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Determination methadone in hair using directly suspended droplet three phase microextraction technique with UV-visible spectroscopy

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ABSTRACT

In this study, we are used Liquid–liquid–liquid microextraction (LLLME) with directly suspended droplet using uv-visible spectroscopy for extraction and determination of methadone in hair. The target compound was extracted from the aqueous sample solution (donor phase, pH 9) into an organic phase and then was back-extracted into a directly suspended droplet of an acidic aqueous solution (acceptor phase, pH 1). In this method, without using a microsyringe as supporting device, an aqueous large droplet is freely suspended at the top-center position of an immiscible organic solvent, which is laid over the aqueous sample solution while being agitated. Then, the droplet was withdrawn into the microsyringe and determined with UV detection at λ=205 nm. 1-octhanol (350μL) used as organic phase. The results show extraction time: 15 min, back-extraction time: 5 min and stirring rate: 600 rpm. Also, these conditions used for determination methadone in hair samples of abusers were collected from 13 men ranging from 16 to 45 years old. The calibration graph was linear in the range of 0.2 -10 μg mL^{-1} with r = 0.995(N=5). The limit of detection and the enrichment factor were 1.7 ng mL^{-1} and 159.92, respectively. The relative recovery was in the range of 82–98% with an average of 91.50%. Also the relative standard deviation (RSD, n = 5) obtained 4.3%. All experiments were carried out at room temperature (25±0.5 °C).

Keywords: Methadone, Directly suspended droplet three phase microextraction, Spectroscopy

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INTRODUCTION

Methadone [(*R, S*)-6-(Dimethylamino)-4, 4-di phenyl heptan-3-one)] is a synthetic long-acting analgesic promoted as a treatment in detoxification and maintenance programs of heroin addiction [1, 2]. However, methadone use results also in habit formation [3, 4]. Consequently, the use of methadone represents a serious problem and indicates the necessity of control. Because of low concentration of drugs like methadone in biological samples a pre-concentration step is generally required for determination of trace amounts of drugs in the different matrixes. On the other hand, the most of techniques for the determination methadone is time-consuming (e.g., drug detection windows may be months to years for hair) therefore recent research activities are oriented forward the development of efficient, economical, and miniaturized sample preparation methods for extraction and determination of drugs.

Sample preparation is traditionally carried out by liquid–liquid extraction (LLE) or by solid-phase extraction (SPE) techniques [5, 6], which need a substantial amount of organic solvents. Also, both techniques require the evaporation of the solvent to dryness that it will lead to loss of the analytes through evaporation and reconstitution. Therefore, the extraction and clean up of the sample has been performed using a number of different purification techniques such as solid-phase microextraction (SPME) and liquid phase microextraction (LPME). The most

common sample isolation and preconcentration technique is solid-phase microextraction, which is a solvent free technique [7-9]. But this method has some disadvantages such as, the SPME fibers, which are coated with selective polymer, are fragile and still comparatively expensive. Also, SPME techniques suffer extensively from the analyte carry-over due to the incomplete analyte desorption [10]. Because of these problems, an alternative miniaturized sample preparation approach, i.e., liquid phase microextraction (LPME) emerged in the mid-to-late 1990s [11, 12]. In LPME, only a small amount of the solvent (microliter) is needed for concentrating of the analytes from the aqueous samples. This method overcomes many of the disadvantages of LLE and SPME, which are mentioned above. In two-phase LLME, extraction takes place between a small amount of a water-immiscible organic solvent and an aqueous phase containing the analytes. However, if the analytes are further back-extracted into a third (aqueous) phase, the procedure is termed three-phase LLLME or liquid–liquid–liquid microextraction (LLLME) which is usually used for the organic acids and bases [13-15].

In the present work, we used a new design of Liquid–liquid–liquid microextraction (LLLME) with directly suspended droplet using UV-visible spectroscopy for extraction and determination of methadone in hair. The methadone compound was extracted from the aqueous sample solution as donor phase into an organic phase and then back-extracted into a directly suspended droplet of an aqueous solution as acceptor phase. In this method, without using a microsyringe as supporting device, an aqueous large droplet is freely suspended at the top-center position of an immiscible organic solvent. Therefore, a larger droplet with a higher lifetime than conventional one can be used that it makes an increase at the efficiency of extraction.

MATERIALS AND METHODS

Methadone obtained from the Ministry of Health and Cure of Iran, center of North Khorasan. 1-octanol, methanol, benzene, octane, hydrochloric acid and sodium hydroxide (all from them) were purchased from Merck.

Stock solution of Methadone (1 mg mL^{-1}) was prepared in methanol and stored at 4ºC. Standard sample solutions were provided daily at different concentration by diluting the stock standard solution with distilled water.

2.1. Apparatus

The value of absorbance were measured with a spectrophotometer (model; JENWAY 6305), also the pH of the solutions was adjusted with dilute hydrochloric acid or sodium hydroxide solution using pH-meter of HORIBA (F-11).

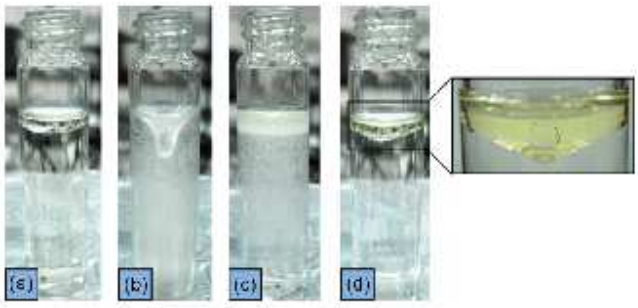

Figure 1. Illustration of the microextraction apparatus for directly suspended droplet LLLME: (a) addition of the organic solvent to the aqueous sample solution, magnetic stirrer is off; (b) the mixture is being agitated, extraction procedure; (c) separation of the tiny drop of the organic solvent and aqueous sample solution and then addition of the acceptor phase into the organic solvent, magnetic stirrer is off; (d) back-extraction procedure, magnetic stirrer is on [16]

2.3. Directly suspended droplet LLLME

5 mL of the sample solution (5µg mL^{-1}) was placed in a 6 mL glass vial. A stirring bar (3mm) was used to facilitate the mass transfer process. Organic solvent was added to the sample solution by a 1000 µL microsyringe. Then the mixture was agitated. After this time the acceptor phase (microdroplet) was delivered to the top-center position of the immiscible organic solvent and again, the mixture was agitated. Methadone was extracted from the aqueous

sample solution (donor phase) into an organic phase and then was back-extracted into a directly suspended droplet of an acidic aqueous solution (acceptor phase). Finally the amount of methadone was measured using UV-visible spectroscopy with detection λ=205 nm (Fig. 1).

RESULTS AND DISCUSSION

2.2. Theory of LLLME

Liquid–liquid–liquid-phase microextraction consists of two processes and three phases: extraction from donor phase (P1) into an organic solvent (P2), and finally back-extraction from the organic phase into an aqueous acceptor phase (P3). In such cases, the pH of the sample is adjusted to make the analytes neutral and thus extractable into the organic solvent. After reaching the equilibrium of phase separation, the analytes that are mostly transferred into the organic phase are back-extracted into the second aqueous phase (acceptor) set to a pH at which, the analytes are charged. This back-extraction step introduces extra selectivity since neutral compounds will preferably stay in the organic phase [17, 18]. The theory of the method is well defined by the others [13, 19].

2.3. Optimization method

2.3.1. Volume of phases

The enrichment factor can be improved by the increase in the volume ratio of the donor and acceptor phases [12]. In this work, the volume of the acceptor phase was changed in the range 3-10 µL in three liquid –phase when the volume of the donor phase was constant (5mL) and observed that the use of larger droplet result in an increase in the analytical response but the enrichment factor decrease due to dilution of the analyte in these large droplets. Also, the results showed with a 4µL droplet the best enrichment factor was obtained (Fig. 2).

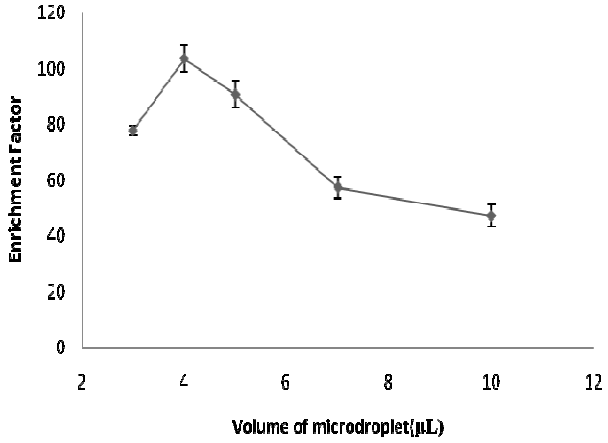

Figure 2. Effect of volume of microdroplet on enrichment factor of methadone. Other extraction conditions: analyte concentration 5 µg mL^{-1}; 1-octanol as the organic solvent(350µL); sample pH 9; acceptor phase pH 1; stirring speed 500 rpm; 5 mL donor sample volume; extraction Time(T1)=15 min and back-extraction Time(T2)= 5 min

On the other hand, because of the design of our extraction device, the volume of the organic phase was also important and must it optimized. The best volume of the organic solvent was found 350µL (Fig. 3). At smaller volume of organic solvent, the stability of the aqueous drop decrease during agitation, also the extraction time is increased when a large volume of organic phase is used.

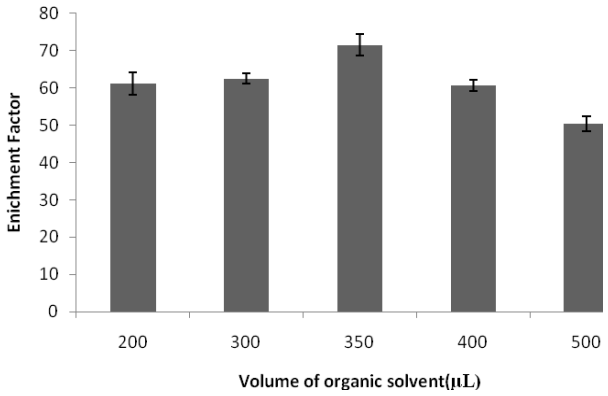

Figure 3. Effect of volume of organic solvent on enrichment factor methadone. Other conditions: analyte concentration 5 µg mL^{-1}; 1-octanol as the organic solvent; sample pH 9; acceptor phase pH 1; stirring speed 500 rpm; 5 mL donor sample volume; extraction Time(T1)=15 min; back-extraction Time(T2)= 5 min and volume of microdroplet 4µL.

3.2.2. The pH of the donor and acceptor phases

For basic analytes, the donor phase should be strongly alkalized to produce molecular forms of analytes and consequently reduce their solubility within the donor phase while the acceptor phase should be acidized in order to ionize analyses. The difference in pH between the donor and acceptor phases can promote the extracted analyte from donor to acceptor phase [20]. Methadone is a moderately basic drug (pK_a = 8.94), therefore the effect of donor phase pH in the range of 5-10 was investigated and the best enrichment factor was observed on pH 9 and then it decrease at pH>9, that it may be due to the methadone decomposed in alkali medium.

The results show that the pH of the acceptor phase also is an important factor on the enrichment factor therefore; it is adjusted between 1 and 12 and the maximum of amount efficiency of extraction was observed in pH 1.

3.2.3. Extraction Time

The extraction of methadone from the aqueous sample into the organic phase can be described as a slow equilibrium process and according to the theoretical model of the mass transfer for the solvent microextraction [21], increasing of the stirring speed causes an increase in the mass transfer coefficient. In this study, the range of extraction times investigated between 2 and 20 min when 600 rpm of stirring speed was applied. The results show, the maximum of enrichment factor obtains at the equilibration time of 15 min (T1). Also, there are no significant differences in the enrichment factor for extraction time more than 15 min.

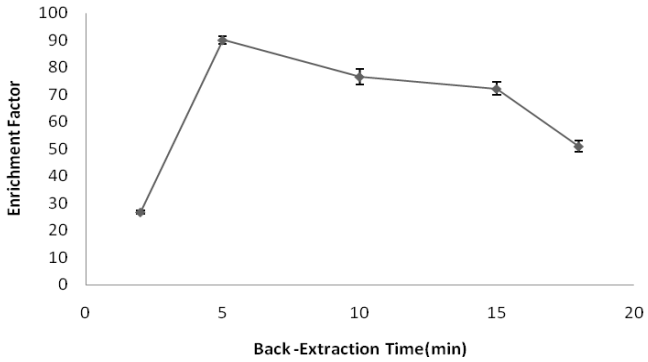

Figure 4. Effect of back-extraction Time on enrichment factor methadone. Other conditions: analyte concentration 5 µg mL^{-1}; 1-octanol as the organic solvent(350µL); sample pH 9; acceptor phase pH 1; stirring speed 500 rpm; 5 mL donor sample volume; extraction Time(T1)=15 min; and volume of microdroplet 4µL.

3.2.4. Back-extraction time

The back-extraction from the organic solvent (1-octanol) into the aqueous microdroplet (4µL) should not be too long because the droplet will not stable due to the dissolution, loss or fall. Therefore; according to the results it was

selected 5min for the back-extraction to attain equilibrium. The enrichment factor decrease after 5 min that it can relate to instability of the droplet and fell down in the vortex (Fig. 4).

3.2.5. Organic solvent selection

There are several requirements for obtaining the selected organic solvent in directly suspended droplet LLLME, the organic solvent should have lower density than the water to flat on the top aqueous sample solution and be immiscible with water to avoid dissolution in two aqueous phases. Also, the organic solvent should have high viscosity to hold the micro droplet at its top-center position.

Five organic solvents 1-octanol, benzene, n-hexane, ethyl acetate and octane have been examined. 1-octanol had the best extraction efficiency than the other solvents; the microdroplet was unstable when the stirring speed 700 rpm at 5 min was applied. The stability of microdroplet in 1-octanol solvent can be due to if the viscosity of it (6.490) [22] are higher than the other solvents.

3.2.6. Stirring speed

Increasing of the stirring speed caused an increase in the mass transfer and the extraction kinetic. In the present work, the procedure adopts a symmetrical rotated flow field created by a stirring bar, placed at the bottom of the cylindrical sample cell and the single drop is delivered at the top-center position of the organic solvent. Thus, it forms a self-stable single microdroplet system, easy to operate and control. Furthermore, the rotation of the microdroplet around a symmetrical axis may cause an internal recycling and intensify the mass transfer process inside the droplet. Therefore, the stirring speed was also optimized for better extraction, while the back-extraction was performed. The range of stirring speed was 400–700 rpm. Agitation increases the extraction efficiency but in speeds more than 600 rpm the extraction efficiency was not dramatic changes in speed. Consequently, the stirring speed was selected at 600 rpm for further analysis.

3.3.Real sample analysis

3.3.1. Hair Treatment

A bulk of blank hair is necessary for method development and validation. This blank sample was obtained from a men hairdresser's shop. The absence of opiate was verified in this blank sample. Hair samples of abusers were collected from 13 men ranging from 16 to 45 years old. They were obtained from addiction therapeutic center of Ibn Sina, Bojnourd, Iran. Also some of the addicted persons were under therapeutic treatment.

3.3.2. Washing and digestion of hair matrix

The hair was washed with different solvents as follow: 20 ml dichloromethane, 15 ml acetone, 15 ml methanol, 10 ml methanol, at room temperature for 5 min and then it was dried. The washed and dried hairs were finally cut into approximately 1mm pieces and digested by the following procedure; 2ml methanol as an extracting solvent was added to 50 mg of hair, in a 10ml screw-cap tube. The pH was adjusted to 7.4 by phosphate buffer solution. The samples were incubated at 50 ºC for 5h [23]. In case of a remaining solid matrix, extracts were filtered. The remaining was rinsed with 0.5 ml ethanol and both fractions were evaporated to dryness at 40 ºC under a steam of nitrogen.

3.3.3. Preparation working solutions

Standard solutions were obtained by adding calculated amounts of the stock solution (1mg mL^{-1}) into the blank hair solutions which were prepared .These working samples were used at optimization conditions for drawing of calibration curve. All solutions were stored at 4°C and protected from light.

3.3.4. Analytical performance

After optimization of all affective parameters, optimal conditions have been set to evaluate the performance of microextraction. The calibration curves were linear in the range 0.2-10 μg mL^{-1} for methadone in hair, with correlation coefficient r=0.995(N=5), so a direct proportional relationship between the extracted amount of compound and the initial concentration of the sample was demonstrated. The limit of detection (LOD) was estimated on a signal-to-noise ratio of 3 (S/N = 3) and was 1.7 ng mL^{-1} (n = 5). Also, the relative standard deviation value obtained 4.3% (N=5) for methadone in hair. According to data from addiction therapeutic center of Ibn Sina, the real value of methadone in the hair abusers was reported in Table 1. As can be seen from this Table, the relative recovery is obtained in the range 82-98% with an average of 91.50% using this method.

For calculation of enrichment factor, response of analysis after extraction by the investigated method should be divided to response of it before extraction. For reporting of this factor, we calculated enrichments of three standards solution (in the initial, mid and final reign of linear range) and the enrichment factor of the method was reported as the average of these calculates. We obtained enrichment factor 152.92.

Table 1. The result of methadone determination in the difference hair samples with relative recoveries

No	Age	Sex	Color	Methadone used (ng mL^{-1})	Methadone Founded (ng mL^{-1})	Recovery %
1	16	M	black	5	4.3	86
2	16	M	black	5	4.8	96
3	28	M	black	5	4.3	86
4	30	M	black	30	28.8	96
5	32	M	black	15	13.0	86.6
6	33	M	black	5	4.1	82
7	37	M	black	5	4.3	86
8	38	M	black	10	9.5	95
9	38	M	black	8	7.4	93
10	40	M	black	4	3.9	97
11	42	M	black	50	45.6	91
12	43	M	black	10	9.7	97
13	45	M	black	20	19.5	98

The review of some method [24- 27], which were used for determination of methadone in the biological samples is showed in Table 2. The low detection limit and relative standard deviation are achieved in this work than other methods.

Table 2. Comparison between current methods for determination of methadone with this work

Matrix	Method	Detection	LOD	[a]DLR	RSD	R	Recovery	[b]EF	Reference
Human hair	HS-SPME	GC-MASS	0.03 ng mg^{-1}	0.1-3 ng mg^{-1}	9.2%	-	-	-	26
Human hair	SPME	GC-MASS	-	1-50 ng mg^{-1}	13.30%	0.99	-	-	27
Human hair	SD-LLPME	UV	1.7ng mL^{-1}	0.2-10 µg mL^{-1}	4.3%	0.995	96%	159.92	This work

[a] *Dynamic linear range*
[b] *Enrichment factor*

CONCLUSION

The aim of the present study was to develop and validate a rapid, sensitive, robust and reliable method for the determination methadone in human hair using SD-LLLME technique with uv-visible spectroscopy.

In this method contrary to the ordinary microextraction used the large droplet due to that it is freely suspended on the surface of the organic solvent, without using a microsyringe as supporting device, therefore, this causes an increase in mass transfer process and decrease in equilibrium time. Also, the enrichment factor, good linearity and low detection limit are reasonable relative recovery have been obtained.

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REFERENCES

[1] V. P, Dole, M. E, Nyswander., *HCI. JAMA*. **1965**, 193, 646.
[2] H. Isbell, V. H. Vogel, *Am J Psychiatry*., **1949**, 105, 909.
[3] P. Symond, *Br Med J.*, **1977,** 1,512.
[4] D. S. Ettinger, P. J. Vitale, D.I. Trump, *Cancer Treat Rep.*, **1979**, 63,457.
[5] D. Puig, D. Barcelo, *Trends Anal. Chem*., **1996**, 15, 362.
[6] J. Patsias, E. Papadopoulou-Mourkidou, *J. Chromatogr. A.*, **2000**, 904, 171.
[7] L. Müller, E. Fattore, E. Benfenati, *J. Chromatogr. A.*, **1997**, 791, 221.

[8] L. S. DeBriun, P. D. Josephy, J. B. Pawliszyn, *Anal. Chem.*, **1998**, 70, 1986.
[9]A. Fromberg, T. Nilsson, B. R. Larsen, L. Montanarella, S. Facchetti, J.Q. Madsen, *J. Chromatogr. A.*, **1996**, 746, 71.
[10] R. Doong, S. Chang, Y. Sun, *J. Chromatogr. A.*, **2000**, 879, 177.
[11] M. A. Jeannot, F.F. Cantwell, *Anal. Chem.*, **1996**, 68, 2236.
[12] H. Liu, P. K. Dasgupta, *Anal. Chem*., **1996**, 68, 1817.
[13] A.S. Yazdi, Z. Es'haghi, *J. Chromatogr. A.*, **2005**, 1082, 136.
[14] L. Xu, C. Basheer, H. K. Lee, *J. Chromatogr. A.*, **2009**, 1216, 701.
[15] Y. Tao, J. F. Liu, T. Wang, G. B. Jiang, *J. Chromatogr. A.*, **2009**, 1216, 756.
[16] A. S. Yazdi, F. Mofazzeli, Z. Es'haghi, *J. Chromatogr. A.*, **2009**, 1216, 5086.
[17] L. Zhao, L. Zhu, H. K. Lee., *J. Chromatogr. A.*, **2002**, 963, 335.
[18] L. Hou, H. K. Lee, *Anal. Chem*., **2003**, 75, 2784.
[19] J. A. J◌onsson, L. Matheson, *J. Sep. Sci*., **2001**, 24, 495.
[20] R. J. Lewis, Sax's Dangerous Properties of Industrial Materials, Van Nostrand Reinhold, New York, **1992**, 8th ed..
[21] Jeannot, M.A.; Cantwell, F.F. *Anal. Chem*., **1997**, 69, 235.
[22] Howard, P.H.; Meylan, W.M. Handbook of Physical Properties of Organic Chemicals, CRC Press/Lewis Publishers, Boca Raton, FL, USA, **1997**.
[23] P. Kintz, V. Cirimele, F. Vayssette, P. Mangin, Mangin, *J. Chromatogr. B.*, **1996**, 67, 241.
[24] H. Ebrahimzadeh, E. Khezri, F. Kamarei, *Chromatographia.*, **2010**, 72, 231.
[25]A. C. Dos Santos tucas, A. Bermejo, P. Fernlindez, M. J. Tabernero., *J. Anal. Toxical*., **2000**, 24, 93.
[26] F. Sporkert, F. Pragst, *J Chromatogr B Biomed Sci Appl*., **2000**, 764, 255.
[27] AC. Lucas, AM. Bermeio, MJ. Tabernero, P. Fernandez, S. Strano-Rossi., *Forensic Sci Int.* **2000**, 107, 225.