Spectrofluorimetric determination of the herbicide bentazone: microenvironment effects on the analytical signal

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Abstract

A spectrofluorimetric method for the determination of bentazone residues has been developed. The method is performed in N,N-dimethylformamide as solvent. The results were compared with those obtained by incorporation of the compound in α-cyclodextrin and liquid chromatography with spectrophotometric detection at 340 nm. The fluorescence intensity is linearly related to bentazone concentration with a detection limit of 3.3 ng ml⁻¹ and a relative standard deviation of 1% at the 0.55 µg ml⁻¹ level. The method was applied to herbicide added (0.2 µg ml⁻¹) to beans with recoveries of 100.8% compared with 96% by the liquid chromatographic method.

Keywords: Fluorimetry, Bentazone, Herbicides, Pesticides

The interaction between a molecule and its environment has important effects on luminescence behaviour. An important environmental consideration is solvent polarity, as increasing solvent polarity stabilizes the energy levels involved in fluorescence. On the other hand, complexation between organic molecules and cyclodextrins isolates the molecules from the surrounding environment and largely eliminates the non-radiative energy dissipation pathways [1]. Cyclodextrins in aqueous media have received considerable attention in recent years and their ability to enhance luminescence has been exploited in the determination of a wide variety of organic compounds, mainly pesticides and drugs [2].

Bentazone is a contact herbicide that is currently used for broad-leaved weed control in several bean crops. It is absorbed by the leaves and, having little effect on germinating seeds, is not used pre-emergence. Its herbicide activity is brief. Levels found in stream water are ca. 0.5 µg l⁻¹ [3]. It has toxic properties, is a biologically hardly decomposable substance [4] and its residues can cause problems. Hence, there is increasing interest in the determination of its concentration in vegetables. Several methods are available, e.g., liquid chromatography (LC) with UV spectrophotometric [5–8], fluorescence [9–11] and electrolytic detection [7,11], gas chromatography with flame ionization [12–16], electron capture [12,17], nitrogen–phosphorus [12] and mass spectrometric detection [18], thin-layer chromatography [19–21] and polarography [22], but no direct fluorimetric methods have been reported.

In this work, α- and β-cyclodextrins and a set of organic solvents with different polarities were used to study the influence of the microenvironment of bentazone on the analytical signal arising.

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from fluorescence measurements. The results were used to establish a spectrofluorimetric method to determine bentazone. The optimized method was used to determine bentazone added to beans, and the results obtained were compared with those of an LC method with UV-visible spectrophotometric detection.

EXPERIMENTAL

Apparatus

Emission measurements were made with a Perkin-Elmer LS-50 luminescence spectrometer equipped with a xenon discharge lamp and two monochromators. Instrumental parameters were controlled by the Perkin-Elmer FL Data Manager (FLDM) software. Information was sent via the RS232C interface of the fluorescence instrument to an external computer. For graphical recording, an NEC Silentwriter2 S60P laser printer was connected to the spectrofluorimeter.

The LC equipment included a Merck–Hitachi L-6200 pump, with injection using a Merck–Hitachi AS-4000 autosampler. A Model L-4250 UV-visible detector (Merck–Hitachi) was used. The analogue signals were converted to digital by a Merck–Hitachi D-6000 interface. Integration was made with a PC/AT computer and the instrumental parameters were controlled by Hitachi–Merck HM software. A reversed-phase column (12.5 × 0.4 cm i.d.) and a precolumn of LiChrospher 100 RP-18 (5 μm) were used, with acetonitrile–water–methanol (60:20:20, v/v/v) as the mobile phase at a flow-rate of 1 ml min⁻¹.

Absorbance was measured with a Shimadzu UV-240 Graphcord spectrophotometer.

Reagents and solvents

Bentazone [3-isopropyl-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2 dioxide] (99%) was obtained from Dr S Ehrenstorfer (Augsburg, Germany). The solvents used were purchased from Merck Solutions (0.01 M) of α- and β-cyclodextrin (Sigma, St Louis, MO), recrystallized once from boiling water, were prepared in doubly deionized, deionized water. A buffer solution (pH 9.3) was prepared from 0.2 M Na₂CO₃ and 0.2 M NaHCO₃ solutions.

Stock solutions of bentazone (4.16 × 10⁻³ M) were prepared in N,N-dimethylformamide (DMF) and stored in the dark at 4°C. This solution was stable for at least 4 weeks.

Solutions of bentazone with α- and β-cyclodextrin were prepared from aliquots of the herbicide in DMF and buffer solutions and made up to a final volume of 3 ml with cyclodextrin solution. These samples were sonicated for 20 min.

For the LC method, methanolic solutions of bentazone (4.16 × 10⁻³ M) were used.

Spectrofluorimetric procedure for a DMF solution

The fluorescence intensity of aliquots of bentazone solutions was measured at 430 nm with excitation at 353 nm, against a reagent blank, at 50–800 ng ml⁻¹ using 2.5 nm slit widths for excitation and emission.

Spectrofluorimetric procedure for an α-cyclodextrin solution

Aliquots of bentazone solutions sufficient to ensure final concentrations between 0.1 and 1 μg ml⁻¹ were added to 0.01 M α-cyclodextrin solutions and 0.3 ml of buffer solution (pH 9.3) to a final volume of 3 ml and were sonicated for 20 min to ensure completion of the complex inclusion process. The fluorescence spectra were measured at the emission maximum of 458 nm, with excitation at 334 nm with a 2.5 nm excitation slit width and a 10 nm emission slit at levels between 0.1 and 1 μg ml⁻¹.

LC procedure

Standard methanolic solutions of bentazone were prepared and injected (10 μl) by the autosampler. Detection was based on absorbance at 340 nm. The mobile phase was acetonitrile–water–methanol (60:20:20, v/v/v). Bentazone had a retention time of 0.76 min and the beans matrix a time retention of 1.20 min under these conditions at a flow-rate of 1 ml min⁻¹. The linear dynamic range was 1–10 μg ml⁻¹. 
SPECTROFLUORIMETRY OF BENTAZONE

Sample extraction

Chopped bean tissue (5 g), previously spiked with bentazone, was blended a high speed for 5 min in 25 ml of methanol. The blended sample was filtered through a fritted glass Buchner funnel (coarse porosity) under reduced pressure, and the filter cake was washed with 6 ml of methanol. The filtrate was then diluted to volume (50 ml) with methanol.

The methanol extract (8 ml) was transferred into a 100-ml separating funnel and 0.1 ml of 2 M sulphuric acid was added, followed by 20 ml of 5% sodium sulphate solution. The mixture was extracted twice with 10 ml of dichloromethane, with each extract being passed through a Whatman No. 1 filter-paper containing charcoal and then 6 ml of anhydrous sodium sulphate into a 50-ml round-bottomed flask, followed by washing of the sodium sulfate with 5 ml of dichloromethane. The combined dichloromethane extracts were concentrated just to dryness by using a rotary evaporator. The residue was diluted with the appropriate solvent (DMF or methanol) and aliquots of these solutions were analysed by the spectrofluorimetric or LC method.

Recovery assay

A 100-μl volume of bentazone solution in DMF was transferred into a 10-ml flask, with different amounts of atrazine, MCPA or dichlorprop solutions in DMF, with final weight ratios of 1:10, 1:50 and 1:100 and pre-fixed experimental conditions.

RESULTS AND DISCUSSION

Solvent effects on spectral data

The effects of various solvents with different polarities and hydrogen-bonding capacities on the fluorescence and absorption spectra of \(4.16 \times 10^{-5}\) M bentazone solution were studied. The most relevant characteristics found are summarized in Table 1. Some spectra are shown in Fig 1. The results showed that the fluorescence intensity, which is dependent on the molar absorptivity (\(ε\)), is the highest in DMF. It also gives the largest relative efficiency (RE = relative intensity/\(ε\)), and greatly increases the values with respect to aqueous solution. The absorption spectrum of bentazone is slightly shifted to the red on going from dioxane to water. Also, the highest molar absorptivity and relative fluorescence intensity (RFI) are achieved in polar solvents, but with an anomalous behavior with water, in which the RFI is minimum. The results indicate that, although the probability of absorption transition is greater in polar solvents, this is not the case for the fluorescence intensity in water because in this solvent other processes such as internal conversion and intersystem crossing probably compete with fluorescence. Therefore, it can be deduced that the best results are obtained in solvents with high dielectric constants such as methanol or DMF, in which a very high relative efficiency of fluorescence is obtained. For this reason, DMF was selected as solvent in the determination of bentazone.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dielectric constant</th>
<th>λ(abs) a (nm)</th>
<th>λ(em) (nm)</th>
<th>Relative intensity</th>
<th>Log ε b (\times 10^{-3})</th>
<th>Relative efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>78.54</td>
<td>335</td>
<td>440</td>
<td>2.1</td>
<td>3.69</td>
<td>0.43</td>
</tr>
<tr>
<td>DMF</td>
<td>36.70</td>
<td>353</td>
<td>430</td>
<td>1043.5</td>
<td>3.78</td>
<td>170.0</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.63</td>
<td>339</td>
<td>450</td>
<td>91.9</td>
<td>3.41</td>
<td>35.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.30</td>
<td>308</td>
<td>425</td>
<td>25.4</td>
<td>3.31</td>
<td>12.0</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>9.08</td>
<td>305</td>
<td>470</td>
<td>21.5</td>
<td>3.35</td>
<td>9.6</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>6.02</td>
<td>303</td>
<td>435</td>
<td>10.0</td>
<td>3.16</td>
<td>6.9</td>
</tr>
<tr>
<td>Dioxane</td>
<td>2.21</td>
<td>300</td>
<td>370</td>
<td>25.0</td>
<td>3.21</td>
<td>15.4</td>
</tr>
</tbody>
</table>

a Wavelength of maximum absorbance; b Logarithm of the maximum molar absorptivity measured in \(1\) \(\text{mol}^{-1}\) \(\text{cm}^{-1}\)
Fig 1 Fluorescence emission spectra of bentazone in (1) water, (2) DMF, (3) \( \beta \)-cyclodextrin and (4) \( \alpha \)-cyclodextrin 

\[ [\text{Bentazone}] = 4.16 \times 10^{-2} \text{ M}, [\alpha-\text{CD}] = [\beta-\text{CD}] = 10^{-2} \text{M}, \text{pH 9.3} \]

Even as little as 0.5% of water greatly decreased the fluorescence of bentazone in DMF, indicating that when water is present, rapid deactivation of the excited states take place. Hence the method must be applied in anhydrous DMF. The effect of herbicide concentration was tested and showed a linear increase in signal up to 2.08 \( \times \) 10\(^{-3} \) M bentazone, with a slight decrease at higher concentrations (Fig 2). Because cyclodextrins can solubilize hydrophobic molecules in aqueous media through complex formation, separate experiments were conducted to study the behaviour in \( \alpha \)- and \( \beta \)-cyclodextrin solutions. The inclusion process promotes changes in the fluorescence spectra. An enhancement of the fluorescence emission is observed in the presence of \( \alpha \)- or \( \beta \)-cyclodextrin, but for the bentazone–\( \alpha \)-cyclodextrin complex a shift to longer wavelengths is registered. Inclusion decreases the rotational freedom of the bentazone included because it is bonded to the interior of the cyclodextrin cavity in a particular orientation. This physical constraint also decreases the constant rate of non-radiative processes, and so enhances fluorescence.

In Fig 1 the emission spectra of 4.16 \( \times \) 10\(^{-7} \) M bentazone in aqueous, DMF and \( \alpha \)- and \( \beta \)-cyclodextrin solutions in pH 9.3 are shown. The maximum intensity in 100% DMF solution was achieved at 430 nm with excitation at 353 nm. The bentazone–\( \alpha \)-cyclodextrin complex showed maxima at excitation and emission wavelengths of 334 and 458 nm, respectively, and for the \( \beta \)-cyclodextrin complex at 330 and 441 nm, respectively. As can be seen, there is little difference in intensity between the complexes. The emission

Fig 2 Effect of bentazone concentration on the analytical signal. Conditions as under Experimental.
maxima displacements in cyclodextrins with respect to that in water, 5 and 35 nm for β- and α-cyclodextrin, respectively, also support the inclusion of bentazone in cyclodextrin. The inner diameter is 5.7 and 7.8 Å for α- and β-cyclodextrin, respectively [23], so geometric factors are probably decisive in determining the inclusion.

Optimization of reversed-phase LC was carried out with a column and a precolumn using acetonitrile–water–methanol (60:20:20, v/v/v) as the mobile phase at a flow-rate of 1 ml min⁻¹. Various proportions of acetonitrile, water and methanol were tried, 60:20:20 gave the maximum peak height. The most favourable UV wavelength of 340 nm, where the signal of the blank was minimum, was selected.

**Analytical characteristics**

A linear calibration graph was obtained by plotting the fluorescence intensity against standard bentazone concentration between 50 and 800 ng ml⁻¹ in DMF. For α-cyclodextrin, a linear calibration graph was established for the range 0.1–1 μg ml⁻¹. The calibration graph obtained in the DMF method by least-squares treatments is described by

\[ I_f = 180[B] + 5.89 \quad (r = 0.990, \ n = 5) \]

where \( I_f \) is the relative fluorescence intensity, \( r \) the correlation coefficient and \([B]\) the bentazone concentration in μg ml⁻¹. For α-cyclodextrin, the corresponding expression is

\[ I_f = 92.2[B] - 7.11 \quad (r = 0.9996, \ n = 5) \]

Reproducibility was measured on seven replicates of bentazone in DMF at the 0.55 μg ml⁻¹ level and on four replicates of bentazone–α-cyclodextrin complex at the 0.50 μg ml⁻¹ level.

The detection limit was calculated as \( 3\sigma_B/m \), where \( \sigma_B \) is the standard deviation of the blank signal and \( m \) is the slope of the linear plot. The limit of quantification, \( C_Q = 10\sigma_B/m \), was employed to establish the lower limit of the linear dynamic range. The relative sensitivity of the method is reported as \( s_A = \sigma_{n-1}/m \), where \( \sigma_{n-1} \) is the standard deviation of the analytical signal.

Table 2 gives the results obtained, together with other details of the precision and accuracy of the method.

Using the LC method, linear calibration graphs (standard solutions of bentazone in methanol) were obtained in the range 3–10 or 1–3 μg ml⁻¹ with correlation coefficients of 0.9999 (n = 3) and 0.9532 (n = 3), respectively. The relative standard deviations of the peak area and retention time were 1.29% and 1.5%, respectively (n = 7).

**Interferences**

To evaluate the selectivity of the proposed spectrofluorimetric method, the effect of some other pesticides on the determination of 1 μg ml⁻¹ of bentazone was studied. The pesticides selected were those currently included in formulations together with bentazone. Various volumes of stock solutions of the different potential interferents in DMF were added to bentazone standard solution (1 μg ml⁻¹) in order to obtain different interferent to analyte ratios in the final.

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**Table 2**

Characteristics of the method

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection limit (ng ml⁻¹)</th>
<th>( C_Q ) (ng ml⁻¹)</th>
<th>Relative sensitivity (ng ml⁻¹)</th>
<th>Error (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>33</td>
<td>11</td>
<td>5.8</td>
<td>100 a</td>
<td>1.08 a</td>
</tr>
<tr>
<td>α-Cyclodextrin</td>
<td>570</td>
<td>191</td>
<td>20.7</td>
<td>872 b</td>
<td>4.05 b</td>
</tr>
</tbody>
</table>

a At 0.55 μg ml⁻¹ (n = 7) b At 0.50 μg ml⁻¹ (n = 4)
TABLE 3
Results of interference study (bentazone = 1 μg ml⁻¹)

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration (μg ml⁻¹)</th>
<th>Recovery of bentazone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>MCPA</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Dichlorprop</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>102</td>
</tr>
</tbody>
</table>

The solutions were treated as described under Experimental.

The results obtained are given in Table 3, where it can be seen that acceptable recoveries are obtained, except perhaps for dichlorprop at a 10:1 interferent to analyte ratio.

**Application to the determination of bentazone in beans**

Bentazone is used as a contact herbicide in the control of pests affecting maize, peas, rice, soybeans, cereals and other crops. The proposed DMF method was applied to the determination of bentazone added to beans following the sample extraction procedure. In Table 4, the results obtained for bean samples spiked at two levels are summarized. To confirm the results they were compared with those obtained using the LC method described previously, the recovery was 96% at a concentration of 0.2 μg ml⁻¹.

TABLE 4
Determination of bentazone added to different beans samples

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Taken (μg ml⁻¹)</th>
<th>Found (μg ml⁻¹)</th>
<th>Recovery (%)</th>
<th>Mean recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.200</td>
<td>0.195</td>
<td>98.0</td>
<td>100.8</td>
</tr>
<tr>
<td>2</td>
<td>0.200</td>
<td>0.202</td>
<td>101.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.200</td>
<td>0.207</td>
<td>103.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.550</td>
<td>0.546</td>
<td>99.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.550</td>
<td>0.553</td>
<td>100.7</td>
<td>99.8</td>
</tr>
<tr>
<td>3</td>
<td>0.550</td>
<td>0.547</td>
<td>99.5</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

The results show that the proposed spectrofluorometric procedure is a simple and useful method for the determination of bentazone at trace levels in samples such as beans. The cyclodextrin method does not give an improved analytical performance compared with the DMF method and, because the previous extraction procedure incorporates organic solvents, no additional complication is introduced in the DMF procedure.

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