Indonesian Journal of Science and Education
Volume 07, Nomor 01, 2023, pp: 29-35
p-ISSN:2598 –5205, e-ISSN: 2598-520, DOI: 10.31002/ijose.v7i1.207
e-mail: ijose@untidar.ac.id, website: https://journal.untidar.ac.id/index.php/ijose/index

GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GCMS) QUALITATIVE ANALYSIS FOR MORPHINE, HEROIN AND CODEINE USED IN DOPING

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Received: August, 24th 2022 *Revised: February, 20th 2023* *Accepted: April, 22th 2023*

ABSTRAK

Three types of doping drugs, morphine, heroin, and codeine were analyzed qualitatively in urine using GCMS: HP5MS column containing a nonpolar substance, phenylmethyl siloxane, with a temperature range of 90-250ºC and a rate of increase of 10ºC/minute. Diethyl ether was used to extract morphine, codeine, and heroin. The extractions were performed at pH 8, the organic phase was evaporated, followed by derivatization. Morphine and its derivatives were treated with a solution of N-methyl-N-trimethylsilyl-trifluoroacetamide and N-methyl-bis-trifluoroacetamide. The results of the gas chromatographic analysis showed that the retention times of morphine, heroin, and codeine the retention times were 8.01, 7.32, 6.21 minutes, while those of their derivatives were 8.10, 8.10, 7.95 minutes respectively. The identification of each drug using mass spectrometry revealed that their mass spectra were identical to the standard. The mass spectra of the derivatives: morphine-TMS and codeine-TMS showed a resemblance index of 93% respectively compound to the standard, with a detection limit of 2 ug/ml.

Kata Kunci: doping, gas chromathography mass spectroscopy (GCMS), codeine, morphine, heroine

INTRODUCTION

Doping was defined as the administration of an abnormal amount of a foreign substance or physiological substance to a competing athlete by any means or administered improperly with the specific purpose of dishonest artificial ability enhancement, according to the results of the International Scientific Congress of Sports in (Handelsman, 2020; Reardon & Creado, 2014). In the classification of doping drugs, amphetamines and ephedrine are stimulants that increase physical and mental strength, increase alertness, and reduce fatigue and drowsiness, whereas morphine-derived compounds are narcotic analgesics that relieve pain and cause a sense of calm (Bahrir, 2019; Bird et al., 2016). In general, the analytical methods recommended by the International Olympic Committee were high performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography mass spectrometry (GCMS) for doping drug analysis (Cadwallader & Murray, 2015; Kementerian Kesehatan RI, 2020; Politi et al., 2005). The results of a GCMS analysis of a mixture of 40 types of standard doping drugs from the stimulant, analgesic-narcotic, and beta-blocker groups using a column of 17 m x 0.2 mm (5% phenylmethylsiloxane) are non-polar. The selective derivatives reagents used were N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and N-methyl-bis-trifluoroacetamide (MBTFA), which provided for good separation of each compound based on their retention times

(Ahrens et al., 2016; Darmapatni et al., 2016; Thevis & Schanzer, 2005).

Urine is a bodily fluid containing organic and inorganic compounds excreted by the kidneys (Rose et al., 2015). The drugs will exist as a metabolite or bound form, with relatively small amounts of the intact form present. Identification of doping drugs is generally indicated for drugs in unchanged form, though the detection of metabolites will help strengthen conclusions about the drug given (Handelsman, 2020; Shen et al., 2016). Derivatization was used to improve the analysis, particularly in the identification of doping drugs from urine using GCMS, by changing the polarity of the analyte so that it evaporated easily and eluted as separate peaks on the column while remaining stable to heating (Candraningrat et al., 2021; Darmapatni et al., 2016; Politi et al., 2005). Only qualitative analysis was performed in the doping analysis for the majority of the compounds, and only a few compounds needed to be determined (Candraningrat et al., 2021).

The use of mass spectrometry as a detector in gas chromatography can increase the specificity and sensitivity of the analysis results. The molecules separated in the chromatographic column are bombarded with electrons, resulting in the formation of molecular ion fragments unique to each molecule of the substance. Because the fragmentation pattern of each molecule of a particular substance serves as a fingerprint for identification by referring to the reference spectrum in the GCMS library (Darmapatni et al., 2016; HimaBindu & Parameswari, 2013; Loos et al., 2016; Rahayu et al., 2020; Waters & Tadi, 2021). The focus of this research was to use GCMS with an HP5MS capillary column in urine to identify doping substances such as morphine, heroin, and codeine compounds.

METHODS

Materials and Instrumentations

The materials were doping user's urine, amphetamine sulfate, ephedrine hydrochloride, morphine hydrochloride, codeine hydrochloride, heroin hydrochloride, all of which were obtained from PPOM (Center for Drug and Food Examination). Methanol, diethyl ether, ethyl acetate, chloroform, sodium hydroxide, ammonium hydroxide, sodium sulfate, cysteine, acetonitrile, acetic acid anhydride, methyl orange, trifluoroacetic acid (TFA), trifluoro-acetic-anhydride (TFAA), N-methyl-N-trimethylsilyl-trifluoro-acetamide (MSTFA), N-methyl-bis-trifluoro-acetamide (MBTFA). Analytical scale (Mettler Toledo), GCMS type HP 1800 C GCD series II (Hewlett Packard), 1 µl microsyringe, and glasswares.

Preparation

Morphine, heroin, and codeine derivatives, each weighing exactly 50 mg, were dissolved in methanol in a 50 ml volumetric flask and diluted to a concentration of 1000 ppm. In a 50 ml volumetric flask, 1 ml of this solution was pipetted and diluted with methanol. Each was adjusted in volume to achieve a concentration of 20 ppm. To determine the detection limit, dilution was performed until the smallest detectable concentration was obtained.

Determination of Optimum Conditions

GCMS was run for 2-3 hours before being tested for optimization to determine the best conditions. The fixed parameters are as follows: carrier gas: helium, gas flow rate: 1.0 ml/min, inlet temperature: 230°C, detector temperature: 250°C, column temperature: 90-250°C (length 25 m), detector activation time: 3.0 minutes, solution volume injected: 1 µl. Each standard solution contained 20 ppm, and the temperature increased at a rate of 10°C/min. To test the system, perfluorotributylamine compounds were injected. At 190°C and

200°C, morphine, heroin, and codeine compounds can be separated and detected. The final result is a chromatogram of each compound showing the retention time and ionic fragments.

Extraction

Organic solvents were used in extraction to separate interfering compounds from the analyte. Derivatization with selective reagents aims to change the polarity and make the analyte heat stable. Five ml of urine were added to an erlenmeyer flask, followed by one ml of 6 N HCl and one hundred mg of cysteine, which was heated for 30 minutes at 105°C. After cooling, 5 ml of diethyl ether was added, shaken for 10 minutes, and centrifuged for 5 minutes at 2500 rpm. The aqueous phase was separated, and pH 8 borate buffer was added, along with 5 ml of diethyl ether and 3 grams of Na_2SO_4, before being shaken for 20 minutes and centrifuged for 5 minutes. The organic phase was evaporated to 1 ml in volume.

Derivatization by Gas Chromatography Mass Spectrometry

Forty µl of evaporation yield, 50 µl of the acetonitrile-trifluoroacetic acid mixture (60:40) containing 200 ppm methyl orange were added. Then add drops of N-methyl-N-trimethylsilyl-trifluoro-acetamide until the solution changes colour from red to yellow, then heat for 5 minutes at 80°C of N-methyl-N-bis-trifluoro-acetamide and heat again for 10 minutes, then identify.

Mass Spectrum

Morphine, heroin, and codeine compounds were identified by gas chromatography, the separation peaks were obtained on the chromatographic column. The pattern of ion fragmentation in the mass spectrum was typical for each compound. The mass spectra were directly compared with the GCMS literature.

RESULTS AND DISSCUSCION

The analysis of heroin, codeine, and morphine extracted with diethyl ether and derivatizing reagents N-methyl-N-bis-trimethylsilyl-trifluoro-acetamide and N-methyl-N-bis-trifluoro-acetamide. These are selective derivatization compounds for doping agents (Pratiwi et al., 2021). The retention time and chromatogram pattern of these compounds were the same as the comparison and database on GCMS instruments, according to the results of the analysis. The first compound produced heroin, the retention time of the analysis revealed differences in the comparison standard, and the derivatization results were 7.32 and 8.09 minutes.

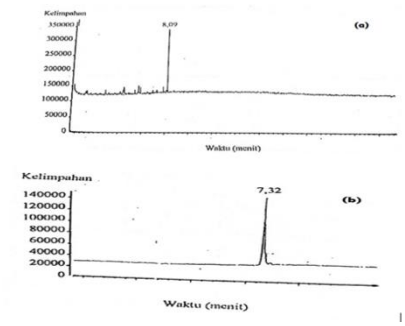

Figure 1. The retention time of (a) sample derivatization result and (b) heroin standard

The mass spectrum of heroin compounds from standardized urine was analysed, and the urine sample extracted from the derivatization showed a fragmentation pattern that was similar to the comparison standard and the Base data on GCMS instruments. The analysis yielded the following mass spectrum pattern:

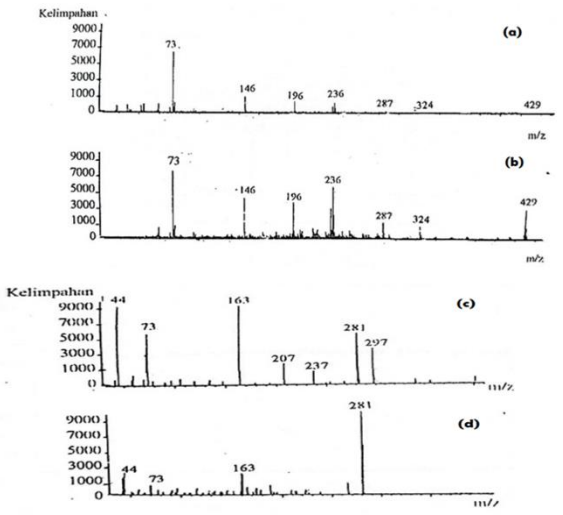

Figure 2. (a) The standard mass spectrum for comparison of heroin compounds (Zuccaro et al., 1997) (b) The blank mass spectrum with heroin (c-d) The urine sample mass spectrum from extraction and derivatization

The retention time of the comparison standard and urine sample was 6.21 minutes, while the sample from the user and the derivatization result was 7.95 minutes, according to the results of the GCMS analysis for the second compound codeine. The chromatogram's findings are as follows:

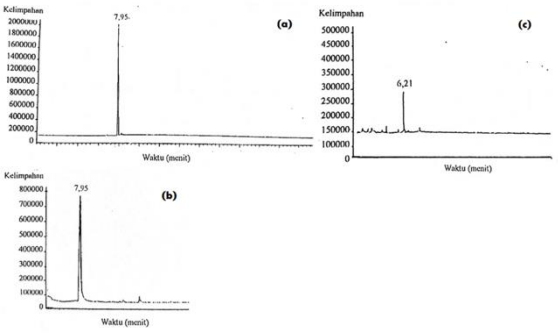

Figure 3. The retention time of (a) codeine standard, (b) sample extraction result and derivatization, and (c) user's urine sample

Mass spectrum analysis of codeine compounds from blank urine with added standard reagents and user urine samples that had been derivatized revealed a relatively similar fragmentation pattern with the comparison standard and database. The analysis resulted in the following mass spectrum pattern:

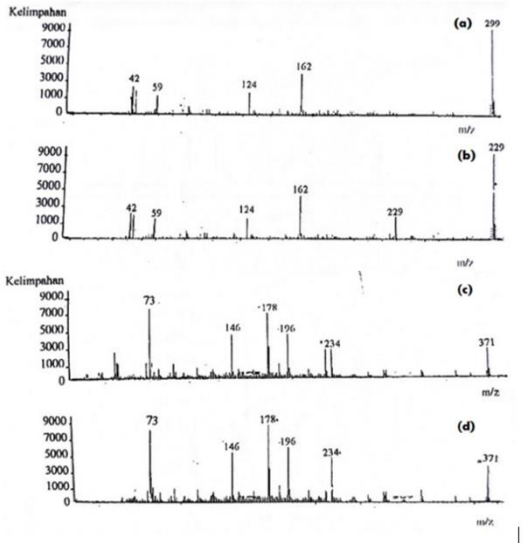

Figure 4. The mass spectrum of (a) codeine standard (Zuccaro et al., 1997), (b) derivatization result, (c) urine sample, and (d) GCMS database

The chromatogram of the third compound, morphine, is shown next. The retention time was 8.01 minutes. The following are the findings of the analysis:

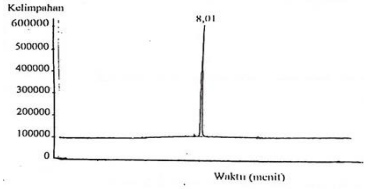

Figure 5. Chromatogram and retention time of morphine standard

The mass spectra of morphine compounds are produced by a chromatogram pattern from GCMS analysis of morphine compounds. The results showed the same compound fragments in

the extracted and derivatized samples as in the standard morphine comparison.

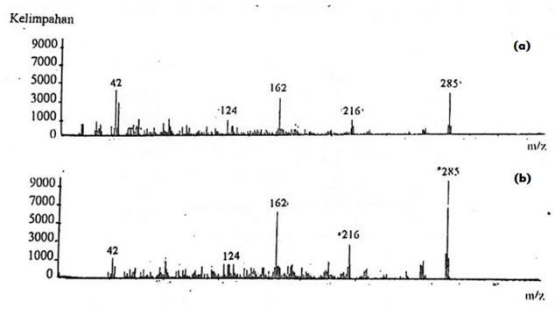

Figure 6. The mass spectrum of (a) morphine standard (Zuccaro et al., 1997) and (b) extraction results and derivatization.

Morphine, heroin, and codeine compounds can be analysed using GCMS, column HP5MS, 25 m long, with 5% phenyl methyl siloxane designed for alkaloids, drugs, and halogen-containing compounds. In mass spectrometry, the separated compounds in the column containing the N-base group are fragmented into separate molecular ions based on the m/z characteristics for each compound, and the pattern can be compared with the fragmentation of ions in the GCMS library and the similarity index is known, molecular ion similarity, and the base peak of at least 6 signals with a relative intensity above 5%. The instrument was optimized by programming the injection temperature and detector, selecting a column (Bhardwaj et al., 2016), increasing the temperature by 10ºC/min, and injecting 1 µl. The compound was 190ºC without derivatization and 200ºC after derivatization. Perfluorotributylamine was injected to test the detector (Fiehn, 2016).

Morphine, heroin, and codeine were first extracted at pH 8 for greater solubility, with borate used to maintain a stable pH throughout the extraction process. The derivatization treatment with N-methyl-N-trimethylsilyl-trifluoro-acetamide and N-methyl-N-bis-trifluoro-acetamide, produced separation results similar to those reported in the GCMS literature (Pratiwi et al., 2021), for change polarity, makes it easier to gas and is relatively stable to heating.

Morphine and heroin derivatives are the same compounds, namely morphine-TMS with m/z values of 73, 146, 196, 236, 285. The m/z value for codeine 42, 59, 124, 162, 229 were changed to 73, 146, 178, 196, 234, 371. The m/z value in the user's urine sample is the same as in the mass spectrometry-chromatography library. The structure fragment pattern is shown in figure 7.

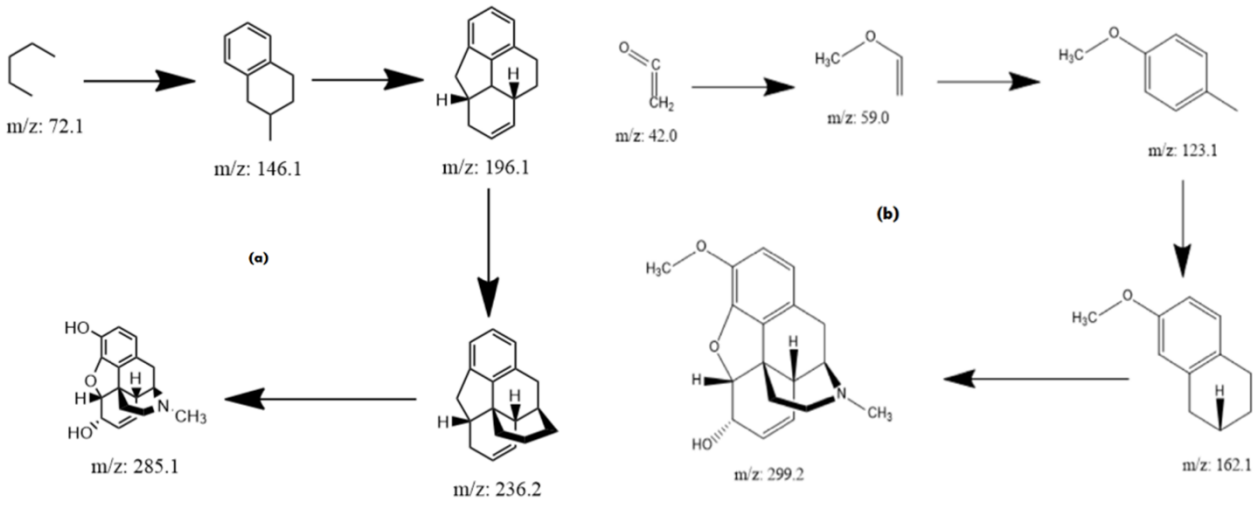

Figure 7. The structure fragment pattern of (a) morphine-TMS and (b) codeine

Derivatization results revealed a shift in the retention time and m/z value of each ion fragment but did not eliminate specificity. Dilution was used to determine the detection limit, and the result for each compound was 2 g/mL.

CONCLUSION

Morphine, heroin, codeine compounds in the user's urine can be separated properly using the GCMS technique, the HP5MS capillary column contains 5% phenylmethylsiloxa, 25 m long, 0.2 mm in diameter, non-polar. The derivatizing reagents N-methyl-N-trimethylsilyl-trifluoro-acetamide and N-methyl-bis-trifluoro-acetamide can increase the sensitivity of detection and the results show a shift in retention time and have the same ion fragmentation pattern as the Library of GCMS, with the similarity index was 93% and the detection limit for each compound was 2 g/ml.

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