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Application Note

Analysis of Catechins in Decaffeinated Green Tea by TLC Using TLC Explorer

Ajay Kaparwan
R&D, APAC (Jigani, Bangalore, India)

Abstract

Catechins in decaffeinated green tea were separated and quantified on a silica gel 60 F_{254} TLC plate aligned to the USP monograph method. Analysis and documentation were performed using the TLC Explorer instrument. The method performance was assessed against the system suitability criteria specified in the monograph for identification and was subsequently extended to the quantitative determination of a representative catechin in powdered decaffeinated green tea extract.

Introduction

Camellia sinensis is an evergreen shrub or small tree belonging to the family Theaceae, with its leaves, leaf buds, and stems used for tea production. Commonly referred to as the tea plant, tea shrub, or tea tree,[1] tea derived from *Camellia sinensis* is categorized into four major types according to the processing method applied, namely green, black, white, and oolong tea.

Green tea is produced from the leaves of *Camellia sinensis* without fermentation, thereby limiting the oxidation of its polyphenolic constituents. The predominant polyphenols present in green tea are catechins.[2] These naturally occurring polyphenolic phytochemicals are recognized for their potential health benefits, including favorable effects on metabolic health, such as obesity related to high-fat diets and type II diabetes, as well as a reduced risk of coronary diseases.[3] Additional beneficial effects of green tea have been reported for various types of cancer as well as cardiovascular and liver diseases, effects that are largely attributed to the catechin content of green tea.[4]

Decaffeinated green tea is obtained from tea leaves subjected to a decaffeination process to eliminate caffeine. When a natural water-based decaffeination process is applied, the tea retains approximately 95% of its antioxidants, thereby preserving most of the health benefits of regular green tea. Consequently, naturally processed decaffeinated tea retains most of the health-related benefits while minimizing caffeine content.[5] As the decaffeination process may impact the polyphenol composition, determination of catechin content in the final product is of interest.

The catechins specified in the USP monograph for powdered decaffeinated green tea extract include (-)-epigallocatechin-3-O-gallate, (-)-epigallocatechin, (-)-epicatechin-3-O-gallate, and (-)-epicatechin (**Figure 1**).[6]

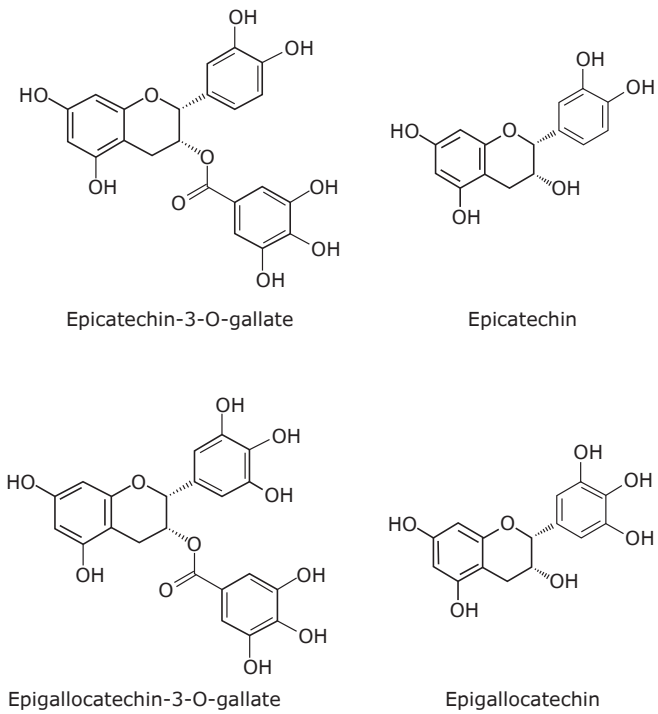

Figure 1. Catechins analyzed in the study

In this study, the separation and quantification of the most intense chromatographic band corresponding to (-)-epigallocatechin-3-O-gallate in a powdered decaffeinated green tea extract, are performed using a silica gel 60 F_{254}, 20 x 20 cm, thin layer chromatography (TLC) plate. While the USP monograph for decaffeinated green tea specifies HPTLC as the method for qualitative analysis (identification) and HPLC as the quantitation method, the present work extends the TLC-based approach beyond identification to enable quantification of (-)-epigallocatechin-3-O-gallate, in the presence of the sample matrix and other catechins naturally occurring in the decaffeinated green tea.

The USP monograph for decaffeinated green tea recommends the use of an HPTLC plate with an average particle size of 5 µm for qualitative determination of catechins. In contrast, this study uses a TLC plate with a larger particle size of 10–12 µm and demonstrates its applicability for both catechin identification and quantitative determination of epigallocatechin-3-O-gallate as a representative analyte.

The identification and imaging of catechin compounds together with the quantification of (-)-epigallocatechin-3-O-gallate were performed by video densitometry with the TLC Explorer instrument. This analysis and documentation system (**Figure 2**) provided an easy-to-use and reliable approach for TLC plate imaging and data handling in analytical routine, method development, in-process control, or daily research activity.

Figure 2. TLC Explorer used for the analysis of catechins in decaffeinated green tea.

The TLC Explorer documentation system enables digital and automated evaluation of TLC plates, enhancing the efficiency and accuracy of thin layer chromatography analyses. The instrument offers three illumination modes based on LED light sources, namely white light (VIS), UV-A (366 nm), and UV-C (254 nm), allowing detection and rapid measurement of compounds of interest. The associated software offers special features like automated track recognition, simultaneous measurement of multiple plates, and background signal correction. The TLC Explorer offers accurate TLC imaging to support video densitometric measurements, thereby enabling quantitative analysis and reliable data interpretation. (read more at **SigmaAldrich.com/tlc-explorer**).

The method developed method using the TLC Explorer instrument was partially validated as per the ICH Q2 R2 guidelines.[7]

Experimental

Reagent, Standard and Sample Preparations

- **Immersion reagent**: Dissolve 140 mg of Fast Blue B salt in 10 mL of water, then add 140 mL of methanol and 50 mL of dichloromethane.
- **Diluent**: Ethyl alcohol/water (4:1 v:v)
- **Standard solution USP RS (4 mg/mL)**: Weigh and transfer 40 mg of USP powdered decaffeinated green tea extract RS into a 10 mL volumetric flask. Add 5 mL of diluent and sonicate for 10 min with intermittent shaking. Make up to volume with diluent, mix thoroughly, and centrifuge. Use the clear supernatant.
- **(-)-Epigallocatechin-3-O-gallate reference solution (2 mg/mL)**: Weigh and transfer 10 mg of (-)-epigallocatechin-3-O-gallate RS into a 5 mL volumetric flask. Add 4–5 mL of diluent and sonicate for 10 min with intermittent shaking. Mix thoroughly and make up to volume with diluent.
- **(-)-Epigallocatechin reference solution (2 mg/mL)**: Weigh and transfer 10 mg of (-)-epigallocatechin RS into a 5 mL volumetric flask. Add 4–5 mL of diluent and sonicate for 10 min with intermittent shaking. Mix thoroughly and make up to volume with diluent.
- **(-)-Epicatechin-3-O-gallate reference solution (2 mg/mL)**: Weigh and transfer 10 mg of (-)-epicatechin-3-O-gallate RS into a 5 mL volumetric flask. Add 4–5 mL of diluent and sonicate for 10 min with intermittent shaking. Mix thoroughly and make up to volume with diluent.
- **(-)-Epicatechin reference solution (2 mg/mL)**: Weigh and transfer 10 mg of (-)-epicatechin) RS into a 5 mL

volumetric flask. Add 4–5 mL of diluent and sonicate for 10 min with intermittent shaking. Mix thoroughly and make up to volume with diluent.

Sample solution commercial extract: Weigh and transfer 40 mg of powdered decaffeinated green tea extract into a 10 mL volumetric flask. Add 5 mL of diluent and sonicate for 10 min with intermittent shaking. Make up to volume with diluent, mix thoroughly, and centrifuge. Use the clear supernatant.

TLC Method

In deviation from the USP monograph, which specifies the use of a HPTLC plate with a particle size of 5 µm, the separation was performed on silica gel 60 F_{254} TLC plate on aluminum support with a particle size of 10–12 µm (**Table 1**). After development and drying, the plate was dipped in the immersion reagent solution and subsequently examined under white light.

Table 1. TLC conditions used for catechin determination

TLC Parameter	
Chromatographic Conditions	
Plate:	TLC aluminum plate silica gel 60 F_{254}, 20 x 20 cm (**1.05554**)
Plate pretreatment:	Prewash plate with methanol and allow to dry in air before use.
Sample application:	Camag Linomat 5 using band length = 8 mm, track distance = 15 mm, distance from left edge = 15 mm, distance from lower edge = 20 mm. Allow to dry completely before plate development. See **Table 2** and **Table 3** for applied volumes
Plate preconditioning:	Condition plate at 30% humidity (e.g. in desiccator with saturated $MgCl_2$ solution)
Mobile phase:	Acetone:toluene:formic acid 9:9:2 (v:v:v)
Chamber conditions:	Unsaturated
Migration distance:	15 cm (develop chromatograms over ¾th of the plate)
Drying:	Dry plate at 100 °C and immediately examine under white light
Staining/derivatization (immersion reagent):	Dipped in immersion reagent (Fast Blue B salt in methanol/dichloromethane), dry in a current of cold air
Detection:	Examine under white light, UV at 366 nm and 254 nm

Table 2. Applied spots and sample application volume for identification (Plate A)

Track No	Spot Name Used in TLC Explorer	Application Volume (µL)
1 & 2	Decaffeinated green tea extract USP RS 4 µg/spot-1	1
	Decaffeinated green tea extract USP RS 4 µg/spot-2	1
3 & 4	Sample decaffeinated green tea extract 4 µg/spot-1	1
	Sample decaffeinated green tea extract 4 µg/spot-2	1
5 & 6	Epigallocatechin-3-O-gallate 2 µg/spot-1	1
	Epigallocatechin-3-O-gallate 2 µg/spot-2	1
7 & 8	Epigallocatechin 2 µg/spot-1	1
	Epigallocatechin 2 µg/spot-2	1
9 & 10	Epicatechin-3-O-gallate 2 µg/spot-1	1
	Epicatechin-3-O-gallate 2 µg/spot-2	1
11 & 12	Epicatechin 2 µg/spot-1	1
	Epicatechin 2 µg/spot-2	1

Table 3. Applied spots and application volume for quantification, linearity, and recovery (Plate B)

Track No	Spot Name Used in TLC Explorer	Application volume (µL)
1	Epigallocatechin-3-O-gallate 1 µg/spot	0.5
	Epigallocatechin 1 µg/spot	0.5
	Epicatechin-3-O-gallate 1 µg/spot	0.5
	Epicatechin 1 µg/spot	0.5
2	Epigallocatechin-3-O-gallate 2 µg/spot	1.0
	Epigallocatechin 2 µg/spot	1.0
	Epicatechin-3-O-gallate 2 µg/spot	1,0
	Epicatechin 2 µg/spot	1.0

Track No	Spot Name Used in TLC Explorer	Application volume (µL)
3	Epigallocatechin-3-O-gallate 3 µg/spot	1.5
	Epigallocatechin 3 µg/spot	1.5
	Epicatechin-3-O-gallate 3 µg/spot	1.5
	Epicatechin 3 µg/spot	1.5
4	Epigallocatechin-3-O-gallate 4 µg/spot	2.0
	Epigallocatechin 4 µg/spot	2.0
	Epicatechin-3-O-gallate 4 µg/spot	2.0
	Epicatechin 4 µg/spot	2.0
5	Epigallocatechin-3-O-gallate 5 µg/spot	2.5
	Epigallocatechin 5 µg/spot	2.5
	Epicatechin-3-O-gallate 5 µg/spot	2.5
	Epicatechin 5 µg/spot	2.5
6	Epigallocatechin-3-O-gallate 6 µg/spot	3.0
	Epigallocatechin 6 µg/spot	3.0
	Epicatechin-3-O-gallate 6 µg/spot	3.0
	Epicatechin 6 µg/spot	3.0
7	Epigallocatechin-3-O-gallate 7 µg/spot	3.5
	Epigallocatechin 7 µg/spot	3.5
	Epicatechin-3-O-gallate 7 µg/spot	3.5
	Epicatechin 7 µg/spot	3.5
8	Green Tea extract sample	1.0
9	Sample + epigallocatechin-3-O-gallate 2 µg/spot	1.0
	Sample + epigallocatechin 2 µg/spot	1.0
	Sample + epicatechin-3-O-gallate 2 µg/spot	1.0
	Sample + epicatechin 2 µg/spot	1.0
10	Sample + epigallocatechin-3-O-gallate 5 µg/spot	2.5
	Sample + epigallocatechin 5 µg/spot	2.5
	Sample + epicatechin-3-O-gallate 5 µg/spot	2.5
	Sample + epicatechin 5 µg/spot	2.5
11	Sample + epigallocatechin-3-O-gallate 7ug/spot	3.5
	Sample + epigallocatechin 7 µg/spot	3.5
	Sample + epicatechin-3-O-gallate 7 µg/spot	3.5
	Sample + epicatechin 7 µg/spot	3.5
12	Epigallocatechin-3-O-gallate in USP standard 2 µg/spot	1.0
	Epigallocatechin in USP standard 2 µg/spot	1.0
	Epicatechin-3-O-gallate in USP standard 2 µg/spot	1.0
	Epicatechin in USP standard 2 µg/spot	1.0

Results and Discussion

Identification of Catechins

Identification of catechins was achieved by individual application of decaffeinated green tea extract (USP RS), decaffeinated green tea extract sample, and four reference standards, namely (-)-epigallocatechin-3-O-gallate, (-)-epigallocatechin, (+)–epicatechin-3-O-gallate, and (-)-epicatechin, onto a silica gel 60 F_{254} TLC plate. The resulting chromatograms obtained under UV (254 nm) and white light are shown in **Figure 3**. Details of applied spots and application volumes are provided in **Figure 3** and **Table 1**. The retardation factor (R_f) values for the compounds in both standard and sample solutions are listed in **Table 4**.

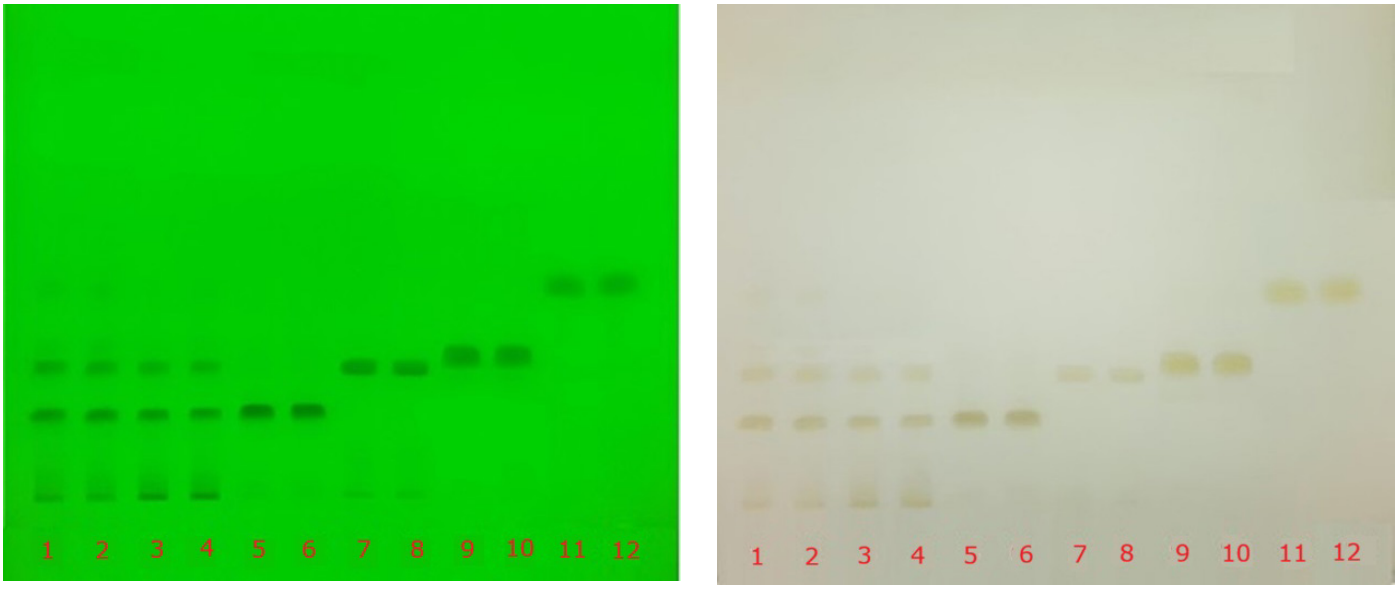

Figure 3. TLC chromatograms (Plate A) recorded under UV (254 nm, left) and visible light (right) for the identification of catechins in powdered decaffeinated green tea extract. Tracks 1 & 2 correspond to decaffeinated green tea standard solution (USP RS), tracks 3 & 4 to decaffeinated green tea extract sample solution, tracks 5 & 6 to (-)-epigallocatechin-3-O-gallate, tracks 7 & 8 to (-)-epigallocatechin, tracks 9 & 10 to (+)–epicatechin-3-O-gallate, and tracks 11 & 12 to (-)-epicatechin.

Table 4. Retardation factors (R_f) determined from **Figure 3** for the decaffeinated green tea standard solution (RS), decaffeinated green tea extract sample solution, and individual catechins standards, in tea samples and reference solutions

Track no.	Sample	R_f			
		(-)-epi gallocatechin-3-O-gallate	(-)-epi gallocatechin	(+)-epi catechin-3-O-gallate	(-)-epi catechin
1	Decaffeinated green tea standard solution (USP RS)	0.34	0.45	0.49	0.59
2	Decaffeinated green tea standard solution (USP RS)	0.34	0.45	0.49	0.59
3	Decaffeinated green tea extract sample solution	0.35	0.45	0.49	0.59
4	Decaffeinated green tea extract sample solution	0.34	0.46	0.50	0.60
5	(-)-epigallocatechin-3-O-gallate reference solution	0.34	-	-	-
6	(-)-epigallocatechin-3-O-gallate reference solution	0.35	-	-	-
7	(-)- epigallocatechin reference solution	-	0.45	-	-
8	(-)-epigallocatechin reference solution	-	0.45	-	-
9	(+)–epicatechin-3-O-gallate reference solution	-	-	0.49	-
10	(+)–epicatechin-3-O-gallate reference solution	-	-	0.48	-
11	(-)-epicatechin reference solution	-	-	-	0.59
12	(-)-epicatechin reference solution	-	-	-	0.59

Comparison to USP Monograph Criteria

The USP monograph specifies the identification of key catechins by the presence of four prominent brownish-orange bands observed under white light with approximate R_f values on an HPTLC plate with a particle size of 5 µm. In the present study, the R_f values obtained using a TLC plate with particle size of 10–12 µm particle were compared with the monograph criteria and are summarized in **Table 5**. A comparable band pattern was observed. The monograph acceptance criterion, requiring correspondence between the key bands of the sample solution and those of the standard solution, was fulfilled.

Table 5. Comparison of R_f values obtained for decaffeinated green tea standard solution (USP RS), decaffeinated green tea extract sample solution, and individual catechin standards with the approximate R_f values specified in the USP monograph

Compound	R_f			
	USP Monograph approx. on HPTLC plate (5 µm)	Experimentally acquired on TLC plate for Standard Solution USP RS	Experimentally acquired on TLC plate for individual catechin RS	Experimentally acquired on TLC plate for commercial sample
(-)-epigallocatechin-3-O-gallate	0.38	0.34	0.34	0.34
(-)-epigallocatechin	0.48	0.45	0.45	0.45
(+)–epicatechin-3-O-gallate	0.52	0.49	0.49	0.49
(-)-epicatechin	0.62	0.59	0.59	0.59

Calibration & Recovery

Quantification, linearity, and recovery studies were conducted using reference standards and over-spiked sample spots applied on Plate B, as described in **Table 3**.

The resulting chromatograms were visualized under white light (**Figure 4**) and used for calibration, quantification, and recovery assessment. Additional documentation was obtained under UV light at 254 and 366 nm (**Figure 5**).

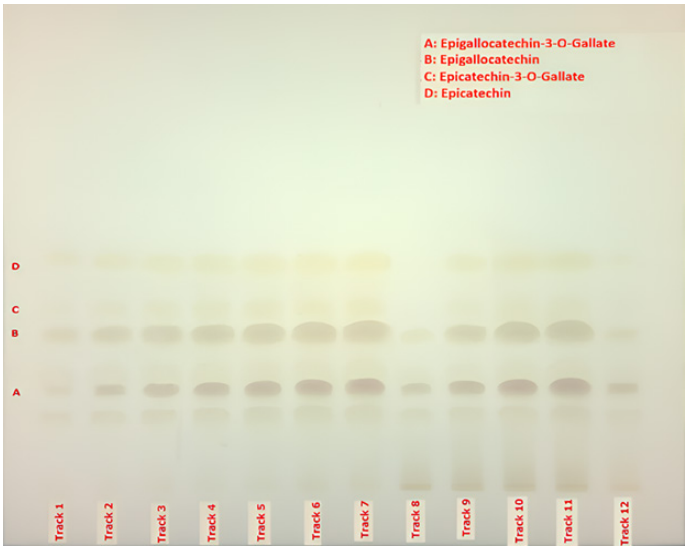

Figure 4. TLC chromatogram (Plate B) for catechins present in powdered decaffeinated green tea extract recorded under visible light. A. (-)-epigallocatechin-3-O-gallate, B. (-)-epigallocatechin, C. (+)–epicatechin-3-O-gallate, D. (-)-epicatechin.

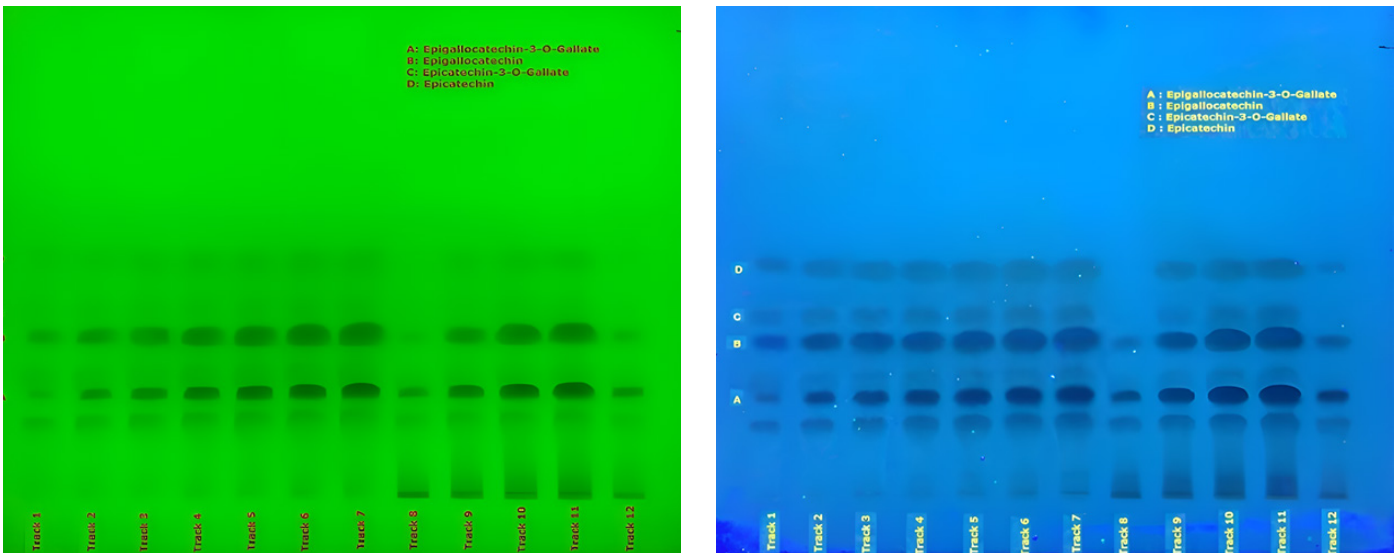

Figure 5. TLC chromatogram (Plate B) for catechins present in powdered decaffeinated green tea extract recorded under UV 254 nm (left) and 366 nm (right). A. (-)-epigallocatechin-3-O-gallate, B. (-)-epigallocatechin, C. (+)–epicatechin-3-O-gallate, D. (-)-epicatechin.

Calibration

Calibration was performed using the most intense chromatographic band corresponding to (-)-epigallocatechin-3-O-gallate (B) observed on the TLC plate under white light. A calibration range from 1 µg per spot to 7 µg per spot was established by applying increasing volumes of the standard stock solution, as described in **Table 3**. A representative densitogram for (-)-epigallocatechin-3-O-gallate (3 µg/spot) is shown in **Figure 6**. This approach was used to determine the peak areas of (-)-epigallocatechin-3-O-gallate in all samples listed in **Table 6**. The resulting calibration curve, shown in **Figure 7**, demonstrated a polynomial fit with a R^2 value of 0.9968.

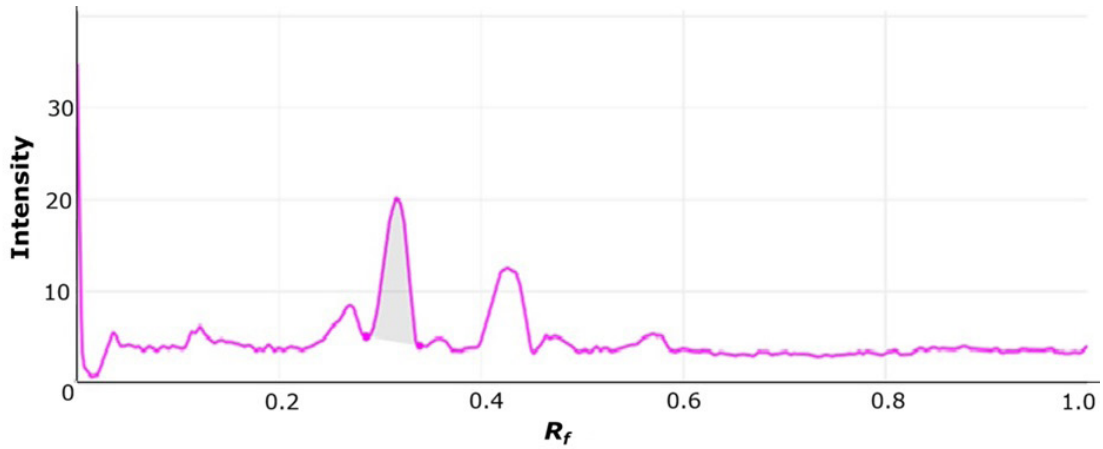

Figure 6. Representative densitogram recorded under white light for the standard solution applied at 3 µg of (-)-epigallocatechin-3-O-gallate per spot (for details see **Table 3**).

Table 6. Results for quantification, retardation factor, and recovery of the most intense band corresponding to (-)-epigallocatechin-3-O-gallate (R_f 0.34) in the concentration range of 1 µg to 7 µg per spot

Applied solution	Track no.	Concentration (µg per spot)	R_f	Area (-)-epigallocatechin-3-O-gallate peak
Calibration Standards	1	1	0.34	107.3
	2	2	0.34	353.6
	3	3	0.34	521.4
	4	4	0.34	633.1
	5	5	0.34	733.6
	6	6	0.34	825.3
	7	7	0.34	861.2

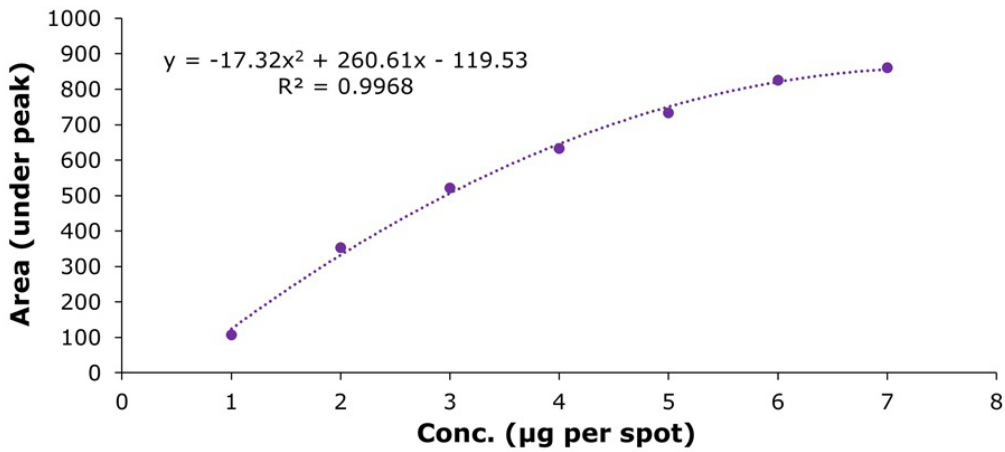

Figure 7. Calibration curve using a polynomial function in the concentration range of 1 µg to 7 µg per spot of (-)-epigallocatechin-3-O-gallate.

Recovery

Recovery was evaluated using commercial decaffeinated tea extract sample spots that were over-spiked with 2, 5, and 7 µg/spot of analyte. The results of the recovery study are shown in **Table 7** (tracks 9–11). Analysis of the unspiked sample indicated that the sample spiked at 7 µg per spot (Plate B, Track 11) exceeded the applicable calibration range of 1–7 µg/spot. Consequently, this data point was excluded from recovery calculations. Nevertheless, the higher concentration spot showed consistency with respect to the R_f value. The recoveries obtained for the samples over-spiked with 2 µg and 5 µg per spot were 92.0% and 95.7%, respectively.

Table 7. Recovery results for the most intense band corresponding to (-)-epigallocatechin-3-O-gallate at over spike concentrations of 2, 5, and 7 µg per spot

Experimental parameter	Track no.	Concentration (added µg per spot to sample)	R_f	Peak Area (-)-epigal locatechin-3-O-gallate peak with Sample	Peak Area (-)-epigal locatechin-3-O-gallate peak after deducting sample response	(-)-Epigal locatechin-3-O-gallate spike per spot (µg)	Spiked (-)-epigal locatechin-3-O-gallate per spot found (µg)	Recovery (%)
Sample solution	8	Sample (commercial extract)	0.34	312.7	0	0	NA	NA
Recovery study	9	Sample + 2 µg per spot	0.34	629.8	317.1	2	1.9	95.0
	10	Sample + 5 µg per spot	0.34	1053.6	740.9	5	4.6	92.0
	11	Sample + 7 µg per spot	0.34	1195.3*	n.a.*	7	n.a.*	n.a.*
Standard solution	12	2 µg per spot	0.34	316.7	NA	2	1.92	96.0

* data not applicable as outside the calibration range of 1-7 µg/spot.

Commercial Extract Sample Result

For the unspiked commercial sample extract spot (**Table 7**, Tack 8) a content of 1.9 µg per spot of (-)-epigallocatechin-3-O-gallate was determined with an applied sample volume of 1 µL. This corresponds to a concentration of 475 mg/g in the original extract powder.

Conclusion

Using TLC plates along with the TLC Explorer instrument and with reference to the USP monograph method for powdered decaffeinated green tea extract, the acceptance criteria for catechin identification were fulfilled using TLC plated with a particle size of 10–12 µm. The chromatogram of the sample solution exhibited major bands comparable in color and size to those observed for the standard solution.

Although the USP monograph describes the use of HPTLC solely for qualitative identification of catechins, the method developed in this study was extended to enable quantitative determination of the most intense band corresponding to (-)-epicatechin-3-O-gallate. Calibration demonstrated a good correlation over the range of 1 to 7 µg per spot with R^2 values greater than 0.996. Recovery studies performed by over-spiking sample spots yielded recoveries between 92% to 95.7%.

Overall, the presented method demonstrates that the combination of TLC plates and the TLC Explorer system is suitable for both qualitative identification and quantitative determination of catechins in powdered decaffeinated green tea extract.

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Formic acid 98 - 100% for HPLC LiChropur™	**5.43804**
Magnesium chloride hexahydrate, for analysis EMSURE® ACS,ISO,Reag. Ph Eur	**1.05833**
Reference Materials	
Powdered Decaffeinated Green Tea Extract, United States Pharmacopeia (USP) Reference Standard, 300 mg	**1298401**
(-)-Epigallocatechin-3-O-gallate, USP reference standard, 20 mg	**1236700**
(-)-epicatechin-3-O-gallate, primary reference standard, 10 mg	**03950590**
(-)-Epigallocatechin, analytical standard, 1 mg	**08108**
(-)-Epigallocatechin, primary reference standard, 10 mg	**03960590**
(-)-Epicatechin, primary reference standard, 10 mg	**03940590**

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