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RESEARCH ARTICLE

A simple Stability-Indicating UV Spectrophotometric Method developed and Validation for Detection of Paracetamol in (liquid, solid) Dosage form and Biological fluid: Application and Comparison

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ABSTRACT

A new stability indicating method UV-Vis Nanodrop 2000c has been developed and validated for detection of paracetamol (PCM) in (liquid, solid) dosage form and biological fluid. The maximum absorption of paracetamol was shown at 247 nm using methanol as solvent. The developed method was found to be linear R2 0.9999 within the concentration range from 0.2 to 30 µg/mL. High precision level % of RSD less than 1%. The LOD and LOQ values are 0.47 1.44 µg/mL, respectively. The accuracy and recovery of the method at three levels showed good results more than 100%± 0.5 with % RSD less than 2% and small percent of SEM. The proposed method was employed to assess the stability of both locally-produced and commercially-available tablets. This research analyzed the stability of their drugs under various conditions, including heat, humidity, acidity, alkaline, oxidative, and photolytic conditions. The method was successfully verified in compliance with the requirements established by the International Council for Harmonization (ICH). Afterwards, the approved method was used to detection of PCM in pharmaceutical dosage forms that are commercially available.

INTRODUCTION

Paracetamol (acetaminophen, N-acetyl-p-amino phenol) is the most drugs widely used in the worldwide, commonly used for analgesic and antipyretic drugs used for the relief of mild to moderate pain and fever (Rittau and McLachlan, 2012). It is readily available and inexpensive. As analgesic, paracetamol is better than the non-steroidal anti-inflammatory drugs (NSAIDs) although it may be somewhat less efficacious(Graham et al., 2013). Analgesics such as PCM are widely used drugs, not only as pain relievers but also in several diseases (musculoskeletal, join disorder, rheumatic disorders, arthritis, and rheumatism (Hadad et al., 2009).

Paracetamol PCM is the de-ethylated active metabolite of phenacetin, with molecular formula of $C_8H_9NO_2$ and M.wt, 151.16. Its chemical structure is given in figure 1. The mechanism of action of PCM

has not been completely elucidated till now. However, recent findings suggest that it has inhibitory effects on cyclooxygenase (COX) enzymes, i. e., COX-1 and COX-2, with stronger selectivity to COX-2, and this leads to the inhibition of prostaglandin synthesis in the central nervous system and eventually results in antipyretic and analgesic effects (Aminu et al., 2019).

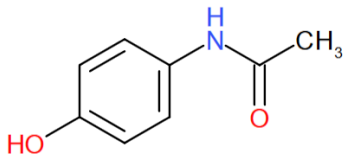

Figure. 1: Chemical Structure of Paracetamol ($C_8H_9NO_2$)

In literature reviewing many analytical technique have been developed to described PCM can be determined individual or in combination with other medicine, such as UV-Vis spectrophotometry(Hussein, 2020), titrimetric (Okai et al., 2016)(Srivastava et al., 1985), GC-MS, HPTLC, UPLC-MS, and HPLC-UV in its single type.

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enables recommendation of storage conditions, retests periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug, and degradants generated, during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines and USP 26(Gupta et al., 2010),(Qasim et al., 2024).

The UV-Vis Nanodrop 2000C is a spectrophotometer designed for quantifying the absorbance of DNA, RNA, and protein samples. It can detect samples as small as 0.5 liters, perform dual modes for UV-Vis spectroscopy and cuvette measurements, and measure absorbance across a broad wavelength range from 190 to 840 nm. The tool delivers fast and accurate results within less than 5 seconds per sample, and comes with integrated software for various study procedures.

(Qasim and Mohammed, 2021),(Rutherford, 2020),(von Thaden et al., 2020).

The Nanodrop 2000C has many Applications, such as a precision instrument used for nucleic acid quantification, protein quantification, purity evaluation, and cell culture monitoring. It accurately measures DNA, RNA, and oligonucleotides, and can evaluate purity ratios. Its ease, precision, and minimal sample requirements make it an essential tool for molecular biology, biochemistry, and biotechnology applications.(Desjardins and Conklin, 2011),(Qasim et al., 2024).

Therefore the objective of this research is to develop and validate new analytical method for detection of paracetamol in solid/liquid dosage form and in biological fluid, furthermore using this method for stability indicating of paracetamol.

MATERIALS AND METHODS

Chemicals and Reagents

Pure pharmaceutical active ingredient paracetamol were obtained from Awa medical company Erbil, Kurdistan, Iraq, and all tablets and syrup were randomly selected from pharmacies. Solvent are grad

(methanol, ethanol, and water) from Merck Germany. Reagent was HCl, NaOH, and H_2O_2 from Scharlau Spain. Per-chloric acid to remove proteins from serum, obtained from local laboratory.

Instrumentation

The spectrophotometric measurement was conducted using the Thermo Scientific nanodrop spectrophotometer (2000C Micro volume). The photo degradation investigation utilized a UV lamp with the specifications UVC-215 TS 8W, 220-240v, 50/6 Hz. The analytical balance used was the Voyager® model. The water bath shaker used was the Elmasonic P, which had a power of 100W and operated at a frequency of 80 KHz. The oven used was the Lab Tech LVO-2030. Centrifugation to mix and remove protein from biological fluid.

Preparation of Standard and Sample Solutions

Precisely measured a solution of 100 mg of pure paracetamol was dissolved in methanol and then transferred to a 100 mL volumetric flask. The remaining volume in the flask was filled with solvent to reach the mark, resulting in a stock standard solution with a concentration of 1000 µg/mL. The conventional solution was stored in a refrigerator and utilized to create various concentrations of working solutions.

Preparation of Paracetamol standard working solutions:

A total of twenty tablets were measured and then crushed into a fine powder. 100 mg of Paracetamol tablet powder was precisely weighed and put into a 100 mL volumetric flask. The powder was dissolved by sonication for 30 minutes using an adequate amount of methanol. The volume was adjusted to the mark using methanol containing 1000 µg/mL of PAC. The content was passed through a 0.45 µm nylon filter using Whatman filter paper. Working solutions at various concentrations were produced from the stock solution of the formulation.

Sample collection and preparation (biological fluid)

To determine the concentrations of paracetamol (PCM) in biological fluids using a Nanodrop 2000c UV spectrophotometric method, blood samples (approximately 4 ml) were collected from healthy human volunteers in ethylene diamintetraacetic acid (EDTA) tubes. The serum was separated by centrifuging the samples at 2500 × g for 10 minutes at 0°C. The plasma was maintained at a temperature of -20 °C until it was required. In order to obtain calibration standards of 0.2-30 µg/mL range of paracetamol concentrations. The absorbance of the standards was then measured at 247 nm.

Method Development

The development of a method to determine the presence of paracetamol (acetaminophen) in various materials, such as tablets and serum, is critical for both drug analysis and clinical diagnostics. The objective is to develop a dependable, precise, and replicable analytical technique capable of measuring the amount of paracetamol in various sample types. The UV-Vis Spectrophotometry Nanodrop 2000c was chosen for this investigation due to its simplicity and cost-effectiveness.

Selection of Wavelength:

Identifying the most effective wavelength for analyzing paracetamol is a critical step in developing a dependable spectrophotometric method. The wavelength at which a material demonstrates maximum absorbance (λmax) is selected for quantitative investigation due to its superior sensitivity and accuracy. The Nanodrop 2000c is a micro volume UV-Vis spectrophotometer that provides accurate measurement capabilities, even when using small sample quantities. This investigation uses

methanol as a solvent due to its compatibility with paracetamol and its ability to provide a distinct spectral baseline.

Types of Solvents:

The selection of a solvent in analytical chemistry is critical because it has a significant impact on the solubility of the analyte, the stability of the solution, and the analysis results. Depending on the analytical method, sample matrix, and specific analysis needs, many solvents can dissolve paracetamol. Some solvents often used include methanol, ethanol, water, acetonitrile, phosphate buffer (pH 7.4), dimethyl sulfoxide (DMSO), and aqueous acetic acid.

Method Validation:

Technique validation is an essential process in analytical chemistry that verifies the reliability, accuracy, and suitability of an analytical technique for its intended use. The method for analyzing paracetamol in various substances such as tablets and serum requires validation to ensure consistent and reliable results within specific parameters. The essential factors in method validation are accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), robustness, and stability.

Stability-Indicating Studies:

Stability-indicating investigations are crucial in the field of analytical chemistry to verify that a pharmacological ingredient or product retains its effectiveness, safety, and quality over the course of its storage period. These investigations aim to create and verify analytical techniques that can precisely identify the breakdown substances of a medication, namely paracetamol, when exposed to many types of stress circumstances, including thermal stress, photolytic stress, acidic hydrolysis, basic hydrolysis, and oxidative stress. The goal is to differentiate between the unaltered (active) medication and any probable breakdown substances, guaranteeing that the technique is particular and dependable.

Acid degradation:

For acid hydrolysis Working solution were used to prepare solution contain 50 ppm of PCM. 2.5 ml were added to 50 ml volumetric flask, therefore adding 2.5 ml of different concentration (N) of HCl starting from 0.1 N to 1.2 N. Furthermore the solution was neutralized by the equal concentration of NaOH, and after that adding methanol to made final volume.

Alkaline degradation:

For alkaline hydrolysis Working solution were used to prepare solution contain 50 ppm of PCM. 2.5 ml were added to 50 ml volumetric flask, therefore adding 2.5 ml of different concentration (N) of NaOH starting from 0.1 N to 1.2 N. Furthermore the solution was neutralized by the equal concentration of HCl, and after that adding methanol to made final volume.

Oxidative Degradation:

For Oxidative Degradation Working solution was used to prepare solution contain 50 ppm of PCM. 2.5 ml were added to 50 ml volumetric flask, therefore adding 2.5 ml of different concentration (%) of H_2O_2 starting from 1% to 13%. Methanol was added to made final volume.

Thermal Degradation:

For thermal degradation, 100 mg of PCM were precisely weighed and maintained at 30°C to 80°C for 24 hours. Then, the required quantity was dissolved in methanol to create a solution of 50µg/mL. The absorbance was measured at 247nm on a Nanodrop UV spectrophotometer using methanol as a blank.

Photolytic Degradation:

The medication sample, which was free from impurities, was precisely measured to weigh 50 mg of PCM. It was then subjected to direct sunlight for durations of 6, 12, 18, 24, 30, to 60 hours. A 50 µg/mL dilution was generated by dissolving the necessary quantity in methanol. The absorbance was then measured at 247 nm using methanol as a blank.

RESULTS AND DISCUSSION

Method optimization

Wavelength Selection:

Paracetamol absorbance spectrum in methanol showed a clear peak, with the highest absorbance occurring at 247 nm as shown in figure 2. This wavelength corresponds to the electronic transition occurring within the aromatic ring of the paracetamol molecule. These transitions are characteristic of several phenolic compounds. The distinct and clearly defined peak observed at this specific wavelength suggests that there is very little interference from the solvent, demonstrating that methanol is a suitable medium for this investigation.

The choice of 247 nm as the analytical wavelength is justified due to its closeness to the λmax, which ensures maximum sensitivity. Furthermore, the absorbance measurement at this specific wavelength fell within the instrument's linear range, enabling precise determination of paracetamol concentration in different sample matrices.

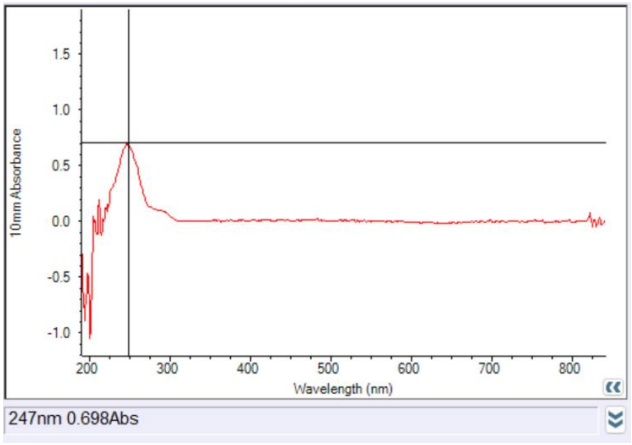

Figure 2: Spectrum of paracetamol 20 µg/mL

Types of Solvents:

The choice of solvent for paracetamol analysis is dependent on the analytical method's specific needs and the characteristics of the sample being examined. Methanol is commonly chosen due to its compliance with UV spectrophotometry and its ability to provide the highest maximum absorbance of PCM. On the other hand, acetonitrile is preferred in HPLC because of its effective elution properties and low UV absorbance. Ethanol and water are comparatively safer options; however, they may have restrictions in some analytical situations. Gaining knowledge about the characteristics of each solvent aids in choosing the most suitable one for precise and dependable analysis of paracetamol.

Method Validation:

The nanodrop spectrophotometric method was validated in accordance with the International Conference on Harmonization (ICH) guideline in terms of system, linearity (calibration), precision, recovery, LOD, LOQ, accuracy, specificity, robustness, and stability.

Linearity:

The analytical method's linearity is its capacity to generate test results that are directly proportional to the concentration of the analyte in the sample. The linearity of this method was investigated between 0.2- 30 µg/mL of concentration of PCM. The result of the calibration curve showed an acceptable value of R^2 (0.9999) as shown in figure 3 and table 3. Depending on these results of calibration curve, indicating that the present method was linear according to ICH criteria.

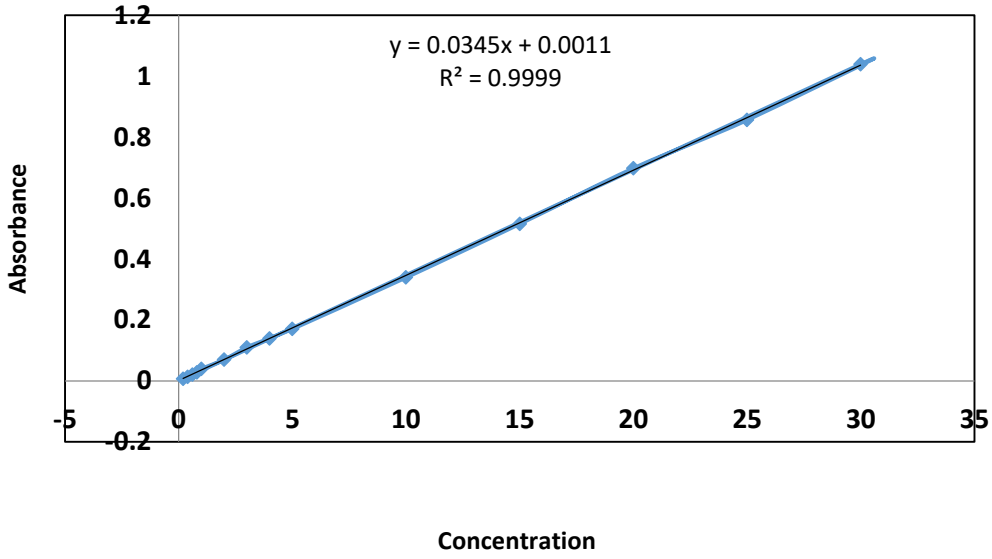

Figure 3: Calibration curve of PCM

Precision:

The precision pf the proposed method was evaluated by using deferent concentration of PCM, each concentration repeated three times according to the condition of the types of precision, such as Intra-day, Inter-day, and repeatability. According to standard criteria of ICH the method showed very good of RSD% value, less than 2%, the low values of the RSD% indicating the good precision of the method.

Table 1: Evaluation of precision study

Precision study	Sample Conc.	RSD%
Intra-day	5 µg/mL	0.34
	10 µg/mL	1.68
	15 µg/mL	0.11
Inter-day	5 µg/mL	1.13
	10 µg/mL	1.67
	15 µg/mL	0.29
Repeatability	5 µg/mL	0.33
	10 µg/mL	1.67
	15 µg/mL	0.30

Accuracy and Recovery:

An analytical technique's accuracy refers to how well its test findings align with the real value produced through the procedure. Initially, the calculation involved the recovery of predetermined amounts of PCM working standard that were applied to the samples. The experiment involved determining five duplicates of three sample concentration: 5, 10, and 15. The three sample of accuracy showed an acceptable RSD% value less than 2%, with small value of standard error, as shown in table 2.

Table 2: Evaluation of accuracy study

Sample Conc.	Mean of Recovery%*	RSD%	SEM
5 µg/mL	100.29	0.58	0.26
10 µg/mL	100.31	1.59	0.71
15 µg/mL	99.94	0.32	0.14

*Mean of five observations.

Specificity and Selectivity:

The specificity and selectivity of an analytical method is determined by its ability to reliably quantify the analyte's response, even in the presence of other substances such as impurities, degradation products, and matrix. The placebo solution, containing all excipients except PCM, was produced following the sample preparation process. In order to assess the effects of these inert chemicals, a validated method was employed to examine a mixture of standard solutions and commercially available pharmaceutical formulations of PCM. The method was further evaluated to verify the absence of any interference products that may arise from forced deterioration. The current Method exhibited relative standard deviation (RSD %) less than 1%. Indicating that the proposed method was selective and specified.

Sensitivity: Limit of detection (LOD) and Limit of quantification (LOQ):

The parameters employed in the sensitivity calculation were the Limit of Quantification and the Limit of Detection. The term LOD, or Limit of Detection, refers to the minimum concentration of an analyte in a sample that can be identified, but not accurately measured. The LOQ, or Limit of Quantification, refers to the minimum level at which an analyte may be quantified with satisfactory accuracy and precision. The proposed analytical method using calibration curve for determination of LOD and LOQ value, as shown in table 3. The following formulae were used to calculate LOD and LOQ.

LOD = 3.3× standard deviation of response/slope of the calibration curve

LOQ = 10× standard deviation of response/slope of the calibration curve

Table 3: Results of LOD and LOQ of the method

Parameters	**Value**
Range	0.2- 30 µg/mL
Regression eq.	y = 0.0345x + 0.0011
R^2	0.9999
Slope	0.0345x
Intercept	0.0011

SD	0.005
LOD µg/mL	0.47
LOQ µg/mL	1.44

Robustness:

In the proposed UV method, a small modification in wavelength (±1nm) was used to validate the robustness parameter. The data will be shown in Table 4. The relative standard deviation (%RSD) was below 1%, indicating a high level of robustness for the method.

Table 4: Robustness study of the method

Absorbance	245	246	248	249
Mean of abs.	0.168	0.171	0.168	0.165
SD	0.0013	0.0009	0.0010	0.0013
%RSD	0.78	0.52	0.60	0.79

Analysis of marketing formulation:

The validated method was implemented to evaluate the concentration of PCM in commercial tablets and syrup. Table 6 illustrates the results of the experiment. The RSD% was 0less than 2%, and the average of three determinations for the PCM in tablets and syrup dosage formulation that shown in table 5.

Table 5: Application PCM in both solid and liquid dosage form

S. D. Form	weight use	abs	weight obtained	recovery %	mean	SD	RSD
Tablet 1	20 µg/mL	0.689	19.97	99.86	99.71	0.81	0.81
		0.682	19.77	98.84			
		0.693	20.09	100.43			
Tablet 2	20 µg/mL	0.7	20.29	101.45	99.52	1.67	1.68
		0.68	19.71	98.55			
		0.68	19.71	98.55			
Tablet 3	20 µg/mL	0.71	20.58	102.90	100.97	1.67	1.66
		0.69	20.00	100.00			
		0.69	20.00	100.00			
Syrup 1	20 µg/mL	0.689	19.97	99.86	100.10	0.22	0.22
		0.691	20.03	100.14			
		0.692	20.06	100.29			
Syrup 2	20 µg/mL	0.71	20.58	102.90	100.97	1.67	1.66
		0.69	20.00	100.00			
		0.69	20.00	100.00			

Syrup 3	20 µg/mL	0.686	19.88	99.42	99.61	0.22	0.22
		0.687	19.91	99.57			
		0.689	19.97	99.86			

Additionally, the proposed method was successfully applied to detection of PCM in biological fluid. The results of this study are illustrated in Table 6. As can be seen from the concentration profiles in serum, the method is sufficiently sensitive for the analysis of paracetamol.

Table 6. determination of PCM in biological fluid

Serum S.	Dosage of PCM			Patient	
	µg/mL	Last dosage time	µg/mL found	Sex	Age
No. 1	1000	24 Hrs.	52 µg/mL	M	30±3
No. 2	1000	24 Hrs.	71 µg/mL	M	30±3
No. 3	1000	24 Hrs.	57 µg/mL	M	30±3
No. 4	1000	24 Hrs.	39 µg/mL	M	30±3
No. 5	1000	24 Hrs.	84 µg/mL	M	30±3
No. 6	Long time	-	No PCM	M	30±3
No.7	1000	24 Hrs.	85 µg/mL	F	30±3
No. 8	1000	24 Hrs.	36 µg/mL	F	30±3
No. 9	1000	24 Hrs.	41 µg/mL	F	30±3
No. 10	1000	24 Hrs.	12 µg/mL	F	30±3
No. 11	1000	24 Hrs.	31 µg/mL	F	30±3
No. 12	Long time	-	No PCM	F	30±3

Forced degradation studies

Acid degradation:

Under acidic conditions, the stress degradation investigation involved dissolving the PCM in solution containing 0.1 to 1.2 N of HCl and subjecting it to reflux for 12 hours. It was noted that the drug highly degraded when the concentration of acidic conditions increased over time, as shown in Figure 4 and table 7.

Alkaline degradation:

A research on alkaline hydrolysis of PCM was conducted by combining the medication with a solution containing 0.1 to 1.2 N of NaOH and subjecting it to reflux for 12 hours at a temperature of 60°C. The medication was discovered to undergo significant deterioration in an alkaline condition, with the rate of degradation increasing gradually after reaching 0.7 N in this particular stress situation, as shown in Figure 4 and table 7.

Oxidative degradation:

Under circumstances of oxidative stress, the drug is exposed to a solution containing 1-13% H_2O_2 for duration of 12 hours at room temperature. According to the study, the drug had a highly degradation under the conditions, as shown in figure 4 and table 7.

Thermal degradation:

In a research on stress degradation under temperature conditions, PCM was subjected for 24 hours at 30 to 90 ℃. As seen in figure 4, the degradation was determined to be less than 2.5% in this stress situation, indicating that the drug was stable, as shown in Figure 4 and table 7.

Photolytic degradation:

In this study Photolytic stress condition carried out in dry form. Here the drug was directly exposed to the sunlight for 6 to 60 hrs on a hot sunny day. The drug was found to be stable to sunlight and degraded less than 2.5% under this stress condition, as shown in Figure 4 and table 7.

Table 7: Results of Forced degradation study of PCM

Different stressed conditions	Percentage degradation of PCM	Remarks
Acidic (0.1 – 1.2) N	8-42 %	Significant degradation
Alkaline (0.1 – 1.2) N	12- 22 %	Significant degradation
Oxidation (1 – 13) %	4.5 – 37 %	Significant degradation
Thermal (30-90)°C	0.5-1.8 %	No degradation
Photolysis (6- 60) hrs.	0.3- 1.25 %	No degradation

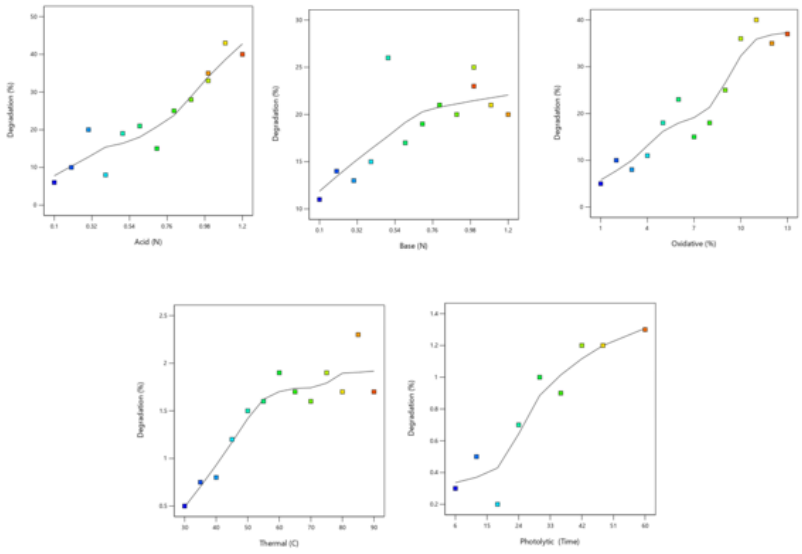

Figure 4: Stress degradation study of PCM

Comparison for the present method with other methods in the literature:

A comparative analysis with other established methods, such as High-Performance Liquid Chromatography (HPLC), Ultra-performance liquid chromatography (UPLC), and traditional UV-Vis spectrophotometry, was conducted as shown in table 8.

Table 8: Comparative Analysis of Nanodrop 2000c for detection of PCM with other established methods.

Method (system)	R^2	Precision (%RSD)	Rec. %	LOQ	LOD	Degradation %					Ref.
						Acid	Alkaline	Oxi.	Photo.	Thermal	
HPLC	0.9998	1.231	99.50	0.3	0.5	Degrade	Degrade	Degrade	No change	No change	(Abdelaleem and Abdelwahab, 2013)
LC	0.9997	0.20	98	-	-	Degrade	Degrade	Degrade	Degrade	No change	(Rajesh And Shrawa, 2012)
HPLC	1	1.28	96.55	0.01	0.02	Degrade	Degrade	Degrade	No change	No change	(Aminu et al., 2019)
UV	0.9905	1.87	99.09	0.59	2	-	-	-	-	-	(Murtaza et al., 2011)
RP-HPLC	0.999	≤ 2.0	100 ±3	2.3	7.9	Degrade	Degrade	Degrade	No change	No change	(Jahan et al., 2014)
UV Nano.	0.9999	< 1	100 ±0.3	0.47	1.44	Degrade	Degrade	Degrade	No change	No change	P. method

CONCLUSION

The quality of pharmaceutical products is crucial for patient safety, and drug stability ensures effective administration of therapeutic dosages, as degrading substances can significantly impact their effectiveness.

This study developed a stability-indicating UV approach that is straightforward, sensitive, accurate, linear, precise, reproducible, repeatable, specific, and robust for detecting PCM in the presence of its degradants. An investigation was conducted to examine the response of PCM under various stress conditions. The method is sufficiently sensitive to quantitatively detect the analytes in pharmaceutical preparations and biological fluid. Therefore, it can be utilized for routine analysis, quality control, and checking the quality during stability studies of pharmaceutical preparations and biological fluid containing these drugs.

REFERENCES

Kamble, R. M., & Singh, S. G. (2012). Stability-Indicating RP-HPLC Method for Analysis of Paracetamol

and Tramadol in a Pharmaceutical Dosage Form. Journal of chemistry, 9(3), 1347-1356.

Abdelaleem, E.A. and Abdelwahab, N.S. (2013), "Validated stability indicating RP-HPLC method for determination of paracetamol, methocarbamol and their related substances", *Analytical Methods*, Vol. 5 No. 2, pp. 541–545.

Aminu, N., Chan, S.Y., Khan, N.H., Farhan, A.B., Umar, M.N. and Toh, S.M. (2019), "A simple stability-indicating hplc method for simultaneous analysis of paracetamol and caffeine and its application to determinations in fixed-dose combination tablet dosage form", *Acta Chromatographica*, Vol. 31 No. 2, pp. 85–91.

Desjardins, P.R. and Conklin, D.S. (2011), "Microvolume quantitation of nucleic acids", *Current Protocols in Molecular Biology*, No. SUPPL.93, pp. 1–16.

Graham, G.G., Davies, M.J., Day, R.O., Mohamudally, A. and Scott, K.F. (2013), "The modern pharmacology of paracetamol: Therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings", *Inflammopharmacology*, Vol. 21 No. 3, pp. 201–232.

Gupta, K.R., Likhar, A. and Wadodkar, S.G. (2010), "Application of Stability Indicating HPLC Method for Quantitative Determination of Etoricoxib and Paracetamol in Pharmaceutical Dosage Form Health risk assessment and estimation of Essential trace elements in drinking water View project Application of Stab", *Eurasian J. Anal. Chem*, Vol. 5 No. 3, pp. 218–226.

Hadad, G.M., Emara, S. and Mahmoud, W.M.M. (2009), "Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms", *Talanta*, Vol. 79 No. 5, pp. 1360–1367.

Hussein, A.D. (2020), "UV Spectrophotometric Development and HPLC Methods for Estimation of Paracetamol in Oral Syrup Formulation: Validation, Comparison and Application", *International Journal of Psychosocial Rehabilitation*, Vol. 24 No. 4, pp. 6308–6316.

Jahan, M.S., Islam, M.J., Begum, R., Kayesh, R. and Rahman, A. (2014), "A study of method development, validation, and forced degradation for simultaneous quantification of paracetamol and ibuprofen in pharmaceutical dosage form by RP-HPLC method", *Analytical Chemistry Insights*, Vol. 9 No. 1, pp. 75–81.

Murtaza, G., Hussain, I., Khan, S.A., Shabbir, A., Mahmood, A., Asad, M.H.H. Bin, Farzanal, K., et al. (2011), "Development of a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets", *Scientific Research and Essays*, Vol. 6 No. 2, pp. 417–421.

Okai, C., Orman, E. and Agyenim–Boateng, A. (2016), "Validation of Titrimetric-UV Spectrophotometric Method for the Simultaneous Quantification of Paracetamol, Caffeine and Ibuprofen in Pharmaceutical Dosage Forms", *British Journal of Pharmaceutical Research*, Vol. 12 No. 4, pp. 1–14.

Qasim, F.O., Hami, M.A. and Yousif, N.I. (2024), "developing and validating a stability-indicating uv-vis nanodrop 2000c method for the simultaneous determination of the anti-hypertensive drug hydrochlorothiazide (hctz) in both bulk and tablet dosage forms", Vol. 12 No. 3, Pp. 375–381.

Qasim, F.O. and Mohammed, N.M.S. (2021), "A Nanodrop Spectrophotometric Method and Stability Indicating for Determination of Amlodipine Besylate in Pharmaceutical Formulations of Kurdistan of Iraq", *Science Journal of University of Zakho*, Vol. 9 No. 1, pp. 25–29.

Rittau, A.M. and McLachlan, A.J. (2012), "Investigating paracetamol pharmacokinetics using venous and capillary blood and saliva sampling", *Journal of Pharmacy and Pharmacology*, Vol. 64 No. 5, pp. 705–711.

Rutherford, C.Z. (2020), "Optimization of DNA Extraction from Human Milk", available at: https://digitalcommons.winthrop.edu/cgi/viewcontent.cgi?article=1121&context=graduatetheses.

Srivastava, M.K., Ahmad, S., Singh, D. and Shukla, I.C. (1985), "Titrimetric determination of dipyrone and paracetamol with potassium hexacyanoferrate(III) in an acidic medium", *The Analyst*, Vol. 110 No. 6, pp. 735–737.

von Thaden, A., Nowak, C., Tiesmeyer, A., Reiners, T.E., Alves, P.C., Lyons, L.A., Mattucci, F., et al. (2020), "Applying genomic data in wildlife monitoring: Development guidelines for genotyping degraded samples with reduced single nucleotide polymorphism panels", *Molecular Ecology Resources*, Vol. 20 No. 3, pp. 662–680.