

## Simultaneous Estimation of Aspirin and Vonoprazan in Pure and Pharmaceutical Dosage Form by UV Spectroscopy

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### Abstract

A simple, accurate, and precise UV-visible spectrophotometric method was developed for the simultaneous estimation of Aspirin and Vonoprazan in pure and pharmaceutical formulations. The method utilized the simultaneous equation method based on their respective absorbance maxima at 225 nm Aspirin and 247 nm Vonoprazan. Validation as per ICH Q2(R1) guidelines showed linearity over 10–50 µg/ml for Aspirin and 2–10 µg/ml for Vonoprazan, with correlation coefficients of 0.9968 and 0.9991, respectively. The method demonstrated excellent specificity, precision (%RSD < 2%), accuracy (mean recovery ~100%), and sensitivity, with low LOD and LOQ values. The developed method was successfully applied to marketed formulations with high accuracy.

**Keywords:** Aspirin, Vonoprazan, UV-Visible Spectroscopy, Simultaneous Equation Method, Validation, ICH Q2(R1)

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### Introduction

Aspirin is a widely used analgesic and anti-inflammatory drug, while Vonoprazan is a potassium-competitive acid blocker used for treating acid-related disorders. Their combination is found in several pharmaceutical formulations, necessitating an accurate simultaneous estimation method. This study proposes a UV-visible spectrophotometric method employing simultaneous equations for their estimation in bulk and dosage forms, validated as per ICH guidelines [1-3]

### Materials and Methods

**Materials:** Active Pharmaceutical Ingredients of Aspirin as a gift sample from Spark Lifesciences and Vonoprazan as a gift sample Bio-Synth

### Chemicals Used

Chemical used in the research are Acetonitrile AR Grade (Fisher Scientific Ltd. India), Buffer Capsules, Methanol, Ortho Phosphoric Acid GR (Merk, India), Potassium Di Hydrogen Phosphate GR (Merk, India), DMSO.

### Equipments

Analytical Balance (Model, UV-Vis Spectrophotometer 1800 (Shimadzu Corporation, Kyoto, Japan), Syringe filter (Vertipure Nylon syringe Filter, 133 mm diameter, 0.45 µm pore size, Vertical, Thialand, Sonicator (Sonapros Ultrasonic Processor / Sonicator Oscar ultrasonics Pvt. Ltd Maharashtra, India), pH Meter (Instrument India, Mumbai), Indicator paper pH 1.0-14.0

### Instrumentation and Chemicals

UV-visible spectrophotometer with quartz cells (1 cm) was used for the analysis. Pure samples of Aspirin and Vonoprazan and pharmaceutical formulations were procured from commercial sources.

### Preparation of Standard Stock Solutions

Accurately weighed quantities of Aspirin (100 mg) and Vonoprazan (100 mg) were transferred into two separate 100 mL volumetric flasks. Each drug was

dissolved in 20 mL of dimethyl sulfoxide (DMSO) and the volume was made up to the mark with distilled water to obtain stock solutions containing 1000 µg/mL of Aspirin and Vonoprazan, respectively.

### Preparation of Working Standard Solutions

Aliquots from the above stock solutions were further diluted with distilled water to prepare working standard solutions of appropriate concentrations for method development and validation.

### Selection of Detection Wavelength

Solutions of each drug in methanol were scanned in the range of 200–400 nm using a UV–Visible spectrophotometer. It was observed that Aspirin and Vonoprazan showed significant absorbance maxima at 225 nm and 247 nm, respectively. These wavelengths were selected for simultaneous quantification.

### Analysis of Marketed Formulation

Five tablets were weighed accurately, finely powdered, and a portion equivalent to 100 mg of Aspirin and 10 mg Vonoprazan was transferred into a 100 mL volumetric flask. Methanol (20 mL) was added and the solution was sonicated for 30 minutes. The mixture was filtered through Whatman filter paper No. 41 and the filtrate was diluted to the mark with distilled water.

### Preparation of Calibration Curve

Appropriate aliquots of the working standard solutions of Aspirin and Vonoprazan were transferred to a series of 10 mL volumetric flasks and diluted to the mark with distilled water to obtain final concentrations in the range of 10–50 µg/mL for Aspirin and 2–10 µg/mL for Vonoprazan. The absorbance values were recorded at 225 nm and 247 nm.

### Preparation of Synthetic Mixture

Synthetic mixtures of Aspirin and Vonoprazan were prepared in a 10:1 ratio. Aliquots (1–5 mL) were transferred to separate 10 mL volumetric flasks and diluted with distilled water. The absorbances were measured at 225 nm and 247 nm.

### METHOD DEVELOPMENT

The UV spectra of both drugs were scanned from 200–400 nm. Aspirin showed λ<sub>max</sub> at 225 nm and Vonoprazan at 247 nm. These two wavelengths were selected for the development of the simultaneous equation method. Absorptivity values (a<sub>x1</sub>, a<sub>x2</sub>, a<sub>y1</sub>, a<sub>y2</sub>) were calculated at the selected wavelengths using Beer-Lambert's law.

### METHOD VALIDATION

As per ICH Q<sub>2</sub> (R1) guidelines, the method was validated for Linearity, Accuracy, Precision, Specificity, LOD, LOQ, and Robustness.

#### Linearity

A good linear relationship between concentration and absorbance over a concentration range of Aspirin and Vonoprazan were found 10–50 µg/mL for Aspirin and 2–10 µg/mL for Vonoprazan. The correlation coefficient was found to be 0.9968 for Aspirin and 0.9991 for Vonoprazan these are near about 0.999, ensure that a good correlation existed between the absorbance and concentration. The regression data shows in (Table 1, 2, 3, 4 and 5). (Fig. 4, 5, 6 & 7).

#### Specificity

Specificity was evaluated by spiking the solution with common excipients (8% starch, 7% magnesium stearate, and 15% lactose) at 1000 µg/mL, followed by filtration. No significant interference in absorbance was observed.

#### Estimation of Aspirin and Vonoprazan in synthetic mixture

The simultaneous equations were applied on the data obtained by the uv spectra. By using the above obtained absorptivity coefficient values given in table for the both drugs at wavelength at 225 nm and 247nm for Aspirin and Vonoprazan respectively.

The synthetic mixture of the combination of both the drugs was prepared in the ratio of 3:1 (Aspirin and Vonoprazan). This ratio of synthetic mixture was selected on the basis of dosage strength of formulation in combination, which is available in the market. Now the absorbance of the synthetic mixtures were measured at two wavelengths and the concentration of Aspirin and Vonoprazan were calculated using following two equations.

#### Simultaneous Equation Method

The concentrations of Aspirin (C<sub>x</sub>) and Vonoprazan (C<sub>y</sub>) in mixtures were calculated using:

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

Where A<sub>1</sub> and A<sub>2</sub> are absorbances at 225 nm and 247 nm. The concentration of synthetic mixture (3:1 Aspirin to Vonoprazan) was found to be 15 µg/mL (Aspirin) and 5 µg/mL (Vonoprazan) (Tables 12 & 13).

#### Precision

The %RSD obtained for repeatability study was found to be 0.153 and 0.747 for Aspirin and Vonoprazan respectively. These values are below

2% indicates that the repeatability of method is satisfactory (Table 6).

#### Intra day

The % RSD values for intraday precision were found to be 0.13 for Aspirin and 0.202 for Vonoprazan in different concentrations.

The % RSD values for intraday precision were found to be 0.13 & 0.010 for Aspirin and 0.202 & 0.019 for Vonoprazan with different analysts. (Table 7 & 8)

The % RSD values of data obtained are under the acceptance limit, less than 2% indicates precise of method.

#### Inter day

The % RSD values for interday were found 0.013 for Aspirin and 0.076 for Vonoprazan. The % RSD values of data obtained are well below 2% indicates that is precise. (Table 9 & 10)

#### Limit of detection and limit of quantitation

The LOD values were found for Aspirin 3.94  $\mu\text{g/ml}$  and 3.38 $\mu\text{g/ml}$  at 225nm and 247 nm. The LOD values for Vonoprazan were found to be 0.36 $\mu\text{g/ml}$  and 0.3 $\mu\text{g/ml}$  at 225nm and 247 nm.

The LOQ values for Aspirin were found to be 11.95 $\mu\text{g/ml}$  and 10.26 $\mu\text{g/ml}$  at 225nm and 247nm. The LOQ values for Vonoprazan was found to be 1.08 $\mu\text{g/ml}$  and 0.9 $\mu\text{g/ml}$  at 225nm and 247nm. The obtained results of LOD and LOQ represents that sensitivity of method is very high. (Table 5)

#### Accuracy

The mean % recovery were found to be 99.98 % for Aspirin and 100.73 %for Vonoprazan .These mean recovery values are well within the 98-102% indicates the method is accurate (Table 13).

#### Analysis of Marketed Formulation

Marketed formulation containing 100 mg Aspirin and 10 mg Vonoprazan was analyzed. Tablets were powdered, dissolved in methanol, filtered, and analyzed. The assay values were found to be 99.96 % for Aspirin and 101.84 for Vonoprazan. The % assay was found to be within 98-102% for both drugs. (Table 14).

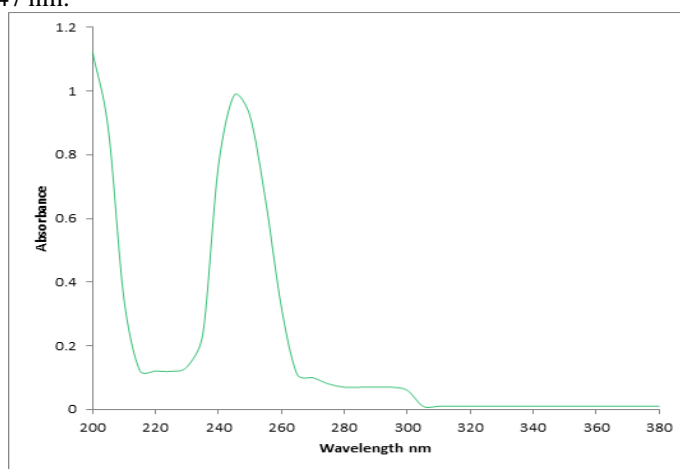


Fig. 1 UV spectra of Vonoprazan

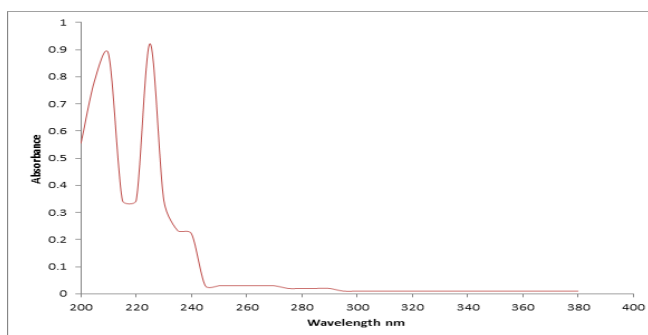


Fig. 2 spectra of Aspirin

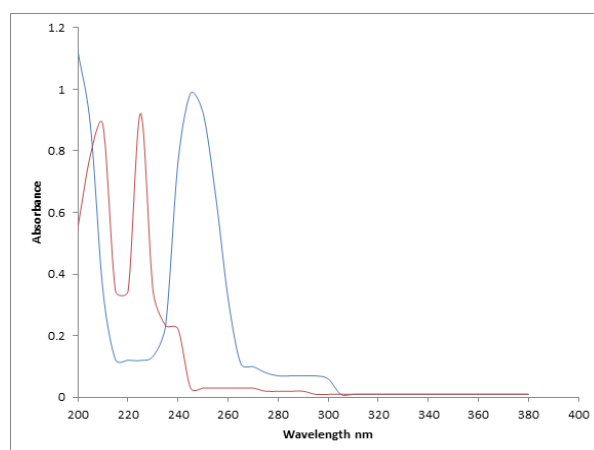


Fig. 3 Overlaps spectra of Vonoprazan and Aspirin

Table No. 1 Linearity Data of Aspirin at 225nm

| Aspirin at 270nm |                          |            |                         |
|------------------|--------------------------|------------|-------------------------|
| S. No.           | Actual Concentration PPM | Absorbance | Concentration Found PPM |
| 1                | 10                       | 0.187      | 9.38                    |
| 2                | 20                       | 0.376      | 19.83                   |
| 3                | 30                       | 0.576      | 30.90                   |
| 4                | 40                       | 0.762      | 41.15                   |
| 5                | 50                       | 0.898      | 48.71                   |

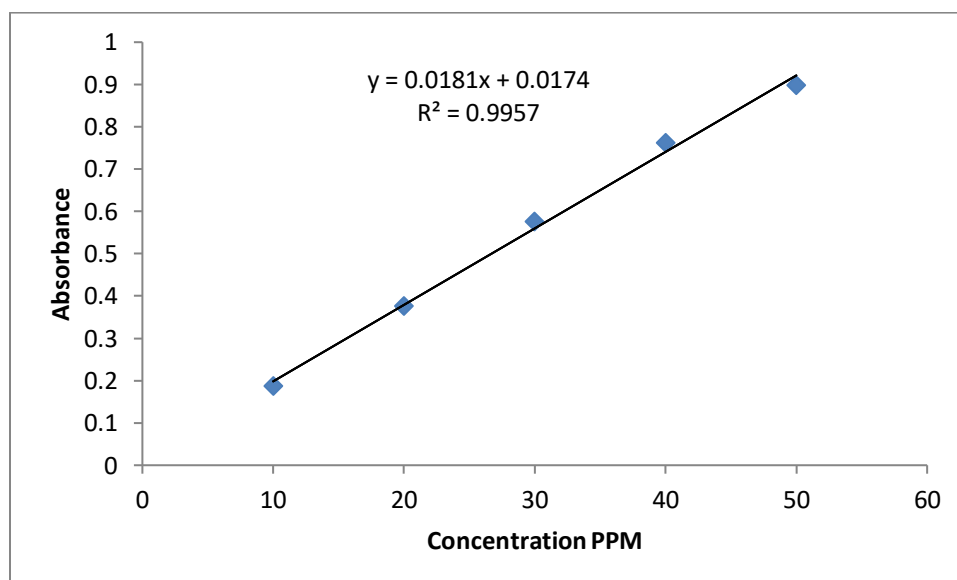


Fig. 4 Calibration curve of Aspirin at 225nm

Table No.2 Linearity Data of Aspirin at 247nm

| S.No. | Actual Concentration PPM | Absorbance | Concentration Found PPM |
|-------|--------------------------|------------|-------------------------|
| 1     | 10                       | 0.032      | 9.06                    |
| 2     | 20                       | 0.066      | 20.51                   |
| 3     | 30                       | 0.098      | 31.28                   |
| 4     | 40                       | 0.123      | 40.41                   |
| 5     | 50                       | 0.152      | 49.46                   |

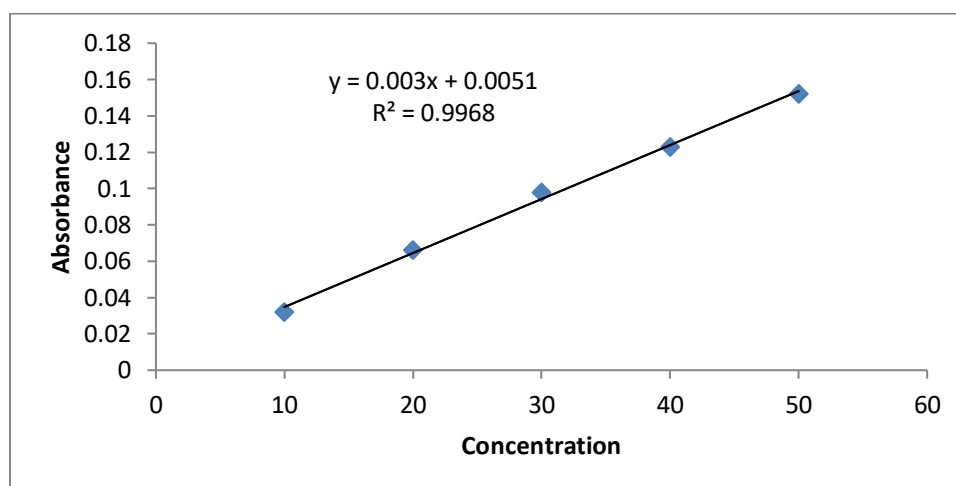


Fig. 5 Calibration curve of Aspirin at 247 nm

Table No. 3 Linearity Data of Vonoprazan at 247nm

| S. No. | Actual Concentration | Absorbance | Concentration Found |
|--------|----------------------|------------|---------------------|
| 1      | 2                    | 0.089      | 1.97                |
| 2      | 4                    | 0.176      | 3.97                |
| 3      | 6                    | 0.266      | 6.04                |
| 4      | 8                    | 0.356      | 7.18                |
| 5      | 10                   | 0.434      | 9.90                |

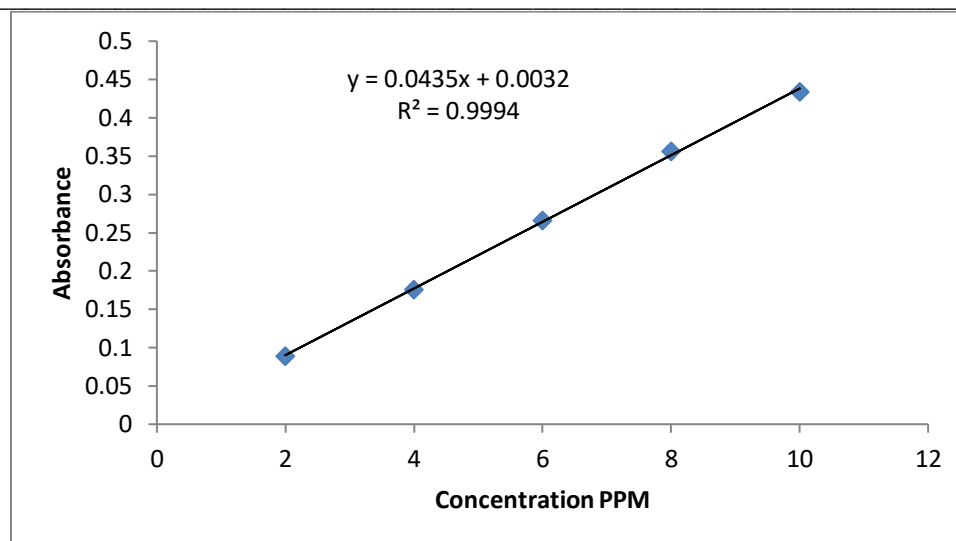


Fig. 6 Calibration curve of Vonoprazan at 247nm

Table No 4 Linearity Data of Vonoprazan at 225nm

| S.No. | Actual Concentration PPM | Absorbance | Concentration Found PPM |
|-------|--------------------------|------------|-------------------------|
| 1     | 2                        | 0.101      | 1.89                    |
| 2     | 4                        | 0.21       | 4.07                    |
| 3     | 6                        | 0.312      | 6.10                    |
| 4     | 8                        | 0.409      | 7.16                    |
| 5     | 10                       | 0.503      | 9.91                    |

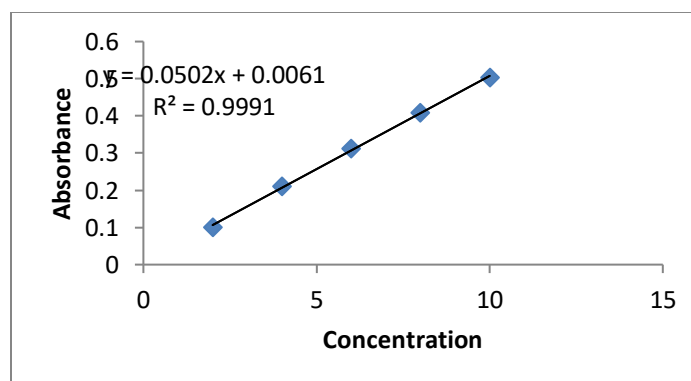


Fig. 7 Calibration curve of Vonoprazan at 225nm

**Table No.5 Regression analysis data for Aspirin and Vonoprazan**

| S. No | Parameter                        | Aspirin |        | Vonoprazan |        |
|-------|----------------------------------|---------|--------|------------|--------|
|       |                                  | 225nm   | 247nm  | 225nm      | 247nm  |
| 1     | Linearity (µg/ml)                | 10-50   | 10-50  | 2-10       | 2-10   |
| 2     | Correlation coefficient( $r^2$ ) | 0.9957  | 0.9968 | 0.9991     | 0.9994 |
| 3     | Slope                            | 0.018   | 0.003  | 0.050      | 0.0435 |
| 4     | Intercept                        | 0.0174  | 0.0051 | 0.0061     | 0.0032 |
| 5     | LOD(µg/ml)                       | 3.94    | 3.38   | 0.36       | 0.3    |
| 6     | LOQ(µg/ml)                       | 11.95   | 10.267 | 1.08       | 0.9    |

**Table No. 6 Repeatability for Aspirin and Vonoprazan**

| S. No | Con ASPIRIN | Absorbance | Concentration found | Con VONOPRAZAN | Absorbance | Concentration found |
|-------|-------------|------------|---------------------|----------------|------------|---------------------|
| 1     | 20          | 0.376      | 19.83               | 4              | 0.176      | 3.97                |
| 2     | 20          | 0.377      | 19.89               | 4              | 0.177      | 4.00                |
| 3     | 20          | 0.376      | 19.83               | 4              | 0.179      | 4.04                |
| 4     | 20          | 0.377      | 19.89               | 4              | 0.178      | 4.02                |
| 5     | 20          | 0.376      | 19.83               | 4              | 0.179      | 4.04                |
|       |             | Mean       | 19.856              |                | Mean       | 4.014               |
|       |             | STDV       | 0.030               |                | STDV       | 0.030               |
|       |             | %RSD       | 0.153               |                | %RSD       | 0.747               |

**Table No. 7 Repeatability for Aspirin and Vonoprazan by Analyst I**

| S. No. | ASPIRIN  |          |         | VONOPRAZAN |         |         |
|--------|----------|----------|---------|------------|---------|---------|
|        | 10 µg/ml | 20 µg/ml | 30µg/ml | 2 µg/ml    | 4 µg/ml | 6 µg/ml |
| 1      | 0.187    | 0.367    | 0.576   | 0.089      | 0.176   | 0.265   |
| 2      | 0.178    | 0.377    | 0.58    | 0.09       | 0.177   | 0.263   |
| 3      | 0.179    | 0.376    | 0.583   | 0.087      | 0.179   | 0.263   |
| 4      | 0.182    | 0.367    | 0.583   | 0.092      | 0.178   | 0.264   |

|            |        |        |        |       |       |       |
|------------|--------|--------|--------|-------|-------|-------|
| 5          | 0.183  | 0.376  | 0.582  | 0.092 | 0.485 | 0.265 |
| MEAN       | 0.18   | 0.3726 | 0.5808 | 0.09  | 0.239 | 0.264 |
| STDV       | 0.0036 | 0.01   | 0.003  | 0.002 | 0.138 | 0.001 |
| %RSD       | 0.020  | 0.014  | 0.005  | 0.024 | 0.575 | 0.004 |
| Mean % RSD | 0.013  |        |        | 0.201 |       |       |

Table No. 8 Repeatability for Aspirin and Vonoprazan by Analyst II

| S. No.     | ASPIRIN  |          |         | VONOPRAZAN |         |         |
|------------|----------|----------|---------|------------|---------|---------|
|            | 10 µg/ml | 20 µg/ml | 30µg/ml | 2 µg/ml    | 4 µg/ml | 6 µg/ml |
| 1          | 0.192    | 0.376    | 0.584   | 0.084      | 0.173   | 0.266   |
| 2          | 0.191    | 0.368    | 0.583   | 0.089      | 0.176   | 0.265   |
| 3          | 0.193    | 0.373    | 0.586   | 0.084      | 0.169   | 0.267   |
| 4          | 0.194    | 0.378    | 0.588   | 0.086      | 0.178   | 0.27    |
| 5          | 0.195    | 0.387    | 0.587   | 0.087      | 0.176   | 0.273   |
| MEAN       | 0.19     | 0.37     | 0.58    | 0.09       | 0.1744  | 0.2682  |
| STDV       | 0.0016   | 0.01     | 0.002   | 0.002      | 0.004   | 0.003   |
| %RSD       | 0.008    | 0.019    | 0.004   | 0.025      | 0.020   | 0.012   |
| Mean % RSD | 0.010    |          |         | 0.019      |         |         |

Table No. 9 Inter day precision Aspirin

| S. No. | Day 1    |         |         | Day 2    |         |         | Day 3    |         |         |
|--------|----------|---------|---------|----------|---------|---------|----------|---------|---------|
|        | 10 µg/ml | 20µg/ml | 30µg/ml | 10 µg/ml | 20µg/ml | 30µg/ml | 10 µg/ml | 20µg/ml | 30µg/ml |
| 1      | 0.187    | 0.367   | 0.576   | 0.188    | 0.369   | 0.564   | 0.177    | 0.357   | 0.573   |
| 2      | 0.178    | 0.377   | 0.58    | 0.186    | 0.372   | 0.563   | 0.175    | 0.367   | 0.569   |
| 3      | 0.179    | 0.376   | 0.583   | 0.185    | 0.378   | 0.556   | 0.173    | 0.359   | 0.573   |
| 4      | 0.182    | 0.367   | 0.583   | 0.178    | 0.373   | 0.562   | 0.179    | 0.36    | 0.567   |
| 5      | 0.183    | 0.376   | 0.582   | 0.186    | 0.366   | 0.566   | 0.179    | 0.376   | 0.573   |
| MEAN   | 0.18     | 0.3726  | 0.5808  | 0.18     | 0.3716  | 0.5622  | 0.18     | 0.3638  | 0.571   |
| STDV   | 0.0036   | 0.01    | 0.003   | 0.004    | 0.005   | 0.004   | 0.0026   | 0.0078  | 0.0028  |
| %RSD   | 0.020    | 0.014   | 0.005   | 0.021    | 0.012   | 0.007   | 0.015    | 0.021   | 0.005   |



|            |       |       |  |  |       |  |  |
|------------|-------|-------|--|--|-------|--|--|
| Mean % RSD | 0.013 | 0.013 |  |  | 0.014 |  |  |
|------------|-------|-------|--|--|-------|--|--|

Table No. 10 Inter day precision Vonoprazan

| S. No.     | Day 1   |         |         | Day 2   |         |         | Day 3   |         |         |
|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|            | 2 µg/ml | 4 µg/ml | 6 µg/ml | 2 µg/ml | 4 µg/ml | 6 µg/ml | 2 µg/ml | 4 µg/ml | 6 µg/ml |
| 1          | 0.089   | 0.176   | 0.265   | 0.084   | 0.176   | 0.267   | 0.088   | 0.178   | 0.273   |
| 2          | 0.09    | 0.177   | 0.263   | 0.087   | 0.175   | 0.264   | 0.087   | 0.177   | 0.273   |
| 3          | 0.087   | 0.179   | 0.263   | 0.089   | 0.174   | 0.266   | 0.086   | 0.178   | 0.276   |
| 4          | 0.092   | 0.178   | 0.264   | 0.089   | 0.176   | 0.261   | 0.083   | 0.175   | 0.274   |
| 5          | 0.092   | 0.485   | 0.265   | 0.09    | 0.178   | 0.263   | 0.083   | 0.176   | 0.275   |
| MEAN       | 0.09    | 0.239   | 0.656   | 0.09    | 0.1758  | 0.2642  | 0.09    | 0.1768  | 0.2742  |
| STDV       | 0.0021  | 0.138   | 0.001   | 0.002   | 0.001   | 0.002   | 0.0023  | 0.0013  | 0.0013  |
| %RSD       | 0.024   | 0.575   | 0.002   | 0.027   | 0.008   | 0.009   | 0.027   | 0.007   | 0.005   |
| Mean % RSD | 0.200   |         |         | 0.015   |         |         | 0.013   |         |         |

Table No. 11 Linearity and absorptivity Aspirin and Vonoprazan mixture

| Name of Drug | Concentration PPM | Absorbance $\lambda$ 1 225 | Absorbance $\lambda$ 2 247 | Absorptivity $\lambda$ 1 225 | Absorptivity $\lambda$ 2 247 |
|--------------|-------------------|----------------------------|----------------------------|------------------------------|------------------------------|
| Aspirin      | 10                | 0.187                      | 0.032                      | 0.003                        | 0.019                        |
|              | 20                | 0.376                      | 0.066                      | 0.003                        | 0.019                        |
|              | 30                | 0.576                      | 0.098                      | 0.003                        | 0.019                        |
|              | 40                | 0.762                      | 0.123                      | 0.003                        | 0.019                        |
|              | 50                | 0.898                      | 0.152                      | 0.003                        | 0.018                        |
|              |                   |                            |                            | ax1                          | ax2                          |
|              | Mean              |                            |                            | 0.003176333                  | 0.018742                     |
| Vonoprazan   | 2                 | 0.045                      | 0.101                      | 0.0505                       | 0.0225                       |
|              | 4                 | 0.098                      | 0.21                       | 0.0525                       | 0.0245                       |
|              | 6                 | 0.145                      | 0.312                      | 0.052                        | 0.024166667                  |
|              | 8                 | 0.201                      | 0.409                      | 0.051125                     | 0.025125                     |
|              | 10                | 0.265                      | 0.503                      | 0.0503                       | 0.0265                       |
|              |                   |                            |                            | ay1                          | ay2                          |

|         |            |       |       |          |             |
|---------|------------|-------|-------|----------|-------------|
|         | Mean       |       |       | 0.051285 | 0.024558333 |
| Mixture | Absorbance | 0.343 | 0.433 |          |             |

Table No 12 Determination of concentration of unknown mixture

|                 |                 |                 |                 |
|-----------------|-----------------|-----------------|-----------------|
| aX <sub>1</sub> | aX <sub>2</sub> | aY <sub>1</sub> | aY <sub>2</sub> |
| 0.0031          | 0.018           | 0.051           | 0.024           |
| A1              | 0.34            | A2              | 0.433           |
| CX mcg/ml       | 15              | Cy mcg/ml       | 5               |

Table No. 13 Recovery study of Aspirin and Vonoprazan

| Parameters         | Aspirin |       |        | Vonoprazan |        |       |
|--------------------|---------|-------|--------|------------|--------|-------|
| Accuracy Level     | 80%     | 100%  | 120%   | 80%        | 100%   | 120%  |
| Amount present PPM | 10      | 10    | 10     | 4          | 4      | 4     |
| Amount Added PPM   | 8       | 10    | 12     | 3.2        | 4      | 4.8   |
| Amount recovered*  | 17.95   | 19.98 | 22.06  | 7.24       | 8.15   | 8.78  |
| % Recovery         | 99.74   | 99.90 | 100.29 | 100.61     | 101.85 | 99.73 |
| Mean               | 99.98   |       |        | 100.73     |        |       |

\*Mean of three determinations in each level

Table No.14 Analysis of Aspirin and Vonoprazan in combination dosage form

| Formulation | Drug       | Label claim(mg/tab) | % Drug found |
|-------------|------------|---------------------|--------------|
| Tablet      | ASPIRIN    | 100                 | 99.96        |
|             | VONOPRAZAN | 10                  | 101.84       |

## Conclusion

The UV-visible spectrophotometric simultaneous equation method for Aspirin and Vonoprazan offers a simple, precise, accurate, and cost-effective tool for quality control. Validated as per ICH Q2(R1) guidelines, this method can be routinely used for determination in bulk drugs and combined pharmaceutical formulations with excellent specificity and sensitivity.

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