Analysis of Hydrolysed Vegetable Protein for Chloropropandiols Using Selected Ion Storage

GC/MS

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Discussion

Hydrolysed Vegetable Protein (HVP) is a long standing food ingredient used to flavor a variety of savory foods such as soups, gravy mixes, and bouillon cubes. In Europe, HVP is more usually traded as a liquid (40% solids content), particularly in Germany where it is used directly as a traditional sauce/seasoning, whereas in the UK the dry powder variant is mainly used for food processing.
In the 1980's, it was realized that contaminants known as chloropropanols (mono and di) may result from the interaction of hydrochloric acid (used during hydrolysis) with lipid molecules associated with the protein source. One of the most important chloropropanols. and the subject of extensive toxicological testing, is 3-monochloropropandiol (3-MCPD). Analysis of 3-MCPD is currently based upon capillary GC of a heptafluorobutyrate derivative with electron capture detection (ECD) at an overall limit of detection of about 1.5 mg/kg, depending on the sample type (dry weight basis). A very complex matrix and the insufficient specificity of the detector employed have made identification at lower levels impractical. With levels of 3-MCPD in samples now at or below current detection limits, a more sensitive and specific method of analysis was sought.

Selected Ion Storage (SIS) was used to increase the detection (150 fold) and provide positive identification of 3-MCPD. One of the features of this mode of analysis is full scan acquisition while eliminating the known matrix ions that would otherwise corrupt the spectrum. In HVP samples, a coeluting compound has an intense ion at m/z 271 that cannot be removed by background subtraction. Figures 1A shows the full scan spectrum of 3-MCPD from a typical sample of hydrolysed vegetable protein. In Figure 1B Selected Ion Storage is used to acquire full scan data while eliminating the large background ion at m/z 271.

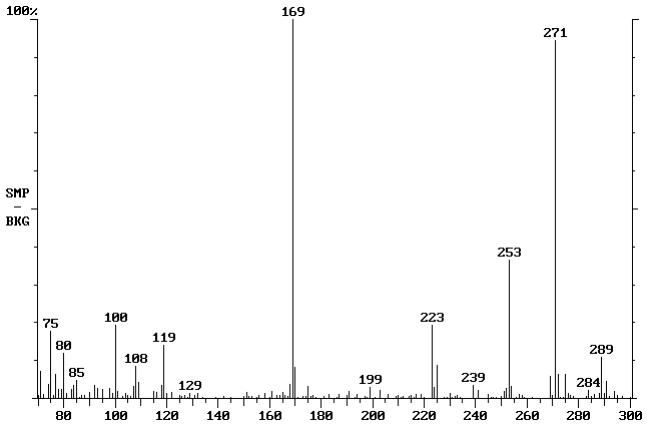

Figure 1A: Full scan spectrum of 3-MCPD coeluting with a compound containing the ion at m/z 271.

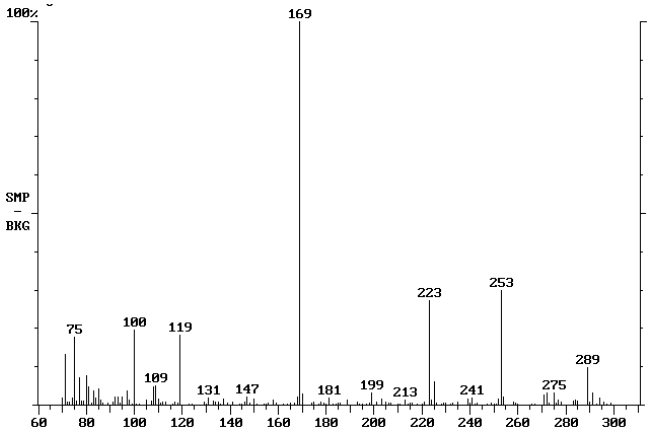

Figure 1B: Spectrum of 3-MCPD using Selected Ion Storage to eliminate the ion at m/z 271.

Figure 2 shows the mass chromatogram obtained for a sample contaminated with 0.03mg/kg of 3-MCPD using Selected Ion Storage. Table 1 shows the recovery data collected from 6 spikes of a typical sample. The limit of detection is about 0.01 mg/kg based on a spike of 0.05 mg/kg. Recoveries of 84-110% were obtained, based on 6 separate spikes at the 0.05 mg/kg level. All quantitation was based on internal standard analysis using p-dichlorobenzene as the internal standard.

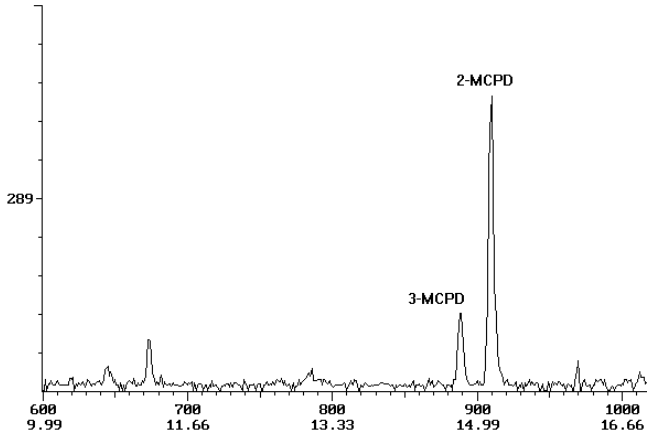

Figure 2: Mass chromatogram m/z 189 of an HVP sample contaminated with 0.03mg/kg of 3-MCPD using Selected Ion Storage (mass range 70-300).

Experimental

HVP, 8g, was weighed into a 100mL beaker. The sample was stirred with 5M sodium chloride (12g) then mixed thoroughly with the contents of an 'Extralut 20' sachet for 5 minutes before transferring to a chromatography column (20mm x 600mm with PTFE stopcock). The sample was eluted with hexane/ether 90:10 (80mL discarded) and diethyl ether (250mL collected). The ether fraction was dried over anhydrous sodium sulphate and reduced to 5mL by rotary evaporation. The sample volume was raised to 10mL for derivatisation. 1mL of the sample was blown to dryness followed by addition of 1mL of internal standard solution (0.200ug/mL p-dichlorobenzene in hexane) and 50µL of heptafluorobutyrylimidazole (HFBI). The sample vial was capped and incubated at 70°C for 30 minutes. The sample was then cooled, and 2mL of deionized water was added. The hexane layer was removed and dried over anhydrous sodium sulphate. Samples are stable for 1 week in the deep freeze.

Instrument Conditions

Gas Chromatograph

Column: BPX5 25M X 0.2mm X 0.25µm
Flow Rate: 1 mL/min.
Oven Program: 50°C and hold for 2 min., then 2°C/min to 90°C, followed by 25°C/min to 280°C and hold for 5 min. Injector: 1077 in Splitless mode
Relay: closed at injection and opened 0.75 min.
Transfer Line: 250°C
Injection volume: 2µL

Mass Spectrometer

Mass Range: 70-300u
Sec/scan: 1
Multiplier Delay: 10 min.
Threshold: 0
Ion Trap Temperature: 190°C
Mode: Selected Ion Storage (70-300u with 271 ejected)

Table 1: Recovery data from samples spiked with 3-MCPD.

Sample	Spike (mg/kg)	Recovered (mg/kg)
1	0.05	0.042
2	0.05	0.052
3	0.05	0.050
4	0.05	0.055
5	0.05	0.042
6	0.05	0.051

Conclusion

Selected Ion Storage GC/MS allows measurements of 3-MCPD in hydrolysed vegetable protein at 0.01 mg/kg. This is about 150 times lower than the existing procedure with electron capture detection. Recoveries range from 84-110% with spiked samples using this procedure. Spectral confirmation is also possible with the wide range of ions collected.

These data represent typical results.
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