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High-performance liquid chromatography of seized drugs at elevated pressure with 1.7 μm hybrid C18 stationary phase columns

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Abstract

High-performance liquid chromatography (HPLC) separation of drugs at elevated pressure with 1.7 μm hybrid C18 stationary phase columns was investigated. This technique, which uses instrumentation engineered to handle the narrow peaks and high back pressures generated by 1.7 μm particle columns, provided significantly better resolution and/or faster analysis than conventional HPLC and capillary electrophoresis (CE). The use of 2 mm internal diameter (i.d.) columns of 3–10 cm length has been evaluated for the separation of basic and neutral drugs, drug profiling, and general screening (including acidic drugs). For these applications, compared to conventional HPLC and CE, it provided up to 12× and 3× faster analyses, respectively. Precision was excellent for both isocratic and gradient analyses. For retention time and peak area, RSDs of ≤0.1% were obtainable. Fifteen anabolic steroids and esters were well separated in a 2.5 min gradient. For drug profiling, compared to HPLC and CE, approximately twice as many peaks were resolved. HPLC at elevated pressure is also well suited as a general screening technique. Twenty-four solutes of varying drug classes including narcotic analgesics, stimulants, depressants, hallucinogens, and anabolic steroids were fully separated in a 13.5 min gradient.

Keywords: Elevated pressure; High-performance liquid chromatography; Seized drugs

1. Introduction

The analysis of seized drugs is important for legal and intelligence purposes. To this end, various high performance separation techniques are used including capillary gas chromatography (GC) [1], high-performance liquid chromatography (HPLC) [2] and capillary electrophoresis (CE) [3]. Although capillary GC offers the greatest peak capacity, it can be problematic for solutes that are thermally degradable, highly polar or nonvolatile. A significant number of seized drugs and related compounds of varying drug classes fall under these categories. Liquid phase separation techniques, such as HPLC and CE, which have a lower peak capacity than GC, are amenable to the analysis of seized drugs including solutes that are problematic by GC. Although HPLC has a smaller peak capacity than CE, it offers lower limits of UV detection than CE due to the higher detection path lengths used.

HPLC, using instrumentation engineered to handle the narrow peaks and high back pressure generated by 1.7 μm particle columns, appears well suited for the analysis of drugs. Compared to conventional HPLC, which typically uses columns packed with ≥3 μm particles on pumps operating at pressures of ≤6000 psi, 1.7 μm particle columns operating at pressures ≤15,000 offer significantly improved speed and peak capacity [4]. This improved performance arises due to the reduced plate height of the smaller particle size columns, the inverse proportionality between optimum velocity and particle size, and the ability to use longer 1.7 μm columns [4]. In addition, the smaller particles exhibit a flatter van Deemter curve at linear velocities higher than the optimum [4]. The commercially available HPLC systems at elevated pressure are based on the work of Jorgenson and co-workers [5,6]. These authors first used 30 μm fused-silica capillaries containing 1.5 μm C18-modified silica particles with lengths up to 66 cm on instrumentation capable of performing isocratic separation at operating pressures up to 60,000 psi [5]. Subsequently, 33 μm fused silica capillaries containing 1.0 μm C18-modified non-porous silica particles were used for isocratic and gradient elution on instrumentation capa-

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ble of operating at pressures up to 130,000 psi [6]. A system with similar capabilities was described by Lee and co-workers [7]. An injection system for practical use was introduced by Lee and co-workers [8] that is capable of operating at pressures up to 18,000 psi. Mellors and Jorgenson [9] found that 1.5 μm C18-modified ethyl-bridged hybrid porous particles can withstand pressures up to 65,000 psi.

In this study, the utility of HPLC at elevated pressures with photodiode array (PDA) UV detection for the analysis of drugs is investigated. The separation of basic drugs at low and high pH using a 1.7 μm hybrid C18 column is discussed. Next, the analysis of neutral drugs is described on this same stationary phase. In addition, the use of a small particle hybrid C18 column for drug profiling is investigated. Finally, the use of HPLC at elevated pressure with the above stationary phase for drug screening is described. The utility of the above technology for drug classes including narcotic analgesics, stimulants, depressants, hallucinogens and anabolic steroids is shown. For the above applications, a comparison between HPLC at elevated pressure and conventional HPLC and CE is described.

2. Experimental

2.1. Chemicals and reagents

All drug standards were obtained from the reference collection of the Drug Enforcement Administration Special Testing and Research Laboratory (Dulles, VA, USA). Phosphoric acid and trifluoroacidic acid (TFA) were obtained from Aldrich (Milwaukee, WI, USA). Sodium hydroxide and 3-(cyclohexylamino)-1-propane-sulfonic acid (CAPS) were obtained from Sigma (St. Louis, MO, USA). High-purity, deionized water was obtained from a Millipore Milli-Q-Gradient A10 water system (Bedford, MA, USA). HPLC-grade methanol and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI, USA). Sodium phosphate buffers were prepared by diluting with water a stock solution containing 189 mM sodium phosphate, pH 1.7. This buffer was prepared by adding 870 mL of water, 10 mL of phosphoric acid and 30 mL of 1 M sodium hydroxide.

2.2. Instrumentation

The elevated pressure HPLC instrumentation consisted of a Waters Acquity Ultra Performance LC system equipped with a diode array detector (Milford, MA, USA). All separations were carried out at 30 °C using Acquity UPLC BEH C18 1.7 μm particle columns (30–100 mm × 2.1 mm).

The conventional HPLC instrumentation consisted of an Agilent Model 1100 HPLC system equipped with a quaternary pump and a 1100 series diode array detector (Waldbronn, Germany). A Whatman 5 ODS-3 column (125 mm × 3.2 mm) (Clifton, NJ, USA) was used at ambient temperature.

2.3. Procedures

For the HPLC experiments for basic drugs and drug profiling, the solutes were dissolved in the mobile phase buffer component of the mobile phase, unless specified otherwise. For the elevated pressure HPLC experiments for anabolic steroids and drug screening, the solutes were dissolved in methanol.

Chromatographic parameters including retention factor k'; selectivity factor α; USP resolution R; USP tailing factor T and USP plate number N were determined as described below using Waters Empower system suitability software (Milford, MA, USA). The time of the void volume was calculated from the first disturbance of the baseline after injection.

$$\alpha = \frac{t_2 - t_0}{t_1 - t_0}$$

where t_1 is the retention time of the first peak and t_2, the retention time of the second peak

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where w_1 is peak width of the first peak and w_2, the peak width of the second peak. Peak widths at baseline between tangent lines drawn at 50% peak height.

$$T = \frac{w_{0.05}}{2f}$$

where $w_{0.05}$ is the peak width at 5% of peak height and f, the time from width start point at 5% of peak height to retention time of peak

$$N = 16\left(\frac{t}{w}\right)^2.$$

3. Results and discussion

3.1. Separation of basic drugs

The operating pH range of the 1.7 μm Acquity UPLC BEH C18 stationary phase used in these studies is 1–12 [10]. It has been reported that the presence of bridged ethylsiloxane/silica hybrid (BEH) particles in combination with a tri-functional C18 bonding chemistry allows for an extended pH range of 1–12 and improved peak shapes for basic solutes [10,11]. An earlier generation hybrid stationary phase, containing a methylsiloxane/silica hybrid, has been shown to have an extremely low content of residual silanol groups up to pH 10 [12]. Therefore, it was of interest whether highly basic drugs, such as phenethylamines (pK_a approximately 10) could be analyzed in the cationic form at low pH without silanol masking (e.g., amines) or lipophilic ion-pair (e.g., alkylsulfonates) reagents, or better analyzed at high pH in their free base form. For this study, the solutes included a mixture of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA).

A test mixture of phenethylamines was analyzed at low pH with phosphate buffer. The effect of phosphate concentration at a solute concentration of 0.01 mg/mL is shown in Table 1 for the under 3 min separations. As shown in Table 1 k' and α does not significantly change with buffer concentration. Increasing the

Table 1
Effect of phosphate buffer concentration on chromatographic parameters for phenethylamines (0.01 mg/mL)

Solute	Buffer	k'	α	USP resolution	USP tailing	USP N ($\times 10^3$)
Amphetamine		3.1			1.7	5.5
MDA		3.7	1.2	2.2	1.6	5.7
Methamphetamine	10 mM phosphate (pH 2.2)	4.1	1.1	1.6	1.7	5.3
MDMA		4.6	1.1	1.7	1.7	5.0
MDEA		6.9	1.5	5.9	1.6	5.6
Amphetamine		3.0			1.4	7.1
MDA		3.6	1.2	2.6	1.3	7.1
Methamphetamine	50 mM phosphate (pH 2.0)	4.0	1.1	1.7	1.4	6.5
MDMA		4.5	1.1	2.0	1.3	6.8
MDEA		6.7	1.5	6.7	1.3	7.3
Amphetamine		3.1			1.3	8.6
MDA		3.7	1.2	2.9	1.2	8.5
Methamphetamine	100 mM phosphate (pH 1.8)	4.1	1.1	1.9	1.2	8.2
MDMA		4.6	1.1	2.3	1.2	8.1
MDEA		6.8	1.5	7.3	1.2	8.2
MDA		2.8			1.2	8.8
Amphetamine		3.2	1.2	2.8	1.4	8.0
MDMA	10 mM CAPS (pH 11.5)	4.6	1.4	6.6	1.4	9.3
Methamphetamine		5.6	1.2	3.1	1.2	8.4
MDEA		8.2	1.5	8.0	1.3	15.0

Conditions: injection size, 5 μl (partial fill mode); column, 5.0 cm × 2.1 mm 1.7 μm Acquity UPLC BEH C18; 10% acetonitrile, 90% phosphate buffer; flow rate 0.375 mL/min; temperature 30 °C; UV detection 205 nm. Conditions high pH buffer, same as low pH buffers except for 30% acetonitrile.

phosphate concentration improved peak shapes, peak efficiencies and resolution (see Table 1). Higher solute concentrations (0.10 mg/ml) led to poorer peak shapes with lower peak efficiencies.

The above results could be explained by a two site adsorption model recently described by Gritti and Guiochon using a XTerra MS C18 column [13]. As described by these authors, the density of the less abundant high energy sites (first populated at low cation solute concentrations) is increased with increase in salt concentration. This would limit the population of both sites leading to decreased tailing and increased peak efficiencies. At the higher solute concentration, the contribution of lower energy sites may increase. Free silanol groups are not believed to be involved in the formation of the higher energy site, while the lower energy site corresponds to simple interactions with the bonded alkyl group [13].

An overlay of an isocratic separation of seven injections of our test mixture plus a structurally related internal standard *N*-butylamphetamine (NBA) which is used for conventional HPLC and CE analysis is shown in Fig. 1A. All solutes are well resolved in under 6 min. For this separation, a 20 μL injection in the overfill mode was employed to optimize peak area precision. In addition, a flow rate of 0.750 mL/min. was used with a resultant operating pressure of 9900 psi to speed up analysis. As expected, compared to the earlier conditions, some loss in chromatographic performance was observed for the phenethylamines. For this separation, the use of a relatively short 50 mm 1.7 μm particle column coupled with the higher flow rate allows for the isocratic separation of these solutes including NBA with a k' of 26 in under 6 min. Due to the high k' value of *N*-butylamphetamine, a gradient was required for a previous HPLC separation of this mixture [14]. As shown in Fig. 1A, for an overlay of seven injections, excellent precision is obtained for retention time (RSD ≤ 0.12%), relative retention time (RSD ≤ 0.10%), peak area (RSD ≤ 0.12%), and relative peak area (RSD ≤ 0.18%). An acetonitrile, phosphate buffer gradient separation of this same mixture resulted in a similar separation as above for the first five solutes (see Fig. 1B). In addition, a faster overall analysis time of 3 min (including 1.0 min gradient re-equilibration) was obtained, with improved peak height for NBA (see Fig. 1A and B). For this separation a 20 μL injection in the overfill mode was again employed with a flow rate of 0.750 mL/min, which resulted in an operating pressure of 9900–10,150 psi. As shown in Fig. 1B, for an overlay of seven injections, similar to isocratic analysis, excellent precision is obtained for retention time (RSD ≤ 0.10%), relative retention time (RSD ≤ 0.14%), peak area (%RSD ≤ 0.14), and relative peak area (RSD ≤ 0.20%).

It was of interest to investigate the separation of the above solutes at high pH. For this purpose, a CAPS buffer at the highest recommended concentration by the column manufacturer was used i.e., 10 mM (pH 11.5). The high pH buffer contained 30% acetonitrile due to the presence of the more lipophilic free bases. As shown in Table 1, all peaks were well resolved using the additional buffer. It is interesting that the solute pairs amphetamine and MDA, and methamphetamine and MDMA switched retention order using the high pH buffer. Again, a two site mechanism could explain this result, with the free base interacting primarily with the bonded alkyl group. Compared to 100 mM phosphate buffer (pH 1.8), higher overall peak efficiencies and resolutions, and comparable peak shapes, were obtained with 10 mM CAPS (pH 11.5) (see Table 1).

An overlay of seven injections of an isocratic separation of the test mixture of phenethylamines plus NBA at high pH is shown in Fig. 2A. All solutes are fully resolved in less

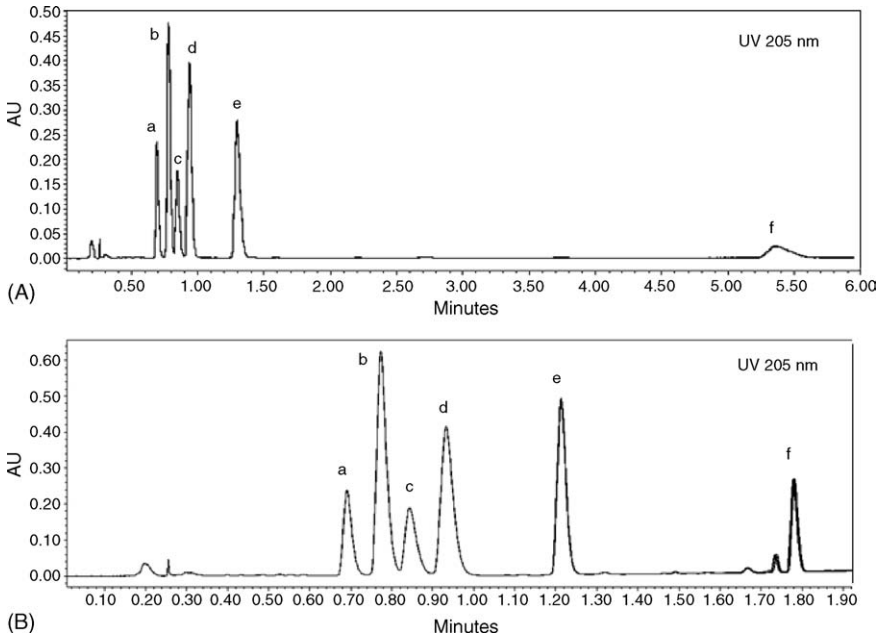

Fig. 1. (A) Overlay of seven injections of an elevated pressure isocratic HPLC separation of phenethylamines at solute concentrations of 0.010 mg/mL. Conditions: injection size, 20 μL (overfill mode); column, 5.0 cm × 2.1 mm 1.7 μm Acquity UPLC BEH C18; 10% acetonitrile, 90% 100 mM phosphate buffer (pH 1.8); flow rate 0.750 mL/min; temperature 30 °C. Peaks: (a) amphetamine, (b) MDA, (c) methamphetamine, (d) MDMA, (e) MDEA, and (f) *N*-butylamphetamine. (B) Overlay of seven injections of an elevated pressure gradient HPLC separation of phenethylamines at solute concentrations of 0.010 mg/mL. Conditions and peaks same as (A) except for mobile phase. Initial conditions: 10% acetonitrile, 90% phosphate buffer (pH 1.8). Intermediate conditions: 15% acetonitrile, 85% phosphate buffer (pH 1.8). Final conditions: 30% acetonitrile, 85% phosphate buffer (pH 1.8). Hold initial conditions for 0.75 min, 0.25 min linear gradient to intermediate conditions, 0.5 min linear gradient to final conditions, hold for 0.24 min at final conditions, 1 min gradient re-equilibration.

than 7.5 min. As shown in Figs. 1A and 2A, operation at the higher pH allows for significantly higher solute concentrations (0.1 mg/mL versus 0.01 mg/mL) with slightly improved overall resolution. The higher solute concentrations are offset by the lower UV response at 254 nm necessitated by the presence of CAPS.

It is of interest to compare the above separations for the phenethylamine test mixture plus *N*-butylamphetamine with those obtained using conventional HPLC and CE. For conventional HPLC of the same solute mixture using a low pH phosphate buffer with an amine modifier, all solutes were fully resolved using a 12.5 cm long 5 μm Luna C18 column with

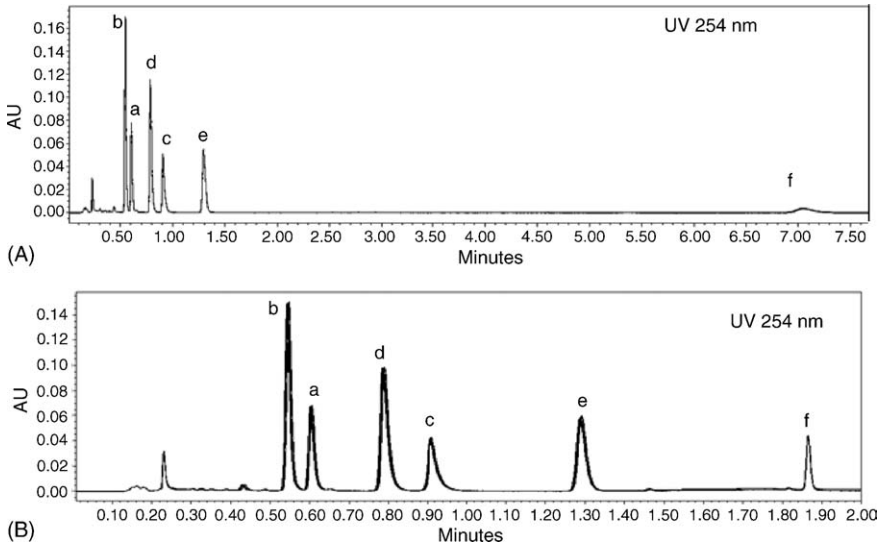

Fig. 2. (A) Overlay of seven injections of an elevated pressure isocratic HPLC separation of phenethylamines at solute concentration of 0.10 mg/mL. Conditions and peaks same as Fig. 1A except for mobile phase. 30% acetonitrile, 70% 10 mM CAPS buffer (pH 11.5). (B) Overlay of seven injections of an elevated pressure gradient HPLC separation of phenethylamines at solute concentrations of 0.10 mg/mL. Conditions and peaks same as Fig. 1B except for mobile phase. Initial conditions: 30% acetonitrile, 70% CAPS buffer (pH 11.5). Final conditions: 60% acetonitrile, 40% CAPS buffer (pH 11.5). Hold initial conditions for 1.0 min, 0.50 min linear gradient to final conditions, hold for 0.49 min at final conditions, 1.0 min gradient re-equilibration.

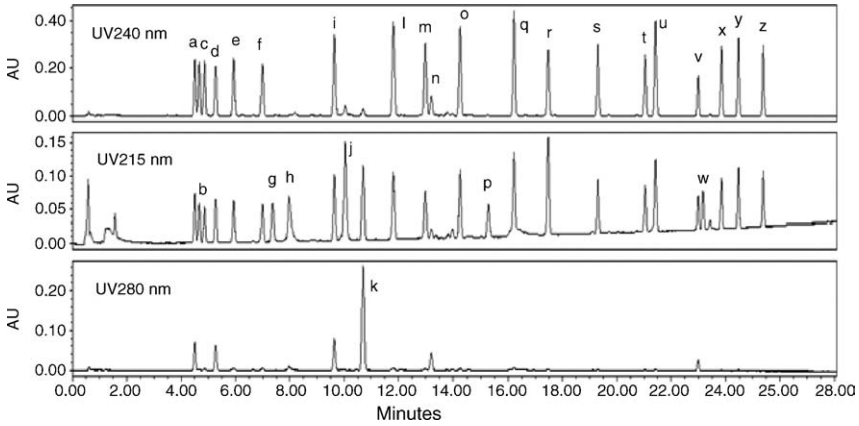

Fig. 3. An elevated pressure gradient separation of anabolic steroids. Conditions: injection size, 10 μL (partial fill mode); column, 10.0 cm × 2.1 mm 1.7 μm Acquity UPLC BEH C18. Initial conditions: 50% methanol, 50% water. Final conditions: 100% methanol, 28 min linear gradient, 1.0 min gradient re-equilibration; flow rate 0.375 mL/min; temperature 30 °C. Peaks: (a) boldenone (0.025 mg/mL), (b) fluoxymesterone (0.025 mg/mL), (c) nandrolone (0.025 mg/mL), (d) methandrostenolone (0.025 mg/mL), (e) testosterone (0.25 mg/mL), (f) methyltestosterone (0.25 mg/mL), (g) methandriol (0.16 mg/mL), (h) stanolone (1.0 mg/mL), (i) boldenone acetate (0.040 mg/mL), (j) stanolozol (0.11 mg/mL), (k) danazol (0.040 mg/mL), (l) testosterone acetate (0.040 mg/mL), (m) nandrolone propionate (0.040 mg/mL), (n) clostebol acetate (0.040 mg/mL), (o) testosterone propionate (0.040 mg/mL), (p) methandriol-3-acetate (0.040 mg/mL), (q) testosterone isobutyrate (0.040 mg/mL), (r) nandrolone phenylpropionate (0.040 mg/mL), (s) testosterone isocaproate (0.040 mg/mL), (t) testosterone enanthate (0.040 mg/mL), (u) testosterone cypionate (0.040 mg/mL), (v) boldenone undecylenate (0.040 mg/mL), (w) methandriol dipropionate (0.16 mg/mL), (x) nandrolone decanoate (0.040 mg/mL), (y) testosterone decanoate (0.040 mg/mL) and (z) testosterone undecanoate (0.40 mg/mL).

a 22 min gradient (15 min gradient re-equilibration) [14]. The 37 min total run time by conventional HPLC is approximately 12× greater than that obtained by HPLC at elevated pressure. Using a previously reported dynamically coating capillary zone electrophoresis (CZE) procedure [15] for the same solute mixture, all compounds were well resolved with an approximately 3× greater total run time than HPLC at elevated pressure. Excellent time and area precision is obtainable for the described techniques.

Since excellent peak area precision was obtained for the phenethylamines in the test mixture using HPLC at elevated pressure (RSD ≤ 0.66%), an internal standard would not appear to be necessary using this technique if care is exercised making dilutions. In this vein, amphetamine, methamphetamine, MDA, MDMA and MDEA were well resolved in under l.5 min using isocratic analysis at low and high pH. This total run time is approximately 3.5× faster than a previously reported gradient reversed phase HPLC method for a similar mixture (sans methamphetamine) using a highly permeable 4.6 mm i.d. monolithic C18 column operating at 4.0 mL/min [16]. HPLC at elevated pressure is also approximately 2.5× faster than CZE techniques that use short capillaries at high field strengths in both the aqueous [17] and non-aqueous mode [18] for the separation of the phenethylamines in the test mixture.

3.2. Separation of neutral drugs

Unlike the separation of basic solutes, which require buffers, neutral drugs, such as anabolic steroids can be analyzed using mobile phases containing water as the base solvent. As shown in Fig. 3, all 26 target anabolic steroids were well resolved using a 10 cm 1.7 μm C18 column with a 28 min water, methanol gradient (2 min gradient equilibration). This is in contrast to conventional HPLC, where using a 25 cm long 5 μm C18 column [19]. For 24 of these solutes, 20 were well resolved using a 30 min water, methanol gradient (15 min gradient equilibration).

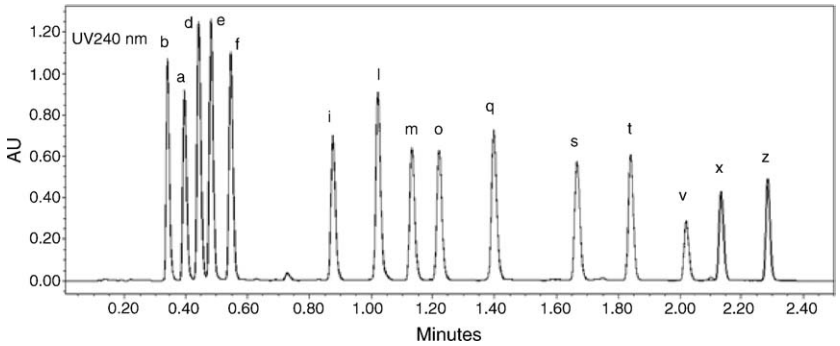

Fig. 4. Overlay of seven injections of an elevated pressure gradient HPLC separation of anabolic steroids at solute concentrations of 0.030 mg/mL. Conditions: injection size, 10 μL (partial fill mode); column, 3.0 cm × 2.1 mm 1.7 μm Acquity UPLC BEH C18. Initial conditions: 35% acetonitrile, 65% water. Final conditions: 100% acetonitrile, 2 min linear gradient, hold for 0.49 min, 0.5 min gradient re-equilibration; flow rate 0.750 mL/min; temperature 30 °C. Peak identity same as Fig. 3.

For micellar electrokinetic chromatography (MEKC), using a 72 cm long uncoated capillary (50 cm to detector), 12 of the 24 anabolic steroids separated by conventional HPLC were well resolved during a 40 min run (pre-run flushes 2 min) [19]. Multi-wavelength UV detection is used for anabolic steroids to enhance signal to noise of certain solutes depending on their extinction coefficients. The enhanced HPLC gradient system, coupled with PDA UV detection, is excellent for screening to determine which anabolic steroids may be present in seized drugs. As described previously, there are significant differences in the UV spectra depending whether an enone, dienone, ketone, isolated double bond, etc. are present [19]. In practice, only a single anabolic steroid would be present in most seized exhibits. Therefore, the separation of anabolic steroids using a fast gradient on a 3 cm 1.7 μm C18 column was investigated. This system would be particularly useful for providing an additional confirmation of the presence of an anabolic steroid and for quantitative analysis. An overlay of seven injections of a 2.5 min water, acetonitrile gradient separation of 16 of the anabolic steroids is shown in Fig. 4. For this separation, a 10 μL injection in the partial loop mode was employed with a flow rate of 0.750 mL/min and a gradient equilibration time of 0.5 min. This resulted in an operating pressure of 2600–6500 psi. For this separation, excellent precision was obtained for retention time (RSD ≤ 0.16%), and peak area (RSD ≤ 0.17%). Retention data for all 26 anabolic steroids are shown in Table 2.

Table 2
Relative retention times anabolic steroids[a]

Anabolic steroid	Relative retention time
Fluoxymesterone	0.689
Boldenone	0.822
Nandrolone	0.872
Methandrostenolone	0.919
Testosterone	1.000 (0.48 min)
Methandriol	1.050
Methyltestosterone	1.130
Stanozolol	1.298
Stanolone	1.323
Boldenone acetate	1.827
Danazol	1.830
Testosterone acetate	2.118
Methandriol 3 acetate	2.308
Nandrolone propionate	2.363
Clostebol acetate	2.476
Testosterone propionate	2.532
Testosterone isobutyrate	2.902
Nandrolone phenylpropionate	2.979
Testosterone isocaproate	3.473
Testosterone enanthate	3.810
Testosterone cypionate	3.886
Boldenone undecylenate	4.182
Methandriol dipropionate	4.211
Nandrolone decanoate	4.461
Testosterone decanoate	4.547
Testosterone undecanoate	4.779

[a] Relative to testosterone.

3.3. Drug profiling

Drug profiling for forensic analysis can entail the analysis of trace impurities in synthetic drugs (e.g., from MDMA precursor chemicals) or the constituents of natural products (e.g., from opium) for tactical and strategic intelligence [20]. The use of HPLC at elevated pressure with 1.7 μm particles, which can provide increased peak capacity over conventional HPLC, appears well suited for this task.

A comparison of HPLC at elevated pressure and conventional HPLC for impurity profiling of MDMA using a TFA, pH 2.2, acetonitrile gradient is shown in Fig. 5. For the former system, a 10 cm 1.7 μm C18 column was used in contrast to a 12.5 cm 5 μm C18 column for the latter technique. Based on the number of peaks detected, HPLC at elevated pressure has approximately twice the peak capacity of conventional HPLC for MDMA impurity profiling. It is interesting to note, although, for the same

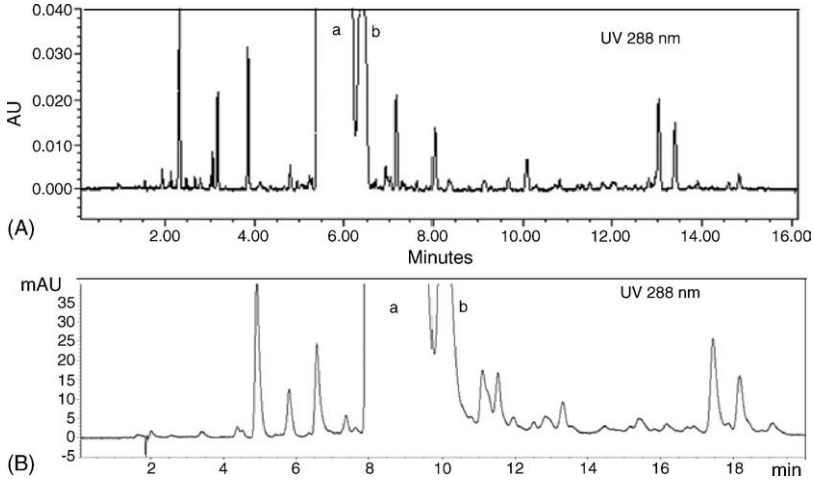

Fig. 5. Comparison of elevated pressure gradient HPLC (A) and conventional gradient HPLC (B) for the drug profiling of MDMA (1.0 mg/mL). Elevated pressure HPLC conditions: injection size, 20 μL (overfill mode); column, 10.0 cm × 2.1 mm Acquity UPLC BEH C18. Initial conditions: 2% acetonitrile, 98% 0.1% TFA buffer (pH 2.2). Final conditions: 40% acetonitrile, 60% 0.1% TFA buffer (pH 2.2), 15 min linear gradient, hold for 1.2 min, 2.0 min gradient re-equilibration; flow rate 0.375 mL/min; temperature 30 °C. Conventional HPLC conditions: injection size, 100 μL; column, 12.5 cm × 3.2 mm 5 μm Partisil 5 ODS3. Initial conditions: 2% acetonitrile, 98% 0.1% TFA (pH 2.2). Final conditions: 40% acetonitrile, 60% 0.1% TFA (pH 2.2), 15 min linear gradient, hold for 1.2 min, 12.0 min gradient re-equilibration; flow rate 0.375 mL/min; temperature 30 °C. Peaks: (a) MDMA (b) dimethyl-MDMA.

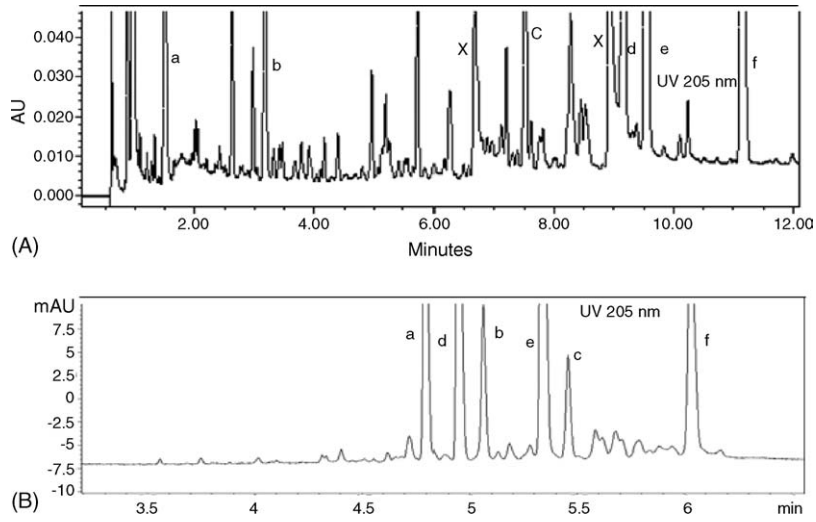

Fig. 6. Comparison of elevated pressure gradient HPLC (A) and CZE (B) for the drug profiling of opium (for sample preparation see Ref. [21]). Elevated pressure HPLC conditions: injection size, 4 μL (partial fill mode); column, 10.0 cm × 2.1 mm Acquity UPLC BEH C18. Initial conditions: 5% acetonitrile, 95% 100 mM phosphate buffer (pH 1.8). Final conditions: 29% acetonitrile, 65% 100 mM phosphate buffer (pH 1.8), 12 min linear gradient, 2.0 min gradient re-equilibration; flow rate 0.375 mL/min; temperature 30 °C. CZE conditions as in reference 18. Peaks: (a) morphine, (b) codeine, (c) thebaine, (d) papaverine, (e) noscapine, (f) tetracaine (internal standard) and (x) gradient artifacts.

sample, the injection size for the former technique is 1/5th of conventional HPLC (20 μL versus 100 μL), and the path lengths of both UV cells are both 10 mm. The signal to noise is similar for both techniques.

A comparison of HPLC at elevated pressure and a dynamically coated capillary CZE approach [21] with dual neutral cyclodextrins in the run buffer, for profiling opium is shown in Fig. 6. Again, based on the number of peaks detected, the former technique using a phosphate (pH 1.8), acetonitrile gradient with a 10 cm 1.7 μm C18 column exhibited approximately twice the peak capacity as a low pH CZE approach [21]. For the latter technique, a 33 cm dynamically coated capillary (24.5 cm to detector) was used with a run buffer containing a mixture of two neutral cyclodextrins. As expected for the opium sample, HPLC at elevated pressure exhibited significantly greater signal to noise than CE (approximately 15×). For both profiling applications, the operating pressure was between 7720 and 8930 psi using the 10 cm 1.7 μm column.

3.4. Drug screening

Seized drugs are derived from divergent drug classes, such as narcotic analgesics, stimulants, depressants, hallucinogens, and anabolic steroids. Therefore, a highly efficient, universal and reasonably fast separation technique with a suitable detection system would be desirable for drug screening. The use of HPLC at elevated pressure with 1.7 μm particle columns appears well suited for this purpose. As shown in Fig. 7, 24 solutes of the varying drug classes are fully separated in under 13.5 min (2 min gradient equilibration) using a 10 cm 1.7 μm C18 column and a

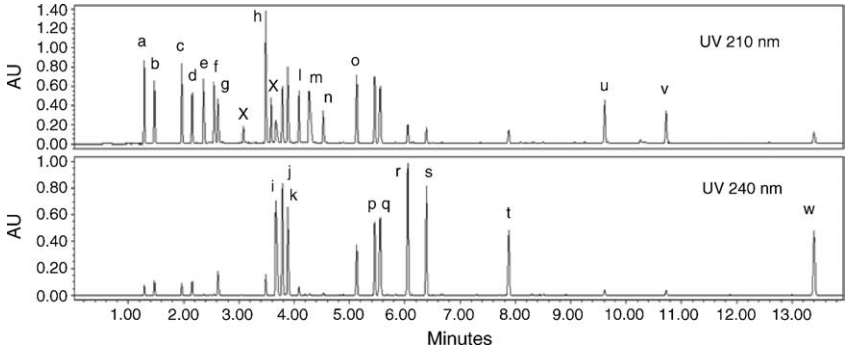

Fig. 7. An elevated pressure HPLC gradient separation of a wide variety of seized drugs. Conditions: injection size, 1 μL (partial fill mode); column, 10.0 cm × 2.1 mm 1.7 μm Acquity UPLC BEH C18. Initial conditions: 2% acetonitrile, 98% 100 mM phosphate buffer (pH 1.8). Final conditions: 100% acetonitrile, 12 min linear gradient, hold for 2.0 min, 2.0 min gradient re-equilibration; flow rate 0.430 mL/min; temperature 30 °C. Peaks: (a) psilocybin (0.10 mg/mL), (b) morphine (0.10 mg/mL), (c) psilocin (0.10 mg/mL), (d) codeine (0.20 mg/mL), (e) amphetamine (0.20 mg/mL), (f) methamphetamine (0.20 mg/mL), (g) MDMA (0.20 mg/mL), (h) heroin (0.20 mg/mL), (i) cocaine (0.40 mg/mL), (j) librium (0.20 mg/mL), (k) LSD (0.20 mg/mL), (l) phenobarbital (0.10 mg/mL), (m) PCP (0.80 mg/mL), (n) fentanyl (0.40 mg/mL), (o) methaqualone (0.10 mg/mL), (p) lorazepam (0.10 mg/mL), (q) diazepam (0.20 mg/mL), (r) testosterone (0.40 mg/mL), (s) methyltestosterone (0.40 mg/mL), (t) boldenone acetate (0.40 mg/mL), (u) cannabidiol (0.10 mg/mL), (v) Δ9-THC (0.10 mg/mL), (w) testosterone undecanoate (0.80 mg/mL) and (x) gradient artifacts.

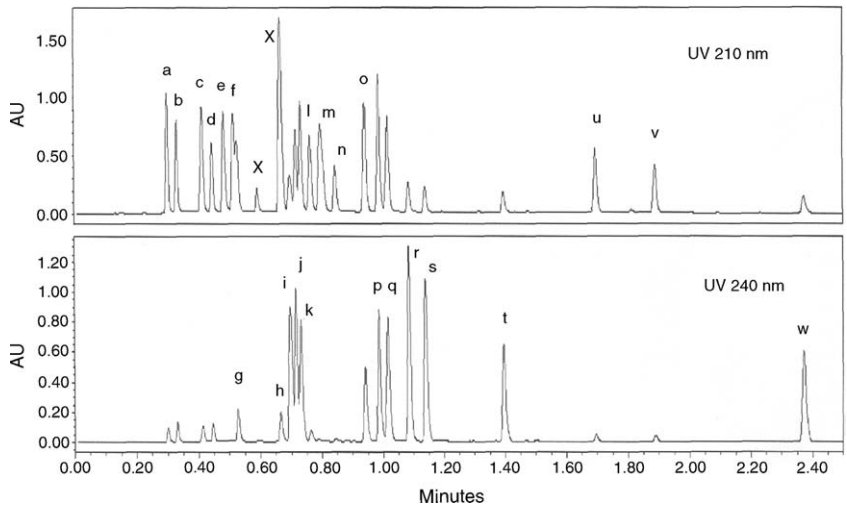

Fig. 8. An elevated pressure gradient separation of a wide variety of seized drugs. Conditions: injection size, 1 μL (partial fill mode); column, 3.0 cm × 2.1 mm 1.7 μm Acquity UPLC BEH C18. Initial conditions: 2% acetonitrile, 98% 100 mM phosphate buffer (pH 1.8). Final conditions: 100% acetonitrile, 2 min linear gradient, hold for 0.5 min, 0.5 min gradient re-equilibration; flow rate 0.750 mL/min; temperature 30 °C. Peak identity and solute concentrations are same as in Fig. 7.

phosphate (pH 1.8), acetonitrile gradient with PDA UV detection. For this separation, the operating pressure is between 4200 and 9600 psi. The use of automatic UV library searches would facilitate and enhance the specificity of analysis. Mass spectrometric detection in combination with PDA UV detection would further enhance specificity of analysis and allow the detection of solutes which lack a UV chromophore.

It is interesting to compare the enhanced HPLC technique with another highly efficient technique, MEKC, for drug screening. Weinberger and Lurie previously separated all the solutes in the test mixture except for anabolic steroids [22]. For these MEKC conditions, the more lipophilic anabolic steroids, such as testosterone undecanoate would stick to the micelle and migrate as a group. For the MEKC separation, 17 out of the 19 solutes were fully resolved (MDMA and methamphetamine co-migrated) in 40 min (pre-run flushes 2 min).

As shown in Fig. 8, using a shorter 3 cm 1.7 μm C18 column and a faster gradient allowed for all solutes to be at least partially resolved in 2.4 min (gradient equilibration 0.5 min). For this separation, operating pressure is between 2800 and 6200 psi. Detection at a higher UV wavelength (240 nm versus 210 nm) allows for the selective detection of MDMA in the presence of methamphetamine (see Fig. 8).

4. Conclusion

This work demonstrates the high separation power of HPLC at elevated pressure with 1.7 μm hybrid C18 stationary phase columns for the analysis of drugs. The system investigated, in terms of resolution and speed of analysis, offers the best liquid phase separations to date for the commonly encountered phenethylamines and anabolic steroids. Using either isocratic or gradient analysis, excellent retention time and peak area precision is obtainable. The high resolving power of HPLC at elevated pressure with relatively long 1.7 μm particle columns is excellent for drug profiling and appears superior to existing liquid phase techniques for this purpose. The general applicability of the described technique to a wide variety of drugs including narcotic analgesics, stimulants, depressants, hallucinogens and anabolic steroids was demonstrated.

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