

ON LINE HPLC+HRGC COUPLING: A NEW FULLY AUTOMATED METHOD FOR THE DETERMINATION OF PESTICIDES IN VEGETABLE SAMPLE

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OVERVIEW

In this work, a new application of the patented TOTAD® Interface for on-line HPLC-HRGC is presented. The interface coupling an HPLC to HRGC in the KONIK K2 system allows the direct analysis of pesticides in fruits and vegetables.

The pesticides are extracted from the grounded sample by a simple stirring and with a few ml's of organic solvent placed directly into the injection vial. After the extraction, the supernatant is injected into the HPLC and the fraction of interest is transferred to the HRGC system. With the addition of the KONIK Robokrom Autosampler and the full control through the Konikrom® Plus Software, the complete analysis can be easily automated and performed in a few minutes.

INTRODUCTION

Determination of Organic contaminants is regulated by governments all over the world and has been included as priority pollutants in the European Union lists. Pesticides constitute a very important group of these compounds owing to their high toxicity and their extended use in agriculture practice.

The standard method for the analysis of pesticides in vegetable samples is Chromatography with selective detectors and eventually GC-MS. Due to the complex matrixes, a complicated sample cleaning procedures are needed, previously to the HRGC analysis. Classical methods consist in a pesticide extraction with different organic solvents such as Acetone or Ethyl Acetate, clean-up techniques including Gel Permeation Chromatography (GPC), Solid Phase Extraction (SPE) or even Supercritical Fluid Extraction (SFE).

These clean-up procedures are time-consuming and are susceptible of compound loosing and contamination, particularly when operating at trace levels. The use of K2 on-line HPLC+HRGC system allows the direct injection of the compounds of interest from the HPLC to the HRGC without other sample preparation step, keeping the sample integrity, being a rapid, easy and sensitivity analytical method for the analysis of Organophosphorous Pesticides in vegetables.

METHOD

Instrumentation



The innovative KONIK K2 HPLC-HRGC system marries in synergy the separation and fractionation potential of normal and reversed HPLC, to the separation and selective detection of HRGC.

The flow diagram (Figure 1) describes the interface operating principles. This patented interface allows the trapping of the analytes in the K2 Interface. The trap is held at the chosen temperature while a digitally controlled continuous flow of Helium maintains the columns flow and eliminates the solvent from the trap.

LC-GC Transfer

Figure 1-a Stabilization step

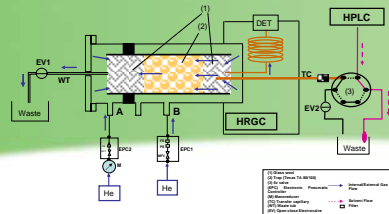
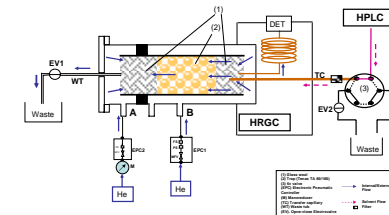


Figure 1-b Transfer step



Stabilization:	Transfer:	Remaining solvent elimination:	Thermal desorption:
Helium enters the packed liner through both the external flow (500 mL/min) and the internal flow (500 mL/min) (A and B in Figure 1). Eluent coming from HPLC pump is sent to waste. K2 injector temperature stabilizes at 100°C. Oven temperature is set at 40°C. EV1 is opened and EV2 is closed.	The solvent with the analytes of interest reaches the liner at 0.1 mL/min (FID) or 0.05 mL/min (NPD). Helium pushes the solvent through the adsorbent. Analytes are retained, and the solvent is vented to waste through the waste tubing (WT in Figures 1 and 2).	LC solvent coming from the pump is sent to waste. Helium pushes the remaining solvent in the capillary tubing to waste. These conditions are maintained for 2 min in order to achieve complete elimination of the solvent. EV2 is closed. EV1 is opened.	Helium enters only through the external gas inlet (A in figure 1) to the column. K2 interface is heated for 5 min and the retained analytes are desorbed and transferred to the capillary GC column. EV1 and EV2 are closed.

System conditions

Table 1: Chromatographic Conditions

Samples: Vegetable samples purchased on the local market. Tomato Field Sample previously treated with the target pesticides.
Standards: Organophosphorous pesticides (OPP) from Chem-Service: Dimethoate, Chlorpyrifos, Diazinon, Fenitrothion, Malathion, Chlorfenvinphos, Methidathion, Fenitrothion and Tetrachlorvinphos.
Reactives: Pesticide-grade Ethyl Acetate from Merck. Pesticide-grade Methanol from Pestican.

HPLC Conditions

Injection volume: 20 µL

Mobil Phase: Methanol

K2 Interface Conditions

Adsorbent: TENAX TA 80/100 mesh

Adsorption Temp: 100 °C

Desorption Temp: 275 °C

Transfer Flow: 0.1 mL/min (FID) / 0.05 mL/min (NPD)

Carrier Flow: 500 mL/min (A) + 500 mL/min (B)

HRGC Conditions

Column: KAP-5 (5% Phenyl Methyl Silicone); 30m x 0.32mm x 0.25µm

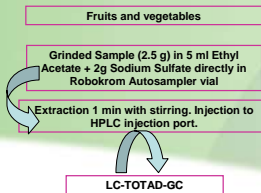
Carrier: Helium at 1.8 mL/min

Oven: 40°C (30 min); 20°C/min to 170°C; 3°C/min to 210°C; 5°C/min to 230°C (5 min)

Detector: FID at 250°C; Detector Gas: H₂ at 38 mL/min, Air at 220 mL/min, N₂ at 25mL/min

NPD at 250°C; detector Gas: H₂ at 4mL/min, Air at 110mL/min, N₂ at 25mL/min

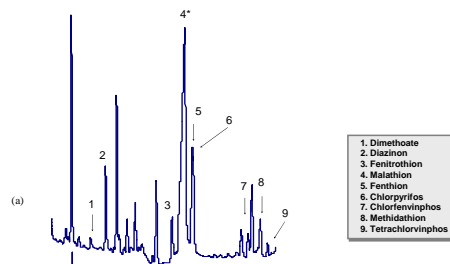
Sample analysis methodology



RESULTS

a. Fortified Samples

Figure 1



1. Dimethoate
2. Diazinon
3. Fenitrothion
4. Malathion
5. Fenitrothion
6. Chlorpyrifos
7. Chlorfenvinphos
8. Methidathion
9. Tetrachlorvinphos

Figure 2

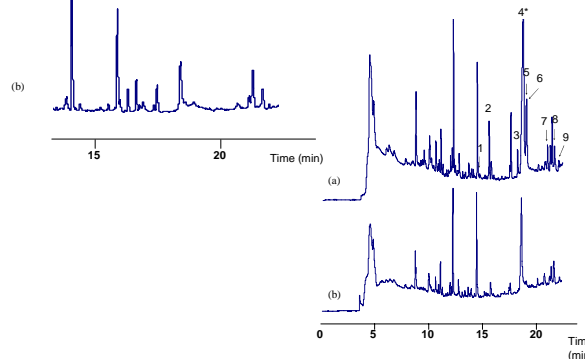
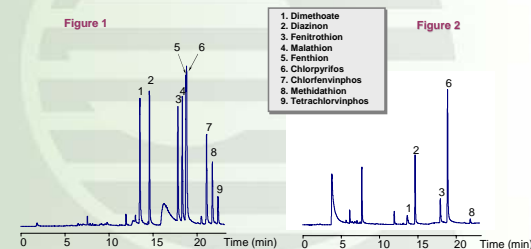


Figure 1: Cucumber Sample spiked at 10mg/Kg (a), Cucumber Blank Sample (b), NPD Analysis.

Figure 2: Tomato Sample spiked at 10mg/Kg (a), Tomato Blank Sample (b), FID analysis.

b. Real Samples

Figure 1



1. Dimethoate
2. Diazinon
3. Fenitrothion
4. Malathion
5. Fenitrothion
6. Chlorpyrifos
7. Chlorfenvinphos
8. Methidathion
9. Tetrachlorvinphos

Figure 2

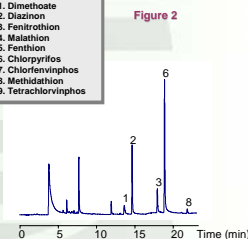


Figure 1: Tomato Sample spiked at 0.05mg/Kg, NPD analysis

Figure 2: Real Tomato Sample previously treated with the target compounds two weeks before harvest.

c. K2 Quality Parameters

LOD, precision and linearity for the selected pesticides subjected to KONIK K2 HPLC-HRGC system coupled with NPD detector

	LOD (µg/Kg) S/N=3	Precision (n=6)		Linearity (R ²)
		RSD Rt (%)	RSD area (%)	
Dimethoate	0.07	0.16	2.4	0.996
Diazinon	0.07	0.17	0.3	0.977
Fenitrothion	0.08	0.20	4.4	0.969
Malathion	0.07	0.21	3.6	0.991
Fenitrothion	0.06	0.21	8.4	0.969
Chlorpyrifos	0.06	0.20	2.0	0.977
Chlorfenvinphos	0.10	0.19	4.7	0.989
Methidathion	0.15	0.20	4.3	0.987
Tetrachlorvinphos	0.34	0.17	2.4	0.988

Precision was determined with a Tomato sample fortified at 0.05mg/Kg. Linearity was obtained with Tomato samples fortifies in the range from 0.01 to 0.1 mg/Kg. Limit of detection values were determined using the 0.01mg/Kg fortified Tomato Sample.

CONCLUSION

⚡ Rapid Extraction with Ethyl Acetate and On-line K2 HPLC+HRGC system allows the determination by direct injection of Organophosphorous Pesticides in different kind of vegetables.

⚡ The method reduces the use of organic solvents in the sample Preparation step.

⚡ The method eliminates the time-consuming sample preparation step.

⚡ The ROBOKROM autosampler in combination with K2 interface is highly suitable for the automatization of HPLC-GC systems avoiding errors caused by sample manipulation.

⚡ The described method shows good precision, linearity and high sensitivity.