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DEVELOPMENT AND VALIDATION OF SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF SELEXIPAG IN API AND ITS BULK DOSAGE FORM

Kotwal T.S*, Patwardhan D. M., Amrutkar S. S., Wagh M. P.
Department of Quality Assurance, MVP Samaj's College of Pharmacy, Nasik, Maharashtra, India.

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ABSTRACT

The present study was undertaken to develop and validate a simple, accurate, precise, reproducible and cost effective UV-Visible spectrophotometric method for the estimation of Selexipag. The solvent used throughout the experiment was Dimethyl sulphoxide (DMSO). Absorption maximum (λmax) of the drug was found to be 306 nm. The quantitative determination of the drug was carried out at 306 nm and Beer's law was obeyed in the range of 5-25µg/mL. The approach of this work includes preliminary literature survey followed by the practical method development, applicable to be used on regular basis. The major outcomes of this method includes following: method was shown linear in the mentioned concentrations having line equation y =0.045x-0.039 with correlation coefficient R^2 of 0.9963. The recovery values of Selexipag for 80%, 100% and 120% were found to be 99.16%, 99.7% and 99.00% respectively. The percent relative standard deviation (RSD %) of interday precision was 0.490% and intraday precision was 0.28%. The limit of detection and limit of quantification was 0.477µg/mL and 1.44µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 1.78 and 0.38% respectively. It can be concluded that proposed method was precise, accurate and cost effective and it could be applicable for quantitative determination of the bulk drug as well as dosage formulation.

Corresponding author
Kotwal Tanuja Suresh
MVP Samaj's College of Pharmacy,
KTHM Campus, Gangapur Road,
Nasik, Maharashtra, India – 422002.
tanukotwal123@gmail.com

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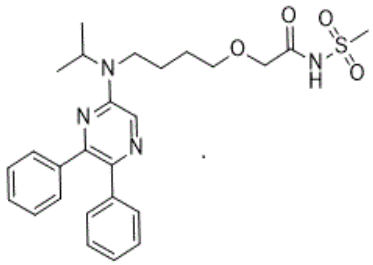
INTRODUCTION

Selexipag is pale yellow coloured crystalline substance having chemical name 2-{4-[5,6-diphenylpyrazin-2-yl)(propan-2-yl)amino]butoxy}-N-methanesulfonylacetamide. It is soluble in Dichloromethane Dimethyl sulphoxide, insoluble in aqueous solution, (insoluble at pH 2 to 4, freely soluble at pH 8 and very soluble from pH 9 to pH 12)[1,2].

Selexipag is used as antihypertensive agent[3]. Selexipag is a selective prostacyclin (IP, also called PGI2) receptor agonist. The key features of pulmonary arterial hypertension include a decrease in prostacycline and prostcycline synthase (enzyme that helps to produce prostcyclin) in the lung. Prostacyclin is a potent vasodilator with anti-proliferative, anti-inflammatory, and antithrombotic effects; Therefore, there is strong rationale for treatment with IP receptor agonist. Selexipag is chemically distinct as it is not PGI2 or a PGI2 analogue and has high selectivity for the IP receptor. It is metabolized by carboxylesterase 1 to yield an active metabolite (ACT333679) that is approximately 37 times more potent than selexipag. Both selexipag and its metabolite are selective for the IP receptor over other prostanoid receptor[1,4].

Statistical parameters like mean, mode, standard deviation were used to analyse the obtained data and draw suitable conclusions based on their results.[5,6]

The main problem being tackled is that there is no such reported simple UV spectrophotometric method yet for the analysis of Selexipag. So the development of such method justifies the research and its need. The purpose of this investigation was to develop and validate a simple, rapid, sensitive, precise, accurate and spectrophotometric method for the estimation of Selexipag in bulk and dosage form.



Selexipag.

The objectives of this work includes:

1. To develop rapid, sensitive and precise UV spectrophotometric method for estimation of Selexipag.
2. To validate this method as per ICH Q2 R1 guidelines.
3. To be able to estimate the drug in API as well as in its Bulk dosage form via recovery studies.

MATERIALS AND METHODS

API:

Selexipag was kindly procured as gift sample from Megafine pharmaceuticals, Nashik. Dimethyl sulphoxide was purchased from Modern chemical laboratory, Nashik, Maharashtra, India.

Instruments:

For Weighing, a calibrated weighing balance (Make- Shimadzu) of 1mg sensitivity was used. For analytical purpose Shimadzu- 1800 UV Spectrophotometer and Shimadzu 2501 PC UV-Vis Recording Spectrophotometry was used.
All other glasswares and apparatus were made of Borosilicate and were calibrated.

Experimental Work:

Method Development:

Preparation of API standard Stock solution:

The standard stock solution of 1000 µg/mL of Selexipag was prepared by weighing 100 mg of the drug, taken in 100 mL volumetric flask and diluted with Dimethyl sulphoxide.

By appropriate dilution of standard stock solutions with Dimethyl sulphoxide, different solutions containing different concentration (5, 10, 15, 20, & 25µg/mL) of Selexipag were prepared.

Preparation of tablet stock solution:

Ten tablets were triturated in a mortar and weighed to take 10 mg equivalent of API. This was dissolved in 100 ml of Dimethyl Sulfoxide solution to get 100ppm solution. This solution was sonicated for 5 minutes and then filtered. The filtered solution was then accurately diluted further as needed.

Determination of wavelength of maximum absorption: [7,8]

The API stock solution further diluted was scanned in the range of 200-400 nm to determine the wavelength of maximum absorbance. Selexipag has shown maximum absorption at 306 nm.

Validation:[9,10]

The proposed method was validated according to ICH Q2 (R1) guidelines for validation of analytical procedures. As per the ICH guidelines the method was validated for Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification Robustness and Ruggedness.

Linearity:

Different volumes of stock solutions were suitably diluted with corresponding medium (5, 10, 15, 20 and 25 µg/ml) to get the desired concentrations. Each solution was analysed in triplicate. The absorbance values were plotted against the corresponding concentrations to obtain the linear calibration curve with equation:

$$y = mx + c$$

Along with that regression is calculated, to establish linearity.

The dilutions for linearity were prepared as follows:

Table 1: Linearity.

Sr. no	Concentration in ppm	Absorbance
1	5	0.181
2	10	0.401
3	15	0.653
4	20	0.892
5	25	1.064

Precision

It is the measure of repeatability of the results. For precision studies dilutions of 10ppm were prepared in six replicates and absorbance of each was checked. It was performed for intra as well as interday precision. Standard deviation and relative standard deviation was calculated for all these results. The precision should be within limit of 2% RSD (Relative Standard Deviation).[11,12] Concentration of 10 ppm was selected for the precision studies. Dilutions were prepared as follows:

Table 2: Precision.

Sr. no	Concentration in ppm	Absorbance
1	10	0.403
2	10	0.407
3	10	0.408
4	10	0.408
5	10	0.402
6	10	0.403

Intraday precision:

The same dilutions were used on same day again to note the absorbance and calculate the relative standard deviation.

Table 3: Intraday Precision.

Sr. no	Concentration in ppm	Absorbance
1	10	0.401
2	10	0.399
3	10	0.402
4	10	0.405
5	10	0.396
6	10	0.401

Interday precision:
Dilutions of the precision taken above were kept overnight and analysed on the next day for standard deviation.

Table 4: Interday Precision.

Sr. no	Concentration in ppm	Absorbance
1	10	0.409
2	10	0.408
3	10	0.405
4	10	0.411
5	10	0.407
6	10	0.411

Accuracy:
The accuracy studies were performed by using 80%, 100% and 120% of the solution 0f 10 ppm. Thus 8, 10 and 12 ppm concentration were used in triplicates for accuracy.

Table 5: Accuracy.

Sr. No	% concentration	Concentration in ppm	Volume of API Stock in ml	Volume of Tablet stock in ml	Absorbance
1	80	8	0.5	0.3	0.317 0.319 0.319
2	100	10	0.5	0.5	0.412 0.404 0.414
3	120	12	0.5	0.7	0.498 0.496 0.496

Limit of Detection and Limit of Quantification:
The Limit of Detection (LOD) is the smallest concentration of the analytes that gives the measurable response. LOD was calculated using the following formula

$$\mathbf{LOD = 3.3\ \sigma / S}$$

The Limit of Quantification (LOQ) is the smallest concentration of the analytes, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$\mathbf{LOQ = 10\ \sigma / S}$$

Where, σ is standard deviation of the response and S is the slope of the calibration curve.
The slope used in this equation is from the linearity curve and the standard deviation is obtained from precision.

Robustness
In robustness the capacity of method to remain unaffected by the small deliberate changes is calculated. The study includes change of wavelength of maximum absorbance and analysing the absorbance. The results are interpreted with respect to relative standard deviation.

Table 6: Robustness.

Sr. no	Wavelength in nm	Absorbance
1	301	0.320
2	302	0.326
3	303	0.330
4	304	0.334
5	305	0.336
6	306	0.340
7	307	0.340
8	308	0.338
9	309	0.335
10	310	0.333
11	311	0.329

Ruggedness

The ruggedness study includes change of analyst and change of UV Spectrophotometer for determining the capacity of method to remain unaffected by any external environment changes.

For both the cases, the precision was performed by a different analyst on a different UV Spectrophotometer.

Table 7: Ruggedness.

Sr. no	Concentration in ppm	Absorbance
1	10	0.401
2	10	0.398
3	10	0.399
4	10	0.402
5	10	0.400
6	10	0.402

RESULT AND DISCUSSION

Determination of wavelength of maximum absorption:

The wavelength of maximum absorption was found to be 306 nm.

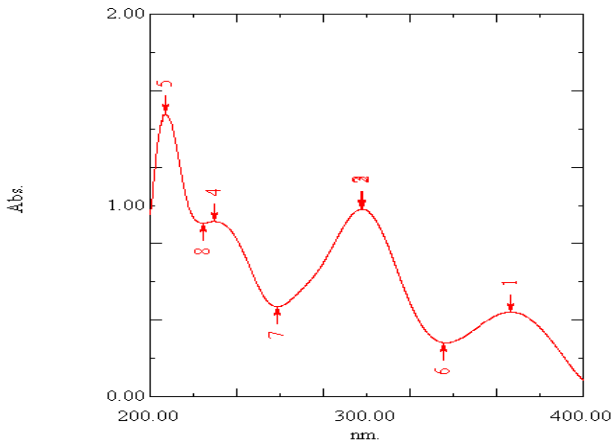
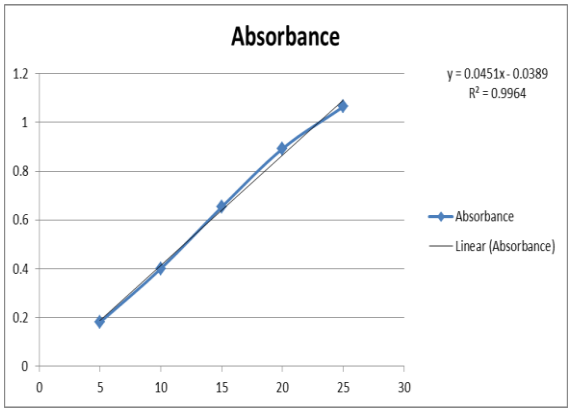


Fig 1: Ultra Violet Spectrum of Selexipag.

Linearity:

Table 8: Linearity.

Sr. no	Concentration in ppm	Absorbance
1	5	0.181
2	10	0.401
3	15	0.653
4	20	0.892
5	25	1.064



The method for Selexipag was found to be linear in the range of 5-25 ppm with $R^2 = 0.9964$ and the straight line equation as:

y = 0.0451x – 0.0389

Precision

The study was performed and relative standard deviation was calculated.

Table 9:% RSD of results of Precision.

Sr. no	Concentration in ppm	Absorbance (y)	(y-ȳ)	$(y-\bar{y})^2$	S.D	%RSD
1	10	0.403	0.002	0.000004		
2	10	0.407	0.002	0.000004		
3	10	0.408	0.003	0.000009		
4	10	0.408	0.003	0.000009	0.0025	0.61
5	10	0.402	0.003	0.000009		
6	10	0.403	0.002	0.000004		
		ȳ= 0.405		Σ= 0.000039		

Intraday Precision:

Table 10: % RSD of results of Intraday Precision.

Sr. no	Concentration in ppm	Absorbance (y)	(y-ȳ)	$(y-\bar{y})^2$	S.D	%RSD
1	10	0.401	0.002	0.000004		
2	10	0.399	0.002	0.000004		
3	10	0.402	0.003	0.000009		
4	10	0.405	0.003	0.000009	0.0025	0.61
5	10	0.396	0.003	0.000009		
6	10	0.401	0.002	0.000004		
				Σ= 0.000039		

Interday Precision:

Table 11: % RSD of results of Interday Precision.

Sr. no	Concentration in ppm	Absorbance (y)	(y-ȳ)	$(y-\bar{y})^2$	S.D	%RSD
1	10	0.409	0.001	0.000001		
2	10	0.408	0.000	0.000000		
3	10	0.405	0.003	0.000009		
4	10	0.411	0.003	0.000009	0.0021	0.49
5	10	0.407	0.001	0.000001		
6	10	0.411	0.003	0.000009		
		ȳ=0.408		Σ= 0.000029		

Accuracy:

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.0451x - 0.0389

% Recovery=

$$\frac{x}{8} * 100$$

Table 12: % Recovery (1).

Sr. no	% concentration	Concentration in ppm	Absorbance	% Recovery
1	80	8	0.317 0.319 0.319 Ȳ=0.318	99.12

Table 13: % Recovery (2).

Sr. no	% concentration	Concentration in ppm	Absorbance	% Recovery
2	100	10	0.412 0.404 0.414 Ȳ=0.410	99.12

Table 14: % Recovery (3).

Sr. no	% concentration	Concentration in ppm	Absorbance	% Recovery
3	120	12	0.498 0.496 0.496 Ȳ=0.496	

Limit of Detection and Limit of Quantification:

It is calculated by using slope and standard deviation from linearity and precision respectively:

Limit of detection (LOD):

$$LOD = 3.3 \times SD/Slope$$

$$LOD = 0.182\ ppm$$

Limit of quantification (LOQ):

$$LOQ = 10 \times SD/Slope$$

$$LOQ = 0.554\ ppm$$

Robustness:

Table 15: % RSD of results of Robustness.

Sr. no	Wavelength in nm	Absorbance (y)	(y-ȳ)	$(y-\bar{y})^2$	S.D	%RSD
1	301	0.320	0.012	0.000144		
2	302	0.326	0.006	0.000036		
3	303	0.330	0.002	0.000004		
4	304	0.334	0.002	0.000004		
5	305	0.336	0.004	0.000016		
6	306	0.340	0.008	0.000064	0.0059	1.78
7	307	0.340	0.008	0.000064		
8	308	0.338	0.006	0.000036		
9	309	0.335	0.003	0.000009		
10	310	0.333	0.001	0.000001		
11	311	0.329	0.003	0.000009		
		ȳ= 0.332		Σ= 0.000387		

The % relative standard deviation for robustness was found to be 1.78%.

Ruggedness:

Table 16: % RSD of results of Ruggedness.

Sr. no	Concentration in ppm	Absorbance (y)	(y-ȳ)	$(y-\bar{y})^2$	S.D	%RSD
1	10	0.401	0.001	0.000001		
2	10	0.398	0.002	0.000004		
3	10	0.399	0.001	0.000001		
4	10	0.402	0.002	0.000004	0.0015	0.38
5	10	0.400	0.000	0.000000		
6	10	0.402	0.002	0.000004		
		ȳ=0.400		Σ= 0.000014		

CONCLUSION

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Selexipag in in API and its bulk dosage form without any interference from the excipients. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations. Hence it can be effectively applied for the routine analysis of Selexipag in bulk drug. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy. It can be concluded that proposed method was precise, accurate and cost effective and it could be applicable for quantitative determination of the bulk drug as well as dosage formulation.

Abbreviations

UV : Ultra Violet
µm : micrometer
nm : nanometer
ml : millilitre
UV- Vis : UltraViolet-Visible
API : Active Pharmaceutical Ingredient
% : Percentage
Ppm : Parts per mole
API : Active Pharmaceutical Ingredient

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Conflict of Interest:

The authors do not report any conflict of interest.

REFERENCES

1. www.ema.europa.eu/../WC500207175.pdf (02/09/2016,8:16PM)
2. https://www.drugs.com>uptravi
3. www.drugbank.ca/drugs/DB11365 (19/08/2016, 3:40PM)
4. Tripathi K. D., Essentials of Medical Pharmacology, 6th edition, Jaypee Brothers Medical Publishers Pvt. Ltd., 2008 pp 800-802
5. Best W.J., Kahn J.V., Research in Education, 10th edition, Eastern Economy Edition, PHI Learning PVT. LTD. New Delhi 2009, pp.359-365
6. Kothari C.R., Research Methodology, 2 revised edition, New Age International Publishers, 2004, pp.132-135
7. Skoog D.A., West D.M., Principles of Instrumental Analysis, Saunders golden sunburst series, 2nd edition.1980. pp. 176-189
8. Kemp W., Organic Spectroscopy. Palgrave publication, 3 rd edition, 1999. pp. 251-153.
9. International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures, March 1995.
10. International Conference on Harmonization (ICH),Q2 R1, Validation of analytical procedures: text and methodology, 1994, http://www.ich.org
11. Chung C.C., Lee Y.C., Lam H., Zhang X.M., Analytical Method Validation and Instrument Performance Verification, John Wiley and sons.2004, pp.16-21
12. Quality Assurance of Pharmaceuticals, 2002, Pharma Book Syndicate. vol-1pp. 119-123.