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Analysis of Paracetamol, Diclofenac Sodium and Caffeine in Mixture by Validated First Derivative Spectrophotometric Method

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Abstract: : ***For the simultaneous measurement of paracetamol (Para), diclofenac sodium (Diclo), and caffeine (Caff) in their combination without previous separation, a straightforward, precise, and economical first-derivative UV spectrophotometric approach has devised and validated. The approach is based on the first-derivative spectra and unique UV absorption properties of the three medications. In order to reduce mutual interference, paracetamol was measured at 209 nm, diclofenac at 283 nm, and caffeine at 318 nm. The method's linearity, accuracy, precision, limit of detection, and limit of quantification were all verified in accordance with ICH recommendations. With correlation coefficients less than 0.99, the calibration curves were found to be linear in the concentration ranges of 10-60 microgram per milliliter for paracetamol, 4–24 microgram per milliliter for diclofenac, and 2–12 microgram per milliliter for caffeine. While precision experiments produced %RSD values less than two percent for both intra-day and inter-day measurements, recovery studies verified accuracy in the range of 98–102%. For regular quality control analysis of combination Para–Diclo–Caffeine formulations in pharmaceutical dosage forms, the established first-derivative approach is quick and does not need chromatographic separation.***

Keywords: Paracetamol, Diclofenac Sodium, Caffeine, UV Spectrophotometry, First Order Derivative, Validation.

1. Introduction

Now days, fixed dose combinations of drugs are becoming popular in the pharmaceutical field, as these combinations are proving to have synergistic effects, as they attack different aspects of the disease[1]. In the context of analgesic and migraine treatments, the combination of Paracetamol (PARA), Diclofenac Sodium (DICLO), and Caffeine (CAFF) is very useful. PARA is the source of pain relief in this combination, while DICLO, a powerful non-steroidal anti-inflammatory medication, acts by blocking COX, and CAFF facilitates the passage of the drug over the blood-brain barrier and amplifies the effects of the other two substances [2].

Paracetamol ($C_8H_9NO_2$; MW: 151.16 g/mol) is basically a Para-aminophenol with an OH group and acetamide part, coming as white crystals that dissolve nicely in methanol but not so much in water. Diclofenac Sodium ($C_{14}H_{10}Cl_2NNaO_2$; MW: 318.13 g/mol) has this dichlorophenyl-acetic acid linked to an indole, sold as the sodium salt so it mixes well in water (~50 mg/mL) and stays stable in pills. Caffeine ($C_8H_{10}N_4O_2$; MW: 194.19 g/mol) is a methylated xanthine that jumps into methanol easily and has strong UV-absorbing parts [3].

Quantifying multiple active pharmaceutical ingredients (APIs) in combined formulations demands analytical methods that resolve spectral interferences while maintaining cost-effectiveness, simplicity, and regulatory compliance [4]. UV-Visible spectrophotometry serves as the cornerstone technique in pharmaceutical quality control due to its rapidity, affordability, and minimal sample preparation [5]. However, overlapping absorbance profiles necessitate advanced derivative mathematical transformations to enable accurate simultaneous determination without physical separation [6].

First derivative UV spectroscopy fixes overlap issues by turning flat absorbance lines into slopes ($dA/d\lambda$), creating spots where one drug's signal peaks while others flat line at zero [4]. Peak of special wavelengths 209 nm for PARA, 283 nm for DICLO, 318 nm for CAFF where each one's derivative height matches its amount exactly, no interference from the rest. Skip the chemicals or columns; it's perfect for everyday testing [7].

In methanol, these spots give clean readouts: PARA at 209 nm ignores the others' wiggles; DICLO at 283 nm stays clear of PARA and CAFF noise and CAFF at 318 nm reads true. Measure those peak heights against standard lines, and concentrations over real ranges PARA 10-60 microgram per milliliter, DICLO 4-24 microgram per milliliter, CAFF 2-12 microgram per milliliter with sharp detection [5].

Studies cover HPLC or HPTLC for pairs like paracetamol-caffeine, but nobody's published a checked-out first derivative UV method for this exact three-drug mix. With these combos popping up more in pain meds, labs need easy ways to test stability, release batches, or run dissolutions without blowing budgets on fancy gear. That's the hole we're filling here [4].

Building and testing of a fresh first derivative UV method to measure PARA, DICLO, and CAFF together in lab mixes and tablets. Full ICH Q2 (R1) checks showed great straight lines, spot-on recovery (98-102%), tight precision (%RSD fewer than 2%), low detection limits, durability, and clean separation ready for real pharma QC work [8].

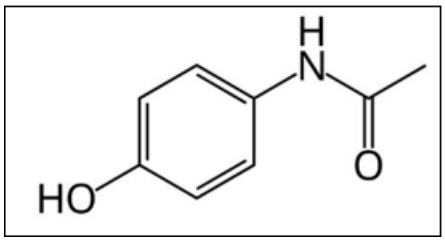

Figure 1: PARA

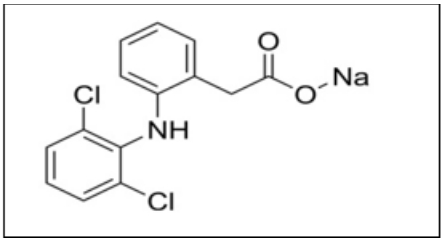

Figure 2: DICLO

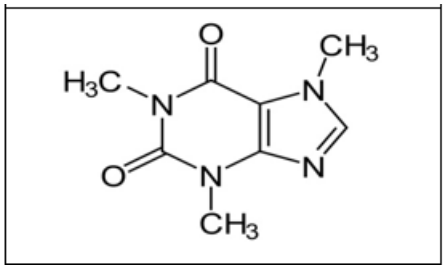

Figure 3: CAFF

2. Materials & Experimental Procedures

2.1 Equipment

The UV–Visible double-beam spectrophotometer (UV-1800, Shimadzu, Japan) used in this investigation has a wavelength accuracy of ±0.5 nanometer and a spectral band width of 2 nanometer. Using matching quartz cuvettes with a 10 millimeter route length, the analysis was carried out. UV-Probe 2.0 software was used to gather and evaluate the spectra's data. Additionally, an ultrasonic bath was employed in the experiment, and the materials were weighed using an electronic analytical balance [6].

2.2 Material

Standards of PARACETAMOL, DICLOFENAC SODIUM, and CAFFEINE were obtained from SPENSUS Pharmaceuticals Ltd., Ganeshpura, Gujarat, India, as gift samples and directly used without further purification. AR-grade methanol served as the experiment's solvent. The remaining substances and reagents utilized in the investigation were all analytical grade.

2.3 Solution Preparation

2.3.1 Standard Stock

In separate 100 mL volumetric flasks, precisely 10.0 mg of each reference standard (PARA, DICLO, and CAFF) is dissolved to prepare stock solutions of all the analytes. The solution totally dissolved after adding around 50 mL of methanol and ultrasonicating for five minutes. Each flask was filled with methanol until the volume reached 100 mL after it had cooled to room temperature. Stock solutions with a final concentration of 100 microgram per millimeter were produced as a result.

2.3.2 Standard Solution

To make working standards, stock solutions were mixed with methanol in 100 mL volumetric flasks at the right amounts. We got PARA of 10 to 60 microgram per millimeter, DICLO concentrations of 4 to 24 microgram per millimeter, and CAFF concentrations of 2 to 12 microgram per millimeter by taking the right amount from each stock solution. We made all working solutions fresh every day and tested them right away to make sure the results were accurate and stable.

2.4 Synthetic Mixture

A laboratory-prepared synthetic blend was formulated based on the labeled claim ratio of 325:50:30 for Paracetamol, Diclofenac sodium, and Caffeine, respectively. Accurately weighed quantities of 65 mg of Paracetamol, 10 mg of Diclofenac sodium, and 6 mg of Caffeine were taken and combined with suitable excipients, to obtain a total blend of approximately 100–150 mg. To create a consistent and well-homogenized powder blend, all the ingredients were properly combined. First-order derivative spectrophotometric analysis was then performed using the synthetic combination that had been created as the sample.

2.5 Sample Solution

A volumetric flask that has capacity of 100 mL was used for mixing the drugs where the amount of Para was 325 mg, Diclo 50 mg, and Caff 30 mg. To ensure that all drugs were completely dissolved and there was no undissolved material, 50 mL of meOH solution has add to mixture, followed by its sonication in the ultrasonic bath for 10 minutes. The mixture was filtered in order to separate the undissolved excipients, which might cause interference in the results obtained during experimentation. Finally, the methanol solution was added to reach the concentration of 3250 microgram per millimeter of Para, 500 microgram per millimeter of Diclo, and 300 microgram per millimeter of Caff. The following step was to prepare the working solution; for this purpose, 0.5 mL of the solution has add to 100 mL V.F. containing methanol and diluted up to the mark. It gave 16.25 microgram per millimeter of Paracetamol, 2.5 microgram per millimeter of Diclofenac, and 1.5 microgram per millimeter of Caffeine.

2.6 Determination of Zero Crossing Points

Taking methanol as a blank, solutions containing paracetamol, diclofenac, and caffeine (each at 10 µg/mL) were analyzed using the spectrophotometer within the wavelength range of 200–400 nanometer in order to establish their analytical wavelengths. It was established that the zero-order absorption spectrum of the drugs overlapped significantly, and it was not possible to estimate them directly in one run.

Zero-order spectra of the drug were differentiated into first-order derivative spectra by means of the Δλ parameter of the UV Probe software.

Each of the medicines has specific zero-crossings in the first-order derivative spectra. Analytical wavelengths for diclofenac, caffeine, and paracetamol were determined to be 283 nm, 318 nm, and 209 nm respectively. It was established these wavelengths because they enabled analysis of these three drugs in one measurement and without interfering with one another.

3. First-Order Derivative Method

This method has applied to estimate concentration of paracetamol, diclofenac, and caffeine. The zero-crossing technique was applied to the method. The three drugs were estimated simultaneously. The unique zero-crossing values were obtained for the three drugs. The zero-crossing values were obtained by taking the derivative spectrum of the three drugs. The zero-crossing values were obtained at 209 nm for diclofenac and caffeine. Paracetamol showed a derivative spectrum. Paracetamol and caffeine passed the zero-crossing point. However, diclofenac showed a clear derivative spectrum at 283 nm. Similarly, paracetamol and caffeine passed the zero-crossing point. However, diclofenac showed a clear derivative spectrum. Caffeine was estimated at 318 nm. There was very little interference from the other two drugs.

4. Development of Calibration Curve

Calibration curves for PARA, DICLO, and CAFF were plotted at concentrations within their suitable ranges. In order to create the solution of working, a series of 10 mL V.F. was employed in creating the adequate dilutions of the std solutions. Paracetamol solutions have created at concentrations of 10-60 µg/mL. Diclofenac solutions were prepared at concentrations ranging from 4-24 µg/mL, whereas caffeine solutions were made at concentrations of 2-12 µg/mL. All solutions has then diluted to volume with methanol.

5. Method Validation

The validation of this Method was performed in accordance with the ICH Q2 (R2) guidelines by determining the important performance parameters, including linearity, range, precision, accuracy, LOD, and LOQ.

5.1 Linearity

Calibration plots were prepared using concentration ranges from 10 to 60 microgram per millimeter for PARA, 4 to 24 microgram per millimeter for DICLO, and 2 to 12 microgram per millimeter for CAFF. Each of the ten-milliliter capacity volumetric flasks held different concentrations of the accurate stock solution of PARA, DICLO, and CAFF, that is, 10 to 60 microgram per millimeter for PARA; 4to 24 microgram per millimeter for DICLO; and 2 to 12 microgram per millimeter for CAFF.

5.2 Limit of Detection

This is a measure of the smallest amount of the compound that is detectable but not always quantifiable using specific analytical procedures at a particular sensitivity level. The following formula was used in determination of the detection limit of this absorbance correction procedure according to ICH guidelines:

$$LOD = 3.3\ \sigma / S$$

Here, σ denotes standard deviation in the response and S stands for gradient of linear calibration graph.

5.3 Limit of Quantification

The minimum measurable quantity is the least quantity of analyte that will provide sufficiently precise and accurate measurements. In the case of absorbance correction method, the LOQ is obtained by the following formula in accordance with ICH guidelines:

$$LOQ = 10\sigma / S$$

Here, σ denotes standard deviation in the response and S stands for gradient of linear calibration graph.

5.4 Range

Range is defined as the range of concentrations from minimally to maximally, where a linear response can be obtained by the method. In the proposed method, linear correlation was obtained at a concentration range of 10-60 microgram per millimeter for PARA, 4-24 microgram per millimeter, and 2-12 microgram per millimeter for CAFF.

5.5 Precision

5.5.1Repeatability

It refers to degree of similarity between the outputs produced when the experiment is conducted with similar conditions within a very short period of time. For this particular experiment, the proper amount of the standard solution for PARA, DICLO, and CAFF was made and analyzed in one day, and all of the parameters remained constant. Precision of the procedure has determined through evaluating absorbance in the UV spectrophotometer using %RSD.

5.5.2 Reproducibility:

In addition, accuracy of established method has checked with the help of intraday and interday variations. Intraday precision was checked by determining the three concentration levels of drugs at various times during a day. On the other hand, inter-day precision was checked by analyzing for three consecutive days by maintaining the same concentration levels of drugs. The values obtained were Intermediate and expressed as %RSD.

5.6 Drug Recovery Study

Accuracy of developed technique has studied via these experiments employing the traditional technique of additions. In the test solution, the content of para, diclo, and caff was 16.25 microgram per millimeter, 2.5 microgram per millimeter, and 1.5 microgram per millimeter, respectively. The studies involved solutions having different concentrations at the following three levels: 80%, 100%, and 120%. Absorbance of each solution was evaluated at the appropriate wavelength (λ), and then corresponding concentration was determined according to the regression

equation. Percentage recovery was calculated for all concentrations, and the value of recovery has verified the correctness of the suggested method.

6. Experimental Results and Discussion

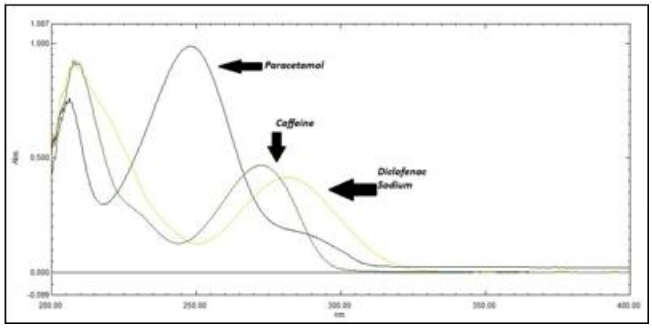

Figure 4: Zero Order Overlay UV Spectra of PARA, DICLO and CAFF (10 µg/mL).

For wavelength scanning of standard solutions of Paracetamol, Diclofenac sodium and Caffeine containing 10 µg/mL concentrations within a range of 200-400nm using meOH as blank for reference. This spectrum superimposed of the mixture revealed peaks at 249nm for Paracetamol, 209nm for Diclofenac and 210nm for Caffeine in Figure 3. Conversion of the zero order spectrums to first order derivative spectrum was achieved via UV Probe 2.70 where the user can select certain wavelength range. In the first order derivative spectrum of Paracetamol, Diclofenac and Caffeine showed zero-crossing point at 209nm, 283nm and 318nm respectively in Figure 4. These wavelength ranges were used in determining Paracetamol, Diclofenac and Caffeine in both drug mixture and bulk drug forms.

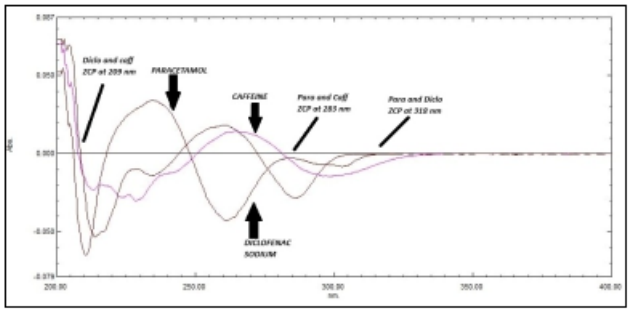

Figure 5: First Derivative Spectrum of PARA, DICLO and CAFF (10 µg/ML)

6.1 Linearity

In order to quantitatively determine all three drugs at the same time in combination formulations, a method based on first derivative UV spectroscopy has been developed. For better discrimination and minimization of the influence of spectral overlap, the zero derivative spectra of drugs have been converted to first derivative spectra. The measurements were carried out at particular zero crossing wavelengths where one drug has zero absorbance but another gives an analytical signal.

Table 1: Result of Linearity

Parameters	*PARA*	*DICLO*	*CAFF*
Wavelength (nm)	209	283	318
Concentration range (µg/mL)	Oct-60	Apr-24	02-Dec
Regression Equation y=mx+c	y = 0.0018x + 0.0475	y = 0.0008x + 0.0002	y = 0.003x – 0.0026
Slope	0.00135	0.0084	0.0029
Intercept	0.0499	0.0031	0.045
Correlation Coefficient	0.9993	0.9992	0.9977

For paracetamol at 10-60 microgram per millimeter, diclofenac at 4-24 microgram per millimeter, and caffeine at 2-12 microgram per millimeter, the method followed Beer-Lambert law. In the case of all the three drugs in the specified concentration range, Drug concentration and derivative absorbance had a linear relationship. The calibration curve's linearity and strong R2 values around one demonstrated the method's dependability.

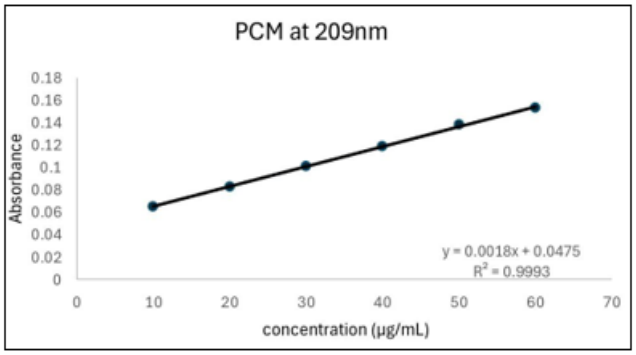

Figure 7: Linearity (PARA)

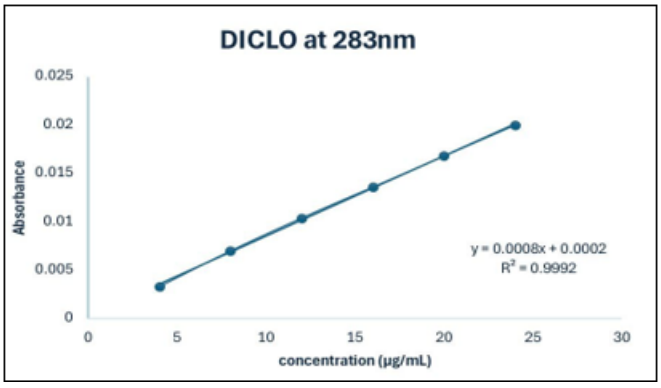

Figure 8: Linearity (DICLO

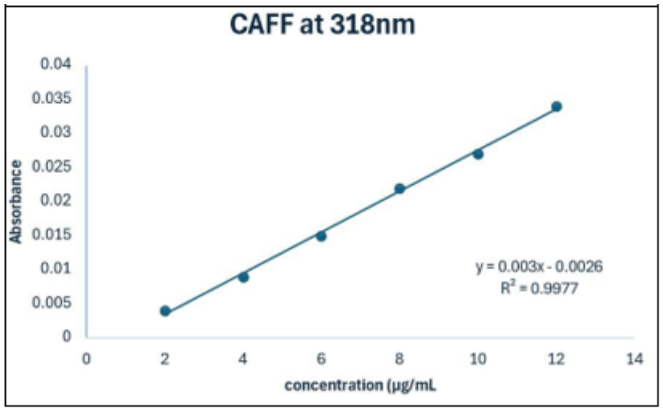

Figure 9: Linearity (CAFF)

6.2 LOD and LOQ

Sensitivity analysis was performed based on the calculation of the LOD and LOQ according to ICH guidelines based on the S. D. and slope of the calibration curve. For all three drugs, the LOD and LOQ values were found to be quite low, indicating the satisfactory sensitivity of the proposed first-order derivative spectrophotometry method. The LOD and LOQ values for paracetamol at 209 nm were estimated to be about 2.22 microgram per millimeter and 6.67 microgram per millimeter. The LOD and LOQ values of diclofenac at 283 nm were estimated to be about 1.42 microgram per millimeter and 4.15 microgram per millimeter, respectively, while for caffeine at 318 nm, the same values were about 0.907 microgram per millimeter & 3.35 microgram per millimeter.

6.3 Precision

6.3.1 Repeatability

Table 2: Result of Repeatability

S. No.	*PARA*	*DICLO*	*CAFF*
1	0.1012	0.0101	0.0151
2	0.1008	0.0103	0.0149
3	0.1015	0.0102	0.015
4	0.1009	0.01	0.0152
5	0.1011	0.0102	0.0148
6	0.101	0.0101	0.015
mean	0.1011	0.01015	0.015
S.D.	0.00025	0.00011	0.00014
RSD	0.24	1.08	0.93

6.3.2 Intermediate Precision

The produced first-order derivative spectroscopic technique of UV spectrophotometry was used to conduct the intraday and interday precision experiments, and it was found that the values of RSD% for caffeine, sodium diclofenac, and paracetamol has obtained to fall within allowable range, and hence, this technique showed high precision. The consistency and reliability of the proposed method under various analytical conditions can be established from the small variations observed in results. Hence, the proposed method may be considered reliable and appropriate for routine analysis of the selected drugs.

Table 3: Result of Intermediate Precision

Sr.no	drug	*%RSD (n=3)*	
		intraday	*interday*
1	PARA	0.51-0.36	0.57-0.39
2	DICLO	1.12-0.88	1.21-0.92
3	CAFF	0.68-0.91	0.88-0.97

6.4 Recovery study

Accuracy of this technique has determined through recovery experiments at the levels of 80%, 100%, and 120% utilizing the addition method. Caffeine, diclofenac sodium, and paracetamol were spiked into previously analyzed solutions, showing recovery values of 98-102%. This is an indication that the method is accurate and reliable.

Table 4: Result of Accuracy (PARA, DICLO & CAFF)

(% Recovery Level)	Sample Amt. Taken (ug/ML)			Standard Amt. Added (ug/mL)			Amount Recovered (ug/mL)			% Recovery ± SD (n=3)			%RSD
	PARA	DICLO	CAFF	PARA	DICLO	CAFF	PARA	DICLO	CAFF	PARA	DICLO	CAFF	(n=3)
I 80%)	16.25	2.5	1.5	13	2	1.2	29.1	4.47	2.69	99.48 ± 0.41	99.22 ± 0.38	99.63 ± 0.44	0.41
II (100%)	16.25	2.5	1.5	16.25	2.5	1.5	32.54	4.99	3	100.12 ± 0.33	99.86 ± 0.27	100.09 ± 0.30	0.3
III (120%)	16.25	2.5	1.5	19.5	3	1.8	35.88	5.52	3.32	100.37 ± 0.29	100.41 ± 0.35	100.52 ± 0.28	0.31

6.5 The synthetic mixture assay

A first-order derivative UV spectrophotometric method measured paracetamol, diclofenac sodium, and caffeine in a synthetic mixture. Each drug was analyzed at its specific wavelength. Results demonstrate the method's reliability and repeatability for simultaneous estimation of all three components. The assay confirms its suitability for routine quantitative analysis, free from interference by other formulation ingredients.

Table 5: Assay result of PARA, DICLO & CAFF in synthetic mixture

Sample no.	Drug Amount (mg)			Amount found (mg)			% Assay		
	PARA	DICLO	CAFF	PARA	DICLO	CAFF	PARA	DICLO	CAFF
1	325	50	30	324.2	49.7	29.8	99.75	99.4	99.33
2	325	50	30	326.1	50.2	30.1	100.33	100.4	100.33
3	325	50	30	323.8	49.9	30.2	99.63	99.8	100.67
4	325	50	30	327	50.4	29.9	100.62	100.8	99.67
5	325	50	30	324.5	49.8	30	99.85	99.6	100
6	325	50	30	325.6	50.1	29.7	100.18	100.2	99
Mean							100.06	100.03	99.83
SD							1.21	0.26	0.19
%RSD							0.37	0.52	0.63

7. Conclusion

For all three drugs within the selected concentration range, the proposed first order derivative UV spectrophotometry method to measure paracetamol, diclofenac sodium, and caffeine together was found to be linear. This could be confirmed through the recovery percent close to 100% and %RSD being within permissible limits. For this reason, it was confirmed that the method provides accurate and precise data. Precision was established through experiments run within-day and between-day. Therefore, it was proved that the method is reliable and reproducible. Being relatively simple, cost-effective, and rapid, the method can be considered suitable for routine quantitative analysis. Hence, it is applicable to determine content of paracetamol, diclofenac, and caffeine in dosage form.

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