Bull. Environ. Contam. Toxicol. (1984) 32: 310–315

Bulletin of Environmental Contamination and Toxicology

Quantification of Carbaryl in Pineapples by HPLC and GCMS-CI-NH_3

Thomas Cairns, Emil G. Siegmund, Gregory M. Doose, William S. Langham, and Kin S. Chiu

Department of Health and Human Services, Food and Drug Administration, Office of the Executive Director of Regional Operations, 1521 West Pico Boulevard, Los Angeles, CA 90015

INTRODUCTION

Pineapples imported into the USA from Mexico have been analyzed by this laboratory and found to consistently contain carbaryl [1-naphthyl-N-methylcarbamate], a cholinesterase inhibiting pesticide, at low ppm levels. The analysis and sample preparation are conducted according to current federal regulations (Code of Federal Regulations 1982). The only part of the pineapple discarded before analysis is the crown and leaves. An analytically supportable determination of this residue is necessary to permit consideration for compliance action, i.e. based on unregistered use.

To provide acceptable analytical data for trace levels (Cairns & Rogers 1983), the analytical chemist must respond by developing methodology to satisfy the immediate need. Usually, no official methods exist, or, if they do, the limit of detection is frequently insufficient. Additionally, analyses by two independent methods are normally required to confirm the quantitative data presented as being reliable.

This paper describes the quantitation of carbaryl in pineapples by two techniques, high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GCMS) using ammonia chemical ionization and single ion monitoring (SIM).

METHODS AND MATERIALS

All spectra were obtained on a Finnigan 3300 quadrupole mass spectrometer equipped with a CI source and INCOS data system; operating conditions: 45 cm x 2 mm i.d. glass column packed with 2% DEGS on 80/100 mesh Chromosorb W; carrier gas, 25 mL methane/min, column inlet 250°C, column temperatures 180°C, isothermal; electron energy 150 eV and a source pressure of 0.8 Torr (adjusted to maximize the intensity of the $[NH_4]^+$ ion at m/z 18).

The liquid chromatograph employed was a Spectra Physics Model SP-8000 with microprocessor, printer-plotter and 100 uL sample loop and equipped with a 25 cm x 3.2 mm i.d. Ultrasphere ODS 10 u with guard column operated as follows: acetonitrile/water, 40/60 at 1.5 mL/min (equivalent to about 3100 psi). The detector was a Schoeffel FS 970 LC Fluorometer with excitation set at 288 nm with a Corning glass 7-60 filter to give an emission window of 310 nm to 402 nm. Photocell voltage was 920 volts with a 6 second time constant and a range of 0.02 uA. This procedure was from a previously published method (Krause & August 1983) with some modification.

For analysis by GCMS, 100 g portions from homogenized 50 Kg sampled lots of pineapples (crown and leaves removed) were extracted (Luke et al. 1981) and cleaned up using Florisil (Luke & Doose 1983). The 50% petroleum ether/diethyl ether elution was combined with the 100% diethyl ether elution and concentrated to approximately 0.1 mL with a stream of dry nitrogen and then diluted up to 1.5 mL with acetone depending on suspected concentration to allow sample injection to fall on centroid of calibration curve.

For analysis by HPLC, the same sample preparation was followed with the sample extract being taken up in acetonitrile before injection (3 to 5 mL as final volume for injection).

RESULTS AND DISCUSSION

Five samples were analyzed by GCMS and HPLC and the results are tabulated in Table 1.

Table 1. Carbaryl Levels found in Pineapples

Sample[a]	Level of Carbaryl (ppm) GCMS-CI-NH_3	HPLC
1	0.21	0.54
2	0.42	0.40
3	1.80	1.60
4	0.26	0.22
5	0.16	0.15

[a] 100 g samples extracted from composite of 20 pineapples.
Recoveries were 81% and 93% for GCMS and HPLC respectively.

The isocratic conditions selected for the analysis (1.5 mL/min 40% acetonitrile 60% water) resulted in a retention time of about 6 minutes (Figure 1). A blank sample of domestic pineapples (obtained locally) was also examined and found to contain no interfering peaks in the retention zone expected for carbaryl. Having established a true blank, the homogenized pineapples were then spiked with carbaryl standard at a concentration level of 0.149 ppm and then carried through the analytical protocol. The recovery of carbaryl by the method was 93%. A calibration curve (using peak area measurements) established using the injected concentration levels of 4.63, 9.25, 23.1, 46.3, 69.5 and 92.5 ng resulted in a coefficient of correlation (r^2) of >0.99 (Figure 2). Samples were then analyzed and carbaryl quantitated via the calibration curve with no corrections applied due to blank or recovery data (Table 1).

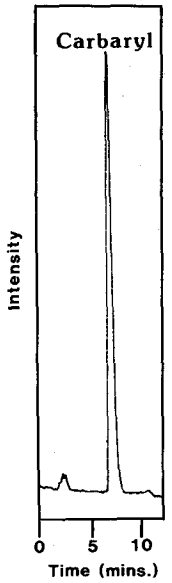

Figure 1. HPLC chromatogram of pineapple extract.

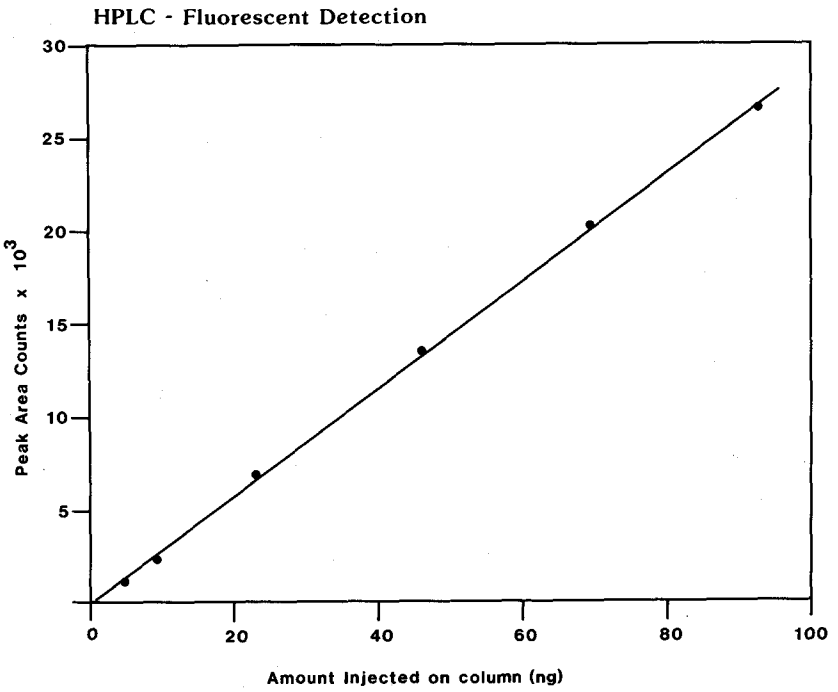

Figure 2. Calibration curve established for carbaryl by HPLC.

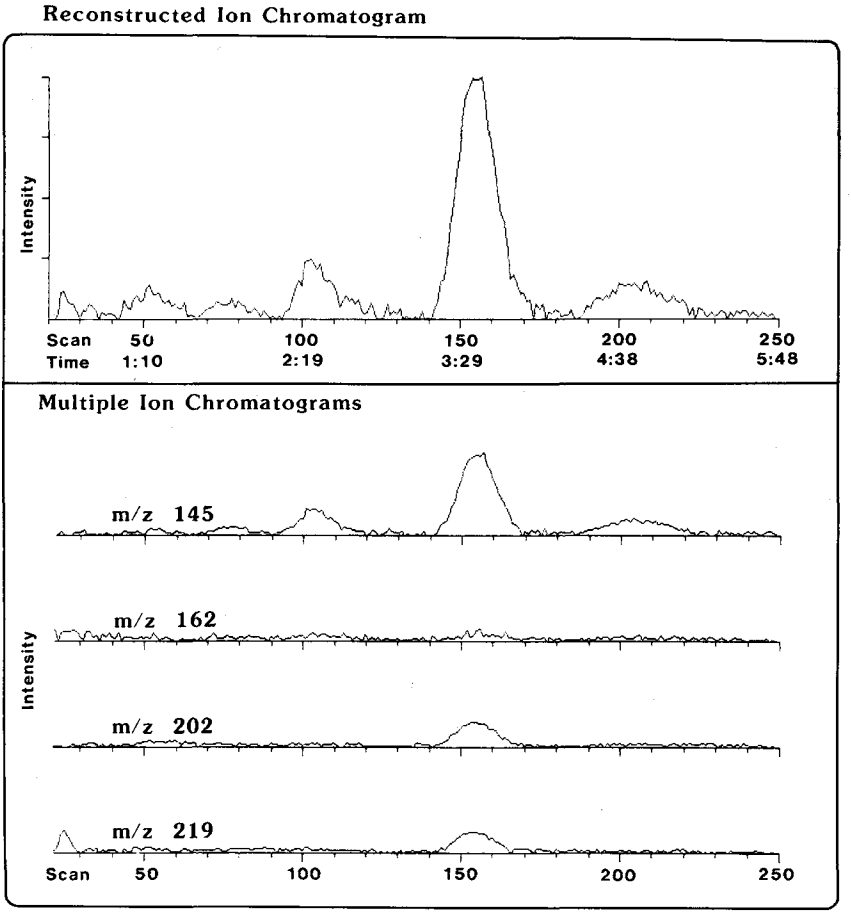

Figure 3. Ammonia chemical ionization GCMS data on pineapple extract.

Previous confirmation of carbaryl in marionberries (Cairns et al. 1983) via chemical ionization using ammonia as reagent gas resulted in a base peak at m/z 145 representing the protonated naphthol from loss of the methylcarbamate side chain. Relying on more than one ion for confirmation, three other ions were also monitored, namely m/z 162, 202, and 219 representing [naphthol + NH_4]$^+$, MH^+ and [M + NH_4]$^+$ respectively (Figure 3). Quantitation, however, was achieved by single ion monitoring of the ion at m/z 145 due to the level of sensitivity required. The other ions representing other products of ion-molecule reactions were less abundant and hence deemed unsuitable for the level of quantitation required. A calibration plot was established using three concentration levels with measurements in triplicate (Figure 4). The coefficient of correlation (r^2) was 0.95. Results of quantitation of samples are listed in Table 1.

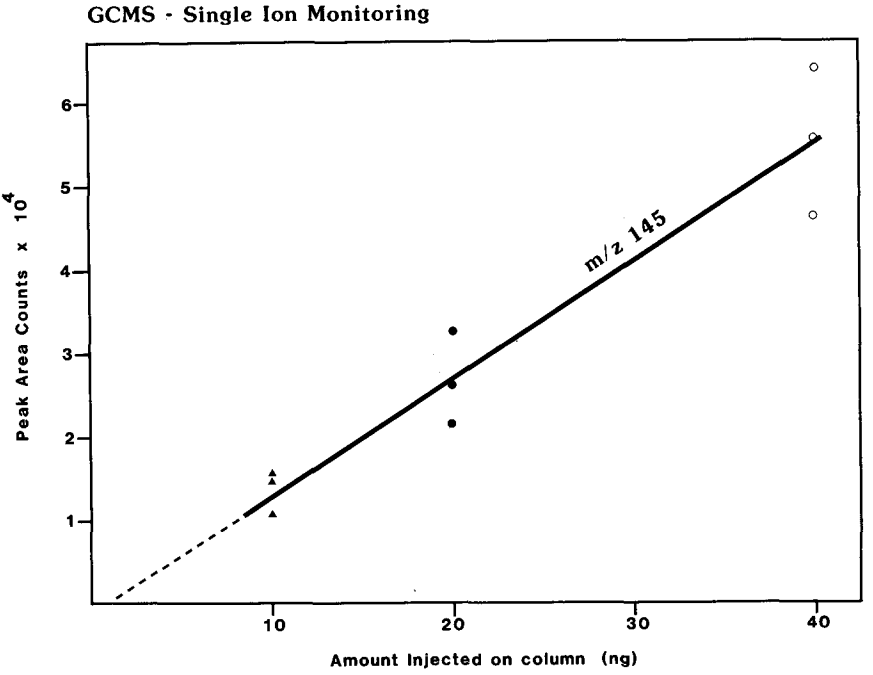

Figure 4. Calibration curve for carbaryl established by GCMS-SIM-NH_3.

In the earlier confirmation of carbaryl in marionberries (Cairns et al. 1983), the proposed fragmentation mechanism to explain the ion at m/z 145 was outlined as occurring via production of the neutral naphthol moiety and subsequent protonation. Preliminary studies using ND_3 as reagent gas have now indicated that the amide hydrogen is transferred to the naphthol moiety. With this new information, three other possible mechanisms to explain the ion at m/z 145 must also be now considered. It is possible to envisage amide proton transfer via 4- and 6-membered cyclic transition states after initial protonation at the ether oxygen and the ortho-aromatic site, respectively. While these mechanisms are classical in concept (i.e. bond forming before bond breaking), a non-classical route might also be responsible. Recently, a qualitative picture for such intramolecular hydrogen rearrangements has been advanced (Morton 1982) whereby the side chain is first lost by bond fission as a cation which immediately bonds electrostatically to the resultant neutral molecule formed by the original bond fission (i.e. naphthol). Due to low barriers, the cation can rotate to provide the amide hydrogen for subsequent transfer. Studies are currently under way to determine which of the proposed mechanisms might be responsible.

In comparing the quantitative results obtained by the two techniques employed for the analyses (Table 1), it is evident that the experimental degree of correlation is high with the sole exception of sample number 1. At the present time there is insufficient data to suggest a possible source of error. Such an approach has provided reliable and acceptable analytical data for trace levels of carbaryl. While the coefficient of correlation by HPLC was excellent (>0.99), the lower value of 0.95 by GCMS did not effectively reduce the observed reliability or impact of the data. On the contrary, the confirmation gained by the synergistic results added to the acceptability.

Acknowledgements. The authors wish to express their thanks to M. Bennett, H. Masumoto and D. Kodama for their technical assistance.

REFERENCES

Code of Federal Regulations (1982) Title 40 part 180.1: 159

Cairns T, Siegmund EG, Doose GM (1983) Potential identification problems with carbaryl. Bull Environ Contam Toxicol 30: 93-98

Cairns T, Rogers WM (1983) Acceptable analytical data for trace analysis. Anal Chem 55: 54A-57A

Krause RT, August EM (1983) Applicability of a carbamate insecticide multiresidue method for determining additional types of pesticides in fruits and vegetables. J Assoc Off Anal Chem 66: 234-240

Luke MA, Doose GM (1983) A modification of the luke multiresidue procedure for low moisture non fatty products. Bull Environ Contam Toxicol 30: 110-116

Luke MA, Froberg JE, Doose GM, Masumoto HT (1981) Improved multiresidue gas chromatographic determination of organo-phosphorus, organonitrogen, and organohalogen pesticides in produce using flame photometric and electrolytic conductivity detectors. J Assoc Off Anal Chem 64: 1187-1195

Morton, TH (1982) Gas phase analogues of solvolysis reaction. Tetrahedron 35: 3195-3243

Received August 23, 1983; Accepted September 14, 1983