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Registry No. *p*-Coumaric acid, 7400-08-0; ferulic acid, 1135-24-6.

Determination of Several Pesticides with a Chemical Ionization Ion Trap Detector

Gregory C. Mattern, George M. Singer,[†] Judy Louis,[‡] Mark Robson,[§] and Joseph D. Rosen*

Department of Food Science, Cook College, Rutgers University, New Brunswick, New Jersey 08903

A total of one hundred (twenty five each) apple, peach, tomato, and potato samples were analyzed for twelve pesticides and two pesticide metabolites with a slightly modified Luke multiresidue extraction procedure, separation by capillary column gas chromatography with cold on-column injection, and detection by mass chromatography with an ion trap mass spectrometer in the chemical ionization mode (GC/CIMS). Residues of carbaryl, captan, dichloran, dimethoate, methamidophos, phosmet, and tetrahydrophthalimide were found in several samples, with peaches containing the most residues. None of the residues found were above legal tolerances. Recovery studies were performed at the 0.5 ppm fortification level of each pesticide and metabolite at least three times in each of the four crops. Recoveries were between 73 and 120%, with an average coefficient of variation of 11%. Because the computer can be programmed to search for several hundred targeted ions, the use of capillary column GC/CIMS is a promising method that should be explored by regulatory agencies for the analysis of pesticide residues.

The Luke extraction procedure (Luke et al., 1981; AOAC, 1985) is, at last count, capable of extracting 234 pesticides and pesticide metabolites (Luke et al., 1988) and is, consequently, extensively used by regulatory agencies for multiresidue analyses. This method requires no column chromatography cleanup step as interfering chromatographic peaks are minimized by the use of specific detectors such as the flame photometric and the Hall electrolytic conductivity detectors. Current methodology makes use of packed gas chromatography columns of various polarities. Thus, an analyst determining all the possible pesticide residues extracted by the Luke extraction procedure needs to make from four to six separate determinations for each sample.

We believe that these analyses could be vastly improved by the use of a single capillary gas chromatographic column whose effluent is detected and quantified by mass chromatography after chemical ionization mass spectrometry. We now report our initial results using this system for the analysis of twelve selected pesticides and two pesticide metabolites in four commonly consumed commodities.

[†] Present address: FMC Corp., P.O. Box 8, Princeton, NJ 08540.

[‡] Permanent address: Department of Science and Research, Department of Environmental Protection, CN 409, Trenton, NJ 08625.

[§] Permanent address: Division of Rural Resources, Department of Agriculture, CN 330, Trenton, NJ 08625.

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EXPERIMENTAL SECTION

Chemicals. Acephate, azinphos-methyl, carbaryl, carbofuran, captafol, captan, chlorothalonil, dimethoate, methamidophos, and phosmet reference standards were purchased from Chem Service, Inc. (West Chester, PA). *cis*- and *trans*-permethrin reference standards were supplied by FMC Corp. (Princeton, NJ). Dichloran, 1-naphthol, tetrahydrophthalimide, *p*-bromonitrobenzene, and fluorene were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sodium chloride, anhydrous sodium sulfate, and high-purity HPLC grade acetone, petroleum ether, and dichloromethane were purchased from Fisher Scientific (Springfield, NJ).

Samples. One hundred (twenty five each) samples of apples, peaches, tomatoes, and potatoes were collected. New Jersey grown samples were collected from various farms in New Jersey and consisted of thirteen samples for each of the four crops. Out-of-state samples (twelve of each crop) were obtained from supermarket warehouses in New Jersey. The apple samples consisted of nine from New York, one from Pennsylvania, one from California, and one from Vermont. The tomato samples consisted of seven from Mexico, one from Israel, and four from Florida. The potato samples consisted of nine from Florida and three from California. The peaches consisted of five from Georgia and seven from South Carolina.

Instrumentation. A Varian Model 3400 gas chromatograph (Varian Associates, Walnut Creek, CA) interfaced to a Finnigan MAT ion trap detector equipped with chemical ionization (CI) (Finnigan MAT, San Jose, CA) and controlled by an IBM PC/AT was used. The analyses and quantifications were performed with Finnigan ion trap software Version 3.15. A splitless on-column injector held at 50 °C was used, and a 2 m × 0.53 mm (i.d.) deactivated fused silica precolumn was fitted between the injector and capillary column. Approximately 45 cm of tubing is removed from the precolumn after every five injections due to the accumulation of nonvolatile sample components. A new, 2-m precolumn is used after 20 injections. A 30 m × 0.25 mm (i.d.) J&W (Rancho Cordova, CA) DB-1 methyl silicone fused silica capillary column (1-μm film thickness) was held at 50 °C for 2 min and then temperature-programmed from 50 to 300 °C at 15 °C/min. Carrier gas (He) velocity was 25 cm/s, and the injection volume was 1 μL. A 15-cm syringe needle was used for the on-column injections. The mass spectrometer was operated in the chemical ionization mode using methane reagent gas at a source pressure that gave a 10/1 ratio for m/z 17 to m/z 16. Some chemical ionization experiments were carried out later using isobutane reagent gas at a pressure that gave a 2/1 ratio for m/z 43 to m/z 57. The filament voltage and current were 70 eV and 80 μA, respectively. Electron multiplier gain was 10^5. Scan range was from 70 to 420 amu at 1 s/scan. Transfer line and manifold temperatures were 250 and 220 °C, respectively.

Preparation of Calibration Curves. A 10-mg portion of each reference standard was dissolved in 100 mL of dichloromethane to give a 0.1 mg/mL stock solution. The stock solution was then serially diluted, and the appropriate amount of the internal standard stock solution was added. The standard solutions then contained 2, 5, 10, 20, 25, 30, and 50 ng/μL of each pesticide and metabolite and 25 ng/μL of each internal standard. Fluorene was the internal standard for nonhalogenated pesticides; *p*-bromonitrobenzene was used as internal standard for the halogenated pesticides. These solutions were analyzed by GC/CIMS three to four times at each concentration level by determining the areas of peaks obtained by mass chromatography (a computerized plot of specific ions vs time) at the correct retention times. The resulting calibration curves were used for all calculations. The calibration curves were linear within this concentration range, with linear correlation coefficients between 0.92 for carbofuran and 0.99 for methamidophos. The following ions were used for quantification: acephate, m/z 143; azinphos-methyl, m/z 160; *p*-bromonitrobenzene, m/z 202; captafol, m/z 312; captan, m/z 264; carbaryl, m/z 202; carbofuran, m/z 222; chlorothalonil, m/z 267; dichloran, m/z 207; dimethoate, m/z 230; fluorene, m/z 167; methamidophos, m/z 142; 1-naphthol, m/z 145; *cis*- and *trans*-permethrin, m/z 183; phosmet, m/z 318; tetrahydrophthalimide, m/z 152.

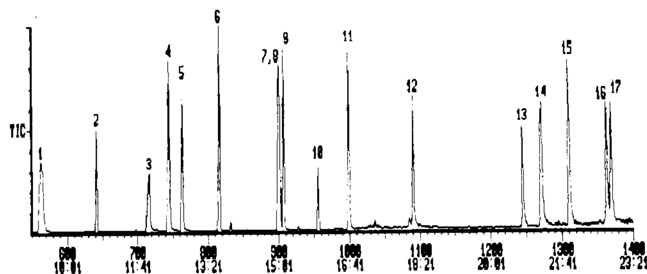

Figure 1. Total ion chromatogram of a standard solution containing 10 ng/μL of the fourteen pesticides and metabolites and 25 ng/μL of the two internal standards: methamidophos (1), *p*-bromonitrobenzene internal standard (2), acephate (3), tetrahydrophthalimide (4), 1-naphthol (5), fluorene internal standard (6), carbofuran (7) and dimethoate (8) coelute, dichloran (9), chlorothalonil (10), carbaryl (11), captan (12), captafol (13), phosmet (14), azinphos-methyl (15), *cis*-permethrin (16), *trans*-permethrin (17).

Sample Preparation. The Luke procedure was followed exactly up to the step where petroleum ether and acetone are added through a Snyder column, and the solution is reconcentrated. Instead, 50 mL of dichloromethane was added through the Snyder column, and the solution was reconcentrated to approximately 2 mL. (There is no need to partition the pesticides from dichloromethane into acetone because the chlorine-specific Hall detector is not used in the determinative step. Furthermore, dichloromethane is preferred over acetone in CIMS as the latter is a good proton acceptor that limits complete protonation of the compounds of interest.) The internal standard stock solution (40 μL) containing 2.5 mg/mL each *p*-bromonitrobenzene and fluorene was added, and the final volume was adjusted to 4 mL.

Sample Analyses. Samples were analyzed by GC/CIMS. At the completion of the analysis, the computer searched for each analyte at its retention time by mass chromatography of the analyte's quantification ion. If a mass chromatographic peak was present in the retention time window (relative retention time ±5 s), the methane CI mass spectrum of that peak was compared to the known methane CI mass spectrum of the analyte stored in the search file. All analytes that were found were then listed and quantified by the computer. Confirmation of the computer results was performed manually either by observing the mass spectrum or by plotting multiple mass chromatograms for the pesticide. This entire process takes only 2–5 min/sample, depending on the number of pesticides present.

Confirming ions used: acephate, m/z 184; azinphos-methyl, m/z 132; captafol, m/z 314, 280; captan, m/z 266, 236, 152; carbaryl, m/z 145; carbofuran, m/z 165; chlorothalonil, m/z 265, 269; dichloran, m/z 209; dimethoate, m/z 199; *cis*- and *trans*-permethrin, m/z 365; phosmet, m/z 160, 286. Methamidophos and 1-naphthol gave no additional ions.

Recovery Studies. Recovery studies were performed at the 0.5 ppm fortification level of each pesticide and metabolite at least three times in each of the four crops. These samples were prepared by adding 0.5 mL of the 0.1 mg/mL pesticide stock solution to 100 g of chopped fruit or vegetable before extraction. The extracts were then analyzed as previously described, and recoveries of the pesticides and metabolites were calculated by the instrument software, where the relative response factors of each pesticide (area of pesticide/area of internal standard) were applied to the calibration curves and the amount of each pesticide (ng/μL) was calculated. The amounts of each pesticide (ppm) in the crops were calculated as in the Luke procedure. The average volumes of the initial extract for potatoes, apples, peaches, and tomatoes were 238, 242, 249, and 252 mL, respectively.

Sensitivity Determinations. To 4-mL extracts of tomato, potato, apple, and peach samples previously determined to be free of any of the pesticides in this study were added 32 μL of the stock solution containing 0.1 mg/mL of each reference standard. Assuming 100% extraction efficiency, these solutions represent extracts of crops spiked at approximately the 0.1 ppm level for each pesticide and metabolite.

Table I. Recovery of Analytes at the 0.5 ppm Fortification Level

analyte	recovery, % (% coefficient of variation)			
	apples[a]	peaches[a]	potatoes[b]	tomatoes[c]
acephate	107.7 (3.5)	104.8 (8.7)	116.3 (4.9)	109.5 (11.9)
azinphos-methyl	87.1 (11.0)	80.0 (1.5)	94.7 (25.5)	82.3 (9.6)
carbaryl	91.7 (14.2)	94.0 (15.1)	93.4 (15.6)	95.6 (9.8)
carbofuran	107.4 (4.4)	107.5 (4.7)	100.8 (13.2)	112.3 (3.1)
captafol	93.6 (12.3)	73.8 (6.0)	86.8 (24.1)	91.1 (16.4)
captan	107.7 (14.8)	81.4 (15.4)	82.0 (22.3)	82.9 (10.3)
chlorothalonil	117.5 (5.9)	79.1 (14.0)	98.0 (14.0)	101.1 (12.4)
dichloran	95.6 (11.2)	102.7 (18.9)	87.2 (15.4)	95.9 (9.8)
dimethoate	114.7 (8.0)	120.0 (2.8)	115.2 (8.4)	114.5 (12.9)
1-naphthol	72.6 (8.7)	84.7 (21.2)	76.6 (8.4)	95.7 (11.6)
methamidophos	79.9 (7.3)	75.3 (1.3)	84.5 (14.4)	89.1 (8.1)
permethrins[d]	73.6 (6.9)	73.6 (15.1)	82.5 (10.4)	82.1 (12.3)
phosmet	105.3 (9.1)	105.3 (3.8)	91.1 (9.7)	94.1 (15.3)
tetrahydrophthalimide	113.1 (7.1)	113.1 (12.7)	103.6 (15.6)	115.2 (6.2)

[a] Analyses performed in triplicate. [b] All potato samples performed in quintuplicate except carbofuran, 1-naphthol, and phosmet, which were done in quadruplicate. [c] All analyses performed in quintuplicate, except for carbofuran, captan, dichloran, phosmet, and tetrahydrophthalimide, which were done in quadruplicate. [d] Spiked with 0.5 ppm each *cis*- and *trans*-permethrin but analyzed at 1.0 ppm because of incomplete GC separation.

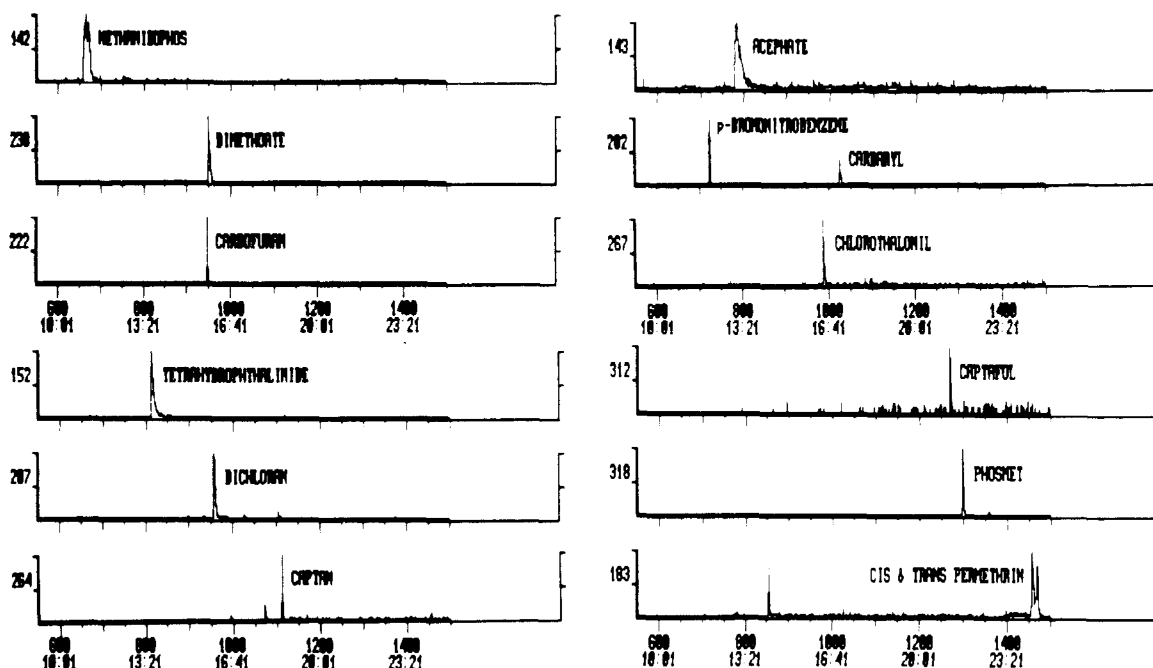

Figure 2. Methane chemical ionization mass chromatograms for some of the analytes spiked at the 0.5 ppm level before extraction: methamidophos, dimethoate, and carbofuran in potatoes; tetrahydrophthalimide, dichloran, and captan in peaches; acephate, carbaryl and chlorothalonil in tomatoes; captafol, phosmet, and the permethrins in apples.

RESULTS AND DISCUSSION

The four crops analyzed in this study were chosen because they are of economic importance to New Jersey agriculture and they comprise a large segment of the dietary intake of fruits and vegetables. The pesticides that were targeted in this study were chosen because they are suspected oncogens, mutagens, or teratogens or are frequently used on the four crops of interest.

Captan and captafol are classified as B_2 (probable human carcinogen) carcinogens by the EPA; acephate, chlorothalonil, permethrin, and phosmet are classified as C (possible human carcinogen) carcinogens; azinphos-methyl is a D carcinogen (not classified due to no or inadequate data). Methamidophos is used as a pesticide but is also a metabolite of acephate. Dichloran has been found to be a carcinogen in one animal study but not in another (EPA, 1980) and is unranked. Tetrahydrophthalimide is a metabolite of captan and captafol; 1-naphthol is a metabolite of carbaryl.

The chromatographic separation of a 10 ng/μL standard solution of the pesticides and metabolites studied and the two internal standards is shown in Figure 1. Although carbofuran and dimethoate elute together, one can still be analyzed in the presence of the other because carbofuran gives no response at m/z 230 while dimethoate gives no response at m/z 222. The results of the recovery studies in each of the four commodities for all the targeted analytes at the 0.5 ppm level are given in Table I. Recoveries were between 73 and 120%, and coefficients of variation were in the range of 1–26%, with an average coefficient of variation of 11% for the 56 analyses. With few exceptions, the targeted analytes could be detected (most with signal/noise ratios much greater than 5) at the 0.1 ppm level by methane chemical ionization mass chromatography. Methamidophos and captafol were detectable at 0.1 ppm in tomatoes, apples, and peaches but could not be detected at 0.1 ppm in potatoes. Subsequent sensitivity determinations at the 0.1 ppm level using isobutane instead of methane as the reagent gas

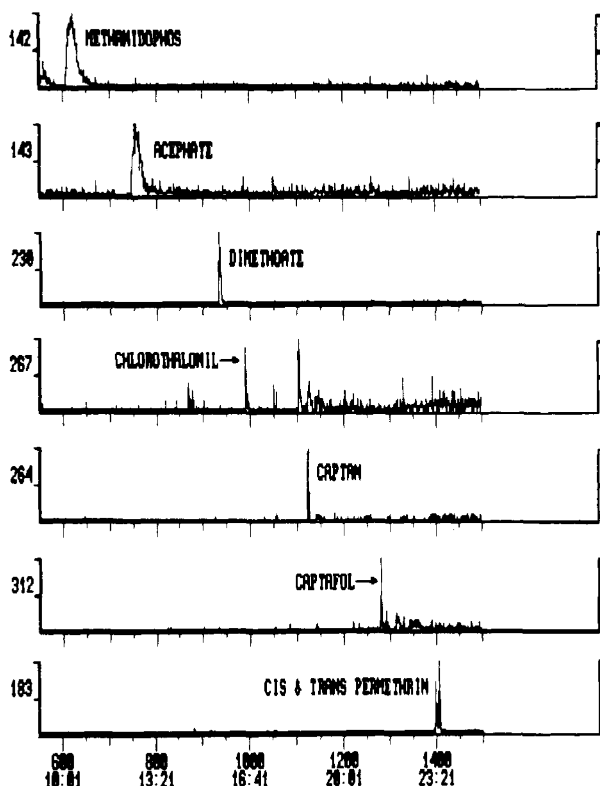

Figure 3. Isobutane chemical ionization mass chromatograms of some of the pesticides added to a tomato extract to simulate a 0.1 ppm determination.

demonstrated that all the analytes could be detected at the 0.1 ppm level with an estimated limit of detection of 0.05 ppm. Methane was originally chosen as the reagent gas because the Food and Drug Administration has an extensive compendium of methane chemical ionization spectra for pesticides and because we felt that some of the other ions produced during the methane chemical ionization process could serve to better confirm the identity of those pesticides found. The gentler ionization conditions afforded by isobutane, in general, result in a lower number of fragment ions and a more intense M + 1 ion that translates into improved sensitivity. In general, the higher the mass of the ion upon which detection and quantification is performed, the better the signal to noise ratio because the chances of interferences from matrix components are much reduced at higher masses. Some of the mass chromatograms obtained at the 0.5 and 0.1 ppm levels are shown in Figures 2 and 3, respectively. The ion trap detector, using isobutane in the chemical ionization mode, is very sensitive, with on-column sensitivities of 100–300 pg, depending on the pesticide analyzed.

The results of our analyses are summarized in Table II, and it is quite obvious that none of the targeted pesticides were found in concentrations above the tolerance limits set by the Environmental Protection Agency. Of the pesticides with known or suspected oncogenic and/or mutagenic activity, traces of dimethoate were found in one apple sample, phosmet (trace [2] and 0.37 ppm) was found in three of the apple samples, and captan (trace and 0.43, 0.44, and 0.70 ppm) was found in four of the peach samples. Dichloran was found in sixteen of the twenty-five peach samples (trace [3] and 0.29, 0.45, 0.79, 1.03, 1.16, 1.23, 1.28, 1.64, 3.55, 4.26, 4.78, 6.01, and 6.21 ppm).

Other pesticides with demonstrated or suspected oncogenic activity in laboratory animals (daminozide, linuron, benomyl) were also targeted for analysis and will be discussed here only briefly because they were determined by other methods. Linuron (limit of detection 0.1 ppm) was not found in any of the twenty-five potato (tolerance 1 ppm) samples analyzed (Mattern et al., 1989). The three other commodities were not analyzed because linuron is not used for their protection. Daminozide (limit of detection 0.05 ppm) was found in two of the twenty-five apple (tolerance 20 ppm) samples tested at concentrations of 1.04 and 0.32 ppm (Liu et al., 1989). Benomyl (limit of detection 0.025 ppm) was detected in eighteen of the twenty-five peach (tolerance 15 ppm) samples tested at concentrations of trace to 3.48 ppm with an average concentration, based on the twenty-five samples, of 0.84 ppm (Liu et al., 1990). Benomyl was also detected in six of the apple (tolerance 7 ppm) samples (trace (2) and 0.15, 0.16, 0.56, and 0.59 ppm). Three tomato (tolerance 5 ppm) samples contained trace levels (approximately 0.025 ppm) of benomyl.

Table II. Summary of Positive Findings

crop[a]	pesticide	no. pos findings	range, pm	tolerance, ppm
apple	dimethoate	1	tr	2.0
	phosmet	3	tr–0.37	10.0
	tetrahydrophthalimide	3	tr–0.20	
peach	captan	4	tr–0.70	50.0
	carbaryl	4	tr–0.20	10.0
	dichloran	16	tr–6.21	20.0
	tetrahydrophthalimide	12	tr–3.30	
tomato	methamidophos	2	0.72–0.80	1.0
	tetrahydrophthalimide	1	tr	
potato		0		

[a] Twenty-five samples of each crop were analyzed.

Our results confirm, at least in this limited study, that residues of most of the pesticides that are of major concern in terms of chronic effects are far below tolerance levels. Similar results have been obtained by others (Luke et al., 1988; Hundley et al., 1988) in far more extensive surveys. The results of these studies indicate that it is misleading to generate risk data on the basis of tolerance levels as was done in a recent well-publicized study (National Research Council, 1987).

In spite of these findings and those of others, it is important, for a variety of reasons, that regulatory agencies throughout the world continue to monitor the food supply for the presence of pesticides and that improved, more rapid analytical methods be used. We believe that the methods we have outlined in this paper could be expanded into a procedure which, at the very least, could be used for those pesticides that are extractable by the Luke procedure. The major advantages of our procedure over presently used methods are (1) the substitution of cold-on column injection capillary gas chromatography for hot-injector packed-column chromatography technology to permit the analysis of polar as well as some thermally labile pesticides and (2) the substitution of a single specific detector (based on mass) for the array of specific detectors currently used. The major disadvantage of our method is that autoinjectors cannot be used. The trade-off for this serious inconvenience is the ability to determine many more pesticides with only one injection. In order to determine just the twelve pesticides in this study by current methods, one chromatographic analysis would have to be made for those determined by the flame photometric detector (acephate, dimethoate, methamidophos, phosmet) and a second for those pesticides determined by the Hall detector (captafol, captan, chlorothalonil, dichloran) and a liquid chromatography procedure

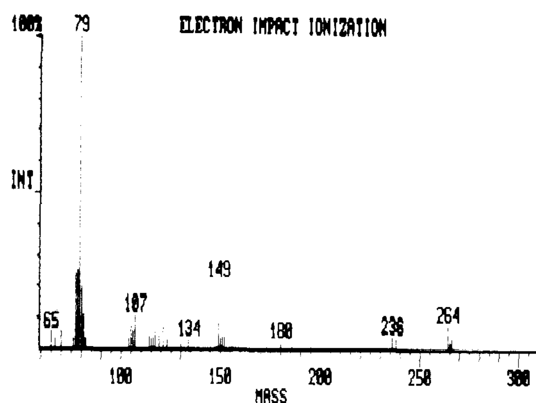

Figure 4. Comparison of electron impact (upper trace) and methane chemical ionization (lower trace) mass spectra of captan.

would be required for carbaryl and carbofuran. Finally, separate high-temperature runs would be required for azinphos-methyl (flame photometric) and permethrin (Hall) because they are late eluters.

Chemical ionization mass spectrometry (especially when isobutane is the reagent gas) produces only one or just a few different ions for each analyte and is important for obtaining the desired sensitivity. Of even greater importance, chemical ionization produces mainly M + 1 ions in contrast to the extensive fragmentation usually obtained under electron ionization conditions. The higher the mass that the analysis is carried out, the less chance there is for interferences from ions derived from the sample matrix. McLafferty (1963) compiled a list of the most abundant ions found in a file of 4000 mass spectra. Using his data for the three most abundant ions, we have calculated that the relative probabilities of interfering masses in the 40–99, 100–159, 160–219, 220–279, and 280–339 mass ranges are 187/83/49/13/1. As a practical example, the electron ionization (EI) and methane chemical ionization spectra of captan are compared in Figure 4. Use of EI forces one into detecting the m/z 79 ion while CI allows one to monitor the ion at m/z 264. Figure 5 compares the results obtained for the analysis of 0.4 ppm captan in one of our peach samples. The signal to noise ratio advantage of chemical ionization over electron impact is quite obvious.

The use of mass chromatography allows for the detection of several hundred ions of choice in any given analysis. The presence of one to three ions at a specific retention time is presumptive evidence that a specific pesticide is present. Although these two pieces of data do not by themselves confirm the presence of the pesticide, neither do present methods of analysis; an electron ion-

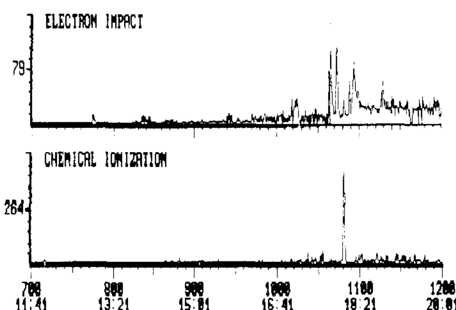

Figure 5. Comparison of mass chromatograms obtained from a peach sample with an incurred residue of 0.4 ppm captan under electron impact (upper trace) and methane chemical ionization (lower trace) conditions.

ization mass spectrum must be obtained for confirmation in both cases.

The cost of ion trap mass spectrometer including the chemical ionization option is much less than the cost of conventional mass spectrometers and compares favorably to the total cost of the three specific detectors currently used and the extra labor costs needed to make a complete analysis by conventional methods. It is easy to learn to operate the ion trap, and the instrument can be maintained easily. Because of its unique design, the ion trap is very sensitive in the full-scan mode and is well-suited to detection of analytes in subnanogram amounts, especially when mass chromatography is used to dramatically increase signal to noise ratios.

ACKNOWLEDGMENT

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Registry No. Methamidophos, 10265-92-6; acephate, 30560-19-1; tetrahydrophthalimide, 85-40-5; 1-naphthol, 90-15-3; carbofuran, 1563-66-2; dimethoate, 60-51-5; dichloran, 99-30-9; chlorothalonil, 1897-45-6; carbaryl, 63-25-2; captan, 133-06-2; captafol, 2425-06-1; phosmet, 732-11-6; azinphos-methyl, 86-50-0; *cis*-permethrin, 61949-76-6; *trans*-permethrin, 61949-77-7.

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Determination of Phytic Acid in Cottonseed by Near-Infrared Reflectance Spectroscopy

Frederick W. Parrish, John P. Madacsi, Brian Q. Phillippy,* Antonia G. Wilfred, and Steven M. Buco[†]

Southern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124

A rapid and simple method was developed to determine phytic acid (from 1.64% to 2.99%) in dehulled cottonseed kernels. Ground samples of 14 varieties of cottonseed were directly analyzed by near-infrared reflectance and by an ion chromatography reference method. Analysis of variance revealed that the highest F value of 60.6 was obtained with a wavelength combination of 1594 and 1226 nm. A correlation, R, of 0.88 was obtained between phytic acid values obtained by near-infrared reflectance and those obtained by ion chromatography of acid extracts with a standard error of 0.118. The new method obviates the need for an extraction step, which may take several hours, that is required in all previous methods.

The market value of grains and oilseeds is based on their proximate composition. Water, protein, and lipid contents of many agricultural commodities in worldwide commerce are now determined by near-infrared analysis (Wetzel, 1983). The phytic acid content of the seed could be used as an additional factor in pricing, because phytate [myoinositol hexakis(phosphate)], which is present in all seeds, can function as an antinutrient when consumed in excess. The major products derived from cottonseed are oil, cake, and meal. In 1985 the United States produced 546 500 tons of cottonseed oil and 1 587 000 tons of cake and meal (USDA, 1987). The cake and meal are currently used in animal feeds and in the future may be used to provide protein for human consumption.

In humans, major concern is over the bioavailability of minerals such as zinc, calcium, and iron, which are not readily absorbed when insolubilized as calcium phytate complexes (Wise, 1983). Phytic acid has also been linked to the inhibition of digestive enzymes such as protease (O'Dell and De Boland, 1976), lipase (Knuckles, 1988), and α-amylase (Knuckles and Betschart, 1987). In poultry feeds, a primary concern is the utilization of phytate phosphorus (Nelson, 1967; Scheideler and Sell, 1987).

In recent years the literature has been voluminous with methods for measuring phytic acid. Originally iron precipitation methods were used (Thompson and Erdman, 1982). A step-gradient ion-exchange procedure has been adopted as the official method of the Association of Official Analytical Chemists (Harland and Oberleas, 1986). While these also measure polyphosphates other than phytate (Phillippy et al., 1988), specific methods utilizing HPLC (Phillippy and Johnston, 1985) and NMR (Mazzola et al., 1986) are also available. All of the above methods require a time-consuming acid extraction prior to analysis. We report here a rapid, direct near-infrared (near-IR) analysis method for the determination of phytic acid in ground cottonseed that is simple to perform.

EXPERIMENTAL SECTION

Materials. Nineteen samples of whole fuzzy cottonseed were obtained from the National Cotton Variety Testing Program and represent fourteen varieties from three crop years grown in four regions of the United States (Eastern, Delta, Plains, and Arizona). Three of the samples were glandless seed. Sample preparation included delinting and dehulling of the fuzzy seed and grinding the cottonseed kernels in a Krups home-style coffee mill to a fine powder. The powder was sieved through a 590-μm screen to obtain uniform particle distribution. Sodium phytate, obtained from Sigma Chemical Co., was used as a reference to characterize the absorbance of phytic acid in the cottonseed powder.

Determination of Phytic Acid by Near-Infrared Reflectance. About 8 g of the milled sample was required to fill the sample cup for presentation to the instrument. Care was taken to ensure consistency of sample density when loading each sample. A Neotec 6350 scanning monochromator (Pacific Scientific) was used to collect the spectra in the near-infrared region. Spectral data for each sample, after 50 scans, were recorded at 2-nm intervals from 1100 to 2498 nm. Absorbance from the near-IR reflectance mode is described in terms of log $(1/R)$. Second derivatives of the log $(1/R)$ data were used to determine the wavelengths and equation constants for the calibration sample set.

[†] Present address: Statistical Resources, Inc., 7332 Highland Rd., Baton Rouge, LA 70808.

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