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APPLYING OF TOXICOLOGY SCREENING IN ANALYSIS OF POST MORTEM MATERIAL – CASE SERIES OF LETHAL OVERDOSES BY HEROINE AND DRUGS

PRIMENA TOKSIKOLOŠKOG *SCREENING*-A U ANALIZI *POST MORTEM* MATERIJALA – PRIKAZ SERIJE LETALNIH PREDOZIRANJA HEROINOM I LEKOVIMA

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Ključne reči

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Key words

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Abstract

Toxicology screening is process which includes different analytical techniques for determination of the toxic agents. These tests are often done in emergency medical situations. They can be used to evaluate possible accidental or intentional overdose or poisoning. Also, they may help identification the cause of acute drug toxicity, to monitor drug dependency and to determine the presence of substances in the body for medical or legal purposes. The aim of this work was to present liquid chromatographic technique with mass spectrometric (LC-MS) and photodiode array (HPLC-PDA) detection and using computer library in toxicology screening of post mortem material of heroine abusers. LC-MS method has used for determination of heroine metabolites (morphine, 6-monoacethylmorphine and codeine) in samples. Samples also were analyzed by HPLC-PDA method to determine if other drugs exist. Thanks to computer library of UV spectrums over 1000 drugs and their metabolites, described HPLC-PDA technique is very useful in toxicology screening, and could be used in analysis of post mortem material when cause of death is unknown.

INTRODUCTION

A toxicology screening is a set of standard blood and urine tests for the most common pharmaceuticals and illegal drugs of abuse. When a patient shows symptoms that indicate poisoning or overdose, a toxicology screening is usually the first diagnostic test ordered by the physician. However, it does have some weaknesses. It takes time to process the results, although the test is designed to be completed as quickly as possible. Moreover, it does not test every possible cause of intoxication.

Even a clear toxicology screening is not a definitive indication that the patient is not suffering from some form of poisoning (1).

The most applying biological matrices in toxicology screening are blood and urine. Blood analysis is the most used for determination of drug concentration which is responsible for pharmacological and toxicology effects on human organism. Urine analysis is use for monitoring of drug elimintion. It contains higher concentrations of drugs ant their metabolites in comparison to blood, and it is suitable for qualitative screening. Gastric content also could be used for identification of intoxicant. Qualitative analysis can be easier according to residue of tabletes and capsules. Quantitative analysis of this sample does not have significance.

Vitreous humor can be used for toxicology analysis. It is easy to collect and as it is contained within the eye it is almost completely unaffected by post-mortem redistribution (2).

For forensic purpose saliva, hair and nails can also be analized (3).

Saliva is alternative sample which can be used for both therapeutic drug monitoring and determination of drugs of abuse which has a big importance in forensic cases (4-5).

Toxicology screening is using for determination of drugs of abuse (heroin, cannabinoids, ampetamines, cocain, benzodiazepines) in urine and alcohol and the most applying psyhotropic drugs.

Different analytical technique can be using in toxicology screening. For fast and simple detection of group of compounds with similar chemical structure and properties, the most applyed are immuno assays (immunochromatographic test strips for identifikation of drugs of abuse, EMIT, FPIA etc). The modified EMIT immunoassay system described by Hino et al. can be useful for screening of drugs of abuse in post-mortem blood samples, especially when urine is not available [6]. Chromatographic methods are using for identification of specific drug or drug of abuse (gas chromatography with flame-ionization (FI) or mass spectrometric (MS) detector or liquid chromatography with ultraviolet (UV), photo diode array (PDA) and mass spectrometric (MS) detektor) [7-8].

Essential thing for every analytical methods is to be fast and reliable in both qualitative and quantitative analysis.

The aim of this work was to describe importance of HPLC-PDA and LC-MS method in toxicology screening of post mortem material.

MATERIALS

Analytical standards of morphine and codeine (99,5 % s.s.), were obtained from Australian Government, National Measurement Institute.

Analytical standard of 6-acetylmorphine (1 mg/mL) was obtained from Cerilliant.

Acetonitril, sodium hydrogen phosphate, methanol, ammonium acetate, phosphoric acid, acetic acid, chloroform and 2-propanol were of HPLC, MS or p.a. purity, obtained from MERCK. Water was purified by Millipore Milli-Q system.

Immunochromatographic test strips for detection of drugs of abuse (opiates, benzodiazepines, cocaine, cannabinoides and amphetamines), were obtained from Biognost, Zagreb.

Serum, urine and tissue samples after four autopsies were analyzed.

Chromatography

The LC-MS system for determination of opiates consisted of a Waters 2695 separation module interfaced to a ZQ mass spectrometer equipped with an electrospray ionisation source. The apparatus was managed with a Masslynx software.

Analyses were run in positive mode (electrospray) with capillary and cone voltages set to 3.8 kV and 30V. Temperature of the source and desolvatation temperature were 125°C and 430°C, respectively, and nitrogen desolvatation gas and cone gas flow were 400 L/h and 50 L/h, respectively. The mobile phase consisted of a mixture of acetonitrile/acetic acid 1% and acetate buffer pH 3.5 in the ratio of 20:80, with a flow rate of 0.3 mL/min. Run time was 30 min. Identification were performed after separation on ODS® RP18 3,5µm 2,1mm x 150mm, Waters column in a fool scan mode (m/z= 100 – 500) by selecting the characteristic ions m/z = 286, m/z = 300 and m/z = 328 for morphine, codeine and 6-monoacethylmorphine, respectively. The retention times for morphine, codeine and 6-acetylmorphine were 8.5, 16.1 and 18.7 min. respectively Compounds of matrix did not interfere analysis after extraction.

The method used high performance liquid chromatograph Waters Aliance 2695 XE Separations Module pump with Waters 2696 Photodiode Array Detector and Empower Login Software.

The mobile phase was mixture of acetonitrile (A) and phosphate buffer pH 3.6 (B).

Ratio of mobile phases A and B and flow are in gradient Table 1.

Table 1. *Ratios of mibile phases A and B for HPLC-PDA method*

Time (min.)	flow (mL/min)	A %	B %	curve
	1.0	85	15	
3.0	1.0	65	35	6
9.0	1.0	20	80	6
28.0	1.5	20	80	6
31.0	1.5	20	80	6
31.5	1.5	85	15	6
35.0	0.3	85	15	6

The method was used column Symmetry® C8 (wat 054270) 4,6 x 250mm (Waters) with guard column Sentry Guard Symmetry® C18, at the temperature of 30°C, with injector loop volume of 50 µL.

Sample preparation

Serum and urine samples were prepared by alkaline liquid-liquid extraction with mixture of chloroform-2-propanol (9:1). After extraction and evaporation of organic solvent, dry residue reconstituted in methanol and analized by LC-MS and HPLC-PDA method.

Tissue samples prepared by Stas' method and alkaline extracts were analized by LC-MS and HPLC-PDA method.

RESULTS

Table 2. shows concentrations of morphine, 6-monoacethylmorphine (6-MAM) and codeine after LC-MS analysis.

Table 2. *Concetrations of opiates in post mortem materia*l

SAMPLE	MORPHINE	6-MAM	CODEINE
1. Blood	0,166 mg/L	0,052 mg/L	0,103 mg/L
Urine	0,337 mg/L	0,396 mg/L	0,114 mg/L
Liver	6,43 ìg/kg	2,24 ìg/kg	2,01 ìg/kg
Kidney	6,71 ìg/kg	0,68 ìg/kg	3,26 ìg/kg
Brain	3,21 ìg/kg	2,38 ìg/kg	2,53 ìg/kg
2. Blood	0,058 mg/L	-	0,009 mg/L
Urine	0,011 mg/L	0,136 mg/L	0,004 mg/L
Liver	3,892 ìg/kg	-	3,477 ìg/kg
Kidney	13,171 ìg/kg	1,181 ìg/kg	7,217 ìg/kg
Brain	3,585 ìg/kg	5,588 ìg/kg	2,525 ìg/kg
3. Blood	0,016 mg/L	-	-
Urine	0,644 mg/L	0,878 mg/L	2,115 mg/L
Liver	27,292 ìg/kg	1,967 ìg/kg	1,247 ìg/kg
Kidney	1,089 ìg/kg	-	-
Brain	0,299 ìg/kg	-	-
4. Blood	0,221 mg/L	-	0,031 mg/L
Gastric content	0,381 mg/L	0,559 mg/L	0,098 mg/L
Liver	11,39 ìg/kg	-	1,37 ìg/kg
Kidney	59,70 ìg/kg	5,78 ìg/kg	6,23 ìg/kg
Brain	14,03 ìg/kg	16,67 ìg/kg	7,41 ìg/kg

Table 3. *Identified drugs in post mortem materials*

	Other drugs
1.	Diazepam
	Clonazepam
	Nitrazepam
	Carbamazepine
	Fluoxetine
	Haloperidol
	Chlorpromazine
2.	Bromazepam
3.	Bromazepam
	Sertralin
4.	Diazepam
	Maprotilin
	Bromazepam
	Sertralin

DISCUSSION

Posmortem toxicology very often is required identification of unknown cause of intoxication and death. It is used to determine whether alcohol, drugs or other poisons which may have caused or contributed to the death of a person. It usually starts with a drug screen. In most forensic laboratories a drug screen consist of a panel of immunoassay tests and head space analysis of alcohol, combined with one or more HPLC or GC procedures. The most HPLC system use ultraviolet and mass spectrometric detection.

Like other [9-10], we also applied gas chromatography with flame ionization detector for determination of alcohol. Only one of four analyzed material was positive.

For fast identification of drugs of abuse we have used immunochromatographic test strips, which were positive for opiates and benzodiazepines in all of tested samples. Immunochromatographic tests (test strips for detection of drugs of abuse) are only presumptive indications of the possible presence of the drugs of abuse. It has advantage that they are rapid and inexpensive, but must be followed up by other test which offers confirmation of the presence of a drug. [11]

Confirmation and quantitation of opiates positive samples was performed by validated LC-MS method, and confirmation and determination of benzodiazepines and screening for other psychotropic drugs by HPLC-PDA method.

Because of its rapid hydrolysis, heroin is difficult to detect in blood. It is metabolized into 6-acethylmorphine and morphine. Small quantities of codeine also could be detected in biological material of chronic users [3]. Codeine was detected in 96% of the subjects. In the majority of cases the forensic investigation indicated polydrug use, the most common additional findings being alcohol and benzodiazepines [12].

Literature data of morphine, 6-monoacethylmorphine and codeine post mortem concentration are various.

In femoral vein blood of 17-years old girl who founded dead concentrations of morphine, 6-monoacethylmorphine and codeine were 0.28 mg/L, 0.004 mg/L and 0.02 mg/L respectively [13].

Darke et Ross described 10 fatalities resulting from heroin overdoses. The mean blood concentration of morphine was 0.31 mg/L (range from 0.06 to 0.99). Drugs other then opiates were also detected in 7 cases (alcohol, cocaine, paracetamol, methadone and dosulepin) [14].

A 46-year-old man died after heroin abuse. The post mortem morphine concentrations were 0.68 mg/L, 0.49 mg/L and 0.32 mg/L in blood, urine and bile, respectively [3].

Of 10 death involving heroin body-packers, 4 had morphine blood concentrations < 1 mg/L. Morphine concentrations in the others were 4.4, 6.7, 35.8, 39.4 and 52.6 mg/L, respectively [15].

We determined concentrations of morphine, codeine and 6-acethyl morphine and results has shown in Table 2. Our results are in compliance with literature.

Literature data shows that heroin overdose often combined with legal drugs.

43-year-old female was reported to inject heroin, which led to her rapid death. Initial toxicology analysis detected morphine (0.78 mg/L), amitriptyline (2.91 mg/L), and nortriptyline (2.80 mg/L) in femoral blood [16].

In the majority of cases the forensic investigation indicated polydrug use, the most common additional findings being alcohol and benzodiazepines. In one-quarter of the cases other drug combinations were found [12].

High performance liquid chromatography is a simple and reliable method for analysis of the most drugs. It is both accurate and precise for qualitative and quantitative analysis.

Described HPLC-PDA method with library of UV spectrums could be used for toxicology screening of biological matrices (urine, blood, tissues). This method enable simultaneous determination several compounds from different chemical and pharmacological groups. Thanks to library of more than thousand UV spectrums, it enable identification not only drugs or drugs of abuse, but their metabolites. Computer library contains UV spectrums of drugs and their metabolites which is pharmacological group of benzodiazepines, antiepileptics, phenothiazines, tricyclic antidepressants, diuretics, calcium antagonists, β-blockers, nonsteroidal anti-inflammatory drugs, narcoanalgetics, hypnotic, anesthetics and group of drugs of abuse such us opiates, amphetamines, cannabinoides and cocaine and their metabolites. Applying of this method in toxicology screening, ensure detailed information about the sample in relative short time period (about 30 minutes).

Results of analysis (Table 3.) have shown presence not only the benzodiazepines, but also other psychotropic drugs, which are often abusing. This is in compliance with fact that majority of heroin addictor abuse many other drugs.

CONCLUSION

Analysis of post mortem material is challenge for every analyst. In those cases very useful and fast screening method for identification of drugs of abuse is immunochromatographic test. Described LC-MS method is reliable for determination of small quantities of morphine, 6-monoacethylmorphine and codeine in biological samples. HPLC-PDA system with computer library of UV spectrums is applicable for toxicology screening, especially when cause of intoxication and death is unknown.

Apstrakt

Toksikološki skrining je proces koji podrazumeva primenu različitih analitičkih tehnika za određivanje toksičnih agenasa. Ovi testovi se često primenjuju u urgentnim medicinskim stanjima. Mogu se primeniti da dokažu eventualno mogućnost slučajnog ili namernog predoziranja ili trovanja. Takođe, mogu pomoći u identifikaciji uzročnika akutnog trovanja, u praćenju zloupotrebe droga i za dokazivanje prisustva supstanci bilo u medicinske svrhe ili u cilju zloupotrebe. Cilj ovog rada bio je da prikaže tečnu hromatografiju sa maseno-spektrometrijskom (MS) i UV skenirajućom (PDA) detekcijom i korišćenje kompjuterske biblioteke UV spektara u toksikološkom skriningu post mortem materijala heroinskih zavisnika. LC-MS metoda je korišćena za određivanje metabolita heroina (morfina, 6-monoacetilmorfina i kodeina) u uzorcima. Uzorci su takođe analizirani i HPLC-PDA metodom da se dokaže eventualno prisustvo drugih lekova. Zahvaljujući biblioteci UV spektara više od 1000 lekova i njihovih metabolita, opisana HPLC-PDA metoda je veoma korisna u toksikološkom skriningu i može se primeniti u analizi post mortem materijala, naročito kada je uzrok smrti nepoznat.

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