APPLICATION INFORMATION

Capillary Electrophoresis

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 the 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/NP.

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/NP 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 P/ACE™ System MDQ with DAD from Beckman Coulter. We chose the sim-

plest possible system: uncoated fused-silica capillaries, 50 or 75 micron I.D., with an unmodified run buffer (100 mM/L phosphate, pH 2.38). We normally use electrokinetic injection, typically eight seconds at 10 kV, and a separation voltage of 18 to 20 kV. Run time is typically 25 minutes per sample. Detection is by UV monitoring at 200 nm. Complete details have been published elsewhere.[5,6]

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/NP 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.

Mobility Is Highly Reproducible

This approach requires that migration behavior be expressed in a way that gives reproducible results from 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 cm^2/Vs) 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 for 20 different drugs run every day for four months. This high degree of reproducibility means two things: 1) in most cases, the list of possibilities truly is a short list; and 2) a given peak can be eliminated as a possibility with a high degree of confidence.

First of all, this means 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.

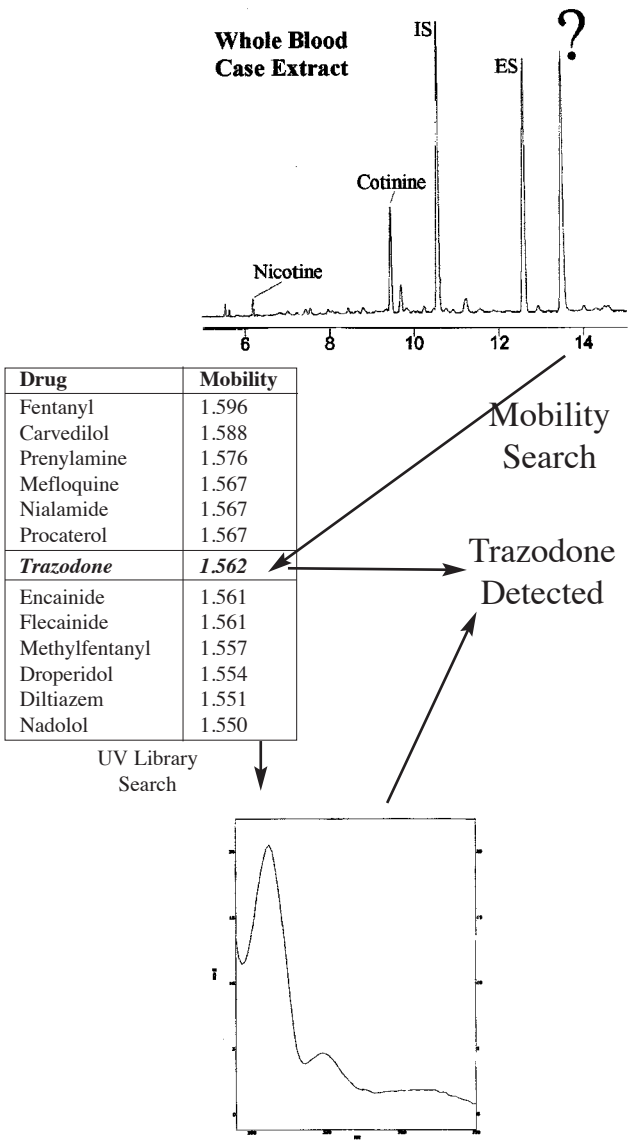

Figure 1. *Screening blood for basic drugs.*

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 of 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.

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/NP, there must be some pretreatment of blood samples to concentrate the analyte, if nothing else.

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) 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.

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 200 nm 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 (*e.g.*, Paxil from SmithKline Beecham). This compound is one that is known to behave erratically in our chromatography system.

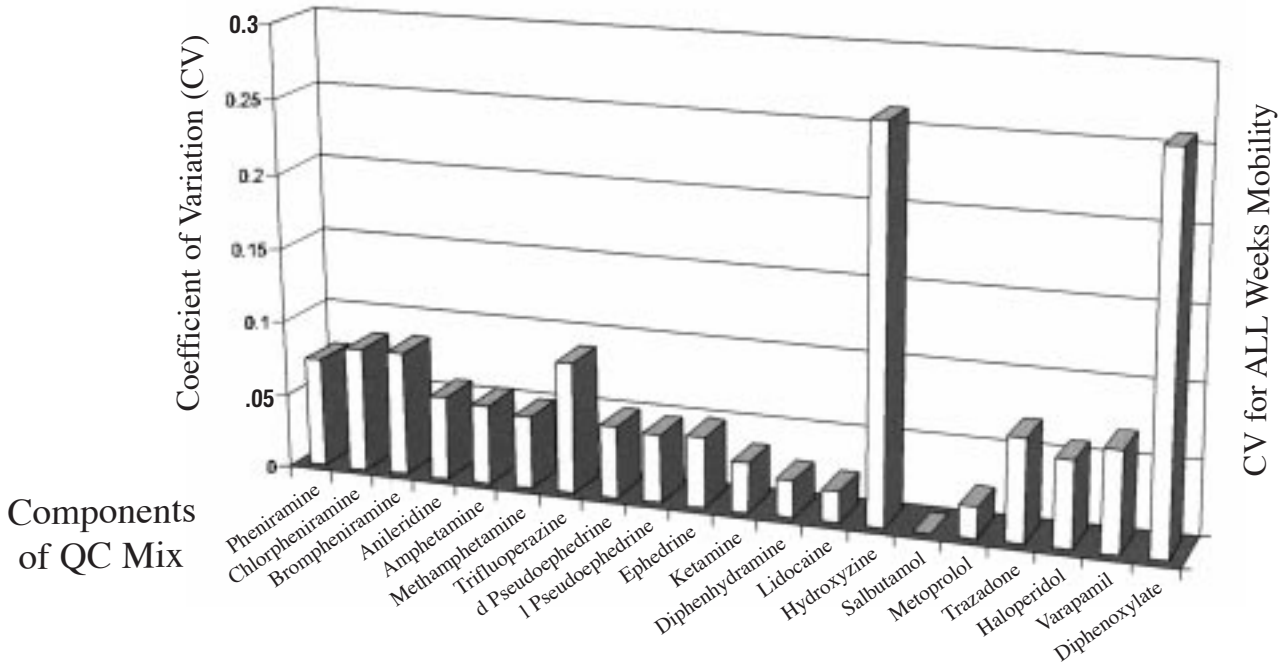

Figure 2. *CVs of migration parameters for QC mixture.*

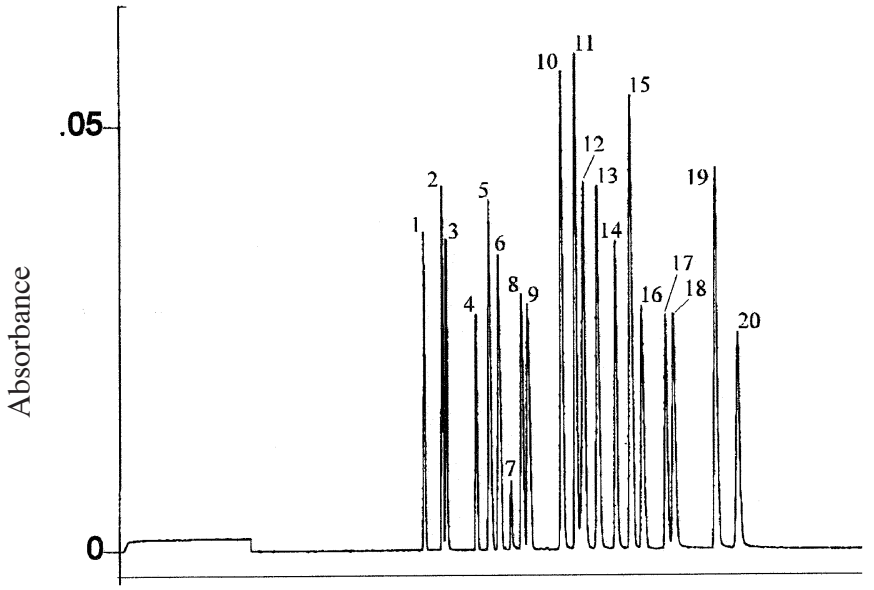

Figure 3. *Quality control sample 1 µg/mL in water 8-second 10 kV injection.*

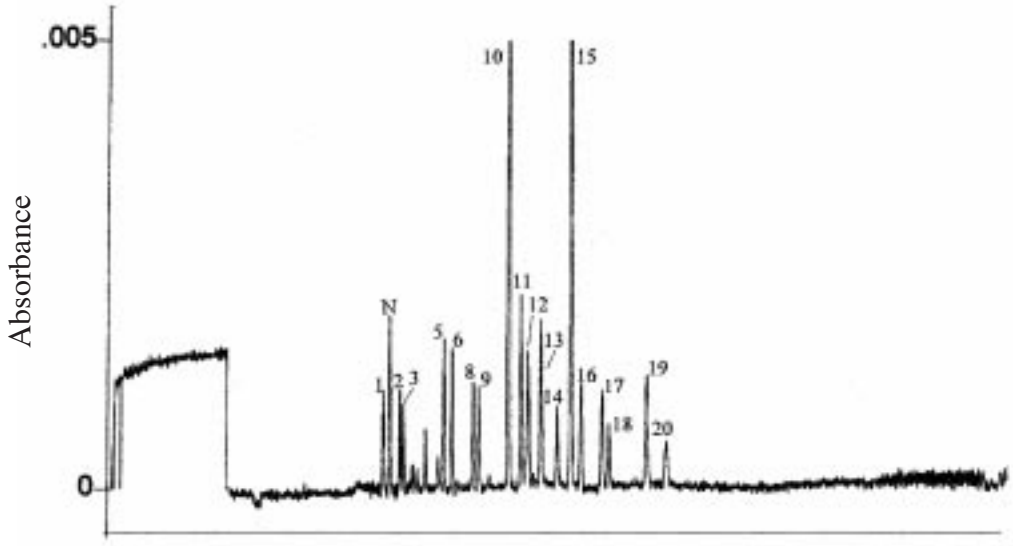

Figure 4. *Quality control extract, 10 ng/mL porcine blood.*

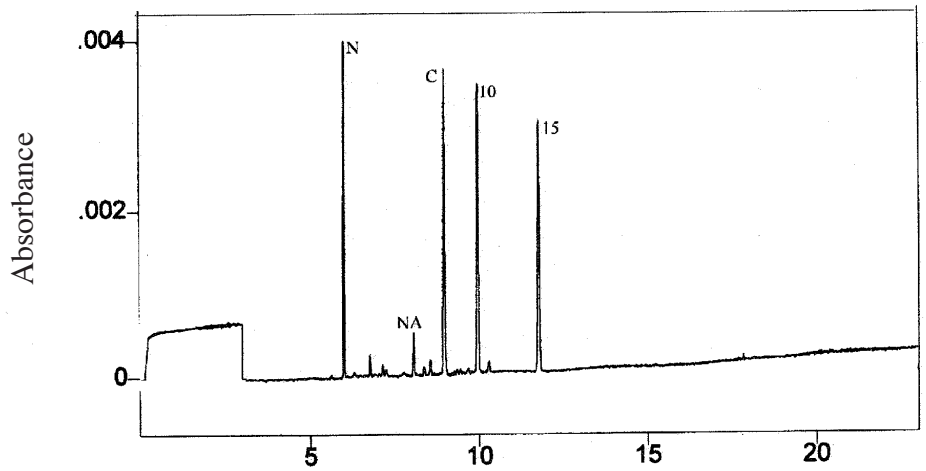

Figure 5. *"Normal" extract whole blood, heavy smoker.*
IS - 50 ng/mL. Residue in 30 µL ES. Es - 1 ng/µL.

Table 1. Legend/QC Mixture for Figures 3-5

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.

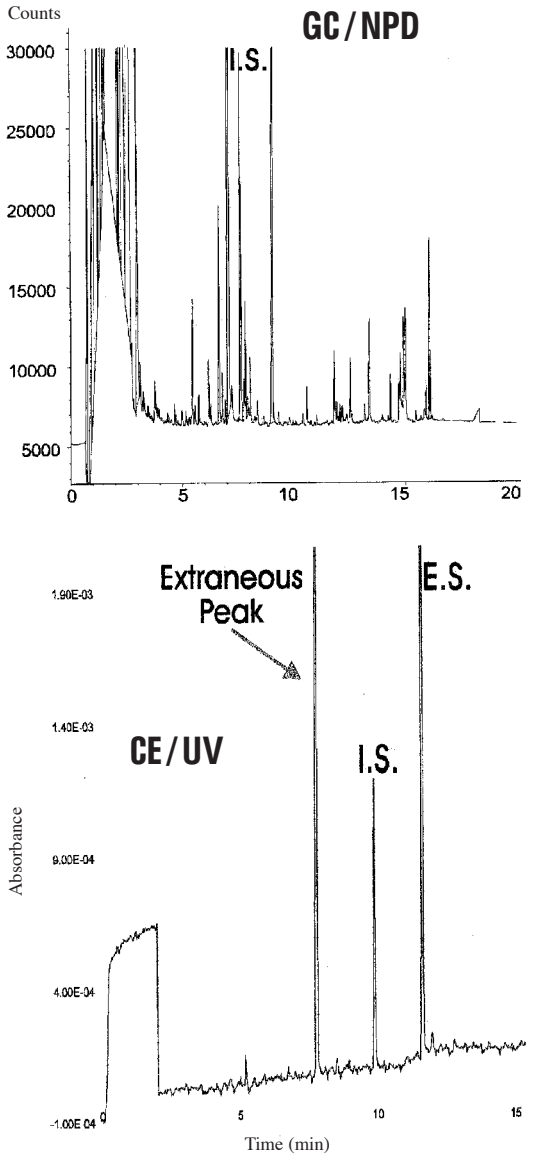

Figure 6. *Putrefaction of whole blood in vitro, 20°C. Day 56.*

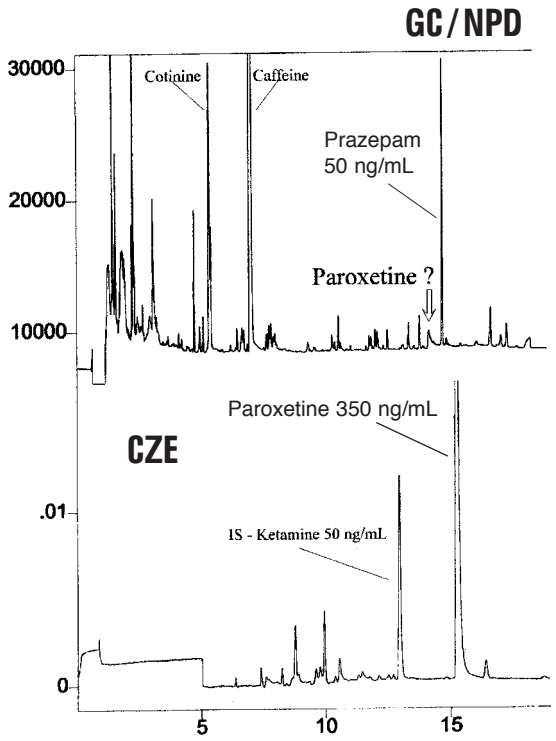

Figure 7. *Paroxetine (Paxel) by CZE and GC/NPD.*

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 is easily identified and quantified in the electropherogram.

Case Example: 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 (*e.g.*, Effexor from Wyeth-Ayerst). The second chart shows a negative case that 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.

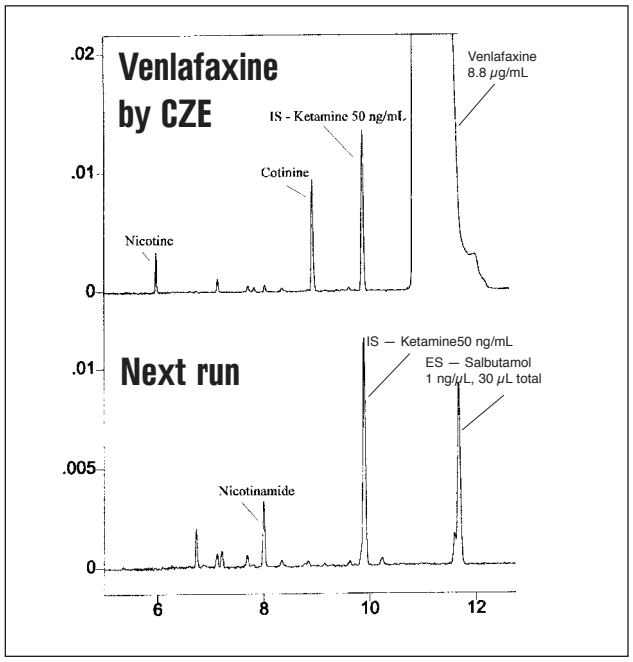

Figure 8. *Robustness of CZE/Venlafaxine.*

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 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.

References

1. Sharp, M. E. Evaluation of a Screening Procedure for Basic and Neutral Drugs: n-Butyl Chloride Extraction and Megabore Gas Chromatography. *Can. Soc. Forens. Sci. J. 19(2),* 83-101 (1986)
2. Lurie, I. S. Analysis of seized drugs by capillary electrophoresis, in *Analysis of Addictive and Misused Drugs*, pp. 151-219. New York: Marcel Dekker, 1994.
3. Thormann, W. Capillary electrophoresis and electrokinetic capillary chromatography of drugs in body fluids. *Chromatogr. Sci. Ser. 64,* 693-704 (1993)
4. Chee, G. L., Wan, T. S. M. Reproducible and High-Speed Separation of Basic Drugs by Capillary Zone Electrophoresis. *J. Chromatogr. 612,* 172-177 (1993)
5. Hudson, J. C., Golin, M., Malcolm, M. Capillary Zone Electrophoresis in a Comprehensive Screen for Basic Drugs in Whole Blood. *Can. Soc. Forens. Sci. J. 28(2),* 137-152 (1995)
6. Hudson, J. C., Golin, M., Malcolm, M., Whiting, C. F. Capillary Zone Electrophoresis in a Comprehensive Screen for Drugs of Forensic Interest in Whole Blood: An Update. *Can. Soc. Forens. Sci. J. 31(1),* 1-19 (1998)
7. Williams ,B. A., Vigh, G. Fast, accurate mobility determination method for capillary electrophoresis. *Anal. Chem. 68,* 1174-1180 (1996)
8. Brunner, L. J., DiPiro, J. T., Fedman, S. High Performance Capillary Electrophoresis in the Pharmaceutical Sciences. *Pharmacotherapy 15(1),* 1-22 (1995)
9. Malcolm, M. J., Whiting, C., Green, S. J. Possible Impairment by Venlafaxine: A Case Report. *Can. Soc. Forens. Sci. 31(1),* 31-33 (1998)