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An efficient method for simultaneous detection of Pheniramine, Pentazocine and cotinine in urine by Gas Chromatography in De-addiction program

Raka Jain[1], Shayani Ghosh[1], Nizamuddin Saifi[1]

Original Article

Abstract

Background: Nonmedical use of prescription drugs for recreational purposes is a major health problem that raises high concerns for public health. Recently, several laboratory studies have reported the misuse of pentazocine, an agonist-antagonist opioid in combination with antihistamines in opioid addicts. Illicit self-administration of prescription drugs has been increasingly reported in India. Urinalysis as an adjunct to self-report plays a key role in providing additional information in the treatment of drug users. This paper aims to discuss a simple, convenient, and rapid capillary column gas-liquid chromatography method for simultaneous detection of pentazocine, pheniramine, and cotinine in urine.

Methods: The sample was extracted with chloroform and isopropanol (3:1,v/v) and evaporated to dryness. After reconstitution with methanol, it was directly subjected to gas chromatography analysis. Method performance was evaluated and validated in terms of sensitivity, precision, the limit of detection (LOD), and the limit of quantification (LOQ).

Findings: The linearity obtained was in the range of 50–1000 ng/ml with a correlation coefficient (r) above 0.999 for each drug. Good LOQ (50ng/ml) was obtained with each drug. Also, the developed method was effective in analyzing samples from patients with suspected abuse of these drugs.

Conclusion: The technique was found to be simple, robust, sensitive, and precise in the simultaneous analysis of drugs (pentazocine, pheniramine, and cotinine). This method was proved to be useful and cost-effective in treating and monitoring patients seeking help for addiction in clinical settings.

Keywords: Pheniramine; Pentazocine; Cotinine; Urine; Gas chromatography

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1- Department of Psychiatry, National Drug Dependence Treatment Centre, All India Institute of Medical Sciences, New Delhi-110029, India
Correspondence to: Raka Jain; Department of Lab Medicine, All India Institute of Medical Sciences, New Delhi-110029, India,
Email: rakajain2009@gmail.com

Introduction

The abuse of prescription drugs has gained popularity among substance users worldwide. In India, many people use psychoactive substances, and substance use affects all the population groups.[1] Increasing addiction cases have become a major clinical and public health concern.

Pheniramine maleate is an anti-histamine H1 receptor antagonist, widely used in the treatment of allergies such as hay fever, runny nose, itching skin, and rashes, and symptomatic relief of hypersensitivity reactions.[2] In India, several cases of pheniramine abuse have been reported.[3,4] Pentazocine, a mixed agonist-antagonist opiate of the benzomorphan class, has been widely used in the management of moderate to severe pain in patients with postoperative pain or early carcinogenic pain.[5] Pentazocine is used among substance users because pharmacological investigations has shown that its subjective effects are somewhat euphoric or even dysphoric at times.[6-8] A few published reports also suggest that most people abuse a combination of buprenorphine with injectable diazepam and antihistamines (pheniramine, promethazine), sometimes pentazocine, to enhance the quality and duration of "high".[9,10] Similarly, the use of pentazocine in combination with antihistamines has been frequently reported.[11] Additionally, the prevalence of smoking remains high among substance users. Cotinine, an alkaloid of tobacco, acts as a biomarker for both passive and active tobacco use.[12] The use of these drugs alone or in combination is being reported in increasing numbers. Numerous techniques have been reported for the identification of pheniramine maleate, pentazocine, or cotinine in urine. Among them, thin-layer chromatography-densitometry, high-performance liquid chromatography (HPLC), and ultra-violet spectrophotometry are commonly used for pheniramine maleate detection.[2,13-15] Similarly, gas chromatography (GC), as well as HPLC, is used for the detection of pentazocine in various biological matrices.[16-18] Pheniramine maleate and pentazocine can be analyzed from direct pharmaceutical formulations or biological specimens either alone or in combination with other drugs, by such methods. Likewise, techniques such as gas chromatography (GC) combined with mass spectrometry (MS), liquid chromatography combined with tandem mass spectrometry (LC-MSMS), and capillary electrophoresis (CE) with HPLC are used for the detection of nicotine and its major metabolite cotinine in urine.[19-22]

Screening for patients involved in substance use is based on self-reports, which may lack validity and have often been questioned.[23] In response to concerns about the potential inaccuracies of self-reported data, urinalysis provides an objective approach along with the self-reports to monitor patients in clinical settings. With this in view, the present study was designed to develop a simple, efficient, and economical method for simultaneously detecting pentazocine, pheniramine, and cotinine in the urine of substance users using GC technique. The developed method was validated and applied to individuals indulging in substance use. This technique supported minimum sample preparation, eliminating the need for an extra hydrolysis step.

Methods

Chemicals and reagents: Pheniramine Maleate (Avil) as a standard reference material was obtained from Sanofi India Limited (New Delhi, India). Pentazocine hydrochloride as a standard was obtained from Ranbaxy Laboratories Limited (New Delhi, India). Cotinine as a standard was obtained from Sigma Aldrich (Missouri, USA). Chloroform, methanol, isopropanol, and sodium hydroxide were procured from Merck (Darmstadt, Germany). All of the chemicals and reagents used were of analytical grade. The study was approved by the institution's ethics committee. (IEC/NP-222/05.06.2015)

Urine sample preparations: Five adult volunteers with no history of drug use or smoking provided blank urine samples (5ml). After centrifugation, the supernatant from each urine sample was decanted. The supernatant was pooled and the urine was kept at −20°C until it was analysed. A stock solution of 10µg in methanol was prepared for pheniramine maleate, pentazocine hydrochloride, and cotinine.

One milliliter of blank urine sample was taken in three individual tubes for analysis, and the pH was adjusted to 11-12 with drops of 10 M sodium hydroxide. Two milliliters of a freshly prepared solution of chloroform and isopropanol (3:1, v/v) was added to each blank urine sample. The tubes were tightly closed, vortexed for one minute, and centrifuged at 2000 rpm for five minutes. The organic layer was separated and each blank urine sample was spiked with appropriate volumes of

pheniramine maleate, pentazocine hydrochloride, and cotinine standards, resulting in final concentrations of 1,000, 500, and 50 ng/mL. At room temperature, the samples were evaporated to dryness under a stream of nitrogen, then reconstituted in 200µl of methanol before being injected into the GC.

GC Conditions: The analysis was carried out on GC (7890A series, Agilent Technologies) system equipped with a nitrogen phosphorus detector and an automatic injector. An Agilent HP-5 fused silica column cross-linked with 5% diphenyl and 95% polydimethylsiloxane (30m x 0.32 mm ID, 0.25 µm film thickness) was used for separation. Nitrogen was used as the carrier gas, with a constant flow rate of 8 mL/min. In splitless mode, samples (2 µL) were injected at a temperature of 250°C. The oven temperature was set to 90°C for 1 minute, then increased at a rate of 10°C/min to 250°C and held for 3 minutes. The total duration of the run was 19 minutes. The detector was maintained at a temperature of 250°C. The flow rates for air and hydrogen were 60 ml/min and 2.5 ml/min respectively.

Method Validation: The method's selectivity, linearity, the limit of detection (LOD), lower limit of quantification (LLOQ), recovery, and precision were all validated (intra-day and inter-day).[24,25]

Results

Selectivity: The selectivity of the method was analyzed in pooled urine specimens. No interference peak was observed in the blank (Figure 1) as well as spiked urine samples of pheniramine maleate, pentazocine hydrochloride, or cotinine (Figure 2) indicating good selectivity. The retention times for cotinine, pheniramine maleate, and pentazocine hydrochloride were 11.05, 12.34, and 16.70 minutes, respectively.

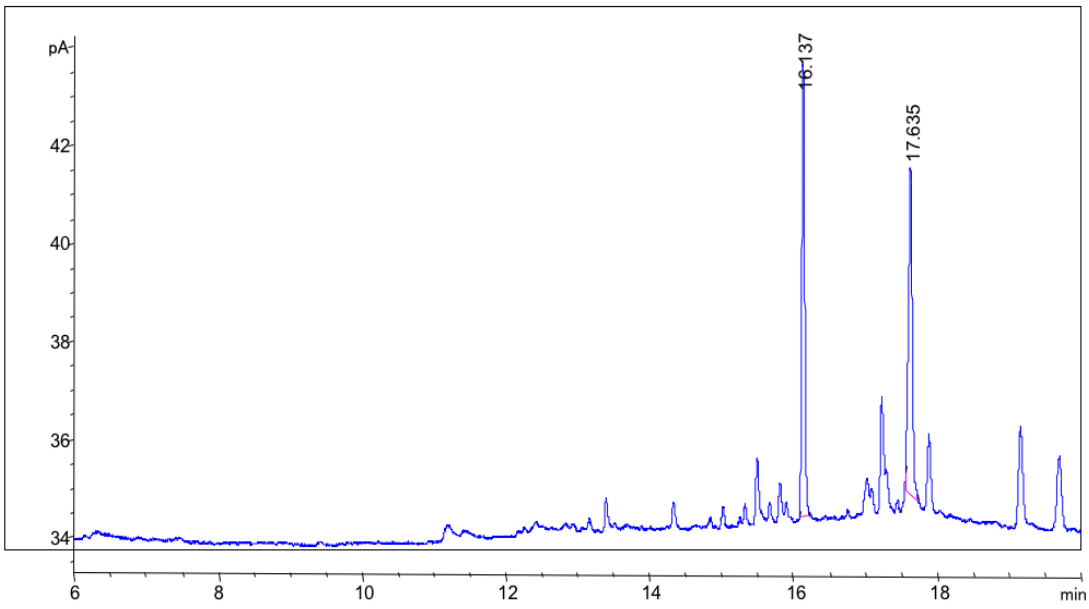

Figure 1. Chromatogram of a blank urine sample

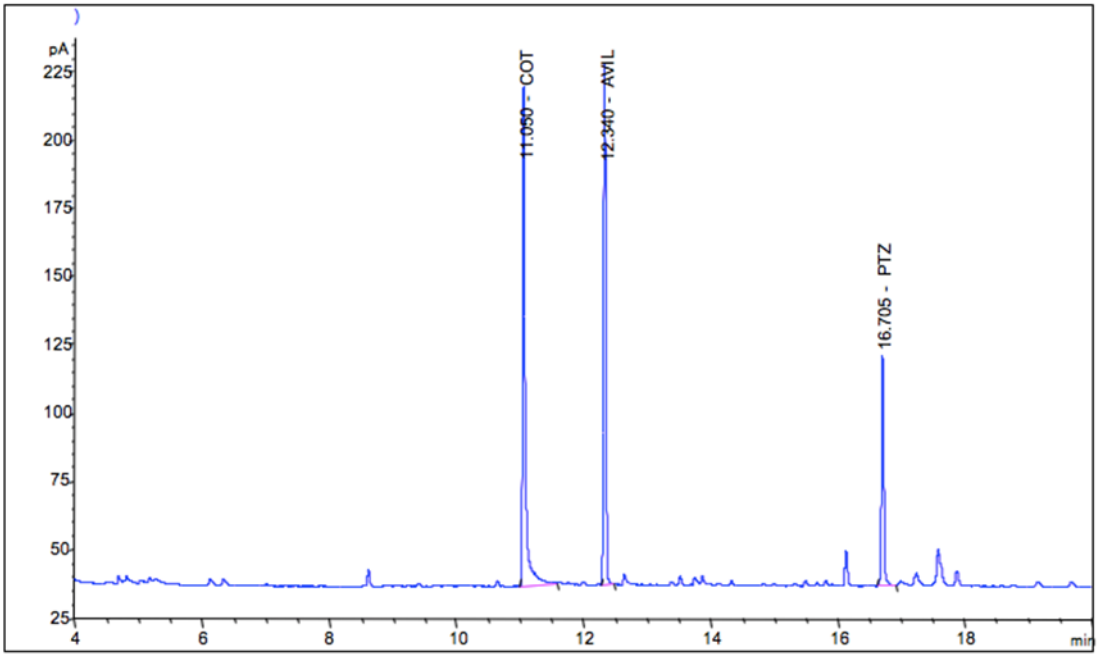

Figure 2. Chromatogram of control urine sample spiked with pheniramine maleate, pentazocine, and cotinine

Applicability of the method: The developed method was successfully used to analyze pheniramine, pentazocine, and cotinine in 20 urine samples from patients with suspected abuse of these drugs treated at a tertiary de-addiction centre in northern India. The patients' urine samples showed no external interferences (Figure 3).

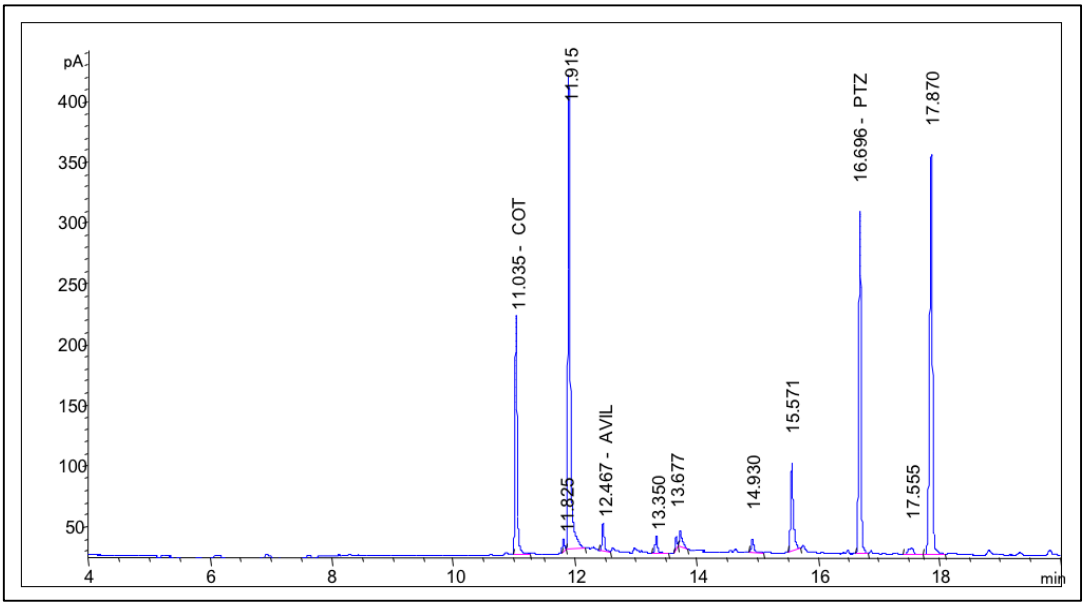

Figure 3. Chromatogram of patients' urine showing the presence of pheniramine maleate, pentazocine, and cotinine

Linearity/Calibration curves: A minimum of five calibrators was prepared by spiking blank urine with definite concentrations (1,000, 500, and 50 ng/mL) of all three drugs. Good linearity was observed for peak intensity within the *specified* concentration range for all three drugs and the correlation coefficients of the calibration curves were consistent >0.999.

***Limit of Detection and Lower Limit of Quantification*:** The LLOQ was defined as the lowest point on the calibration curve and met the requirement of LLOQ signal-to-noise ratio of 10:1.[24,25] The LLOQ of all three drugs was 50ng/mL. The detection limit was also systematically evaluated.[8,9] The LOD for pheniramine maleate, pentazocine hydrochloride, and cotinine were 18 ng/mL, 8ng/mL, and 6ng/mL, respectively.

Recovery: To calculate the extraction recovery, peak areas of extracted standards were compared to those of pure reference standards at three different concentrations: 50, 500, 1000 ng/mL. The extraction recovery rates for pheniramine maleate, pentazocine hydrochloride, and cotinine at these concentrations were 85 ± 1.8, 82.5 ± 2.3, and 90 ± 1.5, respectively.

Intra-Day and Inter-Day Precision: For all three drugs, precision assays were expressed as the coefficient of variation (CV). The intra-day and inter-day precisions were determined at the three controlled concentrations: 50, 500, and 1,000 ng/mL. Table 1 and Table 2 show the acquired precision values for intra-day and inter-day.

The intra-day precision was determined by calculating the CV of three replicates of the urine matrix at three concentrations on the same day. Similarly, CV was calculated for three replicates of the urine matrix at three different concentrations over three consecutive days to determine the inter-day precision.

Table 1. Intra- day Precision for drug spiked urine sample

Concentration (ng/mL)	Pheniramine maleate (average)	CV %	Pentazocine hydrochloride (average)	CV %	Cotinine (average)	CV (%)
50	33.53±2.35	7.0	13.16±1.07	8.13	25.96±0.96	3.69
500	238.33±10.40	4.36	117.43±6.21	5.28	225±7.80	3.46
1000	436±8.28	1.89	223.8±9.56	4.27	472.22±6.98	1.47

Table 2. Inter- day Precision for drug spiked urine sample

Concentration (ng/mL)	Pheniramine maleate (average)	CV %	Pentazocine hydrochloride (average)	CV %	Cotinine (average)	CV (%)
50	33.38±1.40	4.19	12.88±0.42	3.27	27.22±1.44	5.2
500	238.44±11.50	4.82	119.41±4.90	4.10	232.3±7.02	3.02
1000	444.76±20.6	4.63	230.63±7.17	3.10	460.67±10.04	2.17

Discussion

Over the last decade, multidrug use has always been a major concern for healthcare professionals and policy-makers. Substance co-use is associated with risky behaviors and adverse health outcomes and has been found to be more dangerous than single drug use.[26,27] Recently, immunoassays have become more popular in clinical settings due to their ease of use and rapid results. Even with their popularity, they remain presumptive and are highly influenced by external variables. Clinicians cannot certainly rely upon this technique to monitor patients. For confirmation, more reliable techniques such as GC or GC-MS are being successfully used to screen patients based on urinalysis.

Pheniramine, pentazocine, or cotinine have been

already determined with various methods, such as UV-spectrometry, thin layer chromatography, and high-performance liquid chromatography. The current study aimed to develop a simple technique for simultaneous detection of pheniramine, pentazocine, and cotinine in urine specimens of patients who are suspected of abusing such drugs. The validation of this method was achieved on gas-chromatography using a nitrogen phosphorus detector. The linearity of the method was determined in the 50–1000 ng/mL range by the least square's regression method, with R2 over 0.999. The LOQ of all three drugs was 50 ng/mL. The intra- and inter-day precision and CV % were always below 10% in QC samples and below 20% for the LOQ.

The method's applicability was demonstrated by analyzing 20 urine samples from individuals suspected of abusing these medications and receiving treatment at a tertiary de-addiction centre in northern India. As shown in Figure 3, no external interferences were detected in the urine samples of the patients. This technique encouraged a small sample volume size which was advantageous because many clinical and forensic toxicology laboratories have limited sample volume because of polydrug use history. Despite the small sample volume, a LOQ of 50 ng/mL was achieved. The sample was prepared using a liquid-liquid extraction method in an alkaline condition and showed more than 80% recovery. The separation of the three analytes was achieved in less than 20 minutes. Acceptable sensitivity was achieved without using any hyphenated expensive techniques such as tandem mass spectrometry. However, the failure to use the internal standard as a reference for method validation was one of the limitations of the study.

The strength of the current method is that it was selective, robust, and showed no compromise in the separation of otherwise interfering peaks. This study proves to be cost-effective as it reduces the demand for individual drug testing. The method shows its usefulness as it allows rapid detection of substances commonly encountered in addiction cases. Being robust and simple, it can easily be adapted in any clinical setting.

Conclusion

The current study has been shown to be effective for simultaneous detection and quantification of pheniramine, pentazocine, and cotinine in urine samples. This method is capable of identifying all three drugs from a single urine sample using simple GC technology without using any higher-grade techniques. The method can be successfully used by clinicians in analyzing such drugs in de-addiction programs to diagnose, treat, and monitor patients seeking treatment for drug addiction.

Conflict of Interests

None.

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Authors' Contribution

RJ contributed to the study's conception and design. Experimental work was carried out by NS, and RJ and SG drafted the manuscript. All of the authors read and approved the final version of the manuscript.

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روشی کارآمد برای تشخیص همزمان فنیرامین، پنتازوسین و کوتینین در ادرار با کروماتوگرافی گازی در برنامه ترک اعتیاد

راکا جین[1]، شایانی قوش[1]، نظام الدین سیفی[1]

مقاله پژوهشی

چکیده

مقدمه: استفاده غیرپزشکی از داروهای تجویزی برای اهداف تفریحی یک مشکل عمده بهداشتی است که نگرانی‌های زیادی را برای سلامت عمومی ایجاد می‌کند. اخیراً، چندین مطالعه آزمایشگاهی استفاده نادرست از پنتازوسین، یک گونیست- آنتاگونیست اپیوئیدی، در ترکیب با آنتی هیستامین‌ها را در معتادان به مواد افیونی گزارش کرده‌اند. مصرف غیرقانونی داروهای تجویزی به طور فزاینده‌ای در هند گزارش شده است. آزمایش ادرار به عنوان تشخیص کمکی , نقشی کلیدی در ارائه اطلاعات بیشتر در درمان مصرف کنندگان مواد مخدر دارد. هدف این مقاله ارائه یک روش کروماتوگرافی گازی مایع ستون مویرگی ساده، راحت و سریع برای تشخیص همزمان پنتازوسین، فنیرامین و کوتینین در ادرار است. هدف این مقاله ارائه یک روش کروماتوگرافی گازی- مایع ستون مویرگی ساده، راحت و سریع برای تشخیص همزمان پنتازوسین، فنیرامین و کوتینین در ادرار است.

مواد و روش‌ها: نمونه با کلروفرم و ایزوپروپانول (۳:۱) استخراج و تا خشک شدن تبخیر شد. پس از بازسازی با متانول، مستقیماً تحت آنالیز کروماتوگرافی گازی قرار گرفت. عملکرد روش از نظر حساسیت، دقت، حد تشخیص (LOD) و حد کمیت (LOQ) ارزیابی و اعتبارسنجی شد.

یافته‌ها: رابطه خطی به دست آمده در محدوده ۵۰ تا ۱۰۰۰ نانوگرم در میلی لیتر با ضریب همبستگی (r) بالای ۰/۹۹۹ برای هر دارو بود. LOQ خوب (۵۰ng/ml) با هر دارو به دست آمد. همچنین، این روش در آنالیز نمونه‌های بیماران مشکوک به سوءمصرف این داروها مؤثر بود.

نتیجه‌گیری: این تکنیک در تجزیه و تحلیل همزمان داروها (پنتازوسین، فنیرامین و کوتینین) ساده، قوی، حساس و دقیق است. بـه نظـر می‌رسد این روش در درمان و نظارت بر بیمارانی که به دنبال کمک برای نرک اعتیاد در محیط‌های بالینی هستند مفید و مقرون به صـرفه باشد.

واژگان کلیدی: فنیرامین؛ پنتازوسین؛ کوتینین؛ ادرار؛ کروماتوگرافی گازی

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۱- مرکز ملی درمان وابستگی به مواد مخدر، گروه روانپزشکی، دانشکده علوم پزشکی هند، دهلی نو، هند

نویسنده مسئول: راکا جین؛ مرکز ملی درمان وابستگی به مواد مخدر، گروه روانپزشکی، دانشکده علوم پزشکی هند، دهلی نو، هند

Email: rakajain2009@gmail.com