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DILEMMAS

Chromatography as Method for Analytical Confirmation of Paracetamol in Postmortem Material Together with Psychoactive Substances

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ABSTRACT

Introduction: Paracetamol (Acetaminophen) in addition to aspirin is the most commonly used analgesic and antipyretic medication by millions of patients worldwide. It is an example that paracetamol as medicine that in the world is provided without a doctor's prescription, can lead to death. Today paracetamol became an integral part of a heroin mixture and is very popular at the street market. The main reason for this is that it can be obtained without a prescription, it is cheap, and by most people well tolerated without side effects. It is probably used for "cutting" the pure heroin, as it says in the jargon, and in that manner from small amount of pure drug is obtained greater amount, which is then sold on the street. The goal is to identify presence of paracetamol, by analytical method of gas chromatography mass spectrometer (GC-MS) in postmortem material together with psychoactive substances. **Material and methods:** For chemical-toxicological analysis is used biological material collected trough autopsy of 20 deceased people, suspected to have died due to psychoactive substance overdose. All received samples are stored at -20 ° C until analysis at our laboratory. From processed 47 samples that were analyzed in the period from 2014 to 2015, 19 are blood samples, urine 19, 3 samples of stomach contents, and 6 samples of bile content. Deceased were middle-aged, of which only 7 were female. The tested samples were processed according to two methods of extraction. Extraction by XAD-2 resin, and the extraction by the method of salting out with sodium tungstate. Extracts of the samples were then dissolved in chloroform and continued analysis at the analytical instrument. Identification of the paracetamol presence, in the test biological samples is demonstrated by the technique of gas chromatography with mass spectometry (hereinafter referred to as GC-MS). The technique of GC-MS is a selective, sensitive and reliable, and is therefore considered a "gold standard" for determining the drug, and the drug substance. Used GC-MS instrument was an Agilent 7890A with helium as the carrier gas. **Results:** The analysis of blood samples, urine, bile and stomach contents, obtained after the autopsy of deceased persons, by using gas chromatography with mass spectrometry, in analytical manner confirmed the fact that paracetamol is a very common component of psychoactive substances poisoning. In our assay of samples we detected psychoactive substances (heroin, codeine, morphine, sertraline, diazepam), and almost all were found in the combination with paracetamol, indicating the poor quality of illicit drugs sold on the market. **Discussion:** Paracetamol (Acetaminophen) is a very common component in mixtures of street drugs. Such mixtures almost anyone can afford, but the very quality of these drugs has become extremely low, because it does not sell the pure substance, but is mixed with various medications. According to research Pantazia et al. the heroin mixture proportion of the heroin is very small so a lot of that mixture has only 3% of heroin, a large number of cases can be only 1% of pure heroin. Most of the time it replaces caffeine and paracetamol. According to the Risser et al. reason why acetaminophen component is present in these mixtures is because it can be purchased without a prescription, it is cheap, well tolerated by most people and shows no side effects. **Conclusion:** When we talk about illegal drugs, we must emphasize the fact that there is no quality control, or the composition of the drug. The composition of the drug purchased on the black market is still unknown to potential user. While reaching the final drug users it pass through many hands, and at each step something is added to increase earnings. Most often present additives or impurities in narcotic drugs that are added are caffeine, ephedrine, acetaminophen, acetylsalicylic acid (aspirin) and additives such as powders, cement and chalk.

Keywords: paracetamol, psychoactive substances (PAS), gas chromatography with mass spectrometry

1. INTRODUCTION

Paracetamol (Acetaminophen) besides aspirin is the most commonly used analgesic and antipyretic medication, used by millions of patients worldwide. While safe and effective in therapeutic doses, paracetamol is one of the most common causes of acute drug poisoning. It is an example that medicines that are in the world sold without a doctor's prescription can lead to death. Paracetamol from mid 1950 is widely used. It has emphasized security, safety of therapeutic dose with (hepato) toxicity of high doses especially in combination with alcohol (1). It is a chemical compound that belongs to the group of para-aminophenol compounds, phenacetin and acetanilide (2). It is an active metabolite of phenacetin, but unlike it in therapeutic doses has no carcinogenic effect (3). Acetaminophen and paracetamol have their roots in the common name N-acetyl-para-aminophenol, APAP (4,5). It is rapidly absorbed from the gastrointestinal tract and reaches a maximum plasma concentration about 1 hour after ingestion. Higher doses are absorbed more slowly. Only 2% of the dose is excreted in urine, 94% is conjugated with glucuronide and sulphate and thus as non-toxic excreted in urine (1).

To date, the mechanism of paracetamol action is not yet fully understood. It is believed that the main effect is by the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for cyclooxygenase-2 (COX-2). While its analgesic and antipyretic properties are comparable to those of aspirin and other NSAIDs, its anti-inflammatory properties and activities are often limited by several factors, one of which is the high level of peroxide present in inflammatory lesions. However, in some cases, are also observed some of peripheral anti-inflammatory activity which is comparable to other NSAIDs. Because of its selectivity for COX-2 it does not inhibit the production of thromboxane in significant extent which promotes blood clotting (6).

Today paracetamol became an integral part of a heroin mixture and is very popular at the street market. Pure heroin is white, of bitter taste and is soluble in water. However, the street "heroin" (hors, yellow) is usually a brownish color and contains only about 7-10% to a maximum of 20 percent of heroin, while the rest are other substances: paracetamol represented tremendously, more than 50% in the "street" heroin–dealers mix it with heroin because of its analgesic and antipyretic properties (relieves pain and reduces fever–calms). It does not show side effects, as is case with other analgesics–painkillers, such as e.g. Valium. The most commonly used routes of administration are sniffing, inhaling fumes heroin heated on foil or so called "Catching dragon", smoking or injecting. When ingested, produces very quickly strong psychological and physical dependence (7).

Due to the high concentration of not intoxicating substances (paracetamol) in relation to the content of diamorphine, abuse and overdose of "street" heroin is more dangerous than the pure form of opiate overdose (8). Consultants providing first aid in case of overdose has to pay attention to the effect of each component of the drug because the contents and concentrations of individual components vary between batches and must wait for the completion of toxicological analysis to know each of the effects of individual components and initiate the treatment. Acetaminophen presence causes serious, irreversible damage to the liver when taken in high doses for a long time. Very high doses of acetaminophen are capable of producing acute liver failure and death within hours, patients who survived the acute phase of this toxicity generally required dialysis and at the end liver transplant. Because of the numerous methods of preparing the user cannot know how much acetaminophen in each series and therefore cannot reliably determine safe dose. Doses of the last batch, which is not produced by the toxic effect of the lethal effect, can be produced in the next series (9).

2. GOAL

To identify paracetamol by analytical method of gas chromatography mass spectrometer (GC-MS) in postmortem material, together with psychoactive substances.

3. MATERIAL AND METHODS

For chemical-toxicological analysis was used biological material collected trough autopsy of 20 deceased, suspected to have died due to a psychoactive substances overdose. All received samples are stored at -20 °C until analysis at our laboratory. From processed 47 samples that were analyzed in the period 2014 to 2015 there was 19 blood samples, urine–19, 3 samples of stomach contents and 6 samples of bile content. Deceased were middle-aged, of which only 7 were female.

The tested samples were processed according to two methods of extraction. Extraction by XAD-2 resin and the extraction by the method of salting out with sodium tungstate. Extracts of the samples were then dissolved in chloroform and continued analysis at the analytical instrument. The reagents which are used both for extraction and for the instrument were obtained from Sigma-Aldrich manufacturer.

Identification of paracetamol and substances that can be found in the composition of a mixture of heroin, in the test biological samples has proven technique of gas chromatography with mass spectrometry (hereinafter referred to as GC-MS). GC-MS is a technique that combines the possibility of separation of gas chromatography with mass spectrometry detection capability. Mass spectrometry is an analytical technique widely involving the production and subsequent separation and identification of charged species according to their mass to charge ratio. The technique of GC-MS is a selective, sensitive and reliable, and is therefore considered a "gold standard" for determining the illicit drugs, medicines and psychoactive substances.

Used GC-MS instrument was an Agilent 7890A with he-

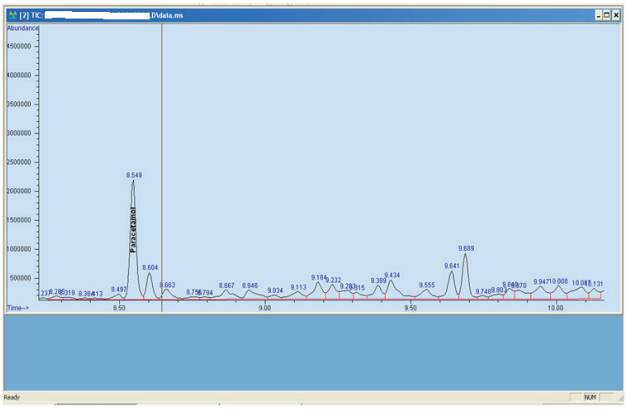

Figure 1. Blood sample chromatogram indicating the peak of paracetamol

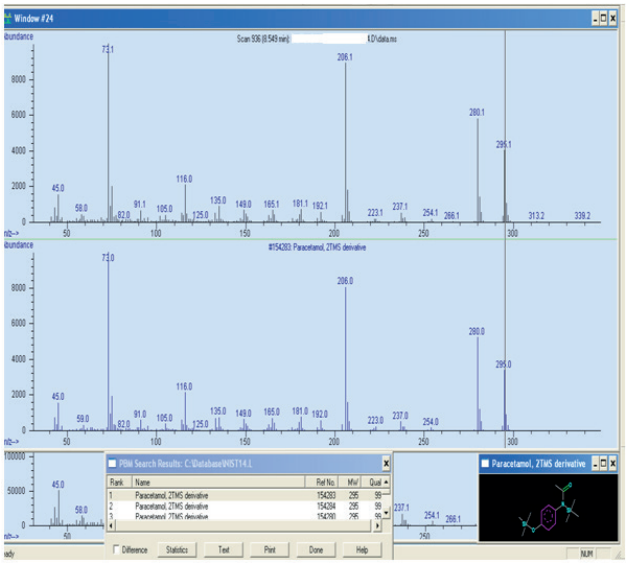

Figure 2. The upper part of the figure shows the mass spectrum of the peak of paracetamol from the blood sample, which was compared with mass spectrum of paracetamol library software instrument, which is shown in the lower part of the image.

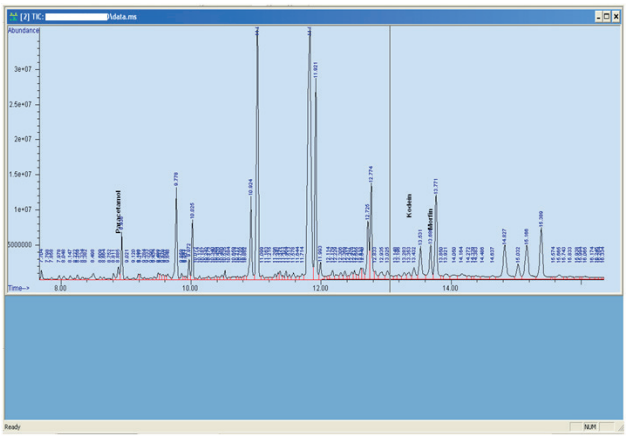

Figure 3. Blood samples chromatogram for all three components (paracetamol, codeine and morphine)

lium as the carrier gas. Used capillary column HP5-MS (30m x 0.25mm x 0,25µm) composition of 5% phenyl-methyl siloxane. The used injection volume was 1µl.

4. RESULTS

By the analysis of blood samples, urine, bile and stomach contents, applying gas chromatography with mass spectrometry, we aimed to develop a study that will validate analytical fact that paracetamol is very common component of psychoactive substances poisoning, although it has the appearance of harmless drug. Analysis confirmation of paracetamol found post-mortem material can be seen in Figure 2 where the upper part of the image is shown a mass spectrum of the peak of paracetamol from the sample is blood, which was compared with mass spectrum of paracetamol library software instrument, which is shown in the lower part of the image. By comparing ions the lower and upper part of the image, it is clear matching the major ions of paracetamol (206, 73, 280, 295, 116) which identify acetaminophen in the sample.

In our sample we detected psychoactive substances, and almost all, we found the combination of paracetamol with

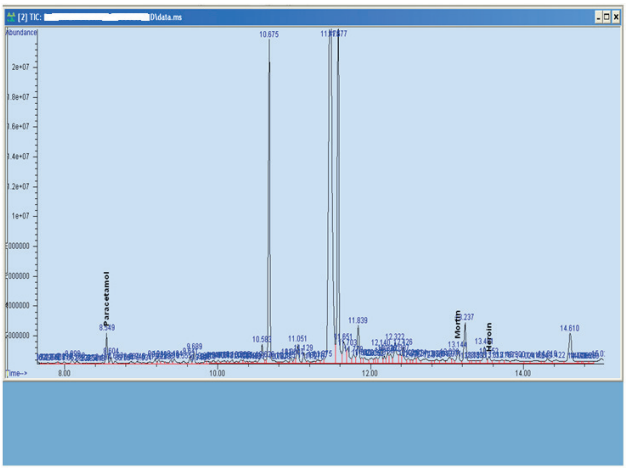

Figure 4. Chromatogram of second processed biological sample, with paracetamol, morphine and heroin.

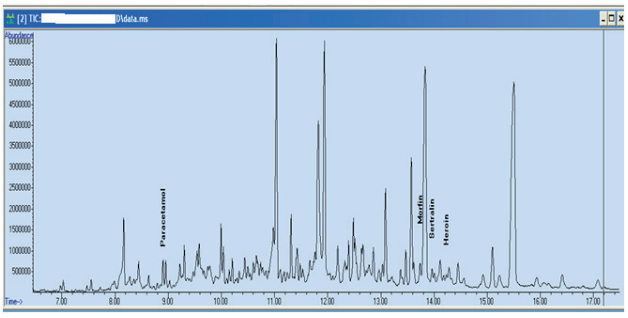

Figure 5. Urine sample chromatogram positive on paracetamol, morphine, heroin and sertraline

them, indicating the poor quality of drugs being sold on the market. For biological samples (blood, urine, bile and stomach contents) which we presented in Figures 3, 4 and 5 shows the presence of paracetamol together with codeine, morphine and heroin, and even in some cases sertraline.

5. DISCUSSION

Paracetamol (Acetaminophen) is a very common component in mixtures of street drugs. Such mixtures almost anyone can afford, but the quality of these drugs has become extremely low, because dealers do not sell the pure substance, but it is mixed with various medications. According to research of Pantazia et al. in the heroin mixture proportion of the heroin is very small so a lot of that mixture has only 3% of heroin, and in large number of cases can be only 1% of pure heroin. Most of the time it is replaced by caffeine and paracetamol (7,8). According to the Risser et al. reason why acetaminophen is component of these mixtures is that it can be purchased without a prescription, it is cheap, and well tolerated most people without side effects. It is probably used for "cutting" the heroin, as it says in the jargon, so from small amount of pure drug is obtained greater amount, which is sold on the street (9). According to Hendrickson and colleagues, except that paracetamol, sugar and chalk are used to obtain a larger quantity of heroin, they increase the toxicity to the central nervous system, which is becoming more of a problem (10,11). Dark and Rose describe also lethal poisoning by heroin, part of which is a paracetamol along with methadone and caffeine. In our test sample we showed that

the quality of drugs in our market is no different from other countries in Europe.

6. CONCLUSION

Drug use is growing in the world every year, together with the number of deaths from overdose. Number of drug addicts in Bosnia and Herzegovina is growing together with the amount of drugs that occurs in the country. A sudden increase in the number of consumers of drugs, including people who sell drugs, in Bosnia and Herzegovina was particularly obvious during the period after the war. In Bosnia and Herzegovina there is about 7,500 registered " injecting drug use," which is less than half compared to the countries in the region.

The reaction to the drug depends on the amount of the drug, its quality, or purity, or whether it is mixed with other, less expensive substances, which can be extremely dangerous. The person who takes illegal drugs practically never knows what he/she introduce into the body. When we talk about illegal drugs, we must emphasize the fact that there is no quality control and the control of drug composition. The composition of the drug to be purchased on the black market is still unknown potential user. While reaching the final drug users pass through many hands, with her each by something added to increase earnings. Most often present additives or impurities in narcotic drugs that are added to the more expensive drugs sold as caffeine, ephedrine, acetaminophen, acetylsalicylic acid (aspirin) and additives such as powders, cement and chalk. In our State the biological samples, we confirmed the presence of paracetamol as part of psychoactive substances (PAS).

CONFLICT OF INTEREST: NONE DECLARED

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