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Chemo-Metric Evaluation of Levocetrizine Dihydrochloride, Paracetamol, Ambroxol Hydrochloride and Phenylephrine Hydrochloride in Pharmaceutical Dosage Forms.

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Research Article

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ABSTRACT

The study explains multivariate analytical methods named Classical Least Square (CLS), Inverse Least Square (ILS), and Principle Component Regression (PCR). In general, the term multivariate analysis leads to solving the problem having number of variables and in here it leads to evaluate the different drugs in various combinations. So to evaluate Ambroxol Hydrochloride (AMB), Paracetamol (PCM), Levocetrizine Dihydrochloride (LCZ) and Phenylephrine Hydrochloride (PHE) in pharmaceutical dosage form whether the later contains two, three or all of them. Thus, these methods were also called as multipurpose methods. To reach the goal first 36 different composite mixtures of all drugs were prepared and analyzed through UV- spectrophotometer and above mentioned all the three models (CLS, ILS and PCR) were depicted. The LOD and LOQ values for AMB, PCM, LCZ and PHE from these models were found to be 0.32 µg/mL, 0.29 µg/mL, 0.43µg/mL and 1.15µg/mL and 0.96 µg/mL, 0.87 µg/mL, 1.29 µg/mL and 3.45 µg/mL respectively which is very less than previous conventional methods. Then these models were applied on five different dosage forms having above drugs in binary, tertiary or quaternary combinations and mean recoveries of 101.4%, 100.9 %, 100.2%, 101.3% for CLS; 101.8%, 100.4 %, 100.2%, 100.7% for ILS; 100.8%, 100.3 %, 100.2%, 100.1% for PCR; for AMB , PCM, LCZ and PHE respectively. The statistics were applied to conclude that there is no significant difference between the methods. The accepted t-value (less than 5.42) of marketed samples confirms that these all methods are suitable for routine assay of selected drugs in binary/ tertiary or quaternarycombination.

INTRODUCTION

Chemometrics is the methodology which forms its own space in the world of analysis from last few years. This method was originated by S.wold in 1972 who is physical organic chemist, jointly with B.R Kowalski who is American analytical chemist, S.wold combine to create the international chemometrics community or society [1]. Thus, chemometrics can be explained as the science which gives the information that how the chemical measurement can be done with the help of mathematical and statistical method. The term chemometrics obtained from chemistry and measurement. Thus this method helps to obtain the hidden information from the chemical data [2].Now in this world of technology the chemometrics method is the expansion as computer software that contribute to getting final recognisition from the unsolved information that present in term of raw or crude data [3,4]. Some physicist or analyst reviewed about the chemometrics method as a sub field for modern analytical chemistry. But the chemometricans themselves consider chemometrics set the new directions for analysis [5] .Ambroxol (AMB) chemically it is Trans-4-[(2-

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Amino-3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride [6]. It is a mucolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. By decreasing the viscosity of sputum it enhances its removal from respiratory tract [7]. Figure 1 shows the structure, molecular formula and molecular weight of AMB.

Levocetirizinedihydrochloride (LCZ) is (*R*)-2-[2-[4-[(4-chlorophenyl)phenylmethyl] piperazin-1-yl] ethoxy] aceticacid dihydrochloride [8]. It is an active enantiomer of cetirizine. It works by blocking histamine receptors [9].Figure 1 shows the structure, molecular formula and molecular weight of LCZ.

Paracetamol (PCM) is *N*-(4-Hydroxyphenyl)acetamide [10] (PCM). It is a non-steroidal anti-inflammatory, analgesic and antipyretic drug and most commonly used over-the-counter remedy for minor aches and pains and relief of headaches and used along with various cold preparations. It may act by inhibiting cyclo-oxygenase (COX-3, a linked variant of COX-1) [11]. Figure 1 shows the structure, molecular formula and molecular weight of PCM.

Phenylephrine hydrochloride (PHE) is (1*R*) -1-(3-Hydroxyphenyl)-2-(methyl amino) ethanolhydrochloride [12] (PHE). It is selective alpha agonist used as nasal decongestant and sympathomimetic bronchodilator [13]. Figure 1 shows the structure, molecular formula and molecular weight of PHE.

As per literature survey there is no method reported regarding the selected combination. Therefore the aim of our research work is develop and validate different chemometric method to estimate AMB, LCZ, PCM and PHE in as binary, tertiary and quaternary combinations.

EXPERIMENTAL

Instruments

UV-Vis double beam spectrophotometer perkinelmer version 6.0.3 was used for all spectrophotometric measurements, having slit width of 1 nm, installed with UV Probe data processor and viewer software.Ultra bath sonicator (PCI analytics 3.5 Lcapacity) was used for proper mixing of stock solutionsand sonication.

Reagents and materials

All chemicals used were analytical grade (Central Drug House Chemicals, New Delhi, India). Pure samples of Amboroxol hydrochloride (AMB), Levocetrizinedihydrochloride (LCZ), Paracetamol (PCM), and Phenylephirine hydrochloride (PHE) were obtained from LABORATE Pharmaceuticals, Panipat (HARYANA) India and used after their identification and assessment of percentage purity. Marketed formulation named as 1-AL (Total) which is a tablet formulation, akums drugs and pharmaceuticals Ltd, batch no. NYA3082 was procured from local market.

Stock Solutions

Pure samples stock solution 1 mg/mL of AMB, LCZ, PCMand PHE were freshly prepared individually in 0.1N HCl and methanol (1:9)and further dilutions were made in methanol (forspectrophotometric measurement blank was keptconstant in the ratio of 1:9 0.1N HCl: methanol).

Extraction Procedure

For preparation of marketed dilutions, the equivalentweight were calculated and according to the calculated weight, firstly required suspension extracted in 0.1N HCl and methanol (1:9)by using sonication process for 1 hour at room temperature. After the completion of process the resultant solution was filtered through Whatman filter paper number 41. Thenfurther dilutions were made in methanol A.R and absorbencies were measured and analyzed.

Standard Laboratory Mixture

Standard laboratory mixture was prepared to obtainprecise result by making the concentration same as inmarketed formulation.

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Software's

Design of experiment v 5.8 (DoE) was used to design the calibration set of concentrations then these prepared mixtures of different concentrations were scanned in complete range i.e from 400 nm to 200 nm.Unscrambler v 10.2, 32 –bit was used for chemometric calculations in order to solve different matrices calculations in method CLS, ILS, PCR, NAS etc. Graph pad Prism v 5.01 was used to apply on statistical parameters such as two way ANOVA and, student's t-test in order to determine the final results.

METHODS

Optimization ofparameters

There are different solubility properties of each drug therefore, depending on the solubility and U.V absorption maxima, linearity for all drugs were determined. All parametersof physical properties of AMB, LCZ, PCM and PHE wereestimated accordingly (Table 1 and 2).

Calibration Model

By using DoE, a response surface D-optimal design wasoptimized. This design contains 6 levels of 4 variables studies. 6 levels indicate the different concentration range as suggested by DoE and 4 variables indicates to the 4 drugs. Design comprises of 36 differentcomposite mixtures of all 4 drugs indifferent concentrations. Here the concentration model was consideredas concentration (C) matrix in multivariate analysis. All these 36 composite mixtures were scanned in U.V. range(400 nm - 200 nm) after that each spectrum was autocorrectedby subtracting spectrum of blank. Then absorbenciesreading was takenat interval of 1nm from each spectrum, from these absorbencies thematrix is formed which is referred as A- matrix.

Optimization of Stability

Stability of stock solutions were determined by measuring absorbencies of freshly prepared dilution of sameconcentration from same reserved stock. Stock solutions were found to be stable for three days when stored in refrigerated conditions. The results were concluded in Table no. 3

MULTIVARIATE ANALYSIS

Classical Least Squares (CLS) [14, 15]

This method is used for quantitative determination and also consider as K- matrix. This method is based on Beer Lambert law. In the very complex mixture this method found to be very advantageous. The absorptivity coefficients can be calculated than the much simpler least square regression method.

As discussed above this method depends upon Beer Lambert law, it consists of different variables such as:-

The absorbance (A), the molar absorptivity (€), the path length of sample (b), the concentration of the compound in solution (C).For the most quantitative experiments the path length of the sample and the molar absorptivity must be remain constant.

$$A = C \times K \quad \text{..... (1)}$$

Where K is the constant of molar absorptivity and path length.

For the calibration the classical least squares solution to above equation

$$K = (C^T \times C)^{-1}. C^T \times A \text{..... (2)} \ [16]$$

The above equation can be taken into consideration to determine the concentration of the unknown sample with the help of absorbencies was taken having known concentration.

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Inverse least squares [17-19]

This method is used for quantitative determination and also considers as P- matrix

By rearrange the Beer Lambert law the equation can be written as

$$C = A \times P \quad \text{..... (3)}$$

Where, (C) concentration and (A) absorbance is same as in CLS and (P) is the constant of inverse of molar absorptivity and path length.

For the calibration the inverse least squares solution to above equation

$$P = (A^T \times A)^{-1} A^T \times C \quad \text{..... (4)} \ [20]$$

The above equation can be taken into consideration to determine the concentration of the unknown sample with the help of absorbencies was taken having known concentration.

The advantage of the method is that the equations or unsolved matrix can be easily calculated. On the basis of recognition of constituents of interest this model helps to predict the very complex mixture. Thus, those complex mixtures that cannot be resolved by CLS method can easily predict by ILS method.

Principle component regression [21]

Principal component regression is the method that is the combination of inverse least square and principal component analysisand also called as multiple linear regression.

The PCR method divided into following steps:

- Principal components were obtained on the consideration of data matrix by performing PCA for different variables. Then the subset was selected on suitable basis on theses basis the principal component can be considered for further use.
- Now the observed vector that is the outcome of principal component by application of linear regression on co-variate estimated regression coefficient to get a vector.
- Now with the help of PCA loading that is the eigen vector of selected principal components the vector now transformed into actual co-variates to get the final PCR estimation which gives the characterization of the original model [22].

The highest values of eigen values gives the optimal numbers of principal components. The concentration of unknown sample can be easily determined by the application of linear regression equation:

$$C = a + b \times A \quad \text{..... (5)}$$

We calculate the coefficients a and b: coefficient

$$b = P \times Q \quad \text{..... (6)}$$

Where P is the matrix of eigenvectors and Q is C-loadings given by

$$Q = D \times T^T \times A_0 \quad \text{..... (7)}$$

Where, T^T is the transpose of the score matrix T, D is a diagonal matrix having on the diagonal components the inverses of the selected eigenvalues.

Knowing b, we found a using formula:

$$a = C_{mean} - A^T_{mean} \times b \quad \text{..... (8)}$$

Where, C_{mean} represents the mean concentration of the calibration set and A^T_{mean} is the transpose of the matrix having the entries of mean absorbance values.

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Evaluation of data

The above data is further evaluated by application of two way ANOVA. It is done with the help of graph pad prism.

Method validation

Validation is the process that gives the conformation that the method adopted for any test is working properly. Positive results obtained after validation confirms about the uniformity or trueness of the analytical method. Thus, the validation is the procedure which provides high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics.

In terms of chemometrics the validation parameters are named as figure of merits [23]. It includes: NAS, SENSITIVITY, SELECTIVITY, LOD, LOQ.

Application of methods on marketed formulation

The five marketed formulations,having selected drugs in binary\ tertiary and\or quaternary combinations,were evaluated by these above methods such as CLS, ILS, and PCR. These methods are used to determine the percentage label claim. The results were shown in Table 15 and Table 16.

RESULT AND DISCUSSION

Stock solution stability

The stock solution was prepared under the conditions of room temperature.These solutions were kept under refrigerator for three days. Then check their stability under room temperature. The results were shown under table 3.

Linearity of drugs

Linear ranges of all four standard drugs such as AMB, LCZ, PCM and PHE were taken according to beer's Lambert law. The linear range of drugs was shown in table 4. The linearity of all four drugs was shown in figure 2 and 3.

Calibration model

With the help of design of experiment 36 different mixtures were taken for all the four drugs AMB, LCZ, PCM and PHE. The concentration matrix 36 × 4 (row × column) were taken and scanned in U.V under the linear ranges as shown in the table 5. Then the absorbances were taken for all 36 mixtures. The absorbencies we got are termed as A- matrix. Theautocorrection was done by subtracting the each spectrum with the spectrum of blank. The dimensions of A- matrix 36 × 200 (row × column).

Multivariate Analysis

Classical Least Square (CLS) Regression Method

As explained in equations 1 and 2 the K-matrix is calculated. TheCross Validation (CV) for the method has been done. Sum oferrors in calibration were calculated fromCV study. Theresults were summarized in Table 6.

Inverse Least Square (ILS) Regression Method

Almost same as in CLS theILS P-matrix was calculated. This method was used to predictconcentrations of constituents in unknown mixtures.TheCross Validation (CV) for the method has been done. Sum oferrors in calibration were calculated fromCV study. The results ofcalculated SEC were given in Table 7.

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Principle Component Regression (PCR) Method

After pretreatment of absorbance and Concentration matrixscores and loadings were calculated in graphical form. Thisis shown in Figures 4 (a) and 4 (b). The cross validation of the method has been done. By the help of graphical representation the PC- matrix were calculated. The results are summarized in table 8.

Statistical Evaluation of Calibration Set

From the calibration model (shown in Table 5) the mean recoveries(%), RSD (%) obtained when two way ANOVA was applied between them. For the estimation of AMB, PCM, LCZ and PHE two way ANOVA was applied between the mean recoveries (%), RSD (%) as shown in the table 9. In the show results in table 10 the calculated F-value was found less than the theoretical value therefore the prediction ability of designed tools does not differ significantly.

Validation

The validation can be done by designed multivariate tools that consist the checking of predictive ability of all models in set of synthetic mixtures called prediction set. Thus, predictionset consist of 12 different mixtures having different concentrations of alld rugs. The concentrations of drugs inprediction set were shown in Table 11.The consisting mixtures were scanned under UV range and the resulting absorbencies were reported as unknown to predict the concentration of synthetic mixtures. The mean recoveries(%) for each drug were calculated. The results were shown in Table 12.

Figure of Merits (Validation Parameters)

Figure of merits (FOM) were calculated from prediction set and is also called as validation parameters. All the parameters were followed according to ICH guidelines. The parameters were summarized in Table 13.The compliance of Figure of merits and statistical data concludes the effective use of designed multivariate tools in estimation of AMB, PCM, LCZ and PHE in mixture. Further these tools were applied to marketed formulations.

Statistical Evaluation of Prediction Set

Two way ANOVA was applied for the estimation of AMB, PCM, LCZ and PHE between the mean recoveries (%), RSD (%) and the multivariate tools. The calculated F value was found less than the theoretical value. Thus, the prediction ability of designed tools does not differ significantly. The statistical results of prediction set were given in Table 14.

Application of Model to Marketed Formulation

The five different marketed formulations were analyzed through all multivariate analytical tools for amount of all the drugs present in them. The results were shown in Table 15. Then student t- test were applied on label claim (%) found for each drug in each method. The results were shown in Table 16. The each calculated t- value was found lesser than tabulated t-values for 95 % confidence limit. As mentioned already the marketed formulations werebinary/tertiary or quaternary combinations of selected drugs. The accepted t-value concludes all the designed multivariate tools are multipurpose methods to estimate AMB, PCM, LCZ and PHE in their binary tertiary or quaternary mixtures.

Table 1: Identification Test for AMB and PCM.

Physical Properties	Description	
	LCZ	PHE
Appearance	White powder	White, crystalline powder
Melting point mean (n=3)	205 - 208 °C	141 - 142 °C
(Limits)	(204 - 210 °C)	(140-145°C)
Solubility	Freely soluble in Methanol, slightly soluble in ether.	Freely soluble in Water, Alcohol.
λ_{max}	230 nm	275 nm

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Table 2: Identification Test for LCZ and PHE.

Physical Properties	Description	
	AMB	PCM
Appearance	A white to yellowish crystalline powder	White, crystalline powder
Melting point mean (n=3)	79 - 80 °C	168-172ºC
(Limits)	(78-82°C)	(78-82 °C)
Solubility	Slightly soluble in Chloroform, Ether. Freely soluble in Water, Methanol.	Free soluble in methanol, chloroform. Sparingly Soluble in Ethanol (95%).
λ_{max}	245 nm	249 nm

Table 3: Stability of Stock Solution (least concentration).

Days	Drugs absorbencies at λ max (n=3)			
	AMB	PCM	LCZ	PHE
1	0.385	0.485	0.467	0.334
11	0.383	0.484	0.464	0.331
111	0.386	0.485	0.462	0.328

Table 4: Linear Range of AMB, PCM, LCZ and PHE.

Drugs	Range (µg/mL)
AMB	8– 24
PCM	3–8
LCZ	6– 18
PHE	20 – 60

Table 5: Calibration model / C-matrix.

S.NO	LCZ (µg/mL)	AMB (µg/mL)	PCM (µg/mL)	PHE (µg/mL)	S.NO	LCZ (µg/mL)	AMB (µg/mL)	PCM (µg/mL)	PHE (µg/mL)
1)	12	0	8	60	19)	12	16	8	40
2)	9	0	0	40	20)	18	24	0	40
3)	18	24	8	60	21)	0	24	8	60
4)	18	12	5	30	22)	0	24	5	30
5)	0	0	0	0	23)	0	24	0	60
6)	0	12	8	30	24)	18	24	8	0
7)	18	0	8	0	25)	0	0	5	60
8)	18	0	0	60	26)	0	0	8	0
9)	0	24	0	60	27)	12	24	0	60
10)	18	24	0	0	28)	12	16	0	0
11)	6	8	5	20	29)	18	0	8	40
12)	15	16	8	60	30)	18	24	8	0
13)	0	0	8	50	31)	18	0	0	0
14)	0	24	6	0	32)	0	0	0	60
15)	12	8	8	0	33)	15	20	7	50
16)	0	24	0	0	34)	12	12	6	40
17)	18	16	6	60	35)	9	12	5	30
18)	12	0	6	0	36)	6	8	3	20

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Table 6: SEC found in CLS.

Multivariate Analysis				
	CLS			
	AMB	PCM	LCZ	PHE
Mean Recovery (%)	99.4	101.8	100.6	99.8
Percent (%)R.S.D	1.18	1.02	1.23	0.97
SEC (sum of errors in calibration)	0.65	0.55	0.56	0.39

Table 7: SEC found in ILS.

Multivariate Analysis				
	ILS			
	AMB	PCM	LCZ	PHE
Mean Recovery(%)	99.7	98.1	99.4	99.1
Percent (%)R.S.D	1.22	1.12	1.41	0.93
SEC	0.76	0.56	0.87	0.39

Table 8: SEC found in PCR.

Multivariate analysis				
	ILS			
	AMB	PCM	LCZ	PHE
Mean Recovery(%)	99.7	98.1	99.4	99.1
Percent (%)R.S.D	1.22	1.12	1.41	0.93
SEC	0.76	0.56	0.87	0.39

Table 9: Mean Recoveries from Calibration Set.

Parameters	Calibration Set											
	CLS				ILS				PCR			
	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE
Mean Recovery (%)	99.4	101.8	100.6	99.8	99.7	98.1	99.4	99.1	100.7	99.1	98.4	100.1
% RSD	1.18	1.02	1.23	0.97	1.22	1.12	1.41	0.93	0.72	0.72	0.59	0.36

Table 10: Two way ANOVA in Calibration Set.

TWO WAY ANOVA ANALYSIS		
Methods	F- calculated	F- theoretical
CLS	9.7	19.2
ILS	10.8	19.2
PCR	8.9	19.2

Table 11: Concentrations of drugs in Prediction Set.

Sample	Conc. (µg/mL)			
	AMB	PCM	LCZ	PHE
1	8	3	6	20
2	12	6	9	60
3	16	2	12	30
4	20	7	15	50
5	24	5	18	40
6	8	2	15	60
7	12	7	12	20
8	16	3	9	50
9	20	6	6	30
10	25	5	9	60
11	8	7	12	20
12	24	3	15	40

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Table 12: Mean Recoveries (%) in Prediction Set.

SAMPLE	PREDICTION SET CONC (µg/mL)				Conc. Recovered (µg/mL)											
					CLS				ILS				PCR			
	A	P	L	E	A	P	L	E	A	P	L	E	A	P	L	E
1	8	3	6	20	8.02	2.96	6.01	19.99	7.98	2.99	5.99	20.01	8.12	2.99	6.10	19.93
2	12	6	9	60	12.04	6.02	8.98	59.98	11.98	6.01	8.99	60.01	12.04	6.11	8.8	59.36
3	16	2	12	30	15.98	2.04	12.01	29.99	16.02	2.02	12.01	30.02	15.92	2.12	11.8	28.99
4	20	7	15	50	20.01	6.97	15.0	49.98	19.98	7.02	14.98	50.01	20.09	7.03	15.1	48.95
5	24	5	18	40	23.97	5.04	18.0	40.01	24.01	4.98	17.99	40.02	23.93	4.96	18.0	36.49
6	8	2	15	60	8.02	2.01	15.04	59.99	7.98	2.02	14.99	59.99	8.11	2.11	15.4	59.36
7	12	7	12	20	12.04	6.98	12.01	19.98	11.98	7.02	11.97	20.02	12.34	7.12	12.1	18.94
8	16	3	9	50	16.02	3.01	9.02	49.97	15.99	2.98	8.98	50.01	16.12	2.99	9.11	48.36
9	20	6	6	30	19.98	6.02	5.98	29.96	20.02	5.99	6.02	29.99	19.94	5.99	5.94	29.96
10	25	5	9	60	24.96	5.01	9.02	59.96	25.01	4.99	9.01	59.98	24.86	4.93	9.21	59.96
11	8	7	12	20	8.02	6.99	12.01	19.99	7.98	7.02	11.99	20.02	8.09	7.06	12.1	19.86
12	24	3	15	40	23.96	3.02	14.99	39.98	24.02	3.01	15.01	39.97	23.84	3.11	14.8	39.48

Where E = Phenylephrine hydrochloride

Table 13: Figure of Merits (validation parameters).

Parameters	CLS				ILS				PCR			
	A	P	L	PH	A	P	L	PH	A	P	L	PH
Wavelength (nm)	220-260	230-260	210-260	250-330	220-260	230-260	210-260	250-330	220-260	230-260	210-260	250-330
Mean Recovery (%)	100.2	98.8	99.3	99.2	100.6	98.2	99.1	98.1	99.2	100.4	98.4	99.1
(%) RSD	1.34	0.82	0.98	0.97	1.26	1.09	0.82	0.97	0.87	0.87	0.67	0.56
SEP	0.67	0.29	0.31	0.27	0.65	0.74	0.89	0.34	0.29	0.34	0.59	0.30
SEN (mL/ µg)	A = 0.11, P = 0.12 , L = 0.04, PH = 0.16											
SEL	A = 0.03, P = 0.03 , L = 0.06, PH = 0.0015											
LOD (µg/mL)	A = 0.32, P = 0.29 , L = 0.43, PH = 1.15											
LOQ (µg/mL)	A = 0.96, P = 0.87 , L = 1.29, PH = 3.45											

Table 14: Statistical evaluation of prediction set.

Methods	CLS				ILS				PCR			
Drugs	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE
Mean Recovery (%)	100.2	98.8	99.3	99.2	100.6	98.2	99.1	98.1	99.2	100.4	98.4	99.1
(%) RSD	1.34	0.82	0.98	0.97	1.26	1.09	0.82	0.97	0.87	0.87	0.67	0.56
F-calculated	9.2				12.9				7.6			
F-theoretical	19.7											

Table 15: Application of CLS, ILS, PCR to the Marketed Formulations.

BRAND NAMES	LABEL CLAIM FOUND (%)											
	CLS				ILS				PCR			
	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE
AMBRODIL	99.2	-	-	-	98.4	-	-	-	99.6	-	-	-
ABCET	100.1	-	100.3	-	99.6	-	100.1	-	100.4	-	99.7	
ALERCY AX	99.18	-	99.98	99.7	99.4	-	99.8	98.6	99.4	-	99.4	100.1
L CITRIMIN PLUS	100.8	99.3	100.4	-	100.24	99.7	100.1	-	99.93	99.9	99.7	-
1-AL(TOTAL)	101.4	100.9	100.2	101.3	101.8	100.4	100.2	100.7	100.8	100.3	100.2	100.1

Table 16: Results of t- test between the Marketed Formulations.

CALCULATED t-values between both methods and combination.												
S. No	CLS				ILS				PCR			
	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE	AMB	PCM	LCZ	PHE
1	2.43	-	-	-	2.41	-	-	-	2.44	-	-	-
2	2.45	-	2.45	-	2.44	-	2.45	-	2.46	-	2.44	-
3	2.43	-	2.45	2.44	2.43	-	2.44	2.41	2.43	-	2.43	2.45
4	2.47	2.43	2.46	-	2.45	2.44	2.45	-	2.44	2.44	2.44	-
5	2.48	2.47	2.45	2.48	2.49	2.46	2.45	2.46	2.47	2.45	2.45	2.45

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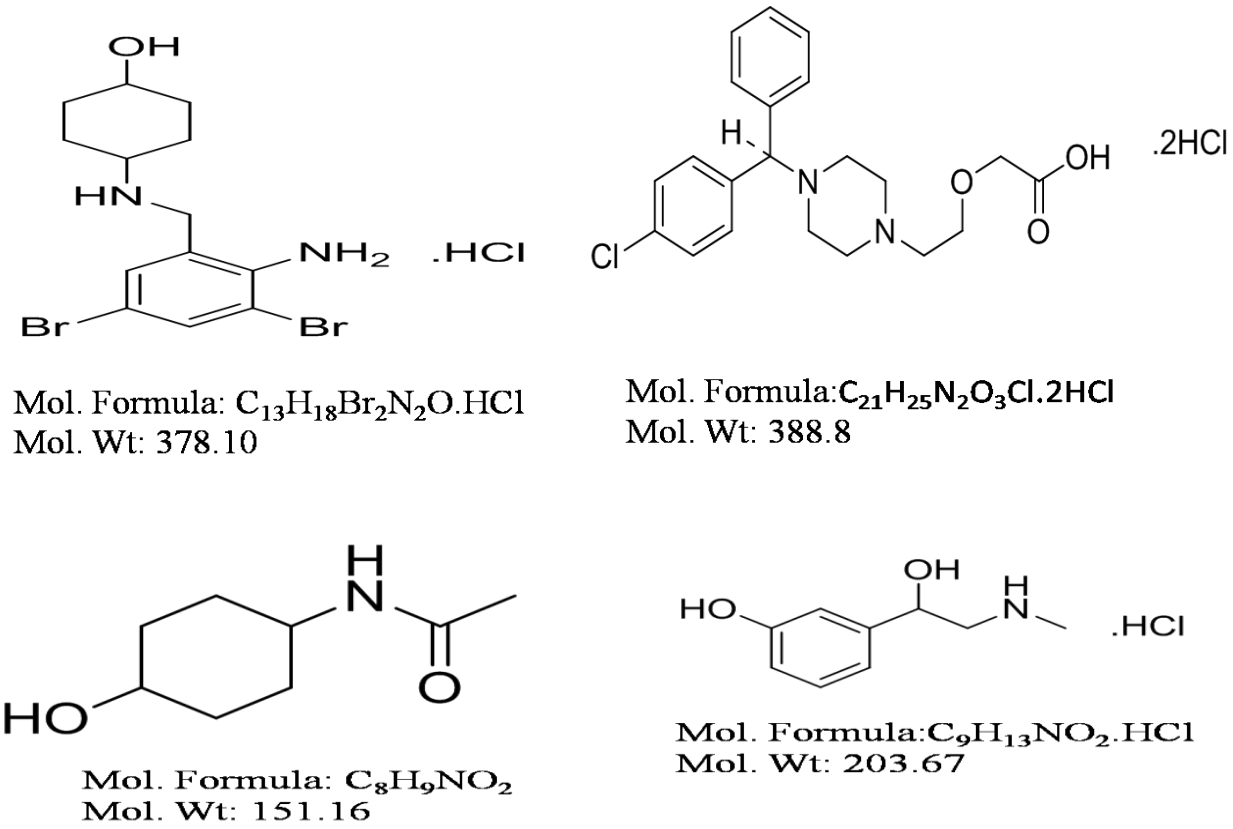

Figure 1: Chemical Structure of Ambroxol hydrochloride, Levocetrizine dihydrochloride, Paracetamol and Phenylephrine hydrochloride.

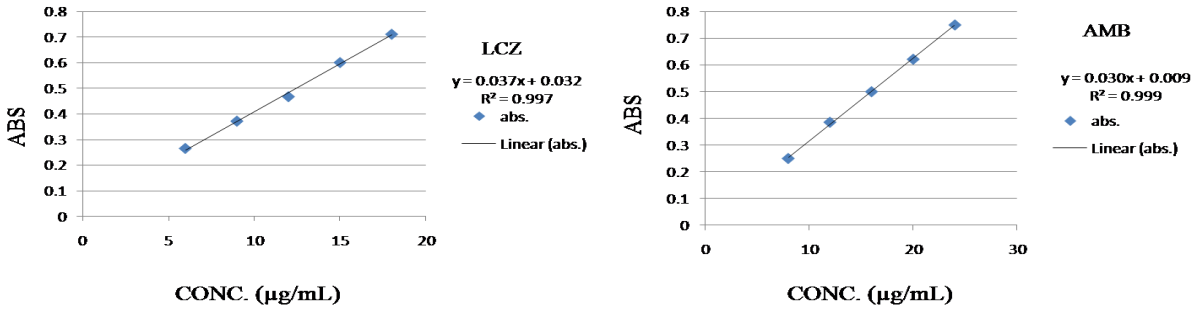

Figure 2: Calibration curve of LCZ and AMB.

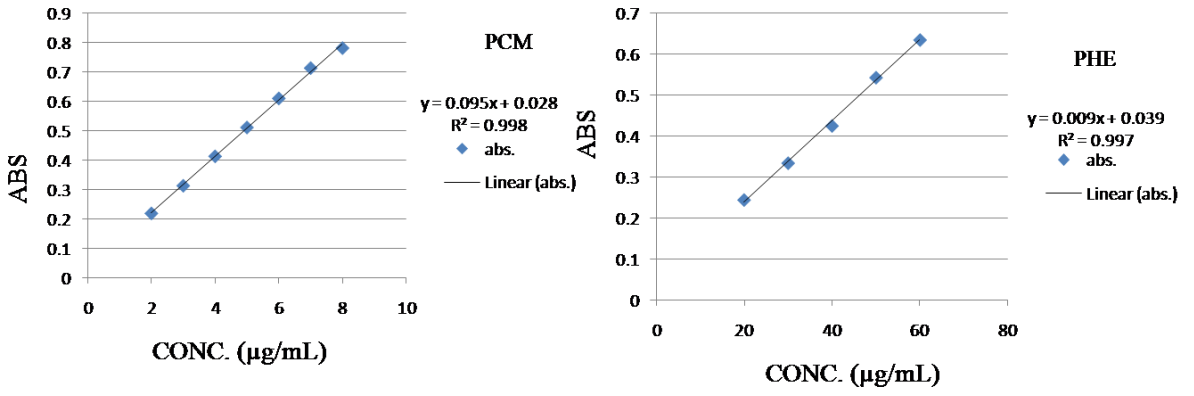

Figure 3: Calibration curve of PCM and PHE.

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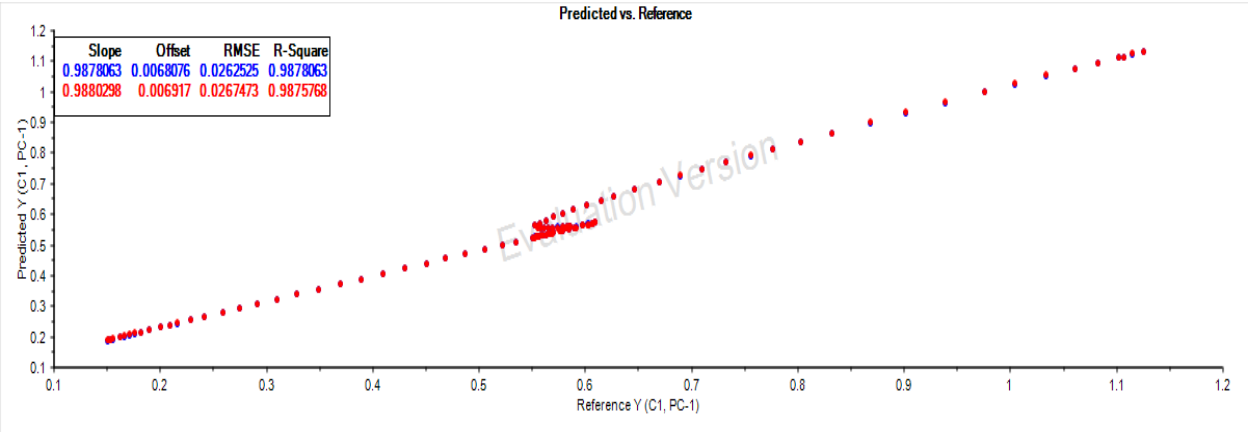

Figure 4(a): Computation of predicted data.

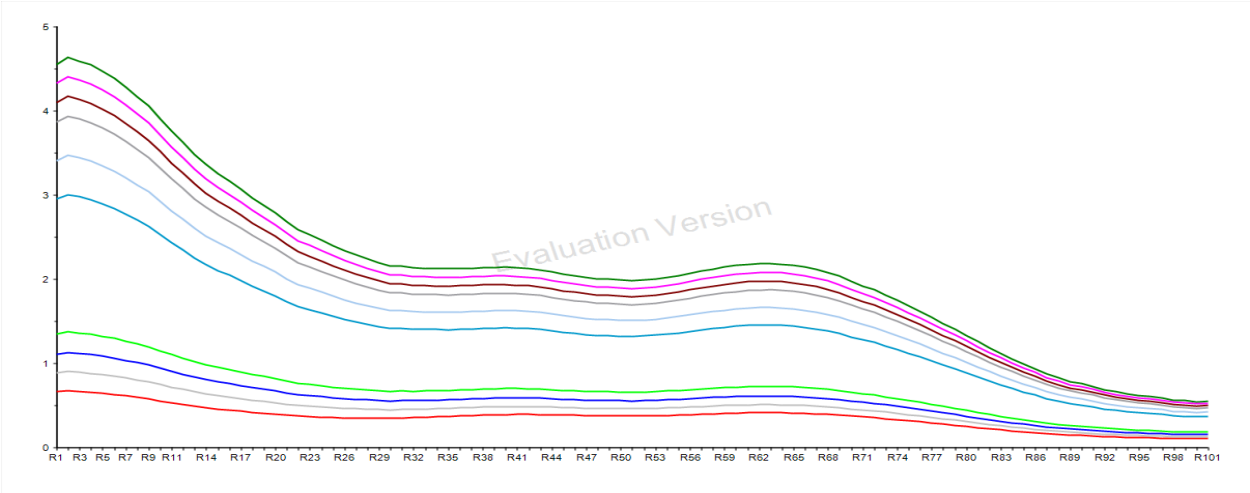

Figure 4(b): Computation of explained data.

CONCLUSION

The different multivariate analytical models CLS, ILS, and PCR were constructed and their prediction ability was checked through CV. The prediction ability of all the models does not differ significantly as the accepted F-value was calculated through two way ANOVA applied on mean recovery percent from cross validation step. Furthermore, brief study on 5 marketed formulations containing AMB, PCM, LCZ, and PHE in different combinations with different amount of drugs was carried out and student t-test was applied on each label claim (%) found for each drug in every marketed formulation. The accepted t-valves conclude that all the developed methods were multipurpose methods.

REFERENCES

1. M Ortiz, and L Sarabia. Quantitative determination in chromatographic analysis based onn-way calibration strategies. J Chromatogr. 2007;1158:94-110.
2. L Xu, LJ Tang, and CB Cai. Chemometric methods for evaluation of chromatographic separation quality from two-way data—A review. Anal Chem Acta. 2008;613:121-134.
3. A Siouffi, and R Phan-Tan-Luu. Optimization methods in chromatography and capillary electrophoresis. J. Chromatogr. 2000;892:75-106.
4. P Vanbel. Development of flexible and efficient strategies for optimizing chromatographic separations. J Pharm Biomed Anal. 1999;21:603-610.
5. YZ Liang, P Xie, and KC Chan. Quality control of herbal medicines. J Chromatogr. 2004;812:53-70.
6. British Pharmacopoeia, B.medicinal and pharmaceutical substances, Ambroxol hydrochloride. Department of Health and Social Services for Northern Ireland. 2009, 265.
7. KD Tripathi, Essential of Medical Pharmacology.Jaypee Brothers Medical Publishers (P) Ltd, New Delhi. 2003, 5

8. Indian Pharmacopoeia, The Indian pharmacopoeia commission Ghaziabad. 2007, 2, 660- 661.
9. HP Rang, MM Dale, JM Ritter, and PK Moore. Rang and Dale's Pharmacology. New York: Churchill Livingstone Elsevier. 2007, 213
10. British Pharmacopoeia, B.medicinal and pharmaceutical substances, Paracetamol. Department of Health and Social Services for Northern Ireland. 2009, 483.
11. HP Rang, MM Dale, JM Ritter, and PK Moore. Rang and Dale's Pharmacology. New York: Churchill Livingstone Elsevier. 2007, 236.
12. British Pharmacopoeia, B.medicinal and pharmaceutical substances, Phenylephrine Hydrochloride. Department of Health and Social Services for Northern Ireland. 2009, 3, 4683.
13. HP Rang, MM Dale, JM Ritter, and PK Moore. Rang and Dale's Pharmacology. New York: Churchill Livingstone Elsevier. 2007, 186.
14. A Lorber. Features of quantifying chemical composition from two-dimensional data array by the rank annihilation factor analysis method. Anal Chem. 1985;57:2395-2397.
15. N Faber, . Buydens, and G Kateman. Generalized rank annihilation method: derivation of eigenvalue problems. J Chemo. 2005;8:147-154.
16. H Martens, and T Naes, Multivariate calibration. John Wiley & Sons: Chichester. 1989, 223- 225
17. E Sanchez, and BR Kowalski. Generalized rank annihilation factor analysis. Anal Chem. 1986;58: 496-499.
18. M Maeder. Evolving factor analysis for the resolution of overlapping chromatographic peaks. Anal Chem. 1987;59:527-530.
19. CJ Xu, JH Jiang, and YZ Liang. Evolving window orthogonal projections method for two-way data resolution. Analyst. 1999;124:1471-1476.
20. MJ Adams, Chemometrics in Analytical Spectroscopy. Graham House Science Park, Cambridge. 1995, 2, 187.
21. YZ Liang, OM Kvalheim, and HR Keller. Heuristic evolving latent projections: resolving two-way multicomponent data. Anal Chem. 1992;64:946-953.
22. KR Beebe, and BR Kowalski. An introduction to multivariate calibration and analysis. Anal Chem. 1987;59:1007-1017.
23. AC Olivieri, NM Faber, J ferré, R boqué, JH Kalivas, and H Mark. Uncertainty estimation and figures of merit for multivariate calibration. Pure Appl Chem. 2006;78:633–661.