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Integrated resonance Rayleigh scattering approach utilizing Box–Behnken experimental design for the facile quantification of prucalopride in pharmaceutical tablets and human urine with sustainability assessment

Khalid M. Badr El-Din,[a] Sayed M. Derayea,[a] Fatma F. Mohammed *[a] and Ahmed Abdulhafez Hamad[b]

Prucalopride (PCP) is one of the recent drugs used for the regulation of gastrointestinal tract motility and the treatment of constipation. A new, highly sensitive and fast resonance Rayleigh scattering (RRS) approach was suggested for PCP determination. The approach was based on its reaction of PCP with eosin Y in buffered medium (pH 3.5) to form an ion pair association complex which had a significant enhancement in RRS compared to that of eosin Y or PCP alone. The enhancement of RRS intensity had straight correlation to PCP concentration ranging from 150 to 2000 ng mL^{-1} with 38 ng mL^{-1} as LOD and 125 ng mL^{-1} as LOQ. The measurements were done at a wavelength of 365 nm that provided the maximum sensitivity. All the experimental parameters were studied carefully and optimized *via* Box–Behnken experimental design. The International Council for Harmonization (ICH) guidelines were employed to validate the suggested method and the obtained results proved the appropriate method performance. The method was efficiently utilized to determine PCP in pure form, pharmaceutical tablets and spiked urine samples with no interferences from the surrounding matrices. Furthermore, the greenness of the suggested procedure was confirmed using different green metric approaches.

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1. Introduction

Prucalopride (PCP) is a selective agonist for 5-hydroxytryptamine receptors (especially 4), which normalizes bowel function in patients with constipation.[1] The documented IUPAC name for PCP (Fig. 1) is 4-amino-5-chloro-*N*-[1-(3-methoxypropyl) piperidin-4-yl]-2,3-dihydro-1-benzofuran-7-carboxamide; butanedioic acid. The medication is employed for the therapeutic management of constipation through stimulation of high amplitude propagated contraction in the colon, the enhancement of colonic propulsion and the acceleration of right colon emptying.[2] PCP is orally active and well tolerated. It is more efficient and safer for the treatment of patients with severe chronic constipation compared with its predecessors (*e.g.* cisapride and tegaserod).

According to the literature, the quantification of PCP was accomplished through the utilization of separation methodologies, specifically high-performance liquid chromatography (HPLC).[3–6] Electro-analytical techniques, such as voltammetry[7] and potentiometry[8] were also employed for its estimation. The reported chromatographic and electrochemical methods required tedious sample pretreatment steps, less green and expensive solvents and sophisticated instruments. On the other hand, few spectroscopic techniques were reported, including UV spectrophotometry[9–13] and luminescence-based reaction.[14–16] The spectrophotometric methods suffered from limited sensitivity. While, the procedure of the first spectrofluorimetric method[14] was tedious, lengthy, utilized costly reagent (terbium chloride) and an environmental contaminating one (8-hydroxyquinoline). Although the second method[15] had simple procedure, its sensitivity was less than the proposed method. The linear range of the reported method[15] was 0.75–5.5 μg mL^{-1} with 0.75 μg mL^{-1} as a lower limit of quantitation. Meanwhile these values for the proposed method were 150–2000 and 125 ng mL^{-1}, respectively. Thus, the present method is 6 times more sensitive. The third spectrofluorimetric method[16] was utilized for simultaneously determination of PCP and empagliflozin.

The analytical strategy based on Resonance Rayleigh Scattering (RRS) is gaining prominence due to its combination of high sensitivity and simplicity, making it an essential analytical approach. This technique has been applied in the analysis of various chemical and therapeutically active agents,[17] a diverse

[a]*Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia, Egypt. E-mail: fatma.farouk@mu.edu.eg*

[b]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut, Egypt*

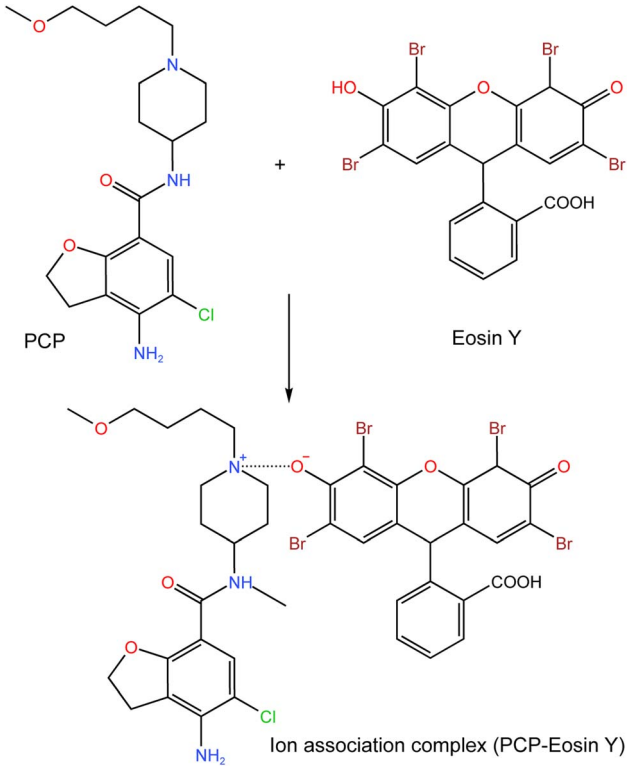

Fig. 1 The suggested ion association reaction between PCP and eosin Y in buffered acidic medium (pH 3.5).

range of biological macromolecules, such as nucleic acids[18] and proteins,[19] in addition trace metal ions.[20]

A variety of chemical dyes and pigments were employed as probes in the development of RRS approaches.[17] These dyes have either acidic or basic nature, and some of them have highly conjugated system with rigid structures that naturally emit light. At the optimal pH, the dye is ionized, then complexed with the analyte. This process causes the RRS spectrum of the dye to be augmented with appearance of a new band. The observed phenomenon has the potential to be utilized for the determination of very low concentration of the desired analyte down to a few nano-grams.[17]

The dye known as eosin Y (Fig. 1) is a compound belonging to the xanthene family which is chemically described as 2,4,5,7-tetrabromo-fluoresceine. This dye was utilized in the chemical analysis relied on forming an ion-coupling complexes between the dye and basic chemicals. Eosin Y, has been employed for the purpose of protein identification and investigation,[21] as well as for the analysis of numerous medicinal substances.[22–26]

There is a growing demand for an environmentally sustainable, efficient, rapid, user-friendly, and highly responsive technique for the analysis of the PCP. The previously mentioned objectives can be effectively tackled through the utilization of the RRS method. This method entails the reaction of the targeted pharmaceutical compound with self-fluorescent dyes within an acidic solution. In terms of environmental sustainability and sensitivity, the designed approach exhibited more positive results than the previously reported spectroscopic methods. Thus, the present article provides a new sensitive, rapid and feasible method for the determination of PCP based on measuring the RRS intensity of the ion association complex formed between the cited drug and the dye in buffered acidic medium.

2. Experimental

2.1. Apparatus

The RRS measurements were done utilizing FS-2 SCINCO spectrofluorometer with a 150 W Xe-arc as a light source lamp (Korea). ADWA AD11 pH meter was utilized for pH adjustments (Hungary).

2.2. Statistical methods and software

Microsoft Excel 365 and GraphPad Instat software (version 3.05) were exploited for statistical calculations. Linear regression models were adopted for PCP calibration. Design expert software (version 13) was used for producing Box–Behnken experimental design model and calculating its statistical parameters. Student's *t*-test and *F* test were utilized for comparison between the proposed and reported methods.

2.3. Materials and reagents

PCP succinate was obtained generously from Al Andalous Company for Pharmaceutical Industries (Cairo, Egypt), and the drug was used without pretreatment. Eosin Y (Merck, Darmstadt, Germany) was prepared as 0.024% w/v (3.47×10^{-4} M) aqueous solution. Sodium acetate and acetic acid were purchased from ElNasr Company for Chemicals (Cairo, Egypt) and both were prepared as 0.2 M solution then mixed in adequate amounts to prepare acetate buffer (pH 3.5). The main solvent utilized throughout the work was distilled water. The grade of all used chemicals was analytical.

2.4. Pharmaceutical tablets

The analysed commercial tablets were Prucasoft® (Delta Grand Pharma, Cairo, Egypt) that was labelled to contain 2.64 mg PCP succinate per tablet (equivalent to 2 mg PCP).

2.5. Standard drug solutions

A stock solution of PCP succinate (100 μg mL^{-1}) was obtained by dissolving 10.0 mg of the drug with distilled water into 100.0 mL calibrated flask. Then further dilutions were done with the same solvent to produce working solutions of PCP in the concentration ranges of 1.5–20.0 μg mL^{-1}. Finally, 1.0 mL of the prepared working solutions was taken in the general procedure.

2.6. Procedures

2.6.1. General analytical procedure. Aliquot of 0.8 mL of acetate buffer (pH 3.5) was moved into a series of 10 mL calibrated flasks, then 1.0 mL of PCP working solutions was added followed by the addition of 2.1 mL eosin Y solution (0.024% w/v). The solutions were completed to 10 mL with distilled water. Finally, the RRS intensity of the solutions was monitored

without standing at 365 nm. A blank solution was treated in the same way but neglecting the drug solution.

2.6.2. Analysis of pharmaceutical tablets. For preparation of commercial tablets for analysis, ten units of Prucasoft® tablets were weighed carefully, grinded well and mixed. Then an amount of the resulting powder that was equivalent to 10 mg PCP was dissolved in adequate amount of distilled water. After sonication for 5 min, the solution was filtered into a 100 mL volumetric flask and completed to volume using the same solvent (100 μg mL^{-1}). Further dilutions were done to produce working solution in the concentration level of 10.0 μg mL^{-1}. Finally, 1.0 mL of that solution was analyzed according to the suggested method.

2.6.3. Preparation of spiked urine samples. Into 10.0 mL closed adjusted tube, 1.0 mL of fresh urine sample was spiked with 1.0 mL PCP solution (100.0 μg mL^{-1}) then 2.0 mL methanol was added followed by dilution with distilled water to 10.0 mL to produce a 10.0 μg mL^{-1} spiked solution. After that, centrifugation at 3500 rpm for 10 minutes was done. Finally, the general procedure was applied on different volumes of the clear supernatant giving three final concentration levels (600, 800 and 1000 ng mL^{-1}). A blank was treated similarly on a free urine sample without adding the drug solution.

3. Results and discussion

Rayleigh scattering is a type of elastic light scattering that have the same wavelength to that of the incident light. RRS is special type that happens if the wavelength of Rayleigh scattering is equal or close to the molecular absorption wavelength. It could be measured using synchronous spectrofluorimetry when $\lambda_{excitation} = \lambda_{emission}$ ($\Delta\lambda = 0$ nm). This analytical technique acquired much attention due to its sensitivity and simplicity. It was utilized widely for the analysis of many types of compounds including pharmaceutical agents, metal ions, anions, surfactants, proteins, biological samples. Many pharmaceutical compounds could react in weak acidic solutions with acidic xanthene dyes such as erythrosine B, eosin Y and Rose Bengal to produce ion-pair associates. These associates attain significant enhancement in the RRS intensity which could be measured and exploited for the analysis of the target analytes.[17]

It was reported that forming of association complexes (binary or ternary types) between eosin Y dye and many pharmaceutical compounds causes significant enhancement of RRS spectrum and the emerging of new RRS bands. This augmentation was ascribed to the enlarged molecular volume and molecular weight, the increased rigidity and planarity of the molecule, and improvement of the hydrophobicity because of complex formation.[26] This increase in the RRS intensity was in direct proportion with the analyte concentrations. In the suggested method, PCP reacted with eosin Y in weak acidic medium pH 3.5 resulting in ion association binary complex (Fig. 1) that caused high augmentation of the RRS intensity compared to either eosin Y or PCP alone in the same pH (Fig. 2). By observing the produced spectra, the wavelength 365 nm was chosen for measurements as it provided the greatest enhancement.

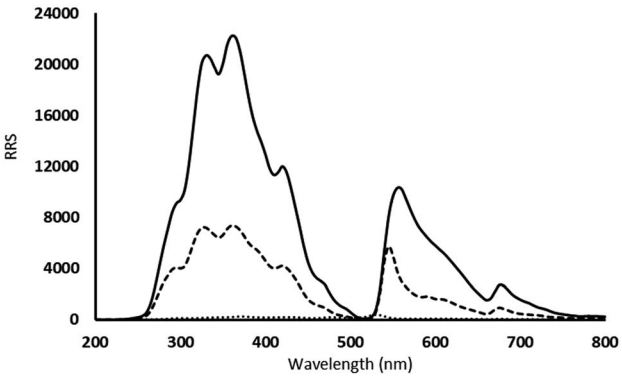

Fig. 2 The RRS spectra of (—) the complex formed between PCP succinate (1200 ng mL^{-1}) and eosin Y (0.024% w/v) compared with (---) blank eosin Y solution and (.....) PCP succinate solution (1200 ng mL^{-1}) in acetate buffer pH 3.5.

When the pH is sufficiently acidic, the tertiary amino group of PCP will undergo protonation, forming dye anion. At the same pH, PCP was fully ionized in the cationic form. The two oppositely charged ions interact with each other resulting in an ion-pair complex formation. The PCP-dye system exhibited augmentation in the RRS spectral bands, providing a way for the quantification of the drug. Due to the low pricing of the reagents and the widespread availability of the required equipment in quality control laboratories, this method is regarded as cost-effective.

3.1. Optimization of experimental parameters

Different experimental parameters were investigated and adjusted to attain maximum sensitivity. The parameters were the pH and volume of acetate buffer, volume of eosin Y and the diluting solvents. Experimental design using Box–Behnken surface response design[27] was adopted for optimization.

Box–Behnken design is considered as a highly fractionalized three-level factorial design, with the treatment combinations being the centre point and the midpoints of the factor levels' edges. These designs require three levels of each factor being studied and are rotatable, or almost rotatable. Box–Behnken designs have advantages over other designs as it requires fewer tests than the central composite design with only three levels. It is also simpler to arrange and interpret than other designs. Furthermore, it can be expanded, contracted, or even translated; and since midpoints of factor edges are always used, it avoids combined factor extremes and can suit full quadratic response surface models.[28]

Table 1 Experimental levels utilized to build the Box–Behnken optimization

	Coded variable		
Variable	(+1)	(0)	(−1)
pH of acetate buffer	5	4	3
Volume of acetate buffer (mL)	1.2	0.7	0.2
Volume of eosin (mL)	3.1	1.6	0.1

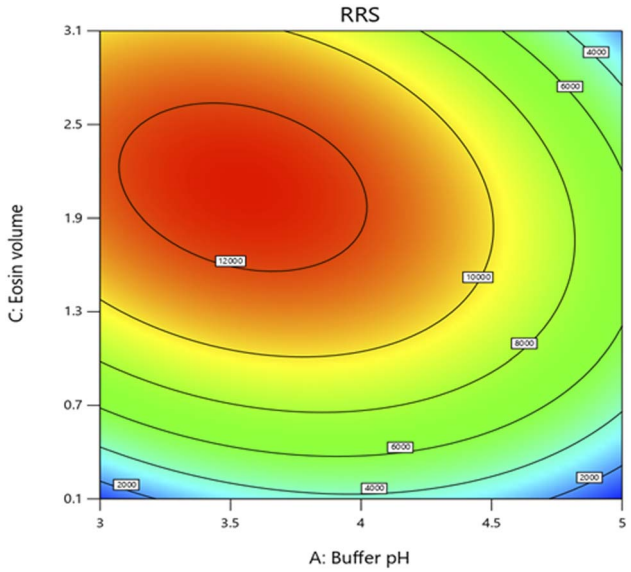

Fig. 3 Box–Behnken design results *via* contour plot.

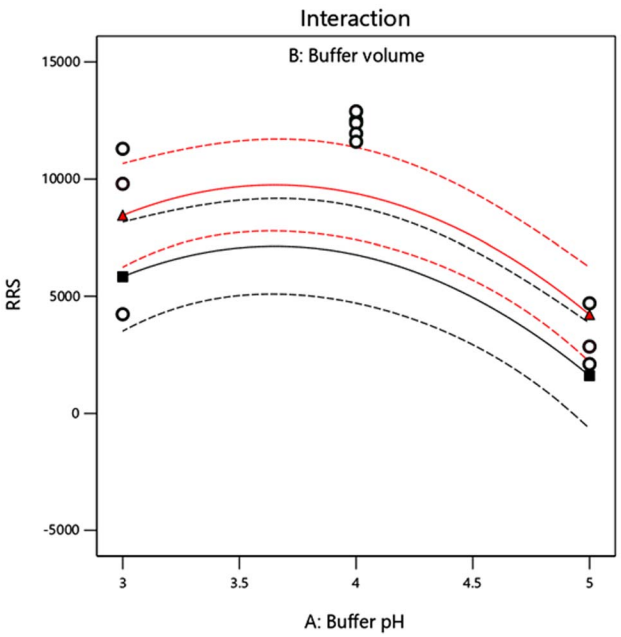

Fig. 5 Box–Behnken design *via* interaction plot.

The design was exploited for the optimization of pH of acetate buffer solution, the volume of the buffer and the volume of eosin Y solution. The chosen levels of the evaluated variables are described in Table 1. The number of runs were 15 and they were augmented by additional five runs with central points. The quadratic fit was found to get the optimal response. Analysis of Variance (ANOVA) was used to confirm all proposed models with 95.0% confidence limits. In models with quadratic terms, ANOVA was run first, and then insignificant variables were eliminated backwards (α to remove = 0.5). This resulted in the creation of a "basic" model that only included the significant variables and the terms required to keep the model harmonious. The following equation summarized the response surface derived using Box–Behnken design which is displayed in Fig. 3–5 by contour plot, 3D response surface plot and interaction plot, respectively.

$$\begin{aligned}\mathrm{RRS} = {} & 11700.77 - 2118.85 \times A + 1307.68 \times B \\ & + 2671.98 \times C - 1922.86 \times AC - 3048.98 \times A^2 \\ & - 3624.45 \times B^2 - 5323.77 \times C^2\end{aligned}$$

where A, B and C are pH, volume of acetate buffer and eosin Y volume, respectively.

ANOVA for quadratic models proved the validity of the model through the obtained statistical data. The lack of fit F-value was 8.43 indicating that there was a 5.31% chance of lack of fit which could be due to noise and non-significant. The adjusted and predicted R^2 values were >0.8 and the difference between them was less than 0.2 and the adequate precision values was greater than 4 confirming the validity of the models (Table 2).

To determine the optimum factors, the numerical optimization was performed and individual desirability was studied. The target was to maximize the RRS response. The best factorial blend was shown by a desirability value that is ~1.0000 that was achieved using pH of 3.5, volume of buffer 0.8 mL and volume of eosin Y 2.1 mL (Fig. 6) and the highest response was practically confirmed.

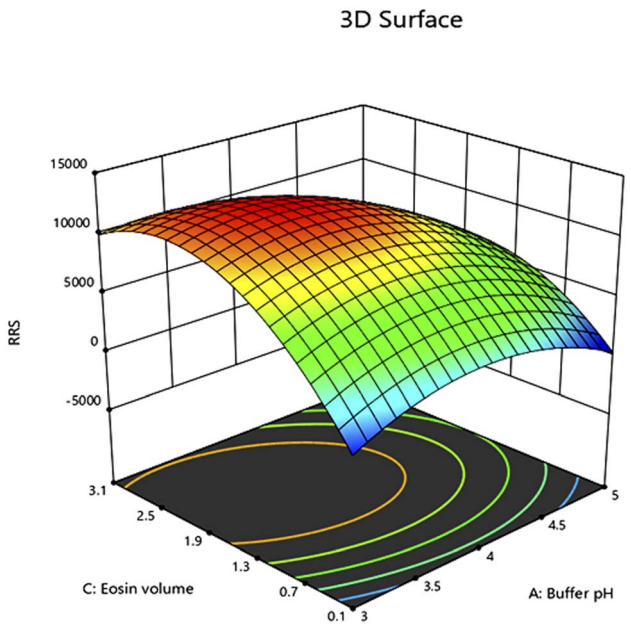

Fig. 4 Box–Behnken design results *via* 3D response surface plot.

3.2. Causes for RRS augmentation

3.2.1. RRS enhancing effect. During RRS phenomena, absorption-re-scattering occurs due to the resonance between the Rayleigh scattering process and the light absorption at the same frequency if both Rayleigh scattering and absorption band are located at the same wavelength. Thus, visual inspection could identify a close relation between RRS and absorption

Table 2 The statistical parameters of the suggested Box–Behnken model for the suggested RRS method

Source	Sum of squares	df	Mean square	*F*-Value	*p*-Value	
Block	9.830×10^6	1	9.830×10^6			
Model	4.341×10^8	7	6.201×10^7	27.84	<0.0001	Significant
A-Buffer pH	4.694×10^7	1	4.694×10^7	21.07	0.0006	
B-Buffer volume	1.447×10^7	1	1.447×10^7	6.50	0.0255	
C-Eosin volume	6.322×10^7	1	6.322×10^7	28.39	0.0002	
AC	1.659×10^7	1	1.659×10^7	7.45	0.0183	
A^2	4.756×10^7	1	4.756×10^7	21.35	0.0006	
B^2	5.647×10^7	1	5.647×10^7	25.35	0.0003	
C^2	1.341×10^8	1	1.341×10^8	60.21	<0.0001	
Residual	2.673×10^7	12	2.227×10^6			
Lack of fit	2.571×10^7	9	2.857×10^6	8.43	0.0531	Not significant
Pure error	1.017×10^6	3	3.389×10^5			
Cor total	4.707×10^8	20				

R^2	0.9420
Adjusted R^2	0.9082
Predicted R^2	0.8024
Adeq. precision	14.1588

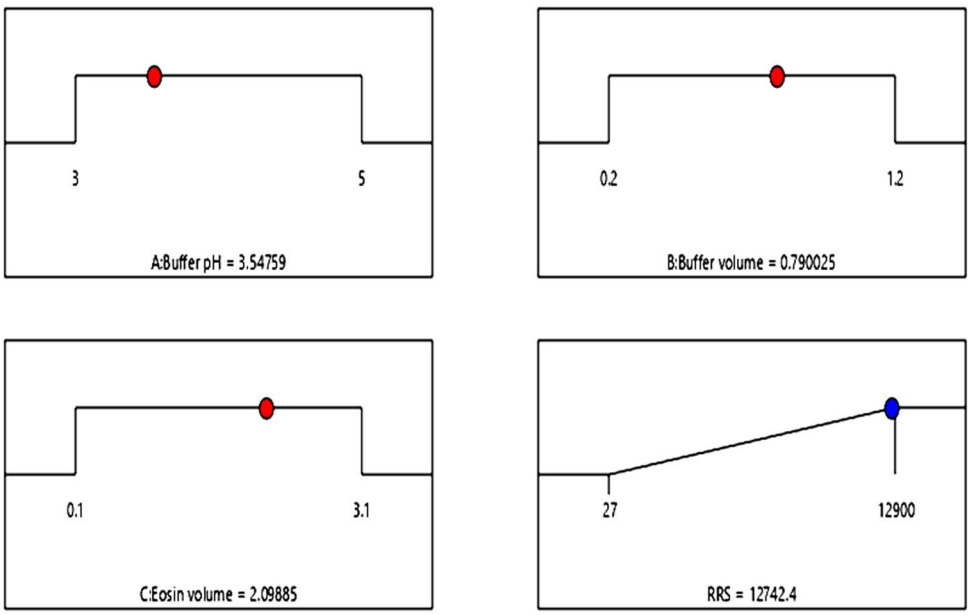

Fig. 6 The ramp view numerical optimization for solutions. Red points show optimal factor settings while blue points show optimal response prediction values.

spectra. Nearly the same bands in the RRS spectrum of the eosin Y–PCP complex could be clearly observed in the absorption spectrum. This confirmed the enhancement effect of the absorption in the resonance scattering.

3.2.2. Increasing volume of the molecule. It was previously reported that increasing the volume of the scattering molecule is beneficial for the augmentation of RRS intensity.[29] PCP carries a positive charge at pH 3.5 buffer solution, while eosin Y will be in the mono anion form and negatively charged. Thus, it feasible for PCP molecules to bind to the eosin Y through the electrostatic attraction, resulting in the formation of combined complex with increased molecular volume. It should be noted that, the individual spectrum of either of eosin Y and PCP showed very low RRS intensity. Upon combining of the two reactants producing binary complex, a great augmentation in the RRS intensity was observed (Fig. 2). Consequently, increasing the volume of the molecule could be considered one of the important reasons for the RRS augmentation.

3.2.3. Formation of a hydrophobic interface. Upon the interaction of the dye with the drug a hydrophobic interface is formed which could be directly linked to augmentation of the RRS signal.[30] If the two reactants (dye and drug) are in separated

Table 3 The statistical parameters of the suggested RRS method for the determination of PCP succinate

Parameter	Value
Linear range (ng mL^{-1})	150–2000
Slope	5.368
Standard deviation of slope (S_b)	0.0638
Intercept	8000
Standard deviation of the intercept (S_a)	5.568
Correlation coefficient*	0.9995
Standard deviation of residuals ($S_{y,x}$)	124.77
Limit of detection (LOD, ng mL^{-1})	37.916
Limit of quantitation (LOQ, ng mL^{-1})	126.388

solution at pH 3.5, both would be in the ionized form. PCP would be in the cationic form while eosin Y dye is in the mono anionic form. Thus, the two compounds exist as water soluble ions. Hence, they could be converted to the hydrated forms which possess very low RRS intensity. If the solution of the drug and the dye are brought in contact, the combined complex (neutral form) could be produced leading to the generation of a hydrophobic interface between the complex and the surrounding aqueous solution. The formed hydrophobic interface is because of the existence of the hydrophobic aryl moiety and the neutralization of the two opposite charges of the components in the combined complex.

3.3. Validation of the proposed method

After optimization of the experimental parameters, the suggested approach was validated according to the guidelines of the International Council for Harmonization[31] regarding linearity, range, precision, accuracy, robustness, limits of detection and quantitation.

3.3.1. Linearity and range. The RRS intensity of the association complex between PCP and eosin Y measured at 365 nm was directly related to the concentration of PCP in the concentration range of 150–2000 ng mL^{-1}. The calibration curve for the RRS intensity–concentration plot was built and excellent correlation coefficient value was obtained ($r = 0.9995$). The data of the calibration curve underwent linear regression analysis with calculation of the statistical parameters (Table 3).

3.3.2. Detection and quantitation limits. According to ICH, the limits of detection (LOD) and quantitation (LOQ) were calculated to assess the sensitivity of the developed RRS approach. The following equations were applied.

Table 4 Evaluation of the accuracy of the suggested RRS method for determination of PCP succinate

Taken concentration (ng mL^{-1})	% Recovery[a]	SD	% RSD
400	98.85	1.69	1.77
700	102.73	1.41	1.37
1000	100.60	1.61	1.60
1200	98.53	0.72	0.73
2000	100.68	0.89	0.88

[a] The value is a mean of three determinations.

Table 5 Intraday and interday precision of the suggested RRS method for the determination of PCP succinate

Concentration level (ng mL^{-1})	% recovery[a] ± RSD	
	Intra-day precision	Inter-day precision
700	99.54 ± 0.96	100.96 ± 1.61
1000	98.74 ± 1.89	98.68 ± 1.04
1200	102.58 ± 1.69	101.87 ± 1.90

[a] The value is a mean of three determinations.

Table 6 Robustness of the suggested RRS method by trying minute variations in method parameters

Optimization factor	Value	% recovery [a]	SD	% RSD
pH of acetate buffer	3.3	95.13	0.95	1.00
	3.5	98.65	0.57	0.58
	3.7	99.65	1.16	1.16
Volume of acetate buffer	0.7	95.24	0.84	0.88
	0.8	97.44	1.11	1.14
	0.9	96.98	1.74	1.79
Volume of eosin Y	2.0	97.85	0.55	0.56
	2.1	101.89	1.14	1.12
	2.2	103.84	1.53	1.47

[a] the value is a mean of three determinations.

$$\mathrm{LOD} = 3.3\sigma/S \text{ and } \mathrm{LOQ} = 10\sigma/S$$

where S is the slope and σ is the standard deviation of intercept. The method showed high sensitivity for PCP determination as the calculated LOD and LOQ values were 38 and 125 ng mL^{-1}, respectively.

3.3.3. Accuracy and precision. Testing the accuracy of the suggested method was carried out through analysing three replicates of five chosen concentrations of standard PCP solutions (400, 700, 1000, 1200, 2000 ng mL^{-1}). Excellent percentage recoveries (98.53–102.73%) were obtained which revealed the excellent accuracy of the method (Table 4).

Precision of the suggested method was validated by testing intraday and inter-day precision where the relative standard deviation was calculated in both cases. Three replicate determinations at three levels of concentration (700, 1000 and 1200 ng mL^{-1}) were done using the assay procedure within the same day and in three successive days for intraday and interday precision levels, respectively. The resulting values of % RSD (0.96–1.90) were lower than 2% proving the suggested method's high precision (Table 5).

3.3.4. Robustness. The method was proved robust by changing some parameters by minute values and testing their effect on the obtained results. The tested parameters were the volume, pH of acetate buffer, and the volume of eosin Y. These changes had no significant effect on the results of the suggested method. Good % recoveries (95.13–103.84%) with low % RSD below 2% were produced proving the high robustness of the method (Table 6).

Table 7 Application of the suggested approach on Prucasoft® 2 mg commercial tablets in comparison with a previously reported spectrophotometric method

Method	Proposed method	Reported method
% recovery	100.10	96.58
	101.39	101.44
	98.47	97.84
	98.64	97.12
	100.43	99.24
Mean recovery	**99.73**	**98.44**
SD	1.42	1.95
RSD	1.42	1.98
t-Value[a]	1.316	
F-Value[a]	2.483	

[a] Tabulated values at 95% confidence limit are $t = 2.306$, $F = 6.338$.

Table 8 Application of the suggested method on urine samples spiked with PCP succinate ant three concentration levels

Concentration level (ng mL^{-1})	% recovery[a] ± RSD
600	98.69 ± 1.81
800	98.47 ± 1.72
1000	96.07 ± 0.44

[a] The value is the mean of three determinations.

3.4. Application of the method

3.4.1. Application to pharmaceutical commercial tablets. The suggested approach was applied on Prucasoft® tablets and the extracted solutions from tablets were analysed according to the general procedure in five replicates. Good % recoveries were obtained. The tablet solutions were also analysed by a reported spectrophotometric method.[10] The student's *t*-test and *F* test were performed in order to compare the two methods. The estimated *t*- and *F* values were lower than the tabulated. Consequently, there was no significant difference between the suggested approach and the reported one (Table 7) in respect to accuracy and precision. So, quality control units in pharmaceutical factories could make use of the suggested approach.

3.4.2. Application to spiked urine samples. The method was utilized for the analysis of urine samples spiked with PCP solutions at three concentrations (600, 800 and 1000 ng mL^{-1}). Excellent % recoveries with % RSD lower than 2% were obtained that confirmed the high accuracy, precision, and selectivity of the suggested RRS method (Table 8).

3.5. Assessment of the sustainability and greenness of the method

3.5.1. Eco score scale method. To assess the greenness level of the suggested procedure, eco score scale method[32] was utilized. In this approach, a penalty point was calculated for each aspect of the analysis such as the utilized reagents, solvent, experimental conditions such as pH and time, energy consumption and the amount of the formed wastes. Each of these parameters was assigned a value which corresponds to its level of toxicity. Null or low value indicates full or high agreement with green chemistry principals. To get the total number of penalty points, the individual values were summed. Then total number was subtracted from 100 (optimum green Eco score) to get the Eco Score of the tested method. As presented in Table 9, the present method was assigned a value of 8 as the total penalty points, therefore, the Eco score of the current methodology was 92. This confirmed the high level of greenness of the proposed RRS method.

Table 9 The greenness evaluation of the present method through penalty points calculation

Item	Parameter	Word sign	PP score
Instrument	Spectrofluorimeter	LSH[a]	1
Reagent (s)	Erythrosine B	LSH[a]	1
Amount of reagent	>10 mL		1
Solvent (s)	Water (for dilution)	LSH[a]	1
Heating	No heating		0
Temperature	Ambient temperature		0
pH	3.5		1
Cooling	No cooling		0
Energy (kW h per sample)	>1.0		0
Waste	1–10 mL		3
Occupational hazards (TPPs)[a]			0
Eco-scale total score = 100 − TPP			8
			92

[a] LSH for the less severe hazard, and TPPs for the total penalty points.

3.5.2. The analytical greenness metric (AGREE). Pena-Pereira *et al.* generated a free and downloadable program in 2020 for greenness evaluation called "AGREE".[33] The program could evaluate the twelve green analytical chemistry (GAC) principles and assign both a number (from 0 to 1) and a color for each principle which demonstrates its degree of greenness. Three color codes were used in this tool including red, green, and yellow with different color level. The output of the AGREE program is in the form of pictogram composing of 12 circular sections, in addition a central part. The total green impact of the method could be taken from both the color and the number presented in the central part. The best method will show the green color in the center of AGREE pictogram. The AGREE algorithm was used to evaluate the suggested method. The results shown in Fig. 7 indicated the acceptable greenness of the proposed method as the number in the central part of the pictogram is 0.76.

3.5.3. Red, green blue 12 metric. In 2021, Nowak *et al.*, published a new green approach called, Red Green Blue 12 algorithm (RGB12). The output of this tool composed of three sections. Each section assigned a unique color and corresponded for the assessment of certain characters of the analytical procedure of the investigated method. Red section represents the analytical validity of the suggested procedure

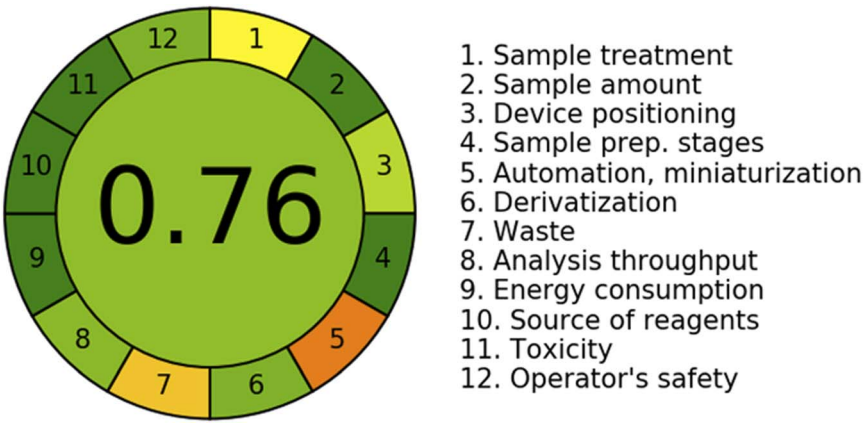

Fig. 7 The pictogram of AGREE for evaluation of the greenness of the suggested RRS method.

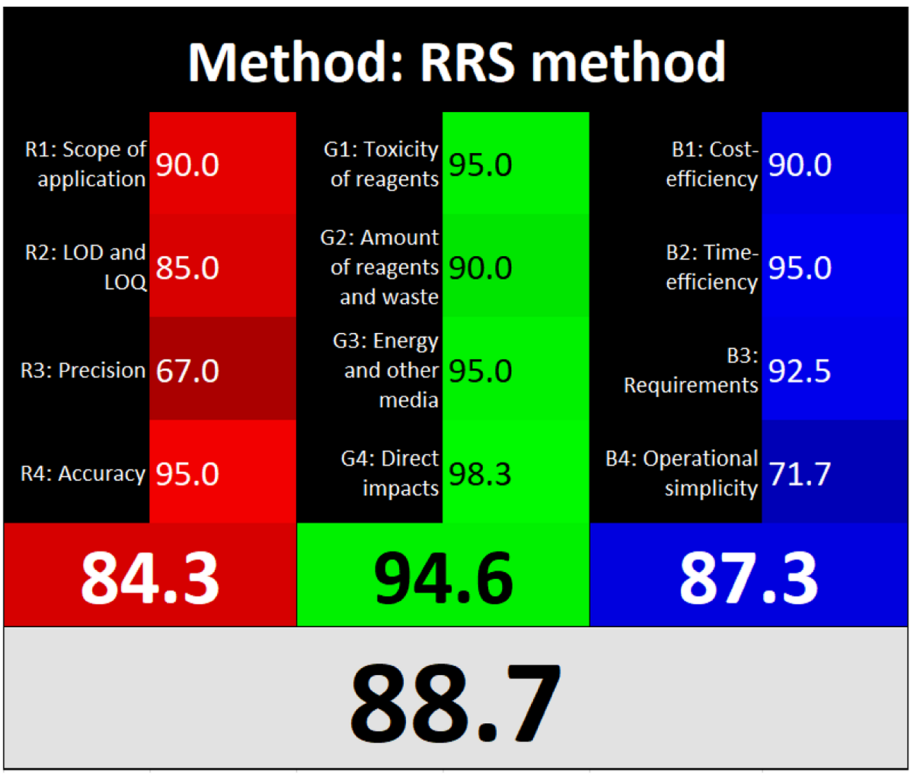

Fig. 8 RGB12 whiteness assessment evaluated by using the analytical RGB 12 algorithm.

including precision, accuracy, limits of detection, and quantification, in addition to its analytical applicability. While the green section represents the fitness of the method to 12 GAC principles. The blue section is related to the productivity of the method. This third section represents the cost effectiveness, time saving, limited practical requirements, and ease of procedure. Finally, the whiteness is the fourth section in this tool and reflects the degree of sustainability based on the 12 white analytical chemistry (WAC) principles. The method may be considered "white" according to the WAC approach considers if the analytical procedure is balanced and suitable for the intended analytical use. A free Excel spreadsheet is available for generating RGB 12 output and its use is simple and quick. Fig. 8 illustrates the output of RGB 12 for the evaluation of the suggested RRS method which proves its sustainability.

4. Conclusion

The suggested method presented a highly sensitive, green, rapid and simple method for the determination of PCP based on RRS measurements. The method had higher sensitivity than spectrophotometric methods. It is also simpler and greener compared with chromatographic methods that employ expensive and hazardous organic solvents and require sophisticated

instruments. Furthermore, the method was successfully applied on pharmaceutical tablets and with no errors from the added excipients proving the method's high selectivity and allowing its utilization in quality control laboratories. Moreover, spiked urine samples were analyzed successfully using the suggested approach with no significant interferences from the surrounding matrix. In addition, three green analytical chemistry metrics were employed to ensure the sustainability of the proposed method.

Conflicts of interest

There are no conflicts to declare.

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