

Environmental toxics and metabolites (endocrine disrupters) in urine by direct injection into patented HPLC- GC-ECD system

Abstract

The system KONIK K2 HPLC-HRGC instrument has been successfully applied as a direct method for the analysis of pesticides in biological matrices at trace levels.

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Introduction

It is widely known that exposure to many pesticides is cause of undesirable hazards and risks of human and animal health. For that, biological monitoring to determine those chemicals and/or their metabolites directly in biological fluids is required and consequently, analytical methods are necessary for the estimation of human exposure to the target analytes of interest.

The determination of pesticides residues in biological samples usually requires the application of clean-up procedures (such as liquid-liquid or solid-phase extraction) to remove interferences and reduce the detection limits.

In this study a method for direct on-line HPLC-GC determination of some Organochlorine Pesticides in urine using the automated K2 HPLC-HRGC Multidimensional system (based on the patented TOTAD interface) is proposed. Automated HPLC-HRGC practically eliminates the time-consuming sample preparation step as raw urine is loaded directly into the HPLC. The sample is injected with no sample pre-treatment step other than a simple filtration. Pesticides are retained in a first HPLC step in a C18 column while salts and other interferences are removed. Afterwards, pesticides are eluted from the column and the fraction of interest is transferred to the GC, where the separation and detection of these compounds is performed.

Experimental

The innovative KONIK K2 HPLC-HRGC system (Figure 1) marries in synergy the separation and fractionation potential of normal and reversed HPLC, to the separation and selective detection of HRGC.

Figure 1: KONIK K2 HPLC-HRGC System



LC-GC Transfer

The flow diagram (Figure 2) describes the interface operating principles. This patented interface allows the trapping of the analytes in the modified injector of the HRGC system. 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.

Figure 2-a:
Stabilization step

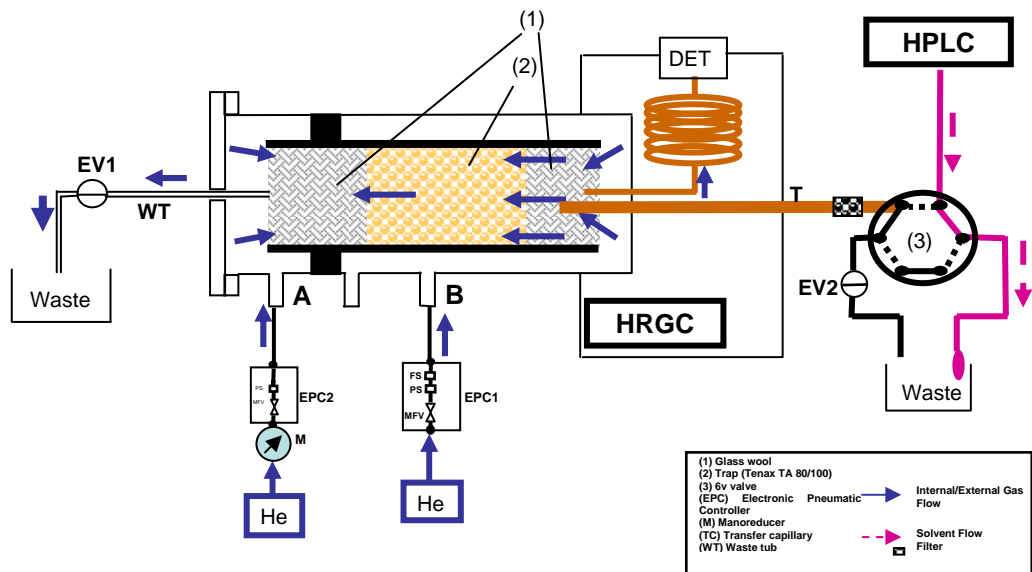
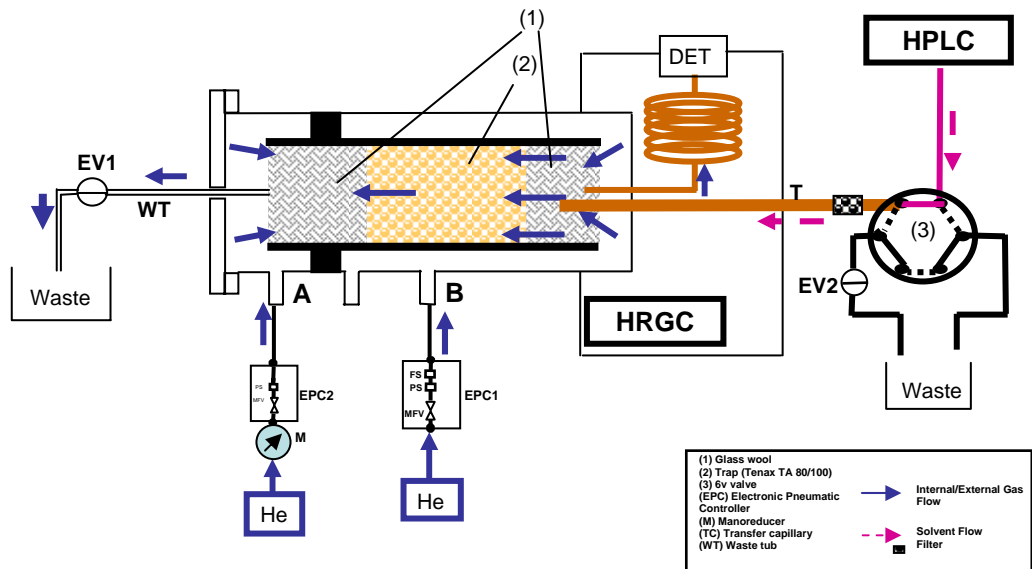


Figure 2-b:
Transfer step



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1- Stabilization

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 2-a). Eluent coming from HPLC pump is sent to waste. K2 Injector temperature stabilizes at 80°C. Oven temperature is set at 40°C. EV1 is opened, EV2 is closed.

2- Transfer

The solvent with the analytes of interest reaches the liner at 0.1 mL/min. Helium pushes the solvent through the adsorbent. Analytes are retained, and the solvent is vented to waste through the waste tubing (WT in Figure 2-b).

3- Remaining solvent elimination

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 opened.

4- Thermal desorption

Helium enters only through the external gas inlet (A in figure 2) 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.

Chromatographic Conditions

Standards, samples and the necessary Konik HPLC-HRGC system conditions for this analysis are listed in Table 1. For this study, control of the GC, data acquisition, reduction, and analysis were done using Konikrom® Chromatography Data System.

Table 1: Chromatographic Conditions

Samples: Urine samples spiked with OCP solution. Final concentration: 100 ng/mL (LOD 10ng/mL).

Standards: Organochlorine pesticides (OCP) solution mix from Supelco; containing 20µm/mL of α-BHC, β-BHC, γ-BHC, δ-BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, p,p'-DDE, dieldrin, endrin, endosulfan II, p,p'-DDD, endrin aldehyde, endosulfan sulfate, p,p'-DDT, methoxychlor.

HPLC Conditions

Column: C18 Kromasil 100, 100x4.6 mm, 5µm

Injection volume: 100 µL (LOD: 1000 µL)

Detector: UV-VIS 220 nm

Mobil Phase: H2O 100% (0-10 min at 1 mL/min); Isopropanol (10-30 min at 0.1 mL/min)

K2 Interface Conditions

Adsorbent: TENAX TA 80/100 mesh

Adsorption Temp: 80 °C

Desorption Temp: 275 °C

Transfer Flow: 0.1 mL/min / 20 min

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

HRGC Conditions

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

Carrier: Helium at 11.5 psi

Oven: 40°C (30.5min); 30°C/min to 180°C; 8°C/min to 210°C; 4°C/min to 270°C (10min)

Detector: ECD at 330°C; Detector Gas: N2 at 50mL/min

MS Conditions

Ionization mode: EI (+)

Electron Energy: -70eV

Source Temp.: 160°C

Transfer line Temp.: 270°C

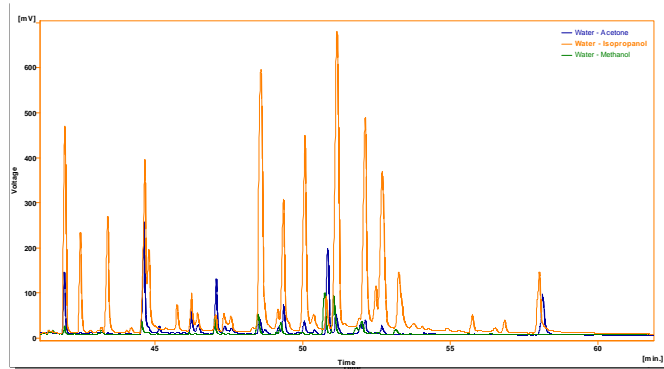
Scan Rate.: 100 ms/ion (SIM)

Results

a. Method Optimization: Choice of HPLC solvent

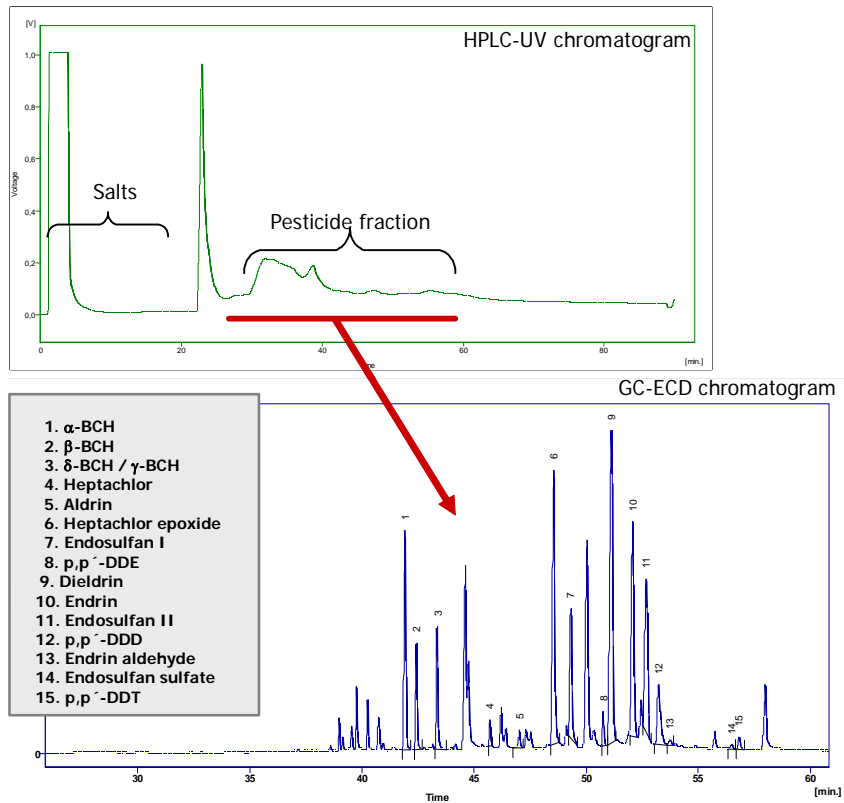
The Konik K2 HPLC+HRGC system handles all type of column and all types of solvent, as well as normal or reversed phase methods. Figure 3 shows the increase of sensibility using the same conditions with 100 ng/mL OCPs urine spiked, by changing the HPLC solvent: Acetone, Methanol or Isopropanol.

Figure 3:
Choice of HPLC eluent:
Acetone (blue), Methanol (green) or Isopropanol (orange).



b. Method Optimization: Choice of fraction to transfer; optimization of GC/ECD conditions

Figure 4:
Choice of fraction to transfer

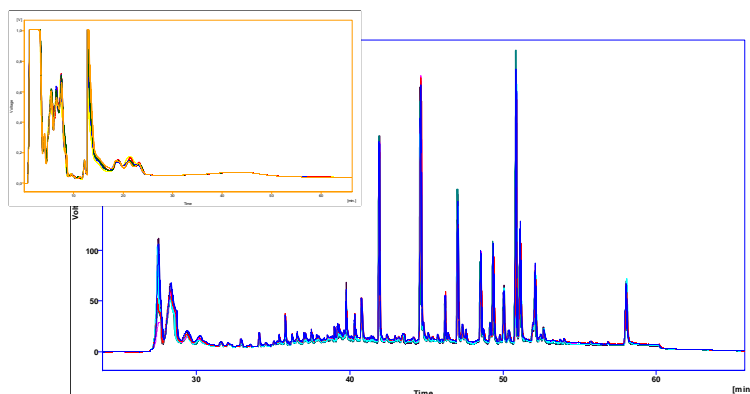


c. Quality Parameters

Table 2 shows the results of LOD, precision and linearity for the selected pesticides subjected to KONIK K2 HPLC-HRGC system coupled with ECD:

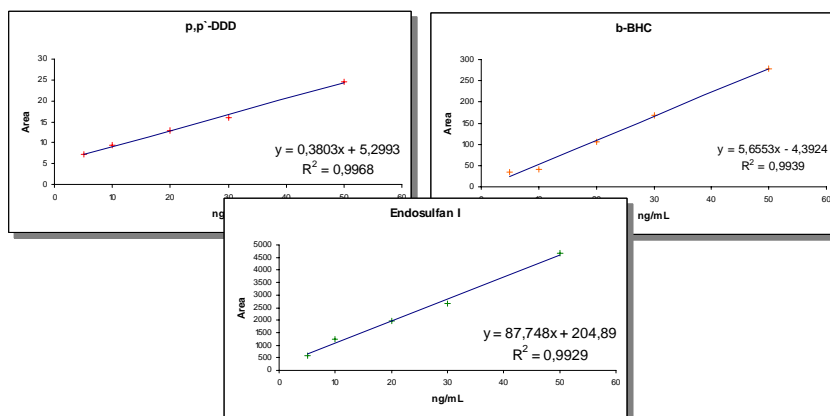
	LOD (ng/mL) S/N=3	Precision (n=6)		
		RSD Rt (%)	RSD area (%)	Linearity (r ²)
a-BHC	0.03	0.02	6.1	0.994
β-BHC	0.5	0.03	12.5	0.994
?-BHC / d-BHC	0.4	0.11	12.8	0.986
Heptachlor	0.4	0.04	5.2	0.991
Aldrin	1.0	0.03	11.2	0.999
Heptachlor epoxide	0.03	0.03	8.2	0.994
Endosulfan I	0.03	0.05	6.5	0.993
p,p'-DDE	0.09	0.03	7.0	0.995
Dieldrin	0.01	0.04	7.4	0.993
Endrin	0.02	0.02	7.8	0.990
Endosulfan II	0.24	0.04	10.8	0.991
p,p'-DDD	8.3	0.04	2.3	0.997
Endrin aldehyde	1.4	0.04	12.0	0.983
Endosulfan sulfate	15	0.02	10.1	0.989
p,p'-DDT	2.7	0.06	12.2	0.995
Methoxychlor	---	----	---	---

Figure 5:
Precision – 6
chromatograms
overloaded



Linearity

Linearity has been established by the analysis of five different concentration of the standard solution. Table 2 shows the result obtained for the correlation coefficient and figure 6 shows some examples of the calibration curve for different compounds.

Figure 6:
Linearity -
Examples

CONCLUSION

- KONIK K2 HPLC-HRGC instrument have been proved to be suitable for the determination of pesticides in biological samples.
- The method allows the automated analysis of organic components of a water matrix such as urine.
- The method eliminates the time-consuming sample preparation step as no pretreatment is required other than a simple filtration step.
- K2 interface is highly suitable for the automatization of HPLC-GC systems avoiding errors caused by sample manipulation.
- The described method shows good precision and high sensitivity.
- HPLC as a sample preparation method is a good alternative to traditional techniques such liquid-liquid extraction or SPE.