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Strategies for the on-line preconcentration and separation of hypolipidaemic drugs using micellar electrokinetic chromatography

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ABSTRACT

Three strategies were investigated for the simultaneous separation and on-line preconcentration of charged and neutral hypolipidaemic drugs in micellar electrokinetic chromatography (MEKC). A background electrolyte (BGE) consisting of 20 mM ammonium bicarbonate buffer (pH 8.50) and 50 mM sodium dodecyl sulfate (SDS) was used for the separation and on-line preconcentration of the drugs. The efficiencies of sweeping, analyte focusing by micelle collapse (AFMC), and simultaneous field-amplified sample stacking (FASS) and sweeping, were compared for the preconcentration of eight hypolipidaemic drugs in different conductivity sample matrices. When compared with a hydrodynamic injection (5 s at 50 mbar, 0.51% of capillary volume to detection window) of drug mixture prepared in the separation BGE, improvements of detection sensitivity of 60-, 83-, and 80-fold were obtained with sweeping, AFMC and simultaneous FASS and sweeping, respectively, giving limits of detection (LODs) of 50, 36, and 38 μg/L, respectively. The studied techniques showed suitability for focusing different types of analytes having different values of retention factor (k). This is the first report for the separation of different types of hypolipidaemic drugs by capillary electrophoresis (CE). The three methods were validated then applied for the analysis of target analytes in wastewater samples from Hobart city.

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1. Introduction

Hypolipidaemic drugs are a diverse group of pharmaceutical compounds which are used to treat different forms of hyperlipidaemia. These drugs are mainly suited for reducing levels of low-density lipoprotein (LDL) in blood. Elevated levels of LDL are responsible for manifestation of coronary artery disease [1] which is one of the main causes of mortality around the world [2]. Analytical methods have been developed for the determination of some hypolipidaemic drugs in different matrices; these include high performance liquid chromatography (HPLC) [3–7], gas chromatography (GC) [8–10], and capillary electrophoresis (CE) [11–15]. Interestingly, only a few of these methods were designed for the separation of a mixture of drugs [5,7]. This could be due either to the close structural similarities between such drugs, or to the fact that hypolipidaemic drugs are not prescribed in combination with each other during normal treatment of patients [16]. However, there is an important need for analytical methods for the determination of mixtures of these drugs, especially in environmental applications involving the monitoring of these species in wastewaters.

There is a wide range of hypolipidaemic pharmaceuticals currently available around the world. Of particular importance to their separation by CE is the fact that some are neutral while others are negatively charged and if a large range of hypolipidaemics are to be separated, then micellar electrokinetic chromatography (MEKC) must be used. MEKC is a variant of CE introduced in 1984 by Terabe et al. [17] to facilitate the separation of neutral species. Analytes are separated because of their differential partitioning of between a moving micellar phase, called a pseudostationary phase, and the aqueous background electrolyte (BGE). Of importance here is the fact that charged analytes can also be separated by MEKC in which their migration is a combination of their own electrophoretic mobility and their interaction with the PSP.

Similar to other CE modes, MEKC suffers from poor detection sensitivity. This is due to the very small optical path-length for on-line spectrophotometric detection (typically 50–100 μm) and the limited amount of sample that can be introduced into the capillary (typically <1 μL). Different on-line preconcentration techniques have been developed in order to overcome this drawback [18–22]. These on-line preconcentration strategies can be classified into two main approaches. The first includes techniques that are mainly dominated by electric field strength difference between the sample and the BGE zones; the so-called stacking techniques. Being dependent on field strength differences, stacking occurs at the boundary between the high electric field sample zone and the low electric field BGE zone. Because of this difference in field

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strength, stacking results from the rapid change in electrophoretic velocity of analytes when they pass from the low conductivity sample zone to the high conductivity BGE zone. Quirino and Terabe have extensively examined factors affecting stacking modes in MEKC and have presented different stacking models including normal stacking mode (NSM) [23], reversed electrode polarity stacking mode (REPSM) [24], stacking with reverse migrating micelles (SRMM) [25], field-enhanced sample injection (FESI) [26], field-enhanced sample injection with reverse migrating micelles (FESI–RMM) [27], and stacking using reverse migrating micelles and water plug (SRW) [28]. These approaches are all very similar, involving stacking of the micelles on the sample/BGE boundary, but differ in terms of the magnitude and direction of the EOF and whether or not electrokinetic injection is used.

The second approach is dominated by the interaction of the analytes with the PSP, which is called sweeping. This is where analytes are picked up and accumulate at the front of the PSP as it moves and penetrates the sample zone upon application of the separation voltage [19]. Unlike stacking, the process of sweeping is theoretically independent of the sample and BGE zone conductivities [29], and the only requirement for sweeping to take place is the absence of PSP in the sample. As is well documented, sweeping is especially suitable for high retention factor analytes [30] and is also partly responsible for some of the concentration effects observed in a number of the MEKC stacking techniques mentioned above [31].

Recently, analyte focusing by micelle collapse (AFMC), was introduced by Quirino and Haddad [32] as an alternative approach for on-line concentration. This technique is based on the transport, release, and accumulation of analytes bound to the micelles of the PSP, that collapse when they reach the BGE zone. In contrast to sweeping, the sample solution in AFMC is prepared in a micellar sample matrix. Analytes that have a reasonable affinity for the PSP are carried through the sample upon the application of the voltage, and upon reaching the sample/BGE boundary, the micelles collapse as their concentration is adjusted according to the Kohlrausch regulating function to below the critical micelle concentration (CMC), and as a result, the analytes are released. As the analytes cannot move unless associated with the micelle, they remain on the boundary while the remaining analytes are deposited and thus their concentration is increased. In essence, AFMC is the reverse of sweeping.

In the current work, three on-line preconcentration strategies were investigated for the analysis of charged and neutral hypolipidaemic drugs by MEKC with UV detection. Sodium dodecyl sulfate (SDS) was used as a PSP, which selectively interacts with the analytes and consequently facilitates the separation and the subsequent on-line preconcentration via different mechanisms. The studied strategies, namely sweeping, AFMC, and simultaneous field-amplified sample stacking (FASS) and sweeping, were operated under low EOF conditions using linear poly-acrylamide (LPA)-coated capillaries to reduce band broadening arising from laminar flow induced from the different field strengths in the sample and BGE zones. The validated methods were applied for the determination of the target drugs in the effluent of a sewage treatment plant (STP) in Hobart city. To the best of our knowledge, these strategies show the first separation and/or on-line preconcentration of different types of hypolipidaemic drugs using CE.

2. Experimental

2.1. Standards and reagents

The compounds studied, atorvastatin, fluvastatin, gemfibrozil, lovastatin, mevastatin, pravastatin, and rosuvastatin (all >98%) were purchased from Sequoia (Oxford, UK), while simvastatin was kindly supplied by Merck & Co., Inc. (NJ, USA). SDS was from Sigma–Aldrich (St. Louis, MO, USA). Ammonium bicarbonate (98%) was from Ajax Chemicals (Sydney, Australia). Ammonium hydroxide solution (28%) was from Fluka (Buchs, Switzerland). HPLC-Grade Methanol was from Ajax Finechem (Seven Hills, Australia). Water was treated with a Millipore (North Ryde, Australia) Milli-Q water purification system. Stock standard solutions of 1 mg/mL of each drug were prepared in methanol. A mixed standard solution of the eight hypolipidaemic drugs was prepared at a concentration of 0.1 mg/mL in methanol. Subsequent standards were prepared daily by diluting the mixed standard with Milli-Q water. All solutions were stored in dark containers at 4 °C. The working BGE was 20 mM ammonium bicarbonate (pH 8.50) containing 50 mM SDS unless otherwise stated. The BGE solutions were prepared freshly daily, sonicated for 5 min and filtered through a 0.45 μm membrane filter.

2.2. Instrumentation

Electrophoretic separations were performed using an Agilent^{3D} CE (Agilent Technologies, Waldbronn, Germany) equipped with a UV diode-array detection (DAD) system operated at 214 nm with a bandwidth of 10 nm. Separations were carried out using LPA-coated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 70 cm total length (61.5 cm effective length) and 50 μm i.d. The capillary temperature was set at 25 °C. A separation voltage of −28 kV was applied through the study.

2.3. Sweeping

For sweeping, the hypolipidaemic drugs (10 and 1 μg/mL) were prepared in a solution having the same composition as the separation BGE, but containing no micelles. The conductivity of the sample solution was adjusted to be the same as that for the BGE by the addition of bicarbonate BGE. Samples were injected hydrodynamically at 50 mbar for different times ranging between 10 and 400 s.

2.4. AFMC

For AFMC, the hypolipidaemic drugs (10 and 1 μg/mL) were prepared in a solution containing 3.2 mM SDS with a conductivity 2.2 times higher as the separation BGE, which was achieved by adding more ammonium bicarbonate buffer to the sample. This was introduced into the capillary by hydrodynamic injection at 50 mbar for different times ranging between 10 and 400 s.

2.5. Simultaneous FASS-sweeping

The hypolipidaemic drugs (1 μg/mL) were prepared in Milli-Q water. Sample was introduced into the capillary by applying hydrodynamic pressure at 50 mbar for different times ranging between 10 and 120 s.

2.6. Preparation of water samples

Wastewater was collected from the effluent of Self Point STP (Hobart, Australia). Prior to analysis, the samples were filtered through a 0.45 μm nylon membrane syringe filter (Phenomenex, Australia) in order to eliminate particulate matter. The samples were stored in dark glass containers and kept in the refrigerator at −4 °C for 15 months prior to analysis.

3. Results and discussion

Previous experiments in our laboratory have demonstrated the separation of a mixture of five charged hypolipidaemics by CE–MS with electrokinetic supercharging (EKS) for on-line concentration [33]. However, it is well known that the simultaneous separation of neutral and charged analytes is not possible by conventional CE and thus it was not possible to completely separate all of the hypolipidaemics of interest using this approach. Here, we have used MEKC for the separation of charged and neutral hypolipidaemics and have studied the performance of different on-line preconcentration strategies.

3.1. Optimisation of separation selectivity

The structures and pK_a values of the eight hypolipidaemics are shown in Fig. 1, where it can be seen that these analytes fall into two distinct groups. Atorvastatin, fluvastatin, gemfibrozil, pravastatin, and rosuvastatin have a carboxylate moiety and are weak acids. Lovastatin, mevastatin, and simvastatin are neutral drugs under all practical conditions due to the presence of lactone moiety instead of a carboxylic acid. The log *P* values for the studied drugs [37,38] are shown in Fig. 1. To separate all of these species, SDS was selected as the PSP and the concentration was varied from 10 to 70 mM. Ammonium bicarbonate was selected as a separation buffer due to our previous use of this electrolyte to separate the five charged hypolipidaemic drugs [33]. The concentration used (20 mM) together with the selected separation voltage (−28 kV) provided the shortest separation time. Higher amounts of SDS were found to provide a very high and unstable separation current, increased noise and a loss in efficiency due to joule heating. With the different concentrations of SDS, and assuming that the EOF is zero in the LPA-coated capillary, the retention factor (k) for neutral analytes was calculated according to the following equation [34]

$$k = \frac{t_{mc}}{t - t_{mc}} \tag{1}$$

where t_{mc} is the migration time of the micelle forming agent SDS (using methylene blue as micellar marker), and t is the migration time of the analyte in seconds. The retention factor (k) for charged analytes was calculated according to the following equation [35].

$$k = \frac{1 - t/t_0}{t/t_{mc} - 1} \tag{2}$$

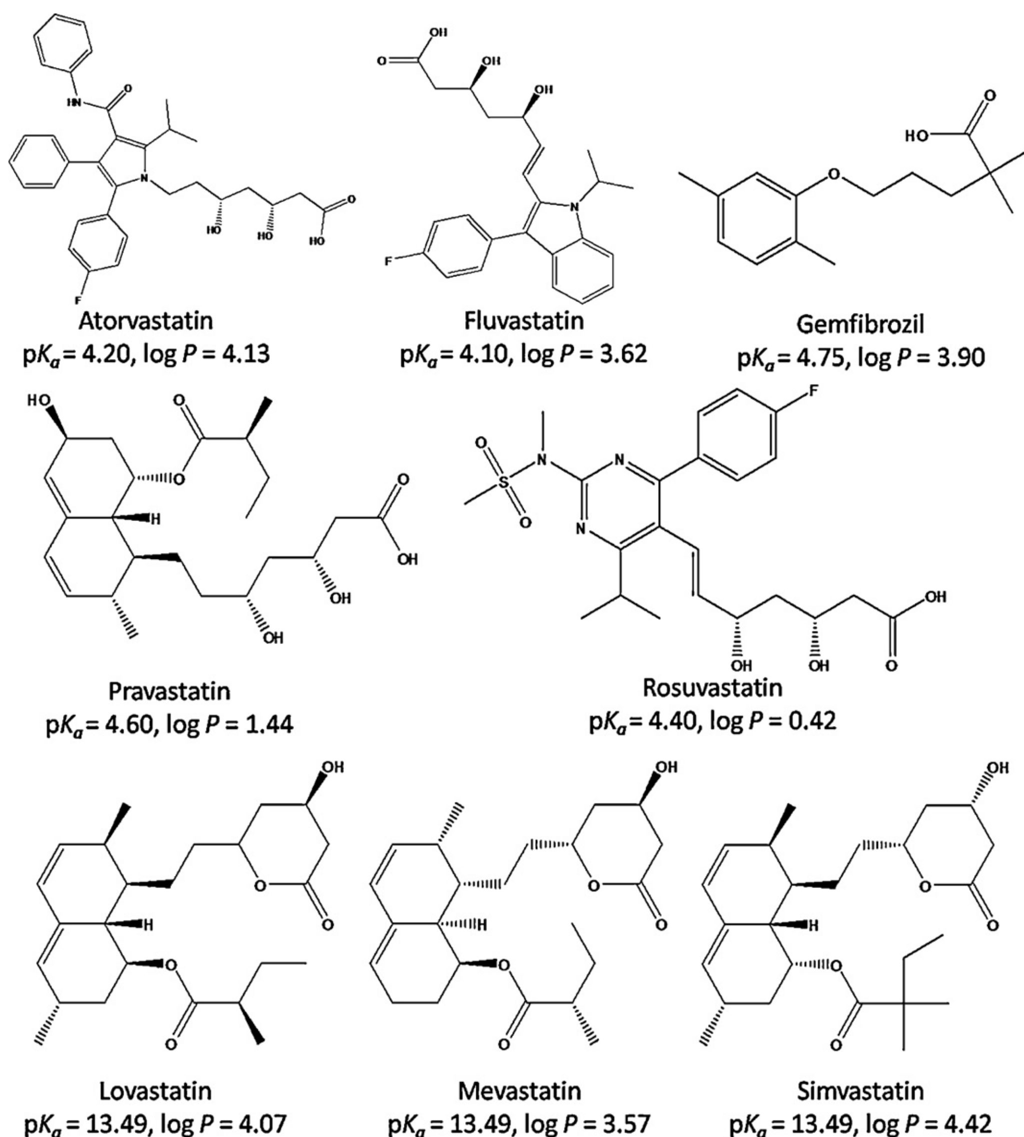

Fig. 1. Chemical structures, pK_a, and log *P* values of the studied hypolipidaemic drugs.

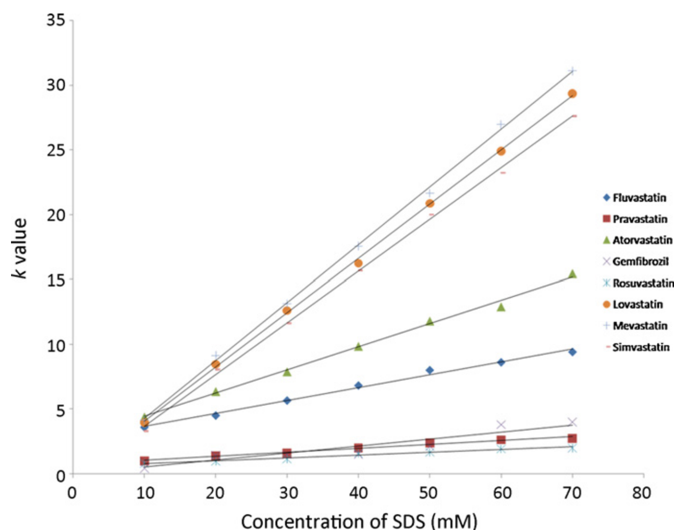

Fig. 2. Effect of the concentration of SDS on the retention factor (k) of the hypolipidaemic drugs.

where t is the migration time of the analyte in the MEKC system, and t_0 is the migration time of the analyte in the absence of the PSP (calculated in CZE). Fig. 2 shows the change in retention factor of the analytes with different concentrations of SDS with the slope of the trendline being directly proportional to the strength of interaction. The figure shows that the analytes fell into three groups according to mass distribution ratio (k_{MEKC}). High k_{MEKC} values were observed for lovastatin, mevastatin, and simvastatin, moderate k_{MEKC} values for atorvastatin, and fluvastatin, and low k_{MEKC} values for rosuvastatin, gemfibrozil, and pravastatin. Importantly, those analytes with a moderate or low k_{MEKC} are charged and this theoretically allows all eight drugs to be separated, albeit by differing mechanisms, with the neutral analytes separated mainly based on their interaction with the PSP and the negative analytes separated by a combination of electrophoretic mobility and interaction with the PSP. From Fig. 2, the optimal concentration of SDS that afforded baseline separation of the eight drugs was 30–50 mM, but the higher concentration was selected due to the potential for superior enhancements in sensitivity with the various preconcentration approaches to be coupled with the separation. Using 50 mM SDS in 20 mM ammonium bicarbonate at pH 8.50 as the BGE and performing the separation in a LPA-coated capillary at −28 kV, baseline separation of the eight drugs was obtained in less than 15 min.

3.2. Sweeping

Sweeping is the most widely used sample focusing technique in MEKC. It takes place whenever the sample is prepared in a matrix that is void of the PSP. As the PSP moves through the sample, it picks up and concentrates the analytes, or ‘sweeps’, the analytes into a very sharp zone [18]. As depicted from Fig. 2, the eight drugs show a wide range of interaction with SDS and this will have some impact upon the ability with which they can be swept. Analytes with a k_{MEKC} will be swept better than those with a low k_{MEKC}. To investigate the potential of sweeping to concentrate the hypolipidaemics, the drugs were prepared in the BGE without SDS and having the same conductivity as the separation BGE solution. This sample was injected into the capillary hydrodynamically at 50 mbar for different times ranging between 10 and 400 s. Different separation and on-line preconcentration trends were obtained with the different injection times. When short injection times (10–60 s) were used, a linear focusing effect was obtained for analytes with a large k_{MEKC} values (mevastatin, lovastatin, and simvastatin) and moderate k_{MEKC} values (atorvastatin, and fluvastatin). Analytes with a low k_{MEKC} values (pravastatin, gemfibrozil, and rosuvastatin) were not focused at all and migrated as small, broad peaks, because they do not have sufficient interaction with the micelles for proper accumulation to occur. When the injection times were increased (60–100 s) the peak areas increased linearly for analytes with large and moderate k_{MEKC} values, but the resolution between atorvastatin and fluvastatin decreased significantly, making their quantification difficult. Increasing the injection time up to 200 s resulted in complete loss of resolution for these analytes, while the three large k_{MEKC} analytes were still focused and well-resolved. Higher injection times (>200 s) resulted in lower resolution of these analytes, and while they could still be quantified, their resolution was compromised and the separation efficiency was affected. Fig. 3 shows the electropherograms for separation of the analytes using sweeping with an injection time of 40 s (10 μg/mL standard drugs solution was used), which was the maximum injection that provided acceptable resolution of five of the hypolipidaemics, and 200 s (1 μg/mL standard drugs solution was used), which was the maximum injection that provided resolution of the three analytes that interacted most strongly with the SDS. Under these conditions, peak heights were improved by 7-fold for the 40 s injection and up to 60-fold were obtained with a 200 s injection, when compared with a hydrodynamic injection occupying 0.51% of the capillary.

3.3. AFMC

AFMC is the newest approach for on-line concentration in MEKC. The basic requirements for AFMC are to have the conductivity of the micellar sample solution higher than that of the BGE, and the concentration of the surfactant just above the CMC [32,39]. When the separation voltage is applied the micelles will move from the higher conductivity sample zone towards the lower conductivity BGE zone where they enter the micellar dilution zone which is located between the sample and the BGE. Micelles then collapse as the surfactant concentration falls below the CMC and the analytes are released and accumulate on the sample/BGE boundary. In order to facilitate micelle collapse, the conductivity of the sample should be at least twice that of the BGE and the surfactant concentration in the sample should be only slightly above the CMC [39,40]. Based on the mechanism of action and on literature findings [32], AFMC should be applicable to neutral and charged analytes, based

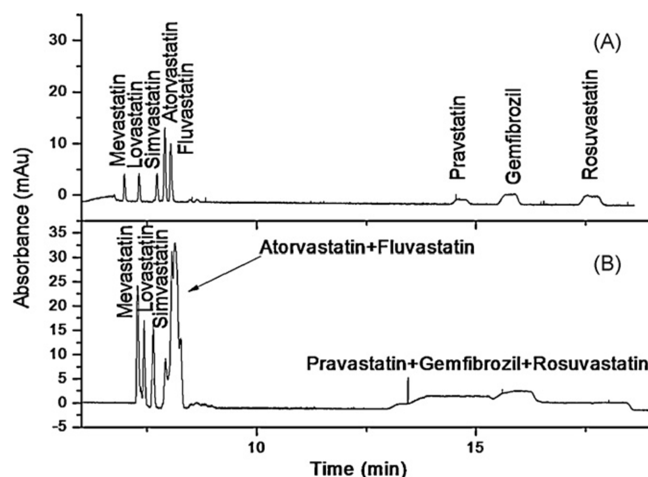

Fig. 3. Sweeping of standard mixture of hypolipidaemic drugs in ammonium bicarbonate with the same conductivity as the BGE CE conditions: LPA-coated capillary 78 cm × 50 μm i.d.; BGE 20 mM ammonium bicarbonate pH 8.50 containing 50 mM SDS. Voltage −28 kV, hydrodynamic injection of sample at 50 mbar for (A) 40 s of 10 μg/mL drugs mixture and (B) 200 s of 1 μg/mL drugs mixture; detection, UV at 214 nm.

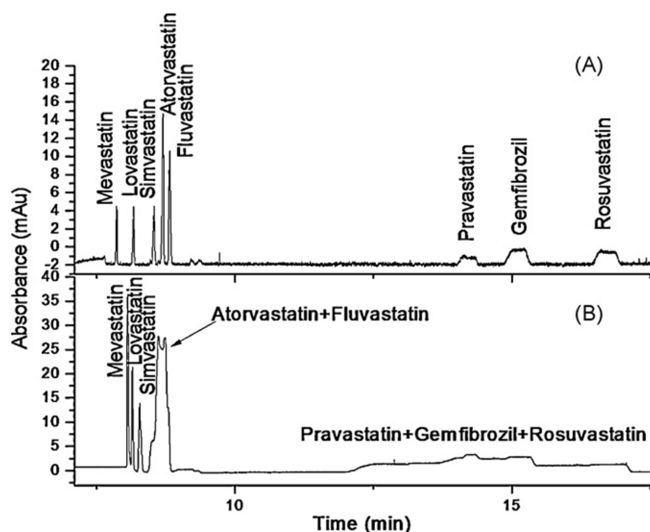

Fig. 4. AFMC of standard mixture of hypolipidaemic drugs in ammonium bicarbonate containing 3.2 mM SDS and having a conductivity of 2.2 times as the BGE. (A) Injection for 40 s of 10 μg/mL drugs mixture. (B) 220 s of 1 μg/mL drugs mixture. CE conditions as in Fig. 3.

on their degree of interaction with the micelles. Although this technique is still in its infancy, it is capable of producing improvement in detection sensitivity of more than two orders of magnitude [32] and while this is inferior to that which can be obtained with sweeping, AFMC shows more potential for the concentration of analytes with low and moderate k_{MEKC} values than sweeping.

In our system, the analytes were prepared in the ammonium bicarbonate buffer pH 8.50 containing 3.2 mM SDS and the conductivity was adjusted to be 2.2 times higher than that of the BGE by adding more bicarbonate BGE. In attempts to optimise AFMC, different concentrations of SDS ranging between 3 mM (which is the CMC of SDS in the buffer used for sample preparation for AFMC, evidenced by no analyte retention with micellar buffers containing less than 3 mM SDS in MEKC) and 4 mM were examined. As the concentration of SDS increased, the conductivity ratio of the sample and BGE zones needed to be increased in order to permit SDS micelles to collapse and to obtain the desired focusing effect. The best on-line preconcentration was obtained with a concentration of 3.2 mM SDS and a conductivity ratio of 2.2 between the sample and BGE solutions. Under these conditions, injection times of 10–400 s at 50 mbar were examined for the hydrodynamic injection of sample. Similar to sweeping, injection times ranging between 10 and 60 s gave a linear increase in peak area for the high and moderate k_{MEKC} analytes, while analytes with a low k_{MEKC} were not focused at all. Again, this could be attributed to insufficient interaction of the weakly interacting analytes with the SDS micelles. When the injection time was increased further (60–120 s) the focusing of analytes with a large k_{MEKC} was increased, but the resolution between atorvastatin and fluvastatin decreased dramatically. Longer injection times (120–220 s) caused improved focusing of the high k_{MEKC} analytes, but led to complete loss of resolution between atorvastatin and fluvastatin. Injection times higher than 220 s compromised the resolution between the analytes that interacted most strongly with the SDS. Fig. 4 shows the electropherograms for AFMC of the eight hypolipidaemic drugs with injection times of 40 s (10 μg/mL standard drugs solution was used) which was the maximum injection time that gave acceptable resolution of the five analytes with large and moderate k_{MEKC} values and 220 s (1 μg/mL standard drugs solution was used) which was the maximum injection time which provided baseline resolution of the three large k_{MEKC} analytes. Under these conditions the detection sensitivity was improved by 8-fold for the 40 s injection, and by up to 83-fold for the 220 s injection.

3.4. Simultaneous FASS-sweeping

Sweeping and AFMC showed improvements in sensitivity for analytes having a strong interaction with the SDS micelles, but both were unsuitable for the charged analytes. In order to preconcentrate all of the hypolipidaemic drugs, it is therefore necessary to use a combination of preconcentration mechanisms. Since the analytes that interact only weakly with the SDS are charged, it should be possible to concentrate them by stacking under conditions in which sweeping will also occur to concentrate the neutral drugs. For FASS, the sample must have a conductivity at least ten times lower than that of the BGE, and the best results are typically obtained using water as the sample solvent. Sweeping has been observed to occur with samples prepared in water although performance is usually better when the conductivity of the sample is matched to the BGE. Nevertheless, it is possible that simultaneous FASS and sweeping will allow the preconcentration of all of the analytes, which was not possible by sweeping or AFMC used separately.

A sample of the eight hypolipidaemics in water was injected hydrodynamically at 50 mbar using injection times between 10 and 120 s to determine whether sensitivity could be enhanced. Maximum peak height while maintaining the separation efficiency was obtained with injection for 80 s. Injection times from 80 to 100 s resulted in serious peak broadening and loss of resolution, especially for the charged drugs. This could be due to overloading of the stacking boundary with the charged analytes. Injection times higher than 100 s led to serious instabilities of the electric current due to the introduction of large volumes of the low conductivity sample matrix. Fig. 5 shows the electropherogram for the separation of the eight hypolipidaemic drugs using simultaneous FASS-sweeping with an injection time of 80 s. Under these conditions enhancement in detection sensitivity of up to 80-fold was obtained. Although the volume of the injected sample solution in the FASS-sweeping experiments was smaller than that used in studies on sweeping or AFMC, the enhancement in sensitivity was still high. This can be attributed to the difference in focusing mechanism among the three approaches. While the degree of interaction

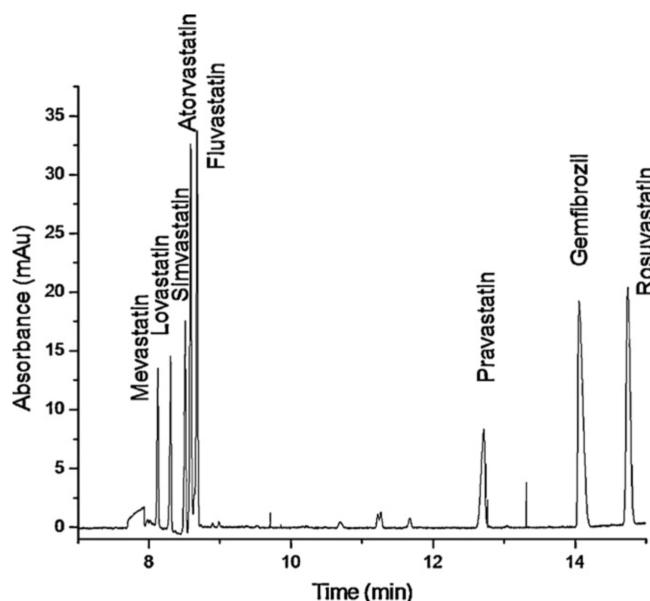

Fig. 5. FASS-sweeping of standard mixture of hypolipidaemic drugs. Injection of 1 μg/mL drug mixture in Milli-Q water at 50 mbar for 80 s. CE conditions as in Fig. 3.

Table 1
Within-day and between-day repeatabilities (RSD%) of migration times, peak areas, and peak heights for the three on-line preconcentration methods.

Method	Compound	Within-day RSD% ($n = 5$)			Between-day RSD% ($n = 5$)		
		Migration time	Peak area	Peak height	Migration time	Peak area	Peak height
Sweeping	Lovastatin	0.28	0.98	0.96	0.89	1.44	2.02
	Mevastatin	0.24	0.88	0.92	0.88	1.67	1.88
	Simvastatin	0.32	0.55	0.62	1.21	1.33	2.12
AFMC	Lovastatin	0.22	0.78	1.12	0.69	2.12	2.08
	Mevastatin	0.28	1.00	1.02	0.99	1.22	2.00
	Simvastatin	0.89	1.23	1.87	1.99	2.22	2.72
FASS-sweeping	Atorvastatin	2.40	2.31	2.89	3.45	4.40	4.83
	Fluvastatin	1.99	2.12	2.19	3.33	4.45	4.93
	Gemfibrozil	2.88	3.44	3.59	4.89	4.11	4.99
	Pravastatin	3.40	4.00	4.42	5.12	4.59	6.46
	Rosuvastatin	4.12	4.01	4.46	5.12	5.33	6.00
	Lovastatin	2.22	2.56	2.99	2.98	3.23	3.80
	Mevastatin	2.02	2.24	2.88	3.00	3.08	3.68
	Simvastatin	2.28	3.46	3.98	4.02	4.22	4.66

of the analytes with the micelles is solely responsible for focusing in sweeping and AFMC, the addition of a FASS component enables preconcentration of the charged analytes. The effectiveness of this latter mechanism is dependent not only on the volume of sample solution, but also on the differences in field strength between the sample and the BGE zones.

3.5. Analytical performance characteristics of preconcentration methods

The repeatability of the three on-line preconcentration techniques was investigated by determining the within-day and between-day precision values. Within-day precision was evaluated by performing five replicate separations of the hypolipidaemic drugs at a concentration of 1 μg/mL. Between-day precision was evaluated by performing the same separations for five different days. Precision (expressed as percentage relative standard deviation (RSD%)) was calculated for migration times, peak areas, and peak heights employing the optimal conditions capable of producing the best focusing effect for each one of the three methods. RSD% values for within-day and between-day precision for sweeping, AFMC, and simultaneous FASS-sweeping are presented in Table 1. RSD% values for sweeping and AFMC were calculated for the neutral drugs only because these were the only analytes that were sufficiently well-resolved for reliable quantification. Sweeping showed the lowest RSD% values, and while FASS-sweeping showed applicability to the higher number of analytes, its repeatability was the lowest. The relatively high RSD% values for FASS-sweeping is understandable because this technique depended strongly on the field strength difference between the sample and BGE zones, and this difference changes with the length of the sample zone. This zone length can be expected to show some variations associated with change in viscosity of different solutions due to temperature fluctuations in an unregulated laboratory.

The signal enhancement factors for each analyte obtained by each preconcentration technique are listed in Table 2. The average enhancement factors were found to be 26.8, 29.8, and 46.3 for sweeping, AFMC, and simultaneous FASS-sweeping, respectively. These average enhancement factors were calculated by dividing the sum of enhancement factors for each technique by the number of the drugs. It is noteworthy that these signal enhancement factors were calculated for different injection times, with the injection time being determined by analyte resolution (as discussed in Sections 3.2 and 3.3). The high average signal enhancement factor obtained with simultaneous FASS-sweeping is also influenced by the fact that all the analytes could be resolved at the optimum injection time for preconcentration, which was not the case for sweeping and AFMC.

The limits of detection (LODs) achieved by each preconcentration technique are also listed in Table 2. These values were calculated at signal/noise ratio of 3. As discussed above for calculation of sensitivity enhancement factors, the LOD for each drug was calculated at the injection time which gave highest signal while maintaining the base-line resolution of that drug. High LODs obtained with sweeping and AFMC for the analytes that interacted only weakly with the SDS are because of inability of these approaches to preconcentrate any of these analytes.

The linearity range for the three preconcentration techniques was determined over the whole range where the detector response was linear for peak heights of the eight analytes. It was observed that the best linearity range (where linear response was obtained over the widest range) was obtained with sweeping followed by AFMC. As performed for calculation of LODs, the linearity range was calculated at the injection time which gave the highest response while maintaining the base-line separation of the drugs. It is noteworthy that higher linearity range was obtained for peak areas, but

Table 2
LODs (μg/L) and sensitivity enhancement factors (SEFs) achieved by the three on-line preconcentration methods.

Compound	Sweeping (μg/L) (SEF)	AFMC (μg/L) (SEF)	FASS-sweeping (μg/L) (SEF)
Atorvastatin	116 (36)*	104 (40)*	64 (65)
Fluvastatin	208 (20)*	189 (22)*	38 (80)
Gemfibrozil	2592 (1)*	2592 (1)*	48 (54)
Pravastatin	2964 (1)*	2964 (1)*	57 (52)
Rosuvastatin	2958 (1)*	2958 (1)*	51 (58)
Mevastatin	50 (60)	36 (83)	46 (35)
Lovastatin	70 (42.5)	54 (55)	78 (38)
Simvastatin	57 (52.5)	85 (35)	65 (46)

* Values obtained under different injection times as explained in the text.

Table 3
Linearity range (mg/L) for peak heights achieved by the three on-line preconcentration methods.

Compound	Sweeping	AFMC	FASS-sweeping
Atorvastatin	0.34–2.11	0.32–2.09	0.20–1.31
Fluvastatin	0.62–4.67	0.60–4.10	0.13–0.71
Gemfibrozil	0.79–6.41	0.76–6.01	0.15–0.81
Pravastatin	0.91–8.76	0.90–7.61	0.19–0.91
Rosuvastatin	0.90–8.61	0.92–7.92	0.16–0.90
Mevastatin	0.16–0.99	0.13–0.80	0.14–0.81
Lovastatin	0.23–1.18	0.18–0.81	0.24–1.02
Simvastatin	0.18–1.01	0.31–2.41	0.18–0.92

as peak height was used for calculation of the LODs, it was also used for the assessment of linearity range as listed in Table 3.

3.6. Application to water samples

To demonstrate the potential of the developed on-line preconcentration methods for the analysis of hypolipidaemic drugs in water samples, the proposed methods were applied for the analysis of the target analytes in wastewater collected from the effluent of Self Point STP (Hobart, Australia). Wastewater sample spiked with 1 μg/mL of the hypolipidaemic drugs mixture was analysed using the three on-line preconcentration techniques.

When sweeping was performed, the conductivity of the sample was adjusted to that of the BGE by the addition of ammonium bicarbonate buffer pH 8.50. This required 1.4-fold dilution of the sample. Upon injection of the wastewater sample for 200 s at 50 mbar, the same enhancement in detection sensitivity was obtained for mevastatin, lovastatin, and simvastatin as was observed for standard solutions, which suggests the suitability of sweeping process for the analysis of target drugs in wastewater samples. For AFMC, 3.2 mM SDS was added to the sample and the conductivity was adjusted to 2.2 times that of the BGE by the addition of ammonium bicarbonate buffer pH 8.50. This required 1.6-fold dilution. When the optimal conditions for AFMC were applied, the separation of the neutral analytes was lost and the three neutral drugs were detected as one peak. It has been reported that there is evidence of a decrease of the CMC of surface active agents with high salt content [41], which would explain the observed results. However, we believe that the high salt content in the water samples (mainly sodium chloride) produces an ITP boundary, which could account for the stacking of micelles at the back of the boundary, such that the analytes might not be separated before detection. For FASS-sweeping, the water sample was injected directly. A significant reduction in signal enhancement (2 times) was obtained for the charged analytes in comparison to the standard solutions tested earlier. This reduction can be attributed to the increased conductivity of the sample, which affects the FASS process. Surprisingly, the resolution of the neutral analytes was decreased markedly, and the three neutral drugs were detected as one sharp peak. We believe that this may also be due to the salt content of the sample producing an ITP boundary at which the micelles will concentrate and then not be separated before reaching the detector. Fig. 6 shows the electropherogram of wastewater samples spiked with hypolipidaemic drugs (1 μg/mL) and analysed by the three approaches.

3.7. Comparison of the performance of the preconcentration techniques

Although sweeping is the most widely used preconcentration technique in MEKC, it showed considerable limitations for analysis of most of the studied drugs. Analytes with a weak interaction with SDS were not concentrated under any of the experimental conditions used for sweeping. Analytes that interacted moderately were only preconcentrated to a significant extent when a small sample plug (<6% of capillary volume to detection window) was used. The presented results show that sweeping was suitable only for the preconcentration of analytes with a large k_{MEKC}. Adequate sweeping of these analytes could be obtained with sample plugs up to 20% of the capillary volume to detection window, with larger sample plugs leading to loss of resolution. Sweeping exhibited the lowest RSD% values of the three techniques.

Although AFMC resembles sweeping in that it relies on the interaction of analytes with the micelles, slight improvement in detection sensitivity was obtained compared to sweeping. This can be attributed to its ability to tolerate slightly larger sample plugs (22% of capillary volume) than sweeping. However, AFMC was only suitable for preconcentration of analytes that interacted strongly with the SDS. Moreover, the operating conditions required for AFMC bring limitations when dealing with real samples. As discussed above, the two key factors for successful AFMC are the concentration of the micelle forming agent and the conductivity ratio between the sample and the BGE. A change in one of these factors will require optimisation of the other parameter [39], making the operating conditions of AFMC more difficult to optimise. Since the CMC of SDS is dependent on the salt content in the sample

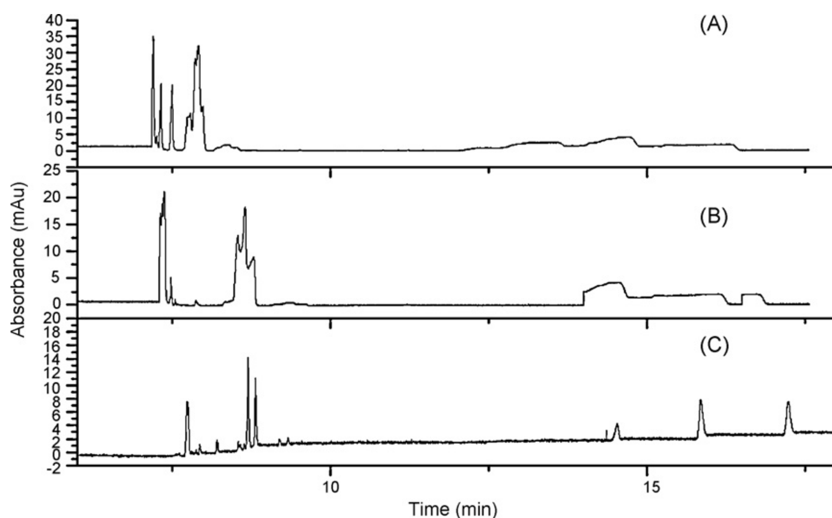

Fig. 6. Electropherograms of wastewater samples spiked with hypolipidaemic drugs (1 μg/mL) and analysed by (A) sweeping, (B) AFMC, and (C) FASS-sweeping. CE conditions as in Figs. 3B, 4B, and 5B, respectively.

[41], considerable obstacles arise in the application of the method to water samples because of the need to optimise the conditions for different samples.

Simultaneous FASS-sweeping is suitable for preconcentration of a wide range of analytes, but showed limited applicability for analysis of target analytes in wastewater samples. Moreover, the repeatability of the method was poorer than the other enrichment techniques. On the other hand, selection of the operating conditions for simultaneous FASS-sweeping was most straightforward because there was no requirement to adjust the conductivity ratios between the sample and the BGE.

4. Conclusions

This study has investigated three on-line preconcentration techniques for the analysis of eight hypolipidaemic drugs in water samples. All of the methods involved interaction of the analytes with SDS micelles as the PSP. Sweeping proved to be well suited for the preconcentration of analytes that interact strongly with SDS in both standard solutions and wastewater samples with up to 60-fold improvement in detection sensitivity and low RSD% values. However, this method could be used for only three of the drugs examined. AFMC showed a high potential for the preconcentration of large k_{MEKC} analytes and gave a similar enhancement in sensitivity to sweeping, but was again applicable to only three of the drugs. AFMC did not operate successfully for wastewater samples, presumably due to the additional salt present in the sample. Simultaneous FASS-sweeping was suitable for the on-line concentration of the widest range of analytes when prepared in a low conductivity sample matrix. FASS enriched the charged analytes, with sweeping enriching the neutral analytes. Improvements in detection sensitivity of up to 80-fold were obtained with FASS-sweeping, with baseline separation for all eight of the drugs. However, the application of the method to wastewater samples showed decreased sensitivity due to the high salt content of this sample. Off-line extraction methods, such as solid-phase extraction or liquid-liquid extraction could be used with all of the above approaches to make the method more applicable to wastewater samples.

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