SHORT COMMUNICATIONS

Quantitative Determination of Epigallocatechin Gallate Present in GreenTea Extract by HPTLC

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A high performance thin layer chromatographic method was developed and described for the quantitative estimation of epigallocatechin gallate in green tea extract.

Green tea has been prized as a traditional tonic for keeping the body and soul in good condition[1]. The tea plant, technically known as *Camellia sinensis* is cultivated in more than thirty different countries especially in the higher altitudes of China, India, Japan and Sri Lanka[1].

Green tea extract is produced from young green tea leaves. Today, scientific research work supports the benefits of green tea extract for reducing cancer[2] and heart complaints. Catechins present in the green tea extract interfere with the binding of cancer causing substances to the DNA of healthy cells[3]. It is also reported that green tea reduces the platelet aggregation and reduces blood clots[4].

Among the catechins, epigallocatechin gallate (EGCG) is the most active ingredient in tea extract[5]. Catechins can be estimated by titration method[6], thin layer chromatography (TLC)[7] and gas chromatography (GC)[8]. High performance liquid chromatography (HPLC) profile of green tea catechins is also published[9].

Green tea leaves were collected from Munnar area of Idukki district in Kerela. All the solvents used were of AR (Analytical Reagent) grade. The reference standard, pure EGCG was purchased from Sigma Chemical Company, USA.

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A HPTLC system consisting of a TLC sample applicator device (Desaga applicator AS-30), a TLC plate development chamber (Desaga) and TLC plate scanner connected to a computer was used for the experiment. Silica gel 60 F_{254} 10 x 10 cm HPTLC glass plate (Merck) was used. Approximately 120-150 µg/ml solution of EGCG was prepared in methanol. Filtered and 3 to 24 µl solution (1 to 8 µg material) was loaded on the HPTLC plate. The mobile phase used was toluene:acetone:formic acid, 7:5:1. After developing the chromatogram up to 9 cm, the plate was dried at 100° for 5 min and scanned at 280 nm.

Fifteen grams of fresh pulverised green tea was refluxed with 150 ml methanol for 3 to 4 h. The methanolic extract was cooled, filtered and concentrated. The residue was again refluxed with 50 ml methanol for 1 to 2 h. The methanol solutions were combined and concentrated under 60° temperature. This crude extract was decaffeinated by extracting with chloroform and purified by extracting with different solvents. The purified EGCG rich extract was used for HPTLC analysis. Fifty milligrams of this purified extract was dissolved in methanol and made up to 25 ml in a standard flask. Ten milliliters aliquot was pipetted out into a 25 ml standard flask and diluted to 25 ml.

Six bands (0.3 cm length) of the standard and 2 bands (0.3 cm length) of the sample solution were loaded about 10 cm from the edge of HPTLC plate. The distance (centre to centre) between two bands was about 9 cm.

Approximately 5 to 8 µg extract was loaded on each band. The plate was developed upto 9 cm in the mobile phase, dried in the oven at 100° for 10 min, cooled and scanned at 280 nm.

UV spectra obtained for solutions of EGCG in acetate buffer at slightly acidic pH showed peaks at 273 nm for EGCG and there are no peaks in the visible region of these spectra[10]. Under the HPTLC conditions mentioned in the experimental part the Rf value obtained for both pure EGCG and EGCG in the green tea extract was 0.12. The green tea extract used was EGCG enriched green tea extract in which the major peak corresponded to EGCG. The same procedure was used for pure catechin, epicatechin and epigallocatechin for which the Rf values obtained were different.

This method is very specific, less time consuming and accurate. By this method we can determine the EGCG content of fresh green tea as well as purified green tea extracts and tablets or capsules containing EGCG. Since HPLC method and HPTLC methods are comparable this analysis procedure is helpful to industries and professionals who are more particular about epigallocatechin gallate (EGCG) rich green tea extract.

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High Performance Thin Layer Chromatographic Method for Estimation of Lovastatin from Tablets

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A new simple, specific and precise high performance thin layer chromatographic method has been developed for estimation of lovastatin in its tablet formulations. In this method, standard solutions and sample solution of lovastatin were applied on precoated silica gel G60 F_{254} TLC plate and developed using toluene:methanol (75:25 v/v) as mobile phase. The plate was scanned and quantified at 239 nm for lovastatin. The method was validated for precision, accuracy and reproducibility.

Lovastatin is an antihyperlipidemic drug, widely used in the treatment of hypercholestremia. It acts by competitively inhibiting HMG Co-A reductase, the key enzyme in cholesterol biosynthesis[1]. It is an official drug in USP[2]. Various methods like spectrophotometry[3], spectrofluorimetry[4], HPLC[5-8] have been reported for the estimation of lovastatin from its formulations. In the present article, we report a simple, specific high

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