Method for the Quantitative Determination of 1-Naphthaleneacetic Acid in Spiked Canned Pineapple Samples by Micelle-Stabilized Room Temperature Phosphorescence

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This paper presents a convenient method for the determination of 1-naphthaleneacetic acid (NAA) plant growth regulator in spiked canned pineapple samples by micellar-stabilized room temperature phosphorescence with sodium dodecyl sulfate as a micellar medium, thallium nitrate as an external heavy atom, and sodium sulfite as an oxygen scavenger. A multivariate optimization approach using the type of central composite blocked cube-star design was carried out. The analytical curve of NAA gives a linear dynamic range of 70−500 ng mL^{-1} with a detection limit of 21 ng mL^{-1} and precision of 2.37% ($n = 7$). A mean recovery value of 98.6% was obtained for 250 ng mL^{-1} NAA in the pineapple samples.

Keywords: *1-Naphthaleneacetic acid; room temperature phosphorescence; experimental design*

INTRODUCTION

1-Naphthaleneacetic acid (NAA) has been employed for 40 years as a plant growth regulator for control of preharvest fruit drop and flower induction or as a fruit-thinning agent in different crops such as apples, potatoes, olives, and citrus fruits (Shindy et al., 1973; Liñán and Vicente, 1985).

To produce a sufficient biological effect, this plant growth regulator must be applied in a concentration of 20−100 μg mL^{-1} to the different crops, so it has become increasingly important to have sensitive analytical procedures for its detection.

Several investigators have published methods for the determination of NAA by different techniques such as electron affinity (Bache et al., 1964), ultraviolet absorption (Bache et al., 1962; Young et al., 1963; Randall, 1970; Muñoz de la Peña et al., 1993), HPLC (Cochrane and Lanouette, 1979; Moye and Wheaton, 1979), or GC with photometric detection, in potatoes (Zweig et al., 1962) and olives (Zweig et al., 1964), or with mass spectroscopy detection (Heberer and Stan, 1996).

The problems related with the most popular technique used to determine NAA, the spectrophotometric technique, is that procedures are long and require a great amount of cleanup or the recoveries obtained are too low.

Most of these problems have been overcome by employing another molecular technique, the luminescence technique based on the native fluorescence properties of this plant growth regulator, which has given some of the fluorometric determinations of these phytohormones in the literature (Horstein, 1958; Jolliffe and Coggins, 1970; García Sánchez and Cruces Blanco, 1989; Coggins et al., 1972).

The lack of selectivity when fluorescence detection is used has been overcome, in most cases, by using separation techniques, when the determination of NAA in real samples was needed (Cochrane and Lanouette, 1979; Archer and Stokes, 1983) with detection limits of nanograms per milliliter.

Taking into account the native fluorescence of NAA, the possibility of applying another luminescence method such as phosphorescence has not been considered previously. The purpose of this work was to exploit the possibilities offered by the native phosphorescence of NAA using of the advantages offered by this technique to increase the selectivity of its determination without using any cleanup procedures or separation techniques.

Micellar-stabilized room temperature phosphorescence (MS-RTP) has been observed from a wide variety of lumiphors to offer an improved selectivity for the analysis of many organic mixtures, so it has been employed, for the first time, for the determination of this plant growth regulator.

However, the traditional MS-RTP method of using N_2 purging to remove oxygen from solution is limited in application, because of foam generation and other concomitant problems, but we have made use of the proposal of Díaz-García and Sanz-Medel (1986) concerning the use of sulfite ion for MS-RTP as an efficient O_2 scavenger in micellar solution, representing a major technical advance in the use of MS-RTP, which during the past decade has been development by several authors (Sanz-Medel et al., 1987; Nugara and King, 1989; WeiJun and ChangSong, 1993; Segura Carretero et al., 1996).

Owing to the numerous experimental factors that influence the phosphorescence response of any analyte, when this luminescence technique is to be employed, a statistical model of central composite design (Box et al., 1978) was used to evaluate the significance of each factor in addition to the interaction between variables that affect the phosphorescence response of NAA.

This paper demonstrates that chemical deoxygenation micellar-stabilized room-temperature phosphorimetry using SDS as a micellar medium and thallium nitrate as an external heavy atom may be successfully applied

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to the quantitative determination of NAA in fruit 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 MS-RTP intensities were carried out with an Aminco Bowman Series 2 luminescence spectrometer (Rochester, NY) equipped with a 7 W pulsed xenon lamp. Measurements required a personal computer with 4 MB of RAM, DOS 6.0, OS/2 version 2.0, and a GPIB(IEEE-488) interface card for computer-instrument communication. The spectrometer was equipped with a thermostated cell holder. Statgraphics Statistical Institution Edition version 6.0 (Statistical Graphics Corp., Rockville, MD).

Reagents. NAA (Sigma) was used as received. A stock standard solution was prepared by dissolving 5 mg of NAA in 50 mL of 0.5 mol L^{-1} surfactant sodium dodecyl sulfate (SDS). This surfactant, together with the heavy atom thallium(I) nitrate, anhydrous sodium sulfite, and sulfuric acid (all from Sigma, St. Louis, MO) were used as received and were all of analytical reagent grade. Aqueous solutions were prepared with doubly distilled water. The sodium sulfite solutions were prepared daily and kept in tightly stoppered containers.

The chronic toxic nature of thallium salts has to be taken into account in using and handling this material, and the corresponding precautions must be taken.

Procedure. A 50 μL aliquot of the NAA stock solution, 0.61 mL of 0.5 mol L^{-1} SDS, 1.01 mL of 0.25 mol L^{-1} thallium nitrate, 0.51 mL of 0.1 mol L^{-1} sodium sulfite, and 0.81 mL of 0.02 mol L^{-1} sulfuric acid were introduced into a 10 mL calibrated flask and diluted to volume with water. If a precipitate appears after the addition of the thallium nitrate, the flask was warmed until the precipitate disappeared, before the other reagents were added. After thorough mixing, the flask was placed in a water bath at 25 ± 1 °C for 10 min. Standard 10 mm fused silica cells were filled with this solution.

Relative phosphorescence intensities (RPI) were measured at 528 nm with excitation at 288 nm. Reagent blanks lacking NAA were prepared and measured following the same procedure.

Extraction Procedure. Two hundred and fifty grams of canned pineapples in their own juice bought in the local supermarket was chopped in a food processor, and 10 g was transferred to a blender cup. A certain amount of NAA was added to 10 g of pineapple pulp so that the final concentration was included in the range of the calibration graph. Twenty milliliters of chloroform containing 1 mL of 10% H_2SO_4 was added as extractive, and the mixture was blended for several minutes. The sample residue has to be reextracted with another 20 mL of $CHCl_3$ plus 1 mL of 10% H_2SO_4, and the mixture was filtered with a 30 mL medium-porosity fritted-glass Büchner flask under suction. The blender and filter were washed with three 5-mL portions of $CHCl_3$, and 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 0.5 M SDS. The basic procedure was applied to this solution.

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

RESULTS AND DISCUSSION

Optimization of Experimental Variables. To achieve these critical conditions in an easy way, a four-variable, composite hypercube-star design was applied. A multiple optimization approach for the different factors affecting the response was designed. For the proposed method, the dependent variable is NAA phosphorescence intensity and the independent variables used in this multivariate optimization were SDS, $TlNO_3$, Na_2SO_3, and H_2SO_4 concentrations. Different levels of these variables were selected to maximize the information that could be extracted from the experimental data by carrying out 29 experiments dictated by the experimental matrix of the type composite blocked hypercube-star design applied.

The experimental data were fitted to a polynomial mathematical model so that the variables were adjusted until the calculated values were in close agreement with the experiment values. For the relationship between independent variables, model fitting methods gave the quadratic equation

$$\begin{aligned}\mathrm{RPI} = &-12.4[\mathrm{SDS}] + 18.9[\mathrm{TlNO_3}] + \\ &1554.1[\mathrm{Na_2SO_3}] + 717.5[\mathrm{H_2SO_4}] + \\ &112.5[\mathrm{SDS}][\mathrm{TlNO_3}] - 125.0[\mathrm{SDS}][\mathrm{Na_2SO_3}] + \\ &14062.5[\mathrm{SDS}][\mathrm{H_2SO_4}] + 3250.0[\mathrm{TlNO_3}][\mathrm{Na_2SO_3}] + \\ &9375.0[\mathrm{TlNO_3}][\mathrm{H_2SO_4}] + \\ &681250.0[\mathrm{Na_2SO_3}][\mathrm{H_2SO_4}] - 209.14[\mathrm{SDS}]^2 - \\ &1072.7[\mathrm{TlNO_3}]^2 - 267823.0[\mathrm{Na_2SO_3}]^2 - \\ &1.49681 \times 10^5[\mathrm{H_2SO_4}]^2 - 3.409 \quad (1)\end{aligned}$$

The different surfaces for all of the variables at the optimum values obtained are presented in Figure 1.

As a result of these experiments, the optimum experimental variables to obtain the highest phosphorescence response have been obtained using a SDS concentration of 3.04×10^{-2} mol L^{-1}, a thallium nitrate concentration of 2.52×10^{-2} mol L^{-1}, a sodium sulfite concentration of 5.12×10^{-3} mol L^{-1}, and a sulfuric acid concentration of 1.63×10^{-3} mol L^{-1}, which have been kept constant for the rest of the experimental work.

As these variables greatly affect the phosphorescence response, the variance of the data set, as accounted for by the variables as they appear in the model, is larger than the variance of the residuals. This was confirmed by the Fisher variance ratio (F) for the significance of the variables effect, $F = 35.45$ [the tabulated critical value at a given level of probability ($F_{crit} = 2.48$)], which is significant at the 95% confidence level.

As replicate experiments were carried out on the central point of the system, it was possible to evaluate the variation attributed to the lack of fit of the model and the purely experimental uncertainty. As F_{lof} (variance due to the lack of fit) = 2.84 ($F_{crit} = 5.96$), which is not significant, there is not a significant amount of variation in the measured and predicted responses and the measured responses can be explained by the model.

The coefficient of multiple determination (r^2) is 0.925, indicating that the variables explain the data fairly well. If the degrees of freedom are considered, the adjusted r^2 is 0.850.

Once the experimental variables have been selected, a detailed selection of the different instrumental parameters related to the luminescence technique has to be carried out. As it is seen in Figure 2, the excitation and emission wavelengths of greatest phosphorescence are observed at 288 and 528 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 300 μs, a detector sensitivity of 1100 V, and a minimum period pulse or time between flashes of 5 ms. All of these instrumental variables were kept constant for the rest of the experimental work.

Effect of Temperature on RTP Intensities. The RTP intensities decrease almost linearly with an increase in temperature. A decrease of 30% in the RPI

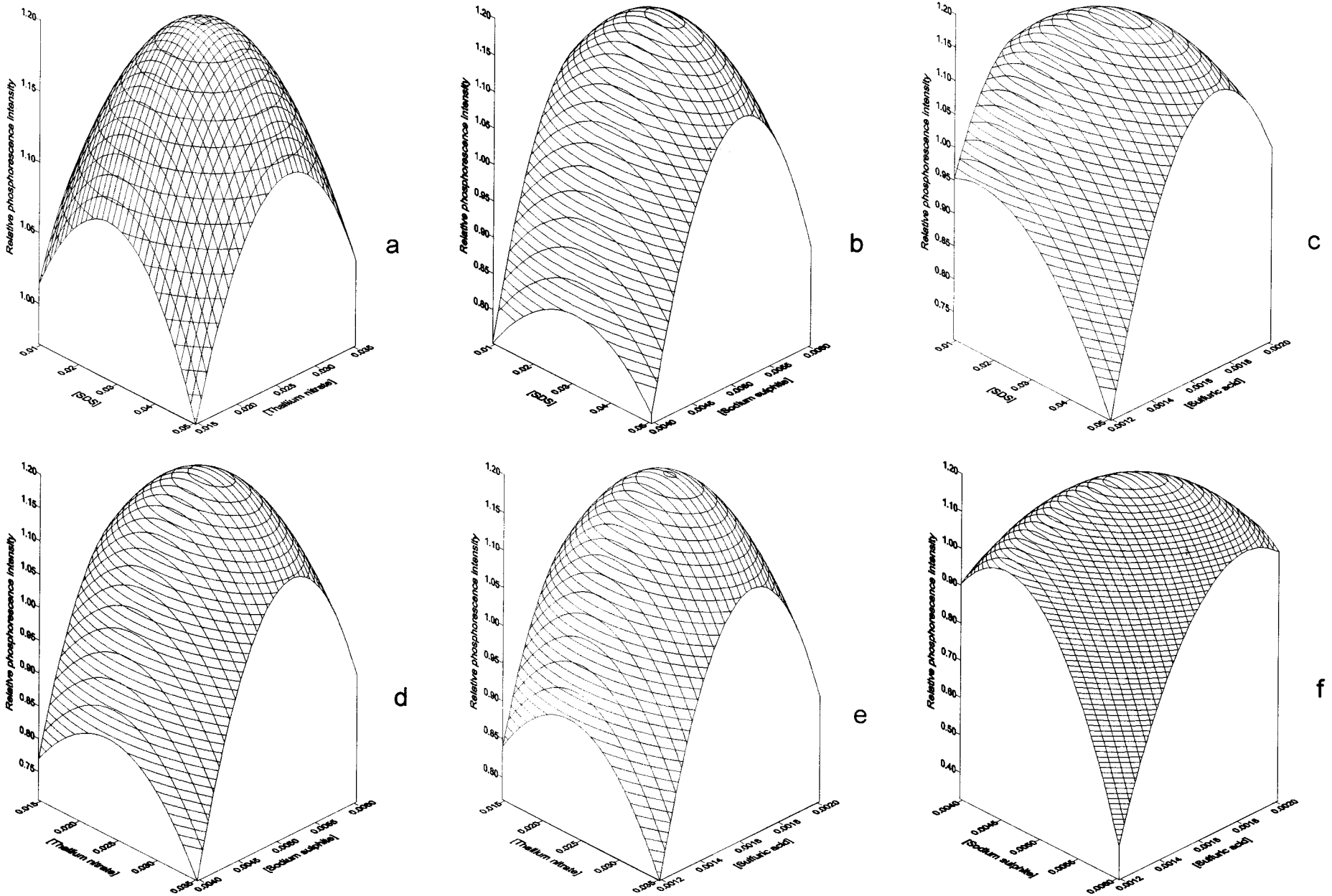

Figure 1. Surface response of NAA for (a) SDS and $TlNO_3$ concentrations at 5.12×10^{-3} mol L^{-1} Na_2SO_3 and 1.63×10^{-3} mol L^{-1} H_2SO_4; (b) SDS and Na_2SO_3 concentrations at 2.52×10^{-2} mol L^{-1} $TlNO_3$ and 1.63×10^{-3} mol L^{-1} H_2SO_4; (c) SDS and H_2SO_4 concentrations at 2.52×10^{-2} mol L^{-1} $TlNO_3$ and 5.12×10^{-3} mol L^{-1} Na_2SO_3; (d) $TlNO_3$ and Na_2SO_3 concentrations at 3.04×10^{-2} mol L^{-1} SDS and 1.63×10^{-3} mol L^{-1} H_2SO_4; (e) $TlNO_3$ and H_2SO_4 concentrations at 3.04×10^{-2} mol L^{-1} SDS and 5.12×10^{-3} mol L^{-1} Na_2SO_3; and (f) Na_2SO_3 and H_2SO_4 concentrations at 3.04×10^{-2} mol L^{-1} SDS and 2.52×10^{-2} mol L^{-1} $TlNO_3$.

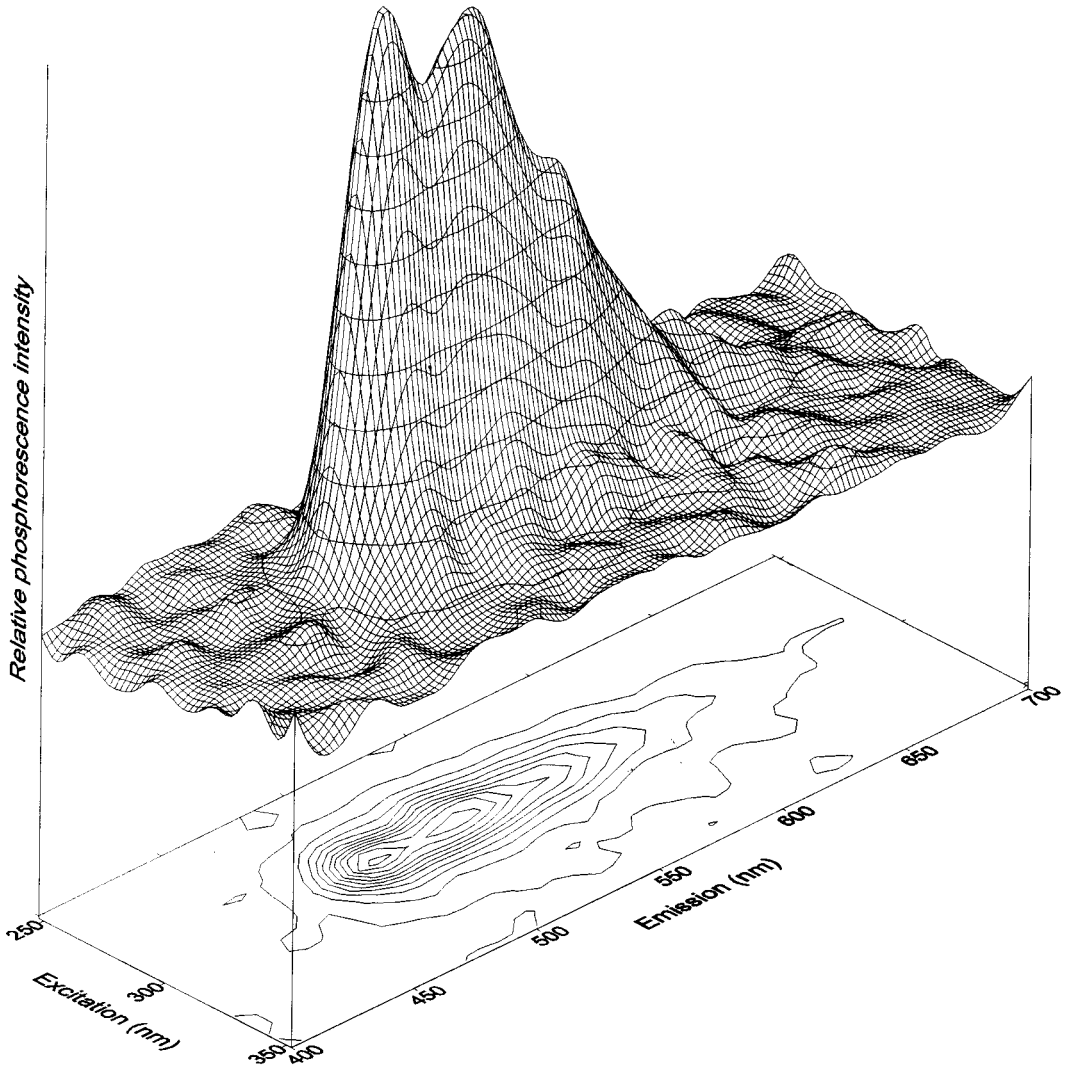

Figure 2. Projected three- and two-dimensional spectra of NAA. [NAA] = 250 ng mL^{-1}; [SDS] = 3.04×10^{-2} mol L^{-1}; [$TlNO_3$] = 2.52×10^{-2} mol L^{-1}; [Na_2SO_3] = 5.12×10^{-3} mol L^{-1}; [H_2SO_4] = 1.63×10^{-3} mol L^{-1}; emission at 400−700 nm; excitation at 250−350 nm; scanning speed, 2 nm s^{-1}; delay time, 100 μs; gate time, 300 μs; and detector sensitivity, 1100 V.

signals was observed with a temperature increase from 20 to 35 °C. These effects were mainly related to molecular thermal motion and intermolecular energy conversion. The molecular thermal motion causes collisional deactivation of the phosphors. Hence, a temperature of 25 ± 0.1 °C was used in the rest of the experimental work.

Stability. The MS-RTP signal of the system can reach stability in 2 min under the condition of chemical deoxygenation and remain stable for at least 1 h.

Analytical Curve and Precision. The method was tested for linearity, precision, repeatability, and specificity. Phosphorescence response was linear in relation to the concentration of NAA over the range 0−500 ng mL^{-1}, calculated in the final solution. The regression equation was

$$\mathrm{RPI} = 0.356 + (3.2 \times 10^{-3}C) \qquad (2)$$

where C is the concentration of NAA in ng mL^{-1}. A correlation coefficient (r) = 0.999 (n = 7) was obtained, indicating excellent linearity. A detection limit of 21.1 ng mL^{-1} was determined (Cuadros Rodríguez et al., 1993).

The precision of the method was determined at two different concentrations (250 and 500 ng mL^{-1}) obtaining relative standard deviations (RSD) (n = 7) of 2.37 and 1.41%.

The selectivity of the MS-RTP method was established by the determination of 250 ng mL^{-1} of NAA in the presence of different phytohormones and insecticides that could be present in real samples. The greatest interferences were observed for α-naphthol and 2-(4-thiazolyl)benzimidazole with a tolerance level >40 ng mL^{-1}. For carbendazim and 2-aminophenol, the tolerance levels were of >2000 ng mL^{-1}, while for other compounds such as *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, and 3-amino-1,2,4-triazole, the tolerance levels were >20 000 ng mL^{-1}.

From these results, it can be concluded that the proposed method for the determination of NAA shows very good selectivity, due to the intrinsic characteristics of the micelle-stabilized phosphorescence measurements.

Analysis for NAA in Canned Pineapple. Recoveries of NAA added to the canned pineapple samples prior to extraction were studied at a 250 ng mL^{-1} level. The mean recovery value was 98.6% (n = 3), with an RSD of 4.50%.

While we have demonstrated with this application the adequacy of the method to this kind of fruit, we believe

that it might also be applicable to other crops and to the study of the natural disappearance of NAA in numerous citrus fruits, because it is rapid and simple and the results obtained showed good precision.

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