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ANALYSIS OF DICAMBA IN TOBACCO BY ON-LINE COUPLED LIQUID CHROMATOGRAPHY–GAS CHROMATOGRAPHY

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SUMMARY

A new analysis procedure for determining dicamba in dried tobacco is presented. The method consists of a simple sample extraction followed by esterification and a final analysis using on-line coupled liquid chromatography–gas chromatography (LC–GC). Clean-up and preseparation of a sample is by LC and the fraction of interest is analysed using GC with electron-capture detection. The conditions during sample transfer produce concurrent solvent evaporation and vapours are vented out through a solvent vapour exit placed after the separation column. The detection limit is low and the method is simple and sensitive.

INTRODUCTION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a herbicide, widely used for selective post-emergence purposes. It acts on broad-leaved weeds but not on grasses. The reported oral LD_{50} in rats is 1 g per kg. The compound is sold under a wide range of commercial names and is used in the culture of maize, cereals, sorghum, sugar cane, pastures and also in the early stages of the culture of flax, rape cabbage oil palm and rice.

This compound should not be found in tobacco. The latter is susceptible to it, and the only reason for its presence on tobacco is careless application to other crops or, as some say, for accelerated yellowing of the leaves. No maximum allowable limit for tobacco is foreseen in, *e.g.*, the legislation of the Federal Republic of Germany. However, in foods of plant origin the limit is 0.05 mg per kg (1982). The rules on tobacco inspection issued by the US Department of Agriculture state a maximum allowable residue level for dicamba of 0.5 mg per kg (1986).

The commonly applied methods for determining dicamba in tobacco involve repeated liquid–liquid extractions to isolate the acids from the rest of the sample, followed by esterification with diazomethane and clean-up by column liquid chromatography. The final analysis is generally by gas chromatography (GC) with electron-

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capture detection (ECD)[1]. These methods are lengthy. Furthermore, the recoveries of dicamba from tobacco tended to be low and poorly reproducible.

The method proposed here reduces the procedure to a simple extraction, followed by esterification. The sample clean-up is carried out by normal-phase liquid chromatography (LC), coupled on-line to a gas chromatograph for the final analysis. This greatly reduces the work involved in the analysis of a sample, but also improves the recovery of dicamba and reduces detection limits through more efficient removal of interfering by-products.

Application of coupled LC–GC to pesticide analysis was first described by Majors[2] for the determination of atrazine in sorghum. More recently, Ramsteiner[3] applied a similar technique for the analysis of the plant protection agent Folpet in hop samples. In both cases, transfer was achieved through a conventional GC autosampler, injecting up to a few microlitres into a vaporizing injector. This transfer technique, providing a heart-cut analysis of the LC peak, sensitively relies on accurate LC retention times, as the transfer should occur at the maximum of the LC peak. Furthermore, only a small proportion of the LC fraction is transferred (at most a few percent), either resulting in modest sensitivity or necessitating loading a large amount of sample material onto the LC column.

The transfer technique applied here involves introduction of the complete LC fraction into the gas chromatograph by the concurrent solvent evaporation technique[4].

Concurrent eluent evaporation means evaporation during transfer. This avoids a flow of liquid into a GC column, which occurs when the retention gap transfer technique is applied[5]. Concurrent eluent evaporation has the important advantage that large fraction volumes can be transferred to the gas chromatograph by employing short (2–3 m) precolumns before the analytical column. An inherent problem is peak broadening of early eluted peaks, *i.e.*, peaks eluted up to 50–80°C above the GC column temperature used during eluent transfer[6]. Using a moderately volatile eluent, such as *n*-pentane and diethyl ether, the first sharp peaks can be expected at elution temperatures of 100–140°C. The methyl ester of dicamba is eluted within the critical temperature range, considering peak broadening, when standard thin-film GC columns are used.

To achieve sharp dicamba peaks with the concurrent eluent evaporation technique the following factors must be considered. Broadening of the dicamba peak depends on the column oven temperature used during eluent transfer. The minimum transfer temperature is determined by the requirement that the column temperature must exceed the eluent point to produce a vapour pressure exceeding the pressure of the carrier gas pushing the liquid into the column. As the eluent boiling point increases with pressure (10–20°C/bar), the lowest transfer temperature is possible at low inlet pressure. However, high inlet pressure is of interest for efficient discharge of the eluent vapours through the column. For the analysis of dicamba, this problem was solved, first, by applying a minimum inlet pressure during transfer, and secondly, by use of a GC column of intermediate polarity and somewhat elevated film thickness, which increased the elution temperature of dicamba.

EXPERIMENTAL

Apparatus

The LC system consisted of a Orlita membrane-type pump, a Waters U6K injector and a Perkin-Elmer LC-75 UV detector. Valco W-type valves were applied for interfacing the liquid and gas chromatographs. The gas chromatograph was a Carlo Erba Fractovap GI, equipped with an electron-capture detector (Model 40) and a loop-type interface as described in ref. 7.

Sample preparation

A 6-g amount of tobacco taken from different brands of cigarettes was extracted by mixing the sample for 15 min with 40 ml of *n*-hexane (E. Merck, Darmstadt, F.R.G.; p.a. grade)–diethyl ether (May & Baker, Dagenham, U.K.; HPLC grade) (1:1), containing 2 ml of 10% H_2SO_4 (Merck) in ethanol, in an ultrasonic bath. After removal of the extract, the tobacco was rinsed with *n*-hexane–diethyl ether (1:1) to give a final extract volume of 100 ml. A 15-ml volume of water was added to the extract to allow separation of the liquid phases. Half of the organic phase (equivalent to 3.0 g of tobacco) was evaporated to dryness, using a rotary evaporator. The residue was dissolved in 5 ml of methyl *tert.*-butyl ether (MTBE, Merck, HPLC grade). Methylation with diazomethane was carried out as described in ref. 8. After addition of 2 ml of *n*-heptane (Merck, p.a.), the MTBE was evaporated in a stream of nitrogen. The final solution was made up to 3 ml with *n*-heptane and centrifuged at 670 *g* for 5 min.

LC preseparation

The LC preseparation was carried out on a 100 mm × 2 mm I.D. column (Knauer, Bad Homburg, F.R.G.) packed with 5-μm Spherisorb S-5-W silica. *n*-Pentane (technical grade, distilled over sodium) containing 1.5% diethyl ether (May & Baker, HPLC grade) was the eluent at a flow-rate of 300 μl/min. A 10-μl volume of the centrifuged crude sample was injected. After the elution of dicamba methyl ester, the LC column was backflushed with 1 ml of MTBE to remove remaining polar material. The LC fraction of interest was cut out by a 250-μl stainless-steel sample loop placed in the ten-port sample valve (Fig. 1).

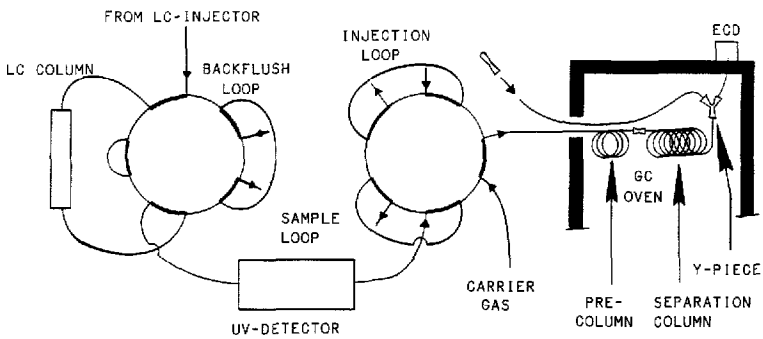
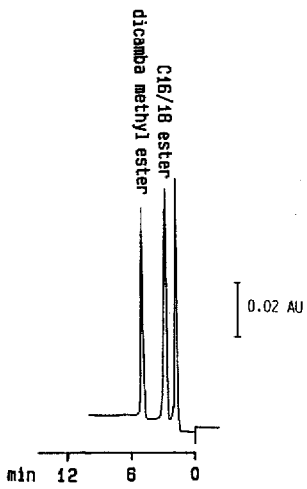


Fig. 1. Schematic diagram of the LC–GC set-up: ——— , LC separation and GC analysis; ———, LC backflush and sample introduction into the gas chromatograph.



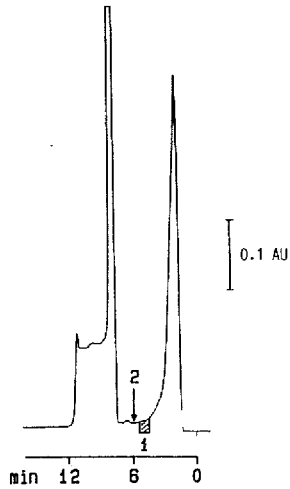


Fig. 2. LC separation of dicamba methyl ester and the C_{16-18} wax ester.

Fig. 3. Liquid chromatogram of a tobacco extract (tobacco spiked with 10 μg/kg dicamba), detected at 220 nm. Backflushing with 1 ml of MTBE occurred at the point indicated. The dicamba methyl ester fraction (250 μl), determined by previous coinjection, is marked. 1 = fraction; 2 = start of backflush.

Removal of wax esters by LC

With the simple extraction method applied, the most important by-products are the wax esters from the tobacco leaves. They must be removed as they would overload the GC capillary column and cause severe broadening of the early-eluted peaks (such as dicamba) and necessitate baking out of the column at high temperatures after each experiment. The proposed LC preseparation of dicamba and the wax esters was checked by using a mixture of dicamba and hexadecyl stearate. Fig. 2 shows an LC chromatogram, obtained with 1.5% diethyl ether in *n*-pentane as the eluent (10 μg of dicamba methyl ester and 1 mg of hexadecyl stearate in 1 μl of *n*-hexane, 10 μl injected). Fig. 3 shows an LC chromatogram of a tobacco extract with the dicamba fraction cut out, using the apparatus described in Fig. 1.

Memory effects

Special care must be taken to avoid memory effects, since large amounts of dicamba methyl ester (up to 10 μl of 10 μg/ml solution) must be injected into the LC system to determine the LC retention time (< 10 ng of dicamba methyl ester cannot be reliably detected by an UV detector). Injections of LC standard solutions and real samples should be carried out by using different syringes. It should be regularly checked for possible memory effects originating, *e.g.*, from the LC injector, by injecting eluent into the LC system and analyzing the fraction with the retention time of dicamba.

GC separation

The GC separation was carried out on a 15 m × 0.32 mm I.D. glass capillary column, coated with OV-61-OH of film thickness 0.3 μm. The exit of this column was

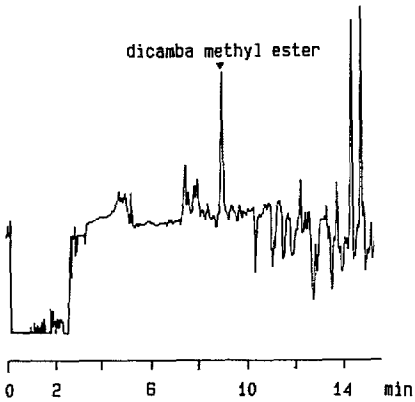


Fig. 4. LC–GC–ECD chromatogram of a tobacco sample containing 10 μg/kg of dicamba (the 250-μl fraction from Fig. 3).

connected to a press-fit Y-piece made of glass, which served as a solvent vapour exit during solvent evaporation. This Y-piece was connected to the detector by a 30 cm × 0.1 mm I.D. fused-silica capillary. It was closed by a stopper (made of a press-fit connector) after completion of the solvent evaporation step. The column inlet was equipped with a 3 m × 0.32 mm I.D. fused-silica precolumn (retention gap), deactivated by phenyldimethyl silylation. The precolumn was connected to the separation column using a press-fit connector. Transfer of the LC fraction was carried out at an inlet pressure of an 1.5 bar (rather low pressure to keep the transfer temperature low) with an oven temperature of 66°C. The flow regulator was set to a flow-rate of 3 ml/min (hydrogen). After completion of solvent evaporation, the oven temperature was programmed at 8°C/min to 280 °C where it was kept for 10 min. The elution temperature of dicamba methyl ester was 134°C.

RESULTS AND DISCUSSION

Fig. 4 shows a LC–GC–ECD chromatogram of a tobacco sample corresponding to 100 pg of dicamba injected. It shows that the achievable detection limits are very low, despite the short analysis procedure. Recoveries of dicamba added to tobacco samples before extraction ranged between 50 and 70%. Considering the recovery and sensitivity obtained, our analyses showed no traces of dicamba in commercial cigarettes. The proposed method for analysing dicamba in tobacco is simpler and more convenient compared to commonly applied procedures. The sensitivity is rather good, although the recovery might possibly be improved by optimizing the sample extraction step, which would improve both the sensitivity and reliability of the analysis.

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