



Davorka Sutlovic^{1*}, Maja Versic Bratincec² and Marija Definis-Gojanovic²

¹Department of Pathology and Forensic Medicine, University Hospital Centre Split, Spinciceva 1, 21 000 Split, Croatia

²Department of Forensic Medicine, University of Split School of Medicine, Soltanska 1, 21 000 Split, Croatia

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***Corresponding author:** Davorka Sutlovic, Department of Pathology and Forensic Medicine, University Hospital Centre Split, Spinciceva 1, 21 000 Split, Croatia. Tel: +385 21 556 717; Fax: +385 21 556 834; E-mail: dsutlov@kbsplit.hr

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Research Article

Amphetamine in postmortem liver sample?

Abstract

The aim of this paper is to present the formation of false positive amphetamine in postmortem liver samples and to determine conditions that are responsible for its formation.

Postmortem samples used in the experiment underwent toxicological analysis and all liver samples gave positive results for amphetamine. There was no evidence that patients consumed amphetamine or any other substance that could have produced such finding. We tested liver tissue for better understanding of false positive amphetamine. The influence of different temperatures in different time periods was studied on liver samples, which were divided into several pieces with identical weight. Samples were qualitatively and quantitatively determined and analyzed using gas chromatography-mass spectrometry technique (GC/MS).

Liver samples gave false positive results for amphetamine after ten days at the 32.5 0C. After derivatization, the results on amphetamine have not been positive. Toxicological finding for a presence of a positive amphetamine in liver sample is not sufficient and should be confirmed on other biological samples. Also, there is a need for derivatization of biological samples to confirm and eliminate the suspicion of false positive findings.

Introduction

Amphetamine is a psychostimulant drug, known to produce increased wakefulness and focus in association with decreased fatigue and appetite. Clinical uses of amphetamine include chronic administration for the treatment of narcolepsy in adults and attention-deficit/hyperactivity disorder in children [1]. It is also used as a drug of abuse [2]. Amphetamines are well absorbed from the gastrointestinal tract and metabolized by deamination, oxidation and hydroxylation. Approximately 90% of the dose is excreted in the urine within 3-4 days [1]. An amphetamine overdose is rarely fatal but can lead to a number of different symptoms, including psychosis, chest pain and hypertension. Cardiovascular effects include increased heart rate and blood pressure, chest pain, myocardial ischemia or infarction, dysrhythmias, cardiovascular collapse and death [2].

A typical postmortem toxicological analysis is identification of the drugs or chemicals presence in postmortem specimens [3]. Blood, urine, bile, liver and kidney are routinely screened for alcohols, volatile organic compounds, drugs and the drugs abuse. The main purpose of toxicological data in a postmortem investigation is to help the forensic pathologist to determine the cause of death. The findings may yield very important

information on contributing factors and general circumstances of the death. Sometimes, the drug detection in postmortem cases can provide some specific difficulties and can lead to misinterpretation of obtained results. Therefore, this study helps to describe the stability of drugs in biological samples as potential factor that can provide false findings. Drummer reviews particular toxicological issues associated with more common drugs of abuse such as amphetamines, cannabinoids, cocaine, opioids and the benzodiazepines [4]. The problem can also be the formation of some substances, especially in degraded and decomposed bodies, in which there are no biological fluids for analysis. Biological tissues, liver and kidney, are usually analyzed in those situations.

Reynolds et al., in their unpublished in vitro study showed that rat liver homogenates convert several drugs to amphetamine and methamphetamine. They described that the proportion of amine, excreted after deprenyl ingestion, was comparable with excreted amount of amine detected after methamphetamine oral consumption [5]. To our knowledge, there are a few drugs and their metabolites which can cause a false-positive result for amphetamines in postmortem liver drug screen, but there is still a lack of explanation of conditions in which amphetamine formation occurs.

Postmortem biological samples (tissues and fluids) were analyzed from thirteen suspected poisoning cases, characterized as a suicide. The degraded bodies were found several days after death occurred in summer period in Split-Dalmatia County, Croatia. All the samples were subjected to routine toxicology analysis. If the larvae were found on the skin surface (Figure 1A) and/or from inside the body (Figure1B), it will be also collected for toxicological analysis. Amphetamine was identified in all thirteen liver samples. Only six samples gave false positive results, which was confirmed by analysis of other biological tissues and fluids that gave negative amphetamine result (Table 1). There was no evidence that the patients were taking any substances that could give these false results. Those data give rise for further research of conditions that affects post-mortem liver amphetamine formation. This study also shows that the ration of time and temperature is a key for understanding the amphetamine formation. Table 1, Figure 1A,B.

Materials and Methods

Materials

The study was carried in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Hospital Split (No. 2181-147-01/06/J.B.-14-2). Postmortem samples were collected into sterile and chemically clean containers during the autopsy, which was performed on putrefied bodies, found several days after death. Samples were taken and stored at 4 °C, until extraction and analysis. After the completion of routine toxicological analysis, the samples were stored at -20 °C.

The effect of various parameters on amphetamine occurrence

Postmortem samples, including liver, blood and urine, underwent routine toxicological analysis, and all gave negative results for amphetamine. Negative liver samples were divided into several pieces with identical weight and were subjected to influence of different temperatures: the room temperature (15–20°C) and the temperatures in thermostat (30 °C and 32.5 °C) in time period of 20 days under controlled conditions shown in Table 2. Samples were analyzed periodically, every second or third day from samples collection, in a predetermined order. Samples were extracted and analyzed using GC/MS. The samples with amphetamine positive results were evaporated and derivatized with 30 µL BSTFA+1% TMCS for 30 min. at the temperature (~70 °C) and analyzed using GC/MS. All analyses were performed in duplicate.

Methods

A solid-phase extraction was performed using Amberlite XAD-2, polyaromatic adsorbent resin Supelco; SIGMA ALDRICH, Taufkirchen, Germany. The GC/MS drug analysis was performed using a Shimadzu GC-2010 with an ion trap mass spectrometer mass selective detector, MSD. The chromatographic column was RTX-5MS (5% diphenyl-95%

dimethyl polysiloxane, length 30 m, diameter 0.25 mm, film thickness 0.25 µm). An initial column temperature of 90 °C was held for 3 min, then programmed to rise to 270 °C at 20 °C min⁻¹, and held for 25 min. Ultra-pure grade helium was used as a carrier gas at the flow rate of about 1.5 ml min⁻¹.

Results and Discussion

Toxicological analyses, including extraction, evaporation and derivatization prior to GC/MS analysis, were performed on thirteen postmortem cases, including the larvae. All the postmortem liver samples gave positive amphetamine results (RT~5.5 min; m/z 91, 65 and 121). Six liver samples gave a false positive amphetamine finding, proven by analysis of other excluded postmortem samples that gave negative amphetamine results (Figure 2). In all six samples, followed by the completion of derivatization, the presence of amphetamines wasn't proved. That makes derivatization an inevitable part of the toxicological analysis. All these analysis are needed to avoid false conclusions.

It is well known that many substances can give a false positive amphetamine result, including amphetaminil, benzphetamine, clobenzorex, dimethylamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylamine, fenproporex, furfenorex, lisdexamfetamine, mefenorex, mesocarb,



Figure 1: Female death body found after 19 days after death. 1A - before autopsy; 1B - in time of autopsy.

Table 1: Details of the cases and received specimens.

Case	Male/ Female	Age	A place of finding	Reputed number of days from death to discovery	Season	Collected samples		
						liver	kidney	larvae
1	Male	43	Flat	21	Winter			
2	Male	59	Flat	20	Winter			
3	Female	61	Beach	13	Autumn			
4	Male	47	Flat	5	Summer			
5	Male	41	Flat	11	Autumn			
6	Female	51	Flat	19	Spring			

Table 2: Incubation conditions and results: **ND** amphetamine not detect; **+** slightly positive amphetamine; **++** positive amphetamine; **+++** strongly positive amphetamine; **MAX** the highest amphetamine concentration.

Temperature condition (°C)	Number of incubation days							
	3	5	7	10	12	14	17	19
room	ND	ND	ND	ND	ND	ND	+	++
30	ND	+	++	++	+	+	+	+
32.5	ND	+	++	MAX	+++	+++	+	+

prenylamine, propylamphetamine, and selegiline, among others [6]. Also, it is known that under degradation process in postmortem tissues amphetamine can occur. It is assumed that amino acid phenylalanine, with catalyst activity, may convert into amphetamine [7]. It is unknown which processes occur in postmortem liver tissues that can lead to false positive amphetamine results.

The interpretation of postmortem toxicology data is a crucial factor in determination of the cause of death. In case of body degradation, biological fluids can be inconvenient or even non-existent for toxicology purpose, therefore the liver and other biological tissues are only available samples for toxicology analysis. The quality of the postmortem liver specimen can also be deficient: watery, putrefied and degraded. Still, post-mortem liver samples are of great importance because they contain many substances in higher concentrations than other biological samples [8]. Some drugs may vanish or arise due to bacterial activity and hydrolysis, especially if the body is exposed to outdoor factors [9]. Butzbach investigated problems associated with postmortem drug concentration changes and the significance of microbial influences during the postmortem interval and sample storage [10]. The author indicates that numerous biochemical and biological processes occurs after death, and may have a significant influence on postmortem drug concentrations. Sutlovic et al. demonstrated increase of ethanol in the postmortem urine sample due to microbial activity [11] Figure 2.

Recognizing false amphetamine occurrence and for better understanding, a putrefaction process was initiated on negative liver tissues under controlled conditions, at different temperatures and in different time period. Amphetamine concentrations were compared by the area under the resulting peak. Liver samples with negative amphetamine findings gave positive results after five days at the temperature of 30 °C and after three days at the temperature of 32.5 °C. In all liver samples the highest concentration of amphetamine was reached between 7th and 10th day. After 10th day the concentration

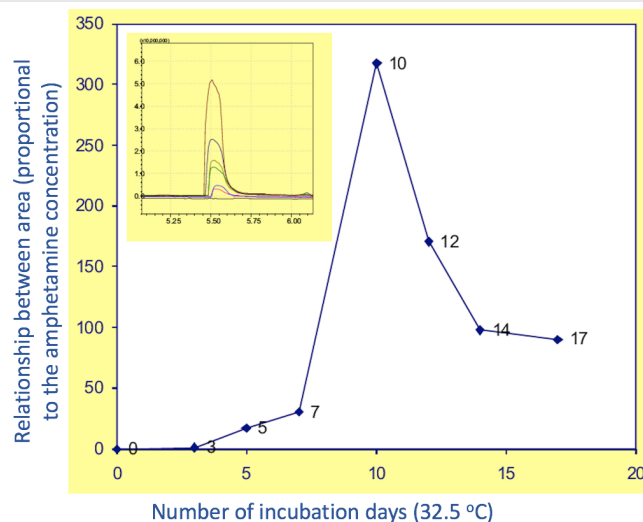


Figure 3: Single ion chromatogram of relationship between false positive amphetamine concentrations and number of incubation days.

of amphetamine started to decrease. In comparison of two temperatures (30 °C and 32.5 °C) significantly higher concentration of amphetamine was obtained at the temperature of 32.5 °C (Table 2, Figure 3).

The results of our study indicates that it could be possible to predict how many days have passed from death to autopsy by determining the number of days needed to achieve the maximum amphetamine concentration, in correlation with the environmental temperature.

Finally, it is very important, due to correct interpretation of the postmortem toxicological results, to identify false positive amphetamine, emerged as a result of subsequent postmortem activities.

These findings believed to have a great significance in forensic toxicology practice. The positive amphetamine finding should be confirmed in all excluded post-mortem samples including biological fluids and tissues. Furthermore, all the samples, especially the positive ones, are necessary to be derivatized in order to eliminate any possible doubt in interpretation of toxicological findings.

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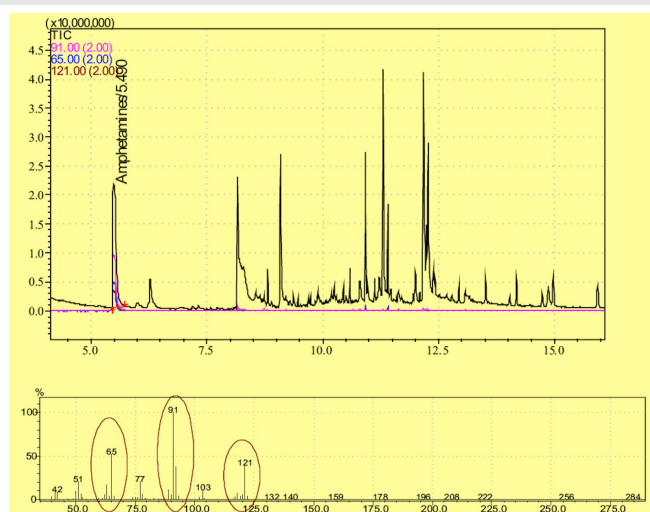


Figure 2: Total ion chromatogram and characteristic spectra of amphetamine.



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12. Figure 1. Female death body found after 19 days after death. **1A** - before autopsy; **1B**- in time of autopsy.