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EVALUATION OF CONTENT UNIFORMITY AND DISSOLUTION TEST OF PARACETAMOL (SDI TABLETS) BY OXIDIZING COUPLING REACTION WITH *O*-PHENYLENEDIAMINE

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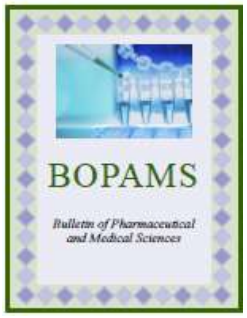

ABSTRACT

In this study, paracetamol tablets were evaluated using the dissolution test and content uniformity for each tablet, the method based on oxidation by potassium chromate and followed by reaction with *O*-phenylenediamine (O-Ph) as a coupling agent, in acidic medium, to form a yellowish-orange dye soluble in water and stable at room temperature and was followed spectrophotometriclly at λ_{max}= 446 nm. The method was precise and accurate. The concentrations obeyed Beer's law from (8-40) µg/ml. The molar absorptivity (ε_{max}) of the colored product was found to be (8316) l.mole-1 .cm-1 and Sandel's index 0.0182µg.cm-2 with relative standard deviation percent (R.S.D%) between 1.824- 0.495% and the recovery percent, 100.29-100.09%.

Keywords: Paracetamol, Spectrophotometric, content uniformity, dissolution test

1. Introduction

Paracetamol, also known as acetaminophen or N-Acetyl-p-aminophenol APAP,its molecular formula is $C_8H_9NO_2$ [1] and consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the *para* (1,4) pattern. It is characterized by white crystalline powder. Its molecular weight is 151.17 g / mol. It has a melting point of 168- 172 ºC, very soluble in ether or methyl chloride. It dissolves partially in water (0.1-0.5 g / 100 ml) and has high solubility in alcohol [2].

Paracetamol is widely used as analgesic and antipyretic, a non-narcotic and also used for reducing fever in people of all ages [3]. It is used instead of Aspirin is especially common in patients with gastric diseases such as stomach ulcers[4].Osteoarthritis[5], Low back pain[6], Headaches[7], Postoperative pain[8], Dental use[9] and do not increase detection in congenital malformations associated with paracetamol during pregnancy[10].

There are different methods have been studied for determination of Paracetamol in pharmaceutical and biological samples. These include flow injection voltammetry[11], thin layer chromatography [12] and Fluorescence methods [13], high performance liquid chromatography [14]. UV-Visible Spectrophotometric methods and colorometric methods [15]. and indirect determination method of paracetamol depending on acidic or basic paracetamol hydrolysis and its conversion to p - amino phenol and the hydrolysis product reaction with different organic coupling reagents and with different oxidizing agents [16,17].

The aim of the present research is development of simple and sensitive spectrophotometric method for the quantitative determination of paracetamol in pharmaceutical tablets and evaluation the method by content uniformity for each tablet (SDI product) and dissolution test.

2. Materials and methods

2.1.Instruments

Spectrophotometric measurements are performed using UV-530 UV-Visible JascoSpectrophotometer and using 1-cm quartz cells. Type (II) dissolution instrument Erweka/ Germany. The pH measurements are performed on pH meter type HANNA 211 pH-Ion meter.

2.2.Materials and Reagents

All Chemicals used were of high degree of purity and used without furtherpurification and prepared as follow:

Solutions of 100µg/ml PA, 0.01M O-Ph and potassium chromate are prepared separately by dissolving 0.01g (in 2ml of ethanol for PA) , 0.1081g and 0.1942g in distilled water and the volume completed to the mark in a 100ml volumetric flask. Also a solution of 1M hydrochloric acid was prepared from concentrated acid of 37% and 0.01M HCl prepared by dilution.

3. Results and discussion

The effect of various variables on the color development of 16µg/ml of PA, 1ml of O-Phynelene and1ml of K_2CrO_4 in 1ml HCl was tested in final volumetric flask of 25ml to establish the optimum conditions.

3.1. Optimum conditions for product formation

3.1.1. Choice the type and concentration of oxidizing agent

Different types of oxidizing agents were used to select the best, which gives the highest color intensity (see Table1)

Table 1: Choice the type and concentration of oxidizing

1ml Oxidizing agent 0.01M	Absorbance
$FeCl_3$	0.08
KIO_4	0.09
$(CH_2CO)_2NBr$	0.03
K_2CrO_4	0.14
$K_3Fe(CN)_6$	0.02

The results illustrated in Table 1 indicated that K_2CrO_4 give the highest intensity of colored product.

The effect of different volumes (0.1-2.5ml) of 0.01MK_2CrO_4 solution on the color intensity has been studied, it was observed that 2ml ofK_2CrO_4, is the most suitable amount, since it gives the highest intensity of the formed product therefore it is chosen for further studies (see Figure 1)

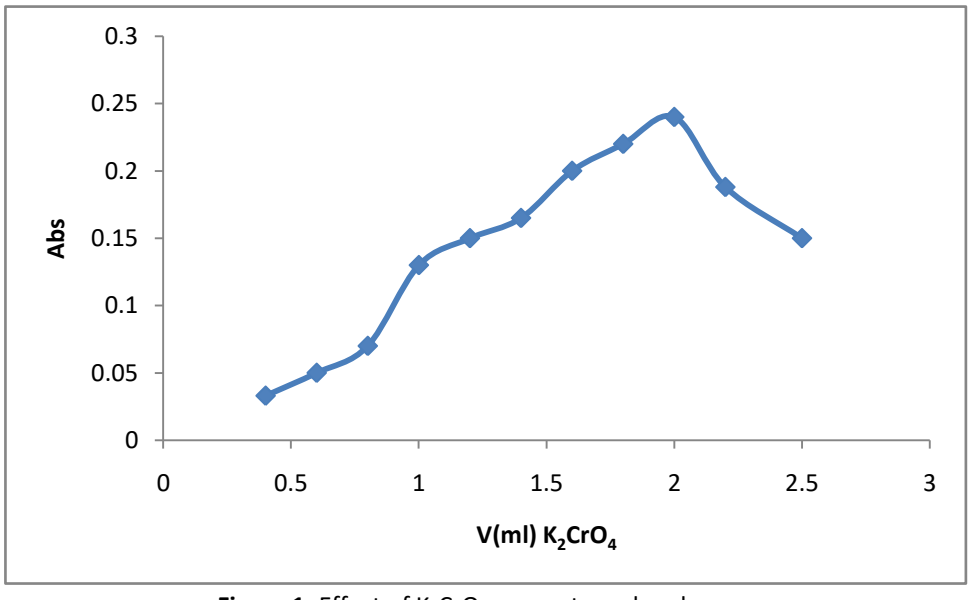

Figure 1: Effect of K_2CrO_4 amount on absorbance.

3.1.2. Effect of the coupling reagent.

The effects of the different volumes (0.1 – 2.5) ml of 0.01M O-Phenylenediamne solution on the formation of the colored product were examined. Figure2 shows that 1.5 ml of the solution was optimum and was used in the subsequent experiments

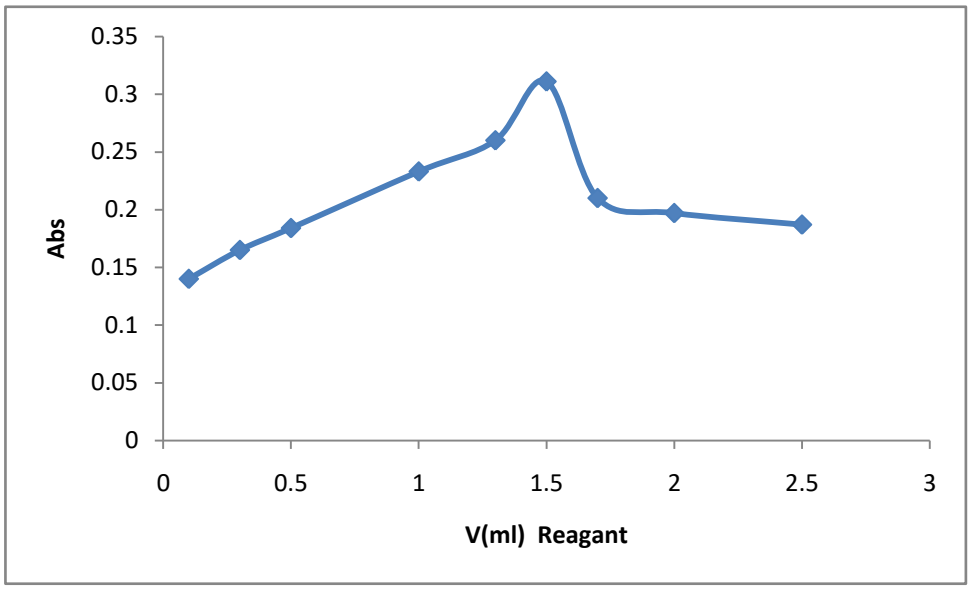

Figure 2: Effect of reagent amount.

3.1.3.Effect of acid amount on absorbance

Different volumes of HCl (0.1 – 2.0 ml) was examined to show its effecting on the maximum absorbance and complete the other additions of PA, K_2CrO_4 and O-Ph, 1.5 ml found the best volume gave optimum absorbance Figure 3 and it was used in the subsequent experiments.

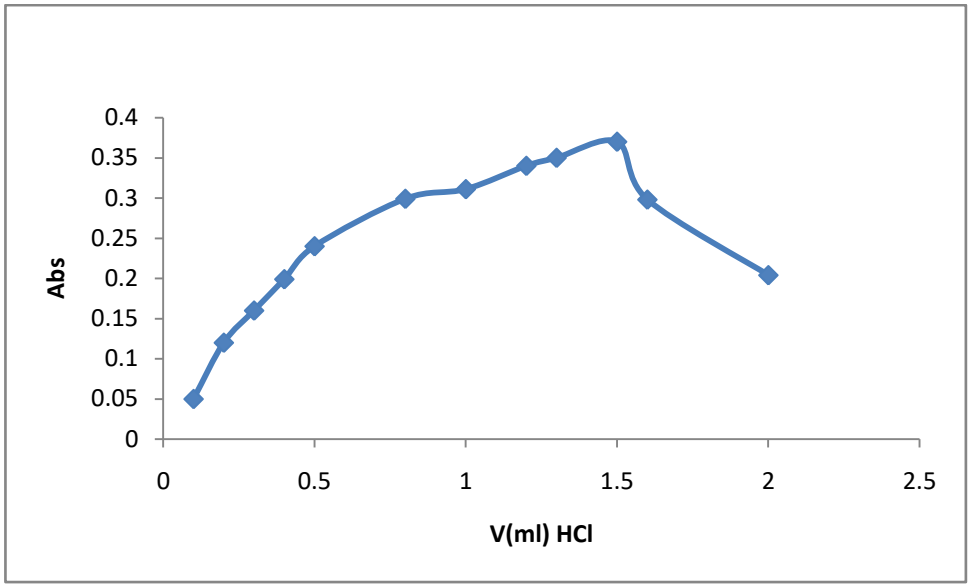

Figure 3: Effect of acid amount on absorbance.

3.1.4. Effect of order of addition

The effect of different orders of reagents addition were investigated(see Table 2).It was found that the order **(II)** of addition give highest color intensity, otherwise a loss in color intensity takes place

Table 2: Order of addition

Order number	Order of addition	Absorbance
I	K_2CrO_4+ O-Ph + HCl +PA	0.196
II	PA + K_2CrO_4+ O-Ph + HCl	0.42
III	PA+ O-Ph + K_2CrO_4+ HCl	0.147
IV	O-Ph+ K_2CrO_4+ HCl+ PA	0.122
V	HCl+ O-Ph + K_2CrO_4+ PA	0.210
VI	HCl+ K_2CrO_4+ PA+ O-Ph	0.149
VII	HCl+ PA+ K_2CrO_4+ O-Ph	0.233

3.1.5. Effect of temperature

Different temperatures were studied (10-60) **°C** to select the best one that gives best color intensity and found that the best temperature was at laboratory temperature (25°C).(see Table 3).

Table 3: Effect of temperature

Temp C°	10	15	20	25	30	40	45	50	55	60
Absorbance	0.021	0.031	0.34	0.45	0. 31	0.044	0.063	0.057	0.051	0.049

3.1.6. Development time and stability period

The stability time of the formed colored complex is investigated under the optimum conditions for the determination of PA, the experimental results (see Table 4) showed that the formation of colored complex is attained after 5 minutes.

Table 4: Effect of time on color stability

Time (min)	0	5	10	15	20	25	30	40	50	60
Absorbance	0.371	0.45	0.44	0.44	0. 41	0.40	0.40	0.398	0.378	0.376

3.2. Calibration curve

To a series of 25-ml volumetric flasks, 2-8 ml of 100 µg.ml-1 PA solution are transferred then 2 ml of potassium chromate and 1.5ml of 0.01M O-Ph reagent, after that a 1.5ml of HCl solution was added. then the volumes we recompleted to the mark with distilled water and left to stand for 5 minutes at room temperature, after that the absorbance are measured at 446 nm against the reagent blank. The calibration graph is linear over the concentration range of 8-40 µg / ml(see Figure 4).

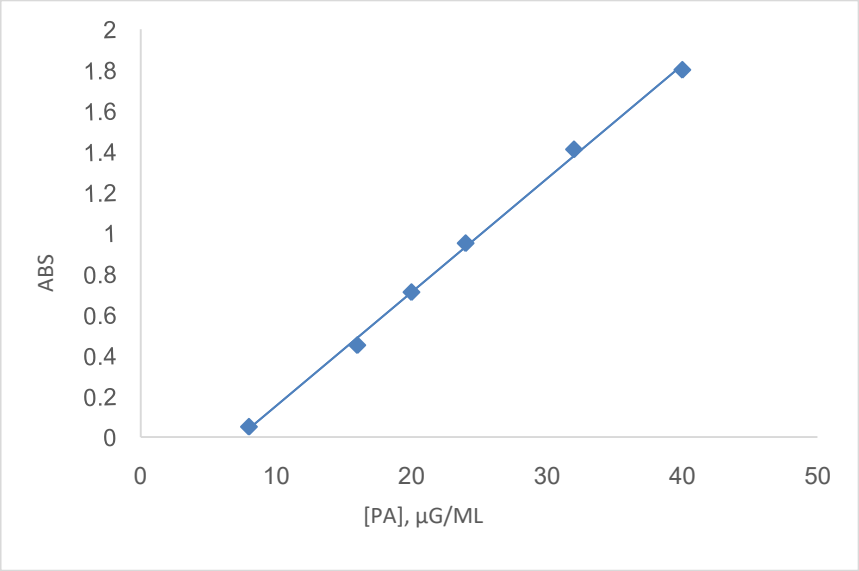

Figure4: Calibration Curve for Determination of PA

3.3. Final absorption spectra

The absorption spectra of the colored formed by reaction of PA with O-Ph in presence of potassium chromate in acidic medium shows a maximum absorption at 446 nm against blank solution that has not absorption at this wavelength (see Figure5).

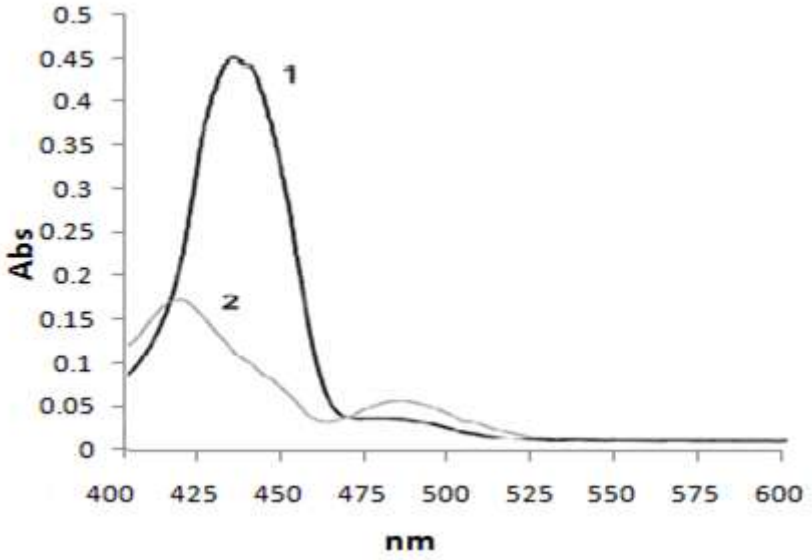

Figure 5: Final absorption spectrum at 446nm, (1) sample verses blank, (2) Blank verses water

As shown in the calibration curve of PA some variables were studied, the linearity of calibration graph, molar absorptivity, Sandell's sensitivity, and limit of detection **Table (5).**The slope, intercept and the correlation coefficient were also calculated.

Table (5): Statistical treatments for the calibration Curve of proposed method.

Parameter	Value
Regression equation	y = 0.055 x - 0.405
Slope	0.055
Intercept	0.405
Linearity range (µg ml^{-1})	8-40
Molar absorptivity ε (l. $mol^{-1}.cm^{-1}$)	8.316×10^3
Sandell's sensitivity S (µg. cm^{-2})	0.0182
Limit of detection, LOD (µg.ml^{-1})	6.732
Correlation coefficient	0.9989

3.4. Accuracy and the Precision

The percentage of recovery (Rec%), relative error (E%) as an expression of the accuracy and relative standard deviation(RSD%) as an expression of precision were studied for PA determination, five measurements of each concentration of PA containing 20, 24, and 32µg.ml^{-1} were studied. The results were of good accuracy and precision (see Table 5).

Table 5: The Accuracy and the Precision.

Conc., µg ml		E%	Rec.%	RSD%
Present	Found			
20	20.018	+0.090	100.09	1.824
24	24.078	+0.325	100.325	0.728
32	32.095	+0.296	100.296	0.495

3.5. Nature of the reaction product

By measuring the absorbance at 446nm, Job's method of the continuous variations indicates that the colored product has a composition of 1:1 PA to O-Ph reagent (see Figure 6).

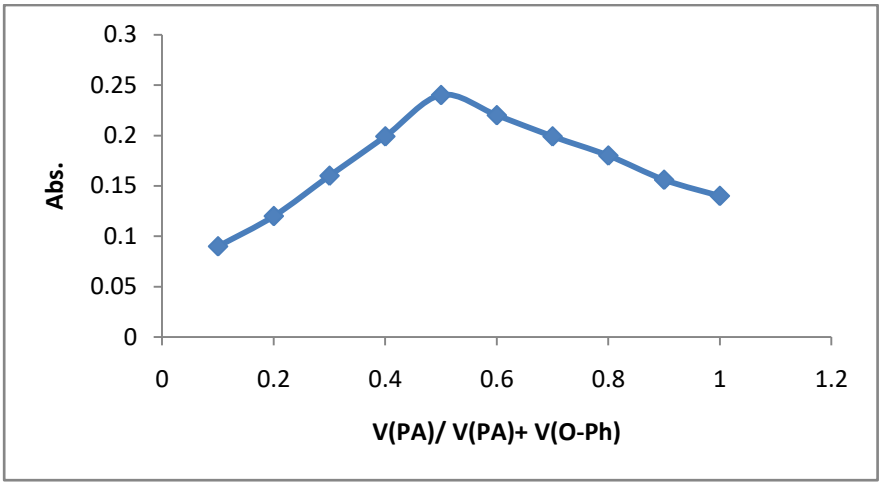

Figure6: Job's plot for PA-*O*-Ph colored product.

Therefore, the probable reaction path might be written as follows:

3.6. Effect of interferences

The effect of some organic and inorganic pharmaceutical additives was studied as interfering materials. The below **Table 6** shows no effect on the proposed method.

Table6: Effect of excipients on the recovery of 20µg ml-1 of PA

Excipient, µg ml^{-1}	**Conc.of PA µgml^{-1}Found**	**E%***	**Rec.%***
Starch	20.030	+0.150	100.150
PVP	20.0.41	+0.205	100.205
Sucrose	19.970	-0.150	99.850
Magnesium stearate	19.957	-0.215	99.785
Lactose	20.050	+0.250	100.250

***** Average of five determinations.

3.7. Pharmaceutical applications

The proposed method was applied to pharmaceutical tablets (SDI Tablets 500 mg to determine PA by direct method by using three different concentrations. The below **Table 7** shows a good value of Rec% and RSD%.

Table 7: Application of the proposed method for determination of PA in pharmaceutical tablets

Pharmaceutical tablets	Prepared Conc. of PA,µg ml^{-1}		Rec.%	* RSD%
	Present	Found*		
Paracetol 500	10	10.01	100.1	0.567
	20	19.78	98.9	0.681
	30	29.90	99.7	0.488

* Average of five determinations.

3.8. Content Uniformity of PA Tablets:

The content uniformity of the investigated drug in its tablets was carried out using the proposed spectrophotometric method. Ten individual tablets were dissolved in 250 ml distilled water. Then 0.5 mlof this solution is pipette in 25 ml volumetric flask and complete the procedure according to calibration curve. To evaluate the content uniformity, the mean absorbance was recorded and the content of the drug in each tablet was calculated from the calibration graph.The mean obtained result as % recoveries ± standard deviations was 99.19±1.16. (see Figure 7).

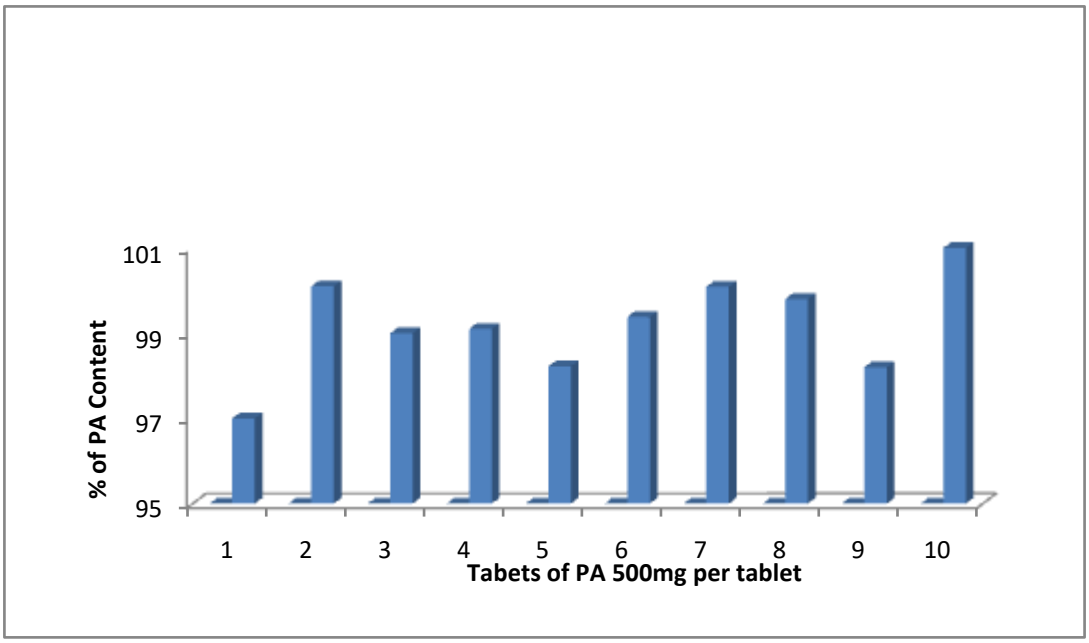

Figure 7: Content uniformity of Paracetamol tablets

3.9. Dissolution Test

PA tablets (® 500 mg/ tablet) were tested by the dissolution test according to USP using Type (II) dissolution instrument. The dissolution volume was 900 mlof phosphate buffer (pH 5.8). The temperature was adjusted at 37.0±0.5˚C and the rotation was 50rpm for 60 minutes. After 30 min, the amounts of PA released were calculated from the calibration curve and represented by the dissolution profile as shown in **Figure 8**. The results obtained were 82 %. The results were identical to those reported in the US Pharmacopoeia (at least 80% released within 30 minutes)[17].

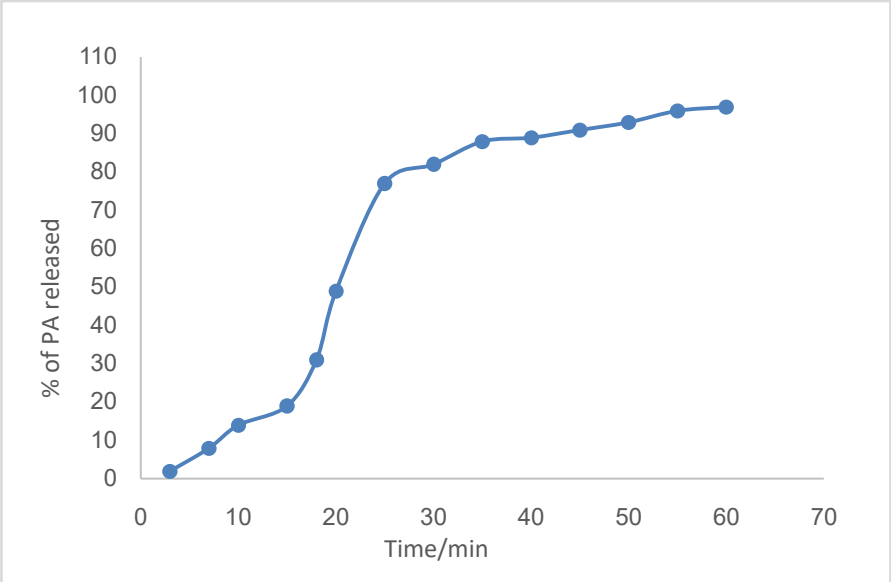

Figure 8: Dissolution profile of (PA SDI ® 500 mg/ tablet) using the proposed methods

4. Conclusion

The developed proposed spectrophotometric method is a simple and sensitive method for the determination of trace amount of paracetamol in aqueous solution based on the reaction PA with O-Ph in the presence of potassium chromate in acidic medium. The proposed method has been successfully applied to the assay of paracetamol, content uniformity and dissolution test of Paracetamol (SDI Tablets)has been evaluated.

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