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Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Glecaprevir and Pibrentasvir in Drug Product

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Abstract:

The aim of the method was to develop and validate a rapid, sensitive and accurate method for simultaneous estimation of Pibrentasvir and Glecaprevir in drug product by liquid chromatography. The chromatographic separation was achieved on C8 column (Hypersil BDS-C8 100*4.6, 3.5um) at ambient temperature .The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Methanol: Acetonitrile (30:60:10). The flow rate was 0.8ml/ minute and ultra violet detector at 225nm. The average retention time for Pibrentasvir and Glecaprevir found to be 2.107 min and 2.341 min. The proposed method was validated for 2.341selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 40.0 – 120.0µg/mL for Pibrentasvir and 100.0 - 300.0µg/mL of Glecaprevir.

Key words: Pibrentasvir, Glecaprevir, Isocratic, HPLC, C8, Trifluoro acetic acid, Acetonitrile, Methanol and validation

1. INTRODUCTION

Pibrentasvir

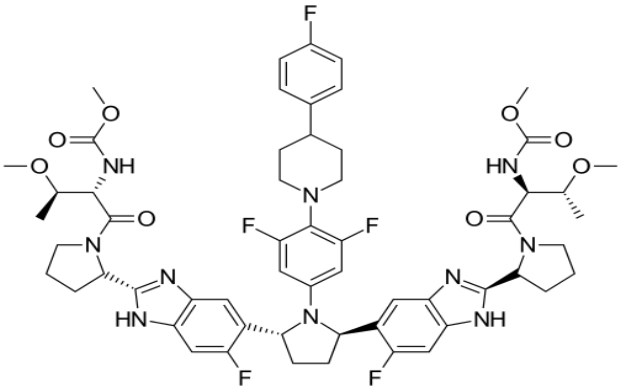


Fig.1. Chemical structure: Pibrentasvir

Pibrentasvir is an antiviral agent. In the United States and Europe, it is approved for use with glecaprevir as the combination drugglecaprevir/pibrentasvir (trade name *Mavyret* in the US and *Maviret* in the EU) for the treatment of hepatitis C.

Pibrentasvir is chemically designated as Methyl {(2*S*,3*R*)-1-[(2*S*)-2-{5-[(2*R*,5*R*)-1-{3,5-difluoro-4-[4-(4-fluorophenyl)-1-piperidinyl]phenyl}-5-(6-fluoro-2-{(2*S*)-1-[*N*-(methoxycarbonyl)-*O*-methyl-L-threonyl]-2-pyrrolidinyl}-1*H*-benzimidazol-5-yl)-2-pyrrolidinyl]-6-fluoro-1*H*-benzimidazol-2-yl}-1-pyrrolidinyl]-3-methoxy-1-oxo-2-butanyl}carbamate. Its molecular formula is $C_{57}H_{65}F_5N_{10}O_8$, and its molecular weight is 1,113.20 g·mol^{-1}.

Glecaprevir

Glecaprevir (INN,[1]) is a hepatitis C virus (HCV) nonstructural (NS) protein 3/4Aprotease inhibitor that was identified jointly by AbbVie and EnantaPharmaceuticals. It is being developed as a treatment of chronic hepatitis C infection in co-formulation with an HCV NS5A inhibitor pibrentasvir. Together they demonstrated potent antiviral activity against major HCV genotypes and high barriers to resistance *in vitro.*

Glecaprevir is chemically designated as (3aR,7S,10S,12R,21E,24aR)-7-tert-Butyl-N-{(1R,2R)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl)carbamoyl]cyclopropyl}-20,20-difluoro-5,8-dioxo-2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1H,10H-9,12-methanocyclopenta[18,19][1,10,17,3,6]trioxadiazacyclonon adecino[11,12-b]quinoxaline-10-carboxamide. Its molecular formula is C38H46F4N6O9S, and its molecular weight is 838.87 g·mol−1.

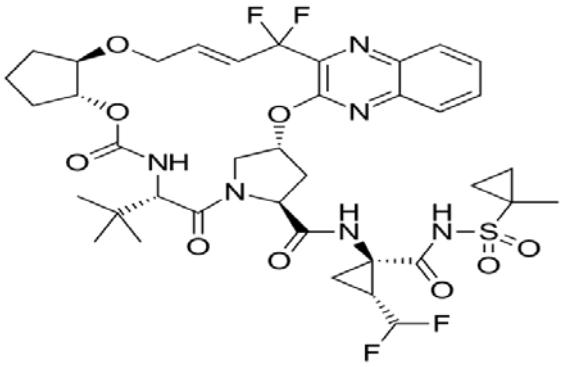


Fig.2. Chemical structure: Glecaprevir

2. MATERIALS AND METHODS

2.1 Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C8 column (Zorbax SB-C8 100*4.6, 3.5µm) as stationary phase ,Ultrasonic cleaner, Scaletech analytical balance and Vacuum micro filtration unit with 0.45µ membrane filter.

2.2 Materials: Pharmaceutically pure sample of Pibrentasvir/Glecaprevir were obtained as gift samples from Fortune pharma training institute, Sri Sai nagar colony, KPHB, Hyderabad, India.

HPLC-grade Methanol and Acetonitrile were obtained from qualigens reagents pvt ltd. Trifluoro acetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions The sample separation was achieved on a (Hypersil BDS-C8 100*4.6, 3.5µm) C8 column, aided by mobile phase mixture of 0.1%v/v Trifluoro acetic acid in water: Methanol:Acetonitrile (30:60:10). The flow rate was 0.8 ml/ minute and ultra violet detector at 225nm that was filtered and degassed prior to use, Injection volume is 10µl and ambient temperatures.

Preparation of mobile phase:

Buffer Preparation: Taken accurately 1ml of Trifluoro acetic acid in 1000mL of water

Mobile phase: Then added 20 volumes of buffer and 80 volumes of Methanol mixed well and sonicated for 5 min.

Diluents: Water: Acetonitrile: 50:50 v\v

2.4 Preparation of solutions

2.4.1 Standard solution: 40mg of pure Pibrentasvir and 100mg of Glecaprevir were weighed and transferred to 25 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to

mark with water to give a solution containing 80.0µg/ml of Pibrentasvir and 200.0µg/ml Glecaprevir .

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 40mg of Pibrentasvir and 100mg of Glecaprevir sample and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 80.0µg/ml of Pibrentasvir and 200.0µg/ml Glecaprevir .

2.5 Method validation

2.5.1. System suitability

The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system

2.5.2. Linearity

Linearity was studied by analyzing five standard solutions covering the range of 40.0 -120.0µg/ml for Pibrentasvir and 100.0 -300.0µg/ml Glecaprevir. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.50 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 40.0 µg /mL, 60.0µg/mL, 80.0µg/mL, 100.0µg/mL and 120.0µg/mL of Pibrentasvir and 100.0g/mL, 150.0µg/mL, 200.0µg/mL, 250.0µg/mL and 300.0µg/mL of Glecaprevir.

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.5.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

LOD = 3.3 δ/S

LOQ =10 δ/S

Where,

δ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

2.5.4. Method precision

The precision of the method was checked by repeated preparation(n=6) of 80.0µg/ml of Pibrentasvir and 200µg/ml Glecaprevir without changing the parameter of the proposed chromatographic method. And measured the peak areas and retention times.

2.5.5. Accuracy

The accuracy of the method was determined by calculating the recoveries of Pibrentasvir and Glecaprevir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Pibrentasvir and Glecaprevir.

2.5.6. Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min.

3. RESULTS AND DISCUSSIONS:

Determination of Working Wavelength (λ max): 10 mg of the Pibrentasvir and Glecaprevir standard drug is taken in a 10 ml volumetric flask and dissolved in diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using Water as blank. The λmax was found to be 225nm

After several initial trails with mixtures of methanol, water, Acetonitrile and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoro acetic acid in water: Methanol (20:80). At flow rate was 0.8mL/ minute brought sharp peaks. The chromatogram was shown in Fig 3**.**

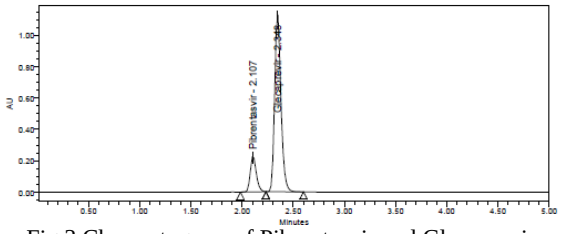


Fig 3 Chromatogram of Pibrentasvir and Glecaprevir

System suitability

The system suitability of the method was checked by repeated preparations for Glecaprevir and Pibrentasvir. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system, System suitability data of Glecaprevir and Pibrentasvir are shown in Table 1

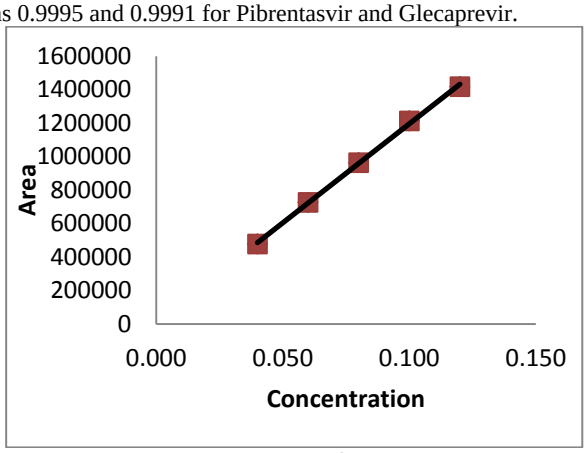
Parameter	Pibrentasvir	Glecaprevir	Acceptance criteria
Retention time	2.102	2.340	+-10
Theoretical plates	6132	6799	>3000
Tailing factor	1.16	1.21	<1.50
% RSD	0.44	0.39	<2.00

Table 1 System suitability data of Pibrentasvir and Glecaprevir

Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 40.0 -120.0µg/ml for Pibrentasvir and 100.0 -300.0µg/ml Glecaprevir. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.50 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 40.0 µg /mL, 60.0µg/mL, 80.0µg/mL, 100.0µg/mL and 120.0µg/mL of Pibrentasvir and 100.0g/mL, 150.0µg/mL, 200.0µg/mL, 250.0µg/mL and 300.0µg/mL of Glecaprevir in Table 2 and Table 3

A linear relationship between peak areas versus concentrations was observed for Pibrentasvir and Glecaprevir in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9995 and 0.9991 for Pibrentasvir and Glecaprevir.



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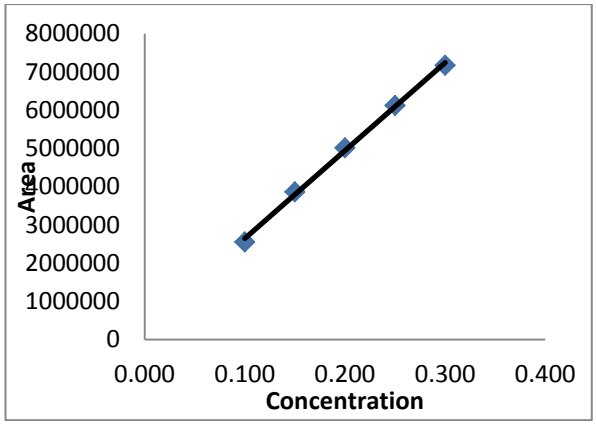

B

Fig. 4 Calibration curve: (A) Pibrentasvir: (B) Glecaprevir

Level	Concentration (mg/mL)	Peak area
50%	0.040	478329
75%	0.060	726397
100%	0.080	964059
125%	0.10	1212822
150%	0.120	1417571
Correlation		**0.9995**

Table 2: Linearity data of Pibrentasvir

Level	Concentration (mg/mL)	Peak area
50%	0.10	2549114
75%	0.15	3861635
100%	0.20	5014167
125%	0.25	6121442
150%	0.30	7172492
Correlation		**0.9991**

Table 3: Linearity data of Glecaprevir

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 σ /S ……. (1)

LOQ =10 σ /S …….. (2)

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

	Pibrentasvir mg	Glecaprevir mg
LOD	**0.0040**	**0.0129**
LOQ	**0.0120**	**0.0392**

Table 4: LOD and LOQ values Calculated from calibration curve

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 40.0µg/ml of Pibrentasvir and 200.0µg/ml Glecaprevir without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times. The precision of the method (% RSD) was found to be <1% showing good repeatability. The values of percentage RSD for Pibrentasvir and Glecaprevir are shown in Table 5 and Table 6.

Sample. NO	Retention time	Peak area	% Assay
1	2.104	951512	100.1
2	2.101	949881	100.5
3	2.112	958921	100.3
4	2.101	957891	100.0
5	2.108	962871	100.3
6	2.099	962259	100.6
Mean	2.104	957223	100.3
%RSD	**0.24**	**0.57**	**0.24**

Table 5: Summary of peak areas for method precision of Pibrentasvir

Sample No	Retention time	Peak area	% Assay
1	2.342	4932749	100.1
2	2.338	4945205	100.3
3	2.350	4978168	100.4
4	2.338	4982442	99.9
5	2.350	4997416	100.8
6	2.336	5022640	100.7
Mean	2.342	4976437	100.4
%RSD	**0.27**	**0.67**	**0.35**

Table 6: Summary of peak areas for method precision of Glecaprevir

Accuracy (recovery study):

The accuracy of the method was determined by calculating the recoveries of Pibrentasvir and Glecaprevir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Pibrentasvir and Glecaprevir. The percentage recovery results obtained are listed in Table 7 &8

LEVEL	S.NO	%Recovery of Pibrentasvir	Average
50	1	98.3	99.3%
	2	99.5	
	3	100.2	
100	1	100.1	100.3%
	2	100.5	
	3	100.3	
150	1	100.8	100.6%
	2	99.9	
	3	101.0	

Table 7: Recovery data of Pibrentasvir

LEVEL	S.NO	%Recovery of Glecaprevir	Average
50	1	99.0	99.6%
	2	99.7	
	3	100.2	
100	1	100.1	100.3%
	2	100.3	
	3	100.4	
150	1	98.7	99.5%
	2	99.6	
	3	100.3	

Table 8: Recovery data of Glecaprevir

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table 9&10)

the results of Robustness of the present method had shown that changes are not significant was found to be the method is Robust.

parameter	Rt of Pibrentasvir	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	2.392	6388	1.22
Increased flow rate (0.9ml/min)	1.882	5622	1.11
Wave Length 223nm	2.107	5682	1.22
227nm	2.101	6307	1.15

Table 9: Results of Pibrentasvir

parameter	Rt of Glecaprevir	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	2.666	7381	1.24
Increased flow rate (0.9ml/min)	2.091	5850	1.22
Wave Length 223nm	2.348	6896	1.19
227nm	2.339	6708	1.21

Table 10: Results of Glecaprevir

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table 11&12.
The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

		%Assay	%RSD
Analyst-1	**PIBRENTASVIR**	100.1	0.29%
Analyst-2		100.5	

Table 11: Ruggedness data for Pibrentasvir

		%Assay	%RSD
Analyst-1	**GLECAPREVIR**	100.1	0.18%
Analyst-2		100.3	

Table 12: Ruggedness data for Glecaprevir

Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Both drugs are sensitive to acid, alkali, UV light and thermal conditions. The results of forced degradation studies were given in Table 13

S.No	Degradation Condition	Pibrentasvir		Glecaprevir	
		Assay (%)	Degradation (%)	Assay (%)	Degradation (%)
1	0.1N HCl 24h	78.68	25.1	15.82	25.5
2	0.1N NaOH 24h	93.2	6.8	92.8	7.2
3	5% H_2O_2 24h	98.6	1.4	95.5	4.5
4	Heat 24 h	98.3	1.7	99.58	0.2
5	UV 24h	98.7	1.3	99.1	0.9

Table 13 Results of Forced degradation study

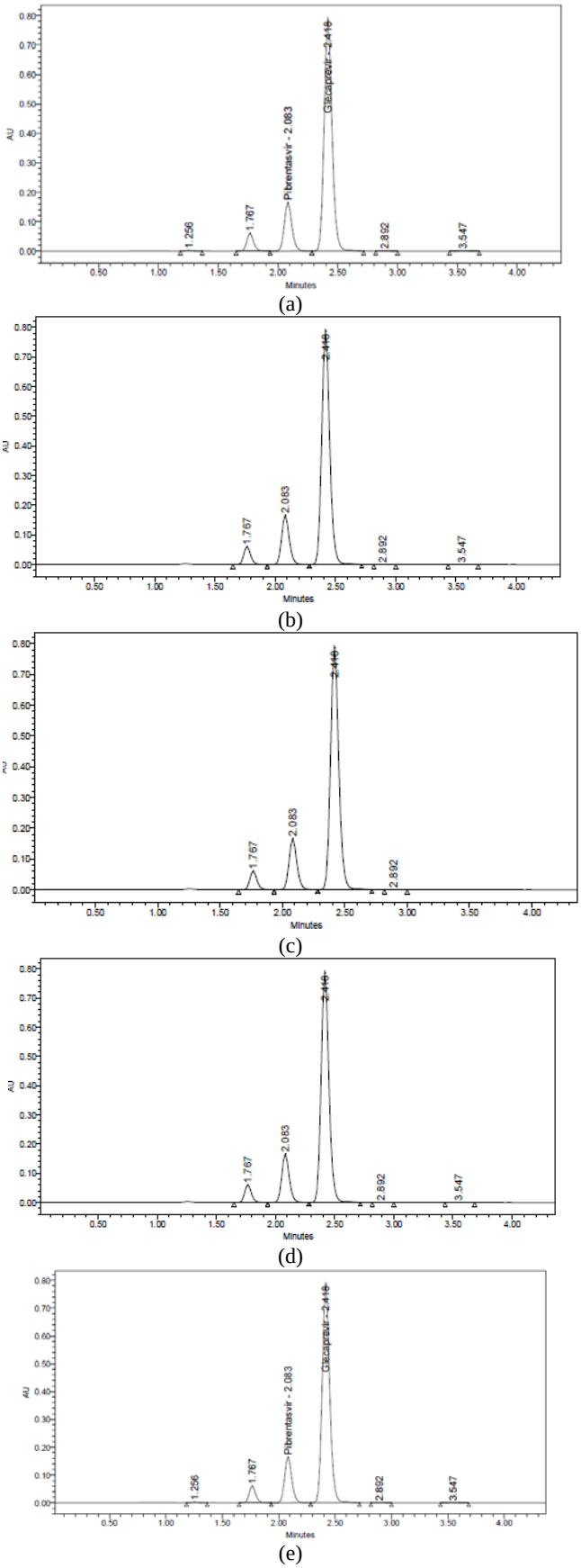

Fig. 5: Chromatograms of after forced degradation: (a) Acid degradation; (b) base degradation; (c) peroxide degradation; (d) photolytic degradation; and (e) thermal degradation

CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of PIBRENTASVIR and GLECAPREVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

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8. SIMULTANEOUS ESTIMATION OF NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GLECAPREVIR AND PIBRENTASVIR BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. www.innovatpublisher.com ISSN No. 2456-8694 Research Article K. HEMALATHA , DR.C.KISTAYYA, N.D.NIZAMUDDHIN * , D DASTIAGIRIAMMA