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# Evaluation of illicit drugs in pediatric emergency patients using LC-MS/MS

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## Abstract

**Introduction:** Illicit drug use is an ever-increasing problem all over the world also reflected in emergency services as drug-induced toxicities. There is limited data about illicit drugs that pose pediatric emergency cases in our country which of most are based on immunochemical screening test results that are prone to false positivity and negativity or insufficient for detection of some drugs.

**Material-Method:** We established validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods for 39 illicit drug analytes and used them to evaluate blood and urine samples of pediatric emergency patients (n=50, mean age: 15.6 y, 72% male; %28 female) along with an easy and short sample preparation step.

**Results:** Acceptable method validation results by means of linearity, repeatability, accuracy, sensitivity, and selectivity were achieved. In analyzes using this validated method illicit drugs were detected in 60% of patients (of these 71.4% were male). Forty percent of patients showed mixed drugs. Amphetamine-type drugs and synthetic cathinones were the most found illegal drugs in samples (12 as a single drug and 12 as a mixed drug component).

**Conclusions:** This study was the first to use LC-MS/MS for the determination of 39 illicit drug analytes in pediatric emergency patients in our country. LC-MS/MS is a reliable and sensitive tool for the evaluation of drug-suspected emergency patients. In this way, illicit substance use profiles that cause urgent health problems can be determined accurately, and current findings can be shared through a national network, informing physicians and toxicologists as well as authorities who make regulations regarding illicit substance policy.

**Keywords:** Illicit drug, Pediatric emergency, Liquid chromatography-tandem mass spectrometry, Method development

## Introduction

Drug abuse among children and adolescents causes deaths and urgent health problems by both direct overdose and indirectly drug-related diseases, accidents, violence and suicide. The 2018 National Drug Report revealed that illegal drug seizures increased 214% for heroin, 20% for cannabis, 75% for cocaine, 128% for ecstasy, 162% for methamphetamine, and 53% for synthetic cannabinoids in comparison to the previous year[1]. According to the European Drug Report it is estimated that one in four students, aged between 15-16 years, uses illegal drugs, since 2011. Generally, 7% of students report multiple illegal substances for lifetime use[2]. The European School Survey Project on Alcohol and Other Drugs (ESPAD) conducted a survey on 96043 students, with the average age of 15.8, in 35 countries and found that, alcohol and tobacco use (80% and 46%, respectively) were more frequently than cannabis but cannabis users also took alcohol (96%) and tobacco (91%) simultaneously[3]. Beside these data, the emergence of synthetic cannabinoids is an even greater question. Although they bind to the same receptors as cannabis (CB1 and CB2) they show a full agonist effect causing more serious acute health problems than cannabis[4]. Cocaine, amphetamine and ecstasy are common illegal stimulant drugs used among youths; while piperazines and synthetic cathinones were less reported in the past but became more popular in recent years. Stimulants lead to serious health consequences such as cardiovascular, neurological, mental, and infectious diseases or deaths[5-9].

According to the Drug Use Survey for the Young Population, conducted by the Turkish Drug Addiction Monitoring Centre (TUBIM), drug use frequency was found as 1.5% with the average age of 13 years10. The number of deaths attributable to direct substance abuse was 500 in 2013, rising to over 900 in 2017 in the general population, while 10% of deaths included 15-19 years old individuals. The most commonly used drugs were cannabis and synthetic cannabinoids, amphetamines (mostly ecstasy), opiates (heroin, morphine, codeine) and cocaine.

There are fairly little reports about the use of illicit drug testing in poisoned patients and those trials have a number of constraints. One of the most notable is that almost all were carried out with immunochemical test kits, which

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can only determine a limited number of substances and are well-known to have a high false-positive and false-negative ratio[11]. Mass spectrometry techniques such as gas and liquid chromatography - tandem mass spectrometry (e.g., GC-MS/MS and LC-MS/MS) have more advantages in terms of detecting definitively hundreds of toxicants/drugs by a single sample analysis with high accuracy. LC-MS/MS can establish exact superiority in an emergency by shortening the time for extraction because GC-MS requires additional derivatization steps which lengthen the time[12].

In this study, we aimed to establish validated LC-MS/MS methods for the analysis of illicit drugs and then, for the first time in our country, to evaluate the biological samples (blood and urine) of patients admitted to pediatric emergency service in order to determine the prevalent drug related toxicity cases.

## Material and methods

### *Chemicals*

All reference standard materials including opiates, amphetamines, cocaine, cannabis, synthetic cannabinoids, synthetic cathinones, and internal standards (IS) were purchased from Lipomed (Arlesheim, Switzerland) (Table 1). High purity acetonitrile, methanol and isopropyl alcohol were purchased from Merck (Darmstadt, Germany); ammonium formate, ethyl acetate, dichloromethane and beta-glucuronidase (85000i, Helix pomatia), were purchased from Sigma Aldrich (Taufkirchen, Germany); ultrapure water was produced by MP Minipure water system (MES Medical, Turkey).

### *Instrumental Conditions*

We used ultra-high-pressure liquid chromatography combined with a tandem mass spectrometry (LC-MS/MS 8030-plus, Shimadzu, Japan) with an electrospray ionization (ESI) unit employed in positive mode. The chromatographic separation was performed using a Shim-Pack Column FCODS (150 mm x 2.0 mm, 3 µm, Shimadzu). The aqueous mobile phase consisted of 10 mM ammonium formate in water, while the organic mobile phase consisted of methanol. The column oven temperature was maintained at 40ºC, the flow rate was 0.4 mL/min and the injection volume was 10 µL. The flow rates of nebulizing and drying gas were 1.5 L/min and 10 L/min, respectively. The gradient flow program was optimized for each of three groups of drugs; common drugs (CD), synthetic cannabinoids (SCb), and synthetic cathinones (SCt), with the total analyzing time of 15, 22, and 12 min respectively (Table 2). Multiple reaction monitoring (MRM) method parameters were optimized by direct injection of standard solutions. The most abundant MRM transition was selected for quantification along with qualifier ions and the retention times (RT) were determined for schedule time of all substances (Table 3).

**Table 1:** Sensitivity, recovery and r2 values of analytes

| Analyte | LOD (ng/mL) | LOQ (ng/mL) | Recovery (%) | $r^2$ |
|---|---|---|---|---|
| Synthetic cannabinoids (SCb) | | | | 1 – 20 ng/mL |
| JWH-018-N-pentanoic acid | 0.87 | 2.89 | 98.02 | 0.9985 |
| UR-144-N-pentanoic acid | 0.7 | 2.33 | 100.9 | 0.9994 |
| JWH-018-N-5-OH-pentyl | 1.07 | 3.56 | 100.3 | 0.9994 |
| JWH-073-N-2-OH-butyl | 0.9 | 2.99 | 98.93 | 0.9990 |
| JWH-200 | 1.03 | 3.44 | 101.2 | 0.9994 |
| UR-144-N-5-OH-pentyl | 1.06 | 3.53 | 100.2 | 0.9974 |
| AM-2201 | 0.71 | 2.36 | 102.3 | 0.9974 |
| RCS-4 | 0.9 | 2.99 | 100.2 | 0.9989 |
| JWH-250 | 1.03 | 3.45 | 102.7 | 0.9993 |
| XLR-11 | 0.82 | 2.74 | 101.8 | 0.9991 |
| JWH-073 | 0.86 | 2.86 | 99.28 | 0.9999 |
| JWH-018 | 0.9 | 2.98 | 101.2 | 0.9997 |
| JWH-081 | 0.8 | 2.66 | 102.2 | 0.9997 |
| UR-144 | 0.7 | 2.32 | 101.2 | 0.9991 |
| JWH-122 | 0.66 | 2.19 | 101.4 | 0.9991 |
| Synthetic cathinones (SCt) | | | | |
| Methedrone | 0.72 | 2.41 | 94,41 | 0.9972 |
| A-PVP | 0.7 | 2.35 | 96,51 | 0.9963 |
| Buphedrone | 0.48 | 1.61 | 85.5 | 0.9971 |
| Bupropion | 1,8 | 5,98 | 96,96 | 0,9962 |
| Mephedrone | 0.8 | 2.68 | 100.1 | 0.997 |
| d.l-4-EMC | 0.97 | 3.24 | 95.89 | 0.9966 |
| Common drugs (CD) | | | | 1 – 400 ng/mL |
| Amphetamine | 0.61 | 2.03 | 101.2 | 0.992 |
| MBDB | 0.41 | 1.36 | 101.3 | 0.9955 |
| MDA | 0.85 | 2.85 | 99.2 | 0.9939 |
| MDEA | 0.72 | 2.4 | 102 | 0.9981 |
| MDMA | 0.71 | 2.37 | 100.8 | 0.9946 |
| Methamphetamine | 0.57 | 1.89 | 100.1 | 0.9965 |
| Codeine-6-ß-D-glucuronide | 1.11 | 3.71 | 102.4 | 0.9987 |
| Norcodeine | 0.93 | 3.11 | 99.2 | 0.9927 |
| Codeine | 0.64 | 2.13 | 98.4 | 0.9842 |
| Dihydrocodeine | 0.69 | 2.32 | 101.9 | 0.9929 |
| Heroin | 0.66 | 2.21 | 101.5 | 0.9914 |
| Morphine | 0.62 | 2.07 | 98 | 0.9932 |
| Morphine-3-ß-D-glucuronide | 0.55 | 1.83 | 99.6 | 0.9986 |
| Buprenorphine | 0.77 | 2.57 | 100.7 | 0.9918 |
| Norbuprenorphine | 0.82 | 2.72 | 100.8 | 0.9863 |
| 6AM | 0.81 | 2.69 | 101.6 | 0.9802 |
| BEC | 0.59 | 1.97 | 98.8 | 0.9933 |
| THC-COOH (+) | 0.9 | 2.99 | 99.6 | 0.9962 |

LOD: Limit of Detection, LOQ: Limit of Quantification, EMC: 1,4-Ethylmethcathinone, 6AM: Monoacetyl morphine, BEC: Benzoylecgonine, THC-COOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, MBDB: N-methyl-1,3-benzodioxyl-butanamine, MDA: 3,4-methylenedioxyamphetamine, MDEA: 3,4-Methylenedioxy-N- ethylamphetamine, MDMA: 3,4-Methylenedioxy-N-methylamphetamine, A-PVP: alpha-Pyrolidinovalerophenone.
LOD and LOQ are the mean values obtained from the analysis of the lowest plasma and urine QC samples. Recovery is expressed as the mean value of both plasma and urine three level QC analysis results.

**Table 2:** Data obtained from patients' questionnaires

| | | Number (n) | Percent (%) |
|---|---|---|---|
| Drug History | Substance use/abuse (total) | 36 | 72 |
| | Alcohol | 4 | 8 |
| | Glue | 4 | 8 |
| | Heroin | 4 | 8 |
| | Ecstasy | 8 | 16 |
| | Cannabis | 4 | 8 |
| | Bonzai* | 2 | 4 |
| | Cocaine | 2 | 4 |
| | Mix drug | 12 | 24 |
| | Unknown substance | 8 | 16 |
| | No drug use | 12 | 24 |
| Education | High school | 22 | 44 |
| | Primary school or dropped | 12 | 24 |
| | Dropped out at unknown level | 4 | 8 |
| | Not studying | 10 | 20 |
| | Special education | 2 | 4 |
| Living with/at | Family | 38 | 76 |
| | Mother or Father | 4 | 8 |
| | Grandmother | 2 | 4 |
| | Dormitory | 2 | 4 |
| | Apart from family | 2 | 4 |
| | Homeless | 2 | 4 |
| Drug application way | Oral | 16 | 32 |
| | Inhalation / smoking | 8 | 16 |
| | Nasal sniffing / snorting | 8 | 16 |
| | Injection | 4 | 8 |
| Gender | Male | 36 | 72 |
| | Female | 14 | 28 |
| | Mean age (10-17y, median age: 16) | 15.6 ± 1.81 | |
| Parents | Together | 34 | 68 |
| | Divorced | 7 | 14 |
| | Separated | 9 | 18 |

* synthetic cannabinoid

**Table 3:** Drugs in patients based on LC-MS/MS analysis

| | n | % | Descriptions |
|---|---|---|---|
| Drug positivity | 30 | 60* | 73% (n=22) male, 26% (n = 8) female |
| Multidrug use | 12 | 40 | ecstasy + a-PVP(n = 2)<br>amphetamine + cannabis (n = 2)<br>ecstasy + methamphetamine + methedrone (n = 2)<br>ecstasy + methedrone (n = 2)<br>ecstasy + cocaine + methedrone (n = 2)<br>amphetamine + codeine (n = 2) |
| Single drug use | 18 | 60 | codeine (n = 2)<br>a-PVP (n = 4)<br>amphetamine (n = 8)<br>cannabis (n = 2)<br>cocaine (n = 2) |
| Drugs | | | |
| Ecstasy | 8 | 26,7 | MDMA, MDA, and/or MBDB positive |
| Amphetamine | 12 | 40 | Methamphetamine and amphetamine positive |
| Methamphetamine | 2 | 6,7 | |
| a-PVP | 6 | 20 | |
| Methedrone | 6 | 20 | |
| Cocaine | 4 | 13,3 | BEC positive |
| Codeine | 4 | 13,3 | CG and NC positive |
| Cannabis | 4 | 13,3 | THC and/or THC-COOH positive |

a-PVP: alpha-Pyrolidinovalerophenone; BEC: benzoylecgonine; CG: Codeine 6-beta-D-glucuronide; MBDB: N-methyl-1,3-benzodioxyl-butanamine; MDA: 3,4-methylenedioxyamphetamine; MDMA: 3,4-Methylenedioxy-N-methylamphetamine; NC: Norcodeine; THC: Δ9-tetrahydrocannabinol; THC-COOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.
*of 50 patients in total

***Preparation of calibrator and control samples***

Urine and blood plasma samples collected from healthy and non-drug users (n=5) were verified as blank matrixes. The stock solutions (1µg/mL) of reference standard materials and IS were prepared in methanol and were stored at -20oC. Blank blood and urine samples fortified with standard materials to obtain seven serial concentrations between 1 - 400 ng/mL for CD, 1 - 20 ng/mL for SCb and SCt were used to construct the calibration curves. Positive urine and blood quality control (QC) samples with three different concentrations of 10, 75, and 300 ng/mL (CD) and 2, 8, and 20 ng/mL (SCb and SCt) were prepared daily and freshly, separately from calibrators. Each calibrator and QC sample was fortified with appropriate IS with the final concentration of 100 ng/mL (for CD) and 40 ng/mL (for SCb and SCt). Negative and two positive (low and high concentration) urine or plasma QC samples were included before and after every batch of analysis.

***Method validation studies***

We used QC solutions prepared in three different concentrations for the validation of three methods according to international and national guidelines[13,14]. Linearity was defined in the concentration range 1- 400 ng/mL and 1-20 ng/mL for CD and for SCb and SCt respectively, and expressed as calibration regression coefficients (r2). Selectivity and specificity were evaluated by determining the lack of interfering peaks at the interested retention times in fortified (with standard solutions) and non-fortified plasma and urine samples. Imprecision (RSD) and accuracy (bias) were calculated for intra- and inter-day, up to 5 days with five replicates of each level with the accepting criteria below 20% for both. The recovery was evaluated from the results of QC samples compared to those of neat standards with 100% recovery. Sensitivity by means of limit of detection

(LOD) and limit of quantification (LOQ) were calculated from consecutive measurements (n=10) of the lowest QC samples. Signal-to-noise ratio of the analyte response was ≥3 for LOD and ≥10 for LOQ.

***Collection of blood and urine samples***

The ethical approval was obtained from Clinical Research Ethics Committee (reference number: 2015-026). 140 patients (9-18 years old) out of a total of 3270 patients admitted to the pediatric emergency service between October 2017 and March 2018 were evaluated for suspected substance use (having symptoms such as unconsciousness, clouding of consciousness, agitation, tachycardia, hypertension, hypotension, nausea and vomiting). Fifty patients (35.7%) and their relatives volunteered to participate in the study and gave written informed consent. Urine and blood plasma (obtained by centrifuging of blood at 1500 xg for 10 min) samples collected from all participants were recorded anonymously and stored at -20°C until analysis. The medical history, education, and sociodemographic information of each patient were also noted after a short questionnaire.

***Sample preparation***

Plasma and urine samples, fortified with IS were first mixed with acetonitrile (v/v=1/1), then centrifuged for 5 min at 14000 rpm. The supernatant (200 µL) was transferred to the auto sampler vial. For evaluating THC and synthetic cannabinoids, urine samples were also subjected to enzymatic hydrolysis by using sequentially beta-glucuronidase (0.5 mL, 30 min incubation) and dichloromethane/ethyl acetate/isopropyl alcohol (1:1:3, v/v) (2.5 mL), which then evaporated under nitrogen, dissolved in methanol (50 µL) and transferred to LC vials.

***Data analysis***

Microsoft Excel (2017, version 15.39) program was used for all calculations and method validation studies. Calibration coefficients (r2) and sample concentrations (by comparing the signal peak area values of analytes with the peak area values of internal standards) were defined by LC-MS/MS software (Lab Solutions Version 5.80, Shimadzu). Analyte results higher than LOQ levels were accepted as positive.

## Results

We achieved appropriate method validation results for all analytes individually (Table 1, 4). LOD and LOQ were defined in the range of 0.4 – 1.8 and 1.36 – 5.98 ng/mL respectively. Recovery was estimated between 85.5 – 102.7%. The average $r^2$ of calibration curves was calculated above 0.98. Intra- and inter-day accuracy and precision were found within acceptable ranges, all values were below 15%. Total ion chromatograms of three different methods are presented as supplemental figures (Figure 1-3). We tested sample stability by repeated analysis of the same samples with three different concentrations within day (n=3, after every 3 hours) and between days (3 days) by keeping them on the autosampler (4°C) and found the accuracy and precision below 15%. Dilution of samples up to ten times did not affected the results significantly (bias <15%). In the case of repeated high drug results in ten-fold diluted samples, we did not recalibrate the method because these high results were already sufficient to show us the high-level drug positivity.

**Table 4:** Amounts (ng/mL) of drugs detected in patients' samples

| Drug | Urine | Blood |
|---|---|---|
| Amphetamine | 48.6 - 21602.8 | 24.3 - 36.5 |
| MDMA | 11391.8 - 61570.1 | 259.56 - 889 |
| MDA | 315.2 - 1302.7 | 87 - 91 |
| MBDB | 68.2 | < LOQ |
| Methamphetamine | 42.1 | < LOQ |
| a-PVP | 4.31 - 56.9 | 9.2 |
| Methedrone | 21.9 - 33.2 | 17.7 |
| Cocaine (BEC) | 386.7 | 12.4 - 26.5 |
| THC-COOH | 2.9 | 6.1 |
| CG | 88.6 -281.2 | < LOQ |
| NC | 20.4 | < LOQ |
| SCb | < LOQ | < LOQ |

a-PVP: alpha-Pyrolidinovalerophenone; BEC: Benzoylecgonine; CG: Codeine 6-beta-D-glucuronide; MBDB: N-methyl-1,3-benzodioxyl-butanamine; MDA: 3,4-methylenedioxyamphetamine; MDMA: 3,4-Methylenedioxy-N-methylamphetamine; NC: Norcodeine; THC-COOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol; SCb: Synthetic cannabinoids.
Data are presented as amount ranges for those results obtained from more than one sample and as a single result for those results obtained from one sample.

Seventy-two percent (n=36, 72%) of patients stated using any substance including volatiles during their questionnaire (Table 2). LC-MS/MS analysis (volatiles were not evaluated) revealed that 60% out of 50 patients were drug positive of which 73% were male (Table 3). Forty percent of drug positive patients showed multidrug. Detected substances were amphetamines, (amphetamine, methamphetamine, ecstasy) synthetic cathinones (alpha-PVP, methedrone), cocaine, codeine and cannabis. Ecstasy (MDMA, 3,4-Methylenedioxy-N-methylamphetamine) was the drug with the highest amount in the samples (Table 4). Synthetic cannabinoids were not detected above LOQ levels.

All patients brought to the emergency department with suspected illegal drug misuse were observed in accordance with the advice of the National Poison Control Centre. They were monitored and vital parameters were followed at frequent interval. None of them required any indication of intervention or intensive care follow-up. All patients were discharged from the emergency room after the normalization of their vital signs and test results. Patients with their relatives were directed to the Department of Social Work and the Department of Child and Adolescent Psychiatry for consultation.

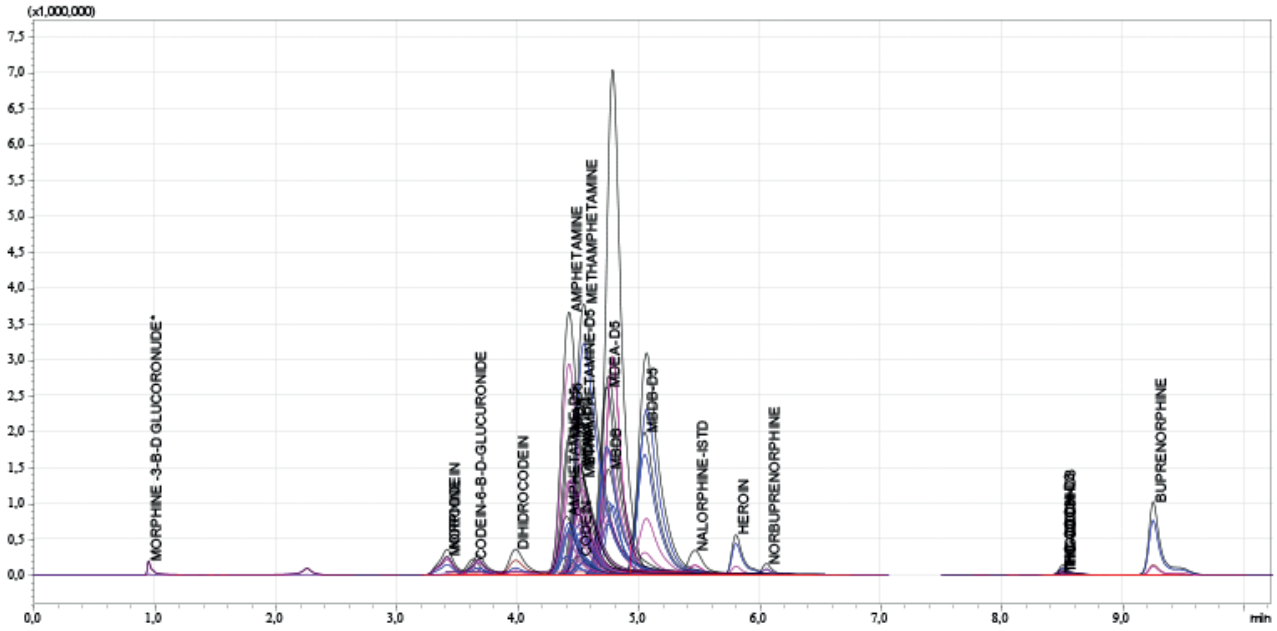


**Figure 1:** Total ion chromatogram of common drugs

## Discussion

Regarding our method development and validation studies, similar results are also reported in literature[15-21]. We did not perform freeze and thaw studies in our validation experiments. We held our patients' samples maximum for 6 weeks at -20°C until analysis, control samples for 4 weeks at 4°C, and all samples for 6 hours at 4°C (in the autosampler) during LC-MS/MS analysis. Neither of these conditions appears to cause a significant change in the stability of the analytes according to published articles (16, 22-26).

The previous survey data on drug use preferences in adolescents indicated that amphetamines were less frequently

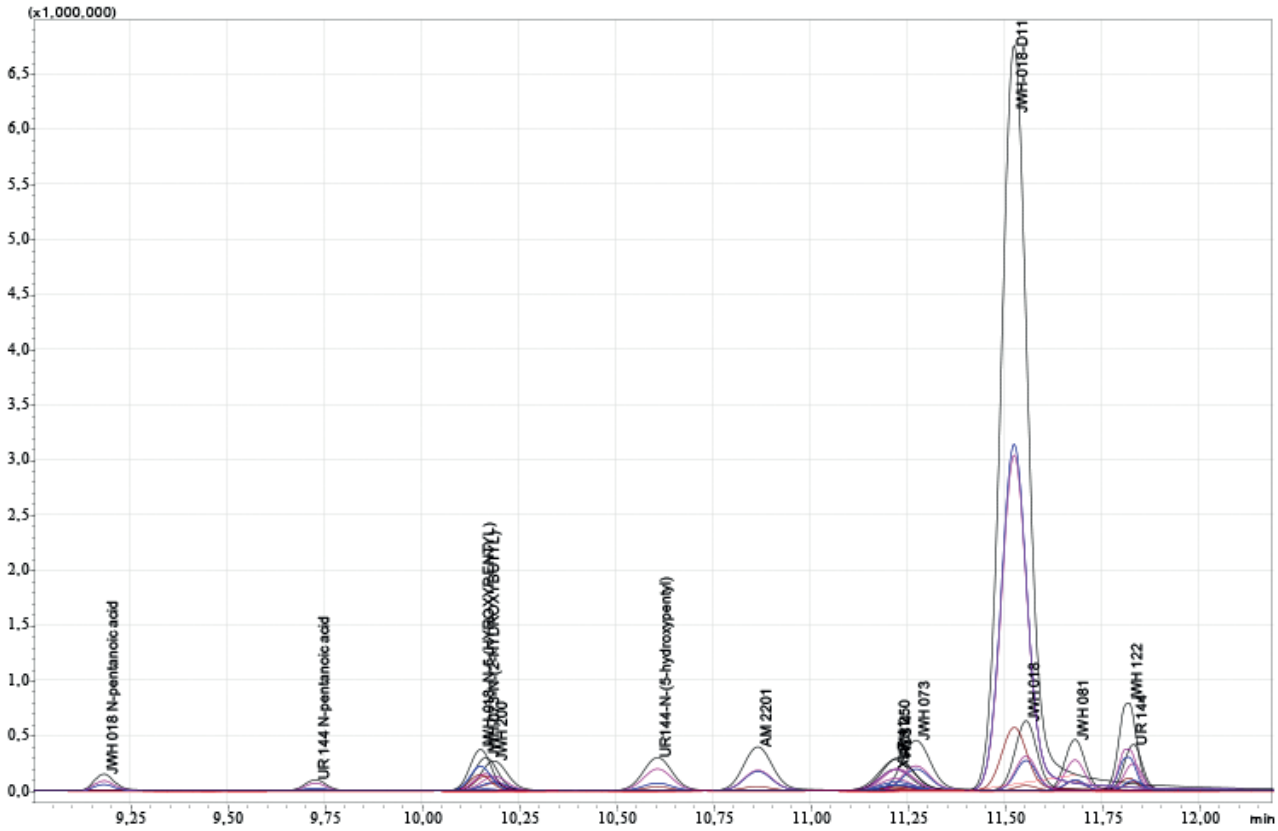


**Figure 2:** Total ion chromatogram of synthetic cannabinoids

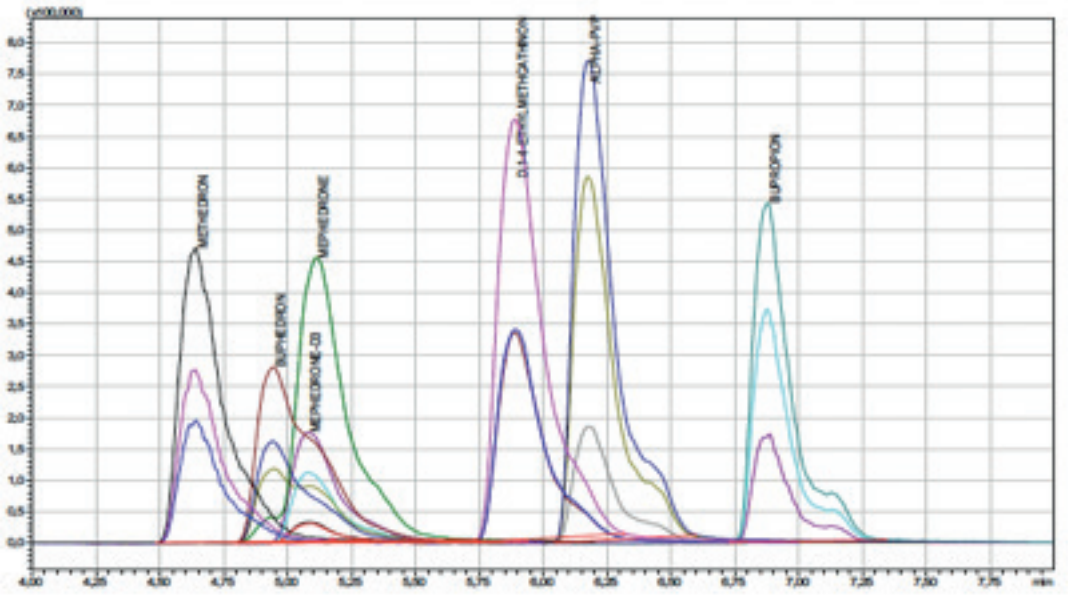


**Figure 3:** Total ion chromatogram of synthetic cathinones

preferred substances (22.7%) than cannabis/cannabis derivatives (84.1%) and volatiles (32.9%)1. Cannabis is still among the most widely misused drugs both in youth and adults. However, synthetic designer drugs cause more acute health problems due to their potent side effects. In addition, the production and variety of these synthetics are constantly increasing. The number of ecstasy seizures in Europe rose up from 3 million tablets in 2010 to over 15 million tablets in 2017, and our country attracts the attention where MDMA was seized more than double the amount in the last year (8.6 million in 2016)[10], which could explain why amphetamines were frequently detected in patients' samples.

In patients with heroin use history we found codeine metabolites, which indicates that they might have been taken codeine or heroin, since illegal heroin (also called diacetylmorphine, is derived from the opium alkaloid morphine, where other alkaloids such as codeine, thebaine and papaverine are also ingredients of opium) is mainly found mixed with codeine. Besides codeine is a natural alkaloid found in opium poppy[27] (legally consumed as food, but opium alkaloids are regarded to be reduced during food processing), it is also a medicine used in pain treatment that can be abused. In these cases, heroin (involving codeine) might have taken days ago, that's why we could not detect its metabolites such as 6AM (6-Monoacetyhlmorphine) (specific for heroin) and morphine, but we detected codeine in urine appeared as codeine-6-glucuronide and norcodeine, confirming that the body was exposed to codeine recently and metabolized it. In two of heroin use-stated patients, we also found amphetamine. Amphetamine might have been detected due to the use of illegal amphetamine or other drugs that were metabolized to amphetamine; but, none of the patients stated an intake of such drugs (e.g. selegiline, benzphetamine, chlorobenzorex, dimethylamphetamine, ethylamphetamine etc). Two patients with the statement of stone (crack cocaine) use have been confirmed with the results of analysis both in urine and blood. We detected multidrug in 12 patients (40%) as told by the same number of patients initially, where amphetamines were combined with cathinones, cocaine, codeine, and cannabis (Table 3). With these findings we determined the real drug type(s) taken or abused by the patients by using validated LC-MS/MS methods. Most of these drugs such as synthetic cathinones could not be detected if immunochemical drug screening would have been applied, because there are no test kits for these analytes.

Looking at reports from different countries; in a prospective cohort of Israeli adolescents (n: 138, median age: 16 years, gender: 47% male) admitted to emergency department 28% had a history of substance abuse, but the laboratory results showed a positivity of 5% for THC, 4% for opioids, 4% for MDMA (revealed after immunochemical urine drug screen test), and 29% for ethanol (in blood)[28]. Among patients presented to US pediatric emergencies between 1997-2010, 58.8% was found positive for illicit drugs, from which 4.5% was opioids, 5% stimulants (cocaine, amphetamine etc.), 6% cannabis, and 43.5% other (unspecified or combined), indicating that stimulants were most frequent drugs as found in our current study[29]. Among 30 drug positive patients we found 73.4% positivity for amphetamines, 40% for synthetic cathinones, and 13.3% for cocaine, codeine, and cannabis, and many of these drugs were used in combination (40%).

Showing the presence of synthetic cathinones (a-PVP and methedrone) in our patients' samples was interesting because there were almost no confirmed antemortem data about cathinone use in our country among children or adolescents. Just as in synthetic cannabinoids, cathinones

are also being produced with new chemical formulas and novel drugs are being marketed under the name of plant, incense and bath salt as legal substances. These synthetic drugs can attract the attention of children because of their cheapness and especially easy availability on the internet. As reported in an article, 51 patients under 20 years of age were admitted to the Texas Poison Center between the years 2010 and 2011 with synthetic cathinone exposure which of 60.8% were male with a mean age of 17.5 (age range 12-19 years), 74% had serious health problems30. The European Early Warning System (EWS) Report revealed that 60% of the substances reported in 2014 were new psychoactive substances (NPS) (especially synthetic cannabinoids and cathinones), but much reduced and remained similar in the years 2017 and 2018 (55 NPS)[10]. In two of our patients having a history of bonzai use, we detected alpha-PVP and amphetamine in their urine samples respectively, confirming the knowledge that bonzai besides synthetic cannabinoids also may involve other synthetic designer drugs such as cathinones, and amphetamines. None of our patients showed a positive result (most were between LOD and LOQ levels) for synthetic cannabinoids. It is possible to say that synthetic cannabinoids are no more preferred because of their serious acute and fatal side effects experienced by users during the last years; or less likely, newly produced synthetics, not included in our test panel, might have been missed. But confirming our findings, the number of hospitalizations in pediatric emergency departments due to using NPS decreased by more than a half in 2018 compared to 2016[31].

The fact that amphetamine-type drugs were predominantly detected in our patients' samples, suggests that adolescents may have used these substances for purposes such as keeping fit, making their minds open, and facilitating learning because they may be worried or stressed about the university exam held at the end of the high school. More importantly, pubertal changes, entertainment (for getting high), social and family problems should not be ignored as they may lead the teens to experience illegal drugs, unfortunately.

The limitation of this study may be that we did not include therapeutically used (legal) but abused drugs such as benzodiazepines, barbiturates, synthetic opioids, and other some newly produced NPS in our research panel, mainly because of our limited budget. We intended to evaluate illegal drugs that were currently seized by the national police and were widely abused, and are more prone to cause emergent health problems.

## Conclusion

We developed and validated LC-MS/MS methods for the determination of multiple illegal drugs simultaneously in both blood and urine human samples with easy and short sample preparation and analysis time which have been applicated to drug suspected pediatric emergency cases for the first time in our country. It would take approximately one hour (except hydrolysis) to report the results when using these methods in an emergency laboratory, not much longer than an immunochemical drug screening test. Our findings emphasized that stimulant synthetic drugs such as ecstasy, amphetamine, alpha-PVP (colloquially called "flakka"), methedrone, and cocaine (crack) in turn were currently the most drugs related to urgent health problems, which should be taken into account by emergency physicians and toxicologists. To control and follow up the drug use, repeated studies should be performed by applying sensitive laboratory techniques, and also important preventive measures (against abused drugs and drug trafficking) should be provided by the authorities.

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## References

1. EMCDDA (European Monitoring Centre for Drugs and Drug Addiction). European Drug Report 2018. 2018.Access date: 05.12.2021 http://www.narkotik.pol.tr/kurumlar/narkotik.pol.tr/Arsiv/TUBIM/Documents/Avrupa%20Uyu%C5%9Fturucu%20Raporu%202018%20EN.pdf
2. EMCDDA (European Monitoring Centre for Drugs and Drug Addiction). European drug report 2015. 2015.Access date: 06.12.2021 https://www.emcdda.europa.eu/system/files/publications/974/TDAT15001ENN.pdf
3. ESPAD (European School Survey project on Alcohol and Drugs) Report 2015 - Results from the European School Survey Project on Alcohol and Other Drugs. 2016:99.Access date: 25.12.2021 https://op.europa.eu/en/publication-detail/-/publication/2373452c-7975-11e6-b076-01aa75ed71a1/language-en
4. Tait RJ, Caldicott D, Mountain D, Hill SL, Lenton S. A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. Clin Toxicol (Phila). 2016;54(1):1-13.
5. Richards JR, Garber D, Laurin EG, Albertson TE, Derlet RW, Amsterdam EA, et al. Treatment of cocaine cardiovascular toxicity: a systematic review. Clin Toxicol (Phila). 2016;54(5):345-64.
6. Swepson C, Ranjan A, Balasubramaniam M, Pandhare J, Dash C. Cocaine Enhances HIV-1 Transcription in Macrophages by Inducing p38 MAPK Phosphorylation. Front Microbiol. 2016;7:823.

**7.** Paratz ED, Cunningham NJ, MacIsaac AI. The Cardiac Complications of Methamphetamines. Heart Lung Circ. 2016;25(4):325-32.
**8.** Sanchez-Ramos J. Neurologic Complications of Psychomotor Stimulant Abuse. Int Rev Neurobiol. 2015;120:131-60.
**9.** Shelton CP, Rosini JM. Multisystem Organ Failure and Death Resulting From Ingestion of "Molly" (3,4-Methylenedioxymethamphetamine). J Emerg Nurs. 2015;41(5):447-50.
**10.** EMCDDA. European Drug Report 2019. 2019.
**11.** Saitman A, Park H-D, Fitzgerald RL. False-positive interferences of common urine drug screen immunoassays: a review. Journal of analytical toxicology. 2014;38(7):387-96.
**12.** Van Wijk XM, Goodnough R, Colby JM. Mass spectrometry in emergency toxicology: Current state and future applications. Critical reviews in clinical laboratory sciences. 2019;56(4):225-38.
**13.** Scientific Working Group for Forensic Toxicology (SWGTOX), Standard practices for method validation in forensic toxicology. Journal of Analytical Toxicology. 2013;37(7):452-74.
**14.** Ministry, of, Health. The Working Procedures and Principles of Confirmation Laboratories. Official Gazette of Republic of Turkey. 2015(Circular 14).
**15.** Knittel JL, Holler JM, Chmiel JD, Vorce SP, Magluilo J, Jr., Levine B, et al. Analysis of Parent Synthetic Cannabinoids in Blood and Urinary Metabolites by Liquid Chromatography Tandem Mass Spectrometry. J Anal Toxicol. 2016;40(3):173-86.
**16.** Jang M, Shin I, Kim J, Yang W. Simultaneous quantification of 37 synthetic cannabinoid metabolites in human urine by liquid chromatography-tandem mass spectrometry. Forensic Toxicology. 2015;33(2):221-34.
**17.** Marinetti LJ, Antonides HM. Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. J Anal Toxicol. 2013;37(3):135-46.
**18.** Yang HS, Wu AH, Lynch KL. Development and Validation of a Novel LC-MS/MS Opioid Confirmation Assay: Evaluation of beta-glucuronidase Enzymes and Sample Cleanup Methods. J Anal Toxicol. 2016;40(5):323-9.
**19.** Schaefer N, Peters B, Schmidt P, Ewald AH. Development and validation of two LC-MS/MS methods for the detection and quantification of amphetamines, designer amphetamines, benzoylecgonine, benzodiazepines, opiates, and opioids in urine using turbulent flow chromatography. Analytical and bioanalytical chemistry. 2013;405(1):247-58.
**20.** Berg T, Lundanes E, Christophersen AS, Strand DH. Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC–MS/MS. Journal of Chromatography B. 2009;877(4):421-32.
**21.** Scheidweiler KB, Desrosiers NA, Huestis MA. Simultaneous quantification of free and glucuronidated cannabinoids in human urine by liquid chromatography tandem mass spectrometry. Clinica chimica acta. 2012;413(23-24):1839-47.
**22.** Ammann D, McLaren JM, Gerostamoulos D, Beyer J. Detection and Quantification of New Designer Drugs in Human Blood: Part 2 Designer Cathinones. Journal of Analytical Toxicology. 2012;36(6):381-9.
**23.** Wohlfarth A, Scheidweiler KB, Chen XH, Liu HF, Huestis MA. Qualitative Confirmation of 9 Synthetic Cannabinoids and 20 Metabolites in Human Urine Using LC-MS/MS and Library Search. Anal Chem. 2013;85(7):3730-8.
**24.** Coles R, Kushnir MM, Nelson GJ, McMillin GA, Urry FM. Simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and 6-acetylmorphine in urine, serum, plasma, whole blood, and meconium by LC-MS-MS. Journal of Analytical Toxicology. 2007;31(1):1-14.
**25.** Jimenez C, De la Torre R, Ventura M, Segura J, Ventura R. Stability studies of amphetamine and ephedrine derivatives in urine. Journal of Chromatography B. 2006;843(1):84-93.
**26.** Clauwaert KM, Van Bocxlaer JF, De Leenheer AP. Stability study of the designer drugs "MDA, MDMA and MDEA" in water, serum, whole blood, and urine under various storage temperatures. Forensic science international. 2001;124(1):36-42.
**27.** Ozbunar E, Aydogdu M, Doger R, Bostanci HI, Koruyucu M, Akgur SA. Morphine Concentrations in Human Urine Following Poppy Seed Paste Consumption. Forensic Sci Int. 2019;295:121-7.
**28.** Kozer E, Bar-Hamburger R, Rosenfeld NY, Zdanovitch I, Bulkowstein M, Berkovitch M. Illicit drug and alcohol users admitted to the pediatric emergency department. Age (yrs, median and range). 2008;16:12-8.
**29.** Fahimi J, Aurrecoechea A, Anderson E, Herring A, Alter H. Substance abuse and mental health visits among adolescents presenting to US emergency departments. Pediatric emergency care. 2015;31(5):331.
**30.** Forrester MB. Adolescent synthetic cathinone exposures reported to Texas poison centers. Pediatric emergency care. 2013;29(2):151-5.
**31.** Jalali R, Dmochowska P, Godlewska I, Balmas J, Młynarska K, Narkun K, et al. Designers Drugs—A New Challenge to Emergency Departments—An Observational Study in Poland. Medicina. 2020;56(7):354.