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VALIDATION OF A CHROMATOGRAPHIC METHOD FOR THE MCPD DETERMINATION IN VEGETABLE OILS

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Abstract. Today, esters of 3-monochloropropane-1,2-diol (3-MCPD-E) and glycidol (GE) remain one of the most pressing food safety issues, so a thorough study of their structure, formation mechanisms and control methods is an urgent issue. 3-MCPD and GE are fat-soluble toxic substances that can be formed in vegetable oils during their production and processing. Chloropropanols are by-products formed as a result of acid hydrolysis of glycerol lipids at elevated temperatures, during production or storage, and in the process of deodorization of edible oils. As a rule, 3-MCPD and GE are formed as a result of high-temperature exposure to fats, and they have carcinogenic, genotoxic, nephrotoxic and other types of negative effects on the human body. That is why their content is strictly regulated and must be controlled both in raw materials and in final products based on them. The direct determination of glycidol esters and fatty acid esters of 3-MCPD is performed by HPLC/MS/MS, but this method is difficult to use in practice due to the large number of components to be determined (84) and the lack of analytical standards. The most common indirect methods of control are when all esters of glycidol and 3-MCPD decompose to just glycidol and 3-monochloropropanediol, respectively. The purpose of the work is to validate the method ISO 18363-1:2015 "Animal and vegetable fats and oils – Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS. Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol" on an Agilent 8890B gas chromatograph under the conditions of Kotekna Ukraine Limited LLC. It has been determined the limits of detection (LOD) and quantification (LOQ) of MCPDs, proved the linearity of the method; assessed the precision of measurements, provided an estimate of the extraction and bias coefficients, calculated the expanded uncertainty. This study may contribute to the correct determination of the content of MCPDs and glycidol in vegetable oils.

Key words: 3-MCPD, hazards, detection, validation, standartisation, plant oils.

Introduction. Formulation of the problem

Esters of 2,3-monochloropropane-1,2-diol (3-MCPDE) and glycidol (GE) remain one of the most pressing food safety problems, therefore a thorough study of their structure, formation mechanisms, and control methods is an urgent issue [1-4].

MCPDs and GE are fat-soluble toxic substances that can be formed in vegetable oils during their manufacture and processing. Chloropropanols are by-products formed as a result of the acid hydrolysis of glycerol of lipids at elevated temperatures, during production or storage and during the deodorization process of edible oils. As a rule, MCPDs and GE are formed as a result of high-temperature exposure to fats, they have carcinogenic, genotoxic, nephrotoxic and other types of negative effects on the human body. That is why their content is strictly regulated and must be controlled both in raw materials and in final products based on them [3-4].

Analysis of recent research and publications

During industrial fat processing, MCPDEs and GE can be formed during the deodorization process, which is a necessary step in the production of refined oils, to remove undesirable taste, color, or odor. The highest concentrations of 3-MCPDE and GE are usually found in refined palm oil and palm olein oil, but they are also found in other refined vegetable oils (such as safflower, coconut, sunflower, and soybean oils). Karel Hrncirik et al. has been studied the impact of refining conditions of palm and rapeseed oils on 3-MCPDE and GE accumulation [2]. The experiments

showed that 3-MCPD esters and glycidyl esters were formed during the deodorization of palm oil, but not rapeseed oil. The level of 3-MCPD esters in the refined palm oils (3.5–4.9 mg/kg) was independent of the deodorization conditions. No correlation was found between the level of 3-MCPD esters formed and the content of the potential precursors, partial acylglycerols and chlorides. In contrast, the formation of glycidyl esters was affected by the deodorization conditions (both temperature and residence time). Higher levels of glycidyl esters (up to 3.8 mg/kg) were found in palm oil deodorized at temperatures above 230°C [2].

Glycidol esters are considered potential precursors of MCPD esters, and in the human body they break down into glycidol and fatty acids. Therefore, all these structurally different substances are classified as one group of toxic pollutants. The LD_{50} value for 3-MCPD varies according to different studies from 170 μg/kg to 290 μg/kg. In rodent studies, 3-MCPD caused adverse effects on the kidneys and male reproductive organs, and both 3-MCPD and glycidol cause cancer[1].

The first reports on the toxicity of 3-MCPD were published more than 20 years ago, and their content in certain types of products is also controlled under European legislation. However, over time, the permissible daily intake of these contaminants has been reassessed and changed. New maximum permissible limits for their content are being introduced and the list of foods in which they are controlled is being expanded [5-8].

In 2020, Commission Regulation (EU) 2020/1322 amended Regulation (EC) No 1881/2006 as regards maximum levels of 3-monochloropropanediol (3-MCPD), 3-MCPD fatty acid esters and glycidyl fatty acid esters in certain foodstuffs, including edible fats and oils. The acceptable daily intake of 3-MCPD is 2 μg/kg body weight and has not been generally exceeded for adults, but the ADI has been observed to be exceeded for younger age groups, especially infants fed exclusively with formula. This has led to a change towards stricter control of these contaminants in foodstuffs intended for infants and children under three years of age [9-10].

According to the regulation, the content of 3-MCPD in vegetable protein hydrolysates and soy sauce should not exceed 20 μg/kg; in vegetable oils and fats, fish oil and oils of other marine organisms placed on the market for the final consumer or for use as an ingredient in foodstuffs, with the exception of foodstuffs specified in the following paragraph and virgin olive oils – no more than 1000 μg/kg; in vegetable oils and fats, fish oil and oils of other marine organisms intended for the production of baby food and processed cereal-based products for infants and young children – no more than 500 μg/kg; in infant formulae (starter) and follow-on formulae, as well as foodstuffs for special medical purposes intended for infants and young children (sold in powder form) - no more than 50 μg/kg [11-12].

FDA is also working with industry to share information about the health effects of 3-MCPD and GE and the availability of mitigation methods for refined cooking oils and infant formulas to help reduce levels of these contaminants. In addition, the Codex Alimentarius Commission, in which FDA participates, has developed guidance for industry to reduce 3-MCPD and GE in refined cooking oils and foods. As chair of the Codex working group, the FDA helped develop a description of agricultural, sunflower processing, post-refining and processing measures that industry can use to reduce levels of 3-MCPD and GE in refined oils and foods made from refined oils [13-18].

For the reduction of 3-monochloro-1,2-propanediol fatty acid esters (3-MCPD esters) and related compounds in edible fats and oils three different strategies are conceivable: removal of critical reactants from the raw material, changing of the refining process or removal of formed 3-MCPD esters and related compounds from the refined product [13]. Bertrand Matthäus et al. have been shown that different raw materials have different capabilities to form 3-MCPD esters and related compounds, whereas palm oil, but also corn oil or coconut oil possess the highest potential for the formation. Washing of the raw material before the refining process with water or ethanol (75%) reduced the capability for the formation of 3-MCPD esters and related compounds in palm oil for about 20 and 25% respectively. Chloride and DAGs seem to be important precursors for the formation of 3-MCPD esters and related compounds. A hypothesis was defined that a content of 4% DAGs could be a threshold for a higher potential of the raw material for the formation of the esters. Using acid solutions (formic acid) instead of water for the generation of strip steam during deodorization resulted in a reduction of the formation of glycidyl esters for about 35% [13].

Nowadays, the method based on determining the derivative of this compound by using gas chromatography and mass spectrometry is widely used. However, there is still a big need for developing new methods that would produce repeatable results [1].

In the study [11] the 3-MCPD and glycidol levels in 9 types (46 brands) of edible fat and oils were determined by making some modifications to the DGF C VI 18 (10) (GC/MS) method. The highest levels of 3-MCPD and glycidol levels were detected in hazelnut oils, riviera olive oils, margarines, and shortenings. As expected, these contaminants were not observed in extra-virgin olive oils, while they were detected at low levels in fish oils. The highest 3-MCPD levels were found in the range of 0.06–2.12 $mg \cdot kg^{-1}$ in hazelnut oil, 0.16–1.69 $mg \cdot kg^{-1}$ in riviera olive oils, and 0.17–1.17 $mg \cdot kg^{-1}$ in margarines. The highest glycidol levels were found in the shortenings in the range of 1.98–6.46 $mg \cdot kg^{-1}$, followed by hazelnut oil (0.54–2.63 $mg \cdot kg^{-1}$) and riviera olive oil (0.19–3.53 $mg \cdot kg^{-1}$).

In the study [16] the determine 3-MCPD in consumed edible oils (palm, palm olein, extra virgin olive, corn, sunflower, soybean, olive pomace) and blend of 5% sunflower oil with extra virgin olive oil was done using selective and sensitive gas chromatography tandem triple quadrupole mass spectrometry (GC-MS/MS) with employing deuterated 3-MCPD (3-MCPD-d5) as internal standard during the entire analytical procedure to obtain precise and accurate results. The occurrence and variation of 3-MCPD contents among the studied oils were found in different levels ranged from 93.1 μg/kg to 5634.1 μg/kg oil samples, with maximum value assigned for palm oil (5634.1 μg/kg) followed by palm olein (5576.8 μg/kg), corn oil (2447 μg/kg), sunflower oil (1817.3 μg/kg), soybean oil (1486.1 μg/kg), olive pomace oil (572.5 μg/kg), blend of 5% sunflower oil with extra virgin olive oil (210 μg/kg) and extra virgin olive oil (93.1 μg/kg). Palm, palm olein, corn, sunflower and soybean oils were found out of the limits recommended by the Commission Regulation (EU) 2020/1322, whereas, extra virgin olive oil, olive pomace oil and blend of 5% sunflower oil with extra virgin olive oil were in compliance and within the limits recommended by EU [16].

Nur Aainaa Syahirah Ramli et al. have been developed the modification of AOCS Official Method Cd 29a-13 to suit the nature of fatty acids, method validation, and quantification of 2-MCPDE, 3-MCPDE and GE in commercial fatty acids [19]. The sample preparation step was modified during the extraction of 2-MCPDE, 3-MCPDE, and 3-MBPDE from the aqueous phase, through the addition of tetrahydrofuran to facilitate the extraction process. It was founded that the calibration curves for 2-MCPDE, 3-MCPDE and GE showed good linearity with coefficient of correlation (R^2) >0.999. The attained limit of detection (LOD) was 0.02, 0.01 and 0.03 mg/kg^{-1} for 2-MCPDE, 3-MCPDE, and GE, respectively. Recovery, repeatability and intermediate precision were then evaluated using spiked fatty acids at three levels of 2-MCPDE, 3-MCPDE, and GE. Satisfactory recoveries were achieved, ranging from 100.6 to 108.4%, 96.8–100.9%, and 99.5–103.0% for 2-MCPDE, 3-MCPDE, and GE, respectively. Repeatability and intermediate precision (expressed as relative standard deviation) were less than 9%, 7%, and 6% for 2-MCPDE, 3-MCPDE, and GE, respectively. Analyses conducted on various commercial fatty acids from oleochemical plants showed that 2-MCPDE, 3-MCPDE and GE were lesser than the LOD of the respective compounds [19].

Some improvements for simultaneous determination 2-MCPDE, 3-MCPDE and GE were established in the study [20] using GC-MS/MS method. GE were converted into 3-monobromopropanediol monoesters (3-MBPDE) through bromination, then, 3-MBPDE, 2-MCPDE and 3-MCPDE were converted into their free forms through acid catalysis. The three target analytes were purified by matrix solid phase dispersion extraction and then derivatized with heptafluorobutyrylimidazole. The derivatives were detected by GC-MS/MS, and key parameters including NaBr concentration, bromination time and temperature, and transesterification time were optimized. Finally the new procedure was fully validated. The limits of detection for 3-MCPDE, 2-MCPDE and GE in vegetable oil were 2.0, 5.0, and 10 μg/kg, respectively. The average recoveries ranged from 85.4% to 110% with relative standard deviation of 1.3–7.4% when the spiked levels varied from 0.05 to 1.0 mg/kg [20].

Scientific research allows to improve methods for determining the content of 2-MCPDE, 3-MCPDE and GE. To determine the content of MCPDEs in vegetable oils, independent accredited testing laboratories usually use standardized methods from the ISO series. One of the earliest and most widely used methods for the determination of MCPDEs, which was adopted by the international organization for standardization, is the ISO 18363-1:2015 method “Animal and vegetable fats and oils – Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS. Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol”. ISO 18363-1:2015 describes a procedure for the indirect determination of 3-MCPD esters (bound 3-MCPD) and possible free 3-MCPD after alkaline catalysed ester cleavage and derivatization with phenylboronic acid (PBA). Furthermore, this part of ISO 18363 enables the indirect determination of glycidyl esters (bound glycidol) under the assumption that no other substances are present that react at room temperature with inorganic chloride to generate 3-MCPD.

Method ISO 18363-4:2021 “Animal and vegetable fats and oils – Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS. Part 4: Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS, який забезпечує точне кількісне визначення, 2- MCPD та гліцидолу” specifies a rapid procedure for the simultaneous determination of 2-MCPD esters (bound 2-MCPD), 3-MCPD esters (bound 3-MCPD) and glycidyl esters (bound glycidol) in a single assay, based on alkaline catalysed ester cleavage and derivatization of cleaved (free) analytes with phenylboronic acid (PBA) prior to GC-MS/MS analysis. Glycidyl ester overestimation is corrected by addition of an isotopic labelled ester bound 3-MCPD which allows the quantification of 3-MCPD induced glycidol during the procedure. This method is applicable to solid and liquid fats and oils. This document also applies to animal fats and used frying oils and fats, but these matrices were not included in the validation.

ISO 18363-3:2024 “Animal and vegetable fats and oils – Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS. Part 3: Method using acid transesterification and measurement for 2-MCPD, 3-MCPD and glycidol”

specifies a procedure for the simultaneous determination of 2-MCPD esters (bound 2-MCPD), 3-MCPD esters (bound 3-MCPD) and glycidyl esters (bound glycidol) in a single assay, based on acid catalysed ester cleavage and derivatization of cleaved (free) analytes with phenylboronic acid (PBA) prior to GC/MS analysis.

The purpose of the work is to validate the method ISO 18363-1:2015 "Animal and vegetable fats and oils – Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS. Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol" on an Agilent 8890B gas chromatograph under the conditions of Kotekna Ukraine Limited LLC.

Research objectives: to determine the limits of detection (LOD) and quantification (LOQ) of MCPDs; to prove the linearity of the method; to assess the precision of measurements; to provide an estimate of the extraction and bias coefficients; to calculate the expanded uncertainty

Research materials and methods

Validation object ISO 18363-1:2015 «Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS – Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol»

The study was conducted using an Agilent 8890B gas chromatograph (Single quadrupole mass spectrometer: Agilent 5977B; Gerstel Mps Dual Head automatic sample preparation system). The analytical equipment used in the validation was verified and calibrated. The study was conducted in the testing laboratory of Kotekna Ukraine Limited LLC.

General metrological requirements for the method of analysis according to Commission Regulation No. 333/2007 "Establishing methods of sampling and analysis for the control of levels of trace elements and technological contaminants in foodstuffs" are presented in Table 1. Table 2 presents the data of Annex ISO 18363-1:2015 regarding metrological characteristics for a sunflower oil sample.

Statistical data processing was carried out according to ISO 16269 "Statistical interpretation of data" Part 4: Detection and treatment of outliers; Part 6: Determination of statistical tolerance intervals; Part 7: Median – Estimation and confidence intervals.

Table 1 – General metrological requirements for the analysis method

№	Parameter	Methodology suitability criterion
1	Linearity	In the working concentration range, the linear dependence of the analyte response on its concentration should be observed. The correlation coefficient R can be ≥ 0.98
2	Precision (repeatability)	The relative standard deviation RSD of the results of the analysis of the same sample, performed under the same conditions by the same analysts, should not exceed the RSDs calculated according to the Hurwitz equation for a given concentration level and multiplied by a factor of 0.66: for the level <100 μg/kg RSD 14.5%; for the level 100–750 μg/kg RSD ≤ 14.5–11.0%; for the level 750–1500 μg/kg RSD ≤ 11.0–9.9%.
3	Precision (reproducibility)	The relative standard deviation RSDR of the results of the analysis of the same sample, performed under the same conditions by different analysts, should not exceed the RSDR calculated by the Hurwitz equation for a given concentration level: for the level <100 μg/kg = RSDR 22% for the level 100–750 μg/kg RSDR 22–16.7%; for the level 750–1500 μg/kg = RSDR 16.7–15.05%.
4	Accuracy (trueness)	The values of the recovery coefficients (Recovery, %) should be in the ranges: 70–125%
5	Limit of quantitation (LOQ)	LOQ≤ 100 μg/kg
6	Expanded uncertainty	The expanded uncertainty (U) should be calculated using a coverage factor of 2 and a confidence interval of 95%.
7	Selectivity	The method must distinguish between the analyte and other substances under the experimental conditions. The minimum acceptable retention time for the analyte of interest is twice the retention time corresponding to the void volume of the column.

Table 2 – Metrological characteristics for sunflower oil samples (ISO 18363-1:2015).

Indicators	Sample A	Sample B
Average value of samples, X (mg/kg)	1.07	0.62
Standard deviation of repeatability S, (mg/kg)	0.09	0.04
Relative standard deviation of repeatability RSD, (%)	8.1	5.7
Repeatability, (mg/kg)	0.24	0.10
Standard deviation of reproducibility, SR (mg/kg)	0.21	0.18
Relative standard deviation of reproducibility, RSD_g (%)	20.0	28.8
Reproducibility, R (mg/kg)	0.6	0.5

Results of the research and their discussion

Results of measurement and calculation of acceptance criteria. The limits of detection and quantification were calculated under the conditions of intermediate precision of ISO 16269-4:2010. For this, samples with a content of 3-MCPD/2-MCPD/Glycidol at a level of 100/100/65 µg/kg, respectively, were analyzed for 10 days. The calculation of the limits of detection and quantification was carried out in accordance with ISO 16269-4:2010 using formulas 1,2. To analyze the input data for the presence of "outliers", the Grubbs criterion was used, respectively. The results of the calculations are presented in Table 3.

$$LOD = 3 \cdot SD \quad (1)$$

$$LOQ = 10 \cdot SD \quad (2)$$

where *SD* is the standard deviation calculated based on the results obtained within 10 days of analysis of samples with a 3-MCPD/2-MCPD/Glycidol content of 100/100/65 µg/kg.

To confirm the linear relationship between concentration and signal, calibration curves were constructed for 5 concentration levels. When checking linearity, the R^2 approximation coefficient was used, and the linear and quadratic approximations were compared using Fisher's exact test. Therefore, the obtained calibration curves are linear over the entire working concentration range for all analytes (Table 4).

Therefore, the obtained values of the detection and quantification limits for all samples meet the requirements presented in Table 1.

Evaluation of measurement precision. Intra-laboratory repeatability and reproducibility were assessed under intermediate precision conditions. For this, samples containing 3-MCPD/2-MCPD/Glycidol at levels of 750/750/488 µg/kg and 1500/1500/975 µg/kg, respectively, were analyzed over a period of 10 days. The standard deviation (SD_r and SD_R), relative standard deviation (RSD_r and RSD_R), repeatability (r) and reproducibility (R) were calculated using formula 3-8, respectively. The Grubbs criterion was used to analyze the input data for the presence of "outliers".

$$SD_r = \sqrt{\frac{\sum_{j=1}^{j=n/2}\left(\sqrt{\frac{\sum_{i=1,j=1}^{i=2,j=n/2}\left(X_{i,j}-\overline{X}_j\right)^2}{(2-1)}}\cdot\frac{1}{\sqrt{2}}\right)}{n/2}} \quad (3)$$

$$RSD_r = \frac{SD_r}{\overline{X}} \cdot 100\% \quad (4)$$

$$r = 2.8 \cdot SD_r \quad (5)$$

$$SD_R = \sqrt{\frac{\sum_{i=1}^{i=n}\left(X_i-\overline{X}\right)^2}{(n-1)}}\cdot\frac{1}{\sqrt{2}} \quad (6)$$

$$RSD_R = \frac{SD_R}{\overline{X}} \cdot 100\% \quad (7)$$

$$R = 2.8 \cdot SD_R \quad (8)$$

where $\overline{X}$ – average value, $\overline{X}_j$ – average value of the j-th series, $X_{i,j}$ – i-th value j-th series, n – total amount of data.

The results of the calculations are presented in Tables 5, 6. Therefore, the obtained precision values for all samples meet the requirements presented in Table 1.

Table 3 – Results of determination of the limit of detection (LOD) and quantification (LOQ) of 3-MCPD/2-MCPD/Glycidol in refined sunflower oil samples

Analyte	*n*	*m*	*X*, µg/kg	*SD*, µg/kg	*LOD*, µg/kg	*LOQ*, µg/kg
3-MCPD	6	0	107.4	4.24	13	42
2-MCPD	6	0	96.0	6.11	18	61
Glycidol	6	0	56.7	2.55	8	26

n – number of input data; m – number of "outliers" X – mean value, SD – standard deviation

Table 4 – Determination of linearity of the method

Analyte	Linear range, ng/ml	Equation of a line	R^2	*F*	F_{cp}	Conclusion
3-MCPD	10–500	A=806.7C+11954	0.9978	1.11	9.55	Acceptable
2-MCPD	10–500	A=1218C-1645	0.9949	1.78	9.55	Acceptable
Glycidol	10–500	A=447C+3957	0.9992	2.04	9.55	Acceptable

Table 5 – Results of calculation of precision parameters for determination of 3-MCPD/2-MCPD/Glycidol at the level of 750/750/488 µg/kg in samples of refined sunflower oil

Analyte	Sample	*n*	*j*	*m*	*X*, µg/kg	SD_r, µg/kg	RSD_r, %	*r*, µg/kg	SD_R, µg/kg	RSD_R, %	*R*, µg/kg*
3-MCPD	Refined sunflower oil	10	6	0	776.3	15.5	2.0%	43.4	22.3	2.9%	62.5
2-MCPD		10	6	0	723.6	25.1	3.5%	70.2	35.4	4.9%	99.2
Glycidol		10	6	0	492.5	41.2	5.1%	115.5	32.1	6.5%	89.8

Table 6 – Results of calculation of precision parameters for determination of 3-MCPD/2-MCPD/Glycidol at the level of 1500/1500/975 µg/kg in samples of refined sunflower oil

Analyte	Sample	*n*	*j*	*m*	*X*, µg/kg	SD_r, µg/kg	RSD_r, %	*r*, µg/kg	SD_R, µg/kg	RSD_R, %	*R*, µg/kg
3-MCPD	Refined sunflower oil	10	6	0	1491.7	20.0	1.3%	56.0	20.8	1.4%	58.1
2-MCPD		10	6	0	1351.2	50.1	3.7%	140.2	48.5	3.6%	135.8
Glycidol		10	6	0	964.0	70.7	5.2%	197.9	58.8	6.1%	164.7

*n – number of input data; m – number of "outliers"; j – number of data series; X – average value; SD_r – standard deviation of repeatability; RSD_r – relative standard deviation of repeatability; r – repeatability; SD_R – standard deviation of reproducibility; RSD_R – relative standard deviation of reproducibility; R – reproducibility.

Estimation of the extraction and displacement coefficient. The estimation of the extraction coefficient (Recovery) and the relative shift (bias(%)) was carried out under conditions of intermediate precision. For this purpose, samples containing 3-MCPD/2-MCPD/Glycidol at levels of 100/100/65 µg/kg, 750/750/488 µg/kg and 1500/1500/975 µg/kg were analyzed for 10 days. The recovery coefficient (Recovery) and bias (%) were calculated using formulas (9-10). The Grubbs criterion was used to analyze the input data for the presence of "outliers".

$$Recovery = \frac{\overline{X}}{X_{spike}} \cdot 100\% \quad (9)$$

$$bias(\%) = \frac{\overline{X} - X_{spike}}{X_{spike}} \quad (10)$$

where $\overline{X}$ – average value of the analyte found; X_{spike} – amount of added analyte.

The results of the calculations are presented in Tables 7, 8. Therefore, the obtained values of the extraction coefficient and relative displacement for all samples meet the requirements presented in Table 1.

Uncertainty of the method. The expanded uncertainty estimation method was performed under intermediate precision conditions. To calculate the expanded uncertainty, data were used at low (100/100/65 µg/kg), medium (750/750/488 µg/kg) and high (1500/1500/975 µg/kg) levels of 3-MCPD/2-MCPD/Glycidol additives in refined sunflower oil samples. The Grubbs criterion was used to analyze the input data for the presence of "outliers". The calculation of the expanded uncertainty was performed using formula 11.

$$U = k \cdot \sqrt{RSD_R^2 + bias(\%)^2}, \% \quad (11)$$

where k is the coverage coefficient, RSD_R is the relative standard deviation of reproducibility, *bias* (%) is the relative bias.

The calculations are presented in Tables 9 and 10. Therefore, the obtained values for the expanded uncertainty for all samples meet the requirements presented in Table 1.

Table 7 – Results of calculation of the *recovery* coefficient for the determination of 3-MCPD/2-MCPD/Glycidol in refined sunflower oil samples

Analyte	Sample	$Recovery_{low\ level}$, %*	$Recovery_{middle\ level}$, %**	$Recovery_{high\ level}$, %***
3-MCPD	Refined sunflower oil	107	104	99
2-MCPD		96	96	90
Glycidol		87	101	99

Table 8 – Results of calculation of relative shift (*bias*(%)) for determination of 3-MCPD/2-MCPD/Glycidol in refined sunflower oil samples

Analyte	Sample	$bias_{low\ level}$, %*	$bias_{middle\ level}$, %**	$bias_{high\ level}$, %***
3-MCPD	Refined sunflower oil	7	4	-1
2-MCPD		-4	-4	-10
Glycidol		-13	1	-1

*$bias_{low\ level}$ – extraction coefficient values for 3-MCPD/2-MCPD/Glycidol at 100/100/65 µg/kg
**$bias_{middle\ level}$ – extraction coefficient values for 3-MCPD/2-MCPD/Glycidol at 750/750/488 µg/kg
**$bias_{high\ level}$ – extraction coefficient values for 3-MCPD/2-MCPD/Glycidol at 1500/1500/975 µg/kg.

Table 9 – Results of calculation of expanded uncertainty (*U*) for determination of 3-MCPD/2-MCPD/Glycidol in refined sunflower oil samples (at k – 2)

Analyte	Sample	$U_{low\ level}$, %*	$U_{middle\ level}$, %**	$U_{high\ level}$, %***
3-MCPD	Refined sunflower oil	17	9	3
2-MCPD		15	12	21
Glycidol		27	13	12

*$U_{low\ level}$ – extraction coefficient values for 3-MCPD/2-MCPD/Glycidol at 100/100/65 µg/kg
**$U_{middle\ level}$ – extraction coefficient values for 3-MCPD/2-MCPD/Glycidol at 750/750/488 µg/kg
**$U_{high\ level}$ – extraction coefficient values for 3-MCPD/2-MCPD/Glycidol at 1500/1500/975 µg/kg.

Table 10 – Relative contribution to the expanded uncertainty of the parameters *RSD*, and *bias*(%) in the determination of 3-MCPD2-MCPD/Glycidol in refined sunflower oil samples (at *k* – 2)

Analyte	Sample	Low level		Middle level		High level	
		RSD_R,%	*bias*(%)	RSD_R,%	*bias*(%)	RSD_R,%	*bias*(%)
3-MCPD	Refined sunflower oil	22	78	40	60	86	14
2-MCPD		72	28	66	34	12	88
Glycidol		11	89	98	2	97	3

Conclusion

Considering the harmful effects of MCPDs and glycidol on the human body, market operators of the production of refined vegetable oils should pay considerable attention to the control of these substances in the oil production process and in the final product. Not all enterprises have equipment in their production laboratories to determine the content of MCPDs and glycidol, so they usually use the services of independent accredited testing laboratories.

The validation study conducted in this work of the method ISO 18363-1:2015 "Animal and vegetable fats and oils – Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS. Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol" on an Agilent 8890B gas chromatograph can help implement this method in various testing centers. It has been determined the limits of detection (LOD) and quantification (LOQ) of MCPDs, proved the linearity of the method; assessed the precision of measurements, provided an estimate of the extraction and bias coefficients, calculated the expanded uncertainty. This study may contribute to the correct determination of the content of MCPDs and glycidol in vegetable oils.

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ВАЛІДАЦІЯ ХРОМАТОГРАФІЧНОГО МЕТОДУ ВИЗНАЧЕННЯ MCPD В РОСЛИННИХ ОЛІЯХ

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Анотація. На сьогоднішній день складні ефіри 3-монохлорпропан-1,2-діолу (3-MCPD-E) та гліцидолу (GE) залишаються однією з найактуальніших проблем безпеки харчової продукції, тому досконале вивчення їхньої структури, механізмів утворення та методів контролювання є нагальним питанням. 3-MCPD та GE – жиророзчинні токсичні речовини, які можуть утворюватись в рослинних оліях у процесі їхнього виготовлення та переробки. Хлорпропаноли – побічні продукти, що утворюються внаслідок кислотного гідролізу гліцерину ліпідів при підвищеній температурі, під час виробництва або зберігання та в процесі дезодорації харчових олій. Як правило, 3-MCPD та GE утворюються в результаті високотемпературного впливу на жири, вони мають канцерогенну, генотоксичну, нефротоксичну ті інші види негативних дій на організм людини. Саме тому їхній вміст суворо регламентується та повинен контролюватися як в сировині, так і в кінцевій продукції на її основі. Пряме визначення гліцидолових ефірів та ефірів жирних кислот 3-MCPD проводиться методом ВЕРХ/МС/МС, але цей метод складний для практичного використання внаслідок великої кількості компонентів, що визначаються (84), та відсутності аналітичних стандартів. Найбільш поширеними є непрямі методи контролю – коли відбувається реакція розкладання всіх ефірів гліцидолу та 3-MCPD до відповідно просто гліцидолу та 3-монохлоропропандіолу. Метою роботи є валідація методу ISO 18363-1:2015 «Жири та олії тваринного та рослинного походження. Визначення зв'язаних жирними кислотами хлорпропандіолів (MCPD) та гліцидолу методом ГХ/МС. Частина 1. Метод із використанням швидкої лужної переетерифікації та вимірювання для 3-MCPD та диференціального вимірювання для гліцидолу» на газовому хроматографі Agilent 8890B. в умовах ТОВ «Котекна Україна Лімітед». Визначено межі виявлення (LOD) та кількісного визначення (LOQ) MCPDs, доведено лінійність методу; оцінив точність вимірювань, надав оцінку коефіцієнтів вилучення та зміщення, розрахував розширену невизначеність. Це дослідження може сприяти коректному визначенню вмісту MCPD та гліцидолу в рослинних оліях.

Ключові слова: 3-MCPD, небезпечні фактори, виявлення, валідація, стандартизація, рослинні олії.