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Analyses of non-steroidal anti-inflammatory drugs by on-line concentration capillary electrochromatography using poly(stearyl methacrylate–divinylbenzene) monolithic columns

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ABSTRACT

This study describes the ability of on-line concentration capillary electrochromatography (CEC) coupled with UV or mass spectrometry (MS) for the determination of nine common non-steroidal anti-inflammatory drugs (NSAIDs) in water samples. A series of poly(stearyl methacrylate–divinylbenzene) (poly(SMA–DVB)) monolithic columns, which were prepared by single step *in situ* polymerization of divinylbenzene (DVB), stearyl methacrylate (SMA) and vinylbenzenesulfonic acid (VBSA, charged monomer), were developed as separation columns for the first time. The effects of polymerization condition of monolithic columns on analyte separations were examined, and the results indicated that separation performances were markedly improved in monolithic columns prepared with short reaction time (3 h) and low SMA:DVB ratio (40/60 ratio of SMA:DVB). Subsequently, an on-line concentration step of step-gradient elution was combined to this CEC system, and by optimizing the difference in eluent strength between the sample matrix and mobile phase, all NSAIDs detection sensitivity were improved (limit of detection (LOD) was 3.4–10 μg/L for UV, and 0.01–0.19 μg/L for MS). When compared to the best CE and LC reports on NSAIDs analyses so far, this on-line concentration CEC method provided better detection ability within shorter separation time (12 min) when either UV or MS detector was employed. This is the first report for on-line concentration CEC with MS detection applied in trace solute analyses of real samples.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of analgesic, antipyretic and anti-inflammatory drugs, which have been used extensively in human and farm animals to treat inflammations and other related clinical effects. It has been well documented that the use of NSAIDs does not produce sedation, respiratory depression or addiction, so that some of them are available without prescription. However, NSAIDs are regarded as being one of the emerging chemical contaminants because of their high environmental distribution and potential ecotoxicological effects [1–3]. Recently, several reports indicated that the NSAIDs residues in diverse aquatic environments have reached at the ng/L to μg/L level [4,5]. In response to the increasing unpredictable threat to public health, some national and international environment authorities have begun monitoring NSAIDs residues in environmental samples. Consequently, the development of highly sensitive and simple methods for NSAIDs determination have received considerable attention in recent years.

Most literatures on NSAIDs analyses of pharmaceutical and environmental samples have mainly utilized high performance liquid chromatography (HPLC) [6–11], free solution capillary electrophoresis (CE) [12,13], micellar electrokinetic chromatography (MEKC) [14–16], microemulsion electrokinetic chromatography (MEEKC) [17–21] and capillary electrochromatography (CEC) methods [22–29]. CEC is a hybrid separation technique which combines the features of HPLC and CE, and has gained much attention in recent years [30–34]. Many studies have demonstrated the potential applications of CEC for the separation of a wide range of compounds including charged and neutral analytes [35–37]. CEC reports of NSAIDs analyses mostly used C_{18} packed capillaries as separation columns with the exception of the polyacrylamide- and polymethacrylate-based monolithic capillaries that were proposed by Hoegger and Freitag and Yan et al., respectively [24,38], in these two cases, no analytical performances as well as real sample applications were evaluated.

Narrow internal diameter of the capillary tube, however, has made all CE derived techniques including CEC, to generally have

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lower sample capacity and concentration sensitivity. Therefore, a suitable sample concentrating step or a combination of highly sensitive detector such as mass spectrometer coupled to CEC separation is urgently needed to extend CEC potential in trace solute analyses of "real world" samples [39–49]. While a wall-coated histidine capillary column coupled to an on-line concentration step has already been successfully developed in improving NSAIDs detection wherein a retention time of 76 min was required for seven NSAIDs separation [28], to our knowledge the analysis of NSAIDs in environmental water by on-line concentration CEC method using polymeric monolith column has never been documented.

In this study, analytical methods for the simultaneous separation and identification of NSAIDs residues in water samples were developed based on both high sensitivity on-line concentration CEC-UV and CEC–MS methods. A series of poly(stearyl methacrylate–divinylbenzene) (poly(SMA–DVB)) monolithic columns prepared by a simple *in situ* polymerization with different polymerization time and SMA–DVB ratio were used first as separation column, and their effects on NSAIDs separations were compared. Furthermore, several variables such as compositions of sample matrix (pH and organic solvent ratio), sample injection time and MS parameters, were examined in order to achieve optimal NSAIDs analyses. Finally, the proposed on-line concentration CEC methods were also employed for the determination of NSAIDs in water samples with as low as 50 μg/L or 2 μg/L level, and with no complicated sample pretreatment was necessary. This work demonstrates that the combination of on-line concentration CEC with MS detection is feasible in trace solute analyses of "real world" samples for the first time.

2. Experimental

2.1. Chemicals and reagents

Sulindac (SUL, pK_a 4.2) and indoprofen (INP, pK_a 4.4) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ketoprofen (KEP, pK_a 4.2), naproxen (NAP, pK_a 4.8), flurbiprofen (FLB, pK_a 4.1), ibuprofen (IBP, pK_a 4.4), indomethacin (IND, pK_a 4.0), diclofenac (DIC, pK_a ~4.2) and stearyl methacrylate (SMA) were purchased from TCI (Tokyo, Japan). Fenoprofen (FEP, pK_a 4.2) was purchased from MP (Illkirch, France). Divinylbenzene (DVB) and vinylbenzenesulfonic acid (VBSA) were obtained from Aldrich (Steinheim, Germany). Azobisisobutyronitrile (AIBN) and ammonium formate were bought from Showa (Tokyo, Japan). *N*-Methyl-2-pyrrolidone (NMP) was purchased from Mallinckrodt Baker (Paris, KY, USA). Uncoated fused-silica capillaries with 100 μm I.D. and 375 μm O.D. were purchased from Reafine Chromatography Ltd. (Hebei, China). All other chemicals were of analytical-reagent grade, and were used as received except for DVB, which was washed with 10% (w/v) aqueous sodium hydroxide to remove the inhibitors prior to use. The NSAIDs standards were individually dissolved in methanol at a stock concentration of 2 mg/mL and then stored at 4 °C. Mobile phases of pH 3 were prepared by mixing acetonitrile (ACN) with phosphate buffer (5 mM) (CEC-UV) or with ammonium formate buffer (5 mM) (CEC–MS) in different volume ratios, in which 1.0 M HCl (CEC-UV) or formic acid (CEC–MS) was added until the desired pH was achieved.

2.2. Apparatus

All CEC-UV experiments were performed with a Beckman Coulter MDQ CE system equipped with a photodiode array detector (Fullerton, CA, USA). Beckman Coulter MDQ 32 Karat software was used for CEC-UV instrumental control and data analysis. The CEC–ESI-MS experiments were performed with a configured in-house CE coupled to a Bruker Daltonics TOF mass spectrometer model microTOF II (Bremen, Germany) with an Agilent ESI source (model G1607-6001). The setup in this configured CE consisted of a platinum electrode in a vial containing a running buffer connected to CZE1000R high-voltage power supply (Spellman, Plainview, NY, USA). The microTOF control and Data Analysis™ software were used for mass instrumental control and data analysis.

2.3. Preparation of polymeric monolithic column

Prior to the preparation of a polymeric monolithic column, the inner wall of a 100 μm ID capillary column was treated according to the procedure described in our previous article [50]. After conditioning, a solution composed of monomers (DVB and SMA), porogenic solvents (water, cyclohexanol and NMP), charged monomer (VBSA) and initiator (AIBN) was used to prepare the polymeric columns. The polymerization procedure was optimized by univariate and multivariate approaches by varying four parameters (the reaction temperature, the reaction time, the monomer–porogenic solvent ratio and the SMA–DVB ratio) likely to have the most significant effect on the NSAIDs separation. In this study, the conditions of each of the four parameters were varied (i.e. the reaction temperature with 50, 60 and 70 °C, the reaction time with 3, 7 and 15 h, the monomer–porogenic solvent ratio with 18/82%, 24/76% and 30/70% and the SMA–DVB ratio with 33/67%, 40/60% and 50/50%) either by the univariate or multivariate approach. In the univariate approach, only one parameter was changed for each column preparation, but two or three parameters were varied simultaneously in the multivariate approach. Finally, the optimal polymerization condition obtained by both approaches was the same as described below. 0.0155 g of AIBN (0.67%, w/v) and 0.0448 g of VBSA (1.93%, w/v) were dissolved in 2318 μL of monomer mixture, which included 40% SMA (v/v, 927 μL) and 60% DVB (v/v, 1391 μL). Ternary porogenic solvent, which consisted of water (375 μL; 5%, v/v), cyclohexanol (4180 μL; 57%, v/v) and NMP (2787 μL; 38%, v/v), was slowly added to the monomer mixture. The solution was sonicated for 15 min until it became homogeneous, then it was used to fill the preconditioned capillary (33-cm) to a total length of 20- or 30-cm by syringe injection. The remainder of the homogeneous mixture was sealed in a glass vial. After both ends of the capillary were sealed with ethylene vinylacetate (EVA) adhesive resin, the capillary and the glass vial were submerged in a 70 °C water-bath for 3 h. The monolithic column was then washed with methanol and mobile phase by an LC pump. For CEC-UV system, a detection window was fabricated by using a microtorch to remove the polyimide coating at the 20-cm position on the column, where a polymer bed was absent. The monolithic polymer formed in the vial was Soxhlet extracted with methanol for 24 h, and then vacuum dried overnight. The polymers produced in glass vials were used to obtain surface analyses. A surface area analysis equipment model Tri-star 3000 from Micromeretics (Norcross, GA, USA) was employed for surface area measurement of the monoliths. A scanning electron microscope S-4700 type II from Hitachi (Tokyo, Japan) was used for morphology observation of the monolithic columns.

2.4. Operation condition for CEC

The monolithic column was placed in the CE instrument and was equilibrated with the mobile phase under 10 kV applied voltage with 40 psi pressure at both ends of the column until a stable baseline was obtained in CEC-UV, while no pressure was applied at both ends of the monolithic column in CEC–MS. CEC-UV separations were performed in 30.2-cm total length (20-cm length filled with polymer) of 100-μm I.D. fused-silica capillaries, and the same capillaries of 30-cm total length filled with polymer were used for CEC–MS, which is the shortest distance from the inlet end of this

configured CE instrument to the ESI interface. Samples and standards were electrokinetically injected into the capillary for 3 s at a voltage of 10 kV for normal CEC mode. CEC separations were carried out using electrical voltage of 20 kV. The temperature of the capillary and the detection wavelength in CEC-UV system was maintained at 25 °C and 200 nm, respectively. On the other hand, MS detection was performed in the selected ion mode. Since all NSAIDs had relatively strong molecular ion signals which exhibited as $[M-H]^-$ form except for SUL with $[M+H]^+$ form, these accurate-mass molecular ion peaks (i.e. 357.096 *m*/*z* for SUL, 280.096 *m*/*z* for INP, 253.085 *m*/*z* for KEP and 229.085 *m*/*z* for NAP, 241.085 *m*/*z* for FEP, 243.081 *m*/*z* for FLB, 205.122 *m*/*z* for IBP, 356.068 *m*/*z* for IND and 294.008 *m*/*z* for DIC) were selected as monitored mass signals in the following CEC–MS experiments. Negative ions (or positive ion for SUL) were generated through the application of 3.8 kV (or −4.5 kV) to the probe tip, and end plate off-set was fixed at −0.5 kV. Nitrogen gas was used as drying gas at 180 °C with a flow rate of 4 L/min. Nitrogen nebulization gas for electrospray was supplied at 0.4 psi. The sheath liquid (isopropyl alcohol (IPA)/water (90/10, v/v) containing 0.1% 1 M NH_3) was delivered to electrospray at 220 mL/h. Scanning mass range was from *m*/*z* 50 to 1500.

2.5. On-line concentration step

A step gradient elution on-line sample concentration was used to enhance the detection sensitivity of NSAIDs. First, the CEC column was filled with a pH 3 mobile phase (50% phosphate or ammonium formate solution (5 mM), 50% ACN), and standards or samples which were first mixed with phosphate (CEC-UV) or ammonium formate (CEC–MS) solution (20% ACN, pH 3, 5 mM) in a volume ratio of 1:24 (i.e. the volume ratio of acidic solution, methanol and ACN was 76.8%:4%:19.2%), were then electrokinetically injected into the capillary for 15 min at a voltage of 10 kV. After sample injection, a voltage of 20 kV was applied with the original mobile phase in the inlet vial, and then the CEC separation proceeded.

2.6. Real sample and pretreatment

10 mL of water samples, with or without the spiked NSAIDs standards (50 μg/L for UV mode or 2 μg/L for MS mode), were filtered through a 6 μm filter followed with a 0.45 μm nylon membrane. For CEC-UV mode, the clear solution was oven-dried at 100 °C, and then the final dry residues were vortex-mixed with a 10 mL of phosphate or ammonium formate solution (pH 3, 5 mM) diluted with methanol and ACN in a volume ratio of 76.8%:4%:19.2% for 30 min. The resulting liquid was filtered with a 0.2 μm nylon membrane prior to CEC-UV analysis.

For CEC–MS mode, the filtered water sample was then acidified to pH 2 with 12 M HCl solution, and was ready for solid-phase extraction (SPE) procedure described below. A C-18 SPE column (LC-18; Supelco, Bellefonte, PA, USA) was conditioned prior to use by washing with methanol (6 mL), deionized water (6 mL) then followed with HCl aqueous solution (pH 2, 3 mL). After the addition of the acidified water sample, the SPE column was dried by N_2 flush, and then was eluted with methanol. The eluted methanol solution was mixed with an ammonium formate solution (pH 3, 5 mM) and ACN in a volume ratio of 4%:76.8%:19.2%, and then was directly analyzed by on-line concentration CEC–MS.

3. Results and discussion

3.1. Optimal polymerization condition of poly(SMA–DVB) column

Since this is the first time that this novel *in situ* polymerized poly(SMA–DVB) monolithic column was used as the stationary phase in CEC, polymerization conditions were examined. Four parameters were studied and the conditions of each parameter were varied (i.e. reaction temperature: 50, 60 and 70 °C, reaction time: 1, 3, 7 and 15 h, monomer–porogenic solvent ratio: 18/82%, 24/76% and 30/70%, and SMA–DVB ratio: 33/67%, 40/60% and 50/50%). Among these parameters, the polymerization time and the SMA–DVB ratio were found to significantly influence the NSAIDs separation, and this is reported below.

3.1.1. Polymerization time

The effect of varying the polymerization time of poly(SMA–DVB) monolithic columns on the NSAIDs separation was examined and results are shown in Fig. 1. Most of the NSAIDs compounds were separated well on the monoliths polymerized for shorter reaction time (1 or 3 h), while the broad and overlapped signals were obtained at the polymerization time of 7 or 15 h. The surface area of poly(SMA–DVB) monolith was measured to be 86.7, 37.6, 29.5 and 31.4 (m^2/g) for 1, 3, 7 and 15 h reaction time, respectively. Moreover, morphological observation from the scanning electron microscope (SEM) displayed a smaller granular structure of the poly(SMA–DVB) monolith produced at shorter reaction time. Therefore, a better resolution was observed since the monolith provided a more predominant sieving effect and larger surface area to interact with the NSAIDs.

On the other hand, evaluation of the conversion yield by comparing the weights of the poly(SMA–DVB) monolith and the original monomer at different reaction times indicated that higher conversion yields were obtained with increasing reaction times (i.e. 24.8%, 65.6%, 82.7% and 88.4% (w/w) for 1, 3, 7 and 15 h, respectively). This is consistent with the observation of the optical microscope image where a dense and homogeneous monolith was formed at 15 h, but few amounts of poly(SMA–DVB) monolith and many voids were produced at 1 h. Upon examination of the profiles in Fig. 1, it indicated that the NSAIDs retention increased by almost two-fold when the polymerization time was reduced from 15 h to 1 h. Basically, solute migration in the CEC system is determined by the interaction between the analyte and the stationary phase as well as the EOF magnitude; the latter is highly dependent upon the amount of charged monomers (for example, VBSA) carried on the stationary phase. Therefore, the longer NSAIDs retention in columns polymerized for 1 or 3 h was not only due to the more significant interaction (sieving effect) between the monolith and the NSAIDs but also to the smaller EOF brought by the few amounts of polymer produced at 1 or 3 h polymerization. Consequently, the poly(SMA–DVB) column prepared for 3 h, which had adequate EOF and sieving effect, and provided a baseline separation for all analytes within the shortest time, was chosen as the optimal condition.

3.1.2. SMA–DVB monomer ratios

Following the study on the optimum polymerization time, the effect of SMA–DVB monomer ratio on NSAIDs separation was examined. A series of monomer solution composed of SMA and DVB in various volume ratios (33/67, 40/60 and 50/50) was used to prepare the poly(SMA–DVB) columns. Results indicated that as the amount of DVB was increased, the peak resolutions improved markedly though all analytes had relatively longer retention (Fig. S1, supplementary data). For example, the retention times of 6, 11 and 19 min were observed at 50%, 60% and 67% DVB levels, respectively. This was likely due to the smaller nodule polymer produced in the higher DVB amount resulting to a significant sieving effect that enhanced the NSAIDs retention and resolution on the poly(SMA–DVB) column. In addition, each tested compound carries one or two benzene moieties on its structure. Once the polymeric stationary phase carries more benzene rings, a stronger π–π interaction between the analytes and the stationary phase would possibly increased NSAIDs retention on the poly(SMA–DVB). By

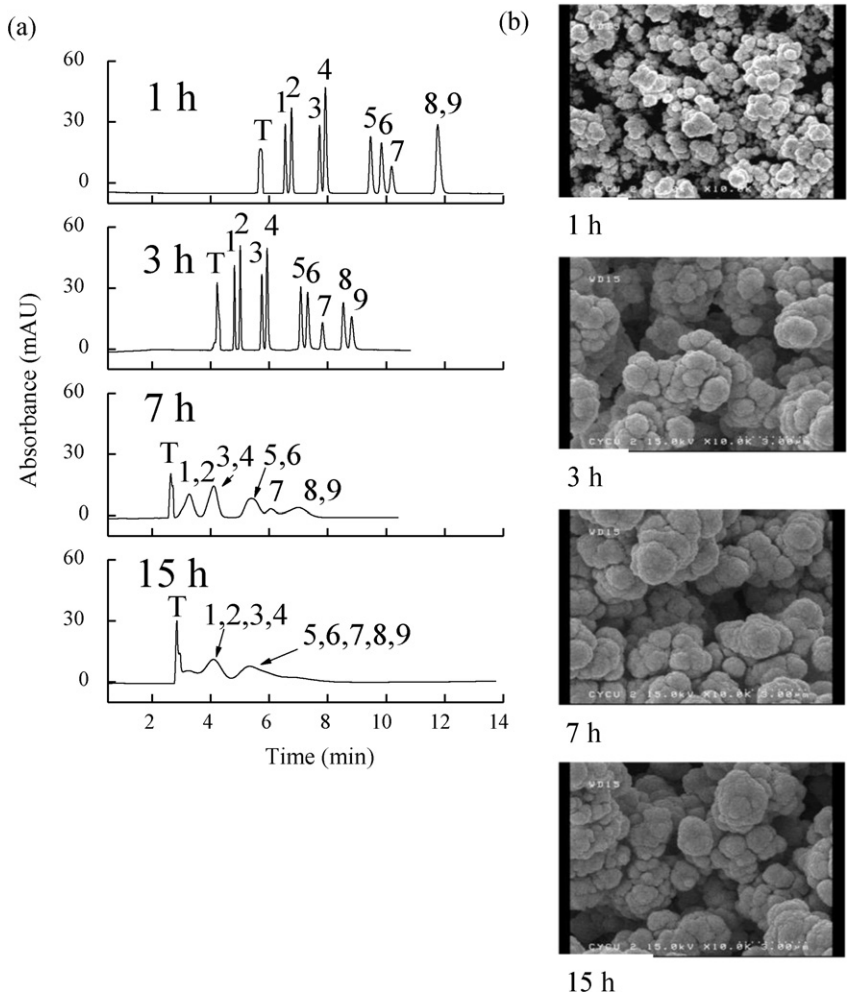


Fig. 1. (a) Electrochromatograms of 9 NSAIDs separated in poly(SMA–DVB) stationary phase. 24% (v/v) monomers, SMA and DVB in the volume ratio of 40%:60%; 76% (v/v) porogenic solvent (NMP, cyclohexanol and H_2O). Mobile phase, 50% phosphate buffer and 50% ACN (5 mM, pH 3.0). 20 kV was applied to a 30-cm capillary tube (20 cm active length filled with monolithic stationary phase). 250 μg/ml NSAIDs was electrically injected at 10 kV for 3 s; T (thiourea), 1 (sulindac), 2 (indoprofen), 3 (ketoprofen), 4 (naproxen), 5 (fenoprofen), 6 (flurbiprofen), 7 (ibuprofen), 8 (indomethacin), and 9 (diclofenac). (b) SEM micrographs of poly(SMA–DVB) at different polymerization time.

comparison, the polymeric monoliths prepared with the SMA–DVB ratio of 40/60 had the best resolution ($R > 1$) for IND and DIC within 12 min, and thus was regarded as the best monomer ratio in the study.

3.2. Optimization of on-line concentration step for NSAIDs analyses

On-line sample focusing effects have been reported in CEC using either C_{18} particles packed columns or monolithic columns [39–49] wherein either the step gradient elution (i.e. chromatographic zone-sharpening effect) or the field amplified sample injection (FASI) (i.e. using sample matrix with low electrolyte amounts) was regarded as the most effective strategy to lower the detection limit of neutral and charged solutes. In this study, these two focusing modes, FASI and step gradient elution, were examined subsequently to enhance the NSAIDs sensitivity.

3.2.1. Effect of sample matrix pH on NSAIDs sensitivity

All NSAIDs with pK_as around 3.9–5.0, are present as anions at sample matrix pH higher than 5. Thus, an anion-selective injection (ASI) step that has an advantage of low sample solvent introduced into the CEC column was first used to concentrate these NSAIDs. Several phosphate buffers (pH 5–7) were used as sample matrices to convert all NSAIDs to anion form, and an injection voltage of −10 kV was used to introduce these analyte anions into the column; however, no analyte signals were observed. This could be due to the presence of sulfonic groups on the charged monomer, VBSA; as a consequence, the poly(SMA–DVB) column produced a normal EOF that migrated from the positive to the negative electrode at a wide pH range. When a negative injection voltage (−10 kV) was employed, this normal EOF caused all NSAID anions to be pushed back to the inlet end, thus no NSAIDs were detected. The above results demonstrated that the ASI step was not feasible to improve the NSAIDs sensitivity in the poly(SMA–DVB) column. Alternatively, when the acidic phosphate buffer (pH 3) and the positive voltage (10 kV) were employed as the sample matrix and injection voltage, respectively, the neutral NSAIDs were successfully introduced into the column by the normal EOF driving (Fig. 2(a), 0% ACN). In contrast to the normal sample injection (10 kV, 3 s), the detection sensitivity was obviously enhanced by a longer injection time (10 kV for 180 s) and a slight field amplified condition.

3.2.2. Effect of ACN level in the sample matrix on NSAIDs sensitivity

Previous study on the step-gradient elution has demonstrated that a larger difference in the eluent strength between the mobile phase and the injection solvent creates a better compression of sample zone [39,40]. Because of markedly different acetonitrile (ACN) level between the sample matrix and the mobile phase (e.g.

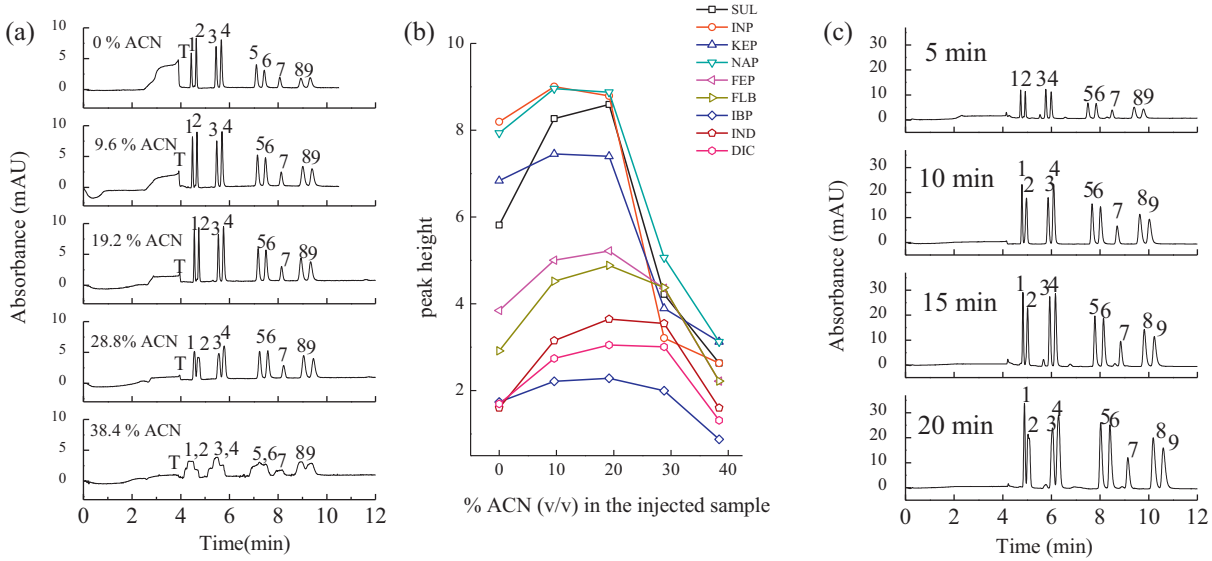


Fig. 2. Effect of (a and b) ACN level in the sample matrix, and (c) sample injection time on NSAIDs signals for the on-line concentration step. Polymerization time was kept at 3 h. 1 μg/ml NSAIDs was electrically injected at 10 kV for 3 min (a and b). All other conditions were the same as in Fig. 1.

0% and 50% ACN, respectively), the enhancement of NSAIDs sensitivity as shown in Fig. 2(a) (0% ACN) was partly contributed to the chromatographic zone-sharpening effect. In order to clarify the influence of eluent strength on the NSAIDs sensitivity, the optimal ACN level in both mobile phase and sample matrix was examined. Since a higher ACN level in the mobile phase provided inadequate resolution for the nine analytes (e.g. 60% ACN), a phosphate buffer containing 50% ACN had to be used as the mobile phase, as a result, only the ACN level in the sample matrix was optimized.

Fig. 2(a) shows the electrochromatograms derived from different ACN level of the sample matrix (0–38.4% ACN in phosphate solutions of pH 3), in which the NSAIDs standards (1 μg/mL) were electrokinetically injected at 10 kV for 180 s. As can be seen in Fig. 2(b), in which the peak height of NSAIDs is a function of ACN level in the sample, a slight increase in the peak height as ACN percentage in the sample matrix is in the range of 0–19.2% (sensitivity enhancement factor in terms of peak height (SEF_{height} = dilution factor × (peak height obtained with on-line concentration step/peak height obtained with normal injection)) changed from 29–67 (0% ACN) to 59–76 (19.2% ACN)), while the peak intensities, as well as resolution, worsen when the ACN amount was further increased to 28.8% or 38.4% (SEF_{height} changed from 22–75 (28.8% ACN) to 18–32 (38.4% ACN)). This observation is consistent with previous reports on on-line concentration CEC using C_{18} packed column [41,47]. Similar to the pre-wetting effect of SPE material with an activating solvent (methanol or ACN), increasing the ACN amount in the sample (0–19.2%) improved the surface interaction between the poly(SMA–DVB) and the solutes, and thus enabled the NSAIDs to accumulate in the column entrance in higher concentrations leading to a more enhanced signal. As the ACN concentration in the sample is increased further (28.8% or 38.4%), the eluent strength in the sample solution strengthened, and the solute affinity to the poly(SMA–DVB) material was reduced. Moreover, the subsequent eluting step was unable to further compress the sample band that spread during injection; thus, a significant decrease in the focusing effect of the mobile phase elution (28.8% ACN), as well as a wider peak width (38.4% ACN), was obtained (Fig. 2(a) and (b)).

By comparing the separation ability and peak intensities in Fig. 2(a) and (b), the phosphate buffer (pH 3, 9.3 mM) composed of 19.2% ACN that provided the highest sensitivity for most NSAIDs, was used as the optimal sample matrix in the on-line concentration CEC method.

3.2.3. *Effect of sample injection time on NSAIDs sensitivity*

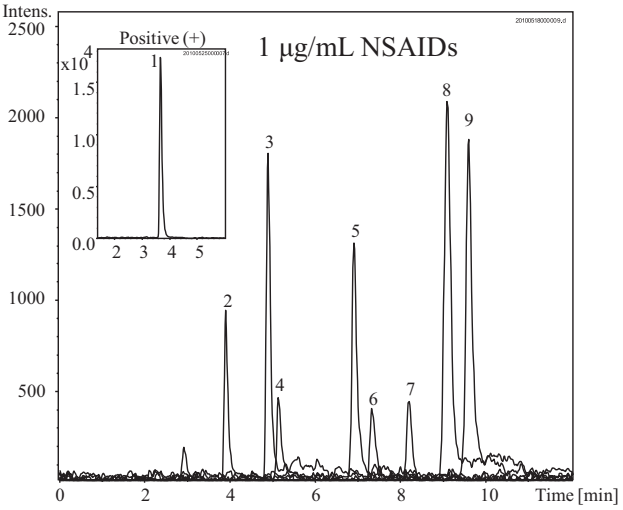
From the above results, an acidic sample matrix (pH 3) containing lower ACN content (19.2%) provided better on-line enrichments for NSAIDs compounds. Next, it was necessary to examine the sample injection time to maximize the NSAIDs sensitivity. The results shown in Fig. 2(c) indicated that all peak heights increased greatly as the injection time was raised from 5 to 15 min, but above 15 min, a significant decrease in peak height and resolution of INP, KEP and NAP was observed. Obviously, if the injection time was over 15 min, the NSAIDs compounds would not have accumulated on the column inlet; instead, a sample band spreading could possibly happen. This sample band spreading would otherwise limit the focusing effect in the following mobile phase elution, as a result, the resolution and efficiency of NSAIDs will worsen. Therefore, the 15 min sample injection time was chosen in this study since it provided better detection sensitivities and resolutions for all NSAIDs (SEF_{height} = 161–306).

3.3. *CEC method coupled with mass spectrometer for NSAIDs analyses*

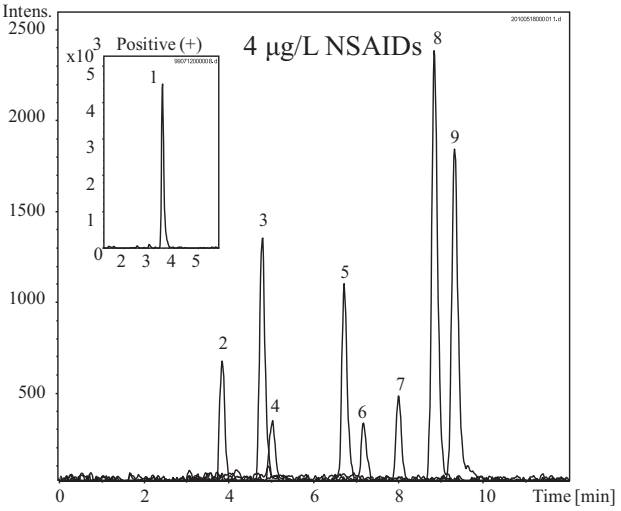
3.3.1. *The optimal mass parameters*

Next, an attempt was made to develop a poly(SMA–DVB) CEC separation combined with mass spectrometric detection for the nine NSAIDs. When the phosphate electrolyte in the original mobile phase was replaced with ammonium formate (5 mM), the optimal mobile phase for NSAIDs separation proposed in the above CEC-UV system (i.e. Section 3.2) also provided good mass sensitivities and resolutions, and thus it was employed as the mobile phase in the CEC–MS system.

Several instrumental parameters of the mass spectrometer such as capillary voltage, flow rate and composition of sheath liquid were examined to obtain the highest mass signals for the tested compounds. The results indicated that the use of 3.8 kV capillary voltage in the ESI source led to an increase in negative ions for most NSAIDs, however, the ion signals of SUL (i.e. positive ions) were only generated with the application of −4.5 kV capillary voltage. Since there were different charged states in the ESI source for the nine NSAIDs, both positive and negative modes ($[M+H]^+$ form for SUL and $[M-H]^-$ for the rest of the analytes) were employed to monitor the NSAIDs in the CEC–MS experiment. Subsequently, the flow rate of the sheath liquid was varied (140, 180, 220 and



(a) normal injection (10 kV for 3 s)



(b) on-line concentration injection (10kV for 15min)

Fig. 3. Electrochromatograms of 9 NSAIDs separated using poly(SMA–DVB) monolithic column in CEC–MS by (a) normal injection (10 kV for 3 s) and (b) on-line concentration injection (10 kV for 15 min). Mobile phase, ammonium formate (5 mM, pH 3) and ACN in the volume ratio of 50:50. 20 kV was applied to a capillary tube of 33 cm (30 cm active length filled with monolithic stationary phase). Sheath liquid: 220 mL/h, IPA/water (90/10, v/v) containing 0.1% 1 M NH_3; 4 L/min dry gas flow rate; 180 °C dry gas temperature.

240 μL/h), and its influence on the NSAIDs mass signals was compared. Although the mass signals of NSAIDs compounds rarely varied with the flow rate of the sheath liquid, all analytes had the most stable mass signals at the flow rate of 220 μL/h. The effect of the sheath liquid composition on analyte signals was also evaluated. Several organic solvents (isopropyl alcohol and methanol) mixed with water in various ratios (9:1, 8:2 and 7:3, v/v) were employed as sheath liquids, but no mass signals were detected. But, when an ammonium solution was added to the sheath liquid of isopropyl alcohol/water mixture, a reversed effect was found. This implied that most of the analytes existed in neutral forms in the CEC mobile phase (pH 3), thus the addition of ammonium base to the sheath liquid can improve the ionization ability of NSAIDs analytes. On the other hand, the ammonium concentration in the sheath liquid also needed optimization to achieve the highest mass signals. The results showed that the mass signals of NSAIDs obviously varied with the ammonium amount, and the use of 0.5 mM (vs. 0.25 mM) led to an increase in the signal to noise (S/N) ratio for IND and DIC by 162% and 86%, respectively, but the addition of 1 mM ammonium (vs. 0.25 mM) decreased the S/N ratio by 34% for the former and 38% for the latter. Consequently, the mixture solution of isopropyl alcohol and water in the ratio of 9:1 (v/v) containing 0.5 mM ammonium electrolyte provided the highest mass signals for most NSAIDs, and thus was chosen as the optimal sheath liquid.

3.3.2. NSAIDs analyses by normal injection and on-line concentration CEC–MS

The electrochromatograms of NSAIDs standards (1 ppm each) using the previously optimized operation conditions for CEC–MS methods are shown in Fig. 3(a). From the results obtained, the baseline separation for the nine NSAIDs was still acquired within 10 min even if a longer monolithic column (30-cm column length filled with stationary phase) was used in the combination of CEC separation and MS detection.

As mentioned in Section 3.2, an on-line concentration step of step-gradient elution has been demonstrated to increase effectively the NSAIDs signals in CEC-UV (Fig. 2(c), 15 min). To further enhance their signal sensitivities in MS detector, the feasibility of the optimal CEC–MS method combined with the proposed on-line

Table 1
Limits of detection, repeatability of retention time, calibration curves and coefficients of determination obtained for NSAIDs standards in on-line concentration CEC method with UV detection.[a]

NSAID compounds	LOD (μg/L) (S/N = 3)	Retention time (min) (RSD%)	Calibration curves[b]	Coefficient of determination for calibration curves (r^2)
(1) Sulindac	3.4	5.02 (0.30)	$y = 90{,}006x + 13{,}429$	0.9982
(2) Indoprofen	4.5	5.21 (0.30)	$y = 108{,}223x + 12428$	0.9986
(3) Ketoprofen	4.0	6.25 (0.46)	$y = 114{,}270x + 11508$	0.9988
(4) Naproxen	3.4	6.52 (0.46)	$y = 151{,}860x + 18221$	0.9985
(5) Fenoprofen	4.9	8.38 (0.61)	$y = 123{,}263x + 10781$	0.9994
(6) Flurbiprofen	5.2	8.79 (0.67)	$y = 136{,}304x + 11471$	0.9993
(7) Ibuprofen	10	9.56 (0.64)	$y = 65{,}776x + 1971$	0.9999
(8) Indomethacin	6.7	10.68 (0.70)	$y = 129{,}842x + 11961$	0.9989
(9) Diclofenac	8.3	11.11 (0.68)	$y = 115{,}378x + 13811$	0.9982

[a] Separation conditions: the volumes of acetonitrile and 5 mM phosphate buffer in the mobile phase was in a ratio of 50:50. Standards were electrokinetically injected into the capillary at a voltage of 10 kV for 15 min. The data of LOD and calibration curves did not include sample pretreatment. Values were means of three intra-day replicates on the same column. The value in parenthesis indicates the RSD of retention time in percentage.
[b] The calibration curves were constructed from three replicate measurements at each concentration in the range of 0.1–10 μg/mL (0.1, 0.5, 1, 5 and 10 μg/mL).

concentration step in the CEC-UV system was also evaluated. Fig. 3(b) is the electrochromatogram of the on-line concentration CEC–MS, in which the NSAIDs standards (4 μg/L) were first prepared in an acidic sample matrix (i.e. the volume ratio of ammonium formate solution, methanol and ACN was 76.8%:4%:19.2%), and then electrokinetically injected for 15 min at a voltage of 10 kV. It obviously demonstrated that this on-line concentration step of step-gradient elution with a longer injection time indeed improved the mass sensitivity of all tested analytes without a loss in separation velocity and resolution.

3.4. Comparison of on-line concentration CEC-UV and CEC–MS for NSAIDs analyses

The qualitative and quantitative performances of the proposed CEC-UV and CEC–MS methods under optimal conditions are shown in Tables 1 and 2, respectively. The RSDs of retention time in the CEC-UV method were in the range of 0.3–0.7% ($n = 3$), while in the CEC–MS method, these were 0.0–1.77%, ($n = 3$). The LODs of NSAIDs were in the range of 3.4–10 μg/L for UV mode, and 0.01–0.19 μg/L for MS mode (S/N = 3). Compared to the detection sensitivity of NSAIDs obtained with UV detection, the CEC–MS method provided about 18–429-fold reduction in the detection limit, nevertheless without loss in separation velocity ($t_R < 12$ min) and resolution ($R \sim 1$). Previous CE studies on the analyses of these NSAIDs compounds reported the best detection limits was around 3–57 μg/L in CEC system [28], and around 0.05–0.18 μg/L in CE system when an on-line concentration step coupled to an UV detector was employed [21]. Furthermore, the use of MS detector in CE or CEC for the NSAIDs identification has not been reported except for Desiderio and Fanali; in this case, however, no quantitative data (detection limits) were presented [22]. Consequently, the CEC methods from this study did provide better detection ability and faster separation velocity for the NSAIDs analysis when either UV or MS spectrometer was employed.

3.5. Real sample analyses

Finally, the proposed on-line concentration CEC methods were used to analyze water samples (river water). The results indicated that no NSAIDs residues were found in the samples. In order to examine the separation and detection ability of the proposed CEC methods, the water sample (NSAIDs-free) spiked with nine NSAIDs compounds (50 μg/L for CEC-UV, and 2 μg/L for CEC–MS) was also analyzed by the optimal CEC conditions. Their electrochromatograms are shown in Fig. 4 (chromatograms shown on the left were measured by UV detection, and on the right was obtained by MS detection). The result indicated that all trace-amount NSAIDs compounds, except for IND, in the water sample were detected by the CEC-UV method (Fig. 4(a) and (b)), while the CEC–MS method detected successfully all tested NSAIDs (Fig. 4(c)). Note that sample treatments were different for the CEC-UV and CEC–MS methods. Water samples can be analyzed directly by this on-line concentration CEC-UV method when they were dissolved in the optimal sample matrix (acidic solution (pH 3, 5 mM): methanol: ACN = 76.8%:4%:19.2%, v/v) after an oven-drying step. The recoveries of these spiked analytes were between 75.0% and 96.1% except for IND that was not detectable. This was likely because the above

Table 2
Limits of detection, repeatability of retention time, calibration curve and coefficients of determination obtained for NSAIDs standards in on-line concentration CEC method with TOF-MS detection.[a]

NSAID compounds	LOD (μg/L) (S/N = 3)	Retention time (min) (RSD%)	Calibration curves[b]	Coefficient of determination for calibration curves (r^2)
(1) Sulindac	0.01	3.76 (1.53%)	$Y = 31{,}186x + 32912$	0.9925
(2) Indoprofen	0.05	3.80 (0.00%)	$Y = 284x + 638$	0.9907
(3) Ketoprofen	0.05	4.73 (1.22%)	$Y = 1115x + 2149$	0.9980
(4) Naproxen	0.19	4.97 (1.16%)	$Y = 335x + 252$	0.9944
(5) Fenoprofen	0.08	6.63 (0.87%)	$Y = 1045x + 1994$	0.9993
(6) Flurbiprofen	0.10	7.07 (1.63%)	$Y = 639x + 532$	0.9998
(7) Ibuprofen	0.02	7.87 (1.47%)	$Y = 10{,}158x + 688$	0.9999
(8) Indomethacin	0.02	8.64 (1.77%)	$Y = 906x + 883$	0.9963
(9) Diclofenac	0.04	9.13 (1.67%)	$Y = 1789x + 1879$	0.9997

[a] Separation conditions: the volumes of acetonitrile and 5 mM ammonium formate in the mobile phase was in a ratio of 50:50. Standards were electrokinetically injected into the capillary at a voltage of 10 kV for 15 min. The data of LOD and calibration curves did not include sample pretreatment. Values were means of three intra-day replicates on the same column. The value in parenthesis indicates the RSD of retention time in percentage.
[b] The calibration curves were constructed from three replicate measurements at each concentration in the range of 0.5–50 μg/L (0.5, 2, 4, 10 and 50 μg/L).

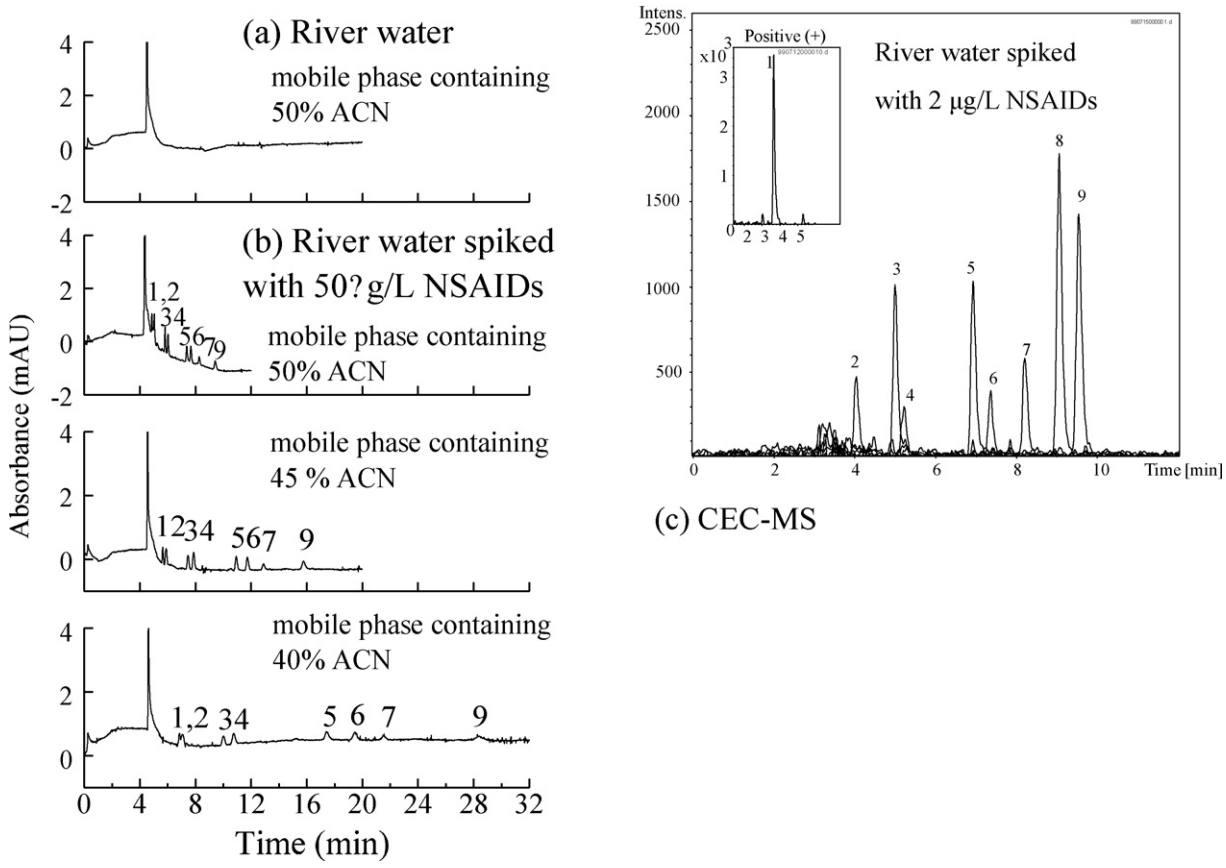


Fig. 4. Electrochromatograms of river water samples determined by on-line concentration CEC methods. NSAIDs signals were acquired by (a and b) UV (214 nm), and (c) MS detection. Sample injection at 10 kV for 15 min. Samples were prepared by the procedure described in Section 2. All other conditions for CEC-UV and CEC–MS were the same as in Figs. 1 and 3, respectively.

sample matrix had a poor dissolving ability for IND compound once it has been absorbed in the sample vial wall during drying treatment. When methanol was used to dissolve the dry residues and then it was analyzed by on-line concentration CEC-UV method, all spiked NSAIDs including IND compound can be detected, but with poor detection sensitivities. As a result, the optimal sample matrix mentioned above was still used in order to maintain good sensitivity enhancement for the analytes. Compared with UV detection, however, sample treatment was a little complicated in the MS mode (Section 2.2) because the river water sample resulted in serious matrix interferences with NSAIDs detection. A C_{18}-SPE was necessary to reduce matrix interference prior to CEC–MS, and all tested NSAIDs were clearly detected by mass spectrometer; meanwhile, this SPE method also overcomes the poor dissolving problem of IND compound that was mentioned above. The recoveries of these spiked analytes in CEC–MS method were between 69.6% and 100.4%. The above results demonstrated that the proposed on-line concentration CEC method either in the commonly used UV or the highly sensitive MS detection really possessed high potential to analyze trace NSAIDs residues in water samples after a simple sample pretreatment.

4. Conclusion

In this paper, a highly sensitive CEC method using poly(SMA–DVB) monolithic column was developed for analyzing trace non-steroidal anti-inflammatory related drugs present in water samples. Polymerization time and the SMA–DVB ratio of poly(SMA–DVB) column were found to have strong influence on the NSAIDs separation. In addition, an on-line concentration step of step-gradient elution can effectively increase NSAIDs sensitivity. The proposed CEC method was successfully coupled to TOF-MS detector. Finally, this proposed on-line concentration CEC–MS methods have combined several advantages such as high separation resolution, high detection sensitivity and an abundant structural information for real sample analyses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.11.040.

References

[1] A. Gentili, Trends Anal. Chem. 26 (2007) 595.
[2] S.D. Richardson, Anal. Chem. 80 (2008) 4373.
[3] S.D. Richardson, Anal. Chem. 81 (2009) 4645.
[4] Z. Yu, S. Peldszus, P.M. Huck, Water Res. 42 (2008) 2873.
[5] H. Söderström, R.H. Lindberg, J. Fick, J. Chromatogr. A 1216 (2009) 623.
[6] S.H. Hoke II, J.D. Pinkston, R.E. Bailey, S.L. Tanguay, T.H. Eichhold, Anal. Chem. 72 (2000) 4235.
[7] P.S. Bonato, M.P.F.M. Del Lama, R. de Carvalho, J. Chromatogr. B 796 (2003) 413.
[8] I. Ferrer, E.M. Thurman, Anal. Chem. 77 (2005) 3394.
[9] K. Suenami, L.W. Lim, T. Takeuchi, Y. Sasajima, K. Sato, Y. Takekoshi, S. Kanno, J. Chromatogr. B 846 (2007) 176.

[10] P. Gallo, S. Fabbrocino, F. Vinci, M. Fiori, V. Danese, L. Serpe, Rapid Commun. Mass Spectrom. 22 (2008) 841.
[11] M. Farré, M. Petrovic, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1203.
[12] M. Fillet, I. Bechet, V. Piette, J. Crommen, Electrophoresis 20 (1999) 1907.
[13] A. Macià, F. Borrull, C. Aguilar, M. Calill, Electrophoresis 24 (2003) 484.
[14] C. Mardones, A. Ríos, M. Valcárcel, Electrophoresis 22 (2001) 484.
[15] C. Martínez-Algaba, L. Escuder-Gilabert, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Pharm. Biomed. Anal. 36 (2004) 393.
[16] A. Macià, F. Borrull, M. Calull, C. Aguilar, J. Chromatogr. A 1117 (2006) 234.
[17] K.D. Altria, M.F. Broderick, S. Donegan, J. Power, Electrophoresis 25 (2004) 645.
[18] A. Macià, F. Borrull, C. Aguilar, M. Calull, Electrophoresis 25 (2004) 428.
[19] A. Macià, F. Borrull, M. Calull, C. Aguilar, Electrophoresis 26 (2005) 970.
[20] A. Macià, F. Borrull, M. Calull, C. Aguilar, Trends Anal. Chem. 26 (2007) 133.
[21] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, J. Chromatogr. A 1189 (2008) 278.
[22] C. Desiderio, S. Fanali, J. Chromatogr. A 895 (2000) 123.
[23] G.S. Chirica, V.T. Remcho, Anal. Chem. 72 (2000) 3605.
[24] D. Hoegger, R. Freitag, J. Chromatogr. A 914 (2001) 211.
[25] Y.F. Pai, C.Y. Liu, J. Chromatogr. A 982 (2002) 293.
[26] A. De Rossi, C. Desiderio, J. Chromatogr. A 984 (2003) 283.
[27] S. Fanali, P. Catarcini, C. Presutti, J. Chromatogr. A 994 (2003) 227.
[28] Y.F. Pai, C.C. Lin, C.Y. Liu, Electrophoresis 25 (2004) 569.
[29] D. Mangelings, I. Tanret, N. Matthijs, M. Maftouh, D.L. Massart, Y.V. Heyden, Electrophoresis 26 (2005) 818.
[30] M. Wu, R. Wu, F. Wang, L. Ren, J. Dong, Z. Liu, H. Zou, Anal. Chem. 81 (2009) 3529.
[31] S. Eeltink, F. Svec, Electrophoresis 28 (2007) 137.
[32] N.W. Smith, Z. Jiang, J. Chromatogr. A 1184 (2008) 416.
[33] D. Schaller, E.F. Hilder, P.R. Haddad, J. Sep. Sci. 29 (2006) 1705.
[34] F. Svec, E.C. Peters, D. Sykora, J.M.J. Frechet, J. Chromatogr. A 887 (2000) 3.
[35] M. Bedair, Z. El Rassi, Electrophoresis 23 (2002) 2938.
[36] W. Jin, H. Fu, X. Huang, H. Xiao, H. Zou, Electrophoresis 24 (2003) 3172.
[37] I. Gusev, X. Huang, C. Horvath, J. Chromatogr. A 855 (1999) 273.
[38] M.-M. Wang, H.-F. Wang, D.-Q. Jiang, S.-W. Wang, X.-P. Yan, Electrophoresis 31 (2010) 1666.
[39] Y. Zhang, J. Zhu, L. Zhang, W. Zhang, Anal. Chem. 72 (2000) 5744.
[40] T. Tegeler, Z.El. Rassi, Anal. Chem. 73 (2001) 3365.
[41] J.P. Quirino, M.T. Dulay, R.N. Zare, Anal. Chem. 73 (2001) 5557.
[42] D.A. Stead, R.G. Reid, R.B. Taylor, J. Chromatogr. A 798 (1998) 259.
[43] J.P. Quirino, M.T. Dulay, B.D. Bennett, R.N. Zare, Anal. Chem. 73 (2001) 5539.
[44] Z. Liu, K. Otsuka, S. Terabe, Electrophoresis 22 (2001) 3791.
[45] S. Oguri, H. Tanagaki, M. Hamaya, M. Kato, T. Toyooka, Anal. Chem. 75 (2003) 5240.
[46] G. Ping, Y. Zhang, W. Zhang, L. Zhang, L. Zhang, P. Schmitt-Kopplin, A. Kettrup, Electrophoresis 25 (2004) 421.
[47] Z. Liang, L. Zhang, J. Duan, C. Yan, W. Zhang, Y. Zhang, Electrophoresis 26 (2005) 1398.
[48] V. Augustin, G. Proczek, J. Dugay, S. Descroix, M.C. Hennion, J. Sep. Sci. 30 (2007) 2858.
[49] S. Wang, L. Jia, D. Chen, J. Sep. Sci. 32 (2009) 388.
[50] H.-Y. Huang, Y.-C. Liu, Y.-J. Cheng, J. Chromatogr. A 1190 (2008) 263.