

Analysis of 3-chloro-1,2-propanediol in soy sauce (hydrolysed vegetable protein – HVP)

Novalina Lingga, Ph.D. Customer Support Centre, Shimadzu (Asia Pacific), Pte. Ltd.

Hydrolysed Vegetable Protein (HVP) is widely used as a savoury flavour ingredients in processed food products such as soy sauce, oyster sauce, soups, etc. HVP is commonly produced by hydrolysis of vegetable proteins using hydrochloric acid.

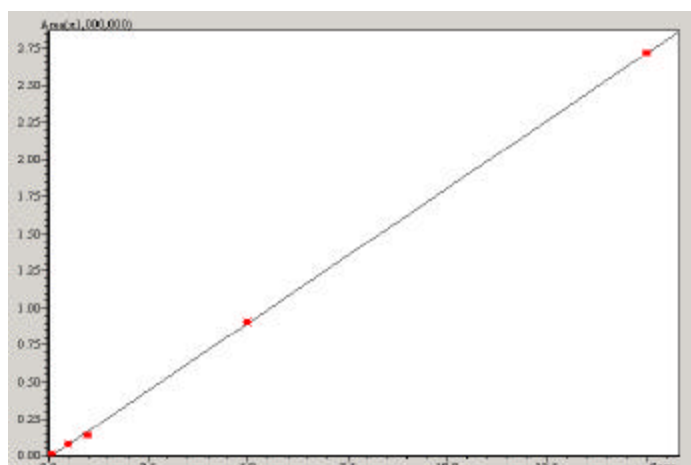
Around 1980, studies¹ demonstrated the presence of several chloropropanols in HVP that was obtained by acid hydrolysis. The chloropropanols may have resulted from the reaction between the lipid components of the protein source and hydrochloric acid.

One of the most important of the chloropropanols is 3-chloropropane-1,2-diol (3-MCPD). 3-MCPD is known to cause cancer in animals and the EC Scientific Committee for Food concluded that “3-MCPD should be regarded as a genotoxic carcinogen”.²

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. This regulation is to come into force in April 2002.²

The limit of detection of 3-MCPD in HVP based on GC/ECD analysis was 0.05 – 0.1 mg/kg.³ GCMS proves to give a more sensitive and specific detection in the Selected Ion Monitoring (SIM) mode, giving a limit of quantitation of 0.01 mg/kg.⁴

Presented here is the analysis of 3MCPD in soy sauce using N(heptafluorobutyryl)imidazole (HFBI) derivatisation and a Shimadzu QP5050A gas chromatograph-mass spectrometer (GCMS), in the SIM mode.



Y = 182042.1X - 11318.13
R² = 0.9997526
R = 0.9998763

External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None

Mean RF : 123354.1
RF SD : 42,621.43
RF %RSD : 34.55209

Figure 1. Calibration curve for HFBI-derivative of 3-MCPD in the concentration range of 0.005 – 15 µg/mL. Correlation coefficient (r) = 0.9999.

Figure 1 shows the calibration curve for HFBI-derivative of 3-MCPD in the concentration range 0.005 – 15 µg/mL, obtained by external standard calibration method using n= 5 for each level. A correlation coefficient (r) of 0.9999 was obtained.

A limit of detection of 0.002 µg/mL was obtained from a calibration plot obtained in the concentration range of 0.005 – 0.1 µg/mL, having r = 0.9999. This limit of detection was defined as the concentration that corresponds to a signal derived by multiplying the residual standard deviation of the regression line by a coefficient of 3.

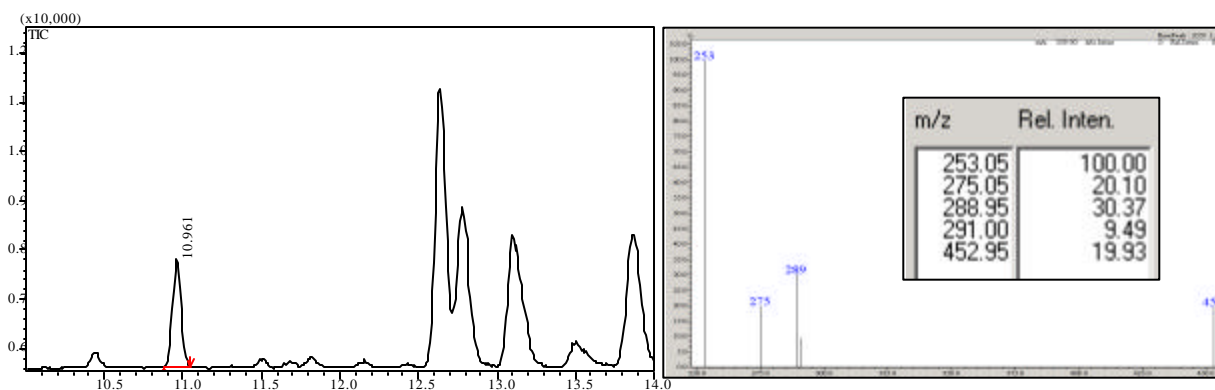


Figure 2. Total Ion Chromatogram (TIC) and SIM mass spectrum of derivatised 3-MCPD in liquid HVP sample containing 0.05 mg/kg 3-MCPD (retention time at 10.961 min). Column: DB5ms, 30m×0.25mm, 0.25µm), carrier gas pressure 100kPa Helium, oven temperature program: 50°C (1 min) to 90°C at 2°C/min, then to 270°C (for 10.5 min) at 40°C/min..

Figure 2 shows the total ion chromatogram (TIC) and the SIM mass spectrum of HFBI derivative of 3 MCPD in soy sauce sample spiked with 0.05 mg/kg 3-MCPD.

The identity of the HFBI-derivative of 3MCPD in the sample was confirmed by comparing its retention time and mass spectrum to those of a standard. In this case, the library search (Similarity Search) function was used to determine the degree of similarity between the relative intensities of the characteristic ions in the sample spectrum and those in the standard spectrum.

A user-defined library was created that contains the scan, as well as the SIM, mass spectra of the HFBI-derivative of 3-MCPD standard.

The sample spectrum shows a high degree of similarity to the scan mass spectrum of the HFBI-derivatised 3MCPD (similarity index (SI) = 87) as well as to the SIM mass spectra of the standard.

Experimental

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. The sample was sonicated to achieve homogeneity and was subsequently mixed thoroughly with the contents of an Extrelut NT 20 column. The mixture was then transferred into a 40 cm × 2 cm glass chromatography column. The non-polar components were eluted with a 90:10 mixture of hexane-diethylether, and the 3-MCPD was eluted with diethylether. The 3-MCPD extract was concentrated using rotary evaporation at 35°C to a small volume. 2 mL of this extract was evaporated to dryness and re-dissolved in 1 mL of 2,2,4-trimethylpentane. The 3-MCPD was subsequently derivatised using N-(heptafluorobutryl)imidazole.

1 µL of the derivatised sample was then injected into the GCMS..

Instrument Parameters

Gas Chromatograph

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

Mass Spectrometer

Ionization	: Electron Impact
Acquisition mode	: Selected Ion Monitoring (SIM)
m/z	: 253, 275, 289, 291, 453

Conclusion

GCMS in the Selected Ion Monitoring mode, combined with the validated method of sample extraction and derivatisation using N-(heptafluorobutryl)imidazole, provide a sensitive measurement of 3-MCPD in soy sauce.

Reference:

1. Velisek et al., *J. Agric. Food Chem.*, 28 (1980) 1142–1144.
2. Food Surveillance Information Sheet No. 14/01, Food Standards Agency, UK.
3. P.D. Collier *et al.*, *J. Chromatog.*, 589 (1992) 109–119.
4. Brereton, P. et al., *J. AOAC Int.*, 84 (2001) 455–465.