lemon juices. Their particle size distributions are plotted in Figures 1 through 5; note that the scale of the ordinate for juice concentrate differs substantially from the ordinates of the fresh juice plots. The plots of particle-size distribution show that the fresh, unpasteurized juices varied considerably, and that the properties of the reconstituted juice prepared from commercial concentrate were strikingly different. Although the turbidity of the reconstituted juice was higher than that of the unpasteurized fresh juices, its concentration of detectable particles was the smallest found. This is the clearest case yet encountered of the correlation of high turbidity with small modal diameter and narrow particle-size distribution.

The lack of correlation (Table V) between concentration of particles larger than 0.8 μm and turbidity index is not surprising when the distribution widths are considered. The two lowest turbidities correspond not only with the highest particle concentrations but also with the greatest distribution widths. Since the small particle portions of the particle-size distributions differ only slightly, these large widths indicate higher proportions of large particles and hence lower overall light-scattering capability. In juice from the SK lemons (Figure 4), modal diameter and distribution width were considerably greater than for any of the other juices. More work is needed to indicate how common such broad distributions may be.

Sedimentation Experiments. The differential sedimentation work with KBr indicated that the density of most of the juice particles ranged from 1.10 to 1.20 g/ml, and the median was about 1.17 g/ml. These results suggested that a gradient ranging from 10 to 60% sucrose would be suitable for isopyenic fractionation of lemon juice particles. The zonal rotor experiment, using such a sucrose gradient, provided evidence of the heterogeneity of the juice particles. Particles larger than 1.1 μm displayed a dominant band that spread from about 1.09 to about 1.21 g/ml. Most of the detectable particles were smaller than 1.1 μm. When all particles larger than 0.7 μm were counted, the band for particles larger than 1.1 μm was completely obscured by the bands of smaller particles, which were spread over all the fractions with no band dominating. The apparent density range of the smaller particles was about 1.01 to 1.26 g/ml. These results with particles from fresh, unpasteurized juice suggest that, in general, a narrow band of effective diameters includes a wide variety of densities. Such density variability is consistent both with the biological origin of the juice and with certain aspects of juice production. The bulk of the juice sac cell consists of the vacuole, whose contents are mixed with the cell sap during extraction. It is not known if the organelles originally present in the cell sap survive or if growth processes such as agglomeration occur. Nevertheless, it is probable that material originally present as organelles contributes significantly to juice particulates. The fragility of juice particles may be responsible for the contrast between Figure 2 and the other particle-size distributions. Apparently the concentration process led to the loss of large particles. This manifestation of particle fragility could be plausibly attributed to organelles that had remained intact prior to juice concentration but subsequently suffered membrane rupture or loss.

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Comparison of Extraction Methods for Triazine Herbicides in Root Crops Using Electrolytic Conductivity Detection

James F. Lawrence

A number of extraction methods for the analysis of triazine herbicides (atrazine, propazine, simazine, sencor, prometone) in foods, soil, and water were compared for gas chromatographic electrolytic conductivity determination in root crops (potato, carrot, turnip, beet, and parsnip). All of the methods produced similar results although they differed in the use of reagents and cleanup techniques. The use of the nitrogen-specific detection system made the hexane partition and column cleanup steps unnecessary, thus shortening analysis time considerably. The method of choice consisted of methanol extraction, followed by partitioning between water and chloroform. The chloroform extract was concentrated for direct glc analysis. Interferences appeared only as irregular changes in baseline. No interfering peaks were observed for any of the triazines in the crops studied at 0.02 ppm or greater.

The use of the Coulson electrolytic conductivity detector (CCD) for the determination of residues of nitrogen-containing pesticides has increased greatly in recent years. The utility of the CCD for the selective gas chromatographic analysis of S, Cl, or N-containing pesticides has been investigated by a number of workers (Cochrane and Greenhalgh, 1973; Cochrane and Wilson, 1971; Cochrane *et al.*, 1972; Greenhalgh and Cochrane, 1972; Laski and Watts, 1973; Lawrence, 1973; Patchett, 1970). These authors examined the response of a variety of pesticides as well as the effects of a number of operating parameters in the oxidative, reductive, and pyrolytic modes. As little as 0.1 ng of organic nitrogen may be detected in the reductive mode (Patchett, 1970). The determination of triazine herbicides in samples by CCD has been carried out by a few workers. Westlake *et al.* (1970) analyzed for ACD 15M

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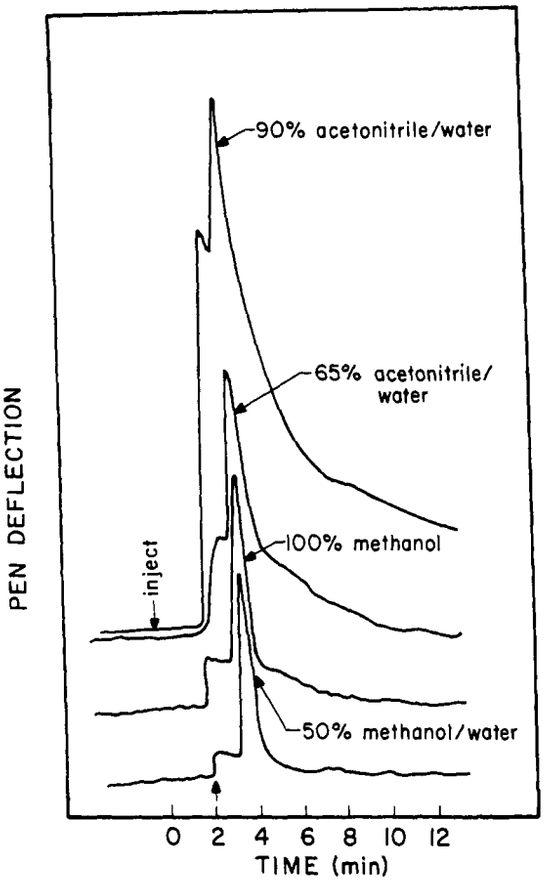

Figure 1. GLC results of various extractions of 0.1 ppm of atrazine in potatoes. A 20-ml aliquot of each extract was taken for partitioning and analysis. The vent valve was closed at 2 min after injection. Attenuation, 1×.

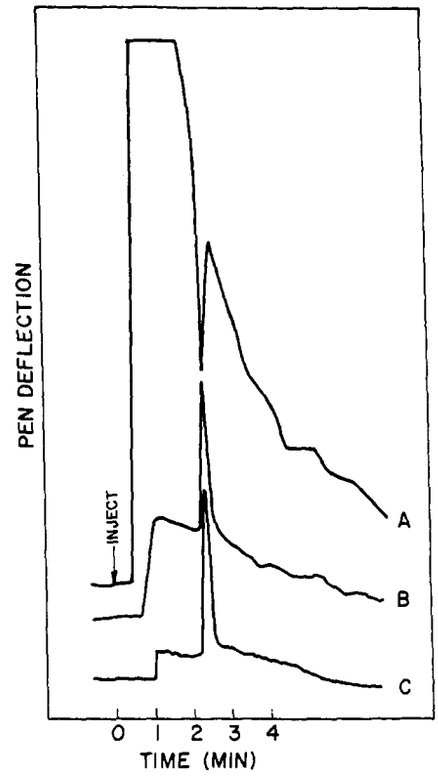

Figure 2. Influence of venting time on background for 0.2 ppm of atrazine in potatoes. 10 ng atrazine injected. A, negative peak obtained with 30-sec vent; B, 45-sec vent; C, 60-sec vent. Attenuation, 2×.

(2-chloro-4-isopropylamino-6-hydroxymethylamino-*s*-triazine) in corn plants. Eberle *et al.* (1969) and Hormann *et al.* (1972) developed an automated system for triazine analysis in soil and formulations. Purkayastha and Cochrane (1972) determined atrazine in soil, water, and corn by CCD. Coulson (1965) and Coulson *et al.* (1960) compared the CCD with ec detection for organochlorine compounds and concluded that the CCD was superior to ec detection. Purkayastha and Cochrane (1972) came to a similar conclusion for atrazine analysis in corn samples.

The analysis of triazines in root crops is of particular interest at present. These herbicides are usually applied to the soil before plant emergence. Thus, root crops may accumulate significant quantities of these compounds by penetration into the root system or by adsorption on the root surface. Thus, it was decided to investigate the use of the CCD for triazine analysis in these vegetables. The present work deals with the results of evaluating several existing extraction methods for triazines for application to the analysis of root crops using gas chromatography with electrolytic conductivity detection. The methods were standardized for sample size and quantities of solvents used.

MATERIALS

Apparatus. An Aerograph HY-FI Model 600C gas chromatograph equipped with a Coulson conductivity detector (Tracor Inc., Austin, Tex.) and a 6 ft × 6 mm o.d. glass column packed with 4% SE 30 on Chromosorb W/HP (80–100 mesh) was used for the determinations. (A 2% Reoplex 400 column was used to separate and identify each of the triazines studied; Mattson *et al.*, 1965.) Operating conditions were: column temperature, 195°; transfer unit, 200°; pyrolysis furnace temperature, 780°; helium carrier flow, 60 ml/min; helium sweep flow, 60 ml/min; hydrogen flow, 50 ml/min; D.C. bridge potential, 30 V. A 1-cm plug of strontium hydroxide coated glass wool was inserted at the exit of the quartz pyrolysis tube and a 50-strand nickel wire catalyst was placed in the center of the tube. The reservoir water level was maintained just above the pump entrance. A 1.0 mV strip-chart recorder operating at 0.25 in./min was employed. Peak height was used for quantitation.

Reagents. The herbicides studied were: atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine], propazine [2-chloro-4,6-bis(isopropylamino-*s*-triazine], prometone [2-methoxy-4,6-bis(isopropylamino-*s*-triazine], and sencor (4-amino-6-*tert*-butyl-3-(methylthio)-*s*-triazin-5-(4*H*)-one], all of which, except sencor (Chemagro), were obtained from CIBA-Geigy. Solutions of these were prepared in methanol. All organic solvents were analytical grade, glass distilled, and residue free. The crops used were potatoes, carrots, turnips, parsnips, and beets.

ANALYTICAL PROCEDURE

Sample Extraction. The washed sample (100 g) was chopped, placed in a Waring blender, and fortified with atrazine. The mixture was blended with 150 ml of methanol (225 ml for parsnips) (Delley *et al.*, 1967; Shultz, 1970; Westlake *et al.*, 1970). Other extracting solvents examined were: 80% (v/v) and 50% v/v methanol-water (Beynon, 1972); 90% (v/v) acetonitrile-water (Hormann *et al.*, 1972); 35% (v/v) acetonitrile-water (Purkayastha and Cochrane, 1972); methanol-dichloromethane-ammonia (40:75:1) (Benfield and Chilwell, 1964); 65% v/v acetonitrile-water. The macerate was suction filtered through a medium porosity 600-ml sintered glass funnel. The blender jar was rinsed with 25 ml of methanol and the washings were transferred to the filter funnel for collection. A 10-ml aliquot of the filtrate (which must be warmed to room temperature) was added to 100 ml of distilled water in a 250-ml separatory funnel for partitioning.

Table I. Percent Recovery from Root Crops[a]

Crop	ppm added	% recovered				
		Atrazine	Simazine	Propazine	Sencor	Prometone
Potato	10					94
	1	98[b]	103[b]	100	92	107
	0.1	95[b]	102[b]	100	93	96
	0.05	90[b]	98			
	0.02	94[b]	90	99	103	90
Carrot	1	101[b]	94[b]	105	90	89
	0.1	96[b]	95[b]	89	82	91
	0.05	98[b]	90[b]			
	0.02	100[b]	87	96	89	85
Turnip	1	97	84	99	104	98
	0.1	95	88	102	90	
	0.02	106	99	89	87	83
Beet	1	97	100	95	95	
	0.1	90	96	91	89	91
	0.02	98	90	105	82	78
Parsnip	1	106	94	92	99	
	0.1	91	82	88	95	93
	0.02	85	105		98	88

[a] Methanol extraction, no hexane partition or column cleanup. Blanks indicate that no samples were analyzed. [b] Average of three values.

Partitioning. The diluted aliquot was partitioned with 100 ml of hexane (Purkayastha and Cochrane, 1972; Shultz, 1970; Westlake *et al.*, 1970) which was reextracted with 50 ml of water and then discarded. Then 50 ml of water was added to the original aqueous phase. The aqueous layer was partitioned with 3 × 50 ml of dichloromethane (McKane *et al.*, 1972; Purkayastha and Cochrane, 1972; Westlake *et al.*, 1970). The combined extracts were evaporated to about 1 ml by rotary vacuum evaporation at 30°. Other partitioning solvents used were: chloroform or ethyl acetate (Beynon, 1972); diethyl ether (Delley *et al.*, 1967; Shultz, 1970); hexane–ether (2:1) (Hormann *et al.*, 1972).

Column Chromatography. The evaporated extract was quantitatively transferred to the top of an 8-cm 13% deactivated basic alumina (Woelm) column. The column was eluted with 50 ml of 2% diethyl ether in CCl_4, which was discarded. Following this, 100 ml of 6% diethyl ether in $CHCl_3$ was passed through the column and collected (Purkayastha and Cochrane, 1972). Other column chromatography systems examined were: a 16-cm 7.5% deactivated basic alumina column eluted with 100 ml of hexane–ether (1:1) discarding the first 15 ml (Shultz, 1970); a 16-cm 6% deactivated basic alumina column eluted with 100 ml of diethyl ether–0.5% water (Delley *et al.*, 1967); and an 8-cm Florisil column (activated at 130° for 1 hr) eluted with 100 ml of methylene chloride (Westlake *et al.*, 1970). The collected eluants were evaporated to dryness as above and dissolved in 1 ml of ethyl acetate for gas chromatography.

Comparisons were made between results of cleanup with and without the hexane partition and the column chromatography.

RESULTS AND DISCUSSION

Extractions. All extracting solvents quantitatively removed atrazine from the samples. However, the time of filtration of the macerates increased considerably with the increased water content of the extracting solvent. The 50% methanol–water and 35% acetonitrile–water systems took 20–30 min to filter, while the methanol and the 90% acetonitrile–water took 5–10 min. However, the CCD results showed that the extracting solvents containing a high proportion of water decreased the amount of background in the chromatograms. Figure 1 compares chromatograms of the different extracts from potatoes (0.1 ppm of atrazine) which were partitioned with dichloromethane but without the hexane partition and column chromatography cleanup. The 50% methanol–water produced the least interferences. The column cleanup reduced the high backgrounds obtained with the 90% and the 65% acetonitrile–water systems.

Partitions. The hexane partitioning was found unnecessary for the CCD analysis of the triazines in the root crops studied. This partitioning also necessitated a reextraction of the hexane to recover removed herbicides.

The second partition was accomplished with equal efficiency using chloroform or dichloromethane. Hexane–diethyl ether (1:1), diethyl ether and ethyl acetate removed more impurities from the crops than the first two, resulting in higher baselines in the chromatograms. The appearance of emulsions was greater with the samples which were extracted with the water-containing solvents. The 50% methanol–water and the 35% acetonitrile caused the most persistent emulsions in the partitioning, while the methanol and 90% acetonitrile–water extractions produced little or no emulsion formation.

Column Chromatography. All column cleanup systems removed the pigments from the residue extracts. Background interferences were decreased for all extraction systems. The acetonitrile extractions still exhibited more background interference than the methanol system.

Gas Chromatography. Interferences in results at low levels of herbicides (<0.1 ppm) were observed as high or irregular baselines after venting due to sample background. No interfering peaks were observed for any of the herbicides in any of the samples analyzed down to 0.2 ppm. At 0.02 ppm occasional peaks appeared in the turnip and parsnip samples after the triazine peak but did not interfere. The use of the vent valve to remove interferences at the solvent front was necessary at lower levels of herbicide for which concentration of the final extract was required. The vent valve was maintained in the vent position for 2 min after injection. This practice kept the pyrolysis tube and detector system free from contamination by materials at the solvent front, which often caused a decrease in sensitivity and efficiency. Figure 2 shows the increase in background as the vent time is shortened. When 30-sec venting was employed, occasional negative peaks appeared for the triazines, indicating that the interferences were acidic and had overloaded the scrubber. At high concentrations (0.2–1 ppm) venting was less critical.

The peaks were generally narrow and symmetrical. Peak height was found to be satisfactory for quantitating the herbicides.

Choice of Method. Most methods examined were suit-

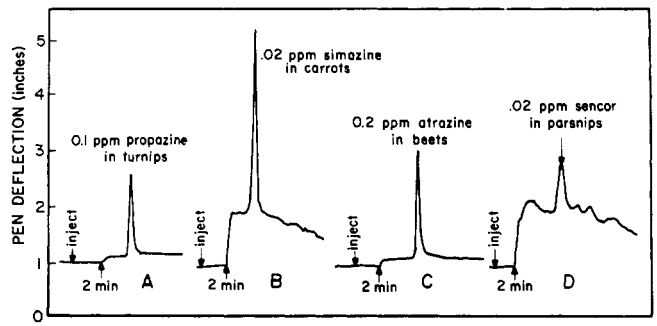

Figure 3. Chromatograms of some root crops analyzed by the developed method. Chromatograms were obtained on different days and are not comparable. Attenuation, 2×. A, 4 ng propazine; B, 6 ng simazine; C, 4 ng atrazine; D, 6 ng sencor.

able for the quantitative glc–CCD analysis for triazines in root crops. With the methanol extraction methods, the hexane partition and column cleanup were not required and thus a considerable saving of analysis time resulted. Methanol was chosen as the extraction solvent because it filtered rapidly from the macerate and produced no emulsions in the partitions. Chloroform or dichloromethane was chosen over the other partitioning solvents because of fewer impurities extracted. These extracts were dried over anhydrous Na_2SO_4, evaporated to dryness, and dissolved in ethyl acetate for glc analysis. Although the extracts were yellow, no significant interferences were encountered. Figure 3 illustrates results from several vegetables at 0.2–0.02 ppm using the above method. Table I shows the recoveries obtained using this method for a number of triazines down to 0.02 ppm. The triazines are essentially quantitatively extracted from the crops down to the 0.02-ppm level. At the lower concentrations, it was possible to take a 20-ml aliquot of the methanol for partitioning and analysis. However, larger volumes required evaporation to about 10–20 ml before partitioning. The methanol extracts and the final ethyl acetate extracts were kept for 2 weeks at −2° with no effect on quantitative results for any of the herbicides studied.

CONCLUSIONS

The Coulson electrolytic conductivity detector has proven to be very useful for the analysis of triazines in root crops. The detector makes hexane partitioning and column cleanup unnecessary. Analysis time is decreased greatly while quantitation for low levels of triazine (<0.1 ppm) is equal to other detection methods.

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Hematological Effects of Injected Gossypol and Iron in Rats

Jim N. Tone* and D. Reed Jensen

Studies designed to determine the hematological effects of intraperitoneally injected gossypol acetic acid and iron in rats were carried out. A total of 185 animals were divided into four groups. One experimental group was injected with corn oil containing 2 mg of gossypol acetic acid/ml at a dosage level of 1.5 mg/100 g of body weight; control animals were injected with 1.2 ml of corn oil. Another group was injected with the same dosage of gossypol and intramuscularly with 2 ml of iron sorbitex containing 50 mg of iron/ml; control animals were injected with 1.2 ml of corn oil and 2 ml of iron sorbitex. Gossypol injection resulted in an increase in erythrocytes, packed cell volumes, and hemoglobin concentrations during the first 7 days of postinjection; these same hematologic parameters were reduced below normal values by the 14th day postinjection. On the other hand, iron-injected animals had a normal erythrocyte population at the 14th day postinjection. Free and bound gossypol accumulated in the livers of gossypol-injected animals and was progressively eliminated as the postinjection time was increased.

A toxic effect of ingested gossypol on some blood constituents of nonruminant animals has been established. Gossypol has a hemolytic effect on erythrocytes and inhibits the dissociation of oxyhemoglobin (Menaul, 1922, 1923). Pigs which were fed high levels of gossypol developed a hypoprothrombinemia and a decreased hemoglobin concentration (Clawson *et al.*, 1962; Harms and Holley, 1951). Rats which were fed high levels of free gossypol for 28 days developed a microcytic-hypochromic anemia (Danke and Tillman, 1965). Supplemental iron in the diet alleviated this condition.

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