



INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH



SIMULTANEOUS ESTIMATION OF AMBROXOL, CHLORPHENERAMINE MALEATE AND GUAIPHENESIN IN BULK AND LIQUID DOSAGE FORM BY CHEMOMETRIC ASSISTED SPECTROPHOTOMETRIC METHODS.

Bhavana. Nagireddy^{*}, A. Elphine Prabhahar, P.V.Suresh, Rama Rao Nadendla

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur.

ARTICLE INFO

Article history

Received 05/06/2017
Available online
20/10/2017

Keywords

Chemometrics,
UV-Visible,
Simultaneous,
Ambroxol,
Chlorpheneramine Maleate
And
Guaiphenesin.

ABSTRACT

A simple UV-visible spectroscopic method was developed and Chemometric designs were applied for the simultaneous estimation of Ambroxol (AMB), Chlorpheneramine maleate (CPM) and Guaiphenesin (GPN) in bulk and liquid dosage form. The spectroscopic method was developed by using methanol as solvent for the three drugs and the data generated from the spectra were mined by using Chemometric methods such as trilinear regression analysis, Cramer's matrix method, Method of least squares, Multivariate calibration methods such as partial least square regression (PLS) and Principle component regression (PCR). The wavelengths selected for all the above methods were 248 nm (wavelength of maximum absorption; λ_{\max} of AMB), 261 nm (wavelength of maximum absorption; λ_{\max} of CPM) and 274 nm (wavelength of maximum absorption; λ_{\max} of GPN). Results: The methods hold good linearity for AMB from 10-30 $\mu\text{g/ml}$, for CPM from 2-10 $\mu\text{g/ml}$ and GPN from 10-80 $\mu\text{g/ml}$ with regression coefficient values of 0.999, 0.998 and 0.999 respectively. The intraday and inter-day precision was found to be less than 2% RSD. The percentage recovery and percentage assay was in the range of 95-105% for Ambroxol (AMB), Chlorpheneramine maleate (CPM) and Guaiphenesin (GPN) by all the methods. Conclusion: The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the three drugs and moreover they are effective in minimizing the errors in analysis, simple and economical.

Corresponding author

N. Bhavana

Department of Pharmaceutical Analysis,
Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur.
nagireddybhavana02@gmail.com

Please cite this article in press as **N. Bhavana et al. Simultaneous Estimation of Ambroxol, Chlorpheneramine Maleate and Guaiphenesin in Bulk and Liquid Dosage form by Chemometric Assisted Spectrophotometric Methods. Indo American Journal of Pharmaceutical Research. 2017;7(09).**

Copy right © 2017 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Chemometrics is a branch of science which derives the data by the application of mathematical and statistical tools for the extraction of useful information from the physical and chemical phenomenon involved in a manufacturing process. Chemometrics¹⁻⁵ is used for calibration, signal correction and compression, pattern classification and recognition, multi variate data collection and analysis protocols, process modelling and statistical process control. To overcome the significant problems in the analysis of intricate multi component formulations by conventional UV-spectroscopy⁶⁻⁸, HPLC⁹⁻¹⁷ methods Chemometric assisted analytical methods¹⁸⁻²¹ are designed to perform analytical investigation of such complex formulations.

Ambroxol hydrochloride is *trans*-4-[(2-amino-3,5-dibromobenzyl) amino]cyclohexanol hydrochloride. It acts as mucolytic and was used in treatment of respiratory diseases such as cough.

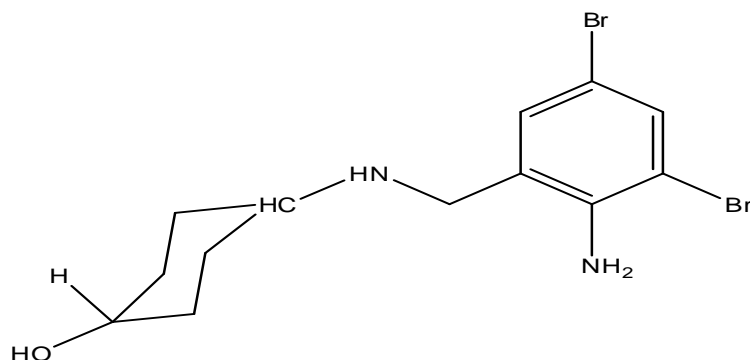


Fig: 1 Structure of Ambroxol.

Chlorpheniramine maleate is chemically (RS)-3-(4-chlorophenyl)-3-(pyridin-2-yl) propyl dimethyl amine hydrogen maleate. It acts as anti-histamine and is used in cough syrups.

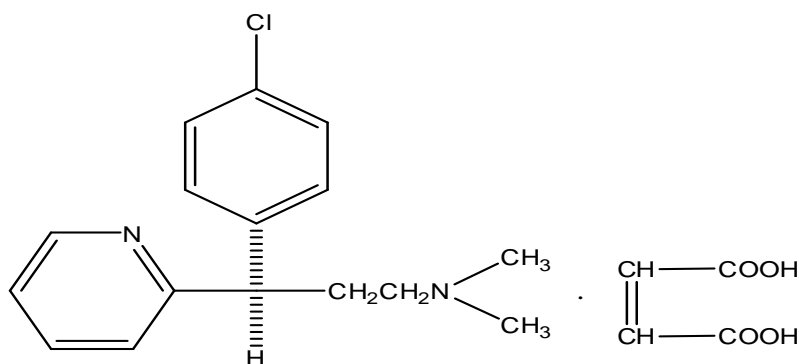


Fig: 2 Structure of Chlorpheniramine maleate.

Guaiphenesin is chemically known as (RS)-3-(2-methoxyphenoxy) propane-1,2-diol. It comes under the category of expectorant and is used to reduce cough.

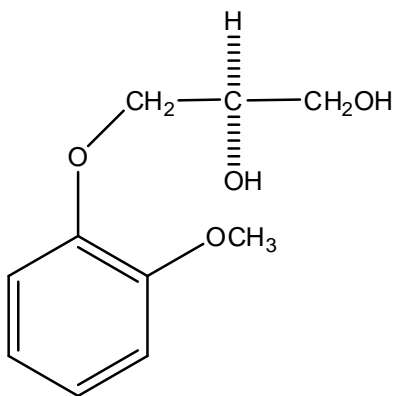


Fig: 3 Structure of Guaiphenesin.

The combination of these three drugs was widely used in the preparation of cough syrups to treat respiratory disorders.

Literature survey revealed that very few analytical methods like UV-spectroscopy and HPLC methods were reported and no Chemometric methods were reported for the analysis of above combination. The present study aims to design chemometric assisted spectroscopic methods for the intricate analysis of Ambroxol, Chlorpheniramine and Guaiphenesin.

MATERIALS AND METHODS:

Instruments used:

Analytical balance
UV-Visible spectrophotometer (Lab India -3072)

Data handling systems:

UV-win for the handling of spectrophotometer.
The Unscrambler X
Microsoft excel.

Materials used:

Working standards of drugs were procured from Dr. Reddy s laboratory.
Commercial formulation of drugs were purchased from local market. Methanol AR grade was procured from Merck (India) ltd, Mumbai.

PREPARATION OF SOLUTIONS:

Preparation of Ambroxol standard solutions:

10 mg of Ambroxol standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using 5 ml methanol and volume was made up to the mark with methanol. Further dilutions were made with the methanol to get required concentrations of 10,15,20,25 and 30 µg/ml.

Preparation of Chlorpheniramine maleate standard solutions:

10 mg of Chlorpheniramine maleate standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using 5 ml methanol and volume was made up to the mark with methanol. Further dilutions were made with the methanol to get required concentrations of 2,4,6,8 and 10 µg/ml.

Preparation of Guaiphenesin standard solutions:

10 mg of Guaiphenesin standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using 5 ml methanol and volume was made up to the mark with methanol. Further dilutions were made with the methanol to get required concentrations of 10,20,40,60 and 80 µg/ml.

Preparation of Ambroxol, Chlorpheniramine maleate, Guaiphenesin:

Stock solution was prepared by diluting 5 ml of marketed liquid formulation to 50 ml with methanol. Required quantity of this stock solution was pipetted into volumetric flask to get 15 µg/ml, 2 µg/ml, 50 µg/ml of Ambroxol, Chlorpheniramine maleate, Guaiphenesin respectively.

Design of chemometric models:

Chemometric models were designed for the developed spectrophotometric methods for the simultaneous estimation of Ambroxol (AMB), Chlorpheniramine maleate (CPM), Guaiphenesin (GPN).

Trilinear regression analysis (TLRC):

In this method three wavelengths were considered for the analysis of the component mixture [AMB(X), CPM(Y), GPN (Z)].The three linear regression equations were obtained by using the absorbance measured at three wavelengths against concentrations of standard solutions for each component. The slope values obtained from the linear regression analysis for each component were used for the formation of matrix set.

The wavelengths selected for analysis were 248nm (λ_{\max} of AMB), 261 nm (λ_{\max} of CPM), 274nm (λ_{\max} of GPN).
Equations for the formation of matrix are:

$$A_{\text{mix1}} = b_{x1}C_x + b_{y1}C_y + b_{z1}C_z + a_{xy1}$$

$$A_{\text{mix2}} = b_{x2}C_x + b_{y2}C_y + b_{z2}C_z + a_{xy2}$$

$$A_{\text{mix3}} = b_{x3}C_x + b_{y3}C_y + b_{z3}C_z + a_{xy3}$$

Where, A_{mix1} , A_{mix2} , A_{mix3} are the absorbance of the mixture of X, Y, Z analytes at three wavelengths set. a_{xy1} , a_{xy2} , a_{xy3} are the sum of intercepts of the linear regression equation at the three wavelengths.
Conversion of equation into matrix form:

$$\begin{bmatrix} Amix1 - axyz1 \\ Amix2 - axyz2 \\ Amix3 - axyz3 \end{bmatrix} = \begin{bmatrix} bx1 & by1 & bz1 \\ bx2 & by2 & bz2 \\ bx3 & by3 & bz3 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \\ Cz \end{bmatrix}$$

Cramer's Matrix Method

Molar absorptivity (ϵ) values were calculated by using the absorbance measured at 248nm, 261 nm, and 274nm for each compound in the ternary mixture. The selected wavelength values were λ_{max} of AMB, CPM and GPN respectively. By using absorptivity (ϵ) values, a system of equations with three unknowns in the ternary mixture have been written as follows:

$$A_{m, 248} = \epsilon_{AMB, 248} C_{AMB} + \epsilon_{CPM, 248} C_{CPM} + \epsilon_{GPN, 248} C_{GPN}$$

$$A_{m, 261} = \epsilon_{AMB, 261} C_{AMB} + \epsilon_{CPM, 261} C_{CPM} + \epsilon_{GPN, 261} C_{GPN}$$

$$A_{m, 274} = \epsilon_{AMB, 274} C_{AMB} + \epsilon_{CPM, 274} C_{CPM} + \epsilon_{GPN, 274} C_{GPN}$$

Where A_m denotes the absorbance of the ternary mixture and ϵ represents the values of molar absorptivity for the calculated AMB, CPM and GPN respectively at 248, 261 nm and 274 nm. C is the molar concentration of AMB, CPM and GPN. The matrix simplifies and solves the system of equations with three unknowns as follows:

$$\begin{bmatrix} Am, 248 \\ Am, 261 \\ Am, 274 \end{bmatrix} = \begin{bmatrix} \epsilon_{AMB, 248} & \epsilon_{CPM, 248} & \epsilon_{GPN, 248} \\ \epsilon_{AMB, 261} & \epsilon_{CPM, 261} & \epsilon_{GPN, 261} \\ \epsilon_{AMB, 274} & \epsilon_{CPM, 274} & \epsilon_{GPN, 274} \end{bmatrix} \times \begin{bmatrix} C_{AMB} \\ C_{CPM} \\ C_{GPN} \end{bmatrix}$$

This matrix can be solved and each compound was determined by solving the following operations (Δ = Determinant value of matrix)

$$\Delta = \begin{bmatrix} \epsilon_{AMB, 248} & \epsilon_{CPM, 248} & \epsilon_{GPN, 248} \\ \epsilon_{AMB, 261} & \epsilon_{CPM, 261} & \epsilon_{GPN, 261} \\ \epsilon_{AMB, 274} & \epsilon_{CPM, 274} & \epsilon_{GPN, 274} \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} Am, 248 & \epsilon_{CPM, 248} & \epsilon_{GPN, 248} \\ Am, 261 & \epsilon_{CPM, 261} & \epsilon_{GPN, 261} \\ Am, 274 & \epsilon_{CPM, 274} & \epsilon_{GPN, 274} \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} \epsilon_{AMB, 248} & Am, 248 & \epsilon_{GPN, 248} \\ \epsilon_{AMB, 261} & Am, 261 & \epsilon_{GPN, 261} \\ \epsilon_{AMB, 274} & Am, 274 & \epsilon_{GPN, 274} \end{bmatrix}$$

$$\Delta_3 = \begin{bmatrix} \epsilon_{AMB, 248} & \epsilon_{CPM, 248} & Am, 248 \\ \epsilon_{AMB, 261} & \epsilon_{CPM, 261} & Am, 261 \\ \epsilon_{AMB, 274} & \epsilon_{CPM, 274} & Am, 274 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration AMB, CPM AND GPN can be found by:

$$C_{AMB} = \Delta_1 / \Delta, \quad C_{CPM} = \Delta_2 / \Delta, \quad C_{GPN} = \Delta_3 / \Delta$$

Method of Least Squares

The standard stock solutions of AMB (15 μ g/ml), CPM (2 μ g/ml) and GPN (50 μ g/ml) were measured at 240nm, 244nm, 248nm, 252nm, 256 nm, 260nm, 264nm, 268nm, 272nm, 276 nm, 280nm and their absorbances were recorded (acts as calibration set) and tabulated in MS- Excel. The individual drug absorbances of known concentrations of AMB, CPM and GPN were added and synthetic mixture (as validation set) was created and absorbances were recorded. Similarly the test sample was also measured at same wavelengths and absorbances were recorded and tabulated. By applying method of least squares using Solver add-in in MS-Excel, the actual concentration of AMB, CPM and GPN were predicted in test samples.

Multivariate calibration methods:

Calibration was performed by using the wavelength range 240 – 280 nm at 4nm interval. Cross-validation of the final models was performed with respect to the number of factors affecting the prediction of each of the compounds. The optimum number of factors was found to be three for both AMB, CPM and GPN in the both PCR and PLS models.

Validation of spectrophotometric method:**Linearity and range:**

The linearity of analytical method is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

The range of analytical procedure is the interval between the upper and lower concentrations of the sample for which the analytical procedure has a suitable level of Precision, Accuracy and Linearity.

Precision:

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Accuracy:

The accuracy of analytical procedure express the closeness or agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by adding known quantities of analyte (pure drug) to the drug product and applying the developed methods to determine the quantity of the drug present in the spiked sample.

Samples were spiked with 50,100,150% level solutions of the standards and analysed. The experiment was performed triplicate (n=3). Percent recovery values were reported.

$$\text{Accuracy} = \frac{\text{Amount of Sample Conc. found} - \text{Amount of Test Conc. taken}}{\text{Amount of Standard Conc. added}} \times 100$$

Assay:

The commercial marketed formulation containing 15mg of Ambroxol, 2mg Chlorpheniramine maleate and 50mg guaiphenesin. The sample solution was treated same as standard solution. The resulting solution scanned under UV using methanol as blank.

$$\text{Percent Assay} = \frac{\text{Calculated qty of test sample (mg)}}{\text{Weight of test sample (mg)}} \times 100$$

RESULTS AND DISCUSSION**TRILINEAR REGRESSION ANALYSIS:**

Table No.1: Absorbance of Ambroxol at 248 nm, 261 nm and 274 nm.

Conc. ($\mu\text{g/ml}$)	248 nm	261 nm	274 nm
10	0.209	0.062	0.015
15	0.311	0.095	0.024
20	0.409	0.122	0.031
25	0.511	0.155	0.040
30	0.599	0.189	0.0495
Linear Equation	$y = 0.0196x + 0.0158$	$y = 0.0063x - 0.001$	$y = 0.0017x - 0.0021$
R^2	0.9994	0.9986	0.9977

Table No.2: Absorbance of Chlorpheniramine at 248 nm, 261 nm and 274 nm.

Conc. ($\mu\text{g/ml}$)	248 nm	261 nm	274 nm
2	0.044	0.051	0.024
4	0.064	0.078	0.028
6	0.087	0.109	0.037
8	0.112	0.140	0.045
10	0.138	0.174	0.058
Linear Equation	$y = 0.0118x + 0.0182$	$y = 0.015 + 0.018$	$y = 0.0043x + 0.0129$
R^2	0.9974	0.998	0.9695

Table No. 3: Absorbance of Guaiphenesin at 248 nm, 261 nm and 274 nm.

Conc. ($\mu\text{g/ml}$)	248 nm	261 nm	274 nm
10	-0.039	0.014	0.098
20	-0.023	0.075	0.226
40	-0.002	0.185	0.461
60	0.402	0.314	0.702
80	0.056	0.413	0.927
Linear Equation	$y = 0.0014x - 0.0526$	$y = 0.0058x - 0.0415$	$y = 0.0118x - 0.0144$
R^2	0.9772	0.9985	0.9997

$$\begin{bmatrix} \text{Amix1} - \text{axyz1} \\ \text{Amix2} - \text{axyz2} \\ \text{Amix3} - \text{axyz3} \end{bmatrix} = \begin{bmatrix} \text{bx1} & \text{by1} & \text{bz1} \\ \text{bx2} & \text{by2} & \text{bz2} \\ \text{bx3} & \text{by3} & \text{bz3} \end{bmatrix} \times \begin{bmatrix} \text{Cx} \\ \text{Cy} \\ \text{Cz} \end{bmatrix}$$

$$\begin{bmatrix} 0.369 - (-0.0186) \\ 0.390 - (-0.0245) \\ 0.620 - (-0.00036) \end{bmatrix} = \begin{bmatrix} 0.0196 & 0.0118 & 0.0014 \\ 0.0063 & 0.015 & 0.0058 \\ 0.0017 & 0.0043 & 0.0118 \end{bmatrix} \times \begin{bmatrix} \text{Cx} \\ \text{Cy} \\ \text{Cz} \end{bmatrix}$$

$$\begin{bmatrix} 0.388 \\ 0.414 \\ 0.624 \end{bmatrix} = \begin{bmatrix} 0.0196 & 0.0118 & 0.0014 \\ 0.0063 & 0.015 & 0.0058 \\ 0.0017 & 0.0043 & 0.0118 \end{bmatrix} \times \begin{bmatrix} \text{Cx} \\ \text{Cy} \\ \text{Cz} \end{bmatrix}$$

$$\begin{bmatrix} \text{Cx} \\ \text{Cy} \\ \text{Cz} \end{bmatrix} = \begin{bmatrix} 15.055 \\ 1.942 \\ 50.005 \end{bmatrix}$$

The concentration of Ambroxol (C_x), Chlorpheniramine maleate (C_y) and guaiphenesin (C_z) present in the given formulation sample were found to be 15.055 $\mu\text{g/ml}$, 1.942 $\mu\text{g/ml}$ and 50.005 $\mu\text{g/ml}$ respectively.

Cramer's matrix method:

$$A_{\text{mix1}} = b_{x1}C_x + b_{y1}C_y + b_{z1}C_z + a_{\text{xyz1}}$$

$$A_{\text{mix2}} = b_{x2}C_x + b_{y2}C_y + b_{z2}C_z + a_{\text{xyz2}}$$

$$A_{\text{mix3}} = b_{x3}C_x + b_{y3}C_y + b_{z3}C_z + a_{\text{xyz3}}$$

$$\begin{bmatrix} \text{Am, 248} \\ \text{Am, 261} \\ \text{Am, 274} \end{bmatrix} = \begin{bmatrix} \varepsilon_{\text{AMB, 248}} & \varepsilon_{\text{CPM, 248}} & \varepsilon_{\text{GPN, 248}} \\ \varepsilon_{\text{AMB, 261}} & \varepsilon_{\text{CPM, 261}} & \varepsilon_{\text{GPN, 261}} \\ \varepsilon_{\text{AMB, 274}} & \varepsilon_{\text{CPM, 274}} & \varepsilon_{\text{GPN, 274}} \end{bmatrix} \times \begin{bmatrix} \text{C AMB} \\ \text{C CPM} \\ \text{C GPN} \end{bmatrix}$$

By substituting the values in matrix and it was solved and each compound was determined by solving the following operations (Δ = Determinant value of matrix).

$$\Delta = \begin{bmatrix} 20733 & 22000 & 40 \\ 6333 & 25500 & 5000 \\ 1600 & 12000 & 12400 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} 0.369 & 22000 & 40 \\ 0.390 & 25500 & 5000 \\ 0.645 & 12000 & 12400 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} 20733 & 0.369 & 40 \\ 6333 & 0.390 & 5000 \\ 1600 & 0.645 & 12400 \end{bmatrix}$$

$$\Delta_3 = \begin{bmatrix} 20733 & 22000 & 0.369 \\ 6333 & 25500 & 0.390 \\ 1600 & 12000 & 0.645 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration of ATR, EZT and FNF were found as follows

$$C_{AMB} = \Delta_1 / \Delta$$

$$= 15.58 \mu\text{g/mL}$$

$$C_{CPM} = \Delta_2 / \Delta$$

$$= 1.99 \mu\text{g/mL}$$

$$C_{GPN} = \Delta_3 / \Delta$$

$$= 48.07 \mu\text{g/mL}$$

The concentration of Ambroxol (C_x), Chlorpheniramine maleate (C_y) and guaiphenesin (C_z) present in the given formulation sample were found to be 15.58 $\mu\text{g/ml}$, 1.99 $\mu\text{g/ml}$ and 48.07 $\mu\text{g/ml}$ respectively.

Method of least squares:

The standard stock solutions of AMB (15 $\mu\text{g/mL}$), CPM (2 $\mu\text{g/mL}$), GPN (50 $\mu\text{g/mL}$) were measured at 240-280 nm with 4 nm interval. Molar absorptivity's are calculated and tabulated. Further calculations are done as shown below

Wavelength	Absorbances			Am	Absorptivities			A calc	Acalc-Am	(Acalc-Am) ²
	AMB	CPM	GPN		AMB	CPM	GPN			
240	0.225	0.045	0.066	0.336	15000	22500	1320	0.336253	0.000252651	6.38327E-08
244	0.281	0.043	0.001	0.325	18733	21500	20	0.325237	0.000237483	5.63984E-08
248	0.311	0.044	0.001	0.356	20733	22000	20	0.356243	0.0002435	5.92921E-08
252	0.279	0.046	0.035	0.36	18600	23000	700	0.360259	0.000259054	6.71087E-08
256	0.197	0.047	0.103	0.347	13133	23500	2060	0.347258	0.000258269	6.6703E-08
260	0.112	0.051	0.204	0.367	7466	24500	4080	0.365263	-0.001737029	3.01727E-06
264	0.058	0.046	0.329	0.433	3866	23000	6580	0.433246	0.0002455	6.02704E-08
268	0.036	0.04	0.463	0.539	2400	20000	9260	0.539222	0.000221942	4.92584E-08
272	0.025	0.031	0.563	0.619	1666	15500	11260	0.619162	0.000161958	2.62305E-08
276	0.022	0.021	0.558	0.601	1467	10500	11160	0.601122	0.00012157	1.47792E-08
280	0.023	0.014	0.453	0.49	1533	7000	9060	0.490073	7.28489E-05	5.30667E-09
										3.48645E-06
Actual Concentration										
AMB=	15				AMB=	1.50002E-05				
CPM=	2				CPM=	2.01107E-06				
GPN=	50				GPN=	5E-05				
J4=(G4*H17)+(H4*H18)+(I4*H19)										

Fig No.4: Screen shot of arranging data into excel sheet.

Microsoft Excel 15.0 Answer Report

Worksheet: [New Microsoft Excel Worksheet (2).xlsx]Sheet1

Report Created: 26-04-2017 14:38:49

Result: Solver found a solution. All Constraints and optimality conditions are satisfied.

Solver Engine

Engine: GRG Nonlinear

Solution Time: 0.031 Seconds.

Iterations: 3 Subproblems: 0

Solver Options

Max Time Unlimited, Iterations Unlimited, Precision 0.000001, Use Automatic Scaling

Convergence 0.0001, Population Size 100, Random Seed 0, Derivatives Forward, Require Bounds

Max Subproblems Unlimited, Max Integer Sols Unlimited, Integer Tolerance 1%, Assume NonNegative

Objective Cell (Min)

Cell Name	Original Value	Final Value
\$J\$15	2.57068E-05	3.48645E-06

Variable Cells

Cell Name	Original Value	Final Value	Integer
\$H\$1 amb=AMB	1.50002E-05	1.50002E-05	Contin
\$H\$1 cpm=CPM	2.08163E-06	2.01107E-06	Contin
\$H\$1 gpn=GPN	0.00005	5E-05	Contin

Fig No.5: Screen shot of solver report.

The concentration of Ambroxol (C_x), Chlorpheniramine maleate (C_y) and guaiphenesin (C_z) present in the given formulation sample were found to be 15.00 $\mu\text{g/ml}$, 2.01 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ respectively.

Table No.4: Percentage assay for the three methods.

	TLR			CRM		MLS	
	<i>Actual concentration (µg/mL)</i>	<i>Predicted concentration (µg/mL)</i>	<i>Assay %</i>	<i>Predicted concentration (µg/mL)</i>	<i>Assay %</i>	<i>Predicted concentration (µg/mL)</i>	<i>Assay %</i>
AMB	15	15.06	100.40	15.58	103.86	15.00	100.00
CPM	2	1.94	97.00	1.99	99.50	2.01	100.50
GPN	50	50.01	100.02	48.07	96.14	50.00	100.00

Multi variate calibration techniques:

Experimental design for the calibration set.

Table No.5: Calibration set containing 15 synthetic mixtures of AMB, CPM and GPN.

<i>Mix. No.</i>	AMB	CPM	GPN
<i>Mix 1</i>	20	6	40
<i>Mix 2</i>	20	2	10
<i>Mix 3</i>	10	2	80
<i>Mix 4</i>	10	10	20
<i>Mix 5</i>	30	6	80
<i>Mix 6</i>	15	4	40
<i>Mix 7</i>	30	4	20
<i>Mix 8</i>	20	8	20
<i>Mix 9</i>	15	10	60
<i>Mix 10</i>	15	8	80
<i>Mix 11</i>	25	10	60
<i>Mix 12</i>	30	8	40
<i>Mix 13</i>	25	6	80
<i>Mix 14</i>	20	10	80
<i>Mix 15</i>	30	10	10

Experimental design for the validation set.

Table No. 6: Validation set containing 10 synthetic mixtures of AMB, CPM and GPN.

<i>Mix. No.</i>	AMB	CPM	GPN
<i>Mix 16</i>	30	2	60
<i>Mix 17</i>	10	8	10
<i>Mix 18</i>	25	2	40
<i>Mix 19</i>	10	6	60
<i>Mix 20</i>	20	8	60
<i>Mix 21</i>	25	8	20
<i>Mix 22</i>	25	4	10
<i>Mix 23</i>	15	2	20
<i>Mix 24</i>	10	4	40
<i>Mix 25</i>	15	6	10

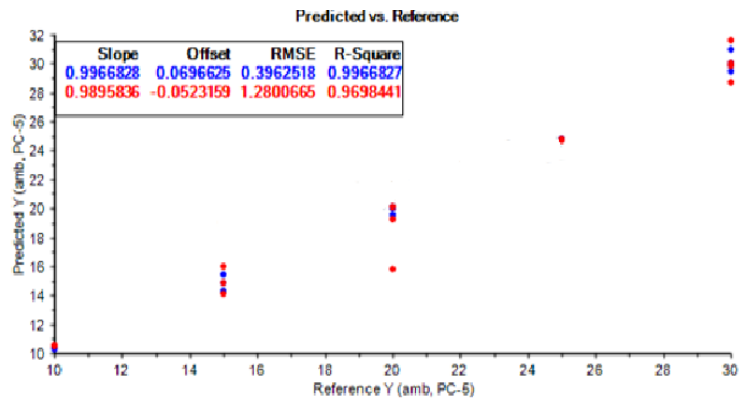


Fig No.6: Predicted Vs Reference Concentrations of AMB by PCR method.

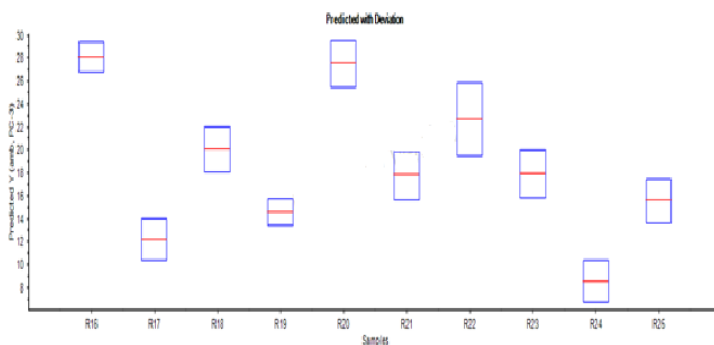


Fig No.7: Predicted Vs Reference Concentrations of AMB by PCR method showing deviation from Mean.

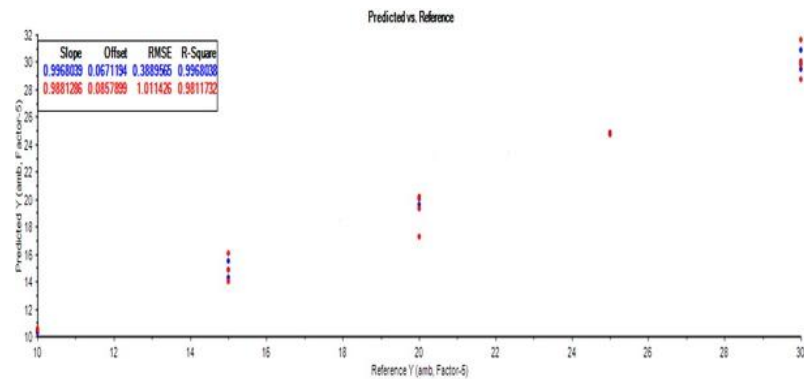


Fig No.8: Predicted Vs Reference Concentrations of AMB by PLS method.

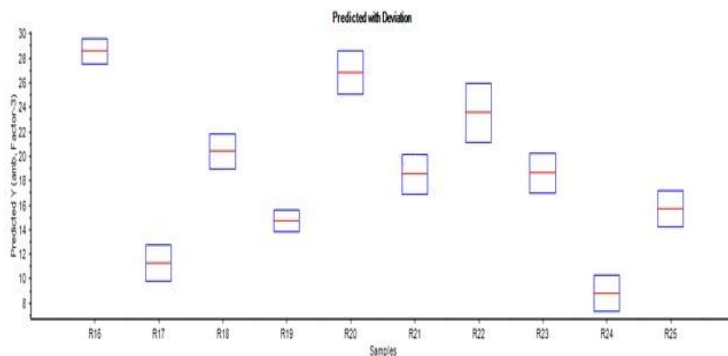


Fig No.9: Predicted Vs Reference Concentrations of AMB by PLS method showing deviation from Mean.

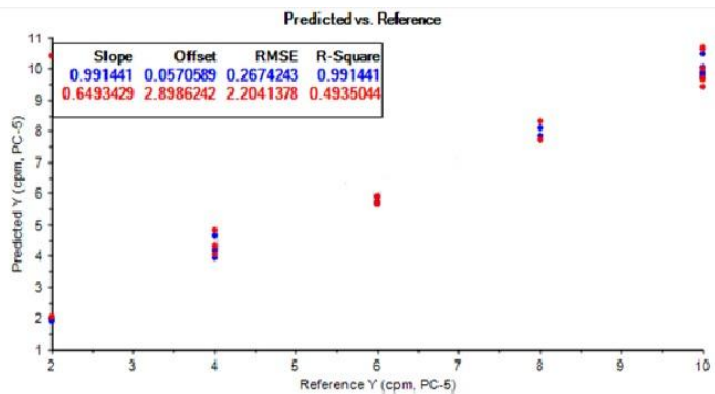


Fig No.10: Predicted Vs Reference Concentrations of CPM by PCR method.

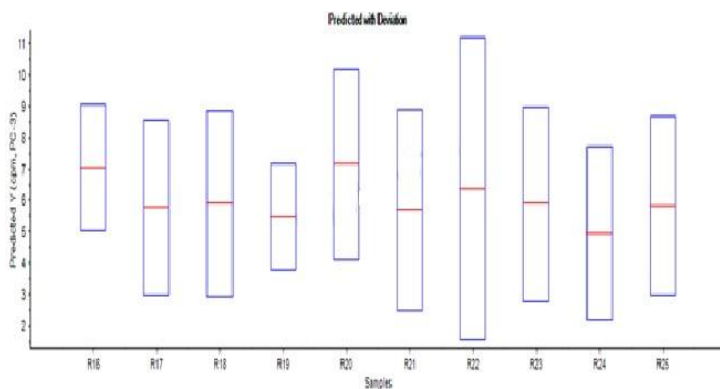


Fig No.11: Predicted Vs Reference Concentrations of CPM by PCR method showing deviation from Mean.

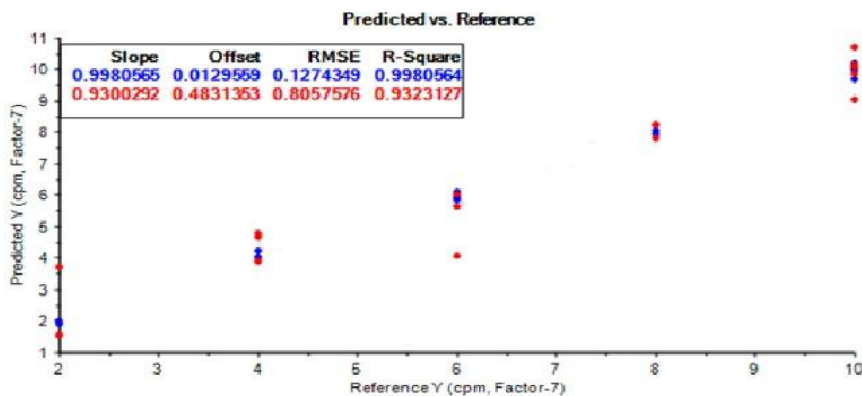


Fig No.12: Predicted Vs Reference Concentrations of CPM by PLS method.

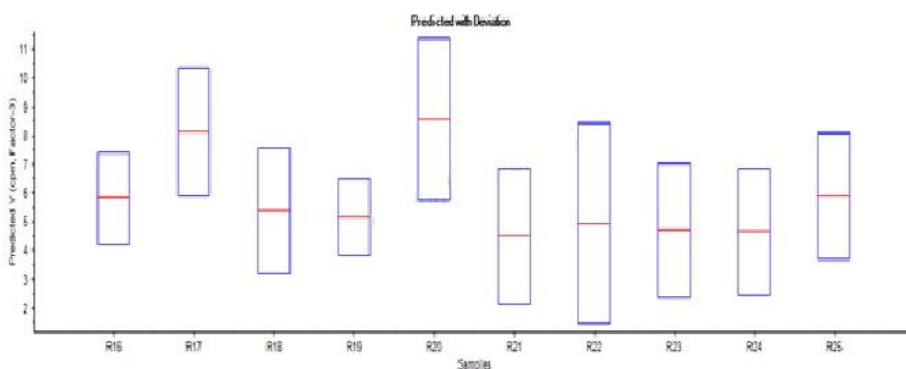


Fig No.13: Predicted Vs Reference Concentrations of CPM by PLS method showing deviation from Mean.

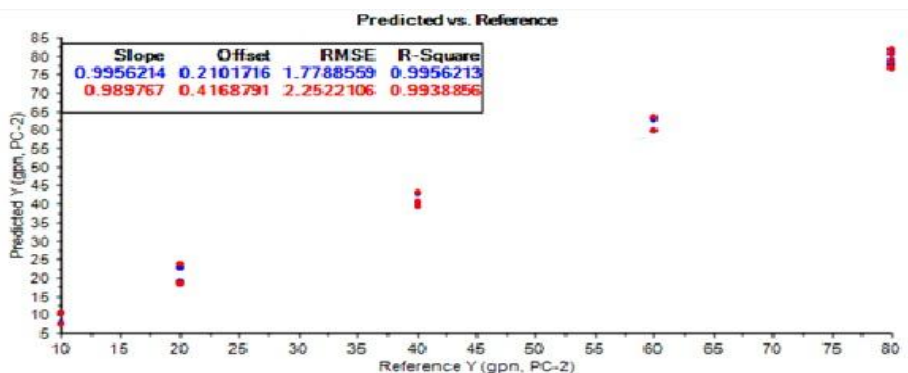


Fig No.14: Predicted Vs Reference Concentrations of GPN by PCR method.

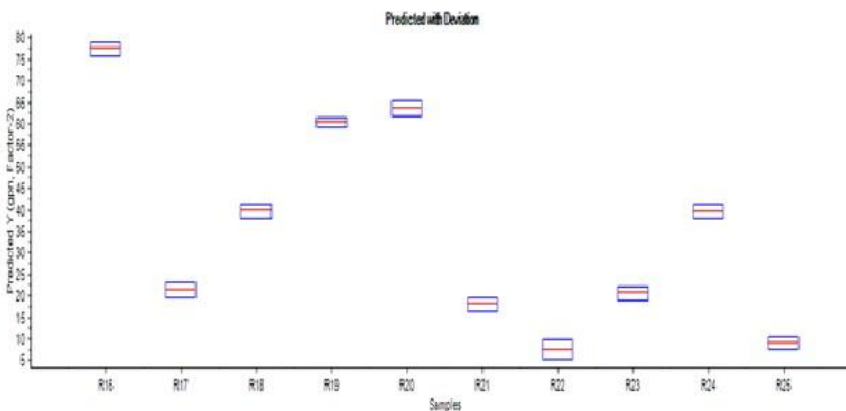


Fig No.15: Predicted Vs Reference Concentrations of GPN by PCR method showing deviation from Mean.

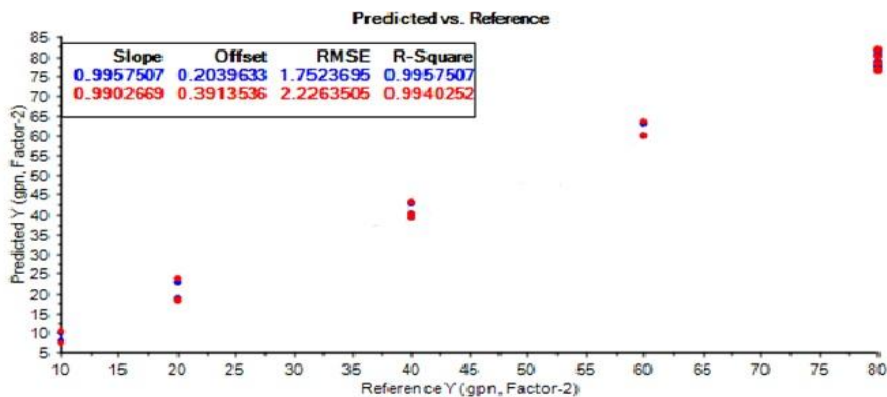


Fig No.16: Predicted Vs Reference Concentrations of GPN by PLS method.

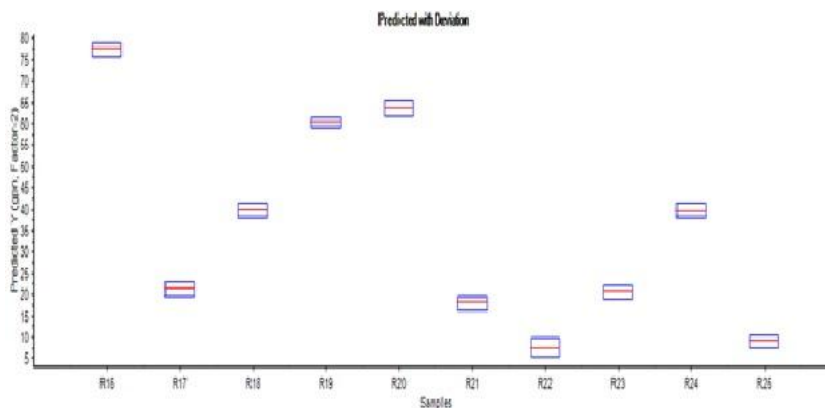


Fig No.17: Predicted Vs Reference Concentrations of GPN by PLS method showing deviation from Mean.

When the calibration models were applied to the prediction set, the concentrations predicted by the models were found to be very close to the nominal concentrations, confirming the validity of both methods. The obtained results were summarized as shown below

Table No.7: Predicted concentrations from PCR and PLS models for validation set.

Mix. No	Actual Concentration (in $\mu\text{g/mL}$)			Predicted Concentration (in $\mu\text{g/mL}$)					
	AMB	CPM	GPN	PCR			PLS		
	AMB	CPM	GPN	AMB	CPM	GPN	AMB	CPM	GPN
16	30	2	60	28.0058	7.0210	77.2659	28.5243	5.7885	77.2907
17	10	8	10	12.1181	5.7575	21.2461	11.2096	8.0928	21.2107
18	25	2	40	20.0100	5.8669	39.4994	20.3418	5.3857	39.5209
19	10	6	60	14.5073	5.4459	60.0585	14.6674	5.1313	60.0788
20	20	8	60	27.4651	7.1371	63.4211	26.7767	8.5420	63.3937
21	25	8	20	17.7310	5.6732	17.7607	18.4564	4.4739	17.7963
22	25	4	10	22.6361	6.3563	7.3268	23.5089	4.9174	7.3599
23	15	2	20	17.8593	5.8693	20.3504	18.5565	4.6667	20.3826
24	10	4	40	8.4624	4.9189	39.4464	8.7275	4.6353	39.4699
25	15	6	10	15.4791	5.8067	9.8412	15.6384	5.8636	9.8495

Assay of Pharmaceutical formulation

From the precise prediction ability of both PCR and PLS methods the concentrations of AMB, CPM and GPN were found as follows

Table No. 8: Predicted concentrations from PCR and PLS in Assay of Formulation.

	PCR			PLS		
	Actual concentration ($\mu\text{g/mL}$)	Predicted concentration ($\mu\text{g/mL}$)	Assay %	Actual concentration ($\mu\text{g/mL}$)	Predicted concentration ($\mu\text{g/mL}$)	Assay %
AMB	15	15.47	103.13	15	15.64	104.27
CPM	6	5.81	96.83	6	5.89	98.17
GPN	10	9.84	98.41	10	9.85	98.50

Acceptance criteria: 95- 105% (w/v)

METHOD VALIDATION:**Accuracy****Table No. 9: Percentage recovery for all the methods.**

DRUG	PERCENTAGE	% RECOVERY				
		FOR TLRC	FOR CRM	FOR MLS	FOR PCR	FOR PLS
AMB	75%	98.66	100.13	99.85	96.65	96.56
	100%	99.10	99.75	99.72	97.12	97.26
	125%	100.44	99.84	99.15	96.78	97.72
CPM	75%	98.89	100.44	99.12	96.54	97.67
	100%	99.16	100.50	100.26	96.68	96.92
	125%	100.26	99.86	98.98	97.56	97.16
GPN	75%	99.50	99.26	99.64	98.12	99.22
	100%	99.90	100.30	99.86	98.72	98.95
	125%	99.96	100.46	100.12	97.95	99.16

Linearity and range**Table No. 10: Linear equation parameters.**

Drug	Wave length nm	For TLRC Method			For Cramer's matrix method(CRM)		
		Linear equation	R ²	RANGE µg/mL	Linear equation	R ²	RANGE µg/mL
AMB	248	$y = 0.0196x + 0.0158$	0.9994	10-30	$y = 0.0196x + 0.0158$	0.9994	10-30
	261	$y = 0.0063x - 0.001$	0.9986		$y = 0.0063x - 0.001$	0.9986	
	274	$y = 0.0017x - 0.0021$	0.9977		$y = 0.0017x - 0.0021$	0.9977	
CPM	248	$y = 0.0118x + 0.0182$	0.9974	2-10	$y = 0.0118x + 0.0182$	0.9974	2-10
	261	$y = 0.015x + 0.018$	0.998		$y = 0.015x + 0.018$	0.998	
	274	$y = 0.0043x + 0.0129$	0.9695		$y = 0.0043x + 0.0129$	0.9695	
GPN	248	$y = 0.0014x - 0.0526$	0.9772	10-80	$y = 0.0014x - 0.0526$	0.9772	10-80
	261	$y = 0.0058x - 0.0415$	0.9985		$y = 0.0058x - 0.0415$	0.9985	
	274	$y = 0.0118x - 0.0144$	0.9997		$y = 0.0118x - 0.0144$	0.9997	

PRECISION**Table No. 11: Percentage RSD for all the methods.**

DRUG	Concentration	Inter day precision (% RSD)					Intraday precision (% RSD)					
		TLRC	CRM	MLS	PCR	PLS	TLRC	CRM	MLS	PCR	PLS	PCR
AMB	15	1.1	1.7	1.5	1.4	1.2	1.7	1.3	1.2	1.8	1.4	1.2
	20	1.2	1.5	1.4	1.4	1.4	1.5	1.5	1.3	1.6	1.6	0.9
	25	1.1	1.4	1.2	1.6	1.5	1.8	1.1	1.5	1.5	1.5	1.1
CPM	04	1.4	1.8	1.2	1.6	1.1	1.8	1.5	1.6	1.8	1.6	1.5
	06	1.2	1.6	1.3	1.5	1.2	1.7	1.7	1.5	1.7	1.7	1.6
	08	1.5	1.2	1.5	1.7	1.2	1.8	1.6	1.6	1.8	1.6	1.5
GPN	20	1.2	1.6	1.4	1.5	1.6	1.6	1.6	1.5	1.8	1.5	1.2
	40	1.1	1.3	1.3	1.2	1.4	1.7	1.6	1.7	1.7	1.8	1.4
	60	1.2	1.2	1.4	1.5	1.2	1.6	1.8	1.5	1.6	1.6	1.6

The proposed spectrophotometric method was found to be linear and the data is presented in the Table No 10. The intra-day and inter-day precision values for both the chemometric designs were presented in Table No 11. Accuracy was performed in terms of the Percent recovery values and the values for Ambroxol, Chlorpheneramine maleate and guaiphenesin by all the chemometric designs were presented in Table No 9. The assay of the commercial formulation of the drugs were performed and their percentage assay values were presented in Table No 4 and 8.

CONCLUSION

The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the three drugs and moreover they are effective in minimizing the errors in analysis, simple and economical. Finally it is concluded that the developed methods were simple and accurate can be used in routine analysis.

ACKNOWLEDGEMENT

We acknowledge the management and the principal of Chalapathi institute of pharmaceutical sciences, Lam, Guntur for providing the facilities to carry out this research work. We also Dr.Reddy's laboratories, Pvt. Ltd, Hyderabad for providing the gift samples of the drugs.

CONFLICT OF INTEREST

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

REFERENCES

1. Sanni Matero. Chemometric methods in pharmaceutical tablet development and manufacturing unit operations; Publications of the University of Eastern Finland, 2010; p. 5-12.
2. Mike. J. Adams. *Chemometrics in Analytical Spectroscopy*; Royal society of chemistry; 1995; p.155-197.
3. Naveen Kumar, Ankit Bansal, G.S. Sarma and Ravindra K. Rawal. Chemometrics tools used in analytical chemistry: An overview; *Talanta*, 2014 (123); p. 186–199.
4. J. N. Miller, J. C. Miller. *Statistics and Chemometrics for analytical chemistry*. 6th ed. Pearson Education Limited; 2010; p. 52-58.
5. Angus M. Brown. A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet. *Computer Methods and Programs in Biomedicine*, 2001; 65; p. 191-200.
6. P. Y. Pawar, Ankita R. Bhagat., Sonu R. Lokhande. and Amruta A. Bankar U.V. spectrophotometric method *Der Pharma Chemica*, 2013
7. Chintan P. Patel, Rajesh R. Parmar, Dushyant A. Shah, Amit B. Gadhavi U.V. spectrophotometric method 10-03-2012
8. V. Aparna, D. Sireesha, Dr. Vasudha bakshi UV- Spectrophotometry *pharma reserch library*;
9. Londhe SV, Mulgund SV, Jain KS. Reversed-phase HPLC JAN 2011
10. Mr. JIVAN RAJARAM PATIL RP-HPLC 2011-2012
11. Harshad O. Kaila UPLC Method *Saurashtra University*; 2011
12. S.M.Patole 1 , L.V.Potale , A.S.Khodke , M.C.Damle HPLC 2015
13. A. Sankar, Thangarasu Vetrichelvan, Devashya Venkappaya UV-spectrophotometric *Degruyter*; Sept 2011
14. A.K.M. Pawar , A.B.N. Nageswara Rao, D. Gowri Sankar Isocratic RP-HPLC *Scholars Research Library, Der Pharmacia Lettre*, 2011
15. H. O. Kaila, M. A. Ambasana 1 and A. K. Shah 1 Ultra-Performance Liquid Chromatographic Assay Method Jan-Mar 2011
16. D. Kealey, P. J. Haines. *Instant notes on analytical chemistry*. 2nd ed. bios Scientific Publishers Ltd; 2002; p. 1-7.
17. David Harvey, *Modern analytical chemistry*, 1st ed. McGraw-Hill; 2000; p. 5-9.
18. Satinder Ahuja, Stephen Scypinski. *Handbook of modern pharmaceutical analysis*, Academic Press. Vol.3; 2001; p. 420-426.
19. James W. Robinson. *Undergraduate instrumental analysis*. 6th ed. Marcel Dekker, 2005; p. 329-45.
20. Prashant S. Devrukhakar LC Method *American Journal of Analytical Chemistry* (01.09.2010)
21. S. Shetty, K.Surendranath, P.Radhakrishnanand, R. Borkar, P. Devrukhakar, J.Jogul UHPLC method 2011.



54878478451170606



Submit your next manuscript to **IAJPR** and take advantage of:

- Convenient online manuscript submission
- Access Online first
- Double blind peer review policy
- International recognition
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in **Scopus** and other full-text repositories
- Redistributing your research freely

Submit your manuscript at: editorinchief@iajpr.com

