SHIMADZU

Application Report

GCMS-QP5050A with GCMSsolution workstation

Improving the Sensitivity of Detection of 3-chloropropane-1,2-diol (3-MCPD) using Gas Chromatography with Negative Chemical Ionisation Mass Spectrometry detection

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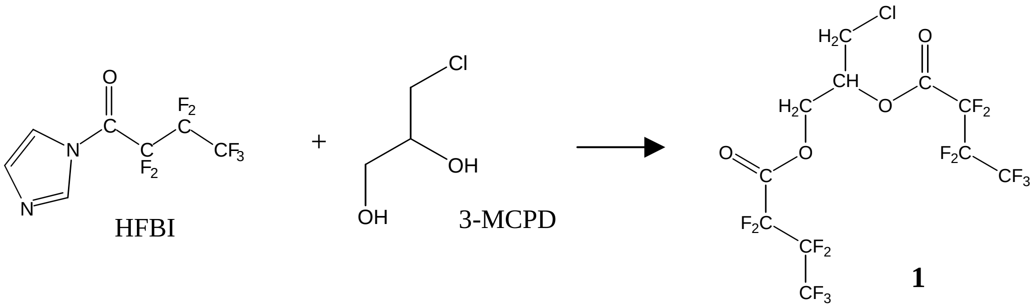
3-Chloropropane-1,2-diol (alpha-chlorohydrin; 3-monochloropropanediol; 3-MCPD) is a toxic contaminant that has been found in soy sauce and other food products that use acid-hydrolysed vegetable protein (acid-HVP) [***Collier, 1992; SCF, 1995***]. Acid-HVP is a widely used ingredient in savoury foods, produced from the hydrolysis of plant protein-containing raw materials, such as wheat, soybeans or peanuts, at high temperature in the presence of hydrochloric acid. During the manufacturing of acid-HVP, residual lipid components in the protein source can also undergo hydrolysis to form 3-MCPD [***Velisek, 1980, Collier, 1991***].

Some toxicological studies have shown that 3-MCPD has carcinogenic effects in animals [***Lynch, 1998***]. Although there is no direct evidence for toxic effects of 3-MCPD in humans, its occurrence in human diet should be minimised, and monitoring of 3-MCPD in food products is required. In fact, in March 2001, the European Commission adopted a regulatory limit of 0.02 mg/kg (based on 40% dry matter content) for 3-MCPD in soy sauce and acid-HVP, which is to be implemented in April 2002.

Gas chromatography is usually the analytical method of choice for the quantitative determination of 3-MCPD. Brereton et al. recently published a validated method capable of measuring 3-MCPD ≥0.01 mg/kg, based on gas chromatography with mass spectrometric detection [***Brereton et al., 2001***]. This method is based on extraction of 3-MCPD followed by its derivatisation using *N*-heptafluorobutyrylimidazole (HFBI) to give compound (**1**).



HFBI

3-MCPD

1

Negative chemical ionisation mass spectrometry (NCI-MS) provides a specific and sensitive detection for electronegative compounds. The technique is based on the formation and detection of negative ions. The presence of CF_n moieties in the 3-MCPD derivative (**1**) renders the molecule capable of forming negative ions in the mass spectrometer.

The data presented here demonstrates that NCI-MS is suitable for the detection of 3-MCPD as its derivative (**1**). In Scan mode, NCI-MS provides qualitative information of 3-MCPD, and a comparable sensitivity to EI-MS in Selected Ion Monitoring (SIM) mode. In SIM mode, NCI-MS provides a lower detection limit than EI-MS.

Experimental

3-Chloro-1,2-propanediol was obtained from Chem Service. 3-Chloro-1,2-propane-d_5-diol was obtained from C/D/N Isotopes. *N*-Heptafluorobutyrylimidazole (HFBI) was obtained from Tokyo Kasei Kogyo. Organic solvents used were HPLC grade, except ethyl acetate, which was organic residue grade. Extrelut™ 20 columns were obtained from Merck.

Samples were analysed on a Shimadzu GCMS-QP5050A (Japan) in electron-impact ionisation (EI) and in negative chemical ionisation (NCI) mode, equipped with a Shimadzu AOC-20i/s auto-injector/ auto-sampler (Japan). Data were acquired and processed using GCMSsolution software.

Standards preparation

EI-MS experiment: Calibration standards were obtained by adding 100 μL of 3-MCPD-d_0 solution (concentration range of 0.05–150 μg/mL, in ethyl acetate) into 900 μL of 2,2,4-trimethylpentane, followed by derivatisation with HFBI.

NCI-MS experiment: Calibration standards were obtained by adding 100 μL of the 3-MCPD-d_5 internal standard solution (0.10 μg/mL, in ethyl acetate) and 100 μL of the 3-MCPD-d_0 solution (concentration range of 0.02–0.10 μg/mL, in ethyl acetate) into 800 μL of 2,2,4-trimethylpentane, followed by derivatisation with HFBI.

Sample preparation [*Brereton et al., 2001*]

8 g of a soy sauce sample was weighed into a 250mL beaker and 5M NaCl solution was added to give a total weight of 20 g. This was followed by sonication to achieve homogeneity.

After sonication, the contents of Extrelut™20 was mixed with the sample and the mixture was transferred into a glass chromatography column.

The non-polar components were eluted using 75 mL of hexane-diethylether (90:1). The 3-MCPD was subsequently eluted using 250 mL diethylether. The 3-MCPD extract was concentrated to 10 mL and 2 mL of this extract was evaporated to dryness using a gentle stream of nitrogen gas. 1 mL of 2,2,4-trimethylpentane was added to the dried extract and derivatisation was carried out.

Derivatisation

To a 1 mL solution of standard or extract was added 50 μL HFBI. The mixture was then heated at 70°C for 20 min. After cooling the mixture to room temperature, 1 mL of water was added. The mixture was then stirred on a Vortex stirrer for 30 s and the phases were allowed to separate; the step was repeated. The organic upper layer was subsequently extracted to new vial, dried over anhydrous Na_2SO_4, and injected into the GCMS (1 μL).

Gas chromatograph operating parameters

Column	:	DB-5, 30m × 0.25 mmID × 0.25 μm (J&W Scientific, USA)
Injector temperature	:	270ºC
Oven temperature	:	50ºC (1 min) → 90ºC at 2ºC/min → 270ºC at 40ºC/min
Carrier gas	:	Helium
Inlet pressure	:	100 kPa
Injection	:	Splitless, sampling time 0.6 min
Injection volume	:	1 μL

Mass spectrometer operating parameters

Ionisation mode	:	Electron Impact
Interface temperature	:	270ºC
Acquisition mode	:	Selected Ion Monitoring (SIM)
m/z	:	253, 275, 289, 291, 453
Ionisation mode	:	Negative Chemical Ionisation
Reagent gas	:	Isobutane
Reagent gas pressure	:	0.5 bar
Interface temperature	:	200°C
Acquisition mode	:	Full-scan and SIM
m/z range (Scan)	:	154 – 514 amu
m/z (SIM)	:	446, 482, 502 (3-MCPD-d_0) 449, 486, 507 (3-MCPD-d_5)

Results and Discussion

GC/EI-MS

The EI full-scan mass spectrum of the HFBI derivative of 3-MCPD-d_0 is shown in Figure 1. The ions used for SIM acquisition and quantitation, and the possible chemical formula for the ion species, are shown in Table 1.

Calibration and detection limit

The detection limit for the EI-SIM acquisition was estimated to be 2 ng/mL. This detection limit was calculated using the QAQC function of the GCMSsolution software; it is based on the residual standard deviation of the regression line ($s_{y/x}$) for the external standard calibration plot (Figure 2a) in the concentration range 5–50 ng/mL, with *m/z* 253 ion as the quantitation ion. The detection limit was defined as the concentration that give a signal, *S*, equal to

$$S = S_0 + 3\,s_{y/x} \qquad eq.(1)$$

where S_0 is the signal for zero concentration and is estimated by the *y*-intercept of the calibration plot.

The analysis also gave a good linearity in the concentration range 5–15000 ng/mL with a correlation coefficient (*r*) of 0.99 or better (Figure 2b).

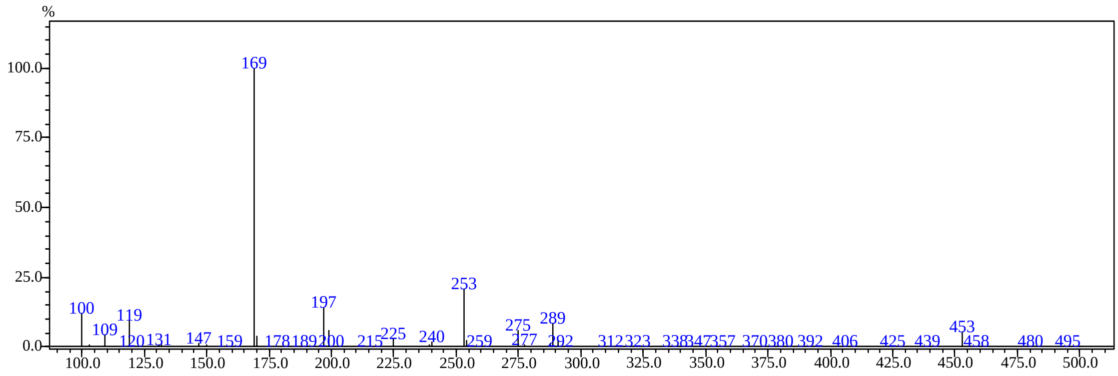


Figure 1. Full scan EI mass spectrum of HFBI derivative of 3-MCPD. (ref. 3MCPD6/003)

Table 1. Ions used for detection and quantitation of 3-MCPD (Parent compound: M = [$CH_2(COOCF_2CF_2CF_3)CH(COOCF_2CF_2CF_3)CH_2Cl$], M.W. = 502) and the possible chemical formula for the ion species.

Ion Species	***m/z***
$[M\text{-}CH_2\text{-}Cl^{35}]^+$	453
$[M\text{-}(COOCF_2CF_2CF_3)]^+$ ($Cl = Cl^{37}$)	291
$[M\text{-}(COOCF_2CF_2CF_3)]^+$ ($Cl = Cl^{35}$)	289
$[M\text{-}(COOCF_2CF_2CF_3)\text{-}CH_2]^+$ ($Cl = Cl^{35}$)	275
$[M\text{-}(COOCF_2CF_2CF_3)\text{-}HCl^{35}]^+$	253
$[CF_3CF_2CF_2]^+$	169

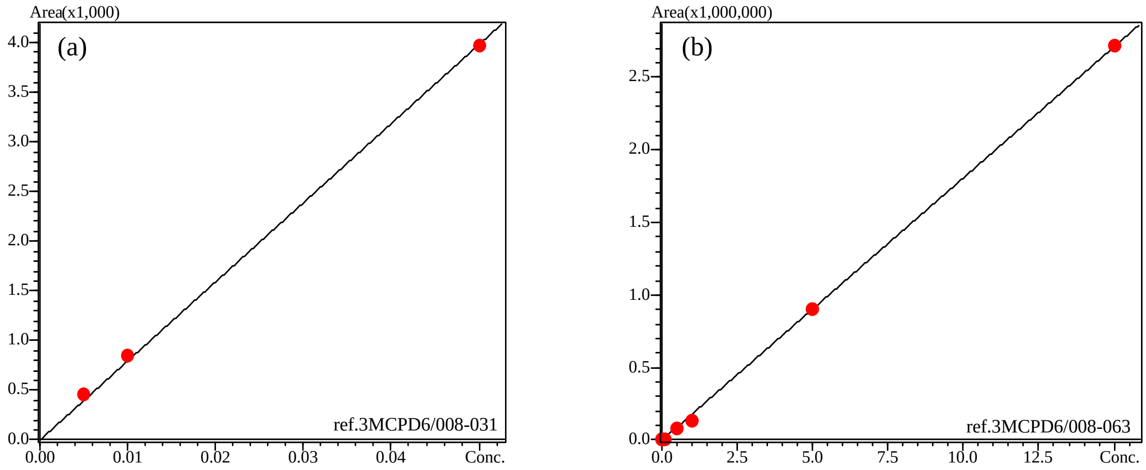


Figure 2. (a) External standard calibration plot of 3-MCPD in the concentration range 5–50 ng/mL (n=5). $y = 79775.81x$. $r^2 = 0.9999963$. r = 0.9999981. Mean RF : 84609.75. RF SD : 5,942.116 . RF %RSD : 7.022969 (ref. 3MCPD6/008-031).

(b) External standard calibration plot of 3-MCPD in the concentration range 5–15000 ng/mL. $y = 181066.2x$. $r^2 = 0.9997526$. r = 0.9998763. Mean RF : 124315.5. RF SD : 42,711.84. RF %RSD : 34.35762 (ref.3MCPD6/008-063).

Figure 3 shows the SIM mass chromatogram for the *m/z* 253 ion (5 ng/mL 3-MCPD-d_0). The signal-to-noise (S/N) ratio is >20, calculated using the GCMSsolution software. This ion has the highest intensity among the characteristic ions in the EI mass spectrum of 3-MCPD.

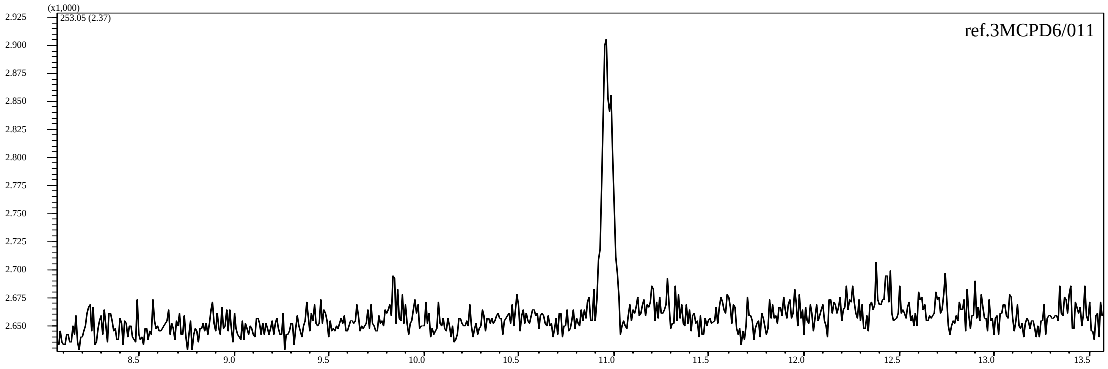


Figure 3. Mass chromatogram of *m/z* 253 ion (3-MCPD-d_0 derivative = 5 ng/mL) obtained by Electron impact Ionisation in SIM mode. (ref. 3MCPD6/011)

GC/NCI-MS

Isobutane NCI mass spectra & identification of 3-MCPD

The isobutane NCI full-scan mass spectra of the HFBI derivatives of 3-MCPD-d_0 and 3-MCPD-d_5 standards are shown Figures 4 and 5. The main ions observed and the possible chemical formula for the ion species, are shown in Table 2.

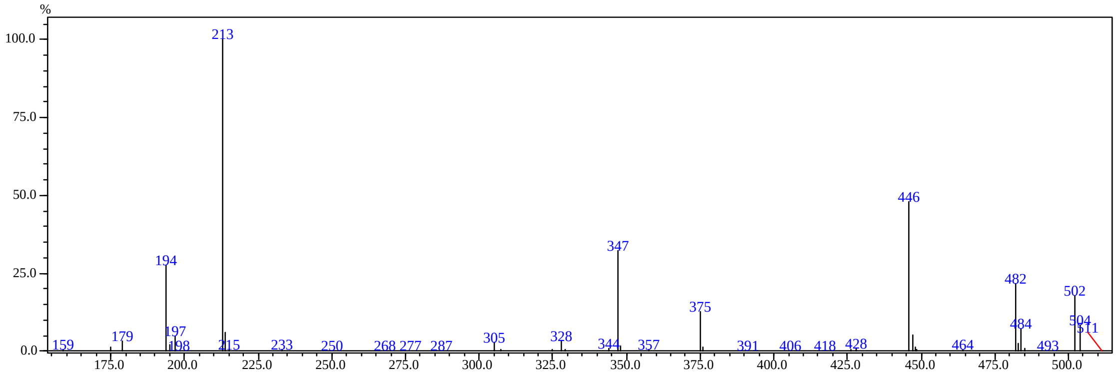


Figure 4. NCI isobutane mass spectrum of 3-MCPD-d_0 derivative, M = [$CH_2(COOCF_2CF_2CF_3)CH(COOCF_2CF_2CF_3)CH_2Cl$]. The region from *m/z* 220–514 is magnified 20x. (ref. 3MCPD7/138)

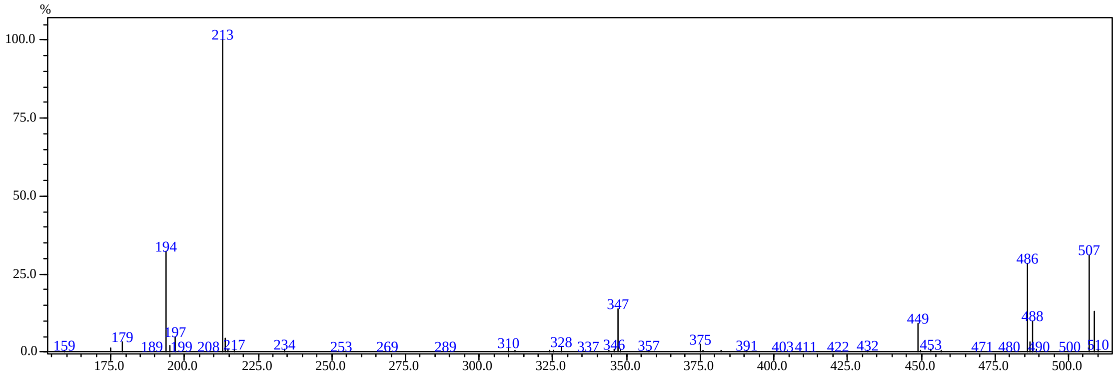


Figure 5. NCI isobutane mass spectrum of 3-MCPD-d_5 derivative, M' = [$CD_2(COOCF_2CF_2CF_3)$ CD(COOCF2CF2CF3)CD_2Cl], at interface temperature 200°C. The region from *m/z* 220–514 is magnified 20x. (ref. 3MCPD7/138)

Table 2. Ions observed in the NCI spectra of 3-MCPD-d_0 derivative (M = [$CH_2(COOCF_2CF_2CF_3)CH(COOCF_2CF_2CF_3)CH_2Cl$], M.W. = 502) and 3-MCPD-d_5 derivative (M' = [$CD_2(COOCF_2CF_2CF_3)CD(COOCF_2CF_2CF_3)CD_2Cl$], M.W. = 507).

3-MCPD-d_0			**3-MCPD-d_5**		
Ion Species	***m/z***	**Rel. Int.(%)**	**Ion Species**	***m/z***	**Rel. Int.(%)**
$[M]^-$ ($Cl = Cl^{35}$)	502	37	$[M']^-$ ($Cl = Cl^{35}$)	507	100
$[M]^-$ ($Cl = Cl^{37}$)	504	17	$[M']^-$ ($Cl = Cl^{37}$)	509	42
$[M-HF]^-$ ($Cl = Cl^{35}$)	482	45	$[M'-HF]^-$ ($Cl = Cl^{35}$)	486	91
$[M-HF]^-$ ($Cl = Cl^{37}$)	484	15	$[M'-HF]^-$ ($Cl = Cl^{37}$)	488	32
$[M-HF-HCl]^-$	446	100	$[M'-HF-HCl]^-$	449	29
$[CF_3CF_3CF_2COO]^-$	213	4181	$[CF_3CF_3CF_2COO]^-$	213	6405

3-MCPD (0.01 mg/kg) in a spiked soy sauce extract could be conveniently identified by using a private NCI mass spectral library. This library was created by recording the NCI mass spectra of the derivatives of 3-MCPD-d_0 and 3-MCPD-d_5 standards. A library search for the spiked sample conducted against this private library gave a Similarity Index value of 90 or better for 3-MCPD-d_0. Similar results were obtained for 3-MCPD-d_5.

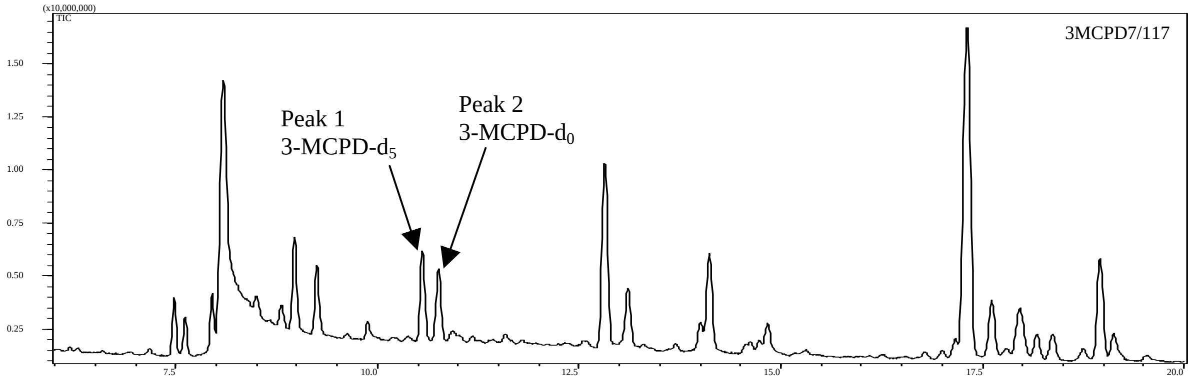


Figure 6. Chromatogram of soy sauce extract spiked with 3-MCPD-d_0 (0.01 mg/kg) and 3-MCPD-d_5 (0.05 mg/kg). (ref. 3MCPD7/117)

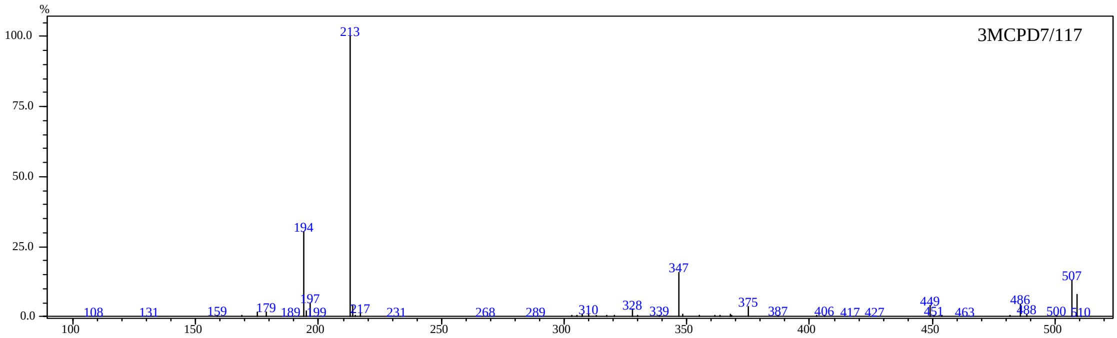


Figure 7. Mass spectrum of Peak 1, identified as 3-MCPD-d_5 derivative. (ref. 3MCPD7/117)

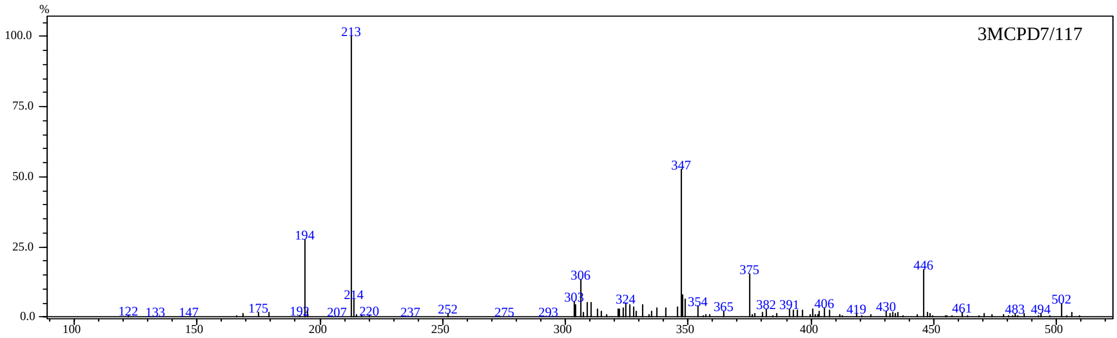


Figure 8. Mass spectrum of Peak 2, identified as 3-MCPD-d_0 derivative. (ref. 3MCPD7/117)

Calibration and detection limits

The detection limit for 3-MCPD-d_0 based on NCI-Scan mode of detection is estimated to be ~0.25 ng/mL when *m/z* 213 ion is used as the quantitation ion, and ~1 ng/mg when *m/z* 347 is used as the quantitation ion. The detection limits were calculated based on the residual standard deviation of the regression line ($s_{y/x}$) of the internal standard calibration plot in the concentration range 2–10 ng/mL (Figure 6). Correlation coefficients (r) of better than 0.99 were obtained in both cases.

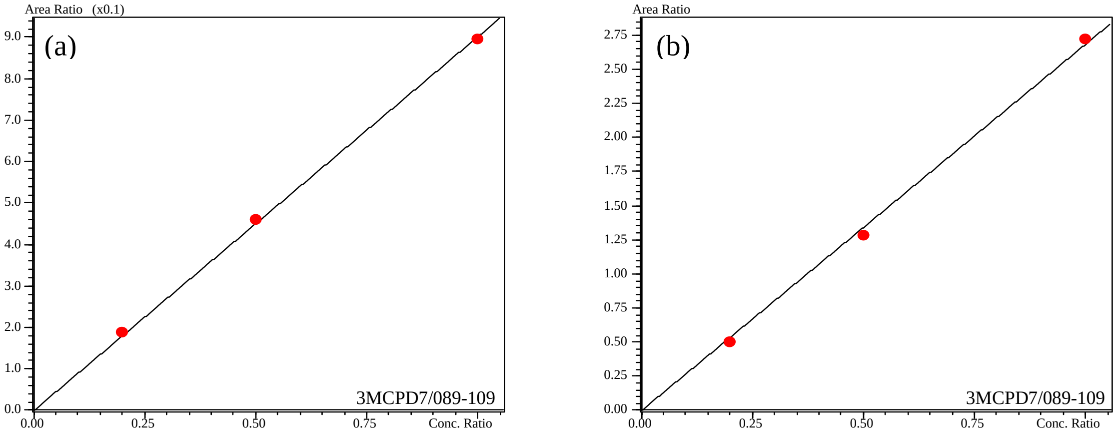


Figure 6. Internal standard calibration plot for 3-MCPD analysis by NCI-Scan in the concentration range of 2–10 ng/mL: (a) using *m/z* 213 ion as the quantitation ion. $y = 0.9023328x$. $r^2 = 0.9999215$. r = 0.9999607. Mean RF : 0.9193472. RF SD : 0.0268817. RF %RSD : 2.923998. (b) using *m/z* 347 ion as the quantitation ion. $y = 2.686379x$. $r^2 = 0.9991832$. r = 0.9995915. Mean RF : 2.600782 . RF SD : 0.3157530. RF %RSD : 12.14069. (ref. 3MCPD7/089-109)

When *m/z* 213 ion was used as the quantitation ion, the detection limit is about 10 times lower than that obtained using EI-SIM mode. However, this ion is not specific to 3-MCPD. Analysis of sample matrix revealed that there are other components of the sample contain *m/z* 213 ion. Quantitation based on *m/z* 213 ion may not be accurate if other components in the sample that have this ion elute at or near the elution time of 3-MCPD.

The detection limit is comparable to that obtained using EI-SIM mode, when *m/z* 347 ion was used. However, the *m/z* 347 ion is more specific to 3-MCPD, even though its intensity is not so high in the NCI mass spectra of 3-MCPD-d_0.

To achieve a better sensitivity and specificity of detection and quantitation, NCI-SIM mode was used. In this case, *m/z* 446 was used as the quantitation ion for 3-MCPD-d_0, and *m/z* 507 as the quantitation ion for 3-MCPD-d_5.

The calibration curve in the 2-10 ng/mL range, plotted by the method of least squares, shown in Figure 7, shows a good linearity with r > 0.99. The detection limit is estimated to be ~0.1 ng/mL.

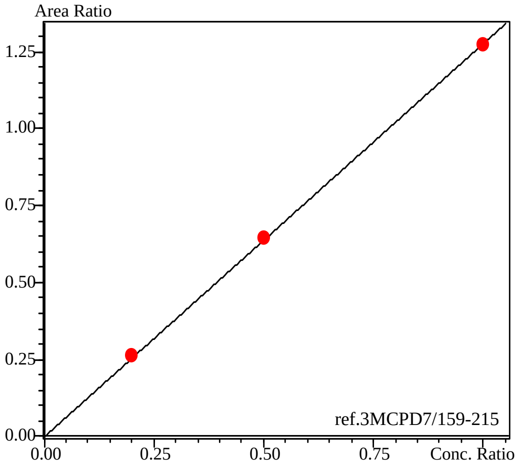


Figure 7. Internal standard calibration plot for 3-MCPD analysis by NCI-SIM in the concentration range of 2–10 ng/mL using *m/z* 446 as the quantitation ion. $y = 1.27777x$. $r^2 = 0.9999971$. r = 0.9999986. Mean RF : 1.304162. RF SD : 0.0390569. RF %RSD : 2.994791. (ref.3MCPD7/159-215)

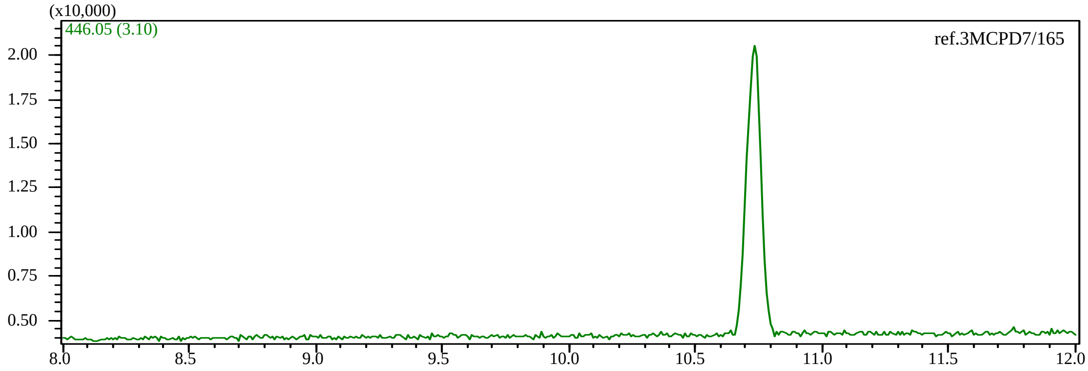


Figure 8. Mass chromatogram of *m/z* 446 ion (3-MCPD-d_0 = 5 ng/mL) obtained by Negative Chemical Ionisation in SIM acquisition mode. (ref.3MCPD7/165)

The mass chromatogram for *m/z* 446 ion (5 ng/mL), obtained in SIM acquisition mode, is shown in Figure 8.

The S/N ratio is calculated to be better than 150.

Quantitation

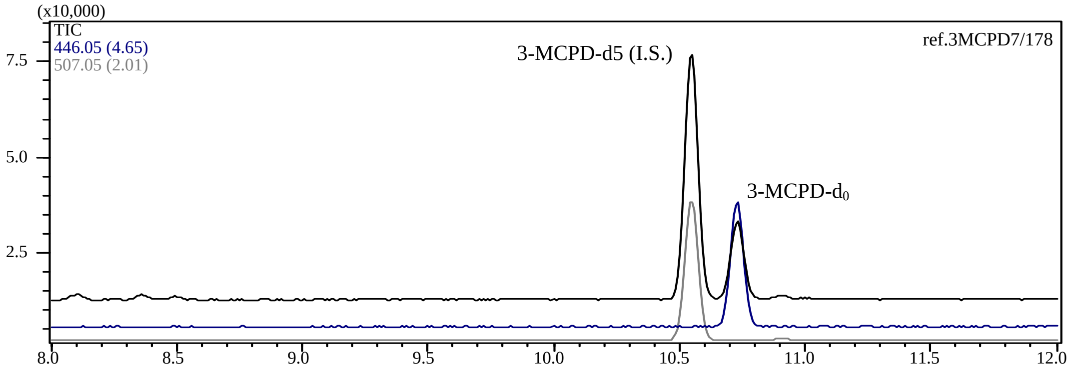


Figure 9. Chromatogram of sample spiked with 10 µg/kg of 3-MCPD-d_0 obtained in NCI-SIM mode. (ref. 3MCPD7/178)

Table 3. Quantitation of soy sauce samples each spiked with 0.01 mg/kg (10 ppb) 3-MCPD-d_0.

n	3-MCPD-d_0 spike amount calculated (ppb)	Recovery (%)	ref.
1	13.8	138	3MCPD7/174-5
2	15.0	150	3MCPD7/177-8
3	13.5	135	3MCPD7/180-1

CONCLUSION

Negative Chemical Ionisation Mass Spectrometry (NCI-MS) provides a more sensitive detection for 3-MCPD (as its HFBI derivative) than Electron impact Ionisation Mass Spectrometry (EI-MS). The NCI-MS (Scan) detection limit is estimated to be ~0.25 to 1 ng/mL, while the NCI-MS (SIM) detection limit is estimated to be ~0.1 ng/mL for 3-MCPD-d_0; compared to the EI-MS (SIM) detection limit of ~2 ng/mL.

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