CASE REPORT

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Determination of Fentanyl in Hair: The Case of the Crooked Criminalist

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ABSTRACT: When a State Crime Laboratory Director found that fentanyl patches were missing from a case submission stored in his evidence vault, he performed an investigation of those members of his staff who had access to the materials. As part of this investigation, two staff members were forced to submit to hair testing for fentanyl and other opiates and opioids. This unusual testing protocol was used to identify a senior Criminalist as a chronic abuser of fentanyl, and led to the rapid resolution of the case. To the best of our knowledge, this is the first report of the forensic analysis of hair for drugs of abuse in which the technology was used to identify an individual as a chronic fentanyl abuser.

KEYWORDS: toxicology, fentanyl, forensic hair drug testing, internal investigation

Fentanyl and its analogues are potent synthetic morphine substitute analgesics which are widely used as surgical anesthesia adjuncts [*1*]. Although strictly controlled, fentanyl and its analogues are subject to abuse, especially among health care professionals involved in anesthesia. Fentanyl and its analogues have also been identified as drugs of abuse on the street, with access to these drugs broadly derived through trafficking of diverted pharmaceutical products and through the clandestine production of fentanyl and analogues like alpha-methylfentanyl ("China White").

As a class of drugs, fentanyl and its analogues have short pharmacologic half-lives and are metabolized rapidly to (primarily) inactive metabolites [*1*]. Our laboratory has reported on the use of urine testing for the determination of fentanyl and one of its principle metabolites, norfentanyl [*2*]. This testing protocol has been effectively used for testing medical professionals who may have exhibited signs of abuse of these drugs. However, this urine testing approach gives rise to a relatively short "window of detectability" for the drug or metabolite. For example, a dose of ≥200-mcg of fentanyl was only detected (as norfentanyl) for a maximum of 96 hours in the urine of one of four patients given a single perioperative IV dose [*2*].

We had previously been asked to develop a testing approach which could be used to detect long-term abuse of fentanyl and its analogues by interns, residents and senior staff in a teaching hospital. In response to this request, we developed and validated a hair drug testing procedure which allowed for the identification and quantitative estimation of fentanyl, sufentanil, alfentanil and carfentanil (a veterinary analgesic) by Gas Chromatography-Mass Spectrometry (GC-MS). The method validation demonstrated linearity of response for spiked hair concentrations of these drugs from 8- to 400-nanogram drug/gram (ng/g) of hair, using 50 milligram (mg) aliquots of hair, and provided limits of quantitation of 8 ng of fentanyl or analogue/g hair. (With respect to the validation data, injections of extracts derived from hair spiked with methanolic solutions of drug at concentrations of 2, 10, 50, 200 and 400 ng/g were analyzed, and gave rise to the linearity equation "Response" = 0.010,06 × [Fentanyl] − 0.005 (r^2 = 0.9993)). Although our original application of the validated method did not detect any problems among the medical staff tested, we have subsequently used forensic hair drug testing in another case to identify fentanyl in the hair of an anesthesiologist suspected of diverting the drug for his own use.

In the recent past, fentanyl has become available for use in the form of transdermal patches. These patches, which are available in 25-, 50-, 75- and 100-mcg/hour dosages (containing 2.5-, 5-, 7.5- and 10-mg fentanyl, respectively), are often prescribed for the treatment of chronic pain [*3*]. Our laboratory has been asked in other cases to characterize the body burden associated with the use or misuse of these patches in postmortem body fluids. However, until the case reported herein, we had not been involved in any cases where patches were diverted for personal use and where forensic hair drug testing played a significant role in case resolution.

Case Report

In late October 1992, a State Police Investigative team was notified by the Director of the State's Crime Laboratory that evidence was missing from the laboratory's evidence vault. An investigation was initiated, and quickly focused on a chemist within the laboratory who had access to the evidence vault during the time that a case submission of Fentanyl Transdermal patches, a syringe

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with hypodermic needle and a solution of morphine were delivered for testing. In the course of multiple interviews with this chemist (hereafter known as "Chemist #1"), the following information was obtained:

1. The evidence was initially delivered on 24 September 1992 in a sealed evidence bag and consisted of one (1) open foil package containing one (1) fentanyl patch and four (4) unopened fentanyl patch packages. When the evidence bag was examined by Chemist #1 on 21 October 1992, it was discovered that the bottom of the evidence bag had been opened and reglued and contained only four empty foil packages.
2. In 1989 and 1990, a total of six other cases of missing drug evidence—in each case involving heroin—were internally investigated by the laboratory. Three of these cases were resolved when another chemist ("Chemist #2") found the items in a cabinet which had been thoroughly searched before his own search began. The other three cases were "written off" as accidental destructions which had not been documented in the disposition records maintained by the laboratory.
3. Chemist #1 suspected that Chemist #2 had a problem with heroin. However, he did not initially voice this concern to the investigators because he had previously diverted cocaine evidence bound for destruction for his own use, and didn't want to draw attention to himself.
4. During the period December 1989 through April 1992, Chemist #2 had at least eight prescriptions for hydrocodone or oxycodone (written by three different physicians) filled by his local pharmacist.
5. By the time of the investigation, Chemist #2 had not worked on drug cases for over three years. This case represented the first time that fentanyl had been submitted to this laboratory. Chemist #2 stated that he had seen fentanyl only in the course of his studies, that he had no contact with the fentanyl in the case under investigation, and that he had no other known exposure to fentanyl.
6. Chemist #1 volunteered to have his blood, urine or hair tested for drugs. Shortly after he volunteered, Chemist #2 had his hair cut shorter than the investigating officer had ever seen it.

Based on this and other information, our laboratory was asked to determine the feasibility of testing for fentanyl in blood, urine and hair. Based on the time frame and case details available, it was our opinion that forensic hair testing represented the best approach for potentially identifying which, if either, of the chemists had chronically abused opiates or opioids. The investigator obtained court orders to collect hair samples from both chemists. These samples were submitted to our laboratory, and were tested for fentanyl and its analogues (fentanyl/analogues—specifically alfentanil, sufentanil and carfentanil), as well as opiates, opioids and their metabolites (including among other substances hydrocodone, oxycodone, morphine, codeine, 6-monoacetyl-morphine and heroin).

Methods

We define a "forensic" hair drug testing protocol as one in which two separate aliquots (equivalent portions) of a sample test positive, each using a complementary analytical technique, while an intact, written chain of custody for the entire process is maintained. In the described case, testing for fentanyl and its analogues was performed using Gas Chromatography—Nitrogen Phosphorus Detection (GC-NPD) and GC-MS. Testing for other opiates, opioids and their metabolites was performed using enzyme immunoassay (EMIT) and GC-MS.

Our procedure for hair preparation prior to testing has been previously described [*4*]. To summarize, this process begins with the entire hair sample being weighed and measured. Depending on the analysis, 100-mg or 50-mg of hair is used. (In the present case, "whole hair" analysis was performed, rather than "segmented" analysis in which a particular portion and length of hair are selected to represent an approximate time period in the donor's life). The hair is then rinsed with reagent ethanol, with vigorous vortexing for 2 minutes. This alcohol rinse is discarded, but is followed by four pH 7 phosphate buffer rinses which are saved for later analysis to evaluate the efficiency of rinsing in removing external traces of drugs. This rinsing protocol follows the convention of many within the consortium of laboratories who participate in the National Institute of Standards and Technology (NIST) round-robin hair drug testing working group [*5*].

After drying, the hair aliquot is pulverized to create a fine powder, which is then extracted using dilute hydrochloric acid with overnight heating. The incubate is subjected to testing, along with the first and fourth buffer rinses. In the case of fentanyl/analogue testing, extraction with a mixed organic solvent from basified samples is followed by a back-extraction into dilute hydrochloric acid, readjustment of the sample to basic pH, re-extraction into methylene chloride, and finally dry-down and reconstitution in preparation for GC-NPD or GC-MS [*6*]. Enzyme immunoassay is performed using the modified methods previously published for blood and serum [*7,8*]. The extraction of hair incubates for opiates, opioids and their metabolites is very similar to that used for fentanyl, except that trimethylsilyl derivatization is performed prior to GC-MS [*9*]. In all GC-NPD and GC-MS procedures, internal standard quantitation is used (8-methoxyloxapine), and the GC-MS identification involves three-ion selected ion monitoring (m/z 146, 189 and 245 for fentanyl; 287 and 274 for 8-methoxy loxapine). Internal standard quantitation is based on the ion pair m/z 245 and 287.

The batch composition in the analysis of fentanyl/analogues in the present case included spiked serum calibrators (at 20-, 5-, 1- and 0.2-ng/mL fentanyl, sufentanil, and alfentanil), serum negative and two levels of serum positive controls, a negative hair control and a positive hair control spiked with 2-ng of fentanyl, sufentanil, alfentanil and carfentanil. Serum base was used for the preparation of calibrators and controls, to allow for the evaluation of QC acceptability against historical serum fentanyl controls. 100-mg aliquots of the suspect hairs were analyzed for fentanyl/analogues, while 50-mg aliquots were tested by EMIT and GC-MS for opiates, opioids and their metabolites. Finally, the first and fourth buffer rinses were also included in the batches for analysis.

Findings

Analysis of the head hair collected from Chemist #1 did not reveal any findings of toxicological significance for fentanyl and its analogues, or opiates, opioids and their metabolites. On the other hand, GC-NPD and GC-MS analyses of the 5.5-cm length head hair from Chemist #2 identified fentanyl, at a level of 20-ng/g (quantitative estimation by GC-NPD). This identification was based on retention time and ion ratio correspondence with the calibrators, with acceptable limits of ±2% about the retention time and ±20% about the ion ratio mean for the qualifier ion pairs.

Chromatograms for the hair sample from Chemist #2 and a Negative hair are shown in Fig. 1.

All rinses tested were negative for fentanyl/analogues, and there was no evidence of opiates, opioids or their metabolites by either enzyme immunoassay or GC-MS. The reporting limits for opiates, opioids and their metabolites are in the range of 0.4- to 10-mcg/g. These results were interpreted to mean that Chemist #2 had chronically or repetitively used or been exposed to fentanyl during the time period represented by the collected hair (approximately mid-June to the beginning of December 1992) [*11*]. This time period included both the date of submission of the patches to the State laboratory, as well as the date that the patches were found to be missing.

Discussion

Whenever a new forensic technology is applied in a new way or to a new analytical challenge, it is important to recognize that the technology must be validated for that application before it can be used to answer the questions posed. In our experience with hair testing, we have come to realize that, beyond the normal analytical figures of merit which must be demonstrated (for example, accuracy, precision, sensitivity and linearity), hair testing approaches have a built-in set of unique presumptions which apply when the results are evaluated. Therefore, once the method has been demonstrated to be free from interferences by endogenous materials in hair, and predictable recovery of drug from spiked hair has been realized, the following analytical presumptions must be evaluated for the new method:

1. *Analyte(s) is(are) incorporated into hair with some relationship to circulating levels of drug.* The positive correlation of analyte incorporation in hair with absorption/elimination curves in blood has been observed for many drugs and their metabolites [*10–14*]. And although some controversy remains [*15–17*], it is generally agreed that higher levels of circulating drug are associated with higher amounts of drug incorporated into hair, when compared to incorporations of drug into hair during periods of low concentrations of circulating drug. This consideration includes the presumption that, if drug(s) is(are) present in blood, the hair will contain traces of the drug(s).
2. *Incorporated analyte(s) can be released from hair through chemical/physical means in an extraction.* Although this release of incorporated drug may not lead to recoveries which are identical to those realized from the optimized method for spiked hair, the presumption is made that the precision of this recovery is acceptable and that irreversible binding of drug to hair constituents will not pose a major impediment. The controlled dosing studies necessary for conclusive evaluation of this presumption have not been performed for many important drugs and metabolites.
3. *If the drug is incorporated into hair and released through extraction or dissolution (for example, presumptions 1 and 2 are correct), the level recovered is within the analytical capabilities of the method and instrumentation used.* Evaluation of this presumption is governed to some degree by the availability of information regarding the number of doses, amount of each dose, and time period containing the doses (length of the hair) for the case.
4. *Differentiation of apparent use from apparent environmental contamination can be sufficiently demonstrated.* Another popular way to describe the effect of this presumption is "Do the results of testing allow for the evaluation of evidentiary false positives?" Two common areas of related concern include exposure by an individual to a drug through passive means (unknowing inhalation of volatilized drug, inadvertent transdermal absorption, transfer and ingestion through contaminated currency, etc.), and the contamination of hair by external/exogenous means (for example, by deposition of volatilized drug vapors or inadvertent transfer from contaminated hands).

In the present case we were able to demonstrate that our negative control hair did not have any endogenous materials which interfered with the GC-NPD or GC-MS determination of fentanyl, sufentanil, alfentanil and carfentanil. In addition, linearity of response for these drugs from 0.4- to 20-ng/50-mg hair was realized, with a reporting limit of 0.4-ng/50-mg hair. Acceptance of the first presumption above—incorporation of drug from systemic circulation—seemed prudent in light of the previous report of positive radioimmunoassay findings for fentanyl in the hair of patients who had been dosed with the drug [*18*]. Our own experience with basic drugs suggested that, if present, fentanyl should be recoverable through the use of an acidic digest of hair, so the second presumption also seemed tenable. The GC-NPD and GC-MS methods represent the most sensitive approaches available to our laboratory for the detection of fentanyl and its analogues, so the third presumption seemed appropriate.

The issues of unknowing exposure and environmental contamination, and their possible contributory role to the fentanyl findings in the hair of Chemist #2, were discussed in-depth with the lead investigator on the case. Even though rinsing protocols—involving multiple ethanol and phosphate buffer rinses and testing of the rinses—were followed, and the rinses were determined to be negative, this did not necessarily preclude the possibility that the Criminalist was inadvertently and repetitively exposed to fentanyl in the laboratory environment. Such repetitive and inadvertent exposure, if leading to circulating levels of the drug, could explain the findings in the hair from the individual. As noted above, however, Chemist #2 denied any previous or current contact or exposure to fentanyl during questioning. In addition, there were no known previous submissions of fentanyl to the laboratory, and Chemist #2 reported no other known sources of potential exposure. Therefore, we were comfortable that the sufficiency of the method to be free of sources of evidentiary false positives was adequately defined in this case.

There are several interesting investigative features which somewhat serve as an epilogue to this case. First, Chemist #2 was given an opportunity to take a polygraph examination, and (seemingly without consulting his attorney) chose to undergo the test. He did not pass the examination. Shortly thereafter, when faced with the results of the hair testing, he claimed that he had not, in fact, diverted the fentanyl patches from the evidence vault. Rather, he claimed that he had stolen and consumed a 10 mg portion of 3-methylfentanyl analytical standard. This led to some in-depth toxicologic and chemical literature searches, as well as review of library mass spectra. In the end it was determined that fentanyl is not an expected metabolite of 3-methylfentanyl, and that there are significant differences between the chromatographic and mass spectral characteristics of the two compounds. In addition, Chemist #2's consumption of 10 mg of 3-methylfentanyl analytical standard would have required his use of over 2000 fatal doses of ca. 5 mcg each [*1*] in a period of less than 100 days. This would require the use of an average of more than 20 fatal doses per day, a highly

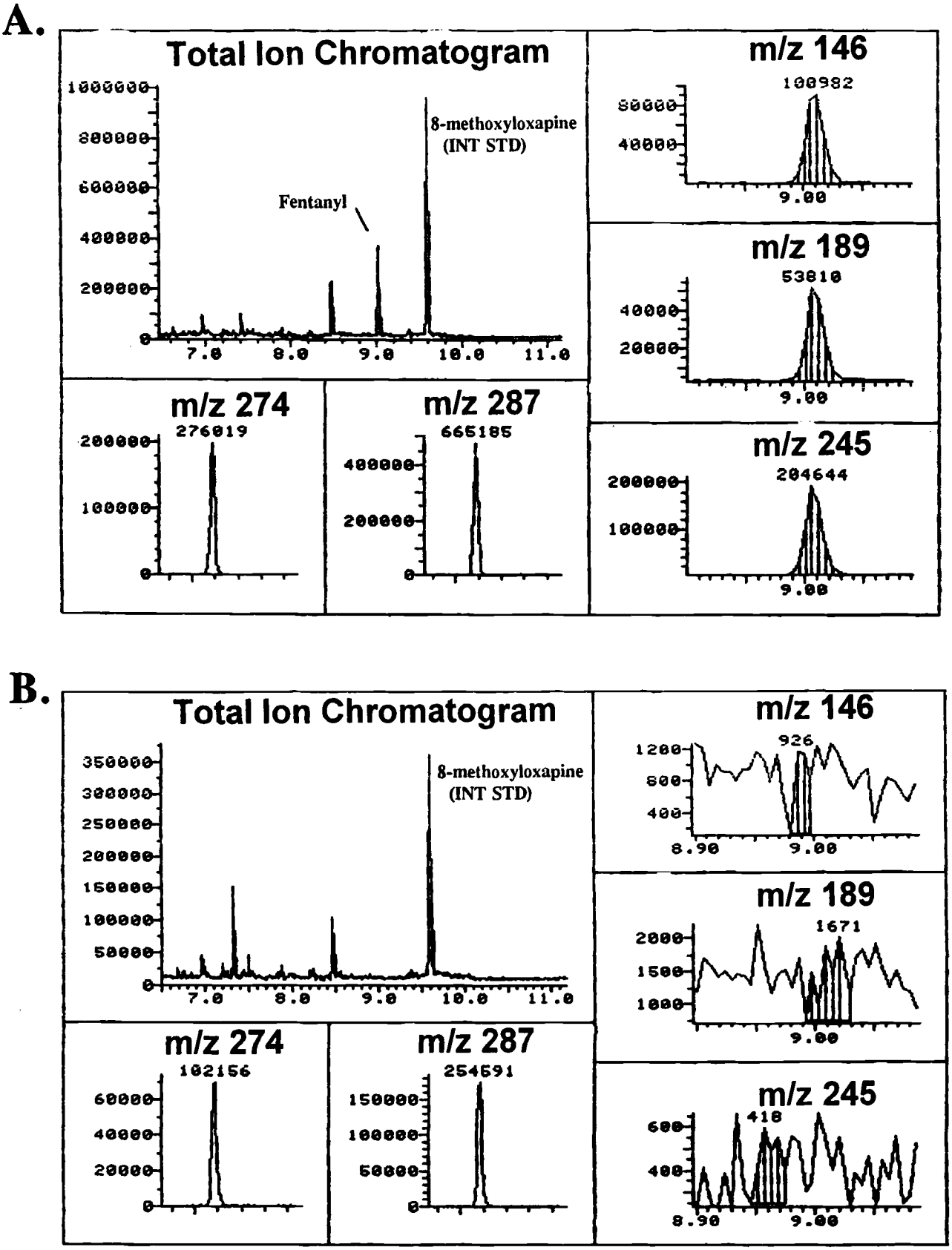

FIG. 1—*Total Ion Chromatogram (TIC) and Selected Ion Monitoring (SIM) data for the GC-MS analysis of extracts from* (A) *the hair from Chemist #2, and* (B) *a Negative hair.*

improbable scenario. Eventually, Chemist #2 pled Guilty or No Contest to counts of Possession of a Regulated Drug and Larceny.

The use of hair testing for fentanyl led directly to the rapid resolution of this case. Although urine and blood methods are available for the determination of fentanyl and its analogues, they would not have provided positive findings for the "Crooked Criminalist" because of the rapid clearance of the drugs from the body. As an historical record of drug abuse, hair represents a unique opportunity for fact finding in cases such as that described here. This is especially true when internal investigations of law enforcement and laboratory personnel are conducted, and there has been a significant delay since the time of the suspected misuse or diversion of controlled substances. Hair testing for drugs of abuse is an extremely effective tool for assisting investigators in bringing these sensitive matters to rapid resolution. When adequate analytical and interpretive guidelines and safeguards are built into the testing protocol, the results will withstand administrative and judicial scrutiny.

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