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# Determination of Chloroxuron in Strawberries Using Liquid Chromatography with Electron-capture Detection

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The phenylurea herbicide chloroxuron ($N^1$-dimethyl-$N^3$-4-(4-chlorophenoxy)phenylurea; Cx, see Fig. 1) is used in agriculture as a selective herbicide on strawberries. In the Netherlands, the residue tolerance for Cx in strawberries, until recently, was 200 ppb (Ministerie van Landbouw en Visserij, 1981); in 1984, this limit has been reduced to 50 ppb (Ministerie van Landbouw en Visserij, 1984). Obviously, reliable determination of Cx at the sub-ppm level requires a selective and sensitive method of analysis.

All phenylureas can, in principle, conveniently be determined via reversed-phase column liquid chromatography (LC) with UV detection (De Kok et al., 1984a; De Kok et al., 1984b). Sensitivity and selectivity are, however, generally too low to solve trace-level problems. In such cases, gas chromatography (GC) often is an attractive alternative. However, most phenylureas rapidly decompose into their isocyanates at the temperatures prevalent in GC. Analysis via GC can also be based on the hydrolysis of a phenylurea to its corresponding aniline, which is then derivatized for sensitive electron-capture detection (ECD) (De Kok et al., 1984a; De Kok et al., 1984b). Hydrolysis is, however, time-consuming and it should be realized that all procedures based on hydrolysis have an inherent lack of selectivity, because they cannot distinguish between anilines initially present in the sample, and those originating from the herbicide.

Recent research has shown that the phenylureas can rapidly be determined via direct derivatization with heptafluorobutyric anhydride (HFBA) (Brinkman et al., 1984). These derivatives - for their structure, see Fig. 1 - are sufficiently stable to allow analysis by GC-ECD excepting, however, the HFB derivatives of Cx and difenoxuron which do not show up in GC-ECD. A successful alternative for all phenylureas is the combination of column liquid chromatography with electron-capture detection (LC-ECD) (Brinkman et al., 1984). In the present communication, this technique is applied for the residue analysis of Cx in strawberries.

Chloroxuron Chloroxuron-HFB

Figure 1: Structures of chloroxuron, Cx, and the direct HFB derivative of chloroxuron, HFB-Cx.

## MATERIALS AND METHODS

Chloroxuron was a gift from the Food Inspection Service (Amsterdam, the Netherlands). Stock solutions were made in toluene, hexane or acetone. All solvents were analytical-grade products from Baker (Deventer, the Netherlands). Hexane and toluene were distilled over sodium before use. HFBA from Merck (Darmstadt, F.R.G.) was used as received. Inland and foreign cultivated strawberries were used.

The LC system consisted of an Orlita (Giessen, F.R.G.) Model 034 sRC reciprocating pump, a Valco (Houston, TX, U.S.A.) six-port injection valve with a 10-µl loop, and a 25 cm x 4.6 mm I.D. stainless-steel column packed with 10-µm LiChrosorb $NH_2$ (Chrompack, Middelburg, the Netherlands). Mixtures of hexane and toluene were used as mobile phase. For detection, the LC column was connected with a Pye Unicam (Philips, Eindhoven, the Netherlands) $^{63}$Ni electron-capture detector via a vaporization interface. The interface (De Kok et al., 1982) essentially consists of a 75 cm x 0.25 mm I.D. capillary enclosed in a massive stainless-steel block and kept at a temperature of 300°C. For the rest, the set-up was slightly different from that used in most of our previous work: the interface was simply installed in the injection port of a Pye Unicam Model GCV GC apparatus and directly connected with the detector via a stainless-steel capillary situated in the GC oven (cf. Krull et al., 1980).

20 g of strawberries were spiked with a suitable amount of Cx. Sample extraction was done by mixing the strawberries with 30 ml of dichloromethane in a Waring blender, and subsequent centrifugation. The organic layer was separated and the residue washed with 10 ml of dichloromethane. The organic phases were combined and their volume reduced to 10 ml under a stream of nitrogen, then washed with 10 ml of demineralized water and dried over anhydrous magnesium sulphate. Next, the solvent was gently evaporated and the residue reconstituted in 5 ml of hexane. A 1-ml aliquot in hexane was mixed with 20 µl of HFBA in a centrifuge tube, and shaken for 30 sec after stoppering the tube. Derivatization took a further 60 min at a temperature

of 60°C. After derivatization, the excess of reagent was destroyed by shaking the organic solution for 30 sec with 2 ml of a phosphate buffer of pH 8. The layers were then separated and the aqueous layer washed three times with hexane. The hexane phases were collected, evaporated to 1 ml and dried over anhydrous magnesium sulphate. The dry hexane extract was used for injection.

After derivatization and the final wash step, samples should not be stored for a prolonged period of time, because signal intensity typically decreases some 20% within 24 h. For more prolonged storage, the sample solutions must be kept at a temperature of -24°C.

## RESULTS AND DISCUSSION

Preliminary experiments were carried out with solutions of Cx in hexane, the final solvent in the procedure used with real samples. At a concentration of 2.4 µg of Cx per ml - which corresponds with a concentration of 500 ppb in the case of the analysis of strawberries - the repeatability of the derivatization procedure with HFBA was shown to be satisfactory, with a rel. S.D. of 1.0% (n = 6). Calculation was done by means of peak area measurements. With standard solutions, the detection limit of HFB-Cx is about 15 pg.

Residue analyses were performed using strawberry samples spiked at the 200-1000 ppb level with Cx. After extraction, derivatization, and immediate injection onto the LC column, average recoveries of between 70 and 95% were obtained. Illustrative examples of LC-ECD chromatograms are shown in Fig. 2, which also includes a chromatogram of a blank (non-spiked) strawberry sample.

The detection limit of 15 pg referred to above should, in principle, allow the determination of 0.5 ppb of Cx in strawberries. However, the small baseline discontinuity observed in the chromatogram of the non-spiked sample in the HFB-Cx position - and also in those of other non-spiked strawberry samples - actually corresponds with a Cx level of 5-10 ppb, so that a reliable estimate of the lower limit of detection in real samples is 15-30 ppb. This value is well below the residue tolerances quoted in the introduction to this paper.

No essential differences were noted between the various strawberry samples tested. Repeatability was satisfactory in all cases down to a spiking level of about 200 ppb (1-3%; n = 6). It deteriorated at lower levels. Probably, this is at least partly due to the decomposition of the HFB-Cx already referred to above.

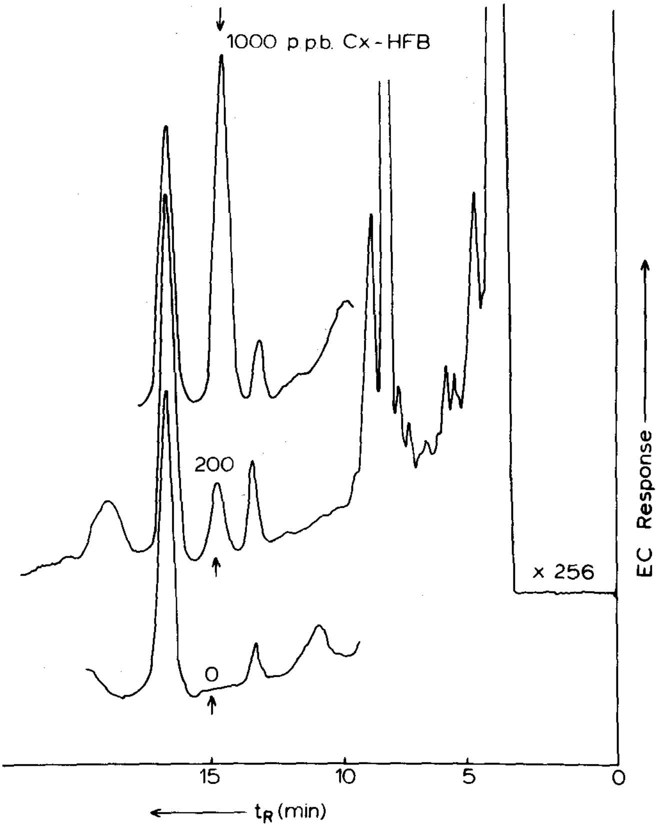

Figure 2: Normal-phase LC-ECD of Cx-spiked strawberry samples after extraction with dichloromethane, reconstitution in hexane and derivatization with HFBA. Spiking level (from top to bottom): 1000 ppb, 200 ppb, no spike added. The injected amounts correspond with a 34-mg strawberry sample. LC system: 10 µm LiChrosorb $NH_2$/ hexane-toluene (60:40); flow-rate, 1 ml/min; 0.5 ml/ min to ECD. ECD current, 1 x $10^{-10}$ A; attenuation, x256.

The present study demonstrates that the trace-level determination of chloroxuron in strawberries can conveniently be carried out by combining rapid extraction into dichloromethane, reconstitution into hexane and subsequent HFBA derivatization with normal-phase LC of the HFB derivative formed, using the electron-capture detector for sensitive and selective on-line detection.

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