Technical Note

The Analysis of Methadone in Nail Clippings from Patients in a Methadone-Maintenance Program*

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Abstract

This study offers an analytical scheme for methadone in fingernail clippings. Nail specimens (0.18–16.33 mg) were collected from 30 consenting adults participating in a methadone-maintenance program along with questionnaires regarding their drug-use histories. The nail clippings were stored in plastic bags and transferred to the laboratory for analysis. They were decontaminated by sonication for 15-min intervals successively in 0.1% sodium dodecyl sulfate, water (three times), and methanol (three times). The methanolic washes were collected and screened for methadone by enzyme immunoassay (EIA). Three washes were found sufficient to provide EIA negative results. The decontaminated nail clippings were hydrolyzed in 1M NaOH. Aliquots of the hydrolysates were screened for methadone by EIA and confirmed by gas chromatography–mass spectrometry (GC–MS). The mean methadone concentrations in fingernail clippings determined by EIA and GC–MS were 32.8 and 26.9 ng/mg, respectively. Hydrolysates of the equivalent of 10 mg of blank nail clippings were spiked with known concentrations of methadone and analyzed by the developed procedures in order to determine extraction recoveries and limits of detection of the two techniques. Based on our results, fingernails appear to be a potentially useful biological specimen for the analysis of methadone and the monitoring of patient compliance to methadone-maintenance programs.

Introduction

Methadone is a synthetic narcotic analgesic possessing pharmacological properties similar to those of morphine and when administered parenterally it is approximately equipotent (1). It produces sedative effects with chronic use as a result of drug accumulation. Methadone was not used to advantage until 1966 when Dole and Nyswander (2) began narcotic-maintenance treatment of former heroin users by administering large daily oral doses.

The analysis of biological samples for methadone and its major metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP), has been extensively studied. All three compounds have been identified by gas chromatography–flame ionization detection without derivatization (3–5), gas chromatography–mass spectrometry (GC–MS) (6–8), and liquid chromatographic techniques (9). Blood and urine are not the only specimens for which analytical protocols for the detection of methadone have been offered in the literature. Methadone detection in hair has also been published (10–12).

The present study was conducted in order to determine the usefulness of nail as an analytical specimen for methadone detection and quantitation. The experiments were carried out using nail clippings from drug users already on a methadone-maintenance program. Adaptations of a blood methadone enzyme immunoassay (EIA) method for screening and a GC–MS method for confirmation were successfully employed.

Materials and Methods

Standards and reagents

All organic solvents, sodium dodecyl phosphate (SDS), and NaOH were high-pressure liquid chromatography grade, and all other chemicals were reagent grade. Methadone was purchased from Alltech-Applied Science Labs (State College, PA), and methadone-d_3 was purchased from High Standard Products Corp. (Inglewood, CA).

Samples

Nail clippings (0.18–16.33 mg) were collected with informed consent from adults attending the methadone-maintenance clinic of the Edinburgh Drug Addiction Study. The procedures followed were in accordance with the ethical standards of the responsible regional committee on human experimentation. At the time of sampling, the participants were asked to provide answers to a short questionnaire regarding their drug-use patterns. Nail clippings were obtained using commercially available

* This paper was presented in part at the 1999 meeting of the Society of Forensic Toxicologists, Inc. (SOFT) in Puerto Rico.

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cosmetic nail clippers. The nail clippings of each participant were pooled and stored in a plastic bag at room temperature until the time of analysis. Demographic and epidemiological data for each participant are shown in Table I. The sample population studied comprised 29 Caucasians, 19 males and 10 females. Their average age was 34.3 years with a range of 22 to 47 years.

In addition, five sets of fingernail clippings from individuals who were unlikely to be methadone users (i.e., laboratory personnel, postgraduate research students, and academics) were obtained and used as negative controls.

Sample decontamination

Using an ultrasonic bath, the sets of nail clippings were sonicated once in 10 mL of 0.1% SDS for 15 min and three times in 10 mL of deionized water for 15 min each time, and each of the four resulting washes was discarded by decanting. Next, the samples were sonicated in 10 mL methanol three times for 15 min each time. These washes were collected in separate vials, evaporated to dryness on a hot plate set at 50°C under a stream of nitrogen, reconstituted in phosphate buffer (pH 7.0), and screened by EIA for methadone. All third and final methanolic washes tested negative for methadone, and as a result, the analysis of the nail clippings proceeded.

Table I. Demographic and Epidemiological Data of the Human Subjects Participating in the Methadone Study as Reported at the Time of Sampling and Methadone Levels in Nail by EIA and GC–MS

No.	Gender	Age (years)	Daily methadone dose*	Other drugs taken†	Nail weight (mg)	Methadone by EIA (ng/mg)	Methadone by GC–MS (ng/mg)
1	F	35	50 mL	D	5.58	15.8	25.5
2	M	32	65 mL	At, Ap	2.12	60.4	97.6
3	F	47	100 mL	Ap, D	7.07	39.7	15.7
4	F	32	100 mL	At, Cn, T	0.79	89.5	92.6
5	F	22	N/A	Ap, Cn, D, Dc	15.58	3.0	2.43
6	F	33	140 mL	D, T	7.09	8.6	0.55
7	M	29	N/A	Cn, D, Dc	6.28	9.8	13.9
8	M	42	80 mL	D	0.18	577.8	362.5
9	M	32	N/A	Cn, D, Dc	5.89	14.1	12.9
10	M	29	N/A	Cn, D, Dc	9.54	3.7	1.43
11	F	35	N/A	Ap, Cn, D, Dc	10.40	5.8	4.93
13	F	34	N/A	Ap, Cn, Dc, T	7.33	10.6	32.2
14	F	35	N/A	D, Mo	4.27	9.70	7.04
15	M	31	N/A	Cn, D, H	9.25	9.06	9.53
16	M	38	70 mL	Cn, D, Dc	15.29	4.0	5.76
17	M	N/A	70 mL	Ap, Cn, D	12.17	2.95	1.15
18	M	31	N/A	Cn, D, Dc	11.28	4.44	4.63
19	M	32	N/A	Ap, Cn, D, Dc, H	7.90	1.15	N/D
20	F	37	65 mL	Cn, D	6.43	7.77	7.64
21	F	47	90 mL	Cn, D	3.97	2.19	0.81
22	M	43	140 mL	D	8.26	5.32	3.08
23	M	30	80 mL	Cn, D	5.38	12.6	15.1
24	M	23	60 mL	Cn, D	16.33	1.59	0.88
25	M	37	N/A	Cn, Dc	5.58	5.63	4.53
26	M	38	N/A	H	6.47	7.14	1.98
27	M	31	N/A	Ap, Cl, Cn, Dc	8.06	4.56	N/D
28	M	45	N/A	Cn, D, Dc	9.26	2.81	0.51
29	M	36	50 mL	Cn, D	9.01	N/D	11.6
30	M	26	N/A	At, Ap, Cn, D, Dc, LSD	6.31	N/D	17.5
B-1‡	M	29	N/A	N/A	3.89	0.00	0.00
B-2	F	28	N/A	N/A	7.45	0.00	0.00
B-3	F	29	N/A	N/A	12.87	0.00	0.00
B-4	M	44	N/A	N/A	4.78	0.00	0.00
B-5	M	34	N/A	N/A	15.92	0.00	0.00

* The methadone elixir concentration is 1 mg/mL (physeptone).
† Abbreviations: At, amitriptyline; Ap, amphetamines; Cl, chlorpromazine; Cn, cannabis; D, diazepam; Dc, dihydrocodeine; H, heroin; LSD, lysergic acid diethylamide; Mo, morphine sulfate; T, temazepam; N/A, not answered; and N/D, not detected.
‡ Negative control specimens (blanks).

Sample extraction and EIA

The decontaminated sets of nail clippings were allowed to air dry overnight and weighed. Nail clippings were incubated for 30 to 40 min at 90°C in the presence of 1 mL of 1M NaOH. The resulting nail hydrolysates were then divided into two 500-µL halves; one intended for methadone screening by EIA and the other for methadone confirmation by GC–MS. The latter aliquots were stored at 4°C until such confirmation was carried out.

To the aliquots intended for EIA, 500 µL of phosphate buffered saline solution were added and the samples were analyzed in duplicate using the methadone microplate EIA forensic application by Cozart Bioscience Ltd. (Oxfordshire, U.K.). The EIA analyses were carried out using a MARK-5 sample processor by DPC for all precision pipetting and Dynatech MRW and Dynatech MRX instruments for the rinsing and reading of the microplates, respectively. The EIA kits used had been tested for a wide range of non-related drugs at 10,000 ng/mL in serum with no cross-reactivity found according to their manufacturers, whereas low cross-reactivities have been reported to EDDP (0.01–0.69%) and EMDP (0.01–0.59%).

Standard curves used in the analyses were prepared from methadone-containing protein matrices as supplied by the manufactures. Those specimens which tested at levels beyond the linear range of our standard curves, were diluted and retested.

SPE

To the 500-µL aliquots intended for GC–MS confirmation, 50 µL of internal standard solution (100 ng methadone-d_3) was added, and the volume was brought up to 1 mL with 100mM phosphate buffer (pH 6.0). The samples were then vortex mixed and extracted using 200-mg Clean Screen® solid-phase extraction columns by Worldwide Monitoring Corp. (Congleton, U.K.). The extractions took place on a VARIAN Vac-Elut™ vacuum workstation using a Millipore® vacuum pump. The columns were washed, conditioned, and eluted as described by Alburges et al. (8) except that 2 mL of 100mM HCl was substituted for acetic acid in the wash step. The eluates were evaporated to dryness on a hot plate set at

40°C under a stream of nitrogen, and the resulting residues were reconstituted in 100 µL ethyl acetate before confirmatory GC–MS.

GC–MS

A 2.0-µL portion of each sample was injected in duplicate through an HP-5 capillary column (cross-linked 5% phenylmethyl silicone, 30 m × 0.32-mm i.d., 0.25-µm film thickness) in a Hewlett-Packard HP 5890 GC coupled to a VG Analytical 70-250S double-focusing MS. The injector temperature was 280°C, and splitless injection was employed with a split-valve off-time of 0.7 min. The MS was operated at a resolution of 1000, and data were acquired and processed using a Mass Spectrometry Services Maspec I data system. Helium was used as the carrier gas at 5 psi, linear velocity approximately 50 cm/s. The column temperature was initially 180°C and was programmed to rise to 280°C at a rate of 10°C/min immediately after injection. The ion source was set at a temperature of 200°C and the transfer line at 275°C, and the MS was operated in the selected ion recording mode with electron impact ionization at an electron energy of 70 eV. It was tuned daily using PFK according to the manufacturer's recommendations. For methadone, qualitative and quantitative analyses were obtained using the selected ion recording mode and comparison of retention times (t_R) and relative abundance of qualifier ions with methadone-d_3. The ion monitored for methadone was the 72.0814, which eluted at 5.12 min, whereas the ion monitored for methadone-d_3 was the 75.1002, which eluted at 5.11 min. Quantitative results were obtained after determination of the response factor of methadone against methadone-d_3.

Method validation

In EIA, linear standard curves (mean R^2 = 0.927; N = 4) were prepared at concentrations of 0.0, 1.5, 2.5 and 5.0 ng/mg. In GC–MS, linear calibration curves (mean R^2 = 0.945; N = 6) were constructed using standard solutions of methadone (0.0, 1.0, 2.5, 5.0, 10.0, and 25.0 ng/mg) in ethyl acetate to test and confirm the linearity of the analytical method. These standards were subjected to the same extraction as the nail specimens described earlier.

As no currently method available tests intact nail matrix spiked with methadone, the limits of detection and extraction recoveries for methadone were determined after dissolution of the nail in NaOH. Ten different nail hydrolysates in NaOH (each equivalent to 10 mg of nail) were spiked with methadone and then analyzed as usual in order to determine the extraction recovery of the method. Furthermore, nail hydrolysates in NaOH (each equivalent to 10 mg of nail) were spiked with decreasing amounts of methadone from 0.01 to 0.001 ng/mg and then analyzed as usual in order to determine the limits of detection of the methods.

Results and Discussion

For the specimens examined in this study, the SDS wash, the three water washes and the three methanol washes were sufficient to remove any superficial contamination and to produce a negative final (i.e., third) methanol screen for methadone. In 90% of our cases, the second methanolic washes were also negative for methadone.

Under the analytical conditions used, there was no interference in the analysis of methadone due to any extracted endogenous material present in nail. Chromatograms of analyses of a nail extract and a standard mixture are shown in Figure 1.

Concentrations as low as 0.01 ng/mg methadone could be detected by EIA and at least 0.005 ng/mg methadone could be detected by GC–MS if the equivalent of at least 10 mg of nail was used for the extractions. The extraction recovery for methadone was determined by spiking hydrolysates of drug-free nail clippings with known quantities of the drug, followed by extraction and analysis using the instrumental methods described earlier. The extraction recovery calculated in this way was 87.3% for methadone by EIA and 90.2% by GC–MS. All blank samples and their methanolic washes tested negative for methadone by EIA and GC–MS.

The results of EIA screening and GC–MS confirmation for the nail extracts and blanks are summarized in Table I. The two methods employed (EIA for screening and GC–MS for confirmation) agreed for the majority of specimens but substantially different results were found in approximately 15% of the specimens analyzed even though the analyses were performed in duplicate. The difference in methadone concentrations as determined by EIA and GC–MS in 15% of our population indicates that further statistical considerations must be addressed.

The relatively small size of the sample population examined in the present study (N = 29), although allowing for proper identification and quantitation of methadone in nail, does not allow for the construction of meaningful dose-response relationships. Furthermore, the study relied on participants consuming the daily dose prescribed to them rather than conducting a controlled-dosage study. This added at least two potential sources of variation within the group studied: variations in the methadone-consumption patterns of the volunteers and in the length of methadone use. Finally, the mechanisms of substance incorporation into the nail matrix are not yet understood, and there may be substantial interindividual variation because of physiological, biochemical, and pharmacokinetic factors.

The compliance of methadone users participating in a long-term methadone-maintenance program could eventually be determined by analysis in nail. Further work could be carried out to establish dose-response relationships for methadone in the nail and to determine whether the nail can provide incremental drug usage information. In this study nail clippings were successfully evaluated as analytical specimens for the detection and quantitation of methadone.

As has been determined earlier, the nail matrix is useful for the detection of many drugs of abuse including amphetamines (13), cannabinoids (14), and opiates (15) and offers several advantages to the forensic toxicologist. Drugs appear to remain trapped in the nail matrix for extensive periods of time thus allowing the determination of exposure for periods ranging from months to years. The collection of nail clippings is a noninvasive procedure as compared to the collection of blood or urine, and only a small sample size is required as demonstrated in this study (sample weight range: 0.18–16.33 mg). Nails are easily stored in plastic bags at room temperature, allow for increased stability of drugs, and are less likely to suffer any melanin race bias (16).

Conclusions

The main achievement of this study was to offer the forensic toxicologist an analytical protocol to determine methadone presence in nail clippings from long-term methadone users. This was achieved by initially screening nail clipping hydrolysates by EIA and confirming the presence of methadone by GC–MS. Methadone was determined in hydrolysates of decontaminated nail clippings by EIA (mean 32.8 ng/mg) and confirmed by GC–MS (mean 26.9 ng/mg).

The advantages of nail clipping usage as described, combined with the relative ease with which small samples may be analyzed for the presence of drugs, renders nail a potentially useful analytical specimen for the detection of methadone presence and for monitoring patients' compliance to their methadone-maintenance programs.

Acknowledgments

The authors would like to acknowledge the skillful technical assistance of Ms. Janice Adcock and Ms. Maxine MacDonald of the Department of Forensic Medicine and Science of the University of

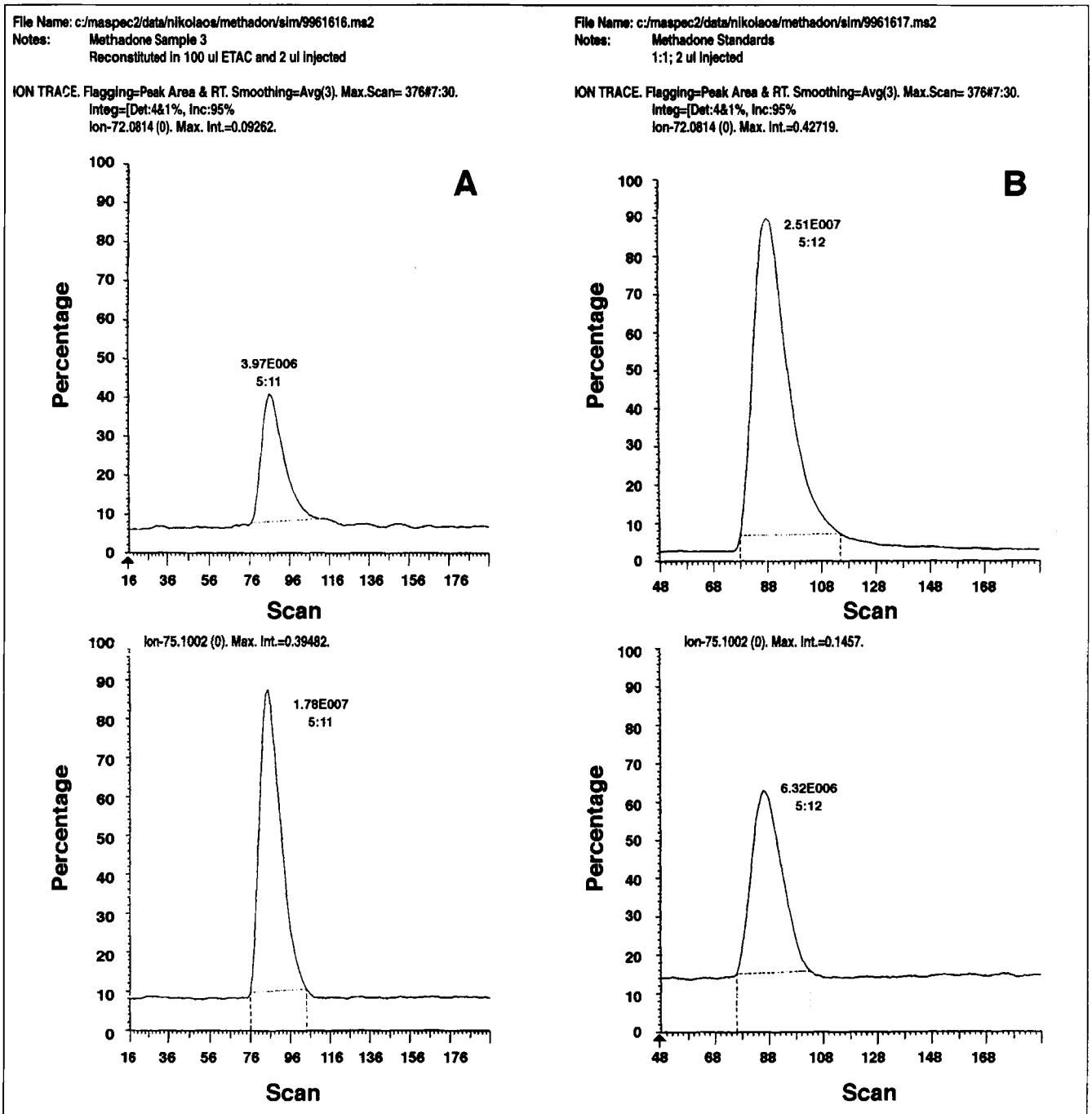

Figure 1. Selected ion chromatograms of a methadone-positive nail specimen (number 3; determined methadone concentration, 15.7 ng/mg) (A) and the methadone and methadone-d_3 standards used in the determination of the drug in nail by GC–MS (B).

Glasgow. The Partners of the Muirhouse Medical Group are hereby acknowledged for their support, and in particular, Ms. Lorraine Copeland at the Muirhouse Practice is gratefully acknowledged for organizing the behavioral data collection.

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Manuscript received March 13, 2000;
revision received May 19, 2000.