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Design of a compressed air modulator to be used in comprehensive multidimensional gas chromatography and its application in the determination of pesticide residues in grapes

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ABSTRACT

In this study, a new modulator that is simple, robust and presents low operation costs, was developed. This modulator uses compressed air to cool two small portions in the first centimeters of the second chromatographic column of a comprehensive multidimensional gas chromatography (GC × GC) system. The results show a variation in the peak area less than 3 and 5% to alkanes and pesticides, respectively. The standard deviations for the retention times in the first and second dimension are around 0.05 min and 0.05 s for all the compounds. The system was optimized with *n*-alkanes. The GC × GC system proposed was applied in the determination of pyrethroid pesticides (bifenthrin, cypermethrin, deltamethrin, fenvalerate, esfenvalerate, *cis*- and *trans*-permethrin) in grape samples. Samples were extracted by the mini-Luke modified method and pesticides were quantified by comprehensive multidimensional gas chromatography with micro electron-capture detection (μECD). The values of method limit of quantification (LOQ) were 0.01–0.02 mg kg^{-1} for all studied pyrethroid and the values of recovery were between 94.3 and 115.2%, with good precision (RSD < 18.4%), demonstrating that the performance of the total method consisting of a modified Luke extraction method and determination by GC × GC-μECD are satisfactory. This study also showed that the system using a modulator with a double jet of compressed air has the potential for application in the analysis of a wider range of pesticide residues in other commodities since it provides low values of LOQ with acceptable accuracy and precision.

1. Introduction

Over the years, dramatic progress has been made in conventional gas chromatography (1D-GC; one-dimensional) with capillary columns offering high peak capacities. The technique can, typically, separate around 100–150 peaks during one chromatographic run. However, it fails to separate all individual constituents of very complex samples from each other and also from matrix constituents [1].

About fifteen years ago, comprehensive two-dimensional gas chromatography (GC × GC) began to attract attention. GC × GC has emerged as a powerful method for the separation of complex mixtures of volatile and semi-volatile organic compounds [2]. GC × GC is a technique that uses two connected columns, with different separation mechanisms. In most applications, samples are first separated in one 15–30 m × 0.25–0.32 mm I.D., 0.1–1 μm film thickness column containing a non-polar stationary phase. After the modulation, each individual fraction is released into a much shorter and narrower column—with dimensions of, typically, 0.4–2 m × 0.1 mm I.D., 0.1 μm, containing a medium to polar or a shape-selective stationary phase [3]. In principle, the second-dimension separation should be finished before the release/injection of the next fraction, otherwise a wrap-around could occur (when the second-dimension retention time exceeds the modulation time). This allows the simultaneous analysis of a sample by means of two different and independent column combinations placed in the same oven [4]. All kinds of stationary phases can be used in the first dimension of a GC × GC system. However, generally non-polar phases are preferred for reasons of orthogonality [5,6].

In conventional 1D-GC using a non-polar column, the main separation mechanism is volatility and, consequently, a boiling point

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separation is obtained. In all other types of columns, whether medium-polar, polar or a shape-selective, separations will be based on both volatility and on specific interactions of the selected column. In GC × GC with, for instance a non-polar first-dimension and polar second-dimension column, for analytes with similar volatility, but different polarity or shape, there will be no boiling point contribution in the second dimension. Hence, only the specific interactions with the stationary phase will govern the retention. Commonly, this is called an orthogonal separation [7-9].

Due to the extremely narrow peaks eluting in GC × GC from the short second-dimension column, detection has to be very fast. In addition, the detector should not influence the peak width or shape, which means that the detector cell should be as small as possible. Considering that the detection volume in the flame ionization detection (FID) is limited to the flame, this is the detector that least contributes to band broadening and, therefore, is the most popular detector for GC × GC. More recently, nitrogen–phosphorus detection (NPD) [10], micro electron-capture detection (μECD) [11] and time-of-flight mass spectrometry (TOF-MS) [12] were coupled to GC × GC for the analysis of diverse samples.

The main difference between GC–GC heart-cut and GC × GC, is that this second technique uses a modulator and needs only one GC oven. The modulator is responsible for the necessary modulation to obtain two-dimensional (2D) chromatograms. An essential aspect of GC × GC is the modulation that has three main purposes; that is, collecting and focusing fractions of peaks eluting from the first-dimension column and re-injecting the collected fractions into the second-dimension column for separation [1].

The modulators used to perform GC × GC separations can broadly be classified into two main categories: thermal and valve-based (see for a quick overview the most recent review article [13]). Over the years, many types of modulators have been developed, of which majority is based on cryogenic focusing and thermal desorption. Modulation is then achieved by cooling with a longitudinally modulated cryogenic system or with several jet-type cryogenic modulators [1,4].

Even though GC × GC is a powerful technique for complex matrix separations, its use in routine analysis is not so attractive. This, in part, may be due to the fact that most commercially available GC × GC systems use cryogenic modulators; only recently a flow modulator became commercially available. The use of CO_2 or N_2 for modulation brings the best results at this moment. However, liquid nitrogen is not easily available in all laboratories and needs bulky insulation when transported through tubing. Moreover, liquid CO_2 or N_2 are quite expensive, especially in less fortunate regions of the world.

This paper describes the redesign and adaptation of an earlier non-moving dual-stage air modulator [14], which was evaluated with a mixture of alkanes (C_7–C_{29}) and some pesticides using a GC × GC-FID. The proposed modulator was evaluated in the determination by GC × GC-μECD of pyrethroid pesticides (bifenthrin, cypermethrin, deltamethrin, fenvalerate, esfenvalerate, permethrin *cis* and *trans*) in grape samples. Samples were extracted by the mini-Luke modified method (acetone method) [15] and pesticides were quantified by GC × GC-μECD.

2. Experimental

2.1. Chemicals and reagents

Dichloromethane, light petroleum (b.p. 40–60 °C) and acetone (all pesticide grade) and anhydrous sodium sulphate (analytical reagent grade) were purchased from Merck (Darmstadt, Germany) and ethyl acetate (pesticide grade) was purchased from Lab-scan Analytical Science (Dublin, Ireland). Pesticides references standards (purity > 96%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Ridel-de Haën (Seelze, Germany) and Zeneca (Wilmington, DE, USA).

During the design stage of the project, a mixture containing 16 *n*-alkanes in the region C_9–C_{29} (exceptions C_{15}, C_{16}, C_{19}, C_{26} and C_{27}) at 30 mg L^{-1} in *n*-hexane was used to evaluate the modulation performance. To evaluate the proposed modulator for pesticide analysis, a mixture in methanol was prepared containing trifluralin, fenitrothion, fipronil, trifloxystrobin, bifenthrin, permethrin, cyfluthrin, cypermethrin and esfenvalerate (all from Dr. Ehrenstorfer) at 5 mg L^{-1}. For quantification purposes this mixture was diluted into ethyl acetate or in grape extracts (free of pesticides) to the indicated levels below.

2.2. Instrumentation

2.2.1. Chromatographic conditions GC × GC-FID

A HP 6890 GC (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph equipped with a FID system, a split/splitless injector and an autosampler was used. The injector was kept at 280 °C with a splitless time of 1 min. The injection volume was 1 μL. The first-dimension column VF-5 (30 m × 0.25 mm I.D., 0.25 μm film thickness, Varian, Middelburg, The Netherlands) was coupled through a glass press-fit connector to a second-dimension column VB-50 (0.4 m × 0.1 mm I.D., 0.1 μm film thickness, Valco Bond; Vici AG International, Schenkon, Switzerland). The temperature was programmed from 100 to 320 °C at 3 °C min^{-1}. The total run time was 73.3 min. Helium (99.999% purity) was used as a carrier gas and the GC was operated at constant flow (flow rate 0.5 mL min^{-1}). The data acquisition rate of the FID system was set to 100 Hz; it was operated at 300 °C at the following gas flow rates: hydrogen, 30 mL min^{-1}; air, 300 mL min^{-1} and make up gas (nitrogen), 15 mL min^{-1}. The modulation time used was 3.5 s.

2.2.2. Chromatographic conditions GC × GC-μECD

For GC × GC-μECD the gas chromatograph and column-set used were the same used in the GC × GC-FID. The oven temperature programme was: 80 °C at 20 °C min^{-1} to 200 °C, 200 °C at 5 °C min^{-1} to 300 °C; the injection volume and temperature were set at 1 μL and 280 °C, respectively, and all the injections were carried out in the splitless mode. The μECD system was operated at 300 °C with a nitrogen make-up gas flow rate of 60 mL min^{-1} and a data acquisition rate of 50 Hz. Helium was used as carrier gas at a constant flow rate of 1.0 mL min^{-1}. The modulation time used was 5 s.

2.3. Software

HP Chemstation software (Agilent, Revision - A.07.01) was used to control the GC instruments, to acquire the data and to peak integration; 2D Converter Program (beta version 1.0, Gerard Sharp) was used to convert the data matrix in image and Transform software (version 3.4, Fortner software) was used to view the image in the contour plots.

2.4. Performance of the dual-stage compressed air modulator

The evaluation of the new modulator performance was carried out by injecting standard solutions of the *n*-alkane (around 1 mg L^{-1} each) and pesticide (2.5 mg L^{-1} each) mixtures with the emphasis of data analysis on peak shape, retention time repeatability, and day-to-day repeatability (intermediate precision).

2.5. Sample preparation (grape extraction)

A homogenized portion of 10 g of grape sample was weighed in a 250 mL PTFE centrifuge tube. Subsequently, acetone (30 mL) was