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# METHOD DEVELOPMENT AND VALIDATION BY UV SPECTROPHOTOMETRY FOR ESTIMATION OF SULFADOXINE & PYRIMETHAMINE, SIMULTANEOUSLY

**Rama Krishna Veni P[1*], Kusuma K[2], Hari Babu B[3]**
[1*]Department of Applied Sciences and Humanities, Sasi Institute of Technology & Engineering, Tadepalligudem - 534101, Andhra Pradesh, India.
[2]Department of Chemistry, Andhra Christian College, Guntur - 522002, Andhra Pradesh, India.
[3]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur 522510, Andhra Pradesh, India.
*Email: prk.veni@gmail.com*

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**Abstract**

UV-spectrophotometric method for simultaneous estimation of Sulfadoxine and Pyrimethamine was described. In UV method, the two wavelengths used for the analysis of the drugs were 254 nm for Sulfadoxine and 275 nm for Pyrimethamine. A simultaneous equation method was developed as per ICH guidelines using a mixture of ethanol and methanol in 1:1 ratio as diluent and validated with respect to various parameters. The proposed method was found to be simple, rapid, accurate, precise, and economical and can be used successfully in the quality control of pharmaceutical formulations and for routine laboratory analysis.

**Keywords:** Methanol, Pyrimethamine, Sulfadoxine and UV-spectrophotometry.

## Introduction

Sulfadoxine [SDX], chemically known as 4-Amino-*N*-(5,6-dimethoxy-4-pyrimidinyl) benzene sulfonamide belongs to the amino benzene sulfonamide a long acting sulfonamide that is used, usually in combination with other drugs, for respiratory, urinary tract, and malarial infections [1]. It is also used in combination with other drugs, to treat or to prevent various infections in livestock. SDX and pyrimethamine were used for the treatment of *Plasmodium falciparum* malaria in those patients in whom chloroquine resistance is suspected [2].

SDX competitively inhibits plasmodium dihydropteroate synthase and dihydrofolate reductase, interfering with folate synthesis [3, 4]. Sulfa drugs or Sulfonamides are antimetabolites. The action of sulfonamides exploits the difference between mammal cells and other kinds of cells in their folic acid metabolism. Side effects with the pyrimethamine and SDX includes skin rash, a severe blistering, peeling, and red skin rash, pale skin, easy bruising or bleeding etc.

Pyrimethamine [PYR] chemically known as 5-(4-chlorophenyl)-6-ethyl- 2,4-pyrimidinediamine belongs to the phenylpyrimidines used for medication of protozoal infections. It is a folic acid antagonist that is used as an anti-malarial or with a sulfonamide to treat toxoplasmosis. It is commonly used as an anti-malarial drug (for both treatment and prevention of malaria), and is also used (combined with the sulfonamide antibiotic sulfadiazine) in the treatment of *toxoplasma gondii* infections in immune compromised patients, such as HIV-positive individuals.

PYR is an antiparasitic. It works by killing the parasites or preventing their growth. PYR interferes with tetrahydrofolic acid synthesis from folic acid by inhibiting the enzyme dihydrofolate reductase (DHFR) [5]. Tetrahydrofolic acid is needed for DNA and RNA synthesis in many species, including protozoa. Side effects with the drug includes rash and if higher doses are used it can cause symptoms like nausea, vomiting, abdominal cramps, dry mouth, weight loss and diarrhoea, effects like headache, ataxia and rarely seizures and haematologic side effects like leucopenia and anaemia.

Literature survey reveals that spectro-photometric [6-12], HPLC [13-18], LC-MS [19-20] and HPLC/ESI-MS/MS [21] methods were reported for the estimation of SDA and TMP either individually, as a combination of both or with other drugs in bulk and biological samples. The aim of the present work is to develop and validate simultaneous equation method for the estimation of SDX and PYR in pharmaceutical formulations which is simple, rapid, accurate, precise and economical.

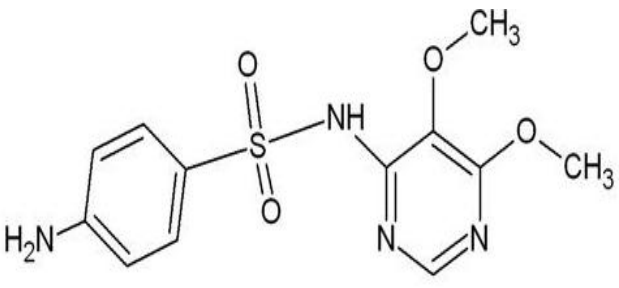


**Figure 1: Structure of SDX.**

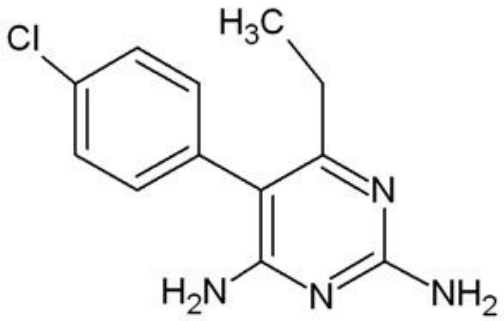


**Figure 2: Structure of PYR.**

**Materials and Methods**

**Instrumentation**

Teccomp UV-2301 double beam UV/Vis spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10 mm path length are used for analysis. Sonicator (1.3 L) ultrasonicator was used to sonicate the standard and formulation sample. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

**Chemicals and reagents**

The working standard drug SDX and PYR were obtained as gift sample from Lupin pharma limited. The pharmaceutical formulation CROYDOXIN (SDX- 500mg; PYR- 25mg) was purchased in local pharmacy. HPLC grade methanol, ethanol and water were purchased from Merck Chemicals Private Limited, Mumbai.

**Preparation of standard drug solution**

10 mg of standard drug SDX and PYR was accurately weighed separately and dissolved in 5 mL diluents, a mixture of ethanol and methanol in 1:1 ratio, then transferred to a 10 mL volumetric flask, sonicated it for 5 min.

Finally volume was made up to the mark with same solvent to make 1000 µg/mL stock solution. From this 1 mL was again diluted to 10 mL to get a concentration of 100 µg/mL solution of SDX and PYR separately. From the solution, required concentration were prepared separately, then 1 mL from each of the solution was mixed to obtained a combined solution for the simultaneous estimation of SDX and PYR.

**Preparation of formulation solution**

10 tablets of SDX and PYR (CROYDOXIN; SDX-500 mg and PYR-25 mg) were powdered and the average weight of the powder was calculated. From the powder an amount equivalent to 10 mg of standard drug

SDX was accurately weighed and dissolved in 5 mL diluent then transferred to a 10 mL volumetric flask sonicated it for 5 min, finally volume was made up to the mark with same solvent to make 1000 µg/mL stock solution.

From this 1 mL was again diluted to 10 mL to get a concentration of 100 µg/mL solution of SDX. From this, by proper dilution, a concentration of 60 µg/mL of SDX was prepared. As per the label claim of the two drugs a PYR concentration of 3µg/mL was obtained. The resultant solution was used for the simultaneous estimation of SDX and PYR in combined dosage forms.

**Selection of Method and Wavelength**

Standard solution of SDX and PYR were scanned in the wavelength range of 200-400 nm against diluent as blank. Specific prominent wavelengths maxima were obtained for both the drugs. SDX shows maximum wavelength at 254 nm and PYR at 275 nm, hence the obtained wavelength maxima were used for the simultaneous analysis of both the drugs in a single method. The resultant wavelength scanning spectra were overlay and iso-absorption wavelength was determined. The overlay spectra of SDX and PYR was given in Figure 3.

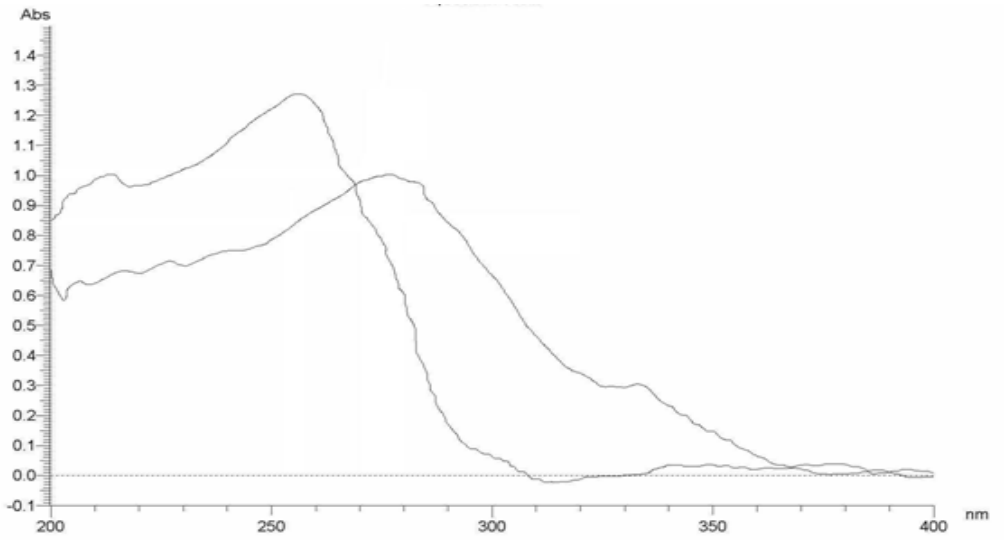


**Figure 3: Overlay spectrum of SDX and PYR.**

**Results**

The method was validated according to ICH guidelines [22].

**Linearity**

The absorbance of the standard solution was measured by diluting the solution in systematic way to satisfy the label clime ratio of the drugs. The absorbance of the diluted solution was measured triplicates in the

corresponding wavelength of both the drugs. The calibration curve was plotted by taking the concentration prepared on X-axis and the obtained average absorbance on Y-axis. Accurate fit linear graph was observed within the concentration range of 10-60 µg/mL for SDX and 0.5-3.0 µg/mL for PYR. The calibration curve was found to be suitable for the accuracy study also. Regression equation was found to be y = 0.0223x - 0.0316 ($r^2$ = 0.9996) for SDX and y = 0.3221x - 0.0472 ($r^2$ = 0.999) for PYR. Hence the obtained linearity range was found to be suitable for the analysis of SDX and PYR. Results of the linearity were given in Table 1 and linearity graphs were given in Figure 4 for SDX and 5 for PYR.

**Table 1: Linearity results for SDX and PYR.**

| S.No. | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| | **Concentration** | **Absorbance** | **Concentration** | **Absorbance** |
| 1 | 10 µg/mL | 0.191 | 0.5 µg/mL | 0.121 |
| 2 | 20 µg/mL | 0.414 | 1.0 µg/mL | 0.276 |
| 3 | 30 µg/mL | 0.636 | 1.5 µg/mL | 0.434 |
| 4 | 40 µg/mL | 0.861 | 2.0 µg/mL | 0.581 |
| 5 | 50 µg/mL | 1.101 | 2.5 µg/mL | 0.756 |
| 6 | 60 µg/mL | 1.297 | 3.0 µg/mL | 0.931 |
| | **Slope** | **0.0223** | **Slope** | **0.3221** |
| | **Intercept** | **0.0316** | **Intercept** | **0.0472** |
| | **$r^2$** | **0.9996** | **$r^2$** | **0.999** |

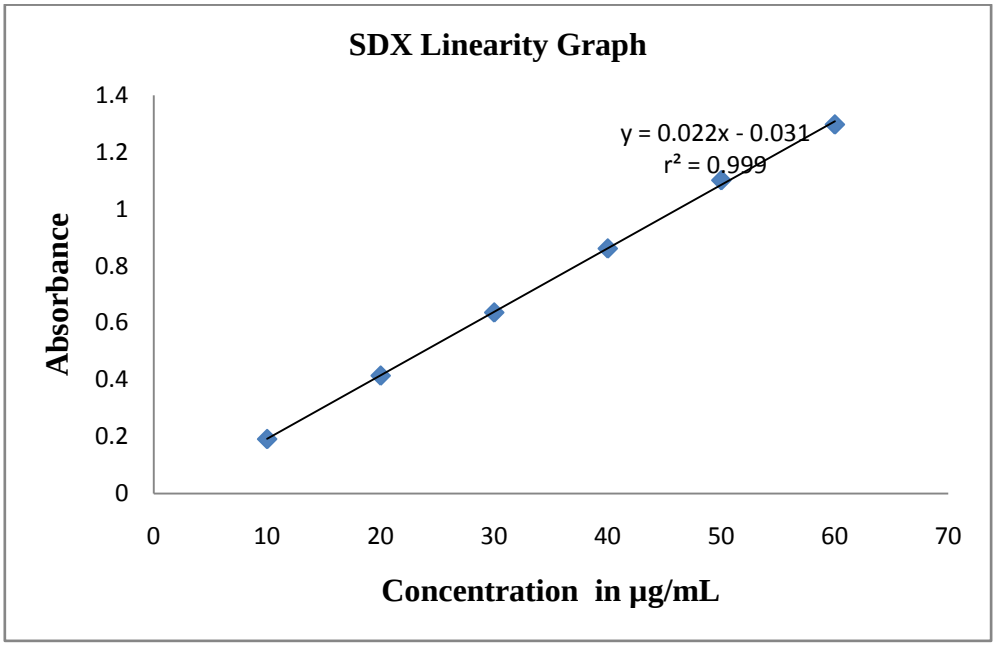


**Figure 4: Linearity graph for SDX.**

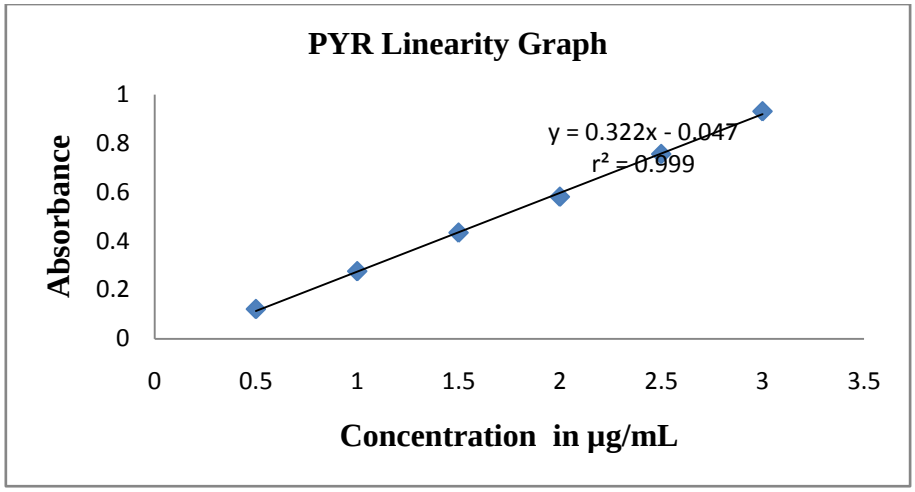


**Figure 5: Linearity graph for PYR.**

**Recovery**

Accuracy of the method was further assured by applying the standard addition technique at different levels of 50 %, 100 % and 150 %, where known amounts of the studied drugs were separately added to the pre-analyzed CROYDOXIN tablets powder and the percentage recoveries and % RSD in each spiked level was then calculated. Good percentage recoveries in the acceptable range of 98-102 % and % RSD in each spiked level was found to be < 2. % recovery was found to be in the range of 98.00-99.57 for SDX with a % RSD of 0.185, 0.164 and 0.365 in 50 %, 100 % and 150 % spiked level respectively and 98.40-99.60 for PYR with % RSD of 0.398, 0.505 and 0.618 in 50 %, and 100 % and 150 % spiked levels, respectively. In the recovery study good acceptable results were obtained, confirming the accuracy of the proposed method. Recovery results were given in Table 2 and 3 for SDX and PYR respectively.

**Table 2: Recovery results for SDX.**

| % of Recovery | Target Conc., (µg/mL) | Spiked conc., (µg/mL) | Final Conc., (µg/mL) | Conc., Obtained | % Recovery | RSD of recovery |
|---|---|---|---|---|---|---|
| 50 % | 20 | 10 | 30 | 29.87 | 99.57 | **0.185** |
| | 20 | 10 | 30 | 29.76 | 99.20 | |
| | 20 | 10 | 30 | 29.81 | 99.37 | |
| 100 % | 20 | 230 | 40 | 39.61 | 99.02 | **0.164** |
| | 20 | 230 | 40 | 39.54 | 98.85 | |
| | 20 | 230 | 40 | 39.67 | 99.17 | |
| 150 % | 20 | 30 | 50 | 49.51 | 99.02 | **0.365** |
| | 20 | 30 | 50 | 49.15 | 98.30 | |
| | 20 | 30 | 50 | 49.35 | 98.00 | |

**Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision experiments were carried out by intra-day measurement for intra-day precision and three days for inter-day precision. The precision of the analytical method was checked by repeated scanning and measurement of absorbance of solutions (n=6) for SDX and PYR at a concentration of 60 µg/mL and 3 µg/mL respectively without changing the parameter of the proposed spectrophotometry method. % RSD in both the cases for both the drugs was measured. % RSD was found to be 0.113, 0.316 for SDX and 0.451, 0.231 for PYR in intra-day and inter-day precision respectively. Very low % RSD indicates that the precision of the method was acceptable. Table 4 and 5 shows the intra-day and inter-day precision results, respectively.

**Table 5: Inter-day precision results for SDX and PYR.**

| S.No. | Sulfadoxine at 60 µg/mL | Pyrimethamine at 3 µg/mL |
|---|---|---|
| 1 | 1.289 | 0.933 |
| 2 | 1.284 | 0.936 |
| 3 | 1.291 | 0.934 |
| 4 | 1.287 | 0.937 |
| 5 | 1.288 | 0.935 |
| 6 | 1.296 | 0.931 |
| **RSD** | **0.316** | **0.231** |

**Ruggedness**

The effect of change in the results due to the change in the analyst was checked by ruggedness study. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. A concentration of 60 µg/mL and 3 µg/mL of SDX and PYR respectively was prepared by six different analysts in the same laboratory conditions. The absorbance of the prepared solutions was measured by following the same method. % RSD of the absorbance obtained was calculated. The percentage RSD was found to be 0.302 for SDX and 0.419 for PYR. The percentage RSD was found to be very lower than the acceptance limit of < 2. Hence the developed method was found to be rugged. Results of the ruggedness were given in Table 6.

**Table 6: Ruggedness results for SDX and PYR.**

| S.No. | Sulfadoxine at 60 µg/mL | Pyrimethamine at 3 µg/mL |
|---|---|---|
| 1 | 1.299 | 0.936 |
| 2 | 1.297 | 0.937 |
| 3 | 1.302 | 0.933 |
| 4 | 1.306 | 0.934 |
| 5 | 1.307 | 0.941 |
| 6 | 1.304 | 0.943 |
| **RSD** | **0.302** | **0.419** |

**Sensitivity of the method**

The LOD was found to be 5.00µg/mL and 0.03µg/mL for SDX and PYR, respectively whereas LOQ was found to be 0.01µg/mL and 0.15µg/mL for SDX and PYR, respectively. Hence the proposed method was found to be very sensitive and can be used for determination of SDX and PYR at very low concentrations. Results (Table 7) indicate that the proposed method was found to be very sensitive.

**Table 7: Sensitivity results for SDX and PYR.**

| Drug | LOQ | LOD |
|---|---|---|
| **Sulfadoxine** | 0.15 µg/mL | 5.00 µg/mL |
| **Pyrimethamine** | 0.01 µg/mL | 0.03 µg/mL |

**Formulation analysis**

The absorbance of the prepared formulation solution of 60 µg/mL of SDX and 3 µg/mL of PYR measured in the selected wavelength of 254 nm and 375 nm for both the drugs. By using the resultant absorbance values and absorptivity values % assay was calculated. The absorptivity's (A1%, 1 cm) of both the drugs at the wavelengths were determined using the following equations.

Equation 1: Simultaneous equation for the estimation of SDX

$$C_x = A_2a_{y1}\text{-}A_1a_{y2}/a_{x2}a_{y1}\text{-}a_{x1}a_{y2}$$

Equation 2: Simultaneous equation for the estimation of PYR

$C_y = A_1a_{x2} - A_2a_{x1} / a_{x2}a_{y1} - a_{x1}a_{y2}$

Where:

$ax_1$= Absorptivity of SDX at 254 nm

$ax_2$= Absorptivity of *SDX* at 275 nm

$ay_1$= Absorptivity of *PYR* at 254 nm

$ay_2$= Absorptivity of *PYR* at 275 nm

$A_1$ and $A_2$ are the absorbance of the diluted sample at 254 nm and 275 nm respectively.

A percentage assay of 99.067 % for SDX and 98.367 % for PYR was obtained in the formulation analysis study. This confirms that more than 98 % assay was observed for both the drugs in the proposed method and the formulation excipients doesn't interfere and the results were unaffected by excipients. This confirms that the method was found to be suitable for the routine analysis of SDX and PYR in fixed dosage forms. Results of the formulation analysis were given in Table 8.

**Table 8: Formulation analysis results for SDX and PYR.**

| S.No. | Drug | Brand name | Label claim | Amount prepared | Amount found | % Assay |
|---|---|---|---|---|---|---|
| 1 | **Sulfadoxine** | CROYDOXIN | 500 mg | 60 µg/mL | 59.440 µg/mL | 99.067 |
| 2 | **Pyrimethamine** | | 25 mg | 3 µg/mL | 2.951 µg/mL | 98.367 |

**Discussion**

A simple, accurate and reproducible spectrophotometric method have been developed and validated for simultaneous estimation of SDX and PYR in combined dosage forms. In simultaneous equation method, the primary requirement for developing a method for analysis is that the spectra shows specific wavelength maxima for both the drugs and should follow the Beer's law at the wavelength, which was fulfilled in case of both these drugs. The two wavelengths used for the analysis of the drugs were 254 nm for SDX and 275 nm for PYR. SDX and PYR obeyed linearity in the concentration range of 10-60 µg/mL for SDX and 0.5-3.0 µg/mL for *PYR* at their respective λmax with linear equation of y = 0.0223x - 0.0316 ($r^2$ = 0.9996) for

SDX and y = 0.3221x - 0.0472 ($r^2$ = 0.999) for PYR. In proposed method precision was studied as repeatability (% RSD < 2) and inter and intra-day variations (% RSD < 2) for both drugs; shows the high precision of the method (Table 4 and 5). The accuracy of method was determined by calculating mean percentage recovery. It was determined at 50, 100 and 150 % level and data was presented in Table 2 and 3. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The developed method for estimation of SDX and PYR in tablet dosage form was found to be simple, accurate, reproducible, sensitive and economic. For projected method we used ethanol and methanol (AR grade) in the ratio 1:1 as solvent, the developed method of simultaneous estimation not required any expensive and satisfactory apparatus in contrast to reported chromatographic and hyphenated techniques. So it shows that proposed method is simple, economic and rapid for estimation of SDX and PYR in combined dosage forms.

**Table 3: Recovery results for PYR.**

| % of Recovery | Target Conc., (µg/mL) | Spiked conc., (µg/mL) | Final Conc., (µg/mL) | Conc., Obtained | % Recovery | RSD of recovery |
|---|---|---|---|---|---|---|
| 50 % | 1 | 0.5 | 1.5 | 1.48 | 98.67 | 0.398 |
| | 1 | 0.5 | 1.5 | 1.49 | 99.33 | |
| | 1 | 0.5 | 1.5 | 1.48 | 98.67 | |
| 100 % | 1 | 1 | 2 | 1.97 | 98.50 | 0.505 |
| | 1 | 1 | 2 | 1.99 | 99.50 | |
| | 1 | 1 | 2 | 1.98 | 99.00 | |
| 150 % | 1 | 1.5 | 2.5 | 2.47 | 98.80 | 0.618 |
| | 1 | 1.5 | 2.5 | 2.46 | 98.40 | |
| | 1 | 1.5 | 2.5 | 2.49 | 99.60 | |

**Table 4: Intra-day precision results for SDX and PYR.**

| S.No. | Sulfadoxine at 60 µg/mL | Pyrimethamine at 3 µg/mL |
|---|---|---|
| 1 | 1.297 | 0.931 |
| 2 | 1.296 | 0.933 |
| 3 | 1.299 | 0.928 |
| 4 | 1.298 | 0.926 |

| | | |
|---|---|---|
| 5 | 1.296 | 0.922 |
| 6 | 1.295 | 0.924 |
| **RSD** | **0.113** | **0.451** |

**Conclusion**

UV-spectrophotometric method for simultaneous estimation of SDX and PYR was developed. The results of analysis were validated statistically according to ICH (International Conference on Harmonization) guidelines. The proposed simultaneous equation method was simple, rapid, accurate, precise, reproducible, sensitive and economical which can be successfully used in the quality control of pharmaceutical formulations and routine laboratory analysis.

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**Conflicts of Interests:** Authors have none to declare.

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**Corresponding Author:**

**Rama Krishna Veni P*,**

Department of Applied Sciences and Humanities, Sasi Institute of Technology & Engineering,

Tadepalligudem - 534101, Andhra Pradesh, India.

*Email: prk.veni@gmail.com*