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Pharmacy

STUDY OF AMPHETAMINES IN DISCIPLINE "FORENSIC CHEMISTRY" BY UKRAINIAN STUDENTS OF PHARMACY

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Abstract: Quantity of pharmaceutical preparations, which used at the medicine and different areas of economy increased during the development of chemistry. These substances can provide poisonings in certain causes. "Forensic chemistry" — is a science, which studies methods for isolation, purification, qualitative and quantitative analysis of toxic and poisonous substances and their metabolites in objects of various origins: in the body, animal and plant biological materials, industrial waste, emissions of waste water, soil and agricultural crops, etc. The aim of this study is to show the importance of usual chemical substances as amphetamines – derivatives of phenyl alkyl amines (*ATS*), which are strong toxic substances, during studying of "Forensic chemistry" at the higher medical and pharmaceutical universities in Ukraine.

UDC Classification: 54.01:615 (075)

Keywords: forensic chemistry, phenyl alkyl amines (*ATS*), amphetamines, poison.

Introduction

Conclusions of toxicological chemists on the presence and the amount of poison in the investigational material provide a great support for forensic experts. Conclusions of toxicological chemists, hygienists, pharmacologists about high toxicity of certain pharmaceutical products and substances used in the national economic enterprise, serve as the basis for raising the issue of removal of these substances from wide use or introduction changes into storage conditions and their over-the-counter dispense to the population.

Methods of forensic chemistry helps to define and control the maximum permissible concentration (MPC) of toxic substances presents in water and air and develop standards of pesticide residues and other toxic substances in food according to Moffat et al (2011), Bayerman (1987), Busari et al (2009), Ellenhorn (2003), Knunyants et al (1992), Welchinska (2017). The strategic line of pharmaceutical graduates' preparing is a complex approach used for the studying of pharmaceutical chemistry and forensic chemistry courses together. It is caused by the fact that future professional activities of new pharmacists will deal with solving the tasks related to all mentioned courses (European Association for Quality Assurance in Higher Education. Helsinki, 2005;

Communiqué of Conference European countries' Ministers which responsible for higher education, 2010).

One of the most important groups of toxic substances being studied at the forensic chemistry is the group of substances, which are isolated from biological material by extraction – medications and poisons Amphetamines.

Amphetamines (chemical structure — derivatives of phenyl alkyl amine: amphetamine, methamphetamine and others) — are a group of chemical compounds, many of which have been used as medications with a stimulating effect, characteristic of which are euphoria and the development of tolerance to them, and exceeding the dose causes aggressiveness and the threat of antisocial behavior.

Representatives of this group of compound are amphetamines: amphetamine (Benzedrine, biphetamine, phenyl isopropyl amine), methyl amphetamine, methamphetamine («batu», desoxyn, phenyl isopropyl-N-methyl-amine hydrochloride), dextro-amphetamine (dexamyl, dexedrine), MDA (3,4-methylenedioxyamphetamine), MDMA («ecstasy», 3,4-methylenedioxymethamphetamine), MDEA (3,4-methy-lenedioxy-N-ethylamphetamine), MMDA (3-methoxy-4,5-methylenedioxyamphe-tamine) and other.

Amphetamines — are compounds with structure similar to phenyl ethylamine or phenyl isopropyl amine, added side chains promote different levels of catecholamine and serotonin activity. Amphetamine was discovered in 1887 and exists as two enantiomers (*D*- and *L*-). It properly refers to a specific chemical, the raceme free base, which is equal parts of the two enantiomers, left-amphetamine and write-amphetamine, in their pure amine forms. Historically, it has been used to treat nasal congestion and depression according to Holstege et al (2009), Reisene et al (1996), United Nations Office on Drugs and Crime (2006).

Dextroamphetamine, methylphenidate are used in the treatment of narcolepsy and attention-deficit hyperactivity disorder (ADHD). Fenfluramine, dexfenfluramine were used for weight loss but later recalled due to cardiopulmonary toxicity when used in combination with Phentermine. MDMA began to used therapeutically in 1970s after the chemist *Alexander Shulgin* introduced it to psychotherapist *Leo Zeff*.

Methamphetamine in a smokable form is referred to as «ice». Amphetamines are popular among the persons, poorly adaptable to social conditions, it is difficult to get used to a new lifestyle. These drugs activate the internal excitation mechanisms. Antisocial individuals and people with schizophrenia are particularly prone to amphetamine use. The use of amphetamines as not for medical purpose is typical for individuals seeking to improve their performance. Trying to reduce the dose of taking amphetamines causes a person drowsiness and depression.

MDMA is a psychoactive amphetamine derivative, when amphetamine abuse observed reactions: dysphoria, tachycardia, hypertension, high blood pressure. MDMA is in 1-6 times more potent than mescaline and 1.5—3 times less toxic than MDA. Mescaline is a hallucinogenic alkaloid present in peyote that is structurally related to amphetamines and is a 5-HT_2 agonist.

Methods

The basic criteria and requirements for the specialist accreditation in higher medical institution with the specialty "Pharmacy," under conditions of the Bologna System in Ukraine and according to instructions on quality assurance (ENQA), are: staff provision for specialists' training by declared specialty; material and technical base; educational and instructional support; information support; qualitative characteristics of specialists' training.

The main methods used during the discipline studying are pedagogical, psychological, statistical, chemical, analytical, biochemical ones. The testing control by basic topics contains the questions related to general characteristics of poisons: objects of study, the ways of studied material isolation, general regularities of toxic dynamics and toxic kinetics, general methods of methods of qualitative detection and quantitative definition, the scheme of metabolism on the phase's I and II. The test control by specific topics contains the questions related to each representative of the class.

Results and Discussions

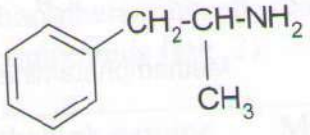
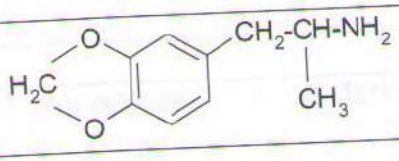
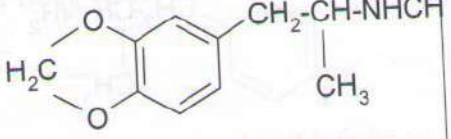
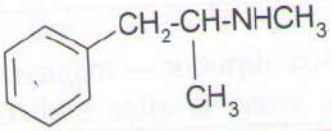
It should be noted that these reactions were not presented schematic in the literature. In the academic literature (Welchinska, 2017) for students of medical university for the first time describes the processes of metabolism and reaction qualitative detection and quantification in the form of the proposed schemes by author.

The main consideration of the material begins to explore: metabolism, qualitative and quantitative methods for analysis of biological material for the content of amphetamines and some metabolites.

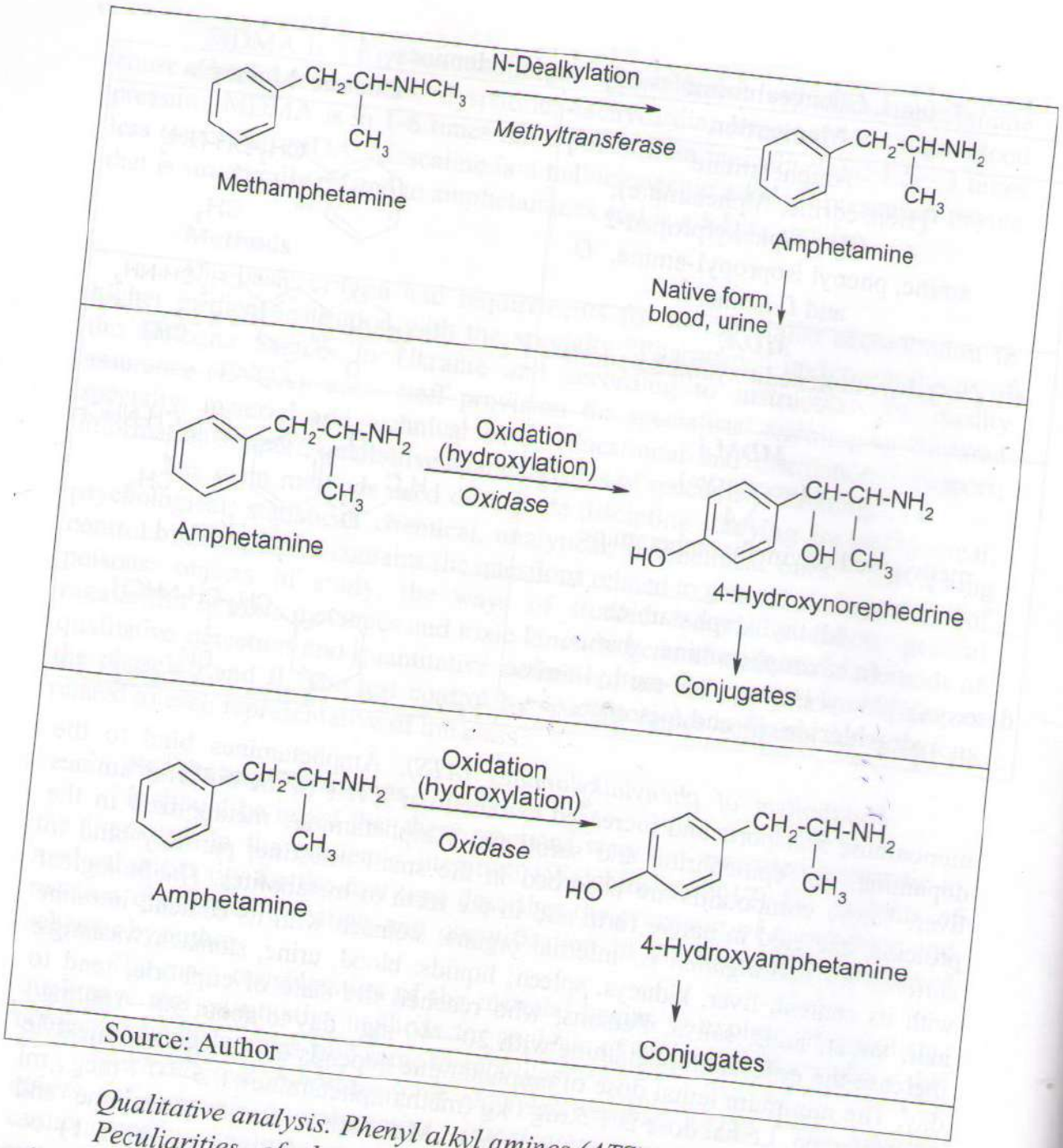
Toxicological significance of phenylalkylamines (ATS). The adverse effects of chronic amphetamine intoxication are anorexia, paranoia, cardiomyopathy, pulmonary hypertension, vasculitis, aortic and mitral regurgitation.

The clinical signs of acute amphetamine intoxication are agitation, anxiety, AMS (altered mental status), mydriasis, hypertension, tachycardia, diaphoresis, tremor, muscle rigidity, hyperthermia, and seizures. There is the sympathomimetic toxidrome. Chemical formulas of these medications showed in tab. 1.

Table 1. Chemical formulas of phenylalkylamines

Medication	Chemical formula
Amphetamine (Benzedrine, Biphetamine), (*RS*)-1-phenylpropan-2-amine, phenyl isopropyl-amine, *D*- and *L*-isomers	
MDA, 3,4-methylenedioxyamphetamine	
MDMA («ecstasy»), 3,4-methylenedioxymethamphetamine	
Methyl amphetamine (methamphetamine, «batu», esoxyn), phenyl isopropyl-N-methyl-amine hydrochloride, *D*- and *L*-isomers	

Metabolism of phenylalkylamines (ATS). Amphetamines bind to the monoamine transports and increased extracellular levels of the biogenic amines dopamine, nor epinephrine and serotonin. Amphetamines metabolized in the liver. These compounds are absorbed in the small intestine; partially bind to proteins, excreted in native form and in the form of metabolites. *The biological material for investigation is:* internal organs: stomach with its content, intestine with its content, liver, kidneys, spleen; liquids: blood, urine, stomach washings; hair, sweat, nails, saliva. Persons, who reached the state of euphoria, tend to increase the dose of amphetamine with 20—40 mg / day to about 50—150 mg / day. The minimum lethal dose of amphetamine depends on the age and lifestyle of the person. Lethal dose is 1.5 mg / kg (methamphetamine); 0.5—0.7 mcg / ml in blood, 28 mg / kg (amphetamine). Metabolism for amphetamine and methamphetamine takes place in the liver in the following ways: 1) N-dealkylation; 2) oxidation (hydroxylation); 3) deamination; 2) conjugation (glucuronides, sulfates):



Source: Author

Qualitative analysis. Phenyl alkyl amines (ATS).

Peculiarities of the isolation: amphetamines extracted by organic solvents from aqueous alkaline solutions.

Reactions of qualitative analysis of phenyl alkyl amines

Color tests: with Marqui's reagent, with Simon's reagent, with Chen's reagent, with Gallic acid.

With Marqui's reagent test — allows the distinction between amphetamine and its ring-substituted analogues.

With Simon's reagent ' test — it is for secondary amines (methamphetamine).

With Chen's reagent test — amphetamine, methamphetamine, MDA,

MDMA do not react with Chen's test reagent, but it used to distinguish ephedrine, pseudoephedrine, norephedrine.

With Gallic acid test — provides a simple means for the distinction of MDMA, MDA, MDEA from amphetamine or methamphetamine. It reacts specifically with methylenedioxy substituted aromatic compounds (tab. 2):

Table 2. Color tests for phenylalkylamines

	Amphetamine	Methamphetamine	MDA	MDMA
With Marqui's reagent	An orange,	An orange,	A dark-blue → black	
With Simon's reagent	—	A deep blue	—	A deep blue
With Chen's reagent	—	—	—	—
With Gallic acid	—	—	A bright to dark green	

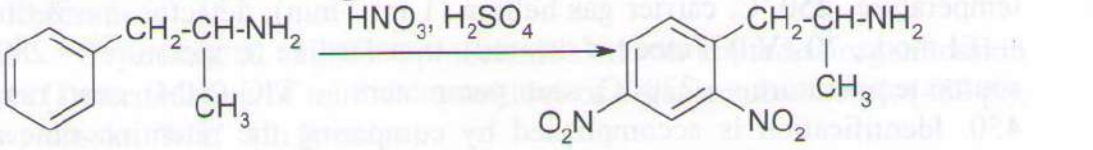
For compare: ***ephedrine*** gives test with Chen's reagent — a purple color.

Anion tests (the solubility of compounds and their salts in water and solvents systems) (tab. 3):

Table 3. The solubility of phenyl alkyl amines

Amphetamine base: slightly soluble in H_2O, methanol, ethanol, diethyl ether, chloroform
Amphetamine hydrochloride: soluble in H_2O, methanol, ethanol, chloroform; insoluble in diethyl ether
Amphetamine phosphate: soluble in H_2O; slightly soluble in methanol, ethanol; insoluble in diethyl ether, chloroform
Amphetamine sulfate: soluble in H_2O; slightly soluble in methanol, ethanol; insoluble in diethyl ether, chloroform

Reactions on benzene ring: the reaction of nitration:



Amphetamine — Nitro derivatives of amphetamine, an yellow colour

Source: Author

Quantitative determination of amphetamines (ATS) is performed by the following instrumental methods:

— Chromatography: ***TLC, HPLC,*** gas chromatography — flame ionization detector ***(GC—FID),*** gas chromatography — mass spectrometry ***(GC—MS),*** solid phase-micro extraction — gas chromatography ***(SPME—GC),*** gas chromatography — Fourier transform infrared spectroscopy ***(GC—FIR);***

— Spectral methods: Fourier transform infrared spectroscopy ***(FTIR),*** ^{1}H-nuclear magnetic resonance ***(NMR)***;

— *Capillary electrophoresis* ***(CE)***.

Thin layer chromatography (ATS). TLC plates (stationary phases): silica gel G with a layer thickness of 0.25 mm, and containing an inert indicator, which fluoresces under UV light wavelength 254 nm; solvent system or mobile phases (methanol—ammonia conc., 100:1.5; ethyl acetate—methanol—ammonia conc., 85:10:5; cyclohexane—toluene—diethyl amine, 75:15:10). ***Visualization reagent***: UV light at 254 nm — purple spots on an otherwise green-fluorescent plate; ninhydrin reagent — result in violet or pink spots; acidified potassium iodoplatinate reagent — result in light blue spots; Fast Black K — result in violet to orange or orange-red spots; fluorescamine reagent (Fluram) — result in fluorescence spots.

Gas chromatography — flame ionization detector ***(GC—FID).*** Detector: FID (or NPD); column: DB-5 (5 % phenyl 95 % dimethylpolysiloxane), DB-1 (100 % dimethylpolysiloxane); length: 10—30 m, ID 0.20—0.53 mm; 0.10—0.50 μm; carrier gas: nitrogen (0.8 ml / min), helium, hydrogen (1—1.2 ml / min for He or H_2); split ratio: 20:1 to 50:1; column temperature: 60—90 °C to 280 °C; injector temperature: 210—250 °C; detector temperature: 310 °C. Identification is accomplished by comparing the retention time of the analyte with that of a reference standard.

The elution order is as follows:

Amphetamine < methamphetamine < pseudoephedrine < ephedrine < MDA < MDMA < MDEA < MBDB.

Gas chromatography — mass spectrometry ***(GC—MS).*** Operating conditions. GC oven conditions: same as for GC analysis; column: DB-5 (5 % phenyl 95 % dimethylpolysiloxane), DB-1 (100 % dimethylpolysiloxane), 0.25 mm x 30 m x 0.25 μm; inlet: mode Split/Splitless (constant flow or pressure), temperature 250 °C, carrier gas helium (1 ml / min); detector: ionization mode — EI mode, 70 eV (Cl mode if desired), transfer line temperature — 280 °C, ion source temperature — 230 °C, scan parameters — TIC (SIM), scan range 35—450. Identification is accomplished by comparing the retention time at mass spectrum of the analyte and a reference standard.

(HPLC). Detector: diode array detector (rapid scanning or variable wavelength, UV 200—210 nm); stationary phase: C_8 or C_{18} with 5 μm particle size; column length and diameter: ≤ 30 cm and ≤ 5.0 mm; pre-column: diameter 2—4 mm, length 25 mm, reversed phase C_8 or C_{18}; column temperature 15—35

; mobile phase buffer: phosphate buffer pH 2.0—3.2; mobile phase organic odifier: methanol or acetonitrile between 2 % and 20 %; flow rate: 0.1—2.0 ml min; injection volume 1—100 μm. Identification is accomplished by mparing the retention time of the analyte with that of a reference standard and using multiple UV wavelengths or diode array or rapid scanning UV etection.

(CE). It is similar to ***HPLC***, requires no derivatisation or extraction eps. In contrast to ***HPLC, CE*** offers higher resolving power for the analysis of ese solutes, which translates into faster analysis.

IR-spectroscopy: in the 800—1600 cm^{-1} region.

^{1}H-nuclear magnetic resonance (***NMR***): enables the analyst to nequivocally distinguish between different ring-substituted amphetamine lerivatives; although certain substitution patterns resemble one another in the rea corresponding to the protons of the alkyl side chain, the integrated spectrum nd the pattern of the aromatic proton singles allow their distinction from one nother. Liberate the free base of the ***ATS*** in situ by the addition of 20—30 mg of solid K_2CO_3 and 0.5 ml $CDCl_3$ and record the spectrum of the free base.

Immunoassay and radioimmunoassay methods. Test on reduction of a number of leucocytes.

Conclusion

Thus, Amphetamines are a well-known medical preparations and poisons that are studied in the discipline «Forensic chemistry» as a «medicinal» poisons, which requires from the future specialists the carefully study the methods of qualitative and quantitative determination of this substance not only in medical forms of preparations and in substance, but also in biological objects. We propose a logically consolidated system in the study of these medications, starting their consideration with data of use in medicine, toxicological significance, metabolism, and ending with chemical and physicochemical methods of determination. A modern pharmacist should be armed with a wide range of knowledge about the activity of medications. Deep knowledge of the possible toxic effects of drugs, the interaction of drugs, toxic metabolites, which are products of «lethal» synthesis, the symptoms of poisoning with "medicinal" poisons will help prevent poisoning and, even, the death of the patient.

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