Original Research Article

Chemical screening for codeine and tramadol in urine of anonymous students attending university sick bays

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ABSTRACT

In this study, anonymous chemical screening of psychoactive substances (codeine and tramadol) was carried out on urine collected from some students in two Nigerian Universities, coded UN1 and UN2 for a Federal a State University, respectively. Chloroform was used as the extractant and sodium borate was used to adjust the pH of the urine samples to 9. Thin-layer chromatography (TLC) and UV-Visible spectrophotometer was used as the primary separation and quantification methods, respectively. The concentrations of codeine in the urine samples collected from UN1 sick bay ranged from 2.822 ppm to 44.756 ppm, while that of UN2 ranges from 0.289 ppm to 4.434 ppm. The respective concentrations of tramadol for the institutions are within the range of 0.015 ppm to 34.833 ppm and 0.181 ppm to 37.030 ppm. There was a statistical difference (p<0.05) in the use of codeine and tramadol among students attending the selected clinics. It was suggested that the University students be subjected to continuous routine screening and counseling.

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Introduction

Man has used and abused certain substances since time immemorial. For cultures, there has been a desire for man, consciously or unconsciously to escape from monotony, frustration and pains and to seek euphoria or a sense of well-being when taking part in different achievement tasks. Substances such as alcohol, marijuana, and tobacco are no exception. These substances are psychoactive substances. According to psychology dictionary, psychoactive drugs are the chemical substances that affect the brain functioning, causing changes in behavior, mood, and consciousness. These substances are also known as designer drugs, legal highs, spice, herbal incense, bath salts, internet drugs, club drugs research chemical, and synthetic drugs. Part of the problem in controlling the proliferation of these substances lies in their variety, their ease of synthesis, low cost, being undetectable by standard toxicology screens, and resourceful marketing. In addition to being available from drug traffickers, they are often sold on the internet as well as in neighborhood head shops [1]. Most of these substances are synthesized by adjusting or manipulating the molecular structure of previous popular psychoactive agents such as cocaine, cannabis, lysergic acid diethylamide (LSD), methylenedioxymethamphetamine (or MDMA, commonly known as ecstasy), methadone and synthetic cathinones which are sold as “legal highs,” “research chemicals,” “party pills,” “herbal highs,” or “plant food”. [2].

The name codeine is derived from a Greek word _kodeia_ for ‘poppy head’ and is found naturally in the poppy plant ‘_Papaver somniferum var. album_.’ Codeine is a phenanthrene derivative extracted from opium or produced synthetically by the methylation of morphine. Codeine or 3-methylmorphine is the most commonly consumed opiate worldwide and is used for its analgesic, anti-tussive, and anti-diarrheal properties [3, 4].

Codeine is an opioid analgesic which is also utilized in the management of pain and diarrhoea. Like other opioids, it is widely abused due to its potential to produce euphoria (high mood) when consumed in large quantities. Hence, codeine abusers consume large
quantities of codeine-containing cough syrup which ultimately leads to adverse effects like dependence, sedation, euphoria, and tolerance [5].

Ramadol was first synthesized in 1962 by Grünenthal GmbH [6] by coupling of the corresponding cyclohexanon with 3-methoxyphenylmagnesiumbromide in a Grignard reaction. Recently, chemical synthesis of tramadol and two of its metabolites have been described by the same coupling reaction using organo-lithium derivatives [6] and narrates that drug abuse. The primary reasons for continuous drug usage were to relieve and to prolong the time of sexual intercourse [7]. Many ugly practices such as smoking/sniffing of lizard dungs, dried human dungs, sniffing of pit toilet/soak away bio generic gas, concoction of unimaginable substances known as goskolo, pharmaceutical formulations such as codeine, tramadol, rohypnol, and many more.

Traditionally, urine is one of the choices used for screening and identification of unknown drugs due to high concentration of drugs and their metabolites in urine. Both identification and quantification can be performed in one matrix [8]. Another great advantage is that drugs can be detected just after intake prior to metabolism or filtration. Review of relevant literature revealed that TLC and UV spectrophotometry are suitable for detection and quantification of drugs in both pharmaceutical formulations and biological samples.

According to [9], more than 500,000 bottles of codeine are consumed daily by young Nigerians across the country, same with the intake of tramadol, rohypnol, marijuana and other opioids, an alarming trend that has subtly eaten deeply into the students’ fabric with youths of all classes having a field day abusing these drugs. In Nigeria, the use of psychoactive substances is mostly prevalent in the northern region [10]. No wonder [9], posit psychoactive substances are commonly used and abused, especially among the uneducated youths in the Northern part of Nigeria.

Materials and Methods

Chemicals and Materials

All the reagents used in the course of this analysis were analytical grade. Separating funnel, measuring cylinder, UV-Vis spectrophotometer (shimadzu model 1800), thin-layer chromatography (TLC), and UV Lamp (λ=254 nm) analysis were used in this study.

Sampling

The sampling was carried out in two Nigerian Universities (names withheld), situated in the same city. Urine sample handling was strictly followed to ensure no alteration and contamination of the samples. Sample collection bottles (screw-cap-sterile plastic sample bottles) were used for hundred (100) different urine samples (fifty from each of the study area). To preserve the samples from deterioration, one drop of concentrated nitric acid was added to each of the samples. The collected samples were then refrigerated (kept cool) at 2°C to 8°C prior to analysis using UV-Visible and TLC plates. The sample were collected using screw-cap-sterile plastic sample bottles after which was wrapped with aluminum foil to prevent contamination, and kept in ice chest, transported to the laboratory and stored at (2°C to 8°C) in a refrigerator for later use [11].

pH determination of urine samples

The pH of the urine samples was determined following the documented suggestion [12], using digital pH meter (model pHep).
Sample preparation and extraction procedure

A liquid-liquid extraction technique was used adopted for drug extraction [13]. Before the extraction, 10 mL of each urine sample was diluted with 10 mL distilled water (10:10; v/v) in a beaker. The pH of the samples was adjusted to alkaline range of 9-10 [14, 15] by adding 4 mL of saturated sodium borate and then stirred for 5 min [15]. The two targeted psychoactive substances and their metabolites were extracted from the urine samples using chloroform as the extraction solvent. The mixture was then transferred into a separating funnel which was followed by the addition of 10 mL of the extraction solvent (chloroform), shaken for twenty minutes after which it was exposed to air to allow for the evaporation of the extraction solvent. The supernatant (organic phase) was collected [16], and kept for UV-Vis and TLC analysis.

Preparation of stock and standard solutions

Standard curves were derived, using pure and analytical grade drug standards, for screening and quantification of drugs in urine samples. Working solutions, between 10 to 50 ppm were prepared, using serial dilution.

Chemical screening for codeine and tramadol

Thin layer chromatography (TLC) screening

For TLC screening, silica gel 60 F\textsubscript{254} TLC plates were utilized [17]. The sample used on the TLC plate was prepared by dissolving a small amount of the sample in a vial containing chloroform and methanol (80:20, v/v) [18]. A pencil was drawn on the coated TLC plate 2 cm from the bottom of the plate above the solvent level. Reference standards of codeine and tramadol solutions were spotted next to unknown sample onto the TLC plate with the help of micro capillary tube which was placed in the developing tank. Iodine solution was prepared and sprayed on the TLC plates in order to aid visualization [19]. With the lid on, the TLC plate was developed by allowing the solvent to run up the plate for a period of up to 10 min. After development, the TLC plate was removed and visualized under UV lamp at a wavelength of 254 nm [19].

![Figure 1. Typical of separation using TLC Plates (a and b) and Rf measurement (c) [29].](image)

UV-Vis analysis

The UV-Vis profile of codeine and tramadol were determined by the intensity of electromagnetic radiation absorbed by the analytes using UV-Vis spectrophotometer (shimadzu model 1800). The predetermined wavelengths of 284.0 nm and 271.0 nm were used for codeine and tramadol, respectively. The standard solutions were prepared using the stock solution at the intervals of 10, 20, 30, 40, and 50 ppm for codeine; 10, 20, 30, and 40 ppm for tramadol. The absorbance values at which these selected substances absorbed were read from the regression equations of standard calibration curve [20-23].
Statistical test of significance

Analysis of variance (ANOVA) at 95% confidence level (Welch test and TukeyHSD) was used as statistical tool [24] to compare drug use level between federal and state universitis; and codeine and tramadol use levels in both universities.

Results and discussion

Physico-chemical parameter

The pH of the urine sample was determined as a preliminary step into screening of urine samples for codeine and tramadol.

Determination of pH of urine samples

The results of the pH of the urine samples determined are shown in Table 1.

| Sample Code | pH of Urine | Qualitative (TLC) Screening | Quantitative Uv-vis. Analysis |
|-------------|-------------|----------------------------|------------------------------|
|             | UN1 | UN2 | UN1 | UN2 | Codeine | Tramadol |
| 1           | 2.3 | 6.5 | +   | +   | -       | 2.822     | 0.015 |
| 2           | 4.7 | 2.4 | -   | -   | +       | 36.333    |       |
| 3           | 5.2 | 2.8 | -   | -   | +       | 0.289     | 0.181 |
| 4           | 8.0 | 5.1 | -   | -   | -       |           |       |
| 5           | 3.1 | 6.3 | +   | -   | -       | 44.756    |       |
| 6           | 3.2 | 2.1 | -   | +   | +       | 1.140     | 9.258 |
| 7           | 6.9 | 3.0 | -   | -   | +       | 13.591    |       |
| 8           | 2.7 | 2.9 | +   | -   | -       | 33.136    | 5.091 |
| 9           | 8.2 | 2.8 | -   | -   | +       | 2.240     |       |
| 10          | 7.2 | 8.4 | -   | +   | -       |           |       |
| 11          | 2.9 | 2.3 | +   | +   | -       | 11.516    | 0.167 |
| 12          | 3.0 | 2.5 | +   | +   | -       | 2.474     | 43.818 |
| 13          | 2.6 | 2.9 | -   | -   | +       | 4.434     | 18.500 |
| 14          | 6.1 | 8.3 | -   | -   | -       |           | 9.364 |
| 15          | 6.8 | 6.7 | -   | -   | -       |           |       |
| 16          | 8.3 | 5.0 | -   | -   | -       |           |       |
| 17          | 2.5 | 5.3 | -   | +   | -       |           | 0.015 |
| 18          | 2.4 | 8.1 | -   | +   | -       |           | 27.576 |
| 19          | 6.9 | 8.2 | -   | -   | -       |           |       |
| 20          | 7.8 | 8.0 | -   | -   | -       |           |       |
| 21          | 8.4 | 3.1 | -   | -   | +       | 3.391     |       |
| 22          | 2.8 | 3.3 | -   | +   | -       | 0.030     | 0.015 |
| 23          | 2.9 | 7.5 | +   | -   | -       | 22.491    |       |
| 24          | 8.3 | 2.7 | -   | -   | +       | 2.231     |       |
| 25          | 2.5 | 7.9 | +   | -   | -       | 22.334    |       |
| 26          | 6.5 | 5.8 | -   | -   | -       |           |       |
| 27          | 3.1 | 6.6 | -   | +   | -       | 34.833    |       |
| 28          | 4.9 | 3.4 | -   | -   | -       |           | 18.894 |
| 29          | 2.5 | 4.9 | -   | +   | -       | 27.379    |       |
| 30          | 2.6 | 7.1 | -   | +   | -       | 0.197     |       |
| 31          | 2.8 | 3.1 | +   | -   | +       | 44.495    | 4.427 |
| 32          | 7.1 | 7.7 | -   | -   | -       |           |       |
- Absent, + Present of the targeted drugs, UN1 = Student attending Federal University sick bay, UN2 - Student attending state university sick bay, Cod- Codein, Trama – Tramadol, Empty cell – Below detection limit.

**Uv-Vis. quantitative screening**

The absorbances at 284.0 nm and 271.0 nm for codeine and tramadol respectively, and their level of concentrations were determined by UV-Vis spectrophotometer. Table 1 shows the UV quantitative analytical report for codeine in urine samples from UN1. Table 1 also shows report for codeine in urine samples from UN2.

![Figure 2](image.png)  
*Figure 2. Drug (Codeine; C\textsubscript{18}H\textsubscript{21}NO\textsubscript{3}) Intake level by UN1 and UN2 students.*
Discussion

Visual inspection of urine

The physical inspection of urine samples carried out showed some characteristic colors. These colors observed ranges from amber, dark yellow, orange, syrup or brown ale, transparent yellow, pink to reddish. Some of the samples also shown no color (colourless), while some are foam or fizzy. The different colors exhibited by the urine samples can be traced to some medications such as laxatives and chemotherapy drugs. The normal and healthy urine colors, according to [25] are transparent yellow and dark yellow. This assertion best explains that urine exhibiting color(s) other than the normal and healthy colors named are said to be infected by disease(s), or alteration due to food and drug intake.

pH of urine samples

The preliminary identification of drugs in urine samples was carried out by measuring the pH before the proper screenings and quantization. Healthy and normal human urine is slightly acidic and slightly alkaline with a pH range of 4.5 to 8.0. Urine pH may change depending on the diet, certain diseases, and the medications. Urine pH tends to be more acidic when it is collected in the morning than any other time of the day. pH of urine samples ranges from 2.1 to 8.8. Urine samples showing pH values below 4.5 were assumed to be contaminated with drugs while samples showing values above 8.0 also show signs of contamination with drugs. Urines are more acidic on drugs such as methenamine mandelate, ammonium chloride while alkaline urine is caused by drugs like acetazolamide to treat glaucoma.

Detection of drugs

Drug prevalence in urine samples of both UN1 and UN2 students are presented in Figures 2 and 3. In this study, two major drugs (codeine and tramadol) were screened to detect the presence of the drugs in the urine samples students of UN1 and UN2. The quantification of drug level for codeine and tramadol was carried out using the positive detection from the
qualitative screening results. The screening procedure was based on thin layer chromatography (TLC) and the quantitative determination was performed using the Uv-Vis spectroscopy approach.

**Detection of codeine**

The detection of codeine was carried out using the TLC and Uv-Vis spectroscopy. Many drugs include codeine are active in the range of UV light. The TLC test and visualization showed the spots of compound at different location as shown in plates 1. In comparison to positions of movement of spots of standard reference sample of tramadol, a number of separations of spot were recorded. These separations arise due to the presence of drugs and other compounds.

Table 1 revealed that the codeine is actually used and abused by University students in the study area as shown by the qualitative (TLC) and confirmed by quantitative (UV) screenings. From the results presented, it was recorded that 11 students representing 22% out of 50 were recorded positive to codeine in UN1 at different concentrations, while 10 students representing 20% out of 50 were tested positive to codeine in Benue State University at different concentrations.

**Detection of tramadol**

Identification and quantitation of tramadol was also carried out using the TLC and Uv-Vis spectroscopy analysis. The TLC test and visualization revealed spots of compound at different location as seen in Figure 1. In comparison to positions of movement of spots of standard reference sample of tramadol, a number of separations of spot were recorded. 14 urine samples representing 28% each from both UN1 and UN2 recorded positive result to tramadol use. The positive result was confirmed by a second dependent method that is as sensitive as the screening test.

**Qualitative screening of codeine**

In Table 1, eleven urine samples from UN1 were recorded positive for the presence and use of codeine, while 10 urine samples were recorded positive from UN2. This brought to a total of 21 urine samples which presented positive results of drugs use. Detection of these drugs in test urine samples was an indication of drug use by students.

**Uv-Vis. quantification of codeine**

In this quantitative screening, concentration levels of the codeine were discussed. After detecting the codeine in the urine samples, the codeine that was detected in 11 urine samples from UN1 showed different concentration levels (measured in ppm by Uv-Vis spectrophotometer). Also 10 urine samples tested positive from UN2 recorded different concentrations. As seen in Table 1, concentration of the codeine intake from UN1 was at the range of 2.474 ppm to 44.756 ppm, while that of UN2 took the range of 0.289 ppm to 4.434 ppm. The different levels of concentration showed different levels of the codeine intake by the two universities. From the results in Table 1, codeine abuse by students of tertiary institution is eminent. Concentration of the codeine in urine sample was 44.756 ppm can be dangerous to human health in many ways. It was reported that, at a concentration of 1.00 ppm, codeine starts developing adverse reaction in man [26].

**Qualitative Screening for Tramadol**

Table 1 reveals a high number of tramadol intake as detected by the TLC. The result showed that 14 urine samples recorded positive
test for tramadol in the both Universities (UN1 and UN2). Reported high level of tramadol intake reported for UN1 and UN2 agrees with a that of a similar study [27], which opined that students misuse the drug against prescription. This shows that tramadol has attracted a considerable attention from young people compared with any other drugs. Another view on high rate of tramadol intake among the students under the area of this study may result from the weak or absence of legislation on these drugs and non-enforcement by regulatory agencies such as the National Drug Law Enforcement Agency (NDLEA) and the National Agency for Food and Drug Administration and Control (NAFDAC).

Uv quantitation of tramadol

The UV quantitation of tramadol was carried out after the positive results of tramadol used by students were detected from urine samples to ascertain the concentration levels of individual intake. As seen in Table 1, urine samples from both UN1 and UN2 recorded different concentration levels of tramadol. The concentration in UN1 found to be at the range of 0.015-43.818 ppm. While that of UN2 ranges from 0.181 to 37.030 ppm of concentrations. High concentrations of tramadol were recorded in the urine samples. In addition, there are records of high concentrations up to 37.030 ppm from UN2 and 43.818 ppm from UN1 which was observed as a threat to human health. This view is supported by [28] that oral tramadol is eliminated in the urine (90%) and the faeces (10%), and that peak concentrations of tramadol after single oral administration (100 mg) are 0.31± 0.08 ppm. In addition, according to [28] therapeutic dose of tramadol shows linear pharmacokinetics. The analgesic effect dose dependent and serum concentration of 0.1 to 0.3 ppm is considered effective. At a concentration higher than the stated one, drug may likely pose adverse effects on the human health.

Statistical analysis at 95 % confidence interval

The p-value obtained was 0.001 (p < 0.05) at a degree of freedom of 3 and at a confidence interval of 95%. This implies that there is a statistically significant difference in the means of the compared groups (UN1 and UN2 drugs). In addition, from the output result, codeine intake in UN1 and UN2 was compared and the p-value of 0.018 (p-value < 0.05) at 95% confidence interval. This p-value (0.018) revealed strong evidence that there is a statistically significant difference between the codeine in UN1 and UN2 levels. Therefore, the null hypothesis was rejected and the alternate hypothesis was accepted. This reveals that the codeine intake in UN1 and UN2 are different. From multiple comparison output, UN1 codeine and UN2 tramadol were compared and the p-value of 0.970 (p-value > 0.05) at 95% confidence interval. This shows that there is a strong evidence that there is no statistically significant difference between UN1 codeine intake and UN2 tramadol. Hence, the null hypothesis is accepted. From the multiple comparison output, UN1 tramadol and UN2 tramadol was compared and the p-value of 0.456 (p-value > 0.05) at 95% confidence interval. This p-value shows that there is a strong evidence that there is no statistically significant difference between the UN1 tramadol intake and UN2 tramadol intake. So, the null hypothesis is accepted. The codeine used in UN1 was found to be significantly higher than that of the UN2.

Conclusion

This research study provided invaluable information on the types of the widely-used
Psychoactive substances. The drug used by the students may not be limited to codeine and tramadol. Various degrees of concentrations of these drugs were identified in this study. The results revealed important implications for drug education intervention programs on the various campuses in tertiary institutions in Nigeria. Based on the findings of this study, tramadol and codeine were detected to be used among the university students in Nigeria.

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Conflict of interest

We have no conflicts of interest to disclose.

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