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Flow Injection Analysis for the Determination of Thiabendazole and Fuberidazole in Water by Spectrofluorimetry

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Abstract. Flow-injection analysis (FIA) is proposed for determining thiabendazole (TBZ) and fuberidazole (FBZ) by spectrofluorimetry. A pH 2 aqueous solution was found to be the optimal solvent for the rapid, precise and sensitive fluorescence analysis of both fungicides. Linear dynamic graphs were established over a concentration range of two orders of magnitude. Limits of detection were 0.7 ng/ml for TBZ and 0.1 ng/ml for FBZ. Relative standard deviations were 0.5 and 0.8% for TBZ and FBZ, respectively. The method was applied to the determination of both compounds in spiked river and tap water samples, with satisfactory recoveries.

Key words: flow injection, spectrofluorimetry, thiabendazole determination, fuberidazole determination, water analysis.

2-Substituted benzimidazoles are the most important class of benzimidazoles used in agriculture. Fuberidazole (2-(2-furanyl)benzimidazole, FBZ) has been applied as a fungicide [1], as an antihelmintic [2] and against enteroviruses [3]. Residue analysis in plants and soil is usually performed by gas chromatography (GC) [4, 5]. Recently, fluorimetric methods have been described for FBZ determination in stationary solutions; applications to analysis of FBZ in fruits and water have been described [6, 7]. Thiabendazole (2-(4-thiazolyl)-1 *H*-benzimidazole, TBZ) has been widely used as a broad-spectrum anthelmintic agent for domestic animals [8–10] and as a pre- or postharvest fungicide for the control of a wide range of fungi affecting field crops and stored fruits and vegetables [11]. Because TBZ is frequently spread on crops in large quantities [12], it may constitute an important entry route for residues into the human food chain and natural waters. Several analytical methods using fluorescence, spectrophotometry and liquid chromatography (LC) have been reported for quantitation of TBZ in plant and animal products [13–19]. Determination of FBZ in the presence of TBZ and benomyl also has been carried out by GC after derivatization of the fungicides [20].

The high requirements on water purity set by the European Community (0.1 ng/ml for each individual pesticide in drinking water) has resulted in an increasing need to develop sensitive analytical methods for determining pesticides in water matrices. In general, fluorescence spectrometry is considered as a powerful, highly sensitive analytical technique and is widely used for environmental routine analysis; its advantages are its simplicity, rapidity and low detection limits, which fit perfectly the natural conditions in which the environmental impact must be studied. Flow injection analysis (FIA) is a technique well suited to automation, since it possesses the required features of rapid analysis, robustness, simplicity, versatility and minimal operating cost. In recent years the potential and application of FIA for on-line process monitoring have been discussed by several authors [21–25]. Moreover, FIA is a technique that satisfies the requirements to determine noxious species in waters in a fast and cheap way without sacrificing precision.

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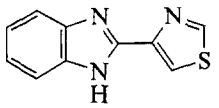
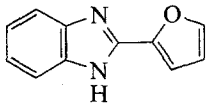


TBZ



FBZ

The aim of this work was to develop a simple, sensitive and rapid procedure for the analysis of fungicide residues in water samples, using a flow-injection system coupled to a spectrofluorimetric detector. Successive passage of the sample through the flow-cell and continuous fluorimetric monitoring of the process provided the analytical information needed to determine TBZ and FBZ. The proposed method allows the automatic on-line monitoring of these compounds in water. In addition, the system is simple and easily assembled.

Experimental

Apparatus

Fluorescence measurements were performed on a Kontron SFM-25 spectrofluorimeter, equipped with a Hellma 18-µl flow-fluorescence cell, and connected to a GEOCOM microcomputer model CPC 1420E. Data acquisition and data analysis were performed by using an SFM data acquisition program.

The manifold of the FI system included an Ismatec IPN-4 peristaltic pump to pump the solution. The aqueous carrier solution was pumped at a flow rate of 2.5 ml/min. The samples (50–300 µl) were injected into the carrier stream by means of a six-way rotary injection valve (Omnifit No. 1106) equipped with a volume control loop. Throughout the system, poly(tetrafluoroethylene) (PTFE) tubing of 0.5 mm i.d. was used.

Reagents

All chemicals were analytical-reagent grade, and deionized water was used throughout. Thiabendazole (2-(4-thiazolyl)-1*H*-benzimidazole, TBZ) was obtained from Merck & Co. (USA) and fuberidazole (2-(2-furanyl)benzimidazole, FBZ) was a gift of Bayer (FRG). Ethanol (Merck) was used for preparing standard solutions of fungicides. Fluka buffer solutions between pH 2 and 11 were used.

Procedure

A stock solution of 200 µg/ml of each fungicide was prepared from the corresponding compound by dissolving it in ethanol, and stored under light-protected conditions in a refrigerator to avoid photodecomposition. Serial dilutions were performed to prepare solutions at selected concentrations, using pH 2 aqueous buffer solutions to give the optimal conditions for measurement of both TBZ and FBZ. All diluted solutions had an ethanol content lower than 4% and 15% for TBZ and FBZ, respectively. These working solutions were utilized for measuring analytical parameters such as the limit of detection (LD), the linear dynamic range (LDR) and the relative standard deviation (RSD).

Table 1. Analytical parameters for the flow-injection fluorimetric determination of fungicides

Analytical parameter	Studied range	TBZ	FBZ
λ_{ex} (nm)[a]	–	299	312
λ_{ex} (nm)[a]	–	347	350
Flow-rate (ml/min)	1.9–4.2	2.5	2.5
Injected volume (µl)	50–300	300	300
Reactor length (cm)	20–200	20	20
Sensitivity[b]	1–10	10	10
Voltage (V)[b]	400–500	500	500

[a] Analytical excitation and emission wavelengths.
[b] Instrumental characteristics.

A 300-µl volume of sample solution containing TBZ or FBZ was injected directly into the solution carrier, which was water in all instances. The solution was carried to the flow cell, and the fluorescence intensity was measured at constant excitation and emission wavelengths (Table 1). Each solution was assayed in triplicate. As many as sixty FIA measurements could be performed per hour. The sensitivity of the spectrofluorimeter was maintained at 10, and a voltage of 500 V was used for the PM tube.

Calibration curves, consisting of plots of fluorescence intensity vs. concentration of each standard, were obtained. These linear dynamic graphs were established over a concentration range of two orders of magnitude for both fungicides studied. Recoveries from water samples (Seine river and tap water) were evaluated by using the standard addition procedure.

Results and Discussion

Preliminary Bulk Solution Studies

In order to select the optimum spectroscopic and physicochemical conditions for fluorescence measurements, a bulk solution procedure was utilized. Fluorescence excitation and emission spectra of TBZ and FBZ were recorded in a pH 2 buffer aqueous solution at room temperature (Fig. 1). Excitation and emission maxima were located at 299 and 347 nm for TBZ and 312 and 350 nm for FBZ. The effect of pH on the fluorescence intensity as well as the stability and photoreactivity of TBZ and FBZ standard solutions were examined.

We found that no significant fluorescence spectral shift occurred for pH values between 1.5 and 11.0. At all pH, excitation and emission maxima were identical to those reported in Fig. 1. As can be seen in Fig. 2, sigmoidal fluorescence intensity (I_F) vs pH curves were found for both compounds, with a much higher I_F value at acidic pH. Therefore, a pH 2.0 aqueous solution was chosen as the optimal medium for analytical studies.

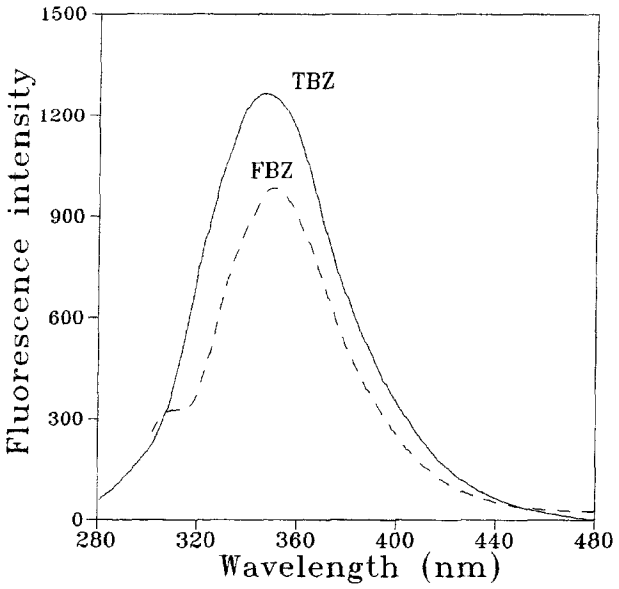


Fig. 1. Fluorescence emission spectra of TBZ ($\lambda_{ex} = 299$ nm) and FBZ ($\lambda_{ex} = 312$ nm) in a pH 2 buffer aqueous solution

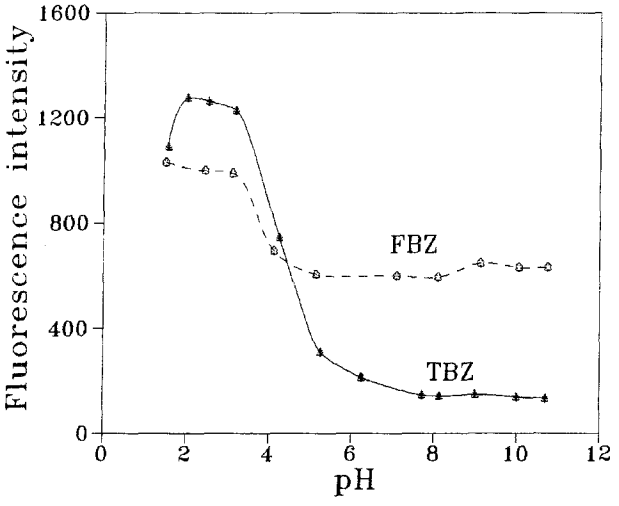


Fig. 2. Effect of pH on the fluorescence intensity of 400 ng/ml TBZ and 16.6 ng/ml FBZ, in aqueous solution

In contrast to our previous photochemical-fluorescence studies of other pesticides [26], no analytically-useful effect of methanol or ethanol percentage on the fluorescence intensity of TBZ and FBZ was found. Consequently, a purely aqueous pH 2.0 medium was selected to carry out the proposed method of determination of both fungicides.

The stability of the TBZ and FBZ ethanolic stock solutions and pH 2.0 aqueous solutions was studied by recording their fluorescence spectra at several periods of time. Ethanolic stock solutions were found to be stable for at least 15 days when stored in the dark in a refrigerator. Dilute aqueous solutions were satisfactorily stable for above 2 h when kept in the dark.

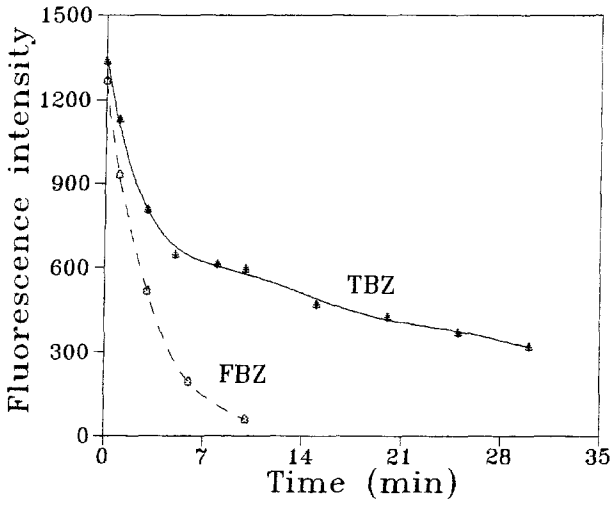


Fig. 3. Effect of the ultraviolet irradiation time on the fluorescence intensity of TBZ (400 ng/ml) and FBZ (160 ng/ml) in aqueous solution

In another work on the fluorescence determination of aromatic pesticides [27] and chlorophenoxyacid herbicides [26], we had found that the sensitivity of the method could be improved by irradiating these compounds under UV light. To determine whether the fluorescence signals of TBZ and FBZ could be increased upon UV irradiation, the photoreactivity of both compounds was investigated in bulk pH 2.0 aqueous solutions. It was observed that the intensities of their fluorescence bands diminished markedly after exposing their corresponding solutions to light from a mercury lamp under the experimental conditions used (Fig. 3). No significant change of the excitation and emission wavelengths occurred upon irradiation. These results confirm the described photochemical behaviour of TBZ and FBZ, which indicate photodegradation of these compounds [28–31].

Effect of FIA Variables

For FIA studies, all fluorescence measurements were carried out at the excitation and emission wavelengths listed for both compounds in Table 1.

The FIA system was optimized by changing each variable in turn while keeping all others unchanged. Thus, the effect of injected volume and flow-rate on the fluorescence signals were examined at concentrations of 108 ng/ml for TBZ and 9.3 ng/ml for FBZ, in order to provide maximum response and minimum band broadening.

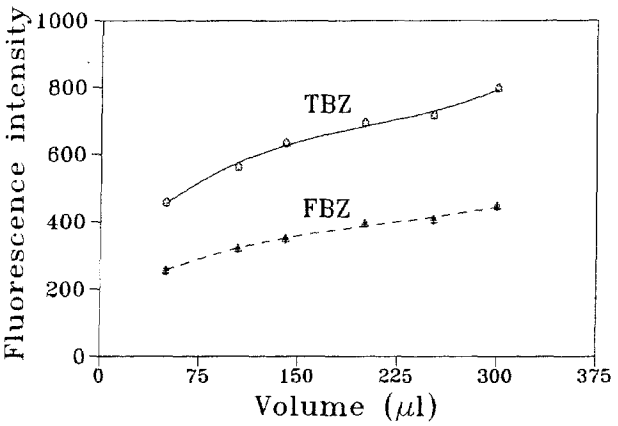


Fig. 4. Effect of sample injection volume on the fluorescence intensity of TBZ and FBZ in the FI system. [TBZ] = 108 ng/ml; [FBZ] = 9.3 ng/ml; flow rate = 2.5 ml/min; reactor length = 20 cm

Table 1 gives the range of investigation of these parameters and the optimum analytical values. The volume injected was varied between 50 and 300 μl. Figure 4 shows that fluorescence intensity increased significantly upon increasing the injected volume. A 300-μl volume was selected as a suitable value for both fungicides.

Contrary to previous FIA photochemical-fluorescence studies on other pesticides [32], no photoreactor was necessary, since the native fluorescence of the fungicides was measured. Therefore, a 20-cm tube (minimum length of reactor required for operating the rotary injection valve) was used. Changes in flow-rate produced a slight variation of fluorescence intensity for both compounds. As is observed in Fig. 5, an increase in the flow-rate led to a decrease in fluorescence signal. For TBZ and FBZ, the optimum flow-rate was 2.5 ml/min. A smaller flow-rate resulted in an increase of the peak broadening.

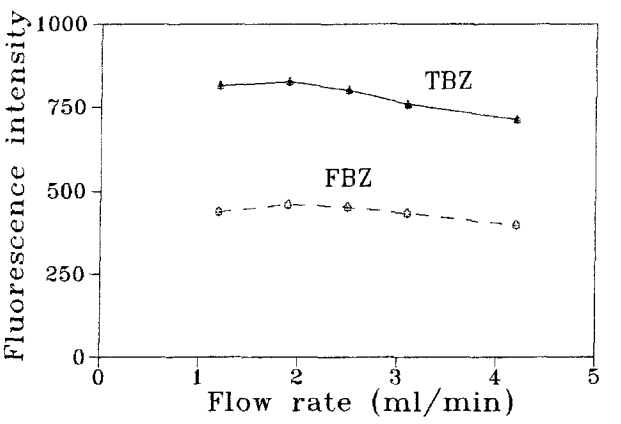


Fig. 5. Effect of flow rate on the fluorescence intensity of TBZ and FBZ in the FI system. [TBZ] = 108 ng/ml; [FBZ] = 9.4 ng/ml; sample volume injection = 300 μl; reactor length = 20 cm

Analytical Figures of Merit

The analytical figures of merit for the determination of both TBZ and FBZ under optimum conditions are presented in Table 2. These figures were obtained from measurements performed for at least ten concentrations of each compound. A typical set of FIA peaks, obtained under optimum conditions, is given for each compound in Fig. 6, showing the reproducibility of the triplicate measurements.

The linearity of typical calibration graphs for these analytes, which cover more than two orders of magnitude, is clearly evidenced by correlation coefficients close to unity.

In order to determine the precision of the recommended procedure, two series of ten standard samples containing respectively 5.6 and 16.2 ng/ml of fuberidazole, and another two series of an equal number of samples containing respectively 24.2 and 121.1 ng/ml of thiabendazole, were prepared and the fluorescence measurement were made according to the proposed method. The relative standard deviations (RSD), are shown in Table 2.

Since the blanks used for the determination of these compounds showed no fluorescence signal, the limit of detection (LD) was calculated on the basis of the variation of the analyte response at low concentrations [33]. Thus, the LD was defined as the concentration giving a fluorescence signal three times the standard deviation of the fluorescence signal for 20 determinations, for a sample containing one of the smallest analyte concentrations. We found LD values of 0.7 ng/ml for TBZ and 0.1 ng/ml for FBZ. These values

Table 2. Analytical figures of merit for the FIA fluorimetric determination of TBZ and FBZ

	TBZ	FBZ
Concentration range (ng/ml)	2–242	0.2–36
Regression equation[a]	$I_F = 7.47c - 2.95$	$I_F = 47.32c + 7.66$
Correlation coefficient	0.9999	0.9998
Limit of detection[b] (ng/ml)	0.7	0.1
RSD (%)[c]	0.5	2.1
RSD (%)[d]	0.5	0.8

[a] I_F = relative fluorescence signal; c = analyte concentration (ng/ml).

[b] Defined as the concentration of analyte giving a signal-to-noise ratio of 3.

[c] RSD = relative standard deviation for a TBZ concentration of 24 ng/ml and an FBZ concentration of 5.6 ng/ml ($n = 10$).

[d] RSD for a TBZ concentration of 121 ng/ml and a FBZ concentration of 16 ng/ml ($n = 10$).

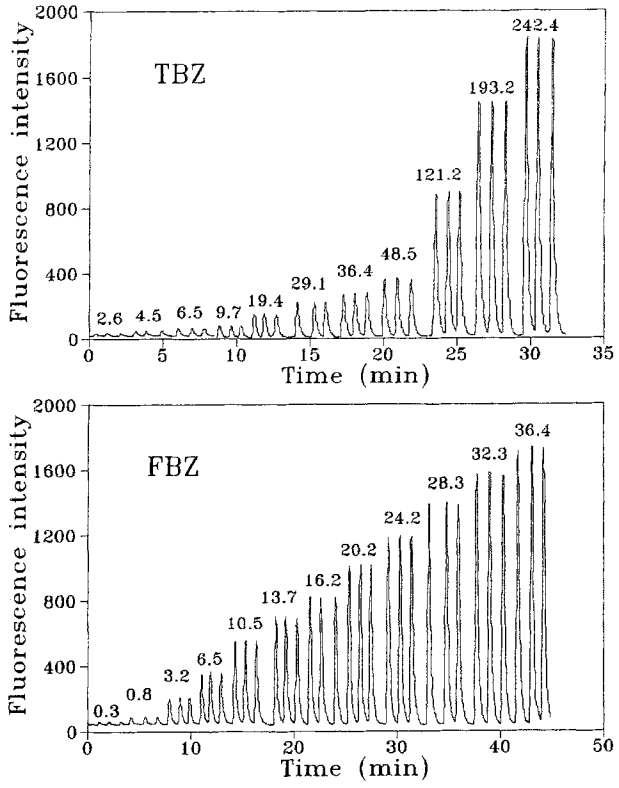


Fig. 6. Typical recorder output for both series of TBZ and FBZ standards under the proposed conditions by FI technique (numbers above the peaks are concentrations in ng/ml)

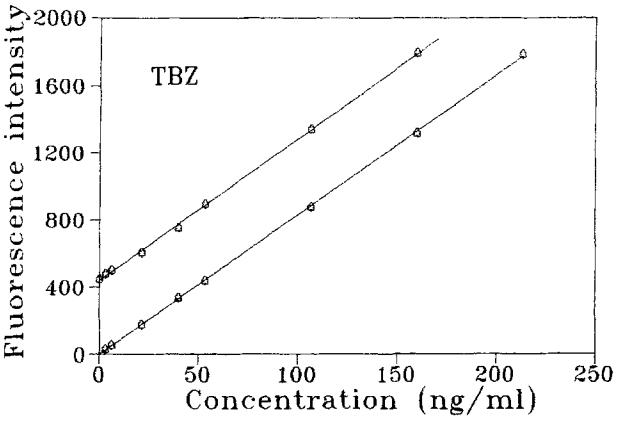


Fig. 7. Standard addition procedure for the determination of TBZ in a river water sample. Lower line: pure sample; upper line: spiked river water

compare favorably with literature values of 1–50 ng/ml for TBZ [13–15] and 0.08–50 ng/ml for FBZ [6, 7] obtained by direct fluorescence or LC methods.

Applications

In order to evaluate the applicability of our method to authentic samples, TBZ and FBZ were determined in river water (Seine river) and tap water samples. The river water samples were filtered on a Whatman filter paper. Both water samples were spiked with a fungicide stock solution (200 µg/ml), since a preliminary study showed that these compounds were initially absent in both river and tap water samples studied.

To estimate the response of the added compounds, the standard addition procedure was used (Fig. 7). The results are summarized in Table 3. As can be seen, satisfactory recoveries were obtained for both real water samples. Moreover, direct measurements based on the appropriate calibration graph, led to similar recovery values for the determination of both TBZ and FBZ. Consequently, river and tap water matrices do not show significant interferences in the proposed FIA method for determining residues of these fungicides.

It should be noted that a preconcentration step might be required for the determination of real samples containing very low levels of fungicides, as is generally the case for most analytical methods.

Conclusions

We can conclude from this study that fungicide residues can be determined in water by using a simple FIA system with spectrofluorimetric detection. The method is rapid, reproducible and sufficiently sensitive for the determination of very low level fungicide residues. The flow-injection analysis technique, which has been developed in this work, allows fast and precise determinations of TBZ and FBZ at low operating cost. The

Table 3. Determination of TBZ and FBZ in real water samples[a]

Type of sample	TBZ			FBZ		
	Added (ng/ml)	Found (ng/ml)	Recovery (%)	Added (ng/ml)	Found (ng/ml)	Recovery (%)
Tap water	49.9	51.9	104.0	10.9	11.1	101.8
Seine river	49.9	52.3	104.8	10.9	11.7	107.3

[a] Triplicate measurements with RSD ca. 1% for TBZ and ca. 3% for FBZ.

proposed method should be suitable for automated use in a routine monitoring system for fungicide residues in water.

The similarity of analytical emission wavelengths (347 and 350 nm) for TBZ and FBZ means that it is impractical to determine one in the presence of the other with this set-up. Derivative and/or synchronous fluorescence spectra may enable them to be resolved.

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References

[1] J. F. Jenkyn, R. D. Prew, *Ann. Appl. Biol.* **1974**, *75*, 241; *Chem. Abstr.* **1974**; *81*, 488u.
[2] Merck and Co. Inc. (Inv. J. J. Netta, J. R. Egerton) U. S. 3.549.754 1970; *Chem. Abstr.* **1971**, *74*, 146378d.
[3] I. Tamm, H. J. Eggers, R. Bablanian, A. F. Wagner, K. Folkers, *Nature* **1969**, *223*, 785.
[4] W. Specht, M. Tillkes, *Pflanzenschutz-Nachr.* **1980**, *33(1)*, 61.
[5] R. Brennecke, K. Vogeler, *Pflanzenschutz-Nachr.* **1984**, *37(1)*, 46.
[6] F. Garcia Sanchez, A. Ramos, V. Cerda, M. T. Oms, *Anal. Chim. Acta* **1990**, *228*, 293.
[7] F. Garcia Sanchez, A. Aguilar Gallardo, *Microchem. J.* **1994**, *50* 161.
[8] M. Sittig, *Veterinary Drug Manufacturing Encyclopedia*, Noyes Publications, Park Ridge, NJ, 1981, pp. 456-457.
[9] C. E. Lanusse, R. K. Prichard, *Drug Metab. Rev.* **1993**, *25*, 235.
[10] R. K. Prichard, S. Ranjan, *Vet. Parasitol.* **1993**, *46* 113.
[11] *Farm Chemicals Handbook*, Meister Willoughby, OH, 1993, C339.
[12] E. Papadopoulou-Mourkidou, *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 745.
[13] R. V. Arenas, N. A. Johnson, *JAOAC Int.* **1994**, *77*, 710.
[14] R. J. Bushway, L. Li, L. R. Paradis, L. B. Perkins, *J. AOAC Int.* **1995**, *78*, 815.
[15] R. V. Arenas, N. A. Johnson, *JAOAC Int.* **1995**, *78*, 642.
[16] N. Motohashi, H. Nagashima, R. Meyer, *J. Liq. Chromatogr.* **1990**, *13*, 345.
[17] D. M. Gilvydis, S. M. Walters, *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 753.
[18] N. Motohashi, H. Nagashima, R. Meyer, *J. Liq. Chromatogr.* **1991**, *14*, 3591.
[19] L. W. LeVan, C. J. Barnes, *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 487.
[20] L. Ogierman, *J. Chromatogr.* **1981**, *210*, 83.
[21] J. Ruzicka, *Anal. Chim. Acta* **1986**, *190*, 155.
[22] M. Gisin, C. Thommen, *Anal. Chim. Acta* **1986**, *190*, 165.
[23] R. L. Benson, P. J. Worsfold, F. W. Sweeting, *Anal. Proc.* **1989**, *26*, 385.
[24] M. D. Luque de Castro, *Talanta* **1989**, *36*, 591.
[25] K. N. Andrew, N. J. Blundell, D. Price, P. J. Worsfold, *Anal. Chem.* **1994**, *66*, A916.
[26] S. A. Eremin, B. Laassis, J. J. Aaron, *Talanta* **1996**, *43*, 295.
[27] A. Coly, J. J. Aaron, *Analyst* **1994**, *119*, 1205.
[28] M. J. Melo, F. Pina, A. L. Macanita, E. C. Melo, C. Herrmann, R. Förster, H. Koch, H. Wamhoff, *Z. Naturforsch.* **1992**, *47B*, 1431.
[29] M. J. Melo, A. Macanita, E. Melo, H. Wamhoff, F. Pina, *J. Photochem. Photobiol. A: Chem.* **1994**, *83*, 237.
[30] T. A. Jacob, J. R. Carlin, R. W. Walker, F. J. Wolf, W. J. A. Vanden-Heuvel, *J. Agric. Food Chem.* **1975**, *23*, 704.
[31] G. Crank, A. Mursyidi, *Aust. J. Chem.* **1982**, *35*, 775.
[32] A. Coly, J. J. Aaron, *Analusis* **1996**, *24*, 107.
[33] J. A. Glaser, D. L. Foerdt, G. d. Mckee, S. A. Quave, W. L. Budde, *Environ. Sci. Technol.* **1981**, *15*, 1426.

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