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Original Article

Simultaneous Determination of Amitriptyline Hydrochloride and Propranolol Hydrochloride in Commercial Formulation by Multitudinal UV and Multivariate FT-IR Spectroscopic Methods

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ABSTRACT

Aim: In the current study, a multivariate FTIR method and two different UV spectrophotometric methods were employed for the simultaneous determination of Amitriptyline Hydrochloride (ATH) and Propranolol Hydrochloride (PPH) in their combined formulation. **Materials and Methods:** The multivariate FTIR method based on the use of Classical Least Squares (CLS) was developed and executed in the lab solutions software for the simultaneous determination of ATH and PPH. CLS model was performed in the wavenumber range of 2550.04-3600.12 cm^{-1} and 969.64-1757 cm^{-1}. In addition, two UV spectroscopic methods, namely absorbance correction method and crammer's matrix method were developed and validated in the concentration ranges of 2-10µg/mL and 5-55µg/mL for ATH and PPH respectively. **Results:** The statistical parameters obtained from the applied multivariate FTIR-CLS model revealed the model accuracy and the assay results of ATH and PPH were found to be 92.32 (%w/w) and 97.35 (%w/w) respectively. The developed UV spectroscopic methods were validated as per ICH guidelines and all the validation parameters were found to be within the acceptance criteria. **Conclusion:** The methods employed in the current study were found to simple, economical, accurate and do not require any prior separation.

Keywords: Classical least squares, Absorbance correction method, Crammer's matrix method.

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INTRODUCTION

Amitriptyline Hydrochloride (ATH), N,N-dimethyl-3-(2-tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaenylidene) propan-1-amine;hydrochloride is a tricyclic antidepressant and Propranolol hydrochloride (PPH), 1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol;hydrochloride is a nonselective beta-adrenergic receptor blocker. The combination of ATH and PPH is available in tablet dosage form in different strengths and prescribed in the treatment of migraine. The commercial formulation TRIPTOLOL (Centaur Pharmaceuticals Pvt. Ltd.,) containing 10 mg of ATH and 40 mg of PPH per tablet was used in the present study. Reviewing the literature revealed that the quantification of ATH and PPH in formulation was done by three HPLC methods and one HPTLC method.[1-4] However, simultaneous estimation of ATH and PPH by using spectroscopic methods was not reported. Hence, an attempt was made to develop multivariate FTIR method and various UV spectrophotometric methods for their simultaneous determination.

Classical Least Squares (CLS) is a multivariate FT-IR spectroscopic technique which can be used in the quantification of analytes in IR spectra.[5-12] When studying complicated multiple spectra which include broad and overlapping bands, this technique is especially suitable. In comparison to other widely used quantitative techniques, spectroscopic methods have the benefit of being simple to use and relatively less expensive. They have also been the most widely used techniques in quantitative analysis.[13-19]

Secondly, a basic spectrophotometric technique called the "Absorption Correction Method" (ACM) involves simultaneously estimating both drugs at their respective absorption maxima and

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is a modified version of simultaneous estimation method.[20-22] In this method, quantitative determination of one drug is carried out by A (1%, 1 cm) and quantitation of other drug is carried out by subtracting the absorbance of the other drug using absorption factor.

Thirdly, the Cramer's matrix approach employs the Cramer's rule. In this method, a matrix was formed by taking the molar absorptivity of the drugs at two wavelengths and the matrix is solved using the Crammer's rule to get the absorbance of the drugs.[23]

MATERIALS AND METHODS

Instruments and software

Shimadzu FTIR (IR-SPIRIT) is used for the determination of analytes by multi-variate FTIR-CLS method. Multitudinal UV spectroscopic methods were carried out by using Shimadzu UV-1800 spectrophotometer with matched pair of 10 mm quartz cells. UV Probe software was used for data acquisition. Shimadzu electronic balance (AY 220) was used for weighing of samples.

Materials and solvents

The pure samples of Amitriptyline Hydrochloride (ATH) and Propranolol Hydrochloride (PPH) were purchased from TCI Chemicals (India) Pvt. Ltd., Chennai. Methanol was used as the solvent in the current analysis. The marketed formulation TRIPTOLOL (Centaur Pharmaceuticals Pvt. Ltd.) containing 10 mg of ATH and 40 mg of PPH per tablet was purchased from local pharmacy (Tirupati, Andhra Pradesh, India) and was employed as test sample for the study.

Multivariate FTIR-CLS spectroscopic method development and validation

The method applied for the quantification of ATH and PPH was Classical Least Squares (CLS) method. In this CLS method, series of mixtures containing different ratios of ATH and PPH were prepared and their IR spectra were recorded. A Series of 15 mixtures were prepared with different concentrations in the linearity ranges of ATH and PPH. Out of these, 10 mixtures were taken as a calibration set and 5 mixtures were taken as prediction set or validation set. CLS method was executed in the software LAB SOLUTIONS between wavenumber ranges of 2550.04-3600.12 cm^{-1} and 969.64-1757.07 cm^{-1}. The statistical parameter obtained from calibration set was used to evaluate the method reliability and then the validated method is applied for the formulation analysis.

UV spectrophotometric method development and validation

The UV absorption spectra of the standard solutions of ATH and PPH in methanol were recorded in the UV region of 200-400 nm and the spectra showed maximum absorption at 239 nm for ATH and 289 nm for PPH. The calibration curves of ATH and PPH were plotted in the ranges of 2-10 µg/mL and 5-45 µg/mL respectively at absorption maxima of 239 nm for ATH and 289 nm for PPH using methanol as solvent. The sample solution was prepared from the formulation using methanol to get a concentration of 10 µg/mL and 40 µg/mL of ATH and PPH respectively. Two methods, namely absorbance correction method and crammer's matrix method were developed and validated for the analysis of ATH and PPH in their combined commercial formulation.

Absorbance correction method

The two wavelengths selected for absorption correction method are 295 and 239 nm. At the wavelength of 295 nm, PPH showed absorbance whereas ATH had no interference. The absorbance of PPH working standard solution at 295 nm was calculated using equation (1) and followed by the subtraction of PPH absorbance from total absorbance of sample solution at 239 nm wavelength. This calculated absorbance is the corrected absorbance of ATH and is used in the estimation of ATH at 239 nm. The estimation of ATH and PPH was done by using the equations mentioned below.

$$c_x = \frac{A_{295}}{ax_{295}} \dots\dots\dots EQ(1)$$

$$c_y = \frac{A_{239} - c_x * a_{x239}}{a_{y239}} \dots\dots\dots EQ(2)$$

Where,

Cx=Concentration of PPH,

Cy=Concentration of ATH,

A_{239}=Absorbance of Sample solution at 239 nm,

A_{295}=Absorbance of Sample solution at 295 nm,

a_{x239}=Corrected absorbance of ATH at 239 nm,

a_{x295}=Corrected absorbance of ATH at 295 nm,

a_{y239}=Absorbance of ATH at 239 nm.

Crammer's matrix method

Molar absorptivity (ε) values were calculated by using the absorbances measured at 239 nm and 289 nm for each compound in the binary mixture. The selected wavelength values were λ_{max} of ATH and PPH respectively. In this method, a mathematical matrix employing crammer's rule is constructed in which determinant values are calculated by using absorptivity (ε) values.

The concentrations of the drugs can be calculated by using the following equations;

$$\begin{bmatrix} Am_{239} \\ Am_{289} \end{bmatrix} = \begin{bmatrix} \varepsilon ATH_{289} & \varepsilon PPH_{289} \\ \varepsilon ATH_{239} & \varepsilon PPH_{239} \end{bmatrix} X \begin{bmatrix} C_{ATH} \\ C_{PPH} \end{bmatrix} \dots\dots\dots EQ(3)$$

$$\Delta = \begin{bmatrix} \varepsilon ATH_{289} & \varepsilon PPH_{289} \\ \varepsilon ATH_{239} & \varepsilon PPH_{239} \end{bmatrix} \dots\dots\dots EQ(4)$$

$$\Delta 1 = \begin{bmatrix} Am_{289} & \varepsilon ATH_{289} \\ Am_{239} & \varepsilon ATH_{239} \end{bmatrix} \dots\dots\dots EQ(5)$$

$$\Delta 2 = \begin{bmatrix} \varepsilon PPH_{289} & Am_{289} \\ \varepsilon PPH_{239} & Am_{239} \end{bmatrix} \dots\dots\dots EQ(6)$$

Where:

A_m denotes the absorbance of the binary mixture.

ε represents the values of molar absorptivity for the calculated ATH and PPH respectively at 239 nm, and 289 nm.

C is the molar concentration of ATH and PPH.

By applying Cramer's matrix rule, the concentration of ATH and PPH can be found by

$$C_{ATH} = \frac{\Delta_1}{\Delta} \ldots\ldots\ldots EQ(7)$$

$$C_{PPH} = \frac{\Delta_2}{\Delta} \ldots\ldots\ldots EQ(8)$$

RESULTS AND DISCUSSION

Multivariate FTIR-CLS spectroscopic method development and validation

Multivariate FTIR-CLS spectroscopic method was developed with algorithm of MLR evolution via K-matrix for mixtures within the range of 2500.04-3600.12 cm^{-1} and 969.64-1757.07 cm^{-1}. The FTIR spectrum of calibration set of ten mixtures was shown in Figure 1. The actual and predicted values of the calibration set were found to be within the acceptance criteria as shown in Table 1 and minimum PRESS values were noticed in the developed method. The developed CLS calibration set is further applied to a test set containing five mixtures and the results obtained from the test set depicted the accuracy of the developed model (Table 2). Hence this method is optimized and applied to analyse the commercial formulation. The assay values for FTIR-CLS method were found to be 92.32% and 97.35% for ATH and PPH respectively.

UV-spectrophotometric method development and validation

The calibration curves of ATH and PPH were plotted in the ranges of 2-10 µg/mL and 5-45 µg/mL respectively at absorption maxima of 239 nm for ATH and 289 nm for PPH using methanol as solvent and the linearity spectra of both the drugs was shown in Figure 2a and 2b.

Table 1: Results obtained for calibration set of ATH and PPH by FTIR-CLS.

Sl. No.	Calibration set	ATH		% Recovery	PPH		%Recovery
		Actual	Predicted		Actual	Predicted	
1	Mixture 1	10	9.958	99.58	25	24.513	98.05
2	Mixture 2	10	10.043	100.43	35	34.719	99.20
3	Mixture 3	4	3.927	98.17	45	44.391	98.65
4	Mixture 4	8	8.091	101.13	25	24.552	98.21
5	Mixture 5	6	6.056	100.93	15	15.123	100.82
6	Mixture 6	12	12.331	102.75	25	24.723	98.89
7	Mixture 7	8	8.257	103.21	15	14.98	99.87
8	Mixture 8	6	5.976	99.60	45	44.345	98.54
9	Mixture 9	4	3.929	98.20	55	55.533	100.97
10.	Mixture 10	4	3.921	98.02	15	15.369	102.46
Mean % recovery				100.202	99.565		
%RSD				1.8411	1.4447		

Table 2: Results obtained for test set of ATH and PPH by FTIR-CLS.

Sl. No.	Calibration set	ATH		% Recovery	PPH		%Recovery
		Actual	Predicted		Actual	Predicted	
1	Mixture 1	10	10.059	100.59	55	55.429	100.78
2	Mixture 2	6	6.114	101.9	25	25.364	101.45
3	Mixture 3	8	8.174	102.175	45	45.37	100.82
4	Mixture 4	8	8.039	100.4875	55	56.329	102.41
5	Mixture 5	10	9.829	98.29	45	44.347	98.54
Mean % recovery				100.685			100.08
%RSD				1.5289			1.4126

Table 3: Results obtained for Absorption correction method of ATH and PPH.

Conc. (µg/mL)	λ_1 (295 nm)	ax_1	λ_2 (239 nm)	ax2	Conc. (µg/mL)	λ_1 (295 nm)	ay_1	λ_2 (239 nm)	ay_2
2	0.246	0.0492	0.409	0.0818	5	0.059	0.0295	0.145	0.0725
4	0.419	0.0279	0.618	0.0412	15	0.002	0.0005	0.339	0.0847
6	0.606	0.0242	0.844	0.0241	25	0.000	0.000	0.516	0.086
8	0.777	0.0222	1.077	0.0307	35	0.000	0.000	0.713	0.0891
10	0.942	0.0209	1.261	0.0280	45	0.003	0.0003	0.911	0.0911

Table 4: Validation parameters obtained for Absorption correction method and Crammer's matrix method of ATH and PPH.

Validation Parameters	Absorbance correction method		Crammer's matrix method	
	ATH	PPH	ATH	PPH
Wavelength for analysis	239 nm	295 nm	239 nm	289 nm
Linearity and range	2-10 µg/mL	5-45 µg/mL	2-10 µg/mL	5-45 µg/mL
Correlation coefficient	R^2=0.9996	R^2=0.9996	R^2=0.9996	R^2=0.9992
Precision (Repeatability and Inter day)	<2%	<2%	<2%	<2%
Accuracy (% Recovery)	98.4	98.23	98.5	99.4
Assay (%w/w)	98.2	97.4	98.6	98.15

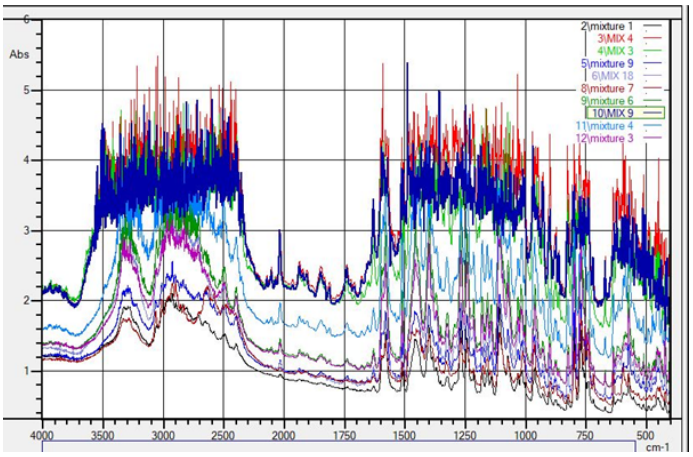

Figure 1: Overlay FTIR absorption spectra of ATH and PPH.

Absorbance correction method

The overlay absorption spectra of ATH and PPH depicting the absorbance of PPH at the wavelength of 295 nm and no interference of ATH was shown in Figure 3. Estimation of PPH was done at 295 nm and estimation of ATH was done by using the absorption correction factor. The parameters required for calculations in the absorbance correction method were presented in Table 3. The obtained values were substituted in the respective equations to get the concentrations of ATH and PPH. All the validation parameters like linearity, range, accuracy and precision were executed as per the ICH guidelines and all the obtained results including assay values were found to be within the acceptance criteria.

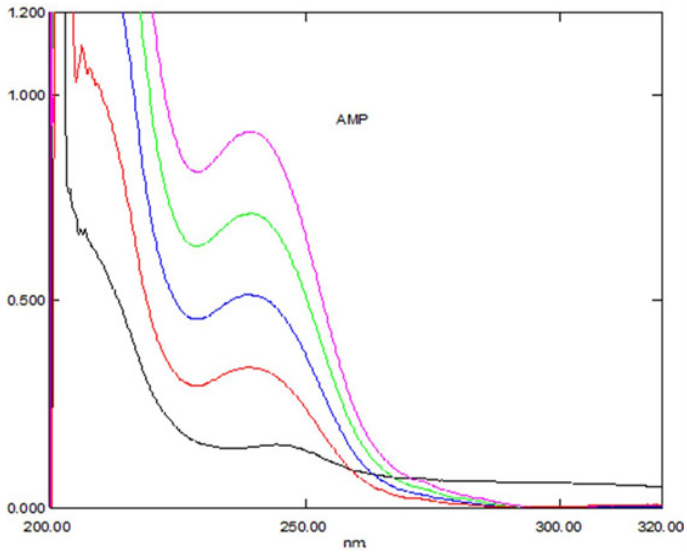

Figure 2a: Linearity UV absorption spectra of ATH.

Crammer's matrix method

In Crammer's matrix method, a matrix was formed by considering the molar absorptivity of the drugs at 239 nm and 289 nm. By substituting the molar absorptivity (ε) values in the respective crammer's rule equations, the concentrations of ATH and PPH were calculated. Linearity was established between concentration and absorbance of working standard solutions of ATH and PPH individually at 239 and 289 nm respectively and the results depicted a good linear regression between the concentration and absorbance. Hence, the method was found to be linear in the range of 2-10 µg/mL for ATH and 5-45µg/mL for

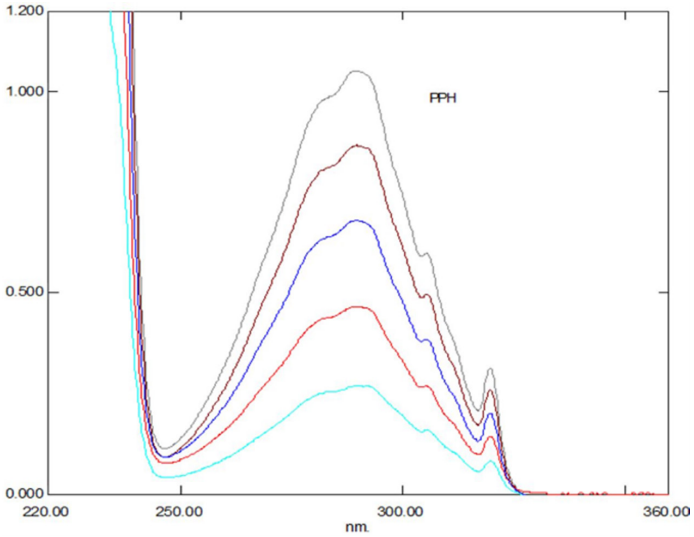

Figure 2b: Linearity UV absorption spectra of PPH.

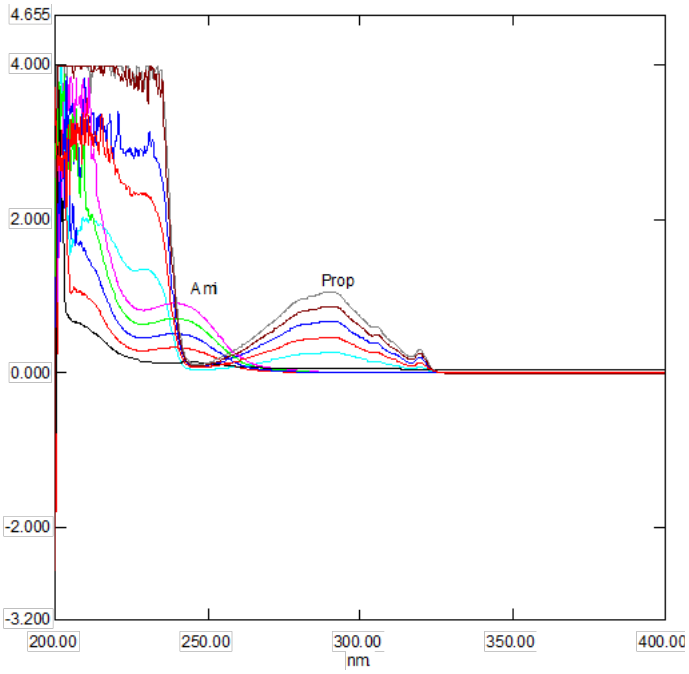

Figure 3: Overlay UV absorption spectra for Absorption correction method of ATH and PPH.

PPH. The method precision results showed a %RSD value of less than 2 for both the drugs, which is acceptable. The % recoveries for the spiked standard concentrations of ATH and PPH at all the three levels were calculated and all the validation results were presented in Table 4.

Analysis of Commercial formulation

The optimized and validated FTIR-CLS, UV absorption correction method and Crammer's matrix methods were applied to estimate the concentrations of ATH and PPH in the sample solution and the assay results obtained for the methods were found to be within the acceptance criteria which depicted that all the three developed methods could be executed for the simultaneous estimation of drugs without any prior separation.

CONCLUSION

Three analytical methods namely FTIR-CLS, UV absorption correction method and Crammer's matrix methods were successfully developed for the estimation of ATH and PPH in tablet dosage form. All the developed analytical methods were validated as per ICH guidelines, which make them reliable for global acceptance. The results obtained for all the developed methods were satisfactory and the methods developed were simple, accurate, precise, specific, reproducible and economical which can be further employed in the routine analysis by various quality control laboratories.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

ACM: Absorption Correction Method; **ATH:** Amitriptyline Hydrochloride; **CLS:** Classical Least Squares; **FTIR:** Fourier Transform Infra Red; **HPLC:** High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **ICH:** International Council for Harmonization; **MLR:** Multiple Linear Regression; **PPH:** Propranolol Hydrochloride; **PRESS:** Predicted residual Error of Sum of Squares; **UV:** Ultra-Violet.

SUMMARY

In the present study, three methods namely multivariate FTIR-CLS method and UV spectrophotometric methods like absorbance correction method and crammer's matrix method were developed and validated for the simultaneous determination of Amitriptyline Hydrochloride (ATH) and Propranolol Hydrochloride (PPH) in their combined commercial formulation. The commercial formulation TRIPTOLOL (Centaur Pharmaceuticals Pvt. Ltd.,) containing 10 mg of ATH and 40 mg of PPH per tablet prescribed for the treatment of migraine was used in the present study. All three optimized methods were validated as per ICH guidelines and the validation parameters including the assay results were found to be within the acceptance criteria and all the methods can be employed in regular QC analysis.

REFERENCES

1. Khalid M, Ahmad S. Box–Behnken design used to optimize the simultaneous quantification of amitriptyline and propranolol in tablet dosages by RP-HPLC-DAD method and their stability tests. Separations. 2022; 9(12): 1-15. doi: 10.3390/separations9120421.
2. Siddiqua S, Prerana J. RP-HPLC method development and validation for the simultaneous estimation of amitriptyline hydrochloride and pantoprazole sodium in bulk and capsule dosage form. J Drug Deliv Ther. 2019; 9(4): 37-42.
3. Gajmal A, Andhale GS, Neharkar VS, Hol K, Nagare SM. Development and validation of RP-HPLC method for simultaneous estimation of amitriptyline hydrochloride and propranolol hydrochloride in pharmaceutical dosage form. NeuroQuantology. 2022; 20(13): 1401-10.
4. John C, Chainulu C, Ghosh P, Srivastava S, Shukla S, Satyanarayana S. Validated high performance thin layer chromatography method for the simultaneous determination of amitriptyline, atenolol and propranolol. Asian J Res Chem. 2011; 4(7): 1059-63.
5. Haaland DM, Easterling RG. Improved sensitivity of infrared spectroscopy by the application of least squares methods. Appl Spectrosc. 1980; 34(5): 539-48. doi: 10.1366/0003702804731258.
6. Haaland DM, Easterling RG. Application of new least-squares methods for the quantitative infrared analysis of multicomponent samples. Appl Spectrosc. 1982; 36(6): 665-73. doi: 10.1366/0003702824639114.
7. Haaland DM, Easterling RG, Vopicka DA. Multivariate least-squares methods applied to the quantitative spectral analysis of multicomponent samples. Appl Spectrosc. 1985; 39(1): 73-84. doi: 10.1366/0003702854249376.
8. Kramer R. Chemometric techniques for quantitative analysis. 1st ed. New York: Marcel Dekker; 1998.
9. Hamilton WC. Statistics in physical science. 1st ed. New York: Ronald Press Company; 1964.
10. Diwan A, Linford MR. An Introduction to Classical Least Squares (CLS) and Multivariate Curve Resolution (MCR) as Applied to UV-vis, FTIR, and ToF-SIMS. Vacuum Tech. and Coating. 2014; 1-9.
11. Pottel H. The use of partial least squares (PLS) in quantitative FTIR: determination of gas concentrations in smoke gases of burning textiles. Fire Mater. 1995; 19(5): 221-31. doi: 10.1002/fam.810190505.
12. Sim SF, Kimura ALJ. Partial least squares (PLS) integrated Fourier transform infrared (FTIR) approach for prediction of moisture in transformer oil and lubricating oil. J Spec. 2019; 2019: 1-10.
13. Keerthisikha P, Charan AS, Bharathi K. Development and validation of chemometric assisted UV methods for the simultaneous determination of ambroxol hydrochloride, terbutaline sulphate and guaiphenesin in pharmaceutical dosage form. Indian J Pharm Educ Res. 2023; 57(1): 242-9.
14. Charan AS, Keerthisikha P, Sravya A, Vinay B, Kadiyala Keerthi PKP, Ranganayakulu D. A review on chemometric assisted UV spectroscopic and RP-HPLC methods for multicomponent analysis. Ymerdigital. 2023; 22(6): 761-72.
15. Keerthisikha P, Charan AS, Bharathi K. Chemometric assisted UV spectrophotometric and RP-HPLC methods for simultaneous determination of paracetamol, diphenhydramine, caffeine and phenylephrine in tablet dosage form. Spectrochim Acta A Mol Biomol Spectrosc. 2020; 243: 1-8.
16. Keerthisikha P, Charan AS, Bharathi K. Development and validation of chemometric assisted UV methods for the simultaneous determination of ambroxol hydrochloride, terbutaline sulphate and guaiphenesin in pharmaceutical dosage form. Indian J Pharm Educ Res. 2023; 57(1): 242-9.
17. Charan AS, Keerthisikha P. Arava Praveen Kumar. Multivariate analytical methods for simultaneous estimation of atenolol and hydrochlorothiazide in bulk and tablet dosage form. "IP Int J Comp. Adv Pharmacol. 2021; 6(4): 205-8.
18. Keerthisikha P, Charan AS, Bharathi K. Development and validation of chemometric assisted analytical methods for simultaneous estimation of atorvastatin calcium and Aspirin in capsule dosage form. Int J Res Pharm Sci. 2019; 10(3): 1692-7.
19. Palur K, Koganti B, Archakam SC, Chenchugari S, Nagireddy B, Devabhaktuni MB, *et al.* Simultaneous estimation of atorvastatin and Aspirin in bulk and capsule dosage form by chemometric assisted spectrophotometric methods. J Young Pharm. 2016; 8(4): 424-9. doi: 10.5530/jyp.2016.4.19.
20. Kant R, Bodla R, Bhutani R, Kapoor G, Goswami S. Spectrophotometric absorbance correction method for the estimation of tazobactam and cefepime in combined tablet dosage forms. J Chem Pharm Res. 2015; 7(6): 648-56.
21. Patel K, Patel A, Dave J, Patel C. Absorbance correction method for estimation of telmisartan and metoprolol succinate in combined tablet dosage forms. Pharm Methods. 2012; 3(2): 106-11. doi: 10.4103/2229-4708.103891.
22. Viswanath V, Shanmugasundaram P, Ravichandiran V. UV-spectrophotometric absorbance correction method for the simultaneous estimation of tenofovir disoproxil Fumerate and emtricitabine in combined tablet dosage form. Int.J.Pharm. J Technol Res. 2013; 5(3): 1179-85.
23. Attia KAM, Elabasawy NM, Abolmagd E. Simultaneous equation and area under the curve spectrophotometric methods for estimation of cefaclor in presence of its acid induced degradation product; A comparative study. Future J Pharm Sci. 2017; 3(2): 163-7. doi: 10.1016/j.fjps.2017.06.001.

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