Journal of Applied Pharmaceutical Science Vol. 7 (10), pp. 125-130, October, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.71018
ISSN 2231-3354

Validation Analysis Methods of α-Mangostin, ϒ-Mangostin and Gartanin Mixture in Mangosteen (*Garcinia mangostana* L.) Fruit Rind Extract from West Java with HPLC

Muchtaridi Muchtaridi[1*], Nadia Ananda Puteri[1], Tiana Milanda[2], Ida Musfiroh[1]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy Universitas Padjadjaran, Sumedang, Indonesia.
[2]Department of Pharmacology and Biology Pharmacy, Faculty of Pharmacy Universitas Padjadjaran, Sumedang, Indonesia.

ARTICLE INFO

Article history:
Received on: 25/05/2017
Accepted on: 09/08/2017
Available online: 30/10/2017

Key words:
α-mangostin, γ-mangostin, gartanin, HPLC, Solid Phase Extraction (SPE).

ABSTRACT

The quantitative analysis methods for bioactive compounds in mangosteen pericarp have been reported. The purpose of this study is to validate the methods of analysis for α-mangostin, γ-mangostin, and gartanin in mangosteen rind extract derived from Bogor, Purwakarta, Subang and Tasikmalaya using High Performance Liquid Chromatography (HPLC) for routine analysis. The methods employed were Enduro C-18 reverse-phase (250 mm x 4.6 mm) column chromatography systems with Photo Diode Array detector 375 nm and acetonitrile: water containing 0.1% phosphoric acid (95:5) as the mobile phase at the flow rate of 1.0 mL/min and validation methods with parameters of linearity, limit of detection and quantification, precision, and accuracy. Mangosteen rind extracts were pre-treated with the technique of solid-phase extraction (SPE). The results of this study show that the validation results meet the requirements of the standard retention time of α-mangostin at 5.801 minutes, γ-mangostin at 4.707 minutes and gartanin at 5.290 minutes. The correlation coefficient (R) for each standard were 0.999, 0.999, and 0.999, respectively. The value of recovery for the α-mangostin, γ-mangostin, and gartanin were 100.32%, 102.31%, and 101.48%, respectively. The analysis shows that the levels of α-mangostin, γ-mangostin, and gartanin from Bogor are 13.87%, 8.28% and 10.44%, respectively. The results from mangosteen pericarp extract from Purwakarta are 10.07% for α-mangostin, 6.33% for γ-mangostin, and 8.76% gartanin. Mangosteen pericarp extract from Subang has concentrations of α-mangostin at 10.88%, γ-mangostin at 6.01%, and gartanin at 8.08%. The contents of α-mangostin, γ-mangostin, and gartanin from Tasikmalaya are 8.53%, 6.07%, 17.28% respectively. This study concludes that the methods are valid and can be used for routine analysis.

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) as a tropical queen of fruits has become one of the Indonesian important export commodities because of its sweet-sour and pleasant taste. Fruit rinds which become waste, are usually used as traditional medicine in Thailand to alleviate diarrhoea or treatment for skin infection and wounds (Pothitirat *et al.*, 2009). Many compounds have been reported to be isolated from *G. mangostana* rind extract, such as: α-mangostin, gartanin, γ-mangostin, 3-isomangostin (Walker, 2007; Mahabusarakam *et al.*, 1987). These isolated xanthones provide diverse pharmacological uses such as antimicrobial, antimalarial, antioxidant, and anti-inflammatory functions (Iinuma *et al.*, 1996; Mahabusarakam *et al.*, 2006; Mahabusarakam *et al.*, 2000; Chen *et al.*, 2008). In the current study, xanthones of mangosteen have inhibitory effects against neuraminidase of *C. perferingens* (Ryu *et al.*, 2010). The number of studies to determine valid analytical methods to identify xanthone derivatives are still lower than those in isolating xanthone compounds (Walker, 2007).

* *Corresponding Author*
Muchtaridi Muchtaridi, Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy UniversitasPadjadjaran, Jl. Bandung-Sumedang KM-21, 45363, Jatinangor, Indonesia.
E-mail: muchtaridi @ unpad.ac.id

Some studies in analytical methods for the determination of xanthones in extracts of mangosteen pericarp have been performed as reported in some literatures (Yodhnu *et al*., 2009; Walker, 2007; Li *et al*., 2013). The development in analytical methods ensure that the methods used are valid and replicable. Validation methods of simultaneous determination of compound contained in *Garcinia mangostana* rind extract also facilitate methods to specify more than one compound in mixtures samples.

MATERIALS AND METHODS

Chemicals

The standard xanthones (α-mangostin, gartanin and γ-mangostin) used as references were purchased from Chengdu *Biopurified*, China. The solvents are HPLC grade, acquired from JT Baker®.

Plant material and extraction

Mangosteen rinds obtained from Bogor, Purwakarta, Tasikmalaya, and Subang (West Java, Indonesia) were identified by Drs. Joko Kusmoro, MS., a scientist in Department of Biology, the Faculty of Mathematics and Natural Sciences. Samples were cleaned, cut, air-dried, and finally powdered. Fruit rinds were extracted by maceration using 900 mL of 70 % ethanol for 72 hours. The extract was dried in an oven at 40 °C.

UV Spectral Analysis

Standard mixture consisting of α-mangostin, γ-mangostin and gartanin are diluted into three concentrations. Concentrations used for α-mangostin are 2.5, 5 and 10 ppm; for γ-mangostin are 20, 40, and 80 ppm; and for gartanin are 10, 20, and 40 ppm. Each standard was analyzed with UV Spectroscopy. UV spectra collected from UV spectrophotometer (*Analytikjena specord* 200®) and performed in range 200-400 nm resulting 375 nm for chromatograms.

Preparation of standard mixture

Stock solution of the standard mixture consisting of α-mangostin, γ-mangostin and gartanin was prepared at 2 mg/mL in HPLC grade methanol. The solution was further diluted to obtain 200, 100, 25, 10, and 5 µg/mL. The sample extracts were prepared at 500 µg/mL in the same solvent, and were further diluted to obtain 100 µg/mL. The stock solutions were filtered through 0.45 µm syringe filters.

Preparation of Samples

Pre-treatments were conducted to extract samples with Solid Phase Extraction (SPE) to obtain ± 92.50% recovery. Samples are diluted into methanol to 100 µg/mL, followed by SPE processes with C-18 cartridges 47 mm Supor®-450 membrane purchased from Pall Corp (Michigan, US). SPE processes include conditioning them with 1 mL of methanol, sample loading (diluting extract to 100 ppm, 1 mL), washing them with 1 mL of double-distilled water, and eluting them with methanol.

RESULTS AND DISCUSSIONS

Validation Methods

System suitability such as column efficiency, resolution, plate number, and tailing factor are applied to ensure that the HPLC system is capable of providing adequate data for analysis (Ermer, 2001; Ermer and Ploss, 2005). Plate number, resolution, tailing factor, and column efficiency are parameters in this study set to carry out analysis as shown in Table 1. According to Table 1, the suitability of system has met the requirements. We further analyzed the linearity of methods using five concentration variation: 5; 10; 20; 40; and 80 µg/mL. Each variation is injected into HPLC with three replications thus producing coefficient correlation value for α-mangostin, γ-mangostin, and gartanin of 0.999, 0.999, and 0.999, respectively. All of the values which exceed the value of coefficient correlation of 0.99 was valid (ICH, 1996) (as shown in Figure 1).

Table 1: Results of parameters of system suitability experiments.

	Retention Time	N (Plate Number)	HETP (L/N)	*Tailing factor*	k'	Rs
α-mangostin	5.805	15,385.92	0.016	0.032	0.871	10.74
γ-mangostin	4.707	15,385.92	0.016	0.031	0.517	6.40
Gartanin	5.290	15,385.92	0.016	0.011	0.705	13.467

* L = HPLC Column Length (25 cm)

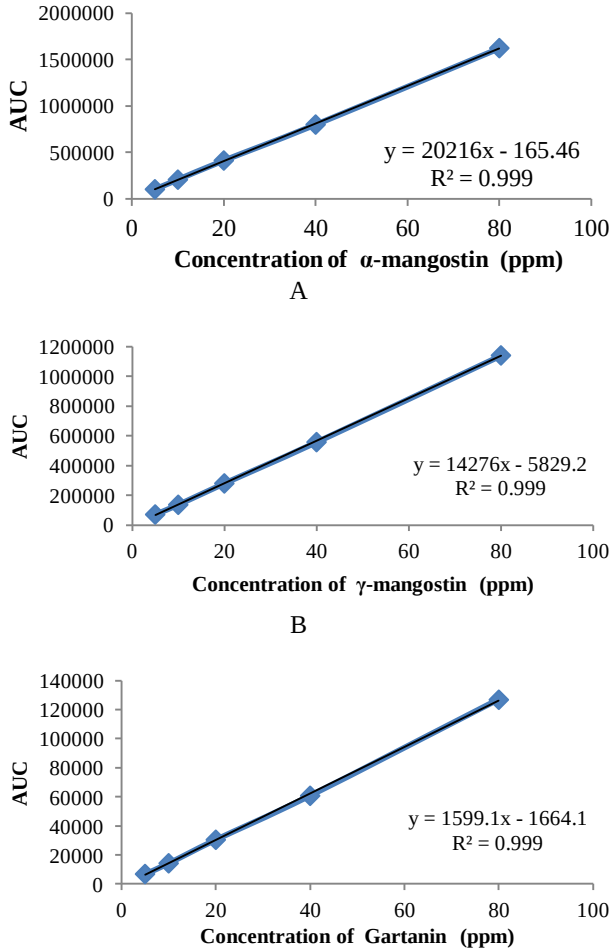


Fig. 1: Calibration curve of γ-mangostin (a), α-mangostin (b), and gartanin (c).

Table 2: Accuracy of α-mangostin, γ-mangostin, Gartanin.

A- Accuracy of γ-mangostin.

Concentration(ppm)	AUC	Measured Concentration (ppm)	% Recovery	% Average of Recovery
5	100,226	4.965	99.318	
5	97,963	4.979	99.583	100.63
5	105,406	5.149	102.989	
10	203,854	10.091	100.919	
10	209,237	10.221	102.210	101.54
10	210,801	10.148	101.483	
20	410,185	20.298	101.491	
20	414,588	19.894	99.473	100.17
20	416,538	19.906	99.531	
40	797,421	39.453	98.633	
40	837,319	39.808	99.521	99.05
40	831,765	39.600	99.000	
80	1,620,895	80.187	100.233	
80	1,692,494	80.093	100.117	100.20
80	1,687,662	80.194	100.243	

B- Accuracy of γ-mangostin.

Concentration (ppm)	AUC	Measured conc. (ppm)	% Recovery	% Average of Recovery
5	68,4481	5.202	104.058	
5	63,025	4.823	96.461	99.88
5	70,211	4.955	99.108	
10	136,019	9.936	99.361	
10	141,635	10.329	103.295	101.28
10	147,712	10.118	101.180	
20	279,612	19.994	99.972	
20	284,346	20.326	101.630	99.77
20	289,146	19.539	97.697	
40	557,220	39.440	98.600	
40	593,747	41.998	104.997	100.56
40	584,860	39.237	98.094	
80	1,140,105	80.269	100.337	
80	1,215,064	85.520	106.900	102.60
80	1,203,380	80.439	100.549	

C- Accuracy of Gartanin.

Concentration (ppm)	Area (AUC)	Measured conc. (ppm)	% Recovery	% Average of Recovery
5	6,329	4.998	99.997	
5	6,543	5.132	101.603	102.52
5	6,812	5.301	106.010	
10	14,334	10.004	100.044	
10	16,071	10.564	105.649	100.97
10	15,848	9.720	97.204	
20	30,535	20.135	100.678	
20	30,891	18.902	94.510	97.82
20	33,515	19.652	98.262	
40	60,802	39.063	97.658	
40	67,627	39.568	98.920	99.04
40	70,086	40.211	100.529	
80	126,917	80.408	100.510	
80	140,176	80.381	100.476	100.32
80	140,838	79.986	99.983	

Accuracy of α-mangostin, γ-mangostin, and gartanin were studied at concentration of 5, 10, 20, 40 and 80 μg/mL of each individual reference compound. The accuracy ranges between 90 and110 % with results presented in average ± SD (n = 3). Accuracy results show a range between 99.05 and 101.54% for α-mangostin, 99.77and 99.98 % for γ-mangostin, and 97.82 and 99.98% for gartanin which comply with the validation qualification in the range from 80 to 110 % for accuracy in unit concentration ranging from 1 to 10 ppm measured, and 90-107 % for accuracy in unit concentration from 10 to 100 ppm measured (AOAC, 2002). The percentages of variation coefficient were measured for the precision analysis which considered retention time and peak area of the curves. The standard mixture was analyzed at a concentration of 20 μg/mL (n=6). Precision results indicated good reproducibility. The results of precision in method validation for α-mangostin, γ-mangostin, and gartanin based on AUC stated as RSD (%) were 1.591%, 1.663%, and 1.912%, respectively. Based on retention time, the results of variation coefficient were 0.596%, 0.439%, and 0.393% for α-mangostin, γ-mangostin, and gartanin consecutively. The precision was determined based on the Limit of Detection (LOD) and the Limit of Quantification (LOQ).

Table 3: Precision method validation of α-mangostin (A), γ-mangostin (B), Gartanin (C).

A- α-mangostin.

Concentration (ppm)	AUC	Retention Time (minutes)
20	148,427	5.767
20	145,992	5.838
20	143,025	5.817
20	145,276	5.829
20	149,587	5.752
20	146,138	5.806
Total	878,445	34.809
Average	146,407.5	5.801
SD	2,330.428	0.034
%RSD	1.592	0.597

B- γ-mangostin.

Concentration (ppm)	AUC	Retention Time (minutes)
20	148,427	4.687
20	145,992	4.731
20	143,025	4.715
20	145,276	4.723
20	149,587	4.678
20	146,138	4.708
Total	1,302,569	28.242
Average	217,094.8	4.707
SD	3611.359	0.020
%RSD	1.663	0.439

C- Gartanin.

Concentration (ppm)	AUC	Retention Time (minutes)
20	16,584	5.272
20	16,926	5.315
20	16,100	5.302
20	16,286	5.303
20	16,187	5.26
20	16,628	5.292
Total	98,711	31.744
Average	16,451.83	5.290
SD	314.566	0.020
%RSD	1.912	0.393

LOD and LOQ are calculated to analyze the amount of α-mangostin, γ-mangostin and gartanin in mangosteen rind extract statistically calculated based on the equation of the calibration curve to the area under the peak area ratio. In method validation the values of LOD and LOQ are important in terms of characteristics. The values of α-mangostin were 0.161 ppm and

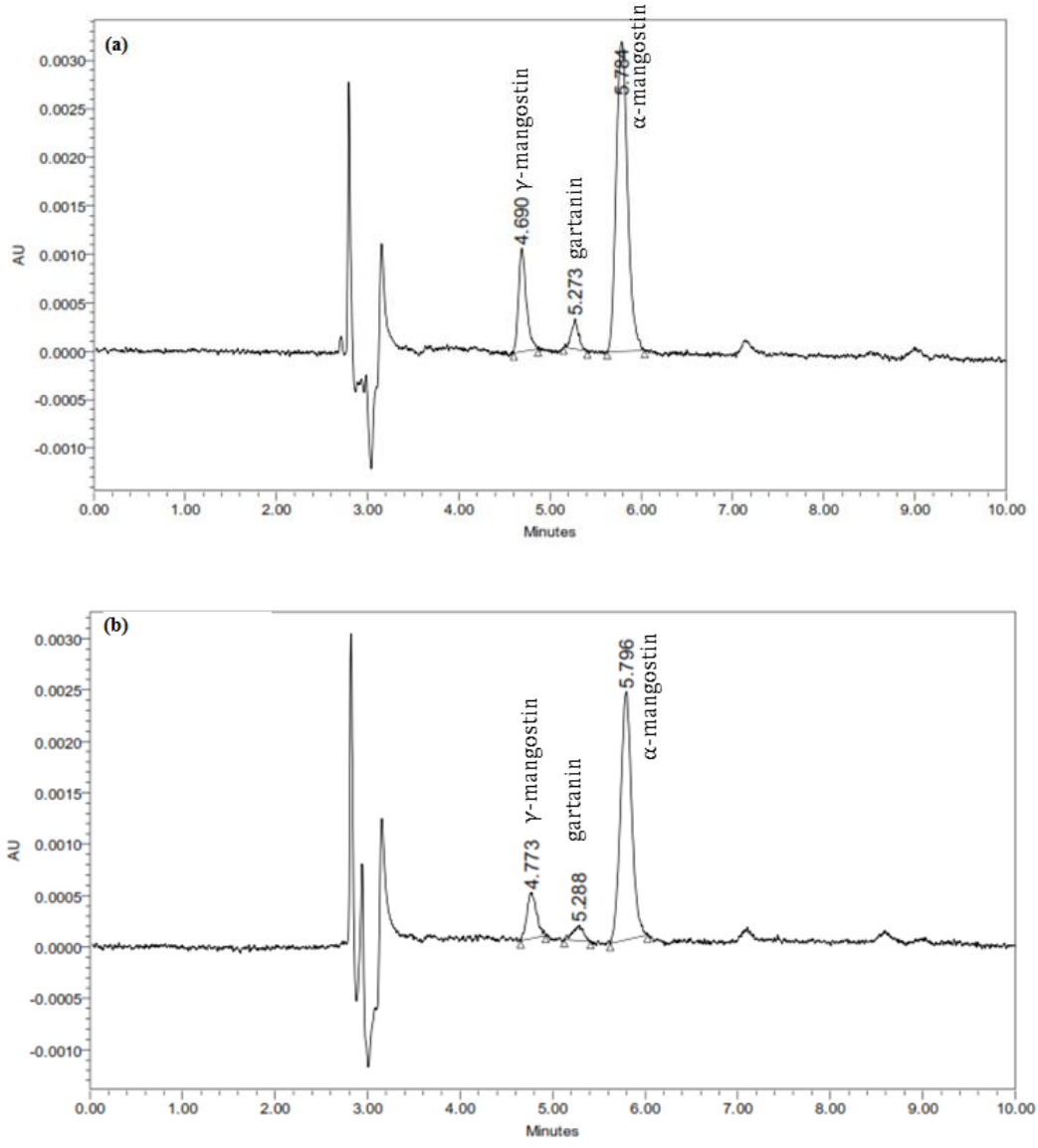
0.488 ppm, γ-mangostin 0.013 ppm and 0.039 ppm, and gartanin 0.019 ppm 5 and 0.060 ppm. These results are better than those in the previous analysis which obtained higher LOD and LOQ for α-mangostin (Popp *et al.*, 2000).

Results of α-mangostin, γ-mangostin and Gartanin Analysis in Mangosteen Rind Extract of Four Districts in West Java

Mangosteen pericarp extracts are given SPE pre-treatment which mainly distinguishes analytes in a complex matrix by the differences of hydrophobicity. The SPE technique has been used successfully for separating organic compounds from complex samples (Zhang *et al.*, 2016; Otles and Kartal, 2016).

C-18 cartridges are used for hydrophobic (strongly non-polar) types of analyte . Each step of the SPE pre-treatment required a solvent matching the hydrophobic analyte type.

For the eluting solvent, methanol may be used with intermediate polarity (Muchtaridi and Musfiroh, 2012). Mangosteen pericarp extract which has been given SPE pre-treatment and eluted with methanol is injected to an HPLC instrumentation with a validated condition. Chromatogram results were obtained and the concentration of α-mangostin, γ-mangostin and gartanin are calculated by *Area Under Curve* (AUC) at each standard retention time. The analysis showed that the levels of α-mangostin, γ-mangostin, and gartanin from Bogor respectively are 13.87%, 8.28% and 10.44%. The results for mangosteen pericarp extract from Purwakarta are 10.072% for α-mangostin, 6.33% for γ-mangostin, and 8.77% for gartanin. The resuts for mangosteen pericarp extract from Subang are 10.88% for α-mangostin, 6.012% for γ-mangostin, and 8.54% for gartanin. Lastly, the α-mangostin, γ-mangostin, and gartanin contents of pericarp extracts from Tasikmalaya are 8.53%, 6.07%, and 8.64%, respectively.



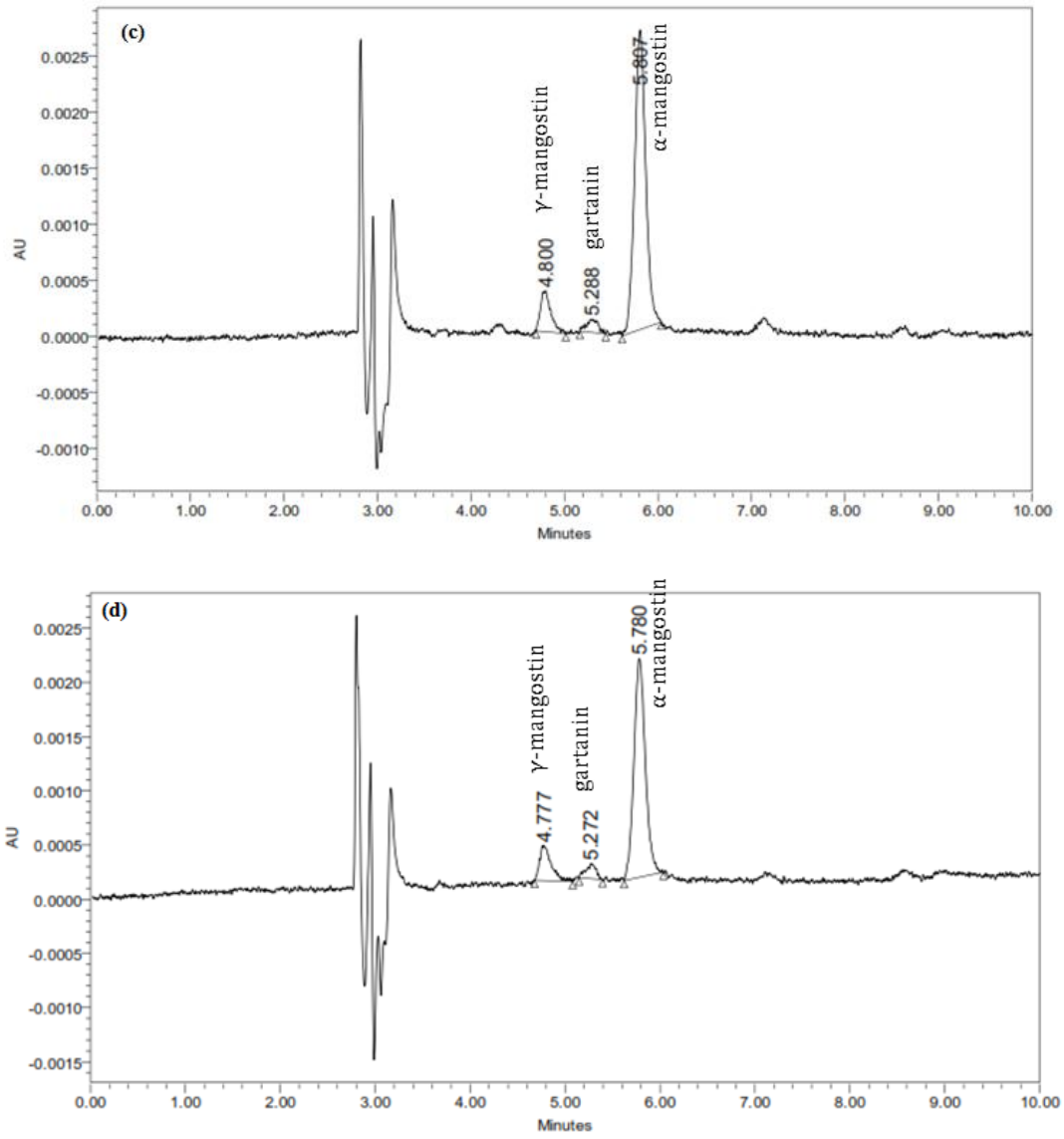


Fig. 2: Chromatograms of mangosteen rind extracts from Bogor (a), Purwakarta (b), Subang (c), and Tasikmalaya.

CONCLUSION

These results showed that this HPLC method can be used for routine analysis of α-mangostin, γ-mangostin and gartanin from mangosteen. The optimum condition was performed using Enduro column C-18 reverse phase (250 mm x 4.6 mm), Photo Diode Array detector 375 nm, acetonitrile mobile phase and water containing 0.1 % phosphoric acid (95: 5) with a flow rate of 1.0 mL/min.

ACKNOWLEDGEMENT

The authors are thankful to Basic Science Research Centre and Pharmaceutical Analysis and Medicinal Chemistry laboratory (Faculty of Pharmacy), Universitas Padjadjaran and the Ministry Research, Technology, and Higher Education of Indonesia for providing the necessary facilities for the research.

Financial support and sponsorship: Penelitian Unggulan Perguruan Tinggi (PUPT) 2017 for financial support.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Muchtaridi M, Puteri NA, Milanda T, Musfiroh I. Validation Analysis Methods of A-Mangostin, Γ-Mangostin, AndGartanin Mixture in Mangosteen (*Garcinia mangostana* L.) Fruit Rind Extract from West Java with HPLC. J App Pharm Sci, 2017; 7 (10): 125-130.