Analysis of Polymer Additives in High-Temperature Liquid Chromatography

Byoung-Hyoun Kim*, D.-K. Yang, and **Jong Hoa Ok**

Analytical Science, Corporate R&D / LG Chemical Research Park, LG Chemical Ltd., 104-1, Moonji-dong, Yusong-gu, Daejeon, 305-380, South Korea

Abstract

A high-temperature liquid chromatographic technique is employed for the separation of commercially available polymer additives to enhance the resolution and speed. Separation efficiencies and elution behaviors for seven phthalate plasticizers and five antioxidants are evaluated at elevated column temperatures and with a thermal gradient. Diamondbond C_{18} (octadecylsilica), Zirchrom PS (zirconia-based polystyrene), and Zirchrom PBD (zirconia-based polybutadiene) columns are selected for the study because of their thermal stability. The temperature programming is controlled with a column oven in conjunction with an independent mobile phase preheater and a post-column effluent cooling assembly. Van't Hoff plots show that the reverse-phase liquid chromatography mechanism is maintained over a wide range of column temperatures. A 1% increase of acetonitrile in the mobile phase is estimated to have a comparable effect as a 7–7.5°C column temperature increase on the retention time changes.

Introduction

Recently, a high column temperature technique in reversed-phase (RP) liquid chromatography (LC) has been frequently employed for separation analysis. A high column temperature in RPLC increases the mass transfer rate and reduces the column backpressure; therefore, less organic modifier in the mobile phase is required, and the time for the analysis can be significantly reduced. There are, however, some limitations in column selection because the requirement for the stationary phase to remain stable enough to maintain the separation ability at a high temperature is not always satisfied. Zirconia-based stationary phases have often been employed for high temperature column materials because of their appropriate thermal stability. However, even with this kind of column material, column bleeding is frequently observed because of the leaching of packing material at high temperatures. An evaluation report on the use of graphitic–carbon, polystyrene–divinylbenzene, and polydentate–silica as stationary phases show that they had more stable behaviors at high temperatures (1). The advantages and disadvantages of using a high column temperature in an HPLC analysis have been well demonstrated by several groups (2,3). The high-temperature effects on the retention time, selectivity, and separation efficiency in LC were demonstrated with both an aqueous mobile phase and a non-aqueous mobile phase using a narrow-bore column (3). Several research groups have also described the use of superheated water as a mobile phase for high-temperature separation analysis (4,5). Sanagi employed high-temperature (HT) LC and used superheated water as the mobile phase for the separation of several triazole fungicides (4). They were able to acquire good separation efficiency with the limit of detection (LOD) down to the picogram level. Fields could also differentiate the selectivity of several steroids with superheated water as the mobile phase in a polybutadiene-coated zirconia column at 200°C (5). However, these applications with superheated water were limited to the analysis of polar or medium polar compounds. Superheated water did not have enough elution strength for the separation of nonpolar compounds. Modified superheated water, with additional organic solvent, should be employed in the mobile phase for the sufficient separation of nonpolar compounds. There have also been some reports on HTLC methods that used packed capillary columns (7,8). Trones separated organo-lead compounds with packed capillary columns at high column temperature in association with the inductively coupled plasma (ICP) mass spectrometry (MS) as the detector. Good peak height repeatability was successfully achieved for the organo-lead compounds with the LOD in the sub picogram range (7). Many HTLC investigations were also carried out and reported in combination with MS detectors and flame-ionization detectors (9,10). For the routine applications, however, HTLC should cope with some instrumental limitations. The traditional block heater was not efficient enough to transfer the necessary heat fast enough to the standard 4.6-mm i.d. column for temperature programming; thus, the column efficiency and reproducibility were susceptible to severe deterioration (1). Mobile phase pre-heating was also crucial in order to avoid peak splits because of the thermal mismatch (2,5,11). It

*Author to whom correspondence should be addressed: email bhkime@lgchem.

was also necessary to cool down the effluent after the column to avoid any possible damage to the ordinary detector by hot effluent. Equipment integrated with these vital functions for HTLC recently became commercially available, and implementation for the routine analysis became much more applicable than ever before (1). In this work, the separation of phthalate plasticizers and antioxidants in polymer additives is carried out with HTLC to enhance the resolution and speed. The following details the experimental results and discussions of the separation efficiencies and elution behaviors of the additives at high column temperatures and with a thermal gradient elution.

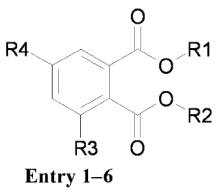
Experimental

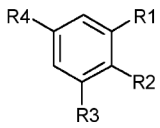
Instrumentation and reagents

An HPLC system consisting of a Waters 2695 and a 996 photodiode array detector (Waters, MA) was used for the experiments. The column temperature was controlled with a Polartherm series 9000 with an independent mobile phase pre-heater (Selerity Technologies, UT). The Polartherm series 9000, a forced air oven, was equipped with a dynamic mobile phase pre-heater and a post-column effluent cooling assembly. The mobile phase pre-heater was controlled to set the temperature equal to the column oven temperature, and the post-column effluent temperature was cooled down to 30°C. Deionized water was purified by a Milli-Q water purification system (Millipore, MA). The acetonitrile was of high-performance liquid chromatography (HPLC) grade and was purchased from J.T. Baker (Phillipsburg, NJ). The phthalate plasticizer and antioxidants used in this study were of technical grade. All compounds were used without further purification. The chemical structures of each compound are shown in Figure 1.

HTLC conditions and columns

Diamondbond C_{18} (octadecylsilica), Zirchrom PS (zirconia-based polystyrene), and Zirchrom PBD (zirconia-based polybutadiene) columns were purchased from Zirchrom separations (Anoka, MN). Separations were carried out on a column with a 4.6-mm internal diameter, 100-mm length, and 3-µm particle size. HTLC conditions are shown in Table I. Organic solvent proportions under each HTLC condition were tested to give adequate retention time for the additives. For the analysis of





Entry	Plasticizer	R1	R2	R3	R4
1	Dipropyl phthalate (DprP)	- Propyl	- Propyl	- H	- H
2	Dibutyl phthalate (DBP)	- Butyl	- Butyl	- H	- H
3	Butyl benzyl phthalate (BBP)	- Butyl	- Benzyl	- H	- H
4	Dicyclohexyl phthalate (DCHP)	- Cyclohexyl	- Cyclohexyl	- H	- H
5	Dioctyl phthalate (DOP)	- Octyl	- Octyl	- H	- H
6	Diisononyl phthalate (DINP)	- Isononyl	- Isononyl	- H	- H
7	Trioctyl trimellitate (TOTM)		- H		

Entry	8	9	10	11	12
Anti-oxidants	Irganox 245	Irganox 1098	Naugard XL-1	Irganox 1081	Irganox 1035
Structure					

Figure 1. Chemical structures of phthalate plasticizer and antioxidants used in this study.

Table I. Analysis Conditions for HTLC Used in This Study

Analytes	Conditions	Diamondbond C_{18}	Zirchrom PS	Zirchrom PBD
Plasticizer	Organic modifier	70%, 75%, and 80% Acetonitrile in water	40% Acetonitrile in water	40% Acetonitrile in water
	Isothermal	40°C, 70°C, 100°C	–	–
	Thermal gradient	40–150°C at 5°C/min, 10°C/min, and 15°C/min		
	Flow rate	2.0 mL/min	1.0 mL/min	1.0 mL/min
Antioxidants	Organic modifier	75% Acetonitrile in water	40% Acetonitrile in water	48%, 52%, and 55% Acetonitrile in water
	Isothermal	–	–	40°C, 70°C, 100°C
	Thermal gradient	40–150°C at 5°C/min, 10°C/min, and 15°C/min		
	Flow rate	1.0 mL/min	1.0 mL/min	1.0 mL/min

phthalate plasticizers and a Diamondbond C_{18} column was used, and the elution behaviors of the analytes were evaluated according to the organic solvent proportions and column temperature variations. The effect of a thermal gradient on the retention time and resolution of the analytes was examined with Diamondbond C_{18}, Zirchrom PS, and Zirchrom PBD columns, and the column temperatures were controlled from 40°C to 150°C by increasing the temperature at a rate of 5°C/min, 10°C/min, and 15°C/min. The flow rate was adjusted to 2.0 mL/min for the Diamondbond C_{18} column, and to 1.0 mL/min for the Zirchrom PS and Zirchrom PBD columns. The solvent gradient effect was also examined and compared along with the column temperature changes. The Diamondbond C_{18} column was used, and temperatures were adjusted to 40°C, 70°C, and 100°C, with the gradient elution of acetonitrile proportion varying from 70% to 100%. For the analysis of antioxidants, a Zirchrom PBD column was used, and the elution behaviors of the analytes were evaluated according to the organic solvent proportions and column temperature variations. The effect of the thermal gradient on the retention time and resolution of the analytes was examined with the same conditions used in the analysis for the plasticizers. The injection volume was 20 µL, in which the samples were dissolved in acetonitrile by the concentration of 0.2 mg/mL. The detection wavelength was set to 270 nm for phthalate plasticizers and 225 nm for antioxidants.

Results and Discussion

In order to analyze the baseline rise that is primarily caused by a thermal gradient, the column temperature was increased from 40°C to 150°C at the rate of 5°C/min and 15°C/min with 50% acetonitrile as the mobile phase. As shown in Figure 2, severe column bleedings were observed from all three kinds of columns, which was because of column material leaching at high column temperatures (> 100°C). Among the three types of columns used in this experiment, column bleeding appeared the worst when the Diamondbond C_{18} column was used. A high column temperature caused the mass transfer rate to increase, the column backpressure to reduce, and the analysis time to significantly reduce. In the conventional temperature HPLC application, the corresponding effect can be achieved with a stronger solvent proportion. Retention time changes were examined at a few sets of column temperatures, and the whole experiment was repeated and compared with different organic eluent proportion. Column temperature varied from 40°C, 70°C, and 100°C, and the acetonitrile proportion in the mobile phase was adjusted accordingly to 70%, 75%, and 80% for the analysis of a phthalate plasticizer with the Diamonbond C_{18} column. For the analysis of antioxidants, the Zirchrom PBD column was used, and the organic solvent proportion was adjusted to 48%, 52%, and 55%, respectively. As is shown in Table II, all of the analytes show the tendency to retain on the stationary phase as the acetonitrile proportion in the mobile phase decreases at the fixed column temperature. The equivalent tendency in the retention time was observed when the column temperature was lowered with a fixed eluent proportion. These results verify that the changes in the retention time of each analyte, along with the temperature changes, do not break out from the conventional RPLC mechanism. As indicated in Table II, a 1% increase of the acetonitrile proportion in the mobile phase was estimated to have a comparable effect as a 7–7.5°C column temperature increase on the retention time changes for the analysis of polymer additives. Sanagi also reported the similar comparison of the correlations between the solvent composition and column temperature, where a 1% increase in acetonitrile showed a similar effect as a 4°C increase in temperature for the analysis of triazole fungicides (4). Horvath showed that a 1% increase in acetonitrile had the same effect as a 5°C increase in column temperature for the separation of *n*-alkylbenzenes (12). Van't Hoff plots were also conducted based on the data from Table II and the corresponding data is presented in Table III.

$$\ln k' = \frac{-\Delta H^{o}}{RT} + \frac{\Delta S^{o}}{R} + \ln \phi \qquad \text{Eq. 1}$$

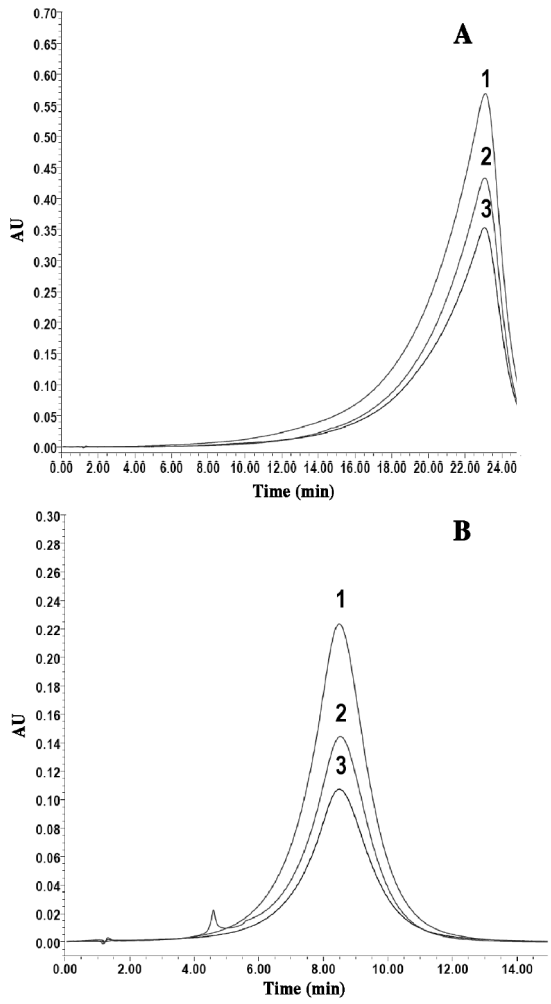


Figure 2. Baseline rise caused by a thermal gradient on each column. The chromatographic conditions were 50% acetonitrile in water at 225 nm, with a temperature range from 40–150°C. A 5°C/min temperature increase was used (A), and a 15°C/min temperature was used (B). The peak numbers pertain to the column used: Diamondbond C_{18}, 1; Zirchrom PS, 2; and Zirchrom PBD, 3.

Where DH^o is the enthalpy change related with the transfer of the solute between the stationary and the mobile phase, DS^o is the corresponding entropy change, k' is the retention factor of the analyte, R is the gas constant, T is the absolute temperature in Kelvin, and f is the volume ratio of stationary phase to mobile phase. The Van't Hoff plots show excellent linearity for all of the polymer additives. Negative enthalpy values for all the analytes, as shown in Table III, indicate that the exothermic process is more favorable. Hence, retaining to the stationary phase was, thermodynamically, more preferable. Their preference to the stationary phase rather than the mobile phase showed that the RPLC mechanism was maintained under the HTLC conditions.

Figure 3 demonstrates the effects of a thermal gradient for the analysis of phthalate plasticizers. Three types of high-temperature columns (Diamondbond C_{18}, Zirchrom PS, and Zirchrom PBD) were examined for the comparison. As shown in Figure 3, both the Zirchrom PS and Diamondbond C_{18} columns demonstrated good resolution and peak shapes for most of the phthalate plasticizers; however, they could not elute trioctyl trimelliatate (TOTM) with the 40°C isothermal condition. The Zirchrom PBD column was not so efficient for the analysis of plasticizers. Column temperature was elevated from 40°C to 150°C under an isocratic elution to enhance the separation by using a thermal gradient. With the Diamondbond C_{18} and Zirchrom PS columns,

Table II. Retention Data of Phthalate Plasticizers and Antioxidants as a Function of Temperature Using Different Proportion of Organic Solvent in Mobile Phase

(Diamondbond C_{18})								
		Retention time (min)						
Temperature (°C)	Acetonitrile (%) in water	DprP	DBP	BBP	DCHP	DOP	DINP	TOTM
40	70	1.068	1.500	1.917	2.775	10.229	24.126	–
	75	0.942	1.223	1.506	2.065	6.204	13.617	–
	80	0.857	1.044	1.242	1.619	3.951	8.000	35.705
70	70	0.910	1.159	1.366	1.888	5.135	10.130	–
	75	0.824	0.987	1.131	1.472	3.248	5.878	22.836
	80	0.768	0.874	0.975	1.203	2.172	3.564	11.388
100	70	0.797	0.937	1.047	1.351	2.737	4.558	15.391
	75	0.737	0.834	0.903	1.107	1.859	2.819	7.873
	80	0.708	0.768	0.805	0.952	1.361	1.855	4.248

(Zirchrom PBD)						
		Retention time (min)				
Temperature (°C)	Acetonitrile (%) in water	Irganox 245	Irganox 1098	Naugard XL-1	Irganox 1081	Irganox 1035
40	55	2.110	2.558	3.804	7.684	10.600
70	55	1.694	2.000	2.689	5.069	6.368
	52	2.005	2.558	3.669	7.299	10.126
100	55	1.505	1.733	2.099	3.755	4.244
	48	1.961	2.607	3.635	7.052	9.977

Table III. Enthalpy Data of Phthalate Plasticizers and Antioxidants in HTLC

(Diamondbond C_{18}, Temperature range: 40–100°C)						
	Enthalpy, ΔH^o (kJ/mol) / Correlation (r)					
Acetonitrile (%) in water	DprP	DBP	BBP	DCHP	DOP	DINP
70	–13.96 / 0.9939	–15.80 / 0.9956	–17.45 / 0.9976	–17.17 / 0.9965	–24.31 / 0.9975	–28.80 / 0.9982
75	–14.74 / 0.9913	–13.96 / 0.9939	–17.68 / 0.9958	–17.13 / 0.9960	–24.12 / 0.9977	–28.59 / 0.9985
80	–13.99 / 0.9962	–15.69 / 0.9971	–15.69 / 0.9971	–17.16 / 0.9967	–23.96 / 0.9985	–28.68 / 0.9990
(Zirchrom PBD, Temperature range: 40–100°C)						
	Enthalpy, ΔH^o (kJ/mol) / Correlation (r)					
Acetonitrile (%) in water	Irganox 245	Irganox 1098	Naugard XL-1	Irganox 1081	Irganox 1035	
55	–18.08 / 0.9978	–15.48 / 0.9985	–17.51 / 0.9927	–15.39 / 0.9973	–18.57 / 0.9937	

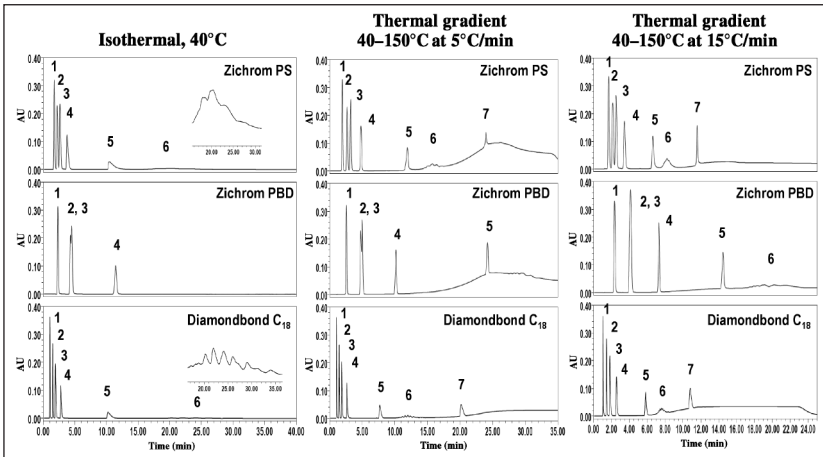


Figure 3. HTLC chromatograms of phthalate plasticizer according to the column temperature variation.

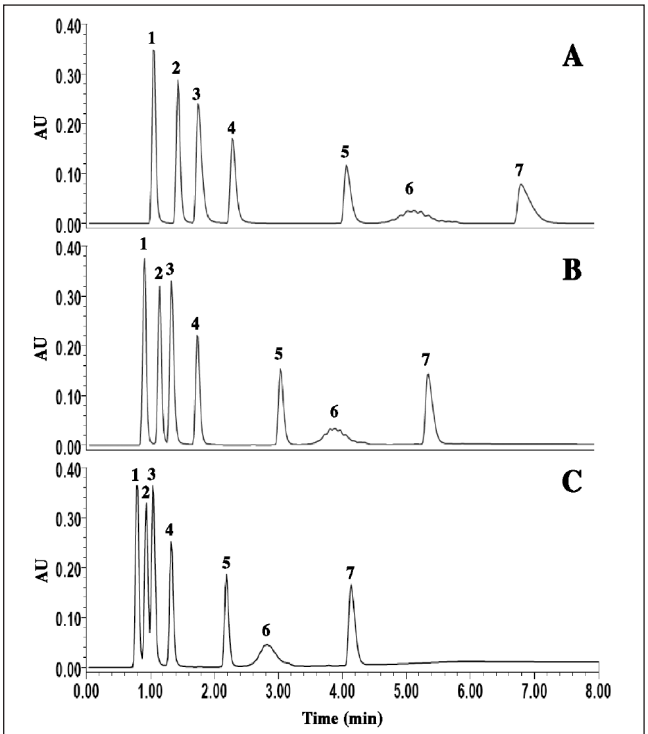


Figure 4. Effect of solvent gradient with column temperature variation 40°C (A), 70°C (B), and 100°C (C) for phthalate plasticizer (Diamondbond C_{18}, 70–100% acetonitrile for 5 min).

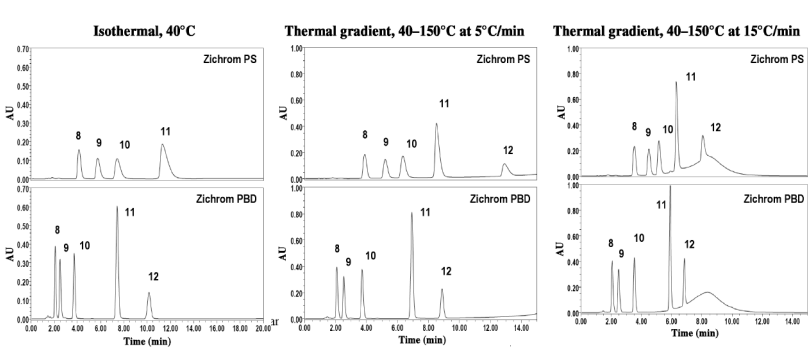


Figure 5. HTLC chromatograms of antioxidants according to column temperature variation.

TOTM was successfully eluted and the peak shape of di-isononyl phthalate (DINP) was improved. Thermal gradient conditions with the rate of 5°C/min and 15°C/min showed similar peak resolution, respectively, and the elution time was decreased further with the rate of 15°C/min as expected. Under these thermal conditions, the Zirchrom PBD column did not present any acceptable separation results.

Solvent gradient effects were added and compared to further enhance the separation efficiency, as seen in Figure 4. The Diamondbond C_{18} column temperature was adjusted isothermally to 40°C, 70°C, and 100°C, and the acetonitrile proportion in the mobile phase was varied from 70% to 100%. As shown in Figure 4, the combination of a solvent gradient and a high column temperature additionally enhances the separation efficiency and significantly reduces the elution times by increasing the column temperature without any deterioration in resolution.

Figure 5 demonstrates the effects of a thermal gradient for the analysis of antioxidants. Zirchrom PS and Zirchrom PBD columns were employed for the comparison. Good resolution and peak shapes were observed for most of the antioxidants, even with isocratic elution at 40°C. Irganox 1035 was, however, not eluted through the Zirchrom PS column. With the help of a thermal gradient from 40°C to 150°C, Irganox 1035 was eluted under a Zirchrom PS column and the peak shape of the analytes were also improved. The thermal gradient elution reduced the elution time and enhanced the separation efficiency, but generated a little baseline distortion with the high gradient rate.

Mobile phase pre-heating was expected to eliminate the thermal mismatch inside the column that could deteriorate peak shapes; however, as seen in Figure 6, there was no significant effect observed for the analysis of plasticizers and antioxidants. Figure 6 shows the effect of mobile phase pre-heating when the column temperature was 100°C under a Zirchrom PBD column. It seems that the column oven used for this study could minimize the possible thermal gradient inside the column, even without the preheating, so that the thermal mismatch was negligible.

This study demonstrated that the separation efficiencies and the elution behaviors for seven phthalate plasticizers and five antioxidants were enhanced at elevated column temperatures and with a thermal gradient. The RPLC mechanism was estimated to maintain over a wide range of column temperatures based on Van't Hoff plots. A 1% increase of the acetonitrile proportion in the mobile phase was estimated to have a comparable effect as a 7–7.5°C column temperature increase on the retention time for the analysis of polymer additives. A high-temperature technique is applicable even for the routine analysis, and the separation efficiency could be further enhanced without much trouble.

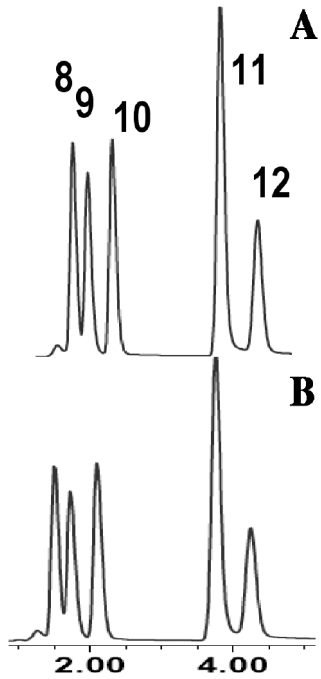


Figure 6. Effect of mobile phase pre-heating under high column temperature with the pre-heater off (A) and on (B). A Zirchrom PBD column, column temperature at 100°C, 55% acetonitrile in water, antioxidants.

References

1. S.J. Marin, B.A. Jones, W.D. Felix, and J. Clark. Effect of high-temperature on high-performance liquid chromatography column stability and performance under temperature-programmed conditions. *J. Chromatogr. A* **1030:** 255–62 (2004).
2. J.W. Dolan. Temperature selectivity in reversed-phase high performance liquid chromatography. *J. Chromatogr. A* **965:** 195–205 (2002).
3. T. Greibrokk and T. Andersen. High-temperature liquid chromatography. *J. Chromatogr. A* **1000:** 742–55 (2003).
4. M.M. Sanagi, H.H. See, W.A.W. Ibrahim, and A.A. Naim. High temperature liquid chromatography of triazole fungicides on polybutadiene-coated zirconia stationary phase. *J. Chromatogr. A* **1059:** 91–101 (2004).
5. S.M. Fields, C.Q. Ye, D.D. Zhang, B.R. Branch, X.J. Zhang, and N. Okafo. Superheated water as eluent in high-temperature high-performance liquid chromatographic separations of steroids on a polymer-coated zirconia column. *J. Chromatogr. A* **913:** 197–204 (2001).
6. A Jones and Y. Yang. Separation of nonpolar analytes using methanol-water mixtures at elevated temperatures. *Anal. Chim. Acta.* **485:** 51–55 (2003).
7. R. Trones, A. Tangen, W. Lund, and T. Greibrokk. Packed capillary high-temperature liquid chromatography coupled plasma mass spectrometry. *J. Chromatogr. A* **835:** 105–12 (1999).
8. R. Trones, T. Andersen, I. Hunnes, and T. Greibrokk. Modified laser light-scattering detector for use in high temperature micro liquid chromatography. *J. Chromatogr. A* **814:** 55–61 (1998).
9. G. Vanhoenacker and P. Sandra. High temperature liquid chromatography and liquid chromatography-mass spectroscopy analysis of octylphenol ethoxylates on different stationary phases. *J. Chromatogr. A* **1082:** 193–202 (2005).
10. D. Guillarme, S. Heinisch, J.Y. Gauvrit, P. Lanteri, and J.L. Rocca. Optimization of the coupling of high-temperature liquid chromatography and flame ionization detection application to the separations of alcohols. *J. Chromatogr. A* **1078:** 22–27 (2005).
11. D. Guillarme, S. Heinisch, and J.L. Rocca. Effect of temperature in reversed phase liquid chromatography. *J. Chromatogr. A* **1052:** 39–51 (2004).
12. M.H. Chen and C. Horvath. Temperature programming and gradient elution in reversed-phase chromatography with packed capillary columns. *J. Chromatogr. A* **788:** 51–61 (1997).

Manuscript received November 24, 2005;
revision received August 20, 2006.