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Pretreatment-free fast ultraviolet detection of melamine in milk products with a disposable microfluidic device

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ABSTRACT

A new method for sensitive and fast screening of melamine (MEL) in milk products was developed with a low-cost disposable microfluidic device coupled with ultraviolet (UV) detection. This method avoided the need of sample pretreatment prior to the separation process, thus was simple and green. Due to the advantages of the device and fracture sampling technique, milk sample could be directly sampled through the fracture to achieve baseline separation from amino acids, and proteins in the sample did not interfere with the detection. Using 20 mM phosphate running buffer (pH 9.0), a sampling time of 3 s at +180 V and a separation voltage of +1800 V (240 V/cm), this method could detect MEL in milk within 75 s. At the detection wavelength of 202 nm, the linear range for MEL was from 1.0 to 100 μg mL^{-1} with a detection limit of 0.23 μg mL^{-1} ($S/N = 3$). The novel protocol had been successfully used to screen MEL in milk samples with recovery more than 82%. The environmentally friendly methodology for pretreatment-free sensitive screening of MEL provided promising applications in monitoring the quality of different foods.

1. Introduction

Recently, melamine (MEL), a triazine-based industrial chemical, has been found in pet food and many milk products. Due to the harm to health caused by its illegal addition in milk products, its determination has attracted considerable attention [1–4]. Many methods have been developed for the screening or detection of MEL in various matrices. The traditional method for screening MEL in milk products or animal tissues was high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [4–6]. Methods such as gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) have been developed for more authoritative determination of MEL [7–13]. The ability of MS techniques for component verification and high sensitive detection enabled them to be extensively used for MEL analysis. For example, a tandem mass spectrometric method combined with ambient ionization using a low-temperature plasma probe has been applied for high-throughput trace melamine analysis in complex mixtures [14], a MS method by surface desorption atmospheric pressure chemical ionization has been used to detect MEL in Milk [15], and a matrix-assisted laser desorption/ionization MS technique has been developed for analyzing melamine cyanurate in urine [16].

Recently two capillary zone electrophoresis (CZE) methods coupled with UV detection [17] or diode array detection [18], two Raman spectroscopic methods [19,20], and a visual detection method by hydrogen-bonding recognition-induced color change of gold nanoparticles [21] have been developed for melamine detection in raw milk and infant formula. Several commercial enzyme-linked immunosorbent assay (ELISA) test kits for the detection of triazines have also been available [22]. All these methods mentioned above need complex sample pretreatments such as extraction, preconcentration and derivatization. The sample pretreatment procedure is time-consuming, and always needs toxic solvents, e.g. dichloromethane, nitrile, methanol or trichloroacetic acid. Moreover, the extraction process often leads to low recovery of melamine [22]. Although several pretreatment-free methods such as extractive electrospray ionization MS [23] and near-/mid-infrared spectroscopic method [24] have recently been developed for in situ analysis of MEL, they are relatively expensive for general investigation of MEL in milk products. Moreover, the infrared spectroscopy is relatively weak in quantitative and sensitive analysis. Thus it is still urgent to develop cheaper, faster and green techniques for extensive screening, particularly in situ detection, of MEL in milk products. This work presented a novel pretreatment-free method for sensitively screening MEL by using a disposable microfluidic device developed in our group [25].

Microfluidic electrophoresis device (MED) is a powerful tool for analytical application due to its low consumption of reagents, short separation time, high separation efficiency, and low cost [26–28].

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Its fabrication is also cheap and convenient by using polymer materials such as polydimethylsiloxane [29], polymethylmethacrylate (PMMA) [30], or polycarbonate [31] as matrix. In view of the shortage of these matrices in UV detection [32], our previous work used a fused-silica capillary as separation channel to develop a hybrid quartz capillary/PMMA MED (HQM) by using PMMA as support substrate [33]. This technique avoided the requirements of cleanroom facilities, corrosive etchants and time-consuming bonding for the preparation of sampling and separation channels [34]. Based on the integration of all advantages of the HQM [25,33,34] with the properties of fracture sampling technique [34], such as negligible sample leakage, efficient sample self-stacking and high separation efficiency, this work developed a fast and environmentally friendly strategy for low-cost and sensitive UV screening of MEL by directly sampling milk sample into separation channel. The ultra narrow sampling fracture and high separation efficiency led to excellent analytical performance of the proposed method, thus it could be widely applied in fast and low-cost in situ screening of MEL in different milk products.

2. Experimental

2.1. Reagents and materials

MEL with 99% purity was purchased from Acros. All aqueous solutions were prepared using ≥18 MΩ ultrapure water (Milli-Q, Millipore). The phosphate running buffer was passed through a membrane filter (0.22 μm pore size) and dealt with ultrasonic for removing air bubbles prior to use. Fused-silica capillaries (360 μm o.d., 50 μm i.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China). All other chemicals were of analytical grade. Milk samples were commercially available in Nanjing. The MEL contents in the samples were measured according to the standard methods [4] by the Center for Analysis and Testing, Nanjing Normal University.

2.2. Equipments

The UV microfluidic workstation employed in this work was home manufactured in cooperation with Beijing Cailu Scientific Instrument Limited Company. It was composed of an eight-port high-voltage power supply, a UV detector and data processor [33]. Ultrasonic disintegrator with a 2-mm o.d. probe from Ningbo Scientz Biotechnology Co., Ltd (Ningbo, China) was used to prepare the sampling fracture. An inverted fluorescence microscope (Nikon Eclipse TE2000-U) was used to observe the fracture. Scanning electron microscopic (SEM) images of the sampling fracture were obtained on a Hitachi S-4800 scanning electron microscope (Japan).

2.3. Fabrication of HQMs

The HQMs were fabricated according to a modified process reported previously [33]. Briefly, a printed circuit board (PCB, 85 mm × 35 mm × 1 mm for length × width × thickness) and a PMMA board (85 mm × 10 mm × 2 mm for length × width × thickness) with a groove were firstly prepared with usual techniques, and four holes were drilled on the PMMA board as buffer reservoir (BR, 3-mm i.d.), sample reservoir (SR, 3-mm i.d.), detection reservoir (DR, 2-mm i.d.) and waste reservoir (WR, 4-mm i.d.), respectively. The machined PMMA board was then glued on the PCB with the groove outside for fixing a pretreated capillary by using 705 silicone glue, and three reservoirs were adjusted at the electric positions, by which BR, SR and WR were respectively connected with E1, E2 and E3 on PCB for applying separation and sampling voltages (Fig. 1). The sampling voltage was applied between E2 and E1 with E3 in floating. The separation voltage was applied between E1 and E3 with E2 in floating. Before putting into the groove, the fused-silica capillary (7.5-cm length) was cut with potsherd at 0.5 cm from one end, and the polymer coatings at 0.6 cm from the other end were removed for UV detection. After the capillary was fixed on the groove, the cut at the position of sample reservoir was ultrasonated by an ultrasonic probe at 150 W with an action frequency of 12 times/min and a distance of 2 mm for 1 min to form a perfect sampling fracture [25].

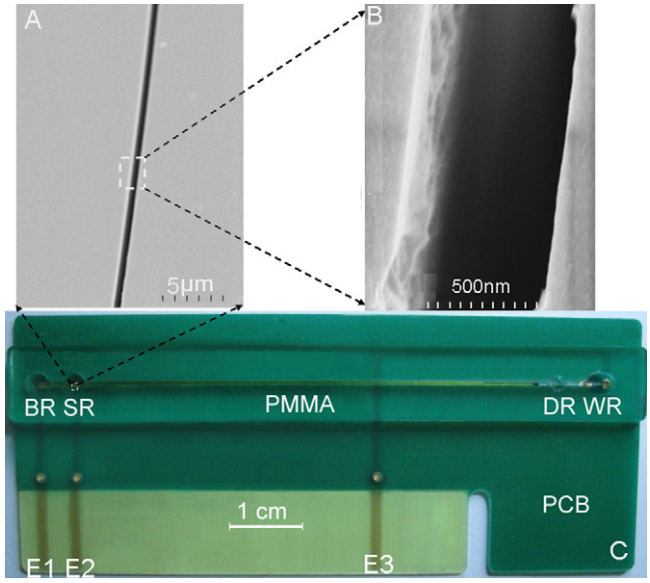

Fig. 1. SEM photos of sampling fracture (A and B) on the HQM (C). BR, buffer reservoir; SR, sample reservoir; DR, detection reservoir; WR, waste reservoir; E1, E2 and E3, electrodes for applying sampling and separation voltages.

3. Results and discussion

3.1. Properties of HQM

The ultrasonic method for formation of sampling fracture on a capillary excluded the subjective handling influence on the fracture quality [25]. Thus the preparation reproducibility of sampling fracture was good, which could be verified by the reproducible results for MEL detection. The enlarged images of sampling fracture were shown as Fig. 1A and B, at which the width of the sampling fracture was measured to be about 800 nm. Both the two fracture edges and the inner surface were quite smooth and uniform. Sampling from the ultra narrow fracture could produce a very narrow sample plug, thus suppressing effectively sample leakage and zone broadening, improving greatly the separation efficiency, and reducing largely the sample consumption [35]. The 800-nm width and 155-μm depth of sampling channel (the wall thickness of capillary) produced a prohibitive behavior to the entrance of microparticles in sample suspension to the separation channel, thus excluding their interference with the UV detection of MEL. Thus milk suspension could be directly used for sample injection through the sampling channel for screening without any pretreatment. The uniform sampling channel provided repeatable flow condition and led to good reproducibility of sampling. In addition, different from the traditional MED, both the PMMA and PCB boards of HQM were relatively isolated from the separation channel. The fused-silica capillary could be easily peeled off from the HQM. Thus both the PMMA and the PCB boards could be conveniently recycled after screening, which could reduce the screening cost.

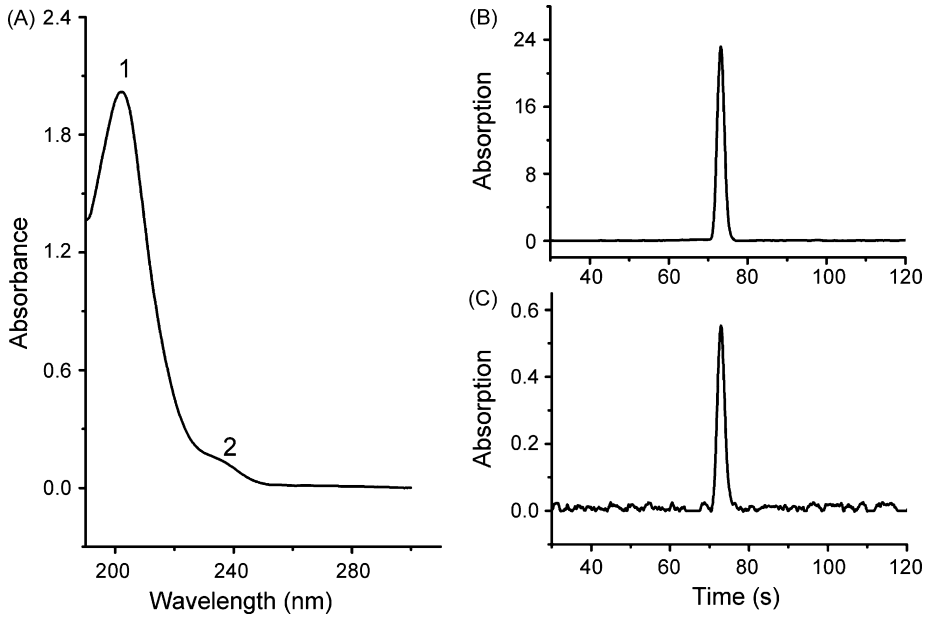

Fig. 2. UV spectrum of 5 μg mL^{-1} MEL aqueous solution (A) and electropherograms for 25 μg mL^{-1} MEL detected at 202 (B) and 240 nm (C) under the same conditions. Separation voltage, +1800 V; sampling voltage, +180 V for 3 s; running buffer, 20 mM phosphate (pH 9.0).

3.2. Optimization of detection wavelength and running buffer

The UV spectrum of MEL from 190 to 300 nm was shown in Fig. 2A. Two obvious absorption peaks could be observed at 202 and 240 nm. The electropherograms of 25 μg mL^{-1} of MEL detected at both 202 and 240 nm occurred at the same migration time (t_M) (Fig. 2B and C), and their baselines were also similar. The absorbance at 202 nm was 14.4 times that at 240 nm, thus this work used 202 nm for UV detection of MEL contents. The signal for 25 μg mL^{-1} of MEL detected at 202 nm was about 40 times stronger than that at 240 nm, indicating that a much more sensitive method could be obtained at 202-nm detection wavelength.

Usually, both borate buffer and phosphate buffer are applicable to the UV detection at 202 nm because of their weak absorption. However, obvious increase of the absorption near 195 nm could be observed when using borate buffer as running buffer [36], which would interfere with the detection of MEL. Thus, phosphate buffer was chosen as the running buffer.

The pH value of the running buffer can strongly affect the t_M of amino acids [25,37]. Moreover, tryptophan (Trp), tyrosine (Tyr) and phenylaniline (Phe) all had obvious absorption of UV light, and the t_M of Trp was closest to that of MEL [25]. When the pH was less than 9.0 Trp and MEL showed the almost same t_M, however, MEL and Trp could be completely separated with a resolution (R_s) more than 6 at pH 9.0 (Fig. 3). Although higher pH value could produce larger R_s between MEL and Trp, sugars would produce higher absorption of UV in strong alkaline solution [38], which would not benefit to the UV detection of MEL. Thus, pH 9.0 was used as the optimum pH value of phosphate buffer.

The phosphate buffers with different concentrations such as 10, 20, 30 and 40 mM were used to perform the separation. High buffer concentration could suppress the electroosmotic flow (EOF) and thus resulted in long t_M. Moreover, high concentration of running buffer would produce more Joule heat in the separation channel to worsen the separation and decrease the reproducibility of the analysis. When the concentration of running buffer was less than 20 mM, the buffer capacity was not enough for keeping a stable baseline. Considering the analytical time, stability of separation and buffer capacity, 20 mM of phosphate buffer was used as the optimal condition.

3.3. Effects of separation voltage and sampling conditions

The separation voltage affected the t_M and separation efficiency by altering the EOF. Low separation voltage resulted in long t_M, while high separation voltage led to high Joule heat in separation channel, which resulted in unstable baseline and decreased the reproducibility of the analysis. At the separation voltage of +1800 V (240 V/cm), a stable baseline could be obtained and the t_M of MEL was about 75 s (Figs. 2 and 3), thus, it was selected as separation voltage to obtain fast separation.

The sampling voltage and time were related to the detection sensitivity and separation efficiency. Low sampling voltage and short sampling time were allowed in the fracture sampling technique [25,33,34], which indicated the utilization of low-cost power and the limitation for the sample zone diffusion. When the sampling voltage was higher than +180 V, the baseline became unstable. However at the sampling voltages less than +180 V, long time sampling should be carried out for the need of sensitive detection, at which the detection sensitivity of MEL was not high enough

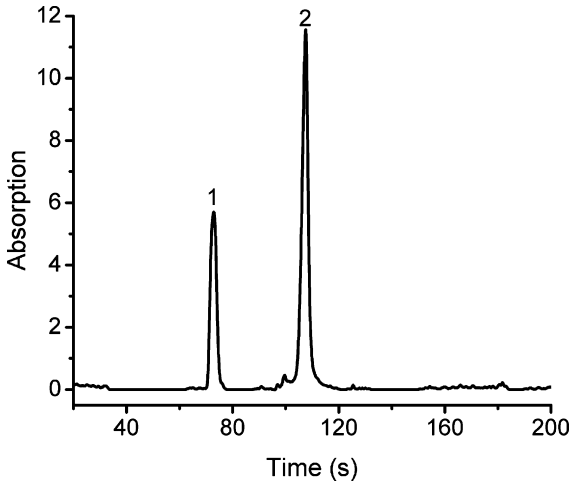

Fig. 3. Electropherogram for 8 μg mL^{-1} MEL (1) and 100 μg mL^{-1} Trp (2) detected at 202 nm. Other conditions were the same as in Fig. 2.

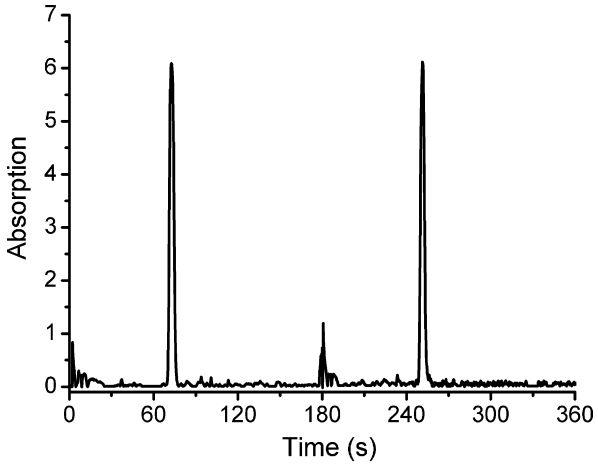

Fig. 4. Electropherogram for two consecutive sampling of 10 μg mL^{-1} MEL under optimal conditions as described in Fig. 3. Peaks at 0 and 180 s were the system peaks caused by the startup and switch of power.

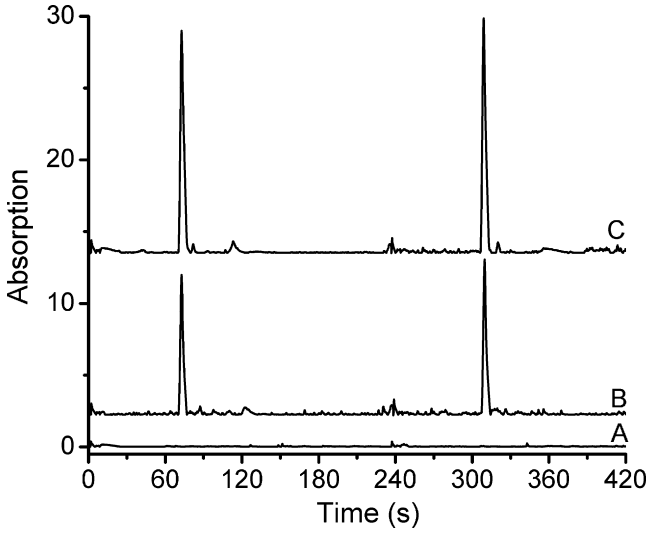

Fig. 5. Electropherograms for two consecutive sampling of pooled blank milk (A), milk sample 1 (B) and sample 1 spiked with 12.5 μg mL^{-1} MEL (C).

for detection of MEL at low concentration, e.g. 2.5 μg mL^{-1} when the sampling time was less than 3 s. Contrarily, when the sampling time was more than 3 s, the long sampling time resulted in serious broadening of sample zone, thus led to low separation efficiency. Considering the sensitivity and the separation efficiency, a sampling time of 3 s at +180 V was used for detection of MEL.

3.4. Separation and detection of MEL

A series of MEL solutions with different concentrations was prepared by gradual dilution of standard solutions with running buffer (20 mM phosphate, pH 9.0). The electropherogram for two consecutive sampling of 10 μg mL^{-1} of MEL was shown in Fig. 4. The t_M of 75 s for MEL was much shorter than those of 13.6 min [4], 3.8 min [6] and 5 min [8] for HPLC analysis. The relative standard deviation (RSD) of t_M (n = 6) was 1.8% for run-to-run and 2.6% for device-to-device, indicating acceptable reproducibility of the separation and the fabrication of HQMs. The RSD (n = 6) of peak area measured at the MEL concentration of 10 μg mL^{-1} was 6.6% for run-to-run, and 7.5% for device-to-device. These results indicated both the designed HQM and the proposed method including the sampling fracture and UV microfluidic workstation had acceptable reproducibility and stability.

The calibration curve, plot of peak area *vs.* MEL concentration, for UV detection of MEL showed a linear range from 1.0 to 100 μg mL^{-1} with a relative coefficient of 0.999. The detection limit was 0.23 μg mL^{-1} at S/N of 3, which was about 2 orders of magnitude lower than the safety limit of melamine permitted by the U.S. Food and Drug Administration (FDA). The detection limit was also lower than those with a pretreatment and/or extraction step such as 0.5 mg kg^{-1} for CZE-UV detection [17], 1% (w/w) [19] and 0.7% [20] for Raman spectroscopic detections.

3.5. Analysis of MEL in milk samples

The designed method for screening of MEL was tested by analyzing the commercially available milk samples. Since natural milk usually contains electrolytes, the milk sample solutions were prepared in pure water so that the solutions had almost the same ion strength as that of the standard solutions. In order to identify the MEL peaks, a standard solution of MEL was spiked into the samples. Fig. 5 shows the typical electropherograms with two consecutive sampling. The peaks for MEL occurred at the same t_M as that of the standard solutions (Fig. 4). After spiking MEL to the sample, the enhanced absorption peaks were also observed at the same t_M. From the peak areas and calibration curve, the MEL contents in the samples and the recovery for the spiked MEL could be obtained. The results and a comparison to those from a professional MEL testing agency were illustrated in Table 1. The recovery of MEL was from 82.4% to 85.7%. The use of standard solutions prepared with pooled blank milk might obtain more satisfactory recovery. Although it was lower than that of CZE-UV detection [17], it was comparable to that of HPLC-UV detection [6], and much better than those of 70–78% for commercial extraction-needed ELISA test [22], indicating satisfactory accuracy.

As seen from Fig. 5, the milk sample showed stable baseline without other interference peaks, indicating that the microparticles, macromolecules such as proteins and fat, and sugars in the milk suspension did not interfere with the separation and detection. The interference of sugars had been excluded by optimizing the separation conditions. In view of the UV absorption of these macromolecules in the detection window, it could be concluded that the sampling channel with a depth-to-width ratio of more than 190 acted as a sieve to prohibit the entrance of macromolecules to the separation channel. Furthermore, the bias effect of elec-

Table 1
Screening results of MEL in milk samples on HQM (n = 6).

Sample	Content[a] (mg kg^{-1})	Content[b] (mg kg^{-1})	RSD (%) of t_M[c]	RSD (%) of peak area[c]	Spiked (mg kg^{-1})	Obtained (mg kg^{-1})	Recovery (%)
1	72.1	77.4	3.2	8.2	12.5	82.4	82.4
2	5.1	5.3	2.9	7.9	2.8	7.5	85.7
3	Undetected	Undetected	–	–	1.5	1.25	83.3

Conditions: separation voltage, +1800 V; sampling voltage, +180 V for 3 s; running buffer, 20 mM phosphate (pH 9.0); detection wavelength, 202 nm.
[a] Detected on HQM.
[b] Given by professional MEL testing agency.
[c] Device-to-device.

trokinetic injection of the macromolecules was also beneficial to the prohibition. Thus the milk samples could be directly used for sampling for screening of MEL without any pretreatment.

4. Conclusions

A fast and low-cost approach was developed for the screening of MEL using a disposable microfluidic device with UV detection. The proposed strategy could exclude the interference of microparticles, macromolecules and sugars in the milk suspension, thus avoid large amount of toxic solvents used for the troublesome and time-consuming pretreatments. The ultrasonically formed sampling fracture showed a uniform and smooth surface for producing a very narrow sample plug to achieve highly efficient separation and good sampling and separation reproducibility. The environmentally friendly method showed a relatively high sensitivity, acceptable recovery and satisfactory accuracy for milk samples. It could be used in both a professional laboratory and the market for fast, green and convenient in situ monitoring of the quality of different foods.

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References

[1] U.S. Food and Drug Administration, Melamine Pet Food Recall of 2007. http://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/ucm129575.htm.
[2] U.S. Food and Drug Administration, Melamine Contamination in China, October 18, 2008. http://www.fda.gov/ohrms/dockets/ac/08/briefing/2008-4386b3.pdf.
[3] U.S. Food and Drug Administration, Analytical Methods for Melamine and Triazine Analogs. http://www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/ucm135002.htm.
[4] GB/T 22388-2008 (China), Determination of Melamine in Raw Milk and Dairy Products. Available at http://www.cnca.gov.cn/cait/htbz/jyjybzh/images/20081013/6188.pdf.
[5] R.L. Epstein, V. Randecker, P. Corrao, J.T. Keeton, H.R. Cross, J. Agric. Food Chem. 36 (1988) 1009.
[6] R.E. Baynes, G. Smith, S.E. Mason, E. Barrett, B.M. Barlow, J.E. Riviere, Food Chem. Toxicol. 46 (2008) 1196.
[7] 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.
[8] M.S. Filigenzi, B. Puschner, L.S. Aston, R.H. Poppenga, J. Agric. Food Chem. 56 (2008) 7593.
[9] Y.-T. Wu, C.-M. Huang, C.-C. Lin, W.-A. Ho, L.-C. Lin, T.-F.g Chiu, D.-C. Tarng, C.-H. Lin, T.-H. Tsai, J. Chromatogr. A 1216 (2009) 7595.
[10] M.S. Filigenzi, E.R. Tor, R.H. Poppenga, L.A. Aston, B. Puschner, Rapid Commun. Mass Spectrom. 21 (2007) 4027.
[11] W.-C. Cheng, S. Chen, T.-J. Lin, I.-J. Wang, Y.-M. Kao, D.Y.C. Shih, Rapid Commun. Mass Spectrom. 23 (2009) 1776.
[12] A. Desmarchelier, M. Cuadra, T. Delatour, P. Mottier, J. Agric. Food Chem. 57 (2009) 7186.
[13] M. Ibanez, J.V. Sancho, F. Hernandez, Anal. Chim. Acta 649 (2009) 91.
[14] G.M. Huang, O.-Y. Zheng, R.G. Cooks, Chem. Commun. (2009) 556.
[15] 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.
[16] H.-W. Tang, K.-M. Ng, S.-S.Y. Chui, C.-M. Che, C.-W. Lam, K.-Y. Yuen, T.-S. Siu, L.C.L. Lan, X.-Y. Cheo, Anal. Chem. 81 (2009) 3676.
[17] C.W. Klampfl, L. Andersen, M. Haunschmidt, M. Himmelsbach, W. Buchberger, Electrophoresis 30 (2009) 1743.
[18] N. Yan, L. Zhou, Z.-F. Zhu, X.-G. Chen, J. Agric. Food Chem. 57 (2009) 807.
[19] S. Okazaki, M. Hiramatsu, K. Gonmori, O. Suzuki, A.T. Tu, Forensic Toxicol. 27 (2009) 94.
[20] M. Lin, L. He, J. Awika, L. Yang, D.R. Ledoux, H. Li, A. Mustapha, J. Food Sci. 73 (2008) T129.
[21] K.-L. Ai, Y.-L. Liu, L.-H. Lu, J. Am. Chem. Soc. 131 (2009) 9496.
[22] E.A.E. Garber, J. Food Prot. 71 (2008) 590.
[23] M. Li, B. Hu, J.-Q. Li, J.-Q. Li, R. Chen, X. Zhang, H.-W. Chen, Anal. Chem. 81 (2009) 7724.
[24] L.J. Mauer, A.A. Chernyshova, A. Hiatt, A. Deering, R. Davis, J. Agric. Food Chem. 57 (2009) 3974.
[25] C. Zhai, W. Qiang, J.-P. Lei, H.-X. Ju, Electrophoresis 30 (2009) 1490.
[26] D.R. Reyes, D. Iossifidis, P.A. Auroux, A. Manz, Anal. Chem. 74 (2002) 2623.
[27] W.R. Vandaveer IV, S.A. Pasas-Farmer, D.J. Fischer, C.N. Frankenfeld, S.M. Lunte, Electrophoresis 25 (2004) 3528.
[28] G.M. Whitesides, Nature 442 (2006) 368.
[29] J.A. Vickers, M.M. Caulum, C.S. Henry, Anal. Chem. 78 (2006) 7446.
[30] F. Dang, K. Kakehi, J. Cheng, O. Tabata, M. Kurokawa, K. Nakajima, M. Ishikawa, Y. Baba, Anal. Chem. 78 (2006) 1452.
[31] J.S. Buch, C. Kimball, F. Rosenberger, W.E. Highsmith Jr., D.L. DeVoe, C.S. Lee, Anal. Chem. 76 (2004) 874.
[32] B. Ma, X. Zhou, G. Wang, Z. Dai, J. Qin, B. Lin, Electrophoresis 28 (2007) 2474.
[33] W. Qiang, C. Zhai, J.-P. Lei, J. Sheng, C.-J. Song, D.-M. Zhang, H.-X. Ju, Analyst 134 (2009) 1834.
[34] C. Zhai, C. Li, W. Qiang, J.-P. Lei, X.-D. Yu, H.-X. Ju, Anal. Chem. 79 (2007) 9427.
[35] V. Kostal, J. Katzenmeyer, E.A. Arriaga, Anal. Chem. 80 (2008) 4533.
[36] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann, H.M. Widmer, Anal. Chem. 63 (1991) 1541.
[37] J.N. Ye, R.P. Baldwin, Anal. Chem. 66 (1994) 2669.
[38] S. Rovio, J. Yli-Kauhaluoma, H. Sirén, Electrophoresis 28 (2007) 3129.