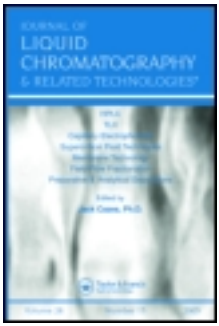

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ljlc20

# Use of High-Temperature Liquid Chromatography with Sub-2 µm Particle $C_{18}$ Columns for the Analysis of Seized Drugs

Ira Lurie [a] & Li Li [a]

[a] Special Testing and Research Laboratory, U.S. Drug Enforcement Administration , Dulles, Virginia, USA

Published online: 05 Oct 2009.

To cite this article: Ira Lurie & Li Li (2009) Use of High-Temperature Liquid Chromatography with Sub-2 µm Particle $C_{18}$ Columns for the Analysis of Seized Drugs, Journal of Liquid Chromatography & Related Technologies, 32:18, 2615-2626, DOI: 10.1080/10826070903245516

To link to this article: http://dx.doi.org/10.1080/10826070903245516

*Journal of Liquid Chromatography & Related Technologies*®, 32: 2615–2626, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903245516



# Use of High-Temperature Liquid Chromatography with Sub-2 μm Particle $C_{18}$ Columns for the Analysis of Seized Drugs

**Ira Lurie and Li Li**

Special Testing and Research Laboratory, U.S. Drug Enforcement Administration, Dulles, Virginia, USA

**Abstract:** The use of high-temperature liquid chromatography with sub-2 μm particle $C_{18}$ columns is described for the analysis of seized drugs. The drug compounds investigated include narcotic analgesics, stimulants, depressants, hallucinogens, and anabolic steroids. Although retention times for 20 solutes of various drug classes decreased with increased temperature (15–95°C) under constant gradient conditions, relative retention times increased, decreased, or remained the same. No significant sample degradation at elevated temperature was observed. The use of rapid gradients at 95°C was well suited for drug screening and the separation of anabolic steroids.

**Keywords:** Anabolic steroids, High-temperature liquid chromatography, Seized drugs, Sub-2 μm particle $C_{18}$ column

## INTRODUCTION

The use of sub-2 μm particle columns allows for significantly improved high performance liquid chromatography (HPLC) separations, both in terms of faster analysis and/or improved peak resolution.[1,2] This improved performance is a result of the reduced plate height of the

Correspondence: Ira Lurie, Special Testing and Research Laboratory, U.S. Drug Enforcement Administration, Dulles, Virginia, USA. E-mail: ira.s.lurie@usdoj.gov

smaller particle columns and the inverse proportionality between optimum velocity and particle size. The use of high temperature liquid chromatography is further advantageous in that the optimum linear velocity increases with temperature (not the minimum plate height (H) value), resulting in faster separations.[3–6] High temperature liquid chromatography and the use of elevated temperature with sub-2 μm particle columns have been recently reviewed.[7,8]

One major concern for performing separations at elevated temperature is analyte stability. Thompson and Carr[9] demonstrated that decreasing the residence time of thermally labile compounds may decrease the extent of on-column reactions.

Another significant concern is the effect of column temperature on selectivity which could adversely affect the separation at elevated temperature.[6] The analysis of seized drugs is important for legal and intelligence purposes. For this reason, high performance separation techniques are required. In this vein, HPLC[10] and capillary electrophoresis (CE)[11] are excellent for solutes that are thermally degradable, highly polar, or non-volatile. Recently HPLC at elevated pressure with 1.7 μm particle $C_{18}$ columns has been shown to offer significantly better resolution and/or faster analysis than conventional HPLC (pressure $\leq$6000 psi) and CE for the analysis of seized drugs. Veuthey et al.[6] demonstrated the high speed obtainable for the separation of pharmaceutical compounds, including several barbiturates and benzodiazepines, using sub-2 μm particle columns at elevated temperature.

In this study, the utility of high temperature liquid chromatography using sub-2 μm particle $C_{18}$ columns for the analysis of seized drugs is described. The effect of temperature on retention times and relative retention times for different drug classes is detailed. On-column chemical stability of the various solutes is studied. Finally, the use of high temperature for drug screening and the separation of anabolic steroids is described.

## EXPERIMENTAL

### Chemicals

Drug standards were obtained from the reference collection of the Drug Enforcement Administration, Special Testing and Research Laboratory (Dulles, VA, USA). Phosphoric acid was obtained from Aldrich (Milwaukee, WI, USA). Sodium hydroxide was 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 (Musekgon, 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 mixing 870 mL of water, 10 mL of phosphoric acid, and 30 mL of 1 M sodium hydroxide. Mobile phase buffers were mixed with an appropriate amount of acetonitrile or methanol as dictated by experimental conditions.

The more polar standard compounds (i.e., psilocybin, psilocin, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA)) were dissolved in starting mobile phase, while all other reference solutes were dissolved in acetonitrile. The individual concentrations of all solutes were 100 μg/mL.



### Instrumentation

The instrumentation consisted of an Agilent 1200 Series Rapid Resolution system (Waldbronn, Germany) equipped with a 1200 Series vacuum degasser, a 1200 Series binary pump SL, a 1200 Series high performance autosampler SL, a 1200 Series thermostatted column compartment SL, and a 1200 Series SL diode array UV detector. All data was acquired using Chem Station software. The HPLC columns were Zorbax Rapid Resolution HT Stable Bond C18, 1.8 μm, 50–100 mm × 2.1 mm.

## RESULTS AND DISCUSSION

### Effect of Temperature on Retention Times and Relative Retention Times

The effect of temperature on retention times and relative retention times of 20 seized drugs of varying drug classes including stimulants, hallucinogens, narcotic analgesics, depressants, and anabolic steroids is shown in Figure 1. A temperature range of 15–95°C was used with a Zorbax Stable Bond C18, 1.8 μm column[1] and a phosphate pH 1.8, acetonitrile gradient. As shown in Figure 1, retention times decreased with an increase in temperature. As evidenced by the non-parallel plots for adjacent solutes, relative retention times decreased, increased, or remained the same with an increase in temperature (see Figure 1). The decrease in retention times with increased temperature is consistent with those observed in reversed phase HPLC for both isocratic[7] and gradient analysis.[12]

[1]Rated by manufacturer to be stable up to 100°C

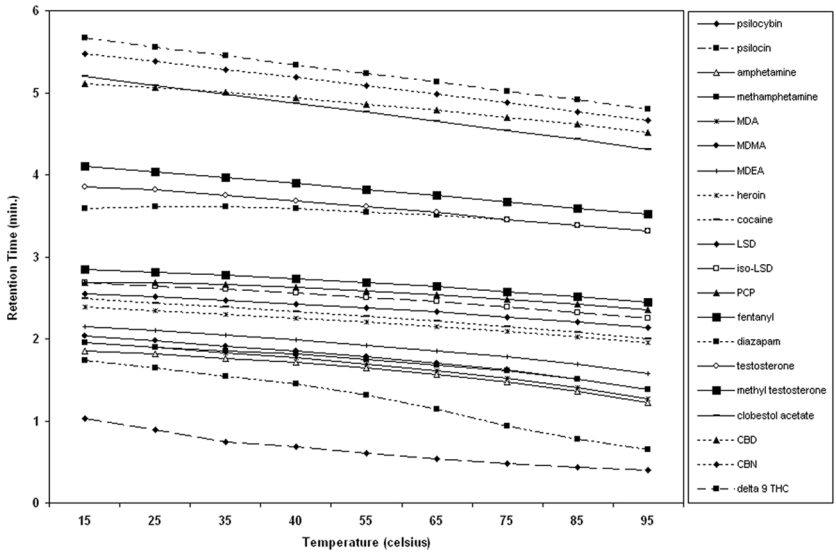


***Figure 1.*** Plot of retention time (minutes) versus temperature (Celsius) for seized drugs of varying drug classes. A Zorbax Rapid Resolution HT Stable Bond C18 2.1 mm × 50 mm, 1.8 μm column was used with a five minute linear gradient from 3% acetonitrile: 97% 100 mM phosphate buffer pH 1.8 to 95% acetonitrile: 5% 100 mM phosphate buffer (pH 1.8) (two minute hold) at flow rate of 0.4 mL/min. 2 μl injections were employed with UV detection at 210 nm.

This effect in both cases is related to the van't Hoff relationship:[12,13]

$$\log k = A + B/T \text{ (isocratic)} \quad (1)^{[12]}$$

$$\log k_0 = A + B/T \text{ (gradient)} \quad (2)^{[12]}$$

$$t_R = (t_0/b)\ \log(2.3k_0b + 1) + t_0 + t_D \text{ (gradient)} \quad (3)^{[12]}$$

The terms $k$ and $k_0$ are the capacity factors for isocratic retention and gradient analysis at the start of the gradient, while $T$ is the absolute temperature. $A$ and $B$ are constants for a given solute and set of experimental conditions. A is a function of the standard state entropy of retention and the phase ratio, while $B$ is proportional to the standard state enthalpy of retention. The quantities $b$, $t_0$, and $t_D$ are the gradient-steepness parameter, the column dead-time, and the gradient dwell time, respectively. For isocratic separations, parallel as well as non-parallel van't Hoff curves, are obtained.[14,15] Such relationships would also be expected for gradient analysis. Zhu et al.[12] have observed both parallel and

non-parallel curves in plots of retention times versus $1/T$ for gradient analysis. Factors such as modifications of stationary phases with solvent molecules at different temperatures, changes in retention mechanism, and changes in pKa values with temperature can contribute to non-classical van't Hoff behavior.[15]

Examples of solute pairs whose relative retention times decrease with temperature include heroin and cocaine, methyltestosterone and clostebol acetate, cannabidiol (CBD) and cannabinol (CBN), and CBN and Δ9-tetrahydrocannabinol (Δ9-THC). In contrast, examples of solute pairs whose relative retention times increase with temperature include methamphetamine and MDA, MMDA and MDEA, and iso-lysergic acid diethylamide (iso-LSD) and phencyclidine (PCP). Solute pairs whose relative retention times remain the same with temperature include LSD and iso-LSD, and testosterone and methyltestosterone. It is interesting that CBD elutes before clostebol acetate at temperatures less than 35°C and then elutes after the ester at higher temperatures.

Although at elevated temperatures faster separations are obtainable in terms of kinetic considerations, Figure 1 clearly indicates that this effect may be negated by decreases in relative retention times. Conversely, increases in relative retention time, in conjunction with decreases in retention factors, enhance the favorable kinetics at higher temperatures.



## On-Column Degradation of Solutes at Elevated Temperature

A major concern of using elevated temperature is the on-column stability of compounds. In this vein, the on-column stability of various solutes was studied at constant gradient steepness (b) (increased flow rate, decreased time of gradient).

The gradient steepness parameter $b$ is given by

$$b = V_m \Delta\Phi S/(t_G F) \qquad (4)^{[12]}$$

where $V_m$ is the column dead-volume (mL), $\Delta\Phi$ is the change in volume fraction of the B-solvent (Φ) from the start to the end of the gradient, $S$ is a constant for each solute, $t_G$ is gradient time (*min*), and $F$ is flow rate (mL/min).

As shown in Figure 2, there is a significant change in column residence time under constant b conditions. The autosampler was kept at 4°C to maximize solute stability in the injection solvent.

The peak area for a concentration dependent detection such as the diode array UV detector is given by

$$Area_\phi = \varepsilon b_1 W_a/F \qquad (5)^{[9]}$$

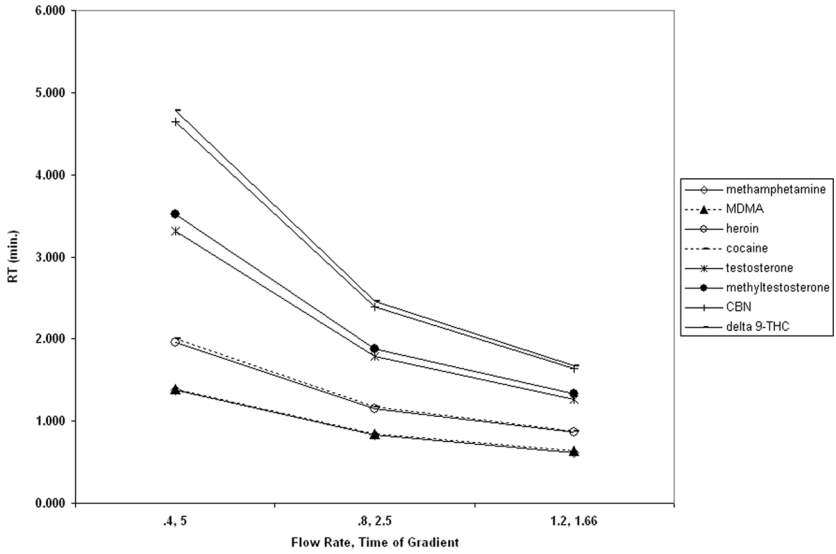


***Figure 2.*** Plot of retention time (minutes) versus flow rate (mL/min) and gradient time (min) at constant b for selected drug pairs. Conditions same as Figure 1 except for a temperature of 95°C, flow rate, and gradient time.

where $\phi$ refers to a specific eluent, $\varepsilon$ is the molar absorptivity for the analyte, $b_1$ is the flow cell path length, $W$ represents the total mass of the analyte, and $F$ is the flow rate.

As indicated in Equation (5), a change in normalized peak area (area × flow rate) with column residence time for a given solute would indicate the presence of a new compound due to on column degradation. For all solutes, there was no significant change in normalized peak area with gradient time at constant $b$. This effect is shown in Figure 3 for thermally labile compounds[2] such as diazepam, psilocybin, LSD, heroin, cocaine, PCP and clostebol acetate. As Equation (5) indicates, the above experiments would not be indicative of stability for solutes that have identical or very similar extinction coefficients to their degradant at the measured UV wavelength of 210 nm. As pointed out by Thompson and Carr,[9] it is possible to effect highly reliable separations even when the parent peak is not separated from the degradant, so long as the peak area is reproducible as was the case in the present study.

Evidence for on-column reaction can also be obtained by observing peak shape.[9] In the present study, the peak shapes did not indicate the presence of degradants. In addition, no additional peaks were observed.

[2]Decompose using gas chromatography (GC)

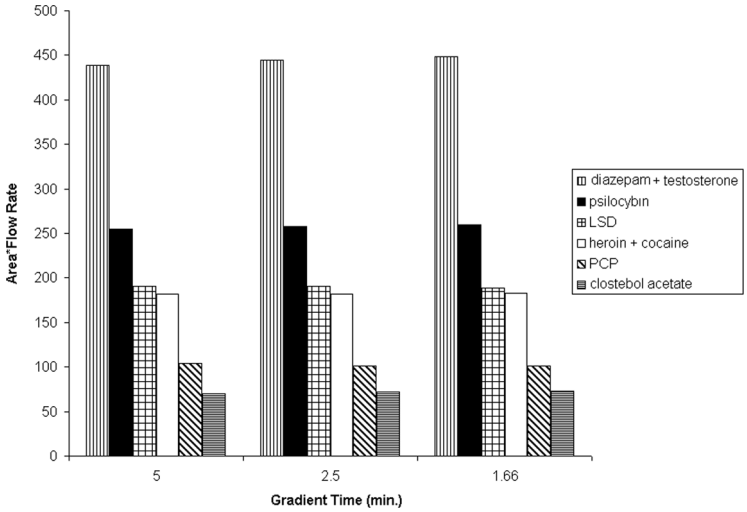


*Figure 3.* Plot of area*flow rate versus gradient time (min) at constant *b*. Conditions same as Figure 2.

The lack of apparent sample degradation could be the result of relatively fast retention times.

## Drug Screening

Screening for the presence of seized drugs with a rapid and fairly specific separation technique is desirable. The use of high temperature with a sub-2 μm particle $C_{18}$ column and diode array UV detection offers highly efficient and rapid separations with reasonable specificity, and, therefore, is well suited for drug screening. As shown in Figures 2 and 3 and described in previous sections, at 95°C, rapid analysis is possible without apparent solute degradation. The separation of 20 solutes representing divergent drug classes (i.e., narcotic analgesics, stimulants, depressants, hallucinogens, and anabolic steroids) is shown in Figure 4. The flow rate and gradient time chosen (0.8 mL/min., 2.5 min.) were a compromise between values giving optimum relative retention times and optimum plate height (H). As shown in Figure 2, relative retention times at constant *b* usually decrease with an increase in flow rate and decrease in gradient time. For a sub-2 μm, 2.1 mm I.D., C18 column, it was previously shown that the optimum H corresponds to a flow rate of 1.2 mL/min.[6] As shown in Figure 4, 16 out of the 20 compounds are at least partially resolved (resolution $\geq 1$) in less than 2.6 minutes. This

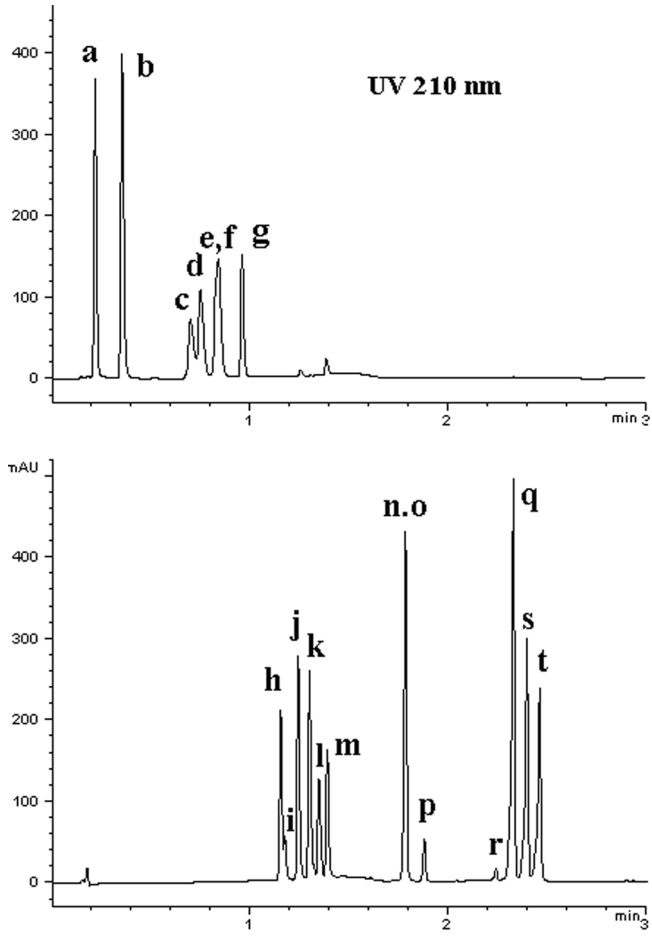


***Figure 4.*** A high temperature gradient separation of a wide variety of seized drugs. Conditions same as Figure 1 except for a temperature of 95°C, flow rate 0.80 mL/min, gradient time 2.5 min, and a 0.5 min hold; maximum pressure 3375 psi. Two sets of standard mixtures injected as described in experimental section. Peaks: (a) psilocybin, (b) psilocin, (c) amphetamine, (d) MDA, (e) methamphetamine, (f) MDMA, (g) MDEA, (h) heroin, (i) cocaine, (j) LSD, (k) iso-LSD, (l) PCP, (m) fentanyl, (n) diazepam, (o) testosterone, (p) methyltestosterone, (q) CBD, (r) clostebol acetate, (s) CBN, (t) Δ9-THC.

separation aided by the lower mobile phase viscosity at elevated temperature (maximum operating pressure of 3375 psi) could be acquired on a conventional HPLC instrument (pressure limit $\leq$6000 psi). Each of the poorly separated compounds (which could be present without the interfering solute in a drug exhibit) has easily distinguishable UV spectra.

UV analysis at 288 nm would distinguish MDMA from methamphetamine (methamphetamine UV cutoff 275 nm). Mass spectrometric (MS) detection would further enhance the specificity of analysis and distinguish between any co-eluting solutes and partially resolved solutes. In this instance replacing the phosphate buffer with a volatile buffer would be required. A minimum amount of method development may suffice. Replacing 100 mM phosphate, pH 1.8 buffer, with either 1% formic acid, pH 2.2, or 10 mM ammonium formate, pH 3.8, gave a similar separation for amphetamine, methamphetamine, MDA, MDMA, and MDEA using a 1.7 μm BEH $C_{18}$ column.[16]

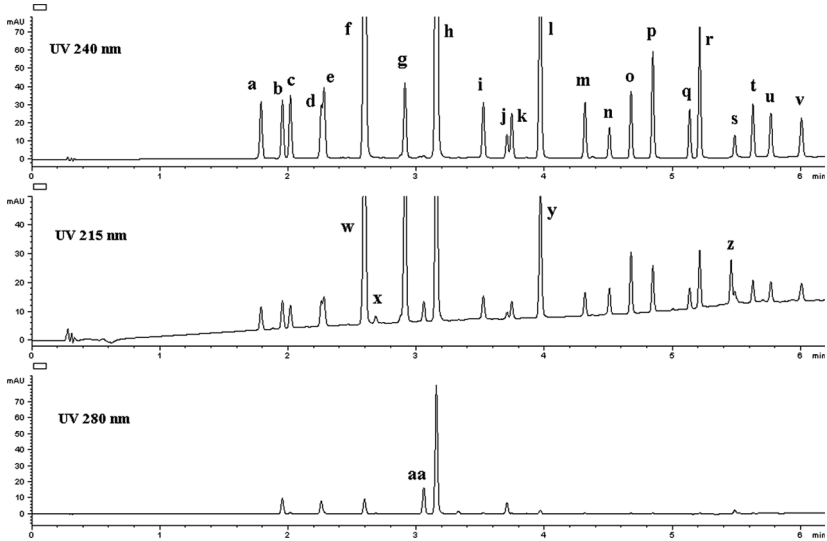


***Figure 5.*** A high temperature gradient separation of anabolic steroids. A Zorbax Rapid Resolution HT StableBond C18 2.1 mm × 100 mm, 1.8 μm column was used with a five minute linear gradient from 40% methanol: 60% 25 mM phosphate buffer (pH 2.4) to 90% methanol: 10% 25 mM phosphate buffer (pH 2.4) (one minute hold) at flow rate of 0.8 mL/min and a temperature of 95°C; maximum pressure 7650 psi. 2 μl injections were employed with muti-wavelength UV detection. Peaks: (a) fluoxymesterone, (b) boldenone, (c) nandrolone, (d) methandrostenolone, (e) testosterone, (f) methyltestosterone, (g) stanozolol, (h) boldenone acetate, (i) testosterone acetate, (j) clostebol acetate, (k) nandrolone propionate, (l) testosterone propionate, (m) testosterone isobutyrate, (n) nandrolone phenylpropionate, (o) testosterone phenylpropionate, (p) testosterone isocaproate, (q) testosterone enanthate, (r) testosterone cypionate, (s) boldenone undecylenate, (t) nandrolone decanoate, (u) testosterone decanoate, (v) testosterone undecanoate, (w) methandriol, (x) stanolone, (y) methandriol acetate, (z) methandriol diproprionate, (aa) danazol.

### Separation of Anabolic Steroids

The separation of 27 anabolic steroids at 95°C is shown in Figure 5. Using a 5 minute phosphate (pH 2.4), methanol gradient followed by a short hold, 23 of these compounds are at least partially resolved in approximately 6 minutes. Each of the poorly separated compounds has easily distinguishable UV spectra.[17] Since, in practice, only a single anabolic steroid would be present in most seized exhibits, the use of sub-2 μm particle columns at an elevated temperature with diode array UV detection is well suited for anabolic steroid analysis. This would include screening, providing an additional confirmation, and quantitative analysis. Multi-wavelength detection was employed (see Figure 5) to take advantage of the different UV maxima of the various anabolic steroids.[17] MS detection with the proper buffer additive could allow the deconvolution of co-eluting or partially resolved compounds. The flow rate was limited to 0.800 mL/min in order to safely operate under the maximum pressure of the instrument (9000 psi). Although not required for the chromatography of the neutral solutes, the acidic buffer was added to stabilize the column at high temperatures. The use of the Stable Bond $C_{18}$ column with water as the weak solvent led to peak shapes that significantly deteriorated over time. This effect is likely due to dissolution of the silica backbone, which is accelerated at $pH > 6$ and elevated temperature.[18] This phenomena could also be exacerbated by the presence of an organic modifier which may increase the apparent pH [19], depending on the strength of the acid present in the mobile phase.

## SUMMARY

High temperature liquid chromatography with small particle size columns can be a useful separation technique to obtain high speed separations of seized drugs. The change in separation selectivity with temperature is a very important factor governing the separations when operating at elevated temperature. In contrast to GC, high temperature liquid chromatographic analysis demonstrated no significant evidence of solute degradation at elevated temperature for compounds which are thermally labile. Liquid chromatographic separations at 95°C are well suited for the rapid separation of complex mixtures encountered in drug screening and anabolic steroid analysis.

## ACKNOWLEDGMENT

The authors would like to thank Michael Woodman of Agilent for useful discussions.

## REFERENCES


1. Swartz, M.E. UPLC. An introduction and review. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 1253–1263.
2. Lurie, I.S. High-performance liquid chromatography of seized drugs at elevated pressure with 1.7 μm hybrid $C_{18}$ stationary phase columns. J. Chromatogr. A **2005**, *1100*, 168–175.
3. Anita, F.; Horvath, Cs. High-performance liquid chromatography at elevated temperatures: examination of conditions for the rapid separation of large molecules. J. Chromatogr. A **1988**, *435*, 1–15.
4. Plumb, R.S.; Rainville, P.; Sith, B.W.; Johnson, K.A.; Castro-Perez, J.; Wilson, I.D.; Nicholson, J.K. Generation of ultrahigh peak capacity LC separations via elevated temperatures and high linear mobile-phase velocities. Anal. Chem. **2006**, *78*, 7278–7283.
5. Plumb, R.; Mazzeo, J.R.; Grumbach, E.S.; Rainville, P.; Jones, M.; Wheat, T.; Neue, U.D.; Smith, B.; Johnson, K.A. The application of small porous particles, high temperatures, and high pressures to generate very high resolution LC and LC/MS separations. J. Sep. Sci. **2007**, *30*, 1158–1166.
6. Nguyen, D.T.T.; Guillarme, D.; Heinisch, S.; Barrioulet, M.P.; Rocca, J.L.; Rudaz, S.; Veuthey, J.L. High throughput liquid chromatography with sub-2 μm particles at high pressure and high temperature. J. Chromatogr. A **2007**, *1167*, 76–84.
7. McNeff, C.V.; Yan, B.; Stoll, D.R.; Henry, R.A. Practice and theory of high temperature liquid chromatography. J. Sep. Sci. **2007**, *30*, 1672–1685.
8. Wu, N.; Thompson, R. Fast and efficient separations using reversed phase liquid chromatography. J. Liq. Chromatogr. & Rel. Technol. **2006**, *29*, 949–988.
9. Thompson, J.D.; Carr, P.W. A study of the critical criteria for analyte stability in high-temperature liquid chromatography. Anal. Chem. **2002**, *74*, 1017–1023.
10. Caddy, B. The Use Of High Performance Liquid Chromatography for the Detection and Quantiation of Abused Drugs. in *The Analysis of Drugs of Abuse*; Gough, T., Ed.; Wiley: Chichester, UK, 1991; 121–174.
11. Lurie, I.S. Analysis of Seized Drugs by Capillary Electrophoresis. in *The Analysis of Addictive and Misused Drugs*; Adamovics, J.A., Ed.; Marcel Dekker: New York, USA, 1995; 151–220.
12. Zhu, P.L.; Snyder, L.R.; Dolan, J.W.; Djordjevic, N.M.; Hill, D.W.; Sander, L.C.; Waeghe, T.J. Combined use of temperature and solvent strength in reversed-phase gradient elution. I. Predicting separation as a function of temperature and gradient conditions. J. Chromatogr. A **1996**, *756*, 21–39.
13. Melander, W.; Campbell, D.E.; Horvath, Cs. Enthalpy-entropy compensation in reversed-phase chromatography J. Chromatogr. **1978**, *158*, 215–225.
14. Guillarme, D.; Heinisch, S.; Rocca, J.L. Effect of temperature in reverse phase liquid chromatography. J. Chromatogr. A **2004**, *1052*, 39–51.
15. Waksmundzka-Hajnos, M.; Petruczynik, A.; Ciesla, G.J. Temperature the tool in separation of alkaloids by RP-HPLC. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30*, 2473–2484.
16. Unpublished data.

17. Lurie, I.S.; Sperling, A.R.; Meyers, R.P. The Determination of anabolic steroids by MECC, Gradient HPLC, and Capillary GC. J. Forensic Sci. **1994**, *39*, 74–85.
18. Agilent Zorbax SB-C18 datasheet, www.agilent.com (accessed April 2008).
19. McCalley, D.V. Is hydrophilic interaction chromatography with silica columns a viable alternative to reversed-phase liquid chromatography for analysis of ionisable compounds? J. Chromatogr. A **2007**, *1171*, 46–55.

Received March 21, 2009
Accepted June 4, 2009
Manuscript 6508