UV-Chemometric Method Development for Resolving The Overlapped Spectra of Aspirin, Caffeine and Orphenadrine Citrate in Their Ternary Pharmaceutical Dosage Form

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Abstract

A simple, specific, accurate and precise UV-chemometric methods have been developed for the analysis of aspirin, caffeine and orphenadrine citrate in their ternary mixture form without prior separation. CLS, PLS and PCR were developed for simultaneous determination of the three drugs together by using different sets of data in which better results were produced. Unfortunately, PLS method was the only method which was able to determine them accurately. The validity of these chemometric methods has been carried out by 8 synthetic mixtures for determination of the power of prediction for each method. Latent variable number is different from one model to another with changing the set of data. Predicted residual error sum of squares (PRESS) and root mean square error of prediction (RMSEP) were used for comparison between different methods and for determination the predictive power of each set of data. In addition, statistical comparison between the proposed chemometric methods was performed.

1. Introduction

Acetylsalicylic acid, [2-acetoxybenzoic acid] (Fig. 1) also known as aspirin (ASP), is a salicylate drug which is rapidly hydrolyzed in the body of the human to produce its pharmacological activity and can acts as an analgesic, antipyretic and anti-inflammatory drug. ASP can be used also to reduce cardiovascular morbidity and mortality in high-risk patients with myocardial infarction or stroke [1].

Caffeine (CAF); 1,3,7-trimethylxanthine (Fig. 1) is a psycho stimulant purine alkaloid that can increase alertness. CAF can increase the effect of analgesic and antipyretic drugs so it is combined with them in many pharmaceutical preparations [2].

Orphenadrine citrate (ORP); (±)-N,N-Dimethyl-2-[o-methyl-a-phenylbenzyl]oxy]ethylamine citrate (Fig. 1) is a centrally acting skeletal muscle relaxant which prevents impulses of the somatic nerves from being generated by inhibiting a specific neurons in the nervous system. The combination of an analgesic drug and a skeletal muscle relaxant is better than single agents alone [2].

Literature survey revealed that ASP & CAF & ORP were determined in some of their different binary mixtures by electrochemical methods [3, 4], spectrophotometric methods [5–13] and HPLC methods [13, 14]. Also, the literature demonstrated that few methods have been carried out for the analysis of ASP & CAF & ORP in their ternary mixtures and pharmaceutical formulations. They were determined by spectrophotometric method [15], a TLC method [16] and two HPLC methods [17, 18].

To the best of our knowledge, no UV-Chemometric method has been developed for the analysis of this ternary mixture. The novelty of this research is based on developing a new chemometric technique using UV-Spectrophotometer instrument in analysis of this mixture without prior separation or sample treatment.

The aim of this work is to develop simple, easy, economic, accurate, fast and non-complicated UV-chemometric methods with different sets of data for the determination of ASP, CAF & ORP in their combined tablet formulation without any interference and through comparing the results of each set of data to determine which one would be having the most powerful predictive power. The obtained results have been statistically compared and interpreted.

2. Experimental

2.1. Apparatus

JASCO dual beam UV-visible spectrophotometer (Japan) model V-630, connected to a compatible computer (ACER) with spectra manager II software was used. The spectral slit width is 2 nm with scan speed up to 8000 nm/min. At room temperature, 1 cm quartz cell was used for all measurement over wavelength range of 200 ~ 400 nm.

2.2. Software

Matlab® 7.0.1 Software program was used to carry out the chemometric analysis. PASW statistics 18® software program was used for statistical analysis.

2.3. Materials and Reagents

2.3.1. Pure standard materials

ASP was obtained from El-Nasr Pharmaceutical Co., Abu Zaabal, Cairo, Egypt. ORP was obtained as a gift from EIPICO, located in 10th of Ramadan city, Egypt. Their purity was reported to be 99.70%. CAF was obtained from LABORT FINE CHEM, located in 602/A, President Plaza, Near RTO, Ring Road, Surat-395001, Gujarat, India, and its purity was reported to be 99.00%.

2.3.2. Pharmaceutical formulations

Relatic® tablets were obtained from the market (label claim: ASP 770 mg, CAF 60 mg and ORP 50 mg) manufactured by Sigma for pharmaceutical International (SPI), Egypt for Horizone Pharma, Egypt.

2.3.3. Solvents

HPLC grade Methanol was purchased from, Merck (Darmstadt Germany). Spectral measurements have been carried out using 90% Methanol (methanol: Distilled water, 9:1).

2.3.4. Standard solutions preparation
ASP, CAF, ORP stock standard solutions of 1 mg/mL have been prepared in 90% methanol. All working standard solutions of 50 µg/mL have been prepared by
dilution from the stock solution with 90% methanol. 25 mixture solutions of ASP, CAF & ORP in the range of 4-25 µg/mL for ASP, 5-35 µg/mL for CAF and 5-50 µg/mL for ORP in the same solvent have been symmetrically prepared from the previous stock solutions respectively and the concentration set design was
demonstrated in Fig. 2.

2.4. Calibration set

17 synthetic mixtures in the range of 4-25 µg/mL for ASP, 5-35 µg/mL for CAF and 5-50 µg/mL for ORP were prepared as a calibration set according to the
multifactor and the multilevel design [19] in 10 mL volumetric flasks. Calibration set was chosen according to stratified random sampling technique [20]. UV absorption spectra in its raw form and in its manipulated spectral data sets were used to construct the chemometric models of CLS, PLS and PCR.

2.5. Prediction set

8 synthetic mixtures in the same range of calibration set of the three drugs were also prepared as a validation set according to the multifactor and the
multilevel design [11] in 10 mL volumetric flasks to evaluate the accuracy and precision of the constructed models.

2.6. Data preprocessing

Absorption spectra were scanned from 200 to 400 nm while the region 200–215 nm was neglected to avoid the noise interference. Several wavelengths have been tried and the wavelength range of 260-285 nm was chosen due to its superior and accurate results over other ranges.

First and Second derivatives of the absorption spectra were processed before building some models to assess their effect on the validation of the analysis. Ratio spectra were calculated using divisors of ASP (25ug), CAF (25ug) and ORP (25ug), separately then the analysis was continued using only CAF (25ug) as a divisor and its ratio derivatives as it gave more precise values than ASP and ORP.

2.7. Application to pharmaceutical formulation

10 Tablets of Relatic® were weighed and crushed then an amount equivalent to 385 mg ASP, 30 mg CAF and 25 mg ORP in each tablet was transferred into a
50 mL volumetric flask (equivalent to 7700 µg/mL ASP, 600 µg/mL CAF and 500 µg/mL ORP) and diluted with 90% methanol as follow: First, 35 mL of 90%
methanol were added and sonicated then dilution was carried out to the mark and filtered. Second, 1 mL of the dilution was transferred into a 100 mL volumetric flask to give a concentration equivalent to 77 µg/mL ASP, 6 µg/mL CAF and 5 µg/mL ORP. Third, any further dilutions were done in 10 mL volumetric flasks and treated in the same way as described under the proposed methods.

3. Results And Discussion

3.1. Method Optimization

Trials of three simple chemometric methods CLS, PLS & PCR have been used for simultaneous determination of ASP, CAF & ORP in their pharmaceutical
dosage form. Absorption spectra in its raw form and in its manipulated forms (First derivative, Second derivative, Ratio spectra, First derivative of ratio spectra
and Second derivative of ratio spectra) to make different sets of data (Fig. 3) were used to build CLS, PLS and PCR models in the range of 260-285nm. 90%
Methanol (HPLC grade methanol; Distilled water 9:1) was used as a solvent in which all drugs showed good solubility. Fig. 4 displays the absorption spectra of Zero, First derivative, Second derivative, Ratio spectra, First derivative of ratio spectra and Second derivative of ratio spectra of ASP, CAF & ORP and their mixture. CLS, PLS and PCR models were constructed by using a calibration set consisting of different ratios of ASP, CAF & ORP as shown in Table 1.

Cross-validation and Scaling were carried out on the calibration set through leaving out one at a time cross-validation and mean center scaling for PLS and PCR models. The number of latent variables is varied from one model to another. Wavelength range from 260-285 nm with Δλ = 0.1 nm for zero, first and
second derivative and $\Delta \lambda = 1$ nm for Ratio spectra and its derivatives was used in all measurements as it is found to give better and more accurate results. Parameters used in the construction of PLS and PCR models were demonstrated in Table 2. The optimal number of latent variables is different from one model to another and is demonstrated for PLS in Fig. 5 and for PCR in Fig. 6.

### Table 2

| Method               | Range (nm) | Interval (nm) | Scaling     | Cross Validation |
|----------------------|------------|---------------|-------------|------------------|
| Zero                 | 260-285    | 0.1           | Mean center | Leave one out    |
| First derivative     | 260-285    | 0.1           | Mean center | Leave one out    |
| Second derivative    | 260-285    | 0.1           | Mean center | Leave one out    |
| Ratio spectra        | 260-285    | 1             | Mean center | Leave one out    |
| Ratio derivative     | 260-285    | 1             | Mean center | Leave one out    |
| Ratio second derivative | 260-285    | 1             | Mean center | Leave one out    |

### 3.2. Method validation

The Validation of CLS, PLS and PCR models were calculated by the analysis of their predictive ability on the validation (prediction) set for assessment of the accuracy and precision. The predicted values and actual values of both calibration and validation sets were compared then predicted residual error sum of squares (PRESS) and root mean square error of prediction (RMSEP) were calculated for various models as follow:

- PRESS = Calculate the difference between expected values and predicted values for all the samples and square them then sum them together.
- RMSEP = Divide PRESS by number of mixtures and calculate the root of the resulted value.

Results for different sets of data by using PLS are shown in Table 3.

### Table 3

| Spectra order    | PLS Parameter | ASP  | CAF  | ORP  | ASP  | CAF  | ORP  | ASP  | CAF  | ORP   |
|------------------|---------------|------|------|------|------|------|------|------|------|-------|
|                  |               | Calibration set | Validation set |               |               | Mean | Mean | Mean | Mean | Mean  |
|                  |               | ASP   | CAF  | ORP  | ASP  | CAF  | ORP  | ASP  | CAF  | ORP   |
| Zero             | Mean          | 99.78 | 100.32 | 99.51 | 99.81 | 100.00 | 100.20 | 99.97 | 100.31 | 100.56 |
|                  | RMSEP         | 0.2304 | 0.2271 | 0.2571 | 0.2303 | 0.1699 | 0.2356 | 0.3074 | 0.3154 | 0.3228 |
|                  | PRESS         | 0.9023 | 0.8771 | 1.1237 | 0.9019 | 0.4905 | 0.9432 | 1.6063 | 1.6910 | 1.7711 |
| First derivative | Mean          | 100.58 | 99.44 | 101.18 | 100.54 | 100.02 | 99.80 | 100.33 | 99.60 | 99.12 |
|                  | RMSEP         | 0.1504 | 0.1503 | 0.3161 | 0.1743 | 0.1324 | 0.2590 | 0.2728 | 0.2459 | 0.2179 |
|                  | PRESS         | 0.1808 | 0.1806 | 0.7996 | 0.2431 | 0.1402 | 0.5368 | 0.5954 | 0.4837 | 0.3798 |
| Second derivative| Mean          | 100.58 | 99.44 | 101.18 | 100.54 | 100.02 | 99.80 | 100.33 | 99.60 | 99.12 |
|                  | RMSEP         | 0.2304 | 0.2271 | 0.2571 | 0.2303 | 0.1699 | 0.2356 | 0.3074 | 0.3154 | 0.3228 |
|                  | PRESS         | 0.9023 | 0.8771 | 1.1237 | 0.9019 | 0.4905 | 0.9432 | 1.6063 | 1.6910 | 1.7711 |

Unfortunately, CLS and PCR gave inaccurate results and as such, they would not be used in determination of this ternary mixture unlike PLS which gave very accurate values.

In respect of PLS, Zero absorption spectra, First derivative spectra and Second derivative spectra can be used for determination of ASP, CAF & ORP in which Zero absorption spectra has the most powerful prediction for ASP and First derivative spectra has the most powerful prediction for both CAF & ORP while Ratio spectra, Ratio derivative spectra and Ratio second derivative spectra can’t be used for determination of ASP, CAF & ORP.

Although the Raw data set (Zero spectra) is the simplest method but manipulation of the spectra to have different sets of data led to a great difference with improving the results. Although the ratio spectra set of data require an extra-process before carrying out the measurements, First and Second derivative of ratio spectra sets of data requires more work as it needs more extra process than the ratio spectra.

### 3.3. Application to Pharmaceutical Formulation

The proposed chemometric method (PLS) was successfully applied for determination of ASP, CAF & ORP in their tablet formulation (Relatic® tablets). The results were in the acceptable range concurrent with the labeled amounts. The standard addition technique was applied for accuracy and demonstrated that no interference of the excipients was observed (Table 4).
### Table 4
Pharmaceutical preparation (Relatic® tablets) and standard addition results from using PLS chemometric

| Spectra order | Parameter | Zero | First derivative | Second derivative | Ratio spectra |
|---------------|-----------|------|------------------|-------------------|--------------|
|               | ASP       | CAF  | ORP              | ASP               | CAF          | ORP          | ASP  | CAF  | ORP  | ASP    | CAF  | ORP  |
| PCR           | Mean      | 100.02 | 100.03           | 100.08            | 100.01       | 99.99        | 100.03 | 100.12 | 99.81 | 103.75 | 102.42 | 103.66 |
|               | SD        | 0.05  | 0.14             | 0.19              | 0.15         | 0.04         | 0.42   | 0.42   | 0.62  | 6.37   | 9.15    | 7.64   |
| Pharmaceutical formulation | Mean | 100.04 | 100.03           | 100.12            | 100.01       | 99.99        | 100.05 | 99.52  | 99.85 | 99.93  | 102.75 | 102.33 | 103.71 |
|               | SD        | 0.08  | 0.16             | 0.12              | 0.18         | 0.08         | 0.07   | 0.52   | 0.41  | 0.75   | 8.22   | 8.64   | 6.20   |

### 3.4. Statistical Analysis

Statistical comparison between different sets of data in PLS model has been carried out by One-way ANOVA method through PASW statistics 18® software program. The calculated F values were less than the theoretical ones indicating that there was no significant difference between the proposed methods as reported in Table 5.

### Table 5
Statistical comparison of the results obtained by the proposed methods using One-way ANOVA.

| Models | Drugs | Sum of Squares | df | Mean Square | F    | Sig.  |
|--------|-------|----------------|----|-------------|------|-------|
| PLS    | ASP   | Between Groups | 59.606 | 5 | 11.921 | .109 | .988 |
|        |       | Within Groups | 1315.070 | 12 | 109.589 |
|        |       | Total         | 1374.676 | 17 |       |
| CAF    | Between Groups | 75.240 | 5 | 15.048 | .218 | .948 |
|        | Within Groups | 827.777 | 12 | 68.981 |
|        | Total | 903.016 | 17 |       |
| ORP    | Between Groups | 75.240 | 5 | 15.048 | .218 | .948 |
|        | Within Groups | 827.777 | 12 | 68.981 |
|        | Total | 903.016 | 17 |       |

### 4. Conclusion

A UV-Chemometric PLS technique can be used for simultaneous determination of ASP, CAF & ORP in their mixture and pharmaceutical formulation. By applying different sets of data, we can deduce that different prediction powers are obtained from using different sets of data by manipulating the spectra in which First derivative set of data is best used for determination of both CAF & ORP while Zero absorption set of data is best used in determination of ASP alone. Also, we can deduce that PLS model has the most powerful prediction in determination of ASP, CAF & ORP in their mixture form. On the other hand, Ratio spectra and their derivatives set of data has the least prediction powers in all models and therefore can't be used in such drug determination. Statistical comparison showed that there was no significant difference between the proposed methods.

### Declarations

#### Ethics approval and consent to participate
Not applicable.

#### Consent for publication
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#### Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article [and/or] its Additional file.

#### Competing interests
The authors declare that they have no competing interests.
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Contributions
Amr A. Mattar, and Mahmoud M. Sebaiy designed and wrote the research work, and Sobhy M. El-Adl revised the manuscript and supervised the research. All authors read and approved the final manuscript.

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Figures

Figure 1

Chemical structures of aspirin (ASP), caffeine (CAF) and orphenadrine citrate (ORP).

Figure 2

Concentration set design for ASP, CAF & ORP for CLS, PLS and PCR methods.
Figure 3

Different sets of data used for construction of CLS, PLS and PCR models.
Figure 4

Different absorption spectra of ASP (25ug), CAF (25ug) and ORP (25ug) and their mixture (12ug each).
Figure 5

Different Latent Variables (RMSECV vs LV) for different sets of data for construction of PLS models.
Figure 6

Different Latent Variables (RMSECV vs LV) for different sets of data for construction of PCR models.

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