https://doi.org/10.31925/farmacia.2026.4.14 **ORIGINAL ARTICLE**

ANALYTIC TOXICOLOGICAL STUDY IN POLYDRUG USERS

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Abstract

Drug abuse and addiction are major public health challenges, increasingly complicated by the emergence of new psychoactive substances (NPS) among adolescents. This study characterized the demographic, clinical, and toxicological profiles of 17 adolescents (mean age 16.0 ± 1.22 years; 82.4% male) presented to the emergency department for acute voluntary drug intoxication and compared the exposure history, biochemical, multidrug immunoassay, and qualitative GC-MS findings on urine and plasma samples. Although no classical toxidrome was established, common manifestations included loss of consciousness (58.8%), vomiting (52.9%), and drowsiness (41.2%), with dehydration prominent in self-reported NPS exposure. GC-MS qualitative analysis identified classical drugs of abuse, such as MDMA, ketamine, and its metabolites, as well as mass spectra matching NPS, including a cathinone-type compound. In this patient series, matrix comparison indicated that urine offered superior detection capabilities over plasma, owing to prolonged detection windows and the identification of excreted metabolites. Overall, analytical toxicology provides valuable qualitative support for clinical assessments. Despite the small group limiting statistical generalizations, integrating clinical manifestations with qualitative GC-MS identification remains essential for optimizing emergency care in adolescent drug intoxications.

Rezumat

Abuzul și dependența de droguri reprezintă provocări majore de sănătate publică, fiind din ce în ce mai mult complicate de apariția noilor substanțe psihoactive (NPS) în rândul adolescenților. Acest studiu a caracterizat profilurile demografice, clinice și toxicologice pentru 17 adolescenți (vârsta medie 16,0 ± 1,22 ani; 82,4% bărbați) prezentați la departamentul de urgență pentru intoxicație acută voluntară cu droguri și a comparat istoricul de expunere, analizele biochimice, testele imunologice multidrog și rezultatele calitative GC-MS pe probele de urină și plasmă. Deși nu a fost stabilit un toxidrom clasic bine definit, manifestările clinice comune au inclus pierderea conștienței (58,8%), vărsăturile (52,9%) și somnolența (41,2%), iar deshidratarea a fost evidențiată în cazul expunerii auto-raportate la NPS. Analiza calitativă prin GC-MS a identificat substanțe de abuz clasice, precum MDMA, ketamina și metaboliții săi, precum și spectre de masă corespunzătoare NPS, incluzând un compus de tip catinonă. În această serie de pacienți, compararea matricilor a indicat faptul că urina a oferit capacități superioare de detecție comparativ cu plasma, datorită ferestrelor de detecție prelungite și identificării metaboliților excretați. În ansamblu, toxicologia analitică oferă un sprijin calitativ valoros pentru evaluările clinice. În pofida grupului mic care limitează generalizările statistice, integrarea manifestărilor clinice cu identificarea calitativă prin GC-MS rămâne esențială pentru optimizarea îngrijirii de urgență în cazul intoxicațiilor cu droguri la adolescenți.

Keywords: acute intoxication, adolescents, new psychoactive substances, qualitative GC-MS

Introduction

In recent years, the global prevalence of polysubstance use has increased markedly, representing a serious threat to public health and child safety, with adverse consequences that extend into adolescence. Early initiation of substance use disrupts normal development processes and can profoundly alter the trajectory of a child's future. Epidemiological data indicate that most individuals who consume drugs report their

first exposure during childhood. Such early involvement in substance use has been consistently associated with long-term physical, behavioural, social, and health-related risks (1, 2).

Adolescence is a critical developmental period characterized by vulnerability to substance use, which may lead to substance use disorders and psychiatric conditions such as depression, anxiety, conduct disorder, and suicidal behaviour (3).

Among young people aged 15 to 24, about 19% reported using cannabis in the past year, and about 10% using it in the past month (4, 5).

More recently, polysubstance use, involving two or more substances during the same episode, has been increasingly described among adolescents and may contribute to complex presentations in paediatric emergency departments.

In recent years, new psychoactive substances (NPSs) have become a growing concern. In 2024, the EU Early Warning System (EWS) on new psychoactive substances reached a significant milestone by formally notifying delta-9-THC-methylcarbonate as its 1000th monitored substance. Among the NPSs tracked by the system, nitazene opioids account for 23 substances, while 24 are semi-synthetic cannabinoids (6).

A significant increase in NPS-related incidents was also reported in Europe in 2024, a trend that has increasingly affected Romania as well, leading to a high number of medical emergencies.

The prevalence of other illicit drugs is also striking. In the European Union, surveys suggest that almost 2.5 million young adults (aged 15 - 34) used cocaine and 1.5 million used amphetamines in 2023. In addition, 2.2 million young adults reported using methylenedioxymethamphetamine (MDMA, ecstasy) during the same period (7).

In Romania, official data show that among individuals aged 15 - 34, the rates of lifetime use, last-year use, and last-month use of any illicit drug are higher than those in the general population, at 16.9%, 10%, and 6.6%, respectively. Among 16-year-old students, lifetime use of any illicit drug is 9.5%, with 9% reporting use in the past year. Notably, among those who used cannabis in the last 12 months, more than one-quarter also used ecstasy (29.3%) and cocaine (26.3%), and a significant proportion also reported the use of new psychoactive substances (NPSs) (16%) (8).

Analytical toxicology enables the identification of psychoactive substances and supports clinicians in adopting individualized and comprehensive treatment strategies, particularly for underage patients.

This study evaluated the demographic, clinical, and toxicological profiles of underage patients presenting to the Emergency Department with acute voluntary intoxication, focusing on the correlation between self-reported history, routine urine immunoassay screening, and qualitative GC-MS data.

The investigation was designed as a prospective case series. Urine immunoassay was used as an initial class-based screen, followed by qualitative GC-MS analysis of available urine and plasma samples. Concurrently, a methodological evaluation was performed to compare different urine sample preparation procedures (liquid-liquid *vs.* solid-phase extraction) and to assess thin-layer chromatography-densitometry (TLC-densitometry) as a cost-effective alternative screening tool with higher confidence than routine immunoassays.

The study also analysed the patterns of common clinical manifestations to identify potential symptom clustering and compared the practical utility of urine and plasma matrices for qualitative substance identification within this emergency care setting.

These findings can contribute to a better understanding of current adolescent substance abuse, offering a practical basis for optimizing targeted clinical strategies and emergency responses.

Materials and Methods

Study design and setting

This single-centre prospective case series included 17 patients aged between 13 and 17 years who presented to the emergency department of the Emergency Clinical Hospital for Children "Grigore Alexandrescu" in Bucharest, Romania, with symptoms suggesting acute intoxication caused by substances of abuse during a 12-month period (starting from February 2024).

Study population

The inclusion criteria required patients aged 13 to 17 years presenting to the emergency department with acute voluntary intoxication associated with the suspected use of alcohol, traditional illicit drugs, psychoactive medication, or unknown novel products. Additionally, formal informed consent and an adequate volume of biological samples (urine and plasma) for confirmatory toxicological analysis were mandatory for inclusion.

Cases involving a documented suicide attempt, therapeutic overdose, refusal of consent or insufficient biological sample volume were excluded.

No a priori sample-size calculation was performed due to the descriptive nature of this prospective case series. The selection process consecutively evaluated all adolescent presentations for suspected acute intoxication involving substances of abuse over the 12-month period. Driven by the increasing prevalence of novel drugs among youth, the analytical workflow was designed to specifically capture and characterize emergent exposures, including new psychoactive substances (NPS). Application of the inclusion and exclusion criteria resulted in a final study group of 17 patients who met all criteria for clinical and toxicological analysis.

The study was approved by the Hospital Ethics Committee (approval no. 5/12.02.2024) and conducted in accordance with the Declaration of Helsinki (DoH). Informed consent was systematically documented in the clinical records following the approved hospital procedure, including both parent or legal guardian consent and adolescent assent.

Data collected

The study group was thoroughly characterized based on demographic data and clinical and paraclinical assessments. The monitored indicators included age, sex, suspected substance, toxicological analysis of biological samples, and routine laboratory tests aimed at evaluating the changes associated with drug use.

Patient management and emergency treatment were guided by the self-reported exposure history, the clinical evaluation conducted by a paediatrician specialized in toxicology, and the results of toxicological tests. All cases were managed exclusively within the Emergency Department, where patients received immediate observation, symptomatic treatment, and stabilization before being safely discharged.

Biological samples

Urine and blood were taken from patients for toxicological analysis. Urine specimens were immediately frozen and stored at - 20°C. Blood was drawn into EDTA (ethylenediaminetetraacetic acid) vacutainers, then centrifuged to separate the plasma. The resulting plasma samples were also preserved at - 20°C until further analysis. All samples were collected immediately upon admission during the acute phase of intoxication.

Reagents and materials

n-hexane and ethyl acetate, used as solvents for extraction, were obtained from Merck Schuchardt OHG (Germany) and Sigma-Aldrich Chemie (Germany) respectively. Sodium hydroxide (p.a.), used for the preparation of a 2 M solution, was obtained from Lach-Ner (Czech Republic). Methanol (HPLC grade, Sigma-Aldrich Chemie) was used to reconstitute the residues.

For the TLC-densitometry analysis, the following reagents were used: methanol (HPLC grade Sigma-Aldrich Chemie), ethyl acetate (Sigma-Aldrich Chemie) and ammonia (Chimopar SA).

Two types of extraction cartridges were evaluated: HyperSep C18 (Thermo Fisher Scientific, USA) and Discovery DSC-8 (Supelco, USA).

Ready-to-use glass TLC plates (Silica gel 60 F254, 20 × 10 cm, layer thickness 200 μm) containing a fluorescent indicator at 254 nm were obtained from Merck KGaA (Germany).

Toxicological screening (immunoassay)

The screening was performed on urine samples using a rapid multi-drug urine test (Triage – Tox Drug Screen, QuidelOrtho, USA).

This screening test provides simultaneous qualitative determination of drugs of abuse and/or their primary metabolites in urine above established threshold concentrations. The panel evaluated nine separate tests across eight different drug classes, configured as follows: amphetamines (AMP, 500 ng/mL), methamphetamines (mAMP, 500 ng/mL), barbiturates (BAR, 200 ng/mL), benzodiazepines (BZO, 200 ng/mL), cocaine (COC, 150 ng/mL), the methadone metabolite (EDDP, 100 ng/mL), opiates (OPI, 300 ng/mL), cannabinoids (THC, 50 ng/mL), and tricyclic antidepressants (TCA, 1000 ng/mL).

Sample preparation/extraction

Liquid-liquid extraction procedure

For the extraction of the analytes of interest from urine and plasma samples, a liquid-liquid extraction method was applied using a mixture of organic solvents (n hexane: ethyl acetate, 9:1, v/v) under alkaline conditions (pH 10). Specifically, 1 mL of plasma or 5 mL of urine were alkalized with 2 M NaOH to pH 10 and subsequently subjected to extraction by vortex agitation for 15 minutes, followed by centrifugation for 15 minutes at 3500 rpm and 15°C. The organic upper layer was then separated and evaporated under a nitrogen stream at 40°C. The resulting residues were reconstituted in 100 μL of methanol. A volume of 1 μL of the final extract was injected into the GC-MS system.

Solid-phase extraction procedure

The urine sample (1 mL) was alkalized using 2 M NaOH. The cartridge was conditioned by washing with methanol and water before the sample was slowly passed through the stationary phase. Subsequently, the analytes were eluted with 1 mL of methanol. The eluate was evaporated to dryness under gentle stream of nitrogen at 40°C, and the resulting residue was reconstituted in 0.5 mL of methanol for subsequent analysis.

Instrumentation and experimental conditions

GC-MS conditions

A Focus gas chromatograph from Thermo Electron Corporation, coupled with a DSQII mass spectrometer equipped with a quadrupole and electron ionization (EI) source, was utilized in the analysis. The separation of compounds was carried out using a TR-5MS capillary column (stationary phase: 5% phenyl polysilphenylene siloxane) with dimensions of 30 m × 0.25 mm × 0.25 μm film thickness. Data acquisition and processing were performed using Xcalibur software version 1.2, integrated with the NIST 02 mass spectral library.

The analyses were performed under the following temperature gradient program: an initial column temperature of 100°C was held constant for 2 minutes, then it was increased by 15°C/minute to 160°C, held for 1 minute, followed by increase by 15°C/minute to 280°C, held for 5 minutes. The carrier gas (helium) flow rate was set at 1 mL/min. Mass spectrometer parameters: solvent delay: 6 min; mass range: m/z 20 - 500; positive ionization, injector temperature 200°C;

working in split mode, transfer line temperature 250°C, ionization source temperature 200°C.

The acquisition was performed in full scan.

As reference standards were unavailable, compound identification was performed via qualitative analysis based on mass spectral features, including characteristic fragments and fragmentation patterns. Identification was established by systematically correlating the obtained full-scan mass spectra and diagnostic fragments with validated data from current scientific literature and reference databases, such as the National Institute of Standards and Technology (NIST) library (9-13).

TLC-densitometry

Chromatographic development was performed on ready-to-use glass plates utilizing vertical developing chambers with lids. Samples were applied in bands under a nitrogen stream using a Linomat 5 semiautomatic sample applicator equipped with a 100 µL Hamilton micro-syringe (Hamilton Company, USA; CAMAG, Switzerland). The mobile phase consisted of ethyl acetate, methanol, and ammonia solution (25%) in a ratio of 85:10:5 (v/v/v). Prior to plate insertion, chamber saturation with mobile phase vapours was performed for 20 min at room temperature. Chromatographic development was carried out using the ascending technique. Once the solvent front reached the designated height, the plate was removed and thoroughly dried under a fume hood.

Densitometric evaluation was performed using a CAMAG TLC Scanner 3 system equipped with a dark chamber and UV lamps, controlled by winCATS Manager software (version 1.4.4, CAMAG, Switzerland). The instrument was operated in absorbance mode. Qualitative evaluation was carried out at a detection wavelength of 254 nm. Subsequently, *in situ* UV-Vis spectra were recorded for each band within the spectral range of 200 - 400 nm to support an assessment of analyte identity and spectral consistency.

Biochemical and electrolyte analysis

Biochemical and electrolyte parameters were evaluated descriptively to characterize the laboratory findings observed in the study group.

Biochemical and electrolyte tests (including sodium, potassium, chloride, urea, creatinine, and blood glucose concentrations) were performed using commercial diagnostic kits on automated analysers. Specifically, analysis was performed using an AU480 Chemistry Analyzer equipped with an Ion-Selective Electrode (ISE) module (Beckman Coulter, USA) and XN-1500 and XN-1000 automated haematology analysers (Sysmex Corporation, Japan).

Statistical analysis

Descriptive statistics were the primary analytical approach. Categorical variables are presented as counts and percentages, and age and biochemical variables as mean ± standard deviation, with ranges or numbers outside the laboratory reference interval where available.

One two-sided Fisher's exact test was used as a secondary exploratory comparison between current-episode polysubstance exposure and single-substance exposure with respect to loss of consciousness. Current-episode polysubstance exposure was defined as more than one suspected or declared substance in the same episode; a history of chronic use alone was not counted as a concurrent exposure. A p value < 0.05 was used as the nominal threshold.

All inferential results were considered exploratory and underpowered, and a non-significant p value was not interpreted as evidence of no relationship. Calculations were performed using Python 3.13.5 and SciPy 1.17.0.

Results and Discussion

Results

The characterization of the study group is summarized in Table I.

Table I

Characterization of the study group

Parameter	Group characteristics
Total cases	17
Sex	Males: 14 (82.4%); Females: 3 (17.6%)
Male/Female ratio	4.67 (14/3)
Age (years) (mean ± SD)	Total: 16.00 ± 1.22 (min 13; max 17); Males: 16.21 ± 1.05; Females: 15.00 ± 1.73
Age range/categories	13 years: 1 (5.9%); 14 years: 1 (5.9%); 15 years: 3 (17.6%); 16 years: 4 (23.5%); 17 years: 8 (47.1%)
Residential environment	Urban 82.35% (14/17); rural 17.65% (3/17)
Exposure pattern/history	Single-substance exposure in the current episode: 8/17 (47.1%); polysubstance exposure: 9/17 (52.9%); chronic use: 9/17 (52.9%); occasional use: 5/17 (29.4%); unknown: 3/17 (17.6%)

The male-to-female ratio was 4.67, showing a clear predominance of male patients. The average age of the studied group was 16 ± 1.22 years (16.21 ± 1.05 years for males and 15.00 ± 1.73 years for females), with an age range of 13 to 17 years. The 17-year-old age category was the most represented, accounting for 47.1% of cases. Clinical evaluations revealed both single-substance and polysubstance abuse, reflecting the high variability of substance exposure among adolescents presenting to the emergency department with acute voluntary intoxication.

An individual profile was established for each patient, summarizing demographic characteristics (sex, age, and residential area), the suspected substances used, and the clinical manifestations observed following substance exposure, as summarized in Table II.

Table II

Individual patient profile: demographic characteristics, suspected/declared exposure and clinical manifestations

Patient code	Sex (F/M)	Age (years)	Residence (Urban/Rural)	Suspected/ declared substance(s) used*	Clinical manifestations
1	M	17	Urban	THC, benzodiazepines, opioids, amphetamines, methamphetamines	Hallucinations, paresthesias, loss of consciousness, tonic-clonic contractions, dehydration
2	F	13	Urban	THC, new psychoactive substances	Vomiting, dehydration, loss of consciousness
3	M	15	Urban	THC	Vomiting, balance disorders, dehydration
4	F	16	Urban	THC, alcohol	Vomiting, loss of consciousness, balance disorders
5	M	16	Urban	Ketamine, clonazepam	Drowsiness, euphoria, agitation
6	M	16	Rural	Unidentified cigarette (smoked)	Seizures
7	M	17	Urban	Alcohol, THC	Vomiting, balance disorders, loss of consciousness, drowsiness
8	M	17	Urban	Alcohol	Vomiting, loss of consciousness
9	M	17	Urban	Alcohol, chronic user of psychoactive substances	Vomiting, balance disorders, dehydration, loss of consciousness
10	M	17	Urban	Alcohol, benzodiazepines, THC	Vomiting, balance disorders
11	F	16	Urban	Chronic user of psychoactive substances, benzodiazepines	Drowsiness, balance disorders
12	M	15	Urban	THC	Vomiting, tremors, dizziness
13	M	17	Urban	Alcohol, THC	Drowsiness, vomiting
14	M	17	Urban	Alcohol, white powder (smoked)	Drowsiness, agitation, loss of consciousness
15	M	15	Rural	White powder (smoked)	Drowsiness, loss of consciousness
16	M	17	Urban	Alcohol, unknown substance	Drowsiness, loss of consciousness
17	M	14	Rural	Unidentified cigarette (smoked)	Dizziness, loss of consciousness

*Suspected or declared substances were extracted from the medical record and exposure history and should not be interpreted as analytically confirmed

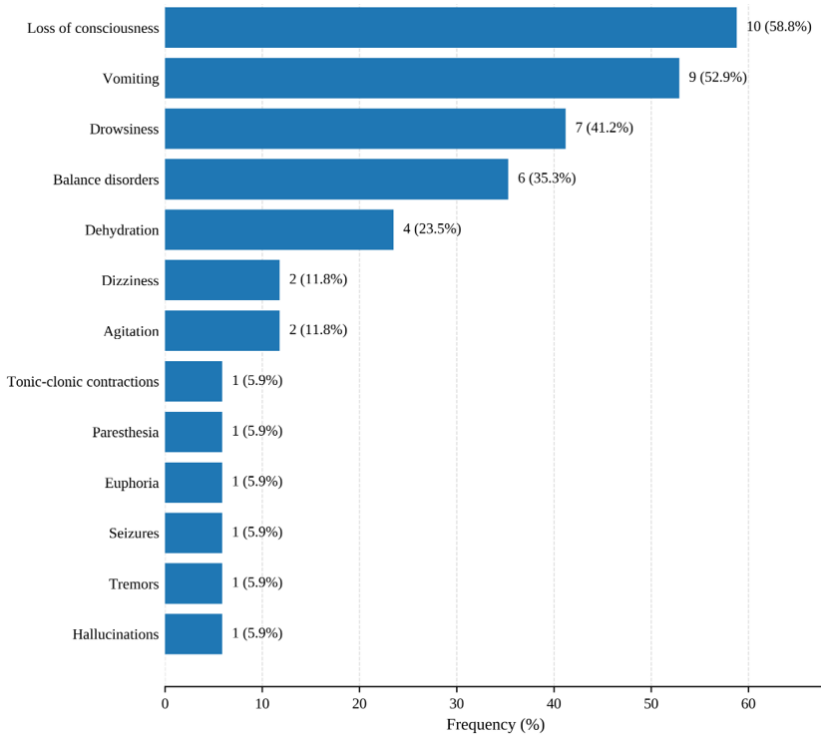

Figure 1.

Frequency of clinical manifestations among patients (n = 17). Values are shown as n (%)

Analysis of the 17 cases of acute intoxication reveals several distinct characteristics regarding the substances involved, patient profiles, and associated clinical manifestations.

The most frequently suspected substances were THC and alcohol, each involved in 8 cases, followed by benzodiazepines in 4 cases. Using a current-episode definition based on Table I, polysubstance exposure was recorded in 9/17 patients (52.9%), and single-substance exposure in 8/17 (47.1%).

Clinical manifestations are summarized in Figure 1. The most frequent manifestations were loss of consciousness (10/17; 58.8%), vomiting (9/17; 52.9%), drowsiness (7/17; 41.2%), and balance disorders (6/17; 35.3%). Dehydration occurred in 4/17 (23.5%), while dizziness and agitation each occurred in 2/17 (11.8%).

Comparisons by sex and residence were omitted because the groups were highly unbalanced and the study was not powered to evaluate these associations.

Loss of consciousness occurred in 6/9 patients (66.7%) with current-episode polysubstance exposure and in 4/8 (50.0%) with single-substance exposure. The two-sided Fisher's exact test was not statistically significant ($p = 0.637$). This exploratory, underpowered result is inconclusive and does not demonstrate the absence of a clinical association.

Urine immunoassay results were available for 15/17 patients. Available urine and plasma extracts were subsequently evaluated by qualitative GC-MS. The findings are reported as identifications based on mass spectral data alignment with the published literature and reference databases.

The case-level comparison of suspected exposure, urine immunoassay and qualitative GC-MS findings are presented in Table III.

The discrepancies between the initial immunoassay screening and the qualitative GC-MS findings reflect differences in analytical principles, specific detection thresholds, and target analytes. The positive screens for amphetamines and methamphetamines are consistent with immunoassay cross-reactivity driven by the presence of MDMA, an amphetamine-derivative structurally flagged by the rapid tests. Conversely, the non-detection of THC by GC-MS is directly explained by the lack of a derivatization step in the extraction workflow, which is necessary to volatilize cannabinoids for gas chromatographic analysis. Regarding the benzodiazepine-positive screens that remained unconfirmed by GC-MS, these discordances likely stem from antibody cross-reactivity or the analytical limitation of extracting highly polar glucuronide-conjugated metabolites without a prior enzymatic hydrolysis step. Finally, emerging compounds like ketamine and synthetic cathinones were completely missing from the routine immunoassay panel design, explaining why they were only captured by the untargeted GC-MS analysis.

Table III

Patient-level comparison of suspected/declared exposure, urine immunoassay and qualitative GC–MS findings

Patient	Suspected/declared exposure	Urine immunoassay	Urine qualitative GC-MS	Plasma qualitative GC-MS
1	THC, benzodiazepines, opioids, amphetamines, methamphetamines	Positive: THC, AMP, mAMP, BZO	MDMA, ketamine	Not available
2	THC, new psychoactive substances	Negative	MDMA	Not available
3	THC	Positive: THC	No reportable analyte	Cathinone-type signal
4	THC, alcohol	Negative	No reportable analyte	No reportable analyte
5	Ketamine, clonazepam	Negative	Ketamine, norketamine	No reportable analyte
6	Unidentified smoked cigarette	Negative	No reportable analyte	No reportable analyte
7	Alcohol, THC	Positive: THC	No reportable analyte	No reportable analyte
8	Alcohol	Not available	Not available	Not available
9	Alcohol; history of chronic psychoactive-substance use	Positive: THC	No reportable analyte	No reportable analyte
10	Alcohol, benzodiazepines, THC	Positive: THC	Venlafaxine, non-target finding	No reportable analyte
11	Benzodiazepines; history of chronic psychoactive-substance use	Positive: BZO	No reportable analyte	No reportable analyte
12	THC	Positive: THC	No reportable analyte	No reportable analyte
13	Alcohol, THC	Negative	No reportable analyte	No reportable analyte
14	Alcohol, unidentified white powder	Negative	No reportable analyte	No reportable analyte
15	Unidentified white powder	Negative	No reportable analyte	No reportable analyte
16	Alcohol, unknown substance	Not available	Not available	Not available
17	Unidentified smoked cigarette	Negative	No reportable analyte	No reportable analyte

AMP, amphetamines; BZO, benzodiazepines; mAMP, methamphetamines; THC, cannabinoids. "No reportable analyte" indicates that no analyte was reported in the available case-level result; "not available" indicates that the specific analysis was not performed. All GC-MS findings are qualitative

The present study is qualitative, relying on the GC-MS analysis method which allows identification based on the mass spectra of each individual analyte. The GC-MS technique (electron ionization, 70 eV) remains

the gold standard in analytical toxicology due to the uniqueness and reproducibility of the mass spectra generated through molecular fragmentation, providing highly specific fragmentation profiles for compound identification. In untargeted screening analyses of biological matrices, to ensure analytical reliability, identification was carried out through spectral interpretation and systematic comparison of the fragmentation patterns against validated data from current scientific literature.

Qualitative GC-MS analysis achieved unambiguous identification of MDMA in two urine samples and ketamine in two urine samples, while providing a class-level assignment for a cathinone-type compound in one plasma sample. These findings illustrate the critical complementary information provided by mass spectrometry for compounds that are completely unrepresented in routine immunoassay panels.

MDMA was identified in the urine samples of Patients 1 and 2 at a retention time (tR) of 5.18 min (Figure 2), with key ions at m/z 58 and 135 confirming the presence of the compound. Furthermore, ketamine was identified in the urine of Patient 1 (unreported exposure); its presence was consistently verified through cross-case spectral matching and retention time correlation, as it exhibited a peak at the same retention time and a mass spectrum identical to that of Patient 5, whose ketamine exposure was clinically declared. Additionally, the primary metabolite norketamine was identified in only one of these two positive urine samples, further characterizing the metabolic profile.

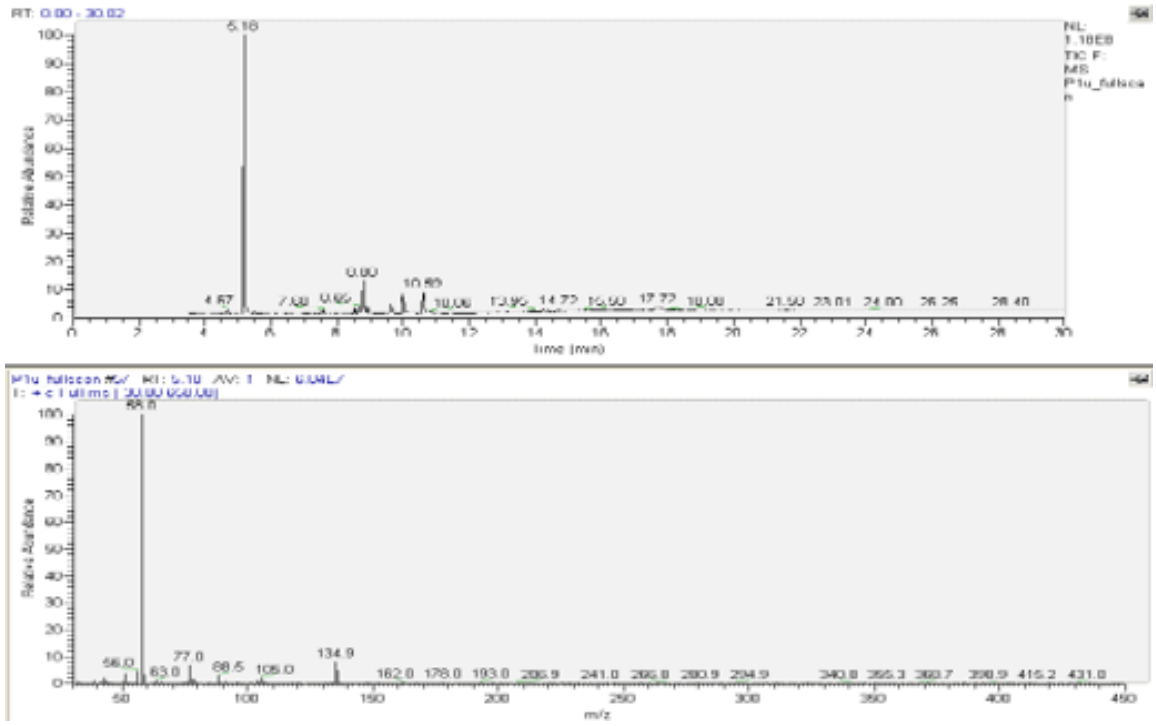

Figure 2.

Typical chromatogram and MS spectrum for the qualitative analysis of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) in the urine sample of Patient 1 (t_R = 5.18 min)

For Patient 1, the urine sample was further analysed using thin-layer chromatography-densitometry to complement the GC-MS findings. Two extraction techniques-liquid-liquid extraction and solid-phase extraction (using C8 and C18 cartridges) – were compared. Under all extraction conditions, chromatographic analysis revealed a reproducible spot at R_f = 0.28.

In the absence of a commercial analytical standard, the *in situ* UV absorption spectrum of the observed spot was compared with reference spectra reported in the literature. The highly consistent and superimposable spectral profiles obtained across all three extraction procedures confirmed the reproducibility of the method, providing independent data that corroborated the identification of MDMA obtained through the GC-MS (Figure 3).

For Patient 5, two distinct chromatographic peaks were identified in the urine sample: one at tR = 8.83 min, with characteristic ions at m/z 180 and 152, and another at t_R = 8.53 min, with a major fragment ion at m/z 166. These spectral data achieved clear identification of ketamine and its primary metabolite, norketamine, respectively.

For Patient 1, an additional chromatographic peak was observed at (t_R = 8.80 min.), displaying identical mass spectral ions at m/z 180 and 152. Despite the absence of a self-reported history or a reference

standard, this substance was identified as ketamine based on its precise spectral and chromatographic alignment with the confirmed positive sample from Patient 5 (Figure 4).

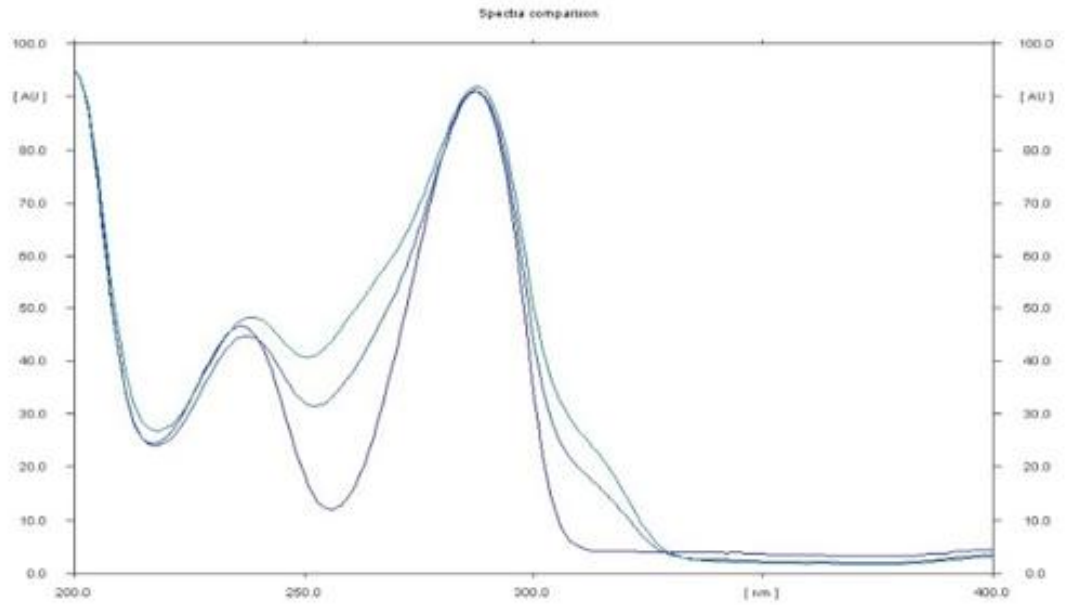

Figure 3.

Overlapping *in situ* UV spectra of the MDMA chromatographic spot obtained from the urine sample of Patient 1, comparing liquid–liquid extraction and solid-phase extraction (C8 and C18 cartridges)

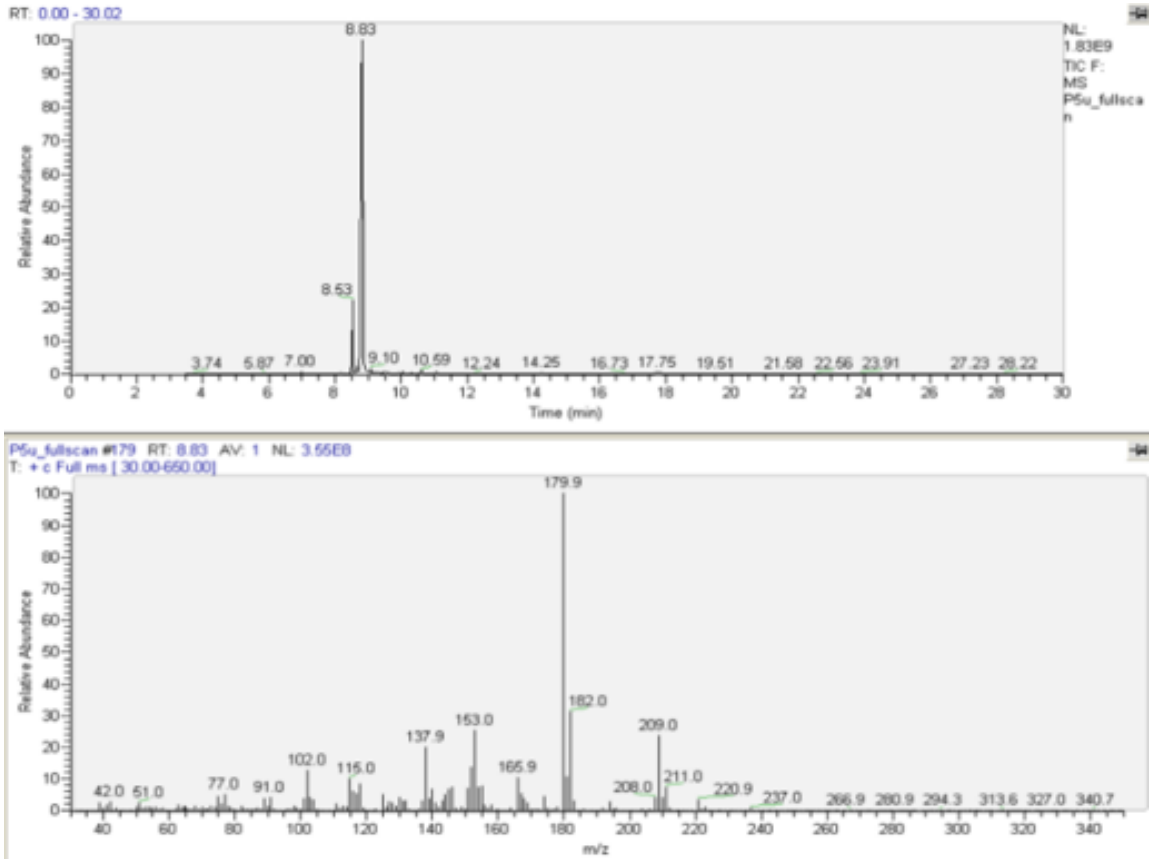

Figure 4.

Typical chromatogram and MS spectrum for the qualitative analysis of ketamine in the urine sample of Patient 5 (t_R = 8.83 min)

While no drugs of abuse were identified in the urine sample of Patient 10, venlafaxine, an antidepressant, was reported as a non-target finding (characteristic ions: m/z 58, 134, 179).

In the absence of sample derivatization, THC could not be identified in the urine samples collected from patients who had a positive rapid test for THC.

Regarding blood biochemistry and electrolytes, the results are summarized in Table IV.

For biochemical and electrolyte parameters, the percentage of those who are outside the normal range is as follows: 17.65% for sodium, 11.76% for potassium, 11.76% for urea, 23.53% for creatinine, 29.41% for chloride, and 41.18% for blood sugar.

Table IV

Average of biochemical and electrolytes test values compared to normal values

Biochemical parameters	Mean ± standard deviation	Laboratory reference interval	Outside interval, n/N
Creatinine (mg/dL)	0.65 ± 0.17	0.6 - 1.2	4/17
Urea (mg/dL)	23.35 ± 5.78	15 - 40	2/17
Blood sugar (mg/dL)	90.24 ± 16.80	70 - 99	7/17
Sodium (mmol/L)	136.82 ± 2.67	135 - 145	3/17
Potassium (mmol/L)	3.82 ± 0.33	3.5 - 5.0	2/17
Chlorine (mmol/L)	104.18 ± 2.79	98 - 106	5/17

Several biochemical and electrolyte values were outside the laboratory reference intervals; however, because the study group was small, individual-level values were not available for all planned comparisons, and polysubstance exposure was frequent, these abnormalities could not be statistically linked to specific substances.

The present study included 17 adolescents who presented to the emergency department with acute intoxication involving various substances of abuse. Qualitative GC-MS analyses of urine and plasma were used to obtain analytical findings, in accordance with methodological approaches described in previous studies (14, 15). This complementary approach may be useful when clinical manifestations are nonspecific and routine screening panels have limited analyte coverage.

The findings obtained in this study highlight several important aspects regarding patterns of substance use and the analytical challenges encountered in emergency settings. The study revealed a male-to-female ratio of 14:3, consistent with global trends showing higher prevalence among males. Therefore, the predominance of male adolescents aligns with previously reported trends indicating earlier initiation, higher risk-taking behaviour, and greater likelihood of experimenting with illicit substances among males (16). This distribution, although based on a relatively small cohort, reflects broader epidemiological observations documented in adolescent substance-use research.

The study group had a mean age of 16 ± 1.22 years and an age range of 13 - 17 years, corresponding to a developmental stage in which experimentation with psychoactive substances may become more common. This age profile is consistent with adolescent substance-use literature describing late adolescence as a high-risk period for initiation or escalation (17). The present 12-month case series was not designed to evaluate annual or seasonal trends, and the dataset did not contain sufficiently complete information on the location of substance use to support a case-level analysis.

Both single-substance and polysubstance exposure were recorded. Loss of consciousness was numerically more frequent in polysubstance cases, but the exploratory Fisher's exact test was inconclusive because of the small sample size and wide uncertainty.

Polysubstance exposure was recorded in 9/17 cases (52.9%), frequently involving alcohol. Given the descriptive nature of this study group, no formal statistical associations regarding sex, residence, substance dependence, or clinical severity were inferred from the data.

Overall, these results underline the vulnerability of adolescents in this age range and the need for targeted preventive and educational interventions.

The most frequent clinical manifestations were vomiting, loss of consciousness, drowsiness, and balance disorders. Because polysubstance use was common, these manifestations could not be attributed reliably to individual substances.

Psychomotor agitation and seizure-related manifestations were uncommon and occurred in heterogeneous exposure contexts. The small number of events did not permit a substance-specific toxidrome to be defined.

THC and alcohol were each suspected in 8/17 cases (47.1%), followed by benzodiazepines in 4/17 cases (23.5%). Current-episode polysubstance exposure was recorded in 9/17 patients (52.9%). NPS were suggested by case history or by qualitative toxicological findings, but the small single-centre study group did not permit estimates of prevalence, urban–rural differences, or substance-specific risk.

These observations can be considered alongside national and international reports describing cannabis, NPS, opioids, and hallucinogens among adolescents (8, 17).

Among the qualitative GC-MS findings, MDMA was reported in two urine samples, ketamine in two urine samples (with norketamine identified in one), and a

cathinone-type compound in one plasma sample. These qualitative findings illustrate the practical utility of GC-MS in identifying substances that remain unconfirmed or undetected by routine immunoassay panels, thereby supporting clinical management.

Internationally, the NPS landscape has evolved considerably. The EUDA reported a decline in newly detected substances in recent years, attributed to stricter legislative measures (16). Similarly, the United Nations Office on Drugs and Crime (UNODC) recorded in 2022 the lowest number of new NPS identified in more than a decade, suggesting that control strategies are producing positive effects (18). The European Drug Report 2025 also emphasized legislative policies designed to limit the emergence of new psychoactive substances on the European market.

The present 12-month case series was not designed to assess long-term temporal variations, estimate the epidemiological incidence of cannabis or NPS exposure, or evaluate the direct impacts of legislation and market accessibility. Given the compact sample size and the absence of a multi-year comparator, annual fluctuations, sex-based disparities, and broader temporal variations could not be inferred from the data; continuous, large-scale surveillance reports remain necessary for that purpose (19, 20). While MDMA remains highly relevant in toxicological reports of substance use among youth, particularly in urban nightlife settings (3, 17), these localized findings serve as a descriptive clinical baseline rather than evidence of national prevalence patterns.

At present, no standardized urine or blood screening assays exist for the specific detection of NPS, as highlighted in the latest EUDA reports and also in scientific literature (3, 21-24). These substances may also produce false-positive results in routine immunoassay urine screens; for instance, synthetic cathinones can cross-react with methamphetamine immunoassays due to structural similarities (24). Therefore, the use of GC-MS remains clinically relevant for improving toxicological identification and supporting evidence-based management protocols in paediatric emergency settings. In this study, qualitative GC-MS provided essential complementary data, delivering substance identification based on specific mass fragmentation patterns in cases where routine screening panels lacked coverage.

This study, based on cases presenting to a paediatric toxicology centre, provides a focused overview of acute intoxications in adolescents, highlighting the substances involved, clinical manifestations, and available toxicological findings. By integrating demographic, clinical, and analytical data, the study strengthens current understanding of adolescent intoxication characteristics and supports the development of more accurate identification strategies and evidence-based management protocols in emergency settings.

Because the study includes only adolescents who presented to the emergency department-excluding those managed in other healthcare settings or outside the medical system-the sample reflects acute, clinically significant intoxications, but does not capture the full spectrum of adolescent substance use in Romania.

On a laboratory level, the investigation encountered a further limitation regarding matrix-dependent detection, as successful identification was more frequently accomplished in urine, while plasma samples presented higher analytical challenges and a lower identification rate. This discrepancy is primarily attributed to the short half-life and rapid clearance of psychoactive substances from the bloodstream, resulting in trace levels in plasma, compared to the accumulation of compounds and metabolites in urine which extends the detection window. Within this framework, GC-MS was utilized to achieve qualitative identification. For the detected compounds, identification relied on the detailed interpretation of unique fragmentation pathways and comparisons against validated scientific literature. This approach proved particularly essential for aligning mass spectra with literature profiles to propose a class-level assignment when available data did not permit a specific molecule-level identification, as observed with the synthetic cathinone-type signals.

Despite these combined clinical and analytical limitations, the findings offer meaningful contributions for the Romanian emergency care framework by emphasizing the importance of precise toxicological confirmation, targeted symptomatic management, and coordinated multidisciplinary care for adolescents experiencing acute intoxication.

Conclusions

Substance-related intoxication represents a critical public health issue among underage populations. Data from this single-centre case series show a frequent occurrence of alcohol exposure, alongside cannabis and benzodiazepines detected via multidrug immunoassay screening. Although this high frequency of cannabis mirrors general epidemiological trends, the small sample size precludes broader statistical generalization.

Clinical manifestations presented a consistent core of common features across the studied group, dominated by loss of consciousness, vomiting, and drowsiness. A distinct, substance-specific toxidrome could not be established, primarily due to the overlapping clinical effects induced by multiple concurrent exposures.

Analytical toxicology provided valuable qualitative support. Within this framework, GC–MS confirmed the presence of classical drugs such as MDMA and ketamine – along with its excreted metabolites – and

identified signals compatible with novel psychoactive substances, including a cathinone-type compound. Complementing these findings, TLC-densitometry analysis demonstrated highly reproducible and superimposable *in situ* UV spectra across distinct extraction techniques, thereby corroborating the detection of MDMA.

From an analytical perspective, urine indicated a distinct advantage over plasma for qualitative investigation due to prolonged detection windows and the presence of excreted metabolites. On the other hand, the non-detection of THC by GC-MS is directly explained by the lack of a derivatization step in the extraction workflow.

Taken together, the findings support an integrated interpretation of clinical assessment, exposure history, urine immunoassay, and qualitative GC-MS. This integrated approach optimizes therapeutic strategies and significantly enhances emergency care management for underage patients presenting with acute drug intoxication.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

The other data generated in the present study may be requested from the corresponding author.

Ethics approval and consent to participate

All experimental procedures were approved by "Grigore Alexandrescu" Children's Clinical Hospital Ethics Committee (approval No. 5/2024). Informed consent was obtained from all patients included in the study.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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