FOOD COMPOSITION AND ADDITIVES

Determination of 3-Chloro-1,2-Propanediol in Foods and Food Ingredients by Gas Chromatography with Mass Spectrometric Detection: Collaborative Study

PAUL BRERETON, JANET KELLY, COLIN CREWS, SARAH HONOUR, and ROGER WOOD
Ministry of Agriculture, Fisheries and Food, Central Science Laboratory, Norwich Research Park, Colney Ln, Norwich NR4 7UQ, UK
ANN DAVIES
Ministry of Agriculture, Fisheries and Food, Ergon House, 17 Smith Square, London SW1P 3JR, UK

Collaborators: E. Anklam; B. Baigrie; H. Diserens; P. Drake; C. Ebden; N. Fukue; L. Hageman; H. Louter; M. McBreen; G.A. Perfetti; P. Sayer; I.R. Slaiding

The results of a collaborative study are reported for the determination of 3-chloro-1,2-propanediol (3-monochloropropane-1,2-diol; 3-MCPD) in a wide range of foods and food ingredients, using gas chromatography with mass spectrometric detection and incorporating the use of a deuterated internal standard. After a pretrial study, 12 laboratories (6 United Kingdom, 1 Switzerland, 1 Japan, 2 United States, 1 The Netherlands, and 1 from the European Commission) were asked to analyze 12 test materials (as known duplicates or split-level samples) by using a prescribed procedure. The test materials consisted of duplicate samples of acid-hydrolyzed vegetable protein (containing 3-MCPD at 0.029 mg/kg), malt extract (0.055 mg/kg), wholemeal bread crumbs (0.030 mg/kg), salami (0.016 mg/kg), cheese alternative (0.043 mg/kg), and soup powder (split levels at 0.045 and 0.041 mg/kg). Repeatability ranged from 0.005 to 0.013 mg/kg and reproducibility, from 0.010 to 0.027 mg/kg, for the samples tested. Precision values were well within statistically predicted levels (HORRAT values of <1 for 5 of the 6 matrixes tested) and within method criteria prepared by a joint working group composed of the United Kingdom Ministry of Agriculture, Fisheries and Food and industry representatives. The study demonstrated the satisfactory validation of the method for quantifying 3-MCPD at levels of ≥0.010 mg/kg. The limit of detection derived from separate in-house studies was estimated to be 0.005 mg/kg. The method was adopted First Action by AOAC INTERNATIONAL.

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The recommendation was approved by the Methods Committee on Additives, Beverages, and Food Process-Related Analytes and was adopted by the Official Methods Board of AOAC INTERNATIONAL. *See* "Official Methods Board Actions," (1999) *Inside Laboratory Management*, November/December issue.

Carcinogenic effects have been attributed to 3-chloro-1,2-propanediol (3-monochloropropane-1,2-diol; 3-MCPD; 1), and the compound has been identified by the European Union's Scientific Committee for Food (SCF) as a suspected genotoxic carcinogen (2). It is formed as a product of the acid hydrolysis of vegetable proteins during the manufacture of the food seasoning ingredient hydrolyzed vegetable protein (acid-HVP; 3, 4). 3-MCPD is produced by the reaction of hydrochloric acid with the vegetable triglycerides (5). Producers of acid-HVP have modified the hydrolysis conditions and posthydrolysis treatments over recent years to achieve substantial reductions in the levels of 3-MCPD present in acid-HVP. However, the potential exists for the formation of 3-MCPD in other foods and food ingredients during the storage or processing of foods with high fat and salt content.

The United Kingdom Food Advisory Committee, in response to the SCF advice, recommended in November 1996 that industry should reduce levels of 3-MCPD in foods and food ingredients to <0.01 mg/kg within 18 months (6, 7).

3-MCPD has been determined in HVP by direct derivatization with phenylboronic acid (8, 9), but Extrelut® extraction with ethyl acetate or diethyl ether has also been used (3, 10–12). The technique has recently been extended to some foods (13).

The work described here was commissioned by the United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF) with the objective of validating a method for the determination of 3-MCPD in foods and ingredients with a limit of detection of <0.01 mg/kg. The method is based on published methods (3, 13) and was refined and validated internally at the Central Science Laboratory before this study.

Collaborators from 12 laboratories in The Netherlands, Japan, United States, United Kingdom, and the European Com-

mission took part in the study. The laboratories comprised commercial laboratories, research institutes, and control laboratories.

Pretrial

Before the main collaborative study, 9 participants were sent a detailed method protocol and 2 acid-HVP test materials to be analyzed in duplicate by using the prescribed method. For the pretrial test materials, the repeatability values were 0.003 and 0.005 mg/kg, and the reproducibility was 0.022 mg/kg for both test materials. Precision values were well within statistically predicted levels and within the method criteria prepared by a MAFF and food industry working group. Minor amendments were made to the method at this stage. The results of the pretrial study were sufficient to proceed to the main study. Analytes from 3 UK Public Analyst laboratories, who had attended a 2-day training workshop on the 3-MCPD method organized by the coordinating laboratory, were deemed to have sufficient expertise to be included in the main study.

Collaborative Study

Each participant was sent 12 test materials (5 sets of known duplicates and one split-level duplicate), a vial of deuterated internal standard, a method protocol, an instruction sheet, a results sheet, and an acknowledgment sheet. Participants were instructed to perform only one analysis of each test material.

Sample Scheme

The test materials used are described in Table 1. Because the sample pretreatment procedures in the method varied with the matrix, participants were informed of the identity of the test materials before analysis. Therefore, it was not possible to incorporate blind duplicate samples in the design of the collaborative trial. However, unknown to the participants, an element of split-level design was included (2 soup samples with slightly different levels of 3-MCPD) to preserve the integrity of the trial.

Sample Preparation

Seven bulk samples, including 2 split-level samples (C1 & C2), labeled A–F were used to prepare the 12 test materials used for the main collaborative study. The approximate 3-MCPD content of these samples had been determined previously in the laboratory. (*See* Table 1 for details of the sample scheme.)

Sample A, Test Materials 01 & 02 (Acid-HVP)

Commercially manufactured acid-HVP was mixed in a tumble mixer for approximately 1 h. Portions of approximately 10 g material were transferred to 28 mL plastic vials.

Sample B, Test Materials 03 & 04 (Malt Extract)

Two samples of commercially manufactured malt extract were combined in one container and mixed by rolling for approximately 3 h. Portions of approximately 22 g material were transferred to 28 mL plastic vials.

Sample C1, Test Material 05, and Sample C2, Test Material 06 (Soup Powders)

Packeted retail oxtail soup powder was mixed by rolling for approximately 3 h. Portions of approximately 6 g material were transferred to 28 mL plastic vials.

Sample D, Test Materials 07 & 08 (Bread Crumbs)

Granary malted wholemeal bread, purchased at a retail outlet, was blended to form bread crumbs in a food processor; the bread crumbs were dried in a drying cabinet for a few hours to reduce the moisture content and facilitate mixing, and then were roller-mixed for approximately 16 h. Portions of approximately 11 g were transferred to 28 mL plastic vials.

Sample E, Test Materials 09 & 10 (Salami)

Retail salami was blended to a smooth consistency in a food processor for approximately 5 min. Portions of approximately 21 g were transferred to 28 mL plastic vials.

Sample F, Test Materials 11 & 12 (Cheese Alternative)

Retail "cheese alternative" was grated in a food processor and then roller-mixed for approximately 16 h. Portions of approximately 21 g material were transferred to 28 mL plastic vials.

Homogeneity

The test materials were tested for homogeneity in most cases according to an internationally agreed protocol (14). Because of the limited sample size, a range of regimens was used. For sample A, 5 sample vials were selected at random, and the 3-MCPD was extracted. The extracts were then derivatized in duplicate. For sample B, 5 sample vials were selected at random, and the 3-MCPD was extracted in duplicate. For each of samples C–F, 10 sample vials were selected at random and analyzed individually. All samples had acceptable homogeneity; i.e., they passed the established test used in the

Table 1. Test materials used in the collaborative study

Sample code	No.	Material	MCPD level assigned from homogeneity testing, mg/kg
A	01/02	Acid-HVP	0.025
B	03/04	Malt extract	0.073
C1	05	Soup powder	0.045
C2	06	Soup powder	0.038
D	07/08	Bread crumbs	0.026
E	09/10	Salami	0.011
F	11/12	Cheese alternative	0.037

preparation of materials for proficiency testing (14), or they had relative standard deviations (RSDs) of <10%.

Sample Storage

Participants were instructed to store test materials 01, 02, 05, and 06 at room temperature; test materials 03, 04, 11, and 12 at 4°C; and test materials 07–10 at –20°C.

AOAC Official Method 2000.01 Determination of 3-Chloro-1,2-Propanediol in Foods and Food Ingredients

Gas Chromatography/Mass Spectrometric Detection First Action 2000

[The method is applicable to the determination of 3-chloro-1,2-propanediol (3-monochloropropane-1,2-diol; 3-MCPD) in hydrolyzed vegetable protein (HVP), soups and stocks, stock cubes, soy sauce, malt extract, salami, fish, cheese, flour, starch, cereals, and bread.]

Caution: This work should be performed under a fume hood. Wear laboratory coat, gloves, and eye/face protection. Use double-containment systems for handling concentrated solutions of 3-MCPD-d_5. Take care to avoid ignition of flammable reagents by sparks or static discharge.

See Table **2000.01** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Internal standard 3-chloro-1,2-propanediol-d_5 (3-MCPD-d_5) is added to the test portion, followed by salt solution, and the mixture is blended to a homogeneous consistency. After sonication, the contents of an Extrelut™ refill pack are added and mixed thoroughly. The mixture is transferred to a glass chromatographic tube, and the nonpolar components are eluted with a mixture of hexane and diethyl ether. The 3-MCPD is eluted with diethyl ether, and the extract is concentrated to a small volume. A portion of the concentrated extract is derivatized and analyzed by gas chromatography with mass spectrometric detection (GC/MS). The concentration of 3-MCPD is expressed in mg/kg.

B. Apparatus

(**a**) *Gas chromatograph*.—Fitted with a split/splitless injector. Column: nonpolar, 30 m × 0.25 mm, 0.25 μm film thickness (J&W Scientific, 91 Blue Ravine Rd, Folsom, CA 95630-4714, USA) DB-5ms, or equivalent. Suggested temperature program: initial temperature 50°C for 1 min, increase temperature at 2°C/min to 90°C; increase temperature at maximum rate to 270°C; hold for 10 min. Operating conditions: injector temperature, 270°C; transfer line temperature, 270°C; carrier gas, He at 1 mL/min; and injection volume, 1.5 μL in splitless mode with 40 s splitless period.

(**b**) *Mass spectrometer*.—Capable of multiple-ion monitoring or full scanning at high sensitivity. Conditions: positive electron ionization with selected-ion monitoring of *m/z* 257 (internal standard), 453, 291, 289, 275, and 253 (3-MCPD), or full scanning over the range 100–500 amu.

(**c**) *Ultrasonic bath*.

(**d**) *Glass chromatography tube*.—40 × 2 cm id, with sintered glass disk and tap.

(**e**) *Syringe*.—Gas-tight, 1 mL.

(**f**) *Centrifuge*.—Capable of 3500 rpm.

(**g**) *Filter paper*.—No. 4 (Whatman International Ltd., Whatman House, St. Leonard's Rd, 20/20 Maidstone Kent, ME16 OLS, UK).

(**h**) *Rotary evaporator*.

(**i**) *Vortex shaker*.

(**j**) *Aluminum block heater*.—Pierce Reactitherm (Rockford, IL) or equivalent.

C. Reagents

(**a**) *3-Chloro-1,2-propanediol (3-MCPD)*.

(**b**) *3-Chloro-1,2-propanediol-d_5 (3-MCPD-d_5)*.—Minimum 98% isotopic purity. Available from Isotec, Inc., 3858 Benner Rd, Miamisburg, OH 45342, USA.

(**c**) *Sodium sulfate.*

(**d**) *Heptafluorobutyrylimidazole*.

(**e**) *Extrelut™ 20 mL refill packs*.—EM Science, 480 S Democrat Rd, Gibbstown, NJ 08027, USA.

Table 2000.01. Interlaboratory study results for the determination of 3-chloro-1,2-propanediol in foods and food ingredients

Sample ID	$\bar{x}$, mg/kg	No. of labs[a]	s_r, mg/kg	RSD_r, %	s_R, mg/kg	RSD_R, %	HORRAT
A, HVP	0.029	10(2)	0.002	7.5	0.004	12.8	0.5
B, Malt extract	0.055	11(1)	0.003	4.9	0.007	13.3	0.5
C1 & C2, Soup powder	0.043	11(1)	0.004	8.9	0.008	18.6	0.7
D, Bread crumbs	0.030	12(0)	0.003	8.4	0.006	20.8	0.8
E, Salami	0.016	12(0)	0.002	11.6	0.006	38.6	1.3
F, Cheese alternative	0.043	11(1)	0.005	10.6	0.010	22.3	0.9

[a] Each value is the number of laboratories retained after elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

Table 2. Collaborative results (mg/kg) for sample A, test materials 01 and 02 (acid–HVP)

Laboratory	01	02
1	0.031	0.035
2[a]	0.11	0.034
3	0.029	0.027
5	0.031	0.036
7	0.023	0.026
9	0.033	0.028
10	0.029	0.028
12	0.031	0.032
13	0.025	0.026
15[a]	0.008	0.027
16	0.029	0.026
17	0.024	0.025
In-house value (homogeneity testing)	0.025	
Mean[b]	0.029	
n[c]	12	
Outliers[d]	2	
n_1[e]	10	
r[f]	0.006	
s_r[g]	0.002	
RSD_r[h]	7.5	
Ho_r[i]	0.4	
R[j]	0.010	
s_R[k]	0.004	
RSD_R[l]	12.8	
Ho_R[m]	0.5	

[a] Outlier by the Cochran test (P <0.01); not used in the calculation of statistical parameters.
[b] Mean of all data used in the statistical analysis.
[c] Total number of data sets submitted.
[d] Number of results excluded from statistical analysis on the basis of the Cochran test, the Grubbs test, or noncompliance.
[e] Number of results used in statistical analysis.
[f] Repeatability limit (within-laboratory variation), i.e., the value below which the absolute difference between 2 single test results obtained with the same method for identical test materials under the same conditions may be expected to lie, with a probability of 95%.
[g] Repeatability standard deviation.
[h] Repeatability relative standard deviation ($s_r \times 100$/mean).
[i] HORRAT value for repeatability, i.e., the observed RSD_r divided by the RSD_r value estimated from the Horwitz equation by using the assumption r = 0.66R.
[j] Reproducibility limit (between laboratories variation), i.e., the value below which the absolute difference between 2 single test results obtained with the same method for identical test materials under different conditions may be expected to lie, with a probability of 95%.
[k] Reproducibility standard deviation.
[l] Reproducibility relative standard deviation ($s_R \times 100$/mean).
[m] HORRAT value for reproducibility, i.e., the observed RSD_R divided by the RSD_R value calculated from the Horwitz equation.

Table 3. Collaborative results (mg/kg) for sample B, test materials 03 and 04 (malt extract)

Laboratory	03	04
1	0.064	0.063
2	0.058	0.05
3	0.056	0.056
5	0.064	0.064
7	0.047	0.042
9	0.057	0.062
10	0.06	0.059
12	0.057	0.054
13	0.041	0.042
15[a]	0.065	0.039
16	0.056	0.062
17	0.052	0.053
In-house value (homogeneity testing)	0.073	
Mean	0.055	
n	12	
Outliers	1	
n_1	11	
r	0.008	
s_r	0.003	
RSD_r	4.9	
Ho_r	0.3	
R	0.021	
s_R	0.007	
RSD_R	13.3	
Ho_R	0.5	

For definitions, *see* footnotes in Table 2.
[a] Outlier by the Cochran test (P <0.01); not used in the calculation of statistical parameters.

(**f**) *Ethyl acetate*.
(**g**) *2,2,4-Trimethylpentane*.
(**h**) *Diethyl ether*.—Glass-distilled grade.
(**i**) *Hexane*.—Glass-distilled grade.
(**j**) *Sodium chloride*.
(**k**) *Diethyl ether–hexane*.—(1 + 9). Add 100 mL diethyl ether, (**h**), to 900 mL hexane, (**i**), and mix well.
(**l**) *Sodium chloride solution*.—5M. Dissolve 290 g NaCl, (**j**), in 1 L water.
(**m**) *3-MCPD stock solution*.—(1 mg/mL). Weigh 25 mg 3-MCPD, (**a**), dissolve in ethyl acetate, (**f**), in 25 mL volumetric flask, and dilute to volume.
(**n**) *3-MCPD intermediate standard solution*.—(100 μg/mL). Dilute 3-MCPD stock solution, (**m**), 1 + 9 with ethyl acetate, (**f**), to 100 μg/mL.

(**o**) *3-MCPD spiking solution*.—(2 μg/mL). Pipet 2 mL intermediate standard solution (100 μg/mL), (**n**), into 100 mL volumetric flask, and dilute to volume with ethyl acetate, (**f**).

(**p**) *3-MCPD-d_5 internal standard stock solution*.—(1 mg/mL). Weigh 25 mg 3-MCPD-d_5, (**b**), dissolve in ethyl acetate, (**f**), in 25 mL volumetric flask, and dilute to volume.

(**q**) *3-MCPD-d_5 internal standard working solution*.—(10 μg/mL). Prepare 100 mL internal standard working solution by diluting 1 mL internal standard stock solution (1 mg/mL), (**p**), with ethyl acetate, (**f**).

(**r**) *3-MCPD calibration solutions*.—Pipet 100 μg/mL intermediate standard solution, (**n**), in aliquots of 0, 12.5, 25, 125, 250, and 500 μL into 25 mL volumetric flasks, and dilute to volume with 2,2,4-trimethylpentane, (**g**), to obtain 3-MCPD concentrations of 0.00, 0.05, 0.10, 0.50, 1.00, and 2.00 μg/mL, respectively.

(**s**) *Nitrogen gas*.

Table 4. Collaborative results (mg/kg) for split-level samples C1 and C2, test materials 05 and 06 (soup powder)

Laboratory	05	06
1	0.045	0.043
2	0.037	0.037
3	0.056	0.049
5	0.045	0.037
7	0.037	0.033
9	0.049	0.039
10	0.055	0.047
12	0.049	0.047
13[d]	0.036	—
15	0.026	0.029
16	0.049	0.04
17	0.046	0.053
In-house value (homogeneity testing)	0.045, 0.038	
Mean	0.045, 0.041	
n	11	
Outliers	0	
n_1	11	
r	0.011	
s_r	0.004	
RSD_r	8.9	
Ho_r	0.5	
R	0.022	
s_R	0.008	
RSD_R	18.6	
Ho_R	0.7	

For definitions, *see* footnotes in Table 2.

[d] Full results were not supplied; therefore, results were excluded from statistical analysis.

D. Preparation of Test Samples

(**a**) *Dry materials such as stock cubes and cereals*.—Grind to a fine consistency. Mince or grate bread, cheese, salami, and fish to a homogeneous mixture. Store in air-tight containers, and freeze if necessary. Mix thoroughly before analysis.

(**b**) *Hydrolyzed vegetable protein (HVP), soy sauce, soups, stocks, malt extracts, soup powders, and stock cubes*.—Weigh to the nearest 0.01 g 8 g test portion of HVP or soy sauce, 10 g of soup, stock, or malt extract, or 5 g of soup powder or stock cubes. Add 100 μL 3-MCPD-d_5 internal standard working solution (10 μg/mL), **C**(**q**). Add 5M NaCl solution, **C**(**l**), to a total weight (HVP + salt solution) of 20 g, and blend to a homogeneous mixture, using a spatula and crushing all small lumps. Place in an ultrasonic bath for 10 min. Proceed to preparation of test extract, (**e**).

(**c**) *Salami, cheese, and fish*.—Weigh 20 g test portion to the nearest 0.01 g. Add 100 μL 3-MCPD-d_5 internal standard working solution (10 μg/mL), **C**(**q**). Add 5M NaCl solution,

Table 5. Collaborative results (mg/kg) for sample D, test materials 07 and 08 (bread crumbs)

Laboratory	07	08
1	0.035	0.031
2	0.034	0.029
3	0.042	0.039
5	0.03	0.029
7	0.024	0.025
9	0.032	0.028
10	0.037	0.034
12	0.032	0.034
13	0.018	0.015
15	0.032	0.037
16	0.03	0.028
17	0.03	0.024
In-house value (homogeneity testing)	0.026	
Mean	0.030	
n	12	
Outliers	0	
n_1	12	
r	0.007	
s_r	0.003	
RSD_r	8.4	
Ho_r	0.5	
R	0.018	
s_R	0.006	
RSD_R	20.8	
Ho_R	0.8	

For definitions, *see* footnotes in Table 2.

C(l), to give a total weight of 70 g, and blend to a homogeneous mixture. If necessary to enable blending, add another 10 g NaCl solution, **C(l)**. Transfer entire mixture to a centrifuge tube, and centrifuge at 3500 rpm for 20 min. Pour the supernatant layer into a beaker, avoiding the transfer of solid material and visible fat. Weigh 20 g of the supernatant into a 250 mL beaker and proceed to preparation of the test extract, (**e**).

(**d**) *Flour, starch, cereal, and bread*.—Weigh 10 g test portion to the nearest 0.01 g. Add 100 µL 3-MCPD-d_5 internal standard working solution (10 µg/mL), **C(q)**. Add 5M NaCl solution, **C(l)**, to a total weight of 40 g, and blend to a homogeneous mixture, using a spatula and crushing all small lumps. Place in an ultrasonic bath, **B(c)**, for 15 min. Cover and let soak for 12–15 h. Proceed to preparation of the test extract, (**e**).

(**e**) *Test extract*.—To 20 g prepared product, add the contents of an Extrelut™ refill pack, **C(e)**, and mix thoroughly with spatula. Add the mixture to the chromatography tube, **B(d)**, briefly agitate by hand to compact contents, top with a 1 cm layer of Na_2SO_4, **C(c)**, and leave for 15–20 min. Elute nonpolar components with 80 mL diethyl ether–hexane (1 + 9). Allow unrestricted flow except for powdered soup, for which the flow should be restricted to about 8–10 mL/min. Close the tap when the solvent reaches the Na_2SO_4 layer, and discard the collected solvent. Elute the 3-MCPD with 250 mL diethyl ether, **C(h)**, at a flow rate of about 8 mL/min. Collect 250 mL eluate in a 250 mL volumetric flask. Add 15 g anhydrous Na_2SO_4, **C(c)**, to the flask and leave for 10–15 min. Filter the eluate through a filter paper, **B(g)**, into a 250 mL round-bottom or pear-shape flask. Concentrate the extract to about 5 mL by rotary evaporation at 35°C. Do not allow to dry. Transfer the concentrated extract to a 10 mL volumetric flask with diethyl ether, **C(h)**, and dilute to volume with diethyl ether, **C(h)**. Add a small quantity (spatula tip) of anhydrous Na_2SO_4, **C(c)**, to the flask, shake flask well, and leave for 5 to 10 min. Using a 1 mL gas-tight syringe, **B(e)**, transfer 1 mL extract to a 4 mL vial. Evaporate the solution just to dry-

Table 6. Collaborative results (mg/kg) for sample E, test materials 09 and 10 (salami)

Laboratory	09	10
1	0.015	0.012
2	0.025	0.021
3	0.021	0.019
5	0.016	0.012
7	0.031	0.028
9	0.016	0.016
10	0.015	0.013
12	0.012	0.011
13	0.008	0.004
15	0.012	0.012
16	0.014	0.016
17	0.017	0.019
In-house value (homogeneity testing)	0.011	
Mean	0.016	
n	12	
Outliers	0	
n_1	12	
r	0.005	
s_r	0.002	
RSD_r	11.6	
Ho_r	0.6	
R	0.017	
s_R	0.006	
RSD_R	38.6	
Ho_R	1.3	

For definitions, *see* footnotes in Table 2.

Table 7. Collaborative results (mg/kg) for sample F, test materials 11 and 12 (cheese alternative)

Laboratory	11	12
1	0.044	0.051
2	0.044	0.044
3	0.064	0.054
5	0.041	0.040
7[a]	0.087	0.081
9	0.045	0.046
10	0.034	0.031
12	0.036	0.026
13	0.035	0.032
15	0.049	0.062
16	0.041	0.041
17	0.042	0.038
In-house value (homogeneity testing)	0.037	
Mean	0.043	
n	12	
Outliers	1	
n_1	11	
r	0.013	
s_r	0.005	
RSD_r	10.6	
Ho_r	0.6	
R	0.027	
s_R	0.010	
RSD_R	22.3	
Ho_R	0.9	

For definitions, *see* footnotes in Table 2.

[a] Outlier by the single Grubbs test (P <0.025); not used in the calculation of statistical parameters.

Table 8. Summary of statistical parameters calculated for 3-MCPD

Parameter	Sample (test material) and matrix					
	A (01 & 02) HVP	B (03 & 04) Malt extract	C1 & C2 (05 & 06) Soup powder	D (07 & 08) Bread crumbs	E (09 & 10) Salami	F (11 & 12) Cheese alternative
Assigned value	0.025	0.073	0.045, 0.038	0.026	0.011	0.037
Mean	0.029	0.055	0.045, 0.041	0.030	0.016	0.043
n	12	12	12	12	12	12
Outliers	2	1	1	0	0	1
n_1	10	11	11	12	12	11
r	0.006	0.008	0.011	0.007	0.005	0.013
s_r	0.002	0.003	0.004	0.003	0.002	0.005
RSD_r	7.5	4.9	8.9	8.4	11.6	10.6
Ho_r	0.4	0.3	0.5	0.5	0.6	0.6
R	0.010	0.021	0.022	0.018	0.017	0.027
s_R	0.004	0.007	0.008	0.006	0.006	0.010
RSD_R	12.8	13.3	18.6	20.8	38.6	22.3
Ho_R	0.5	0.5	0.7	0.8	1.3	0.9

For definitions, *see* footnotes in Table 2.

ness below 30°C under a stream of N, **C**(**s**). Immediately add 1.0 mL 2,2,4-trimethylpentane, **C**(**g**), and 0.05 mL heptafluorobutyrylimidazole, **C**(**d**), and seal vial. Shake vial, using a vortex shaker, **B**(**i**), for a few seconds, and heat at 70°C for 20 min in a block heater, **B**(**j**). Let the mixture cool to <40°C, add 1 mL distilled water, shake on a vortex shaker, **B**(**i**), for 30 s, let the phases separate, and then repeat shaking. Remove the 2,2,4-trimethylpentane phase to a 2 mL vial, add a small quantity (spatula tip) of anhydrous Na_2SO_4, **C**(**c**), shake vial, and let stand for 2–5 min. Transfer the solution to a new 2 mL vial for GC/MS. Parallel method blanks comprising 20 g 5M NaCl solution, **C**(**l**), should be run with each batch of tests.

(**f**) *Calibration standards*.—To a set of 4 mL vials, transfer 0.1 mL of each of the calibration solutions, **C**(**r**), 10 μL 3-MCPD-d_5 internal standard working standard (10 μg/mL),

Table 9. Summary of chromatography conditions used by collaborative study participants

Lab	Mass spectrometer	Column	Column length, m	Column dia., mm	Column film thickness, μm	Temperature program
1	MD800	BPX5	25	0.32	1	50°C for 1 min, 2°C/min to 90°C
2	Trio 2	SGE BPX-5	30	0.25	0.25	50°C for 1 min, 4°C/min to 100°C
3	QMD 1000	DB-5 (J&W)	30	0.32	1	50°C for 1 min, 2°C/min to 90°C
5	GCQ	Optima 5 MS	30	0.25	0.25	50°C for 1 min, 2.5°C/min to 73°C, 5°C/min to 100°C
7	HP-5972	HP-5	25	0.2	0.33	50°C for 1 min, 2°C/min to 84°C
9	GCQ	DB-5MS	30	0.25	0.25	40°C for 0.6 min, 0.4°C/min to 60°C, 1°C/min to 75°C
10	HP5890	5%-Phenylmethylpolysiloxane	30	0.25	0.25	50°C for 1 min, 2°C/min to 90°C
12	MD800	HP-5MS	30	0.25	0.25	50°C for 1 min, 2°C/min to 90°C
13	Trio 1000	RTX5-MS	30	0.25	0.25	50°C for 1 min, 2°C/min to 90°C
15	3400 GC/Saturn 3 MS	SGE BPX-5	30	0.25	0.25	50°C for 1 min, 2°C/min to 90°C
16	Thermoquest Voyager	DB-5MS	25	0.25	0.25	40°C for 1 min, 2°C/min to 90°C
17	HP	5% Phenylmethypolysiloxane	30	0.25	0.25	50°C for 1 min, 2°C/min to 90°C

C(**q**), and 0.9 mL 2,2,4-trimethylpentane, **C**(**g**). Proceed with the derivatization as above, (**e**).

E. Calculations

(**a**) Measure the areas of the 3-MCPD-d_5 (*m/z* 257) and 3-MCPD (*m/z* 253) derivative peaks. Calculate the ratio of the area of the 3-MCPD (*m/z* 253) derivative peak to the area of the 3-MCPD-d_5 (*m/z* 257) derivative peak. Construct a calibration graph for the standards by plotting the peak area ratio versus the weight in micrograms of the 3-MCPD in each vial. Calculate the slope of the calibration line.

$$\text{3-MCPD, mg/kg} = \frac{(A \times 10)/(A' \times C)}{\text{sample, g}}$$

where A = peak area for the 3-MCPD derivative; A′ = peak area for the 3-MCPD-d_5 derivative; and C = slope of the calibration line.

(**b**) *Confirmation of peak identity*.—For full-scan spectra, ensure that a minimum library fit of 800 versus a standard is achieved for a background-subtracted spectrum. For selected-ion monitoring, measure the ratio of the responses at *m/z* 291, 289, 275, and 253 relative to the response at *m/z* 453 for the standards and tests. At least 2 of the 4 ion abundance ratios should be within ±20% of the mean of the ion abundance ratios of the standards.

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Results and Discussion

Statistical Analysis of Results

The pretrial and trial results were examined for evidence of individual systematic error (p <0.025) by using the Cochran and Grubbs tests progressively, by procedures described in the internationally agreed protocol for the design, conduct, and interpretation of method-performance studies (15).

Only 4 statistical outliers were identified in the 71 (Laboratory 13 only sent in one result for sample C) pairs of results. No cause was identified for the aberrant results. The results of the collaborative trial are given in Tables 2–7 and summarized in Table 8.

Repeatability and reproducibility.—Repeatability (r) and reproducibility (R) as defined by the protocol (15) were calculated for the results remaining after removal of outliers. Repeatability ranged from 0.005 to 0.013 mg/kg, and reproducibility ranged from 0.010 to 0.027 mg/kg.

Horwitz predicted precision parameters.—When a new method is assessed, there is often no validated reference or statutory method with which to compare precision criteria; thus, it is useful to compare the precision data obtained from a collaborative trial with "predicted" levels of precision. These "predicted" levels are calculated from the Horwitz equation. Comparison of the trial results and the predicted levels gives an indication as to whether the method is sufficiently precise for the analyte level being measured (16).

The Horwitz predicted value is calculated from the Horwitz equation (16):

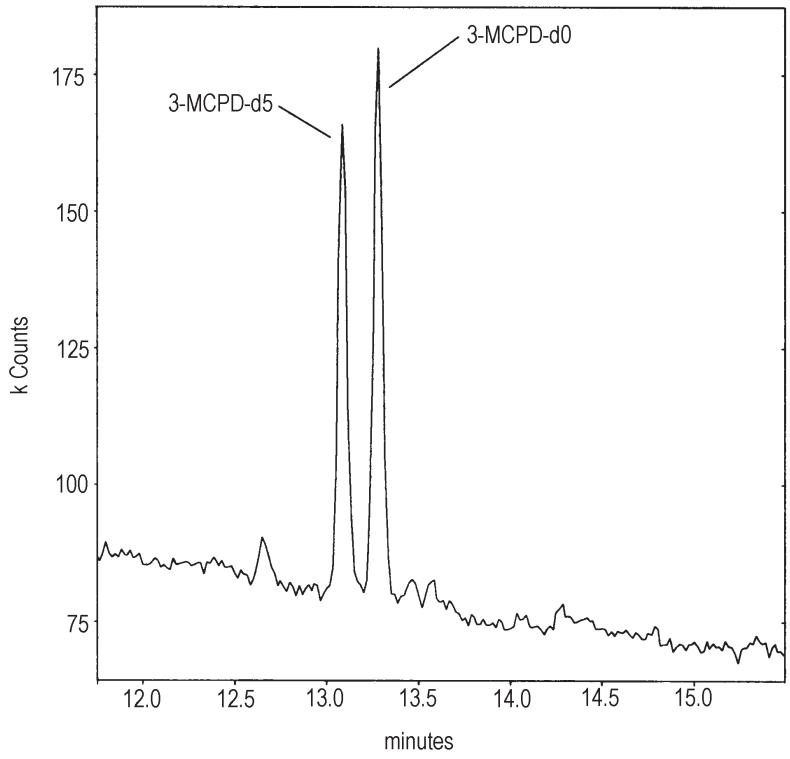

Figure 1. Total ion current chromatogram of 3-MCPD-d_0 and 3-MCPD-d_5 derivatives.

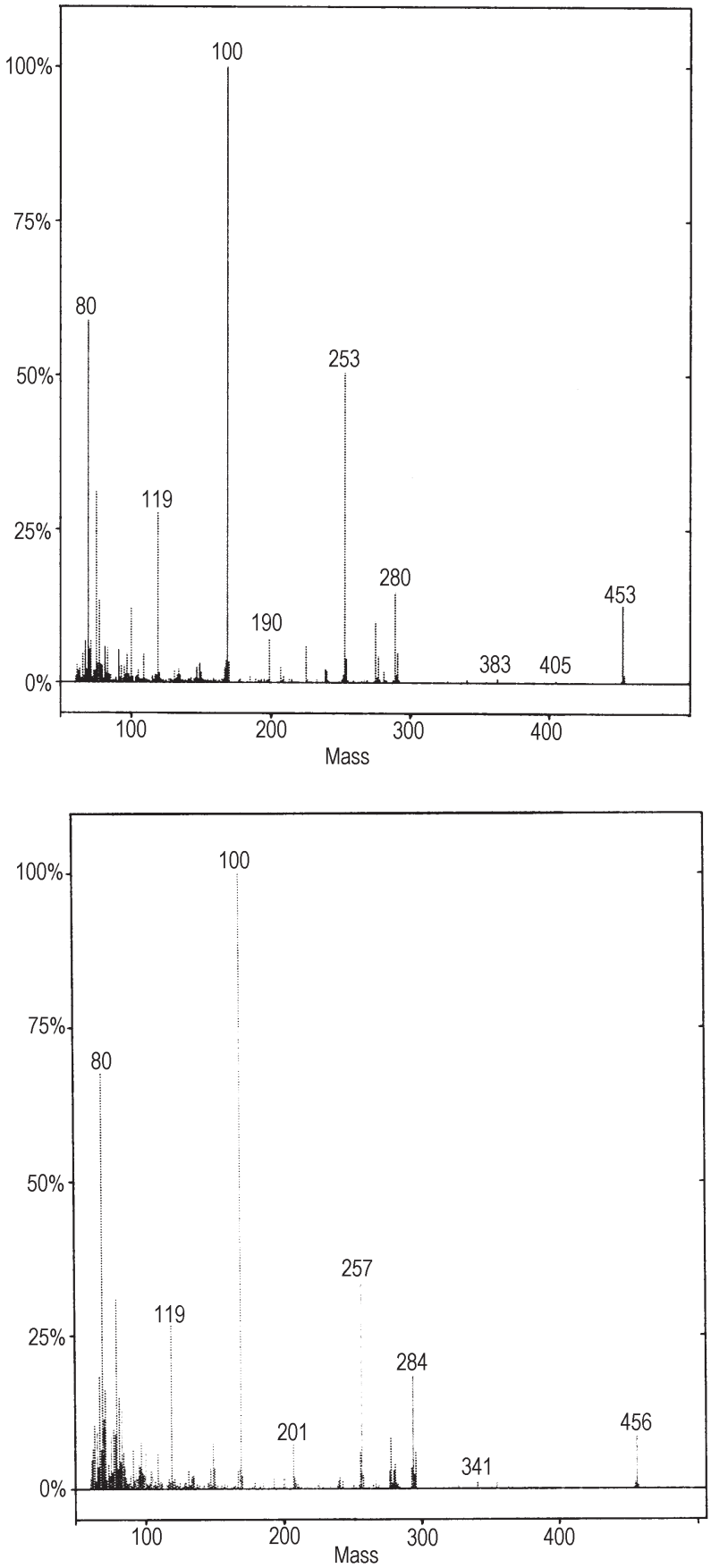

Figure 2. Mass spectrum of 3-MCPD-d_0 (top) and 3-MCPD-d_5 (bottom) derivatives.

$$RSD_R = 2^{(1-0.5\ \log C)}$$

where C = measured concentration of analyte expressed as a decimal (e.g., 1 g/100 g = 0.01).

The HORRAT value (17) gives a comparison of the actual precision measured with the precision predicted by the Horwitz equation for a method that measures the analyte at that particular level. It is calculated as follows:

$$Ho_R = \frac{RSD_R\ (\text{measured})}{RSD_R\ (\text{Horwitz})}$$

An Ho_R value of 1 usually indicates satisfactory interlaboratory precision, whereas a value of >2 usually indicates unsatisfactory precision, i.e., the precision is too variable for most analytical purposes or the variation obtained is greater than that expected for the type of method employed. Ho_r, used to assess intralaboratory precision, is calculated by using the following approximation:

$$RSD_r(\text{Horwitz}) = 0.66\ RSD_R(\text{Horwitz})$$

(This assumes the approximation r = 0.66 R.)

Pretrial

No significant problems were encountered with the method at the pretrial stage. Minor changes were made to the method before the collaborative study. The scope of the method was increased to take into account the range of matrixes to be tested, the calibration range was extended slightly, and the treatment for powdered soup was modified to remove interferences from coeluting compounds.

Collaborative Study

Most of the Ho_R values were <1, and all values were <1.4, demonstrating that the method gave acceptable levels of precision for each of the duplicate and split-level samples.

The salami, sample E, test materials 09 and 10, had the highest RSD_R value (38.6) of all the matrixes tested. This result probably reflected the more complex nature of the matrix as well as the lower 3-MCPD concentration involved. All of the other matrixes had relative precision values (RSD_R) between 12.8 and 22.3%.

All participants submitted satisfactory chromatograms and were deemed to have obtained satisfactory chromatographic performance. The chromatography columns and instrumental conditions used by the participants in the study are given in Table 9. Figure 1 shows the total ion current chromatogram for a derivatized mixture of 3-MCPD-d_0 and 3-MCPD-d_5 standard solutions at the concentration employed. Baseline chromatographic separation of the peaks was obtained, with the 3-MCPD-d_5 eluting earlier.

Figure 2 shows the mass spectra of derivatized 3-MCPD-d_0 and 3-MCPD-d_5. The 3-MCPD-d_5 spectrum does not contain the major ions (*m/z* 253, 275, 289, and 453) of 3-MCPD-d_0 at significant levels of intensity. Production batches of 3-MCPD-d_0 may vary in isotopic purity, and any potential contribution to the 3-MCPD-d_0 should be considered. Addition of 3-MCPD-d_5 of 98% isotopic purity at the level described in the method (0.0125 mg/kg in an HVP sample) could contribute 3-MCPD-d_0 at 0.003 mg/kg.

Method Criteria Requirements

Method quality assurance criteria were drawn up by an MAFF/industry working group; they required quantification by using the response at *m/z* 253, together with confirmation of peak identity by comparison of retention times and ion abundances at *m/z* 253, 289, and 291 relative to *m/z* 453 with corresponding values for calibration standards. Participants 1, 13, and 15 reported difficulty in obtaining sufficient sensitivity to measure the signal at *m/z* 453 in some cases. In general, most participants were unable to comply absolutely with the quality assurance criteria regarding ion abundance ratios specified in the method. It may be appropriate to modify the quality assurance criteria because the participants were able to obtain satisfactory quantitative data without fulfilling the criteria in all respects. The relative peak retention time criteria were met for all reported results.

Robustness

Participants used a range of columns, conditions, and instrumentation in the study, demonstrating the robustness of the method.

Conclusions

A robust method for the determination of trace levels (i.e., 0.010 mg/kg) of 3-MCPD in a wide range of foods and food ingredients was successfully validated by a collaborative study conducted according to internationally agreed procedures.

Recommendation

It is recommended that the method be adopted First Action by AOAC INTERNATIONAL.

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