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Simultaneous Analysis of the Active Ingredients in Ecstasy Tablet as Hyper-Active Drug

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Abstract: Ecstasy is used with various ingredients, such as methamphetamine (MET), acetaminophen (ACE), caffeine (COF), and ephedrine (EPH). These active compounds can cause problems in determining tablet levels in the laboratory. So a method is needed that is cheaper, effective, and fast to determine the tablet content. This study aims to develop a spectrophotometric subtraction of ratio spectra method for the simultaneous analysis of MET, EPH, COF, and ACE on ecstasy tablets without separation. The research was conducted using a spectrophotometric method with the subtraction of ratio spectra method using methanol and a validity test with linearity, accuracy, precision, detection limit (DL), and quantitative limit (QL) parameters. This method was applied to determine MET, EPH, COF, and ACE levels simultaneously in ecstasy tablets. The results showed that the reduction of the ratio spectra method for MET, EPH, COF, and ACE was determined simultaneously at 258 nm, 257nm, 273 nm, and 246 nm. The percentage level is 38.83% for MET, 32.13% for EPH, 19.05% for COF, and 10.39% for ACE. The subtraction of ratio spectra method met the validation parameters, including linearity, accuracy, precision, DL, and QL. It can be concluded that the can subtraction of ratio spectra method is used for simultaneous determination of levels for ecstasy tablets containing MET, EPH, COF, and ACE using methanol as a solvent and meets the validation parameter requirements.

Keywords: methamphetamine, ephedrine, caffeine, acetaminophen, the subtraction of ratio spectra method.

興奮劑搖頭丸中有效成分的同時分析

摘要： 搖頭丸與各種成分一起使用，如甲基苯丙胺、對乙酰氨基酚、咖啡因和麻黃鹼。這些活性化合物可能會導致在實驗室中確定片劑水平時出現問題。因此需要一種更便宜、有效且快速的方法來確定片劑含量。本研究旨在開發一種分光光度比減法光譜方法，用於在不分離的情況下同時分析搖頭丸片上的甲基苯丙胺、麻黃鹼、咖啡因和對乙酰氨基酚。該研究使用分光光度法和使用甲醇的減法比光譜法以及具有線性、準確度、精密度、檢出限和定量檢測參數的有效性測試進行。該方法用於同時測定搖頭丸中的甲基苯丙胺、麻黃鹼、咖啡因和對乙酰氨基酚水平。結果表明，甲基苯丙胺、麻黃鹼、咖啡因和對乙酰氨基酚的還原比譜法在 258 納米儀表、257 納米儀表、273 納米儀表和 246 納米儀表處同時測定。甲基苯丙胺的百分比水平為 38.83%，麻黃鹼為 32.13%，咖啡因為 19.05%，對乙酰氨基酚為 10.39%。減比譜法滿足驗證參數，包括線性、準確度、精密度、檢出限和數量限制。可以得出結論，採用罐減比譜法以甲醇為溶劑同時測定含有甲基苯丙胺、麻黃鹼、咖啡因和對乙酰氨基酚的搖頭丸片的含量，符合驗證參數要求。

关键词：甲基苯丙胺、麻黃鹼、咖啡因、對乙酰氨基酚、比值減法光譜法。

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1. Introduction

Nowadays, ecstasy is becoming a major drug abuse among nightclub patrons. The abused drugs are given orally by intentionally adding ecstasy tablets into patrons' drinks [1]. The results of examinations of drugs in several places in Medan by the Medan Police, North Sumatra, Indonesia, after being investigated by the Narcotics Division of the Forensic Laboratory, are tablets containing four active ingredients, namely methamphetamine (MET), ephedrine (EPH), caffeine (COF), and acetaminophen (ACE) [1].

Amphetamines are hazardous chemicals that can cause addiction. However, amphetamines are used for treatment [2]. Amphetamines help people with attention deficit hyperactivity disorder (ADHD) focus more on activities, control appetite, and control weight [2, 3, and 4].

Methamphetamine HCl (MET) with IUPAC names (2S)-N-methyl-1-phenylpropan-2-amine hydrochloride [5], is the main component in ecstasy [1]. MET is a psychostimulant drug [4, 6] used for people with hyperactivity disorder due to inattention and abuse of the drug alone or in combination with several sympathomimetic drugs [7, 8]. After nine days of a death, MET cannot be detected in the body and can only be detected in the hair [9].

Various studies on the content of MET found in blood, plasma, serum, urine, and hair have been published using various methods, namely AUC [1], GC-MS [5], CSP-LC-MS/MS [9], HPLC [10-12], LC-MS-MS [13], LC-ESI-MS/MS [14], and MIP-PSI-MS [15].

Ephedrine (EPH) with IUPAC name (1R,2S)-2-(Methylamino)-1-phenylpropan-1-ol, hydrate, is a bronchodilator. Caffeine (COF) or 1.3.7-Trimethylpurine-2.6-dione is a stimulant drug, and Acetaminophen (ACE) or N-(4-Hydroxyphenyl) acetamide is an antipyretic and analgesic drug [2, 3, and 5]. Similarly, these components are found in ecstasy and whose intended use supports drug abuse [9, 10].

Various methods are used to determine component levels, one of which is spectrophotometry, a simple, effective, fast, and relatively inexpensive method compared to others [12]. The determination of MDMA, MET, ketamine, and amitriptyline simultaneously has been carried out using an SPE and LC-MS-MS [9]; meanwhile, ecstasy containing MET, EPH, COF, and ACE is carried out with multiple wavelengths [1].

As one of the applications of the spectrophotometric method, it is possible to directly analyze drug mixtures with adjacent wavelengths by spectrum ratio reduction (SRS) [16, 17, and 18].

This method was applied to the analysis of mixtures of four drugs, which have overlapping spectra. The resulting spectrum will give a new curve. In this method, the mixed spectrum will be separated into one spectrum. The resulting spectrum is plotted with a calibration curve [15, 16].

According to some of the publications above, studies have been conducted on the four components of tablets containing ecstasy.

1.1. Hypothesis

Several factors above suggest that it may be measured simultaneously using the subtraction system of ratio with spectrophotometric for four drug components in ecstasy tablets.

2. Material and Method

2.1. Apparatus

UV-Vis Spectrophotometer 1800 (Shimadzu), Personal Computer (PC), UV-Probe 2.42 software, Matlab® version R2016, a sonicator (Branson 1510), pH meter (Hanna) were used.

2.2. Material and Reagent

Methamphetamine Raw (Cerilliant®), Ephedrine HCl Raw material (Malladi), Caffeine raw material (Sigma-Aldrich), Acetaminophen (Anqluan), Ecstasy Tablets from confiscated evidence at the North Sumatra Police forensic laboratory. Methanol (E-Merck), HCL (E-Merck).

2.3. Preparation of MET, EPH, COF, and ACE Standard Absorption Spectrum

Pipetted 18 ml of 1000 g/ml MET solution was put into a 50 ml volumetric flask, filled with methanol so that MET is 360 g/ml, weighed 50 mg of standard EPH, added the marked amount methanol to a 50 ml volumetric flask. Then 18.05 ml of the EPH solution was pipetted into a 50 ml volumetric flask, and methanol was added to the marked line, resulting in a solution containing 361 ug/ml of EPH. The same process is followed for producing COF and ACE solutions. After that, 5 ml of COF solution and 5 ml of ACE solution were used, and 100 ug/ml of COF and 100 ug/ml of ACE solutions were obtained.

Then solutions of 1 ml of EPH, 0.85 ml of COF, and 0.65 ml of ACE were dissolved in a 10 ml volumetric flask, and their absorption was measured in the wavelength range of 200-400 nm. The absorption spectra of MET, EPH, COF, and ACE are superimposed.

2.4. Preparation of Standard Absorption Spectrum

MET solution was pipetted are (10.0-26.0) ml, and for EPH solution are (9.75-26.3) ml, and for COF as much as (0.45-1.25) ml, while for ACE (0.35-0.95) ml was sequential. Then these were put into 50 ml volumetric flasks separately and diluted with methanol. For each solution containing MET with a concentration of (200-520) g/ml, EPH solution with (195-527) ug/ml, COF solution with (4.5-12.5) ug/ml, and solution with ACE (3.5–9.5) ug/ml, the absorbance was measured at a wavelength of 200-300 nm, respectively [16, 17, and 18].

2.5. Validation Test

Validation tests were carried out on standard solutions for MET, EPH, COF, and ACE for absorption spectra made at the selected wavelength point 257.8 nm for MET, 257.2 nm for EPH, 273 nm for COF, while ACE used a wavelength of 245.6 nm. The predetermined zero order is used after manipulation to obtain the regression equation for each component.

2.5.1. Precision

The precision of the methods was provided by repeating them six times: intra-day and inter-day precision methods and measurements with simultaneous samples. Determination of inter-day and intra-day precision was seen from its relative standard deviation < 2.5% [20].

2.5.2. Accuracy

The accuracy test was calculated by measuring recovery percentage in three specific points: 80%, 100%, and 120%. The tests used 70% from the sample and 30% from the pure active substances (standard addition method) [20].

2.6. Construction of SRS Calibration Curve

The absorption spectra of the MET, EFD, KFN, and PCT mixture were divided by the absorption spectrum of the MET at a concentration of 361 g/mL as the divisor. It is possible to manipulate zero-order spectrum MA, EFD, KFN, and PCT with UV Probe 2.42 software. EPH, COF, and ACE have been evaluated in the same way. The results obtained are then plotted at various concentrations of each component [15, 16]. Then the regression equation or calibration curve of MET, EPH, COF, and ACE is obtained after receiving the spectrum data for the calibration curve [19]. The data obtained are calculated for linearity, detection limits, and quantitative limits [20].

2.7. Sample Solution Preparation

Crushed tablets weighing 53.4 mg were set into a 50 mL volumetric flask. The next step is to dilute with methanol and then homogenize it in a volumetric flask of 50mL. Five ml of the sample solution were transferred to a volumetric flask and diluted with methanol to the mark. Measurement of absorbance should take place between 200 and 300 nm.

The results obtained were determined by SRS for components of COF, MET, EPH, and ACE levels by using the UV Probe 2.43 application to calculate the manipulated data and system divisor [15, 16].

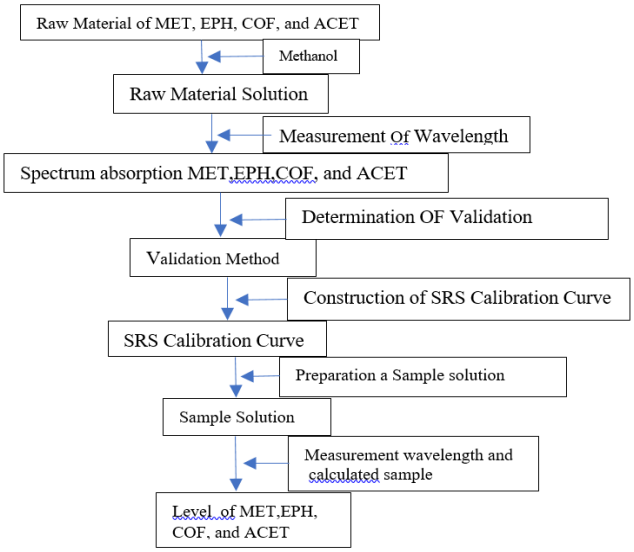


Fig. 1 Flow chart of research methodology of SRS method for determination of ecstasy tablet

3. Results and Discussion

A corresponding study was carried out using ecstasy tablets with a pink color. A certain fine ground ecstasy powder was weighed and placed in a 50 ml volumetric flask with methanol added to the marking. This ecstasy tablet was obtained from the confiscation of the Narcotics Section of the North Sumatra Police in Medan [1].

The tablet was the most criminally used tablet until 2018. Based on the qualitative tests in the forensic laboratory of POLDA, this tablet contains four components, namely MET, EPH, COF, and ACE. The SRS spectrophotometer was used to conduct quantitative studies on ecstasy tablets.

3.1. Analysis of SRS Process

The analysis was conducted to confirm the accuracy in determining MET, EPH, COF, and ACE in the sample. Based on Food and Drug Administration 2020, we evaluated linearity, detection limit (DL), quantitative limit (QL), accuracy, and precision [20].

Characterization of MET, EPH, COF, and ACE was verified by analyzing the absorption spectrum of the standards in methanol and comparing the literature study. Spectrum absorption ecstasy is shown in Fig. 2, while MET, EPH, COF, ACE, and ecstasy tablets overlaps are shown in Fig. 3.

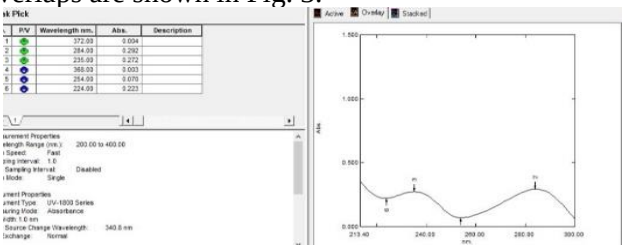


Fig. 2 Spectrum absorption ecstasy tablet at 200-300 nm

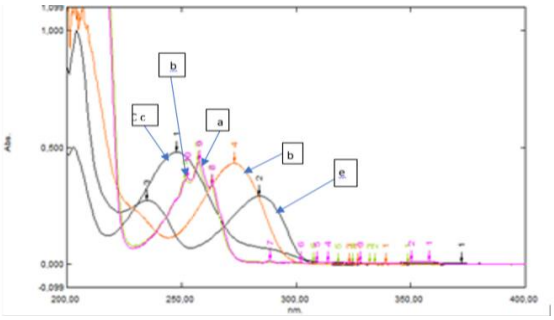


Fig. 3 Overlapping spectrum absorption of: a – MET, b –EPH, c – ACE, d – COF, and e – ecstasy tablet

The overlap spectrum is obtained from the maximum absorption spectrum of 360 ug/ml MET, 361 ug/ml EPH, 8.5 ug/ml COF, 7.5 ug/ml ACE, and a mixture of the four components.

In this study, with methanol as a single solvent, absorption was obtained at a maximum wavelength for MET 257.8 nm, EPH 257.2 nm, COF 273 nm, and ACE before manipulating the spectrum 245.6 nm. While in the literature with acid solvents are MET at 257 nm, EPH at 257 nm, COF at 273 nm, and ACE at 245 nm [5]. Using the subtraction of ratio spectra method (SRS), Kamal et al. determined that the study of overlapping spectra is the starting point for determining levels [15].

Before proceeding to the next step in the SRS method, it is important to determine the wavelength used for the initial analysis of the drug used as the divisor to determine X, Y, or Z [16].

This method will be developed to determine the levels of four components, namely MET, EPH, COF, and ACE. In this method, the initial divisor (D°) used to manipulate is selected by examining the overlapping spectra of the four single drugs.

The rule for selecting the initial divisor (D°) is based on the area and the effect on the concentration. From the observed spectrum, it was found that EPH has a longer area than other compounds, so EPH is used as the initial divisor, namely D°. Determination of the initial divisor is very influential on the results of the drug mixture regression plot. Because the results of the regression plot will be different if an unsuitable compound is used as the initial divisor, it causes poor linearity results [15, 16].

3.2. Validation Report

For the determination of the ecstasy tablet, the SRS method was used for validating the component values of MET, EPH, COF, and ACE. These validation values can be seen in Table 1.

Table 1 Result of validation parameter for MET, EPH, COF, and ACE

No	Parameter	MET	EFD	COF	ACE
1.	Linearity	0.9995	0.9997	0.9998	0.9995
2.	Accuracy (%)	101.03	100.18	100.20	100.08
3.	Precision (%)	1.82	9.92	1.43	4.12
4.	DL (ug/mL)	21.99	17.29	0.35	0.40
5.	QL (µg/mL)	66.66	52.41	1.07	1.22

Table 1 above shows the Validation results of the SRS analysis that the linearity value described is a good correlation coefficient value for SRS ≤ 1. A correlation between the drug concentration and the absorbance value also indicates that as the concentration increases, the absorbance value will also increase.

Accuracy, one of the parameters, is defined by adding the standard method to a certain range in the sample. Both are measured, the added standard is recalculated. The accuracy value obtained shows that this method meets the requirements for method validation (requirements for accuracy value are 98%-102%) [17].

Precision also as a parameter shows the closeness of the drug analysis results carried out in several repetitions. The precision shows that the method gives results that are close to each other even though it has been tested in several replications, as reflected in the resulting RSD calculation value that meets the validation requirements (RSD < 2%) [17].

The detection limit and quantification limit are calculated from the regression equation obtained from the calibration curve. In this study, samples were tested in the absorption spectrum area of 0.2-0.6 and the calibration curve area that met the requirements for the DL and QL parameters [17].

3.3. Determination with the Subtraction of Ratio Spectra Method (SRS)

The subtraction of the ratio spectra method begins by creating a ratio spectrum and selecting the initial divisor concentration (D°). The determination of the concentration of the divisor is a concentration range that satisfies the Lambert-Beer law. The spectrum overlapped the ratio spectrum of MET, EPH, COF, and ACE with various concentrations. The initial divisor (D°) was used based on the study of the overlapping spectrum found that the initial divisor was EPH.

Spectrum processing of the raw drug mixture was manipulated using UV probe 2.42 with division operation with EPH 361 g/ml. Then the result is subtracted with a constant is EPH 361 g/ml divided by the divisor; the result of the subtraction spectrum produces a new spectrum, namely MET+COF+ACE/EPH°. The spectrum is multiplied by the same divisor to obtain the MET spectrum +COF+ACE, and then proceed with the second division.

The MET+COF+ACE spectrum is divided by 6.5 ug/ml ACE, and then the result is subtraction with a constant 6.5 ug/ml ACE divided by the divisor. The result of the subtraction produces a new spectrum, namely MET+COF/ACE°. Then the spectrum is multiplied by the same divisor so that the MET+COF spectrum is obtained and then divided by 360 ug/ml of MET.

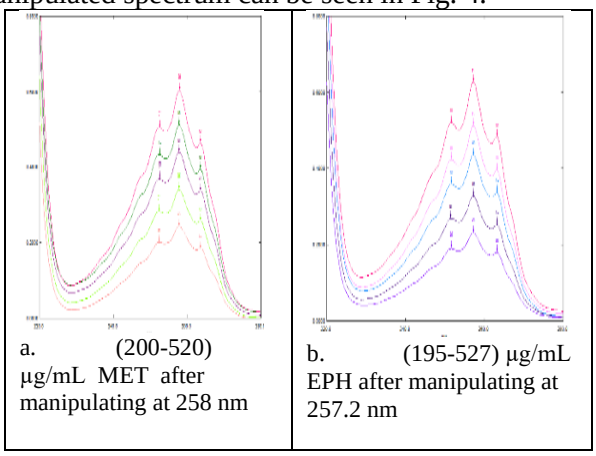
The result is subtraction with a constant where the constant is 360 ug/ml MET divided by the divisor; the subtraction produces a new spectrum, namely

COF/MET°. Then the spectrum is multiplied by the same divisor so that a single COF spectrum is obtained from the mixture. The spectral results of each step are set to zero using a regression equation or calibration curve of COF.

The MET component can be determined by dividing its spectrum by the zero-order spectrum, used as the divisor COF°, then subtracted by the constant COF° so that a new spectrum will be obtained (MET+EPH+ACE/COF°) and then multiplied by COF° and obtained the spectrum MET+EPH+ACE, and divided by 361 ug/ml EPH.

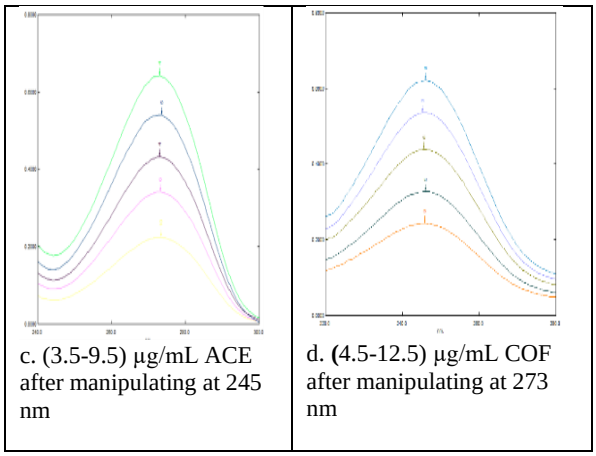
This result is subtraction with the constant 361 ug/ml EPH, which is divided by the divisor, and produces a new spectrum, namely MET+ACE/EPH°, then multiplied by EPH° so that the spectrum of MET+ACE is obtained and then divided by 6.5 ug/ml ACE. The results are shown in subtraction with a constant 6.5 ug/ml ACE divided by the divisor, the result of the subtraction produces a new spectrum, namely MET/ACE°. The spectrum is multiplied by the same divisor to obtain a single MET spectrum from the mixture. The determination of EPH and ACE can be continued by dividing the MET+EPH+ACE Spectrum from the divisor with COF then the spectrum is shared with MET (D°) to get the EPH+ACE spectrum.

Fig. 4 shows that the maximum absorption of the component was changed to 258 nm for MET, 257 nm for EFD, 246 nm for ACE, and 273 nm for COF; this means that the manipulation process in this method made changes to the maximum wavelength. The spectrum result was plotted to obtain a linear regression by the relationship between absorbance versus concentration. The following summarizes the process, and the manipulated spectrum can be seen in Fig. 4.



a. (200-520) µg/mL MET after manipulating at 258 nm

b. (195-527) µg/mL EPH after manipulating at 257.2 nm



c. (3.5-9.5) µg/mL ACE after manipulating at 245 nm

d. (4.5-12.5) µg/mL COF after manipulating at 273 nm

Fig. 4 Absorption spectrum of MET, EPH, COF, and ACE after manipulate

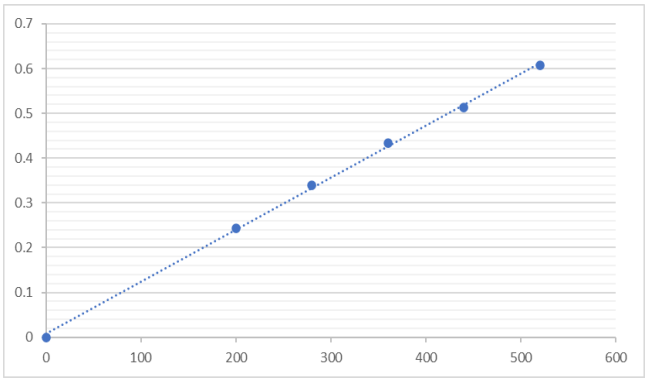


Fig. 5 Calibration curve of (200-520) µg/mL MET

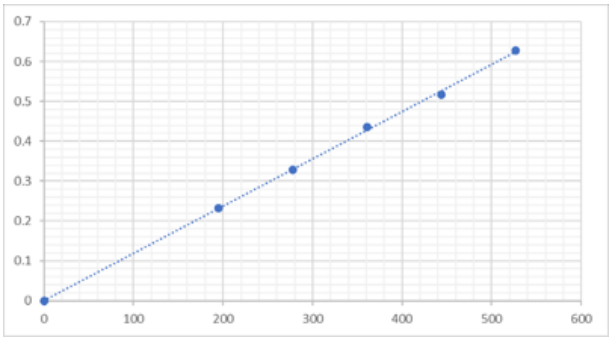


Fig. 6 Calibration curve of (195-527) µg/mL EPH

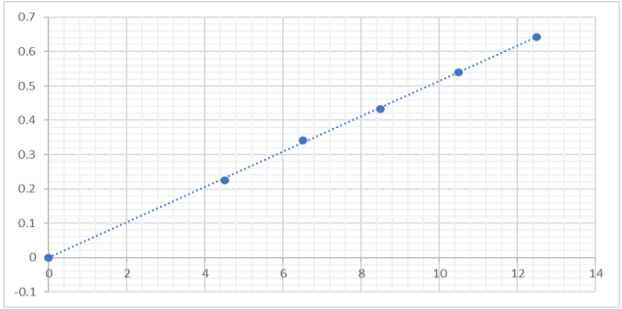


Fig. 7 Calibration curve of (4.5-12.5) µg/mL COF

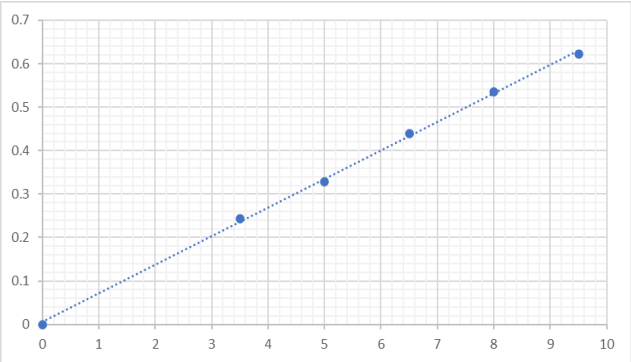


Fig. 8 Calibration curve of (3.5-9.5) µg/mL ACE

After calculating the data on the calibration curves of Figs 5-8, the regression equation for MET is Y = 0.0011X - 0.0070 with a value of r = 0.9994, EPH is Y = 0.0011X + 0.0005 at a value of r = 0.9996, COF is Y = 0.0514X - 0.0008 with a value of r = 0.9997, and ACE is Y = 0.0657X + 0.0051 at a value of r = 0.9994.

So it can be concluded that based on the regression equation and the value of r since r = 0.9950, it is possible to use the subtraction of ratio spectra method to determine the concentration of ecstasy.

3.4. Determination of MET, EPH, COF, and ACE in Ecstasy Tablets

In this study, the determination of levels was corresponding using the subtraction of ratio spectral method on evidence tablets from the Lab. Forensic POLDASU contains the composition of MET, EPH, COF, and ACE.

After manipulating the sample, calculating each regression equation, the results can be seen in Table 2 below.

Table 2 Levels of MET, EPH, COF, and ACE in ecstasy tablets

No	Component	Level Percentage (%)
1	Methamphetamine	38.83
2	Ephedrine HCl	32.13
3	Caffeine	19.05
4	Acetaminophen	10.39

Based on Table 2 above, the results of determining the levels of MET, EPH, COF, and ACE with the subtraction of ratio spectra method, that the terms of the value in the validation test. As a result of the analysis of samples of ecstasy tablets, the SRS method is likely to be used for routine drug analysis, especially for those containing multiple drugs. This method is easy to work, especially for routine analysis, and does not require special skills.

Despite some monographs in the pharmacopeia, some of the contents of ecstasy tablets are not considered therapeutic doses, as shown in Table 2 [2, 3]. These components do not follow the way of treatment but are based on illegal effects that cause health effects on hyperactivity. So have been used drug components for illegal abuse.

4. Conclusion

Based on the discussion and observations during the research, it can be concluded:

1. The subtraction of ratio spectra (SRS) with the ultraviolet spectrophotometry method can be used to determine the components of the ecstasy mixture containing MET, EPH, COF, and ACET in methanol. The absorption spectrum of the two main components in this study was for MET and EPH, though their percentages were similar.

In order to determine the level of ecstasy, it successfully demonstrated its analytical performance by utilizing a linear calibration curve, a DL, and a QL, which is similar to what other researchers do when testing an analytical technique's validity. Moreover, other validation parameters such as precision and accuracy values are below the required limit, demonstrating that subtraction of ratio spectra is a method that has been validly tested against the validation parameters and has met the regulatory agency requirements for method development and validation.

The subtraction of ratio spectra method can be applied to the simultaneous determination of a mixture of four components without being separated. This method is green chemistry, effective, efficient, and reliable in determining MET, EPH, COF, and ACE mixture levels in ecstasy tablets, and meets validation requirements.

2. In general, other researchers who study ecstasy implement qualitative research on how much methamphetamine is in the drug and other components that can increase the negative effects of amphetamine. Ecstasy has been studied by many researchers based on qualitative content using the Molecularly Imprinted Polymer Assisted Paper Spray Ionization Mass Spectrometry, HTPTLC-Densitometry, Modified LC-ESI-MS/MS Method, SPE, and LC-MS-MS method, chiral stationary phase liquid chromatography-tandem mass spectrometry method.

Quantitative research on preparations containing methamphetamine, ephedrine HCL, caffeine, and paracetamol has been carried out using ultraviolet spectrophotometry with the dual-wavelength method. However, the results give fairly high paracetamol content, indicating that the composition of the ecstasy tablet content is not based on its effect but possibly based on the manufacturer.

In terms of the components contained in the ecstasy studied, there is no comparison between the methods used because the compositions contained in each ecstasy studied only have the same methamphetamine, while the supporting components are not the same.

However, based on the tools used and the chemicals used, research using ultraviolet spectrophotometry and the SRS method is simpler.

3. The above implies that the components contained in ecstasy tablets can be determined in methanol solvent, and the absorption spectrum of each standard solution of

the component measured. Then one SRS method can be derived by mathematics using UV Probe 2.42 software connected to a spectrophotometer. Validation of this method has been done. This method can simultaneously determine the four components of MET, EPH, COF, and ACET.

Due to the similar wavelengths of the MET and EPH, using a divisor, the calibration curve of each component can be obtained with a value of r2 > 0.99 so that this method can be implicated in the simultaneous assay of a mixture of Ecstasy tablets.

In the SRS method, it is crucial that a component whose concentration is determined to be soluble in the same solvent, even if its solubility is different.

4. Subtraction of ratio spectra is based on the divisor system and zero-order spectrum of the prepared standard solutions. This method in determining ecstasy levels is one of the spectrophotometric methods to analyze mixtures of several substances directly without their separation. It is easy to use without derivatization and works well with adjacent wavelengths.

The limitation of SRS is the skill of analysts who can handle complex software. UV Probe 2.42 software requires that the analyst working on it understand mathematics. It is necessary to conduct preliminary research to determine how many and what types of components are added to illegal ecstasy tablets because each ecstasy tablet, besides amphetamine or methamphetamine, is also added with components with different compositions.

5. The method of subtraction of ratio spectra can simultaneously analyze four components in ecstasy tablets without separating the components. It is accurate, measurable, and valid. This method is recommended for studies of ecstasy content with different compositions and using different solvents. This method can be used to assay mixtures in laboratories that still have limited sophisticated tools and analysts. It is recommended that the laboratory manager prepare software that can support a spectrophotometer in determining the concentration of a mixture of compounds. Various applications of spectrophotometric methods have been widely used in other studies to determine ecstasy levels. These are Mean Centering Ratio Spectra, Double Divisor Ratio Spectra, Successive Ratio Spectra, Area Under Curve, and Chemometric methods because all are fast, cheap, and represent green chemistry.

In future research, the spectrophotometric methods can be developed to study mixtures not limited to preparations but also for biological components such as plasma and urine - development of various methods from new combinations of spectrophotometer tools with up-to-date mathematical software.

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References

[1] HUTAGAOL D.M, MUCHLISY A.M., and SINAGA S.M. Application of Ultraviolet Spectrophotometry with the Dual Wavelength Method for Simultaneous determination of ecstasy Tablet Content, Asian *Journal of Pharmaceutical Research and Development*, 2020, 8(5): 01-04. http://dx.doi.org/10.22270/ajprd.v8i5.823

[2] UNITED STATES PHARMACOPEIAL CONVENTION. *The USP 43–NF 38*. 2020, 1-5: 661, 1641, 2840.

[3] MINISTRY OF HEALTH OF THE REPUBLIC OF INDONESIA. *Indonesian Pharmacopeia V Edition*. Jakarta, 2020: 348,734, 1046.

[4] INDONESIAN MINISTRY OF HEALTH. *Regulation of the Minister of Health of the Republic of Indonesia. About Changes in Narcotics Classification*. 2018, 50: 5-6.

[5] MOFFAT A.C., OSSELTON M.D., and WIDDOP B. Clarke's analysis of drugs and poisons. 4th ed. Pharmaceutical Press, London, 2011: 1226-27, 1337-40, 1639-43, 1857-58.

[6] REGINA T., and NOVITA C. Impaired Cognitive Function Due to Amphetamine Abuse. *Majority*, 2017, 7(1): 49-53.

[7] GUNAWAN, and SULISTIA G. *Pharmacology and Therapy*. 6th ed. FKUI Publishing Agency. Jakarta, 2016: 64-74.

[8]THANOS P.K., KIM R., DELIS F., ANANTH M., CHACHATI G., and ROCCO M.J. Chronic Methamphetamine Effects on Brain Structure and Function in Rats. *PLoS ONE*. 2016 11(6): e0155457. https://doi.org/10.1371/journal.pone. 0155457

[9] YEONG E.S., BEOM J.K., and JIN Y.K. Determination of methamphetamine and amphetamine enantiomers in human urine by CSP-LC-MS/MS. *Analytical Science and Technology*, 2019, 32(5): 163-72. https://doi.org/10.5806/AST.2019.32.5.163

[10] KAR-WENG C., and SURAYA H.R. Development of an in-house HPLC method for the analysis of ecstasy-laced beverages. *Egyptian Journal of Forensic Sciences*, 2018, 8(18). https://doi.org/10.1186/s41935-018-0048-8

[11] LOANDA R.C., JAMIE P.S., KHALED Y.Z., OLIVER B.S., DEVANEY R.C., and CRAIG E.B. Forensic electrochemistry: simultaneous voltametric detection of MDMA and its fatal counterpart "Dr. Death." *Analitical Methods*, 2016, 8: 142-152. https://doi.org10.1039/c5ay02924d.

[12] CHU-AN Y., HSIU-CHUAN L., DONG-LIANG L., RAY H.L., YOU-ZUNG H., and SHU-PAO W. Simultaneous Quantitation of Methamphetamine, Ketamine, Opiates and their Metabolites in Urine by SPE and LC-MS-MS, *Journal*

of Analytical Toxicology, 2017, 41(8): 679–87. https://doi.org/10.1093/jat/bkx057
[13] YANG XU L., JUAN D., HUADI X., LING Z., XUNCAI C., ANDE M., and QIZHI L. Simultaneous Determination of Methamphetamine and Its Isomer N-Isopropylbenzylamine in Forensic Samples by Using a Modified LC-ESI-MS/MS Method. *Journal of Nanomaterials*, 2021, 6679515. https://doi.org /10.1155/2021/6679515
[14] DE BRITO T.P., DEBORAH DE AGUIAR V.A., PEREIRA I., and VAZ B.G. Determining Methamphetamine in Urine by Molecularly Imprinted Polymer Assisted Paper Spray Ionization Mass Spectrometry. *Journal of the Brazilian Chemical Society*, 2021, 32(2): 269-276. https://dx.doi.org/10.21577/0103-5053.
[15] KAMAL A.H., EL-MALLA S.F., and HAMMAD S.F. A Review on UV Spectrofotometric Methods for Simultaneous Multicomponent Analysis. *European Journal of Pharmaceutical and Medical Research*, 2016, 3(2): 348-360.
[16] SEBAIY M., and MATTAR A. Ratio Subtraction Coupled with Extended Ratio Subtraction Method for Simultaneous Determination of Paracetamol and Orphenadrine Citrate in their Combined Pharmaceutical Dosage Forms. *International Journal of Modern Pharmaceutical Research*, 2020, 4(1).
[17] BAHRI M., MASFRIA M., SYAHPUTRA S., and HAFID H. Quantitative Estimation of Anti Hypertension Combination by Subtraction of Ratio Spectra Spectrophotometry Method. *Open Access Macedonian Journal of Medical Sciences*, 2019, 7(17): 2940-2946. DOI: 10.3889/oamjms.2019.750.
[18] LOTFY H.M., and HAGAZ M.A.M. Comparative Study of Novel Spectrophotometric Methods Manipulating Ratio Spectra: An Application on Pharmaceutical Ternary Mixture of Omeprazole, Tinidazole and Clarithromycin. *Molecular and Biomolecular Spectroscopy*, 2012, 96: 259-270. DOI: 10.1016/j.saa.2012.04.095
[19] SOUMIA B., FATIMA H., SAÏD B., BOUCHAÏB B., SOUAD T., SOUAD H., AHMED B., and ABDELMJID A. Statistical Tools and Methods for Validation of Analysis Methods: Methodology and Examples, *International Journal of Metrology and Quality Engineering*, 2017, 8. https: //doi.org/10. 1051 /ijmqe/2016030
[20] FOOD AND DRUG ADMINISTRATION OFFICE OF REGULATORY AFFAIRS. *ORA Laboratory Manual Methods, Method Verification and Validation*. 2020, II, 27-29.

参考文:

[1] HUTAGAOL D.M、MUCHLISY A.M. 和 SINAGA S.M. 紫外分光光度法與雙波長法同時測定搖頭丸含量的應用, 亞洲藥物研究與開發雜誌, 2020, 8(5): 01-04. http://dx.doi.org/10.22270/ajprd.v8i5.823
[2] 美國藥學公約。美國藥典 43 – 國家處方集 38. 2020, 1-5: 661, 1641, 2840。
[3] 印度尼西亞共和國衛生部。印度尼西亞藥典伏版。雅加達，2020 年：348,734、1046。
[4] 印度尼西亞衛生部。印度尼西亞共和國衛生部長條例。關於麻醉品分類的變化。 2018，50：5-6。
[5] MOFFAT A.C.、OSSELTON M.D. 和 WIDDOP B. 克拉克的分析毒品和毒藥。第 4 版。製藥出版社，倫敦，2011 年：1226-27、1337-40、1639-43、1857-58。
[6] REGINA T. 和 NOVITA C. 濫用苯丙胺導致認知功能受損。多數, 2017, 7(1): 49-53。
[7] GUNAWAN 和 SULISTIA G. 藥理學和治療。第 6 版。印度尼西亞大學醫學院出版社。雅加達，2016 年：64-74。
[8] THANOS P.K.、KIM R.、DELIS F.、AANTH M.、CHACHATI G. 和 ROCCO M.J. 慢性甲基苯丙胺對大鼠腦結構和功能的影響。公共圖書館一。 2016 年 11（6）：e0155457。 https://doi.org/10.1371/journal.pone。 0155457
[9] YEONG E.S.、BEOM J.K. 和 JIN Y.K.通過光熱發電-液相色譜串聯質譜測定人尿中的甲基苯丙胺和苯丙胺對映體。分析科學與技術，2019, 32(5): 163-72. https://doi.org/10.5806/AST.2019.32.5.163
[10] KAR-WENG C. 和 SURAYA H.R. 開髮用於分析含搖頭丸的飲料的內部高效液相色譜方法。埃及法醫學雜誌，2018，8(18)。 https://doi.org/10.1186/s41935-018-0048-8
[11] LOANDA R.C.、JAMIE P.S.、KHALED Y.Z.、OLIVER B.S.、DEVANEY R.C. 和 CRAIG E.B. 法醫電化學：同時伏安檢測 3,4-亞甲二氧基甲基苯丙胺及其致命對應物“博士。死亡”。分析方法，2016，8：142-152。 https://doi.org10.1039/c5ay02924d。
[12] CHU-AN Y.、HSIU-CHUAN L.、DONG-LIANG L.、RAY HL、YOU-ZUNG H. 和 SHU-PAO W. 尿液中甲基苯丙胺、氯胺酮、阿片類藥物及其代謝物的同時定量血清蛋白電泳和液相色譜-質譜法液相色譜串聯質譜，分析毒理學雜誌，2017 年，41(8)：679 – 87。 https://doi.org/10.1093/jat/bkx057
[13] YANG XU L.、JUAN D.、HUADI X.、LING Z.、XUNCAI C.、ANDE M. 和 QIZHI L. 使用改進的液晶显示器同時測定法醫樣品中的甲基苯丙胺及其異構體 N-異丙基苄胺-電子光譜成像-串聯質譜方法。納米材料雜誌，2021，6679515。https://doi.org/10.1155/2021/6679515
[14] DE BRITO T.P.、DEBORAH DE AGUIAR V.A.、PEREIRA I. 和 VAZ B.G. 通過分子印跡聚合物輔助紙噴霧電離質譜法測定尿液中的甲基苯丙胺。巴西化學會雜誌，2021 年，32(2)：269-276。 https://dx.doi.org/10.21577/0103-5053。
[15] KAMAL A.H.、EL-MALLA S.F. 和 HAMMAD S.F. 用於同時多組分分析的紫外分光光度法綜述。歐洲藥物與醫學研究雜誌，2016 年，3(2)：348-360。
[16] SEBAIY M. 和 MATTAR A. 比例減法與擴展比例減法相結合，用於同時測定複方藥物劑型中的撲熱息痛和檸檬酸奧芬那君。國際現代藥物研究雜誌，2020，4(1)。
[17] BAHRI M., MASFRIA M., SYAHPUTRA S., 和 HAFID H. 抗高血壓組合的定量估計減比光譜分光光度法。開放存取馬其頓醫學科學雜誌, 2019, 7(17): 2940-2946。 DOI：10.3889/oamjms.2019.750。
[18] LOTFY H.M. 和 HAGAZ M.A.M. 新型分光光度法控制比譜的比較研究：在奧美拉唑、替硝唑和克拉黴素的藥物三元混合物中的應用。分子和生物分子光譜學，2012，96：259-270。DOI：10.1016/j.saa.2012.04.095
[19] SOUMIA B.、FATIMA H.、SAÏD B.、BOUCHAÏB B.、SOUAD T.、SOUAD H.、AHMED B. 和 ABDELMJID A. 用於驗證分析方法的統計工具和方法：方法論和實例，國際計量與質量工程雜誌，2017，8。 https://doi.org/10。 1051 /ijmqe/2016030

[20] 食品藥品監督管理局監管事務辦公室。監管事務辦公室實驗室手冊方法、方法驗證和驗證。 2020，二，27-29。