Fiona J. Couper,[1] B.Sc. (Hons); Iain M. McIntyre,[1] Ph.D.; and Olaf H. Drummer,[1] Ph.D.

Extraction of Psychotropic Drugs From Human Scalp Hair

REFERENCE: Couper, F. J., McIntyre, I. M., and Drummer, O. H., **"Extraction of Psychotropic Drugs from Human Scalp Hair,"** *Journal of Forensic Sciences,* JFSCA, Vol. 40, No. 1, January 1995, pp. 83–86.

ABSTRACT: A comparison of techniques for the extraction of antidepressant and antipsychotic drugs in human scalp hair is described. Human scalp hair was obtained from cadavers known to be taking psychotropic drugs prior to their death. Following a washing step, hair was either solubilized in sodium hydroxide, or treated with dilute hydrochloric acid, methanol or subtilisin. Digests were treated with a solvent and the extracted drugs quantified by high performance liquid chromatography. The alkaline digestion procedure was found to be significantly more effective ($P < 0.01$) in recovering a range of antidepressant and antipsychotic drugs from hair than either the acidic, methanolic or enzymatic treatments.

KEYWORDS: toxicology, human scalp hair, antidepressant drugs, antipsychotic drugs, HPLC

The application of hair analysis for the detection of drug use in the human body has become important since it can provide evidence of drug use over a much longer period than conventional testing in blood and urine [*1*]. The incorporation of therapeutic and illicit drugs into human scalp hair may also provide a valuable specimen for forensic use in establishing long-term exposures to drugs, particularly in decomposed cases and in skeletal remains, where there are often no other suitable fluids or tissues available for analysis.

In the past decade, particular attention has been given to the use of hair for the detection of drugs of abuse. Numerous studies have shown that cocaine, heroin, amphetamines and barbiturates, amongst others, can be quantitatively measured in human hair following their use [*1–4*]. It is now also known that therapeutic drugs such as antidepressants [*5,6*], benzodiazepines [*6,7*] and haloperidol [*8,9*] can be detected in human hair.

Various extraction techniques have been used by investigators to detect these drugs in hair, the most common being alkaline, acidic, methanolic and enzymatic treatments [*2,10–12*]. However, it is not clear which, if any of the procedures, provide optimal recovery of drugs. We have compared four methods for their ability to extract a range of psychotropic drugs from human scalp hair.

Methods

Reagents and Materials

Methanol and hexane (Mallinckrodt, Aust.), and acetonitrile (Millipore/Waters, Aust.) were of HPLC grade. Trizma base and Subtilisin carlsberg were obtained from Sigma (U.S.A) and diethylamine was obtained from Fluka (Aust.). Butyl chloride (BDH Chemicals, Aust.) was redistilled prior to use. All other chemicals were of analytical reagent grade (Ajax Chemicals, Aust.).

Subtilisin was made fresh daily in 2 *M* tris buffer, pH 9.6, as a 10 mg/mL solution. Buffer A was prepared by adding 30 mL diethylamine to 1200 mL 0.1 M sodium dihydrogen orthophosphate (pH 7.0) and Buffer B was 10 *m*M potassium dihydrogen orthophosphate (pH 3.0).

Glass extraction tubes were silanized with a 5% solution of Surfasil® (Pierce, IL) in toluene for 1 h, and then rinsed twice in methanol prior to drying.

Drug Standards

Pure standards of all drugs were obtained either from Sigma Chemical Co. or from the curator of standards from the Australian Government Analytical Laboratories.

Stock solutions for all drugs (1 mg/mL) were prepared daily in methanol. Working standards were prepared by diluting stock solutions with deionized water to give concentrations of 100 μg/mL and 10 μg/mL. For calibration curves, working standards were added to drug-free hair digests to give drug concentrations ranging from 0.1 to 10.0 μg/mL.

Sample Collection and Washing Procedure

In postmortem cases, hair samples (approximately 20 to 100 mg) were pulled from the vertex posterior or the nape of the neck. Tape was placed around the root ends until their use. To remove surface contamination, samples were soaked in distilled water for five min then rinsed three times with methanol. Hair samples were dried and subsequently weighed.

To analyze the methanol wash for the presence of drugs, the three eluants from the methanol rinses were combined, internal standard added and the mixture evaporated to dryness in a sample concentrator (model SVC 200H, Savant Industries, Melb., Aust.). The residue was reconstituted in 100 μL of 0.2% orthophosphoric acid and analyzed using high performance liquid chromatography (HPLC).

Extraction Methods

Hair from cases known to contain psychotropic drugs were each divided into four portions of approximately equal weight (5 to 25 mg). All portions were washed identically to remove surface contamination then incubated under four separate conditions. The first hair sample was solubilized by the addition of 1 mL of 1 M sodium hydroxide (NaOH), and incubated at 70°C for 30 min. The second and third hair samples were treated with 1 mL of 0.1 M hydrochloric acid (HCl) and 1 mL of methanol, respectively. The fourth sample was soaked in distilled water for 2 h to 'swell' the hair, prior to adding 1 mL of the subtilisin solution. The latter three

Received for publication 24 Jan. 1994; revised manuscript received 15 May 1994; accepted for publication 10 June 1994.

[1] Honors Student, Chief Toxicologist, and Assistant Director (VIFP) and Honorary Associate Professor in Forensic Medicine, respectively, Victorian Institute of Forensic Pathology and Department of Forensic Medicine, Monash University, South Melbourne, Victoria, Australia.

mixtures were incubated at 55°C for 18 h. Following incubation, the pH of the alkaline, acidic and methanolic treatments were adjusted to between 9.5 and 10.

The liquid phase of the extractions were then simultaneously prepared for quantitative analysis by HPLC. Drug-free hair samples and spiked drug standards were similarly treated in each of the four incubation conditions. Internal standard (specific for each drug class) was added to all samples prior to incubation.

Stability Experiments

In order to study the stability of drugs, studies were conducted in 1 M NaOH, 0.1 M HCl, methanol and subtilisin on a range of antidepressant, antipsychotic and benzodiazepine drugs. Drug solutions of known concentration were prepared in the four digestion conditions, as outlined above. The concentrations were compared after set periods of time to zero time controls of the same concentration and in the same digestion solutions. Both controls and incubated samples were extracted and analyzed as per the quantitative procedures described as follows.

Quantitation Procedures

For antidepressant drugs and haloperidol, 1 mL of hair digests, standards and controls (all containing 1 μg protriptyline as internal standard) were added to freshly silanized 12 mL glass extraction tubes. One mL of distilled water and 1 mL of 0.2 M sodium carbonate buffer were successively added. The tubes were then mixed and the contents extracted with 6 mL of 95% hexane: 5% butanol for 20 min. The solvent layer was then transferred to clean extraction tubes containing 100 μL of 0.2% orthophosphoric acid and the tubes mixed for 20 min. The solvent layer was discarded by aspiration and the acid layer transferred into a 200 μL autosampler vial (Kimble, Aust.). An aliquot (30 μL) was injected into the HPLC system.

For chlorpromazine, thioridazine and benzodiazepines, 1 mL of hair digests, standards and controls (all containing 0.5 μg of trimipramine or clobazam as internal standard) were added to freshly silanized 12 mL glass extraction tubes. One mL of 2% sodium tetraborate buffer was added. The tubes were then mixed and the contents extracted with 8 mL of butyl chloride for 20 min. The solvent layer was then transferred to clean extraction tubes containing 200 μL of 0.2% orthophosphoric acid, and the tubes mixed for 20 min. The solvent layer was discarded by aspiration and the acid layer was transferred into a 200 μL autosampler vial (Kimble, Aust.). An aliquot (50 μL) was injected into the HPLC system.

Chromatographic Conditions

The HPLC system consisted of an auto injector (Shimadzu SIL-6B), liquid chromatograph pump (LC-6AD, Shimadzu Oceania, Melb., Aust.), UV detector (SPD-6AV UV-VIS, Shimadzu) and a single channel integrator (C-R4A, Shimadzu). For the quantitation of antidepressant drugs and haloperidol, the guard column used was a RP-18 Newguard cartridge, 3.2 × 15 mm I.D, 7 μm particle size

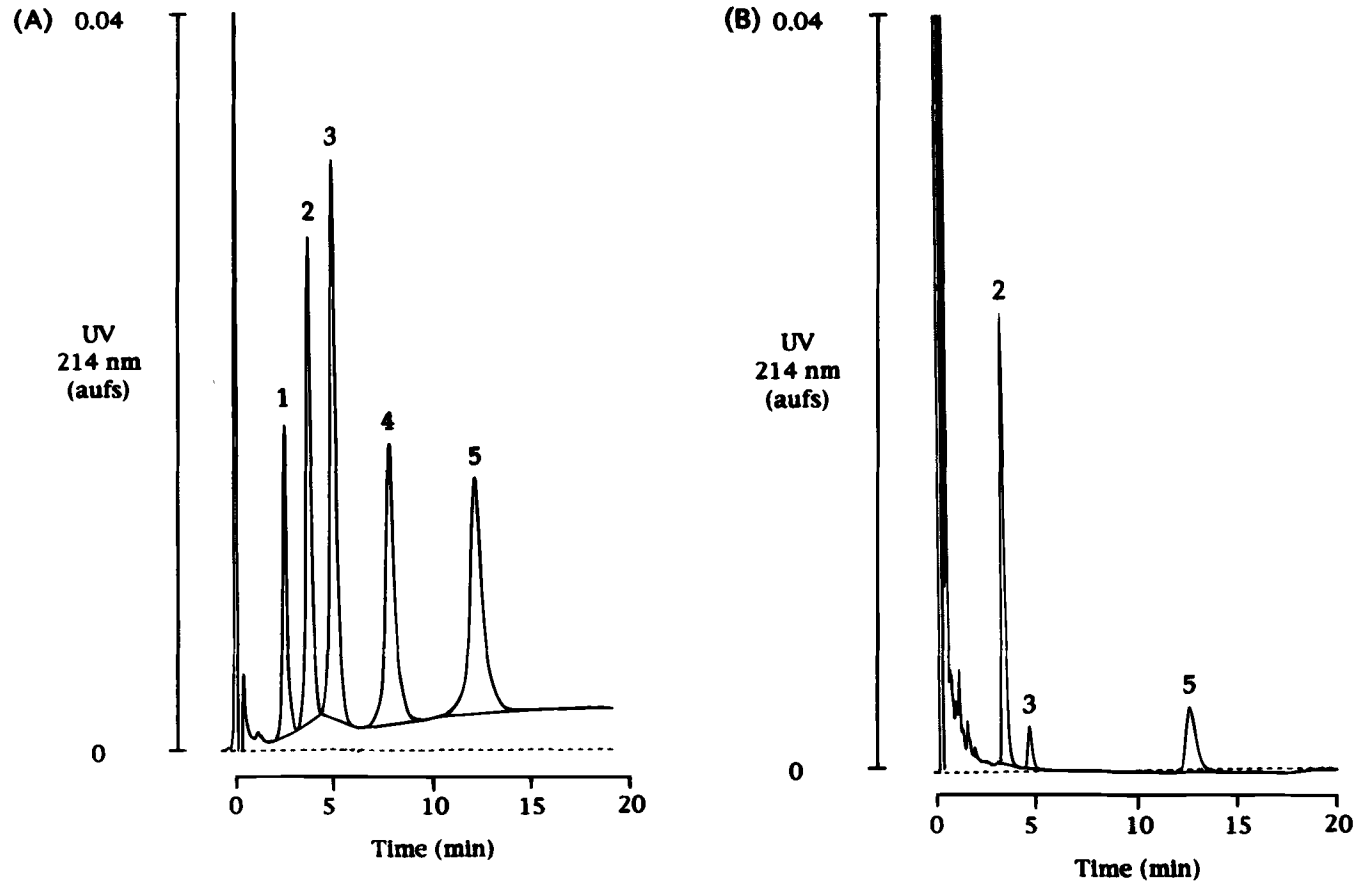

FIG. 1—*HPLC chromatogram showing the profile of* (a) *an extracted 'control' hair sample, containing 1.0 μg/mL of haloperidol (1), protriptyline (2), nortriptyline (3), dothiepin (4) and amitriptyline (5), and* (b) *an extracted postmortem hair sample (24.2 mg), containing 3.8 and 14 ng/mg of nortriptyline and amitriptyline, respectively.*

TABLE 1—*Stability of psychotropic drugs incubated in sodium hydroxide at 70°C for 30 min, or 0.1 M hydrochloric acid (HCl), methanol and subtilisin at 55°C for 18 h (means ± S.D of 6–10 separate experiments).*

Drug	Percentage recovery[a]			
	NaOH	HCl	Methanol	Subtilisin
Antidepressants				
Amitriptyline	99 ± 6.25	99 ± 4.84	100 ± 4.50	99 ± 5.91
Nortriptyline	87 ± 4.11[c]	98 ± 6.56	97 ± 6.73	97 ± 3.35
Dothiepin	99 ± 3.63	100 ± 4.51	96 ± 4.07	101 ± 3.05
Imipramine	94 ± 3.15	101 ± 5.68	102 ± 3.86	108 ± 7.13
Desipramine	96 ± 3.43	95 ± 5.65	98 ± 5.77	98 ± 6.07
Clomipramine	103 ± 4.37	—	—	—
Doxepin	101 ± 5.50	—	—	—
Mianserin	95 ± 3.78	—	—	—
Antipsychotics				
Haloperidol	88 ± 4.52[c]	97 ± 2.77	99 ± 6.94	99 ± 0.58
Chlorpromazine	103 ± 4.78	—	—	—
Thioridazine	96 ± 4.14	—	—	—
Benzodiazepines				
Diazepam	8.0 ± 2.43[b]	96 ± 3.11	99 ± 5.27	100 ± 5.31
Flunitrazepam	4.6 ± 2.82[b]	64 ± 6.46[b]	93 ± 4.03	27 ± 8.71[b]
Nitrazepam	1.9 ± 0.28[b]	47 ± 5.44[b]	101 ± 5.50	49 ± 15.16[b]
Oxazepam	0 ± 0.0[b]	5.8 ± 8.67[b]	90 ± 8.21[c]	59 ± 5.29[b]
Temazepam	2.4 ± 1.22[b]	7.5 ± 2.50[b]	98 ± 4.57	99 ± 3.76

[a]Percentage recovery compared to zero-time controls.
[b]$P < 0.001$, compared to control.
[c]$P < 0.05$, compared to control.

(Applied Bio Systems, Aust.), whilst the analytical column was a Spheri-5, RP-C18 column, 4.6 × 100 mm I.D (Applied Bio Systems, Aust.). Both were operated at ambient temperature and the UV wavelength was set at 214 nm. The mobile phase was 40% acetonitrile: 60% Buffer A, pumped at a flow rate of 2.0 mL/min.

For the quantitation of chlorpromazine and thioridazine, the column was a Nova-Pak Phenyl column, 3.9 × 150 mm I.D, 5 μm particle size (Millipore/Waters, Aust.) operated at ambient temperature. The UV wavelengths were 255 nm and 265 nm for the detection of chlorpromazine and thioridazine, respectively. The mobile phase was 55% acetonitrile: 45% Buffer B, pumped at a flow rate of 1.5 mL/min.

Statistics

Statistical evaluation of stability data was carried out using InStat™ Version 2.01 on an Apple MacIntosh. Analysis of variance was used for statistical evaluation of the extraction procedure data, using the Minitab program CLR-ANOVA (Clear Lake Research Inc., Version 1.1).

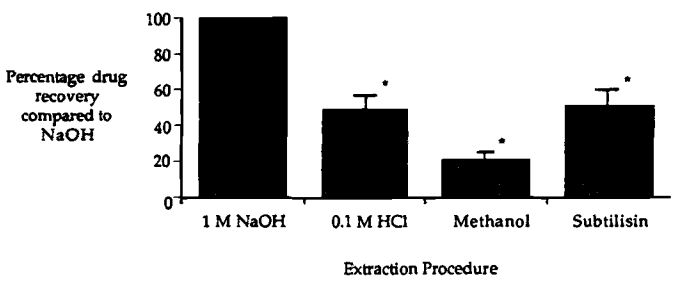

FIG. 2—*Percentage recovery of drugs from hair compared to NaOH treatment for three alternative extraction procedures. Values shown are the mean percentages of 13 drugs from 9 cases.* *P < *0.01, ANOVA.*

Results and Discussion

Stability studies on a range of psychotropic drugs were conducted in NaOH, HCl, methanol and subtilisin. A number of benzodiazepines were found to be chemically unstable in acid and subtilisin, while under alkaline conditions all benzodiazepines were degraded (Table 1). The methanol treatment did not affect the concentration of benzodiazepines, expect for oxazepam which was only slightly degraded. The loss of parent drugs when incubated in NaOH may explain why previous studies obtained low levels of benzodiazepines in hair using NaOH digestion [6]. Alternative procedures to NaOH digestion are therefore required for the optimal detection of benzodiazepines in hair.

Nortriptyline and haloperidol showed a small but significant degradation in strong alkaline conditions over 30 min, with a percentage recovery of 87% and 88%, respectively. The rest of the antidepressant and antipsychotic drugs showed no significant losses in NaOH, nor during the other three treatments (Table 1).

The acidic, methanolic and enzymatic extraction procedures were compared to the alkaline digestion procedure to determine their ability to extract psychotropic drugs from the hair. Nine hair cases known to contain the antidepressant and antipsychotic drugs dothiepin, haloperidol, amitriptyline, nortriptyline, imipramine and desipramine were investigated. In three cases the benzodiazepines diazepam, nitrazepam and oxazepam were also known to be used by the deceased.

For the HPLC extraction method described, the coefficients of variation for inter assay variability ranged from 3.6–6.4% at 0.25 μg/mL and from 9.3–13% at 1.00 μg/mL, for all drugs. Calibration curves (from 0.1 to 10.0 μg/mL) were analyzed by regression analysis and found to be linear with $r^2 \geq 0.99$. The limit of detection was 0.1 to 0.25 ng/mg hair, for all drugs. Figure 1 shows typical chromatograms for an extracted spiked hair sample and an extracted postmortem hair sample. The chromatograms show no interferences in the areas of the drugs.

The recovery of all drugs were expressed as a percentage of drug recovered using NaOH digestion (Fig. 2). The absolute antidepressant and antipsychotic drug concentrations obtained using NaOH were as follows: dothiepin 37, 95, 109 and 137 ng/mg, haloperidol 17 and 242 ng/mg, amitriptyline 3.5 and 14 ng/mg, nortriptyline 3.8 and 9.2 ng/mg, imipramine 104 ng/mg, and desipramine 88 ng/mg hair. The acidic, methanolic and enzymatic treatments were significantly less effective ($P < 0.01$) than the alkaline digestion procedure in recovering antidepressant and antipsychotic drugs from case hair. The acidic and enzymatic treatments were effective in recovering 48.8% (S.E.M ±9.5) and 51.3% (±10.1) of the drugs, respectively, while the methanolic extraction procedure recovered only 20.8% (±5.8) of the drugs as compared to NaOH. There was no significant difference in the relative recovery of individual drugs for the four treatments ($P < 0.05$).

An explanation for the large difference in extraction efficiency for the four treatments is that NaOH was the only substance that resulted in the complete solubilization of the hair. The acid, methanol and enzyme treatments left the hair structure relatively intact and possibly only extracted drugs that were bound to the outer layers of the hair. However, a previous report has suggested that methanol extraction was equally effective to NaOH digestion in recovering haloperidol from hair samples [*9*]. In this case the authors used a 48 h incubation period, rather than 18 h, and included a 24 h period of continuous sonication.

Although diazepam, nitrazepam and oxazepam were known to be taken in three of the cases, no benzodiazepines were detected in the hair samples by either of the four treatments. Since diazepam was shown to be stable in acid, methanol and subtilisin, and nitrazepam was stable in methanol (Table 1), it is not known whether the recovery of the drugs using these treatments was too poor to allow their detection, or whether the hair did not contain these drugs. Complete solubilization using an enzymatic digestion procedure has been reported for the detection of cocaine, also unstable under strong alkaline conditions [*10*]. In this case, hair was digested overnight in a mixture of Proteinase K, sodium dodecyl sulfate and dithiothreitol. The effectiveness of this treatment for the detection of benzodiazepines needs to be investigated.

The washing of hair samples is essential to remove any surface contamination. Traces of blood from the root ends or environmental contamination may produce false positive detections [*2*]. However, extensive washing of hair may remove drugs from within the hair. The presence of cocaine metabolites, nor-cocaine and cocaethylene, have been observed in the methanolic wash of hair from cocaine users [*11*]. Since these metabolites are only produced within the body, their presence in the methanolic wash provides evidence that drugs incorporated into hair following absorption into the body may be removed during washing procedures. Although a variety of washing procedures are employed in hair analysis, a relatively simple methanolic washing step was used in our study [*2*]. This methanolic washing of hair samples visibly removed surface contamination from the hair and produced clean chromatograms without interfering peaks. In addition, there was no evidence of antidepressant and antipsychotic drugs, nor their metabolites, in these methanolic washes. This suggests that the brief methanolic washing of hair effectively removed surface contamination without removing significant amounts of drug bound to hair.

In conclusion, for the complete recovery of drugs from hair to occur, either more extensive procedures or complete solubilization of hair is recommended. Alternatively, relatively mild non-destructive techniques such as methanol and subtilisin may allow only 50% or less of drug to be detected, however these procedures have the advantage of preserving chemically sensitive molecules such as the benzodiazepines, cocaine and heroin. In any case, we advise the need to determine the chemical stability of drugs in the extraction medium and to provide an assessment of extraction recovery for the measurement of drug concentrations in hair in order that hair results can be properly interpreted.

Acknowledgments

This work formed part of an Honours Thesis in Pharmacology and Toxicology from Monash University for Fiona Couper. The assistance provided by the forensic technicians in the collection of hair samples is appreciated.

References

[*1*] Baumgartner, W. A., Hill, V. A., and Blahd, W. H., "Hair Analysis for Drugs of Abuse," *Journal of Forensic Sciences*, Vol. 34, No. 6, Nov. 1989, pp. 1433–1453.

[*2*] Goldberger, B. A., Caplan, Y. H., Maguire, T., and Cone, E. J., "Testing Human Hair for Drugs of Abuse. III. Identification of Heroin and 6-Acetylmorphine as Indicators of Heroin Use," *Journal of Analytical Toxicology*, Vol. 15, Sept./Oct. 1991, pp. 226–231.

[*3*] Suzuki, O., Hattori, H., and Asano, M., "Detection of Methamphetamine and Amphetamine in a Single Human Hair by Gas Chromatography/Chemical Ionisation Mass Spectrometry," *Journal of Forensic Sciences*, Vol. 29, No. 2, April 1984, pp. 611–617.

[*4*] Smith, F. P. and Pomposini, M. S., "Detection of Phenobarbital in Bloodstains, Semen, Seminal Stains, Saliva Stains, Perspiration Stains, and Hair," *Journal of Forensic Sciences*, Vol. 26, No. 3, July 1981, pp. 582–586.

[*5*] Ishiyama, I., Nagai, T., and Toshida, S., "Detection of Basic Drugs (Methamphetamine, Antidepressants, and Nicotine) from Human Hair," *Journal of Forensic Sciences*, Vol. 28, No. 2, April 1983, pp. 380–385.

[*6*] Kintz P., Tracqui, A., and Mangin, P., "Detection of Drugs in Human Hair for Clinical and Forensic Applications," *International Journal of Legal Medicine*, Vol. 105, 1992, pp. 1–4.

[*7*] Sramek, J. J., Baumgartner, W. A., Ahrens, T. N., Hills V. A., and Cutler, N. R., "Detection of Benzodiazepines in Human Hair by Radioimmunoassay," *Annals of Pharmacotherapy*, Vol. 26, April 1992, pp. 469–472.

[*8*] Uematsu, T., Sato, R., Suzuki, K., Yamaguchi, S., and Nakashima, M., "Human Scalp Hair as Evidence of Individual Dosage History of Haloperidol: Method and Retrospective Study," *European Journal of Clinical Pharmacology*, Vol. 37, 1989, pp. 239–244.

[*9*] Matsuno, H., Uematsu, T., and Nakashima, M., "The Measurement of Haloperidol and Reduced Haloperidol in Hair As an Index of Dosage History," *British Journal of Clinical Pharmacology*, Vol. 29, 1990, pp. 187–194.

[*10*] Offidani, C., Carnevale, A., and Chiarotti, M., "Drugs in Hair: A New Extraction Procedure," *Forensic Science International*, Vol. 41, 1989, pp. 35–39.

[*11*] Strang, J., Marsh, A., and Desouza, N., "Hair Analysis for Drugs of Abuse," *The Lancet*, Vol. 24, March 24, 1990, p. 740.

[*12*] Fritch, D., Groce, Y., and Rieders, F., "Cocaine and Some of Its Products in Hair by RIA and GC/MS," *Journal of Analytical Toxicology*, Vol. 16, March/April 1992, pp. 112–114.

[*13*] Harkey, M. R., Henderson, G. L., and Zhou, C., "Simultaneous Quantitation of Cocaine and Its Major Metabolites in Human Hair by Gas Chromatography/Chemical Ionisation Mass Spectrometry," *Journal of Analytical Toxicology*, Vol. 15, Sept./Oct. 1991, pp. 260–265.

[*14*] Cone, E. J., Yousefnejad, D., Darwin, W. D., and Maguire, T., "Testing Human Hair for Drugs of Abuse. II. Identification of Unique Cocaine Metabolites in Hair of Drug Abusers and Evaluation of Decontamination Procedures," *Journal of Analytical Toxicology*, Vol. 15, Sept./Oct. 1991, pp. 250–255.

Address requests for reprints or additional information to
Olaf H. Drummer, Ph.D.
Dept. of Forensic Medicine
Monash University
57-83 Kavanagh St.
South Melbourne
Victoria, Australia 3205