Abstract

Objective: Morphine is one of the important narcotics which constitutes one of the alkaloid and opium components. If this substance is prepared defectively, it will appear in a variety of colors. Therefore, it is not possible to identify this substance by its color. **Method:** In this study, drug addicts were invited to take urine tests. After morphine extraction from urine samples by chromium toxicity method, different standard concentrations were injected into HPLC device and the resultant diagrams were analyzed. Then, some changes were made into the methodology for the optimality of measurement process and morphine determination in human urine. **Results:** It was found that the amount of morphine available in the urine samples was measureable through high-performance liquid chromatography and the amount of impurities added to drugs could be determined. **Conclusion:** This method can be used for diagnosis.

Keywords: Drugs, Morphine, Chromatography, Addiction

On the Measurement of Morphine Level and Specification of Consumption of Different Drugs in People's Urine at Different Ages through High-Performance Liquid Chromatography

Saeed Shahabi, Ali Morad Karami , Mohammad Reza Ba Ezzat , Ali Asghar Amiri

Saeed Shahabi
Department of Biology, Islamic Azad University of Arsanjan
E-mail: shahabi1391@yahoo.com

AliMorad Karami
Drug Control Headquarters
Fars Province
Shiraz
Iran

MohammadReza BaEzzat
Drug Control Headquarters, Fars Province, Shiraz
Iran

AliAsghar Amiri
Department of Chemistry, Faculty of Sciences, Islamic Azad University of Firozabad

Research on Addiction Quarterly Journal of Drug Abuse
Presidency of the I. R. of Iran
Drug Control Headquarters
Department for Research and Education

Vol. 9, No. 33, Spring 2015
http://www.etiadpajohi.ir

Introduction

Nowadays, drug addiction is no longer considered a social ill, but it has become a problem threatening global security. Production of chemicals and evolution of drug use model from natural modes to chemical modes is also a great risk. Undoubtedly, breaking geographical boundaries and the boundaries of human knowledge has led to the qualitative and quantitative proliferation of narcotics despite all its benefits and advantages in other fields. For example, synthetic drugs are generated in different places of the world in addition to poppy cultivation and heroin production in Afghanistan (Taheri Nokhost, 1999; Ehsanmanesh & Karimi, 1999). Morphine is categorized in alkaloids and is one of the important components of opium that makes up 7 to 14 percent of opium. This chemical is insoluble in water and is found as crystalline powder, white to cream in color and sometimes brown. If this substance is prepared in an incomplete mode, it will have different colors. Therefore, it is not possible to identify it by color. Heroin is another narcotic drug that is extracted from morphine by distillation. About 900 grams of heroin is extracted from a kilo of morphine while heroin is 3 to 5 times more potent than morphine (Akhtar Mohagheghi, 2006).

In the present study, high performance liquid chromatography (HPLC) method with a series of changes was experimented to extract morphine from urine samples. This method has overcome the problems pertaining to the sample analysis by gas chromatography such as destruction of samples at high temperatures and also the problems in the analysis in impurity profiling techniques. Successful progress in HPLC has started since the beginning of 1980 and attracted a lot of fans and applicants who wanted good isolation. Most HPLC columns are made of stainless steel type 316 which is austenitic steel chrome - nickel-molybdenum, is resistant to HPLC, and is relatively inert to chemical corrosion (Skoog, Holler & Nieman, 1998; Rouessac & Rouessac, 2007; Shafii, 1994).

To date, many methods have been used to extract morphine and codeine from human urine and blood (Yamada & Oguri, 2005). Taghavi, et al. (2002) used high recycling liquid - liquid phase to extract codeine from human urine and quantitatively investigated the urine by chromatography gas (Taghavi, Nazeri, Sabzevari, Fekri & Afshar, 2002). They concluded that it is possible to benefit from this method for extracting codeine (and morphine) due to high recycling and acceptable sensitivity. Taghavi, et al. (2003) used liquid-liquid phase and solid-phase to extract morphine from human urine and compared them via scanning densitometry. In both methods, the concentration of extracted morphine in hydrolyzed state was higher than that in non-hydrolyzed state (Taghavi, Nazeri, Sabzevari, Fekri & Afshar, 2003). Today, HPLC with minor changes and other lateral methods are used to diagnose and measure morphine (Ruzilawati, Yusuf, Ramli, Hussain & Rasool, 1994; Schönberg, Grobosch,

Lampe & Kloft, 2006). In the present study, the amount of morphine in the samples obtained from individuals addicted to drugs, as well as lifetime of morphine compounds after consumption were measured by high performance liquid chromatography; and the metallization resulting from opium use and heroin use were compared to each other. In fact, this study aimed at examining and testing high performance liquid chromatography method in order to extract morphine from human urine with respect to the consumed narcotic drug and age. Another purpose of this study was to examine and test this method in order to diagnose the type of used drug and detect the impurities of the consumed substance in addicts. Generally, the current study was an attempt to investigate the application of this method in drug discovery by police and explore the relationship between different discovered samples and the arrested individuals.

Method

Population, sample, and sampling method

Morphine and codeine standards and methanol acetonitrile with high purity were purchased from Merck Company. Other solvents, such as ethanol, and acetone were used without any purification. All the experiments were performed using deionized distilled water (twice distillation). The devices used here were as follows: high performance liquid chromatography system containing degassing system with helium, a six-part injection valve with 10-microliter loop, a multi-wavelength fluorescence detector, and an analytical column (60Å, 4μm, 150×3.9mm) with protective columns (60 Å and 20 × 3 mm). Full glass filtration system was used to filter all mobile phase solvents. PTEFE-SUPER- 450 was used for filtering solvents and .45-micrometer syringe filter was used for filtering the samples.

Although the preparation of samples for experiments was a very difficult task in this study, anti-drug police in Fars Province cooperated and issued research permit among the patients at that center. Therefore, research began. These individuals were fully informed on the research topic and the sampling was done with their consent. It should be noted that no drugs were offered to these people, but these people were those who had previously used drugs and were under the control of anti-drug police. Indeed, the selection of these people took about three months and all the people who were transferred to this center during this period were researched.

In this study, some individuals meeting the requirements for taking the sample were invited; in fact, these requirements were reflected in the following items: amount of drug use, type of drug, time of drug use, age, etc. Among them, only five people aged 24, 26, 30, 50, and 52 years were selected. The 30-year old person took urine test 5, 12, 24, and 36 hours after drug use, respectively. The 24-year-old person took urine test about 76 hours after the consumption of heroin, the 26-year-old took urine test 36 hours after the consumption of opium,

the52-year-old person took urine test 36 hours after the consumption of heroin, and the 50-year-old person took urine test 24 hours after the consumption of opium.

The amount of urine sample for each test was 10 milliliters. The sample temperature is considered a very important issue only in the laboratory diagnosis of addiction and should be approximately equal to the temperature of the human body. The sampler should make sure that the sample is free of additional colors and the sample must be without and colors or additives. In this study, the samples were tested with different PHs, in which the most favorable one ranged between 1.5 and 8.5. To this end, the special weight of the sample should not be smaller than 1.004 and the concentration of creatinine in it should not exceed 20 dL /mg.

In this study, chromium poison (morphine-codeine Detection Kit) was used wherein the following drugs were examined and no drug interactions were observed in any of them. These drugs include: Adult Cold, Chlordiazepoxide, Diclofenac Sodium, Antihistamines, Decongestant, amitriptyline, Acetaminophen codeine, imipramine, cocaine, hashish, Fluoxetine, Perphenazine, Diazepam, atenolol, theophylline, Pentazocine, phenobarbital, cimetidine, ranitidine, Diphenoxylate, Spironolactone, methadone, caffeine, and phenybutazone. In the case of cimetidine, a stain near the top of morphine is created at high concentrations (about 20µg/ml) that may cover the morphine. It is noteworthy that the color of these stains is yellow and quite different from that of morphine. If such stains appear, it will be better to go for another complementary test using ethyl acetate, methanol, and ammonia (85: 10: 5) to ensure the accuracy of the answers.

For the extraction of morphine from the sample, the sample must be inserted in the chromatography column along with a series of chemical solutions that will be mentioned below and, then, it should pass through the column. In this study, chromatography columns of Chrome poison (morphine-codeine diagnostic kit) were used. Each package of Chrome poison kit containing powder morphine diagnosis A1, A2 is an activator and powder B is a fixation substance.

For the preparation of buffer A, a vial of powder A1 was resolved in 100 ml of distilled water at room temperature. In the same way, A2 solution was also formed. Then, 10 ml of solution A1 and 90 ml of solution A2 were mixed and stirred in order to prepare buffer A. For the preparation of buffer B, a vial of powder B was resolved in Chrome poison and 100 ml of distilled water. Buffers should be prepared one hour before the start of the experiment. These buffers are stable for one week at 25 degrees of centigrade of laboratory conditions. Chromatography columns were placed in the holes of the vacuum chamber vent. Pressure pump was set about .2-.3 loading and, then, the pump was turned off. The distance between cotton and resin into the column was slowly removed by a glass rod (use of glass rod was because of lack of electric charge). An amount of 3 ml of buffer prepared by PH of approximately 8.5 was added to the column. After about 2 minutes, the vacuum pump was turned on again and its pressure

was set to1.0 bar. Then, 10 ml of the available samples (samples were coded) was added to each of the columns and the pressure of the vacuum pump was fixed to the previous amount until the full discharge of the sample (1.0 bar). After the complete discharge, the cotton inside each column was removed and the amount of 5 ml buffer B with PH of 8 was added to each column (pressure for the previous amount was fixed). After discharge of buffers, the pressure of vacuum pump was increased to 3.0 bar and 15 minutes took to correctly accomplish discharge and drying. Extraction columns were put in a special tube and their tips were set in the epithelial thimbles that were arranged on the hot plate. Then, three ml of ethanol was added to each column so that extraction can be performed by evaporation. Extraction lasted approximately 15 minutes and, then, beakers were removed from the hot place. The result of extraction along with a few drops of ethanol was injected into the HPLC device.

All separation and measurement were conducted on the Novapak column 18 at room temperature. Isocratic elution mode with acetonitrile-water phase (35-65% V / V) was applied with a flow rate of one ml per minute. All mobile phases were filtered through suitable PTEFE-SUPER- 450. Excitation and emission wavelengths were selected 235 and 350 nm, respectively. All samples were passed through 4-μmsyringe filters before injection into HPLC.

The most common solvents used in reversed-phase high performance liquid chromatography were water, acetonitrile, methanol, and tetrahydrofuran that are usually used as mobile phases in double or triple modes. In this regard, different mobile phases in different volume ratios of water-methanol and water-acetonitrile were investigated in order to achieve lower analysis time and desired separation between peaks. Volume ratios of 30-80, 30-75, 35-65, 50-50, and 40-60 (v / v%) of binary mixtures of water-acetonitrile and water-methanol system were injected into the analytical column by the pumps and the chromatograms obtained from morphine compounds were investigated.

Results

The results showed that the mobile phase of water-acetonitrile with the volume ratio of 35-65 (v / v%) results in the best the separation between peaks. On the other hand, the polarity of mobile phase is in such a state that unwanted polar compounds are quickly washed from the column and do not overlap with the peak of analytes. Therefore, acetonitrile solvent was selected as the modifier of the organic mobile phase despite being more expensive than methanol. Tetrahydrofuran solvent was not used due to its high toxic properties. In mobile phases where the amount of water is more than 40% volumetric, the separation and distinction between peaks are performed in higher degrees, but 35% water was selected since analysis takes a longer period of time and peaks are influenced by broadening. The flow rate of mobile phase is also an important factor to achieve a better separation; therefore, flow rates of .8, .9, 1.1, and 1.2 ml per

minute were evaluated and, in conclusion, the flow rate of 1 ml per minute was selected as the optimal flow rate. It should be mentioned that this flow rate is used in most of HPLC analyses. In higher flow rates, the peaks overlap while separation between the peaks is better performed in lower flow rates, but it is not used because the analysis took longer. The results of the injection of morphine extracted from different samples into HPLC device are presented in the table below.

Table 1: Results of the injection of morphine extracted from different samples into HPLC device

Sample	*Age*	*Substance / time spent after use-hours*	*First iteration*		*Second iteration*		*Third iteration*		*RSD%*		*Amount of morphine in ppm*
			Peak level of morphine	*Retention time-minute*	*Below the peak level of morphine*	*Retention time-minute*	*Peak level of morphine*	*Retention time-minute*	*Peak level of morphine*	*Retention time-minute*	
A	30	5 hours-opium	58920	9.89	58290	9.91	57941	9.71	.85	1.12	79.31
B	30	12 hours-opium	44880	9.85	44986	.10	43999	10.07	1.21	1.47	67.61
C	30	24 hours-opium	37860	9.98	37015	9.90	37986	10.20	1.40	1.55	52.69
D	30	36 hours-opium	10760	9.93	10410	9.77	10228	9.97	2.58	1.07	17.88
E	26	48 hours-opium	9780	.11	9100	9.87	9690	9.79	3.88	1.68	16.67
F	24	76hours-heroin	20395	.05	19591	9.66	20130	9.78	2.15	2.03	30.14
G	50	36 hours-opium	22260	9.83	22849	9.71	21882	9.92	2.18	1.07	33.09
H	52	36 hours-heroin	31840	9.84	31231	9.91	32252	10.12	1.52	1.46	45.20

The different concentrations of standard morphine sample were initially injected into HPLC device so that the figures 1-A, 1-B, 1-C, 1-H, and 1-5 were obtained during 10 minutes in concentrations 10 ppm, 30 ppm, 50 ppm, 70 ppm, and 90 ppm, respectively. Thereafter, Figure 1-D, known as calibration curve, using the area under curve peak based on morphine concentration. In this curve, y-axis and x-axis were respectively drawn based on millivolt and morphine concentration, and a straight line was obtained whose equation is -3480*780=y. It is noteworthy that y is the curve under the peak in different injected morphine concentrations which is obtained with the calculation of the curve under the peak. Then, x (morphine concentration) is obtained by the placement of y in calibration curve equation.

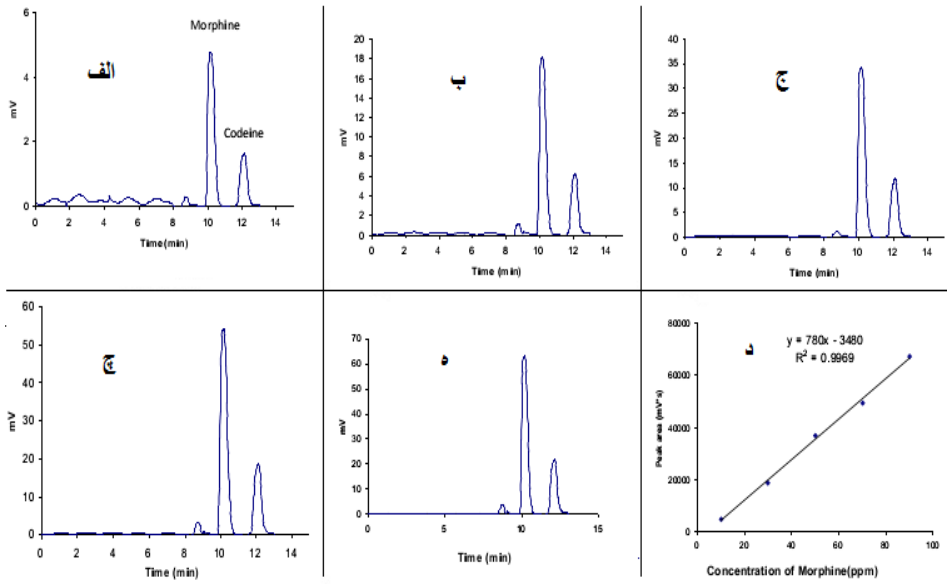

Figure 1: a) standard solution chromatogram with 10-ppm morphine b) 30-ppm morphine, c) 50-ppm morphine, d) 70-ppm morphine, e) 90-ppm morphine, and) calibration curve (flow rate of 1 ml per minute, mobile phase 65: 35 water-acetonitrile, Novapak column c18)

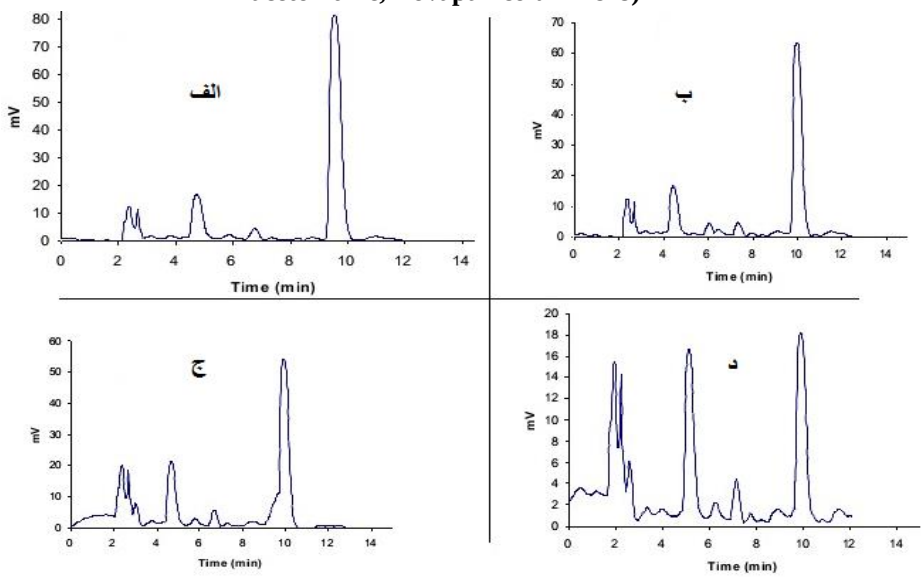

Figure 2: Chromatogram of 30-year-old person a) 5 hours, b) 12 hours, c) 24 hours, and d) 36 hours after opium use (flow rate of 1 ml per minute, mobile phase 65: 35 water-acetonitrile, Novapak column c18)

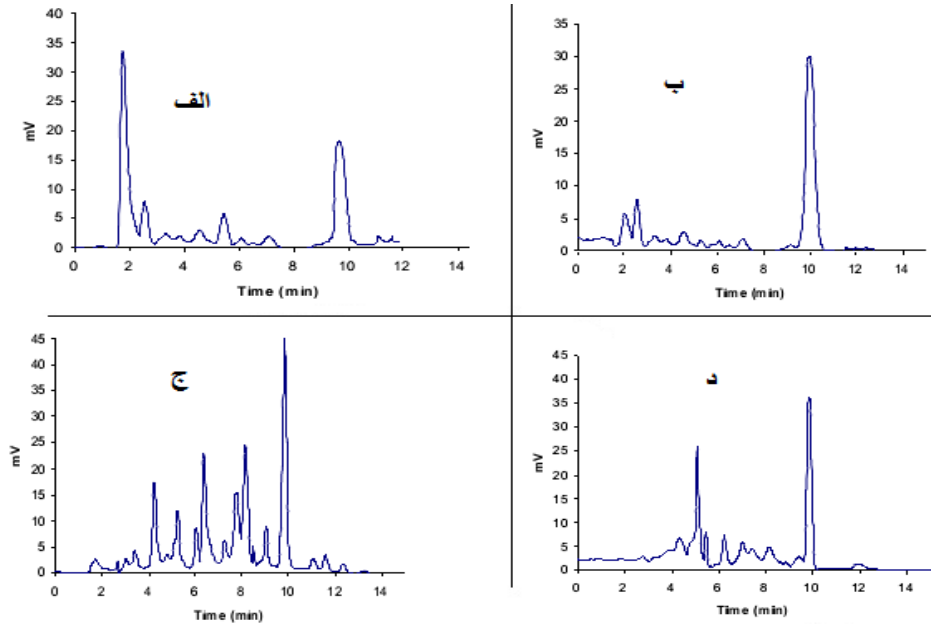

Figure 3: a) Chromatogram of 24-year-old person 72 hours after opium use, b) Chromatogram of 26-year-old person 48 hours after heroin use, c) Chromatogram of 52-year-old person 36 hours after heroin use, and d) Chromatogram of 50-year-old person 36 hours after opium use (flow rate of 1 ml per minute, mobile phase 65: 35 water-acetonitrile, Novapak column c18)

As is clear from the obtained figures, the consumed food materials (according to the difference of individuals) in the sample have the least impact on the extracted product. Figures 2-A, 2-B, 2-C, and 2-D show the chromatogram obtained from the injection of morphine into HPLC in the samples extracted from 30-year-old individuals 5, 12, 24, and 36 hours after opium use, respectively. Figure 3 shows the chromatogram of 24-, 26-, 50-, and 52-year-old individuals.

Discussion and Conclusion

The results of this study showed that the amount of morphine in the sample with high performance liquid chromatography is measurable. It was also revealed that the amount of impurities in narcotic drugs and the level of morphine in the body of drug users can be estimated in specific time intervals. In addition, it is possible to obtain the relationship between the amount of morphine and individuals' age over a period of time after the use of drugs. In terms of the results obtained from the amount of morphine in the sample, it was concluded that there is a direct relationship between the time spent after morphine use and the time duration morphine stays in the body. Hence, the

amount of morphine extracted at intervals after opium consumption has experienced a descending mode. For example, after the passage of five hours from the consumption, the amount of morphine reached 79.31 ppm; the amount of extracted morphine in the sample reached 61.67 ppm 12 hours after opium use; this amount reached 52.69 ppm 24 hours after opium use; and this amount reached 17.88 ppm 24 hours after opium use. These results pertained to a 30-year-old person who took opium and the pertinent chart shows the obtained results in four different time intervals. Figure 3 shows chromatograms of morphine extracted in a 50-year-old person and a 52-year-old person who are closely related in age. The 52-year-old person had taken heroin and his sample was tested 36 hours after consumption; his morphine was extracted which reached 45.20 ppm. However, the 50-year-old person had used opium and the morphine extracted from his sample was calculated after 36 hours which was equal to 33.09 ppm. Therefore, the amount of morphine in the 52-year-old was about 12 ppm higher than that in the 50-year-old person although they were similar to each other in terms of age and they both took tests in the same time interval after drug use. This can be accounted for by the difference in the amount of morphine in heroin and opium since the 52-year-old person had taken heroin but the 50-year-old person had taken opium. In fact, the amount of morphine in heroin outweighs that in opium heroin metabolization is performed in the body more quickly than opium metabolization in the body.

In terms of police investigation, the data analysis can lead to some in drug discovery scenes and to the relationship between different discovered samples and arrested individuals. The results of this study can be helpful and effective in the following areas: recognition of the possession of discovered drugs in various locations, estimation of geographic location of drug distribution and production based on the analysis of compounds of discovered drugs, prioritization of sampling from individuals in certain circumstances, acceptance or rejection of the statements of offenders in the consumption of psychotropic substances and investigation of their abnormal states at the time of committing crime, identification of the type of drugs and the association of suspects with the criminal scene. Opium and its derivatives such as heroin, morphine, and codeine are obtained from the opium gum, heroin is rapidly metabolized and converted to morphine. Thus, it is possible to find both compounds of morphine and glyco urinate morphine in urine samples of heroin users.

Therefore, the presence of morphine and/or its metabolites in the samples taken from people suggest heroin and morphine use. At the beginning of chemistry as a science, humans have made different uses of chemistry, one of which has been regarding the recognition of opium alkaloids, especially morphine in biological samples. Morphine disposal by different parts of the body decreases with aging; in other words, as one gets older, the metabolism of different body parts has a descending order and its function is reduced. Therefore, morphine disposal is also reduced which can be due to the functional

decrease of body parts with aging. Investigation a person of the amount of morphine excreted in such people suggests that the amount of morphine in is reduced with the passage of time. This is due to the decomposition of morphine to other substances and reduction of initial amount of morphine in the body. In terms of the comparison of the excreted morphine in people of similar ages but with consumption of different drugs, it can be concluded that as the amount of morphine in raw materials is higher, the amount of excreted morphine in the biological samples will be higher, too. Therefore, it is possible to guess the type of raw materials to some extent. With the investigation of the type and amount of morphine available in biological samples, it is possible to find the relationship between unknown criminal scenes and unknown police crimes. The conduct of similar studies on people with certain diseases, on the amount of morphine over time on the basis of gender, etc. in different environmental conditions and with blood or saliva samples is strongly recommended.

Reference

Akhtar Mohagheghi, M. (2006). *Sociology of Addiction*. Tehran: Mo'allef Publication.

Ehsanmanesh, M. & Karimi, I. (1999). A look at the history of and some research in the field of addiction in Iran, *Iranian Journal of Psychiatry and Clinical Psychology*, 5 (3), 62-100.

Rouessac, F & Rouessac, A. (2007). "*Chemical Analysis Modern Instrumentation Methods and Techniques"* 2nd Edition, England, John Wiley & Sons Ltd.

Ruzilawati, A. B., Yusuf, W. W., Ramli, N., Hussain, Z., & Rasool, A. H. G. (2013). Determination of Morphine in Human Urine by A Simple Reverse Phase High-Performance Liquid Chromatography Method with UV Detection. *International journal of pharmaceutical Sciences and Drug Research*.5 (1), 18-22.

Schönberg L, Grobosch T, Lampe D, Kloft C. (2006). New screening method for basic compounds in urine by on-line extraction–high performance liquid chromatography with photodiode array detection. *Journal of Chromatography A*, 1134 (1–2), 177–85.

Shafii, A. (1994). *Chromatography and Spectroscopy*. Tehran: Publication of Tehran University.

Skoog, D. A., Holler, F. J., & Nieman, T. A. (1998). *Principles of Instrumental Analysis*. Philadelphia: Saunders College Pub

Taheri Nokhost, H. (1999). Global trend of drug abuse, *Advances in Cognitive Science*, 1 (2), 22-30.

Taghavi, A., Nazeri, A., Sabzevari, O., Fekri, M. & Afshar, M. (2002). Codeine extraction from human urine using high recycling liquid-liquid phase and quantitative analysis of it by gas chromatography, *Journal of Research in Medical Sciences*, 26 (4), 287-290.

Taghavi, A., Nazeri, A., Sabzevari, O., Fekri, M. & Afshar, M. (2003). Morphine extraction from human urine using liquid-liquid phase and solid phase and comparison of them by scanning densitometry, *Journal of Research in Medical Sciences*, 27 (1), 23-27.

Yamada, H and Oguri. K. (2005). "*Morphine and its analogues." Drugs and Poisons in Humans.* Springer Berlin Heidelberg, 195-206.