



Screening of Morphine & Codeine in Urine of Opioid Abusers by Rapid and TLC Analysis

Rezai-Basiri M¹, Ghazi-khansari M¹, Faghiih A², Sadeghi M², Lotfalizadeh N¹, Eghbal M², Mohajell-Nayebi A², Rezazadeh H², Arshad Zadeh M¹

¹East Azarbayjan province, welfare organization, Tabriz/Iran, Faculty school of Pharmacy, University of Medical Sciences, ²Tabriz/Iran, Drug Applied research center, Tabriz/Iran. Pharmacology Department of Medical Sciences University, Tehran/Iran

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ABSTRACT

Aim: one hundred urine samples of abusers were examined for the presence of alkaloid substances in urine. Alkaloids of urine were confirmed with rapid and TLC methods. A thin-layer chromatography (TLC) procedure for the screening and confirmation of urinary codeine and morphine has been developed.

Method: Urine samples were daily collected from abusers of Tabriz/Iran. Urine extracts were prepared using silica-gel absorbent resin in column. The resin was rinsed with two buffers. These procedures make PH appropriation, then residual water was removed by vacuum aspiration and adsorbed alkaloid substances were eluted with a organic solvent of methanol and concentrated. After evaporation to dryness with heater, the residue was dissolved in organic solvent of methanol to reach 10 to 40 fold concentration. For screening, the extraction residues were spotted on TLC plates which are developed in chloroform: methanol: Ammoniac (12:1.4:0.2). For confirmation the spots for the two opioids were visualized with acidified iodoplatinate. Codeine and morphine standards are well separated from one another and comparison with samples urinary substances on plates. The limit of detection was a concentration of 300ng/mL.

Result: The result of this study has shown that 100 percent of urine samples from addicted in Tabriz (Iran) were contained alkaloid substances and 25 percent of these samples have shown drug interaction.

Conclusion: This study describes importance of urine drug testing such as TLC analysis in comparison of other rapid tests.

Key words: TLC, alkaloid substances, urine extracts, abusers.

Correspondence: Rezai-Basiri M
Tabriz/Iran pharmacy school of medical university-Drug Applied research center.
East Azarbayjan province-Tabriz/Iran welfare organization,
Tel/Fax: +98 411 5569777
E-mail: basiri@razi.tums.ac.ir

Rapid ve TLC Analizi ile Opioid Bağımlılarının İdrarında Morfin & Kodein Taraması

Amaç: Yüz bağımlının idrar örneği idrarda alkaloid maddeleri varlığı açısından incelendi. İdrar alkaloidleri rapid ve TLC yöntemleri ile araştırıldı. Bir ince tabaka kromatografi (TLC) yöntemi idrar kodein ve morfin tarama ve doğrulaması için geliştirildi.

Metod: Tebriz/İran'daki bağımlılardan idrar örnekleri günlük olarak toplandı. İdrar özütleri sütunda silika jel emici reçine kullanılarak hazırlandı. Reçine iki tampon ile durulandı. Bu işlemlerle PH uygunluğunu sağlandı, daha sonra kalan su vakum aspirasyonu ile ortadan kaldırıldı ve emilen alkaloid maddeler metanolün bir organik çözücüsü ile ayrıştırıldı ve yoğunlaştırıldı. Isıtıcı ile kuruluk için buharlaştırma sonrası, kalan madde metanolün organik çözücüsünde 10-40 kat konsantrasyona ulaşana kadar çözüldü. Tarama için, özüt kalıntıları kloroform:metanol:amonyakla (12:1.4:0.2) geliştirilmiş TLC tabakalarında noktalandı. Doğrulama için iki opioid için noktalar asidifiye iodoplatinate ile gözlemlendi. Kodein ve morfin standartları birbirinden ve tabakalardaki karşılaştırılan örnek idrar maddelerinden iyice ayrıldı. Tespit limiti 300ng/mL 'lik bir konsantrasyondur.

Bulgular: Bu çalışmanın bulguları gösterdi ki Tebrizdeki (İran) bağımlıların idrar örneklerinin yüzde yüzü alkaloid madde içermekteydi ve bu örneklerin yüzde 25'i ilaç etkileşimi göstermekteydi.

Sonuç: Bu çalışmada diğer hızlı testlerin karşılaştırılmasında TLC analizi gibi idrar ilaç testlerinin önemini anlatmaktadır

Anahtar kelimeler: TLC, alkaloid maddeler, idrar özütleri, bağımlılar

INTRODUCTION

Opioid substances use and abuse is globally perceived as one of the alarming public health problems. No effective treatment and prevention related policy issues can be arrived at without adequate information regarding the nature and distribution of drug abuse in population and their causative factors (1). Widespread data in drug abuse is lacking and it is difficult to make generalizations because of regional variations. The geographical location of Afghanistan makes transit of opium drugs possible across the east Azerbaijan province in Iran. The main of this study refer to diagnosis and detoxification of opioid abusers. Testing for presence or absence of drugs of abuse in urine also helps to screening improvement of patients in treatment (2). Because opioids and their metabolites detections are feasible in urine samples, therefore in this study we have just done to opioid confirmation by TLC method analysis in urine of abusers. Thin layer chromatography (TLC) has been used as a broad spectrum screen for detection of drug abuse (3). As a part of this recent, large-scale, well-designed drug abuse epidemiological study supported by the clinical laboratory of welfare organization has been extremely helpful in quick diagnosis and rapid detoxification of suspected substance opioid abusers who had denied history of any drug use or abuse at first contact with the treatment team. Several screening studies including screening and confirmation of opioids by thin-layer chromatography (2), an evaluation of TLC system for opioid analysis (3) and also use of thin layer chromatographic analysis in forensic medical analysis of urine for opioids have been reported (4). With this background we carried out screening of morphine and codeine of opioid abusers at toxicology laboratory of welfare organization in Tabriz/ Iran.

MATERIALS AND METHODS

Opioid abusers take opioid drugs on street; they refer for their treatment to welfare organizations. The morphine-codeine collection kits were designed for TLC analysis from some companies in Iran. Then according below mention to the followings these methods for opioid confirmation were examined. Urine samples were daily collected from opioid abusers of Tabriz/İran on Jan and Feb 2008. Besides providing urine samples, agreed to complete a questionnaire focusing on their conditions and addicted history and life conditions characteristics. Table-1 shows content of this questionnaire. Data confounding factors were such as medication with methadone, bupronorphine, naloxan, nalterxon, acetaminophen codeine, cimetidine, dextromethorphan and theophylline syrup and tobacco consumption and other small interaction factors were also collected. Overall 100 urine samples were daily collected. All of were male aged 20 to 60 (mean 35) years. All of them smoke. The study was submitted to and approved by the local ethical committee. All gave their informed consent prior to their in the study. All of urine samples were daily collected from abusers and stored in refrigerator, they were centrifuged with 4000 run for 4min, then pH of upper clear section of urine samples changed to alkaline condition with some ammoniac droplet added. The pH regulated with pH paper for each sample (5). Extraction columns were filled with 1 g from one kind of silica-gel between two layers of cotten.

Resins were activated with 3ml of buffer A (pH=9±0.2), Urine samples were deforested at room temperature and centrifuged. Solid-liquid extraction was performed on resins by applying the volume of urine (50 ml,

Table 1. Opioid Abuser Questionnaire

<i>Tabriz welfare Organization Opiates Distinguish Laboratory</i>
Date:
First name/Surname:
The Physician's name of the center:
Gender:
Age:
How long have you been addicted?
Which kind of opioid do you usually use?
When have you started detoxifying?
Other prescribed drugs:

depending of the total volume available). Resins were then rinsed with 5ml buffer B (pH=8.5±0.2). All buffers (A and B) were prepared from company kits. Residual water was removed by vacuum aspiration. Adsorbed substances were eluted with an organic solvent of methanol. After evaporation to dryness with heater, the residue was dissolved in organic solvent of methanol to reach a 10-40 fold concentration. Urine extracts were kept to plates spotted use (6,7). All of plates were pre-coated with silica-gel and provided on kit collection (3). For screening, the extraction residues were spotted on TLC plates which are developed in saturated chloroform: methanol: Ammoniac (12:1.4:0.2). For confirmation the spots for the two opioid were visualized with acidified iodoplatinate. For drug interaction problems resolution we changed saturated phase to Ethyl acetate:Methanol:Ammonic (85:10:5) and continued the process of TLC technique on plates spot tests. Codeine and morphine standards are well separated from one

another and comparison with samples urinary substances on plates and detected at a concentration of 300 nanograms/mL (2,8,9).

RESULTS

First, All urine samples were examined with strip rapid test then all urine extracts were screened with TLC analysis method. The results of urine strip test for drug abusers while they were under detoxification and treatment were positive although, these urine TLC results must be negative. So these results explain drug interaction, because abusers take some other medication under their physicians' orders. All calculation were performed using SPSS. The data were analyzed by using of variance (ANOVA ONE WAY) by post hoc comparison between groups were examined with student Newman-kehus test. Differences in which $p < 0.05$ were considered statistically significant. The results of this study show that 100 percent of urine samples from opioids abusers in Tabriz (Iran) were contained alkaloid substances and drug interaction happened in 25 percent of these samples. Some of positive and also drug interactions results were reported on Table 2 and Table 3.

DISCUSSION

Common clinics and General Medical setting of Welfare Organization support abusers in Iran. There are three types of opioids. First, the poppy alkaloids: morphine,

Table 2. Compared results from urine samples between Rapid strip tests and TLC technique.

Samples	Age	Morphine & Codeine Positive control	Normal Negative control	Rapid test	TLC	Drug interaction
1	30	+	-	+	+	-
2	45	+	-	+	+	-
3	59	+	-	+	+	-
4	51	+	-	+	+	-
5	53	+	-	+	+	±
6	32	+	-	+	+	-
7	45	+	-	+	+	-
8	40	+	-	+	+	±
9	31	+	-	+	+	-
10	46	+	-	+	+	-
11	55	+	-	+	+	±
12	30	+	-	+	+	-

No 5; the patient has taken methadone, the drug interaction is seen. No8; the patient has taken chlordizpoxide, drug interaction is seen. No11; the patient has taken cimitidine, drug interaction was seen. *Detection limit of morphine & codeine is 300ng/mL for standards and samples. *All urine sample tests are compared between positive and negative controls

Table 3. Compared results of urine samples between Rapid strip tests and TLC technique.

Samples	Age	Morphine & Codeine Positive control	Normal Negative control	Rapid test	TLC	Drug interaction
1	41	+	-	+	+	-
2	39	+	-	+	+	±
3	45	+	-	+	+	-
4	49	+	-	+	+	-
5	33	+	-	+	+	-
6	52	+	-	+	+	-
7	55	+	-	+	+	-
8	44	+	-	+	+	±
9	35	+	-	+	+	-
10	49	+	-	+	+	-
11	55	+	-	+	+	-
12	58	+	-	+	+	-
13	36	+	-	+	+	±

No2; the patient has taken Acetaminophen codeine, drug interaction is seen. No8; the patient has taken Amitriptyline, drug interaction is seen. No13; the patient has taken DifenoXilate, drug interaction is seen *Detection limit of morphine & codeine is 300ng/mL for standards and samples. *All urine sample tests are compared between positive and negative controls.

codeine, thebaine, noscapine and papaverine. Also the semi-synthetic and synthetic derivatives used in therapy as antitussives and analgesics, such as pholcodine, ethylmorphine and dextrometorphan. Opioids, diacetylmorphine (heroin) and opioids are used as substitutes in treatment of addiction also buprenorphine and methadone. Opium is usually consumed by Iranian abusers (1). At the first step of treatment, patients take naloxon & naltrexon for own detoxification, then they continue their medication with methadone and buprenorphine, but at the same time they take also other drugs such as adult cold, chlorodizpoxide, natrium-diclofenac, antihistamine-deconjuant, amitriptyline, acetaminophen codeine, imipramine, cocaine, cannabis, flouxetine, perphenazine, diazepam, atenolol, phenobarbital, theophiline, pantazosine, cimitidine, ranitidine, difenoXilate, spironolactone, caffeine, phenilbutazone. Some of these drugs are used under physicians' orders and some of them are used intentionally. Which one of the drugs, mentioned above, or any other substance can cause drug interaction in the results of Rapid Strip Tests and leads them to false positive or false negative. The term of drug interaction refers to other drugs except of opioid which overlap on plates of TLC at the same time. For resolving drug interaction we must repeat tests on the new urine samples of abusers by TLC method, but probably we can't see drug interaction results on TLC plates so we should continued TLC plate spot tests on Ethyl acetate: Methanol: Ammonic (85:10:5)saturated phase. Since all samples were screened by rapid tests, the rapid strip screening tests has less specificity and

a high likelihood of false positives and false negatives results. The brand test of this study commercially available by inc.P.R China products (11-13). Toxicologist recommended that positive results should be considered as presumptive and be tested by TLC and to be confirmed. Studying of the results for identifying the opioids (morphine, codeine and their synthetic analogue heroin) in urine samples during the toxicology chemical examinations by using the method of thin-layer chromatography (TLC) are described, and this study attends to this topic. The main found of this study is the comparison of the results taken from Urine Rapid Strip Test and Thin Layer Chromatography Test to distinguish the presence of opioid in abusers' urine samples for their detoxifications and treatment. Several studies show these results in abusers urine samples False positive & false negative results must be repeated by laboratory diagnostic team, which in this way, results taken by TLC will help physicians & abusers in all steps of detoxification & medication process (10,11).

In conclusion, this study describes the importance of urine drug tests such as TLC analysis in comparison with other rapid tests. Since 25% of these urine samples have shown drug interaction, for final confirmation we used TLC analysis test to be able to remove this problem.

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REFERENCES

1. Weatherburn D, Lind B. The impact of law enforcement activity on a heroin market. *Addiction* 1997;92(5):557-69.
2. Budd RD, Mathis DF, Leung WJ. Screening and confirmation of opiates by thin-layer chromatography. *Clin Toxicol* 1980;16(1):61-6.
3. Rajananda V, Nair NK, Navaratnam V. An evaluation of TLC systems for opiate analysis. *Bull Narc* 1985;37(1):35-47.
4. Gorbacheva NA, Orlova AM. Use of thin layer chromatographic analysis in forensic medical analysis of urine for opiates. *Sud Med Ekspert* 2003;46(3):34-8.
5. John Bernard Henry. *Clinical diagnosis and management by laboratory methods, clinical chemistry chapter*, 2000.
6. Andre V, et al, Henry-Amar M, Gauduchon P. Biomonitoring of urine mutagenicity with the Ames test: improvement of the extraction/concentration method. *Mutat Res* 2002;520(1-2):199-205.
7. Gómez MJ, Petrović M, Fernández-Alba AR, et al. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. *J Chromatogr A* 2006;1114(2):224-33.
8. Moffat AC, Osselton MD, Widdop B, Clark S. *Analysis of Drugs and poisons*. Pharmaceutical Press, London, 2005.
9. Cone EJ, Clarke J, et al. Prevalence and disposition of drugs of abuse and opioid treatment drugs in oral fluid. *J Anal Toxicol* 2007;31(8):424-33.
10. Galer-Tatarowicz K, Wierowski M, Szpiech B, Reguła K, Jankowski Z. Drug addiction in the medico-legal certification of the Department of Forensic Medicine of Medical University of Gdańsk in the years 1996-2005. *Arch Med Sadowej Kryminol* 2007;57(3):277-84.
11. Cone EJ, Dickerson S, Paul BD, Mitchell JM. Forensic drug testing For opiates. V. Urine Testing for Heroine, Morphine, and Codeine With commercial Immunoassays. *J Anal Toxicol* 1993;17:156-64.
12. *Urine Testing for Drugs of Abuse*. National Institute on Drugs of Abuse (NIDA Research Monograph) 1986, 73.
13. *Opiates (opi/morphine) one step test strip (urine)*, ©Advanced Quality™, for invitro diagnostic, Inc Tec Products, inc. P.R China, EXP 2010