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Extractive Spectrophotometric Estimation of Sitagliptin Phosphate

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Abstract : Two simple, accurate, sensitive and reproducible Ion-Pair Spectrophotometric methods (A & B) have been developed for the determination of Sitagliptin Phosphate (SGP) in bulk drugs and also in tablets and spiked human urine. The proposed methods are based on complexation of the drug with Patent blue vf (Method 1) &Methyl Orange (Method 2), extracted with chloroform, showing absorbance maxima at **633 nm** and **415 nm** respectively. Beer's law is obeyed over a concentration range of 30-120 µg/ml and 20-80 µg/ml for Method 1 and 2 respectively. Results of analysis for the two methods established were validated statistically and also by ICH guide lines. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipients. The validity of the methods was tested by analyzing the drug in its pharmaceutical preparations. Good recoveries were obtained.
Keywords : ion-pair color complex, SGP, MO, Patent blue vf& Molar Absorptivity.

Introduction:

Sitagliptin Phosphate is chemically 7-[(3R)-3-Amino-1-oxo-4-(2,4,5 Trifluorophenyl) butyl]-5,6,7,8-Tetrahydo-3-(Trifluoromethyl)-1,2,4-Triazolo [4,3-a] pyrazine phosphate (1:1)monohydrate **(Figure 1)**. Sitagliptin Phosphate is the first and only prescription medication in a new class of oral antihyperglycemic agents, which enhance the body's own ability to lower blood glucose when it is elevated**.** The therapeutic combination in Type II is the use of the orally active Dipeptidyl Peptidase-4 (DPP - IV) inhibitors (1-3) like Sitagliptin Phosphate. It is an oral anti-diabetic drug (4-8) that helps to control blood sugar levels by regulating the levels of insulin in the body.

A survey of literature reveals that, the analytical methods reported for Sitagliptin Phosphate were based upon Spectrophotometry, HPLC, HPTLC (9-20) and other relatedanalytical techniques like capillary electrophoresis and nuclear magnetic resonance spectroscopy(21). As highlighted earlier, the use of the above drug has become, very wide spread. The present article seeks to bridge this gap by developing a new, simple, highly sensitive, accurate, rapid and economical visible spectrophotometric method in the pure form and its tablet formulation as per ICH guides.

Experimental

Materials and Method

Instrument used: A Shimadzu UV-Vis Double beam Spectrophotometer (Pharmaspec-1700) with 1cm matched quartz cells was used for all spectral measurements. Microprocessor based colorimeter (Elico CL 220), UV-visble spectrophotometer double beam (Elico SL 220), Metzer colorimeter, Metzer pH meter and single

electronic pan balance (Contech) were used in the assay procedure.Acid Phthalate buffer (pH-2.4), Patent blue solution vf (0.1%) aqueous, double distilled water,

Method 1:

Standard stock solution: Standard stock solutions of SGP (100 µg/ml) was prepared in Acid Phathalate buffer (pH 2.4) and used for the analysis.

Working Standard Stock Solution: 4, 5, 6, 7, 8 and 9 ml of standard stock solutions were taken in 06(Six) different 10ml volumetric flasks and then diluted up to mark with Acid Phthalate buffer (pH 2.4) in order to get 40, 50, 60, 70,80 and 90µg/ml drug concentration respectively.

Selection of λ max: 60µg/ml concentration of drug solution was scanned between 350 to 750 nm to find out **λ** max (i.e. the wavelength at which amplitude is maximum) 633.0 nm.

Stability of colour complex: It has been observed that the green colour complex was stable for more than 4 hours. The absorbance was measured at an every 30mins for 4hours and found that S.D. is 0.056.

Table 2: Linearity data of Sitagliptin

Sl. No.	Concentration (µg/ml)	Absorbance at (633nm)
0	0	0
1	30	0.019
2	40	0.099
3	50	0.217
4	60	0.419
5	70	0.628
6	80	0.818
7	90	1.023

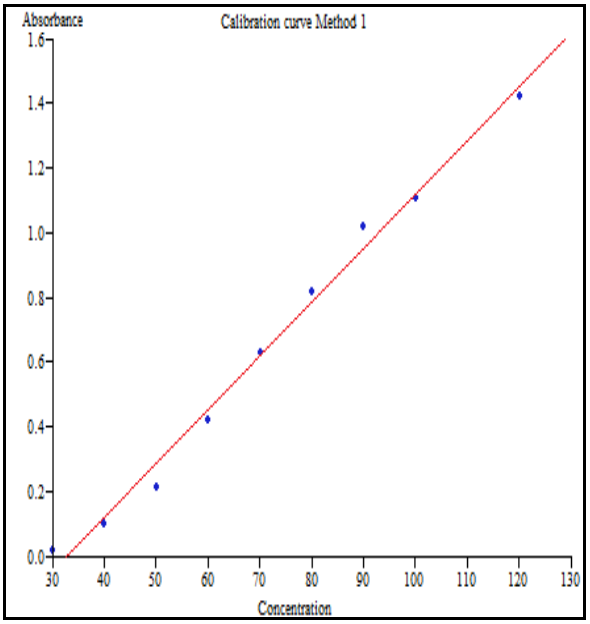


Fig 1: Linearity curve of Sitagliptin

Construction of calibration curve: Entire content of working standard stock solution were transferred to six different 125ml separating funnels. 1ml of Patent blue solution (0.1%) was transferred into each separating funnel and shaken well for 5mins and kept aside for 5mins. 15ml of chloroform was added into each separating funnel and shaken well for 3mins and the drug was extracted into the chloroform layer and it was separated into 6 different 25ml volumetric flasks. The organic layer was then passed over anhydrous sodium sulphate and the maximum absorbance was measured at 633.0nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The calibration curve was plotted using concentration Vs. absorbance. The linear regression equation was found to be**Y =0.0172x– 0.5691** and the Co-relation coefficient $\mathbf{r}^2 = \mathbf{0.9860}$. The linearity curve is shown in the Fig 1. The overlay absorption spectrum of the drug is shown in Fig 2.

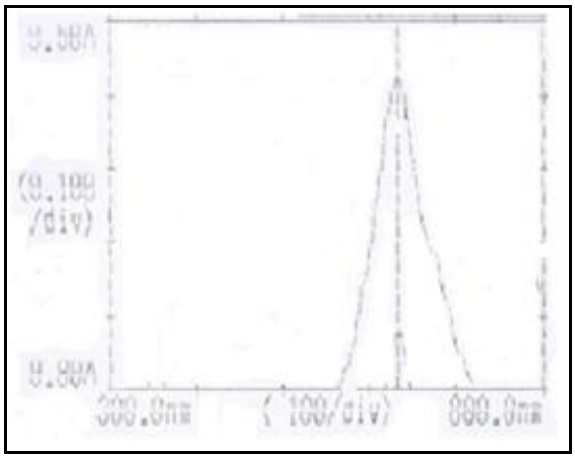


Fig 2: Visible absorbance spectrum of Sitagliptine Phosphate

Assay of Tablets:

Twenty tablets are weighed accurately and ground into a fine powder. An amount of powder equivalent to 10mg of Sitagliptin was weighed into a 100ml volumetric flask, about 40ml of freshly prepared acid phthalate buffer pH 2.4 was added and sonicated thoroughly for about 5mins, then the volume was made up to the mark with the phthalate buffer, well and filtered using Whatman filter paper No 42 and the first few milliliters of the filtrate were discarded. 5ml of filtered tablet sample solutions were transferred into four different 10 ml volumetric flasks and the volume was made upto the mark with the buffer.The contents of the volumetric flasks were transferred into six different 125ml separating funnels and 1ml of patent blue vf solution (0.1%) was added into each funnel.15ml of chloroform was added into each separating funnel and shaken for 5mins and kept aside for 3mins.The chloroform layers were collected in volumetric flasks and measured the absorbance at **633nm**.The concentration of the drug was calculated by employing the linear regression equation.The results of tablet analysis are shown in the Table 2.

Table 2: Results of tablet analysis (istavel ®)

Formulation	Label claim of tablet (mg/tab)	Amount found (mg/tab)	C.I.	SD	SE	t
I	50	50.62	101.12±3.2	1.82	0.91	1.22

Accuracy: It was found out by recovery study using standard addition method. Known amounts of standard Sitagliptine was added to pre-analysed samples at a level of 80%, 100% and 120% and then subjected to the proposed extractive spectrophotometric method. Results of recovery studies are shown in Table 3.

Table 3: Recovery study of Sitagliptine

Analyte	% Level of recovery	Formulation (µg/ml)	Pure drug added (µg/ml)	Found Conc. (µg/ml)	C.I.	SD	SE	t
SGP	80	50	40	90.01	100.01±1.15	0.72	0.36	0.02
	100	50	50	100.532	100.53±2.69	1.69	0.84	0.62
	120	50	60	109.585	99.62±2.08	1.30	0.65	0.57

SD: Standard deviation, SE: Standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis of Recovery study (n = 4). Theoretical't' values at 95% confidence level for n-1 degrees of freedom t (0.05, 3) = 3.182.

Urine sample

Drug-free human urine was obtained from a healthy male aged about 28 years.

Procedure for assay in spiked urine (Pure drug)

10 mL of urine, 5 mL of acetonitrile, and 10 mL of 50 µg/mL Sitagliptin solutions [in buffer (pH 2.4)] were added into a 25 mL volumetric flask. The resulting solution was filtered through a Whatman No. 42 filter paper and then transferred into a 125 mL separating funnel. Then, 1mL of Patent blue vf (% 0.1) was transferred into a separating funnel and it was shaken for 3 minutes. 15 mL of chloroform was added into the separating funnel and shaken well for 5 min and kept aside for 5 min. The drug was extracted into the chloroform layer and it was separated into 25 mL volumetric flasks. The organic layer was then passed over anhydrous sodium sulfate, and the maximum absorbance was measured at 633nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The concentration of SGP in urine was found by using the linear regression equation. The results are given in the Table 4.

Table 4: The results of pure drug in spiked urine

Pure drug (µg/mL)	Amount found (µg/mL)	C.I.	SD	SE	t
50	50.11	100.220±3.23	2.03	1.01	0.21

Procedure for assay in spiked urine (formulation, i.e tablet)

In a 25 mL volumetric flask, 10 mL of urine, 5 mL of acetonitrile, and 10 mL of 50 µg/mL tablet sample solution [in acetate buffer (pH 2.4)] were added. The resulting solution was filtered through a Whatman No. 42 filter paper, and then transferred into a 125 mL separating funnel. Then, 1 mL of patent blue vf solution (0.1%) was transferred into a separating funnel and 15 mL of chloroform were added into the separating funnel and shaken well for 5 min and kept aside for 5min. The drug was extracted into the chloroform layer, and it was separated into 25 mL volumetric flasks. The organic layer was then passed over anhydrous sodium sulfate, and the maximum absorbance was measured at 633 nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The concentration of SGPin tablet urine was found by using the linear regression equation. The results are given in the Table 5.

Formulation (µg/ml)	Label claim of tablet (mg/tab)	Amount found (mg/tab)	C.I.	SD	SE	t
50	50	50.50	101.01±2.0	1.29	0.64	1.56

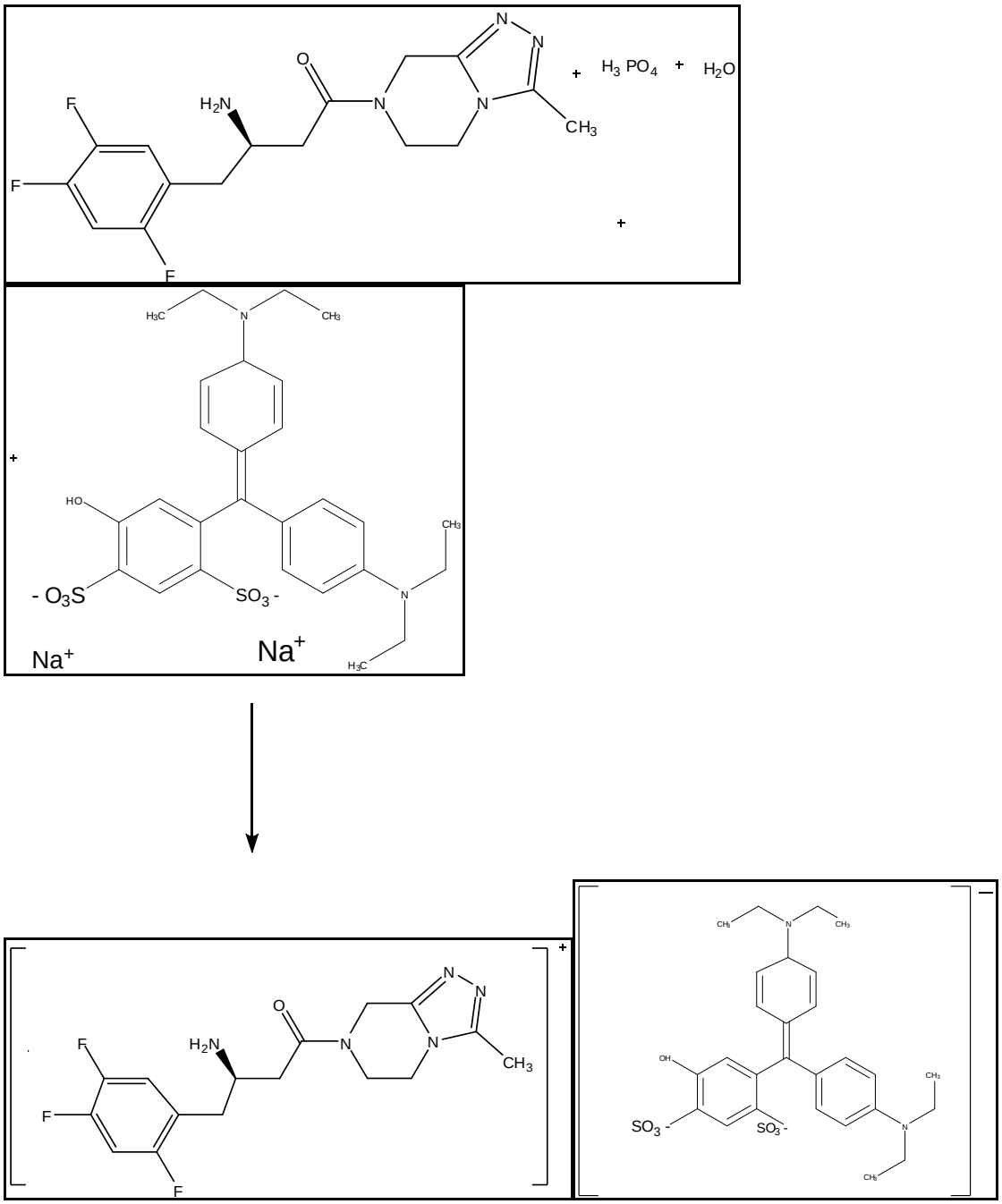


Fig: 3: Proposed reaction pathway for Sitagliptin phosphate and Patent Blue VF

Robustness and ruggedness

The robustness of the method was evaluated by making small incremental changes in volume of dye and contact time, and the effect of these changes on the absorbance of the colored systems was studied. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (≤ 2 %). Method ruggedness was demonstrated by having the analysis done by a single analyst performing analysis on four different instruments in the same laboratory. Intermediate precision values (%RSD) of this study were in the range 2.16 – 3.99% indicating acceptable ruggedness.

Method-2:

Standard Stock Solution: Standard stock solutions of SGP (100 µg/ml) was prepared in double distilled water and used for the analysis.

Working Standard Stock Solution: 2, 3, 4, 5, 6, 7 and 8ml of standard stock solutions were taken in 7 different 10ml volumetric flasks and diluted up to mark with double distilled water in order to get 20, 30, 40, 50, 60, 70 and 80µg/mL drug concentration respectively.

Selection of λ max:30 µg/ml concentration of drug solution was scanned between 350 to 700 nm to find out λ max (i.e. the wavelength at which amplitude is maximum). The λ max was found to be having yellow color chromogen 415nm.

Stability of colour complex: The absorbance was measured at an every 30mins for 5hours and found that S.D. is 0.059.It has been observed that the yellow colour complex was stable for more than 5 hours.

Construction of Calibration curve: Entire content of working standard stock solution were transferred to seven (7) different 125ml separating funnels. 2ml of buffer solution was transferred into each separating funnel. 1ml of methyl orange solution (0.2%) was transferred into each separating funnel and shaken well for 5mins and kept aside for 5mins. 07ml of chloroform was added into each separating funnel and shaken well for 3mins and the drug was extracted into the chloroform layer and it was separated into seven (7) different 25ml volumetric flasks. The organic layer was then passed over anhydrous sodium sulphate and the maximum absorbance was measured at 415nm against the reagent blank. The blank solution was prepared by utilizing all the above reagents excluding the drug solution. The calibration curve was plotted using concentration Vs. absorbance. The linear regression equation was found to be **Y = 0.0307x – 0.5856** and **r^2 = 0.9924.** The linearity data of Sitagliptine Phosphate is given in the Table 6. The linearity curve is shown in the Fig. 4. The overlay visible absorption spectrum is shown in the Fig 5.

Table 6: The linearity data of SGP (Method 2)

SL. No.	Concentration (µg/ml).	Absorbance (415 nm)
1	20	0.115
2	30	0.319
3	40	0.548
4	50	0.931
5	60	1.224
6	70	1.602
7	80	1.897

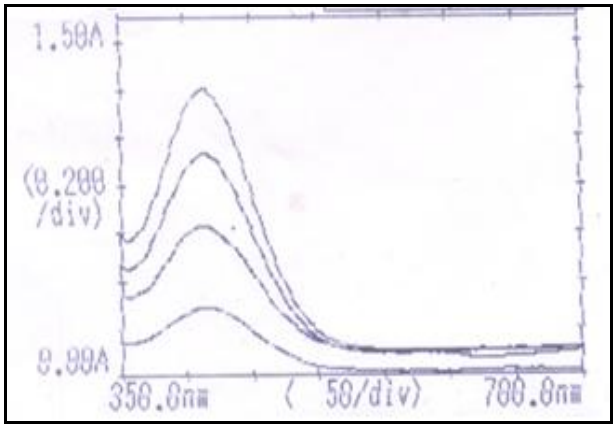
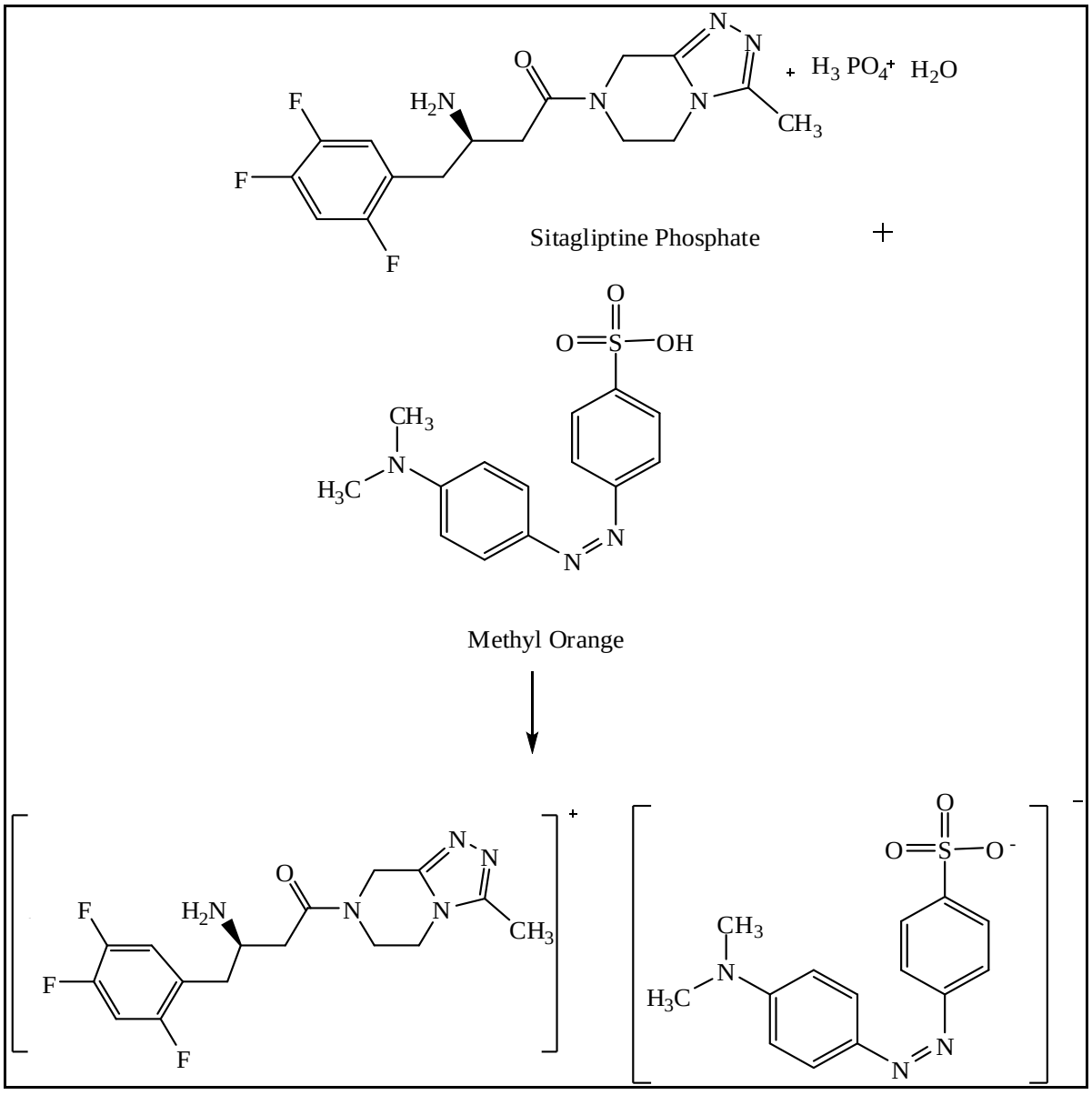


Fig 5: The Overlay Visible absorption spectrum of Sitagliptin Phosphate



Protonated form of drug **Anion form of dye**

Fig No. 5:Proposal for reaction pathway for Sitagliptine phosphate and Methyl Orange

Assay of Tablet:

Formulation (µg/mL)	Label claim (50mg/tab)	Found Concentration. (mg/tab)	C.I.	SD	SE	t
I	50	50.33	100.67±3.17	1.9	0.95	0.67

Table No. Results of Recovery study

Analyte	% Level of recovery	Formulation (µg/ml)	Pure drug added (µg/ml)	Found Conc. (µg/ml)	C.I.	SD	SE	t
SGP	80	30	24	53.89	99.80±0.74	0.46	0.23	0.81
	100		30	60.51	100.85±2.73	1.71	0.85	0.99
	120		36	66.14	100.21±3.18	1.99	0.99	0.21

Table 11: The results of pure drug in spiked urine

Formulation (µg/mL)	Label claim (50mg/tab)	Found Concentration.* (mg/tab)	C.I.	SD	SE	t
I	50	50.8025	101.65±2.59	1.62	0.81	2.08

Table 12: The results of assay in spiked urine (formulation, i.e. tablet)

Formulation (µg/mL)	Label claim (50mg/tab)	Found Concentration. (mg/tab)	C.I.	SD	SE	t
30	50	50.44	100.88±3.0	1.91	0.95	0.91

Results and Discussion and Conclusion:

The results of analysis of average recoveries obtained in each instance were compared with 100 percent theoretical value of Students‘t’ test. As the calculated‘t’ values are less than theoretical‘t’ values, it is concluded that the results of recoveries obtained are in accordance with 100 percent for each analyte. So the percentage recovery experiments revealed good accuracy of the data. Less than 1.5 SD was observed in both the methods. So the method is precise. The molar ratio of the drug: dye was determined by Job’s method and found to be 1:1. It was determined by taking identical molar ratios i.e. 0.15M for drug and dye. In this ratio it is producing maximum absorbance in the methods. Below and above the mentioned buffer strength pH does not produce any stable colour complex. The recovery results of the proposed method were well agreed with the reported RP-HPLC method for the drug. So both the proposed methods are simple, economy, accurate, precise and reproducible and highly sensitive methods and can be used for quality control laboratory.

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