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Review

Determination of tea catechins

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Abstract

An overview of analytical methods for the measurement of biologically important tea catechins is presented. Liquid chromatography and capillary electrophoresis are the most cited techniques for catechin separation, identification and quantitation. Liquid chromatography with ultraviolet detection is frequently used; however, mass spectrometry, electrochemical, fluorescence and chemiluminescence detection are also utilized in cases where more sensitive or selective detection is needed. Two modes of capillary electrophoresis, capillary zone electrophoresis and micellar electrokinetic capillary chromatography, have been employed for the determination of catechins. Both modes of capillary electrophoresis are based on ultraviolet detection. Additional analytical techniques, such as gas chromatography, thin-layer chromatography, paper chromatography, spectrophotometry, biosensing, chemiluminescence and nuclear magnetic resonance spectroscopy have also been utilized for the determination of catechins and are reviewed herein. © 2000 Published by Elsevier Science B.V.

Keywords: Tea; Food analysis; Reviews; Catechins; Phenolic compounds

Contents

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1. Introduction

Tea is one of the most widely consumed beverages in the world [1]. Although consumption of tea has primarily been associated with countries in Asia and Europe, the heightened popularity of this beverage throughout the world in recent years may be due in part to evidence of a relationship between tea consumption and prevention of certain forms of human disease. Most recently, the role of tea consumption and tea polyphenols in the prevention of cancer and cardiovascular disease has received a great deal of attention [2–10], although epidemiological studies to support this relationship have been inconsonant [8,11]. Nevertheless, several recent laboratory studies have provided evidence supporting a role for tea and tea polyphenols (catechins), in the inhibition of cancer in animal models [3,11]. The eight most abundant naturally occurring tea catechins, (+)-catechin (C), (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-catechin gallate (CG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG) are illustrated in Fig. 1. These natural products have strong antioxidant activity [4,12,13], and have numerous potentially beneficial medicinal properties including inhibition of carcinogenesis [11,14,15], tumorigenesis [16,17], and mutagenesis [13,18,19], as well as the inhibition of tumor growth and metastasis [7,20]. In addition, these bioflavonoids have antibacterial [21], antiviral [22], and antiallergic [23] properties, and have been demonstrated to induce apoptosis [24,25], inhibit platelet aggregation [26], and inhibit human immunodeficiency virus (HIV) reverse transcriptase [27]. Detailed discussions of the chemistry and health effects of tea and tea catechins are recommended to the interested reader [2,4,5,8,11,28,29].

The current interest in the health effects of tea and the investigation of natural materials as a source of chemotherapeutic agents [30,31] has necessitated the development of new analytical methods for the determination of natural products such as the tea catechins listed above. Typical catechin content in a green tea infusion is approximately 60 mg/g tea leaves (range: 9 mg/g to 117 mg/g). A discussion of the variety of methods developed for the measurement of tea catechins is the focus of this review.

2. Liquid chromatographic determination of tea catechins

A summary of liquid chromatography (LC)-based methods for the measurement of catechins in tea and biologically derived mixtures is presented in Table 1. The existing literature on this subject is replete with methods for the measurement of catechins, however, the quality of the separations and data presented in these reports vary greatly. This variance necessitates a critical perspective that highlights only those reports that present complete and efficient separation of tea catechins and support identification and/or quantification of these compounds in tea or at low levels in biofluids. Reports that describe methods which provide inadequate separation of the catechins or that do not present advantages over previously described separation systems for the same analytes will not be discussed.

2.1. Liquid chromatography with UV absorbance detection for the determination of tea catechins

The method of choice for the analysis of catechins in tea has traditionally been reversed-phase LC with UV absorbance detection. The use of LC for the determination of tea constituents was first reported in 1976 by Hoefler and Coggon [32]. The report demonstrated identification of five catechins (C, EGC, EC, EGCG and ECG) directly in a green tea infusion. The separation was somewhat lacking in resolution, but represented an early and significant achievement toward the identification and measurement of catechins in tea. No significant improve-

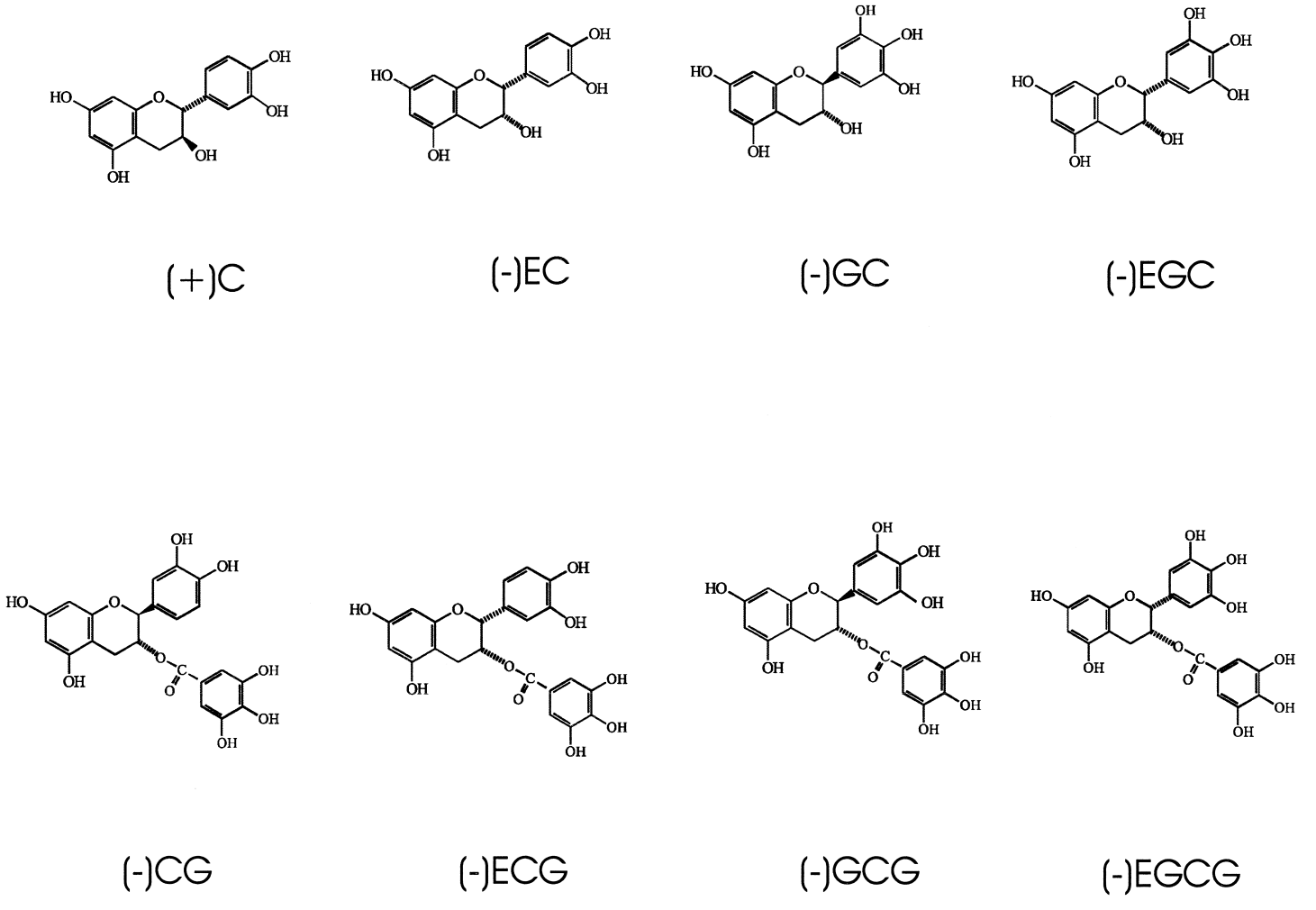

Fig. 1. Structures of eight catechins from green tea. (+)-Catechin (C); (−)-epicatechin (EC); (−)-gallocatechin (GC); (−)-epigallocatechin (EGC); (−)-catechin gallate (CG); (−)-epicatechin gallate (ECG); (−)-gallocatechin gallate (GCG); (−)-epigallocatechin gallate (EGCG).

Table 1
Summary of liquid chromatography based methods for the determination of tea catechins

Method	Sample	Ref.
LC–UV	Green tea extract[a]	[32–35,70–74]
LC–UV	Green tea infusion[b]	[8,37,75–78]
LC–UV	Commercial tea drinks	[79]
LC–UV	Saliva	[36]
LC–coulometric electrode array detection	Plasma/urine	[43–45]
LC–fluorescence detection	Plasma	[46]
LC–electrochemical detection	Green tea extract	[80]
LC–fluorescence detection	Plasma	[47]
LC–fluorescence detection	Plasma/saliva	[48]
LC–chemiluminescence	Green tea extracts	[49]
LC–thermospray MS	Green tea extract/infusion	[39]
LC–electrospray MS	Green tea infusions/plasma	[40]
Electrospray MS	Tea extracts	[42]
Electrospray, electron ionization, and fast atom bombardment mass spectrometry	Tea extracts	[41]

[a] Catechins extracted with organic or organic/aqueous solvent.
[b] Catechins extracted in hot water as in brewing a cup of tea.

ments were reported for the measurement of catechins by LC until 1996 when Goto and co-workers developed an LC–UV method capable of separating the eight naturally occurring tea catechins shown in Fig. 1 [33–35]. This system consisted of a C_{18} reversed-phase LC column (details regarding the chemistry of the C_{18} solid support were not provided) utilizing a water–acetonitrile–phosphoric acid mobile phase composition. Although the mobile phase and gradient system utilized for the separations were complex, and the quality of the separations was shown to be dependent on column temperature, the report represents the benchmark for the comprehensive separation of tea constituents. A typical chromatogram showing the determination of eight catechin standards and the effect of column temperature on the separation is illustrated in Fig. 2. Separation and identification of catechins in 85 samples of commercially available green teas [35] indicated that four catechins (EGC, EGCG, EC and ECG) represented

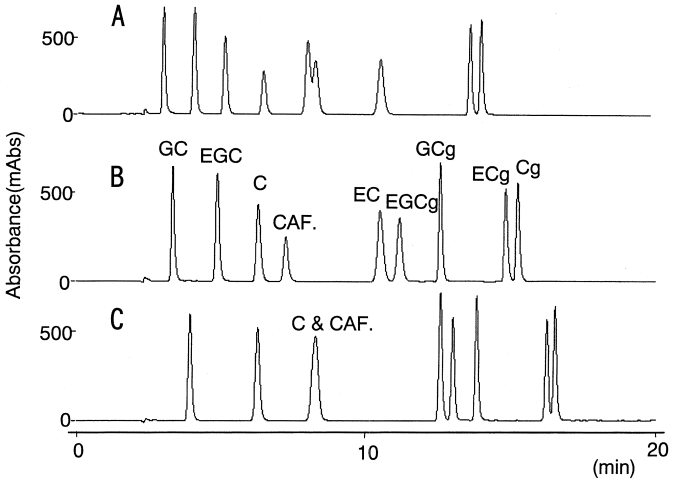

Fig. 2. Chromatographic analysis of standard catechins and caffeine and the effect of column temperature on their separation. (A) 50°C; (B) 40°C; (C) 30°C. Reproduced with permission from Ref. [34].

the largest percentage of catechin content in tea, although GC, C and GCG are also present in appreciable amounts. The determination of catechins in human saliva using LC–UV has also been reported [36], as catechins are believed to play a role in the prevention of dental caries. Detection of eight catechins extracted from human saliva after mouth-rinsing with a green tea extract was accomplished. The chromatographic separation and efficiencies for the reported analyses were excellent, displaying baseline resolution of all eight catechins. However, the catechin concentrations in saliva were reported with poor reproducibility [relative standard deviations (RSDs) in the range 20% to 25%].

A systematic study of the effect of column selection and mobile phase composition on the separation of six prominent green tea catechins and caffeine

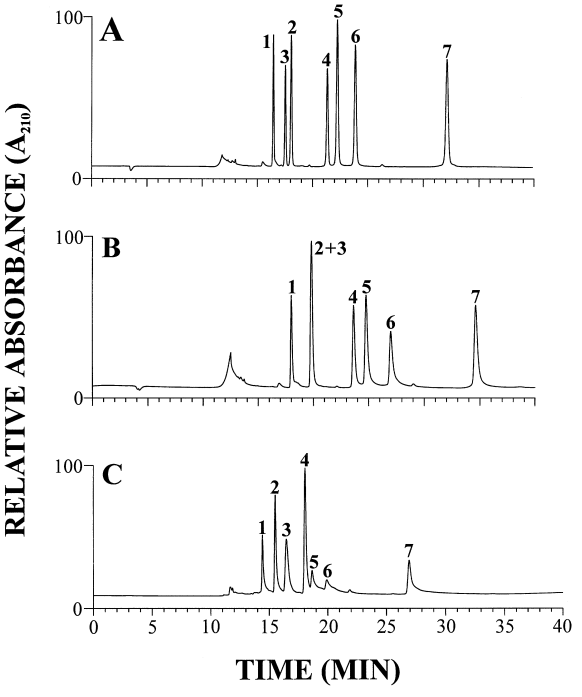

Fig. 3. The effect of stationary phase and acid-containing mobile phase on the separation of a standard catechin mixture. (A) Separation utilizing endcapped, deactivated C_{18} reversed-phase stationary phase and acid-containing mobile phase; (B) separation utilizing standard monomeric C_{18} stationary phase and acid-containing mobile phase; (C) separation utilizing endcapped, deactivated C_{18} stationary phase without acid-containing mobile phase. Reproduced from Ref. [37].

appeared in 1998 [37]. Emphasis was placed on achieving baseline resolution of six catechins and caffeine present in green tea, on chromatographic efficiency, and on the resolution of the structural isomer pairs GCG/EGCG and C/EC to assure accurate quantification of all species. The study described the development of two systems for the LC measurement of catechins that use well-defined commercially available columns and uncomplex mobile phases making the method easily accessible and adaptable to techniques such as directly combined liquid chromatography–mass spectrometry (LC–MS). Two notable points emerged during the development of these systems: (1) complete separation of the catechins and chromatographic quality were column-dependent, with endcapped, deactivated, monomeric C_{18} columns preferable over non-deactivated monomeric or polymeric C_{18} columns (Fig. 3A, 3B) and (2) the presence of acid in the mobile phase was essential to both complete resolution of the catechins and efficient chromatography of these compounds, specifically the elimination of peak tailing (Fig. 3A, 3C). The efficacy of one of the developed systems was demonstrated by the quantitative measurement of the six biologically active catechins in aqueous infusions of green tea (*Camellia sinensis*). Overall precision values for the analyses were within the range 0.3% to 1% RSD.

2.2. Liquid chromatography with MS detection for the determination of tea catechins

Atmospheric pressure ionization mass spectrometry, including the techniques of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and thermospray ionization (TSI), is characterized by the formation of ions from solutions that are sprayed from a needle held at high voltage [38]. This feature makes these techniques particularly compatible with liquid separation methods such as liquid chromatography. The first report of directly combined LC–MS for the identification of catechins isolated from tea appeared in 1993 [39]. This report demonstrated the separation of an isolated catechin mixture (EC, EGC, ECG, EGCG) with detection employing TSI-MS. Tandem mass spectrometry with collisionally-induced dissociation of the catechin ions was also utilized for identification

and assignment of fragment ions of these analytes. LC separations illustrated in this report were characterized by relatively poor chromatographic resolution and efficiency. The selectivity of mass spectrometric detection, however, minimizes problems associated with inadequate separation quality. Directly combined LC–ESI-MS for the separation and detection of catechins in green tea and human plasma has been reported [40]. In that study, the determination (≅20 ng) of six catechins in a green tea infusion, and the most extensively studied catechin, (−)-epigallocatechin gallate (EGCG), in human plasma was demonstrated by capillary LC–ESI-MS with selected ion monitoring of protonated molecular ions (Fig. 4). The overall quality of the analysis was shown to be dependent on the use of a capillary column with a deactivated, monomeric C_{18} stationary phase. The high chromatographic separation efficiency of this packed-capillary column, combined with the high sensitivity and selectivity afforded by the mass spectrometer as detector, provide a reliable approach to the analysis of picomolar quantities of catechins in complex matrices. Direct mass spectrometric characterization of catechins in tea extracts without the use of LC has also been demonstrated [41,42]. Electrospray ionization [42], electron impact [41], and fast atom bombardment MS [41] have been employed to provide both molecular mass and structural information for selected catechins.

2.3. Determination of catechins in biological fluids

The application of LC methods to the measurement of catechins in blood plasma, urine and saliva has been pursued as a means to understand better the bioavailability and pharmacokinetics of these bioflavonoids, as well as links between tea consumption and prevention of chronic disease. Levels of catechins in biofluids such as plasma and urine following tea consumption have been measured in the range of 50 ng/ml to 300 ng/ml [43], and require highly sensitive detection methods for accurate quantification in these matrices. A number of methods have been described including LC with coulometric electrode array detection (coularray) [43–45], fluorescence detection [46–48], and chemiluminescence detection [49]. Lee and co-workers presented the first report of the analysis of plasma and urinary tea polyphenols in human subjects employing LC with coularray detection [43,45]. Detection limits on the order of 1.0 ng/ml for three catechins (EGC, EC, EGCG) were estimated. Quantification of these three catechins in human plasma and urine following tea consumption was reported with overall precision values within the range 5% to 11% RSD. A similar study of the bioavailability of four catechins (EGC, EGCG, EC, ECG) in the plasma and urine of patients after consumption of black tea has been described by Warden et al. [44]. Determination of

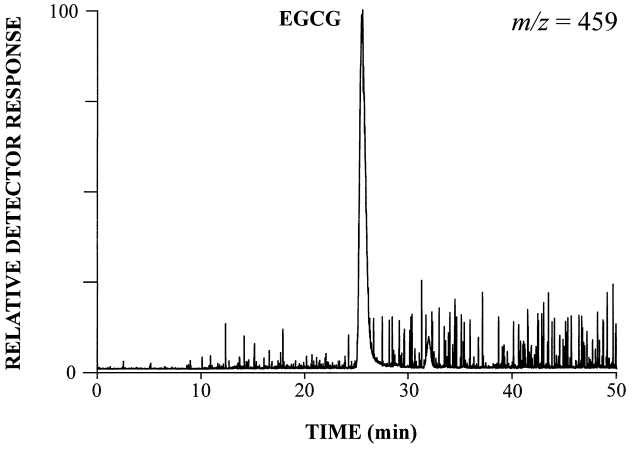

Fig. 4. Determination of EGCG in human plasma using capillary LC–MS. Column: 30 cm×506 μm×256 μm capillary column packed with Zorbax eclipse monomeric C_{18}. Approximately 100 ng of EGCG injected. Reproduced from Ref. [40].

(+)-catechin in plasma by LC with fluorescence detection was reported the same year [46]. Limits of detection of 20 ng/ml were reported for this method, and RSDs for quantitation of (+)-catechin spiked in plasma ranged from 0.5% to 11%. Finally, Tsuchiya et al. report the use of borate complex extraction of catechins followed by LC with fluorescence detection for the determination of C and EC spiked into plasma [48].

3. Capillary electrophoretic determination of tea catechins

At the time of this review, a total of seven capillary electrophoresis (CE) reports have been published on the separation and/or quantification of tea catechins. Capillary zone electrophoresis (CZE) [50,51] and micellar electrokinetic capillary chromatography (MEKC) [52–56] with UV absorbance detection are the CE methods of choice for the determination of catechins. In all instances, uncoated fused-silica capillaries have been used to effect the separations. In general, the MEKC methods provide better separation, resolution and quantitation for a larger number of catechins than do the rudimentary CZE methods. Only two out of seven CE reports describe the separation of GCG from EGCG [52,55] in standards or in samples, however, all of the reports describe the identification of EGCG. The GCG isomer differs only from the EGCG isomer in the spatial orientation of the pyrogallol group on the parent structure. This subtle difference in structure makes it difficult to resolve these two catechins from each other, and may affect quantitative values reported for EGCG if the species co-migrate. It is likely that the authors who do not resolve GCG from EGCG, yet who give quantitative data for EGCG in real tea samples, are in fact reporting incorrect (high) values for EGCG.

3.1. CZE of tea catechins

The reported CZE methods employ borate-based running buffers for the determination of five catechins (C, EC, EGC, ECG, EGCG) and in each case, the separation between several of the catechin peaks is inadequate (peaks not baseline resolved). The method reported by Horie et al. uses a simple 20 mmol/l borax run buffer, pH 8.0 to analyze catechin standard mixtures and green tea samples [50]. Data for caffeine, theanine and ascorbic acid were also included. The total analysis time was just under 11 min. The resolution among EGC/EC/C, in particular, is noticeably poor, however the authors were able to report quantitative results presumably based upon external standards for all five catechins. The precision of all catechin determinations ($n=3$) was better than 9.0% RSD. A typical electropherogram of a CZE separation from Horie et al. is shown in Fig. 5.

A more recent CZE method reported by Arce et al. uses a 150 mmol/l boric acid run buffer, pH 8.5 to separate five catechins, caffeine, adenine, theophylline, gallic acid, quercetin and caffeic acid within 20 min [51]. The authors describe the use of a flow injection (FI) system interfaced to a CZE system (FI–CZE) in which the FI system is used to perform on-line sample extraction, filtration and dilution in a single step. The analytical performance of the method (limit of detection, limit of quantitation, calibration regression equations, precision) is detailed in the report. While the analytical performance values for individual species reported in the method appear to be good, the overall separation of the catechins is poor. In these types of analyses, catechins tend to separate into two groups depending upon the presence or absence of the gallic acid moiety. Within these groups, ECG/EGCG and C/EC/EGC, the catechins are incompletely resolved. The authors report quantitative data for eight different types of green tea samples in which external standard results are consistent with standard addition results. The precision of all catechin determinations ($n=3$) was quite variable, ranging anywhere from 0.5% to 36.5% RSD.

3.2. MEKC of tea catechins

The five reported MEKC methods for the determination of catechins all utilize sodium dodecyl sulfate (SDS) micelles in the presence of a borate-based running buffer. The reports describe the separation of five, six, or seven catechins with total analysis times in the range of 10 min to 50 min. Larger et al. have developed a strictly qualitative

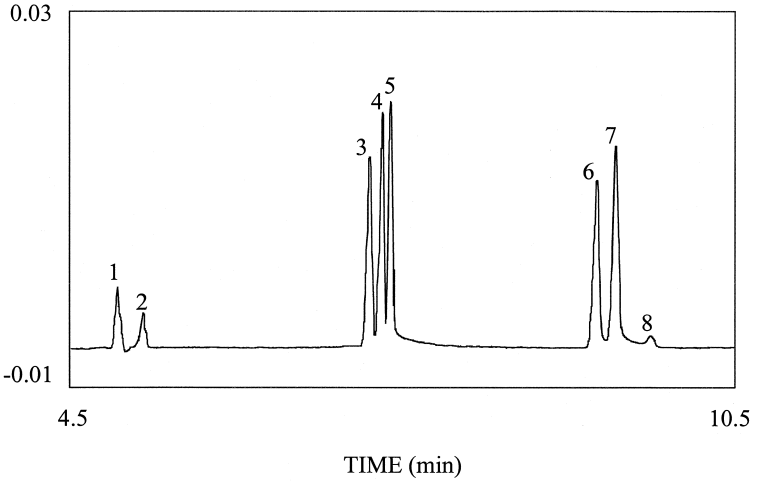

Fig. 5. CE separation of a catechin standard mixture. The concentrations of the eight compounds were 50 mg/l each. Peaks: 1=caffeine, 2=theanine, 3=EGC, 4=EC, 5=C, 6=EGCG, 7=ECG, 8=ascorbic acid. Experimental conditions: fused-silica capillary, total length 77 cm, length to detector 70 cm; buffer 20 mmol/l borax (pH 8.0); voltage 30 kV; detection 200 nm; temperature 23°C; injection time 5.0 s. Reproduced with permission from Ref. [49].

method for the separation of five catechins (C, EC, EGC, ECG, EGCG), theobromine, caffeine, chlorogenic acid and other flavonols using 50 mmol/l dihydrogenphosphate, 50 mmol/l tetraborate, 20 mmol/l SDS, 10% volume fraction of acetonitrile in water, pH 6.0 [54]. The total analysis time for this method is 50 min for all components, but the catechins can be separated within 20 min. The authors claim that a systematic optimization of various method parameters, including inorganic buffer concentration, micelle concentration, presence or absence of borate ions, and organic solvent concentration was performed, however, many essential details are left out of the report. A notable exclusion is a reference electropherogram illustrating the separation of polyphenolic (catechin) standards. The separations shown for typical tea (green and Darjeeling) samples suffer from high backgrounds as the teas were not filtered. For the green tea sample that is shown, C and EGC are not resolved, and EGCG and ECG migrate in a high background.

The MEKC method of Watanabe et al. describes an impressive separation of seven catechins (C, EC, EGC, ECG, EGCG, GCG, CG), caffeine and ascorbic acid in under 10 min [55]. This is the largest number of catechins separated using CE. The authors use a 25 mmol/l phosphate, 50 mmol/l borate, 25 mmol/l SDS, pH 7.0 running buffer to effect the efficient resolution of five out of seven catechins. EGCG and GCG are separated from each other but not baseline resolved. The resulting MEKC separation is compared to a CZE separation and to an LC separation in terms of overall analysis time and resolving power. In general, the analysis time of the MEKC method (10 min) is shorter than the LC method (20 min) and the resolution by the MEKC method is better than the resolution obtained by CZE. Quantitative data based upon external standard calibration were collected for two different types of teas (black and green) and these data compared favorably with data collected by an independent LC method. The precision of all catechin determinations ($n=3$) by MEKC was better than 4% RSD.

A qualitative MEKC method developed by Horie and Kohata focuses on the separation of four catechins (EC, EGC, ECG, EGCG), theanine, caffeine and ascorbic acid [56]. The authors use a run buffer consisting of 20 mmol/l tetraborate, 80 mmol/l boric acid, 50 mmol/l SDS, pH 8.4. Even though the analysis is complete within 11 min, the separation is poor. All four of the catechin peaks are broad and tail strongly. Additionally, EC/EGC and EGCG/ECG are not resolved. Finally, the authors state that an internal standard (*p*-hydroxybenzoic acid) was

used but why this internal standard was employed cannot be determined.

Nelson et al. developed a MEKC separation of six catechins (C, EC, EGC, ECG, EGCG, GCG) and caffeine [52]. Separation parameters such as micelle charge type, surfactant type, organic solvent buffer modifier, micelle concentration, buffer pH and cyclodextrin buffer modifier were individually evaluated. The optimized run buffer consisted of 20 mmol/l tetraborate, 110 mmol/l SDS, a volume fraction of 14% methanol in water, 1.5 mol/l urea, 1.0 mmol/l β-cyclodextrin, pH 8.0. The method produced a high resolution separation of all six catechins; however, the overall run time (30 min) was longer than most CE methods (Fig. 6). Quantitative data (based on internal standard calibration) were given for three different types of green tea samples and the results agreed well with results from an independent LC method [37]. The precision of all catechin determinations ($n=3$) by MEKC was better than 4.0% RSD.

The most recent MEKC method is described by Barroso and van de Werken and uses 4 mmol/l tetraborate, 12 mmol/l phosphate, 40 mmol/l SDS, pH 7.0 [53]. The essential details of the method development process are given (effect of pH, effect of SDS concentration, optimization of injection volume, etc.) and the results are well described. Five catechins (C, EC, EGC, ECG, EGCG) and caffeine are well separated in less than 20 min and all of the catechins are fully baseline resolved. Analytical data for the method performance (migration time/peak area reproducibility, regression line data, inter-/intra-assay precision, analyte stability, limits of detection) are provided and the data support the ruggedness of the method. Quantitative data (based on standard additions calibration) are given for two tea samples. It should be noted that all of the RSDs for the individual catechin determinations ($n=4$) were less than or equal to 8.0%, except for the determination of C in black tea (black tea=19.6% RSD vs. green tea=6.8% RSD). The authors report that following the analyses of black teas, the capillary had to be specially cleaned to remove irreversibly adsorbed theaflavines.

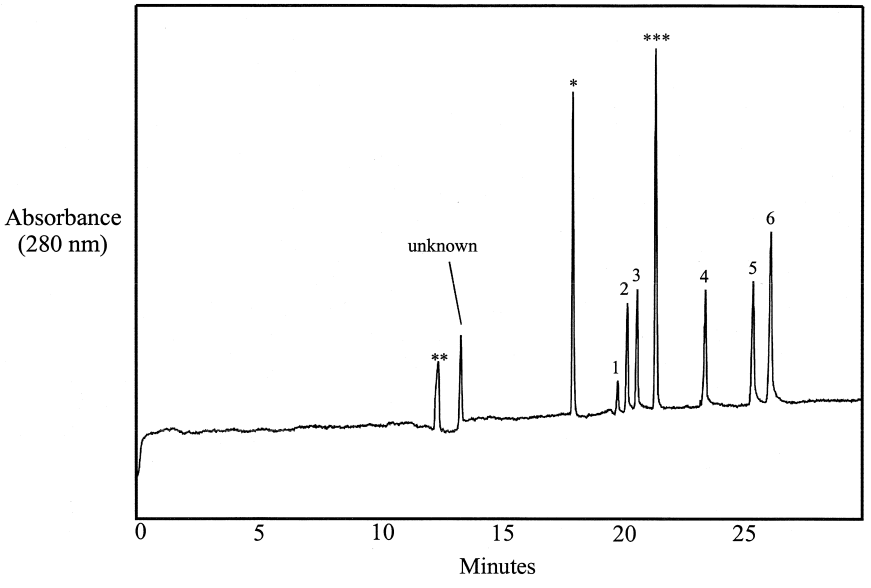

Fig. 6. MEKC separation of a catechin standard mixture. Run buffer: 20 mmol/l tetraborate, 110 mmol/l SDS, 14% methanol (volume fraction), 1.5 mol/l urea, 1.0 mmol/l β-CD, pH 8.0. Peak identification: caffeine (*), methanol (**), L-tryptophan (internal standard) (***), EGC (1), C (2), EC (3), GCG (4), EGCG (5), ECG (6). All peaks 0.075 mg/ml, except for methanol (unknown). Experimental conditions: fused-silica capillary, total length 67 cm, length to detector 60 cm; voltage 20 kV; detection 280 nm; temperature 20°C; injection time 4.0 s. Reproduced from Ref. [51].

4. Additional techniques for the determination of tea catechins

Several other recent physical and chemical techniques have been applied to the separation and/or quantification of tea catechins. The most effective techniques include gas chromatography (GC) [57–60], thin-layer chromatography (TLC) [61,62], paper chromatography (PC) [63], visible spectrophotometry [63,64], biosensing [65], chemiluminescence [66], and nuclear magnetic resonance (NMR) [67–69]. Some of these techniques have been shown to be generally useful for the measurement of individual catechins while others are only practical for the measurement or identification of ''total'' catechins. A brief review of these additional techniques follows.

4.1. Gas chromatography of tea catechins

GC methods utilizing both glass columns and fused-silica capillary columns have been developed for the determination of catechins. In all cases, a derivatization step is needed to convert the catechins to volatile compounds. Pierce et al. [57] and Collier and Mallows [58] have separated five catechins (C/EC/EGC/ECG/EGCG) as their trimethylsilyl (TMS) derivatives on glass columns packed with a mass fraction of 3% OV-1. The method of detection in each case is based on flame ionization detection (FID). The method of Pierce et al. requires two separate isocratic temperature runs (20 min each) to separate all five catechins in tea samples [57]. Quantitative results necessitate the use of a different internal standard (triphenylbenzene or coronene) for each isocratic run, due to the difference in chromatographic profiles at the different isocratic temperatures. Method precision for the catechin determinations was not assessed. The method gives good separation of the catechins but the derivatization step, along with the individual isocratic runs, makes the method time consuming and labor intensive. By contrast, Collier and Mallows developed a temperature programmed GC method that separates all five catechins in one run in less than 32 min [58], however EC and C are not baseline resolved (Fig. 7). The authors report quantitative results for a black tea sample even with incomplete resolution of EC and C. The precision of all catechin determinations (n=3) ranges from 3.8% to 12.5% RSD.

Capillary column GC–FID and GC–MS have been applied to the separation of the TMS derivatives of catechins [59,60]. At the time of this review, no literature exists describing the simultaneous separation of the eight biologically important catechins by capillary GC. Current reports emphasize the separation of C and EC only. Stremple utilizes capillary GC–FID combined with temperature pro-

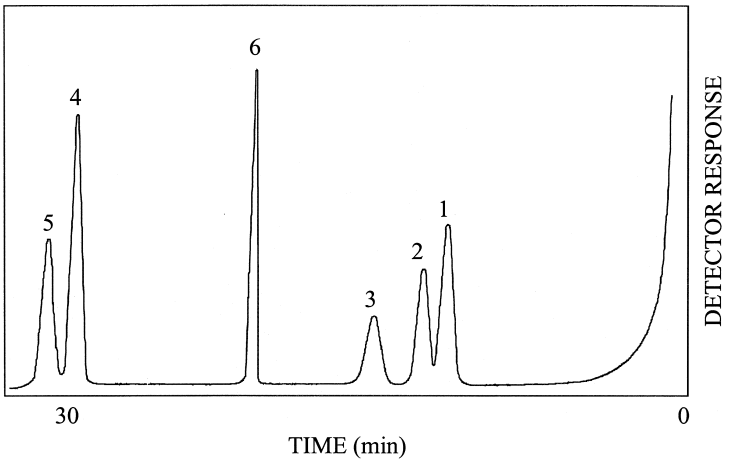

Fig. 7. GC separation of catechins as TMS derivatives. 1=EC, 2=C, 3=EGC, 4=ECG, 5=EGCG, 6=quercetin. GC column=3% mass fraction of OV-1 on 100 mesh to 200 mesh Diatomite CQ. Temperature program=235°C isothermal for 22 min, then 48°C/min to 310°C. Reproduced with permission from Ref. [57].

gramming to separate C and EC in less than 22 min [59]. The developed method is used to qualitatively separate C and EC in a black tea extract. Other compounds that are separated include kaempferol, quercetin and myricetin. The method shows satisfactory separation of C and EC, however C is incompletely resolved from an unknown component in the tea. Luthria et al. used GC–MS combined with temperature programming to separate and quantitate (based on an internal standard) C and EC in human plasma in less than 25 min [60]. The authors report a limit of detection lower than 10 ng/ml.

4.2. Thin-layer chromatography of tea catechins

TLC has been used for both qualitative and quantitative determination of individual catechins [61,62]. Zhu and Xiao used silica gel plates and a chloroform–ethyl formate–*n*-butanol–formic acid mobile phase to separate five catechins (C/EC/EGC/ECG/EGCG) and caffeine in green tea samples [62]. The separated components are visualized with UV radiation (254 nm) and quantitative measurements are made based on the areas of the catechin spots. The authors report the results (% of each catechin) for 11 different samples of green tea. Method precision for the catechin determinations was not assessed. Amarowicz and Shahidi successfully identified the eluates from a Sephadex column catechin separation by TLC [61]. Four catechins (EC/EGC/ECG/EGCG) were separated on a silica gel plate using chloroform–methanol–water. Detection of the catechins was via visible color formation with vanillin–hydrochloric acid reagent.

4.3. Paper chromatography of tea catechins

Singh et al. have developed a simple method for separating, identifying, and quantitating individual catechins based on two-dimensional paper chromatography (2D-PC) [63]. The method has been shown to effectively separate six tea shoot catechins (C/EC/GC/EGC/ECG/EGCG) using inexpensive Whatman No. 1 chromatographic paper. The catechins are identified (using R_F values) as bright yellow spots on the chromatographic paper by spraying the paper with diazotized sulfanilamide (a reagent that is highly specific and selective for catechins). The authors state that the sensitivity of visual detection is <1 μg of C. This represents a simple and rapid method for verifying the presence of catechins in a sample. A representative paper chromatogram is shown in Fig. 8. A quantitative version of this procedure is based on rinsing the separated yet unreacted catechins from the chromatographic paper into individual test tubes. The catechins are then reacted in the test tubes with diazotized sulfanilamide, which forms a yellow complex (λ=425 nm). The concentration (mg/g tea shoot) of the catechin is estimated via spectrophotometry and external standard calibration graphs based upon C. Satisfactory results are given in terms of the measurement precision (RSD<6%) for samples, however, the authors' results for summed individual catechins do not agree with the results for their total catechin (see Section 4.4) measurement in the samples. The summed total is lower by no less than 10% for all samples.

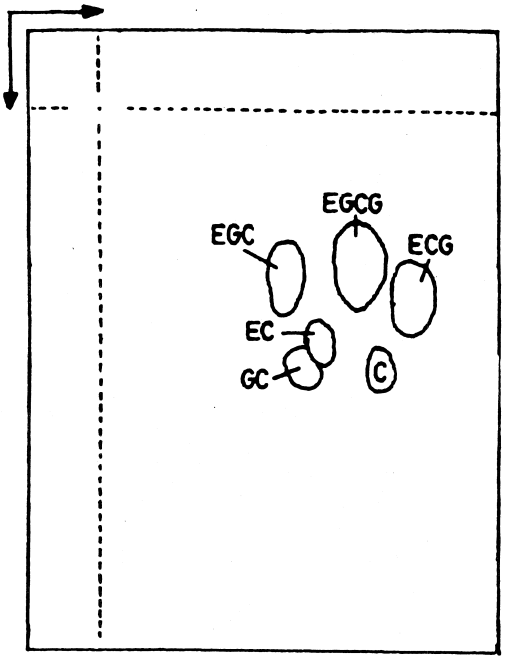

Fig. 8. Two-dimensional PC separation of catechins. Solvent 1=10% volume fraction of acetone in water. Solvent 2=butanol–acetic acid–water (4:1:5, v/v, organic phase). Sulfanilamide reagent revealed spots of the catechins alone. Reproduced with permission from Ref. [62].

4.4. Spectrophotometry of tea catechins

Singh et al. [63] and Kivits et al. [64] have developed visible spectrophotometric methods for the measurement of total catechins. Kivits et al. were able to determine total catechins (C/EC/EGC/ECG/EGCG) in human plasma after consumption of green tea [64]. The determination is based on the spectrophotometric measurement of the colored complex between 4-dimethylaminocinnamaldehyde (DMACA) and the catechins. DMACA is a specific and selective reagent for the detection of catechins; a green complex (λ=637 nm) forms between DMACA and the reported catechins in approximately a 1:1 equimolar ratio. The method uses external standard calibration graphs based upon C to determine the relative amount of total catechins in plasma. Analytical parameters such as recovery, within- and between-day precision, possible interferents, linear regression results and limits of detection are reported. The method does appear to have problems with recovery, as the listed analytical recovery for catechins is only 78%. The precision of within-day catechin determinations (n=6) is better than 2.0% RSD. Singh et al. use the spectrophotometric measurement of a colored complex (λ=425 nm) between diazotized sulfanilamide and the catechins (C/EC/GC/EGC/ECG/EGCG) to determine total catechins in extracts from tea shoots [63]. This method also uses external standard calibration graphs based upon C to estimate the relative amount of total catechins in the samples. The precision of total catechin determinations (n=3) is better than 0.1% RSD.

4.5. Tea catechin biosensors

Total catechins have also been measured via the use of a biosensor. Burdock tissue (*Arctium lappa L.*, a biennial plant) contains polyphenol oxidase, which is an enzyme that catalyzes the oxidation of polyphenols (catechins). Horie et al. have constructed a catechin biosensor that uses a slice of burdock tissue as the biological element and an oxygen electrode as the transducer element [65]. As the catechins in a sample are oxidized, the oxygen electrode measures the amount of oxygen consumption (as a decrease in electric current). This catechin biosensor was found to respond to five catechins (C/EC/EGC/ECG/EGCG), gallic acid, catechol and ascorbic acid. The authors report the application of the biosensor to the determination of total catechins in green tea infusions. The precision of the measurements is good (<3% RSD) and the biosensor shows no interference from major amino acids or carbohydrates in the infusions. One limitation of this approach, however, is that the biosensor is inadequate for accurate quantitation of total catechins because of the severe variability in the relative biosensor response to the different catechins (EGC=50%, C=100%, EC=380%).

4.6. Chemiluminescence of tea catechins

The qualitative determination of three catechins (EC/EGC/EGCG) based on their chemiluminescent emission has been reported by Miyazawa and Nakagawa [66]. Catechins are reacted with hydrogen peroxide–acetaldehyde–horseradish peroxidase, which results in a distinct chemiluminescent emission at 630 nm. The authors report on the specific identification of EGCG in Sprague–Dawley rat mucosal cells.

4.7. NMR of tea catechins

Davis et al. have assigned the absolute structures of ten different green tea catechins (C/EC/GC/EGC/CG/ECG/GCG/EGCG/EGCMG/EZ) using ^{1}H- and ^{13}C-NMR [69]. EGCMG and EZ are (−)-epigallocatechin methylgallate and (−)-epiafzelechin, respectively. The NMR assignments were based upon direct and long-range proton-carbon correlation experiments. Sakata et al. used ^{13}C-NMR to identify two catechins (EGC/EGCG), caffeine, theanine, sucrose and quinic acid in green tea [67]. NMR was described as a qualitative tool for classifying different types of tea. Gong et al. have described the use of ^{13}C-NMR to identify four catechins (EC/EGC/ECG/EGCG), caffeine, theanine, glucose, sucrose, gallic acid, inositol glycoside and quinic acid in extracts of green tea [68]. NMR was used purely as a qualitative tool to profile different stages of tea processing. The NMR profiles showed how the microbial fermentation process caused the levels of the catechins to decrease from the beginning processing stage to the concluding processing stage.

5. Conclusions

Natural products have played an invaluable role in the drug discovery process in recent years, particularly in the area of cancer treatment. It has been estimated that greater than 60% of the approved drugs and pre-NDA (new drug application) candidates in this area developed from 1989 through 1995 are of natural origin [30,31]. The current interest in natural materials as a source of chemotherapeutic agents necessitates the development of highly sensitive and selective methods for the analysis of compounds such as tea catechins, to support preclinical efficacy and toxicity testing studies. Approaches presented in this paper demonstrate the sensitivity and selectivity necessary for the determination of catechins in tea and complex matrices. Further, the application of these methods to the measurement of catechins in tea and blood plasma should be very useful to clinical researchers investigating the bioavailability and pharmacokinetics of these bioflavonoids, as well as links between tea consumption and disease prevention.

Note: Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

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