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Original Article

A Rapid and Sensitive Method for Determination of Morphine in Saliva of Opium Addicts

K. Javidnia, R. Miri, D. Miri

Abstract

Background: Saliva is an alternative biological matrix in drug testing. It has several advantages in comparison with other biological fluids such as blood and urine. Collection of saliva is simple, noninvasive and can be controlled under direct observation in drug abusers, or opium addicts.

Methods: In the present study we report our experience with saliva collection, liquid-liquid extraction and quali-quantitation of morphine abuse. The GC-MS method was applied to the analysis and determination of morphine in saliva samples obtained from addicted persons.

Results: LOD of the method was 1ng/ml and LOQ 5 ng/ml, with acceptable within-and between-day reproducibility and a linearity (r^2=0.998) over a concentration range from 5 to 500ng/ml.

Conclusion: The results of this study indicated that saliva may have potential use for identification of patients suspected to drug abuse.

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Keywords ● Morphine ● saliva ● GC-MS ● detection

Introduction

During the last decade, the use of saliva as a diagnostic tool in therapeutic drug monitoring, detection of suspected drug abusers and pharmacokinetic studies has been increased.[1-4] There are several advantages of using saliva, as an analysis tool, to obtain appropriate results similar with other biological fluids. Collection of saliva is simple, noninvasive and its collection can be controlled under direct observation in drug abusers. Multiple saliva sampling for drug monitoring, especially in pediatric and neonatal patients, is easy, convenient, and painless.

Laboratory services play an important role in initial diagnosis of addicted persons. Many laboratories and clinics use radioimmunoassay as a routine procedure for the analysis of morphine, while it is not selective and has false positive results due to the presence of unrelated compounds.[1] Gas Chromatography-Mass Spectrum (GC-MS) is a highly sensitive and selective method, which do not have any false positive results.[5] It also provides the mass spectrum of the peak which is important for legal purposes.[5]

In many reports morphine-d_3 has been used as an internal standard to detect mophine.[5,7] In these methods a solid phase extraction is used for purification of morphine from the saliva.[2,6]

Department of Medicinal Chemistry,
School of Pharmacy and
Medicinal & Natural Products Chemistry
Research Center,
Shiraz University of Medical Sciences,
Shiraz, Iran.

Correspondence:
Katayoun Javidnia PhD,
Department of Medicinal Chemistry,
School of Pharmacy,
Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel: +98 711 2303872
Fax: +98 711 2332225
E-mail: javidniak@sums.ac.ir

Some methods use one step liquid-liquid extraction for obtaining morphine from plasma or saliva samples which is expensive and cumbersome.[5,7] In the present study, we have introduced a new standard way which is cheaper and more cost benefit and can be used for detection of very low levels of morphine in the internal milieu without affecting the accuracy and validity of the method. We improved the method of extraction by using two steps of liquid-liquid extraction followed by derivatisation with Bis (trimethylsilyl)-tri fluoroacetamide (BSTFA). Then we applied this method for the determination of morphine concentration in saliva samples taken from opium addicted individuals.

Materials and Methods

A 5973 N Hewlett-Packard mass spectrometer coupled to a HP 5890 gas chromatograph (GC) was used in EI mode (70 eV). The GC oven was equipped with crossed linked 5% phenyl methyl siloxane (HP-5, 25 m* 0.25 mm i.d.) with He as the carrier gas and split less mode. The flow rate of the carrier gas was 1.0 ml/min and the injector temperature was 250°C. The programming temperature was performed from 180°C for 3 min and to 260°C at 12°C/min, and finally holding it at 260°C for 5 min.

The stock solutions of morphine and metronidazole (BP grade) were prepared separately at concentration of 1mg/ml in methanol and stored at 4°C. The working solutions then were prepared by serial dilution of the stock solution in distilled water. Standard calibration samples were prepared by spiking the 1 ml blank saliva samples with known morphine concentrations (5-500 ng/ml) and 50 µl of Internal Standard (IS, 0.2 mg/ml). The percentage recoveries were determined by comparing the peak area of neat and extracted samples.

Extraction from saliva was performed by liquid-liquid extraction. One ml of saliva samples, spiked with 50 µl of IS (0.2 mg/ml), were washed with 5 ml of hexane and the organic layer was discarded. The aqueous phase was made alkaline by addition of carbonate buffer (pH= 9.2), then shaken with 5 ml ethyl acetate for 5 min. The organic layer was separated after centrifugation, and dried under nitrogen. BSTFA (Merck, Darmstadt; Germany) was added and the mixture was heated at 80°C for one hour and finally 0.2 µl of it was injected to GC-MS.

The GC-MS method was validated over the concentration range of 5-500 ng/ml. The inter- and intra-day assay precision and accuracy were determined from the analysis of controlled saliva.

Three different concentrations were prepared and analyzed in the first day and three replicates per concentration were analyzed over 3 days for calibration the curve. This method was also applied to determine the morphine concentration in 12 opium addicted persons.

Results

One ml of saliva samples was needed in this study and LOD of 1ng/ml and LOQ 5 ng/ml were achieved. The linear range for morphine (n=4; r^2=0.998) were over the concentrations of 5-500 ng/ml. The extraction recoveries were calculated as 91±2.9%. The extraction procedure was selective for morphine and no interfering peaks were observed (Fig 1 A). Metronidazole was also used as an internal standard for the presence of one hydroxyl group, which can be salicylated as morphine (Fig 1B) in derivatisation procedure and obtained results, confirmed the validity of the method.

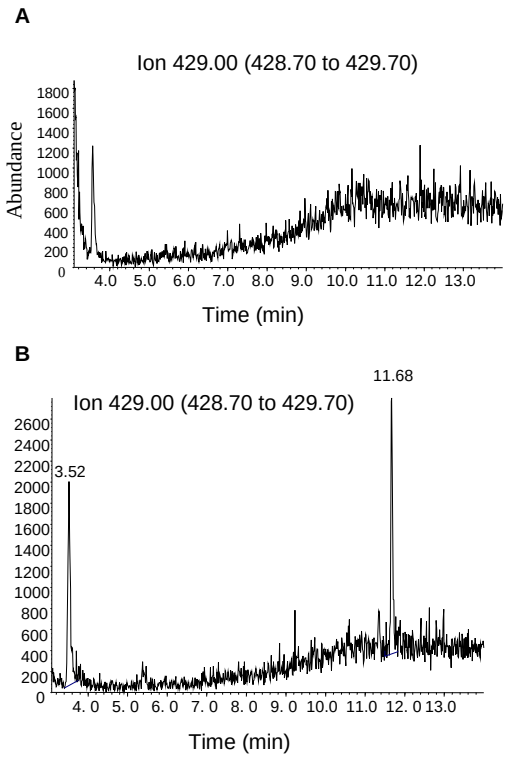

Fig 1: Extracted ion 429 chromatogram of blank saliva sample (A), and Saliva spiked with 5ng/ml of morphine (B). Rt=11.68: silylated morphine

The within precision and the accuracy of the method were determined at three different concentrations (n=3) for intra- and inter-assay analyses (Table 1). Finally the results of determination of morphine in 12 opium addicts are presented in Table 2.

Table 1: Intra- and inter- day variations of morphine assayed in human saliva

Concentrations				
	Intra- day		Inter- day	
Added (ng/ml)	found (ng/ml)	% Ac (CV)	found (ng/ml)	% Ac (CV)
10	9.7	97.0 (9.2)	10.5	110.0 (7.0)
50	47.9	95.8 (10.8)	50.3	100.6 (15.7)
100	98.6	98.6 (16.1)	98.5	98.5 (11.2)

Table 2: Concentration (conc) of morphine in saliva samples of 12 opium addicted individuals identified as 1 to 12.

No	Age (yr)	PA (yr)	LT (hr)	Conc. (ng/ml)
1	21	4	39	178.0
2	25	4	40	146.0
3	26	3	48	46.6
4	30	3	30	56.0
5	20	3	24	116.6
6	32	4	340	10.1
7	48	3	40	27.0
8	30	4	30	38.6
9	31	4	400	2.4
10	35	5	120	4.9
11	40	3	30	100.1
12	27	3	2	498.7

PA= period of addiction; LT= last time abused

Discussion

Saliva is a filtrated fluid of the blood but less intricate and has interferences from endogenous compounds. Its sampling is easier and noninvasive. The collection of a saliva sample is relatively easy to perform and can be achieved under close supervision.[8] Although, most patients prefer donating saliva rather than blood or urine. Many laboratories and clinics use radioimmunoassay as a routine method for the analysis of morphine, while it is not selective and has false positive results due to the presence of unrelated compounds. GC-MS is a highly sensitive and selective method, which do not have any false positive results as we can observe in RIA.[5] It also provides the mass spectrum of the peak which is documented and is important for legal purposes.[5]

Many methods used for extraction of morphine or other drugs from saliva contain solid phase.[2,6] Whereas, in this study we used hexane for liquid-liquid extraction of morphine and the clean up process from saliva. As showed in Figs 1A and 1B the extraction procedure for morphine was selective.

The GC-MS method was applied to analysis and determination of morphine in saliva samples obtained from opium addicted persons. Twelve subjects suspected to drug abuse among a pool of volunteers were studied. A history of patients including what they use, the last time of abuse and the amount of opium they used at the last time, was obtained. The morphine concentration in saliva samples of the persons were determined by this method. As shown in table 2 there is a significant correlation between the last time of abuse and morphine concentration in saliva. The results show that the method is useful for determination of morphine in saliva sample even after 400 hours after the last time of abuse.

Acknowledgments

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