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Aqueous two-phase extraction for determination of triazine herbicides in milk by high-performance liquid chromatography

Xiao Yang[a], Rui Yu[a], Shaohua Zhang[b], Bocheng Cao[a], Zhongling Liu[a], Lei Lei[a], Na Li[a], Zhibing Wang[a], Liyuan Zhang[a], Hanqi Zhang[a], Yanhua Chen[a,*]

[a] College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, PR China
[b] Department of Rehabilitation, the General Hospital of FAW (the Fourth Hospital of Jilin University), Haoyue Road 3788, Changchun 130011, PR China

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ABSTRACT

A simple extraction method based on acetonitrile-K_2HPO_4 aqueous two-phase system was developed for separation and enrichment of five triazines in milk samples. Acetonitrile was used for extraction of analytes from milk sample and precipitation of milk protein. Deproteinization and extraction were achieved in one single step. Analytes were extracted into the upper phase of the aqueous two-phase system. The parameters affecting the extraction efficiency, such as the volume of acetonitrile, the type and amount of salts, pH value of sample and extraction time were investigated. The limits of detection of atraton, desmetryn, atrazine, terbumeton and terbuthylazine were 2.1, 2.6, 2.3, 2.8 and 2.5 μg/L, respectively. When the present method was applied to the analysis of real milk samples, the recoveries of analytes ranged from 86.3 to 120.6% and relative standard deviations were lower than 7.9%.

1. Introduction

Triazine herbicides are not only widely used in agriculture to control grasses and broadleaf weeds but also applied for nonagricultural purposes such as soil sterilization and road maintenance [1]. The intensive application of triazines has resulted in the contamination of the atmosphere, ground and wastewaters, agricultural products and, consequently, in the direct and indirect pollution of food and food products [2,3]. Milk-producing animals may accumulate residues of triazines through carry over processes from contaminated feed, grass and corn silage, water, top-layer soil and inhaled air. Consequently, triazines may persist at trace levels in milk, being a potential risk to milk consumers in terms of cancer, birth defects and interruption of hormone functions [4,5]. The European Union (EU) legislation harmonizes a maximum residue limits (MRLs) of the pesticides and fixes default value of MRLs at 0.01 mg kg^{-1} for human food and animal feeding stuffs [6]. MRLs of triazines in milk are only extablished for terbuthylazine which is set at 0.05 mg kg^{-1} [7].

In recent years, several extraction methods have been proposed for the extraction of herbicides in milk samples, such as solid-phase extraction (SPE) [8–10], dispersive solid-phase extraction (DSPE) [11], quick, easy, cheap, effective, rugged and safe (QuEChERS) method [12], microwave assisted ionic liquid microextraction (MAILME) [13], cloud point extraction (CPE) [14], hollow fiber membrane-protected solid-phase microextraction (HFM-SPME) [15], pressurized liquid extraction (PLE) [16] and diphasia dialysis extraction (DDE) [17]. The most sample pretreatment methods mentioned above are not suitable for direct extraction of analytes because of the effect of the protein and fat in milk. As a consequent, an extra de-emulsification step is needed to eliminate interference from proteins and fats [14]. Therefore, our research aims at simplifying the extraction procedure, making it possible to directly extract triazines from milk in one single step.

In the study, the aqueous two-phase system (ATPS) was formed in the presence of acetonitrile (ACN) and K_2HPO_4. The inorganic salt has a stronger affinity for water molecules than ACN. Consequently, a "migration" of water molecules away from ACN to the inorganic salt takes place, which results in the decrease of the hydration and hence the solubility of ACN in water [18]. As a consequence, an ACN-rich phase is separated from the rest of the solution. When the ATPS is formed, the upper phase is ACN-rich and the lower phase is salt-rich. The soluble compounds and a suspended precipitated protein layer exist in the lower phase. The analytes are extracted into the ACN-rich phase according to the distribution coefficients of them in the two phases. The ACN-based aqueous two-phase extraction is an easy and rapid sample pretreatment method. ACN is used for extraction of analytes from milk sample and precipitation of milk

* Corresponding author. Tel.: +86 431 85168399; fax: +86 431 85112355.
E-mail address: vivianyxiao@163.com (Y. Chen).

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protein. Deproteinization and extraction are achieved in one single step. Direct extraction of triazines from milk without extra de-emulsification makes the method suitable for preparation of batch samples.

2. Experimental

2.1. Instrument

The 1100 series liquid chromatograph (Agilent Technologies, Palo Alto, USA) equipped with UV detector and quaternary gradient pump was used. Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 3.5 μm) and a C18 guard column (7.5 mm × 2.1 mm, 5 μm) were used. Ultrasonic mixing was performed with a 100 W ultrasonic cleaner (model KQ-100DE, Kunshan Ultrasonic Instrument, Kunshan, China). The phase separation was performed on HC-2066 high-speed centrifuge (Anhui USTC Zonkia Scientific Instruments, Hefei, China).

2.2. Reagents and chemicals

Triazine herbicides, including atraton, desmetryn, atrazine, terbumeton and terbuthylazine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chromatographic grade methanol and acetonitrile were purchased from Fisher Scientific Company (Loughborough, UK) and pure water was obtained with a Milli-Q water purification system (Millipore, Billerica, USA). All other chemicals used, such as hydrochloric acid and K_2HPO_4, were of analytical grade.

2.3. Standard solutions

The individual stock solution of each analyte (200.0 μg mL^{-1}) was prepared by dissolving 2000.0 μg of the analyte in 10 mL of methanol and stored in the refrigerator at 4 °C. The mixed stock solution containing all analytes (10.0 μg mL^{-1}) was prepared with individual stock solutions by diluting with methanol and stored at 4 °C. The mixed working standard solution was prepared by diluting the mixed stock solution with methanol.

2.4. HPLC-UV conditions

The mobile phase consisted of acetonitrile (A) and water (B). The gradient condition was as follows: 0–8 min, 40–50% A; 8–11 min, 55–60% A; 11–13 min, 60–70% A; 13–16 min, 70% A; 16–18 min, 70–40% A. The flow rate of the mobile phase was 0.5 mL min^{-1}. The column temperature of 30 °C was maintained. The injection volume of analytical solution was 20 μL. The detection wavelength was 220 nm.

2.5. Samples

Milk samples, including pasteurized milk (sample 1), low-fat milk (sample 2), skimmed milk (sample 3) and pure milk (sample 4), were purchased from a local market. Except for the experiments mentioned in Section 3.3, which were performed with Samples 1–4, all other experiments were performed with sample 4. The freshly spiked samples were prepared by spiking the mixed working standard solutions into milk samples and shaking for 10 min.

2.6. Aqueous two-phase extraction

5 mL of milk sample was diluted with 2 mL water in 10 mL polytetrafluoroethylene (PTFE) centrifuge tube. The pH of the sample solution was adjusted to 5 with hydrochloric acid. 1.5 mL of ACN was added into the solution and shaken evenly. 1.57 g corresponding to 0.009 molar of K_2HPO_4 was accurately weighed and added in the mixture. After the mixture was ultrasonically shaken for 10 min and centrifuged for 5 min at 8000 rpm, corresponding to relative centrifugal force of 5369 g, the ATPS was formed. The upper phase is ACN-rich and the lower phase is salt-rich. Suspended precipitated protein was retained in the lower phase. Analytes were extracted into the ACN-rich phase. 200 μL of the upper phase was diluted with 50 μL of ACN. The resulting solution was filtered with 0.22 μm PTFE filter membrane and referred to as analytical solution.

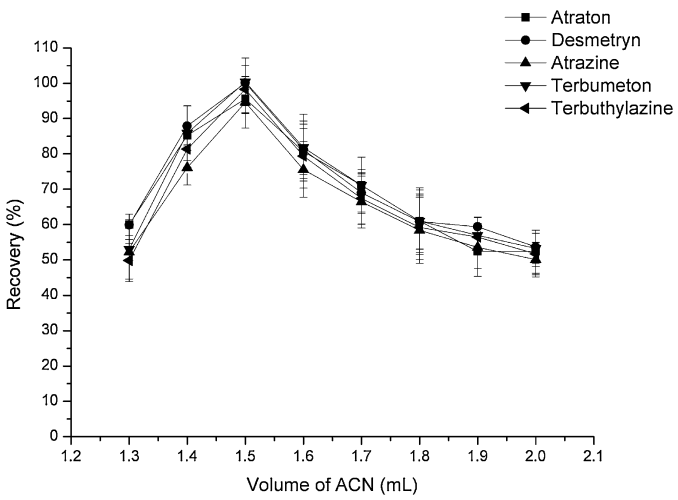

Fig. 1. Effect of volume of ACN. Amount of K_2HPO_4, 0.009 mol; pH value of sample solution, 5; ultrasound extraction time, 10 min. Error bars represent standard deviations.

3. Results and discussion

3.1. Optimization of extraction conditions

In order to obtain high extraction efficiency, the effects of experimental parameters, including the volume of ACN, the type and amount of salts, pH value of sample solution and extraction time were investigated.

3.1.1. Volume of ACN

ACN is used for extraction of analytes from milk samples by forming ATPS along with precipitation of milk protein. When the amount of K_2HPO_4 was fixed at 0.009 mol, ATPS could not form if the volume of ACN is smaller than 1.2 mL. To obtain the optimum volume of ACN, various experiments were carried out by adding different volumes of ACN. The results are shown in Fig. 1. With the increase of the volume of ACN, the volume of upper phase increases. The recoveries of analytes are highest when the volume of ACN is 1.5 mL. Therefore, 1.5 mL was selected as the volume of ACN.

3.1.2. Type and amount of salts

In most extraction procedures, solubility of target analytes in aqueous phase decreases and the concentration of the analytes in extraction phase increases at the same time in the presence of a salt [19]. Addition of salt not only improves the extraction efficiency but also assists in precipitating milk protein and results in the formation of ATPS. On the other hand, with the increase of salt amount, the volume of upper phase increases, and the concentration of analytes in the upper phase decreases. In the research, some salts, including K_2CO_3, $(NH_4)_2SO_4$, K_2HPO_4 and NaCl, were used. All these salts could help forming ATPS. However, K_2HPO_4 is more beneficial to the precipitation of protein than other salts. Therefore, K_2HPO_4 was chosen as the salt. The effect of K_2HPO_4 amount in the range