Development Method for Determination of Aspirin as Sodium Salicylate by UV-VIS Spectroscopy

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1. Abstract
New, easy and quick way to signal U.V. – Vis. spectroscopy was developed and validated for the estimation of Aspirin in pure form, and in respective formulations. The appropriate solubility of the drug and the maximum sensitivity of the assay were found as Salicylate, the peak of maximum absorbance of Salicylate was appear at (535 nm) in the wavelength scope of (300-900 nm); The linear calibration curve of aspirin concentrations range (10-80 ppm) are shown to show the regression equation (Y=0.0086X - 0.0429), and correlation Coefficient (R^2 = 0.9999), the LOQ for Aspirin was up to (5.43742 – 0.306928 mg.L⁻¹), while LOD were (1.794348 - 0.101286 mg.L⁻¹).

This method has been validated and applied to the estimate of aspirin from various companies in Iraqi marts, no intrusions was found from tablet of aspirin excipients at the favored wavelength and conditions of estimate; Concluded that the method developed was perfect, precise, accurate, and replicable. As well as it can be directly applied to determine Salicylate and indirectly for the estimation of Aspirin content in pharmaceutical formulations, by calibration curve method or standard addition method.

2. Introduction
Aspirin is a [2-(acetyloxy) benzoic acid] [1]. It the preparation procedure involves treating acetylsalicylic with acetic anhydride [2, 3]. It belongs to a group of drugs known as non-steroidal anti-inflammatory drugs (NSAIDS) which are characterized for their anti-inflammatory, analgesic and antipyretic effect, however aspirin is widely used for its antithrombotic effect, it reduces platelet aggregation thus reducing the incidence of cardiovascular diseases [4,5,6].

The pharmaceutical market is constantly growing and since new companies and new products are constantly being introduced routine analysis of commercial pharmaceutical products has become essential, the British Pharmacopoeia
describes several methods for the determination of aspirin in commercial tablets one of which is the acid base titration method [7] but numerous other methods are also described in literature Including chromatographic method HPLC and GC/MS [8, 9], the potentiometers, flow-injection spectrophotometric [10], diazotization and coupling [11, 12].

This paper will focus on the colorimetric method which depends on the addition of ferric nitrate to the aspirin solution leading to formation of a violet color the intensity of which depends the concentration of aspirin in solution, this occur due to the fact that aspirin slowly decompose in the presence of moisture to acetic acid and salicylic acid which forms the violet complex with ferric nitrate [13], but quickly decompose in adding sodium hydroxide with heat to sodium acetate and sodium salicylate which forms the violet complex directly at addition ferric nitrate, it is decompose and forms the complex as follows:

Figure 1: Decompose Aspirin to sodium acetate and sodium salicylate

![Figure 1: Decompose Aspirin to sodium acetate and sodium salicylate](image)

Figure 2: Formation violet complex from salicylate anion and ferric ion.

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Since the procedure is dependent upon the reaction between the salicylate anion with the ferric nitrate it is possible to use sodium salicylate as an additional standard solution and to obtain a comparative image about the relationship between the salicylate and aspirin.

Calibration curve, otherwise known as standard curve, is a method that allows us to determine the concentration of unknown samples from a set of standard samples with known concentrations [14]. This is one method for determining the unknown concentration another method includes mixing the standard and the unknown sample leading to internal standard or standard addition method [15, 16].
3. Experimental

Equipment

160 digital Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZU (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version1.10), using 1.00 cm quartz cell was used for measuring the absorption single, digital electrical balance, volumetric flask (BDH) and a pipette (BDH).

Chemicals and reagents

1- Pure sodium hydroxide crystals (Fluka) were obtained.
2- Pure aspirin crystal (Fluka).
3- Pure salicylic acid crystals (Fluka).
4- Aspirin tablets from three different brands, shown in (Table1). 5- Nitric Acid (Fluka).

Solutions preparation

(1%) Ferric Nitrate solution was prepared by dissolved (1g) of Ferric Nitrate with 1mL of HNO₃ (0.1N) then transfer to volumetric flask (100mL) and fill to mark with D.W.

Stock solutions:

a. (1805 ppm) Aspirin solution was prepared by dissolved (0.1805g) of pure Aspirin with (10mL) of (0.1N NaOH) solution was added and gentle heating was used to increase decompose and solubility, then transfer to volumetric flask (100mL) and fill to mark with D.W.

b. (1601 ppm) of Sodium Salicylate solution was prepared by dissolved (0.1601g) of pure Sodium Salicylate with D.W., then transfer to volumetric flask (100mL) and fill to mark with D.W.

Standard solutions:

Dilute both stock solutions (a) and (b) to (90.025 ppm) and (80.05 ppm) respectively.

Absorbance spectrum of Aspirin:

Transfer (6mL) of standard solution (90.025ppm) of aspirin or (80.05ppm) of sodium salicylate by pipette up to (10mL) volumetric flask, add (1mL) of ferric nitrate 1%, then diluted with distal water to the mark, the absorbance of the solution measured at a range (300-900 nm), show the absorbance spectrum and λ_max at (535nm) as in Figure 3.
Samples preparation

10 tablets of (100 mg) aspirin from each brand were weighed the average weight was calculated, then the tablets were crushed and the amount of powder equivalent to 100mg aspirin was prepared to which (10mL) of (0.1N NaOH) solution was added and gentle heating then diluted in (100mL) distal water (∼1000ppm), (or 1805ppm as in the upon) then samples solutions was diluted to (∼90ppm), for analyze; Transfer (6mL) of sample solution (∼90ppm) by pipette up to (10mL) volumetric flask, add (1mL) of ferric nitrate 1%, then diluted with distilled water, measurement of absorbance at $\lambda_{\text{max}}$ (535nm), or using (5mL) volumetric flasks as following in (Table 4) for each the solutions prepared, the volume completed with distal water, the absorbance was measured using UV-VIS spectroscopy at a wavelength (535 nm) and a standard addition curve was plotted for sample (unknown) solutions.

| Brand name | Manufacturing company | Batch no. | Manufacture date | Expiry date | Average Tablets Wt.(gm) * |
|------------|-----------------------|-----------|------------------|-------------|--------------------------|
| aspirin    | Bayer                 | BTAK8L1   | March 2017       | March 2020  | 0.135                    |
| Aswpin     | SDI                   | BiLN15    | May 2017         | May 209     | 0.127                    |
| Aspicot    | Pharmaline            | A17016    | July 2014        | July 2017   | 0.165                    |

* Average ten tablets weight.

Methods Assay 1. Calibration curve method

Using the following (Table2) or (Table3) for each of the solutions prepared using (10mL) volumetric flask and the volume completed using distal water, the absorbance measured using UV-VIS spectroscopy at (535 nm) wavelength and a calibration curve was plotted as shown in Figure 4, and Figure 5. a. For aspirin
Table 2: Standard solutions of Aspirin

| No. | Standard (ml) | FeNO₃(ml) | Concentration (ppm)* | Absorbance |
|-----|---------------|-----------|----------------------|------------|
| Blank | 0             | 1         | 0                    | 0.0        |
| 1    | 2             | 1         | 18.05                | 0.1121     |
| 2    | 4             | 1         | 36.1                 | 0.2667     |
| 3    | 5             | 1         | 45.125               | 0.3419     |
| 4    | 6             | 1         | 54.15                | 0.4231     |
| 5    | 8             | 1         | 72.2                 | 0.5757     |

* The average six aspirin solutions of standard solution.

Figure 4: Calibration Curve of Aspirin

The curve passes in intercept = (+) or (-) of equation of Beer’s Law. Ideal work gives perfect results.

c. For sodium salicylate

Table 3: Standard solutions of Sodium salicylate

| No. | Standard (ml) | FeNO₃ (ml) | Concentration (ppm)* | Absorbance |
|-----|---------------|-----------|----------------------|------------|
| Blank | 0             | 1         | 0                    | 0.0        |
| 1    | 2             | 1         | 16.01                | 0.1116     |
| 2    | 4             | 1         | 32.02                | 0.2661     |
| 3    | 5             | 1         | 40.025               | 0.3412     |
| 4    | 6             | 1         | 48.03                | 0.4227     |
| 5    | 8             | 1         | 64.04                | 0.5748     |

* The average six Sodium salicylate solutions of standard solution.
2. Standard addition method

Using the following (Table 4) and (Table 5) for each of the solutions prepared using a 5ml volumetric flask and the volume completed using distal water, the absorbance was measured using UV-VIS spectroscopy at a wavelength of (535 nm) and a standard addition curve was plotted for standard and sample (as standard) solutions, as shown in Figure 6, Figure 7, and Figure 8.

a. For standard aspirin

Table 4: Standard addition solutions of Aspirin

| No. | Standard (ml) | FeNO₃ (ml) | Unknown (ml) | Concentration (ppm) | Absorbance |
|-----|---------------|------------|--------------|---------------------|------------|
| Blank | 0 | 1 | 0 | 0 | 0 |
| 1 | 0 | 1 | 1 | 0 | 0.0852 |
| 2 | 0.5 | 1 | 1 | 9.025 | 0.1304 |
| 3 | 1 | 1 | 1 | 18.05 | 0.1689 |
| 4 | 1.5 | 1 | 1 | 27.075 | 0.2114 |
| 5 | 2 | 1 | 1 | 36.1 | 0.2557 |
| 6 | 2.5 | 1 | 1 | 45.125 | 0.3008 |

* The average six Aspirin solutions of standard solution.
d. For sodium salicylate

Table 5: Standard addition solutions of Sodium salicylate

| No. | Standard (ml) | FeNO₃ (ml) | Unknown (ml) | Concentration (ppm) | Absorbance |
|-----|--------------|-----------|--------------|---------------------|------------|
| Blank | 0            | 1         | 0            | 0                   | 0          |
| 1    | 0            | 1         | 1            | 0                   | 0.0841     |
| 2    | 0.5          | 1         | 1            | 8.005               | 0.1284     |
| 3    | 1            | 1         | 1            | 16.01               | 0.1681     |
| 4    | 1.5          | 1         | 1            | 24.015              | 0.2105     |
| 5    | 2            | 1         | 1            | 32.02               | 0.2542     |
| 6    | 2.5          | 1         | 1            | 40.025              | 0.2978     |
* The average six Sodium salicylate solutions of standard solution.

![Absorbance versus Concentration](image)

**Figure 8: Standard Addition Curve for Standard Sodium salicylate**

### 4. Results and Discussion

To compare the two formulas and to know their compatibility and differences.

**Calibration curve:**

The method was determined at five concentration levels ranging (18-72 ppm) for Aspirin, and (16-64 ppm) for Sodium salicylate, the calibration curve was constructed by plotting absorbance versus concentrations of Aspirin (ppm), and Sodium salicylate (ppm), and the regression equation was calculated, each absorbance was the average of six determinations, linear absorbance data for calibration curve was shown in *(Table 6, and Figure 4)* for Aspirin and *(Table 7, and Figure 5)* for Sodium salicylate.

**Table 6: Standard solutions of Aspirin**

| Concentration (mg.L⁻¹) | Absorbance | Notes |
|------------------------|------------|-------|
| 0                      | 0.0        | Y = 0.0086X - 0.0429 |
| 18.05                  | 0.1121     | Correlation Coefficient R² = 0.9999 |
| 36.1                   | 0.2667     | Chi Square = 0.00456231 |
| 45.125                 | 0.3419     | Standard Error of Estimate(SEE) = 0.001588 |
| 54.15                  | 0.4231     | Residual Standard Deviation = 0.005849 |
| 72.2                   | 0.5757     | Multiple Correlation Coefficient R² = 0.999936775 |
|                        |            | Slope = 0.008572 |
|                        |            | Intercept = -0.0429 |
Table 7: Standard solutions of Sodium salicylate

| Concentration (mg.L⁻¹) | Absorbance | Notes |
|------------------------|------------|-------|
| 0                      | 0.0        | Y = 0.0097X - 0.0433  |
| 16.01                  | 0.1116     | Correlation Coefficient $R^2 = 0.9999$ |
| 32.02                  | 0.2661     | Chi Square = 0.005309843 |
| 40.025                 | 0.3412     | Standard Error of Estimate (SEE) = 0.001735 |
| 48.03                  | 0.4227     | Residual Standard Deviation = 0.005266721 |
| 64.04                  | 0.5748     | Multiple Correlation Coefficient $R^2 = 0.999924498$ |

Linearity:

The linearity of the drug was obtained for (18-72 ppm) concentration range of Aspirin, the regression analysis was performed for line equation, the linear equation was found to be ($Y = 0.0086X - 0.0429$) and correlation coefficient ($R^2$) 0.9999 (Table 6), the calibration curve was found to be linear in the above stated concentration, and Slope (0.008572), Intercept (-0.0429), Chi Square (0.00456231), Standard Error of Estimate (0.001588), Residual Standard Deviation (0.005849), and Multiple Correlation Coefficient $R^2$ (0.999936775).

The linearity of the drug was obtained for (16-64 ppm) concentration range of Sodium salicylate, the regression analysis was performed for line equation, the linear equation was found to be ($Y = 0.0097X - 0.0433$) and correlation coefficient ($R^2$) 0.9999 (Table 7), the calibration curve was found to be linear in the above stated concentration, and Slope (0.009658), Intercept (-0.04327), Chi Square (0.005309843), Standard Error of Estimate (0.001735), Residual Standard Deviation (0.005266721), and Multiple Correlation Coefficient $R^2$ (0.999924498).

Precision and Accuracy:

The precision of the analytical system was investigated by performing six consecutive replicate solution of the same standard solution, the standard deviation (SD) and relative standard deviation (RSD) obtained are listed in (Table 8), the low RSD values indicated that the method is precise.

The limit of quantification (LOQ), and limit of detection (LOD) were calculated from the standard deviation (SD) of responses and slope ($m = 0.078363$) of Aspirin and ($m = 0.0097$) of Sodium Salicylate, as shown in (Table 8)
Table.8: Precision and accuracy

| Name of compound | Concentration (ppm) | Recovery* % | SD | R.S.D* % | Error* % | LOQ | LOD |
|------------------|---------------------|-------------|----|----------|----------|-----|-----|
| Taken            | Found*              |             |    |          |          |     |     |
| Aspirin          | 18.05               | 18.0232558  | 99.85187 | 0.000263 | 0.00146  | 0.14813 | 0.306928 | 0.101286 |
|                  | 36.1                | 36.000      | 99.72999 | 0.002658 | 0.007384 | 0.27701 | 3.09107  | 1.020053 |
|                  | 45.125              | 44.74419    | 99.15642 | 0.002875 | 0.006426 | 0.84358 | 3.343234 | 1.103267 |
|                  | 54.15               | 54.18605    | 100.0656 | 0.00405  | 0.007474 | -0.0656 | 4.708943 | 1.553951 |
|                  | 72.2                | 71.93023    | 99.62465 | 0.001862 | 0.002588 | 0.37535 | 5.43742  | 1.794348 |
| Sod. Salicylate  | 16.01               | 15.969072   | 99.74287 | 0.001472 | 0.009218 | 0.25713 | 1.517485 | 0.50077  |
|                  | 32.02               | 31.89691    | 99.61534 | 0.001472 | 0.004615 | 0.38466 | 1.517485 | 0.50077  |
|                  | 40.025              | 39.6701     | 99.11264 | 0.001472 | 0.003711 | 0.88736 | 1.517485 | 0.50077  |
|                  | 48.03               | 48.04124    | 100.0239 | 0.002903 | 0.006042 | -0.0239 | 2.992652 | 0.987575 |
|                  | 64.04               | 63.72165    | 99.50291 | 0.001751 | 0.002748 | 0.49709 | 1.805351 | 0.595766 |

* Average of six (Aspirin & Sod. Salicylate) solution of standard solution.

The accuracy of the method (recovery) was investigated by determination of in Aspirin, a solution containing Aspirin (C = 18 – 72 mg/L) with no detectable impurities was spiked with the reference substances at appropriate concentrations, the recovery and relative standard deviations (RSD) obtained are listed (Table.8) confirmed the satisfactory accuracy of the method.

Method validation:

The method was validated through linearity, sensitivity, precision and accuracy, as shown in Table.9

Table.9: Optical characteristics, parameters of the method

| No. | Parameters         | Result of Aspirin | Result of Sod. Salicylate |
|-----|--------------------|-------------------|--------------------------|
| 1   | Absorption maxima(\(\lambda_{\text{max}}\)) | 535 nm            | 535 nm                   |
| 2   | Linear equation    | \(Y = 0.0086X - 0.0429\) | \(Y = 0.0097X - 0.0433\) |
| 3   | Regression co-efficient | 0.9999           | 0.9999                   |
| 4   | Linearity          | 10-80 mg/L        | 10-70 mg/L               |
| 5   | LOD (mg/L)         | 1.794348 - 0.101286 | 0.987575 - 0.50077       |
| 6   | LOQ (mg/L)         | 5.43742 – 0.306928 | 2.992652 - 1.517485      |
Determination of active Aspirin tablets in the Iraqi market:

This validated method was used to analyze commercially available different brands of Aspirin tablets manufactured by three different companies in Iraqi markets. The results revealed the marketed brands are compliance with the amount requirement (90-110%) with respect to the labeled claim in Table 10, it was found that Aspirin from Payer company had higher Wt.% than others while SDI company had lower concentration, in which concentration of each absorbance was determined for six sample solutions of each samples compared to the calibration curve method, or by standard addition method (Table 4), then calculate recovery percent and standard deviation, the validity of the method could be proved by analyzing authentic samples of the drug, as shown in Figure 9, 10 and 11.

Table 10: Determination of Aspirin as Sodium Salicylate from Aspirin companies

| No | Trade name | Company Name | Found* (mg/L) | SD | RSD%  | Wt.%  |
|----|------------|--------------|---------------|----|-------|-------|
| 1  | Aspicot    | Pharmaline Co., Lebanon | 52.7727 | 0.01260 | 0.02388 | 97.7272 |
| 2  | Aswpin     | SDI Co. Iraq | 50.1492 | 0.00875 | 0.01745 | 92.8689 |
| 3  | aspirin    | Payer Co., Garmany | 53.9347 | 0.00692 | 0.01282 | 99.8791 |

* Average of six solutions of the sample solution.
5. Conclusion

The successful method was developed and UV spectroscopy is simple, inexpensive, effective, fast, and accurate, with high values of precision and accuracy, the parameters of validation were evaluated as per I.M.H. guidelines, the satisfactory finding of this work indicates that this analysis technical might be applied for determination of Aspirin from pharmaceutical dosage forms, and may be employed in routine quality control aspects. The results shown that Aspirin tablets is accepted within the normal percentage (90-110%) according I.M.H., U.S.P.30 [17] and BP.2018 [18], when applied this method for determination of Aspirin tablets in Iraqi markets.
The results obtained show that it is possible to use sodium salicylate as a standard for the estimation of Aspirin since no interference in the absorbance for the two compounds in observed.

6. References

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