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Fluorescence Determination of the Pesticide Asulam by Flow Injection Analysis

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This paper presents the analytical determination of the pesticide Asulam based on its native fluorescence. The method was optimized in either a flow injection analysis (FIA) assembly or in batch. The maximum fluorescence intensity was observed for basic pH solutions and at a λ_{ex} of 258 nm and a λ_{em} of 342 nm. The influence of different empirical parameters, such as the pH, the presence of surfactants, solvent polarity or solved oxygen amount, was studied. The calibration range was fitted with a linear equation from 0.01 – 3 mg l^{-1} Asulam and 0.005 – 15 mg l^{-1} Asulam for batch and continuous-flow, respectively. The RSD for both procedures was 1.0%. After testing the influence of a large series of potential interferents, the method was applied to water samples from different locations.

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Introduction

The massive use of pesticides imposes the necessity to find easy, quick and simple analytical methods for the control analysis of pesticides in different samples of environmental interest. This trend is also imposed by strict legal rules for control analysis.

Asulam is a methyl 4-aminobenzenesulfanilyl carbamate, and is used to control broad-leaf weeds, perennial grasses, and non-flowering plants. See the molecular formulae in Fig. 1. The EPA (Environmental Protection Agency)[1] classified Asulam as a carcinogenic agent, and due to the high water solubility of its sodium salts (in commercially available formulations) it is water polluting agent. It can also be accumulated and remain in soil for more than one season.

Asulam is most often determined by diazotization of its amino-group subsequent coupling with Marshall's reagent,[2] resulting in an analytical procedure that is poorly selective. Synchronous fluorometry[3] allows Asulam to be determined following derivatization of the primary amino group with fluorescamine. The determination of Asulam in soil, water or food samples has been largely studied. The vast majority of methods have been separation procedures, such as chromatographic, electrophoresis or a combination of both, and have been provided with spectrophotometric detection (most of the reported methods), fluorometric or electrochemical. Into the chromatographic field can be found many articles concerning thin layer[4,5] as gas chromatography[6] or a relatively large number of procedures concerning liquid chromatography[7–12] on certain occasions coupled with solid-phase extraction.[13–16] Capillary electrophoresis has been applied to Asulam determination by simultaneous spectrophotometric and electrochemical detection.[17,18] Other authors have investigated isotachophoresis along with conductimetric or UV-vis detection[19] or micellar electrokinetic capillary chromatography.[20] For complex matrices, combinations of these methods have been employed: one of them for the sample pretreatment; preparative capillary isotachophoresis for humic matrices[21] and HPLC; or, sample pretreatment for soil samples followed by capillary electrophoresis.[22]

Other non-separation procedures have been published for Asulam determination. FIA with an amperometric detector has been applied to water samples[23] and multicommutation (continuous flow-manifold based on solenoid valves) combined with chemiluminescence[24] after on-line photodegradation. Asulam has also been quantified electrochemically (using a glassy carbon electrode)[25] and by square-wave voltammetry and FIA-amperometry. The mechanism behind the electro-oxidation of Asulam was elucidated by voltammetry studies. The quantitation range afforded by both methods only allows the determination of relatively large amounts of the herbicide Asulam.

The fluorometric behavior of Asulam has been widely analytically exploited with previous chemical reactions of the analyte with fluorescamine. In thin layer chromatography the product from the fluorescamine reaction[4] is observed under a UV lamp; this reagent has also been proposed for pre or postcolumn chromatographic derivatization[26–28] by measuring the fluorescence emission at 484 nm (excitation at 380 nm). Other authors have proposed ion-pair reverse-phase liquid chromatography using sodium cholate as an organic counter ion.[29]

Other non-chromatographic methods also used the fluorescamine reaction after liquid extraction[30] applied to

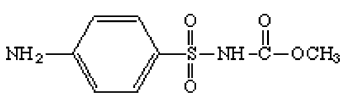

Fig. 1 Molecular structure of Asulam.

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chopped peaches. Working in batch and after a solid-phase extraction to remove any interference from the matrix, the EDTA treatment (metallic ions interferences), methanol elution and pH adjusted to 8 – 10 allowed the native fluorescence of Asulam to be measured.[31]

The present work was focused on studying the influence of surfactants on the native fluorescence of Asulam (no bibliographic information was available) as an effect of ultraviolet irradiation on the fluorescence of Asulam by forming photo-fragments with high emission intensity. On the other hand, a second goal was to design a simple automated procedure for Asulam to be applied to water samples. Therefore, the flow injection analysis (FIA) was selected as a versatile usual analytical technique for the determination of Asulam. To reach this target, the study started in batch by testing the influence of different parameters, and then the procedure was applied to water samples. The work was implemented along with a new study on the influence of chemical parameters applied to continuous flow.

Experimental

Reagents and apparatus

All chemical were of analytical reagent grade and solved in purified water by reverse osmosis and deionized (18 MΩ cm) with the aid of Sybron/Barnstead Nanopure II. Asulam was from Dr. Ehrenstorfer (94.0% purity). Other reagents were: NaOH, ethanol, acetonitrile, *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide from Merck; CH_3COOH, HCl, methanol, from Baker; KH_2PO_4, $Na_2B_4O_7{\cdot}10H_2O$, NH_4Cl, ammonia, NaCl, dimethylformamide, Triton X-100, Tween 80 from Panreac; glycine, quinine sulfate, benzalkonium chloride from Guinama; isopropanol from Scharlau; hexadecylpyridine, sodium dodecylsulfate, β-cyclodextrine from Fluka.

Two fluorometers were used: Jasco FP-6200 (batch procedure and study of pH in flow) and F-4500. The flow manifold comprised PTFE tubing of 0.8 mm internal diameter, a peristaltic pump (Minipuls 2) from Gilson and an injection valve from Rheodyne (Model 5041). The flow cell was a Hellma 176.052-QS (inner volume 125 µl).

An external standard solution was used to test the fluorometer reproducibility. As a standard solution, 25 µg l^{-1} quinine in 0.1 mol l^{-1} H_2SO_4 was used. The test was performed twice every day, before and after the Asulam study.

Solution preparation

An aqueous stock solution of 100 mg l^{-1} of Asulam (Dr. Ehrenstorfer) was prepared by exactly weighing and dissolving it in purified water with the help of an ultrasonic bath. The stock solution was protected against room light and stored in a refrigerator. This solution was stable for at least for one week.[24] The working standard solutions were prepared daily by diluting the stock solution and protected against light.

For the batch procedure, the required amount of Asulam was added to 5 ml of the buffer NH_4^+/NH_3 at pH 9 in a 25 ml volumetric flask. For the flow procedure, dilution was made in deionized water.

Sample preparation

Several water samples of different type were collected from different places, namely: irrigation channel, river, bottled and underground. Samples were collected in plastic flasks at 4˚C and were analyzed within 24 h. No sample pretreatment was required, except for filtering when the sample presented a turbid appearance. All samples were spiked with the required volume of the stock solution (100 mg l^{-1} of Asulam) to obtain samples containing 0.5 mg l^{-1}.

Results and Discussion

Batch procedure

Influence of the pH. The pH of aqueous solutions containing 14 mg l^{-1} Asulam was adjusted over the range 1 to 11 by potentiometric control and by dropping 0.1 mol l^{-1} of HCl or NaOH. Then, the excitation and emission spectra were recorded. The maximum absorbance appeared at 275 nm in acidic media; this maximum was slightly moved to a minor wavelength when the pH was increased. Native fluorescence was observed at any tested pH, with higher intensity in the pH range over 5 to 6 and presenting the maximum absorbance (λ_{ex}) around 256 nm and the maximum emission at λ_{em} 342 nm.

Preliminary experiments

Effect of UV light on Asulam fluorescence. UV irradiation has the properties that make an "ideal", universal reagent, since, depending on its chemical structure; a compound may undergo oxidation, reduction, hydrolysis or a variety of other transformations under its action. Photochemical reactions offer a powerful means for obtaining derivatives with improved chromophoric, fluorophoric or electrolytic properties. All of these facts mean that light is a powerful tool for fluorometric measurements,[32-34] affecting the fluorometric emission intensity, increasing or inhibiting even the native fluorescence. A photo-reactor was prepared with a low-pressure mercury lamp (Sylvana, 15 W, commercially available for germicide use) and PTFE tubing (0.8 mm i.d. and 173 cm) helically coiled around the lamp. The Asulam solution was irradiated during a period of 3 min (this period was obtained by controlling the solution flow-rate). The photo-degradation process was performed at different pH values over the range of 1 to 11 (0.1 mol l^{-1} NaOH or HCl).

The irradiation resulted in a relevant decrease of the native fluorescence of the Asulam.

Effect of buffer solutions. The pH influence on the fluorescence was shown to be an important parameter. For this reason and trying to obtain a system as robust as possible (chemical robustness), different buffers were tested over the pH range of 4.7 to 10.8. The tested buffers were: phosphate, acetic acid–acetate, glycine and ammonia–ammonium. No relevant differences were observed in the fluorescence intensity; however, higher emission was observed with glycine or ammonia–ammonium in the pH range from 8.0 to 10.8. Therefore, ammonia–ammonium (pH 9.0) was selected for further work.

Influence of other chemical parameters: solvent polarity, presence of surfactants and solvent oxygen amount

The presence of organic solvents can affect the fluorescence of Asulam solutions. Different concentrations (1, 10, 20 and 40%) of the following solvents were tested: ethanol, methanol, *iso*-propanol and acetonitrile. All solutions contained 12.5 mg l^{-1} of Asulam. Since no important increase was found with any of the tested solvents (small increases with methanol and *iso*-propanol), their non-use was found to have a simpler chemical system.

A rigid environment often increases the intensity of fluorescence by inhibiting the probability of deactivation of the excited molecule by non-emitting routs. Different solutions

containing surfactants at concentration over the micellar critical concentration and with Asulam 12.5 mg l^{-1} were studied. The tested substances were: hexadecylpyridine (0.22%), *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (0.25%), benzalkonium chloride (0.62%), sodium dodecylsulfate (1.29%), Triton X-100 (0.075%) and β-cyclodextrine (1.30%). No relevant increases in the signals were observed.

The oxygen amount was varied by two different procedures: a) to increase the oxygen amount, 25 ml of the solution was aerated by bubbling air during 30 min; b) to remove oxygen, a solution of the pesticide was put in an ultrasonic bath for 20 min. Fluorescence measurements were compared with the non-pretreated solution. No clear differences were observed.

Flow procedure

Optimization process. The optimization (or re-optimization) of empirical parameters in the continuous-flow system was designed according to the obtained results in batch. The chemical parameters (pH, surfactants, polar solvents, temperature) were optimized using the univariant method. All other FIA variables (volume, length, flow rate) were optimized by using a multivariate strategy: the modified simplex method (MSM).[35–37]

The pH and buffer presence were the first variables to be re-optimized. An assembly was prepared with a mono-channel configuration with pure water circulating at 6.3 ml min^{-1} as the carrier-stream, in which the sample volume was inserted. The resulting sample to be inserted (296 µl) was obtained by merging a solution containing 4 mg l^{-1} of Asulam with a medium solution, both flowing at the same flow rate (mixture at 50%). The tested buffers were: phosphate, tetraborate, glycine and ammonia-ammonium in the pH range over 5.8 to 10.1. The observed results were similar for all tested media (slightly higher with glycine or ammonia-ammonium). Therefore, to avoid the risk of evolution of gas bubbles into the system, a glycine buffer (pH 9.0) was selected for further work.

The effect of surfactants (Tween was added to the list) was similar to the reported results in batch. Such comments can also be applied to the results observed concerning the solvent polarity (dimethylformamide was also checked).

The influence of the temperature was studied by immersing parts of the flow-manifold (carrier line, 150 cm; sample loop, 100 cm) into a water bath at temperatures of 20, 40, 60 and 80˚C. The temperature increase resulted in minor outputs: from 45.73 a.u. (arbitrary units) at 20˚C down to 40.44 a.u. at 80˚C. The results were as expected in most of the systems based on native fluorescence; it is said, the emission decreases through an increased likelihood of deactivation *via* external conversions as the result of an increased frequency of collisions between the molecules.

The hydrodynamic parameters were optimized by using a multiparametric method, known as MSM (Modified Simplex Method), and preparing a simple manifold by removing the confluence of the sample and the medium solutions; a sample formerly adjusted with the required medium (glycine buffer at pH 9.0) was directly prepared and injected into the carrier stream. The empirical procedure involved two simplex series; after the first series the intervals of each parameter were adjusted to the optimum neighbour according to the results observed in the first series. The optimized parameters were the sample volume, the flow-rate of the carrier and the distance from the injection valve to the flow-cell. The limits for each of them (in the same order) were as follows: a) first series, 0 – 100 cm, 300 – 950 a.u., and 22 – 150 cm; b) second series, 65 – 120 cm, 700 – 900 a.u., and 50 – 100 cm. Selected results were: sample volume, 670 µl; carrier flow-rate, 6.5 ml min^{-1}; distance injection valve-flow-cell, 94 cm.

Re-optimization of the pH, from 8.6 to 10.0, with the new flow parameters resulted in no special differences, thus confirming the chemical robustness of the method.

Table 1 Analytical figures of merit for batch and continuous-flow procedures

Parameter	Batch procedure	FIA procedure
Limit of detection/µg l^{-1}	10	5
Lineal interval/mg l^{-1}	0.01 – 3	0.005 – 15
Relation between intensity (I) and concentration (C)/mg l^{-1}	$I = (480 \pm 20)C - (80 \pm 30)$; $r^2 = 0.995$	$I = (530 \pm 40)C - (0 \pm 30)$; $r^2 = 0.997$
Repeatability (DSR), %	1.2 (n = 12; 0.5 mg l^{-1})	1.0 (n = 26; 0.5 mg l^{-1}); 0.8 (n = 26; 4 mg l^{-1})
Sample throughput/h^{-1}	—	78

Analytical figures of merit

Table 1 depicts the obtained figures in sensitivity, repeatability and reproducibility for studied procedures, batch and continuous-flow. The detection limit was established as the minimum concentration of Asulam resulting in an output not minor compared to the average of the blank solution plus three-times the standard deviation. The repeatability was established by a series of 30 consecutive insertions of the same concentration samples.

The influence of interfering substances was studied by preparing solutions containing 0.5 mg l^{-1} of Asulam and the tested compound. The results obtained with the FIA system, as an average of at least four reproducible determinations, were compared with those obtained from pure Asulam solutions of the same concentration; differences of less than 5% were considered not interfering. Potential interferents were considered to be inorganic ions usually present in water samples, and other pesticides chemically related with Asulam. The relative errors are depicted in Table 2 and, as can be observed, no interferences were found.

Both procedures (batch and flow) were applied to the determination of Asulam in spiked water samples; the final Asulam concentration was 0.5 mg l^{-1}. All of the obtained (average of five determinations) results are given in Table 3.

Conclusions

The present study describes a fluorometric determination of Asulam based on the native fluorescence emission of the pesticide. The advantages of the FIA system are rapidity and cost-effectiveness.

The influence of empirical parameters, like the pH, presence of surfactants, solvent polarity or amount of solved oxygen, has been studied and reported in the present paper. The proposed procedure shows competitive sensitivity *vs*. other published procedures (LOD 5 µg l^{-1}). Also, a study on the influence of inorganic interfering compounds demonstrates the required selectivity for many applications.

The flow procedure allows to insert 78 samples h^{-1} and in a wide linear range, up to 15 mg l^{-1} with an RSD of less than 1%.

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Table 2 Influence of foreign substances

Interferent	Conc./mg l^{-1}	Er, %	Interferent	Conc./mg l^{-1}	Er, %
Cation			Anion		
K^+	1000	–0.3	Cl^-	2000	–0.1
Na^+	1000	–0.6	SO_4^{2-}	2000	–2.3
Ca^{2+}	2000	–1.1	CH_3COO^-	2000	+1.9
NH_4^+	200	–1.7	HCO_2^-	2000	+0.6
Mg^{2+}	200	–0.1	$H_2PO_4^-$	2000	+1.7
Ni^{2+}	50	+4.9	NO_3^-	200	+4.7
Zn^{2+}	200	+2.5	I^-	2000	+4.2
Mn^{2+}	200	+2.0			
Pb^{2+}	10	+3.8			
Cd^{2+}	10	–5.1			
Other pesticide					
Ferbam	0.5	–2.1			
Aldicarb	0.5	–2.4			
Karbutilate	0.5	–1.5			
Isoprocarb	0.5	+0.7			
Cycloate	0.5	+0.3			
Fenobucarb	0.5	+2.6			

Table 3 Determination of Asulam in water samples by batch and flow procedures

Water sample	Found batch procedure/ mg l^{-1}	Found flow procedure/ mg l^{-1}
Tap water	0.49 ± 0.03	0.540 ± 0.005
Mineral water	0.500 ± 0.020	0.490 ± 0.020
River water	0.500 ± 0.010	0.525 ± 0.008
Well water	0.48 ± 0.03	0.492 ± 0.005
Irrigation water	0.500 ± 0.006	0.509 ± 0.006

The spiked amount was 0.5 mg l^{-1} of Asulam.

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