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Flow-through optosensing of 1-naphthaleneacetic acid in water and apples by heavy atom induced–room temperature phosphorescence measurements

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Abstract

A sensitive and selective phosphorimetric method for the determination of 1-naphthaleneacetic acid (1-NAA) based on a flow-injection system connected to a flow cell packed with a solid support and placed in the sample compartment of a conventional luminescence spectrometer is described. A non-ionic solid polymeric resin Amberlite XAD-7 is used for the packing. After injection of the sample, 1-NAA is on-line retained in the packed resin and measurements of the heavy atom induced (HAI)–room temperature phosphorescence (RTP) emission ($\lambda_{ex}/\lambda_{em}$ = 292/490 nm) from this native luminescent compound are taken.

The optimum experimental conditions were investigated by injecting 2 ml samples of an aqueous solution of 1-NAA in the flow system. A concentration 0.15 mol l^{-1} of thallium(I) ions, as heavy atom, both in the samples and the carrier flow, was finally selected. Also, a concentration of 6 mmol l^{-1} of sulphite was optimal for ensuring the necessary deoxygenation of the system at the selected flow rate of 0.8 ml min^{-1}. After measurement, the solid support was efficiently regenerated by injecting 1 ml of a mixture water:acetone in a ratio 1:1 (v/v) into the flow.

The detection limit (3σ criterion) was 1.2 ng ml^{-1} of 1-NAA. The repeatability (R.S.D.) for five replicates of a sample containing 50 ng ml^{-1} of analyte turned out to be ±3% and the calibration graphs proved to be linear up to 500 ng ml^{-1} of 1-NAA (maximum concentration assayed). The effect of potential interferences from other organic species which can be also used as plant growth regulators, as well as from various inorganic cations and anions, has been investigated as well.

The method was successfully applied to the determination of low levels of this plant growth regulator in natural waters (river and fountain waters) and apples.

Keywords: Heavy atom induced–room temperature phosphorescence; 1-Naphthaleneacetic acid; Flow injection analysis; Waters; Apples

1. Introduction

1-Naphthaleneacetic acid (1-NAA) is widely employed in agriculture as a plant growth regulator (a phytohormone or synthetic "auxin", from Greek auxein which means to grow). Among other effects, NAA prevents premature flowering, fruit drop and it controls regrowth of tree sprouts after trimming.

To produce an appropriate biological effect, this compound must be applied at concentrations of 20–100 mg l^{-1} in the spraying solution [1]. Consequently, trace amounts in surface and underground waters may be expected to arise from agricultural operations. Besides, EPA regulations establish tolerance limits for this pesticide in fruits such as apples, oranges, pears, etc.

The determination of 1-NAA can be carried out by high performance liquid chromatography [2] or gas chromatography [3]. Alternatively, this phytohormone has also been routinely determined by molecular absorption spectropho-

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tometry after appropriate clean-up procedures [4,5]. Besides, the measurement of the native fluorescence of 1-NAA has formed the basis for sensitive fluorimetric methods based on approaches such as synchronous derivative spectra [6], formation of inclusion complexes with β-cyclodextrin [7] and solid support preconcentration/measurement [8,9], all of them aiming to enhance the sensitivity and/or selectivity of these determinations.

Room temperature phosphorescence (RTP) offers usually interesting advantages over fluorimetric-based methods [10]. In the phosphorescence process, the analytical signal is a low-noise emission measured after any short-lived background luminescence or scattered light has ceased and so lower detection limits (DLs) could be expected. Additionally, the larger singlet-triplet splitting and the long emission wavelength of the phosphorescence phenomena favours the design of robust and inexpensive instrumentation [11].

Many published RTP determinations resort to the use of organized media to protect the radiative deactivation from the triplet state. Along these lines, RTP methods for 1-NAA based on the use of micelles [12] (sodium dodecyl sulphate as surfactant), and on the formation of host-guest combinations with β-cyclodextrin [13] have been described.

Moreover, previous studies [14] showed that RTP emission of several naphthalene derivatives can be directly induced in aqueous solutions by the addition of high concentrations of a heavy atom perturber and sodium sulphite as chemical deoxygenator. This new type of RTP emission was named as "heavy atom induced–RTP" (HAI–RTP). Further work investigating the potential of this new methodology for a variety of analytes [15] showed that 1-NAA can be determined by HAI–RTP with a DL of about 15 ng ml^{-1}.

On the other hand, it is important to mention that flow-through optosensing strategies [16,17] have demonstrated to be a successful approach to increase the sensitivity of many methodologies because they allow to simultaneously preconcentrate and detect the analyte. In particular, the combination of RTP measurements with flow-through optosensing offers a great analytical potential [17].

Aiming at improving further the sensitivity obtained for 1-NAA by HAI–RTP, the combination of this RTP methodology with a flow system allowing the on-line retention of the analyte on a solid surface packed on the flow cell [18,19] has been investigated for the determination of 1-NAA in natural water samples and apples.

2. Experimental

2.1. Reagents and materials

Analytical reagent grade chemicals were employed for the preparation of the standards and solutions. All standards and solutions were prepared with ultrapure deionized Milli-Q Water (Millipore, Molsheim, France).

A stock standard solution of 50 mg l^{-1} of 1-NAA (Sigma, Madrid, Spain) was prepared by dissolution of the appropriate amount of the solid in Milli-Q water with the help of an ultrasonic bath. This stock solution was freshly prepared after 7 days and stored in the refrigerator at 4 °C (in these conditions it is stable for at least 1 week).

A 0.1 M sulphite solution was daily prepared in Milli-Q water from sodium sulphite (Sigma). A 0.25 M thallium nitrate solution was prepared by dissolving thallium(I) nitrate (Sigma) in Milli-Q water. Also, a 2 M iodide solution was prepared by dissolving potassium iodide (Panreac quimica, Barcelona, Spain) in Milli-Q water.

Sodium chloride, calcium chloride, sodium sulphate, sodium nitrate, magnesium sulphate, sodium acetate trihydrate and di-sodium hydrogen phosphate (all of them from Merck, Darmstadt, Germany) were used for the study of the interferences. Also, 1-naphthaleneacetamide (NAD), indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA) (all of them from Sigma) were investigated as potential interferents.

Florisil (magnesium silicate, activated) (Sigma), chloroform (Romil, Cambridge, England), sulphuric acid, acetonitrile, diethyl ether, ammonia solution, and acetic acid (all of them from Merck, Darmstadt, Germany), were used for the extraction of the analyte from the apples in the real sample analyses.

The materials investigated for the selection of the solid support were: Dowex 1 × 2 100–200 mesh (Fluka, Buchs SG, Switzerland), Dowex 1 × 4 100–200 mesh (Supelco, USA), Dowex 1 × 8 100–200 mesh (Fluka), Sephadex-QAE A-25 (Aldrich, Milwaukee, WI, USA), Sephadex-DEAE A-25 (Aldrich), all of them anionic solid supports. Dowex 50 W × 2 100–200 mesh (Fluka), Dowex 50 W × 4 100–200 mesh (Fluka), Dowex 50 W × 8 50–100 mesh (BDH, Garden City, NY, USA), Sephadex-SP C-25 (Aldrich) and Sephadex-CM C-25 (Aldrich), all of them cationic solid supports, and Silica gel Davisil 35–60 mesh (Aldrich), Silica gel merck 35–70 mesh (Aldrich), Amberlite XAD-4 (Supelco, Bellefonte, PA, USA) and Amberlite XAD-7 (Supelco) as non ionic solid supports. The solids were cleaned thoroughly before use with 2 M HCl to remove trace metal impurities, then with de-ionized water and finally with ethanol to displace air from the pores of the resin and to remove residual monomers and solvents.

To obtain different grain-size supports, solids were crushed in an agate mortar and then sieved.

Acetone (Fluka) was used for the elution of the analyte and the regeneration of the solid supports.

It should be taken into account that thallium nitrate is freely soluble in water and is very toxic to aquatic life. It can also be toxic to plant life and other terrestrial organisms in soils. Thus, thallium nitrate solutions were stored in suitable, labelled containers in cool and well-ventilated designated area and gloves were used.

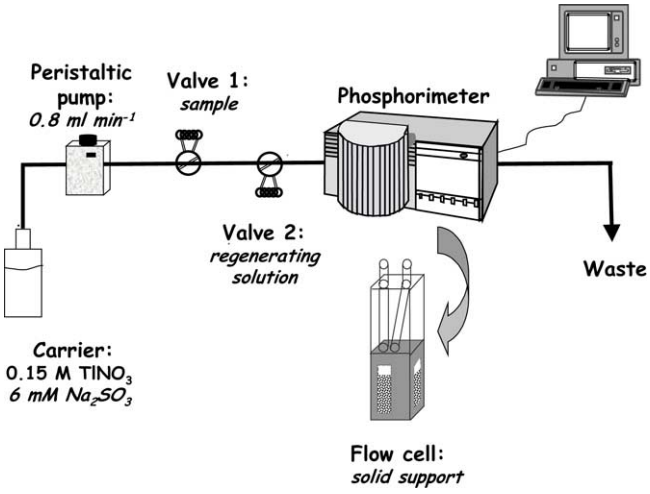

Fig. 1. Schematic diagram of the flow system used.

2.2. Instrumentation

Phosphorescence measurements were performed with a Varian Cary Eclipse spectrometer (Varian Iberica, Madrid, Spain) equipped with a pulsed xenon discharge lamp (peak power equivalent to 75 kW), Czerny–Turner monochromators, and a R-928 photomultiplier tube. Excitation and emission wavelengths were set at 292 and 490 nm, respectively.

A peristaltic pump (model HP4, Scharlau Science, Barcelona, Spain) was used to pump the flow of the carrier solution through the system. Two six-way injection valves were employed (see Fig. 1). Valve 1 was used to inject the sample solution and valve 2 was employed for the introduction of the regenerating solution. 0.8 mm i.d., PTFE tubes were used throughout the flow system. A conventional luminescence flow through quartz cell (Hellma Model 176.052-QS, Mullheim, Germany) of 1.5 mm light path was employed in all the experiments.

A centrifuge Biofuge Stratus (Heraeus, Hanau, Germany) was employed for the pre-treatment of the real samples.

2.3. General procedure

The carrier solution was prepared by adding 60 ml of the stock solution of thallium(I) nitrate, 6 ml of the sodium sulphite stock solution and then made-up to 100 ml with Milli-Q water.

For the preparation of the calibration graphs, 6 ml of thallium(I) nitrate, 600 μl of sodium sulfite, and an aliquot of 1-NAA standard solution were introduced into a 10 ml calibrated flask, diluted to volume with ultrapure deionized water (Milli-Q) and then shook vigorously for homogeneity.

Samples or standards (2 ml) were injected into the flow system via valve 1, and the regenerating solution (1 ml) was injected through valve 2 (see Fig. 1). After injection, the pesticide passes the flow-through measurement cell containing the resin Amberlite XAD-7 (where it is on-line immobilized) and RTP is measured (292 nm for excitation and 490 nm for emission). The gate time was kept constant at 5 ms and the delay time at 120 μs. Excitation and emission slits of 10/20 nm were used, respectively.

Reagent blanks were prepared and measured following the same procedure but without adding the phytohormone. All the analytical measurements were carried out at room temperature ($20 \pm 3\,^{\circ}C$).

2.4. Real sample analyses

The proposed RTP method was investigated for the determination of 1-NAA in apples and natural waters.

2.4.1. Apples

1-NAA in apples was determined by following the general procedure after a pre-treatment step. First, the samples were chopped in the laboratory with a food-mixer and liquidized with a domestic blender. The total volume of the liquid portion was measured and then 100 ml mixed with 20 ml of 1 N H_2SO_4 and 400 ml of chloroform. The mixture is centrifuged during 10 min at 1200 rpm, and a 200 ml aliquot from the chloroform layer is taken for the cleanup of the sample.

For this purpose, a glass wool plug was placed into a chromatographic tube, 10 cm of florisil (in chloroform) was added and at the top a glass wool plug was located. Two hundred millilitres of the organic phase was transferred to the column. Then, the tube was rinsed twice with 5 ml of $CHCl_3$ and the column was eluted, in order, with 100 ml portions of CH_3CN, diethyl ether, NH_3-saturated $CHCl_3$, and $CHCl_3$ and the eluates discarded.

The 1-NAA was eluted with 100 ml of 1% acetic acid in $CHCl_3$ followed by 100 ml of $CHCl_3$. Then 50 ml 1N H_2SO_4 were added to this solution and then vigorously shaken. The $CHCl_3$ layer was transferred to a 250 ml separator containing 50 ml of water and it was again shaken. Finally, the $CHCl_3$ layer was transferred to a 250 ml separator containing exactly 50.0 ml 0.5 M Na_2HPO_4, shook, and the $CHCl_3$ layer discarded. This pretreatment procedure corresponds to method 970.54 (analysis of 1-NAA in apples) of the official methods of analysis of the AOAC [5].

A 0.5 ml aliquot of this aqueous phase is mixed with 6 ml of 0.25 M Tl(I) solution and 0.6 ml of 0.1 M sulphite solution into a 10 ml calibrated flask. Due to the sample pretreatment, the calibration graph was prepared following the general procedure but adding 0.5 ml of 0.5 M di-sodium hydrogen phosphate into the calibration flasks (to get there a similar concentration of phosphate as in samples).

Chloroform is a probable human carcinogen. Inhalation and ingestion are harmful and may be fatal. When handling it avoid contact with eyes, skin and clothing. Do not use in poorly ventilated spaces. Safety glasses and solvent-resistant gloves were used. Chloroform was stored in sealed containers in a cool, dry, well-ventilated area.

2.4.2. Natural waters

The determination of traces of 1-NAA in river and fountain waters was evaluated by following the general procedure after a filtration through a 45 μm filter to remove material in suspension. Three milliliters of 1-NAA spiked waters were mixed with 6 ml of the thallium nitrate solution, 0.6 ml of sulphite solution and then made-up to 10 ml with Milli-Q water.

3. Results and discussion

3.1. Selection of the heavy atom and the concentration of the deoxygenant

Two heavy atom salts, KI and $TlNO_3$, were investigated as heavy atom perturbers (to be added both in the sample and in the carrier solution) to give rise to the HAI–RTP emission of 1-NAA. RTP analytically useful emission signals were observed for 1-NAA in thallium nitrate deoxygenated solutions (see Fig. 2 where an emission spectrum from the immobilized 1-NAA is collected), while no RTP signals were detected when measuring the emission from deoxygenated iodide solutions of 1-NAA. Fig. 3(a) shows the effect of the concentration of thallium ions on the net RTP intensity obtained for a sample containing 100 ng ml^{-1} of 1-NAA. A concentration 0.15 mol l^{-1} of thallium nitrate was selected for further experiments.

Sodium sulphite was selected as chemical oxygen scavenger [20] and Fig. 3(b) shows the effect of the concentration of sulphite ions. As can be seen, no RTP emission signals were observed in the absence of sulphite and a concentration of 6 mmol l^{-1} sulphite was selected.

3.2. Selection of the solid support and the eluent

In the search of a proper solid phase, anionic supports (Dowex 1 × 2, Dowex 1 × 4, Dowex 1 × 8, Sephadex-QAE A-25, Sephadex-DAE A-25), non-ionic supports (Silica Davisil, Silica gel merck, Amberlite XAD-4 and Amberlite XAD-7) and cationic supports (Dowex 50 W × 2, Dowex 50 W × 4, Dowex 50 W × 8, Sephadex-SP C-25, Sephadex-CM C-25) all of them with a grain size 80–120 μm were investigated for the RTP detection of 100 ng ml^{-1} of 1-NAA using the proposed procedure. The analytical signals obtained when using anionic supports were negligible. Concerning the non ionic and the cationic supports, best results were achieved for those with a highly hydrophilic nature. Fig. 4(a) shows the results obtained for those non-ionic sup-

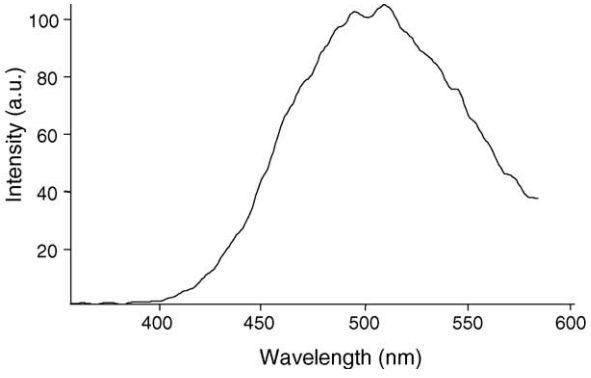

Fig. 2. Room temperature phosphorescence emission spectrum of 1-NAA immobilized onto Amberlite XAD-7 (λ_{exc} = 292 nm).

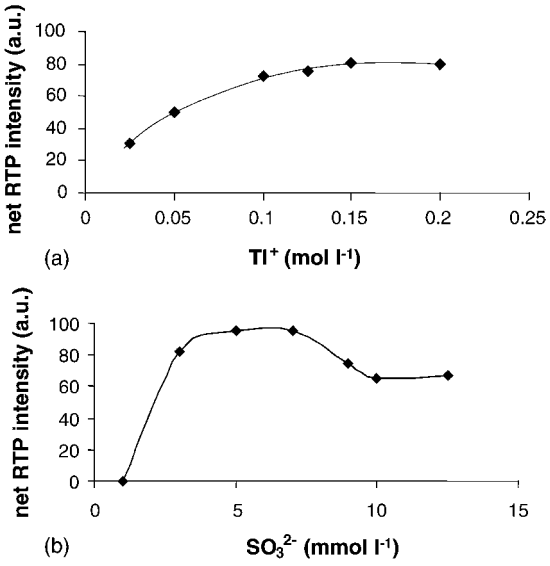

Fig. 3. Effect of the thallium nitrate and sulphite concentrations on the RTP signal of 100 ng ml^{-1} of 1-NAA, using Amberlite XAD-7 as solid support. (a) Concentration of thallium(I) nitrate. (b) Concentration of sodium sulphite.

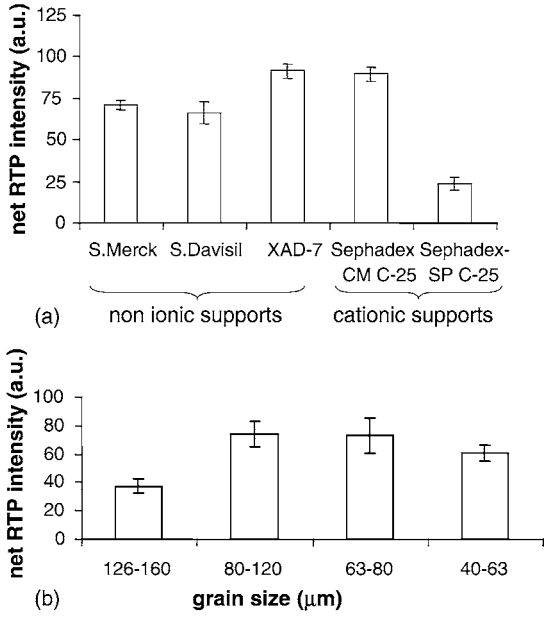

Fig. 4. Effect of the solid support (packed on the flow cell) on the RTP signal of 100 ng ml^{-1} of 1-NAA. (a) Solid supports with 80–120 μm grain size. (b) Grain size of the Amberlite XAD-7.

ports and cationic supports which provided higher analytical signals. The Amberlite XAD-7 resin (a cross-linked polymer of methylmethacrylate) was selected for further experiments.

Fig. 4(b) shows the effect of the resin particles size on the 1-NAA analytical signal. For further experiments the particles 80–120 μm were selected because they gave rise to the higher signals and, on the other hand, backpressure problems were not observed.

After each analysis, the solid phase was efficiently regenerated by injecting in the flow 1 ml of a 1:1 mixture acetone:water (v/v).

3.3. Effect of the flow rate and the sample loop

Fig. 5(a) shows the influence of the flow rate on the net RTP intensity of a 100 ng ml^{-1} 1-NAA standard solution. As can be seen, higher net RTP emission signals were obtained at lower flow rates. Aiming at minimizing the analysis time but maintaining a good sensitivity, a flow rate of 0.8 ml min^{-1} was selected.

On the other hand, sample injection loops of 0.5, 1, 2 and 4 ml were assayed for a 100 ng mg^{-1} 1-NAA standard solution and results are collected in Fig. 5(b). As expected, results showed that the higher the sample loop the higher the analytical signal. In order to not lengthen too much the analysis time, a sample loop of 2 ml was selected for further experiments. In any case, when higher sensitivity is required, it can be used of the 4 ml sample volume loop (or even higher volumes).

3.4. Analytical performance characteristics

The analytical performance characteristics of the proposed RTP method were evaluated under optimum experimental conditions. Calibration graphs were prepared from the results of triplicate measurements of the RTP emission from 1-NAA standard water solutions of increasing concentration. Results showed to be linear at least up to 500 ng ml^{-1} of analyte (maximum concentration assayed). The DL, calculated as the concentration of 1-NAA which produced an analytical signal three times the standard deviation of 10 injections of a blank, turned out to be 1.2 ng ml^{-1} of 1-NAA. The DL reported with a method based on micelle-stabilized RTP [12] was 21.1 ng ml^{-1}, while the DL reported by the HAI–RTP methodology [15] was 14.6 ng ml^{-1}. Therefore, the approach proposed in the present work, based on the combination of HAI–RTP and solid-phase sensing, allows to noticeably improve the detection limit obtained for 1-NAA by RTP measurements. The precision of the proposed method, evaluated as the relative standard deviation for five replicates of a sample containing 50 ng ml^{-1} of 1-NAA, resulted to be ±3%.

Following the general procedure, the effect on the 1-NAA RTP signal of common inorganic cations (calcium and magnesium), common anions (chloride, sulphate, nitrate, acetate), and organic species which can be also used as plant growth regulators (NAD, IPA, IBA) were investigated. Potential interferences were added to a standard 1-NAA solution containing 50 ng ml^{-1} of the analyte. The maximum concentrations assayed for such species (see Table 1) did not produce significant interference on the determination of 1-NAA. Di-sodium hydrogen phosphate was used in the pretreatment of the apple samples. In our study of interferences, no effect was observed for phosphate up to a concentration of 200 mg l^{-1}. However, concentrations of phosphate in the interval 200–4000 mg l^{-1} (maximum assayed) gave rise to a decrease about 20% of the net RTP analytical signals for 1-NAA, and so matching of phosphate concentration above 200 mg l^{-1} is recommended.

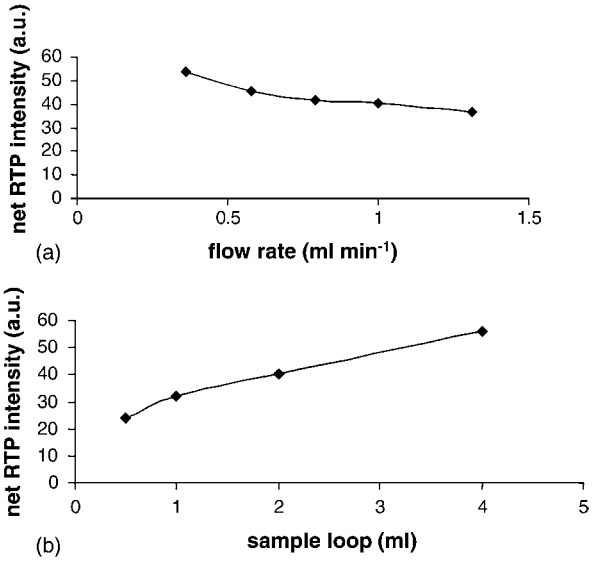

Fig. 5. Influence of the carrier flow rate and the volume of the sample loop on the RTP signal of 100 ng ml^{-1} of 1-NAA. (a) Carrier flow rate. (b) Sample loop.

3.5. Analysis of apple and water samples

The usefulness of the proposed method for the determination of traces of 1-NAA in natural waters (river and

Table 1
Species and maximum concentrations assayed for interferences[a] in the analysis of 50 μg l^{-1} of 1-NAA

Species assayed	Maximum concentration assayed (mg l^{-1})
Chloride	50
Sulphate	50
Nitrate	50
Acetate	50
Magnesium	50
Calcium	50
NAD (1-naphthaleneacetamide)	2
IPA (indole-3-propionic acid)	2
IBA (indole-3-butyric acid)	2

[a] The presence of the potential interferent up to this concentration level (maximum assayed) results in deviations of the phosphorescence signal lower than 3%.

Table 2
Results of the analysis of 1-NAA in river water, fountain water and apples (each result corresponds to the mean of five replicates)

Sample	[1-NAA] added ($\mu g\ l^{-1}$)	[1-NAA] measured ($\mu g\ l^{-1}$)	Recovery (%)
(a)			
Moral river "1"	167	180 ± 24	108
Moral river "2"	333	352 ± 16	106
Bendicion river "1"	500	488 ± 24	98
Bendicion river "2"	667	649 ± 24	97
Tiroco fountain"1"	167	164 ± 17	98
Tiroco fountain "2"	333	360 ± 21	108
Sample	[1-NAA] added ($ng\ g^{-1}$)	[1-NAA] measured ($ng\ g^{-1}$)	Recovery (%)
(b)			
Apples "1"	250	250 ± 9	100
Apples "2"	125	128 ± 4	102

Table 3
Comparison of the analytical performance of published RTP methods for the determination of 1-NAA

References	RTP methodology	DL ($\mu g\ l^{-1}$)	R.S.D. (%)	Linear range ($\mu g\ l^{-1}$)
[13]	β-Cyclodextrin	Not given	10	900–9000
[12]	SDS micelles[a]	21	2.4	up to 500
[15]	HAI	15	4	up to 300
This work	Solid phase-HAI	1.2	3	up to 500 (max. concentration assayed)

[a] SDS: sodium dodecyl sulphate.

fountain water) and in apples was evaluated. Water samples were analysed after filtration through a 45 μm filter without any other sample pre-treatment. The analysis of apples was carried out following the pre-treatment procedure detailed in the Section 2.

None of the analysed samples contained detectable 1-NAA and, therefore, they were spiked in the laboratory with 1-NAA at different concentrations (in the case of the apples the spikes were added right after liquidizing the fruit with a domestic blender). Table 2 collects the results for the RTP determinations of 1-NAA. As can be seen, good recoveries in the interval investigated (167–667 $\mu g\ l^{-1}$) were obtained for the water samples, thus indicating the applicability of the proposed RTP method. Besides, very good recoveries were obtained for the analysis of apples at the 1-NAA concentrations investigated (0.125 and 0.250 $\mu g\ g^{-1}$) which were lower than the tolerances established in apples for this plant growth regulator according to EPA regulations (the limit fixed for 1-NAA in apples is 1 $\mu g\ g^{-1}$).

4. Conclusions

The proposed on-line RTP method offers interesting advantages for a selective determination of 1-NAA in real samples, including simplicity of the methodology, low-cost, large separation between the wavelengths for excitation and emission (about 200 nm), and a good detection limit (1.2 $ng\ ml^{-1}$ for 2 ml sample injections). Besides, the sensitivity can be improved by using higher sample volumes. As can be seen in Table 3 , the analytical performance characteristics achieved for the proposed method compare favourably, in overall terms, with other RTP methodologies published for 1-NAA determination.

The practical utility of the method has been demonstrated by determining this phytohormone in apples (1-NAA contents below the limit given by EPA regulations). For possible environmental contamination monitoring, low levels of 1-NAA in river and fountain waters were satisfactory determined.

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