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FIA-Fluorimetric Determination of the Pesticide 3-Indolyl Acetic Acid

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The paper presents the determination of the pesticide 3-indolyl acetic acid based on its native fluorescence; the method has been optimised either in a flow injection analysis (FIA) assembly or in batch. Maximum fluorescence intensity was observed at basic pH solutions at $\lambda_{exc} = 280$ nm and $\lambda_{em} = 364$ nm. The influence of different empirical parameters as pH, surfactants presence, solvent polarity, solved oxygen amount, and temperature was studied; highest outputs only required the pH in aqueous solutions to be adjusted over the range 9.5–10.5. Different calibration ranges were obtained by working with three different sensitivity scales of the fluorimeter either in flow injection analysis or batch. With the high sensitivity scale and FIA, the linear dynamic range was from 0.005 to 0.6 mg L^{-1} 3-indolyl acetic acid; with an relative standard deviation (RSD) of 4.9% inter-day reproducibility. A large series of potential interferents was studied and finally, the method was applied to several water samples.

KEY WORDS: 3-Indolyl acetic acid; pesticides; fluorescence; FIA.

INTRODUCTION

The massive use of pesticides to fight against plague (in animals or plants) resulted in environmental pollution. Most of these pesticides are water soluble expanding easily through the environment. All countries are trying to protect the environment through new and strict legal rules. This results in the search of new, quick and cost-effective procedures for control analysis of environmental samples.

3-Indolyl acetic acid [1], or β-indoleacetic acid is a white crystalline powder with molecular weight 175.19 and with a molecular structure as depicted in Fig. 1. It is soluble in water (1.5 g L^{-1} at 20°C) and remain stable in an alkaline and neutral media protected from room light; it is also soluble in some organic solvents like ethanol, acetone, diethyl ether and chloroform. It is a plant growth regulator of the auxins family. These products help the vegetative propagation of plants. On a cellular level auxins influence cell elongation, cell division, and the formation of adventitious roots. Some auxins are active at extremely low concentrations. 3-Indolyl acetic acid is being used on potato, tomato, lettuce, onion, etc. It presents acute toxic effects when inhaled or absorbed through the skin; it causes skin irritation, eyes, mucous membranes and respiratory tract.

Most of the reported methods for the determination of 3-indolyl acetic acid are applied to plant material and involve its chromatographic (HPLC) separation. It has been find along with other plant growth regulators (gibberellic acid and 3-indolyl acetic acid) in earth worm samples [2], gibberellic, 3-indolyl acetic and abscisic acids in seeds of wheat [3], with indolic compounds (triptophan, 3-indolyl acetic acid,. . .) in pork's fat sample with a previous solid–liquid extraction [4], in plant internal secretions by using a colorimetric detector [5], analysis of serotonine and indols in human fluids [6], rice hormones [7], determination of 3-indolyl acetic acid, triptophan and metabolites in grape-fruit jugs and wine by fluorimetric detection [8]. The biosynthetic way of determining 3-indolyl acetic acid in the Klebsiella oxytoca has been

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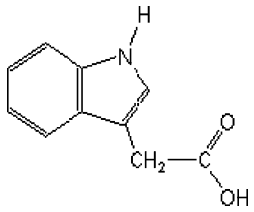

Fig. 1. Molecular structure of 3-indolyl acetic acid.

reported by means of gas chromatography. The capillary electrophoresis was used to separate 3-indolyl acetic acid from its primary metabolite 3-acetylaspartic acid with fluorimetric detection in plant extracts [9], phyto-hormones in tomato samples by using the UV-vis diode array detector [10], a radio-immunoassay method has been developed for the pesticide determination in extracts of the pine tree seeds [11]; an amperometric procedure based on a carbon electrode has been also developed for determination of 3-indolyl acetic acid [12].

The present work was focused to design a simple and quick automated procedure for 3-indolyl acetic acid to be applied to water samples by means of a flexible and versatile analytical technique, the flow injection analysis (FIA). The influence of any physico-chemical and hydrodynamic parameters influencing the fluorimetric outputs was tested. Finally, the paper also includes the study of the batch procedure by testing the influence of different parameters. Both analytical procedures (continuous flow and batch) were applied for the analysis of water samples.

To the author's knowledge, this is the first report dealing on the native fluorescent-based determination of 3-indolyl acetic acid and also the first using a continuous-flow methodology for its automated quantitation.

EXPERIMENTAL

Reagents

All chemicals were of analytical reagent grade and were dissolved in purified water by reverse osmosis and deionised (18 MΩ cm) by adding Sybron/Barnstead Nanopure II. 3-Indolyl acetic acid was from Dr. Ehrenstorfer GmbH (99.0%, Germany). Other reagents were glycine for buffer solutions, ethanol, *iso*propanol, acetonitrile and dimethylformamide all from Scharlau (Spain); NaCl, sodium tetraborate and NH_4Cl from Panreac (Spain), ammonia and HCl from Probus (Spain); acetonitrile, *N*-cethyl *N*,*N*,*N* trimethyl-ammonium 0.2% all from Merck; hexadecylpiridinium 98% and β-cyclodextrine form Fluka (Switzerland), benzalkonium chloride from Guinama (Spain); sodium dodecyl sulphate, Triton X-100 and arabic gum from Panreac (Spain).

Inorganic salts tested as potential interferents were NaCl, KCl, $Na_2SO_4{\cdot}10H_2O$, KCN, $NaNO_3$, $FeSO_4{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$ and CrO_3 (Panreac), $Na_2H_2PO_4$ (UCB), Na_2CO_3 (Prolabo), KI (Guinama), $ZnCl_2$ (Scharlau), $Pb(CH_3COO)_2{\cdot}3H_2O$ and $MnCl_2{\cdot}4H_2O$ (D'Hémio), $Co(CH_3COO)_2{\cdot}4H_2O$ (Riedel de Haëdel, Germany), $Cr(CH_3COO)_3{\cdot}nH_2O$ and $CuSO_4{\cdot}5H_2O$ (Scharlau), $CaCl_2{\cdot}2H_2O$, $NaNO_2$, $FeCl_3$, $NaCH_3COO{\cdot}3H_2O$, and $NiCl_2$ all from Probus. The pesticide Fenoprop (from Dr. Ehrenstorfer GmbH) was tested also as interferent.

The flow manifold comprised PTFE tubing of 0.8 mm internal diameter, peristaltic pump Minipuls 2 from Gilson and injection valve from Rheodyne, model 5041 and the detector was the Fluorimeter Jasco FP-6200 provided with a flow-cell Hellma 176.052-QS (inner volume 125 μL). Data collection was performed by means of the corresponding software prepared for the fluorimeter "Spectra Manager for Windows 95/NT," type 1.53.00. Manifold is depicted in Fig. 2.

An external standard solution was used to test the fluorimeter reproducibility. As standard solution 25 μg L^{-1} quinine in 0.1 M H_2SO_4 was used. The test was performed twice every day, before and after the studies with the pesticide.

RESULTS AND DISCUSSION

Flow Preliminary Assays (Screening)

Preliminary assays were performed on the auxins pesticide family. The pH of analyte solution at about 20 mg L^{-1} was previously adjusted by potentiometry over the range 2–12 and by dropping 0.1 mol L^{-1} HCl or NaOH. Excitation and emission spectra were recorded at each tested pH. Tested pesticides were brompyrazon

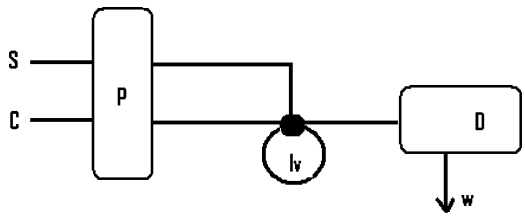

Fig. 2. FIA assembly for the fluorimetric determination of 3-indolyl acetic acid. S: aqueous solution of analyte; C: carrier; P: peristaltic pump; Iv: injection valve; w: waste; D: fluorimeter FP-750 (λ_{exc} = 280 nm and λ_{em} = 364 nm).

(20.4 mg L^{-1}), buminafos (20.0 mg L^{-1}), metobromuron (25.4 mg L^{-1}) and 3-indolyl acetic acid (18.3 mg L^{-1}).

No fluorescence was observed for metobromuron ($\lambda_{exc} = 246$ nm) and brompyrazon ($\lambda_{exc} = 226$ nm). Small fluorescent emission outputs were observed for buminafos with maximum emission at different wavelengths according the pH, namely: at pH 9.2 maximum at 321.6 nm with a relative intensity of 111.7 and at pH 5.32 the relative maximum intensity was 262.9 at the wavelength 322.6 nm

Promising results were observed with 3-indolyl acetic acid; the relative output was over 1000 at $\lambda_{exc} =$ 280 nm and $\lambda_{em} = 364$ nm. Obviously, 3-indolyl acetic acid was selected for further studies, as it presented the most interesting native fluorescent outputs.

The stability of aqueous solutions of the pesticide was tested by preparing a solution containing 20 mg L^{-1} of 3-indolyl acetic acid which was kept refrigerated at 4°C up to 53 days. The absorption spectra excitation maximum at 280 nm and emission maximum at 364 nm) was obtained every day and no changes were observed (Fig. 3).

Influence of pH

The pH could be an important parameter in the fluorimetric output; it was adjusted in solutions containing 19 mg L^{-1} of 3-indolyl acetic acid, over the range 2–12 by dropping 0.1, 0.5 and 1.0 mol L^{-1} of HCl or NaOH and potentiometric control. Pure water was used as a carrier. Then, excitation and emission spectra were recorded. Native fluorescence was observed at any tested pH, with higher intensity at pH range over 9.5–10.5.

Effect of Buffer Solutions

As the pH was shown to be an important parameter and in trying to obtain a system as robust as possible (chemical robustness) three different buffers were tested, namely: glycine/NaOH, sodium tetraborate/NaOH and NH_4Cl/NH_3. No relevant differences were observed in fluorescence intensity; the calculated RSD (in %, 10 replicates) was: 0.9, 0.6 and 0.7 for ammonia, glycine and sodium tetraborate buffers, respectively. Results are depicted in Fig. 4 to select the suitable medium different concentrations of the pesticide (precalibration graph) were prepared at pH 10.0 in glycine/NaOH and sodium tetraborate/NaOH; An aqueous pesticide solution was used as reference. The presence of ammonia was not tested due to the higher observed RSD and also to avoid as much as possible, the formation of gas bubbles into a continuous-flow assembly.

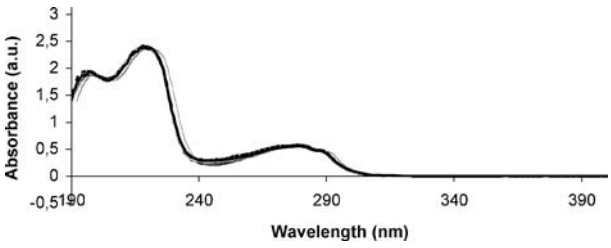

Fig. 3. Daily variation of the UV-visible absorption spectra of solutions containing 20 mg L^{-1} of 3-indolyl acetic acid, from 0 to 53 days (at 4°C).

The resulting pre-calibration graphs presented an increase of 104.0% and 64.2% as compared to the aqueous reference, in the slope average (three replicas) for glycine and sodium tetraborate, respectively. The amount of glycine buffer solution (5, 10 and 20%) revealed the 10% as the concentration with best results.

A final assay consisted in testing the influence of the buffer in the carrier stream. The new carrier was an aqueous mixture at 10% of buffer and the inserted sample contained 5 mg L^{-1} of analyte. Slightly smaller outputs (−2.8%) were obtained with the buffered carrier as compared to the obtained with pure water as the carrier. Selected conditions for further work were, sample solution containing 10% of the glycine/NaOH buffer at pH 10.0 and pure water as carrier.

Influence of Other Chemical Parameters: Solvent Polarity, Presence of Tensoactive Agents and Solvent Oxygen Amount

The presence of dissolved oxygen usually reduces the fluorescent emission, some authors think it is due to the photo-induced oxidation of the fluorescent compounds; generally usual it is due to the paramagnetic characteristics of the dissolved molecular oxygen in inter systems crossing resulting in the deexcitation of to the triplet state.

The influence of oxygen amount was studied in 100 mL of the solution containing 5 mg L^{-1} of 3-indolyl acetic acid and buffered with the glycine/NaOH buffer solution (10 mL). Two different procedures were applied; to increase the oxygen amount the solution was aerated by bubbling air during 30 min. To remove oxygen the solution of the pesticide was put in vacuum for 30 min. Fluorescence measurements were compared with the non-pre-treated solution. Small differences were observed, +1.98% and −1.0% for oxygen increase and decrease, respectively.

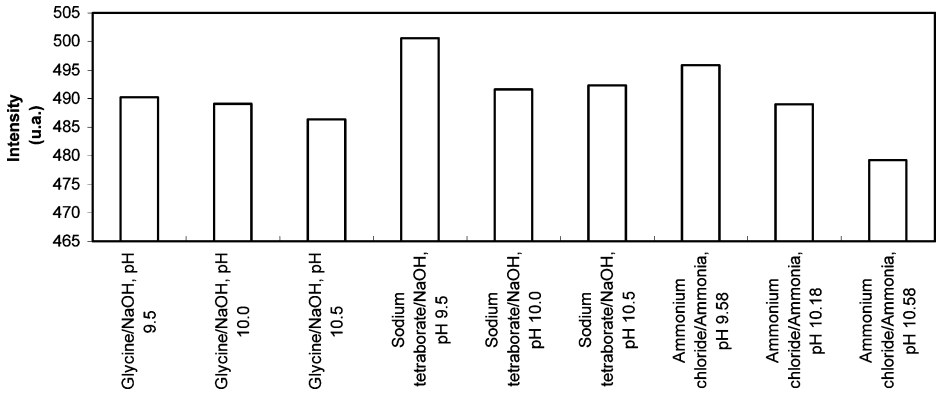

Fig. 4. Variation of fluorimetric outputs at different pH and buffer solutions (0.55 mg L^{-1} of 3-indolyl acetic acid).

Influence of the Temperature

Usually in the analytical systems based on native fluorescence, the temperature increases results the fluorimetric light emission decrease through an increased likelihood of deactivation via external conversions as a result of an increased frequency of collisions between molecules.

The influence of temperature was studied by immersing the flasks containing sample and carrier into a water-bath (TECTRON 200) with testing temperatures from 20 to 75°C.

The performed study resulted in a relevant decrease of the fluorescence emission compared with the outputs at room temperature; a difference of about 39.5% at 75°C were observed. Room temperature was selected for further work.

Study of the Medium: Solvent Polarity and Presence of Tensoactive Agents

The solvent polarity, viscosity, presence or absesce of an organized media or the mass atoms can affect the fluorimetric emission.

Several organic solvents were tested at 5% in a buffered solution containing 20 mg L^{-1} of pesticide. No higher outputs were observed as compared to the aqueous reference solution. Results for each tested solvent were as follows: acetonitrile, −5.5%; ethanol, −3.8%; *iso*propanol, −2.7%. A lower value with dimethylformamide or methanol was observed. A further experiment in which different concentrations of organic solvent were used (from 1 to 10%) resulted in smaller outputs due to the higher concentration of the organic solvent. Pure aqueous solutions were selected.

The organized media, usually surfactant agents, can increase the light emission as they protect the excited molecule from surrounding influences. The study was performed with solutions containing 3 mg L^{-1} of pesticide, 10% of the buffer solution and tensoactive concentration over the micellar critical concentration. Added compounds and amounts were (the final volume was levelled to 50 mL): 0.1 g of 98% hexadecyl pyridinium; 0.3 g of 5% of benzalkonium; 0.1 g of 0.2% *N*-cethyl *N,N,N* trimethyl-ammonium; 0.6 g of Tween 80; 0.6 g of dodecyl sodium sulphate (SDS); 0.3 g of Triton X-100; 0.6 g of β-cyclodextrine and 0.05 g of Arabic gum.

No output increases were observed; some of them resulted in relevant decreases; however, as β-cyclodextrine and SDS presented analytical signals similar than the observed with the pesticide without tensoactive agents, these two were tested again by varying the concentrations. No output increases were however observed. No tensoactive agents were selected for further optimization experiments.

Multiparametric Optimization of the FIA Parameters

FIA parameters were optimized by using the multiparametric method known as modified simplex method (MSM) [13]. The optimized parameters and the ranges studied were: sample volume; length of the external loop of the injection valve, 0–100 cm; flow-rate, 100–900 (arbitrary units in the pump display); and, distance from injection valve to the flow-cell 15–100 cm. Pesticide concentration was 3 mg L^{-1}.

The complete empirical procedure consisted two empirical series; after the first simplex series the vertices resulting in higher outputs were selected to test the best compromise sensitivity (peak height)—reproducibility

Table I. Multiparametric Optimization of the FIA Parameters

Vertices	Parameters						
	Volume	Flow-rate	Distance	Peak height	RSD (%)	Peak base	RSD (%)
Multiparametric series							
1	0	100	20	131.6	3.0		
2	94	288	39	291.3	0.8	86	1.4
3	24	854	39	209.1	0.8		
4	24	288	95	130.6	0.4		
5	39	414	33	245.5	0.4		
6	36	391	44	220.8	0.4		
7	30	300	20	193.5	0.8		
8	96	465	27	164.7	0.7		
9	46	960	27	148.3	1.1		
10	46	465	48	123.6	1.2		
11	57	575	25	125.7	0.8		
12	52	522	36	232.0	0.4		
13	10	300	20	159.2	1.3		
14	76	441	27	290.8	0.5	70.8	0.9
15	26	866	27	240.1	0.8		
16	26	441	48	196.5	1.5		
17	43	583	34	256.3	0.6		
18	76	866	48	296.5	0.9	41	0.9
19	43	583	34	244.0	0.3		
20	59	724	34	272.2	0.7	36.6	1.8
21	50	645	38	277.1	0.4	39.9	1.6
22	67	651	38	284.5	0.3	41.5	0
23	53	729	34	272.7	1.3		
24	67	651	38	261.1	1.2		
25	60	693	36	256.4	0.7		
Re-optimization of the highest outputs							
2	94	288	39	269.41	1.47	102.50	1.48
14	76	441	27	274.99	1.18	59.68	1.31
18	76	866	48	279.50	1.09	32.75	1.44
20	59	724	34	280.64	1.64	35.79	1.37
22	67	651	38	266.37	1.22	39.50	8.01

Note. Selected FIA parameters from vertices 20: sample volume, 359 μL;. flow-rate, 357 mL min^{-1}; distance to detector, 34 cm.

(RSD)—sample throughput (peak base wide). The result of each vertices (both series) was the average of 20 peaks. Results are depicted in Table I.

Chemical Study of 3-Indolyl Acetic Acid in Batch

The continuous-flow procedures differ from the corresponding in batch in fact the continuous flow is a kinetic analytical procedure in which the output is obtained at fixed time before reaching the chemical and dynamic equilibriums; the batch performs the analytical measurements after reaching the chemical equilibrium. This relevant difference requires to repeat the optimization of all physico-chemical parameters. In fact it was a re-optimization following similar criteria as reported for the continuous-flow procedure. The work was performed at $\lambda_{exc} = 280$ nm and an emission range from 300 to 500 nm.

The pH influence, tested over the range 6.2–11.5 and at 0.5 mg L^{-1} of 3-indolyl acetic acid, presented empirical results slightly different from the one reported for the flow procedure. It made convenient to preselect the range from 8.0 to 9.0. The study of buffers sodium tetraborate/HCl and glycine/NaOH resulted in the selection of sodium tetraborate/HCl at pH 8.0.

The influence of organic solvents (0.5 mg L^{-1} of pesticide) was seen in difference such as differences with the flow procedure where the increase of the output with the presence of ethanol (up to 31.5% at ethanol concentration of 30%) was observed; however, the reproducibility was minor compared to the observed with aqueous solution. No organic solvent was selected for further work.

Table II. Influence of Foreign Compounds (0.05 mg L^{-1} of 3-Indolyl Acetic Acid)

Interferent	Concentration (mg L^{-1})	Error (%)
(a) Influence of foreign compounds		
Na^+	500[a]	2.7
NH_4^+	500[a]	1.1
Mg^{2+}	100	−1.4
K^+	500[a]	−0.6
Cu^{2+}	10	−3.8
Ca^{2+}	500[a]	−2.2
Zn^{2+}	500[a]	−4.2
Pb^{2+}	5[b]	−2.7
Ni^{2+}	100[a]	1.9
Mn^{2+}	10[b]	−5.2
Fe^{2+}	10[a]	−63.4
Fe^{3+}	50[b]	0.2
Co^{2+}	100[a]	−1.6
Cr^{3+}	10[a]	−0.5
Cl^-	500[a]	2.3
CH_3COO^-	500[a]	−5.5
CO_3^{2-}	500[a]	−4.1
$H_2PO_4^-$	500[a]	−4.6
CN^-	500[a]	0.6
I^-	500[a]	1.2
NO_2^-	500[a]	−29.4
SO_4^{2-}	500[a]	0.4
CrO_4^{2-}	10[a]	−50.6
Fenoprop	1	−0.2
(b) Removing interferences		
NO_2^-	500[c]	−3.6
Cu^{2+}	500[d]	0.4
Pb^{2+}	500[d]	−2.4
Mn^{2+}	500[d]	−3.2
Fe^{2+}	500[d]	5.7
Fe^{3+}	500[d]	5
Cr^{3+}	500[d]	2.1

Note. Anions were prepared from the potassium or sodium salts and metallic cations from its chloride salts.
[a]Maximum tested concentration.
[b]Filtered sample to remove precipitates.
[c]By gentle heating.
[d]Solid-phase extraction with the aid of a SAX cartridge.

No qualitative differences were observed with the study of the influence of organic solvents (SDS and β-cyclodextrine) and temperature. Both of these showed negative influences.

Analytical Figures of Merit

Flow Injection Analysis

Due to the large dynamic ranges presented in emission procedures the calibration range was tested in the three different sensitivity scales of the fluorimeter. Linear dynamic ranges: Fitted equations and correlation coefficients were: (a) 0.005–0.6 ppm; $I = 1393x+3.218$; $r^2 = 0.9998$; (b) 0.10–12 ppm; $I = 81.937x+20.561$; $r^2 = 0.9971$ and (c) 2.0–60 ppm; $I = 4.609x+3.721$; $r^2 = 0.9976$.

The reproducibility of the slope of the calibration curve (or relative standard deviation in experiments performed in different days and freshly prepared solutions), was determined from 0.03 to 0.6 mg L^{-1} (six dots). The mean slope from seven independent calibrations of 3-indolyl acetic acid was 1432.23 (RSD = 4.9%).

The RSD for the peaks, which is a measure of repeatability or reproducibility, was determined by using 21 consecutive insertions of the same solution of 3 mg L^{-1} and the RSD was 1.6%. The experiment was repeated on seven different nonconsecutive days, the RSD was 4.8% at 0.1 mg L^{-1}.

The maximum sample throughput was calculated from the average of base-peak wide (21 replicates) containing 3 mg L^{-1} of 3-indolyl acetic acid; obtained result was 100 hr^{-1}. The limit of detection, which was taken to be the lowest pesticide concentration that yielded a signal equal to the blank signal plus three times its standard deviation, was 0.03 μg L^{-1}.

The analytical features of the proposed method and its tolerance to potential interferences accompanying 3-indolyl acetic acid in water samples and preparations were studied for a concentration of pesticide of 0.05 mg L^{-1}. Foreign compounds were not considered as interference when the calculated relative error , compared to the reference (solution containing only 0.05 mg L^{-1} of pesticide), was less than ±5% (see results in Table II). The most critical interference was due to ions with redox activity as nitrite and chromate; these interferences can be easily removed. The interference from cations arise at concentrations higher than that usually present in residual water samples. To remove the interferences solutions containing 0.05 mg L^{-1} of the pesticide and 500 mg L^{-1} of the corresponding interferent to be tested were prepared. The solution containing nitrite only required gentle heating to eliminate the presence of the interferent. The solutions containing a metallic cation were passed through an ion exchange column (Duolite C20 and A-102-D Probus); erroneous results were observed due to the pesticide was partially retained on the resin. The separation of the pesticide from the matrix by means of a cartridge SAX (Bond Elut SAX, Varian) resulted in minor relative errors; the solution containing pesticide and interferent was forced through the column and then it was eluted by 1 mL of 0.1 mol L^{-1} of HCl, then, and after adjusting the pH, the native fluorescence was measured (see the results in Table II).

The applicability of the FIA-fluorimetric method was checked on water samples collected from two different origins. All samples were spiked with the buffered pesticide solution with a final amount of 0.2 mg L^{-1}. Analysed samples and recoveries were as following: residual water (collected in Chirivella, Valencia; 0°26′19.057″W and 39°27′10.642″N), 97,7%; and, mineral bottled water (trade mark Agua de Bejìs, Castellón), 99.3%.

Batch Procedure

The study was performed according to the reported criteria in the above paragraphs; and, the obtained results were:

A. Calibration graphs (linearity ranges, equations and correlation coefficients) were:

a. 0.005–0.1 ppm; $I = 8370x+2.735$; $r^2 = 0.9831$;
b. 0.05–1 ppm; $I = 524.97x+3.672$; $r^2 = 0.9996$; and,
c. 0.5–60 ppm; $I = 28.12x+5.089$; $r^2 = 0.9996$.

B. The reproducibility of the slope (RSD) from four independent calibrations from 0.05 to 1.6 mg L^{-1} of 3-indolyl acetic acid was 2.9%.
C. The RSD for the peaks, was determined by using 17 consecutive insertions of the same solution of 1.0 mg L^{-1} and the RSD was 3.5%.
D. The limit of detection was 0.01 μg $L^{-1.}$

CONCLUSIONS

A new and simple fluorimetric determination of 3-indolyl acetic acid is presented by means of a flow injection assembly or in batch. The method is based on the native fluorescence of the pesticide and it is applied at pH 10, at room temperature and in aqueous solutions.

The method presents a competitive sensitivity with an L.O.D of 0.03 ppb, RSD 1.6% and sample throughput 100 hr^{-1}. The required selectivity from metallic cations is easily obtained by a solid-phase extraction.

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