Research Article

Chemical Derivatization UV Spectrophotometric Method for Detection of P-Aminophenol and Energy of Activation Approach to Set Degradation Protocol for Forced Degradation Studies

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ABSTRACT

A spectrophotometric method has been developed for the determination of paracetamol in a dosage form and in Pharmaceutical preparation. The method is based on the reaction of p-aminophenol group of the drug with ninhydrin in N, N. dimethylformamide (DMF) medium producing a coloured complex which absorbs maximally at 547 nm. Beers law is obeyed in the concentration range 1-3µg/ml and with set parameter the method is validated, LOD and LOQ were found to be 0.254, 0.770 and 0.2849, 0.863 by standard deviation of blank and calibration curve method respectively. As it is having least sum square error that is 1.448667 × 10 -5. Method is found to be specific for the para amino phenol, data supported by ANOVA test with at P ≤ 0.05. For accuracy and precision we are 99% sure that the results lie between 100.32-99.26% and 100.19 to 100.07% respectively. From the principle of energy of activation the force degradation of Paracetamol in tablet dosage form is carried out at 100°c for 3 days, degradation of Paracetamol was found to be 0.7% Hence the method is developed and validated for the detection of p-amino phenol, a degradation product of paracetamol and by using concept of energy of activation p-amino phenol from paracetamol drug product is estimated the results were validated statistically.

Keywords: Ninhydrin, paracetamol, pharmaceutical preparation, spectrophotometry

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1. INTRODUCTION

Paracetamol, acetaminophen or N-acetyl-p-aminophenol is commonly used analgesic and antipyretic drug, present in different pharmaceutical formulations such as tablets, soluble powder syrups and suppositories. It is administered to both children and adults. [1, 2] The primary degradation product of paracetamol is 4-aminophnol or 1-hydroxy-4-aminobenzone.It is formed during the synthesis of paracetamol or during the storage condition of pharmaceutical formulation such as heat, pH, temp etc. [3, 4] Paracetamol degrades slowly forming a mixture of contaminants such as 4-aminophenol and acetic acid. [3, 4]

It is reported that p-aminophenol have significant nephrotoxic and tetratogenic effects, therefore its amount should be strictly controlled. [5] It is limited to a low level of 0.005 % (w/w) i.e.50 ppm in the drug substance by European pharmacopoeia .The significant limit of p-aminophenol may vary in different products depends on dosage forms and formulations. The monograph of paracetamol tablet in BP allows 0.1 %. For drug product containing paracetamol often less tight limits are applied such as 1000 ppm or 0.1 % (w/w). Internally the lower drug substances specification limit is applied to product 50 ppm is equivalent to 25µg of p-aminophenol per 500 mg tablet. [5] The present work describes simple, sensitive, accurate, precise, economical visible spectrophoto-meteric method using ninhydrin reagent for estimation of p aminophenol in paracetamol dosage form. Ninhydrin is a 2, 2, dihydroyindane 1,3-dione [6] which react with the primary amino group of p-

aminophenol to yield purple color derivative called Ruhemans purple and hydroquinone. [7]

1.1. Chemical Derivatization

Derivatization is a technique which transforms a chemical compound into a product of similar chemical structure. It changes chemical and physical properties. Chemical derivatives may be used to facilitate analysis. Chemical Derivatization modifies or converts substances with a low, UV absorption into highly sensitive products. [8, 19]

1.1.1. Types of Chemical Derivatization Methods

1. **Diazotisation and coupling of primary aromatic amines**

The amine is diazotised with aqueous solution of Nitrous acid, the colorless diazonium salt is very reactive and when treated with suitable coupling reagent like aromatic amine or phenol undergoes an electrophilic substitution reaction to produce an azo derivative. The azo derivatives are colored and have absorption maximum in UV visible region at λ_{max}550 nm. [8]

2. **Condensation Reaction**

Many Colorimetric procedures are based on rapid reaction that occurs under suitable condition between amines and carbonyl compounds. The reaction involves the nucleophilic attack by the amine on the carbonyl carbon with the elimination of water. [8]

3. **Reduction of tetrazolium salts**

Tetrazolium salts are reduced to their colored form azan derivatives in the presence of a Steroid with α-ketol (21-hydroxy-20-keto) side chain group. [8]

4. **The acid-dye method**

The addition of an amine in its ionized form to an ionized acidic dye, i.e. methyl orange or bromocresol purple, yields a salt (ion-pair). [8]

5. **Oxidation methods**

Oxidation of side chain of weakly absorbing compounds containing a simple phenyl group produces a carbonyl derivative that has a much greater absorptivity than the parent compound. [8]

6. **Metal-ligand complexation**

Many organic reagents that are called ligands forms complexes with metal atoms by the formation of coordination (in which both electrons are donated by the ligand) and covalent bonds ligands with two or more donating groups may share more than one pair of electrons with a single metal atom by coordinating to two or more positions. [8]

1.2. Ninhydrin

The chemistry of ninhydrin has been studied extensively. Ninhydrin is a triketo compound; much of the work has been directed towards the reaction of amines with ninhydrin. This pigment serves as the basis of detection and quantitative estimation of amino acids, the reaction is usually carried out by heating for short time in a mixture of water and organic solvents. [7, 9, 10]

1.3. Energy of Activation

Activation energy is a term introduced in 1889 by the Swedish scientist Syante Arrhenius that is defined as the energy that must be overcome in order for a chemical reaction to occur. Activation energy may also be defined as the minimum energy required starting a chemical reaction. The activation energy of a reaction is usually denoted by Ea and given in units of kilojoules per mole. [11]

$$Ea = 2.303 \frac{[T1\ T2\ R]}{T2 - T1}\ X\ LOg\ K2/K1$$

Where

Ea is the activation energy of the reaction in J/mol

R is the ideal gas constant = 1.98 cal / deg· mol

T1 and T2 are absolute temperatures

Convert T1 and T2 in Kelvin

K1 and K2 are the reaction rate constants at T1 and T2

2. MATERIALS AND METHODS

2.1. Apparatus

A UV-visible spectrophotometer (Shimadzu, model 1800) with 1 cm quartz cells was used for the absorbance measurements.

2.2. Materials

Para amino phenol in the form of powder was provided by Thomas Baker, Mumbai, and DMF in the form of liquid was provided by LOBA Chemie, India, which was used in

analytical method development and validation.

2.3. Determination of wavelength of maximum absorption

A standard stock solution of Para Amino Phenol (100 µg/ml) was prepared using DMF as diluents and 1 to 5 ml of standard solution was then diluted to obtain 10 ml with DMF to obtain 10 to 50 µg/ml Para Amino Phenol. An UV spectroscopic scanning (200– 600nm) was carried out to determine the λ max.

2.4. Method

2.4.1. Preparation of stock solution

10mg of the pure drug was weighed and transferred to a 100ml volumetric flask; 50ml DMF was added to the above flask and dissolved, the volume was made up with the DMF.

2.4.2. Preparation of ninhydrin reagent

2gm of the ninhydrin was weighed and transferred to a 100ml volumetric flask, 50ml DMF was added to the above flask and dissolved, the volume make up 100ml with the DMF.

2.5. Method validation

Analytical method development and validation for detection of p-aminophenol in the paracetamol bulk dosage form by ICH guideline.

2.5.1. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

2.5.1.1. LOD

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. [12-15]

2.5.1.2. LOQ

Lowest concentration or amount of analyte that can be detected quantitatively with an acceptable level of repeatability, precision and trueness. [12-15]

2.5.2 Method based on Standard Deviation of blank

Analysis were carried out using 2% w/v ninhydrin reagent; 3 ml were added and mixed, heated on water bath at 70^0 C ± 2^0 C for 30 minutes, cooled at room temperature and volume in each were adjusted to 10 ml with DMF, The absorbance of resulting solution was measured 10 times at 547nm.

2.5.3. Method based on Calibration Curve

Prepare 0.001ppm to 2.0 ppm of para aminophenol solution from stock solution respectively. Analysis were carried out using 2% w/v ninhydrin reagent; 3 ml were added and mixed, heated on water bath at 70^0 C ± 2^0 C for 30 minutes, cooled at room temperature and volume in each were adjusted to 10ml with DMF, The absorbance of resulting solution was measured 10 times at 547nm.

In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations

$$LOD = 3.3\ \delta/S$$

$$LOQ = 10\ \delta/S$$

Where,

δ = Standard Deviation

S = Slope

Cut off range was found to be 0.03

Table 1: Determination LOD and LOQ based on calibration curve calculations

Sr. No	Conc. µg/ml	Abs at 547nm
1	0.03	0.00209
2	0.04	0.0025
3	0.05	0.0035
4	0.06	0.0037
5	0.07	0.0045
6	0.08	0.00507
7	0.09	0.0056
8	0.1	0.0061
9	0.2	0.0077
10	0.3	0.01012
11	0.4	0.01774
12	0.5	0.01906
13	0.6	0.02381
14	0.7	0.02792
15	0.8	0.03302
16	0.9	0.03697
17	1.0	0.04062
18	1.1	0.04554
19	1.2	0.0494
20	1.3	0.0532
21	1.4	0.05762
22	1.5	0.062
23	1.6	0.06821
24	1.7	0.07252
25	1.8	0.07751
26	1.9	0.0797
27	2.0	0.0868

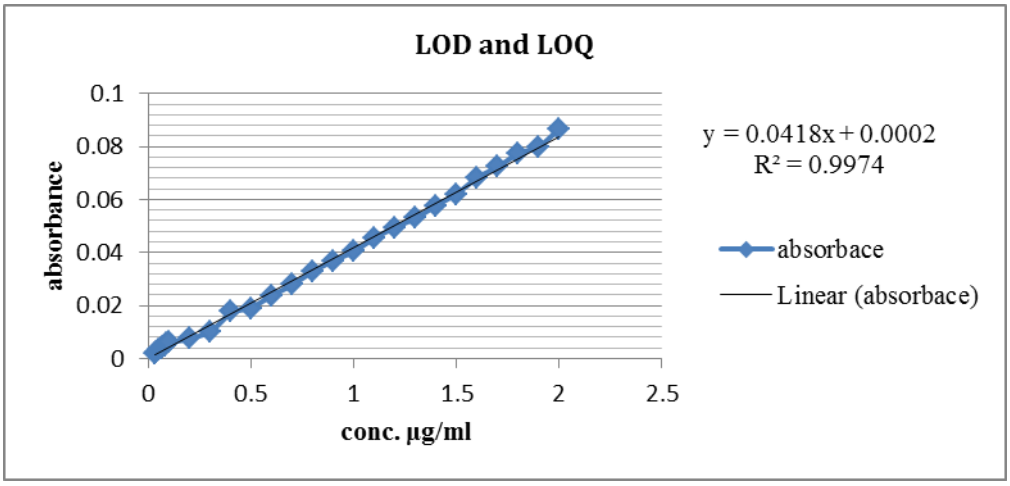

Figure 1: Calibration Curve for p-amino phenol for LOD & LOQ (0.03 to 2µg/ml)

$$LOD = 3.3\ \delta/S$$

$$\text{LOD} = \frac{3.3 \times 0.00388597}{0.045}$$

LOD = 0.284971 µg/ml

$$LOQ = 10\ \delta/S$$

$$\text{LOQ} = \frac{10 \times 0.00388597}{0.045}$$

LOQ = 0.8635 µg/ml

2.5.4. Linearity and Range

2.5.4.1 Linearity

Linearity indicates the ability to produce results that are directly proportional to the concentration of the analyte in samples. [12, 13, 15, 16]

2.5.4.2 Range

Range is an expression of the lowest and highest levels of analyte that have been demonstrated to be determinable for the product. [12, 13, 15, 16]

Standard stock solution para amino phenol (1.0 ml) were transferred to a 10ml of ambered colored volumetric flask, 2% w/v ninhydrin reagent; 3.0 ml were added and mixed. The flask were immediately immersed in a water bath 70⁰ C ± 2⁰ C for 30 minutes, cool to room temperature and the volume were adjusted to 10 ml with DMF. The absorbance of resulting solution was measured at 547nm against blank.

The absorbance of the samples in the range of 2–12µ g/ml was linear with a correlation coefficient (R2) 0.999.

Table 2: Data for calibration curve

Sr. no	Concentration (µg /ml)	Absorbance at 447nm
1	2	0.0812
2	4	0.1801
3	6	0.2682
4	8	0.3593
5	10	0.4449
6	12	0.5392

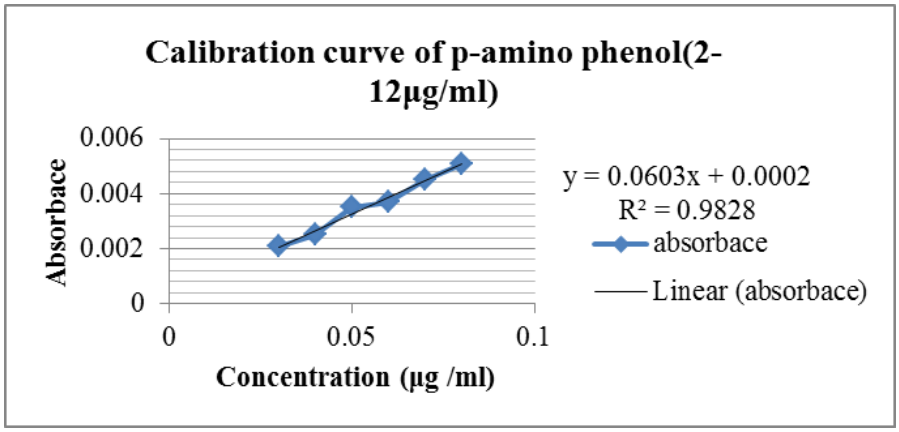

Figure 2: Calibration Curve for p-amino phenol (2 to 12µg/ml)

2.5.5. Specificity

Specificity (selectivity) is the ability to measure unequivocally the desired analyte in the presence of components such as excipients and impurities. [12, 13, 17, 18]

Standard solution of para amino phenol (0.4 ml) from stock solution A. Add 3.0 ml of 2% w/v ninhydrin reagent and mixed, immediately heated on water bath 70^0 C ± 2^0 C for 30 minutes, cooled at room temperature. Now spiking Paracetamol in 3 different levels 50,100,150% respectively from stock solution B, make up the volume to 10ml with DMF, The absorbance of resulting solution was measured at 547nm against blank.

Spiking Paracetamol in 3 different levels 50,100,150% respectively from stock solution B to determine the amount of %recovery at 547nm.

2.5.6. Accuracy

Accuracy is the degree of agreement of test results with the true value, or the closeness of the results obtained by the procedure to the true value. [12, 13, 15, 17, 18]

Prepare 20 µg/ml of para aminophenol solution in three sets from stock solution. Spike standard para amino phenol in concentration of 50, 100,150% respectively. Analysis were carried out using 2% w/v ninhydrin reagent; 3.0 ml were added and mixed, immediately heated on water bath 70^0 C ± 2^0 C for 30 minutes, cooled at room temperature and volume adjusted to 10 ml with DMF, The absorbance of resulting solution was measured at 547nm against blank. Solution stability was also performed simultaneously for 2 hours highlighted in (**Table 4**).

Table 3: % Recovery studies for specificity

Sr. No	Conc. of API µg/ml	Add % level	Spike stock sol. µg/ml	Abs. at 547nm	Drug found µg/ml	% Recovery
1	4	50	2	0.1812	4.14	103.5
2	4	100	4	0.1812	4.136	103.4
3	4	150	6	0.1816	4.146	103.7

Table 4: Accuracy Study with Solution Stability

Sr. No	conc. Of API	ml of stock sol.	Level of add. %	Spike API in ml	Total conc. µg/ml	Abs. at 547 nm	Drug found µg/ml	% recovery
After 1 Hour								
1	20	2	50	1	30	1.334	29.76	99.31
2	20	2	100	2	40	1.797	40.03	100.1
3	20	2	150	3	50	2.242	49.93	99.91
After 2 Hours								
1	20	2	50	1	30	1.336	29.8	99.42
2	20	2	100	2	40	1.799	40.66	100.2
3	20	2	150	3	50	2.243	49.86	99.97

2.5.7. Precision

Precision is the degree of agreement among individual results. The complete procedure should be applied repeatedly to separate, identical samples drawn from the same homogeneous batch of material. [12, 13, 15, 17, 18]

Prepare 20µg/ml of para aminophenol solution in three sets from stock solution. Spike standard para aminophenol in concentration of 50,100,150% respectively. Analysis were carried out using 2% w/v ninhydrin reagent; 3.0 ml were added and mixed, immediately heated on water bath 70^0 C ± 2^0 C for 30 minute, cooled at room temperature and volume adjusted to 10 ml with DMF, The absorbance of resulting solution was measured at 547nm against blank. The same complex solution were taken and the absorbance were measured at 547nm at 4 different time intervals (i.e. at 30 minutes, 60 minutes, 90 minutes, 120 minutes).

The interday precision and intraday precision are applied to determine the sample stability. The interday precision and

intraday precision was found RSD is less than 2%.

3. Calculation

3.1. Energy of Activation E_a [11, 20]

3.1.1. Water bath

Table 5: Calibration Curve of Paracetamol in 0.01M H_2SO_4

Sr. No	Concentration µg/ml	ml of Stock Solution	ml of 0.01M H_2SO_4	Absorbance
1	2	0.2	9.8	0.2349
2	3	0.3	9.7	0.2383
3	4	0.4	9.6	0.3140
4	5	0.5	9.5	0.4028
5	6	0.6	9.4	0.4761
6	7	0.7	9.3	0.5410
7	8	0.8	9.2	0.6035
8	9	0.9	9.1	0.6835

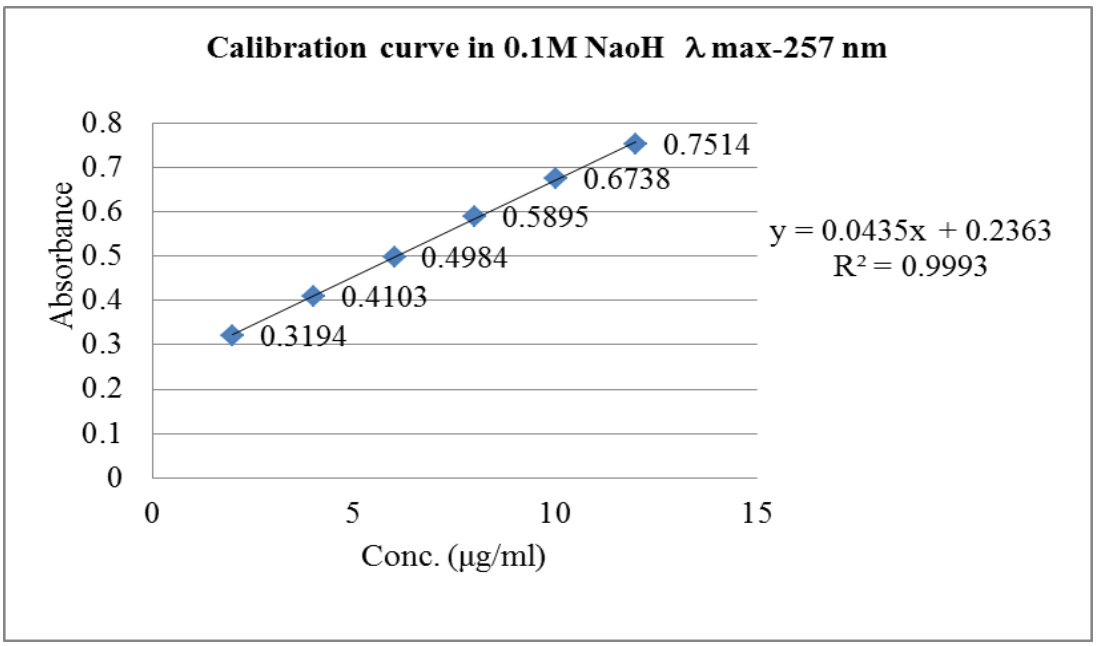

Figure 3: Calibration curve of Paracetamol in 0.01M H_2SO_4 (λ max-372 nm)

Table 6: Determination of specific reaction rate constant (K1, at room temperature)

Sr. No	Time Minutes	Abs (a-x)	Conc. (a-x) µg/ml	Log (a-x)	$K_1 = \frac{2.303}{t} \log \frac{a}{(a-x)}$
1	Initial	0.3208	4.0112	0.60327	---
2	30	0.3450	4.3521	0.63869	0.27188×10^{-4}
3	60	0.3135	3.9084	0.59199	4.3292×10^{-4}
4	90	0.3129	3.90	0.59106	3.12331×10^{-4}
5	120	0.3123	3.8915	0.59011	2.5254×10^{-4}
6	150	0.2905	3.5845	0.55442	7.49847×10^{-4}
7	180	0.2866	3.5295	0.54771	7.106124×10^{-4}
8	210	0.2627	3.1929	0.50418	0.108602×10^{-4}
9	240	0.2614	3.1746	0.50168	9.748373×10^{-4}

Mean = 9.041325×10^{-4}/min

Table 7: Determination of specific reaction rate constant (K2, at 60ºC)

Sr. No	Time Minutes	Abs (a-x)	Conc. (a-x) µg/ml	Log (a-x)	$K_2 = \frac{2.303}{t} \log \frac{a}{(a-x)}$
1	Initial	0.3553	4.4971	0.65293	---
2	30	0.3265	4.0915	0.61188	3.1509×10^{-3}
3	60	0.2932	3.6225	0.5590	3.60503×10^{-3}
4	90	0.1584	1.72394	0.23652	0.010651
5	120	0.1527	1.64366	0.21579	8.3883×10^{-3}
6	150	0.1580	1.71830	0.23509	6.41384×10^{-3}
7	180	0.1350	1.39436	0.14437	6.5044×10^{-3}
8	210	0.0611	0.35352	- 0.45158	0.012105
9	240	0.0507	0.20704	- 0.68394	0.012828

Mean = 7.561975×10^{-3}/min

Table 8: Determination of specific reaction rate constant (K3, at 80⁰C)

Sr. No	Time minutes	Abs (a-x)	Conc. (a-x) µg/ml	Log (a-x)	$K_3 = \frac{2.303}{t} \log \frac{a}{(a-x)}$
1	Initial	0.3400	4.2816	0.65293	---
2	30	0.1924	2.2080	0.61188	0.0220769
3	60	0.1326	1.3605	0.5590	0.01910947
4	90	0.0099	- 0.3676	0.23652	5.03874×10^{-3}
5	120	0.0071	- 0.4070	0.21579	4.62862×10^{-3}
6	150	0.0067	- 0.4126	0.23509	3.79344×10^{-3}
7	180	0.0026	- 0.47042	0.14437	3.8893×10^{-3}
8	210	- 0.0418	- 1.09577	- 0.45158	6.49056×10^{-3}
9	240	- 0.0013	- 0.5253	- 0.68394	3.3778281×10^{-3}

Mean = $8.55060725 \times 10^{-3}$/min

3.1.1.1. Calculation for Energy of Activation (E_a): For room temp and 60⁰C

$$Ea = 2.303 \frac{[T1\ T2\ R]}{T2-T1}\ X\ LOg\ K2/K1$$

Where K_1 and K_2= Mean of Room temperature and 60⁰C
Convert T_1 and T_2 in Kelvin, hence add 273

$$E_a = 2.303 \frac{[(20 + 273)\ (60 + 273)\ 1.98]}{[(20 + 273) - (60 + 273)]} \quad X \quad \text{Log } \frac{0.007561975}{0.0009041325}$$

$$E_a = \frac{444908.7859}{40} \quad X \quad (-2.121364763) - (-3.043767919)$$

E_a= 11122.71965 X 0.922403156 = 10.25963171 Kcal/mole

3.1.1.2. Energy of Activation (E_a): For room temp and 80⁰C

$$Ea = 2.303 \frac{[T1\ T2\ R]}{T2 - T1}\ X\ LOg\ K3/K1$$

Where K_1 and K_3 = Mean of Room temperature and 80⁰C
Convert T_1 and T_3 in Kelvin, hence add 273

$$E_a = 2.303 \frac{[(20 + 273)\ (80 + 273)\ 1.98]}{[(20 + 273) - (80 + 273)]} \quad X \quad \text{Log } \frac{0.00855060725}{0.0009041325}$$

$$E_a = \frac{471630.0343}{60} \quad X \quad (-2.068003054) - (-3.043767919)$$

E_a= 7860.500571 X 0.975764865 = 7.670000278 Kcal/mole.

3.1.2. Hot air oven

Table 9: Calibration Curve of Paracetamol in 0.01M NaOH

Sr. No	Concentration µg/ml	ml of Stock Solution	ml of 0.01M H_2SO_4	Absorbance
1	2	0.2	9.8	0.3194
2	4	0.3	9.7	0.4103
3	6	0.4	9.6	0.4984
4	8	0.5	9.5	0.5895
5	10	0.6	9.4	0.6738
6	12	0.7	9.3	0.7514

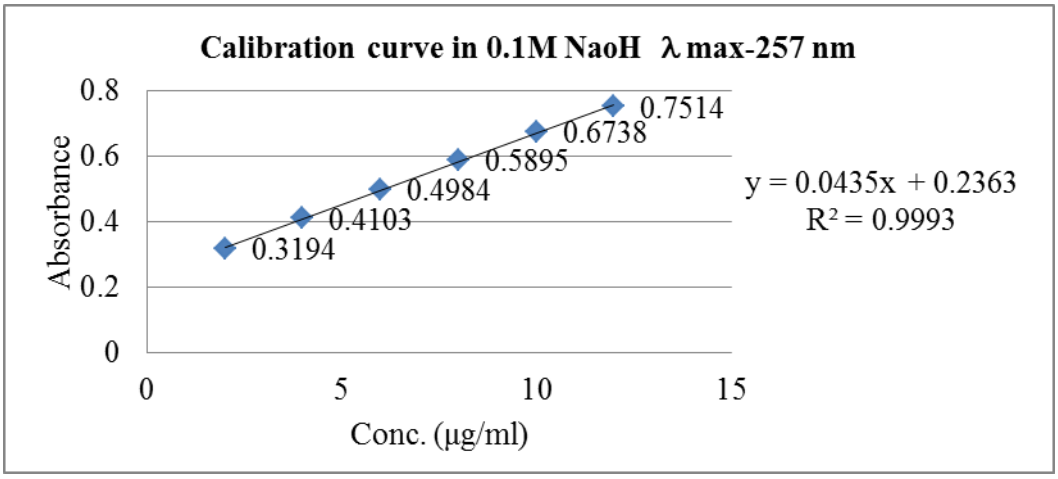

Figure 4: Calibration curve of Paracetamol in 0.1M NaOH (λ max-257 nm)

Table 10: Specific reaction rate constant Paracetamol Degradation in Bulk in hot air oven (K_1, at 120⁰ C)

Sr. No	Time Minutes	Abs (a-x)	Conc. (a-x) µg/ml	Log (a-x)	$K_1 = \frac{2.303}{t} \log \frac{a}{(a-x)}$
1	Initial	0.5995	8.4534	0.9270	---
2	60	0.5979	8.4162	0.9251	7.25382×10^{-5}
3	120	0.5648	7.6460	0.8834	8.36684×10^{-4}
4	180	0.5413	7.10	0.8512	9.69482×10^{-4}
5	240	0.5281	6.7930	0.8321	9.106414×10^{-4}
6	300	0.5211	6.630	0.8215	8.0989185×10^{-4}

Mean=$7.06790614 \times 10^{-4}$/min

Table 11: Specific reaction rate constant Paracetamol Degradation in Bulk in hot air oven (K_2, at140⁰ C)

Sr. No	Time Minutes	Abs (a-x)	Conc. (a-x) µg/ml	Log (a-x)	$K_2 = \frac{2.303}{t} \log \frac{a}{(a-x)}$
1	Initial	0.5995	8.4534	0.9270	---
2	60	0.5990	8.4418	0.9264	2.3028×10^{-5}
3	120	0.5460	7.2093	0.8579	1.326029×10^{-3}
4	180	0.5152	6.4930	0.8124	1.466192×10^{-3}
5	240	0.4739	5.5326	0.7429	1.766592×10^{-3}
6	300	0.4337	4.5977	0.6625	2.0304872×10^{-3}

Mean = 1.3183206×10^{-3}/min

Table 12: Specific reaction rate constant Paracetamol Degradation in Bulk in hot air oven (K_3, at 160⁰ C)

Sr. No	Time Minutes	Abs (a-x)	Conc. (a-x) µg/ml	Log (a-x)	$K_3 = \frac{2.303}{t} \log \frac{a}{(a-x)}$
1	Initial	0.5995	8.4534	0.9270	---
2	60	0.5619	7.57907	0.8796	1.819212×10^{-3}
3	120	0.5266	6.7581	0.82983	1.8646923×10^{-3}
4	180	0.4740	5.5349	0.7431	$2.35289016 \times 10^{-3}$
5	240	0.4214	4.31163	0.63464	$2.805436859 \times 10^{-3}$
6	300	0.3688	3.0884	0.48973	$3.356790609 \times 10^{-3}$

Mean $= 2.439804386 \times 10^{-3}$/min

3.1.2.1. Calculation for Energy of Activation (E_a): For 120⁰ C and 140⁰ C

$$Ea = 2.303 \frac{[T1\ T2\ R]}{T2 - T1}\ X\ LOg\ K2/K1$$

Where K_1 and K_2 = Mean of 120⁰ C and 140⁰ C
Convert T_1 and T_2 in Kelvin, hence add 273

$E_a = 2.303 \left[\frac{(120 + 273)\ (140 + 273)\ 1.98}{[(120 + 273) - (140 + 273)]}\right] \text{ X Log } \frac{0.0013183206}{0.000706790614}$

$E_a = \frac{740119.3015}{20}$ X (-2.879978962) – (-3.150709226)

E_a= 37005.96507 X 0.270730266 = 10.01863477 Kcal/mole

3.1.2.2. Calculation for Energy of Activation (E_a): For 140⁰ C and 160⁰C

$$Ea = 2.303 \frac{[T1\ T2\ R]}{T2 - T1}\ X\ LOg\ K3/K1$$

Where K_2 and K_3 = Mean of 120⁰ C and 140⁰ C
Convert T_2 and T_3 in Kelvin, hence add 273

$E_a = 2.303 \left[\frac{(140 + 273)\ (160 + 273)\ 1.98}{[(160 + 273) - (140 + 273)]}\right] \text{ X Log } \frac{0.0024398043}{0.0013183206}$

$E_a = \frac{815449.5103}{20}$ X (-2.612644992) – (-2.879978962)

E_a= 40772.47551 X 0.26733397 = 10.89986774 Kcal/mole.

3.1.2.3. Calculation for Energy of Activation (E_a): For 160⁰ C and 120⁰ C

$$Ea = 2.303 \frac{[T1\ T2\ R]}{T3 - T1}\ X\ LOg\ K3/K1$$

Where K_1 and K_3 = Mean of 120⁰ C and 160⁰ C
Convert T_1 and T_3 in Kelvin, hence add 273

$E_a = 2.303 \left[\frac{(120 + 273)\ (160 + 273)\ 1.98}{[(160 + 273) - (120 + 273)]}\right] \text{ X Log } \frac{0.0024398043}{0.0013183206}$

$E_a = \frac{775960.4299}{40}$ X (-2.612644992) – (-3.150709226)

E_a= 19399.01075 X 0.538064234 = 10.43791386 Kcal/mole.

Energy of Activation for paracetamol was found to be 10 Kcal/mol

Assuming Energy of 12 Kcal/mol affords nearly doubling of reaction rate for every 10ºC rise in temperature.

With this relationship one can calculate the amount of time a sample should be stored at a specified temperature to achieve the energy equivalent of exposure at accelerated stability conditions (40 ºC for 6 months) so to keep at 80 ºC (every 10 ºC rise 2^4)

180 days (6 months) / 2^4 = 11.25 = 12days.

A sample of bulk drug substance stored at 80 ºC would be stored for 12 days is kinetically equivalent to 40 ºC for 6 months.

Using the same concept paracetamol drug product was kept at 100 ºC for 3 days considering the energy of activation to be 10 Kcal/mole. The degraded product was estimated by the prepared method. [20]

3.2. Paracetamol Degradation

Take 9 tablets of Calpol 500 mg, covered with aluminum foil was kept in hot oven at 100 ^{0}C for 3 days. [5, 10]

After heating each tablet was triturated, Weight equivalent to 5 mg of paracetamol, 5.7 mg of tablet powder was for analysis using 2% w/v ninhydrin reagent; 3.0 ml were added and mixed, immediately heated on water bath 70^0 C ± 2^0 C for 30 minutes, cooled at room temperature and volume adjusted to 10 ml with DMF, The absorbance of resulting solution were measured at 547nm against blank.

Table 13: $\bar{x}$ Found (For tablet degradation) at 100^{0}C for 3 days

Tab. Powder mg	Abs 547 nm	Mean $\bar{x}$
5.7	1.7	
5.7	1.8	
5.7	1.7	
5.7	1.6	
5.7	1.5	1.6412
5.7	1.4	
5.7	1.5	
5.7	1.4	
5.7	1.6	

Absorbance of degraded paracetamol i.e. P-amino phenol is 1.6412,

Concentration of p-amino phenol =

Y = 0.0454x – 0.0054

1.6412 = 0.0454 x – 0.0054

X= 36.2687 µg/ml

Weight equivalent to 5 mg of paracetamol, 5.7 mg of tablet powder was analyzed and P- amino phenol was found to be 36.2687µg/ml, thus the degradation is 0.7% from the tablet dosage form.

4. RESULTS AND DISCUSSION

A spectrophotometric method was developed for quantitative determination of degraded amino group in sample and reduces unnecessary tedious sample preparation.

The ninhydrin has been known as a reagent for detection of amino acid and amines. It was suggested that the reaction of ninhydrin with amine, amino acid and imino acids all produced by the mechanism to give the Ruhemans purple.

In present study by using chemical Derivatization, UV method is developed for the determination of the Para -Amino phenol, which is a degraded product of paracetamol. For the preparation of stable complex of para amino phenol solvents like Water, Methanol, Acetone, Ethanol, DMF are used, and DMF showing stable readings among all other solvents. Accordingly concentration of Ninhydrin 3ml of 2% w/v, heating time 30 minutes at 70 ºC decided for formation of stable complex.

With set parameter the method is validated, LOD and LOQ were found to be 0.254(**Table 1,** and **Fig. 1**), 0.770 and 0.2849 (**Table 1,** and **Fig. 1),** 0.863 by standard deviation of blank and calibration curve method

respectively. Among the entire range 1 to 30 µg/ml Linearity range was found to be 2 to 12 µg/ml (**Table 2,** and **Fig. 2**) as it is having least sum square error i.e. 1.448667 x 10^{-5}. Method is found to be specific for the para amino phenol, data supported by ANNOVA test with at $P \leq 0.05$. For accuracy and precision confidence interval of 99% was considered, indicates that the results lie between 100.32-99.26% and 100.19 to 100.07% respectively (**Table 4**). From the principle of energy of activation the forced degradation of paracetamol in tablet dosage form is carried out at 100ºC for 3 days, degradation of paracetamol was found to be 0.7% (**Table 13**).

Table 14: Result of various validation parameters

Parameters	Values
Linearity and range (µ/ml)	2-12 µg/ml
Precision (% RSD)	RSD< 2%
Accuracy(% Recovery)	99.86 %
ANOVA	$P \leq 0.05$
LOD (µg/ml)	0.284971
LOQ (µg/ml)	0.8635
Energy of Activation	10.43791386 Kcal/mole
Degradation of Paracetamol	0.7 %

5. CONCLUSION

In present study by using chemical derivatization, UV method is developed for the determination of the Para -Amino phenol, which is a degraded product of paracetamol for the preparation of stable complex of Para amino phenol and DMF showing stable readings among all other solvents.

The developed spectrophotometric method was simple, sensitive and specific for the detection of amino group in bulk and pharmaceutical formulation. It could be precisely LOD was found to be0.284971µg/ml and LOQ was found to be 0.8635 µg/ml. The calibration curve shows linear relationship between the absorbance and concentration and the coefficient correlation was higher than 0.99. Precision was found to be less that 2%. The % recovery was found to be 99% and sample solution stable for 2 hrs and the degradation of Paracetamol was found to be 0.7% At 100 ºC for 3 days.

Hence the method is developed and validated for the detection of p-amino phenol, a degradation product of paracetamol and by using concept of energy of activation p-amino phenol from paracetamol drug product is estimated.

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