Journal of Chromatography A, 1217 (2010) 415–418

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Short communication

Determination of tetracyclines in surface water and milk by the magnesium hydroxide coprecipitation method

Wen-Hsien Tsai[a,b], Tzou-Chi Huang[a], Ho-Hsien Chen[a], Joh-Jong Huang[b], Min-Hsien Hsue[b], Hung-Yi Chuang[c,d,*], Yuh-Wern Wu[e]

[a] Department of Food Science, National Pingtung University of Science and Technology, Pingtung 912, Taiwan
[b] The Health Bureau of Kaohsiung County Government, Kaohsiung County 833, Taiwan
[c] Department of Occupational and Environmental Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, 807, Taiwan
[d] Department of Public Health, and Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung City 807, Taiwan
[e] Department of Chemical Engineering, I-Shou University, Kaohsiung County 840, Taiwan

ARTICLE INFO

Article history:
Received 31 July 2009
Received in revised form
29 November 2009
Accepted 2 December 2009
Available online 4 December 2009

Keywords:
Coprecipitation
Sample preparation
Milk analysis
Water analysis
Tetracyclines

ABSTRACT

A simple coprecipitation method was developed for the determination of tetracyclines (TCs) in surface water and milk by high-performance liquid chromatography with diode-array detection (HPLC–DAD). Magnesium ion was added into the surface water or the acetonitrile (MeCN) extract of milk. After alkalinization, magnesium hydroxide precipitates which had been formed can be separated from the matrix solution easily by centrifuging and then a dissolution step was performed by adding a small amount of acid. The final solution could be introduced directly into HPLC system for the determination of the analytes. Under optimal conditions, recoveries for the analysis of spiked surface water samples ranged from 83.6% to 95.1% with relative standard deviation of 2.0–5.5%. For milk samples, relative recoveries were 95.9–104.6% with relative standard deviation of 3.4–6.7%. The enrichment factors ranged from 41.5 to 48.1 for 10 mL water samples, and from 3.6 to 4.4 for 1 mL MeCN extracts of milk. Limits of detection ranged from 0.13 to 0.51 ng/mL, and from 3.0 to 8.5 ng/g for four TCs in surface water and milk samples, respectively.

1. Introduction

Tetracyclines are broad-spectrum antibiotics that are active against both gram-positive and gram-negative bacteria. Improper use of these compounds may result in unsafe residue levels in the tissues of food-producing animals. Moreover, the widespread use of TCs in food animals has led to concerns regarding the public health impact of the occurrence of these antibiotics in the aquatic environment [1,2] due to the possible enhancement of resistance formation in bacteria.

Liquid–liquid extraction (LLE) is one of the oldest preconcentration and matrix isolation techniques in analytical chemistry and it remains a popular choice. However, conventional LLE is time-consuming, tedious and requires large amounts of organic solvent. Solid-phase extraction (SPE) has gradually replaced classical LLE to become the most common sample preparation technique [3,4], but this can be relatively expensive and it normally requires an extra step of concentrating the extract down to a small volume. Moreover, using parent TCs as model compound, a number of solvent-minimized sample extraction methods include using in-tube solid-phase microextraction (SPME) [5], molecularly imprinted SPME [6], hollow fiber based liquid phase microextraction (HF-LPME) [7], magnetic molecularly imprinted polymers [8], carbon nanotubes [1], restricted access materials (RSM) [2] and dispersive micro solid-phase extraction [9] for the determination of TCs have been developed. Despite their advantages, these methods are usually instrument-based techniques or still require time-consuming extraction steps.

Recently, coprecipitation has been accepted as a useful technique to preconcentrate traces of heavy metals rapidly and easily because it has some advantages including, simplicity, rapidity, low expense, high preconcentration factor and low consumption of organic solvent. Unlike traditional methods, the coprecipitation method does not entail a solvent evaporation step to further concentrate the analytes in the final extract prior to analysis. Moreover, the final aqueous solution could also be directly introduced into the HPLC system. However, the determination of organic compounds by coprecipitation has undergone only limited application [10]. Several studies have revealed that TCs could form chelate complexes with multivalent cations and have the greater complex

* Corresponding author at: Department of Occupational and Environmental Medicine, Kaohsiung Medical University Hospital, Kaohsiung City 807, Taiwan. Tel.: +886 7311 5974; fax: +886 7311 5948.
E-mail address: ericch@kmu.edu.tw (H.-Y. Chuang).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.12.006

formation with magnesium [11], therefore, the aim of this study was to assess the feasibility in the application of coprecipitation with magnesium hydroxide to determine TCs in surface water and milk samples by HPLC–DAD.

2. Experimental

2.1. Reagents and materials

Analytical standards of oxytetracycline (OTC) hydrochloride and doxycycline (DC) hyclate and analytical grade oxalic acid dehydrate were obtained from Riedel-de Haen (Sigma–Aldrich Laborchemikalien, Seelze, Germany). Tetracycline hydrochloride (TC) and Chlortetracycline (CTC) hydrochloride were supplied by Sigma–Aldrich Chemie (Steinheim, Germany). HPLC-grade acetonitrile (MeCN), methanol, sodium hydroxide solution (1 M), phosphoric acid (85%) and perchloric acid (70–72%) were purchased from Merck KGaA (Darmstadt, Germany). Sodium sulfate anhydrous was from Shimakyu's Pure Chemical (Osaka, Japan) and sodium chloride was form Nihon Shiyaku Reagent (Tokyo, Japan).

2.2. Instrumentation

For TCs analysis, the Thermo Separations HPLC system (ThermoFinnigan Italia, Rodano, Milan, Italy) was used consisting of a P4000 pump and a highly sensitive UV6000LP diode-array detector. The chromatographic separation was performed on an Intersil ODS-2, 5 μm, 150 mm × 4.6 mm analytic column (GL Sciences Inc., Tokyo, Japan) operated at 40 °C. The separation gradient for water sample analysis was an initial isocratic step with an 89:11 (v/v) 10 mM oxalic acid: MeCN mixture during 9 min, MeCN percentage was raised to 17% in 0.1 min and maintained for 15 min. The initial conditions were reestablished in 0.1 min and held for 10 min. For milk, an initial step with a 90:10 (v/v) 10 mM oxalic acid: MeCN mixture during 9.5 min, MeCN percentage was raised to 16% in 0.1 min and maintained for 15 min. The flow-rate was 1 mL/min and the detector was set at 360 nm. Injection volume was 50 μL.

2.3. Sample preparation

Twenty-five microliter of 10% (w/v) magnesium sulfate was added to 10 mL water sample. The pH of the solution was adjusted by the addition of 1 M NaOH. Then the solution was mixed immediately on a vortex mixer for 30 s and centrifuged at 1500 rpm for 3 min. The supernatant was removed. The remaining precipitates were sonicated for 30 s min after adding 10 μL of phosphoric acid. For neutralization and bringing the final solution to pH 4–6, 100 μL of 1 M NaOH was added. Deionized water was used to adjust the final solution volume to 200 μL.

For milk samples, 5 mL of MeCN and 200 μL of perchloric acid were added to 4 g of milk in a 50 mL centrifuge tube and the mixture was mixed for 1 min by vortex mixer. Two grams of anhydrous Na_2SO_4 and 1 g of NaCl were added and mixed on a vortex mixer immediately for 1 min, and then centrifuged for 4 min at 10,000 rpm. A 1.0 mL aliquot of upper MeCN layer was transferred into a 2 mL minicentrifuge vial with 0.5 mL water containing 0.5 mg magnesium (II) and mixed to form a homogeneous solution. Then 175 μL of 1 M NaOH was added, mixed immediately for 30 s and centrifuged at 6500 rpm for 1 min. The precipitates were sonicated for 30 s after adding 10 μL of phosphoric acid. For neutralization, 100 μL of 1 M NaOH was added. Deionized water was used to adjust the final solution volume to 200 μL and then filtered by 0.45 μm membrane.

3. Results and discussion

3.1. Influences of amounts of magnesium as carrier element

Magnesium (II) was selected as carrier element for the present work and the influences of magnesium (II) on the coprecipitation of TCs were investigated in the range of 0.1–0.6 mg for 10 mL deionized water. Increasing recoveries for TCs were observed with increased amounts of magnesium (II). After 0.4 mg of magnesium (II), the recoveries of TCs were kept constant and high enough for the quantitative extraction of analytes. However, the use of a large amount of magnesium (II) increased the volume of the hydroxide and thus reduced the concentration factor. A smaller volume of final solution is needed if the enrichment factors are important. In this study, in order to be compatible with HPLC automatic injector, the volume of final solution after the centrifuging and dissolving steps was finally adjusted to 200 μL even with the sacrifice of some sensitivity and then transferred to an autosampler vial insert. When >1 mg magnesium (II) is used, the volume of final solution after the dissolving step will readily exceed 200 μL. Therefore, 0.5 mg magnesium (II) was used in the following experiments.

3.2. Effects of pH and centrifuging time

The influence of pH of the aqueous solutions on the coprecipitation efficiency of TCs within the range of 8.5–12.0 were investigated by adding the volumes of 1N NaOH from 20 to 200 μL. TCs showed similar behavior with an increase in recovery at the beginning and then reaching a plateau from pH 11 to 12. From the obtained results, 150 μL of 1 M NaOH was chosen to get the pH value to about 11.4. After coprecipitation by the alkalinization step, the collector was easily separated from the matrix solution by centrifuging. However, a problem encountered in this method was that TCs were not stable under alkaline conditions [11]. To overcome this problem, the performance between the alkalinization step for the formation of precipitate and the dissolving step should be achieved as quickly as possible. Our results show that the variations of recovery were not remarkable in the range 1.5–6 min at 1500 rpm. Therefore, 3 min was enough for the collector separation because a longer time would not give much larger recoveries.

3.3. Effect of the sample volume and sample matrix

The ratio of the volume of the sample to the final extract solution (200 μL) governs enrichment of analytes in the final extract solution. A high volume ratio promotes high enrichment. For the purposes of cost and ease for sample handling, 10 and 1.5 mL water in 15 and 2 mL centrifuge tubes, respectively, were used to examine the coprecipitation efficiencies of the analytes. The results for this study are given in Fig. 1. The results with 10 mL sample volume were similar to those with 1.5 mL sample volume for all TCs. To achieve the demand for ultra-trace analysis of water samples, 10 mL sample was required for the adequate sensitivity of TCs by using HPLC–DAD. The enrichment factor was defined as the ratio between the analyte concentration in the final extract solution and the initial concentration of analyte. The enrichment factors obtained from 10 mL water were from 41.5 to 48.1.

For milk samples, the higher metal ions (such as magnesium and iron) levels in milk will increase the volume of the hydroxides formed and thus reduce the concentration factor. Therefore, we tried to extract TCs from milk into MeCN phase using a salting-out assisted liquid–liquid extraction (SALLE) step with Na_2SO_4 and NaCl before concentrating by the coprecipitation step. After the SALLE step, a 1 mL of aliquot of the MeCN extract was added to a small vial (2 mL) with 0.5 mL water containing 0.5 mg magnesium (II) to form a homogeneous solution for coprecipitation. To

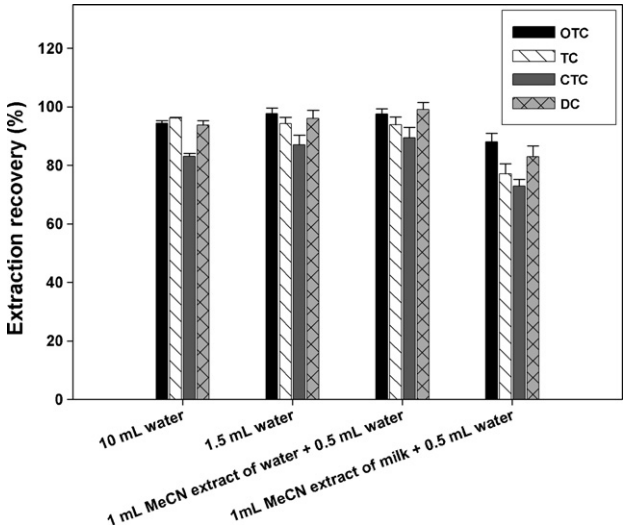

Fig. 1. Influences of sample volume, organic solvent and sample matrix on the coprecipitation efficiencies of 100 ng TCs.

assess sample matrix effects, the extraction efficiencies of TCs from the 1 mL MeCN extracts of water and milk were evaluated and the results are shown in Fig. 1. There were obvious differences for the extraction efficiencies of TCs from the 1 mL MeCN extracts between water and milk, which reflected the influences of the sample matrix on the proposed procedure. The enrichment factors for milk were from 3.6 to 4.4.

Since phase separation induced by the addition of inorganic salts into a mixture of water and a water-miscible organic solvent was revealed in 1914 [12], the SALLE approach with MeCN was also applied in the LC-UV analysis of biological samples first time in 1989 [13]. Recently, besides SPE, SALLE has been investigated as an alternative to the conventional LLE for sample preparation. Extractions using MeCN followed by salting-out has been used more extensively for the analysis of pesticide residues [14], antibiotics in animal tissues [15], and pharmaceuticals in biological samples [16,17]. Following SALLE step, Anastassiades et al. introduced a dispersive solid-phase extraction (dSPE) step for clean-up and named this procedure as "QuEChERS" method [14]. For QuEChERS, after SALLE and dSPE clean-up steps, the main disadvantage compared to other common methods is that the 1 g/mL final extract concentration is lower than the 2–5 g/mL concentrated extracts of most traditional methods. Moreover, the organic phase formed after centrifuging is not suitable for directly injecting into the conventional HPLC. Recently, dispersive liquid–liquid microextraction (DLLME) method has been used to concentrate the final MeCN extract form SALLE for GC analysis [18] and a reverse-DLLME method was also introduced for HPLC analysis [19]. In the present work, for milk sample, SALLE was used not only for extracting analytes but also for getting rid of intrinsic interferences ions for coprecipitation. Moreover, compared to dSPE of QuEChERS, the coprecipitation step was also used to concentrate the MeCN extract obtained from SALLE for HPLC analysis.

Table 2
Analysis of milk samples fortified at 50 (0.5 MRL), 100 (1.0 MRL)and 150 (1.5 MRL) ng/g ($n = 5$).

	Added (ng/g)	Recovery (%)	RSD (%)
Oxytetracycline	50	97.2	4.2
	100	96.3	4.1
	150	100.2	3.4
Tetracycline	50	97.0	4.1
	100	100.6	4.9
	150	99.6	4.6
Chlortetracycline	50	100.4	3.7
	100	104.6	6.7
	150	95.9	5.0
Doxycycline	50	97.7	4.8
	100	102.9	5.2
	150	96.2	3.8

3.4. Validation and analysis of four TCs in water and milk samples

The calibration curves were evaluated for extracting the TCs from 10 mL of deionized water spiked at the range from 0.75 to 8 ng/mL using the proposed method. The coefficients of estimation were ≥0.998 for the four TCs, thus confirming the linearity of the method. The limits of detection of the proposed method, based on the signal-to-noise ratio (S/N) of 3, were as follows: 0.13 ng/mL (OTC), 0.13 ng/mL (TC), 0.44 ng/mL (CTC) and 0.51 ng/mL (DC). For the analysis of real samples, the surface water samples were collected from an artificial lake (Cheng-ching Lake, Kaohsiung County, Taiwan) and revealed the absence of TCs. Table 1 lists the results obtained from the analysis of spiked samples. The method detection limit (MDL) values, according to 40 CFR 136 Appendix B, were also estimated: 0.10 ng/mL (OTC), 0.12 ng/mL (TC), 0.23 ng/mL (CTC) and 0.31 ng/mL (DC). Fig. 2A shows the chromatogram of a surface water sample spiked with 5 and 0.5 ng/mL of TCs and no interferences were observed. For the analysis of milk samples, calibration curves isolated from spiked milk samples were linear over the range 25–200 ng/g for all TCs (the coefficients of estimation >0.996). The LODs were as follows: 3.0 ng/g (OTC), 3.3 ng/g (TC), 7.4 ng/g (CTC) and 8.5 ng/g (DC). The relative recoveries of TCs from milk samples are summarized in Table 2. Fig. 2B shows the chromatogram of a milk sample spiked with 100 and 10 ng/g of TCs.

Table 1
Analysis of spiked deionized water and surface samples ($n = 5$).

	Added (ng/mL)	Deionized water		Lake	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Oxytetracycline	2	98.6	2.9	89.1	3.4
	5	97.8	3.2	87.0	2.0
Tetracycline	2	100.9	4.6	89.9	5.5
	5	99.8	2.7	87.6	4.7
Chlortetracycline	2	108.4	4.1	83.9	4.0
	5	99.3	4.4	83.6	4.5
Doxycycline	2	98.4	2.4	93.9	3.5
	5	100.4	3.0	95.1	5.0

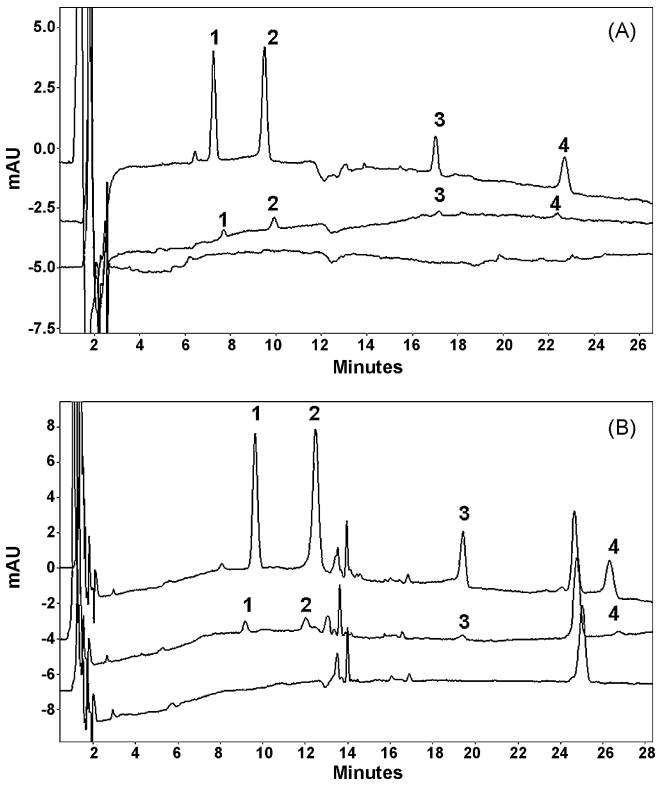

Fig. 2. (A) HPLC chromatograms of blank surface water sample (bottom curve) and of a sample spiked with 0.5 ng/mL (middle curve) and 5 ng/mL (top curve) of TCs. (B) HPLC chromatograms of blank milk sample (bottom curve) and of a sample spiked with 10 ng/g (middle curve) and 100 ng/g (top curve) of TCs. The peaks were (1) OTC; (2) TC; (3) CTC and (4) DC.

4. Conclusions

The proposed extraction technique based on the magnesium hydroxide coprecipitation system was demonstrated to be an excellent strategy for the rapid concentration of traces of TCs in water. For milk sample, the coprecipitation method could be also applied to the MeCN extract after a SALLE step. Compared with conventional methods, the proposed method was simple and the final solution from dissolving the hydroxide precipitates could be introduced directly into HPLC system for the determination of the analytes.

References

[1] B. Suárez, B. Santos, B.M. Simonet, S. Cárdenas, M. Valcárcel, J. Chromatogr. A 1175 (2007) 127.
[2] J. Chico, S. Meca, R. Companyó, M.D. Prat, M. Granados, J. Chromatogr. A 1181 (2008) 1.
[3] A. Jia, Y. Xiao, J. Hu, M. Asami, S. Kunikane, J. Chromatogr. A 1216 (2009) 4655.
[4] H. De Ruyck, H. De Ridder, Rapid Commun. Mass Spectrom. 21 (2007) 1511.
[5] Y. Wen, Y. Wang, Y.Q. Feng, Talanta 70 (2006) 153.
[6] X. Hu, J. Pan, Y. Hu, Y. Huo, G. Li, J. Chromatogr. A 1188 (2008) 97.
[7] S. Shariati, Y. Yamini, A. Esrafili, J. Chromatogr. B 877 (2009) 393.
[8] L. Chen, J. Liu, Q. Zeng, H. Wang, A. Yu, H. Zhang, L. Ding, J. Chromatogr. A 1216 (2009) 3710.
[9] W.H. Tsai, T.C. Huang, J.J. Huang, Y.H. Hsue, H.Y. Chuang, J. Chromatogr. A 1216 (2009) 2263.
[10] T. Saitoh, S. Matsushima, M. Hiraide, Colloid Surf. A-Physicochem. Eng. Aspects 299 (2007) 88.
[11] C.R. Anderson, H.S. Rupp, W.H. Wu, J. Chromatogr. A 1075 (2005) 23.
[12] G.B. Frankforter, L. Cohen, J. Am. Chem. Soc. 36 (1914) 1103.
[13] A.M. Rustum, J. Chromatogr. 490 (1989) 365.
[14] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenk, J. AOAC Int. 86 (2003) 4121.
[15] C.K. Fagerquist, A.R. Lightfield, S.J. Lehotay, Anal. Chem. 77 (2005) 1473.
[16] J. Zhang, H. Wu, E. Kim, T.A. El-Shourbagy, Biomed. Chromatogr. 23 (2009) 419.
[17] H. Wu, J. Zhang, K. Norem, T.A. El-Shourbagy, J. Pharm. Biomed. Anal. 48 (2008) 1243.
[18] E. Zhao, W. Zhao, L.,S.J. Han, Z. Zhou, J. Chromatogr. A 1175 (2007) 137.
[19] W.H. Tsai, T.C. Huang, H.H. Chen, Y.W. Wu, J.J. Huang, H.Y. Chuang, J. Chromatogr. A 1217 (2010) 250–255.