Specific Determination of Urea Herbicide Residues by EC Gas Chromatography After Hydrolysis and Iodine Derivative Formation

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The classical residue method for urea herbicides includes alkaline hydrolysis of the parent compounds to the corresponding anilines, followed by steam distillation/extraction and colorimetric measurement (1, 2). Provided limited method sensitivity (0.1-0.2 p.p.m.) is acceptable, the anilines may alternatively be determined by gas chromatography using thermal conductivity or flame ionisation detection (1, 3). Improved sensitivity (about 0.02 p.p.m.) has been reported by Gutenmann and Lisk (4, 5) who brominated the anilines in aqueous acid media before detecting them by EC gas chromatography. These bromination techniques, when applied to a variety of urea compounds and crop materials, have given erratic results in our laboratories. Bromination of 4-monosubstituted anilines was more successful when carried out in ethereal solution, as described by van Middelem et al. (6) for carbamates. However, when the same procedure was applied to 3- or disubstituted compounds, a mixture of brominated derivatives was consistently observed.

We wish to report an aniline-derivative/gaschromatographic technique which is based on the Sandmeier iodination reaction and which has been found to be applicable

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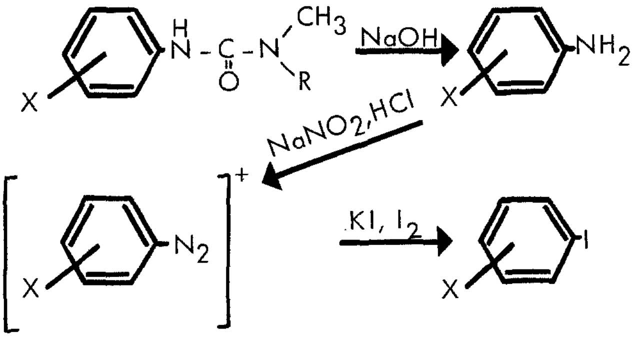
to all phenylurea herbicides at present available[1]. In this method, the anilines, after hydrolysis and steam distillation/extraction, are diazotized and the diazo-moiety is then exchanged for iodine by potassium iodide treatment, using iodine as a catalyst:



The iodinated derivatives so obtained are easily gas chromatographed with a minimum detectability of around 0.01 to 0.05 p.p.m.

[1] Compounds mentioned in the text:

Common name/ code number	X	R
Fenuron	-	CH_3
Monuron	4-Cl	CH_3
Monolinuron	4-Cl	OCH_3
Buturon	4-Cl	$CH(CH_3)C\equiv CH$
Metobromuron	4-Br	OCH_3
Diuron	3,4-Cl,Cl	CH_3
Linuron	3,4-Cl,Cl	OCH_3
Neburon	3,4-Cl,Cl	C_4H_9
C-6313	3-Cl,4-Br	OCH_3
Fluometuron	$3-CF_3$	CH_3
Chloroxuron	4-phenoxy-(4'-Cl)	CH_3

Experimental

Hydrolysis, Steam Distillation/Extraction. Crop and soil samples (20-60 g) were subjected to alkaline hydrolysis[2] and steam distillation/extraction according to the standard procedure (1, 2). The distillation/extraction head originally designed by Bleidner *et al.* (7) was modified to permit more rapid operation (Fig. 1). Operating time for the different ureas were as follows: 2 hours for fluometuron, monolinuron, metobromuron, and monuron; 3 hours for C-6313, diuron, and linuron; 5 hours for chloroxuron.

Diazotisation. A 20 ml aliquot of the isooctane (hexane) phase, normally representing 10 g of plant or soil material, was extracted with two 5-ml portions of 1N hydrochloric acid in a 50-ml separatory funnel. The combined acid extracts were transferred to a glass- or Teflon-stoppered 50-ml Erlenmeyer flask and cooled to 0°C in an ice bath. After adding 2 ml of 1 % aqueous sodium

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[2] Normal foam formation was suppressed by adding 4-5 ml of liquid Nopco NXZ antifoam (Nopco Chemical Co. Newark, N.J.) to the hydrolysis mixture. With a number of still troublesome starchy plant commodities (potatoes, corn, wheat, and oat grains, etc.) persistent foaming was drastically reduced by subjecting the material to one hour acid refluxing with 0.5N hydrochloric acid prior to alkaline hydrolysis. After the acid treatment, the suspension was allowed to cool to room temperature. The sodium hydroxide solution was then added through the condenser and the mixture was again allowed to cool before connecting the flask to the distillation/extraction head

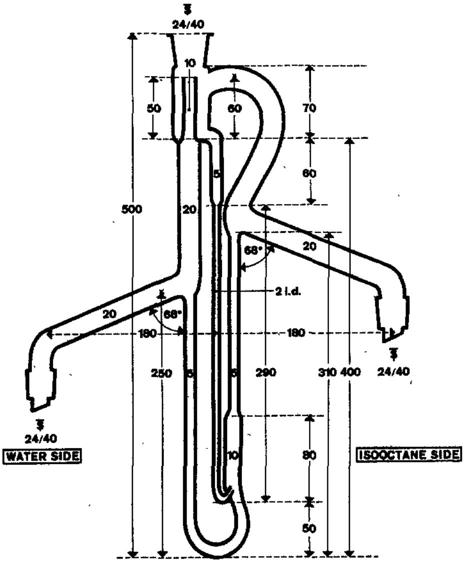

Fig. 1. Dimensions (in mm) of distillation/extraction head according to Bleidner *et al.* (7), as modified by W. Heizler of CIBA Ltd.

nitrite, the mixture was allowed to stand at 0°C for 25 minutes. Excess nitrite was then destroyed by adding 2 ml of 10 % aqueous sulfamic acid. The flask was vigorlusly shaken until nitrogen generation stopped.

Iodination Reaction. To the ice-cold diazonium salt solution, 0.5 ml of potassium iodide/iodine solution3) was added and the mixture was allowed to stand at room temperature for 25 minutes. It was then transferred to a room-temperature water bath which was heated to boiling

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3) Dissolve 2.5 g iodine in 50 ml of a 10 % aqueous potassium iodide solution.

within 15 minutes (the stoppers were periodically loosened to prevent excess pressure). After 5 minutes in boiling water, the flasks were removed from the bath and allowed to cool to room temperature. Excess iodine in each flask was reduced by adding about 200 mg of sodium sulfite powder, whereupon the solutions turned colorless. Each reaction mixture was made alkaline with 1.1 ml of 10N sodium hydroxide solution and extracted with 20 ml of n-hexane. After vigorous shaking, a few ml of the upper, organic phase was transferred to a 10-ml screw-capped vial with a pipette.

To avoid interferences in subsequent gas chromatographic analyses, hexane extracts derived from plant or soil samples were further cleaned up by shaking them with about the same volume of 1 % potassium permanganate in 1N sodium hydroxide solution. This mixture was allowed to stand for 1 to 2 hours before agitating it again and transferring the hexane to a clean 10-ml vial.

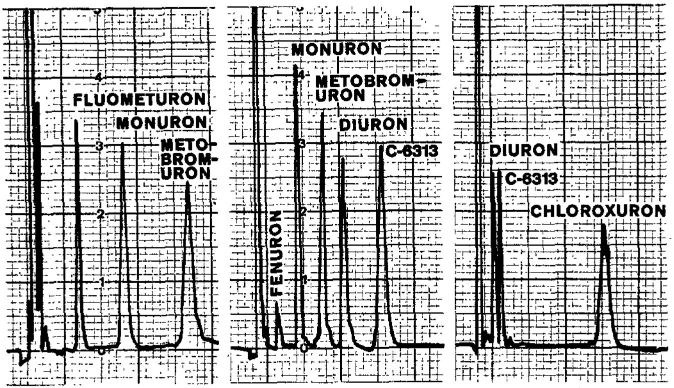
Calibration Curve. A 10 μg-quantity of each aniline (1 μg for fluometuron) in 10 ml of 1N hydrochloric acid was subjected to the diazotisation/iodination procedures as described above. Each hexane solution was diluted to permit injection of aniline equivalents ranging from 10 to 500 pg in no more than 4 μl of solvent. The peak heights were plotted against pg aniline equivalents on a log - log scale (Fig. 3).

Gaschromatographic Analysis. An F & M Model 402 High Efficiency Gas Chromatograph equipped with a pulsed type electron capture detector was used for chromatography and detection. The 120 cm x 3 mm i.d. borosilicate glass column packed with 10 % QF-1-0065 (Dow Chemical Co.) on 72 - 96 mesh Celite 545 (Johns-Manville Corp.) was maintained as follows: oven-150°C (120°C for fluometuron, 200°C for chloroxuron); inlet-155°C (125°C for fluometuron, 210°C for chloroxuron); detector-200°C, pulse interval 150 µsec., range x attenuation 10x64(x32, x128), purge gas 5 % methane in argon at 37 ml/min. The carrier gas was 5 % methane in argon at 23 ml/min. (57 ml/min. for chloroxuron). With the chromatograph stabilized under the conditions described above, 1 - 4 µl of sample or standard hexane solution was injected.

Retention times relative to iodoform were as follows for their respective iodine derivatives: fluometuron = 0.7 (120°C), fenuron = 0.8, monuron (monolinuron, buturon) = 1.4, metobromuron = 2.0, diuron (linuron, neburon) = 2.5, C-6313 = 3.5, chloroxuron = 15.7 (200°C, 57 ml/min.).

Results

Standards. The gas chromatographic separation and detector responses of the standard iodine derivatives prepared from the anilines of the various urea herbicides are demonstrated in Fig. 2.



t = 120°
f = 23 ml./min.
r x a = 10 x 64

t = 150°
f = 23 ml./min.
r x a = 10 x 128

t = 200°
f = 57 ml./min.
r x a = 10 x 128

Fig. 2. Electron capture gaschromatograms demonstrating separation and detector response of standard iodine derivatives prepared from aniline moieties of some urea herbicides. Quantities injected = 100 pg aniline equivalents (50 pg fluometuron; 300 pg chloroxuron); t = column temperature, f = carrier gas flow rate, r x a = range x attenuation settings.

In addition to the standard gaschromatographic conditions applied to the majority of the compounds examined (center chromatogram) it was found advantageous to use a lower column temperature for the specific detection of fluometuron (left-hand chromatogram) and a higher column temperature and increased carrier gas flow for the detection of chloroxuron (right-hand chromatogram). Depending on the type and number of halogen ring substituents, quantities of 50 to 300 pg in terms of aniline equivalents were found to give a detector response of 40-60 % full scale deflection under the particular conditions applied. A slightly lower response was observed for the iodine derivative of unsubstituted aniline (hydrolysis product of fenuron). Standard calibration curves for the CIBA-herbicides metobromuron, fluometuron, C-6313 and chloroxuron obtained by plotting peak height versus picograms of aniline equivalents on a log - log scale are shown in Fig. 3. The curves were found to be linear within the range (extended to 10 - 500 pg) examined.

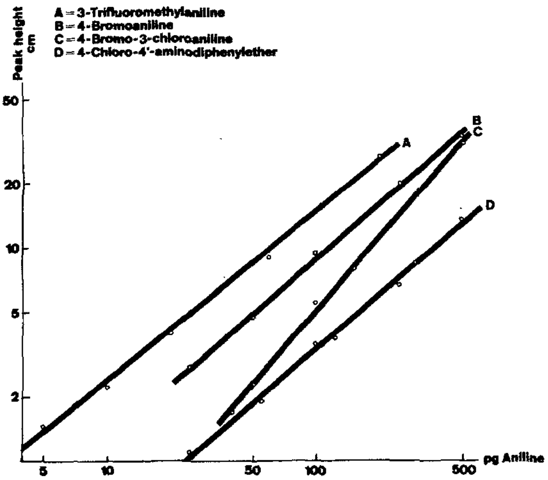


Fig. 3. Calibration curves for iodine derivatives of 3-trifluoromethylaniline (fluometuron), 4-bromoaniline (metobromuron), 4-bromo-3-chloroaniline (C-6313) and 4-chloro-4'-aminodiphenylether (chloroxuron).

Crop samples. Control (untreated) samples of apples, plums, potatoes and wheat and samples of the same crops fortified with known quantities of fluometuron, metobromuron, C-6313 and chloroxuron ranging from 0.05 to 0.5 p.p.m. were carried through the entire procedure described above. Typical examples of chromatograms of these untreated and fortified crops are in Fig. 4.

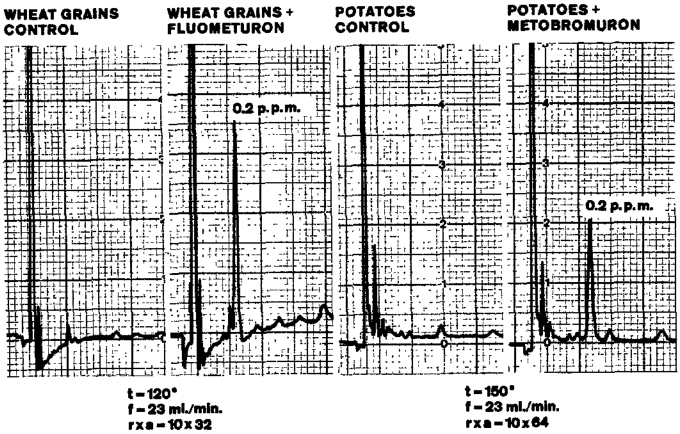


Fig. 4. Electron capture gaschromatograms obtained after diazotisation/iodination of distillates derived from untreated and fortified crop materials; t = column temperature, f = carrier gas flow rate, r x a = range x attenuation settings.

Recoveries varied between 85 and 102 %. None of the control samples so far examined has shown any significant false peaks interferring with those of fluometuron, metobromuron, C-6313 or chloroxuron. Consequently, the limit of detection of the method was not determined by the standard deviation of the controls but by the smallest peak height, which could be measured with reasonable accuracy. Admitting this minimal peak height to be 0.6 to 1.0 cm, the limits of detection for the different compounds in terms of the original herbicide in crops or soils were determined to be as follows : fluometuron 0.01 p.p.m., metobromuron 0.03 p.p.m., C-6313 0.05 p.p.m., chloroxuron 0.07 p.p.m. If required, this detectability may be increased by starting with a larger aliquot of the original isooctane extract.

For serial analyses, 10 to 12 samples at a time were carried through the diazotisation/iodination procedure within a period of about 2 hours.

Discussion

The specificity of the gaschromatographic method described above is superior to that of the standard colorimetric procedure (8) or of the recently described thin-layer chromatographic separation of the anilines (9). It permits satisfactory separation of mixtures of such closely related compounds as 4-chloroaniline and 4-bromoaniline or 3,4-dichloroaniline and 3-chloro-4-bromoaniline. However, it has to be remembered that the present method will distinguish only between urea herbicides having differing phenyl moieties. Compounds with identical substituents (e.g. monuron, buturon and monolinuron or diuron, neburon and linuron) have to be separated before separate hydrolysis to their anilines.

Potential application of the present iodine-derivative gaschromatographic method is not limited to urea herbicides ; it may be extended to other pesticides, which upon hydrolysis or reduction hydrolysis yield aromatic amines (e.g. phenylcarbamates, anilides, parathion, etc.). It has been successfully applied for residue determinations of the acaricide chlorphenamidine [3-(2-methyl-4-chlorophenyl)-1,1-dimethylformamidine] .

Acknowledgment

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