Spectrophotometric Determination of Paracetamol Drug by Use Methyl 2- Phenyl Salponyl Acetate (M10) Lap Reagent Prepared

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KEYWORDS

Azo dye, determination, hydrazine, paracetamol, spectrophotometric

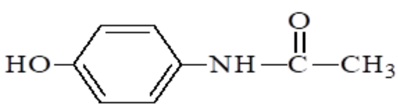
ABSTRACT

Sensitive, fast, accuracy spectrophotometric method developed to determination of paracetamol drug depend on reaction between drug and sodium nitrite solution in acid medium to produced dizonium salt and react with M10 reagent in basic solution, the reaction produced a final yellow and stable product that remained unchanged for over 60 minutes, providing sufficient time to analyze multiple samples. The highest absorption occurred at 432 nm, and the reaction follows Beer's law applies to concentrations between 5 and 40 µg/ml. The molar absorption coefficient is 2262.21 liters/mol/cm, and the Sandel sensitivity is 0.0568 µg/cm^2, with a correlation coefficient (r) of 0.9996. The Limit of Detection (LOD) is 0.2522 µg/ml, and the Limit of Quantification (LOQ) is 0.7641 µg/ml. This method was successfully used to analyze paracetamol in pharmaceutical preparations.

1. Introduction

Paracetamol, also known as acetaminophen, Paracetamol is a common pain reliever and fever reducer. Its chemical name is N-acetyl-p-aminophenol, and its chemical formula is C8H9NO2. Paracetamol was first developed by the American chemist Harmon Northrop Morse. (2) in 1878, as a combination of acetylacetone and ammonia. It is marketed under various brand names, including Panadol, Tylenol (derived from N-acetyl-para-amino phenol), and Panadol Extra (3). Paracetamol is often an ingredient in many cold and flu remedies and is available in tablet, liquid, capsule, injection, and syrup forms (5). When taken in appropriate doses, paracetamol is generally safe for adults, with the maximum recommended daily dose being 3 or 4 grams (6,7). However, high concentrations can lead to poisoning, with potentially serious consequences. In some cases, overdose of paracetamol can result in cytolytic hepatitis, caused by a toxic metabolite known as N-acetyl-p-benzoquinone-imine causes dose-dependent damage to liver cells and, in some cases, death (1).

Paracetamol is used to relieve severe pain, including cancer-related and postoperative pain, in combination with opioid-based treatments (8). It specifically affects the brain, blocking the enzyme involved in pain transmission. Its mechanism of action differs from other pain relievers, yet it effectively alleviates pain throughout the body (9). Paracetamol is listed on For a healthcare system, the most effective and safest option is the World Health Organization's List of Essential Medicines.. (10). High-performance liquid chromatography is commonly used to evaluate paracetamol in pharmaceuticals, while spectrophotometric technology remains popular (11). Various methods, including HPLC, RP-HPLC, HPTLC, voltammetry, electrical techniques, and spectrophotometry, have been documented for determining paracetamol in pharmaceutical preparations (12-29). The literature includes several spectrophotometric methods for measuring paracetamol (30).



Scheme 1: Paracetamol structure

Experimental

Instruments

All spectrophotometric measurements were performed using a T90 UV-Visible Spectrophotometer manufactured by PG Instrumental Ltd. (UK). A 10mm quartz cell was used for these measurements. The Sartorius Balance 210S Kern was utilized for precise weight measurements.

2. Methodology

Chemicals

Solvents and chemicals were sourced from American companies Aldrich and Fluka for the project. Stuart melting point apparatuses were used for the purpose of measuring melting points. To get the infrared spectra, a Shimadzu FTIR-8100 spectrophotometer was used in conjunction with KBr discs. The 13C-NMR and 1H-NMR spectra were acquired using the same MHZ spectrometer in conjunction with DMSO-d6 as the solvent. The purity of compounds was determined using TLC on alumina sheets percolated in silica gel (type 60 F254 Merck, Darmstadt, Germany). No additional purification was performed before using the chemicals and solvents. The State Company for Drug Industries and Medical Appliance (SDI), Samarra, Iraq, supplied the paracetamol. In order to make all of the solutions, distilled water was used. Paracetamol from Panda, Joswe, Piodol, Pioneer, Panadol, and GlaxoSmithKline, Ireland, were also used. Distilled water was also used to prepare these solutions.

Solutions

In a volumetric flask, 0.25 g of paracetamol powder was dissolved in distilled water to create a 1000 µg/ml solution. The volume was subsequently increased to 250 ml. To make the reagent solution, a 250 ml volumetric flask was filled to the mark with ethanol after dissolving 0.535g of the reagent that had been made in the lab. To make the sodium nitrite solution, dissolve 0.1725 grams of sodium nitrite powder in distilled water. Fill a 250 ml volumetric bottle with more distilled water until it reads the mark. In addition, 100 mL volumetric flasks were used to dilute hydrochloric acid, nitric acid, and sulfuric acid solutions with distilled water.

Additionally, solutions of 1.0 M Sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, prepared in 100 mL volumetric flasks.

Solutions of interfering substances

A solution (glucose, fructose, lactose, sucrose, magnesium citrate, sodium sulfate, starch, cellulose) was prepared 1000 µg/ml by dissolving 0.1000 grams of it in 5 ml of ethanol, then adding the volume to 100 ml of distilled water.

Sample Preparation

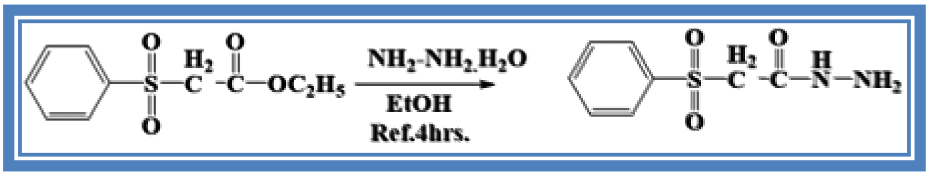
The pharmaceutical solution was prepared at a concentration of 1000 µg/ml by taking ten tablets of four different pharmaceutical preparations containing Paracetamol (Paracetamol, Panda, Piodol, Panadol). The weights of the tablets were 6.5847g, 5.5231g, 6.4360g, and 6.2676g, respectively. After grinding the tablets, 0.521g, 0.542g, 0.532g, and 0.551g were taken from them, respectively. These amounts were dissolved in distilled water, filtered, washed several times, and then the volume was made up to 500 ml in a volumetric flask using distilled water.

Preparation of hydrazide Methyl 2-(Phenyl Salponyl acetate)

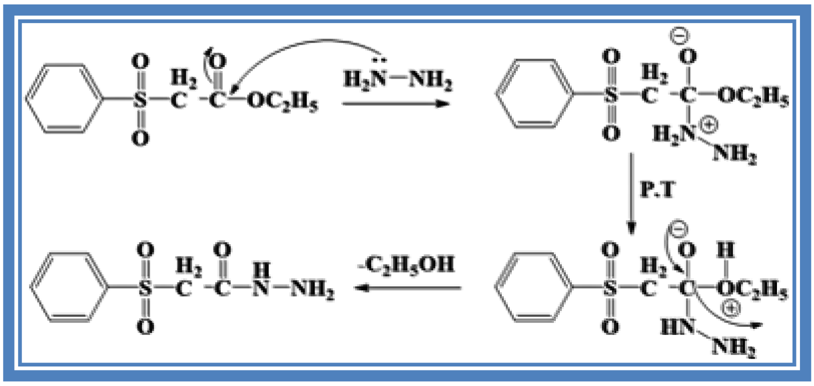
It was synthesized by incorporating (0.004 mol 1 g) of 2-(phenylsulfonyl) methyl acetate into (25 ml) of absolute ethanol, followed by adding (0.08 mol, 16.72 ml) of hydrazine (99%) in droplets with continuous stirring. The mixture was elevated for approximately (4) hours, then the mixture was concentrated by one third and passed through a filter, the precipitate was dried and placed on filter paper, then the product percentage was calculated (148). The melting point was 137-138 °C, and the resulting compound was white in color.

Preparation and diagnosis of hydrazide Methyl 2-(Phenyl Salponyl acetate)

Hydrazide was prepared by combining 2-(phenylsulfonyl)ethyl acetate with hydrazine, and the solvent was chosen as absolute ethanol, as shown in the following equation.



The process or mechanics was as follows: [31]



3. Result and Discussion

The physical properties of the compound (M10) were found to be similar, including a lower melting point and a change in the compound (M10). Furthermore, the data was subjected to a flow rate test, which revealed a single discrete spot on a Japanese TLC plate. And by analyzing the 1H-13C-NMR and infrared spectra, we were able to identify the hydrazide molecule (M10). The stretching of the aromatic (CH) bond caused an absorption band at (3053) cm-1 to form in the infrared spectrum of the hydrazide molecule (M10). Since the (NH) bond was stretched, an absorption band at (3174) cm-1 emerged. There were two absorption bands at (3301) cm-1 and (3375) cm-1, which were caused by the stretching of the (NH2) bond. The ester carbonyl group (C=O) seemed to have a lower frequency, appearing at (1668) cm-1, because it was associated with the [NH-NH2] group. This was because its association with the [NH-NH2] group caused the force constant for the double bond to be lower, which in turn reduced its frequency. It was also observed that two absorption bands appeared at (1456) cm-1 and (1508) cm-1 due to the stretching of the aromatic (C=C) bond, and the results were close to what is found in Literature(32); when measuring the 1H-NMR spectrum of compound (M10), a double signal was observed at ppm (3.69) due to the protons of the (NH2) group, as well as a signal at ppm (3.47) due to the protons of the (CH2) group, in addition to the appearance of a multiple signal in the range of ppm (7.43-7.87) due to the protons of the aromatic ring, as well as the appearance of a signal at the range of ppm (9.36) due to the proton of the (NH) group, and a signal at the position ppm (2.51) due to the protons of the solvent d6(DMSO). In the 13C-NMR analysis of the compound M10, a signal was detected at ppm (165.23) corresponding to the carbonyl group, another signal at ppm (57.40) corresponding to the carbon of the (CH2) group, numerous signals in the range (134.73-121.31) ppm corresponding to the aromatic ring's protons, and signals in the range (40.48-39.23) ppm corresponding to the solvent's carbons (DMSO) (Figure1,2,3.(

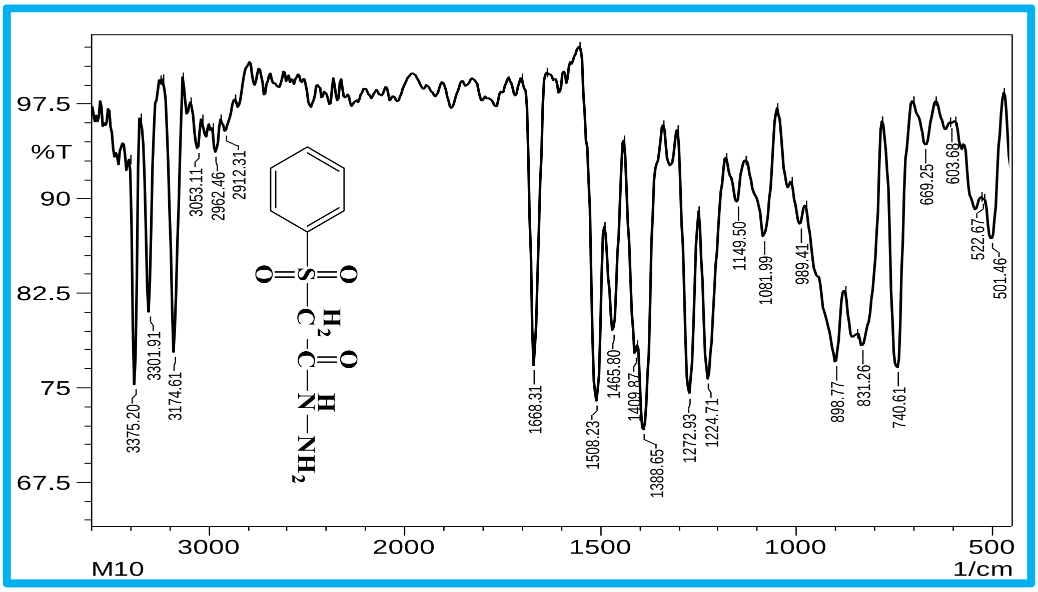


Figure (1) FT-IR spectrum of the detector

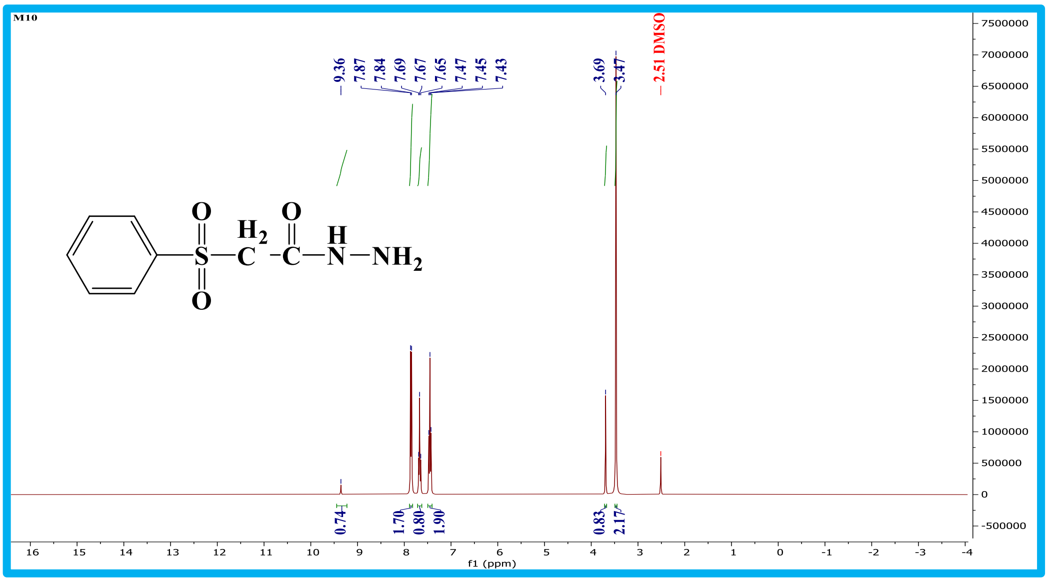


Figure (2) 1H-NMR spectrum of the detector

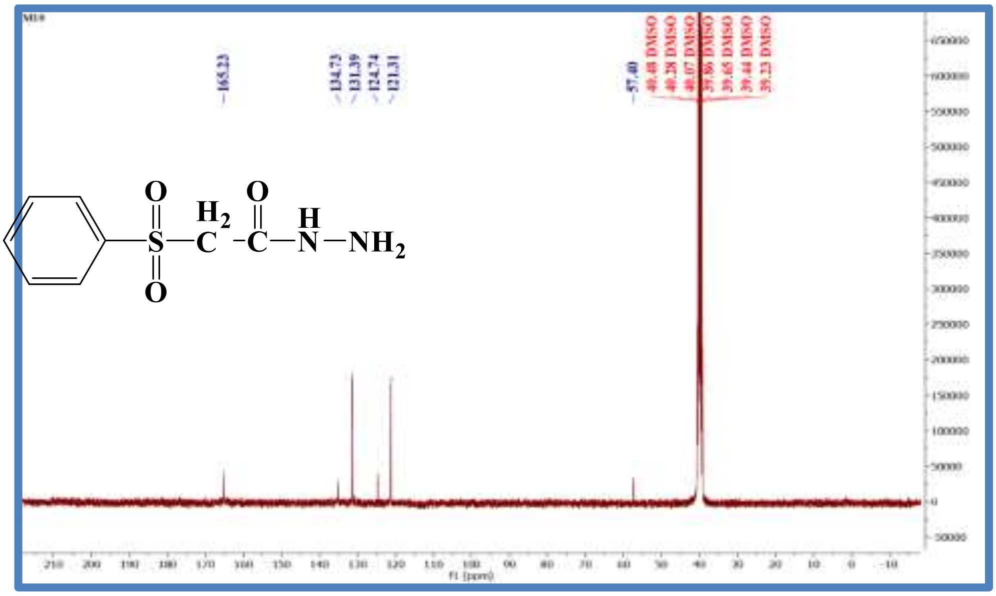


Figure (3) 13C-NMR spectrum of the detector

Effect of the amount of sodium nitrite

The absorbance of the colored Azo dye product was affected by various quantities of sodium nitrite solution (0.25-2 mL), as shown in Figure 4

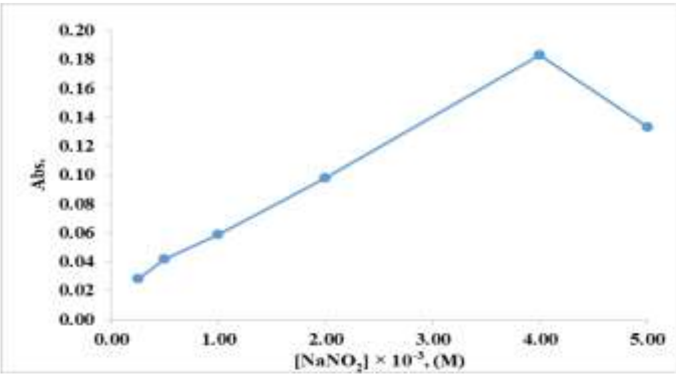


Figure (4): Effect of the amount of sodium nitrite

Effect of the amount of coupling reagentM10

The absorbance of the colored product was tested by increasing quantities of M10 solution, with all other conditions held constant (Figure 5).

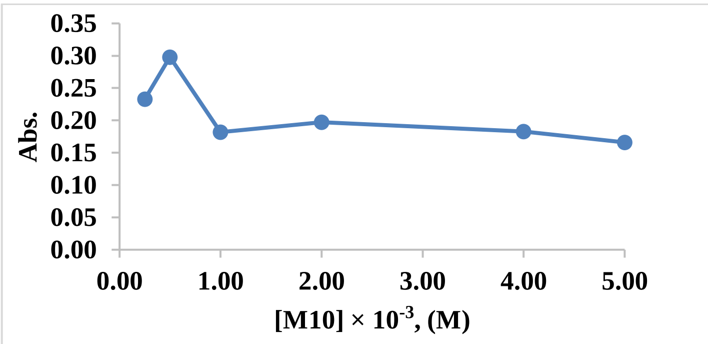


Figure (5): Effect of reagent quantity reagentM10

Effect of acid type

Figure 6 shows the greatest absorption value, which was obtained by reacting a paracetamol sample with three different acids, each of which was taken at a concentration of 1 molar.

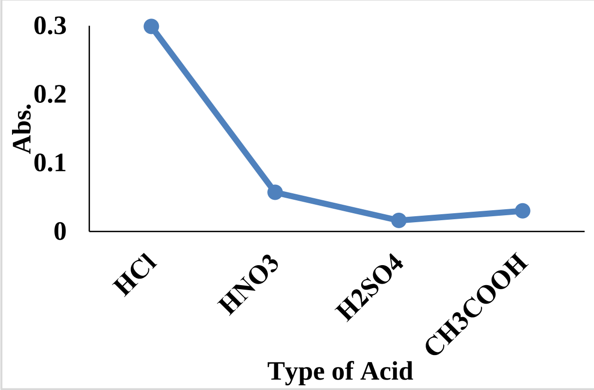


Figure (6): Effect of acid type

Effect of acid amount

Figure 7 shows the results of an investigation into how varying concentrations of a 1M HCl solution affected the final color outcome.

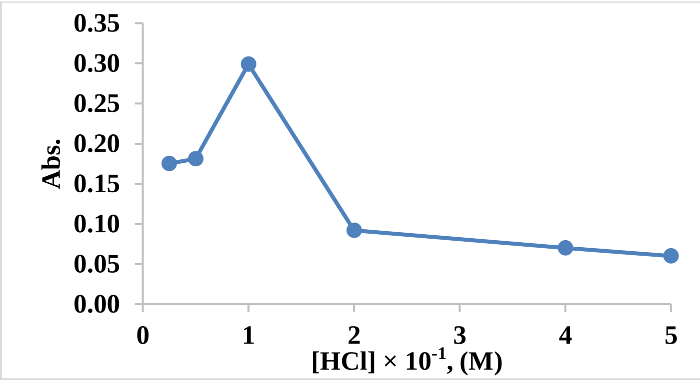


Figure (7): Effect of the amount of acid

Effect of the type of basic solution

Sodium hydroxide, ammonium hydroxide, potassium hydroxide, and sodium carbonate were among the alkaline solutions tested for their effects at 1M. The solution color stability of sodium hydroxide was found to be the highest (Figure 8).

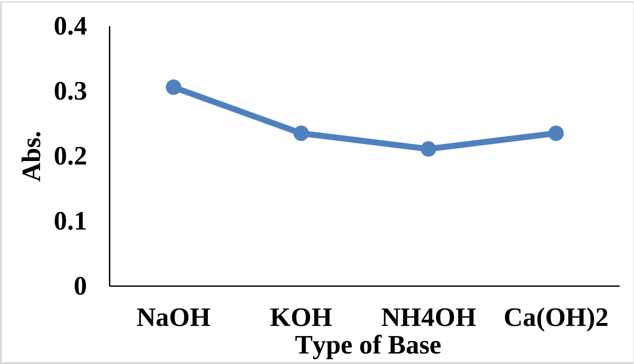


Figure (8): Effect of the type of basic solution

The quantity of basic solution and its impact

They looked at how different amounts of basic solution affected things. Various quantities were evaluated, with absorbance measured at 432 nm, ranging from 2 to 0.25 ml. Figure 9 shows that 1 ml was the ideal volume to be added.

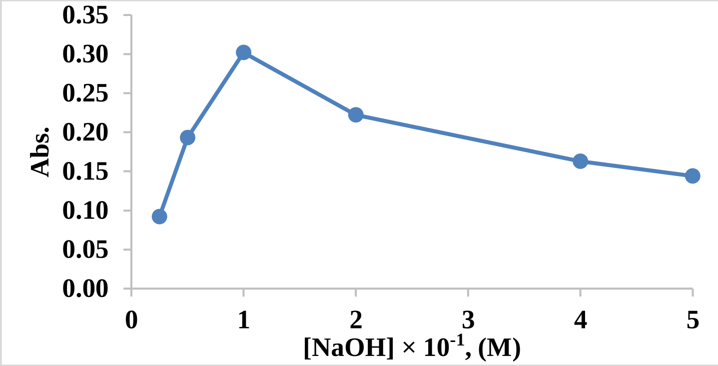


Figure (9): Effect of the amount of basic solution

Final absorption spectrum

After the experimental conditions were optimized, the final absorption spectra were measured. A 1000 µg/ml concentration of paracetamol, 4 ml of a 10-2 M concentration of sodium nitrite, and 1 ml of a 1 M concentration of hydrochloric acid were used in the procedure. The remaining 10 minutes were used to let the solution finish forming the diazonium salt. After that, in accordance with the reaction's optimal conditions, 0.5 ml of a 10-2 M M10 reagent and 1 ml of a 1 M sodium hydroxide were added. A volumetric bottle was used to get the volume down to 10 ml by adding distilled water. At a wavelength of 432 nm, the sample solution and the yellow product were tested for absorption (Figure 10).

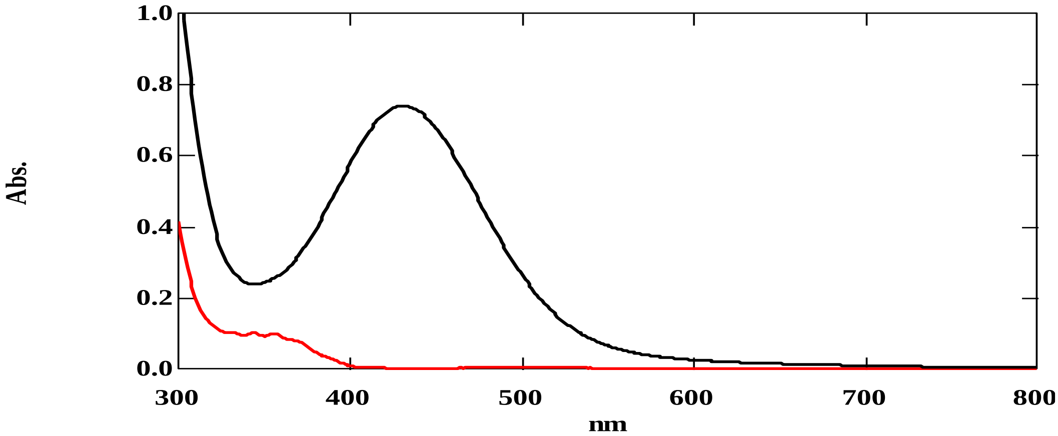


Figure (10): Final absorption spectrum for determination paracetamol

Procedure for construction of calibration curve

Paracetamol concentrations ranging from 5-40 mcg/ml were contained in a set of 10 ml volumetric vials. These values are equivalent to 1000 mcg/ml in quantities ranging from 0.05 to 0.4 ml. The vials were supplemented with 4 milliliters of sodium nitrite (10-2 M) and 1 milliliter of hydrochloric acid (1 M). Half a milliliter of the 10-2 M M10 reagent and one milliliter of 1 M sodium hydroxide were added after 10 minutes. The solutions were subsequently thinned with distilled water and their absorbance was compared at a 432 nm wavelength to the sample solution.

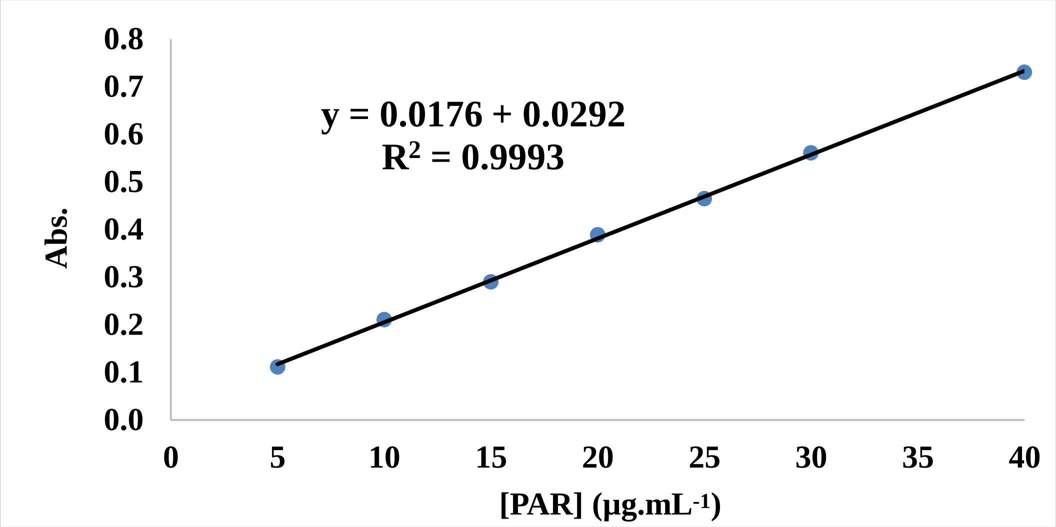


Figure (11): Calibration curve for paracetamol

The suggested method's precision and compatibility

By plugging the results of three separate paracetamol concentration measurements taken under the ideal circumstances outlined in the working method into the aforementioned equations, we were able to determine the accuracy and compatibility of the suggested approach. The acquired findings demonstrate the method's high level of accuracy and compatibility. Tables 1 and 2.

Table (1): Accuracy and compatibility of the proposed method

D (ppm)		Rec%	RSD%
Present μ	**Found $\bar{x}$**		
15	14.87	99.14	0.344
20	19.78	98.90	0.141
30	30.23	100.77	0.203

***n=3**

Table (2): Results of Detection limits

Concentration μg /mL	b	S	LOD μg/mL	LOQ μg/mL
blank	0.0176	0.0292	0.2522	0.7641

Effect of additives

The effect of adding an increase in the concentrations of additives present with paracetamol in the form of tablets as a pharmaceutical preparation, and in a volumetric vial of capacity (10 ml), was studied. The results show that there is no interference of these substances in the paracetamol with the reagent, by adding three concentrations of each substance, which It confirms the possibility of successfully applying this method to pharmaceutical preparations. Table 3).

Table (3): Effect of additives

Type of Interference	Conc. of Interferences (ppm)	Present Conc. of D (ppm)	Abs.	Found Conc. of D (ppm)	Erel%	Rec%
Standard	non	20	0.389	20.44	2.19	102.19
Glocose	10	20	0.388	20.38	1.91	101.91
	20	20	0.388	20.38	1.91	101.91
	40	20	0.385	20.21	1.06	101.06
Frectose	10	20	0.38	19.93	-0.36	99.64
	20	20	0.382	20.04	0.20	100.20
	40	20	0.382	20.04	0.20	100.20
Socaros	10	20	0.384	20.15	0.77	100.77
	20	20	0.383	20.10	0.49	100.49
	40	20	0.384	20.15	0.77	100.77
Lactose	10	20	0.388	20.38	1.91	101.91
	20	20	0.387	20.32	1.62	101.62
	40	20	0.387	20.32	1.62	101.62
Starch	10	20	0.39	20.50	2.48	102.48
	20	20	0.391	20.55	2.76	102.76
	40	20	0.39	20.50	2.48	102.48
Celelos	10	20	0.38	19.93	-0.36	99.64
	20	20	0.382	20.04	0.20	100.20
	40	20	0.381	19.98	-0.08	99.92
Sodium Sulfate	10	20	0.379	19.87	-0.65	99.35
	20	20	0.378	19.81	-0.93	99.07
	40	20	0.378	19.81	-0.93	99.07
Maghnesume Citrate	10	20	0.383	20.10	0.49	100.49
	20	20	0.384	20.15	0.77	100.77
	40	20	0.384	20.15	0.77	100.77

Applications

The amount of paracetamol in a pharmaceutical tablet composition was determined using the suggested approach. Ten pills containing the active ingredients paracetamol, paracetamol, piodol, paracetamol, and pandol, paracetamol were measured and found to be 6.5847, 5.5231, 6.4360, and 6.2676 grams, respectively. They were ground to a fine consistency, and then weighed at (0.521, 0.542, 0.532, 0.551) grams each. They were mixed with distilled water, filtered, and then rinsed multiple times to remove debris. Finally, a volumetric bottle containing 500 ml of distilled water was filled to the mark. In order to determine the amount of paracetamol in the pharmaceutical formulation, three different concentrations were extracted from the filtrate. Figure 4.

Table (4): Application of the proposed method for the determination of paracetamol in pharmaceutical preparations.

Industrial application	Conc. of D (ppm)		Erel %	Rec %	Abs.
	Present	Found			
Paracetol, Paracetamol (500 mg), SDI, Iraq, Tablets	10	10.33	3.27	103.27	0.211
	20	20.44	2.19	102.19	0.389
	30	30.66	2.21	102.21	0.569
Panda, Paracetamol (500 mg), Joswe, Jordan, Tablets	10	10.27	2.70	102.70	0.210
	20	20.55	2.76	102.76	0.391
	30	30.72	2.40	102.40	0.570
Piodol, Paracetamol (500 mg), Pioneer, Iraq, Tablets	10	10.55	4.54	104.54	0.215
	20	20.27	1.34	101.34	0.386
	30	30.38	1.26	101.26	0.564
Panadol, Paracetamol (500 mg), Glaxosmithkline, Ireland, Tablets	10	9.76	-2.41	97.59	0.201
	20	19.81	-0.93	99.07	0.378
	30	29.87	-0.44	99.56	0.555

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