Journal of Applied Pharmaceutical Science Vol. 9(09), pp 092-097, September, 2019
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2019.90913
ISSN 2231-3354

Chemometric-assisted RP-HPLC method for the simultaneous determination of ambroxol hydrochloride, terbutaline sulfate, and guaiphenesin in combined dosage form

Keerthisikha Palur[1*], Bharathi Koganti[2], Sreenivasa Charan Archakam[1]

[1]Assistant Professor, Sri Padmavathi School of Pharmacy, Tiruchanoor, India.
[2]Professor, Sri Padmavthi Mahila University, Tirupati, India.

ARTICLE INFO

Received on: 01/04/2019
Accepted on: 10/07/2019
Available online: 01/09/2019

Key words:
Chemometric model, central composite design, response surface methodology, Derringers' desirability function.

ABSTRACT

An isocratic reverse-phase High performance liquid chromatography (HPLC) method using a chemometric model was developed and validated for the simultaneous determination of ambroxol hydrochloride, terbutaline sulfate, and guaiphenesin in their combined dosage form. Central composite design which is a subset of response surface methodology was used as the chemometric model. The separation of three drugs was carried out by using a Phenomenex C_{18} column and detection at 220 nm using a UV detector. Based on initial trials, the three factors selected for the design were methanol (MN) concentration, mobile phase pH, and flow rate in the range of 55%–65% (v/v), 4–5 units, and 0.6–1 ml/min, respectively. The impact of the selected factors on the responses like retention time of first peak (tR_1), resolution of 2nd and 3rd peak ($Rs_{2,3}$), and theoretical plates of the first peak (TP_1) were evaluated. Derringers' desirability function was used to optimize the responses. The conditions which were optimized for the assay of drugs were MN, ACN, and 50 mM KH_2PO_4 (pH 5) in the ratio of 55:10:35 at a flow rate of 0.8 ml/minute. The developed and optimized method was validated as per the International conference on harmonization (ICH) guidelines and can be used for routine analysis in quality control laboratories.

INTRODUCTION

Ambroxol hydrochloride (AMB), [trans-4-(2-Amino-3, 5-dibrombenzylamino)—cyclohexanol hydrochloride] (Fig. 1A), which belongs to the category of mucolytics, is used to decrease surfeit mucus and to treat respiratory system diseases. Guaiphenesin (GUA) is an expectorant and used to expel sputum from the respiratory tract. (Pubchem, 2018) .Chemically, it is 3-(2-methoxyphenoxy) propane-1, 2-diol. (Fig. 1B). Terbutaline sulfate (TER), tert-butyl-[2-(3, 5-dihydroxyphenyl)-2-hydroxyethyl] azanium; sulfate belongs to bronchodilator category which relaxes the vascular and bronchial muscles (Pubchem, 2018). The combination of these three drugs AMB, GUA, and TER is available in an expectorant dosage form containing the drugs in divergent proportions and is specified in the treatment of chronic bronchitis and asthma.

Only two RP-HPLC and one spectrophotometric methods were reported for the analysis of three drugs in their combined dosage form (Ghosh *et al*., 2016; Lakshmi Narasimha Rao *et al*., 2014; Ritu Kimbahune *et al*., 2011). Hence, it was aimed to develop a RP-HPLC method using a chemometric model and to validate and apply the method for the quantification of the drugs in their dosage form.

MATERIALS AND METHODS

Chemicals and reagents

The active pharmaceutical ingredients (API) of AMB (98.9%), TER (98.7%), and GUA (98.7%) were supplied by Raffles Pharmaceuticals Pvt Ltd., Tirupati. HPLC-grade methanol, acetonitrile, and analytical reagent grade potassium

*Corresponding Author
Keerthisikha Palur, Assistant Professor,
Sri Padmavathi School of Pharmacy, Tiruchanoor, India.
E-mail: keerthi8spsp @ gmail.com

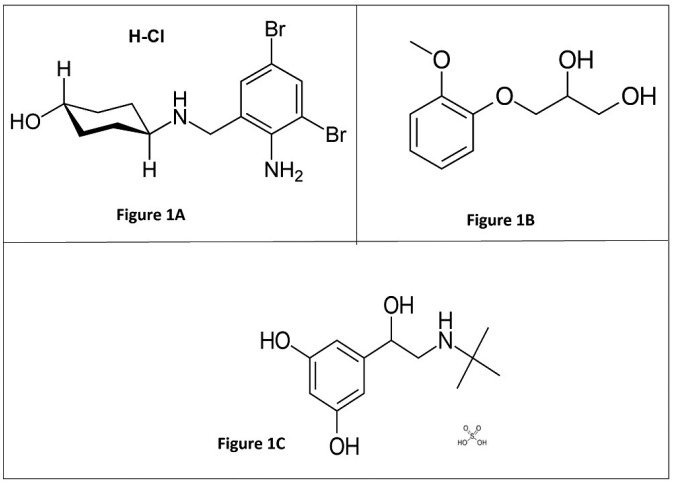

Figure 1. Chemical structures of analyte.

dihydrogen phosphate (KH_2PO_4) and orthophosphoric acid were acquired from S.D fine chemical Ltd., Mumbai, India, and HPLC grade water from Millipore were used in the analysis. NORVENT expectorant containing AMB 15 mg, TER 1.25 mg, and GUA 50 mg was purchased from the local medical store.

Instrumentation and software

HPLC system used was Shimadzu Prominence LC system with LC-20AT pump with UV-VIS detector and with 20 µl loop volume Rheodyne injector. The software used in data processing is LC Solution. An Elico pH meter (Model LI-120) was used to measure and adjust the pH of the buffer. Ultra-sonicator (PCI Analytics) was used for degassing the solutions. Design-Expert, version 11.0.0. (Stat-Ease Inc., Minneapolis, MN) software was used to select the design for optimization of the data and for further processing.

Chromatographic conditions

RP-HPLC method was developed using a Phenomenex C18 analytical column (4.5 × 250 mm, 5 µ) at room temperature with a mobile phase containing methanol (MN), acetonitrile (CAN), 50 mM KH_2PO_4 buffer pH adjusted to 5 in the ratio 55:10:35 (v/v/v) . The flow rate of the mobile phase was 0.8 ml/min. and the detection was carried using a UV detector and wavelength selected was 220 nm. The injection volume was 20 µL.

Preparation of stock and standard solutions

Stock solutions of AMB, TER, and GUA containing 1 mg/ml were prepared individually by dissolving 50 mg in 50 ml of methanol. A mixture containing the three standard solutions were prepared from the stock solution to get a concentration of AMB (20 µg/ml), TER (20 µg/ml), and GUA (20 µg/ml), respectively, using mobile phase.

Preparation of phosphate buffer

6.8 gm of potassium di-hydrogen orthophosphate (50 mM) was dissolved in sufficient water (HPLC grade) and volume was made up to 1,000 ml. The solution was kept for sonication and pH was adjusted to 5.0 using orthophosphoric acid.

Sample preparation

Sample enrichment technique was used in sample preparation. Syrup sample equivalent to 20 mg of GUA (2 ml of syrup), 0.5 mg of TER, and 6 mg of AMB was transferred to a 50 ml volumetric flask containing 20 ml of the mobile phase. To this 19.5 mg of TER API, 14 mg of AMB API were added and this sample solution was kept for sonication for 15 minutes and then the volume was adjusted up to the mark with mobile phase to get a concentration of 400 µg/ml of the three drugs. Filtration was carried out by using vacuum filter using 0.45 µ filter papers. 0.5 ml of the filtrate was taken and transferred into a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase to get a concentration of AMB (20 µg/ml), TER (20 µg/ml), and GUA (20 µg/ml), respectively.

Design of experiments

The widely used chemometric model in the optimization of RP-HPLC method is the central composite design which is a subclass of response surface methodology (Giriraj *et al*., 2014; Sivakumar *et al*., 2007; Sree Janardhanan *et al*., 2016). In central composite design (CCD), three factors at two levels were used to optimize HPLC separation. Based on the trials performed, the factors were selected and in the variation ranges of 55–65(% v/v) of MN concentration (A), 4–5 of mobile phase pH (B), and 0.6–1.0 ml/minute of flow rate (C), respectively. The retention time of first peak (tR_1), resolution of 2nd and 3rd peak ($Rs_{2,3}$), and theoretical plates of the first peak (TP_1) were selected as responses and measured in 20 runs in randomized order according to the CCD.

Method validation

Specificity

Specificity of the method was studied for the interference of mobile phase (blank) with that of the sample chromatogram. For this, blank and sample chromatograms were compared for any interference.

Linearity and Range

The linearity of the proposed method was established at 50%, 70%, 100%, 130%, and 150% of the target concentrations of all the drugs. Calibration curves were plotted for each drug in the range of 10–30 µg/ml and the regression parameters were assessed.

Precision

For the developed RP-HPLC method, both system precision and method precision were performed. System precision was carried out by injecting six replicate injections of the standard mixture at its target concentration. Various chromatographic parameters like retention time, peak area, theoretical plates, and tailing factor were assessed and their % relative standard deviation (RSD) were calculated. Method precision was carried out by injecting six replicate injections of the assay sample and % RSD of the peak area and % assay values were calculated.

Accuracy

Accuracy in terms of % recovery was performed by using standard addition method for 70%, 100%, and 130% levels of test concentration.

Table 1. Central composite design of experiments and responses.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	A:A	B:B	C:C	tR_1	$Rs_{2,3}$	TP_1
1	65	4	1	2.426	3.236	3737
2	60	4.5	1.13636	2.158	2.848	3316
3	65	5	0.6	4.018	5.919	4719
4	65	4	0.6	4.012	4.345	5083
5	60	4.5	0.8	3.038	3.237	4029
6	68.409	4.5	0.8	3.036	3.917	4470
7	55	4	0.6	4.006	2.804	4706
8	60	4.5	0.8	3.046	3.235	4065
9	60	4.5	0.8	3.046	3.279	4085
10	60	4.5	0.8	3.028	3.224	4038
11	60	4.5	0.8	3.04	3.18	4044
12	60	4.5	0.463641	5.232	3.608	4886
13	60	5.3409	0.8	3.055	11.959	4064
14	55	4	1	2.431	2.931	3624
15	55	5	0.6	4.085	12.097	3852
16	60	4.5	0.8	3.037	3.233	4069
17	55	5	1	2.454	9.904	3406
18	60	3.6591	0.8	3.023	1.385	4365
19	51.591	4.5	0.8	3.063	6.881	4043
20	65	5	1	2.439	2.871	4126

RESULTS AND DISCUSSION

Development and optimization

The factors A, B, and C and their ranges were given as input to the CCD employed. The software generated 20 runs in total and all the experiments were performed. The experiments conducted and their obtained responses are shown in Table 1. Three responses were selected based on their effect on the separation of drugs. The design model chosen was Quadratic mathematical model as its *p*-value is less than 0.05 and this model was suggested by the design. Different statistical parameters, such as adjusted r^2, adequate precision, and coefficient of variation (C.V.) obtained from analysis of variance (ANOVA) are shown in Table 2 and all the values were found to be within the acceptance criteria (Beg *et al*., 2003; Lundstedt *et al*., 1998; Parajo *et al*., 1992).

Interaction between the factors and responses and the impact of factors on responses were given by the design in the form of Perturbation plots and response surface plots which are shown in Figures 2 and 3, respectively (Myers *et al*., 1995). Perturbation plots revealed that factor C has the most important effect on tR_1 (Fig. 2A), whereas factors A, B, and C had the effects on $Rs_{2,3}$ and TP_1 (Figs. 2B and C). The interaction of the three factors on responses was predicted by response surface plots as shown in Figure 3 (% MN concentration in Perturbation plots and response surface plots revealed that factors B and C had a large significant effect on separation, whereas factor A, i.e., is MN concentration, is of little significance.

Derringer's desirability function (*D*) is used as a critical parameter for optimization. D value near to 1 state that response values are near to the criteria values (Derringer *et al*., 1980).

Table 2. Statistical parameters obtained from ANOVA for CCD.

Responses	*p* value	% CV	Adequate precision	Adjusted R^2
tR_1	< 0.0001	2.26	55.7879	0.9906
$Rs_{2,3}$	< 0.0001	15.52	19.3484	0.9444
TP_1	< 0.0001	2.13	27.3581	0.9637

The criteria for each response which was used in optimization are presented in Table 3. The diagrammatic representation of the response surface obtained for the global desirability function according to the criteria is shown in Figure 4. The maximum desirability value (D = 0.944) produced by the conditions (MN concentration of 55%(v/v), mobile phase pH of 5, and flow rate at 0.8 ml/minute) was chosen for optimization of the method. The experiment was performed using optimized conditions and the obtained and predicted responses are shown in Table 4 and the chromatogram is shown in Figure 5.

Method validation

The optimized method was validated according to ICH guidelines. Specificity was performed by comparing the chromatograms of the sample and blank and it was found that there were no interferences. Hence, the observed peaks in the sample solution revealed that the method was specific for the three drugs. Linearity was constructed at five levels in the range of 10–30 µg/ml for all the three drugs with R^2 of more than 0.999. Standard addition method was used to determine the accuracy and the percent recovery values were found to be 101.62%, 99.42%, and 99.52% for TER, GUA, and AMB, respectively, which were within acceptable

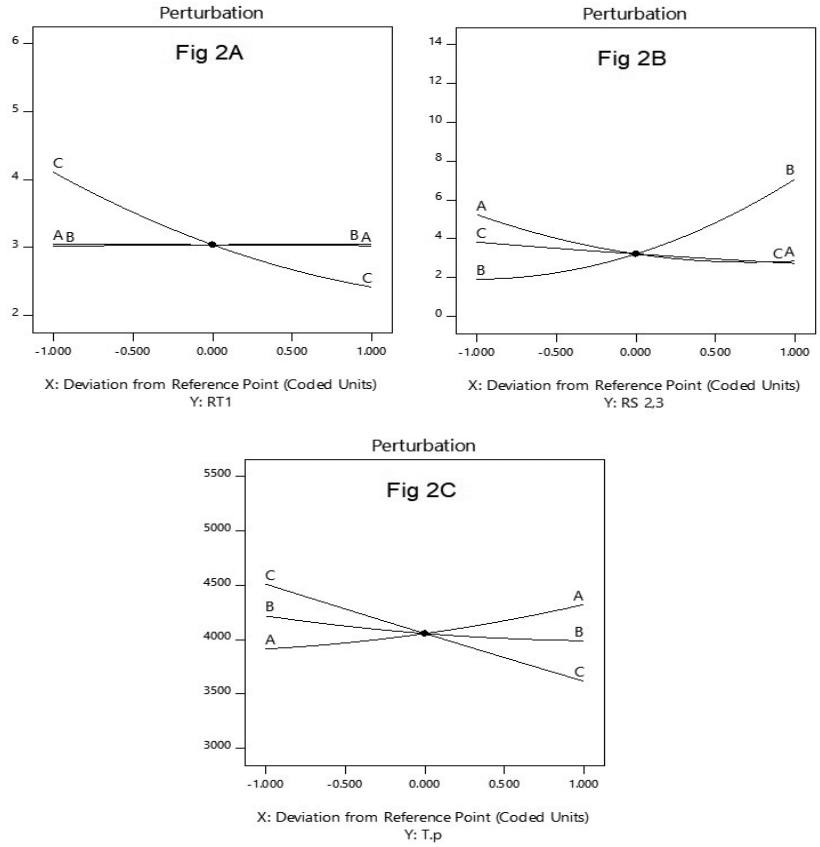

Figure 2. Perturbation plots.

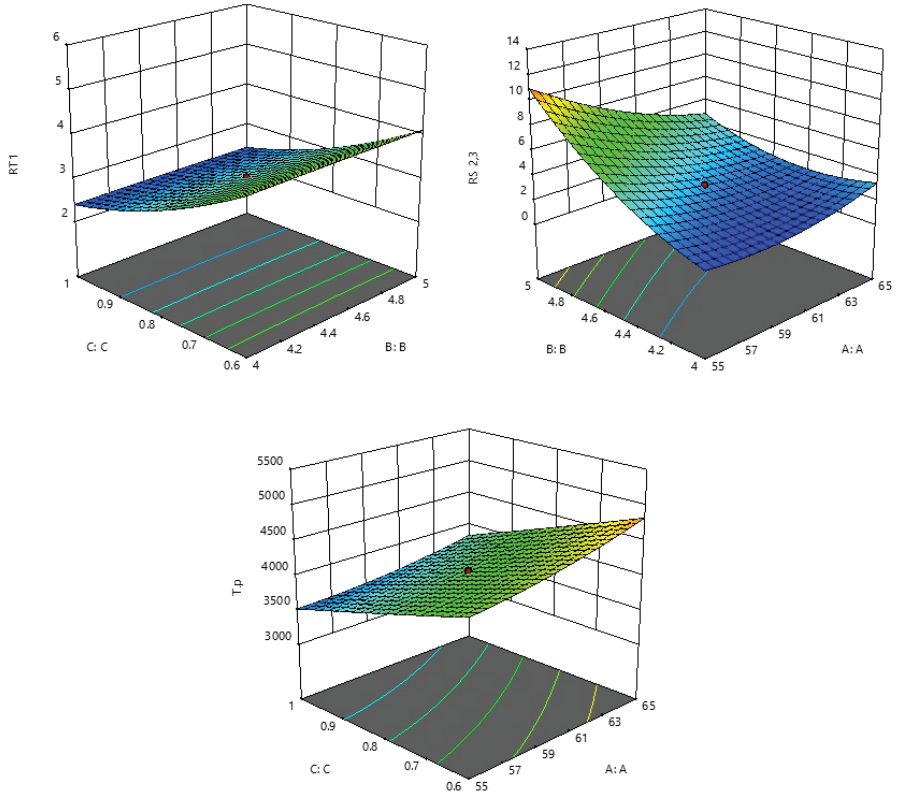

Figure 3. Response surface plots.

Table 3. Criteria for optimization of individual responses.

Response	Lower limit	Upper limit	Criteria
tR_1	2.158	5.232	Target – 3.00
$Rs_{2,3}$	1.385	12.097	Maximize
TP_1	3316	5083	In Range

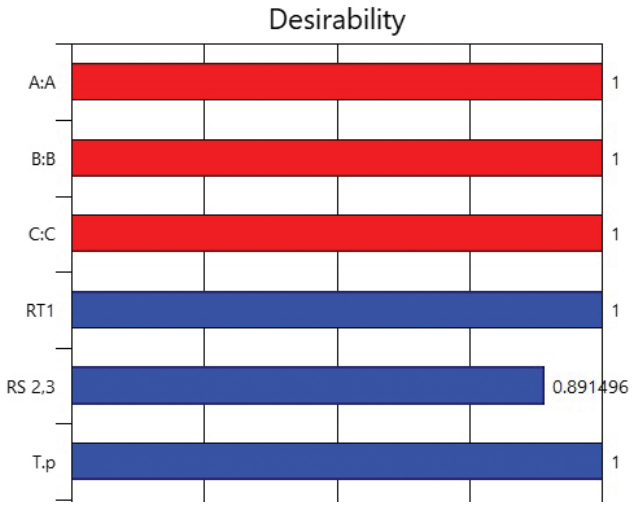

Figure 4. Response surface obtained for the global desirability function.

Table 4. Comparison of experimental and predictive values under optimum condition.

Optimum condition	Responses	Experimental	Predictive	Percentage error
MeOH-55 %(v/v)	tR_1	3.080	3.000	2.66
Mobile phase pH -5	$Rs_{2,3}$	10.971	10.935	0.32
Flow rate-0.8 ml/minute	TP_1	3799	3695	2.81

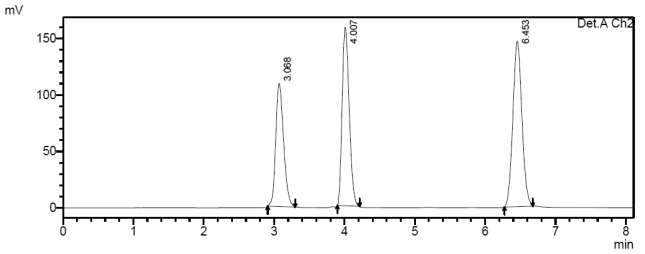

Figure 5. Test sample chromatogram.

ranges of 100 ± 2%. System precision and method precision were performed and the % RSD values were found to be less than 2.0. The experiments conducted as per the CCD and their responses revealed the information about robustness. The results obtained by the validation parameters revealed that the developed and optimized method was specific, suitable, linear, precise, accurate, and robust for the simultaneous determination of ambroxol hydrochloride, terbutaline sulfate, and guaiphenesin in their combined dosage form.

Table 5. Assay of marketed formulation by proposed methods.

Marketed formulation	Samples[a]	Assay %(w/w)		
		AMB	**TER**	**GUA**
NORVENT	Sample 1	99.96	102.10	98.80
	Sample 2	98.48	100.52	98.2
	Sample 3	99.56	101.08	99.10
	Mean ± SD[1]	99.33± 0.77	101.23± 0.89	98.70± 0.46

[a]Three replicate samples of a single syrup container.

Application of the developed method to combined dosage form

The developed and validated chemometric-assisted RP-HPLC method was applied for simultaneous determination of AMB, TER, and GUA in the syrup dosage form. The sample solutions (n = 3) were injected into the HPLC system and their assay results were calculated. The assay results obtained were found to be within the acceptable limits for all the three drugs and are presented in Table 5.

CONCLUSION

A chemometric-assisted RP-HPLC method was successfully developed and applied for the simultaneous determination of AMB, TER, and GUA in their combined dosage form and this was considered as the first chemometric method for this combination. The developed method has the advantage of being rapid, precise, accurate, simple, and direct and can be applied for routine analysis in quality control laboratories.

ACKNOWLEDGMENTS

The authors would like to thank the management and the principal of Sri Padmavathi School of Pharmacy, Tiruchanoor, for providing the facilities to carry out this research work.

CONFLICT OF INTEREST

This is a non-funding research work. There were no conflicts of interest.

REFERENCES

Beg Q, Sahai, V, Gupta R. Statistical media optimization and alkaline protease production from Bacillus mojavensis in a bioreactor. Process Biochem, 2003; 39(2):203–9.

Derringer G, Suich R. Simultaneous optimization of several response variables. J Qual Technol, 1980; 12(4):214–9.

Ghosh A ,Mandal S. K, Ghosh S, Deb S, Development and validation of RP-HPLC method for simultaneous determination of Terbutaline sulphate, Guaiphenesin and Ambroxol hydrochloride from an oral liquid. Indian Drugs, 2016; 53:53–6.

Giriraj P, Sivakkumar T. A rapid—chemometrics assisted RP-HPLC method with PDA detection for the simultaneous estimation of ofloxacin and nimorazole in pharmaceutical formulation. J Liq Chromatogr Relat Technol, 2015; 38(8):87–9.

Kimbahune R, Sunil K, Kabra P, Delvadiya K, Surani S. Spectrophotometric simultaneous analysis of ambroxol hydrochloride, guaifenesin and terbutaline sulphate in liquid dosage form. Int J Pharm Sci Rev, 2011; 8:004.

Lakshmi Narasimha Rao L, Krishnaiah C, Sudheer Babu K, Padmaja Reddy K. Development and validation of a stability-indicating LC method for simultaneous determination of related compounds of guaifenesin,

terbutaline sulphate and ambroxol HCl in cough syrup formulation. J Saudi Chem Soc, 2014; 18:593–600.

Lundstedt T, Seifert E, Abramo L, Thelin B, Nystrom A, Pettersen J, Bergman R. Chemom. Intell Lab Syst, 1998; 42(1–2):3–40.

Myers RH, Montgomery D. Response surface methodology. John Wiley & Sons Inc., New York, NY, 1995.

National Center for Biotechnology Information. PubChem Compound Database; CID=3516, https://pubchem.ncbi.nlm.nih.gov/compound/3516 (Accessed 18 December 2018).

National Center for Biotechnology Information. PubChem Compound Database; CID=31620, https://pubchem.ncbi.nlm.nih.gov/compound/31620 (Accessed 18 December 2018).

Parajo JC, Alonso JL, Lage MA, Vazquez D. Empirical modeling of eucalyptus wood processing. Bioprocess Eng, 1992; 8:129–36.

Sivakumar T, Manavalan R, Muralidharan C, Valliappan K. 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.

Sree Janardhanan V, Manavalan R, Valliappan K. Chemometric technique for the optimization of chromatographic system: simultaneous HPLC determination of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin used in combined cardiovascular therapy. Arab J Chem, 2016; 9:S1378–87.

How to cite this article:
Palur K, Koganti B, Archakam SC. Chemometric-assisted RP-HPLC method for the simultaneous determination of ambroxol hydrochloride, terbutaline sulfate, and guaiphenesin in combined dosage form. J Appl Pharm Sci, 2019; 9(09): 092–097.