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Green Approaches in Sample Preparation for the Estimation of Atenolol, Aspirin, Atorvastatin Calcium and Losartan Potassium in Bulk and its Pharmaceutical Dosage Form Prior to Chromatographic Technique

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ABSTRACT

Objective: A multivariate statistics method, simple, economical and eco friendly method for simultaneous estimation of Atenolol, Aspirin, Atorvastatin and Losartan potassium in bulk and its Pharmaceutical dosage form using liquid chromatographic method.

Method: The method has been developed by using C18 column (250 • 4.6 mm, 5 lm) with UV detection at 237 nm. The developed method optimized by using Quality by design (QbD) technique and Derringer's desirability function was applied. The sample solution is prepared by using dispersive liquid –liquid extraction (DLLME).

Results: Central Composite Design was used to study the effect of independent factors. Derringer's desirability function was applied to simultaneously optimize the retention time of last eluting peak (Atorvastatin calcium), capacity factor and resolution between Aspirin and Losartan potasium. The optimized method was validated according to ICH guidelines. The another important aspect is DLLME that the method uses microlitre amounts of organic solvent, produces low levels of waste and minimizing effluent treatment, which contributes to the environment and implements methods aimed green chemistry, making economic and environment safe for the people.

Conclusion: The method can be employed as an alternative in quality control routine analysis of pharmaceutical industry for quantification of Atenolol, Aspirin, Atorvastatin and Losartan potassium in bulk and its Pharmaceutical dosage form using liquid chromatographic method.

Keywords: optimization, QbD,DLLME, method validation, green chemistry

INTRODUCTION

Atorvastatin calcium (AC) is chemically described as [R-(R_,R_)]-2-(4- fluorophenyl)-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1Hpyrrole-1-heptanoic acid calcium salt trihydrate. It is an antilipidemic agent acting by inhibiting HMG-CoA reductase. Losartan potassium (LP) f2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol mono potassium salt is an angiotensin II receptor antagonist and Atenolol(AT) f4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzene acetamide is a beta blocker. AT and LP is used in the treatment of hypertension. Aspirin (AS) f2-acetyloxy-1-benzoic acid has an anti-platelet effect at the dose included in the selected combined dosage form [1,2] **Figure 1 a**, **b**, **c**, and **d** represented the structure of AS, AT, AC and LP.

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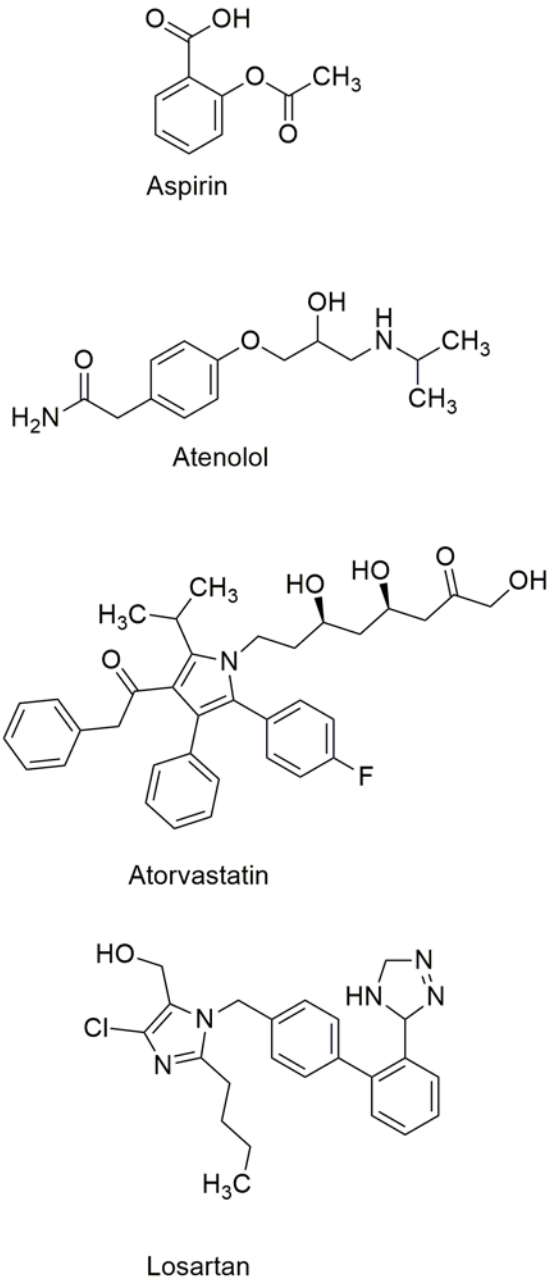


Figure 1. Chemical structure of (a) Aspirin, (b) Atenolol, (c) Atorvastatin and (d) Losartan

The literature survey reveals that a variety of spectrophotometric [3] and chromatographic [4-7] methods for stability indicating LC method has been reported for determination of AS and AC in pharmaceutical preparations in combination with other drugs. In the present paper an attempt has been made to develop an accurate, rapid, eco-friendly Quality by design method for the estimation of AS, AC, AT and LC pharmaceutical dosage form along with application of Dispersive liquid- liquid micro extraction (DLLME) International Conference on Harmonization (ICH) Q8 (R2), ICH Q9 guidelines provides specified analytical target profile (ATP) for identifying method operable design region (MODR) by analytical quality by design (AQbD) approach. ATP is a prospective summary of measurement requirements that ensure that the method is fit for the purpose where as MODR is based on a multivariate approach to evaluate the effects of various method inputs variable on method performance.

In accordance to FDA requirement of 2013, MODR needs to be conducted together with method validation [8, 9]. But a conference on Analytical QbD by FDA in 2014, the current (OFAT) approach in method development phase is not an appropriate method approach for routine analysis to be considered under regulatory flexibility [10, 11]. The Analytical QbD [12-14] is highly desired for a drug substance like AS, LP, AT and AC. The AC is poorly soluble in water and AS, AT, LP has reversed solubility profile between methanol and water that significantly

Table 1. Quality Target Product Profile (Starpill) Tablet

S. No	Quality Attributes	Target	Criticality
1	Dosage form	Tablet	Not applicable
2	Potency	75mg of Aspirin,10 mg of Atorvastatin, 50mg of Atenolol and Losartan potassium.	Not applicable
3	Physical description	Colorless round shape tablet	Not applicable
4	Appearance	Fine powder	Critical
5	Identity	Positive	Critical
6	Assay	98.00-102%	Critical
7	Impurities	Total impurity ≤ 1.0%	Critical
8	Content uniformity	98-102%	Critical
9	Water	0.5%	Critical

Table 2. Identification of Method of Assessment of Critical Quality Attributes

S. No	Critical Quality Attributes	Target	Method of Assessment
1	Appearance	Clear not more intense color than reference	Visual methods
2	Identity	Positive	FT-IR,UV, Melting point
3	Assay	98%-102%	HPLC(assay method), HPTLC
4	Impurities	Total impurity ≤ 1.0%	HPLC(Qualitative method), LC-MS
5	Heavy metals	≤ 10ppm	Atomic absorption Spectroscopy

affects the robustness of the LC method. The solubility character of AS, AC and LP and dual peak recommends the optimization is desirable for selecting a mobile phase ratio and pH. Hence, the pH, % aqueous phase, flow rate of mobile phase were considered as critical method variables in the experimental design. The DLLME [15,16] application are successfully utilized by Iverson NM et al. [17], Fatih Sen et al. [18], Yağmur Koskun et al. [19], Birgütay Şahin et al. [20], Sait Bozkurt et al. [21], Gaye Başkaya et al. [22], Birgütay Şahin et al. [23], Selda Sen et al. [24] and Zachary W et al. [25].We also used DLLME process for The preparation of sample solution. The CCl_4 is used as an extraction solvent and acetonitrile is act as a dispersing solvent. The volume of this solvent we are used only microlitre level. The DLLME process reduce the solvent volume litre level in to microlitre level. The DLLME process help to safe our environment.

MATERIALS

HPLC grade methanol, acetonitrile and water were purchased from Qualigens, Mumbai, India. AS, AT, AC and LP are procured from Bafna pharmaceuticals and formulation (Starpill tablet contains 75 mg of AS, 10 mg of AC and 50 mg of each AT and LP) procured from local market. All the drugs are 99.91% purity and authenticated by FT-IR method. A Shimadzu HPLC system consists of a LC-20AD solvent delivery system (pump), SPD-M20A photodiode array detector, Rheodyne injector with 20 μL loop volume, and LC-Solution assisted for data collections and processing. The Chromatographic separation was performed using phenomenex C_{18} 150 x 4.6 mm, 5µ column, detection wavelength is 237 nm and run time is 6.5 min. Data analysis was performed by using Design Expert (Version 7.0.1.0 Stat-Ease Inc., Minneapolis, MN, USA) trial version statistical software.

METHOD AND RESULTS

The quality target product profile (QTPP) has been used to identify critical quality attributes (CQA) of the product at **Table 1**. QTPP determines the quality of the product, which helps us to define targeted responses to be optimized in the life cycle of the product. Based on the CQAs, Analytical target profile (ATP) was designed with accuracy, precision, specificity, linearity were selected as method performance characteristics for assay (Q-parameter). Further the method of assessment of each CQA of the product has been identified and a representative method assessment for CQA is shown in **Table 2**. This work designed for development of robust HPLC methods to apply to determination 'Assay' component of QTPP and ATP. This type of approach is recommended in QbD based analytical methods to apply food and drug administration (FDA) and ICH Guidelines [26,27]. So far, no analytical method has been reported with ATP, risk assessment and MODR concepts.

Preparation of Standard Solution

A stock solution of standard (7.5 mg/mL of AS, 1 mg/mL of AC, 5.0 mg/ml each of the AT and LP) was prepared in diluent. Working solutions 7.5 µg mL^{-1} of AS, 1 µg mL^{-1} of AC, 5 µg mL^{-1} each of AT and LP were prepared from above stock solution by using diluent for assay determination.

Preparation of Sample Solutions

Twenty tablets of Starpill™ were weighed and their average weight was calculated. The tablets were crushed to a homogeneous powder and a quantity equivalent to one tablet (75 mg of AS, 10 mg of AC, 50 mg of each AT and LP) was weighed and transferred in a 100-mL volumetric flask. The (1 mL) was quantitatively transferred to a 10-mL volumetric flask, and solution was diluted to volume with the methanol.

Extraction by using DLLME Method

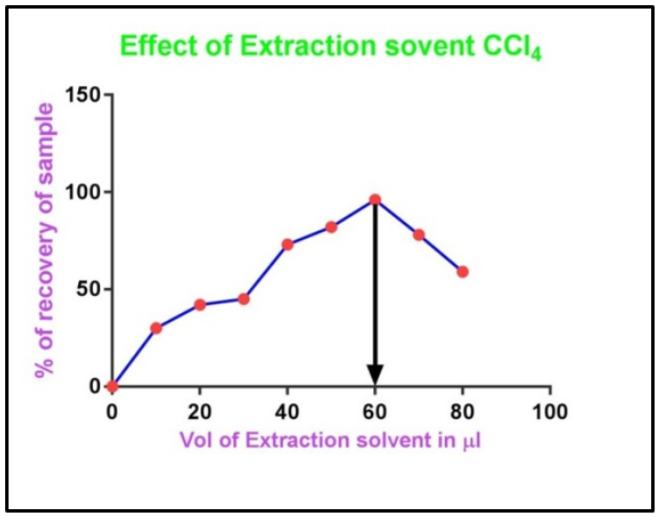
The 10 ml aliquots of the pretreated sample were placed in a 15 mL conical test tube. The 600 µL of carbon tetrachloride (CCl_4) and 1.5 ml acetonitrile (optimal values) was injected rapidly into the sample using a 2 mL syringe. A cloudy solution formed in the test tube and the analyte in drug sample was extracted into the fine droplets of CCl_4. Then, the mixture was centrifuged for 10 min at 5000 rpm. After centrifuging, the dispersed fine droplets of extraction were sediment and whitish interface was observed between the settled drop of CCl_4 and the upper aqueous phase in the test tube. The upper aqueous solution was removed with a syringe and the residual phase was dissolved in 500 µL acetonitrile. Finally, 20 µL of the extract was injected into the HPLC.

Effect of the Extraction Solvent Type and Volume

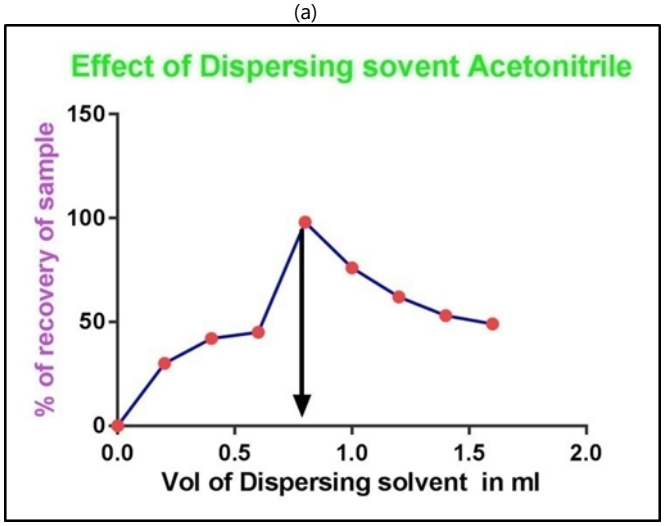
Carbon tetrachloride (CCl_4), chlorobenzene (C_6H_5Cl) and dichloromethane (CH_2Cl_2) were tried as extraction solvents. The series of experiments were performed by using 2000 µL acetonitrile as the dispersed solvent and 600 µL of several halogenated hydrocarbons. The results shows that the CH_2Cl_2, and C_6H_5Cl more soluble in aqueous solution compared to CCl_4. The volume of CCl_4 was varied in the range of 100-1000 µL, with other experimental conditions being constant. The extraction recovery increased by increasing the volume up to 600 µL. However, a reduction in the extraction recovery for compound occurred when the volume of CCl_4 exceeded 600 µL. This is probably due to the variation of the volume ratio between the dispenser and the extraction organic solvents. The decreased ratio lowers the amount of droplet formation available for extraction, thereby lowering the extraction efficiency. Based on the experimental results, 600 µL of CCl_4 was chosen as the optimal volume for the extraction solvent. The **Figure 2 a** shows the effect of Extraction solvent during sample preparation.

Effect of the Dispersed Solvent Type and Volume

The selection of the dispersed solvent is based on its miscibility in the organic (extraction solvent) and aqueous (sample solution) phases. The acetonitrile has the highest efficiency compared with acetone, methanol and ethanol. Therefore acetonitrile was selected as the dispersed solvent in the subsequent experiments. The influence of the volume of dispersed solvent was investigated by using 0.5, 1.0, 1.5, 2.0 and 2.5 mL .The extraction efficiency increases by increasing the volume of acetonitrile up to 1.5 ml and then decreases in volumes more than 1.5 ml. The increase in extraction efficiency was attributed to the much finer droplets and larger surface area of extraction solvent, obtained by increased acetonitrile volume. Decrease in extraction efficiency was related to the increase in the solubility of the analyte in the aqueous phase. Therefore, based on the obtained results, 1.5 ml of acetonitrile was chosen as optimum volume for dispersed solvent. The **Figure 2 b** shows the effect of dispersing solvent sample preparation.



(a)



(b)

Figure 2. (a) shows the effect of extraction solvent volume, (b) shows the effect of Dispersing solvent volume

Table 3. shows the experimental factors and levels used in central composite design

Factor	Name	Level (-1)	Level (0)	Level (+1)
A	Flow rate (mL min^{-1})	0.8	1.0	1.2
B	Buffer pH	2.5	3	3.5
C	Methanol (%v/v)	45	50	55

Experimental Design

The present AQbD work was carried out as per the cited literatures to investigate the impact of different variables on retention time (as method response) and to verify method performances. The levels of these variables are as follows: the flow rate (A) of mobile phase (0.8 and 1.2 ml/min), pH (B) of aqueous phase used in the mobile phase (2.5 and 3.5) and the proportion of the methanol (C) in the mobile phase (45% and 55%) which are given in **Table 3**. The retention time, capacity factor and resolution between II and III compound peak were used as response in experimental design as controlling response, which are expected to affect and control method responses. A Central composite design consisting of 3 factors at 3 levels was considered for experimental plan initially and after confirming that the process is a central composite design (CCD) was used. The experimental observations along with Factorial Design (DOE) plan are shown in **Table 4** and the statistical analysis is given in **Table 5**. The MODR was defined using all three variables. From MODR suitable method conditions were selected and subjected to verification of method performance like accuracy and precision (less than 2% RSD) and robustness as targeted response.

Table 4. Central composite rotatable design arrangement and responses for Atenolol, Aspirin, Atorvastatin calcium and Losartan potassium

Run	Type	Factor 1 A:flow rate in ml	Factor 2 B:pH	Factor 3 C:methanol concentration in mobile Phase	Response 1 Capacity factor	Response 2 Resolution b/w II and III peak	Response 3 last Retention time in min
1	Axial	1	2.1	50	1.4	3.15	12.7
2	Center	1	3	50	1.4	3.21	9.62
3	Axial	1	3.8	50	1.5	3.23	6.95
4	Center	1	3	50	1.4	3.23	9.7
5	Axial	1	3	58.40	1.57	3.42	6.98
6	Axial	0.66	3	50	1.38	3.23	9.55
7	Center	1	3	50	1.43	3.33	6.6
8	Fact	1.2	3.5	55	1.38	3.08	14.8
9	Fact	0.8	2.5	45	1.38	3.12	9.9
10	Fact	0.8	2.5	55	1.3	3.2	15.3
11	Center	1	3	50	1.35	3.28	9.84
12	Fact	1.2	2.5	55	1.57	3.54	7.96
13	Axial	1	3	41.59	1.37	3.16	12.2
14	Fact	0.8	3.5	45	1.5	3.56	7.85
15	Fact	0.8	3.5	55	1.43	3.23	12.54
16	Fact	1.2	2.5	45	1.6	3.45	8.16
17	Center	1	3	50	1.43	3.12	9.16
18	Fact	1.2	3.5	45	1.32	3.26	10.56
19	Axial	1.33	3	50	1.43	3.12	9.56
20	Center	1	3	50	1.43	3.23	9.16

Table 5. Regression model and statistical parameters obtained from ANOVA

Response	Reduced Regression equation	Adjusted R2	Model p Value (Not more than 0.05)	% CV	Adequate precision (NLT 4)
Capacity factor	Capacity = +0.34187 +1.70098 A 0.64241 B-0.034332C-0.90000AB +0.022500AC+5.00000E-003BC-2.00852 B^2+0.19676C2	0.863	0.0173	4.10	7.952
Resolution	Resolution b/w II and III peak - 5.10657+3.21282A+3.09334B+0.08342C-1.40000AB+0.020000 AC -0.03400BC	0.850	0.0313	3.19	7.633
Last Retention Time	last retension time = +52.68715-16.37358A-27.65493B+0.27511C+17.56250AB-0.75625AC +0.18650BC	0.85	0.0456	5.15	6.541

Statistical Analysis

The behavior of the system was explained by the following polynomial equation.

$$Y = b_0 + b_1A + b_2B_2 + b_3C_3 + b_{12}A_1B_2 + b_{23}B_2C_3 + b_{13}A_1C_3 + b_{123}A_1B_2C_3 \quad \text{(Eqn.1)}$$

where, Y is the response, b_0 is the intercept, b_1,b_2, b_3 are the regression coefficients of variables for X_1, X_2 and X_3, respectively. b_{12}, b_{23}, b_{13} are the regression coefficients for two factor interactions between variables and b_{123} is the coefficient for three factor interaction between $A_1B_2C_3$. Design expert software was used for the statistical analysis of the experimental observations and the analysis is given in **Table 5**.

It can be seen from **Table 6** that the coefficient of A (flow rate), B (pH), C (% of methanol phase) coefficients of variables and interactions are significantly contributing at more than 99% confidence level (P<0.1). B i.e. % aqueous phase has positive highest SS % contribution of 45%. The methanol concentration in the mobile phase and flow rate are the predominant factor to control retention time. pH of buffer phase B has negative coefficient and hence by increasing the B level retention time can be reduced. The strategy of optimization is to reduce A,C and increase B to control the retention time. ANOVA indicated that the process model with A1, B2, C3 along with interactions is highly significant at 99% Confidence level (P<0.1). Since the Curvature effect is significant and says it has a nonlinear relationship between Y and A,B,C, it requires going for CCD i.e. central composite design and

Table 6. Comparison of experimental and predictive values of different experimental runs under optimum conditions

Optimum Conditions	Selected Optimized Run						
1	A: flow rate (mL/min)	B: buffer pH	C:methanol mL/min	Response 1.Capacity factor	Response 2 Resolution	Response 3 last Retention time	Desirability Value
	0.8 ml	3.5	45ml	1.452	3.364	6.62 min	0.96 (limit NLT 0.8 and NMT 1)
	• Experimental Value						
	• Predictive Value			1.4836	3.4894	6.50 min	
	• % of Predicted Error			0.0102	0.0061	0.052	

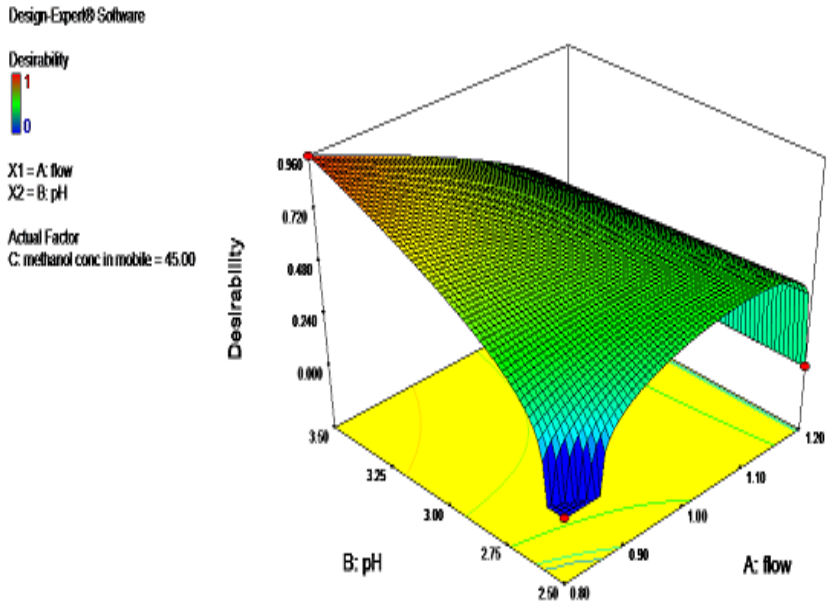


Figure 3. 3D Graphical representation of Derringers Desirability function (D=0.960)

accordingly the CCD plan and observed data are given below in **Table 6**. The following Quadratic model was obtained on application of Design expert software,

$$Y = 5.8778 - 0.0025A_1 + 2.9925B_2 - 0.8088C_3 - 0.4925A_1B_2 - 0.075A_1C_3 - 0.125B_2C_3 + 0.1178A_{12} + 1.1803B_{22} + 0.2768C_{32} \quad \text{(Eqn.2)}$$

The coefficient of determination (r^2) for the above process model was 0.9999. Hence the Process model is well valid to predict the behavior of the process and can be used for simulation of the process model. The design space or MODR region for robustness was achieved from contours **Figure 3** these regions offer robust processes parameters.

Contours

There could be different combinations, which may give a number of feasible solutions for robust process. A vs B with C as constant, B vs C with A as constant, A vs C with B as constant. Out of these combinations, whichever is the most desirable from the point of retention time that can be selected as a robust process. This contour space is called as design space in products and MODR in analytical works. The MODR that control the variation in response is obtained from contours a three dimensional plot and it resembles for other variable combinations. These three combinations are shown at **Table 6**, the contour gave the best design space covering the entire range of variables and retention time of 6 to 15 and was taken for verification purpose. It was also noted that the optimized 45% of (C) at flow rate 0.8 ml/min (A), the pH(B) was kept at 3.5 gives significant results, which offered several method advantages like column long life, mobile phase stability of analyte and symmetric elution. The mathematical model, the proposed contours was validated by experimental verification of predicted retention time (true) .The results are shown in **Table 7** and graph in **Figure 4 a**, **b**, **c**.

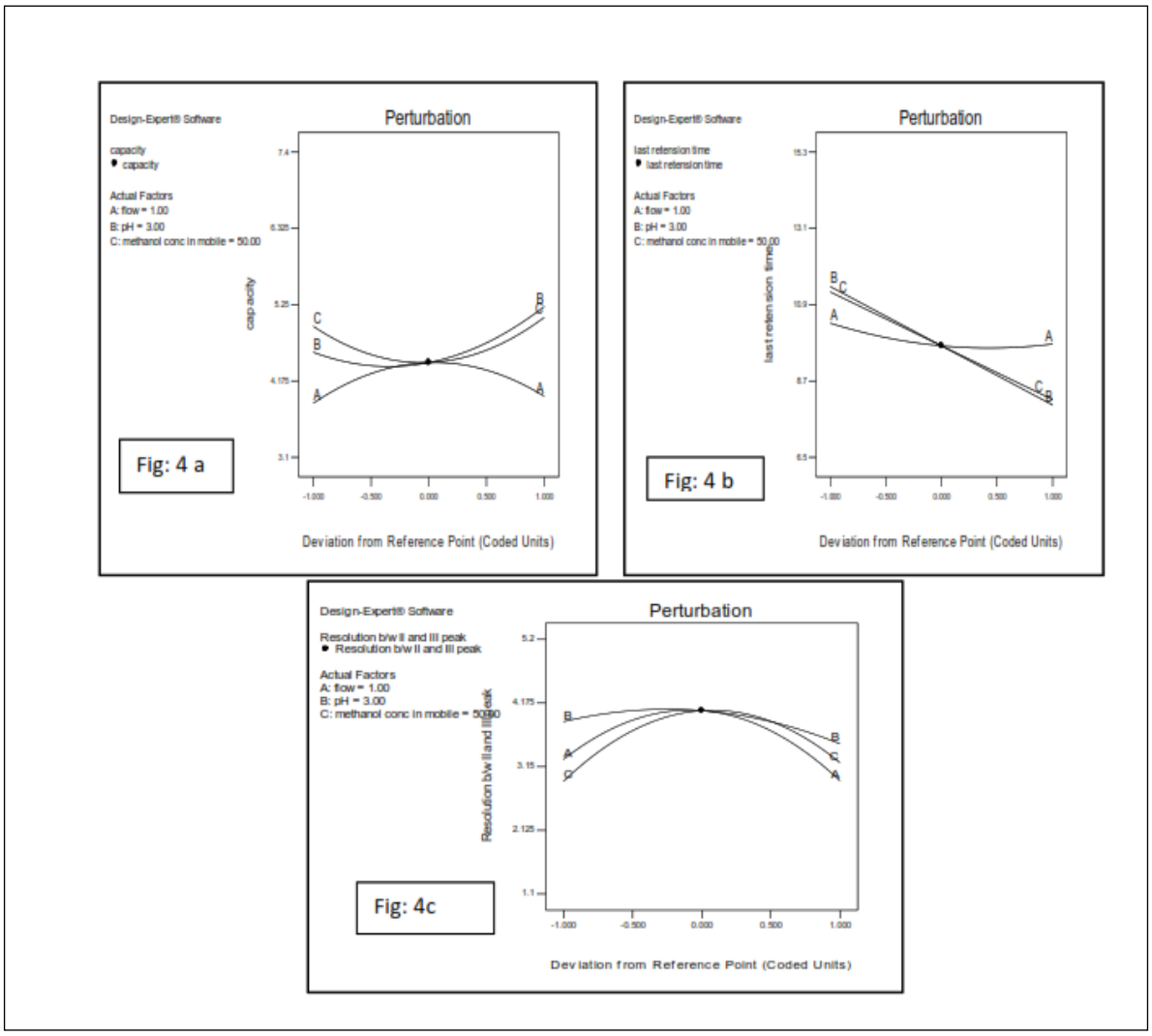


Figure 4. 4a,4b,4c Perturbation graph showing the each factor A,B and C

Chromatographic conditions after optimization

The trial method, HPLC was carried out by using a flow rate 1mL/min, pH adjusted to 3 and methanol concentration in the mobile phase (50%) was used, the time required for analysis is 12 min. After robust process was obtained, the HPLC analysis was carried out by using methanol (45, %v/v) as mobile phase, pH adjusted to 3.5 and flow rates of 0.8 ml/min on C18 analytical column, UV-PDA detection wavelength at 237 nm and 20 µl of injection volume, which gave a retention time (t_R) about 6.59 min. These parameters are within MODR and hence this design space has been validated and the time required for analysis of the four drugs reduced by nearly 50% (6.49 min). The results are shown chromatogram **Figure 5 a**, **b**.

Verification of method by method transfer and Validation of the robust method

The most robust method was verified on another manual RP-HPLC instrument in different laboratory and the robustness and other system suitability parameters were compared. The % assay result and its %RSD value were calculated. Accuracy and precision were compared. Method parameters for robust process were obtained from MODR of contour, and verified experimentally. The verified method was validated as per ICH Q2 (R2) [17] guidelines for assay method. Method performance like assay, precision and robustness was considered as target response.

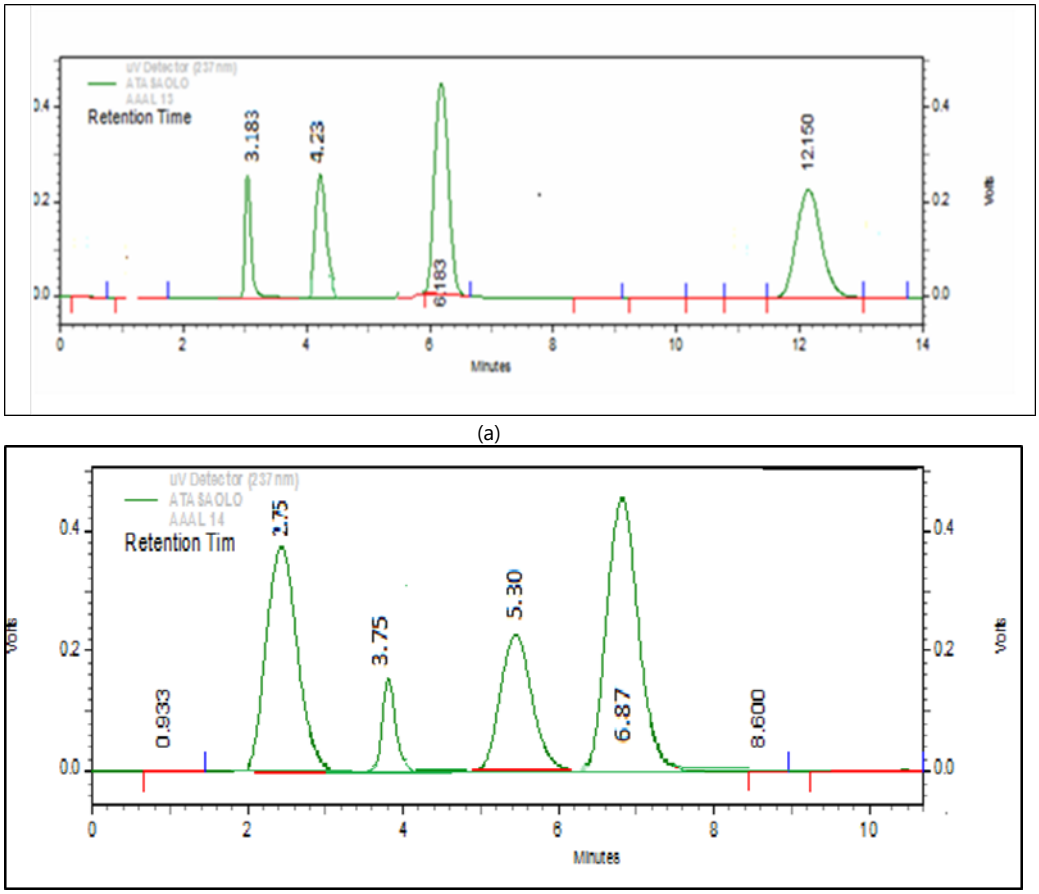


Figure 5. (a)Chromatogram of Atenolol (Rt 3.18min),Aspirin(Rt 4.23min),Losartan potassium (Rt 6.183min) ,Atorvastatin (Rt 12.152min) before the optimization, (b) Chromatogram of Atenolol (Rt 2.75min),Aspirin(Rt 3.75min),Losartan potassium (Rt 5.30min) ,Atorvastatin (Rt 6.87min) after the optimization

System Suitability Parameters (SST)

Chromatographic conditions were tested for SST in laboratories. 10 µg/ml of was injected in replicates through the column it can be detected at retention time 6.5 min with theoretical plates more than 8000 and a tailing factor of 1.12. SST parameters are within the limit in both laboratories.

Linearity

The linearity of peak area responses versus concentrations were studied from 4 to 14 µg mL^{-1} atorvastatin, 5 to 25 µg mL^{-1} Aspirin and 1 to 10 µg mL^{-1} for Atenolol and losartan potassium. A linear response was observed over the examined concentration range and the regression equation was observed and it was good against the targeted value and the values are shown on **Table 7**.

Table 7. System suitability parameters and validation parameters of proposed method

S. no	Parameter	Validation result
1	Stationary phase (Column type)	Phenomenex C_{18} (150 x 4.6 mm, 5µ column
2	Mobile phase	Acetonitrile: phosphate buffer (pH 3):methanol(25:30:45)
3	Flow rate	0.8mL/min
4	Detection wavelength	237nm
5	Retention time	2.3±0.1 min- Atenolol,3.7±0.1 min- Aspirin, 5.1 ±0.1 min Losartan and 6.5±0.1 min Atorvastatin.
6	Theoretical plates*	>8000
7	Tailing factor	1.12
8	Assay (%)*	98-102%
9	Precision *(% RSD) Interday Intraday	 (1.55 Atenolol,0.94-Losartan,1.44atorvastatin,0.88 aspirin) Atenolol-1.26,Losartan-0.76,Atorvastatin-1.36,Aspirin-0.55
10	Repeatability *(%RSD)	0.56

*Average of five determinations

Table 8. Parameters of linear regression equations for each drug compound

Parameters	Atenolol	Atorvastatin	Losartan potasium	Aspirin
Calibration range (µg mL^{-1})	1-10	4-14	1-10	5-25
Correlation coefficient (r)	0.9998	0.9995	0.9999	0.9998
Slope	47922.21	376839	64525.33	40652.2
Intercept	-230.92	4698107	4633.00	221309
LOD (µg mL^{-1})	0.097	0.2685	0.092	0.3682
LOQ (µg mL^{-1})	0.330	0.8137	0.310	0.2732

Table 9. Accuracy study by using the proposed method

% Level	Amount Added (µg mL^{-1})				Amount found* in mg mL^{-1} (Mean ± S.D)			
	Atorvastatin calcium	Atenolol	Aspirin	Losartan potasium	Atorvastatin calcium	Atenolol	Aspirin	Losartan potasium
50	4	4	4	4	3.98 ± 0.04	3.99 ± 0.03	3.96± 0.04	4.02± 0.02
75	5	5	5	5	4.96 ± 0.02	4.98 ± 0.05	5.03± 0.02	4.99± 0.04
100	6	6	6	6	5.97 ± 0.05	6.03 ± 0.02	5.99± 0.05	5.97± 0.02

* Average of three determinations

Repeatability

The system repeatability was calculated from five replicate Atorvastatin, Atenolol, Losartan potassium and Aspirin (Starpill tablet) at the analytical concentration of about and the %RSD found was 0.56. LOD and LOQ were determined based on signal to noise ratio. The S/N ratio of 3:1 was taken as LOD and S/N of 10:1 was taken as LOQ. LOD. It can be shown on **Table 8**.

Accuracy and Precision Studies

Accuracy was studied using three different solutions, containing 50, 75 and 100% level. The obtained values were within the range of 98 102 %, mean %RSD was 0.29, satisfying the acceptance criteria for the study. The results are shown in **Table 9**. Both Intraday and inter day precisions were studied at different levels in linearity levels are reported in **Table 10**, **11**. The % RSD was within the limit (below 2%). The precision was tested for the optimized method in two different laboratories, the %RSD was below 2%.

Table 10. Precision study (Interday) by using the proposed method

Drug sample name	Sample no	Labelled amount in mg	Amount found* (mg/tablet)	% purity	Average (%)	% RSD
Atenolol	1	50	49.62	99.24	100.53	1.55
	2	50	49.50	99.00		
	3	50	50.90	101.80		
Losartan potasium	1	50	49.34	98.68	99.5	0.94
	2	50	50.26	100.52		
	3	50	49.65	99.30		
Atorvastatin calcium	1	10	9.85	98.50	99.56	1.44
	2	10	10.12	101.20		
	3	10	9.90	99.00		
Aspirin	1	75	74.02	98.66	99.46	0.88
	2	75	74.54	99.33		
	3	75	75.03	100.4		

Table 11. Precision study (Intraday) by using the proposed method

Drug sample name	Sample no	Labeled amount in mg	Amount found* (mg/tablet)	% purity	Average (%)	% RSD
Atenolol	1	50	49.61	99.22	100.67	1.26
	2	50	50.80	101.6		
	3	50	50.60	101.2		
Losartan potasium	1	50	49.94	99.88	100.76	0.76
	2	50	50.56	101.12		
	3	50	50.65	101.3		
Atorvastatin calcium	1	10	9.95	99.50	100.43	1.35
	2	10	10.20	102.00		
	3	10	9.98	99.80		
Aspirin	1	75	74.62	99.49	99.68	0.55
	2	75	74.44	99.25		
	3	75	75.73	100.30		

DISCUSSION

There are a few works reported on the implementation of quality by design in analytical method development. But the sequence of implementation has to be considered as per the FDA. Beg et al. [28] and Kurmi et al. [29] have reported a method based on stability assay by considering resolution, as a method response to support the specificity in robustness. However, method verification in design space, method performance has to be added. The knowledge based QTPP for the product of AS, AT, LP and AS (Starpill tablet) was constructed with the assessment of criticality for its critical attribute. Analytical target profile (ATP) was derived based on QTPP profile and then objective of this analytical QbD work was considered as an essay component of QTPP of product specifications. The derived QTPP is shown in **Table 1**. The method assessment for attaining CQA of the product is shown in **Table 2**. To initiate the QbD work, the ester nature of chemical structure, pKa and solubility profile of Atorvastatin, atenolol, losartan potassium and aspirin (Starpill tablet) was considered in the selection of input variables (A, B and C) for factorial design (2^3). Midpoints were added to find the curvature effect. Once the curvature effect was significant, CCD was adopted to get response surface to optimize design. C18 column was chosen as stationary phase due to wide acceptability pharmaceuticals and high reproducibility. In the initial run, acetonitrile was chosen to eliminate the tailing effect. In this design, the concentration of acetonitrile was not considered as a quantitative variable. Because, the concentration range that is being used in chromatographic condition is very narrow. So, flow rate, organic phase concentration and buffer pH were considered as qualitative variable at 0.2% in water and were controlled. In order to achieve complete scientific understanding between method results (Y; such as t_R) and input variables, a central composite design was designed and performed. The various variables and their levels were shown in Tables (**Table 3** and **4**). The obtained experimental results was subjected various statistical parameters for better understanding and was found to be a nonlinear relationship between input variable and response. The statistical data and ANOVA analysis are shown in **Table 5**. The curvature effect was significant, so the Quadratic model (Eqn. 2) was obtained using of Design expert 7.1.0.1 software.

The above model was validated by the coefficient of determination (R^2). The value was near to 1.00 (0.96) indicated the process model is valid for predicting the behavior of the process and it was used for simulation of the

process model and the contours were obtained. The design space or MODR region for robustness was achieved from contours. These regions offer robust process parameters and shown in **Table 7**. In the optimized model pH was indicated the suitability for ester in the column because lower pH or even pH 7.0 may hydrolyze drugs in the sample or in column. The flow rate was optimized at 0.8 ml/min, and % organic phase is lower. The method was validated for accuracy and precision during method transfer between instrument I (automatic HPLC) and II (manual HPLC). The result was satisfactory.

CONCLUSIONS

The robust AQbD approach for analysis AS, AT, AC and LP in drugs and combined dosage forms was developed by using RP-HPLC with DLLME technique. Huiquan Wu et al. [30], S., Huaixia Chen et al. [31], S. Armenta, et al. and Mojtaba Shamsipur et al. [32] are reported AQbD with DLLME application. We are used DLLME for minimizing the volume of organic solvent (Instead of litres volume of solvent we have used µl amount. This type of concept also coming under one of the green approaches as mentioned in reference articles).The predicted developed method also based on AQbD with DLLME is more precise, accurate, and robust during method transfer and cost effective. The prediction form MODR has been verified by actual experimental results indicating its robustness. This method satisfies the design space concept for analytical method (MODR) and suitable for regulatory submission under regulatory flexibility. We are successfully attempted the DLLME and AQbD application for estimation of synthetic drugs. Our future scope is to apply the DLLME and AQbD for analysis of biological sample estimation.

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REFERENCES

1. The internet drug index. Specialized information services: http://www.rxlist.com/atenolol-drug.htm (accessed on March 25, 2016)
2. Martindale. The Extra Pharmacopoeia. 32nd ed, UK: Pharmaceutical Press 2014.
3. Rao L, Rajeswari KR. Spectrophotometric Method for Simultaneous Estimation of Atorvastatin and Amlodipine in Tablet Dosage Form. Res. J. Pharm. Biol. Chem. Sci., 2010;1(2):66-9.
4. Kumar P, Shukla SBB. Development and Validation of a Novel Isocratic RP-HPLC Method for Simultaneous Determination of Atenolol and Aspirin in Fixed Dose Combinations. Der Pharm. Chim, 2011;3(2):13–21.
5. Malavika V, Utam D. Simultaneous Estimation of Aspirin, Atorvastatin, Atenolol and Losartan Potassium from their combination dosage form by RP-HPLC. Global journal of Analytical chemistry. 2011;2(3):133-40.
6. Sathiyasundar R, Valliappan K. New Stability Indicating Assay Method by Liquid Chromatographic Separation of Aspirin, Atorvastatin and Clopidogrel in Pharmaceutical Dosage Form. Indo American Journal of Pharmaceutical Research. 2014;4(12):5919–27.
7. Talluri MVNK, Kalyankumar A, Ragampeta S. Synchronized separation of atorvastatin—an antihyperlipidemic drug with antihypertensive, antidiabetic, antithrombotic drugs by RP-LC for determination in combined formulations. Journal of pharmaceutical analysis. 2012;2(4):285-92. https://doi.org/10.1016/j.jpha.2012.02.006
8. ICH Topic Q8 (R2); "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '09): Pharmaceutical Development, 2009.
9. ICH, "Validation of analytical procedures and methodology," in Proceedings of the International Conference on Harmonization, Geneva, Switzerland, 2005.
10. Chatterjee S. QbD Consideration for Analytical Methods – FDA Perspectives, US IFPAC Annual Meeting; Baltimore. 2013.
11. Schmidt AH. Validation and Transfer, Berlin, 4th Annual Conference on Analytical Method Development, Germany. 2014.
12. Zar JH. Biostatical analysis: Pearson Education Inc. New Jersey, 2010.
13. Karmakar S, Garbar R. Quality by design (QbD) based development of stability indicating HPLC method for drug and impurities. J Chromatogr Sci. 2011;49:439–46. https://doi.org/10.1093/chrsci/49.6.439

14. Hockman KK, Berengut D. Determination of pharmaceutical excipients by design of experiments in chemical engineering. Chromatogr Res Int. 1985;102:142–8.
15. Rezaee M, Yamini Y, Shariati S, Esrafili A, Shamsipur M. Dispersive liquid-liquid microextraction combined with high-performance liquid chromatography-UV detection as a very simple, rapid and sensitive method for the determination of bisphenol A in water samples. J. Chromatogr. A. 2009;12(16):1511–4. https://doi.org/10.1016/j.chroma.2008.12.091
16. Xiong C, Ruan J. Extraction and determination of some psychotropic drugs in urine samples using dispersive liquid-liquid microextraction followed by high-performance liquid chromatography. J. Pharm. Biomed. Anal. 2009;49:572–8. https://doi.org/10.1016/j.jpba.2008.11.036
17. Iverson NM, Barone PW, Shandell M, Trudel LJ, Sen S, Sen F, Ivanov V, et al. In vivo biosensing via tissue-localizable near-infrared-fluorescent single-walled carbon nanotubes. Nature nanotechnology. 2013;8(11):873–80. https://doi.org/10.1038/nnano.2013.222
18. Sen F, Boghossian AA, Sen S, Ulissi ZW, Zhang J, Strano MS. Observation of Oscillatory Surface Reactions of Riboflavin, Trolox, and Singlet Oxygen Using Single Carbon Nanotube Fluorescence Spectroscopy. ACS Nano. 2012;6(12):10632–45. https://doi.org/10.1021/nn303716n
19. Koskun Y, Şavk A, Şen B, Şen F. Highly sensitive glucose sensor based on monodisperse palladium nickel/activated carbon nanocomposites, Analytica Chimica Acta. 2018;1010:37-43. https://doi.org/10.1016/j.aca.2018.01.035
20. Şahin B, Aygün A, Gündüz H, Şahin K, Demir E, Akocak S, Şen F. Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 cell line, Colloids and Surfaces B: Biointerfaces. 2018;163:119-24. https://doi.org/10.1016/j.colsurfb.2017.12.042
21. Bozkurt S, Tosun B, Sen B, Akocak S, Savk A, Ebeoğlugil MF, Sen F. A hydrogen peroxide sensor based on TNM functionalized reduced graphene oxide grafted with highly monodisperse Pd nanoparticles. Analytica Chimica Acta. 2017;989:88-94. https://doi.org/10.1016/j.aca.2017.07.051
22. Başkaya G, Yıldız Y, Savk A, Okyay TO, Eriş S, Sert H, Şen F. Rapid, sensitive, and reusable detection of glucose by highly monodisperse nickel nanoparticles decorated functionalized multi-walled carbon nanotubes,Biosensors and Bioelectronics. 2017;91:728-33. https://doi.org/10.1016/j.bios.2017.01.045
23. Şahin B, Demir E, Aygün A, Gündüz H, Şen F. Investigation of the effect of pomegranate extract and monodisperse silver nanoparticle combination on MCF-7 cell line. Journal of Biotechnology. 2017;260:79-83. https://doi.org/10.1016/j.jbiotec.2017.09.012
24. Sen S, Sen F, Boghossian AA, Zhang J, Strano MS. Effect of Reductive Dithiothreitol and Trolox on Nitric Oxide Quenching of Single-Walled Carbon Nanotubes J . Phys. Chem. C. 2013;117(1):593–602. https://doi.org/10.1021/jp307175f
25. Ulissi ZW, Sen F, Gong X, Sen S, Iverson N, Boghossian AA, Godoy LC, Wogan GN, Mukhopadhyay D, Strano MS. Spatiotemporal Intracellular Nitric Oxide Signaling Captured Using Internalized, Near-Infrared Fluorescent Carbon Nanotube Nanosensors Nano Lett. 2014;14(8):4887–94. https://doi.org/10.1021/nl502338y
26. International Conference on Hormonization (ICH), Tripartite Guidelines, ICH Q2 (R1): Validation of Analytical Procedures: Text and Methodology, London. 2009.
27. Sivakumar T, Manavalan M. Multi-criteria decision making approach and experimental design as chemometric tools to optimize HPLC separation of domperidone and pantoprazole. J. Pharm. Biomed. Anal. 2007;43:1842–8. https://doi.org/10.1016/j.jpba.2006.12.007
28. Beg S, Sharma G. Development and validation of stability-indicating liquid chromatographic method for estimating olmesartan medoxomil using quality by design. J Chromatogr Sci. 2015;53:1048–59. https://doi.org/10.1093/chromsci/bmu165
29. Kurmi M, Kumar S. Implementation of design of experiments for optimization of forced degradation conditions and development of a stability-indicating method for furosemide. J Pharm Biomed Anal. 2014;96:135–43. https://doi.org/10.1016/j.jpba.2014.03.035
30. Wu H, White M, Khan MA. Quality-by-Design (QbD): An integrated process analytical technology (PAT) approach for a dynamic pharmaceutical co-precipitation process characterization and process design space development. International Journal of Pharmaceutics. 2011;405(1–2):63-78. https://doi.org/10.1016/j.ijpharm.2010.11.045
31. Chen H, Chen H, Ying J, Huang J, Liao L. Dispersive liquid–liquid microextraction followed by high-performance liquid chromatography as an efficient and sensitive technique for simultaneous determination of chloramphenicol and thiamphenicol in honey. Analytica Chimica Acta. 2009;632(1):80-5. https://doi.org/10.1016/j.aca.2008.10.068

32. Shamsipur M, Fattahi N. Extraction and determination of opium alkaloids in urine samples using dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. Journal of Chromatography B. 2011;879(28):2978-83. https://doi.org/10.1016/j.jchromb.2011.08.033
33. Armenta S, Garrigues S, de la Guardia M. Green Analytical Chemistry. TrAC Trends in Analytical Chemistry. 2008;27(6):497-511. https://doi.org/10.1016/j.trac.2008.05.003