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METHOD DEVELOPMENT AND VALIDATION OF EPIGALLOCATECHIN GALLATE BY UPLC

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ABSTRACT

A simple and selective UPLC method is described for the determination of Epigallocatechin Gallate. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture Potassium di-hydrogen phosphate : Methanol(70:30) with detection of 266 nm. Linearity was observed in the range 50-150 µg /ml for Epigallocatechin Gallate (r^2 =0.995) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Epigallocatechin Gallate, UPLC method

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INTRODUCTION

UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time. According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or Linear velocities. Therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography,orUPLC. The technology takes full advantage of chromatographic principles to run separations. Using columns packed with smaller particles(less than2.5µm) and/or higher flow rates for increased speed, this gives superior resolution and sensitivity.

Epigallocatechin gallate is anti-tumor activity, EGCG promotes apoptosis through mitochondrial damage, membrane depolarization, and cytochrome c release. This apoptotic action of EGCG is inhibited by NAC or catalase suggesting that excess hydrogen peroxide may contribute to mitochondrial damage-induced cell death [1-4].Aim is to develop new stability indicating RP-UPLC method for the estimation of Epigallocatechin Gallate in bulk and its pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax)

Preparation of standard stock solution of Epigallocatechin gallate

10 mg of epigallocatechin Gallate was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of samples for Assay (6-8)

Preparation of Standard solution

10 mg of epigallocatechin Gallate was weighed and transferred in to 100 ml volumetric flask and Dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

Preparation of Sample solution

Weigh 20 capsules by removing the shell then crush with mortar and pestle then weigh a quantity of powder equivalent to 10mg of Temozolomide and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{\text{WS}}{DS} \times \frac{\text{DT}}{WT} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{LC} \times 100$$

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig-1and the absorption curve shows characteristic absorption for epigallocatechin gallate.

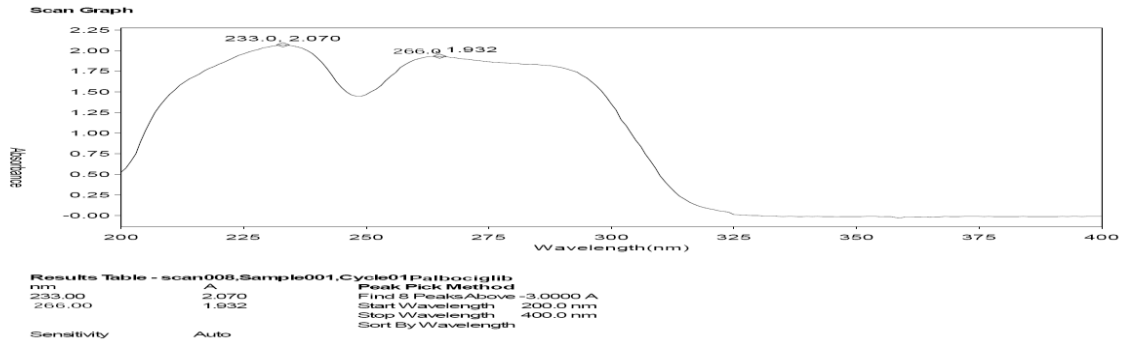

Fig-1 UV-VIS spectrum of Epigallocatechin Gallate(266 nm)

The plate count and tailing factor results were found to be within the limits andthe % RSD was found to be 0.4. so system is suitable and giving precise results (Table-1).

Table-1 Results for system suitability of epigallocatechin gallate

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.157	562.49	30521	1.22
2	2.154	563.11	30554	1.22
3	2.158	563.25	30541	1.25
4	2.158	563.58	30563	1.26
5	2.157	563.25	30568	1.24
6	2.156	563.3	30581	1.27
Mean	2.157	563.16	-	-
SD	0.00	0.36	-	-
%RSD	0.1	0.1	-	-

The %RSD of Assay for 6 Samples determinations of Epigallocatechin gallate found to be within the acceptance criteria (less than 2.0%). %Assay Also within the limits (95.0 to 105.0) hence method is precise (Table-2).

Table-2 Method precision results for epigallocatechin gallate

Injection	EPIGALLOCATECHIN GALLATE	
	Area	**%Assay**
1	558.29	99.0
2	558.73	99.1
3	559.02	99.1
4	559.15	99.1
5	558.82	98.9
6	559.44	98.9
Average		99.0
SD		0.09
%RSD		0.1

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparation 0.9996. The results % Assay and %RSD obtained acceptance criteria so method is rugged (Table-3).

Table-3 RuggednessResults of epigallocatechin gallate

IntermediatePresicion/Ruggedness		
Name of the Standard	**Area**	**%Assay**
Intermediate Precision_01	559.63	99.4
Intermediate Precision_02	559.68	99.3
Intermediate Precision_03	559.67	99.2
Intermediate Precision_04	559.74	99.4
Intermediate Precision_05	559.66	99.1
Intermediate Precision_06	558.41	97.7
	Average	99.0
	Std Dev	0.67
	%RSD	0.7
% RSD Between %Assay of both Analysts		0.3

CONCLUSION

A new precise, accurate, rapid method has been developed for the estimation of Abiraterone acetate in bulk and its pharmaceutical dosage form by UPLC.From the above experimental results and parameters it was concluded that, this newly developed method for the estimation Abiraterone acetate 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 meant in industries, approved testing laboratories studies in near future. From results the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Epigallocatechin Gallate Educational institutions and quality control laboratories.

REFERENCES

1. U.S. Food and Drug Administration. Guidance for Industry, Analytical Procedures and Methods
2. Validation for Drugs and Biologics; U.S. Food and Drug Administration: Silver Spring, MD, USA, 2015.
3. Skoog DA, West DM, Holler FJ. Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers, 1996, 1-5.
4. Sharma B K. Instrumental method of chemical analysis Meerut, 1999, 175-203.
5. International conference on the harmonization. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2 (R1); November 2005.
6. United States Pharmacopoeia. Validation of Compendial Procedures; USP32–NF27; The United States Pharmacopeial Convention: Rockville, MD, USA, 2007;
7. International conference on the harmonization. ICH Harmonized Tripartite Guideline. Stability Testing of New Drug Substances and ProductsQ1A (R2); November 2003.
8. International conference on the harmonization. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and MethodologyQ2 (R1); November 2005.