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Research Article

Simultaneous Determination Of Metamizole, Thiamin And Pyridoxin In Multicomponent Tablet by RP-HPLC

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ABSTRACT

The aim of the study was to develop and validate high performance liquid chromatography (HPLC) assay for the simultaneous determination of Metamizole, Thiamine and Pyridoxin in multicomponent tablet dosage form. The experimental procedure involved reversed-phase-HPLC with a Atlantis T3 C_{18} column (5 µm particle size, 4.6 ID x 150 mm), PIC solution-metanol-acetic acid volumetric solution (700: 300 : 4, v/v/v) mobile phase, UV detection at 275 nm. The flow rate of the mobile phase was 1.2 mL/min. The method was validated with respect to specificity, precision, accuracy and linearity. Due to its simplicity and accuracy, the assay method is suitable for routine analysis of multiomponen tablet formulation.

Key words : metamizole, thiamine, pyridoxin, HPLC

1. Introduction

Metamizole (MET), Thiamine (B_1) and Pyridoxin (B_6) are active pharmaceutical ingredients frequently combined and widely used to relieve pain complaints caused by neuritis and neuralgia, especially on severe pain (Indonesian Pharmacist Association, 2013). MET is pirazolon derivative having analgesic and antipiretic effects. It is commonly used to relieve acute pain (Zukowski and Kotfis, 2009). Thiamine and Pyridoxin are neurotropic vitamins which play an important role in formation of energy metabolism needed by brain cells. The combination of MET with vitamin B complex (ie. thiamine and pyridoxin) will increase the potential synergistic analgesic effect (Rosales et al., 2006). The chemical structure of MET, B_1 and B_6 are shown in Fig1.

Some analytical methods have been reported for determination of MET, B_1 and B_6, either alone or in combination with other medicines in pharmaceutical products. Several analytical methods for determination MET such as electrochemical and electrophoretic (Basaez et al., 2008), reflectometric (Weinert et al., 2007), spectrophotometry (Salih and Al-Sharook, 2008), HPLC (Altun, 2002) and LC/MS for bioequivalence study

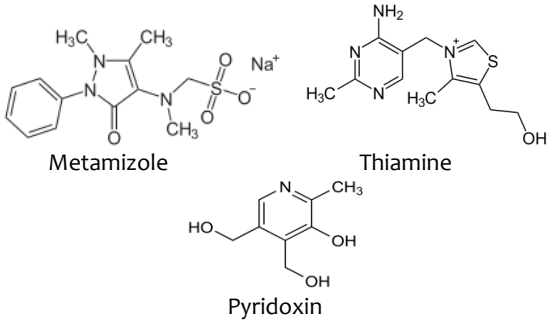

Fig 1. The chemical structure of Metamizol, Thiamine, and Pyridoxin

(Shep et al., 2012). Furthermore several analytical methods of quantification B_1 and B_6 such as densitometry and spectrophotometry multivariat (Elzanfaly et al., 2010), capilary zone electrophoresis (Franco et al., 2012), HPLC (Yantih, et al., 2011; USP, 2013) and LC/MS (Chen, *et al.*, 2006). The combination of MET, B_1 and B_6 is commercially available in tablet dosage form and most of the drugs in multicomponent dosage forms can be analyzed by HPLC.

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The objective of this study was to develop and validate a specific, accurate, precise HPLC method for simultaneous determination MET, B_1 and B_6 in multicomponent tablet dosage form.

2. Material and method

2.1. Material

The standards of MET, B_1 dan B_6 were of reference standard of Indonesian Pharmacopeia and were obtained from the National Agency of Drug and Food Control, Republic of Indonesia. The chemicals and reagents used were analytical grade. Methanol (HPLC grade, Merck), pentan sulphonic acid sodium salt (Merck), heptan sulphonic acid sodium salt (Merck), acetic acid (Merck) and bi-distillated water (Ikapharmindo) were used to prepare the dilute solution and mobile phase.

The tablet dosage form was obtained from pharmacy in Yogyakarta, labeled to contain 500 mg, 50 mg, 100 mg and 100 µg of MET, B_1, B_6 and B_{12} respectively in each tablet.

2.2. Aparatus

The method development was performed with a LC system consisting of Shimadzu LC 20AD solvent delivery system, a SPD-M20A photo diode array detector and samples were injected with a 7725i Rheodyne injector system with a 20 µL sample loop. The assay were performed with another LC system consisting of Shimadzu LC 20 AD solvent delivery system, SPD 20A uv/vis detector and SIL 20A autosampler using 20 µL sample loop. The detector was set at 275 nm.

Separation was carried out at ambient temperature using an Atlantis T3 C_{18} colomn (5µm, 150 x 4,6 mm I.D., Waters, Milford, USA). All the calculations of quantitative analysis were performed with external standarization by the measurement of peak area.

2.3. Method

2.3.1. Preparation dilute solution

Dilute solution were 0,5% acetic acid in water

2.3.2. Preparation of mobile phase

Prepare PIC solution by dissolving a mixture of 0.522 g pentane sulphonic acid sodium salt and 0.404 g heptane sulphonic acid sodium salt into bi-destilated water.

A mixture of PIC solution-Methanol-Acetic acid glacial (700:300:4) (v/v/v) was used for mobile phase. The mobile phase were filtered through a 0.45 µm milipore filter before use and degassed in an ultrasonic bath.

2.3.3. Standart stock solution and sample stock solution

Standard stock solution composed of a mixture of 400 µg/mL MET, 40 µg/mL B_1 and 80 µg/mL B_6, while the stock sample solution made by dissolving the sample in order to obtain the composition of 1500 µg/mL MET, 150 µg/mL B_1 and 300 µg/mL B_6)

2.3.4. Working standart solution

Working standard solution was made by dissolving standard stock solution to obtain 200 µg/mL MET, 20 µg/mL B_1 and 40 µg/mL B_6. Six replicate of 20 µL injection were made for system suitability test.

2.3.5. Sample solution preparation

Sample solutions were made by weighing tablets that have been crushed an equivalent to 10 mg MET, 1 mg B_1 and 2 mg B_6 in 50 mL volumetric flask and dilute with acetic acid 0.5%. Then, the solution was shaken vigorously for 30 minutes. The solution was filtered using Whatman microfilter Ø 0,45µm, and supernatant was taken. Standart and sample solution were subjected to HPLC measurement as describe above. The concentration of DIP, B_1 and B_6 in tablet dosage forms was calculated based on single point calibration. All determinations were performed six times.

3. Result and Discusion

Reference method of this study from NDFC 2001 namely methampiron assay in a mixed multicomponent tablet. Flow rate between 1 mL/min and 1.2 mL/min were studied. A flow rate 1.2 mL/min chosen because it provides good separation in reasonable time. The separation of MET, B_1 and B_6 shown in figure 2.

3.1. System Suitability Test (SST)

The performance qualification of HPLC was determined with the system suitability to verify system performance under actual running conditions with a well-characterized analyte mixture, column, and mobile phase. The evaluation of SST based on the value of relative standart deviation (RSD) of peak area and retention time, and it performed every time when the analysis was began.

The table 1 showed that RSD of retention time and peak area MET, B_1 and B_6 were lower than 2%, that means performance of HPLC is good.

3.2. Specificity

ICH defines specificity as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. specificity determination can be done by calculating the resolution and peak purity index values obtained using the PDA detector (Snyder et al., 1997, Ahuja and Dong, 2005).

In this study, the resolution of MET, B_1 and B_6 were greater than 2 and peak purity index values were close to 1 as shown at Table 1, thus it can be concluded that the method is specific.

3.3. Precision

ICH defines the precision of an analytical procedure as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The aim of repeatability test is determine

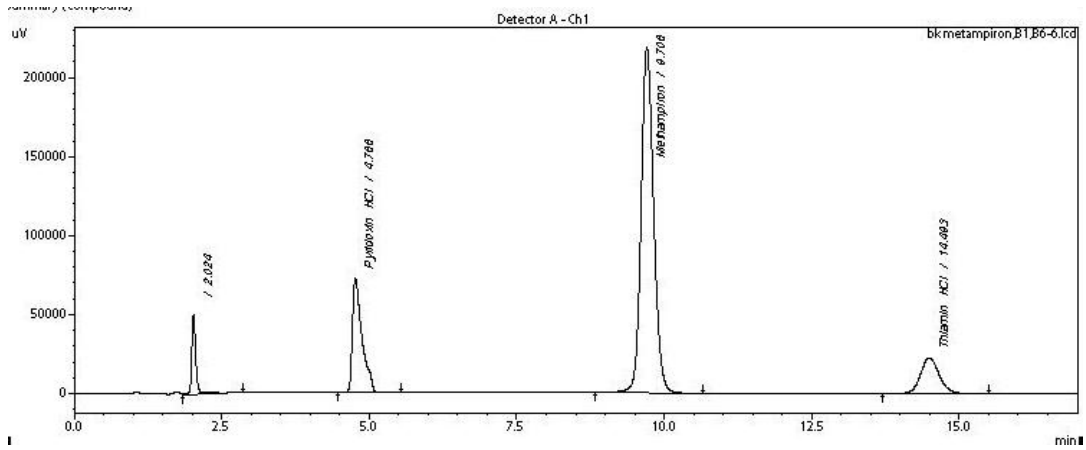

Fig 2. Chromatogram of the mixture of pyridoxine (Rt = 4.81), metamizole (Rt = 9.82) and Thiamine (Rt = 14.69). HPLC condition : coloumn: Waters, Atlantis T_3, C-18, 150 x 4.6 mm, 5 µm; mobile phase : PIC solution : methanol : Acetic acid (700 : 300 : 4 v/v/v); flow rate : 1.2 mL/minute ; injection volume 20 µL ; detector 275 nm

Table 1. performance parameters of Metamizole, Thiamin (B_1) and Pyridoxin (B_6) in system suitability test

	Retention time		Peak Area		Resolution	Peak purity index
Compound	Mean (n=6)	RSD (%)	Mean (n=6)	CV (%)	Mean (n=6)	
B_6	4.81	0,89	822432	0.06	13,90	1.000
MET	9.82	1.17	3188671	0.95	14.99	0.999
B_1	14.69	1.54	496160	0.14	10.15	0.999

Table 2. Precision and Accuration result

	% Recovery (n=3, mean) ± % RSD		
Conc (µg/mL)	B_6	MET	B_1
260	99.31± 0.05	98.43 ± 0.57	98.84 ± 0.52
230	99.45 ± 0.05	98.60 ± 0.44	99.41 ± 1.31
200	99.39 ± 0.10	98.44 ± 0.41	99.32 ± 0.97
170	99.60 ± 0.05	98.64 ± 0.58	99.39 ± 0.97
140	99.43 ± 0.65	98.27 ± 0.29	99.44 ± 1.16

Tabel 3. Linearity results

Compound	Equation	r
Metamizol	Y = 232,513.69 x + 2,672.38	0.9998
Thiamine	Y = 466,939.20 x + 4,092.21	0.9992
Pyridoxin	Y = 232,513.69 x – 2,672.39	0.9998

Table 4. The level of Metamizole (MET), Thiamin (B_1) and Pyridoxine (B_6) of tablet dosage form obtained by HPLC and by uv spectrophotometry in combination with PLS

Compound	Concentration mg/tab (mean,n=6) ± %RSD	
	HPLC	Uv-PLS
B_6	98.96 ± 0.67	98.44 ± 1.66
MET	480.32 ± 0.76	482.15 ± 0.38
B_1	49.68 ± 1.56	50.24 ±1.82

ability of the method to analyze samples at the same operational conditions within a short time. Repeatibility should be assed using a minimum 9 determination covering the specified range for the procedure or a minimum of 6 determination at 100% of the test concentration.

Repeatibility test on this study done by determination 5 concentrations on 3 replicates each, and expressed by %RSD. The precision result shown at table 2. RSD concetration of MET, B_1 and B_6 at each concentration were lower than 2%, so it can be concluded that the analytical method was precision.

3.4. Accuracy

Accuracy is a parameter to indicate the closeness between the values obtained with the true value, expressed in percent recovery. ICH recommends that the accuracy was done by 9 times assay at 3 different concentrations. In this study the accuracy performed at 5 concentrations by spiking method, namely by adding a certain amount of the standard solution to the sample solution from stock (2.3.3) with a ratio of 3 : 7.

The accuracy result shown at table 2. Recovery of MET, B_1 and B_6 were in the range of 98-102%, which means that the method has good accuracy.

3.5. Linearity

Linearity test conducted by injecting 5 series concentrations in the range 70-130% of the target concentration with 3 times replication. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation.The linearity result shown at table 3.

The linearity result of MET, B_1 and B_6 showed that coefficient correlation (r) greater than 0.999, so it can be stated that the method produces peak area that is proportional to the analyte concentration.

3.6. Samples analysis using HPLC

The results of determination MET, B_1 and B_6 in tablet dosage form shown in the table 4, and when compared with the result of spectrophotometric method combined multivariate calibration patial least square (PLS) are not significantly different. Thus it can be concluded that both methods can be used to determination MET, B_1 and B_6 with precision and accuracy as good.

4. Conclusion

The simultaneous determination of metamizol, thiamine and pyridoxin was performed on a C_{18} column of (4.6x150mm) dimension and 5 µm of particle size. A mixture of PIC : Methanol : acetic acid (700 : 300 : 4) (v/v/v) as mobile phase with flow rate of 1.2 mL/minute and monitored at 275 nm. That method was simple, accurate, precise, and could be successfully applied for the analysis of metamizol, thiamine and pyridoxin in multicomponent tablet dosage form.

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