

# DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION METHOD FOR ESTIMATION OF EMPAGLIFLOZIN AND SITAGLIPTIN PHOSPHATE MONOHYDRATE IN BULK AND DOSAGE FORM BY UV SPECTROSCOPY

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## ABSTRACT:

For measuring EMPA and SPM in bulk and pharmaceutical forms to created a accurate and affordable UV spectrophotometric method. Both tested medications in a water and methanol mix (70:30). EMPA absorbs light at 224 nm and SPM at 267 nm, which we found by scanning light wavelengths from 200 to 400nm. The method shows results for both EMPA and SPM across certain concentration ranges: 4–20 µg/mL for EMPA with a correlation coefficient of 0.99579 and 16–80 µg/mL for SPM with a correlation coefficient of 0.99295. And checked that our method meets ICH requirements for Linearity, Accuracy, Precision, Robustness our method is precise with a percentage standard deviation (%RSD) of less than 2% for all test parameters. This technique is suitable, for quality control analysis of mixed dose forms containing EMPA and SPM.

## KEYWORDS:

SPM , EMPA, UV spectroscopy, ICH guidelines.

## INTRODUCTION:

Sitagliptin phosphate monohydrate is a type of medicine used to treat Diabetes. It works by blocking an enzyme called DPP-IV. This helps increase the levels of hormones, GLP-1 and GIP which reduce blood sugar levels.

Empagliflozin is another medicine for Diabetes. It belongs to a group of medicines called SGLT2 inhibitors.

<sup>(1)</sup>Empagliflozin helps the kidneys remove glucose through urine, which lowers blood sugar levels. This medicine also has benefits. Studies have shown that empagliflozin reduces the risk of heart-related deaths, heart attacks and strokes in people, with Diabetes and heart disease.<sup>(2-3)</sup>

When we looked at what people have written about this we found that there are many ways to measure sitagliptin phosphate monohydrate and empagliflozin on their own or, with other drugs.<sup>(4)</sup> We did not find any simple way to measure sitagliptin phosphate monohydrate and empagliflozin together using ultraviolet light.<sup>(5)</sup> This is because you cannot buy sitagliptin phosphate monohydrate and empagliflozin together in one product. So we tried to create an

accurate way to measure sitagliptin phosphate monohydrate and empagliflozin together using ultraviolet light. We were able to make this method work. We made sure it followed the rules set by the ICH guidelines.<sup>(6,7,8)</sup>

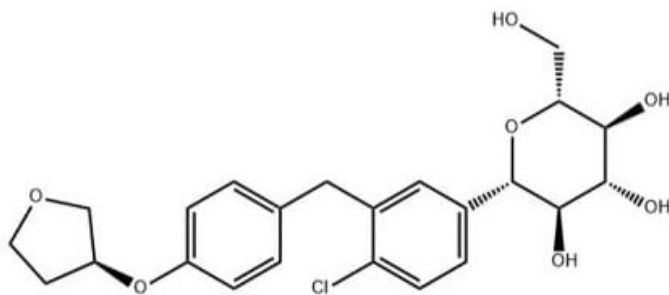


Fig 1: Sitagliptin phosphate monohydrate

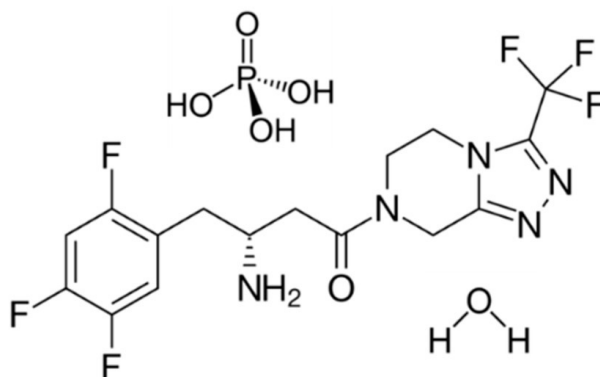


Fig 2: Empagliflozin

#### SYMPTOMS:

Feeling very thirsty, Needing to urinate often, Blurred vision, Feeling tired, Losing weight without trying, Slow healing sores.<sup>(10,12)</sup>

## 2. MATERIALS AND METHODS:

### 2.1 INSTRUMENTATION:

All measurements were done using a Shimadzu 1650 UV/VIS double beam spectrophotometer with 1cm cells whose quartz cells were matched. Another instrument that it utilized in the analysis was an Ultra sonicator Fast clean model (LMUC9D).

**2.2 CHEMICALS AND REAGENTS:**

Empagliflozin (EMPA) and sitagliptin phosphate monohydrate (SPM) were given as gift samples by Reine Life Science. The study used HPLC-grade methanol that was acquired in Merck Chemicals, and distilled water. The experimental analysis was done on the received samples.

**2.3 METHOD DEVELOPMENT:****CHOICE OF SOLVENTS:**

The analysis used a selection of an appropriate solvent based on the analysis of various mixtures of methanol and water (water:methanol (70:30)) mixture was identified.

**SELECTION OF WAVELENGTH:**

The solvent was a mixture of methanol and water, which was used to prepare the standard solutions of SPM and EMP. A UV spectrophotometer was used to analyze the solutions in the wavelength of 200-400 nm. Methanol and water mixture was taken as a blank to correct the baseline. The absorbance spectra was measured, and the corresponding wavelength graphs of both SPM and EMP were determined. Based on the overlain spectra, the isosbestic point was determined as the wavelength where the absorbance of the two drugs were equal because of the intersection point that was observed between the spectra.

**2.4 SPECTROSCOPIC CONDITIONS:**

Optimal analytical conditions that were applied while performing the study.

The analysis was done using a solvent mixture of methanol and water in a ratio of 30:70. The measurements were done in spectrum mode. The light ranged to scan was 200-400 nm. The range of absorbance was kept at 0.0 and 2.0 absorbance. The speed of scan was set at medium.

**2.5 DETECTION WAVELENGTHS:**

Three wavelengths are detected at 267nm which is the maximum wavelength of SPM, 224nm which is the maximum wavelength of EMP and 277nm which is the isobestic point.

**2.6 PREPARATION OF STANDARD STOCK SOLUTION:**

EMPA 4mg and SPM 16mg were measured separately. The individual ones were placed in volumetric flasks. They were diluted in a solution of methanol and water. The solvents were then added to the volume, in each flask to a certain point of 50ml. Further dilutions were done to obtain the concentrations of both EMPA and SPM. This was done to obtain concentrations of EMPA & SPM.

**2.7 SELECTION OF WAVELENGTH:**

Look independently at either of the two solutions in the 200 - 400 nm range to determine which wavelength each of the drugs absorbs as most. The absorbance at 267 nm and 224 nm is the maximum absorbance wavelength of SPM and EMPA.

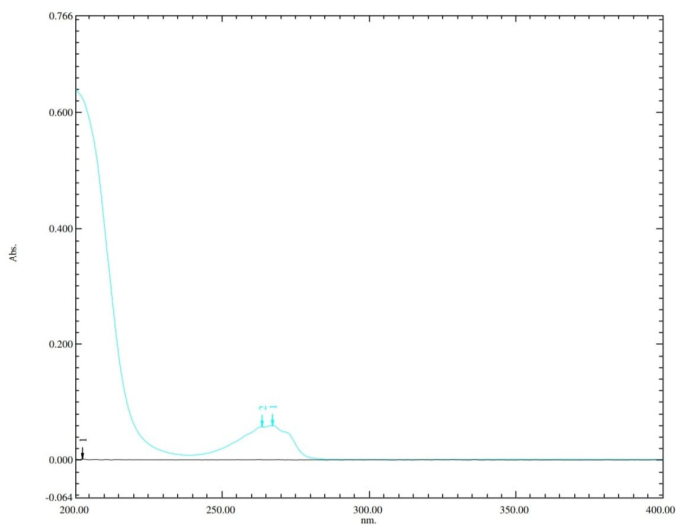
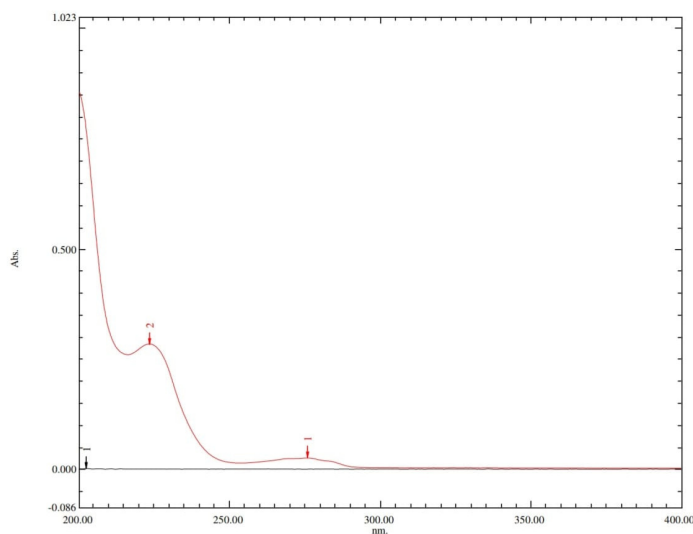


Fig 3: Wavelength of Empagliflozin

Fig 4: Wavelength of Sitagliptin phosphate monohydrate

## 2.8PREPARATION OF SAMPLE SOLUTION:

To prepare the sample solution to first determine the weight of two tablets containing SPM & EMPA. This mass was 419mg and crushed these tablets into fine. Next took 67.04 mg of this powder. Put it in a flask which would be able to hold 100 mL of liquid. To the flask, 60-70 mL of water and methanol were added. Mixed everything in the sonicator for 25 minutes. More liquid was put in until the flask was full of liquid to the mark of 100 mL. Then filtered the solution with Whatman filter paper grade 1 and diluted the solution where it was necessary to do so that it was just suitable to analysis. This can be done by adding liquid to the solution. In this manner we might obtain the solution, to the concentration we wanted in our tests.

## 2.9 SIMULTANEOUS EQUATION METHOD:

To find both drugs at one time we use the equation method. To measure how much X absorbs light at two wavelengths,  $\lambda_1$  and  $\lambda_2$ . Let's call these absorptivities  $a_{x1}$  and  $a_{x2}$ .<sup>(9)</sup> Y absorbs light at these wavelengths too with absorptivities,  $a_{y1}$  and  $a_{y2}$ . When we dilute the samples and measure how light they absorb at  $\lambda_1$  and  $\lambda_2$  we get values  $A_1$  and  $A_2$ . The method helps us figure out the amounts of X and Y.<sup>(11)</sup>

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

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

## 3.0 METHOD VALIDATION

HERE ARE THE VALIDATION METHODS I USE:

1. Linearity
2. Precision
3. Accuracy
4. Sensitivity
5. Limit of detection
6. Limit of quantification
7. Ruggedness
8. Robustness

### 1. LINEARITY:

Linearity is the ability of the UV method to produce results that are directly proportional to the concentration of analyte within a given range.<sup>(15)</sup> It is evaluated by plotting concentration vs absorbance. A straight-line graph with a good correlation coefficient ( $R^2 \approx 1$ ) indicates good linearity. The concentration range of EMPA and SPM is, between 4-20  $\mu\text{g/ml}$  and 16-80  $\mu\text{g/ml}$ .<sup>(16)</sup>

### 2. PRECISION:

Precision refers to the closeness of agreement between repeated measurements under the same conditions. It is usually expressed as %RSD (Relative Standard Deviation). It includes repeatability (same day) and intermediate precision (different days/analysts). Low %RSD indicates high precision of the method.<sup>(13,14)</sup>

### 3. ACCURACY:

Accuracy is the closeness of the measured value to the true or accepted reference value. It is often determined by recovery studies (e.g., adding known amounts of analyte). Results are expressed as % recovery. Values close to 100% indicate good accuracy.<sup>(18)</sup>

### 4. SENSITIVITY:

Sensitivity is the ability of a UV analytical method to detect and measure small changes in analyte concentration accurately. It is mainly indicated by the slope of the calibration curve—steeper slope means higher sensitivity.<sup>(17)</sup> It also involves LOD (Limit of Detection) and LOQ (Limit of Quantification), which represent the lowest detectable and quantifiable concentrations. A highly sensitive method can reliably detect very low levels of the analyte with good precision.

**5. LIMIT OF DETECTION:**

The Limit of detection is the amount of a drug that can be found in a sample. The analytical method can detect this amount of the drug. It may not be able to measure it exactly. This limit tells us the minimum amount of the substance that will give a response from the instrument. <sup>(19,20)</sup>

FORMULA:

$$\text{LOD} = 3.3 \times \sigma / S$$

This formula is used to calculate the Limit of detection of a drug.

**6. LIMIT OF QUANTIFICATION:**

The Limit of Quantification of a drug is the amount that can be measured accurately. This means the analytical method can measure the amount of the drug in a sample, with accuracy and precision. The Limit of Quantification is the amount of a drug that can be reliably measured. <sup>(19,20)</sup>

FORMULA:

$\text{LOQ} = 10 \times (\text{deviation} / \text{Slope of calibration curve})$ . This formula is used to calculate the Limit of Quantification of a drug.

**7. RUGGEDNESS:**

The thing, about ruggedness is that it is when a test can still work properly even if different people do it. <sup>(22)</sup> They use different equipment or do it in different places. This means the test gives the results every time it is used normally.

**8. ROBUSTNESS:**

Robustness is when a test is not affected by changes that are done on purpose like using a slightly different light wavelength or changing the mix of solvents or the temperature. This shows that the test is reliable and works well. <sup>(21)</sup>

**4.0 RESULTS AND DISCUSSION:**

To simultaneously measure EMPA and SPM applied the method of analysis. The findings indicated that EMPA and SITA are both good at the wavelengths we used and did not have any interference. The technique was effective in the case when it used with EMPA and SPM amounts. The findings were highly stable as an indicator that the method is reliable and also determined whether the method was accurate or not by testing it using (80, 100 and 120) percent of amount of EMPA and SPM. The findings were satisfactory hence we believe that the procedure is challenging, accurate and precise. This renders it an effective option in EMPA and SPM testing that is in medicines.

**4.1 ANALYSIS OF FORMULATION:**

The method was developed and tested and using the marketed formulation.

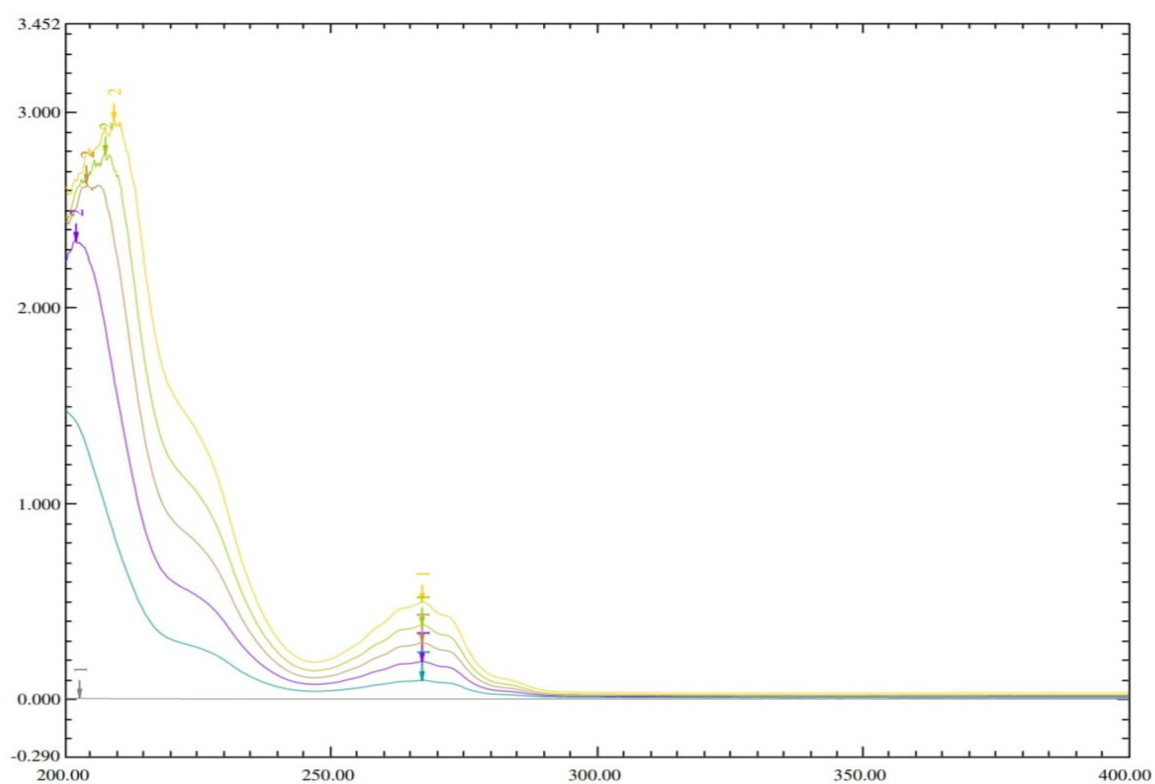
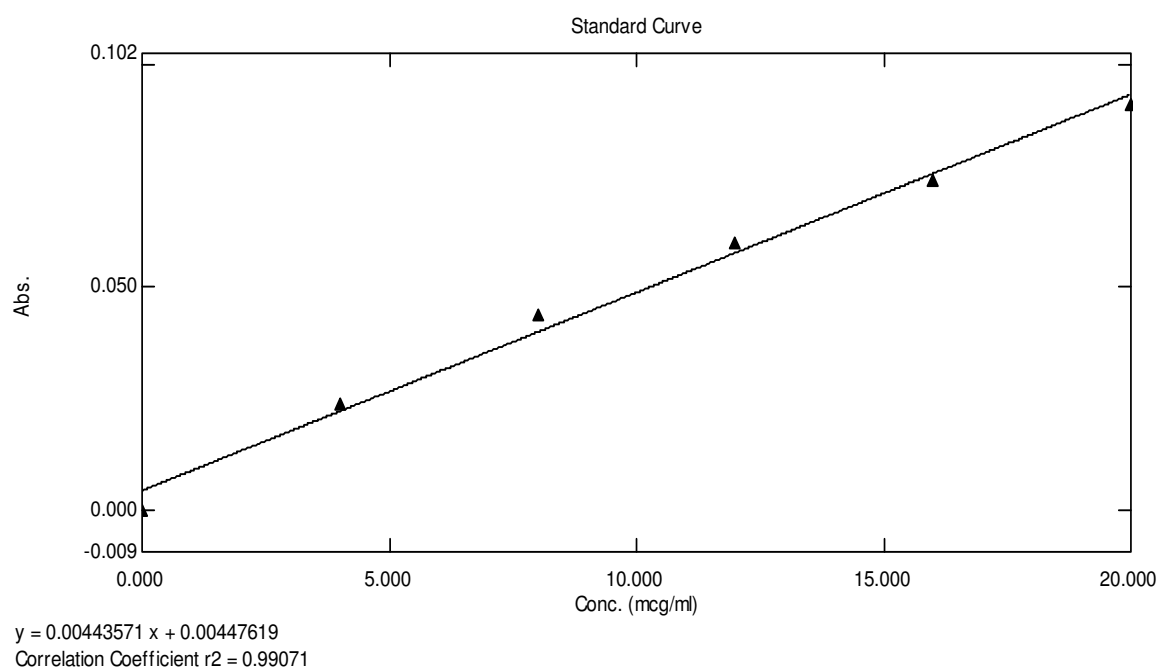
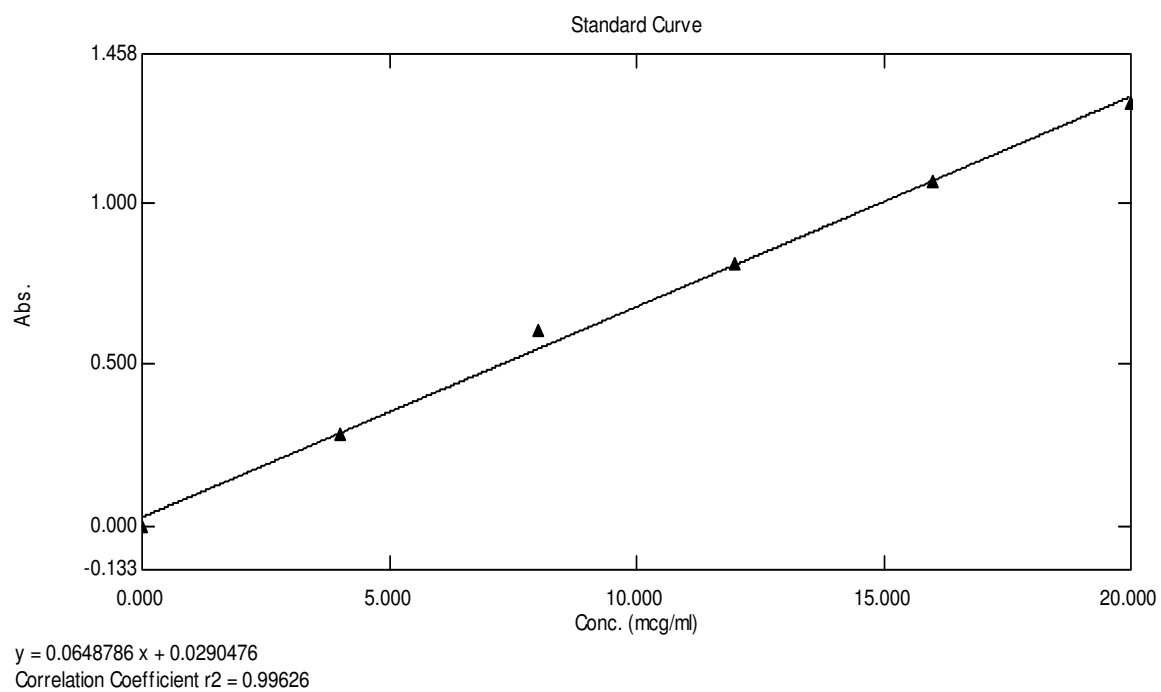


Fig 5: Formulation spectrum

#### 4.2 LINEARITY

EMPAGLIFLOZIN BOTH WAVELENGTH:

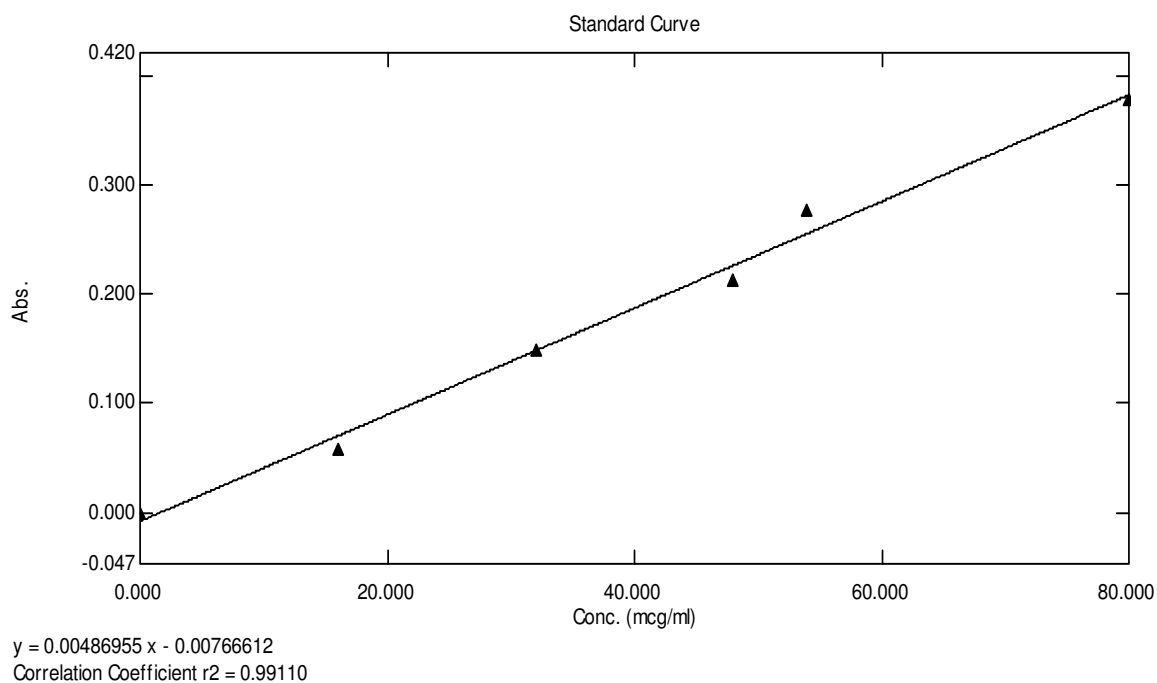
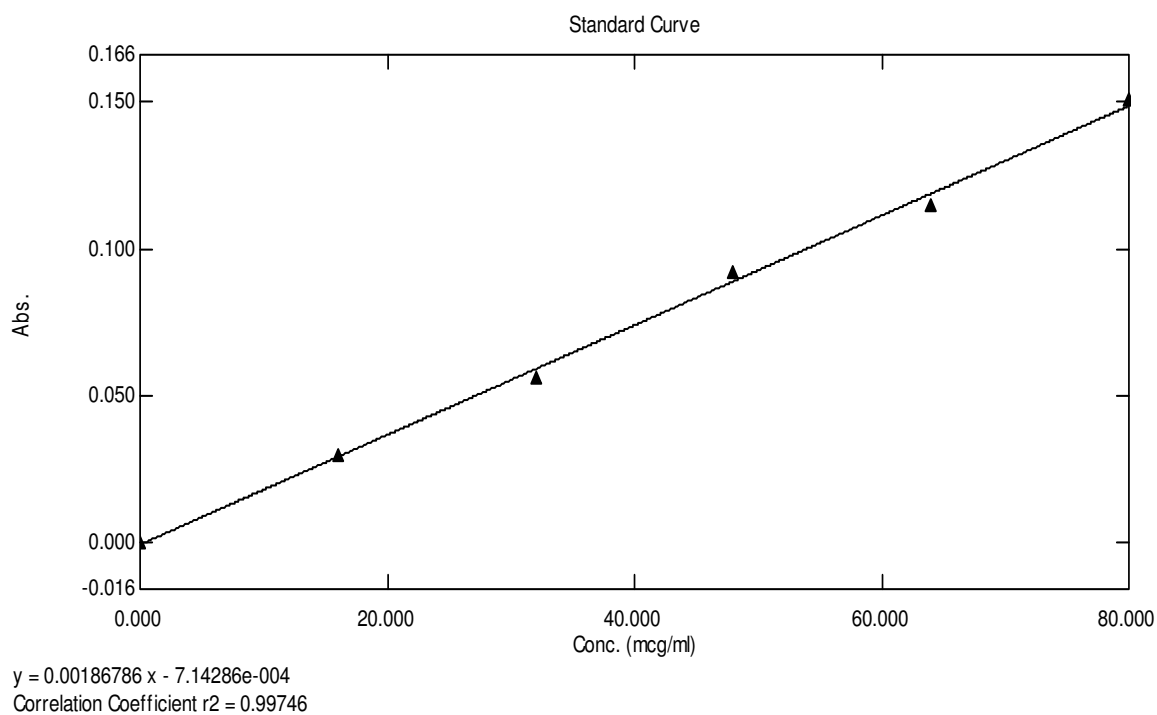
Fig 6: Linearity graph of EMPA at 224 nm and 267 nm





## SITAGLIPTIN PHOSPHATE MONOHYDRATE BOTH WAVELENGTH:

Fig 7: Linearity graph of SITA at 267 nm and 224 nm



**4.3 PRECISION:**

Table 2: Results of precision study of EMPA &amp; SPM

| Level              | Concentration (µg/ml) |     | Absorbance |       |       |       | % RSD* |        |        |        |
|--------------------|-----------------------|-----|------------|-------|-------|-------|--------|--------|--------|--------|
|                    | EMPA                  | SPM | 224nm      |       | 267nm |       | 224nm  |        | 267nm  |        |
|                    |                       |     | EMPA       | SPM   | EMPA  | SPM   | EMPA   | SPM    | EMPA   | SPM    |
| Intraday precision |                       |     |            |       |       |       |        |        |        |        |
| 1                  | 12                    | 48  | 0.919      | 0.202 | 0.058 | 0.410 | 0.0515 | 0.2329 | 0.8081 | 0.4983 |
| Interday precision |                       |     |            |       |       |       |        |        |        |        |
| 1                  | 12                    | 48  | 0.931      | 0.208 | 0.057 | 0.420 | 0.0505 | 0.2262 | 1.616  | 0.2957 |

\*RSD of three observations

**4.4 REPEATABILITY**

Table 3: Repeatability results for EMPA &amp; SPM

| S.NO | Concentration (µg/ml) |     | Absorbance |       |       |       | % RSD* |        |        |        |
|------|-----------------------|-----|------------|-------|-------|-------|--------|--------|--------|--------|
|      | EMPA                  | SPM | 224nm      |       | 267nm |       | 224nm  |        | 267nm  |        |
| 1    | 12                    | 48  | 0.916      | 0.202 | 0.058 | 0.412 | 0.0892 | 0.2337 | 0.8081 | 0.5188 |

\*RSD of six observations

**4.5 RECOVERY STUDY**

Table 4: Results of recovery study for EMPA &amp; SITA

| Level | % Recovery |      |
|-------|------------|------|
|       | EMPA       | SPM  |
| 80%   | 99%        | 101% |
| 100%  | 100%       | 102% |
| 120%  | 98%        | 99%  |

#### 4.4 LOD & LOQ:

Table 5: Results of LOD &amp; LOQ

| Parameters              | EMPA at 224nm         | EMPA at 267nm         | SITA at 267nm         | SITA at 224nm         |
|-------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| LOD( $\mu\text{g/ml}$ ) | 0.88 $\mu\text{g/ml}$ | 0.68 $\mu\text{g/ml}$ | 2.52 $\mu\text{g/ml}$ | 2.42 $\mu\text{g/ml}$ |
| LOQ( $\mu\text{g/ml}$ ) | 2.70 $\mu\text{g/ml}$ | 2.55 $\mu\text{g/ml}$ | 7.60 $\mu\text{g/ml}$ | 7.30 $\mu\text{g/ml}$ |

#### 4.6 OVERLAY SPECTRUM OF EMPA AND SITA:

The overlay spectrum shows how EMPA and SPM absorb UV light between 200 and 400 nm. EMPA and SPM have their special absorption peaks at different wavelengths. This helps us analyze them at the time. The overlay spectrum also shows the wavelengths used for analysis so they measure both EMPA and SPM accurately without any issues. It is useful for getting results, for both EMPA and SPM. EMPA and SPM can be analyzed together using these wavelengths.

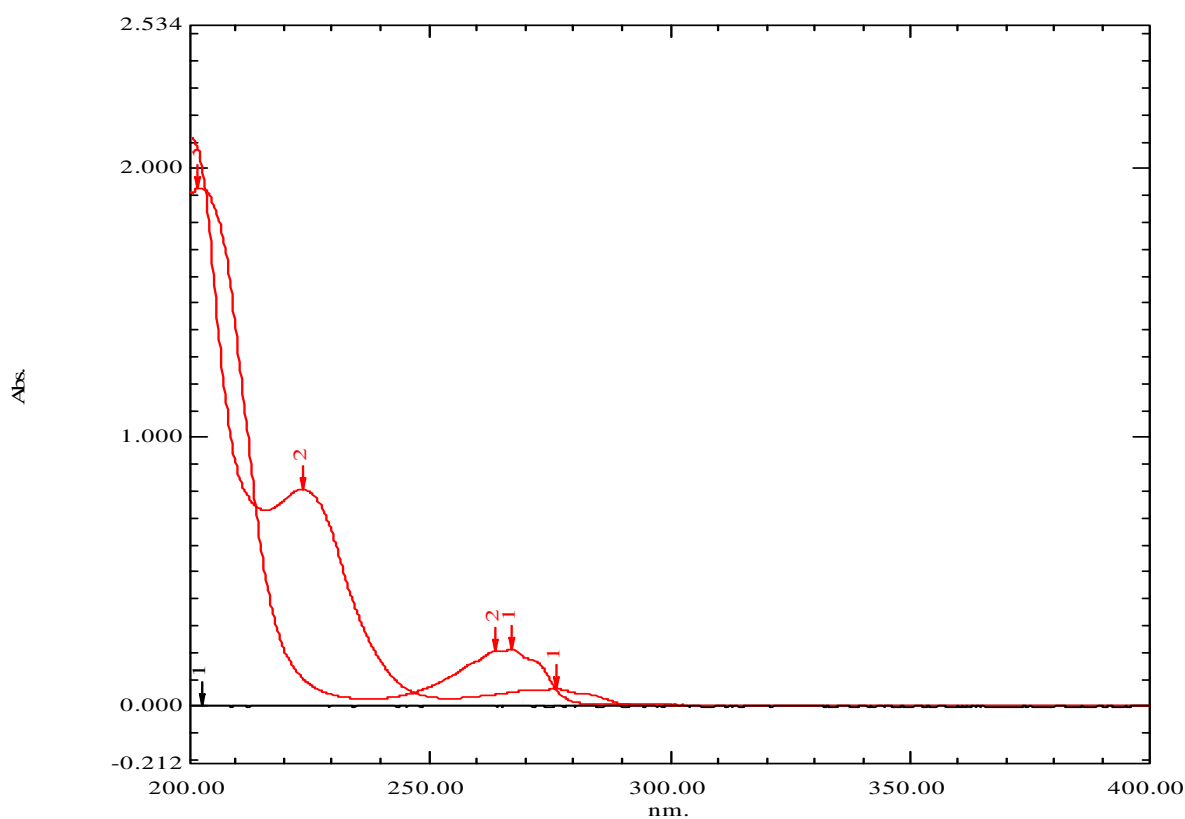


Fig 8: Overlay spectrum of both drugs

## 5. CONCLUSION:

The results have seen far show that the method are talking about is really simple and it gives accurate results. It is also very precise and sensitive which is what the ICH guidelines say it should be. So used this method to figure out how much EMPA and SPM in a batch of the drug or, in the pills that people take. The method works well for both EMPA and SPM.

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