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Method Development and Validation of Ivacaftor in Bulk and Pharmaceutical dosage form by UV Spectrophotometry

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ABSTRACT

The easiest, precise, accurate means of analysis of the drug is by UV Spectrophotometry. In this regard, Ivacaftor drug is analyzed by method development and validation for UV spectrophotometric study. The linearity studies obeyed Beer and Lambert's law where the linear regression coefficient value was found to be 0.9973 at λmax 202nm. The linearity range considered for the present study being 1-5 µg/ml. The test validation parameters and the recovery value satisfy the acceptance criteria by the method developed. Therefore the present method developed can be used for routine analysis of Bulk product and formulated dosage forms of Ivacaftor.

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INTRODUCTION

Ivacaftor is an aromatic compound which belongs to aromatic anilides. It contains an anilide group where an aromatic group substitutes the carboxamide group. The general structure of Ivacaftor is RNC (=O) R', where R= benzene, and R = aryl group. IUPAC name of Ivacaftor is N-(2,4ditert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxamide. Its molecular formula is $C_{24}H_{28}N_2O_3$ and molecular weight 392.499 g/mol (Pub Chem CID 16220172). Ivacaftor, a CFTR potentiator, is a gene-based therapy approved for treating cystic fibrosis. It is also known as Kalydeco. Food and Drug Administration (FDA) has approved Ivacaftor for treating the patients of age 6 years or above, with minimum one G551D mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Ivacaftor alters the activity of the CFTR channel. G551D mutation is a class III mutation, which impairs the ability of the opening of ion channel involved in the transportation of chloride and sodium ions across the cell membranes of various organs such as lungs and pancreas. Hence, even though the CFTR protein reaches the cell surface, it can enter the channel leading to multi-organ dysfunction. At the cell surface, Ivacaftor increases the ions flow via activated CFTR thus increases the chance of opening of the channel. Ivacaftor and its metabolite M1 show significant inhibition of f CYP3A and P-glycoprotein and lead to the increase in its therapeutic values and the likelihood of adverse events. When is administered with strong CYP3A inhibitors, its exposure is approximately nine-fold increased and its exposure is decreased nine fold when administered with CYP3A inducers. When given with moderate CYP3A inhibitors, Ivacaftor exposure is increased three-fold. CYP3A enzyme metabolizes Ivacaftor in liver and M1 and M6, two

major metabolites are produced (Michelle *et al*.,

2013). One-sixth of the parent drug potency in retained in M1, thus making it pharmacologically active, whereas M6 which retains only one-fiftieth of the parent drug potency is pharmacologically inactive. Its half-life is approximately 12 hours Ramsey *et al*., 2011). Development of HPLC and LC-MS/MS methods for the analysis of ivacaftor, its major metabolites and lumacaftor in plasma and sputum of cystic fibrosis patients treated with Orkambi or Kalydeco was carried out for the quantitative determination of the drug effectively (Schneider *et al*., 2016). Therefore the present study emphasises on the method development and validation of Ivacaftor by UV- Spectroscopy

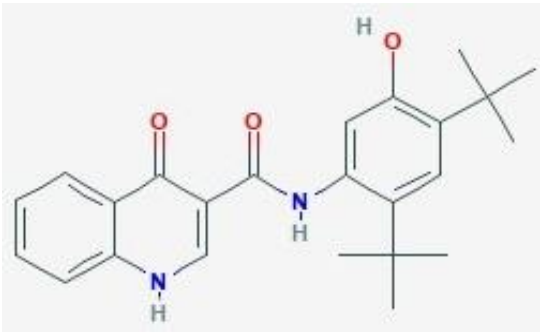

Figure 1: Structure of Ivacaftor

Materials

Ivacaftor was gifted. The commercial tablets of these drugs are available in the Indian market. Other chemicals used were analytical grade and HPLC grade.

Instruments

Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. Analytical balance used for weighing standard and sample was SHIMADZU AUX 220 Uni Bloc PAT 1987.

Preparation of standard stock solution

Ivacaftor has weighed 10mg accurately. This was transferred into the 10ml volumetric flask. The volume was made up to the mark with the diluent ethanol. This solution was ultrasonicated for 8mins. The solution is considered as stock solution whose concentration is 1000µg/ml. From this working solution is prepared by pipetting 1ml to 10ml volumetric flask and volume was made up to the mark with ethanol. The concentration of this working solution is 100µg/ml (ICH 2005).

Selection of λmax

From working solution different concentration of solution for linearity range was prepared. The solution was considered for determining the **λmax.** This solution was scanned in the UV range of 200nm-400nm in an electromagnetic spectrum. Ethanol is taken as blank reading.

Standard Linear Curve of Ivacaftor

The calibration curve for linearity for Ivacaftor was prepared by using aliquots of the standard working solution. The standard stock solution was prepared by weighing 10mg of the drug dissolved in 10ml (1000µg/ml) volumetric flask with the diluent ethanol. The working solution is prepared by the above solution by transferring 1ml of the aliquot 10ml standard volumetric flask (100 µg/ml) and volume was made up to 10ml with ethanol. Now, the linearity range was fixed which should obey Beer-Lamberts law. The linearity range fixed for Ivacaftor is from 1–5 µg/ml is prepared by diluting 0.1ml,0.2ml,0.3ml,0.4ml and 0.5ml from the standard working solution to respective 10ml volumetric flask and the volume was made up to the mark by ethanol. This read in triplicates to get statistical data at λmax of Ivacaftor using ethanol as blank. The calibration graph is plotted taking absorbance on y-axis and concentration on the x-axis. LOD and LOQ are calculated from the linear curve of the respective standards based on their linear regression coefficient values.

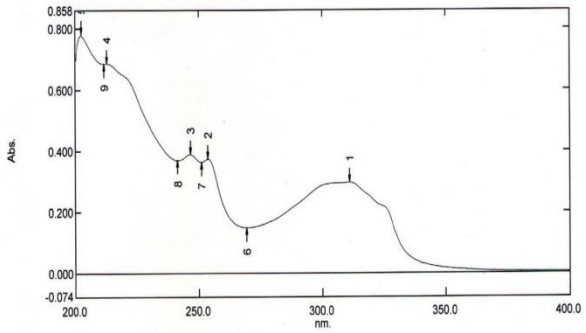

Figure 2: UV spectrum of Ivacaftor in the standard solution

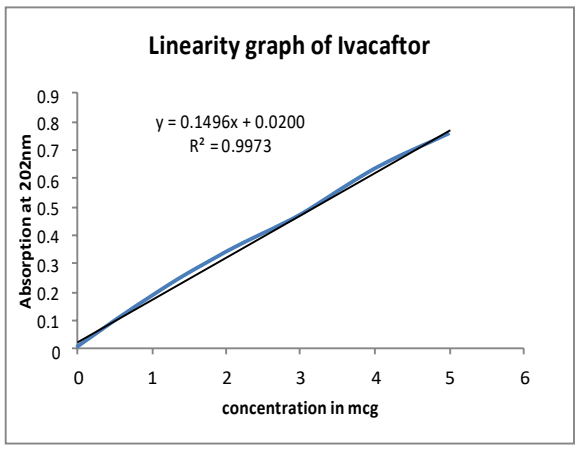

Figure 3: Linearity curve of Ivacaftor

Accuracy

2.4µg/ml, 3µg/ml and 3.6µg/ml were spiked at an accuracy level at 80%, 100% and 120%

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Table 1: Study of linearity

Trial I	Absorption at 202 nm	Trial II	Absorption at 202 nm	Trial III	Absorption at 202 nm
1	0.181	1	0.182	1	0.181
2	0.334	2	0.333	2	0.338
3	0.465	3	0.467	3	0.469
4	0.625	4	0.628	4	0.628
5	0.755	5	0.752	5	0.753

respectively. Recovery concentration and percentage of recovery was calculated at 80%, 100% and 120% level and was statistically determined.

Table 2: Average Absorption values

Concentration in mcg	Average Absorption at 202nm
1	0.181
2	0.335
3	0.467
4	0.627
5	0.753

Table 3: Limit of detection and Limit of quantification of Ivacaftor

Sl. no.	Slope	Intercept
1	0.1497	0.0192
2	0.1495	0.02
3	0.1496	0.0208
Mean	0.1496	0.02
STDEV	0.0001	0.0008
LOD	0.016042781	
LOQ	0.160427807	

Table 4: Intraday Precision study of Ivacaftor

Sl. no.	Peak absorption at 202nm
1	0.489
2	0.486
3	0.494
4	0.494
5	0.498
6	0.481
Mean	0.490
StDev	0.006
%RSD	1.268

Precision

In this method the drug of same concentration that is 3µg/ml of Ivacaftor was analyzed at λ max that is at 202nm repeatedly for 6 times for intraday precision. And in the three consecutive days for interday precision. At every stage absorbance is recorded at 202nm and is statically reported.

Table 5: Interday Precision study of Ivacaftor

Sl.No	Day-1	Day-2	Day-3
1	0.475	0.452	0.468
2	0.47	0.463	0.472
3	0.474	0.469	0.462
4	0.473	0.468	0.465
5	0.481	0.468	0.463
6	0.479	0.456	0.459
Mean	0.475	0.462	0.464
StDev	0.004	0.007	0.004
%RSD	0.848	1.544	0.994

Robustness

The changes were done in the wavelength by ±2nm. And for every change the absorbance were recorded in triplicates. The changes were done in the concentration at ±0.5µg/ml. And for every change the absorbance were recorded in triplicates.

Assay Value

Amount of drug content present in tablet formulation is calculated. The tablet form of these drugs was procured from the market.

RESULTS AND DISCUSSION

The method developed and validated of Ivacaftor by UV spectroscopy is accurate and falls under the accepted criteria. The validation parameters were carried out by following ICH guidelines. The method obeyed Beer's law. The method proved to be effective and efficient with a high degree of reproducibility. The method showed 100% recovery studies. This method can be effectively used to validate the drug and its pharmaceutical dosage forms in the regular quality control process.

CONCLUSION

The developed new method for UV spectroscopic studies of Ivacaftor is fast, easy, highly reproducible, efficient, accurate and inexpensive study. The present method was validated according to the ICH guidelines. As the results were within the acceptance criteria, it is found to be satisfactory and hence can be used in the routine analysis of Ivacaftor in the bulk form and the dosage forms.

Table 6: Study of Robustness

Sl.No	Robustness parameter	Mean	Standard deviation	% RSD
1	Concentration			
	2.5µg/ml	0.435	0.006	1.507
	3µg/ml	0.432	0.002	0.582
	3.5µg/ml	0.440	0.007	1.763
2	Wavelength			
	200nm	0.405	0.004	0.99
	202nm	0.447	0.001	0.341
	204nm	0.427	0.001	0.357

Table 7: Study of Accuracy

Level of recovery	Spiked in mcg/ml	Theoretical absorbance	Practical absorbance	% of release	Found in mcg/m
80	2.4	0.559	0.571	101.978	3.671
100	3	0.467	0.466	99.914	2.997
120	3.6	0.373	0.374	100.281	2.406

Table 8: Assay value

Tablet	Label claim	Assay value of tablet	% Recovery
Ivacaftor	150mg	149.67mg	99.78%

Table 9: Study of Ruggedness

Analyst 1		Analyst 2	
Sl. no.	Peak absorption at 202nm	Sl. no.	Peak absorption at 202nm
1	0.433	1	0.430
2	0.44	2	0.432
3	0.441	3	0.436
Mean	0.438	Mean	0.432
StdDev	0.004	StDev	0.003
%RSD	0.995	%RSD	0.706

Table 10: Summary of Method Development and Validation of Ivacaftor

Sl. No	Parameters	Results	
1	Beer's law limitµg/ml	1-5 µg/ml	
2	Absorption maxima in nm	202nm	
3	Standard regression equation	y = 0.1496x + 0.0200	
4	Correlation coefficient (r^2)	0.997	
5	Accuracy	99%-103%	
6	Precision –Intraday % RSD	1.26%	
7	LOD	0.016	
8	LOQ	0.16	
9	Robustness % RSD	Wavelength 0.99,0.34,0.35	Wavelength 0.99,0.34,0.35
10	Ruggedness % RSD	0.995 and 0.706	
11	Assay	99.78%	

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