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ARTICLE

Determination of Amitrole in Environmental Water Samples with Precolumn Derivatization by High-Performance Liquid Chromatography

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Amitrole is a nonselective polar herbicide that can easily pollute ground and surface waters because of its high solubility in water. A precolumn derivatization high-performance liquid chromatographic method for amitrole analysis has been developed. Derivatization of amitrole was performed with 4-chloro-3,5-dinitrobenzotrifluoride (CNBF). The derivatization conditions and the influence of elution composition on the separation were investigated. In pH 9.5 $H_3BO_3-Na_2B_4O_7$ media, the reaction of amitrole with CNBF was complete at 60 °C after 30 min. The stability of the derivative under light irradiation and room temperature in methanol−water samples was demonstrated. The derivatized amitrole was separated on a K C_{18} column (250 mm × 4.6 mm, 5 μm) at room temperature, and UV detection was applied at 360 nm. The separation of derivatized amitrole was achieved within 18 min by gradient elution mode. The method correlation coefficient was 0.9995, in concentrations ranging from 1.59 to 318 mg L^{-1}. The detection limit of amitrole was 0.16 mg L^{-1} with a signal-to-noise ratio of 3. The proposed method was applied to the quantitative determination of amitrole in environmental water with recoveries of 92.0−103.0% and RSDs of 2.22−6.26, depending on the sample investigated. This method has good accuracy and repeatability that can be used to quantify amitrole in environmental water.

KEYWORDS: Amitrole; precolumn derivatization; 4-chloro-3,5-dinitrobenzotrifluoride (CNBF); high-performance liquid chromatography

INTRODUCTION

The intensive use of pesticides in recent years has increased agricultural productivity, but at the same time it has generated pesticide residues in natural waters at levels that exceed the legal limits and have caused high-risk problems in environment and food safety. Cases of incidental pesticide pollution of water reservoirs have become more numerous in recent years. Amitrole (3-amino-1,2,4-triazole) is a nonselective herbicide used for the control of a wide range of weeds (*1*, *2*). This herbicide, although potentially carcinogenic, is of low toxicity to mammals. Because of the good solubility of amitrole in water, leaching may occur and can lead to polluted ground and surface waters under certain conditions such as sandy soils with a low content of organic carbon and a high water level (*3*−*5*). Because of its low volatility, losses by photodecomposition or volatilization are negligible (*6*). The low volatility and high solubility of this herbicide residue in water indicate a possible food contamination through plants, fruits, and water media (*7*). Analytical procedures for the determination of amitrole are complicated due to the high polarity and good water solubility of the herbicide. In this actual situation, a rapid and reliable method for the determination of amitrole in environmental samples is therefore a must for this research.

Analytical methods for the analysis of amitrole include thin-layer chromatography (TLC) (*8*), capillary electrophoresis (CE) (*9*−*11*), gas chromatography (GC) (*12*−*14*), and liquid chromatography (LC) after derivatization (*15*−*17*). Because of amitrole's suitability for aqueous samples, HPLC is the analytical technique of choice for polar compounds. However, for LC with conventional detection systems, such as UV−vis or fluorescence detectors, amitrole needs to be derivatized because of the lack of chromophore or fluorophore. For these reasons, chemical derivatization or labeling becomes a necessary procedure to transform the analytes into derivatives that can be more easily isolated, separated, and detected (*18*).

4-Chloro-3,5-dinitrobenzotrifluoride (CNBF) is a common derivatization reagent, which has been known to react with primary or secondary amines in the presence of base to produce stable *N*-substituted-2,6-dinitro-4-(trifluoromethyl)benzamine derivatives, which display satisfactory ultraviolet absorption (*19*−*22*). In the present work, we proposed a simple, sensitive, and selective HPLC method for the determination of amitrole by precolumn derivatization with CNBF. The derivatization process was fast and required minimal consumption of solvents. The detection limit was 0.16 mg L^{-1}, which was comparable to or better than that reported for existing detection methods. The proposed method was applied to the determination of amitrole in environmental water. To the best of our knowledge there is no method

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available describing the HPLC detection of amitrole with CNBF derivatization.

MATERIALS AND METHODS

Instrumentation and Conditions. A high-performance liquid chromatography system, which consisted of two LC-10ATvp pumps and a SPD-10Avp ultraviolet detector (Shimadzu), was used for analysis and separation. A reversed-phase K ODS C_{18} column (250 mm × 4.6 mm i.d., particle size = 5 μm) was used for separation at ambient temperature, and a Chromato Solution Light Chemstation for LC system was employed to acquire and process chromatographic data.

Chemicals and Reagents. Amitrole was purchased from Hangzhou Fude Chemical Co., Ltd. (Zhejiang, China). A standard solution of 0.01 mol L^{-1} amitrole was prepared in water and further diluted to the required concentration when used. Working standard was prepared by mixing an aliquot of the stock solution and water. The stock and working standard were stored under dark conditions at 4 °C when not in use. Acetonitrile and methanol were of HPLC grade and purchased from J. T. Baker (Phillipsburg, NJ). Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA). CNBF was obtained from Alfa Aesar (Ward Hill, MA), and its solution was prepared in methanol and filtered through a 0.45 μm nylon membrane filter and refrigerated when not in use. Cetyltrimethylammonium bromide (CTAB) was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). All other chemicals and solvents were of analytical grade and from commercial sources. H_3BO_3−$Na_2B_4O_7$ buffer was prepared by mixing 0.2 mol L^{-1} H_3BO_3 solution with 0.05 mol L^{-1} $Na_2B_4O_7$ solution to the required pH value. The phosphate buffer was prepared by dissolving K_2HPO_4 in water, and then the pH was adjusted to the required value by adding concentrated H_3PO_4.

Chromatographic Method. Before the analysis, the C_{18} column equipped with a guard column (4 mm × 3 mm i.d.) was pre-equilibrated with the mobile phase for 30 min. HPLC separation of amitrole derivative was carried out on the K ODS C_{18} column. Acetonitrile−0.01 M CTAB solution (5:5, v/v, eluent A) and phosphate buffer (pH 4.5 with phosphoric acid) (eluent B) were used as mobile phases. All of the solvents were filtered with a 0.45 μm membrane filter. The program was set for a linear gradient starting from 40% of solvent A to 90% of the solvent at 15 min. The injection volume was 20 μL, and the detection wavelength, 360 nm. The flow rate was constant at 0.8 mL min^{-1}, and the column was at room temperature.

Derivatization Procedure. To a 1.0 mL vial containing an appropriate amount of amitrole solution were added 300 μL of H_3BO_3−$Na_2B_4O_7$ buffer (pH 9.5) and 100 μL of CNBF methanol solution. After the whole solution was diluted to 1.0 mL with water, it was incubated at 60 °C for 30 min, and 2 M HCl (10 μL) was added to quench the reaction. The resulting solutions were filtered through 0.45 μm nylon filters and injected in the chromatographic system. Each sample was assayed in triplicate, and all of the assays were carried out at ambient temperature.

Analysis of Real Samples. Environmental water samples were centrifuged at 4000 rpm for 10 min to remove solid particles and then the supernatant was filtered through a 0.45 μm membrane filter before analysis. An aliquot of 1 mL of samples or 1 mL of amitrole standards was transferred to a vial, adjusted to pH 9.5 with reaction buffer, and water was added to 3 mL. After thorough mixing on a vortex mixer, 2 mL of CNBF solution was added and mixed again. The mixture was heated in a water bath for 30 min at 60 °C, shaking by a vortex mixer at 10 and 20 min. The reaction was quenched by the addition of 50 μL of 2 M HCl. The resulting solution was filtered through 0.45 μm nylon filters and injected in the chromatographic system.

RESULT AND DISCUSSION

Optimization of Derivatization Conditions. The reaction of CNBF with the amino group on the amitrole molecule is represented in **Figure 1**. CNBF is known to have good activity and selectivity for amino compounds and to be employed as an excellent active group. It can react with amines in low concentration to form stable derivatives under basic conditions, and the excess reagent is hydrolyzed to the corresponding phenol.

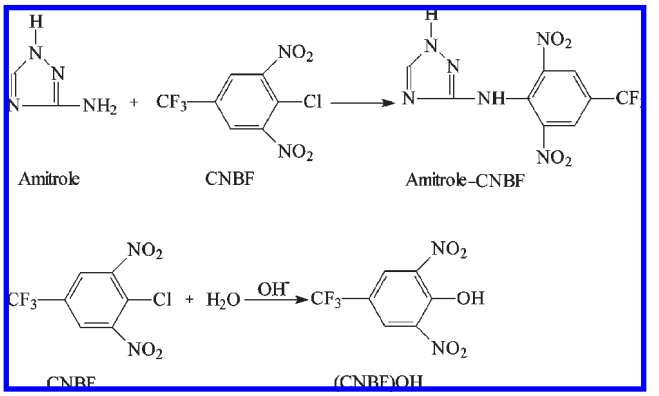

Figure 1. Reaction scheme of CNBF with amino group on amitrole molecule and H_2O.

The hydrolysis compound can be written as (CNBF)OH. Because CNBF has relatively poor solubility in water, organic solvent should be added to the derivatization medium to avoid the precipitation of the reagent and derivative. Therefore, at least 100 μL of methanol should be added to the derivatization medium. There is a competition between the labeling and the hydrolysis, so excess labeling reagent should be used.

The effect of amitrole/CNBF ratio, derivatization pH value, derivatization reaction temperature, and derivatization reaction time on the peak area of amitrole−CNBF derivative is presented in **Table 1**. First, the influence of the amount of reagent on the derivatization was investigated. An aliquot of amitrole was reacted with various concentrations of CNBF (1.0×10^{-3}, 2.0×10^{-3}, 3.0×10^{-3}, and 4.0×10^{-3} mol L^{-1}). The results showed that the peak areas of derivative are highest and unchangeable when the concentration of reagent reached 3.0×10^{-3} mol L^{-1}, and there was not a statistically significant difference between 3.0×10^{-3} and 4.0×10^{-3} mol L^{-1}. Therefore, 3.0×10^{-3} mol L^{-1} was selected as the optimal concentration. The reaction of CNBF with amitrole was also found to be pH dependent. The influence of various pH values on the peak area was also studied by using borate buffer. The optimum reaction pH was determined by derivatizing amitrole at pH values ranging from 7.5 to 11.0. The results showed that the peak areas of the derivative were almost stable at pH 8.5−11.0. This was probably due to deprotonation of amitrole at the basic condition, which can promote the nucleophilic addition, as observed in the case of aliphatic diamines (*23*). Also, as the pH rose, the peak areas of CNBF−OH increased more quickly than those of amitrole−CNBF. Hence, an optimum derivatization pH of 9.5 was selected for all subsequent experiments. Temperature is a very important factor in optimizing the derivatization rate. Therefore, the values ranging from 40 to 70 °C were performed to find the best derivative temperature. It was found that peak areas of the derivative reached a plateau at 60 °C. The reaction time was a critical factor for the derivatization reaction. The effect of reaction time on derivatization was studied over the period from 10 to 40 min while all other parameters were kept constant. It was clear that peak areas reached an optimum value over a period of 30−40 min. To keep the total analysis time short, a reaction time of 30 min was chosen.

Optimization of Separation Conditions. The mobile phase composition was optimized to achieve fast and optimum separation of amitrole derivative, CNBF, and (CNBF)OH. Chromatographic separations were carried out under gradient reversed-phase condition on the K C_{18} column. Acetonitrile−CTAB solution (5:5, v/v, eluent A) and phosphate buffer (eluent B) were used as mobile phases. The retention time of

Table 1. Effect of Amitrole/CNBF Ratio, Derivatization pH Value, Derivatization Reaction Temperature, and Derivatization Reaction Time on the Peak Area of Amitrole−CNBF Derivative

amitrole/CNBF ratio[a]	peak area ($\times 10^4$)	pH	peak area ($\times 10^4$)	temperature (°C)	peak area ($\times 10^4$)	time (min)	peak area ($\times 10^4$)
1:1	8.6218	7.5	8.2856	40	7.3563	10	7.4243
1:2	8.6761	8.5	8.5237	50	8.5852	20	8.6659
1:3	8.7163	10.0	8.7162	60	8.7148	30	8.7183
1:4	8.7171	11.0	8.7156	70	8.7166	40	8.7206

[a] The amitrole concentration used was 1.0×10^{-3} mol L^{-1}; the CNBF concentration selected was 3×10^{-3} mol L^{-1} for the effect of pH, temperature, and reaction time on the peak areas of amitrole−CNBF derivative. Each sample was determined in triplicate injections.

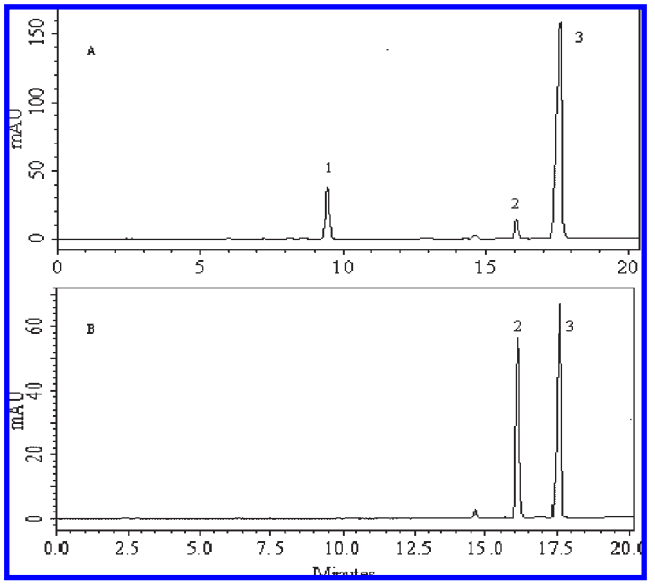

Figure 2. HPLC chromatogram of amitrole (50 mg L^{-1}) standard solution after derivatization (**A**) and CNBF hydrolysis without amitrole (**B**). Peaks: 1, amitrole−CNBF; 2, CNBF; 3, (CNBF)OH.

derivative was 2.85 min without CTAB in mobile phase; the derivative could not be separated completely with some interferences. To reduce the interferences, CTAB was used as ion-pairing reagent to delay the retention time of derivative and the effect of CTAB concentration was also studied. It was found that appropriate addition of CTAB (10 mM) could improve separation efficiency and the peak shape of amitrole; a higher concentration of CTAB caused the delay of retention time with peak broadening. In this method, 10 mM CTAB was used in subsequent experiments. The program was set for a linear gradient starting from 40% of solvent A to 90% of the solvent at 15 min, which gave the best separation within the shortest analysis time. The pH value of buffer in the mobile phase was studied. In this experiment, the retention time of derivative had no obvious change as the pH value varied from 3.0 to 6.0 and the peak areas of derivative also indicated that CNBF derivative was pH-insensitive and stable. In this experiment, pH 4.5 was used. The chromatograms of amitrole derivative with CNBF and CNBF hydrolysis obtained in gradient elution mode are shown in **Figure 2**.

Stability of the Derivatives. The stability of amitrole derivative with CNBF in methanol−water (1:9, v/v) at room temperature was investigated over 4 days without light irradiation. No significant change in peak area of the derivatives was found. Derivative in methanol−water (1:9, v/v) also showed no significant change in the absolute peak areas when exposed to ordinary light from a 100 W bulb for about 24 h. It appeared that the derivative of amitrole was very stable, as evidenced by the fact that the derivative showed $< 5\%$ degradation when analyzed by HPLC after 7 days of standing at room temperature.

Table 2. Linear Calibration Range, Regression Equation, and Detection Limit of Amitrole

parameter	amitrole
calibration range (mg L^{-1})	1.59−318
regression equation,[a] Y	$2736X + 165$
coefficient regression, R^2	0.9995
RSD (%), $n = 6$, within-day	2.86
RSD (%), $n = 6$, between-day	3.78
detection limit[b] (mg L^{-1})	0.16

[a] X = concentration of amitrole (mg L^{-1}); Y = peak area of amitrole derivative. [b] S/N = 3, per 20 μL injection volume.

Validation of the Method. Test solutions with different concentrations in the range of 1.59−318 mg L^{-1} of standard amitrole were prepared and analyzed by using the optimized derivatization procedure and separation conditions for the determination of amitrole. The peak areas of the standards were recorded. The slope and intercept of the calibration graph were obtained by linear regression of peak area versus concentration: $y = ax + b$, where a is the slope, b is the intercept, x is the concentration, and y is the peak area. The coefficient regression (R^2) is 0.9995. The relative standard deviations (RSDs) of both within-day and between-day were calculated. The detection limit of amitrole was calculated as the amount of amitrole that resulted in a peak 3 times higher than that of the baseline noise. The linear calibration range, regression equation, and detection limit of amitrole were calculated, and the results are listed in **Table 2**. The detection limit for amitrole was 0.16 mg L^{-1}. It was shown that the quantification of amitrole could be done well with this method. Several RP-HPLC methods for amitrole analysis were developed using precolumn derivatization and either fluorescence or UV detection (*15*−*17*). Dugay and Hennion (*15*) diazotated amitrole in the native aqueous sample and were able to separate the derivative with HPLC, reaching a detection limit of 1.0 mg L^{-1} with UV detection. García Sánchez et al. (*16*) derivatized amitrole with fluorescamine and analyzed the samples with RP-HPLC using fluorescence detection. The best achieved detection limit was 0.375 mg L^{-1}. Bobeldijk et al. (*17*) developed a method for the determination of amitrole in water by precolumn derivatization, liquid chromatography, and tandem mass spectrometry with 9-fluorenylmethyl chloroformate (FMOC-Cl) as labeling reagent. The calculated LOD reached 0.025 mg L^{-1}. By comparison of methods for determination of amitrole given in the literature, the detection limits of a few reagents were higher than that of CNBF, except FMOC-Cl using fluorescence and mass spectrometry. Considering the detection properties (such as detection wavelength, derivatization time, and temperature, and detection limits) in the determination of amitrole, CNBF was more advantageous than other reagents.

Application to Sample Analysis. The applicability of the proposed method was evaluated in environmental water samples. Amitrole was identified by adding standard to the samples. The results obtained from the analysis of samples are shown in **Table 3**.

Table 3. Average Recovery of Amitrole from Water Samples by Using Proposed Method

	tap water[a]		pond water[b]		river water[c]	
amitrole added (mg L^{-1})	amitrole found (mg L^{-1})	recovery (%) ± RSD [d]	amitrole found (mg L^{-1}	recovery (%) ± RSD [d]	amitrole found (mg L^{-1}	recovery (%) ± RSD [d]
2.0	1.88	94.0 ± 2.56	1.84	92.0 ± 4.16	2.06	103.0 ± 5.96
5.0	4.96	99.2 ± 6.26	4.93	98.6 ± 3.63	5.12	102.4 ± 3.56
10.0	9.80	98.0 ± 4.61	10.02	100.2 ± 3.89	9.91	99.1 ± 3.24
20.0	19.68	98.4 ± 2.65	19.56	97.8 ± 3.16	19.62	98.1 ± 3.51
50.0	49.50	99.0 ± 2.22	49.46	98.9 ± 2.34	49.38	98.7 ± 2.42

[a] Collected from China Agricultural University, Beijing, China. [b] Collected from Shangzhuang, Haidian District, Beijing, China. [c] Collected from Xiaojiahe River, Haidian Distric, Beijing, China. [d] Mean value of six determinations.

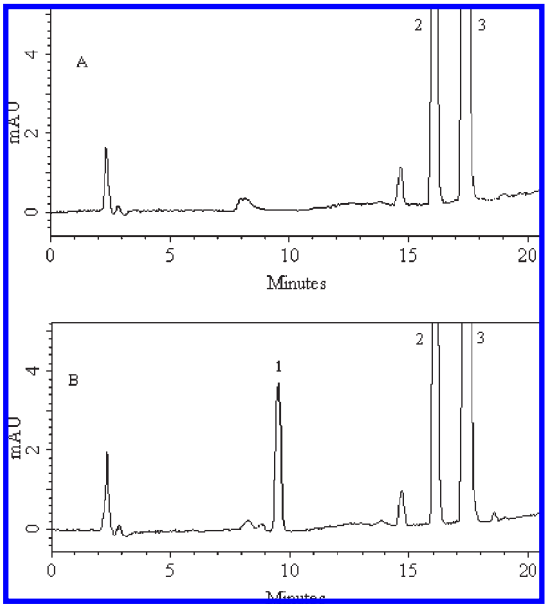

Figure 3. Chromatograms obtained from (**A**) river water and (**B**) river water spiked with 5 mg L^{-1} of standard amitrole. Peaks: 1, amitrole–CNBF; 2, CNBF; 3, (CNBF)OH.

The chromatograms of sample and spiked standard are presented in **Figure 3**. The recoveries of amitrole were from 92.0 to 103.0% and RSDs from 2.22 to 6.26, depending on the sample investigated.

A method for the detection of amitrole in different types of water was developed in this study. The application of CNBF as a derivatizing reagent seemed to be an attractive choice for the determination of amitrole with HPLC and had a good performance in tap, pond, and river waters. The reaction of CNBF with amitrole lead to a stable derivative. The proposed method showed good repeatability (better than 6.26% in all matrices), low detection limit (0.16 mg L^{-1}), and excellent linearity (0.9995) that could be used as the quantification method for amitrole in environmental water.

LITERATURE CITED

(1) Fontecha-Cámara, M. A.; López-Ramón, M. V.; Pastrana-Martínez, L. M.; Moreno-Castilla, C. Kinetics of diuron and amitrole adsorption from aqueous solution on activated carbons. *J. Hazard. Mater.* **2008**, *156*, 472–477.

(2) Nader, S.; Swanton, C. J.; Hamill, A. S.; Vyn, J. D.; Sikkema, P. H. Effect of amitrole and 2,4-D applied preplant and pre-emergence in soybean (*Glycine max*). *Weed Biol. Manag.* **2008**, *8*, 139–144.

(3) Fontecha-Cámara, M. A.; López-Ramón, M. V.; Álvarez-Merino, M. A.; Moreno-Castilla, C. Effect of surface chemistry, solution pH and ionic strength on removal of herbicides diuron and amitrole from water by an activated carbon fiber. *Langmuir* **2007**, *23*, 1242–1247.

(4) Scholz, K.; Spiteller, M. *Seventh International Congress of Pesticides Chemistry*, Hamburg, 1990.

(5) López-Ramón, M. V.; Fontecha-Cámara, M. A.; Álvarez-Merino, M. A.; Moreno-Castilla, C. Removal of diuron and amitrole from water under static and dynamic conditions using activated carbons in form of fibers, cloth, and grains. *Water Res.* **2007**, *41*, 2865–2870.

(6) Jensen-Korte, U.; Anderson, C.; Spiteller, M. Photodegradation of pesticides in the presence of humic substances. *Sci. Total Environ.* **1987**, *62*, 47–54.

(7) Oesterreich, T.; Klaus, U.; Volk, M.; Neidhart, B.; Spiteller, M. Environmental fate of amitrole: influence of dissolved organic matter. *Chemosphere* **1999**, *38*, 379–392.

(8) Pribyl, J.; Herzel, F.; Schmidt, G. Beitrag zur Ruckstandsanalytik des Aminotriazols. *Fresenius' Z. Anal. Chem.* **1978**, *289*, 81–85.

(9) Chicharro, M.; Moreno, M.; Bermejo, E.; Ongay, S.; Zapardiel, A. Determination of amitrole and urazole in water samples by capillary zone electrophoresis using simultaneous UV and amperometrical detection. *J. Chromatogr., A* **2005**, *1099*, 191–197.

(10) Takeda, S.; Fukushi, K.; Chayama, K.; Nakayama, Y.; Tanaka, Y.; Wakida, S. Simultaneous separation and on-line concentration of amitrole and benzimidazole pesticides by capillary electrophoresis with a volatile migration buffer applicable to mass spectrometric detection. *J. Chromatogr., A* **2004**, *1051*, 297–301.

(11) Chicharro, M.; Zapardiel, A.; Bermejo, E.; Moreno, M. Determination of 3-amino-1,2,4-triazole (amitrole) in environmental waters by capillary electrophoresis. *Talanta* **2003**, *59*, 37–45.

(12) Achiraman, S.; Archunan, G. Characterization of urinary volatiles in Swiss male mice (*Mus musculus*): bioassay of identified compounds. *J. Biosci.* **2002**, *27*, 679–686.

(13) Dzygiel, A.; Masiukiewicz, E.; Rzeszotarska, B. Acetylation of 5-amino-1*H*-[1,2,4]triazole revisited. *J. Agric. Food Chem.* **2002**, *50*, 1383–1388.

(14) Pepich, B. V.; Prakash, B.; Domino, M. M.; Dattilio, T. A.; Munch, D. J.; Price, E. K. Development of U.S. EPA method 527 for the analysis of selected pesticides and flame retardants in the UCMR survey. *Environ. Sci. Technol.* **2005**, *39*, 4996–5004.

(15) Dugay, J.; Hennion, M. C. Evaluation of the performance of analytical procedures for the trace-level determination of aminotriazole in drinking waters. *Trends Anal. Chem.* **1995**, *14*, 407–414.

(16) García Sánchez, F.; Navas Díaz, A.; García Pareja, A.; Bracho, V. Liquid chromatographic determination of asulam and amitrole with pre-column derivatization. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20*, 603–615.

(17) Bobeldijk, I.; Broess, K.; Speksnijder, P.; van Leerdam, T. Determination of the herbicide amitrole in water with pre-column derivatization, liquid chromatography and tandem mass spectrometry. *J. Chromatogr., A* **2001**, *938*, 15–22.

(18) Sahasrabuddhey, B.; Jain, A.; Verma, K. K. Determination of ammonia and aliphatic amines in environmental aqueous samples utilizing pre-column derivatization to their phenylthioureas and high performance liquid chromatography. *Analyst* **1999**, *124*, 1017–1021.

(19) Callahan, H. L.; Kelley, C.; Pereira, T.; Grogl, M. Microtubule inhibitors: structure–activity analyses suggest rational models to identify potentially active compounds. *Antimicrob. Agents Chemother.* **1996**, *40*, 947–952.

(20) Pitzer, K. K.; Werbovetz, K. A.; Brendle, J. J.; Scovill, J. P. Synthesis and biological evaluation of 4-chloro-3,5-dinitrobenzotrifluoride

analogues as antileishmanial agents. *J. Med. Chem.* **1998**, *41*, 4885–4889.

(21) Tang, T.; Shi, T. Y.; Qian, K.; Li, P. L.; Li, J. Q.; Cao, Y. S. Determination of biogenic amines in beer with pre-column derivatization by high performance liquid chromatography. *J. Chromatogr., B* **2009**, *877*, 507–512.

(22) Qian, K.; Tang, T.; Shi, T. Y.; Wang, F.; Li, J. Q.; Cao, Y. S. Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3,5-dinitrobenzotrifluoride. *Anal. Chim. Acta* **2009**, *635*, 222–226.

(23) Zhang, L. Y.; Liu, Y. M.; Wang, Z. L.; Cheng, J. K. Capillary zone electrophoresis with pre-column NDA derivatization and amperometric detection for the analysis of four aliphatic diamines. *Anal. Chim. Acta* **2004**, *508*, 141–145.

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