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Heavy Atom Induced Room Temperature Phosphorescence Method for the Determination of the Plant Growth Regulator β-Naphthoxyacetic Acid

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A simple, selective, and sensitive heavy atom induced room temperature phosphorimetric method (HAI-RTP) is described for the determination of the plant growth regulator β-naphthoxyacetic acid (NOA) in spiked apple samples. A careful selection of the different variables (heavy atom and sodium sulfite) to obtain the phosphorescence signal constitutes the basis of a HAI-RTP method for the determination of NOA. The analytical curve of NOA gives a linear dynamic range of 0−625 ng mL^{-1} and a detection limit of 30.5 ng mL^{-1}. A recovery of 108.8% was obtained for 500 ng mL^{-1} NOA in spiked apple samples.

Keywords: *β-Naphthoxyacetic acid; heavy atom induced room temperature phosphorescence (HAI-RTP)*

INTRODUCTION

Various plant growth regulating hormone type chemicals have been investigated in the past years on different crops for promoting early fruit-set. Among these substances β-naphthoxyacetic acid (NOA) has been demonstrated to be useful as a plant hormone to prevent fruit from falling prematurely and to promote the growth of roots on dipping, specially on grapes, pineapples, apples, strawberries, and tomatoes.

The main objective in the analysis of plant growth regulators is the control of the agricultural crops usually consumed by human beings. Several country regulations permit relatively high tolerance levels, due to their low toxicity. Despite being not very toxic substances, they could be hazardous if their consummption is carried out in high quantities.

After a careful review of the methods published in the literature for the analytical determination of this hormone, it is deduced that there are only a few methods available, being mostly based on fluorescence measurements (Pease and Gardiner, 1969; Davidson, 1970; García Sánchez and Cruces Blanco, 1988).

Although phosphorimetry is sensitive and more selective than fluorimetry for the analysis of many compounds, until now, it has not been frequently used when the methodology of low temperature was the only one to be developed, because of the time-consuming sample conditions required. Recently, different methodologies such as sensitized room temperature phosphorescence (RTP), micelle-stabilized RTP, or the use of cyclodextrins combined with sodium sulfite as oxygen scavenger have allowed for development of RTP methods for numerous compounds in solution (Díaz-García and Sanz-Medel, 1986; Sanz-Medel et al., 1987; Hurtubise, 1990; Cruces Blanco et al., 1996; Segura Carretero et al., 1996, 1997). Only a few phosphorimetric methods have been proposed for this compound, mainly at low temperature (Sanders and Winefordner, 1972; Aaron et al., 1979; Trautwein and Guyon, 1983), with only three at room temperature with solid support (Aaron and Winefordner, 1979), cyclodextrin (Muñoz de la Peña et al., 1993), or micellar medium (Segura Carretero et al., 1996).

We have recently observed that it is possible, for some compounds, to observe phosphorescence signals in solution by using exclusively aqueous solutions of the analytes in the presence of a heavy atom and salt sodium sulfite as oxygen scavenger, which is mentioned as heavy atom induced room temperature phosphorescence (HAI-RTP) (Segura Carretero et al., 1998).

The aim of this work is the development of the first application of the heavy atom induced room temperature phosphorimetric method in solution for the determination of NOA in spiked apple samples. The work presented here competes favorably in simplicity and detection limit with those methods proposed earlier in the literature.

MATERIALS AND METHODS

Apparatus. All recordings of uncorrected luminescence spectra and measurements of HAI-RTP intensities were carried out with an Aminco Bowman series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp, equipped with a thermostated cell holder. The system was controlled with a personal computer with a 40 MB hard disk, a 4 MB RAM memory, a 3.5 in. 1.44 MB floppy disk drive, a VGA color monitor with VGA graphics adapter card, serial two-button mouse, DOS 6.0, OS/2 version 2.0, and a GPIB(IEEE−488) interface card for computer instrument communication. An ultrasonic bath ULTRASONS (Selecta) was used for sample sonication.

Reagents. The reagent grade thallium(I) nitrate, potassium iodide, potassium bromide, potassium chloride, sodium iodide, sodium bromide, and anhydrous sodium sulfite (Sigma Chemical Co.) were used as received. Aqueous solutions were made with doubly distilled water. The sodium sulfite solutions were prepared daily and kept in tightly stoppered containers.

NOA (Sigma) was used without further purification. Stock solutions were prepared by dissolving 2.50 mg of NOA in 25 mL of doubly distilled water.

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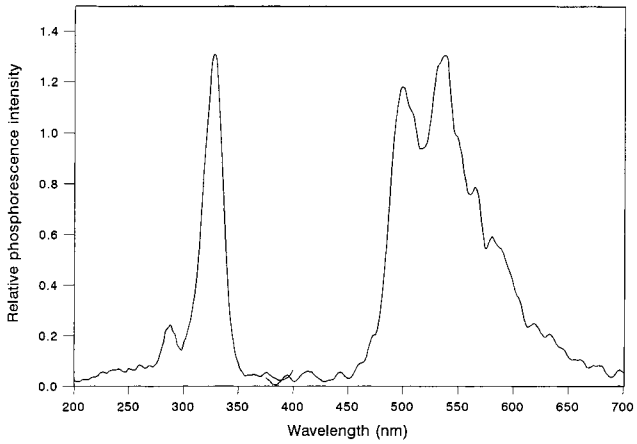

Figure 1. Excitation and emission spectra of NOA: [NOA] = 500 ng mL^{-1}; [$TlNO_3$] = 0.25 mol L^{-1}; [Na_2SO_3] = 0.010 mol L^{-1}; delay time, 100 μs; gate time, 500 μs; slits, 16/16 nm; detector sensitivity, 1100 V.

Procedure. A 50 μL aliquot of NOA stock solution with 5.00 mL of 0.5 mol L^{-1} thallium was introduced into a 10 mL calibrated flask and made up to volume with water. Standard 10 mm fused silica cells were filled with these analyte solutions. The intensities of the samples and the corresponding blanks were measured at phosphorescence wavelength maxima $\lambda_{ex}/\lambda_{em}$ = 326/540 nm (fluorescence wavelength maxima of NOA are 326/346 nm). The half-life time of the phosphorescence emission in the experimental conditions was 307 μs.

Precautions must be taken with the use and handling of thalium salts, due to its chronic toxic nature.

Samples. A 250 g sample of apples was chopped in a food processor, 15 g was transferred to a blender cup, and 20 mL of acetone containing 0.3 mL of orthophosphoric acid was added and blended for several minutes. The mixture was filtered with a 30 mL medium-porosity fritted-glass Büchner flask under suction, and the blender and filter were washed three times with 5 mL portions of acetone; all of the extracts were mixed and taken to dryness in a rotary evaporator at 40 °C. The concentrate was diluted to 25 mL with distilled water. The basic procedure was applied to this solution.

Due to the toxicity of acetone vapors, the extraction and blending should be carried out under a vacuum dispositive.

RESULTS AND DISCUSSION

HAI-RTP Spectra. Different instrumental parameters related to the luminescence technique could also affect the phosphorescence response, so they should be carefully selected. As is seen in Figure 1, the excitation and emission wavelengths of greatest phosphorescence are observed at 326 and 540 nm, respectively, using slits of 16 nm, a scan speed of 2 nm s^{-1}, a delay time of 100 μs, a gate time of 500 μs, a detector sensitivity of 1100 V, and a minimum period pulse or time between flashes of 5 ms. All these instrumental variables were kept constant for the rest of the experimental work.

Influence of Heavy Atom. The influence of heavy atom salt (KI, NaI, KBr, NaBr, KCl, $TlNO_3$, $Pb(NO_3)_2$, and $AgNO_3$) concentration on the HAI-RTP of NOA was studied. $Pb(NO_3)_2$ and $AgNO_3$ precipitated in the presence of SO_3^{2-}, so they cannot be used, while, with KCl, no phosphorescent signals were observed.

The study of the rest of the heavy atom salts is represented in Figure 2. In all cases, it has been proved that no phosphorescence responses of the analytes are obtained in the total absence of a heavy atom, while, in general, the HAI-RTP intensity increased with increasing heavy atom concentration. A concentration of 0.25 mol L^{-1} for $TlNO_3$ was found as optimal, because the maximum intensity was obtained and was selected for the rest of the experimental work.

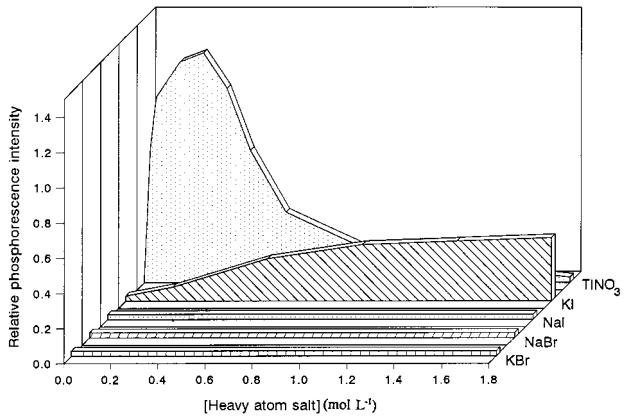

Figure 2. Influence of different heavy atoms on the HAI-RTP of NOA: [NOA] = 500 ng mL^{-1}; [Na_2SO_3] = 0.010 mol L^{-1}; $\lambda_{ex}/\lambda_{em}$, 326/540 nm; delay time, 100 μs; gate time, 500 μs; slits, 16/16 nm; detector sensitivity, 1100 V.

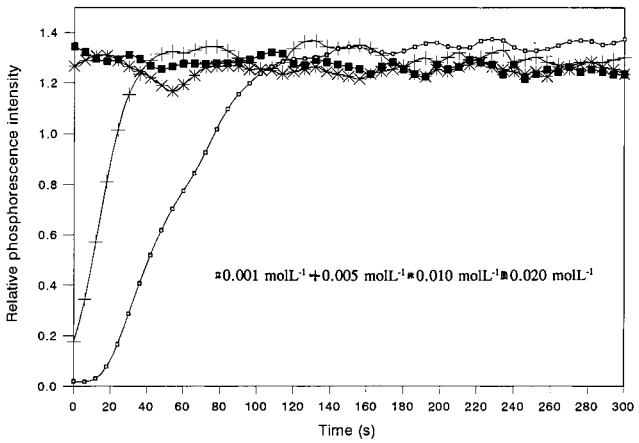

Figure 3. Influence of sodium sulfite concentration on the HAI-RTP of NOA: [NOA] = 500 ng mL^{-1}; [$TlNO_3$] = 0.25 mol L^{-1}; $\lambda_{ex}/\lambda_{em}$, 326/540 nm; delay time, 100 μs; gate time, 500 μs; slits, 16/16 nm; detector sensitivity, 1100 V.

Influence of Sodium Sulfite Concentration. Various amounts of sodium sulfite were added to a solution with a fixed amount of NOA and heavy atom salt, while the concentration of sodium sulfite was varied from 0.001 to 0.020 mol L^{-1}. The concentration of NOA was 500 ng mL^{-1}, and the concentration of $TlNO_3$ was 0.25 mol L^{-1}. These solutions were transferred into a 10 mL flask, with the appropriate amounts of 0.1 mol L^{-1} sodium sulfite stock solution to obtain the final desired concentration.

The development of the HAI-RTP signals has been performed by monitoring at 540 nm the phosphorescence emission as a function of time until the HAI-RTP signal was stable for, at least, 5 min.

Figure 3 shows the influence of sodium sulfite concentration on the phosphorescent emission from NOA in the presence of $TlNO_3$. It has been observed, in both cases, that Na_2SO_3 concentration does not influence the intensity of phosphorescence; however, it influences the time of appearance of the emission in such a manner that increasing sodium sulfite concentration and phosphorescence signals are more rapidly obtained due to a more effective deoxygenation. A 0.010 mol L^{-1} sodium sulfite concentration was selected for the rest of the experimental work because less quantity would be used

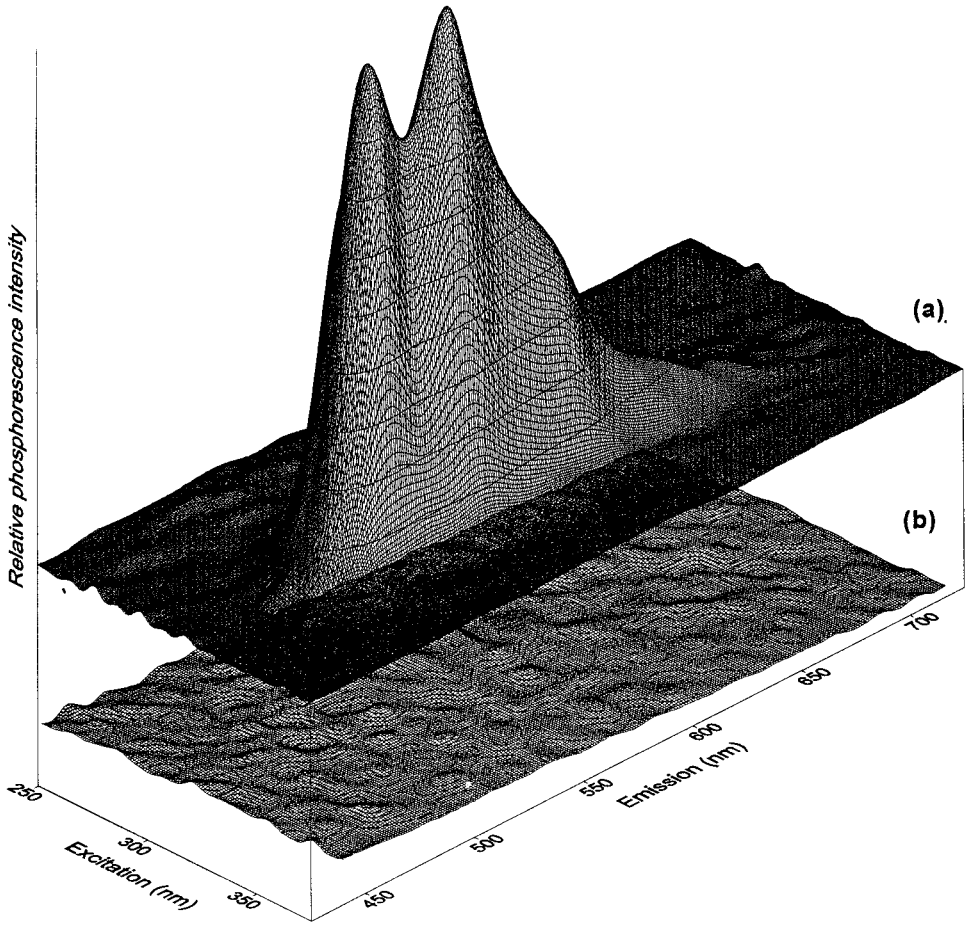

Figure 4. Three-dimensional spectra for a 500 ng mL^{-1} NOA spiked sample (a) and blank apple sample (b): delay time, 100 μs; gate time, 500 μs; slits, 16/16 nm; detector sensitivity, 1100 V.

and the intensities as statistically equivalent for 0.020 and 0.010 mol L^{-1} maximum RTP signal is reached in 0 s.

Effect of Temperature on HAI-RTP Intensity. For obtaining the HAI-RTP signal of NOA, a detail study of temperature was carried out. The HAI-RTP intensity of NOA was decreased by 30% upon increasing the temperature from 20 to 35 °C. It can be deduced from these results that higher phosphorescence signals would be obtained at low temperatures, but, due to the fact that ambient temperature is around 25 °C, to obtain more reproducible results, this value has been chosen for the rest of the experimental work.

Stability. The HAI-RTP signal of the system can reach stability instantaneously under these experimental conditions and remain stable at least 1 h.

Analytical Curve and Precision. The method was tested for linearity, precision, reproducibility, and specificity. Phosphorescence response was linear in relation to the concentration of NOA over the range 0.03−625 ng mL^{-1}.

The regression equation was

$$\mathrm{RPI} = 0.022 + 0.0023C$$

where RPI is the relative phosphorescence intensity and C the concentration of NOA in nanograms per milliliter. A slope of 0.0023, a correlation coefficient of (r) 0.998 for $n = 7$, indicates good linearity. The detection limit ($3S_c$) was obtained by the methodlogy of Cuadros Rodríguez et al. (1993) with a final value of 30.5 ng mL^{-1}.

The precision of the method was determined at two different concentrations. The relative standard deviation (RSD; $n = 7$) was 3.57 and 1.61% for concentrations of NOA of 250 and 625 ng mL^{-1}, respectively.

The specificity of the method was investigated by observing the possible interference caused from other plant growth regulators and insecticides such as *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, carbendazime, 3-amino-1,2,4-triazole, α-naphthol, and 2-aminophenol. The effect of these foreign species indicates that tolerance levels greater than 6000 ng mL^{-1} are obtained for most of those compounds. The greatest interferences are observed for α-naphthol with a tolerance level > 250 ng mL^{-1} and for 2-aminophenol with a tolerance level > 20 ng mL^{-1}. With these results, it can be concluded that the proposed method presents very good selectivity, due to the intrinsic characteristics of the room temperature phosphorescence measurements.

Analysis for NOA in Apple. In order to reproduce a real sample situation, the analysis of trace quantities of NOA has been carried out in fresh apples bought in the local market.

These samples were spiked with NOA by adding appropriate volumes of a standard solution, the final concentration to be analyzed being of 500 ng mL^{-1}. The recovery experiments of the NOA standard in the spiked apple samples were performed.

To know the matrix effect of the plant material, two three-dimensional spectra have been compared, one for

the spiked apple samples and the other for a blank plant sample, which are shown in Figure 4.

The mean recovery results, obtained when seven spiked samples containing the 500 ng mL^{-1} NOA had been analyzed, were of 108.8%, with a relative standard deviation of 3.80%.

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