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Development of UV-Chemometric techniques for resolving the overlapped spectra of aspirin, caffeine and orphenadrine citrate in their combined pharmaceutical dosage form

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Abstract

A UV-chemometric approach has been developed to analyze a ternary combination of aspirin, caffeine, and orphenadrine citrate without the need for previous separation. The method is easy, specific, accurate, and highly precise. The three medications were evaluated simultaneously utilizing CLS, PLS, and PCR, which were generated based on separate data sets that yielded superior findings. Regrettably, their accurate estimation could only be achieved using the PLS approach. In order to determine the prediction power of each chemometric approach, its validity has been tested using 8 synthetic mixes. The latent variable number varies across various models as the dataset changes. The comparison of various methodologies and the assessment of the predictive capacity of each set of data were done using the predicted residual error sum of squares (PRESS) and the root mean square error of prediction (RMSEP). The created approach was also used to statistically compare the performance of PLS in a datasets. The environmental impact of the created approach was assessed to determine the overall ecological sustainability of the designed methodology. According to the new Blue Applicability Grade Index (BAGI) evaluation methodology, the suggested technique was also found to be practicable.

Keywords Aspirin, Caffeine, Orphenadrine citrate, AGREE, BAGI, GAPI, PLS

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Introduction

Nonmalignant chronic pain (NMCP) is regarded as a matter of public health significance [1]. Every year in the United States, almost 100 million Americans suffer from chronic pain. The United States incurs an estimated added cost of \$261-\$300 billion in health care expenditure associated with chronic pain [2, 3]. The American Pain Society has advocated the use of polytherapy to achieve efficient pain management while minimizing the risk and occurrence of side effects [4]. The combination of aspirin (ASP), caffeine (CAF), and orphenadrine citrate (ORPH) is used to alleviate the discomfort associated with musculoskeletal pain. Acetylsalicylic acid, commonly known as aspirin (ASP), is a salicylate drug that rapidly proceeds hydrolysis in the human body to produce its therapeutic effects. It serves as a pain reliever, fever reducer, and anti-inflammatory medication (Fig. 1). ASP may also be utilized to decrease death from cardiovascular disease in high-risk individuals who have had a heart attack or stroke [5, 6]. The psychostimulant purine alkaloid caffeine (CAF), scientifically known as 1,3,7-trimethylxanthine, may heighten one's state of alertness. (Fig. 1). It is common practice to mix CAF with analgesic and antipyretic medications because of its ability to enhance their effects [7]. Orphenadrine citrate (ORP) is a chemical compound with the chemical formula (±)-N, N-Dimethyl-2-[(o-methyl-a-phenylbenzyl)oxy] ethylamine citrate is a centrally acting skeletal muscle relaxant that inhibits certain neurons in the brain's neurological system, hence preventing the generation of impulses in the somatic nerves (Fig. 1). The synergistic effect of combining an analgesic medication with a skeletal muscle relaxant surpasses the individual efficacy of either agent alone [7].

Through a literature study, it has been found that the determination of ASP and CAF in their binary combination was accomplished using electrochemical techniques [8, 9] and HPLC methods [10, 11]. Moreover, the existing literature has shown that there have been limited approaches used to analyze the combination of ASP, CAF, and ORP in their ternary mixes and pharmaceutical formulations. The determination was conducted using the spectrophotometric technique [12], the TLC method [13], and the HPLC methods [14, 15]. UHPLC, TLC, and HPLC are analytical approaches that provide enhanced sensitivity and selectivity. Nevertheless, these approaches are distinguished by their heightened intricacy, elevated costs associated with equipment care, and analysis duration. Furthermore, it is imperative to do sample purifying prior to the injection. UV-spectrometry is an inexpensive, quick, and sensitive analytical instrument; nevertheless, the direct UVspectrophotometric methods are unsuitable for analyzing multicomponent drug formulations as a result of spectral overlap and lack of specificity. Chemometric is currently a captivating area of study that combines mathematical and computational methods to effectively analyze complicated mixtures and separate overlapping spectra by eliminating noise in the recorded signal [16].

Recently, the scientific sector has been actively adopting environmental chemistry and green analytical chemistry (GAC) ideas into their work methodologies. Consequently, a set of criteria was developed to measure ecological compatibility and sustainability in order to assess the analytical methodologies [17, 18]. Two metrics, GAPI and AGREE, were used in this research to evaluate the proposed method's sustainable profile. Greenness of the procedure was shown by the findings [19, 20]. Additionally, the procedure's practicability was evaluated using the newly introduced Blue Applicability Grade Index (BAGI) methodology [21].

As far as we know, there is currently no UV-Chemometric technique available for analyzing this ternary combination. The innovation of this study is in the development of a new chemometric approach that utilizes UV-Spectrophotometer equipment to analyze this combination without the need for separation or sample treatment.

The objective of this study is to create efficient and environmentally-friendly UV-chemometric techniques that are cost-effective, precise, rapid, and user-friendly. These methods will be used to analyze ASP, CAF, and ORP in their combined tablet formulation, without any interference. By comparing the results obtained from different sets of data, we will identify the set with the highest



Fig. 1 Chemical structures of aspirin (ASP), caffeine (CAF) and orphenadrine citrate (ORP)

predictive capability. The data have been subjected to statistical analysis and then compared and evaluated.

Experimental

Apparatus

The JASCO dual beam UV-visible spectrophotometer model V-630, manufactured in Tokyo, Japan, was used in conjunction with an ACER-compatible computer running spectra management II software. The instrument's spectral slit width was 2 nm and it could scan at different speeds up to 8000 nm/min. Every measurement has been conducted in a 1 cm quartz cell at normal temperature, spanning a wavelength range of 200 to 400 nm.

Software

The chemometric analysis was performed using the MATLAB[°] 7.0.1 software (https://www.mathworks.com/products/matlab.html). The statistical analysis was performed utilizing the PASW Statistics 18° software (http://www.spss.com.hk/statistics/).

Materials and reagents

Pure standards

ASP was obtained from El-Nasr Pharmaceutical Co., Abu Zaabal, Cairo, Egypt. ORP was obtained as a gift from EIPICO, located in the 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- 395,001, Gujarat, India, and its purity was reported to be 99.00%.

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.

Solvents

HPLC grade Methanol (LiChrosolv, Merck KGaA, Germany). All of measurements have been carried out by using 90% Methanol (HPLC grade methanol: Distilled water 9:1).

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 μ g/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 μ g/mL for ASP, 5–35 μ g/mL for CAF and 5–50 μ g/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.



Fig. 2 Concentration set design for ASP, CAF & ORP for CLS, PLS and PCR methods

Calibration set

17 synthetic mixtures in the range of $4-25 \ \mu g/mL$ for ASP, $5-35 \ \mu g/mL$ for CAF and $5-50 \ \mu g/mL$ for ORP were prepared as a training set according to the multifactor and the multilevel design [22] in 10 mL volumetric flasks. Calibration set was chosen according to stratified random sampling technique [23]. 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.

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 [22] in 10 mL volumetric flasks to evaluate the accuracy and precision of the constructed models.

Data preprocessing

Absorption spectra were scanned from 200 to 400 nm while the region 200–215 nm was neglected to avoid 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.

The 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.

Application to pharmaceutical formulation

10 Tablets of Relatic^{\circ} 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 μ g/mL ASP, 600 μ g/mL CAF



Fig. 3 Different sets of data used for construction of CLS, PLS and PCR models represent the range of absorption for each set of data

and 500 μ g/mL ORP) and diluted with 90% methanol as follow: First, 35 mL of 90% methanol were added and sonicated then filtered and dilution was carried out to the mark. Second, 1 mL of the dilution was transferred into a 100 mL volumetric flask to give a concentration equivalent to 77 μ g/mL ASP, 6 μ g/mL CAF, and 5 μ g/mL ORP. Third, any further dilutions were done in 10 mL volumetric flasks (Sample enrichment must be done to CAF and ORP) and treated in the same way as described under the proposed methods.

Results and discussion 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 their raw form and in their 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 260–285 nm. 90% Methanol (HPLC grade



Fig. 4 Different absorption spectra of ASP (25ug), CAF (25ug) and ORP (25ug) and their mixture (12ug each) represent the range of absorption for each set of data

methanol: Distilled water 9:1) was used as a solvent in which all drugs showed good solubility. Figure 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 by 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 to 285 nm with Δ_{λ} =0.1 nm for zero, first, and second derivative and $\Delta\lambda$ =1 nm for Ratio

spectra and its derivatives were 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 are 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.

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 the predicted residual error sum of squares (PRESS) and

Set	Calibra	tion set		Set	Calibra	tion set		Set	Validat	ion set	
Mix No.	ASP	CAF	ORP	Mix No.	ASP	CAF	ORP	Mix No.	ASP	CAF	ORP
C1	12	12	12	C10	12	16	16	V1	8	16	10
C 2	12	8	8	C11	16	16	8	V 2	10	16	12
C 3	8	8	16	C12	8	14	8	V 3	10	14	16
C 4	16	10	16	C13	8	12	14	V 4	16	14	12
C 5	16	12	10	C14	12	14	14	V 5	16	8	14
C 6	12	10	10	C15	14	14	10	V 6	14	8	12
C 7	10	10	14	C16	10	8	10	V 7	14	10	8
C 8	14	16	14	C17	10	12	8	V 8	8	10	12
C 9	14	12	16								

Table 1 Chemometric design for calibration and validation sets for ASP, CAF & ORP

• Concentrations used for ASP, CAF and ORP are 8 ug, 10 ug, 12 ug, 14 ug and 16 ug for each

 Table 2
 Chemometric parameters used for construction of PLS

 & PCR models
 PCR models

Method	Range (nm)	Inter- val (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

root mean square error of prediction (RMSEP) were calculated for various models as follows:

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.

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 as shown in Tables S1–S4.

In respect to PLS, Zero absorption spectra, First derivative spectra, and Second derivative spectra can be used for the determination of ASP, CAF & ORP in which Zero absorption spectra have the most powerful prediction for ASP and First derivative spectra have 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 requires an extra process before carrying out the measurements, the First and Second derivatives of ratio spectra sets of data require more work as they need more extra process than the ratio spectra.

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 (a known amount of standard was added to the formulation and then measured) was applied for accuracy and demonstrated that no interference of the excipients was observed (Table 4).

Assessment of the proposed approaches level of eco-friendliness

Green analytical chemistry is concerned with determining how to put a numerical value on the many environmental impacts of chemical analysis. Researchers and practitioners in the chemical sciences have enormous hurdles in determining whether chemical processes are environmentally friendly and in avoiding unintended consequences. The level of greenness of the intended method was assessed using two contemporary metrics, namely GAPI and AGREE. The use of GAPI here allows for a comprehensive representation of the established technique (https://cdn.mostwiedzy.pl/73/48/4c/f1/0_2 02010301350285966291_FME/gapi-chart-generator-sf x.exe). The influence on the environment is quantified using five pentagrams. The objects are categorized into three color-coded groups: green, yellow, and red, indicating low, medium, and significant environmental consequences, respectively [24-26]. AGREE (http://mostwiedz y.pl/AGREE) is a cutting-edge measure designed to evaluate the environmental and occupational hazards associated with the analytical process, focusing on 12 main factors. The final score is a fractional value ranging from



Fig. 5 Different Latent Variables (RMSECV vs. LV) for different sets of data for construction of PLS models (Blue, green and red lines represent ASP, CAF and ORP, respectively)



Fig. 6 Different Latent Variables (RMSECV vs. LV) for different sets of data for construction of PCR models (Blue, green and red lines represent ASP, CAF and ORP, respectively)

Table 3 Resu	ults obtained	d from F	² LS moc	dels for	determi	ination c	of ASP, C	AF & OF	RP in cal.	ibratior	n and valid	lation sets							
Spectra order		Zero			First d€	rivative		Second	derivati	ive	Ratio spe	ctra		Ratio deri	vative		Ratio 2 nd	derivative	
PLS	Parameter	ASP	CAF	ORP	ASP	CAF	ORP	ASP	CAF	ORP	ASP	CAF	ORP	ASP	CAF	ORP	ASP	CAF	ORP
Calibration set	Mean	99.78	100.32	99.51	99.81	1 00.00	100.20	99.97	100.31	100.56	98.02	103.50	102.55	98.72	104.04	103.45	99.63	106.17	105.22
	RMSEP	0.2304	0.2271	0.2571	0.2303	0.1699	0.2356	0.3074	0.3154	0.3228	3.4975	2.4447	3.1148	3.6164	2.6107	3.1372	2.9212	2.7890	2.9719
	PRESS	0.9023	0.8771	1.1237	0.9019	0.4905	0.9432	1.6063	1.6910	1.7711	207.9551	101.5981	164.9332	222.3275	115.8640	167.3122	145.0681	132.2380	150.1522
Validation set	Mean	100.58	99.44	101.18	100.54	100.02	99.80	100.33	99.60	99.12	117.87	104.36	105.69	116.20	102.68	104.09	118.97	104.83	107.89
	RMSEP	0.1504	0.1503	0.3161	0.1743	0.1324	0.2590	0.2728	0.2459	0.2179	3.3262	4.1954	1.8864	2.9421	3.4651	1.7904	2.8550	2.6931	2.4248
	PRESS	0.1808	0.1806	0.7996	0.2431	0.1402	0.5368	0.5954	0.4837	0.3798	88.5084	140.8140	28.4672	69.2457	96.0542	25.6439	65.2098	58.0221	47.0382

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PLS Parameter ASP CAF ORP ASP CAF ORP ASP CAF ORP ASP CAF OR OR ASP CAF OR ASP CAF OR OR ASP CAF OR OR <thor< t<="" th=""><th>First derivative</th><th>Second de</th><th>erivative</th><th>Ratio sp</th><th>ectra</th><th>Ra</th><th>tio derivati</th><th>ve</th><th>Ratio 2</th><th>nd deriva</th><th>itive</th></thor<>	First derivative	Second de	erivative	Ratio sp	ectra	Ra	tio derivati	ve	Ratio 2	nd deriva	itive
Pharmaceutical formulation Mean 100.02 100.03 100.03 100.01 99.99 100.03 100.01 99.81 103.75 102.42 11 Standard addition technique Mean 100.03 0.19 0.15 0.04 0.04 0.42 0.62 6.37 9.15 7.	ORP ASP CAF O	RP ASP C	AF ORP	ASP	CAF 0	RP AS	P CAF	ORP	ASP	CAF	ORP
SD 0.05 0.14 0.19 0.15 0.04 0.04 0.42 0.42 0.62 6.37 9.15 7. Standard addition technique Mean 100.04 100.03 100.12 100.01 99.99 100.05 99.52 99.85 99.93 102.75 102.33 10	03 100.08 100.03 100.01 95	99 100.03 11	0.12 99.81	103.75	102.42 10	03.66 10.	3.49 105.4	0 102.75	103.74	103.42	108.93
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	03 100.12 100.01 99.99 10	0.05 99.52 9	9.85 99.93	102.75	102.33 10	03.71 10.	7.1 105.7	7 102.86	108.45	102.91	106.37
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Fig. 7 Environmental friendliness evaluation of the technique

zero to one, which is located in the center of the AGREE icon. The resulting color in the center is a blend of colors that represent the performance of the 12 AGREE pictogram areas. The optimal approach is assigned a score of 1 and is represented by a dark green color. Hence, the AGREE metric is regarded as user-friendly, comprehensive, easily applicable, and highly efficient [27, 28]. The results shown in Fig. 7 illustrate the effectiveness of an environmentally friendly approach, as indicated by the computed GAPI pictogram and the satisfactory AGREE score of 0.58.

Assessment of the practicability of the proposed techniques

The Blue Applicability Grade Index (BAGI) is proposed as an innovative tool for measuring the applicability of an analytical approach (git.pg.edu.pl/p174235/bagi). Ten essential variables are evaluated by this tool: analysis kind, hourly sample analysis productivity, analytical technique, reagents and materials used, needed instrumentation, capacity to treat samples concurrently, the necessity of preconcentration, automating level, sample setup method, size of sample, and the capability for concurrent determination of analytes. A visual depiction of an asteroid and its associated score is generated by evaluating



Fig. 8 Evaluating the practicality of the proposed strategy utilizing BAGI tools

the aforementioned characteristics. The following color scheme is used to symbolize different degrees of acceptance: white for no acceptance, light blue for limited acceptance, blue for moderate acceptance, and dark blue for ideal acceptance. To be considered "practical," it is advised that the technique reaches a minimum of 60 points [21, 29]. The assigned BAGI rating of 77.5 indicates the strategy's level of practicality (Fig. 8).

Statistical analysis

Statistical comparison between PLS in zero absorption set of data and the reference reported method [12] was carried out and statistical comparison between the proposed chemometric methods in different sets of data 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 in both analysis indicating that there was no significant difference between the proposed methods and no significant difference between different sets of data of the proposed method as reported in Tables 5 and 6.

Models	Drugs		Sum of Squares	df	Mean Square	F	Sig.
PLS	ASP	Between Groups	0.019	1	0.019	0.032	0.867
		Within Groups	2.431	4	0.608		
		Total	2.450	5			
	CAF	Between Groups	0.608	1	0.608	1.757	0.256
		Within Groups	1.385	4	0.346		
		Total	1.993	5			
	ORP	Between Groups	0.032	1	0.032	0.083	0.788
		Within Groups	1.561	4	0.390		
		Total	1.594	5			

 Table 5
 Statistical comparison of the results obtained by PLS and the reported method using One-way ANOVA

						5 /	
Models	Drugs		Sum of Squares	df	Mean Square	F	Sig.
PLS	ASP	Between Groups	59.606	5	11.921	0.109	0.988
		Within Groups	1315.070	12	109.589		
		Total	1374.676	17			
	CAF	Between Groups	75.240	5	15.048	0.218	0.948
		Within Groups	827.777	12	68.981		
		Total	903.016	17			
	ORP	Between Groups	186.335	5	37.267	0.368	0.861
		Within Groups	1213.590	12	101.132		
		Total	1399.925	17			

Table 6 Statistical comparison of the results obtained by the proposed methods in different sets of data using One-way ANOVA

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. Statistical comparison showed that there was no significant difference neither between the proposed methods nor between different sets of data of the proposed method. The study of the environmental sustainability of the technique using several greenness appraisal methods has revealed that the suggested methodology is eco-friendly. The assessment of the BAGI methodology also shows that the newly designed method is practicable.

Supplementary Information

The online version contains supplementary material available at https://doi.or g/10.1186/s13065-025-01429-x.

Supplementary Material 1

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Author contributions

Amr A. Mattar: Writing the main manuscript text, methodology, Figures preparation, analysis of all data. Sobhy M. El-Adl& Mahmoud M. Sebaiy: Conceptualization, writing review and editing. Omar M El-Abassy: writing review and editing. All the authors read, reviewed and approved the manuscript.

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Data availability

The data used and/or analyzed during this study are available from the corresponding author on a reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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