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THREE-WAY DATA ANALYSIS OF COPPER-PARACETAMOL COMPLEX FORMATION FOR THE QUANTIFICATION OF PARACETAMOL IN PHARMACEUTICAL MATRICES

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ABSTRACT. We present a chemometric strategy to study complex formation reactions using parallel factor analysis (PARAFAC) of three-way spectral data.UV spectra of PAR, $CuSO_4$, and their complex were recorded at five different pH levels. Reorganizing the spectra produced a wavelength × sample × pH array, to be decomposed by PARAFAC into spectral, pH, and concentration modes. This strategy achieved the resolution of contributing species and their profiles without requiring advanced instrumentation. PAR quantification was achieved using the relative concentration mode of the PARAFAC model. The calibration curve showed linearity in the range of $1.10x10^{-5}$ M-$8.90x10^{-5}$M, and standard-addition validation yielded recoveries of 96.70-99.00% for tablets and 96.48-99.67% for syrups. Crucially, this strategy achieved accurate quantification of PAR in syrup formulation even with uncalibrated interferences in syrup matrix. The application of PARAFAC to the pH-dependent UV dataset acquired according to Job's method revealed a 1:1 stoichiometric ratio between PAR and Cu^{2+}. Overall, the results demonstrate that three-way decomposition of pH-resolved UV measurements offers a practical and reliable alternative for studying Cu-PAR complex formation and quantifying PAR in different pharmaceutical matrices. To the best of our knowledge, this is the first PARAFAC application to study PAR-Cu complex formation for accurate quantification of PAR in the presence of uncalibrated interferences.

Keywords: *chemometrics, paracetamol, spectral analysis, ultraviolet spectrophotometry*

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INTRODUCTION

Paracetamol (PAR), chemically named N-acetyl-p-aminophenol, consists of a phenol ring substituted with an amide group at the para position, forming a conjugated system (Figure 1). It is widely recognized as an effective analgesic and antipyretic for both adults and children. PAR is the principal active pharmaceutical ingredient in numerous over-the-counter and prescription medications, either alone or in combination with other active substances [1]. Although paracetamol is generally safe at therapeutic doses, intentional or accidental overdoses can lead to severe hepatotoxicity and other adverse effects. Therefore, its accurate determination in pharmaceutical formulations for quality control and in biological fluids for overdose monitoring is of critical importance [2].

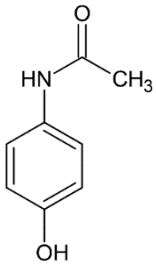

Figure 1. Molecular structure of paracetamol

There is an increasing interest in metal-drug complexes due to their potential biological, pharmaceutical, and catalytic applications. Several studies have reported the ability of PAR to form complexes with metal ions through coordination involving the hydroxyl and amide functional groups [3-6]. It has been shown that PAR exhibits affinity for transition metals via the lone pairs on the carbonyl oxygen, hydroxyl oxygen, or amide nitrogen atoms [7]. Recently, Al-Abbasi and co-workers characterized a Cu-PAR complex using potentiometric, conductometric, spectrophotometric, and computational techniques [8, 9]. In the present study, we aim to investigate the spectroscopic characteristics of the Cu-PAR complex using a chemometric approach and to exploit three-way data analysis for the quantification of PAR in different pharmaceutical formulations.

Chromatographic techniques such as high-performance liquid chromatography (HPLC) have been extensively employed for the analysis of complex samples across a wide range of disciplines including analytical chemistry, pharmaceuticals, medicine, food, agriculture, and environmental sciences. Numerous studies have reported the determination of paracetamol

using HPLC [10-13] and high-performance thin-layer chromatography (HPTLC) [13-15]. However, chromatographic techniques often require extensive method development for optimal separation, time-consuming optimization processes, dependence on organic solvents, and the use of costly and sophisticated instrumentation.

In contrast, ultraviolet-visible (UV-Vis) spectrophotometry is widely favored due to its simplicity, rapidity, cost-effectiveness, and minimal sample preparation requirements. UV-Vis methods have been commonly used to quantify PAR in relatively simple pharmaceutical matrices [16-20]. However, traditional UV-Vis techniques may not suffice when dealing with complex mixtures containing multiple active and inactive components due to spectral overlap. Thus, alternative analytical strategies are needed to address these challenges in a cost-effective and efficient manner.

To overcome the limitations of classical analytical methods, multi-way data analysis offers a promising alternative for identifying spectral features, monitoring reaction kinetics, and evaluating complex formation between drugs and metal ions. In chemometrics, multi-way analysis techniques have gained considerable attraction for addressing complex analytical challenges. Spectrophotometric measurements inherently generate multidimensional data without the need for sophisticated or expensive instrumentation. The spectral characteristics of metal complexes vary depending on analyte concentration and solution pH. These data can be structured as three-way arrays, encompassing wavelength, pH, and concentration dimensions, making them ideally suited for multi-way analysis. Integrating such methods with studies of metal-drug interactions can provide valuable insights into system behavior, chelation mechanisms, and ligand specificity, with potential implications for chelation therapy and drug development [21].

Among multi-way techniques, parallel factor analysis (PARAFAC) has emerged as a powerful tool for resolving overlapping signals and extracting meaningful information from complex datasets. The theoretical framework of PARAFAC has been well-documented [22-24] and its applications in spectroscopic data analysis include multicomponent quantification [25-29], spectral characterization [29-34], and kinetics, [35-38] and complexation studies [39].

In this study, we developed a novel analytical strategy based on three-way UV spectral data analysis to investigate Cu-PAR complex formation and to quantify PAR in commercial tablet and syrup formulations. Calibration, validation, and commercial sample solutions containing varying concentrations of PAR and $CuSO_4$ at different pH levels were prepared. Cu^{2+} precipitation as $Cu(OH)_2$ in alkaline conditions was avoided by using a methanol-aqueous buffer mixture. Under these co-solvent conditions, all solutions remained

clear up to pH 10, with no turbidity. Metal-ligand complexation was facilitated via sonication followed by vortex mixing. The resulting UV absorbance spectra were assembled into a three-way data array and decomposed using PARAFAC to resolve the individual spectral, concentration, and pH profiles of PAR, $CuSO_4$, and the Cu-PAR complex. The validity of the proposed strategy was confirmed using independent validation samples, yielding satisfactory recovery results. Application of the PARAFAC model to real pharmaceutical samples demonstrated accurate and reliable quantification of PAR in both tablet and syrup formulations.

RESULTS AND DISCUSSION

The proposed spectrophotometric method for the determination of paracetamol is based on its complexation with copper(II), followed by spectral three-way analysis, without the need for extraction or chromatographic separation. The application of the PARAFAC model as a three-way data analysis tool to the pH-dependent absorbance dataset provides an effective strategy for resolving spectral interferences. This approach enables accurate quantification of paracetamol in pharmaceutical formulations, offering a low-cost, rapid, and reliable alternative to conventional analytical techniques.

Construction of spectral datasets

The proposed PARAFAC calibration method for monitoring complex formation and quantifying paracetamol (PAR) in pharmaceutical formulations began with the construction of a calibration matrix composed of PAR and $CuSO_4$. As previously described in the Experimental section, the calibration set included 40 different sample solutions, combining five pH conditions with eight concentration levels. The UV spectrum of a solution containing a metal ion, a ligand, and their corresponding coordination complex yields a vector of absorbance values as a function of wavelength. When spectra of the analyte at varying concentrations are recorded, they can be arranged into a two-dimensional matrix. Because complex formation reactions are pH-dependent, acquiring spectra at different pH values enables the generation of a three-way data array (tensor), which enhances the system's modeling capacity. Figure 2 illustrates the pH-dependent UV absorbance matrices for the calibration samples across increasing paracetamol concentrations. These matrices were organized into a three-way calibration dataset with dimensions of 231×8×5 (wavelength × concentration × pH), which served as the input for PARAFAC modeling.

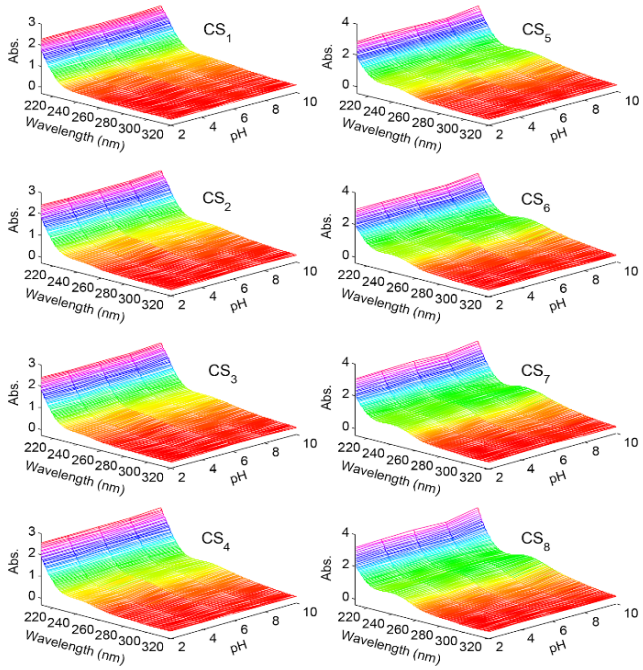

Figure 2. Three-dimensional representation of the pH-dependent UV absorbance matrices of calibration samples prepared at increasing paracetamol concentrations (8 levels) across five pH conditions. The dataset forms the basis of the calibration tensor used in PARAFAC modeling (231×8×5; wavelength×concentration×pH).

In this study, two independent datasets were constructed and analyzed using PARAFAC to quantify PAR in two types of pharmaceutical formulations: tablet and syrup formulations. Unlike tablet excipients, which were not expected to produce observable signals in the UV region, the excipients in the syrup formulations were anticipated to contribute additional spectral components and thus appear as distinct factors in the PARAFAC model. For the tablet analysis, a three-way dataset was constructed using eight calibration samples, four tablet standard addition samples, and five commercial tablet samples, at each pH conditions. These were combined into a data tensor of dimensions 231×17×5 (wavelength × sample × pH). The UV absorbance matrices corresponding to the five tablet samples at different pH conditions are shown in Figure 3a. Similarly, the syrup dataset was composed of the same eight calibration samples, four syrup standard addition samples, and five syrup samples at each pH level. This data was also structured as a tensor with dimensions 231×17×5, as shown in Figure 3b. These tensors enabled the simultaneous decomposition of spectral, pH, and sample modes, facilitating selective quantification of paracetamol even in the presence of complex excipient backgrounds.

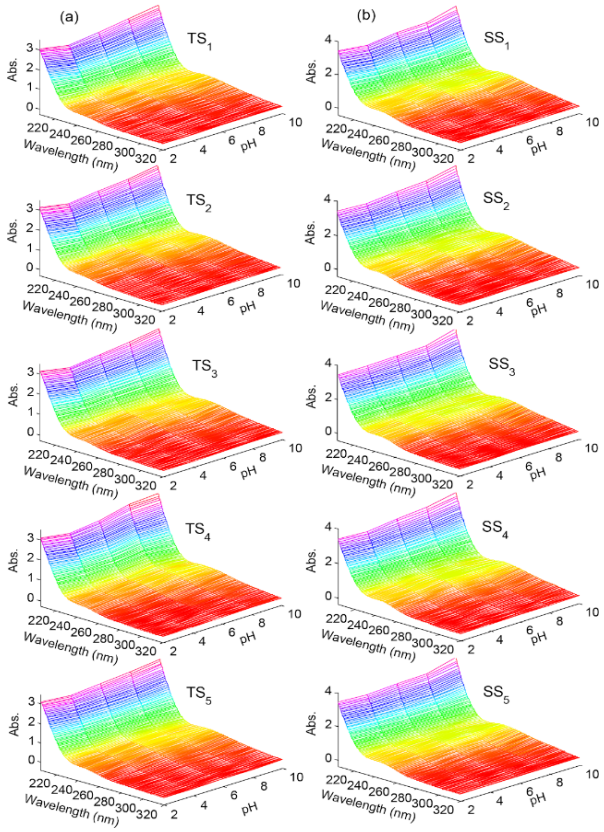

Figure 3. pH-dependent UV absorbance matrices of (a) tablet samples (TS_1-TS_5) and (b) syrup samples (SS_1-SS_5) recorded at five different pH levels. These matrices were used to construct the sample mode of the three-way data tensors for PARAFAC analysis.

Application of PARAFAC model

The three-way dataset constructed from the tablet analysis was subjected to PARAFAC modeling using the alternating least squares (ALS) algorithm. A three-component model was selected based on explained variance and residual analysis, and non-negativity constraints were applied to all modes to ensure chemical interpretability. The decomposition of the tensor yielded three distinct profiles:

- the relative spectral profile across the wavelength dimension (Figure 4a),
- the relative pH distribution profile (Figure 4b), and
- the relative concentration profile across the sample dimension (Figure 4c).

These profiles corresponded to three chemical species: paracetamol (PAR), $CuSO_4$, and the Cu-PAR complex. As shown in Figure 4a, the spectral

profile of PAR estimated by the model closely aligned with the experimental spectrum of pure PAR (represented by a dashed line), confirming the validity of the model. In Figure 4b, the Cu-PAR complex signal was found to intensify with increasing pH, indicating that complex formation is favored under alkaline conditions. The relative concentrations of the three species across samples, shown in Figure 4c, were used for the quantitative determination of PAR in tablet samples.

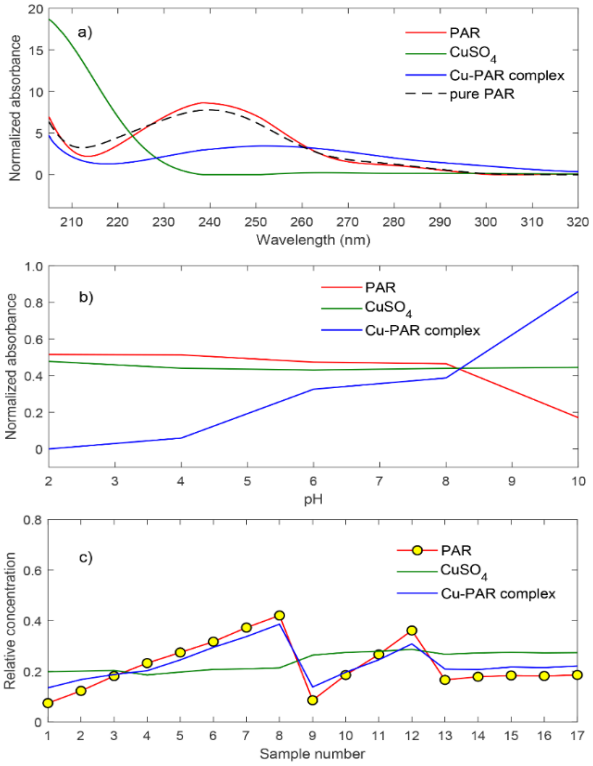

Figure 4. PARAFAC model results (three-component solution) for the tablet dataset: (a) Relative spectral profiles of PAR, $CuSO_4$, and Cu-PAR complex across the 205-320 nm range; the dashed line shows the experimental spectrum of pure PAR for comparison. (b) pH-dependent absorbance contributions of each component (pH 2-10) (c) Relative concentration profiles of the three species across 17 samples (8 calibration, 4 standard addition, and 5 tablet samples).

In contrast, the three-way dataset obtained from the syrup analysis required a four-component PARAFAC model to adequately explain the variance and capture additional sample complexity. Non-negativity constraints were also

applied. Figure 5a-5c illustrate the decomposed profiles from this four-component model. In addition to PAR, $CuSO_4$, and the Cu-PAR complex, a fourth profile was extracted corresponding to syrup excipients, which was not observed in the tablet dataset.

This additional component is a clear manifestation of the second-order advantage of the PARAFAC model, enabling the isolation and resolution of uncalibrated interferents without prior standardization. The presence of this excipient profile did not hinder the accurate resolution of PAR, demonstrating the model's robustness and selectivity. Figure 5c shows the relative concentration profiles of all four species across the syrup samples, from which the PAR concentrations were quantitatively predicted.

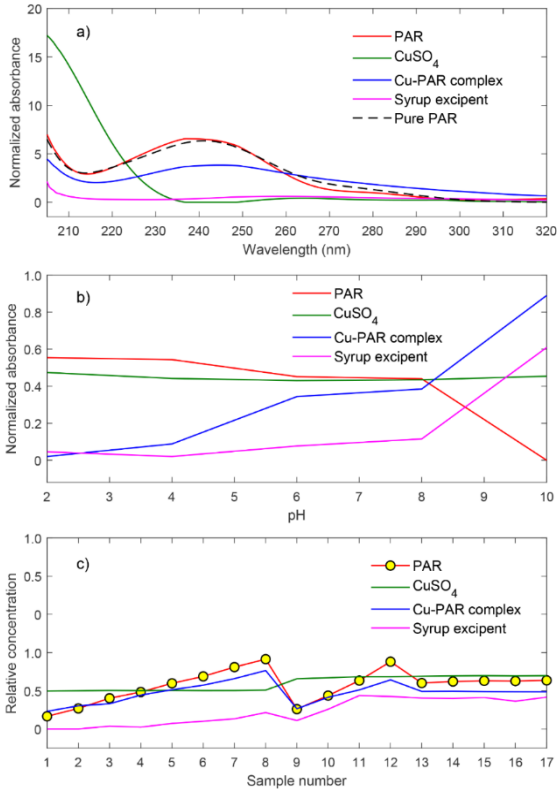

Figure 5. PARAFAC model results (four-component solution) for the syrup dataset: (a) Estimated spectral profiles of PAR, $CuSO_4$, Cu-PAR complex, and syrup excipient. (b) Corresponding pH profiles showing signal variation across (pH 2-10) (c) Relative concentration profiles of the four species across 17 samples (8 calibration, 4 standard addition, and 5 syrup samples). The fourth component, attributed to syrup excipients, illustrates the model's ability to resolve interfering matrix effects.

For both datasets, alternating least squares (ALS) algorithm was used for PARAFAC modeling applying non-negativity constraints. While unconstrained models can sometimes reveal deeper insights into mathematical interactions, UV absorbance and concentration values of chemical species in the sample are inherently non-negative physical quantities, meaning they are impossible to have a negative value in the physical world. Hence, applying non-negativity constraints ensured physically realistic and chemically interpretable PARAFAC solutions.

To ensure solution uniqueness and avoid convergence to local minima, the PARAFAC models were initialized multiple times using different random starting points. Specifically, the tablet dataset was subjected to 172 independent initializations, while the more complex syrup dataset required 1420 initializations to ensure stable convergence. The presence of rotational ambiguity or degeneracy was assessed by evaluating the chemical interpretability of the resolved profiles. In particular, the spectral profile of paracetamol (PAR) resolved by the model (solid red line) showed close agreement with the experimentally recorded spectrum of pure PAR (dashed line). If significant rotational ambiguity were present, such precise spectral recovery would be highly unlikely.

Model parameters were optimized by systematically testing different numbers of components (two to six) guided by chemical knowledge of the system and evaluating the explained variance and residual structure. Although core consistency diagnostics are widely used for component validation, they were not relied upon in this study because matrix heterogeneity and complexation-induced non-idealities may lead to misleading core consistency values despite chemically valid solutions. The final number of components was selected based on the best compromise between model simplicity, variance explained, and chemically interpretable profiles.

For the tablet dataset, three components corresponding to paracetamol (PAR), $CuSO_4$, and the Cu-PAR complex were considered chemically meaningful. Models with three or more components were evaluated, and increasing the number of components beyond three did not yield interpretable or chemically relevant profiles. The explained variance reached 99.8788% for the tablet dataset using a three-component model.

For the syrup dataset, which exhibits higher matrix complexity due to excipients, a four-component model was required, achieving an explained variance of 99.9300%. In addition to matrix effects, the metal–drug complexation reaction itself further increased system complexity by introducing strongly pH-dependent and overlapping spectral contributions, which can adversely affect conventional diagnostic metrics.

PARAFAC model enabled the resolution of three chemical species (PAR, $CuSO_4$, and Cu-PAR complex) in both datasets. Unlike the tablet dataset, the syrup dataset required an additional component attributed to syrup

excipients, indicating the presence of uncalibrated interferences. Despite this added complexity, the model successfully separated the contributions of all components, allowing reliable interpretation of the identified chemical species. The validity of the resolution was confirmed by the close agreement between the spectral profile of PAR obtained by PARAFAC and the experimentally recorded spectrum of pure PAR, as shown in Figures 4 and 5.

Calibration and Quantitative Analysis Based on PARAFAC Profiles

Quantitative calibration and prediction of paracetamol (PAR) were performed using the relative concentration values obtained from the PARAFAC model. Specifically, the PAR signal extracted from the sample mode (Figure 4c for tablets, Figure 5c for syrup) was used as the dependent variable in a least-squares regression against the nominal concentrations of PAR in the calibration set (first 8 samples).

To evaluate the validity of the proposed method, the linearity of the calibration model was assessed. A highly linear regression equation was obtained with a correlation coefficient (R) of 0.9994, confirming the strong agreement between the actual and estimated values. The independent variable was the known PAR concentrations ranging from 1.67 µg/mL to 13.44 µg/mL, and the dependent variable was the corresponding PARAFAC-derived relative concentration values.

The calibration parameters, including the slope, intercept, and correlation coefficient of the regression equation, along with their associated standard errors, are summarized in Table 1. The computed values of limit of detection (LOD) and limit of quantitation (LOQ) were also given in the same table. They were calculated by the equations $LOD = (3 \times SD)/m$ and $LOD = (10 \times SD)/m$, where SD represents the standard deviation of the intercept and m is the slope of the calibration curve.

These results validate the robustness and accuracy of the PARAFAC-assisted quantification strategy and confirm its suitability for routine application in pharmaceutical analysis.

Table 1. Linear regression analysis of tablet and syrup formulation

Parameter	Tablet	Syrup
slope	0.0291	0.0252
intercept	0.0305	0.0258
correlation coefficient	0.9994	0.9992
standard deviation of slope	4.11×10^{-4}	4.13×10^{-4}
standard deviation of intercept	3.49×10^{-3}	3.50×10^{-3}
standard deviation of correlation coefficient	5.76×10^{-3}	6.68×10^{-3}
limit of detection (µg/mL)	0.36	0.42
limit of quantitation (µg/mL)	1.20	1.39

Calibration and prediction of the analyte were based on the relative concentration values of PAR in the corresponding profile after PARAFAC decomposition. A least-squares regression was performed with the estimated relative concentration values and the nominal concentration values from the calibration set (first 8 samples in Figure 4c and 5c).

Analysis of Standard Addition Samples

In the next step of validation, the PARAFAC model was applied to the analysis of standard addition samples in order to evaluate the effect of excipients from both tablet and syrup formulations on the accuracy of drug quantification. As detailed in the Experimental section, standard addition samples were prepared at three concentration levels by spiking known amounts of paracetamol (3.49 µg/mL, 6.99 µg/mL, and 10.48 µg/mL) into fixed volumes of tablet or syrup samples, containing approximately 1.68 and 2.67 µg/mL of paracetamol for tablet and syrup, respectively.

In this validation approach, the prediction of total paracetamol concentration in standard addition samples (samples 9-13 in Figure 4c and 5c) was carried out using the relative concentration values obtained from PARAFAC decomposition and the calibration equations derived in Table 1. The added recovery values were calculated by subtracting the estimated PAR concentration of the non-spiked commercial sample (sample 9 in Figure 4c and 5c) from the total PAR concentration of the spiked samples at the three respective levels (samples 10-12 in Figure 4c and 5c). The results of these analyses are summarized in Table 2.

Table 2. Analysis results and recovery values of standard addition samples

PFT*	Spiked sample no	Added (µg/mL)	Found (µg/mL)	Added recovery (%)
tablet	1	3.49	3.43	98.3
	2	6.99	6.92	99.0
	3	10.48	10.13	96.7
syrup	1	3.49	3.48	99.67
	2	6.99	6.95	99.50
	3	10.48	10.11	96.48

*PFT: Pharmaceutical form type

The data in Table 2 show that excipients in both tablet and syrup formulations did not affect the accuracy of paracetamol determination. Although the tablet formulation did not exhibit significant spectral interference, the syrup formulation presented a noticeable interference signal due to the presence of excipients. Nevertheless, the PARAFAC model successfully resolved the contributions of all components, including the interfering excipient illustrated as the pink line in Figure 7, across the spectral, pH, and concentration dimensions. As the contribution of syrup excipients was extracted efficiently

by PARAFAC, the relative concentration profile of PAR in Figure 7 was free from interferences. This ability to correctly quantify analytes in the presence of uncalibrated and unknown interfering species is a characteristic of the second-order advantage. It allows for accurate, selective estimation of the analyte without requiring any preliminary separation or extraction steps.

Furthermore, the added recovery values for all standard addition levels in both formulations were found to be close to 100%, confirming the selectivity and reliability of the developed method. The strong agreement between predicted and actual concentrations, further validates the robustness of the method. These results demonstrate that the proposed PARAFAC-based spectrophotometric strategy is highly effective for the analysis of paracetamol in complex pharmaceutical matrices.

Quantitative Estimation of Paracetamol in Commercial Samples

The proposed PARAFAC-based spectrophotometric methods were applied to the determination of paracetamol (PAR) in commercial tablet and syrup formulations to evaluate their practical applicability. Sample preparation procedures for both dosage forms were performed as described in Section 2.3.5 (tablet) and 2.3.6 (syrup). The corresponding pH-dependent UV spectral datasets, recorded using a UV-Vis spectrophotometer, are shown in Figure 3a and Figure 3b.

PARAFAC decomposition of the datasets for both formulations was carried out as previously described in Section 3.2. Application of the PARAFAC Model. The estimated relative concentration values of PAR from the PARAFAC concentration profiles for five commercial samples (samples #13-17 in Figure 4c and 5c, for tablet and syrup, respectively) were used to compute the predicted concentrations using the calibration equations (see Table 1).

The calculated concentrations of paracetamol in the analyzed pharmaceutical products are listed in Table 3, along with the corresponding recovery values, which were calculated based on the label claims of the respective products.

Table 3. Amount of paracetamol for tablet and syrup samples obtained by PARAFAC model

Sample No.	Tablet assay (mg/tablet)	Recovery (%)	Syrup assay (mg/5mL)	Recovery (%)
1	492.8	98.56	127.5	106.25
2	507.8	101.56	127.4	106.17
3	525.2	105.04	128.3	106.92
4	507.8	101.56	126.3	105.25
5	506.2	101.24	128.9	107.42
Average	507.96	101.59	127.68	106.4
Standard deviation	11.5	2.30	1.0	0.82
Relative standard deviation	2.27	2.27	0.77	0.77

The results demonstrated excellent agreement with the expected values. Specifically, the mean recovery was found to be 101.59% for tablets and 106.4% for syrups, with corresponding relative standard deviations (RSD) of 2.27% and 0.77%, respectively (Table 3). Although the mean recovery of commercial syrup samples (106.4%) is slightly above the commonly accepted range for pharmaceutical assays (approximately 95-105%), this deviation may arise from sample preparation and dilution steps (e.g., minor pipetting errors) and/or matrix effects associated with liquid formulations containing excipients. The standard-addition recoveries (96.48-99.67%) confirm that the method is accurate and does not demonstrate a positive bias. These findings confirm the accuracy and precision of the developed method for the routine quantification of paracetamol in commercial formulations, including those containing potentially interfering excipients.

Multi-way analysis techniques such as PARAFAC provide a powerful strategy for investigating metal-drug interactions by enabling the simultaneous resolution of spectral, environmental (e.g., pH), and concentration-dependent variations. In the context of chelation therapy, this capability allows the direct extraction of the spectral signature of the metal-drug complex from overlapping contributions of the free metal ion and ligand, without requiring physical separation. Such information is critical for understanding complex stability, formation conditions, and environmental sensitivity (e.g., pH dependence, which are key factors in evaluating chelation efficiency and selectivity in situ.

In syrup samples, the total contributions of all individual excipients were modelled as a single additional component. On the other hand, no extra component was required for the tablet dataset, indicating that tablet excipients did not contribute significantly to the spectral, pH, or concentration modes. The explained variance reached its maximum with three components for the tablet dataset, while a four-component model was required for the syrup dataset. The presence of fourth component in the syrup dataset demonstrates the increased complexity of the formulation and the necessity of three-way analysis in spectroscopy for selectively resolving the drug and drug-metal complex in such matrices. The successful extraction of this excipient-related profile indicates that future studies can reliably analyze syrup and similar liquid formulations even without prior knowledge of the specific additives in the formulation.

Since the proposed method was shown to resolve the uncalibrated interferences in real samples, it may be extended to different types of pharmaceutical formulations. However, if a matrix component exhibits spectral behavior that is fully collinear with the analyte across all modes, mathematical resolution would not be feasible. Other limitations might occur depending on the formulation. For example, semi-solid formulations such as creams and

ointments would likely require an extraction step to create a clear solution suitable for UV spectrophotometric measurements, as turbidity and light scattering effects cause non-linear behavior that violates the trilinear model assumption. On the other hand, the application of PARAFAC to nasal sprays and eye drops formulations would be similar to application on syrup formulation described in this work. Nevertheless, the presence of excipients with strong and pH-dependent UV absorbance may still hinder accurate component resolution.

Application of the PARAFAC Model to the Job Plot Method

To determine the stoichiometry of Cu-PAR complex, the Job's method of continuous variation was applied. Based on the spectral behavior observed within the pH range of 2-10, it was assumed that only one type of complex species is predominantly formed, making the application of Job's method appropriate under these conditions.

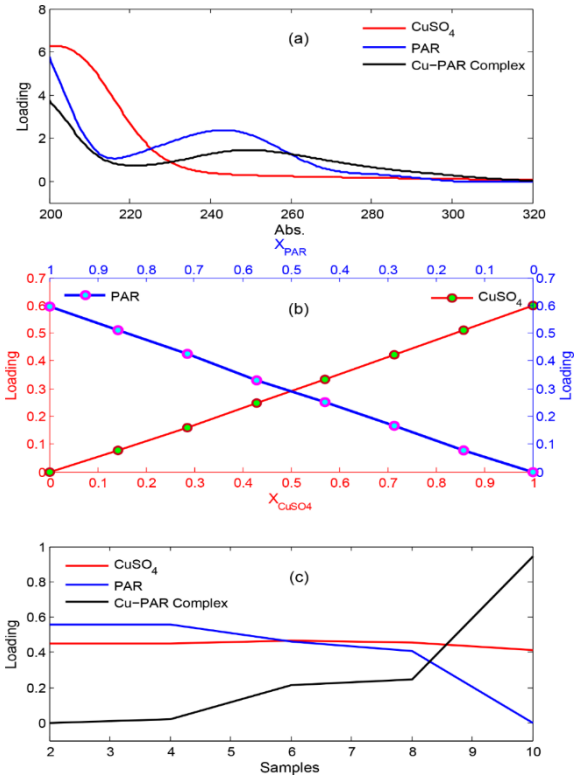

Figure 6. (a) Spectral profile, (b) mole fraction profile, and (c) relative concentration profile obtained by PARAFAC decomposition using Job's plot method

Eight solutions containing Cu(II) and PAR were prepared with a constant total molar amount of 7.0×10^{-4} mmol, but varying molar fractions of the two components. The pH of each solution was adjusted using Britton-Robinson (BR) buffer to fall within the 2-10 range. The UV absorption spectra of the resulting mixtures were recorded over the 200-320 nm range.

The spectral dataset was arranged into a three-way tensor and subjected to PARAFAC decomposition. The resulting component profiles, including the mole fraction contributions, are shown in Figure 6. As illustrated in Figure 6b, the Job plot obtained from the PARAFAC concentration mode exhibits a clear maximum at a molar fraction of 0.5, indicating a 1:1 stoichiometric ratio between PAR and Cu(II) ions as reported in the literature [8, 9].

In addition to the PARAFAC-based interpretation, the Job method was also applied using the classical univariate approach. For this purpose, absorbance values at the characteristic wavelength were recorded for a series of solutions with constant total molar concentration of Cu(II) and PAR but varying molar fractions. The corresponding Job plot is shown in Figure 7, where absorbance is plotted as a function of the molar fraction of paracetamol.

As seen in Figure 7, the plot exhibits a clear maximum at a molar fraction of 0.5, which corresponds to a 1:1 stoichiometric ratio between PAR and Cu(II). This result is fully consistent with the PARAFAC-based Job plot analysis, confirming that one paracetamol molecule coordinates with one copper ion to form the Cu-PAR complex. The agreement between the classical and multiway approaches provides additional confidence in the validity of the complexation model proposed in this study.

The formation of a single dominant complex over pH 2-10 was assumed in this work. The assumption was justified by symmetrical Job's plot and the analysis of the residuals. The Job's plots (both classical and PARAFAC-based) exhibit a clear, symmetric maximum at exactly 0.5 molar fraction. The presence of higher-order species (e.g., 1:2 or 2:1) would typically cause a shift in the peak position or asymmetry in the plot. In addition, the tablet and syrup datasets were adequately described by three- and four-component PARAFAC models, respectively. If significant higher-order species were present, high residuals would be observed, or inclusion of an additional component would be required to model the data. Furthermore, to prevent the formation of hydroxo complexes such as $Cu(OH)_2$, a mixed methanol–aqueous buffer system was employed. Under these conditions, solutions remained optically clear even at pH 10, supporting the negligible presence of insoluble hydroxo species and the assumption of a single dominant Cu-PAR complex.

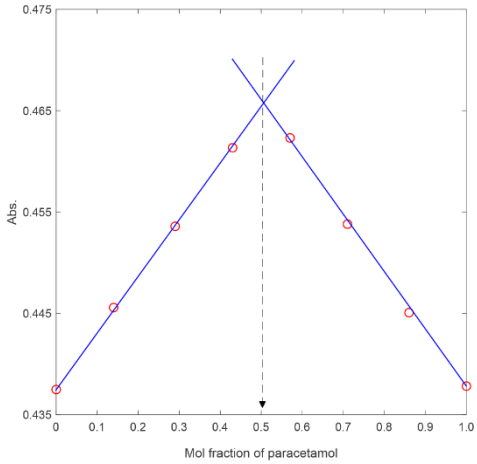

Figure 7. Classical Job plot for the Cu-PAR system.

CONCLUSIONS

In this study, a chemometric method based on three-way analysis of pH-dependent UV absorbance data was developed and successfully applied for the quantification of paracetamol in commercial tablet and syrup formulations. The approach combines simple UV measurements with PARAFAC decomposition of wavelength × sample × pH tensors, enabling resolution of overlapping signals and reliable estimation of PAR concentrations without chromatographic separation. The method demonstrated high accuracy with recovery rates between 96.48% and 99.67% in standard addition validation. LOD values 0.36 µg/mL for tablets and 0.42 µg/mL for syrups were reported. While HPLC can often achieve lower LODs, the sensitivity in this work is sufficient for pharmaceutical quality control where PAR is a major active ingredient. The main advantages of the PARAFAC approach over traditional chromatography are its efficiency and green profile while proving sufficient sensitivity. The second-order advantage, which enables accurate quantification even with uncalibrated interferences, provide mathematical separation of components in the sample. Whereas traditional chromatographic methods require extensive method development as well as more time, effort, and consumables to physically separate components to achieve the accurate quantification. The method exhibited excellent selectivity for both solid and liquid dosage forms. In tablet samples, no excipient-related component was detected in the PARAFAC model, confirming that the excipients do not significantly contribute to the studied spectral region. In syrup samples, an additional factor attributable to excipients or additives was clearly identified, yet the model was still able to selectively isolate the PAR signal and

accurately quantify the drug. The ability to separate the Cu-PAR complex absorbance from excipient-related signals illustrates the selectivity and second-order advantage of the three-way PARAFAC approach.

Furthermore, the stoichiometry of the Cu-PAR complex was investigated using both PARAFAC-assisted and classical Job plots, and in both cases a 1:1 complex was confirmed. The study reveals that the Cu-PAR complex signal intensifies with increasing pH, indicating that complex formation is favored under alkaline conditions. The 1:1 ratio confirmed by Job's method implies a specific binding mechanism. A plausible explanation is the involvement of the deprotonated phenolic group of paracetamol, given its higher availability in alkaline conditions, although further mechanistic studies would be required to confirm this hypothesis. This pH-dependence has practical implications for both complex stability and analytical selectivity, and it underlies the effectiveness of the second-order advantage exploited by the PARAFAC model. Overall, the proposed three-way modeling strategy provides a reliable, cost-effective, and chromatography-free alternative for the quantification of paracetamol, particularly in syrup formulations where classical UV methods often fail due to matrix interferences. Its simplicity and efficiency make it a promising tool for routine quality control in pharmaceutical analysis.

EXPERIMENTAL SECTION

Apparatus and Software

A Perkin Elmer Lambda 750 UV dual-beam spectrophotometer with a constant slit width (0.5 nm) and equipped with a 1.00 cm optical path quartz cell was used for all absorbance measurements. The scan was carried out in the range of 205 to 320 nm at 0.5 nm intervals. PARAFAC modelling performed through N-way Toolbox [40] in Matlab platform (MathWorks, MA, USA). The pH values were measured on a Mettler Toledo pH meter, with glass electrode.

Chemicals and Reagents

Paracetamol standard material was supplied from Nobel Pharmaceuticals. Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) (≥98%), and ACS spectrophotometric grade methanol (MeOH) were procured from Sigma-Aldrich (St. Louis, Missouri, USA). CH_3COOH, H_3BO_3, H_3PO_4, and NaOH were of analytical grade. For real sample analysis two commercial pharmaceutical formulations, Parol Tablet (produced by Atabay Kimya, Türkiye, containing 500 mg of paracetamol per tablet) and Parol Oral Suspension (produced by Atabay Kimya, Türkiye, containing 120 mg of paracetamol per 5 mL) were procured from a local pharmacy in Siirt, Türkiye.

Buffer solutions

Buffer solutions at five pH levels (2, 4, 6, 8, and 10) were prepared by adjusting a mixture of 0.04 M CH_3COOH, 0.04 M H_3BO_3 and 0.04 M H_3PO_4 with 0.1 M NaOH, while monitoring the pH value with the pH meter.

Stock solutions

PAR stock solution ($1.0x10^{-3}$ M, equivalent to 151 µg/mL) was prepared in methanol due to its limited solubility in water. $CuSO_4$ solution was prepared in ultrapure water at a concentration of $1.0x10^{-3}$ M. All stock solutions were freshly prepared and protected from light prior to use.

Experimental design for complex formation

The solution sets were designed to investigate the complexation between copper(II) sulfate ($CuSO_4$) and paracetamol (PAR) under varying pH conditions and analyte concentrations. For the preparation of sample sets, five pH levels and eight PAR concentration levels were planned. The complexation of PAR with Cu(II) was monitored by an increasing amount of PAR while $CuSO_4$ concentration, buffer concentration, and methanol: aqueous solution ratio was kept constant.

For each solution:

- 5.00 mL of the appropriate buffer solution (corresponding to pH 2-10) was first introduced into a conical polypropylene tube.
- Sequentially, 0.90 mL of $CuSO_4$ stock solution and required volume of PAR stock solution (0.00 to 0.80 mL) were added.
- Since the PAR solution was prepared in methanol, additional methanol was added to ensure that the total MeOH content in each solution was fixed at 0.80 mL.
- 0.10 mL PAR + 0.70 mL MeOH
- 0.20 mL PAR + 0.60 mL MeOH
- …
- 0.80 mL PAR + 0.00 mL MeOH
- Then, 2.30 mL of ultrapure water was added to each tube to reach a total volume of 9.0 mL.

Samples were prepared in the order of increasing pH, and each was manually shaken for 1 minute to ensure complete mixing. Immediately after mixing, UV absorption spectra were recorded over the range 205.0–320.0 nm with an increment of 0.5 nm. Each spectrum, corresponding to a vector of 231 elements was transferred to a Microsoft Excel file as a column vector, resulting in a matrix with wavelength and sample dimensions. Each pH condition was organized in a separate worksheet as the third dimension.

The precipitation of Cu^{2+} as $Cu(OH)_2$ in alkaline media was prevented by the use of a mixed medium of methanol and aqueous buffer. To ensure a constant matrix composition, Cu(II) concentration (1.0×10^{-4} M) and MeOH concentration (~8.9% *v/v*) were kept constant across all solutions within the pH range of 2-10. The fixed methanol content was sufficient to dissolve paracetamol in all solutions without significantly affecting pH or Cu^{2+} speciation. Under these controlled co-solvent conditions, all solutions remained optically clear even at pH 10 (no turbidity or baseline lift in the 205–320 nm range), and the spectra were highly reproducible.

Preparation of calibration set

A total of 40 calibration samples were systematically designed across five pH values (2, 4, 6, 8, and 10), with eight different PAR concentrations evaluated at each pH. Required volumes of buffer, methanol, water, stock solutions of $CuSO_4$ and PAR to prepare the portion of calibration set with pH 2 is presented in Table 4. The design was replicated with pH=4, pH=6, pH=8, and pH=10 conditions resulting in 40 calibration solutions.

Table 4. The composition of calibration set at the level of pH 2

Sample	Buffer		$CuSO_4$	PAR	MeOH	Water	Total	PAR
no	pH	mL	mL	mL	mL	mL	mL	µg/mL
1	2	5.0	0.90	0.10	0.70	2.30	9.00	1.68
2	2	5.0	0.90	0.20	0.60	2.30	9.00	3.36
3	2	5.0	0.90	0.30	0.50	2.30	9.00	5.04
4	2	5.0	0.90	0.40	0.40	2.30	9.00	6.72
5	2	5.0	0.90	0.50	0.30	2.30	9.00	8.40
6	2	5.0	0.90	0.60	0.20	2.30	9.00	10.08
7	2	5.0	0.90	0.70	0.10	2.30	9.00	11.76
8	2	5.0	0.90	0.80	0.00	2.30	9.00	13.44

Solutions of tablet formulation

A tablet stock solution was prepared in order to be used for PAR determination in tablet samples and tablet standard addition studies. Ten tablets with a label claim of 500 mg PAR were weighed to calculate the average weight of one tablet. The tablets were finely powdered in a mortar, and a mass theoretically containing $1x10^{-3}$ mol (151 mg) paracetamol was weighed and dissolved in 100 mL of methanol in a volumetric flask. After 30 min of mechanical shaking, the solution was filtered by Whatman No. 42 filter paper. The filtered solution was diluted to a ratio of 1:10 to obtain a tablet stock solution. Considering the label claim, PAR concentration of tablet stock solution was $1.0x10^{-3}$ M, equivalent of 151 µg/mL PAR.

Tablet sample solutions were prepared in a similar manner with calibration set samples across five pH values (2, 4, 6, 8, and 10). For each pH value, five replicate solutions were prepared by mixing 5.00 mL buffer, 0.90 mL $CuSO_4$ stock solution, 0.30 mL tablet stock solution, 0.50 mL methanol and 2.30 mL water. Required volumes of stock solutions to prepare a representative tablet sample set of each pH level is presented in Table 5. The design was replicated with five different buffers (pH=2, pH=4, pH=6, pH=8, and pH=10) resulting in 25 tablet sample solutions.

Table 5. Experimental design for the tablet sample solutions

Sample No	Buffer (mL)	$CuSO_4$ (mL)	Tablet stock (mL)	MeOH (mL)	Water (mL)	Total volume (mL)
1	5.0	0.90	0.30	0.50	2.30	9.00
2	5.0	0.90	0.30	0.50	2.30	9.00
3	5.0	0.90	0.30	0.50	2.30	9.00
4	5.0	0.90	0.30	0.50	2.30	9.00
5	5.0	0.90	0.30	0.50	2.30	9.00

In order to determine whether the excipients in the tablet formulation have an impact on the determination of the drug, standard addition technique was used. A portion of tablet standard addition solutions was designed as depicted in Table 6. All tablet standard addition solutions contained a fixed volume of tablet stock solution (0.10 mL), increasing volume of PAR stock solution (0.00 mL, 0.20 mL, 0.40 mL, 0.60 mL). The same table was used to prepare 5 tablet solutions in each pH value resulting in 20 tablet standard addition solutions.

Table 6. Experimental design of the tablet standard addition samples

Exp. no	Buffer (mL)	$CuSO_4$ (mL)	Tablet stock (mL)	PAR (mL)	MeOH (mL)	Water (mL)	Total volume (mL)
1	5.0	0.90	0.10	0.00	0.70	2.30	9.00
2	5.0	0.90	0.10	0.20	0.50	2.30	9.00
3	5.0	0.90	0.10	0.40	0.30	2.30	9.00
4	5.0	0.90	0.10	0.60	0.10	2.30	9.00

Solutions of syrup formulation

A syrup stock solution was prepared in order to be used for PAR determination in syrup formulation and syrup standard addition studies. The oral suspension formulation was prepared by adding distilled water to the marked line and hand mixing for 2 minutes. PAR concentration in the suspension was 120 mg/5 mL (0.159 M) according to the label claim. A portion of 2 mL from the suspension was diluted to 100 mL with methanol, mechanically

stirred for 10 minutes then diluted to a ratio of 1:10. The diluted solution was filtered through a syringe filter (Merck Millipore, USA) to obtain the syrup stock solution, approximately containing 240 µg/mL PAR (1.59 $x10^{-3}$ M) according to the label claim.

Syrup sample solutions were prepared in a similar manner with tablet sample solutions across five pH values (2, 4, 6, 8, and 10). For each pH value, five replicate solutions were prepared by mixing 5.00 mL buffer, 0.90 mL $CuSO_4$ stock solution, 0.30 mL syrup stock solution, 0.40 mL methanol and 2.30 mL water. The total number of syrup sample solutions were 25.

The effect of excipients in syrup formulation was studied by standard addition technique. A set of syrup standard addition solutions was designed similar to the tablet standard addition set. All syrup standard addition solutions contained a fixed volume of syrup stock solution (0.10 mL), and an increasing volume of PAR stock solution (0.00 mL, 0.20 mL, 0.40 mL, 0.60 mL) at each pH level.

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