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Determination of bentazon residues in water by high-performance liquid chromatography Validation of the method

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Abstract

A method for determination of bentazon residues in water has been developed. The method involves solid-phase extraction with C_{18} extraction tubes and high-performance liquid chromatographic analysis. A C_{18} column and guard column were used with UV detection at 230 nm, a mobile phase of methanol–water (60:40, v/v) at pH 4.6 (phosphoric acid) and a flow-rate of 0.8 ml/min. After optimization of the extraction and separation conditions, the method was validated. The method developed can be used for determination of bentazon in water, within the international limits of 0.1 μg/l, with a 500-fold pre-concentration.

Keywords: Water analysis; Validation; Bentazon; Pesticides

1. Introduction

Around the world, pesticides of different chemical structures have been applied on agricultural lands. Although these pesticides are considered to be essential for agricultural development, some of them can cause serious ambient contamination [1–6], principally in water.

The acidic herbicides are of interest within the European Union countries. A recent report shows that the acidic herbicides, benazolin, bentazon, 2,4-D, 4-chloro-2-methylphenoxyacetic acid (MCPA), MCPP (2-(4-chloro-2-methylphenoxy)propionic acid) and TCA (trichloroacetic acid) are being used in Europe in amounts over 5×10^5 kg/year. Since most of these pesticides do not attach to soil particles, they probably move easily through the soil in the ground water, and hence may pose problems for resources such as drinking water [7].

Because of this, the European Union and the US Environmental Protection Agency (EPA) established a maximum admissible concentration of 0.1 μg/l for individual pesticides in drinking water [1,3,4,8–12]. This rigorous standard for drinking water purity requires the availability of suitable analytical methods with high sensitivity, selectivity, accuracy and precision.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are good options for pesticide monitoring in water [12]. HPLC is favored over GC in the case of acidic pesticides, with high polarities, low volatilities and thermal instabilities because GC can only be used following a prior derivatization step [5,13–15]. For example, bentazon

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can be analyzed by GC only after diazomethane derivatization, as *N*-methylbentazon [7,16]. Thus, reversed-phase HPLC, with UV or diode-array detection (DAD), is widely used for the analysis of bentazon [7,16–18].

Because of the rigorous limits for water purity, methods for extraction and pre-concentration of the pesticides present in water have become necessary. For this purpose, solid-phase extraction (SPE) is replacing traditional methods [8] such as liquid–liquid extraction (LLE), and has been widely used for extraction of water samples prior to analysis. SPE reduces sample handling, labor and solvent consumption [1,9,11,12]. The most popular SPE sorbent for pesticides in water is octadecyl (C_{18}) bonded silica.

In this work, a simple, rapid, and efficient method was developed for the determination of bentazon in water. Bentazon is a thermally unstable polar herbicide having an acidic character (Fig. 1). It belongs to the tiodiazine family, giving the following poisoning symptoms: apathy, tumors, vomiting and diarrhea. Bentazon is a herbicide used exclusively in post emergence application because of its reduced radicular absorption. In Brazil it is mainly used on peanuts, rice, beans, corn, soy-beans and wheat. In others countries it is used on these and several other, such as grassy, leguminous and others leafy cultures [19–21]. The principal bentazon transformation products are 6- and 8-hydroxybentazon [7,18].

After a method is developed, a most important step is method validation [22,23]. In this work, method validation was applied to the HPLC determination of aqueous residues of the herbicide bentazon, after optimization of the extraction and chromatographic separation conditions. The parameters involved were precision (repeatability), accuracy, recovery, detection and quantification limits and linearity.

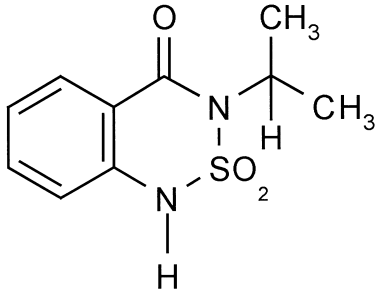

Fig. 1. Structure of bentazon herbicide.

2. Experimental

2.1. Chemicals and reagents

Bentazon standard was obtained from BASF (99.9%). The methanol (Ominosolv, Merck) was chromatographic grade. Sodium chloride (Mallinkrodt) and phosphoric acid (Synth) were analytical reagent grade. Water was purified with a Millipore Milli-Q Plus System.

The extraction tubes were Envi C_{18}, Supelclean (Supelco), packed with 500 mg silica-octadecyl C_{18}.

2.2. Instrumentation

Chromatography was performed with a modular HPLC system equipped with a Rheodyne 7725i injector with a 10-μl loop, a Waters 510 pump, a UV–Vis absorbance detector (Waters Model 486) coupled to a Chrom Perfect for Windows, version 3.03, program in a microcomputer, for acquisition and treatment of data. The pH of the mobile phase was adjusted with use of a Digimed, model DM21, pH meter, with glass and thermal compensation electrodes. The column was a Waters Nova-Pak C_{18} (150×3.9 mm I.D.) and the guard column was also Nova-Pak C_{18} (20×3.9 mm I.D.).

The spectrum of bentazon in mobile phase was taken using a Hewlett-Packard series II/M liquid chromatograph, with a 25-μl loop, a diode array detector and a ChemStation system for data acquisition.

2.3. Procedure

All measurements were carried out at ambient temperature.

The mobile phase was prepared volumetrically from individually measured aliquots of methanol and water. The mobile phase flow-rate was set at 0.8 ml/min and the detection at 230 nm.

The column dead time, t_M, was determined using methanol as the unretained compound.

The stock solution for calibration was prepared in methanol at 0.13 g/l. The samples were diluted in mobile phase and stored in the refrigerator (4°C).

Aqueous samples (250 ml) were fortified by addition of an established volume of stock solution of bentazon, resulting in two levels of fortification, 0.13 and 1.3 μg/l. After adjusting the pH to <2 by addition of phosphoric acid, to increase the bentazon retention, and the ionic strength with 5 g of sodium chloride, the samples were mixed well and forced to percolate through the SPE column under vacuum at a rate of 3 ml/min. Before sample application, the SPE column was conditioned with 10 ml of methanol and equilibrated with 10 ml of Milli-Q water. After the sample had passed through the column, the column was washed with 5 ml of Milli-Q water, the eluate discarded and the sorbent bed dried under vacuum for 5 min. The analyte was then eluted with 1 ml of methanol. The solvent was evaporated to dryness under a stream of nitrogen and the residues were dissolved in 0.5 ml of mobile phase.

3. Results and discussion

Reversed-phase HPLC, with UV detection, has proven to be a good alternative for bentazon determination because no derivatization step is needed.

The position and intensity of the maximum absorption band depends on the solution pH and the solvent. Orinák et al. [16] used 219 nm as the maximum absorption wavelength of bentazon in methanol while Hogendoorn et al. [2] used 229 nm for analysis of bentazon in water. Fig. 2 shows the spectrum of a standard solution of bentazon dissolved in the mobile phase methanol–water (60:40, v/v). The spectrum showed a maximum at 225 nm. However, we choose 230 nm because it is a good wavelength for analyses of bentazon, permitting good sensitivity, while methanol has lower absorption at this wavelength than at 225 nm. The most intense absorption band of bentazon is assumed to belong to the $\pi \rightarrow \pi^*$ transition of the C=O group [16].

Because of its polar character, bentazon does not interact strongly with the C_{18} reversed phase, the most important stationary phase utilized in HPLC. Thus, it is necessary to add phosphoric acid to the mobile phase to increase the bentazon retention, both in the chromatographic column and in the extraction tube. Silva and Jardim [24] showed that methanol–water (60:40, v/v), pH 4.6 is a good mobile phase

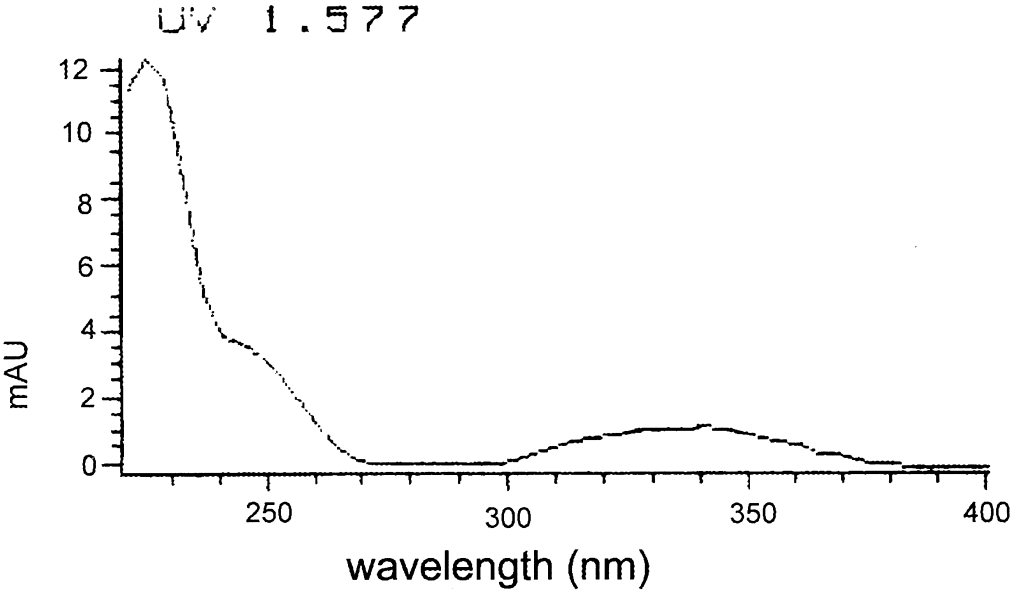

Fig. 2. UV absorption spectrum for bentazon in methanol–water (60:40, v/v), taken with DAD.

for this determination, because it permits retention of bentazon with an adequate retention time. Phosphoric acid was selected because it does not damage either the chromatographic system or the column, has a low absorption in UV and is a stronger acid than acetic acid. Under the chosen conditions, the retention time of bentazon is 1.59 min, permitting the analysis of bentazon in the presence of other herbicides.

Table 1 shows the results for the parameters used in method validation, as described below.

3.1. Precision (repeatability) and accuracy

Accuracy and precision determine, respectively, the analysis error and the deviation, and are the most important criteria for evaluating analytical method performance. Precision reflects the variation in results when repetitive analyses are made on the same sample. The numerical value used is the relative standard deviation (R.S.D.), Eq. (1):

$$\text{R.S.D.} = 100s/x_{\text{m}} \quad (1)$$

where $s=[\Sigma(x_{\text{i}}-x_{\text{m}})^2/n-1]^{1/2}$; $x_{\text{m}}=\Sigma\, x_{\text{i}}/n$; n is the total number of measurements; and x_{i} is number of the individual measurements.

Accuracy is the concordance between true value of analyte in the sample and the value measured by the analytical process. Accuracy is calculated by the Eq. (2):

$$\text{Accuracy} = \left(\frac{\text{measured value} - \text{true value}}{\text{true value}}\right) \times 100 \quad (2)$$

Table 1
Results for validation and extraction of bentazon

Validation parameters	Values
Precision	0.9%
Accuracy	2.1%
Linearity	6.4–1330.0 μg/l
LOD	6.4 μg/l
LOQ	19.0 μg/l
LOD[a]	0.013 μg/l
LOQ[a]	0.038 μg/l
Recovery fortification of 0.13 μg/l	70.1% (R.S.D.=13.5%)
Recovery fortification of 1.3 μg/l	88.1% (R.S.D.=0.30%)

$n=5$ for all measurements.
[a]After 500-fold pre-concentration.

The precision and accuracy are very good (Table 1) because both measurements should be within ±15% at all concentrations [25].

3.2. Calibration

For most chromatographic procedures a linear relation is observed between detector response (y) and analyte concentration (x). This can be expressed as a linear regression equation: $y=a+bx$. The parameters obtained for bentazon calibration are: $a=-1626$ and $b=71.1$ with $r=0.99921$.

3.3. Linearity

The linearity of a method is a measure of range within which the results are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in a sample [25].

Throughout the analytical curve, obtained in the range of three orders of magnitude of concentration, the linearity was evaluated by means of the ratio between signal (S) and concentration (Q), defined by $(S/Q_i)=(S_i-b)/Q_i$, where the ratio signal/concentration for the ith point of the analytical curve, $(S/Q)_i$, is calculated from the corresponding measured signal S_i, of the corresponding concentration Q_i and slope of the analytical curve (a).

In the absence of undetermined errors, i.e., with $r^2=1$, and inside the linear range, it can be shown that $(S/Q)_i=a$ for all pairs of experimental values used to construct the curve. In the presence of undetermined errors ($r^2<1$), the real situation in the experimental condition, and within the linear range, $(S/Q)_i\approx a$. If $(S/Q)_i<a$ or $(S/Q)_i>>a$, then it can be assumed to be out of the linear range. Points were considered to be in the linear range if their $(S/Q)_i$ values were in the interval $(1.00\pm0.05)a$, i.e., points whose signal/concentration ratios do not differ more than 5% from the slope. This tolerance interval is based on the IUPAC chromatography standards.

For bentazon the linearity extended up to 1330.0 μg/l.

3.4. Detection and quantification limits

The detection limit (LOD) is the lowest solute concentration detectable by an analytical method and

is expressed in concentration units. The quantification limit (LOQ) is the lowest solute concentration that can be determined with acceptable precision and accuracy, under experimental conditions. It is also expressed in concentration units. In this study LOD and LOQ were determined according to the definition of Francotte et al. [23], Eqs. (3) and (4):

$$\mathrm{LOD} = 2h_n C_s / h_s \quad (3)$$

$$\mathrm{LOQ} = 6h_n C_s / h_s \quad (4)$$

where C_s is amount of analyte injected; h_s is peak height of the analyte; h_n is the largest deviation of detector signal from the average baseline level, measured at the retention time of the analyte.

To measure these parameters a series of diluted bentazon standard samples were used. From this series, the peak is selected whose height h_s is about 2–10 times larger than the signal-to-noise ratio h_n ($C_s = 13.3$ μg/l). The h_s value is the height of the analyte measured from the average baseline level to the top of the peak, in mV, while h_n is measured over 10 peak widths in the absence of analyte.

3.5. Recovery

After defining the analytical conditions, tests were made on the recovery of bentazon with C_{18} SPE extraction tubes.

Recovery is measured as the response of a processed spiked matrix standard, expressed as a percentage of the response of a pure standard which has not been subjected to sample pre-treatment. It indicates whether the method provides a response for the entire amount of analyte that is present in the sample. It is best established by comparing the replicate responses of extracted samples at matrix concentrations with those of non-extracted standards which represent 100% recovery. The recovery was calculated with the Eq. (5) [25]:

$$\mathrm{Recovery} = \frac{\text{mass of analyte afterextraction} \times 100}{\text{mass of analyte added}} \quad (5)$$

The recovery tests were carried out on five replicates, permitting calculation of the relative estimated standard deviation (R.S.D.), also called the variation coefficient.

The average results obtained for bentazon recovery (see Table 1) are very good, because a 70–110% recovery range has been considered acceptable, along with a 15% range for R.S.D. [25].

4. Conclusion

The mobile phase methanol–water (60:40, v/v), adjusted to pH 4.6 with phosphoric acid, is adequate for accurate analyses of bentazon. The wavelength utilized (230 nm) permits good detection of the herbicide.

The results obtained for calibration, recovery, linearity, precision and accuracy show that this is a rapid, efficient and simple method for the determination and quantification of bentazon in water samples.

The results of LOD and LOQ are, respectively, 6.4 and 19.0 μg/l. Considering the 500-fold pre-concentration step, the effective LOD and LOQ are, respectively, 13 and 38 ng/l.

Thus, using appropriate SPE pre-concentration, it is possible to determinate bentazon in water at concentrations lower than 0.1 μg/l, satisfying the international limits.

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References

[1] M. Knuteson, G. Nilvé, L. Mathiasson, J.A. Jonson, J. Chromatogr. A 754 (1996) 197.
[2] E.A. Hogendoorn, R. Hoogerbrugge, R.A. Baumann, H.D. Meiring, A.P.I.M. de Jong, P. van Zoonen, J. Chromatogr. A 754 (1996) 49.
[3] S. Hartrík, J. Tekel, J. Chromatogr. A 733 (1996) 217.
[4] J.R. Dean, G. Wade, I.J. Barnabas, J. Chromatogr. A 733 (1996) 295.

[5] C. Aguilar, F. Borrull, R.M. Marcé, Chromatographia 43 (1996) 592.
[6] S. Rodolino, R. Giovinazzo, M. Mosconi, Bull. Environ. Contam. Toxicol. 38 (1997) 644.
[7] S. Chiron, E. Martinez, D. Barceló, J. Chromatogr. A 665 (1994) 283.
[8] I. Tolosa, J.W. Readman, L.D. Mee, J. Chromatogr. A 725 (1996) 93.
[9] C. Aguilar, F. Borrull, R.M. Marcé, LC·GC 14 (1996) 1048.
[10] C. Hidalgo, J.V. Sancho, F. Hernández, Anal. Chim. Acta 338 (1997) 223.
[11] A. Balinova, J. Chromatogr. A 754 (1996) 125.
[12] A. Junker-Buchneit, M. Witzenbacher, J. Chromatogr. A 737 (1996) 67.
[13] I. Liska, J. Slobodník, J. Chromatogr. A 733 (1996) 235.
[14] V. Gokmen, J. Acar, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 1917.
[15] H.S. Kim, S.K. Lee, D.W. Lee, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 871.
[16] A. Orinák, K. Holovská, V. Budínská, Fresenius J. Anal. Chem. 339 (1991) 436.
[17] G. Durand, V. Bouvot, D. Barceló, J. Chromatogr. 607 (1992) 319.
[18] S. Chiron, S. Papilloud, W. Haerdi, D. Barceló, Anal. Chem. 67 (1995) 1637.
[19] P.C. Kearney, D.D. Kaufman, Herbicides: Chemistry, Degradation and Mode of Action, 2nd ed., Marcel Dekker, New York, 1969.
[20] WSSA Herbicide Handbook Committee, Herbicide Handbook, 3rd ed., Weed Science Society of America, 1974.
[21] F.S. Almeida, B.N. Rodrigues, Guias de Herbicidas, 2nd., Livroceres, Londrina, 1988, 603 pp.
[22] R. Causon, J. Chromatogr. B 689 (1997) 175.
[23] E. Francotte, A. Davatz, R. Richert, J. Chromatogr. B 686 (1996) 77.
[24] G.M.F. Silva, I.C.S.F. Jardim, Seleção de Fase Móvel para Separação de Herbicidas, por Cromatografia Líquida de Alta Eficiência (CLAE), em Amostras de Água, 7th Latin-American Congress on Chromatography and Related Techniques, March 1998, Águas de São Pedrro, SP, Brazil, Abstr. P189, p. 202.
[25] Roteiro para Validação de Metodologia Analítica Visando a Determinação de Resíduos de Pesticidas, Laboratório Vegetal do Ministério da Agricultura, GARP, ANDEF, versão 1, 1997.