The Green Analytical Method of Detecting Analgesic and Anti-inflammation Drugs in Pain Reliever Herbal Using Spectroscopy FTIR-ATR Combined with Multivariate Analysis

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Abstract. Green analytical method performed optimal result to detecting any adulterant in many pharmaceutical products. The increasing level of herbal traditional consumption, especially pain reliever herbal medicine insists on several irresponsible parties to intentionally add illegal drugs into pain reliever herbal to make false positive effect on consumers. The aim of this study is developing the combination between spectroscopy FTIR-ATR and multivariate analysis in identifying synthetic drugs adulteration in pain reliever herbal. Pain reliever herbal product (AJSP), ten antiinflammation drugs, and mixture model of AJSP and each drug. All samples were recorded using FTIR-ATR. The spectra data obtained were analysed using multivariate analysis Principal Component Analysis (PCA) and Linear Discriminant analysis to know the capability of FTIR-ATR in determining synthetic drugs. Those method exhibited suboptimal result while distinguishing those adulteration model. This result needs more research to find the optimal method for detecting synthetic drugs in large matrix herbal product.

1 Introduction

The growing trend of herbal medicines being marketed as safe alternatives to synthetic drugs has raised concerns about adulteration and the illegal addition of pharmaceutical substances[1]. To ensure public safety and the authenticity of herbal products, rapid and reliable analytical techniques are crucial. Among the emerging methods, green analytical spectroscopy**,** particularly Fourier Transform Infrared (FTIR) spectroscopy, combined with chemometrics**,** offers a sustainable, non-destructive, and efficient approach to identifying synthetic drugs in herbal medicine [2,3].

FTIR spectroscopy is widely used to obtain molecular fingerprints based on the vibrational frequencies of chemical bonds. When coupled with chemometric techniques such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), and other multivariate methods. This combined approach not only enhances sensitivity and accuracy but also aligns with the principles of green chemistry by reducing the need for hazardous solvents, reagents, and extensive sample preparation [4]. The combination of both technical methods exhibited an optimal result to distinguish the synthetic drugs in ternary mixture between pain reliever herbal and antiinflammation drugs [5].

The purpose of this study is developing a fast, effective, and reliable method to determine the drugs adulterant as its usual dose in the pain reliever herbal model mixture. In this context, FTIR-chemometrics provides a promising tool for ensuring the safety, efficacy, and authenticity of herbal medicines, enabling the rapid identification of synthetic drug adulteration while adhering to sustainable analytical practices.

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2 Materials and Methods

2.1 Materials

The traditional herbal powder claimed as pain reliever with registered number coded by AJSP were purchased from one of certified traditional herbal industry in Indonesia. The analgesic drug was paracetamol given by Zenith Pharmaceutical Industry. Antiinflammation drugs were metamizole, prednisone, diclofenac sodium, mefenamic acid, ibuprofen, dexamethasone, piroxicam granted from Zenith Pharmaceutical Industry. Meloxicam and ketoprofen were presented by Dexa Medica Pharmaceutical Industries. Acetone p.a. was obtained from Merck with catalogue number 100014.2500.

2.2 Sample preparation

Each drug was intentionally added to one pack of AJSP (7 grams). The design of each drug supplemented into one pack of AJSP were shown at Table 1.

Table 1. List of the amount of supplemented drug into AJSP

Drug's Name	The amount of drug added (mg) into one pack AJSP
Paracetamol	500
Metamizole	500
Mefenamic Acid	500
Ibuprofen	400
Ketoprofen	100
Diclofenac Sodium	50
Meloxicam	15
Piroxicam	10
Prednisone	60
Dexamethasone	10

2.3 FTIR-ATR

The first step before using the FTIR-ATR was cleaning the diamond crystal ATR to reduce the fluctuating data caused by any possible noise during the data collection and this step was repeated before every sample measured. Type of spectrophotometer FTIR-ATR for this study was Nicolet iS10 embedded with deuterated triglycine sulphate as detector. Collecting background was applied before sample located in diamond crystal ATR to minimize the unwanted signal from the environment. The wavenumber range, resolution, and the number scan every minutes of FTIR-ATR spectrophotometer were conditioned subsequently as 4000-650 cm^{-1}, 8 cm^{-1}, and 32 scans/minute. AJSP, each drug, and the mixture of AJSP-each drug were located around 10 mg above the diamond crystal and pressed to ensure the sample was adhesive enough. The software used for collecting and preparing the spectrum FTIR data was Omnic. Every sample were recorded in absorbance and measured in triplicate.

2.4 Analysis Data

The obtained FTIR spectra were conditioned first using Omnic software such as automatic baseline. The file extension had to be changed to csv files first before analysis multivariate were employed. The multivariate analysis for this study were PCA and LDA. PCA was carried out by Minitab 18 and LDA was visualized by TQ Analyst.

3 Results and Discussion

3.1 FTIR Spectra Data Analysis

The use of FTIR spectroscopy is widely developed to analyze the presence of adulterants in herbal products. The advantages of FTIR-ATR analysis such as no sample preparation required, simple, fast, and economical made this method chosen to detect the presence of drugs in herbal products. The principle of FTIR-ATR analysis is that the sample was placed in the ATR compartment scanned, then the molecules in the sample would absorb energy at specific wave numbers with vibrations of functional groups contained in the sample molecule. The energy absorption in the molecule would be proportional to the absorbance intensity in the FTIR spectra in accordance with Lambert-beer law [6].

The results of the FTIR spectroscopy analysis of AJSP could be seen in Figure 1. AJSP had absorption at wave numbers 3100-3600 cm^{-1} for the absorption of the stretching vibration group of O-H. The characteristic peak at wave number 1633 cm^{-1} was the absorption of the C=O (carbonyl) group. The C-O group could be seen in the peak at wave number 1016 cm^{-1} [7]. The composition of AJSP herbal medicine product was exhibited in table 2. FTIR spectrum interpretation data of AJSP appeared in table 3.

Table 2. The Ingredients of AJSP

No.	Sample	Ingredients
1.	AJSP	*Curcuma xanthorrhizae rhizoma, Languatis galangae rhizoma, Zingiberis aromaticae rhizoma, Imperatae cylindrica radix, Zingiberis purpurei rhizoma, Moschomosmae polystachii folium, Orthosiphonis aristatus folium, Piper retrofracti fructus, Smilacis chinesis rhizome, Piperis nigri fructus,* Anise ADS

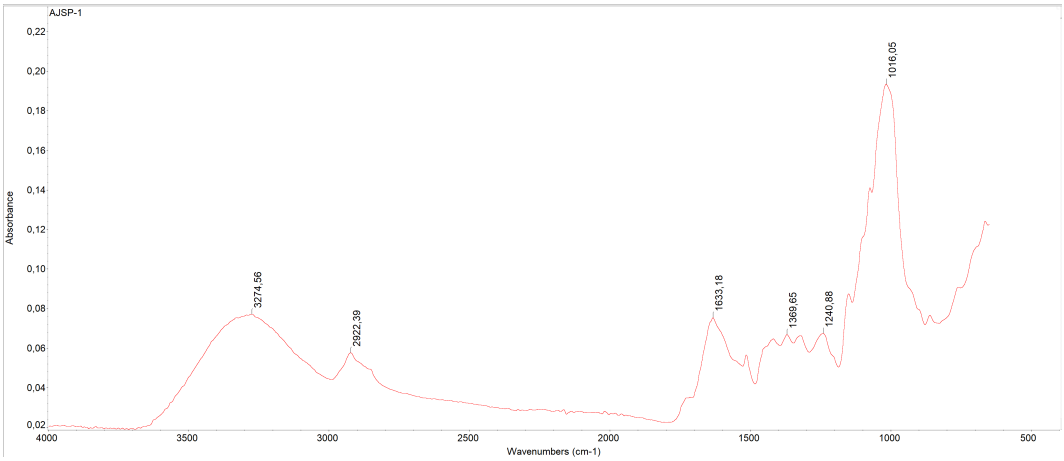

Fig 1. AJSP FTIR Spectrum

Table 3. Data Interpretation of AJSP

Wavenumber (cm^{-1})	Functional groups
3100-3600	O-H symmetric stretching
2922	C-H asymmetric stretching
1633	C=O symmetric stretching
1369	C=C stretching
1240	$=CH_2$ stretching
1016	C-O symmetric stretching

FTIR spectrum of Paracetamol (Figure 2) showed a typical peak at wave number 1650 cm^{-1} which is the vibrational absorption of C=O carbonyl group. The peaks at wave numbers 3321 and 3158 cm^{-1} were the stretching vibrations of the OH and NH groups. The absorption of the C-O group was appeared at wave number 1326 cm^{-1} [8].

The results of Mefenamic acid scanning by FTIR spectroscopy displayed in Figure 73 The peak at wave number 3306 cm^{-1} was responsible for the absorption of the NH group. The broadened peak in the 3200-2500 cm^{-1} region showed the absorption of the OH group. The peak at wave number 1645 cm-1 was the absorption of C=O group. The

FTIR spectrum of Ibuprofen (Figure 2) displayed a typical peak for the absorption of C=O (carboxylate) group at wave number 1707 cm^{-1}. The peak at wave number 1506 cm^{-1} is the absorption for C-O group. The absorption of the C=O group of carboxylic acid and ketone in the Ketoprofen spectra (Figure 2) respectively appeared at wave numbers 1693 cm^{-1} and 1652 cm^{-1}.

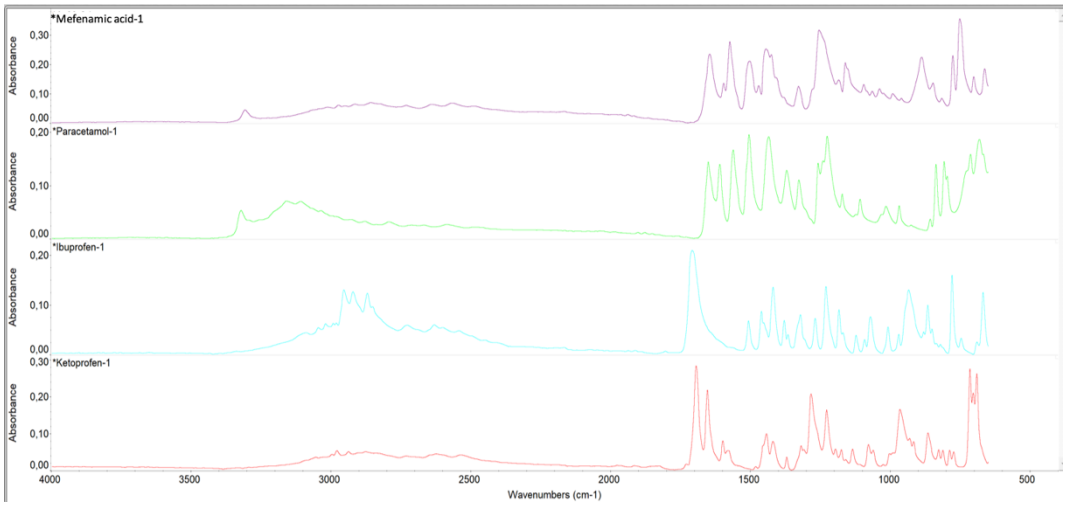

Fig 2. FTIR Spectra data of Mefenamic acid, Paracetamol, Ibuprofen, Ketoprofen

The FTIR spectrum of dexamethasone (Figure 3) showed a peak at wave number 3469 which is the absorption of the OH functional group. The absorption of C=O (carbonyl) ketone group appeared at the peak of 1702 cm^{-1}. Vibration of C-F group appeared with strong intensity at wave number 1055 cm^{-1}. The peak at wave number 3284 cm^{-1} in the spectrum of meloxicam (Figure 3) was the absorption of stretching vibrations of the NH group (secondary amine). A typical peak in the FTIR scan of meloxicam is found at wave number 1150 cm-1 which is the absorption of the S=O functional group. The FTIR spectrum of piroxicam (Figure 3) exhibited the absorption of C=O amide group at wave number 1628 cm^{-1}. The wave number region of 1576-1560 cm^{-1} is the peak of the absorption of the C=C stretching group and the peak at wave number 1433 cm^{-1} represented the absorption of the stretching vibration of C=N [9–11].

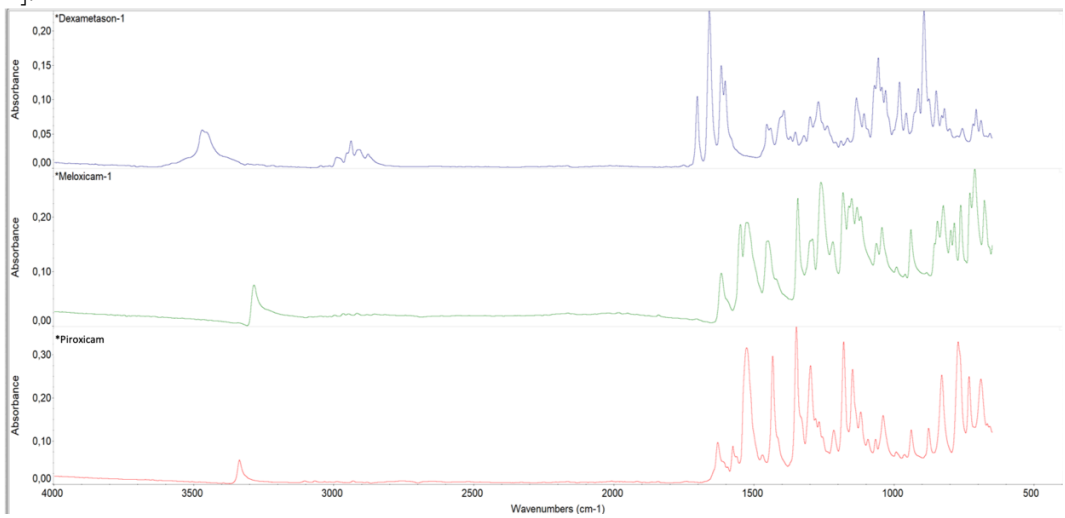

Fig 3. FTIR Spectra data of Dexametasone, Meloxicam, and Piroxicam

The FTIR spectra absorption of metamizole showed peaks at wave numbers 1656, 1625, 1172, and 1052 cm^{-1}, respectively the peaks showed C=O stretching group, C=C vibration, O=S=O stretching, S-O stretching. There was a slightly widened absorption at wave numbers 3550-3400 cm^{-1} with low intensity, the peak was a stretching vibration of the O-H group. C-N group absorption was seen at wave number 1333 cm^{-1}. The FTIR spectrum of diclofenac sodium displayed peaks at 3385, 3254, 1572, 1555, and 743 cm^{-1}, respectively, these peaks were secondary N-H stretching vibrations, O-H groups from carboxylic acids, C=O stretching vibrations, C=C groups from the ring, and C-Cl stretching vibrations [12,13].

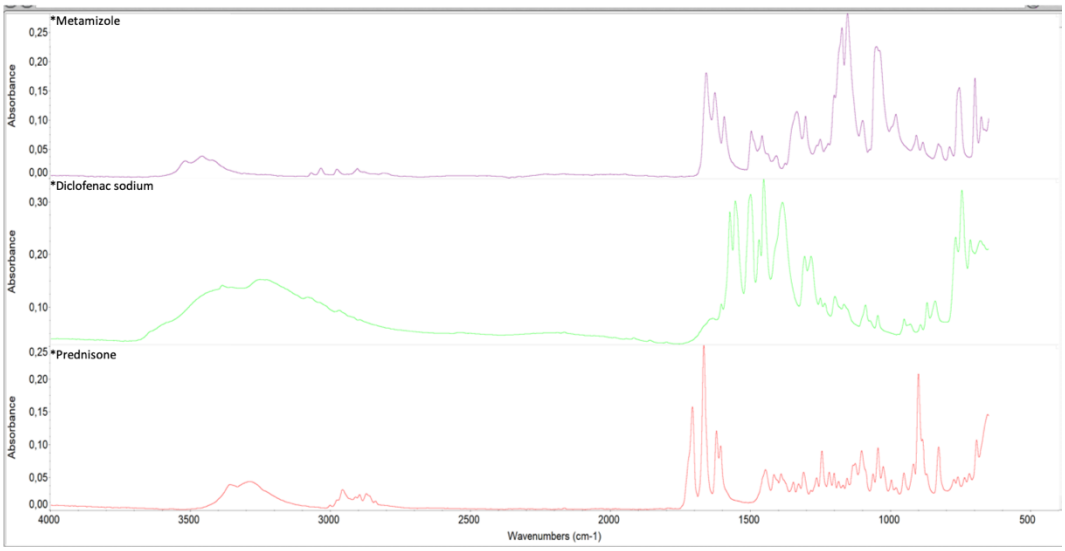

Fig 4. FTIR Spectra data of Metamizole, Diclofenac sodium, Prednisone

3.2 Multivariate Analysis

The FTIR spectra of each sample were further analyzed by chemometrics qualitatively. The multivariate analysis method in FTIR spectra processing of prevalent dose modeling was PCA with Minitab 18 and LDA using TQ Analyst. Multivariate analysis aimed to see the similarity and relationship between variables simultaneously. The spectra of a single sample of herbal medicine code AJSP and each drug analyzed by PCA were visualised by score plot results (Figure 5). The score plot aimed to show the correlation of the two components (component 1 and component 2) by projecting the observation data on a new coordinate system as the main component [14,15].

The score plot results of the AJSP and each drug were located far from each other. The difference absorption energy of functional groups in each sample makes the score plot results far apart from each other. Ibuprofen and Ketoprofen had similar spectral patterns so they were located close to each other. This also applied to Piroxicam and Meloxicam, the similarity of the molecular structure between the two drugs turned out the energy absorption of each functional group appeared at similar wavenumbers [16].

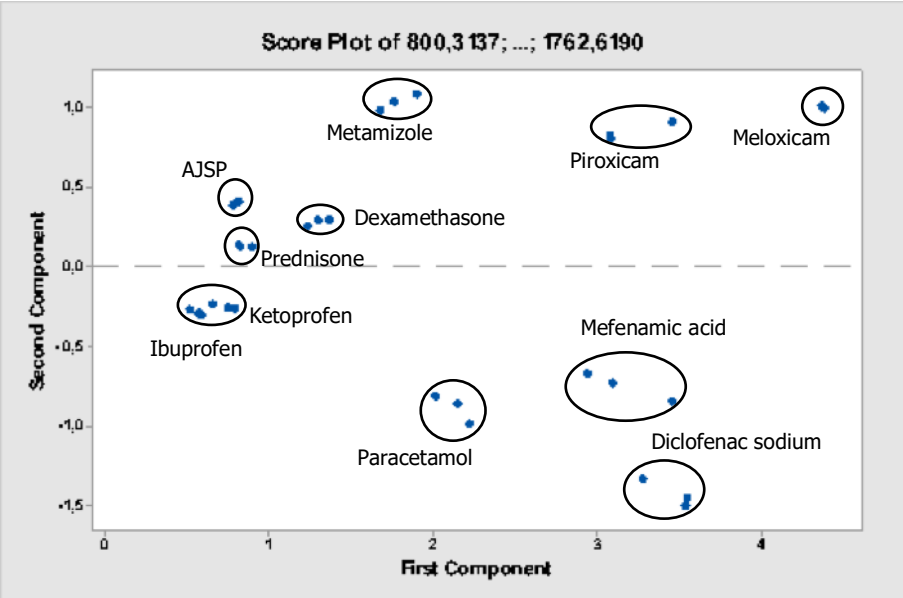

Fig 5. Score plot AJSP and each drug sample

Based on the score plot of PCA AJSP-each drug, PCA analysis for drug with a low dose was less able to distinguish between herbal medicine and mixture samples between AJSP and each drug because FTIR-ATR spectroscopy scanned more functional groups contained in herbal medicine molecules. Drugs with a higher dose in the modelling of AJSP-drug mixture with a prevalent dose showed optimal score plot results in distinguishing between single herbs and AJSP-drug mixture. The mixing model of AJSP and 10 drugs displayed the score plot results in Figure 6 while Figure 7 was the score plot results between the overall mixing model and also the AJSP-10 drugs model. AJSP and

the mixture sample model were located close to each other, indicating that the PCA score plot was not optimal for detecting the presence of drugs at the usual dose in herbal products. The reason between these results was the lack of FTIR spectroscopy sensitivity detecting the small amounts of drugs when it contained in large herbal matrix. The amount ratio between traditional herbal medicine powder and drugs in a mixture sample has a major influence on the results of PCA analysis [17].

LDA analysis was performed with TQ-Analyst software. The results of the LDA analysis in the wavenumber region 3933-716 cm^{-1} was visualized by Cooman's plot curve (Figure 8). The results of the LDA analysis of the modelling mixture spectra showed that several samples were misclassified. Hence, this method was not optimal in discriminating between AJSP and model mixtures. The combined analysis method between FTIR spectroscopy and LDA multivariate analysis was not optimal for separating between the two samples. It caused the amount of matrix in AJSP was larger than the amounts of drugs added in sample [18].

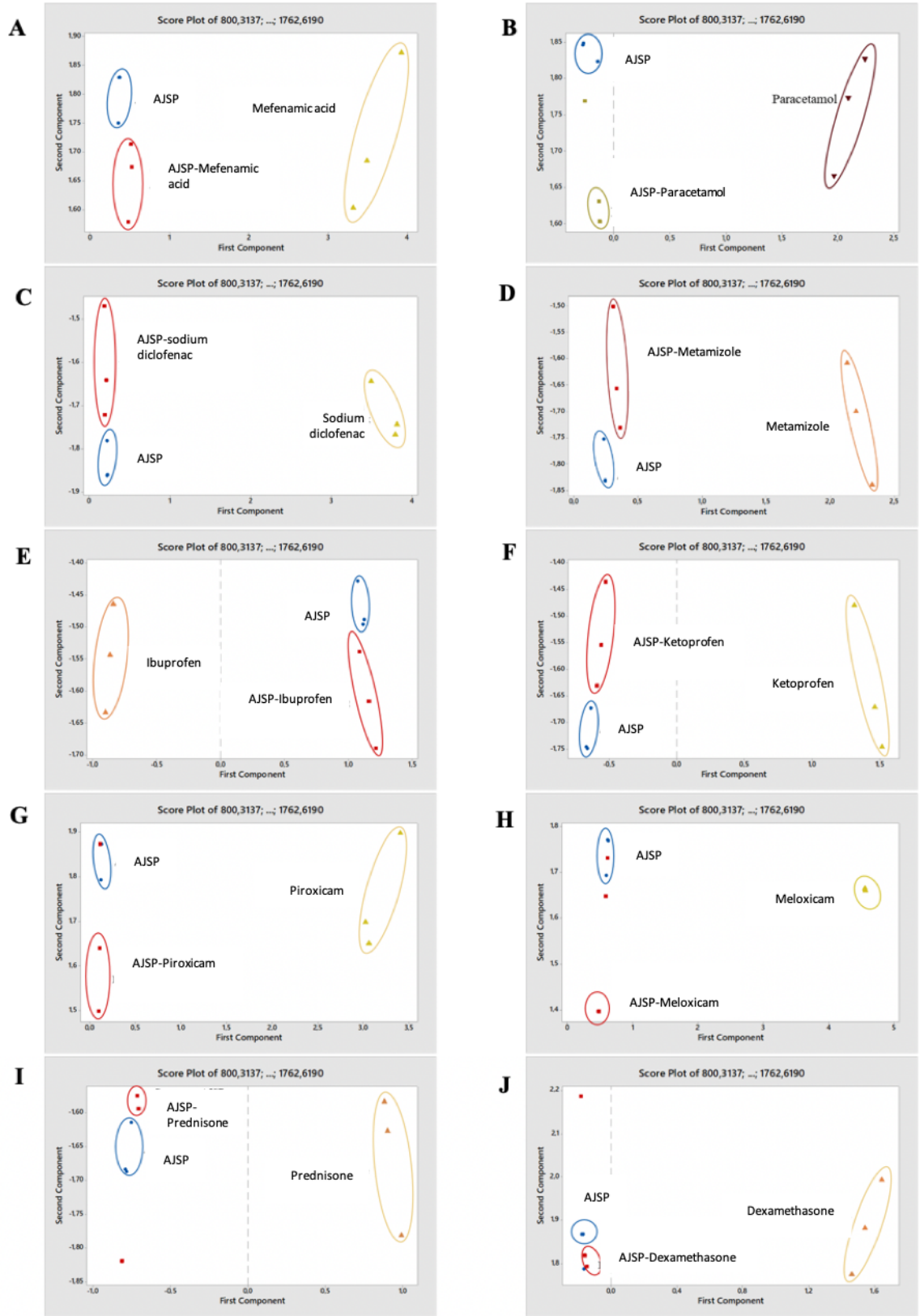

Fig 6. PCA Score plot AJSP-Drugs. (A) AJSP-Mefenamic acid. (B) AJSP-Paracetamol, (C) AJSP-Sodium Diclofenac, (D) AJSP-Metamizole, (E) AJSP-Ibuprofen, (F) AJSP-Ketoprofen, (G) AJSP-Piroxicam, (H) AJSP-Meloxicam, (I) AJSP-Prednisone, (J) AJSP-Dexamethasone

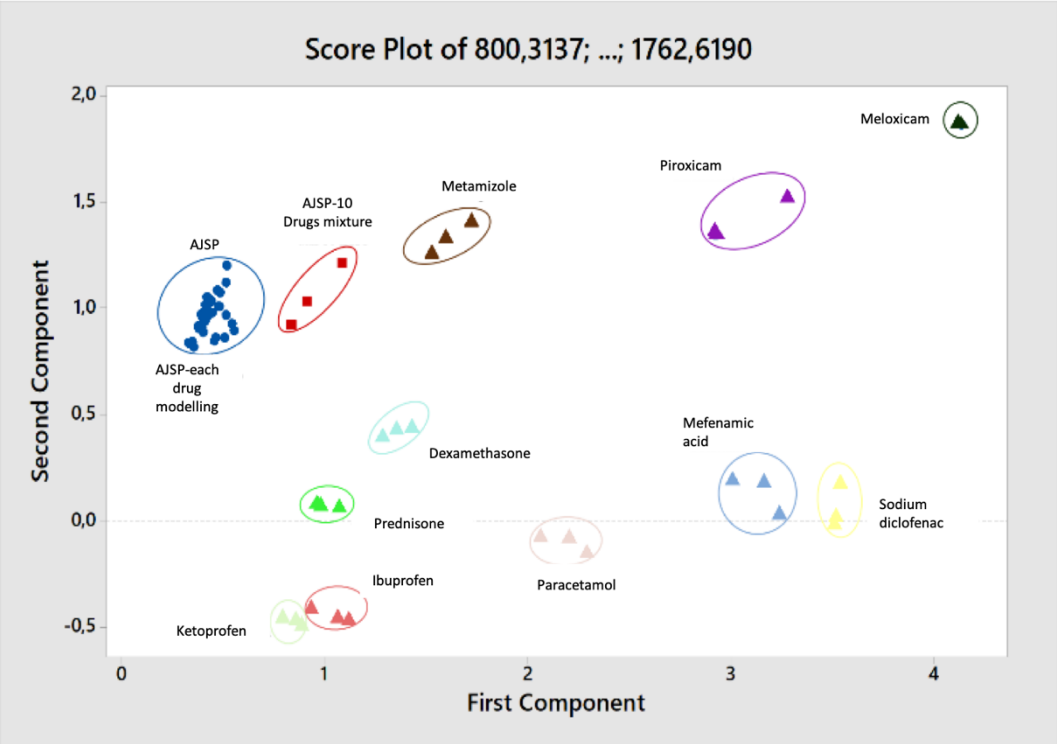

Fig 7. PCA Score plot of AJSP, Each Drug Sample, AJSP-Drugs Modelling

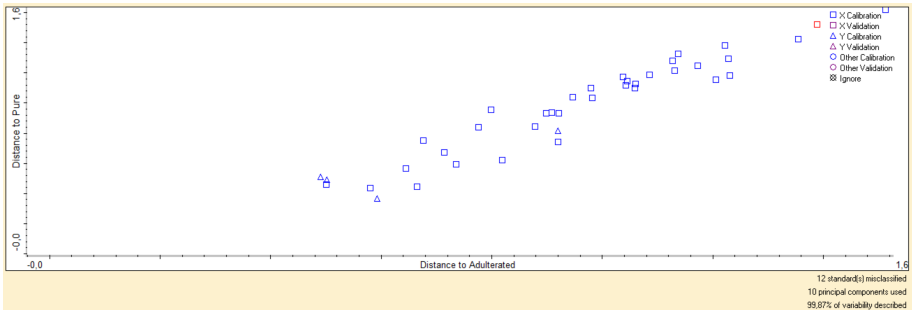

Fig 8. Cooman's Plot Model of of AJSP, Each Drug Sample, AJSP-Drugs Modelling

4 Conclusions

As shown the data above, the combination of spectroscopy FTIR-ATR and unsupervised pattern recognition (PCA) also supervised pattern recognition (LDA) in this study indicated the suboptimal method to distinguish synthetic drugs adulterant in pain reliever herbal. The ratio of synthetic drugs and matrix pain reliever herbal became the strongest factor for this result. This study needed to be scrutinized more to be developed as fast and reliable method to be used in traditional herbal medicine quality control.

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