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DEVELOPMENT OF DISSOCIATION CONSTANT VALUE OF THE SITAGLIPTINE IN PURE DRUG AND IN THE TABLET

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ABSTRACT

Background: The most crucial factor in the production and enhancement of pharmaceuticals is the dissociation constant since it enables us to comprehend a variety of chemical phenomena, such as biological activity, absorption, and the degree of ionisation of a substance at different pH levels. The pKa of a material is the pH at which it is 50% protonated.

Methods: The aim of this study is to have a better understanding of sitagliptine's molecular behavior. The above study was performed by using UV-Visible Spectrophotometer. At first, alkaline buffer of different pH range was prepared by adding 50.0 ml of 0.2M boric acid and potassium chloride in a 200-ml volumetric flask. The volume was then raised to the necessary level with double-distilled water after adding the prescribed quantity of 0.2M sodium hydroxide. The pH of the buffer was measured using a pH metre. For Preparation of standard stock solution, 10mg of Sitagliptine was measured and transferred into 100ml volumetric flask containing 40ml of alkaline borate buffer at pH 8. Then it was sonicated for two minutes to dissolve and then the volume was adjusted to 100 ml with same buffer to get 100µg/ml. 2ml of the SSS were transferred into a second 10ml volumetric flask, and the volume was diluted with buffer pH 8 until the required concentration was achieved. Then this solution was scanned in the range of 200 to

400nm with the help of UV-visible spectrophotometer (Pharmaspec-1700). The λmax was found to be 267nm. For Preparation of Tablet sample solution (TSS), 10 tablets of SGP was weighed and ground into a fine powder. An accurately weighed portion of the powder equivalent to 10 mg was transferred into different 12 nos of 100mlvolumetric flask containing 50ml of buffer and sonicated for 3 minutes and then the volume was made up to the mark with 0.1 N HCl, 0.01 N HCl, 0.1 N NaOH, 0.01 N NaOH, alkaline borate buffer pH 8, 8.4, 8.8 & 9.2. Then content of the flask was sonicated for 3minutes and then the volume was made with the respective buffer. Whatmann filter paper 41 was used to filter the solutions. Then 2ml of the tablet sample solution was transferred into different 12 nos 10ml volumetric flasks and diluted up to the mark with the respective buffer to produce the sample solution strength of 20µg/ml. Measure the absorbance reading of the tablet sample solution at 267nm. The Dissociation constant value of the drug can be calculated by utilizing the below equation, i.e.,

$$\mathbf{pKa = pH + \log \frac{dm - d}{d - d1}}$$

Where pH is the pH meter value, dm is the unionised molecule absorbance, di is the ionised molecule absorbance, and d is the molecule absorbance in each of the tested buffers.

Results: The entire experiment was replicated on two different spectrophotometers by two different people, along with the inter-day validation with same drug concentrations. The Log P experiment was performed three times in order to acquire accurate and trustworthy data. Sitagliptine's pKa was roughly calculated to be 9.2, since it is where the graph of the absorbance of the drug solution vs. pH's inflection point was found to be at a pH value of 9.2. The presence of a single value of pKa for sitagliptine was demonstrated by the absorbance curve at 267 nm. A single equilibrium in the Sitagliptine ionisation state is supported by the absorbance diagram's typically linear appearance. The experiment also shows that sitagliptine exhibits pH-dependent UV-absorption at buffers with a pH range of 8 to 12, which comprise drugs at a concentration of 20 g/ml. The ruggedness of the investigation was confirmed in accordance with the analytical method validation parameter.

Conclusion: The aforementioned experiment revealed that additional understanding of Sitagliptine's (SGP) molecular behaviour was required. In the current work, the pKa and log P of sitagliptine were calculated experimentally for the first time. pKa is the main physicochemical variable employed in drug development. According to our findings, sitagliptine's pKa is inferred to be at 9.2. The fact that all of these figures fall within a range of ± 0.25 shows that the data is accurate.

Keywords: Sitagliptine, pKa, Calibration Curve, Standard Stock Solution, Dissociation constant

INTRODUCTION

Dissociation constant:

The most crucial factor in the production and enhancement of pharmaceuticals is the dissociation constant since it enables us to comprehend a variety of chemical phenomena, such as biological activity, absorption, and the degree of ionisation of a substance at different pH levels. The pKa of a material is the pH at which it is 50% protonated **[1, 2]**. The relation of acid/base strength, pKa and site of absorption can be displayed in **Figure 1**. **Figure 1** represents the site of absorption of different types of acidic and basic drugs with their pKa value.

Sr.no.	Acid/ base strength	pKa	Site of absorption
1.	Very weak acids	> 8.0	Unionized at all pH values; absorbed along the entire length of GIT.
2.	Moderately weak acids	2.5 to 7.5	Unionized in gastric pH and ionized in intestinal pH, better absorbed from stomach
3.	Stronger acids	< 2.5	Ionized at all pH values; poorly absorbed from GIT.
4.	Very weak bases	< 5.0	Unionized at all pH values; absorbed along the entire length of GIT.
5.	Moderately weak bases	5 - 11	Ionized at gastric pH, relatively unionized at intestinal pH; better absorbed from intestine.
6.	Stronger bases	> 11.0	Ionized at all pH values; poorly absorbed from GIT.

Figure 1: Comparison between the Acid/Base Strength with pKa and site of absorption [3]

MATERIALS AND INSTRUMENTS

Sitagliptine was purchased from Dr. Reddy's Laboratories in Hyderabad as a pure medication. All spectrum measurements were performed using a Shimadzu UV-Vis Spectrophotometer (pharmaspec-1700) with 1 cm matched quartz cells. The assay method used a single electronic pan balance, a Metzer pH metre, an Elico CL 220 colorimeter with a microprocessor, and an Elico SL 220 double-beam UV-visible spectrophotometer (Contech). The solvents and reagents were all analytical-grade (AR).

METHODS

Preparation of Alkaline Borate Buffer: Alkaline Borate Buffer pHs 8 to 9.2 were prepared as per the Indian Pharmacopoeia (IP). The different buffer pH was prepared by transferring 50.0 ml of 0.2M boric acid and potassium chloride in a 200-ml volumetric flask **[4]**. The volume was then raised to the necessary level with double-distilled water after adding the prescribed quantity of 0.2M sodium hydroxide **(see Table 1)**. The pH of the buffer was measured using a pH metre **[5]**.

Table 1: Amount of 0.2 M Sodium Hydroxide (in ml) mixed to prepare Alkaline Borate Buffer Solution [6]

pH	0.2MNaOH, ml	pH	0.2MNaOH, ml
8.0	3.9	9.2	26.4
8.2	6.0	9.4	32.1
8.4	8.6	9.6	36.9
8.6	11.8	9.8	40.6
8.8	15.8	10.0	43.7
9.0	20.8		

Preparation of Standard stock solution (SSS):

10mg of Sitagliptine was measured and transferred into 100ml volumetric flask containing 40ml of alkaline borate buffer pH 8. Then it was sonicated for 2-minutes to dissolve and then the volume was adjusted to 100 ml with same buffer to get 100µg/ml[7]. 2ml of the SSS were transferred into a second 10ml volumetric flask, and the volume was diluted with buffer pH 8 until the required concentration was achieved. Then this solution was scanned in the range of 200 to 400nm with the help of UV-visible spectrophotometer (Pharmaspec-1700). The λmax was found to be 267nm **[7]**.

Table 2: Absorbance data of Sitagliptine in the variousbuffers:

Sl. No	pH	Absorbance
1	0.1 N HCl1	0.766
2	0.01 N HCl2	0.692
3	8	0.698
4	8.4	0.777
5	8.8	0.807
6	9.2	0.841
7	9.4	0.768
8	9.8	0.623
9	10	0.413
10	12	0.334
12	0.01 N NaOH13	0.327
14	0.1N NaOH14	0.398

Dissociation constant value of the drug can be calculated by utilizing the below equation:

The following formula is used to determine the pKa of a weakly basic drug:

pKa =pH +log $\frac{dm-d}{d-d1}$

Where pH is the pH meter value, dm is the unionised molecule absorbance, di is the ionised molecule absorbance, and d is the molecule absorbance in each of the tested buffers **[8]**.

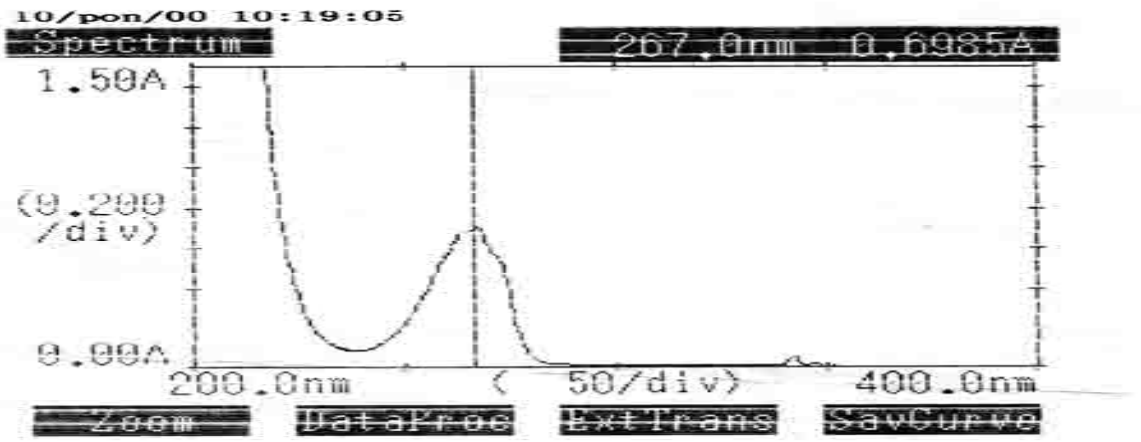

Figure 2: UV Spectrum of the drug in buffer pH 8

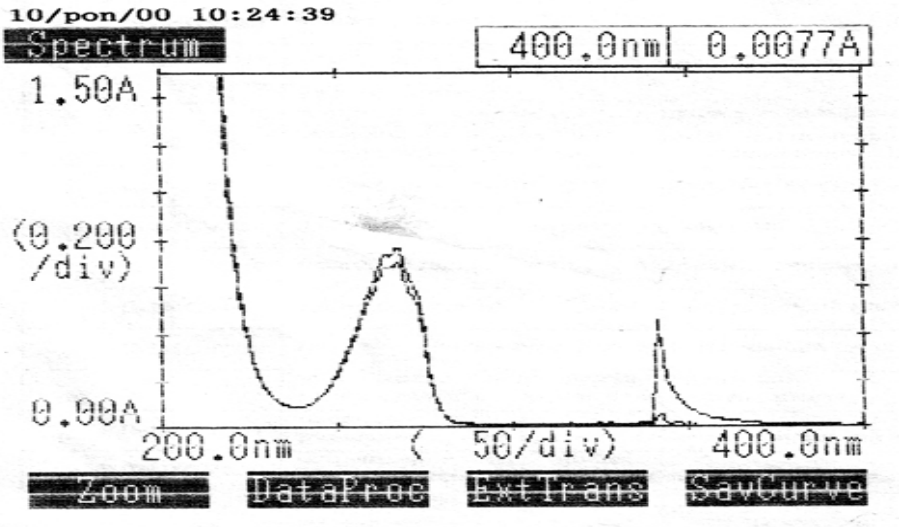

Figure 3: UV Spectrum of the drug in buffer pH 8.4

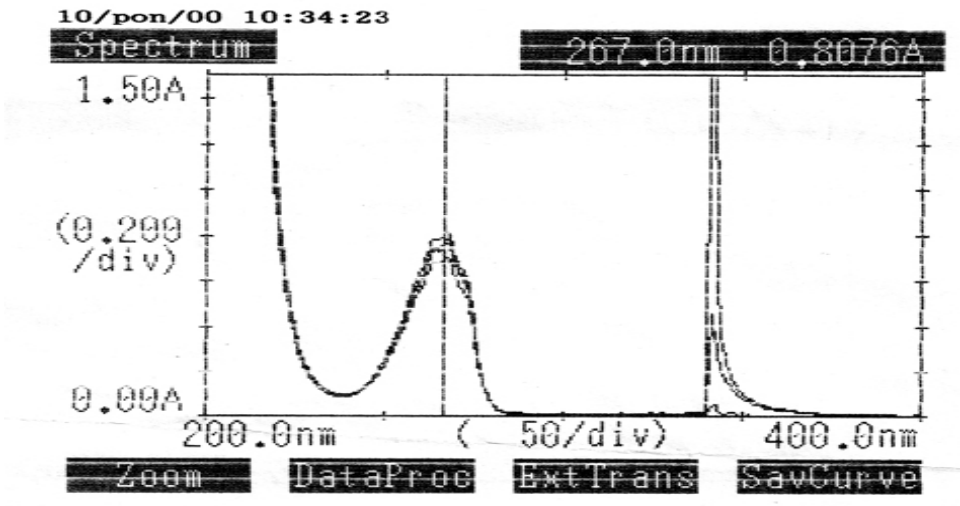

Figure 4: UV Spectrum of the drug in buffer pH 8.8

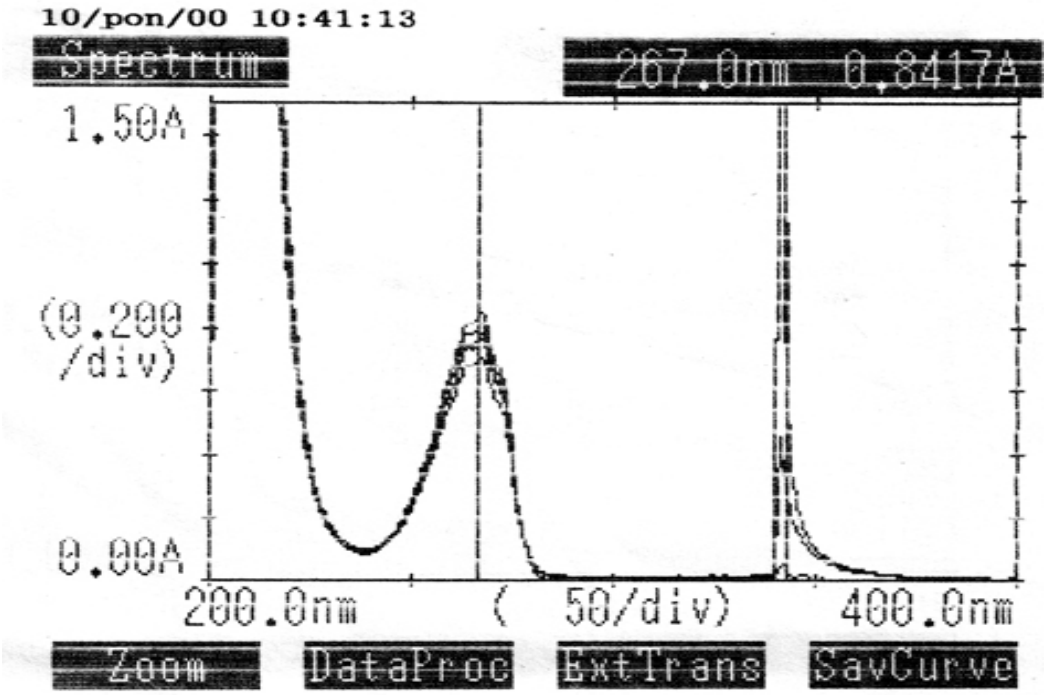

Figure 5: UV Spectrum of the drug in buffer pH 9.2

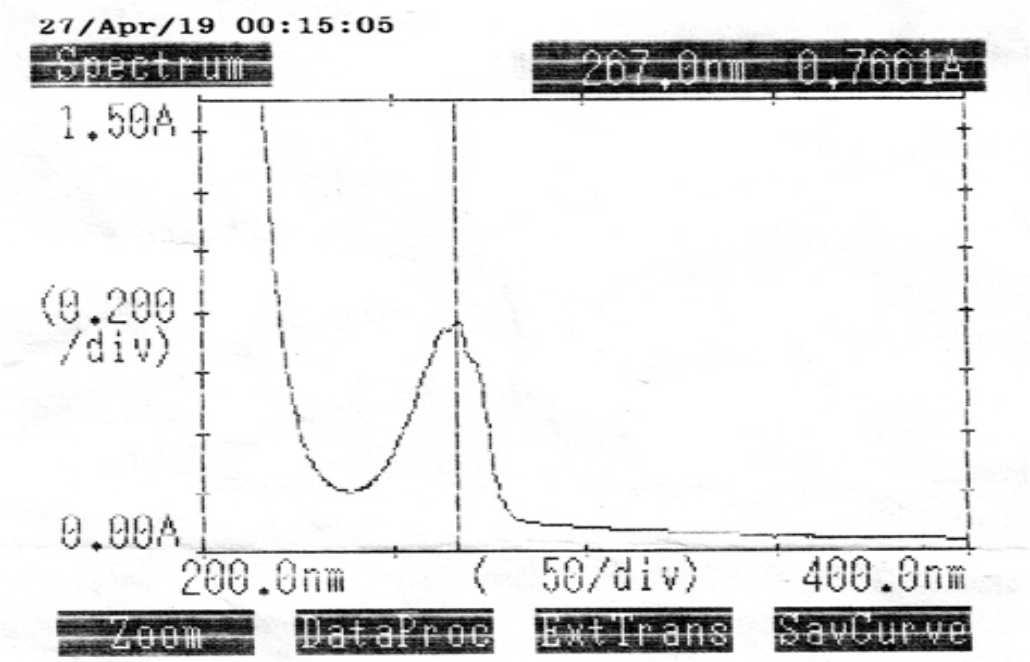

Figure 6: UV Spectrum of the drug in 0.1 N HCl

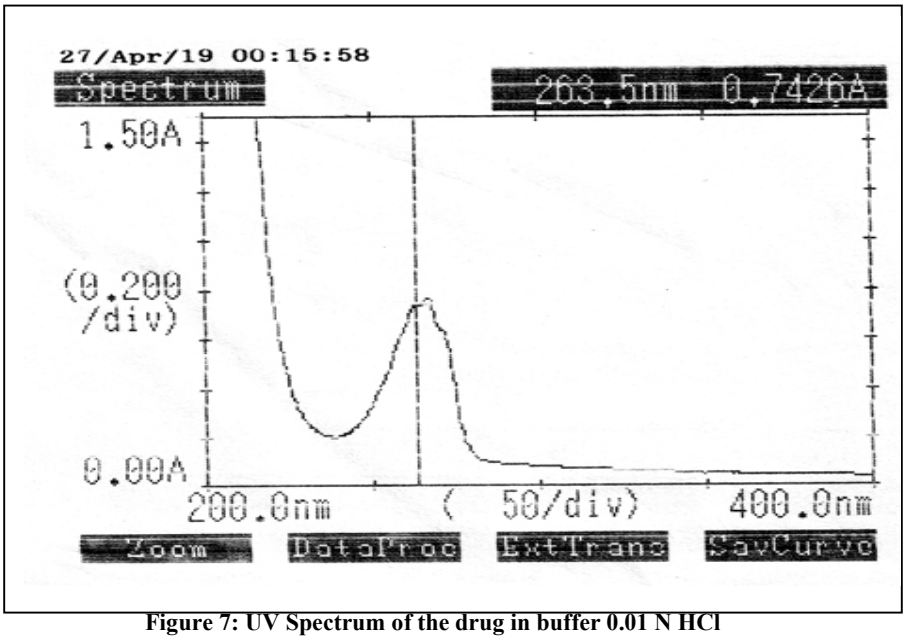

Figure 7: UV Spectrum of the drug in buffer 0.01 N HCl

Preparation of Tablet sample solution (TSS):

Ten 10 tablets of SGP were weighed and ground into a fine powder. An accurately weighed portion of the powder equivalent to 10 mg was transferred into different 12-nos of 100ml volumetric flask containing 50ml of buffer and sonicated for 3-minutes and then the volume was made up to the mark with 0.1 N HCl, 0.01 N HCl, 0.1 N NaOH, 0.01 N NaOH, alkaline borate buffer pH 8, 8.4, 8.8 & 9.2 **[9]**. Then content of the flask was sonicated for 3minutes and then the volume was made with the respective buffer **[10]**. Whatmann filter paper 41 was used to filter the solutions. Then 2ml of the tablet sample solution was transferred into different 12- nos 10ml volumetric flasks and diluted up to the mark with the respective buffer to produce the sample solution strength of 20µg/ml[11]. Measure the absorbance reading of the tablet sample solution at 267nm.

Table 3: Absorbance data of tablet sample (20µg/ml) in the various buffers:

Sl. No	pH	Absorbance
1	0.1 N HCl1	0.796
2	0.01 N HCl2	0.692
3	8	0.698
4	8.4	0.777
5	8.8	0.807
6	9.2	0.841
7	9.4	0.768
8	9.8	0.623
9	10	0.413
10	12	0.334
12	0.01 N NaOH13	0.364
14	0.1N NaOH14	0.428

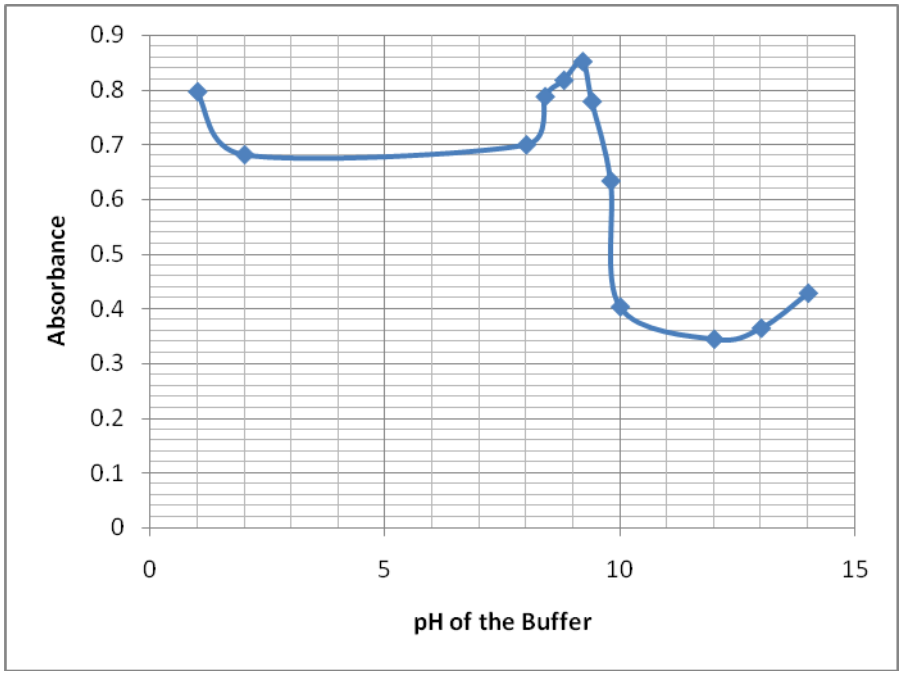

Figure 8: Graph between pH of the buffer and its Absorbance of the tablet sample

Validation of ruggedness of the pKa value

The entire experiment was repeated on two different spectrophotometers by two different people, along with the inter-day validation using the same drug concentration **[12]**. pKa experiment was repeated thrice to get the accurate and precise results **[13]**.

Table 4: Validation of ruggedness of the pKa value

Instrument 1		Instrument 2	
Person 1 day 1	Person 1 day 2	Person 2 day 3	Person 2 day 4
Drug strength= 20µg/ml	Drug strength= 20µg/ml	Drug strength = 20µg/ml	Drug strength = 20µg/ml
pKa:9.164± 0.2570	pKa:9.261±0.2550	pKa:9.095±0.2510	pKa:9.0018±0.2490

RESULTS

The result analysis of different absorbance data of Sitagliptine in the various buffer solutions has been displayed in the **Table 2**. The UV spectrum of drug in different pH ranging from 8.0 to 9.2, 0.1N HCl and 0.01N HCl has been displayed in **Figure 2, 3, 4, 5, 6 and 7**. The Absorbance data of tablet sample (20µg/ml) in the various buffers has been displayed in **Table 3**. The graph between pH of the buffer and its absorbance of the tablet sample has been displayed in **Figure 8**. The validation of ruggedness of the pKa value has been shown in **Table 4**.

DISCUSSION

Sitagliptine's pKa was roughly calculated to be 9.2 since it is where the graph of the absorbance of the drug solution vs. pH's

inflection point was found to be at a pH value of 9.2. The presence of a single value of pKa for sitagliptine was demonstrated by the absorbance curve at 267 nm. A single equilibrium in the Sitagliptine ionisation state is supported by the absorbance diagram's typically linear appearance. The experiment also shows that sitagliptine exhibits pH-dependent UV-absorption at buffers with a pH range of 8 to 12, which comprises drug at a concentration of 20 g/ml. The ruggedness of the investigation was confirmed in accordance with the analytical method validation parameter.

CONCLUSION

The aforementioned experiment's goal is to gain deeper understanding of sitagliptine's (SGP) molecular behavior. In the current work, the pKa of sitagliptine were calculated experimentally for the first time. pKa is the most important physicochemical variable employed in drug development. According to our findings, sitagliptine's pKa is inferred to be at 9.2. The fact that all of these figures fall within a range of ± 0.25 shows that the data is accurate.

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