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Method evaluation study on the determination of the chloropropanols 3-monochloro-1,2-propanediol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP) in cold water extracts of paper, board and fibre using GC-MS(/MS)

NRL-FCM-DE 01/2025

Joint report of the BfR and the BVL

This Method Evaluation Study (MES), NRL-FCM-DE 01/2025, was organised by the German National Reference Laboratory for Food Contact Materials (NRL-FCM-DE). The NRL-FCM-DE is part of the Reference Centre for Food and Feed Analysis at the German Federal Institute for Risk Assessment (BfR). The MES was carried out in the framework of the § 64 Working Group "Consumer products" of the Federal Office of Consumer Protection and Food Safety (BVL). According to §64 of the German Food and Feed Code (LFGB), the BVL publishes an official collection of methods for sampling and analysis (ASU). In this MES, the concentrations of 3-monochloro-1,2-propanediol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP) in cold water extracts of paper, board, and fibre according to the standard DIN EN 645 had to be determined using GC-MS(/MS).

The participating laboratories received two solutions. One containing the test solution (cold water extract spiked with 3-MCPD and 1,3-DCP) and another containing a blank solution.

As part of this MES, the influence of various parameters on the quantification of chloropropanols had to be investigated. This included the influence of derivatization reagent (MSTFA or BSTFA), the method of solvent removal from the eluate, sample dilution prior to analysis, and the choice of internal standard (IS) for the quantification of 1,3-DCP (1,3-DCP-d_5 or CMP).

Thirteen laboratories reported results for the 3-MCPD analysis of samples prepared with both derivatization reagents and vacuum evaporation systems. Eleven laboratories reported results for samples prepared using evaporation

systems with nitrogen. After removing the outliers, eight to twelve results were available for evaluation for each preparation method, including the diluted samples.

Regardless of the derivatization reagent utilised, more laboratories quantified 1,3-DCP for sample preparation methods employing a vacuum evaporator system than for those methods that employed an evaporator system with a nitrogen stream. The utilisation of 1,3-DCP-d_5 as IS was found to be more frequently for the quantification of 1,3-DCP, in comparison to CMP.

The results reported by the laboratories were evaluated quantitatively based on the reported concentrations in accordance with ISO 5725-2 [1], and the interlaboratory repeatability (s_r) and reproducibility (s_R) standard deviations were calculated. For the convenience of the participating laboratories, z scores were also calculated in accordance with ISO 13528 [2].

The use of the provided method led to comparable and reproducible results for both analytes and for all sample preparation methods when their respective deuterated analogues were used as IS. The calculated s_R values ranged from 8% to 16%, and the calculated s_r values ranged from 2% to 8%.

When CMP was used as IS for the determination of 1,3-DCP, the s_R and s_r values were much higher for all sample preparation methods (20-40% and 4-20%, respectively) except for the diluted samples prepared using MSTFA (9-16% and 1-10%, respectively). In consideration of the scientific data generated in this MES, it is recommended that the utilisation of CMP as an IS be discontinued. Consequently, it will be removed from the final method description.

The majority of the results were acceptable in terms of *z* scores. A particularly good performance was achieved in the determination of 1,3-DCP using 1,3-DCP-d_5 in samples prepared using MSTFA with acceptable *z* scores for all laboratories.

For both analytes, it was demonstrated that sample dilution prior to analysis did not significantly improve reproducibility between laboratories when their respective deuterated analogues were used for quantification.

Combining all reported values for undiluted solutions quantified using their respective deuterated analogues results in a much larger dataset, reducing the uncertainty in the statistical estimation of the method's reproducibility and repeatability. This results in s_R and s_r values of 12% and 4% for 3-MCPD, and 12% and 6% for 1,3-DCP, respectively.

These findings demonstrate a high degree of interlaboratory reproducibility and repeatability for the determination of both 3-MCPD and 1,3-DCP in cold water extracts of paper, board, and fibre according to DIN EN 645 at concentrations approximating those recommended in BfR Recommendation XXXVI [3].

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List of abbreviations and symbols

3-MCPD	3-Monochloropropane-1,2-diol
1,3-DCP	1,3-Dichloro-2-propanol
BfR	Bundesinstitut für Risikobewertung - German Federal Institute for Risk Assessment
BSTFA	*N,O*-bis(trimethylsilyl)trifluoroacetamide
CL	Commercial laboratory
CMP	3-Chloro-1-methoxy-2-propanol
CWE	Cold water extract
DIN	Deutsches Institut für Normung - German Institute for Standardisation
FCM	Food contact material
GC	Gas chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MES	Method evaluation study
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSTFA	*N*-methyl-*N*-(trimethylsilyl)-trifluoracetamid
MU	Measurement uncertainty
ND	Not detectable
NRL	National Reference Laboratory
OCL	Official Control Laboratory
RV	Recommended value
RWR	Recommended working range
SML	Specific migration limit
k	Coverage factor
n_i	Number of results of the laboratory *i*
p	Number of laboratories after elimination of outliers
s_i	Standard deviation of the results of the laboratory *i*
s_L^2	Variance between the laboratories
s_r	Interlaboratory repeatability standard deviation
s_R	Interlaboratory reproducibility standard deviation
x_i	Mean value, calculated from single values reported by the participant *i*
x_{pt}	Assigned value
$\overline{x}$	Overall mean value of the mean values of all the laboratories after elimination of outliers
z	Score used for proficiency assessment
σ_{pt}	Standard deviation for proficiency assessment

1 Introduction

This Method Evaluation Study (MES), NRL-FCM-DE 01/2025, was organised by the German National Reference Laboratory for Food Contact Materials (NRL-FCM-DE). The NRL-FCM-DE is part of the Reference Centre for Food and Feed Analysis at the German Federal Institute for Risk Assessment (BfR). The MES was carried out in the framework of the § 64 Working Group "Consumer products" of the Federal Office of Consumer Protection and Food Safety (BVL). According to §64 of the German Food and Feed Code (LFGB), the BVL publishes an official collection of methods for sampling and analysis (ASU). The study assesses the parameters for a new method for the ASU for determining the presence of the chloropropanols 3-monochloro-1,2-propanediol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP) in cold water extracts of paper, board, and fibre according to the standard DIN EN 645.

According to the BfR Recommendation XXXVI [3] for food contact materials 1,3-dichloro-2-propanol must not be detectable in water extracts of the finished product (detection limit 2 µg/L, as of 01.02.2023). The transfer of 3-monochloro-1,2-propanediol into the water extract of the finished products must be as low as technically achievable, a limit of 12 µg/L (as of 01.02.2023) must not be exceeded in any case.

This MES was open to European Union Member State National Reference Laboratories (NRL), Official Control Laboratories (OCL) and commercial laboratories (CL). 14 laboratories registered for this MES and 13 laboratories submitted results. One laboratory submitted results for individual samples from both an GC-MS and an GC-MS/MS systems.

The final results of the MES are summarized in this report.

2 Scope

The objective of the present MES was to evaluate and assess the performance criteria for a draft version of a new method for the ASU for determining the presence of the chloropropanols 3-MCPD and 1,3-DCP in cold water extracts of paper, board, and fibre according to the standard DIN EN 645.

3 Set-up of the study

3.1 Time frame of the MES

The invitation for NRL-FCM-DE 01/2025 was dispatched to the participating German labs on June 17, 2025. The invitation was also distributed on July 28, 2025, within the EURL-FCM network. The registration period remained open until August 22, 2025. Samples were dispatched to the participants on October 8, 2025 and the deadline for reporting of results was set to November 7, 2025. However, a deadline extension has been granted to two laboratories, allowing them until November 14, 2025 to comply. The last results were received on November, 14 2025.

3.2 Confidentiality

The procedures used to organize this MES guarantee that the identity of the participants and the information they provide is treated as confidential. Participants in this MES were given a unique laboratory code, which is used throughout this entire report.

3.3 Distribution

In order to ascertain the presence of 3-MCPD and 1,3-DCP, each participant was provided with two well-sealed headspace vials. One containing approximately 60 mL of the test solution (cold water extract spiked with 3-MCPD and 1,3-DCP), while the other contained a blank solution. All test solutions were prepared in the TÜV Rheinland LGA Products GmbH laboratories and already contained the necessary amount of sodium chloride. All participating laboratories received their parcels containing two samples successfully, and no problems (e.g. loss of volume) were reported.

3.4 Instructions to participants

The draft version of the ASU method for the determination of 1,3-DCP and 3-MCPD in CWEs of paper, board and cardboard using GC-MS(/MS) was sent to the participants in advance. Some further information to the participants were given in the instructions document (see 11.4).

The results and general information about the analytical procedure had to be submitted via a reporting website using a unique laboratory token.

4 Sample material - Cold Water Extract (CWE) test solutions

4.1 Concentrations of the chloropropanols in the CWE test solution

The concentrations of the chloropropanols in the test solution (x_{pt}) were based on the BfR Recommendation XXXVI "Paper and board for food contact" [3] and the recommended working ranges (RWR) for compliance testing. The RWR-values were selected according to the JRC guidelines for analytical methods in food control [4]. Herein, a working range of at least 20 to 200% of the corresponding legal limit (equivalent to recommended value of the BfR recommendation) is recommend for enforcement laboratories.

Table 1: Concentrations of chloropropanols (x_{pt}) in CWE test solution, the recommended values (RV, [3]) and the recommended working ranges (RWR, [4]) for testing.

Analyte	x_{pt} [µg/L]	x_{pt} [% of RV]	RV [µg/L]	RWR [µg/L]
3-MCPD	16.32	136	12	2.4-24.0
1,3-DCP	2.26	113	2*	0.4-4.0

*Not detectable (ND), a limit of 2 µg/L is currently applicable

4.2 Preparation of the CWE test solutions

The CWE test solutions were prepared in the laboratories of the TÜV Rheinland LGA Products GmbH from CWEs of paper muffin cups that were combined and spiked to the target concentrations of 3-MCPD and 1,3-DCP.

4.3 Stability

A stability study was performed in the laboratories of the TÜV Rheinland LGA Products GmbH. The solutions were stable for the entire MES duration.

5 Assigned values and standard deviations for proficiency assessment

The assigned values (x_{pt}) for 3-MCPD and 1,3-DCP are shown in Table 2. The values for the standard deviations for proficiency assessment (σ_{pt}) were set to 20% for the analysis of 3-MCPD and 1,3-DCP by perception of experts.

Table 2: Assigned values (x_{pt}) and standard deviations for proficiency assessment (σ_{pt})

Analyte	x_{pt} [µg/L]	σ_{pt} [% of x_{pt}]
3-MCPD	16.32	20
1,3-DCP	2.26	20

6 Evaluation

6.1 Evaluation criteria and scores

The evaluation of a standard measurement method in terms of interlaboratory repeatability (s_r) and reproducibility (s_R) standard deviations was performed according to ISO 5725-2 [1]. The following equations were used for the calculations:

$$s_r = \sqrt{\frac{\sum_{i=1}^{p}(n_i - 1) \cdot s_i^2}{\sum_{i=1}^{p}(n_i - 1)}} \qquad \text{Equation 1}$$

where:

- n_i Number of individual results of the laboratory *i*
- s_i Standard deviation of the results of the laboratory *i*
- p Number of laboratories after elimination of outliers

$$s_R = \sqrt{s_r^2 + s_L^2} \qquad \text{Equation 2}$$

$$s_L^2 = \frac{s_d^2 - s_r^2}{\bar{n}} \qquad \text{Equation 3}$$

$$s_d^2 = \frac{1}{p-1} \cdot \sum_{i=1}^{p} n_i \cdot (x_i - \bar{x})^2 \qquad \text{Equation 4}$$

$$\bar{n} = \frac{1}{p-1} \cdot \left(\sum_{i=1}^{p} n_i - \frac{\sum_{i=1}^{p} n_i^2}{\sum_{i=1}^{p} n_i} \right) \qquad \text{Equation 5}$$

where:

- s^2_L Variance between the laboratories
- x_i The mean value of the results of the laboratory *i*
- $\bar{x}$ The overall mean value of the mean values of all the laboratories after the elimination of the outliers

The reproducibility limit *R* shows the maximum expected absolute difference between two single laboratory results. For a 95% probability:

$$R = k \cdot \sqrt{2} \cdot s_R = 2.8 \cdot s_R \qquad \text{Equation 6}$$

The repeatability limit *r* shows the maximum expected absolute difference between two single test results obtained under repeatability conditions. For a 95% probability:

$$r = k \cdot \sqrt{2} \cdot s_r = 2.8 \cdot s_r \qquad \text{Equation 7}$$

The Horwitz equation is an empirical formula used in analytical chemistry to estimate the expected reproducibility of a measurement method based on analyte concentration [5]. Thompson modified the model to better fit very low and very high concentrations [6].

$$\sigma\,[g/g] = \begin{cases} 0.22 \cdot c & \text{if } c < 1.2 \cdot 10^{-7} \\ 0.02 \cdot c^{0.8495} & \text{if } 1.2 \cdot 10^{-7} \leq c \leq 0.138 \\ 0.01 \cdot c^{0.5} & \text{if } c > 0.138 \end{cases} \qquad \text{Equation 8}$$

Where c is a mass fraction of the analyte in $g_{Analyte}/g_{Matrix}$.

The expected reproducibility can be calculated as a percentage σ [%] by multiplying the calculated mass fraction σ [g/g] by 100%.

$$\mathrm{SD}_{\mathrm{Horwitz}}[\%] = \sigma\,[\%] = \sigma\,[g/g] \cdot 100\% \qquad \text{Equation 9}$$

In analytical chemistry, the Horwitz ratio (*HorRat*) is a performance parameter used to evaluate the method's reproducibility.

$$HorRat_R = \frac{s_R[\%]}{\sigma[\%]} \qquad \text{Equation 10}$$

$HorRat_R$ values between 0.5 and 2.0 are considered acceptable for the routine method [7].

In addition, the individual laboratory performance was expressed in terms of *z* scores according to ISO 13528 [2]. The *z* score describes the deviation between the participants' mean and the assigned value in terms of the standard deviation for proficiency assessment (σ_{pt}). The z scores for the proficiency test results x_i were calculated as follows:

$$z_i = \frac{x_i - x_{pt}}{\sigma_{pt}} \qquad \text{Equation 11}$$

The interpretation of the *z* performance scores is done according to ISO 13528 [2]:

$\lvert z_i \rvert \leq 2.00$	acceptable performance	(green in Tables)
$2.00 < \lvert z_i \rvert < 3.00$	questionable performance	(orange in Tables)
$\lvert z_i \rvert \geq 3.00$	unacceptable performance	(red in Tables)

6.2 Statistical parameters

The evaluation of the MES results in terms of interlaboratory repeatability (s_r) and reproducibility (s_R) standard deviations is summarized in Table 3 to Table 5.

The results presented below are grouped according to the type of sample preparation technique applied in this study:

Evaporation: use of a vacuum evaporator system (ES), such as a Syncore, or an evaporator system that uses a nitrogen stream (NS), such as a TurboVap.

Derivatization: using MSTFA or BSTFA

Dilution factor: 1 (direct analysis with no dilution) or 5 (dilution 1:5)

The instructions for the sample preparation can be found in the Annex (see 11.4)

The majority of participating laboratories (10-13) reported results for the 3-MCPD analysis, using both derivatization reagents and evaporation techniques.

Regardless of the derivatization reagent used, more results for the quantification of 1,3-DCP were reported for sample preparation methods that used a vacuum evaporator system (ES; 7-11 laboratories) than for methods that used an evaporator system with a nitrogen stream (NS; 4-8 laboratories). 1,3-DCP-d_5 was used more frequently for the quantification of 1,3-DCP (ES: 10-11, NS: 7-8), as compared to CMP (ES: 7-8, NS: 4-5).

The reproducibility standard deviations (s_R) for the determination of 3-MCPD and 1,3-DCP for all sample preparation methods were within the range of 8.0-16.4% when their respective deuterated analogues were used as internal standards. When CMP was used as the internal standard for the determination of 1,3-DCP, the s_R-values were much higher for all sample preparation methods, ranging from 20.1% to 40.4%, except for the diluted samples prepared using MSTFA with values of 8.5% (NS) and 15.7% (ES).

6.3 Results for the determination of 3-MCPD using 3-MCPD-d_5

Table 3: Statistical parameters for the determination of 3-MCPD using 3-MCPD-d_5 as the internal standard.

Evaporation	**Nitrogen Stream (NS)**				**Vacuum Evaporator (ES)**			
Derivatization	**MSTFA**		**BSTFA**		**MSTFA**		**BSTFA**	
Dilution Factor	**1**	**5**	**1**	**5**	**1**	**5**	**1**	**5**
No. of labs	11	10	11	10	13	12	13	13
No. of labs without outliers	10	8	9	8	12	11	11	11
Mean [µg/L]	16.80	16.40	17.07	16.65	17.28	16.82	16.69	17.30
Repeatability SD* (s_r) [%]	5.2	2.3	4.5	2.5	2.6	2.7	3.6	2.4
Reproducibility SD* (s_R) [%]	13.7	9.4	16.4	13.6	9.7	12.7	10.9	12.8

* SD: Standard deviation

6.4 Results for the determination of 1,3-DCP using 1,3-DCP-d_5

Table 4: Statistical parameters for the determination of 1,3-DCP using 1,3-DCP-d_5 as the internal standard.

Evaporation	**Nitrogen Stream (NS)**				**Vacuum Evaporator (ES)**			
Derivatization	**MSTFA**		**BSTFA**		**MSTFA**		**BSTFA**	
Dilution Factor	**1**	**5**	**1**	**5**	**1**	**5**	**1**	**5**
No. of labs	7	8	8	8	11	11	10	11
No. of labs without outliers	7	8	7	7	11	11	9	9
Mean [µg/L]	2.30	2.30	2.37	2.49	2.35	2.41	2.40	2.44
Repeatability SD* (s_r) [%]	7.3	3.9	3.7	5.1	8.0	7.0	4.2	4.9
Reproducibility SD* (s_R) [%]	12.0	8.0	13.1	11.7	14.2	9.9	8.7	14.0

* SD: Standard deviation

6.5 Results for the determination of 1,3-DCP using CMP

Table 5: Statistical parameters for the determination of 1,3-DCP using CMP as the internal standard.

Evaporation	**Nitrogen Stream (NS)**				**Vacuum Evaporator (ES)**			
Derivatization	**MSTFA**		**BSTFA**		**MSTFA**		**BSTFA**	
Dilution Factor	**1**	**5**	**1**	**5**	**1**	**5**	**1**	**5**
No. of labs	5	5	4	4	8	8	7	7
No. of labs without outliers	5	4	4	4	8	7	6	7
Mean [µg/L]	2.29	2.08	2.25	2.23	2.97	2.50	2.36	2.00
Repeatability SD* (s_r) [%]	3.5	0.9	6.1	20.3	14.7	9.5	8.1	14.3
Reproducibility SD* (s_R) [%]	29.0	8.5	28.3	31.4	36.9	15.7	20.1	40.4

* SD: Standard deviation

6.6 Combined results for undiluted solutions, scientific justification and reasoning

In general, the method using CMP as IS for 1,3-DCP results in significantly higher s_R values. Consequently, this approach is not advised for this particular analytical method. For both analytes, it was demonstrated that sample dilution prior to analysis did not significantly improve reproducibility between laboratories when their respective deuterated analogues were used for quantification. Furthermore, the dilution of the sample has been shown to reduce the concentration of target analytes in the analysis solution, which may result in an increase in the LOQ of the method.

Therefore, all reported values for undiluted solutions were combined into a single data set for statistical analysis, except for the 1,3-DCP data quantified using CMP. This results in a much larger dataset, reducing uncertainties in the statistical estimation of the method's reproducibility and repeatability. Furthermore, the parameters estimated using the combined dataset include potential deviations related to differences in derivatization

reagents (MSTFA or BSTFA) and evaporator systems (NS or ES). This makes the entire method even more robust. The estimated statistical parameters are summarized in Table 6.

Table 6: Final statistical parameters for the determination of 3-MCPD and 1,3-DCP estimated using all reported values for undiluted CWE test solutions quantified using their respective deuterated analogues.

Analyte **Internal standard**	**3-MCPD** **3-MCPD-d_5**	**1,3-DCP** **1,3-DCP-d_5**
No. of results*	46	37
No. of results without outliers	42	34
Mean [µg/L]	16.97	2.36
Repeatability SD (s_r) [µg/L]	0.68	0.15
Repeatability SD (s_r) [%]	4.0	6.3
Repeatability limit (r) [%]	11.3	17.6
Reproducibility SD (s_R) [µg/L]	2.08	0.28
Reproducibility SD (s_R) [%]	12.3	11.9
Reproducibility limit (R) [%]	34.3	33.3
Reproducibility SD ($SD_{Horwitz}$) [%]	22.0	22.0
$HorRat_R$ value	0.56	0.54

*Number of results submitted by laboratories. The great majority of these results were analysed in duplicates.

According to the t-test at the 95% confidence level, no significant difference was found between the total mean of participants and the spiked value for both analytes.

The relative reproducibility standard deviation values for 3-MCPD of 12.3% and for 1,3-DCP of 11.9 % are within the range of 8.0-16.4% determined for the individual sample preparation methods (see above). This demonstrates that the distribution of values in the combined dataset is similar to those of the individual sample preparation methods, indicating that there is no significant bias between these methods. Otherwise, an increase in reproducibility standard deviation due to variation in individual biases would be expected. This assumption was confirmed through variance analysis. The homogeneity of variances requirement was met, and the one-way ANOVA revealed no significant differences among the groups. The calculated values of reproducibility standard deviation are significantly lower than the corresponding Horwitz reproducibility standard deviation ($SD_{Horwitz}$) value of 22%, often used as a benchmark for collaborative trials [2,6]. This results in acceptable Horwitz ratios ($HorRat_R$) of 0.56 and 0.54 for 3-MCPD and 1,3-DCP, respectively. These values demonstrate that the method is performed with a high degree of precision and consistency in participating laboratories, and that it is robust and yields highly consistent results.

A similar situation is observed for the relative repeatability standard deviation values. The values of 4.0 for 3-MCPD and 6.3 for 1,3-DCP are within the previously observed range of 2.3-8.0% for the individual sample preparation methods.

6.7 Performance of the laboratories according to *z* score

6.7.1 Determination of 3-MCPD using 3-MCPD-d_5

All except one laboratory reported acceptable results for the determination of 3-MCPD in diluted and undiluted solutions using both evaporation techniques with MSTFA. All except two of the results for the diluted solutions prepared using BSTFA were acceptable, while four of the results for undiluted solutions (two per evaporation method) were unacceptable and one was questionable. One of the laboratories reported unacceptable results for all solutions.

For samples prepared using vacuum evaporator systems (ES), all laboratories reported results for all sample preparation methods, except for one laboratory that did not report results for undiluted solutions. One laboratory reported an additional result for a diluted solution that was prepared using a BSTFA and a vacuum evaporator system. Regarding samples prepared using nitrogen stream evaporators (NS), two laboratories did not report any results and one laboratory only reported results for undiluted solutions.

Table 7: *z* scores for the quantification of 3-MCPD using 3-MCPD-d_5 as the internal standard. The value of σ_{pt} was set to 20 % of the corresponding x_{pt} value of 16.32 µg/L. According to ISO 13528 [2], |*z* score| ≤ 2.00 is acceptable (highlighted in green), 2.00 < |*z* score| < 3.00 is questionable (highlighted in orange) and |*z* score| ≥ 3.00 is unacceptable (highlighted in red).

Evapor.	**Nitrogen Stream (NS)**								**Vacuum Evaporator (ES)**							
Derivat.	**MSTFA**				**BSTFA**				**MSTFA**				**BSTFA**			
Dilut. F.	**1**		**5**		**1**		**5**		**1**		**5**		**1**		**5**	
Lab 1	16.61	0.09	17.37	0.32	17.21	0.27	17.66	0.41	16.44	0.04	16.86	0.17	17.00	0.21	16.76	0.13
Lab 2	-	-	-	-	-	-	-	-	19.95	1.11	20.55	1.29	19.26	0.90	19.75	1.05
Lab 2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17.41	0.33
Lab 3	21.74	1.66	22.08	1.77	22.88	2.01	22.19	1.80	19.16	0.87	19.37	0.94	19.50	0.97	19.51	0.98
Lab 4	18.51	0.67	18.96	0.81	28.01	3.58	20.44	1.26	18.52	0.67	18.62	0.70	30.84	4.45	20.13	1.17
Lab 5	-	-	-	-	-	-	-	-	18.22	0.58	16.64	0.10	15.61	-0.22	14.90	-0.44
Lab 7	15.01	-0.40	14.71	-0.49	15.47	-0.26	15.98	-0.10	16.34	0.00	14.78	-0.47	14.65	-0.51	16.35	0.01
Lab 8	14.05	-0.70	14.55	-0.54	13.33	-0.92	13.12	-0.98	16.28	-0.01	15.22	-0.34	14.58	-0.53	13.83	-0.76
Lab 9	17.15	0.25	16.35	0.01	17.30	0.30	16.65	0.10	17.40	0.33	16.90	0.18	16.70	0.12	16.95	0.19
Lab 10	14.98	-0.41	15.57	-0.23	15.57	-0.23	14.80	-0.47	15.15	-0.36	15.94	-0.12	15.76	-0.17	15.15	-0.36
Lab 11	17.41	0.33	-	-	18.20	0.58	-	-	17.68	0.42	-	-	16.92	0.18	-	-
Lab 13	17.21	0.27	17.61	0.40	18.64	0.71	18.49	0.66	17.95	0.50	17.06	0.23	18.48	0.66	19.02	0.83
Lab 14	15.32	-0.31	16.10	-0.07	15.08	-0.38	16.11	-0.06	14.28	-0.63	13.14	-0.97	15.13	-0.37	19.60	1.00
Lab 15	2.50	-4.23	2.51	-4.23	2.52	-4.23	2.68	-4.18	2.50	-4.23	2.22	-4.32	2.59	-4.21	2.68	-4.18

* This laboratory submitted results for individual samples from both an GC-MS and an GC-MS/MS systems
'-' indicates that no value was submitted

6.7.2 Determination of 1,3-DCP using 1,3-DCP-d_5

All of the reported results for the determination of 1,3-DCP using the 1,3-DCP-d_5 internal standard and MSTFA were acceptable. All except one laboratory reported acceptable results for samples prepared using BSTFA.

For samples prepared using vacuum evaporator systems (ES) and BSTFA, two laboratories did not report any results, and one laboratory did not report a result for the undiluted sample. For samples prepared using ES and MSTFA, one laboratory did not report any results, and one laboratory reported results for either diluted or undiluted samples. Regarding samples prepared using a nitrogen stream evaporator (NS), four laboratories did not report any results, and one laboratory did not report result for an undiluted sample produced using MSTFA.

Table 8: *z* scores for the quantification of 1,3-DCP using 1,3-DCP-d_5 as the internal standard. The value of σ_{pt} was set to 20 % of the corresponding x_{pt} value of 2.26 µg/L. According to ISO 13528 [2], |*z* score| ≤ 2.00 is acceptable (highlighted in green), 2.00 < |*z* score| < 3.00 is questionable (highlighted in orange) and |*z* score| ≥ 3.00 is unacceptable (highlighted in red).

Evapor.	**Nitrogen Stream (NS)**								**Vacuum Evaporator (ES)**							
Derivat.	**MSTFA**				**BSTFA**				**MSTFA**				**BSTFA**			
Dilut. F.	**1**		**5**		**1**		**5**		**1**		**5**		**1**		**5**	
Lab 1	-	-	-	-	-	-	-	-	2.65	0.87	2.71	1.00	2.56	0.66	2.20	-0.12
Lab 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab 2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.90	-0.80
Lab 3	-	-	-	-	-	-	-	-	2.45	0.41	2.34	0.19	-	-	-	-
Lab 4	1.96	-0.67	2.30	0.08	1.80	-1.03	2.96	1.55	1.84	-0.93	2.68	0.92	1.89	-0.82	3.07	1.79
Lab 5	-	-	-	-	-	-	-	-	2.99	1.62	2.52	0.56	2.63	0.82	2.33	0.15
Lab 7	2.12	-0.32	2.25	-0.03	2.28	0.04	2.47	0.46	2.10	-0.37	2.22	-0.10	2.31	0.11	2.35	0.20
Lab 8	-	-	2.33	0.14	2.51	0.55	2.30	0.08	-	-	2.29	0.06	2.41	0.32	2.44	0.40
Lab 9	2.31	0.11	2.17	-0.20	2.41	0.33	2.40	0.30	2.42	0.35	2.45	0.41	2.42	0.35	2.58	0.71
Lab 10	2.12	-0.31	2.13	-0.30	2.28	0.04	2.21	-0.12	2.11	-0.34	2.06	-0.44	2.31	0.10	2.37	0.24
Lab 11	-	-	-	-	-	-	-	-	2.22	-0.10	-	-	-	-	-	-
Lab 13	2.64	0.83	2.67	0.91	2.74	1.05	2.76	1.11	2.48	0.48	2.65	0.86	2.65	0.86	2.74	1.06
Lab 14	2.46	0.44	2.20	-0.14	2.61	0.76	2.31	0.11	2.28	0.04	2.35	0.19	2.42	0.35	2.16	-0.22
Lab 15	2.53	0.59	2.41	0.34	0.51	-3.87	0.50	-3.89	2.38	0.27	2.29	0.06	0.74	-3.36	0.62	-3.63

* This laboratory submitted results for individual samples from both an GC-MS and an GC-MS/MS systems
'-' indicates that no value was submitted

6.7.3 Determination of 1,3-DCP using CMP

All of the reported results for the determination of 1,3-DCP using the CMP internal standard and nitrogen stream evaporator (NS) in the diluted solutions were acceptable, while the results from one laboratory for undiluted solutions were questionable for both of the derivatization agents. Regarding samples prepared using vacuum evaporator systems (ES), three results were unacceptable for sample preparation methods using MSTFA (two for undiluted solutions and one for diluted solutions). Additionally, there was one unacceptable result (undiluted solution) and three questionable results (diluted solutions) for sample preparation methods using BSTFA.

Seven laboratories did not report results for sample preparation methods using NS. Two laboratories reported results only for MSTFA: one for a diluted solution and one for an undiluted solution. Regarding samples prepared using vacuum evaporator systems (ES), five laboratories did not report results, and one laboratory reported results for an undiluted sample prepared using MSTFA.

Table 9: *z* scores for the quantification of 1,3-DCP using CMP as the internal standard. The value of σ_{pt} was set to 20 % of the corresponding x_{pt} value of 2.26 µg/L. According to ISO 13528 [2], |*z* score| ≤ 2.00 is acceptable (highlighted in green), 2.00 < |*z* score| < 3.00 is questionable (highlighted in orange) and |z score| ≥ 3.00 is unacceptable (highlighted in red).

Evapor.	**Nitrogen Stream (NS)**								**Vacuum Evaporator (ES)**							
Derivat.	**MSTFA**				**BSTFA**				**MSTFA**				**BSTFA**			
Dilut. F.	**1**		**5**		**1**		**5**		**1**		**5**		**1**		**5**	
Lab 1	-	-	-	-	-	-	-	-	2.51	0.56	2.68	0.93	3.10	1.86	1.19	-2.37
Lab 2	-	-	-	-	-	-	-	-	2.54	0.61	2.09	-0.39	2.46	0.44	2.38	0.25
Lab 2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab 4	2.00	-0.58	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab 5	-	-	-	-	-	-	-	-	5.00	6.05	5.44	7.04	2.32	0.12	1.90	-0.81
Lab 7	2.02	-0.54	2.73	1.03	2.03	-0.52	3.16	1.99	2.52	0.58	2.53	0.60	2.53	0.59	3.45	2.62
Lab 8	-	-	1.83	-0.96	-	-	-	-	-	-	2.32	0.13	-	-	-	-
Lab 9	3.34	2.39	2.21	-0.11	3.19	2.05	1.85	-0.91	4.11	4.09	2.74	1.05	3.90	3.63	1.14	-2.49
Lab 10	1.72	-1.21	2.10	-0.37	1.81	-1.01	2.06	-0.44	1.79	-1.04	2.11	-0.33	1.86	-0.88	2.03	-0.52
Lab 11	-	-	-	-	-	-	-	-	2.41	0.33	-	-	-	-	-	-
Lab 13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lab 14	2.40	0.31	2.18	-0.18	2.00	-0.59	1.87	-0.87	2.87	1.35	3.06	1.76	1.92	-0.76	1.91	-0.79
Lab 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* This laboratory submitted results for individual samples from both an GC-MS and an GC-MS/MS systems
'-' indicates that no value was submitted

6.8 Limits of detection and quantification as well as used analytical techniques

Seven laboratories used GC-MS/MS and six used GC-MS to quantify chlorpropanols. One laboratory also submitted GC-MS results for individual sample preparation combinations in addition to GC-MS/MS results.

The limits of detection (LODs) and quantification (LOQs) for the determination of 3-MCPD and 1,3-DCP submitted by the participants are shown in Table 10. Thirteen laboratories submitted the required information, while two did not. The LOD values range from 0.2 to 10 µg/L, and the LOQ values range from 0.5 to 20 µg/L for the determination of 3-MCPD. For the determination of 1,3-DCP, the LOD values range from 0.03 to 2 µg/L, and the LOQ values range from 0.13 to 4 µg/L, when 1,3-DCP-d_5 is used as the IS. Slightly higher values were reported for the determination of 1,3-DCP using CMP as the IS, resulting in LOD and LOQ ranges of 0.05-2 µg/L and 0.5-4 µg/L, respectively.

Table 10: The limits of detection (LODs) and quantification (LOQs) for the determination of 3-MCPD and 1,3-DCP, together with the m/z ratios used for quantification, which were submitted by the participants.

Lab Code	3-MCPD 3-MCPD-d_5			1,3-DCP 1,3-DCP-d_5			1,3-DCP CMP			**	Analytical technique
	LOD	LOQ	m/z	LOD	LOQ	m/z	LOD	LOQ	m/z		
	[µg/L]										
Lab 1	0.67	2	239 > 147	0.13	0.4	185 > 93	ND	ND	185 > 93	(1)	GC-MS/MS
Lab 2	0.2	1.2	119	ND	ND	ND	0.05	0.5	151	(3)	GC-MS
Lab 3	2	10	239	0.5	2	151	0.5	2	93	(1)	GC-MS
Lab 4	1	2	239	ND	ND	ND	0.4	1.2	93	(3)	GC-MS
Lab 5	0.52	1.87	116	0.03	0.13	93	0.16	0.54	93	(1)	GC-MS
Lab 6	-	-	-	-	-	-	-	-	-	-	-
Lab 7	0.37	1.11	101 > 59	ND	ND	185 > 93	0.27	0.92	185 > 93	(1)	GC-MS/MS
Lab 8	0.71	2	116 (119)	0.32	1	93 (93)	ND	ND	93 (89)	(2)	GC-MS
Lab 9	0.564	2.05	239.0>73.1	0.069	0.254	185.0>93.0	0.388	1.604	185.0>93.0	(1)	GC-MS/MS
Lab 10	10	20	116>101@5	2	4	185>93@5	2	4	183>93@5	(5)	GC-MS/MS
Lab 11	ND	2	239	ND	2	185	ND	ND	185	(4)	GC-MS
Lab 12	-	-	-	-	-	-	-	-	-	-	-
Lab 13	ND	2	116>59	ND	0.4	185>93	ND	ND	ND	(3)	GC-MS/MS
Lab 14	1.25	4.17	101>59	0.21	0.69	93>65	0.16	0.53	93>65	(4)	GC-MS/MS
Lab 15	ND	0.5	116 > 59	ND	0.5	185 > 93	ND	ND	ND	(6)	GC-MS/MS

ND: indicates that no LOD or LOQ value has been determined in the lab
'-' indicates that no value was submitted
** Method of LOD/LOQ determination:
(1): DIN 32645 (calibration method)
(2): DIN 32645 (blank value method)
(3): Signal-to-noise Ratio (S/N)
(4): Standard Deviation of the Response (σ) and Slope (S) of the Calibration Curve [e.g. LOD=3σ/S]
(5): Lowest calibration level
(6): Reproducibility and repeatability on the LOQ below limits from standard BVL B 80.56-2

6.9 Acidity of the analyzed solution

Pursuant to the evaluated method herein, it is imperative that the pH value of the CWE is ascertained prior to analysis. Thirteen laboratories submitted the pH values, while two did not (see Table 11). Most of the submitted pH values (7 out of 13) were determined using a pH meter. Six laboratories used pH indicator paper to determine the pH value of the test solution. The pH range of 5-7.5 was determined using indicator paper. The following pH values were reported: two laboratories reported the correct value of 6, two reported too low values of 5, and one reported too high value of 7.5. The much narrower range was determined using a pH meter. All received results, except one (6.98), were within the range of 5.93-6.11, which is very close to the consensus value of 6 determined by expert laboratories.

However, results obtained using indicator paper should be analyzed critically. For instance, consider whether the indicator paper's range is appropriate and whether the pH value resolution is acceptable (at least 0.5 pH steps). In any case, if the pH analysis using indicator paper results in a value greater than 7.5, it is necessary to determine the pH using a pH meter to ensure that the solution does not require acidification (see method).

Table 11: The pH values of the test solution submitted by the laboratories. pH=6 is a consensus value determined by expert laboratories.

Lab Code	pH	Method	Result
Lab 1	5	pH indicator paper (MQuant Strips)	Not OK
Lab 2	5	pH indicator paper	Not OK
Lab 3	5,95	pH meter	OK
Lab 4	5,93	pH meter	OK
Lab 5	< 6	pH indicator paper (6.0 - 8.1)	OK
Lab 6	-	-	-
Lab 7	6	pH meter	OK
Lab 8	6	pH meter	OK
Lab 9	6.01	pH meter	OK
Lab 10	6	test strip	OK
Lab 11	7.5	pH indicator paper	Not OK
Lab 12	-	-	-
Lab 13	6.98	pH meter	Not OK
Lab 14	6.11	pH meter	OK
Lab 15	6	pH Indicator Paper	OK

6.9.1 Sample preparation and analysis

Thirteen laboratories provided the necessary information. Of these, eleven used non-activated MSTFA for derivatisation, while two used activated MSTFA. Seven laboratories subtracted the blank values, while six did not. Of the thirteen laboratories, only one used weighting by calibration (1/x), while the others used non-weighted calibration.

The majority of the reported issues were associated with the utilisation of the CMP as an internal standard or the low sensitivity of the method for the analysis of diluted samples. Inquiries were made regarding the method and/or suggestions for improvement were requested, and most laboratories provided them. The following problems, suggestions, and comments were reported:

- Problems arise when evaluating results using CMP as the IS. These problems are mainly related to significant CMP losses, which result in a low peak area, poor linearity of the calibration, and inconsistent results when different ions are used for quantification. Additionally, the loss of CMP in the autosampler was observed when samples were left at room temperature for more than six hours.
- Dilution of the samples results in a low peak area. In some laboratories, this leads to poor linearity of the calibration and poor peak shape.
- In some laboratories, high blank values for 1,3-DCP are observed, particularly in samples prepared using evaporator systems with a nitrogen stream, or when BSTFA is used for derivatisation.
- The proper choice of ion used for the quantification of 1,3-DCP is especially important when using 1,3-DCP-d_5 as an IS. The ion with an m/z of 93 is highly abundant in the spectra of both 1,3-DCP and 1,3-DCP-d_5. Therefore, if these substances are not well-separated by chromatography, reliable quantification using m/z=93 is not possible, and other ion should be used for quantification.
- When using BSTFA for derivatization, one laboratory reported poor linearity, while another reported a double peak for MCPD-d_5.
- Long time for the solvent removal using a nitrogen stream (5h) and hard conditions when a vacuum evaporator is applied. Therefore, it was proposed to use a combination of these two systems to reduce the time required for solvent removal.
- Other proposals included reducing the elution volume to 20 mL, increasing the amount of ethanol used for quenching to 500 µL, and using LLE extraction instead of LLE extraction with diatomaceous earth to reduce costs.

The complete list of problems, suggestions, and comments reported by participants can be found in Annex (see Table A 3).

7 Conclusions

The primary aim of this MES was to assess and evaluate the performance criteria for a new method for the ASU for the determination of the chloropropanols 3-MCPD and 1,3-DCP in cold water extracts of paper, board and fibre by means of DIN EN 645 using GC-MS(/MS) systems.

In total, 13 laboratories submitted results. One laboratory submitted results for individual samples from both an MS and an MS/MS mass-analyzer. After removing the outliers, eight to twelve results were available for the evaluation of 3-MCPD for each preparation method. Seven to eleven results were available for the evaluation of 1,3-DCP with the 1,3-DCP-d_5 internal standard, and only four to eight were available with CMP.

The statistical evaluation of the data submitted by the participating laboratories was performed in terms of relative repeatability (s_r) and relative reproducibility (s_R) standard deviations.

The use of the provided method led to comparable and reproducible results for both analytes and for all sample preparation methods when their respective deuterated analogues were used as internal standards. The calculated s_R values were of 8.0-16.4%, and the calculated s_r values were of 2.3-8.0%. Much higher values were obtained for all sample preparation methods (20.1-40.4% and 3.5-20.3%, respectively), except for diluted samples prepared using MSTFA (8.5-15.7% and 0.9-9.5%, respectively), when CMP was used for 1,3-DCP quantification. For both analytes, sample dilution prior to analysis did not significantly improve reproducibility between laboratories when their respective deuterated analogues were used for quantification. However, due to dilution, the limits of detection and quantification may be reduced. Therefore, the dilution step and the use of CMP for the quantification of 1,3-DCP do not improve the method and will be removed from the method procedure.

Combining all reported values for undiluted solutions that were quantified using their respective deuterated analogues results in a much larger dataset. This reduces the uncertainty in the statistical estimation, resulting in s_R and s_r values of 12.3% and 4.0% for 3-MCPD, and 11.9% and 6.3% for 1,3 DCP. Because these parameters were estimated using a combined dataset that included data for both derivatization reagents (MSTFA or BSTFA) and all applied evaporator systems (NS or ES), they can be applied to the method regardless of which of these conditions is used.

The majority of the submitted results of the labs were acceptable in terms of *z* scores. A particularly good performance was achieved in the determination of 1,3-DCP using 1,3-DCP-d_5 in samples prepared using MSTFA with acceptable *z* scores for all laboratories.

The performance demonstrated in the determination of 3-MCPD and 1,3-DCP in undiluted solutions at concentrations close to those in the current BfR recommendation [3] was found to be highly satisfactory. The same can be said of the performance in 1:5 diluted solutions at much lower concentrations.

In general, the results of this method evaluation study demonstrated a high interlaboratory reproducibility and repeatability for the determination of both 3-MCPD and 1,3-DCP when using their deuterated analogues as internal standards. Finally, it can be concluded from the assessed and statistically evaluated dataset that this method for the determination of

chloropropanols (3-MCPD and 1,3-DCP) in cold water extracts of paper, board, and fibre according to DIN EN 645 using GC-MS(/MS) can clearly be recommended.

8 Acknowledgments / List of participating laboratories

The 13 laboratories listed here are kindly acknowledged for their participation in the MES. The laboratory codes were allocated randomly to the participants and do not correspond to the alphabetical order shown here.

Table 12: List of participating laboratories.

Organization	Country
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (OCL)	Germany
Bundesinstitut für Risikobewertung (NRL)	Germany
Chemisches und Veterinäruntersuchungsamt Stuttgart (OCL)	Germany
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (OCL)	Germany
Universidade Católica Portuguesa (NRL)	Portugal
GALAB Laboratories GmbH (CL)	Germany
ISEGA GmbH (CL)	Germany
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (NRL)	Italy
Landesbetrieb Hessisches Landeslabor (OCL)	Germany
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (OCL)	Germany
Thüringer Landesamt für Verbraucherschutz (OCL)	Germany
TÜV Rheinland LGA Products GmbH (CL)	Germany
Wessling GmbH (CL)	Germany

Special thanks go to TÜV Rheinland LGA Products GmbH, whose laboratories kindly provided all the necessary test solutions.

9 References

[1] ISO 5725-2:2019; Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

[2] ISO 13528:2022, Statistical methods for use in proficiency testing by interlaboratory comparison.

[3] German Federal Institute for Risk Assessment (BfR), BfR Recommendation: XXXVI. Paper and board for food contact. 2022, as of 01.02.2023, https://www.bfr.bund.de/cm/349/XXXVI-Paper-and-Board-for-Food-Contact.pdf

[4] Bratinova S., Raffael B., Simoneau C. Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. EUR 24105 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC53034

[5] Horwitz, W., Evaluation of analytical methods used for regulation of foods and drugs. Analytical Chemistry, 1982, 54(1), 67A–76A. https://doi.org/10.1021/ac00238a765

[6] Thompson M., Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst, 2000, 125, 385–386. https://doi.org/10.1039/B000282H

[7] Horwitz W., Albert R. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J AOAC Int., 2006, 89(4), 1095-109. https://doi.org/10.1093/jaoac/89.4.1095

10 List of Tables

11 Annex

11.1 Invitation letter

Dear colleagues,

together with TÜV Rheinland and the Federal Office of Consumer Protection and Food Safety (BVL) we will organize a method evaluation study (MES) of a §64 method (LFGB) for the analysis of chloropropanols. On behalf of the organizers, we would like to invite you to participate in this MES! Start will not be before the end of Q3 this year. A draft of the method is attached as a pdf.

The scope of the MES is to investigate the influence of different parameters on the quantification of chloropropanols. In detail, we will focus on the choice of internal standard for 1,3-DCP (CMP/DCP-d_5), the derivatization reagents (MSTFA/BSTFA) and the method for solvent removal of the eluate (vacuum evaporation/nitrogen stream). Please find below the registration form for participation in the MES as well as some additional questions regarding your laboratory equipment, for which we need some information for the preparation of the final study design. If you have time and the possibility to help us with our method you can register for a participation under the following link:

https://akademie.bfr.berlin/277614?lang=en

Deadline for the registration is the 22nd of august 2025, as we can only offer a limited number of participations (about 20) registration might be closed earlier if this number has been reached. Registration/Participation is free of costs and open for all NRL/OCL.

Yours sincerely,

NRL-FCM-DE Team

11.2 Registration form

The registration process was completed online (https://akademie.bfr.berlin/277614?lang=en)

Registration Form

for a method evaluation study on the determination of

1,3-dichloro-2-propanol (1,3-DCP) and 3-monochloro-1,2-propanediol (3-MCPD)

using GC-MS(/MS)

Dear colleagues,

together with TÜV Rheinland and the Federal Office of Consumer Protection and Food Safety (BVL) we will organize a method evaluation study (MES) for the analysis of chloropropanols. On behalf of the organizers, we would like to invite you to participate in this MES! Start will be at the end of Q3 this year.

The scope of the MES is to investigate the influence of different parameters on the quantification of chloropropanols. In detail, we will focus on the choice of internal standard for 1,3-DCP (CMP/DCP-d_5), the derivatization reagents (MSTFA/BSTFA) and the method for solvent removal of the eluate (vacuum evaporation/nitrogen stream). Please find below the registration form for participation in the MES as well as some additional questions regarding your laboratory equipment, for which we need some information for the preparation of the final study design.

Deadline for the registration is the 22th of august 2025, as we can only offer a limited number of participations (about 20) registration might be closed earlier if this number has been reached. Registration/Participation is free of costs.

Yours sincerely,

NRL-FCM Team

There are 4 questions in this survey.

1) Name of the institution: _____

2) Name and contact details of the contact person
- Title: _____
- First name: _____
- Surname: _____
- E-mail address: _____
- Telephone number: _____

3) If you already have an established method for chloropropanol analysis in your laboratory, does it allow sufficient chromatographic separation of 1,3-DCP and 1,3-DCP-d_5 and thus individual integration of the two peaks?

*Choose **one** of the following answers:*
- o Yes
- o No
- o Unknown

4) Which of the following equipment for solvent removal is available in your laboratory? Please specify all available options (multiple selections possible).

Comment only when you choose an answer.
- Vacuum evaporator (e.g. rotary evaporator, SynchorePlus (Büchi) etc.):
  _____
- Evaporators using nitrogen stream (e.g. Turbovap or devices with manual settings):
  _____
- Other devices (please specify):
  _____

Thank you very much for your participation!
We will evaluate all feedback received and then contact you again with more detailed instructions on the MES procedure.
NRL-FCM Team

Print your answers.

11.3 Accompanying letter to participants

Dear [Participants' Name and Surname],

The samples for our method evaluation study have been dispatched with UPS, your tracking number is:

[Tracking Number]

In the package you will find two headspace vials, one with the sample and one with a blank solution. Please check the vials and if there are any problems, please contact us immediately.

To submit your results and to answer some questions please use the following link:

https://akademie.bfr.berlin/775199?lang=en

For the submission you need your lab code and a token, these can be found in the attached pdf including the instructions. The submission can be saved and resumed at any time. When saving the submission, you will be asked for a username and password (please choose your own). To resume your submission later, you will need to login again with your token and select "Load unfinished survey" in the lower left corner. In the next step, you will be asked for your username and password.

After successfully submitting your results, use the 'Print your answers' button and select 'queXML PDF export'. Check the resulting PDF for the correctness of the results (long sentences are not completely readable in the PDF, but are readable in the submitted form). Please send this PDF digitally signed to NRL-FCM@bfr.bund.de or alternatively the PDF with the statement in the e-mail whether all data are correct.

Please contact us if you have any further questions.

Yours sincerely,

NRL-FCM-DE Team

11.4 Instructions

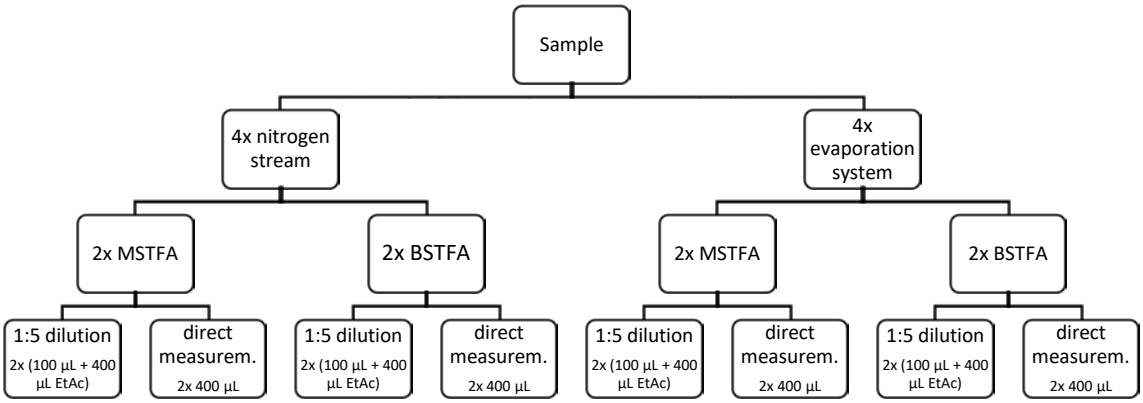
Instructions

Thank you, for participating in our method evaluation study (MES) for the quantification of chlorpropanoles in cold water extracts from paper, cardboard or fiber casting. In this MES only the analytical part is evaluated, the extraction procedure will be part of another study.

You should have received a headspace vial with about 60 mL of sample, and another one with a blank solution. Please check, if everything is well sealed, if not please send us an email to nrl-fcm@bfr.bund.de. Please keep the sample in the refrigerator. It is a spiked cold-water extract and NaCl has been added already, so please start the sample preparation with the pH-measurement step of the method draft.

Please use all three internal standards (CMP, DCP-d_5 and MCPD-d_5) for sample preparation but make sure that you can chromatographically separate DCP and DCP-d_5 or use a unique quantifier for DCP. Prepare each sample as close as possible to the draft that we sent to you, of course calibration and the methods described for the chromatography are just suggestions and should be adjusted for the systems you use. If you do not have the draft please contact us immediately.

We ask you to do four liquid-liquid extractions with each (if available) of the evaporation techniques mentioned in the method draft (7.2.3) i.e. four with evaporation under a nitrogen stream (e.g. Turbovap or comparable) and four with an vacuum evaporator (like the Büchi system). For each of the techniques perform two derivatizations using MSTFA (preferably non-activated) and two with BSTFA. Overall, you should now have 8 samples with 500 µL each, four MSTFA derivatized and four BSTFA derivatized. Each of these samples should now be divided in 100 µL and 400 µL portions. The 400 µL samples can be directly measured while the 100 µL ones have to be diluted 1:5 with ethylacetate for the measurement.



In the end we need 16(+8) results from you: 8 for 3-MCPD and 8 for 1,3-DCP with DCP-d_5 as ISTD (+8 with CMP as ISTD), report of these will be done online. Please fill out the results page very thoroughly. You should check the page before to make sure that you have all the information we need for a proper evaluation of the method. A link, an individual lab code, and a personalized token will be sent with a separate email. Please keep in mind that the submission of results is finalzed only when you sent us a signed pdf of your questionnaire. The preparation of the pdf is described in the questionnaire after digital submission.

Deadline for the submission of results will be the **7th of November, 1 p.m.**

Dear [Participants' Name and Surname],

You can find the questionnaire here:

https://akademie.bfr.berlin/775199?lang=en

Your Lab-Code is: Lab-[Number]

Your Token is: [Token]

Best regards from Berlin,

NRL-FCM-DE

11.5 Questionnaire and Results

The process was completed online (https://akademie.bfr.berlin/775199?lang=en)

NRL-FCM-DE-01/2025 (MES-Chloropropanols) Questionnaire and Results

Please answer the questions of the questionnaire and present your results.
There are 21 questions in this survey.

- **General**

1) Lab Code: _____

2) Please identify yourself. You are ....

*Please choose **only one** of the following:*
- o National Reference Laboratory (NRL)
- o Official Control Laboratory (OCL)
- o Private laboratory

3) Which analytical technique was used for analysis?

*Please choose **only one** of the following:*
- o GC-MS (quadrupole)
- o GC-MS/MS (triple quadrupole)

Please provide the pH value for the analyzed solution, as well as the method used to determine the pH (pH meter, pH indicator paper, etc.)
Please use a dot as the decimal separator.

4) pH of the solution: _____

5) Determination method: _____

- **Limits of detection and quantification**

Please use a dot as the decimal separator, and report LOD and LOQ values with two digits after the dot.
If no LOD or LOQ value is determined, please use 'ND'.

6) Limits of detection and quantification *

	LOD [µg/L]	LOQ [µg/L]	Mass or Reaction [m/z or m/z > m/z]
3-MCPD (MCPD-d_5)			
1,3-DCP (DCP-d_5)			
1,3-DCP (CMP)			

7) Please provide the information about the method of LOD/LOQ determination. *

*Please choose **only one** of the following:*
- o Signal-to-noise Ratio (S/N)
- o Standard Deviation of the Response (σ) and Slope (S) of the Calibration Curve [e.g. LOD=3σ/S]
- o DIN 32645 (blank value method)
- o DIN 32645 (calibration method)
- o Other _____

- **Experimental procedure**

8) Was the MSTFA used activated or non-activated? *

*Please choose **only one** of the following:*
- o activated
- o non-activated

9) Did you subtract process blank values? *

*Please choose **only one** of the following:*

o yes
o no

10) Did you apply any special treatment to the provided solutions? *

*Please choose **only one** of the following:*

o yes
o no

If yes, please specify. _____

11) Was the calibration line weighted? If yes, which weighting factor was used? (e.g. 1/x) *

*Please choose **only one** of the following:*

o yes
o no

If yes, please provide the weighting factor. _____

12) Other deviation from the suggested method? *

*Please choose **only one** of the following:*

o yes
o no

If yes, please specify. _____

- **Results**

Please use a dot as the decimal separator and report results with two digits after the dot.

The workout steps can be found in the instructions (see the workout tree).
The following abbreviations are used:

- *For methods of solvent removal from the sample: using a nitrogen stream (Nitrogen stream) or a vacuum evaporator (Evaporation system).*
- *Compound used in derivatization step: MSTFA or BSTFA.*
- *Analysis of the sample after derivatization: without dilution (direct measurement) or after 1:5 dilution with ethyl acetate (1:5 dilution).*

13) Nitrogen stream / MSTFA / direct measurement

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

14) Nitrogen stream / MSTFA / 1:5 dilution

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

15) Nitrogen stream / BSTFA / direct measurement

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

16) Nitrogen stream / BSTFA / 1:5 dilution

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

17) Evaporation system / MSTFA / direct measurement

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

18) Evaporation system / MSTFA / 1:5 dilution

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

19) Evaporation system / BSTFA / direct measurement

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

20) Evaporation system / BSTFA / 1:5 dilution

	Concentration I [µg/L]	Concentration II [µg/L]
3-MCPD (MCPD-d_5)		
1,3-DCP (1,3-DCP-d_5)		
1,3-DCP (CMP)		

21) Do you have any comments or suggestions regarding the method?

_____

Thank you for submitting the results.

Please use the '**Print your answers**' button and select 'queXML PDF export'. Check the resulting PDF for correctness.

If everything is ok please send this PDF digitally signed (if possible) or, alternatively, the PDF with a confirmation in the e-mail text whether all data is correct to:
NRL-FCM@bfr.bund.de
Please contact us if you have any further questions.

Yours sincerely,
NRL-FCM-DE Team

11.6 Results reported by the participating laboratories

Table A 1: Results reported by the participating laboratories

Analyte (ISTD)	Lab 1	Lab 2	Lab 2*	Lab 3	Lab 4	Lab 5	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 13	Lab 14	Lab 15
Nitrogen stream / MSTFA / direct measurement														
3-MCPD	16.6902	-	-	20.253	18.11	n.d.	15.26	14.00	18.3	15.00	17.20	17.35	15.21	2.456
(MCPD-d_5)	16.5342	-	-	23.22	18.91	n.d.	14.75	14.10	16.0	14.96	17.61	17.07	15.43	2.553
1,3-DCP	ND	-	-	-	2.22	n.d.	2.07	ND	2.30	2.16	ND	2.63	2.56	2.653
(1,3-DCP-d_5)	ND	-	-	-	1.69	n.d.	2.16	ND	2.32	2.08	ND	2.64	2.36	2.404
1,3-DCP	ND	-	-	-	2.00	n.d.	1.94	ND	3.28	1.73	ND	-	2.46	n.a.
(CMP)	ND	-	-	-	-	n.d.	2.09	ND	3.40	1.70	ND	-	2.34	n.a.
Nitrogen stream / MSTFA / 1:5 dilution														
3-MCPD	17.9049	-	-	20.556	18.79	n.d.	14.53	14.44	16.3	15.55	ND	18.00	15.90	2.661
(MCPD-d_5)	16.8382	-	-	23.61	19.13	n.d.	14.89	14.66	16.4	15.59	ND	17.22	16.29	2.361
1,3-DCP	ND	-	-	-	2.21	n.d.	2.22	2.37	2.26	2.08	ND	2.71	2.14	2.326
(1,3-DCP-d_5)	ND	-	-	-	2.38	n.d.	2.27	2.28	2.08	2.17	ND	2.63	2.25	2.499
1,3-DCP	ND	-	-	-	-	n.d.	2.33	1.85	2.21	2.10	ND	-	2.17	n.a.
(CMP)	ND	-	-	-	-	n.d.	3.12	1.80	2.21	2.09	ND	-	2.19	n.a.
Nitrogen stream / BSTFA / direct measurement														
3-MCPD	17.1246	-	-	21.845	29.06	n.d.	15.26	13.81	17.4	15.61	18.28	19.23	16.08	2.363
(MCPD-d_5)	17.2946	-	-	23.910	26.96	n.d.	15.67	12.85	17.2	15.53	18.12	18.05	14.08	2.672
1,3-DCP	ND	-	-	-	1.82	n.d.	2.34	2.46	2.43	2.28	ND	2.63	2.51	0.604
(1,3-DCP-d_5)	ND	-	-	-	1.77	n.d.	2.22	2.56	2.39	2.28	ND	2.84	2.70	0.417
1,3-DCP	ND	-	-	-	-	n.d.	2.04	ND	3.04	1.70	ND	-	1.92	n.a.
(CMP)	ND	-	-	-	-	n.d.	2.01	ND	3.33	1.91	ND	-	2.07	n.a.
Nitrogen stream / BSTFA / 1:5 dilution														
3-MCPD	17.1750	-	-	20.610	20.49	n.d.	15.95	13.02	17.2	14.60	ND	18.54	15.78	2.738
(MCPD-d_5)	18.1363	-	-	23.760	20.38	n.d.	16.01	13.21	16.1	14.99	ND	18.44	16.44	2.624
1,3-DCP	ND	-	-	-	3.09	n.d.	2.48	2.20	2.47	2.23	ND	2.66	2.43	0.474
(1,3-DCP-d_5)	ND	-	-	-	2.83	n.d.	2.46	2.39	2.32	2.18	ND	2.86	2.19	0.530
1,3-DCP	ND	-	-	-	-	n.d.	3.49	ND	1.49	2.04	ND	-	1.45	n.a.
(CMP)	ND	-	-	-	-	n.d.	2.83	ND	2.21	2.08	ND	-	2.28	n.a.
Evaporation system / MSTFA / direct measurement														
3-MCPD	16.4877	20.74	-	19.104	18.34	18.05	15.80	16.31	17.0	15.14	17.65	17.88	14.55	2.521
(MCPD-d_5)	16.3851	19.16	-	19.22	18.69	18.39	16.87	16.25	17.8	15.15	17.70	18.02	14.00	2.474
1,3-DCP	2.6069	ND	-	2.351	2.18	2.93	2.10	ND	2.57	2.10	2.32	2.45	2.12	2.435
(1,3-DCP-d_5)	2.6953	ND	-	2.543	1.5	3.05	2.09	ND	2.27	2.11	2.11	2.50	2.44	2.325
1,3-DCP	2.5581	2.60	-	-	-	4.92	1.92	ND	3.81	1.76	2.40	-	2.32	n.a.
(CMP)	2.4654	2.47	-	-	-	5.07	3.12	ND	4.41	1.82	2.42	-	3.42	n.a.
Evaporation system / MSTFA / 1:5 dilution														
3-MCPD	17.1960	21.20	-	19.030	18.55	16.26	14.82	15.32	16.5	15.80	ND	16.95	12.84	2.291
(MCPD-d_5)	16.5213	19.89	-	19.714	18.68	17.02	14.73	15.12	17.3	16.08	ND	17.17	13.44	2.144
1,3-DCP	2.6230	ND	-	2.25	2.52	2.22	2.13	2.26	2.44	2.06	ND	2.70	2.47	2.238
(1,3-DCP-d_5)	2.7977	ND	^-	2.439	2.83	2.81	2.30	2.31	2.45	2.06	ND	2.60	2.22	2.336
1,3-DCP	2.5813	2.10	-	-	-	5.22	2.13	2.16	2.72	2.12	ND	-	3.00	n.a.
(CMP)	2.7829	2.07	-	-	-	5.66	2.93	2.48	2.75	2.10	ND	-	3.11	n.a.
Evaporation system / BSTFA / direct measurement														
3-MCPD	17.2708	18.99	-	19.41	30.84	15.53	15.30	14.62	16.3	15.79	17.45	17.49	15.09	2.619
(MCPD-d_5)	16.7220	19.53	-	19.59	-	15.68	13.99	14.54	17.1	15.72	16.39	19.46	15.16	2.560
1,3-DCP	2.5193	ND	-	-	1.89	2.70	2.33	2.38	2.27	2.34	ND	2.73	2.48	0.596
(1,3-DCP-d_5)	2.5952	ND	-	-	-	2.56	2.29	2.43	2.57	2.27	ND	2.57	2.36	0.890
1,3-DCP	3.1728	2.41	-	-	-	2.56	2.34	ND	3.06	1.79	ND	-	1.87	n.a.
(CMP)	3.0325	2.51	-	-	-	2.07	2.71	ND	4.74	1.93	ND	-	1.96	n.a.
Evaporation system / BSTFA / 1:5 dilution														
3-MCPD	16.8141	19.46	17.77	19.040	20.4	14.85	16.58	13.74	17.3	15.05	ND	17.49	20.07	2.774
(MCPD-d_5)	16.7021	20.04	17.05	19.972	19.86	14.95	16.12	13.91	16.6	15.24	ND	20.54	19.12	2.582
1,3-DCP	2.1485	ND	1.88	-	2.94	2.16	2.44	2.41	2.62	2.41	ND	2.79	1.71	0.517
(1,3-DCP-d_5)	2.2613	ND	1.92	-	3.2	2.50	2.26	2.47	2.54	2.33	ND	2.69	2.61	0.719
1,3-DCP	1.3314	2.46	-	-	-	1.64	3.54	ND	0.76	2.10	ND	-	1.71	n.a.
(CMP)	1.0419	2.29	-	-	-	2.15	3.35	ND	1.51	1.95	ND	-	2.10	n.a.

'-' indicates that no value was submitted

* This laboratory submitted results for individual samples from both an GC-MS and an GC-MS/MS systems

11.7 Additional information extracted from the questionnaire

Additional information extracted from the questionnaire is presented in Table A 2 and Table A 3.

Table A 2: Additional information regarding the method extracted from the questionnaire. The complete wording of the questions can be found by referring to the number in brackets in Chapter 11.5.

Lab Code	MSTFA used (8)	Blancks subtracted? (9)	Weighting factor? (11)		Deviations from the suggested method? [12]	
Lab 1	non-activated	no	no	-	no	-
Lab 2	activated	yes	no	-	yes	Only one evaporation technique was used. Evaporation under nitrogen stream was practically impossible due to the high volume of extraction agent.
Lab 3	activated	yes	no	-	no	-
Lab 4	non-activated	no	no	-	yes	Samples were evaporated to 300 µL instead of 450 µL. After evaporation 150 µL ethylacetate was added.
Lab 5	non-activated	no	no	-	no	-
Lab 7	non-activated	no	no	-	no	-
Lab 8	non-activated	yes	yes	1/x	no	-
Lab 9	non-activated	yes	no	-	no	-
Lab 10	non-activated	no	no	-	no	-
Lab 11	non-activated	yes	no	-	no	-
Lab 13	non-activated	no	no	-	no	-
Lab 14	non-activated	yes	no	-	no	-
Lab 15	non-activated	yes	no	-	yes	CMP was not used as a standard. Instead, deuterated MCPD and DCP were used

'-' indicates that no information was submitted

Table A 3: Problems with the analysis, as well as suggestions and comments regarding the method reported by participants.

Lab Code	Did you encounter any problems with the analysis?		Do you have any comments or suggestions regarding the method?
Lab 1	yes	The blind value of the analysis of 1,3-DCP using nitrogen evaporation is too high. It is not possible to evaluate these results. Therefore 'ND' was entered in the corresponding fields of the result table.	Reduction of the elution volume of ethyl acetate to 20 mL. It is not necessary to use such a large volume of ethyl acetate for the elution.
Lab 2	yes	Tests were made using ISTD DCP-d5. It turnes out that DCP-d5 has a lot of not deuterated DCP, which made quantification impossible. The 1:5 dilution with MSTFA was not evaluable, because of bad peak shapes and not linear calibration curves. Values with MSTFA in 1:5 dilution are only semi-quantitive, because the calibration curve was not linear and only the last calibration point had more than 1000 counts.	We have measured the samples by using GC-MS and GC-MS/MS. Calibration curves with MSTFA looked bad with both analytical methods when measuring 1:5 dilution. Results with BSTFA in 1:5 dilution with GC-MS/MS in µg/L: 1,3-DCP (1,3-DCP-d5): I: 1.88 and II: 1.92 and 3-MCPD (MCPD-d5): I: 17.77 and II: 17.05 Results with MSTFA 1:5 dilution with GC-MS/MS were not evaluable due to bad calibration curves.
Lab 3	yes	We had significant blank value problems with the Turbovap processing. Therefore, we cannot report any DCP values for it. We also experienced some DCP issues with BSTFA, so only a few values are available here as well. We routinely work with the Evaporator and MSTFA.	We had significant blank value and derivatisaion problems with the Turbovap/BSTFA processing fot DCP. Therefore, we cannot report any DCP values for it.
Lab 4	yes	- low recovery rates using evaporator - double peak for MCPD-d5 using BSTFA - sensitivity of mass spectrometer too low for cal. 1 and cal. 2 using 1:5 dilution	- solvent removal using a nitrogen stream takes 5 hours, which is too long - solvent removal using a vacuum evaporater is too hard (loss of analytes and internal standards) -> using a combined version (first vac evaporator then nitrogen steam might be the best option) - peak areas/signals after a 1:5 dilution are too small

Lab Code	Did you encounter any problems with the analysis?		Do you have any comments or suggestions regarding the method?
			when using our single quad mass spectrometer (sensitivity is too low)
Lab 5	yes	When calculating the DCP results using the internal standard CMP, we obtained widely differing results depending on which ion was used for the calculation (GC-MS). MSTFA derivatized: Ion 181 approx. 5 µg/l, Ion 89 approx. 0.5 µg/l BSTFA derivatized Ion 181 approx. 2.3 µg/l, Ion 89 approx. 2.0 Depending on the derivatization reagent, different ions had to be used for the calculation of DCP and MCPD results.	When measuring the samples and calibration, we noticed that the DCP values in particular varied significantly when left in the device for a longer period of time (> 6 h to approx. 10 h at 23 °C room temperature). If the silylated sample is measured within approx. 6 h immediately after removal from the freezer, the values remain stable. We suspect that the amount of ethanol used for quenching (5 µl to a total volume of 500 µl) is insufficient. We were unable to carry out the evaporation under a nitrogen stream because we do not have the necessary equipment.
Lab 7	no	-	pH of Blank solution: 6.66
Lab 8	yes	An evaluation for 1,3-DCP via 3-CMP was only possible with MSTFA and dilution, as 3-CMP was masked by the matrix in all other sample solutions. With MSTFA without dilution, no evaluation for 1,3-DCP was possible, as 1,3-DCP and d5-DCP eluted from the column in a mountain and m/z 93 is used for the quantification of both substances.	-
Lab 9	no	-	We have tested and validated the method with 2.5ml instead of 5ml extract. We used smaller columns for the extraction (EXtrelut NT 3) and only used 25ml instead of 80ml Ethylacetate for elution. To evaporate the 25ml Ethylacetate we only used a nitrogen stream. We tested this also for high concentrated samples. We can provide data for this purpose.
Lab 10	yes	some 1/5 dilutions: loss of area across all compounds incl. ISTD; CMP can not compensate the loss of area and therefore leads to 5-6 times higher concentration when calculation 1,3-DCP; in contrast, 1,3-DCP-d5 & 3-MCPD-d5 will give roughly the same final concentraton of their corresponding non labeled compounds;	-
Lab 11	yes	Significant losses of CMP; the solvent ethyl acetate has a boiling point 5 °C higher than CMP. Internal investigations with 25 mL of tert-butyl methyl ether/ethyl acetate (50/50 v/v) resulted in complete elution of the analytes. This solvent mixture has a lower boiling point, resulting to lower losses of analyte and internal standard during concentration step. Unclear dimensions of the column to be used for liquid-liquid extraction with silica. Blank values of DCP in the used BSTFA; it was not tested previously to ensure it was free of DCP. We always use MSTFA for derivatization and we dont had any problems with blank values. Is quenching with ethanol really necessary? We dont perform this additional step. This additional step poses a risk of introducing water. This also increases the probability of damaging the unstable trimethylsilyl ether derivatives. No information is provided regarding the purity of the ethanol to be used.	1:5 diluton was not performed due of GC-MS measurement. according due blank values in BSTFA no valid concentrations for DCP was estimated. with nitrogen evaporation high losses of DCP was observed.
Lab 13	yes	Very long elution and evaporation times due to high volume of solvent used	-
Lab 14	yes	Linearity of 1,3 DCP/CMP calibration was not good and one or more calibration point were excluded to have a proper fit.	-
Lab 15	yes	• The derivatization needed to be done by hand. In our routine workflow this is done automatically. • The lab uses MSTFA in routine analysis. Regarding the BSTFA derivatization, the R^2 in the calibration curve was not good enough for quantification. BSTFA-derivatized samples were therefore quantified via the MSTFA calibration curve.	- We're not sure whether the used MSTFA (Article No. 701270.110) was activated or non-activated - In our routine analysis workflows we prefer a LLE instead of the expensive Extrelut columns - In our routine analysis workflows we prefer the deuterated standards instead of CMP

Lab Code	Did you encounter any problems with the analysis?		Do you have any comments or suggestions regarding the method?
		• Extrelut columns are extremely expensive and not used in our routine workflow	

'-' indicates that no information was submitted

11.8 Submitted results for the determination of 3-MCPD using 3-MCPD-d_5

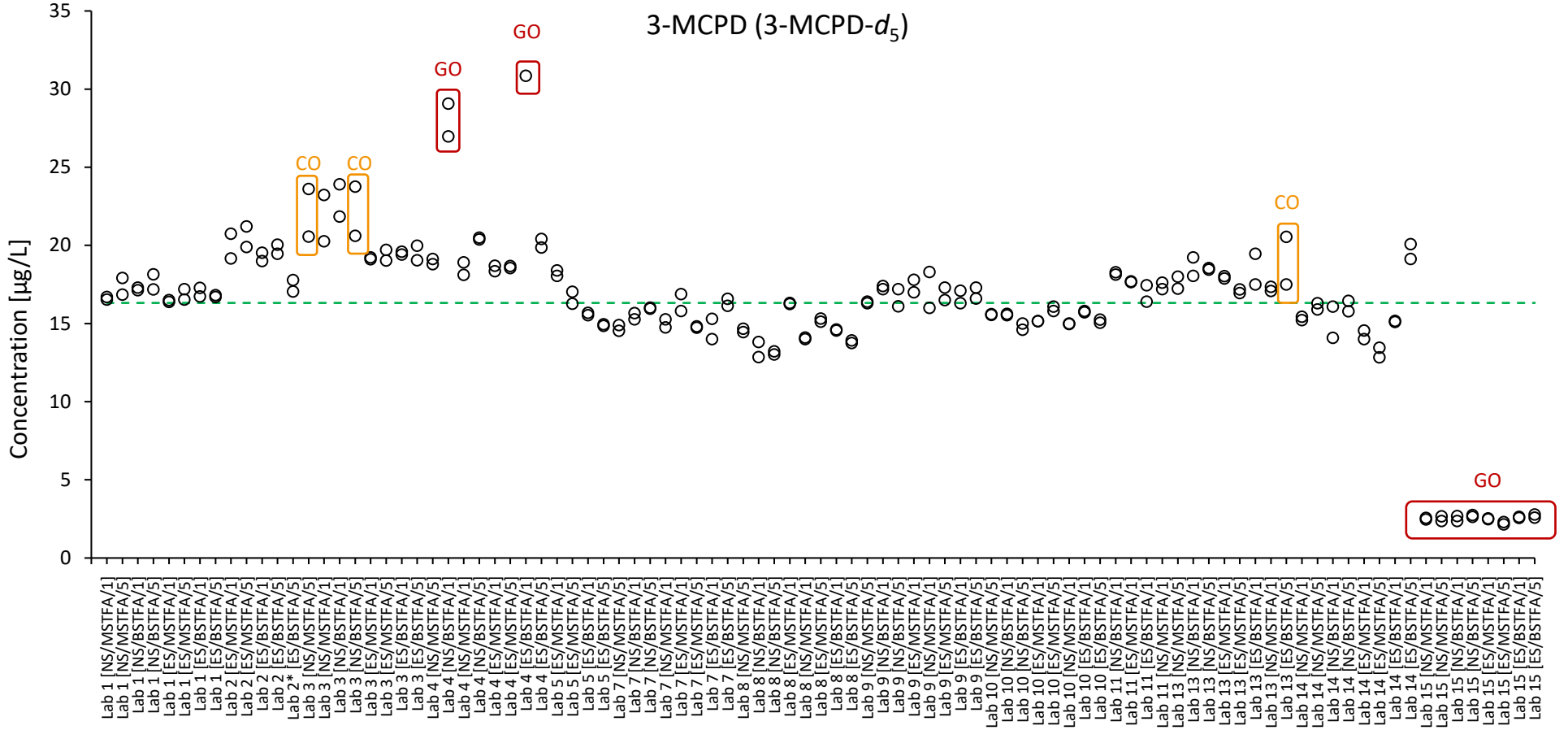


Figure A 1: Submitted results for the determination of 3-MCPD using 3-MCPD-d_5. The dotted green line shows the spiked concentration of 3-MCPD. Outliers according to Grubbs (GO) and Cochran (CO) are shown in red and orange rounded rectangles, respectively. An outlier test has been performed on the results of each sample preparation combination separately. Sample preparation information is provided in brackets after the lab number. This includes details of whether the sample was prepared using a nitrogen stream (NS) or a vacuum evaporator (ES), and whether derivatisation was performed using MSTFA or BSTFA. Numbers 1 and 5 reflect the dilution factor.

11.9 Submitted results for the determination of 1,3-DCP using 1,3-DCP-d_5

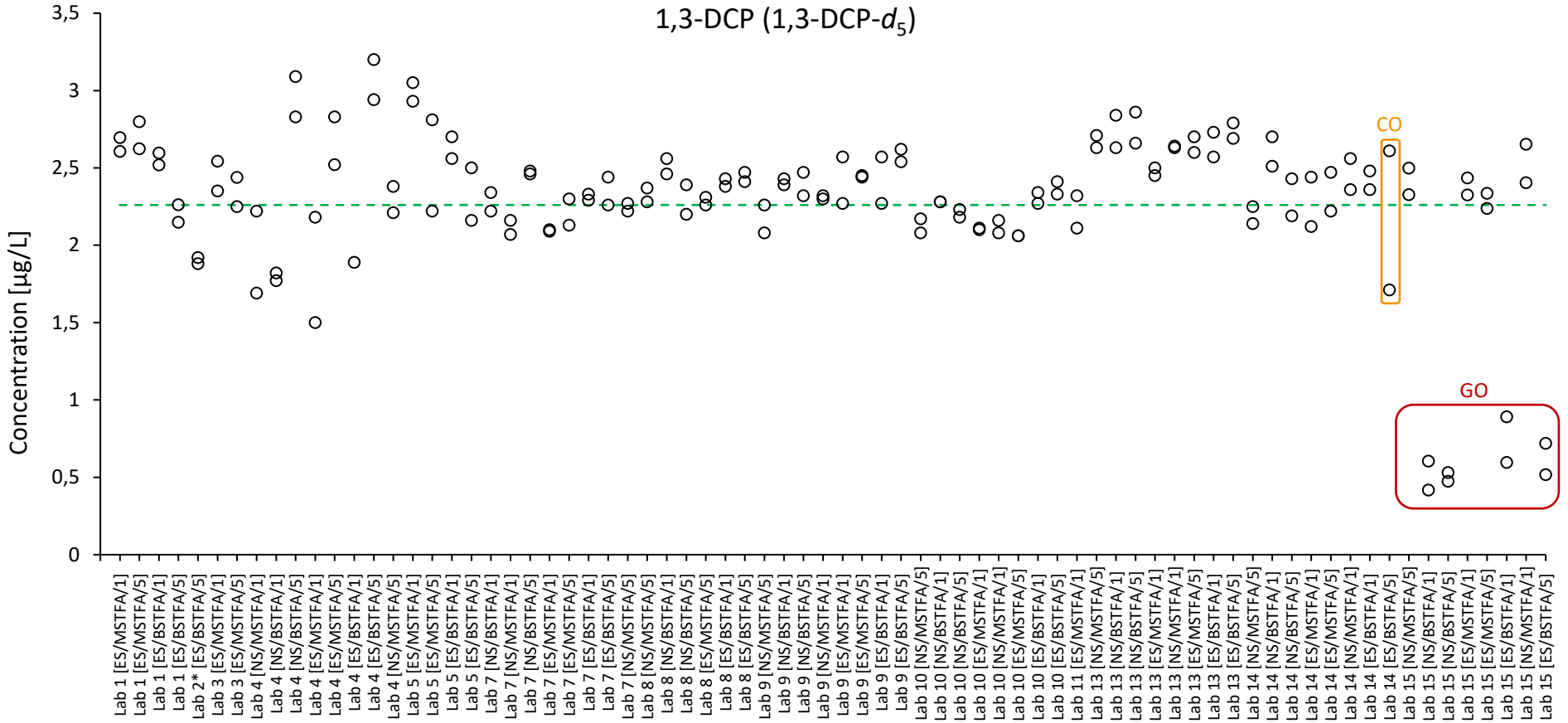


Figure A 2: Submitted results for the determination of 1,3-DCP using 1,3-DCP-d_5. The dotted green line shows the spiked concentration of 1,3-DCP. Outliers according to Grubbs (GO) and Cochran (CO) are shown in red and orange rounded rectangles, respectively. An outlier test has been performed on the results of each sample preparation combination separately. Sample preparation information is provided in brackets after the lab number. This includes details of whether the sample was prepared using a nitrogen stream (NS) or a vacuum evaporator (ES), and whether derivatisation was performed using MSTFA or BSTFA. Numbers 1 and 5 reflect the dilution factor.

11.10 Submitted results for the determination of 1,3-DCP using CMP

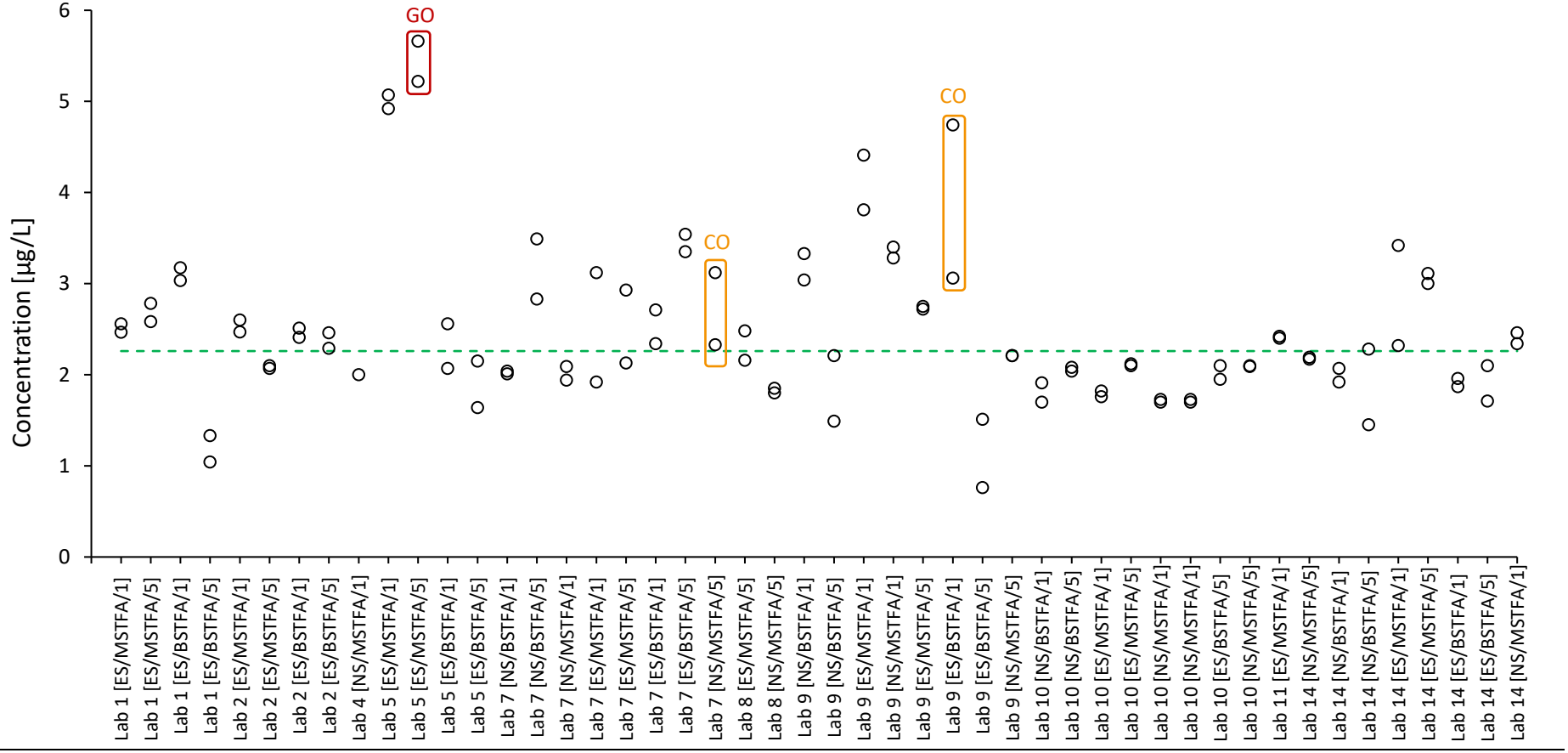


Figure A 3: Submitted results for the determination of 1,3-DCP using CMP. The dotted green line shows the spiked concentration of 1,3-DCP. Outliers according to Grubbs (GO) and Cochran (CO) are shown in red and orange rounded rectangles, respectively. An outlier test has been performed on the results of each sample preparation combination separately. Sample preparation information is provided in brackets after the lab number. This includes details of whether the sample was prepared using a nitrogen stream (NS) or a vacuum evaporator (ES), and whether derivatisation was performed using MSTFA or BSTFA. Numbers 1 and 5 reflect the dilution factor.

11.11 Results for the determination of 3-MCPD using 3-MCPD-d_5, used in the statistical analysis

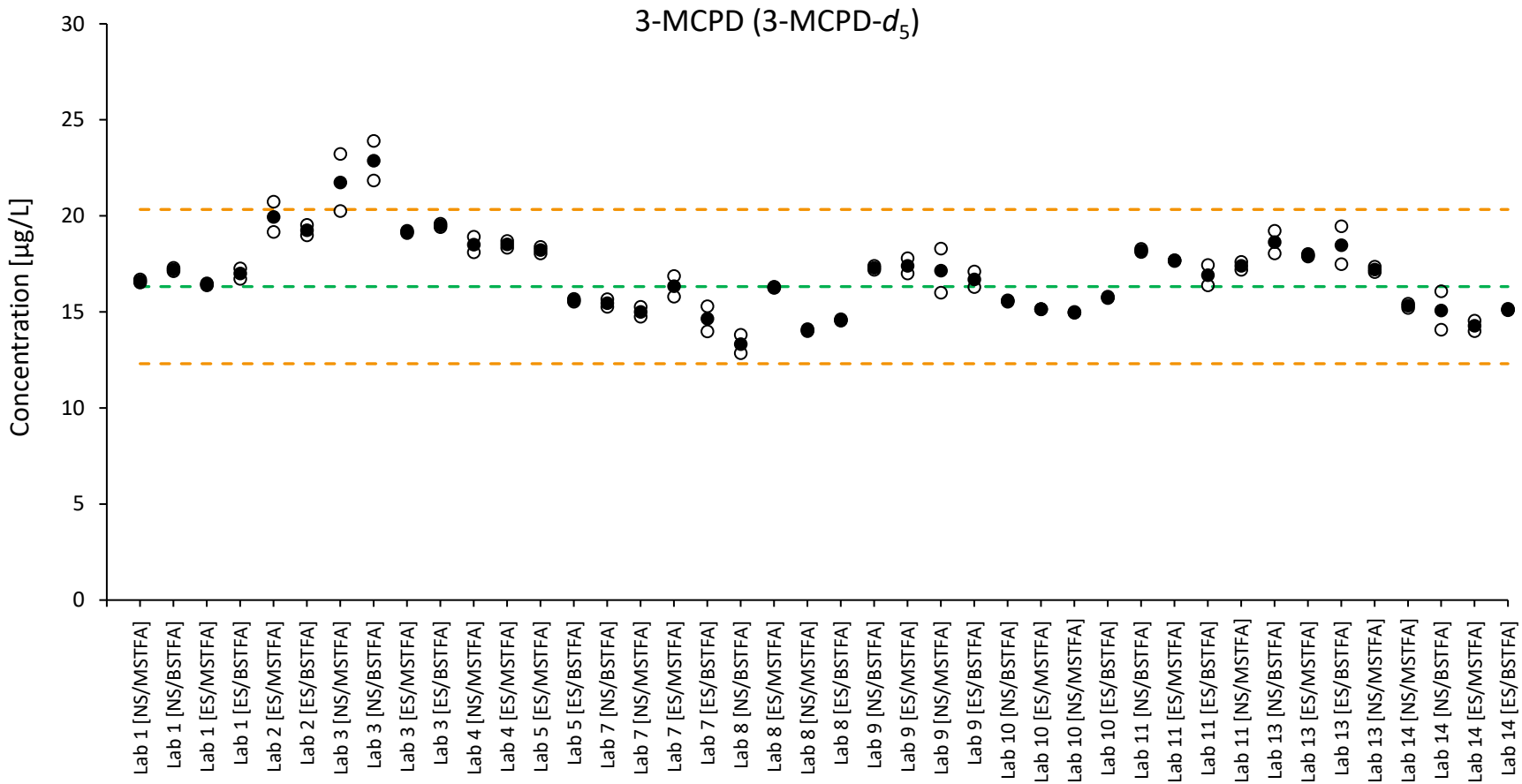


Figure A 4: Results for the determination of 3-MCPD using 3-MCPD-d_5, used in the statistical analysis. The empty black circles represent the submitted results, while the solid black circles represent the corresponding mean value. The dotted green line shows the spiked concentration of 3-MCPD (x_{pt}) and dotted orange lines demonstrates the range of x_{pt}-2·s_R to x_{pt}+2·s_R (~95% confidence level). Sample preparation information is provided in brackets after the lab number. This includes details of whether the sample was prepared using a nitrogen stream (NS) or a vacuum evaporator (ES), and whether derivatisation was performed using MSTFA or BSTFA.

11.12 Results for the determination of 1,3-DCP using 1,3-DCP-d_5, used in the statistical analysis

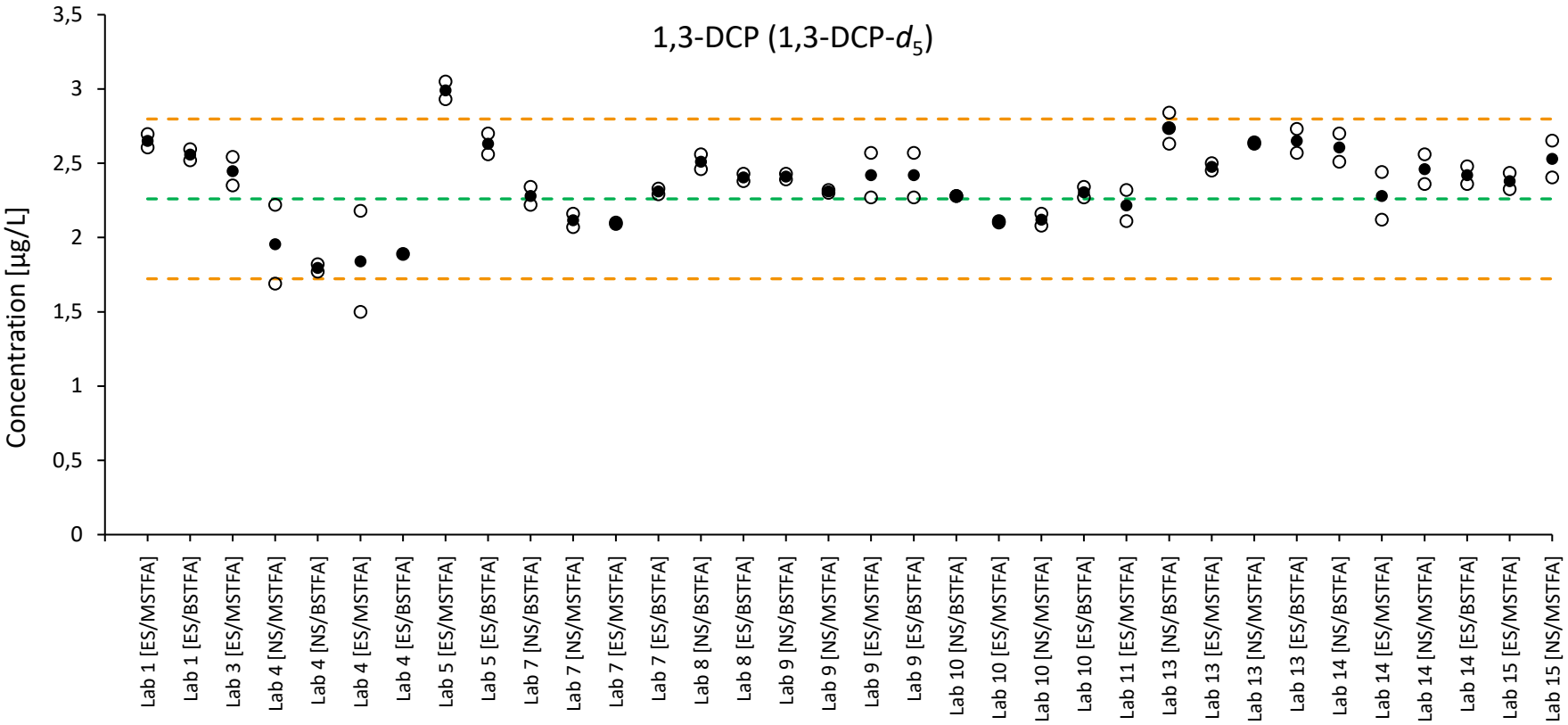


Figure A 5: Results for the determination of 1,3-DCP using 1,3-DCP-d_5, used in the statistical analysis. The empty black circles represent the submitted results, while the solid black circles represent the corresponding mean value. The dotted green line shows the spiked concentration of 1,3-DCP (x_{pt}) and dotted orange lines demonstrates the range of x_{pt}-2·s_R to x_{pt}+2·s_R (~95% confidence level). Sample preparation information is provided in brackets after the lab number. This includes details of whether the sample was prepared using a nitrogen stream (NS) or a vacuum evaporator (ES), and whether derivatisation was performed using MSTFA or BSTFA.

11.13 The draft version of the evaluated § 64 method

DRAFT XX dated 05/12/2024

Official collection of methods of analysis according to § 64 LFGB		
B	Examination of consumer products Determination of **1,3-dichloro-2-propanol** and **3-monochloro-1,2-propanediol** in **cold water extracts of paper, board** and **cardboard** using GC-**MS(/MS)**	XX.XX XX

1 Purpose and scope of application

This official method describes a procedure for the determination of the chloropropanols 1,3-dichloro-2-propanol (1,3-DCP) and 3-monochloro-1,2-propanediol (3-MCPD) in cold water extracts of paper, board and cardboard.

2 Term

The use of epichlorohydrin in the production of manufacturing additives for paper, board and cardboard can lead to the formation of migratable fractions of 1,3-DCP and 3-MCPD in the finished product. 1,3-DCP is an intermediate product of epichlorohydrin production that can remain as an impurity in the additives. 3-MCPD is formed as a hydrolysis product from unreacted residual amounts of epichlorohydrin.

3 Brief description

The analytes are extracted from the cold water extract using solid-phase-based liquid-liquid extraction. 1,3-DCP and 3-MCPD are derivatised with *N*-methyl-*N*-(trimethylsilyl)trifluoro-acetamide (MSTFA) or *N*,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The analysis is carried out using gas chromatography coupled with (tandem) mass spectrometry (GC-MS(/MS)).

4 Chemicals

Unless otherwise specified, chemicals pure for analysis and, if possible, certified analyte standards must be used. Water must be at least ultrapure water type II (1 MΩ· cm at 25 °C, according to DIN ISO 3696).

4.1 Sodium chloride (NaCl)

4.2 Ethyl acetate, for residue analysis

4.3 Derivatisation reagent

4.3.1 *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA)

4.3.2 Alternative: *N*,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)

4.4 Liquid-liquid extraction column with diatomaceous earth, e.g. Extrelut®

4.5 1,3-Dichloro-2-propanol (1,3-DCP)

4.6 3-Monochloro-1,2-propane diol (3-MCPD)

4.7 3-Monochloro-1,2-propanediol-d_5 (3-MCPD-d_5), internal standard for 3-MCPD

4.8 3-Chloro-1-methoxy-2-propanol (CMP), internal standard for 1,3-DCP

4.9 Alternative: 1,3-dichloro-2-propanol-d_5 (1,3-DCP-d_5), internal standard for 1,3-DCP

4.10 Diluted hydrochloric acid, e.g. 5 M

4.11 Production of stock and working solutions

The stock and working solutions (analyte mix and internal standard mix (ISTD mix)) can be prepared as described in the following chapters. Adjustments, e.g. with regard to the concentrations, can be made. If exact purity specifications for the analyte standards used are given in the certificate, the exact concentration of the solutions prepared should be calculated from the weights, taking the purity into account.

4.11.1 Preparation of stock solutions for 3-MCPD and 1,3-DCP and for the internal standards 3-MCPD-d_5 and CMP/1,3-DCP-d_5

A solution with a concentration of approximately 5 mg/mL is prepared in ethyl acetate. Intermediate dilutions can be prepared for the following steps if required.

4.11.2 ISTD mix (3-MCDP-d_5 and CMP/1,3-DCP-d_5)

When preparing the ISTD mix, make sure that the concentration of 3-MCPD-d_5 is 1250 ng/mL and of CMP/1,3-DCP-d_5 is 300 ng/mL. When using 1,3-DCP-d_5 for the determination *keep in mind that both 1,3-DCP and 1,3-DCP-d_5 form the same main fragment m/z 93*. Accordingly, complete chromatographic separation of the two substances must be ensured when using this fragment. Alternatively, another fragment can be selected (if it allows to achieve a sufficient

LOQ for 1,3-DCP) or CMP can be used.

4.11.3 Analyte mix

When preparing the analyte mix, make sure that the concentration of 3-MCPD is 500 µg/L and of 1,3-DCP 100 µg/L.

4.12 DCP-MCPD calibration solution en

Table 1 contains an example of a 7-point calibration series. For the calibration solutions, ethyl acetate, the analyte mix (4.11.3) and the ISTD mix (4.11.2) are combined as described inTable 1 in a suitable vessel, e.g. an amber glass vial. Prior to analysis, the analytes and internal standards in the calibration solutions are derivatised directly by adding the derivatisation reagent (4.3.1 MSTFA or 4.3.2 BSTFA) according to 7.2.4 . In addition, it is recommended to measure a blank solution without analytes, but with an internal standard (ISTD), which is not part of the calibration series.

N o t e s : All stock and calibration solutions can be stored underivatised for 7 months in the refrigerator at <8 °C or derivatised for 3 months at< -15 °C in the freezer (see appendix).

If the chemicals used are available in containers with very small quantities, it makes sense to calculate the weight by weighing. To do this, the vessel is weighed before the substance is transferred to a volumetric flask by rinsing the vessel with ethyl acetate. The vessel is then dried and weighed. However, this can lead to significantly increased uncertainties. The use of larger quantities is therefore recommended

Table 1 : Example of a 7-point calibration series (the zero point is not to be integrated into the calibration series). The concentrations refer to values after addition of the derivatisation reagent and before any quenching/dilution after derivatisation.

Calibration point	Volume ethyl acetate [µL]	Volume analyte mix (4.11.3) [µL]	Volume ISTD-Mix (4.11.2) [µL]	c* 3-MCPD [µg/L]	c* 1.3-DCP [µg/L]	c* 3-MCPD-ds [µg/L]	c* CMP/1,3-DCP-ds [µg/L]
Blank	400	0	50	0	0	125	30
1	380	20	50	20	4	125	30
2	360	40	50	40	8	125	30
3	320	80	50	80	16	125	30
4	280	120	50	120	24	125	30
5	220	180	50	180	36	125	30
6	150	250	50	250	50	125	30
7	100	300	50	300	60	125	30

c*- concentration in the measuring solution, i.e. after addition of the derivatisation reagent (50 µL; according to 7.2.4)

5 Devices and tools

In addition to the usual laboratory equipment, you will need, for example

5.1 Scales with an accuracy of at least 0.1 mg

5.2 Erlenmeyer flask with ground glass joint

5.3 Nutsche filter

5.4 Glass fibre filter

GF/C; 1.2 µm

5.5 Volumetric flask

5.6 Glass column with frit

Length: 30 cm, inner diameter: 1 cm

5.7 Piston or positive displacement pipettes with disposable tips, various sizes

5.8 Bulb pipette, 5 mL

5.9 Vacuum evaporator/nitrogen evaporator

5.10 Evaporator glass, at least 80 mL, with graduated tip for 1 mL

5.11 Test tubes with ground glass

5.12 Kuderna-Danish vaporiser with nitrogen

5.13 Suitable vials for derivatisation and for GC-MS(/MS) measurements, e.g. amber glass vials with screw cap, e.g. with butyl rubber PTFE septa

5.14 Insert, 250 µL

5.15 pH meter + pH paper

5.16 Drying cabinet, 60 °C ± 5 °C

5.17 GC-MS(/MS) device with electron ionisation source

5.18 GC column

5 % phenyl-methylpolysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness

N o t e s : To avoid contamination, it is recommended that the glassware is either rinsed with ethyl acetate or baked in a drying oven/muffle furnace at 380 °C for one hour before use.

6 Sampling

The sampling procedure is not part of the testing procedure described in this official method.

Care must be taken to ensure that a representative sample is given to the laboratory for analysis. The laboratory sample must be stored and handled in such a way that damage and/or changes are avoided.

7 Implementation

7.1 Sample preparation

The sample is prepared according to DIN EN 645 [1] with the specifications for preparing cold water extracts from the BfR's collection of methods for paper [2], .i.e. the sample is cut, not torn. The chopped sample can be stored in a glass bottle with a screw cap. The sample is weighed for extraction in accordance with DIN EN 645 [1].

N o t e s : If the total sample quantity is small, the sample weight can be reduced. In this case, the volume of water for extraction, the final volume and the amount of NaCl to be added to the volumetric flask (see 7.2.1) must be adjusted accordingly.

7.2 Determination of chloropropanols

7.2.1 Extraction

Add 200 mL ultrapure water to the weighed sample in a ground-glass Erlenmeyer flask. Ensure that the sample is completely covered with water. The cold water extract is prepared according

to the precise specifications of DIN EN 645 [1, 2] for 24 hours at 23 °C ± 2 °C in the sealed Erlenmeyer flask. The extract is then vacuum-filtered using a glass fibre filter. The solution should be transferred as completely as possible to the filter flask. The Erlenmeyer flask is rinsed twice with 20 mL ultrapure water each time and the rinsing solutions are poured over the filter cake one after the other. If the material is very swellable, carefully squeeze the remaining water out of the filter cake. If the material does not swell strongly, it is also possible to decant the extract instead of vacuum filtration, also rinsing the residue twice with 20 mL ultrapure water.

29.25 g of NaCl are placed in a 250 mL volumetric flask, into which the filtrate is then transferred. After complete dissolution of the NaCl, fill up to the mark with ultrapure water. Immediately after extraction, check the pH value using a pH meter or suitable pH paper. If the extract has an alkaline pH value (>7.5), a test with pH paper is no longer sufficient and a more precise test with a pH meter is necessary. At a pH >7.5 and ≤8.5, the extract must be adjusted to a pH range of 6.0 ± 0.5 with diluted hydrochloric acid so that the chloropropanols are not further degraded. At a pH value >8.5, 1,3-DCP is no longer sufficiently stable over a period of 24 hours, so that there may be significant underestimation of 1,3-DCP in the cold water extract according to DIN EN 645! For 3-MCPD, this applies accordingly for pH values >9.0. From a pH value >8.5, the cold water extract is therefore repeated with an extraction time of only 1 hour and the analysis is carried out after neutralisation as described in this method.

Notes: The cold water extract must be used to analyse chloropropanols [3, 4] . The preparation of a hot water extract for testing for chloropropanols is expressly no longer recommended by the BfR, as the cold water extract already represents the worst conditions to be assumed. The hot water extracts can lead to underestimation [5]. NaCl is added to the water extract to ensure better transfer of the chloropropanols during the liquid-liquid extraction. This requires an approx. 2 M NaCl solution; a more precise determination of the NaCl concentration is not necessary for the quantification of the chloropropanols, as this value is not relevant to the result.

7.2.2 Liquid-liquid extraction with diatomaceous earth

The extraction columns are packed with 5 g of extraction material. Alternatively, commercially available packed extraction columns can be used. Care must be taken to ensure that the correct packing size is used for the extract volume of 5 mL (e.g. EXtrelut® NT 20) or that the volumes are adjusted and taken into account accordingly when calculating the concentrations. First, 50 µL ISTD mix (4.11.2 with 5.7), followed by 5 mL of the extract (with 5.8) are loaded on the extraction column. After 15 minutes, the analytes and internal standards are slowly eluted with 80 mL ethyl acetate into an evaporator tube.

7.2.3 Removal of solvent from the eluate

The eluate is concentrated to a volume of approx. 1 mL using a vacuum evaporator. The remaining eluate is transferred to a test tube with a ground joint. The evaporator tube is then rinsed with approx. 1 mL ethyl acetate and the combined eluate (extract and rinsing solution) is concentrated to approx. 450 µL under a nitrogen stream. An example programme for evaporation on the evaporator can be found in Appendix A2. Alternatively, the eluate can also be concentrated exclusively under a nitrogen stream. It is also possible to omit the last step of concentration under the nitrogen stream and concentrate to 450 µL in the vacuum evaporator. However, this can lead to distorted recoveries when using CMP as an internal standard due to the significantly different boiling points of CMP and DCP.

Note: When using the vacuum evaporator, it is essential to ensure that the final pressure does not fall below 90 mbar, as this can lead to losses of analytes. The use of a backflush system is also recommended. If there are problems with recovery, checking the seals of the vacuum evaporator has often proved helpful in practice.

7.2.4 Derivatisation

For derivatisation, the extract is transferred to a suitable vessel, e.g. an amber glass vial. Then, 50 µL of MSTFA (4.3.1) or BSTFA (4.3.2) are added using a piston-operated pipette. The vial is sealed, shaken and the solutions are incubated for at least 5 minutes at room temperature. After the reaction time has elapsed, the sample is quenched with 5 µL ethanol and mixed briefly. To avoid matrix interferences, it is recommended for difficult matrices to dilute the derivatised and quenched sample 1:5 with ethyl acetate (100 µL extract + 400 µL ethyl acetate). In this case, the calibration must also be diluted. The extract is then transferred to a suitable vessel, e.g. an amber glass vial with insert.

Note: During derivatisation, ensure that the tube is completely sealed. When using crimp-on vials/screw-on vials, caps with suitable septa material must be used. Butyl rubber PTFE septa, for example, have proven to be suitable for this purpose.

8 Quality assurance

The laboratories are responsible for quality assurance and control.

8.1 Blank values

A blank sample must be included in each sample preparation. Load 50 µL ISTD mix (4.11.2) and 5 mL ultrapure water (with NaCl in a ratio of 29.25 g NaCl to 250 ml) on the extraction column and process in the same way as the samples (7.2.1 to 7.2.4)

Each laboratory must choose its own approach when dealing with blank values. Blank values may have to be subtracted from the analysis results. There are various possible approaches to this, e.g. the FDA (US Food and Drug Administration) recommends subtracting the blank value if it is more than 20% of the limit of quantification [6, 7].
A reprocessing should be considered if the blank value accounts for a significant proportion of the analysed value and at the same time does not allow a clear classification above or below the respective limit or guide value.

Note: Possible sources of contamination include the column material, the salt and the ethyl acetate. It is therefore advisable to test new batches of these materials in advance.

9 Evaluation

9.1 Calculation

The mass content of the individual chloropropanols in the sample is calculated using the internal standard method. The determined content must be stated in µg/L; if necessary, a blank value must be subtracted.

To calculate the linear regression (y=mx+b), the ratios of the peak areas of the analyte to the internal standard from the calibration solutions are plotted against the concentrations of the analyte in the calibration solutions. The chloropropanol concentration in the extract is calculated using the equation:

$$x = \frac{\left(\frac{y-b}{m}\right)}{F}$$

x: Concentration of chloropropanol in the cold water extract in µg/L
y: Integrated peak area of the chloropropanol in the extract/integrated peak area of the respective ISTD in the extract
b: y-axis intercept
m: Slope
F: Conversion factor; ratio of the ISTD concentration in the calibration solutions and the extract ($c_{ISTD[calibration]}$ / $c_{ISTD[extract]}$)

Example calculation for the factor F:
When carrying out the liquid-liquid extraction as described in 7.2.2, 5 mL of the extract is concentrated to approx. 450 µL. During derivatisation according to 7.2.4 with MSTFA, 50 µL of reagent is added, resulting in a final volume of approx. 0.5 mL. This results in

$$F = \frac{50\ \mu L\ (ISTD)}{0{,}5\ mL\ (Kalibrierlösung)} / \frac{50\ \mu L\ (ISTD)}{5\ mL\ (Extrakt)} = 10$$

9.2 Reliability of the method

The statistical data shown in Table X were determined in a round robin test involving XX laboratories at XXX.

Table 1: Statistical data of the round robin test

Parameters	XXX	XXX	XXX	XXX
Number of participating laboratories				
Number of laboratories after elimination of outliers				
Number of				
Mean value $\bar{x}$, mg/kg				
Repeatability limit *r*, mg/kg				
Repeatability standard deviation s_r, mg/kg				
Relative repeatability standard deviation s_r, %				
Relative repeatability limit r_{rel}, %				
Comparison limit *R*, mg/kg				
Comparison standard deviation s_R, mg/kg				
Relative comparison standard deviation s_R %				
Relative comparison limit R_{rel}, %				

10 Report

The report must contain at least the following information with reference to this official method:

- Type, origin and name of the sample
- Type and date of sampling
- Date of receipt and investigation
- Test result
- Reasons if deviation from this official method has been made.

11 Explanations and notes

Reference is made to the general explanations provided in the introductory section of the official collection as part of the "Instructions for drafting the methods".

The method was developed by the working group "XXX" of the Federal Office of Consumer

Protection and Food Safety (BVL) for the implementation of § 64 LFGB and tested in a round robin test with XXX participants on the matrices XXX.

All trade names listed in this official method or product descriptions referring to individual suppliers are only for information purposes of users of this official method and do not imply recognition of the named product by the BVL. Equivalent products may be used if they demonstrably lead to comparable or better results.

12 Literature

1. DIN EN 645, *Paper and board intended to come into contact with foodstuffs - Preparation of a cold water extract*, 1994.
2. *Methods for the analysis of paper, cardboard and paperboard for food packaging and other consumer goods*. 04/2022. Federal Institute for Risk Assessment.
3. *XXXVI/1. Cooking and hot filter papers and filter sheets* .. 01.022023 . Federal Institute for Risk Assessment.
4. *XXXVI/2. Paper, cardboard and paperboard for baking purposes*. .01.022023 . Federal Institute for Risk Assessment.
5. R. Korte, S. Schulz, and B. Brauer, *Chloropropanols (3-MCPD, 1,3-DCP) from food contact materials: GC-MS method improvement, market survey and investigations on the effect of hot water extraction*. Food Additives & Contaminants: Part A, 2021.38 (6): p. 904-913.
6. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM), *Bioanalytical Method Validation - Guidance for Industry*. 2018.
7. European Medicines Agency, *ICH guideline M10 on bioanalytical method validation and study sample analysis - Step 5*. 2022.

Appendix

Determination of 1,3-dichloro-2-propanol and 3-monochloro-1,2-propanediol in the cold water extract of paper, board and cardboard using GC-MS(/MS)

A1 Example of system settings for a GC-MS/MS system

A1.1 Chromatographic settings

Injection volume:	1.5 µL, splitless
Separating column:	5% phenyl-methylpolysiloxane; 30 m (length)× 0.25 mm (inner diameter) × 0.25 µm (film thickness)
Pre-column:	Fused silica, deactivated; 10 m (length) × 0.25 mm (inner diameter)
Column flow:	1.2 mL/min He
KAS/PTV Liner:	Glass swirl liner

Oven programme:	Heating rate [°C/min]	Temperature [°C]	Holding time [min]
		50	2
	6	100	0
	45	285	11

Duration of the measurement: 25.4 min

Temperature programme:	Starting temperature: 45 °C	Equilibration time: 0.50 min	Starting time: 0.01 min
	Heating rate [°C/s]	Temperature [°C]	Holding time [min]
	12	220	20
	12	340	4

Retention times:

	Time (min)
CMP-TMS ether	10.36
1,3-DCP-TMS ether	10.76
1,3-DCP-d_5-TMS-Ether	10.71
3-MCPD-d_5-TMS ether	12.09
3-MCPD-TMS ether	12.11

A1.2 Detector settings

Ion source	EI 70 eV
Source temperature:	230 °C
Transfer line temperature:	320 °C
Quadrupole temperature:	150 °C
Collision gas	N_2
Mode:	MRM

A1.3 Quantifier and qualifier for MS

Substance or trimethylsilyl ether (TMS ether)	Quantifier	Qualifier
1,3-DCP TMS ether	93*	151, 185
CMP/DCP-Methoxy TMS ether (ISTD)	181	151
1,3-DCP-d_5 TMS ether	93*, 190	154
3-MCPD TMS ether	239	101, 116
3-MCPD-d_5 TMS ether (ISTD)	244	119

* Ensure complete chromatographic resolution of DCP and DCP-d_5

A1. Example of transitions and collision energies (CE) at the MS/MS

	Precursor ion	Product ion (M-X)+	Dwell time (msec)	CE (eV)
3-MCPD-TMS ether	101	59	5	8
	116	59	5	17
	116	73	5	7
	151	73	5	7
	239	147	5	7
1,3-DCP-TMS ether	185	93	5	8
	151	73	5	7
	93	65	5	17
1,3-DCP-d_5-TMS-Ether	190	93	5	5
	154	73	5	5
	192	93	5	5
3-MCPD-d_5-TMS ether	104	60	5	8
	119	60	5	17
	154	73	5	7
	119	73	5	12
	244	147	5	7
CMP-TMS ether	151	73	5	7
	181	89	5	7
	181	59	5	30

A2 Additional examples and tips for reprocessing

A2. 1 Example programme for vacuum evaporation

Table2 : Example programme for vaporisation on the evaporator

Level	Initial pressure [mbar]	Final pressure [mbar]	Duration [min]	Temperature cooling thermostat [°C]	Temperature thermoblock [°C]	Shaker speed [rpm]
1	550	250	2	10	50	270
2	250	200	3	10	50	270
3	200	150	5	10	50	270
4	150	125	5	10	50	270
5	125	100	7	10	50	270
6	100	90	approx. 40	10	50	270

A3 Stability of analytes

A3. 1 Shelf life in the refrigerator at 5 ± 3 °C

Data from the LAVES in Lüneburg: Comparison of a working solution in ethyl acetate from 06/12/2023 with a working solution in ethyl acetate from 04/07/2024; prepared from separately prepared stock solutions in ethyl acetate. The calibration solutions prepared from this were **derivatised on the same day**. Deviation of the concentrations < 10 % for 1,3-DCP and 3-MCPD. The **non-derivatised** solutions in ethyl acetate are therefore stable for at least 7 months.

A3. 1 Shelf life in the freezer at -18 °C

The stability of various analyte solutions when stored at -18 ± 5 °C was confirmed for the following times:

- Storage of derivatized (quenched) calibration series for 3 weeks
- Storage of spiking solutions on pH-neutral KWE for 6 months
- Storage of calibration and stock solution mixes for 6 months

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The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the German Federal Ministry of Agriculture, Food and Regional Identity (BMLEH). It protects people's health preventively in the fields of public health and veterinary public health. The BfR provides advice to the Federal Government as well as the Federal States (‘Laender’) on questions related to food, feed, chemical and product safety. The BfR conducts its own research on topics closely related to its assessment tasks.

About the BVL

The Federal Office of Consumer Protection and Food Safety (BVL) as a higher federal authority under the jurisdiction of the Federal Ministry of Food and Agriculture (BMEL) and fulfils many tasks in the area of food safety. The aim of the BVL is to improve coordination between the Federal Government and the Federal States in the area of consumer health and to make the communication of risks more transparent and to manage risks before they turn into crises.

The BVL is responsible for publishing the Official Collection of Methods of Analysis according to § 64 LFGB, § 38 TabakerzG, § 28b GenTG.

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