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Qualitative Examination Of Amfetamine And Ephedrine In Urine In Doping Using Gas Chromatography-Mass Spectrometry Method

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ABSTRACT

Amphetamines and ephedrine are stimulant class doping drugs which have the effect of reducing fatigue and drowsiness, increasing alertness, physical strength, mental sense of competition and competitive attitude. Analysis of doping drugs in urine that has been recommended by the International Olympic Committee, including Gas Chromatography-Mass Spectrometry. Derivatization is carried out to change the polarity of the analyte, making it easier to become a gas and stable to heating. This study was conducted for qualitative analysis of amphetamine and ephedrine, using an HP5MS column containing non-polar phenyl methyl siloxane, temperature 100-250oC with a temperature increase of 10oC/minute. Then it was extracted with a mixture of diethyl ether-ethylacetate (1:1) in an alkaline medium pH 8, the organic layer was evaporated then derivatized with trifluoro-acetic acid-anhydride and ephedrine with acetic-anhydride-pyridine (3:2). The final results showed that the retention times of amphetamine and ephedrine were 10.47 and 4.9 minutes. While the derivatives are 13.34 and 5.50 minutes. Identification using mass spectrometry for each compound showed that the mass spectra matched the Ref. and the amphetamine and ephedrine structural similarity index was 96% and the detection limit for each compound was 2 ug/ml.

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1. INTRODUCTION

In the field of sports, doping is used to increase ability artificially so that victory is obtained in a match. Doping includes (a) drug use, (b) doping methods such as blood doping, chemical, physical and pharmacological manipulation, (c) restrictions on the use of certain drugs such as alcohol, local anesthetics and corticosteroids. (3,11). Amphetamines are one of the strong sympathomimetic amines in stimulating the central nervous system. While ephedrine is a stimulant class used for people with narcolepsy, eye drops and nose drops.

Doping checks can be carried out on various body fluids such as urine, blood, saliva, sweat and vomit. The body fluid that is often used is urine, which contains dissolved organic and inorganic compounds and is excreted through the kidneys. The largest part of the substances in the urine is the result of metabolism in the body and a small portion is non-metabolic. Analysis of doping drugs for amphetamine and ephedrine which belong to the group of nitrogen-containing compounds that are freely excreted in the urine. Derivatization is carried out to improve the performance of an analysis by changing molecules with polar functional groups into less polar derivatives, then evaporate and eluted

as symmetrical peaks. The type of reaction is trimethylsilylation(TMS), the reactant is trimethylchlorosilane. The reaction for trimethylsilyl derivatives occurs without the formation of by-products. (3,8,11)

The results of the analysis of a mixture of 40 standard types of doping drugs from the stimulant, analgesic-narcotic and beta-blocker groups using gas chromatography-mass spectrometry (KG-MS) technique using a 17 m x 0.2 mm column (5% phenylmethylsiloxa) are non-polar, derivatizing reagent The selective ones used were N-methyl-N-trimethylsilyl-trifluoro-acetamide (MSTFA) and N-methyl-bis-trifluoro-acetamide (MBTFA) providing good separation of each compound according to its retention time.(7,16).

In the doping analysis for most of the compounds, only qualitative analysis was carried out and only certain compounds needed to be determined. The use of mass spectrometry as a detector in gas chromatography can increase the specificity and sensitivity of the analysis results. The molecules that have been separated in the chromatography column are bombarded with electrons to form ionic molecular fragments that are characteristic for each molecule of the substance. Because the fragmentation pattern of each molecule of a characteristic substance is a fingerprint for identification with reference to the reference spectrum found in the gas chromatography-mass spectrometry library. (5,10,12). The purpose of this study was to identify substances used for doping such as amphetamine, ephedrine by gas chromatography-mass spectrometry using an HP5MS capillary column in urine.

2. METHOD

A. Material

The samples were amphetamine sulfate, ephedrine hydrochloride, all obtained from the Food and Drug Administration (PPOM). Ingredients: methanol, diethylether, ethylacetate, chloroform, sodium hydroxide, ammonium hydroxide, sodium sulfate, cysteine, acetonitrile, acetic acid anhydride, methyl orange, trifluoroacetic acid(TFA), trifluoro-acetic-anhydride (TFAA).

B. Tools

The tools needed consist of an electric analytical balance (Mettler), KG-SM type HP 1800 C GCD seriesII (Hewlett Packard), 1 ul micro syringe and glassware.

Preparation of mother liquor

A number of amphetamine sulfate and ephedrine hydrochloride were weighed exactly 50 mg each, then dissolved with methanol in a 50 ml volumetric flask, diluted until a concentration of 1000 ppm was obtained.

Preparation of standard solutions

Pipette 1 ml of each of the 1000 ppm mother liquor, dilute with methanol in a 50 ml volumetric flask. The volume of each was adjusted until a concentration of 20 ppm was obtained. To determine the limit of detection, dilution is carried out until the smallest concentration that can be detected is obtained.

Optimum conditions determination

To determine the optimal conditions, the tool is run for 2-3 hours, then tested for optimization as required. The fixed parameters are carrier gas: helium, gas flow rate: 1.0 ml/minute, inlet temperature: 230oC, detector temperature: 250oC, column temperature: 90oC-250oC (25 m long), detector activation time: 3.0 minutes, volume of solution injected: 1 ul. Standard solution of 20 ppm each was injected, temperature rise 10oC/minute. System verification was carried out by injecting perfluorothributylamine compound. Amphetamine and ephedrine compounds can be separated and detected at 190oC and 200oC. The end result is a chromatogram of each compound.

Mass spectrum

The amphetamine and ephedrine compounds after being identified by gas chromatography obtained the separation peaks on the chromatography column. The pattern of ion fragmentation in the mass spectrum

is unique for each compound. The mass spectrum was compared directly to a gas chromatography-mass spectrometry library.

Extraction

Extraction was carried out to separate interfering compounds from analytes using organic solvents. Derivatization by selective reagent aims to change the polarity and stability of the analyte to heating.

Extraction of amphetamine and ephedrine compounds

Pipette 10 ml of urine sample into a separatory funnel and add 10 ml of saturated sodium sulfate. 1 ml sodium hydroxide IM and extracted twice with 10 ml each of the diethylether-ethylacetate mixture (1:1) for 3 minutes. The organic phase was separated and centrifuged at 2500 rpm, then acidified with a few drops of 37% hydrochloric acid, evaporated to a volume of approx.
1 ml.

Derivatization

A total of 40 ul of the evaporated extract was flowed with nitrogen gas, then derivatized with 40 ul of trifluoro-acetic-anhydride and 40 ul of ethyl acetate, heated for 15 minutes at 60oC. After being derivatized, evaporated with nitrogen gas and dissolved with isopropanol, injected into gas chromatography-mass spectrometry.

3. RESULTS AND DISCUSSION

Analysis of doping drugs based on their physical and chemical properties, for group one, namely volatile compounds containing nitrogen which are excreted freely in the urine (stimulants, narcotics and beta blockers). After extraction with organic solvents under alkaline conditions without water then identified. Stimulant class drugs such as amphetamine and ephedrine can be detected by gas chromatography-mass spectrometry. The reaction is as follows:

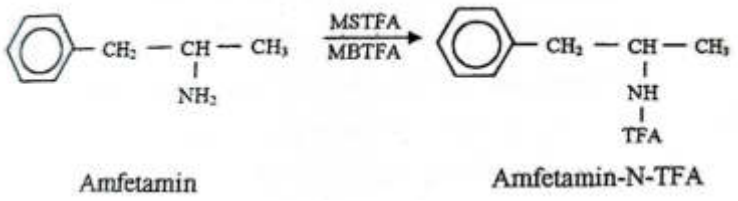


Figure 1. Amphetamine Derivatization

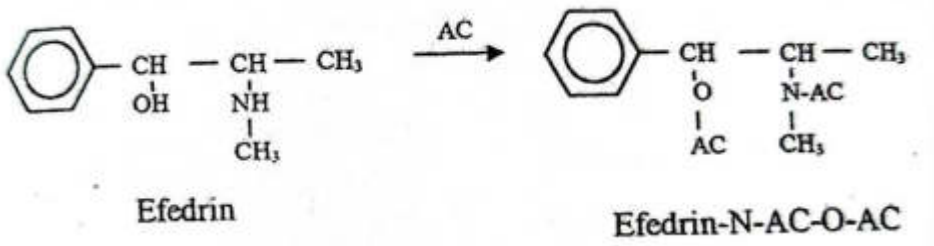


Figure 2. Derivatization of Ephedrine

Amphetamine and ephedrine in urine can be determined by gas chromatography-mass spectrometry method, HP5MS column, 25 m long, containing 5% phenyl methyl siloxane, specifically intended for alkaloids, drugs and compounds containing halogens, then proceed to the extraction and derivatization stages. Identification of the mass spectrum was carried out on standard solutions, blanks of urine added with standards and user urine samples. The result is as follows:

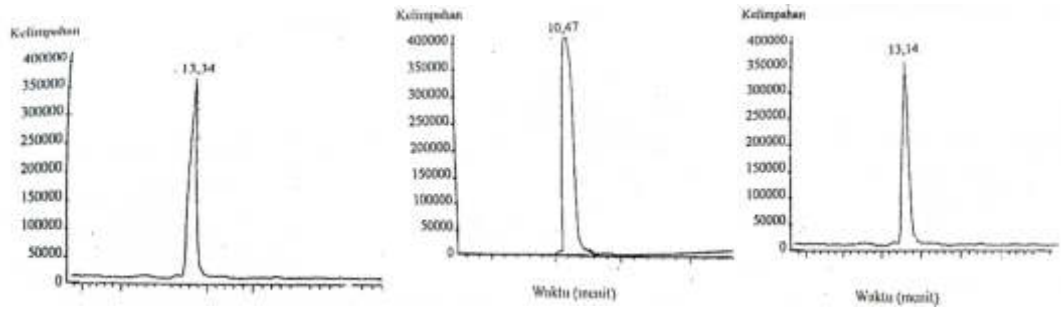


Figure 2. Chromatogram and standard retention time of amphetamine

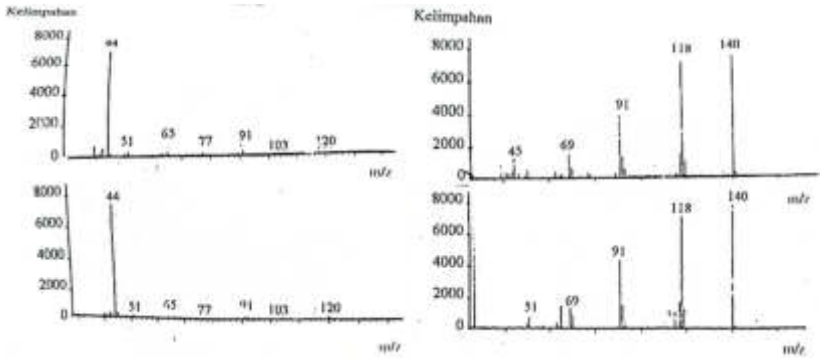
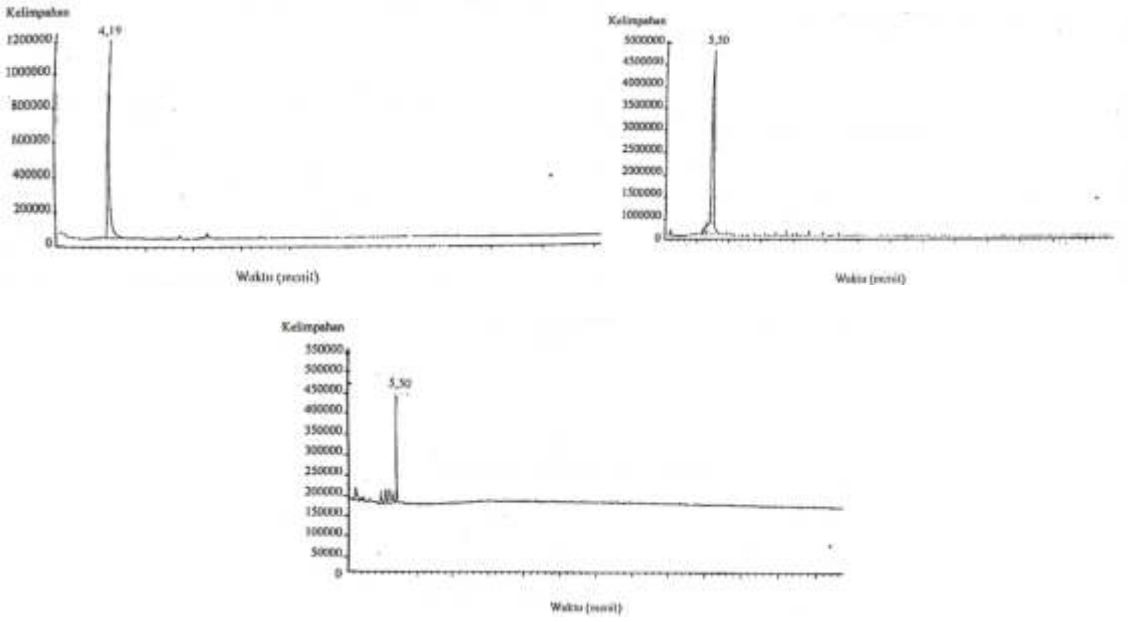


Figure 3. Standard mass spectrum of amphetamine and mass spectrum from gas chromatography-mass spectrometry library



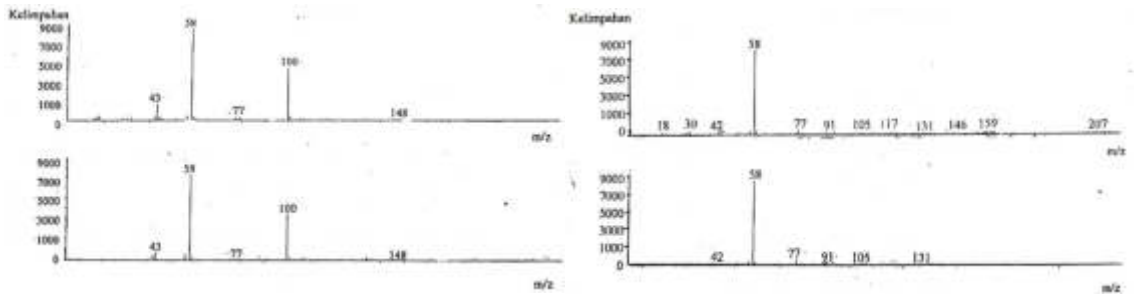


Figure 4. Chromatogram and standard retention time of ephedrine

Discussion

Analysis using gas chromatography technique is based on the distribution of the sample between the two phases. One of the phases is the stationary phase which has a relatively large surface area and the other phase is the gas eluting the separated molecules which are retained on the stationary phase by virtue of their coefficient of distribution so that a number of distinct bands are formed in the carrier gas. These component bands leave the column with the carrier gas stream to reach the detector and are recorded as a function of time and thus show, set many separate components.

Derivatization aims to improve the performance of an analysis by changing polar functional group molecules into less polar derivatives, which then evaporate and eluted as symmetrical peaks. In addition, it can also increase the thermal stability of compounds and their ability to be detected.

Mass spectrometry acts as a detector which has high sensitivity and selectivity and can be operated on various methods. The mass spectrum of each detected molecule is stored in a computer system which is characteristic for a particular compound. The fragmentation pattern shows peaks related to electrons and the loss of certain groups in the molecule such as CO2, NH and others which provide information about a molecule. The mass spectrometer can provide library data in its computer system regarding thousands of mass spectra of compounds so that the spectra of unknown compounds can be compared.

In this study amphetamine and ephedrine in urine were identified by gas chromatography-mass spectrometry through extraction and derivatization steps. Optimum conditions are determined by varying the column temperature, namely the initial temperature, temperature rise rate and isothermal time. The instrument was run for 2-3 hours, the selection of the column temperature program was carried out by injecting a 20 ppm standard solution and each peak was compared with the mass spectrum in the gas chromatography-mass spectrometry library data. Identification of the mass spectrum was carried out on a standard solution, a blank of urine added with a standard, then a sample of the user's urine. Sampe was extracted with diethylether-ethylacetate (1:1) and the reagents for amphetamine were trifluoro acetate (TFAA) and for ephedrine acetic acid anhydride-pyridine (3:2). The limit of detection was obtained by diluting various concentrations from 20 ppm standard salt to the smallest detectable concentration.

Analysis of the mass spectrum of compounds containing n-base groups is fragmented into separate molecular ions based on the characteristic m/z for each compound. Amphetamine and ephedrine standards as well as blank urine added to standards and user urine samples resulting from extraction and derivatization show the same molecular ion fragmentation pattern as Ref. on gas chromatography-mass spectrometry. For amphetamine-TFA it has a retention time of 10.47 to 13.34. Then the m/z values were from 77, 65, 91 to 140, 118, 91, 69. For ephedrine-AC the retention time was from 4, 19, to 5.50, and the m/z values were from 58, 77, 91, 105, 117 to 58, 100, 43, 134. From the user's urine sample, the m/z value is the same as in the Gas Chromatography-Mass Spectrometry Library. To improve the performance of the analysis, derivatization was carried out with the aim of changing polarity, making it easier to become gas and relatively stable to heating, the results showed that there was a shift in the retention time and the m/z value of each ion fragment but did not eliminate

the specificity. The similarity index for amphetamine and ephedrine is 96% and the detection limit is 2 ug/ml.

4. CONCLUSION

Amphetamine and ephedrine compounds in urine were identified qualitatively using gas chromatography-mass spectrometry with HP5MS capillary column containing 5% phenylmethylsiloxane, 25 m long, 0.2 mm in diameter, non-polar with trifluoro-acetic-anhydride and acetic anhydride-pyridine derivatizers (3:2) to increase the sensitivity and each compound has a shift in retention time and ion fragmentation pattern exactly the same as the reference from gas chromatography-mass spectrometry with a similarity index of 96% and a detection limit of 2% each.

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