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Assessment of critical steps of a GC/MS based indirect analytical method for the determination of fatty acid esters of monochloropropanediols (MCPDEs) and of glycidol (GEs)

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ABSTRACT

Fatty acid esters of 2- and 3-chloropropanediol (MCPDEs) and fatty acid esters of glycidol (GEs) are commonly monitored in edible fats and oils. A recommendation issued by the European Commission emphasizes the need of generating data on the occurrence of these substances in a broad range of different foods. So far, analytical methods for the determination of MCPDEs and GEs are fully validated only for oils, fats and margarine. This manuscript presents the assessment of critical steps in the AOCS Cd 29a-13 method for the simultaneous determination of MCPDEs and GEs in the fat phase obtained from bakery and potato products, smoked and fried fish and meat, and other cereal products. The trueness of the method is affected by the additional formation of 3-MBPD esters from monoacylglycerols (MAGs), which are frequently present in food. The overestimation of GE contents for some samples was confirmed by the comparison of results with results obtained by an independent analytical method (direct analysis of GE by HPLC-MS/MS). An additional sample pre-treatment by SPE was introduced to remove MAGs from fat prior to the GEs conversion, while the overall method sensitivity was not significantly affected. Trueness of the determination of GEs by the modified analytical procedure was confirmed by comparison with a direct analysis of GEs. The potential impact on accuracy of results of the final sample preparation step of the analytical procedure, the derivatization of free forms MCPD and MBPD with PBA, was evaluated as well. Different commercial batches of PBA showed differences in solubility in a non-polar organic solvent. The PBA derivatization in organic solvent did not affect precision and trueness of the method due to the isotopic standard dilution. However, method sensitivity might be significantly compromised.

1. Introduction

Fatty acid esters of 2-/3-chloropropane-1,2-diol (2-/3-MCPDEs) and of glycidol (GEs) might be generated during food processing (Hamlet et al., 2011; Hrncirik & Duijn, 2011). The presence of chlorinated propanols, particularly 3-MCPD, in food is well known since 1970's when this substance was discovered by Velisek et al. (1978) in acid-hydrolysed vegetable proteins (acid-HVP). Esters of MCPD were also found in acid-HVP (Davidek, Velisek, Kubelka, Janicek, & Simicova, 1980), but the majority of investigations has started quite recently after reporting high levels in foods and in particular in refined edible oils (Weisshaar, 2008; Zelinkova, Svejkovska, Velisek, & Dolezal, 2006). GEs have been detected in the frame of MCPDEs analysis in vegetable oils (Weisshaar & Pere, 2010). Free forms of these substances (3-MCPD and glycidol) released from their esterified forms during digestion have been classified as carcinogenic to humans (group 2B and 2A, respectively) (IARC, 2000; IARC, 2012).

Preliminary exposure assessment of the European Food Safety Authority (EFSA) on 3-MCPD in food identified margarine and vegetable fats and oils as major contributors to dietary exposure, followed by bread and fine bakery wares (EFSA, 2013). EFSA concluded that for 3-MCPD a tolerable daily intake (TDI) of 0.8 μg/kg body weight is appropriate, whereas a margin of exposure of 25000 was considered of low health concern in case of glycidol (EFSA, 2016).

The European Commission issued in 2014 Commission Recommendation 2014/661/EU on the monitoring of free MCPD, MCPDEs

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and GEs in food. MCPDEs and GEs were recommended to be monitored in several food groups, comprising fine bakery ware, bread and rolls, smoked meat and fish, potato- and cereal-based snacks, fried potato products and vegetable oil containing foods. Analytical methods standardised by the American Oil Chemists Society (AOCS) were suggested to be used as basis for analysis, but these methods covered only edible oils and fats (EC, 2014).

In general, analytical methods for the determination of MCPDEs and GEs follow two distinct routes. The direct analysis of fatty acid esters by HPLC-MS comprises one possibility, which however entails the measurement of a large number of substances (individual fatty acid esters of MCPD/glycidol) (Haines et al., 2011; Hori et al., 2012). The strong similarity of target compounds with major matrix constituents (in particular mono- and diacylglycerols) hampers the separation of MCPDEs and GEs from the oil matrix. However, satisfactory separation can be achieved for GEs by applying SPE or gel permeation chromatography (GPC) clean-up (Dubois et al., 2011; Musukawa, Shiro, Kondo, & Kudo, 2011). Consequently, a direct analytical method for the determination of GEs in fats and oils was standardised by AOCS (AOCS/JOCS, 2012). The direct analysis of MCPDEs on routine basis is so far hardly applied and analytical methods for the direct determination of MCPDEs in whatever food have not been fully validated yet.

The second route consists of the indirect determination of MCPDEs and GEs via the MCPD/glycidol moieties. The analytical methods entail the cleavage of MCPD/glycidol from its esterified form, and determination of the total amount of the so called bound MCPD/glycidol (Divinova, Svejkovska, Dolezal, & Velisek, 2004; Ermacora & Hrncirik, 2013; Kuhlmann, 2011; Küsters et al., 2010; Weisshaar, 2008). Several methods have been developed for the indirect analysis of MCPDEs, however all are following a similar protocol (cleavage of MCPD, clean-up, derivatization, GC/MS analysis). The important two steps are the cleavage of MCPD from their esterified form (transesterification) and derivatization prior to GC/MS analysis. Both of these steps have been already well optimized and the performance of analytical methods was evaluated by collaborative studies (Fry, Schödel, These, & Preiß-Weigert, 2013; Karasek, Wenzl, & Ulberth, 2013). The cleavage of MCPD (transesterification) is carried out under acidic or alkaline conditions in the presence of methanol to form fatty acids methyl esters and MCPD. Due to the low volatility and high polarity of MCPD, derivatization prior to the GC/MS analysis is necessary.

Relatively new is the methodology for indirect GE determination. The determination of GEs has been incorporated into the existing indirect methods for MCPDE determination and follows the same analytical procedure. This was achieved by conversion and thereby stabilisation of GEs to either a compound structurally similar to MCPD - bromopropandiol (MPBD) or to MCPD itself (DGF, 2011; Ermacora & Hrncirik, 2013; Kuhlmann, 2011). In the first case the phenylboronic acid (PBA) derivative of 3-MBPD is determined by GC/MS as an equivalent of glycidol, in the second case the PBA derivative of MCPD is determined as a sum of bound MCPD and bound glycidol. Indirect methods were considered more suitable for routine application.

A number of analytical methods had been standardised for the indirect analysis of MCPDEs and GEs (AOCS, 2013a, 2013b; AOCS, 2013c) in fats and oils and oil-based emulsions (AOCS, 2015). A standardised method for the determination of MCPDEs and GEs in foods other than fats and oils does not exist yet. Our group has investigated the performance of the AOCS methods mentioned in the Commission Recommendation for foods other than fats and oils, considering the broad scope of the monitoring plan issued by the European Commission and consequently the variety of matrices that has to be dealt with. AOCS Cd 29a-13 was selected, as this method allows the determination of MCPDE and GE content within a single assay (AOCS, 2013a). The analytical procedure consists of the conversion of GEs to MBPDEs, followed by acid catalysed transesterification and cleavage of MCPDEs and MBPDEs. The released free forms of MCPD and MBPD are further derivatized with PBA and determined by GC/MS.

For certain groups of foods, in particular those containing partial glycerides used as emulsifiers we observed somewhat elevated levels of GEs and speculated that reactions carried out in the course of sample preparation may affect the trueness of the method as artefact formation might occur (additional formation of MBPD, transformation of GEs into MCPDEs and vice versa). Ermacora and Hrncirik (2013) already reported an unfavourable influence of partial glycerides on the artefact formation of MBPD.

The aim of the presented study was to critically evaluate the applicability of the selected method to the fat phase obtained from different food matrices. Several aspects were considered including the impact of sample composition and content of potential precursors on the accuracy of the analytical results. Main focus was given to the artefact free conversion of GEs into 3-MBPDEs, which was identified as a critical step having a potential impact on the trueness of the method. The final sample preparation step, the derivatization with PBA, and the influence of the particular batch of commercial derivatization reagent were evaluated as well.

2. Materials and methods

2.1. Food samples

A set of 12 food samples representing different food categories was purchased in Belgian retail markets. Extra virgin olive oil, used as a blank sample, was obtained from a local producer in Greece and palm oil from the European Federation of the Oil and Proteinmeal Industry (FEDIOL). A spiked soybean oil was used for analytical quality control purposes. It contained the following analyte amounts, expressed as equivalents to the free forms: 3-MCPD 2.88 ± 0.29 mg/kg; 2-MCPD < 0.10 mg/kg; glycidol 4.25 ± 0.68 mg/kg (glycidyl laurate 2.01 mg/kg; glycidyl palmitate 5.74 mg/kg; glycidyl stearate 1.53 mg/kg; glycidyl oleate 6.56 mg/kg, glycidyl linoleate 0.49 mg/kg; glycidyl linolenate 1.39 mg/kg). All samples were homogenized and kept according to the labelled storage recommendations.

2.2. Reagents and materials

All solvents were of at least analytical grade, purchased from either Sigma-Aldrich (Diegem, Belgium) or VWR (Leuven, Belgium). Sodium polyacrylate cross-linked, sand 50–70 mesh particle size, sulphuric acid (≥95%), sodium hydrogen carbonate, anhydrous sodium sulphate and sodium bromide were obtained from Sigma-Aldrich (Diegem, Belgium). Aminopropyl (NH_2) SPE cartridges (Extract Clean™, 500 mg, 4.0 mL) and HPLC syringe filters (regenerated cellulose, 13 mm, 0.2 μm) were purchased from Grace Davison Discovery Science (Deerfield, IL, USA). Four different batches of phenylboronic acid (PBA) reagent were obtained for comparison purposes from different suppliers, three from Sigma-Aldrich (Diegem, Belgium) and one from ACROS (Geel, Belgium).

The standard compounds 1,2-dipalmitoyl-3-chloropropanediol (diP-3-MCPD, CAS#51930-97-3); 1,3-distearoyl-2-chloropropanediol (diS-2-MCPD, CAS#26787-56-4); glycidyl laurate (GE-L, CAS#1984-77-6); glycidyl palmitate (GE-P, CAS#7501-44-2); glycidyl stearate (GE-S, CAS#7460-84-6) as well as the isotopically labelled compounds 1,2-dipalmitoyl-3-chloropropanediol-d_5 (diP-3-MCPD-d_5); 1,3-distearoyl-2-chloropropanediol-d_5 (diS-2-MCPD-d_5) and glycidyl oleate-d_5 (GE-O-d_5) were obtained from Toronto Research Chemicals Inc.