DEVELOPMENT AND VALIDATION OF A GC-FID ASSAY FOR DETERMINATION OF QUERCETIN IN A PHARMACEUTICAL PREPARATIONS

Dr. Kurapati. Swarna Latha[1*], Ganji. Veena Madhuri[2], Aleti. Chandrika[3], Peram. Usha[4], Kotipalli.Manasa[5], Yericherla. Samyuktha[6], Chikkala. Angeline Jyothirmayee[7], Manne Rama[8], Vedula Nagalakshmi[9], Nanduri Gayathri[10]

1,2,3,4,5,6,7,8,9,10 Department of Chemistry, Ch.SD.St.Theresa's College for Women, Eluru – 534 002, AP, India.

kurapatisl1971@gmail.com

Abstract:

A simple, rapid, precise, sensitive, reproducible, GC-FID method has been developed for the quantitative analysis of Quercetin in pharmaceutical dosage form. Chromatographic separation of Quercetin was achieved on Omega 700 series, GC system by using, DB624 column length and width 30x0.53.

The flow rate was 40ml/min, detection was carried out by absorption at 232nm using a flame ionisation detector at 250^0c temperature. The percentage of relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guide lines. The method was found to be simple, economical, suitable, precise, accurate for quantitative analysis of quercetin.

Keywords: GC-FID detector, Quercetin, Development

Introduction:

Gas Chromatography is a widely used analytical technique used to separate & analyze the gaseous & volatile compounds. In 1952, Modern Gas Chromatography was invented by James & Martin. Since early 1950's this technique was first used for the separation of amino acids[1] now GC has large number of applications as this technique is rapid & has a great sensitivity. Both qualitative & quantitative analysis can be done through GC. Even minute quantity sample can be analyzed through GC. In gas chromatography, the sample is dissolved In a solvent and vaporized in order to separation the chromatography (GLC). The criteria for the compounds to be analyzed in GC is volatility & thermostability. analytes. The sample is distributed between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas such as helium, nitrogen etc[2]. Gas chromatography is one of the unique forms of chromatography that does not need the mobile phase for interacting with the analyte. The stationary phase is either a solid adsorbent, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC)[3]. The criteria for the compounds to be analyzed in GC is volatility & thermostability.

Molecular formula: C10H15O7

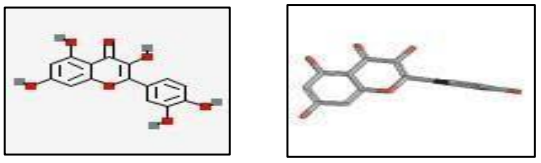
Molecular weight: 302.23

Solubility: Very soluble in ether, methanol; soluble in ethanol, acetone, pyridine, acetic acid.

Soluble in alcohol and glacial acetic acid; insoluble in water.

Melting point: 601 to 603 °c.

Appearance: Yellow crystalline powder.



Structure of Quercetin

Uses: Quercetin suggested uses include for seasonal allergies, prevention of diabetic cataracts, viral infections, cardiovascular disease, as adjunctive therapy in cancer, to treat chronic prostatitis, and interstitial crystalline. Quercetin is possibly effective in the treatment of nonbacterial chronic prostatitis, and interstitial cystitis

Common side effects of quercetin include: Headache, Numbness and tingling, Shortness of breath, Nausea and vomiting, Kidney damage.

Quercetin belongs to a group of plant pigments called flavonoids that give many fruits, flowers, and vegetables their colours.

Flavonoids, such as quercetin, are antioxidants. They scavenge particles in the body known as free radicals which damage cell membranes, tamper with DNA, and even cause cell death. Antioxidants can neutralize free radicals. They may reduce or even help prevent some of the damage free Radicals cause. In test tubes, quercetin has strong antioxidant properties. But researchers are not sure whether taking quercetin (and many other antioxidants) has the same effects inside the body.

Quercetin may help protect against heart disease and cancer. Quercetin can also help stabilize the cells that release histamine in the body and thereby have an anti-inflammatory and antihistamine effect.

EXPERIMENTAL WORK:

OPTIMIZED METHOD CONDITIONS

Software – N 2000, Column – DB624, Company – Netel chromatographs, Model – make Omega, Carrier Gas-Nitrogen, Column flow – 3.3ml/min, Particle size – 3.0 microns, Sample volume – 10 micro litres Nitrogen flow rate – 30 ml/min, Hydrogen flow rate – 40 ml/min, Zero air – 400ml/minute, Run time – 10 min, Oven temperature – 80°c, Injector temperature – 180°c, Detector temperature – 250°c, Split ratio – 1:5.

PREPARATION OF STANDARD SOLUTION:-

Accurately weight 5mg of drug Quercetin and transferred into 10 ml volumetric flask and add few drops of Methanol (diluent) to dissolve the drug. later the volumetric flask are kept in sonicator, for 5minutes for removal of air bubbles and for dissolving of drug completely. ofter removing from sonication the solution in the volumetric flask are made up to the mark with methanol (this will be stock of our drug). For further dilution

1ml of Quercetin is transferred in to 10 ml volumetric flask with the help of graduated pippete.this solution is made upto the mark with methanol. This is the standard solution of our drug quercetin.

PREPARATION OF SAMPLE

The label claim of Quercetin is 100 mg.

Firstly we have to calculate the sample weight they have given the 3 capsules of Quercetin. The average of the three capsules are 309mg.So this the weight of the capsule,the weight of the drug quercetin is 5mg.So that the Sample weight 309X5/100=15mg.This 15mg is used for the preparation of assay. This 15mg is weighed accurately and transfer into 10ml volumetric flask and few drops of methanol is added to dissolve later it is kept in sonication for 30min to remove air bubbles. After removing from the Sonication it is make up to the mark. This will be stock of our sample.

PROCEDURE:

ASSAY:

From the stock solution 1ml is taken by using pipette and transferred into another 10ml volumetric flask and make up to the mark.

Spiking method:

1ml is taken from standard stock and sample stock and it is transferred into another 10ml volumetric flask and make up to the mark. Then the concentration of the solution is 100ppm[4].

Specificity:

The blank solution is injected into GC.

System precision:

The standard solution is injected into the GC for 6 times.

Linearity:

From standard solution various concentration are taken and injected into GC

Method precision:

The sample is weighed accurately for 6 times and the 6 standard solutions are prepared and then injected into the GC.

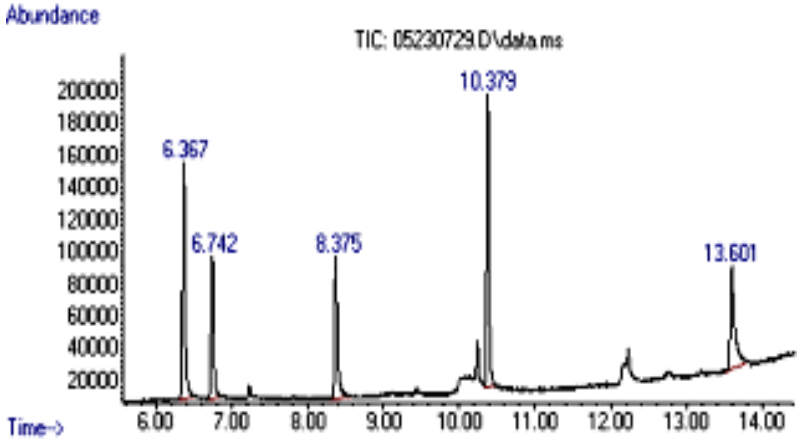
Intermediate precision:

The sample is weighed accurately for 6 times and the 6 standard solutions are prepared but the analyst is changed and then injected into the GC.

Robustness:

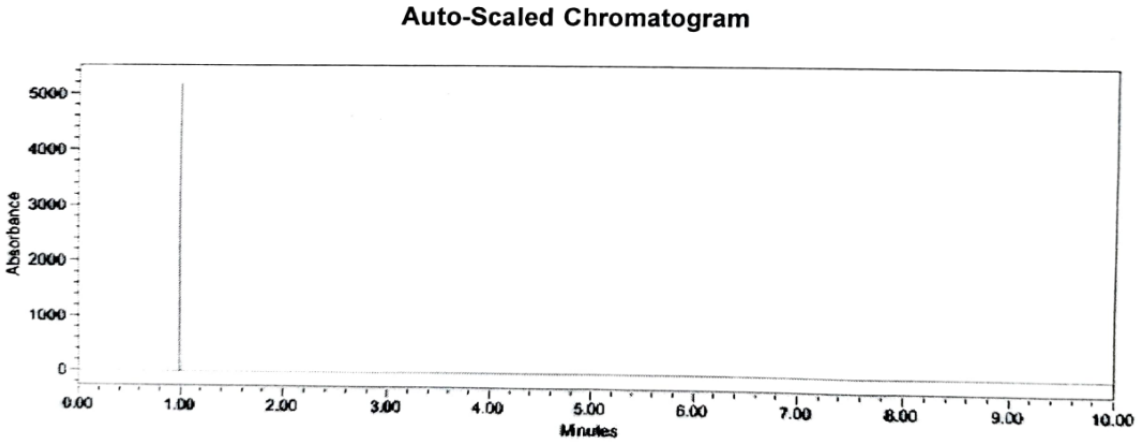
The drug 5mg is weighed accurately for 3 times and the sample 15mg is also weighed for 3 times, then these solutions are injected into the GC.

REULTS AND DISCUSSIONS:



QUERCETIN SPECTRA

SPECIFICITY:



CHROMATOGRAM OF BLANK

ACCEPTANCE CRITERIA: Chromatogram of blank should not show any peak at the retention time of analytic peak.

SYSTEM PRECISION:

TABLE-1 :SYSTEM PRECISION DATA FOR QUERCETIN

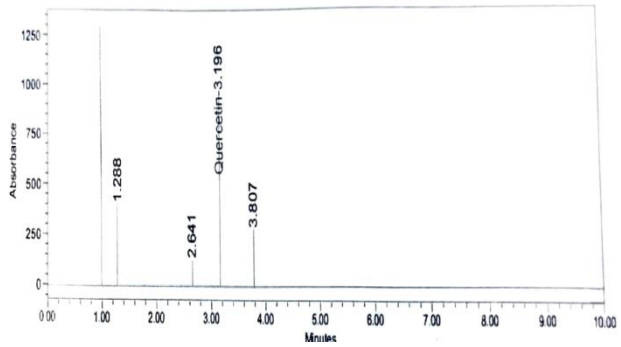
INJECTIONS	1.291	2.645	QUERCETIN	3.814
1	871485	498014	1649572	817714
2	872236	493951	1650636	814569
3	872954	497011	1645221	815692
4	871840	493562	1649585	818506

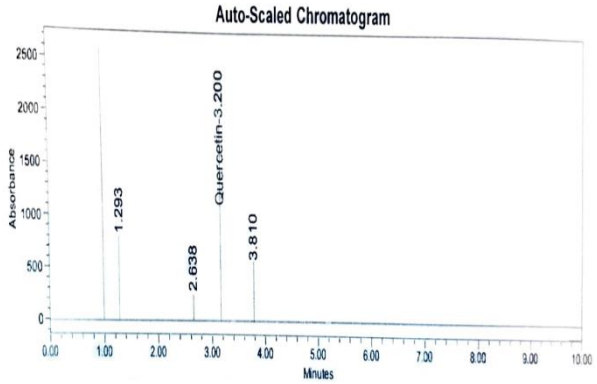
5	870871	498495	1653847	817121
6	873266	497702	1661253	816023
Mean			1651686	
SD			5440.21	
%RSD			0.329	

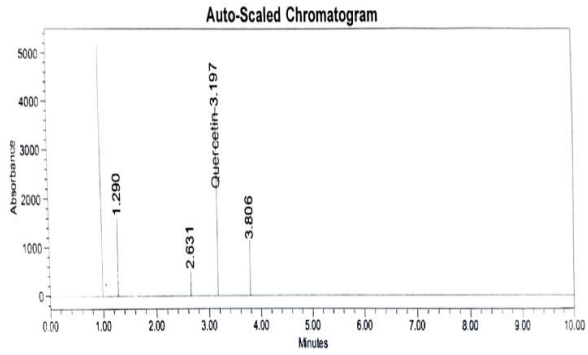
Acceptance criteria:

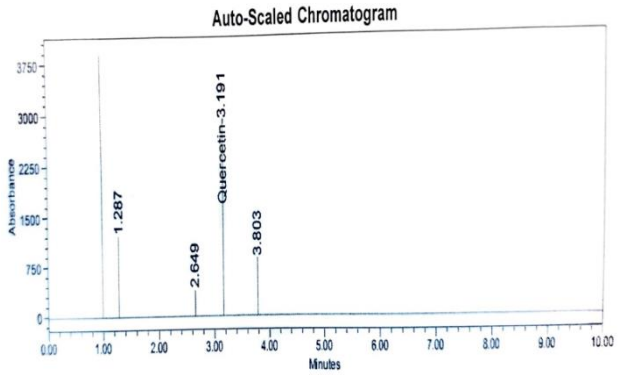
%RSD-less than 2

LINEARITY:









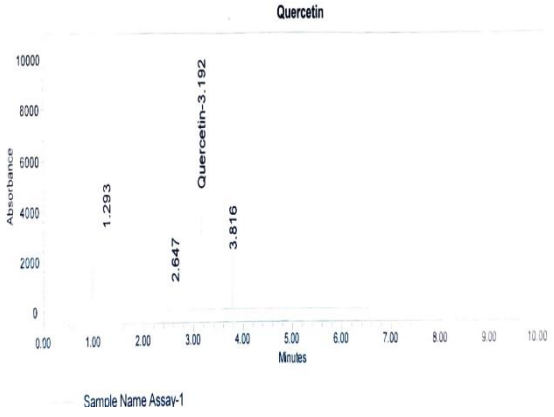
CHROMATOGRAMS OF LINEARITY

TABLE-2: LINEARITY DATA FOR QUERCETIN

LINEARITY	**1.291**	**2.645**	**QUERCETIN**	**3.814**
25%	217504	139812	413481	204606
50%	435008	255624	826962	409212
75%	652511	368435	1240443	613817
100%	870015	499247	1653924	817423
120%	1050519	598059	2027405	1002029
150%	1305023	733871	2357286	1158535
Regression equation			Y =31790.48x+24928.57	
Slope			31790.48	
Intercept			24928.57	
R^2			0.999	

Acceptance criteria: Correlation coefficient – Greater than or equal to 0.999

CHROMATOGRAMS OF ASSAY



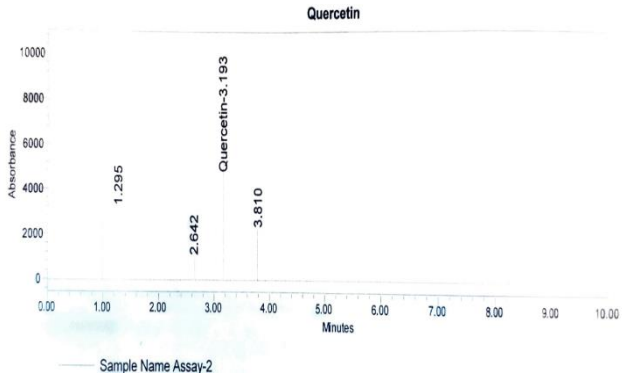


TABLE-3: ASSAY DATA FOR QUERCETIN

SNO	ASSAY	1.291	2.645	QUERCETIN	3.814	% ASSAY
1	ASSAY-1	1741693	995417	3292510	1632489	100%
2	ASSAY-2	1734085	996032	3299684	1627650	100%

Acceptance criteria: Correlation coefficient – Greater than or equal to 0.999

%Assay: 98-102%

METHOD PRECISION:

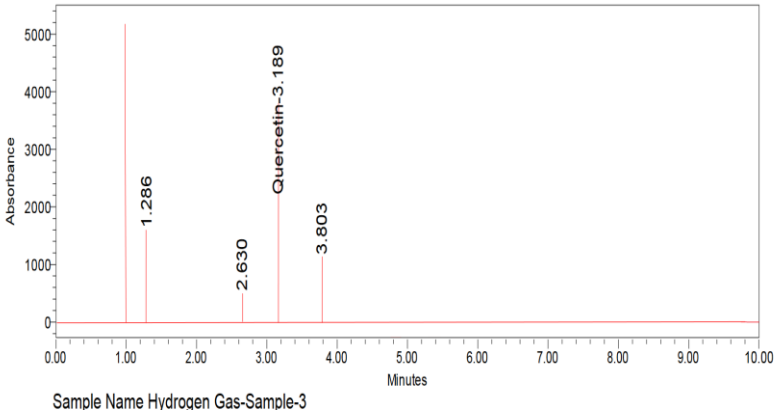
TABLE-4 METHOD PRECISION DATA FOR QUERCETIN

SNO	METHOD PRECISION	1.286	2.633	QUERCETIN	3.803	%ASSAY
1	METHOD PRECISION-1	871210	498514	1653924	818024	100.1%
2	METHOD PRECISION-2	872942	496932	1648520	819526	100%
3	METHOD PRECISION-3	871044	497408	1666987	818823	101%
4	METHOD PRECISION-4	872281	496158	1650213	819247	100%
5	METHOD PRECISION-5	870597	498033	1641695	817862	99.3%

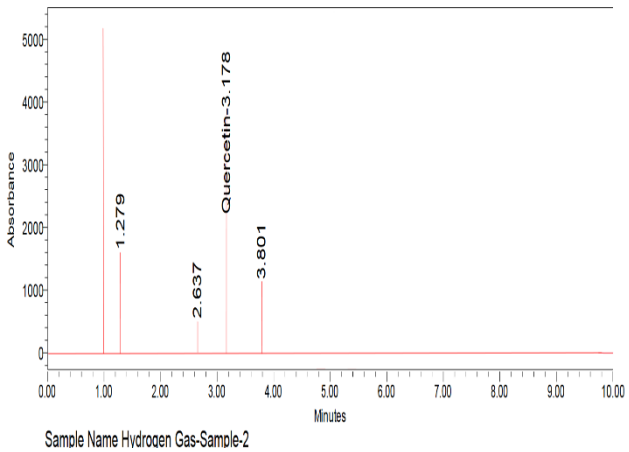
6	METHOD PRECISION-6	872470	497642	1645406	818431	100%
7	Mean			1651124.167		
8	SD			8817		
9	%RSD			0.533		

Acceptance criteria : Correlation coefficient – Greater than or equal to 0.999

%Assay : 98-102% , **%RSD:** less than 2



R0BUSTNESS



CHROMATOGRAMS OF ROBUSTNESS

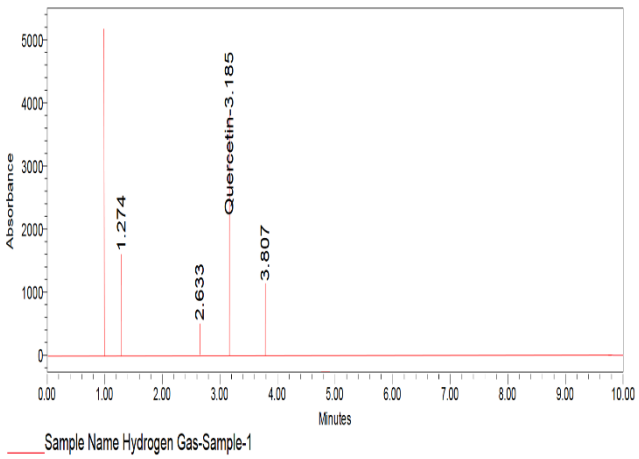


TABLE-5: ROBUSTNESS DATA FOR QUERCETIN

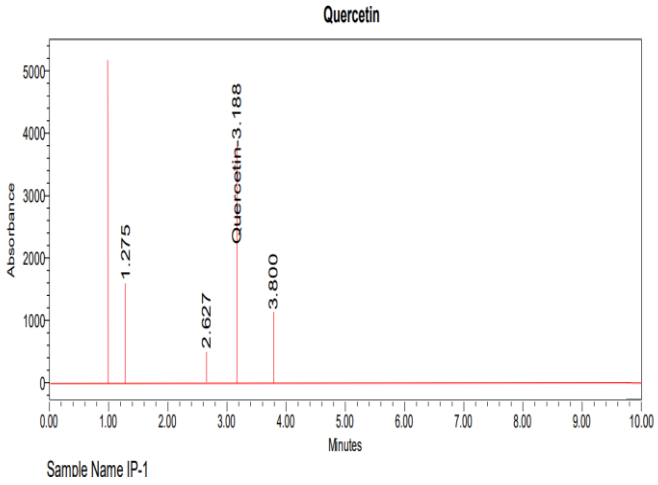
SNO	SAMPLE NAME	1.274	2.633	QUERCETIN	3.807	%ASSAY
1	H_2SAMPLE -1	872415	498411	1653278	818041	100%
2	H_2SAMPLE-2	871087	496029	1639201	819832	101%
3	H_2SAMPLE-3	873469	497236	1664557	817505	99%
4	MEAN			1652345		
5	SD			12703.7		
6	%RSD			0.7688		

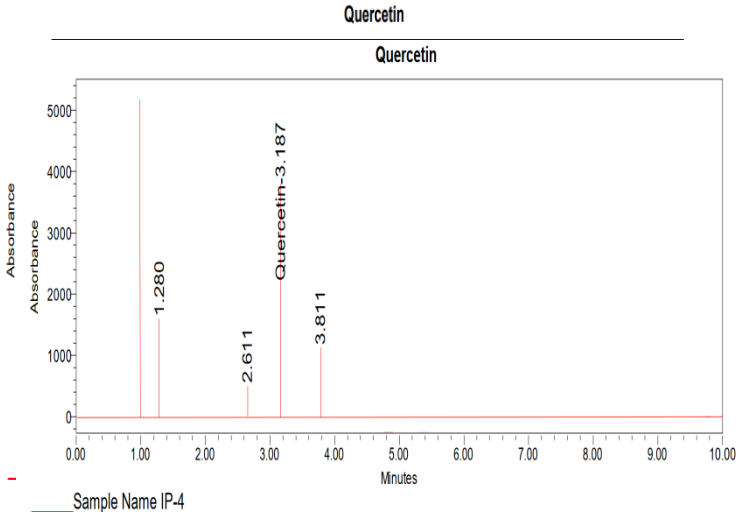
Acceptance criteria:

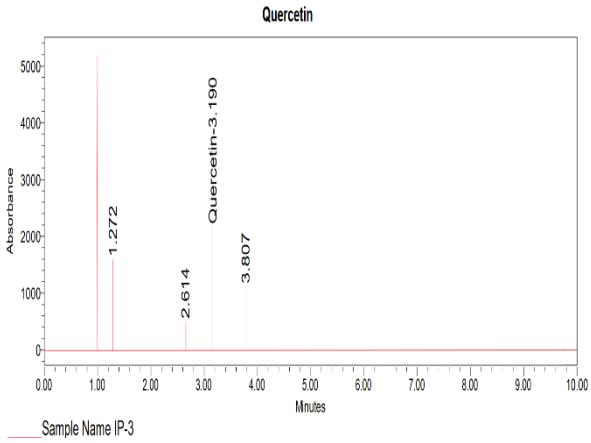
%Assay 98-102%

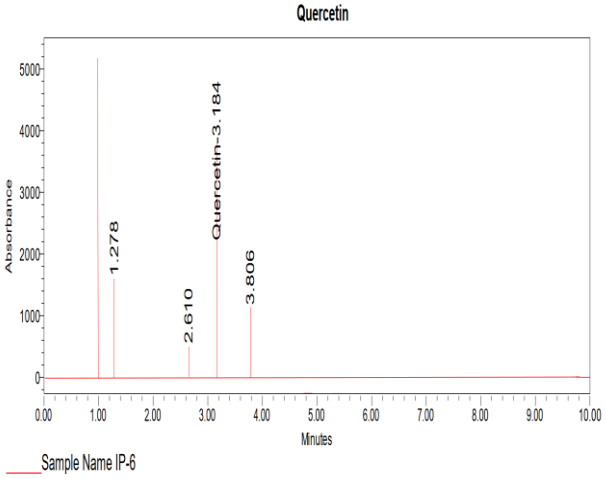
%RSD-less than 2

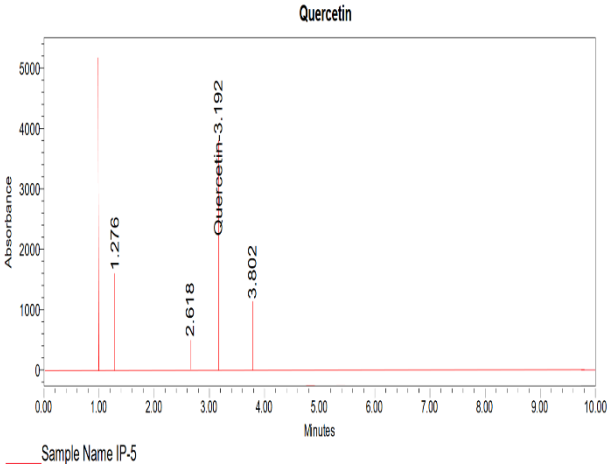
INTERMEDIATE PRECISION: CHOMATOGRAMS OF INTERMEDIATE PRECISION











PEAK RESULTS:

TABLE-6: INTERMEDIATE PRECISION DATA FOR QUERCETIN

S.NO	SAMPLE NAME	1.275	2.627	QUERCETIN	3.800	%ASSAY
1	IP-1	871174	496427	1642379	817724	99%
2	IP-2	871895	497749	1653960	819085	100%
3	IP-3	870247	497130	1659845	817255	100%
4	IP-4	872196	495963	1661581	819342	100%
5	IP-5	870471	498031	1641683	818561	99%
6	IP-6	871508	497248	1660798	817432	100%
7	MEAN			1653374.3		
8	SD			9189.74		
9	%RSD			0.558		

Acceptance criteria: Correlation coefficient – Greater than or equal to 0.999

%RSD less than 2

%ASSAY: 98-102%

CONCLUSION:

Development and validation of GC-FID method for the estimation of Quercetin in bulk and pharmaceutical dosage forms with the facilities and the results are incorporated in this thesis.

In conclusion, a validated GC-FID method has been developed for determination of Quercetin in bulk and pharmaceutical dosage form. The results show that the method was found to be specific, simple, accurate, precise, and sensitive. The method was successfully applied for the determination of Quercetin tablet dosage form. Several analytical procedures have been proposed for the quantitative estimation of Quercetin separately and in combination with other drugs.

So, attempt was taken to develop and validate a GC-FID method for the quality control of Quercetin in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

References:

1. Bartle KD, Myers P. History of gas chromatography. TrAC Trends in Analytical Chemistry. 2002 Sep 10;21(9-10):547-57. Retrieved 23 January 2017. "Slide 11 on "Gas Chromatography" presentation". slideplayer.com. Retrieved 2016-03-08.
2. Chavan V, Kamble HV, Waghmare SA. A SHORT REVIEW ON GAS CHROMATOGRAPHY.f cardiovascular disease and stroke: hypertension at the core". The Canadian Journal of Cardiology. 31 (5): 569–571. doi:10.1016/j.cjca.2015.01.009. PMID 25795106. Mendis S, Puska P, Norrving B (2011).
3. Conder JR. Physicochemical measurements: gas chromatography. Encyclopedia of separation science. Academic Press, Detroit. 2000 Jan 1:3808-15.
4. Fernandez-Fernandez M, Rodriguez-Gonzalez P, Anon Alvarez ME, Rodriguez F, Menéndez FV, Alonso JI. Simultaneous Determination of Creatinine and Creatine in Human Serum by Double-Spike Isotope Dilution Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography–Mass Spectrometry (GC-MS). Analytical chemistry. 2015 Apr 7;87(7):3755-63.