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Validation of Metamizole, Thiamin and Pyridoxine Simultaneous Analysis Methods in Tablet Preparations Using HPLC

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ABSTRACT

The aim of the present study was to 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 precence 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 and 361 nm for cyanocobalamin. 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. In the robustness study, the small changes of mobile phase pH yielded unsymmetrical peaks and lower resolution. The validated method was successfully applied for simultaneous assay of metamizole, thiamine and pyridoxine in tablet.
Keywords: validation; metamizole; thiamine; pyridoxine; hydrolysis of metamizole; HPLC

INTRODUCTION

Metamizole works by inhibiting prostaglandin synthesis. Prostaglandin is substance that can cause pain, so that by inhibiting the synthesis of this substance with metamizole, pain can be relieved. The normal dose of metamizole as an analgesic is 500 mg to 1000 mg, one to four times a day. For oral preparations, metamizole has low toxicity thus many oral preparations of metamizole are circulating on the market[(1)].

Thiamin and pyridoxine (Vitamins B1 and B6) are components of the B complex vitamin that play a role in the metabolism of carbohydrates, proteins and fats. This vitamin is also an important part of new cell information, DNA, RNA and myelin. These three B vitamins are used to reduce pain. Vitamin B provides a neuroprotective effect that is used as an adjunct analgesic therapy in neuropathic pain[(2),(3)].

Thiamin and pyridoxine have anti-nociceptive activity that can fight pain. Metamizole together with these two B vitamins has a synergistic effect so as to relieve acute pain accompanied by low side effects. The synergistic effect between metamizole with thiamine and pyridoxine and the low side effects that occur make the combination of metamizole and vitamin B are widely used for pain therapy, especially neuropathic pain[(4)] .

Metamizole is a crystal powder, white or yellowish white, containing not less than 99.0% and not more than 101.0% $C_{13}H_{16}N_3NaO_4S$ calculated on the dried substance. Metamizole is a substance that is soluble in water and HCl. The chemical properties of metamizole which are easily hydrolyzed cause problems in the analysis of preparations containing metamizole, thiamine and pyridoxine. This is because the sample preparation is generally with water solvents[(5),(6)].

Simultaneous analysis methods in the preparation of a mixture of metamizole, thiamin and pyridoxine have obstacles where metamizole will be hydrolyzed by water solvents. Hydrolysis of metamizole by water solvents causes instability of metamizole at the time of analysis, so that it can affect the results of the analysis. Metamizole hydrolysis occurs in the presence of water and oxygen with a possible degradation result of 1-phenyl-2, 3-dimethyl-4-methyl-aminopyrazol-5-ketone.

Metamizole hydrolysis in the presence of water and oxygen occurs very quickly and can affect the stability of metamizole at the time of analysis. To prevent this from happening, an analytical method needs to be developed to keep metamizole stable. One method for maintaining the stability of metamizole in preparations mixed with vitamins B1 and B6 is by inhibiting its hydrolysis. To inhibit the hydrolysis of metamizole at the time of analysis Na_2SO_3 can be added. Garcia et al in 2009 published the use of 0.5 mg/mL Na_2SO_3 in water in its mobile phase to prevent hydrolysis of metamizole in a mixture of tramadol, metamizole, ropivacain and bupivacain analgesics. The use of Na_2SO_3 at the time of analysis carried out on this analgesic mixture sample

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can significantly reduce the degradation reaction of metamizole in water solvents, so the use of Na_2SO_3 to prevent degradation of metamizole allows use in preparations of a mixture of metamizole, thiamin, pyridoxine and cyanocobalamin[7],[8].

No scientific publications have yet been found regarding the qualitative and quantitative analysis of metamizole, thiamine, pyridoxine and cyanocobalamin simultaneously on the mixed preparations using HPLC. Therefore it is necessary to develop analytical methods for this purpose. The use of HPLC in the analysis of these preparations is due to the selectivity, sensitivity, reproducibility and speed of analysis. The development of analytical methods must be followed by validation of methods to prove that the method is appropriate for its intended use[7].

This research aims to: 1) determine the effective concentration of Na_2SO_3 to inhibit the hydrolysis of metamizole in a mixture of metamizole, thiamin and pyridoxine preparations; 2) optimizing HPLC for simultaneous determination of levels of the mixture of metamizole, thiamin and pyridoxine preparations; 3) validate the optimized HPLC method for simultaneous determination of levels of metamizole, thiamin and pyridoxine preparations.

METHODS

Materials and Tools

The materials used were methanol for HPLC (Merck, Germany), Distilled water (Otsuka), Sodium Hexansulfonate (pro analysis), KH_2PO_4 (pro analysis), Na_2SO_3 (pro analysis), HCl (pro analysis), metamizole, thiamin, pyridoxine, and blank samples. The tool used was a set of Agilent type 1100 series HPLC instruments equipped with an array diode detector and autosampler, Phenomenex luna column C8 (2) with a length of 150 mm diameter 4.6 mm particle size 5.0 µm Agilent brand, membrane filter 0.22 µm porous (Whatmann), 0.45 µm porous filter membrane (Whatmann), ultrasonification devices, analytical scales, and glassware.

Mobile Phase Manufacturing

The mobile phase was manufactured with a mixture of 35mM phosphate buffer solution pH 3.0: methanol in a ratio of 80:20. Phosphite buffer solution was prepared by weighing 2.0 grams of sodium hexansulfonate and 4.76 grams of KH_2PO_4 in 1000 ml of distilled water. Then, we adjusted the pH with H_3PO_4 until it reaches pH 3.0. Followed by filter with a 0.45 µm porous membrane filter (Whatmann), then ultrasonify for ± 10 minutes, let it cool.

Solvent Manufacturing

The solvent used was water added with 1.5 mg//ml Na_2SO_3.

Optimization of HCV Conditions Reversed Phase

Stationary Phase

The column used was Phenomenex luna C8 (2): length=150 mm, diameter=4.6 mm, particle size = 5.0 µm.

Mobile Phase

The mobile phase used was a solvent (section 4.3.1) consisting of a mixture of 35mM phosphate buffer solution pH 3.0 and methanol in a ratio of 80:20. To produce an optimal condition, variations in the elution system can be made in the form of isocratic or gradient. Na_2SO_3 was added to the buffer mixture with various concentrations starting at 0.5 mg/ml. Flow rates at 1-2 ml/minute were carried out from low speeds slowly to obtain optimal separation conditions.

Column Temperature

Column temperature was set at 25 °C (room temperature which was used for HPLC).

Injection Volume

The volume of injection of the sample solution into the HPLC system was 10µl.

Analysis Method Validation

The system suitability test included parameters: resolution (R or Rs) with R> 1.5, number of theoretical plates (N) with N > 2000, RSD (Relative Standard Deviation) repeated injections related to repeatability of retention time (t_R) with RSD ≤1.0% (n= 5), and the following factors: 0.9 ≤1.1 [8] .

Specificity

Specificity test was conducted by comparing the retention time between the results of standard chromatograms (metamizole, thiamin and pyridoxine) and mixed preparation samples containing metamizole,

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thiamin and pyridoxine. The standard parent solution (metamizole, thiamin and pyridoxine), sample solutions, and blank solution after that were filtered with a 0.22 µm porous filter membrane and injected into the HPLC system. The resulting chromatogram must be the same and no other chromatogram should appear on the results of the parent standard chromatogram or sample if it was not present on the results of the blank solution chromatogram. The resolution (Rs) between the components qualifies if it was 1.5-2.0. Whereas for qualitative verification was performed an evaluation using a DAD detector by measuring Match Factor and Peak Purity. The value of Match Factor and Peak Purity must be close to 1 (> 0.9500).

Linearity

Linearity was performed with a mixture containing metamizole, thiamin and pyridoxine. The standard solution for metamizole was diluted to a concentration of 150 ppm; 200 ppm; 250 ppm; 300 ppm; and 350 ppm. The standard parent solution for thiamine was diluted until a concentration of 40 ppm; 50 ppm; 60 ppm; 70 ppm; and 80 ppm were obtained. Moreover, pyridoxine standard solution was diluted to obtain concentrations of 80 ppm, 90 ppm, 100 ppm, 110 ppm and 120 ppm. The sorting and dilution was performed quantitatively. After that it was filtered with a 0.22 µm porous filter membrane and injected into the HPLC system. Do 5 repetitions.

Accuracy

Accuracy was accepted if it meets the criteria: recovery obtained in the range 80-110%.

Precision

Precision was performed by measuring repeatability. This test was carried out with the same sample preparation with 6 times the accuracy test. Then the SD and RSD values were calculated.

Concentration Determination

Determination of the concentration was carried out on a sample of a mixture of metamizole, thiamin, pyridoxine, cyanocobalamin that had been determined then the sample preparation and injection were carried out in the HPLC system with replication of injections 3 times. The resulting chromatogram was observed and the concentration calculation was conducted using a calibration curve.

RESULTS

Optimization of HPLC conditions

The selected HPLC conditions were those that provide good separation between analytes. Good analytical separability was with several parameters, namely a resolution value of more than 1.5 (Rs> 1.5), symmetrical peak shape (follow-up factor or TF <2) and good column efficiency (number of theoretical plates or N > 2000).

Table 1. Analytical condition in several solvents, composition and flow rate of mobile phases

No	Solvent	Mobile phase	Flow rate	Note
1	HCl 0.1 N	Phosphate buffer 35 mM pH 3.0 : methanol (77:23) + Na_2SO_3 0.5 mg/ml	1.0 ml/minute	The degraded metamizole peak appeared at minute 13.809
2	Water + Na_2SO_3 0.5 mg/ml	Phosphate buffer 35 mM pH 3.0 : methanol (77:23) + Na_2SO_3 0,5 mg/ml	1.0 ml/minute	The degraded metamizole peak appeared at minute 13
3	Water + Na_2SO_3 0.7 mg/ml	Phosphate buffer 35 mM pH 3.0 : methanol (77:23) + Na_2SO_3 0,5 mg/ml	1.0 ml/minute	The degraded metamizole peak appeared at minute 14
4	Methanol	Phosphate buffer 35 mM pH 3.0 : methanol (77:23) + Na_2SO_3 0.5 mg/ml	1.0 ml/minute	Only pyridoxine was detected
5	Water + Na_2SO_3 1.0 mg/ml	Phosphate buffer 35 mM pH 3.0 : methanol (77:23) + Na_2SO_3 0.5 mg/ml	1.0 ml/minute	Metamizole is still experiencing degradation
6	Water + Na_2SO_3 1.0 mg/ml	Phosphate buffer 35 mM pH 3.0 : methanol (80:20) + Na_2SO_3 0.5 mg/ml	1.0 ml/minute	Metamizole is still experiencing degradation
7	Water + Na_2SO_3 1.5 mg/ml	Phosphate buffer 35 mM pH 3.0 : methanol (80:20) + Na_2SO_3 0.5 mg/ml	1.0 ml/minute	Optimum conditions (Rs value meets and metamizole is no longer degraded)

From the table 1 the selected conditions of the analyte were with a water solvent added 1.5 mg/ml Na_2SO_3, a mobile phase of the mixture containing phosphate buffer 35mM pH 3.0 and methanol with a composition of 80:20, and a flow rate of 1.0 ml/min. In this condition, metamizole, thiamine, and pyridoxine were separated with good resolution values (> 1.5), it gave a good chromatogram shape, a tailing factor that meets the requirements (<2.0) and the number of theoretical plates that meet the requirements (N> 2000) (Table 2 & Figure 1).

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Table 2. Analytical chromatography parameters in selected conditions

Analyte	tR (minute)	Resolution (Rs)	Number of theoretical plates (N)	Tailing factor (TF)
Metamizole	5.238	14.90	4780	0.935
Thiamin	12.985	8.48	7625	1.235
Pyridoxine	5.981	2.18	4013	0.980

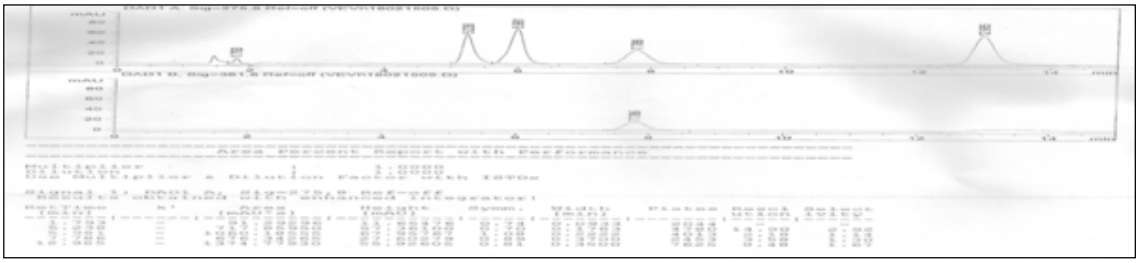

Figure 1. Analytically selected condition chromatograms at 100 ppm

System Conformity Test (SCT)

System Conformity Test (SCT) was performed by injecting 250 ppm metamizole. 50 ppm thiamin and 100 ppm pyridoxine standard solutions into the HPLC system with the selected analysis conditions five times (replication). The peak of the analyte must show repeatability (precision of tool) and good separability. The results of the SCT can be seen in Table 3.

The SCT results pointed out that the HPLC system used meets the tool's repeatability (precision) with RSD values ≤1.0%. resolution (>1.5). and tailing factors (≤2) as well as the number of theoretical plates that meet general requirements (> 2000).

Table 3. Results of SCT repeatability resolution, retention time, number of theoretical plates and tailing factors

Analyte	Replication	Resolution (Rs)	Retention time (tr)	Number of Theoretical Plates (N)	Tailing factor (TF)
Metamizole	1	20.61	5.177	6315	1.205
	2	20.7	5.183	6470	1.190
	3	20.63	5.19	6486	1.205
	4	20.64	5.203	6379	1.176
	5	19.05	5.212	5879	1.176
	RSD		0.312		
Thiamin	1	16.15	12.679	8345	1.229
	2	16.15	12.707	8214	1.231
	3	16.19	12.73	8244	1.219
	4	16.16	12.778	8141	1.254
	5	15.97	12.811	8349	1.223
	RSD		0.419		
Pyridoxine	1	2.15	5.778	6000	1.136
	2	2.18	5.787	6175	1.010
	3	2.18	5.794	6190	1.042
	4	2.18	5.809	6223	1.042
	5	2.07	5.82	5515	1.163
	RSD		0.291		

Specificity

A solvent chromatogram showed a peak at tR (minutes) = 1.359. Chromatograms of standard solutions showed the results of tR of metamizole at 5.177 minutes; thiamine at 12.679 minutes and pyridoxine at 5.778 minutes. The sample chromatogram showed the results of the metamizole tR at 5.226 minutes; thiamine at 12.871 minutes and pyridoxine at 5.876 minutes.

Based on the results of the samples chromatogram. it can be concluded that there was no disturbance of other peaks to the analytes peak. Disturbance peaks have tR = 1.357 (solvent). Peak Purity Index (Peak Purity Index) of metamizole. thiamine and pyridoxine were 0.999722; 0.999373; and 0.998997. respectively. The value of the Match Factor from metamizole. thiamine and pyridoxine were 0.9996495; 0.9995125 and 0.9882339. The value of Peak Purity and Match Factor met the requirements of approaching > 0.95. Here is a picture of Peak Purity from the analyte.

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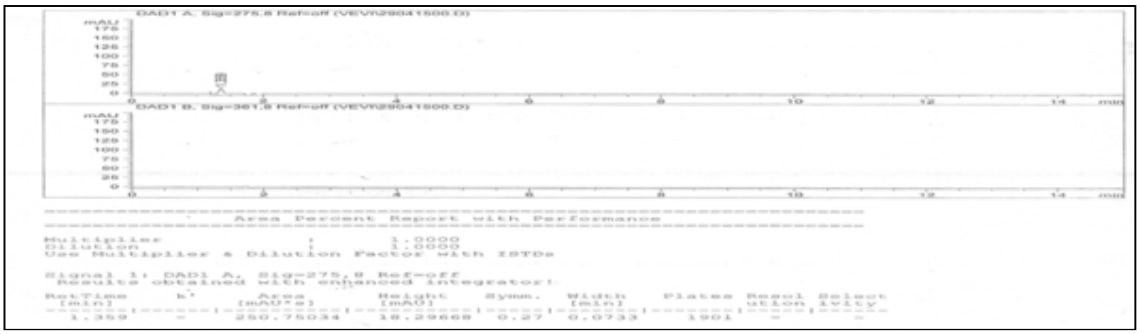

Figure 2. Solvent chromatogram

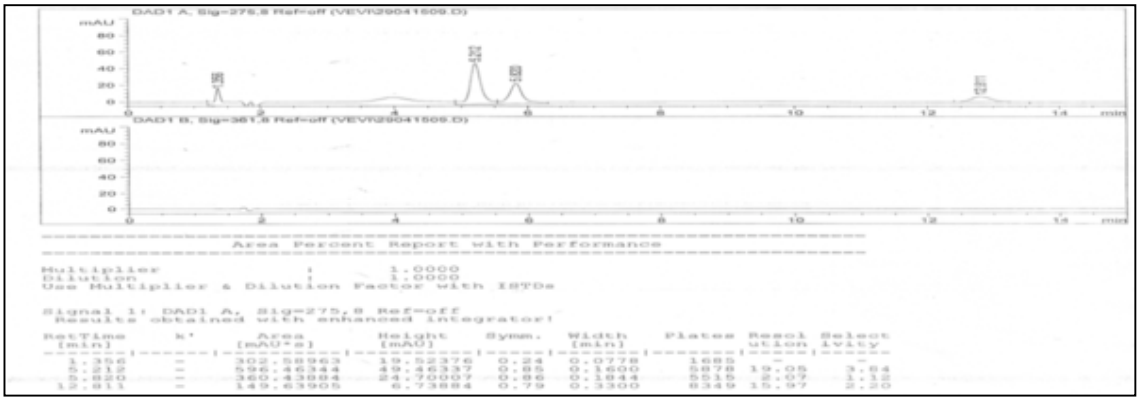

Figure 3. Standard chromatogram

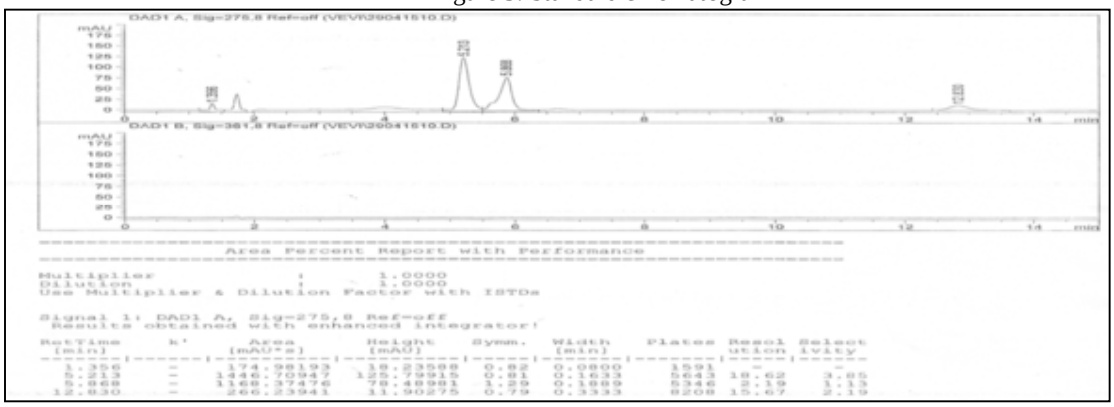

Figure 4. Samples chromatogram

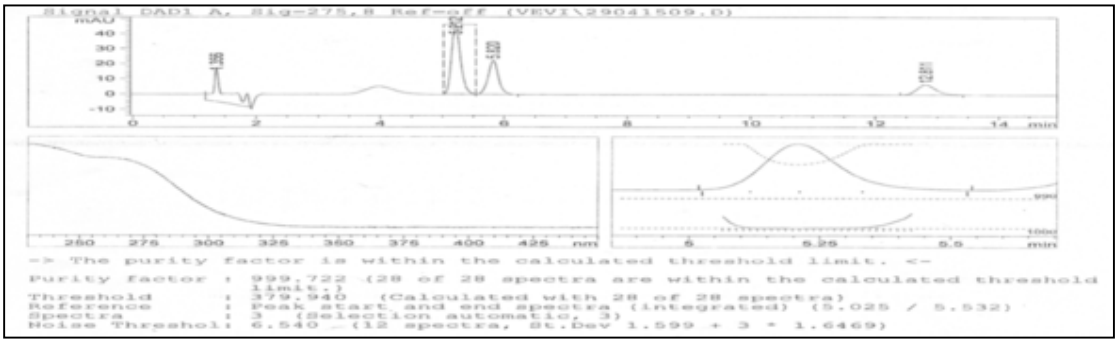

Figure 5. Peak purity of metamizole

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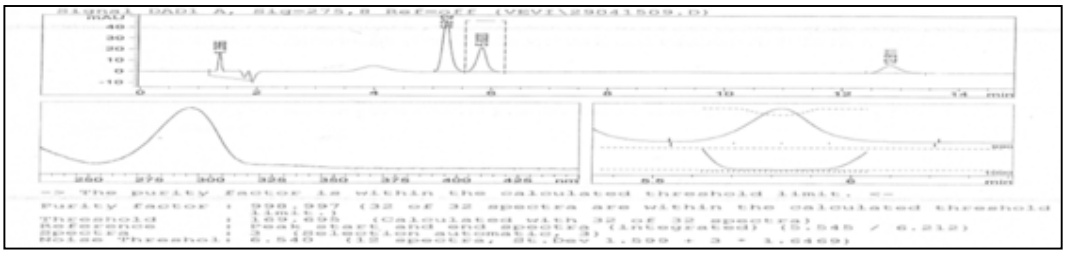

Figure 6. Peak purity of pyridoxine

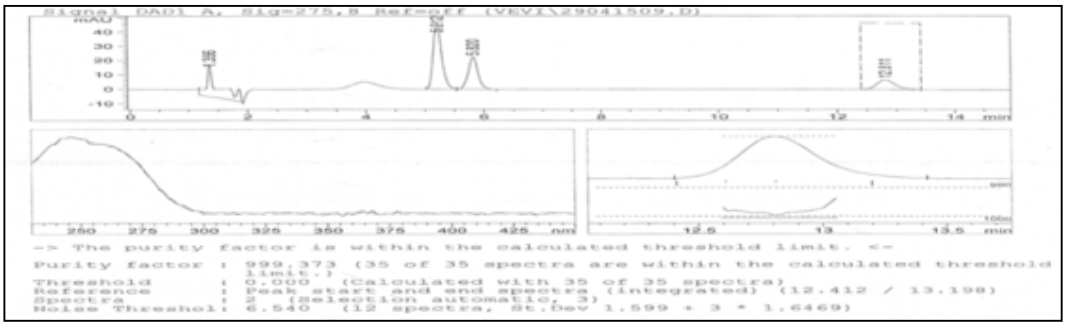

Figure 7. Peak purity of thiamin

Linearity

Linearity tests were carried out by injecting metamizole. vitamin B1 and vitamin B6 as standard solutions with 6 different concentrations. The following linearity test results:

Table 4. Standard metamizole linearity results

No	Concentration (ppm)	Area
1	156	1025.94373
2	208	1319.86865
3	364	2134.38403
4	416	2421.7417
5	468	2672.62476
6	520	2899.42285

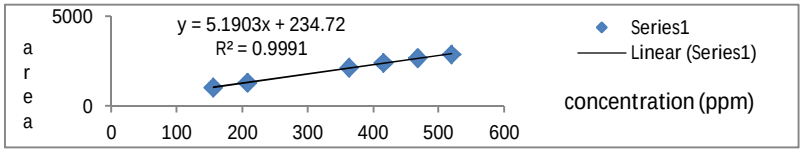

Figure 8. Metamizole linear curve

Table 5. Results of standard thiamin linearity

No	Concentration (ppm)	Area
1	30.3	342.78696
2	40.4	426.34836
3	50.5	551.2547
4	80.8	832.7323
5	90.9	942.69147
6	101	1021.10272

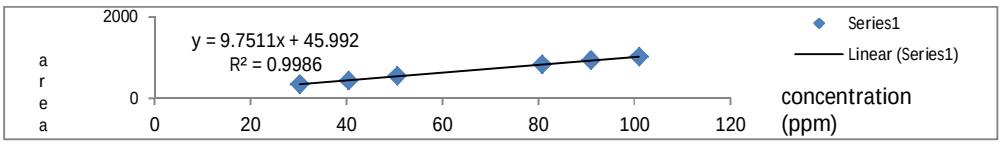

Figure 9. Thiamin linear curve

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Table 6. Results of standard pyridoxine linearity

No	Concentration (ppm)	Area
1	84	928.85522
2	94.5	1047.49854
3	105	1182.83447
4	115.5	1296.51416
5	126	1431.92761
6	147	1679.953

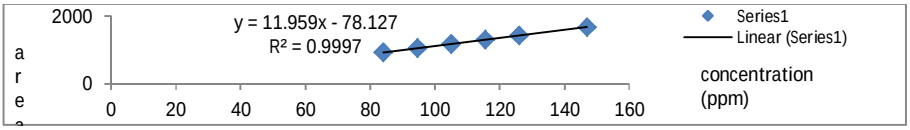

Figure 10. Pyridoxine linearity curve

Table 7. Results of standard linearity tests

Analyte	Range of concentration (ppm)	Regression formula	r	Vxo(%)
Metamizole	156-520	Y = 5.190x + 234.7	0.9995	0.72
Thiamin	30.3-101	Y = 9.751+ 45.99	0.9989	1.26
Piridoksin	84-147	Y = 11.95x – 78.12	0.9995	0.39

The results of the linearity calculation showed that the r value of metamizole and pyridoxine > 0.999 while the r value of thiamine was 0.9989 and the V_{X0}X5% value for all three analytes. It can be concluded that the three analytes provide a linear response between concentration and area.

Accuracy

Table 8. Accuracy test results at 80% analytic concentration

Analyte	Replication	Amount added (ppm)	Amount obtained (ppm)	% Recovery
Metamizole	1	206	209.99	101.94
	2	206	205.76	99.88
	3	206	202.71	98.40
Thiamin	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 9. Accuracy test results at 100% analytic concentration

Analyte	Replication	Amount added (ppm)	Amount 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
Thiamin	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 10. Accuracy test results at 120% analytic concentration

Analyte	Replication	Amount added (ppm)	Amount 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
Thiamin	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

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The accuracy of the test results showed that the three analytes namely metamizole. thiamin and pyridoxine were included in the accuracy range. The accuracy range for metamizole and pyridoxine was 98% -102%. while thiamine was 97% -103%. The accuracy test results were in accordance with AOAC standards.

Precision

The precision test was carried out by determining the levels of metamizole. thiamine. pyridoxine and cyanocobalamin in a placebo matrix solution that had been standardized at 100% level of analyte concentration and replicated 6 times. The precision test results are presented in Table 11:

Table 11. Test results for precision analysis methods

Replication	Concentration (%)		
	Metamizole	Thiamin	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%

The precision test showed that the RSD values for determining levels of metamizole. thiamin and pyridoxine were 2.0912%. 1.4489% and 0.8418%. respectively. The values on the precision test were in accordance with AOAC standards.

Concentration Determination

Determination of the levels of metamizole. thiamin and pyridoxine in tablet samples was carried out three times. The results of the determination of levels in the sample can be seen in Table 12.

Table 12. Results of determination of concentration in samples

Replication	Analyte	Concentration in Samples (ppm)	Regained levels (ppm)	% Recovery
1	Metamizole	250	254.87	101.95
	Thiamin	50	48.56	97.12
	Pyridoxine	100	98.34	98.34
2	Metamizole	250	253.4	101.36
	Thiamin	50	51.48	102.96
	Pyridoxine	100	100.87	100.87
3	Metamizole	250	242.74	97.10
	Thiamin	50	49.88	99.76
	Pyridoxine	100	101.16	101.16

DISCUSSION

The development of analytical methods starts from determining the selected wavelength by injecting a standard amount into the HPLC system with a PDA detector whose wavelength range is set between 200-400 nm. In this study wavelengths were selected based on journals and literature. Based on a journal written by Chotimah, *et al* in 2014 metamizole, thiamine and pyridoxine were analyzed at λ 275 nm. For this analysis we used two wavelengths, where metamizole, thiamin and pyridoxine were observed at λ 275 nm. [9]

The selection of solvents was performed by trying several types of solvents. The solvent used was water, 0.1 N HCl, buffer and buffer-methanol mixture. The choice of solvent was intended to inhibit the hydrolysis of metamizole. The chosen solvent was water and 0.1 N HCl + Na_2SO_3 selected for further optimization methods. The use of 0.1 N HCl + Na_2SO_3 as a metamizole solvent was not able to inhibit the hydrolysis of metamizole. This is because HCl actually accelerates the hydrolysis of metamizole, so the addition of Na_2SO_3 is not able to inhibit the hydrolysis of metamizole. The selected solvent used was water added with Na_2SO_3. The addition of Na_2SO_3 to water solvents was carried out with various concentrations ranging from 0.5mg/mL, 0.7mg/mL and 1.0mg/mL. At these three concentrations Na_2SO_3 has not been able to inhibit the hydrolysis of metamizole. Eventually, at a concentration of 1.5 mg/mL Na_2SO_3 can inhibit the hydrolysis of metamizole.

The column as the stationary phase in HPLC is a very important. The column will be one of the determinants of successful separation with K HPLC. The column used for the analysis of metamizole, thiamine, pyridoxine and cyanocobalamin was Phenomenex luna C8 (2) with a length of 150 mm in diameter 4.6 mm and particle size of 5.0 µm. Using column C8 takes 15 minutes to separate the four analytes. In the study of Chotimah et al,

the analysis of metamizole, thiamin and pyridoxine used column C18 with an analysis time longer than 15 minutes. The use of column C8 further shortens the separation time and was able to provide good selectivity.[9],[10],[11]

The mobile phase is important in the separation of analytes other than columns. The eluent composition and flow rate as the mobile phase to be used in the HPLC system were tested by injecting a certain amount of standard solution using a mobile phase with several compositions and flow rates. The choice of composition and flow rate in the mobile phase was also combined with suitable solvents to obtain good separation and prevent metamizole from being degraded during the analysis. The initial composition tested was a phosphate buffer mixture of 3.0-methanol (77:23) with a flow rate of 1.0 mL/min with 0.1 N HCl solvent, where cyanocobalamin was not detected. The second composition was phosphate buffer pH 3.0-methanol (80:20) + Na_2SO_3 0.5 mg/mL with a flow rate of 1.0 mL/min, which was the chosen condition for the mobile phase in this study, where the resolution value > 1,5 and metamizole had no hydrolysis. The third composition was phosphate buffer pH 3.0-methanol (82:18) plus Na_2SO_3 0.5 mg/mL with a flow rate of 1.0 ml/min, in this condition the resolution value of cyanocobalamin and thiamine did not meet (<1.5). The fourth composition that was tried was phosphate buffer pH 3.0-methanol (80:20) plus Na_2SO_3 0.5 mg/mL with a flow rate of 0.9 ml/min, in this condition the value of pyridoxine and cyanocobalamin resolution did not meet (<1.5). The addition of 0.5 mg/mL Na_2SO_3 to prevent hydrolysis of metamizole during the analysis took place as was done by Garcia *et al*, who used 0.5 mg/mL Na_2SO_3 in the eluent to prevent hydrolysis of metamizole but in this study required a concentration of Na_2SO_3 1.5 mg/mL in solvents and 0.5 mg/mL in the mobile phase which effectively inhibits the hydrolysis of metamizole. This is because the peak that appears is a single peak and the recovery obtained meets the requirements.

SCT on the HPLC system was carried out to ensure that the instrument used can provide data that meets the requirements. The resulting analytic peaks must show good repeatability and separability. The results obtained from the system suitability test carried out showed the peak area that produced repeatability and separability of each analyte fulfilled the requirements, namely RSD <2%; resolution> 1.5; TF ≤2; and N> 2000. In the SCT test the levels of metamizole were 250 ppm, thiamine 50 ppm and pyridoxine 100 ppm.

Method validation was conducted to ensure that the analysis method was accurate, specific, reproducible, and resistant to the range of analytes to be analyzed. A method must be validated when a new method is developed to overcome certain analytical problems. According to the United States Pharmacopeia (USP) the validation method for the main ingredients of the drug falls into category 1. In this category the validation parameters that must be met are specificity, linearity, range, accuracy and precision.

The validation of the analytical method was carried out after the optimum analysis conditions were obtained and the system suitability test conducted under these conditions meets the requirements. In this study the validation parameters performed were parameter 1 consisting of specificity, linearity, accuracy and precision. In addition to the four parameters, the robustness test was also conducted to determine the robustness of the analytical method developed.

Specificity test was performed by injecting a solvent, a standard solution of a mixture of metamizole, thiamine, pyridoxine and cyanocobalamin; and samples. In this specificity test only metamizole, thiamine and pyridoxine were detected, while cyanocobalamin was not detected. The results obtained by the peaks between analytes (metamizole, thiamin and pyridoxine) were completely separate. The peak purity of metamizole, thiamin and pyridoxine each 0.999761; 0,999342; and 0.999269, respectively. From the results obtained it can be seen that the peak of the analyte produced was free from other component intruders because its purity value was close to 1 of the requirement of purity value> 0.9500. The value of the match factors of metamizole, thiamine and pyridoxine were 0.9996495; 0.9995125; and 0.9882339. From the results obtained it can be seen that the peak of the analytes produced was almost the same as the standard.

Linearity tests were carried out by injecting the standard solution of metamizole, thiamin, and pyridoxine in five concentrations. The results obtained from this test r values for metamizole and pyridoxine> 0.999 while the r value for thiamine was 0.9989. It appears that the analytes provide a fairly linear response to the content and.

Accuracy test was performed by analyzing placebo samples added metamizole, thiamine and pyridoxine with three different concentration levels namely 80%, 100% and 120%. Each concentration was replicated 3 times. Accuracy test results are stated in% Recovery. The result of the recovery test was the accuracy of metamizole at a concentration of 80% between 98.40% -101.94%, at a concentration of 100% between 98.18% -100.88% and at a concentration of 120% between 98.33% -101.69%. In the sample levels of 250 mg of metamizole from the average weight of 1000 mg tablets, so the concentration of analytes to the sample was 25%. According to the AOAC analyte concentration ≥10% the value of% recovery was 98-102%, so the% recovery for metamizole meets the requirements.

The results of the recovery test of thiamine accuracy were at 80% concentration between 97.76% -101.69%, at 100% concentration between 98.08% -100.85% and at 120% concentration between 97.47% -102.40%. In the

sample thiamin levels were 50 mg from the average weight of the 1000 mg tablet, so the concentration of the analyte to the sample was 5%. According to the AOAC analyte concentration ≥1% the value of % recovery was 97-103%, so the % recovery for thiamine meets the requirements.

The results of recovery test of pyridoxine accuracy at 80% concentration between 100.25% -101.87%, at 100% concentration between 99.69% -100.22% and at 120% concentration between 98.05% -100.37%. In the sample pyridoxine content of 100 mg from the average weight of 1000 mg tablets, so that the concentration of the analyte to the sample was 10%. According to the AOAC analyte concentration ≥10% the value of % recovery was 98-102%, so the% recovery for pyridoxine meets the requirements. Cyanocobalamin which was standardized at concentrations of 80%, 100% or 120% cannot be detected. [12]

The precision test was carried out by determining the levels of metamizole, thiamine, pyridoxine and cyanocobalamin in a placebo matrix solution that had been standardized at 100% level of analyte concentration and replicated 6 times. The results of the precision test showed that the RSD values for the determination of levels of metamizole, thiamine and pyridoxine were 2.0912%, 1.4489%, and 0.8418% respectively, while cyanocobalamin was not detected in the presence of standardized placebo matrix solutions. The precision requirements using the AOAC reference are RSD≤2.7 for metamizole and pyridoxine while for thiamin RSD≤ 2.8. So it can be concluded that the analysis method meets the precision criteria.[12], [13]

The results of this study prove that HPLC with this method can be used to determine levels of metamizole, thiamine and pyridoxine. This method has good precision and faster analysis time compared to previous studies. In this method the hydrolysis of metamizole can be overcome by adding 1.5 mg/mL Na_2SO_3 to the solvent and 0.5 mg/mL in the mobile phase.

CONCLUSION

The conclusion were: 1) The concentration of Na_2SO_3 1.5 mg/mL in aqueous solvents and 0.5 mg/mL in the mobile phase of a 35mM phosphate buffer mixture with a pH of 3.0 methanol at a ratio of 80:20 can effectively inhibit hydrolysis of Metamizole, Thiamin and Pyridoxin mixtures; 2) Optimum HPLC conditions used include aqueous solvents added with 1.5 mg/mL Na_2SO_3, mobile phase composition of the 35mM phosphate buffer mix pH 3.0-methanol in a ratio of 80:20 plus 0.52 mg/mL Na_2SO_3. The flow rate used is 1.0 ml/min with an analysis wavelength (λ) of 275 nm for Metamizole, Thiamin, and Pyridoxine; 3) The selected HPLC conditions meet the validation requirements of the analysis method with validation parameters including specificity, linearity, accuracy and precision so that this method can be used for the analysis of the content of Metamizole, Thiamin, Pyridoxine in tablet preparations.

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