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Research Article

# METHOD DEVELOPMENT FOR SIMULTANEOUS ANALYSIS OF STEROID AND NON STEROID ANTIINFLAMATORY SUBSTANCES IN JAMU PEGAL LINU USING TLC-SPECTROPHOTODENSITOMETRY

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**ABSTRACT**

Objective: Illegally added chemical adulteration of jamu become a problem that more difficult to stop. Therefore method development of simultaneous detection of chemical adulteration must be conducted. The aim of this research is to develop analytical method to detect simultaneously chemical adulteration in jamu pegal linu.

Methods: Stationary phase, mobile phase, sample preparation and instrument condition were optimized.

Results: The result of optimization was validated for specificity according to International Conference on Harmonization. The validated methods were applied to analyze of three jamu pegal linu products in the market for the qualitative analysis of chemical adulteration. Analytical method optimization showed that the best separation of five substances, acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam was performed on silica gel $GF_{254}$, using chloroform – methanol (9:1) as a mobile phase. Samples were prepared by extraction using ethanol as a solvent. Extraction was done for 30 minutes using 3D shaker. Chamber saturation was done for 60 minutes. Detection of chemical adulteration was performed using CAMAG TLC (Thin Layer Chromatography) Scanner at λ 254 nm for simultaneous detection of acetaminophen, dexamethasone, prednisone, mefenamic acid, piroxicam.

Conclusion: A new validated methods for qualitative analysis (acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam was deeloped) by thin layer chromatography (TLC) at λ 254 nm. The separation was performed on silica gel $GF_{254}$, using chloroform – methanol (9:1) as mobile phase. The methods can be used to analyze jamu pegal linu in the market.

**Keywords:** TLC-spectrophotodensitometry, Jamu pegal linu, Simultaneous detection.

## INTRODUCTION

Jamu is a Indonesian traditional medicine. According to Regulation of Ministry of Health Indonesia No. 007, 2012, jamu is prohibited for chemical adulteration including isolate from natural product, and chemical substances from synthesis, ethanol more than 1.0%. Nowadays, illegally added chemical adulteration in jamu becomes a problem that difficult to stop. According to public release data from NADFC, there always chemical adulteration finding in Indonesia. From 2001 to 2007, illegally added chemical adulteration in jamu, include antiinfamatory drug substances like acetaminophen, dexamethasone, prednisone, mefenamic acid and phenylbutazone [1,2]. Since 2007, chemical adulteration included sildenafil, tadalafil, and sibutramin HCl [1,2]. In 2012, NADFC report there were more than one chemical substances were added to jamu pegal linu. The chemical substance trends were back to antiiflamatory substances like acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam [3].

Acetaminophen, $C_8H_9NO_2$, chemical name: 4-hydroxyacetanilide; p-hydroxyacetanilide; p-acetamidophenol; p-acetaminophenol; p-acetylaminophenol; N-acetyl-p-aminophenol [4]. Description: white crystalline powder, odorless, slightly bitter. Solubility: freely soluble in alcohol, soluble in boiling water and NaOH 1 N [5].

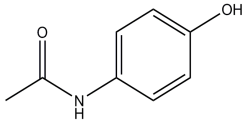



**Fig. 1: Molecular structure of acetaminophen**

Dexamethasone, $C_{22}H_{29}FO_5$, description: white to almost white powder, odorless, crystalline powder, stabile at room temperature, decompose at 250 $^0$C. Solubility: sparingly soluble at acetone, alcohol, dioxane and methanol, slightly soluble in chloroform, very slightly soluble in ether, practically insoluble in water [5].

Mefenamic acid, $C_{15}H_{15}NO_2$, chemical name benzoic acid, 2-(2,3-dimethylphenyl) amino, N-2,3-cylylantranilate acid. Description: white to white off crystalline powder, melting point 230 $^0$C, with decomposition. Solubility: soluble in alkali hydroxide solution, sparingly soluble in chloroform, slightly soluble in alcohol and methanol, and practically insoluble in water [5].

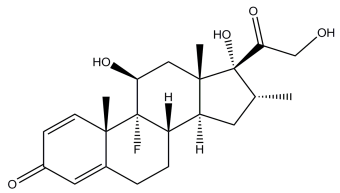



**Fig. 2: Molecular structure of dexamethasone**

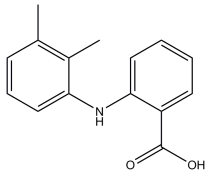



**Fig. 3: Molecular structure of mefenamic acid**

Piroxicam, $C_{15}H_{13}N_3O_4S$, chemical name 4-hidroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzotiazine-3-carboxamide-1,1-dioxid.
Description: crystalline powder, colorless, odorless, bitter. Solubility: insoluble in water and chloroform, sparingly soluble in toluene and diisopropyl ether, slightly soluble in aliphatic alcohol, such as methanol, ethanol, isopropanol [6].

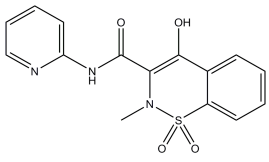



**Fig. 4: Molecular structure of piroxicam**

Prednisone, description: white to almost white, crystalline powder, odorless, melt at 230 $^0$C with decomposition. Solubility: slightly soluble in alcohol, chloroform, dioxane, methanol, very slightly soluble in water [5].

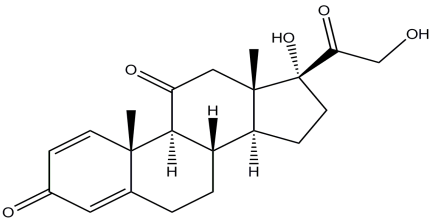



**Fig. 5: Molecular structure of prednisone**

## MATERIALS AND METHODS

### Materials

Analytical reference standard of acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam were purchased from NADFC (The National Agency of Drug and Food Control). All reagents, both for preparation of mobile phase and also for the other procedure, were analytical grades. TLC analysis was performed on precoated TLC plates with silica gel $GF_{254}$ (Merck)

### Crude drug

The crude drug were *Curcumae rhizome*, *Curcuma domesticae rhizome*, and *Zingiberis rhizome*, were purchased from herbal store in Pasar Baru, Bandung, Indonesia. The crude drug was purchased as a powder.

### Instrumentation

CAMAG TLC Scanner III in reflectance-absorbance mode and operated by WinCATS software (CAMAG). Source of radiation was deuteurium lamp emitting a continuous UV spectrum between wavelength 200 nm and 400 nm. The slit dimension were 6.00 x 3.00 mm, scanning speed was 20 mm/s.

### Methods

### Microscopic examination of crude drug

Before using as a matrix of jamu, crude drugs were microscopically analyzed for marker characteristic using Microscope Olympus DP 21.

### Preparation of Jamu Pegal Linu Matrices

1.75 gram of *Curcumae rhizome*, 1.75 gram *Curcuma domesticae rhizome* and 1.5 gram *Zingiberis rhizome*, were mixed in mortar, so we had a homogenous mixture of jamu pegal linu matrices.

### Plate activation

Before use, TLC plates were washed with methanol, dried in oven 120 ºC for 30 minutes. The activated plates were equilibrated and stored in dessicators.

### Mobile phase optimization

Mobile phase optimizations were started using medium strength solvent, dichlormethane. After that optimizations were continued with addition of methanol in every ratio. Optimizations were continued by replacing dichlormethane with ethyl acetate and chloroform. Mobile phase from solvent combinations were also tested such as ethyl acetate 100%, ethyl acetate-methanol-ammonia (85:10:5), dichlormethane-ether-methanol-water (77: 15:8:1.2)[7], and heptanes-2-propanol-acetic acid (15:5:1)[8].

### Standard preparation

10 mg of acetaminophen was weighed as an analytical standard by using an analytical balance. Then, it was dissolved and add to 5 mL using ethanol on a volumetric flasks. 40, 80, 120, 160, 200, 240 µL of the solution was transferred to 5 mL volumetric flasks using volumetric pipette. After that, ethanol was added to a volume, standard solution with concentration 0,04; 0,08; 0,12; 0,16; 0,20; 0,24 mg/mL were obtained.

### Sample preparation

Samples of jamu pegal linu were weighed approximately 500 mg. 10 mL of ethanol was used for the extraction. The sample was shaking for 30 minutes by using 3D shaker, and filtered through Whatman No.1. The filtrate was diluted to 1/5 proportion. Then the filtrate was ready for application on TLC plates.

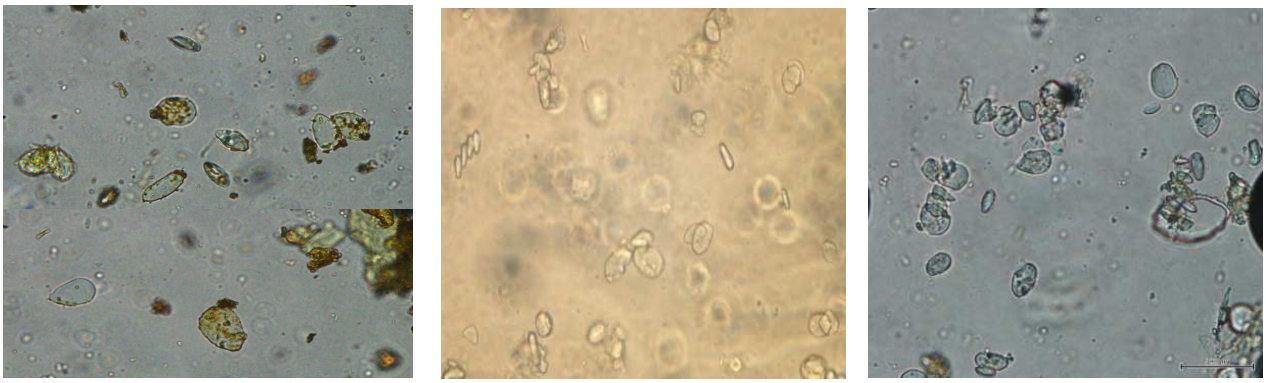
### Determination of scan wavelength

Each of substance was scanned using CAMAG TLC Scanner III to determine the wavelength which gave the maximum absorbance. Then, the wavelength for simultaneous analysis of five substances was determined too.

## RESULT AND DISCUSSION

Based on microscopic analysis of crude drugs, all of crude drug that were used in this research are *Curcumae rhizome*, *Curcuma domesticae rhizome*, and *Zingiberis rhizome*. The crude drug was showed marker characteristic for each crude drug, such as starch, if we used glycerin as a microscopic reagent. It can be seen in Figure 6.

Based on mobile phase optimization, the optimum mobile phase to separate and analyze five substances, acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam, in jamu pegal linu, was chloroform-methanol (9:1). This mobile phase can separate each substances both in standard mixture and also in sample mixture. It can be seen in Figure 7.



**(a)** **(b)** **(c)**

**Fig. 6: Microscopic examination of crude drugs, (a) *Curcuma domesticae rhizome*, (b) *Curcumae rhizome*, dan (c) *Zingiberis rhizome*, using glycerin**

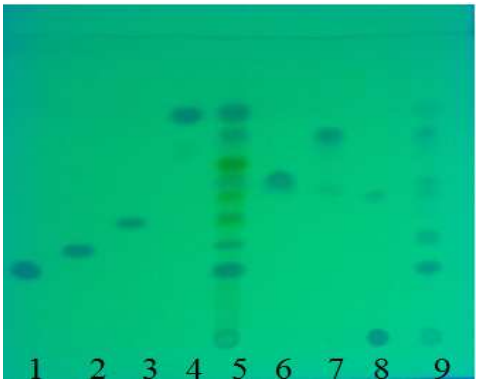



**Fig. 7: Thin layer chromatogram of analytical standard, stationary phase silica gel $GF_{254}$ using chloroform- methanol (9:1) as a mobile phase, (1) acetaminophen, (2) dexamethasone, (3) prednisone, (4) phenylbutazon, (5) simulation of jamu and chemical adulterance, (6) mefenamic acid, (7) piroxicam, (8) methampiron, (9) mixture of standard, using UV 254 nm as a derivatization agent**

The resolution of each peak in densitogram, was showed good separation (Figure 8). It can be seen on the resolution, Rs, which was more than 1.0. Resolution more than 1.0, means that the two spots is well separated [9, 10,11,13,14,15]. The resolution for each peak to the other was showed in Table 1.

Sample preparation was chose using ethanol, because all of the five substances had a good solubility in ethanol. All of five substances can be extracted in one way using ethanol. Sample preparation using ethanol also considered efficiency of the methods.

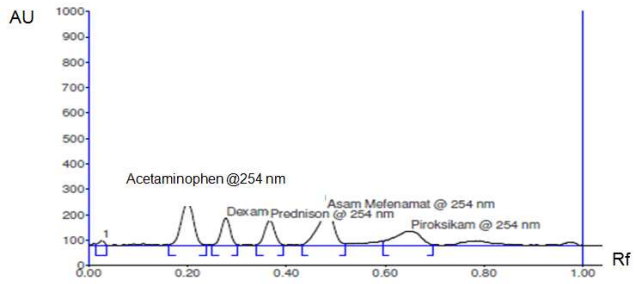



**Fig. 8: Densitogram of standard solution mixture**

**Table 1: Resolution of the substances**

| Chemical Adulterance | Resolution (Rs) |
|---|---|
| Acetaminophen-Dexamethasone | 1.23 |
| Dexamethasone-Prednisone | 1.63 |
| Prednisone-Mefenamic Acid | 1.47 |
| Mefenamic Acid-Piroxicam | 1.79 |

Each of substance spectrums were overlaid with the standard spectrum to assure the identity of the substance in jamu pegal linu matrices. The overlaid spectrum showed that no difference between standard spectrum and sample spectrum. It means that the standard spectrum was similar with sample spectrum (Figure 9).

Simultaneous analysis was done at wavelength 254 nm, because all of the substances gave a good absorbance in this wavelength. So, we could analyze it simultaneously in one way procedure. This method had a good specificity [12], because it still could detect a substance even in a jamu matrices environment. So, this method was valid to detect those substances in jamu pegal linu sample.

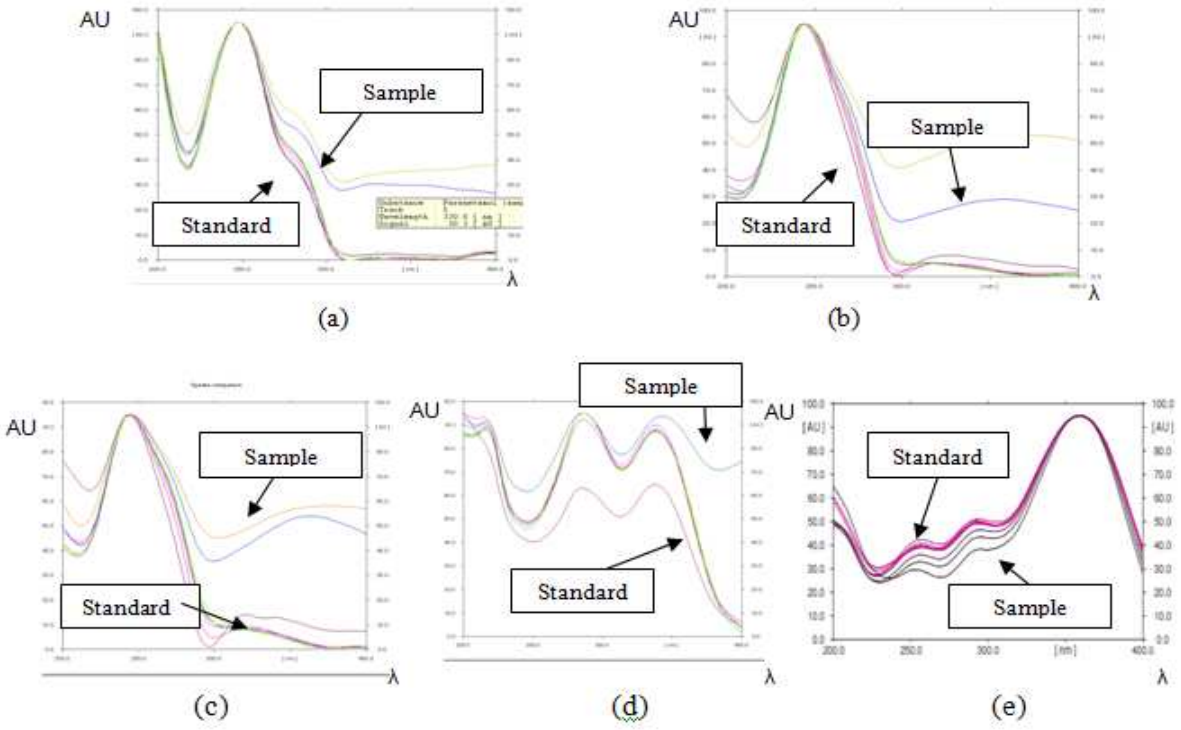



**Fig. 9: Overlaid spectrum between standard and sample, (a) acetaminophen, (b) dexamethasone, (c) prednisone, (d) mefenamic acid, (e) piroxicam**

This method was used to analyze sample of jamu pegal linu in the market. Jamu pegal linu sample was taken from two different herbal stores in Bandung. Those three jamu pegal linu, samples, A, B, C, were produced by different kind of jamu industry. From three samples that were analyzed, one samples were illegally added by acetaminophen, one samples were illegally added by piroxicam, and one samples were illegally added by acetaminophen and piroxicam. This can be assured by thin layer chromatogram, densitogram and spectrum overlaid between standard and sample (Figure 10, 11, 12, 13, 14).

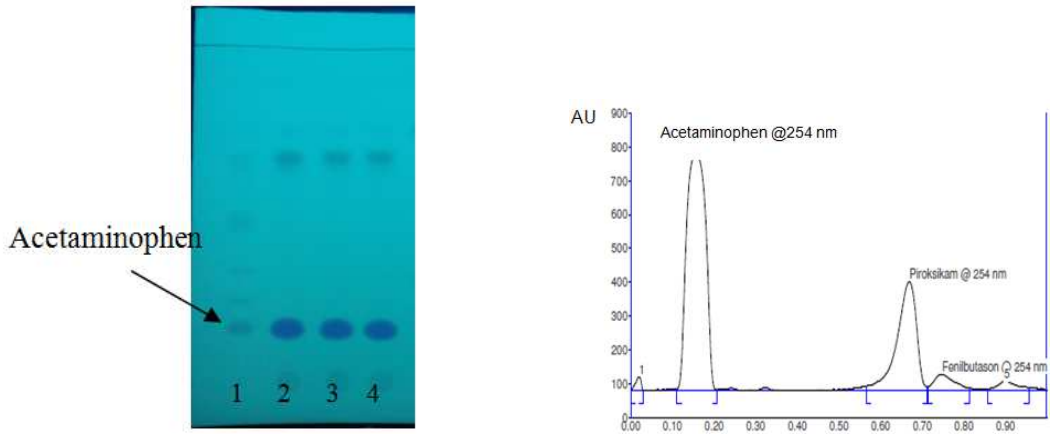



**Fig. 10:Thin layer chromatogram of sample A and standard mixture, (a) using UV λ 254 nm, (b) densitogram, (1) standard mixture of acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam, (2) to (4) sample A**

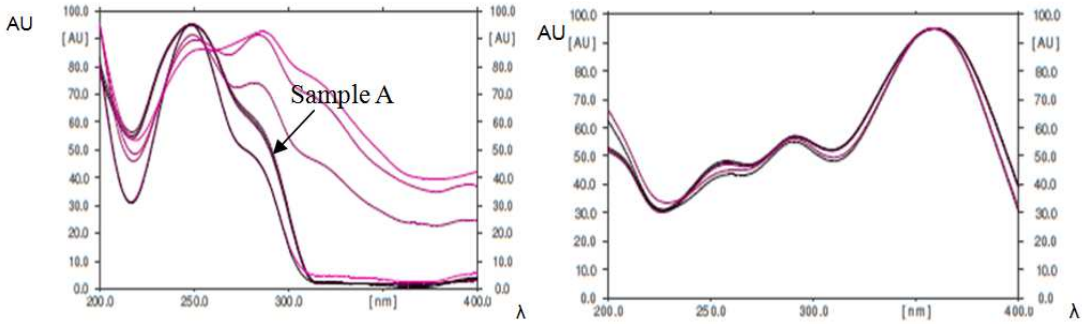



**Fig. 11: Overlaid spectrum between standard and sample, (a) acetaminophen, (b) piroxicam**

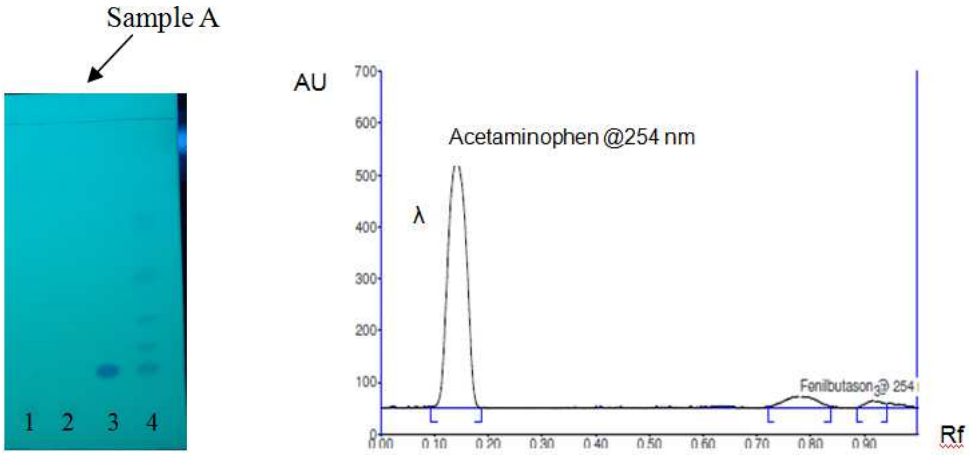



**Fig. 12:Thin layer chromatogram for sample B and standard mixture, (a) using UV λ 254 nm, (b) densitogram, (1) to (3) sample D, (4) standard mixture of acetaminophen, dexamethasone, prednisone, mefenamic acid, and piroxicam**

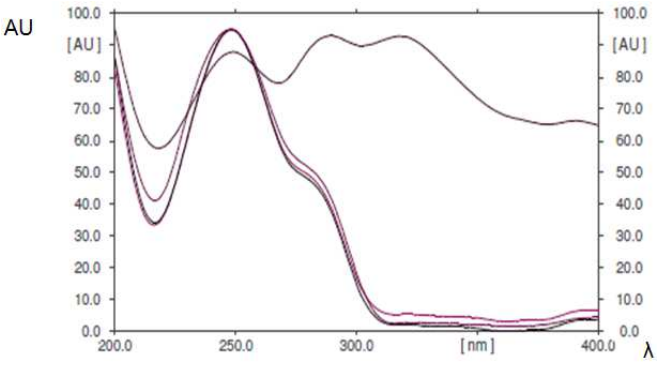
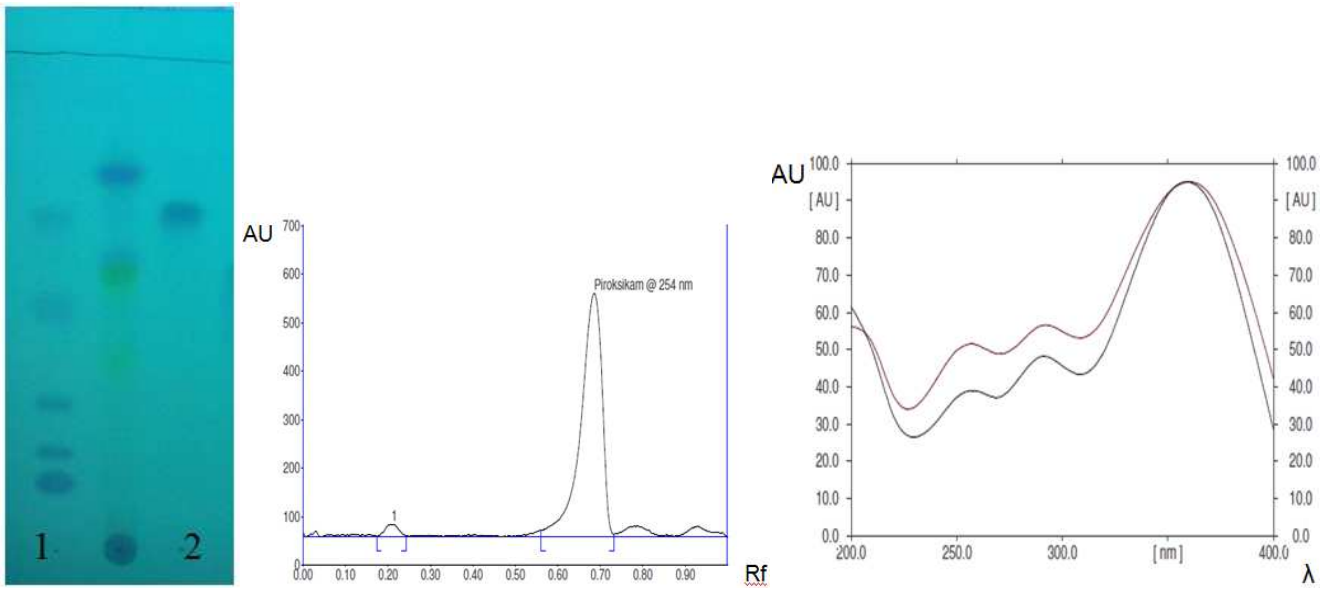



**Fig. 13: Overlaid spectrum between acetaminophen standard and sample B**




(a) (b) (c)

**Fig. 14:Thin layer chromatogram of sample C and standard mixture, (a) using UV λ 254 nm, (b) densitogram, (c) overlaid spectrum between sample C and piroxicam standard, (1) standard mixture of acetaminophen, dexamethasone, prednisone, mefenamic acid, and piroxicam, (2) sample C**

## CONCLUSION

A new validated methods for qualitative analysis (acetaminophen, dexamethasone, prednisone, mefenamic acid and piroxicam was developed) by TLC-spectrophotodensitometry at λ 254 nm. The separation was performed on silica gel $GF_{254}$, using chloroform – methanol (9:1) as mobile phase. The methods can be used to analyze jamu pegal linu in the market.

## ACKNOWLEDGEMENTS

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