1 000 ml. The solution should be clear and colourless.

5.2. **Eluent solution, pH 6,0**

Dissolve 1,74 g of dipotassium hydrogen phosphate (K_2HPO_4), 12,37 g of potassium dihydrogen phosphate (KH_2PO_4) and 21,41 g of sodium sulphate (Na_2SO_4) in about 700 ml of water. Adjust, if necessary, to pH 6,0, using a solution of phosphoric acid or potassium hydroxide.

Make up to 1 000 ml with water and homogenise.

Note: The composition of the eluent can be updated to comply with the certificate of the standards or the recommendations of the manufacturer of the column packing material.

Filter the eluent solution, prior to use, through a membrane filter with a 0,45 μm pore diameter.

5.3. **Flushing solvent**

Mix one volume acetonitrile (CH_3CN) with nine volumes water. Filter the mixture prior to use through a membrane filter with a 0,45 μm pore diameter.

Note: Any other flushing solvent with a bactericidal effect which does not impair the columns' resolution efficiency may be used.

5.4. **Standard samples**

5.4.1. *Skimmed milk powder meeting the requirements of this Regulation (i.e. [0])*

5.4.2. *The same skimmed milk powder adulterated with 5 % (m/m) rennet-type whey powder of standard composition (i.e. [5])*

6. APPARATUS

6.1. **Analytical balance**

6.2. **Optional centrifuge capable of attaining a centrifugal force of 2 200 g, fitted with stoppered or capped centrifuge tubes of about 50 ml capacity**

6.3. **Mechanical shaker**

6.4. **Magnetic stirrer**

6.5. **Glass funnels, diameter about 7 cm**

6.6. **Filter papers, medium filtration, diameter about 12,5 cm**

6.7. **Glass filtration equipment with 0,45 μm pore diameter membrane filter**

6.8. **Graduated pipettes allowing delivery of 10 ml (ISO 648, Class A, or ISO/R 835) or a dispensing system capable of delivering 10,0 ml in two minutes**

6.9. **Dispensing system capable of delivering 20,0 ml water at ca. 50 °C**

6.10. **Thermostatic water bath, set at 25 ± 0,5 °C**

6.11. **HPLC equipment, consisting of:**

6.11.1. *Pump*

6.11.2. *Injector, hand or automatic, with a 15 to 30 µl capacity*

6.11.3. *Two TSK 2 000 -SW columns in series (length 30 cm, internal diameter 0,75 cm) or equivalent columns (e.g. single TSK 2 000 -SWxl, single Agilent Technologies Zorbax GF 250) and a precolumn (3 cm × 0,3 cm) packed with I 125 or material of equivalent effectiveness*

6.11.4. *Thermostatic column oven, set at 35 ± 1 °C*

6.11.5. *Variable wavelength UV detector, permitting measurements at 205 nm with a sensitivity of 0,008 Å*

6.11.6. *Integrator capable of valley-to-valley integration*

Note: Working with columns kept at room temperature is possible, but their power of resolution is slightly lower. In that case, the temperature should vary by less than ± 5 °C in any one range of analyses.

7. SAMPLING

7.1. Samples shall be taken in accordance with the procedure laid down in International Standard ISO 707. However, **[**F4the appropriate authority**]** may use another method of sampling provided that it complies with the principles of the abovementioned standard

Textual Amendments

F4 Words in Annex 5 Pt. 1A Appendix 2 para. 7.1 substituted (31.12.2020) by The Market Measures Payment Schemes (Amendment) (EU Exit) Regulations 2019 (S.I. 2019/823), regs. 1, **6(47)(a)(i)**; 2020 c. 1, **Sch. 5 para. 1(1)**

7.2. Store the sample in conditions which preclude any deterioration or change in composition

8. PROCEDURE

8.1. **Preparation of the test sample**

Transfer the milk powder into a container with a capacity of about twice the volume of the powder, fitted with an airtight lid. Close the container immediately. Mix the milk powder well by means of repeated inversion of the container.

8.2. **Test portion**

Weight 2,000 ± 0,001 g of test sample into a centrifuge tube (6.2) or a suitable stoppered flask (50 ml).

8.3. **Removal of fat and proteins**

8.3.1. *Add 20,0 ml of warm water (50 °C) to the test portion. Dissolve the powder by shaking for five minutes using a mechanical shaker (6.3). Place the tube into the water bath (6.10) and allow to equilibrate to 25 °C*

8.3.2. *Add 10,0 ml of the trichloroacetic acid solution (5.1) of ca. 25 °C in two minutes, while stirring vigorously with the aid of the magnetic stirrer (6.4). Place the tube in a water bath (6.10) and leave for 60 minutes*

8.3.3. *Centrifuge (6.2) for 10 minutes at 2 200 g, or filter through paper (6.6), discarding the first 5 ml of filtrate*

8.4. **Chromatographic determination**

8.4.1. *Inject 15 to 30 µl of accurately measured supernatant or filtrate (8.3.3) into the HPLC apparatus (6.11) operating at a flow rate of 1,0 ml of eluent solution (5.2) per minute*

Note 1. Another flow rate may be used, dependent of the internal diameter of the columns used or the instructions of the manufacturer of the column.

Note 2. Rinse the columns with water during each interruption. Never leave the eluent solution in them (5.2).

Prior to any interruption of more than 24 hours, rinse the columns with water then wash them with solution (5.3) for at least three hours at a flow rate of 0,2 ml per minute.

8.4.2. *The results of chromatographic analysis of the test sample [E] are obtained in the form of chromatogram in which each peak is identified by its retention time RT as follows:*

Peak II:	The second peak of the chromatogram having an RT of about 12,5 minutes.
Peak III:	The third peak of the chromatogram, corresponding to the CMP, having an RT of 15,5 minutes.

The choice of the column(s) can affect the retention times of the individual peaks considerably.

The integrator (6.11.6) automatically calculates the area A of each peak:

$\mathbf{A_{II}}$:	area of peak II,
$\mathbf{A_{III}}$:	area of peak III,

It is essential to examine the appearance of each chromatogram prior to quantitative interpretation, in order to detect any abnormalities due either to malfunctioning of the apparatus or the columns, or to the origin and nature of the sample analysed.

If in doubt, repeat the analysis.

8.5. **Calibration**

8.5.1. *Apply exactly the procedure described from point 8.2 to point 8.4.2 to the standard samples (5.4)*

Use freshly prepared solutions, because CMP degrade in an 8 % trichloroacetic environment. The loss is estimated at 0,2 % per hour at 30 °C.

8.5.2. *Prior to chromatographic determination of the samples, condition the columns by repeatedly injecting the standard sample (5.4.2) in solution (8.5.1) until the area and retention time of the peak corresponding to the CMP are constant*

8.5.3. *Determine the response factors R by injecting the same volume of filtrates (8.5.1) as used for the samples*

9. EXPRESSION OF RESULTS

9.1. **Method of calculation and formulae**

9.1.1. *Calculation of the response factors R:*

Peak II:	$R_{II} = 100/(A_{II}\ [0])$

where:

R_{II} = the response factors of peaks II,
$A_{II}\ [0]$ = the areas of peaks II of the standard sample [0] obtained in 8.5.3.

Peak III:	$R_{III} = W/(A_{III}\ [5] - A_{III}\ [0])$

where:

R_{III} = the response factor of peak III,
$A_{III}\ [0]$ and $A_{III}\ [5]$ = the areas of peak III in standard samples [0] and [5] respectively obtained in 8.5.3,
W = the quantity of whey in standard sample [5], i.e. 5.

9.1.2. *Calculation of the relative area of the peaks in the sample [E]*

$S_{II}\,[E] = R_{II} \times A_{II}\,[E]$

$S_{III}\,[E] = R_{III} \times A_{III}\,[E]$

$S_{IV}\,[E] = R_{IV} \times A_{IV}\,[E]$

where:

$S_{II}\ [E]$, $S_{III}\ [E]$, $S_{IV}\ [E]$ = the relative areas of peaks II, III and IV respectively in the sample [E],
$A_{II}\ [E]$, $A_{III}\ [E]$ = the areas of peaks II and III respectively in the sample [E] obtained in 8.4.2,
R_{II} , R_{III} = the response factors calculated in 9.1.1.

9.1.3. *Calculation of the relative retention time of peak III in sample [E]:*

$RRT_{III}\,[E] = (RT_{III}\,[E])/(RT_{III}\,[5])$

where:

$RRT_{III}\ [E]$ = the relative retention time of peak III in sample [E],
$RT_{III}\ [E]$ = the retention time of peak III in sample [E] obtained in 8.4.2,
$RT_{III}\ [5]$ = the retention time of peak III in control sample [5] obtained in 8.5.3.

9.1.4. *Experiments have shown that there is a linear relation between the relative retention time of peak III, i.e. RRT_{III} [E] and the percentage of whey powder added up to 10 %*

— The $RRT_{III}\ [E]$ is < 1,000 when the whey content is > 5 %;

— The $RRT_{III}\ [E]$ is ≥ 1,000 when the whey content is ≤ 5 %.

The uncertainty allowed for the values of RRT_{III} is ± 0,002.

Normally the value of $RRT_{III}\ [0]$ deviates little from 1,034. Depending on the condition of the columns, the value may approach 1,000, but it shall always be greater.

9.2. **Calculation of the percentage of rennet whey powder in the sample:**

$W = S_{III}[E] - [1,3 + (S_{III}[0] - 0,9)]$

where:

W	=	the percentage m/m of rennet whey in the sample [E];
S_{III} [E]	=	the relative area of peak III of test sample [E] obtained as in 9.1.2;
1,3	=	represents the relative average area of peak III expressed in grams of rennet whey per 100 g determined in non-adulterated skimmed milk powder of various origins. This figure was obtained experimentally;
S_{III} [0]	=	represents the relative area of peak III which is equal to $R_{III} \times A_{III}$ [0]. These values are obtained in 9.1.1 and 8.5.3 respectively;
(S_{III} [0] – 0,9)	=	represents the correction to be made to the relative average area 1,3 when S_{III} [0] is not equal to 0,9. Experimentally the relative average area of peak III of the control sample [0] is 0,9.

9.3. **Accuracy of the procedure**

9.3.1. *Repeatability*

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst using the same apparatus on identical test material shall not exceed 0,2 % m/m.

9.3.2. *Reproducibility*

The difference between two single and independent results, obtained in two different laboratories on identical test material shall not exceed 0,4 % m/m.

9.4. **Interpretation**

9.4.1. *Assume the absence of whey if the relative area of peak III, S_{III} [E] expressed in grams of rennet whey per 100 g of the product is ≤ 2,0 + (S_{III} [0] – 0,9)*

where

2,0	is the maximum value allowed for the relative area of peak III taking into account the relative average area of peak III, i.e. 1,3, the uncertainty due to variations in the composition of skimmed milk powder and the reproducibility of the method (9.3.2),
(S_{III} [0] – 0,9)	is the correction to be made when the area S_{III} [0] is different from 0,9 (see point 9.2)

9.4.2. *If the relative area of peak III, S_{III} [E] is > 2,0 + (S_{III} [0] – 0,9) and the relative area of peak II, S_{II} [E] ≤ 160, determine the rennet whey content as indicated in point 9.2.*

9.4.3. *If the relative area of peak III, S_{III} [E] is > 2,0 + (S_{III} [0] – 0,9) and the relative area of peak II, S_{II} [E] ≤ 160, determine the total protein content (P %); then examine graphs 1 and 2.*

9.4.3.1. *The data obtained after analysis of samples of unadulterated skimmed milk powders with a high total protein content have been assembled in graphs 1 and 2.*

The continuous line represents the linear regression, the coefficients of which are calculated by the least squares method.

The dashed straight line fixes the upper limit of the relative area of peak III with a probability of not being exceeded in 90 % of cases.

The equations for the dashed straight lines of graphs 1 and 2 are:

$S_{III} = 0{,}376\ P\ \% - 10{,}7$	(graph 1),
$S_{III} = 0{,}0123\ S_{II}\ [E] + 0{,}93$	(graph 2),

respectively where:

S_{III}	is the relative area of peak III calculated either according to total protein content or according to the relative area of peak S_{II} [E],
P %	is the total protein content expressed as a percentage, by weight,
S_{II} [E]	is the relative area of sample calculated in point 9.1.2.

These equations are equivalent to the figure of 1,3 mentioned in point 9.2.

The discrepancy (T_1 and T_2) between the relative area S_{III} [E] found and the relative area S_{III} is given by means of the following: $T_1 = S_{III}[E] - [(0{,}376\ P\% - 10{,}7) + (S_{III}[0] - 0{,}9)]$ $T_2 = S_{III}[E] - [(0{,}0123\ S_{II}[E] + 0{,}93) + (S_{III}[0] - 0{,}9)]$

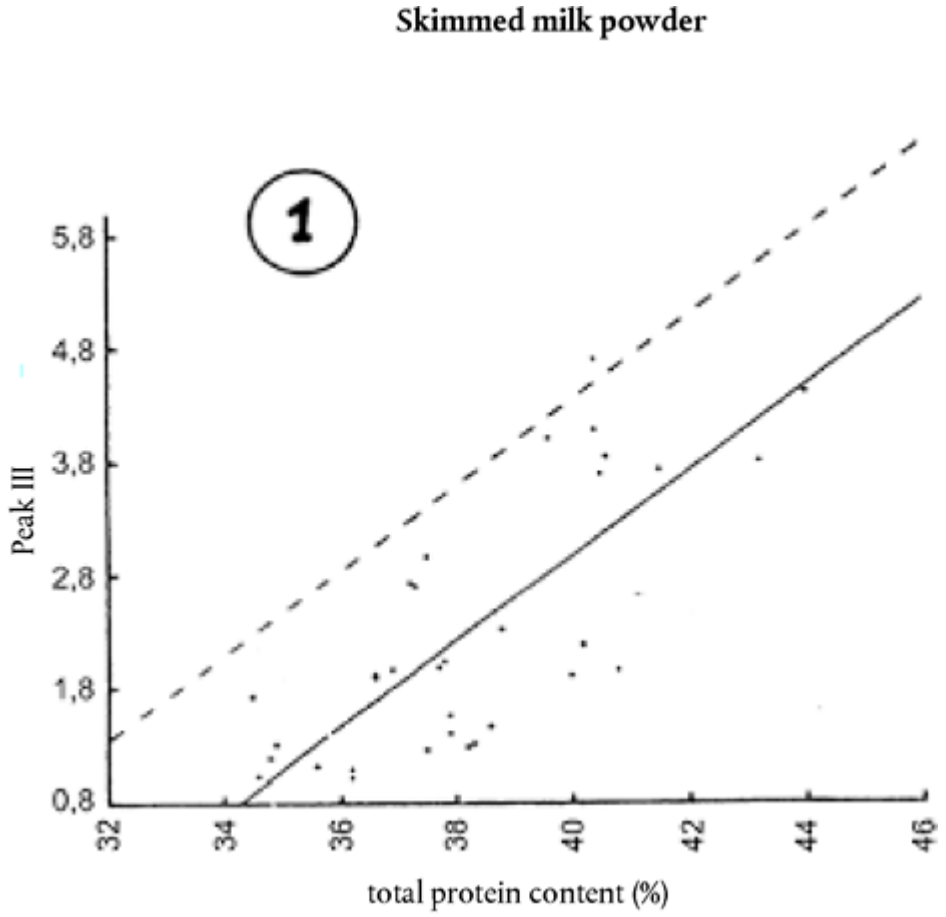
9.4.3.2.

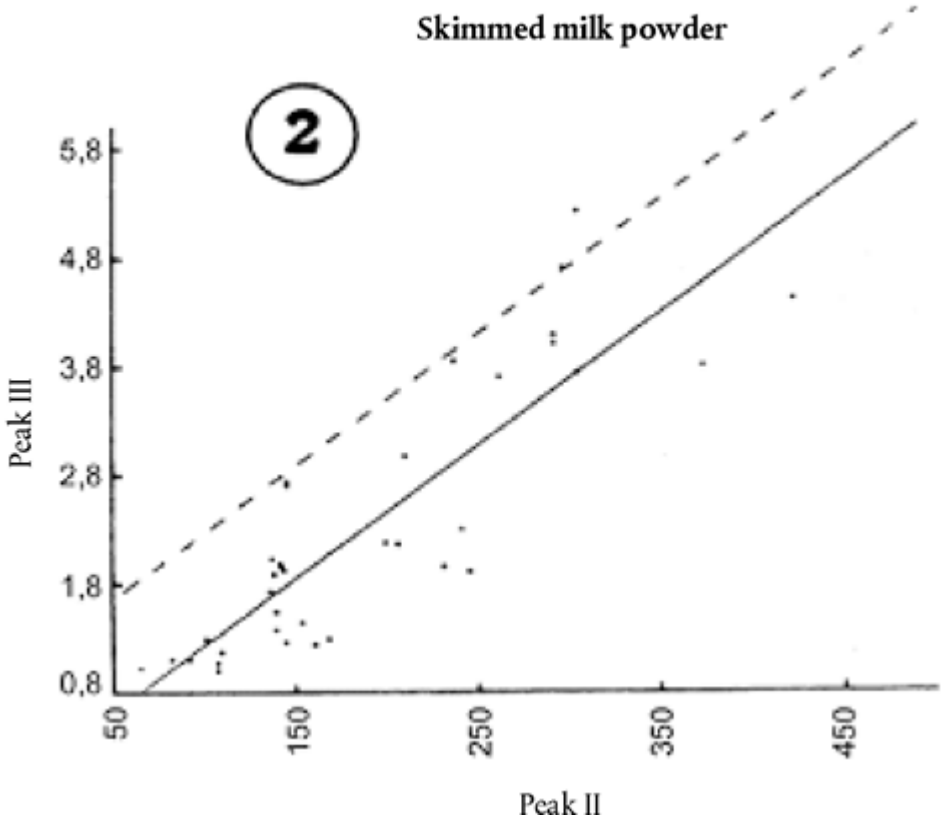
If T_1 and/or T_2	are zero or less, the presence of rennet whey cannot be determined.
If T_1 and T_2	exceed zero, rennet whey is present.

The rennet whey content is calculated according to the following formula: $W = T_2 + 0{,}91$

where:

0,91 is the distance on the vertical axis between the continuous and dotted straight lines.





Appendix III

DETERMINING RENNET WHEY SOLIDS IN SKIMMED MILK POWDER

1. PURPOSE: DETECTING THE ADDITION OF RENNET WHEY SOLIDS TO SKIMMED MILK POWDER

2. REFERENCES: INTERNATIONAL STANDARD ISO 707

3. DEFINITION

The content of rennet whey solids is defined as the percentage by mass as determined by caseinomacropeptide content by the procedure described.

4. PRINCIPLE

Samples are analysed for caseinomacropeptide A by a reversed-phase high-performance liquid chromatography procedure (HPLC procedure). Evaluation of the result is obtained by reference to standard samples consisting of skimmed milk powder with and without a known percentage of whey powder. Results higher than 1 % (m/m) show that rennet whey solids are present.

5. REAGENTS

All reagents shall be of recognised analytical grade. The water used shall be distilled water or water of at least equivalent purity. Acetonitrile should be of spectroscopic or HPLC quality.

5.1. **Trichloroacetic acid solution**

Dissolve 240 g of trichloroacetic acid (CCl_3COOH) in water and make up to 1 000 ml. The solution should be clear and colourless.

5.2. **Eluents A and B**

Eluent A: 150 ml of acetonitrile (CH_3CN), 20 ml of isopropanol ($CH_3CHOHCH_3$), and 1,00 ml of trifluoroacetic acid (TFA, CF_3COOH) are placed in a 1 000 ml volumetric flask. Make up to 1 000 ml with water.

Eluent B: 550 ml of acetonitrile, 20 ml of isopropanol and 1,00 ml of TFA are placed in a 1 000 ml volumetric flask. Make up to 1 000 ml with water. Filter the eluent solution, prior to use, through a membrane filter with a 0,45 µm pore diameter.

5.3. **Conservation of the column**

After the analyses the column is flushed with eluent B (via a gradient) and subsequently flushed with acetonitrile (via a gradient for 30 minutes). The column is stored in acetonitrile.

5.4. **Standard samples**

5.4.1. *Skimmed milk powder meeting the requirements for public storage (i.e. [0]).*

5.4.2. *The same skimmed milk powder adulterated with 5 % (m/m) rennet-type whey powder of standard composition (i.e. [5]).*

5.4.3. *The same skimmed milk powder adulterated with 50 % (m/m) rennet-type whey powder of standard composition (i.e. [50])*

6. APPARATUS

6.1. **Analytical balance**

6.2. **Optional centrifuge capable of attaining a centrifugal force of 2 200 g, fitted with stoppered or capped centrifuge tubes of about 50 ml capacity**

6.3. **Mechanical shaker**

6.4. **Magnetic stirrer**

6.5. **Glass funnels, diameter about 7 cm**

6.6. **Filter papers, medium filtration, diameter about 12,5 cm**

6.7. **Glass filtration equipment with 0,45 µm pore diameter membrane filter**

6.8. **Graduated pipettes, allowing delivery of 10 ml (ISO 648, Class A, or ISO/R 835), or a dispensing system capable of delivering 10,0 ml in two minutes**

6.9. **Dispensing system capable of delivering 20,0 ml water at ca. 50 °C**

6.10. **Thermostatic water bath, set at 25 ± 0,5 °C**

6.11. **HPLC equipment, consisting of:**

6.11.1. *Binary gradient pumping system*

6.11.2. *Injector, hand or automatic, with a 100 µl capacity*

6.11.3. *Agilent Technologies Zorbax 300 SB-C3 column (length 25 cm, 0,46 cm internal diameter) or an equivalent wide-pore silica based reversed-phase column*

6.11.4. *Thermostatic column oven, set at 35 ± 1 °C*

6.11.5. *Variable wavelength UV detector, permitting measurements at 210 nm (if necessary, a higher wavelength up to 220 nm may be used) with a sensitivity of 0,02 Å*

6.11.6. *Integrator capable of setting the integration to common baseline or valley-to-valley*

Note : Operation of the column at room temperature is possible, provided that the room temperature does not fluctuate more than 1 °C, otherwise too much variation in the retention time of CMP_A takes place.

7. SAMPLING

7.1. **Samples shall be taken in accordance with the procedure laid down in International Standard ISO 707. However, [F5the appropriate authority] may use another method of sampling provided that it complies with the principles of the abovementioned standard**

Textual Amendments

F5 Words in Annex 5 Pt. 1A Appendix 3 para. 7.1 substituted (31.12.2020) by The Market Measures Payment Schemes (Amendment) (EU Exit) Regulations 2019 (S.I. 2019/823), regs. 1, **6(47)(a)(ii)**; 2020 c. 1, **Sch. 5 para. 1(1)**

7.2. **Store the sample in conditions which preclude any deterioration or change in composition.**

8. PROCEDURE

8.1. **Preparation of the test sample**

Transfer the milk powder into a container with a capacity of about twice the volume of the powder, fitted with an airtight lid. Close the container immediately. Mix the milk powder well by means of repeated inversion of the container.

8.2. **Test portion**

Weigh 2,00 ± 0,001 g of test sample into a centrifuge tube (6.2) or suitable stoppered flask (50 ml).

Note: In the case of mixtures, weigh such an amount of the test sample that the defatted sample portion corresponds to 2,00 g.

8.3. **Removal of fat and proteins**

8.3.1. *Add 20,0 ml of warm water (50 °C) to the test portion. Dissolve the powder by shaking for five minutes using a mechanical shaker (6.3). Place the tube into the water bath (6.10) and allow to equilibrate to 25 °C*

8.3.2. *Add 10,0 ml of the trichloroacetic acid solution of ca. 25 °C (5.1) constantly over two minutes, while stirring vigorously with the aid of the magnetic stirrer (6.4). Place the tube in a water bath (6.10) and leave for 60 minutes*

8.3.3. *Centrifuge (6.2) 2 200 g for 10 minutes, or filter through paper (6.6), discarding the first 5 ml of filtrate*

8.4. **Chromatographic determination**

8.4.1. *The reversed-phase HPLC method excludes the possibility false-positive results due to the presence of acid buttermilk powder.*

8.4.2. *Before the reversed phase HPLC-analysis is carried out, the gradient conditions should be optimised. A retention time of 26 ± 2 minutes for CMP $_A$ is optimal for gradient systems having a dead volume of about 6 ml (volume from the point where the solvents come together to the volume of the injector loop, inclusive). Gradient systems having a lower dead volume (e.g. 2 ml) should use 22 minutes as an optimal retention time*

Take solutions of the standard samples (5.4) without and with 50 % rennet whey.

Inject 100 µl of supernatant or filtrate (8.3.3) into the HPLC apparatus operating at the scouting gradient conditions given in Table 1.

TABLE 1

Scouting gradient conditions for optimisation of the chromatography

Time (min)	Flow (ml/min)	% A	% B	Curve
Initial	1,0	90	10	*
27	1,0	60	40	linear
32	1,0	10	90	linear
37	1,0	10	90	linear
42	1,0	90	10	linear

Comparison of the two chromatograms should reveal the location of the peak of CMP_A.

Using the formula given below, the initial solvent composition to be used for the normal gradient (see 8.4.3) can be calculated % B = 10 – 2,5 + (13,5 + (RT_{cmpA} – 26) / 6) * 30 / 27 % B = 7,5 + (13,5 + (RT_{cmpA} – 26) / 6) * 1,11

Where:

RT_{cmpA}	:	retention time of CMP_A in the scouting gradient
10	:	the initial % B of the scouting gradient
2,5	:	% B at midpoint minus % B at initial in the normal gradient
13,5	:	midpoint time of the scouting gradient
26	:	required retention time of CMP_A
6	:	ratio of slopes of the scouting and normal gradient
30	:	% B at initial minus % B at 27 minutes in the scouting gradient
27	:	run-time of the scouting gradient.

8.4.3. *Take solutions of the test samples*

Inject 100 µl of accurately measured supernatant or filtrate (8.3.3) into the HPLC apparatus operating at a flow rate of 1,0 ml of eluent solution (5.2) per minute.

The composition of the eluent of the start of the analysis is obtained from 8.4.2. It is normally close to A:B = 76:24 (5.2). Immediately after the injection a linear gradient is started, which results in a 5 % higher percentage of B after 27 minutes. Subsequently a linear gradient is started, which brings the eluent composition to 90 % B in five minutes. This composition is maintained for five minutes, after which the composition is changed, via a linear gradient in five minutes to the initial composition. Depending on the internal volume of the pumping system, the next injection can be made 15 minutes after reaching the initial conditions.

Note 1. The retention time of the CMP_A should be 26 ± 2 minutes. This can be achieved by varying the initial and end conditions of the first gradient. However, the difference in the % B for the initial and end conditions of the first gradient shall remain 5 % B.

Note 2. The eluents should be degassed sufficiently and should also remain degassed. This is essential for proper functioning of the gradient pumping system. The standard deviation for the retention time of the CMP_A peak should be smaller than 0,1 minutes (n = 10).

Note 3. Every five samples the reference sample [5] should be injected and used to calculate a new response factor R. (9.1.1).

8.4.4. *The results of the chromatographic analysis of the test sample (E) are obtained in the form of a chromatogram in which the CMP_A peak is identified by its retention time of about 26 minutes*

The integrator (6.11.6) automatically calculates the peak height H of the CMP_A peak. The baseline location should be checked in every chromatogram. The analysis or the integration should be repeated if the baseline was incorrectly located.

Note: If the CMP_A peak is sufficiently separated from other peaks valley-to-valley baseline allocation should be used, otherwise use dropping perpendiculars to a common baseline, which should have starting point close to the CMP_A peak (thus not at t = 0 min!).Use for the standard and the samples the same type integration type and check in case of common baseline its consistency for the samples and the standard.

It is essential to examine the appearance of each chromatogram prior to quantitative interpretation, in order to detect any abnormalities due either to malfunctioning of the apparatus or the column, or to the origin and nature of the sample analysed. If in doubt, repeat the analysis.

8.5. **Calibration**

8.5.1. *Apply exactly the procedure described from point 8.2 to point 8.4.4 to the standard samples (5.4.1 to 5.4.2). Use freshly prepared solutions, because CMP degrades in an 8 % trichloroacetic acid environment at room temperature. At 4 °C the solution remains stable for 24 hours. In the case of long series of analyses the use of a cooled sample tray in the automatic injector is desirable*

Note: 8.4.2. may be omitted if the % B at initial conditions is known from previous analyses.

The chromatogram of the reference sample [5] should be analogous to Figure. 1. In this figure the CMP_A peak is preceded by two small peaks. It is essential to obtain a similar separation.

8.5.2. *Prior to chromatographic determination of the samples inject 100 µl of the standard sample without rennet whey [0] (5.4.1)*

The chromatogram should not show a peak at the retention time of the CMP_A peak.

8.5.3. *Determine the response factors R by injecting the same volume of filtrate (8.5.1) as used for the samples.*

9. EXPRESSION OF RESULTS

9.1. **Method of calculation and formulae**

9.1.1. *Calculation of the response factor R:*

CMP_A peak: $R = W/H$

Where:

R	=	the response factor of the CMP_A peak
H	=	the height of the CMP_A peak
W	=	the quantity of whey in the standard sample [5].

9.2. **Calculation of the percentage of rennet whey powder in the sample**

$W(E) = R \times H(E)$

Where:

W(E)	=	the percentage (m/m) of rennet whey in the sample (E).
R	=	the response factor of the CMP_A peak (9.1.1)
H(E)	=	the height of the CMP_A peak of the sample (E)

If W(E) is greater than 1 % and the difference between the retention time and that of the standard sample [5] is smaller than 0,2 minutes then rennet whey solids are present.

9.3. **Accuracy of the procedure**

9.3.1. *Repeatability*

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst using the same apparatus on identical test material shall not exceed 0,2 % m/m.

9.3.2. *Reproducibility*

Not determined.

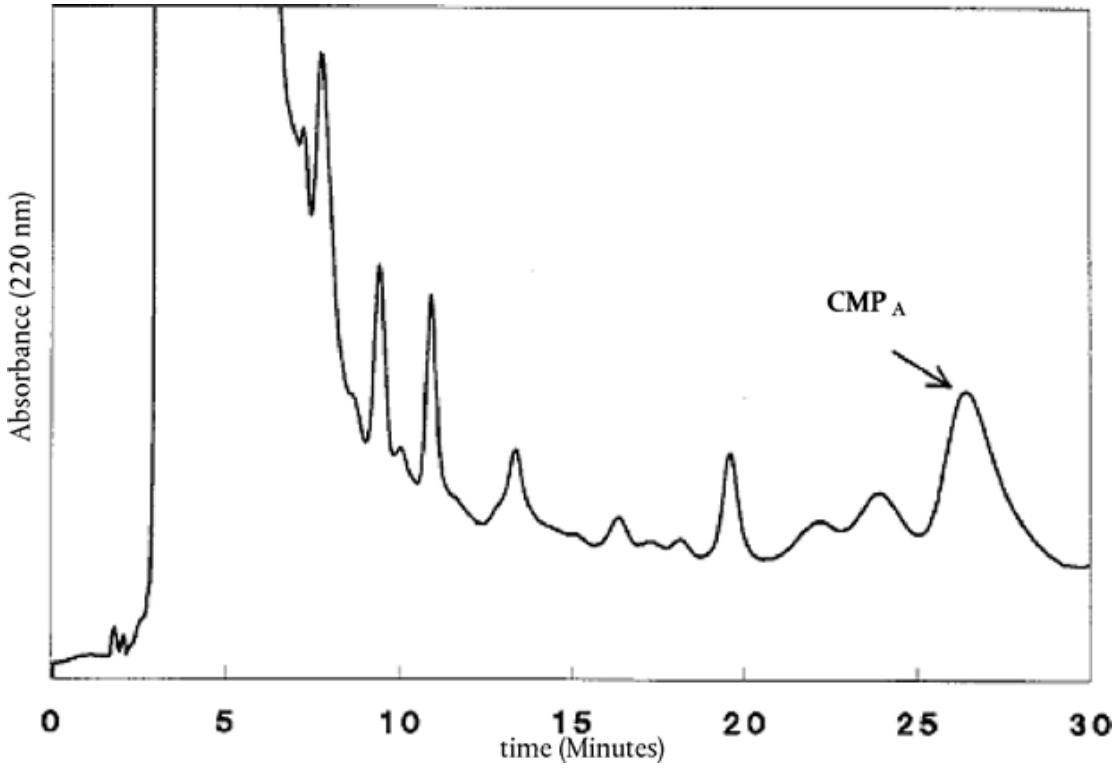
9.3.3. *Linearity*

From 0 to 16 % of rennet whey a linear relationship should be obtained with a coefficient of correlation > 0,99.

9.4. **Interpretation**

The 1 % limit includes the uncertainty due to reproducibility.

Figure 1 **Ni—4.6 standard**



(*) International IDF Standard 135B/1991. Milk and milk products. Precision characteristics of analytical methods. Outline of collaborative study procedure.**]**

PART II

Delivery and packaging of skimmed milk powder

1. Skimmed milk powder shall be packed in new, clean, dry and intact bags meeting the following requirements:

(a) the bags shall have at least three layers, which together correspond to at least 420 J/m^2 TEA average;

(b) the second layer shall be covered with a layer of polyethylene of at least 15 g/m^2;

(c) inside the paper layers, a polyethylene bag at least 0,08 mm thick shall be fused to the bottom;

(d) bags shall conform to standard EN 770;

(e) when filling, the powder should be well pressed down. Loose powder must on no account be allowed to penetrate between the various layers.

2. The bags shall show the following particulars, where appropriate in code:

(a) the approval number identifying the factory and **[**F6indicating that the skimmed milk powder was produced in the United Kingdom**]**;

(b) the date or, where appropriate, the week of production;

(c) the number of the production batch;

(d) the description ‘spray skimmed-milk powder’.

Textual Amendments

F6 Words in Annex 5 Pt. 2 para. 2(a) substituted (31.12.2020) by The Market Measures Payment Schemes (Amendment) (EU Exit) Regulations 2019 (S.I. 2019/823), regs. 1, **6(47)(b)**; 2020 c. 1, **Sch. 5 para. 1(1)**

3. The storekeeper shall keep a register in which the particulars referred to in point 2 are recorded on the date of entry into storage.

Changes to legislation:
There are currently no known outstanding effects for the Commission Implementing Regulation (EU) 2016/1240, ANNEX V.