

ABSTRACT

Validation of HPLC for Simultaneous Determination of Epigallocatechin Gallate, Epigallocatechin, Epicatechin Gallate and Epicatechin in Black Tea Product

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The aim of this study was to develop a valid HPLC method for simultaneous determination of Epigallocatechingallate (EGCG), Epigallocatechin (EGC), Epicatechingallate (ECG) and Epicatechin (EC) in black tea product and compare two extraction technique of black tea product based on EGCG recoveries. Extraction technique A was an extraction using chloroform for matrix extraction, ethyl acetate wich was continued with for EGCG extraction. Extraction technique B was an extraction using chloroform for matrix extraction and the water fraction containing EGCG was directly injected in the HPLC.

The result of this study showed that standard EGC, ECG and EC were not detected using photodiode arrays (PDA) detector because of low molar absorptivity or the standards were expired. The instrument used in this study was HPLC with RP C-18 Waters uBondapak 10 μ m, 3.9x300mm and PDA detector. HPLC optimum condition were as followed. Mobile phase was methanol:water:acetic acid 2% with ratio 35:60:5, flow rate at 0,45 ml/minute, column temperature was 30°C and wavelength detection at 276 nm. The validation result obtained that extraction technique A were selective for EGCG peak with retention time at 10,9 minute and the EGCG recovery was 88,32% with a coefficient of variation of 10,94%. While the validation result of extraction technique B showed that EGCG peak was not selective with retention time at 10,9 minute. But the EGCG recovery was 96,04% with a coefficient of variation of 3,68%. The SPSS program showed that there was a significant difference between EGCG recovery of extraction technique A and extraction technique B. Extraction technique B had a better EGCG recovery.

Keywords: HPLC, validation, black tea extract, recovery, EGCG.