Journal of Chromatography A, 1217 (2010) 6267–6273

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of melamine and cyanuric acid in powdered milk using injection-port derivatization and gas chromatography–tandem mass spectrometry with furan chemical ionization

Shin-Hwa Tzing[a], Wang-Hsien Ding[b,*]

[a] Department of Chemical Engineering, Army Academy ROC, Chung-Li 320, Taiwan
[b] Department of Chemistry, National Central University, Chung-Li 320, Taiwan

ARTICLE INFO

Article history:
Received 8 June 2010
Received in revised form 29 July 2010
Accepted 30 July 2010
Available online 11 August 2010

Keywords:
Melamine
GC–MS/MS
Injection-port derivatization
Powdered milk analysis

ABSTRACT

A reliable, sensitive and eco-friendly injection-port trimethylsilylated (TMS) derivatization and gas chromatography–tandem mass spectrometry (GC–MS/MS) with furan chemical ionization (furan-CI) method was developed to determine melamine and cyanuric acid in powdered milk samples. The effects of several parameters related to the TMS-derivatization process (i.e., injection-port temperature, residence time and volume of silylating agent) and of various CI agents were investigated. Addition of a solution (3 μL) of bis(trimethyl)silyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) reagent to a 20-μL extract from the powdered milk sample gave an excellent yield of the tris-TMS-derivatives of melamine and cyanuric acid at an injection-port temperature of 90 °C. Furthermore, using furan as the CI agent in conjunction with tandem mass spectrometry provided the greatest sensitivity and selectivity of detection. The limits of quantitation (LOQs) for melamine and cyanuric acid were 0.5 and 1.0 ng/g in 0.5-g of powdered milk samples, respectively. The recoveries from spiked samples – after simple ultra-sonication with 5% dimethyl sulfoxide in acetonitrile coupled with *n*-hexane liquid–liquid extraction – ranged from 72% to 93% with relative standard deviations of lower than or equal to 18%. In three of four real powdered milk samples, melamine was detected at concentrations ranging from 36 to 1460 ng/g; and cyanuric acid was detected in two of these samples at concentrations of 17 and 180 ng/g.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, $C_3H_6N_6$) is a white, odorless, crystalline N-heterocyclic organic base that is commonly found in tableware, paints, building materials, and flame-resistant products, and used widely in textile industries, and in the production of pesticides and plant fertilizers. Recently, it has been added illegally to milk and dairy products to suggest deceptively misleading "high" protein content when measured using Kjeldahl nitrogen analysis. In February 2007, the U.S. Food and Drugs Administration (FDA) investigated the cause of a rash of deaths among pets in the USA. The investigation revealed that melamine and related compounds (ammeline, ammelide, cyanuric acid) were present in pet food samples and that the interaction between melamine and cyanuric acid produced a crystalline precipitate in the kidneys, which led to acute renal failures in the animals [1]. Moreover, in 2008, melamine-contaminated dairy products were found in Taiwan, leading to a melamine scare in which many dairy products, such as infant formula, biscuits, candies and powdered milk, were pulled out the market and destroyed.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) with electrospray ionization is the most commonly employed method for the determination of melamine and related compounds in animal feeds, dairy products, sea foods and animal tissues [2–7]. However, gas chromatography–mass spectrometry (GC–MS) is more readily available in many commercial analytical laboratories, and provides higher chromatographic resolution and greater sensitivity with a capillary column. To date, GC or GC–MS methods have also been reported on the detection of melamine and related compounds by various research groups and US-FDA [8–13], however, the limits of detection (LODs) are relatively high and off-line TMS-derivatization is laborious and time-consuming. In previous studies, we have developed direct injection-port derivatization for the determination of a wide range of organic pollutants in various matrices; this approach reduces solvent waste, simplifies sample preparation, and avoids the need for hazardous reagents [14–18]. Furthermore, we have found that using furan as a CI agent in conjunction with MS or MS/MS can

* Corresponding author. Tel.: +886 3 4227151x65905; fax: +886 3 4227664.
E-mail addresses: wding@cc.ncu.edu.tw, wanghsiending@gmail.com (W.-H. Ding).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.07.081

Table 1
CI-MS ARC optimal conditions for the applied reagents.

ACR parameter	CI reagent				
	Methane	Dichloromethane	Acetonitrile	Methanol	Furan
CI maximum reaction time (μs)	80	120	100	100	120
CI ionization storage level (*m*/*z*)	5	30	25	15	15
Reagent ion eject amplitude (V)	9	7.5	9	10	7.5
CI reaction storage level (*m*/*z*)	13	30	25	15	15
CI background mass (*m*/*z*)	45	95	65	45	75

improve the sensitivity and selectivity for analytes containing carboxyamide, amino, or phosphorus groups [19–21].

As part of an effort to evaluate the impact of residues of melamine and cyanuric acid found in dairy products consumed in Taiwan, in this study we developed a simple, reliable and sensitive method for the routine determination of trace levels of these compounds in powdered milk samples. The effects of the injection-port derivatization parameters (i.e., injection-port temperature, residence time and volume of silylating agent) were systematically investigated. Additionally, this work also examined the feasibility of using furan as a CI agent in conjunction with MS/MS to increase the sensitivity and selectivity for the quantitation of melamine and cyanuric acid in dairy products. Accuracy and precision of this method were evaluated, and its effectiveness at determining these compounds in powdered milk samples at trace levels was also examined.

2. Experimental

2.1. Chemicals and reagents

Unless noted otherwise, all chemicals and solvents were obtained at high purity and used without further purification. Melamine (>99%), cyanuric acid (>99%), 2,6-diamino-4-chloropyrimidine (used as an internal standard [11]) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Bis(trimethyl)silyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (known by the trade name Sylon BFT) was purchased from Supelco (Bellefonte, PA, USA). Dimethyl sulfoxide (DMSO) and furan of analytical grade were purchased from Merck (Hawthorne, NY, USA). Acetonitrile, methanol, hexane, and dichloromethane of HPLC-grade were purchased from Tedia (Fairfield, OH, USA). Stock solutions of each analyte (1.0 mg/mL) were prepared in acetonitrile containing 5% DMSO. Mixtures of the analytes for working standard preparation and sample fortification were prepared in acetonitrile with 0.5% pyridine. All stock solutions and mixtures were stored in the dark at 4 °C. Deionized water was further purified using a Millipore water purification device (Billerica, MA, USA).

2.2. Sample preparation

Four powdered milk samples were purchased from nationwide wholesale markets and stored unopened until analysis at 4 °C. The procedure for sample extraction through simple ultra-sonication has been reported elsewhere [11], it was used with some modifications. Briefly, a portion of powdered milk sample (0.5 g) was dissolved in a solution (10 mL) of acetonitrile containing 5% DMSO. The sample solution was mixed for 20 s, and then subjected to ultra-sonication for 20 min at room temperature. The solution was then centrifuged for 10 min at 6000 rpm, the supernatant (2 mL) was filtered through an 0.45-μm membrane filter (Gelman Scientific, Ann Arbor, MI, USA), and then the filtrate was liquid–liquid extracted three times with n-hexane (5 mL each) to remove the fat, which was discarded. The fat-free acetonitrile solution was evaporated to dryness under a gentle stream of nitrogen at 70 °C. The residue was redissolved in a solution (100 μL) of acetonitrile containing 1.0 μg/mL of 2,6-diamino-4-chloropyrimidine (as an internal standard) and 0.5% pyridine, and subjected to injection-port derivatization GC–CI-MS/MS analysis.

2.3. GC–MS and GC–MS/MS with electron-impact ionization (EI) and CI analysis

Analyses were performed using a Varian 3800 CX GC directly connected to a Saturn 2000 ion-trap mass spectrometer (Walnut Creek, CA, USA). A ChromatoProbe (Varian) and a temperature-programmed injector (liner: 3.4 mm i.d.) were used to introduce large-volume samples for injection-port derivatization, as described elsewhere [14,20]. Briefly, a sample solution (20 μL) was mixed with Sylon BFT (3 μL) introduced into a micro-vial (volume: 40 μL), the vial was placed into a ChromatoProbe vial holder, and then positioned in the GC injection-port. The temperature was held at 90 °C for 2 min for TMS-derivatization and solvent vaporization, and then the temperature was rapidly increased to 300 °C to allow introduction of the TMS-derivatives into the analytical column. A DB5-MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent, Santa Clara, CA, USA) was used. The following GC temperature program was used: 100 °C for 7 min; a temperature ramp of 10 °C/min to 230 °C, another ramp of 20 °C/min up to 310 °C, and maintaining this temperature for 3 min. The temperature of the transfer line was set at 280 °C. Full-scan EI spectra were acquired under the following conditions: mass range 50–500 *m*/*z*, scan time 1 s, solvent delay 12 min, ion trap temperature 160 °C, manifold temperature 50 °C, emission current 20 μA (at 70 eV electron energy), and an automatic gain control target set at 20,000.

For CI-MS analysis, the same sample volume, GC column and oven temperature program were used as described for the GC–EI-MS analysis. Methane, methanol, acetonitrile, dichloromethane and furan were tested as CI agents in the selected ejection chemical ionization (SECI) to evaluate their sensitivity and reproducibility. Agent ions were ionized for a variable duration set by the automatic reaction control (ARC) of the instrument. The CI full-scan data were acquired under the following conditions: mass range 100–500 *m*/*z*, scan time 1 s, solvent delay 10 min, manifold temperature 50 °C, ion trap temperature 160 °C, ARC ionization time 0.1 ms and CI maximum ionization time 2 ms. The optimal conditions for the other ARC parameters for each CI agent are given in Table 1. The autotune program was used to set most of the instrument parameters with a target of 10,000 at a filament current of 10 μA. The pressure of CI agent in the ion trap was ca. 2×10^{-5} Torr (1 Torr = 133.3 Pa).

For tandem-in-time furan-CI-MS/MS analysis, the scan time was set at 0.5 s, the amplitude range for resonant collision-induced dissociation (CID) was 0.4–0.6 V, and the excitation time was 20 ms. The optimal MS/MS operating conditions were evaluated using a so-called three-step method [11,14,20] with various resonant CID conditions for each precursor ion. The optimal excitation voltage for product ion formation was determined for each precursor ion through incremental changes in the voltage on a scan-by-scan basis

across the eluting peak, using a scan rate of 0.1 s/scan on the Saturn Toolkit software [22].

2.4. Method evaluation

The method was evaluated using spiked powdered milk samples. Three standard solutions of melamine and cyanuric acid were spiked via a glass syringe into a portion of powdered milk samples (each spiked at the final concentrations of 5.0, 50 and 200 ng/g), and the spiked samples were mixed through tumbling for 30 min. They were then stored in tightly closed brown glass vials at room temperature for at least 12 h prior to analysis. The spiked samples were then subjected to sample pretreatment and injection-port derivatization GC–furan-CI-MS/MS analytical procedures for method evaluation. Five-level calibration curves with an internal standard were applied to quantitate the analytes in powdered milk samples, as described elsewhere [10–13]. LODs and LOQs were calculated following the procedure described elsewhere [10,12,13].

3. Results and discussion

3.1. Evaluation of injection-port derivatization

Trimethylsilylation is a commonly used derivatization procedure when analyzing melamine and cyanuric acid using GC–MS [10–13]. The addition of a stimulator to BSTFA can increase the derivatization efficiency for analytes [23–25]. According to our previous experience, the use of BSTFA containing 1% TMCS as a stimulator is a suitable derivatization reagent for the formation of the main TMS-substituted derivatives; it also improves the chromatograms and generates the optimal average peak areas and quantitative results [23,24]. Initially, the effects of three derivatization parameters (injection-port temperature, residence time, and volume of silylating agent co-injected with the sample) were evaluated, and the derivatization efficiency was compared with the abundances of the GC–EI-MS peak areas of the corresponding TMS-derivatives. These TMS-derivatives followed common fragmentation pathways. Since the molecular ion $[M]^+$ (for cyanuric acid) and the $[M\text{-}CH_3]^+$ ion (for melamine) were the base peaks for these two derivatives during EI-MS analysis, these peaks were used as quantitation ions to obtain the maximum detection sensitivity and specificity for evaluation of the derivatization efficiency. Fig. 1(a) reveals that the abundances of the TMS-derivatives increased when the injection-port temperature increased from 75 to 90 °C, but decreased thereafter. Thus, the effect of the residence time was evaluated at a temperature of 90 °C. The highest yield was achieved at a residence time of 2 min (Fig. 1(b)), for residence times longer than 2 min, the abundances of the TMS-derivatives decreased. This phenomenon might be due to the TMS-derivatives escaping from the injection-port at the higher temperatures and longer residence time when the analytes completely converted to their corresponding TMS-derivatives. Next, the effect of the volume of silylating reagent co-injected with the sample was evaluated, we found that the abundances were similar when the volume was within the range from 2 to 5 μL (data not shown). Therefore, the following derivatization conditions for our subsequent experiments were selected: addition of 3 μL of Sylon BFT (a mixture of BSTFA and 1% TMCS) to 20 μL of the sample and then heating the mixture at 90 °C for 2 min in the injection-port.

No retention effect of the silylating reagent, or the thermally degraded components from the sample matrix in the injection-port were detected because we used the ChromatoProbe device with a disposable micro-vial, and because no glass wool was inserted into the inlet glass liner for on-line derivatization. Therefore, this

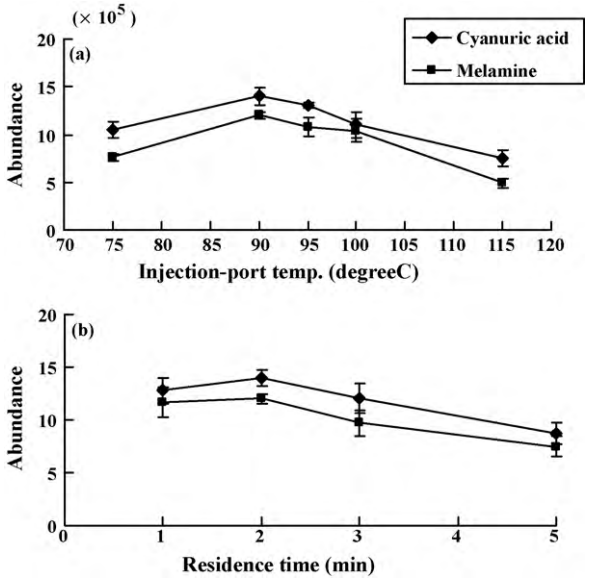

Fig. 1. Effects of (a) injection-port temperature and (b) residence time on the efficiency of injection-port derivatization. Three replicate experiments were performed; the error bars represent standard deviations.

method did not require a routine check of sample carryover through subsequent injection of a different derivatizing reagent after sample injection. Sharp and symmetric peaks remained visible after more than 50 sample injection.

3.2. Evaluation of sensitivity and selectivity by CI-MS

Previous studies have shown that the sensitivity and selectivity of the analytes containing carboxyamide, amino, or phosphorus groups could be improved by using furan as the CI agent in conjunction with MS or MS/MS spectrometric detectors [19–21]. Furthermore, to confirm the determination of the TMS-derivatives and to provide an extra dimension for more certain compound identification at trace-level, we applied the CI-MS technique and monitored the protonated molecular signals or adduct signals. Fig. 2 displays the abundances of the $[M+H]^+$ or adduct signals of the tris-TMS-derivatives of cyanuric acid and melamine obtained when using methane, dichloromethane, acetonitrile, methanol and furan as CI agents; the abundances of the signals for the $[M]^+$ and $[M\text{-}CH_3]^+$ ions obtained using EI-MS are also provided for comparison. Considerably higher abundances of the $[M+H]^+$ or adduct signals were obtained when furan was used as a CI agent; these signals were even higher than those obtained using EI-MS. These

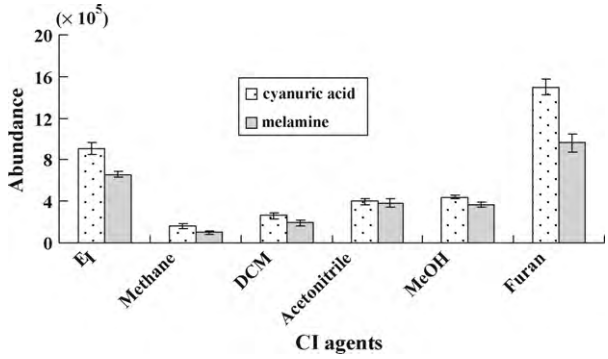

Fig. 2. Abundances of the signals obtained for the $[M+H]^+$ or adduct ions of cyanuric acid and melamine when using EI and various CI agents. Three replicate experiments were performed; the error bars represent standard deviations.

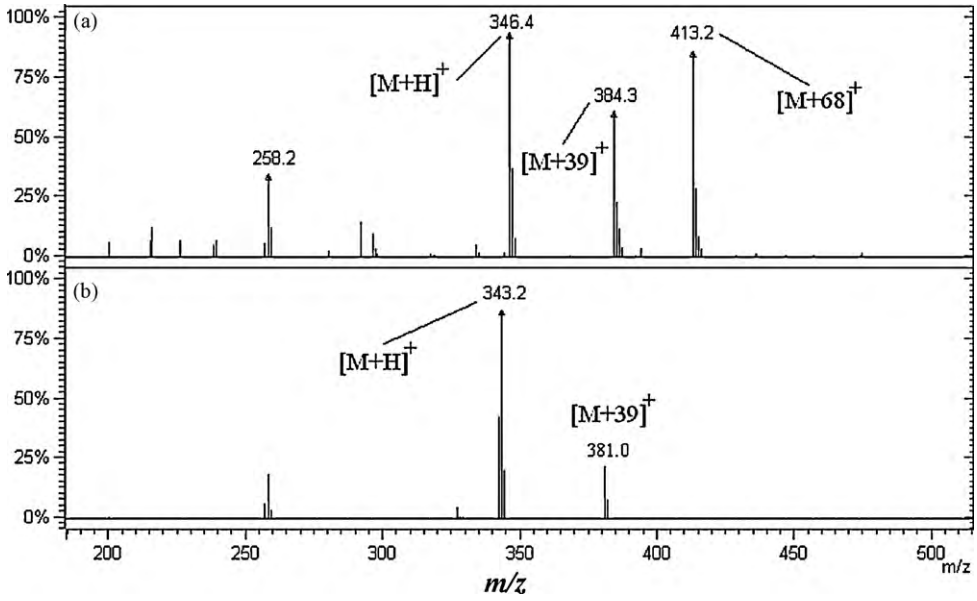

Fig. 3. Furan-CI mass spectra of (a) tris-TMS-cyanuric acid and (b) tris-TMS-melamine.

results indicate that the abundances of the $[M+H]^+$ or adduct signals increased as the protonation exothermicity decreased (Fig. 2), with furan providing the more stable and largest signals, similar to our previous findings [20,21]. Fig. 3 presents the furan-CI mass spectra of the TMS-derivatives of cyanuric acid and melamine; the predominant protonated molecular clusters ($[M+H]^+$) were observed as base peaks. Moreover, the adduct ions of $[M+C_3H_3]^+$ and $[M+C_4H_4O]^+$ were formed, corresponding to ions of *m*/*z* 384 and 413 for tris-TMS-cyanuric acid (Fig. 3(a)), and *m*/*z* 381 for tris-TMS-melamine (Fig. 3(b)). Such adducts formation may improve the selectivity and sensitivity of the ion current signals.

Tandem mass spectrometric techniques have been suggested to reduce the effects of matrix interference and maximize the signal-to-noise ratio for ion-trap mass spectrometry [14,19,20,26]. To optimize the tandem-in-time MS/MS operating conditions, the ions at *m*/*z* 413 and *m*/*z* 343, from the furan-CI mass spectra of tris-TMS-cyanuric acid ($[M+68]^+$) and tris-TMS-melamine ($[M+H]^+$), respectively, were selected as precursor ions. Fig. 4 displays their resonant CID product-ion mass spectra; the resonant CID voltages were 0.6 and 0.55 V, respectively. Table 2 presents an overview of the single product ions used as quantitation ions for GC–furan-CI-MS/MS, and the two or three major ions used as confirmation ions for the GC–furan-CI-MS analysis.

3.3. Method performance and applications

To estimate the efficiency and the feasibility of applying the developed method to the analysis of powdered milk samples, the

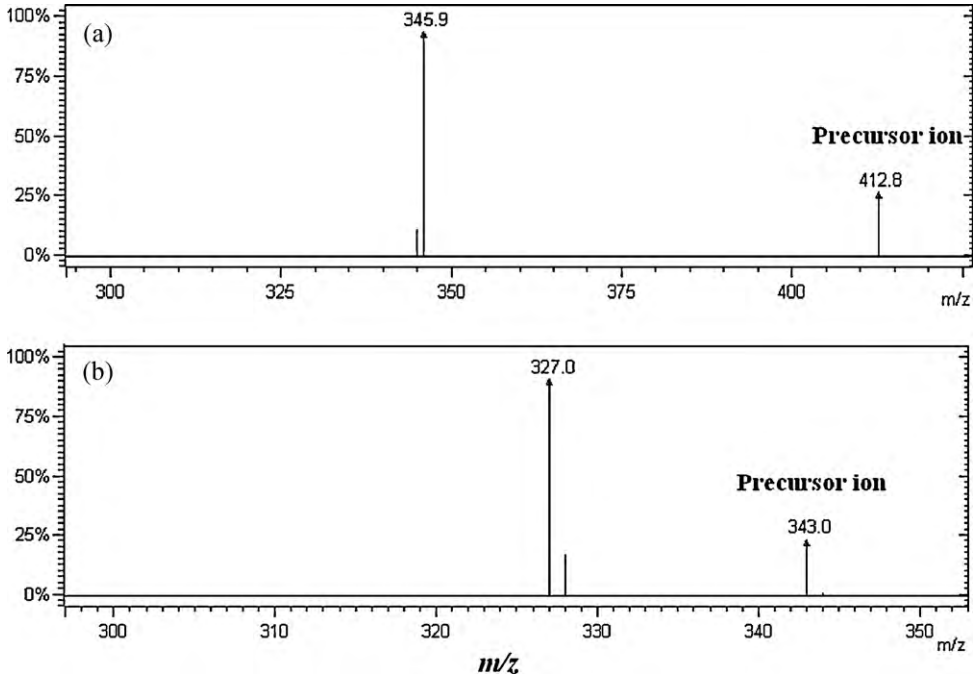

Fig. 4. Resonant CID product-ion mass spectra of the ions at (a) *m*/*z* 413 (formed from tris-TMS-cyanuric acid $[M+68]^+$) and (b) *m*/*z* 343 (formed from tris-TMS-melamine $[M+H]^+$).

Table 2
Detection characteristics, linear ranges, linearity, and limits of detection and quantitation.

Analyte	Retention time (min)	Furan-CI-MS/MS quantitation ion (*m/z*)	Furan-CI-MS confirmation ions (*m/z*)	Linear range (ng/mL)	RSD (%)	r^2	LOD (ng/g)	LOQ (ng/g)
Cyanuric acid	15.49	413 < 346 $[M+68-C_4H_4O]^+$	346, 384, 413	1–500	6.8	0.9980	0.5	1.0
Melamine	18.54	343 < 327 $[M+H-CH_4]^+$	343, 381	1–500	4.1	0.9992	0.2	0.5

Table 3
Spiked recoveries from various spiked concentrations in sample 4, and the concentrations of analytes detected in powdered milk samples.

Analyte	Spiked recovery (%)			Sample 1	Sample 2	Sample 3	Sample 4
	5 ng/g	50 ng/g	200 ng/g		Conc. (ng/g)		
Cyanuric acid	72[a] (18%)[b]	86 (9%)	90 (5%)	n.d. (85%)[c]	180[d]	17 (112%)[c]	n.d. (78%)[c]
Melamine	86 (11%)	82 (8%)	93 (6%)	88 (87%)[c]	1460	36 (82%)[c]	n.d. (104%)[c]

n.d. not detected at LOQ, as listed in Table 2.
[a] Spiked mean recovery (%, $n = 3$) at final concentrations of 5, 50 or 200 ng/g for each analyte.
[b] Relative standard deviations (%RSD) are given in parentheses ($n = 3$).
[c] Spiked recovery at final concentration of 50 ng/g.
[d] Original concentration (ng/g) of analytes found in samples ($n = 1$).

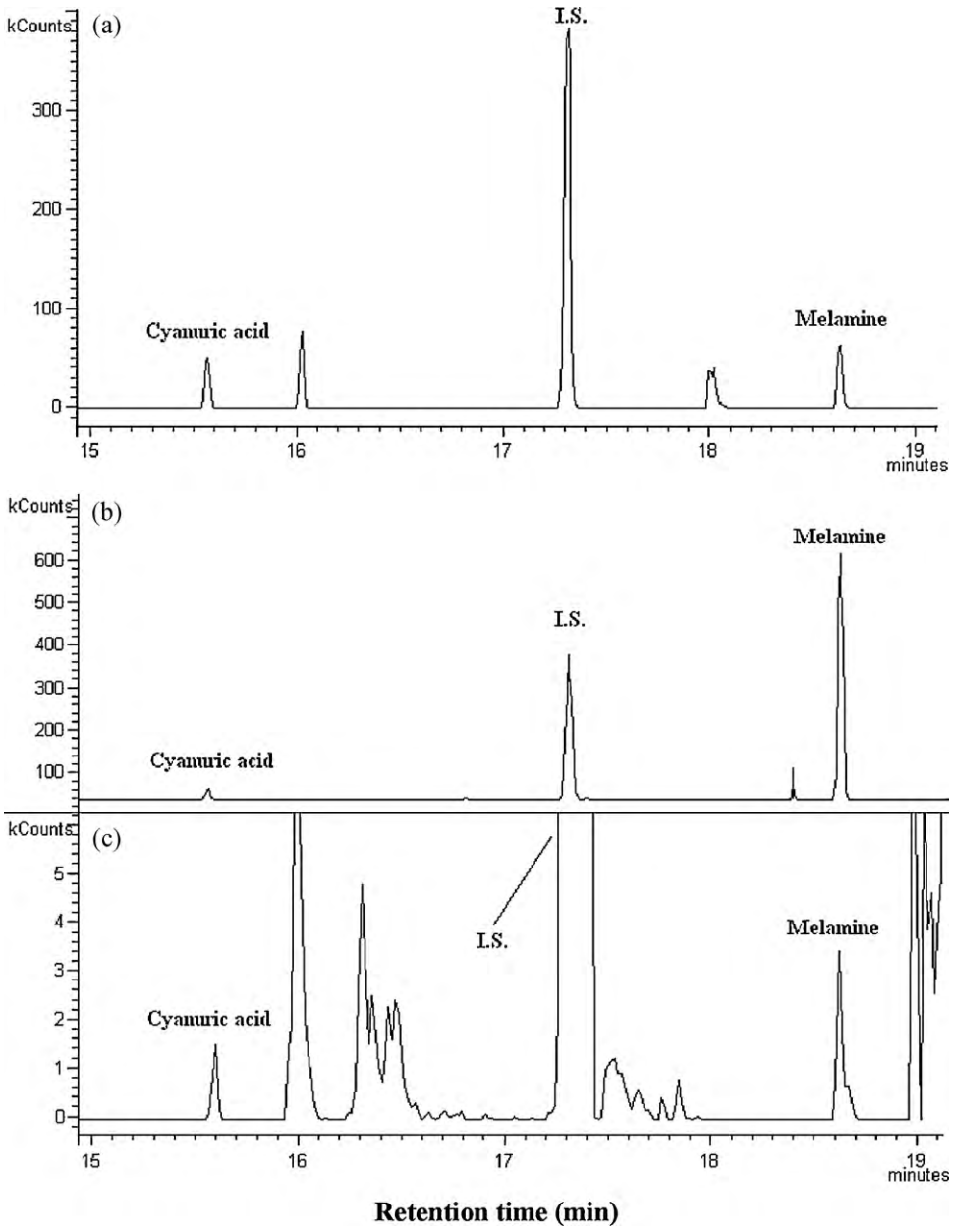

Fig. 5. GC–furan-CI-MS/MS chromatograms of (a) the spiked sample 4, and the non-spiked (b) sample 2 and (c) sample 3.

Table 4
Comparison of the present method with other previously developed techniques for the analyses of cyanuric acid and melamine in milk products.

Matrix	Extraction method	Derivatization	Detection	Recovery	LOQ	Ref.
Powdered milk	Ultra-sonication + n-hexane LLE	Injection-port (on-line) TMS (immediately)	GC–furan-CI-MS/MS	72–93%	0.5 and 1.0 ng/g	This study
Milk, milk products	Ultra-sonication	Off-line TMS (>50 min)	GC–EI-MS/MS	61–117%	5.0 ng/g	[12]
Milk	Hollow fiber sorptive extraction	Off-line TMS (>30 min)	GC–EI-MS-SIM	65–106%	1.0 ng/mL	[13]
Powdered milk	None	None	Surface-desorption APCI-MS/MS	93–116%	25 ng/g[a]	[28]
Liquid milk or yogurt	Ultra-sonication	None	CZE-DAD	93–104%	10–50 ng/mL (LOD)	[29]
Dog food	LLE, sonication, centrifugation, dilution	None	ELISA	74 ± 4%	9 ng/mL (LOD) (melamine)	[30]

LLE: liquid–liquid extraction; APCI: atmospheric pressure chemical ionization. CZE-DAD: capillary zone electrophoresis with diode array detection; ELISA: enzyme-linked immunosorbent assay.

[a] The LOQ was estimated from the calibration curve III, which was recorded from the powdered milk spiked with melamine (Fig. 5 in Ref. [28]).

analytical characteristics of the ultra-sonication sample preparation method coupled with GC–furan-CI-MS/MS in terms of its linear response range, repeatability, LODs and LOQs were investigated; and Table 2 lists the results. The ranges of linearity for cyanuric acid and melamine were calculated from five-level calibration curves (three replicates for each level) that had been calibrated using the internal standard [10–13]. The precisions of the curves, as indicated in terms of the relative standard deviations (RSDs) of the response factors (RFs), were 6.8% and 4.1%, respectively; the coefficient of estimation (r^2) exceeded 0.9980. The curve covered a range equivalent to the concentrations of the analytes in 0.5-g samples of powdered milk after the extracts had been concentrated to 100 μL. As for LODs, defined at a signal-to-noise (S/N) ratio of 3, the values of cyanuric acid and melamine were 0.5 and 0.2 ng/g in a 0.5-g sample, respectively, and the LOQs, defined as an S/N ratio of 10, were 1.0 and 0.5 ng/g, respectively. Method performance was evaluated by analyzing three sets of spiked powdered milk samples (sample 4); and Table 3 lists the results. The precision (RSD) was 18% or less; the accuracy, determined as the mean recovery, ranged from 72% to 93%. These data reveal that the ultra-sonication method coupled with injection-port derivatization GC–furan-CI-MS/MS ensures good repeatability with excellent linearity and sensitivity for the quantitation of cyanuric acid and melamine in powdered milk samples.

Table 3 lists the recovery rates of the matrix-spiked samples and the concentrations of cyanuric acid and melamine residues detected in four powdered milk samples. The recovery rates of the analytes ranged from 78% to 112%. The concentrations of cyanuric acid and melamine residues in these powdered milk samples ranged from undetectable to 1460 ng/g. Fig. 5 displays the GC–furan-CI-MS/MS chromatograms of (a) the spiked sample 4, and non-spiked (b) sample 2 and (c) sample 3. The European Commission Decision 200/657/EC introduced the concept of identification points (IPs) to set up quality criteria for the spectrometric identification and confirmation of organic residues and contaminants [21,27]. In the case of low-resolution MS technique coupled with GC (i.e., GC–MS), the EC Decision states that, at a minimum, three IPs are required for the confirmation of cyanuric acid and melamine residues. In this study, our analysis of the each target compounds involved the monitoring of two or three ions (as listed in Table 2, earning 2 or 3 IPs, respectively) using GC–CI-MS, and one precursor ion plus one product ion (earning 2.5 IPs each) using GC–CI-MS/MS techniques as shown in Table 2, resulting in a total of 5.5 IPs for cyanuric acid and 4.5 IPs for melamine.

Comparisons with the results of previous studies (Table 4) reveal that the LOQs achieved by our present method are lower than those obtained using other methods, but the recoveries of the spiked samples are not significantly different. Moreover, unlike off-line derivatization, injection-port derivatization provides directly TMS-derivatization coupled with sample injection and GC–MS detection; therefore, the additional reaction time is unnecessary. Although using surface-desorption APCI-MS/MS for the detection of melamine in milk products did not require sample pretreatment and derivatization procedures [28], the LOQ was relatively high, and the instrument (i.e., LTQ-MS) and its maintenance are expensive, and not readily available. The LOQs were also relatively high when capillary zone electrophoresis with diode array [29] or enzyme-linked immunosorbent assay (ELISA) [30] were applied to the detection of melamine in dairy products and dog food, respectively.

4. Conclusions

A simple and reliable injection-port derivatization method coupled with GC–furan-CI-MS/MS has been developed to determine cyanuric acid and melamine in powdered milk samples. This method provides good precision, a wide range of linearity, and sub-ng/g level detection limits when analyzing powdered milk samples. Injection-port derivatization appears to be a good alternative derivatization method for the determination of organics containing –OH and/or $–NH_2$ groups because it is a quick, effective, and eco-friendly. Using furan as the agent in CI-MS provides the most sensitive and selective detection, even higher than those obtained using EI-MS. A survey is currently being conducted throughout Taiwan to elucidate the occurrence of cyanuric acid and melamine residues in dairy products.

Acknowledgment

This study was supported by a grant from the National Science Council of Taiwan under contract No. NSC 97-2113-M-008-001-005-MY3.

References

[1] FDA, 2007, Interim melamine and analogues safety/risk assessment, May 25, http://www.cfsan.fda.gov/~dms/melamra.html.
[2] L. Chen, Q. Zeng, X. Du, X. Sun, X. Zhang, Y. Xu, A. Yu, H. Zhang, L. Ding, Anal. Bioanal. Chem. 395 (2009) 1533.
[3] W.C. Andersen, S.B. Turnipseed, C.M. Karbiwnyk, S.B. Clark, M.R. Madson, C.M. Gieseker, R.A. Miller, N.G. Rummel, R. Reimschuessel, J. Agric. Food Chem. 56 (2008) 4340.
[4] C.M. Karbiwnyka, W.C. Andersen, S.B. Turnipseed, J.M. Storey, M.R. Madson, K.E. Miller, C.M. Gieseker, R.A. Miller, N.G. Rummel, R. Reimschuessel, Anal. Chim. Acta 637 (2009) 101.
[5] M.S. Filigenzi, E.R. Tor, R.H. Poppenga, L.A. Aston, B. Puschner, Rapid Commun. Mass Spectrom. 21 (2007) 4027.
[6] Y.T. Wu, C.M. Huang, C.C. Lin, W.A. Ho, L.C. Lin, T.F. Chiu, D.C. Tarng, C.H. Lin, T.H. Tsai, J. Chromatogr. A 1216 (2009) 7595.
[7] Y.C. Tyan, M.H. Yang, S.B. Jong, C.K. Wang, J.T. Shiea, Anal. Bioanal. Chem. 395 (2009) 729.
[8] P.G. Stoks, A.W. Achwartz, J. Chromatogr. 168 (1979) 455.
[9] Y.C. Fiamegos, C.N. Konidari, C.D. Stalikas, Anal. Chem. 75 (2003) 4034.

[10] X. Zhu, S.H. Wang, Q. Liu, Q. Xu, S.X. Xu, H.L. Chen, J. Agric. Food Chem. 57 (2009) 11075.
[11] US FDA, GC–MS Screen for the Presence of Melamine, Ammeline, Ammelide and Cyanuric Acid, 2007, accessed at: http://www.fda.gov/cvm/gcmsmelamine.htm.
[12] H. Miao, S. Fan, Y.H. Wu, L. Zhang, P.P. Zhou, J.G. Li, H.J. Chen, Y.F. Zhao, Biomed. Environ. Sci. 22 (2009) 87.
[13] J. Li, H.Y. Qi, Y.P. Shi, J. Chromatogr. A 1216 (2009) 5467.
[14] W.H. Ding, S.H. Tzing, J. Chromatogr. A 824 (1998) 79.
[15] W.H. Ding, C.H. Liu, S.P. Yeh, J. Chromatogr. A 896 (2000) 111.
[16] W.C. Lin, H.C. Chen, W.H. Ding, J. Chromatogr. A 1065 (2005) 279.
[17] C.Y. Cheng, W.H. Ding, Rapid Commun. Mass Spectrom. 21 (2007) 1687.
[18] C.L. Hsu, W.H. Ding, Talanta 80 (2009) 1025.
[19] S.H. Tzing, A. Ghule, J.Y. Chang, Y.C. Ling, J. Mass Spectrom. 38 (2003) 401.
[20] S.H. Tzing, A. Ghule, J.Y. Liu, Y.C. Ling, J. Chromatogr. A 1137 (2006) 76.
[21] H.W. Chung, W.H. Ding, Anal. Bioanal. Chem. 395 (2009) 2325.
[22] Saturn 2000 GC/MS Advanced MS Techniques, Varian Associates, Inc, 1996.
[23] W.H. Ding, C.C. Chiang, Rapid Commun. Mass Spectrom. 17 (2003) 56.
[24] C.L. Hsu, C.Y. Cheng, C.T. Lee, W.H. Ding, Talanta 72 (2007) 199.
[25] L. Damasceno, R. Ventura, J. Ortuno, J. Segura, J. Mass Spectrom. 35 (2000) 1285.
[26] W.H. Ding, C.Y. Wu, Rapid Commun. Mass Spectrom. 15 (2001) 2193.
[27] EC, Commission Decision 2002/657/EC of 12 August 2002, Off. J. Eur. Commun. L211 (2002) 8–35.
[28] S.P. Yang, J.H. Ding, J. Zheng, B. Hu, J.Q. Li, H.W. Chen, Z.Q. Zhou, X.L. Qiao, Anal. Chem. 81 (2009) 2426.
[29] N. Yan, L. Zhou, Z.F. Zhu, X.G. Chen, J. Agric. Food Chem. 57 (2009) 807.
[30] E.A.E. Garber, J. Food Prot. 71 (2008) 590.