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PACE Setter

The worldwide newsletter for capillary electrophoresis

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Volume 2 • Issue 4 • Fall 1998

Advancements in forensic toxicology

CZE replaces GC/NPD as the screen of choice for basic drugs

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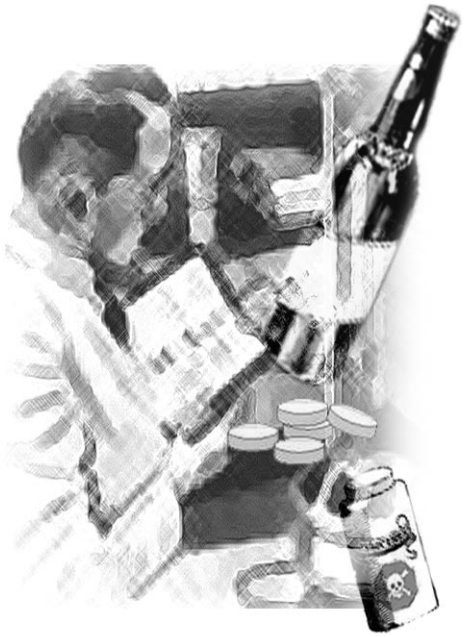
There is no greater analytical challenge in forensic toxicology than the "general unknown," the search for drugs or poisons that might have caused a death, impaired a driver or drugged the victim of sexual assault. Typically, the first step in such an analysis is one or more screening procedures, each designed to detect a number of analytes in a single analytical pass. All else being equal, the more compounds that are detectable in a single pass, the better.

Of all the compounds that are of potential interest forensically, organic bases are the most important. This group includes most of the drugs that affect the central nervous system, in addition to well-known poisons such as strychnine. It is easy to make a list of several hundred such analytes, and of course, a comprehensive screen would test for them all.

GC/NPD

In our laboratory, as in many others, gas chromatography (GC), with nitrogen-phosphorus (NP) or electron capture (EC) detection, has long been the basis for such a comprehensive screen.

Our screening process begins first with direct solvent extraction[1] of 1.0 ml of whole blood (no separation of neutral and basic compounds) followed by the analysis of the extract by GC/NPD, using retention index (RI) to discriminate naturally-occurring compounds from suspicious ones. This discrimination requires a compendium of RIs obtained under standard conditions. The RI of a suspicious peak is compared to values in the compendium and, on the basis of this comparison, the decision is made to write the case off as negative, or to confirm identity of the suspicious peak by mass spectrometry. Although mass spectrometry is ideal for confirmation, its role in the initial screening of complex sample matrices is less practical than with either CZE or GC/NPD.



Problems encountered

Although the GC screen performs reasonably well, it is not without its problems. Some analytes are thermally labile, others are adsorbed irreversibly to the chromatography system, and the complexity of the samples analyzed means constant attention to keep the system working satisfactorily. In addition, a significant fraction of the samples submitted are putrid. The products of putrefaction unfortunately include organic bases, often in sufficient concentration to overwhelm a NP detector. Thus we are always alert to better ways to perform the screen. Could capillary zone electrophoresis solve many of these problems and replace GC/NPD?

The CZE system

A number of workers [2, 3, 4] have demonstrated the possibility of using CZE to analyze drugs in various matrices. For the past five years, we have worked on the development of capillary zone electrophoresis (CZE) as an adjunct to, and a possible replacement for, GC/NPD analysis in our screen for basic drugs. Initially, we used a Beckman Coulter P/ACE™ 5500 with either single-wavelength UV or diode array detection (DAD). More recently, we have used a ▶

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Beckman Coulter P/ACE™ System MDQ with DAD. We chose the simplest possible system: uncoated fused-silica capillaries, 50 or 75 micron i.d. with an unmodified run buffer (100 mmol/L phosphate, pH 2.38). We normally use electro-kinetic injection, typically eight seconds at 10kV, and a separation voltage of 18 to 20kV. Run time is typically 25 minutes per sample. Detection is by UV monitoring at 200 nm. Complete details have been published elsewhere [5, 6].

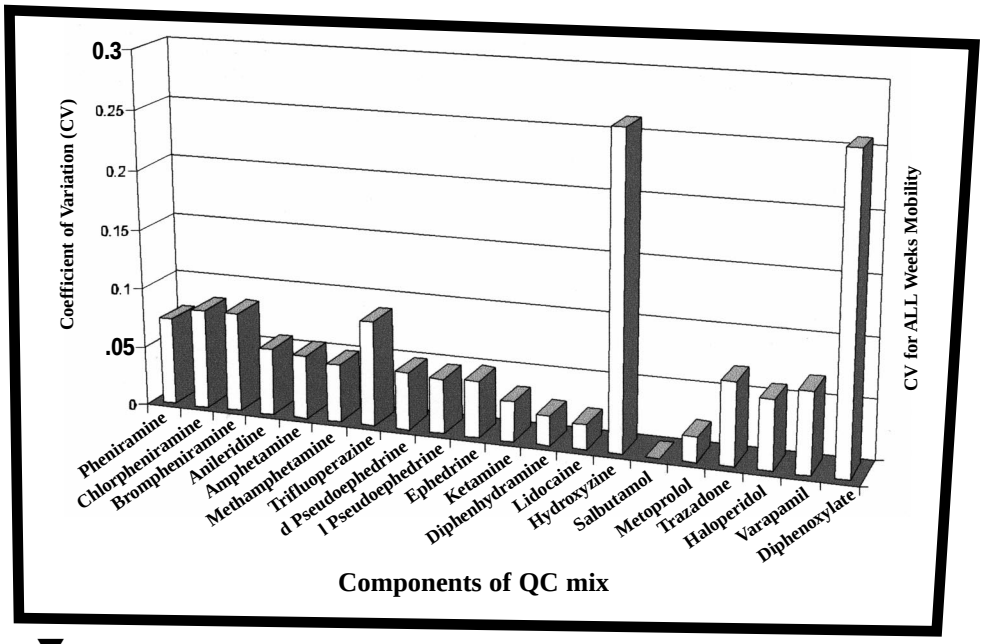

Figure 2: CVs of migration parameters for QC mixture.

Mobility and UV spectral libraries

To develop an effective screening process we needed first to generate a compendium of analytes identifiable by their migration behavior [5,6], just as described above for GC and RIs. In the process, we found that by coupling an analyte's mobility data with UV spectral data, we could arrive upon at least tentative identification more rapidly. This process is illustrated in Figure 1.

The mobility of an unknown peak, "?" is used to create a list of possibilities, and the UV spectrum is used to further shorten this list. Often (although not always), the combination of mobility and UV spectrum will narrow the list of possibilities to a single compound. This raises the question of how much information is actually needed for an identification to be made. The combination of RI and detector selectivity produced by a GC/NPD analysis would not be considered rigorous identification. The combination of mobility and UV spectral data generated by CZE-DAD is much stronger evidence. In some instances, it may be enough.

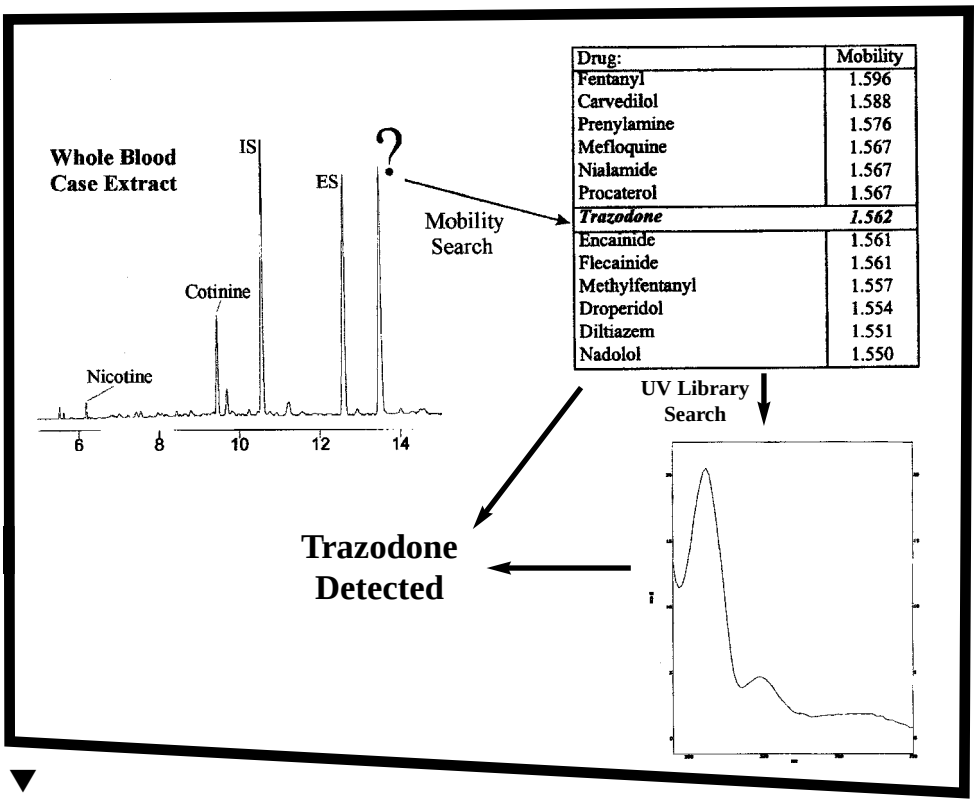

Figure 1: Screening blood for basic drugs.

Mobility is highly reproducible

This approach requires that migration behavior be expressed in a way that gives reproducible results run-to-run and day-to-day, in much the same way that the RI does for GC. Migration behavior expressed as effective mobility [7] meets these requirements. This quantity (called simply mobility in this article) may be described as the mobility of the analyte (with dimensions of cm2/v.s) corrected for the mobility of electroosmotic flow. We have found inter-day mobility to be highly reproducible with coefficients of variation (CV) or relative standard deviation (RSD) less than ±0.3%. Figure 2 illustrates the CVs on mobility on 20 different drugs run every day for four months. This high degree of reproducibil-

ity means two things: 1) In most cases, the list of possibilities truly is a short list; 2) A given peak can be eliminated as a possibility with a high degree of confidence.

This means, first of all, that confirmation of a suspected positive case is simpler because there are fewer possibilities to consider. And, second, it means that the negative case is identified quickly and reliably. Both factors are important in our operation and provide a high degree of confidence that false positives and, more importantly, false negatives can be avoided.

Resolution, sensitivity and real samples

Figure 3 illustrates the resolving power of the CZE screen. The electropherogram shows the analysis of a drug mixture that we use as a check on instrument performance. The mixture consists of 20 drugs in aqueous solution, each at a concentration of 1 µg/mL. This mixture includes several groups of analytes that are closely related, such as amphetamine and methamphetamine, thus providing a measure of the system's resolution (See Table 1 for a legend for Figures 3, 4 and 5.) However, to detect a variety of drugs in pure solution is one thing; to detect them in an extract of whole blood is another matter altogether.

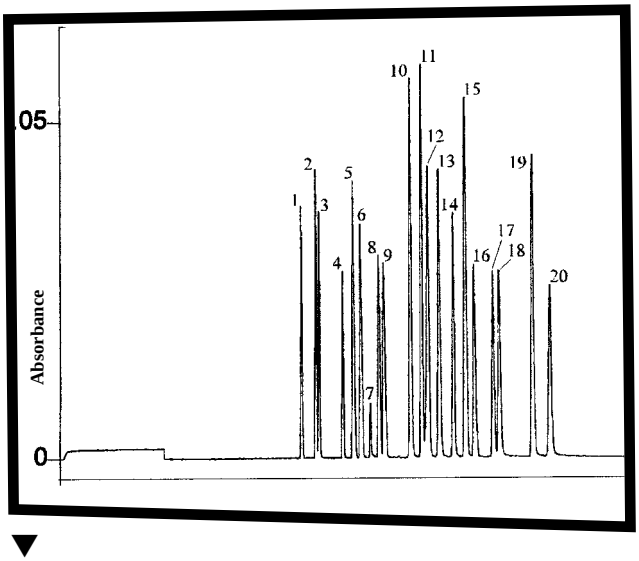

Figure 3: Quality control sample 1 µg/mL in water 8-second 10kV injection.

In whole blood there are two major factors that influence the detection of basic drugs by CZE-DAD. First, analyte concentration in the solution from which injection is made must be at least 0.3 µg/mL for consistent detection. Second, typical therapeutic levels of most basic drugs in blood are on the order of 10-200 ng/mL. These factors simply mean that, as with GC/NPD, there must be some pretreatment of blood samples to concentrate the analyte if nothing else.

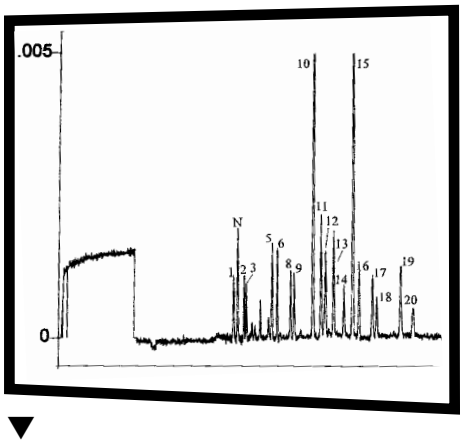

Figure 4: Quality control extract, 10 ng/mL porcine blood.

Figure 4 shows analysis of the residue from direct solvent extraction of whole blood into which a number of drugs were spiked, each at a level of **10 ng/mL** of blood. The mixture of drugs was identical to that shown in the top electropherogram in the figure, except that anileridine and trifluoperazine were not included. It is clear that all the drugs in the mixture are easily detected at 10 ng/mL although our experience has been that analyte concentration has to be about 50 ng/mL before the spectrum can be effectively used. Generally, being able to detect analytes at 10 ng/mL is adequate for detecting forensically significant levels of most basic drugs, and we see such performance as a matter of routine.

Figure 5 shows a negative case. Such drug-free specimens typically show a very simple electropherogram. Peaks are visible for the added reference compounds, methoxamine and salbutamol, as they were in the first two electropherograms. Nicotinamide (NC)▶

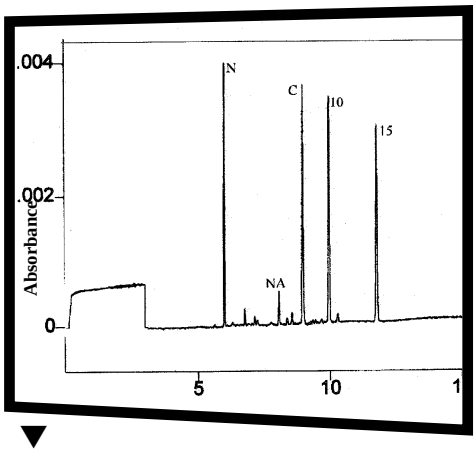

Figure 5: "Normal" extract whole blood, heavy smoker. IS - 50 ng/mL. Residue in 30 µL ES. Es - 1 ng/µL.

QC Mixture*

1 — Pheniramine
2 — Chlorpheniramine
3 — Brompheniramine
4 — Anileridine
5 — Amphetamine
6 — Methamphetamine
7 — Trifluoperazine
8 — Pseudoephedrine
9 — Ephedrine
10 — Methoxamine (IS)
11 — Diphenhydramine
12 — Dextromethorphan
13 — Codeine
14 — Hydroxyzine
15 — Salbutamol (ES)
16 — Metoprolol
17 — Trazodone
18 — Haloperidol
19 — Verapamil
20 — Loperamide

N.NA - Nicotine, Nicotinamide
C —Cotinine
*** Anileridine and Trifluoperazine were not added to the 10 ng/mL spiked blood.**

Table 1: Legend/QC mixture for Figures 3-5.

is often observed, and in samples from smokers nicotine (N) and cotinine (C) are consistently present. No other peaks of significant size are observed. These clean and simple electropherograms are highly desirable in making it easier to detect the presence of a forensically significant compound.

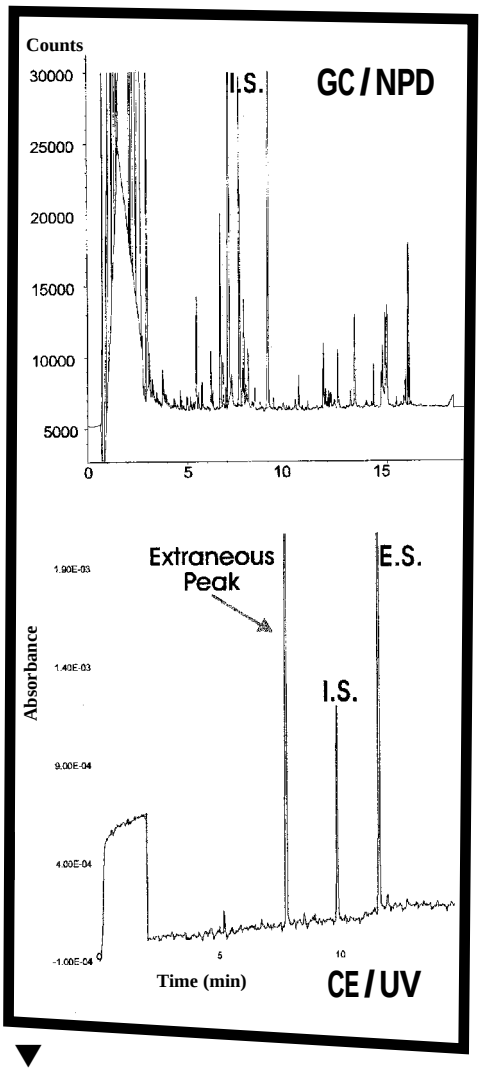

Figure 6: Putrefaction of whole blood in vitro 20ºC. Day 56.

Putrefaction

This simplicity is noted with putrefied samples as well. Figure 6 compares the CZE electropherogram and NP chromatogram for the same sample of decomposed whole blood without preservative. Not surprisingly, beta-phenethylamine (labeled "Extraneous Peak") is detected by CZE, but the electropherogram remains simple, presumably because most of the products of putrefaction do not absorb at 200nm or are weakly basic compounds and are not injected electrokinetically onto the column. By contrast, the NP chromatogram is much more complex and, in our experience with cases of this sort, it is often uninterpretable. Once again the clarity of the electropherogram is highly desirable

Case example: problem drugs

Figure 7 shows an example of a positive screen. The bottom chart is the electropherogram of a specimen containing paroxetine (eg: Paxil® - SmithKline Beecham). This compound is one that is known to behave erratically in our chromatography system. The GC/NP chromatogram for the same blood sample is shown in the upper chart. If this sample were considered a general unknown in which any of a long list of drugs might — or might not — be present, it is clear that paroxetine would almost certainly be overlooked in the NP chromatogram, but easily identified and quantified in the electropherogram.

Case example of CZE robustness

We have also observed that, by contrast with GC, CZE is remarkably robust in the face of sample overload. Figure 8 shows two consecutive electropherograms from a batch of case analyses. The first chart shows the electropherogram of a sample taken from a case of massive overdose[9] with venlafaxine (eg: Effexor® - Wyeth-Ayerst). The second chart shows a negative case which was run immediately after the overdose, separated only by a routine rinse of the capillary. The reference compound, salbutamol (ES), is overwhelmed by the venlafaxine peak in the top chart. However, as the bottom chart shows, carryover of venlafaxine is nil, and that has been our experience consistently.

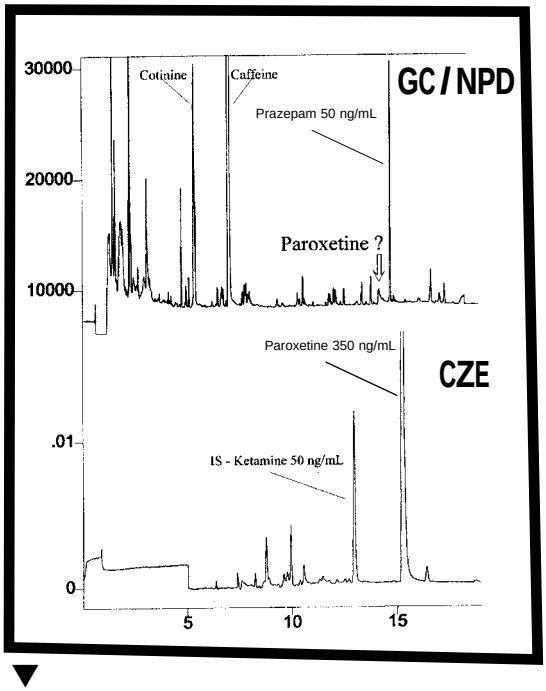

Figure 7: Paroxetine (Paxel) by CZE and GC/NPD.

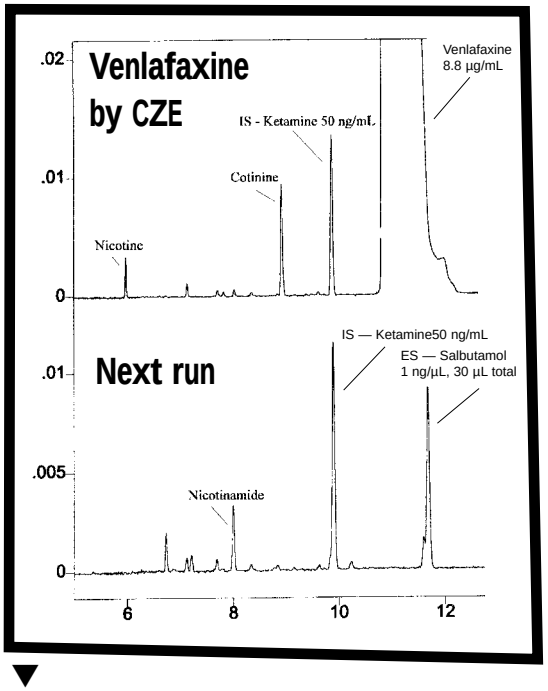

Figure 8: Robustness of CZE/Venlafaxine (Effexor).

The future is bright for CZE

Given all this experience, are there shortcomings in the CZE-DAD approach to screening the 'general unknown'? The only one of any note in our operation is this: the limit of detection; yet 10 ng/mL of blood covers forensically significant levels of most organic bases. For those weakly basic drugs, notably the benzodiazepines such as flunitrazepam and triazolam, where such a cutoff is simply not adequate, a more sensitive method of detection, such as GC/EC, must be applied.

The future truly is bright for CZE. In simple terms, CZE-DAD is the screen of choice for basic drugs by toxicologists at this laboratory.

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Compliance Part II:

Equipment qualification tools

BACK TO SCHOOLS

Jim Schools

Tools to support our customers' compliance with regulations such as GLP and GMP were highlighted in the last issue of P/ACE Setter. This article will focus on a subset of those tools: Equipment Qualification, commonly referred to by its initials, EQ. EQ is a process that is frequently used to assure that equipment used during the drug development and manufacturing process is performing according to its intended purpose. This is keeping in line with the overall process of validation.

Follow sound science

EQ is commonly divided into several phases which provide a timeline approach, documenting that a given piece of equipment is performing according to its intended function. These are typically referred to as DQ (Design Qualification), IQ (Installation Qualification), OQ (Operational Qualification), and PQ (Performance Qualification). While these terms are generally used today, it is interesting to note that 1) the terms themselves are not defined within the GLP or GMP regulations and 2) the definition of the terms often varies depending on who is defining them, sometimes widely. As with most processes that are used to comply with FDA regulations, there is not a "right" or "wrong" way to do it. What is important is to follow sound science, document the process, and continue to follow the defined process.

In the development of useful customer EQ tools, Beckman Coulter followed definitions outlined in a document published by the Laboratory of the Government Chemist.[1]

This document provides a complete and logical description of the EQ process that is compatible with the definitions followed by most users and vendors in the biotechnology and pharmaceutical industries. Briefly, the EQ process is broken down as follows:

DQ: Prior to purchasing equipment, define its intended use in relation to the actual processes that it will be used for. This assures that appropriate equipment will be ordered and placed into operation.

IQ: Verify that the equipment was received as ordered, that it is correctly installed in its selected environment, and that all support documentation is provided.

OQ: Verify that the equipment is properly performing in its selected environment.

PQ: Verify that the equipment is properly performing according to its intended use.

As a company that completely operates under quality system processes (ISO 9001 and GMP for medical devices), Beckman Coulter already provides services for our customers that help com- ▶

ply with these processes. For example, the publication of technical specifications as well as application notes that solve our customers' problems is used in conjunction with consultation to assure that an instrument is selected that is appropriate for the problem or task at hand (DQ). As part of the normal installation process there is verification of receipt of the proper equipment and installation (IQ), verification that the instrument is functioning properly (OQ), and training of the user (not part of EQ, but complies with GMP 211.25). Instrument hardware and software features are provided that can simplify ongoing system suitability and/or control trending (PQ).

The documentation provided from these processes is commonly used to support EQ of instruments such as the P/ACE™ System MDQ. Because of our charter to simplify our customers' processes, we are introducing additional tools that can help the EQ process, or even make it turn-key.

Additional programs to assist in compliance

IQ: An additional data sheet is completed upon installation that documents the equipment received (model and serial numbers), the documentation provided (including safety precautions), and proper installation of the instrument. This procedure can be provided for any P/ACE instrument, and is provided at no charge, when requested, with the purchase of a new system.

OQ1: A datasheet is provided to document that the P/ACE is functioning properly in its installed environment. OQ1 is performed during the installation of the instrument. OQ1 is available for the P/ACE 5000 or P/ACE System MDQ, and is also provided at no charge with the purchase of a new instrument, when requested. The OQ1 procedure could also easily be performed by the user as well. It can also be purchased as an ongoing service from Beckman Coulter.

OQ2: Contains the same procedures as OQ1 with the addition of a software certification procedure that uses a reference disk to verify that the binary program files are correctly installed.

OQ3: A more comprehensive testing procedure using a standardized set of tools to verify proper instrument function.

A variety of OQ tools is offered not to make things confusing, but to provide a choice that will most closely fit the requirements of a particular user. Ongoing experience with the biotechnology and pharmaceutical industry has shown that there is no one way that EQ is performed, and that a product that meets the need of one company may be too complex or too simple for another.

The IQ and OQ phases typically take place upon original receipt of the instrument, with additional OQ's possibly performed at ongoing intervals (such as yearly). While an OQ procedure can verify that an instrument is performing properly at a given moment in time, it does not directly verify continual proper operation. The most important part of the EQ process is PQ, not only because it is ongoing but because it is the process that verifies that the instrument is properly performing on a daily basis, or for a particular data set. The PQ process should be done by the user using known standards and methods that are the same or similar to the samples and their methods. The software provided for P/ACE Systems allows the running of system suitability or control standards for this purpose.

Product information

144867	IQ for all Instruments
144869	OQ1 for P/ACE System MDQ
721110	OQ1 for P/ACE 5000 Series
721112	OQ1 for HPLC
144870	OQ2 for P/ACE System MDQ
721111	OQ2 for P/ACE 5000 Series
721113	OQ2 for HPLC
144688	OQ3 for P/ACE System MDQ
726140	OQ3 for P/ACE 5000 Series
726139	OQ3 for HPLC

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If you would like to see an article on a particular topic in the areas of compliance, please email Jim Schools at jschools@beckman.com

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Detection of psychoactive drugs in human hair

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CE has proved suitable for the investigation of seized products (Lurie, 1997), basic drugs in urine (Schafroth et al., 1994, Taylor et al., 1996) and in whole blood (Hudson et al, 1998). However, only a few studies have been done so far on the CE analysis of extracts from human hair. Only cocaine, morphine and methylenedioxymethamphetamine (MDMA) (Tagliaro et al., 1998) and methadone and its metabolite (Frost et al., 1997) have been determined in hair using CE.

In this study we applied capillary electrophoresis (CE) to detect several psychoactive drugs; three benzodiazepines (diazepam, nordazepam and 7-aminoflunitrazepam), codeine and propoxyphene in human hair and compared our results obtained previously with gas chromatography/mass spectrometry (GC/MS) or high pressure liquid chromatography (HPLC), respectively.

Material and methods

All reagents were of analytical grade. Codeine and propoxyphene were purchased from Radian Corp. (Austin, TX) nordiazepam by Will-Pharma, Brussels (Belgium), diazepam, flunitrazepam by Roche, Reinach (Switzerland). The solid-phase extraction was carried out with Chromabond, C18ec extraction columns (Macherey-Nagel, Dueren, Germany). The ball mill used was purchased from Retsch (Haan, Germany).

Instrumentation

This work was carried out using a PACE™ System 5500 (Beckman Coulter) equipped with a photodiode array detector. CE conditions were the following:

• 50 cm x 75 mm i.d. fused silica capillary with a polyimide coating on the outer surface and an untreated inner surface

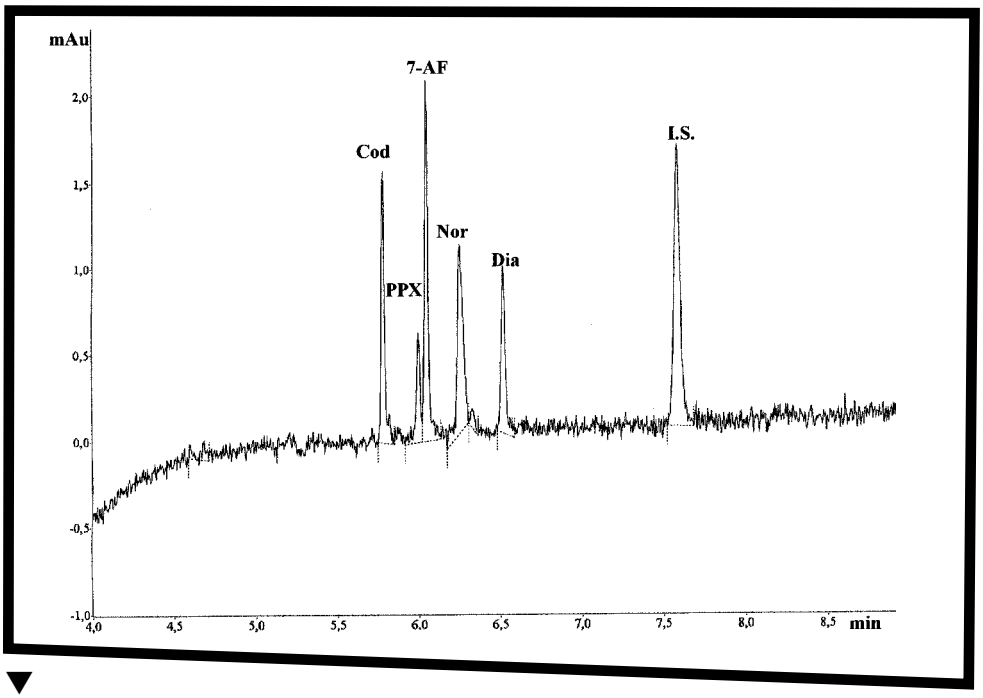

Figure 1: Electropherogram of spiked hair.

• running buffer was a phosphate buffer, CElixir™ pH 2.3 provided by Analis, Namur (Belgium)

• separation voltage was 30 kV

• temperature was 25°C

• injection was performed by 5 second pressure (0.25 psi)

• analytes were detected by UV Absorbance at 200 nm. Photodiode array data was collected over 190 to 350 nm wavelength range.

Specimen collection

Hair specimens were taken from nine subjects (three female and six male aged at time of death from 19 to 26 years) who died from a heroin overdose. Post-mortem tissue analysis previously showed that the subject had consumed concomitantly with heroin benzodiazepines.

Sample preparation

To eliminate external contamination hair was washed with water and acetone, then hair strands were cut into segments of 3 cm and pulverized in a ball mill. Pulverized hair was incubated during two hours at 60° in a urea/thioglycate buffer (8M/0.2M) at pH 3. After solid-phase extraction (Chromabond C18ec columns), extracts were dissolved in running buffer and analyzed by capillary zone electrophoresis. Nitrazepam was used as internal standard at a concentration of 3.3 ng/mg hair. Calibration curves with spiked hair were performed using 1.6, 3.2, 8.3 and 16.7 ng/mg hair of codeine, nordazepam, diazepam, 7-aminoflunitrazepam and propoxyphene.

Results

Diazepam, Oxazepam, Nordazepam and the metabolite of Flunitrazepam (7-Aminoflunitrazepam), codeine and propoxyphene were well separated using our CE conditions at pH 2,3 (Figure 1) in about 8 minutes. Calibration curves were linear in the range used (1.6 to 16.7 ng/mg hair) and the detection limits for the different drugs was about 1 ng/mg hair. ▶

When we compare our CE data with results obtained previously by GC/MS with the same samples (Yegles et al., 1997), we were able to confirm qualitative GC/MS results in 19 of 21 measures. Moreover, the quantitative results obtained by CE were significantly higher than the results obtained by GC/MS. When we compare now our propoxyphene CE measurements with HPLC results obtained previously (Mersch et al., 1997), we notice that qualitatively and quantitatively the HPLC results were relatively similar with CE.

Discussion and conclusions

It can be concluded that the method developed using capillary zone electrophoresis is fast, reliable and accurate, allowing simultaneous and direct determination of several psychoactive drugs in human hair for concentrations higher than 1 ng/mg hair. In most cases we confirm with CE the qualitative results obtained previously with gas GC/MS or HPLC, respectively. However, the quantitative results obtained by CE were higher than the results obtained by GC/MS.

While with propoxyphene, the quantitative results obtained by HPLC were relatively similar with those obtained by CE (higher concentrations — 13.2 to 18.9 ng/mg). These results confirm that the CE technique represents an interesting tool of investigation in forensic and clinical toxicology. It has proven effective, providing simultaneous determinations of different drugs without derivatization.

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A few words on volumes of information

To see the world in a grain of sand
And heaven in a wild flower,
Hold infinity in the palm of your hand,
And eternity in an hour.

— William Blake

CURRENT AFFAIRS

Harry Whatley

While the world in a grain of sand may be a stretch for most of us, the dimensions employed in capillary electrophoresis are much smaller than those with which we are accustomed to working. Capillary diameters are measured in micrometers, the same unit of measure that is applied to microbes such as bacteria. The entire volume of a capillary is typically a few microliters. Injected sample volumes are in nanoliters. At these scales the tolerances required of instrument systems are very small. The ability to handle these very small volumes is of paramount importance to successful CE.

Measuring volume

There is no external sample measuring loop in a CE system like that in an HPLC. Samples and the separation matrix alike are directly delivered to the capillary where the separation will take place. Fluid delivery in capillary electrophoresis is hydrodynamic, i.e., a pressure differential is created between the ends of the capillary. This pressure difference can be accomplished in different ways (positive pressure, vacuum, raising one end of the capillary). The amount of fluid delivered by pressure through a cylindrical tube can be estimated using the Poiseuielle equation:

$$V=(\Delta P d^4 \pi t)/(128 \eta L)$$

This equation has terms for the pressure difference between the ends of the capillary (ΔP, Pascals), the time pressure is applied (t, seconds), capillary diameter (d, meters), length (L, meters), and the viscosity (η, Pascal-seconds) of the fluid being moved. Temperature enters into this calculation indirectly since the viscosity of many common fluids varies with temperature.

Examining this equation we see that the fluid delivery varies linearly with pressure and time, inversely with viscosity and length, and with the fourth power of the diameter. This relationship with the capillary diameter is very important. A variation as little as one or two micrometers can cause a significant change in fluid delivery. Figure 1 illustrates this.

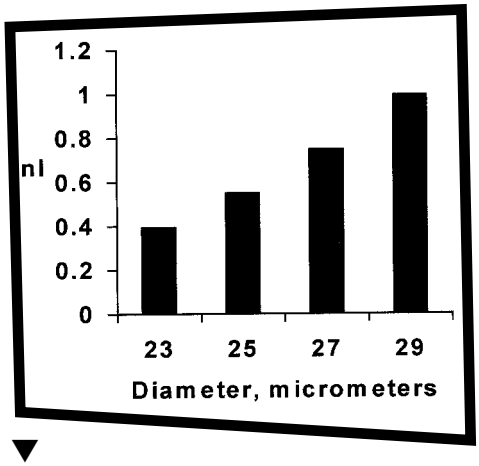

Figure 1: Capillary diameter effect on injection volume.

In this experiment all of the sample injection parameters are kept constant except the internal diameter of the capillary. Using capillary dimensions ranging from 23 to 29 micrometers will result in injection volumes from 0.393 nanoliters to 0.747 nanoliters. It is important to understand that differences in diameter from capillary to capillary may result in significant changes in the peak area calculated.

Effects of viscosity

Viscosity is the most complex factor in the Poiseuille equation. The effect of temperature on viscosity, for example, can be complex. The viscosity of pure water varies in a non-linear manner from 1.519 centipoise (cp) at 5º C to 0.355 cp at 80º C. The addition of solutes or other solvents can completely alter the viscosity profile. A 1:1 mixture of methanol and water, for example, has a higher viscosity than either pure solvent. The velocity of the flow can also be a factor, particularly for solutions of polymers. Linear polymers may tend to line up when forced through a narrow opening like a capillary and actually show a decreasing viscosity with increasing velocity. Because it is complex, viscosity is hard to measure. The value obtained may vary with different measurement techniques (for this reason the technique employed for the measurement of viscosity should always be reported with the result).

For practical purposes we usually assume the viscosity of the sample is insignificant compared with the viscosity of the buffer in the capillary. This is a fair assumption unless the viscosity varies significantly from sample to sample or from sample to standard. Using an external standard made in water to calibrate an instrument for the analysis of a viscous sample can lead to gross errors of quantitation. If it is necessary to operate under these conditions, the inclusion of an internal standard in both the external standards and samples is advisable to normalize for the viscosity differences.

Another parameter that is important is capillary length. This can be measured with a simple ruler. Accuracy to within a millimeter is sufficient for all but the shortest capillaries. It is essential, however, to actually measure the capillary and not to rely on the way a piece of capillary fits into an instrument cartridge. If the capillary cannot be trimmed to length before installation it should at least be measured and marked, installed, then cut to the marks.

Controlling pressure

We depend on the instrument to accurately control pressure and time. The pressures used to inject samples are typically very small, usually less than 1 psi (70 mbar). Regulating such low pressures is an instrument design challenge that, happily, has been met in the P/ACE™ instrument line. Positive pressure can be delivered from a cylinder of compressed gas (as in the P/ACE 5000 Series) or from an on-board pump (as in the P/ACE System MDQ).

Regardless of how the pressure differential is created, a finite time is required for the pressure to reach a steady state. Pressure will change during the injection time as pressure ramps up, stabilizes, and ramps down. Systems that rely on pressure or vacuum must have some sort of feedback mechanism to compensate for these variations. These systems either adjust the delivered pressure or the delivery time to maintain the desired product of pressure and time.

P/ACE instruments monitor the pressure and automatically adjust the time to give the desired value of DP*t. In theory a 3-second injection at 1 psi and a 10-second injection at 0.3 psi should give identical results, because both are 3 psi-second injections. In practice, feedback loops require some time to operate. The longer the time allowed, the more accurate the regulation will be. For this reason better reproducibility is usually obtained with long, low-pressure injections than with short, high-pressure sample delivery.

Effects of sample volume

Sample volume can affect the performance of the separation. One of the factors that accounts for the high peak efficiencies in CE is that fluid movement during the electrophoresis is more or less uniform across the diameter of the capillary. Delivery by pressure is laminar. In laminar flow, fluid moves faster at the center of the tube than at the walls. High-pressure injections can create a concentration gradient in the sample plug that extends both across the capillary and down part of the length of the injection plug. Distorted peak shapes may result.

QC Mixture*

Capillary Diameter	Seconds @ 0.5 psi	Injected Vol (nl)	Injected Plug (mm)
20	5	0.13	0.40
20	60	1.5	4.8
50	5	4.9	2.5
50	60	59	30
75	5	25	5.7
75	30	150	34
75	60	300	68

Table 1: Theoretical injection volumes, 25 °C, viscosity of water, 60 cm total length

As mentioned before, the injected volume in an open capillary separation is measured in nanoliters. Because of the geometry of the capillary even this small volume can occupy a substantial linear dimension. The ability of a method to focus or stack this linear dimension, or plug length, is a major determinant of peak shape and resolution.

Table 1 gives some examples for typical capillary diameters. Transferring the same injection conditions (pressure and time) to different capillary dimensions will result in different volumes being introduced. What is less obvious is that the plug length occupied by a given volume increases less rapidly than does the volume delivered as the capillary diameter increases. This can be exploited to increase sensitivity. Note that a 30-second injection into a 75-micrometer capillary delivers 2-1/2 times more sample than does a 60-second injection into a 50-micrometer capillary while only increasing the plug length from 30 to 34 mm.

P/ACE capillary electrophoresis instruments do a very good job of regulating the mechanical and electrical processes that impact run-to-run reproducibility. An understanding on the scale of the processes involved can simplify method development and lead to greater success with CE.

SAMPLE VOLUME CAN AFFECT THE PERFORMANCE OF THE SEPARATION. ONE OF THE FACTORS THAT ACCOUNTS FOR THE HIGH PEAK EFFICIENCIES IN CE IS THAT FLUID MOVEMENT DURING THE ELECTROPHORESIS IS MORE OR LESS UNIFORM ACROSS THE DIAMETER OF THE CAPILLARY.

To simplify calculations like those shown in this article, Beckman Coulter offers free computer software called CE Expert. Contact your local Beckman Coulter representative to obtain your copy.

Dr. Harry Whatley is a CE Product Planning Specialist for Instruments

HSCDs

A single-method approach to enantiomer analysis

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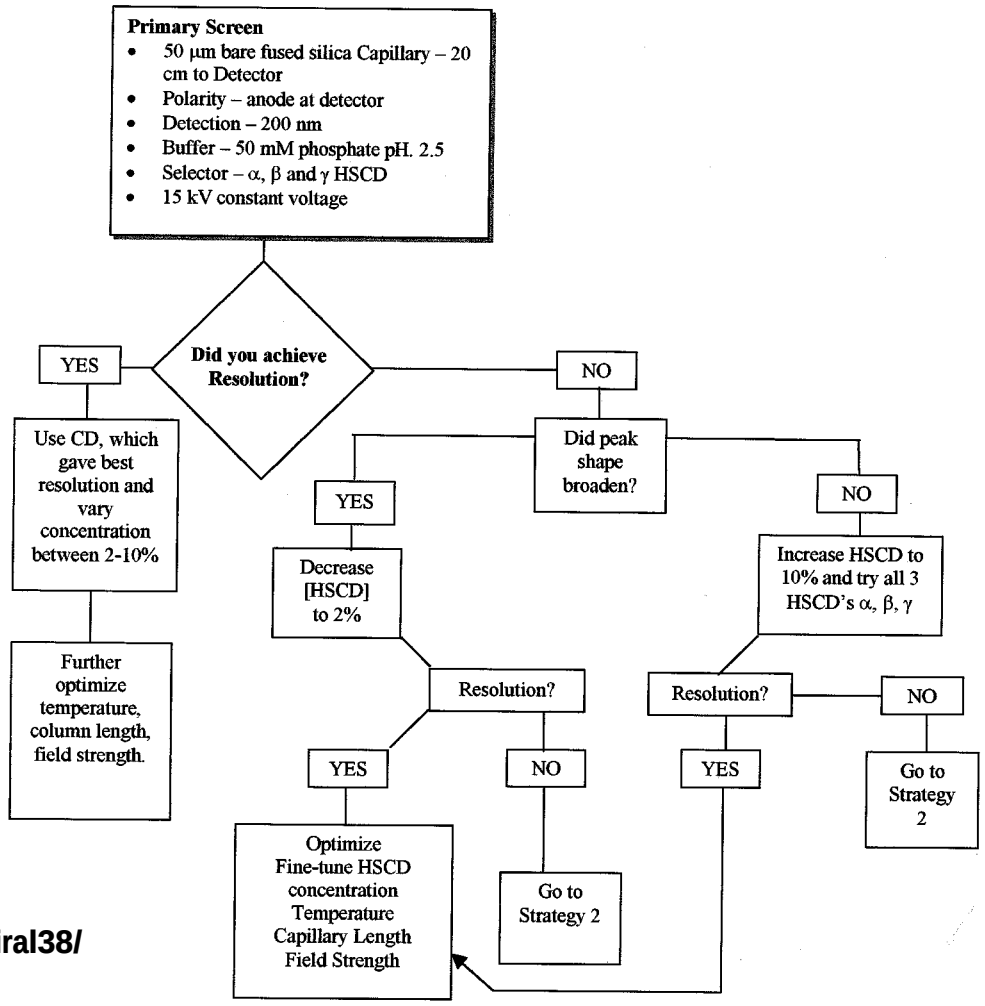

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P/ACE Setter

Palm Springs, Calif., Jan. 25, 1999

Plan to attend 1999 P/ACE User's Meeting at HPCE

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- **have fun**

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