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Development and Validation of UV-Spectrophotometric and HPLC Method determination of Dofetilide in Formulation

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Abstract : A new, simple, specific and economic UV Spectrophotometric method and HPLC method for the estimation of Dofetilide content in bulk and laboratory prepared mixture. UV spectrophotometric detection was carried out at absorption maxima (λ_{max}) at 231nm using methanol as a solvent. The quantitation of drug was carried out using A1% 1cm at 231nm and Beer's law was obeyed in the concentration range of 2.5-20 µg/ml, with correlation coefficient value less than 1.The chromatographic separation was carried on a C-18 (250 mm × 4.6 mm, 5µ) column using an isocratic mode with a mixture of Acetonitrile:Phosphate Buffer (pH-7) in the ratio of 55:45% v/v as a mobile phase. The flow rate was 1.5ml/min, temperature is maintained at ambient and detection was made at 231 nm using Photodiode array (PDA) detector. The developed method was validated according to ICH guidelines and different analytical parameters such as linearity, precision, accuracy, specificity, limit of detection, limit of quantitation were determined. The percent amount of drug estimated was nearly 100%, found to be a good agreement with label claim of prepared laboratory mixture. The proposed method was validated for its accuracy, precision, robustness, ruggedness, linearity, limit of detection, limit of quantitation and was found to be in range (% RSD<2.0 and SD <±2.0). Both methods were validated and found to be simple, sensitive, accurate, and precise. The results of the study and statistical data proved the applicability of the present method in routine analysis of Dofetilide in bulk as well as laboratory prepared mixture.
Keywords : Dofetilide, UV Spectroscopy, HPLC, ICH guidelines.

Introduction:

Dofetilide is a sulfonamide class III antiarrhythmic agent and potassium channel blocker and chemically it is a tertiary amino compound that is N-ethyl-N-methylethanamine substituted by a 4-[(methylsulfonyl)amino]phenoxy and a 4-[(methylsulfonyl)amino]phenyl group at the terminal carbon atoms respectively. It has IUPAC name as N[4[2[2[4(methanesulfonamido)phenoxy]ethylmethylamino]ethyl]phenyl] methanesulfonamide having molecular formula $C_{19}H_{27}N_3O_5S_2$ with molecular weight 441.61 g /mol. The

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chemical structure shown in figure No.1. Dofetilide is a white to off-white powder which is Soluble in methanol, acetonitrile, ethanol and slightly soluble in water [1].It acts by selectively blocking the rapid component of the delayed rectifier outward potassium current (I_{Kr}) result in increase of refractory period of atrial tissue, hence its effectiveness in the treatment of atrial fibrillation and atrial flutter [2].

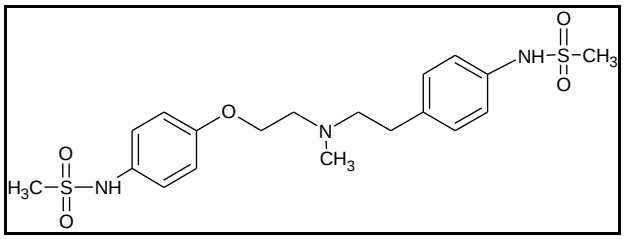

Figure No. 1: Structure of Dofetilide

Literature survey reveals that the drug Dofetilide has been estimated from two analytical methods reported with combination of Dofetilide by using HPLC [3], HPLC method for the analysis of Dofetilide and it's Degradation product [4]and one analytical method developed by using UV and HPTLC[5], but there is no reported method available for the analysis of individual Dofetilide by UV spectrophotometry and HPLC method for the estimation of Dofetilide content in bulk and laboratory prepared mixture. Hence it was thought worthwhile to develop by UV Spectroscopy and HPLC method and validation of developed method for linearity, range, interday and intraday precision, Limit of Quantitation and Limit of Detection according to ICH guidelines. The simplicity of the developed method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS/MS or GC-MS/MS that are complicated, costly, and time consuming rather than a simple UV and HPLC method.[6,7].

Experimental:

Chemicals and Reagents:

Methanol and Acetonitrile (HPLC grade), O-phosphoric acid, Triethylamine, Anhydrous disodium hydrogen phosphate and Potassium hydrogen phosphate all used are of analytical grade. Double Distilled water was used throughout the experiment.

Instruments:

UV-Spectrophotometer (Model JascoV-630 and Shimadzu-1700) double beam with 1 cm quartz cell, HPLC use is Shimadzu HPLC series 1100 and Jasco HPLC PU-2089 Plus, Sonicator are used is PCi Mumbai, Model No.3.5L 100H and the Weighing balance use is Shimadzu AUX220 and analytical balance.

Preparation of Standard Solutions:

❖ **Sample preparation for development of UV-spectrophotometric method for determination of drug:**

Preparation of Standard solution

The stock solution was prepared by dissolving 10.0 mg of Dofetilide in10.0mL volumetric flask with 10.0 mL of methanol to get a concentration of 1 mg/mL(Conc.1000µg/ml of DOFE). From the above stock solution containing 1.0 mL was pipette and diluted to 10.0 mL in a volumetric flask upto the mark with methanol (concentration of 100 µg/mL). Again 2.0 mL of working standard solution was pipetted in a 10.0 mL volumetric flask and volume up to the mark with acetonitrile (concentration 20µg/mL).

Preparation of laboratory mixture

Formulation containing dofetilide was not available in market, therefore the laboratory mixture of dofetilide was prepared.

The formula for laboratory mixture of dofetilide is as follow in Table no. 1

Table no. 1 Formula for preparation of laboratory mixture of Dofetilide

Sr. No.	Ingredients	Quantity (mg)
1.	Dofetilide	0.5
2.	Lactose monohydrate	113.5
3.	Magnessium stearate	3.0
4.	Talc	3.0

❖ **Sample preparation for development of HPLC method for estimation of dofetilide (DOFE)**

Preparation of standard and buffer solutions

- **Standard stock solution (A1)**
  An accurately weighed about 10.0mg DOFE was transferred in a 10.0ml volumetric flask, dissolved in sufficient quantity and volume was made up to the mark with mobile phase. (Conc.1000µg/ml)
- **Working stock solution (A2)**
  A 1.0mL of stock solution (A1) was transferred in 10.0mL volumetric flask and volume was made up to the mark with mobile phase. (Conc. 100µg/mL)
- **Working standard solution (A3)**
  The working stock solution (A2) was appropriately diluted with mobile phase to get the final concentration of 10µg/ml.
- **Phosphate butter (pH-7):**
  Place 100.0ml of 0.2 M potassium dihydrogen phosphate and 0.6 M sodium hydrogen phosphate in 200.0mlvolumetric flask, then was added and make up volume with water, sonicated and filtered through 0.45µ membrane filter paper.

Preparation of standard stock solution

An accurately weighed quantity of DOFE is equivalent to Dofetilide (~10.0mg) was transferred in a 10.0mL volumetric flask, dissolved in sufficient quantity of diluent to get concentration of 1000µg/mL.From the above stock solution containing 1.0 mL was pipette and diluted to 10.0 mL in a volumetric flask upto the mark with methanol (concentration of 100 µg/mL). Further standard solution of 10µg/mL was prepared by appropriate dilution of the stock solution with mobile phase.

Preparation of mobile phase

The mobile phase was prepared by mixing acetonitrile and Phosphate buffer (pH-7.0) in ratio 50:45%v/v.

UV method Calibration curve:

Appropriate dilutions of standard stock solution (A2) were made to get final concentration in the range of 2-20µg/mL and absorbances were measured of each prepared solution at selected wavelength.

HPLC method calibration curve:

Aliquots of working standard solution (A3) were diluted in range of 1-5 mL in 10.0mL Volumetric flask with mobile phase and volume was made up to mark mobile phase to obtain concentration ranging from 1-5µg/mL of Dofetilide.

UV method Assay sample preparation:

An accurately weighed powdered mixture of dofetilide and quantity of powder equivalent to about 1.0mg of dofetilide was transferred to 10.0ml volumetric flask, dissolve in sufficient quantity of methanol, shaken properly and volume was made up to the mark with methanol and filtered through whatmann filter paper. From the above filtrate 2ml solution was pipette out and volume was made up to the 10.0ml with methanol to get concentration 20µg/ml. Above solution was further appropriately diluted with methanol to get the final concentration.

HPLC method Assay sample preparation:

An accurately weighed quantity of powdered mixture of Dofetilide equivalent to about 0.5 mg ofdofetilide were transferred to 10.0mL volumetric flask, sonicated for 15min sufficient quantity of mobile phase and volume was made up to mark with mobile phase. The content of the flask was filtered through 0.45µm nylon filter. A 1.0mL with portion of the filtered was further diluted to 25.0mL with mobile phase. Similarly, five sample solutions were prepared. After equilibration of stationary phase, such six sample solutions were injected separately and chromatograms were recorded.

Validation of Proposed Method

Linearity and Range:

For UV method, accurately measured quantity of Dofetilide equivalent to 80, 100, 120, and 140% label claim were taken and dilution were made as described under laboratory mixture. The absorbances of each resulting solution was measured at selected wavelengths in 1.0 cm cell using solvent blank. Whereas for HPLC method, An accurately weighed capsule powder equivalent to about 80-150% of label claim was taken and dilutions were made and each solution was injected and chromatograms were recorded.

Accuracy:

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. Accuracy of the method was determined by measuring the analyte in the presence of placebo(laboratory formulation).Standard drug substance of known amounts at the 50%, 75% and 100% and 120% levels. The % RSD value was found to be less than 2 , indicating that the methods are accurate.

Precision:

Precision of an analytical method was expressed as SD and %RSD of series of measurements. The carried out repeatability study (n = 5). The precision of the method was expressed in terms of % RSD. The obtained results showed reproducibility of the assay. The % RSD values were found within limit indicates that the methods were found to be precise. For the intermediate precision, a study carried out by the same analyst working on 3 consecutive days (n = 3). Both values were far below 5%, the limit percentage set forthe precision, and indicated a good method precision

Interday and Intraday variation

An accurately measure quantity of lab prepared mixture equivalent to about 1.0mg was transferred to 10.0mL volumetric flask, sufficient quantity of methanol added and sonicated for 15 min. and diluted up to the mark with methanol. The content in each flask was filtered through whattman filter paper. The filtrate was further diluted to with methanol to get final concentration of about 20µg/mL of DOFE. For UV method, the absorbance of the final resulting solution was recorded at 0^{th}hr, 1^{st}hr, 2^{nd}hr, and 3^{rd}hr. Similarly, the absorbance of the same solution was measured on 1^{st} to 3^{rd} day and the percentage label claim was calculated using formulae as described under marketed formulation.

Simillarly, for HPLC method the 20µg/mL of DOFE injected at interval of 0, 2, and 5h and the chromatograms were recorded. The same sample solution was injected on 1^{st}, 2^{nd}, and 3^{rd} day. The chromatograms so recorded and result were calculated. The content of DOFE was calculated by comparing the peak area of sample with that of standard using formula given under laboratory mixture formulation.

Ruggedness:

Ruggedness of proposed methods was performed to examine effect of non procedure related factors such as instruments and analysts. For this study Dofetilide was analyzed by proposed methods using two different analyst restraining similar operational and environmental conditions.

Robustness:

It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The analysis was performed by slightly changing the pH, mobile phase composition, and detection wavelength and flow rate. The mobile phase flow rate was changed to 0.8mL/min & 1.5mL/min (Actual flow rate: 1.0mL/min). The Wavelength was changed to 226nm & 236nm (Actual Wave length: 231nm). The effect change in buffer such as 0.1% TEA: ACN (65:35) and phosphate buffer:ACN (55:45) also evaluated.

Results and Discussion:

Selection of λ_{max} for DOFE:

The working standard solution (A_3) of DOFE (10µg/ml) was prepared and scanned in the range of 200-400nm in 1.0cm matched quartz cell against solvent blank (methanol) and spectrum was recorded. The spectrum so recorded as shown in this Figure No.2

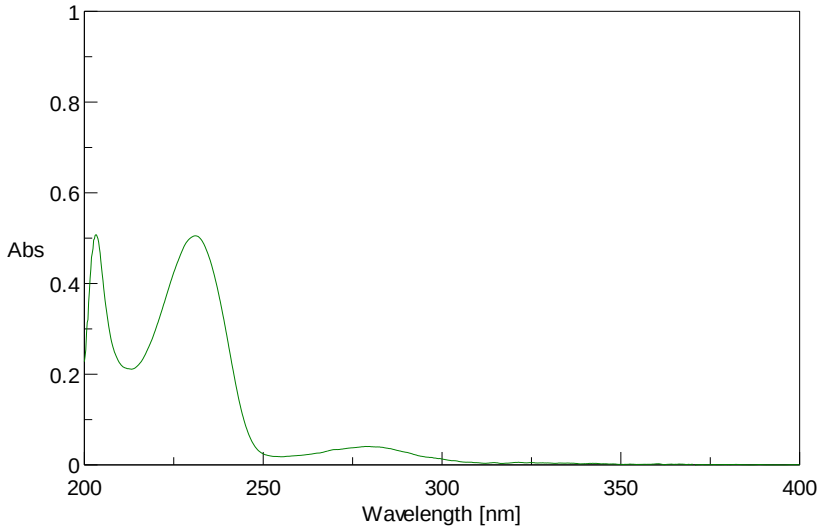

Figure N.2: spectrum of DOFE in methanol.

Preliminary optimization of mobile phase and other chromatographic conditions:

In order to achieve the optimized chromatographic condition, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. Various mobile phases were tried by permutation and combination and also by change in flow rate, buffer and its pH. On the basis of trial study, following optimized chromatographic parameters selected for further study.

Table No.2: Optimized chromatographic parameters

System	Shimadzu HPLC series 1100
Stationary Phase	Phenomenex C-08-04 (5µm), 150x4.60mm
Mobile phase	ACN : phosphate buffer (55:45 % v/v)
Detection wavelength	231
Flow rate	1.5mL/min
pH	7
Temperature	Ambient
Injection volume	20µL
Diluent	Mobile Phase

A 20µL of solution was injected through manual injector and chromatogram was recorded. A mobile Phase containing ACN: Phosphate buffer, pH 7 (55:45 % v/v) gave well-resolved peak and reasonable retention time as shown in Figure No.3.

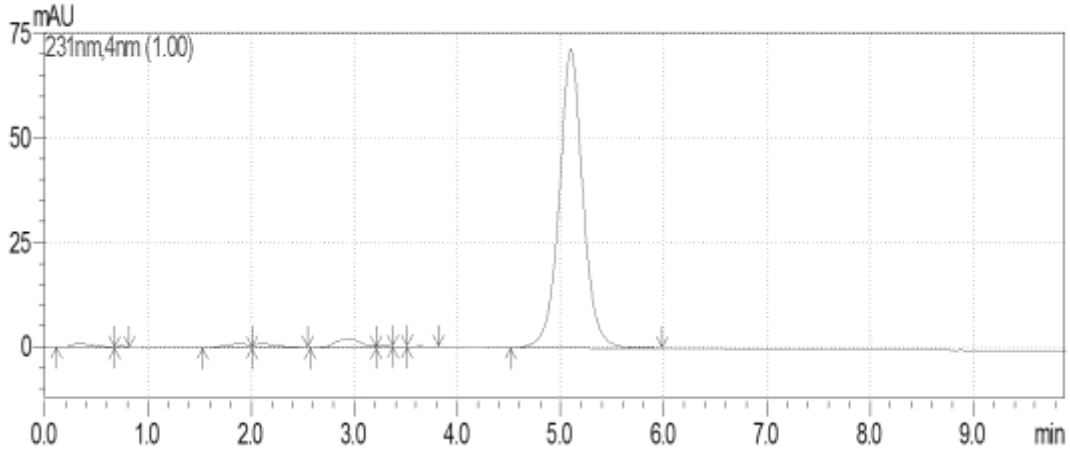

Figure No.3: A chromatogram for standard DOFE

Study of system suitability parameters

After equilibration of column with mobile phase, working standard solution (A3) 2.0 ml portion was further diluted to 10 ml to get the concentration about 2µg/ml, was injected through the manual injector five times and the chromatograms were recorded and the peak area was measured. The recorded results of system suitability parameters are shown in Table No.3.

Table No.3: Observations of system suitability parameters

Sr. No.	Wt. of std. drug taken in (mg)	Peak area (mV)	Retention Time (min)	Tailing Factor	Theoretical Plates/mt
1	10.0	221394	2.321	1.671	15465
2		223341	2.332	1.641	15368
3		238702	2.342	1.642	15367
4		264897	2.343	1.636	15412
5		277817	2.346	1.618	15463

Linearity Study:

For UV method aliquots of working stock solution of standard (A_2) were diluted with methanol to get a concentration in the range 2.5-20µg/ml. The absorbance of each solution was measured at selected wavelength. Similarly 1-5µg/ml concentration samples were prepared and injected in a optimized chromatographic parameters. The correlation coefficient of UV and HPLC were found to b 0.997 and 0.9996 respectively as shown in Table No.4.

Table No.4: Calibration curve data of UV and HPLC method

Calibration Observations for UV method		**Calibration Observations for HPLC method**	
Conc. (µg/mL)	Absorbance at 231	Conc.(µg/mL)	AUC (mV)
2.5	0.1273	1	183556
5	0.2554	2	228283
10	0.4999	3	277740
15	0.8014	4	323397
20	0.9982	5	404246
r^2	0.997	r^2	0.9996

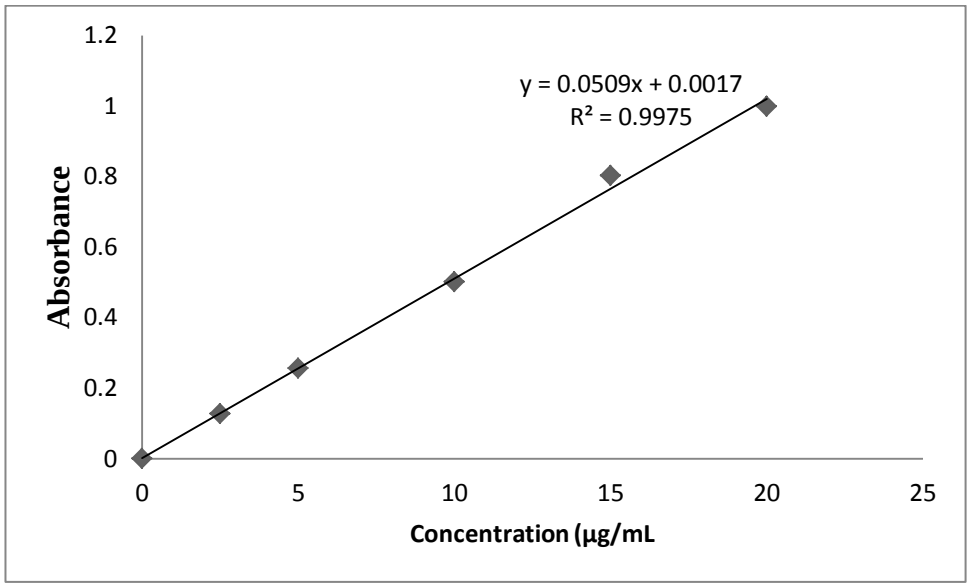

Figure No.4: Beer- Lambert plot of DOFE for UV-method

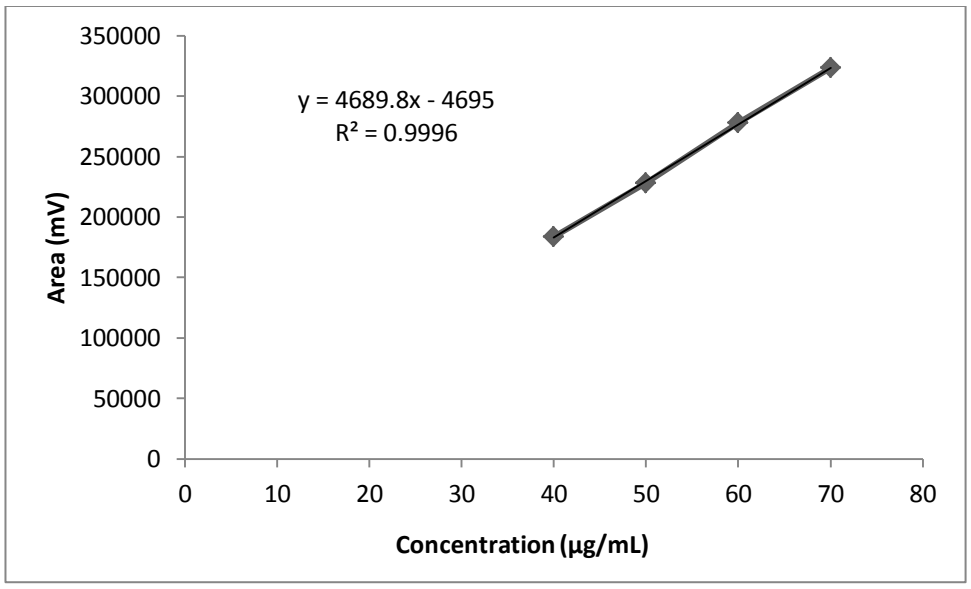

Figure No.5: Calibration curve of DOFE for HPLC method

Statistical comparision of assay data of UV and HPLC method and % recovery by UV and HPLC method:

Assay of drug sample in a laboratory formulation by UV and HPLC method:

Assay sample solutions were prepared as earlier mentioned in sample preparations. Five such replicates were evaluated by UV and HPLC. The obtained results of % labeled claim and estimated concentration by UV and HPLC were shown in Table No.5. The obtained %contents by UV and HPLC were statistically compared for F-test and T-test shown in Table No.7 and 8 respectively.

Table no.5: Assay of drug sample in a laboratory formulation by UV and HPLC method

UV assay data			**HPLC assay data**		
Weight taken (mg)	**Amt estimated (mg)**	**Percent content**	**Weight taken (mg)**	**Amt estimated (mg)**	**Percent content**
240.4	0.9966	99.23	120.7	0.4983	99.66
240.6	0.9878	99.56	119.3	0.4939	98.78
240.1	0.1009	99.44	121.1	0.5043	100.87
240.2	0.9916	99.50	119.6	0.4959	99.16
239.8	0.1008	99.92	120.9	0.5039	100.77
Mean		99.53	**Mean**		99.85
±SD		0.227	**±SD**		0.8418
%RSD		0.25	**%RSD**		0.8431

% Recovery study by UV and HPLC:

For UV study and HPLC method, separately an accurately weighted quantity of laboratory mixture (0.120mg) excluding drug was weighed and transferred to series of 10.0mL volumetric flask and to it known quantities i.e. 0.4, 0.5, 0.6, and 0.7mg of Std. Dofetilide was added at four different levels and sonicated for 15 min. with sufficient quantity of methanol and volume was made up to mark. The contents were filtered and diluted with methanol to get final concentration of Dodetilide.

The amount of drug estimated and the resulting quantities were assured to be recovered from the placebo (lab mixture excluding drug). % Recovery was calculated shown in Table No.6 and results of % recovery were statistically compared for F-test and T-test shown in Table No.7 and 8 respectively.

Table No.6: The observations and results of recovery studies

Recovery observations of UV method				
Sr No.	**Wt. of lab prepared powder**	**Amt. of pure drug added (mg)**	**Amount recovered**	**% Recovery**
1	119.5	0.404	0.3987	98.68
2	119.6	0.505	0.5076	100.52
3	119.52	0.606	0.6125	101.06
4	119.65	0.707	0.7114	101.05
Mean				100.33
±SD				0.9784
%RSD				0.9751
Recovery observations of HPLC method				
Sr No.	**Wt. of lab prepared powder**	**Amt. of pure drug added (mg)**	**Amount recovered**	**% Recovery**
1	119.8	0.408	0.4085	100.12
2	119.3	0.510	0.5080	99.61
3	119.7	0.612	0.6168	101.00
4	119.4	0.714	0.7197	100.80
Mean				100.38
±SD				0.6380
%RSD				0.6356

F-TEST:

Statistical Comparison between % contents and % recovery study of assay sample by UV and HPLC method shown in Table No.7.

Table No.7: Observations and results of F-test for % contents and % recovery study

F- Test for % content and % recovery study					
Parameter	**Variance**		**F-value**	**P(F<=f)one tail**	**F critical one tail**
	UV method	**HPLC method**			
% content	0.0465	1.1666	0.03986	0.01259	0.1078
% recovery	0.09543	0.5647	0.16900	0.14457	0.05263

Discussion:

From the above observations of F-test, it was concluded that in case of % contents F-value was less than F-critical value therefore could not rejected the null hypothesis, whereas % recovery F-value was greater than F-critical value therefore null hypothesis was rejected.

T-Test:

Statistical Comparison between % contents and % recovery study of assay sample by UV and HPLC method shown in Table No.8.

Table No.8: Observations and results of T-test for % contents and % recovery study

Parameters	**t-Stat**	**P(T<=t) one-tail**	**t Critical one-tail**	**P(T<=t) two-tail**	**t Critical two-tail**
% content	-0.5267	0.3175	2.3534	0.6349	3.1824
% recovery	0.8669	0.2249	2.3534	0.4498	3.1824

Discussion:

From the above observations of T-test, it was concluded that the observed P-value is greater than the α-value (P-value generally 0.05). Therefore accept the null hypothesis i,e. there was a statistically significant difference in mean of% contents and % recovery estimation by UV and HPLC method.

Validation of UV and HPLC Method:

The developed method was validated according to ICH guidelines. The developed method was validated for linearity and range, accuracy, precision, ruggedness, LOD, LOQ and robustness.

Table No. 9. Validation Parameters by UV and HPLC Estimation of Dofetilide

Parameters	**UV**		**HPLC**	
	%Mean	**% RSD**	**%Mean**	**% RSD**
Accuracy	100.33	0.978	100.38	0.63
Precision	99.64	0.11	99.72	0.16
Analyst-I	98.87	0.25	98.23	0.12
Analyst-II	98.49	0.39	98.51	0.45
Interday	99.42	0.31	100.73	0.81
Intraday	99.31	0.42	99.76	0.81
Linearity and range	0.9998		0.9996	
Linearity (R^2)	0.997		0.9996	
LOD (µg)	1.6568		0.1504	
LOQ (µg)	5.0217		0.4556	

Robustness of HPLC method:

The robustness of the method was evaluated by injection of the sample at deliberately varying the chromatographic conditions viz. composition of organic phase in mobile phase by 10%, pH of buffer by ±0.2unit, varying flow rate and change in wavelength by ±5nm. The system suitability parameters were evaluated at each varied condition and the observations are tabulated in Table No.10.

Table No.10: Observations for robustness study

Parameters	**Wt. of powder taken (mg)**	**Retention time (min)**	**Theoretical plate**	**Tailing factor**
Standard condition	120.00	2.306	15325	1.256
Wavelength at 236nm		2.336	15378	1.210
Wavelength at 226nm		2.330	15355	1.240
0.1%TEA:ACN (65:35)		2.303	15388	1.251
Phosphate buffer:ACN (55:45)		2.302	15362	1.230
Flow rate (1.00mL/min)		3.202	22231	1.542
Flow rate (1.5mL/min)		2.351	15402	1.216
Mean			**16348.7**	**1.2778**
±SD			**2401.5**	**0.1089**

Conclusion:

The results obtained by UV and RP-HPLC methods for determination of Dofetilide are reliable, accurate and precise. The method does not have any interference of excipients while determining Dofetilide from its laboratory prepared mixture. The developed HPLC method was found to be superior with respect to resolution of drug from its prepared mixture under applied. From the results of statistical tests such as F-test and T-test, it was concluded that HPLC method is more reliable than UV method for estimation of drug with the use of economic, simple, accurate and validated method. Also method was found to be economical and less time consuming. Hence, developed UV and HPLC methods can be employed for routine quality control analysis of Dofetilide in capsule dosage form.

References:

1. https://pubchem.ncbi.nlm.nih.gov/compound/Dofetilide: Access on 2 May 2019.
2. https://en.wikipedia.org/wiki/Dofetilide: Access on 2 May 2019.
3. Kaddar N, Pilote S, Wong S, Caillier B, Patoine D, Drolet B, Simard C. Simultaneous Determination of Dofetilide and Amlodipine in Plasma by HPLC. J Chromat Separation Techniq. 2013;4(6).
4. Rapilane. KS, Thengungal K, Ravi and MuruganathanG, Development of Spectroscopy and HPLC Method for the Analysis of Dofetilide and it's Degradation Product European Journal of Pharmaceutical And Medical Research**.**2017;4(3):520-23
5. Ramesh A and Ashok K., Method Development and Method Validation for the Estimation of Pharmaceutical Dosage Preparation By RP-HPLC, Indo American Journal of Pharmaceutical Research. 2018;8(12):1336-43
6. Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. J Anal Bioanal Techniques. 2012;3(6):151-7.

7. European Medicines Association. Note for Guidance on Validation of Analytical Procedures: Text and Methodology (CGMP/ICH/381/95); ICH topic Q2 (R1). European Medicines Association, London, United Kingdom. 1995.

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