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On-line determination of hexazinone in water by solid phase extraction-UV/Vis spectrophotometry

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Abstract

In this work, the continuous determination of hexazinone in water was carried out by developing an online solid phase extraction by flow injection coupled with spectrophotometric detection. Variables related to hydrodynamic conditions were optimized by a full factorial design 3^2, of which the results were analyzed through an analysis of variance. Under the proposed optimal conditions, the principal figures of merit were a working range between 0.50 to 7.00 μg mL^{-1} of hexazinone; a precision of 4.6% expressed as variance coefficient; a limit of detection of 0.05 μg mL^{-1}, and a limit of determination of 0.16 μg mL^{-1}. Univariant calibrations based on the height or area of transitorial signals were compared to identify the best conditions for quantification. Samples of well and sea water were analyzed, obtaining satisfactory results in all cases in terms of precision and accuracy.

Keywords:

Hexazinone, flow injection, solid phase extraction, UV-Vis spectrophotometry

1. Introduction

Hexazinone, or 3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione according to the IUPAC, is a herbicide that belongs to the triazine group; specifically, it is a triazinone (Figure 1) [1]. It is a solid white crystalline with a melting point of 113.5°C. Its solubility in water at 25°C is 33 g Kg^{-1}, while solubilities in methanol, acetone, and hexane are 2 650, 790, and 3 g Kg^{-1}, respectively. The log K_{oc} is 1.30-1.43, while the log K_{ow} is 1.36 [2].

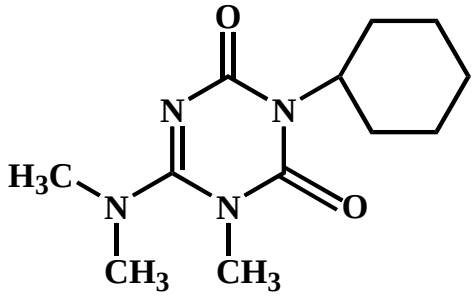

Fig.1. Chemical structure of hexazinone.

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It is considered a selective herbicide of pre-emergence and post-emergence application to control grasses, broadleaf and woody plants, in crops like pineapple, sugarcane, alfalfa, blueberry, Christmas tree plantations, etc. It is a systematic herbicide that inhibits photo-synthesis in the target plants.

Hexazinone may be of concern in groundwater and surface water contamination. It is not hydrolyzed under normal environmental conditions; no significant photo-degradation is observed in aqueous media at pH 7 when exposed to an artificial light source. A half life of 82 days is reported in soil; major routes of dissipation are biodegradation and leaching [3]. Fortunately, acute oral toxicity studies have shown that the oral LD_{50} for this compound in mammals is around 1000 mg kg^{-1}, while long-term exposures at doses of about 5 mg per Kg per day are not associated with evident adverse effects [4].

The herbicide is commercialized as Velpar, a trademark of Dupont, in which it is the unique active ingredient, accompanied by other inert compounds [5]. Combination with other herbicides is not suggested.

For analytical purposes, major analytical methods have been devoted to its simultaneous determination with other pesticides in water and soil samples by using liquid chromatography [6, 7], gas chromatography [8, 9] or capillary electrophoresis [10, 11]. With regard to UV-Vis spectrophotometric methods, derivative modality has been used for its determination in mixtures [12, 13]. As a single pesticide, some immunoassays have been presented with interesting results [14, 15].

In this work, the simple and rapid quantification of hexazinone in water was achieved, based on its cleanup/pre-concentration through a continuous SPE manifold and UV-Vis spectrophotometric detection.

2. Experimental

2.1. Instrumentation

A UV-Visible spectrophotometer (Perkin-Elmer, model lambda EZ 210) was used, controlled by a PC while applying the program PESSW v1.2.E by Perkin-Elmer. The flow injection (FI) system (Figure 2) consisted of: a) a peristaltic pump (Gilson, model Minipuls 3); b) two injection valves for low pressure operation (Rheodyne, model 512); c) a glass column of 50 x 10 mm designed to operate under moderate pressures (Omnifit), d) PTFE tubing of 0.5 mm ID; and d) a flow cell with a 10 mm optical path and 18 µL of internal volume (Hellma). Data treatment was carried out with the software packages OriginPro 8 SR0 v8.0724 by OriginLab Corporate and Statgraphics plus 5.1 by Statpoint Inc.

2.1. Reagents and solutions

All the reagents used were at least analytical grade. Hexazinone (HEXA) was pestanal grade from Riedel-de Häen; sodium salt of humic acids (NaHu) was from Aldrich. Methanol (MeOH) was from J.T. Baker. Water purified with EasyPure equipment (Barnstead) was used throughout. For Solid Phase Extraction (SPE), octadecyl silica (C_{18}, particle size 40-63 µm, mean pore size 60 Å) was acquired from Supelco.

Stock solutions of HEXA containing 100 µg mL^{-1} and NaHu containing 500 µg mL^{-1} were prepared in water and stored at 4°C; under these conditions, HEXA and NaHu solutions were stables for at least one month. The working solutions were prepared daily through adequate dilution.

2.2. Flow Injection manifold and procedure

With the two valves in load position, the 2000 μL loop of the injection valve (V1) was filled with the sample, meanwhile the carrier solution passed through the adsorption mini-column positioned in the loop of the second valve in order to prepare the stationary phase for the retention of HEXA; also, an aqueous MeOH 70% v/v solution (eluent) reached the flow cell, allowing for baseline recording. Then valve 1 was changed to injection position for the pre-concentration of the analyte on the C_{18} support. Later, the change of valve 2 to injection position facilitated that the eluent passed through the column, desorbing the retained species and sending them to the detector. Finally, valves 1 and 2 were returned to the load position to begin the analysis of a new sample. All experiments were done in triplicate.

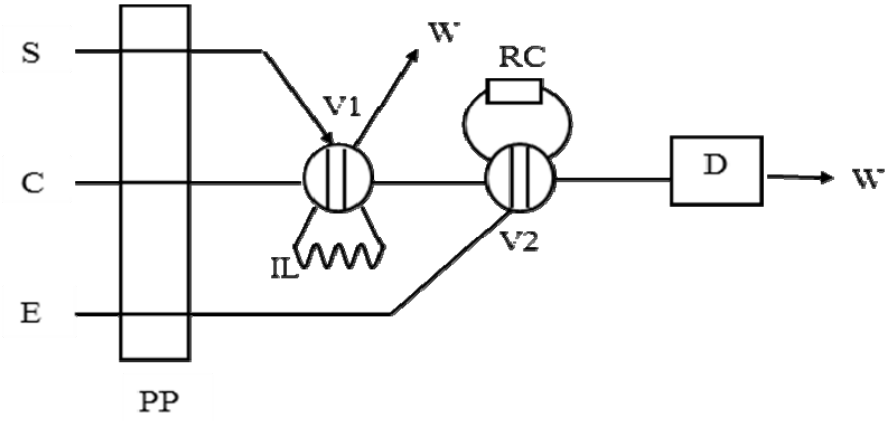

Fig. 2: FI manifold proposed for the continuous determination of hexazinone in water: sample solution (S), carrier solution (C), eluent (E), peristaltic pump (PP), injection valves 1 and 2 (V), injection loop (IL), retention column (RC), detector (D), and wastes (W).

3. Results and discussion

HEXA in water exhibits an absorption maximum at 245 nm, the signal that was used for its quantification in this work. It has a pk_a of 1.2 [16] as a result of the protonation of nitrogen in the azomethine bond, which reflects that in a neutral aqueous medium it remains in a neutral form, just as the present study shows.

3.1. Optimization of variables

Variables and optimization results related to the FI system are shown in Table 1.

Table 1: Variables studied in the optimization of the FI system.

Type	Variable	Studied range	Selected value
Hydrodynamic	Injection volume (mL)	0.5-2.0	2
	Flow rate of injection (mL min^{-1})	1.8-5.1	3.5
	Flow rate of retention and elution (mL min^{-1})	0.7-2.2	1.5
Retention/ Elution	Eluent (% v/v of MeOH in water)	40-100	70
	Elution time (min)	2-13	3

For SPE, C_{18} was used for retention and methanol was considered as eluent, both of which have proven to be useful for HEXA pre-concentration in aqueous environmental

matrices [17, 18]. The glass mini-column was packed with a volume bed of 0.3 cm^3 of C_{18}, and the support was conditioned at the beginning of each work session with ten column volumes of methanol and water, respectively. Pure water was selected as carrier solution, owing to the fact that in this medium the herbicide is highly soluble and remains in the neutral form.

For the selection of the hydrodynamic conditions, Tygon tubing of 1.52 mm of internal diameter (ID) was adapted to the pump head for a sample channel, while Tygon and Viton tubing of 0.89 mm ID was used for carrier and eluent solution channels, the last especially recommended for the use of solvents. Then the injection volume was evaluated, as well as the flow rates required both to fill up the injection loop (flow rate of injection) and to remove the sample from it and to send it to the column (flow rate of retention). As expected, the highest sample volume (2.0 mL) provided the best analytical signal for a given concentration of HEXA, meanwhile flow rates of 3.5 and 1.5 mL min^{-1} showed to be appropriate in the scale time. Due to the fact that the tubing of the carrier and elution solutions adapted to the pump head were of the same ID, the flow rates of retention and elution were the same.

For elution, MeOH/H_2O solutions in the range of 40 to 100% v/v were tested (Figure 3). Low MeOH content gave rise to greater elution times with broad elution peaks as a consequence of a major dispersion of the analyte through the system. In contrast, for solutions reaching 100% of MeOH content, narrow elution peaks were obtained as a consequence of a quick desorption of the analyte. Unfortunately, bubbles were observed in the mixing zone of eluent and carrier solutions, due to exothermic mixing. Therefore, a solution with a content of MeOH/H_2O 70% v/v was selected as a compromise condition in order to obtain well-defined peaks without risk of physical interference (bubbles) in the reasonable time. Under these experimental conditions, the sample throughput was of 9 samples h^{-1}.

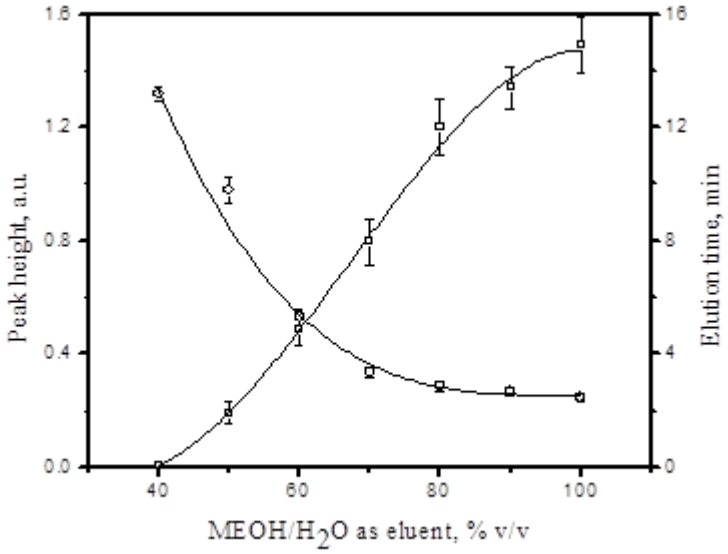

Fig. 3: Influence of eluent composition on the FI system.

3.2. Data analysis and figures of merit

Eight samples with concentrations varying in range from 0.3 to 7 µg mL^{-1} of HEXA were prepared in triplicate to estimate the calibration function, as well as fourteen samples at a given concentration of 3 µg mL^{-1} of HEXA to measure the repeatability of the system. In all cases, the peak height or area of the elution profile was evaluated as analytical signal with or

without baseline correction. Smoothing of the elution profiles was carried out by the Savitzky-Golay procedure (21 points, polynomial function).

The concentration errors (the absolute values of the expected concentrations minus the estimated concentrations through the respective calibration function) were considered as response. The Analysis of Variance (ANOVA) results are presented in Table 2. As can be observed, the consideration of peak area instead of height has a significant effect on the errors obtained during the estimation of HEXA through the proposed method, since the P-value is less than 0.05. However, baseline correction does not show a significant influence. Therefore, peak area without baseline correction was selected as signal for analytical purposes.

Table 2: Description of experimental setup and ANOVA results for concentration errors of HEXA, considering the eight samples of calibration in triplicate and the fourteen samples for repeatability (95% of confidence level).

Experimental design					
Factor		(-)		(+)	
A: Signal type		Height		Area	
B: Baseline correction		With		Without	
ANOVA					
Source	Sum of squares	Degrees of freedom	Mean Square	F-ratio*	P-value
MAIN EFFECTS:					
A	2.8538	1	2.8538	44.09	0.0000
B	0.0074	1	0.0074	0.11	0.7360
RESIDUAL	9.6443	149	0.0647		
TOTAL (Corrected)	12.5055	151			

* All F-ratios are based on the residual mean square error.

Then ten samples were prepared as synthetic mixtures with concentrations varying in range from 0.6 to 6.6 μg mL^{-1} of HEXA for validation of the selected calibration function through the estimation of recoveries expressed as percentages. Also, ten reagent blank samples were made up for the estimation of the limits of detection and quantification. The corresponding figures of merit are shown in Table 3.

Table 3: Figures of merit estimated for the on-line determination of HEXA. All samples were injected in triplicate.

Parameter	Value
Linear range (n=8)	0.50 – 7.00 μg mL^{-1}
Regression coefficient	0.997
Limit of detection (n=10)[a]	0.05 μg mL^{-1}
Limit of quantification (n=10)[b]	0.16 μg mL^{-1}
Precision or repeatability expressed as relative standard deviation ([HEXA]= 3 μg mL^{-1}, n=14)	4.6 %
Exactitude expressed as mean recovery ± confidence limits (P=0.05, n=10, two tailed test)	101 ± 1 %

[a, b] Estimated as 3 and 10 times the blank signal divided by sensitivity, respectively [19].

3.3. Analysis of water samples

The validity of the FI-SPE-spectrophotometric method was checked for its application to the analysis of well and sea water samples taken within a radius of thirty kilometers around the Campus of the University, in which the absence of the herbicide was presumed, considering that the soil of the region is not used for agricultural purposes. Samples were taken in triplicate and fortified with HEXA in a range of 1.3 to 4.5 μg mL^{-1}. Likewise, each sample was injected in triplicate in the FI system.

Recovery results are shown in Table 4. Estimated *vs.* real concentrations of HEXA can be observed in Figures 4 and 5, which correspond to well and sea water samples, respectively. Each symbol represents the mean value of one sample injected in triplicate. As can be appreciated, satisfactory results were obtained in both cases, with exception of the samples with the highest concentration of HEXA, in which the salinity of the matrix probably reduced the aqueous solubility of the herbicide (see Fig. 5).

Table 4: Analysis of well and sea water samples fortified with HEXA (n=15, P=0.05, two-tail test).

Origin	Mean recovery ± confidence limits
Zipolite 1	95 ± 10
Zipolite 2	105 ± 3
El Colorado	97 ± 5
Zipolite Beach	93 ± 8

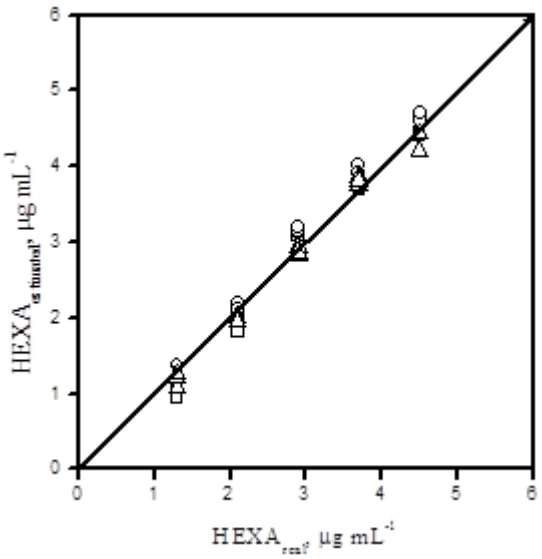

Fig. 4: Correlation of given and found concentrations of HEXA in well water samples: (□) Zipolite 1, (○) Zipolite 2, (△) El Colorado.

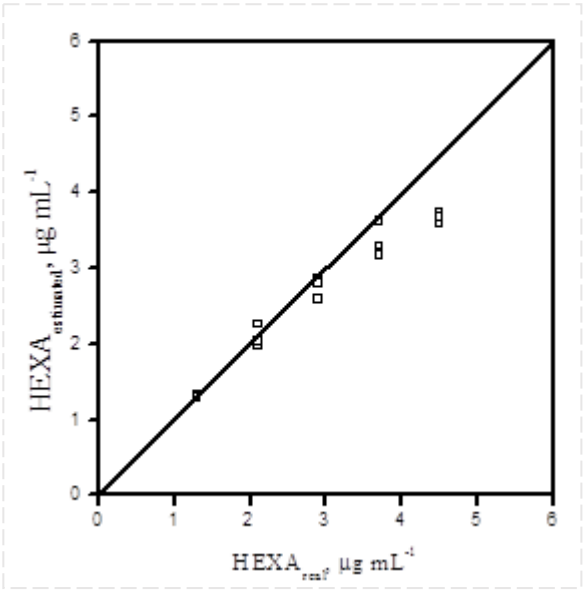

Fig. 5: Correlation of given and found concentrations of HEXA in sea water samples.

4. Conclusions

The proposed method proved to be a suitable tool for the determination of HEXA in water samples. Flow injection manifold facilitates the automated manipulation of environmental samples, in which cleanup and pre-concentration were carried out by means of a solid phase extraction step. Due to the dynamic nature of the process where non-equilibrium conditions prevail during the adsorption on C_{18}, better results were found by using the area instead of the height of the elution profile as analytical signal. Satisfactory results were found in both well and sea water samples fortified with HEXA within the range of a few µg mL^{-1}.

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