SPECTROPHOTOMETRIC MULTICOMPONENT ANALYSIS OF TELMISARTAN, HYDROCHLOROTHIAZIDE AND RAMIPRIL IN PHARMACEUTICAL FORMULATIONS BY CHEMOMETRIC TECHNIQUES

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ABSTRACT

Multivariate spectrophotometric methods offer an extensive application for quantification of multicomponent mixtures that exhibit severe spectral overlapping. Partial least Squares (PLS) and Principal component Regression (PCR) methods were proposed for the spectrophotometric multicomponent analysis of a ternary mixture consisting of Telmisartan (TEL), Hydrochlorothiazide (HCZ) and Ramipril (RAM) without prior separation. In these chemometric techniques, the measurement of the absorbance values were made in the spectral range from 200-350 nm in the intervals of $\Delta \lambda = 1$ nm at 151 wavelengths in the zero order, first and second derivative spectra of the ternary mixtures of these active ingredients in 0.1 M NaOH. The prepared calibrations of both techniques using absorbance data and concentration matrix data sets were used to predict the concentration of the active ingredients in their ternary, binary and single component mixtures. The optimized models were tested on an external validation set containing synthetic mixtures of the mentioned components. The models were finally used to assay the studied drugs in the commercial formulations. The methods are selective for the determination of telmisartan, hydrochlorothiazide and ramipril and overcome the spectral interference. The prediction values for TEL, HCZ and RAM in both models were found satisfactory.

Keywords: Telmisartan, Hydrochlorothiazide, Ramipril, PLS, PCR, Multivariate calibration.
INTRODUCTION

Many quantitative methods for the determination of Telmisartan (TEL), Hydrochlorothiazide (HCZ) and Ramipril (RAM) including spectrophotometry\textsuperscript{[1-6]}, spectrofluorimetry\textsuperscript{[7, 8]} chemometrics \textsuperscript{[9-14]}, high Performance Liquid Chromatography (HPLC)\textsuperscript{[15-35]}, LC-MS \textsuperscript{[36-40]}, capillary zone electrophoresis \textsuperscript{[41]} and High performance thin layer chromatography (HPTLC)\textsuperscript{[42-46]} were reported individually or in combination with other drugs. We could not able to find any chemometric study on the ternary mixture of these drugs in the literature.

Multivariate spectrophotometric methods offer several advantages over univariate methods, as it includes many variables for data analyses. Multivariate calibration methods have been widely applied to the simultaneous quantification of analytes in mixtures. Among multivariate calibration methods, principal component Regression (PCR) and partial least-squares regression (PLS) have been successfully adopted in many quantitative assays of pharmaceutical formulations. These methods are related with the establishment of an association between matricial algorithms and calibration data. The theoretical base of these methods has been fully described by several authors.

The derivative spectrophotometry offers a powerful tool for quantitative analysis of multi component mixtures. When the original spectra are derivatized, the overlapping peaks are resolved with increase in intensity. Hence derivative spectrophotometry offers greater sensitivity than the normal spectrophotometry in the simultaneous determination of two or more components without prior separation. Though derivative spectrophotometry offers many advantages quantitative analysis of three components becomes difficult due to serious spectral overlapping. An attempt has been made to overcome such spectral overlapping by the combined use of derivative spectrophotometry and chemometric techniques.

In this paper, two multivariate procedures based on PCR and PLS algorithms have been applied on the absorbance data of a ternary mixture of drugs of interest. The work was focussed on the comparison of regression methods when applied on ordinary or first and second order derivative spectral data. The calibration models were built by using a novel experimental design on data from a calibration set of 25 reference mixtures. The defined models were optimized and validated to an external validation test by assaying a prediction set of 10 synthetic mixtures, in order to verify their prediction ability in terms of accuracy and precision. The proposed methods were finally applied to the quantitative analysis of
commercial samples containing one to three drugs to confirm their effectiveness in the routine analysis of the real samples.

MATERIALS AND METHODS

Apparatus
A Perkin Elmer (Lamda 25) spectrophotometer controlled by UV winlab software and equipped with 1 cm pathlength quartz cell was used for the spectral data acquisition. The chemometric procedure was carried out using MATLAB software version 7.5 (The Mathworks) and PLS toolbox version 5.0 (Eigen Vector Technologies).

Chemicals
TEL, HCZ and RAM reference standards were kindly supplied by Madras Pharmaceuticals, Chennai, India. The tablets Telista H, Lupin Pharmaceuticals, Mumbai (containing 40 mg of TEL and 12.5 mg of HCZ), Tazloc*R, Hetero drugs, HP (containing 40 mg of TEL and 5 mg of RAM) and Cardace#2.5, Aventi Pharma (containing 2.5 mg of RAM and 12.5 mg of HCZ) were procured from local pharmacies. All other chemicals were of analytical reagent grade and procured from SD Fine chemicals, Mumbai, India.

Standard Solutions
Standard stock solutions (1000 μg/ml) of TEL, HCZ and RAM were prepared separately in the diluent 0.1M NaOH and water in the ratio 20:80. These solutions were taken and then diluted to 10 ml with water to give a final analyte concentration desired.

Sample Preparation
Ten tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred into a 100 ml volumetric flask including the diluent. The flask was sonicated for 15 mins and diluted to the mark with diluents. An aliquot of the solution was centrifuged at 5000 rpm for 10 mins. Appropriate amount of clear supernatant was transferred into a 10 ml flask and diluted with water. Then the absorbance values were measured.

Multivariate calibration methods
These methods are designed based on the relationship between matrices of chemical data. Initially a calibration model is built using a chemical data set i.e. absorbance values and a concentration data set. The constructed calibration model is then used to estimate the
concentration of the unknown samples in the prediction set. In the PLS and PCR methods, the original variables are transformed into a smaller number of orthogonal variables called factors or principal components which are in turn linear with the original variables.

From the prepared reference samples, a relationship between spectral and concentration data, representing the variables of the system can be built using the multivariate calibration techniques. The PCs and scores are built by constituting a new matrix specific to the regression method adopted. PCs represent the absorptivity values of the samples at the various concentration and wavelength values, whereas the scores represent the numerical coefficients. Their combination allows to build the mathematical model representing the reference spectra and able to predict the component concentrations of new samples.

**Determination of PC number**

The optimal number of factors determined helps in improving the prediction power of the selected methods. This can be achieved by employing a full cross validation also known as leave-one-out method. This method employs leaving one sample at a time from the calibration step and performs the calibration with all other samples. The concentration of the sample removed is then predicted with the obtained model. This step is in turn repeated for each sample considered. The procedure can be repeated after fixing a different number of factors. The standard error prediction (SEP) was chosen as an optimizing criterion to select the optimal number of factors. SEP represents an estimate of the error involved in the assay of external samples by using the model. Its value depends on the number of factors used for that calibration. The number of factors giving the minimal SEP was selected as the optimal number of factors.

\[
RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (\hat{C}_i - C_i^2)}{n}}
\]

where \( \hat{C} \) denotes the added drug concentration, \( C_i \) is the predicted drug concentration and \( n \) represents the total number of synthetic mixtures.

**RESULTS AND DISCUSSION**

**Data Processing and model building**

Fig 1 a-c shows the UV absorption spectra of pure TEL, HCZ and RAM in 0.1 M NaOH recorded in the range of 200 – 350nm. The absorption spectra recorded in the ordinary mode (a), first derivative mode (b) and second derivative mode (c) shows a clear overlap between
the curves. A thorough examination of all the spectra was done but no signal proportional to the components was singled out. Multivariate calibration methods appeared to be ideal in order to overcome such a drawback, as they allow extracting of analytical information from the full spectra.

Fig 1 a – Fundamental Absorption spectra of Telmisartan, Hydrochlorothiazide, Ramipril and mixture

Fig 1 b – First derivative spectra of Telmisartan, Hydrochlorothiazide, Ramipril and mixture

Fig 1 c – Second derivative spectra of Telmisartan, Hydrochlorothiazide, Ramipril and mixture
To build PLS and PCR calibration models, a concentration of 25 mixtures of three compounds in the range of 0.5-1.5 µg/ml of TEL, 0.5-3.0 µg/ml of HCZ and 1-6 µg/ml of RAM were prepared in 0.1 M NaOH. A concentration set comprising of 25 synthetic mixtures was prepared corresponding to the above working concentration range. The concentration set and its composition is given in Table 1. The fundamental, first derivative and second derivative spectra of the proposed concentration set were registered in the 200 - 350 nm wavelength range. The absorption values of spectra of the concentration set were measured at the 151 wavelength points with $\Delta \lambda = 1$ nm in the spectral region of 200 –350 nm. The concentration set and absorption data were considered as Y-block (25×3) and X-block (25×151) for the construction of PLS calibration using cross-validation procedure to reach the best calibration model. The calculations were done with PLS Toolbox 5.0 and the optimal factors were selected.

**Table 1 - Concentrations of TEL, HCZ and RAM used as Calibration and Prediction sets**

| Calibration Set (µg/ml) | Prediction Set (µg/ml) |
|------------------------|-----------------------|
| **TEL** | **HCZ** | **RAM** | **TEL** | **HCZ** | **RAM** |
| 0.5 | 0.5 | 5 | 2 | 2 | 1 |
| 1 | 0.5 | 4 | 2.5 | 2.5 | 1.5 |
| 1.5 | 0.5 | 3 | 1 | 1 | 2.5 |
| 2 | 0.5 | 2 | 2 | 2 | 2 |
| 0.5 | 1 | 5 | 0 | 0 | 0 |
| 1 | 1 | 4 | 1 | 1 | 2 |
| 1.5 | 1 | 3 | 2 | 2 | 1 |
| 2 | 1 | 2 | 0 | 0 | 3 |
| 0.5 | 1.5 | 5 | 1 | 1 | 6 |
| 1 | 1.5 | 4 | 0 | 0 | 0 |
| 1.5 | 1.5 | 3 | - | - | - |
| 2 | 1.5 | 2 | - | - | - |
| 0 | 2 | 0 | - | - | - |
| 0.5 | 2 | 5 | - | - | - |
| 1 | 2 | 4 | - | - | - |
| 1.5 | 2 | 3 | - | - | - |
| 0.5 | 2.5 | 5 | - | - | - |
| 1 | 2.5 | 4 | - | - | - |
| 1.5 | 2.5 | 3 | - | - | - |
| 2 | 2.5 | 2 | - | - | - |
| 0.5 | 3 | 1 | - | - | - |
| 1 | 3 | 1.5 | - | - | - |
| 0 | 0 | 6 | - | - | - |
| 3 | 0 | 0 | - | - | - |
| 1.5 | 3 | 2 | - | - | - |
The PCR and PLS models obtained by using this training set were validated by full cross-validation and the SEP values were calculated each time that a new factor was added to the models. Table 2 shows the selected numbers of factors and the corresponding SEP values. The square of the correlation coefficient ($R^2$), which indicates the fraction of the total variance explained by the models, is also reported. The prediction values for TEL, HCZ and RAM in both models were found satisfactory.

Table 2 - Statistical Parameters calculated from application of PLS and PCR methods to calibration samples

| Drug | Parameter | PLS | | PCR | | |
|------|-----------|-----|-----|-----|-----|-----|
|      |           | $D^0$ | $D^1$ | $D^2$ | $D^0$ | $D^1$ | $D^2$ |
| TEL  | Mean      | 100.011 | 100.011 | 100.011 | 100.036 | 100.036 | 100.036 |
|      | RMSEC     | 0.0260  | 0.0260  | 0.0260  | 0.0265  | 0.0265  | 0.0265  |
|      | $R^2$     | 0.9986  | 0.9986  | 0.9986  | 0.9986  | 0.9986  | 0.9986  |
| HCZ  | Mean      | 99.909  | 99.909  | 99.909  | 100.145 | 100.145 | 100.145 |
|      | RMSEC     | 0.0265  | 0.0265  | 0.0265  | 0.0474  | 0.0474  | 0.0474  |
|      | $R^2$     | 0.9992  | 0.9992  | 0.9992  | 0.9973  | 0.9973  | 0.9973  |
| RAM  | Mean      | 100.016 | 100.016 | 100.016 | 100.014 | 100.014 | 100.014 |
|      | RMSEC     | 0.0428  | 0.0428  | 0.0428  | 0.1537  | 0.1537  | 0.1537  |
|      | $R^2$     | 0.9993  | 0.9993  | 0.9993  | 0.9908  | 0.9908  | 0.9908  |

Mean – Mean of 25 samples of calibration set; RMSEC – Root Mean Square Error of Calibration set; $R^2$ – Correlation coefficient obtained by plotting amount present against amount estimated.

**Application of PLS and PCR models to the Prediction set**

One set of 10 synthetic samples containing ternary and binary component mixtures of the three studied drugs were prepared in different ratios was built to perform an external validation for the proposed PLS and PCR models. The construction of the prediction set was designed to test the prediction ability of the models when applied on selected component mixtures. The mixtures were prepared in the same concentration range as in the calibration set. The composition of prediction set is listed in Table 1. The application of proposed PLS and PCR models to the prediction set gave the following results and are indicated in Table 3.
Table 3 - Accuracy (% recovery) and Precision (SD) results from application of optimized PLS and PCR models on the prediction set

| Drug | Parameter     | PLS          | PCR           |
|------|---------------|--------------|---------------|
|      |               | D₀ | D¹ | D² | D₀ | D¹ | D² |
| TEL  | Mean% Recovery| 95.435 | 95.435 | 95.435 | 96.692 | 96.692 | 96.692 |
|      | RMSEP         | 0.1162 | 0.1162 | 0.1162 | 0.0962 | 0.0962 | 0.0962 |
|      | SD            | 4.7389 | 4.7389 | 4.7389 | 5.6094 | 5.6094 | 5.6094 |
| HCZ  | Mean% Recovery| 99.4565 | 99.4565 | 99.4565 | 96.654 | 96.654 | 96.654 |
|      | RMSEP         | 0.0672 | 0.0672 | 0.0672 | 0.0871 | 0.0871 | 0.0871 |
|      | SD            | 4.7322 | 4.7322 | 4.7322 | 3.8777 | 3.8777 | 3.8777 |
| RAM  | Mean% Recovery| 103.390 | 103.390 | 103.390 | 102.750 | 102.750 | 102.750 |
|      | RMSEP         | 0.1471 | 0.1471 | 0.1471 | 0.1691 | 0.1691 | 0.1691 |
|      | SD            | 4.2175 | 4.2175 | 4.2175 | 4.9451 | 4.9451 | 4.9451 |

Mean % Recovery – Mean of 10 samples of prediction set; RMSEP – Root Mean Square Error of Prediction set; SD – Standard Deviation

The results of PLS model includes mean % recovery ranging from 99.768 to 100.127 for all the three drugs in the calibration set and from 95.435 to 103.390 in the prediction set. The SD values were found to increase with increasing spectral mode in the calibration set but seem to decrease with increasing spectral mode in the prediction set. R² value ranges from 0.9986 – 0.9997 in the calibration set and from 0.9936 – 0.9996 in the prediction set. RMSEC value ranges from 0.0116 – 0.0429 for all three drugs in the calibration set. RMSEP value ranges from 0.0443 – 0.1471 for all three drugs in the prediction set.

The results of PCR model includes mean % recovery ranging from 99.002 to 100.746 for all the three drugs in the calibration set and from 96.654 to 104.390 in the prediction set. The SD values were found to increase with increasing spectral mode in the calibration set but seem to decrease with increasing spectral mode in the prediction set. R² value ranges from 0.9908 – 0.9988 in the calibration set and from 0.9921 – 0.9993 in the prediction set. RMSEC value ranges from 0.0246 – 0.1537 for all three drugs in the calibration set. RMSEP value ranges from 0.0426 – 0.1691 for all three drugs in the prediction set. Fig.2 shows the graphical representation of mean recovery obtained for all the drugs by both the methods.
Fig 2: Comparison of Mean Recovery of PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra

Analysis of commercial formulations

The validated PLS and PCR models were applied to the assay of binary pharmaceutical specialities. The assay results are summarized in Table 4. A good coincidence was observed between experimental results and label claim of the pharmaceutical formulations. For simultaneous analysis of TEL, HCZ and RAM, application of models from fundamental to second derivative spectra gave recovery between 98.32 to 101.90% for TEL, 99.44 to 101.04 for HCZ and 98.80 to 102.40 for RAM respectively. The SD for all three drugs by both the methods at three different modes was found to be not more than 1.0. No interference from the excipients used in the dosage forms was observed.
Table 4: Assay Results of optimized PLS and PCR models on Pharmaceutical preparations

| Method | Tablet | Mode | TEL | HCZ |
|--------|--------|------|-----|-----|
|        |        |      | % recovery (mg/tab) ± SD | % recovery (mg/tab) ± SD | % recovery |
|        |        |      | % recovery | % recovery |
| **PLS** | T1 | D0 | 40.54 ± 0.05 | 101.35 | 12.43 ± 0.11 | 99.44 |
|        |     | D1 | 40.43 ± 0.06 | 101.07 | 12.61 ± 0.07 | 100.88 |
|        |     | D2 | 40.76 ± 0.03 | 101.90 | 12.59 ± 0.03 | 100.72 |
| **PCR** | T1 | D0 | 38.92 ± 0.12 | 97.30 | 12.63 ± 0.12 | 101.04 |
|        |     | D1 | 39.43 ± 0.07 | 98.57 | 12.54 ± 0.04 | 100.32 |
|        |     | D2 | 40.23 ± 0.04 | 100.57 | 12.54 ± 0.02 | 100.32 |

| **PLS** | T2 | D0 | 40.46 ± 0.33 | 101.15 | 4.94 ± 0.08 | 98.80 |
|        |     | D1 | 40.43 ± 0.12 | 101.07 | 5.08 ± 0.06 | 101.60 |
|        |     | D2 | 39.77 ± 0.05 | 99.42 | 5.03 ± 0.05 | 100.60 |
| **PCR** | T2 | D0 | 40.68 ± 0.11 | 101.70 | 5.12 ± 0.04 | 102.40 |
|        |     | D1 | 39.33 ± 0.21 | 98.32 | 4.96 ± 0.03 | 99.20 |
|        |     | D2 | 40.62 ± 0.06 | 101.55 | 5.03 ± 0.03 | 100.60 |

| **PLS** | T3 | D0 | 2.53 ± 0.04 | 101.20 | 12.58 ± 0.11 | 100.64 |
|        |     | D1 | 2.47 ± 0.03 | 98.80 | 12.58 ± 0.03 | 100.64 |
|        |     | D2 | 2.51 ± 0.03 | 100.40 | 12.48 ± 0.04 | 99.84 |
| **PCR** | T3 | D0 | 2.49 ± 0.04 | 99.60 | 12.46 ± 0.03 | 99.68 |
|        |     | D1 | 2.53 ± 0.03 | 101.20 | 12.56 ± 0.02 | 100.48 |
|        |     | D2 | 2.52 ± 0.02 | 100.80 | 12.46 ± 0.04 | 99.68 |

* Average of six estimations

T1 – Telista H – Lupin Pharmaceuticals, Mumbai
Telmisartan – 40 mg and Hydrochlorothiazide – 12.5 mg

T2 – Tazloc R – Hetero Labs, HP
Telmisartan – 40 mg and Ramipril – 5 mg

T3 – Cardace # 2.5 – Aventis Pharma, Goa
Ramipril – 2.5 mg and Hydrochlorothiazide – 12.5 mg

CONCLUSIONS
Multivariate methods PCR and PLS by use of spectrophotometric data have proved to be valid analytical tools even when applied to complex mixtures characterized by a severe
overlapping and high ratios in analytes concentration. Application of chemometric methods to derivative UV spectra can magnify the prediction ability of this spectrophotometric technique. The application of PLS and PCR methods on zero to second order derivative spectra of ternary mixtures of TEL, HCZ and RAM has been accomplished. Very satisfactory results were obtained when the optimized models were applied to the synthetic mixtures and commercial formulations. According to these studies, multivariate calibration methods (PLS and PCR) coupled with derivative spectral data can be recommended as a very suitable choice to resolve severe overlapped absorption spectra of drug mixtures. This approach is simple in application, inexpensive, requires an easy treatment of the samples and provides reliable analytical results.

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