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Research article

Development and validation of high performance liquid chromatography methods (HPLC) analysis for simultaneous determination of Acetaminophen, Propyphenazone and Caffeine

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Abstract

Objective: The purpose of this research was to conduct an analysis with a high-performance liquid chromatography (HPLC) method for determining a simultaneously mixture of acetaminophen, propyphenazone and caffeine. **Methods:** This descriptive-experimental study was conducted by the HPLC method. This research was conducted by optimizing the ratio of the mobile phase of methanol: water (10:90), (20:80), (30:70), (40:60), (50:50), (60:40), (70: 30), (80:20), (90:10), flow rates 0.5-2.0 mL/min and column temperatures 30, 40, and 50°C. The analytical parameters in the validation test include accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ). **Results:** The results showed that the mobile phase of methanol: water (70:30) was a good optimization result. The average results of determining the levels of acetaminophen, propyphenazone and caffeine in the preparation of Bodrex Migraine® were 101.35 ± 0.16%, 99.24 ± 0.14%, 97.95 ± 0.16%, and Saridon® were 99.50 ± 0.07%, 100.25 ± 0.12%, 98.94 ± 0.06%. The results of the validation parameters performed on acetaminophen, propyphenazone and caffeine were 99.66%, 98.89% and 100.24% for the average recovery results, and 0.01738, 0.01751, 0.02557 for precision, and 1.00026, 0.69104, 0.38920 for the average LOD results and 3.33423, 2.30347, 1.29735 for LOQ average results. **Conclusion:** The mobile phase of methanol: water (70:30) and the flow rate of 1 mL/min with a column temperature of 30°C was a good optimization result for use in the simultaneous analysis of a mixture of acetaminophen, propyphenazone and caffeine.

Introduction

The combination of drugs in a preparation provides increased potential and onset of action of the drug so that it can relieve pain faster and with fewer side effects [1]. The combination of drugs in multicomponent preparations needs to be balanced with increased quality control, so that the drugs in body circulation can be guaranteed its safety and efficacy. According to Indonesian Health Law no. 36 of 2009 in article 105 paragraph 1, it is stated that "pharmaceutical preparations in the form of drugs and drug raw materials must meet the requirements of the Indonesian Pharmacopoeia V Edition or other standard books". There are many methods carried out still require extraction processes for further separation because they still contain several similar compounds that cannot be separated [2]. One of the methods used to determine levels of a combination of drugs such as acetaminophen, propyphenazone and caffeine was UV-Visible spectrophotometry method, but it need more efficient methods in determining levels of drug

combinations such as high performance liquid chromatography (HPLC).

The use of HPLC method for quality control purposes of preparations containing acetaminophen, propyphenazone and caffeine due to fast analysis time, good separation, sensitivity, selection of columns and eluents varies greatly, columns can be reused, can be used to analyze large and small molecules, easy samples recovered, the detector does not damage the components of the substance being analyzed, it can be used to count samples with very low levels [3].

The condition of the HPLC instrument used in determining the levels of the mixture of acetaminophen, propyphenazone and caffeine must be in the optimum state. The purpose of this optimization is to reduce analysis time, minimize the amount of solvents, and maintain the life of the equipment and columns.

Methods

This descriptive-experimental study was conducted using HPCL with column Zorbax ODS (4.5x150mm). This research was conducted by optimizing the ratio of the mobile phase of methanol: water (10:90), (20:80), (30:70), (40:60), (50:50), (60:40), (70: 30), (80:20), (90:10), flow rate 0.5, 0.75, 1.0, 1.5, 2.0 mL/min and column temperatures 30, 40, and 50°C. The results obtained from optimization are used in the determination of tablet dosage levels and validation test. Analysis parameters in the validation test include accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) [4].

Results and discussion

Optimization of the ratio of the mobile phase

The development of High Performance Liquid Chromatography Method which is carried out is the optimization of the comparison of the mobile phase, flow rate and column temperature. Optimization of the ratio of the mobile phase (methanol: water) is the initial stage carried out in this study. In this study the comparison of mobile phase (methanol: water) which is optimized is (10:90), (20:80), (30:70), (40:50), (50:50), (60:40), (70:30), (80:20), (90:10). Optimal phase ratio results are used for optimization of the flow rate ratio (0.5), (0.75), (1.0), (1.5), (2.0) mL/minute. Optimal phase and flow rate comparisons are used for column temperature optimization of 30°C, 40°C, 50°C.

The selection of optimization results performed in this study is based on short retention time, theoretical plate, tailings factor ≤ 2 and resolution ≥ 1.5 [5]. Flow rate optimization data can be seen in table 1 and column temperature optimization data can be seen in table 2, and the chromatogram in seen in figure 1. The optimization results obtained are used for identification, determination of calibration curves, determination of levels and validation.

The results showed that the comparison of the mobile phase of methanol: water (70:30) and the flow rate of 1 mL/min with a column temperature of 30°C is a good optimization result for use in this study, because of the short retention time of acetaminophen 3.093 minutes, propyphenazone 5.845 minutes, caffeine 3.808 minutes, where as the value meets the requirements of the Tailing factor ≤ 2 and Resolution ≥ 1.5 [5, 6].

Identification of comparative standards of acetaminophen, propyphenazone and caffeine and samples carried out aims as a qualitative analysis. The results of the identification of comparative standards and samples can be seen in figure 2.

The results of identification carried out on the standard of acetaminophen, propyphenazone, caffeine and samples gave almost the same retention time, namely acetaminophen (2.984), propyphenazone (3.566) and caffeine (5.430). The data obtained above shows that the results obtained are true the test sample contains acetaminophen, propyphenazone and caffeine.

Table 1. Flow rate optimization data (0.5), (0.75), (1.0), (1.5) mL/min with mobile phase ratio (methanol: water) 70:30 and column temperature 30°C.

Flow rate (mL/min)	Retention time	Theoretical plate	Tailing factor	Resolution
0.5	5.769	603	-	-
	6.137	2750	-	0.20
	10.401	14336	1.751	4.050
0.75	3.879	5120	-	-
	4.203	998	-	0.335
	6.809	14078	2.082	2.650
1.0	3.093	18991	2.085	-
	3.808	5337	-	1.857
	5.845	31356	2.024	4.634
1.5	2.003	5009	-	-
	2.306	6443	-	1.030
	3.463	15109	2.924	3.938

Table 2. Column temperature optimization data 30°C, 40°C, 50°C with phase comparison (methanol: water) 70:30 and a flow rate of 1.0 mL/min.

Temperature (°C)	Retention time	Theoretical plate	Tailing factor	Resolution
30	3.093	18991	2.085	-
	3.808	5337	-	1.857
	5.845	31356	2.024	4.634
40	2.923	15509	-	-
	3.175	276	-	0.228
	5.050	21981	2.092	1.614
50	2.858	14267	-	-
	3.073	473	-	0.252
	4.850	18832	2.017	1.948

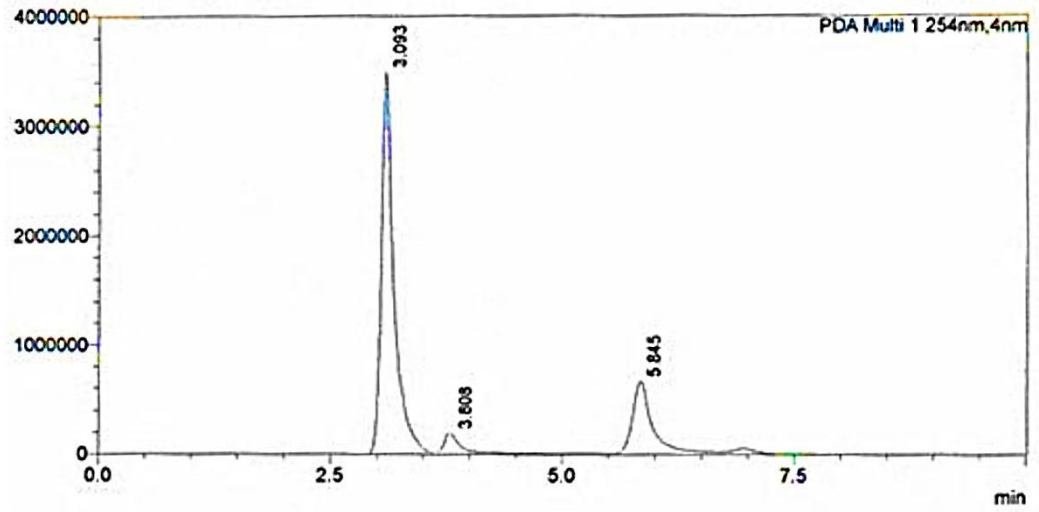

Figure 1. Chromatogram optimization of mobile phase comparison (methanol: water) 70:30, flow rate 1 mL/minute and column temperature 30°C.

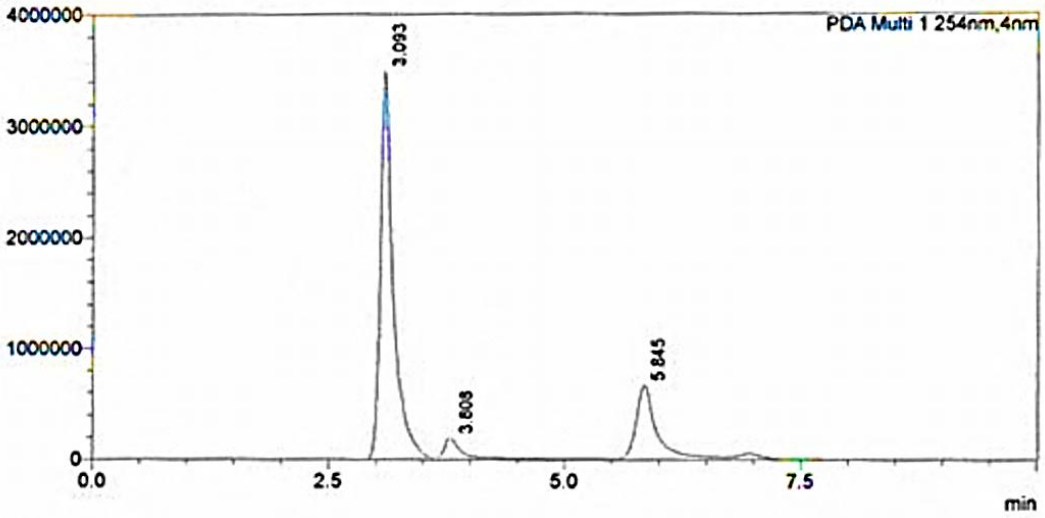

Figure 2(A). Comparative standard chromatogram containing acetaminophen propyphenazone and caffeine.

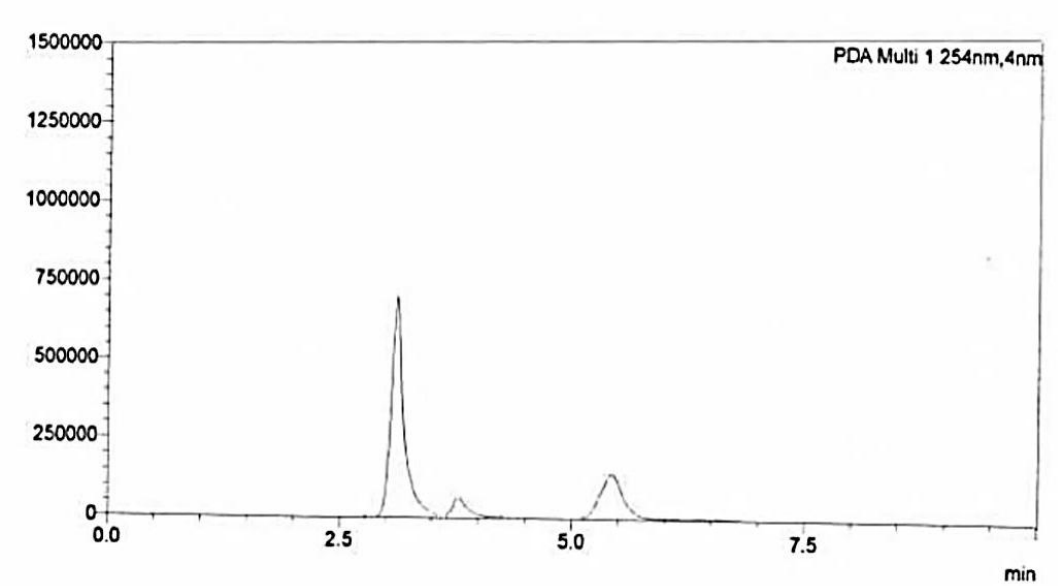

Figure 2(B). Sample chromatograms containing acetaminophen, propyphenazone and caffeine.

Preparation of calibration curve

Preparation of calibration curve is done by making a standard main solution comparing each acetaminophen, propyphenazone and caffeine with various concentrations. Calibration curves can be seen in figure 3, figure 4 and figure 5.

The results of the calculation and determination of the calibration curve linearity obtained by each regression line equation and the correlation coefficient for acetaminophen Y = 84902.95194X + 32179.955 with the correlation coefficient (r = 0.99996), propyphenazone Y = 46474.55631X + 6824.3351 with correlation coefficient (r = 0.99997) and caffeine Y = 30564.12183X-118.1324 with a correlation coefficient (0.99993).

The correlation coefficient (r) is used to determine the existence of a linear relationship between concentration and area. A perfect relationship if the price (r) approaches one. The correlation coefficient (r) obtained is still within the limits of acceptance of the correlation coefficient value that is r = 0.995[7].

Determination of mixed concentration of acetaminophen, propyphenazone and caffeine in samples

In the trade of tablet preparations that contain acetaminophen, propyphenazone and caffeine are found with trade names that contain various compositions. The results of determining the concentration levels of a mixture of acetaminophen, propyphenazone and caffeine in tablet preparations can be seen in table 4 and table 5.

Table 4 and 5 shows that the levels of a mixture of acetaminophen, propyphenazone and caffeine in tablet preparations obtained by several pharmaceutical industries meet the requirements for the general levels of tablet preparations that contain acetaminophen, Propyphenazone and caffeine not less than 90.0% and not more than 110.0% of the amount listed on the label [2].

Validation test of high performance liquid chromatography methods

The validation of an HPLC method is the procedure that shows information to determine whether the operating system is intended to use, precise, reliable and accurate.In this study, a validation test was done using a standard addition method to the Saridon® (Roche) tablet sample which included an accuracy test with a percent recovery parameter with a requirement of 98-102%, a precision test with an RSD parameter (Relative Standard Deviation) with requirements ≤ 2%, linearity with correlation coefficient parameters with requirements r = 0.995, detection limit (LOD) and quantitation limit (LOQ) [8]. The results can be seen in table 6, table 7, and table 8.

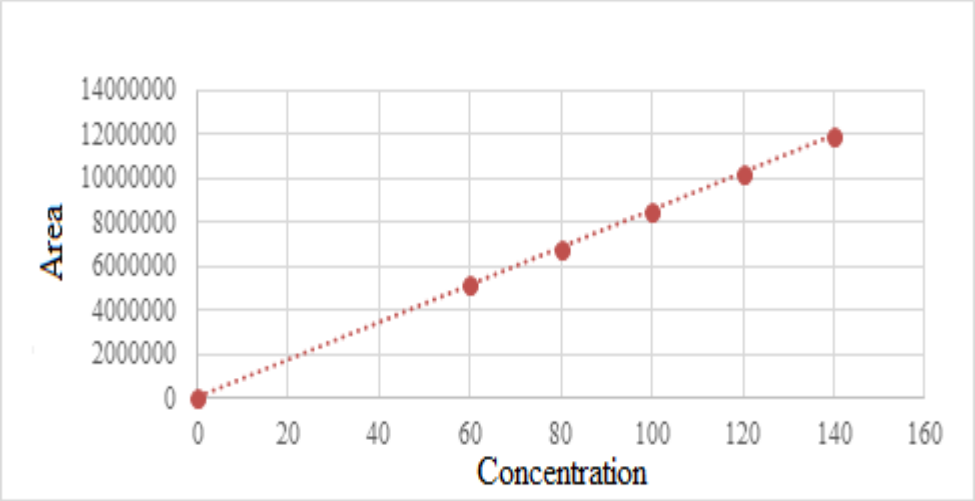

Figure 3. Calibration curve of acetaminophen.

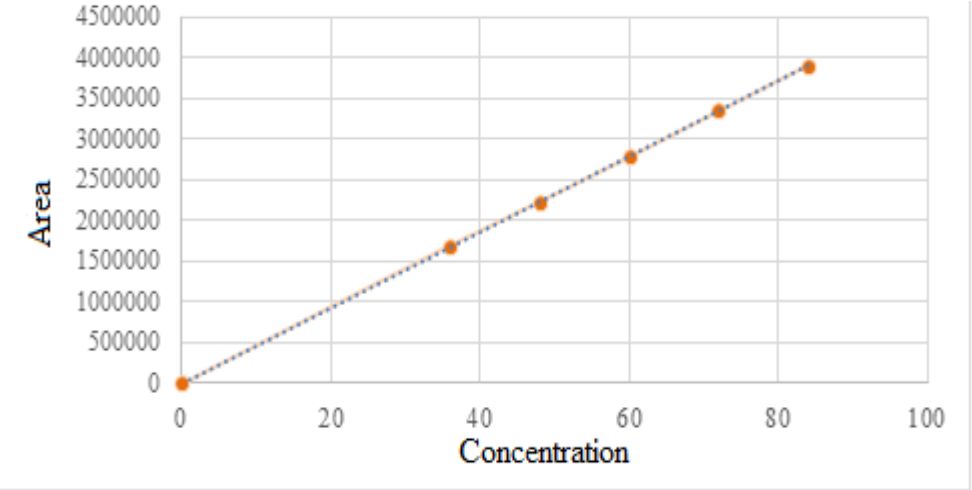

Figure 4. Calibration curve of Propyphenazone.

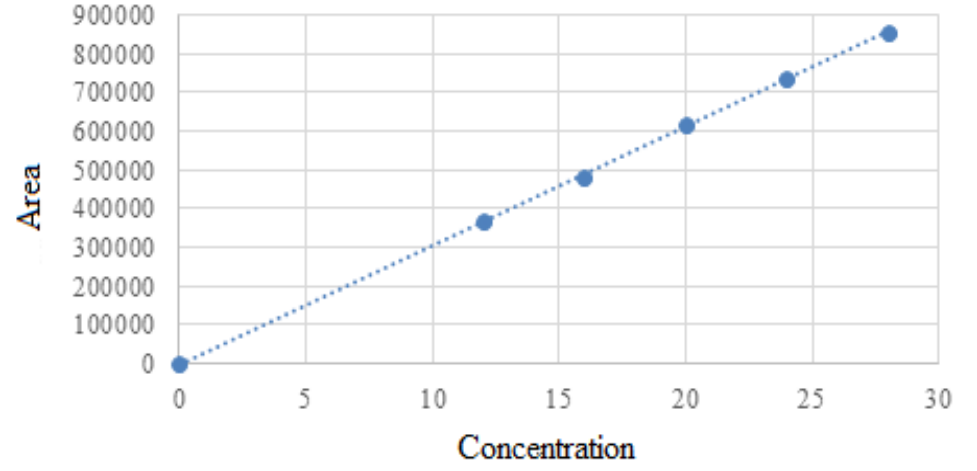

Figure 5. Calibration curve of caffeine.

Table 4. Results of the determination of levels of acetaminophen, propyphenazone and caffeine in Bodrex Migraine® tablets (Pacific Tempo Scan).

Drugs name	Bodrex Migraine®		
	Area	Concentration (%)	Concentration Range (%)
Acetaminophen	12113530	101.23	101.35 ± 0.16
	12138492	101.44	
	12131360	101.38	
	12123039	101.31	
	12118285	101.27	
	12142058	101.47	
Propyphenazone	2782750	99.25	99.24 ± 0.14
	2779403	99.13	
	2784702	99.32	
	2785538	99.35	
	2780519	99.17	
	2781913	99.22	
Caffeine	604807	97.97	97.95 ± 0.16
	604257	97.88	
	604440	97.91	
	603951	97.83	
	605235	98.04	
	605479	98.08	

Table 5. Results of the determination of levels of acetaminophen, propyphenazonee and caffeine in Saridon® tablet (Roche).

Drugs name	Saridon®		
	Area	Concentration (%)	Concentration Range (%)
Acetaminophen	8519079	99.56	99.50 ±0.07
	8512287	99.48	
	8515683	99.52	
	8508890	99.44	
	8514834	99.51	
	8510588	99.46	
Propyphenazone	2813144	100.34	100.25 ±0.12
	2807846	100.15	
	2811192	100.27	
	2812307	100.31	
	2808961	100.19	
	2809798	100.22	
Caffeine	611103	98.99	98.94 ±0.06
	610492	98.89	
	610798	98.94	
	610553	98.90	
	610920	98.96	
	610981	98.97	

Table 6. Validation of high performance liquid chromatography methods at determination acetaminophen levels by standard addition methods (standard addition method).

Specific Range	Concentration (µg/mL)		(% recovery)
	Before	After	
80 %	55.8096	79.7280	99.66
	55.7984	79.7120	99.64
	55.8208	74.7440	99.68
100 %	69.7620	99.6600	99.66
	69.7480	99.6400	99.64
	69.7760	99.6800	99.68
120 %	83.7144	119.5920	99.66
	83.6976	119.5680	99.64
	83.7312	119.6160	99.68
Mean (% recovery)			99.66
Standard Deviation(SD)			0.01732
Relative Standard Deviation (RSD)			0.01738
Linearity (r)			0.99996
LOD			1.00026
LOQ			3.33423

Table 7. Validation of high performance liquid chromatography methods at determinationpropyphenazone levels by standard addition method).

Specific Range	Concentration (µg/mL)		(% recovery)
	Before	After	
80 %	33.2338	47.4763	98.91
	33.2270	47.4672	98.89
	33.2203	47.4576	98.87
100 %	41.5422	59.3460	98.91
	41.5338	59.3340	98.89
	41.5254	59.3220	98.87
120 %	49.8506	71.2152	98.91
	49.8406	71.2008	98.89
	49.8305	71.1864	98.87
Mean (% recovery)			98.89
Standard Deviation (SD)			0.01732
Relative Standard Deviation (RSD)			0.01751
Linearity (r)			0.99997
LOD			0.69104
LOQ			2.30347

Table 8. Validation of high performance liquid chromatography methods at determination caffeine levels by the standard addition method.

Specific Range	Concentration (µg/mL)		(% recovery)
	Before	After	
80 %	11.2606	16.0899	100.61
	11.2640	16.0947	100.64
	11.2673	16.0995	100.67
100 %	14.0777	20.1143	100.61
	14.0819	20.1203	100.64
	14.0861	20.1263	100.67
120 %	16.8947	24.1387	100.61
	16.8998	24.1457	100.64
	16.9048	24.1531	100.67
Mean (% recovery)			100.64
Standard Deviation(SD)			0.02574
Relative Standard Deviation (RSD)			0.02557
Linearity (r)			0.99993
LOD			0.38920
LOQ			1.29735

From the above data, the percent recovery results meet the accuracy test requirements where the average range of recovery results is 98-102% [9]. With Acetaminophen Standard Deviation (SD) of 0.01732, propyphenazone of 0.01732 and caffeine in the amount of 0.02574. The precision test results with the Relative Standard Deviation (RSD) parameters are acetaminophen of 0.01738, propyphenazone of 0.01751 and caffeine of 0.02557 where the Relative Standard Deviation (RSD) results meet the precision requirements, where the RSD value is ≤ 2%. While the results of the detection limit (LOD) and the limit of quantitation (LOQ) obtained acetaminophen 1.00026 and 3.33423, propyphenazone 0.69104 and 2.0347, caffeine 0.38920 and 1.29735 [9, 10]

Conclusion

From the results of the study it was concluded that the determination of levels of a mixture of acetaminophen, propyphenazone and caffeine in tablet preparations with high performance liquid chromatography methods provided good accuracy and precision.

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