Journal of Chromatography A, 1351 (2014) 110–114

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Cloud point sample clean-up and capillary zone electrophoresis with field enhanced sample injection and micelle to solvent stacking for the analysis of herbicides in milk

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ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form 15 May 2014
Accepted 16 May 2014
Available online 27 May 2014

Keywords:
Cloud point
Capillary electrophoresis
Stacking
Herbicides
Milk

ABSTRACT

Sample clean-up by cloud point phase separation and analysis by capillary electrophoresis with stacking was developed for quaternary ammonium herbicides (i.e., paraquat and diquat) in milk. For sample clean-up, a mixture of 845 μL of milk sample, 5 μL of 100 mM phosphoric acid, and 150 μL of Triton X-114 was heated (60 °C for 2 min) and centrifugated (3000 rpm for 2 min) in 2-mL Eppendorf tube. The upper phase was directly analysed by capillary electrophoresis via electrokinetic injection at 10 kV for 150 s. The separation electrolyte was 100 mM phosphate buffer with 20% acetonitrile at pH 2.5. Before sample injection, a micellar solution (10 mM SDS in 80 mM phosphate buffer at pH 2.5) and an organic solvent rich solution (30% ACN) was hydrodynamically introduced into the capillary. These solutions provided the necessary conditions for stacking the cationic herbicides via the combination of field enhanced sample injection and micelle to solvent stacking. The LODs ($S/N = 3$) obtained from the entire strategy for paraquat and diquat in milk was 0.004 and 0.018 μg/mL, respectively. This is 1.5 to >2 orders of magnitude better than the corresponding LODs obtained from the electrophoretic analysis of herbicide standards prepared in the separation electrolyte. The strategy was also successfully applied to 5 milk samples available in the market.

1. Introduction

The cloud point is the temperature at which phase separation of micellar solutions of nonionic surfactants containing polyoxyethylene chains occur. A surfactant-rich micellar phase and a diluted aqueous phase containing low concentration of the surfactant are formed. This phenomenon has been utilized for the extraction or concentration of species into the surfactant-rich phase where the target analytes are more soluble [1–6]. This approach known as cloud point extraction (CPE) can produce high extraction efficiency and a large preconcentration factor if a relatively small volume of surfactant-rich phase is obtained compared to the original aqueous solution. CPE also offers a convenient alternative to more conventional extraction systems because of low cost, use of non-toxic reagents and less toxic organic solvent. The extracted species were analyzed using various analytical instrumental methods such as spectrophotometry [4,5,7–9], inductively coupled plasma mass spectrometry [4,5,10], high performance liquid chromatography (HPLC) [4–6,11–14], and capillary electrophoresis (CE) [14–19].

On one hand, the analysis of the concentrated analytes from CPE by CE or HPLC is quite problematic. This is due to the high concentration of surfactant that could have deleterious effects on the separation. The surfactant rich extract is often diluted (e.g., with organic solvent) prior to sample injection in order to reduce the sample viscosity. In particular, the diluted extracts have been shown to be amenable to the CE modes of capillary zone electrophoresis (CZE) [15–18], nonaqueous CE [19], and micellar electrokinetic chromatography [20,21]. It is noted that the presence of surfactant in the sample extracts, however hinders the application of known stacking techniques to improve on the sensitivity of CE. Another approach to improve compatibility of CPE to CE is by back extraction (dual-cloud point extraction) of the target analytes by an

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http://dx.doi.org/10.1016/j.chroma.2014.05.050
0021-9673/

appropriate aqueous buffer [22–25]. Injection schemes in CE that are compatible to cloud point prepared samples should be developed.

In this work, we demonstrate sample clean-up using cloud point phase separation followed by direct injection of the cleaned sample by CE with stacking. This strategy is applied to the analysis of cationic quaternary ammonium herbicides (i.e., paraquat and diquat) in complex cow milk sample. The tested herbicides are toxic and can migrate from soil to crop and then enter the food chain. Cloud point sample clean-up (CPSC) was performed using the readily available Triton X-114 (TX-114). The non-ionic surfactant TX-114 was chosen for its low cloud-point temperature (22–30 °C) and high density of the surfactant-rich phase, facilitating the phase separation [26]. The unwanted milk proteins that are detrimental in CE analysis were precipitated during phase separation and then removed into the bottom phase by centrifugation. CPSC in contrast to CPE does not concentrate the target analytes and thus a small volume of phase separated sample is not required.

The CPSC prepared sample was directly analyzed by CZE with electrokinetic injection. In order to improve the concentration sensitivity, stacking or online sample concentration [27] was performed by the recently introduced combination of field enhanced sample injection (FESI) and micelle to solvent stacking (MSS) [28]. FESI–MSS was implemented because of its ability to introduce more sample ions but less sample matrix into the capillary. On top of the higher gain in sensitivity, FESI–MSS is found to be compatible with the cloud point prepared sample. We refer the readers to Refs. [27,28] for details regarding general stacking and FESI–MSS, respectively. CPSC was compared with conventional acetonitrile precipitation and was optimized by varying Triton X-114 concentration, acid content, and equilibration temperature. The analytical figures of merit including accuracy by standard addition as well as analysis of different cow milk samples available in a local supermarket were also studied to show the applicability of CPSC and FESI–MSS CZE.

2. Materials and methods

2.1. Reagents and stock solutions

Sodium dodecyl sulfate (SDS), phosphoric acid, and TX-114 were purchased from Sigma-Aldrich (New South Wales, Australia). Methanol (MeOH) and acetonitrile (ACN) were HPLC grade obtained from Merck (Victoria, Australia). Sodium hydroxide was obtained from Chem-Supply (Victoria, Australia). Stock solutions at appropriate concentration were prepared in purified water. 1 M stock phosphate buffer, pH 2.5was prepared by adjusting the pH of phosphoric acid with 5 M sodium hydroxide. All stock solutions were sonicated and filtered using 0.45 μm filter prior to use.

Paraquat was obtained from Chemservice (West Chester, PA, USA). Diquat was purchased from Sigma-Aldrich (New South Wales, Australia). Stock solutions of the standard were prepared in MeOH to a concentration of 1 mg/mL each. The solutions were stored at 2–8 °C when not in use.

2.2. Equipments

Capillary electrophoresis and stacking were performed with a Hewlett Packard 3D capillary electrophoresis system (Waldbronn, Germany). Detection wavelength was performed at 200 nm. Polyimide-coated untreated fused-silica capillaries were obtained from Molex(Phoenix, Arizona). Fused-silica capillaries of 50.0 cm (effective length 41.5 cm) × 50 μm I.D. were thermostated at 20 °C. New capillaries were conditioned with 1 M NaOH (20 min), water (10 min), and CZE electrolyte (15 min). The centrifugation was performed using VWR Microcentifuge Galaxy 7D, VWR (Radnor, PA, USA). The pH was measured using an Activon model 210 pH meter (New South Wales, Australia). Water was purified with a Milli-Q system from Millipore (Bedford, MA).

2.3. General CE procedure

The CZE electrolyte (100 mM phosphate buffer with 20% ACN at pH 2.5) was prepared each day by mixing the appropriate amounts of 1 M phosphate (pH 2.5), ACN, and water. Typical injection of standard sample in CZE electrolyte was performed at 25 mbar for 6 s. Between each run the capillary was conditioned by flushing with water (2 min) and CZE electrolyte (5 min). In the stacking process, the micellar solution (10 mM SDS in 80 mM phosphate buffer, pH 2.5) and organic solvent rich solution (30% ACN) were injected at 50 mbar. The sample was electrokinetically injected at 10 kV. The separation voltage was applied at 20 kV. Application of voltage was performed with the cathode at detector end.

2.4. Cloud point sample clean-up

Commercial milk samples were obtained from a local supermarket in Hobart, Tasmania, Australia. Milk was spiked with standard stock of herbicides. 845 μL of spiked milk, 5 μL of 100 mM phosphoric acid, and 150 μL of TX-114 were mixed well in 2-mL Eppendorf tube. The mixture was equilibrated at 60 °C for 2 min and then centrifuged at 3000 rpm for 2 min. The upper phase was directly injected into CE without any treatments.

2.5. Analysis of milk samples

Four different milk samples labeled as M1 to M5 (ranging from light milk to full cream milk) were spiked with the herbicides at analyte concentrations that were 3× the LOQ. Moreover, standard addition was used to obtain the recovery in spiked milk also at a concentration of 3× the LOQ.

3. Results and discussion

3.1. ACN precipitation versus CPSC—Proof of concept

Milk spiked with 2 μg/mL of each herbicide was subjected to two types of sample clean-up in order to remove milk proteins. First was the conventional sample clean-up method by ACN precipitation. The content of ACN was varied at 50, 60 and70% (v/v) in a total volume of 1 mL. The effective precipitation of protein started at 60% ACN. A photograph of the resulting sample after centrifugation is shown in Fig. 1B. For comparison, Fig. 1A shows a photograph of untreated milk.

The other scheme was the proposed CPSC. The effect of TX-114 concentration was investigated at 0, 10, 15 and 20% (v/v) in a total volume of 1 mL. The resulting solutions were equilibrated at 60 °C for 2 min. No precipitation was observed in the absence of TX-114 and it was found that 10% (v/v) was unsuccessful to complete the clean-up process. A clear upper-phase was obtained at ≥15% (v/v). Thus, to obtain good sensitivity, clear solution, and minimum usage of surfactant, 15% (v/v) TX-114 was selected as the optimum concentration for CPSC of milk. A photograph of the sample after CPSC is shown in Fig. 1C. In addition, the upper phase was found more viscous than the lower phase. Thus, the nonionic surfactant and protein complexes were in the lower surfactant-poor phase. The upper phase is the cleaned-up sample for analysis.

The cleaned-up samples in Fig. 1B and C without further processing were electrokinetically injected at 10 kV for 30 s. This injection is longer than typical and broad peaks were obtained with injections >30 s. The CE results are shown in Fig. 2B and C,