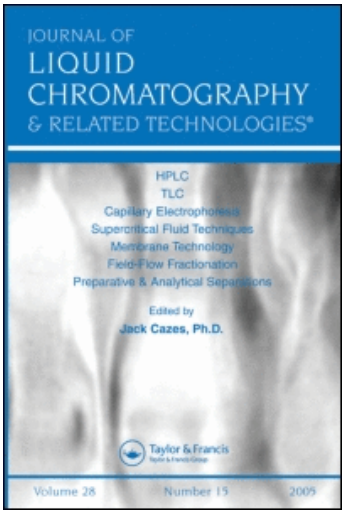

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SIMULTANEOUS CAPILLARY ELECTROPHORESIS DETERMINATION OF BARBITURATES FROM MECONIUM

David C. Delinsky[a]; Karthik Srinivasan[a]; Harvey M. Solomon[b]; Michael G. Bartlett[a]
[a] Dept. of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA, U.S.A. [b] Quintiles Laboratories, Ltd., Smyrna, GA, U.S.A.

Online publication date: 01 November 2002

To cite this Article Delinsky, David C. , Srinivasan, Karthik , Solomon, Harvey M. and Bartlett, Michael G.(2002) 'SIMULTANEOUS CAPILLARY ELECTROPHORESIS DETERMINATION OF BARBITURATES FROM MECONIUM', Journal of Liquid Chromatography & Related Technologies, 25: 1, 113 — 123
To link to this Article: DOI: 10.1081/JLC-100108543
URL: http://dx.doi.org/10.1081/JLC-100108543

J. LIQ. CHROM. & REL. TECHNOL., 25(1), 113–123 (2002)

SIMULTANEOUS CAPILLARY ELECTROPHORESIS DETERMINATION OF BARBITURATES FROM MECONIUM

David C. Delinsky,[1] Karthik Srinivasan,[1] Harvey M. Solomon,[2] and Michael G. Bartlett[1,*]

[1]Dept. of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA 30602-2352, USA
[2]Quintiles Laboratories, Ltd., 5500 Highlands Parkway, Suite 600, Smyrna, GA 30082, USA

ABSTRACT

Meconium is the first stool passed by a new born and, as such, represents a record of the fetal environment during the last two trimesters of pregnancy. We have developed the first capillary electrophoresis (CE) method for the analysis of meconium. This method has the potential to help tremendously in the study of fetal drug exposure. Solid-phase extraction (SPE) was used to extract the drugs (pento-, mepho-, pheno-, seco-, and amobarbital) and the internal standard, hexobarbital, from meconium. The extraction efficiency was studied using C_{18}, C_8, Silica (Si), and polymeric cartridges for samples buffered at pHs 2.5, 7.0, and 9.0. The polymeric (Oasis HLB) SPE cartridge at pH 9.0 was

*Corresponding author. E-mail: Bartlett@mail.rx.uga.edu

selected because it gave clean extractions and high recoveries for most of the studied barbiturates.

The CE system consisted of a 75 µm I.D. 77 cm length fused silica capillary and a UV detector set at 254 nm. The run buffer consisted of 150 mM tris buffer at pH 7.8 and the run voltage was 25 kV (at 25°C). Linear calibration curves show a coefficient of determination of more than 0.99 for all components. The method also showed high between run and within run precision and accuracy. The limit of quantification was 10 µg per gram of meconium. Some common drugs such as aspirin, acetaminophen, and caffeine may be taken in conjunction with barbiturates. The method completely resolved these compounds, along with several other potential interferences, from all the barbiturates in this analysis.

INTRODUCTION

Meconium is the first stool passed by the newborn baby and is, in essence, a record of the drug history of the mother during the later stages of pregnancy. This biological matrix has not been widely studied but can be a sample of choice when drug abuse during pregnancy is suspected. Most of the work that has been done with meconium involves analyzing for cocaine and its metabolites from the meconium of infants born to mothers that abuse cocaine. (1) Other illicit drugs, xenobiotics, and a few heavy metals, have also been studied from meconium. (2–4) The presence of barbiturates in the meconium of neonates born to mothers who have been administered barbiturates has been documented. (5) Thus, development of rugged methods that can quantitate these drugs rapidly and reliably from meconium has assumed importance.

Meconium is a complex biological matrix that contains large amounts of proteins, lipids, and pigments. Due to the high level of these endogenous compounds in meconium, recovery of drugs requires significant sample preparation prior to solid-phase extraction. To date, high performance liquid chromatography (HPLC) and gas chromatography (GC) have been the most widely used techniques for determination of drugs out of biological matrices. (1–4) Currently, there are no methods for the analysis of meconium that use capillary electrophoresis (CE). The advantages of CE, such as low sample volumes, high efficiency, and low cost provide powerful alternatives to existing chromatographic methodologies in the area of therapeutic and drug abuse monitoring. (6–11)

Barbiturates are sedative hypnotics that were introduced in 1903. Despite the fact that these compounds have largely been replaced by benzodiazepines as

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

sedative-hypnotics of choice, (12) several barbiturates maintain widespread use today. Phenobarbital is used as a treatment for epilepsy and is considered a very effective drug for this use. (13) Barbiturates are also used in some prescription sedative products. (14) Pentobarbital has been administered for sedation and to relieve stress prior to surgery. (15)

Barbiturates show some adverse reactions at doses from 0.15 to 1.5 mg/kg and are reported to cause bradycardia, hypotension, and syncope. Detrimental effects in all stages of development in the children of addicts have been observed during pregnancy, birth, during breast-feeding, and throughout maturation. Therefore, controlled regulation, identification, treatment, and rehabilitation of barbiturate exposure may be warranted. These procedures would require rapid, sensitive, and accurate determination of these drugs.

HPLC (16–21) and GC (18,22–24) methods have been devised for the analysis of barbiturates from serum, plasma, and urine. These methods have found immense clinical significance and have contributed considerably to understanding the pharmacokinetics and pharmacodynamics of barbiturates. The analysis of barbiturates from meconium opens an additional window to study maternal to fetal transfer of drugs during the last two trimesters of pregnancy, and would further our understanding of the processes of maternal to fetal drug transport. This paper is also a demonstration of the emerging idea that capillary electrophoresis is a technique which has the capacity to augment, if not replace, other existing chromatographic techniques.

EXPERIMENTAL

Reagents and Chemicals

Pentobarbital, mephobarbital, phenobarbital, amobarbital, secobarbital, and the internal standard, hexobarbital were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphoric acid (85%), sodium dihydrogen phosphate monohydrate, and ammonia solutions were obtained from J.T. Baker (Phillipsburg, NJ, USA). C_{18} and C_8 solid-phase extraction columns (100 mg) were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). Silica solid-phase extraction cartridges (100 mg) were obtained from Alltech Associates Inc. (Deerfield, IL, USA). Polymer based Oasis™ extraction cartridges were obtained from Waters Corp. (Milford, MA, USA). All SPE cartridges were 1 mL capacity. All solutions were filtered through 0.2 μm nylon membrane filters (Fisherbrand, Fisher Scientific, Pittsburgh, PA, USA).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Preparation of Stock and Standard Solutions

Individual stock solutions were prepared in methanol to give a concentration of 1 mg/mL of the analyte. The nominal concentrations of barbiturates studied were 10, 20, 40, 60, 80, 100, 120, 140 µg/g. Appropriate volumes of pentobarbital, mephobarbital, phenobarbital, amobarbital, secobarbital, and the internal standard, hexobarbital were pipetted and volume made up to 1 mL to give the above concentrations. Approximately 0.5 g of meconium was weighed and added to the analyte samples. 2 mL of 25% methanol/acetonitrile was then added to the above and the whole mixture was homogenized. The methanol and acetonitrile added, helps in breaking up the meconium, facilitating sample handling. This mixture was then centrifuged (3000 rpm for 30 min) and the supernatant was removed and evaporated under vacuum. The residue was then reconstituted in 30% (v/v) methanol/buffer and filtered prior to solid-phase extraction. For the extraction studies, sodium dihydrogen phosphate buffer was prepared in double distilled, deionized water and the pH was adjusted to 2.5, 7.0, and 9.0, using 100 mM sodium hydroxide and concentrated phosphoric acid.

Electrophoretic System

All CE experiments were performed using a P/ACE System 5000 (Beckman Inc., Fullerton, CA, USA) equipped with a UV detector. An uncoated fused silica capillary total length 82 cm, effective length 77 cm, 75 µm I.D (Polymicron Technologies, Phoenix, AZ, USA) was used for analysis. The capillary was thermostated at 25°C and the voltage applied was 25 kV. The typical running current was about 100 µA. A 0.5 cm detection window was created by stripping the polyamide coating of the capillary. The detection was 5 cm from the cathode end of the capillary. The run buffer consisted of an aqueous solution of 150 mM tris buffer pH 7.8 (adjusted with concentrated nitric acid). The analytes were monitored at a wavelength of 254 nm.

New capillaries were conditioned by rinsing with 1 M sodium hydroxide for 5 min, followed by 5 min each with 1 M hydrochloric acid, water, and run buffer solutions. The sample introduction was performed using a 5 sec pressure injection (0.5 PSI). Before each analysis, the capillary was rinsed for 2 min, first with 0.1 M sodium hydroxide and 2 min with the run buffer.

Assay Procedure

Sample clean up was attempted using C_{18}, C_8, silica and polymeric solid-phase extraction cartridges with extracted samples buffered at three pHs (2.5, 7.0,

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

and 9.0). Prior to SPE, the cartridge was conditioned using 2 mL of methanol and then with 2 mL of the appropriate phosphate buffer (either pH 2.5, 7.0, or 9.0 matched with the pH of the buffer used to reconstitute the sample). The reconstituted sample containing the drug and internal standard in 1 mL of 30% methanol/buffer (pHs 2.5, 7.0 or 9.0) was added to the cartridges and allowed to flow down under low vacuum. The SPE cartridges were not allowed to dry between the pretreatment and sample application steps. The column was then washed with 2 mL of buffer (corresponding to the respective sample pHs) and allowed to dry for 15 min. The analytes were then eluted with 3 mL of methylene chloride. The samples were then evaporated and reconstituted in 1 mL of 30% methanol/water, filtered using a 0.2 μm nylon filter, and pressure injected into the CE instrument for 5 seconds. Absolute recoveries were calculated by comparing the drug peak height from spiked meconium samples to unextracted stock solutions that had been injected directly into the electrophoretic system.

RESULTS AND DISCUSSION

The analytes eluted after the EOF (analogous to the solvent front in HPLC) in tris buffer at a pH of 7.8, with migration times from 12–19 min. Figure 1A shows an electropherogram of blank meconium. Figure 1B shows the electropherogram of the barbiturates and internal standard spiked into meconium. Figure 2 shows the structures of (A) hexobarbital, (B) phenobarbital, (C) pentobarbital, (D) amobarbital, (E) mephobarbital, and (F) secobarbital.

To increase sensitivity by reducing band broadening, thereby achieving sharper peaks, the sample was prepared in a lower conductivity solvent (methanol/water) than the electrolyte solution. When a voltage of 25 kV is applied across the capillary, a greater field develops across the sample plug. This causes the ions to move faster. When the ions reach the buffer they slow down due to the reduced field to which they are subjected, this results in analyte stacking within a narrow zone of the capillary. (25,26)

SPE was attempted on four different cartridges (polymeric, C_{18}, C_8, and Silica), with samples at three different pHs 2.5, 7.0, and 9.0. The recovery of the barbiturates studied is reported in Table 1. The polymeric (Oasis HLB) SPE cartridge at pH 9.0 was selected because it gave clean extracts and good recovery for most components.

Common drugs, such as aspirin, caffeine, and acetaminophen must not interfere with the separation of the barbiturates as they may also be taken by the mother prior to giving birth. While caffeine and acetaminophen elute in the EOF (electrosmotic flow, difference in migration time [t_Δ] of 2.8 min before the first barbiturate), aspirin has a t_Δ of 5.1 min after the last migrating barbiturate (phenobarbital). Anticonvulsants, such as phenytoin may also be present;

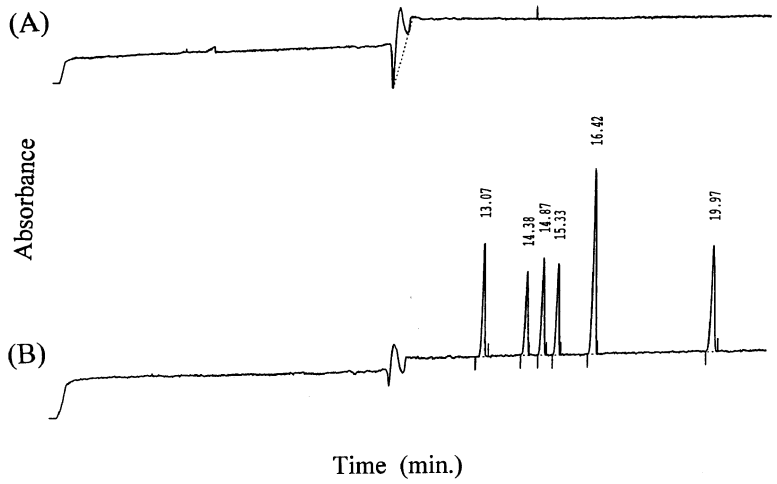

Figure 1. Typical electropherogram of (A) blank meconium and (b) meconium spiked with pentobarbital (14.38 min), secobarbital (14.87 min), amobarbital (15.33 min), mephobarbital (16.42 min), phenobarbital (19.97 min), and internal standard hexobarbital (13.07 min) on a 77 cm, 75 μm fused silica capillary. The run buffer contained 150 mM tris buffer (pH 7.8) with detection at 254 nm. The capillary was thermostated at 25°C and run voltage was 25 kV.

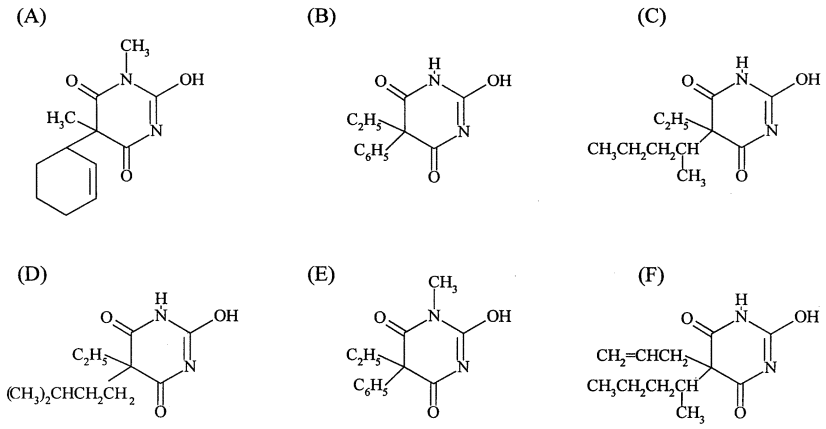

Figure 2. Structures of (A) hexobarbital, (B) phenobarbital, (C) pentobarbital, (D) amobarbital, (E) mephobarbital, and (F) secobarbital.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Extraction Efficiency (%)

SPE	Sample pH	Hex	Pent	Sec	Amo	Meph	Pheno
Polymer	9.0	72.6	99.3	103.6	99.2	82.2	22.9
Polymer	7.0	78.2	76.9	75.5	73.6	73.7	28.2
Polymer	2.5	88.3	82.1	74.5	68.8	70.7	21.6
C8	9.0	67.0	89.9	104.1	90.0	64.2	246.6
C8	7.0	91.1	100.7	100.9	100.8	96.2	33.9
C8	2.5	85.5	97.8	100.9	99.2	89.3	36.2
C18	9.0	73.2	100.1	100.2	109.6	73.4	19.6
C18	7.0	85.5	94.0	99.1	96.8	92.9	35.1
C18	2.5	92.2	97.8	99.1	100.0	98.5	32.6
Silica	9.0	16.2	22.4	25.5	19.2	16.6	52.8
Silica	7.0	0.0	0.0	0.0	0.0	0.0	0.0
Silica	2.5	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Within-Run Data, n = 5 (Run 3)

	Conc. Found				
Spike Conc. (μg/g)	Pent	Sec	Amo	Meph	Pheno
19.93	21.69	20.21	19.81	18.61	19.22
20.13	19.09	20.50	19.01	17.85	23.05
20.00	18.96	19.42	18.91	19.34	20.95
19.94	20.89	20.85	19.36	18.28	20.83
20.20	20.50	20.95	19.01	18.94	19.15
120.70	128.21	125.94	126.16	122.20	111.38
120.31	125.53	122.48	124.40	123.30	110.80
120.89	125.40	123.98	125.97	123.56	109.13
120.14	122.45	119.26	118.39	119.83	134.81
119.26	127.99	127.46	129.04	127.97	121.27
			% Error		
Avg.					
20.04	5.11	2.91	4.06	7.14	6.51
120.26	4.70	3.26	4.36	2.70	7.85
			% RSD		
Avg.					
20.04	4.75	2.24	1.52	2.32	5.64
120.26	1.39	1.91	2.18	1.55	7.19

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. Between-Run Data, n = 15

		Conc. Found				
	Avg. Spike Conc. (μg/g)	Pent	Sec	Amo	Meph	Pheno
Run 1	19.93	18.59	19.83	21.05	20.75	19.22
Run 2	19.86	17.85	20.01	19.20	18.50	19.79
Run 3	20.04	20.23	20.39	19.22	18.60	20.64
Run 1	119.96	124.53	123.31	122.61	119.85	126.41
Run 2	120.14	120.65	119.12	121.33	122.74	115.96
Run 3	120.26	125.91	123.82	124.79	123.37	117.48
		% Error				
Nominal Conc.						
20	7.80	3.84	5.21	6.06	6.99	
120	3.27	3.20	2.66	1.75	7.05	
		% RSD				
Nominal Conc.						
20	5.13	3.58	3.27	3.01	6.19	
120	1.34	2.28	1.54	1.18	6.12	

phenytoin migrates past the detector before the internal standard hexobarbital (first in the migration order), maintaining baseline resolution with a t_Δ of 0.43 min. The difference in migration times allows baseline resolution of the drugs. Atropine, an anticholinergic, also elutes in the EOF.

The calibration curve showed good linearity over the range from 10 to 140 μg/g for all the barbiturates. The coefficient of determination was greater than 0.99 (n = 3). Representative linear regression equations obtained were $y = 0.01604x + 0.01755$ (secobarbital), where y and x were drug to internal standard peak area ratios and concentration, respectively. The within-run (n = 5) and between-run (n = 15) precision and accuracy as expressed by % error and % RSD, are shown in Table 2 and Table 3, respectively. The limit of quantitation for this method is 10 μg/g, and the limit of detection is 5 μg/g.

CONCLUSIONS

The HPCE assay described herein is sensitive and suitable for simultaneous determination of barbiturates from meconium. The solid phase extraction method provides excellent sample clean up with no endogenous interferences and good recovery. This method also shows excellent within-run and between-run linearity, precision, and accuracy in the range of 10–140 μg/g. The method is sensitive and sturdy, and would be a good alternative to existing HPLC or GC methods.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

REFERENCES

1. Xia, Y.; Wang, P.P.; Bartlett, M.G.; Solomon, H.M.; Busch, K.L. An LC-MS-MS Method for the Comprehensive Analysis of Cocaine and Cocaine Metabolites in Meconium. Anal. Chem. **2000**, *72* (4), 764–771.
2. ElSohly, M.A.; Feng, S.X.; Murphy, T.P. Analysis of Methadone and its Metabolites in Meconium by Enzyme Immunoassay (EMIT (R)) and GC-MS. J. Anal. Toxicol. **2001**, *25* (1), 40–44.
3. Ostrea, E.M.; Matias, O.; Keane, C.; Mac, E.; Utarnachitt, R.; Ostrea, A.; Mazhar, M. Spectrum of Gestational Exposure to Illicit Drugs and Other Xenobiotic Agents in Newborn Infants by Meconium Analysis. J. Pediatr. **1998**, *133* (4), 513–515.
4. Ramirez, G.B.; Cruz, M.C.V.; Pagulayan, O.; Ostrea, E.; Dalisay, C. The Tagum Study I: Analysis and Clinical Correlates of Mercury in Maternal and Cord Blood, Breast Milk, Meconium, and Infants' Hair. Pediatrics **2000**, *106* (4), 774–781.
5. Issacson, E.I. Central Nervous System Depressants. In *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 9th Edition; Delgado, J.N., Remers W.A., Eds.; J. B. Lippincott, Co.: Philadelphia, PA, 1991; 368–372.
6. Hobbs, W.R.; Rall, T.W.; Verdoorn, T.A. Hypnotics and Sedatives; Ethanol. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed.; Hardman, J.G, Gilman, A.G., Limbird, L.E., Eds.; McGraw-Hill: New York, 1996; 373–380.
7. Physicians Desk Reference, 51st Edition; Medical Economics Co., Inc.: Montvale, NJ, 1997; 333.
8. Dahlem, P.; Bucher, H.U.; Cuendet, D.; Mieth, D.; Gautschi K. Prevalence of Drugs in Meconium. Monatsschrift Kinderheilkunde **1993**, *141* (3), 237–240.
9. Snopek, J.; Jelinek, I.; Smolkova-Keulemansova, E. Use of Cyclodextrins in Isotachophoresis. 4. The Influence of Cyclodextrins on the Chiral Resolution of Ephedrine Alkaloid Enantiomers. J. Chromatogr. **1988**, *438* (2), 211–218.
10. Guttman, A.; Paulus, A.; Cohen, A.S.; Grinberg, N.; Karger, B.L. Use of Complexing Agents for Selective Separation in High-Performance Capillary Electrophoresis-Chiral Resolution via Cyclodextrins Incorporated within Polyacrylamide-Gel Columns. J. Chromatogr. **1988**, *448* (1), 41–53.
11. Terabe, S. Electrokinetic Chromatography – an Interface Between Electrophoresis and Chromatography. Trac-Trend Anal. Chem. **1989**, *8* (4), 129–134.
12. Ewing, A.G.; Wallingford, R.A.; Olefirowicz, T.M. Capillary Electrophoresis. Anal. Chem. **1989**, *61* (4), 292A.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

13. Terabe, S.; Shibata, M.; Miyashita, Y. Chiral Separation by Electrokinetic Chromatography with Bile-Salt Micelles. J. Chromatogr. **1989**, *480*, 403–411.
14. Fanali, S.; Bocek, P. Enantiomer Resolution by Using Capillary Zone Electrophoresis – Resolution of Racemic Tryptophan and Determination of the Enantiomer Composition of Commercial Pharmaceutical Epinephrine. Electrophoresis **1990**, *11* (9), 757–760.
15. Desiderio, C.; Aturki, Z.; Fanali, S. Separation of Alpha-Hydroxy Acid Enantiomer by High-Performance Capillary Electrophoresis Using Copper(II)-L-Amino Acid and Copper(II)-Aspartame Complexes as Chiral Selectors in the Background Electrolyte. Electrophoresis **1994**, *15* (6), 864–869.
16. Ryan, T.W. Identification of Barbiturates Using High-Performance Liquid-Chromatography Particle-Beam EI/CI Mass-Spectroscopy. J. Liq. Chromatogr. **1994**, *17* (4), 867–881.
17. Lurie, I.S.; Cooper, D.A.; Krull, I.S. High-Performance Liquid-Chromatography Using Continuous Online Post-Elution Photoirradiation with Subsequent Diode-Array UV or Thermospray Mass-Spectrometry Detection. J. Chromatogr. **1993**, *629* (2), 143–151.
18. Gaillard, Y.; Pepin, G. Screening and Identification of Drugs in Human Hair by High Performance Liquid Chromatography Photodiode-Array UV Detection and Gas Chromatography Mass Spectrometry After Solid-Phase Extraction – A Powerful Tool in Forensic Medicine. J. Chromatogr. A **1997**, *762* (1–2), 251–267.
19. Aloba, O.T.; Adusumilli, P.S.; Nigalaye, A.G. High-Performance Liquid-Chromatographic Analysis of a Multicomponent Product Using a Silica Stationary Phase and an Aqueous Organic Mobile Phase. J. Pharm. Biomed. Anal. **1991**, *9* (4), 335–340.
20. Lopez-Rivadulla, M.; Fernandez, P.; Rodriguez, P.; Bermejo, A.M.; Concheiro, L. Simultaneous Identification and Determination of Several Barbiturates in Plasma by Reverse-Phase HPLC. Anal. Lett. **1988**, *21* (12), 2253–2262.
21. Drost, M.L.; Walter, L. Blood and Plasma-Concentrations of Butalbital Following Single Oral Doses in Man. J. Anal. Toxicol. **1988**, *12* (6), 322–324.
22. Laakkonen, U.M.; Leinonen, A.; Savonen, L. Screening of Nonsteroidal Antiinflammatory Drugs, Barbiturates and Mehtyl Xanthines in Equine Urine by Gas-Chromatography Mass-Spectrometry. Analyst **1994**, *119* (12), 2695–2696.
23. Liu, R.H.; McKeehan, A.M.; Edwards, C.; Foster, G.; Bensley, W.D.; Langner, J.G.; Walia, A.S. Improved Gas-Chromatography Mass-Spectrometry Analysis of Barbiturates in Urine Using Centrifuge-Based Solid-

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Phase Extraction, Methylation, with D(5)-Pentobarbital as Internal Standard. J. Forensic Sci. **1994**, *39* (6), 1504–1514.
24. Hall, B.J.; Brodbelt, J.S. Determination of Barbiturates by Solid-Phase Microextraction (SPME) and Ion Trap Gas Chromatography Mass Spectrometry. J. Chromatogr. A. **1997**, *777* (2), 275–282.
25. Burgi, D.S.; Chien, R.L. Optimization in Sample Stacking for High-Performance Capillary Electrophoresis. Anal. Chem. **1991**, *63* (18), 2042–2047.
26. Chien, R.L.; Burgi, D.S. Field Amplified Sample Injection in High-Performance Capillary Electrophoresis. J. Chromatogr. **1991**, *559* (1–2), 141–152.

Received June 2, 2001
Accepted July 5, 2001
Manuscript 5605