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Migration of lubricants from food packagings Screening for lipid classes and quantitative estimation using normal-phase liquid chromatographic separation with evaporative light scattering detection

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Abstract

A normal-phase high-performance liquid chromatography (NP-HPLC) method is introduced for the identification and quantitative estimation of 12 lipid classes (paraffin, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amide) used as lubricantsin food packaging materials. The HPLC separation is carried out on a LiChrospher® Diol (100 Å, 5 μm, 125 mm × 3 mm) column with gradient elution (isooctane/0.1% acetic acid in *tert*-butyl methyl ether) and evaporative light scattering detection (ELSD). The method has been calibrated with representatives of each class in working ranges of about 5–150 mg/l, depending on the lipid class. Intra-day variance for all representatives range from 1.9 to 5.1%, inter-day variances from 7.0 to 26.5% and the limits of detection from 0.79 to 3.65 mg/l (except for two classes). A simple sample preparation could be established for the determination of migrating lubricants obtained from packaging materials containing external or internal lubricants. Since the detector response depends on the chain length and the degree of saturation, the quantification of a lipid class with unknown composition is only semi-quantitative. The amount of migrating lubricants from an epoxy-based can coating could be estimated with 0.3 mg/dm^2 and from a light weight container with 5.5 mg/dm^2.

Keywords: Evaporative light scattering detection; Food packaging; Migration; Lipids; Lubricants

1. Introduction

1.1. Presence of different lipid classes as lubricants in food packagings

Lubricants are used as additives for the production of coated or laminated packaging for different reasons. External lubricants may be applied to the surface of

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the finished packaging in order to enable the forming process and to minimise the adhesion of food components to the packaging. Internal lubricants are added to liquid lacquer-compositions in order to enhance the elasticity, which is required for the deep-drawing process [1]. Slip additives used for plastic films belong to this group of internal lubricants. They are added to plastic formulations where they gradually tend to migrate to the surface preventing the adhesion between films by reducing friction [2,3].

Useful substance classes for internal and external applications are waxes, paraffins, fats and oils as well as partial acyl glycerols or fatty acid amides. Lubricants are mostly technical mixtures of isomers and analogues of one chemical class (e.g. paraffin) or natural products of diverse compositions (e.g. waxes or oils). Natural waxes like carnauba wax can also contain long-chain aliphatic alcohols, free fatty acids, cholesterol or cholesterol esters beside the main class fatty acid ester [4]. Partial acyl glycerols can also include fatty acid methyl esters [5]. In consequence, 12 lipid classes (Table 1) are expected to be found in migrates of food packagings. Their identification and quantitative estimation will enable the elucidation of the composition of the total migrate. Concerning the variety of possible components in each class a chromatographic separation method has to be developed for the determination of these classes avoiding a specific separation into the single substances forming these classes.

Table 1
Investigated lipid classes and standard substances as representatives

	Lipid class	Abbreviation	Representative
1	Paraffin	PAR	Paraffin (liquid)
2	Wax ester	WE	*n*-Hexadecyl palmitate
3	Cholesterol ester	CE	Cholesteryl palmitate
4	Fatty acid methyl ester	FAME	Stearic acid methyl ester
5	Triacyl glycerol	TAG	Glycerol tripalmitate
6	Fatty alcohol	FOH	Hexadecyl alcohol
7	Free fatty acid	FFA	Stearic acid
8	Cholesterol	CHOL	Cholesterol
9	1,3-Diacyl glycerol	1,3-DAG	Glycerol-1,3-dipalmitate
10	1,2-Diacyl glycerol	1,2-DAG	Glycerol-1,2-dipalmitate
11	Monoacyl glycerol	MAG	Glycerol monopalmitate
12	Fatty acid amide	FAA	Erucylamide

1.2. Previous work

Hitherto, examinations have mostly been carried out in order to determine external lubricants like paraffin and waxes using GC–FID [6], LC–GC–FID-coupling [7,8] and SFC–FID-techniques [9,10]. The presence of different slip additives like erucylamide was analysed by GC–FID in food simulants [3]. Non-specific separation methods for lipids have been developed by several authors for different analytical tasks. Bruns [5] determined five lipid classes (FAME, TAG, 1,3-DAG, 1,2-DAG and MAG) on a cyanopropyl-phase in order to control the production of technical partial glycerols. El-Hamdy and Christie [11] analysed the influence of the stationary phase (cyanopropyl-phase) and the eluent on the liquid chromatographic separation of seven lipid classes (CE, TAG, FFA, CHOL, 1,3-DAG, 1,2-DAG and MAG). Based on the optimized method of El-Hamdy and Christie, Foglia and Jones [12] extended the separated lipid classes by FAME and applied this technique to acylglycerides after enzymatic cleavage and subsequent esterification. Nine of the 12 lipid classes mentioned in Table 1 were determined by Elfman-Börjesson and Härröd [13] on a dihydroxypropyl-phase using unusual flash chromatography with a high flow (3 ml/min for a column diameter of 4 mm) and several gradient steps. All of the referenced authors combined the liquid chromatograph with an evaporative light scattering detector (ELSD) to enable the detection of UV-inactive analytes. Additionally, the necessity of gradient elution does not allow the use of a refractive index detector (RID).

The aim of this work was the development of a chromatographic separation for all 12 lipid classes with subsequent ELS-detection using representatives of each class. Within these classes four unpolar lipid classes (PAR, WE, CE and FAME), the medium polar TAG and eight polar lipid classes (FOH, FFA, CHOL, 1,3-DAG, 1,2-DAG, MAG and FAA) are included. The ELS-detection had to be optimized in order to achieve a reasonable precision and low detection limits for all lipid classes. Furthermore, a procedure for the extraction and subsequent purification of both

internal and external lubricants from packagings had to be established.

2. Experimental

2.1. Chemicals

Glycerol monopalmitate (MAG), glycerol-1,3-dipalmitate (1,3-DAG), glycerol-1,2-dipalmitate (1,2-DAG) and glycerol-tripalmitate were obtained from Fluka, Buchs, Switzerland. Cetyl alcohol (FOH), cholesterol (CHOL) and stearic acid (FFA) were obtained from Merck, Darmstadt, Germany whereas cholesteryl palmitate (CE) and *n*-hexadecyl palmitate (WE) were obtained from Acros, Geel, Belgium and paraffin (liquid, PAR) from Riedel-de Haen, Hannover, Germany. Ethanol (99.5%, p.a.) used as simulant and acetic acid were obtained from Merck, Darmstadt, Germany. Isooctane (Biosolve, Valkenswaard, Germany) used as simulant and for chromatography as well as *tert*-butyl methyl ether (MTBE), 2-propanol and *n*-heptane (Merck, Darmstadt, Germany) used for chromatography were HPLC grade and were freshly distilled over NaOH and a vigreux column. The water used was bidistilled. All other chemicals were of analytical grade.

2.2. Samples

Tinplate strips (1 cm × 25 cm) coated with a commercial epoxy-anhydride coating containing carnauba wax and a partial acyl glycerol as internal lubricants were obtained from the Valspar Corporation, Grüningen, Switzerland. Aluminium light weight container prepared with an unknown external lubricant were obtained from the Federal Institute of Military Technique and the Supply of the German Armed Forces.

2.3. Apparatus

Separation was performed on an HP1100 (Agilent, Waldbronn, Germany) system equipped with an autosampler (G1313A), an automatic degasser (G1322A), a binary pump (G1312A), a column oven (G1316A) and a variable wavelength detector (G1314A). Data were assessed by Chemstation® software (Rev. A 08.03). Chromatographic separation was carried out at 25 °C on different HPLC columns, a LiChrospher® Diol (100 Å, 5 μm, 125 mm × 3 mm, Merck, Darmstadt, Germany), a Spherisorb® Si (80 Å, 3 μm, 125 mm × 4 mm, Waters, Eschborn, Germany), a Spherisorb® CN (80 Å, 5 μm, 125 mm × 3 mm, Waters, Eschborn, Germany) and Kromasil® RP1 (100 Å, 5 μm, 125 mm × 3 mm, Eka Nobel, Bohus, Sweden). The ELSD (Sedex 75) and a thermostatisable nebulizer chamber were from Sedere, Alfortville, France. A stainless steel in-line filter (0.5 μm mesh) was inserted between the column and detector. Sterilizations were performed in an autoclave Sanoclav (Wolf, Geislingen, Germany) and centrifuged in a Sigma 3K 30 (Sigma Laboratory, Osterode am Harz, Germany).

2.4. Simulation of migration

For migration experiments, approved EU-simulants and simulation conditions (97/48/EC amending 82/711/EEC, 85/572/EEC) were used. The assumed worst case, a sterilization of fatty food for 1 h at 121 °C, has to be performed using the following solvents (approved substitutes for the fatty simulant olive oil) and conditions: 95% ethanol (4 h at 60 °C) and isooctane (2 h at 60 °C). Therefore, coated tinplate strips were folded like a concertina and a sample of four strips (ca. 1 dm^2) was extracted with 50 ml simulant. In case coated strips are not available, empty cans and light weight containers can be extracted. However, it is important to achieve a high surface–volume ratio.

2.5. Optimized sample preparation and chromatographic conditions

2.5.1. Sample preparation for external lubricants

Four milliliters of isooctane migrate (see Section 2.4) was evaporated to dryness under nitrogen stream. The residue was redissolved in 400 μl isooctane and injected directly into the HPLC.

2.5.2. Sample preparation for internal lubricants

Four milliliters of 95% ethanol migrate (see Section 2.4) was brought to dryness under a nitrogen stream. The residue was dissolved in 400 μl ethanol. Subsequently 400 μl isooctane and 1600 μl water were added. The mixture was stirred vigorously. Centrifugation (2 min, 5000 g) was performed to enhance the

separation of two layers. The upper isooctane layer was used for HPLC analysis.

2.5.3. Chromatographic conditions

The separation of the lipid classes was performed on a LiChrospher® Diol (100 Å, 5 μm, 125 mm × 3 mm) column at 25 °C and a flow rate of 0.5 ml/min with (A) isooctane and (B) MTBE/acetic acid (99.9/0.1, v/v) using following gradient: 100% A held for 1 min, linear decrease to 86% A in 24 min and then linear decrease to 40% A in 10 min, followed by 10 min rinsing of the stationary phase (40% A) and 10 min equilibration to 100% A. Detection of the analytes was done by ELS (20 °C nebulizer, 35 °C vaporizer, 3.5 bar nitrogen and gain 8).

3. Results and discussion

3.1. Development of a lipid class-specific normal-phase (NP)-HPLC separation

As reported in the literature, reversed phases enable the separation of lipid classes mainly by the number of carbon atoms and the degree of saturation [14–19]. In contrast normal-phase chromatography is based on the separation by the polar part of the molecule neglecting mostly the unpolar side chain. Different normal phases were used for separation of the lipid classes: silicagel (Si)- [20–23], alumina- [24], cyanopropyl (CN)- [5,11,12,25,26] and dihydroxypropyl (diol)-phase [13,27]. Due to the long equilibration time and the poor reproducibility of retention times of unmodified phases chemically bonded phases were preferred [5,11,12,26]. PAR exhibited no retention on any of the examined normal phases (Si, CN, diol). The diol-phase gave best results especially for the non-polar lipids (PAR, WE, CE and FAME) and, as the only phase, produced a sufficient selectivity between PAR and WE.

For the separation of the non-polar lipid classes, the choice of the non-polar component in the eluent is important. Isooctane [5,22], *n*-heptane [13] and *n*-hexane [12,20,21,23–27] were used previously, whereas some of the mentioned authors modified the non-polar eluent with up to 1% tetrahydrofuran or acetic acid. Only the use of pure isooctane without modifier provided a baseline separation for all four non-polar lipid classes.

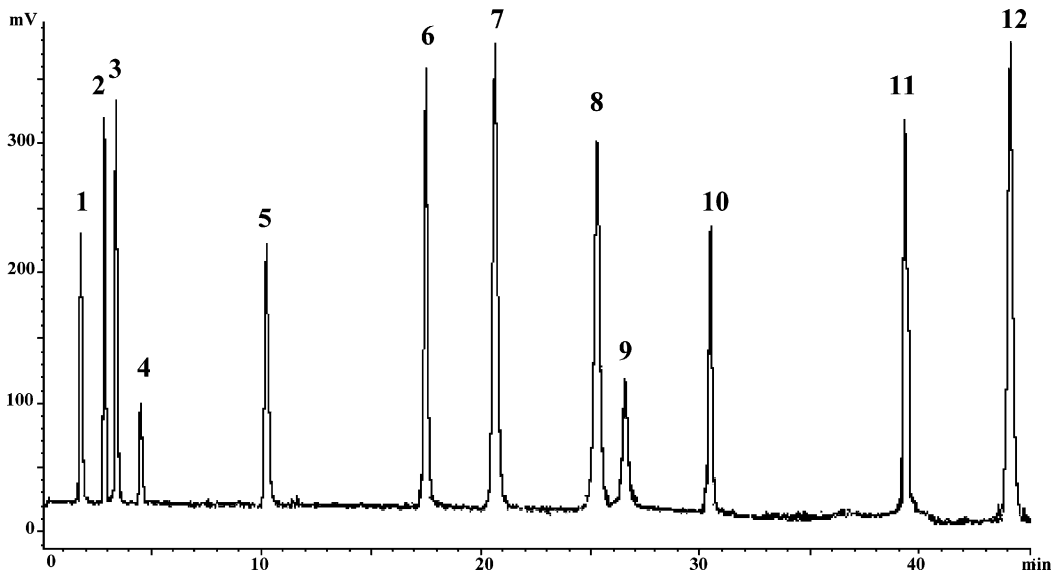

Fig. 1. Separation of lipid classes representatives (1) PAR (paraffin, liquid), (2) WE (*n*-hexyldecyl palmitate), (3) CE (cholesteryl palmitate), (4) FAME (stearic acid methyl ester), (5) TAG (glycerol tripalmitate), (6) FOH (hexadecyl alcohol), (7) FFA (stearic acid), (8) CHOL (cholesterol), (9) 1,3-DAG (glycerol-1,3-dipalmitate), (10) 1,2-DAG (glycerol-1,2-dipalmitate), (11) MAG (glycerol monopalmitate) and (12) FAA (erucylamide), chromatographic conditions see Section 2.5, concentrations 20–50 mg/l, except CHOL 100 mg/l.

A separation for TAG and the seven polar lipid classes required 2-propanol [5,13,21,23,25,27] or MTBE [11,12,25,26] as the polar component in the eluent. We found that very small changes of the eluent composition (99.4/0.6 to 98.8/1.2, isooctane/2-propanol, v/v) led to a coelution of four of five originally baseline separated classes having similar polar properties (FOH, FFA, CHOL, 1,3-DAG, 1,2-DAG). Thus, a development of a gradient with isooctane and 2-propanol failed for not reproducible resolution. The use of MTBE, because of its moderate polarity, enabled the separation of all 12 lipid classes by gradient elution (see Section 2.5 and Fig. 1).

However, the influence of the MTBE content in the mobile phase on the separation is high. The change of the isocratic eluent composition from 92.5/7.5 (isooctane/MTBE, v/v) to 85/15 (isooctane/MTBE, v/v) changed the order of elution of FFA and FOH as well as CHOL and 1,3-DAG.

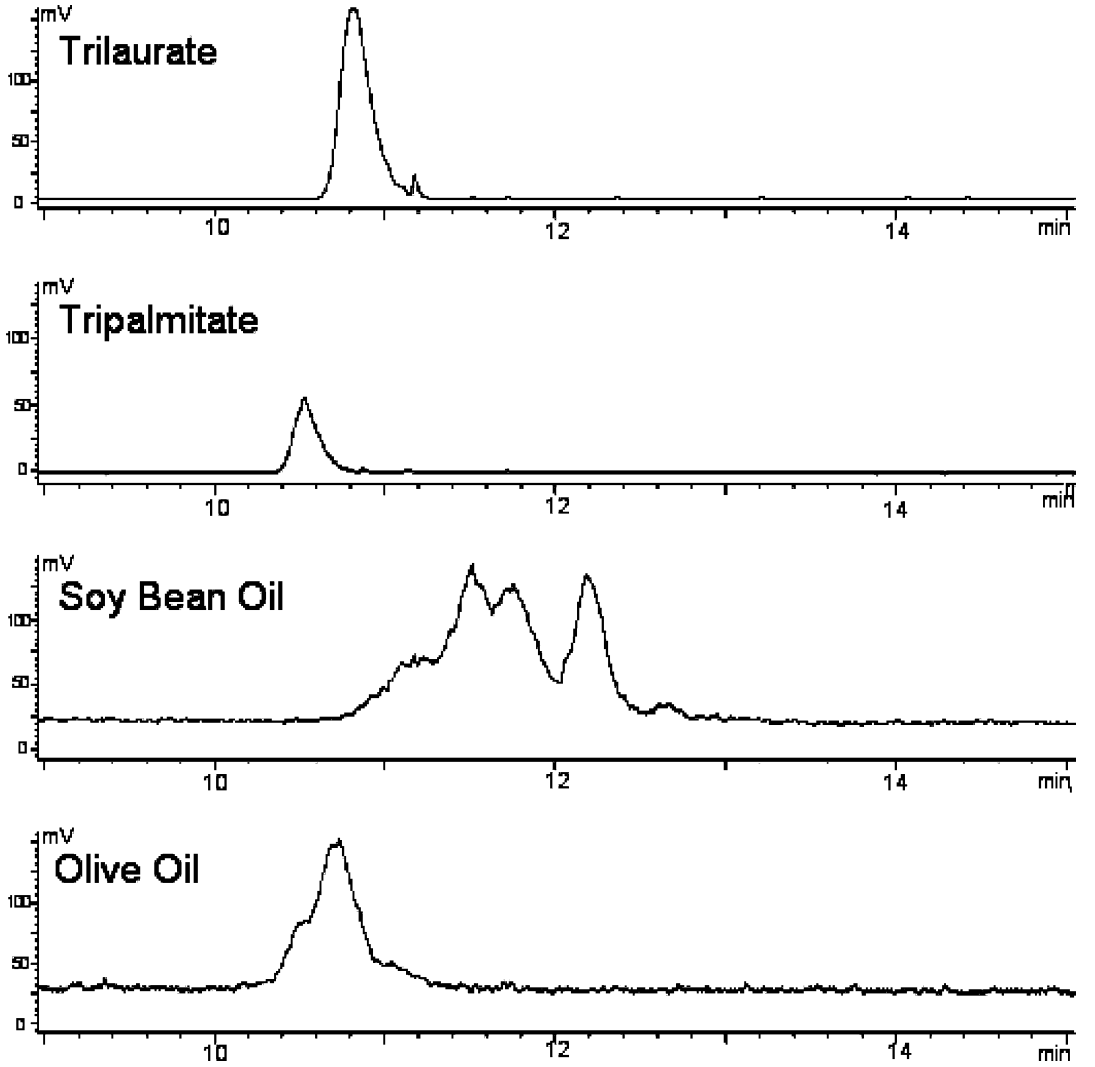

Fig. 2. Peak-splitting of TAGs from soy bean and olive oil compared to defined TAGs (trilaurate and tripalmitate), chromatographic conditions see Section 2.5.

Additionally, a C1-reversed phase was tested in order to achieve a retention of PAR. Even the C1-phase separated by the number of carbon atoms in the molecule and PAR, as a complex mixture of alkanes, appeared as a "hump".

Peak-splitting of lipid classes was also observed on the diol-phase when comparing defined TAGs and two natural oils (see Fig. 2). As proved for four FAME standards (C18:0, C18:1, C18:2, C18:3), the splitting mostly occurred due to the different degrees of saturation.

3.2. Optimization of ELS-detection regarding highest sensitivity

All three parameters of the ELSD (N_2-pressure, nebulizer temperature and vaporizer temperature) were optimized in order to enhance the sensitivity for all lipid representatives. The optimized conditions were found to be: a nebulizer temperature of 20 °C, a vaporizer temperature of 35 °C and a nitrogen pressure of 3.5 bar. Since the ELSD-signal does not linearly increase with substance concentration second order regression (see Fig. 3a) was used to compute the correlation between concentration and detector response. The slope of the calibration curve for all representatives, calculated by the first deviation on a fixed concentration (here 50 mg/l), provides information about the sensitivity of the detector for these substances.

Fig. 3b shows varying sensitivities for all lipid classes whereas Fig. 3c compares the sensitivities of different TAGs with different chain lengths and different degrees of saturation. Sensitivity decreases with decreasing chain length and increasing degree of saturation.

The method has been calibrated with representatives of each class in working ranges of about 5–75 mg/l (except FAME, FOH and CHOL 10–150 mg/l), depending on the lipid class. Coefficients of intra-day variance range from 1.88 to 5.09% and the limits of detection from 0.79 to 3.65 mg/l (except FOH, FAME) as indicated in Table 2. With regard to the substance specific response even within one lipid class, the accuracy of quantification is limited. Without additional information, or other more specific methods, the amounts of migrating species can only be estimated.

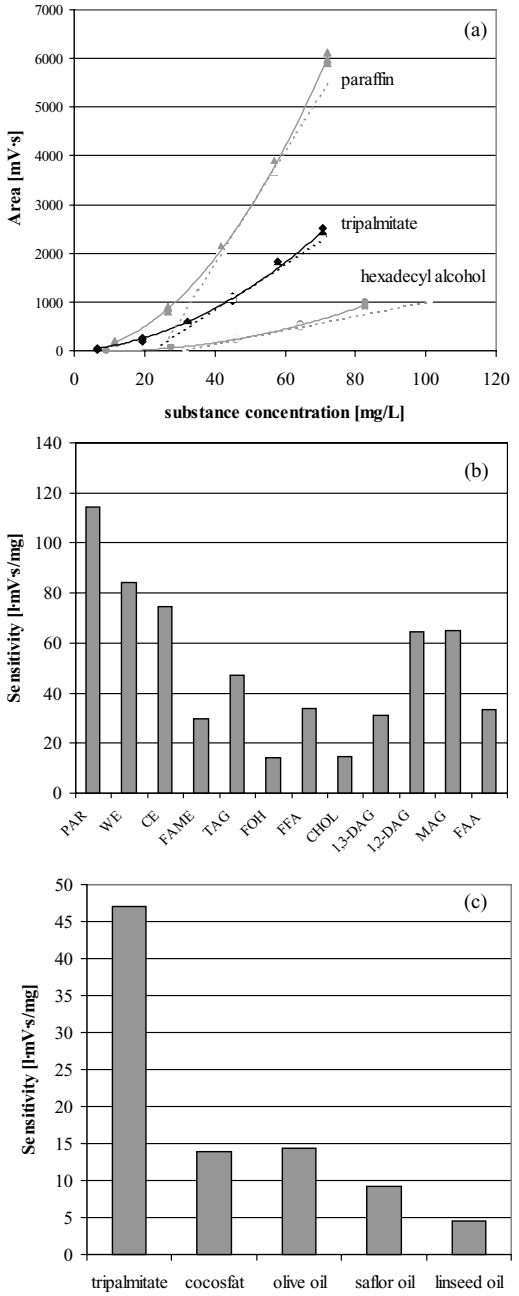

Fig. 3. Determination of the sensitivity of the ELS-detection (a) exemplary second grade regression for the detector responses of PAR, TAG and FOH, sensitivity at 50 mg/l is illustrated by the dashed tangent, (b) comparison of the sensitivity obtained for representatives of 12 different lipid classes at 50 mg/l (c) and for various TAGs (fatty oils and the representative glycerol tripalmitate) at 50 mg/l.

Table 2
Calibration data for representatives of all lipid classes

	Intra-day variances[a] (%)	Inter-day variances[b] (%)	Detection limit[c] (mg/l)	Sensitivity (l mV s/mg)
PAR	4.1	9.9	1	115
WE	3.1	8.6	1	84
CE	4.6	24.7	2	74
FAME	1.9	–[d]	6	30
TAG	4.1	12.9	2	47
FOH	5.1	15.8	6	14
FFA	2.7	7.0	2	34
CHOL	4.7	8.0	4	15
1,3-DAG	5.0	14.3	3	31
1,2-DAG	4.3	19.5	1	64
MAG	3.2	26.5	1	65
FAA	2.4	–[d]	2	33

[a] Intra-day variances are calculated from the standard deviation of the 18-point calibration.
[b] Inter-day variances are calculated from the Sheward control chart (recording one standard with a concentration of the center of the working range at 14 days during 1 month).
[c] Signal-to-noise ratio of 3 is used for the estimation of the detection limit. The respective height is transformed to concentration using second grade standard calibrations (obtained by peaks heights) for every lipid class.
[d] Not determined.

The variances of retention times and the ELSD reponse of the lipid class representatives (except of FAME and FAA) were recorded by Sheward control charts. Retention times for non-polar as well as polar substances varied less than 0.2 min during 1 month. The inter-day variances during this month varied from 7.0 to 26.5% as indicated in Table 2. Although these values are high, they are acceptable for an ELS-detection which normally requires new calibration each day. Time depending deterioration of MTBE developing within 1 week after distillation led to an increasing noise which especially disturbed the quantification of the later eluting components like MAG. The high inter-day variance of MAG can be explained by this effect.

3.3. Sample preparation for food packagings

The sample preparation for migrants from external lubricants differs from the sample preparation for migrants from internal lubricants (see Section 2.5). Being located on the inner surface of the packaging, external lubricants migrate directly (e.g. aluminium laminated with PP) into the simulant isooctane and can be analysed after concentration. While no migration of internal lubricants from coatings into isooctane was measurable, TAG and 1,2-DAG as well as 1,3-DAG could be detected in the 95% ethanol migrate. This result may be traced back to an enhanced partition coefficient between the lacquer and ethanol compared to isooctane. Furthermore, migration of ethanol into the lacquer swells the polymer network and enhances the diffusion of the internal lubricants. Therefore, 95% ethanol was chosen as simulant for internal lubricants in coatings.

However, the direct injection of the 95% ethanol extract leads to loss of resolution of the early eluting non-polar lipid classes. After evaporation of ethanol a dissolution of the residue in isooctane is not possible. Obviously extracted resin components soluble in 95% EtOH but not in isooctane enclose the lubricants in a fixed resin-like structure. Therefore, the residue is dissolved in ethanol with subsequent addition of isooctane. A phase separation was achieved by the addition of water. For the polar lipid classes, especially MAG and FAA, the concentration of the water must be high enough to enable the transfer of these substances to isooctane phase. By using varying amounts of added water (400–1600 μl) it was shown for standards that at least 70% water is necessary for highest recoveries ($62 \pm 3\%$) of MAG and FAA. For standards of less polar lipid classes (tested for 1,2-DAG, 1,3-DAG, TAG and WE) the recovery is for all different amounts of added water $100 \pm 3\%$.

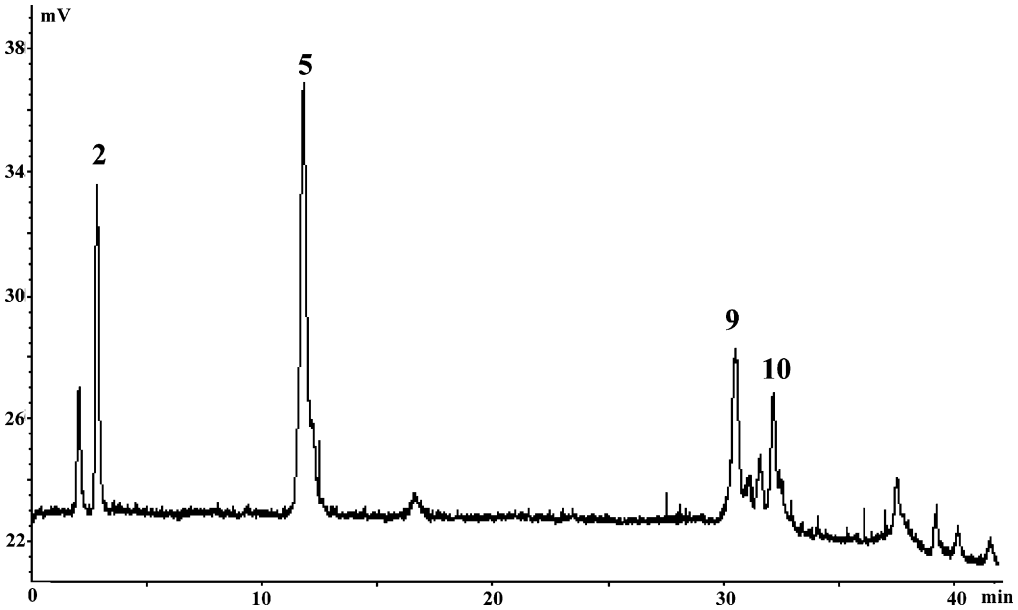

Fig. 4. Chromatogram of the 95% ethanol migrate of a commercial epoxy-anhydride coating, (2) WE (0.05 mg/dm^2), (5) TAG (0.1 mg/dm^2), (9) 1,3-DAG (0.1 mg/dm^2), (10) 1,2-DAG (0.05 mg/dm^2), chromatographic conditions see Section 2.5.

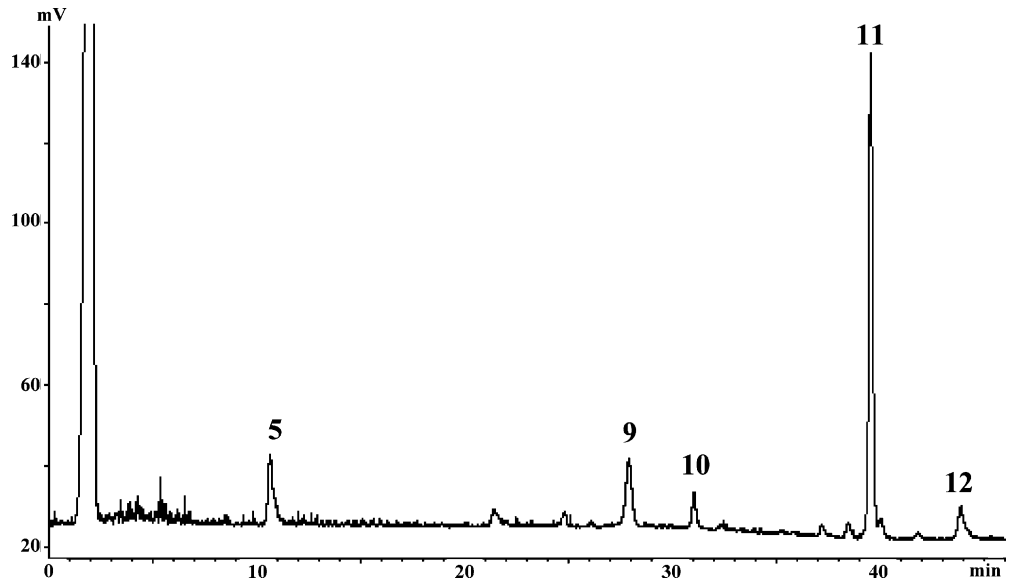

Fig. 5. Chromatogram of the isooctane migrate of a commercial lid made of PP-aluminum laminate, (5) TAG (0.8 mg/dm^2), (9) 1,3-DAG (1.4 mg/dm^2), (10) 1,2-DAG (0.5 mg/dm^2), (11) MAG (2.3 mg/dm^2), (12) FAA (0.5 mg/dm^2), chromatographic conditions see Section 2.5.

The extraction procedure was verified with a coating migrate to which a standard mixture (WE, TAG, 1,2-DAG, 1,3-DAG and MAG) was spiked. Similarly the more polar lipid classes exhibit a smaller recovery than the non-polar classes. However, due to an altered phase separation the recovery rates amounted to about 100 and 130%.

3.4. Application to food packagings

The developed method was applied to a commercial can coating (epoxy-anhydride) including carnauba wax and a partial acyl glycerol as internal lubricants. Consequently, WE, TAG, 1,3-DAG and 1,2-DAG were identified (see Fig. 4) while MAG was not detectable. It is assumed that MAG reacted quantitatively with the epoxy groups of the resin under formation of MAG-resin ethers. The initial peak is possibly PAR, but confirmation should be obtained by specific GC methods for paraffin as described in [6]. The identified migrating lubricants were estimated as 0.3 mg/dm^2.

Fig. 5 shows the chromatogram of a migrate from a laminated commercial light weight container with external and internal lubrication of the polypropylene film. The container was extracted with 100 ml isooctane, which covered a surface of 1.2 dm^2. A partial acylglycerol mixture containing TAG (5), 1,3-DAG (9), 1,2-DAG (10) and MAG (11) used as external lubricant as well as FAA (12) used as slip additive were detected. As already stated for the can coating migrate, the identity of the initial peak must be confirmed by specific method for hydrocarbons. The sum of the identified migrating lubricants was estimated as 5.5 mg/dm^2.

4. Conclusion

A simple screening method of 12 lipid classes commonly used in lubricant formulations for packaging materials was established. The chromatographic system was optimized in order to obtain a separation for all lipid classes whilst minimising the separation of the analogues within one class. However, due to the varying sensitivity of ELSD, especially for different representatives of one lubricant class, the method provides only an approximate quantitative estimation. To ensure identification and provide valid determination of single components in samples with unknown lubricant composition specific methods have to be applied. The presented method is useful in order to confirm the presence of lubricants in the total migrate of packaging materials as well as to estimate their amount.

Acknowledgements

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