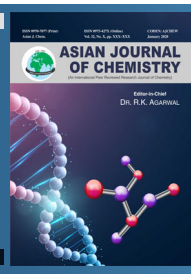
Asian Journal of Chemistry; Vol. 32, No. 12 (2020), 3135-3143

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22635



RP-HPLC Method for Determination of Salbutamol and Bromhexine in Syrup: Modelling and Optimization by Response Surface Methodology

CHUNG DUONG DINH[1,*], YEN NGUYEN NGOC THI[1], KHANH QUAN NGUYEN HUU[1], DUY CHINH NGUYEN[2], UNG THANH DAT[2] and THUY CA THI[3]

[1]Department of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh City, 700000, Vietnam
[2]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, 700000, Vietnam
[3]Faculty of Basic Science, University of Medicine and Pharmacy Ho Chi Minh, 217 Hong Bang, Ward 11, District 5, Ho Chi Minh City, 700000, Vietnam

*Corresponding author: E-mail: ddchung@ntt.edu.vn

Received: 13 January 2020; *Accepted*: 6 October 2020; *Published online*: 7 December 2020; AJC-20152

In present work, the RP-HPLC method was established for the determination of bromhexine and salbutamol in syrup by using a design of experiment approach. The Plackett-Burman design was applied to screen the influence of independent variables (ratio of organic solvent and pH in mobile phase, flow rate, column temperature, sample injection volume and detection wavelength) on the output data of chromatographic signals (peak area, tailing factor, theoretical plates, resolution) of bromhexine and salbutamol. The Pareto diagram shows that the selected variables affect mainly target function. A central composite design has been used to optimize the values of main factors and Design expert® software predicts the interaction and quadratic model to evaluate the impact of input parameters on output. The optimal conditions were determined with the support of response surface methodology for flow rate 0.9 mL/min, temperature 25 °C and 60% methanol in water with 0.06% orthophosphoric acid as the mobile phase. Good linearity was observed in the concentration range of 8-48 µg/mL for bromhexine and 4-24 µg/mL for salbutamol with a significantly high correlation coefficient ($R > 0.999$). The limit of detection and limit of quantitation were 0.32 and 0.96 µg/mL, respectively for bromhexine and 0.08 and 0.25 µg/mL, respectively for salbutamol. This method was validated according to ICH guidelines.

Keywords: Salbutamol sulfate, Bromhexine hydrochloride, Response surface methodology.

INTRODUCTION

The chemical name of bromhexine hydrochloride is *trans*-4-((2-amino-3,5-dibromo-benzyl)amino)cyclohexanol hydrochloride. Clinically, bromhexine is an expectorant ingredient since it enhances the transport of mucus in the airway by reducing the adhesion of mucus on the furry epithelium. This oral mucolytic agent breaks acid mucopolysaccharide of sputum on the airway epithelium and prevents the production of viscous mucus, which is easier to widen the respiratory tract.

Salbutamol sulfate named *bis*[(1RS)-2-[(1,1-dimethyl ethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)-phenyl]-ethanol]sulfate is a selective β-2-adrenergic receptor agonist. Salbutamol is used to relieve bronchospasm through the increase of the intracellular cyclic adenosine monophosphate (cAMP) in asthma or chronic obstructive pulmonary disease (COPD) [1]. The combination of bromhexine and salbutamol is used to treat asthma and bronchitis. It is usually administrated by the inhaled route for a direct effect on the upper respiratory system.

Various approaches have been addressed for separate or simultaneously determinate bromhexine and salbutamol in pharmaceutical dosage such as spectrophotometry [2-5], HPTLC [6,7] and RP-HPLC [6,8-11]. Among these, HPLC was used as a routine analysis for many active ingredients in medicine because of speed and high selectivity, accuracy and reproducibility. However, it still is affected by many parameters concerning instruments and accessories, stationary phase, mobile phase composition and environmental factors. It is common for optimization studies to adopt response surface methodology (RSM) as the tool to select optimal process parameters. RSM involves the establishment of quadratic function

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

with the desired outcome denoted as the dependent variable (response) and process parameters and their interactions as independent variables. The model will then be estimated using real experimental data, constructed by a pre-specified experiment design, to allow calculation of optimal response and its corresponding process parameters. RSM has been employed in various chemical processes such as isolation of natural products [12-18], adsorption [19] or product manufacture [20]. In this study, the Plackett-Burman design and response surface methodology (RSM)-central composite design (CCD) tool were applied to optimize the chromatographic conditions of the quantitative process the bromhexine and salbutamol. Plackett-Burmann design is a useful screening method for identifying the most critical factors that influence output responses [21-28].

The values of these factors are optimized with a few experimental runs as possible by RSM. The new approach helps to shorten analytical time with simple elution process and validate procedure to ensure linearity, precision, the limit of detection, the limit of quantitation and robustness following the guideline of International Conference on Harmonisation (ICH).

EXPERIMENTAL

Drugs and chemicals: Salmoldil® expectorant syrup was manufactured in India by FDC Limited. All chemicals, including phosphoric acid, sodium hydroxide (pure analysis), HPLC-grade ethanol, methanol, acetonitrile, were purchased from Merck (Singapore). Water was deionized by using an Arium® Pro system (Sartorius-Germany). Standard references including salbutamol sulfate, bromhexine hydrochloride (purity ≥ 98%) were purchased from The Institute of Drug Quality Control - Ho Chi Minh City, Vietnam.

Equipments and chromatography conditions: Mettler Toledo analytical balance MS105DU (max 120 g, 0.01 mg). Ultrasonic tank Elma (Germany). Agilent 1260 Infinity HPLC system (USA) with diode-array detector (DAD). The separation was achieved on a Gemini C18 column (250 mm × 4.6 mm, 5 µm). Data were analyzed by Chemstation software. Chromatographic elution with a mobile phase containing methanol and water with 0.06% orthophosphoric acid at the ratio of 60: 40, the flow rate of 0.9 mL/min, kept at 25 °C. Detection was at the wavelength of 225 nm. The injection volume was 20 µL.

Sample preparation: The sample solution: 5 mL syrup was transferred into 50 mL volumetric flask add 35 mL of methanol sonicate for 5 min and made up volume up to the mark using methanol. Using pipette taken 5 mL volume of solution was transferred into 20 mL volumetric flasks and the volume was filled up to the mark with mobile phase to obtain the final concentrations of 10 and 20 µg/mL for salbutamol and bromhexine, respectively. Filter through a 0.45 µ nylon filter paper (Sartorius, Germany).

Preparation of stock standard solution and establishment of the calibration curve: The stock solution was prepared at the concentration of 1 mg/mL salbutamol and 2 mg/mL for bromhexine in methanol. Dilution of the stock standard solution with mobile-phase obtained concentrations of 4-24 µg/mL, 8-48 µg/mL for salbutamol and bromhexine, respectively. Two factors were solution concentrations and peak areas that were used as responses for regression analysis to build up a calibration curve.

Optimization of HPLC parameters: The experiment was designed according to the Plackett-Burman matrix to screen for important factors that affect the chromatographic parameters of bromhexine and salbutamol, such as theoretical plates, retention time, peak area, peak tailing factor, resolution of bromhexine and salbutamol peak. The low (-1) and high (+1) values of the 06 factors in the 12 experiments are listed in (Table-1) [29]. The Pareto charts were established to express the influence of each input variable on the outputs. The three factors (Table-2) that most influenced the results of the survey were chosen to conduct experiments according to RSM to determine the optimum value and were studied at 3 levels in the 20-experiment RSM (Table-3) [30].

Herein, two-order polynomial equations could be constituted to clarify the mathematical relationship between the independent variables (x) and the responses (y) as below equation (eqn. 1):

$$y = f(x) = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (1)$$

TABLE-1
EXPERIMENTAL DESIGN MATRIX BY PLACKETT-BURMAN

Exp. No.	Factors code						Factors actual					
	A	B	C	D	E	F	A. Methanol (%)	B. Orthophosphoric acid (%)	C. Flow rate (mL/min)	D. Sample injector (µL)	E. Wavelength (nm)	F. Column temp. (°C)
1	–1	–1	+1	–1	+1	+1	55	0.01	1.2	5	230	40
2	+1	–1	+1	+1	+1	–1	65	0.01	1.2	25	230	25
3	+1	–1	+1	+1	–1	+1	65	0.01	1.2	25	220	40
4	–1	–1	–1	–1	–1	–1	55	0.01	0.8	5	220	25
5	+1	+1	+1	–1	–1	–1	65	0.10	1.2	5	220	25
6	+1	+1	–1	+1	+1	+1	65	0.10	0.8	25	230	40
7	–1	+1	–1	+1	+1	–1	55	0.10	0.8	25	230	25
8	+1	+1	–1	–1	–1	+1	65	0.10	0.8	5	220	40
9	–1	–1	–1	+1	–1	+1	55	0.01	0.8	25	220	40
10	+1	–1	–1	–1	+1	–1	65	0.01	0.8	5	230	25
11	–1	+1	+1	+1	–1	–1	55	0.10	1.2	25	220	25
12	–1	+1	+1	–1	+1	+1	55	0.10	1.2	5	230	40

TABLE-2
LIST OF VARIABLES FOR OPTIMIZATION

Independent factors	Unit	Code	Levels		
			–1	0	+1
Methanol	% (v/v)	x_1	55	55	65
Orthophosphoric acid in mobile phase	% (v/v)	x_2	0.01	0.55	0.1
Flow rate	mL/min	x_3	0.8	1	1.2

TABLE-3
EXPERIMENTAL DESIGN AND RESPONSES

Run	Independent factors			Retention time (y_1) of bromhexine		Tailing factor (y_2) of bromhexine peak	
	X_1	X_2	X_3	Actual (%)	Predicted (%)	Actual (%)	Predicted (%)
1	1	–1	–1	3.906	3.909	0.838	0.831
2	1	–1	1	2.612	2.634	0.890	0.892
3	1	1	1	2.730	2.726	0.966	0.959
4	1	0	0	3.233	3.203	0.946	0.954
5	0	–1	0	3.889	3.833	0.952	0.940
6	0	0	1	3.400	3.371	1.018	1.016
7	–1	–1	1	4.289	4.285	1.000	0.992
8	1	1	–1	4.120	4.130	0.892	0.904
9	–1	0	–1	6.850	6.874	1.105	1.086
10	–1	1	0	5.659	5.710	1.084	1.076
11	0	0.5	–0.5	4.606	4.652	0.999	1.016
12	–1	–1	–1	6.517	6.516	0.988	1.008
13	–1	1	–1	7.174	7.102	1.073	1.074
14	–1	1	1	4.743	4.742	1.042	1.053
15	0	–1	–1	4.882	4.921	0.919	0.916
16	1	1	1	2.734	2.726	0.963	0.959
17	–1	–1	1	4.282	4.285	0.989	0.992
18	1	–1	1	2.607	2.634	0.886	0.892
19	1	1	–1	4.123	4.130	0.915	0.904
20	1	–1	–1	3.936	3.909	0.831	0.831

where y is the predicted response; are the independent variables. The parameter b_0 is the model constant; b_1, b_2, b_3 is the linear coefficient; b_{11}, b_{22}, b_{33} is the second-order coefficient and b_{12}, b_{13} and b_{23} is the interaction coefficient. The Design-Expert® Software Version 7.0.0 from Stat-Ease, Inc. was used as ANOVA analysis.

Method validation: The optimal procedure was validated to ensure linearity, precision, the limit of detection and limit of quantitation following the guideline of ICH (2005).

RESULTS AND DISCUSSION

Method development and optimization: The optimal process for HPLC analytical method includes two steps: screening factors that affected highly on outputs by Plackett-Burmann design and optimizing their values by RSM. According to the Plackett-Burman matrix, ANOVA analysis of the model has statistical significance for all responses.

Specifically, the theoretical plates of salbutamol values are mainly influenced by three factors: flow rate, the ratio of methanol and orthophosphoric acid (Fig. 1a). The peak area of salbutamol is impacted by flow rate, sample injection volume and column temperature (Fig. 1b). The methanol content, flow rate and sample injection volume determine the peak tailing factor of salbutamol (Fig. 1c). Theoretical plates of bromhexine are affected by the concentration of methanol and orthophosphoric acid (Fig. 1d). The flow rate and sample injection volume have an influence on the peak area of bromhexine (Fig. 1e). The retention time of bromhexine is affected by the % methanol and column temperature (Fig. 1f). Peak tailing factor of bromhexine is influenced by % methanol, % orthophosphoric acid and the flow rate, injection volume, column temperature (Fig. 1g). All the factors surveyed have an effect on the resolution of bromhexine and salbutamol (Fig. 1h). From the analysis of Pareto charts, salbutamol peak satisfied all chromatographic parameters in the experimental range (theoretical plates $N > 3000$, tailing factor > 0.8 and < 1.5). However, bromhexine peak had theoretical plates $N > 3000$, but the tailing factor exceeds the permitted limit. The resolution of bromhexine peak and salbutamol is higher than 3 in the surveyed range. From the observed data, we defined the sample injection volume as 20 mL, detection at 225 nm and column temperature of 25 °C (room temperature) and selected factors such as % orthophosphoric acid, % methanol and the flow rate into the study. The following optimization through RSM is to provide a new solution for fast, specific, high-precision results for the analysis.

Optimization of chromatographic conditions with RSM: Three-factor independent including x_1: concentration of methanol in mobile phase (% v/v), x_2: concentration of orthophosphoric acid (% v/v) and x_3: flow rate (mL/min); ranges selected for independent variables during determination of method were; (55-65), (0.01-0.1) and (0.8-1.2), respectively for x_1, x_2 and

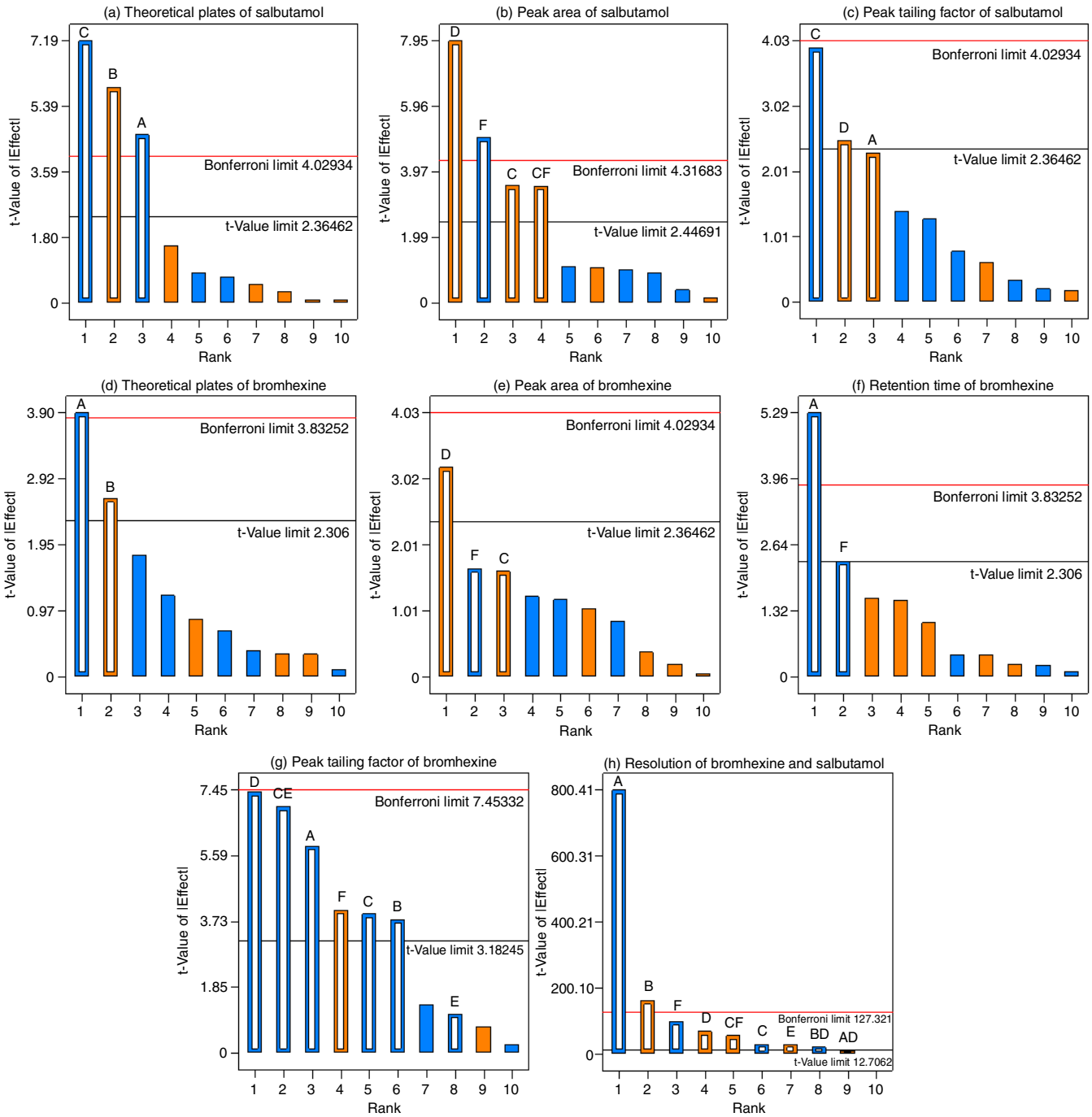


Fig. 1. Effect of variables [(A) methanol (%); (B) orthophosphoric acid (%); (C) flow rate (mL/min); (D) sample injection volume (mL); (E) wavelength (nm) and (F) column temperature (°C)] for responses ($p < 0.05$)

x_3. Important parameters considered responses were retention time (min) (y_1), tailing factor (y_2) of bromhexine. The experimental domain of the selected variables is reported as represented in Table-3. Under the optimized conditions, verification tests were performed to validate the suitability of mathematical models for predicting the optimal response values. The quadratic models were constituted to compare the responses as the guidance of Design-Expert® Software.

Table-3 shows a matrix of observed values and predicted values for testing and responses. After running 20 experi-ments, ANOVA analysis was performed to determine model fitness statistics such as P values, Correlation coefficient (r) and Adeq Precision (AP) ratios at a 95% confidence level. Table-4 indicates an adequate signal and therefore the model is significant for the separation process: P values < 0.05, high r^2 coefficients (> 0.9) and AP ratios > 25. The reproducibility of the model depends on the relative standard deviation (RSD) that is well within the limit of both responses (%RSD < 2). In addition, as shown in Fig. 2, two models exhibited the high predictability since the actual and predicted values were tightly plotted to the distribution line and model residuals also exhibited a random distribution. Therefore, two models for testing and responses

TABLE-4
ANOVA FOR THE RESPONSES MODELS

Source	Retention time (y_1) of bromhexine				Tailing factor (y_2) of peak bromhexine			
	Sum of squares	F-value	P-value	Comment	Sum of squares	F-value	P-value	Comment
Model	32.81	245.77	< 0.0001		0.0125	64.37	< 0.0001	
x_1	18.82	1268.89	< 0.0001		0.0713	368.41	< 0.0001	
x_2	0.35	23.44	< 0.0001		0.0161	83.14	< 0.0001	SD: 0.014
x_3	12.38	835.04	0.0187		0.0015	7.85	0.0187	%RSD:
x_1x_2	0.07	4.63	0.6889	SD: 0.12	0.0000	0.17	0.6889	0.009
x_1x_3	0.70	47.15	0.0006	%RSD: 2.68	0.0048	24.84	0.0006	Adj R:
x_2x_3	0.00	0.07	0.7065	Adj R: 0.991	0.0000	0.15	0.7065	0.9697
x_1^2	0.22	15.01	0.7228	AP: 49.85	0.0000	0.13	0.7228	AP: 25.93
x_2^2	0.07	4.89	0.0006		0.0047	24.31	0.0006	
x_3^2	0.16	10.47	0.2184		0.0003	1.73	0.2184	

Note: Significant at $p < 0.05$; x_1, x_2 and x_3 are the main factors; x_1^2, x_2^2 and x_3^2 are the square factors; x_1x_2, x_1x_3 and x_2x_3 are the interaction factors.

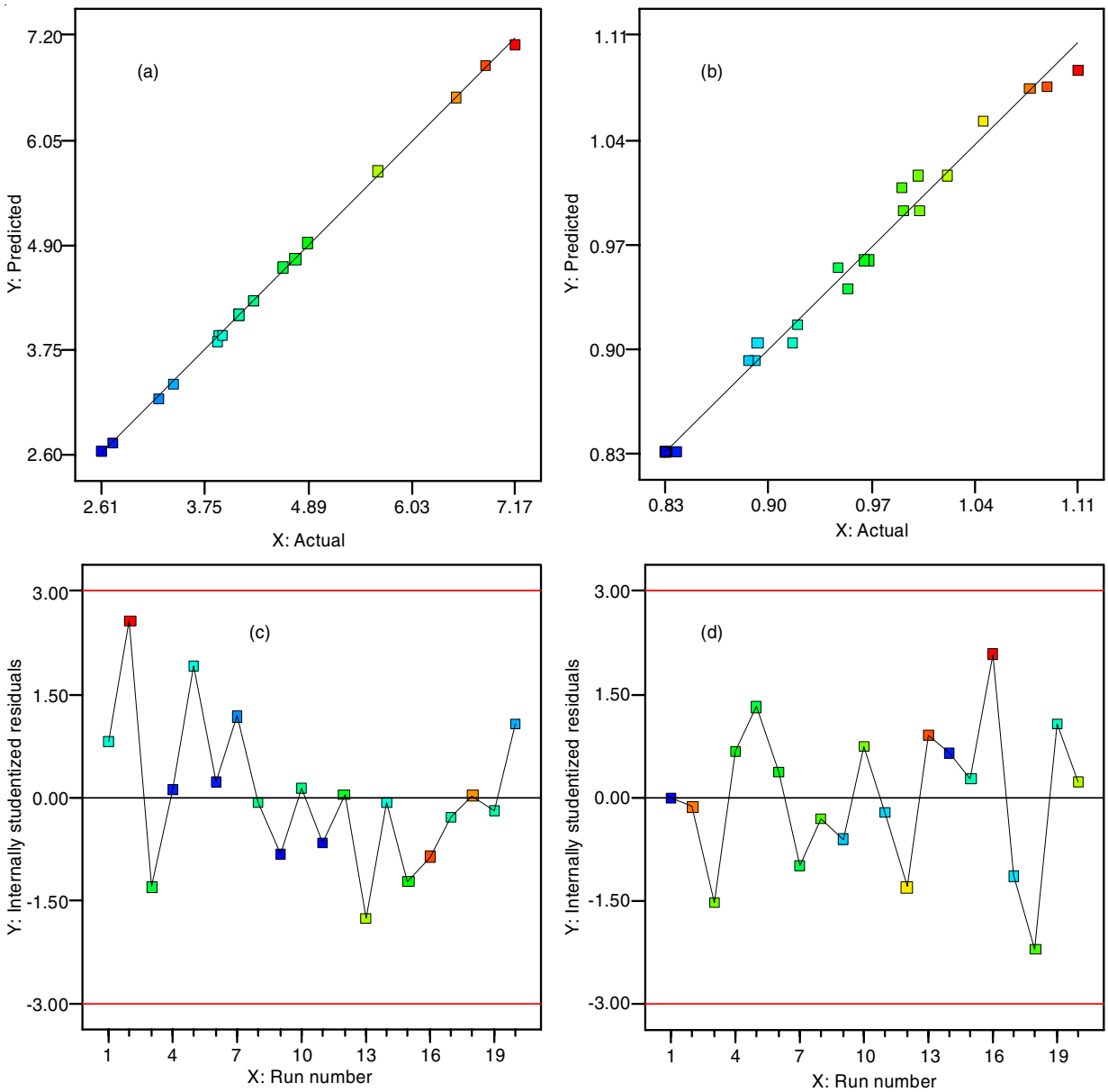


Fig. 2. Actual *versus* predicted plots (a and b) and residuals *versus* runs plots (c and d) for Rt of bromhexine (a and c) and tailing factor of bromhexine (b and d)

(retention time and tailing factor for bromhexine) reached the high compatibility with actual data at the 95% confidence level.

The value range for the optimization of independent variables is listed in Table-5. Analysis time depends on the retention time of each active ingredient, especially bromhexine, since this substance has the last retention peak on the chromatogram. The critical key is reducing the retention time of bromhexine for routine analysis. From the criteria in Table-5, the analysis procedure was optimized using Design-Expert software. The response surface plot for maximum desirability function presented in Fig. 3 shows that the mathematical model is suitable. To obtain maximum objective function, model predicts chromatographic parameters: % methanol 60% (v/v), % orthophosphoric acid 0.06% (v/v) and flow rate 0.9 mL/min.

TABLE-5
OPTIMIZATION OF THE INDEPENDENT VARIABLES

Name	Goal	Lower limit	Upper limit
% Methanol	Range	-1	1
% Orthophosphoric aicd	Range	-1	1
Flow rate	Range	-1	1
Retention time of bromhexine	Target = 4.6	2.607	7.174
Tailing factor of bromhexine	Range	0.831	1.105

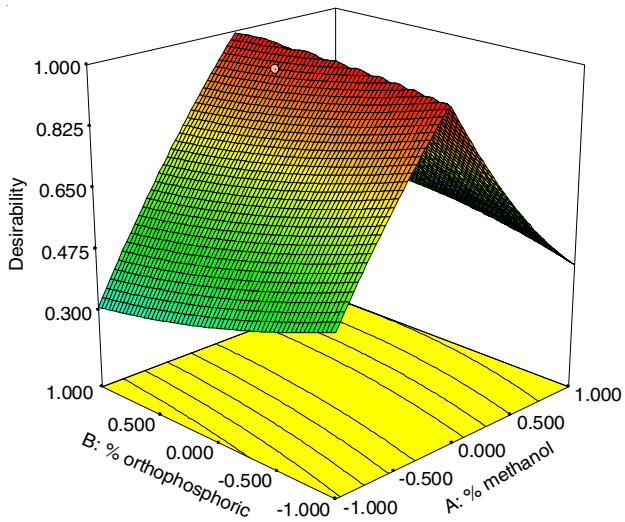


Fig. 3. Graphical representation of the overall desirable function

Selectivity: Fig. 4 shows the selectivity of salbutamol and bromhexine quantification process in syrup. In the method of comparing the relative retention times of the standard samples and test samples, the results of salbutamol peak appeared at 2.18 ± 0.03 (min) and the bromhexine peak appeared at 4.69 ± 0.03 (min). Peak purity was analyzed by DAD probe by comparing the absorption spectrum from appearance to signal end (Fig. 4e, f) and 3D spectrum (Fig. 4b), resulting in greater than 99% for each signal. Thus, the procedure for quantifying salbutamol and bromhexine is selective.

Linearity, accuracy, precision, limit of detection and limit of quantitation: The linearity was established at the concentration range of 4-24 µg/mL for salbutamol and 8-48 µg/mL for bromhexine. The stock standard solution was diluted with the mobile phase. Linearity studies revealed that the values of the coefficient of correlation (r) for the relationship between the concentration of reference standard and peak area are > 0.999. Fig. 5 shows the linearity data.

The accuracy was determined by assessing the repeatability test of an analytical method. The accuracy of the analytical method was determined using standard addition methods in which samples were added with a standard solution of drugs in a known amount in three different levels. In this study, three levels (80, 100 and 120%) from target value, corresponding to 8, 10 and 12 µg/mL (salbutamol) and 16, 20 and 24 µg/mL (bromhexine) were used. The recovery value obtained for salbutamol is 99.2-99.5%, while that of bromhexine is 99.5-99.8% (Table-6). The recoveries of salbutamol and bromhexine at each level were found to lie well within the acceptable criteria of bias 98-102%.

The precision of the analysis procedure was validated by estimate intra-day and inter day precision. The intra-day and inter day assay precision were studied at an analysis of 6 samples by sample treatment and chromatographic analysis according to the proposed procedure. The analytical method gives excellent repeatability with RSD < 2%. Precision is given in Table-7.

The sensitivity of instruments used for the analysis of salbutamol and bromhexine was expressed as the limit of detection (LOD) and limit of quantification (LOQ). Dilute the analyte concentration to the extent the smallest signal is still detected to determine LOD and dilute analyte concentrations.

TABLE-6
ACCURACY STUDY BY USING THE PROPOSED METHOD (n = 3)

% Level	Amount added (µg/mL)		Amount found in µg/mL (Mean ± S.D)		% Recovery (Mean ± S.D)	
	Salbutamol	Bromhexine	Salbutamol	Bromhexine	Salbutamol	Bromhexine
80	8	16	7.98 ± 0.04	15.99 ± 0.03	99.5 ± 0.42	99.8 ± 0.32
100	10	20	9.96 ± 0.02	19.98 ± 0.05	99.2 ± 0.21	99.6 ± 0.53
120	12	24	11.97 ± 0.05	24.03 ± 0.02	99.5 ± 0.54	99.5 ± 0.51

TABLE-7
INTRA-DAY AND INTER-DAY PRECISION FOR SALBUTAMOL AND BROMHEXINE (n = 6)

	Inter-day				Intra-day			
	Con. measured ± SD	Peak area ± SD	% Content*	% RSD	Con. measured ± SD	Peak area ± SD	% Content*	% RSD
Salbutamol	9.97 ± 0.02	166.74 ± 0.38	99.76	0.23	9.98 ± 0.09	166.81 ± 0.14	99.81	0.86
Bromhexine	19.91 ± 0.09	340.99 ± 1.48	98.38	0.57	19.96 ± 0.08	341.84 ± 1.32	99.79	0.39

*Average content compared to label content.

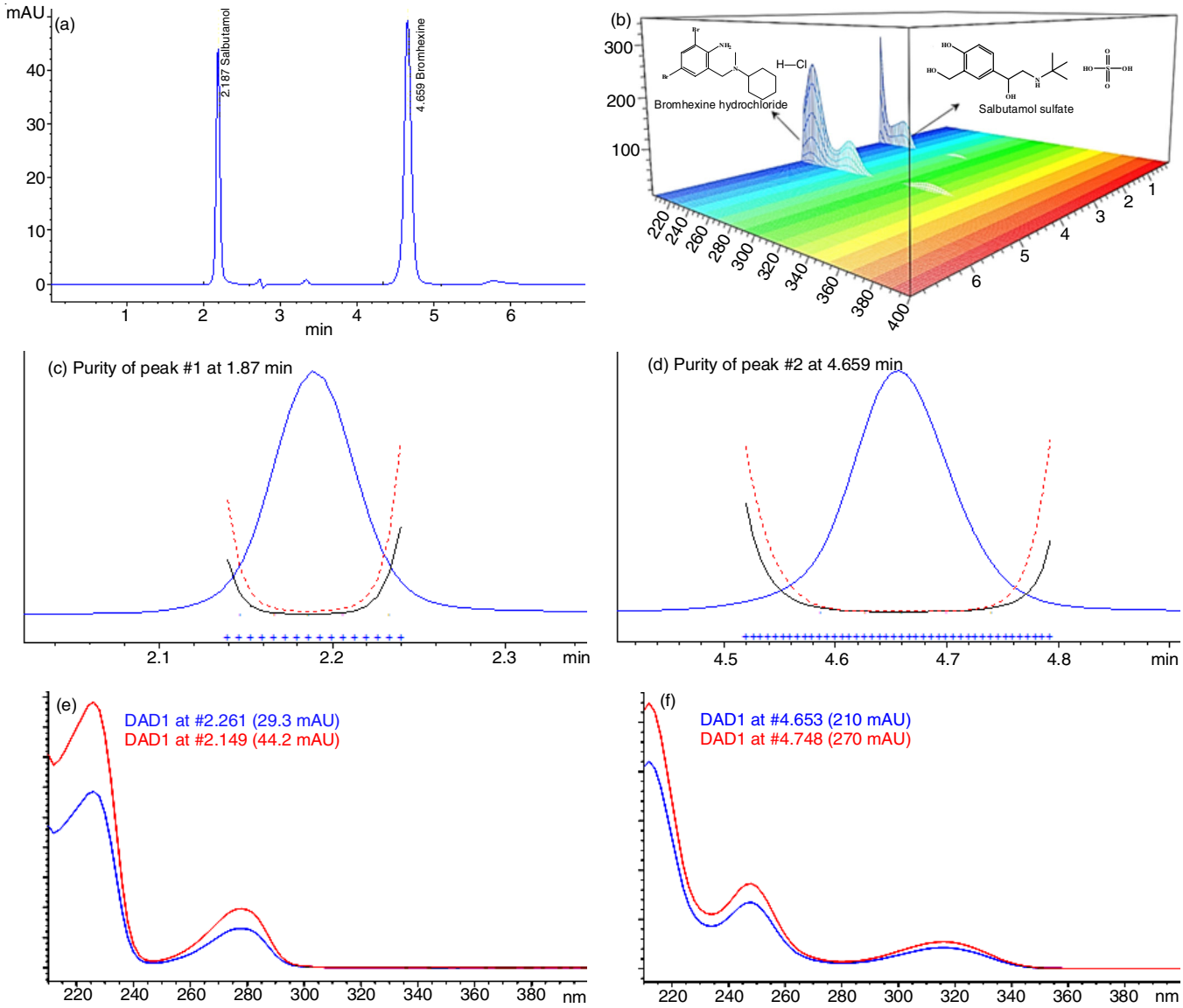


Fig. 4. Validation of selectivity: (a) Sample chromatography, (b) 3D spectrum, (c) Peak salbutamol purity, (d) Bromhexine peak purity, (e) UV spectrum of salbutamol and (f) UV bromhexine spectrum

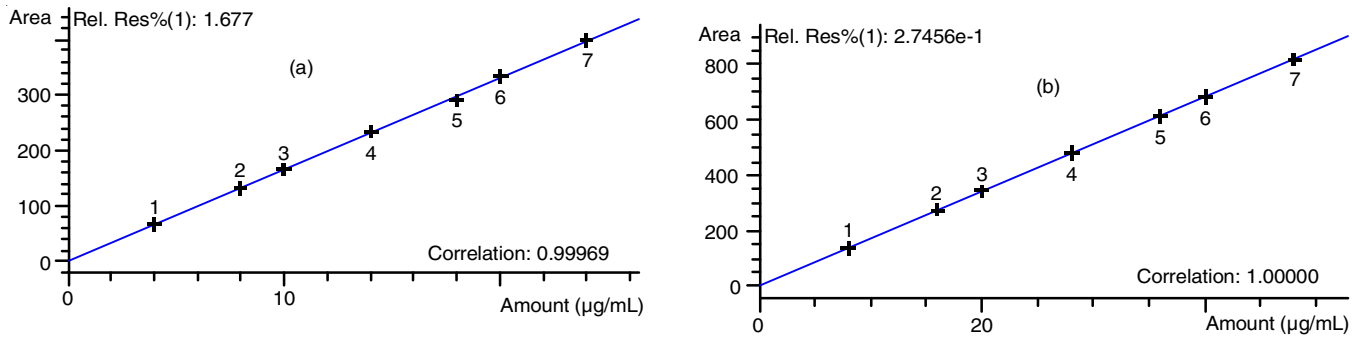


Fig. 5. Calibration curve expresses the correlation between concentration and peak area for (a) salbutamol and (b) bromhexine

The signal level still meets the accuracy and level correct to determine the LOQ. Measure signals obtained from a blank sample (N) and test sample (S). Set the S/N ratio. The concentration of the sample at the point where S/N = 3 and S/N = 10 are LOD and LOQ, respectively. The results are as follows: LOD and LOQ for salbutamol 0.08 & 0.25 µg/mL and 0.32 & 0.96 µg/mL for bromhexine, respectively. Chromatogram Fig. 6 shows concentrations at LOD and LOQ.

Table-8 shows the chromatographic parameters of the analytical method compared to reported works.

Application of the method in marketed formulation: The proposed method was applied to three samples of three

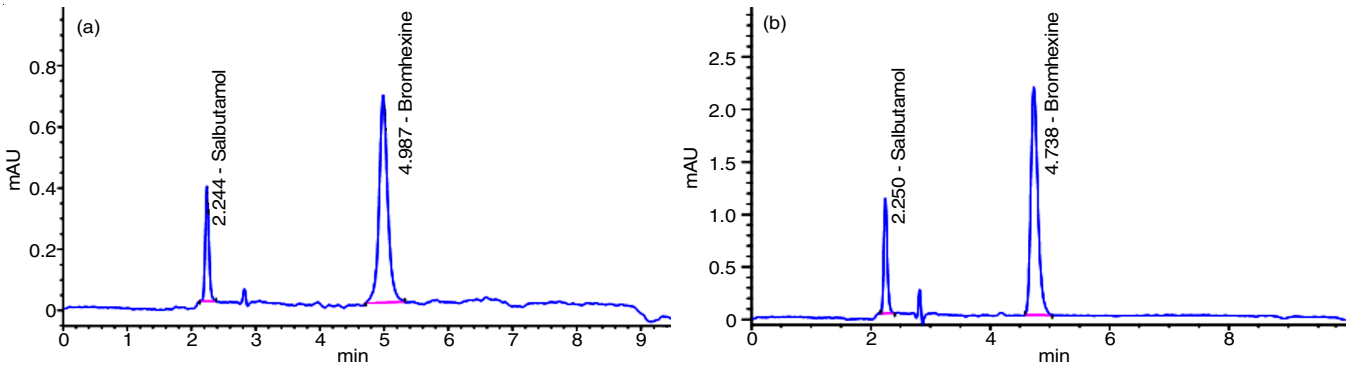


Fig. 6. (a) Chromatogram (a) LOD concentration, (b) LOQ concentration

TABLE-8
CHROMATOGRAPHIC PARAMETER IN COMPARISON TO PUBLISHED METHODS

Articles	Method	Mobile phase	Stationary phase	Wavelength (nm)	Ref.
Salbutamol and bromhexine	RP-HPLC	Isocratic elution: 60% acetonitrile, 20% methanol 20% phosphate buffer (pH 4)	Wakosil-II C18	224	[8]
Salbutamol and bromhexine	HPLC	Isocratic elution: 35% acetonitrile and 65% solution 0.1% triethylamine (pH 3.0)	Zorbax Eclipse C18 (250 × 4, 6 mm, 5 µm)	225	[9]
Salbutamol, bromhexine and etofylline	HPLC	Gradient elution: 30% acetonitrile and 70% solution ammonium acetate buffer 200 mM (pH 4.5)	Spherisorb C18 (250 × 4, 6 mm, 5 µm)	275	[6]
Ambroxol, bromhexine, chlorpheniramine, Salbutamol/terbutamine, guaifenesin, pseudoephedrine and triprolidine	HPLC	Isocratic elution: 35% acetonitrile, 4% sodium hexanesulphonate 250 mM, 10% sodium ammonium acetate 200 mM (pH 3.0) and 51% water	Gemini® NX C18 (250 × 4, 6 mm, 5 µm)	254	[10]
Salbutamol, oxtriphylline and bromhexine	RP-HPLC	Isocratic elution: 35% acetonitrile and 65% phosphate buffer	Kromasil C18 (250 × 4, 6 mm, 5 µm)	260	[11]
Salbutamol, etofylline and bromhexine	HPLC	Isocratic elution: 35% acetonitrile and 65% potassium di-hydrogen phosphate 100 mM (pH 3.0)	Shim Pack C18 (250 × 4, 6 mm, 5 µm)	225	[12]
Salbutamol and bromhexine	RP-HPLC	Isocratic elution: 60% methanol and 40% solution 0.06% orthophosphoric	Gemini® NX C18 (250 × 4, 6 mm, 5 µm)	225	Present study

different batches of marketed Salmoldil® expectorant syrup for determining the content of salbutamol and bromhexine. Each sample was analyzed in three replicates. About 5 mL of syrup formulation was diluted up to mark with the mobile phase in a 50 mL volumetric flask. The dilution process was continued to get a nominal concentration of 10 and 20 µg/mL of salbutamol and bromhexine, respectively. The results showed that the evaluated syrup contained bromhexine and salbutamol in the range as labeled.

TABLE-9
QUANTITATIVE RESULTS OF BROMHEXINE AND SALBUTAMOL IN PHARMACEUTICAL DOSAGE FORMS

Concentration of drugs	
Bromhexine	Salbutamol
3.86 ± 0.08 mg/5 mL	1.91 ± 0.002 mg/5 mL
3.98 ± 0.04 mg/5 mL	1.97 ± 0.001 mg/5 mL
4.01 ± 0.01 mg/5 mL	1.88 ± 0.001 mg/5 mL

Conclusion

Plackett-Burman multi-element experimental design and the surface response method are powerful tools for screening and optimizing the value of system elements in developing the HPLC technique. The results of the study have been modeled, optimized for chromatographic conditions to analyze the content of bromhexine and salbutamol in syrup. An analytical method has been developed and validated in compliance with ICH guidelines. Thus, the HPLC technique can be used as a routine procedure for quality control in a pharmaceutical combination of bromhexine and salbutamol.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. G.K. McEvoy, AHFS Drug Information: Essentials 2006-2007, American Society of Health System (2006)
2. R.V. Rele, *Res. J. Pharmacy Technol.*, **8**, 702 (2015); https://doi.org/10.5958/0974-360X.2015.00111.0
3. H. Dave, R. Mashru and A. Thakkar, *Anal. Chim. Acta*, **597**, 113 (2007); https://doi.org/10.1016/j.aca.2007.06.035
4. I. Habib, M. Hassouna and G. Zaki, *Farmaco*, **60**, 249 (2005); https://doi.org/10.1016/j.farmac.2004.11.007
5. K. Abdel, S.M.Attia, M.W.I. Nassar and A. Osman, *Anal. Chem. Indian J.*, **16**, 475 (2016).

6. A. Tyagi, N. Sharma, K. Mittal, R. Mashru, T. Bhardwaj, J. Malik and A. Thakkar, *Pharm. Anal. Acta*, **6**, 350 (2015); https://doi.org/10.4172/2153-2435.1000350
7. H. Dave, R. Mashru and A. Patel, *J. Pharm. Sci. Res.*, **2**, 143 (2010).
8. P.S. Pai, G.K. Rao, M.S. Murthy, A. Agarwal and S. Puranik, *Indian J. Pharm. Sci.*, **71**, 53 (2009); https://doi.org/10.4103/0250-474X.51957
9. R.V. Rele and R.N. Mali, *Der Pharm. Chem.*, **5**, 273 (2013).
10. P.M. Njaria, K.O. Abuga, F.N. Kamau and H.K. Chepkwony, *Chromatographia*, **79**, 1507 (2016); https://doi.org/10.1007/s10337-016-3158-1
11. K. Chakravarthi and N. Devanna, *Asian J. Chem.*, **29**, 1629 (2017); https://doi.org/10.14233/ajchem.2017.20647
12. N. Rao and K.D. Gawde, *Asian J. Pharm. Clin. Res.*, **11**, 378 (2018); https://doi.org/10.22159/ajpcr.2018.v11i8.26119
13. F.D.O. Riswanto, A. Rohman, S. Pramono and S. Martono, *J. Appl. Pharm. Sci.*, **9**, 125 (2019); https://doi.org/10.7324/JAPS.2019.91018
14. M.W. Nam, J. Zhao, M.S. Lee, J.H. Jeong and J. Lee, *Green Chem.*, **17**, 1718 (2015); https://doi.org/10.1039/C4GC01556H
15. M.K. Ali, N. Outili, A.A. Kaki, R. Cherfia, S. Benhassine, A. Benaissa and N.K. Chaouche, *Foods*, **6**, 64 (2017); https://doi.org/10.3390/foods6080064
16. B. Guldiken, D. Boyacioglu and E. Capanoglu, *Food Anal. Methods*, **9**, 1876 (2016); https://doi.org/10.1007/s12161-015-0370-9
17. H.S. Arruda, G.A. Pereira and G.M. Pastore, *Food Anal. Methods*, **10**, 100 (2017); https://doi.org/10.1007/s12161-016-0554-y
18. N. Anuar, A.F.M. Adnan, N. Saat, N. Aziz, and R.M. Taha, *The Sci. World J.*, **2013**, 810547 (2013); https://doi.org/10.1155/2013/810547
19. J.U. Ani, U.C. Okoro, L.E. Aneke, O.D. Onukwuli, I.O. Obi, K.G. Akpomie and A.C. Ofomatah, *Appl. Water Sci.*, **9**, 60 (2019); https://doi.org/10.1007/s13201-019-0943-7
20. C. Ayabaca and C. Vila, *Materials*, **13**, 373 (2020); https://doi.org/10.3390/ma13020373
21. P.K. Sahu, N.R. Ramisetti, T. Cecchi, S. Swain, C.S. Patro and J. Panda, *J. Pharm. Biomed. Anal.*, **147**, 590 (2018); https://doi.org/10.1016/j.jpba.2017.05.006
22. K. Valliappan, K. Kamarajan, R. Manavalan and C. Muralidharan, *Indian J. Chem.*, **41A**, 1334 (2002).
23. P. Walter, *BioProcess Int.*, **9**, 72 (2011).
24. G. Srinubabu, C.A.I. Raju, N. Sarath, P.K. Kumar and J.V.L.N.S. Rao, *Talanta*, **71**, 1424 (2007); https://doi.org/10.1016/j.talanta.2006.04.042
25. S.L. Ferreira, A.O. Caires, T.S. Borges, A.M.D.S. Lima, L.O.B. Silva and W.N.L. dos Santos, *Microchem. J.*, **131**, 163 (2017); https://doi.org/10.1016/j.microc.2016.12.004
26. M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar and L.A. Escaleira, *Talanta*, **76**, 965 (2008); https://doi.org/10.1016/j.talanta.2008.05.019
27. L.V. Candioti, M.M. De Zan, M.S. Cámara and H.C. Goicoechea, *Talanta*, **124**, 123 (2014); https://doi.org/10.1016/j.talanta.2014.01.034
28. S.N. Politis, P. Colombo, G. Colombo and D. M. Rekkas, *Drug Dev. Ind. Pharm.*, **43**, 889 (2017); https://doi.org/10.1080/03639045.2017.1291672
29. B. Dejaegher and Y. Vander Heyden, *Acta Chromatogr.*, **21**, 161 (2009); https://doi.org/10.1556/AChrom.21.2009.2.1
30. D.B. Hibbert, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **910**, 2 (2012); https://doi.org/10.1016/j.jchromb.2012.01.020