Determination of Aspirin tablets from different industrial drug companies available in Iraqi pharmaceutical market.

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Abstract

Aspirin is commercially available in the form of tablets containing 100 mg for oral administration. Aspirin content of each tablet which is manufactured from different industrial sources companies (SDI, NDI, BAYER, PHARMALINE, RIVA and COCUKLARCI) using high performance liquid chromatography (HPLC) with reversed-phase (ODS-C18) column at low wave length of Uv-visible detection (280 nm). An efficient procedure was used for preparation of aspirin samples. The aspirin was eluted for 5 minute at flow rate 2ml/min and temperature equal to 298 K°. The retention time of aspirin was observed at 3.8 minute. The main absolute recovery of aspirin of all tablets was taken from different above companies were (102, 94, 94, 92, 90 and 88%) respectively. The assay showed good relationship between area under the peak (AUP) and aspirin content (p> 0.001). The Iraqi companies tablets produced an accurate content of ingredients aspirin in each tablet.
Introduction

The common pain reliever Aspirin is known chemically as acetylsalicylic acid and has been in use as a pharmaceutical agent for over 100 years (6). Aspirin is used to relieve mild to moderate pain, reduce fever, to reduce inflammation and swelling in conditions such as arthritis. Aspirin was used in low doses as a blood thinner to prevent the formation of blood clots. It is effective in reducing the risk of stroke and offers a protective effect against heart attacks in men with chest pain. It is unique among Cox-inhibitors (cyclooxygenase) because it covalently modifies the proton of enzymes and irreversibly inhibits them (3).

Aspirin is one of salicylic acid derivatives and widely used drugs for its multi purpose applications and the drug suffer from the side effects, a number of modifications have been carried to overcome this problem (8). Aspirin was first introduced into medicine by dressers in 1899. It is prepared by treating S.A (salicylic acid) with acetic anhydride (2).

Aspirin is the prototype of traditional NSAID (non steroidal anti inflammatory drug), it is the most commonly used and is the drug to

Figure 1: The molecular structure of aspirin, Molecular formula C9H8O4, Molecular weight = 180.2 g/mole [2-(Acetyloxy) benzoic acid].
which all other anti inflammatory agents are compared. It has analgesic, antipyretic, anti-inflammatory effect (4).

Aspirin occurs as white crystalline powder. It is slightly soluble in water \((l: 300)\) and soluble in alcohol \((l: 5)\), chloroform \((l: 17)\), and ether \((l: 15)\). Also it dissolves easily in glycerin, soluble in solutions of acetates and citrates and, with decomposition, in solutions of alkali hydroxides and carbonates (1).

It is stable in dry air, while in the presence of moisture it hydrolyses into acetic acid and salicylic acid (2).

In plasma, salicylic acid about 90% at concentrations below 100µg/ml, decreasing to 50% at concentration above 400µg/ml. The half-life, aspirin about 17 min in plasma. Volume distribution of aspirin about 0.15 L/ Kg, salicylic acid about 0.1 to 0.2 L/ Kg (dose-dependent).

Dose of aspirin usually 1.2 to 4 g daily; doses of up to 8 g daily are given in acute rheumatic disorders.

**Disposition in the Body.**

Readily absorbed after oral administration and rapidly hydrolyzed to salicylic acid this is the active agent. Salicylic acid is conjugated with glucuronic acid and glycine to form acyl and ether glucuronides and salicylic acid; some hydroxylation also occurs to give dihydroxy and trihydroxy derivatives of salicylic acid. Aspirin is excreted almost entirely in the urine with about 50 to 80% of a dose as salicylic acid, 10 to 30% as salicyl o-glucuronide, 5% as salicyl ester glucuronide, and 5 to 10% as free salicylic acid, together with small amounts of gentisic acid, gentisuric acid, and unchanged drug; salicylates are reabsorbed by the renal tubules from acid urine and thus alkaline diuresis increases the rate of salicylate elimination; about 85% of a dose is excreted as free salicylic acid if the urine is made alkaline. Aspirin is a metabolite of aloxiprin and benorilate.

**Therapeutic concentration.**

In plasma, salicylic acid usually in the range 20 to 100 mg/L for analgesia and 150 to 300 mg/L for anti-inflammatory effect. After a single oral dose of 900 mg given to 5 subjects, peak plasma-aspirin concentrations of 16 to 50 mg/L (mean 37) were attained in about 14 min, and peak plasma-salicylic acid concentrations of 47 to 113 mg/L (mean 78) were reported at 0.5 to 1 h. Following daily oral doses of 3.9 g to 8 subjects for 8 days, steady-state plasma-salicylic acid
concentrations of 105 to 227 mg/L (mean 173) were reported; by the 36th day of treatment the steady-state plasma concentrations had declined to 45 to 208 mg/L (mean 129).

**Toxicity:**

The estimated minimum lethal dose is 15 g. Plasma concentrations of salicylic acid greater than 300 mg/L are likely to produce toxic reactions and concentrations greater than 500 mg/L are associated with moderate to severe intoxication. The maximum permissible atmospheric concentration is 5 mg/m³.

A 43-year-old female attempted suicide by self-administration, in the form of an enema, of approximately 700 aspirin tablets dissolved in water. The initial salicylate concentration in the serum was 590 mg/L and rose to 900 mg/L 12 h later; after haemodialysis, serum salicylate concentration fell to 160 mg/L but the patient remained in a coma for more than a year. The patient's poor outcome was attributed to retention of aspirin products in the rectal vault plus poor recognition of the delayed absorption properties of rectally administered aspirin (8).

**CHROMATOGRAPHY:**

1. **CHROMATOGRAPHIC PROCESS:**

   Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase.

   A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface
is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the Chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of Chromatographic methods (2).

2. Modern HPLC:

The separation of analyte mixtures in modern HPLC is performed in the device called the "column." Current HPLC columns in most cases are a stainless steel tube packed with very small (1-5 µm) particles of rigid porous material. Packing material is retained inside the column with special end-fittings equipped with porous frits allowing for liquid line connection (to deliver mobile phase to the column). Stainless steel or titanium frits have a pore size on the level of (0.2-0.5 µm), which allows for the mobile phase to pass through while small particles of packing material are retained inside the column. The column is the "heart" of the chromatographic system; and it is the only device where actual separation of the analyte mixture takes place.

Typical HPLC system consists of the following main components:

**Solvent Reservoirs**: Storage of sufficient amount of HPLC solvents for continuous operation of the system. Could be equipped with an online degassing system and special filters to isolate the solvent from the influence of the environment.

**Pum**: This provides the constant and continuous flow of the mobile phase through the system; most modern pumps allow controlled mixing of different solvents from different reservoirs.

**Injector**: This allows an introduction (injection) of the analytes mixture into the stream of the mobile phase before it enters the column; most modern injectors are auto samplers, which allow programmed injections of different volumes of samples that are withdrawn from the vials in the auto samplers.

**Column**: This is the heart of HPLC system; it actually produces a separation of the analytes in the mixture. A column is the place where the mobile phase is in contact with the stationary phase, forming an interface with enormous surface. Most of the chromatography development in recent years went toward the design of many different ways to enhance this interfacial contact.

**Detector**: This is a device for continuous registration of specific physical (sometimes chemical) properties of the column.
The most common detector used in pharmaceutical analysis is UV (ultraviolet), which allows monitoring and continuous registration of the UV absorbance at a selected wavelength or over a span of wavelengths (diode array detection). Appearance of the analyte in the detector flow-cell causes the change of the absorbance. If the analyte absorbs greater than the background (mobile phase), a positive signal is obtained.

**Data Acquisition and Control System:** Computer-based system that controls all parameters of HPLC instrument (eluent composition (mixing of different solvents); temperature, injection sequence, etc.) and acquires data from the detector and monitors system performance (continuous monitoring of the mobile-phase composition, temperature, backpressure, etc) (2).

**The aim of the study:**

The aim of this study was to determine Aspirin content from different pharmaceutical companies available in Iraqi pharmaceutical market. The study includes the following:

1-Assay of the active ingredient of different aspirin tablets samples, using HPLC-UV method.
2-Estimation of the weight contents of each aspirin tablets and comparing them according to the pharmacopeal requirements.
3-To give information about the products of some pharmaceutical companies available in Iraqi market which may or may not comply with the requirements in the B. P. or U. S. P.

**Experimental Work:**

**Materials:**

1) Acetonitrile of HPLC grade PAI PANREAC, Lot 2621072914, Barcelona-Espna.
2) Glacial acetic acid BDH, Batch No. 28118, England.
3) Formic acid and Sodium heptane sulfonate and other materials are used of HPLC analytical grade were obtained from the National center for drug research and quality control.

**Instruments:**

1- HPLC, Knauer advanced scientific instrument pump 1000 (manager 5000) include the following parts:
   (A) Detector PDA (photo Diod array).
   (B) Auto sampler 3900 computerized /chromgate.
(C) Column LI C18 (ODS) octadecyl silane chemically bound to porous silica or ceramic micro-particles, 3-10 μm in Diameter (250 x 4.6 mm).

2- sonycate/karlkotl vibrator.
3- PH meter microprocessor pH meter. (HANNA No. 210).
4- BUK scientific, model 500, infrared spectrophotometer.
5- Balance mettler Toledo /AB20 y, Switzerland.
6-uv-visible spectrophotometer /Cary 100 cone (Varian).

**Design of the study:**

The specialist brands of aspirin tablets were collected from the Iraqi pharmaceutical market. The table below explains the data obtained concerning the proprietary name, source, manufacture date (M.D.), expire date (E.D.), batch number and average tablet weight of each one.

**Table (1):** Trade name, Source, M.D, E.D, Country and average tablets weight (mg) in the Iraqi markets

<table>
<thead>
<tr>
<th>No.</th>
<th>Trade name</th>
<th>Company</th>
<th>M.D</th>
<th>E.D</th>
<th>Country</th>
<th>Average tablets wt. (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspirin 100 mg</td>
<td>SDI</td>
<td>11-2010</td>
<td>Iraq</td>
<td>250.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Aspirin 100 mg</td>
<td>NDI</td>
<td>11-2010</td>
<td>Iraq</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Aspirin protect 100 mg</td>
<td>BAYER</td>
<td>6-2008</td>
<td>05-2011</td>
<td>Germany</td>
<td>138.3</td>
</tr>
<tr>
<td>4</td>
<td>ASPICOT 100 mg</td>
<td>Pharmaline</td>
<td>4-2008</td>
<td>04-2011</td>
<td>Lebanon</td>
<td>183</td>
</tr>
<tr>
<td>5</td>
<td>RIVO 100 mg</td>
<td>RIVA</td>
<td>10-2007</td>
<td>10-2010</td>
<td>Egypt</td>
<td>118.2</td>
</tr>
<tr>
<td>6</td>
<td>Aspirin 100 mg</td>
<td>Cocuklaricin</td>
<td>7-2008</td>
<td>07-2011</td>
<td>Turkey</td>
<td>119.7</td>
</tr>
</tbody>
</table>

**Identification of aspirin standard powder:**
(1) White crystalline powder.
(2) Melting point: Aspirin decomposed. 134°C-135°C.
(3) On ignition: it burn with smoky blue flame.
(4) Solubility: slightly soluble in water (l: 300), soluble in alcohol (l: 5), chloroform (l: 17), and ether (l: 15).

**Method of assay using HPLC:**
The calibration curve or standard curve was prepared after the aspirin content was determined in tablets from different pharmaceutical companies.
For the preparation of the calibration curve, the external standard aspirin usp reference standard was used, and the stock
solution was prepared, from this stock solution, different dilutions (0.0mg/ml, 0.1mg/ml, 0.2mg/ml, 0.3mg/ml, 0.4mg/ml, 0.5mg/ml and 0.6mg/ml) were made which are then injected into HPLC to obtain the (Aup) of each concentration.

The chromatographic procedure is carried out by using:
1- Stainless steel column with a dimension of (250 x 4.6mm) that contains packing L1 C18. (ODS).
2- Diluent: a mixture of acetonitrile & formic acid (99: l) was prepared.
3- Mobile phase: 2 gram of sodium heptanesulfonate in a mixture of 850 ml of water and 150 ml of acetonitrile was dissolved, and adjust with glacial acetic acid to pH 3.4.
4- Operate in mode LPG.:  
- Flow rate 2ml/min.
- Run time 5min.
- Injection volume 20µl.
- Detector wave length 280nm (UV-Visible detector).

Preparation of standard solution and calibration curve:
Accurately 25 mg of Aspirin, Reference standard USP was weighed. The weighed powder was dissolved in 25 ml volumetric flask containing solvent as diluents, the volume was completed to 25 ml using the same diluents to form a stock solution with a concentration of 1 mg/mL. After that, transfer different volumes from this stock solution in to 10ml volumetric flask and dilute to 10 ml with diluents phase to prepare solutions of different concentrations. Then each one of these dilutions was injected in to HPLC system. Table (2) showing the results which were obtained using area under the peak (Aup) method (5).

Table (2): Dilution of Aspirin and their peak area (Aup)

<table>
<thead>
<tr>
<th>Volume of stock Solution (ml)</th>
<th>Volume of diluents Dilute to 10 ml</th>
<th>The Conc. (mg/ml) x-axis</th>
<th>The Aup y-axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>0.1</td>
<td>56621.9</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.2</td>
<td>118826.6</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0.3</td>
<td>186002</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.4</td>
<td>246754.9</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.5</td>
<td>317516</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.6</td>
<td>365647</td>
</tr>
</tbody>
</table>
Then, draw the calibration curve by plotting the concentration of aspirin versus the area under the peak (Aup) as shown in figure (2).

**Figure (2):** Calibration Curve of Aspirin.

Finally, the above calibration curve follows the straight-line equation \( y = a + bx \), and by substitution the statistical application we get the following data; \( b=617666 \) the slope or regression coefficient, \( r^2 = 0.9989 \) the coefficient of determination, \( r = 0.99945 \) the correlation of coefficient.

Then the straight-line equation that was used in the calculation is rearranged to: \( Y = 617666(X) \).

The highly significant linear correlation of the area under the peak (Aup) on the concentration is indicated by the high value of \( r^2 \) & \( r \), which are close to the highest value of perfect correlation; this ensures the accuracy of the work and the qualification of the HPLC device.

The following chromatogram of 0.5 mg/ml of standard solution of Aspirin is shown below in figure (3).
Assay:

Procedure:

Weigh and powder 20 tablets of each type or source of the drug, to determine the average weight per tablets the combined contents were mixed, an accurately weighed quantity of the powder equivalent to about 50 mg was transferred into 100ml volumetric flask. Add the diluent phase, mix with the aid of sonycate then complete the volume with the same diluent phase and mix. Each one of the six samples will have its AUP which reflect its concentration & tablet content of the drug.

The sample testing by standard curve:

Each one of the six samples is tested using the same conditions that were used in the external standard in the HPLC system to get the Aup. Then the equation of straight line was applied to concentration. The average of Aup and concentrations of the samples are shown in table (3).
Table (3): The Aup of each sample (company), the concentration and the maximum allowed differences.

<table>
<thead>
<tr>
<th>Conc. (mg/ml)</th>
<th>Aup obtain from chromatography</th>
<th>Company</th>
<th>Trade name</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.46</td>
<td>281141</td>
<td>SDI</td>
<td>Aspirin 100 mg</td>
<td>1</td>
</tr>
<tr>
<td>0.47</td>
<td>292255</td>
<td>NDI</td>
<td>Aspirin 100 mg</td>
<td>2</td>
</tr>
<tr>
<td>0.51</td>
<td>346379</td>
<td>BAYER</td>
<td>Aspirin protect 100 mg</td>
<td>3</td>
</tr>
<tr>
<td>0.47</td>
<td>292545</td>
<td>Pharmaline</td>
<td>ASPICOT 100 mg</td>
<td>4</td>
</tr>
<tr>
<td>0.45</td>
<td>276509</td>
<td>RIVA</td>
<td>RIVO 100 mg</td>
<td>5</td>
</tr>
<tr>
<td>0.44</td>
<td>274204</td>
<td>Cocuklaric</td>
<td>Aspirin 100 mg</td>
<td>6</td>
</tr>
</tbody>
</table>

The above results obtained by application of the straight-line equation which was calculated previously or from direct extrapolation from the calibration curve.

Results:
From the data obtained in table (3) in which the cone, of each Aup was determined, we can calculate the weight, percentage of errors and the recovery percent compared to standard which is 250 mg.

\[
Y = 617666 (X) \quad \text{--------- (1)}
\]

\[
X (MG) = \frac{Y (mm2)}{617666} \quad \text{--------- (2)}
\]

Calculated Wt. of sample \(=\) \(
\frac{\text{conce. of the sample} \times 100}{50}
\) \(X 100\)

AMO. of each sample:

Error % \(=\) \(
\frac{\text{calc. Wt.} - \text{strd. Wt.}}{\text{strd. Wt.}} \times 100
\)

Recovery % \(=\) \(
\frac{\text{calc. Wt.}}{\text{strd. Wt.}} \times 100
\)

These Data were arranged according to the higher recovery % or higher weight of the samples as shown in Table (4).
Table (4): Drug Company, weight of each sample, error % and recovery%.

<table>
<thead>
<tr>
<th>Recovery %</th>
<th>Error %</th>
<th>Wt % of each sample (mg)</th>
<th>Drug Company</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>2</td>
<td>102</td>
<td>Bayer</td>
<td>1</td>
</tr>
<tr>
<td>94</td>
<td>-6</td>
<td>94</td>
<td>ASPICOT</td>
<td>2</td>
</tr>
<tr>
<td>94</td>
<td>-6</td>
<td>94</td>
<td>NDI</td>
<td>3</td>
</tr>
<tr>
<td>92</td>
<td>-8</td>
<td>92</td>
<td>SDI</td>
<td>4</td>
</tr>
<tr>
<td>90</td>
<td>-10</td>
<td>90</td>
<td>RIVA</td>
<td>5</td>
</tr>
<tr>
<td>88</td>
<td>-12</td>
<td>88</td>
<td>Coccular</td>
<td>6</td>
</tr>
</tbody>
</table>

Conclusion:

From this study we can conclude the followings:
1- All of the tested tablets within the range of the (maximum allowed difference) except Coccular aspirin and the results indicate that aspirin is accepted within the normal percentage (90%-110%) according to USP 2005.
2- The results obtained from six tested samples, indicated that aspirin Bayer is the highest weight one.
3- Also from the results obtained, it was found that Aspirin Bayer is the most potent one because of close to the 100% recovery.
4. The HPLC quantitative analysis is fast and accurate for Aspirin analysis and can be used for routine work.
References

الحدث

بالcentage

(0.50)

نسبة

(1.88)

نسبة

(8)

(0.001)

(0.50)

الصدمة

(0.88)

(0.001)

(0.88)

(0.50)

(0.88)

(0.001)