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A Nanoparticle-based Solid Phase Extraction Method for Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometric Analysis

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Abstract

A solid-phase extraction (SPE) procedure with the use of superparamagnetic Fe₃O₄ nanoparticles as extracting agent was developed for HPLC-ESI-MS/MS analysis. Four most heavily used triazine pesticides (herbicides) were taken as the test compounds. The NPs showed an excellent capability to retain the compounds tested, and a quantitative extraction was achieved within 10 min under the testing conditions, i.e. 100 µL NP solution was added to 400 mL sample in a beaker with stirring. After extraction, the superparamagnetic NPs were easily collected by using an external magnet. Very importantly, analytes retained on the Fe₃O₄ NPs could be quantitatively recovered by dissolving the NPs with an HCl solution, allowing subsequent HPLC-ESI-MS/MS quantification. A capillary HPLC-ESI-MS/MS method with the present NP-based SPE procedure was developed for the determination of triazines including atrazine, prometryn, terbutryn, and propazine. Atrazine-d₅ was used as internal standard. The method had an LOD of 10 pg/mL atrazine, and a linear calibration curve over a range from 30 pg – 50.0 ng/mL. Simultaneous determination of the four triazine pesticides in water samples taken from local lakes was demonstrated.

Keywords

Fe₃O₄ nanoparticles; Solid-phase extraction; HPLC-ESI-MS/MS; Atrazine; Propazine; Triazine pesticides

1. Introduction

Use of nanometer-sized particles (nanoparticles, NPs) for sample extraction in chemical analysis is gaining research interest [1–10]. Compared with micrometer-sized particles used for solid phase extraction (SPE), NPs offer a significantly higher surface area-to-volume ratio that promises much greater extraction capacity and efficiency [11–12]. Another advantage of NPs is that NPs' surface functionality can be easily modified to achieve selective sample extraction or cell collection [13–14]. From the finding that neutral and modified oligonucleotides exhibited a strong affinity to cationic polystyrene NPs mediated by the combination of hydrophobic and multiple electrostatic interactions, a NP-based SPE method was developed to extract oligonucleotides from human plasma samples [1]. In the procedure,

centrifugation at 24000 g was applied to collect the NPs for subsequent analyte elution. C₁₈-functionalized silica NPs [2] and gold NPs with a chemically modified surface [6–7] were used for enriching/extracting peptides followed by MALDI-MS analysis. In these NP-based extraction procedures, the NPs were collected after extraction either by centrifugation or by filtration, which would be difficult, if not impossible to do in some cases such as with large volume samples.

Fe₃O₄ NPs are the most popular nanomaterials used for immobilizing proteins, enzymes, and other bioactive agents in analytical biochemistry, medical research, and biotechnology [15–16]. These NPs are superparamagnetic, i.e. they are attracted to a magnetic field but retain no residual magnetism after the field is removed. This property makes them particularly suitable for sample extraction in chemical analysis because no centrifugation or filtration of the sample is needed after the treatment. Superparamagnetic NPs can be easily collected by using an external magnetic field placed outside of the extraction container. Actually, Fe₃O₄ NPs have been applied to sample extraction. Vancomycin-bound Fe₃O₄ NPs were employed as affinity agent to selectively trap Gram-positive pathogens from urine samples [3]. Fe₃O₄/TiO₂ core/shell NPs were used as extract agent for phosphopeptides [4]. By immobilizing protein A on the surface of Fe₃O₄/SnO(OH)₂ core/shell NPs, γ -immunoglobulins were selectively extracted from biological media [5]. Purification of Alexandrium DNA from environmental samples was achieved by using silica coated Fe₃O₄ NPs as extract agent [17]. Although the absolute majority of the works reported so far have been focused on the extraction of big biomolecules such as peptides, proteins, and DNAs, isolation of small molecules including salicylamide, mefenamic acid, ketoprofen, flufenamic acid, and triazines by using magnetic NPs was recently reported [9]. In the work, analytes retained on NPs were quantified by using MALDI-MS that is not an ideal technique for quantitative analysis compared with HPLC-ESI-MS. In fact, all the NP-based sample extraction procedures developed so far except one¹ have been for MALDI-MS analysis. This was very likely because there is no need to recover the analytes retained on NPs into solution in MALDI-MS analysis. From our preliminary results, elution of analytes bound to NPs can be very difficult, if not impossible.

Determination of trace level pesticides in aquatic environment is very important due to their intense use in agriculture and to their persistence as well. Triazine pesticides (herbicides) are among the most heavily used pesticides today. Analytical methods often used for the determination of triazines in environmental water samples typically include derivatization followed by GC with electron capture detection (GC/ECD), GC with a nitrogen/phosphorus detector (GC/NPD), or GC with mass spectrometric detection (GC-MS) [18]. The derivatization procedure is often tedious and skill-demanding. More convenient HPLC-MS methods have also been developed [19–21]. In these methods, an extraction/enrichment step, *e.g.* SPE, was normally needed prior to HPLC-MS determination because of the extremely low concentration level of pesticides in water samples. SPE of triazines from water using graphitized carbon black extraction cartridge [19], molecularly imprinted polymers [20], and turbulent-flow chromatography columns (30 – 50 μ m polymeric or carbon particles) [21] were reported.

The aim of this work was to develop a NP-based SPE of small organic molecules for HPLC-EIS-MS/MS analysis, which, to the best of our knowledge, has never been reported before. Superparamagnetic Fe₃O₄ NPs were evaluated for this purpose. Triazine pesticides were taken as the model analytes. The biggest challenge was to elute the analytes retained on NPs into solution for subsequent HPLC-ESI-MS/MS analysis. The problem has been solved by dissolving the NPs with an HCl solution after extraction. A sensitive and specific HPLC-ESI-MS/MS method deploying this effective NP-based sample enrichment/clean-up procedure has thus been developed for simultaneous determination of triazine pesticides at trace levels in aquatic samples.

2. Experimental

2.1. Chemicals and reagents

Prometryn, atrazine, propazine, terbutryn, atrazine-d₅, formic acid, decanoic acid, iron(III) chloride, iron(II) chloride, ammonium hydroxide, acetone, acetonitrile, and hydrochloric acid were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). C₁₈ reversed phase silica particles (5 µm) used for the capillary LC columns were obtained from Restek (Bellefonte, PA, USA). Milli-Q (Millipore Corp., Bedford, MA) water was used throughout the work.

2.2. Capillary LC-ESI-MS/MS

The system consisted of two pumps (LC-10ADvp, Shimadzu, Toyoto, Japan), an autoinjector equipped with a 50 µL sampling loop (SIL-10A, Shimadzu), an on-line degasser (DGU-12A, Shimadzu), and an ion trap mass spectrometer with an ESI source (LCQ Deca, ThermoFinnigan, San Jose, CA, USA). Both the capillary LC and mass spectrometer were controlled by Xcalibur software (ThermoFinnigan). A flow splitter (75cm × 50 µm) was installed prior to the capillary column. Capillary C₁₈ reversed-phase columns (20.0 × 0.25 mm i.d.) were prepared as we described previously [22–23]. A switching-valve was placed after the column directing the eluent either to waste or to the MS detector. Gradient LC elution was carried out with two mobile phases: A) water and B) acetonitrile containing 0.2% formic acid at a flow rate of 40 µL/min. The elution was programmed as following: time 0–20.00 min, 100% mobile phase A to stack and clean up the samples (e.g. to wash away FeCl₃ in the sample matrix) with the eluent directed to waste; time 20.10 – 30.00 min, mobile phase B was linearly increased from 30% to 50% to elute analytes retained on the column; time 30.10–40.00 min, 100% mobile phase A to equilibrate the column for next injection. Multiple-stage mass spectrometry (MS/MS) experiments were performed to isolate and fragment the targeted ions. The operating conditions were optimized in positive mode as following: sheath gas flow rate, 35 units; auxiliary gas flow rate, 0 units; capillary temperature, 220 °C; capillary voltage, 4.0 kV; a relative collision energy of 30% was used for all MS/MS experiments with an isolation width of 1.0 u.

2.3. Preparation of superparamagnetic Fe₃O₄ nanoparticles

The procedure described by Shen and Hatton *et al* [24] was used with minor modifications. Briefly, 1.55 g of FeCl₂ and 1.41 g of FeCl₃ were dissolved under N₂ in 40 mL de-aerated Milli-Q water with vigorous stirring. As the solution was heated to 80 °C, decanoic acid solution (100 mg neat decanoic acid dissolved in 5 mL of acetone) was added, and followed by 2 mL of 28% (w/w) NH₄OH. Further, neat decanoic acid was added to the suspension in five 0.2-g portions over 5 min. The crystal growth was allowed to proceed for 30 min at 80 °C with constant stirring to produce a stable, water-based suspension, which was then cooled slowly to room temperature and ready for use.

2.4. Fe₃O₄ NP-based SPE procedure

A beaker containing 400 mL water sample was added atrazine-d₅ at 0.250 ng/mL as internal standard. With stirring, 0.100 mL Fe₃O₄ NP solution prepared above was added. The mixture was stirred for 10 min. The beaker was placed and rested on a magnet for 10 min to let NPs settle down. The supernatant was removed by using a peristaltic pump, and then by decanting with the external magnet in its place. The residue at the bottom of the beaker was dissolved in 2.00 mL HCl solution (10 M). To neutralize the solution, 2.00 mL NaOH (10 M) was added. The solution was filtered through a 0.45 µm syringe filter. Portions (20 µL) were injected into the HPLC-ESI-MS/MS system for analysis without further purification.

3. Results and discussion

3.1. Extraction of triazines with Fe₃O₄ nanoparticles

The superparamagnetic Fe₃O₄ NPs prepared in this work were stabilized against agglomeration by bilayers of decanoic acid. Therefore, the particle surface was encapsulated with 10 carbons and negatively charged. A strong affinity of triazines to the NPs was expected due to multiple electrostatic interactions between them. A series of tests were performed to investigate the extraction efficiency. Fe₃O₄ NP solution (10 μ L) was added to 10 mL of a test compound solution at 1.00 μ g/mL. After extraction and collection of the NPs, the concentration of the test compound present in the solution was quantified by ESI-MS/MS. Extraction efficiency was calculated by using the equation: $100 \times (1.0 - \text{Conc. } (\mu\text{g/mL}))$. The efficiency was found to be $95.1 \pm 2.2\%$ ($n = 3$) for atrazine. For other small organic compounds, efficiencies were found to be $97.4 \pm 1.7\%$ ($n = 3$) for 1-methyl-6,7-dihydroxy-tetrahydroisoquinoline (alkaloid), and $93.3 \pm 4.2\%$ ($n = 3$) for a tripeptide, Asn-Trp-Phe-NH₂, respectively. Effects of extraction time were also investigated. NP solution (100 μ L) was added to a 400-mL sample solution with stirring. After certain time, NPs were collected, and analyte retained on NPs was quantified by HPLC-ESI-MS/MS. Figure 2 shows the results from extracting triazines. As can be seen, extraction efficiencies remained almost the same for extraction times of 10, 20, and 30 min. Therefore, 10 min was selected for further studies.

3.2. Elution of triazines from Fe₃O₄ nanoparticles

Retrieve of analytes retained on NPs into solution is necessary for subsequent HPLC-ESI-MS/MS, which is different from the case of MALDI-MS/MS analysis. Unfortunately, it was found that elution of triazines as well as the other small organic compounds tested from Fe₃O₄ NPs was very difficult. Elution solvents including methanol, acetonitrile, water, acidified water, and their combinations were tested. Recoveries observed ranged from 20 % to 70% depending upon the chemical structure of the analyte. Fortunately, it was also found later that Fe₃O₄ NPs settled down at the bottom of the extraction beaker could be completely dissolved with 2 mL of 10 M HCl solution within 2 min. The resulting solution was clear and yellow-brownish. Dissolution of the NPs with 3 M HCl solution was not effective as evidenced by poor clarity of the resulting solution and prolonged dissolving time (>10 min). Theoretically, 100% analytes retained on NPs can be retrieved into solution by means of the proposed acid dissolution. To investigate the stability of triazines in 10 M HCl solution, samples obtained from acid dissolution were kept at 5 °C for certain times before they were analyzed by HPLC-ESI-MS/MS. The results showed that triazines except propazine were stable at least for 2.5 hrs. Propazine degraded gradually after 1 hr in 10 M HCl solution.

3.3. Atrazine-d₅ as internal standard

Use of a stable isotope-labeled analyte analog as internal standard is generally advantageous in any quantitative mass spectrometric methods. It is usually considered to be essential in order to correct for matrix effects. Because the physicochemical properties of the analytes are similar to those of the isotope-labeled internal standard, the ratio of signal intensities for analyte/internal standard is ideally independent of the recoveries from chemical processes such as sample extraction and the degree of ionization, thus providing a reliable basis for quantitation. In this work, atrazine-d₅ (Fig. 1, MW=220) was chosen as the internal standard. Atrazine-d₅ was commercially available at a purity of > 99 atom % D. Figure 3 shows the HPLC-ESI-MS/MS chromatograms from analyzing a standard mixture solution of triazines at 0.500 ng/mL each. A 400-mL sample of the solution was first extracted with Fe₃O₄ NPs, and then the analytes were retrieved by acid-dissolution of the NPs and quantified by HPLC-ESI-MS/MS. From Fig. 3B & 3D, atrazine and atrazine-d₅ were co-eluted at 23.2 min indicating they had identical chromatographic behavior. In addition, from the equal peak areas/heights for atrazine and atrazine-d₅ it was suggested that these two compounds had the same extraction efficiency

and MS/MS detection response factor. Based on these results, we expect that the use of atrazine- d_5 as internal standard corrects any effects on the HPLC-ESI-MS/MS quantification from potential variations in extraction efficiency, ionization, and MS/MS detection. Also from Fig. 4, it is worth noting that CID fragmentation of these triazine pesticides produced characteristic predominant ions, which made the MS/MS detection selective and sensitive.

3.4. Analytical figures of merit

Using m/z 221 $\rightarrow$ 179 for atrazine- d_5 , m/z 216 $\rightarrow$ 174 for atrazine, m/z 230 $\rightarrow$ 188 for propazine, m/z 242 $\rightarrow$ 200 for prometryne, and m/z 242 $\rightarrow$ 186 for Terbutryn SRM MS/MS mode, the quantification was carried out by means of the signal ratio of analyte to internal standard. Five-point calibration curves were prepared with authentic triazine solutions at concentrations ranging from 0.030 ng/mL to 50.0 ng/mL while keeping atrazine- d_5 concentration constant at 0.250 ng/mL. Peak areas were used for the calculation. Linear regression analysis of the results yielded the following equations for the four atrazines:

$$\text{atrazine} \quad Y = 2.23 \times 10^5 X + 2.5 \times 10^3, \quad r^2 = 0.999$$

$$\text{terbutryn} \quad Y = 1.96 \times 10^5 X + 2.7 \times 10^3, \quad r^2 = 0.991$$

$$\text{prometryn} \quad Y = 1.19 \times 10^5 X + 1.6 \times 10^3, \quad r^2 = 0.993$$

$$\text{propazine} \quad Y = 2.12 \times 10^5 X + 2.1 \times 10^3, \quad r^2 = 0.993$$

where Y is the peak area ratio of the analyte to atrazine- d_5 , X is the concentration of the analyte in ng/mL, and r^2 is the correlation coefficient. Interday (5 days) precisions of the slope and intercept of the calibration curves were found to be in the range between 2.3% and 3.7% (RSD, $n = 5$). From the calibration curves, the limits of detection were estimated to be in the range from 0.010 ng/mL for atrazine to 0.030 ng/mL for prometryn (signal/noise=3). The method sensitivity is comparable or better than those reported previously [19–21]. The merit of analytical figures also indicates that the present method is sensitive enough for the analysis of atrazine in drinking waters as defined by EPA Method 536.

3.5. HPLC-ESI-MS/MS determination of triazine pesticides in surface water

Triazine pesticides are among the most heavily used pesticides today. Monitoring their levels in aquatic environments is important. Using the present HPLC-ESI-MS/MS method, water samples taken from local lakes were analyzed to determine simultaneously the four triazine pesticides, i.e. atrazine, terbutryn, propazine, and prometryn. Typical chromatograms from such an analysis are shown in Figure 4. As can be seen, peaks corresponding to atrazine, and propazine were well defined. The analytical results are summarized in Table 1. Atrazine and propazine were detected in three of the five water samples at high levels ranging from 7.00 to 17.0 ng/mL (atrazine), and 2.00 – 7.00 ng/mL (propazine), respectively. These results should be an indication of recent uses of these herbicides in the area.

4. Conclusions

Extraction of small organic molecules such as triazine pesticides from large volume samples using Fe_3O_4 nanoparticles as extract agent proved every effective. After extraction, Fe_3O_4 nanoparticles could be easily collected by applying an external magnetic field at the bottom of the extraction container. However, elution of analytes retained on Fe_3O_4 nanoparticles was found very difficult. This problem was solved by dissolving Fe_3O_4 nanoparticles with 10 M HCl solution to retrieve analytes, thus allowing subsequent HPLC-ESI-MS/MS analysis. A sensitive capillary HPLC-ESI-MS/MS method employing the Fe_3O_4 nanoparticle-based SPE was developed for simultaneous determination of triazine pesticides present in surface water.

Analysis of surface water samples taken from local lakes revealed the occurrence of atrazine and propazine at the ng/mL level.

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Fig 1.
Chemical structures of triazine pesticides studied in this work and the internal standard, atrazine-d₅.

Fig 2. Effects of extraction time for extracting triazines from a 400-mL water sample with 0.1 mL Fe_3O_4 nanoparticle solution. Concentrations of triazines were 0.25ng/mL each.

Fig 3.

HPLC-ESI-MS/MS chromatograms from analyzing a standard mixture solution of triazines (A): TIC of m/z 216, m/z 221, m/z 230, m/z 242 and m/z 242; (B) and (C) extracted mass chromatogram of m/z 221 $\rightarrow$ 179 for atrazine- d_5 from A and full scan MS/MS spectrum of m/z 221, respectively; (D) and (E) extracted mass chromatogram of m/z 216 $\rightarrow$ 174 for atrazine from A and full scan MS/MS spectrum of m/z 216, respectively; (F) and (G) extracted mass chromatogram of m/z 230 $\rightarrow$ 188 for propazine from A and full scan MS/MS spectrum of m/z 230, respectively; (H) and (I) extracted mass chromatogram of m/z 242 $\rightarrow$ 200 for prometryne from A and full scan MS/MS spectrum of m/z 242, respectively; (J) and (K) extracted mass chromatogram of m/z 242 $\rightarrow$ 186 for terbutryn from A and full scan MS/MS spectrum of m/z 242, respectively. Concentrations of triazines were 1.50 ng/mL each.

Fig 4.

HPLC-ESI-MS/MS determination of triazine pesticides in a lake water sample: (A) TIC of m/z 216, m/z 221, m/z 230, m/z 242 and m/z 242; (B) extracted mass chromatogram of m/z 221 $\rightarrow$ 179 for atrazine- d_5 from A; (C) extracted mass chromatogram of m/z 216 $\rightarrow$ 174 for atrazine from A; (D) extracted mass chromatogram of m/z 242 $\rightarrow$ 200 for prometryn from A; (E) extracted mass chromatogram of m/z 230 $\rightarrow$ 188 for propazine from A; and (F) extracted mass chromatogram of m/z 242 $\rightarrow$ 186 for terbutryn from A. In this water sample, atrazine and propazine were detected at the ng/mL level, but no prometryn or terbutryn was detected.

Table 1
Analytical Results of Triazine Pesticides in Surface Water Samples

Sample Name	Atrazine (ng/mL)	Propazine (ng/mL)	Prometryn (ng/mL)	Terbutryn (ng/mL)
Lake #1	14.41 ± 1.78 ^a	7.13 ± 1.28	ND	ND
Lake #2	6.66 ± 0.68	2.07 ± 0.56	ND	ND
Lake #3	ND	ND	ND	ND
Lake #4	9.38 ± 0.87	3.81 ± 0.23	ND	ND
Lake #5	ND	ND	ND	ND

^a mean ± SD (n = 3). ND: not detected.