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Journal of Chromatography A, 750 (1996) 391–396

JOURNAL OF CHROMATOGRAPHY A

Short communication

Extraction of maleic hydrazide residues from potato crisps and their determination using high-performance liquid chromatography with UV and atmospheric pressure chemical ionisation mass spectrometric detection

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Abstract

A method was required for the determination of maleic hydrazide residues in potato crisps. A published method for the extraction of the analyte from onions and potatoes was evaluated and found to be inappropriate due to the inability of the extracting solvent to penetrate the oily matrix. A method was developed to overcome this problem; the resulting recovery data (mean=92.9%, R.S.D.=8.3%, $n=16$) confirmed its efficiency, and was used to analyse 48 retail potato crisp samples. To confirm possible residues identified by screening with HPLC–UV, an HPLC–atmospheric pressure chemical ionization MS method was developed. There was good agreement between the data obtained from the two detection techniques ($R^2=0.978$, slope=1.11).

Keywords: Potato crisps; Environmental analysis; Food analysis; Maleic hydrazide; Pesticides

1. Introduction

Maleic hydrazide, 6-hydroxy-3(2H)-pyridazinone (HPZ), is used on potatoes to suppress sprouting and to prevent the growth of potato plants from tubers missed during harvesting. HPZ has a systemic mode of action, translocating to the tubers by way of the vascular system, after being applied to the plant's leaves [1]. The molecular structure of HPZ is shown in Fig. 1. In the UK the maximum residue level (MRL) of HPZ permitted in ware potatoes, i.e. those being grown for storage, is 50 mg/kg [2]. MRLs have not been set for processed products such as potato crisps.

The literature contains several methods for the extraction of HPZ from a range of commodities, including tobacco [3–5], garlic [6], onions and potatoes [7,8]. However, none of them are fully appropriate for the extraction of HPZ from potato crisps. It was found that the high fat content of a potato crisp (~35%, w/w) prevents the extraction

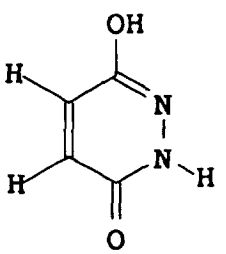

Fig. 1. The chemical structure of maleic hydrazide (molecular mass 112).

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0021-9673/96/$15.00
PII S0021-9673(96)00430-X

Table 1
Recovery of maleic hydrazide from potato crisps

Spiking level (mg/kg)	Recovery (%)	Range (%)	R.S.D. (%)	Number of analyses (*n*)
46.3	93.1	86.0–103.5	6.3	6
23.2	92.0	82.1–102.0	7.9	6
4.6	93.4	86.0–101.1	7.8	4

solvent, methanol, from penetrating the sample matrix. This problem has been overcome by the inclusion of a de-fatting step to remove the crisping oil.

Residues of HPZ can be quantified using a number of techniques, including enzyme-linked immunosorbent assay [9], gas chromatography (GC) with nitrogen–phosphorous [3] and flame ionisation [5] detection and high-performance liquid chromatography (HPLC) [4,6–8]. Sample extracts were routinely screened by closely following the anion-exchange HPLC method described by Vadukul [7]. However, those extracts identified as containing possible residues of HPZ were analysed using a method developed using HPLC–atmospheric pressure chemical ionisation mass spectrometry (APCI-MS). The advantage of this detection technique is that it provides simultaneous quantitative and confirmatory information.

The procedure has been applied to the analysis of 48 retail crisp samples as part of the UK Government's Working Party on Pesticide Residues (WPPR) surveillance programme [10].

2. Experimental

2.1. Extraction of residues from potato crisps

A sample of the crisps (3 g) was weighed into a polythene bottle and water (8 ml) added. The mixture was swirled and allowed to soak for 30 min to ensure complete wetting of the crisp. Hexane (70 ml) was added and the mixture homogenised for 3 min using an Ultra-Turrax homogeniser (Janke and Kunkel, Staufen, Germany). After complete sepa-

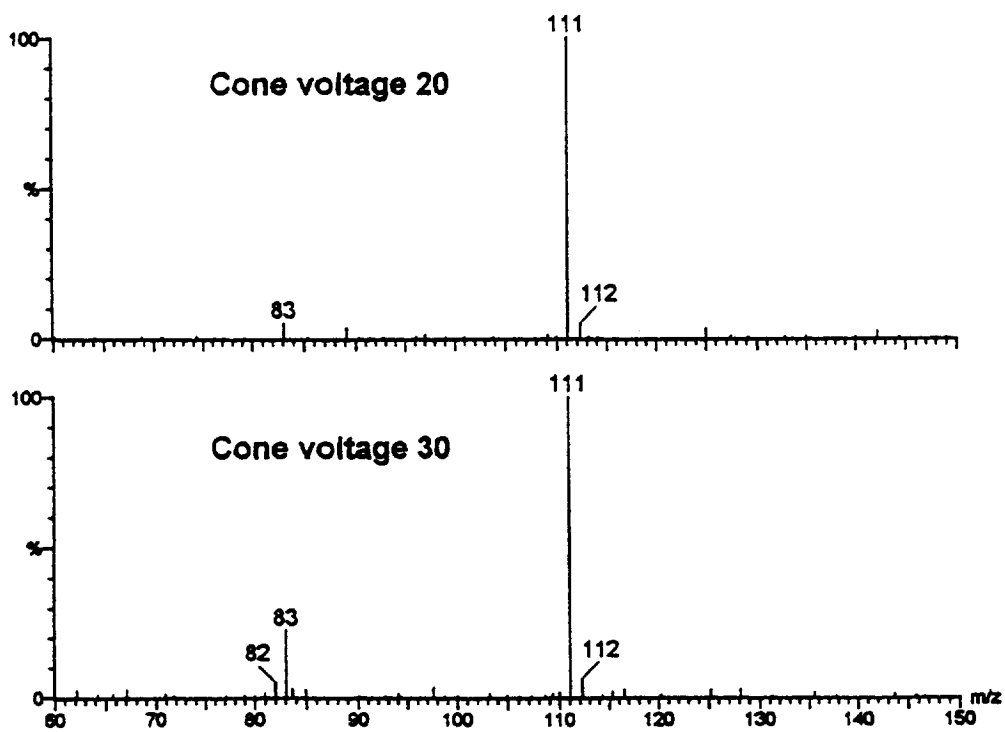

Fig. 2. The spectra for maleic hydrazide at two cone voltages (20 and 30 V).

ration of the layers, the hexane layer, containing the extracted crisp oil, was decanted and discarded. Methanol (70 ml) was added to the de-fatted matrix, the mixture homogenised for 3 min, and allowed to stand for 5 min. The solvent was decanted, and filtered under reduced pressure into a Buchner flask (250 ml) through a filter paper (Whatman No.1). The sample was re-homogenised with methanol (70 ml) and a filter aid, Celite, (5 g) for a further 2 min. The contents of the bottle were filtered and collected into the Buchner flask. The Ultra-Turrax probe and the polythene bottle were rinsed with methanol (25 ml) which was then used to wash the filter cake.

The combined filtrate and washings were transferred to a Kuderna-Danish evaporation flask (500 ml) fitted with a receiving trap. Anti-bumping granules were added to the flask and the extract concentrated until approximately 8 ml remained. The remaining solvent was reduced under a gentle stream of oxygen-free nitrogen until the volume was approximately 4 ml. The extract was quantitatively transferred to a volumetric flask (10 ml) and made up to volume with 0.01 *M* sodium hydroxide.

2.2. Clean-up of extracts by SPE

Clean-up of the extracts was performed using Isolute C_{18} (6 ml, 1 g) SPE columns (Jones/IST, Hengoed, UK). UV-active co-extractants were retained by the column but HPZ was not.

Each column was conditioned with methanol (4

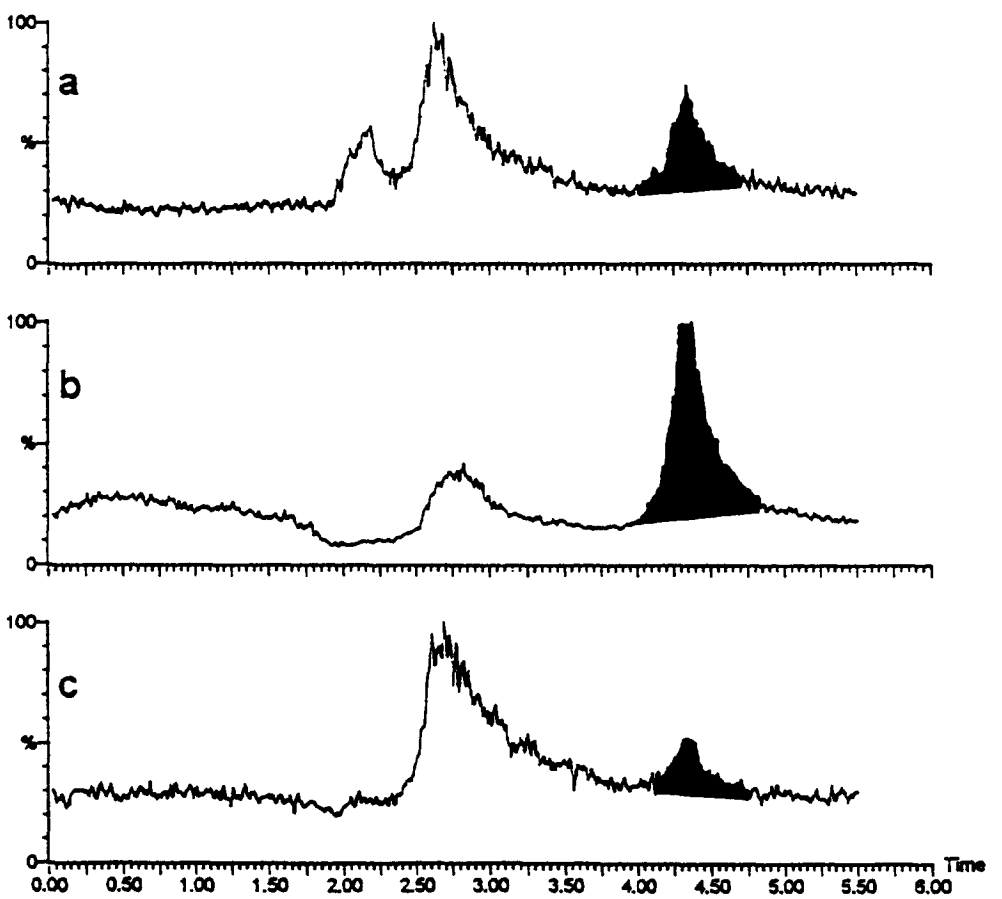

Fig. 3. Chromatograms for a 1.92 mg/l matrix-matched standard at the three ions monitored: (a) the fragment ion at *m/z* 83.00, (b) the quantifying ion at *m/z* 111.02 and (c) its isotope ion at *m/z* 112.02. Time in min.

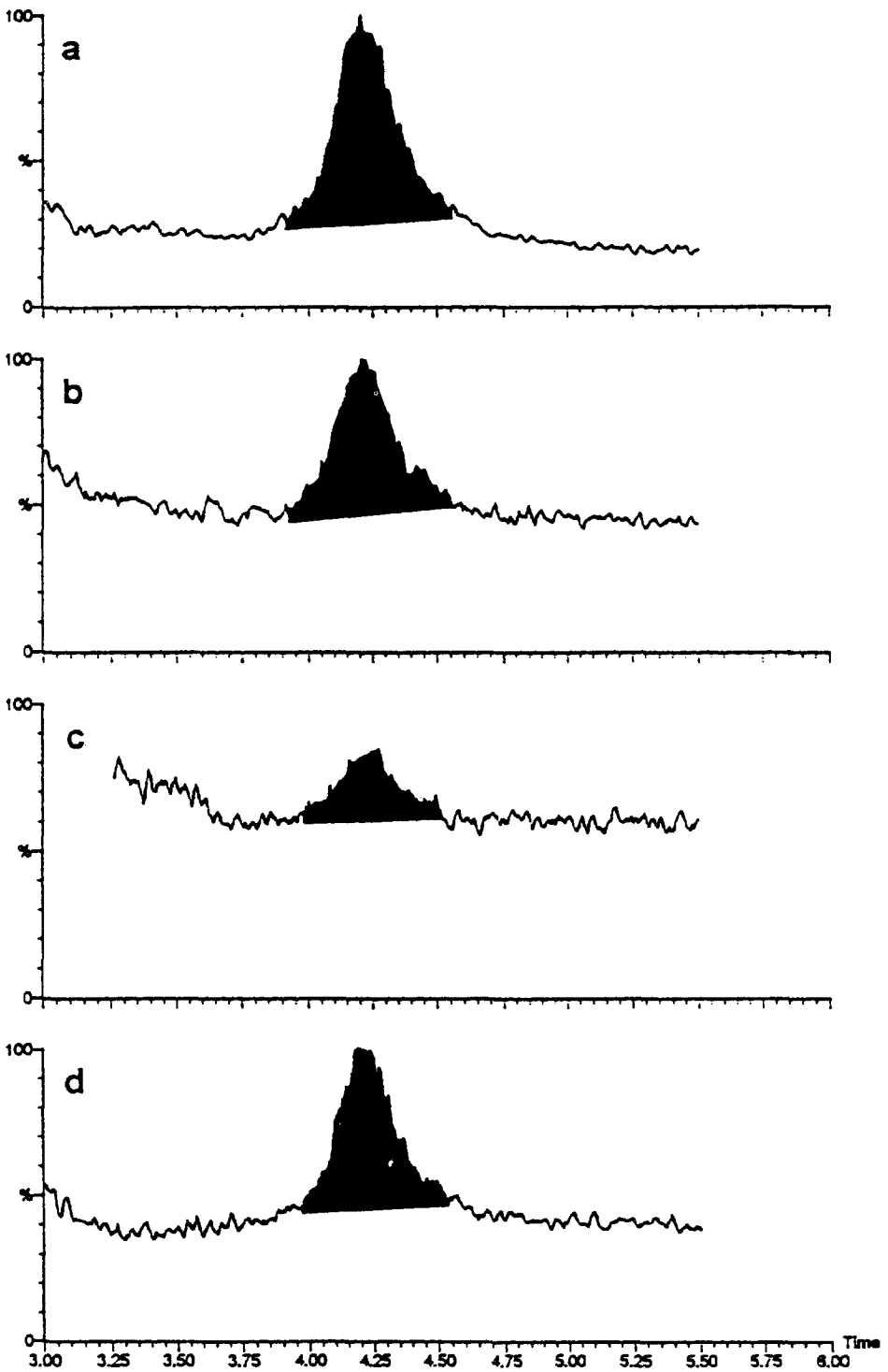

Fig. 4. Chromatograms obtained for a range of samples by monitoring m/z 111.02: (a) sample (0.95 mg/l), (b) spiked sample (0.24 mg/l), (c) blank matrix and (d) matrix-matched standard (0.19 mg/l). Time in min.

ml) followed by 0.01 *M* sodium hydroxide (4 ml). An aliquot of extract (1 ml) was loaded onto the column, the HPZ residues washed through with the sodium hydroxide solution (approx. 8 ml) and collected directly into a volumetric flask (10 ml). The cleaned-up extracts were made up to volume with the sodium hydroxide solution, ready for analysis by HPLC–UV.

2.3. Procedure for the spiking of potato crisps

Potatoes which had not been treated with HPZ were made into crisps and used as blank samples. To ensure the robustness and efficiency of the extraction and clean-up procedures, some of the blank crisps were spiked at three concentrations, 46.3, 23.2 and 4.6 mg/kg, extracted and analysed.

An 1-ml volume of a relevant spike solution, in methanol, was applied to the blank potato crisp. This was done only after the matrix had been fully soaked with the added water, i.e. after 30 min. The mixture was left for a further 5 min and then any remaining solvent from the spike solution removed under a gentle stream of oxygen-free nitrogen.

2.4. HPLC–UV: equipment and conditions for the screening of extracts for maleic hydrazide residues

The HPLC system consisted of a Spectra-Physics AS-3000 autosampler (ThermoSeparations, Stone, UK) fitted with a column oven (operating temperature 40°C) and a Rheodyne 7010 injection valve. Detection was by a Spectra-Physics Spectra-200 variable-wavelength UV detector set at 313 nm. A Spectra-Physics P-2000 tertiary pump delivered a mobile phase consisting of 0.1 *M* acetic acid solution, adjusted to pH 4.8 with 0.1 *M* tetramethylammonium hydroxide solution at a flow rate of 1.3 ml/min. The analytical column was a Hichrom Nucleosil 100 10SB (25 cm×4.6 mm), preceded by a guard column (10 cm×3.2 mm) containing the same packing material. Partial-loop injections of 50 μl (100 μl loop) were made at 13.5 min intervals. The retention time of HPZ was approximately 5 min.

2.5. Confirmation of residues by HPLC–APCI-MS

HPLC–APCI-MS was performed using a VG Platform bench-top mass spectrometer (VG Organic, Manchester, UK). The analytical column and guard column were as described for the HPLC–UV determinations. However, it was necessary to modify the mobile phase because the one used in the UV method produced unpleasant fumes when in contact with the high temperature components of the APCI-MS. A Spectra-Physics SP-8800 tertiary pump delivered the modified mobile phase consisting of 0.05 *M* ammonium acetate (adjusted to pH 4.6 with glacial acetic acid)–methanol (85:15) mixture at a flow rate of 1.3 ml/min.

Partial loop injections (50 μl) were made using a Gilson 231-XL autosampler (Anachem, Luton, UK) fitted with a 100 μl loop on a Rheodyne 7125 injection valve (Cotati, CA, USA). A solid block column heater (Jones Chromatography, Hengoed, UK) maintained the column temperature at 60°C. Injections were made onto the HPLC column at 6 min intervals. The retention time of the maleic hydrazide peak was approximately 4.2 min.

The mass spectrometer was initially tuned on background ions and calibrated in the positive ion mode on a mixture of PEG 300, 600 and 1000. Tuning was then optimised in the negative ion mode on the deprotonated ion, $[M-H]^-$, at *m/z* 111.02. Typical operating conditions were: corona 2.57 kV, high voltage lens 0 kV, cone voltage 20 V, cone voltage offset 5 V, source temperature 150°C, probe temperature 400°C, low mass resolution 15.0, high mass resolution 15.5, ion energy 0.8 V, ion energy ramp 0.0, multiplier 650.

Scanned acquisitions were from *m/z* 60 to 400 with a scan time of 1 s. Selected ion monitoring (SIM) was performed at *m/z* 111.02 and 112.02, for the deprotonated ion and its isotope ion respectively, at a cone voltage of 20 V. At a cone voltage of 30 V, an induced fragment ion at *m/z* 83.00 was also monitored. The dwell time for each channel was 0.1 s, the interchannel delay was 0.02 s and the mass span was 0.5 u. Quantitation was based on the peak area of the *m/z* 111.02 ion relative to external standards and confirmation was based on the ratio of the *m/z* 111.02 ion relative to the other ions monitored.

3. Results and discussion

From the data obtained by HPLC–UV, see Table 1, mean recoveries using the developed extraction procedures were found to be greater than 90% for each of the three spiking levels. This is in line with the accepted quality requirements of the WPPR, recoveries being deemed acceptable if between 80 and 110%.

During the development of the APCI-MS methodology both positive and negative ionisation modes were evaluated. The sensitivity for the deprotonated molecule $[M-H]^-$ produced in negative ionisation mode was found to be three-fold greater than that for the protonated molecule $[M+H]^+$ produced in positive ionisation mode. Hence, negative ionisation was used in this work.

The spectra obtained for the analyte were dominated by the deprotonated molecular ion at m/z 111.02 with the fragment ion at m/z 83.00 only increasing in relative abundance as the cone voltage was increased from 20 to 30 V. Fig. 2 shows the spectra obtained at the two voltages.

Matrix-matching of standards was carried out by using cleaned-up extract from blank crisps rather than solvent to solubilise the maleic hydrazide. The chromatograms presented in Fig. 3 were obtained from a matrix-matched standard (1.92 mg/l) for each of the three ions monitored. The limit of detection for the APCI-MS method was 0.078 mg/l (equivalent to 2.6 mg/kg in the potato crisp). This was based on three times the standard deviation obtained from seven replicate injections of the lowest concentration standard. A linear response to the standards over the range 0.096–1.92 mg/l was obtained from the APCI-MS ($r=0.9994$).

Of the 48 retail potato crisp samples screened, 10 were found to have residues in excess of the agreed reporting level of 3.2 mg/kg. The developed HPLC–APCI-MS method was applied to these, and Fig. 4 illustrates typical chromatograms obtained for a range of samples associated with that survey [10].

A comparison was made between the values obtained from UV and APCI-MS detection techniques and good agreement ($R^2=0.978$, slope=1.11) was found.

4. Conclusion

The extraction and clean-up procedures described here have been successfully applied to the monitoring of HPZ residues in retail potato crisps [10] and may well be applicable to other high-fat-content commodities. The recovery data obtained from a wide range of spike concentrations demonstrates the robustness of the developed extraction procedure. The comparison of data obtained by the two detection techniques shows the validity of applying either technique to this type of analysis. The advantage, however, of APCI-MS is that it provides confirmatory information.

Acknowledgments

The authors would like to thank Dr. Philip Clarke of the CSL Food Science Laboratory for useful discussions throughout the course of the project, and to acknowledge the MAFF Pesticides Safety Directorate for their support in this work.

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