Journal of Chromatography, 638 (1993) 71–74
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 971

Determination of 3-chloropropane-1,2-diol in hydrolyzed vegetable proteins by capillary gas chromatography with electrolytic conductivity detection

G. Spyres

A.E. Staley Manufacturing Co., 2200 E. Eldorado, Decatur, IL 62525 (USA)

(First received October 8th, 1992; revised manuscript received February 10th, 1993)

ABSTRACT

The determination of 3-chloropropane-1,2-diol in hydrolyzed vegetable protein using capillary gas chromatography is improved through the specificity of an electrolytic conductivity detector operated in the halogen mode. The hydrolysate is absorbed onto a Extrelut QE column of kieselguhr, 3-chloropropane-1,2-diol is partitioned into ethyl acetate and is quantitatively measured by gas chromatography using 1-chlorotetradecane as the internal standard. A concentration of 1 mg/kg dry substance is easily determined.

INTRODUCTION

Hydrolyzed vegetable protein (HVP) is commonly manufactured by the hydrolysis of vegetable protein with hydrochloric acid. The protein sources typically contain residual lipids. Without proper control the potential exists for a reaction between triglycerides and hydrochloric acid to produce 3-chloropropane-1,2-diol (3-MCPD) and 2-chloropropane-1,3-diol (2-MCPD).

Pesselman and Feit [1] used gas chromatography with electron-capture detection to quantitatively measure 3-MCPD in a standard aqueous solution after derivatization with *n*-butylboronic acid and extraction into hexane. Rodman and Ross [2] utilized phenylboronic acid to derivatize 3-MCPD in a non-aqueous media for subsequent determination by gas chromatography.

A method to determine 3-MCPD in HVP by gas chromatography using phenylboronic acid derivatization and solvent extraction was reported by Planting *et al.* [3]. Van Bergen *et al.* [4] recently reported a procedure to determine chloropropanols in protein hydrolysates based on gas chromatography of heptafluorobutyrate derivatives. Limitations of derivatization/extraction procedures for measuring 3-MCPD include a potential for incomplete derivatization, inefficient partitioning of the derivative from the aqueous phase into the organic phase, and short term stability of the derivatives.

This paper reports a method to quantitatively determine 3-MCPD in HVP. The liquid hydrolysate is absorbed on a kieselguhr column, 3-MCPD is partitioned from the column into ethyl acetate and selectively measured by gas chromatography using electrolytic conductivity detection.

EXPERIMENTAL

Apparatus

Chromatographic measurements were performed with a Tracor Model 560 gas chromatograph equipped with a packed-column injector and a Hall 700A electrolytic conductivity detector. The packed-column inlet was fitted with a

0021-9673/93/$06.00

SGE on-column adapter to allow direct injection onto a 60 m × 0.75 mm I.D. borosilicate glass Supelcowax 10 column with 1-μm film. The column was fitted with a 50-cm retention gap of 0.53 mm I.D. deactivated fused silica. Helium was used as the carrier gas at a flow-rate of 8 ml/min. The column temperature was 170°C for 5 min, then raised at 5°/min to 250°C and held for 10 min. The injector temperature was 225°C. The electrolytic conductivity detector was operated in the halogen mode.

Hydrogen was used as the reactant gas at a flow-rate of 30 ml/min and 1-propanol was the solvent at a flow through the cell of 0.5 ml/min. Reactor temperature was 900°C with a base temperature of 275°C. Contamination of the reaction tube was minimized by venting flow from the column at all times except for the time during which compounds of interest elute.

Confirmation of peaks observed for 3-MCPD and 2-MCPD was obtained with a Hewlett-Packard Model 5890 II gas chromatograph and a Model 5970B mass-selective detector.

Reagents

3-MCPD was obtained from Eastman Kodak (Rochester, NY, USA), 1-chlorotetradecane was obtained from Aldrich (Milwaukee, WI, USA) and ethyl acetate was Burdick & Jackson capillary grade from Baxter Scientific products (McGraw Park, IL, USA).

Solutions

A stock solution of 3-MCPD was prepared by weighing 100.0 mg into a 100-ml volumetric flask and diluting to volume with ethyl acetate. This solution was diluted 5 ml/100 ml with ethyl acetate to yield a solution containing 50 μg/ml. The stock solution of 1-chlorotetradecane was prepared by weighing 250 mg into a 50-ml volumetric flask and diluting to volume with ethyl acetate. This solution was diluted 5 ml/100 ml with ethyl acetate to provide a solution containing 250 μg/ml.

Procedure

The protein hydrolysate was adjusted as needed with 20% aqueous sodium chloride to obtain a solids content of 36%. A 20-g aliquot was weighed directly into a 20-ml Extrelut column (EM Science, Gibbstown, NJ, USA) and allowed to equilibrate for 15 min. The column was eluted with 150 ml ethyl acetate collecting the eluent in a 250-ml short-neck round-bottom flask with a 24/40 joint. The eluent was concentrated to a volume of approximately 3 ml using a rotary evaporator at 50°C. After the addition of 0.5 ml internal standard solution (250 μg/ml) the eluent was transferred to a 4-dram (1 dram = 3.697 ml) screw cap vial, diluted to a volume of approximately 5 ml, and 1 μl was injected into the gas chromatograph.

Preparation of standards

A standard containing 3-MCPD at 4 μg/ml and internal standard at 30 μg/ml was prepared by pipetting 2.0 ml dilute 3-MCPD solution and 3.0 ml dilute internal standard solution into a 25-ml volumetric flask and diluting to volume with ethyl acetate. A second standard containing

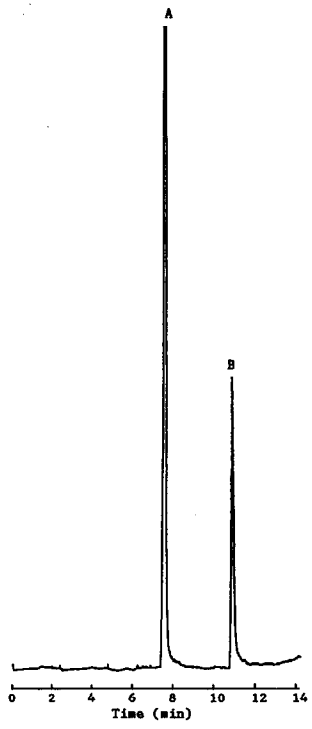

Fig. 1. Chromatogram of standard containing 4 μg/ml of 3-MCPD and 30 μg/ml internal standard. Peaks: A = 1-chlorotetradecane; B = 3-MCPD.

3-MCPD at 20 μg/ml and internal standard at 30 μg/ml was prepared by pipetting 10.0 ml dilute 3-MCPD solution and 3.0 ml dilute internal standard solution into a 25-ml volumetric flask and diluting to volume with ethyl acetate. Calibration was achieved by injecting 1 μl of each standard.

Response factors in reference to the internal standard were calculated for each standard. The amount of 3-MCPD in concentrated sample extracts was calculated using the response factor from a standard exhibiting similar peak areas as the sample.

RESULTS AND DISCUSSION

A spiked protein hydrolysate was used to measure the efficiency for partitioning 3-MCPD from the Extrelut column into diethyl ether, pentane–diethyl ether (80:20) and ethyl acetate.

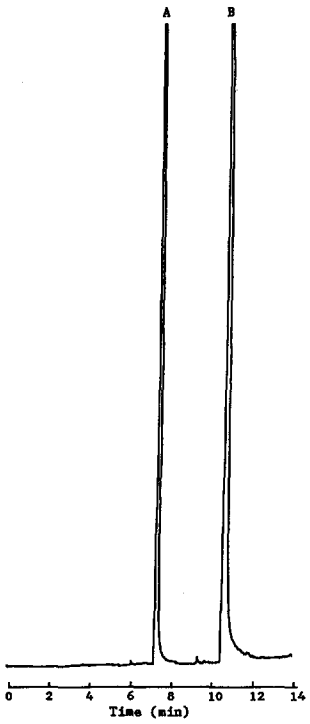

Fig. 2. Chromatogram of standard containing 20 μg/ml of 3-MCPD and 30 μg/ml internal standard. Peaks: A = 1-chlorotetradecane; B = 3-MCPD.

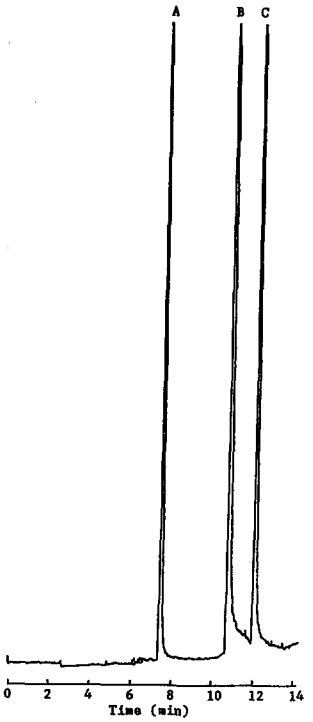

Fig. 3. Chromatogram of concentrated ethyl acetate extract from a sample of HVP. Peaks: A = 1-chlorotetradecane; B = 3-MCPD; C = 2-MCPD.

Highest efficiency was observed with ethyl acetate.

Chromatograms shown in Figs. 1 and 2 are standards containing 3-MCPD at 4 μg/ml and 20 μg/ml with internal standard at 30 μg/ml. The sample chromatogram shown in Fig. 3 exhibits peaks for 3-MCPD and the isomer 2-MCPD.

Standards with 3-MCPD levels between 4 and 2400 μg/ml were chromatographed to determine

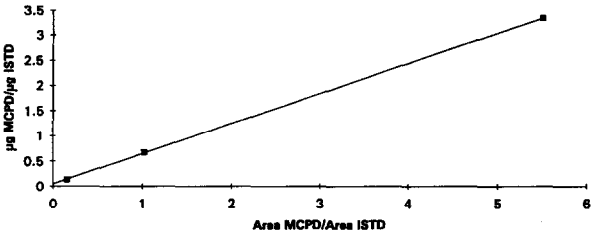

Fig. 4. Plot of the ratio of micrograms 3-MCPD/1-tetrachlorodecane against the ratio of peak area for 3-MCPD/1-tetrachlorodecane.

TABLE I

RECOVERY OF 3-MCPD FROM A SPIKED PROTEIN HYDROLYSATE

Spike level (μg/g)	Found ($x \pm$ S.D.)	Recovery (%)
0	0.82 ± 0.096	–
1	1.80 ± 0.082	98
5	5.65 ± 0.208	96.6

linearity. A plot was made for the ratio of 3-MCPD peak area/internal standard peak area and the ratio for micrograms 3-MCPD/micrograms internal standard. Linearity was observed for the 3-MCPD concentration range of 0 to 100 μg/ml as shown in Fig. 4. This concentration range was satisfactory for the analysis of samples containing 3-MCPD between 0 and 30 mg/kg. When analyzing samples in excess of 30 mg/kg the extracts were diluted to attain 3-MCPD concentrations within the linear range. An appropriate aliquot of internal standard was added to obtain a concentration of 20–30 μg/ml.

Table I shows that recoveries obtained with this method for a protein hydrolysate fortified with 3-MCPD at levels of 1 and 5 mg/kg were greater than 95% when corrected for concentrations in the unspiked sample. The detection limit for the method was 0.25 mg/kg sample.

In conclusion, this method is sensitive and precise for routine determination of 3-MCPD in HVP. The halogen-specific detector provides sample chromatograms free of interfering peaks and allows trace level determinations.

REFERENCES

1 R.L. Pesselman and M.J. Feit, *J. Chromatogr.*, 439 (1988) 448–452.
2 L.E. Rodman and R.D. Ross, *J. Chromatogr.*, 369 (1986) 97–103.
3 W.J. Planting, W.G. van Toorn, and G.H.D. van der Stegen, *J. Chromatogr.*, 555 (1991) 311–314.
4 C.A. van Bergen, P.D. Collier, D.D.O. Cromie, R.A. Lucas, H.D. Preston and D.J. Sissons, *J. Chromatogr.*, 589 (1992) 109–119.