Sudan University for Science and technology

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Development Of Method Of Analysis for Paracetamol Tablet

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الآية الكريمة

قال تعالى:

(الله لا إله إلا هو الحي القيوم لا تأخذه سنة ولا نوم له ما في السموات وما في الأرض من ذا الذي يشفع عنده إلا بإذنه يعلم ما بين أيديهم وما خلفهم ولا يحيطون بشئ من علمه إلا بما شاء وسع كرسيه السموات و الأرض ولا يؤوده حفظهما وهو العلي العظيم)

صدق الله العظيم

Dedication

To our parents, family, friends and all of our relatives

Acknowledgment

First of all, we are so grateful to Allah for lighting our minds and helping us to complete this study.

We would like to express our deep sincerest and thanks for ***D. Salah Ahmed Ibrahim*** *for his supervision valuable time, support and help.*

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Abstract

This research for comparison of pharmaceutical product from two deferent companies using two different method of analysis (HPLC and UV) then compare between two methods by obtained the sensitivity and the accuracy of results.

The results shown that the content of paracetamol in sample one and two respectively by using UV 98.1%, 97.9% and the content for the two sample when using HPLC 99.8%, 100.3% respectively. From the observed results HPLC sensitive method more sensitive and accurate than UV method, this refer to using separation and determination analytical method in the same time, the excipients had been separated from the active ingredient that was been analyzed, then determine the active ingredient on the opposite when using UV method the active ingredient and excipients was not separated. Also HPLC method used a little quantity of sample used for quntitive , qualitative analysis HPLC method was found to be adequate and it should there for be used to obtaing accurate date.

مستخلص البحث

يختص هذا البحث بعمل مقارنة لمنتج دوائي من شركتين مختلفتين باستخدام طريقتين تحليليتين(كروماتوغرافيا السائل ذو الكفاءة العالية والأشعة فوق البنفسجية) ومن ثم مقارنة فعالية الطريقتين بإيجاد نتائج ذات حساسية و دقة عالية. أظهرت النتائج لإيجاد الباراسيتمول للمنتج الأول والثاني على التوالي باستخدام طريقة الأشعة فوق البنفسجية هي 98.1% 97.9%.
كما أوضحت نتائج التحليل لنفس المنتجين باستخدام طريقة كروماتوغرافيا السائل ذو الكفاءة العالية هي 99.8% و 100.3% على التوالي. ومن النتائج السابقة يتضح أن طريقة تحليل محتوى الباراسيتمول للمنتجين أن طريقة كروماتوغرافيا السائل ذو الكفاءة العالية تتمتع بحساسية ودقة عالية أكثر من طريقة الأشعة فوق البنفسجية و يرجع ذلك إلى استخدام طريقة فصل وتقدير في نفس الوقت حيث يتم فصل المواد المضافة عن المادة الفعالة المراد تحليلها ومن ثم يتم تقدير المادة الفعالة والمواد المضافة كل على حده وذلك على العكس في طريقة الأشعة فوق البنفسجية حيث تقدر المواد الفعالة والمواد المضافة في دفعة واحدة.
ويرجع أيضا لاستخدام كمية قليلة من العينة في كروماتوغرافيا السائل ذو الكفاءة العالية.

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1.1 INTRODU TION:

The human body is continually exposed to a wide range of substance which is not natural constituents of the body; indeed, many substances are the product of human industry and are never found in nature. The human digestive tract has evolved to cope with food components, while the internal organs possess limited capacities to metabolize and clear foreign compounds accidentally ingested with foods, or released into the system by parasites.(Kleerebezem 2013). For the past century there has been an enormous increase in both numbers and types of foreign compounds ingested by human populations. Clearly, pharmaceuticals of most type are foreign compounds, though a few contain substance normally present in the human body. There is a wide range of pharmaceutical products used in Medicine, and it was seen that most drugs are used to bring about specific change in some part of the body. A drug may inter the body, generally speaking, in two main ways, eternal (via the gastro-intestinal tract) or parenteral (by-passing the gastro-intestinal tract; which in effect means injection). As the principle routes of drug administration are oral, intravenous and topical, the factors affecting the absorption and uptake by particular receptors of important drugs.(Benet et al 1996), (Briggs, 2014)

1.2 Structure of Paracetamol:

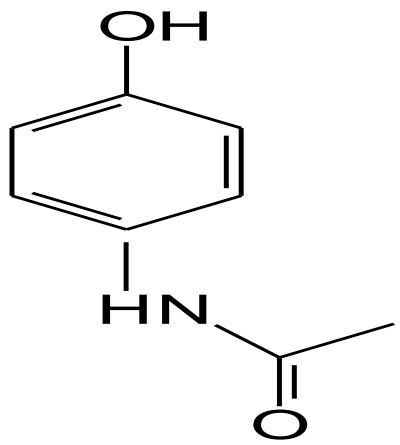


Fig 1.1: Structure of ParacetamoL

l

1.3 Systematic Name:

4-hydroxyacetanilide

1.4 About paracetamol:

Paracetamol is a common analgesic and antipyretic drugthat is used for the relief of fever, headaches, and other minor aches, is accentually and peripherally actingopioidnon- analgesic and antipyretic. Paracetamol lacks many of the side effects of aspirin,unlike other common analgesicsuch as aspirin and ibuprofen, and has no anti-inflammatoryproperties, and so it is not a member of the class of drugs known as non steroidal anti-inflammatory drugs or NSAIDs.
Paracetamol does not irritate the lining of the stomach or affect blood coagulation as compared to aspirin. At normal therapeutic doses, Paracetamol is metabolized very fast and completely by undergoing glucuronidation and sulphonationto inactive metabolites.(Clissold 1986) ,((Turner et al 1998).

1.4.1 Composition:

Each tablet contains paracetamol BP 500 mg

1.4.2 Properties:

Paracetamol is an analgesic antipyretic, which is used generally for the symptomatic management and treatment of mild to moderate pain and pyrexia. It is not related to acetyl salicylic acid and does not produce gastric irritation.

1.4.3 Indications:

It is indicated for the treatment of headache muscular aches, arthritic pain and fever . For children it is considered beneficial for symptomatic treatment of post following immunization. It may also be given prophylactically to infants with a higher than normal risk of febrile convulsions following diphtheria , tetanus, pertussis and polio immunization.

1.4.4 Dosage:

Adults: 1-2 tablets 3-4 times daily. The total daily does should not exceed 8 tablets.

Children: 7-12years old 1/2 -1 tablet 3-4 times daily but not exceeding 4 tablets daily.

1.4.5 Age:

Under 3 months	10mg/kg body weight
3 months 1 year	1/2-1 teaspoonful
1-5 year	1-2 teaspoonful
6-12 year	2-4 teaspoonful

For post immunization pyrexia 1/2 teaspoonful is recommended for children 2-3 months of age followed by a second dose 4-6 hours later . If pyrexia still exists , parents should teak medical advice.

1.4.6 Precaution:

Paracetamol should be given with caution to patients impaired kindly or liver function. It should also be given with care patients taking other drugs that might affect the liver.

1.4.7 Contra-indications:

Paracetamol is contra-indicated for patients having hypersensitivity to the drug.

2.1 Objective:

The aim of conducting study was Comparisons between two products of drugs of paracetamol active ingredient from two different components

2.2.Specific objective:

1/ To determine content paracetemol by HPLC .

2/ To determine content of paracetemol by using UV spectrophotometer.

3/ To Comparison between two samples of paracetemol.

3.1 Equipment& Apparatus required:

-Sensitive balance

- Beakers.

- Pipettes.

- Burette.

- Funnels.

-Mortar and pestle.

-Filter paper.

- Volumetric flasks.

- wash Bottles.

- Conical flasks.

-Graduated cylinder.

- PH meter.

-High Performance Liquid Chromatography.

- UV spectrophotometerInstrument name: UV-1800

- IR Spectrophotometer.

- Magneticstirrer.

3.2 Chemicals:

-0.1 Sodium hydroxide.
- Purified water.
- Paracetamol working standard.
-Paracetamol tablets.

-Phosphate buffer PH 5.8 (prepared by potassiumdihydrogen0rthophosphate and 0.9 mg ofsodiumhydroxide diluting to 6.0 liters with water.

- Potassium bromide (solid).

3.3 Procedure:

3.3.1 Assay (UV spectrophotometer):

Wavelength / 257.0 nm and Date accumulation/ sec: 0.2, with K factor: 278.00 and Slit width /nm 1.0.

3.3.1.1 Preparation of standard:

0.15gm of paracetamol working standard was weighed, 50 ml of 0.1M sodium hydroxide was add, diluted with 100 ml of water,shake for 15 minutes and sufficient water was add to produced 200 ml .mixed, filtered and 10 ml of the filtrate was diluted to 100 ml with water .10 ml of the resulting solution was added to 10 ml of 0.1M sodium hydroxide was diluted to 100 ml with water.(British Pharmacopoeia, 2013)

3.3.1.2 Preparation of sample:

0.19gm. of powder 20 tablets was weighed, 50 ml of 0.1M sodium hydroxide was added, diluted with 100 ml of water, shake for 15 minutes and sufficient water was added to produce 200 ml. Mixed, filtrated and 10 ml of filtrated was diluted to 100 ml with water.
The absorbance was measured at 257 nm; 10 ml of sodium hydroxide0.1M wasdiluted to 100 ml with water as blank.
For measuring the absorbance of UV or visible radiation are made up of the following components; sources(UV and visible), wave length selector (monochromator), sample containers, detector, signal processor and readout.(British Pharmacopoeia,2013)

3.3.2 HPLC:

20 tablets of paracetamol was weighed and powdered, 0.5 mg of the powdered was shaken with 100 ml of the mobile phase for 10 minute , it was diluted to 200 ml with the same solvent and filtered through a glass-fiber filter (whatman GF/C is suitable) . 5 ml of the filtrate was diluted to 250 ml with the mobile phase.

-0.005% w/v of Paracetamol BPCRS in the mobile phase.

Chromatographic Condition:

-Stainless steel column (10 cm * 4.6 mm) was used packed with octadecylsilyl silica gel for chromatography (5µm)(Nucleosil C18 is suitable).

-Isocratic elution and mobile phase were used.

-Flow rate of 1.5 ml per minute .

-Ambient column temperature .

-Detection wavelength of 243 nm .

-20 µl of each solution .

*Mobile Phase:

0.01 sodium pentanesuulfonate in mixture of 22 volumes of methanol and 78 volumes of water, the PH of the solution being adjusted to 2.8 using 2M hydrochloric acid .

3.3.3 Dissolution:

-900 ml of phosphate buffer pH 5.8 was placed in each vessel; temperature was maintain at 37̊ C, one tablet was placed in each vessel, sample was run as per the parameter, at the end of time sample had been withdrawn properly, filtered and diluted 1 ml of filtrated to 100 ml with 0.1M sodium hydroxide, 0.2800 gm of paracetamol working standard was taken and completed to 500 ml V.F with phosphate buffer PH 5.8.

1 ml of this solution was diluted to 100 ml with sodium hydroxide, and then the absorbance was measured at 257 nm using 0.1M sodium hydroxide as blank.

Dissolution is a chemical test used to determine the percentage of hydrolysis active ingredient in dissolution tester.

The main parts: Body, Basket, Paddles, Vessel, Thermometer, Keyboard for setting, Water path.

3.3.4 IR Spectrophotometer:

Preparation of sample:

The sample was prepared in low humidity room ,KBr tablet dwas disassembled and remove the rust – prevented grease in the part of die sample base(3) tablet was framed (5)by CCL4 benzenewell .

The sample base (3) was inserted to concave on the base (1) with its plane surface being upward , the tablet frame (5) was set to the head. Since the tablet frame(5) and the sample base (3) were finished to fit with no gap , the sample frame cannot be inset if it is inclined even a little . When it is inset well the tablet frame (5) can be turned lightly by hand.

KBr powder was shattered into fine particles in the agate Mortar. Next the grain size was equalized by the furnish filter, the powder was heated and dry at 120 – 150 C for more than 24 hour and keep it in a desiccator. 2 – 4 mg was put into 400 mg of KBr powder mixed well in the agate mortar and 200 mg was taken out for forming, the mixed powder was put on forming quantity carefully into the concave of the table frame (5) on the sample base (3) with the spatula.

The another sample base (3) was inserted with its plane surface being downward into the tablet frame (5) and it was pressed with the finger , the powder was make flat by rotated it . As the tablet frame (5) and the sample base (3) were finished to have no gap , was inserted it very straightly . whenthe sample base (3) was turned smoothly in the tablet frame (5) it in was inset well .

The guide frame (6) was put on the base (1) accurately then the guide frame (6) was rotated to see that is was set correctly. Spring (8) was set on the guide frame (6), the plunger (4) was inserted and placed it in the hydraulic press.

The evacuating tap (P) of the guide frame (6) was connected to vacuum pump, and pressed while evacuating was being made; pre-evacuation time is approximately 5minute.Vacuum evacuation was being applied while pressing was made. Theworking pressure 78.5 KN (8 ton) the pressing time 5-10 minute.

When the tablet die was taken out after pressing , the guide frame (6)andplunger(4) were removed together with the spring (8) from the base (1) , then the tablet frame (5) was taken which was still attached with the sample base (3) from the base (1) , and it was put in the punching base (10) as it is .

The spring (8) and plunger (4) were removed and the guide frame (6) was put on punching base (10). The punching rod (11) was inserted to the guide frame (6) and it was put on the hydraulic press, then it was pressed and punched out the tablet. after punching out the punching rod (11) and the guide frame (6) wasremoved from the punching base (10) and then the tablet had been remain in the concave part of the benching base (10) lying between two sample base (3), tablet was taken out and it was set holder then the window of the sample compartment was inset and the measurement was made.(British Pharmacopoeia,2013)

4.1 Assay results & Discussion:

4.1.1 HPLC Result:

HPLC result of two samples were showed that the peak area observed at 3824.511 and 3817.593 respectively with rotation time around 4.82 min, that indicate the sample in range of paracetemol. Table 1 and 2 shows the other parameters of two samples

Table 1 *result of product one*

	Reten.time[min]	Area[Mau]	Height[mAU]	Area [%]	Height[%]	W05[MIN]
1	4.817	3824.511	238.146	100.00	100.00	0.25
	Total	3824.511	238.145	100.00	100.00	

Table 2 *result of product two*

	Reten.tim[mi]n	**Area[Mau]**	**Height[Mau]**	**Area[%]**	**Height[%]**	**W05[min]**
1	4.817	3817.593	238.084	100.0	100.0	0.25
	Total	3817.593	238.084	100.0	100.0	

Table 3 *Standard result*

	Reten.time[min]	**Area[mAU]**	**Height[min]**	**Area[%]**	**Height[%]**	**W05[min]**
1	4.817	3853.133	238.234	100.00	100.00	0.25
2	4.817	3845.485	238.301	100.00	100.00	0.25
3	4.817	3841.951	238.281	100.00	100.00	0.25
	Average	3840.855333	238.272	100.00	100.00	0.25

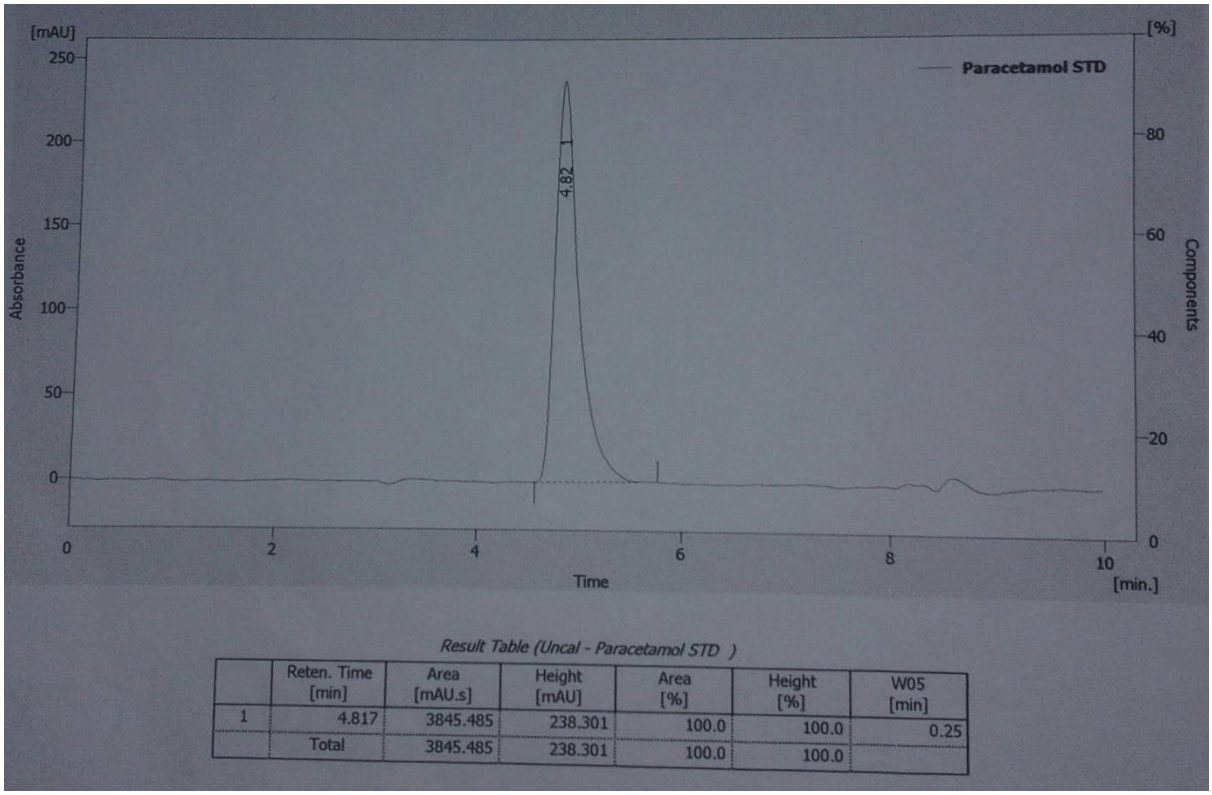
Content%=(Area{mAU.s}sample/Area{mAU.s})*100

Content of product (1) = (3817.593/3824.511)*100

=99.8%

Content of product (2) = (3835.133/3824.511)*100

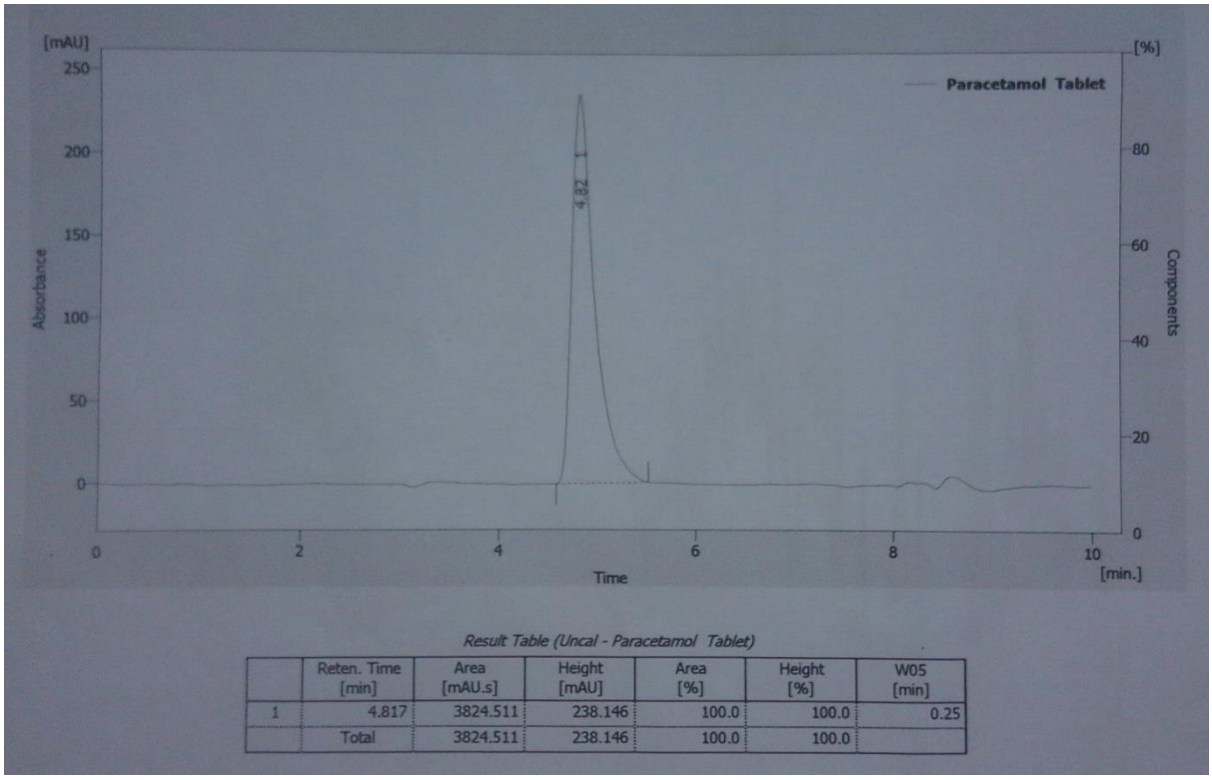
=100.3%



Result Table (Uncal - Paracetamol STD)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]
1	4.817	3845.485	238.301	100.0	100.0	0.25
	Total	3845.485	238.301	100.0	100.0	

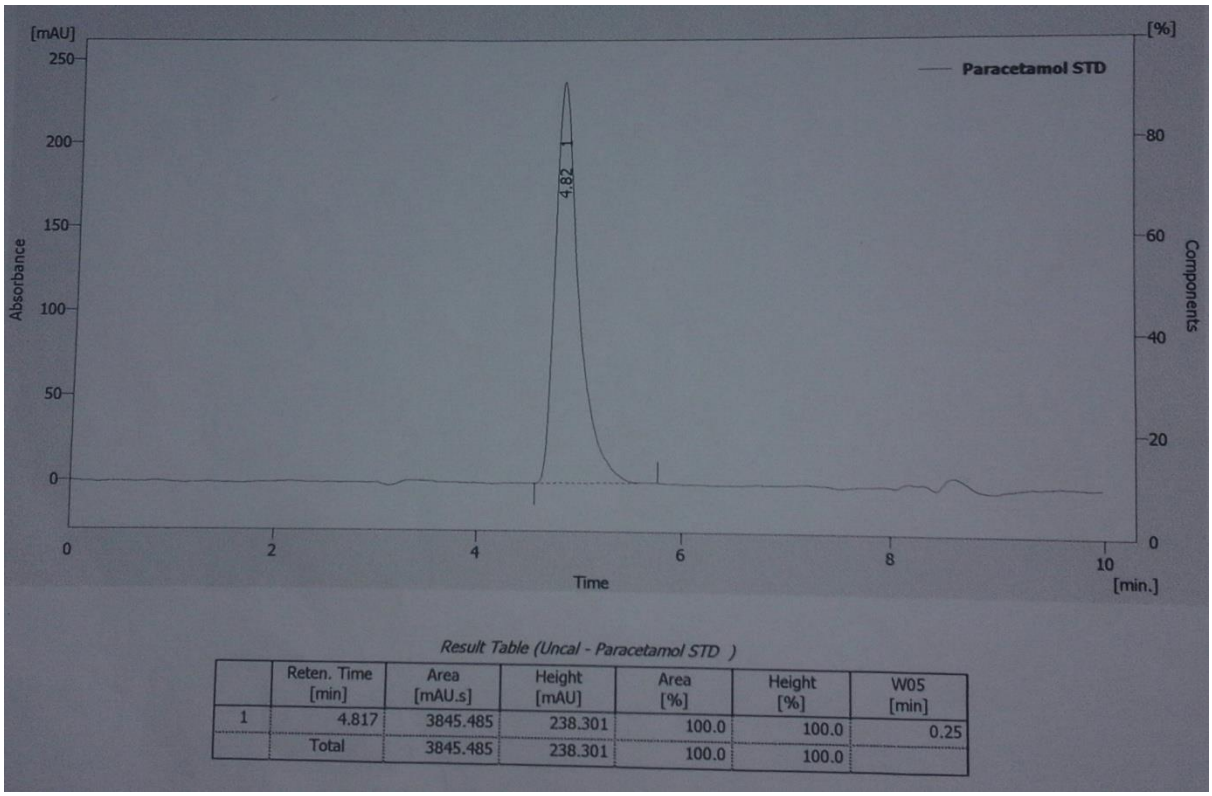
Fig 4-1 HPLC of standard



Result Table (Uncal - Paracetamol Tablet)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]
1	4.817	3824.511	238.146	100.0	100.0	0.25
	Total	3824.511	238.146	100.0	100.0	

Fig 4-2 HPLC of product one



Result Table (Uncal - Paracetamol STD)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]
1	4.817	3845.485	238.301	100.0	100.0	0.25
	Total	3845.485	238.301	100.0	100.0	

Fig 4-3 HPLC of product two

4.1.2 UV results:

The absorption of samples done after standard check, the average of results were 0.526 nm for sample one and 0.525nm for sample two which regarded to the assay of paracetemol. Table 3 present the results of UV

Table 4 *UV Result of two samples*

NO	Abs
1	0.592 (ST)
2	0.593 (ST)
3	0.525(1)
4	0.527(1)
5	0.524 (2)
6	0.525 (2)
7	0.593 (ST)
8	0.593 (ST)

Content%= (abs*100)/ (0.00075*715)

Content of Product(1) = ((0.526)/(0.00075*715))*100

=98.1%

Content of Product (2)=((0.525)/(0.00075*715))*100

=97.9%

The results of HPLC show the percentage of product one close to 99.8% which low than product two 100.3%, however the results of UV observed that product one show percentage about 98.1% and product two about 97.9%.

The determine of paracetamol by HPLC is more suitable according to process of instrument and selectivity.

4.2 Dissolution result:

To calculate the percentage of hydrolysis of active product ingredient (API) first the absorption of API in range with standard, however the result of calculation of product when it compare with standard (mustbe not less than 75% Q) sample one fitted well than sample two 83.8% and 85.9% respectively, Table 5 showed standard and sample one absorbent, and table 6 showed standard and sample two absorbent.

Table 5 *standards and sample one abs*

NO	Abs/nm
1	0.482 ST
2	0.477 ST
3	0.396
4	0.397
5	0.386
6	0.395
7	0.384
8	0.401
9	0.466 ST
10	0.466 ST

Table 6 *standards and sample two abs*

NO	Abs/nm
1	0.476 ST
2	0.475 ST
3	0.403
4	0.409
5	0.411
6	0.409
7	0.411
8	0.396
9	0.463 ST
10	0.464 ST

Content% =(Abs of sample*Wt of STD* 1*900*100)/(Abs of STA *500*100*1*0.5)

Table 7 *content of product (1)*

NO	Content%
1	83.8%
2	84.0%
3	81.7%
4	83.6%
5	81.2%
6	84.8%

Absorbance of standard=0.473 nm.

Table 8 *content of product (2)*

NO	**Content%**
1	85.9%
2	87.2%
3	87.6%
4	87.2%
5	87.6%
6	84.4%

Absorbance of Standard =0.4695 nm

4.3 IR result:

In Fig (2) the peak at 3570 cm^{-} appear due to NH group, the peak at 3400 cm^{-} appear to OH group, the peak at 1780 cm^{-} appear due to theCarbonyl group, the peak at 1150 cm^{-} appear due to C-O group, the two peak at 1400, 1600cm^{-} appear due to benzene ring, and the peak at 2780 cm^{-} appear due to CH (aliphatic) group, this wave length for product (1), and product (2) shown results to product (1).

The results of two products within the range of paracetamol.

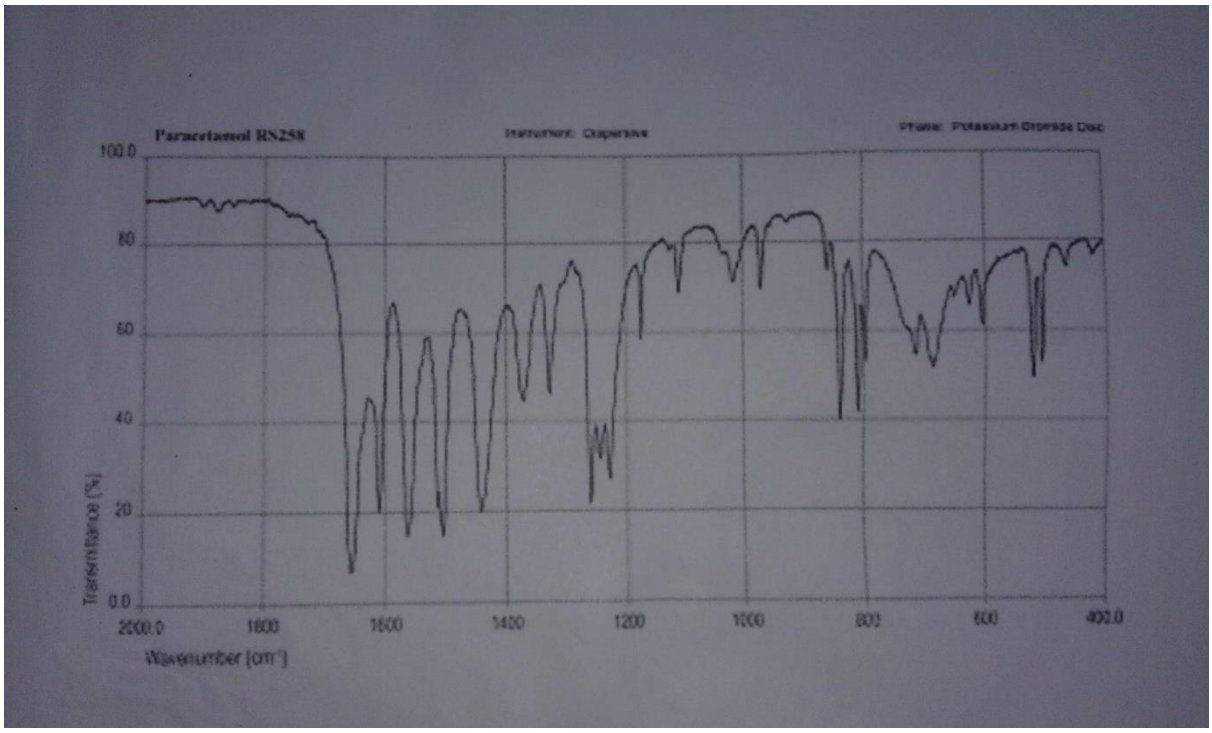


Fig 4-4 IR chart of standard

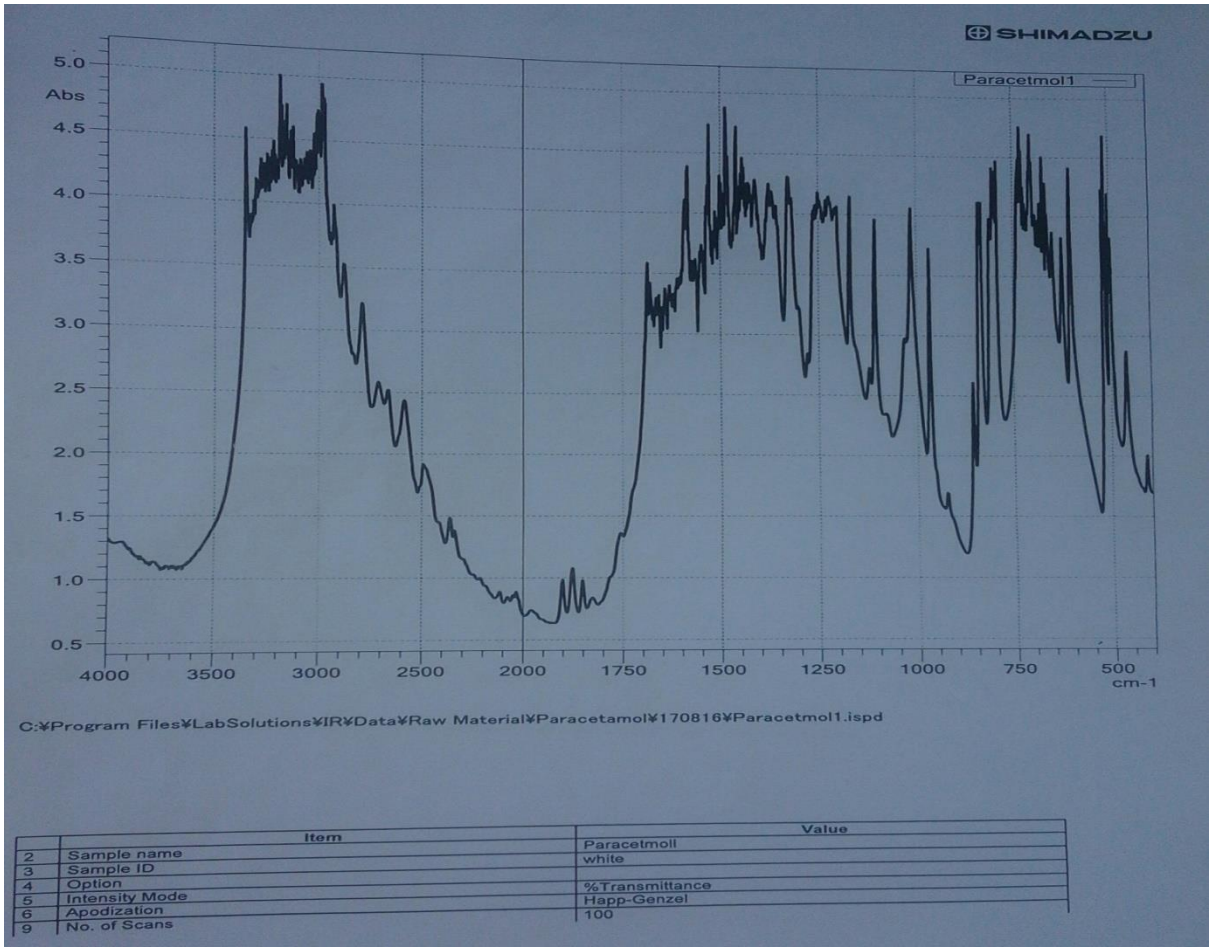


Fig 4-5 IR chart of product one

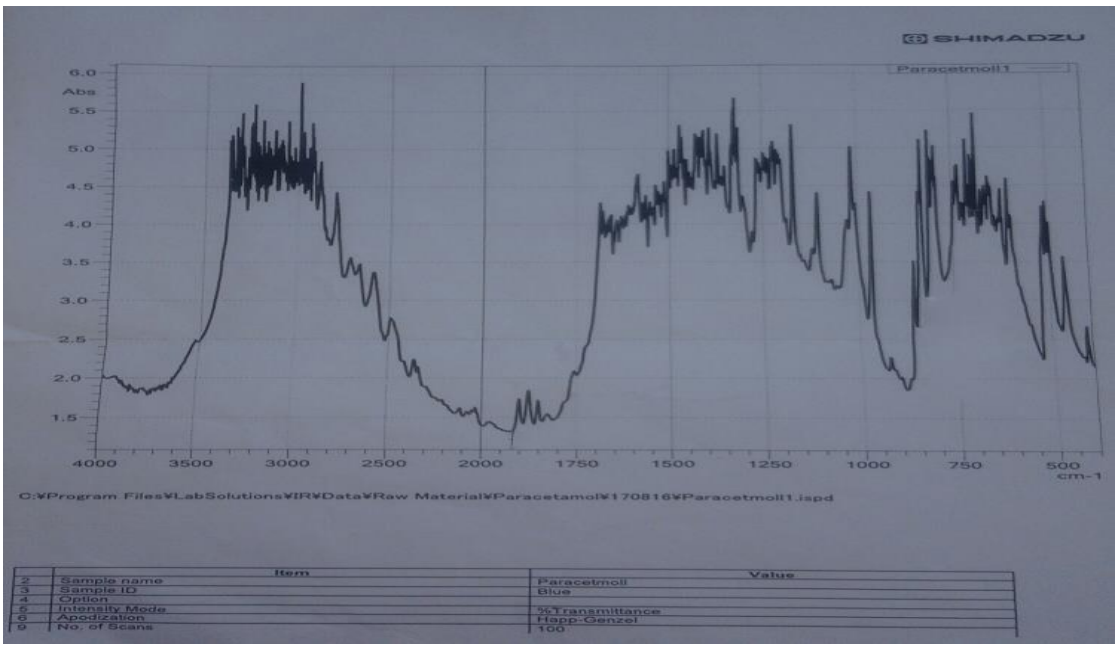


Fig 4-5 IR chart of product two

5.1 Conclusion:

Table 9 Finished Results by percentage

Test	Product (1)	Product (2)
Assay for HPLC	99.8%	100.3%
Assay for UV	98.1%	97.9%
Dissolution	Max%= 87.6%	Max%= 84.8%
	Min%= 84.4%	Min%= 81.2%

The acceptance limit of percentage in HPLC& UV(95% - 105%), and all the obtained results were on the limit.

References

Benet, L.Z., Kroetz, D., Sheiner, L., Hardman, J. and Limbird, L., 1996. Pharmacokinetics: the dynamics of drug absorption, distribution, metabolism, and elimination. Goodman and Gilman's the pharmacological basis of therapeutics, pp.3-27.

Briggs, M. and Briggs, M., 2014. The chemistry and metabolism of drugs and toxins: an introduction to xenobiochemistry. Elsevier.

British Pharmacopoeia Commission, 2011. British Pharmacopoeia 2013.

Clissold, S.P., 1986. Paracetamol and phenacetin. Drugs, 32(4), pp.46-59.

Kleerebezem, M., MICROBIOLOGICAL ,2013, ANALYSIS OF MUCOSA AND FAECES OF WEANED PIGLETS FED WITH PROBI-OTICS LACTOBACILLUS GASSERI LF221 AND K7. DEVELOPMENT, 1, p.5.

Turner, P. and Volans, G., 1998,The drugs handbook. Paul Turner and Glyn.