Research Square

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Assessment and Correction of Ion Suppression Effects in Food Waste Extracts Using LC–MS Analysis

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Abstract

Liquid chromatography–mass spectrometry (LC–MS) is widely employed in food analysis due to its high sensitivity and selectivity. However, matrix effects remain a critical limitation, particularly in complex, minimally processed samples. In this study, the impact of high sugar content on ionization efficiency was evaluated using banana peel extracts as a model matrix and epigallocatechin gallate (EGCG) as a target analyte. UHPLC–Orbitrap analysis revealed that co-extracted carbohydrates, likely including sucrose, dominate the early chromatographic region and significantly interfere with electrospray ionization. A comparative analysis between standard solutions and matrix-spiked samples demonstrated approximately 75% ion suppression of EGCG, indicating a substantial reduction in signal intensity in the presence of the matrix. These results highlight the risk of underestimating or failing to detect minor bioactive compounds in sugar-rich food matrices. The findings emphasize that matrix effects must be systematically evaluated in LC–MS method development, and that mitigation strategies such as sample clean-up, dilution, or matrix-matched calibration are essential to ensure reliable analytical results in complex biomass-derived samples.

Introduction

The evolution of analytical techniques has been challenged by the identification of high-value-added compounds in complex matrices. The combination of mass spectrometry (MS) in combination with gas chromatography (GC) or liquid chromatography (LC) is widely recognized as the “gold standard” for food analysis (Zhao et al 2025). In particular, the use of LC-MS analysis has gained increasing popularity in food science, largely due to improved instrument accessibility and the notable advancements in chromatography. These developments have enabled faster and more effective separations, well as the analysis of polar or ionic compounds (Spacil et al 2010; Hu et al 2018; Hird et al 2014). Over the past decade, the most important change has been the expanded availability and diversity of mass analyzers for LC–MS, including triple quadrupole and high-resolution systems such as Orbitrap and time-of-flight instruments. These innovations have significantly impacted strategies for monitoring chemical contaminants in food (Sun et al 2023; Gómez-Ramos et al 2013).

Discussions on the presence and distribution of bioactive compounds in food extracts and formulations have intensified in recent years. The influence of minor bioactive compounds on the nutritional and biological properties of these extracts is becoming increasingly evident. Spectrometric techniques have emerged as essential tools for detection and quantification of compounds at very low concentrations. However, matrix effects can interfere with the response of the analyte in proportion to analyte concentration, as co-eluting matrix constituents may alter the ionization efficiency of the target analyte (Furey et al 2013; Villagrasa et al 2007). Therefore, the evaluation of ion suppression or matrix effect is an important step in LC-MS method development (Matuszewski et al 2003). These effects can significantly limit the detection of minor constituents in a complex mixture. Depending on the sample composition, major componentes such as polyphenols, triglycerides, fatty acids, and sugars, may suppress or mask the signals of other constituents.

In food analysis, mass spectrometry with electrospray ionization (ESI) remains the most common ionization technique employed for the determination of chemical compounds (Fenn et al 1989). Nevertheless, ion suppression effects in LC-MS analysis of complex food matrices, such as food waste, remain insufficiently understood, particularly regarding its impact on quantitative accuracy. High concentrations of specific compounds can significantly affect ionization efficiency, leading to compromising quantification and cause signal suppression or intensification (Faccin et al., 2016). Recently, it has been recognized that LC-MS analysis can be influenced by interfering substances from the sample matrix. (Larger et al 2005; Antignac et al 2005). Thus, matrix effects evaluation is essential to ensure accurate LC-MS analysis of chemical residues in complex food by-products.

In this context, it is necessary to assess the impact of ion suppression impact in complex biomass-derived matrices, such as banana peel (Santos et al. 2025; Suarez et al. 2008). Banana peel biomass represents a highly complex matrix, characterized by a diverse carbohydrate composition, including soluble sugars (e.g., glucose, fructose, and sucrose) and structural polysaccharides such as cellulose, hemicellulose, and pectin (Tsamo et al 2015). In addition to carbohydrates, it contains polyphenols, organic acids, pigments, and other endogenous compounds that may be co-extracted during sample preparation (Alarcon Flores et al 2012; Becerra-Herrera et al 2014; Faccin et al. 2016). The high sugar content further increases complexity by raising sample viscosity and promoting the co-elution of interfering species, which may adversely affect analyte ionization in LC–MS analyses (Freitas et al 2004).

Bananas (Musa spp.) are among the most widely consumed fruits worldwide. Notably, the largest producing countries primarily supply their domestic markets. This is the case for the five largest global producers: India, China, Indonesia, Nigeria, and Brazil, which are not among the top exporters. As a result of strong domestic consumption, agro-industries waste remains concentrated in the production regions, where it is often discarded as garbage, or alternatively used as animal feed or fertilizer. However, alternative applications that add value to this biomass have gained attention, particularly due to the presence of minor constituents, mainly in the peel. These include highly bioactive polyphenols such as epigallocatechin gallate (EGCG) (Pramote et al 2018). EGCG is one of the biologically active catechins and is widely recognized for its diverse pharmacological properties (Nagle et al 2006). These characteristics have made it a compound of considerable interest in food science, nutrition, and pharmaceutical research, especially due to its strong antioxidant activity (Dixon 1995; Sidhu and Zafar 2018). However, its vulnerability to oxidation and its interactions with complex matrices pose significant analytical challenges, particularly in LC-MS analysis, where matrix effects can compromise affect accurate quantification (Eddahby et al 2025; Mizook et al 2003; Tan et al 2023). Therefore, the study aims to evaluate ion suppression effects in the determination of EGCG in banana peel using LC-MS and to propose strategies to improve analytical reliability.

Materials and Methods

Reagents and materials

Banana peel residues were obtained locally in the commerce of Rio de Janeiro (Brazil) (SisGen A1A6C3D). All solvents and reagents were of analytical grade purity. Methanol GC grades were acquired from Tedia (Fairfield, OH, USA) for sample preparation. The standard Epigallocatechin gallate (≥ 99.5%) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). For UHPLC-HRMS analysis, methanol HPLC grade, formic acid 98%-100%, and ammonium formiate were provided by Tedia (Fairfield, OH, USA), Merck (Darmstadt, Germany), and Spectrum Chemical (Gardena, CA, USA), respectively. Ultrapure water (18.2 MΩ.cm) was obtained from a Millipore Milli-Q purification system (Billerica, MA, USA).

Preparation of standards and solutions

The standard Epigallocatechin gallate stock solution was prepared in MeOH to a final concentration of 1.0 mg/mL (corrected for standard purity). Successive dilutions were prepared in MeOH to a final volume of 1 mL. The following concentrations (µg/mL) were used: 0.2, 0.3, 0.6, 1.2, 2.4, 4.9, 9.7, 24, 49, 97, 146, and 262. The curve was prepared in triplicate, and each point was injected only once. The mean, standard deviation, and coefficient of variation were calculated for each triplicate. The limit of detection (LOD) was determined by diluting each replicate of the lowest calibration point (0.2 ug/mL), performing triplicate injections, and evaluating a signal-to-noise ratio greater than 3. The determined LOD was 0.08 µg/mL (Figure S1).

The volumetric flasks used for preparing the analytical curve had an expanded measurement uncertainty of 0.001 mL. The stock solutions were stored at -30°C. All volumetric materials used are periodically calibrated by LAB CAL, part of LBCD, a laboratory accredited by the Brazilian Calibration Network (CAL 0328 INMETRO).

UHPLC-HRMS parameters

A Dionex Ultimate 3000 ultra-high performance liquid chromatography (UHPLC) system coupled to a QExactive Plus hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray ionization (ESI) source was used. Separation was performed on a reversed-phase column (Kinetex 2.6 µm PS C18, 100 Å, 100 mm × 2.1 mm) at 40°C, with a constant flow rate of 300 µL/min and an injection volume of 5 µL. A gradient chromatographic run started at 5% of mobile phase B (methanol with 0.1% formic acid) and 95% of mobile phase A (water with 5 mM ammonium formiate and 0.1% formic acid). Mobile phase B increased to 10% at 1.0 minutes, 25% at 2 minutes, and 90% at 10 minutes. After reaching 100% B at 14 minutes and maintaining this ratio until 16 minutes, the initial chromatographic conditions were restored from 16.1 to 20.0 minutes.

The LC effluent was pumped to the mass spectrometer, which operated in positive ESI mode, and was calibrated daily with a manufacturer's calibration solution (Thermo Fisher Scientific, Bremen, Germany). ESI parameters were further optimized with the final setup: spray voltage of 2.9 kV, S-lens voltage of 80 V, capillary temperature of 380°C, auxiliary gas heater temperature of 350°C, and nitrogen sheath, auxiliary, and sweep gas set to 30, 10, and 1 arbitrary units, respectively. Full-scan data were acquired in a range of

m/z 70 – *m/z* 1050 at a resolution of 70,000 full widths at half maximum (FWHM), automatic gain control (AGC) of 1 x 10^6, and maximum injection time (IT) of 100 ms.

Sample preparations

To 1 mg of banana peel cut into ~ 1 cm pieces, 5 mL of methanol was added. The mixture was vortexed for 5 min and then centrifuged at 3000 rpm for 5 min. The supernatant was transferred to another test tube and evaporated to dryness under a nitrogen stream at 30°C. The final residue was reconstituted in 1 mL of methanol. Before proceeding with the ion suppression evaluation, all banana peel extracts were screened for the presence or absence of the target analyte.

Matrix Effect Evaluation

The first step in the evaluation was to select a sample lacking EGCG. A standard solution at 49 µg/mL in methanol was then injected. The blank sample was spiked to achieve a final standard concentration of 49 µg/mL in the matrix (banana peel extract without EGCG). The peak area of the standard in methanol was compared with that in the matrix.

Results and Discussion

Banana peel biomass was analyzed by UHPLC-HRMS, and the matrix's high complexity became evident due to its high sugar content. Figure 1 shows the total ion chromatogram (TIC) of a banana peel extract. The peak observed at 0.58 min likely corresponds to sucrose.

However, even with LC–Orbitrap, it remains challenging to unequivocally distinguish a disaccharide such as sucrose from monosaccharides like glucose, even in the presence of analytical standards. This is because these compounds may exist as constitutional isomers and, in the case of sugars, also as stereoisomers. Constitutional isomers share the same molecular formula and, consequently, generate identical exact masses (Gross 2017). Therefore, even the high resolving power of the Orbitrap is insufficient to differentiate such isomeric species, since isomeric compounds cannot be resolved by accurate mass measurement alone (Gautier et al 2016). Only specialized chromatographic columns would allow unambiguous identification of sucrose (Kobayashi et al 2020). Nevertheless, based on literature data, its presence in banana peel can be reasonably inferred (Happi Emaga et al 2007). Under reversed-phase conditions, these highly polar compounds elute at the very beginning of the chromatographic run, often near the dead time, due to their low retention on C18 stationary phases. As a result, reversed-phase chromatography does not provide sufficient retention or selectivity to confirm sugar identity solely on the basis of retention time.

Almost no other substances are observed due to the high sucrose content. It became evident that the ionization of EGCG can be significantly suppressed in the presence of sugars, which are abundant in banana peel extracts. Highly polar carbohydrates such as sucrose, glucose, and fructose tend to co-elute

early in reversed-phase LC, often overlapping with polar analytes or contributing to a dense chemical background at the electrospray interface. These co-eluting species compete for charge and surface activity during electrospray ionization (ESI), thereby reducing the efficiency of EGCG ion formation (Chin et al 2004; Buhrman et al 1996). As a result, signal intensity decreases, leading to ion suppression and potentially underestimating EGCG concentration.

This effect is clearly illustrated in Fig. 2. Figure 2A shows the extracted ion chromatogram of EGCG in a methanolic standard solution (49 µg/mL), while Fig. 2B shows the same analyte in a banana peel extract spiked to a comparable concentration (49 µg/mL). A pronounced reduction in signal intensity is observed in the presence of the matrix, indicating significant ion suppression.

Quantitative evaluation of this effect is presented in Table 1. Comparison of peak areas between the standard solution and the matrix-spiked sample indicates approximately 75% ion suppression. The reproducibility of the measurements is acceptable, with coefficients of variation below 6% for both conditions, supporting the robustness of the observed effect.

Although matrix effects are often evaluated by comparing the slopes of calibration curves, this study used a simplified approach based on peak-area comparisons between solvent- and matrix-spiked samples. This strategy was considered appropriate for a proof-of-concept evaluation, which aimed to demonstrate the magnitude of ion suppression under realistic sample conditions rather than to provide a fully validated quantitative assessment. The observed suppression (~ 75%) clearly indicates a significant matrix effect, supporting the study's conclusions.

Table 1
Results from ion suppression.

Matrix	EGCG (ug/mL)	Replicate	Area	Mean	SD	CV%	Suppression (%)
Methanolic solution	49	R1	166197459	170292970	8193081	4.8	75
	49	R2	179726297				75
	49	R3	164955154				76
Banana peel extract	49	R1	41693368	42192720	2198109	5.2	
	49	R2	44597546				
	49	R3	40287247				

These findings indicate that high concentrations of co-extracted sugars can severely compromise the detection and quantification of EGCG. In minimally processed samples, such as crude biomass extracts, this effect may lead to significant underestimation of analyte concentration or even false-negative results. Therefore, matrix effects must be carefully considered during method development, particularly when analyzing complex food-derived matrices.

To estimate the concentration of EGCG in banana peel, a calibration curve was constructed over a wide concentration range (data are presented in Table S1 of the Supplementary Information). Prior to this, the extract was analyzed qualitatively by comparing the analyte's retention time with that of the methanolic standard and by comparing the exact mass in both cases (Fig. 3).

Figure 4 shows the extracted ion chromatogram of EGCG for a sample containing the target analyte. The presence and concentration of specific compounds in natural products are highly influenced by environmental and agronomic factors. Seasonal variation, soil composition, rainfall patterns, temperature, and sunlight exposure can significantly affect plant metabolism and, consequently, the biosynthesis and accumulation of secondary metabolites. In addition, factors such as plant maturity, geographic origin, and post-harvest handling may further contribute to variability. As a result, the chemical profile of plant-derived materials, including banana peel, may differ substantially depending on when and where the samples are collected, which should be considered when interpreting analytical results (Buhrman et al 1996). The concentration of EGCG in the sample, without accounting for ion suppression, was 4.0 µg/mL. The analysis was performed in triplicate, yielding a coefficient of variation (CV) of 4.6%. When the ion suppression effect was considered, the estimated EGCG concentration increased to 7.0 µg/mL.

Taken together, these results demonstrate that the high sugar content in banana peel extracts plays a critical role in suppressing the ionization of target analytes, such as EGCG, under electrospray conditions. The early co-elution of sugars and their high abundance in the extract create unfavorable ionization conditions, ultimately reducing analytical sensitivity and compromising detection. This effect is not limited to EGCG but is expected to affect a broad range of polar and moderately polar compounds commonly investigated in food matrices. Therefore, analytical strategies based solely on direct injection of minimally processed extracts may yield biased results, particularly when targeting minor constituents. In this context, implementing mitigation strategies, such as sample clean-up, dilution, chromatographic optimization, or matrix-matched calibration, becomes essential to ensure reliable qualitative and quantitative analysis. These findings reinforce the importance of systematically evaluating matrix effects in LC–MS workflows applied to complex biomass-derived samples.

Conclusion

This study clearly demonstrates that high sugar content in banana peel extracts can severely compromise LC–MS analysis by inducing pronounced ion suppression, leading to substantial loss of sensitivity for target analytes such as EGCG. The observed suppression of approximately 75% highlights how co-extracted carbohydrates can interfere with electrospray ionization, potentially masking minor constituents. These findings raise important concerns about the reliability of LC–MS-based screening and quantification of bioactive compounds in complex, sugar-rich food matrices, where false negatives or significant underestimation may occur. Therefore, matrix effects should not be overlooked but should be systematically evaluated as a critical component of method development. The adoption of

appropriate mitigation strategies, including sample clean-up, dilution, and matrix-matched calibration, is essential to ensure analytical reliability when dealing with complex biomass-derived samples.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions Ian G. C. Barcellos Silva: Writing – original draft, Investigation, Methodology, Data curation, Formal analysis. Gustavo Ramalho Cardoso dos Santos: Writing – review & editing, Visualization, Validation, Formal analysis. Monica Costa Padilha: Writing – review & editing, Supervision, Investigation, Data Curation, Visualization, Validation, Funding acquisition. Valdir F. Veiga-Junior: Writing – review & editing, Conceptualization, Supervision, Methodology, Validation, Funding acquisition.

Data Availability Data will be made available on request.

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Figures

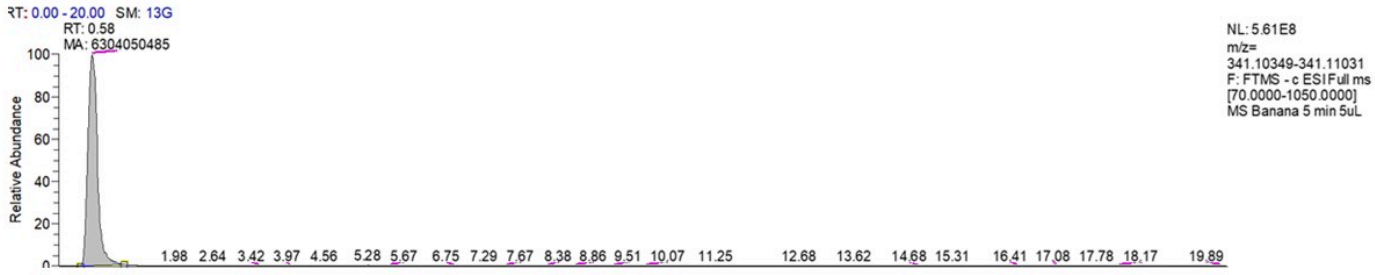


Figure 1

Total ion chromatogram (TIC) of banana peel extract.

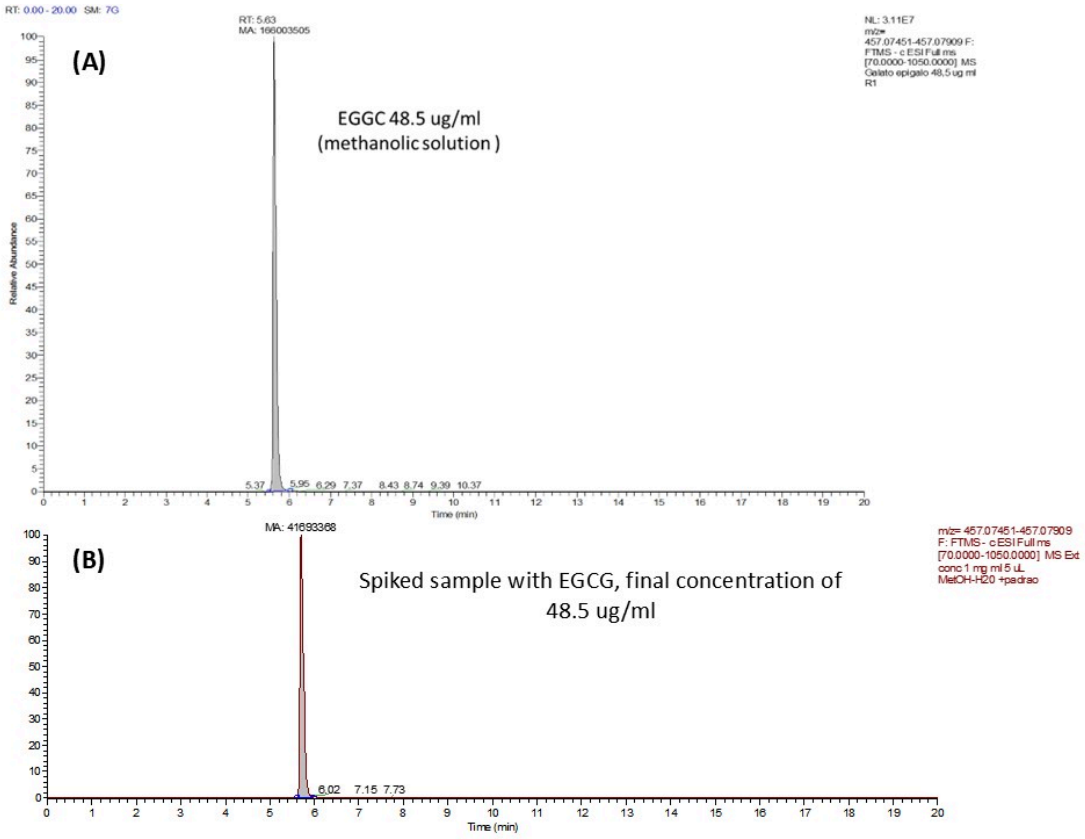


Figure 2

Extracted ion chromatogram of [M–H]⁻ 457.0786: (A) EGCG standard in methanolic solution; (B) matrix spiked with EGCG standard at a final concentration of 48.5 µg/mL.

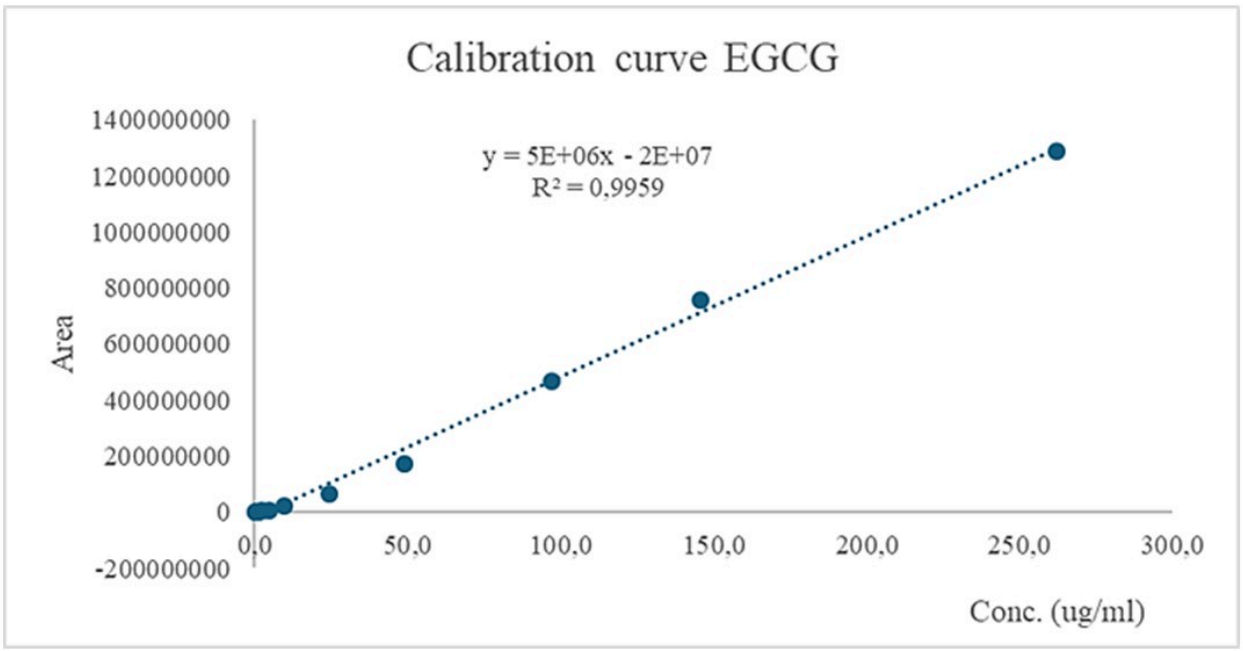


Figure 3

Calibration curve of EGCG.

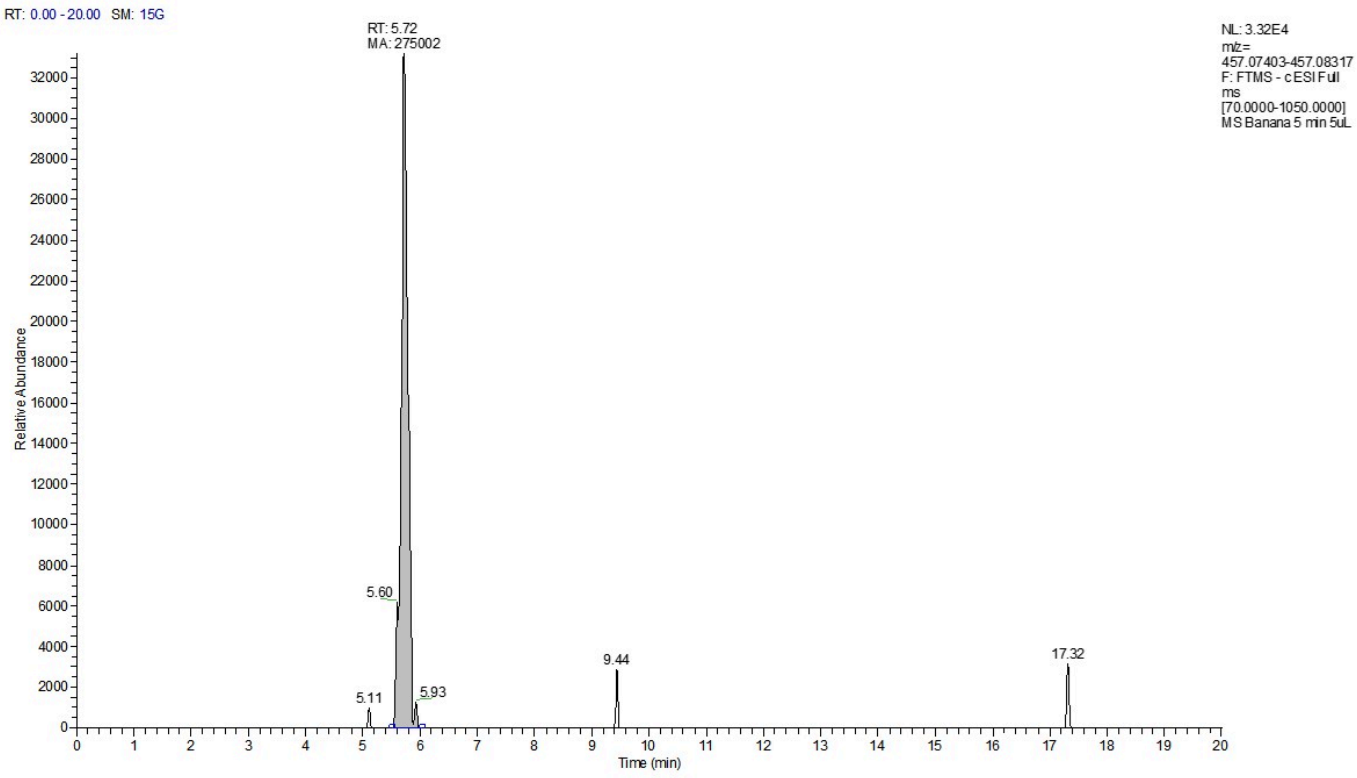


Figure 4

Extracted ion chromatogram of $[M–H]^-$ 457.0786 EGCG in a banana peel extract.

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