BECKMAN COULTER

PACE Setter

The worldwide newsletter for capillary electrophoresis

Volume 7, Issue 2 • September 2003

Table of Contents

BECKMAN COULTER

P/ACE Setter

The worldwide newsletter for capillary electrophoresis

Volume 7, Issue 2 • September 2003

Analysis of Normal and Modified Urinary Nucleosides: Researching Rapid Detection Methods for Cancer

Guo-Wang Xu, Yu-Fang Zheng, Pu-Dun Zhang, Jian-Hui Xiong, Shen Lv
National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, P. R. China
dicp402@mail.dlptt.ln.cn

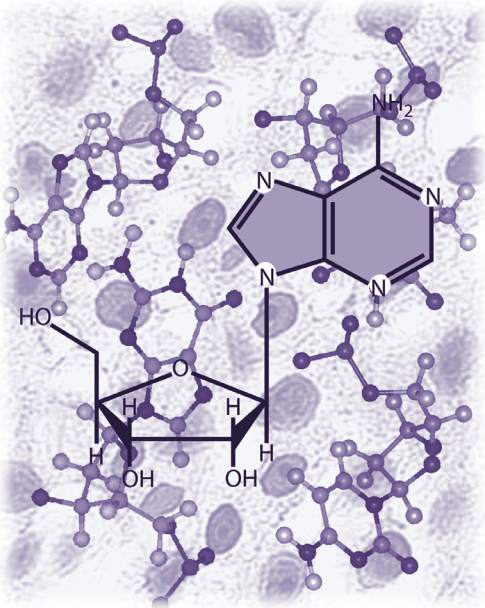
Introduction

Modified nucleosides, derived predominantly from tRNA, have been detected in abnormal amounts in the urine of cancer patients[1-3]. People have been interested in examining their biomedical significance as potential tumor markers. Reversed phase high performance liquid chromatography[4-7] and immunoassays[8-9] are the main analytical methods for these nucleosides. Capillary electrophoresis (CE) has gradually been applied in clinical research due to its high efficiency, high speed, and small sample size requirements. In this paper, we report a capillary electrophoresis (CE) method for the separation of thirteen normal and modified nucleosides and apply it to the analysis of urine from 25 healthy adults and 31 subjects with cancer. A principal component analysis (PCA) technique was used to classify healthy adults from those with cancer.

Experimental Conditions

Equipment and Reagents

All experiments were performed with a P/ACE™ MDQ Capillary Electrophoresis system from Beckman Coulter. This system is equipped with a UV detector/diode array detector and a 50 µm i.d. × 50 cm bare fused-silica capillary with an effective detection length of 40 cm. A 100 × 800 µm aperture was used for detection. Data acquisition was achieved with P/ACE MDQ software version 2.3. A ϕ® 210 pH meter from Beckman Coulter was employed for the preparation of the buffer.



Fourteen nucleoside standards including the internal standard 3-deazauridine (3-Dzu) were obtained from Sigma (St. Louis, Mo, USA). Sodium dodecyl sulfate (SDS) was obtained from HuaMei biological engineering company of China. Affi-Gel* 601 was from Bio-Rad (Richmond, CA, USA). Ammonium acetate, methanol, ammonia, sodium dihydrogenphosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) and sodium tetraborate ($NaB_4O_7 \cdot 10\ H_2O$) were analytical pure reagents purchased from China. Urine specimens were collected from 25 healthy donors from the authors' institute (age range, 25-63 years) and from 31 cancer patients in the first and second affiliated hospital of Dalian Medical University.

Nucleosides were isolated from urine with the use of a phenylboronate gel-affinity column before introduction into the CE system for analysis[5-7].

CE Conditions

The buffer contained 300 mM SDS, 25 mM NaB_4O_7 and 50 mM NaH_2PO_4. The pH value was adjusted to 6.9 with 1 N HCl.

The capillary chamber was thermostatted at 29°C using a recirculating liquid coolant. The sample was introduced under 0.5 psi for 15 seconds at the anode by positive pressure. Electrophoresis was carried out at 7 kV (positive at the inlet end).

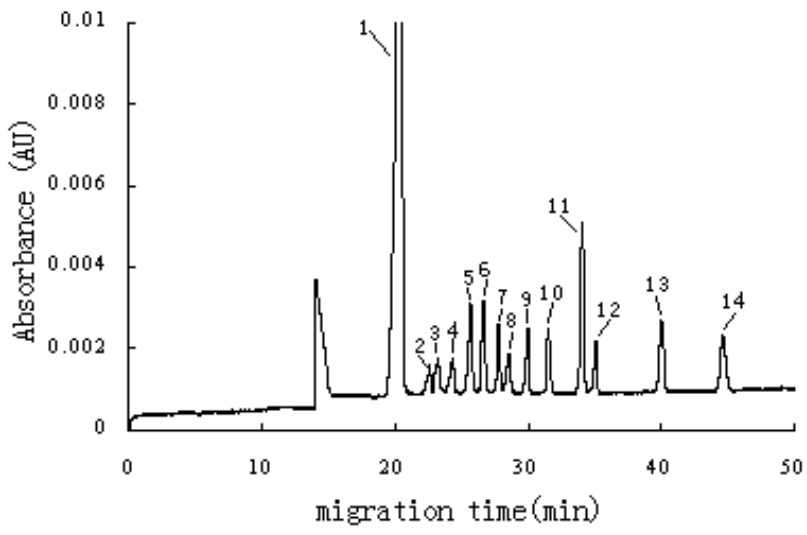


Figure 1. *Capillary electropherogram of standard nucleosides. Capillary: 52 cm × 50 µm i.d.; Vacuum-injection: 0.5 psi × 15 seconds; buffer: 300 mmol/L SDS-25 mmol/L borate-50 mmol/L phosphate (pH 6.9); temperature: 29°; running voltage: 7.0 kV; UV detection: 254 nm; Peak identification—1: Pseu; 2: U; 3-C; 4: mU 5 I ; 6: m1I; 7: ac4C; 8: G; 9: m1G ; 10: A; 11: 3-Dzu; 12: X; 13: m2G; 14: m6A.*

Nucleosides were measured by UV detection at 254 nm with an acquisition data rate of 4 Hz. To ascertain the best separation and reproducibility, the capillary was regenerated by flushing with 0.1 M sodium hydroxide for 1 minute, followed by a 1-minute water rinse and another 2-minute buffer rinse after each run.

The levels of the urinary nucleosides were calculated by the calibration curves; then these were transformed into nmol/µmol creatinine. For the determination of urinary creatinine levels, urine was thawed at room temperature, diluted eight-fold, and then introduced into the capillary. The separation was carried out with 30 mM phosphate buffer (pH 6.0) at 20 kV with diode array detection at 245 nm.

Results and Discussion

Optimization of CE Conditions

In order to establish the method for determination of urinary nucleosides, the separation conditions were optimized by selecting the parameters, including buffer and its concentration, pH, running voltage, and the wavelength of detection[12,15]. Thirteen normal and modified nucleosides were separated using a 50-cm bare fused-silica column (50 µm i.d., 40 cm effective length) with a 300 mM SDS-25 mM NaB_4O_7–50 mM NaH_2PO_4 buffer (pH 6.9), 7 kV running voltage, as shown in Figure 1.

Standard Curve

The concentrations of the nucleosides in urine were calculated based on the calibration curves. The linear correlation between nucleoside concentrations and peak areas, their corresponding correlation coefficients, and the detection limits (expressed as the signal-to-noise ratio of three) are listed in Table 1.

Quantitation of Urinary Nucleosides

The precision of the method was determined by analyzing a standard nucleoside sample six times (Table 2). Using the optimized method, spontaneous urine samples from 25 healthy controls and 31 cancer patients were analyzed. Figure 2 shows the typical electropherogram of urinary nucleosides from a cancer patient using the conditions optimized. The levels of the thirteen urinary nucleosides from normal and cancer subjects and the results of the *t* test are listed in Table 3. By selecting p values <0.05 as statistically significant, it can be seen that the levels of eleven nucleosides were significantly elevated in the cancer

Table 1. The Linear Correlation Between Relative Nucleoside Concentrations and Relative Peak Areas as Well as Their Limits of Detection (LOD)[a]

No	Compound	The linear correlation	R	LOD (µmol/L)
1	Pseu	y = 1.0901x-0.1662	0.9901	64.8
2	U	y = 1.4133x+0.0466	0.9673	12.5
3	C	y = 1.4239x-0.001	0.9978	10.0
4	mU	y = 1.272x+0.0003	0.9985	6.0
5	I	y = 2.0159x-0.008	0.9906	12.0
6	m1I	y = 3.6999x-0.1574	0.9901	8.0
7	ac4C	y = 3.0578x-0.0302	0.9766	7.8
8	G	y = 2.3277x+0.0277	0.9871	5.4
9	m1G	y = 2.8663x+0.0042	0.9832	6.8
10	A	y = 3.0763x+0.0087	0.9970	7.0
11	X	y = 1.5911x+0.0151	0.9786	14.6
12	m2G	y = 4.6331x-0.0238	0.9837	8.4
13	m6A	y = 4.7735x-0.0412	0.9920	15.2

[a] y: peak area relative to that of the internal standard, x: individual nucleoside concentration relative to that of the internal standard.

Table 2. Reproducibility of Migration Times and Peak Areas for a Standard Solution of 13 Nucleosides[a]

Compound	Mean time (min)	RSD%	Mean area	RSD%
Pseu	17.86	0.4	442350	2.4
U	19.51	0.5	14489	2.0
C	20.61	0.6	15772	3.6
mU	21.08	0.6	14077	4.6
I	22.06	0.6	35042	3.0
m1I	22.89	0.7	37494	3.8
ac4C	23.90	0.9	28741	3.4
G	24.44	0.8	15637	4.1
m1G	25.23	0.8	32416	4.0
A	27.22	0.9	27885	3.6
X	29.53	0.6	20946	3.5
m2G	34.58	0.8	35991	4.2
m6A	39.54	1.0	30972	4.0

[a]The results are mean values from six repeated analyses of the standard sample.

patients (except for m6A which had a concentration that was too low). Figure 3 shows this situation clearly.

For an individual urine sample, even in the same kind of cancer, the increase in each nucleoside concentration is different. In this study, we used the PCA technique[5-7,13] to classify cancer and non-cancer. This approach gave us a single value representing the summation of all thirteen nucleosides determined for one person. By using thirteen nucleosides as data vectors, 84% of cancer patients were distinguishable from healthy controls (Figure 4).

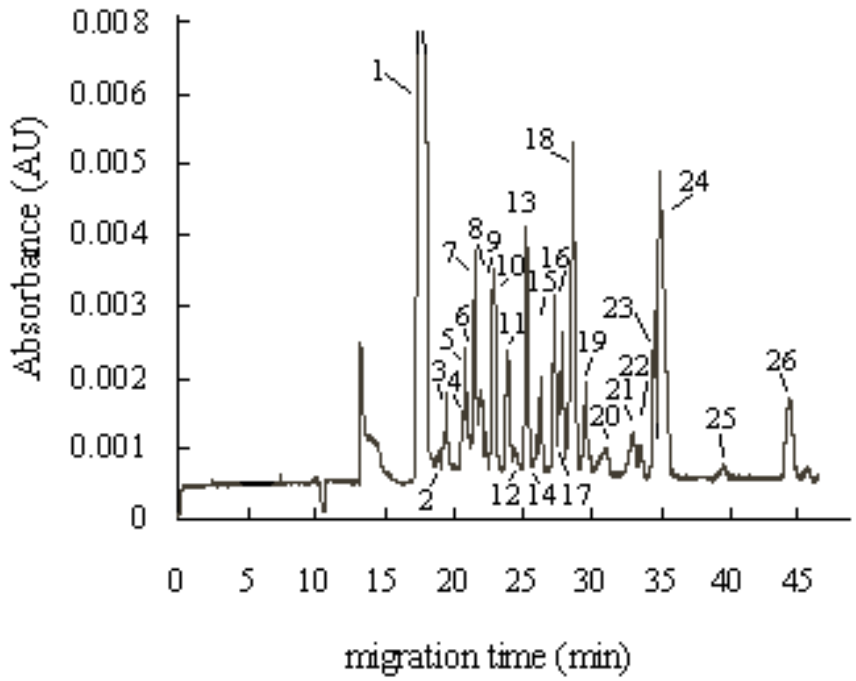


Figure 2. *Capillary electropherogram of nucleosides in extracted urine. Peak identification—1: Pseu; 3: U; 4: C; 5: mU; 8: I; 10: m1I; 11: ac4c; 12: G; 13: m1G; 16: A; 18: 3-Dzu; 19: X; 23: m2G; 25: m6A.*

Conclusion

Our research, although preliminary, shows the potential for combining urinary nucleoside analysis by MECC with principle component analysis to differentiate healthy subjects from those with cancer. The potential of this approach as a diagnostic will be explored further in our laboratory. Further information can be found from references 5-7 and 10-18.

Acknowledgements

The authors would like to thank the National Natural Science Foundation of China (No.29775024), the Knowledge Innovation Program of the Chinese Academy of Sciences, and the Foundation of Dalian City for financial support. We especially thank the students and faculty volunteers from the Dalian Institute of Chemical Physics for the normal urine samples.

References

1. Itoh, K., Konno, T., Sasaki, T., Ishiwata, S., Ishida, N., Misugaki, M. *Clin. Chim. Acta., 206,* 181-189 (1992).
2. Speer, J., Gehrke, C. W., Kwo, K. C., *et al. Cancer, 44,* 2120-2123 (1979).
3. Fischbein, A., Sharma, O. K., Selikoff, I. J., Borek, E. *Cancer Res., 43,* 2971-2974 (1983).
4. Nakano, K., Nakao, T., Schram, K. H., Hammargren, W. M., McClure, T. D., Katz, M., Petersen, E. *Clin. Chim. Acta, 218,* 169-183 (1993).
5. Xu, G., Stefano, C. D., Liebich, H. M., Zhang, Y., Lu, P., J. *Chromatogr. B, 732,* 307-313 (1999).
6. Xu, G., Enderle, H., Liebich, H., Lu, P. *Chromatographia, 52,* 152-158 (2000).
7. Xu, G.W., Lu, X., Zhang, Y. K., Lu, P. C., Stefano, C. D., Lehmann, R., Liebich, H. M. *Chinese J. Chromatogr., 17,* 97-101 (1999).

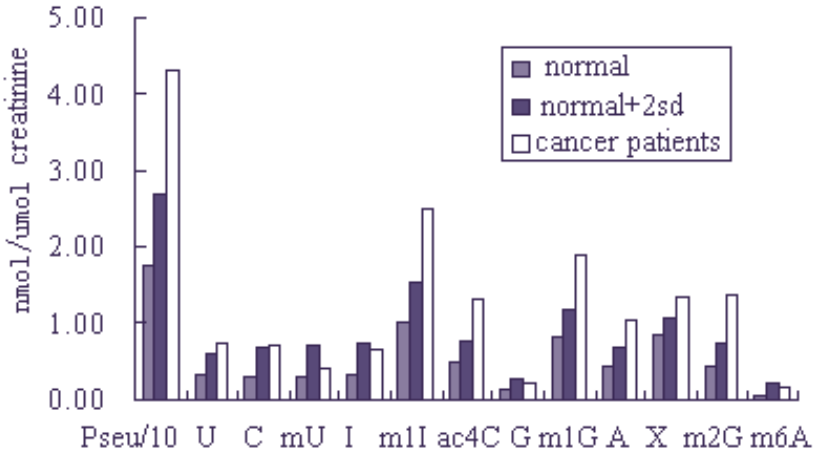


Figure 3. *Mean excretion of normal and modified nucleosides in urine from normal subjects and cancer patients.*

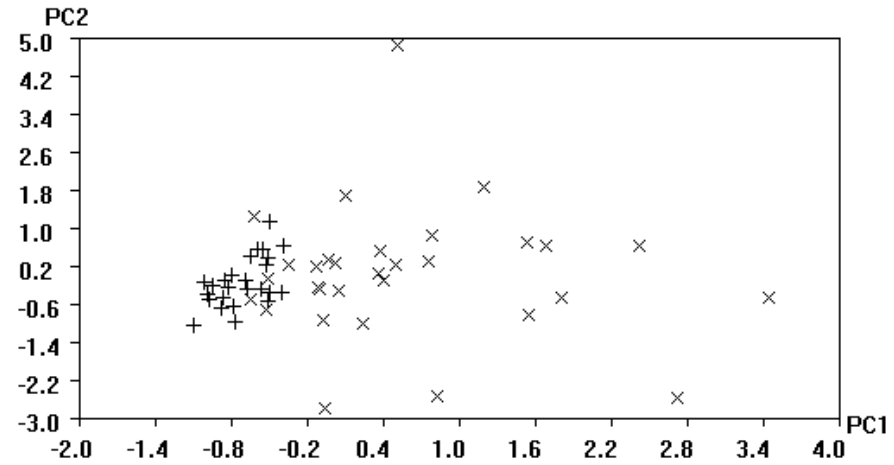


Figure 4. *Principal component analysis (PCA) based on thirteen nucleosides from healthy controls (+) and subjects with cancer (x).*

Table 3. Average Nucleoside Levels Excreted in Urine by Both Normal and Cancer Subjects

	Normal Subjects mean ± sd (nmol/µmol creatinine)	Subjects with Cancer mean ± sd (nmol/µmol creatinine)	P
Pseu	17.55 ± 4.65	43.02 ± 20.79	?0.001
U	0.32 ± 0.15	0.74 ± 0.37	?0.001
C	0.29 ± 0.20	0.69 ± 0.53	?0.001
mU	0.31 ± 0.19	0.39 ± 0.36	?0.05
I	0.34 ± 0.20	0.64 ± 0.38	?0.001
m1I	0.10 ± 0.27	2.49 ± 1.35	?0.001
ac4C	0.49 ± 0.14	1.31 ± 0.69	?0.001
G	0.11 ± 0.07	0.22 ± 0.21	?0.05
m1G	0.81 ± 0.17	1.87 ± 1.04	?0.001
A	0.42 ± 0.13	1.01 ± 0.59	?0.001
X	0.83 ± 0.42	1.33 ± 0.67	?0.05
m2G	0.41 ± 0.16	1.36 ± 0.84	?0.001
m6A	0.05 ± 0.08	0.14 ± 0.26	?0.10

8. Masnda, M., Nishihira, T., Itah, K., *et al. Cancer, 72,* 3571-3578 (1993).
9. Reynaud, C., Bruno, C., Boullanger, P., *et al., Cancer Lett., 61,* 255-262 (1992).
10. Xu, G., Liebich, H., Lehmann, R. *Methods of Molecular Biology, Volume 162—Capillary Electrophoresis of Nucleic Acids, Volume 1: Introduction to the Capillary Electrophoresis of Nucleic Acids.* Keith Mitchelson and Jing Cheng, Eds. Totowa, New Jersey: Humana Press, 2000, 459.
11. Zhao , R., Xu, G., Yue, B., Liebich, H. M., Zhang, Y. *J. Chromatogr. A., 828,* 489 (1998).
12. Liebich, H. M., Xu, G., Di Stefano, C., Lehmann, R. *Chromatographia, V45,* 396-401 (1997).
13. Xu, G., Schmid, H. R., Liebich, H. M., Zhang, Y., Lu, P. *Biomed. Chromatogr. 14,* 1 (2000).
14. Liebich, H. M., Xu, G., Di Stefano, C., Lehmann R. *J. Chromatogr. A., 793,* 341 (1988).
15. Liebich, H. M., Lehmann, R., Xu, G., Wahl, H. G., Haring, H.-U. *J. Chromatogr. B., 745,* 189 (2000).
16. Xu, G., Liebich, H. *Am. Clin. Lab., 20,* 22 (2001).
17. Xu, G., Xin, L. , Zhueng , Y., Liu, D., Zhang, Yun, Zhang, Yukui, Lu, P., Liebich , H. M. *Pittcon 2001,* New Orleans, LA, USA. March 4-8, No.1219.

THE PROTEIN DISCOVERY PATHWAY BEGINS HERE . . .

ProteomeLab™—From Tissues to Targets

Introducing ProteomeLab™—a new initiative that brings Beckman Coulter's life sciences technologies together to address current challenges in proteomics. Since the term 'proteome' was first coined in the mid 1990s, an integrated field of study (proteomics) has emerged that involves the systematic large-scale identification, characterization, and quantification of all proteins involved in a biological pathway. The generation of such a comprehensive data set requires an integrated, multi-technology strategy encompassing cellular isolation, antigen identification, protein fractionation, protein characterization, data evaluation, and ultimately disease diagnosis. ProteomeLab, which springs from a systems biology approach, addresses the range of challenges "from tissues to targets."

As a biomedical research and diagnostic company, Beckman Coulter has been involved in protein research for decades. We are the only company that addresses the complete biomedical testing continuum from research through diagnosis. Building upon key Beckman Coulter technologies including automation, capillary electrophoresis, chromatography, centrifugation, spectrophotometry, and flow cytometry, ProteomeLab provides tools for every important step in protein research.

This program integrates systems, software, and chemistries under the ProteomeLab name and leverages Beckman Coulter's technological strengths in the development of new products for proteomics. The initial offering includes the ProteomeLab PF 2D Protein Fractionation System for automated two-dimensional resolution; the ProteomeLab PA-800 Protein Characterization System for high-resolution protein characterization; ProteomeLab XL-A/XL-I for protein heterogeneity, stoichiometry, interacting/self-associating systems, and molecular conformation studies for lead optimization; and the ProteomeLab DU® 800 System for enzyme kinetic studies.

Featured in this article is our new ProteomeLab PA 800 Protein Characterization System for the automated, high-resolution analysis of proteins. The PA 800 offers a comprehensive range of automated protein characterization solutions including molecular weight determination, peptide mapping, isoelectric focusing, and carbohydrate profiling. Based on Beckman Coulter's well-established automation and capillary electrophoresis technologies, the PA 800 addresses important issues in the characterization of a given proteome—manipulating and resolving low levels of proteins that range from acidic to basic to membrane-bound.

The PA 800 includes specialized chemistry, software, training and support. The system works with the ProteomeLab PF 2D, a new two-dimensional protein fractionation system that resolves thousands of proteins in liquid phase. Protein differences between normal and diseased tissue are resolved and fractionated into a 96-well plate using the ProteomeLab PF 2D system. Wells containing unique fractions are highlighted by the system's differential display and may be easily transferred to the ProteomeLab PA 800 for extensive characterization. The PA 800 and PF 2D systems are also linked by our 32 Karat™ software—a central platform providing instrument control and data analysis for both systems.

The pathway to protein discovery begins here: ProteomeLab—From Tissues to Targets.

For more information, please contact your local representative or visit our ProteomeLab website at www.beckmancoulter.com/proteomelab.

Analysis of Basic Components in Crude Extracts of Natural Products for Pharmaceuticals and Nutriceuticals by CE-MS/MS

Fu-Tai A. Chen

Natural Products for Pharmaceuticals and Nutriceuticals

Natural products of botanical origin (herbal products) have played a major role in health care in human history. They provide a major source for many drugs with well-defined structures, and they continue to be one of the major resources for the modern pharmaceutical industry.

Nutriceuticals are food or food ingredients considered to provide medical or health benefits, including the prevention of diseases. Examples have included:

- Quinine from Cinchona bark (1820)
- Salicylic acid from Willow bark (1860)
- Ephedrine from Ma Huang (1920)
- Taxols from Pacific Yew tree (1971)
- Ginseng as tonic (101 B.C.)

However, the use of herb-based natural products for pharmaceuticals and nutriceuticals poses many analytical challenges since the sample matrix is typically very complex, and the active components are usually not well defined and may be present in trace amounts.

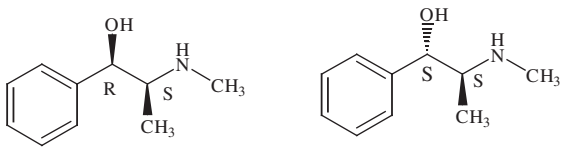
The lack of a well-defined active ingredient as well as simple and reliable sample preparation and analytical processes are the major causes of the quality-related problems found in the herbal medicine market today.

Traditionally, most herbal medicines are "brewed" as aqueous extracts. These hydrophilic species are ideally suited for capillary electrophoresis analysis. Yet, ironically, most scientific publications use an extraction procedure that favors the isolation of neutral and hydrophobic species where the active component is most likely not to be found. Once again, this is because, in most herbal preparation, the "active" is extracted in water and the remaining elements are usually discarded.

In this paper, we propose the use of CE-MS/MS methodologies to manage the characteristics and analysis of natural product extracts.

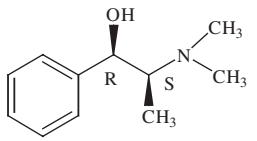
Analytical Procedure

1) Analysis was performed on a CE system (P/ACE™ MDQ, Beckman Coulter, Inc.) with a 75 µm × 80 cm fused-silica capillary. The outlet of the capillary is integrated into the ESI spray needle that is



1R, 2S: l-ephedrine (-)
Bronchodilator, naturally occurring species, 75 to 85%

1S, 2S: d-pseudoephedrin (+)
Decongestant, naturally occurring species, 10 to 25%



1R, 2S: l-N-methyl-ephedrine (-)
Naturally occurring species, < 5%

Figure 1. *Ma Huang structures.*

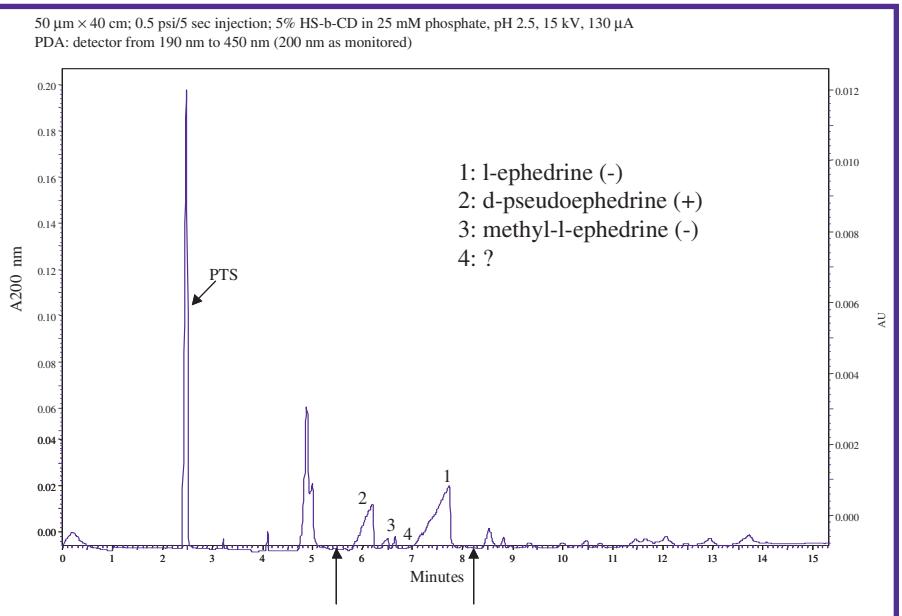


Figure 2. *Chiral Separation of Ephedrine in Crude Extract of Ma Huang.*

CE: 75 µm × 80 cm; 0.5 psi/10 sec injection; 50 mM NH_4OAc in water/methanol (75/25), pH 4.0, 30 kV, 43 µA
LCQ: ESI/Sheath gas: 10, spray voltage: 4.5 kV

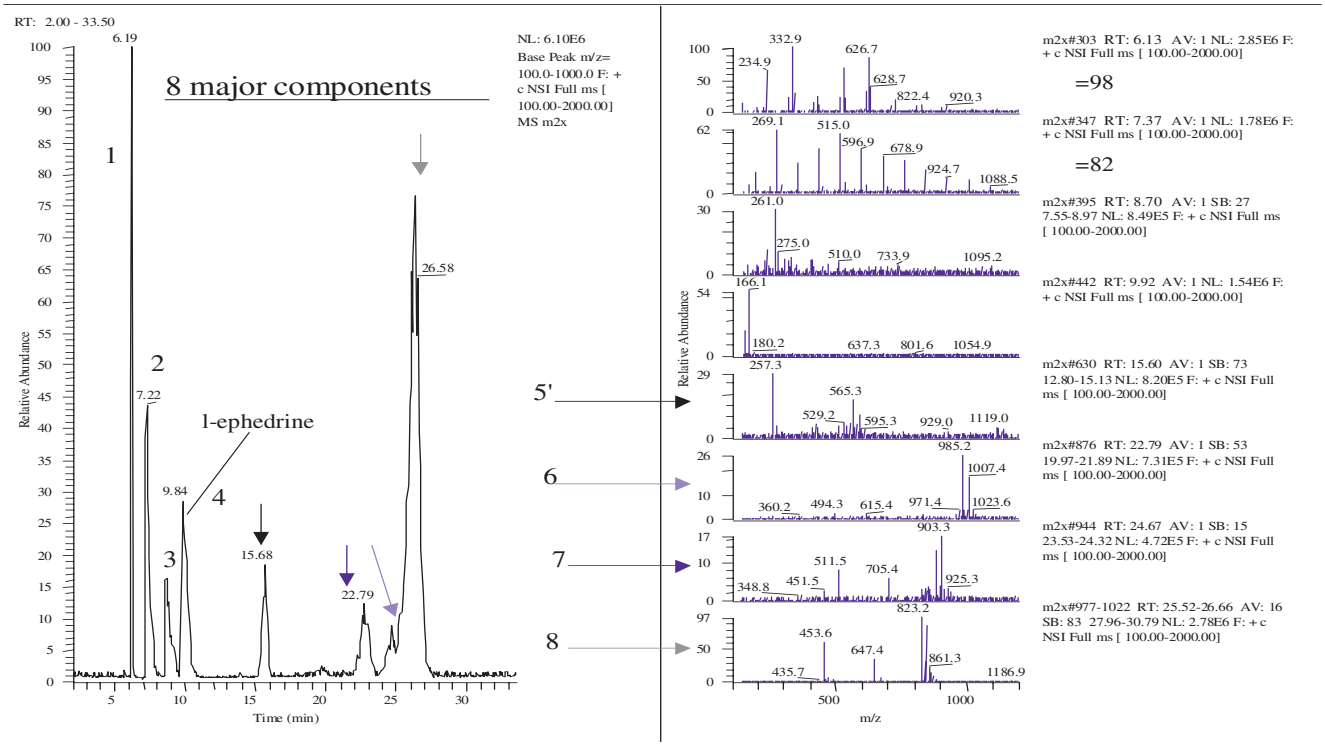


Figure 3. *CE-MS of Crude Extract of Ma Huang Capsule.*

CE: 75 µm × 80 cm; 0.5 psi/10 sec injection; 50 mM NH_4OAc in water/methanol (75/25), pH 4.0, 30 kV, 43 µA
LCQ: ESI/Sheath gas: 10, spray voltage: 4.5 kV

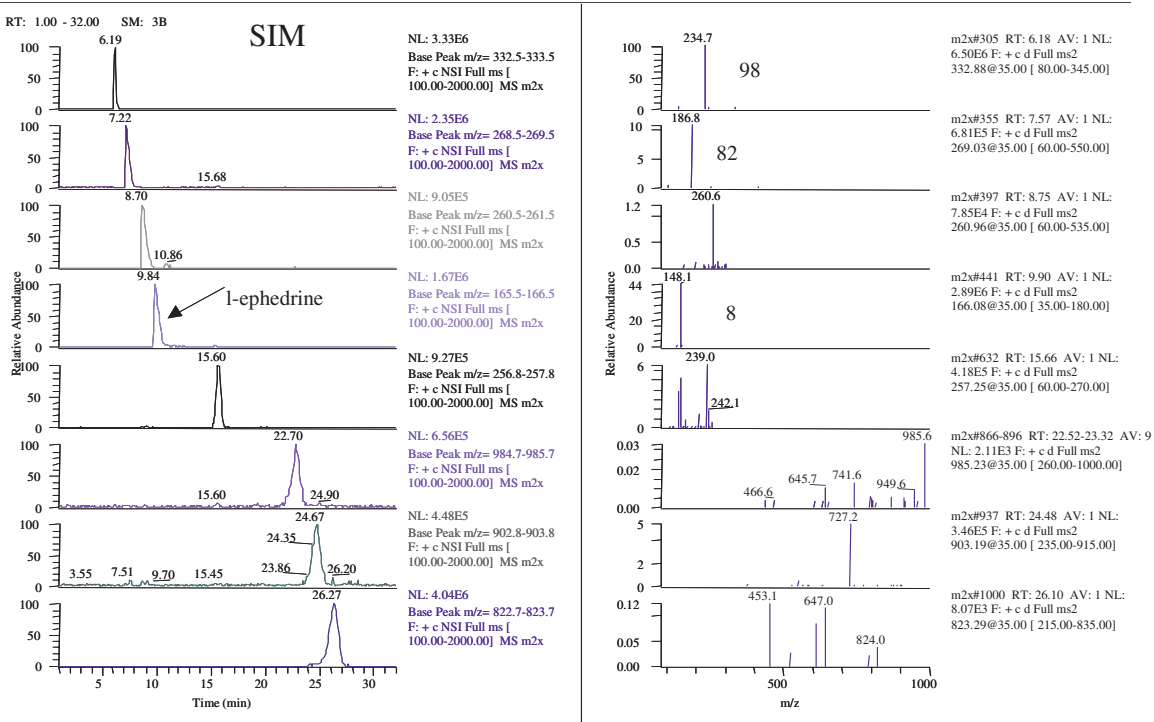


Figure 4. *CE-MS of the Eight Major Components in Ma Huang Capsule.*

CE: 75 µm × 80 cm; 0.5 psi/10 sec injection; 50 mM NH_4OAc in water/methanol (75/25), pH 4.0, 30 kV, 29 µA
LCQ: ESI/Sheath gas: 10, spray voltage: 4.5 kV

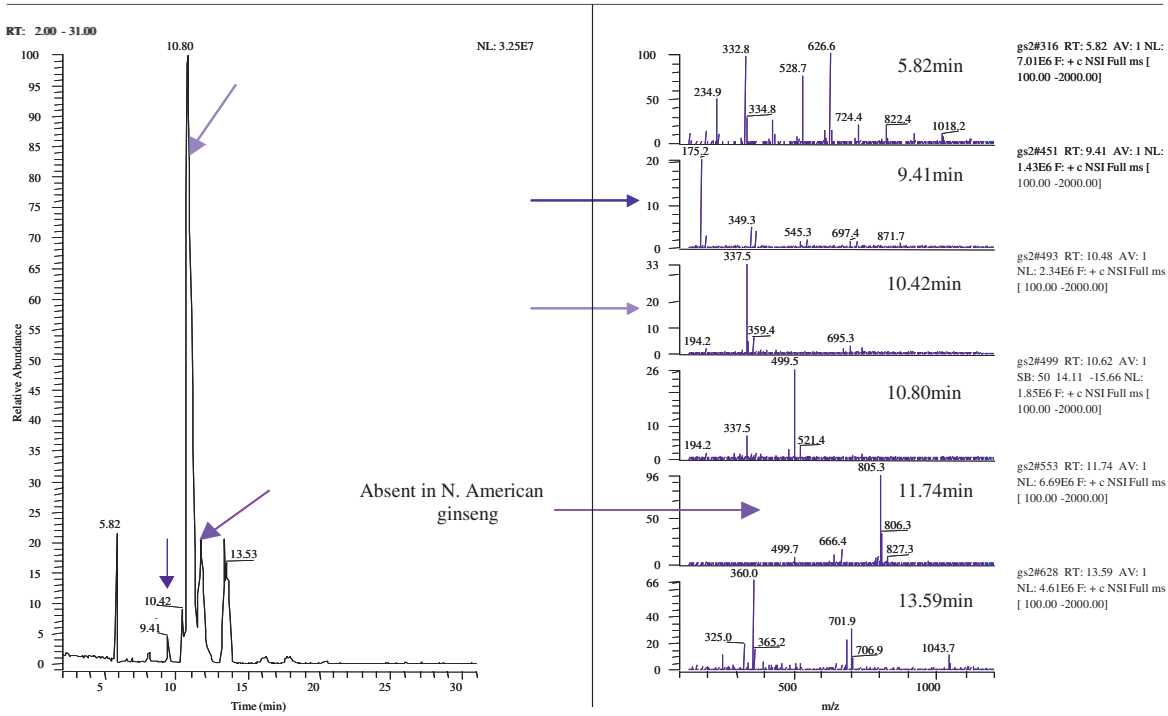


Figure 5. *CE-MS of Crude Extract of Asian Ginseng.*

coupled to an MS/MS system (LCQ* Advantage, Thermo-finnigan, Inc.).

2) The system is controlled by Xcalibur* software (Thermo-finnigan, Inc.) that integrates the CE and MS/MS systems with a single-point software control.
3) Buffer for CE analysis: 50 mM NH_4OAc in 75:25 water:methanol, pH 4.0. Sheath liquid for ESI: 5 mL/min of 1% HOAc in 80:20 methanol:H_2O. Applied potential in CE : 25.5 kV/39-43 mA. ESI potential: 4.5 kV.

Method of Herb Extraction

100 mg of each pulverized sample was dispersed in 1 mL water in a capped 2-mL vial and heated at 100ºC/1 hr. The aqueous extract was filtered and introduced directly to the CE system for analysis. *(Circa 101 B.C., "The herbal classic of the divine plowman" or "Shen Nong Ben Cao Chien" written about 101 B.C.)*

Samples Analyzed

Ma Huang

An antiasthmatic herb that contains the active ingredient ephedrine at about 1.0 to 1.5% of dry weight.

Ginseng

Recognized as the most popular medicinal herb used in traditional oriental medicine. The tonic essences are extracted in hot water.

Two major species: *Panax ginseng* (Asian version) and *Panax quiquefolius* (North American version) were used to compare the distribution of compounds in water extracts.

Results and Discussion

Aqueous extract of herbal product provides water-soluble compounds. Among them, the basic components in the crude extract are the most abundant species that can be readily analyzed in an open-tube capillary electrophoresis system.

The active ingredient in Ma Huang extract is characterized as ephedrine and its isomers by CE-UV using chiral analysis, while the presence of ephedrine is identified by CE-MS/MS analysis.

Major basic components in Ginseng from two species were characterized and some of the basic components were identified. Ginsengosides are neutral species in ginseng extracts and were not detected by the present CE-MS/MS procedure.

The present system combines CE and MS/MS systems with a single-point software control that provides an easy-to-use, robust solution to applications that require high-resolution separation, confirmatory characterization, and quality control.

CE: 75 µm × 80 cm; 0.5 psi/10 sec injection; 50 mM NH_4OAc in water/methanol (75/25), pH 4.0, 30 kV, 39 µA
LCQ: ESI/Sheath gas: 10, spray voltage: 4.5 kV

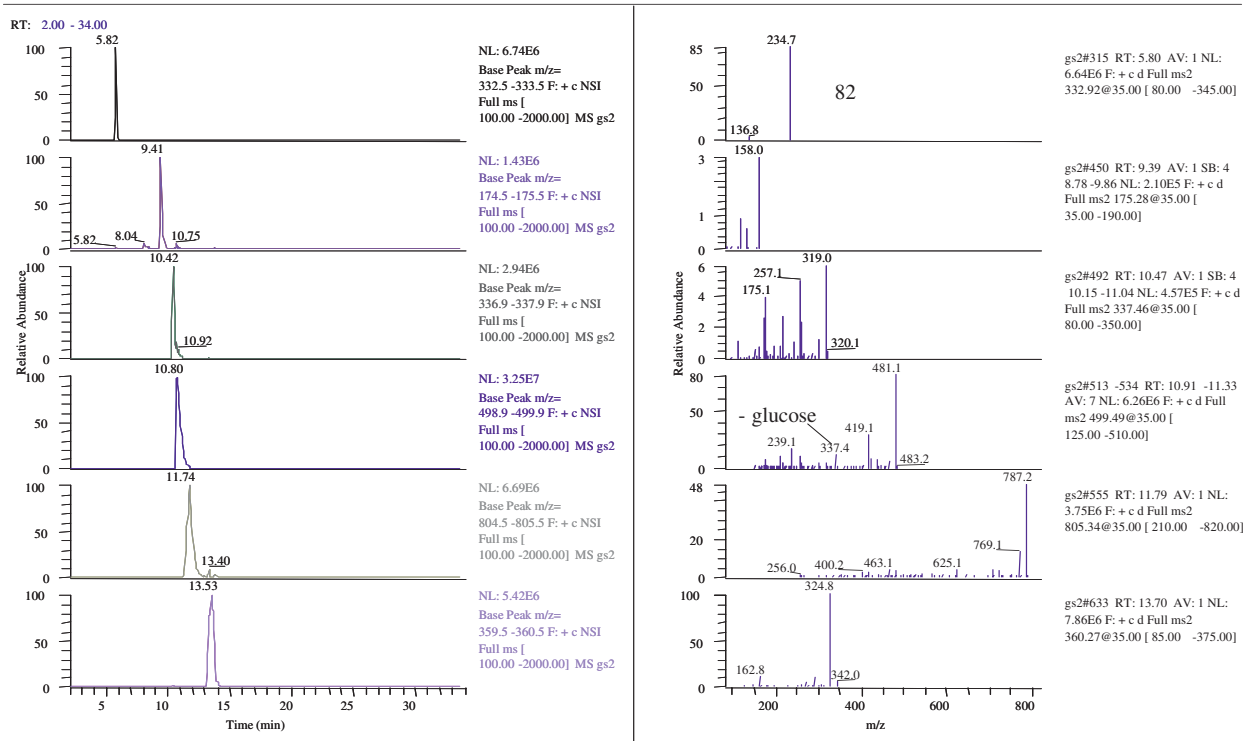


Figure 6. *CE-MS/ of Crude Extract of Asian Ginseng.*

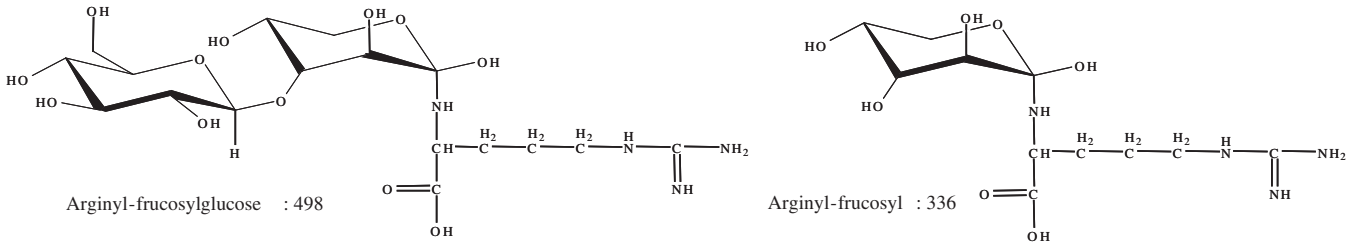


Figure 7. *Structures of Ginseng.*

MDQ Tip: Cleaning the Interface Block and Ejectors

Required Supplies

Before you begin, you will need:

- 2 mL vial with cap
- Tissue towelettes (such as Kimwipes®)
- Mirror
- Pen light
- Cotton swabs
- Distilled and deionized water

Preparation

1. Lift the cartridge cover door.
2. Loosen the two thumbscrews and lift the insertion bar.
3. Remove the capillary cartridge from the interface block.
4. Remove the ejector covers and ejectors for cleaning as shown in Figure 1.

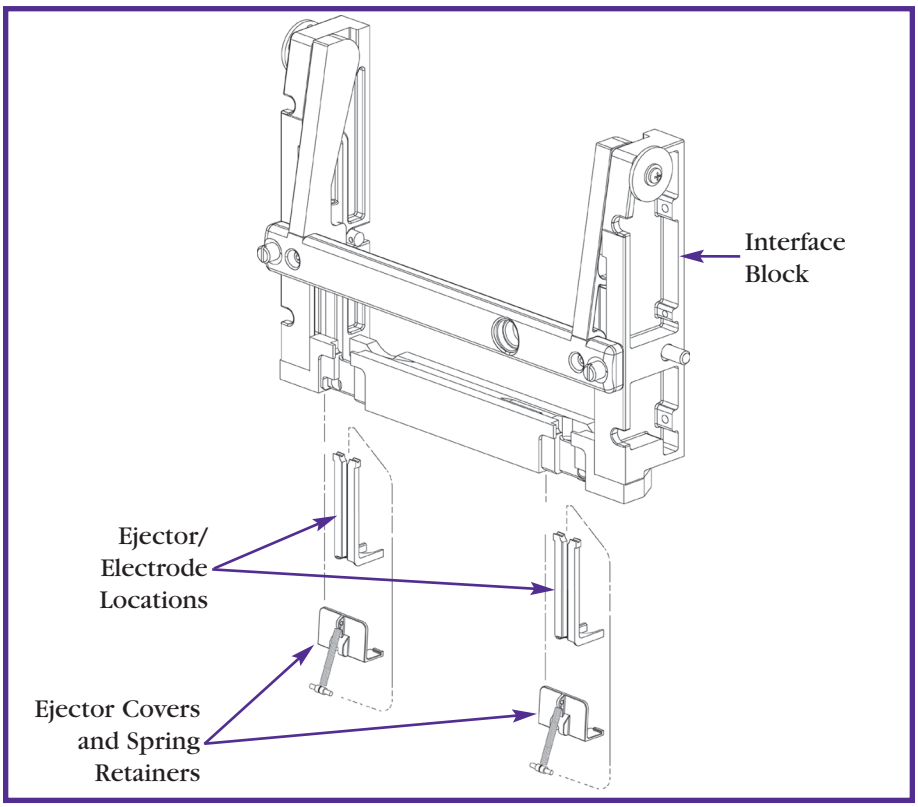


Figure 1. *Interface Block and Ejectors*

Cleaning

1. Using cotton swabs, clean interface block, electrodes and ejector surfaces with water followed by methanol, then allow to dry.
2. Refer to Figure 2. Wet a towelette with DI water. Place the towelette over the top of the capped 2 mL vial. Raise the vial over the electrode and up to the interface block. Rotate the vial so the wet towelette can clean the grooves on the underside of the interface block (A). Remove the vial and inspect the interface block using a mirror and pen light (B). Repeat this process until the interface block is clean.

Reinstallation

1. Reinstall the ejector in front of the electrode.
2. Reinstall the spring retainer and ejector cover.
3. Reinstall the capillary cartridge in the interface block.
4. Lower the insertion bar and tighten the two thumbscrews.
5. Close the cartridge cover door.

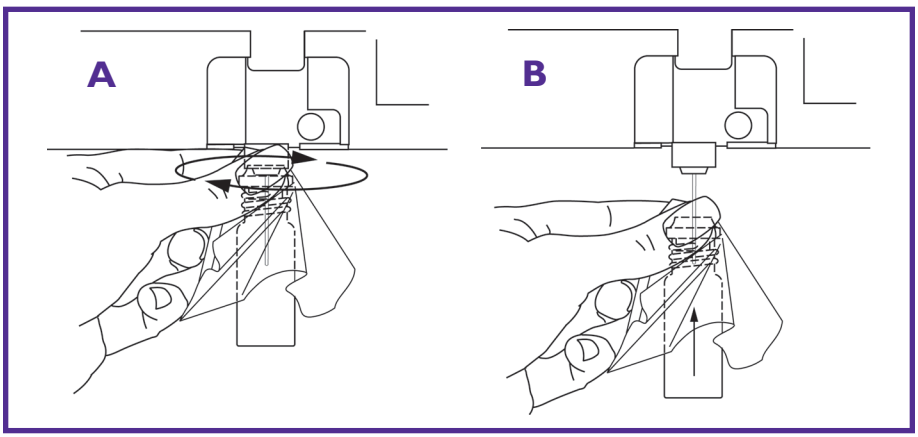


Figure 2. *Cleaning Interface Block*

Winner of the 2003 "Putting CE to Work" Award

Our congratulations go to Laura Bindila from the Institute for Medical Physics and Biophysics, University, of Münster, Germany, for her winning electropherogram titled "Characterization of peptides by capillary zone electrophoresis and electrospray ionization quadrupole time-of-flight tandem mass spectrometry."

The electropherogram was voted upon by those scientists attending the "CE in the Biotechnology and Pharmaceutical Industries 5th Symposium on the Practical Applications for the Analysis of Proteins, Nucleotides, and Small Molecules," August 23–25, San Francisco, CA.

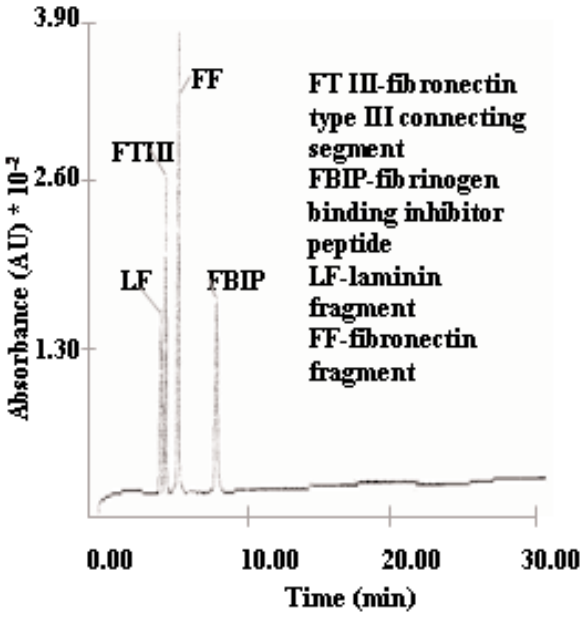


Figure 1. CE/UV profile of an equimolar mixture of FTIII, FBIP, LF and FF peptides

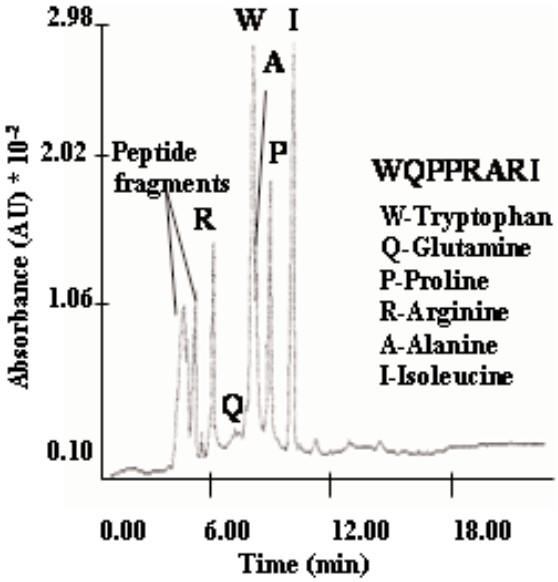


Figure 2. CE/UV profile of the hydrolyzed FAPP

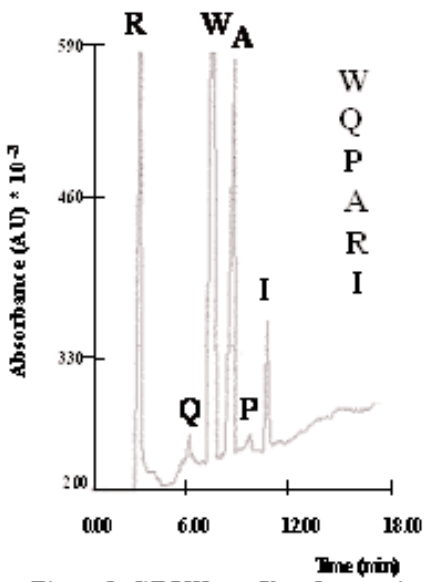


Figure 3. CE/UV profile of an equimolar mixture of W, Q, P, A, R, I amino acids

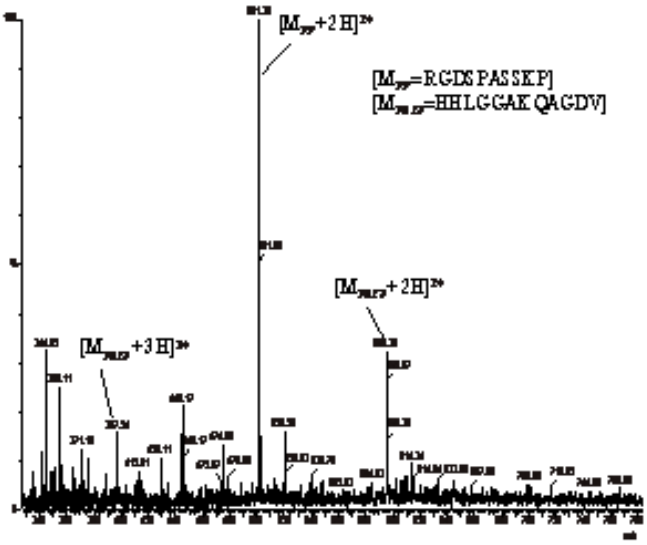


Figure 4. (+) nanoESI-QTOF-MS of the second CE collected fraction between min.6 and 11.0 from the FTIII, FBIP, LF and FF mixture separation

CE-MS IN THE PHARMACEUTICAL, BIOTECHNOLOGICAL, AND BIOMEDICAL ENVIRONMENT
FIFTH BELGIAN CE-USERS GROUP MEETING

JANSSEN PHARMACEUTICA N.V., BEERSE – BELGIUM
OCTOBER 16, 2003

The Belgian CE-Users group within the Royal Flemish Chemical Society (KVCV) is organizing the "Fifth Belgian CE-Users group meeting; CE-MS in the pharmaceutical and bio-technological and -medical environment " on October 16th, 2003, at Janssen Pharmaceutica n.v., Beerse - Belgium.

The meeting is a joint initiative of industrial partners together with academia, the Royal Flemish Chemical Society, and the equipment vendors, highlighting the practical impact.It is aimed to discuss the status and the usefulness of CE-MS through presentations of interesting applications in the pharmaceutical, biotechnological, and biomedical environment (special focus on proteomics).

The presentations will be of high scientific level and provided by renowned world-class experts in the field. The use of CE-MS will always be the key topic of the day in the presentations, however other related techniques may be discussed for comparison and demonstration of suitability.

Please register through our website at:
http:/www.kvcv.be/analytische.htm#CEMS

or contact:
Ilias Jimidar, Ph. D.
Johnson & Johnson Pharmaceutical Research and Development
Beerse, Belgium
Tel: 32(0)14-603387
email: ijimidar@prdbe.jnj.com