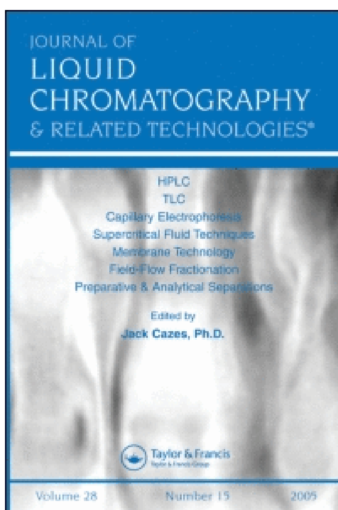
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

The Use of a Concentration Step to Collect Urinary Components Separated by Capillary Electrophoresis and Further Characterization of Collected Analytes by Mass Spectrometry

Norberto A. Guzman[ab]; Maria A. Trebilcock[a]; Juan P. Advis[c]
[a] Protein Research Unit Princeton Biochemicals, Inc. Princeton, New Jersey [b] Pharmaccutical Research and Development, Hoffmann-La Roche, Nutley, NJ [c] Department of Animal, Sciences Rutgers University New Brunswick, New Jersey

To cite this Article Guzman, Norberto A. , Trebilcock, Maria A. and Advis, Juan P.(1991) 'The Use of a Concentration Step to Collect Urinary Components Separated by Capillary Electrophoresis and Further Characterization of Collected Analytes by Mass Spectrometry', Journal of Liquid Chromatography & Related Technologies, 14: 5, 997 — 1015
To link to this Article: DOI: 10.1080/01483919108049300
URL: http://dx.doi.org/10.1080/01483919108049300

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(5), 997-1015 (1991)

THE USE OF A CONCENTRATION STEP TO COLLECT URINARY COMPONENTS SEPARATED BY CAPILLARY ELECTROPHORESIS AND FURTHER CHARACTERIZATION OF COLLECTED ANALYTES BY MASS SPECTROMETRY

NORBERTO A. GUZMAN[1*], MARIA A. TREBILCOCK[1], AND JUAN P. ADVIS[2]
[1]*Protein Research Unit*
Princeton Biochemicals, Inc.
Princeton, New Jersey 08543
[2]*Department of Animal Sciences*
Rutgers University
New Brunswick, New Jersey 08903

Downloaded At: 10:13 25 January 2011

ABSTRACT

Two methods were developed using capillary electrophoresis to concentrate urinary constituents in order to collect adequate amounts of separated analytes. The first method utilizes multiple capillaries arranged in bundles and coupled to a single capillary through a glass connector. The second method consists of the use of an analyte concentrator containing an antibody covalently bound to a solid support material. Both methods allow loading of increased amounts of samples into the capillary, and the continuous collection of purified material in nanogram-microgram quantities. The samples collected by the two methods were further analyzed by mass spectrometry.

*Current address: Pharmaceutical Research and Development, Hoffmann-La Roche, 340 Kingsland Street, Nutley, NJ 07110, (201)-235-4816.

Copyright © 1991 by Marcel Dekker, Inc.

The results are discussed in terms of practical value when using an analyte concentrator coupled to capillary electrophoresis for the analysis of minute quantities of substances, and the semi-preparative collection and further identification of sample components by mass spectrometry or any other relevant technique.

INTRODUCTION

Capillary electrophoresis (CE) is becoming a method with great potential for the analytical determination of a large variety of substances (1-5). Although the vast majority of data available in the literature have been focused in the analysis of highly purified standards (ranging from small molecules to large macromolecules), only a few of them deal with the analysis of substances in a mixture of complex matrices. Slowly, CE is embracing other areas of applied research, such as applications in the field of DNA sequencing (6-9), separation of cells (10) and particles (11,12), clinical chemistry (13-16), determination of active ingredients in pharmaceutical formulations (17), and the monitoring of purity for recombinant-DNA generated proteins (18,19). Nevertheless, the identification of substances present in complex mixtures of biological origin is not a simple task. For example, the complexity of quality control and characterization of proteins, as compared to small molecules, usually requires more than two analytical tests. Standard methods of chemical analysis, such as mass spectrometry, nuclear magnetic resonance, infrared spectroscopy, or elemental analysis, require large sample sizes (i.e., microgram or milligram amounts) that are far above separations commonly carried out by analytical capillary electrophoresis (i.e., nanogram amounts).

One of the major drawbacks of CE is handling very small volumes of substances, producing low possibilities of generating enough material for further analysis of separated sample components. At the present time, four methods are used for the identification of substances separated by CE. The first method consists of spiking known standards with the mixture to be analyzed, and to compare migration times. This method is insufficient by itself as a means of identification and can lead to the erroneous identification of substances. The second method utilizes the measurement of absorbance ratios as an aid in peak identification. This method, however, requires prior knowledge of the absorbance properties of the components in the mixture which are being separated. The third method measures spectral analysis as a sensitive means of identifying separated compounds. This method requires a more sophisticated detector with a

Downloaded At: 10:13 25 January 2011

fast response time (i.e., photodiode array detector) which can give on-the-fly spectral analysis or stopped-flow scanning analysis. The fourth method utilizes an *in tandem* capillary electrophoresis-mass spectrometry (CE-MS) technique which yields more accurate identification of analytes. Although CE-MS has been developed in a few laboratories as a research tool, this method is not yet commercially available, and there are some limitations in sensitivity. Therefore, an alternative for those laboratories which do not own a mass spectrometer to be connected *in tandem* to a CE instrument, is to collect adequate amounts of an analyte separated by CE and obtain off-line mass spectrometry data. In general, two methods have been developed to collect samples by CE: 1) an interrupted (or discontinuous) fraction collection system (20,21), and 2) a continuous fraction collection system (1,22-26). Both methods can generate at least nanogram quantities of collected analytes. Several other methods have been developed to concentrate diluted samples (to be able to reach adequate detectable concentration of an analyte) when using capillary electrophoresis (27,28).

In this paper we report the development of a method for the identification of separated and collected urinary constituents. The method consists of using an analyte concentrating system, a continuous fraction collection device, and an off-line mass spectrometer for further analysis of adequately collected material.

Downloaded At: 10:13 25 January 2011

EXPERIMENTAL

Reagents and samples

High purity sodium tetraborate, potassium hydroxide, uric acid (free acid), (+)methamphetamine hydrochloride, Nonidet P-40, and hydrochloric acid were purchased from Sigma Chemical Company (St. Louis, MO). 3-Aminopropyltriethoxysilane was obtained from Pierce Chemical (Rockford, IL). 1,4-phenylene diisothiocyanate (DITC) was purchased from Eastman Kodak (Rochester, NY). Millex disposable filter units (0.22 μm) were obtained from Millipore Corporation (Bedford, MA). Underivatized controlled-pore glass (CPG) beads (3000 Å pore size, 200-400 mesh, irregular shaped) were purchased from CPG Inc. (Fairfield, NJ). Fused-silica capillary columns were obtained from Polymicro Technologies (Phoenix, AZ) and Scientific Glass Engineering (Austin, TX). HPLC-grade methanol was purchased from EM-Science (Gibbstown, NJ). Other materials were obtained in the highest purity from commercial sources.

Triply distilled and deionized water was used for the preparation of buffer solutions. Both buffer and samples were routinely degassed by sonication and vacuum.

Urine specimens, freshly collected as clean-catch (of fasting morning urine) were obtained from six normal people. All urine donors were adult males of approximately 70-90 Kg body weight.

Preparation of antibodies

Monoclonal antibodies directed against methamphetamine were prepared by the method of Galfre and Milstein (29) with minor modifications described elsewhere (30). More than twenty monoclonal antibodies were produced of which the four with the highest immunological titers were combined into a single pool of sample. The purification of the pooled antibodies from mouse ascites fluid was carried out by HPLC using Protein A affinity chromatography as previously described (30).

Preparation of multiple capillaries

Five capillaries were merged into a single capillary column using a glass connector. For this coupling a sodium-silicate tube was used as a link, having a wider diameter for the multiple capillaries (fused-silica capillaries, 75 μm) at the inlet side, and a thinner diameter for the single capillary at the outlet side. The capillaries, aligned in a uniform and rigid manner by previously tapping them on a flat surface, were glued into the link tube. This connector tube was slowly heated until fusion of the linking terminals (using a controlled incremental increase of temperature), allowing all capillaries to be enclosed into a single compartment. The final product was monitored for imperfections under a stereo microscope (Nikon, Micron Optics, Parsippany, NJ), and then tested for performance aspirating into the capillary column deionized water using a controlled-vacuum pump.

For the fabrication of the grounding micro-device (Figure 1), a tee assembly was constructed using a modification of the fabrication method of Fujimoto *et al.* (25) as described elsewhere (26). The entire concept of the continuous fraction collection system used here is depicted in Figure 2.

Preparation of aminopropyl-glass

Controlled-pore glass beads were silylated by a modification of the procedures described elsewhere (31,32). CPG beads (8 g, 3000 Å, 200-400 mesh) were boiled for 10 min in 10 mM HCl and washed with 400 ml of deionized water, followed by 400 ml of methanol, and dried under vacuum for 2 h over P_2O_5. The beads were then heated for 2 h at 200°C and allowed to cool under vacuum over P_2O_5. A solution of 0.6 ml of 3-aminopropyltriethoxysilane in 60 ml of dry toluene was added, and then the beads were degassed under vacuum

Downloaded At: 10:13 25 January 2011

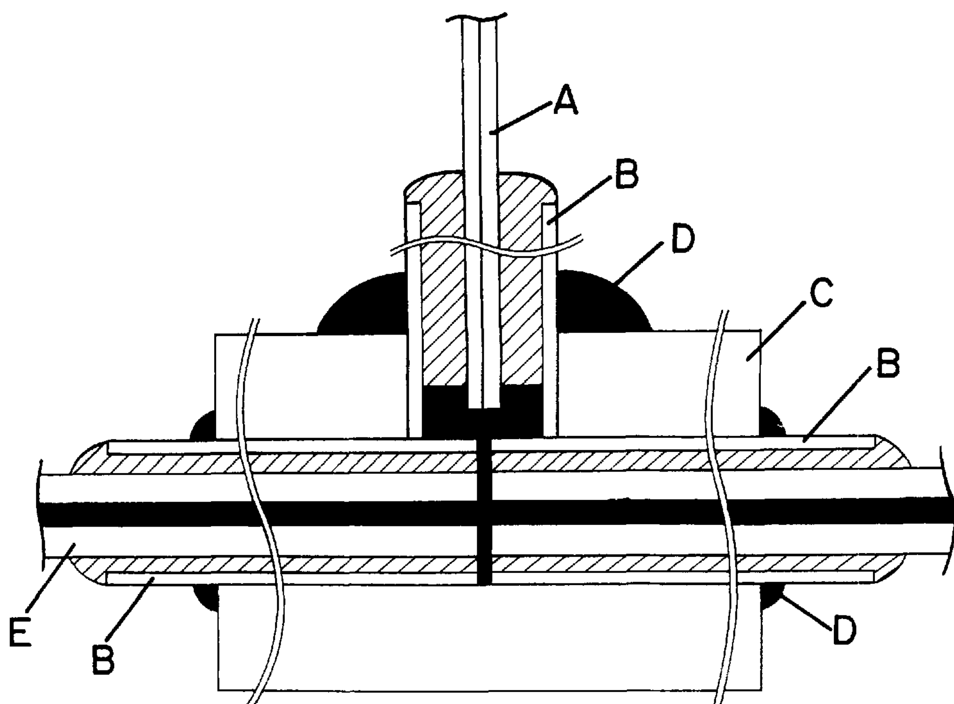


Figure 1. A Schematic Diagram of the Tee Assembly Unit Used as a Grounding Device. **A.** 10 µm i.d., 150 µm o.d. fused-silica capillary; **B.** 530 µm i.d., 660 o.d. fused-silica capillaries; **C.** Teflon tubing; **D.** Epoxy resin; **E.** 75 µm i.d., 375 µm o.d. fused-silica capillary.

Downloaded At: 10:13 25 January 2011

and heated at 56°C for 24 h with occasional stirring. The beads were filtered on a sintered glass filter, washed with methanol, and dried under vacuum at room temperature over P_2O_5. The beads were stored at 4°C under nitrogen.

Preparation of DITC-glass

The preparation of DITC-glass was carried out by a modification of the procedures described elsewhere (31,32). Aminopropyl-glass beads (4 g) were suspended in 12 ml of dry tetrahydrofuran (freshly distilled from sodium and benzophenone) containing 400 mg of DITC. The mixture was kept at room temperature for 2 hr under nitrogen, and was then filtered and washed with 200 ml of benzene. The beads were finally washed with 300 ml of anhydrous methanol

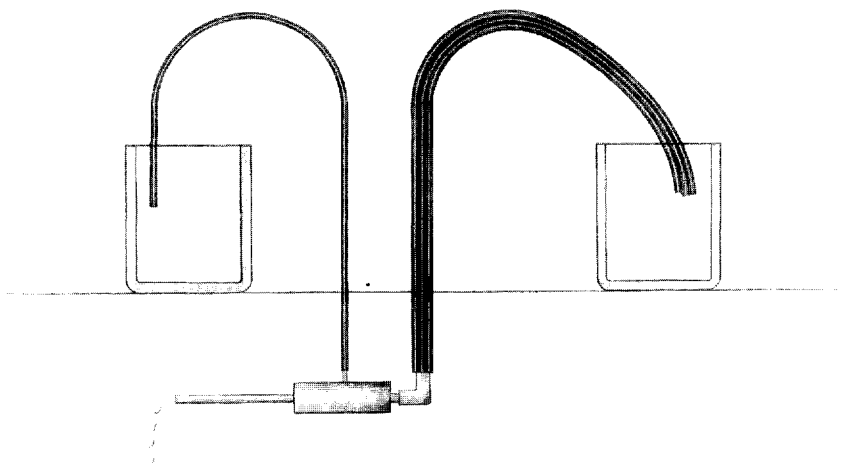


Figure 2. A Schematic Diagram of a Continuous Fraction Collection System for Semi-Preparative Capillary Electrophoresis.

Downloaded At: 10:13 25 January 2011

and were dried under vacuum. The beads were stored at 4°C under nitrogen.

Conjugation of antibodies to DITC-glass

A solution of 1 mg/ml of monoclonal antibody was dialyzed extensively against coupling buffer (0.2 M Na_2HPO_4, pH 7.5, 0.2% Nonidet P-40). Approximately 10 ml of antibody solution were incubated with DITC-glass for 4 hr at room temperature. Alternatively, the coupling can be carried out at pH 9.0 but in order to avoid deterioration of the glass the incubation with the antibody should be performed at 4°C. The conjugated beads were then filtered and washed with the pH 7.5 coupling buffer, and finally stored at 4°C. The entire concept of the chemical conjugation of an antibody to silica-derived glass beads is depicted in Figure 3.

Fabrication of an analyte concentrator

The fabrication of an analyte concentrator was carried out by using a piece of fused-silica capillary (100 μm i.d.) in which the conjugated glass beads were held inside the column by two porous glass frits. Borosilicate beads were dry-packed into one end of the capillary column and then sintered in place by using an arc source. The remaining of the capillary tube was filled with the antibody-glass beads by capillarity effect or by slowly controlled-vacuum aspiration

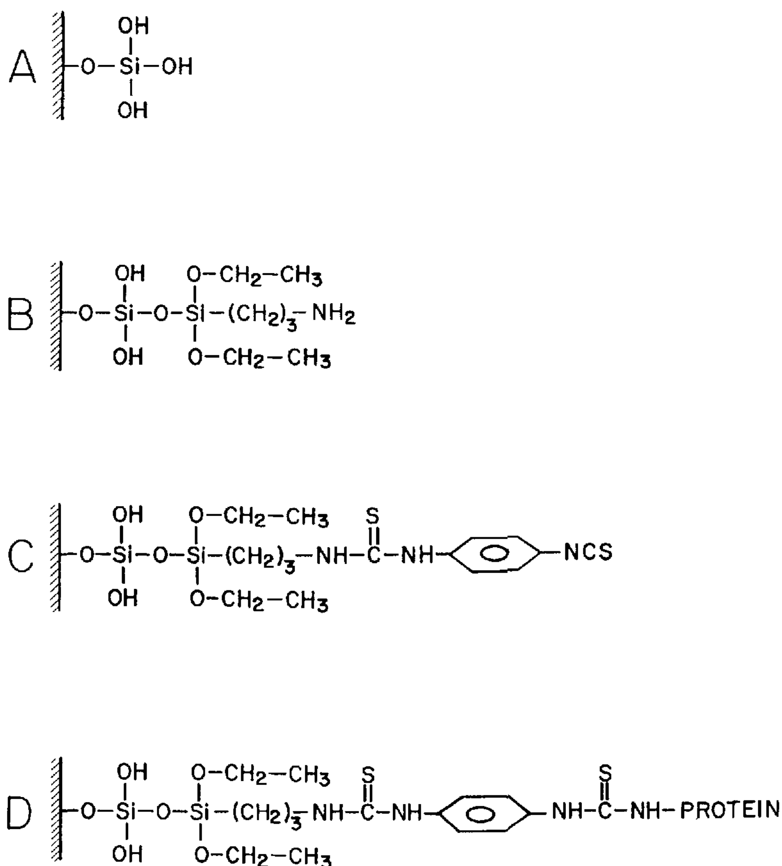


Figure 3. A Schematic Representation of the Various Chemical Reactions Necessary for the Conjugation of an Antibody Protein to the Surface of Glass Beads. For details of the complete chemical process see Materials and Methods.

to reach a depth of 3-5 mm. The capillary tube was carefully tapped to compact the beads, and the other end of the column was sealed with a sintered glass plug as described above. The analyte concentrator was then fused to two longer capillary columns (100 μm i.d.) using a sleeve of larger fused-silica tubing and glued with an epoxy resin. The final product was also monitored under a stereo microscope. The concept of an analyte concentrator is depicted in Figure 4.

Downloaded At: 10:13 25 January 2011

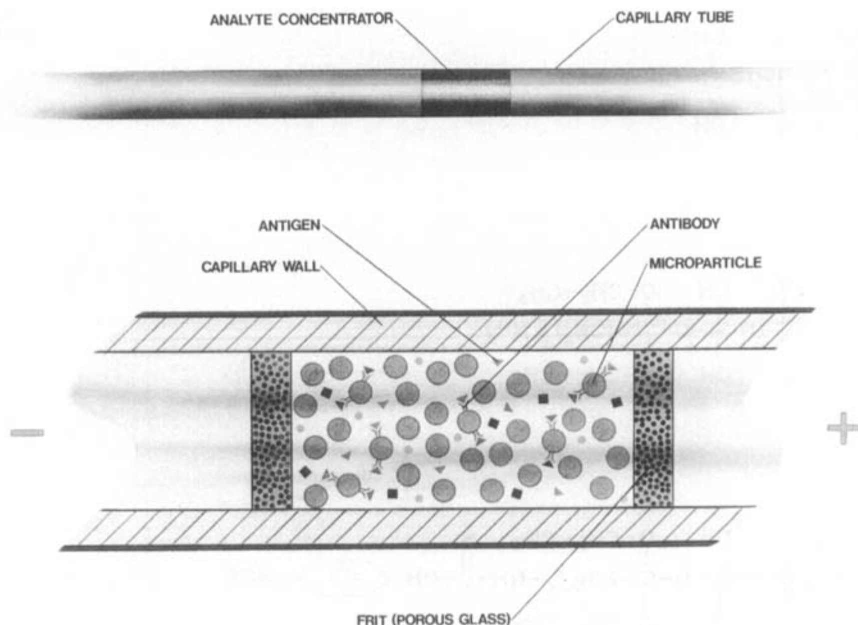


Figure 4. A Schematic Diagram of an Analyte Concentrator. Controlled-pore glass beads were activated, conjugated with a purified antibody, and installed inside a portion of a fused-silica capillary column as described in Materials and Methods.

Downloaded At: 10:13 25 January 2011

Instrumentation

The capillary electrophoresis instrument with ultraviolet detection used was similar to that previously published (1,2). The on-column UV detection system consists of an adjustable wavelength detector (Hitachi Instruments, Inc., Danbury, CT), and the instrument includes a fraction collector. Electropherograms were obtained with a strip chart recorder model L-6512 (Linseis Inc., Princeton Junction, NJ) at 20 cm/h.

The mass spectrometry data was obtained using a Finnigan MAT 8230 mass spectrometer (Bremen, Germany). The samples were analyzed by direct probe Electron Ionization (EI) at a mass range of 35-350 a.m.u., scan rate of 1 sec/decade, and a probe temperature ranging from 25°C to 350°C at a flow rate of 10°C/sec.

Procedure

Urinary constituents were separated and collected by capillary electrophoresis using one of the two concentration systems described

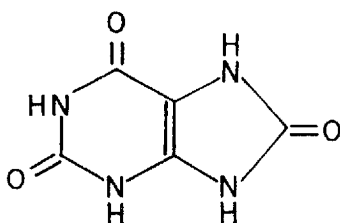
above. When using the method of capillary bundle, five capillaries were connected to a single capillary column. Samples were electrokinetically injected into the five inlet columns, simultaneously, for 30 sec at 17 kV and were separated at 22 kV for 95 min. Detection at 210 nm was carried out in the single outlet capillary. The total length of the capillary column system (from anode to cathode) was approximately 100 cm, and all six capillaries were 75 μm i.d.. Several fractions were collected into small microcentrifuge tubes containing 5 μl sodium tetraborate buffer, and were further reinjected into a single capillary column system for purity confirmation (see below). The separation of the urinary components was carried out in 50 mM sodium tetraborate buffer, pH 8.3. The pH of the pooled urine specimen and the two test substances (uric acid and methamphetamine, see Figure 5) was adjusted to 7.5.

When using the analyte concentrator method, a column containing one or two concentrators was used. Samples were injected electrokinetically or by using a controlled-vacuum pump. For this experiment, a known amount of amphetamine was spiked into an aliquot of urine sample. As previously reported, the flow rate was carefully monitored during sample injection to test for possible effects of the analyte concentrator(s) on operational conditions (1,2). The manufacturing of an optimal analyte concentrator is critical for a good performance and to avoid any plugging of the system. After saturation of the antibody binding sites, the column was extensively washed with 0.05 M sodium tetraborate buffer, pH 8.3, followed by an elution buffer composed of 0.05 M sodium acetate buffer, pH 3.5. Several fractions were collected into small conical microcentrifuge tubes containing 5 μl of sodium tetraborate buffer, and were further reinjected into a single capillary column system for purity confirmation (see below).

Electropherograms of freshly-collected urine, and from fractions collected using the two methods described above, were generated using a single capillary column. The column consisted of a 75 μm x 55 cm fused-silica capillary. Samples were electrokinetically injected at 15 kV for 12 sec and separated at 22 kV for 45 min. A regular cleaning cycle after every injection was carried out using a cleaning device attached to a fluid trap and a miniaturized pump to aspirate the solutions. One minute of 1% (v/v) phosphoric acid, followed by one minute each of water, 0.1 N potassium hydroxide, water, and finally equilibrated with sodium tetraborate buffer.

For mass-spectrometry analysis, samples containing either uric acid or methamphetamine were pooled after several experiments and were then desalted in a (fine) biogel P2 column (200-400 mesh), with a molecular weight range of 100-1800 (BIO-RAD, Richmond, CA), and finally lyophilized.

Downloaded At: 10:13 25 January 2011



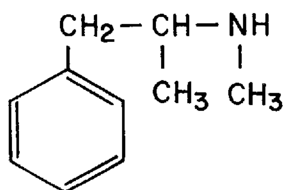


Figure 5. A Schematic Representation of the Molecular Structures of the Two Test Substances Used in this Report: Uric Acid and Methamphetamine.

Downloaded At: 10:13 25 January 2011

RESULTS

Figure 6A depicts an electropherogram of a urine specimen collected from a pool of six normal individuals. Approximately 10 major components were separated, as previously demonstrated (13,15), except the migration time was 45 min rather than 90 min because of the use of a shorter capillary column. Peak 8 (Figure 6A) was originally assigned as phosphate, based on the co-migration of this peak with phosphate standards (13), however, mass spectrometry data (see below) identified this peak as uric acid. When the substance present in this peak was collected and reinjected into a single capillary column, it produced an electropherographic peak having an identical migration time of the uric acid standard (Figure 6B). When

Downloaded At: 10:13 25 January 2011

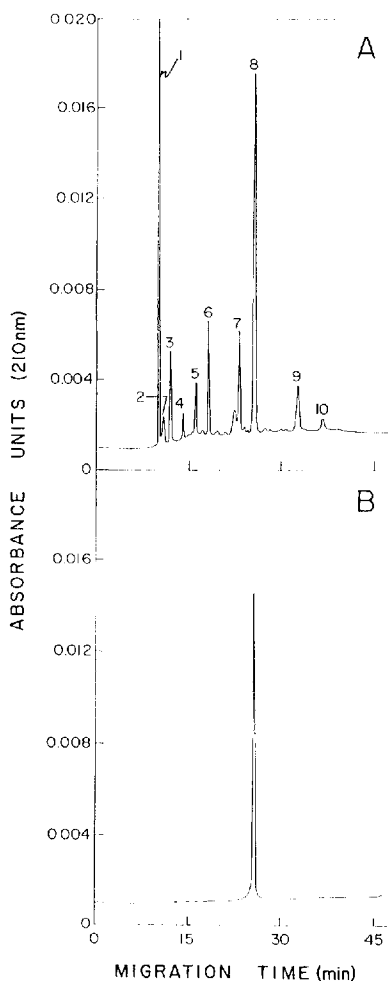


Figure 6. Typical Electropherogram of Human Urine Using Ultraviolet Detection Methods. Approximately 10 major components were observed in the capillary electrophoresis profile of a normal urine specimen. Electropherogram of a freshly-collected clean-catch pooled urine specimen obtained from six normal people (**A**). Electropherogram of a uric acid standard (**B**).

the urine sample was spiked with uric acid, an increase in the height of peak 8 was observed (not shown). Furthermore, when the urine specimen was stored at 4°C a strong precipitation of salts occurred. The supernatant of this precipitate was further analyzed, and a decrease in peak 8 height was found. Conversely, the precipitate-enriched urine specimen produced an electropherogram containing an increase in peak 8 height (13). These precipitated salts were identified as urate salts. When using the five capillary bundle method, it was estimated that approximately 200 ng/40 nl of uric acid was injected into the system with a yield of at least 70% recovery (140 ng of uric acid collected in 95 min). Thus, it is possible to generate more than 1 μg of material after eight sample injections. This process takes approximately 13 hr of total repetitive cycles, including a cleaning cycle after every injection.

Figure 7A depicts an electropherogram of a urine specimen spiked with methamphetamine, and Figure 7B depicts an electropherogram of the same drug solution, obtained as an eluted material when using the analyte concentrator method. Notably, methamphetamine must have a highly positive charge since it migrated faster than any of the urinary constituents present in a normal urine and faster than mesityl oxide used as a flow rate marker (Figure 8). Using this method, the recovery yield varied dramatically from 20% to 65% depending on many factors, including the quality of the analyte concentrator, the quality of the antibody, and the quality of the conjugation process (not shown). Nevertheless, using one analyte concentrator it was possible to generate approximately 200 ng of material after a single injection of methamphetamine-spiked urine. The analyte concentrator device was reusable, and the yield after the second injection was similar to that obtained with the first injection. Using two analyte concentrators, it was possible to generate more than 350 ng of drug per injection. Thus, it was possible to produce more than 1 μg of methamphetamine in one day.

In order to definitively identify urinary constituents collected by either of the two methods, off-site mass spectrometry analysis was performed. As shown in Figure 9A, the mass spectra of the substance collected by the use of the multiple capillaries method corresponds to that of a uric acid standard. As shown in Figure 9B, the mass spectra of the substance collected by use of the analyte concentrator method corresponds to that of a methamphetamine standard.

DISCUSSION

Capillary electrophoresis is becoming a complementary technique to high-performance liquid chromatography (HPLC). In some

Downloaded At: 10:13 25 January 2011

Downloaded At: 10:13 25 January 2011

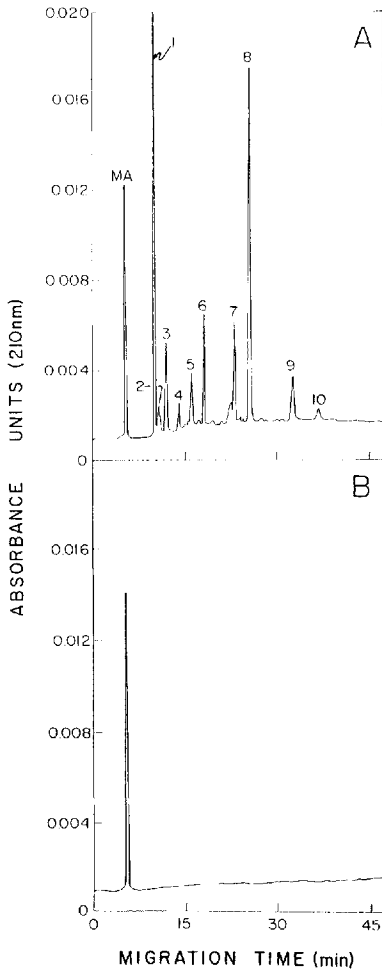


Figure 7. Electropherogram of a Methamphetamine-Spiked Urine Specimen (**A**). Electropherogram of a methamphetamine standard (**B**).

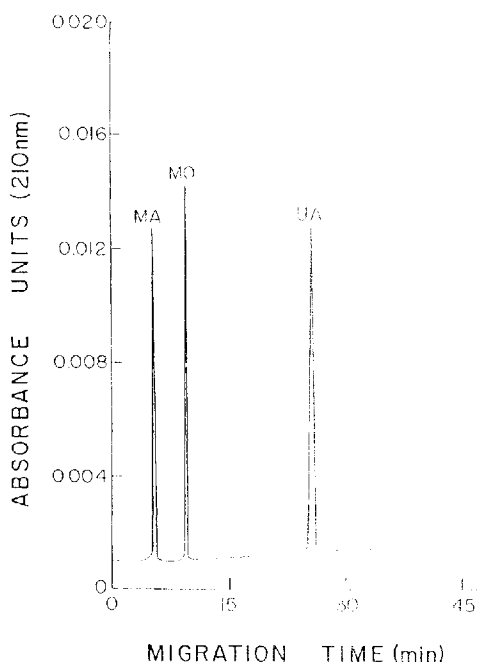


Figure 8. Electropherogram of a Mixture of Methamphetamine (MA), Mesityl Oxide (MO), and Uric acid (UA).

Downloaded At: 10:13 25 January 2011

applications, however, it may even replace HPLC because of the additional amount of information that CE can generate. Nevertheless, CE is still considered by many scientists only as an analytical tool. The option of having adequate fraction collection capabilities is not yet optimized. In this paper we have clearly demonstrated the possibility of increasing sample load into the capillary column, to increase detector response and to generate enough material to perform further analysis for identification purposes, or for any other purpose.

Although we have been successful in generating more than one microgram of collected pure material (obtained from a biological fluid) in one day, it is still possible to optimize the experimental conditions and be able to obtain larger quantities of material. For example, this might be accomplished using more than two analyte concentrators, or using an analyte concentrator with a larger sample capacity. Additionally, it might also be possible to obtain more than one pure substance in a shorter period of time, using a mixture of different antibodies or using different solid support chemistries.

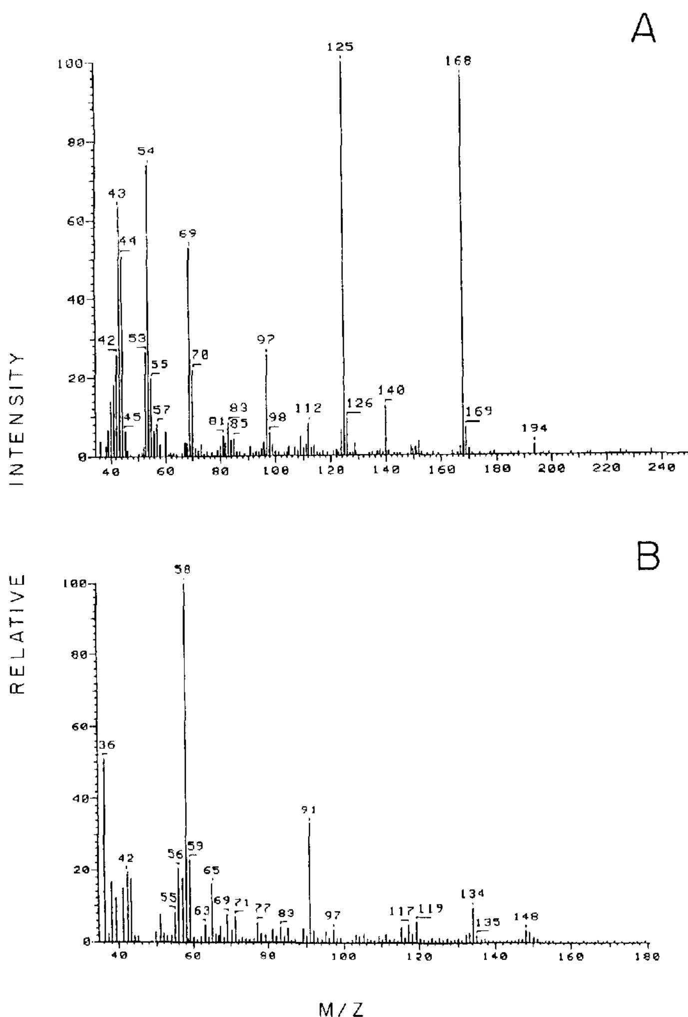


Figure 9. Mass Spectrometry Data Using the Electron Ionization Method. Samples were purified and processed as described in Materials and Methods. (**A**). EI spectra of the compound collected by the bundle of multiple capillaries method (this EI spectra corresponds to that of a uric acid standard). (**B**). EI spectra of the compound collected by the analyte concentrator method (this EI spectra corresponds to that of a methamphetamine standard).

Downloaded At: 10:13 25 January 2011

Finally, the use of the grounding device makes the system very practical to collect substances into a buffer-containing reservoir or just simply onto a flat surface or membrane.

There are specific situations when the sample volume available for biochemical and immunological analysis is limited, among others, biological products derived from cell culture, forensic medicine, and neuroscience. In these cases, CE might be an alternative to traditional chromatographic approaches. In addition, there are also instances when samples having low analyte amounts might benefit by a concentration step previous to the chromatographic detection stage. In these cases, use of a bundle of multiple capillaries and the use of an analyte concentrator might be useful addition to such CE approaches. For example, push-pull cannula (PPC) perfusion and microdialysis are sampling techniques used by neuroscientists to assess *in vivo* release of neuropeptides and neurotransmitters, from discrete brain areas (33-36). Under these sampling conditions, very low volume is available to determine neuropeptides and neurotransmitters, which are usually at very low concentrations. Traditional radioimmunoassay and electrochemical detection measurements require most of each PPC and/or microdialysis sample volume, thus limiting the amount of information which may be obtained from these samples (33-36). CE assessment of such samples show that several neurotransmitters and neuropeptides may be separated in a single electropherogram (33,34). Thus, the use of a bundle of multiple capillaries and the use of an analyte concentrator might increase the sensitivity of CE approaches by increasing the load of sample into the CE system. Further increases in sensitivity may be obtained by coupling the CE system to a fluorescent microscope, thus increasing the detector sensitivity of the CE system (26,37).

Increasing the sample load to the CE system may increase the detectability of specific analytes. This might be achieved by using a bundle of multiple capillaries, an analyte concentrator, or both. For example, we have recently reported a dramatic increase in the detectability of immunoglobulins using CE, by coupling a bundle of multiple capillaries to fluorescence microscope detection (26). A description of the fabrication of such capillary bundles and its associated problems were reported (26). On the other hand, we have presented in this paper evidence for an increased detectability of methamphetamine using CE, when using an analyte concentrator and UV detection. One of the problems encountered during the fabrication of the analyte concentrator used in this report, was the attachment of the second frit to the capillary column. To fuse the porous glass beads to the capillary column (in order to form the frits) it is necessary to heat the beads for a short period of time. When fusing the

Downloaded At: 10:13 25 January 2011

second set of beads to the capillary (to form the second frit), some of the beads carrying covalently-bound antibodies are located near the frit. Unfortunately, the excess amount of heat when fabricating the second frit may have altered the binding efficiency of some of the antigen-antibody binding sites. This might explain the wide range of sample recoveries observed when using the analyte concentrator. Additional problems related to the fusing of the frits might also exist (i.e., alteration of the porosity of the glass material).

Thus, capillary electrophoresis may be used as a semi-preparative technique having capabilities for collecting microgram quantities of pure material. However, this technique faces many improvements in the years to come.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Wetterberg Foundation, New Jersey Experimental Station-Hatch 06108, and USDA 89-37240-4587 to JPA.

Downloaded At: 10:13 25 January 2011

REFERENCES

1. Guzman, N.A., L. Hernandez, and B.G. Hoebel (1989). BioPharm, 2: 22-37.
2. Guzman, N.A., L. Hernandez, and S. Terabe (1990). In: **Analytical Biotechnology. Capillary Electrophoresis and Chromatography.** ACS Symposium Series 434 (C. Horvath, and J.G. Nikelly, Eds.), Chapter 1, pp. 1-35. American Chemical Society, Washington, D.C.
3. Wallingford, R.A., and A.G. Ewing (1990). In: **Advances in Chromatography. Biotechnological Applications and Methods** (J. Giddings, E. Gruska, and P.R. Brown, Eds.), Volume 29, pp. 1-76, Marcel Dekker, Inc., New York and Basel.
4. Kuhr, W.G. (1990). Anal. Chem., 62: 403R-414R.
5. Goodall, D.M., D.K. Lloyd, and S.J. Williams (1990). LC•GC, 8: 788-799.
6. Swerdlow, H., and R. Gesteland (1990). Nucl. Acids Res., 18: 1415-1419.
7. Luckey, J.A., H. Drossman, A.J. Kostichka, D.A. Mead, J.D'Cunha, T.B. Norris, and L.M. Smith (1990). Nucl. Acids Res., 18: 4417-4421.
8. Cohen, A.S., D.R. Najarian, and B.L. Karger (1990). J. Chromatogr., 516: 49-60.

9. Swerdlow, H., S. Wu, H. Harke, and N.J. Dovichi (1990). J. Chromatogr., 516: 61-67.
10. Zhu, A., and Y. Chen (1989). J. Chromatogr., 470: 251-260.
11. Jones, H.K., and N.E. Ballou (1990). Anal. Chem., 62: 2484-2490.
12. VanOrmann, B.B., and G.L. McIntire (1990). Amer. Laborat., 22: 66-67.
13. Guzman, N.A., L. Hernandez, and J.P. Advis (1989). J. Liquid Chromatogr., 12: 2563-2574.
14. Gordon, M.J., X. Huang, S.L. Pentoncy, Jr., and R.N. Zare (1989). Science, 242: 224-228.
15. Guzman, N.A., C.M. Berck, L. Hernandez, and J.P. Advis (1990). J. Liquid Chromatogr., 13: 3843-3858.
16. Weinberger, R., E. Sapp, and S. Moring (1990). J. Chromatogr., 516: 271-285.
17. Guzman, N.A., H. Ali, J. Moschera, K. Iqbal, and A.W. Malick (1991). J. Chromatogr., submitted.
18. Wu, S.-L., G. Teshima, J. Cacia, and W.S. Hancock (1990). J. Chromatogr., 516: 115-122.
19. Wenisch, C. Tauer, A. Jungbauer, H. Katinger, M. Faupel, and P.G. Righetti (1990). J. Chromatogr., 516: 133-146.
20. Guzman, N.A., and F.E. Regnier (1987). The First Symposium of the Protein Society. San Diego, California, August 9-13, Abstract 823.
21. Rose, D.J., and J.W. Jorgenson (1988). J. Chromatogr., 438: 23-34.
22. Guzman, N.A. (1989). Patent No. 33861, Taiwan, Republic of China.
23. Guzman, N.A. (1990). Patent No. 88/8823, South Africa.
24. Huang, X., and R.N. Zare (1990). J. Chromatogr., 516: 185-189.
25. Fujimoto, C., Y. Muramatsu, M. Suzuki, and K. Jinno (1990). Twelfth International Symposium on Capillary Chromatography. Kobe, Japan, September 11-14, pp. 684-692.
26. Guzman, N.A., M.A. Trebilcock, and J.P. Advis (1991). Anal. Chim. Acta, submitted.
27. Dolnick, V., K.A. Cobb, and M. Novotny (1990). J. Microcol. Sep., 2: 127-131.
28. Aebersold R., and H.D. Morrison (1990). J. Chromatogr., 516: 79-88.
29. Galfre, G., and C. Milstein (1981). Meth. Enzymol., 73B: 3-46.
30. Guzman, N.A., and L. Hernandez (1989). In: **Techniques in Protein Chemistry** (T.E. Hugli, Ed.), Chapter 44, pp. 456-467.
31. Wachter, E., W. Machleidt, H. Hofner, and J. Otto (1973). FEBS Lett., 35: 97-102.
32. Liang, S.-P., and R.A. Laursen (1990). Anal. Biochem., 188: 366-373.

Downloaded At: 10:13 25 January 2011

33. Advis, J.P., L. Hernandez, and N.A. Guzman (1989). Peptide Res., **2**: 389-394.
34. Guzman, N.A., L. Hernandez, and J.P. Advis (1990). In: **Current Research in Protein Chemistry: Techniques, Structure, and Function** (J.J. Villafranca, Ed.), Chapter 19, pp. 203-216, Academic Press, San Diego.
35. Current Separations (1989). Bioanalytical Systems (BAS), West Lafayette, Indiana. Special issue (Volume 9).
36. Tossman, U. (1991). LC·GC **9**: 46-52.
37. Hernandez, L., R. Marquina, J. Escalona, and N.A. Guzman (1990). J. Chromatogr., **502**: 247-255.

Downloaded At: 10:13 25 January 2011