Vol. 1/2 (2001) 151 - 154

Short Communication

JOURNAL OF NATURAL REMEDIES

Quantitative and qualitative analysis of (-)-epigallocatechin gallate from Green tea by LC-MS

D. C. Umashankar

Application Laboratory, Waters (India) Pvt. Ltd., 36A, 2nd Phase, Peenya Industrial Area, Bangalore - 560 058

Received 6 June 2001 ; Accepted 25 June 2001

Abstract

Objective: To study the rapid analysis of a natural product using parallel processing of High Performance Liquid Chromatography (HPLC) with on-line mass detection. Materials and methods: The parallel processing of HPLC with mass detection(LC-MS) was used to decrease the cycle time between the analysis, such as (-)-epigallocatechin gallate (EGCG) from green tea. Results and conclusion: The parallel processing LC-MS method provided a rapid procedure for qualitative and quantitative analysis of EGCG from green tea sample.

Key words: Green tea, Natural products, LC-MS, (-)-epigallocatechin gallate

1. Introduction

Green tea contains significant amounts of the polyphenolic compound (-)-epigallocatechin gallate (EGCG, Figure 1) with reported anti-carcinogenic properties [1-7] along with many other biological effects. As interest in the human health benefits of EGCG expands, the need for advanced analytical methods for the qualitative and quantitative analysis of these compounds increases.

Traditional analytical methods for EGCG and related compounds contained in green tea include TCL, GC. While many of the HPLC methods provide useful information, they are incapable of providing confirmational molecular weight information since identification is based solely upon retention time comparisons. Flow injection Mass Spectrometry does provide molecular weight information, however this non-chromatographic technique cannot differentiate between EGCG and isomers of identical mass.

This paper describes the rapid analysis of a natural product (EGCG) contained in biological matrices using parallel processing of HPLC with on-line mass detection.

Corresponding author
E - Mail : Uma_Shankar@Waters.com

2.0 Materials and methods

2.1 HPLC and Mass Spectrometry Conditions:

LC-MS was performed using a Waters Alliance HT HPLC system connected in series to Waters 996 photodiode array (PDA) detector followed by a Waters ZMD Mass Spectrometer fitted with an atmospheric pressure chemical ionisation (Apcl) probe. Gradient chromatography was performed at 35°C using a Waters XterraTM MS C18 column (5µ, 2.1 x 50 mm) at a flow of 1.0ml/minute using 0.10% formic acid (v:v) in water (solvent A) against CH_3CN (solvent B). Seperation and detection conditions are shown in Table 1.

All data were collected and processed using MassLynzTMNT software. Samples were prepared for analysis by adding boiling water (100 ml) to green tea leaves (2 g) with gentle stirring, followed by sample filtration and dilution (20x) with water.

To increase sample throughput (decrease cycle time) and increase compound response (enhance peak concentration) a short, narrow-bore column (2.1 x 50 mm) was used at an analytical flow of 1 ml/min. (Note: Cycle time is defined as the sum of run, system equilibrium, column equilibrium, and vial sampling times). In addition, the high throughput (HT) HPLC system was run in "Parallel Mode" with rapid equilibration (2 min) between analyses.

In Parallel Mode, several processes occur during the chromatographic separation to help speed cycle time (ie., 1: Injector needle is washed and purged and 2: The next sample is drawn and prepared for injection). To further increase sample throughput, the column is taken out of the flow path following sample elution so that the system tubing can be rapidly flushed with initial condition eluent (5 ml/min) before restoring flow for column re-equilibration (1 ml/min).

APcI Mass Spectrometry was performed in the positive ion mode using Single Ion Recording (SIR) at the pseudomolecular weight of EGCG (459.1 m/z). The selected pseudomolecular weight was determined from the MS analysis of an EGCG standard while scanning from 250 to 500 m/z.

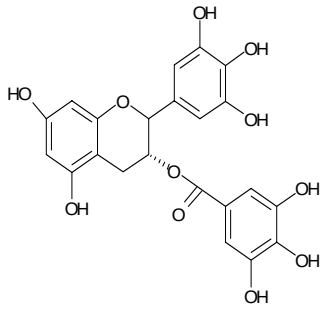

Fig. 1 : (-)-epigallocatechin gallate (EGCG) $C_{22}H_{18}O_{11}$ Mol. Wt. : 458.4

Table 1.
LC-MS Conditions

HPLC Gradient Conditions			Mass Spectrometer (APcI) Conditions	
Time(min)	**%A[a]**	**%B[b]**	Source Temp	140°C
0	95	5	Probe Temp	500°C
3	95	5	Corona Voltage	3.30 kV
6	75	25	Cone Voltage	30V
6.1	95	5	Nebulizing gas	N_2 at 500 L/hr

[a] 0.10% Formic acid (v/v) in water ; [b] Acetonitrile

2.2 Calibration and Quantitation

A calibration curve was made for EGCG from 5.0 to 100.0µg/ml. The resulting data yielded an excellent calibration curve using a quadratic equation (r^2 value greater than 0.9998). To ensure low levels of EGCG carryover between injections, the sample manager of the Alliance HT system was washed with a mixture of acetonitrile and water (1:1) (6 seconds injector port wash and a 15 second exterior needle wash).

Following the wash, the injector was flushed with 600µL of purge solvent (0.10% formic acid). Because this process takes place in parallel during the sample analysis, incorporation of the needle wash step does not increase cycle time. A blank injection performed immediately after the 100µg/mL standard showed no detectable carryover from the EGCG standard.

3. Results and discussions

The Parallel Processing in HPLC with short, narrow-bore columns resulted in higher sample throughput. Typically, HPLC systems running gradient elution methods need to be equilibrated for a total of 3x system volume plus 5x column volume [8].

Using traditional analytical columns (4.6 x 250 mm), column equilibration can add up to 15 minutes (at 1 ml/min) to the total cycle time making the analyses of large numbers of samples difficult. The combination of a short, narrow-bore column (2.1 x 50 mm) at an analytical flow rate combined with the rapid system equilibration, and parallel processing allowed both the system and column to be equilibrated in just under 3 min. In addition, chromatographic separation or column life were not compromised.

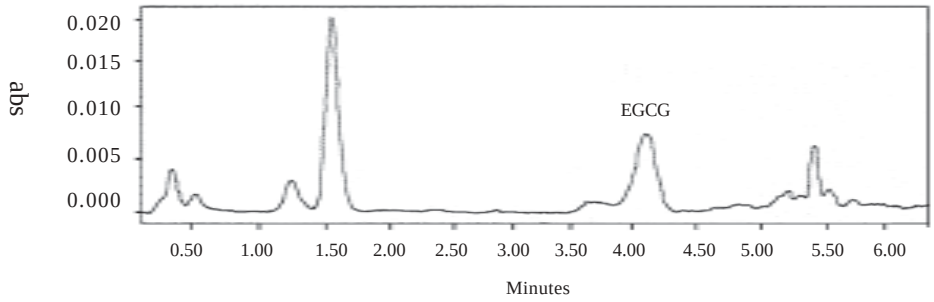

Figure 2. HPLC chromatogram of green tea sample at 274 nm

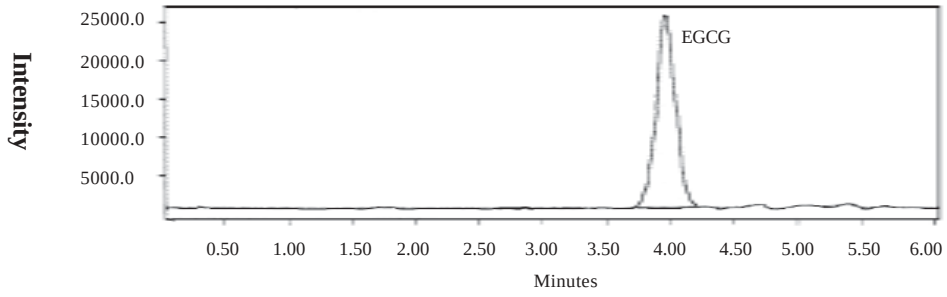

Figure 3. Chromatogram of green tea sample from LC-MS with SIR at 459.1 m/z

The use of Mass Spectrometry yields a high degree of compound specificity in the analyses of complex samples. Figure 2 shows the analysis of a green tea sample using only HPLC with UV detection at 274 nm. While Integration of the EGCG peak is possible, interference from leading and tailing peaks compromises the ability to obtain accurate quantitation. By comparison, integration of the single EGCG peak using SIR at 459.1 yields superior results (Figure 3).

In addition, mass spectrometry provides a confirmational molecular weight information compared to traditional HPLC methods that rely upon retention time comparisons between sample and standard runs for compound identification. The synergistic value of LC/MS technique provides both retention time and molecular weight information for the analysis of EGCG in green tea.

Although direct infusion (flow injection) of the sample into the mass spectrometer would provide accurate quantitation, erroneous values could result due to the presence of isomers in the sample that have the same molecular weight as EGCG.

For example, the compound gallocatechin gallate ($C_{22}H_{18}O_{11}$, mol. wt.=458.4)is also present in green tea. Presence of this compound could interfere with the accurate quantitation of EGCG if a chromatographic separation did not preceed quantitation by mass detection. However, gallocatechin gallate is adequately resolved from EGCG using the described reversed-phase technique (4.70 min for gallocatechin gallate vs. 3.85 min for EGCG)making the accurate quantitation of the HPLC resolved EGCG possible.

4. Conclusion

The Parallel HPLC HT system with online mass detection provides a synergestic approach to the rapid analysis of natural products like EGCG contained in biological matrices. The chromatography prior to SIR mass detection allows isomers to be adequately separated from target compounds resulting in superior EGCG quantitation compared to use of non LCMS techniques (eg. Flow injection analysis).

Use of short, narrow-bore columns at analytical flowrates combined with the advanced capabilities of the parallel HPLC HT system increases sample throughput and peak response by decreasing cycle time between analysis.

References

1. Hamilton-Miller JM.(2001) *J. Med. Microbial.* 50(4): 299 -302.

2. Jung YD, Kim MS, Shin MA, Chay KO, Ahn BN, Liu W, Bucana CD, Gallick GE, Ellis LM. (2001) *Brit. J. Cancer.* 23; 84 (6): 844 -850.

3. Krul C, Luiten-Schuite A, Tenfeelde A, Van Ommen B, Verhagen H, Havenaar R. (2001) *Mutation Res.* 1;474(1-2): 71 - 85.

4. Ismeura M, Saekik, Kimura T, Hayakawa S, Minami T, Sazuka M. (2000) *Biofactors.* 13(1-4): 81-85.

5. Yang CS, Chung JY, Yang GY, Li C, Meng X, Lee MJ. (2000) *Biofactors.* 13 (1-4) : 73-79.

6. Miyazawa T. (2000) *Biofactors.* 13(1-4): 55-59.

7. Fujuki H, Yoshizawa S, Horiuchi T, Suganuma M, Yatsunami J, Nishswaki S, Okabe S, Nishswaki-Matsushima R, Okuda T, Sugimura T. (1992) *Preventive Med.* 21: 503-509.

8. Li JB, Morawski J. (1998) *LC.GC.* 16 (5): 468-476.