Automated Method Development in HPLC for the Quantitative Determination of Catechins in Tea

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ABSTRACT

Method development is always a challenging and time-consuming task for the analyst. In-depth chromatographic knowledge is required for optimization of gradient elution, especially if difficult or complex separations are required. In this work we developed a simple, fast and sensitive high performance liquid chromatography (HPLC) method with ultraviolet detection (UV) for determining catechin levels in different types of tea, by applying a software-based automated method development workflow.

INTRODUCTION

Tea is the most consumed beverage in the world, with black tea and green tea being the most popular types. For years, there has been a trend toward higher consumption of health-promoting foods and beverages [1, 2]. Especially catechins play an important role due to their antioxidant potential. In particular, it is assumed that white tea contains the highest catechin levels because the leaves are processed under relatively mild conditions. Compared to other teas, the leaves of white tea are only dried, and minimally further processed. Green tea, is briefly heated or roasted, which may lead to a reduction in catechin content, while in black tea the lowest catechin levels are expected due to its fermentation process which results in oxidation of catechins [3].

The goal of the current work was to develop a simple, fast and sensitive high performance liquid chromatography (HPLC) method with ultraviolet detection (UV) for the determination of catechin levels in tea types by applying a software-based automated method development workflow.

ChromSwordAuto® 5 Software was selected as a tool for automated method development. Through sophisticated algorithms, this software package is able to optimize methods with complex multi-step gradients and can significantly help to reduce the required lab time of analysts during the process. Four different types of stationary phase and four aqueous mobile phases were screened with either acetonitrile or methanol as the organic solvent. The best condition was then selected for quantification of catechins in tea samples.

SAMPLE PREPARATION

White tea, green tea and black tea were purchased in a local supermarket.

100 mL of hot water was poured over one tea bag and left to stand according to the manufacturer's note on the tea box, as listed in Table 1.

Table 1. Tea samples analyzed.

Tea type	Tea amount [g/ Tea Bag]	Brewing Time [min]
White tea (Jasmin)	1.25	3
Green tea	1.75	5
Black tea	1.5	4

For quantitative analysis the samples were diluted 1:5, 1:10, 1:20, respectively, with water prior to injection. All samples were prepared in triplicate and injected three times each within one day.

CALIBRATION

External calibration of Catechin, Epicatechin, Epicatechin gallate, Epigallocatechin, Epigallocatechin gallate, Gallocatechin and Gallocatechin gallate was performed in the range of 1 to 100 µg/mL by diluting stock solutions (1 mg/mL) with the appropriate volume of water. The limit of detection (LOD) and the limit of quantification (LOQ) were estimated by extrapolation from the signal-to-noise (S/N) ratio of the smallest calibration point with 1 µg/mL.

INSTRUMENTATION AND METHODS

A Thermo Scientific™ UltiMate™ 3000RS system was used for the analysis.

- Solvent rack with 4 Degasser channels SRD-3400 (P/N 5035.9245)
- Quaternary Pump LPG-3400RS (P/N 5040.0036)
- Well Plate Sampler WPS-3000TRS (P/N 5840.0020)
- Thermostatted Column Compartment TCC-3000RS (P/N 5730.0000)
- Photodiode Array Detector DAD-3000RS (P/N 5082.0020)
- Semi-micro flow cell, SST, 2.5 µL (P/N 6080.0300)

Data Analysis

ChromSwordAuto 5 Software 5.0.437.633 with the modules of the ChromSwordAuto Scout for pre-screening experiments, and the ChromSwordAuto Developer for the automated method development and optimization were used. The ReportViewer module of ChromSwordAuto was used for data analysis and evaluation during the method development process and for reporting.

Thermo Scientific™ Chromeleon™ 7.2.8 CDS software was used for data acquisition and processing of the quantitative experiments.

Automated method development workflow

The method development workflow included the following tasks:

- Column and mobile phase scouting
- Rapid optimization
- Fine optimization

The ChromSwordAuto Developer module was used for column and mobile phase scouting, rapid optimization and fine optimization tasks. Column and mobile phase scouting was performed during the rapid optimization task using four different stationary phase, four different aqueous and two organic eluents (Table 2). Afterwards, fine optimization was carried out with the best combination.

Table 2. Columns, aqueous and organic eluents used for the ChromSwordAuto Developer-rapid optimization task.

Columns
Thermo Scientific™ Hypersil GOLD™ aQ column (100 x 2.1 mm, 1.9 µm)
Thermo Scientific™ Acclaim™ VANQUISH™ Polar Advantage II column (150 x 2.1 mm, 2.2 mm)
Thermo Scientific™ Accucore™ Polar Premium LC column (100 x 2.1 mm, 2.6 µm)
Thermo Scientific™ Accucore™ Phenyl-X LC column (100 x 3 mm, 2.6 µm)
Aqueous Eluent
Water + 0.1% acetic acid, pH 3.3
Water + 0.1% formic acid, pH 2.65
20 mM ammonium acetate, pH 3.8
20 mM ammonium formate, pH 3.8
Organic Eluent
Acetonitrile
Methanol

RESULTS

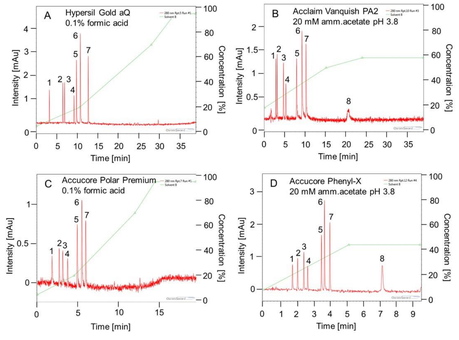
Software-based automated method development

1) ChromsSwordAuto Developer – rapid optimization task

During automated method development four columns, four different aqueous and two organic eluents were tested, as listed in Table 2. A 10 µg/mL standard mixture including the seven catechins served as a sample.

With the rapid optimization algorithm, up to five experiments were performed for each possible combination, resulting in a total of 69 methods. Figure 1 summarizes the best condition of each stationary phase based on the separation results and run time. The results showed that the rapid optimization algorithm could find suitable conditions to separate the analytes on all tested stationary phases, while the combination of Accucore Phenyl-X column, 20 mM ammonium acetate pH 3.8 buffer and acetonitrile provided the best separation performance and the shortest run time.

Figure 1. Summary of best methods obtained on each column, with respect to the ideal eluent composition (buffer/ acetonitrile) after running rapid optimization (green line represents the gradient profile)



The run time of the chosen method was 9.4 min, resulting in a baseline separation of all seven catechins within 4 min (Table 3). Peak 8 in the chromatograms Figure 1B and 1D was identified as an impurity of acetate and formate salts. However the impurity peak is well separated from the target analytes.

Table 3. Peak assignment of the seven catechins of Figure 1D

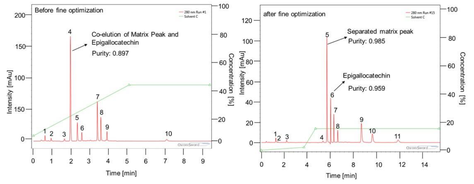
Peak #	Retention Time [min]	Compound	
1	1.7	GC	Gallocatechin
2	2.0	EGC	Epigallocatechin
3	2.3	C	Catechin
4	2.6	EC	Epicatechin
5	3.4	EGCG	Epigallocatechin gallate
6	3.6	GCG	Gallocatechin gallate
7	3.9	ECG	Epicatechin gallate

2) ChromSwordAuto Developer – fine optimization and sample profiling task

For the fine optimization the ChromSwordAuto Developer module in sample profiling mode (gradient and isocratic) was chosen. To include possible matrix interferences all three types of tea were injected and green tea selected for further optimization because it showed the highest number of matrix peaks.

Figure 2 shows chromatograms obtained before and after the fine optimization of the gradient profile for the selected combination of the column and mobile phase

Figure 2. Comparison of separation results before and after fine optimization (green line represents gradient profile)



The peak purity was used to assess the quality of the separation based on UV spectral data. It was found that the purity value for EGC was determined to be 0.897 before the fine optimization step, while all other compounds showed at least a peak purity of 0.944 or better. This leads to the assumption that EGC co-eluted with another analyte with the method obtained by the rapid optimization task. After applying sample profiling, a major matrix component was successfully separated from EGC and the peak purity of EGC increased to 0.959.

In summary, the new method (Table 4) was developed within 5 days (calculation based on 24 hours/ day for instrument time and 8 hours/per day for analyst time).

We assume that for manual method development the time required to find the optimal method for all screened stationary and mobile phase would have been much longer. In addition, the success of a manual method development depends crucially on the experience of the analyst, while software-based automated method development uses sophisticated algorithms to optimize the separation, without user interaction

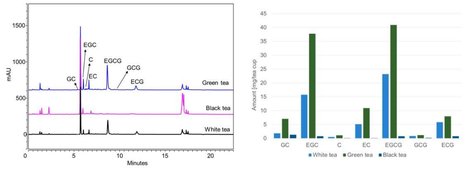
Table 4. Chromatographic Conditions of final optimized method

Parameter	value	
Column	Accucore Phenyl-X (100 x 3 mm, 2.6 µm) (P/N 27926-103030)	
Mobile Phase	A: 20 mM ammonium acetate, pH 3.8 B: Acetonitrile	
Flow rate	1 mL/min	
Gradient	Time [min]	%B
	0	0
	3.7	2
	4.7	15
	15.4	15
	16.0	90
	18.0	90
	18.1	0
	22.5	0
Column Temp	30 °C	
Sampler Temp	10 °C	
UV	λ = 280 nm, data collection rate = 5 Hz, response time = 1 s	
Injection vol.	1 µL	

Quantitation of catechins in tea samples

External calibration was performed in the range of 1 µg/mL – 100 µg/mL. Linearity was found to be excellent with 0.9984 – 0.9995. Low LOD and LOQ values could be achieved (LOD < 0.2 µg/mL and LOQ < 0.7 µg/mL), with the exception of GC and EGC, which show slightly higher values up to 8 µg/mL for LOQ.
Looking at the chromatograms (Figure 3) it can be seen that all seven catechins were detected in all three samples, but in quite different amounts. In order to obtain the content of catechins in one cup of tea, a cup volume of 200 mL was assumed and the brewing time stated by the manufacturer on the tea bag was respected. The results obtained are presented in the bar chart of Figure 3.

Figure 3. Left: Overlaid chromatograms of three tea samples with assigned catechin compounds (blue: Green tea, purple: Black tea; black: White tea)
Right: quantitative results of all seven catechins in each tea sample.



CONCLUSIONS

- ChromSwordAuto 5 software provides fully automated method development on an UltiMate 3000 HPLC system.
- Sophisticated algorithms allow straightforward method development even for non-experienced analysts with significant time-saving compared to manual development.
- The highest total catechin content was found for green tea with 107 mg/tea cup, followed by white tea at 53 mg/ tea cup, and black tea with 4 mg/ tea cup.

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