Solid-Phase Microextraction in the Determination of Methadone in Human Saliva by Gas Chromatography–Mass Spectrometry

A.C. dos Santos Lucas[1], A. Bermejo[2], P. Fernández[2], and M.J. Tabernero[2]

[1]Department of Clinical Analysis and Toxicology, Faculty of Pharmacy, University of Amazonas, Brazil and [2]Department of Legal Medicine, Forensic Toxicology Service, Faculty of Medicine, University of Santiago de Compostela, Spain

Abstract

Solid-phase microextraction (SPME) with a 100-µm polydimethylsiloxane film fiber was applied to the determination of methadone and 2-ethylidine-3,3-diphenylpyrrolidine (EDDP) by GC–MS in human saliva and compared with liquid–liquid extraction. A shorter extraction time of 30 min with the fiber was obtained, speeding up the total analysis time. Linearity was found for SPME from 0.05 to 2.0 µg/mL (r = 0.9976 for methadone; r = 0.9988 for EDDP) with precision between 0.7 and 4.3% for saliva spiked with 0.2 and 1.5 µg/mL of methadone and EDDP. The limit of detection using SPME was 0.04 µg/mL for methadone and 0.008 µg/mL for EDDP. Analytical recoveries of SPME and liquid–liquid extraction ranged from 98.8 to 103.6%. The use of deuterated internal standard by both methods have yielded comparable results. Thus, the SPME method is highly accurate, precise, and useful for determination of methadone and EDDP in saliva.

Introduction

Saliva presents a number of advantages compared to the body fluids traditionally used as substrates for therapeutic drug monitoring, that is, blood and plasma. It is obtained by a painless and noninvasive method of sampling; it does not require specially trained personnel; it is readily available; it contains the free fraction of drugs and, therefore, is a better indicator of intoxication states (1–3).

The quantitative measurement of methadone in saliva was performed in the past using liquid–liquid and solid–liquid extractions (4–7). However, these extraction procedures need at least 1 mL of sample, and the commercial devices for saliva collection do not provide enough sample (2,3).

Solid-phase microextraction (SPME) was initially applied to the determination of pesticides and other analytes of environmental interest, and subsequently used to the determination of many drugs in biological fluids. This extraction method is characterized by its small sample size and short time of analysis (8), but it is relatively nonselective because the validation of SPME methodologies for each drug and biological fluid is necessary.

This work describes the determination of methadone and 2-ethylidine-3,3-diphenylpyrrolidine (EDDP) in saliva by gas chromatography coupled to mass spectrometry (GC–MS) and the comparative study of the use of SPME and liquid–liquid extraction. Finally, the method is applied to the quantitation of both analytes in the saliva from patients in a methadone-maintenance program.

Materials and Methods

Standards and reagents

Methadone hydrochloride and methadone-d_3 hydrochloride were obtained from Sigma Chem. Co. EDDP perchlorate and EDDP-d_3 perchlorate were obtained from Radian Corp. Methanol, n-hexane (high-performance liquid chromatography grade), sodium hydroxide, borax, and sodium chloride (analytical grade) were obtained from Merck Co.

Individual methanolic stock solutions containing 1 mg/mL of methadone, methadone-d_3, EDDP, and EDDP-d_3 were prepared. Working solutions of 0.1 and 0.01 mg/mL for methadone and EDDP and 0.05 and 0.01 mg/mL for the deuterated used as internal standards (IS) were subsequently prepared.

Blank human saliva were spiked with methadone and EDDP at the concentrations of 0.05, 0.1, 0.5, 1.0, 1.5, and 2.0 µg/mL for quantitative comparisons. Quality-control samples for precision evaluation (0.2 and 1.5 µg/mL methadone and EDDP) were prepared. Drug-free saliva was analyzed as the negative control in all assays.

The solutions of 0.05M NaOH and borax buffer (pH 9.2) were prepared with distilled water.

Equipment

GC–MS analysis was performed with a Hewlett-Packard

model 6890 GC equipped with an HP-5 capillary column (12 m × 0.22-mm i.d., 0.33-µm film thickness of cross-linked 5% phenyl methyl silicone). The injection port (splitless) was set at 250°C and the purge time was of 0.75 min for LLE and of 2.0 min for SPME. The column temperature was initially held at 90°C for 2 min, increased to 200°C at 30°C/min, and held at 200°C for 5 min, then increased to 290°C at 30°C/min.

A Hewlett-Packard model 5973 mass selective detector in SIM mode coupled to GC was used for quantitative analysis. The electron impact of 70 eV was used for the ionization of the compounds, and the quantitation was based on target peak (in parentheses) area ratios of methadone and EDDP with their respective internal standards. Ion currents at *m/z* (294), 295, and 223; (297) and 226; (277), 276, and 262; and (280), 279, and 265 were monitored for methadone, methadone-d_3, EDDP, and EDDP-d_3, respectively.

The SPME device equipped with a fiber of 100-µm polydimethylsiloxane film (Supelco®) was used for the microextraction.

SPME

The microextraction was performed at room temperature. To 0.1 mL of the saliva samples, standards and controls were added with 5 µL of each IS solution (0.01 mg/mL methadone-d_3 and EDDP-d_3), 0.4 mL of borax buffer (pH 9.2), and sodium chloride salt, then mixed. The fiber was directly dipped in this solution for 30 min, then inserted into the GC injection port and stripped at 250°C for 5 min.

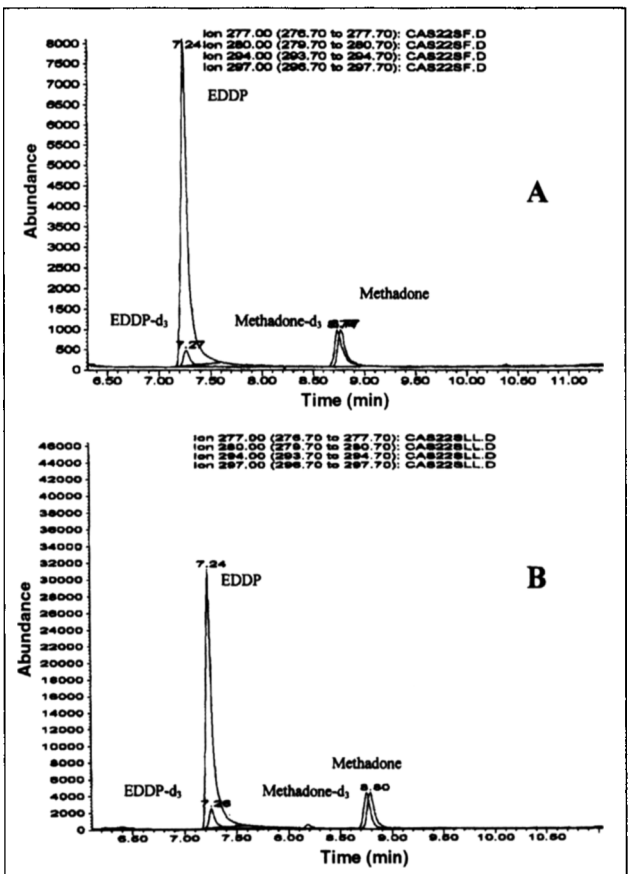

Figure 1. Extracted ions chromatograms of Patient 3 saliva sample using the SPME (A) and liquid–liquid extraction (B).

Liquid–liquid extraction

For a quantitative comparison with the SPME method, a previously reported liquid–liquid extraction for methadone was used (9). To 1 mL of the saliva samples, standards and controls were added with 10 µL of each IS solution (0.05 mg/mL methadone-d_3 and EDDP-d_3), 1 mL of 0.05M sodium hydroxide, and 3 mL of *n*-hexane. The samples were mechanically mixed for 60 min, then centrifuged for 10 min (4000 rpm). The organic upper layer was aspirated and evaporated at 60°C. The dried extract was redissolved in 10 µL of methanol, and 1 µL was injected into the GC–MS.

Results and Discussion

Quantitation

The GC program used in this work was efficient to separate methadone and EDDP (Figure 1). No carryover was observed

Table I. Calibration Curve Equations and Correlation Coefficients (*r*) for Methadone and EDDP in Saliva Using Liquid–Liquid Extraction and SPME

Analyte	Liquid–liquid extraction	SPME
Methadone	$y = 1.7425x + 0.0160$ $r = 0.9988$	$y = 1.7222x + 0.0385$ $r = 0.9976$
EDDP	$y = 2.1741x + 0.0039$ $r = 0.9983$	$y = 2.6425x + 0.0652$ $r = 0.9988$

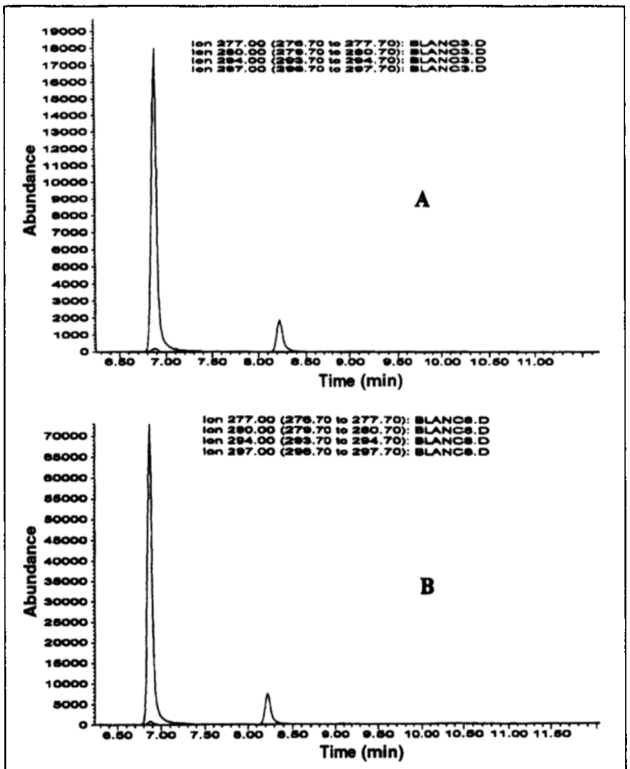

Figure 2. Extracted ions chromatograms of a blank saliva sample using the SPME (A) and liquid–liquid extraction (B).

with the desorption time of 10 min at 250°C for the SPME, and there was no observed degradation of the SPME fiber after at least 90 runs.

The standard calibration curves were obtained in triple runs through the described analysis. Table I shows the calibration curve equations and the correlation coefficients (r) for methadone and EDDP in saliva by using liquid–liquid extraction and SPME. The calibration was found to be linear over the range 0.05–2.0 µg/mL for methadone and EDDP, with both extraction methods. Correlation coefficients, which were between 0.9976 and 0.9983, were found to be quite acceptable.

Figure 2 shows the extracted ion chromatograms obtained from a blank saliva using SPME and liquid–liquid extraction. No interference from the sample matrix was observed.

As can be seen, the equations related to the two extraction procedures for each analyte are very similar, but statistical differences were found when the F-test was applied to compare the slope of the two calibration curves (10) for EDDP (Figure 3). Therefore, the SPME curve is more sensible in concentration changes for EDDP. No statistical differences were found when the F-test was applied at a confidence level of 95% for the two methadone curves (Figure 4).

These results demonstrate that the SPME curves may be used for accurate quantitation of methadone and EDDP. The use of internal standards is believed to correct any variation in the composition or preparation of the sample.

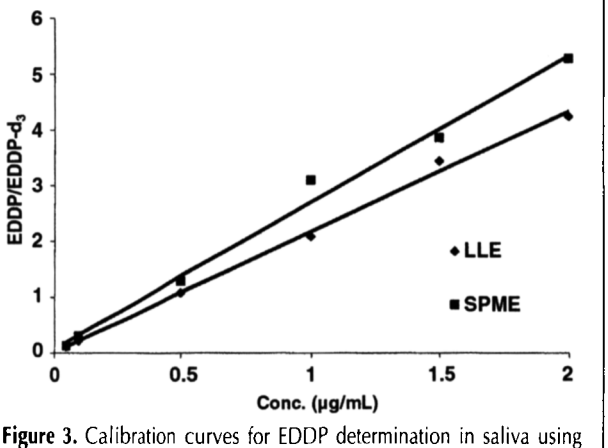

Figure 3. Calibration curves for EDDP determination in saliva using SPME and liquid–liquid extraction (LLE).

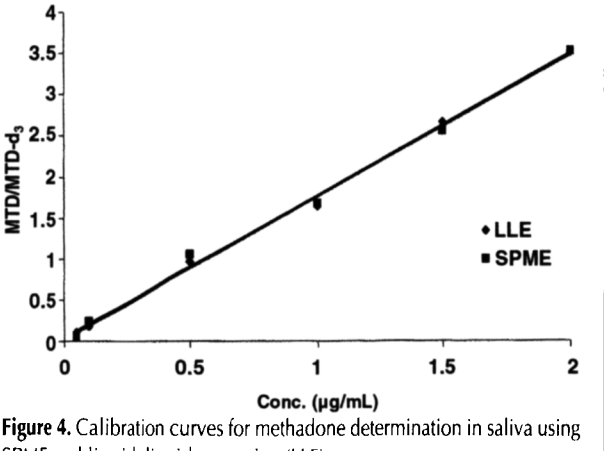

Figure 4. Calibration curves for methadone determination in saliva using SPME and liquid–liquid extraction (LLE).

Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) for methadone and EDDP were determined by replicate analysis of samples devoid of the analytes (N = 11). LOD was defined as the mean value of the apparent analyte concentration in the negative samples plus three times the standard deviation, and LOQ was defined as the mean value plus 10 times the standard deviation (11,12).

We found a lower limit of detection and of quantitation for the SPME assays, which can be seen in Table II.

Precision and analytical recovery

The precision of the methods for methadone and EDDP in saliva was studied through the within-batch precision for 11

Table II. Limits of Detection (LOD) and Quantitation (LOQ) for Methadone and EDDP in Saliva Using Liquid–Liquid Extraction and SPME

		Liquid–liquid extraction	SPME
Methadone	LOD (µg/mL)	0.032	0.004
	LOQ (µg/mL)	0.099	0.045
EDDP	LOD (µg/mL)	0.029	0.008
	LOQ (µg/mL)	0.088	0.018

Table III. Within-Batch Precision* and Analytical Recovery (%) for Methadone and EDDP in Saliva Using Liquid–Liquid Extraction and SPME (N = 11)

		Liquid–liquid extraction		SPME	
Concentration added (µg/mL)		analytical recovery (%)	RSD (%)	analytical recovery (%)	RSD (%)
Methadone	0.2	100.9 ± 2.9	4.3	97.9 ± 2.9	4.3
	1.5	99.4 ± 0.5	0.7	98.8 ± 2.0	3.0
EDDP	0.2	102.1 ± 2.1	3.1	103.6 ± 2.9	4.3
	1.5	99.2 ± 1.1	1.6	99.2 ± 1.8	2.7

* Relative standard deviation, RSD%.

Table IV. Quantitation Results for Methadone and EDDP in MMP Patients' Saliva Using Liquid–Liquid Extraction and SPME

		Liquid–liquid extraction concentration (µg/mL)	SPME concentration (µg/mL)
Patient 1	Methadone	1.60	1.54
	EDDP	0.09	0.12
Patient 2	Methadone	2.58	2.57
	EDDP	0.10	0.06
Patient 3	Methadone	1.24	1.10
	EDDP	0.04	0.08
Patient 4	Methadone	1.20	1.05
	EDDP	0.04	0.08

replicate extractions at two concentration levels, 0.2 and 1.5 µg/mL, prepared with human saliva without any drug and submitted to the extraction procedures (liquid–liquid extraction and SPME). The results (Table III) are expressed as the relative standard deviation, the values of which were lower than 5% for the two analytes at the two extraction procedures, reflecting a good precision for the analysis.

The results of analytical recovery are presented in the same table expressed as the mean recovery obtained for the analytes by each of the extraction procedures for a confidence interval of 95%. As can be observed, adequate analytical recoveries, close to 100%, were achieved with both extraction methods at the two concentration levels studied.

Analysis of saliva from methadone-maintenance program patients

For verification of the SPME method, resting saliva specimens from four patients in a methadone-maintenance program were collected and quantitated by the two extraction procedures. The results of both techniques are in reasonable agreement, as can be seen in Table IV, indicating relatively accurate quantitation results.

Conclusions

The SPME allows the time of extraction to be reduced by half. It needs a small amount of sample for analysis, preserving the sample for another analysis if necessary. It presents an accuracy comparable to the liquid–liquid extraction, but its sensitivity is higher.

This SPME method can be used for the determination of methadone and EDDP in saliva for research in the use of this kind of sample for therapeutic monitoring.

References

1. W. Schramm, R.H. Smith, P.A. Craig, and D.A. Kidwell. Drugs of abuse in saliva: a review. *J. Anal. Toxicol.* **16:** 1–9 (1992).
2. R. Haeckel and P. Hänecke. Application of saliva for drug monitoring: an in vivo model for transmembrane transport. *Eur. J. Clin. Chem. Clin. Biochem.* **34:** 171–191 (1996).
3. D.A. Kidwell, J.C. Holland, and S. Athanaselis. Testing for drugs of abuse in saliva and sweat. *J. Chromatogr. B* **713:** 111–135 (1998).
4. R.K Lynn, G.D. Olsen, R.M. Leger, W.P. Gordon, R.G. Smith, and N. Gerber. The secretion of methadone and its major metabolite in the gastric juice of humans: comparison with blood and salivary concentrations. *Drug Metab. Dispos.* **4(5):** 504–509 (1976).
5. N. El-Guebaly, W.J. Davidson, H.A. Sures, and W. Griffin. The monitoring of saliva drug levels: psychiatric applications. *Can. J. Psychiatry* **26(1):** 43–48 (1981).
6. K. Wolff, A. Hay, and D. Raistrick. Methadone in saliva. *Clin. Chem.* **37(7):** 1297–1298 (1991).
7. G.I. Kang and F.S. Abbott. Analysis of methadone and metabolites in biological fluids with gas chromatography–mass spectrometry. *J. Chromatogr.* **231(2):** 311–319 (1982).
8. J. Pawliszyn. *Solid Phase Microextraction: Theory and Practice.* VHC Publishers, New York, NY, 1997.
9. U. Meresaar, M.I. Nilsson, J. Holmstrand, and E. Änggård. Single dose pharmacokinetics and bioavailability of methadone in man studied with a stable isotope method. *Eur. J. Clin. Pharmacol.* **20:** 473–478 (1981).
10. J.C. Miller and J.N. Miller. *Statistics for Analytical Chemistry,* 2nd ed. Ellis Horwood Ltd., London, England, 1988.
11. D.A. Armbruster, M.D. Tillman, and L.M. Hubbs. Limit of detection (LOD)/limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC–MS assay of abused drugs. *Clin. Chem.* **40(7):** 1233–1238 (1994).
12. G.M. Lawson. Defining limit of detection and limit of quantitation as applied to drug of abuse testing: striving for a consensus. *Clin. Chem.* **40(7):** 1218–1219 (1994).

Manuscript received February 18, 1999;
revision received May 24, 1999.