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Spectrophotometric Method Validation for Estimation of Paracetamol Dosage Form Using 2-Chloro-5-Methoxy Aniline

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Abstract

In this study, a novel spectrophotometric technique has been devised that is quick and easy to use for quantifying paracetamol (PCM.) in tablets and in its pure form. The recommended process consists of two steps: First, a 2-chloro-5-methoxy aniline (2-Cl-5-OMeAn.) reagent's primary amine group is diazotized by reacting with sodium nitrite in an acidic medium to create the corresponding diazonium salt of (2-Cl-5-OMeAn.) Secondly, the diazonium salt reacts with (PCM.) in a basic medium to form a yellow-orange-colored, stable, and water-insoluble azo dye that showed a maximum absorption at 480 nm. In this study, several variables influencing the responses were optimized. The azo dye produced using (PCM.) and (2-Cl-5-OMeAn.) as a coupling reagent follows Lambert Beer's law in the (20–140 µg.mL^{-1}) range with a 0.9995, 1.7337 × 10^{+3} M^{-1}. cm^{-1}, and 0.123 for coefficient of correlation, a molar absorptivity and sandells' sensitivity, respectively. The method has been validated statistically, offering low detection and quantification limits, high recovery percentages (> 98%) and low relative standard deviation values. The method was successfully used to evaluate traces of paracetamol in the dosage formulation (Piodol, 500 mg/tablet, PiONEER Co.).

Keywords: UV Spectrophotometry, Diazo coupling reaction, Paracetamol, Validation method, 2-Chloro-5-Methoxy Aniline.

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Introduction

Acetaminophen (an international phrase used in the US), often known as paracetamol (PCM.), a global term that's used in Europe, has the chemical formula $C_8H_9NO_2$ with a molecular weight of 151.163 g/mol, as shown in figure (1). It is also known by several commercial names, such as panadol, tylenol (which is derived from N-acetyl-paminophenol), and panadol extra [1, 2]. An antipyretic analgesic drug that is commonly used is paracetamol [3]. (PCM.) is among the most well-known chemical antipyretic and non-steroidal anti-inflammatory medications in general use [4]. To determine the amount of paracetamol in pharmaceutical formulations, numerous techniques have been developed. These techniques include the following: voltammetry [5], HPLC [6, 7], voltammetric utilizing ionic liquids and carbon paste electrodes altered with CdO nanoparticles [8], the electrochemical sensor [9-13],

RP-HPLC [14-18], HPTLC and RP-HPLC [19], Quantitative Thin-layer Chromatography (TLC)[20], Gas Chromatography [21], Flow Injection Analysis (FIA) (using different methods of detection) [22], and spectroscopy [22-32]. The purpose of this study was to validate a novel analytical technique for (PCM.) quantification analysis employing (2-Cl-5-OMeAn.)as a chromogenic coupling reagent. The validation process confirmed the method's effectiveness through statistically significant results. The suggested method demonstrates a number of significant advantages, such as, low limits of detection and quantification, high recovery percentages exceeding 98 %, and low relative standard deviations.

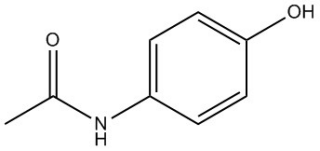

Figure 1: Acetaminophen, or paracetamol, as in its chemical form.

Experimental method

Chemical reagents and equipment

The equipment used was: All spectrophotometric analyses have been taken through a CECIL 7200 UV-Visible double beam spectrophotometer equipped with 1 cm matched quartz cells, and the absorption spectra were recorded. Analytical balance (Shimatzu/AUY220), digital pH meter (DelLab®/DLA-PH), and hot plate magnetic stirrer (Jenway).

Every chemical and reagent used was of the purest grade (analytical grade) that exists. The chemicals were provided by multiple companies, including pure paracetamol from (Awamedica Company for Drug Industries and Medical Applications, Awa, Erbil, Iraq) in pure form, sodium hydroxide and sulfamic acid from (Fluka), hydrochloric acid (37%, Synth®), sodium nitrite was obtained from (BDH), paracetamol (Piodol, with a labeled amount of 500 mg/tablet, PiONEER Co.) purchased from a local market, 2-chloro-5-methoxy aniline (Sigma-Aldrich) with 98% purity, and others such as KOH, HNO_3, C_2H_5OH, HCl, $NaHCO_3$, CH_3COOH, and H_2SO_4, which are bought from numerous companies.

The solutions were prepared as follows:

- By dissolving 0.1 g of authentic, pure sample paracetamol in distilled water (DW) and pouring the mixture into a volumetric flask with a capacity of 100 mL filled with DW, a standard stock of (PCM.) solution with 1000 $\mu g.mL^{-1}$ was created.
- After dissolving 1.0 g of (2-Cl-5-OMeAn.) in 10 ml of absolute ethanol, the mixture was transferred to a volumetric flask with a volume of 250 mL to create a solution (0.025 mol. L^{-1}) of (2-Cl-5-OMeAn.). After adding DW, the flask was shaken ferociously for duration of one to two minutes.
- Using DW, dissolve 3.0 g of $NaNO_2$ and transfer to a 100-ml volumetric flask. Fill to the mark with DW. This is the Sodium Nitrite Solution, 3% (w/v).
- A 25 mL volumetric flask with DW was used to dilute 6 mL of concentrated HCl 37% to the appropriate amount in order to create a hydrochloric acid solution (3M).
- 6.0 g of NaOH was dissolved in a small amount of DW to create sodium hydroxide solution (3M), which was then diluted to the proper amount in a 50 mL volumetric flask utilizing DW.
- 1.5 g of pure sulfamic acid (SA) was dissolved in DW to create a sulfamic acid (3% w/v) solution, which was then transferred to a 50 mL volumetric flask. There was just enough DW in the volume.

General procedure and preparation of the calibration curve.

Depending on the most suitable conditions that several studies have generated, from the standard drug solution (1000 µg/ml), pipette out from 0.5 to 3.5 ml and dilute to 25 ml of volumetric flaks to produce solutions with 20 to 140 µg.mL^{-1} of pure (PCM.), respectively. Then, one milliliter of 3M HCl solution, 1.5 ml of 3% w/v sodium nitrite solution, and then 1.5 ml of 3% w/v sulfamic acid solution to remove the excess nitrite were placed in each flask. The reaction mixtures were thoroughly mixed for 5 minutes and cooled in an ice bath for 2-5 minutes. The reactions were shaken and allowed to stand for 5 minutes. After that, 1 ml of (2-Cl-5-OMeAn.)(0.025 M) solution with 2 ml of 3M NaOH was added and allowed to stand for 10 minutes. The contents of each flask were mixed well and diluted to the mark with distilled water. At 480 nm, using 1-cm quartz cells, the absorbance spectra of each flask solution were measured against the corresponding reagent blank solution (solution made using the same technique without paracetamol). Finally, the graph of absorbance against concentrations was plotted as shown in Figure 2, which presents a graphic representation of the readings, which are reported in Table 1.

Procedure for assaying paracetamol in tablets

To estimate the concentration of (PCM.) in tablet formulation by this method, 12 tablets were weighted and triturated into a fine powder, and an accurately weighted amount of powder equivalent to 0.1 g paracetamol was dissolved in 10 ml of DW, transferred to a 100 ml volumetric flask, and made up to the volume with DW. After that, 0.5 and 0.75 ml of solution were pipetted and transferred into two volumetric flasks of 25 ml to give solutions A and B of 20 and 30 µg.mL^{-1}, respectively. Such a general procedure was carried out, as was carried out for 500 mg (PCM.)/tablets. Similarly, the absorbance of the sample solutions was measured at a wavelength of 480.0 nm, against a blank solution.

Results and Discussion

Optimization of reaction variables

In order to identify the optimal conditions for (PCM.) determination, the effect of several variable factors on the color development of azo day was investigated. In order to perform the optimization procedures, each parameter was changed individually while keeping the others fixed. The experiment's test results, as displayed in tables 2–10, were selected for the reaction because they gave a high intensity, strong constant color for the azo dye with a corresponding low reagent blank absorbance. During the investigation, 140 µg.mL^{-1} of paracetamol in a final volume of 25 mL was used. Some experiments were achieved as follows:

1. **Effect of the Reagent's Volume:** When a study was carried out on the effect of reagent volume (0.2-1.6 mL) on absorbance intensity, it was determined that 1.0 mL was the optimum amount, as shown in Table 2.
2. **Base effects the coupling reaction:** As table 3 shows, research has generally examined various types and its concentrations of strong and weak bases. Based on the results, it can be concluded that strong bases provide high contrast and intensity and that yellow azo dye is stable. On the other hand, sodium bicarbonate produces a colored solution after 30 minutes. The volume chosen for the ensuing tests is 2 mL of 3 M (final reaction mix, pH = 11.7) sodium hydroxide.
3. **Impact of the quantity of sodium nitrite:** The color products' absorbance at 480.0 nm in the range of (0.1–2) mL was measured, as table 4 shows, in order to investigate the impact of the $NaNO_2$ volume (mL). A consistent and maximum color intensity of the azo dye complex was observed at 1.5 mL of 3% solution sodium nitrite, despite the high absorbance values.

4. **The Impact of Various Acids:** For diazotization reactions, a range of acidic solutions (H_2SO_4, HCl, HNO_3, and CH_3COOH) have been investigated at concentrations of 3 M. Based on stability considerations for diazotization and its maximum absorbance values, HCl was determined to be the most effective among them; table 5 illustrates the results of this study.
5. **Acidity volume effects on diazotization:** table 6 illustrates how the diazotization process was affected by a hydrochloric acid volume that was investigated within the range of 0.5–2.5 mL. The addition of 1.0 mL of 3 M HCl produced maximum and continuous absorption intensities; as a result, this effect's optimal value was determined to be 1.0 mL of 3 M.
6. **Impact of Sulfamic Acid Amount:** Adding several amounts of a 3% w/v sulfamic acid solution (0.1 to 2 mL) allowed for the estimation of the ideal sulfamic acid volume; the greatest absorbance value was observed with 1.5 mL of the 3% (m/v) solution. Table 7 illustrates the impact of sulfamic acid concentration, which was applied to eliminate the excess nitrite.
7. **Effect of reagents mixing order:** The investigation of the effects of varying component addition orders on azo dye production involved introducing reactants in four distinct orders, as indicated in table (8). Given that the second mixing order produced the highest absorbance, it is clear from the data that this order should be followed in the next tests.

8. **Coupling reaction time's effect:** By monitoring the development of the color azodye at room temperature, the ideal moment to coupling the reaction was determined. After varying the duration of time, the reaction mixture was left to stand, it was discovered that a full color development took ten minutes; table 9 shows that the color remained stable for at least sixty minutes.

9. **Diazotization Reaction Time's Effect:** By observing the absorbance of the produced azo-dye for a period of 0 to 15 minutes, the optimal diazotization reaction time was determined. It was found that no additional increase in absorbance values was seen, and that a colored product with a maximum absorbance at 480.0 nm occurs instantaneously (Table 10).

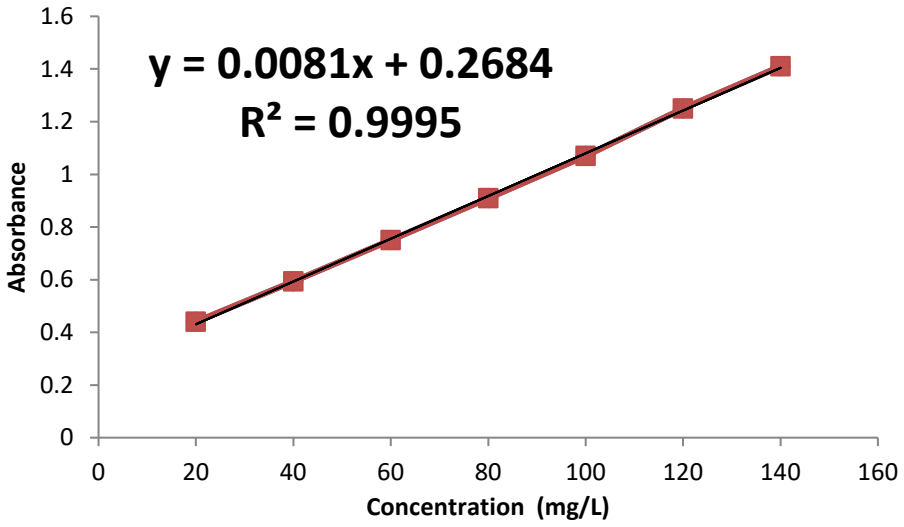

Figure 2: Standard calibration curve for estimation of (PCM.) via ideal condition calculation.

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Table 1: The calibration curve data for the estimation of (PCM.).

S. No.	Concentration of (PCM.) (µg.mL^{-1})	Absorbance*
1	20	0.44
2	40	0.593
3	60	0.75
4	80	0.91
5	100	1.07
6	120	1.25
7	140	1.41

Table 2: Effect of (2-Cl-5-OMeAn.) amount.

Volume (ml) of reagent	Absorbance*
0.2	0.14
0.4	0.198
0.6	0.2
0.8	0.232
1	1.62
1.2	1.57
1.4	1.5
1.6	1.48

Table 3: effects of base type and quantity on color development in 140 µg.mL-1 (PCM.).

Base solution	Absorbance* / ml of 3 M base added									
	pH	1	pH	1.5	pH	2	pH	2.5	pH	3
NaOH	11.6	0.749	11.7	0.76	11.7	0.78	11.8	0.765	11.9	0.758
KOH	11.8	0.734	11.8	0.725	11.9	0.741	12	0.738	12	0.74
$NaHCO_3$	10.5	0.366	10.7	0.441	10.9	0.602	10.9	0.67	11.1	0.69

Table 4: effect of 3% w/v $NaNO_2$ amount.

Volume (mL) of $NaNO_2$ (3% w/v)	Absorbance*
0.1	0.37
0.5	0.56
1	0.67
1.5	0.69
2	0.65

Table 5: influence of diazotization acids on color development during 140 µg.mL^{-1} (PCM.) determination.

Acidic solution with 3M	Absorbance* / mL of acid used
CH_3COOH	0.298
HNO_3	0.520
H_2SO_4	0.585
HCl	0.632

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Table 6: effect of HCl (3 M) amounts.

Volume (mL) of HCl	Absorbance*
0.5	0.23
1	0.42
1.5	0.37
2	0.345
2.5	0.34

Table 7: effect of 3% w/v sulfamic acid amounts.

Volume (mL) of sulfamic acid	Absorbance*
0.1	0.31
0.5	0.35
1	0.35
1.5	0.37
2	0.32

Table (8): Changes in the sequence of reactant in addition and absorbance possess an impact on the color development when determining the PCM of 140 µg/mL.

No.	Sequence	Absorbance*
1	N+M+O+P	1.02
3	M+O+N+P	1.3
2	M+N+O+P	1.26
4	N+O+P+M	0.87

M: (PCM.) N: (2-Cl-5-OMeAn.) O: sodium nitrite P: sodium hydroxide

Table 9: impact of coupling time to reaction.

Time (min)	Absorbance*
0	0.624
10	1.025
20	1.021
30	1.02
40	1.029
50	1.027
60	1.021

Table 10: Effect of diazotization time to reaction.

Time (min)	Absorbance*
0	0.522
5	0.696
10	0.687
15	0.656
20	0.676

Proposed Method Validation and Optical Properties

Table 11 provides a detailed overview of the optical and validation properties of the developed approach and the related results.

Table 11: Optical and validation characteristics of the proposed method for determination of (PCM.).

Parameters		Values
Beer's law limit (µg.mL^{-1})		20-140
Color		Yellow to orange
λmax* (nm)		480
Regression equation (y*=a+bc)	Slope, b (L/mg)	0.0081
	Intercept, a (a=y-bc)	0.2684
Correlation coefficient (r^2)		0.9995
Precision expressed as RSD %	Intra-day	0.25
	Inter-day	0.4
Accuracy (recovery %)		98.72 - 100.35
LOD (µg.mL^{-1})		7.74
LOQ (µg.mL^{-1})		23.47
Sandell's sensitivity (µg.mL^{-1}/0.001 abs. unit)		0.123
Molar absorptivity (M^{-1}. cm^{-1})		$1.7337 \times 10^{+3}$

y = b + ac, where c is the concentration in µg.mL^{-1} and y is absorbance unit.

* Absorbance reading at 480 nm.

The spectrophotometric method for the estimation of (PCM.) was validated for different parameters like:

1. Precision

The precision of the method was demonstrated by performing intra-day and inter-day studies. In this study, three different solutions of the same concentration, which is 40 µg. mL^{-1}, were prepared and analyzed. For the intra-day variation, absorbance was measured three times daily; for the inter-day variation, it was measured three times over three days, and the absorbance was noted. The result was indicated by % RSD (table 12, A).

2. Accuracy

Recovery studies using the standard addition method were used to evaluate the accuracy of the proposed method. A pure standard drug solution was combined with a known amount of riluzole (10 µg/mL) from the dosage form at three different concentrations (20, 25, and 30 µg. mL^{-1}). Following a reanalysis of the resultant solutions using the suggested methodology, the recovery percentage in Table 12, B was determined.

Table 12: Evaluation of the suggested method's accuracy (A) and precision (B) for determining the value of (PCM.).

	Conc. Of STD (µg.mL^{-1}) added	Conc. of sample µg.mL^{-1}	Absorbance*	Total conc. µg.mL^{-1}	Recovery of STD	Recover of STD %
A	20	10	0.512	30.07	20.07	100.37
	25	10	0.549	34.68	24.68	98.72
	30	10	0.591	39.87	29.87	99.57

	Declared conc. (µg.mL^{-1})	Intra-day precision Data		Inter-day precision Data	
		Conc. Found (µg.mL^{-1})	Precision (% RSD)	Conc. Found (µg.mL^{-1})	Precision (% RSD)
B	40	40.1	0.25	39.89	0.4
		39.93		40.2	
		40.11		39.96	

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Application of the suggested method analytically.

By performing the procedure described in the experimental section, the suggested method was used to determine the amount of (PCM.) in pharmaceutical form that was obtained from the local market. There were two form concentrations used: 20 and 30 µg.mL^{-1}. Table 13 displays the results. Low R.S.D. percentage (less than 1%) indicates good precision. Error percentage (0.13 – 0.18) % and Recovery percentage (100.13 – 100.18) % indicate good accuracy, and amount of paracetamol was found to be 500 mg/tablet. The approach can be effectively used to determine (PCM) in drugs, demonstrating the method's good sensitivity.

Table 13: Analytical application of the proposed method to determination of paracetamol concentration in dosage form (tablets).

Concentration (µg.mL^{-1})	**Present[a]**	20	30
	Found[a]	20.01	29.96
		19.98	30.3
		20.09	29.91
Relative error %		0.13	0.18
Mean Recovery %		100.13	100.18
R.S.D %		0.28	0.7

[a] dosage form (Piodol, 500 mg/tablets paracetamol, PiONEER-Sulaimanya Iraq).

Absorption spectra

The primary test for the present method involved diazotization of (2-Cl-5-OMeAn.) in the presence of sodium nitrite in an acidic medium, then the product reacted with PCM in a solution of alkaline to create a colored final product. The experiment was executed out by adding 1.0 mL of 0.025 M (2-Cl-5-OMeAn.), 1 ml of 3% w/v sodium nitrite, 1 ml of 3 M HCl, and 3.5 mL of 1000 µg.mL-1 of PCM, then 1 ml of 3 M sodium hydroxide under shaking conditions in a 25 milliliter volumetric container. The contents were diluted to the mark with distilled water. The lamda max of the colored product was measured against the reagent blank. The greatest absorption has been determined at the wavelength of 480 nm, as Figure 3 illustrates.

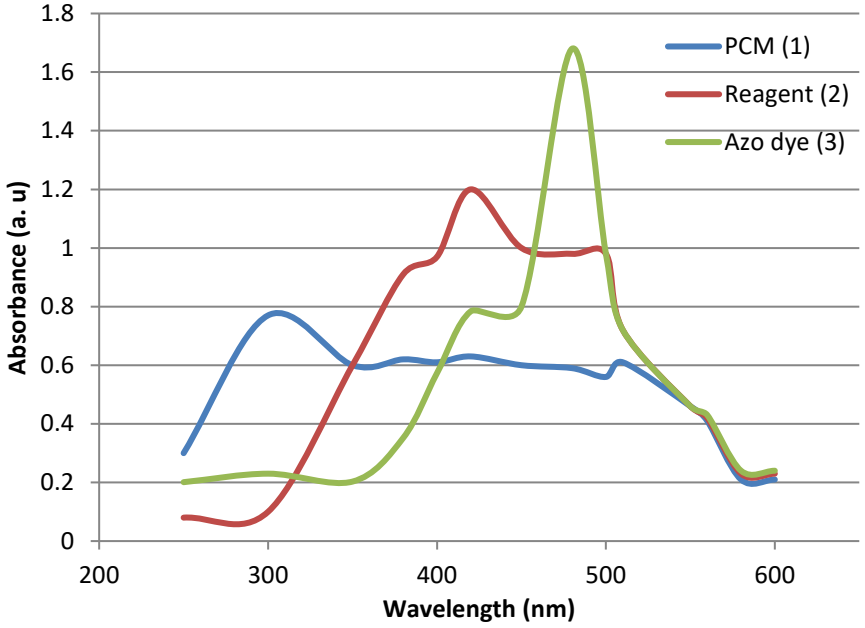

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Figure 3: (1) Absorption spectrum of 140 mg/mL paracetamol (PCM.). (2) Absorption spectrum of (2-Cl-5-OMeAn.) (3) Absorption spectrum of the azo dye formed, (PCM.)/ (2-Cl-5-OMeAn.).

Nature of the Azo dye product.

Using the mole-ratio approach[33], the stoichiometry of the azo dye was investigated at the specified conditions. According to the experimental data method (Figure 4), diazotized 2-chloro-5-methoxy aniline and (PCM.) were combined 1:1 to generate the yellow azo dye. As a result, the dye might have the suggested structure shown in Figure 5.

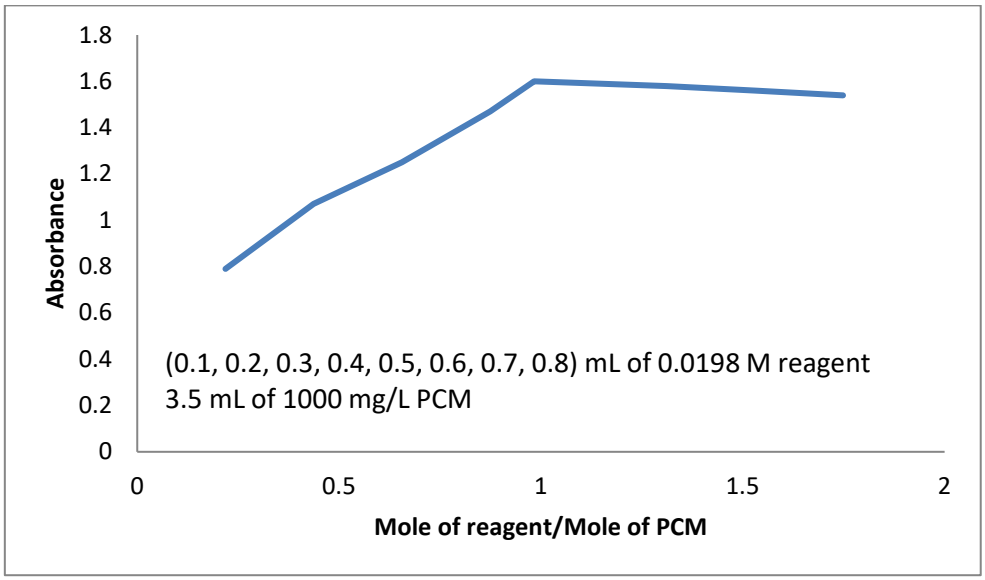


Figure 4: Mole-ratio plot of the reaction between the (PCM.) as well as (2-Cl-5-OMeAn.).

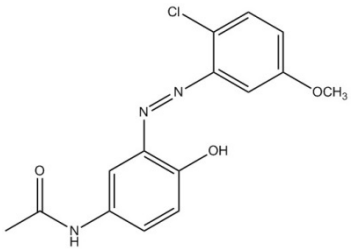
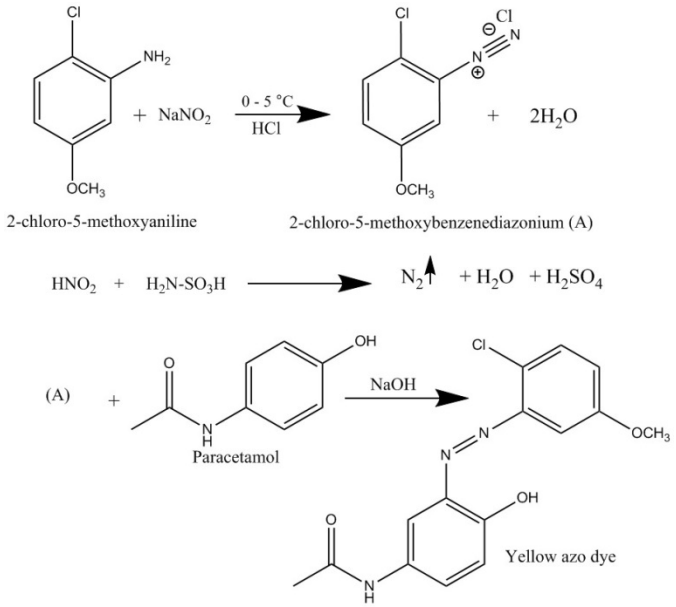


Figure 5: Suggested chemical structure of azo dye.

Route of the Reaction

The reaction process between (PCM.) and (2-Cl-5-OMeAn.) is expected to be followed during the current experiment, according to Scheme 1.



Scheme 1: The reactions suggested mechanism.

Comparison of techniques

Table 14 shows the analysis variables acquired with the current spectrophotometric method and the current approach compared.

Table 14: the comparison of the methods.

Analytical parameters	**Present method**	**Literature method [34]**
pH	11.7	11.77
Temp. (°C)	At room temp.	At room temp.
λ_{max} (nm)	480	470
Medium or reaction	Ethanol : water	Aqueous
Reagent	2-chloro-5-methoxy aniline	Acetophloroglucinol
Beers law rang	20 – 140 µg.mL^{-1}	0.4 – 7.2 µg.mL^{-1}
Molar absorptivity (L. mol^{-1}.cm^{-1})	1.7337 × 10^{+3}	2.16 ×10^{4}
Colour of dye	Yellow to orange	Orange
Nature of dye	1:1	1:1
Type of reaction	Diazotisation and coupling	Diazotisation and coupling
Application of method	Pharmaceutical preparations	Pharmaceutical preparations

The suggested technique appears to have potential for identifying the substance (PCM.) in its dosage form with useful analytical properties. Table 11 indicates that the method exhibits an excellent linear relationship between the concentration and absorbance within the 20–140 µg.mL^{-1} range, in accordance with Beer's law. The method is noteworthy for its ability to detect and quantify at low concentrations, as evidenced by its low limit of detection (LOD) of 7.74 µg.mL^{-1} and low limit of quantification (LOQ) of 23.47 µg.mL^{-1}. Recovery percentages within the tested concentration range, ranging from 98.72 % to 100.37 %, provide evidence for the

method's accuracy. With low relative standard deviation (RSD) values of 0.25% intra-day and 0.4% inter-day, the method also exhibits good repeatability.

Compare the proposed method with another.

Table 15 shows different parameters between the HPLC and spectrophotometry analysis [35].

Parameters	Method 1	Method 2
Reference	1	Proposed
Wavelength (nm)	243	480
Technique	HPLC	UV- VIS. spectrophotometry
Application of method	Tablets, Syrups, drops, Suppositories, and industrial waste water	Tablets
Linear range (µg/ml)	10 – 100 µg/ml	20 – 140 µg/ml
Method accuracy (Recovery %, A) and precision (RSD %, B).	A= > 100% B= 0.962%	A= > 98% B= 0.25 for intra-day, and 0.4 for inter-day
Limit of detection (LOD), and limit of quantification (LOQ)	LOD= 3 ng/ml LOQ= 9 ng/ml	LOD= 7.74 µg/ml LOQ= 23.47 µg/ml
Linear equation	y=3269x -203.8	y=0.0081x+ 0.2684
Temperature	At room temp.	At room temp.

Conclusion

The results of the study showed that the recommended method for determining trace amounts of paracetamol in its pure and pharmaceutical form has good sensitivity and product stability. The recommended spectrophotometric method yields precise and reliable results without requiring a solvent extraction or separation step; it is simple, sensitive, and affordable. The process was based on the reaction of coupling between (PCM.) and diazotized (2-Cl-5-OMeAn.) in a basic medium.

In summary, this study successfully introduced a novel technique for spectrophotometric quantification of acetaminophen utilizing the diazo reaction with (2-Cl-5-OMeAn.)as a chromogenic coupling reagent. This method was demonstrated to be efficient for analyzing acetaminophen (paracetamol) in both its pure and tablet forms.

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