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Development of Captopril Analysis Method in Traditional Antihypertensive Medicine by Densitometry

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ABSTRACT

Introduction: *The utilisation of traditional medicine to address health concerns is on the rise. One such traditional medicine product is antihypertensive herbal medicine. The Indonesian Food and Drug Authority (BPOM) has identified the presence of medicinal chemicals in traditional medicinal products, which has the potential to pose a significant risk to consumers. Captopril is one of the antihypertensive drugs that may be added as BKO in herbal medicine.* ***Aims****: The objective of this study was to develop a method for the analysis of the chemical content of captopril in antihypertensive herbs sold online under the following brands: A, B, C, and D.* ***Method****: The analytical method used was TLC-densitometry, utilising a mobile phase of chloroform: methanol (7:3).* ***Result****: The results show that the method used has fulfilled the requisite validation criteria for linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision parameters. The results of the analysis demonstrated that the sample did not contain any detectable quantities of captopril.* ***Conclusion****: The developed method has been proven to be effective for the analysis of captopril in antihypertensive herbal products and it can be confirmed that there is no presence of captopril medicinal chemicals in samples from brands A, B, C, and D*

KEYWORDS*: Antihypertensive medicine, captopril, TLC-densitometry, antihypertensive,*

INTRODUCTION

The public's inclination towards a return to nature has led to an increasing reliance on natural ingredients to treat health problems, thereby prompting the traditional medicine (OT) industry to expand its production capacity. The use of traditional medicine is perceived as being safer than the use of modern medicine, given that traditional medicine has a relatively lower incidence of side effects. In accordance with the Regulation of the Minister of Health of the Republic of Indonesia Number 007 of 2012, traditional medicine is prohibited from containing ethyl alcohol in excess of 1%. This prohibition does not apply to tincture preparations that are used by dilution, medicinal chemicals that are isolated or synthetic products with medicinal properties, narcotics or psychotropic substances, and other materials that are

based on health considerations and/or based on research and which may endanger health (BPOM, 2021).

The distribution of traditional medicine products, health supplements and cosmetics containing medicinal chemicals (BKO) remains a concern for the POM. A review of the period from July 2020 to September 2021 revealed that 53 traditional medicine products and one health supplement product contained BKO. It is evident that consumers are unaware of the potential risks associated with the consumption of these products, which provide a quick reaction to the body. The consumption of medicinal chemicals without consideration of dosage, method of use, contraindications, or drug interactions that may occur when the user takes other medications simultaneously can pose a risk to the user's health (BPOM, 2021).

The rapid development of information technology has led to an increase in the online circulation of traditional medicines, which has resulted in the easy spread of excessive claims and promotions within the community. This has rendered the public vulnerable to the inappropriate use of traditional medicinal products (BPOM, 2021). The circulation of traditional medicines without BPOM verification can result in adverse health outcomes for the community, as the quality and efficacy of these medicines cannot be guaranteed (Hadimulya et al., 2020). One of the traditional medicinal products sold online is herbal medicine that claims to treat hypertension. A considerable proportion of these antihypertensive herbal powder and knitted products remain unregistered with the BPOM.

Cardiovascular diseases have emerged as a significant global health concern, accounting for the majority of deaths annually. Hypertension is one of the most prevalent and common cardiovascular diseases. According to data from the World Health Organization (WHO) in 2015, approximately 1.13 billion individuals worldwide were affected by hypertension (Ministry of Health, 2019).

One of the pharmaceutical agents utilized for the management of hypertension is captopril. Captopril is classified as an ACE (angiotensin-converting enzyme) inhibitor. The use of ACE inhibitors has been associated with the occurrence of adverse effects, including taste disorders and skin reactions, which are frequently observed in patients undergoing captopril therapy. Other side effects frequently observed in patients undergoing treatment with this pharmacological class include hypotension, dizziness, fatigue, headache,

and indigestion (Brayfield, 2014). ACE inhibitors are the most commonly utilized pharmaceuticals among hypertensive patients between the ages of 40 and 60 (Turana et al., 2019).

One method for identifying captopril is through the use of thin layer chromatography (TLC). TLC is a technique employed for the analysis of samples comprising a mixture of substances, whereby a compound within the mixture is separated. Despite the growing popularity of traditional medicine, quality control remains a significant challenge. While current detection methods are effective, they are often limited in application to complex herbal matrices. This study addresses the need for improved analytical tools by developing a method tailored to the unique properties of traditional medicinal compounds. The TLC method was selected for its numerous advantages, including the fact that it requires compact equipment, is cost-effective, straightforward to use, allows for rapid analysis, and provides highly effective separation of compounds.

MATERIAL AND METHODS

Materials

The equipment employed in this study included a Twin-Through type chromatography chamber (Camag), a TLC Densitometer (Camag), a Linomat (Camag), a Vortex (Maxi Mix II), an analytical balance (Shimadzu AUW-220D), and laboratory glassware. The materials employed included methanol pro analysis (Merck), chloroform pro analysis (Merck), standard Captopril BPFI, iodine pro analysis (Merck), TLC plate silica F254 (Merck), powder samples and knitted antihypertensive herbs.

Methods

Preparation of the standard solution and the calibration curve

A number of captopril standards were weighed and subsequently dissolved in methanol, yielding a parent standard solution with a concentration of 250 ppm. This solution was bottled on a TLC plate with a volume of 1 µL to 5 µL and subsequently eluted. Iodine vapour was employed as a stain spotter (Liliya et al., 2016; Rubiyanto, 2016).

Preparation of samples

250 milligrams of antihypertensive herbal medicine samples were added to 5 ml of methanol, then vortexed and filtered. This process was repeated several times, and the filtrate was added to a certain volume of methanol (Liliya et al., 2016; Rubiyanto, 2016).

Optimisation of the Mobile Phase

Mobile phase optimisation was conducted on the composition of

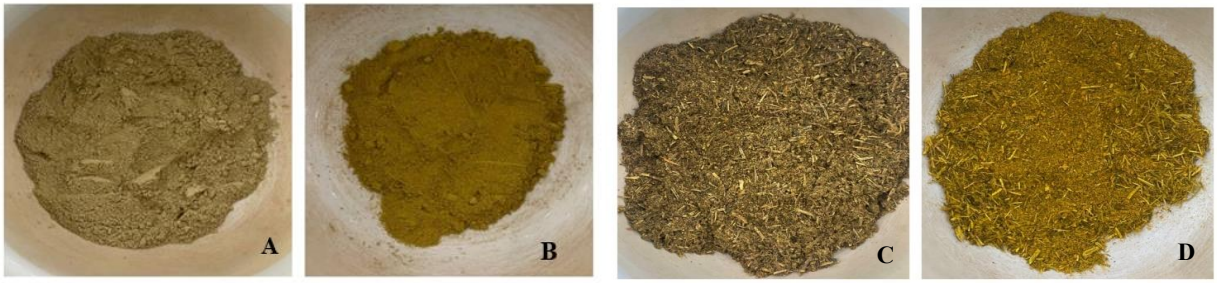

Figure 1. Powder samples

chloroform and methanol, with a chloroform:methanol ratio of 9:1; 8:2; and 7:3. The parent standard solution and the eluted sample solution were then scanned with a densitometer at the wavelength obtained from the reading on the spectrophotometer. The selected mobile phase was determined to be the mobile phase with the best Rf value.

Accuracy and Precision

A total of 2 grams of the sample was extracted with 5 mL of methanol, then vortexed. This process was repeated several times, after which the extraction results were filtered and the filtrate was added to a certain volume of methanol. Subsequently, 2 grams of the sample was added to 250 ppm of the captopril standard. The sample solution and the supplemented sample were each photographed on the TLC plate with a volume of 2 µL to 5 µL (Liliya et al., 2016; Rubiyanto, 2016).

Determination of Captopril Level in Samples.

Prior to determining the levels, the analytical method was validated with regard to linearity, LOD, LOQ, accuracy and precision parameters. This was done to prove that the method used can provide reliable analytical results. Furthermore, the extracted sample solution and the standard solution were each bottled on the TLC plate and eluted in the chromatographic chamber. After elution, the plate was exposed to iodine vapour and readings were taken using a densitometer (Liliya et al., 2016; Rubiyanto, 2016).

RESULTS AND DISCUSSION

The antihypertensive herbs utilized in this investigation were provided in the form of powdered (samples A and B) and knitted (samples C and D) herbal preparations procured from a commercial electronic marketplace. Illustrations of the antihypertensive herbal medicine samples can be observed in Figure 1.

The objective of mobile phase optimisation is to identify the optimal mobile phase composition for the separation of analytes in a given sample. The results of the mobile phase optimisation indicate that the optimal composition is chloroform: methanol (7:3),

Tabel 1. Optimisation of the mobile phase

$CHCl_3$:MeOH	Rf value
9:1	0,13
8:2	0,46
7:3	0,48

as this produces an optimal Rf value and demonstrates the most effective separation (Table 1). An Rf value is deemed satisfactory if it falls within the range of 0.2-0.8 (Wulandari, 2011). Captopril compounds exhibit high solubility in water, methanol, ethanol, and chloroform. The testing of ACE inhibitors demonstrates a reduction in Rf value with an increase in the concentration of non-polar components in the mobile phase, resulting from an enhanced retention of the tested compounds. In the use of mobile phase chloroform:methanol (9:1), the Rf value was less than 0.2, and in the use of mobile phase chloroform:methanol (8:2), the separation was unsatisfactory. Conversely, when mobile phase chloroform:methanol (7:3) was employed, a satisfactory Rf value was attained, ranging from 0.2 to 0.8, thereby ensuring optimal separation. In other words, the greater the polarity of the mobile phase employed, the greater the likelihood of obtaining a higher Rf value. Among the three mobile phases used for optimisation, the chloroform:methanol (7:3) mobile phase has the greatest polarity (Odović et al., 2009). This mobile phase was selected for its ability to provide optimal separation, with a maximum wavelength of 223.8 nm, as determined by the analysis of the maximum wavelength of the captopril standard using a Shimadzu UV-1800 Spectrophotometer.

The use of iodine vapour as a staining technique has been established as an effective method for identifying compounds that act as ACE inhibitors. The iodine vapour detection method does not damage the components or compounds in question and can be used on almost all organic compounds. Following exposure to iodine vapour, the analyte compound will appear as a brownish stain (Rubiyanto, 2016). It has been demonstrated that sulfur atoms in an organic compound can cause an iodine-azide reaction when iodine is used as a stain spotter. Consequently, iodine vapour can be employed on compounds containing sulfur atoms, such as captopril medicinal chemical compounds (Skorupa et al., 2011).

The results of the method validation demonstrate that the method employed has fulfilled the requisite validation criteria for linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision parameters (Table 2). To minimise errors caused by the matrix, the standard addition method was employed for accuracy and precision testing (Suriansyah et al., 2012). The research results for samples A, B, C and D indicated the absence of captopril chemicals, as the

Table 2. Method validation results

Parameters	Result	Reference Value
Linearity	r = 0,9958	r > r tabel = 0,8783 (Wisudyaningsih, 2012)
Accuracy	89,54 – 94,16 %	80-110% (Sugihartini *et al*., 2012; Wisudyaningsih, 2012)
Precision	3,85%	< 5% (Sugihartini *et al*., 2012; Wisudyaningsih, 2012)
LoD	136,24 ng	
LoQ	454,12 ng	

Tabel 3. Determination of captopril level in samples

Samples	Area	Rf	Captopril level (ng)
Captopril standard	5271,11	0,46	939,9819
Sample (A)	580,11	0,46	-276,64
Sample (A)	50,04	0,48	
Sample (A)	113,04	0,48	
Sample (B)	6,67	0,49	-332,64
Sample (B)	30,22	0,50	
Sample (B)	12,67	0,50	
Sample (C)	37,80	0,47	-329,39
Sample (C)	20,33	0,50	
Sample (C)	31,71	0,48	
Sample (D)	ND	ND	-330,68
Sample (D)	28,81	0,49	
Sample (D)	19,04	0,47	

calculated levels of these samples were below the LOD and LOQ (Tabel 3).

The KLT method has been demonstrated to be a viable tool for the determination of captopril levels in mixed samples. This assertion is supported by its extensive application in traditional medicinal samples, which are typically comprised of a diverse array of ingredients. A study was conducted to ascertain the efficacy of KLT in the identification of captopril in pharmaceutical formulations, particularly tablets. The investigation involved the employment of diverse solvent systems as the mobile phase, with a focus on chloroform-methanol (9:1) as it demonstrated optimal Rf characteristics. This study concluded that the chloroform-methanol mobile phase yielded the most precise detection limit of captopril within the system, ranging from 0.4 mcg (Liliya et al., 2016)

CONCLUSION

The developed method has been proven to be effective for the analysis of captopril in antihypertensive herbal products, with method validation results that meet the required standards and based on the results of the determination of levels, it can be confirmed that there is no presence of captopril medicinal chemicals in samples

from brands A, B, C, and D. This approach has the potential to be applied in the context of the supervision of medicinal chemicals (BKO) within traditional medicine, thereby contributing to the assurance of public safety. Further research is necessary to develop other methods of optimizing the mobile phase.

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