Available online on 15.06.2023 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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Research Article

Determination of Temazepam Levels in Urine by Gas Chromatography – Mass Spectrometry

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A B S T R A C T

Temazepam is a benzodiazepine drug belonging to a class IV psychotropic. Temazepam provides a sedative-hypnotic effect that can help with insomnia. Thanks to the sedative-hypnotic effect it produces, many people take this drug to treat various sleep problems. However, if misused for a long period of time this drug can cause dependence. Therefore, to determine the level of temazepam in a person's body, a confirmation test of temazepam in the urine is needed. The test uses the gas chromatography - mass spectrometry method, this method is the most widely used method in drug testing with very specific results, so the results are not in doubt. This test begins with sample preparation by taking the extract using the solid phase extraction (SPE) method followed by enzyme hydrolysis, centrifugation and derivatization. Based on the tests carried out, positive results were obtained for temazepam with levels of 4444.9895 ng/mL.

Keywords: Temazepam, Urine, GC-MS, Solid Phase Extraction (SPE)

A R T I C L E I N F O: Received 23 Feb.2023; Review Complete 12 April 2023; Accepted 19 May 2023; Available online 15 June 2023

Cite this article as:

Sulistiyo J, Determination of Temazepam Levels in Urine by Gas Chromatography – Mass Spectrometry , Asian Journal of Pharmaceutical Research and Development. 2023; 11(3):22-27.DOI: http://dx.doi.org/10.22270/ajprd.v11i3.1223

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INTRODUCTION

Today, along with the times that are happening in society, many problems and deviant behavior arise, one of which is drug abuse (Narcotics, Psychotropics and Addictive Substances). Reported by the website of the Ministry of Social Affairs of the Republic of Indonesia, it is stated that currently 3.6 million people are victims of drug abuse. In a study by the National Narcotics Agency (BNN), the number of drug abuse shows no signs of sloping during the Covid-19 pandemic era, in the past year.[1,2]

The use of dangerous drugs has also begun to be misinterpreted. Drugs that can give hallucinatory effects and disturb the user's thinking are called psychotropics. Psychotropic abuse is rife among young people, especially students or teenagers. The abuse of psychotropics is not only a national problem but also an international problem, because it has a negative impact on the life of the community, nation and state.[3]

Based on Law No. 5 of 1997, psychotropics are substances or drugs, both natural and synthetic, which are not narcotics, which are used as psychoactive drugs through a selective influence on the central nervous system which causes specific changes in mental activity and behavior.[4] One type of psychotropic drug is the benzodiazepine class of drugs which is included in psychotropic class 4. Temazepam is included in the benzodiazepine class of drugs, namely drugs with a hypnotic – sedative effect that helps overcome insomnia.[5,6]

Drug testing in urine specimens aims to detect drugs or their metabolites that indicate the use of prescribed drugs or illegal substances in the near future. Several biological specimens can be used for this purpose, and urine is chosen because it can be examined 1-3 days after use and is easy to collect and can be collected in large quantities.[7]

Based on the description above, the testers carried out a confirmation test on the temazepam substance in the urine to find out whether or not the substance was temazepam and the levels of temazepam contained using the gas chromatography method - mass spectrometry.

METHODS AND MATERIALS

Test method by gas chromatography – mass spectrometry (KG-SM). Principle Testing quantitative analysis of temazepam in urine through the extraction and derivatization process was carried out by gas chromatography – mass spectrometry by comparing ion fractions between standards and samples. The test was carried out on 08 – 09 February 2022 at the Chemical Doping Laboratory, DKI Jakarta Regional Health Laboratory.The Testing Procedure is carried out in stages:

Blank solution

a. Water Blank Solution

Prepare 3 ml of aquabidest in a test tube with a lid and identify it, add 50µl ISTD Nalorphin (10µg/mL). Prepare a Sep-Pak C-18 column and condition it with 3 mL methanol and 3 mL water. Pass the water blank into the column that has been prepared and then wash it with 2 mL of aquabidest. Prepare a new test tube and give an identity then eluted with 3 mL of methanol (contain the methanol in a new test tube that has been given an identity). Dry the methanol with turbo vap. Add 1 mL of 0.2 M phosphate buffer pH 7 and add 50 µL of E.Coli glucuronidase enzyme and incubate at room temperature50^{o}C for 90 minutes. Cool and add 250 µL of 20% carbonate buffer pH 9. Add 5 mL TBME and shake with a rotary shaker for 30 minutes, centrifuge for 5 minutes. Take the organic phase, transfer it to an identical test tube, dry it with a turbo vap at 60^{o}C until dry. Add 50 µL MSTFA then heat with a dry block heater at 60oC for 15 minutes. Put it in an insert vial and ready to inject at GC-MS.

b. Blank urine solution

Prepare 3 ml of blank urine in a test tube with a lid and identify it, add 50µl ISTD Nalorphin (10µg/mL). Prepare a Sep-Pak C-18 column and condition it with 3 mL methanol and 3 mL water. Pass the water blank into the column that has been prepared and then wash it with 2 mL of aquabidest. Prepare a new test tube and give an identity then eluted with 3 mL of methanol (contain the methanol in a new test tube that has been given an identity). Dry the methanol with turbo vap. Add 1 mL of 0.2 M phosphate buffer pH 7 and add 50 µL of E.Coli glucuronidase enzyme and incubate at 50oC for 90 minutes. Cool and add 250 µL of 20% carbonate buffer pH 9. Add 5 mL TBME and shake with a rotary shaker for 30 minutes, centrifuge for 5 minutes. Take the organic phase, transfer it to an identical test tube, dry it with a turbo vap at 60^{o}C until dry. Add 50 µL MSTFA then heat with a dry block heater at 60^{o}C for 15 minutes. Put it in an insert vial and ready to inject at GC-MS.

Standard Solution

Prepare 3 ml of blank urine in a test tube with a lid and identify it, add 15 µL of 100 ppm temazepam standard and then add 50 µl of ISTD Nalorphin (10 µg/mL). Prepare a Sep-Pak C-18 column and condition it with 3 mL methanol and 3 mL water. Pass the water blank into the column that has been prepared and then wash it with 2 mL of aquabidest. Prepare a new test tube and give an identity then eluted with 3 mL of methanol (contain the methanol in a new test tube that has been given an identity). Dry the methanol with turbo vap. Add 1 mL of 0.2 M phosphate buffer pH 7 and add 50 µL of E.Coli glucuronidase enzyme and incubate at 50oC for 90 minutes. Cool and add 250 µL of 20% carbonate buffer pH 9. Add 5 mL TBME and shake with a rotary shaker for 30 minutes, centrifuge for 5 minutes. Take the organic phase, transfer it to an identical test tube, dry it with a turbo vap at 60oC until dry. Add 50 µL MSTFA then heat with a dry block heater at 60^{o}C for 15 minutes. Put it in an insert vial and ready to inject at GC-MS.

Test Solution

Prepare 3 ml of sample in a test tube with a lid and identify it, add 50µl of ISTD Nalorphin (10µg/mL). prepare a C-18 sep-Pak column and condition it with 3 mL methanol and 3 mL water. Pass the water blank into the column that has been prepared and then wash it with 2 mL of aquabidest. Prepare a new test tube and give an identity then eluted with 3 mL of methanol (contain the methanol in a new test tube that has been given an identity). Dry the methanol with turbo vap. Add 1 mL of 0.2 M phosphate buffer pH 7 and add 50 µL of E.Coli glucuronidase enzyme and incubate at 50^{o}C for 90 minutes. Cool and add 250 µL of 20% carbonate buffer pH 9. Add 5 mL TBME and shake with a rotary shaker for 30 minutes, centrifuge for 5 minutes. Take the organic phase, transfer it to an identical test tube, dry it with a turbo vap at 60^{o}C until dry. Add 50 µL MSTFA then heat with a dry block heater at 60^{o}C for 15 minutes. Put it in an insert vial and ready to inject at GC-MS.

Determination Method

The test solutions, standards and blanks were each injected into a gas commatography – mass spectrometry (KG-SM) apparatus.

The steps for determining the level of Temazepam in urine by GC-MS are as follows:

Preparation of Water Blank Solutions and Urine Blanks

a. Prepared 3 ml of water blank put into a test tube (BW)
b. Prepare 3 ml of blank urine, put it in a test tube (BU)
c. Added 50 µL ISTD Nalorphin (10 µg/mL) into each test tube
d. A Sep-Pak C-18 column was prepared, conditioned with 3 mL methanol and 3 mL water
e. Pass the water blank and urine blank into the column, then wash with 2 mL of aquabidest
f. Then eluted with 3 ml of methanol (accommodate in a new test tube that has been given an identity)
g. Methanol was dried with turbo vap at 60˚C
h. Added 1 mL of 0.2 M phosphate buffer pH 7 and 50 µL of E.Coli glucuronidase enzyme
i. Incubated at 50˚C for 90 minutes
j. Cooled and added 250 µL carbonate buffer pH 9 and 5 mL TBME
k. Shaken with a rotary shaker for 30 minutes and centrifuged for 5 minutes

l. The organic phase was taken and transferred to an identified tube, then the solution was dried with a turbo vap at 60˚C
m. Added 50 µL MSTFA, then heated in a dry block heater at 60˚C for 15 minutes
n. Inserted into the insert vial and ready to be injected with GC-MS

Preparation of 500 ppb Temazepam Standard Solution

a. Prepared 3 ml of blank urine put into a tube with a QC identity
b. Added 50 µL ISTD Nalorphin (10 µg/mL) into each test tube
c. Added 15 µL of 100 ppm temazepam standard solution
d. Prepared a Sep-Pak C-18 column, conditioned with 3 mL of methanol and 3 mL of water
e. Pass the water blank and urine blank into the column, then wash with 2 mL of aquabidest
f. Then eluted with 3 ml of methanol (accommodate in a new test tube that has been given an identity)
g. Methanol was dried with turbo vap at 60˚C
h. Added 1 mL of 0.2 M phosphate buffer pH 7 and 50 µL of E.Coli glucuronidase enzyme
i. Incubated at 50˚C for 90 minutes
j. Cooled and added 250 µL carbonate buffer pH 9 and 5 mL TBME
k. Shaken with a rotary shaker for 30 minutes and centrifuged for 5 minutes
l. The organic phase was taken and transferred to an identified tube, then the solution was dried with a turbo vap at 60˚C
m. Added 50 µL MSTFA, then heated in a dry block heater at 60˚C for 15 minutes
n. Inserted into the insert vial and ready to be injected with GC-MS

Preparation of Test Solutions

a. Prepared 3 ml of sample put into an identified tube
b. Added 50 µL ISTD Nalorphin (10 µg/mL) into each test tube
c. A Sep-Pak C-18 column was prepared, conditioned with 3 mL methanol and 3 mL water
d. Pass the water blank and urine blank into the column, then wash with 2 mL of aquabidest
e. Then eluted with 3 ml of methanol (accommodate in a new test tube that has been given an identity)
f. Methanol was dried with turbo vap at 60˚C
g. Added 1 mL of 0.2 M phosphate buffer pH 7 and 50 µL of E.Coli glucuronidase enzyme
h. Incubated at 50˚C for 90 minutes
i. Cooled and added 250 µL carbonate buffer pH 9 and 5 mL TBME
j. Shaken with a rotary shaker for 30 minutes and centrifuged for 5 minutes
k. The organic phase was taken and transferred to an identified tube, then the solution was dried with a turbo vap at 60˚C
l. Added 50 µL MSTFA, then heated in a dry block heater at 60˚C for 15 minutes
m. Inserted into the insert vial and ready to be injected with GC-MS

Blank solution

sample solutions and standard solutions were injected into the Gas Chromatography – Mass Spectrometry (KG-SM) apparatus.

The tools used are gas chromatography – mass spectrometry, C-18 sep-pack column, chamber, incubator, turbo vapp, rotary shaker, centrifuge, dry block heater, closed test tube, reaction tube rack, finn pipette & tip. The ingredients include aquabidest, blank urine, methanol, standard temazepam 100 ppm, ISTD Nalorphine (10 µg/mL), 0.2 M phosphate buffer pH 7, E.Coli Glucoronidase Enzyme, 20% Carbonate Buffer pH 9, Tert-Buthyl-methyl ether (TBME), N-Methyl-N(trimethylsilyl) trifluoro-actamide (MSTFA). Samples were obtained from customers who wanted to test for confirmation of Benzodiazepines with the identity "X"

RESULTS

Based on the measurement results using a gas chromatography – mass spectrometry, the following results were obtained for the urine sample.

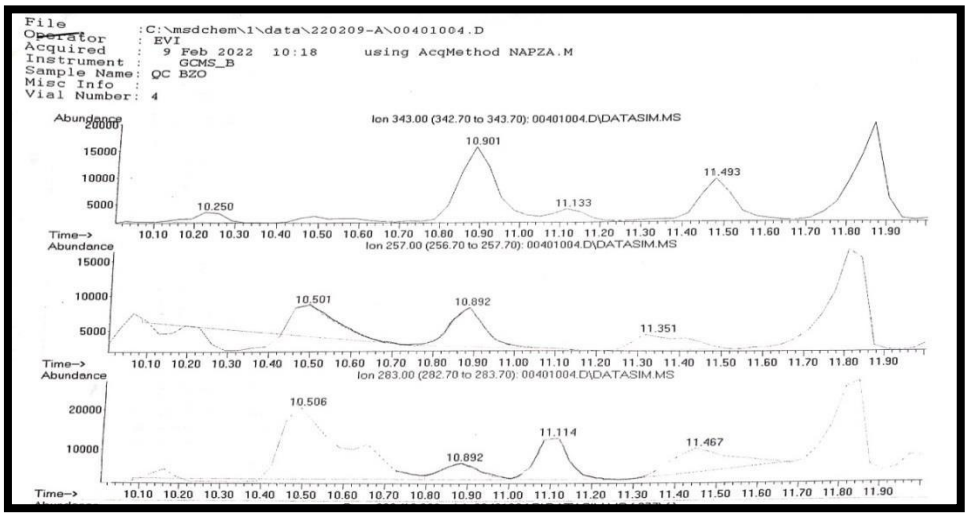

Figure 1: Chromatogram of Temazepam Standard

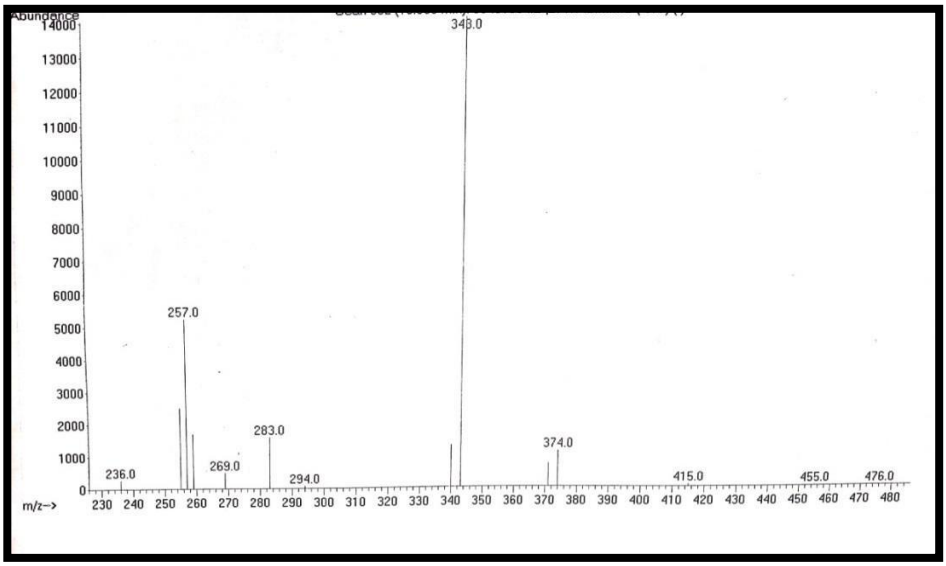

Figure 2: Temazepam Standard Ion Fraction

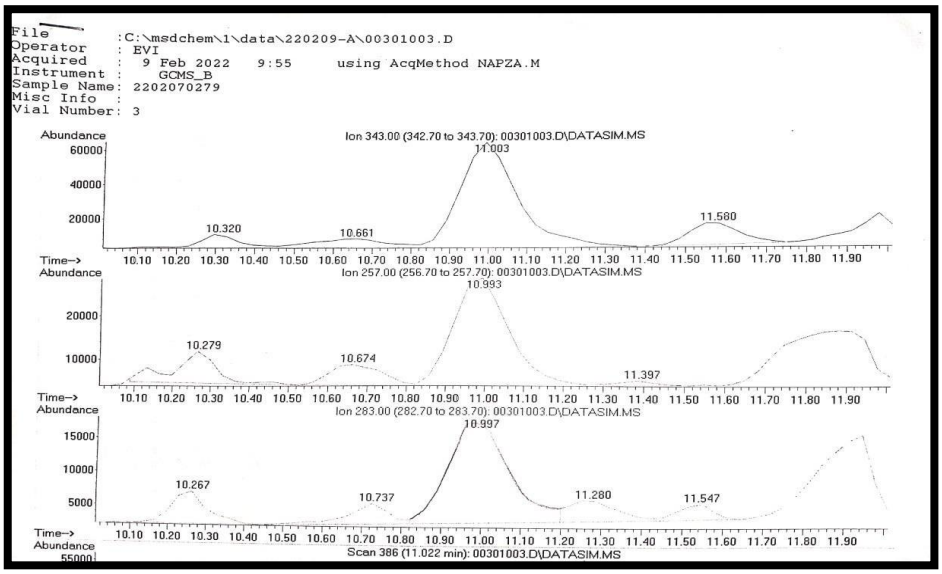

Figure 3: Chromatogram of Sample X

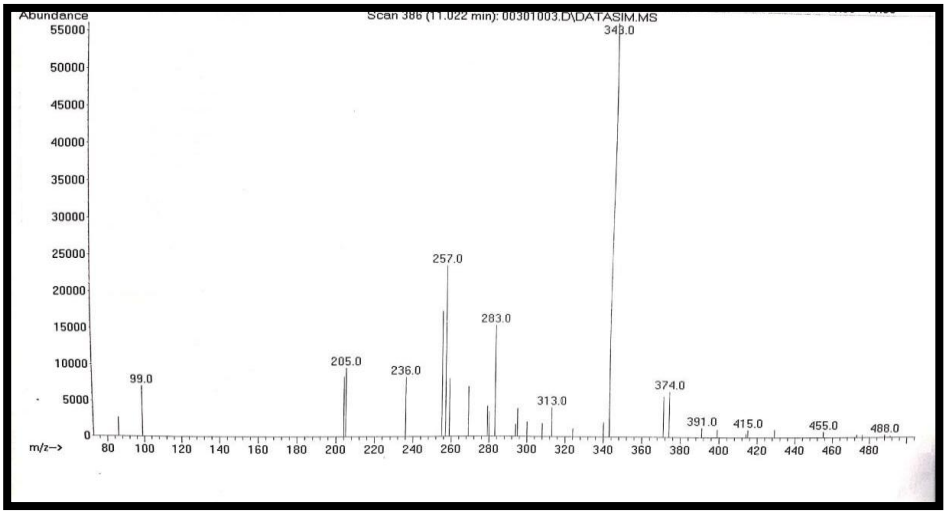

Figure 4: Sample X ion fraction

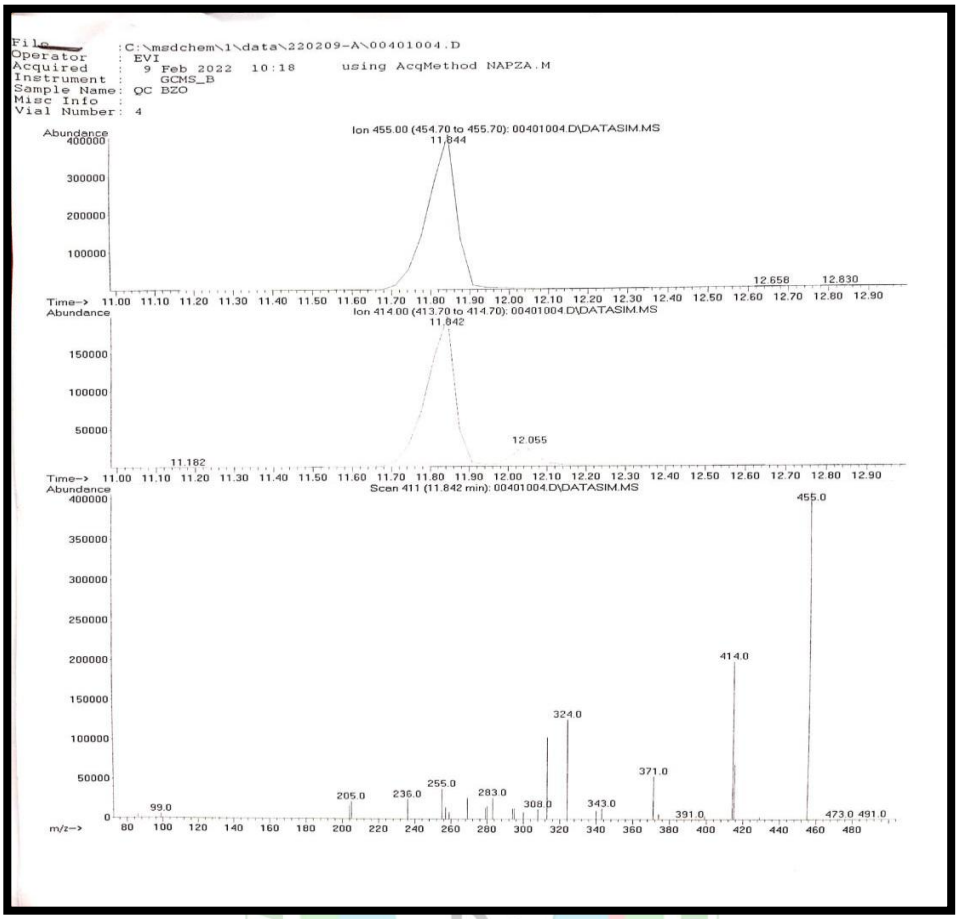

Figure 5: Chromatogram and Internal Ion Fraction of Nalorphine Standard on Temazepam Standard

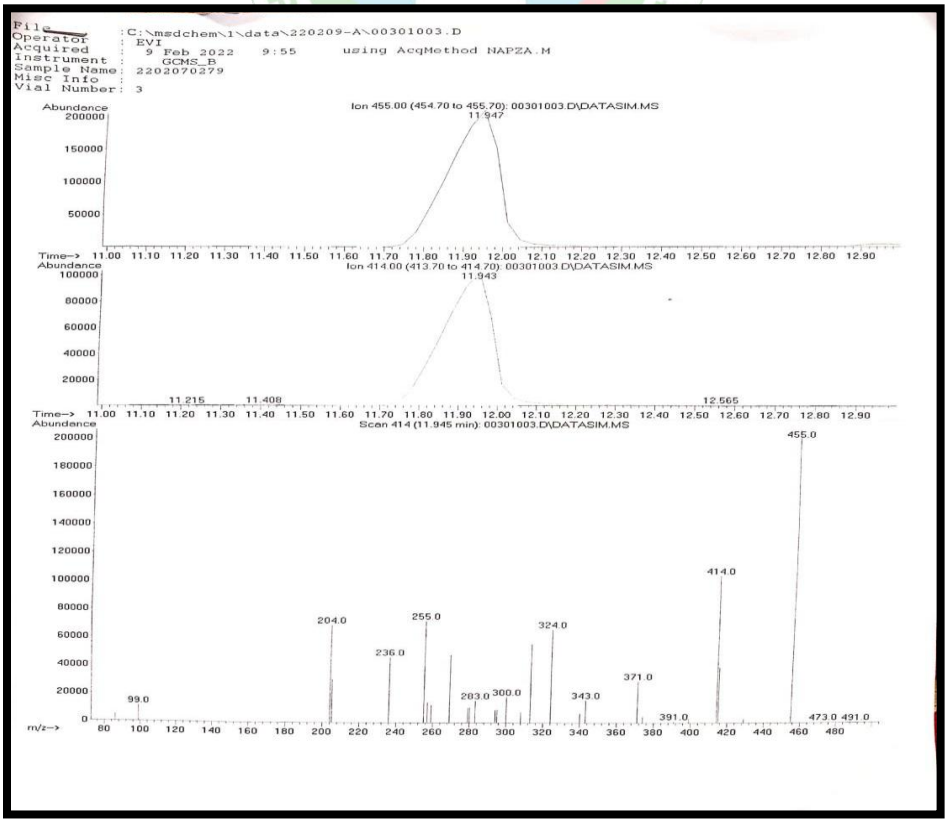

Figure 6: Chromatogram and Internal Ion Fraction of Nalorphine Standard in Sample X

Table 1: Data Height and Ratio of Samples and Standards of Temazepam

NoSample	PeakHeight		Ratio	Content	Result
	ISTD	Temazepam			(ng/mL)
2202070279	194667	60218	0,3093385	4444,9985	4444,9895 ng/mL
Std500 ppb	386995	13466	0,0347963		

DISCUSSION

This assay was carried out using the gas chromatography method mass spectrometry. Before injection, samples, standards and blanks were prepared first. The blanks used were blanks containing aquabidest and blanks containing negative urine. The temazepam standard used as a comparison is 500 ppb taken from the 100 ppm temazepam standard. ISTD or internal standard Nalorphine was added to all solutions, both test solutions, standards and blanks. The function of adding ISTD is as a correction factor. The compounds were isolated by solid phase extraction and then the elution was dried and 0.2M phosphate buffer pH 7 was added which functioned to regulate and maintain the pH of the solution at 7 because E.ColiGlukoronidase enzymes would be added to hydrolyze by breaking down glucuronide compounds to increase the detection of analytes. After that it was followed by incubation at 50oC for 90 minutes to optimize the hydrolysis process. Addition of carbonate buffer pH 9 which functions to maintain an alkaline atmosphere due to increase the solubility of compounds in organic solvents. The addition of TBME functions as an organic solvent which will attract the active substance into the organic phase after centrifugation. After there are two phases, the organic phase or the upper phase is taken carefully and thoroughly and then transferred into a new test tube with an identity. The addition of MSTFA serves to derivatize the temazepam compound so that it is more volatile and stable when in GC-MS.[8–10]

The sample is injected with a syringe into the device and then the sample will pass through the injection gate and will be heated to form a vapor, then the vapor will be carried along with the mobile phase to enter the column for separation according to polarity/adsorption. Furthermore, the separated compounds will be brought to the detector, namely mass spectrometry and ion splitting will be carried out, so that specific ion fractions will occur and conclusions can be drawn from these specific ions.

Based on the results of the temazepam test that was carried out, it was found that the sample was positive for temazepam with levels of 4444.9895 ng/mL. The test results were obtained by comparing the chromatogram of the sample solution with the chromatogram of the standard solution, then viewing the retention time and peak height reports to obtain the levels. Judging from the chromatogram of the specific ion fraction formed, namely ion 343; 257; and 283. This ionic fraction is specific for temazepam, although there are other ionic fractions seen and the ion is small it is possible that it is an impurity.

CONCLUSION

The results of testing the levels of temazepam by GC-MS showed that the positive sample contained temazepam with a level of 4444.9894 ng/mL. As a comparison of the test results, it is further recommended to carry out tests with other methods, such as Liquid Chromatography-Mass Spectrometry (LC-MS).

CONFLICTOFINTEREST

The authors declare that they have no conflictinterests.

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