Disposable microfluidic device with ultraviolet detection for highly resolved screening of illicit drugs†

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A disposable microfluidic device was constructed by conveniently integrating one poly(methyl methacrylate) board with four reservoirs and one fractured fused-silica capillary with 50 μm i.d. and 7.5 cm total length on a printed circuit board for applying sampling and separation voltages. The disposable microfluidic device combined with a home-made ultraviolet workstation could be conveniently used for efficient screening and quantitative detection of μg mL^{-1} illicit drugs. Using eight illicit drugs as models, they could be baseline-separated within 240 s with the separation efficiency up to 600 047 plates m^{-1} at the designed device. The novel device and proposed protocol were successfully used to screen illicit drugs in human urine. This work presented a simple and low-cost method to fabricate the microfluidic device and provided a powerful way for sensitive and specific multi-screening of different drugs with high resolution, fast separation and low-cost.

Introduction

Screening and confirmation of illicit drugs[1–4] in body fluids, especially urine, is very important for identifying drug users in forensic science and abstinence of drug addicts in clinical therapy. For screening tests of illicit drugs, an analytical method needs to be fast, convenient and as accurate as possible due to the mass specimens and broad components. Currently several techniques, including thin-layer chromatographic[5] and immunological methods,[6–16] have been practically applied in screening tests of illicit drugs. However, a rapid paper chromatography method called 'Keystone Diagnostics Quick Test' for on-site testing is only 50% accurate.[5] Immunological methods usually give false positive results—10.7% for cocaine tests, 25% for amphetamines, 32.2% for opiate tests using drug-testing Toxiquick[6] and 9.1% for opiates using fluorescence polarization immunoassay[7]—though they can give results in several minutes.[5,9–16] One reason for the erroneous results of immunological methods is crosstalk interference among the illicit drugs, which may also lead to false negatives. For example, amphetamines have crosstalk with many phenylethylamines because the immunoassay broadly recognizes amines rather than the specific illicit amines.[8] Furthermore, the immunoassay suffers from relatively high-cost, even for multi-analyte tests, which limits its application for screening illicit drugs.[5] In order to eliminate the false-positive, high-performance liquid chromatography/mass spectrometry, or gas chromatography/mass spectrometry has to be further used in confirmation tests with positive results on the screening,[17–22] which greatly increases the labor time and cost. Thus, it is imperative to discover a low false-positive, and even specific, screening method for illicit drugs.

Capillary electrophoresis has been found to be an attractive approach for the analysis of illicit drugs *via* micellar electrokinetic capillary chromatography (MECC).[23–25] Recently, microfluidic electrophoresis devices have been developed as powerful tools for separation and detection due to the small volume, fast separation, and increased portability.[26,27] Furthermore, capillaries have been used for constructing the microchannel of microfluidic devices due to their high surface/volume ratio and low-cost.[28]

Optical detection is generally regarded as superior,[29] particularly for drugs and biomolecules. However, this technique requires special materials such as quartz, and the fabrication of the quartz microchip is high-cost due to the need for expensive clean-room facilities, corrosive etchants, and time-consuming bonding etching. Here, a hybrid quartz/poly(methyl methacrylate) microfluidic device (QPMD) was constructed for screening illicit drugs with ultraviolet (UV) detection. The microfluidic device integrated a poly(methyl methacrylate) (PMMA) board with four reservoirs and a fractured fused-silica capillary[30] on a printed circuit board (PCB) for applying sampling and

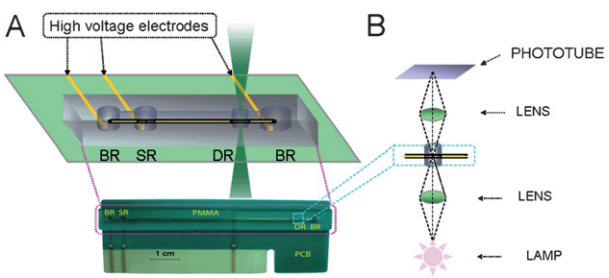

Fig. 1 The structure of the disposable microfluidic device (A) and UV detector (B) with buffer reservoir (BR), sample reservoir (SR) and detection reservoir (DR).

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separation voltages (Fig. 1A). Compared with the conventional quartz microchip and previously reported microdevices,[30,31] QPMD had better fabrication reproducibility, and lower cost, leading to the disposability of the device for screening tests, and the integrated PCB greatly simplified manipulation. Eight illicit drugs were used to examine the practicality of the proposed device. QPMD could perform UV detection on μg mL^{-1} concentrations of illicit drugs. Baseline separation with high resolution was satisfactorily achieved. Furthermore, interference drugs that generally coexist in body fluids could be well separated. This screening approach provided a promising choice for forensic science due to its convenience, practicality, high resolution, good sensitivity and specificity, and low-cost.

Experimental

Chemicals

The illicit drugs including morphine (MOR), 6-monoacetylmorphine (6-AM), ketamine, heroin, ephedrine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and Δ-9-tetrahydrocannabinol (Δ^9-THC) were obtained from Beijing Municipal Public Security Bureau. Standard solutions of drugs were prepared in water at concentrations of 10 mg mL^{-1}, and stored at 4 °C. Urine samples were collected from Nanjing Municipal Public Security Bureau, in which the presence of MOR and ketamine were confirmed respectively. All other chemicals were of analytical grade.

Fabrication of microfluidic device

The PCB (85 × 35 × 1 mm for length × width × thickness) and PMMA board (85 × 10 × 2 mm for length × width × thickness) with a groove were prepared with the usual techniques. Four holes were then drilled on the PMMA board as BR (3 mm i.d.), SR (3 mm i.d.), DR (2 mm i.d.) and BR (4 mm i.d.), respectively (Fig. 1A). The resulting PMMA board was glued on the PCB with the groove outside for fixing a pretreated capillary using 705 silicone rubber, three reservoirs were just above the high voltage electrodes (Fig. 1A) of PCB for applying separation and sampling voltages. The fused-silica capillary (50 μm i.d. and 7.5 cm length) was cut with potsherd at 0.5 cm from one end, and the polymer coat was removed at 0.6 cm from another end for UV detection. Then the capillary was fixed into the groove and the cut at the position of SR was ultra-sonicated to form a perfect sampling fracture.[31]

Apparatus

The UV microfluidic workstation employed in this work was home-manufactured by cooperation with Beijing Cailu Scientific Instrument Limited. It was composed of an eight-port high-voltage power supply, a UV detector and data processor. The high-voltage modules enabled real-time current and voltage monitoring. The designed microfluidic device exactly matched with the groove in the body of the UV microfluidic workstation. After a microdevice was plugged to the groove, the platform loading the microdevice moved into the detector at a certain rate, and a program was used to control the platform to move back and forth until the UV signal reached a maximum value, at which time the UV beam was just aligned to the centre of the separation channel (Fig. 1B). After passing through the channel, the light beam was focused onto the photodiode *via* a lens to collect the signal.

Sample pretreatment and analysis

Prior to screening, the drugs were firstly extracted from urine samples with 500 μL chloroform or cyclohexane for 1 mL MOR- or ketamine-positive samples. After evaporating the organic phase under a gentle stream of nitrogen at room temperature, the residue was dissolved in 100 μL running buffer composed of 40 mM sodium dodecyl sulfate (SDS), 5 mM disodium tetraborate (STB) (pH 9.5), 1 M urea and 5% 1-butanol. The obtained sample solution was then injected into the SR for analysis with a separation voltage of 1800 V and a sampling voltage of 200 V for 2 s.

Results and discussion

Properties of screening microfluidic device and apparatus

The sampling fracture on the capillary was prepared ultra-sonically at 150 W power, which excluded subjective handling influence during the preparation of the fracture. Thus, the preparation reproducibility of the sampling fracture was very good, which was verified from the reproductive detection results for these drugs. The ultra-narrow sampling width produced a very narrow sample plug,[28] thus effectively suppressing sample zone broadening, and greatly improving the separation efficiency, and largely reducing the sample consumption. The quartz capillary as a separation channel not only produced a high electroosmotic flow for separation but also could be integrated with a UV detection system for different analytes.

A UV detector is a suitable choice for determination of illicit drugs because almost all illicit drugs have an absorption at 200 nm, while most lack electrochemical activity or fluorescence. However, the small dimension of separation channel poses a severe problem for sensitive and reliable absorbance measurements due to the very short optical path and the difficulty in aligning the light beam.[32] The home-manufactured apparatus contained an automatic orientation system controlled *via* a program. Once the microfluidic device was plugged into the detector, this system could move parallel to the device until the optical path aligned with the centre of the separation channel of the detection reservoir. Thus, sensitive and convenient detection could be performed.

Optimization of running buffer

SDS concentration was chosen between 30 mM and 50 mM. When the concentration was lower than 30 mM, the analytical results had poor stability and repeatability. As shown in Fig. 2A, with the increasing concentration of SDS, both the retention time of analytes and the resolution increased. Considering the peak broadening and the Joule heat effect at high concentration, 40 mM SDS concentration was used, at which the resolution was good enough for the detection of eight illicit drugs.

With the increasing STB concentration (ionic strength), the electrical double-layer became thinner and the electroosmotic

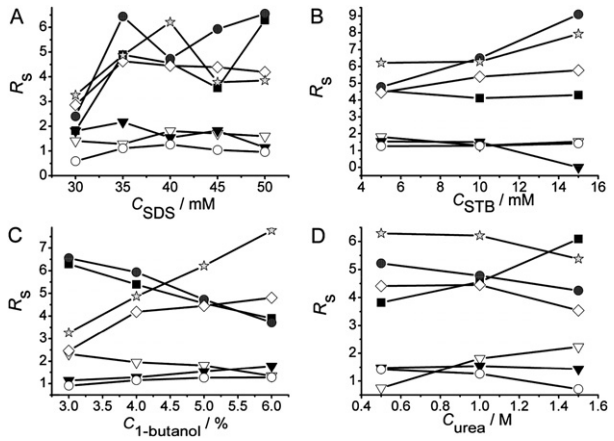

Fig. 2 Effects of SDS (A), STB (B), 1-butanol (C) and urea (D) concentrations on resolution for eight illicit drugs (■ MOR–6-AM, ● 6-AM–ketamine, ▽ ketamine–heroin, ▼ heroin–ephedrine, ◇ ephedrine–MDA, ○ MDA–MDMA, ☆ MDMA–Δ^9-THC). The separation voltage was 1800 V and the sampling voltage, 200 V for 2 s. When changing the concentration of one component, other buffer components are at their optimized concentrations.

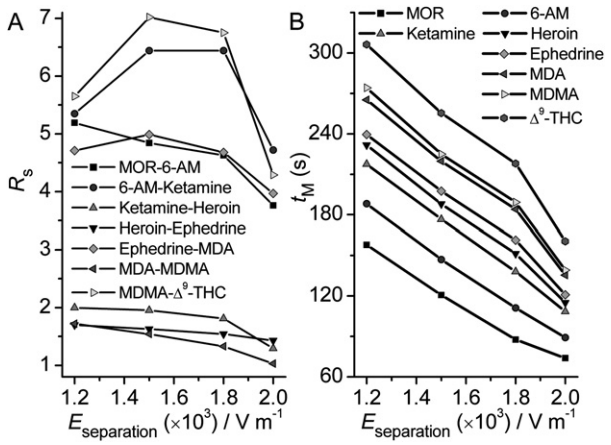

Fig. 3 Effects of separation voltage on resolution (A) and migration time (B) for eight illicit drugs at 10 μg mL^{-1} with a sampling voltage of 200 V for 2 s.

flow (EOF) decreased, leading to longer migration times of these drugs. The long migration time would increase sample zone broadening. Furthermore, high concentration of STB resulted in a large current in the separation channel, which increased the Joule heat and thus could not separate ketamine, heroin and ephedrine (Fig. 2B). Considering the buffer capacity, resolution and analysis time, 5 mM STB was used as the optimal concentration.

An organic additive can expand the migration-time window, alter separation selectivity, achieve gradient elution, and even improve the peak shape.[33,34] 1-Butanol showed a great effect on the resolution of these drugs. In the absence of 1-butanol most of them could not be separated. When the running buffer contained 5% 1-butanol all resolutions were more than 1.26 (Fig. 2C). Higher concentrations of 1-butanol, beyond the miscibility limit of 1-butanol with water, led to heavy noise of the baseline due to the light scattering of microemulsion.

In MECC, urea is usually used to increase the dipolar selectivity of the running buffer for the separation of hydrophobic analytes.[35] Thus it was added into the running buffer for improving the separation of these drugs. The optimal urea concentration was at 1 M because the eight illicit drugs could not be separated completely at 0.5 M or 1.5 M as shown in Fig. 2D. Thus the optimal running buffer is composed of 5 mM STB (pH 9.51), 40 mM SDS, 1 M urea and 5% 1-butanol.

Separation voltage and sampling conditions

The separation voltage or electric field strength altered the EOF and the electrophoretic velocities of analytes and thus affected the migration time (t_M) and separation efficiency. To evaluate the effect of separation voltage, the t_M of each drug and the resolution (R_s) between two adjacent drugs were measured as the function of the separation voltage between 1200 and 2000 V (Fig. 3). Here R_s is defined as $2((t_M)_B - (t_M)_A)/(W_B + W_A)$, where W is the full peak width.[36] With the increasing separation voltage, the t_M decreased due to the increasing EOF. At the separation voltage of 1800 V, eight analytes could be completely separated with the R_s values more than 1.26, at which the total separation time was less than 240 s and the maximum R_s value of 6.75 occurred between MDMA and Δ^9-THC.

The sampling conditions, including injection time and sampling voltage, influence the sensitivity and separation efficiency. The peak area of each drug and the R_s between two adjacent drugs were measured as the functions of the sampling conditions. At a sampling voltage of 200 V, with the increasing injection time the signal increased and tended towards a constant value after 2 s. Although longer injection time led to a larger peak area, in comparison with that at 1 s the separation efficiency decreased by 2.81% and 20.11% in theoretical plates at 2 and 3 s, respectively. Thus, the injection time of 2 s at 200 V was used as the optimal sampling condition. The low sampling voltage would benefit the design of low-cost power.

Separation and screening of drugs

A series of drug solutions with different concentrations were prepared by gradual dilution of standard solutions with running buffer. Fig. 4 shows the electropherogram for 10 μg mL^{-1} of eight illicit drugs. The R_s values for MOR–6-AM, 6-AM–ketamine, ketamine–heroin, heroin–ephedrine, ephedrine–MDA, MDA–MDMA, MDMA–Δ^9-THC were larger than 1.26, showing baseline separation within 240 s, indicating a rapid separation when compared with the previous reports with at least 20 min running time by MECC in capillary electrophoresis.[23,24]

The theoretical plate numbers (N) and separation reproducibility of these drugs were illustrated in Table 1. Here, N is defined as $5.54(t_M/W_{1/2})^2$, where $W_{1/2}$ is the peak width at the half-maximum points.[37] The N values ranging from 134 078 to 600 047 plates m^{-1} contribute to the advantages of the present microfluidic device. The relative standard deviations (RSDs) ($n = 10$) of t_M determination were less than 3.10% for run-to-run and 8.64% for device-to-device, indicating acceptable reproducibility of separation and fabrication of the microfluidic device. The RSD ($n = 10$) of peak area measured at the concentration of

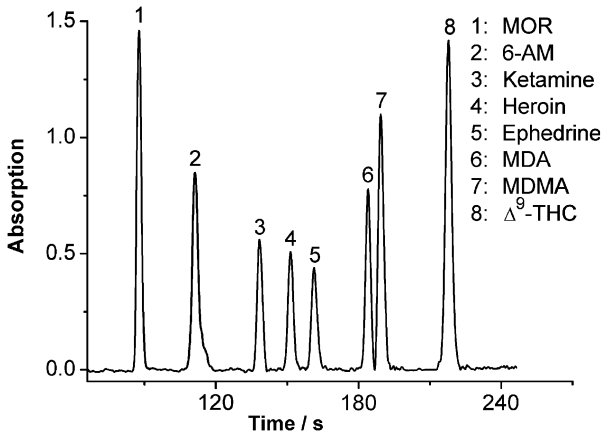

Fig. 4 Electropherogram for eight illicit drugs at 10 μg mL^{-1} in 5 mM STB buffer (pH 9.51) containing 40 mM SDS, 1 M urea and 5% 1-butanol. The separation voltage was 1800 V and the sampling voltage, 200 V for 2 s.

10 μg mL^{-1} was from 0.49% to 5.25% for run-to-run, and 1.91% to 8.47% for device-to-device. Thus, both the designed microfluidic device and the proposed method including the sampling fracture and UV microfluidic workstation had good reproducibility and stability, respectively.

UV detection of these drugs showed the linear ranges from: 5 to 750 μg mL^{-1} for MOR, heroin, MDA and MDMA; 5 to 1000 μg mL^{-1} for 6-AM and ketamine; 5 to 1500 μg mL^{-1} for ephedrine; and 5 to 250 μg mL^{-1} for Δ^9-THC with the relative coefficients ranging from 0.9977 to 0.9997 and the slopes ranging from 62.26 to 115.57 μV s mL μg^{-1} (Table 2). The detection limits at the signal-to-noise ratio of 3 were 1.15, 1.12, 1.62, 1.96, 2.09, 1.94, 1.32, and 1.67 μg mL^{-1} for MOR, 6-AM, ketamine, heroin, ephedrine, MDA, MDMA, and Δ^9-THC, respectively. To our knowledge, this was the first work to simultaneously separate four kinds of illicit drugs including opioids, amphetamines, ketamine, and cannabis using a microdevice. The separation efficiency and sensitivity on the disposable device is much better than those of previous quartz microdevices for other analytes with UV detection.[38–41]

Interference experiments

The interference experiments on the disposable microfluidic device were carried out by adding six calming drugs (amobarbital, barbital, estazolam, diazepam, alprazolam, and nitrazepam), four abstinence drugs (diphenoxylate, caffeine, pethidine and lofexidine), four other opiates (codeine, acetylcodeine, narcotine and pholcodine) which made three test solutions containing the eight illicit drugs. Their t_M values in three different runs and the resolution between the interference and eight adjacent illicit drugs are listed in Table S1 (see ESI†). All resolutions were more than 1.1, indicating good separation. This amazing high separation efficiency provided a powerful ability to avoid false positives from the screening method based on the disposable microdevice.

Minimum detective concentrations in real samples

Prior to analysis, the urine samples were extracted with organic solvents to exclude the effect of salts in the samples on the separation. Chloroform was used as an extractant for MOR, 6-AM, heroin and Δ^9-THC. Ketamine, ephedrine, MDA and MDMA were extracted with cyclohexane. The extraction

Table 1 Separation efficiency and reproducibility of QPMD for illicit drugs at 10 μg mL^{-1} ($n = 10$)

				RSD (%) of t_M		RSD (%) of peak area	
Analytes	N/plates m^{-1}	Plate height/μm	R_s	Run-to-run	Device-to-device	Run-to-run	Device-to-device
MOR	134 078	7.46	4.56	0.55	3.22	0.49	1.91
6-AM	204 594	4.89	4.73	1.96	5.17	5.25	6.15
Ketamine	140 860	7.10	1.81	0.88	2.86	0.86	8.47
Heroin	204 156	4.90	1.54	2.47	8.64	3.17	6.28
Ephedrine	305 453	3.27	4.45	3.10	3.81	2.33	3.99
MDA	600 047	1.67	1.26	1.94	2.97	2.92	3.92
MDMA	548 453	1.82	6.21	1.87	2.75	4.75	7.73
Δ^9-THC	380 359	2.63	—	0.46	1.94	3.85	4.80

Table 2 Linear ranges and detection limits of QPMD for illicit drugs

Analytes	Linear ranges/μg mL^{-1}	Detection limit/μg mL^{-1}	Extraction efficiency (%)	Minimum detective concentration in urine/ng mL^{-1}
MOR	5–750	1.15	91.6 ± 5.3	125
6-AM	5–1000	1.12	47.3 ± 2.1	238
Ketamine	5–1000	1.62	94.5 ± 7.8	172
Heroin	5–750	1.96	95.3 ± 3.2	206
Ephedrine	5–1500	2.09	0[a]	—
MDA	5–750	1.94	45.5 ± 3.8	431
MDMA	5–750	1.32	94.4 ± 5.3	140
Δ^9-THC	5–250	1.67	—[b]	—

[a] Ephedrine could not be extracted by cyclohexane in a real sample. [b] Lack of standard sample.

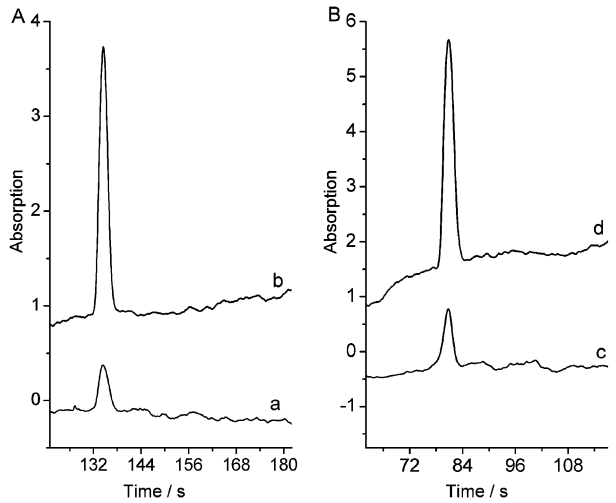

Fig. 5 Electropherograms for (A) ketamine-positive and (B) MOR-positive urine samples (a, c) without and (b, d) with addition of 2.5 μg mL^{-1} ketamine or MOR.

efficiency was calculated by dividing the peak area obtained after extraction with the peak area obtained by direct injection of equal amounts of the drug in the running buffer (Table 2). The low extraction efficiencies of 6-AM and MDA were due to their low solubilities in the extractants. However, 6-AM and MDA are not the main targets in the determination of illicit drugs. The minimum detectable concentrations in a real sample were calculated as shown in Table 2. The minimum detectable concentrations were 125, 140 and 172 ng mL^{-1} for MOR, MDMA and ketamine, respectively, while the cutoffs of screening immunoassay were 300, 1000 and 1000 ng mL^{-1} for the individual drugs which were established in the mid-1980s.[42] Thus, screening of illicit drugs using the designed microfluidic device could lead to less false negatives than the immune colloidal gold label test strips.

Analysis of illicit drugs in human urine

The disposable microfluidic devices were used for analysis of illicit drugs in human urine, in which one sample was confirmed as MOR positive and the other was confirmed as ketamine positive. Thus, they could directly be extracted with chloroform and cyclohexane, respectively. The electropherograms for analysis of the urine samples were illustrated in Fig. 5. The retention time and the increase in peak area after spiking the standard solution identified that the urine samples A and B contained MOR and ketamine, respectively. The concentrations of MOR and ketamine in the samples were 949 and 651 ng mL^{-1} with the RSD ($n = 3$) of 8.66% and 7.09%, respectively, indicating acceptable reproducibility.

Conclusions

A simple approach was developed for fabrication of disposable microfluidic devices and screening of illicit drugs including opioids, amphetamines, ketamine, and cannabis with UV detection. The device with a quartz capillary in a PMMA mould fixed on a PCB could be conveniently fabricated in batch with good reproducibility and low-cost. Combining the advantages of ultra-narrow sampling fracture and the UV detection *via* a home-made apparatus with a programmable automatic orientation system, eight illicit drugs were rapidly separated with high resolution and low detection limits. The proposed device was successfully applied to screening illicit drugs in human urine with acceptable accuracy. This QPMD could give accurate data for each illicit drug with a broad test range of drugs in comparison with immunological methods. This approach and the microfluidic device provided a powerful way for sensitive and specific multi-screening of different analytes.

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References

1 P. Wernly and W. Thormann, *Anal. Chem.*, 1992, **64**, 2155–2159.
2 S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelli and R. Bagnati, *Anal. Chem.*, 2006, **78**, 8421–8429.
3 P. Wernly and W. Thormann, *Anal. Chem.*, 1991, **63**, 2878–2882.
4 R. B. Taylor, A. S. Low and R. G. Reid, *J. Chromatogr., B*, 1996, **675**, 213–223.
5 S. George and R. A. Braithwaite, *Clin. Chem. (Washington, D. C.)*, 2002, **48**, 1639–1646.
6 T. Biermann, B. Schwarze, B. Zedler and P. Betz, *Forensic Sci. Int.*, 2004, **143**, 21–25.
7 W. J. Chen, C. C. Fang, R. S. Shyu and K. C. Lin, *Addict. Behav.*, 2006, **31**, 2304–2308.
8 A. H. Wu, C. McKay, L. A. Broussard, R. S. Hoffman, T. C. Kwong, T. P. Moyer, E. M. Otten, S. L. Welch and P. Wax, *Clin. Chem. (Washington, D. C.)*, 2003, **49**, 357–379.
9 J. L. Zacher and D. M. Givone, *Ann. Pharmacother.*, 2004, **38**, 1525–1528.
10 R. Meatherall and J. Dai, *Ther. Drug Monit.*, 1997, **19**, 98–99.
11 M. Backmund, K. Meyer, M. Von Zielonka and D. Eichenlaub, *Addict. Biol.*, 2000, **5**, 319–320.
12 L. R. Baden, G. Horowitz, H. Jacoby and G. M. Eliopoulos, *JAMA-J. Am. Med. Assoc.*, 2001, **286**, 3115–3119.
13 H. R. Kranzler, J. Stone and L. McLaughlin, *Drug Alcohol Depend.*, 1995, **40**, 55–62.
14 P. D. Camara, L. Audette, K. Velletri, P. Breitenbecher, M. Rosner and W. C. Griffiths, *Clin. Chem. (Washington, D. C.)*, 1995, **41**, 115–116.
15 M. A. Von Mach, C. Weber, M. R. Meyer, L. S. Weilemann, H. H. Maurer and F. T. Peters, *Ther. Drug Monit.*, 2007, **29**, 27–39.
16 M. Laloup, M. D. R. Fernandez, M. Wood, G. De Boeck, V. Maes and N. Samyn, *Forensic Sci. Int.*, 2006, **161**, 175–179.
17 K. A. Mortier, K. E. Maudens, W. E. Lambert, K. M. Clauwaert, J. F. Van Bocxlaer, D. L. Deforce, C. H. Van Peteghem and A. P. De Leenheer, *J. Chromatogr., B*, 2002, **779**, 321–330.
18 T. J. Kauppila, N. Talaty, T. Kuuranne, R. Kostianen and R. G. Cooks, *Analyst*, 2007, **132**, 868–875.
19 O. Quintela, F. L. Sauvage, F. Charvier, J. M. Gaulier, G. Lachatre and P. Marquet, *Clin. Chem. (Washington, D. C.)*, 2006, **52**, 1346–1355.
20 T. Gunnar, K. Ariniemi and P. Lillsunde, *J. Mass Spectrom.*, 2005, **40**, 739–753.
21 T. A. Brettell, J. M. Butler and J. R. Almirall, *Anal. Chem.*, 2007, **79**, 4365–4384.

22 K. Mc Fadden, J. Gillespie, B. Carney and D. O'Driscoll, *J. Chromatogr., A*, 2006, **1120**, 54–60.
23 R. Weinberger and I. S. Lurie, *Anal. Chem.*, 1991, **63**, 823–827.
24 Y. H. Lin, M. R. Lee, R. J. Lee, W. K. Ko and S. M. Wu, *J. Chromatogr, A*, 2007, **1145**, 234–240.
25 P. J. Meng, N. Fang, M. Wang, H. W. Liu and D. D. Y. Chen, *Electrophoresis*, 2006, **27**, 3210–3217.
26 T. Revermann, S. Gotz, J. Kunnemeyer and U. Karst, *Analyst*, 2008, **133**, 167–174.
27 V. Linder, *Analyst*, 2007, **132**, 1186–1192.
28 N. Dendane, A. Hoang, O. Renaudet, F. Vinet, P. Dumya and Eric Defrancq, *Lab Chip*, 2008, **8**, 2161–2163.
29 F. B. Myers and L. P. Lee, *Lab Chip*, 2008, **8**, 2015–2031.
30 C. Zhai, C. Li, W. Qiang, J. P. Lei, X. D. Yu and H. X. Ju, *Anal. Chem.*, 2007, **79**, 9427–9432.
31 C. Zhai, W. Qiang, J. P. Lei and H. X. Ju, *Electrophoresis*, 2009, **30**, 1490–1496.
32 S. Götz and U. Karst, *Anal. Bioanal. Chem.*, 2007, **387**, 183–192.
33 C. Huhn, M. Pütz and U. Pyell, *Electrophoresis*, 2008, **29**, 783–795.
34 A. Dworschak and U. Pyell, *J. Chromatogr., A*, 1999, **848**, 387–400.
35 Z. Liu, H. F. Zou, M. L. Ye, J. Y. Ni and Y. K. Zhang, *J. Chromatogr., A*, 1999, **863**, 69–79.
36 K. Ghowsi, J. P. Foley and R. J. Gale, *Anal. Chem.*, 1990, **62**, 2714–2721.
37 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 1981, **53**, 1298–1302.
38 I. D. Newman, C. B. Giordano, L. C. Copper and E. G. Collins, *Electrophoresis*, 2008, **29**, 803–810.
39 B. Ma, X. M. Zhou, G. Wang, H. Q. Huang, Z. P. Dai, J. H. Qin and B. C. Lin, *Electrophoresis*, 2006, **27**, 4904–4909.
40 R. Jindal and S. M. Cramer, *J. Chromatogr., A*, 2004, **1044**, 277–285.
41 M. Ludwig, F. Kohler and D. Belder, *Electrophoresis*, 2003, **24**, 3233–3238.
42 V. Luzzi, A. N. Saunders, J. W. Koenig, J. Turk, S. F. Lo, U. C. Garg and D. J. Dietzen, *Clin. Chem. (Washington, D. C.)*, 2004, **50**, 717–722.