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Original Article

Detection of Mefenamic Acid in Hyperuricemia Herbal Medicine using UV-Vis Spectrophotometry and FTIR Spectroscopy Combined with Chemometrics

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Abstract: The adulteration of herbal medicines with synthetic drugs poses a serious risk to public health. Mefenamic acid, a non-steroidal anti-inflammatory drug (NSAID), is sometimes illicitly added to herbal products marketed for hyperuricemia to enhance their therapeutic effect. This study aims to detect the presence of mefenamic acid in herbal medicines for hyperuricemia using a combination of UV-Vis spectrophotometry and Fourier Transform Infrared (FTIR) spectroscopy coupled with chemometric. approaches, specifically Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Partial Least Squares (PLS). The UV-Vis method was validated and showed good linearity with an R^2 value of 0.999. Accuracy testing revealed recovery rates ranging from 98.75% to 101.92%, while precision testing produced %RSD values between 0.86% and 1.05%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.235 ppm and 0.711 ppm, respectively. FTIR spectral data were processed using OPLS-DA and PLS to classify and predict adulterated samples. The validated OPLS-DA model demonstrated strong classification performance, effectively distinguishing between adulterated and non-adulterated samples. Furthermore, the detection results from UV-Vis, OPLS-DA, and PLS methods were consistent, confirming the reliability of the integrated approach.

Keywords: mefenamic acid; herbal medicine; UV-Vis spectrophotometry; FTIR spectroscopy; chemometrics

1. INTRODUCTION

Traditional medicines are formulations derived from plants, animals, minerals, or their mixtures that have been used for generations in accordance with local customs [1-3]. In Indonesia, traditional medicines are officially classified into three categories: herbal medicine based on traditional knowledge (in Indonesian known as Jamu), standardized herbal medicines, and phytopharmaceuticals, depending on their preparation, claimed uses, and levels of scientific evidence [4]. Among these, herbal medicine remains the most widely used, with 49.5% of Indonesians reported consuming it and 4.5% consume it daily [5]. This widespread consumption highlights the cultural and therapeutic significance of jamu in Indonesian society. According to BPOM Regulation No. 25 of 2023, jamu refers to natural medicines rooted in traditional or cultural heritage knowledge, intended for health maintenance, disease prevention, and treatment [4].

One of the most popular types of herbals is the anti-gout formulation, commonly used as an alternative therapy for hyperuricemia and gout arthritis. These herbal preparations often contain ingredients such as *Sonchus arvensis* (tempuyung), *Caesalpinia sappan* (secang), *Stelechocarpus burahol* (kepel), *Curcuma xanthorrhiza* (temulawak), *Curcuma longa* (turmeric), and *Phyllanthus niruri* (meniran), which are traditionally believed to help reduce uric acid levels [6]. However, growing consumer demand and expectations for rapid relief have led some irresponsible manufacturers to

adulterate these herbal products with active pharmaceutical compounds, known as *Bahan Kimia Obat* (BKO), including mefenamic acid, in order to enhance their perceived efficacy [7-8].

Mefenamic acid is a nonsteroidal anti-inflammatory drug (NSAID) frequently misused in herbal products for its analgesic and anti-inflammatory properties. While such adulteration may produce faster effects, it also poses serious health risks such as gastrointestinal bleeding, liver and kidney dysfunction, cardiovascular complications, and even death [9-10]. In response, the Indonesian National Agency for Drug and Food Control (BPOM) has issued public warnings about unregistered traditional products containing harmful synthetic drugs. In 2022, 41 traditional medicines and supplements were found adulterated with compounds such as phenylbutazone, dexamethasone, paracetamol, and mefenamic acid, many of which were sold under fake registration numbers [11]. Previous studies in various regions of Indonesia have also reported the presence of mefenamic acid in herbal pain-relief and hyperuricemia herbal medicine [12-20] .

Several analytical techniques have been applied to detect synthetic drug adulterants in herbal medicine, including Thin Layer Chromatography (TLC) [13,17], UV-Vis spectrophotometry [18], FTIR spectroscopy [21], Liquid Chromatography [22-24]. UV-Vis spectrophotometry is favored for its simplicity, rapidity, and cost-effectiveness, particularly for compounds like mefenamic acid, which contain chromophore and auxochrome groups [25-26]. Meanwhile, Fourier Transform Infrared (FTIR) spectroscopy offers a non-destructive approach to analyze molecular structures and chemical compositions and is increasingly combined with chemometric methods such as Partial Least Squares (PLS) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) for more accurate classification and quantification [28-30]. These techniques support a green analytical chemistry approach, minimizing the need for reagents and sample preparation [31].

Based on the results of a literature review and preliminary investigation, no prior studies have been identified that analyze the presence of active pharmaceutical compounds, specifically mefenamic acid, in herbal medicines for hyperuricemia using UV-Visible spectrophotometry and a combination of FTIR spectroscopy with chemometric techniques in five subdistricts of Malang City. Therefore, the authors are interested in exploring and quantifying the levels of mefenamic acid in hyperuricemia herbal medicines sold in traditional herbal shops within the region

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study included powdered simplicia of *Sonchus arvensis herb, Biancaea sappan, Stelechocarpus burahol leaves, Curcuma xanthorrhiza rhizome, Curcuma longa rhizome,* and *Phyllanthus niruri herb* were taxonomically identified at UPT Herbal Laboratory of Materia Medica, Batu City, East Java. The samples in this study consisted of five types of hyperuricemia herbal medicines in the form of powder or capsules, which were obtained from herbal medicine shops located in five subdistricts of Malang City. The samples namely AU1, AU2, AU3, AU4, and AU5. Additional materials included a mefenamic acid reference standard (Ningbo Smart Pharmaceutical®, China), methanol pro analysis grade (Smart Lab®, Indonesia), and distilled water (Merck®, Germany).

2.2. Instrumentations

The instruments used in this study included an analytical balance (Ohaus® PX224, USA), vials, glassware (PYREX®, Arizona), spatulas (Phy Edumedia®, Indonesia), a double-beam UV-Visible spectrophotometer (Shimadzu® UV-1800, Japan), cuvettes (Shimadzu®, Japan), an FTIR spectrometer (Shimadzu®, Japan), and chemometric software (TQ Analyst®, Ireland; SIMCA®, Sweden).

2.3. Methods

2.3.1. Sample preparation

To prepare the herbal matrix, 10 grams of powdered simplicia were weighed, consisting of 10.53% *Sonchus arvensis herb*, 26.32% *Biancaea sappan*, and 15.79% each of *Stelechocarpus burahol leaves*,

Curcuma xanthorrhiza rhizome, *Curcuma longa rhizome*, and *Phyllanthus niruri herb*. The powdered materials were then thoroughly mixed using a Turbula® mixer to obtain a homogeneous herbal matrix.

The negative control herbal extract was prepared by accurately weighing 12.5 mg of the herbal matrix powder using an analytical balance. The weighed sample was transferred into a 15 mL vortex tube, followed by the addition of 15 mL of a hydrochloric acid–methanol solvent mixture. The mixture was vortexed for approximately 15 minutes to ensure complete homogenization, then centrifuged for 10 minutes at 25 rpm. The resulting supernatant was transferred into a 25 mL volumetric flask and brought to volume with the same HCl–methanol solvent mixture, yielding a solution with a concentration of 500 ppm. Subsequently, 5 mL of this solution was pipetted into a 100 mL volumetric flask and diluted to the mark with methanol. The solution was mixed thoroughly to obtain a final working solution with a concentration of 25 ppm.

The mefenamic acid stock solution was prepared by accurately weighing 10 mg of mefenamic acid reference standard and transferring it into a 100 mL volumetric flask. A solvent mixture of hydrochloric acid and methanol was then added to the flask until the calibration mark was reached. The solution was thoroughly mixed to ensure complete dissolution and homogenization, resulting in a stock solution with a final concentration of 100 ppm.

For preparation of positive control herbal powder for FTIR spectroscopy analysis, the samples were prepared at various concentrations of mefenamic acid (5, 7, 9, 10, 13, 15, 17, 19, 20, 21, 23, and 25% w/w). For each concentration, the mefenamic acid standard and herbal matrix were accurately weighed and placed into a mortar. The mixture was thoroughly blended using a mortar and pestle until a homogeneous powder was obtained. The resulting mixture was transferred into a vial for storage.

2.3.2. UV-Vis Spectrophotometry Analysis

a. Method Validation

1. Specificity

Specificity was evaluated by comparing the UV-Vis absorption spectra of the negative control herbal extract, the positive control extract containing mefenamic acid, and a 15 ppm working standard solution of mefenamic acid. The spectra were recorded within a wavelength range of 200–400 nm, and the absorbance peaks of each solution were observed to determine the presence of overlapping or interfering signals.

2. Linearity

Linearity was assessed by constructing a calibration curve based on the absorbance values of mefenamic acid working standard solutions at concentrations of 5, 10, 15, 20, and 25 ppm. The absorbance was measured at the maximum wavelength obtained from the specificity test using UV-Vis spectrophotometry. A linear regression equation in the form of $y = bx + a$ was generated, and the method was considered linear if the correlation coefficient (r) exceeded 0.99.

3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on a series of diluted mefenamic acid standard solutions. First, a 100 ppm stock solution was prepared by dissolving 10 mg of mefenamic acid in 100 mL of a methanol–HCl solvent mixture. Then, 2.5 mL of this stock solution was transferred to a 25 mL volumetric flask and diluted to the mark to yield a 10 ppm standard solution. Working solutions with concentrations of 0.5, 1, 1.5, 2, and 2.5 ppm were then prepared by pipetting respective volumes of the 10 ppm solution into 10 mL volumetric flasks and diluting them to volume with the same solvent mixture. The absorbance values were measured using UV-Vis spectrophotometry at the previously determined maximum wavelength. The limits of detection (LOD) and quantification (LOQ) were determined using the following equations.

$$\text{LOD} = \frac{3.3\ x\ s}{m} \quad (1)$$

$$\text{LOQ} = \frac{10\ x\ s}{m} \quad (2)$$

where **s** is the standard deviation of the response and **m** is the slope of the calibration curve.

4. Accuracy

Accuracy was evaluated using recovery studies at three concentration levels: 12, 15, and 18 ppm. Each solution was prepared by transferring 1.2, 1.5, and 1.8 mL, respectively, of the 100 ppm mefenamic acid stock solution into 10 mL volumetric flasks, and then diluting to volume with the negative control extract. Each concentration level was prepared in triplicate, resulting in nine volumetric flasks in total. The absorbance of each solution was measured at the maximum wavelength using UV-Vis spectrophotometry. Accuracy was evaluated by calculating the percentage recovery (%Recovery) of known spiked concentrations. The recovery value indicates how close the measured concentration is to the true (spiked) concentration. It was calculated using the following equation:

$$\%\text{Recovery} = \frac{\text{C}_{\text{found}}}{\text{C}_{\text{added}}}\ \text{x}\ 100 \quad (3)$$

where $C_{\text{found}}$is the measured concentration of the analyte, and $C_{\text{added}}$is the known amount of analyte added to the sample.

5. Precision

Precision was determined by calculating the relative standard deviation (RSD) from repeated absorbance measurements of a 15 ppm mefenamic acid solution. The solution was measured seven times under identical conditions, and the standard deviation (SD) and RSD were calculated to evaluate repeatability. %RSD was calculated using the following equation:

$$\%RSD = (\frac{SD}{\bar{X}}) \times 100 \quad (4)$$

where $SD$is the standard deviation of replicate measurements, and $\bar{X}$is the mean of the measured values.

b. Quantitative Analysis

Observations were carried out on the wavelength, spectra, and absorbance of the treated samples using UV-Visible spectrophotometry at the previously determined maximum wavelength. The absorbance values of each sample were then inserted into the linear regression equation obtained from the calibration curve to determine the concentration of mefenamic acid, expressed in parts per million (ppm).

2.3.3. FTIR Spectroscopy Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed using a Qatar-S Single Bounce Diamond ATR (Attenuated Total Reflectance) instrument. Prior to sample measurement, the ATR crystal was cleaned with acetone. A background spectrum scan was acquired before analyzing the samples. The samples included pure herbal powder, mefenamic acid standard, and powdered herbal medicine samples for hyperuricemia obtained from five subdistricts in Malang City. Each sample was placed directly onto the ATR crystal for measurement. Scans were carried out at a resolution of 16 cm^{-1} with 32 scans per spectrum, within a wavenumber range of 4000–400 cm^{-1}. The herbal medicine samples were analyzed in triplicate, with three repeated scans per replicate, while the control herbal powder and the mefenamic acid standard were analyzed in five replicates, also with three repeated scans per replicate. All spectra were recorded in absorbance mode. Upon

completion of each measurement, the ATR crystal was cleaned with acetone and gently dried using a lint-free tissue.

2.3.4. Chemometric Analysis

Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was performed using SIMCA 14.1 software (64-bit) (Umetrics, Sweden), while multivariate calibration using Partial Least Squares (PLS) regression was conducted with TQ Analyst software (Thermo Fisher Scientific, Ireland). The OPLS-DA validation was performed by removing group and sample name information from one-third of the total dataset, while the remaining two-thirds were used for model calibration. The excluded one-third of the samples were then projected onto the OPLS-DA model to evaluate whether they were correctly classified according to their respective groups. While, the Partial Least Squares (PLS) model was validated using the Leave-One-Out Cross-Validation (LOOCV) technique.

2. RESULTS AND DISCUSSION

3.1. Analysis using UV-Vis Spectrophotometry

3.1.1. Method Validation

Specificity

The specificity test was conducted by analyzing the negative control herbal extract, the positive control extract containing mefenamic acid, and a 15 ppm mefenamic acid working standard using a UV-Visible spectrophotometer. Spectral observations were performed within the wavelength range of 200–400 nm to determine the characteristic absorption peaks of each solution. The results showed that the maximum absorbance wavelength (λmax) of mefenamic acid was observed at 279 nm. These results are aligned with findings reported in other studies [32-33]. Furthermore, no interfering peaks were detected in the matrix of the positive control herbal extract, indicating that the mefenamic acid peak was well-resolved and free from matrix interference (Figure 1).

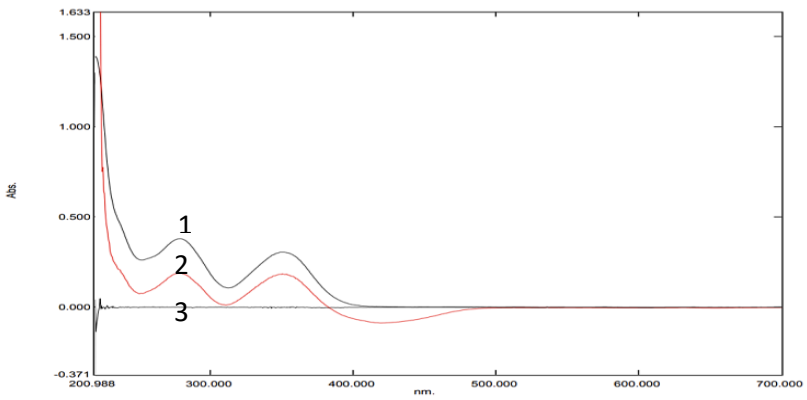

Figure 1. Spectrum of negative control [1], the positive control [2], and a 15 ppm mefenamic acid working standard [3]

Linearity

Linearity testing aims to ensure that the UV-Vis spectrophotometric method provides a proportional response to the concentration of mefenamic acid within a specified range. This test was conducted by preparing standard solutions of mefenamic acid at concentrations of 5, 10, 15, 20, and 25 ppm, which were then analyzed using a UV-Vis spectrophotometer at a wavelength of 279 nm, as determined from the specificity test. The absorbance values at each concentration were used to construct a calibration curve, yielding the linear regression equation $y = 0.0341x + 0.0085$ with a correlation coefficient (r^2) of 0.9999. According to AOAC (2013) guidelines, a method is considered linear if the coefficient of correlation (r^2) is greater than 0.99 [34]. Therefore, the linearity test results

in this study demonstrate that the method exhibits good linearity within the tested concentration range. The results of this test are presented in Figure 2.

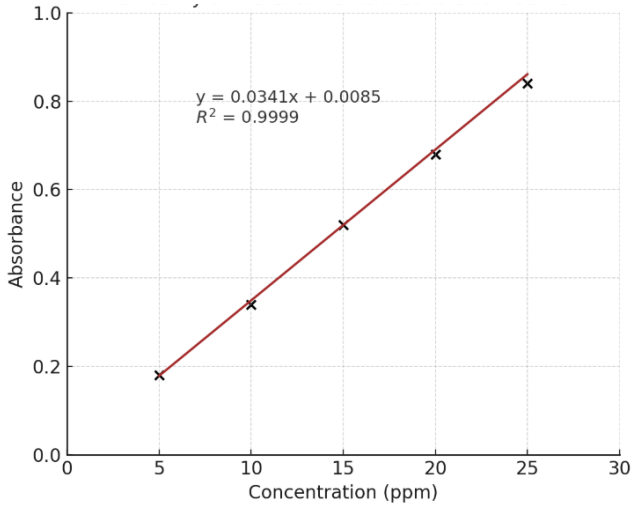

Figure 2. Calibration curve of mefenamic acid standard solution with range concentration 5-25 ppm

LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) tests were performed to determine the lowest concentration of mefenamic acid that can be reliably detected (LOD) and accurately quantified (LOQ) by the UV-Vis spectrophotometric method used. The analysis was carried out using a series of low-concentration standard solutions of mefenamic acid at 0.5, 1, 1.5, 2, and 2.5 ppm. Based on the absorbance measurement data, the LOD and LOQ values were calculated using commonly accepted formulas, which are based on the standard deviation and the slope of the calibration curve. The calculation results showed that the LOD was 0.235 ppm and the LOQ was 0.711 ppm. These values indicate that the method is sufficiently sensitive for detecting and quantifying mefenamic acid at low concentrations.

Accuracy and Precision

Accuracy and precision testing was conducted to evaluate the extent to which the analytical method can produce results that are close to the true value (accuracy) and consistent under repeated conditions (precision). The test involved nine volumetric flasks representing three concentration levels of mefenamic acid: 80% (12 ppm), 100% (15 ppm), and 120% (18 ppm), each prepared in triplicate. The test solutions were prepared by spiking a control herbal matrix with mefenamic acid, and measurements were carried out at 279 nm using a UV-Vis spectrophotometer. The detailed procedure for preparing the accuracy and precision test solutions is provided in Appendix X. The results showed that the percent recovery ranged from 98.75% to 101.92%, while the relative standard deviation (%RSD) values ranged from 0.86% to 1.05%. According to AOAC (2013) guidelines, the acceptable recovery range for an analyte at 100% concentration is 98–101%, and a %RSD value below 1% indicates good precision [34]. Therefore, the results of this study meet the acceptance criteria and demonstrate that the method possesses good accuracy and precision.

3.1.2. Quantitative analysis of mefenamic acid

Quantitative analysis of mefenamic acid content was carried out on five samples. The analysis was performed using UV-Vis spectrophotometry at a wavelength of 279 nm. In this study, none of the five tested samples showed the presence of mefenamic acid. This was indicated by the absence of characteristic absorbance peaks at 279 nm, which is the maximum absorption wavelength of mefenamic acid. This observation is clearly illustrated in Figure 3, which show the spectra of the

five tested samples, all lacking any significant peaks at this wavelength. Therefore, it can be concluded that the five samples analyzed did not contain mefenamic acid, as evidenced by the UV-Vis spectrophotometric results.

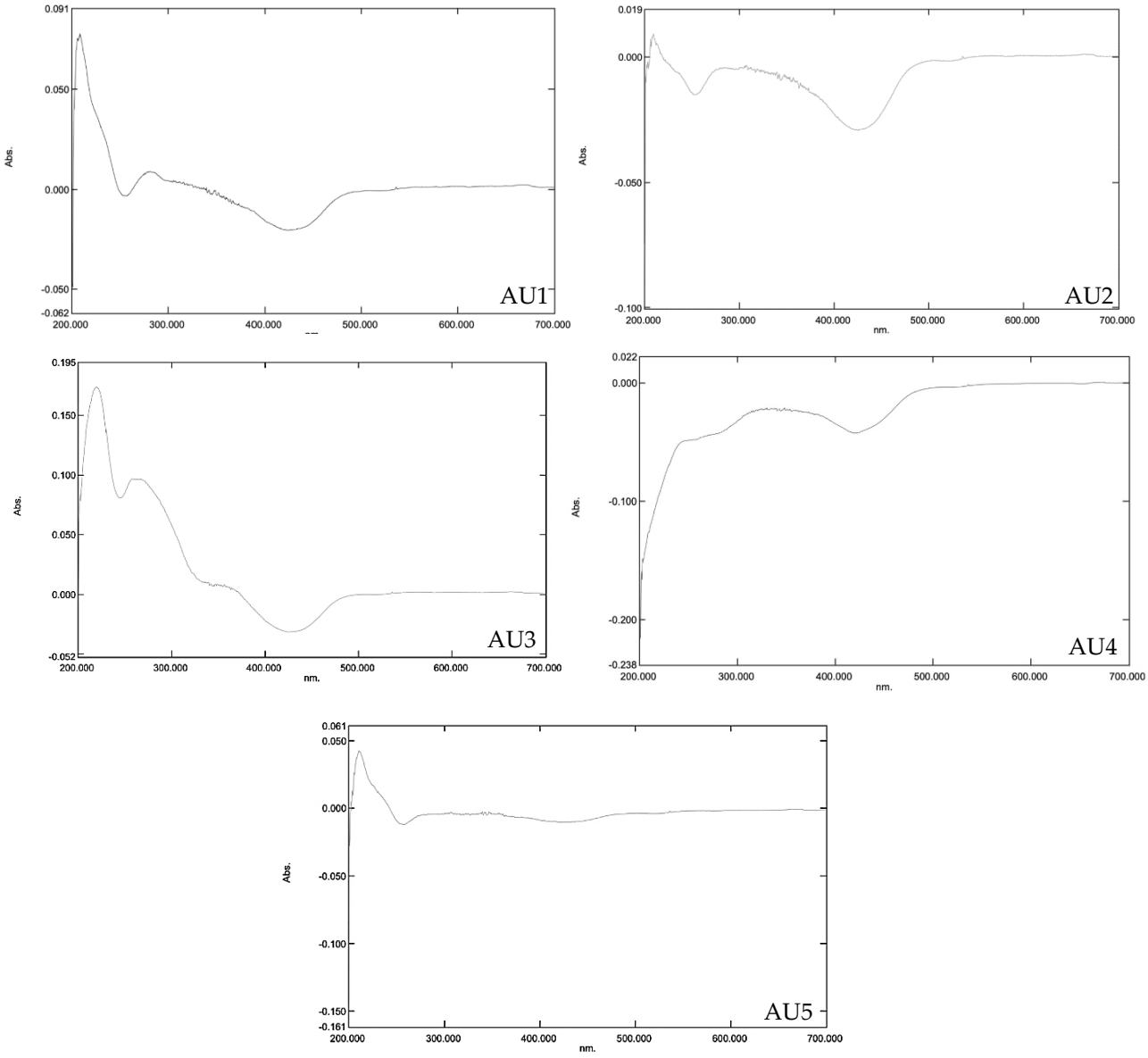

Figure 3. The UV-Vis spectrum of five samples

3.2. Analysis using FTIR Spectoscopy

In this study, analysis of the control herbal powder (anti-hyperuricemia herbal medicine), mefenamic acid, and all samples were conducted using a Shimadzu® Fourier Transform Infrared (FTIR) Spectrophotometer equipped with a QATR-S single bounce diamond ATR accessory. The spectrum was recorded over a wavenumber range of 4000–400 cm^{-1} with a resolution of 16 cm^{-1} and 32 scans. Based on the overlay of FTIR spectra presented in Figure 4, As shown in Figure 4, the FTIR spectra of mefenamic acid and the herbal matrix display distinct differences in their characteristic absorption bands, indicating variations in their functional group compositions. The herbal medicine matrix exhibited characteristic absorption bands corresponding to the functional groups OH (3281.41 cm^{-1}), CH_3 (2920.26 cm^{-1}), C=C (1626.40 cm^{-1}), and C=O (1235.40 and 1021.15 cm^{-1}) [35-37]. The results indicate that mefenamic acid exhibits characteristic absorption bands corresponding to the N–H (3308.73

cm^{-1}), C–H (2859.43 cm^{-1}), C=O (1646.93 cm^{-1}), C=C (1573.42 cm^{-1}), and C–O (1153.59 and 1095.40 cm^{-1}) functional groups [38-40]. The structure of mefenamic acid shown in Figure 5.

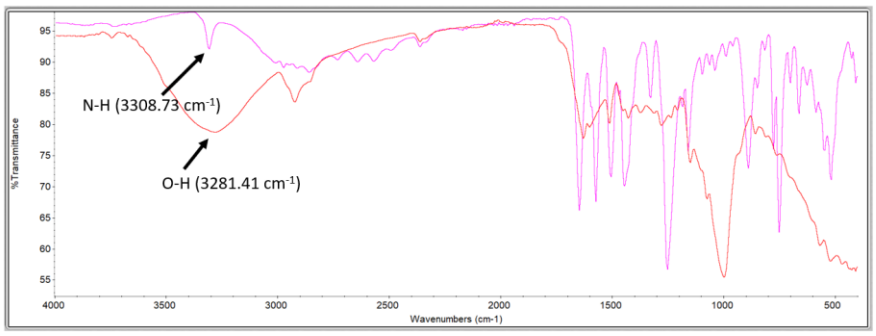

Figure 4. The overlay of FTIR spectra of herbal medicine matrix (red line) and Mefenamic acid (purple line)

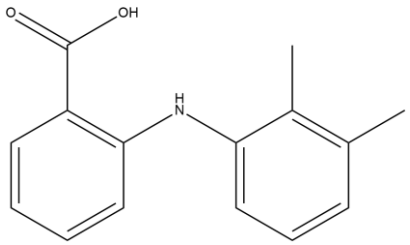

Figure 5. Structure of mefenamic acid

The herbal medicine matrix as negative control, mefenamic acid standard, and positive control (herbal medicine matrix with mefenamic acid) was scan using FTIR spectroscopy to observed the spectrum. The spectrum shown in Figure 6. Based on the results of FTIR spectroscopy analysis, the positive control sample showed an increase in absorbance within the wavenumber range of 1700–1400 cm^{-1} that indicated absoption from functional group of carbonyl group in mefenamic acid [38-40].

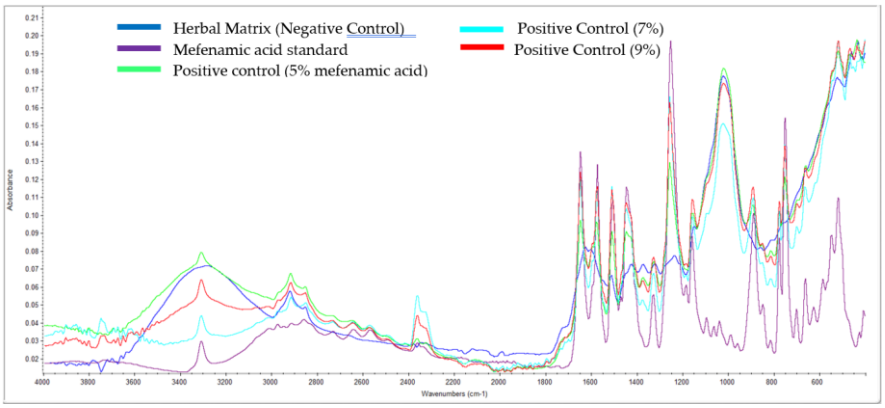

Figure 6. The FTIR spectrum of herbal medicine matrix as negative control, mefenamic acid standard, and positive control (herbal medicine matrix with mefenamic acid)

The five samples were analysis using FTIR spectroscopy. The Figure 7 shows the overlay of FTIR spectrum of five samples. Based from the FTIR Spectrum, no peak indicated the absoption of functional group of mefenamic acid.

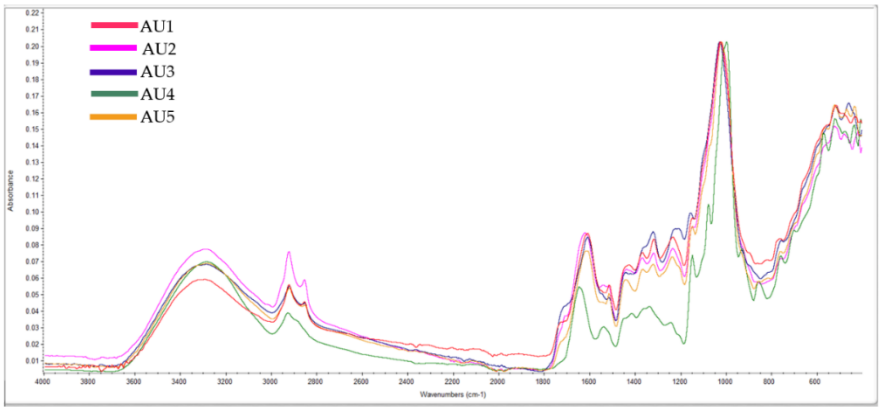

Figure 7. The overlay of FTIR spectrum of five samples

3.3. Chemometric Analysis

3.3.1 OPLS-DA

Sample classification analysis using supervised chemometric modeling (OPLS-DA) was performed by inputting the FTIR spectral data into SIMCA software. Prior to conducting the sample classification, the OPLS-DA model was validated by removing the identity of one-third of the spectral data from the positive and negative control groups. The OPLS-DA was set into two groups, negative control and mefenamic acid groups The validation results are presented in Figure 8 and Table 1. The results demonstrated that the OPLS-DA model was capable of accurately classifying the samples into their respective groups.

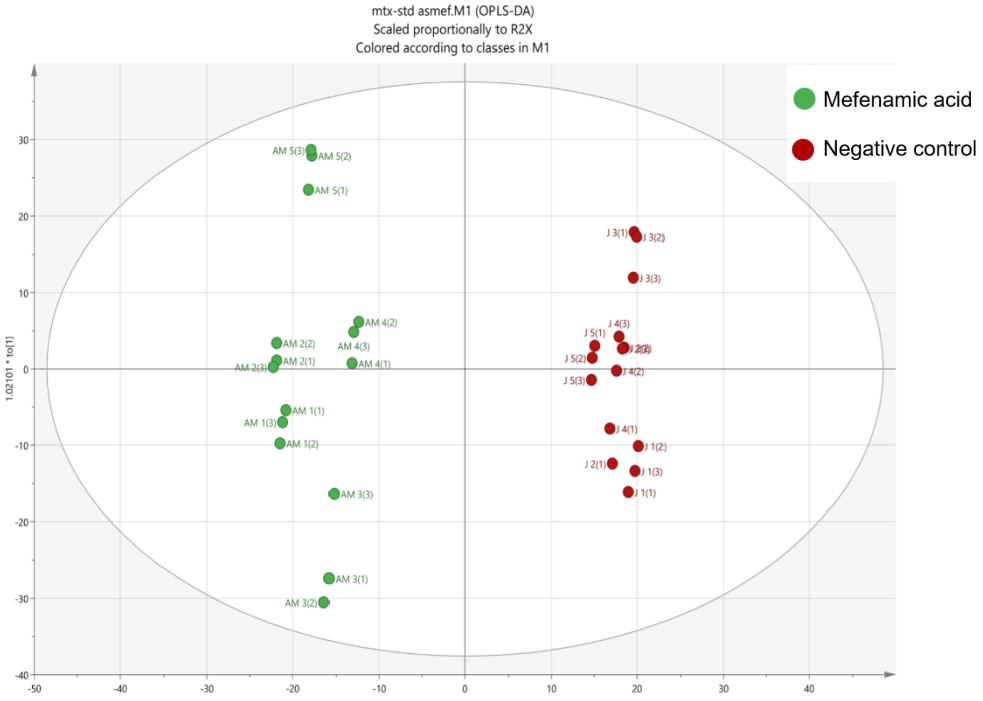

Figure 8. Score Plot of validation the OPLS-DA

Table 1. The results of validation of OPLS-DA

Model	Total	Calibration		Validation	
		Total	Correct	Total	Correct
Control Negative	15	10	100%	5	100%
Mefenamic acid standard	15	10	100%	5	100%

Following the validation stage, prediction of the five test samples was carried out using the OPLS-DA model. The results indicated that none of the five samples contained mefenamic acid. This was evidenced by the classification of all five samples into the negative control group (herbal medicine group). These findings are illustrated in Figure 9.

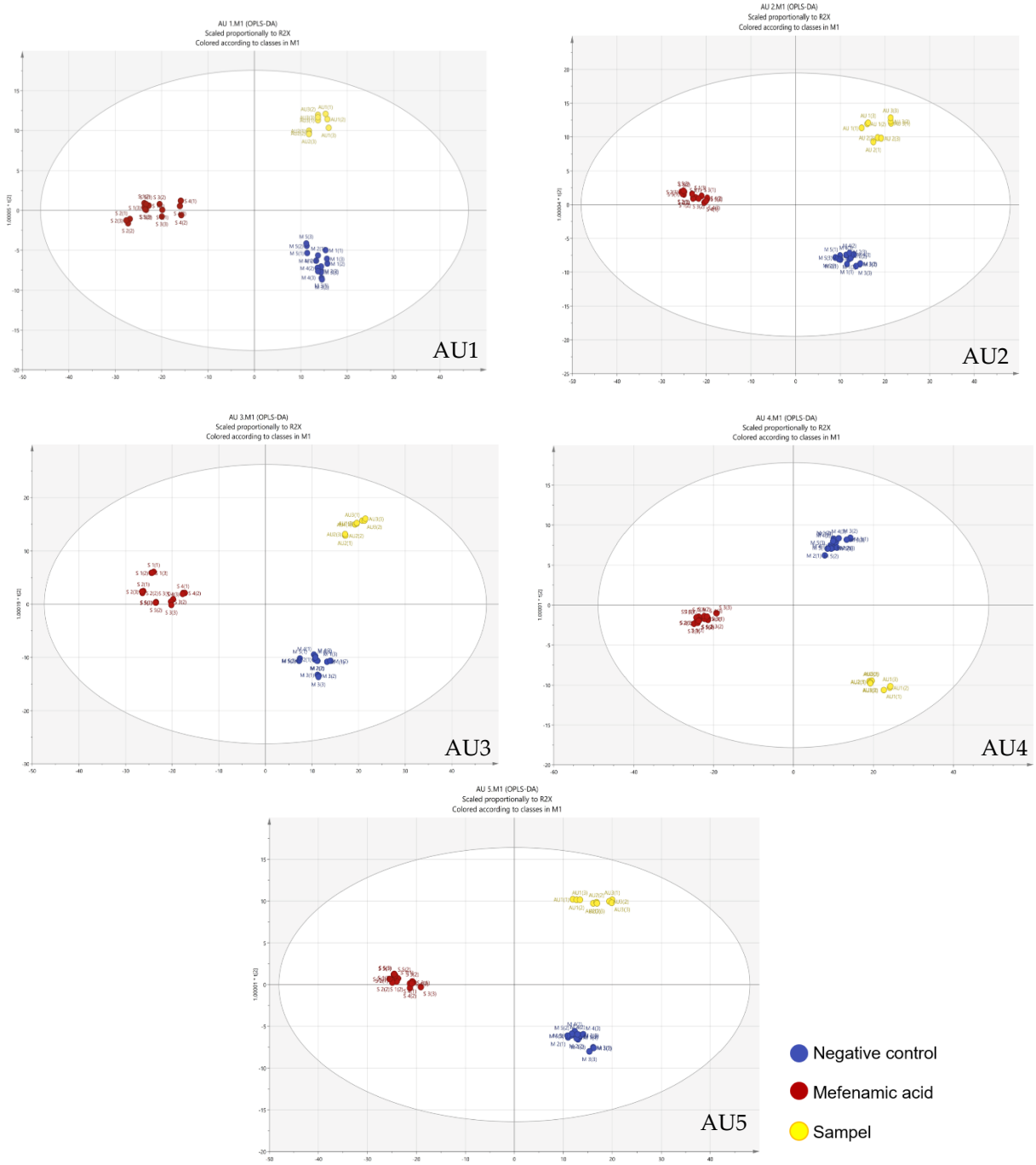

Figure 9. Score Plot of prediction the five samples using OPLS-DA

3.3.2. PLS

Partial Least Squares (PLS) was employed for the quantitative prediction of mefenamic acid content. PLS was conducted using TQ analyst software. Prior to prediction, wavelength optimization was carried out to achieve a coefficient of determination (R^2) greater than 0.99, along with the lowest RMSEC (Root Mean Square Error of Calibration) and RMSEP (Root Mean Square Error of Prediction) values. Model validation was subsequently carried out using the Leave-One-Out Cross-Validation (LOOCV) approach to assess the predictive performance of the PLS model in terms of the validation coefficient of determination (R^2) and the Root Mean Square Error of Prediction (RMSEP). The

optimization results are presented in Table 2. Based on the optimization, the wavenumber range of 1000–600 cm^{-1} yielded the highest R^2 and the lowest error values, and was therefore selected for the quantitative prediction of mefenamic acid using PLS. The calibration and prediction plot was shown in figure 10. The PLS analysis indicated that none of the five samples contained mefenamic acid. This finding is consistent with the results obtained from UV-Vis spectrophotometry and OPLS-DA analysis. These results demonstrate that FTIR combined with chemometric modeling can provide reliable outcomes comparable to those obtained using UV-Vis spectrophotometry.

Table 2. Optimization of Partial Least Square

Wavenumber (cm^{-1})	Calibration		Validation	
	R^2	RMSEC	R^2	RMSEP
4000-400	0.99	0.06	0.98	1.25
3750-1900	0.95	1.89	0.82	3.78
3500-3200	0.87	1.49	0.91	3.82
1800-1000	1.00	0.01	0.99	2.29
1700-1600	0.99	0.69	0.98	1.03
1600-1500	0.99	0.76	0.99	0.73
1300-1000	0.99	0.55	0.99	0.41
1000-600*	**0.99**	**0.24**	**0.99**	**0.10**
900-500	0.99	0.29	0.99	0.27

*Values in bold represent the optimal PLS condition.

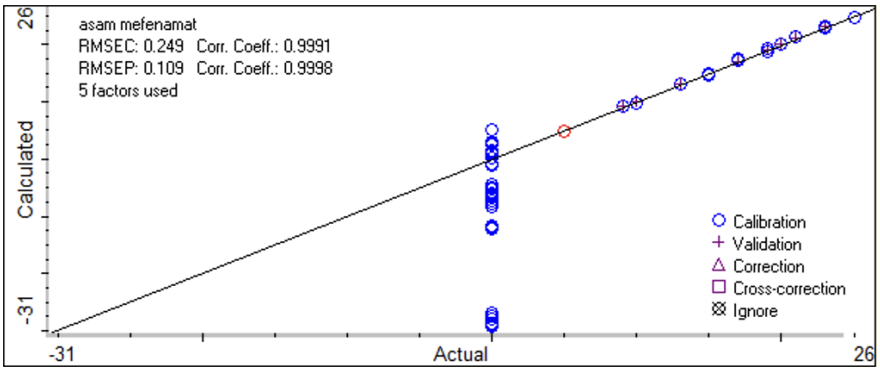

Figure 10. Partial Least Squares (PLS) regression plot for calibration and prediction. The vertically aligned blue dots represent the predicted concentrations of the samples in the PLS model.

3. CONCLUSION

Based on the results obtained, the validation of the UV-Vis spectrophotometric analytical method demonstrated acceptable performance within established criteria. The OPLS-DA validation results indicated that the method was capable of accurately classifying pure herbal samples and adulterated herbal medicine models. Furthermore, the optimization of the PLS method yielded satisfactory outcomes, as evidenced by a high coefficient of determination ($R^2 > 0.99$) and low values of RMSEC and RMSEP, indicating good predictive ability and model robustness. Quantitative analysis using both methods, UV-Vis spectrophotometry and FTIR spectroscopy combined with chemometric approaches produced consistent results, further supporting the reliability and robustness of the applied analytical methods.

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Conflicts of interest: The authors declare no conflict of interest

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