

**Original Article**

**DEVELOPMENT OF HPLC AND CHEMOMETRIC ASSISTED SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF FIVE ACTIVE INGREDIENTS IN COUGH AND COLD TABLETS AND THEIR APPLICATION TO DISSOLUTION STUDY**

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**ABSTRACT**

**Objective:** Chemometric assisted spectrophotometry, and HPLC methods have been developed for the simultaneous determination of phenylephrine hydrochloride (PEPH), paracetamol (PCM), guaifenesin (GNF), chlorpheniramine maleate (CPM) and bromhexine hydrochloride (BRM).

**Methods:** The chromatographic separation was carried out on a Phenomenex RPC18 column. An isocratic elution was carried out with the mobile phase comprising methanol, acetonitrile and 10 mM phosphate buffer (pH 3) in the ratio of 27.5:22.5:50 respectively at a wavelength of 218 nm. Two chemometric methods i.e. principal component regression (PCR) method and partial least squares (PLS) method were also developed to quantify each drug in the mixture using the information included in the UV absorption spectra of appropriate solutions in the range 210–320 nm with the intervals of 2 nm at 51 wavelengths.

**Results:** The three methods were successfully applied to a tablet formulation and the results were compared statistically by applying ANOVA, which showed no significant difference among the three methods. The methods were applied to the dissolution study of the five components in tablet formulation and the percentage release of all the five components was found to be greater than 85% within 45 min by all the three methods.

**Conclusion:** Thus, the proposed methods, i.e., PLS, PCR and HPLC, were found to be suitable and can be successfully used for the determination of the PEPH, PCM, GNF, CPM and BRM in pharmaceutical tablet formulation as well as for dissolution study.

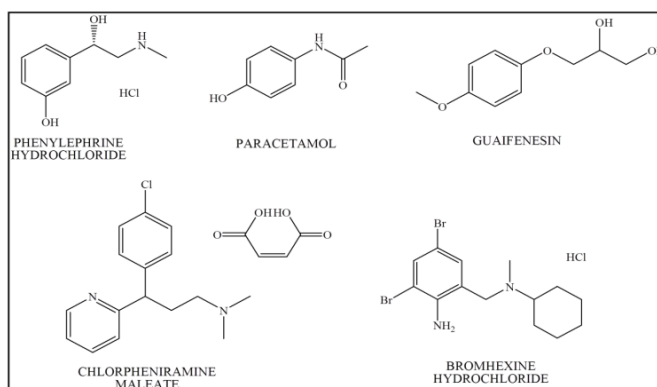
**Keywords:** HPLC, UV, Chemometrics, Cough-cold tablets, Dissolution.

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**INTRODUCTION**

Among the medicines used for the treatment of cough-cold, various formulations consist of combinations of more than two or three drugs (multicomponent system). The analysis of such multi component formulations becomes difficult by conventional analytical techniques like UV spectrophotometry. However, since last few years, the chemometric calibration techniques, such as inverse least squares (ILS), classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS), have widely been applied to the spectrophotometric resolution of such multi component formulations without preliminary separation. PLS and PCR are especially suited for multi component analysis, particularly for mixtures with highly overlapped spectra. Although the HPLC method provides a suitable method for the analysis, but it requires many trials, expensive and high purity solvents and proves to be more time consuming.

The mixture of phenylephrine hydrochloride (PEPH), paracetamol (PCM), guaifenesin (GNF), chlorpheniramine maleate (CPM) and bromhexine hydrochloride (BRM) is the one most widely used the combination for cough and cold therapy. It is mainly used in diseases accompanied by cough, pain and fever, common cold and other viral infections and also used as an analgesic, antipyretic, decongestant, antihistaminic and antitussive. Phenylephrine hydrochloride (PEPH) (fig. 1), (*R*)-3-[1-hydroxy-2-(methylamino)ethyl]phenol, has a decongestant property; paracetamol (PCM) (fig. 1), *N*-(4-hydroxyphenyl)acetamide, is a mild analgesic; guaifenesin (GNF) (fig. 1), (*RS*)-3-(2-methoxyphenoxy)propane-1,2-diol, is used as an expectorant; chlorpheniramine maleate (CPM) (fig. 1), 3-(4-chlorophenyl)-*N,N*-dimethyl-3-pyridin-2-yl-propan-1-amine, is used as an antiallergic and bromhexine hydrochloride (BRM) (fig. 1), 2,4-dibromo-6-[[cyclohexyl(methyl)amino]methyl] aniline, is used as a secretolytic.



**Fig. 1: Structure of PEPH, PCM, GNF, CPM and BRM**

Several analytical methods are available for the determination of PEPH, PCM, GNF, CPM and BRM in various combinations as cough and cold formulations, amongst which some are: high performance liquid chromatography (HPLC) methods [1-8], atomic emission spectrometry [9], mixed ion-pair liquid chromatography [10], liquid chromatography-tandem mass spectrometry [11], non-aqueous capillary electrophoresis [12], gas chromatography [13] and multivariate analytical methods [14, 15]. Chemometric assisted spectrophotometric methods and HPLC method for the combination under study has not been reported yet.

The aim of this study was to investigate the ability of PLS and PCR methods for simultaneous quantification of the five components. The five drug combination (formulation) under study has a wide difference in the weight of components in its tablet formulation, wide differences in the absorptivity of the five components and high UV overlap thus making it a challenging task to develop the simultaneous spectrophotometric method. So UV method in assistance with chemometric methods like PLS and PCR has been developed for simultaneous quantification of the five components. An isocratic HPLC method has also been developed and the results of the chemometric methods have been statistically compared with HPLC method. The proposed methods are simple, accurate, reduce the duration of analysis and are suitable for routine determination of the five components in the commercial formulation.

## MATERIALS AND METHODS

### Instrumentation

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AD and Shimadzu PDA-M20A Diode Array Detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20  $\mu$ l. Data acquisition and integration were performed using LC Solution Software (Shimadzu Corporation, Kyoto, Japan). Separation and quantification were made on a Phenomenex RPC18 column (5  $\mu$ m  $\times$  250 mm  $\times$  4.6 mm i.d.).

Shimadzu UV-1700 double beam spectrophotometer connected to a computer with the Shimadzu UV Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were measured in 1 cm quartz cells over the range of 200-400 nm. PLS and PCR analyses were carried out by using Unscrambler software version 2013. The dissolution was performed on Veego VDA6DR dissolution apparatus.

### Materials and reagents

Bromhexine hydrochloride, paracetamol, phenylephrine hydrochloride, guaifenesin and chlorpheniramine maleate were provided by Ethicare Pharmaceuticals and Alembic Pharmaceuticals (Vadodara, India), as gift samples.

HPLC grade methanol and acetonitrile (Spectrochem), potassium dihydrogen phosphate (AR grade, Loba Chem), phosphoric acid (AR grade, Loba Chem) and triethylamine (HPLC grade, Spectrochem) were used for HPLC analysis. AR grade methanol (Spectrochem) was used for spectrophotometry. AR grade hydrochloric acid (Merck) was used for dissolution. A commercial formulation of Intas Pharmaceutical (Kuff Q tablet) was used for the study. Each tablet contains paracetamol (450 mg), phenylephrine hydrochloride (10 mg), guaifenesin (100 mg), chlorpheniramine maleate (2 mg) and bromhexine hydrochloride (8 mg).

### Experimental conditions

For HPLC, phosphate buffer (0.01 M) was prepared by dissolving 1.36 g of anhydrous potassium orthophosphate ( $\text{KH}_2\text{PO}_4$ ) in 1 L of previously filtered double distilled water, 0.1% of triethylamine was added to it and the pH was adjusted to 3.0 using phosphoric acid. The elution was carried out with a mobile phase composed of the mixture of methanol, acetonitrile and 0.01 M phosphate buffer (pH 3) in the ratio of 27.5:22.5:50. All determinations were performed at ambient temperature. The flow rate was 1 ml/min. The injection volume was 20  $\mu$ l and detection wavelength was 218 nm.

For UV chemometrics, the UV absorption spectra of appropriate solutions (in methanol) were recorded in the wavelength range 200-400 nm. The range of 210-320 nm with intervals of 2 nm ( $\Delta\lambda = 2$  nm) was selected for PLS and PCR model.

The dissolution was carried out by USP paddle method. The dissolution media comprised of 0.01N HCl prepared in single distilled water. The conditions for dissolution were 50 rpm at 37  $^\circ\text{C}$  temperature for the duration of 90 min.

### Standard solutions

For HPLC, standard solutions of each of PEPH, PCM, GNF, CPM and BRM were prepared in the mobile phase within the concentration range of 5-20  $\mu\text{g/ml}$  PEPH, 225-900  $\mu\text{g/ml}$  PCM, 50-200  $\mu\text{g/ml}$  GNF, 1-4  $\mu\text{g/ml}$  CPM and 4-16  $\mu\text{g/ml}$  BRM. The diluted standard solutions with varying concentrations were analysed by HPLC (in triplicate).

Table 1: Training set

Mixture	PEPH	GNF	CPM	PCM	BRM
1	15	1	5	7	12
2	15	5	8	11	2
3	15	12	1	3	9
4	1	15	5	7	12
5	11	15	1	3	9
6	1	5	15	7	12
7	7	12	15	1	6
8	11	1	15	3	9
9	1	5	8	15	12
10	3	8	12	15	2
11	11	1	5	15	9
12	7	12	1	3	15
13	3	5	5	3	6
14	7	8	8	7	9
15	1	5	5	3	6
16	1	8	8	7	9
17	3	1	5	3	6
18	7	1	8	7	9
19	7	8	1	7	9
20	3	5	5	1	6
21	3	5	5	3	1
22	7	8	8	7	1
23	11	12	8	7	9
24	7	8	12	11	9
25	7	8	8	11	12
26	11	8	8	7	12

Table 2: Validation set

Mixture	PEPH	GNF	CPM	PCM	BRM
1	3	5	3	10	6
2	3	5	3	15	6
3	3	5	3	6	12
4	3	5	3	6	15
5	7	8	7	15	9
6	4	4	4	9	4
7	7	8	7	6	6
8	7	8	3	6	9
9	7	3	15	8	9
10	15	3	5	4	4
11	3	15	11	6	4
12	11	4	4	8	6
13	3	11	4	6	6
14	3	12	4	8	6

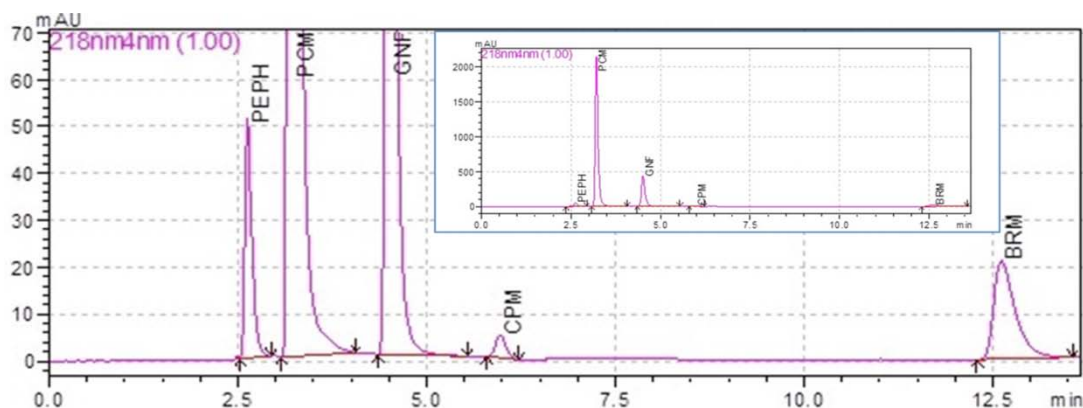


Fig. 2: HPLC chromatogram of sample solution containing 10 ppm PEPH, 450 ppm PCM, 100 ppm GNF, 2 ppm CPM and 8 ppm BRM

For UV chemometrics methods, the standard solutions of each of PEPH, PCM, GNF, CPM and BRM were prepared in methanol. A training set of 26 synthetic mixtures (table 1) and validation set of 15 mixtures (table 2) with different concentrations of each compound were prepared in the range of 3-15 µg/ml PEPH, 4-15 µg/ml PCM, 3-15 µg/ml GNF, 5-15 µg/ml CPM and 4-15 µg/ml BRM. The UV absorption spectra were recorded over the range 200-400 nm.

#### Analysis of tablet formulation

For HPLC, twenty commercial tablets were accurately weighed and powdered in the mortar. An amount of powder equivalent to 45 mg of PCM, 0.8 mg of BRM, 10 mg GNF, 1 mg PEPH and 0.2 mg of CPM was taken in 25 ml of volumetric flask sufficient mobile phase was added and sonicated for 10 min. The volume was then made up to the mark with the mobile phase. This solution was filtered through Whatman filter paper (No. 42) so as to remove undissolved tablet excipients. From this solution, 2.5 ml aliquot was taken and further diluted to 10 ml with the mobile phase. Chromatogram of the sample solution is shown in fig. 2.

For UV chemo metrics, twenty commercial tablets were accurately weighed and powdered. An amount of powder equivalent to 45 mg of PCM, 0.8 mg of BRM, 10 mg GNF, 1 mg PEPH and 0.2 mg of CPM was taken in 25 ml volumetric flask. To this powder, standard addition of 20 mg PEPH, 20 mg CPM and 20 mg BRM was done, owing to their too much low quantity (below LOD of the UV range) in the formulation. This powder mixture was dissolved in sufficient quantity of methanol and sonicated for 10 min. The volume was then made up to the mark with the methanol. This solution was filtered through Whatman filter paper (No. 42) so as to remove undissolved tablet excipients. From this filtered solution, 0.1 ml aliquot was taken and was further diluted to 10 ml with methanol.

#### Dissolution study

The dissolution media was 0.01N HCl, which is the official media reported for the dissolution of PCM and CPM [16]. The dissolution

study was performed for 90 min and the sampling was done at different time intervals of 5, 10, 15, 30, 45, 60 and 90 min. 5 ml of the aliquot was withdrawn from the dissolution vessel at a specific time point. For HPLC analysis, the sample (aliquot) was filtered through 0.2µ membrane filter and injected into the HPLC. For chemometrics analysis, 0.1 ml aliquot was taken in a 10 ml volumetric flask followed by the standard addition of 1 ml (10µg/ml) of PEPH, CPM and BRM each. The solution was made up to the mark with methanol and analysed by UV spectrophotometer.

#### RESULTS AND DISCUSSION

There are various HPLC [1-8] and UV-chemometrics methods [14, 15] available in the literature for the simultaneous estimation of various cough-cold combinations like for the combination of acetaminophen, phenylephrine, chlorpheniramine with cyano column [1], pseudoephedrine, pheniramine, guaifenesin, pyrilamine, chlorpheniramine and dextromethorphan [2], pseudoephedrine hydrochloride, dextrometh orphan hydrobromide, chlorpheniramine maleate and paracetamol [3], but there is no method reported for the combination under study (which is one of the most commonly used formulation). The importance and hence the challenge for this particular combination was that the APIs present in the combination have a wide difference in their weight ratio (i.e. PEPH: PCM: GNF: CPM: BRM in ratio of 5:225:50:1:4 respectively) which makes them difficult to analyse by UV owing to the different detection limits and the different linearity ranges of different components. Moreover, the present method offers the advantage of analysing the five components by a simple UV technique avoiding the difficulties and lengthy procedures of the HPLC method.

#### Optimisation of HPLC method

To optimize the chromatographic conditions, the effect of various chromatographic factors such as: type of buffer (phosphate buffer and formate buffer), pH of buffer (pH 3 to 7), concentration of buffer (10 mM and 25 mM); organic solvent (methanol and acetonitrile);

composition of mobile phase; flow rate (0.8 to 1.2 ml/min) and temperature (25 °C and 40 °C) were studied. Good peak shape and good resolution were observed in phosphate buffer as compared to formate buffer. Acidic pH favoured good peak shapes for all the five drugs. Various combinations of organic and buffer were tried amongst which a ternary mixture of acetonitrile, methanol and buffer gave the best results in terms of peak shape, resolution and other system suitability parameters. The peak shape got a little distorted with high concentrations (25 mM) of the buffer, so 10 mM buffer was used. Temperature and flow rate had no significant effects. Hence, with respect to all these trials, 10 mM phosphate buffer at pH 3 in combination with suitable organic phase (mixture of methanol and acetonitrile) in a ratio of buffer: acetonitrile: methanol as 50:27.5:22.5, at flow rate of 1 ml/min and ambient temperature were selected as the chromatographic conditions.

The developed HPLC method was applied to the simultaneous determination PEPH, PCM, GNF, CPM, and BRM in tablet formulation as well as for the dissolution study of the tablet. A satisfactory separation was obtained with an isocratic elution. Quantitation was achieved based on peak area with UV detection at 218 nm for 20 min. The average retention time in min±standard deviation (for six replicates) for PEPH, PCM, GNF, CPM and BRM was found to be 2.63±0.01, 3.23±0.005, 4.59±0.01, 6.07±0.048, 12.6±0.13 respectively.

#### HPLC method validation

The HPLC method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, specificity and robustness by the ICH guidelines [17].

#### Linearity

The linearity of the HPLC detector response for determination of PEPH, PCM, GNF, CPM and BRM was evaluated by analysing a series of different concentrations of each compound. The calibration range was established with respect to the practical range necessary,

according to the marketed formulation, to give accurate, precise and linear results. In this study, seven concentrations were chosen, in the range of 5-20 µg/ml PEPH, 225-900 µg/ml PCM, 50-200 µg/ml GNF, 1-4 µg/ml CPM and 4-16 µg/ml BRM. Characteristic parameters for regression equations by the HPLC method are given in table 3.

#### Precision

For evaluation of the precision estimates, repeatability and intermediate precision was performed at three concentration levels for each compound. The peak areas of all five drugs were calculated for each trial. The experiment was repeated three times in a day for intra-day precision and on three different days for inter-day precision. The average % RSD (relative standard deviation) of intra-day measurements for determination of PEPH, PCM, GNF, CPM and BRM are given in table 3.

#### Accuracy

The accuracy was performed by the standard addition method. Known amounts of standard APIs were added to a known concentration of the commercial tablet formulation at three levels of standard edition (80%, 100%, and 120%). The resulting mixtures were analysed and the results obtained were compared with the expected results.

The excellent recoveries of standard addition method (table 3) for HPLC suggested the good accuracy of the proposed method. The influence of the commonly used tablet excipients was investigated before the determination of the studied compounds in the tablet. No interference could be observed with the proposed method.

#### Detection and quantitation limits

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the approach based on the standard deviation (S. D.) of the y-intercept and the slope was used and the values obtained are given in table 3 which shows the sensitivity of the method.

**Table 3: Characteristic parameters of the calibration equations for the proposed HPLC method for simultaneous determination of PEPH, PCM, GNF, CPM and BRM**

Parameters	PEPH	PCM	GNF	CPM	BRM
Calibration range(µg/ml)	5-20	225-900	50-200	1-4	4-16
Detection limit (µg/ml)	0.02	1.34	0.087	0.002	0.05
Quantitation limit (µg/ml)	0.074	4.062	0.265	0.008	0.16
Regression equation	y=mx+c				
Slope (m)±SD	5722.51±0.91	25122.09±0.81	6312.33±0.96	15356.89±1.01	11121.77±0.39
Intercept (c)±SD	2353.41±0.62	9002.63±0.49	1611.86±0.74	1421.61±0.49	113.76±0.98
Regression coefficient (r <sup>2</sup> )	0.9994	0.9982	0.9991	0.9992	0.9993
Accuracy (% recovery±SD) <sup>#</sup>					
80%	97.57±1.16	99.6±0.82	99.16±1.25	99.91±0.68	99.18±1.07
100%	98.46±0.94	100.99±1.24	101.32±1.15	101.39±1.15	98.74±0.94
120%	99.65±1.10	100.37±1.01	99.69±0.67	99.66±0.3	99.02±0.6
Precision (%RSD) <sup>*</sup>					
Intraday	0.90	1.18	1.21	1.13	0.89
Interday	1.72	1.58	1.80	1.91	1.70

\*Mean value of n=3 replicates for three concentrations; SD is the standard deviation, <sup>#</sup>Mean recovery of the concentrations at 80%, 100% and 120% level of standard addition for n=3 replicates

#### Robustness

Robustness study was performed by making variations in pH of the phosphate buffer by ±0.2 (2.8, 3, 3.2), change in flow rate by ±0.1 (0.9 ml/min, 1.0 ml/min, 1.1 ml/min) and change in the composition of buffer solution by ±1% (49%, 50% and 51%) did not have significant effects on chromatographic resolution in the HPLC method.

#### Solution stability

The studied compound solutions prepared in the mobile phase exhibited no changes in HPLC or UV data for 24 h when kept at room temperature, and for two days when stored in the

refrigerator (8-25 °C). No additional peak was found in the chromatogram which indicated the stability of the solutions.

#### Specificity

The method was found to be specific as the results were unaffected by the presence of the tablet excipients as shown in fig. 2.

#### System suitability parameters

Theoretical plates, symmetry factor and resolution for PEPH, PCM, GNF, CPM, and BRM in the sample solution were calculated for system suitability of the HPLC method. Satisfactory results were obtained as shown in table 4.

Table 4: System suitability parameters for the five components

Parameters	PEPH	PCM	GNF	CPM	BRM
Retention Time	2.63±0.01	3.23±0.005	4.59±0.01	6.07±0.048	12.6±0.13
Theoretical Plates	3600.81±1.23	3772.68±0.95	7847.76±1.71	7248.06±1.38	9194.40±1.54
Symmetry factor	1.79±0.04	1.67±0.039	1.51±0.003	1.58±0.031	1.83±0.095
Resolution	--	3.11±0.11	6.51±0.28	5.99±0.17	16.16±0.26

Mean (for n=6 determinations)±standard deviation

### UV chemometrics methods (PLS, PCR)

Fig. 3 shows the UV absorption spectra of PEPH, PCM, GNF, CPM, and BRM at their nominal concentrations. The simultaneous determination of PEPH, PCM, GNF, CPM, and BRM in tablet by conventional spectrophotometric methods is hindered by strong spectral overlap throughout the wavelength range and also the wide difference in the ratio of APIs in the formulation. PLS and/or PCR calibration methods are necessary for such determination due to the presence of this spectral interference.

Various HPLC methods have been reported in the literature [14, 15] for a variety of combinations of drugs used as cough-cold formulations. But the present method consists of chemometrics assisted UV method wherein the chemo metric methods like PLS and PCR have been used to resolve the overlapped spectra of the five components under study as shown in fig. 3.

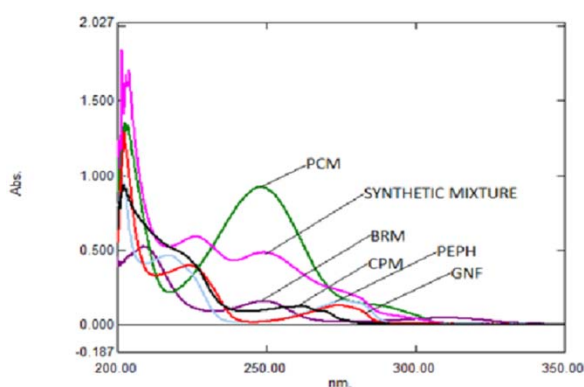


Fig. 3: UV absorption spectra of PEPH, PCM, GNF, CPM and BRM each 10 µg/ml and synthetic mixture of all five drugs

The quality of multi component analysis is dependent on the wavelength range and spectral mode used. In this work, the spectral resolution was assayed with absorbance spectra for PLS and PCR methods, measured at 2 nm intervals over the range 210–320 nm. Wavelengths less than 210 nm were rejected as they were not found to be of much significant contribution for determining the concentration of the five components. Wavelengths more than 320 nm were not used because all the five drugs do not absorb in this region, so any absorbance values obtained at these wavelengths would have introduced a significant amount of noise in the calibration matrix, thereby decreasing the precision.

The predicted concentrations of the components in each sample were compared with the actual concentrations in these training samples and the root mean square error of prediction (RMSEP) was calculated for each method. RMSEP value was used as a diagnostic test to examine the errors in predicting concentrations of the mixtures of the validation set.

Due to the wide difference in the ratio of APIs in the marketed formulation (i.e. PEPH: PCM: GNF: CPM: BRM = 5: 225: 50: 1: 4), a standard addition of some components (i.e. PEPH, CPM and BRM) was required, to bring them at the proper the quantitation limit of UV range.

For PCR and PLS methods, 26 calibration spectra were used for the selection of the optimum number of factors by using the cross-

validation technique. This allowed modelling of the system with the optimum amount of information and avoidance of over fitting or under fitting. The cross-validation procedure consists of systematically removing one of a group of training samples in turn and using only the remaining ones for the construction of latent factors and applied regression [16]. The model should have as low residual variance as possible i.e. explained variance should approach 100%. For this, a number of principal components (in PCR) and the latent factors (in PLS) should be optimised.

### Validation of chemo metric methods

#### RMSEP and PRESS value

The predictive ability, i.e. the validation of PCR and PLS models, was thus assessed by the PRESS value, RMSEP value and residual values of actual concentration and predicted concentration (positive difference of actual and predicted values) [18]. The results are shown in table 5. A satisfactory value of the regression coefficient showed the good predictive ability of the chemometrics models. For the selected principal components (for PCR) and factors (for PLS), the concentration of each sample was then predicted and compared with known concentration (actual value) and the PRESS (prediction residual sum of squares) value was calculated (equation 1) as the difference between the real and the calculated concentrations, squared and summed, over all references for each component. The RMSEP value (root mean square error of prediction) was calculated by using equation 2. The selected model was that with the fewest number of factors such that its RMSEP values were not significantly greater than that for the model, which yielded the lowest RMSEP. A plot of RMSEP values against number of components is shown in fig. 4 which indicates that factor six and seven were optimum for the estimation of the principle ingredients by PLS and PCR. The RMSEP values are indicated in table 5.

#### Accuracy

The accuracy of the chemo metric methods was performed by the standard addition method at three levels (80%, 100%, and 120%). The resulting mixtures were analysed and the results obtained were compared with the true values as shown in shown in table 5. The influence of the commonly used tablet excipients was investigated before the determination of the studied compounds in the tablet. No interference could be observed with the proposed methods.

#### Precision

The precision of PLS and PCR method was performed at three concentration levels for each compound. The solutions were prepared and analysed three times in a day for intra-day precision and on three different days for inter-day precision. The average % RSD of intra-day and inter-day measurements for determination of PEPH, PCM, GNF, CPM and BRM are given in table 5.

#### Solution stability

The stability of the solutions prepared in methanol (for chemo metric study), exhibited no spectrophotometric changes for seven days in the refrigerator (8-25 °C).

$$\text{PRESS} = \sum_{i=1}^n (C_i^{\text{Added}} - C_i^{\text{Found}})^2 \dots\dots\dots (1)$$

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (C_i^{\text{Added}} - C_i^{\text{Found}})^2}{n}} \dots\dots\dots (2)$$

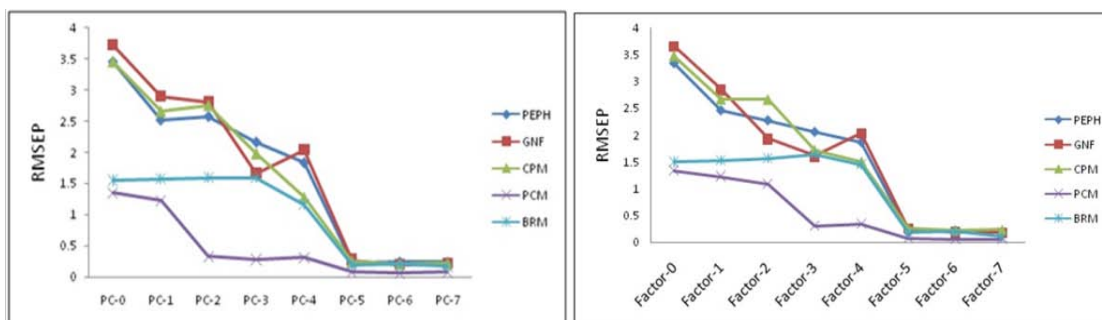


Fig. 4: RMSEP plot of a calibration set prediction (using cross-validation) of PCR model (A) and PLS model (B) for PEPH, PCM, GNF, CPM and BRM

Table 5: Characteristic parameters of the proposed PLS and PCR method for simultaneous determination of PEPH, PCM, GNF, CPM and BRM

Parameters		PEPH	PCM	GNF	CPM	BRM
RMSEP	PLS	0.034	0.022	0.047	0.037	0.0047
	PCR	0.019	0.019	0.017	0.0015	0.0081
$r^2$	PLS	0.9997	0.9994	0.9996	0.9997	0.9998
	PCR	0.9997	0.9998	0.9998	0.9999	0.9996
Regression Equation <sup>#</sup>	PLS	$y = 0.991x + 0.025$	$y = 1.004x - 0.060$	$y = 0.994x + 0.016$	$y = 0.995x + 0.025$	$y = 1.006x - 0.078$
	PCR	$y = 0.999x - 0.007$	$y = 0.998x - 0.008$	$y = 1.005x - 0.028$	$y = 0.993x + 0.017$	$y = 0.996x + 0.011$
Accuracy* 80%	Mean % Recovery $\pm$ SD					
	PLS	99.649 $\pm$ 0.779	100.369 $\pm$ 0.573	100.108 $\pm$ 0.610	99.769 $\pm$ 0.222	100.276 $\pm$ 0.402
100%	PCR	99.649 $\pm$ 0.555	99.875 $\pm$ 0.577	99.441 $\pm$ 0.490	100.458 $\pm$ 0.807	100.075 $\pm$ 0.423
	PLS	100.368 $\pm$ 0.827	99.782 $\pm$ 0.641	99.653 $\pm$ 0.267	100.421 $\pm$ 0.562	99.829 $\pm$ 1.139
120%	PCR	99.621 $\pm$ 0.777	100.165 $\pm$ 0.340	100.376 $\pm$ 0.857	100.825 $\pm$ 0.724	100.442 $\pm$ 0.797
	PLS	99.944 $\pm$ 0.182	100.091 $\pm$ 0.315	99.963 $\pm$ 0.407	100.171 $\pm$ 0.490	100.367 $\pm$ 0.551
Precision <sup>§</sup> Intraday	Mean % Recovery $\pm$ SD					
	PLS	99.67 $\pm$ 0.59	99.33 $\pm$ 1.16	98.88 $\pm$ 0.77	99.59 $\pm$ 0.56	99.36 $\pm$ 0.61
Interday	PCR	99.61 $\pm$ 0.41	99.02 $\pm$ 0.77	98.96 $\pm$ 0.74	99.61 $\pm$ 0.61	99.89 $\pm$ 0.98
	PLS	99.67 $\pm$ 0.85	98.93 $\pm$ 1.45	99.66 $\pm$ 1.11	101.18 $\pm$ 0.72	99.59 $\pm$ 1.39
	PCR	99.31 $\pm$ 0.79	99.26 $\pm$ 1.53	100.65 $\pm$ 0.89	101.71 $\pm$ 0.76	99.31 $\pm$ 1.14

<sup>#</sup> $r^2$  and regression equation for the predicted concentration versus true concentration plot, <sup>§</sup> Mean value of three determinations for three concentrations, \*Mean recovery of the predicted concentrations at 80%, 100% and 120% level of standard addition for n=3 replicates

#### Analysis of tablet formulation

The proposed PLS, PCR and HPLC methods were applied to the simultaneous determination of PEPH, PCM, GNF, CPM, and BRM in the commercial tablet as well as the dissolution of tablets. The determinations were made in six replicates. Satisfactory results were obtained for each compound in good agreement with the label claims (table 6). Results of the proposed PLS and PCR methods were also compared with those of the proposed HPLC method. Statistical comparison between the results of the two chemometric methods with respect to HPLC method was performed with regards to the

recovery results using Student's *t*-test and *F*-ratio at 95% confidence level. There was no significant difference between the results as stated in table 7. The three methods were also applied to the study dissolution of the tablet formulation, and the dissolution profiles are shown in fig. 5. From the dissolution data, it was found that the percentage release of all the five drugs was above 85% in 60 min.

Thus the proposed methods, i.e., PLS, PCR and HPLC, were found to be suitable and can be successfully used for the determination of the PEPH, PCM, GNF, CPM and BRM in pharmaceutical tablet formulation as well as for the dissolution study.

Table 6: Determination of PEPH, PCM, GNF, CPM and BRM in commercial tablet using the proposed methods

Commercial tablet	PLS	PCR	HPLC
PEPH	99.07 $\pm$ 0.99	98.7 $\pm$ 1.22	99.48 $\pm$ 0.54
PCM	100.63 $\pm$ 0.98	99.34 $\pm$ 0.71	98.35 $\pm$ 1.32
GNF	99.51 $\pm$ 0.89	100.17 $\pm$ 0.65	101.32 $\pm$ 1.23
CPM	98.8 $\pm$ 1.19	99.4 $\pm$ 0.71	98.98 $\pm$ 0.76
BRM	98.9 $\pm$ 1.20	98.48 $\pm$ 1.31	98.66 $\pm$ 0.68

mean $\pm$ SD, percentage recovery from the label claim amount

Table 7: Statistical evaluation for the three analytical methods

	Methods	PEPH	PCM	GNF	CPM	BRM
**F-value	PLS	0.18	3.76	0.25	3.24	0.89
	PCR	0.13	1.53	0.77	3.25	0.60
**t-value	PLS	0.43	1.94	0.50	1.80	0.95
	PCR	0.35	0.12	0.87	1.27	0.78

\*\*Theoretical values for F and t are 4.96 and 2.23 respectively.

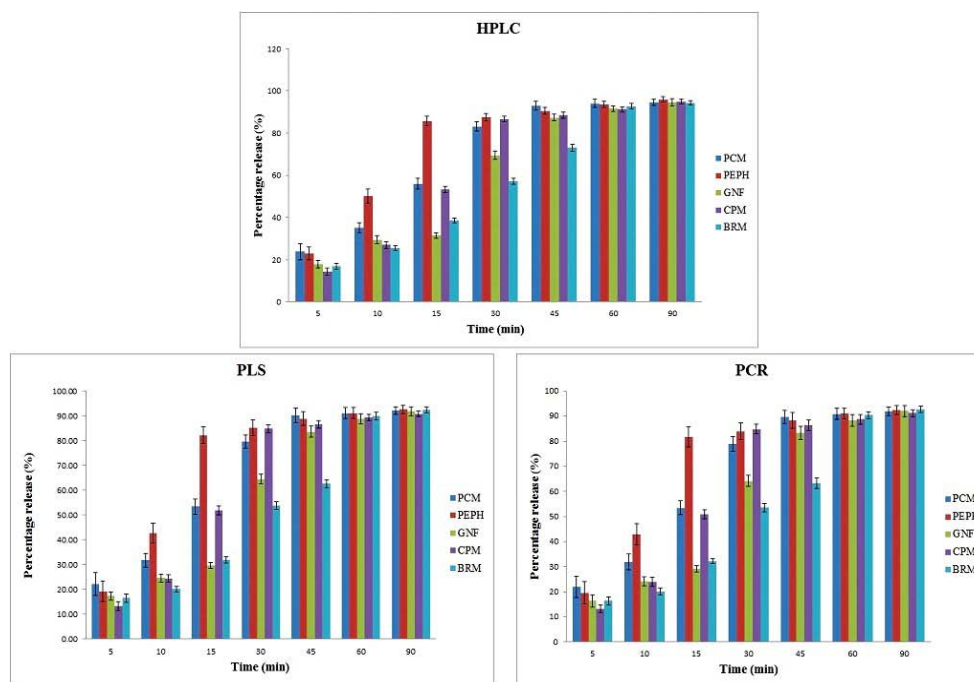


Fig. 5: Dissolution profiles of PEPH, PCM, GNF, CPM and BRM analysed by HPLC, PLS and PCR method showing the percentage release of the five components at different time points (5, 10, 15, 30, 45, 60 and 90 min) for n=6 replicates

## CONCLUSION

The chemometric assisted spectrophotometric methods (PLS and PCR) and RPHPLC method have been proposed and successfully applied for the simultaneous determination of PEPH, PCM, GNF, CPM, and BRM in their commercial tablet formulation. The assay and dissolution results obtained by chemometric methods were found to be in good coincidence with that of HPLC method. The HPLC method is more specific than the chemometric assisted spectrophotometric methods, but it needs expensive equipment and materials such as columns and HPLC grade solvents. Chemometric methods are less expensive and do not require sophisticated instrumentation and any prior separation step. This can be considered as an advantage of the chemometric techniques over HPLC. The proposed methods, i.e., PLS, PCR and HPLC, were found to be suitable and can be successfully used for the determination of the PEPH, PCM, GNF, CPM and BRM in pharmaceutical tablet formulation as well as for the dissolution study.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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