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DEVELOPMENT OF ECONOMICAL ANALYTICAL METHODS FOR THE ESTIMATION OF SITAGLIPTIN PHOSPHATE BY EXTRACTIVE SPECTROPHOTOMETRIC METHODS IN PURE DRUG AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Extractive spectrophotometric methods that are simple, exact, sensitive, and repeatable have been devised for the detection of sitagliptin phosphate (SGP) in bulk drugs, tablets, and spiked human urine. Using a Rhodamine B solution (0.1%) and an acid phthalate buffer with a pH of 2.4, the indicated processes are based on estimation of the medication and tablet formulation. In order to analyse the spiking urine of pure drug and tablet formulation, respectively, Patent Blue vf and Methyl Orange solution are taken. After a chloroform extraction, these solutions showed absorbance maxima at 580 nm for the pure drug and tablets, 633 nm for the pure drug spiked with urine, and 427 nm for the formulations of spiked urine tablets. The analytical results for the above-described methods were statistically validated using statistics and ICH recommendations. All the factors were investigated in order to enhance the reaction circumstances. No influence was seen when common pharmaceutical excipients were present. The efficacy of the approaches was investigated by looking at the material in its therapeutic forms. Recovery rates were very good.

Keywords: Ion-pair color complex, SGP, MO, Rhodamine B, Patent blue vf, methyl orange & Molar Absorptivity

INTRODUCTION

The molecular name for sitagliptin phosphate is 7-[(3R)-3-Amino-1-oxo-4-(2,4,5 Trifluorophenyl) butyl]. 5, 6, 7, and 8-Tetrahydro-3-(Trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine. The first and only prescription medication in a new family of oral antihyperglycemic medications, sitagliptin phosphate, supports the body's innate ability to naturally reduce blood glucose levels as they rise. The use of orally active Dipeptidyl Peptidase-4 (DPP - IV) inhibitors, such as sitagliptin phosphate, is advised as a course of treatment for Type II diabetes **[1]**. It is an oral diabetic medication (for ages 4–8) that balances the body's insulin production to lower blood sugar levels. A literature research revealed that the capillary electrophoresis, nuclear magnetic resonance spectroscopy, and other relevant analytical techniques served as the foundation for the described analytical approaches for sitagliptin phosphate **[2]**. The aforementioned drug is currently widely used, as was previously stated. By creating a brand-new, straightforward, very accurate, quick, and affordable visual spectrophotometric technique in pure form, together with its tablet formulation in accordance with ICH requirements, the current paper aims to close this gap **[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).

EXPERIMENTAL WORK

Estimation of Sitagliptine Phosphate by Rhodamine B

Preparation of Acid Phthalate buffer (pH-2.4): In a 200 ml volumetric flask, combine 50.0 ml of potassium hydrogen phthalate (0.2 M) with 42.2 ml of 0.2 M HCl to create an acid phthalate buffer solution with a 2.4 pH **[4]**.

Preparation of Rhodamine B (0.1%): Rhodamine B was dissolved in 40 millilitres of distilled water and then added to a 100 millilitre volumetric flask. The volume was then sonicated for two minutes before being topped off with distilled water **[5]**.

Preparation of Standard Stock Solution: Aqueous solution of drug (100µg/ml)

Preparation of Working Standard Stock Solution: 2, 3, 4, 5, 6, 7 and 8 ml of standard stock solution were added to seven different 10 volumetric flasks in order to get drug concentrations of 20, 30, 40, 50, 60, 70, and 80 g/ml. After that, double-distilled water was poured into the flasks until they were full **[6]**.

Selection of λ max: A red colour complex at a concentration of 30 g/ml was run between 350 and 800 nm to ascertain the maximum. The wavelength of maximum absorption, which has a complex red colour, is 580 nm **[7]**.

Construction of Calibration Curve:

The full contents of seven 10 ml volumetric flasks were transferred using seven separate 150 ml separating funnels. A pH 2.4 Acid Phthalate buffer containing 2ml was added to each funnel. The Rhodamine B (0.1%) solution was divided into equal amounts in each funnel and shaken vigorously for three minutes **[8]**. 10 ml of chloroform should then be added to each funnel, shaken for 5 minutes, and then held in place for an additional 5 minutes. Divide the chloroform layer across seven distinct 10ml volumetric flasks. The organic layer was then passed through anhydrous sodium sulphate, and the maximum absorbance at 580 nm was determined in comparison to the reagent blank **[9]**. At 498 nanometers, the dye absorbs light most completely. Except for the drug solution, all of the aforementioned reagents were employed to make the blank solution. The calibration curve was drawn as concentration versus absorbance. The linear regression equation, with a co-relation coefficient of 0.9970, was found to be Y = 0.0178x - 0.0447 **[10]**. The data on linearity are shown in **Table 1**. The linearity curve for the drug is shown in **Figure 1**. The drug's visible absorption spectrum is shown in **Figure 2**.

Table 1: The linearity data of SGP

Sl. No.	Concentration (µg/ml)	Absorbance at λmax (580nm)
1	0	0
2	20	0.285
3	30	0.479
4	40	0.648
5	50	0.831
6	60	1.024
7	70	1.158
8	80	1.417

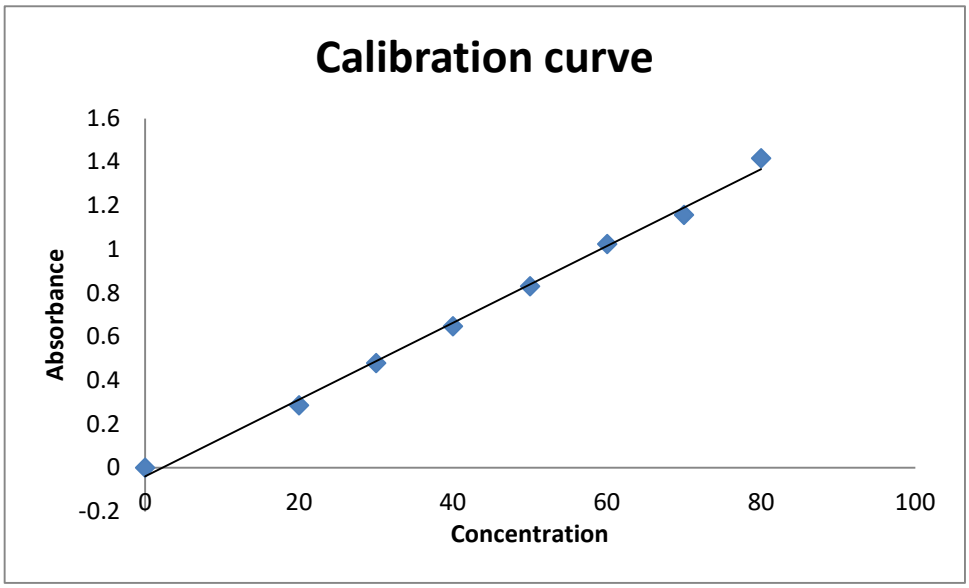

Figure 1: The linearity curve of the drug

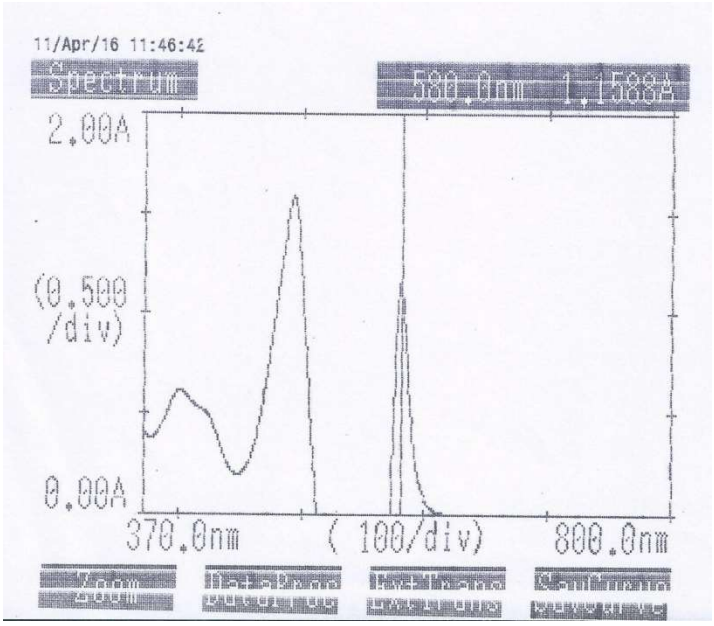

Figure 2: Visible absorption spectrum of the drug (SGP 70 µg/ml)

ASSAY OF TABLET

To make a fine powder, an exact twenty tablet weight is employed. A 100 ml volumetric flask was filled with powder weighing 10 mg of sitagliptine. The mixture was thoroughly sonicated for about 5 minutes after 40 ml of freshly made double distilled water was added. With the DDW, the volume was then brought to the necessary level, thoroughly mixed, and filtered through Whatman filter paper No 42. The filtrate's initial few millilitres were then thrown away.

Three millilitres of the filtered tablet sample solutions were added to each of four separate 10 ml volumetric flasks, and the remaining amount was added with the DDW **[11]**. The contents of the volumetric flasks were divided among four 125 ml separating funnels, each containing 1 ml of Rhodamine B solution (0.1%). 10ml of chloroform were added to each individual funnel, stirred for 5 minutes, and then left undisturbed for 5 minutes. The layers of chloroform were collected in volumetric flasks, and the absorbance at 580 nm was calculated. The concentration of the drug was calculated using the linear regression equation. **Table 2** below shows the results of the tablet analysis **[12]**.

Accuracy: It was identified by using the traditional addition strategy in a recovery research. Standard Sitagliptine was added to pre-analyzed samples in known concentrations of 80%, 100%, and 120% before they were subjected to the advised extractive spectrophotometric technique. The results of the recovery investigation are shown in **Table 3 [14]**.

Table 2: The results of Tablet analysis

Sl. No.	Formulation (µg/mL)	Label claim (mg/tab)	Found Concentration (mg/tab)	C.I.	SD	SE	t
1		50	50.57				
2	30	50	50.55	100.860±2.77	1.74	0.87	0.988
3		50	49.25				
4		50	51.35				

SD: Standard Deviation, S.E.: Standard Error C.I.: Confidence Interval, where R is the mean percent result of the Recovery study analysis (n = 4), and ts is the number of samples, within which a real value may be verified with a 95% confidence level. At a 95% confidence level, the theoretical "t" values for n-1 degrees of freedom are t (0.05, 3) = 3.182 [13]

Table 3: The 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	53.78	101.5±2.7	1.73	0.86	1.32
		30	24	55.15				
		30	24	53.89				
		30	24	55.67				
	100	30	30	61.26	100.85±1.6	1.01	0.50	1.69
		30	30	60.45				
		30	30	59.78				
		30	30	60.56				
	120	30	36	66.19	99.66±2.11	1.36	0.68	0.49
		30	36	65.86				
		30	36	64.5				
		30	36	66.57				

SD: Standard Deviation, S.E.: Standard Error C.I. : Confidence Interval, where R is the mean percent result of the Recovery study analysis (n = 4), and ts is the number of samples, within which a real value may be verified with a 95% confidence level. At a 95% confidence level, the theoretical "t" values for n-1 degrees of freedom are t (0.05, 3) = 3.182 [13]

Urine sample

A healthy individual who was around 28 years old provided us with his drug-free human urine **[15]**.

Procedure for assay in spiked urine (pure drug)

10 mL of urine, 5 mL of acetonitrile, and 10 mL of 30 g/mL Sitagliptine solutions [in buffer (pH 2.4)] were placed in a 25 mL volumetric flask. The final product was filtered through Whatman No. 42 filter paper before being placed into a 125 mL separating funnel **[16]**. After that, 2mL of Patent blue vf (% 0.1) was added to a separating funnel, and it was shaken for three minutes. The separating funnel was filled with 15 mL of chloroform, which was aggressively stirred for 5 minutes before being left alone for the same amount of time. Chloroform was used to dissolve the medicine, which was then distributed into 25 mL volumetric flasks. The highest absorbance at 633 nm was then calculated in relation to the reagent blank after the organic layer had been processed through anhydrous sodium sulphate. All of the aforementioned reagents were used to create the blank solution aside from the drug solution. The linear regression equation was used to determine the SGP concentration in urine **[17]**. The outcomes are displayed in **Table 4**.

Table 4: The results of pure drug in spiked urine

Sl. No.	Pure drug (µg/mL)	Label claim (mg/tab)	Found Concentration. (mg/tab)	C.I.	SD	SE	t
1		50	49.23				
2	100	50	50.55	100.240±3.22	2	1	0.23
3		50	49.35				
4		50	51.35				

SD: Standard Deviation, S.E.: Standard Error C.I. : Confidence Interval, where R is the mean percent result of the Recovery study analysis (n = 4), and ts is the number of samples, within which a real value may be verified with a 95% confidence level. At a 95% confidence level, the theoretical "t" values for n-1 degrees of freedom are t (0.05, 3) = 3.182 [13]

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

A 25 mL volumetric flask was filled with 10 mL of urine, 5 mL of acetonitrile, and 10 mL of the 30 g/mL tablet sample solution [in acetate buffer (pH 2.4)]. After filtering with a Whatman No. 42 filter paper, the resulting mixture was transferred into a 125 mL separating funnel. After that, 15 mL of chloroform and 4 mL of methyl orange solution (0.25%) were poured to a separating funnel and rapidly stirred for 5 minutes before being left alone for 5 minutes **[18]**. Chloroform was used to dissolve the medicine, which was then distributed into 25 mL volumetric flasks. The organic layer was

then passed over anhydrous sodium sulphate, and the maximum absorbance was then determined at 427 nm in comparison to the reagent blank. Except for the drug solution, all of the aforementioned reagents were employed to make the blank solution. The SGP concentration in urine was calculated using the linear regression equation **[19]**. The results are shown in **Table 5**.

Stability of colour complex: The stability of the colour complex was examined by measuring its maximum absorbance every 20 minutes while using a medicine concentration of 30 g/ml. It was found that the SD was within 0.5 **[20]**. Information on the stability of the compound is shown in **Table 6**.

Table 5: The results of assay in spiked urine (formulation, i.e. tablet)

Sl. No.	Formulation (µg/mL)	Label claim (mg/tab)	Found Concentration. (mg/tab)	C.I.	SD	SE	T
1		50	49.59	100.255±1.74	1.09	0.545	0.466
2	100	50	50.35				
3		50	49.78				
4		50	50.79				

SD: Standard Deviation, S.E.: Standard Error C.I. : Confidence Interval, where R is the mean percent result of the Recovery study analysis (n = 4), and ts is the number of samples, within which a real value may be verified with a 95% confidence level. At a 95% confidence level, the theoretical "t" values for n-1 degrees of freedom are t (0.05, 3) = 3.182 [13]

Table 6: Stability data of color complex

Sl. No.	Conc. (µg/ml)	Absorbance (580nm)	SD
1		0.479	0.24
2		0.688	
3		0.831	
4	30	0.731	
5		0.987	
6		1.134	
7		0.892	
8		0.417	

RESULT AND DISCUSSION

Each case's average recoveries were examined, and the outcomes were contrasted with the 100% theoretical value of the Students' test. Because the calculated "t" values are lower than the theoretical "t" values, the results of recoveries obtained are 100% for each analyte. As a result, investigations with % recovery demonstrated that the outcomes were correct. Less than 2 SD was found when utilising this method. Thus, the procedure is precise. The molar ratio of the drug and dye was found to be 1:1 using Job's method. The same molar ratios were utilised in its calculation (0.15M for the medication and dye). The highest absorbance

for the procedures is produced by this ratio. At pH values both below and above the aforementioned buffer strength, no stable colour combination is generated. The Q test equation was used to invalidate a number of conclusions. The process was validated in accordance with ICH criteria Q 2B.

CONCLUSION

The recovery outcomes of the proposed techniques nearly matched those of the previously published RP-HPLC method for this drug. The three suggested methods are therefore novel, easy to use, inexpensive, precise, accurate, resilient, reproducible, and very sensitive, and they can be utilised in quality control labs.

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