Mean centering of ratio spectra and concentration augmented classical least squares in a comparative approach for quantitation of spectrally overlapped bands of antihypertensives in formulations

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**HIGHLIGHTS**
- HCT is commonly co-formulated with IRB or CAN.
- MCR and CRACLS are applied in a comparative approach for resolution of the three drugs.
- One calibration curve and one model were used to predict HCT in both formulations.
- Separation of three drugs was achieved by simple and smart methods.
- Both MCR and CRACLS were successfully determined the three drugs in tablets.

**GRAPHICAL ABSTRACT**

**ABSTRACT**

Two different methods manipulating spectrophotometric data have been developed, validated and compared. One is capable of removing the signal of any interfering components at the selected wavelength of the component of interest (univariate). The other includes more variables and extracts maximum information to determine the component of interest in the presence of other components (multivariate). The applied methods are smart, simple, accurate, sensitive, precise and capable of determination of spectrally overlapped antihypertensives; hydrochlorothiazide (HCT), irbesartan (IRB) and candesartan (CAN). Mean centering of ratio spectra (MCR) and concentration residual augmented classical least-squares method (CRACLS) were developed and their efficiency was compared. CRACLS is a simple method that is capable of extracting the pure spectral profiles of each component in a mixture. Correlation was calculated between the estimated and pure spectra and was found to be 0.9998, 0.9987 and 0.9992 for HCT, IRB and CAN, respectively. The methods were successfully determined the three components in bulk powder, laboratory-prepared mixtures, and combined dosage forms. The results obtained were compared statistically with each other and to those of the official methods.

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**INTRODUCTION**

Hydrochlorothiazide (HCT) (Fig. 1a) or 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide is a diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water and is frequently used for the treatment of
hypertension. Irbesartan (IRB) (Fig. 1b) or 2-butyl-3-\{4-\{2\-(2H-1,2,3,4-tetrazol-5-yl)phenyl\}phenyl\}methyl\}-1,3-diazaspiro[4.4]non-1-en-4-one is an angiotensin II receptor antagonist and used mainly for the treatment of hypertension. Candesartan (CAN) (Fig. 1c) or 2-ethoxy-1-\{4-\{2\-(2H-1,2,3,4-tetrazol-5-yl)phenyl\}phenyl\}methyl\}-1H-1,3-benzodiazole-7-carboxylic acid is an angiotensin II receptor antagonist that is used mainly for the treatment of hypertension. Both IRB and CAN is also available in a combination formulation with a low dose thiazide diuretic, invariably hydrochlorothiazide, to achieve an additive antihypertensive effect [1].

Few methods have been reported for the simultaneous determination of HCT and IRB in their pharmaceutical formulations as HPLC [2–9] and spectrophotometry [2–13]. Different analytical methods have been reported for the determination of HCT and CAN which include HPLC [14–18], HPTLC [19] and spectrophotometry [20–24]. While for IRB and CAN, only two HPLC methods were reported [25,26].

As both IRB and CAN are co-formulated with HCT to achieve maximum therapeutic effect and no method was reported for their determination in ternary mixture. So, our aim was to simultaneously determine the three drugs without prior separation by a simple and smart method that could be applied for determination of any drugs combination. The developed methods can be successfully applied in routine analysis and QC laboratories. Two different techniques were applied; the first is a univariate method, namely mean centering of ratio spectra (MCR) which is based on cancelling the contribution of other components than the analyte. While, the second technique is a multivariate calibration methods namely concentration residual augmented classical least squares (CRACLS) which is based on determination of all components in the presence of each other through spectral resolution.

**Mean centering of ratio spectra method (MCR)**

It is a well-established method that has the advantage of being applicable to binary and ternary mixture, as well it enhances the signal to noise ratio. The theory of the method is well known and based on mean centering as a processing step to eliminate the interference of components present in a mixture rather than the component of interest [27].

**Concentration augmented classical least squares method (CRACLS)**

CRACLS is a recently developed method for the resolution of complex mixtures. Unlike CLS, CRACLS is an alternative method that estimates absorptivity ($\hat{S}$) by a process of repetitive approximation as shown in the following steps [28,29]:

1. $\hat{S}$ is calculated: $\hat{S} = (C_C - 1)A$.
2. $\hat{S}$ is used to predict C: $C = A\hat{S}(\hat{S}\hat{S} - 1)$.
3. Error in C: $E = C - C$.
4. One vector of E is augmented to the original C (E is considered as a new component).
5. Step 5: Step (1) is repeated using the augmented C until no further improvement in prediction is achieved. CRACLS model was built for HCT, IRB and CAN.

**Experimental**

**Samples**

**Pure samples**

Pure samples were kindly donated by October Pharm, the percentage purity was found to be 99.83, 100.28 and 99.67 for HCT, IRN and CAN according to their official methods [1].

**Pharmaceutical formulations**

Kansartan Plus® tablets, Batch No. 120536A (150/12.5), 120537A (300/12.5) of IRB and HCT, respectively, manufactured by CHEMIPHARM. Atacand Plus® tablets Batch No. 130352 (16/12.5) of CAN and HCT, respectively, Manufactured by SANOFI AVENTIS. Both formulations were obtained from local market.

**Solvents**

Methanol (E. Merck, Darmstadt, FRG).

**Apparatus**

Shimadzu (Columbia, MD) 1605 UVPC spectrophotometer using 1.00 cm quartz cells. Scans were carried out in the range of 220–300 nm at 0.5 nm intervals.

**Software**

All computations were performed in Matlab (Natick, MA) for Windows™ Version 6.5 [30]. The PLS procedure was taken from PLS_Toolbox [31] for use with Matlab 6.5.
The absorption spectra of the three compounds in methanol were recorded over the range 200–400 nm, using methanol as a blank.

**Standard solutions and calibration**

Stock standard solutions of HCT, IRB and CAN (1 mg/mL) of each were prepared by accurately weighing and dissolving 100 mg authentic drugs separately in methanol and then diluting to volume in 100 mL volumetric flasks. An aliquot of 10 mL of each of the prepared stock standard solutions was further diluted with methanol to a final volume of 100 mL. The diluted solutions were used as working standard solutions with concentrations of 100 µg/mL for HCT, IRB and CAN.

**For MCR method**

Aliquots of HCT, IRB and CAN were accurately transferred from their respective working standard solutions (100 mg/mL) into 10-mL measuring flask and diluted to volume with methanol. The prepared solutions having the concentration ranges of 1–20 µg/mL HCT and 2–36 µg/mL for IRB and CAN, respectively. The absorption spectra of the resulting solution were measured in the range of 200–400 nm.

The scanned spectra of HCT were divided by the normalized absorption spectrum of IRB, then was mean centered. The mean centered spectra were then divided by the mean centered CAN/IRB. The obtained curves were then mean centered and used for determination of HCT at 331 nm. In the same way, the spectra of IRB were divided by the normalized CAN spectrum and mean-centered. The obtained curves were then divided by the mean centered HCT/CAN. The obtained curves were then mean centered and used for determination of IRB at 237 nm. Similarly, the spectra of CAN were divided by the normalized IRB spectrum and mean-centered. The obtained curves were then divided by the mean centered HCT/IRB. The obtained curves were then mean centered and used for determination of CAN at 255 nm. The mean-centered values at 331, 237 and 255 nm for HCT, IRB and CAN, respectively, were plotted versus the corresponding concentration, and regression equations were computed.

**For the CRACLS method**

Multilevel-multifactor design was used for the construction of the calibration and validation sets [32]. A five-level three-factor calibration design was applied in which different aliquots of the three working standard solutions (100 µg/mL) were combined and diluted to 25 mL with methanol in order to reach the concentration ranges of 1.2–13.2, 8–24 and 4–12 µg/mL for HCT, IRB and CAN, respectively. The concentration details are given in Table 1. The absorption spectra of the prepared mixtures were recorded and transferred to Matlab® for subsequent calculations.

**Assay of laboratory-prepared mixtures**

For MCR method

Different laboratory prepared mixtures were prepared covering all the available ratios and the solutions were scanned in the range of 200–400 nm, stored in the computer and exported to Matlab® for subsequent calculation. The previously mentioned procedure under MCR was followed and the concentrations were calculated from the corresponding regression equations.

**For CRACLS method**

The prediction of the developed model was tested by an external validation set which was randomly chosen from the previously prepared designed set according to multilevel multifactor experimental design.

**Application to pharmaceutical preparations**

The film coat was removed using methanol, then the weight of twenty tablets of each of Kansartan Plus® and Atacand Plus® tablets were accurately determined and then they were finely powdered. An accurate weight of each of the powdered tablets was transferred into a 100-mL volumetric flask; completed to the mark with methanol and sonicated for 10 min. Each solution was then filtered into a 100-mL volumetric flask. The proposed methods were applied for the analysis of the pharmaceutical preparations solutions using the procedures mentioned for each method and the concentrations of the cited drugs were calculated.

**Results and discussion**

The main goal of this work was to introduce and compare two different approaches for quantitation of ternary antihypertensive drugs. This depends mainly on the development and validation of simple, sensitive, and accurate analytical methods for the simultaneous determination of HCT, IRB and CAN in their bulk powders, laboratory-prepared mixtures, and a pharmaceutical dosage form with satisfactory precision for good analytical practice.

As shown in Fig. 2. The absorption spectra of HCT, IRB and CAN are highly overlapped in the range of 200–350 nm which precludes their direct determination. As HCT is commonly co-formulated with either IRB or CAN, so our aim was to apply a method for spectral resolution of the three components as to be applied for both HCT combinations and any future developed dosage form.

Two different techniques were applied for achievement of spectral resolution. One based on mean-centering as a processing step that is capable of removing the interference of all components except for the analyte. The second is a multivariate calibration method which is based on augmented classical least squares in
which resolution of the spectral bands is achieved so, the presence of interfering compound will not affect the prediction of the analyte of interest.

**MCR method**

The scanned spectra of HCT were divided by the normalized absorption spectrum of IRB, then was mean centered. The mean centered spectra were then divided by the mean centered CAN/IRB. The obtained curves were then mean centered and used for determination of HCT at 331 nm, Fig. 3. In the same way, the spectra of IRB were divided by the normalized CAN spectrum and mean-centered. The obtained curves were then divided by the mean centered HCT/CAN. The obtained curves were then divided by the mean centered HCT/IRB. The obtained curves were then mean centered and used for determination of CAN at 255 nm, Fig. 5. The mean-centered values at 331, 237 and 255 nm for HCT, IRB and CAN, respectively, were plotted versus the corresponding concentration, and regression equations were computed, all regression and validation parameters are presented in Table 2.

The effect of divisor concentration on analytical parameters, such as slope, intercept, and correlation coefficient of the calibration graphs was also tested. Different concentrations of divisor were used, but it was observed that changing the concentration had no significant effect on the linear calibration range and calculated analytical parameters. Therefore, a normalized spectrum of HCT, IRB and CAN was used as a divisor spectrum in the proposed method. For analysis of laboratory prepared mixtures and pharmaceutical preparation, the same procedure was used, except that the spectra of the mixtures and dosage form solutions were used instead of those of pure samples, Tables 3.
CRACLS method

Haaland and Melgaard [28] and Melgaard et al. [29] have recently developed a family of augmented classical least squares (CLS) techniques that have many advantages over principal component regression (PCR) and partial least squares (PLS). CRACLS is a recently developed algorithm that allows updating of the model during prediction without recalibration. The CRACLS algorithm is based on CLS, so it retains the qualitative benefits of CLS and the flexibility of PLS modeling when spectrally active components are not explicitly included in the calibration. A calibration set was designed with 25 calibration samples containing the three compounds in the ranges and concentrations shown in Table 1. Seventeen samples were used as a validation set, and another 8 samples as a validation set. The spectral region between 220 and 350 nm was selected for analysis, where each of the three compounds has no spectral absorbance at longer wavelengths. Spectra were digitized each at 0.1 nm so that 1301 experimental points were used in calculations. CRACLS models were built for each component alone in the mixtures. The estimated pure component spectra that resulted by including HCT and augmenting seven times are shown in Fig. 6. The qualitative information obtained from the method will be appreciated when the true pure component spectra are compared with the estimated ones. The estimated pure component spectra were found to improve by including the concentration of the three components or increasing the number of augmentations. Correlation between the estimated spectra and the true spectra was calculated and found to be 0.998, 0.9887 and 0.9982 for HCT, IRB and CAN, respectively. To test the prediction ability of the new method, the model was challenged with the spectra of HCT, IRB and CAN, respectively. To test the prediction ability of the proposed methods was assessed by applying the standard addition technique, Table 3.

The proposed method was also successfully applied to the analysis of HCT, IRB and CAN in a tablet dosage forms. The accuracy of the number of augmentations does not lead to overfitting; after a sharp decrease, the RMSEP decreases gradually until it reaches a constant value.

The proposed method was also successfully applied to the analysis of HCT, IRB and CAN in a tablet dosage forms. The accuracy of the proposed methods was assessed by applying the standard addition technique, Table 3.

For both MCR and CRACLS methods a statistical comparison between the obtained results and those obtained by the reported method for the simultaneous determination of HCT, IRB and CAN and application of standard addition technique.

Table 2
Regression and validation parameters of the proposed MCR method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MCR</th>
<th>CRACLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td>HCT</td>
<td>IRB</td>
</tr>
<tr>
<td>99.56 ± 0.938</td>
<td>99.22 ± 1.30</td>
<td>99.44 ± 1.28</td>
</tr>
<tr>
<td>Range (µg/mL)</td>
<td>1–20</td>
<td>2–36</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0364</td>
<td>2.3004</td>
</tr>
<tr>
<td>SE of slope</td>
<td>0.0030</td>
<td>0.0299</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.1258</td>
<td>1.7126</td>
</tr>
<tr>
<td>SE of intercept</td>
<td>0.0998</td>
<td>0.9995</td>
</tr>
<tr>
<td>SE of regression</td>
<td>0.9144</td>
<td>0.4438</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.93 ± 0.543</td>
<td>100.90 ± 0.901</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.89 ± 0.607</td>
<td>100.98 ± 0.641</td>
</tr>
<tr>
<td>Precision</td>
<td>0.873</td>
<td>0.349</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>0.962</td>
<td>0.721</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>1.90</td>
<td>1.79</td>
</tr>
</tbody>
</table>

Table 3
Application of the proposed methods for the simultaneous determination of HCT, IRB and CAN and application of standard addition technique.

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Assay and standard addition (Mean ± SD)</th>
<th>MCR</th>
<th>CRACLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCT</td>
<td>IRB</td>
<td>CAN</td>
</tr>
<tr>
<td>Kansartan Plus® tablets</td>
<td>Assay</td>
<td>98.54 ± 0.213</td>
<td>101.98 ± 0.459</td>
</tr>
<tr>
<td>B.N. 120536A (150 IRB/12.5 HCT)</td>
<td>Standard addition</td>
<td>101.67 ± 0.213</td>
<td>100.65 ± 0.923</td>
</tr>
<tr>
<td>B.N. 120537A (300 IRB/12.5 HCT)</td>
<td>Assay</td>
<td>99.32 ± 0.491</td>
<td>101.79 ± 0.713</td>
</tr>
<tr>
<td>Atacand Plus® tablets</td>
<td>Assay</td>
<td>97.99 ± 0.741</td>
<td>99.78 ± 0.792</td>
</tr>
<tr>
<td>B.N. 130352 (16 CAN/12.5 HCT)</td>
<td>Standard addition</td>
<td>101.51 ± 0.798</td>
<td>101.54 ± 0.813</td>
</tr>
</tbody>
</table>

Table 4
Regression parameters of the validation data set for the determination HCT, IRB and CAN in the by the proposed CRACLS method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CRACLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (µg/mL)</td>
<td>HCT</td>
</tr>
<tr>
<td>1.2–13.2</td>
<td>1.0210</td>
</tr>
<tr>
<td>8–24</td>
<td>0.0546</td>
</tr>
<tr>
<td>4–12</td>
<td>0.0995</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0811</td>
</tr>
<tr>
<td>SE of slope</td>
<td>0.3634</td>
</tr>
<tr>
<td>SE of intercept</td>
<td>0.2760</td>
</tr>
<tr>
<td>SE of regression</td>
<td>0.0546</td>
</tr>
<tr>
<td>Recovery</td>
<td>100.23 ± 0.891</td>
</tr>
<tr>
<td>RMSEP</td>
<td>0.546</td>
</tr>
</tbody>
</table>

Fig. 6. Pure spectral profiles obtained by deconvolution of spectral data by CRACLS algorithm.
methods showed no significant differences, as seen in Table 5. In order to compare the ability of the proposed methods for the determination of HCT, IRB, and CAN, the results obtained by applying the proposed methods were subjected to statistical analysis using a one-way analysis of variance (ANOVA) test; there was no significant difference between the two methods, Table 6.

Conclusions

The proposed methods are considered simple, very sensitive and precise for the simultaneous determination of HCT, IRB, and CAN in any possible pharmaceutical combination. MCR method has the advantage of eliminating the derivative steps and enhances signal to noise ratio which increases its sensitivity. CRACLS method is considered more selective than MCR method in spite having comparable predictions as it determines each component in the mixture including impurities and noise signals. The proposed methods could be successfully applied for the routine analysis of HCT, IRB, and CAN either in their pure bulk powders or in dosage forms in QC laboratories, without any preliminary separation step which is considered of lower cost and time saving if compared to other HPLC methods.

References