Talanta 88 (2012) 617–622

Contents lists available at SciVerse ScienceDirect

Talanta

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

Validation of an analytical methodology to quantify melamine in body fluids using micellar liquid chromatography

S. Marco-Peiró, B. Beltrán-Martinavarro, M. Rambla-Alegre, J. Peris-Vicente*, J. Esteve-Romero

Química Bioanalítica, QFA, ESTCE, Universitat Jaume I, 12071 Castelló, Spain

ARTICLE INFO

Article history:
Received 6 September 2011
Received in revised form 9 November 2011
Accepted 16 November 2011
Available online 23 November 2011

Keywords:
Direct injection
Melamine
Micellar liquid chromatography
Plasma
Urine

ABSTRACT

Measurement of urine and plasma melamine-concentration is helpful in confirming melamine-associated renal diseases. A chromatographic procedure using a C18 column and a micellar mobile phase of sodium dodecyl sulphate (0.2 M), buffered at pH 3 and detection set at 210 nm, was reported for the resolution and quantification of melamine in plasma and urine. In this work, direct injection was used, thus avoiding long extraction and experimental procedures. Melamine was eluted in nearly 6.3 min without overlapping the protein band or other endogenous compounds. The optimal mobile phase composition was taken by studying the influence of each chromatographic parameter. Validation was satisfactorily performed following the US Food and Drug Administration (FDA), in terms of: linearity (0.25–25 ppm; $r2 > 0.9995$ in both cases), sensitivity, limit of detection (50 ppb), limit of quantification (250 ppb), intra- and inter-day precision (R.S.D. 0.7–10.2% and 1.0–9.1%, respectively) and recovery, calculated as accuracy (85.7–103.8% and 94.8–103.6%, respectively) and robustness (R.S.D. < 7.1%). The suggested methodology has been applied to the analysis of real samples of volunteers, and no melamine was found in any of them.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, MW = 126.12 g/mol) is an inexpensive industrially synthesized organic compound with 66% nitrogen, which is used for a wide variety of applications. Humans are exposed to melamine from a wide number of sources, including food and environmental ones. They range from breakdown of the pesticide cyromazine, which is approved for use in many countries [1], to migration from approved food packaging material to the adulteration of specific foods [2]. A specific source of exposure for which very few data were available is carryover from the (mostly non-approved) presence of melamine in animal feed or feed ingredients. Data have shown carry-over from feed to products of animal origin [3].

In 2008, the intake of contaminated milk and derived products resulted in a large number of renal lithiasis in infants and young children in China. More than 50 000 infants were hospitalized and six deaths were confirmed. Melamine is added to increase the amount of organic-nitrogen, and then apparently augment the amount of protein, providing an economic incentive for their illegal addition [4–6].

Melamine causes crystals in urine. Many of the affected infants in the Chinese incident had stones, or calculi, in the kidney, ureter or bladder. These calculi were composed of uric acid (a normal waste product in human urine) and melamine. Melamine has also shown to be carcinogenic for male rats [7]. Melamine is rapidly absorbed and is eliminated essentially unchanged by the kidney [8]. There is not expectable melamine concentration in urine or plasma, as the amount depends on the degree of contamination of food. Due to the potential transfer of melamine from a remarkable number of different sources authorized plus any illegal tampering directly or indirectly products for human consumption, it is necessary to develop a simple, economical, and efficient method to quantify melamine in body fluids in order to insure human health.

Several analytical methods have been developed for the determination of melamine in food, especially in milk and derived products, mainly using chromatographic instrumentation [6]. In recent years several methods based on: voltammetry [9], GC/MS [5,9], immunoassay analysis (ELISA) [5], HPLC-DAD [10,11] and HPLC–MS [12–15], have been developed for the quantification of melamine in a wide range of biological matrices of body fluids, as urine and plasma of pigs [13], goat blood [14], hen plasma [11], human urine [9,12], chicken body fluids [5], pig plasma [10] and rat plasma [15]. However, due to the complexity of biological matrices, these analytical methodologies involve time-consuming extraction, preconcentration and purification steps, or needs expensive and easy-to-contaminate instrumentation (mass spectrometry).

* Corresponding author. Tel.: +34 964 728099; fax: +34 964 728066.
E-mail address: vicentej@qfa.uji.es (J. Peris-Vicente).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.11.047

Moreover, because of the need of high selectivity, mobile phases are programmed as gradients which therefore make the analysis of a large amount of samples a difficult task.

Micellar solvents and micellar mobile phases can solubilize matrix compounds, thus allowing direct injection of the sample [6]. This avoids extraction and reduces the experimental procedure. Moreover, it allows the resolution of complex mixtures using isocratic mode [16–19]. Micellar liquid chromatography has been used to the quantification of compounds in biological matrices, such as plasma [20], urine and serum [21] and melamine in milk [6]. However, all samples must be filtered, and blood samples should be deproteinized, in order to improve the duration of the column [22].

The aim of this work is to perform an easy, fast, accurate and reliable analytical methodology to quantify the level of melamine in plasma and urine without any previous pretreatment, using solvents and mobile phases containing the anionic surfactant sodium dodecyl sulphate. The analyte has to be resolved from other compounds of the matrix with high sensitivity. The proposed method must be validated following FDA indications in terms of limit of detection, sensitivity, linearity, precision, accuracy, repeatability, reproducibility, robustness and recovery [23].

2. Materials and methods

2.1. Chemical and reagents

Melamine (99% purity) was purchased from Aldrich (St. Louis, MO, USA). Sodium dodecyl sulphates (SDS, 99%purity) were from Merck (Darmstadt, Germany). Sodium dihydrogenophosphate monohydrate and HCl were ordered from Panreac (Barcelona, Spain). NaOH was obtained from Scharlab (Barcelona). Methanol was bought from J.T. Baker (Deventeer, The Netherlands) and 1-propanol came from Scharlab. Ultrapure water was used throughout (Millipore S.A.S., France).

2.2. Instrumentation

Chromatographic separation was performed in an Agilent Technologies Series 1100 system (Palo Alto, CA, USA) equipped with a degasifier, an isocratic pump, an autosampler and a diode-array detector (range 190–700 nm). The stationary phase was a Kromasil C18 column (150 mm × 4.6 mm, 5 μm particle size, Scharlab). A Metter Toledo analytical balance (Greifense, Switzerland) was used to weigh the analyte. The pH was measured with a Crison potentiometer (Barcelona) equipped with a combined Ag/AgCl/glass electrode. The pH was adjusted using HCl 6 M and 0.1 M, as well as, NaOH 1 M and 0.1 M. To dissolve the standards an ultrasonic bath was used (model Ultrasonds-H, Selecta, Abrera, Spain). The mobile phases and the injected solutions were filtered through 0.45 μm nylon membranes.

2.3. Chromatographic conditions

Several mobile phases were tested by varying the SDS concentration and the use of organic modifier. The selected pH was 3 [6]. The optimal mobile phase composition was 0.2 M SDS at pH 3, which was run in the isocratic pump with a flow of 1 mL/min at room temperature. The injection volume was 20 μL and detection was set at UV wavelength 210 nm [6]. Chromatographic signals were acquired and processed with an Agilent ChemStation (REV. B.03.01). The special care of the chromatographic instrumentation using micellar mobile phases is described in [6]. Under these conditions, column was a life span of nearly 1000 injections.

2.4. Mobile phase, standard and sample preparation

Micellar mobile phases were prepared by solving the appropriate amount of SDS and disodium monohydrogen-phosphate in ultrapure water, the pH was adjusted to the desired value and 1-propanol was added, if necessary. Finally, the solution was adjusted to the desired volume with ultrapure water, ultrasonicated and filtrated.

Blood samples were collected using a DB SST tube (BD vacutainer systems, UK). Ethylendiaminetetraacetic acid (EDTA) was added as anticoagulant and samples were centrifuged for 5 min at 3000 rpm at 4 °C, and then precipitated protein was separated from liquid. Obtained plasma was stored at −21 °C, it was then thawed just before use. Plasma samples were provided by the Hematology Department of the “La Plana” Hospital of Castelló. Urine samples were collected in a urine collection cup (BD vacutainer systems). Samples were provided by volunteers in good health from the University Jaume I, which do not intake drugs. For all of them, plasma and urine were taken.

Stock solutions of melamine 1; 100 and 200 ppm were prepared by dissolving the appropriate amount of the solid analyte in methanol.

Spiking the plasma and urine samples were done by adding the appropriate amount to 1 ml of plasma or urine (previously diluted by a factor of 1:5, using 0.05 M SDS at pH 3) and making it up to 10 ml with a solution of 0.05 M SDS at pH 3. Spiked samples were vigorously shaken and kept for 1 day at 5 °C to favor mixture between analyte and matrix [24]. The concentration of the spiked sample refers to the amount in melamine in the undiluted sample of plasma and urine. All samples were filtered directly through 0.45 μm nylon membranes before injection into the autosampler vials.

3. Results and discussion

The optimization and validation was made using plasma and urine voluntarily provided by white Caucasian male of 45 years old in good health.

3.1. Mobile phase selection and chromatographic optimization

The methodology shown in [6] was applied to the determination of melamine in plasma and urine. However, urine shows other endogenous compounds, which overlap with melamine. On the other hand, plasma shows a baseline without other peaks, so that the retention time could be shortened from that in [6]. Then an optimization was necessary to adapt the separation parameters to the studied matrices. The same analytical methodology is developed for both matrices, to allow the clinical laboratory to simultaneously analyze plasma and urine samples.

Melamine is a polar compound ($\log Po/w = -1.14$) [6], then using a C18 column and a pure micellar mobile phases would provide an adequate retention time [6]. Several mobile phases with SDS different concentrations at pH 3 were tested. An organic modifier (1-propanol) was tested, but it does not provide a satisfactory improvement of the separation conditions from other compounds. Finally, it was observed that a 0.2 M SDS concentration provides an adequate resolution of melamine. The absence of an organic modifier also reduces the toxicity and makes less pollutant the mobile phase. Under these conditions, the chromatographic parameters for melamine were: retention time 6.3 min, capacity factor 5.6; efficiency $N = 3246$ (number of theoretical plate and asymmetry $B/A = 1.3$) (ratio between peak apex to back side distance and apex to front side distance at 10% peak height).

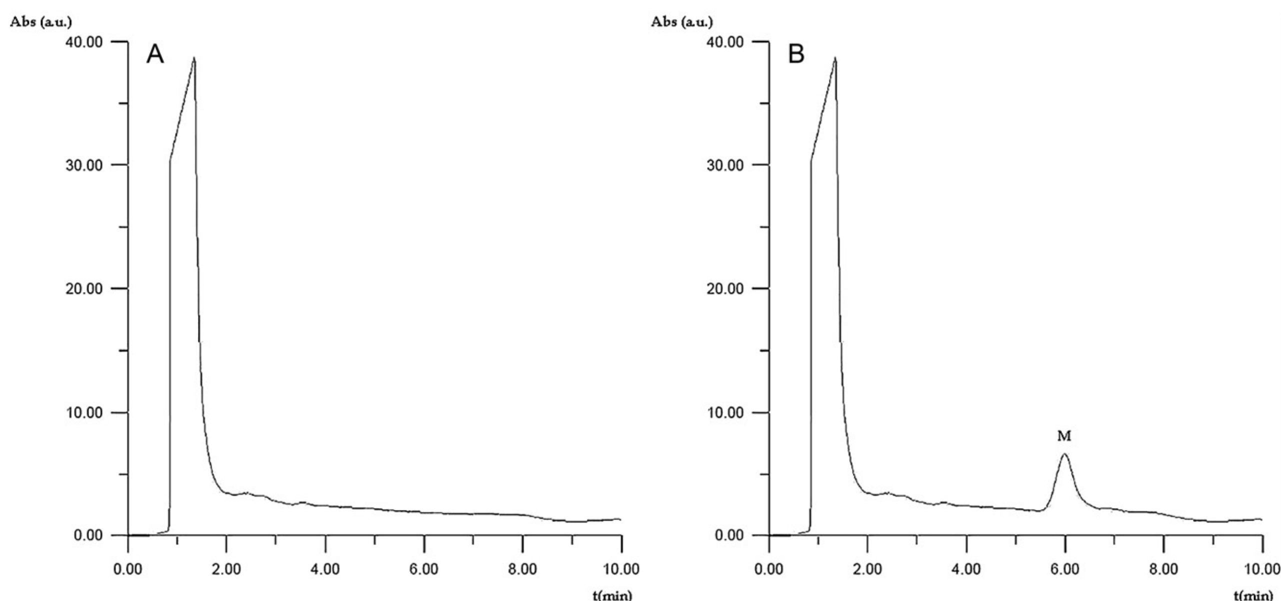

Fig. 1. Chromatograms obtained by the analysis using the proposed methodology of plasma (A) blank and (B) spiked with 2.5 ppm of melamine. Stationary phase was a Kromasil C18 column. The mobile phase was an aqueous solution of 0.2 M SDS buffered with sodium dihydrogenophosphate at pH 3, running at 1 mL/min at room temperature. Detection was set at 210 nm.

3.2. Method validation

Validation was performed according to FDA guidelines [23]. The spiked concentrations indicated in the validation reefer to the amount of melamine in the undiluted sample of plasma or urine.

3.2.1. Selectivity

Melamine-free plasma and urine samples were selected as controls and processed directly in the chromatographic system. They were then analyzed to determine the extent to which endogenous components may contribute to interfere with retention time of the melamine. Then they were compared to spiked samples to evaluate the resolution of the method. No interference from endogenous compounds was found in plasma (Fig. 1) neither in urine (Fig. 2).

The chromatograms obtained for analysis of plasma sample are cleaner than those for urine, despite plasma components are more complicate that those in urine, and no cleaning extraction procedure has been carried out. However, the maximum height of the front of chromatogram was ≈2000 a.u. for plasma samples and ≈70 a.u. for urine samples. Then the total amount of compounds is in fact higher for plasma matrix, but these compounds elute near the dead time. This is caused by the use of micellar mobile phases, which tensioactive interacts with plasma compounds and provoke their fast elution [20,22]. In the case of urine, less amount of compounds are in the matrix, but they interaction with SDS is less effective, so that they show higher retention times.

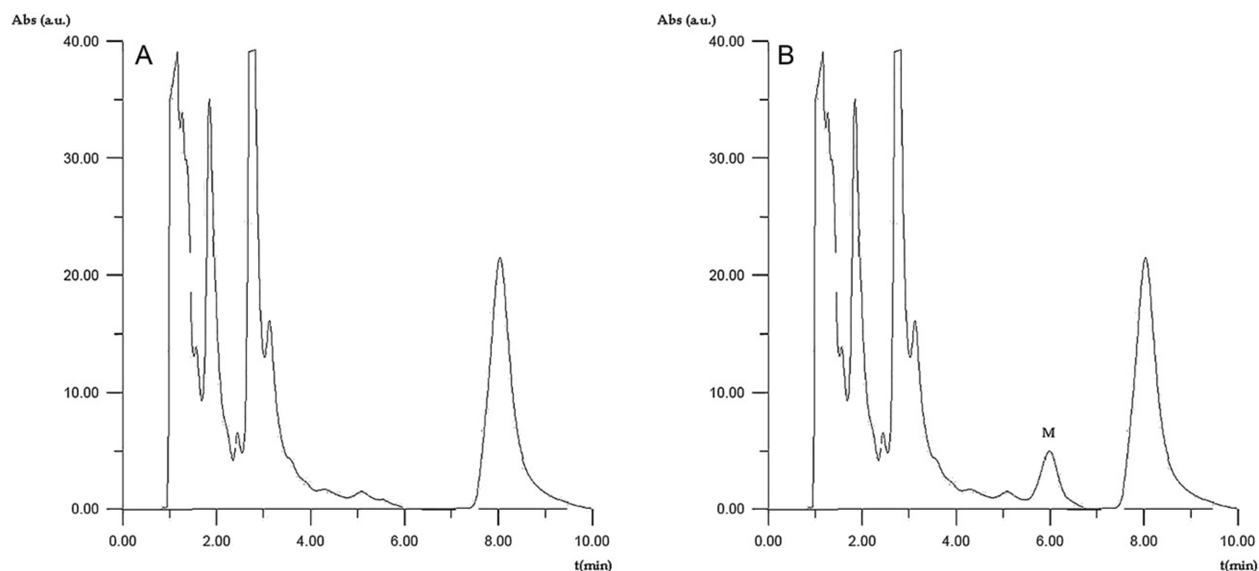

Fig. 2. Chromatograms obtained by the analysis using the proposed methodology of urine (A) blank and (B) spiked with 2.5 ppm of melamine. Chromatographic conditions were as in Fig. 1.

Table 1
Inter-day and intra-day precision and accuracy of melamine in urine and plasma.

Matrix	Added concentration (ppm)	Found[a] (mean ± SD) (ppm)	Accuracy (%)	Intra-day C.V. (%)	Found[b] (mean ± SD) (ng/mL)	Accuracy (%)	Inter-day C.V. (%)
Urine	0.25[c]	0.233 ± 0.009	93.2	9.3	0.25 ± 0.02	98.9	9.1
	1	0.92 ± 0.04	92.2	4.3	0.94 ± 0.04	93.6	4.3
	5	5.02 ± 0.04	100.4	0.8	5.00 ± 0.05	99.9	1.0
	10	10.38 ± 0.07	103.8	0.7	10.35 ± 0.11	103.5	1.1
Plasma	0.25[c]	0.214 ± 0.005	85.7	10.2	0.24 ± 0.04	94.8	9.1
	1	0.948 ± 0.004	94.8	4.2	0.948 ± 0.003	94.8	3.2
	5	5.2 ± 0.3	103.7	5.8	4.98 ± 0.02	99.6	4.0
	10	10.2 ± 0.6	101.7	5.9	10.36 ± 0.17	103.6	4.8

[a] $n = 6$.
[b] $n = 5$.
[c] $n = 9$.

3.2.2. Linearity and sensitivity

Calibration curves were constructed using the area of the chromatographic peak at several different concentrations between 0.25 and 25 ppm (0.25, 0.5, 1, 5, 10, 15, and 25 ppm, considering the dilution factor) in two different matrices: plasma (1:5 dilution factor) and urine (1:5 dilution factor). Each calibration level was analyzed 6 times, except for 0.25 ppm level, which was analyzed nine times. Calibration was repeated five times (preparing the samples on each occasion) in two months. The regression curve, taken as the average of the obtained five calibration curves, was:

$$\text{For plasma}: A = (0.83 \pm 0.04)[\text{melamine}] + (-0.07 \pm 0.02), \quad R^2 = 0.9995$$

$$\text{For urine}: A(0.86 \pm 0.04)[\text{melamine}] + (-0.114 \pm 0.006), \quad R^2 = 0.9996$$

where A is in the arbitrary units and the concentration amount is provided in ppm. LOD was set as the 3 s criteria (three times the standard deviation obtained for the solution with the lowest concentration included in the calibration curve). For both studied matrices, LOD was 50 ppb.

LOQ was the lowest studied concentration in which acceptable precision and accuracy level are obtained, in this case 0.25 ppm for both matrices. These studies were performed intra- and inter-day separately for urine and plasma, by analyzing nine replicates of samples spiked at 0.25 ppm. As seen in Table 1, results were in agreement with FDA guidelines, which establish a maximum range of 80–120% for accuracy and 20% R.S.D. for precision [23]. Fig. 3 shows chromatograms obtained by the analysis of samples of 0.25 ppm-spiked plasma and urine, where it can be seen that the chromatographic peak of melamine is quantifiable. This value was then taken as the lowest concentration of the calibration curve.

3.2.3. Precision, accuracy and recovery

The intra- and inter-day accuracy (calculated as recovery) and precision of the proposed methodology were determined in plasma-SDS (1:5 dilution factor) and urine-SDS (1:5 dilution factor) to four different concentrations (0.25, 1, 5 and 10 ppm); results were very similar in all matrices.

The intra-day analysis was determined by injecting these test solutions six times on the same day, except for LOQ (0.25 ppm), which was analyzed nine times. The inter-day analysis corresponded to the average of five measurements of the intra-day values taken 5 days over a 3-month period. The results, expressed as the percentage of the relative standard deviation, for intra-and inter-day values are depicted in Table 1. In all cases, the precision was R.S.D. <9.3% and accuracy was 85.7–103.8%, proving that the developed methodology is suitable for routine analysis. Results were according to FDA guidelines, which establish a maximum limit for accuracy (error) and precision (R.S.D.) of 20% at LOQ and 15% at higher levels.

3.2.4. Robustness

The robustness of the method was examined by analyzing a spiked plasma and urine (both 1:5 dilution) by making slight changes to the following parameters: SDS concentration, flow rate and pH. The variation of the sensitivity (area) and retention time were considerate, and the results are shown in Table 2. Retention time (R.S.D. < 5.0%) and area (R.S.D. < 7.1%) were in all cases not strongly affected by slight variations in the composition of the mobile phase.

3.2.5. Comparison with an established method

The proposed method was compared with the previous published work developed by the authors about the quantification of melamine in milk [6] (Table 3). It should be indicated that the present paper cannot be considered as an improvement of [6], it is the application of the sample methodology to other matrices, in this case urine and serum.

Retention time was lower in the present work, which allows the analysis of more samples. The calibration curves have been found more reliable. The sensitivity is similar, and the present work was more precise and accurate. Moreover, the resolution and the asymmetry have been improved for the present method.

Table 2
Evaluation of the robustness of the MLC method.

Matrix	Changes of mobile phase parameters	Level	Retention time (min) (R.S.D. (%))	Area (arbitrary unit) (R.S.D. (%))
Urine	Concentration SDS (M)	0.0195–0.0205	6.01 ± 0.17 (2.7%)	4.5 ± 0.3 (6.7%)
	pH	2.9–3.1	6.01 ± 0.02 (0.4%)	4.4 ± 0.2 (4.6%)
	Flow (mL/min)	0.95–1.05	6.0 ± 0.3 (5.0%)	3.81 ± 0.17 (4.5%)
Plasma	Concentration SDS (M)	0.0195–0.0205	3.04 ± 0.16 (2.7%)	4.4 ± 0.2 (4.6%)
	pH	2.9–3.1	6.01 ± 0.8 (0.22%)	4.2 ± 0.3 (7.1%)
	Flow (mL/min)	0.95–1.05	6.1 ± 0.3 (4.9%)	4.2 ± 0.3 (7.1%)

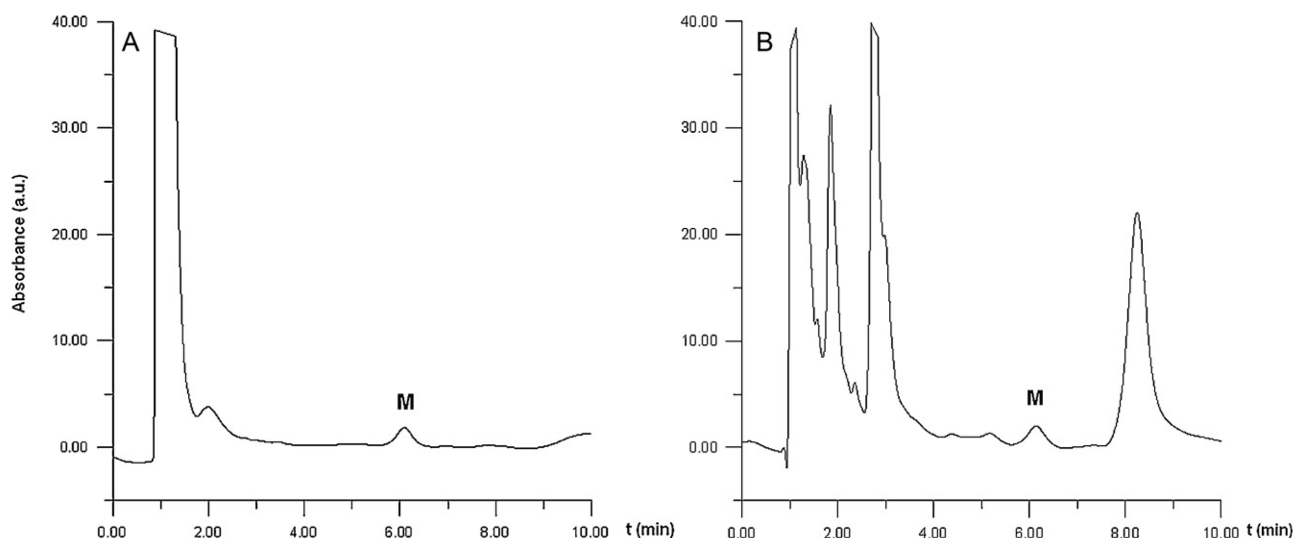

Fig. 3. Chromatograms obtained by the analysis of samples of (A) urine and (B) plasma, spiked at LOQ = 0.25 ppm.

3.3. Analysis of real samples

Samples of volunteers from the University have been taken: five males (31–55 years old, 4 white Caucasian and one Indian) and five females (22–45 years old, 4 white Caucasian and one Indian), all in good health. In all cases, no peak in the range 5.5–7 min was found, so that no melamine has been detected.

3.4. Comparison to other methods

Authors previously develop a useful micellar liquid chromatography based-method to detect melamine in milk [6], also successfully validated following FDA guidelines. The same experimental conditions were initially proposed to the quantification of melamine in urine and plasma. Stationary phase, pH and detection wavelength were kept, but the mobile phase composition would not be able to elute melamine separately from urine matrix compounds. Then a complete study was performed to found the optimal mobile phase, by testing several mobile phases, varying the SDS amount and concentration of 1-propanol, and then comparing the characteristics of the peaks (efficiency, elution time and asymmetry). Selected mobile phase composition was that indicated in section 3.1 (0.2 M SDS without organic modifier). The same mobile phase was proposed for plasma and urine, to allow the clinical laboratories to simultaneously analyze these two kinds of samples. Furthermore, milk has more compounds in suspension than plasma and urine, so that the filtration of the sample can be made reducing the dilution factor. The dilution factor was modified from 1:10 (in [6]) to 1:5, which allows improving the sensitivity. It cannot be considered than the present paper use applies methodology proposed in [6] to other samples, because the main experimental parameters (mobile phase and dilution) have been strongly changed to quantify melamine in human plasma and urine.

Due to the changes in the experimental parameters, the suggested methodology validation was remade: LOD was maintained to 50 ppb (as in [6]), maximal linearity level was reduced, because melamine in biological fluids should be higher than in directly adulterated milk samples, precision was significantly reduced (R.S.D. < 5.9% here and <9.7% in [6] at similar levels), accuracy was higher (92.2–103.8% here, 90.2–106% in [6] at similar levels), and the robustness was improved (R.S.D. < 7.1 here, <7.4% in [6]). Moreover, melamine was eluted at 6.3 min in the methodology proposed and 9.3 min in [6], this allows the analysis of a higher amount of samples at the same time. The here suggested mobile phase do not include modifiers, making these analytical method more environment-friendly.

The suggested methodology was compared with other which analyzes melamine in blood plasma by HPLC–UV [11]. The experimental procedure was significantly shortened, as [11] needs extraction with diethyl ether, vortexation, precipitation with trifluoroacetic acid and lead acetate, sonicated and eluted through a mixed-type cation-exchange column. Sensitivity was found higher, as LOD was 0.2 ppm in [11] and 50 ppb in the present work, and LOQ were 0.5 and 0.25 ppm, respectively. The linearity was more reliable in the present work, as r^2 was 0.9995 here and 0.9989 in [11]. The here proposed method obtains a higher precision (R.S.D. < 5.9%) than in [11] (R.S.D. < 6.1%) and was also more accurate (92.2–103.8%) than [11] (85.8–92.0%), considering similar spiking levels. The here suggested analytical method was more environmental friendly, as no organic solvent is used in mobile phase, while [11] uses acetonitrile. Then the proposed methodology show significant improvements compared to published methodologies.

Table 3
Comparison between the analytical parameters of the present work and those obtained for [6].

Analytical parameters	Present work	[6]
Retention time (min)	6.3	9.3
R^2	>0.9995	0.9990
LOD (ppb)	50	50
LOQ (ppb)	250	250
Precision (R.S.D., %)	<5.9	<9.7
Accuracy (%)	92.2–103.8	91.4–106
Efficiency (N)	3246	2015
Asymmetry (B/A)	1.3	1.5

The results have been shown considering the dilution factor in both cases.

4. Concluding remarks

Micellar liquid chromatography is a suitable technique for the analysis of melamine in plasma and urine with an analysis time below 7 min. Validation was performed according to FDA guidelines with satisfactory results in linearity, selectivity, precision, accuracy, robustness and recovery studies. One advantage of the procedure is the possibility of injecting the diluted samples into the chromatographic system after filtration, thus avoiding long and tedious extractions. This method meets the requirements of the “green chemistry” concept since lower quantity of organic solvents has

been used. Besides, it is relatively inexpensive compared to other methods, thus making it more attractive.

Acknowledgements

This work has been supported by a project from the Spanish Ministry of Education and Science (MEC) CTQ 2007 764473/BQU and the Foundation Caixa-Castelló, Bancaixa P1-1B2006-12. María Rambla-Alegre also wishes to acknowledge the MEC for the FPU grant.

References

[1] R.A. Yokley, L.C. Mayer, R. Rezaaiyan, M.E. Menuli, M.W. Cheung, J. Agric. Food Chem. 46 (2000) 3352.
[2] A.M. Cook, R.S. Hutter, J. Agric. Food Chem. 23 (1981) 1135.
[3] G. Bizzarri, K. Yokose, *Report of a WHO Expert Meeting In collaboration with FAO*, supported by Health Canada, 2008.
[4] S. Yang, J. Ding, J. Zheng, B. Hu, J. Li, H. Chen, Z. Zhou, X. Qiao, Anal. Chem. 81 (2009) 2426.
[5] Z. Wang, X. Ma, L. Zhang, W. Yang, L. Gong, P. He, Z. Li, Anal. Chim. Acta 662 (2010) 69.
[6] M. Rambla-Alegre, J. Peris-Vicente, S. Marco-Peiró, B. Beltrán-Martinavarro, J. Esteve-Romero, Talanta 81 (2010) 894.
[7] M. Okumura, R. Hasegawa, T. Shirai, M. Ito, S. Yamada, S. Fukushima, Carcinogenesis 13 (1992) 1043.
[8] R.W. Mast, A.R. Jeffcoat, B.M. Sadler, Chem. Toxicol. 21 (1983) 807.
[9] T.H. Tsai, S. Thiagarajan, S.M. Chen, J. Agric. Food Chem. 58 (2010) 4537.
[10] R.E. Baynes, G. Smith, S.E. Mason, E. Barrett, B.M. Barlow, J.E. Riviere, Food Chem. Toxicol. 46 (2008) 1196.
[11] X. Bai, F. Bai, K. Zhang, X. Lv, Y. Qin, Y. Li, S. Bai, S. Lin, J. Agric. Food Chem. 58 (2010) 5414.
[12] M. Zhang, S. Li, C. Yu, G. Liu, A.J. Ji, C. Lu, J. He, Y. Ma, J. Zhu, C. Yu, J. Chromatogr. B 878 (2010) 758.
[13] M. Li, L. Gong, H. Wu, L. Zhang, B. Shang, Y. Chen, J. Anim. Vet. Adv. 9 (2010) 2089.
[14] R.E. Baynes, B. Barlow, S.E. Mason, J.E. Riviere, Food Chem. Toxicol. 48 (2010) 2542.
[15] F. Yang, Y. Mao, X. Zhang, Z. Ma, X. Zhang, J. Sep. Sci. 32 (2009) 2974.
[16] M. Rambla-Alegre, J. Peris-Vicente, J. Esteve-Romero, S. Carda-Broch, Food Chem. 123 (2010) 1294.
[17] M. Rambla-Alegre, J. Peris-Vicente, J. Esteve-Romero, M.E. Capella-Peiró, D. Bose, Anal. Chim. Acta 666 (2010) 102.
[18] M.L. Chin-Chen, D. Bose, J. Esteve-Romero, J. Peris-Vicente, M. Rambla-Alegre, S. Carda-Broch, Open Anal. Chem. J. 5 (2011) 22.
[19] M. Rambla-Alegre, S. Marco-Peiró, J. Peris-Vicente, B. Beltrán-Martinavarro, M.A. Collado-Sánchez, S. Carda-Broch, J. Esteve-Romero, Food Chem. 129 (2010) 614.
[20] J. Esteve-Romero, E. Ochoa-Aranda, D. Bose, M. Rambla-Alegre, J. Peris-Vicente, A. Martinavarro-Domínguez, Anal. Bioanal. Chem. 397 (2010) 1557.
[21] M. Rambla-Alegre, M.T. Gil-Agustí, M.E. Capella-Peiró, S. Carda-Broch, J. Esteve-Romero, J. Chromatogr. B 839 (2006) 89.
[22] E.O. Aranda, J. Esteve-Romero, M. Rambla-Alegre, J. Peris-Vicente, D. Bose, Talanta 84 (2011) 314.
[23] Guidance for Industry. Bioanalytical, Method Validation, U.S. Department of Health and Human Services. Food and Drug Administration, Rockville, MD, USA, 2001, http://www.fda.gov/cder/guidance/4252fnl.pdf.
[24] G. Cano-Sancho, S. Marin, A.J. Ramos, J. Peris-Vicente, V. Sanchis, Rev. Iberoam. Micol. 27 (2010) 130.