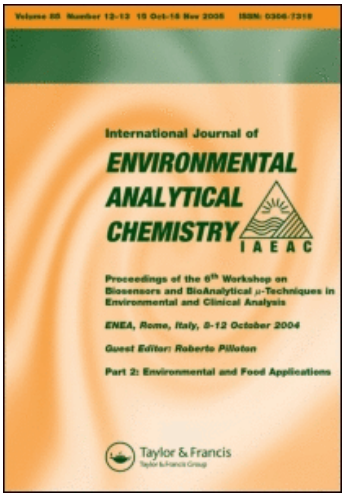

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An Improved Method for the Determination of 1, 1 - Dimethyl Hydrazine in Apple and Cherry Products

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An Improved Method for the Determination of 1,1-Dimethyl Hydrazine in Apple and Cherry Products

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A method, improved with respect to quantitation limit and sample output is described for determining 1,1-dimethylhydrazine in foods by gas chromatography of the pentafluorobenzamide. Recoveries from cherries and apples spiked at from 0.5–100 ppb averaged 84% and 82%, respectively, while apple juice yielded an average of 87% at 6 levels from 0.1–100 ppb. A CV of 4.1% was observed from 11 replicates of apple juice spiked at 10 ppb and analysed over 3 days. The minimum detection limit was 0.04 ppb in apple juice and 0.20 ppb in apples or cherries. A survey of 55 commercial apple and cherry products showed the highest levels and incidence of occurrence in apple sauce and pie filling, with a mean residue of 31 ppb. The identity and amount found in selected samples was verified by mass spectrometry.

INTRODUCTION

1,1-Dimethylhydrazine (UDMH) is a suspected carcinogen[1] and a degradation product of the plant growth regulator daminozide. It has been shown to form on cooking of foods containing the parent compound[2]. A method previously described[2] for determining UDMH involved its isolation on a cation exchange resin followed by pentafluorobenzoylation, cleanup of the derivative on silica and

determination by electron-capture gas chromatography. Although this procedure was capable of quantitating UDMH in apples at levels down to 100 ppb, further development was necessary to reduce the level of detection and enable the survey of foods for smaller amounts of residue.

Two factors in the original procedure placed constraint on the minimum detectable limit attainable; a high background produced by the derivatization reagent, and a low (50%) recovery of UDMH from the ion exchange resin used to isolate it from the sample matrix. In addition, the low absolute recovery obtained in the derivatization necessitated the use of a standard run through the entire method concurrently with the samples to provide accurate quantitation. This requirement reduced the number of samples which could be analysed in a single run.

The present modifications address these deficiencies by (a) substituting hexane for dichloromethane as the derivatizing solvent and use of a more efficient cleanup column to reduce reagent background, (b) replacing the strong cation exchange column with a weak carboxylic acid type to increase recovery, and (c) the use of an external standard to increase sample output.

EXPERIMENTAL

Materials

Weakly acidic ion exchange resin, Amberlite CG-50, 100–200 mesh was obtained from the Sigma Chemical Co., St Louis, MO. An approximately 25% (w/v) slurry of resin was prepared in distilled water and adjusted to pH 8 with 25% NaOH. The resin was permitted to settle overnight, and after decantation of the water resuspended in water. The pH was measured and adjusted to 7.0 if necessary.

Silica, 100–200 mesh was purchased from ICN Biomedicals Inc., K&K Laboratory Division, Plainview, NY. The silica was deactivated by addition of 5% (w/w) distilled water.

Pentafluorobenzolyl chloride reagent was prepared by diluting 0.13 mL of pentafluorobenzoyl chloride (Aldrich Chemical Co., Milwaukee, Wis.) to 25 mL with hexane.

2 M potassium carbonate was prepared by dissolving reagent

grade K_2CO_3 (276 g) in distilled water and making to 1 L. The cooled solution was purified by extracting 3X with 250 mL portions of hexane prior to use.

0.5 N Citrate buffer, pH 6.0 was prepared by dissolving citric acid (70 g) in 800 mL of distilled water, adjusting the pH to 6.0 with NaOH and making to 1 L with distilled water. 0.10 N citrate, pH 6.0 was prepared by a 1:5 dilution of the 0.5 N buffer.

1,1-Dimethylhydrazine (UDMH) used as a standard was purchased from Aldrich Chemical Co., Milwaukee, Wis. Caution: UDMH is a suspected carcinogen. The pure standard should be handled in a fume hood. One hundred mg of UDMH was accurately weighed into approximately 25 mL of 1 N HCl in a 50 mL volumetric and made to volume with 1 N HCl. Sub-dilutions were made in 0.1 N HCl to give a 300 ng/mL standard.

1,1-Dimethyl-2,2-di(pentafluorobenzamido)hydrazine (PFB-UDMH) standard was prepared by stirring pentafluorobenzoyl chloride (2.4 g; 10.3 mmol) and UDMH (206 mg; 3.43 mmol) for 1 h in dichloromethane (5 mL) containing 2 M K_2CO_3 (10 mL). An additional 20 mL of dichloromethane was added and the mixture transferred to a separatory funnel. The organic layer was recovered, washed with 2 M K_2CO_3 then water, and dried by passage through a bed of anhydrous Na_2SO_4. After removal of the solvent on a rotary evaporator, the residue was crystallized from hot hexane to give white crystals (1.03 g).

Crude PFB-UDMH (629 mg) in 15 mL of hexane was added to a 50 g column of 5% deactivated silica, 63–100 mesh, packed in hexane in a 30 cm × 3.2 cm chromatographic tube. The column was eluted with 20% dichloromethane in hexane, the first 125 mL of effluent being discarded and the next 250 mL being collected. Removal of the solvent yielded 189 mg of purified standard, mp 92–93°C. GC-mass spectrometry on a VG 7070 EQ indicated a single compound with ions characteristic of the di-pentafluorobenzoylated compound at m/z 448 ($[M\cdot^+]$, 18); 253 ($[M\cdot^+-F_5PhCO]$, 22); 195 ($[F_5PhCO\cdot^+]$, 100); 167 ($[F_5PhCO\cdot^+-CO]$, 15).

Apparatus

Ion Exchange Columns were prepared by adding a slurry of Amberlite CG-50 to 10 mL of 0.01 N citrate buffer in a 20 × 6 mm id

chromatographic column (Kontes chromaflex K 420100) containing a 50 mL reservoir until a 3 mL bed of resin was obtained. Excess buffer was permitted to flow through the column, resin being added as necessary to maintain the bed at 3 mL.

Silica columns were prepared by adding 2 g of 5% deactivated silica to approximately 10 mL of hexane in a 20 × 6 mm id chromatographic column, and the excess hexane aspirated.

The tube heater used in the derivatization reaction was a Kontes K-720002 (Kontes, Vineland, NJ), containing 45 mm glass shims to decrease the volume of the heater wells and maintained at a temperature of 100°C.

Gas chromatography was conducted using a Varian 3500 fitted with a ^{63}Ni detector, on-column injector, model 8035 autosampler, and controlled by a model 600D data system. Separations were carried out on a 30 m × 0.25 mm id J&W DB-5 fused silica column using helium carrier at a linear velocity of 33 cm sec^{-1}. The detector was maintained at 300°C and purged at 20 mL min^{-1} with nitrogen.

Procedure

Sample extraction Solid samples were homogenized in a Waring blender and sub-samples of 5.0 g weighed into 50 mL round-bottomed centrifuge tubes. Ice-cold 0.02 N HCl (30 mL) was added and the sample mixed in a Polytron mixer at a setting of 5 for 5 sec. The homogenate was filtered through a 1 g bed of Celite 545 on a 4.25 cm disc of Whatman No. 1 paper in a Büchner funnel. The tube and funnel were rinsed with an additional 10 mL of cold 0.02 N HCl, and the filtrate brought to 100 mL with 0.02 N HCl in a volumetric. Cherry samples were more difficult to filter but could be handled satisfactorily by adding 0.5 g of Celite prior to homogenizing, and permitting the homogenate to stand in an ice bath for 5 min prior to filtering through 0.5 g of Celite.

Apple juice was mixed by shaking prior to sampling and 25 g sub-samples weighed into 100 mL volumetric flasks. The solutions were then made to volume with 0.02 N HCl without filtration.

Ion exchange chromatography Aliquots (20 mL) of dilute filtrate were added to the ion exchange columns and the flow commenced at a rate 40 mL h^{-1}. After adsorption of the sample, the column was

washed with 0.1 N citrate buffer (10 mL) and then eluted with 0.5 N citrate buffer (10 mL) to remove the UDMH.

Derivatization An aliquot (1.0 mL) of 0.5 N citrate eluate was added to 9.0 mL of 2 M K_2CO_3 and 2.0 mL of pentafluorobenzoyl chloride reagent in hexane in a 15 mL centrifuge tube. The tube was stoppered tightly and shaken for 30 min at maximum speed on an orbital shaker. An additional 4 mL of hexane was added to the tube, the content shaken, and the hexane layer transferred to a 15 mL centrifuge tube containing 0.1 mL of heavy paraffin. The solvent was evaporated on a rotary evaporator and the stoppered tube placed in a tube heater at 100°C for 15 min. Hexane (1.0 mL) was added to the cooled tube to dissolve the residue for cleanup on silica.

A standard, consisting of 150 ng of UDMH in 0.5 mL 0.1 N HCl was carried in duplicate through the derivatization procedure along with the samples to correct for the incomplete nature of the reaction. At the end of the 30 min reaction, 20 μL aliquots were removed from the 2.0 mL hexane layer, diluted with hexane to 10 mL in a volumetric, and an aliquot injected directly into the GLC.

Cleanup on silica The derivatized sample, followed by a 1.0 mL hexane rinse, was added to a column of deactivated silica. The column was eluted sequentially with 5% (10 mL), 10% (10 mL), and 15% (15 mL) dichloromethane in hexane. The 15% fraction was retained and the solvent removed on a rotary evaporator. The residue was taken up in hexane (3.0 mL) for injection into the GLC, along with the diluted derivatization standard.

Gas chromatography Injections of 1.0 μL were made at 80°C, and after 0.5 min the injector was programmed at 100°C min-^{1} to 200°C. The column was programmed, after a 0.5 min delay, from 80°C to 175°C at 50°C min^{-1} and after 8 min at this temperature to 200°C at 30°C min^{-1}. The column was maintained at the upper temperature for 8.7 min to remove high-boiling contaminents, then cycled to 80°C for a subsequent injection. Under these conditions, a 0.5 ng mL^{-1} standard of PFB-UDMH produced a peak of half-scale deflection (51,000 area counts) at an attenuation of 16.

Since the response of the detector and/or column was slightly curvilinear, a series of standards prepared from crystalline PFB-

UDMH, and ranging from 0.1–5.0 ng mL^{-1} was injected with each batch of samples. The peak areas so obtained were used to calculate the least squares equation for the parabolic curve $y = A + Bx + Cx^2$. This equation was then used to calculate the amount of PFB-UDMH in the samples, and hence the amount of UDMH was derived. The absolute amount of UDMH was obtained by correcting for the yield of derivatization standard as determinded under *Derivatization.*

MS confirmation Residues were confirmed by injection of an aliquot of the same sample as analysed by GC-EC onto an identical column coupled to a VG Analytical 7070 EQ mass spectometer operating in the single ion mode at a resolution of 1000. A splitless injector was used and the temperature program similar to that for GC-EC. The detection limit was 0.1 pg at this resolution, monitoring the molecular ion at m/z 448.0269.

RESULTS AND DISCUSSIONS

The substitution of a weaker carboxylic acid-type ion exchange resin for the strong sulfonic acid resin used previously resulted in a quantitative recovery of UDMH from this step of the chromatographic cleanup. From the data shown in Table 1, it is evident that columns prepared from resin at pH 6.5 and above were superior in adsorbing UDMH from diluted apple juice and desorbing it by elution with 0.5 N citrate.

Table 1 Effect of initial resin pH on recovery of UDMH from apple juice spiked at 0.5 ppb

Resin pH	*Recovery (%)*
5.7	58
5.8	63
5.9	66
6.1	76
6.3	79
6.5	80
7.0	81

In the course of development it was observed that the yield of PFB-UDMH obtained after chromatography on silica was approximately 32% of that obtained by direct injection of the diluted reaction mixture. This loss was not attributable to poor recoveries from the silica, since greater than 95% recovery of the PFB-UDMH standard resulted when it was chromatographed on the silica column. These findings implied that a precursor of the derivative existed in the reaction mixture which converted to PFB-UDMH on injection. Attempts to increase the yield of PFB-UDMH by alterating the reaction time, increasing the reagent concentration, or changing the pH of the reaction mixture were without success. However, a yield equal to that obtained by direct injection resulted when the reaction product was heated at 100°C in paraffin. Heating in the absence of a high-boiling solvent such as paraffin resulted in severe losses of derivative.

As shown by the data in Table 2, the yield of PFB-UDMH obtained after heating the reaction product is constant over the range 0.1–150 ng of UDMH in the reaction. Replication of the derivatization was also satisfactory, a CV of 3% ($n=6$) being obtained with 150 ng of UDMH. This data also suggests a good recovery through the silica column chromatographic and evaporation steps, since the yields are similar to those obtained by direct injection. Since the percentage conversion is constant over the range studied, it provides validity for employing a correction for the efficiency of the derivatization reaction based upon a single concentration. The recovery data shown in Table 3 are corrected for derivatization efficiency, and reflect losses sustained during the remainder of the procedure from extraction and ion exchange chromatography to cleanup on silica. Mean recoveries over the range of concentrations examined were 84, 82, and 87% from cherry,

Table 2 Effect of amount of UDMH on yield of PFB-DMH

UDMH/Reaction (*ng*)	*Yield of PFB-UDMH* (%)
0.1	69.2
1.0	71.3
10.1	74.4
150	68.0
150 (direct injection)	72.8

Table 3 Recovery of UDMH added to various commodities

UDMH added (ppb)	*Recovery (%)*[a]		
	Cherry	*Apple*	*Apple juice*
0.10	—	—	91.0
0.50	106	84.2	80.3
1.0	78.3	81.1	85.9
10	72.8	75.8	84.9
20	—	—	94.9
100	77.8	87.2	85.0

[a]Recoveries are the means of duplicate determinations.

Table 4 Survey of apple and cherry products for UDMH

Commodity	*UDMH found (ppb)*						
	0–10	*11–20*	*21–30*	*31–40*	*41–50*	*51–100*	*Mean*[a]
Apple juice	10	3	0	0	0	0	8.7
Apple sauce[b]	11	2	5	2	1	5	31.2
Fresh apple	3	0	0	0	0	0	3.2
Cherries[c]	7	0	1	0	0	2	30.7

[a]Mean of positive samples.
[b]Includes apple pie filling.
[c]Includes canned, jam, pie filling.

Table 5 Comparison of residues found by GC-EC and GC-MS

Sample	*UDMH found (ppb)*	
	GC-EC	*GC-MS*
Apple juice	1.6	1.9
Apple juice	14.4	15.4
Apple juice	6.0	3.8
Apple pie filling	36.9	34.9
Apple sauce	47.9	43.1
Apple sauce	93.1	89.9
Apple sauce	14.5	17.0

apple, and apple juice, respectively. Eleven samples of apple sauce spiked at 10 ppb and analysed on 3 different days gave a mean recovery of 81% and CV of 4.1%, indicating good repeatability of the method.

Typical chromatograms of apple juice and apple sauce samples with and without the additiona of 1 ppb UDMH are shown in Figure 1. The lower PFB-UDMH response observed with apple sauce is a result of the smaller sample size used. The minimum detection limit, determined as 3 standard deviations above the background obtained from 6 control samples of apple juice was 0.038 ppb, and the minimum quantitation limit, defined as 10 standard deviations above background was 0.098 ppb. Corresponding values determined for apple or cherries were 0.20 and 0.51 ppb respectively. Attempts to improve the detection limit by increasing the volume of aliquot taken after ion exchange chromatography for derivatization were unsuccessful, 62% recovery being obtained with a 2 mL compared to 80% with a 1 mL aliquot for an apple juice sample spiked at 0.58 ppb. Similarly, increasing the amount of juice from 25 g to 50 g resulted in a drop in recovery to 67%. Increases in the amount of apple or cherry used in the initial extraction produced homogenates which were extremely difficult to filter.

As this manuscript was in preparation, an alternative method for UDMH was described by Wright Jr.[3] which involves derivatization with *o*-nitrobenzaldehyde to give a hydrazone. This method does not require as extensive a cleanup and yields good recoveries down to 10 ppb. Although more laborious, the present procedure is capable of a lower detection limit.

A summary of the results obtained in a survey of commercial apple and cherry products for UDMH is shown in Table 4. The highest residues were found in apple sauce and pie filling, with 20 of the 26 samples containing levels above the quantitation limit. Mean residue levels in apple juice were somewhat lower than those in sauce and filling or cherry products, the latter commodities being similar to each other.

GC-MS was used to confirm some of the residues found in the survey. The close agreement of the response obtained with the electron capture detector and the mass spectrometer (Table 5) indicates negligible interference with GC-EC determination.

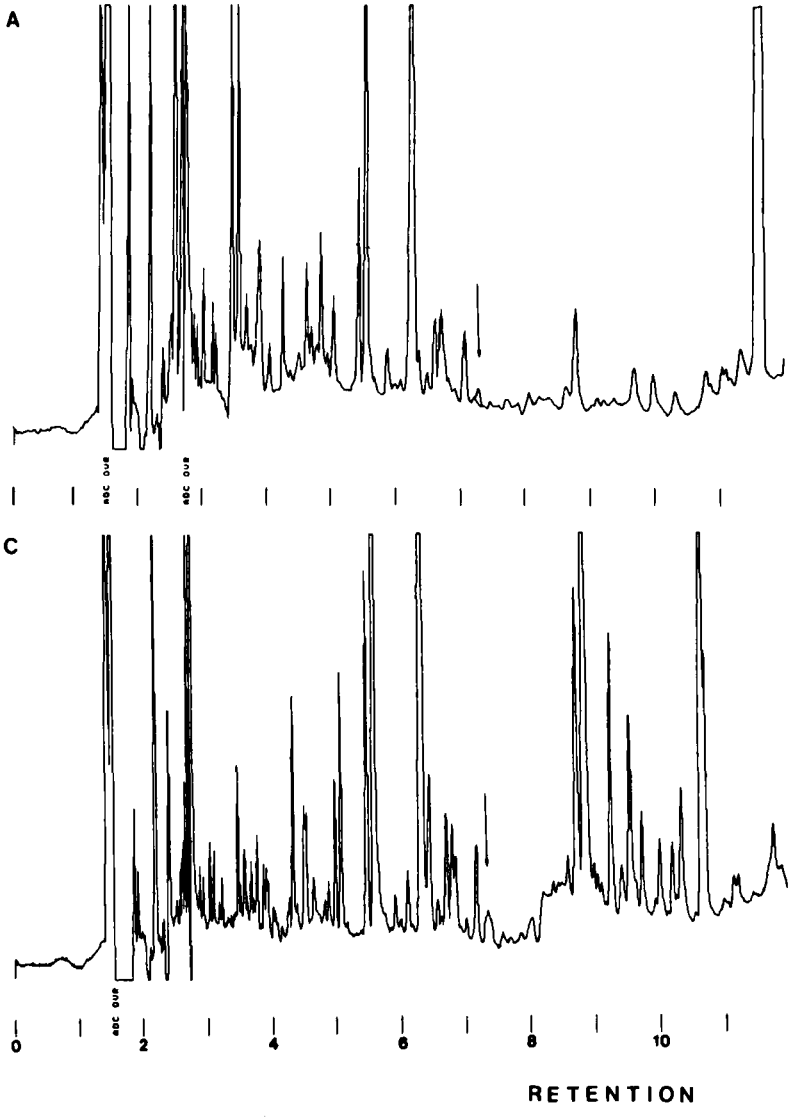

Figure 1 Chromatograms of untreated apple sauce (A), apple sauce spiked with 1 ppb UDMH (B), and untreated apple juice (C) and apple juice spiked with 1 ppb UDMH (D). Arrows indicate the retention time of PFB-UDMH.

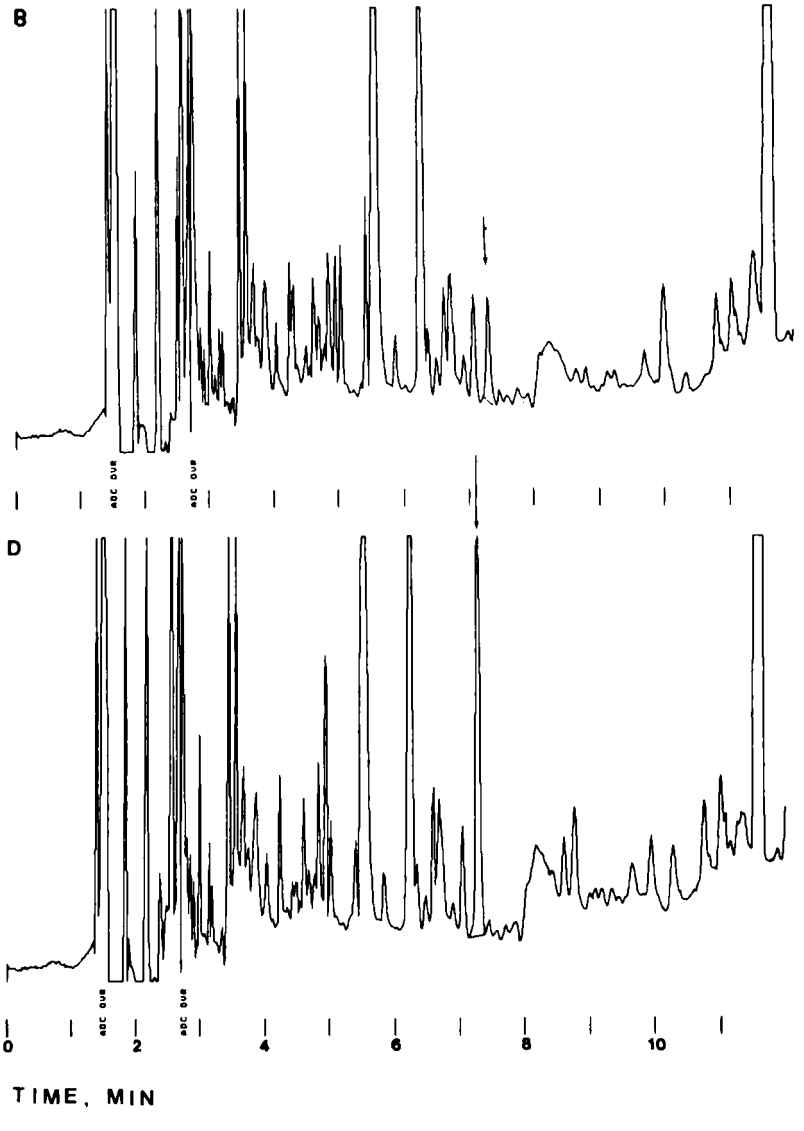

Figure 1 Chromatograms of untreated apple sauce (A), apple sauce spiked with 1 ppb UDMH (B), and untreated apple juice (C) and apple juice spiked with 1 ppb UDMH (D). Arrows indicate the retention time of PFB-UDMH.

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