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FIA-fluorimetric determination of the herbicide Benfuresate

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Abstract

This paper presents the analytical determination of the herbicide Benfuresate based on its native fluorescence in a Flow Injection Analysis (FIA) assembly. The pesticide solution is inserted into the carrier stream of pure water and forced to the fluorimeter flow-cell where the sample solution was excited at 278 nm and the emitted light was measured at 316 nm. The influence of different parameters was investigated obtaining an output increase over 120% compared with the obtained with pure aqueous solution. The calibration range, from 0.001 to 50.0 mg l^{-1}, resulted in three different linear behaviour ranges according to the sensitivity degree of the fluorimeter; the one dealing with minor concentrations was (range in mg l^{-1}, linear equation and correlation coefficient), 0.001 – 0.5 mg l^{-1}, I = 1062.5 X + 36,3, 0.9997. The sample throughput was 210 h^{-1}. After testing the influence of a large series of potential interferents the method was applied to several type of samples.

Key-words: Pesticides, fluorescence, environmental samples

1.Introduction

Benfuresate or 2,3-dihydro-3,3-dimethyl benzofuran-5-yl ethanesulphonate, is a brown viscous liquid which could be solid at room temperature [1]. It is soluble in water (0.261 g l^{-1} at room temperature) and in organic solvents like methanol, acetone, dichloromethane, toluene and ethyl acetate. The molecular structure is depicted in Figure 1.

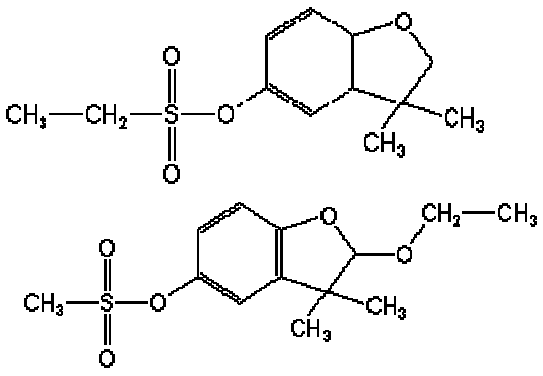

Fig.1 Molecular structure of the herbicides Benfuresate and Ethofumesate

Benfuresate is an herbicide of general use classified as a member of the benzofuranyl alkylsulfonate family [2]; it is commonly used for cotton, tobacco, sugar cane, rice, corn and beans. As herbicide it acts by inhibiting the fat synthesis; it is especially efficient against Digitaria, Setaria, Cyperus, Portulaca, Solanum and Galium. On humans it is moderately toxic by ingestion and skin and eyes irritant. It is commercially available in different types of formulations.

Few and recent papers dealing on Benfuresate determination have been published; the first was published in 2000 and all of them are base on a chromatographic separation (liquid or gas). Nemoto *et al*. [3] published a study on the GC separation and MS detection of 110 pesticides (Benfuresate included); sample was pre-concentrated with the aid of a silica column. During the same year Kaihara *et al*. [4] performed an HPLC separation previously to a supercritical-fluid extraction to determine 27 pesticides on fruits and vegetables. The same group performed a similar study dealing with 18 pesticides on sample of rice, fruits or vegetables [5]. Pesticides (total 87) containing nitrogen or sulphur were determined by gas chromatography [6] on vegetables; and 174 with a

previous micro-extraction by GC-MS [7] in water and 266 in apple juice by GC-MS.

The present paper and as far as the authors known, delays for first time with the FIA-fluorimetric determination of the herbicide Benfuresate. It is a very simple and quick procedure for the determination of a pesticide characterised by the low number of published analytical procedures and most of them dealing on the same type of methods. Alternatively the optimised set of parameters will allow the assembly to be coupled to a post-separation process as an enhanced sensitivity detector.

2. Experimental

2.1. Reagents and apparatus

All reagents used were analytically pure unless stated otherwise and prepared in water purified by reverse osmosis and then deionised (18 MΩ-cm) with a Sybron/Barnstead Nanopure II water purification system provided with a fiber filter of 0.2 μm pore-size. The Benfuresate was acquired from Dr Ehrenstorfer GmbH (Ausburg, Germany), purity. 97.3 %. Other pesticide tested as interferent was Ethofumesate obtained from the same manufacturer.

Strong inorganic acids and alkalis, (from Panreac, Spain); reagents for buffers, tensoactives and sensitizers: Triton X-100, dimethyl-formamide and $Na_2B_4O_7 \cdot 10H_2O$ from Panreac; β-cyclodextrine (Fluka, Buchs, Switzerland) NH_3, Na_2HPO_4, NH_4Cl, and sodium acetate from Probus (Spain); H_2O_2, ethanol and acetonitrile from Prolabo (Spain) and Merck (Germany); KH_2PO_4, sodium dodecyl sulphate and hexadecylpiridinium chloride from Fluka; glycine and acetone from Guinama (Valencia, Spain).

The flow manifold, depicted in Figure 2, consisted of a PTFE coil (from Omnifit, N.J., USA) of 0.8 mm internal diameter; a Gilson (Worthington, OH, USA) Minipuls 2 peristaltic pump provided with flexible tubing from Elkay (Co, USA). The fluorimetric flow-cell (Hellma, GmbH & Co KG, Mullheim, Germany) was of the model 176 050 and the fluorimeter was the FP-6200, type diode array (Jasco Analítica, Spain) provided with the specific software for data collection Spectra Manager model 1.53 for Windows 98 and acquired to the fluorimeter manufacturer.

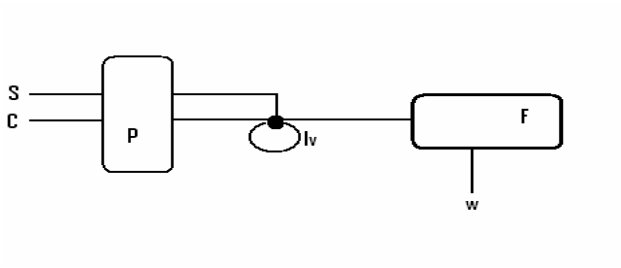

Fig. 2 Flow assembly for determination of the Benfuresate: S, aqueous solution of Benfuresate with pH buffered and presence of β-cyclodextrine; C, carrier, water; P, peristaltic pump (flow-rate 10 ml min^{-1}); F, fluorimeter FP-6200 (excitation 278 nm and emission 316 nm); and, Iv, six-port injection valve.

3. Results and discussion

Figure 3 depicts the native fluorescence spectrum of the aqueous Benfuresate solution with a maximum emission at 316 nm when excited at 278 nm.

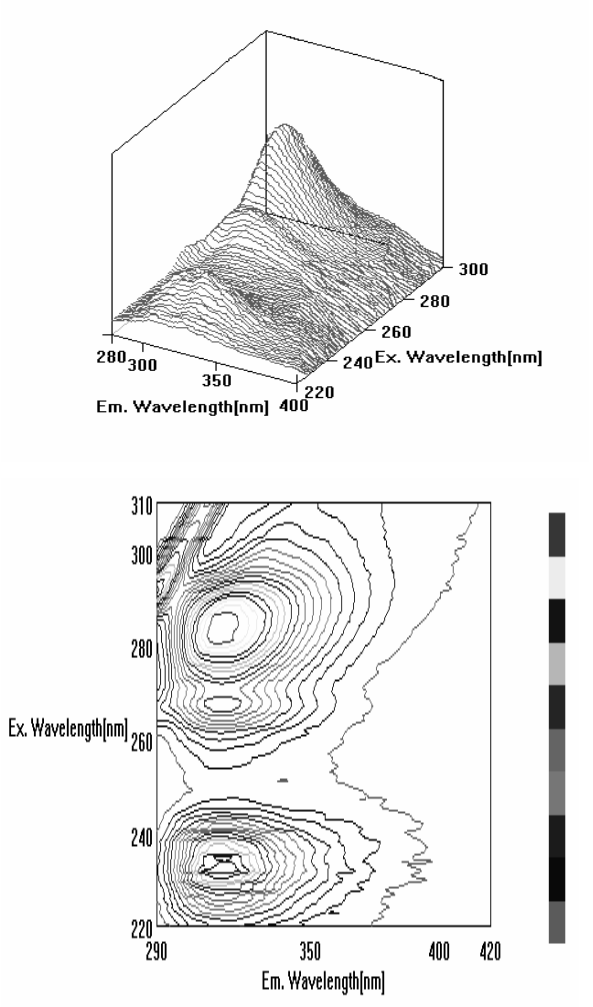

Fig. 3 Three-dimensional spectrum of an aqueous solution of Benfuresate

A preliminary assay consisted in checking the possible increase in the fluorescence emission after a photodegradation process was also performed. The flow assembly included a photo-reactor formed by a low-pressure Hg lamp (Zalux, 15 w) with the PTFE tubing helically coiled around the lamp; the UV-vis irradiation period on a solution containing 20 mg l^{-1} of herbicide was 2 min long. The outputs were compared with the same solution not irradiated (lamp OFF). The transient signals were clearly higher with lamp OFF than the obtained with lamp ON.

3.1. Influence of pH and buffers on the fluorescence emission

The influence of pH on the fluorescence emission spectra could be very important especially for compounds presenting acid or basic functional groups, since changes in pH influence on the dissociation degree, what can affect the aromaticity of the compound and can vary either the wavelength of emission and excitation or the intensity of emission. For that reason the influence of pH on the Benfuresate solution was studied over the range 1,5 - 11,1 by dropping 0.1 mol l^{-1} HCl or NaOH and potentiometric control of the final pH. Pesticide concentration was 5 mg l^{-1}. The pH influence was not critical; however, the higher outputs were observed over the range 5.5 – 7.5. Some buffers were tested on this range (KH_2PO_4 / Na_2HPO_4; acetic acid/ sodium acetate, tartaric acid) and compared with the outputs from a non buffered solution. The selected condition for further work was the KH_2PO_4 / Na_2HPO_4 buffer at pH 7.8; then different concentrations of this buffer were tested and resulted in being not a critical parameter; from the observed results the selected final concentration of buffer in the measured solution was 0.00036 mol l^{-1} /0.0063 mol l^{-1}.

A similar study on the influence of pH was also performed for the carrier stream; this influence shown to be irrelevant; and, due to that pure water was chosen for the carrier stream.

3.2. Influence of solvent characteristics

Fluorescence emission so intensity as the band configuration can be affected by solvent characteristics as viscosity; polarity, the presence of tensoactives, amount of solve oxygen and temperature.

Different organic solvents (ethanol, methanol, iso-propanol, acetonitrile and N,N-dimethylformamide) were added to solutions containing 3 mg l^{-1} of Benfuresate (final concentrations 1, 10, 20 and 40 %) and then excitation and emission spectra were recorded. No important intensity increases were observed and pure water was being used for further experiments.

The influence of tensoactives or the β-cyclodextrine resulted in a relevant increase of the outputs of Benfuresate solution (3 mg l^{-1}) when the cyclodextrine was added (see Figure 4); studied concentration interval up to 1.2%; to avoid solution problems no higher concentrations were tested. The selected concentration was 1.2%.

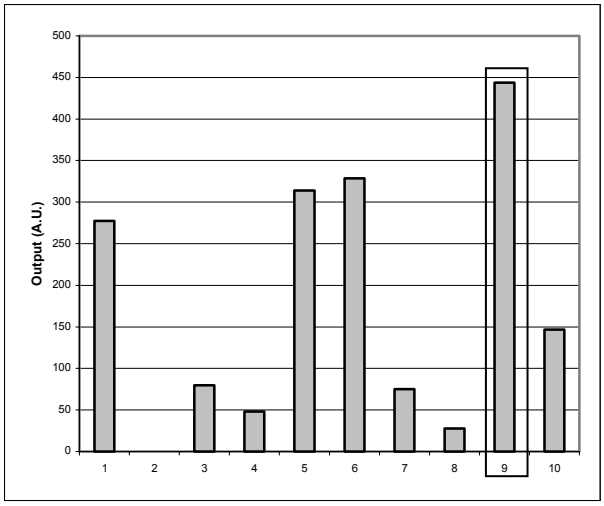

Fig. 4 Effect of presence of organized media and presence of sensitizers on the fluorescent output

1) Benfuresate 3 mg l^{-1}; 2) Rodamine 6G 0.02%; 3) Hexadecilpiridinium chloride 0.2%; 4) Quinine sulphate 10^{-4} M; 5) Benzalconium chloride 0.6%; 6) N-cetyl-N,N,N-trimethylammonium bromide 0.2%; 7) SDS 1.2%; 8) Triton X-100 0.6%; 9) β-cyclodextrine 1.2%; and, 10) Tween 80 0.6%

The amount of dissolved oxygen was varied by means of a vacuum pump; to increase the oxygen amount a flow of air bubbles was forced through the solution during 30 minutes; and, to decrease this concentration was performed by aspirating the air on the solution, also during 30 minutes. Slightly minor signals were observed when the oxygen amount was increased according with the theoretical predictions; however not relevant increases were obtained with minor oxygen amounts. No sample pre-treatment is required.

The influence of the temperature studied from room temperature up to 80ºC (Benfuresate 1 mg l^{-1}) by

immersing the solutions to be processed via the flow assembly into a water bath. Outputs were continuously decreasing with the temperature increased. Room temperature was chosen for ext next experiments.

The Modified Simplex Method (MSM) [9, 10] was applied for the optimisation of the hydrodynamic parameters. Selected parameters and ranges were: sample volume, 10 - 250 cm length of the loop; flow-rate, 100 - 1000 arbitrary units on the pump display and equivalent to 1 - 10 ml min^{-1}; and, distance injection-valve to flow-cell, 30 - 150 cm.

To select the best compromise sensitivity (peak height) – sample throughput (base-peak width) – reproducibility (rsd, %); the two vertices presenting the higher peak-height were selected and a series of 10 consecutive insertions was performed for each of them. According to these results was prepared the finally proposed FIA manifold; sample volume 200 μl; distance injection valve – flow cell, 30 cm; and, total flow-rate, 10 ml min^{-1}.

3.3. Analytical figures of merit

The proposed method was applied into the three instrument sensitivity scales. The calibration range studied, from 0.001 to 50.0 mg l^{-1}, resulted in three linear behaviour ranges according to the sensitivity degree of the fluorimeter: namely (range in mg l^{-1}, linear equation and correlation coefficient), 0.001 – 0.5 mg l^{-1}, I = 1062.5 X + 36,3, 0.9997; 0.025 – 10 mg l^{-1}, I = 71.1 X + 1.1, 0.9999; and, 0.1 – 50 mg l^{-1}, I = 5.5 X + 2.8, 0.9999, for high, medium and low sensitivity, respectively.

The reproducibility of the slope of the calibration curve (or relative standard deviation in experiments performed in different days), was determined over the range 10 – 250 μg ml^{-1} (n 5). The mean slope from 5 independent calibrations of Benfuresate obtained by using fresh solutions, was 0.82 with a calculated rsd of 2.6%.

The rsd for the peaks, which is a measure of repeatability and reproducibility, was determined by using 21 consecutive insertions of the same solution of 0.1 mg $\cdot l^{-1}$ Benfuresate and the rsd was 1.3%. The experiment was repeated on 5 different non consecutive days, the mean rsd obtained being 2.9% at 0.1 mg l^{-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 1 μg l^{-1}. The sample throughput obtained was 210 h^{-1}.

A further experiment was performed to check the robustness of the method; it is said, effect on the results when some parameter is slightly changed. The experiment was carried out by the univariant procedure being tested all parameters (chemical and dynamic) with a variation into the range ±10% around the optimised value. Two parameters resulted critical; flow-rate and distance from the injection valve to the flow-cell; with calculated relative errors of 12 and 14% respectively. All other tested parameters shown errors minor to 1%.

The analytical features of the proposed method and its tolerance to potential interferences accompanying Benfuresate in water samples and preparations were studied for a concentration of Benfuresate of 0.05 mg l^{-1} and are shown in Table 1. No interference was considered when the calculated relative error vs the reference was less than ±5%. The most critical is presented by nitrite down to 1 mg l^{-1}; this is interference can be easily removed; a solution containing 0.05 mg l^{-1} of Benfuresate and 500 mg l^{-1} of nitrite was gently heated during 10 minutes; no error was observed. The phosphate medium partly removed some metallic interference by precipitation [11]; the measurement was performed after filtration.

The applicability of the proposed method was tested on different types of samples, namely: water, formulation, soil and human urine.

Table 1. Influence of foreign compounds (Benfuresate,0.05 mg l^{-1}). Solutions were prepared from sodium salts for anions and chlorides for cations, respectively.

(*) Maximum assayed concentration; (**) Assayed solution after filtering the formed phosphate basic salts; and, (***) After gentle heating

Interferent	C (mg l^{-1})	R.e. (%)	Interferent	C (mg l^{-1})	R.e. (%)
Fe^{3+}	*500	3.4	Ni^{2+}	*500	**2.3
Fe^{2+}	*500	2.5	Co^{2+}	*500	**-1.1
$H_2PO_4^-$	*500	-2.5	CO_3^{2-}	200	4.8
NH_4^+	*500	-2.6	HCO_3^-	*500	-3.5
I^-	*500	0.4	Cr^{3+}	*500	**-5
Mn^{2+}	100	3.6	CrO_4^{2-}	1	-3.2
K^+	*500	1.9	Cd^{2+}	*500	**-3.5
Na^+	*500	2.4	Cu^{2+}	*500	1.5
CH_3COO^-	*500	-1.4	SO_4^{2-}	*500	-3.8
CN^-	*500	-4.5	NO_2^-	*500	***2.6
Zn^{2+}	*500	**1.2	NO_3^-	*500	2.7
Mg^{2+}	*500	-2.6	Ca^{2+}	*500	**1.5
Cl^-	*500	0.4	Urea	*500	-3.9
Pb^{2+}	*500	**1.1	Ethofumesate	0.05	4.1

Water samples were collected from different places and all of them were spiked with 0.05 mg l^{-1} of Benfuresate. Analysed samples (5 replicates), recoveries and r.s.d were as following: river water (río Magro in San Juan, Valencia); 102.1%, 2.3%; residual water (Chirivella, Valencia), 103.4%, 2.9; underground water (well in San Antonio, Valencia), 102.6 %, 1.8%; and, bottled water (trade name Agua de Bejìs, Castellón), 98.7%, 1.2%.

Two samples were collected from two different agricultural soils (argillaceous type from Lliria in Valencia, Spain and the other sample was calcareous from Santa Bárbara in Tarragona, Spain) and both were treated in the lab according to the official rules (an aliquot of the stock solution containing 40% of Benfuresate was diluted to 1/200 with water; 4ml were sprayed at low pressure to 50 g of soil; this sample was extracted with 100 ml of water by magnetic stirring during 20 minutes; Benfuresate was analyzed in the aqueous extract) for the pesticide application to agricultural uses [14]. No fluorescence signals were observed by liquid extraction of the solid sample which was not pre-treated with the pesticide (blank assays). A solid-phase extraction to separate the analyte from the sample matrix as described in the next urine samples paragraph was also required to improve the analytical results. Observed recoveries (and R.S.D. as % for 5 replicates) were 97.8%, 2.0 and 97.5%, 2.5 % for Lliria and Santa Bárbara, respectively.

A formulation containing 40 % of Benfuresate (0.1 mg l^{-1} total amount) was prepared in the lab [11, 12] as no commercial samples can be acquired from local manufacturers. As inert compounds and according to EPA (Environmental Protection Agency XIV) [12] was added 60% acetone. Found (average of 3 determinations) presented relative deviation vs the added amount of 2.8 %.

Human urine simples were from different (male and female) and were directly doped with the pesticide (1 mg l^{-1}); separation of the analyte from the rest of the sample was required due to the native urine fluorescence.

Best results were observed with the cartridge Bond Elut C_{18} (Figure 5) from Varian and elution with acetonitrile (5%, final concentration). The cartridge do not provided a complete elimination of the matrix fluorescence, due to that the standard addition was used with the eluted solutions. Results (%recovery and R.S.D. of 5 replicates) were 97.7, 0.6% and 101.2, 2% for male and female, respectively.

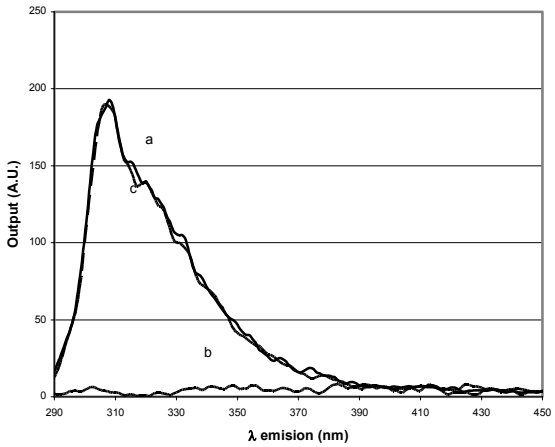

Fig. 5 Separation of Benfuresate (fluorescence spectra) : a, reference Benfuresate solution (1 mg l^{-1}); b, spectrum of the reference solution after being forced through the separation cartridge; and, c) fluorescence of the acetonitrile eluted solution

Table 2. Analytical figures of merit for Ethofumesate.

Ethofumesate, analytical parameters	
Linearity range	10 μg l^{-1} – 2 mg l^{-1}
Linear equation	y = 454,53x + 19,859
Correlation coefficient	R^2 = 0,9998
LOD (μg l^{-1})	6.6
RSD intra-day (n=15), %)	3.0
Recovery, river water (%)	102.1±2.0
Recovery, residual water (%)	99.8±1.8
Recovery, underground water (%)	95.2±3.4
Recovery, bottled water (%)	96.2±1.5

Finally and in order to check the applicability of the method to other members of the pesticide family, the procedure was applied to Ethofumesate, or 2-ethoxy-3,3-dihydro -3 , 3 - dimethylbenzofuranylmethanesulfonate, $C_{13}H_{18}O_5S$, see molecular structure in Figure 1) a compound which is also a member of benzofuranyl alkylsulphonate herbicide group. The obtained analytical figures of merit for this pesticide are depicted in Table 2.

4. Conclusions

A simple and quick procedure is presented for the determination of herbicide Benfuresate based on a FIA assembly provided with the fluorimetric detector.

Outputs were greatly influence by the presence of the KH_2PO_4 / Na_2HPO_4, buffer and 1.2% β-cyclodextrine. The influence of the temperature increase is negative on the peaks height.

The method is competitive and can be applied to water and formulations and also for the determination of Etofumesate.

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