Improvement Of Polyphenolic Separation Of Teas Using HPLC Analysis

Sensus Technical Note (SEN-TN-0014) 02/06/2009

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ABSTRACT

In order to determine accurate concentrations of polyphenolic compounds in various aqueous samples using HPLC, polyphenolic compounds should be properly separated. In the present study, polyphenolic separation was improved using modified gradient mobile phase.

INTRODUCTION

Tea contains various classes of polyphenolics and methylxanthins such as flavan-3-ols, flavanols, phenolic acids, caffeine, and theobromine. When determining concentrations of these compounds, proper separation on an HPLC column is required to prevent co-elution with other chemical compounds. This could be improved using a suitable gradient system and mobile phases. In this study, a new HPLC analytical method was developed to determine the polyphenolic and methylxanthin concentrations in teas.

MATERIALS AND METHODS

Tea preparation: Green and black teas (1g each) were brewed with hot water (90°C) for 10 min. The tea infusion was immediately filtered through cheese cloth followed by Whatman #4 filter paper.

Each tea infusion (green and black tea) was diluted 3-fold with RO water and filtered through a 0.45 µm PTFE syringe filter (Whatman, Clifton, NJ) prior to injection. Polyphenolic separations was conducted on a Agilent 1200 HPLC system using a Agilent G1315B Diode Array Detector (DDA) with a Dionex 250 x 4.6 mm Acclaim 120-C_{18} column with a flow rate of 0.8 mL/min. A gradient mobile phase consisted of Phase A (100% H_2O) and Phase B (60% Methanol and 40% H_2O) each adjusted to pH 2.4 using *o*-phosphoric acid. The gradient started by running 0% Phase B for 1min, 0-30% Phase B over 30min, 30-80% Phase B in 15min, 80-100% Phase B in 15min, followed by a 5 minute equilibration time with 100% phase A for a total run time of 65min. This gradient system was illustrated in Table 1. Phenolic compounds were detected and quantified at 280 nm against external standards of gallic acid, theobromine, (−)-epicatechin, (−)-

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epigallotcatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate, and caffeine, all procured from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO).

Table 1. New gradient system with optimized mobile phases (A and B). A gradient mobile phase consisted of Phase A (100 % H_2O) and Phase B (60 % Methanol and 40% H_2O) each adjusted to pH 2.4 using *o*-phosphoric acid and flow rate was set to 0.8 ml/min.

Time (min)	Mobile A (%)	Mobile B (%)
0	100	0
30	50	50
45	30	70
60	0	100
60.5	100	0
65	100	0

RESULTS AND DISCUSSION

A new gradient system, C_{18} colulmn (Dionex Acclaim 120, C_{18}, 5µm analytical, 4.6x250mm), and mobile phases (combination of water and methanol at pH 2.4) have been introduced for more accurate identification and quantification of green and black teas.

Polyphenolic separation was dramatically improved over the previous method. The peak width was shortened and no peak shoulder was observed.

Figure 1 describes how polyphenolic separation was improved compared to old method.

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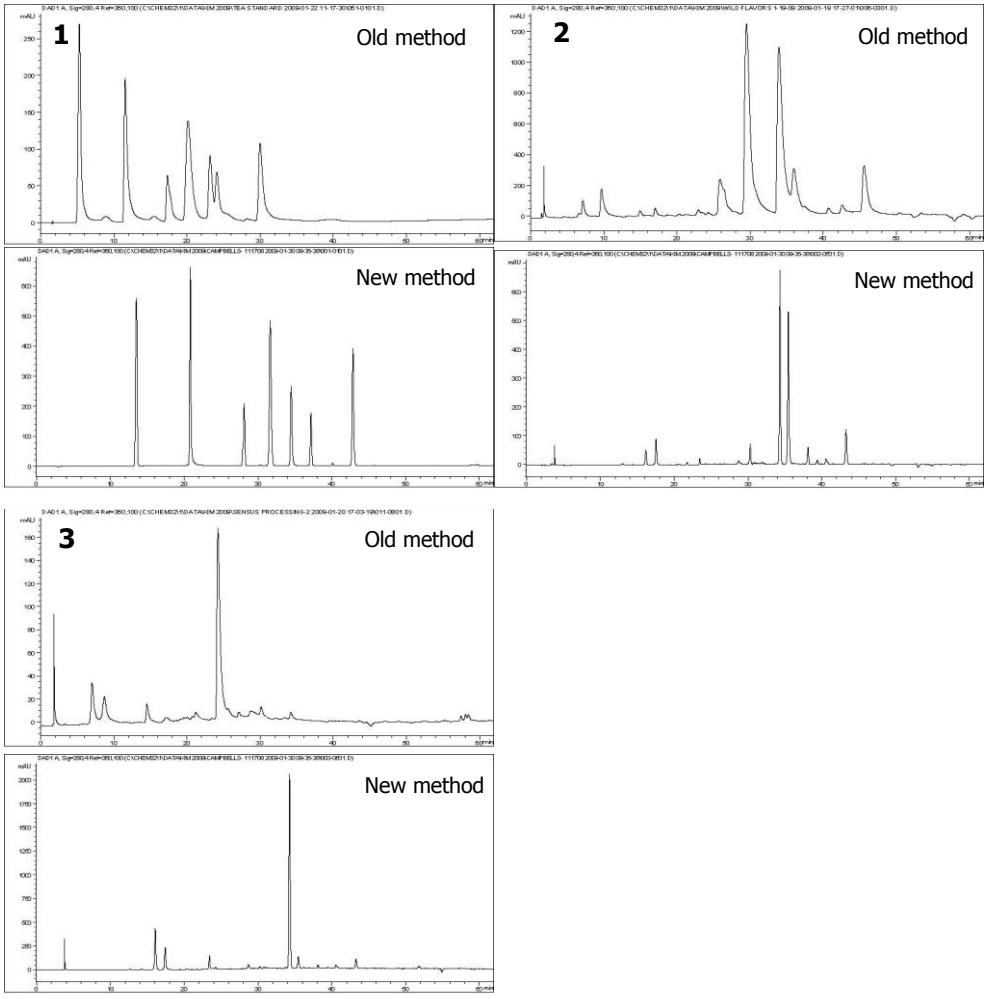
Figure 1. Polyphenolic separation using HPLC at 280nm. 1. Tea catechin standards (gallic acid, theobromine, EGC, caffeine, EGCG, EC, ECG, respectively), 2. Green tea, 3. Black tea.



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