Original Research Article
Annalen der Chemischen Forschung
Vol-2: No-1: 1-9: 2014
www.aocsr.com

Determination of Hexazinone in different water samples by solid-phase extraction and UV spectrophotometry

Amador-Hernández J*[1], Velazquez-Manzanares M [1], Gutierrez-Ortiz MR[2], Marquez-Reyes JM[2]

[1] Instituto de Biotecnología, Universidad del Papaloapan, Circuito Central 200, 68301 San Juan Bautista Tuxtepec, Oaxaca, Mexico.
[2] Ingeniería Ambiental, Universidad del Mar, Ciudad Universitaria S/N, 70902 Puerto Ángel, Oaxaca, Mexico

Abstract

In the present work, an attempt has been made to develop a novel method for the quantification of Hexazinone in water by using solid-phase extraction and UV spectrophotometry. Three different cartridges namely octadecyl and octyl silica, and styrene/divinylbenzeneco-polymer were evaluated. The proposed spectrophotometric method showed a working concentration range from 0.5 to 14.0 µg ml^{-1}, a limit of detection of 0.15µg $ml^{-1}$and a limit of quantification of 0.5 µg ml^{-1}. . Results from the study indicate that under experimental design strategies, best results were obtained when cartridges of octadecyl silica were used. Further, Hexazinone was satisfactorily determined in tap, well and sea water samples previously fortified with the herbicide even in the range of ng ml^{-1}. Results from the experimental data suggested that the proposed method can be efficiently employed for determination of Hexazinone in various sources of water.

Key words: Hexazinone, triazine, solid phase extraction, Spectrophotometry

Cite this article as: Amador-Hernández J, Velazquez-Manzanares M , Gutierrez-Ortiz MR, Marquez-Reyes JM. Determination of Hexazinone in different water samples by solid-phase extraction and UV Spectrophotometry. Annalen der Chemischen Forschung. 2014;2(1):1-8.

1.0 Introduction

Nowadays, water quality is being monitored strictly around the world due to the increased cases of water pollution due to various chemicals like metals, agrochemicals, dyes, etc. Among the agrochemicals group, triazines are used as herbicides very frequently. Generic structure of triazines consists of six-member ring, with three atoms of carbon and nitrogen symmetrical or asymmetrically alternated in it. Triazines are commonly used as pre- and post-emergence weed-killers, in the production of grains (corn), fruits (pineapple, raspberries, apples and peaches), vegetables (potatoes, tomatoes), hardwood and softwood plantations, among others [1].

Hexazinone(3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione), belongs to triazine group of herbicides (Figure-1). It is considered as a wide-spectrum herbicide that is absorbed through the roots or leaves, depending of the type of application and commercial presentation used. Considering the root absorption, it is transported upward through the xylem and acts as photosynthesis inhibitor .

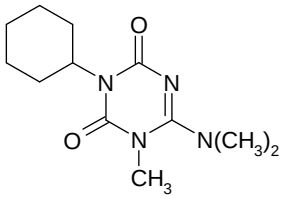

Figure-1: Chemical structure of Hexazinone

It has a solubility in water of 33 g Kg^{-1} at 25°C, while its solubility 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]. It is not hydrolyzed under normal environmental conditions and no significant photo-degradation is observed in aqueous media at pH 7 when exposed to an artificial light source. Literature reports indicate half-life of of Hexazinone 82 days and major routes of dissipation are biodegradation and leaching [3,4]. As it is being used extensively in agriculture, great interest is observed among international community about its presence in the environment and basically in water bodies [5-7].

From an analytical point of view, triazines are traditionally quantified by chromatographic techniques in soil and water [8-10]. Hexazinone is not the exception and some chromatographic methods have been reported for its determination and quantification. [11, 12]. Some spectrophotometric methods are also available in which derivative modality has been used for its determination in mixtures [13, 14].

Solid-phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. The versatility of SPE allows use of this technique for many purposes, such as purification, trace enrichment, desalting, derivatisation and class fractionation. The last few years have been chracterized by a wide interest in this technique and many publications describing SPE methods have been published. Inspite of advantages of SPE, there are no literature reports available about the availability of a reliable analytical method that enables the quantification of this hexazinone in water in a simple way and at low cost. In the present work, an attempt has been done to spectrophotometrically determine the Hexazinone in environmental water samples containing by using the concept of SPE. Three different adsorbents were used in the present study and an attempt is also made to evaluate the experimental design and analysis, in order to establish the optimal conditions for separation.

2.0 Material and methods

2.1 Equipment

A UV-Visible spectrophotometer (Perkin-Elmer, model lambda EZ 210) controlled by a PC which uses the program PESSW v1.2.E by Perkin-Elmer was used during the present study, For SPE, a Visiprep twelve-port vacuum manifold was utilized (Supelco, model 57044). 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.

2.2 Reagents and solutions

All the reagents used during the present study were of analytical grade. Hexazinone (HEXA) was pestanal grade from Riedel-de Häen, sodium salt of humic acids (NaHu) was from Aldrich. Hexane (Hx), methanol (MeOH), and acetonitrile (ACN) were HPLC grade from J.T. Baker. Water which was purified with an EasyPure equipment (Barnstead) was used throughout the study. Stock solutions of HEXA (100 µg mL^{-1}) were prepared in MeOH and stored at 4°C. The working solutions were prepared daily through adequate dilution. A buffer solution of K_2HPO_4/KH_2PO_4 (0.5 $moll^{-1}$, pH 8.0) was also used. Three types of SPE cartridges for environmental applications were evaluated (ENVI, from Supelco): two base silica ($C_{18}$and C_8) and one base styrene/divinylbenzene co-polymer (SDB), all with the same bed weight (0.5 g) and syringe volume (6 mL). C_{18} cartridges of a major bed weight (2 g) were also used. Filtration of water samples was carried out with nylon membranes (0.2 µm of pore size and 47 mm diameter, from Whatman).

2.3 Procedure

A series of HEXA solutions in the concentration range from 0.5 to 14 µg ml^{-1} were prepared for the spectrophotometric determination. For each sample, an adequate volume of the stock solution of the herbicide, the buffer solution (2.5 ml) and MeOH (to complete 10ml)were transferred to a volumetric flask (25 ml), and with the final volume was made with water. The absorption spectrum was recorded from 200 to 340 nm with a resolution of 0.2 nm, against a reagent blank.

For each SPE preconcentration, a C_{18} cartridge (2 g of bed weight) was conditioned with HEXA (10 ml), MeOH(10 ml) and water (20 ml), respectively. Then, the water sample was passed through the sorbent with a constant flow rate (20 $mlmin^{-1}$, approximately). A washing step was carried out with ACN:H_2O (6 % V/V, 5 ml). Finally, the herbicide was eluted with MeOH (8 ml), and was treated as it was mentioned above.

3.0 Results and discussion

3.1 Spectrophotometric determination

Initially, the influence of pH on the absorption spectrum of HEXA was studied. An acid-base equilibrium was observed, deduced from the isosbestic point at 235 nm; as pH increased, a hyperchromic effect was noted at the absorption band with a maximum at 245 nm. After the selection of a pH of 8.0, a phosphate buffer solution was chosen since it did not interfere in the spectral region of work. Concentration of the buffer solution did not show an influence in the absorption spectra, therefore a 0.5 $moll^{-1}$ was selected. MeOH was considered in the preparation of samples, since it is usually the ideal eluent when C_8 or C_{18} cartridges are used. Figure-2 shows the absorption spectrum of HEXA in the conditions proposed in the experimental section.

A linear relationship between HEXA concentration and absorbance at 245 nm was observed for calibration samples. The figures of merit for the spectrophotometric determination of HEXA can be observed in Table-1. Precision under repeatability conditions was expressed as relative standard deviation (7 µg ml^{-1}, n=10). Limits of detection and determination were estimated through a series of ten reagent blank samples, according to:

$$\text{Limit} = k^*s_b/m \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where s_b was the standard deviation of blank samples, m was the slope of calibration curve and k is distinct values (3 for the limit of detection and 10 for quantification).

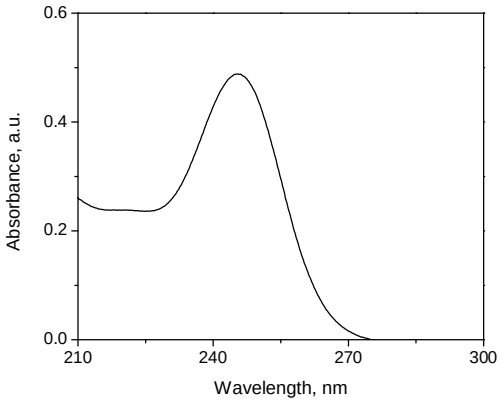

Figure-2: Absorption spectrum of HEXA in water (7 µg ml^{-1})

Table-1:Figures of merit of the spectrophotometric method

Linearity range	0.5 to 14.0 µg ml^{-1}
Equation	A_{245}=0.0701*C + 0.0034
Correlation coefficient, r	0.9997
Precision	1.6 %
Limit of detection	0.15 µg ml^{-1}
Limit of determination	0.5 µg ml^{-1}
Trueness (in absence of organic matter)	98 ± 3% (n=5)
Trueness (in presence of organic matter)	121 ± 3% (n=10)

Trueness was estimated through a series of spiked samples with concentrations varying in the linearity range. Satisfactory results were obtained in absence of organic matter, but if humic substances are present, a major systematic error is observed. Therefore, it was decided to develop a pre-concentration and cleanup step through SPE which could be matched with the spectrophotometric determination, in order to quantify the herbicide in a selective and sensitive way.

3.2 Optimization of SPE

Initially, cartridges of 500 g of bed weight were used as a preliminary step. A categorical multi-factorial model was designed with three factors and one response: a) SPE cartridge, with three levels of SPE (C_{18}, C_8, SDB); b) eluent,with two levels (ACN, MeOH); c) elution volume, with two levels (1.0 and 2.5 ml). As result, 36 trials were generated upon the complete factorial design, by considering three replicates per condition, in a block at random order (26 degrees of liberty). The target response was the recovery percentage of HEXA. In all cases, samples of 250 ml ultrapure water were fortified with HEXA (0.14 µg ml^{-1}) and NaHu (3 µg ml^{-1}). This sample volume did not exceed the infiltration volume of cartridges[25].

Table-2 shows the results of the three-factorial ANOVA[26]. As can be seen, two of the three analyzed factors, the support type and the eluent volume, were statistically significant ($P < 0.05$). Through the analysis of the mean recovery percentages of HEXA for each level of the three factors (see Table-3), it was observed that the highest recoveries were obtained with C_{18} as support and 2.5 ml as eluent volume. Differences were less evident between the eluent type; since MeOH is a solvent of

lower toxicity and cost than ACN, it was used in further studies.

Table-2: Three-factorial ANOVA

Factor	Sum of squares	Degrees of freedom	Mean Square	F	P
Support	27 351.2	2	13 675.6	9.42	*0.0008*
Eluent type	872.3	1	872.3	0.60	0.4451
Eluent volume	6 945.7	1	6 945.7	4.79	*0.0379*

Once the support, eluent, and volume were chosen, the next step was to evaluate the conditioning of the support. For this purpose, the influence of HEXA was studied, as it favors the interaction between the silica and the sample, thus improving the retention of the target analyte, not the interferences [15]. Again, samples of 250 mL of ultra-pure water fortified with HEXA (14 ng ml^{-1}) were prepared (in triplicate), but with a higher content of NaHu (15 µg ml^{-1}). Then, the support was conditioned or not with10 mL of HEXA, followed by 5 ml of MeOH and 10 ml of H_2O. HEXA and NaHu mean recoveries were estimated according to a procedure described elsewhere [16].It could not be observed a statistical difference between the mean recoveries for HEXA; however, a lower amount of organic matter was quantified in the conditioned support. Further experiments with cartridges of 2 g were carried out, from which the final conditioning step was proposed (10 mlof Hx, 10 mlof MeOH and 20 mlof H_2O).

Table-3: Recovery percentages of HEXA obtained from the three-factorial design

Factor	Level	Assays	Mean	Mean error	Lower limit	Higher limit
Support	C_{18}	12	88.44	10.99	65.83	111.04
	C_8	12	76.57	10.99	53.97	99.17
	SBD	12	24.94	10.99	2.34	47.55
Eluenttype	ACN	18	58.39	8.97	39.94	76.85
	MeOH	18	68.24	8.97	49.78	86.70
Eluentvolume	1 ml	18	49.43	8.97	30.97	67.88
	2.5 ml	18	77.21	8.97	58.75	95.66

It was also necessary to define the washing conditions. Based on previous results, ACN seemed to had less capacity to elute HEXA from hydrophobic phases as C_{18} or $C_{8,}$ therefore it was chosen as the solvent for this step. According to the literature, the composition of the washing solution should not reach a final composition of greater than 10% V/V of organic solvent inwater [17]. Thus, a face centered central composite design (2^2 + star) was constructed, considering two factors (volume of MeOH as eluent and percentage of ACN in water for 5 ml of washing solution), one block and one response (recovery of HEXA in percentage); ten trials were done.

The results of the bi-factorial ANOVA are shown in Table-4, using the recovery of HEXA as a response. As can be seen, the volume of the eluent had a significant influence in the recovery of HEXA ($P < 0.05$), but not the composition of the washing solution.

Table-4: Bi-factorial ANOVA

Factor	Sum of squares	Degrees of freedom	Mean Square	F	P
Volume of eluent	1840.20	1	1840.20	145.22	*0.0003*
% ACN:H_2O	0.49	1	0.49	0.04	0.8534

A final washing solution of 6% V/V of ACN in water was proposed, because an acceptable precision was observed (RSD < 5%). From the inspection of the surface response (Figure-3), it was suggested a volume of 8 mlof MeOH as eluent; in that condition, a mean recovery of approximately 100% was obtained, probably because HEXA was eluted but not organic matter.

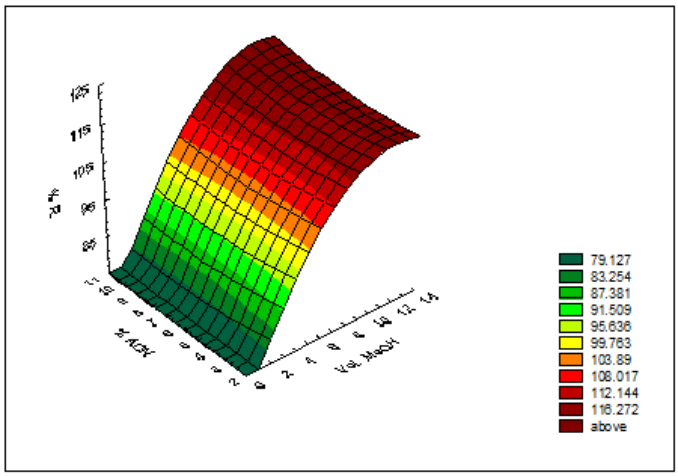

Figure-3: Surface response that shows the influence of eluent volume and composition of washing solution in the recovery of HEXA, for cartridges of 2 g of bed weight.

3.3 Analysis of real samples fortified with HEXA

Once the SPE was optimized in cartridges of (2gm), work was continued with real samples.

Table-5. Analysis of water samples fortified with HEXA(All concentrations are in ng ml^{-1})

Origin	$HEXA_{aded}$	Recovery*
Tap	15	87 ± 30
	35	103 ± 11
	70	100 ± 2
Well 1	40	95 ± 8
	78	107 ± 13
Well 2	40	83 ± 15
Marine	15	71 ± 7

*** Expressed as mean recovery ± confidence interval in percentage (α=0.05, n-1 degrees of freedom, two-sided test)**

Samples of one lot of tap water, well water and marine water were fortified with different concentrations of HEXA and analyzed by the procedure described above. The analysis was carried out in triplicate. According to the results shown in Table-5, a satisfactory quantification of HEXA in terms of precision and accuracy was obtained in tap and well water (mean

recoveries between 87 and 107%), but not in marine water (70%).Also, it was noticed that well II was located near to the beach, which probably influenced the poor results (83%) may be due to the higher salinity of water. The solubility of HEXA could be reduced at high contents of electrolytes in water, reducing its recovery from water samples.

4.0 Conclusion

A simple and reliable method is proposed for the quantification of HEXA in presence of organic matter in water samples. $C_{18}$was identified as a better support than C_8 or SDB for the pre-concentration of the herbicide by SPE, previously conditioned with Hx, MeOH and water. MeOH instead of ACN was selected as eluent. Satisfactory results in terms of precision and accuracy were obtained during the analysis of tap and well water, but not for marine water.

Acknowledgement

The authors gratefully acknowledge financial support from CONACyT (México), project CB-2010/152918.

References

1. Biziu KM. Pesticides, occurrence, determination, and disposal. WNT: Warsaw; 2001**.** (In Polish). Montgomery JH. Agrochemicals desk reference. 2nd ed.Boca Raton FL:CRC Lewis Publishers;2000.
2. US Environmental Protection Agency. Re-registration eligibility decision for hexazinone. Report no. EPA/738-R-94-022 [cited 2013 Dec 20]. Washington: Office of Prevention, Pesticides and Toxic Substances; (1994).Available from: http://www.epa.gov/oppsrrd1/REDs/0266.pdf.
3. Ganapathy C. Environmental fate of Hexazinone Report No. 95814-5624 [cited 2013 Dec 20]. Washington: Department of Pesticide Regulation Sacramento CA; (1996). Available from:http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/hxzinone.pdf.
4. Palma G, Sanchez A, Olave Y, Encina F, Palma R, Barra R. Pesticide levels in surface waters in an agricultural–forestry basin in southern Chile. Chemosphere. 2004; 57: 763-770.
5. Memic M, Vrtacnik M, Vatrenjak-Velagic V, Wissiak-Grm KS. Comparative biodegradation studies of pre-emergence broadleaf and grass herbicides in aqueous medium. Int Biodeter Biodegr. 2005; 55: 109-113.
6. Shaw M, Furnas MJ, Fabricius K, Haynes D, Carter S, Eaglesham G, Mueller JF. Monitoring pesticides in the Great Barrier Reef. Mar Pollut Bull. 2010; 60: 113-122.
7. Zambonin CG, Palmisano F. Determination of triazines in soil leachates by solid-phase microextractioncoupled to gas chromatography–mass spectrometry. J Chromatogr A. 2000;874: 247-255.
8. Gao Y, Zeng Y, Zheng L, Li L. Determination of triazine herbicides in aqueous samples using solidification of a floating drop for liquid-phase micro-extraction with liquid chromatography. Anal Lett. 2009; 42: 1620-1631.
9. Rasul-Jan M, Ara B, Shehzad FN. Quantification of triazine herbicides in soil by microwave-assisted extraction and high-performance liquid chromatography. Environ Monit Assess. 2011; 178:111-119.
10. Baranowska I, Pieszko C. Determination of selected herbicides and phenols in water and soils by solid-phase extraction and high-performance liquid chromatography. J Chromatogr Sci. 2000; 38: 211-218.
11. Lourencetti C, Rodrigues-de-Marchi MR, Ribeiro ML. Determination of sugar cane herbicides in soil and soil treated with sugar cane vinasse by solid-phase extraction and HPLC-UV. Talanta. 2008; 77: 701-709.

12. Baranowska I, Pieszko C. Derivative spectrophotometry in the analysis of mixtures of phenols and herbicides. Analyst. 2000; 125:2335-2338.
13. Baranowska I, Pieszko C. Derivative spectrophotometry in the analysis of bromacil and metoxuron in the presence of simazine or propazine and hexazinone. Anal Lett. 2002; 35: 473-486.
14. Massart DL, Vandeginste BGM, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke J. Handbook of Chemometrics and Qualimetrics: Part A and B.Amsterdam:Elsevier; 1997.
15. Fritz JS. Analytical solid-phase extraction. New York: Wiley-VCH; 1999.
16. Amador-Hernández J, Velázquez-Manzanares M, Márquez-Reyes JM. Determination of hexazinone in environmental samples by UV-Vis spectrophotometry-Partial Least Squares Regression. Res J Chem Sci. 2011; 1: 36-41..
17. Handley AJ. Extraction methods in organic analysis. Sheffield: CRC Press; 1999.