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## Short Communication

# Determination of 3-chloropropane-1,2-diol in liquid hydrolysed vegetable proteins by capillary gas chromatography with flame ionization detection

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ABSTRACT

An improved method for determination of 3-chloropropane-1,2-diol in liquid hydrolysed vegetable proteins using capillary gas chromatography with flame ionization detection is presented. A phenylboronic acid derivative was prepared and extracted with hexane. The identity of the derivative was confirmed by gas chromatography–mass spectrometry. The method showed a standard deviation of 0.019 ppm and a repeatability of 0.05 ppm at a level of 0.84 ppm. The limit of detection was 0.2 ppm.

## INTRODUCTION

3-Chloropropane-1,2-diol (3-MCPD) is a contaminant which can be formed in the manufacture of protein hydrolysates. A routine gas chromatographic (GC) determination of 3-MCPD in such liquid hydrolysed vegetable proteins (HVPs) has not yet been published. As monochloropropanediols often show unsatisfactory chromatographic properties, they are frequently derivatizatized for analysis. Schurig and Wistuba [1] derivatized 3-MCPD in non-aqueous media using *n*-butylboronic acid. Pesselman and Feit [2] showed that quantitative measurements of the diol in standard aqueous solutions is possible, using such a derivatization combined with a hexane extraction. Rodman and Ross [3] described the preparation of a phenylboronic acid derivative of 3-MCPD in a non-aqueous medium and characterized the derivative by Fourier transform infrared spectrometry and its mass spectrum. The phenylboronic acid derivatization and solvent extraction were further explored to develop an analysis suitable for routine purposes in liquid HVPs.

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## EXPERIMENTAL

### *Apparatus*

For GC–flame ionization detection (FID) analyses a Packard Model 439 gas chromatograph equipped with a split injection port and a flame ionization detector was used. For GC–mass spectrometric (MS) identification of the 3-MCPD-phenylboronic acid derivative, a Hewlett-Packard Model 5890A gas chromatograph with a split injection port and a Model 5970B mass-selective detector was used. For both GC–FID and GC–MS analyses the chromatographic separation was carried out using a 50 m × 0.32 mm I.D. CP-SIL 5 CB fused-silica column with 0.12-μm film (Chrompack). The GC operating conditions were as follows: carrier gas, helium; head pressure, 150 kPa; detector make-up gas, nitrogen at 30 ml/min; split flow, 10 ml/min. The oven temperature program was: initial temperature 40°C for 1 min, oven temperature rise 1 = 7.5°C/min for 19 min, oven temperature rise 2 = 39.99°C/min at 20 min for 2.45 min, oven final temperature 280°C for 13 min. The injector and detector temperatures were 200 and 280°C, respectively.

### *Reagents*

3-MCPD and phenylboronic acid were obtained from Aldrich Chemie, acetone (pro analisi grade) from J. T. Baker and *n*-hexane (pro analisi grade), sodium chloride (pro analisi grade) and *n*-heptadecane from E. Merck.

### *Solutions*

The internal standard solution contained 0.01 mg/ml *n*-heptadecane in hexane. The derivatization solution contained 250 mg/ml phenylboronic acid and was prepared by dissolving 5 g phenylboronic acid in 19 ml acetone plus 1 ml distilled water. The sodium chloride solution contained 200 g/l sodium chloride. All solutions except the sodium chloride solution were kept chilled (5°C).

## PROCEDURE

### *Sample preparation*

Into an 100-ml volumetric flask a 75.0-g sample was weighed and diluted with 20% sodium chloride solution.

### *Preparation of standards*

A stock solution of 3-MCPD was prepared by weighing 10.0 mg 3-MCPD into a 100-ml volumetric flask and filling up to volume with 20% sodium chloride solution; 10 ml of the prepared stock solution were diluted to 100 ml with 20% sodium chloride solution (solution 1). Solution 1 contained 10. μg/ml 3-MCPD, which, taking into account the predilution of samples, corresponds to 13.3 ppm 3-MCPD in samples. Calibration solutions corresponding to 0.53, 1.33 and 5.3 ppm 3-MCPD in samples were prepared by dilution of solution 1 using 20% sodium chloride solution.

### *Derivatization and extraction*

Volumes of 5.0 ml of each of the calibration and sample solutions were pipetted into sample vials. After addition of 1.0 ml of derivatization solution, the vials were

sealed with vial caps and left for 20 min at 90°C. After cooling to room temperature, the vial caps were removed, 3.0 ml of internal standard solution in hexane were added and the vials were sealed with a new cap. The vials were shaken for 30 s using a vortex and, after separation of the organic and water layers, 2 μl of the hexane layer were injected.

## RESULTS AND DISCUSSION

Confirmation and identification of the penylboronic acid derivative of 3-MCPD in a sample chromatogram was achieved by matching its mass spectrum with a literature spectrum [3] and by comparing the retention time of the suspected peak with that of the derivatized pure 3-MCPD. The phenylboronic acid derivative of 2-MCPD was identified by interpretation of its mass fragmentation pattern only. The peak gave ions at *m/z* 196 and 104 suggestive of the proposed structure. Using the described chromatographic conditions, the phenylboronic acid derivative of 3-MCPD has a Kovats retention index of 1413 and that of the derivative of 2-MCPD is 1426.

Two different extraction solvents (*n*-hexane and toluene) were evaluated for their influence on the recovery of 3-MCP-phenylboronate in HVP samples. An internal standard (*n*-heptadecane) was added to the extraction solvent to compensate for possible variations in the injection volume. A sample spiked with 21 ppm 3-MCPD was extracted up to six times after derivatization, and each time the 3-MCPD content in the extraction solvent was measured. Toluene proved to have the best recovery: 84% in the first, 13% in the second and 3% in the third extraction step. *n*-Hexane had a lower recovery: about 45% in the first, 27% in the second and 15% in the third step. However, although toluene had the best recovery, its extract produced more peaks with reduced resolution in the chromatogram, which hindered accurate integration and reliable quantification at levels around 1 ppm 3-MCPD. The hexane

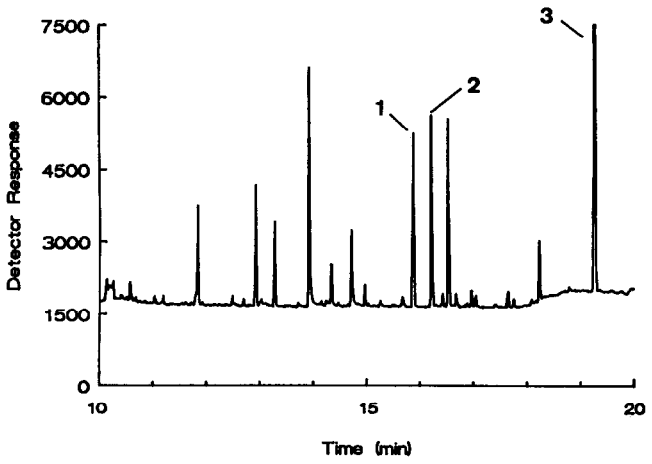


Fig. 1. Chromatogram of MCPD-phenylboronic acid derivatives in a liquid HVP sample containing 5.2 ppm 3-MCPD. 1 = 3-MCPD-phenylboronate; 2 = 2-MCPD-phenylboronate; 3 = *n*-heptadecane.

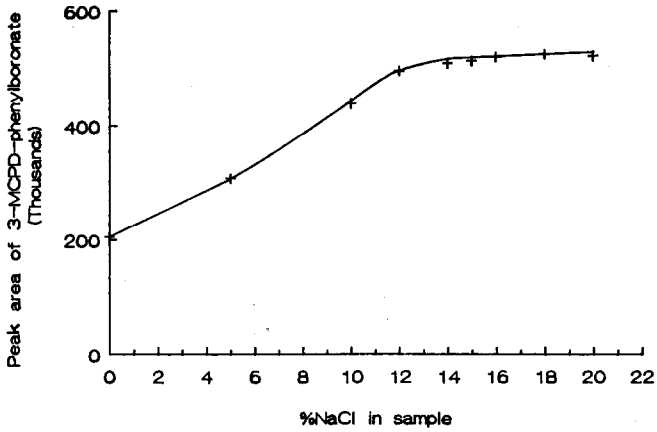


Fig. 2. Influence of the sodium chloride concentration in a sample on the extraction of the 3-MCPD-phenylboronic acid derivative with hexane.

extraction produced a rather clean chromatogram without interfering peaks (Fig. 1). Therefore hexane was selected as the extraction solvent.

The salt concentrations in both test sample and reference sample proved to be quite relevant as there is a clear desalting effect observable in the hexane extraction. A salt concentration in the range 12–20% has been found to be essential. At increasing salt concentrations, the recovery of the phenylboronic derivative of 3-MCPD also increases, reaching a constant level at 12% (Fig. 2). Because most of the samples contain between 13 and 14% salt, dilution of these samples with a 20% sodium chloride solution results in a salt concentration which is always higher then 12%.

The effect of shaking time on extraction was determined. A shaking time of 30 s using a vortex proved to be sufficient.

Linear regression analyses performed on the calibration curve generated when analysing calibration solutions under the described chromatographic conditions revealed excellent linearity (correlation coefficient 0.9999) over the range tested (0.53–13.7 ppm). For sample analyses the standard deviation obtained for 3-MCPD was 0.019 ppm and the repeatability (the maximum absolute difference between two test results, with a probability of 95%, obtained under the same test conditions) was 0.05 ppm, both assessed by eight replicate analyses of a sample containing 0.84 ppm 3-MCPD. The detection limit for the method was 0.2 ppm 3-MCPD.

In conclusion, this proposed method is rapid, sensitive and precise, suited for fast routine determination of 3-MCPD in HVPs.

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