

Comparison of High-Performance Liquid Chromatography and Thin Layer Chromatography for Identification of Amphetamine and Methamphetamine in Human Urine

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Abstract

Background: The use of Amphetamine Type Stimulants (ATS) including amphetamine and methamphetamine is a critical worldwide problem. The development of simple and convenient analytical methods for the detection of amphetamine and methamphetamine is necessary to determine the abuse of illicit drugs in urine. Many useful methods have been developed for qualification and quantification of substance abuse. High-Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) are applied for detection of drugs and poisons for both biological and non-biological materials. The aim of the present study was to compare the power of HPLC and TLC for the detection of amphetamine and methamphetamine in human urine to suggest an appropriate analytical method considering beneficial aspects of it such as validation, simplicity, sensitivity, applicability and economic cost.

Methods: Both HPLC and TLC were used to analyze urine samples of 50 self-reported individuals, whom were referred to Bahar Medical Laboratory and Iranian National Center for Addiction studies.

Results: Screening test showed 22 (amphetamine) and 17 (methamphetamine) percent were false-positive tests in comparison with TLC findings. The results of TLC analysis were consistent with the results from HPLC method.

Conclusion: Based on our results; this study increases the potential validity of TLC as a rapid, inexpensive and simple screening procedure for the detection of amphetamine and methamphetamine in human urine, especially in deprived regions with inexperienced technicians and no advanced laboratory equipment.

Keywords: Amphetamines, HPLC, Methamphetamine, TLC

Introduction

Substance use disorder is a common problem in today's societies; which imposes extensive social and economic consequences¹. Amphetamine Type Stimulants (ATS) such as methamphetamine has been globally known as the second most common drug abused that are responsible for socioeconomic troubles due to different health problems caused by them^{2,3}. It also causes a wide range of adverse effects including depression, hyperthermia, Parkinson's disease, memory impairment and cognitive deficits⁴. Although methamphetamine toxicity is diagnosed by its clinical findings, some laboratory assessments such as urine examination are required too⁵. Analyses of drugs of abuse are important for the prediction of harmful use and prevention of the addictive pattern of abuse, especially in young individuals⁴. Among the biological samples, urine is the primarily preferred specimen for drug testing because specimen collection is simple and non-invasive and drugs and their metabolites may present in relatively high concentrations.

Among different available methods, urine analysis is the most favorite procedure in forensic and health care setting⁶. In general, Thin Layer Chromatography (TLC), and High-Performance Liquid Chromatography (HPLC) are utilized to determine drugs in the urine, after initial clinical screening⁷. Likewise, many clinical laboratories around the world utilize the TLC technique to identify illicit drugs⁸. They can potentially affect the treatment plan for addicted patients. Thus, the accuracy of the assessment is crucial⁸ and misinterpretation of drug tests may cause inappropriate medical treatment in emergencies.

Keeping this in mind, the aim of the present study was to compare the results of TLC and HPLC from urine samples of 50 self-reported individuals, whom were referred to addiction treatment centers and clinical laboratories.

Patients and Methods

Subjects

Urine specimens were solicited from the Bahar Medical Laboratory (Tehran, Iran) and the Iranian National Center for Addiction Studies. These specimens were related to 50 self-reported individuals (40 males and 10 females) with the mean age of 30.1 (16-44) years

old. No further personal information was associated with the specimens. After collection, the urine samples were processed as described below and subsequently were stored at -80°C for further analysis. All procedures were approved by the ethics committee of Islamic Azad University.

Samples preparation

In the first step, urine specimens were removed from the refrigerator and placed in the laboratory environment in order to reach the room temperature. Two milliliters of every sample were poured into each test tube. The pH of the samples was determined to detect the probable changes in the urine samples. Samples with a pH range of 5.5 to 8.4 were used for further study.

Screening test

The urine screening test, as a type of biochemical measure is an immunoassay strategy⁹. Drugs in the urine sample compete against their respective drug conjugate for binding to specific antibody. Urine specimen moves upward by the ability of a liquid to flow in narrow tubes without the presence of external forces, capillary action. The drug in the urine specimen when is lower than its cut off concentration, will not sufficiently occupied the binding sites of the related antibody. When antibody react with its conjugating drug-protein, a colored line will show off in strips region of the test tape. The screening test was utilized for amphetamine, methamphetamine, morphine, codeine, tricyclic antidepressant, benzodiazepines, cannabis, methadone, and ephedrine.

TLC analysis

TLC analysis was performed using the commercial kit according to the manufacturer's instructions (Bahar afshan institute of research and development, Tehran, Iran) for amphetamine, methamphetamine, morphine, codeine, tricyclic antidepressant, benzodiazepines, cannabis, methadone, and ephedrine.

HPLC analysis

HPLC analysis was performed for amphetamine and methamphetamine. A Chrome system CLC300 pump and a Nova pack C18 column filled with ultra-sphere octadesylsilyl (ODS) 3.9; 150 mm, 4 mm) were utilized. The mobile phase was methanol with the eluent

Table 1. The number of positive screening samples

	Screening+	Screening+%	TLC+	(TLC-/Scr+)%
Amphetamine	50	100	39	22
Methamphetamine	48	96	40	17

Screening+, The number of positive screening samples; TLC+, The number of positive TLC samples; (TLC-/Scr+)%, The percent of screening-false positive

flow, 1 ml/min and injection volume of 50 ml. The integrator was a UV detector (Shimadzu SPD-10 AVP UV-VIS Detector, Japan). The concentration of standards substances was determined at 210 nm. Finally 20 µl of methanol were injected to the HPLC equipment.

The standard curve was plotted. For this purpose, 1 mg of amphetamine chloride and methamphetamine sulfate was separately dissolved in 10 ml of methanol and then standard concentrations of 1.25, 2.5, 5 and 10 µg/ml of it were prepared followed by drawing standard curves.

Results

The results of the screening test and TLC of amphetamine, methamphetamine, morphine, codeine, tricyclic antidepressant, benzodiazepines, cannabis, methadone, and ephedrine were depicted in figure 1. The screening test showed 22 and 17 percent of false-positive results for amphetamine and methamphetamine samples respectively (Table 1) in comparison with TLC. The spots of the amphetamine and metham-

phetamine detected in urine samples were shown in figure 2. The TLC-negative samples were later analyzed by HPLC, whereas no peaks were detected.

After plotting the standard curve, all the TLC-positive samples were extracted and in turn were injected into the HPLC system. After repeating tests for many times, the retention time of amphetamine and methamphetamine was identified. As depicted in figure 3, the retention time for amphetamine and methamphetamine were 5:66 and 10:98 min, respectively.

In TLC-negative samples, no peaks were observed in the related area (for amphetamine), but we observed the peaks for the rest of samples. In the same way about methamphetamine in TLC-negative samples, no peaks were observed in the mentioned area, but we had observable peaks for the rest samples. Results are expressed in descriptive terms.

Discussion

Among participants with a false-positive screening re-

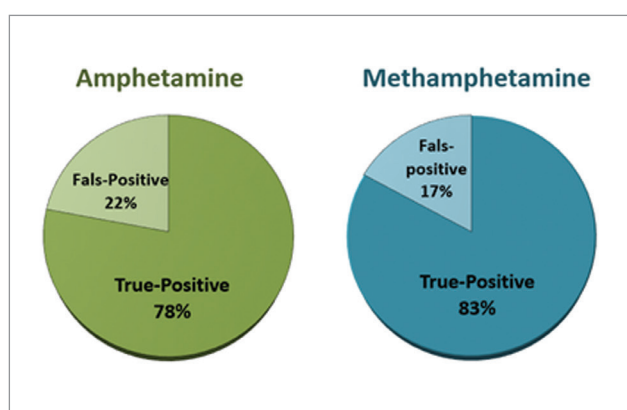


Figure 1. The TLC Method was utilized after urine screening tape-test to detect illicit drugs for all urine specimens (100%). The positive results related to the urine screening tape-test but not the TLC method, were considered as the false-positive results, whereas the positive results obtained by both urine screening tape-test and the TLC method were recorded as true-positive results for amphetamine and methamphetamine.

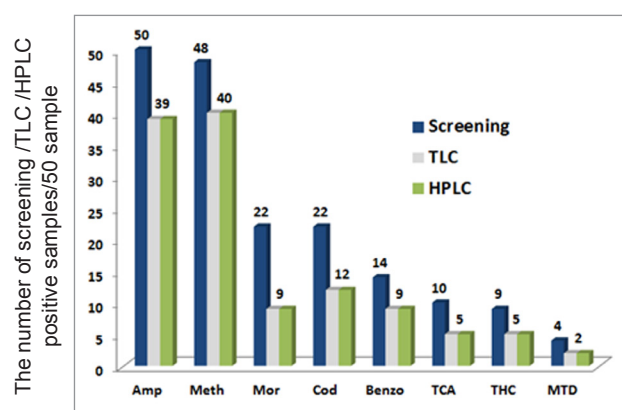


Figure 2. Each column shows the number of screening/TLC positive results from urine samples of 50 self-introducer addicted persons.

Amp, Amphetamine; Meth, Methamphetamine; Mor, Morphine; Cod, Codeine; TCA, Tricyclic Antidepressant; Benzo, Benzodiazepines; Cann, Cannabis; MTD, Methadone.

sults, 22 and 17 % were related to the amphetamine and methamphetamine respectively. The false positive samples may be caused by consumption of cold tablets or diet powders containing ephedrine and pseudoephedrine⁷. According to the manufacture's report, the minimum detectable concentration by the screening test tape was claimed to be 500 ng/ml for amphetamine and 1000 ng/ml for methamphetamine. Diluting urine samples and alteration of pH may produce more false negative results. Since the urine samples were related to the self-reported addicted individuals, no false negative was recorded by screening tests. The main disadvantage of screening test tape is the higher rate of false-positive results. Pseudoephedrine, ephedrine, phenylephrine and decongestants are the common over-the-counter cold medications, with potential cross reaction with amphetamine compounds¹⁰.

By HPLC system, we managed to record the peaks for amphetamine and methamphetamine in all TLC-positive samples. The minimum detected concentrations of amphetamine and methamphetamine in all 50 samples of urine were 0.37 and 0.59 µg/ml, respectively. The data obtained from both methods had no conflict. Lower sensitivity of TLC method was compensated by providing larger sample volume. Although, the HPLC is a precise alternative method in comparison with costly gas chromatography-mass techniques to confirm the existence of amphetamine and methamphetamine in human urine, especially in the clinical care settings¹¹, being in accessible in many small cities and villages across the world, being more costly and its need for skilled operator staffs are the difficulties that limit the use of the chromatography method. Therefore, we decided to evaluate an easier and less expensive method and then we assessed its reliability in the range of substances which are detectable in Iranian population. Finally, through this research, we found some accuracy and validity of TLC method for analyzing amphetamine and methamphetamine in human urine. The advantage of the TLC method as a rapid and precise identification method of toxic substances has been reported in other studies^{12,13} while in another study, the rapid TLC detection of abused tertiary amino drugs has been evaluated¹⁴ and we compared our results with them.

Conclusion

In conclusion, the present assay achieved the simulta-



Figure 3. Chromatogram shows the spot(s) of the amphetamine and methamphetamine standard and a urine sample containing both of them.

neous identification of 2 illicit drugs in human urine. Repeated analysis of TLC samples allowed to provide accurate data and now we may claim that TLC analysis is simple, rapid, sensitive and suitable alternative method for detecting the abuse of amphetamines and methamphetamine in deprived areas of a country with no advanced equipment. This method is potentially sensitive and reliable for drug screening in the clinical care settings too. It can be considered as an alternative for costly and time-consuming HPLC method.

Conflict of Interest

The authors declare that they have no competing financial interests.

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