Multiple Response Optimization of an HPLC Method for Analyzing Resorcinol and 4-n-Butyl Resorcinol in Lipid Nanoparticles

by Rini Dwiastuti

Submission date: 14-Apr-2023 10:42PM (UTC+0700)
Submission ID: 2064502269
File name: IJC_Oktober_2020.pdf (184.25K)
Word count: 5450
Character count: 29220

Short Communication:

Multiple Response Optimization of an HPLC Method for Analyzing Resorcinol and 4-*n*-Butyl Resorcinol in Lipid Nanoparticles

Rini Dwiastuti[1*], Dina Christin Ayuning Putri[1], Maywan Hariono[2], and Florentinus Dika Octa Riswanto[2]

[1]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Sanata Dharma University, Paingan, Maguwoharjo, Depok, Sleman, Yogyakarta 55282, Indonesia*

[2]*Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Sanata Dharma University, Paingan, Maguwoharjo, Depok, Sleman, Yogyakarta 55282, Indonesia*

*** Corresponding author:**

email: rini_dwi@usd.ac.id

Received: August 12, 2020
Accepted: October 10, 2020

DOI: 10.22146/ijc.58537

Abstract: *Resorcinol and 4-n-butyl resorcinol have been used to improve skin health. However, these two compounds were unstable due to the oxidation process. Lipid nanoparticle formulation strategies were reported as the solution to overcome the stability problem for both resorcinol and 4-n-butyl resorcinol. Nevertheless, it is important to determine the content of resorcinol and 4-n-butyl resorcinol in lipid nanoparticle formulation. Aiming to develop the analytical method for resorcinol and 4-n-butyl resorcinol determination, a response surface methodology (RSM) was applied in the HPLC optimization stage. An optimized HPLC condition was obtained by generating a Box-Behnken design followed by multiple response analysis. It was obtained that optimized HPLC conditions due to the predictive multiple response optimization were methanol percentage of 50.0%, acetonitrile percentage of 18.1%, and flow rate of 0.6 mL min^{-1}. This optimized condition was successfully applied and met the requirements of the system suitability test. Quantitative estimation was performed and resulted that the resorcinol and 4-n-butyl resorcinol content in lipid nanoparticles were 70.37 ± 0.47 and 95.07 ± 0.80 μg mL^{-1}, respectively.*

Keywords: *4-n-butyl resorcinol; Box-Behnken design; HPLC; optimization; resorcinol*

■ INTRODUCTION

Resorcinol (benzene-1,3-diol) has been widely used in the formulation of drugs and cosmetics. Resorcinol was formulated as an ointment for skin therapy and medication for acne, seborrheic dermatitis, eczema, psoriatic, and other skin health problems [1]. 4-*n*-Butyl resorcinol, a resorcinol derivative, can be used in melisma therapy [2]. This hygroscopic compound was unstable in the room temperature storage by resulting in a color change. The discoloration of resorcinol and 4-*n*-butyl resorcinol (Fig. 1) can be occurred due to the oxidation process [3]. In the previous study, the physical stability of 4-*n*-butyl resorcinol towards the oxidation process can be enhanced by lipid nanoparticle formulation [4]. Nanoparticles were described as small particles with a size of < 200 nm [5]. In recent years, nanoparticle technology was applied to health since it provided a significant contribution in the drug delivery and therapeutic system [6]. A Lipid nanoparticle, a nanoparticle system that consists of phospholipid molecules, has several advantages, such as lower toxicity, ability to improve drugs' physical properties, flexibility

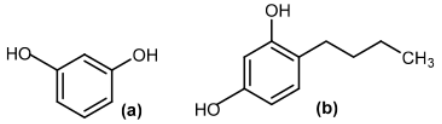

Fig 1. Chemical structures of resorcinol (a) and 4-*n*-butyl resorcinol (b)

to combine hydrophilic and lipophilic properties, stability enhancer, and supporting the drug release in a specific target [7]. Hence, it is possible to improve the stability of 4-*n*-butyl resorcinol by applying lipid nanoparticle formulation [4].

There was a need to determine the content of resorcinol and 4-*n*-butyl resorcinol in the lipid nanoparticle in order to control the quality of the pharmaceutical dosage form. Several modern multivariate analysis techniques for quantitatively determining analytes content in mixtures were applied to analyze food and drug materials [8-10] as well as herbal products [11-13]. However, it is necessary to develop chromatographic separation for determining 'actual value' for chemometrics modeling prediction [14-15]. In the last decade, chromatography techniques have been widely applied to determine chemicals content in foods [16-19], drugs [20-22], and herbals [23-26]. High-performance liquid chromatography (HPLC) can be applied in drug analysis in different matrices and mixtures. There were several publications about resorcinol analysis, but the reported study of resorcinol and 4-*n*-butyl resorcinol determination using HPLC was limited [27-31]. Hence, it is important to develop an appropriate HPLC method as well as its separation condition to analyze resorcinol and 4-*n*-butyl resorcinol in lipid nanoparticles.

Optimization of separation conditions is a crucial stage in analytical method development [32-34]. Chemometrics-assisted chromatographic optimization can be performed using response surface methodologies (RSM) such as factorial, central composite (CCD), Box–Behnken (BBD), and Doehlert designs [35]. The BBD was developed from the design factorial model [36], which has model characteristics of rotatable with three levels for each experimental factor [37]. This design was more effective and economical than other three-level designs [38], with fewer experimental runs compared to full factorial or CCD [39]. In addition, BBD was commonly applied in several studies of HPLC method development [40-43].

The aim of this study was to develop an HPLC method for a simultaneous determination of resorcinol and 4-*n*-butyl resorcinol in lipid nanoparticles. We reported a new optimization model for separating resorcinol and 4-*n*-butyl resorcinol in lipid nanoparticles by HPLC method using a chemometrics approach. BBD was performed to achieve the optimized condition of mobile phase composition of methanol and acetonitrile percentages as well as its flow rate adjustment. Multiple response analysis was conducted to obtain the optimized condition among nine different experimental responses.

■ EXPERIMENTAL SECTION

Materials

Resorcinol standard was purchased from Sigma Aldrich, while 4-*n*-butyl resorcinol standard was purchased from TCI. Solvents used in this study were methanol, acetonitrile (gradient grade for liquid chromatography, Merckmilipore), soy lecithin (Nacalai), and redistilled water (PT. Ikapharmindo Putramas). Lipid nanoparticle was produced in the Faculty of Pharmacy, Sanata Dharma University, Indonesia [43].

Instrumentation and Software

Instrumentation used in this study were HPLC system of Shimadzu® LC-2010 CHT with UV/Vis detector, C18 column of Luna Phenomenex® (250 × 4.6 mm i.d., 5 µm), ultra-micro analytical balance RADWAG® series of UYA 2.3Y (max. 2.1 g, min. 0.01 mg), Scaltec® SBC 22 analytical balance (max. 60/210 g, min. 0.001 g), Gast® vacuum pump model DOA-P504-BN, Retsch® T460 ultrasonicator, sterile syringe filter with a 0.2 µm pore size hydrophilic PTFE membrane (Merckmillipore), and a set of Socorex® micropipettes. Response surface methodology and multiple response optimization were performed using Minitab® 19 for Windows.

Procedure

Standard solution and sample preparation

The resorcinol stock solution was prepared by accurately weighing 1.079 mg resorcinol, transferred into a 5 mL volumetric flask, and diluting into the volume. Meanwhile, a 4-*n*-butyl resorcinol stock solution was prepared by accurately weighing 8.200 mg

4-*n*-butyl resorcinol, transferred into a 10 mL volumetric flask, and then followed by dilution into the volume. Resorcinol and 4-*n*-butyl resorcinol working solutions were prepared by transferring 0.500 and 0.122 mL of resorcinol and 4-*n*-butyl resorcinol stock solution, respectively, into separate 5 mL volumetric flask followed by dilution into the volume. The standard mixture solution was prepared by mixing 0.500 and 0.122 mL of resorcinol and 4-*n*-butyl resorcinol stock solution into a 5 mL volumetric flask followed by dilution into the volume. The sample solution was prepared by transferring 0.5 mL sample solution of soy lecithin lipid nanoparticles containing resorcinol and 4-*n*-butyl resorcinol into a 5 mL volumetric flask followed by dilution into the volume. All solutions were filtered using a sterile syringe filter membrane before injection into the HPLC system.

Response surface methodology

HPLC system optimization was performed by applying the response surface methodology. The preliminary HPLC optimization method in this study was carried out using one variable at time (OVAT) technique. The BBD was applied to observe three different independent variables, namely methanol percentage (X1), acetonitrile percentage (X2), and flow rate (X3). Table 1 presented independent variables with three different levels for generating the BBD model. This experimental design was executed by observing 16 runs, including 12 runs at BBD points and 4 runs at central points.

Multiple response optimization

Dependent variables in this study were separation properties for both resorcinol and 4-*n*-butyl resorcinol (retention time, resolution, tailing factor, and the number of theoretical plates) and HPLC column pressure. It was necessary to perform the multiple response optimization since there were nine different dependent variables with different goals (maximum or minimum) for each variable. This computational optimization process was carried out by employing the response optimizer in Minitab software.

Table 1. Experimental variables with three levels for response surface methodology

Variables	Levels		
	Low	Medium	High
X1: Methanol (%)	50	60	70
X2: Acetonitrile (%)	10	15	20
X3: Flow rate (mL min^{-1})	0.6	0.7	0.8

System suitability test

System suitability test was performed in this study to assess recommended optimization setting resulted from the multiple response optimization stages. Ten microliters of standard mixture solution containing resorcinol and 4-*n*-butyl resorcinol were injected in HPLC system and replicated five times.

Quantitative estimation

Quantitative estimation for resorcinol and 4-*n*-butyl resorcinol were performed using multiple-point calibration method. Each compound's calibration graph was constructed and applied in the quantitative determination for both resorcinol and 4-*n*-butyl resorcinol.

■ RESULTS AND DISCUSSION

Response Surface Methodology

The optimization of HPLC system was necessary to obtain appropriate conditions for achieving sophisticated separation during the analytical process. The preliminary optimization method was carried out using OVAT technique. Three variations of HPLC condition, including the percentage of methanol, percentage of acetonitrile, and flow rate, were applied in the system, followed by the chromatogram observation. Fig. 2 presented three OVAT variations, namely OVAT1, OVAT2, and OVAT3. It was found that OVAT1 and OVAT2 with 70% methanol and 20% acetonitrile in the mobile phase resulted in the separation of both resorcinol and 4-*n*-butyl resorcinol with the retention time less than 10 min. However, the flow rate of 0.8 mL min^{-1} (OVAT1) resulted in a tailing profile for resorcinol peak, while a flow rate of 0.6 mL min^{-1} (OVAT2) resulted in a tailing profile resorcinol peak and baseline drift. In OVAT3 with 50% methanol and 20% acetonitrile in the mobile phase and flow rate of 0.6 mL min^{-1} resulted in better peaks separation with the retention time of 4-*n*-butyl resorcinol was more than 10 min. These OVAT observations were considered to

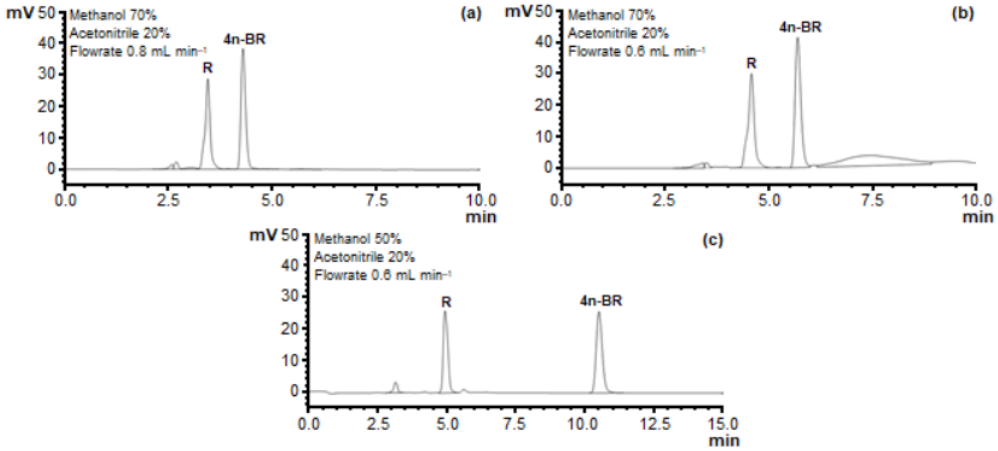

Fig 2. Preliminary HPLC optimization using three variations separation condition of OVAT1 (a), OVAT2 (b), and OVAT3 (b) for standard solution mixture containing resorcinol (R) and 4-*n*-butyl resorcinol (4-*n*-BR). Column: C18 column of Luna Phenomenex® (250 × 4.6 mm i.d., 5 µm). Wavelength detection at 279 nm. Volume injection: 10 µL

Table 2. The Box-Behnken design with experimental responses of resorcinol and 4-*n*-butyl resorcinol separation

Run	X1	X2	X3	Resorcinol				4-*n*-butyl resorcinol				Press.
				ret	res	tai	N	ret	res	tai	N	
1	50	10	0.7	4.613	8.334	1.270	4484	17.116	27.685	1.138	11900	208
2	70	10	0.7	4.049	4.468	1.191	4613	6.315	6.312	1.215	7233	190
3	50	20	0.7	4.277	5.792	1.153	3928	9.158	15.015	1.188	9430	185
4	70	20	0.7	3.937	3.323	0.989	5146	4.917	4.249	1.227	6623	144
5	50	15	0.6	5.148	6.347	1.103	4214	14.142	21.031	1.177	11053	187
6	70	15	0.6	4.643	4.342	0.884	5574	6.374	6.344	1.240	7367	161
7	50	15	0.8	3.874	5.309	1.105	3928	10.653	20.044	1.163	9861	231
8	70	15	0.8	3.496	3.234	1.034	4562	4.814	5.897	1.209	6437	185
9	60	10	0.6	4.957	5.285	1.283	4433	11.129	16.297	1.193	9382	195
10	60	20	0.6	4.722	4.374	1.267	4989	7.323	6.287	1.219	8003	160
11	60	10	0.8	3.750	4.981	1.278	4063	8.365	15.399	1.176	8448	241
12	60	20	0.8	3.557	4.149	1.261	4265	5.516	5.764	1.291	5887	204
13	60	15	0.7	4.143	7.584	1.288	3862	7.541	11.24	1.219	7969	196
14	60	15	0.7	4.141	7.771	1.298	3872	7.532	11.234	1.218	7975	196
15	60	15	0.7	4.140	7.674	1.300	3805	7.525	9.664	1.215	7944	196
16	60	15	0.7	4.138	7.550	1.300	3789	7.519	11.139	1.216	7912	196

Note: X1: methanol percentage (%); X2: acetonitrile percentage (%); X3: flow rate (mL min^{-1}); ret: retention time (min); res: resolution; tai: tailing factor; N: theoretical plate number; Press: pressure (kgf cm^{-2})

develop further optimization stages with the application of RSM. BBD, one of the RSM techniques, has been chosen to optimize HPLC separation conditions since it provided an effective and economic statistical model for optimization purposes [38]. Three levels and three factors BBD model was generated using Minitab software. Methanol percentage, acetonitrile percentage, and flow rate were stated as independent variables of the experiment. While retention time, resolution, tailing factor, and the number of theoretical plates for both

resorcinol and 4-*n*-butyl resorcinol were stated as dependent variables of the chromatographic separation experiment. Column pressure was also stated as the dependent variable since it was important to maintain the separation process and column lifetime. Table 2 presented independent variables and dependent variables as well as their results from 16 runs of BBD model for separating resorcinol and 4-*n*-butyl resorcinol.

Experimental results of BBD observation were analyzed to obtain statistical information for further method optimization. Table 3 presented RSM analytical results of resorcinol and 4-*n*-butyl resorcinol separation, including regression equation, R^2, adjusted R^2, and p-value. It should be noted that independent variables are significantly affected if the value of $R^2 \geq 0.8$ and adjusted $R^2 > 0.8$, while the difference between R^2 and adjusted R^2 must be less than 0.2 [41]. The response of 4-*n*-butyl resorcinol tailing factor has an R^2 of 0.888 and an adjusted R^2 of 0.720. However, all p-values results were less than 0.05 and considered to be statistically significant [33]. Hence, we decided to include all the nine responses to be analyzed further in the multiple response optimization stages.

Multiple Response Optimization

All responses, including retention time, resolution, tailing factor, and the number of theoretical plates for both resorcinol and 4-*n*-butyl resorcinol, and the column pressure, were considered to build model prediction for HPLC separation. Its because they were indicated statistically significant due to their p-values. The goal for retention time, tailing factor, and column

Table 3. Response surface methodology analytical results of resorcinol and 4-*n*-butyl resorcinol separation

Responses	Regression Equation in Uncoded Units	R^2	R^2 (adj)	p-Value
Resorcinol retention time	Y = 19.265 - 0.13471 X1 - 0.12465 X2 - 20.619 X3 + 0.000611 X1*X1 + 0.000695 X2*X2 + 8.862 X3*X3 + 0.001120 X1*X2 + 0.03175 X1*X3 + 0.02100 X2*X3	0.999	0.999	0.000
Resorcinol resolution	Y = -110.1 + 1.010 X1 + 0.783 X2 + 250.4 X3 - 0.01027 X1*X1 - 0.04552 X2*X2 - 180.9 X3*X3 + 0.00699 X1*X2 - 0.018 X1*X3 + 0.039 X2*X3	0.966	0.914	0.001
Resorcinol tailing factor	Y = -7.17 + 0.2057 X1 - 0.0399 X2 + 8.01 X3 - 0.001933 X1*X1 + 0.001900 X2*X2 - 7.17 X3*X3 - 0.000425 X1*X2 + 0.0370 X1*X3 - 0.0005 X2*X3	0.945	0.863	0.004
Resorcinol plate number	Y = 31827 – 419 X1 – 532 X2 – 33702 X3 + 4.214 X1*X1 + 11.57 X2*X2 + 31612 X3*X3 + 5.44 X1*X2 - 181.5 X1*X3 - 177 X2*X3	0.967	0.918	0.001
4-*n*-butyl resorcinol retention time	Y = 156.4 - 2.844 X1 - 3.264 X2 - 60.3 X3 + 0.01380 X1*X1 + 0.01869 X2*X2 + 8.7 X3*X3 + 0.03280 X1*X2 + 0.482 X1*X3 + 0.479 X2*X3	0.993	0.982	0.000
4-*n*-butyl resorcinol resolution	Y = 219.0 - 4.585 X1 - 4.24 X2 - 23.7 X3 + 0.02444 X1*X1 + 0.0021 X2*X2 + 6.6 X3*X3 + 0.0530 X1*X2 + 0.135 X1*X3 + 0.19 X2*X3	0.991	0.977	0.000
4-*n*-butyl resorcinol tailing factor	Y = 0.411 + 0.0371 X1 - 0.0132 X2 - 0.96 X3 - 0.000238 X1*X1 - 0.000050 X2*X2 + 0.400 X3*X3 - 0.000190 X1*X2 - 0.00425 X1*X3 + 0.0445 X2*X3	0.888	0.720	0.028
4-*n*-butyl resorcinol plate number	Y = 56770 – 1325 X1 – 378 X2 + 8060 X3 + 7.980 X1*X1 + 1.94 X2*X2 – 6850 X3*X3 + 9.30 X1*X2 + 65.5 X1*X3 - 591 X2*X3	0.995	0.986	0.000
Pressure	Y = -233 + 17.54 X1 + 7.22 X2 – 415 X3 - 0.1162 X1*X1 - 0.1050 X2*X2 + 662 X3*X3 - 0.1150 X1*X2 - 5.00 X1*X3 - 1.00 X2*X3	0.991	0.977	0.000

pressure were expected to be the minimum value. In contrast, the goal for resolution and theoretical plate numbers were expected to be the maximum value.

General optimization considering all significant factors was generated by calculating the desirability function computed by Minitab software. Desirability calculation resulted in a value with a scale between 0–1. Desirability value of 0 represents a completely undesirable response while 1 represents the most desirable response [44]. Overall desirability in this study was 0.444 indicated several errors in the prediction model. It was found that HPLC system settings due to the predictive multiple response optimization were methanol percentage of 50.0%, acetonitrile percentage of 18.1%, and flow rate of 0.6 mL min^{-1}. Table 4 presented multiple response optimization for resorcinol and 4-*n*-butyl resorcinol along with prediction value for each response and percentage of errors compared with the observational values. Except for resorcinol resolution and plate numbers, all predictions resulted in a minimum error (< 5%) and indicated a good correlation between prediction responses and observational responses [45]. The unexpected percentage of error more than 10% for resorcinol theoretical plate number and a very high percentage of error for resorcinol resolution may contribute to the overall desirability value of 0.444. However, the HPLC peak separation quality will increase with the higher value of resolution [46]. Hence, it was important to perform a system suitability test with the HPLC system suggested by the multiple response optimization models.

System Suitability Test

HPLC system suggestion as an output from the multiple response optimization should be applied to confirm its performance toward analytes separation. For this purpose, HPLC separation properties, including retention time (RT), peak area, resolution (Rs), peak tailing factor (TF), theoretical plate number (N), and column pressure, were assessed in the system suitability test. Table 5 presented the results of the system suitability test. It was found that the system suitability test was met the requirements since the relative standard deviation (RSD) was < 2.0, resolution value greater than 2.0, tailing factor near 1.0, and theoretical plate number more than 2000 [46]. The retention time of 4-*n*-butyl resorcinol of 11.540 min was still acceptable since all analytes can be detected before 15 min while maintaining column pressure of 188 kgf cm^{-2}. Fig. 3 depicted HPLC separation of resorcinol and 4-*n*-butyl resorcinol under the optimized condition. Chromatogram profiles of solvent, standard

Table 4. Multiple response optimization for resorcinol and 4-*n*-butyl resorcinol

Responses	Optimization parameters				Prediction	Error (%)
	Goal	Lower	Target	Upper		
Resorcinol retention time	Minimum	-	3.5	5.148	4.793	4.67
Resorcinol resolution	Maximum	3.23	8.3	-	6.231	87.17
Resorcinol tailing factor	Minimum	-	0.9	1.3	1.154	4.91
Resorcinol plates number	Maximum	3789	5574	-	4095.7	10.94
4-*n*-butyl resorcinol retention time	Minimum	-	4.8	17.116	11.504	0.31
4-*n*-butyl resorcinol resolution	Maximum	4.25	27.7	-	16.947	2.61
4-*n*-butyl resorcinol tailing factor	Minimum	-	1.1	1.291	1.175	1.09
4-*n*-butyl resorcinol plates number	Maximum	5887	11900	-	10335.9	1.02
Pressure	Minimum	-	144	241	179	4.79

Note: Desirability value (D) = 0.444

Table 5. Results of system suitability test (n = 5)

Analytes	RT		Area		Rs	TF	N	Pressure (kgf cm^{-2})
	Mean (min)	RSD (%)	Mean	RSD (%)				
Resorcinol	5.028	0.128	111899.2	0.525	3.329	1.100	4598.6	188
4-*n*-butyl resorcinol	11.540	0.563	153598.4	1.275	17.402	1.188	10442.8	

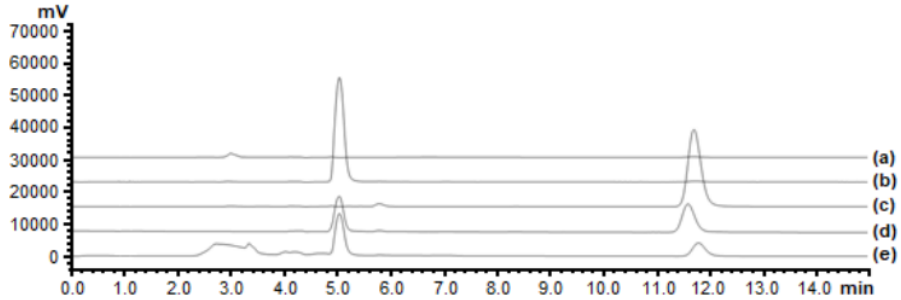

Fig 3. Representative HPLC chromatograms of methanol as solvent (a), standard solution of resorcinol (b), standard solution of 4-*n*-butyl resorcinol (c), standard solution mixture containing resorcinol and 4-*n*-butyl resorcinol (d), and lipid nanoparticle sample solution (e). Mobile phase: methanol-acetonitrile-water (50.0:18.1:31.9 v/v). Flow rate: 0.6 mL min^{-1}. Column: C18 column of Luna Phenomenex® (250 × 4.6 mm i.d., 5 µm). Wavelength detection at 279 nm. Volume injection: 10 µL

of resorcinol, standard of 4-*n*-butyl resorcinol, a mixture of standard solution, and sample lipid nanoparticles were exhibited in a stacked arrangement.

Quantitative Estimation

HPLC optimization followed by quantitative estimation was previously reported using single-point calibration [32,47]. In this study, a multiple-point calibration method was applied for estimating the content of resorcinol and 4-*n*-butyl resorcinol. The calibration equations for resorcinol and 4-*n*-butyl resorcinol were y = 17819x – 13223 (R^2 = 0.994) and y = 22148x – 57082 (R^2 = 0.998), respectively. It was found that contents of resorcinol and 4-*n*-butyl resorcinol in lipid nanoparticle samples (n = 6) were 70.37 ± 0.47 and 95.07 ± 0.80 µg mL^{-1}.

■ CONCLUSION

An HPLC method development to analyze resorcinol and 4-*n*-butyl resorcinol in lipid nanoparticle samples has been successfully conducted. Multiple response optimization was applied to obtain the optimized HPLC condition for methanol and acetonitrile percentage of the mobile phase as well as the flow rate. System suitability test was performed to confirm the HPLC performance for separation purpose followed by quantitative estimation of these two analytes. In the future, we plan to perform analytical method validation for the next stage of our lipid nanoparticles research to achieve comprehensive information both for optimization and validation of the HPLC method.

■ ACKNOWLEDGMENTS

The authors are grateful to the Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia, for the provided laboratory facilities. This research was financially funded by The Ministry of Research and Technology of Higher Education for funding through the Research Grant with contract No: 022b/Penel./LPPM-USD/III/2020 (Amendment letter No.: 038a/Penel./LPPM-USD/VI/2020), awarded to Dr. Rini Dwiastuti. We also thank Ni Kadek Dwi Putri Kusuma Dewi, Eka Yuliana Thonak, Agustine Nita Wulandari, Gede Herdy Cisara Riliansa, Enrico Melvin Setiadi, and Millavenia Pusparini for assistance with preparation of sample solutions.

■ REFERENCES

[1] Chowdhury, M.A.Z., Banik, S., Uddin, B., Moniruzzaman, M., Karim, N., and Gan, S.H., 2012, Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilas in Bangladesh, *Int. J. Environ. Res. Public Health*, 9 (9), 3318–3329.

[2] Kwon, S.H., Yang, J.H., Shin, J.W., Park, K.C., Huh, C.H., and Na, J.I., 2020, Efficacy of liposome-

encapsulated 4-n-butylresorcinol and resveratrol cream in the treatment of melasma, *J. Cosmet. Dermatol.*, 19 (4), 891–895.

[3] Love, A.R., Kerschner, J.L., Barratt, M.J., and Zhou, Y., 2003, Stabilization of resorcinol derivatives in cosmetic compositions, *US Patent*, US20030180234A1.

[4] Dwiastuti, R., Marchaban, Istyastono, E.P., and Riswanto, F.D.O., 2018, Analytical method validation and determination of free drug content of 4-*n*-butylresorcinol in complex lipid nanoparticles using RP-HPLC method, *Indones. J. Chem.*, 18 (3), 496–502.

[5] Reverchon, E., and Adami, R., 2006, Nanomaterials and supercritical fluids, *J. Supercrit. Fluids*, 37 (1), 1–22.

[6] Cooper, D.L., Conder, C.M., and Harirforoosh, S., 2014, Nanoparticles in drug delivery: Mechanism of action, formulation and clinical application towards reduction in drug-associated nephrotoxicity, *Expert Opin. Drug Delivery*, 11 (10), 1661–1680.

[7] Rahmi, D., 2010, Lemak padat; Sintesis dan aplikasi, *JKK*, 32 (1), 27.

[8] Glavanović, S., Glavanović, M., and Tomišić, V., 2016, Simultaneous quantitative determination of paracetamol and tramadol in tablet formulation using UV spectrophotometry and chemometric methods, *Spectrochim. Acta, Part A*, 157, 258–264.

[9] Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M., and Granato, D., 2014, A comparative study of the phenolic compounds and the *in vitro* antioxidant activity of different Brazilian teas using multivariate statistical techniques, *Food Res. Int.*, 60, 246–254.

[10] Salameh, B.A., Al-Degs, Y.S., Abu Safieh, K.A., and AL-Zghool, A.W., 2019, Novel application of multivariate standard addition method based on net analyte signal for quantification of artificial sweeteners in complex food matrices, *J. Food Meas. Charact.*, 14 (1), 78–87.

[11] Wang, P., Zhang, H., Yang, H., Nie, L., and Zang, H., 2015, Rapid determination of major bioactive isoflavonoid compounds during the extraction process of kudzu (*Pueraria lobata*) by near-infrared transmission spectroscopy, *Spectrochim. Acta, Part A*, 137, 1403–1408.

[12] Gómez, J.D., Vital, C.E., Oliveira, M.G.A., and Ramos, H.J.O., 2018, Broad range flavonoid profiling by LC/MS of soybean genotypes contrasting for resistance to *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), *PLoS One*, 13 (10), e0205010.

[13] Riswanto, F.D.O., Rohman, A., Pramono, S., and Martono, S., 2021, Soybean (*Glycine max* L.) isoflavones: Chemical composition and its chemometrics-assisted extraction and authentication, *J. Appl. Pharm. Sci.*, 11 (1), 12–20.

[14] Kayesh, R., Jahan, M.S., and Sultan, M.Z., 2017, Development using response surface methodology and validation of a stability-indicating RP-HPLC method for simultaneous estimation of azilsartan medoxomil and chlorthalidone in solid dosage form, *Chromatographia*, 80 (4), 593–603.

[15] Yusof, N.A.A., Mat Hadzir, N., Ashari, S.E., Mohamad Hanapi, N.S., and Dol Hamid, R., 2019, Optimization of enzymatic synthesis of betulinic acid amide in organic solvent by response surface methodology (RSM), *Indones. J. Chem.*, 19 (4), 849–857.

[16] Cheung, Y., Meenu, M., Yu, X., and Xu, B., 2019, Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources, *Int. J. Food Prop.*, 22 (1), 290–308.

[17] Soto-Zarazúa, M.G., Rodrigues, F., Pimentel, F.B., Bah, M.M., and Oliveira, M.B.P.P., 2016, The isoflavone content of two new alfalfa-derived products for instant beverage preparation, *Food Funct.*, 7 (1), 364–371.

[18] Gliszczyńska-Świgło, A., and Rybicka, I., 2015, Simultaneous determination of caffeine and water-soluble vitamins in energy drinks by HPLC with photodiode array and fluorescence detection, *Food Anal. Methods*, 8 (1), 139–146.

[19] Danezis, G.P., Tsagkaris, A.S., Camin, F., Brusic, V., and Georgiou, C.A., 2016, Food authentication:

Techniques, trends & emerging approaches, *TrAC, Trends Anal. Chem.*, 85, 123–132.

[20] Çelik, B., 2017, Risperidone mucoadhesive buccal tablets: Formulation design, optimization and evaluation, *Drug Des. Dev. Ther.*, 11, 3355–3365.

[21] Razzaq, S.N., Ashfaq, M., Mariam, I., Khan, I.U., and Razzaq, S.S., 2013, Simultaneous RP-HPLC determination of sparfloxacin and dexamethasone in pharmaceutical formulations, *Braz. J. Pharm. Sci.*, 49 (2), 301–309.

[22] Alzoman, N.Z., 2016, A validated stability-indicating and stereoselective HPLC method for the determination of lenalidomide enantiomers in bulk form and capsules, *J. Chromatogr. Sci.*, 54 (5), 730–735.

[23] Syukri, Y., Martien, R., Lukitaningsih, E., and Nugroho, A.E., 2016, Quantification of andrographolide isolated from *Andrographis paniculata* Nees obtained from traditional market in Yogyakarta using validated HPLC, *Indones. J. Chem.*, 16 (2), 190–197.

[24] Kadam, P.V., Yadav, K.N., Bhingare, C.L., and Patil, M.J., 2018, Standardization and quantification of curcumin from *Curcuma longa* extract using UV visible spectroscopy and HPLC, *J. Pharmacogn. Phytochem.*, 7 (5), 1913–1918.

[25] Zhu, J., Fan, X., Cheng, Y., Agarwal, R., Moore, C.M.V., Chen, S.T., and Tong, W., 2014, Chemometric analysis for identification of botanical raw materials for pharmaceutical use: A case study using Panax notoginseng, *PLoS One*, 9 (1), e87462.

[26] Shailajan, S., Hande, H., Singh, D., and Tiwari, B., 2014, Estimation of ursolic acid from *Urtica dioica* L. using validated HPTLC method, *J. Appl. Pharm. Sci.*, 4 (5), 92–95.

[27] Cinier, R., and Guilment, J., 1996, Quantitative analysis of resorcinol in aqueous solution by near-infrared spectroscopy, *Vib. Spectrosc.*, 11 (1), 51–59.

[28] Wu, H.W., Chen, M.L., Shou, D., and Zhu, Y., 2012, Determination of resorcinol and phloroglucinol in environmental water samples using ion chromatography with chemiluminescence detection, *Chin. J. Anal. Chem.*, 40 (11), 1747–1751.

[29] Pistonesi, M.F., Di Nezio, M.S., Centurión, M.E., Palomeque, M.E., Lista, A.G., and Band, B.S.F., 2006, Determination of phenol, resorcinol and hydroquinone in air samples by synchronous fluorescence using partial least-squares (PLS), *Talanta*, 69 (5), 1265–1268.

[30] Boer, J., and Jemec, G.B.E., 2009, Resorcinol peels as a possible self-treatment of painful nodules in hidradenitis suppurativa, *Clin. Exp. Dermatol.*, 35 (1), 36–40.

[31] Narita, M., Murakami, K., and Kauffmann, J.M., 2007, Determination of dye precursors in hair coloring products by liquid chromatography with electrochemical detection, *Anal. Chim. Acta*, 588 (2), 316–320.

[32] Riswanto, F.D.O., Desra, A., Sari, R.M., Thomas, V., Rohman, A., Pramono, S., and Martono, S., 2020, Employing an R software package rsm for optimizing of genistein, daidzein, and glycitein separation and its application for soy milk analysis by HPLC method, *Indones. J. Chem.*, 20 (5), 1184–1198.

[33] Sahoo, D.K., and Sahu, P.K., 2015, Chemometric approach for RP-HPLC determination of azithromycin, secnidazole, and fluconazole using response surface methodology, *J. Liq. Chromatogr. Relat. Technol.*, 38 (6), 750–758.

[34] Bae, H., Jung, H.S., and Jung, J.Y., 2018, Optimization of HPLC-tandem mass spectrometry for chlortetracycline using response surface analysis, *Environ. Eng. Res.*, 23 (3), 309–315.

[35] Hibbert, D.B., 2012, Experimental design in chromatography: A tutorial review, *J. Chromatogr. B*, 910, 2–13.

[36] Giles, H.F., Wagner, J.R., and Mount, E.M., 2014, *Extrusion: The Definitive Processing Guide and Handbook*, 2nd Ed., William Andrew, Inc., Norwich, New York.

[37] Das, A.K., and Dewanjee, S., 2018, "Chapter 3 - Optimization of extraction using mathematical models and computation", in *Computational Phytochemistry*, Eds. Sarker, S.D., and Nahar, L., Elsevier, Inc., Amsterdam, 75–106.

[38] Myers, R.H., Montgomery, D.C., and Anderson-Cook, C.M., 2016, *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, 4th Ed., John Wiley & Sons, Inc., Hoboken, New Jersey.

[39] Ferreira, S.L.C., Bruns, R.E., Ferreira, H.S., Matos, G.D., David, J.M., Brandão, G.C., da Silva, E.G.P., Portugal, L.A., dos Reis, P.S., Souza, A.S., and dos Santos, W.N.L., 2007, Box-Behnken design: An alternative for the optimization of analytical methods, *Anal. Chim. Acta*, 597 (2), 179–186.

[40] Chaudhari, S.R., and Shirkhedkar, A.A., 2019, Design of experiment avenue for development and validation of RP-HPLC-PDA method for determination of apremilast in bulk and in *in-house* tablet formulation, *J. Anal. Sci. Technol.*, 10 (1), 10.

[41] Vera-Candioti, L., De Zan, M. M., Cámara, M. S., and Goicoechea, H. C., 2014, Experimental design and multiple response optimization. Using the desirability function in analytical methods development, *Talanta*, 124, 123–138.

[42] Çankaya, N., Bulduk, İ., and Çolak, A.M., 2019, Extraction, development and validation of HPLC-UV method for rapid and sensitive determination of colchicine from *Colchicum autumnale* L. bulbs, *Saudi J. Biol. Sci.*, 26 (2), 345–351.

[43] Dwiastuti, R., Noegrohati, S., Istyastono, E.P., and Marchaban, 2016, Formulation and physical properties observations of soy lecithin liposome containing 4-*n*-butylresorcinol, *AIP Conf. Proc.*, 1755, 160005.

[44] Amdoun, R., Khelifi, L., Khelifi-Slaoui, M., Amroune, S., Asch, M., Assaf-Ducrocq, C., and Gontier, E., 2018, The desirability optimization methodology; A tool to predict two antagonist responses in biotechnological systems: Case of biomass growth and hyoscyamine content in elicited *Datura stramonium* hairy roots, *Iran. J. Biotechnol.*, 16 (1), e1339.

[45] Kannappan, V., and Mannemala, S.S., 2014, Multiple response optimization of a HPLC method for the determination of enantiomeric purity of S-Ofloxacin, *Chromatographia*, 77 (17), 1203–1211.

[46] Snyder, L.R., Kirkland, J.J., and Dolan, J.W., 2010, *Introduction to Modern Liquid Chromatography*, 3rd Ed., John Wiley & Sons, Inc., New Jersey.

[47] Munawar, T.M., Prakash, D.V.S., and Vangalapati, M., 2019, Development of response surface methodology for optimization of parameters and quantitative analysis of chebulinic acid from composition of medicinal herbs by HPLC, *Saudi J. Biol. Sci.*, 26 (7), 1809–1814.

Multiple Response Optimization of an HPLC Method for Analyzing Resorcinol and 4-n-Butyl Resorcinol in Lipid Nanoparticles

ORIGINALITY REPORT

15%	6%	13%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

3%

★ Riswanto Florentinus Dika Octa, Rohman Abdul, Pramono Suwidjiyo, Martono Sudibyo. "The employment of UV-Vis spectroscopy and chemometrics techniques for analyzing the combination of genistein and curcumin", Journal of Applied Pharmaceutical Science, 2021

Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		