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Assay of Paracetamol in tablet form from different Manufacturing sources in Iraqi market

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الخلاصة:

عقارالبراسيتامول (الاسيتامينوفين) الذي يستعمل كمسكن للالم وخافض للحراره للكبار والاطفال. والذي يمكن أن يحلل بواسطة نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لمعرفة وزن المادة الفعالة للدواء في الصيغة الدوائية المعينة .

في هذه الدراسة تم أخذ خمس عينات من البراسيتامول (500 ملغم) من شركات دوائية مختلفة والموجودة في السوق العراقية لإجراء دراسة مقارنة لمحتوى الدواء في هذه العيناتِ مع النسبة المؤوية المقررة حسب دستور الادوية المعتمد. الدراسة جرت بتشكيلِ منحنى التحديدِ الناتج من استخدامُ محاليلَ قياسيةَ بتراكيز مختلفة من المعيارِ الخارجيِ للبراسيتامول وتحقن هذه المحاليل في نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لتعطي قراءات مختلفة لمساحة ما تحت القمة .ومن معرفة التراكيز ومساحة كل واحد منها يمكننا رسم المنحنى الذي سَيَتْبع معادلةَ الخطِّ المستقيمِ . البراسيتامول استخلصَ مِنْ أقراصِه وبعد ذلك حقنَ في نظامِ (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لمعْرِفة مساحة ما تحت القمّةِ و مِنْ المساحة النَاتِجةِ سيكون بإمكاننا أَنْ نَحْسبَ تركيزَ وبعد ذلك وزن الدواء الفعلي في القرص الواحد بواسطة معادلةِ الخَطِّ المستقيم الناتجة. إن النتائج التي تم الحصول عليها تبين أن جميع العينات الخمس هي ضمن النسبة المئويةِ المَقْبولةُ حسب دستور الأدوية الامريكي (90%–110%).

Abstract:

Paracetamol (Acetaminophen) is an analgesic, antipyretic used in treatment of fever and pain in adult and children. It can be assayed using HPLC to estimate the weight of the drug in the specific dosage form.

In this study we take samples of the Paracetamol (500mg) drug from five different pharmaceutical companies in the Iraqi market to make comparison study of the drug content in these samples. The study carried out by the formation of calibration curve using standard solutions of different concentrations of the external standard paracetamol USP 30, which results in

different readings of the area under the peak. The curve will follow straight line equation. The paracetamol was extracted from its tablets & then injected into HPLC system to determine the area under the peak. From the resulted area we can calculate the concentration and the weight by the straight line equation.

The results show that all of five samples are within the maximum percentage of the differences allowed according USP from (90% - 110%).

Introduction:

Paracetamol or N-(4-Hydroxyphenyl)-acetamide is one of the most popular over-the-counter analgesic and antipyretic drugs[1].

The first observations about the analgesic and antipyretic properties of paracetamol were made back in the late nineteenth century when alternative compounds were being sought to reduce fever in the treatment of infections.

Paracetamol is available in different dosage form: tablet, capsules, drops, elixirs, suspension and suppositories. Dosage form of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias. [2, 3]

Paracetamol is a white, odorless crystalline powder with a bitter taste, 4-hydroxyacetanilide or N-acetyl-p-aminophenol and in the US Pharmacopoeia it is known as acetaminophen as shown in figure (1). It is soluble in 70 parts of water (1 in 20 boiling water), 7 parts of alcohol (95%), 13 parts of acetone, 40 parts of glycerol, 9 parts of propylene glycol, 50 parts of chloroform, or 10 parts of methyl alcohol. It is also soluble in solutions of alkali hydroxides. It is insoluble in benzene and ether. A saturated aqueous solution has a pH of about 6 and is stable (half-life over 20 years) but stability decreases in acid or alkaline conditions, the paracetamol being slowly broken down into acetic acid.

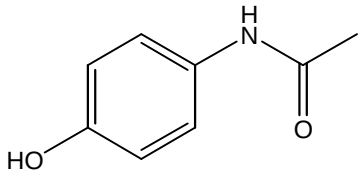

Figure-1: Chemical structure of Paracetamol

Paracetamol is 4-acetamidophenol and may be represented by the following formula ($C_8H_9NO_{2)}$, with molecular weight (151.2), pKa (9.5).

Several papers in the literature describe the assay of Paracetamol and its combination in pharmaceuticals or biological fluids. Determination of Paracetamol using electrical method has been reported[4],

Spectrophotometry[5-8], high performance liquid chromatography (HPLC)[9-13], titration method[14].

In present study a quality control for a Paracetamol from different manufacturing sources in Iraqi market was investigated.

Aim of the study:

The aims of this study were to investigate paracetamol from different pharmaceutical companies in Iraqi market to prove that:

1- The weight of each tablet is within the range of maximum difference allowed.

2 - Assay the active constituent of different samples using HPLC-UV method & comparing the results to obtain the most potent one from the tested samples.

Materials and Methods:

Methanol of HPLC grade (Batch no. 3554) is obtained from labs can TLD, unit T26 still organ, Co-dublin, Ireland. Paracetamol B.P/USP as a standard powder is manufactured by Julphar GULf Pharma (U.A.E). The other entire chemicals were used of pharmacopoeial grade.

Apparatus:

The HPLC with liquid delivery system, KNAUER (Manager type 500), Germany is equipped with auto sampler system (chromgate 3900) , KNAUER, Germany.

UV-Visible spectrophotometer is obtained from (PDA detector type 2800), KNAUER, Germany. Metter balance (Metter, Toledo AB 204), Switzerland and Ultrasonic mixer FRITSCH Laboratte type 17.202 are used.

Study design:

Samples:

The raw material that used for preparation of stock solution obtained from Julphar, GULF Pharma (UEA) and tested in Iraqi National center for quality control by reference standard and the result of quality was (98%). But the samples (paracetamol 500 mg tablet) were taken from the Iraqi pharmaceutical market and (Table -1) explain the data obtained concerning the proprietary name, source, M.D, E.D, batch number and average tablet weight of each sample.

No	Trade name	Company	M.D	E.D	Batch no.	Average tablet wt.(mg)
1.	Paracetamol tablet 500 mg	Dijla under license from SDI	09/2007	09/2012	O4	0.595235
2.	Panadol tablet 500 mg	Glaxo Smithkline/ Ireland	03/2008	02/2012	080276 D	0.594765
3.	Paracetamol tablet 500 mg	Furat Pharm/Iraq	08/2007	08/2010	33	0.604745
4.	Paracetamol 500 mg tablet	SDI/Iraq	Not state	07/2013	P5X	0.629185
5.	Paracetamol tablet 500 mg	Ajanta Pharma India	01/2008	12/2010	ALO11 8A	0.65374

Table-1: Paracetamol 500 mg tablets in the Iraqi market.

Method:

For the preparation of the calibration or the standard curve, we used external standard of different concentrations of Paracetamol U.S.P.. The stock solution was prepared from standard Paracetamol by dissolve an accurately weighed quality (50 mg) standard powder in (100 ml) of the mobile phase to obtain a solution having a known concentration of about 0.50 mg/ml [15].

From this stock solution a different dilutions (20, 50, 100, 200, 500, and 1000 µg/ml) was made respectively to be ready for HPLC study.

The chromatographic procedure is carried out by using:

1 - A stainless steel column(3.9 mm , 30 cm) column that contains packing RP-8.
2 - Mobile phase is composed from degassed mixture of water and methanol (3:1)
3 - UV detector with 243 nm wave length.
4 - 10µl of each solution was injected into HPLC system (injected volume).
5 - The flow rate is about 1.5 ml/min.

After that, calibration curve was made by injected each one of these dilutions into HPLC and the area under the peak (AUP) is measured for each

injection. By plotting the concentration of Paracetamol versus its peak area we get the calibration curve as in figure (2).

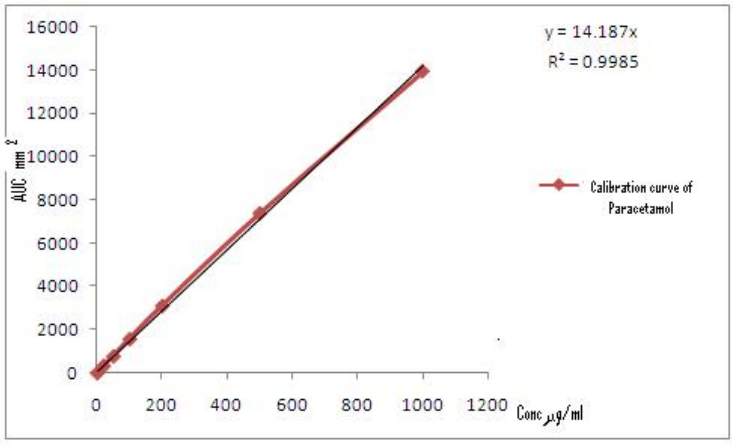

Figure-2: Calibration Curve of Paracetamol.

We find that the calibration curve will follow the straight line equation (Y=a+bx), and by substitution the statistical application we get the following data:

a=0.0the intercept obtained to be applied in the equation.

b= 14.187 the slope or regression coefficient

r^2=0.9985 the coefficient of determination

r= 0.999149 the correlation coefficient

Then the straight-line equation that used in the calculation is rearranged to:

Y=14.187X

The highly significant linear correlation of the area on the concentration is indicated by the high value of r and r^2, which close to the highest value of perfect correlation (1.0), this will ensure that accuracy of the work and qualification of the HPLC device.

The Maximum % difference allowed (within the range), average of AUP and concentration of the five samples are shown in (Table-2).

Let	Type of paracetamol	Max % difference allowed	AUP (mm^2) Y-axis	Concentration (mg/ml) X-axis
A	**Paracetamol Dijla**	0.574465-0.634935	1458.5	0.1028
B	**Panadol Glaxo-smithkline**	0.56506 - 0.62454	1497	0.1055
C	**Paracetamol furat**	0. 574465-0.634935	1522.5	0.1073
D	**Paracetamol SDI**	0. 597645-0.660555	1526.5	0.10759
E	**Paracetamol ajanta**	0.56544 - 0.62496	1464.5	0.1032

Table-2: The Maximum % difference, AUP & Concentration of the five samples.

The chromatogram of 0.01 mg/ml concentration of standard solution of Paracetamol is shown in (figure-3).

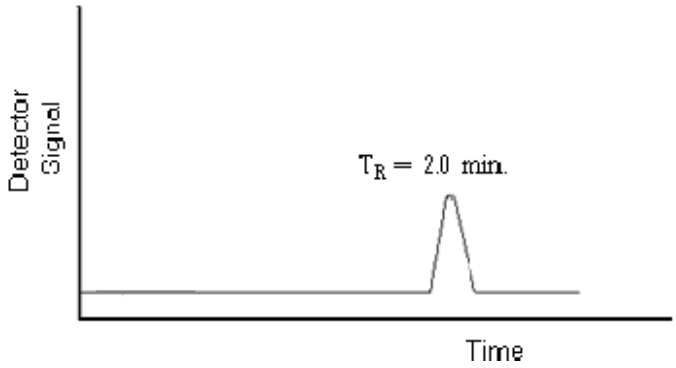

Figure3: Chromatogram of Paracetamol (T_R=2.0 min and AUP= 13966mm^2)

Procedure for sample handling:

Weigh and powder 20 tablets of each type or source of the drug and transfer an accurately weighed portion, equivalent to about 100 mg of Paracetamol, to 200 ml volumetric flask. Add about 100 ml of mobile phase, and shake by mechanical means for 10 min. Dilute with mobile phase to

volume, and mix. Transfer 5 ml of this solution to a 250 ml volumetric flask, dilute with mobile phase to volume, and mix. Pass a portion of this solution through a filter having 0.5 μm or finer porosity, discarding the first 10 ml of filtrate. The final conc. that injected is 0.01 mg/ ml.

Each one of the five samples is tested using the same conditions that used in the external standard in the HPLC system to get the AUP. Then the equation of straight line is applied to calculate Paracetamol concentration & its weight.

Results:

From the data obtained in (Table-3) in which concentration of each AUP was determined ,we can calculate the weight and recovery percent of each sample compared to the standard wt which is 500 mg, also we can calculate the Relative Standard Deviation percent (RSD%) or Sample Coefficient of variation (CV) as shown in the (table-3).

let	Drug Source	Weight of Paracetamol of each sample	Recovery %	Standard deviation	RSD %
A	**Paracetamol Dijla**	488.402 mg	97.680	4.454	3.053
B	**Panadol Glaxo-smithkline**	501.23 mg	100.243	1.828	0.1889
C	**Paracetamol Furat**	509.782 mg	101.956	0.707	0.046
D	**Paracetamol SDI**	511.16 mg	102.232	4.450	2.915
G	**Paracetamol Ajanta**	490.303 mg	98.06	0.707	0.048

Table-3: Data represent the weight of Paracetamol, recovery % and RSD% of the five samples.

Discussion and Conclusions:

From the previous study we can summarize the following:

1- All of the tested tablets were within the range of "Maximum % difference allowed".
2- The quantitative analysis was performed using HPLC with external standard method. The recoveries were close to 100% with acceptable accuracy & precision.
3- The results indicate that paracetamol tablets is accepted within the normal percentage (90%- 110%) according U.S. P.30
4- The HPLC quantitative analysis procedure is fast & accurate for Paracetamol analysis & can be used for routine work.
5- The HPLC method show good accuracy and reproducibility for determination of drug in pharmaceutical dosage forms and biological fluid.
6- From the comparison of the results obtained from the tested five samples, it was found that the panadol (Glaxosmithkline) is the most effective one and close to 100% recovery as shown in the (Table-3)

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