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Sweeping–micellar electrokinetic chromatography for the simultaneous analysis of tricyclic antidepressant and β-blocker drugs in wastewater

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ABSTRACT

The simultaneous analysis of tricyclic antidepressant (amitriptyline, clomipramine, doxepin and nortriptyline) and β-blocker (alprenolol, labetalol and propranolol) drugs in wastewater was developed via sweeping–micellar electrokinetic chromatography (MEKC) together with a simple liquid–liquid extraction step. For sweeping–MEKC, the amount of organic modifier in the separation electrolyte, the concentration of phosphoric acid in the sample matrix and the injection time of the sample were optimized. Sensitivity enhancements of up to 305-fold were achieved via sweeping. This allowed limits of detection (LOD) from 7 to 27 ng/mL. The relative standard deviations of migration time, corrected peak area and peak height were less than 3.2%, 7.8% and 4.5%, respectively. Liquid–liquid extraction using dichloromethane as solvent afforded up to 21-fold enrichment of the drugs from spiked wastewater. No interference of the sample matrix was observed and recoveries were obtained in the range of 77–113% for all analytes except labetalol at three spiking levels of 16, 80 and 160 ng/mL. Detection at the ng/mL level makes this simple, environmentally friendly and cost effective method competitive against recently reported methods using advanced liquid-phase separation techniques for monitoring similar drugs in wastewater.

1. Introduction

Tricyclic antidepressants (TCAs) and β-adrenergic blocking agents (β-blockers) are two classes of small molecule prescription drugs. TCAs are mainly used for the treatment of depression [1]. β-Blockers are mainly used for treatment of hypertension, congestive heart failure, anxiety attacks, and abnormal heart rhythms, to relieve angina, and to prevent cardiac infarctions (heart attacks) [2]. These pharmaceutical drugs are used extensively in most developed countries and can enter the aquatic environment as a result of human excretion [3]. Occurrence of pharmaceutical drugs has been reported in sewage treatment effluents, surface water, and ground water [2,4–8]. Although the ecotoxicological effects are not quite known, it is important to develop methods for the analysis of these drugs in environmental samples for monitoring purposes.

Several methods have been reported for the analysis of TCA and β-blocker drugs including gas-chromatography and high-performance liquid chromatography (HPLC) [1,2,5–9]. Capillary electrophoresis (CE) based methods have also been developed as a good alternative for the analysis of these drugs [10–14]. CE offers several advantages including its speed of analysis, high efficiency of separation, small amount of sample and reagent requirements, and environmentally friendly. In addition, both charged and neutral small molecules can be separated using the CE modes capillary zone electrophoresis (CZE) [15] and micellar electrokinetic chromatography (MEKC) [16], respectively. MEKC can also improve the separation selectivity of charged analytes. Charged analytes are separated based on their affinity with the micellar pseudostationary (PS) phase as well as their electrophoretic migration in the aqueous phase [16–18]. Most reports on CZE and MEKC of TCA and β-blocker drugs dealt with the analysis of standard mixtures, biological or pharmaceutical samples [10–14] and no report on the analysis of wastewater.

CE techniques suffer from poor sensitivity for UV detection due to small sample volume injected (in nanoliters) and narrow optical path length. This drawback limits the application of CE for trace analysis of drugs in environmental samples. On-line preconcentration or stacking methods were employed to address this limitation by enrichment of the analytes before separation in the same capillary [19,20]. These methods include stacking by field amplification or enhancement [21–24], transient isotachophoresis [25–27], analyte focusing by micelle collapse [28] and micelle to solvent stacking (MSS) [29]. Stacking by field amplification and MSS have been applied for β-blockers [22,29] while acetonitrile

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(ACN) stacking and also stacking by field amplification for TCAs [23,24,27].

Another on-line preconcentration technique in CE is sweeping that works for both charged and neutral analytes [30,31]. Like any other stacking method, longer injections of sample can be performed without compromise to separation efficiency. The focusing is based on the interaction of analytes with the additive (i.e., a PS in EKC or a complexing agent in CZE) and on electrophoresis. Remarkable enhancements – up to several thousandfold – in detection sensitivity have been achieved [32,33]. Sweeping–MEKC due to its simplicity and sensitivity is an attractive tool for the trace analysis of small molecules found in different sample matrices. These analytes include corticosterone in mouse plasma [34], carbamazepine in pharmaceutical tablets and human urine [35], alkaloids in *Coptidis rhizoma* [36], voriconazole in patient plasma [37], melamine in infant formula [38], linezolid and its achiral impurities in pharmaceutical samples [39], phenol pollutants in industrial wastewater [40], aromatic amines in river water [41], phenylurea herbicides in environmental waters [42], and hypolipidaemic drugs in wastewater [43]. The sweeping–MEKC methods were capable of detecting the analytes of interest at μg/mL to ng/mL levels.

In this study, sweeping–MEKC was employed for the simultaneous analysis of TCA (amitriptyline, clomipramine, doxepin and nortriptyline) and β-blocker (alprenolol, labetalol and propranolol) drugs that may be present in wastewater. Since both TCA and β-blocker drugs are hydrophobic, have amine moieties in their structure, and are cationic at low pH, simultaneous preconcentration via sweeping using sodium dodecyl sulphate (SDS) can be effectively performed with MEKC separation under acidic conditions in a fused silica capillary. Important parameters affecting the separation and preconcentration efficiency, as well as the analytical performance were investigated. The proposed method was demonstrated to be applicable for the simultaneous monitoring of a mixture of basic pharmaceuticals present at ng/mL levels in wastewater by analysis of a spiked sample after a simple liquid–liquid extraction.

2. Experimental

2.1. Reagents and solutions

Reagents for the separation electrolyte or background solution (BGS) (i.e., SDS, ACN and phosphoric acid) were all obtained from Sigma Aldrich (St. Louise, MO, USA). Stock solutions of 0.2 M SDS or 0.5 M phosphoric acid were prepared by dilution of SDS or phosphoric acid with purified water (Milli-Q system; Millipore, Bedford, MA, USA). All stock solutions were sonicated and then filtered through a 0.45 μm MicroScience membrane filter (MicroAnalytix Pty. Ltd., Australia) prior to use. The micellar BGSs were prepared fresh everyday by mixing appropriate amounts of 0.2 M SDS, 0.5 M phosphoric acid, ACN, and purified water. The sweeping sample matrix was prepared by dilution of 0.5 M phosphoric acid with purified water. The BGS and sample matrix were sonicated prior to use. Standards for TCAs (amitriptyline hydrochloride, clomipramine hydrochloride, doxepin hydrochloride, and nortriptyline hydrochloride) and β-blockers (alprenolol hydrochloride, labetalol hydrochloride and propranolol hydrochloride) were also purchased from Sigma Aldrich. Stock solutions of each standard were prepared in 50 or 100% (v/v) methanol to a concentration of 1 mg/mL each. Working standard solutions were prepared by dilution with either BGS or phosphoric acid. All other reagents including methanol, 1-propanol, dichloromethane (DCM) and sodium hydroxide (NaOH) were analytical or USP grade and purchased from different vendors.

2.2. Apparatus

All experiments were performed using an Agilent 3D capillary electrophoresis system and all electropherograms were obtained using Agilent Chemstation Software (Waldbronn, Germany). Preconcentrations and separations were performed in 50 cm (41.5 cm effective length) long fused-silica capillaries (50 μm i.d. and 375 μm o.d.) obtained from Polymicro Technologies (Phoenix, AZ, USA). Capillaries were thermostated at 20 °C all throughout the study. On-line UV detection was performed at 214 nm where all analytes demonstrated sufficient absorbance.

2.3. Sample preparation

The effluent sample was obtained from a local sewage plant in Hobart, Tasmania, Australia. A 5.0 mL sample was made alkaline with 60 μL of 0.5 M NaOH (pH 12) and was spiked with standard TCA and β-blocker drug mixture. The extraction was performed using 1.8 mL of DCM (0.6 mL, 3×). The mixtures were manually shaken for 2 min and centrifuged for 2 min at 2000 rpm. A 0.9 mL aliquot of the DCM extract was isolated and dried *in vacuo*. The sample was reconstituted with 0.4 mL of 150 mM phosphoric acid and sonicated for 3–5 min. The calculation for recovery was based on the 0.9 mL aliquot.

2.4. General electrophoresis procedure

New capillaries were conditioned with 0.1 M NaOH (30 min), purified water (10 min), and micellar BGS (10 min). The BGS in the optimised sweeping–MEKC was 50 mM SDS with 50 mM phosphoric acid and 27.5% ACN. For the MEKC separation study, the % of ACN was varied in the BGS but the concentration of SDS and phosphoric acid were not changed. All injections were performed at the inlet end of the capillary. Sample solutions were introduced either by hydrodynamic injection at 50 mbar or ~950 mbar. Separation voltage was applied at negative polarity with the BGS at both ends of the capillary, until all peaks were detected. After each run, the capillary conditioning was performed with 0.1 M NaOH (0.5 min), followed by purified water (1 min), methanol (1 min), then again with purified water (1 min) and finally with BGS (4 min) by flushing at ~950 mbar. The applied voltage was kept constant at −15 kV. Other conditions are stated in the figures, tables or texts.

3. Results and discussion

3.1. Reversed migration MEKC optimization

Parameters that influence the separation of the test drugs in reversed migration MEKC using acidic pH and fused silica capillaries were evaluated. In reversed migration MEKC, the migration velocity of the micelle is faster than the electroosmotic flow. Since all the drugs contain an amine group and were cationic at acidic conditions, phosphoric acid was chosen as running buffer. With application of voltage at negative polarity (anode at the detector end), the cationic analytes were separated and carried to the anode by the anionic SDS micelles.

3.1.1. Effect of the amount of organic modifier in the BGS

Addition of organic solvent to the BGS is the simplest way to improve separation selectivity in MEKC. Fig. 1 shows the effect of the addition of ACN in the micellar BGS that contained 50 mM SDS and 50 mM phosphoric acid. The micellar BGS with 0–20% ACN did not separate the tested drugs. Increasing the % of ACN to 25, 27.5 and 30% increased the selectivity and resolution, as well as the migration times. However, labetalol and alprenolol were not separated and co-eluted despite the increase in % of ACN. The marked

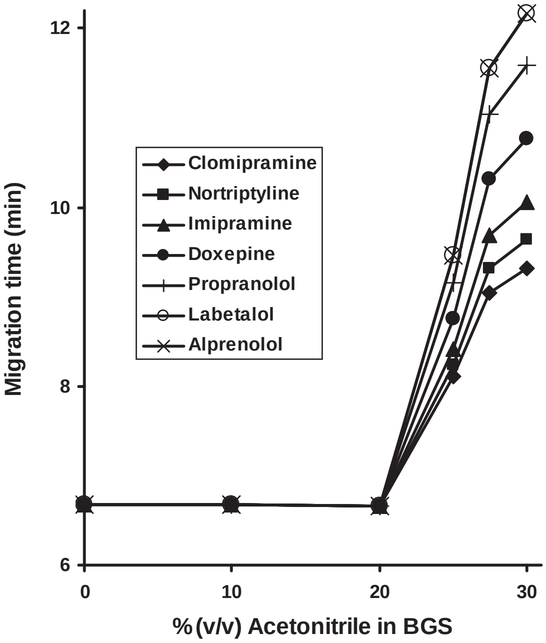

Fig. 1. Effect of the amount of organic modifier (ACN) in the BGS for the separation of TCA and β-blocker drugs in typical MEKC. Conditions: BGS, 50 mM SDS with 50 mM phosphoric acid and (A) 0%, (B) 10%, (C) 20%, (D), 25%, (E) 27.5%, and (F) 30% ACN; sample concentration, 40 μg/mL in BGS; hydrodynamic injection, 3 s at 50 mbar; separation voltage,–15 kV; wavelength, 214 nm.

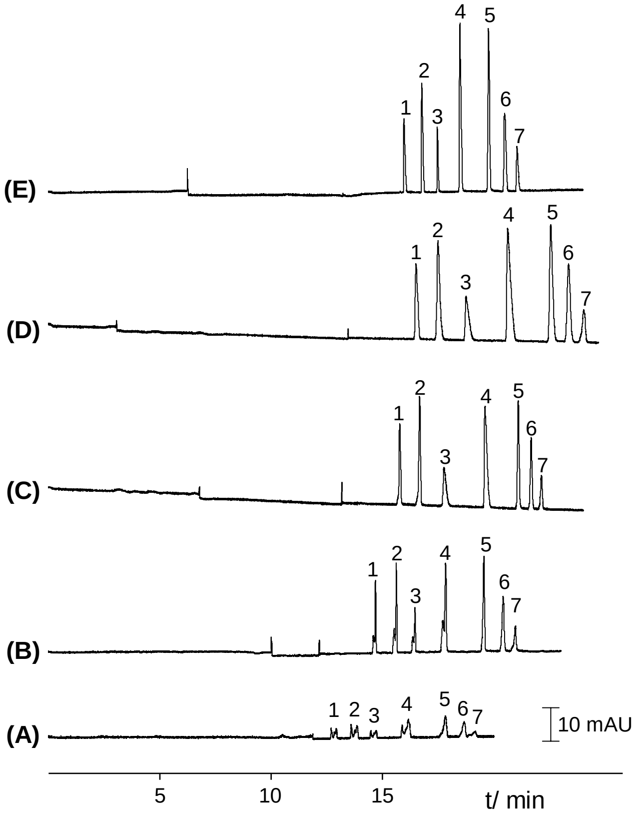

Fig. 2. Effect of sample injection regimen on sweeping–MEKC of TCA and β-blocker drugs. Conditions: BGS, 50 mM SDS with 50 mM phosphoric acid and 27.5% ACN; sample concentration, 0.8 μg/mL in BGS 150 mM phosphoric acid; injection: 50 mbar for (A) 60 s, (B) 150 s, (C) 250 s and (D) 350 s; flushing at ~950 mbar for (E) 14.4 s (equivalent to 250 s at 50 mbar). Other conditions are the same as those used in Fig. 1. Peaks: (1) clomipramine, (2) nortriptyline, (3) imipramine, (4) doxepin, (5) propranolol; (6) labetalol, and (7) alprenolol.

improvement in the separation of analytes is most probably caused by the changes in the structure of the SDS micelle using a %ACN that is greater than 20%. ACN can be incorporated into the SDS micelle [44]. This change in structure reduced the strong interaction between the micelles and cationic drugs. The use of 1-propanol was also investigated but it was unsuccessful in separating all the tested analytes and the migration times are longer compared to that using ACN (data not shown). For best compromise, the 27.5% ACN in the micellar BGS was chosen as the optimum. It is noted that the SDS concentration was chosen at 50 mM because the analyte retention factors at this concentration were high and thus will provide good sweeping efficiencies or performance [30,31]. This was shown by the elution of all the analytes with the micelles in MEKC with 50 mM SDS in the BGS. Also, SDS concentrations greater than 50 mM did not allow any separation and only lead to unacceptably high running currents.

3.2. Sweeping–MEKC condition optimization

Sweeping occurs when the injected sample is free of micelles used in MEKC. In this experiment, a 0.8 μg/mL of TCA and β-blocker mixture dissolved in phosphoric acid was used to study the sweeping performance. Important parameters were investigated including the concentration of phosphoric acid in the sample matrix and the time of sample injection.

3.2.1. Effect of the phosphoric acid concentration in the sample matrix

The standard mixtures of the tested drugs were prepared at different concentrations (50, 100, 150, 200 and 250 mM) of the acid. While the injection time was kept at 60 s at 50 mbar, the peak height intensity and corrected peak area of all analytes increased with the increase in acid concentration from 50 to 150 mM. Corrected peak area was calculated by dividing the peak area with the migration time. In the modes of CE such as MEKC, corrected areas are typically normalized by dividing the peak area by the migration time to ensure correct quantitation [45]. This is because of the differential migration velocity of the analytes through the detector, unlike in HPLC where the velocity is dictated by one flow rate. Note that peak area is proportional to both the analyte concentration and residence time in the detector, which correspond to the peak height and width, respectively. Acid concentrations greater than 150 mM produced broad peaks and a decrease in peak height intensities were observed. In this study, the phosphoric acid concentration of 150 mM in the sample matrix was chosen for further optimisation.

3.2.2. Effect of the sample injection time

Fig. 2 shows the effect of injection time at 50 mbar on the performance of sweeping using 150 mM phosphoric acid as sample matrix. The peak height increased with the increase in injection time from 60 s to 250 s (A–C). When the injection time was further increased (see Fig. 2D), only a slight increase in peak height was observed and the peak widths broadened. In order to reduce the time for sample injection, sample flushing (~950 mbar) for 14.4 s which is equivalent to the 250 s injection at 50 mbar was employed. Sharper and higher peaks were observed (see Fig. 2E). These might be due to less time of contact with the capillary wall thus less adsorption of analytes [46,47].

3.3. Analytical performance

Fig. 3A presents the typical MEKC and sweeping–MEKC electrochromatograms of the TCA and β-blocker drugs. Table 1 lists the

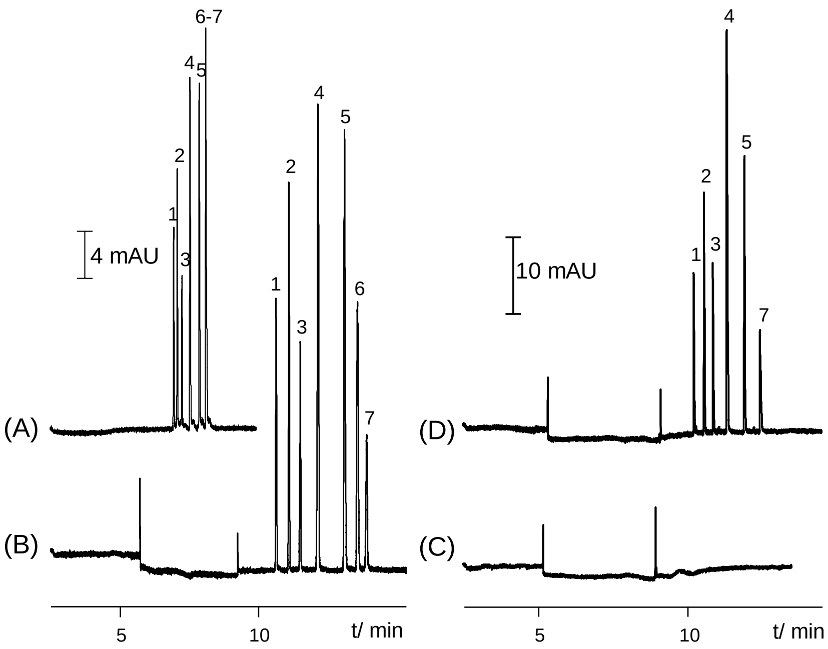

Fig. 3. Typical MEKC analysis with hydrodynamic injection at 50 mbar for 3 s (A) and sweeping–MEKC analysis with hydrodynamic injection at ~950 mbar for 14.4 s (B–D). Samples: 40 μg/mL drug standards in BGS (A), 0.8 μg/mL drug standards in 150 mM phosphoric acid (B), extracted blank wastewater sample (C), extracted wastewater sample spiked with 0.08 μg/mL drugs (D). Other conditions and peak assignments were the same as those in Fig. 2.

Table 1
Sensitivity enhancement factors, method performance, and LODs in sweeping–MEKC method for the analysis of the TCA and β-blocker drugs.

Test analytes	Clomipramine	Nortriptyline	Imipramine	Doxepin	Propranolol	Labetalol	Alprenolol
Sweeping–MEKC							
Regression equation	$y = Ax + B$						
A	7.7115	10.505	5.0208	17.437	14.772	8.2862	10.505
B	0.0362	0.205	0.141	0.2414	0.2403	0.2963	0.205
Correlation coefficient (r^2)	0.9963	0.9977	0.9963	0.9986	0.9984	0.9960	0.9924
Linear range (μg/mL)	0.04–1.2	0.04–1.2	0.04–1.2	0.04–1.2	0.04–1.2	0.04–1.2	0.04–1.2
RSD (%, $n = 10$)							
migration time	2.0	2.1	2.3	2.4	2.8	3.0	3.2
corrected peak area[a]	7.4	7.4	6.5	6.9	7.1	6.2	7.8
peak height	3.8	3.5	2.6	2.3	4.5	4.2	3.8
LOD (S/N = 3, ng/mL)	8	7	15	7	7	14	27
SEF[b]	305	271	213	245	243	260	253
Typical MEKC							
LOD (S/N = 3, μg/mL)	2.4	1.8	2.6	1.1	1.4	2.3	2.3

[a] Corrected peak area = peak area/migration time.

[b] SEF, sensitivity enhancement factor in terms of peak height = dilution factor x (peak height obtained with sweeping–MEKC/peak height obtained with typical MEKC).

analytical performance of the developed sweeping–MEKC method, as well as the limits of detections of the typical MEKC. Compared to typical MEKC, sweeping–MEKC afforded 213–305-fold enhancement in terms of peak height (SEF). These enrichments were obtained due to high affinity of the hydrophobic cationic analytes with the anionic SDS micelles via micellar solubilisation and electrostatic attraction. In addition, the use of sweeping–MEKC (see Fig. 3B) afforded the separation of the last two peaks (labetalol and alprenolol) that co-eluted in typical MEKC mode (see Fig. 3A). The LODs were in the range from 7 to 27 ng/mL. These numbers are at least two orders of magnitude better compared to typical MEKC (see Table 1). In addition, the analytical sensitivity or slope of the calibration graph was highest for nortriptyline, doxepin and propranolol because these analytes have the highest absorption coefficients at the wavelength used for detection (i.e., 214 nm). The LOD obtained for clomipramine was ~2× better compared to imipramine, although both analytes gave quite similar analytical sensitivities. This can be explained by the better focusing effect of sweeping on clomipramine (SEF = 305) compared to imipramine (SEF = 213).

The repeatability of migration time, corrected peak area and peak height of all the tested drugs (0.8 μg/mL) was less than 3.2%, 7.8% and 4.5%, respectively. When one of the analytes was used as internal standard, the repeatability of the results was improved. However, we accepted the repeatability without the use of internal standard, and thus this method was used for further study. The linearity range was examined from 0.04 to 1.2 μg/mL for all analytes and the linearity over the concentration range had a correlation coefficient (r^2) > 0.99. These results indicate that the developed sweeping–MEKC method is reliable and could be used to simultaneously separate the TCA and β-blocker drugs and analyse these drugs with high enrichment efficiencies.

3.4. Analysis of spiked wastewater

The optimized sweeping–MEKC method was applied to the simultaneous determination of TCA and β-blocker drugs in spiked wastewater samples. Electropherograms of wastewater sample and wastewater sample spiked with 0.08 μg/mL of each analyte are shown in Fig. 3C and D, respectively. With higher sample pH, labetalol is anionic due to phenolic OH group [48]. Labetalol (peak 6), therefore, was not extracted from the NaOH treated spiked wastewater sample using DCM and as a result no trace of this drug was detected (see Fig. 3D). Recovery studies were performed by spiking the drugs in wastewater at different concentration levels (16, 80 and 160 ng/mL). Three extraction trials at each level were performed. Average recoveries were obtained in the range of 77–113% for all analytes except for labetalol. The wide interval obtained for the recoveries was due to the laborious sample extraction and the CE analysis at lower concentrations. However, no interference from the sample matrix was observed and enrichment factors of up to 21-fold were achieved using simple liquid–liquid extraction.

4. Conclusion

A high sensitivity sweeping–MEKC method with a liquid–liquid extraction step for the simultaneous analysis of TCA and β-blocker drugs in wastewater was presented. Acceptable recoveries were obtained from spiking wastewater at 16–160 ng/mL levels indicating that the proposed method is comparable to that reported using new and sophisticated liquid-phase separation methods [7–9]. For example, Vázquez et al. reported $\leq$2 mg/mL LOD for five β-blockers in wastewater using coupled-column liquid chromatography and fluorescence detection [7], Nödler et al. reported 5–160 ng/L for 46 micro-contaminants including β-blockers using solid phase extraction followed by HPLC, electrospray ionization with tandem mass spectrometric detection [8], and Lajeunesse et al. reported LODs of 48–100 ng/mL for similar drugs using liquid chromatography with tandem mass spectrometry [9]. It is noted that the proposed method here is much simpler and cheaper to operate compared to the above methods.

The LODs (i.e., 7–27 ng/mL) obtained are also comparable to those reported using other stacking techniques in CE for the analysis of TCA and β-blocker drugs [22–24,27,29]. However, previous methods have only analysed one group of sample, either TCA or β-blocker drugs, and not simultaneously as described here. For β-blocker drugs, LODs of 9.1–23.3 ng/mL and 10–30 ng/mL were obtained in CZE using large volume sample stacking [22] and MSS [29], respectively. For TCA drugs, LODs of ~2 ng/mL were obtained in CZE with stacking by field amplification [23]. Others have utilized a bubble capillary in CZE together with stacking by field amplification and achieved LODs of 3–14 ng/mL [24] or 1.8–5 ng/mL [27].

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