Jurnal Farmasi Indonesia, November 2016, hal 126 - 132 Vol. 13 No. 2
ISSN: 1693-8615 EISSN : 2302-4291 Online : http://farmasiindonesia.setiabudi.ac.id/

HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ASSAY OF METAMIZOLE, THIAMINE AND PYRIDOXINE IN TABLET

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ABSTRACT

The aim of the present study was develop and validate HPLC method for the simultaneous assay of metamizole, thiamine and pyridoxine in tablet. Metamizole is a substance that is easily hydrolyzed in the presence of water and oxygen. To inhibit the hydrolysis of metamizole during sample preparation prior to HPLC analysis. Sodium sulfite is added and its optimum concentration was investigated. The chromatographic system includes a RP C8(2) column (150x4.6 mm, 5 µm particle size) in conjunction with Photo Diode Array (PDA) detector. The optimal chromatographic condition was obtained using a mobile phase consisting of phosphate buffer 35mM pH 3.0: methanol (80:20), flowrate 1.0 mL/min, and 10 µl injection volume. The metamizole, thiamine and pyridoxine were detected at 275 nm. The hydrolysis of metamizole was successfully inhibited by adding solution containing 1.5 mg/mL sodium sulfite to solvent and 0.5 mg/mL sodium sulfite to mobile phase. The validation results indicate a good specificity and a linear detector responses with r>0.999. The accuracy (% recovery) for metamizole, thiamine and piridoxine were 100.26%; 99.09%; and 100.03%, respectively. The method yields good precision with RSD of metamizole, thiamine and pyridoxine were 2.0912%; 1.4489%; and 0.8418%, respectively. The validated method was successfully applied for simultaneous assay of metamizole, thiamine and pyridoxine in tablet.

Keywords: *method development and validation; metamizole; thiamine; pyridoxine; hydrolysis of metamizole, HPLC*.

INTRODUCTION

Metamizole are drugs known as non-steroid anti-inflammatory drug (NSAID) that has analgesic activity, antipyretic and anti spasmodic. Thiamine, and pyridoxine are a vitamine which provides neuroprotective effects and analgesic use as adjunctive therapy in neuropathic pain. Metamizole combined by the thiamine, and pyridoxine used in the treatment of neuropathic pain. Thiamine, and pyridoxine have antinociceptive activity that can fight pain induction. Metamizole by the vitamine B have a synergistic effect of that can relieve acute pain of accompan low side effects. A synergistic effect between metamizole with thiamine and pyridoxine and low side effects that occur make metamizole and vitamie B were widely used the treatment combination of pain, particularly neuropathic pain[1-4].

Metamizole are substances unstable and easily degradable in the presence of oxygen and water. Metamizole chemical properties that the susceptible to hydrolysis cause problems in the analysis of preparations containing metamizole, thiamine, and pyridoxine. Because the

sample preparation solvent generally with water [5-6].

A dosage must be controlled regularly to ensure the quality of the product. Simultaneous analysis methods in preparation metamizole mixture, thiamine, and pyridoxine have barriers where metamizole hydrolyzed by aqueous solvent. Metamizole hydrolysis by aqueous solvent metamizole cause instability at the time of analysis, which could affect the results of the analysis. Metamizole hydrolysis occurs in the presence of water and oxygen with the possible result degradation is1-phenyl-2, 3-dimethyl-4-methyl-aminopyrazol-5-keton [5-6].

Metamizole hydrolysis in the presence of water and oxygen occurs very rapidly and stability affect of metamizole at the time in analysis. To avoid this situation need to develop an analytical method that metamizole remain stable. One method of maintaining the stability of metamizole on mixture preparation with thiamine and pyridoxine to inhibit hydrolysis. To inhibit hydrolysis metamizole at the time of the analysis can be added Na_2SO_3. Na_2SO_3 usage at the time of the analysis conducted on samples of this analgesic mixture can significantly lower solvent degradation reactions metamizole in water, so the use of Na_2SO_3 to prevent the degradation of metamizole allow preparations used in the preparation of a mixture of metamizole, thiamine, and pyridoxine. Use of HPLC in the analysis of this preparation due to factors selectivity, sensitivity, reproducibility and speed of analysis.The development of analytical methods to be followed by validation of methods to prove feasible use intended [6-7].

CHEMICALS AND REAGENTS

The materials in this study ismetanol pro HPLC (Merck, Jerman), aquabidestilata (PT Otsuka), Natrium Hexansulfonat (pro analysis), KH_2PO_4 (pro analysis), Na_2SO_3 (pro analysis) and HCl (pro analysis). Metamizole, thiamine, pyridoxine and sample blanko (PT Interbat, Sidoarjo).

Apparatus

The tools in this research is a set of instruments HPLC Agilent type 1100 series equipped diode array detector and auto sampler, column Phenomenex luna C8(2) with a length of 150 mm diameter 4.6 mm particle size of 5.0 µm for the brand Agilent, 0:22 µm porous membrane filter (Whatmann), 0:45 µm porous membrane filter (Whatmann), ultrasonifikasi tools, analytical balance, and glassware.

Preparation of mobile phase

Made by the carefully mix 35mm phosphate buffer solution pH 3.0: methanol in the ratio of 80:20 Na_2SO_3 plus 0.5 mg / ml. Phosphate buffer solution is made by weighing 2.0 grams and 4.76 grams of sodium hexansulfonat (KH_2PO_4) in 1000.0 ml aquabidestilata. Adjust pH with H_3PO_4 until reaching a pH of 3.0. Make a porous filter membrane filtration with 0:45 µm (Whatmann), then ultrasonifikasi for ± 10 minutes, let it cool**.**

Preparation of solvent

The solvent used water Na_2SO_3 added 1.5 mg/ml.

Preparation of standards

Carefully weighed raw metamizole, thiamine, and pyridoxine respectively of 10.0 mg, then put in a flask and added 10.0 ml of 8 ml solvent. Furthermore ultrasonic for ± 10 minutes. Lastly, the solvent is added ad. limit sign, shake until homogeneous, to obtain the concentration of standard solution of the parent 1.0 mg / ml (1000.0 ppm). Standard solution mixture prepared by pipette each standard solution parent metamizole, thiamine, and pyridoxine as much as 2.5 ml; 0.5 ml and 1.0 ml into 10.0 ml flask, then added solvent ad. mark, shake until homogeneous, in order to get the standard solution mix (metamizole, thiamine, and pyridoxine) each with a concentration of 250.0 ppm, 50.0 ppm and 100.0 ppm.

Preparation of sampel

Weight 20 tablets are containing metamizole, thiamine, and pyridoxine then finely crush and taken the average weight of the tablet. Dissolved in 100.0 ml flask and ultrasonic for 15 minutes. Centrifuge sample solution at 50 rpm for 15 minutes. 1.0 mL samples were taken and put in a 10.0 mL volumetric flask, then added solvent ad sign, shake until homogeneous so that in the sample solution can metamizole, thiamine, and pyridoxine each with a concentration of 250.0 ppm, 50.0 ppm and 100.0 ppm.

Validation of the method

Validation of the method was made in terms of specificity, linearity, accuracy and precision. The specificity of the method was checked using injec solvent, standards and sampel. Specificity was accepted if the no peaks that interfere with analyte. The linierity of the method waschecked using sets of up to six concentration levels. Linierity was accepted if the r-value more than 0.999. Accuracy and precision were estimed by of the recovery value and relative standard deviation (RSD) calculated from 6 replicate mixture samples [8-9].

RESULT AND DISCUSSION

Development of the HPLC method

Optimization of HPLC conditions include the selection of solvent composition and flow rate of the mobile phase. The choice of solvent and mobile phase with the addition of Na_2SO_3 at several concentrations up capable of inhibiting the hydrolysis of metamizole. Composition and flow rate of the mobile phase used in HPLC system was tested by injecting a certain amount of standard solution. Solvents are developed is water coupled with Na_2SO_3 at a concentration of 0.5 mg / mL, 0.7 mg / mL and 1.0 mg / mL have not been able to inhibit hydrolysis metamizole. This can be seen in the presence of another peak in chromatogram results. Solvents are chosen water coupled with Na2SO3 1.5 mg / mL were able to inhibit hydrolysis metamizole, where peaks appear on the chromatogram only a single peak metamizole**.**

The composition of the mobile phase used a mixture of phosphate buffer at *p*H 3.0 with methanol 35mm coupled with Na_2SO_3 with some concentration. Phosphate buffer mixture composition: methanol attempted a comparison 77:23; 80:20; 85:15 and 82:18 with Na2SO3 addition of 0.5 mg / mL. The addition of Na_2SO_3 the mobile phase was intended to inhibit hydrolysis metamizole during analysis. Selected

mobile phase of composition is 35mm phosphate buffer pH 3.0 methanol (80:20) with Na2SO3 plus 0.5 mg / mL, which provides good separation of analytes. The flow rate developed is 1.0 mL / min and 0.9 mL / min. This analysis of the selected flow rate is 1.0 mL / min which is able to provide separation of analytes well.

Validation of the HPLC method

Before the first method validation performed system suitability test (SST) by injecting a standard solution metamizole 250 ppm, 50 ppm thiamin, and pyridoxine 100 ppm five times [7-8]. In this test pointed kansistem HPLC used meet the requirements of repeatability (precision) instrument with a value of RSD ≤1,0%, resolution (> 1.5), and the follow-up factor (≤2) and the number of theoretical plates that meet the general requirements (> 2000).

Validation of analytical methods according to category of the USP is the specificity, linearity, accuracy and precision. In specificity test is done by looking at the value of the resolution and peak purity solvents, raw and samples. This specificity test analyte resolution values> 1.5 as well as the purity of the analyte peak value> 0.95 indicating that this method specific. Chromatogram specificity test results shown in figure 1.

Validation of the second method is linearity. Linearity test is done by injecting a standard solution metamizole, thiamin, and pyridoxine there are 6 kinds of concentration. Linearity calculation results show that the value of the third analyte r> 0.95 and a value VX0≤5% for the three analytes. It can be concluded that all three analytes provide a linear response between levels of the area. Linearity test results shown in Table 1. Validation third method is the accuracy by analyzing samples of placebo were added (addition) metamizole, thiamine, and pyridoxine with three different concentration levels of 80%, 100% and 120%. Each concentration is done 3 times replication. The test results accuracy as a% recovery (recovery). The accuracy of test results in Table 2 until Table 4. Range of accuracy for metamizole and pyridoxine 98% -102%, while thiamin 97% -103%, which means that this method has an accuracy better. Validation of the latter method is presisisi. Precision test carried out by the assay metamizole, thiamin, and pyridoxine in a matrix solution adds across placebo who had a level of 100% raw on the analyte concentration and replication is performed 6 times. The results of precision test performed shown in Table 5. The test results show that the precision of assay value in RSD metamizole, thiamine, and pyridoxine < 2.0%, it is proved this method has in good precision.

Samples analysis using HPLC

The results of determination metamizole, thiamine and pyridoxine in tablet dosage form showed in the table 6 . Results of recovery of the assay meets the requirements.It shows this method can be used for analysis metamizole, thiamine and pyridoxine in tablet dosage form.

Table 1 Linierity

Analyte	Levels Range (ppm)	Regresstion Equation	R	Vxo(%)
Metamizole	156-520	Y = 5,190x + 234,7	0,9995	0,72
Thiamine	30,3-101	Y = 9,751+ 45,99	0,9989	1,26
Pyridoxine	84-147	Y = 11,95x – 78,12	0,9995	0,39

Table 2 The accuracy of the results of the test at concentrations analyte 80%

Analyte	Rep	The amount added (ppm)	The number obtained (ppm)	% Recovery
Metamizole	1	206	209,99	101,94
	2	206	205,76	99,88
	3	206	202,71	98,40
Thiamine	1	42	42,71	101,69
	2	42	41,79	99,50
	3	42	41,06	97,76
Pyridoxine	1	84	85,57	101,87
	2	84	85,52	101,81
	3	84	84,21	100,25

Table 3 The accuracy of the results of the test at concentrations analyte 100%

Analyte	Rep	The amount added (ppm)	The number obtained (ppm)	% Recovery
Metamizole	1	257,5	259,76	100,88
	2	257,5	261,92	101,72
	3	257,5	252,81	98,18
Thiamine	1	50	49,17	98,34
	2	50	50,43	100,85
	3	50	49,04	98,08
Pyridoxine	1	103	102,68	99,69
	2	103	103,23	100,22
	3	103	103,19	100,18

Table 4 The accuracy of the results of the test at concentrations analyte 120%

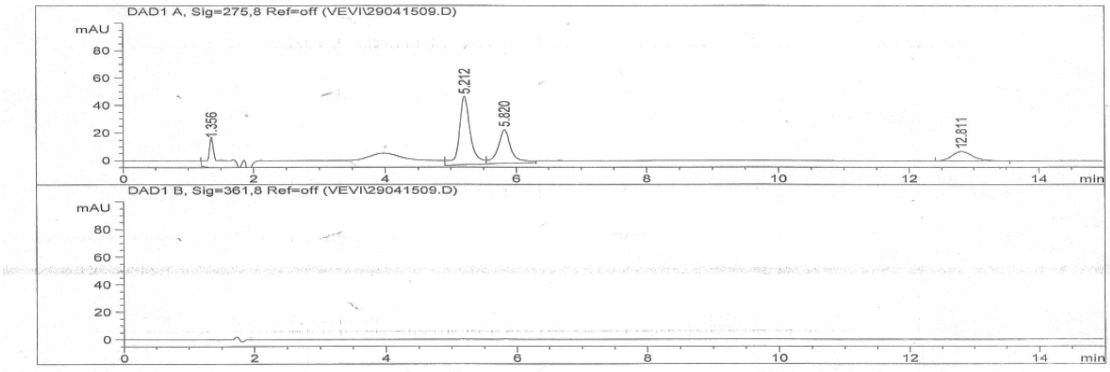
Analyte	Rep	The amount added (ppm)	The number obtained (ppm)	% Recovery
Metamizole	1	301,5	296,46	98,33
	2	301,5	306,6	101,69
	3	301,5	297,41	98,64
Thiamine	1	65,4	63,75	97,47
	2	65,4	66,3	101,38
	3	65,4	66,97	102,40
Pyridoxine	1	126	123,54	98,05
	2	126	124,21	98,58
	3	126	126,46	100,37

Table 5 Precision

Rep	Level (%)		
	Metamizole	Thiamine	Pyridoxine
1	100,88	98,34	99,69
2	101,72	100,85	100,22
3	98,18	98,08	100,18
4	99,66	99,14	99,87
5	99,07	97,50	98,99
6	103,34	100,38	101,34
SD	1,89%	1,33%	0,77%
RSD	2,0912%	1,4489%	0,8418%

Table 6. Determination of levels in samples

Rep	Analyte	Level in samples (ppm)	Level recovered (ppm)	% Recovery
1	Metamizole	250	254,87	101,95
	Thiamine	50	48,56	97,12
	Pyridoxine	100	98,34	98,34
2	Metamizole	250	253,4	101,36
	Thiamine	50	51,48	102,96
	Pyridoxine	100	100,87	100,87
3	Metamizole	250	242,74	97,10
	Thiamine	50	49,88	99,76
	Pyridoxine	100	101,16	101,16



Figur 1. Chromatogram Metamizole, Thiamine and Pyridoxin

CONCLUTION

The simultaneous determination of metamizole, thiamine and pyridoxine was performed on a C8(2) column of (150x4.6 mm, 5 µm particle size). Phosphate buffer 35mM pH 3.0: methanol (80:20) as mobile phase with flowrate 1.0 mL/min, 10 µl injection volume and monitored at 275 nm. The hydrolysis of metamizole was successfully inhibited by adding solution containing 1.5 mg/mL sodium sulfite to solvent and 0.5 mg/mL sodium sulfite to mobile phase. That method was simple, accurate, precise and could be successfully applied for the analysis of metamizole,

thiamine and pyridoxine in tablet dosage form.

REFERENCES

1. Jolivalt, C., Mizisin, L., Nelson, A., Cunha, J., Ramos, K., Bonke, D., and Calcutt, N. 2009. B Vitamins Alleviate Indices of Neuropathic Pain in Diabetic Rats. ***European Journal of Pharmacology***. Vol 612. Pp 41-47
2. Kopruszinski, C., Reis, R., and Chichorro, J. 2012. B Vitamins Relieve Neuropathic Pain Behaviors Induced by Infraorbital Nerve Constriction in Rats. ***Elsevier Science.*** Vol 91. Pp 1187-1195
3. Martinez, J., Vara, J., Lastres, P., Bernabeu, C., Betes, C., and Perez, J. 2002. Effect of Metamizole on Promyelocitic and Terminally Differentiated Granulocytic Cells Comparative Analysis with Acetylsalicylic Acid and Diclofenac. ***Elsevier Science***. Vol 65. Pp 209-217
4. Rosales, F., Santillan, R., Garcia, G., and Soto, V. 2006. Synergis Antinociceptive Interaction Between Achetaminophen or Metamizole and B Vitamins in the Formalin Test. ***Wiley Inter Science***. Vol 66. Pp 286-294
5. Chotimah, C., Sudjadi., Riyanto, S., and Rohman, A. 2014. Simultaneous Determination Of Metamizole, Thiamin and Pyridoxin in Multicomponent Tablet by RP-HPLC. ***Journal of Food and Pharmaceutical Sciences***. Pp 79-82
6. Qian, X., Gang, N., Xian-hua, W., and Gang, C. 2007. Stability and Determination of Metamizolee Sodium by Capillary Electhrophoresis Analysis Combined with Infra-red Spectroscopy. ***Science Direct***. Vol 23(6). Pp 654-658
7. Garcia, A., Navas, N., Martin, A., Roman, E., Cabeza, J., and Vallvey, L. 2009. Determination of Tramadol, Metamizolee, RopiVacaine, and Bupivacain in Analgesic Mixture Samples by HPLC with DAD Detection. ***Journal of Chromatographic Science***. Vol 47. Pp 1-7
8. Yuwono, M., dan Indrayanto, G. 2005. Validation of Chomatographic Methods of Analysis. Profiles of Drug Substances, Exipients,and Related Methodology. ***Elsevier,Inc***. Vol 32
9. USP. 2008. ***USP 32.*** The United States Pharmacopeial Convention.