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A method for the estimation of sucrose esters (E473) in foods using gas chromatography-mass spectrometry

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1 A method for the estimation of sucrose esters (E473) in foods using gas
2 chromatography-mass spectrometry
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Abstract

A method has been developed for estimating the content in foods of the emulsifying additive E473, sucrose esters of fatty acids. The analytical approach taken to estimate the complex mixtures that comprise this additive involved, selective solvent extraction of the intact esters using a mixture of tetrahydrofuran and ethyl acetate, alkaline hydrolysis of the esters to liberate sucrose, and then GC-MS measurement of the liberated sucrose using GC-MS after acidic hydrolysis to glucose and fructose and then silylation. The method was developed to aid future estimates of intake of this food additive. The method determines the total sucrose esters content of a food sample and does not attempt to discrimination between individual sucrose esters when present as a mixture in a food sample. A single (average) factor is used to convert the liberated sucrose content into sucrose ester content. The method was applied to analysis of 8 different food types (including bakery wares, sugar confectionery, dairy product, margarine, meat pies and a sauce) spiked with 0.5 to 1% of a mixture of 3 sucrose esters that spanned the hydrophilic/lipophilic balance (HLB) range 1-16. The limit of quantification was around 50 mg/kg, which is more than adequate for these additives. The analytical recovery was 73 - 106% with an average of 91%. The precision of the method (RSD) was 6 - 18% (n= 3 to 20 for each food type) with an average RSD of 11%. The main analytical uncertainty is the conversion factor used to express sucrose ester content from the amount of sucrose liberated. The method is also applicable to sucroglycerides (E474).

Keywords: food, additives, emulsifiers, analysis, sucrose esters, E473, E474, gas chromatography, mass spectrometry, GC-MS, intake estimates.

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1 Introduction

2 Sucrose esters of fatty acids (SuE, E473) are a group of chemically similar additives
3 controlled in Europe by Directive 95/2/EC (European Parliament, 1995). They may be used
4 as emulsifiers in a wide range of foods, including bakery products, cakes, beverage whiteners,
5 desserts, confectionery, sauces and dairy-based drinks. Sucrose esters are defined in Directive
6 96/77/EC (as amended) as the mono-, di- and tri-esters of sucrose with fatty acids occurring in
7 food fats and oils (EU, 1998). They may be prepared from sucrose and the methyl and ethyl
8 esters of food fatty acids or by extraction from sucroglycerides. Sucroglycerides (E474) are
9 produced by reacting sucrose with edible fat or oil to produce a similar mixture of SuE with
10 fatty acids together with residual mono-, di- and tri-glycerides from fat or oil (Figure 1). For
11 the purposes of this work, E473 and E474 are considered to be chemically equivalent.

13 These two groups of emulsifiers comprise a range of products from stiff gels, soft solids to
14 white or slightly greyish-white powders. They are sparingly soluble in water but soluble in
15 ethanol and other organic solvents, mineral oil and vegetable oils. SuE and sucroglycerides
16 are permitted in a wide range of foodstuffs under Annex IV of Directive 95/2/EC. SuE are
17 also permitted under Annex V as a carrier for food colours and fat-soluble antioxidants, and
18 under Annex VI Parts 1 and 2.

20 These additives have been considered several times by the Joint FAO/WHO Expert
21 Committee on Food Additives (JECFA) and have an ADI of 0-30 mg/kg bw, expressed as
22 sucrose ester content (JECFA, 2004). The European Food Safety Authority has also
23 evaluated E473 and E474 recently and a Group ADI of 0-30 mg/kg bw was established
24 (EFSA, 2004).

1 In a review of food additive intake in the European Union (EU, 2001), several additives
2 including E473 were highlighted as in need of refined intake estimates using actual
3 occurrence and consumption data. Therefore a method was needed to measure levels of E473
4 in foods.

6 **Review of published analytical methods**

7 The quantitative analysis of these emulsifiers in food is difficult, primarily because: (i) the
8 multiple components are similar in structure, (ii) most commercial sources of emulsifiers are
9 quite heterogeneous and (iii) emulsifiers can be difficult to extract from foods that contain
10 significant amounts of lipid, starch and/or protein. Several analytical strategies have been
11 employed for the analysis of SuE as such, but there are very few literature references to their
12 determination in foods.

14 *Analysis of the intact esters*

15 Since SuE are liposoluble their measurement in foods requires differentiation of SuE from
16 other lipids/liposoluble materials. The JECFA specifications describe an assay by
17 solubilization in tetrahydrofuran followed by gel permeation chromatography with refractive
18 index (RI) detection (JECFA, 1995). This method is however applicable only to relatively
19 pure and concentrated SuE additive formulations. SuE can be determined by reversed-phase
20 (RP) high-performance liquid chromatography (HPLC) although interferences from fatty acid
21 esters of glycerol were reported (Brauen, Davidson and Salminen, 1990). Sucrose polyesters
22 are a related class of emulsifiers that are synthesized by esterifying 6 to 8 of the hydroxyl
23 groups of sucrose with fatty acids and they exhibit similar physicochemical properties to SuE.
24 Methods of analysis for sucrose polyesters based on the direct analysis of the liposoluble
25 fraction have been developed using supercritical fluid chromatography (Chester, Innis and

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3 1 Owens, 1985) or high performance gel permeation chromatography (Birch and Crowe, 1976;
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5 2 Chase, Akoh and Eitenmiller, 1995). Thin layer chromatography (TLC) has been used (Xie *et*
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8 3 *al.*, 2000) but RP-HPLC is reported to be superior. RP-HPLC-UV was used for the separation
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10 4 and determination of 18 different SuE and sucrose polyesters, unreacted sugar and other fatty
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12 5 acid esters (Yang *et al.*, 2000). RP-HPLC with light-scattering detection and LC-MS
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14 6 confirmation was used for the separation of SuE isomers (Moh, Tang and Tan; 2000).
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19 8 Specific methods for the analysis of mono- and di-SuE have been developed, notably that
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21 9 reported by Murakami, Marayame and Niiya (1989) who determined mono- and di-SuE in
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23 10 food by HPLC with UV detection. Samples were extracted with THF, the extracts purified by
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25 11 chromatography on silica gel and derivatised with dinitrobenzoyl chloride. SuE were reported
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27 12 to be detected without interference. Recoveries were 75-80% and the detection limit was 10
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29 13 mg/kg for each ester. High temperature (400°C) GC-FID has been used to analyse SuE as
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31 14 trimethylsilyl derivatives (Karrer and Herberg, 1992). The method performance was checked
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33 15 by supercritical fluid chromatography (SFC) and direct chemical ionisation MS of standard
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35 16 compounds, and allowed high molecular weight carbohydrate derivatives (1500 Da) to be
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37 17 determined whilst maintaining high separation efficiency.
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45 19 More recently, Uematsu *et al.* (2001) have reported a GC/ GC-MS method for sucrose
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47 20 monoesters of fatty acids and sucrose acetate isobutyrate (SAIB) in food additive premixes
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49 21 and some foods. A reverse-phase SPE cartridge was used to extract mono-SuE and SAIB
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51 22 from aqueous samples. A silica-gel SPE cartridge removed diglycerides from mono-SuE in
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53 23 THF extracts of solid / fatty samples. Mono-SuEs based on fatty acid residues of C₁₂, C₁₄, C₁₆,
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55 24 C₁₈ and C_{18:1} were acetylated and determined using wide-bore capillary GC with splitless
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57 25 injection and flame-ionization detection. Peak identities were confirmed using GC-MS where
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the mono-SuEs were characterised using 6 fragment ions. The analyte peaks were clearly separated from acetylated derivatives of sucrose, tocopherols, diglycerides and triglycerides. None of these direct methods were considered here to be suitable for the routine analysis of SuE in different types of foods.

Analysis following hydrolysis

Other workers have used indirect approaches following hydrolysis. The liposoluble fraction of foodstuffs was extracted with petroleum ether, which was then saponified by methanolic KOH (Drake, Nagel and Swanson, 1984). The liberated sucrose was extracted with water and quantified colorimetrically using a phenol-sulfuric acid test. Koh *et al.* (1997) determined the composition of commercial SuEs using TLC and gas chromatography (GC) and subsequently developed an indirect method for their analysis in certain foods (crackers, sausage and ice cream) using ion-exchange HPLC with pulsed amperometric detection (PAD) and RI detection of free sucrose following alkaline hydrolysis. The liposoluble fraction of the foods was extracted with a selective mixed solvent of ethyl acetate (EtOAc) and tetrahydrofuran (THF) (7:3 v/v), and purified using diol SPE cartridges prior to hydrolysis. The recovery was 86-99% and the limit of detection for sucrose using PAD was 4 mg/kg. Such methods however did not discriminate between the different mono-, di- and tri- esters nor did any allow for identification of the fatty acid residues associated with the SuE.

For the purposes of estimating concentrations of SuE in a wide range of foods for intake estimates, it was considered that the degradation/analysis approach was likely to be the most successful.

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Materials and methods

Reagents were of recognized analytical grade unless specified otherwise. Tri-Sil[®] Z was obtained from Perbio Science UK Ltd., Cramlington, UK. Fourteen sucrose ester materials were obtained from a European (4 samples) and a Japanese (10 samples) manufacturer. These comprised samples with hydrophilic-lipophilic balance (HLB) values ranging from 1 to 16 (Table 1).

Extraction

Food samples were homogenised prior to weighing 1.0 g (or 0.25 g for margarine) portions directly into a 40 ml glass vial. Samples containing high amounts of gelatin and/or starch were mixed with 2 mL of water prior to solvent extraction. Mixed extraction solvent (tetrahydrofuran: ethyl acetate , 7:3 v/v, 10.0 mL) was added. The mixture was blended using an Ultra Turrax[®] model T-25 probe at 4,000 to 10,000 rpm for 30 seconds. The vial was placed on a horizontal shaker for 30 minutes, then centrifuged at 700-1000 x g at 20°C for 5 minutes. The supernatant was transferred to a 40 ml glass vial containing saturated sodium sulfate solution (10 mL) and the vial placed on the shaker for 20 minutes. The mixture was centrifuged at 700-1000 x g at 20°C for 5 minutes and the supernatant transferred to a 12 ml vial.

Alkaline hydrolysis to liberate sucrose

The solvent was removed under a stream of nitrogen on a dry-block heater set at 70 ± 2°C. The residue was not allowed to remain in the dry state any longer than necessary. A mixed solvent was added (butanol: hexane, 1:1 v/v, 2 mL) and the vial shaken to dissolve the residue. Hydrolysis of the esters was achieved by adding sodium hydroxide solution (4M, 2 mL) and shaking for 40 minutes. The lower aqueous layer was transferred to a 4 ml glass vial and the upper solvent layer discarded.

1 *Acidic hydrolysis to glucose and fructose and their derivatisation*

2 A portion (100 μ L) of the hydrolysed extract was placed in a 4 mL glass vial along with
3 fucose internal standard solution (0.1 mg/mL, 100 μ L) and hydrochloric acid (6M, 125 μ L).
4 The solvent was removed at 50°C under a stream of nitrogen until a dry residue was obtained.
5 The addition of a small volume (1-2mL) of acetonitrile to the vial aided this drying.
6 Immediately on drying, Tri-Sil® Z derivatising agent (250 μ L) was added to the vial which
7 was then capped and heated at 70°C for 30 minutes. The derivatised sample was then cooled
8 and transferred to a GC-MS autosampler vial.

10 *GC-MS analysis*

11 The GC-MS was a Hewlett-Packard Model 5890 series II capillary GC with model 7673
12 autosampler and split/splitless injector, model 5971 mass selective detector (Agilent
13 Technologies UK, Stockport). The column was an HP-5MS (5%-Phenyl-methylpolysiloxane)
14 fused silica column, of dimensions 30m x 0.25mm i.d. x 0.25 μ m d.f. (Esslab, Hadleigh, UK).
15 The column temperature was programmed from 80°C (held 1 min) at 5°C/min to 200°C (held
16 3 min). The carrier gas was helium at 1ml/min. The injection volume was 1 μ l in splitless
17 mode at 280°C. The MS was operated in impact mode and the ions monitored were m/z 204
18 and 217.

20 **Calculation of sucrose levels in samples**

21 Due to isomeric effects, 2 main peaks were observed for each of the substances monitored -
22 glucose and fructose as analytes and fucose as the internal standard. The 2 peaks were
23 integrated and the areas summed to give a single area value for each substance. Using sucrose
24 solutions of 10 and 0.1 mg/mL in water, a series of calibration standards was prepared by

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1 pipetting volumes appropriate to 0, 1, 5, 10, 25, 50 and 125 µg of sucrose into separate 4 mL
2 vials. Internal standard and HCl were added and the standards then treated as for the samples.
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4 The sample/internal standard peak area ratio values derived for calibration standards were
5 plotted against the concentration of sucrose. The correlation coefficient was 0.996 or better.
6 The peak area ratios for the samples were calculated and the sucrose concentration
7 interpolated from the calibration graph. The sucrose ester content was calculated using the
8 following equation: Sucrose ester content (mg/kg) = Sucrose content (mg/kg) x 4.5.

10 **Results and discussion**

11 Since E473 (and E474) emulsifiers are not single substances, it was necessary to chemically
12 characterise appropriate reference materials and a range of additive formulations. The key
13 aspects of available analytical methods were taken forward for development whilst ensuring
14 that the developed method would be readily accessible to a wide base of users. The analytical
15 strategy was based on selective solvent extraction of SuE from food followed by a clean up
16 procedure to remove unwanted co-extractives using solvent partition. Candidate end
17 determination methods considered included colorimetry and gas chromatography, as these
18 offered a lower degree of complexity and were considered as relatively easy to roll out to
19 other laboratories. It was recognised that analysis of intact SuE would be complicated by the
20 wide range sucrose esters that may be present, so hydrolysis of SuE and measurement of the
21 released sucrose was investigated, with derivatisation and gas chromatography considered the
22 upper limit of desired complexity for this aspect.
23
24 The most appropriate way to express the results in terms of SuE content based on sucrose
25 measured, is to apply a conversion factor, based on the sucrose contents of available standard

1 SuE. Theoretically, SuE over the entire HLB range (1-16) could contain between *ca.* 22.3 and
2 52.2% sucrose by weight. The calculated arithmetic mean conversion factor falls around the
3 centre of the HLB scale and could therefore be used as an 'average' conversion factor.
4 However, given that the main purpose of determining the SuE content of foods is for
5 estimating intakes, the use of a conversion factor for higher esters of sucrose (low HLB)
6 would seem appropriate because it would incur an overestimation of the SuE content. Thus a
7 factor of 4.5 was chosen to convert from sucrose content to SuE content (i.e. a ratio of *ca.*
8 100/22.3). This give an upper-bound estimate of SuE content in foods. If information was
9 available for a particular type of SuE used then the more relevant conversion factors could be
10 used.

12 **Preparation and homogeneity testing of an in-house validation material (IHVM)**

13 A pack of (6) mini Cornish pasties were finely chopped in a food processor and a portion (500
14 g) was mixed with water (200 mL) to facilitate further homogenisation. A 250 g portion of the
15 homogenate was spiked with a mixture of 3 different commercial sucrose esters dissolved in
16 THF:EtOAc (7:3 v/v). The spiking details are given in Table 2. The total SuE content was
17 6,060 mg/kg. The IHVM was stored at -20°C .

19 Sucrose was spiked into Cornish pasty homogenate (i.e. blank IHVM) at 1 and 20 mg/g and
20 this was analysed to determine the efficacy of free sucrose removal from the organic extract
21 prior to hydrolysis. No sucrose was detected in either sample and no peaks due to sucrose
22 were observed in the chromatogram from a reagent blank. An example chromatogram from
23 blank IHVM spiked with 20 mg/g sucrose is shown in Figure 2. Two batches of ten replicate
24 sub-samples of the IHVM were extracted on two separate days. The results are given in
25 Table 3.

Statistical analysis of the mean recovery figures from each batch showed that they were not significantly different (t-test at $p=0.05$). A control chart was established over the ± 2 s.d. range based on a mean recovery of 81 % (Figure 3) for use in subsequent batch analyses of test samples. An example of a chromatogram obtained from the IHVM is shown in Figure 4.

Analysis of the blank IHVM (before spiking) showed that the saturated sodium sulfate / solvent partition was effective at removing free sucrose present at high level and that co-extracted lipid from the reasonably high fat product used to prepare the IHVM (Cornish pasty) did not interfere with the subsequent hydrolysis, derivatisation and GC-MS stages. The results from the repeat analyses show that the IHVM was homogeneous and that the analysis was repeatable.

Analysis of food samples

The application range for sucrose esters was categorized into 5 main food commodity groups and at least one commodity was selected to cover the range of applications stated by the major manufacturer. Since SuE usage in the UK is very sparse, commodities were spiked at levels in line with those appropriate to the maximum permitted. Samples were purchased locally and the results are given in Table 4. Recoveries of SuE spiked into foodstuffs at a concentration of between 0.5 to 1.0 % were in the range 73% (soft mint sweets) to 106% (ice cream). The mean recovery for all commodities was 91%, with a mean RSD of 11%.

Quality assurance

Samples were analysed on a batch basis with at least one IHVM sample. The recovery values for the IHVM shown on the chart in Figure 3 show that the method was essentially under analytical

control. The limit of quantitation (LOQ, s:n 10:1) was 12 mg/kg as sucrose, equivalent to 54 mg/kg sucrose ester. The GC-MS chromatograms were free of any significant interferences. During method development, some sample extracts exhibited poor recovery for the internal standard, this was in most instances due to insufficient drying of the acidic hydrolysate prior to derivatisation.

The sample matrices that presented the most difficulties were marshmallow biscuit and margarine. The presence of gelatine and starch in the marshmallow biscuit product caused the sample to congeal during the initial solvent extraction, thereby compromising SuE extraction. This was ameliorated by the addition of 2 mL of water to the 1 g sample and homogenising prior to solvent extraction. Although the mean recovery of 97% for this commodity was very good, the higher RSD value obtained (18%) reflected the difficulty with analysis of this matrix. The high fat content of margarine effectively neutralised the sodium hydroxide and so the hydrolysis of SuE failed. By taking a smaller sample size (0.25 rather than 1 g) the analysis of margarine was successful and the recovery was very good (mean 101%).

The results from this study suggest that E473 and E474 have very limited use in the UK, borne out by the difficulty in obtaining samples. Nevertheless, the developed method provides a means by which the total sucrose ester contents of a limited range of food commodities may be estimated at levels well below the maximum levels specified in the Council Directive. This is particularly relevant to the generation of intake data for E473/474.

The factor for converting the free sucrose content to SuE content was based on the SuE with the lowest sucrose content (i.e. higher degree of esterification) and was adopted on the grounds that its use effectively gives an over estimation of lower esters but does err on the

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1 side of caution with respect to public protection. The alternative was to express the results as a
2 range (based on factors derived from the highest and lowest theoretical sucrose contents
3 across the HLB range). This was considered to be less appropriate for monitoring SuE intake.
4

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5 paper are the authors alone and should not be taken to represent the opinion of the FSA.
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17 esters by HPLC and thin-layer chromatography scanning. *Fenxi Ceshi Xuebao*, **18**, 28-30.

Table 1. Sucrose ester reference materials

SUE standard	Composition	Fatty acid	Source
Monoester P	95% 5%	monopalmitate dipalmitate	Japan
Monoester L-595	29-33 38-42 25-29	monolaurate dilaurate trilaurate	Japan
Sugar Ester L-1695	78-81 14-15 <3	monolaurate dilaurate trilaurate	Japan
Sugar Ester S-170	N/A N/A N/A N/A	hexastearate pentastearate heptastearate tetrastearate	Japan
Sugar Ester S-570	18-20 8-9 23-25 15-17	monostearate dipalmitate distearate tristearate	Japan
Sugar Ester S-1170	37-39 20-22 14-15 7-8 13-19	monostearate distearate monopalmitate dipalmitate alkylate	Japan
Sugar Ester S-1570	46-49 14-15 14-16 5-6 7-16	monostearate monopalmitate distearate dipalmitate alkylate	Japan
Sugar Ester S-1670	N/A		Japan
Sugar Ester P-1670	N/A		Japan
Sugar Ester P-1570	N/A		Japan
Sisterna PS750	N/A N/A	monopalmitate monosteate	Europe
Sisterna SP70	N/A N/A	monosteate monopalmitate	Europe
Sisterna SP50	N/A N/A	monosteate monopalmitate	Europe
Sisterna SP30	N/A N/A	disteate monopalmitate	Europe

[The first letter code L, S or P specifies the fatty acid (lauric, palmitic, or stearic), the first number codes (where four are given) are the HLB value 1-16 and the last two numbers are the percentage of SuE in the formulation. N/A = Not available.]

Table 2. IHVM spiking details

Sucrose ester	HLB value	Amount added (mg)	Spike level in IHVM (mg/kg)
L1695	16	504	2016
S570	5	502	2008
S170	1	509	2036

Table 3. Homogeneity and recovery of SuE in IHVM (n=10)

Batch No.	Mean recovery (%)*	Standard deviation	RSD (%)
1 (week 1)	89	5.8	6
2 (week 2)	73	5.8	6

[*Based on a mean theoretical sucrose content of 45%]

Table 4. Recovery of SuE from spiked food commodities.

Category	Sample	SuE Spike level (%)	n	Mean recovery (%)	RSD (%)
1. Fine bakery wares	(1) Fruit scone	0.5	6	88	6
	(2) Marshmallow biscuit (i)	0.75	3	97	18
	Marshmallow biscuit (ii)	0.75	6	93	15
2. Sugar confectionery	Soft mint sweets	0.5	6	73	12
3. Dairy products	Ice-cream	0.75	3	106	8
4. Processed fats & oils	Margarine	1.0	6	101	12
5. Other	(1) Sausage roll	1.0	5	80	10
	(2) Cornish pastie	0.6	20	81	6
	(3) Stir-fry sauce	0.5	6	96	11

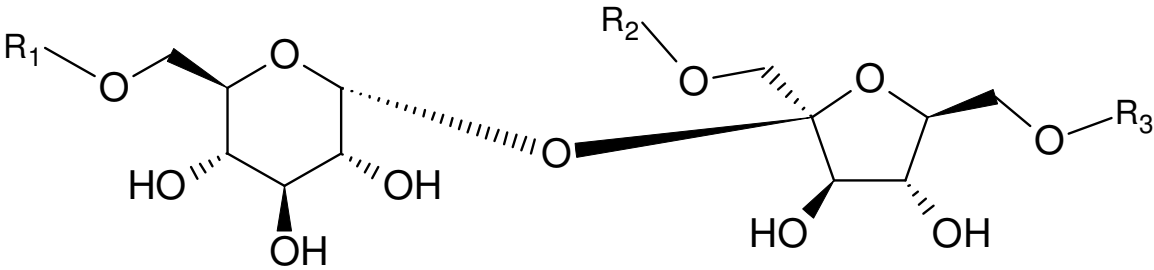
LEGENDS FOR FIGURES

Figure 1. Chemical structure of sucrose esters. R_1 - R_3 are usually mixed residues of $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and/or $C_{18:1}$ fatty acids.

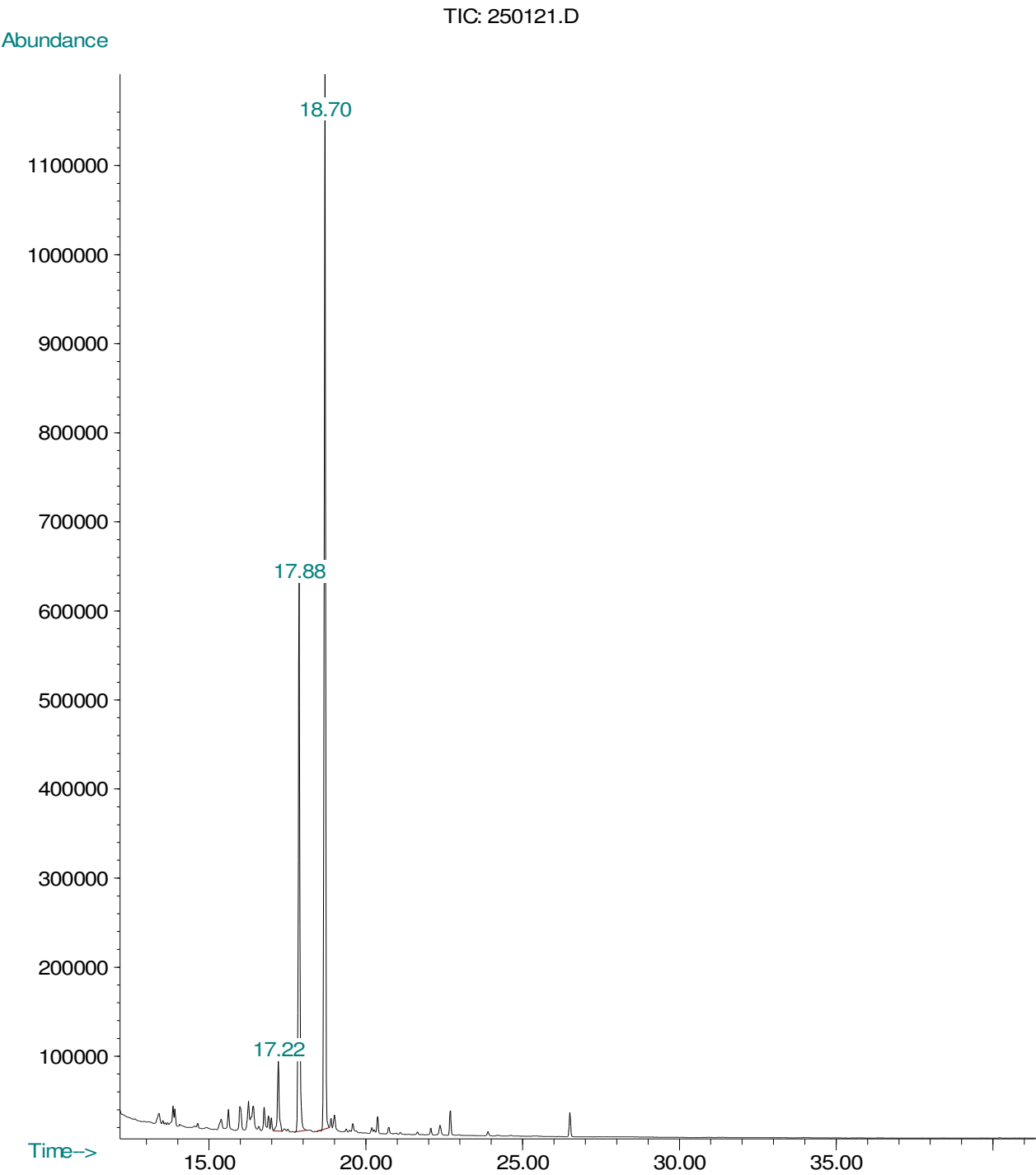
Figure 2. GC-MS analysis of blank IHVM spiked with sucrose at 20 mg/g. The absence of analyte peaks demonstrates that the clean-up was effective. The peaks between 17 and 19 minutes are fucose internal standard.

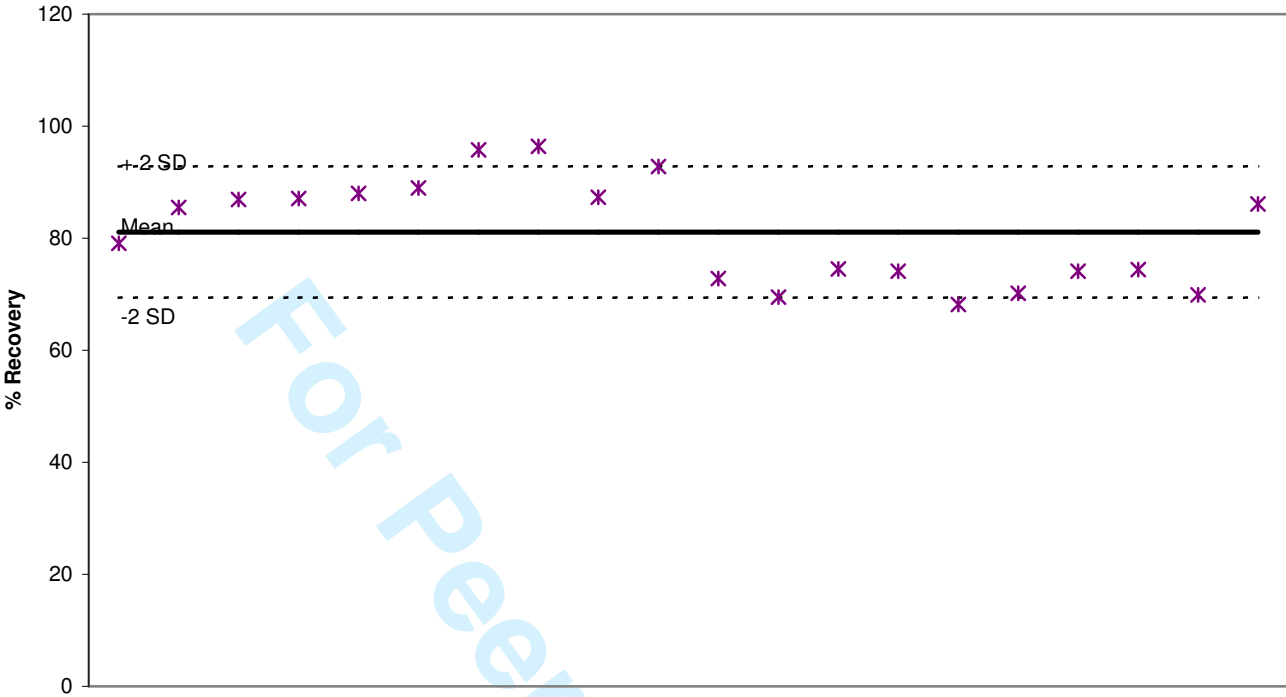
Figure 3. Control chart for batch analysis of sucrose esters based on the IHVM.

Figure 4. GC-MS analysis of IHVM extract. Peak identification: Fructose (20.9 and 21.1 min) and glucose (21.8, 22.7 and 24.6 min).



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