DETECTION OF MORPHINE IN OPIOID ABUSERS HAIR BY GC/MS

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ABSTRACT

Thirty hair samples were collected from the male opioid abusers in which the presence of morphine in their urine samples was confirmed by Thin Layer Chromatography (TLC) analyses. The hair samples were washed, cut into small pieces and extracted in a mixture of methanol-triflouroacetic acid (9:1). The methanolic phase was evaporated to dryness under nitrogen stream and derivitized by addition of N-methyl-N-trimethylsilyl triflouroacetamide (MSTFA) and 1% trimethyl iodosilane (TMIS) with sonication. One micro liter of each derivitized sample was injected into a Gas Chromatograph-Mass Spectrometer (GC/MS) system consisting of a capillary column and finnigan MS with selective ion monitoring (SIM) mode. The selected mass for ions codeine and morphine were 370 and 429, respectively. The limit of detection (LOD) was set at 0.03ng/mg of the hair. By using the above procedure, morphine was detectable in all of the examined samples and this method is capable to detec low levels of morphine in hair for a long period of time following the last intake of the drug.

Key Words: Hair analysis, Opioids, Morphine, Codeine, GC-MS.

INTRODUCTION

Detection of abused drugs in hair samples has been an important tool in forensic toxicology for investigation of the drug addiction history (1,2). Drugs can be detected several months after the last intake, since they enter the hair roots from the capillaries and are embedded in the hair stalk, which grows at a rate of approximately 0.9 –1.2 cm per month (3). Therefore, hair can be used as a "calendar" of the past exposure to drugs. The first case of poison determination in human hair was published by Casper in 1858 (4). He had determined arsenic in the hair of exhumed body after 11 years. Nearly 100 years later in 1954, Goldblum determined amphetamine in hair of a guinea-pig by Radio-Immune Assay (RIA) (5).

The first examination of opiates in human hair by RIA was performed in 1980 (6). RIA is a highly sensitive method but positive results of this method should be confirmed by a chromatographic method. Application of GC-MS was a turning-point in detection of drugs in hair. Detection of opiates and cocaine in hair by GC-MS started in 1980 and since that time the number of papers and newly detected compounds with higher sensitivity and accuracy has rapidly increased (6-8). According to a report of drugs in hair, even if a single hair remaines in the crime seen, is as useful as serological results (9). Following introduction of GC coupled with Mass Selective Detector (MSD), which has increased sensitivity and precision, it has become possible to detect the drugs at low concentration more specifically and with high sensitivity. The results of drug analysis in hair segments indicate that this approach could have an important diagnostic value in studying retrospective abuse history of a subject (9-12).

Opiates are the most abused group of chemicals and morphine is the most important member among them. Opiates, cocaine, amphetamines and other drugs in hair have been found at higher concentrations than their metabolites (13). The purpose of the present study was to identify morphine in human hair by GC-MS, with a simple and highly sensitive method.

MATERIAL AND METHODS

Standards and reagents

All solvents and materials were of analytical grade. Morphine hydrochloride and codeine were dedicated by United Nations International Drug Control Program (UNDCP). Methanol (HPLC grade), triflouroacetic acid, 2-propanole and ammonium iodide (NH_4I) were obtained from Merck (Germany). N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) and ditioerytrytol (DTE) were purchased from Sigma, USA.

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The derivetizing reagent (MSTFA+1%TMIS) was prepared from MSTFA, NH_4I and DTE (14). Standard solutions (morphine and codeine) were prepared in methanol and stored at –15°C until use. Control and calibration samples were prepared by addition of standard solutions to the blank hairs prior to the analysis. All samples were collected from addicts, which were referred to detoxification centers (table 1). All hair samples were stored in an aluminum foil at 0°C until analysis.

Analysis of urine

Urine samples were screened by use of a morphine test strip (Sovo Tec Diagnostics, Inc., Abazar Trading, Iran) with 300 $ng.ml^{-1}$ cut-off, and positive results were further confirmed by TLC following a solid phase extraction (Sam Fanavar Co., Tehran, Iran) (15).

Analysis of the hair specimens

Instrumentation

A Thermoquest 2000 Gas Chromatograph coupled with a Thermo Finnigan Mass Trace MS model (Thermo MassLab Ltd., Manchester, U.K.) was used. The column was a 30 m DB-1 MS (J & W Scientific) with 0.25 mm I.D. and 0.25 μm film thickness. Helium was used as carrier gas at a constant flow of 1.5 $ml.min^{-1}$. Splitless injection was used with a splitless time of 60 s. The injector and interface line temperatures were held at 250 °C and 265 °C, respectively. Oven temperature was held at 150 °C for 2 min and increased to 280 °C at a rate of 10 °C min^{-1} and held at this temperature for 15 min. The selected ions mass for codeine and morphine in SIM mode were 370 and 429, respectively.

Sample preparation

About 50 mg of the hair specimens were weighted and washed consecutively with 2 ml of 2-propanol, 2 ml of deionized water, and at the end with 2 ml of 2-propanol, and then were allowed to be dried of air (16). The samples were cut into small pieces, about 1-2 mm long, and 30 mg of them were weighed (17). Two ml of methanol-triflouroacetic acid (9:1) mixture was added to the specimens as digestion and extraction solvent, and the mixture was heated for 18 h at 40-45 °C in an oven. Sonication was used to improve extraction recovery during incubation time (18). The methanolic phase was transferred to a capped-tube, and evaporated to dryness under nitrogen flow at 50 °C. After evaporation of the solvent, samples were reconstituted and derivetized by addition of 100 μl of MSTFA+ 1% TMIS, and then heating the mixture for 20 min at 70 °C in a sonication bath. After cooling to room temprature, 1 μl of the resulting sample was injected to the GC-MS.

Limit of detection (LOD) for the analyte was determined by decreasing the concentration of spiked blank hair samples until the response was equaled to signal to noise ratio (S/N) of 5 and coefficient correlations percent (CV%) for 6 injection was less than 10. Recovery percent was also determined in three concentrations of methanolic solution and spiked to blank hair sample (table 2).

RESULTS AND DISCUSSION

GC-MS chromatograms of codeine and morphine standards in methanolic solution (A) and hair matrix (C), spiked with morphine and codeine, are presented in figure 1. Limit of detection (LOD) for morphine was $1ng.ml^{-1}$ (approximately 0.033 $ng.mg^{-1}$ hair). Morphine was converted to a trimethylsilyl (TMS) derivate by MSTFA and TMIS. Both hydroxyl groups of morphine were converted to the related trimethylsilyl derivatives and no peak of mono substituted morphine-TMS was present in the corresponding chromatogram. In the absence of trimethyl iodosilan, the reaction of derivatization was not complete and two peaks of mono- and di-morphine-TMS were detectable. The MSTFA+TMIS reagent was used for the first time during this study to derivatize opioids. Application of this highly active reagent and the use of ultrasonic bath during extraction and derivetization period considerably improved the recovery and efficiency of the procedure.

In the above experiment codeine was also detectable in the form of codeine-TMS (figure 1). The simultaneous detection of codeine and morphine may distinguish abusers. It is of worth to notice that heroine abusers may also be identified by hair analysis since monoacethyl morphine is exclusively detectable in the hair of diacethyl morphine (heroine) abusers, while morphine and codeine are present in hair samples of opium abusers. Due to the short half-life of diacethyl morphine, however, its detection in hair samples is not possible but monoacethyl morphine and morphine accumulates in the hair for several months and can be detected using the same method (19,20). A typical hair chromatogram of opium abusers is illustrated in figure 1D. As it can be seen from the chromatogram, codeine-TMS and morphine-TMS are characterized by retention times of 13.55 and 14.10, respectively.

While morphine could be determined in 30 samples hair specimens under study (table 1), it was only detectable in the urine samples of 28 abusers. The two negative samples were obtained from individual volunteers that were used opium several weeks before sampling (periodically abusers). This findings support the value of the GC-MS method for confirmation of the results of urine

Table 1. Characteristic list of individual volunteers who were referred to detoxification center

Code	Age (years)	Daily Dose (g)	Duration (months)	Hair color	Date of urine collection	Date of hair Collection	Urine *	Hair **
1	45	6	72	Black	May 26, 02	June 10, 02	Positive	Positive
2	30	5	36	Black	May 30, 02	June 08, 02	Positive	Positive
3	33	1	26	Blond	May 30, 02	June 06, 02	Positive	Positive
4	51	3	92	White	June 02, 02	June 23, 02	Positive	Positive
5	35	2	22	Blond	June 02, 02	June 15, 02	Positive	Positive
6	36	5	48	Black	June 03, 02	June 22, 02	Positive	Positive
7	21	1	13	Blond	June 06, 02	June 10, 02	Positive	Positive
8	28	2.5	36	Black	June 06, 02	June 15, 02	Positive	Positive
9	26	3	30	Black	June 09, 02	June 20, 02	Positive	Positive
10	35	PA	24	Black	June 09, 02	June 06, 02	*Negative*	Positive
11	41	2	60	Blond	June 10, 02	June 15, 02	Positive	Positive
12	36	2	36	Blond	June 10, 02	June 20, 02	Positive	Positive
13	35	4	36	Black	June 12, 02	June 12, 02	Positive	Positive
14	36	3	42	Black	June 12, 02	June 20, 02	Positive	Positive
15	37	3	36	Black	June 15, 02	June 24, 02	Positive	Positive
16	30	PA	20	Black	June 15, 02	June 15, 02	*Negative*	Positive
17	25	3	24	Black	June 17, 02	July 01, 02	Positive	Positive
18	44	3	18	Blond	June 18, 02	July 08, 02	Positive	Positive
19	32	4	24	Blond	June 20, 02	July 03, 02	Positive	Positive
20	35	2	36	Blond	June 22, 02	June 22, 02	Positive	Positive
21	34	1	12	Blond	June 24, 02	July 06, 02	Positive	Positive
22	46	2	24	Blond	June 24, 02	July 01, 02	Positive	Positive
23	30	2	18	Blond	June 26, 02	June 26, 02	Positive	Positive
24	28	3	36	Black	July 01, 02	July 16, 02	Positive	Positive
25	28	7.5	48	Black	July 01, 02	July 11, 02	Positive	Positive
26	31	4	36	Black	July 01, 02	July 13, 02	Positive	Positive
27	42	3-4	48	Black	July 01, 02	July 18, 02	Positive	Positive
28	33	1	27	Light brown	Aug. 03, 02	Aug. 11, 02	Positive	Positive
29	38	3.5	48	Black	Aug. 06, 02	Aug. 16, 02	Positive	Positive
30	40	4	36	Black	Aug. 13, 02	Oct . 17, 02	Positive	Positive

Samples were collected 2 days after the last opium abuse, PA (samples from Periodically Abusers who abused opium 2 weeks before sampling).

* Result of morphine analysis in urine

** Result of morphine analysis in urine

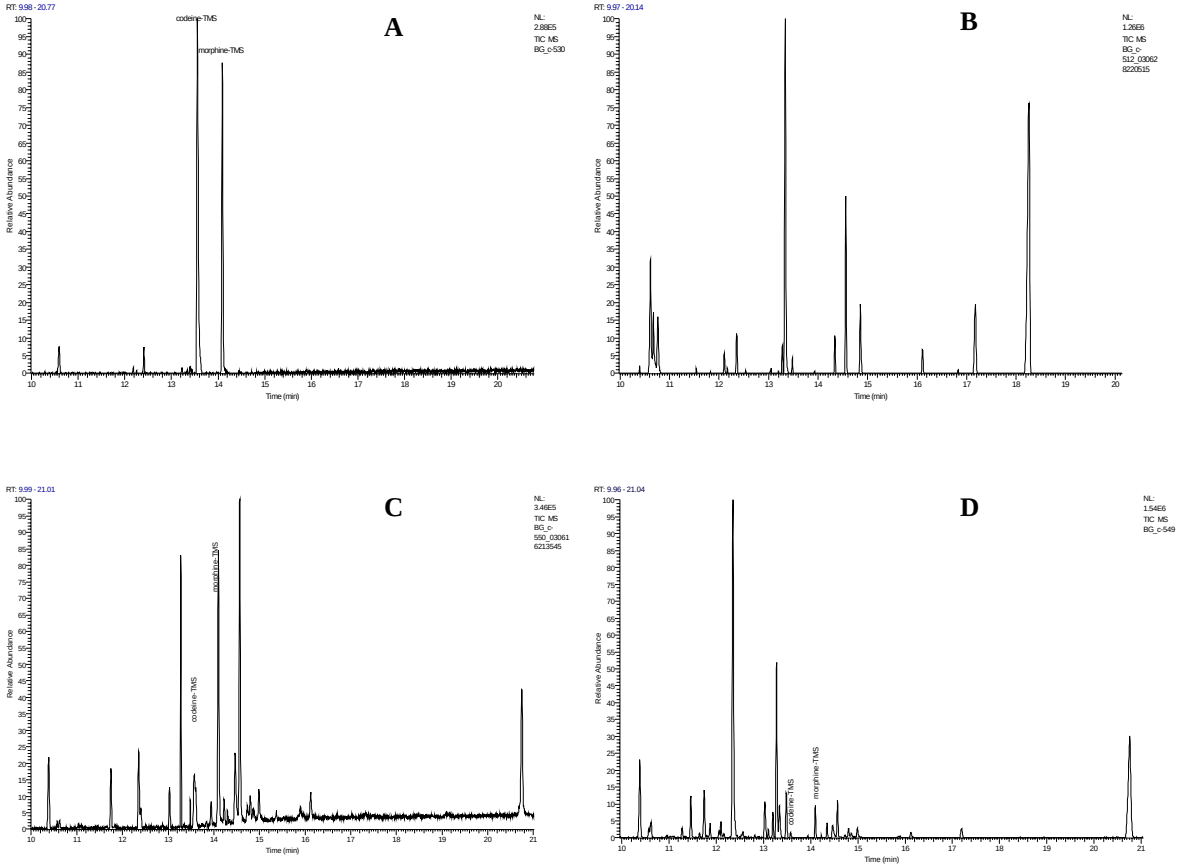

Figure 1.. GC/MS Chromatograms of codeine (RT=13.55) and morphine (RT=14.10) standards in methanolic matrix (A) and blank hair sample (B), spiked codeine and morphine in hair matrix (C) and hair chromatogram of an opium abuser (D) derivatized by MSTFA + %1TMIS.

Table 2. Recovery and CV% results of three concentration for morphine of methanolic solution and spiked blank hair samples (n=6).

Concentration ($ng.mg^{-1}$of hair)	Concentration found mean ± SD	CV %	Recovery %
0.033 (6)	0.032 ± 0.003	9.30	96.97
1.667 (6)	1.624 ± 0.082	4.93	97.42
3.333 (6)	3.254 ± 0.136	4.18	97.63

analysis in suspected cases or even when no urine sample is available. It is noteworthy to mention that morphine and other opioids will last in hair for much longer period of time than urine, *i.e*. several months following the last use (21).

Sensitivity of the employed analytical procedure was 0.033 $ng.mg^{-1}$ of the hair which is more than other reported values of, 0.1-0.5 $ng.mg^{-1}$ hair (10, 22-24). The method is also a simple procedure to use and dose not require solid phase extraction. The reported recovery results for the employed concentrations (0.033-3.333 $ng.mg^{-1}$ hair) were between 96.97 – 97.63 percent in an acceptable range (table 2).

CONCLUSION

The results of this study indicate that the GC-MS method in SIM mode, is a simple, sensitive and reliable procedure for the analysis of morphine (even in periodically opium abuser) and other opium alkaloids, such as codeine in human hair. The method has also important application in medical and forensic toxicology since morphine and codeine can be detected in hair several months after the last intake and as a result hair is considered as a “calendar” of the past exposure to opioids. Therefore, this study further proves the value of hair analysis in suspected urine cases (positive or negative) or when no urine sample is available.

REFERENCES

1. Baumgartner W A, Hill K A, Blahd W H. Hair Analysis for Drugs of abuse. J Forensic Sci 1989; 34:1433-1453.
2. Kintz P, Tracqui A, Mmangin P. Detection of drugs in human hair for clinical and forensic applications. Int J Legal Med 1992; 105:1-4.
3. Kronstrand R, Grundin R, Jonsson J. Incidence of opiates, amphetamines, and cocaine in hair and blood in fatal cases of heroine overdose. Forensic Sci Int 1998; 92:29-38.
4. Casper JL. Practical Handbook of the Forensic Medicine. Berlin: Hirschwald A; 1858. p. 85-100.
5. Moller M. Drug detection in hair by chromatographic procedures. J Chromatogr 1992; 580:125-34.
6. Sachs H. History of hair analysis. Forensic Sci Int 1997; 84:7-16.
7. Baumgartner A M, Jones P F, Baumgartner W A and Black C T. RIA of hair for determining opiate abuser histories. J Nucl Med 1979; 20:748-52.
8. Valente D, Cassani M, Pigliapochi M and Vansetti G. Hair as the sample in assessing morphine and cocaine addiction. Clin Chem 1981; 27:1952-53.
9. Arnold W. The determination of drug and their substitutes in human hair. Forensic Sci Int 1990; 46:17-18.
10. Pushel K, Thomasch P, Arnold W. Opiate levels in hair. Forensic Sci Int 1983; 21:181-86.
11. Nakahara Y N. Detection and diagnostic interpretation of amphetamine in hair. Forensic Sci Int 1995; 70:135-53.
12. Strano-Rossi S, Bermeejo-Barrera A, Chiarotti M. Segmental hair analysis for cocaine and heroine abuse determination. Forensic Sci Int 1995; 70:211-16.
13. Kintz P, Mmangin P. What constitutes a positive result in hair analysis: proposal for the establishment of cut-off values. Forensic Sci Int 1995; 70:3-11.
14. Masse R, Ayotte C, Dugal R. Studies of anabolic steroids. J Chromatogr 1989; 489:23-50.
15. Thghavi SA, Nazeri A, Fekri M, Sabzevari O. Extraction of morphine from human urine by SPE and LLE system for an improved semi-quantitation scanning method. J Faculty of Med, Shahid-Beheshti University of Medical Sciences 2003:27:23-7.
16. Kronstrand R, Grundine R and Jonsson J. Incidence of opiates, amphetamines, and cocaine in hair and blood in fatal cases of heroine overdose. Forensic Sci Int 1998; 92:29-38.
17. Society of hair testing. Forensic Sci Int 1997; 84:3-6.
18. Roth M, progst F. Solvent optimization for the direct extraction. J Anal Toxicol 1995; 19:236-40.
19. Karl A, Sporer MD. Acute heroine overdose. Annual Int Med 1999; 130:584-590.
20. Sawynok J. The therapeutic use of heroine: A review of the pharmacological literature. Can J Physical Pharm 1986; 64:1-6.
21. Kalasinski K, Maluilo J Jr, Schaeler T. Study of drug distribution in hair by infrared microscopy visualization. J Anal Toxicol 1999; 18:337-341.
22. Paterson S, Mclaahlan-Troup N, Dohnal M. Qualitative screening for Drug of Abuse in Hair using GC/MS. J Anal Toxicol 2001; 25:190-97.
23. Kintz P, Bundeli P, Benneisen R, Ludes R. Dose concentration Relationships in Hair from subjects in a controlled Heroine: maintenance program. J Anal Toxicol 2001; 22 :231-35.
24. Montagan M, Polettini A, Stramesi C, Groppi A, Viganali C. Hair analysis for opiates, cocaine and metabolites. Evaluation of a method by interlaboratory comparision. Forensic Sci Int 2002; 128:79.