Research Article

Indian Journal of Science and Research

Molla R *et al.,* Ind. J. Sci. Res. 2025, 5(1), 29-39

ISSN 2583 – 2913

# SPECTROPHOTOMETRIC SIMULTANEOUS QUANTITATIVE ANALYTICAL ANALYSIS METHOD FOR PCM-MET DEVELOPED AND VALIDATED

**Rahul Molla, Arindam Sarkar, Sanchita Mandal***

**Abstract:** Combination of two API Paracetamol (PCM) and Metronidazole (MET) were analysed by simple, particular, accurate and reproducible spectrophotometric method developed and validated following the ICH q2(R1) guideline. MET is a broad-spectrum antibiotic, antiprotozoal and PCM is an analgesic, antipyretic. In method 1 Simultaneous equation method λ-max of PCM and MET were found 243 nm and 320nm respectively. While method 2 in MET and PCM involves formation of "Isoabsorptive point" at 264nm (Q-analysis) and λ-max of MET 320nm were selected. Linearity of both MET and PCM were selected in 2 to10 microgram/ml range. The Beer lambert's law limit for each drug individually, in mixture and marketed formulation was within this concentration series same as linearity range. Current methods were developed and validated depending on the parameters Range and Linearity, Precision (repeatability, interday, intraday, reproducibility), Accuracy, LOD, LOQ, Ruggedness, Robustness, recovery parameters. Range and linearity 2-10, intraday precision for PCM & MET was 1.613 and 0.700 respectively, interday precision of PCM & MET was 4.03 and 3.05 respectively, accuracy (50%, 100%, 150% level of increase, it was found for paracetamol 97.04%, 99.4%, 99.03% and for metronidazole 99.16%, 107%, 99.1% respectively, LOD for PCM & MET was 0.546 and 0.240 respectively, LOQ for PCM & MET was 1.65 and 0.729 respectively. It was found Q absorbance method shows % recovery 96-105% and Simultaneous equation 96-101% in terms of biasness as the deviation is less in Q-absorbance method, more in simultaneous method. So, it was suggested Q absorbance techniques will give more accuracy for routine analysis of both API in combination.

**Key words:** Paracetamol, Metronidazole, Simultaneous equation method, Q-Analysis, ICH guidelines.

**Introduction:** The market is increasingly providing a diverse array of combinational dosage forms, with new options emerging regularly. These multicomponent formulations are gaining popularity due to their enhanced effectiveness, varied actions, quicker relief, reduced side effects, and greater patient acceptance. To ensure these formulations meet the necessary standards for efficacy, safety, and quality, it is essential to have a variety of analytical methods available for their evaluation. The most common OTC drug, PCM (Paracetamol), used for pain relief and fever that has no anti-inflammatory properties(Brigham, 2007). Compared to non-steroidal anti-inflammatory medicines (NSAIDs), PCM is better tolerated and has fewer gastrointestinal adverse effects because it has less peripheral action on prostaglandins(Fokunang, 2018). PCM is often found in combinations with other drugs such as (PCM+ Etodolac)(Baghel & Shah, 2023), (PCM+ Diclofenac)(Baghel & Shah, 2023), (PCM+ Ibuprofen)(Baghel & Shah, 2023), (PCM+ Domperidone) (Baghel & Shah, 2023), PCM+ Aceclofenac ) (Baghel & Shah, 2023), (PCM+ Caffein) (Baghel & Shah, 2023), (PCM+ Aspirin) (Baghel & Shah, 2023), (PCM+ Piroxicam) (Baghel & Shah, 2023), (PCM+ Pentazocine) (Nanda et al., 2024) in the management of cold,

***Corresponding author**

**Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India, 700032**
E-mail: smandal.pharmacy@jadavpuruniversity.in
Sanchitaju2@gmail.com

Article recived on: 03 December 2024
Published on web: 13 February 2025, www.ijsronline.org

more than 600 (OTC) allergy medicine, pain reliever, sleep aids and other products.

MET (Metronidazole), an antiprotozoal drug is widely used in the treatment of invasive amoebiasis. Chemically it is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol. This agent has antibacterial, antiamoebic, antiprotozoal, antiparasitic, and antitrichomonal properties(Ansari et al., 2020). MET acts at low concentrations as a bactericidal agent. The majority of anaerobic (but not aerobic) bacteria, including bacilli, cocci, and gram-ve, gram+ve (*Clostridium diicile*, *Gardnerella vaginalis*), as well as several protozoa and capnophilic organisms including *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Giardia lamblia* are all within its area of activity(Tally & Sullivan, 1981). It has been used in clinical settings for a number of years and is the first-choice medication for the treatment of Crohn's disease, anaerobic infections, parasitic infections, *Helicobacter pylori*, and *Acne rosacea*. MET is available in the market as combination with other API such as (MET+Amoxicilin) (Tiwari et al., 2008), (Norfloxacin+ MET) (Basak et al., 2010), (Ofloxacin + MET)(Glavanović et al., 2016) (MET+ Nalidixic acid) (Baghel & Shah, 2023). Both the drugs are available in Indian pharmacopeia(Welfare, 2018), and United state pharmacopeia(United et al., 2020).

Assessment of PCM, either by itself or in conjunction with other medications carried out by Spectrophotometric method, HPLC(Glavanović et al., 2016), TLC(Borahan et al., 2019), HPTLC(Alam et al., 2022), LC-MS(Vilas & Reddy, 2020), FTIR(Mallah et al., 2015). Amperometric determination(Alagarsamy et al., 2018) and Micellar electrokinetic chromatographic method(Suntornsuk et al., 2003). Many analytical procedure have been explained in the literature for the estimation of MET individually and along with other drugs in different pharmaceutical dosage forms(Basak et al., 2010) (Senta Bharat, 2014) by Spectrophotometric method(Khattab et al., 2010), HPLC(Abdel Hady et al., 2021),TLC(Pyka-Pająk, 2024),HPTLC(Meshram et al., 2009),LC-MS(Kathriarachchi et al., 2018), FTIR(Thanh Xuan & Dang Hoang, 2022). In our previous work we have carried out the simultaneous estimation of MET in combination with norfloxacin(Basak et al., 2010). Since the combination of these two medications is not available in the market and also is not recognized by any standard pharmacopoeia, there is no reported technique for estimating the dosage forms of both the API together. There is no reported article about the simultaneous HPLC or Spectrophotometric determination of two API in combination.

This present work deals with development and then validation of two simultaneous spectrophotometric methods and then a comparison between that two method, which will be helpful for assessment of PCM and MET in marketed combination dosage forms.

The main objective of this research is to explore the pharmacological effects of two medications, PCM and MET, when formulated together in a single dosage form. This combination could serve as a potential treatment for bacterial infections related to fever, such as urinary tract infections, stomach ulcers, skin infections, and lower respiratory tract infections, which are not currently available in the market. Our experiment aimed to create a sustained-release formulation that combines an antibiotic (MET) with an antipyretic (PCM) for use in cases of microbial infection accompanied by hyperthermia. In conclusion, this study contributes to a deeper understanding of the simultaneous estimation of MET in the presence of PCM across pure drug forms, combinations, and existing commercial formulations.

**Material and Methods:**

**Material:** PCM API (Loba Chemie Pvt. Ltd.), Pyregesic 650mg (Local market, B. No DF3004, EAST INDIA PHARMACEUTICAL WORKS LIMITED), MET (Gifted by Holden medical laboratories Pvt. Ltd. Sinnar, Nashik), Metrogyl 400mg (local market, B. No TM823264), J. B. Chemicals & Pharmaceuticals Ltd.), Double distilled water (from our lab), Weighing balance (Denvar weighing system, DWS-223), UV-3200

Double Beam Spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, UV Probe software).

**Methods:**

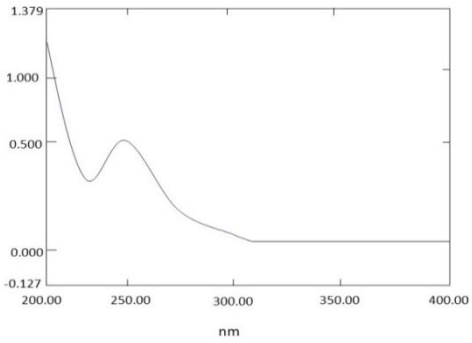
**1. Preparation of stock solution of MET & PCM in green solvent:** Accurately weighed portions of MET and PCM (10mg) were dissolved individually in a 100ml volumetric flask. The volume was made up to the mark with double distilled water to get standard stock solution having conc. of 100µg/ml of both API each.

**2. Preparation of standard curve of MET and PCM:**

The standard stock solution described earlier was created at a concentration of 10 µg/ml. A volume of 10 ml from this solution was examined over a scanning range of 200-400 nm to identify the λ-max of both active pharmaceutical ingredients (APIs). From the stock solution 2, 4, 6, 8, 10ml solutions were taken in 10ml volumetric flask and dilution was done using the double distilled water up to 10ml to prepare working conc. of 2, 4, 6, 8, 10µg/ml solutions. These conc. were then checked in UV-spectrophotometer and the $\lambda_{max}$ were found at 320nm and 243nm for MET(Bader & Aljanaby, 2020) and PCM(Patel et al., 2012) as reported respectively and the absorbances were mentioned in table no 1. Their respective calibration curve and scanned diagram were shown in figure 1 for PCM and Figure 2 for MET.

Table 1: Absorbance of PCM and MET for standard curve:

| Concentration (µg/ml) | Absorbance (PCM) | Absorbance (MET) |
|---|---|---|
| 2 | 0.196 | 0.075 |
| 4 | 0.390 | 0.137 |
| 6 | 0.568 | 0.194 |
| 8 | 0.709 | 0.255 |
| 10 | 0.906 | 0.326 |
| **Regression coefficient ($R^2$)** | **0.9977** | **0.9983** |



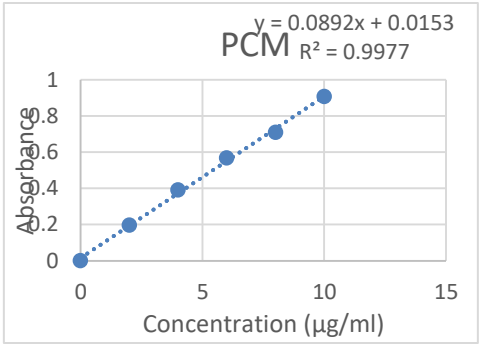
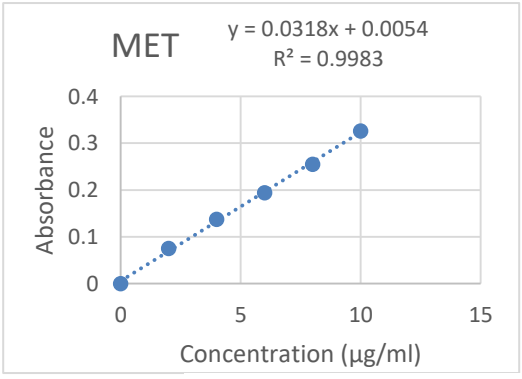




Figure 1: Calibration curve and scanned diagram of PCM.



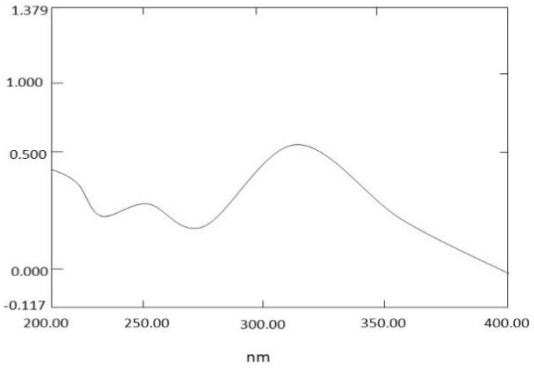




Figure 2: Calibration curve and scanned

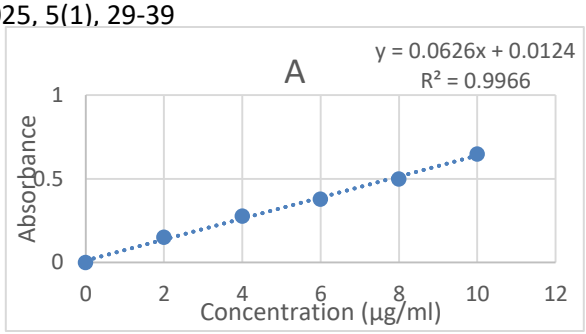
**3. Preparation of stock solution for PCM and MET combined in green solvent:** 10 mg of each active pharmaceutical ingredient (API) was placed in a 100 ml volumetric flask, and the volume was adjusted to the mark with double distilled water to create a stock solution with a concentration of 100 µg/ml.

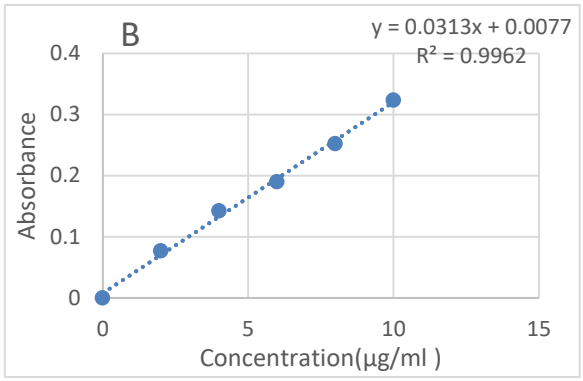
**4.Preparation of standard curve of combination of PCM and MET:**

A 10 ml aliquot of the stock solution was transferred to a 100 ml volumetric flask, and the flask was filled to the mark with double-distilled water to obtain a final concentration of 10 µg/ml for both active pharmaceutical ingredients (APIs). Working solutions were then prepared from this stock by taking 2, 4, 6, 8, and 10 ml into separate 10 ml volumetric flasks, with dilution carried out using a green solvent to obtain concentrations of 2, 4, 6, 8, and 10 µg/ml for each drug.Then the absorbance was checked in both 243nm, 320nm and 264nm (Isoabsorptive point) were shown in table 2, and plotted on figure 3.

Table 2: Absorbance of combined PCM and MET for Standard curve at individual absorbance maximum and at isoabsorptive point.

| Concentration (µg/ml) | PCM absorbance (243nm) in mixture | MET absorbance (320nm) in mixture | Isoabsorptive Absorbance (264nm) in mixture |
|---|---|---|---|
| 2 | 0.151 | 0.077 | 0.083 |
| 4 | 0.277 | 0.142 | 0.154 |
| 6 | 0.377 | 0.190 | 0.219 |
| 8 | 0.499 | 0.252 | 0.284 |
| 10 | 0.647 | 0.323 | 0.369 |
| Regression coefficient ($R^2$) | **0.9977** | **0.9983** | **0.9979** |



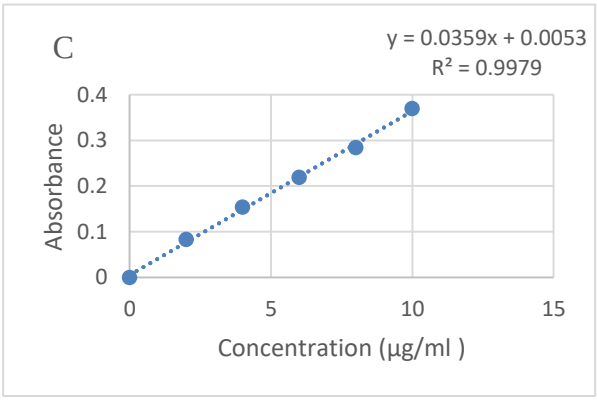




Figure 3: **A**- Absorbance of PCM in mixture at 243 nm, **B**- Absorbance of MET in mixture at 320 nm **C**- Isoabsorptive Absorbance at 264 nm in mixture.

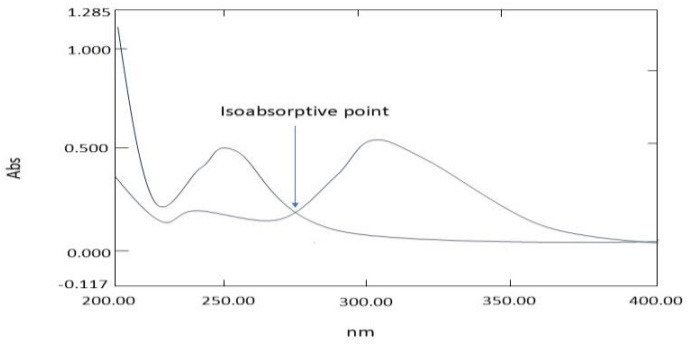



Figure 4: Overlain absorption spectra of PCM and MET (Isoabsorptive point 264nm)

**5. Simultaneous estimation of Combined PCM and MET:** Two different Spectrophotometric procedures were used here for the assessment of both API and their combined form.

**First method: Simultaneous equation method:** After determination of standard curve of both the API absorbance maxima were found 243nm and 320nm for PCM and MET respectively as in IP(Government of India, 2018).

Standard curve was plotted with absorbance on Y axis and conc. on X axis at individual wavelength and regression coefficient was found.

Simultaneous equations used for both drugs concentration calculation by solving these.

Cx = (A1aY2 - A2 Ay1) / (aX1 aY2 - aX2 aY1)….1

Cy = (aX1 A2 - aX2 A1) / (aX1 aY2 - aX2 ay1) …2

Where C x and C y represents the amount of PCM and MET in gm/100ml in their prepared solution.

A1 and A2 are absorbance of mixture at 243nm and 320nm respectively

aX1 and aX2 are absorptivity of PCM at 243nm and 320nm

aY1 and aY2 are the absorptivity of MET at 320nm and 243nm respectively.

**Second method**: **Absorbance Ratio Method (Q-Analysis):** Absorbance ratio method or "Q-analysis" is an analytical method to measure the ratio of two absorbance found at the "isoabsorptive" point of one component and absorbance of the other component at its λ-max.

Following the same process, then determine the "isoabsorptive point and absorbance maxima of both the drugs, found in figure 4 i.e. 264nm, showing isoabsorptive point and same as before at 243nm and 320nm absorbance maxima for PCM and MET respectively.

The Q Absorbance Ratio Method Equation used to calculate concentration of both the drugs simultaneously,

$C_{PCM} = Q_M - Q_y / Q_x - Q_y * A_1/ax1$…….3

$C_{MET} = Q_M – Q_X/Q_X - Q_Y * A_2/ay1$ …...4

$A_1$ and $A_2$ are the absorbances of the mixtures at 264nm and 320nm and

ax1 and ax2, ay1 and ay2 are absorptivity's E (1%, 1 cm) of PCM and MET at 264nm and 320nm.

$Q_M = A_2/A_1$

$Q_Y = ay_2/ay_1$,

$Qx = ax_2/ ax_1$.

**6. Analysis of formulation (marketed):** At first calculate the quantity of PCM and MET in existing marketed formulations, Pyregesic 650mg (From market, B. No DF3004, East India Pharmaceutical Works Ltd), and Metrogyl 400mg (Brought from market, B. No TM823264), J. B. Chemicals & Pharmaceuticals Ltd.) purchased from local market.

10mg of two formulations (marketed) required, to take equivalent powder in a 100ml volumetric flask and volume make up to 100ml with water (double distilled) to get desired conc. Subsequent dilution was done to get required concentration of 10µg/ml solution of both marketed formulations. In Method I, the absorbances, designated as A1 and A2, were measured at 243 nm and 320 nm, respectively. The quantities of the two active pharmaceutical ingredients (APIs) in the dosage forms were then calculated using the equations provided earlier (1) and (2).

In Method II, the absorbances A1 and A2 were recorded at 264 nm (the iso-absorptive point) and 320 nm (the λ-max of MET), respectively. The absorbance ratio, A2/A1, was calculated, and the relative amounts of the two active pharmaceutical ingredients (APIs) in the sample were determined using the equations provided as (3) and (4).

**7. Validation of methods:** Both methods were validated in accordance with ICH guidelines, focusing on parameters such as linearity, precision (both intraday and interday), limit of detection (LOD), and limit of quantification (LOQ).(Elder, 2024).

**-Range and Linearity:** Range and linearity are important parameter to consider when performing simultaneous estimation of pharmaceutical dosage formulations.

The same std conc. of PCM and MET ranging from 2-10 µg/ml was made to study the linearity of both API. Linearity was assessed regarding slope and $R^2$

**- Precision:** The precision of a measurement is determined by comparing the results of several measurements made of the same sample under

specified conditions. For the precision investigations, both intraday & interday precision are taken into account. The accuracy of an analytical procedure is often indicated by the variance, standard deviation, or coefficient of variation derived from a set of measurements.

**i. Intraday precision:** To determine intraday precision, absorbance measurement was done for a single solution. The absorbance of the10 µg/ml concentration sample solutions of both API was taken three times on the same day (morning, afternoon and evening), to calculate their standard deviation and % RSD.

**ii. Interday precision:** The absorbance readings of the sample solutions were recorded.(10 µg/ml) of PCM and MET was checked on three different day means alternative days, and again SD and %RSD was calculated following the same as before.

- **Reproducibility:** Reproducibility, checked by an interlaboratory trial basis. Although a repeatability investigation is typically not necessary for regulatory submission. Reproducibility study is necessary and should be taken into consideration when standardizing an analytical technique like when incorporating the procedure into pharmacopoeias or when conducting the procedure across several locations.

- **Accuracy:** The degree to which the measured values resemble the real ones is known as accuracy. Performing the recovery experiments allowed for the determination of the method's accuracy. The standard drug solution was spiked into the formulation's previously examined sample solution in order to conduct the recovery investigations.

- **Limit of detection:** Limit of detection of an analytical method is usually performed to assess the sensitivity of the method. Limit of detection (LOD) determined by below mentioned formula,

LOD=3.3*SD/SLOPE…...5

- **Limit of quantification (LOQ):** Limit of quantification of any method is usually carried out to identify the contaminants and degradation of the products. The following formula can be used to determine LOQ in accordance with ICH recommendations.

LOQ=10*SD/SLOPE…...6

- **Ruggedness:** Ruggedness of an analytical method is the degree of its reproducibility of the test results obtained under different variables like by different analyst, instrument, laboratories or different days. It was carried out by determining the mean value, %RSD, SD etc.

- **Robustness:** Depending on the type of procedure being studied, the applicability of the analytical procedure within the planned operating environment should be evaluated throughout the development phase and taken into consideration. In addition to demonstrating an analytical procedure's robustness to intentional changes in its parameters, robustness testing should, if applicable, demonstrate the stability of the sample preparations and reagents throughout the process. The robustness evaluation should be available upon request or may be included, on an individual basis, in the development data for an analytical technique.

**Result:**

**1. Determination of λ-max:** The solution of PCM and MET (2-10 µg/ ml) was scanned from 200-400 nm for determination of λ-max. The peak absorbance for PCM and MET was observed at 243 nm and 320 nm, respectively.

2. Determination of λ-max in combination: And it was found that both PCM and MET absorbance curves are crossing at absorbance 264nm which denotes as isoabsorptive point for both the API.

**2. Validation of methods**

**-Linearity and Range:** The range of both the drug i.e. PCM and MET were found to be in the range of concentration 2-10 µg/ml. The linearity of PCM and MET individually as well as in combinations were checked in the range of 2-10 µg/ml and the regression coefficient ($R^2$) were found as 0.9977, 0.9983 and 0.9979 which were greater than 0.9900, which indicate the value passes the criteria of acceptance of ICH guidelines (Elder, 2024). The calibration curve of PCM and MET is shown in the figure 1, 2 respectively.

**-Precision**

**i. Intraday Precision:** The intraday precision was carried out by measuring absorbance of the prepared

solution three times on the same day. It was found that the % RSD for PCM was as 1.613% at 243 nm and for MET, it was found to be 0.700% at 320 nm which meant the %RSD value were not greater than 2%, indicate the intraday precision have criteria of acceptance of ICH guidelines (Elder, 2024).

**ii. Interday Precision:**

The interday precision for PCM and MET was evaluated by measuring the absorbance over three consecutive days. The percentage relative standard deviation (% RSD) was determined to be 1.03% for PCM at 243 nm and 1.65% for MET at 320 nm. Both values were below 2%, which meets the acceptance criteria outlined in the ICH guidelines.

**Accuracy:** Recovery studies are typically conducted to assess the accuracy of the method after spiking the sample solution with standard drug solutions at levels of 50%, 100%, and 150%. The recovery percentages for PCM and MET are presented in Table 4. The simultaneous equation method yielded recovery percentages for PCM and MET ranging from 93% to 107%, while the Q-absorbance ratio method showed recovery values between 95.6% and 105%. According to most guidelines for residue analytical methods, a recovery range of 70% to 120% is considered acceptable, with mean values falling between 70% to 120% or 70% to 110%, depending on the specific regulatory standards at each fortification level.(Schoenau, 2019).

**-LOD:** The value of limit of detection (LOD) for PCM at 243 nm was found to be 0.546 and for MET at 320 nm, LOD was found to be 0.240 (ref……) 0.3275for PCM within the range.

**-LOQ:** The LOQ for PCM and MET were determined and the LOQ for PCM at 243 nm was found to be 1.65 and for MET at 320 nm it was found to be 0.729.

**- Ruggedness and Robustness:** Robustness testing was conducted by using a different instrument (LAB INDIA UV-2300 double beam spectrophotometer) for both drugs, with each concentration being scanned three times (T1, T2, T3) over a span of three days at both specified wavelengths. The percentage relative standard deviation (%RSD) was then calculated. The data from the ruggedness studies can be found in Tables 3 and 4.

**Table 3: Robustness studies of PCM at 243 nm**

| Drug | Wavelength | Conc. (µg/ml) | Day1 | Day2 | Day3 | Mean± SD | %RSD |
|---|---|---|---|---|---|---|---|
| PCM | 243nm | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 0.169 | 0.183 | 0.175 | 0.176±0.00989 | 5.62471 |
| | | 4 | 0.323 | 0.346 | 0.337 | 0.3345±0.01626 | 4.86202 |
| | | 6 | 0.504 | 0.53 | 0.569 | 0.517±0.01838 | 3.55605 |
| | | 8 | 0.702 | 0.733 | 0.718 | 0.7175±0.02192 | 3.05509 |
| | | 10 | 0.933 | 0.966 | 0.951 | 0.9495±0.02333 | 2.45755 |

**Table 4: Robustness studies of MET at 320 nm**

| Drug | Wavelength | Conc. (µg/ml) | Day1 | Day2 | Day3 | Mean± SD | %RSD |
|---|---|---|---|---|---|---|---|
| MET | 320nm | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 0.159 | 0.163 | 0.160 | 0.161±0.0017320 | 1.075 |
| | | 4 | 0.223 | 0.226 | 0.225 | 0.225±0.01626 | 1.945 |
| | | 6 | 0.304 | 0.315 | 0.310 | 0.309±0.00152 | 0.49 |
| | | 8 | 0.372 | 0.382 | 0.375 | 0.376±0.00420 | 1.117 |
| | | 10 | 0.433 | 0.428 | 0.430 | 0.431±0.00224 | 0.512 |

3. Recovery studies are typically performed to assess the method's accuracy by spiking the sample solution with the standard drug solution at concentrations of 50%, 100%, and 150%. The percentage recovery for PCM and MET is presented in Tables 5 and 6. The recovery for PCM and MET was found to be between 96% and 107% using the simultaneous equation method, while the Q-absorbance ratio method yielded recoveries ranging from 95.6% to 105%. According to ICH guidelines, an acceptable recovery range for analytical methods is generally between 70% and 120%, or 70% to 110% based on specific regulatory standards, at each fortification level. Since the recovery data obtained fell within the 70% to 120% range, this indicates the accuracy of the analytical methods employed.

Table 5 Recovery Study of marketed formulation of MET

| Method | Conc. in marketed sample (µg/ml) | Conc. Added pure (µg/ml) | Amt of drug(µg/ml) | Amt found actual (µg) | %RSD | % Recovery |
|---|---|---|---|---|---|---|
| A | 2 | 1 | 3 | 2.974 | 1.63 | 99.16 |
| | 2 | 2 | 4 | 4.08 | 1.72 | 101.1 |
| | 2 | 3 | 5 | 4.955 | 1.50 | 99.1 |
| B | 2 | 1 | 3 | 3.15 | 1.63 | 105 |
| | 2 | 2 | 4 | 2.89 | 1.72 | 96.3 |
| | 2 | 3 | 5 | 2.87 | 1.50 | 95.6 |

Table 6 Recovery Study of marketed formulation of PCM

| Method | Conc. in marketed sample (µg/ml) | Conc. Added pure (µg/ml) | Amt of drug(µg/ml) | Amt found actual (µg) | %RSD | % Recovery |
|---|---|---|---|---|---|---|
| A | 2 | 1 | 3 | 2. 97 | 0.89 | 99 |
| | 2 | 2 | 4 | 4.04 | 0.441 | 101.08 |
| | 2 | 3 | 5 | 5.09 | 0.902 | 101.8 |
| B | 2 | 1 | 3 | 2.88 | 2.98 | 96 |
| | 2 | 2 | 4 | 2.95 | 0.679 | 98.3 |
| | 2 | 3 | 5 | 2.935 | 2.14 | 97.8 |

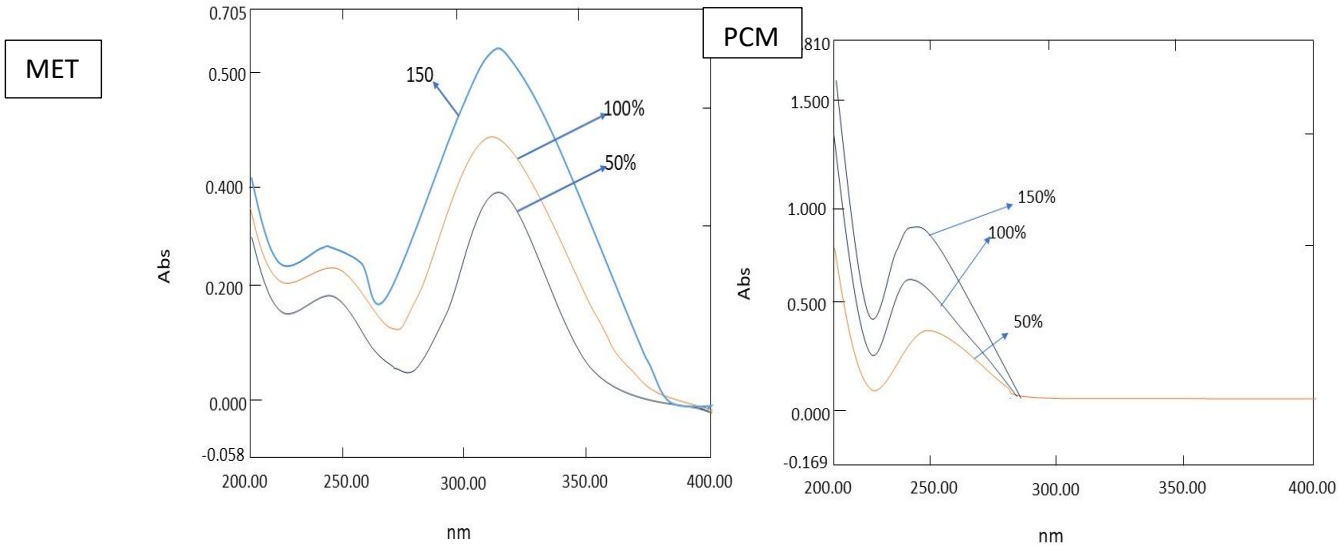



Figure **5**: Recovery study of PCM and MET.

From comparative data,we came to know that the Q-absorbance ratio method had higher $R^2$ value than simultaneous equation method and Y value is closer to 0 so it indicated that the Q-Absorbance ratio method produced better correlation than simultaneous equation method. It is evident that the range of recovery varied from 96% to 105% with a bias of -7 to +7 by simultaneous equation method, the same varied from -3 to +5 by Q-absorbance ratio method. It implies that the Q-absorbance ratio method was more accurate over simultaneous equation method(Dastidar & Sa, 2009).

Table 7: Summary of validation parameters (Q absorbance analysis method)

| Parameters | PCM | MET | Acceptance Criteria ICH |
|---|---|---|---|
| Wavelength (nm) | 243 | 320 | 243nm and 320nm respectively |
| Beer's law limit (µg/ml) | 2-10 µg/ml | 2-10 µg/ml | 2-25 µg/ml |
| Regression equation | Y=0.0892x + 0.0153 | Y=0.0318x+.0054 | |
| Slope | 0.0892 | 0.0318 | |
| Intercept | 0.0153 | 0.0054 | Near to 0 |
| Correlation coefficient ($R^2$) | 0.9977 | 0.9983 | $R^2 \geq 0.99$ |
| Intraday (n=3) (%RSD) | 1.613 | 0.700 | %RSD<2% |
| Interday (n=3) (%RSD) | 1.03 | 1.65 | %RSD<2% |
| LOD (µg/ml) | 0.546 | 0.240 | > 2 times base line |
| LOQ (µg/ml) | 1.65 (3:1) | 0.729 (1.5:1) | Signal-to-Noise=10:1 |
| Accuracy | 93-101.8 | 95.6-107 | 95-105% |

**Conclusion:**This study enhances the understanding of the simultaneous estimation of MET in the presence of PCM across pure drug forms, combinations, and marketed dosage forms. Two simultaneous spectrophotometric methods were developed using environmentally friendly solvents and subsequently validated. It was found Q absorbance method shows % recovery 95.6-105% and Simultaneous equation 96-101.8% in terms of biasness as the deviation is less in Q-absorbance method, more in simultaneous method. So, it was suggested Q absorbance techniques will give more accuracy for routine analysis of both API in combination.

Looking ahead, additional research is needed to investigate the synergistic effects of combining PCM and MET in a single dosage form. This combination could be beneficial for treating bacterial infections associated with fever, such as urinary tract infections, stomach ulcers, skin infections, and lower respiratory tract infections, conditions for which such a formulation is currently not available in the market.

The aim of our experiment was to combine an antibiotic (MET) and an antipyretic drug (PCM) and make a sustained release formulation of the combination to administer in microbial infection with hyperthermia

**Acknowledgement:** Author gratefully acknowledged AICTE m pharm fellowship.

**Funding statement:** One of the author Rahul Molla is getting AICTE M Pharm Scholarship

**Conflict of interest:** The author declare that they have no conflict of interest for current work.

**Author contribution:** Rahul Molla contributed in manuscript writing-original draft, conceptualization, data curation, experimentation, formal analysis, methodology. Dr Sanchita Mandal contributed in calculation, writing, editing and review of the manuscript.

**Reference:**

1. Brigham NVC. N.V. Chandrasekharan. 2007;(1):1–5.
2. Fokunang C. 2018; *Overview of non-steroidal anti-inflammatory drugs (nsaids) in resource limited coun*tries. MOJ Toxicol. 4(1).
3. Baghel S, Shah K. 2023; *A Review on Methods Developed for Estimation of Paracetamol in Combination with Other Drugs*. Int J Pharm Res Allied Sci. 12(1):75–94.
4. Nanda BP, Rani P, Bhatia R. 2024; *Unexplored drug combinations for analytical method development by HPLC*. J Chromatogr Open. 5(December 2023):2023–5.
5. Ansari MF, Inam A, Ahmad K, Fatima S, Agarwal SM, Azam A. 2020; *Synthesis of metronidazole based thiazolidinone analogs as promising antiamoebic agents*. Bioorganic Med Chem Lett [Internet]. 30(23):127549. Available from: https://doi.org/10.1016/j.bmcl.2020.127549
6. Tally FP, Sullivan CE. 1981; *Metronidazole: In Vitro Activity, Pharmacology and Efficacy in Anaerobic Bacterial Infections*. Pharmacother J Hum Pharmacol Drug Ther. 1(1):28–38.
7. Tiwari G, Tiwari R, Srivastava B, Rai AK, Pathak K. 2008; *Simultaneous Estimation of Metronidazole and Amoxicillin in Synthetic Mixture by Ultraviolet Spectroscopy*. Asian Journal of Research in Chemistry. 1(2):91–4.
8. Basak S, Mandal S, Chattopadhyay M. 2010; *Simultaneous spectrophotometric assay for estimation of norfloxacin and metronidazole in tablets*. Asian J Chem. 22(8):5971–5.
9. Glavanović S, Glavanović M, Tomišić V. 2016; *Simultaneous quantitative determination of paracetamol and tramadol in tablet formulation using UV spectrophotometry and chemometric methods*. Spectrochim Acta - Part A Mol Biomol Spectrosc. 157:258–64.
10. Welfare F. INDIAN. Vol. II. 2018.
11. United THE, Pharmacopeia S, Formulary Then. 2020 the National Formulary. 2020.
12. Borahan T, Unutkan T, Şahin A, Bakırdere S. 2019; *A rapid and sensitive reversed phase-HPLC method for simultaneous determination of ibuprofen and paracetamol in drug samples and their behaviors in simulated gastric conditions*. J Sep Sci. Feb;42(3):678–83.
13. Alam P, Shakeel F, Ali A, Alqarni MH, Foudah AI, Aljarba TM, 2022; *Simultaneous Determination of Caffeine and Paracetamol in Commercial Formulations Using Greener Normal-Phase and Reversed-Phase HPTLC Methods: A Contrast of Validation Parameters.* Molecules. 27(2).
14. Vilas D, Reddy S. 2020; *A Rapid LC-MS/MS Method for Quantification of Acetaminophen in Human Whole Blood*. Journal of Chromatography & Separation Techniques, 11(432):1–6.
15. Mallah MA, Sherazi STH, Bhanger MI, Mahesar SA, Bajeer MA. 2015; *A rapid Fourier-transform infrared (FTIR) spectroscopic method for direct quantification of paracetamol content in solid pharmaceutical formulations. Spectrochim Acta - Part A* Mol Biomol Spectrosc. 141:64–70.
16. Alagarsamy P, Settu R, Chen SM, Chen TW, Hong IS, Rao MM. 2018; *Amperometric determination of acetaminophen (paracetamol) using graphene oxide modified glassy carbon electrode*. Int J Electrochem Sci. 13(8):7930–8.
17. Suntornsuk L, Pipitharome O, Wilairat P. 2003; *Simultaneous determination of paracetamol and chlorpheniramine maleate by micellar electrokinetic chromatography*. J Pharm Biomed Anal. 33(3):441–9.
18. Senta Bharat M Ra. 2014; *Development and Validation of Spectrophotometric Method for Simultaneous.* Asian J Pharm Life Sci. 4(2):16–20.
19. Khattab FI, Ramadan NK, Hegazy MA, Ghoniem NS. 2010; *Simultaneous*

*determination of metronidazole and spiramycin in bulk powder and in tablets using different spectrophotometric techniques.* Drug Test Anal. 2(1):37–44.

20. Abdel Hady KK, Abdel Salam RA, Hadad GM, Abdel Hameed EA. 2021; *Simultaneous HPLC determination of vildagliptin, ampicillin, sulbactam and metronidazole in pharmaceutical dosage forms and human urine*. J Iran Chem Soc 18(3):729–38.
21. Pyka-Pająk A. 2024; *TLC-Densitometric Method for Determination of Metronidazole and Tinidazole in Pharmaceutical Preparations.* DOI:10.20944/preprints202402.1722.v1
22. Meshram DB, Bagade SB, Tajne MR. 2009; *Simultaneous determination of metronidazole and miconazole nitrate in gel by HPTLC.* Pak J Pharm Sci. 22(3):323–8.
23. Kathriarachchi UL, Vidhate SS, Al-Tannak N, Thomson AH, da Silva Neto MJJ, Watson DG. 2018. *Development of a LC-MS method for simultaneous determination of amoxicillin and metronidazole in human serum using hydrophilic interaction chromatography (HILIC).* J Chromatogr B Anal Technol Biomed Life Sci 2018;1089(May):78–83.
24. Thanh Xuan D, Dang Hoang V. 2022, *Application of Fourier transform-based algorithms to resolve spectral overlapping for UV spectrophotometric co-assay of spiramycin and metronidazole in tablets. Spectrochim Acta - Part A* Mol Biomol Spectrosc. 277:121253.
25. Bader QA, Aljanaby A. 2020; *Spectrophotometric determination of metronidazole benzoate in pharmaceutical dosage forms*. Int J Pharm Res. 12(03).
26. Patel DM, Sardhara BM, Thumbadiya DH, Patel CN. 2012; *Development and validation of spectrophotometric method for simultaneous estimation of paracetamol and lornoxicam in different dissolution media.* Pharm Methods. 3(2):98–101.
27. Government of India M of H. Indian Pharmacopoeia 2018. Vol. I. Delhi: Manager of Publications, 2018.; 2018.
28. Elder D. 2024; *Validation of analytical procedures – ICH Q2(R2).* Eur Pharm Rev. 29(1):5.
29. Schoenau EA. 2019; *Elements of method design.* ACS Symp Ser. 1300:3–16.
30. Dastidar DG, Sa B. 2009; *A comparative study of UV-spectrophotometry and first-order derivative UV-spectrophotometry methods for the estimation of diazepam in presence of tween-20 and propylene glycol.* AAPS Pharm Sci Tech.10(4):1396–400.