Pure & Appl. Chem., Vol. 62, No. 4, pp. 781–793 1990.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION
COMMISSION ON OILS, FATS AND DERIVATIVES*

DETERMINATION OF THE ESTER-EMULSIFIERS COMPONENTS CONTENT AFTER HYDROLYSIS AND SILYLATION BY GAS CHROMATOGRAPHY

Results of a collaborative study and the standardised method

Prepared for publication by

H. BRÜSCHWEILER[1] and A. HAUTFENNE[2]

[1]Swiss Federal Laboratories for Materials Testing and Research EMPA, Unterstrasse 11, CH-9001 St. Gallen, Switzerland

[2]Université Catholique de Louvain, Unité des Procédés (PRCD) B-1348 Louvain-la-Neuve, Belgium

*Membership of the Commission during the period of the collaborative study (1981–83) was as follows:

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

†Until 1982 ‡Deceased 1982

Determination of ester-emulsifiers components content after hydrolysis and silylation by gas chromatography: results of a collaborative study and the standardised method

Abstract - A method for the gas chromatographic determination of the components of ester-emulsifiers has been elaborated and tested in a collaborative study.
The method involves saponification of the ester-emulsifiers in ethanolic KOH solution, evaporation of the alcohol, silylation of the products of hydrolysis and determination of the components either by packed or capillary gas chromatography. The emulsifiers analysed in this study were mono- and diglycerides, diacetyl tartaric acid ester of mono- and diglycerides and sorbitan monostearate in oil (coconut oil). Emulsifiers present in oil were preliminarily separated by column chromatography.
The hydrolysis-silylation procedure described is applicable not only for the characterization and quantification of functional components of ester-emulsifiers, but also for oils, fats, wax esters and other hydrolysable lipids.

INTRODUCTION

Emulsifiers are one of the most important class of food additives. Methods and procedures to analyse emulsifiers are required by industry for quality control purposes and by regulatory organisations to test if legal requirements are fulfilled. A joint FAO/WHO expert committee on food additives elaborated specifications for these emulsifiers (1).

Emulsifiers are surface-active substances which sustain the formation of emulsions. Emulsions are disperse systems of two liquors which are not or hardly soluble in each other.

Emulsifiers consist of molecules containing a part more readily soluble in apolar solvents having lipophilic properties and another part more readily soluble in polar solvents having lipophobic properties.

Ester-emulsifiers considered in this report are partial esters of edible fatty acids with polyalcohols such as glycol, polyglycol, glycerol, polyglycerols, sorbitan and carbohydrates (sucrose).

The emulsifiers are listed in table 1. They are classified in the order of increasing number of hydroxyl groups in the polar part of the molecule.

Ester-emulsifiers do not consist of one single chemical compound. They are always mixtures of substances with different degrees of esterification, types of fatty acids, positional isomerism etc. For example, monoglycerides of fatty acids may contain 1-mono-, 2-mono-, 1,2-di-, 1,3-di- and triglycerides of various fatty acids. Also free fatty acids, glycerol, di- and polyglycerol may be present. These mixtures can be esterified further with citric-, lactic- and succinic acid.

Emulsifier concentrates and emulsifiers present in oils and fats and in lipid extracts of food may be separated into fractions of different polarity, using chromatographic methods such as thin layer chromatography (TLC) or liquid chromatography (LC, HPLC).

Emulsifiers such as fatty acid esters of polyglycerol (2, 3), sorbitan (4, 5), sugar (6) and other components (1, 7) have been hydrolysed and the components obtained were determined by gas chromatography as trimethylsilyl derivatives.
The products of hydrolysis of these emulsifiers are shown in table 2.

Table 1

CLASSIFICATION OF ESTER-EMULSIFIERS *

1. Carboxylates	Examples:
1.1 Salts of fatty acids	Na-,K-,NH_4-,Ca-,Mg-salts of stearic-, oleic acid etc.
1.2 Esters of fatty acids with hydroxy (mono, poly)carboxylic acids and its salts	Sodium stearoyl citrate Calcium stearoyl-2-lactylate Sodium stearoyl-2-lactylate
1.3 Further carboxylates	Lactic esters of fatty acids Sodium salt of cholic-, deoxycholic acid
2. Sulfates and Sulfonates	
2.1 Sulfated fatty alcohols	Sodium lauryl sulfate
2.2 Sulfonated ester compounds	Sodium dioctyl sulfosuccinate
3. Glycol esters of fatty acids (Glycols)	
3.1 Monoesters of fatty acids and hydroxy fatty acids with glycols	1,2-Propylene glycol mono- and diesters of fatty acids
3.2 Esters of fatty acids with polyoxyethylene and/or polyoxypropylene glycols	Polyoxyethylene(8)stearate
4. Glycerol esters of fatty acids (Glycerides)	
4.1 Mono- and diglycerides of unsaturated and saturated fatty acids	Mono- and diglycerides of fatty acids
4.2 Monoglycerides esterified with	
4.2.1 Acetic acid	Acetylated monoglycerides (Acetoglycerides)
4.2.2 Citric acid	Citric acid ester of monoglyceride (Citroglycerides)
4.2.3 Lactic acid	Lactylated monoglycerides (Lactoglycerides)
4.2.4 Tartaric acid	Tartarylated monoglycerides
4.2.5 Diacetyltartaric acid	Diacetyl tartaric acid esters of monoglycerides
4.2.6 Succinic acid	Succinylated monoglycerides
4.2.7 Mixed acids	Stearyl citric acid ester glycerides
4.3 Esters of glycerol and propyleneglycol	Mixed lactic acid ester of propylene glycol and glycerol
4.4 Ethoxylated monoglycerides	Ethoxylated mono- and diglycerides
4.5 Ester of polyglycerol	Polyglycerol esters of fatty acids
4.6 Polymerised and oxidized fats	Glycerol esters of fatty acids obtained from soja oil oxidized under heat Polyglycerol esters of interesterified ricinoleic acid
4.7 Phosphate-derivatives of mono- and diglycerides	Ammonium phosphates
4.8 Lecithin	Lecithin, hydroxylated lecithin
5. Sorbitan ester of fatty acids	
5.1 Mono-, di- and triesters of fatty acids with sorbitan	Sorbitan monostearate
5.2 Mono-, di- and triesters of fatty acids with sorbitan, ethoxylated	Polyoxyethylene(20)sorbitan monostearate Polyoxyehtylene(20)sorbitan tristearate
6. Sugar esters of fatty acids	
6.1 Mono, di- and triesters of fatty acids with mono- and disaccharides	Sucrose esters of fatty acids (Sucroesters)

* names according to IUPAC recommendations, see Appendix

Table 2

COMPONENTS OF ESTER-EMULSIFIERS AFTER HYDROLYSIS

Alcohols and Polyalcohols	Acids
- Fatty alcohols	- Carboxylic acids acetic-, lauric-, palmitic-, stearic-, oleic acid etc.
- Glycols Polyglycols	
- Glycerols Polyglycerols	- Dicarboxylic acids succinic acid
- Sorbitol and its anhydrides	- Hydroxy carboxylic acid lactic-, tartaric-, citric acid
- Carbohydrates mono- and disaccharides	- Inorganic acids phosphoric-, sulphuric acid

The hydrolysis-silylation procedure proposed simplifies the characterization of complex mixtures of emulsifiers and may be used for the quantification of functional components in emulsifiers.

The collaborative study presented in this report is confined to the gas chromatographic determinations of the products of hydrolysis of samples of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides and sorbitan monostearates in oil. The procedure could be applied to other emulsifiers listed in Table 1 and to oils, fats, wax esters (11) and other hydrolysable lipids.

COLLABORATIVE STUDY

Samples

Samples and the draft method were sent to participating laboratories in April 1981. The substances were internal standard n-tetradecane and the reference substances glycerol, tartaric acid, and furthermore a mixture of $C_{10:0}$-, $C_{12:0}$-, $C_{14:0}$-, $C_{16:0}$-, $C_{18:0}$-, $C_{18:1}$- fatty acids, n-tetradecane and n-tetracosane. The components in the mixture were present in equal weight ratio. The response factors of the silylated reference substance versus n-tetradecane had to be determined. The values were then used for the calculation of the components of the emulsifiers samples 1 to 4 after hydrolysis.

The emulsifiers provided for the study were:
Mono- and diglycerides (sample 1); diacetyl tartaric acid esters of mono- and diglycerides (sample 2); sorbitan monostearate (sample 3); and sorbitan monostearate in coconut oil (sample 4). For sample 4 the participants were asked to perform a preliminary separation of emulsifiers from the oil using silica column chromatography according to method 2.321 "Determination of mono-, di- and triglycerides by column chromatography" (8).

RESULTS AND DISCUSSION

Results obtained by six laboratories are summarized in Tables 3 - 6. Fig. 1 shows typical chromatograms.

Operating conditions

Operating conditions used by collaborators are shown in Table 3. All laboratories used packed columns. Laboratory 1 and 3 additionally applied capillary columns.

Response factors

Response factors of the reference substances vs. n-tetradecane were determined. The response factor for glycerol was high due to the derivatization and introduction of additional $(Me)_3Si$-groups. Response factors for the $C_{8:0}$- to $C_{14:0}$- fatty acids were close to one, but became lower for fatty acids with higher numbers of C-atoms, indicating some discrimination due to instability of the trimethylsilyl fatty acid derivatives at higher column temperatures.

The relative stability of trimethylsilyl derivatives of fatty acids in a gas chromatographic set-up can be tested by the addition of equal amounts of hydrocarbons such as hexadecane (C_{16}), eicosane (C_{20}), tetracosane (C_{24}), octacosane (C_{28}) to the fatty acids to be silylated. The peaks of the corresponding hydrocarbons should have about the same heights as the ones of the silyl derivatives. With packed columns, $C_{18:0}$-, $C_{18:1}$- and $C_{18:2}$-fatty acids silyl derivatives were not separated, but could be quantified applying capillary columns.

Table 3 Operating Conditions Reported by Collaborators

Coll.	Column Dimension m x mm (id)	Material, Support, Phase	Temperatures (°C) Injector	Detector	Initial	Rate °C/min	Final	Carrier Gas Flow	Sample Size µl
Packed columns									
1	2.4x2.0	Glass, Supelcoport AW-DMCS, 80-100 mesh; 3% OV-17	310	310	80	5	275	Helium 30ml/min	1
2	2.0x2.2	Stainless Steel, Chromosorb WHP, 100-200 mesh, 3% Dexil 300 GC.	310	310	80 3 min	5	275	Helium 20ml/min	1.6
3	1.5x2.0	Glass Chromosorb G AW-DMCS, 80-100 mesh, 3% SE 30.	310	310	80 1 min	5	275	Nitrogen 25ml/min 3.5 bar	0.5
4	1.8x3	Glass, Chromosorb W, 80-100 mesh, 5% OV-1.	250	300	130	15	260	Helium 50ml/min 3.4 bar	2-7
5	2x3	Glass, Chromosorb G AW-DMCS, 60-80 mesh, silicone, 2% OV-1.	310	310	100	5	275	Nitrogen 30ml/min 0.5 bar	1-3
6	3x3	Stainless Steel, Chromosorb W AW-DMCS, 100-120 mesh, 3% OV-17.	280	280	100	5	270	Nitrogen 30ml/min 1.95 bar	1
Capillary columns									
1	20x0.32	Glass Capillary, SE 52.	310	310	80	5	275	Helium 1:100 1ml/min	0.5
3	20x0.32	Glass Capillary, Durabond 5, Polymethyl-(5% Phenyl)-Siloxan.	310	310	100	4	300	Nitrogen 0.5ml/min 1.03 bar 1:150	0.5

Statistical evaluation

Statistical analysis of the collaborative study results is presented in Tables 4, 5 and 6. Values of duplicate determinations obtained in participating laboratories are reported. Results were calculated according to the scheme outlined by Pocklington (9) and ISO 5725 (10) which allows rejection of unsatisfactory results using the Cochran's and Dixon's test (95% confidence level) and calculation of standard deviation of repeatability (s_r) and reproducibility (s_R) and their coefficients of variation (CV %).

Analysis of emulsifier samples

Sample 1, mono- and diglycerides

The sum of the mean values of the components glycerol, C_{14}-, C_{16}- and C_{18}-fatty acids as determined by all collaborators was 100.99% (Table 4). Repeatability and reproducibility values are given in Table 4.

Table 4: Results for Sample 1, Mono- and Diglycerides (expressed as a percentage by mass of sample)

Lab.	Determination	Glycerol	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$
Packed column					
1	1	22.19	1.73	23.44	40.76
	2	21.90	1.57	22.63	39.79
2	1	18.65[b]	2.68	24.01	56.54
	2	18.21[b]	2.71	25.10	59.44
3	1	22.32	2.59	24.49	45.63
	2	22.47	2.58	23.81	42.79
4	1	21.35	2.65	24.75	50.15
	2	21.85	2.70	23.85	49.75
5	1	23.80	2.50	29.38	96.93[b]
	2	22.52	2.09	26.48	87.67[b]
6	1	22.25	2.64	27.55	56.30
	2	22.72	2.35	28.88	58.68
Capillary column					
1	1	21.33	2.70	23.43	56.23
	2	21.83	2.88	23.32	52.26
3	1	21.44	3.30	27.66b	58.33
	2	21.03	3.14	24.12b	50.04
n		7	8	8	7
Mean		22.07	2.55	25.18	51.19
Sr		0.432	0.146	1.271	2.774
CV % (r)		1.96	5.72	5.05	5.42
S_R		0.729	0.462	2.172	7.032
CV % (R)		3.30	18.13	8.62	13.74

b Results rejected by Dixon test (95% confidence level)

Sample 2, diacetyl tartaric acid esters of mono- and diglycerides

The sum of the mean values of the components glycerol, tartaric acid, C_{14}-, C_{16}- and C_{18}-fatty acids determined by collaborators was 75.02%. The acetic acid content was analysed by the procedure of Pickett, using saponification, acidification with phosphoric acid, distillation and titration of the acid content. The amount of acetic acid has been found in the co-ordinator's laboratory to be 19.8 %, giving a total of 94.82 %. The recovery is low, due to low values for tartaric acid obtained by some collaborators. This is attributed to salt formation and reduced solubility of the sodium salt of tartaric acid. Collaborator 2 suggested to use ultrasound for dispersion and collaborator 5 to change the solvent, applying a mixed solvent of pyridine and acetonitrile (50:50). It has been found in the co-ordinators laboratory that the use of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and TMCS, the latter acting as a catalyst, completes silylation of salts of lactic-, tartaric- and citric acids. The BSTFA + TMCS-combination has furthermore the advantage that no precipitate of insoluble ammonium chloride is formed, as observed with HMDS + TMCS. Therefore in the final procedure the use of BSTFA + TMCS has been proposed as silylating agent. The performance of this combination has been extensively tested in the co-ordinators laboratory.
Lower carboxylic acids such as formic-, acetic-, butyric- and propionic acid may be analysed by gas chromatography using the same procedure. The peaks of these acids appear between the peaks of the silylating reagents.

Table 5: Results for Sample 2, Diacetyl Tartaric Acid Esters of Mono- and Diglycerides (expressed as a percentage by mass of sample)

Lab.	Determination	Glycerol	Tartaric Acid	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	sum of C_{18}
Packed column									
1	1	14.81	5.76	-	11.02		< 32.90	>	32.90
	2	13.52	7.58	-	13.22		< 34.57	>	34.57
2	1	12.55	13.29	0.71	13.92	9.59	29.67	>	39 26
	2	10.18	7.92	0.70	13.77	9.02	28.88	>	37.90
3	1	13.88	5.81	0.72	14.18		< 50.16	>	50.16[b]
	2	13.88	5.74	0.69	13.41		< 47.46	>	47.46[b]
4	1	14.30	19.85	0.65	11.55		< 35.40	>	35.40
	2	14.10	19.05	0.70	12.15		< 35.80	>	35.80
5	1	13.15	13.63	0.66[b]	12.68	10.86	29.77	>	40.63
	2	12.81	12.01	0.48[b]	11.36	9.37	28.18	>	37.55
6	1	10.29	10.80	0.59	11.84		< 32.77	>	32.77[a]
	2	13.91	16.13	0.80	15.74		< 43.99	>	43.99[a]
Capillary column									
1	1	14.92	5.89	-	12.31	7.17	18.03	7.04	32.24
	2	13.68	7.75	-	11.88	6.79	17.80	7.54	32.13
3	1	12.47	3.97	0.99[b]	15.88		34.20	>	34.20
	2	12.70	6.37	1.03[b]	16.86		38.65	>	38.65
n		8	8	4	8				6
Mean		13.20	10.10	0.70	13.24				35.94
Sr		1.176	2.127	0.077	1.223				1.686
CV % (r)		8.91	21.06	11.10	9.24				4.69
S_R		1.386	5.182	0.077	1.772				2.943
CV % (R)		10.50	51.30	11.10	13.39				8.19

a Results rejected by Cochran test (95% confidence level)
b Results rejected by Dixon test (95% confidence level)

Sample 3, sorbitan monostearate and sample 4, sorbitan monstearate in coconut oil

Sorbitan monostearate was applied as reference to determine the same compound present in coconut oil (sample 4), using the peak areas of sorbitan monoanhydrides and sorbitan dianhydrides for quantification. In the collaborative study sorbitan monostearate was preliminarily separated from the coconut oil triglycerides, by silica column chromatography, applying procedure 2.321 (8). Elution of the triglycerides was performed with benzene and the polar emulsifier fraction was eluted with chloroform/methanol (1:1). Both polar and apolar fractions were separately hydrolysed and silylated in order to see the differences between the two fractions. The interlaboratory study indicated (table 6) that the LC-separation was not very reproducible. This was obviously due to different conditions and the silica gel chosen by collaborators. The polar fraction was generally too high. But this did not affect final determinations of the contents of emulsifiers which were quantified based on the amount of sorbitan dianhydrides in the polar fraction. The amount of sorbitan monostearate added to the coconut oil was 4.5%. The average results were 3.90% and 3.97% (table 6). Recovery was 87.4%. Better recovery were reported if the quantities of polar fraction used for the gas chromatographic determinations were increased.

Further applications

The procedure described was applied in the co-ordinator's laboratory to analyse other emulsifiers such as lactic acid monoglycerides, glycerol monooleates, sucrose esters of fatty acids, polyglycerol stearate, sorbitan tristearate.
Furthermore fats, oils and waxes (11) were analyzed. The one-pot hydrolysis-silylation procedure described allows the determination of components of very small fractions (< 0.1 mg) of emulsifiers, fats, oils, waxes and other hydrolysable lipids.

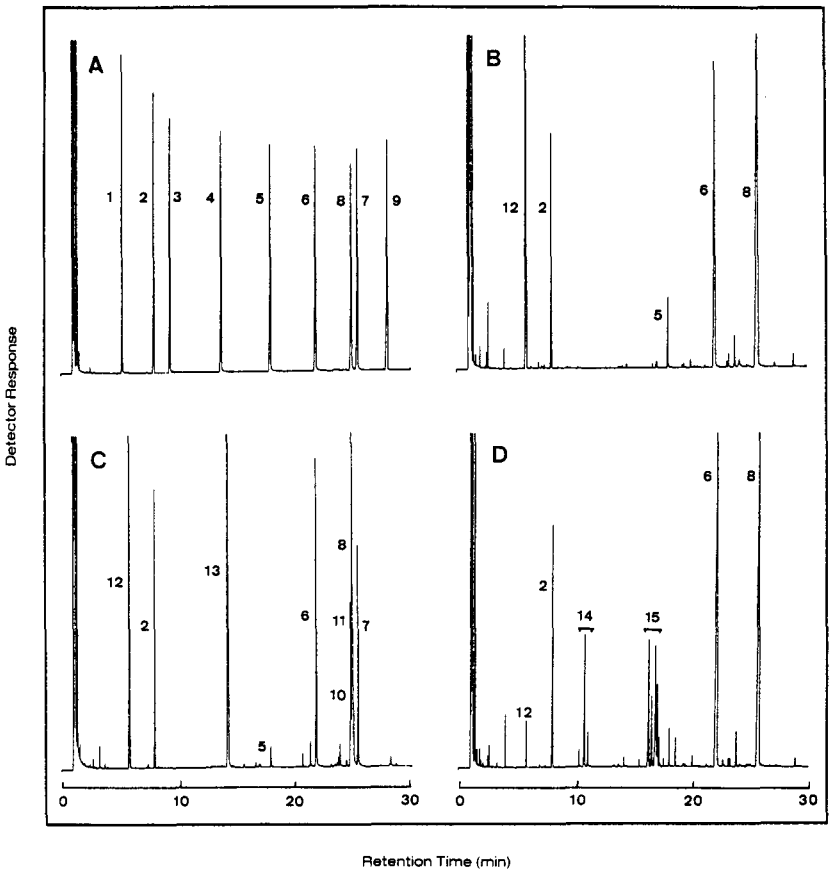

Fig. 1 Gas Chromatograms of Silylated Fatty Acids and Hydrolysed and Silylated Emulsifiers.

A: Mixture of $C_{10:0}$-, $C_{12:0}$-, $C_{14:0}$-, $C_{16:0}$-, $C_{18:0}$-, $C_{18:1}$-fatty acids, n-tetradecane, n-tetracosane, silylated
B: Sample 1: Mono- and diglycerides, hydrolysed and silylated
C: Sample 2: Diacetyl tartaric acid esters of mono- and diglycerides, hydrolysed and silylated
D: Sample 3: Sorbitan monostearate, hydrolysed and silylated

Silylation: Sample: 10 mg; reagents: 0.1ml pyridine containing 1.0 mg n-tetradecane, 0.2ml BSTFA, 0.1ml TMCS; reaction: 20 min at 70 °C.

Hydrolysis: Sample: 10 mg in 2 ml vial, 0.25ml 0.5N ethanolic KOH solution, reaction: 3 h at 80 °C, evaporation of ethanol.

Operation conditions: Ultra #2 (Hewlett-Packard) fused-silica capillary column, 25m x 0.32mm I.D., film thickness: 0.17 μm, col. temp.: 80 ° to 275°C with 5 °C/min, injector temp.: 310 °C, detector temp.: 310 °C, flow: 2.0 ml/min He at 80 °C, det.: FID, sens.: 2 6 (HP 5880A), sample: 2.0 μl, split ratio 1:100.

Peak identification: 1: $C_{8:0}$-fatty acid, 2: n-tetradecane, 3: $C_{10:0}$-, 4: $C_{12:0}$-, 5: $C_{14:0}$-, 6: $C_{16:0}$-, 7: $C_{18:0}$-, 8: $C_{18:1}$- fatty acids, 9: n-tetracosane, 10: $C_{18:2}$-, 11: $C_{18:3}$-fatty acids, 12: glycerol, 13: tartaric acid, 14: sorbitan dianhydrides, 15: sorbitan monoanhydridrides.

Table 6 Results for Sample 4, Sorbitan Monostearate in Coconut Fat, after LC-Separation of Polar Fraction

Lab.	Sample	Test portion	Polar Fraction		Emulsifier calculated from Sorbitan Mono-anhydrid	Sorbitan Di-anhydrid
		mg	mg	%	%	%
				Packed columns		
1	1	1000.0	98.5	9.85	3.00	3.83
	2	1000.0	98.5	9.85	3.93	3.63
2	1	999.2	132.3	13.24	4.96	4.25
	2	1011.2	144.5	14.29	3.82	3.59
3	1	982.2	99.6	10.14	3.19	3.30
	2	1035.6	284.6	27.48	3.39	3.96
4	1	985.4	92.6	9.40	4.63	4.12
	2	1005.6	94.3	9.38	4.77	4.03
5	1	1018.2	93.7	9.20	4.60	4.30
	2	1012.1	93.3	9.22	4.50	4.20
6	1	1110.0	200.0	18.01	4.28	4.50
	2	987.0	220.0	22.23	5.03	4.68
				Capillary columns		
1	1	1000.0	98.5	9.85	3.51	3.59
	2	1000.0	98.5	9.85	3.59	3.59
3	1	982.2	99.6	10.14	2.23	3.57
	2	984.2	127.5	12.95	2.95	4.43
n					8	8
mean					3.90	3.97
Sr					0.456	0.326
CV % (r)					11.69	8.21
S_R					0.845	0.408
CV % (R)					21.68	10.28
Added %					4.50	4.50
Recovery %					86.66	88.2

CONCLUSIONS

On the basis of the results the Commission decided to adopt the method. The text of the standardized procedure is given in the following pages.

6.201 DETERMINATION OF THE ESTER-EMULSIFIERS COMPONENTS CONTENT AFTER HYDROLYSIS AND SILYLATION BY GAS CHROMATOGRAPHY

1. SCOPE

This Standard describes a method for the determination by gas liquid chromatography of ester-emulsifiers components content after hydrolysis (saponification) and silylation.

By this procedure the components determined are:

Acids, or their Na- and K-salts: fatty acids, hydroxy fatty acids, dicarboxylic acids (lactic- , tartaric-, citric acid) glycerol phosphoric acids and inorganic acids (phosphoric-, sulphuric acid) and alcohols and polyalcohols: alcohols (fatty alcohols), glycols, polyglycols, glycerols, polyglycerols, sorbitol and its anhydrides, carbohydrates (mono- and disaccharides).

2. FIELD OF APPLICATION

This Standard is applicable to hydrolysis products of ester emulsifiers concentrates and to emulsifiers separated from oils and fats (Note 1).

The components of the following classes of emulsifiers may be determined:
- esters of fatty acids with hydroxy carboxylic acids (e.g. sodium stearoyl-2-lactylate),
- esters of fatty alcohols with hydroxy carboxylic acids (e.g. sodium stearyl-citrate),
- fatty acid esters of mono- and polyglycols (e.g. 1,2-propylene glycol mono- and diesters of fatty acids, polyoxyethylene-(8)-stearate),
- glycerol esters of fatty acids (mono- and diglycerides, monoglycerides esterified with acetic-, citric-, tartaric-, and lactic acid, esters of polyglycerol)
- sorbitan esters of fatty acids,
- sugar esters of fatty acids,
- lecithin.

3. DEFINITION

The contents of a component of an emulsifier is a quantity determined by the present method and expressed as a percentage by mass relative to the sample.

4. PRINCIPLE

Hydrolysis (saponification) of the emulsifiers with alcoholic KOH solution (Note 1). Evaporation of the alcohol. Silylation of the components. Identification and determination of the trimethylsilyl derivatives by gas liquid chromatography with packed or capillary column.

5. APPARATUS

5.1 Screw cap vials, 2 ml, with conical bottom.
5.2 Screw caps suitable for vials (5.1) with inert plastic faced septa (Notes 2 and 3).
5.3 Volumetric flasks, 10 ml
5.4 Pipettes, 0.1, 0.2, 0.5 ml.
5.5 Microsyringes, 1 - 5 µl.
5.6 Gas liquid chromatograph with flame ionization detector, temperature programming and integrator.
5.7 Packed column, metal or glass, suitable for chromatograph (5.6), about 2 - 3 m long and 2- 4 mm inside diameter filled with either methylpolysiloxane, phenylmethylpolysiloxane or methylphenylvinylpolysiloxane (Note 4) on an acid-washed, silylated, fully deactivated diatomaceous earth (Notes 5 and 6), or
5.8 Capillary column, glass or fused-silica, fully deactivated, suitable for chromatograph (5.6), about 15 - 25 m long and 0.2 - 0.4 mm inside diameter. Film thickness 0.1 - 0.2 µm. Coating: methylpolysiloxane, phenylmethylpolysiloxane or methylphenylpolysiloxane (Notes 4 and 7).
5.9 Heating device for vials (5.1).
5.10 Needles for blowing a gentle stream of nitrogen into the vials (4.1).

6. REAGENTS

6.1 Tetradecane, pure.
6.2 Pyridine, pure, kept over potassium hydroxide (Note 8).
6.3 Trimethylchlorosilane (TMCS).
6.4 Bis(trimethylsilyl)trifluoroacetamide (BSTFA).
6.5 Potassium hydroxide, 0.5 N ethanolic solution.

6.6 Tetradecane in pyridine, internal standard solution.
Accurately weigh about 100 mg of tetradecane (6.1) into a volumetric flask (4.3). Dilute to volume with pyridine (6.2).
6.7 Reference solutions with internal standard.
Accurately weigh about 100 mg of reference substance(s) (alcohols, polyalcohols, fatty acids, hydroxycarboxylic acids, or their sodium or potassium salts) into a volumetric flasks (5.3). Add about 100 mg accurately weighed tetradecane (6.1). Dilute to volume with pyridine (6.2). Several substances (e.g. fatty acids) may be present in a reference solution.
6.8 Carrier gas: helium, dry, free from impurities.
6.9 Auxiliary gases: hydrogen, free from organic impurities, air or oxygen.
6.10 Nitrogen.

7. PROCEDURE

7.1 Hydrolysis (saponification) of sample solutions

Accurately weigh about 10 mg of the homogenized sample into a vial (5.1).
By means of a pipette (5.4) add 0.25 ml of the ethanolic potassium hydroxide solution (6.5) (Note 9). Put the reaction vial into a heating device (4.9) at a temperature of 70 °C. The hydrolysis is completed after about 3 hours. By means of a needle (5.10) evaporate carefully all the ethanol in a stream of nitrogen (6.10).

7.2 Silylation of sample solutions

By means of a pipette or syringe (5.4) add to the dried hydrolysed product 0.1 ml internal standard in pyridine (6.6), 0.2 ml BSTFA (6.4) and 0.1 ml TMCS (6.3) (Note 10). Humidity must strictly be excluded. Close the vial. Shake vigorously. If necessary destroy skin formed and remove substances from the glass wall with a small glass rod which is broken off after use. Heat the reaction mixture for about 20 minutes at 70 °C.

7.3 Chromatography of sample solutions

The supernatant liquor of the silyl derivatives should be injected soon after the derivatization process. Carry out two determinations, each consisting of duplicate injections of the test solutions and record the areas of the peaks.

The operating conditions with gas chromatograph (5.6) and columns (5.7 and 5.8) should be the following:

-injector temperature : 310 °C
-detector temperature : 310 °C
-column temperature : initial 80 °C
program rate 5 - 10 °C/min
final 275 °C.
-with packed column : flow rate of carrier gas 30 ml/min
-with capillary column: flow rate of carrier gas: 1 to 3 ml/min (measured at 80 °C)
-injection by means of
a microsyringe (5.5) : 1 to 5 µl (Note 11).

7.4 Identification

Identify peaks by comparison of retention time of known substances in reference solution (6.7) after silylation and chromatography under the same conditions as the sample or apply coupled gas chromatography and mass spectrometry.

7.5 Silylation and chromatography of reference solution

By means of a pipette (5.4) transfer 0.1 ml of the reference solution (6.7) into a vial (5.1).
With a pipette (5.4) add 0.2 ml BSTFA (6.4) and 0.1 ml TMCS (6.3) (Note 10).
Humidity must strictly be excluded. Close the vial. Shake vigorously.
If necessary remove substances from the glass wall. Heat the reaction mixture for about 20 minutes at 70 °C.
The supernatant liquor of the silylated reference solution should be injected soon after the derivatization process.
Operate under the same conditions as for the sample solution (7.3). Use duplicate injections and concentration ranges of reference substances as to be quantified in sample.
Check response factors periodically.

8. EXPRESSION OF RESULTS

8.1 Response factor

Calculate response factor of the reference substance vs. internal standard using the reference solution chromatogram. The value of the response factor is given by the formula:

$$\underline{R}_x = (\underline{m}_{is}/\underline{m}_x) \times (\underline{A}_x/\underline{A}_{is})$$

where:
$\underline{R}_x$: response factor of reference substance x
$\underline{m}_{is}$: mass, in mg, of internal standard
$\underline{m}_x$: mass, in mg, of reference substance x
$\underline{A}_x$: peak area of reference substance x
$\underline{A}_{is}$: peak area of internal standard

8.2 Calculation of sample component content

Calculate percentage of mass content of component x in the sample by the formula:

$$\underline{m}'_x(\%) = 1/\underline{R}_x \times (\underline{m}'_{is}/\underline{m}'_s) \times (\underline{A}'_x/\underline{A}'_{is}) \times 100\ \%$$

where:
$\underline{m}'_x$: percentage of mass of component x in sample
$\underline{R}_x$: response factor of component x in sample
$\underline{m}'_{is}$: mass, in mg, of internal standard in sample
$\underline{m}'_s$: mass, in mg, of sample
$\underline{A}'_x$: peak area of the component x in sample
$\underline{A}'_{is}$: peak area of the internal standard in sample

9. NOTES

1. If emulsifiers are present in oils and fats, the polar emulsifiers are first separated from the triglycerides by method 2.507 "Determination of polar compounds in frying fats", or by method 2.321 "Determination of mono-, di- and triglycerides by column chromatography". The triglycerides are removed with five 60 ml portions of benzene and the emulsifiers are eluted with five 60 ml portions of dichloromethane/methanol (2/1, V/V) solvents. Finally phospholipids are eluted with three 60 ml portions of methanol. It is recommended that the triglycerides extracted from the column are also hydrolysed and silylated in order to recognize the difference between the polar and apolar fractions.

2. Screw cap vials with magnetic stirrer or ultrasound may be applied.

3. Teflon faced septa are suitable.

4. Silicones OV-1, OV-17, SE-52, SE-54 are suitable.

5. Chromosorbs G, AW-DMCS is suitable.

6. The stationary phase has to be fully deactivated using an excess of silylating agents, otherwise adsorption of trimethylsilyl fatty acid derivatives occurs. The deactivation can be tested with a mixture of hydrocarbons such as hexadecane (C_{16}), eicosane (C_{20}), tetracosane (C_{24}), octacosane (C_{28}) and silylated fatty acids such as decanoic acid (C_{10}), tetradecanoic acid (C_{14}), octadecanoic acid (C_{18}) and docosanoic acid (C_{22}). The peak of the corresponding hydrocarbons and TMS-esters of fatty acids should have about the same height (Donike test). Injection of silylating agents may lead to the required deactivation of the columns.
Higher fatty acids and unsaturated fatty acids may show greater discrimination. Faster heating rates or higher initial temperatures may reduce these effects. Fatty acid methyl esters are to be preferred.

7. Capillary columns are preferred, as the $C_{18\ 0}$-, $C_{18\ 1}$-, $C_{18\ 2}$-fatty acids are well separated and can be quantified.

8. Instead of pyridine solvents such as dimethylformamide, acetonitrile, tetrahydrofurane, hexane or mixtures of them may be used.

9. The amount of ethanolic KOH solution specified is enough to hydrolyse (saponify) an acidic diacetyl tartaric acid glyceride with a saponification value of 490. For better dissolution of some emulsifiers an addition of 0.1 ml of water is advantageous.

10. Salts of hydroxylic acids such as lactic-, tartaric- and citric acids are better and more completely silylated with the BSFTA + TMCS-combination than with hexamethyldisilazane (HDMS) and TMCS.

11. For on-column injection, or direct injection, dilute 50 µl of reaction mixture (8.2; 8.5) with 1 ml hexane and, by means of microsyringe (6.5), inject supernatant (1 µl). In order to lengthen the life time of the column, applying on-column injections, a pre-column is useful.

WARNING – PYRIDINE

The danger from non-purified pyridine is greater than from the pure reagent, the associated homologues and impurities being generally more toxic than pyridine itself. Odour and irritation furnish clear warning of a vapour concentration likely to be dangerous.

APPENDIX

In some cases names commonly used in emulsifier terminology do not conform with IUPAC recommendations. The following table lists IUPAC equivalents for some names used in the present document.

	IUPAC Name
Behenic acid	Docosanoic acid
Stearoyl-2-lactylate	2-*O*-(2-*O*-Stearoyllactoyl)lactate
Capric acid	Decanoic acid
Diacetyl tartaric acid	2,3-Di-*O*-acetyltartaric acid
Polyoxyethylene(20)sorbitan	Mixture of ethoxylated glucitol and anhydroglucitols (molar ratio glucitol + anhydroglucitols/oxirane = 1/20)
Polyoxyethylene(8)stearate	Mixture of ethoxylated stearic acid (molar ratio stearic acid/oxirane = 1/8)
Propyleneglycol	Propane-1,2-diol
Ricinoleic acid	12-Hydroxy-*cis*-octadec-9-enoic acid
Sorbitan	Mixture of glucitol, 1,4-, 2,5-, 3,6-anhydroglucitol, and dianhydroglucitols

REFERENCES

1. Food and Agriculture Organization of the United Nations, Specification for Identity and Purity, Rome 1978.
2. Sahasrabudhe, M.R., J. Am. Oil Chem. Soc. 1967, 44, 376-378
3. Schütze, I., Die Nahrung 1977, 21, 405-415
4. Sahasrabudhe, M.R., J. Am. Oil Chem. Soc. 1969, 46, 8-12
5. Tsuda, T., Nakanishi, H., Kobayashi, S. and Morita, T., J. Assoc. Off. Anal. Chem. 1984, 67, 1149-1151
6. Tsuda, T. and Nakanishi, H., J. Assoc. Off. Anal. Chem. 1983, 66, 1050-1052
7. Buxtorf, U.P., Brüschweiler, H. and Martin, E., Z. Lebensm. Unters. Forschung 1982, 175, 25-28
8. IUPAC, Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th Ed., C. Paquot, Blackwell Sci
9. Pocklington D., Guidelines for the Conducting of Collaborative Study, IUPAC, Commission on Oils, Fats and Derivatives, September 1984.
10. ISO 5725, Precision of Test Methods, Determination of Repeatability and Reproducibility by Inter-Laboratory Test, 1981
11. Brüschweiler, H., Felber, H. and Schwager, F., Fat Sci. Technol, 1989, 91(2), 73-79

Acknowledgements

The Commission wishes to express its thanks to collaborators in Denmark, Germany FR, Japan for their participation and valuable co-operation.