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# Development and Validation of Chemometric Assisted Spectrophotometric Technique for Simultaneous Estimation of Cinitapride and Pantoprazole from Bulk and Combined Dosage Form

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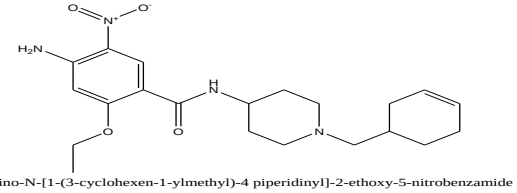
**ABSTRACT**

This paper describes two sensitive, accurate and precise chemometric spectrophotometric methods for the simultaneous determination of Cinitapride hydrogen tartarate (CNT) and Pantoprazole sodium (PANTO) in bulk powder and capsules without prior separation. Multivariate calibration chemometric methods are proposed for simultaneous determination of CNT and PANTO. The chemometric methods applied are Principal Component Regression (PCR) and Partial Least Squares (PLS). These approaches are successfully applied to quantify both drugs using the information included in the absorption spectra of appropriate solutions. In these multivariate methods, calibration sets of standard samples composed of different mixtures of CNT and PANTO have been designed. The methods were validated according to The International Conference on Harmonization (ICH) guidelines. The specificity of the proposed methods was tested using laboratory-prepared mixtures. The developed methods were successfully applied for the determination of CNT and PANTO in bulk powder and dosage form combination.

**Keywords:** Chemometric, Cinitapride hydrogen tartarate, Pantoprazole sodium, Principal Component Regression (PCR), Partial Least Square (PLS).

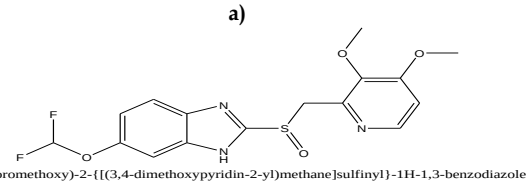
## INTRODUCTION

Cinitapride hydrogen tartarate is chemically designated as 4-amino-N-[1-(3-cyclohexen-1-ylmethyl)-4 piperidinyl]-2-ethoxy-5-nitrobenzamide hydrogen L-(+)-tartarate [1] (Fig. 1 a). It is a new prokinetic agent. It is a substituted benzamide with 5-HT receptor antagonist and 5-HT- receptor agonist activity. Several procedures are reported for quantitative determination of CNT including UV spectrophotometry [2], Extractive spectrophotometry [3], Colorimetric method [4], HPLC [5], HPTLC [6], and also from human plasma. [7]



4-amino-N-[1-(3-cyclohexen-1-ylmethyl)-4 piperidinyl]-2-ethoxy-5-nitrobenzamide

**a)**



6-(difluoromethoxy)-2-{[(3,4-dimethoxypyridin-2-yl)methane]sulfinyl}-1H-1,3-benzodiazole

**b)**

**Fig. 1: Chemical structure of a) Cinitapride hydrogen tartarate b) Pantoprazole sodium**

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Pantoprazole sodium is chemically designated as 6-(difluoromethoxy)-2-{[(3,4-dimethoxypyridin-2-yl)methane]sulfinyl}-1H-1,3-benzodiazole [8] (Fig. 1 b). It is a proton pump inhibitor. It is a substituted benzimidazole indicated for the short term treatment in the healing and symptomatic relief of erosive oesophagitis. It is official in Indian pharmacopoeia and European Pharmacopoeia. Official methods of analysis include chromatographic method. [8-9] Other reported methods include UV spectrophotometric methods [10-12], RP-HPLC methods [13-15], HPTLC method [16], Colorimetric method [17], Titrimetric and spectrophotometric method [18], and stability indicating HPLC method. [19]

Under controlled instrumentation computer-multivariate calibration methods are playing a very important role in the multi- component analysis of mixtures by UV–VIS spectrophotometry. [20-24] These approaches are useful for the resolution of band overlapping in quantitative analysis. Multivariate calibration has been found to be the method of choice for complex mixtures. [24-26] In order to avoid time-consuming procedures, attempts to resolve overlapping spectra by using various chemometric methods have been done. Multivariate statistical analysis methods presume that there is a linear relationship between absorbance and component concentrations. Each method has a calibration step in which the relationship between the spectra and the component concentrations is elucidated from a set of reference samples (calibration set). This step is followed by a prediction step in which the results of the calibration are used to calculate the component concentrations from an "unknown" sample spectrum (Validation set). [23]

Reviewing the literature in hand, there are no reported chemometric determination methods for this combination. The multivariate calibration methods investigated in this manuscript include the two most common methods. These are principal component regression (PCR) and partial least squares (PLS). In this work, multivariate calibration methods were applied to the determination of CNT and PANTO. The proposed procedures were successfully applied for determination of CNT and PANTO in bulk powder and in its pharmaceutical dosage form (capsules).

## MATERIALS AND METHODS

### Instrumentation

Spectrophotometric analysis was carried out on a Shimadzu 1800 double beam spectrophotometer with a fixed slit width (2 nm) using a pair of 1 cm matched quartz cells. The spectrophotometer is connected to an IBM PC. The bundle software, UV-Probe spectroscopy software version 2.42 (Shimadzu, Kyoto, Japan), was used to process absorption.

### Software

Microsoft Excel 2010 was used for handling and storing absorbance data. The computations were made using The Unscrambler X Version 10.3 (64 bit).

### Materials

### Pure samples

Pure drug samples of CNT and PANTO were kindly supplied by RPCP Drug Bank, Charusat Campus, Changa, India.

### Pharmaceutical dosage form

CINTODAC capsules (Cadila Healthcare Ltd), labeled to contain 3 mg Cinitapride hydrogen tartarate and 40 mg Pantoprazole sodium per capsule were purchased from local pharmacies.

### Solvent

Methanol (AR grade, Loba Chemie, India).

### Stock and working standard drug solutions

### Standard stock solutions

CNT and PANTO standard stock solutions (both are 1 mg ml-1), prepared by dissolving 100 mg of CNT and PANTO, each, in a few milliliters of methanol in to two 100 ml volumetric flasks and then completing to the mark with the same solvent.

### Working standard solutions

From the stock solution of CNT 10 ml of solution was transferred to a 100 ml volumetric flask and the volume made up to 100 ml with methanol to give a working standard solution of 100µg/ml CNT.

From the stock solution of PANTO 10 ml of solution was transferred to a 100 ml volumetric flask and the volume made up to 100 ml with methanol to give a working standard solution of 100µg/ml PANTO.

### Procedure

### Spectral characteristics and wavelengths selection

The absorption spectra of 3µg/ml of CNT, 40µg/ml of PANTO and a mixture of both containing the same previous concentration of each drug over the wavelength range of 200–400 nm were recorded.

### Preparation of Calibration set

Multilevel multifactor design was used for the construction of 41 binary mixtures. A five level two-factor design was used. [27] A calibration set of standard mixture solutions containing 1-5µg/ml CNT and 13-65µg/ml PANTO was made from a standard stock solution of 100µg/ml. A calibration set of 25 synthetic mixtures was prepared and made up to the mark with methanol.

### Preparation of Validation set

A validation set of standard mixture solutions containing 1-5µg/ml CNT and 13-65µg/ml PANTO was made from a standard stock solution of 100µg/ml. A validation set of 16 synthetic mixtures was selected on random basis from calibration set and these selected mixtures data has not been utilized for preparation of model.

Final concentration ranges were 1-5µg/ml and 13-65µg/ml for CNT and PANTO, respectively. The ranges of concentrations were selected in order to ensure that the total absorbance will not exceed the linear range of the spectrophotometer. From the 41 samples, 25 samples were chosen for the construction of the calibration set, while 16 samples were used as an

external validation set. Concentrations of the two compounds in both calibration and validation sets are presented in Table (1 a & b).The absorbance of these mixtures were measured between 210 and 330 nm at 10 nm intervals against methanol as blank.

**Preparation of sample solution for assay**

Twenty capsules were accurately weighed and the contents collected by opening the caps. Capsule powder equivalent to 100 mg of Pantoprazole sodium was accurately weighed and transferred to 100 ml volumetric flask and 50 ml methanol was added. The mixture was sonicated for 20 mins and diluted up to the mark with methanol (Solution A), and filtered through Whatman filter paper 41. From this Solution A, 10 ml aliquot was withdrawn into 100 ml volumetric flask and diluted up to mark with methanol (Solution B). From Solution B, 3.9 ml aliquot was withdrawn into 10 ml volumetric flask and diluted up to mark with methanol Solution C (having concentration 39 µg/ml of Pantoprazole sodium and 3µg/ml of Cinitapride hydrogen tartarate.

**Constructing the models**

For the two techniques, the absorbance data matrix for the training set concentration matrix (Table 1) was obtained by the measurement of absorbances between 210.0 and 330.0 nm in the intervals of 10 nm. In these techniques, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for prediction of the unknown concentrations of CNT and PANTO in their binary mixtures and pharmaceutical formulation. For the PCR and PLS models, the training set absorbance and concentration matrices together with The Unscrambler X 10.3 (64 bit) software were used for calculations.

**Table 1 A): Concentrations of CNT and PANTO in calibration set**

| Calibration set No. | Concentration of CNT (µg/ml) | Concentration of PANTO (µg/ml) |
|---|---|---|
| **1c** | 3 | 39 |
| **2c** | 3 | 13 |
| **3c** | 1 | 13 |
| **4c** | 1 | 65 |
| **5c** | 5 | 26 |
| **6c** | 2 | 65 |
| **7c** | 5 | 39 |
| **8c** | 3 | 26 |
| **9c** | 2 | 26 |
| **10c** | 2 | 52 |
| **11c** | 4 | 65 |
| **12c** | 5 | 52 |
| **13c** | 4 | 39 |
| **14c** | 3 | 65 |
| **15c** | 5 | 65 |
| **16c** | 5 | 13 |
| **17c** | 1 | 52 |
| **18c** | 4 | 13 |
| **19c** | 1 | 39 |
| **20c** | 3 | 52 |
| **21c** | 4 | 52 |
| **22c** | 4 | 26 |
| **23c** | 2 | 13 |
| **24c** | 1 | 26 |
| **25c** | 2 | 39 |

**Table 1 B): Concentrations of CNT and PANTO in validation set**

| Validation set No. | Concentration of CNT (µg/ml) | Concentration of PANTO (µg/ml) |
|---|---|---|
| **1v** | 1 | 13 |
| **2v** | 1 | 26 |
| **3v** | 2 | 26 |
| **4v** | 2 | 65 |
| **5v** | 5 | 26 |
| **6v** | 2 | 13 |
| **7v** | 1 | 52 |
| **8v** | 4 | 52 |
| **9v** | 4 | 26 |
| **10v** | 2 | 52 |
| **11v** | 4 | 13 |
| **12v** | 1 | 65 |
| **13v** | 5 | 65 |
| **14v** | 5 | 52 |
| **15v** | 4 | 65 |
| **16v** | 5 | 13 |

**Selection of the optimum number of latent variables to build the PCR and PLS models**

The cross validation method was used, leaving out one sample at a time, to select the optimum number of latent variables (LVs). Given a set of twenty five calibration samples, PCR and PLS calibrations were performed, and using this calibration, the concentration of the sample left out was predicted. The predicted concentrations were then compared with the actual concentrations and the root mean square error of cross validation (RMSECV) was calculated. The maximum number of LVs used to calculate the optimum RMSECV was selected to be ten. The RMSECV indicates both the precision and accuracy of predictions. It was recalculated upon addition of each new LV to the PLS and PCR models.

## RESULTS AND DISCUSSIONS

Multivariate calibration is useful for spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of single wavelength greatly improves the precision and predictive ability. [28] The full-spectrum methods have the ability to achieve improved precision since there is a signal averaging effect when many or all the spectral intensities are included in the analysis making it less susceptible to noise in the spectra.

Haaland and Thomas [24] made a comparison of the different multivariate calibration methods for quantitative spectral analysis. They concluded that it is difficult to generalize about the superiority of one method over another, because the relative performance of the methods is often dependent on particular data set being analyzed. CLS method requires that all components in the calibration samples must be known regarding number of constituents and concentration of every constituent. For PCR and PLS methods, unlike CLS all overlapping spectral components do not have to be known.

The wavelength range 210.0-330.0 nm with 10 nm intervals was chosen as it provides the greatest amount of information about the mixture components.

**Table 2: Results obtained by applying PCR calibration methods to validation set of Cinitapride hydrogen tartarate and pantoprazole sodium**

| Expected Conc. (µg/ml) | | Predicted Conc. (µg/ml) | | % Recovery | | Residual Conc. (Expected-Predicted) (µg/ml) | | (Exp -Pre)² Conc (µg/ml) | |
|---|---|---|---|---|---|---|---|---|---|
| CNT | PANTO | CNT | PANTO | CNT | PANTO | CNT | PANTO | CNT | PANTO |
| **1** | 13 | 0.998 | 12.987 | 99.80 | 99.90 | 0.002 | 0.013 | 4E-06 | 0.00016 |
| **1** | 26 | 1.02 | 26.123 | 102.00 | 100.47 | -0.02 | -0.123 | 0.0004 | 0.01512 |
| **2** | 26 | 1.989 | 26.019 | 99.45 | 100.07 | 0.011 | -0.019 | 0.00012 | 0.00036 |
| **2** | 65 | 1.988 | 64.898 | 99.40 | 99.84 | 0.012 | 0.102 | 0.00014 | 0.01040 |
| **5** | 26 | 4.988 | 25.894 | 99.76 | 99.59 | 0.012 | 0.106 | 0.00014 | 0.01123 |
| **2** | 13 | 1.997 | 12.991 | 99.85 | 99.93 | 0.003 | 0.009 | 9E-06 | 8.1E-05 |
| **1** | 52 | 1.001 | 52.003 | 100.10 | 100.00 | -0.001 | -0.003 | 1E-06 | 9E-06 |
| **4** | 52 | 3.999 | 52.09 | 99.97 | 100.17 | 0.001 | -0.09 | 1E-06 | 0.0081 |
| **4** | 26 | 4.01 | 25.99 | 100.25 | 99.96 | -0.01 | 0.01 | 1E-04 | 0.0001 |
| **2** | 52 | 1.988 | 51.995 | 99.40 | 99.99 | 0.012 | 0.005 | 0.00014 | 2.5E-05 |
| **4** | 13 | 4.012 | 13.012 | 100.30 | 100.09 | -0.012 | -0.012 | 0.00014 | 0.00014 |
| **1** | 65 | 1.004 | 64.889 | 100.40 | 99.82 | -0.004 | 0.111 | 0.00001 | 0.01232 |
| **5** | 65 | 4.979 | 65.014 | 99.58 | 100.02 | 0.021 | -0.014 | 0.00044 | 0.00019 |
| **5** | 52 | 5.017 | 51.967 | 100.34 | 99.93 | -0.017 | 0.033 | 0.00028 | 0.00108 |
| **4** | 65 | 3.987 | 64.989 | 99.67 | 99.98 | 0.013 | 0.011 | 0.00016 | 0.00012 |
| **5** | 13 | 5.002 | 13.002 | 100.04 | 100.01 | -0.002 | -0.002 | 4E-06 | 4E-06 |
| | | | | **Mean %** | | | | **100.020** | **99.988** |
| | | | | **SD** | | | | **0.6249** | **0.1838** |
| | | | | **% RSD** | | | | **0.6248** | **0.1838** |
| | | | | **RMSEP** | | | | **0.0115** | **0.0609** |

**Table 3: Results obtained by applying PLS calibration methods to validation set of Cinitapride hydrogen tartarate and pantoprazole sodium**

| Expected Conc. (µg/ml) | | Predicted Conc. (µg/ml) | | % Recovery | | Residual Conc. (Expected-Predicted) (µg/ml) | | (Exp -Pre)² Conc (µg/ml) | |
|---|---|---|---|---|---|---|---|---|---|
| CNT | PANTO | CNT | PANTO | CNT | PANTO | CNT | PANTO | CNT | PANTO |
| **1** | 13 | 0.988 | 12.879 | 98.8 | 99.06923 | 0.012 | 0.121 | 0.000144 | 0.014641 |
| **1** | 26 | 1.019 | 26.113 | 101.9 | 100.4346 | -0.019 | -0.113 | 0.000361 | 0.012769 |
| **2** | 26 | 1.999 | 26.22 | 99.95 | 100.8462 | 0.001 | -0.22 | 1E-06 | 0.0484 |
| **2** | 65 | 1.978 | 64.888 | 98.9 | 99.82769 | 0.022 | 0.112 | 0.000484 | 0.012544 |
| **5** | 26 | 4.998 | 25.899 | 99.96 | 99.61154 | 0.002 | 0.101 | 4E-06 | 0.010201 |
| **2** | 13 | 1.999 | 12.897 | 99.95 | 99.20769 | 0.001 | 0.103 | 1E-06 | 0.010609 |
| **1** | 52 | 1.011 | 52.214 | 101.1 | 100.4115 | -0.011 | -0.214 | 0.000121 | 0.045796 |
| **4** | 52 | 3.989 | 52.291 | 99.725 | 100.5596 | 0.011 | -0.291 | 0.000121 | 0.084681 |
| **4** | 26 | 4.013 | 25.888 | 100.325 | 99.56923 | -0.013 | 0.112 | 0.000169 | 0.012544 |
| **2** | 52 | 1.998 | 51.905 | 99.9 | 99.81731 | 0.002 | 0.095 | 4E-06 | 0.009025 |
| **4** | 13 | 4.014 | 13.255 | 100.35 | 101.9615 | -0.014 | -0.255 | 0.000196 | 0.065025 |
| **1** | 65 | 1.014 | 64.898 | 101.4 | 99.84308 | -0.014 | 0.102 | 0.000196 | 0.010404 |
| **5** | 65 | 4.989 | 65.015 | 99.78 | 100.0231 | 0.011 | -0.015 | 0.000121 | 0.000225 |
| **5** | 52 | 5.003 | 51.997 | 100.06 | 99.99423 | -0.003 | 0.003 | 9E-06 | 9E-06 |
| **4** | 65 | 3.988 | 64.887 | 99.7 | 99.82615 | 0.012 | 0.113 | 0.000144 | 0.012769 |
| **5** | 13 | 5.011 | 13.123 | 100.22 | 100.9462 | -0.011 | -0.123 | 0.000121 | 0.015129 |
| | | | | **Mean %** | | | | **100.1262** | **100.1218** |
| | | | | **SD** | | | | **0.8035** | **0.7230** |
| | | | | **% RSD** | | | | **0.8025** | **0.7221** |
| | | | | **RMSEP** | | | | **0.0117** | **0.1509** |

**Selection of the optimum number of latent variables for PCR and PLS methods**

Selection of the optimum number of LVs for the PCR and PLS techniques was a very important step before constructing the models. If the number of LVs retained was more than the required, more noise will be added to the data. On the other hand, if the number retained was less than the required, meaningful data that could be necessary for the calibration might be ignored. To select the optimum number of LVs for PCR and PLS methods, a cross- validation method using leave one out, was used. [29-30] Given the set of 25 calibration spectra corresponding to the samples listed in Table 1 a), the PCR and PLS models were constructed using 24 calibration spectra samples. The concentration of the sample left-out during calibration was predicted. This process was repeated 25 times until each calibration sample had been left-out once. The predicted concentration of the compound in each sample was compared with the actual known concentration of the drug. The RMSECV was calculated in the same manner each time. The method described by Haaland and Thomas [23] was used for selecting the optimum number of LVs. The method used an F-test to compare RMSECV values from cross-validation. The procedure starts by finding the smallest RMSECV value, RMSECV (k*) then all the models with fewer LVs (k < k*) are compared with the model with k*LVs.

$$F(k) = RMSECV(h)/RMSECV(k^*)$$

Where, k = 1, 2, 3, 4,…….k*

The number of LVs chosen (k) will be the minimum number having F (k) < Fd, m, m where d is the level of significance and m is the number of calibration samples. As the difference between the minimum RMSECV and other RMSECV values become smaller, the probability that each additional LV is significant

becomes smaller. [31] The maximum number of LVs used to calculate the optimum RMSECV was selected as ten. Seven LVs was found suitable for PCR and PLS respectively, as in Figures 2 and 3. The results predicted by the multivariate methods for the training set model are summarized in Table 2 and 3.

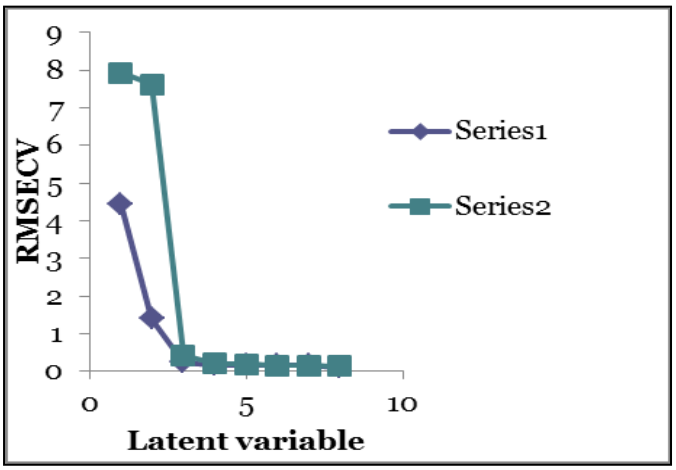


**Fig. 2: RMSEC plot of the cross validation results of the calibration set as a function of the number of latent variables used to construct the PCR calibration.**

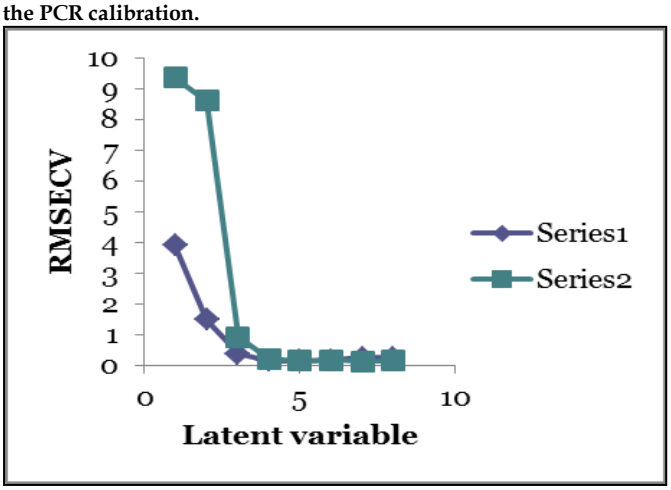


**Fig. 3: RMSEC plot of the cross validation results of the calibration set as a function of the number of latent variables used to construct the PLS calibration.**

**Selection of the optimum number of wavelengths for model building and sample recovery for CLS, PCR and PLS methods**

The absorption spectra of training and validation sets for CPM and ETF mixtures were recorded over the wavelength range of 200–400 nm at an interval of 0.1, 0.5, 1, 2, 5, 7 and 10 nm. But from these satisfactory results were obtained in the range 210-330 nm with 10 nm interval.

**Comparison of the results from the proposed methods**

The results confirm the considerable degree of agreement between the three techniques and indicate that these methods are suitable for this analysis in the given calibration domain for each drug if compared with the official methods. The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations. The results are obtained in Table 4.

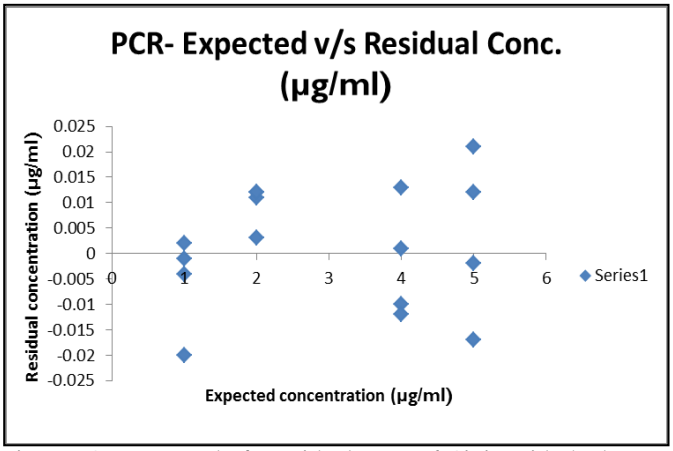


**Fig. 4: PCR- Expected v/s Residual conc. of Cinitapride hydrogen tartarate**

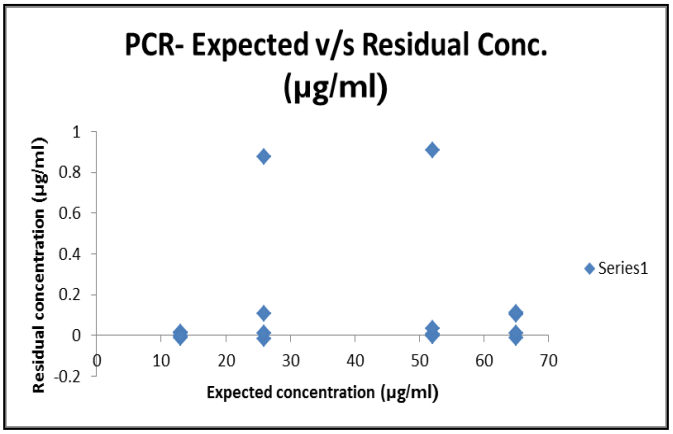


**Fig. 5: PCR- Expected v/s Residual conc. of Pantoprazole sodium**

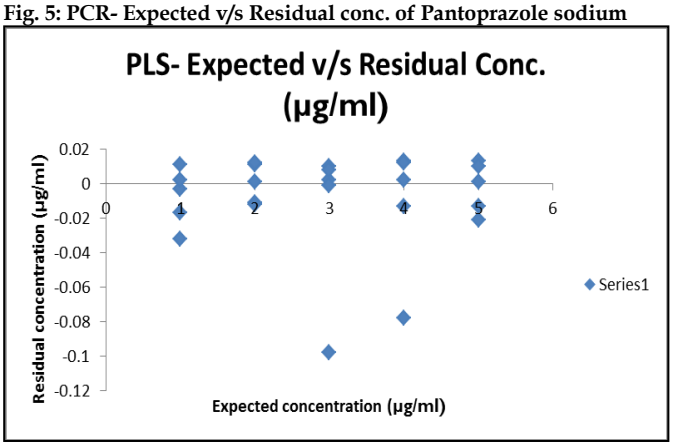


**Fig. 6: PLS- Expected v/s Residual conc. of Cinitapride hydrogen tartarate**

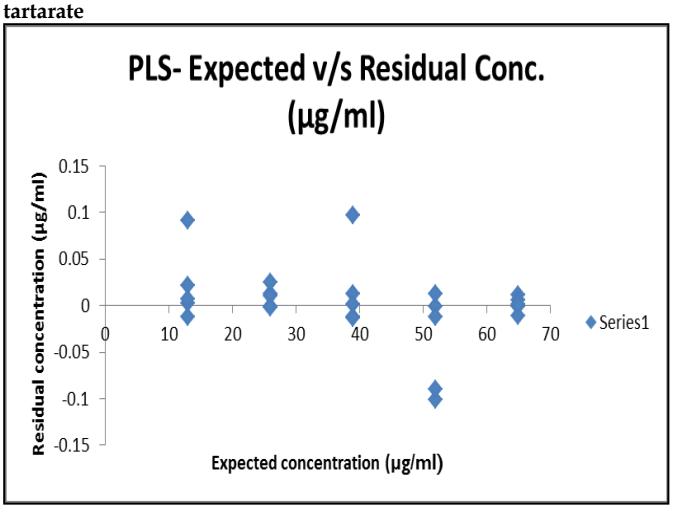


**Fig. 7: PLS- Expected v/s Residual conc. of Pantoprazole sodium**

Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. The residuals appear randomly distributed around zero, indicating adequate models as shown in Figures 4-7. The RMSECV was used as a diagnostic test for examining the error in the predicted concentrations. RMSECV indicates both the precision and accuracy of predictions. RMSECV plays the same role of standard deviation in indicating the spread of the concentration errors. In Table 4, the RMSECV, slope and intercept of predicted Vs. true concentrations are obtained. As can be seen, the results are satisfactory and indicate good predictive abilities of the developed models. The chemometric methods were applied successfully to the analysis of CNT and PANTO in CINTODAC capsules. The interfering species were not included in calibration samples but were present during capsule determination.

**Table 4: RMSECV and statistical parameter values for Cinitapride hydrogen tartarate and Pantoprazole sodium prediction using multivariate calibration methods**

| Parameter | CNT | | PANTO | |
|---|---|---|---|---|
| | PCR | PLS | PCR | PLS |
| **Range** | 1 - 5 µg/ml | | 13 – 65 µg/ml | |
| **Wavelength (nm)** | 210 – 330 | | 210 - 330 | |
| **Δ λ (nm)** | 10 | | 10 | |
| **Factor** | 7 | 7 | 7 | 7 |
| **% recovery** | 100.020 | 100.126 | 99.988 | 101.121 |
| **SD** | 0.624 | 0.803 | 0.183 | 0.723 |
| **% RSD** | 0.062 | 0.802 | 0.183 | 0.722 |
| **Correlation Coefficient ($r^2$)** | 0.9999 | 0.9996 | 0.9998 | 0.9999 |
| **Intercept** | 0.0008 | 0.007 | -0.105 | -0.064 |
| **Slope** | 0.9993 | 0.9996 | 0.9993 | 1.0007 |
| **RMSECV** | 0.0554 | 0.0277 | 0.2958 | 0.0392 |
| **RMSEP** | 0.0115 | 0.0117 | 0.0609 | 1.1509 |

## Method Validation

Validation of the proposed methods was assessed according to ICH guidelines. [32-33]

### Accuracy

The accuracy of the proposed methods was performed by applying the suggested procedures for determination of the validation samples as well as different blind samples of CNT and PANTO. The concentrations were obtained from the corresponding model, from which the percentage recoveries suggested good accuracy of the proposed methods. Results are shown in Table 5 and 6.

**Table 5: Accuracy data of Cinitapride hydrogen tartarate by PCR and PLS methods**

| Level | Amount taken (µg/ml) | Amount found (µg/ml) | | % Mean ± SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| | | PCR | PLS | PCR | PLS | PCR | PLS |
| **80** | 2.4 | 2.401<br>2.399<br>2.397 | 2.398<br>2.403<br>2.402 | 2.399± 0.002 | 2.401± 0.002 | 0.083 | 0.110 |
| **100** | 3 | 3.003<br>3.010<br>2.998 | 2.998<br>3.001<br>2.989 | 3.003± 0.006 | 2.996± 0.006 | 0.200 | 0.208 |
| **120** | 3.6 | 3.599<br>3.602<br>3.604 | 3.603<br>3.597<br>3.598 | 3.601± 0.002 | 3.599± 0.003 | 0.069 | 0.089 |

**Table 6: Accuracy data of Pantoprazole sodium by PCR and PLS methods**

| Level | Amount taken (µg/ml) | Amount found (µg/ml) | | % Mean ± SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| | | PCR | PLS | PCR | PLS | PCR | PLS |
| **80** | 31.2 | 31.199<br>31.201<br>31.204 | 31.197<br>31.199<br>31.201 | 31.201 ± 0.002 | 31.199± 0.002 | 0.008 | 0.006 |
| **100** | 39 | 39.003<br>38.997<br>38.989 | 38.995<br>39.012<br>39.001 | 38.996 ± 0.007 | 39.002± 0.008 | 0.018 | 0.022 |
| **120** | 46.8 | 46.798<br>46.802<br>46.800 | 46.804<br>46.799<br>46.801 | 46.803 ± 0.006 | 46.801± 0.002 | 0.013 | 0.005 |

## Application of the method in assay of capsules

The proposed spectrophotometric multivariate calibration methods were applied for the determination of CNT and PANTO in their combined pharmaceutical formulation (CINTODAC Capsules) as shown in table 7. It shows that the developed methods are accurate and specific for determination of the cited drugs in presence of dosage form excipients.

**Table 7: Assay of Cinitapride hydrogen tartarate and Pantoprazole sodium by PCR and PLS methods**

| Drug | Amount taken (µg/ml) | Amount found (µg/ml) | | % Mean ± SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| | | PCR | PLS | PCR | PLS | PCR | PLS |
| **CNT** | 3 | 2.998<br>3.001<br>3.012<br>2.989<br>2.997<br>3.004 | 2.988<br>3.011<br>3.002<br>2.999<br>2.987<br>3.014 | 100.005 ± 0.2559 | 100.005 ± 0.3755 | 0.2559 | 0.3755 |
| **PANTO** | 39 | 38.997<br>39.012<br>39.008<br>38.999<br>39.015<br>39.019 | 38.987<br>39.002<br>39.018<br>38.989<br>39.005<br>39.020 | 100.021 ± 0.0225 | 100.008 ± 0.0357 | 0.0225 | 0.0357 |

In this manuscript, two chemometric techniques have been investigated to determine which technique is the most suitable for the simultaneous determination of CNT and PANTO without the use of preliminary separation step. The good recoveries obtained in all cases as well as the reliable agreement with the reported procedures proved that the proposed procedures could be applied efficiently for determination of the studied drugs simultaneously in their binary mixtures as well as in the commercial

dosage forms with satisfactory precision. The proposed methods are simple, sensitive, accurate, precise and economical. They could be easily applied in quality control laboratories for the routine analysis of the studied drugs in pure bulk powder and dosage form without any preliminary separation step. The most striking features of the methods are their simplicity and rapidity. Method validation has been demonstrated by accuracy, % recovery and assay of marketed formulation.

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