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Workflow for the isolation of natural product secondary metabolites using off-line SPE guided by gradient reverse-phase liquid chromatography (LCSPE-*fast*)

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The importance of natural products (NPs) as molecular libraries providing diverse three-dimensional structural features and serving as scaffolds for drug discovery is well recognized. However, the isolation of both targeted and untargeted secondary metabolites remains a major challenge in NP research, despite significant advances in analytical and biotechnological tools. To address this, we report a rapid and efficient method for the semipreparative isolation of secondary metabolites from NP extracts using offline C_{18} solid-phase extraction (SPE) cartridges, guided by the retention profile of a reverse-phase liquid chromatography gradient (LCSPE-*fast*). LCSPE-*fast* proved to be fast, cost-effective, and yielded multi-milligram amounts of target-metabolite-enriched fractions (~0.40 g of chlorogenic acid and 0.03–0.103 g of 3,4-Di-O-caffeoylquinic acid) from *Uncaria guianensis* cultivated both *in vitro* and *in situ*.

KEYWORDS

SPE cartridge, semipreparative separation, LC-UV analysis, HPTLC analysis, *Uncaria guianensis*

1 Introduction

It is widely acknowledged that natural products (NPs) research has been a major source of novel bioactive molecules with pharmaceutical applications. Researchers in this field remain committed to the continuous discovery and characterization of such compounds. Despite significant advances in analytical and biotechnological tools, the identification of new molecules continues to pose considerable challenges. Approaches such as metabolomics (Caesar et al., 2021; Salem et al., 2020; Floros et al., 2016) and global natural products (GNPS)-based molecular networking (Huo et al., 2023; Khushi et al., 2023; Quinn et al., 2017) have greatly facilitated the prioritization and dereplication of both known and unknown metabolites. Nevertheless, the isolation and unambiguous structural elucidation of individual molecules remain essential for downstream biological assays and for validating their potential bioactivity. One of the main challenges in NP research is that many compounds occur in trace amounts, requiring large quantities of raw material for isolation (Queiroz et al., 2024).

This raises the question: what is the average number of purification steps needed to obtain a "pure compound"? Pauli et al. (2012) conducted a meta-analysis of 2,000 publications on bioactive NPs from 13 journals published in 1998–1999, 2004–2005, and 2009–2010. They found that in approximately three-quarters of cases (76.3%), NPs were isolated and purified in fewer than three steps, indicating that isolation efforts generally target abundant or easily separable compounds. By contrast, low-abundance metabolites, often requiring six to ten purification steps, are rarely pursued. Similarly, an analysis of 4,859 articles published in the Journal of Natural Products between 1996 and 2009 revealed that 91.5% of the reported NPs were hydrophobic, while only 8.5% were hydrophilic. More recently, 862 articles published between 2017 and 2019 reported that high-performance liquid chromatography (LC) was the final isolation step in most cases, with C_{18} columns being used in 67% of them (Berlinck et al., 2022).

In recent years, solid-phase extraction (SPE) has been increasingly applied for the purification and extraction of diverse analytes from biological matrices such as urine and blood (Gu et al., 2025). The process involves three fundamental steps: conditioning of the sorbent, sample loading, and elution of the target analytes (Bucar et al., 2013). A key advantage of SPE in biological sample analysis is its high recovery rate (80%–100%) with excellent reproducibility, often surpassing liquid–liquid extraction (LLE) methods (Badawy et al., 2022). In NP research, SPE is commonly used as a cleanup step prior to LC analysis (Beltrame et al., 2006; Ranušová et al., 2021) as well as to concentrate samples after LC separation for structure elucidation by nuclear magnetic resonance (LC-SPE-NMR) (Simpson et al., 2004; Thomasi et al., 2017).

Herein, we present a general protocol for the offline semipreparative isolation of secondary metabolites from NP extracts using a multi-milligram C_{18} SPE cartridge. The method employs a reversed-phase elution gradient by LC to define the percentage of organic modifier required for fractionation. This protocol has been used for a while in our group, and to demonstrate the applicability of this protocol, we selected a crude extract of *Uncaria guianensis* (Aubl.) J.F.Gmel. (Rubiaceae) cultivated both *in vitro* and *in situ*. Our group has been investigating *U. guianensis,* commonly known as cat's claw, an Amazonian endemic species valued for its anti-inflammatory and immunostimulatory properties (Honório et al., 2016). These biological activities are associated, at least in part, with quinic acid derivatives such as chlorogenic acid (1) and 3,4-Di-O-caffeoylquinic acid (2) (Figure 1), which were chosen as the target metabolites for isolation.

2 Methods

2.1 Plant material

The specimen was identified by Piero G. Delprete (Herbier de Guyane, Institut de Recherche pour le Développement, Cayenne, French Guiana), and voucher material was deposited in the Herbarium of the Universidade de Ribeirão Preto (UNAERP) under the accession code HPMU 2993 (CGEN/MMA: process number A8ABAE8). Four distinct extracts were obtained from different plant parts and produced through *in situ* and *in vitro* approaches: aerial parts (*in situ*), stem bark (*in situ*), aerial parts (*in vitro* plantlets), and adventitious roots of *U. guianensis*.

2.2 Chemicals

Chlorogenic acid, 3,4-Di-O-caffeoylquinic acid, naphthaleneacetic acid (NAA), benzylaminopurine (BAP), 2-aminoethyl diphenylborinate (NP), sucrose, Murashige & Skoog (MS) basal medium and Woody Plant (WP) basal medium were purchased from Sigma-Aldrich® (St. Louis, MO, United States). Polyethylene glycol (PEG) was purchased from Êxodo® (PG07857RA). LC grade solvents were supplied by J. T. Baker.

2.3 Establishment of *U. guianensis* plantlets and adventitious roots

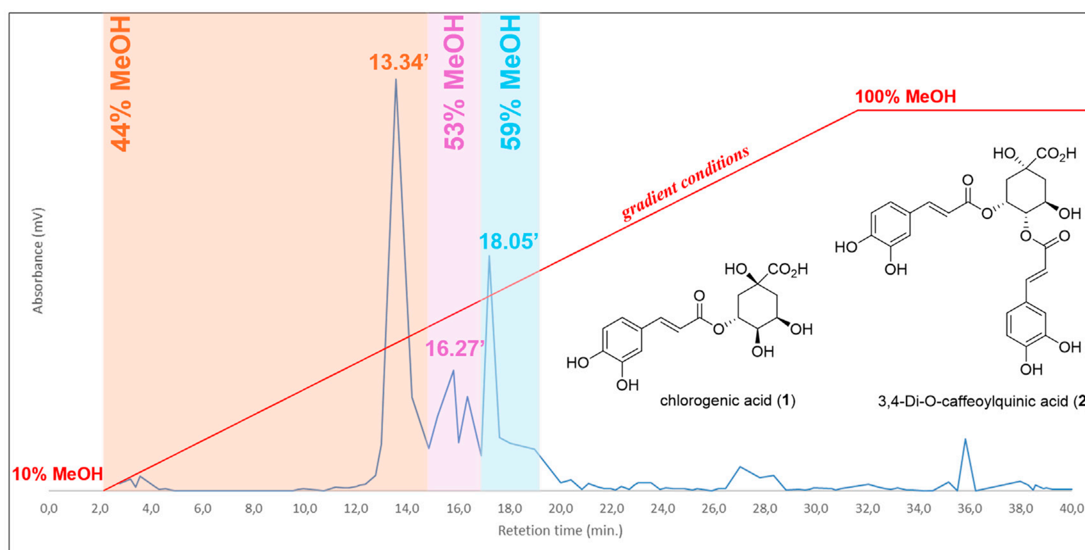
Plantlets were developed by Lopes et al., 2019, as follows. The process of micropropagation involved the use of a WP basal medium (McCown and Lloyd, 1981) that had been supplemented with sucrose (30 $g.L^{-1}$), benzylaminopurine (1 $mg.L^{-1}$), naphthaleneacetic acid (0.5 mg.L), and Gellan Gum (2.5 $g.L^{-1}$). The pH of the medium was then adjusted to 6.0. The explants were maintained in this medium for a period of 3 months (Lopes et al., 2019). *U. guianensis* root cultures derived from micropropagated plantlets were cultivated in Erlenmeyer flasks (250 mL) containing 100 mL of MS basal medium culture (Murashige and Skoog, 1962), with sucrose at a concentration of 3% (w/v), 1 $mg.L^{-1}$ of 1-naphthaleneacetic acid (NAA) and the pH adjusted to 6.0. These cultures were subjected to orbital agitation at a rate of 110 rpm and sub-cultivated over a period of 3 weeks. Plantlets and adventitious roots were maintained in the growth room, where the temperature was maintained at 25 °C ± 2 °C, the relative humidity was 55%–60%, and the photoperiod was 16 h of photoperiod with an intensity of 40 $\mu mol\ m^{-2}s^{-1}$, provided by 85 W cool-white GE fluorescent lamps.

2.4 Preparation of crude extracts from *U. guianensis*

Aerial parts (*in situ*), stem bark (*in situ*), aerial parts (*in vitro* plantlets) and adventitious roots of *U. guianensis* were dried at 45 °C in a circulating air oven for 24 h in order to determine the dry weight. Crude extracts were prepared by macerating the dried powdered plant (1 g) and extracting it using methanol (10 mL) for two separate 24 h periods (two times).

2.5 HPTLC chromatography conditions

All samples, along with chlorogenic acid (**1**) and 3,4-Di-O-caffeoylquinic acid (**2**) standards, were applied to a chromatographic plate (MACHEREY-NAGEL 306179) using a Linomat 5 applicator (Camag, 022.7808) within an HPTLC (High Performance Thin Layer Chromatography) system. The mobile phase consisted of ethyl acetate, water, and anhydrous formic acid (10:1:1, v/v). Chromatographic plate visualization was



Selected fractions obtained after offline C_{18} SPE cartridge fractionation:

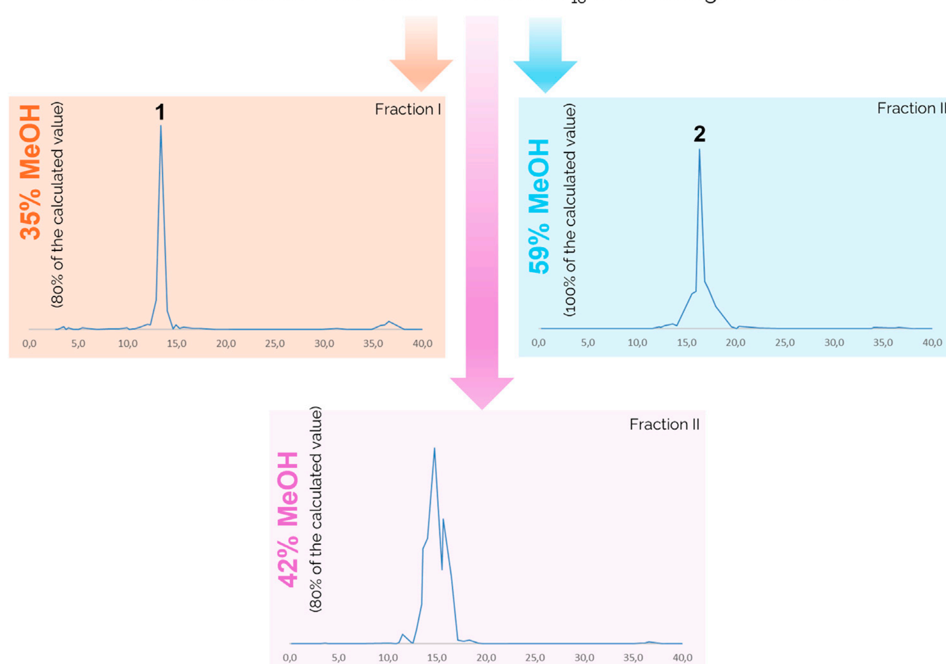


FIGURE 1
LC-UV chromatogram of the matrix under gradient elution, with the percentage of MeOH selected according to the retention times of target metabolites **1** and **2**. Fraction I (35% MeOH), fraction II (42% MeOH) and fraction III (59% MeOH) were obtained after offline C18 SPE cartridge fractionation.

carried out with natural products-polyethylene glycol reagent (NP/PEG).

2.6 LC chromatography conditions

All samples were solubilized in methanol and filtered through Millipore® filters (0.45 μm membrane). Crude extract sample analyses were performed using a Shimadzu LC system, consisting of LC-10AD pumps, DGU-20A3 degasser, SCL-10 system controller, SIL-10AD automatic injector, and an SPD-M10A UV/VIS DAD detector, operated by Class VP software. A Phenomenex Kinetex XB-C_{18} column (250 mm × 4.6 mm, 5 μm) coupled to the respective pre-column was used. The mobile phase composition included water acidified with 0.1% acetic acid (A) and methanol (B). The separation was performed by gradient elution at a flow rate of 1.0 $mL.min^{-1}$ with a ΔB of 10%–100% from 2 to 32 min. To ensure column cleanup, 100% MeOH was maintained for an additional 8 min, then returned to 10% MeOH, and the column was equilibrated for 5 min before the next injection. The injection volume used was 20 μL (8 $mg.mL^{-1}$), with detection occurring at 330 nm.

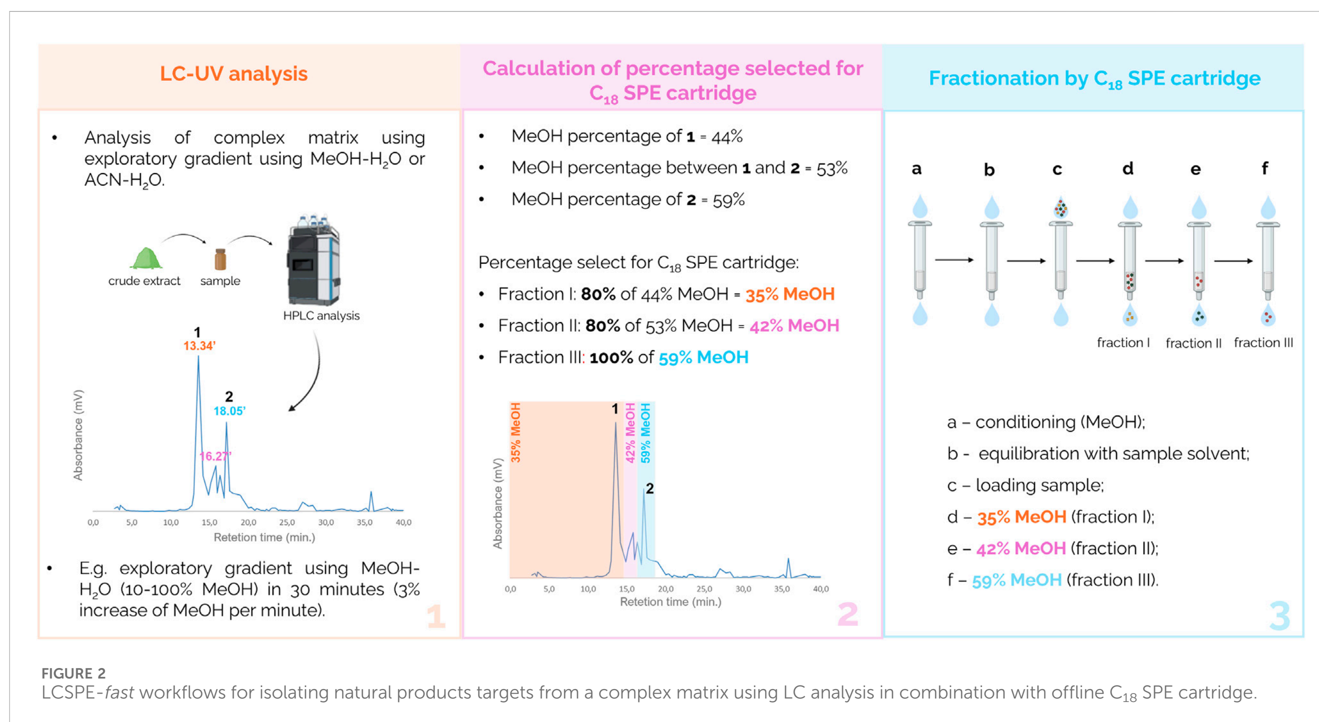


FIGURE 2
LCSPE-*fast* workflows for isolating natural products targets from a complex matrix using LC analysis in combination with offline C_{18} SPE cartridge.

Fractionation of the crude extracts using C_{18} solid-phase cartridge (SPE)

The methanolic extracts were subjected to concentration and fractionation in SPE cartridges. Adventitious roots, aerial parts (*in vitro* plantlets), aerial parts (*in situ*), and stem bark (*in situ*) of *U. guianensis* were submitted to fractionation using a Supelclean™ LC-18 SPE tube (Supelco, 10 g/800 mg). C_{18} SPE cartridges were conditioned in 100% MeOH (40 mL), under gentle vacuum, and column conditioning prepared the sorbent for effective interaction(s) with the compounds of interest. Subsequently, C_{18} SPE cartridges were conditioned in 100% of deionized H_2O (40 mL), the appropriate solvent used for sample preparation. The solvent percentage of each fraction was calculated based on the retention time of each selected metabolites (**1** and **2**) and separating the sample into three fractions: fraction I (44% of MeOH; 13.34 min), fraction II (53% of MeOH; 16.27 min), and fraction III (59% of MeOH; 18.05 min). For fraction I and fraction II, 80% of the calculated MeOH percentage was applied. For fraction III, the exact solvent composition determined by LC was used. Subsequently, the sample (800 mg in 10 mL of deionized H_2O) was applied, and the cartridge was washed with 35%, 42%, 59% and 100% MeOH. Additionally, ethyl acetate solvent (40 mL) was used in the final wash cartridge. The crude extract was fractionated in a 10 g C_{18} SPE cartridge, and the void volume (Vm) of 19.38 mL was calculated according to Equation 1 (Dong, 2019). In each solvent system, a total solvent volume equivalent to twice the Vm (40 mL) was selected, rather than three Vm, as indicated by the manufacturer. The same chromatographic workflow called LCSPE-*fast* was applied to the remaining crude extracts, including aerial parts from *in vitro* plantlets, aerial parts (*in situ*) and stem bark (*in situ*). After fractionation with the C_{18} SPE cartridge, all samples were evaporated to dryness, reconstituted in 100% of MeOH, and subjected to LC-UV analysis.

$$Vm = 0,65\, r^2 L \tag{1}$$

3 Results and discussion

To develop the semipreparative isolation LCSPE-*fast* protocol, the crude extract of *U. guianensis* adventitious roots was first analyzed under linear gradient elution with a gradient slope (*Gs*) of 3%. The resulting chromatogram is shown in Figure 1. Based on this analysis, the organic modifier percentages required for the elution of the target metabolites **1** and **2** were calculated as 44% and 59%, respectively, with retention times of 13.34 and 18.05 min. These retention times values were confirmed by comparison with authentic standards analyzed under the same chromatographic conditions (Supplementary Figure S1). Additionally, an unknown peak at 16.27 min, corresponding to 53% methanol, was also selected for isolation.

For scaling up to the SPE cartridges, 70%–85% of the calculated methanol percentage were evaluated from which 80% was applied to isolate metabolites **1** and the unknown peaks, corresponding to 35% and 42% methanol, respectively. For metabolites **2**, the full calculated percentage (59%) was used as the other fractions had already been isolated. Figure 2 summarizes each step of the generic workflow employed.

Fractions I to III, eluted with 35%, 42%, and 59% methanol respectively, were collected and analyzed by LC-UV. The resulting chromatograms confirmed that metabolites **1** and **2** were isolated as the major components of fractions I and III, respectively (Figure 3). The solvent volume required for washing and elution was directly proportional to the sorbent

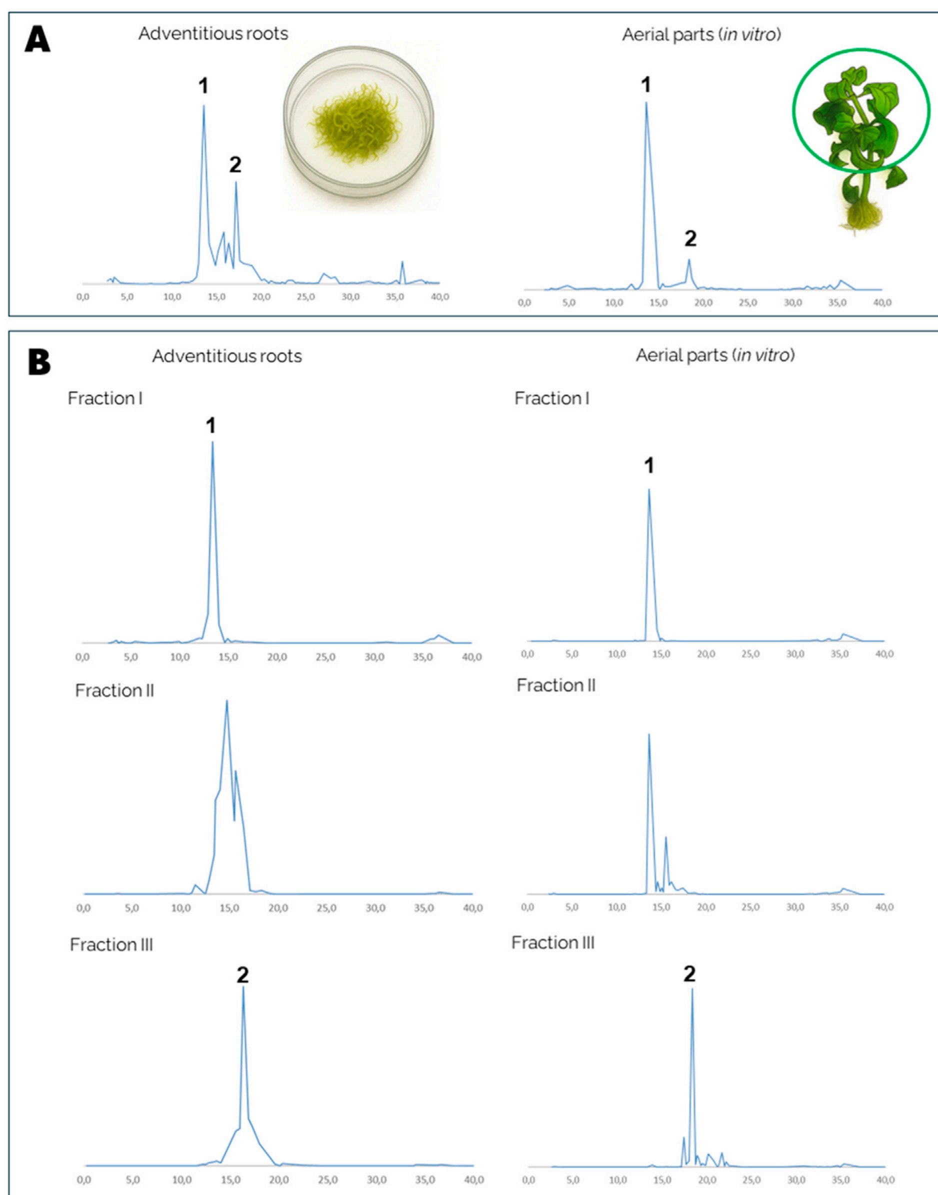


FIGURE 3
LC-UV chromatograms of the crude extracts obtained from of *in vitro U. guianensis* **(A)** and the SPE isolated fractions I-III **(B)** under gradient conditions. Fraction I-III were isolated with 35, 42% and 59% of MeOH respectively. The target metabolites, chlorogenic acid (**1**) and 3,4-Di-O-caffeoylquinic acid (**2**) are highlighted in the crude extracts and in their respective enriched fractions.

mass in the SPE tube and was calculated using Equation 1. It is worth noting that twice the Vm (40 mL) was sufficient for the complete elution of each fraction. To evaluate the general applicability of the protocol, a crude extract of *U. guianensis* aerial parts (*in vitro*) was analyzed under the same gradient conditions. As expected, metabolites **1** and **2** were successfully isolated from the 35% and 59% methanol fractions, respectively (Figure 3). Importantly, the 100% methanol fraction, corresponding to the cartridge washing step, did not contain either of the target metabolites. It is important to mention that metabolites such as chlorophyll are absent from cell and root cultures, facilitating the isolation of target compounds (Karalija et al., 2025; Ochatt et al., 2022).

The same workflow was subsequently applied to *in situ* samples (aerial parts and stem bark). The chromatographic profiles of these samples showed marked differences from the *in vitro* extracts (Figure 4). For *in situ* aerial parts samples, chlorogenic acid (**1**) was isolated from fractions I and II (35% and 43% methanol respectively), while metabolite **2** was isolated from fraction III. HPTLC analysis further evidenced that chlorogenic acid (**1**) was the predominant metabolite in the *in situ* aerial parts extract (Supplementary Figure S2). By contrast, metabolite **2** was absent from the *in situ* stem bark samples, in which chlorogenic acid (**1**) was obtained also from fraction I.

The isolated yields of the enriched fractions containing target metabolite **1** ranged from 46% to 53%, whereas the fractions

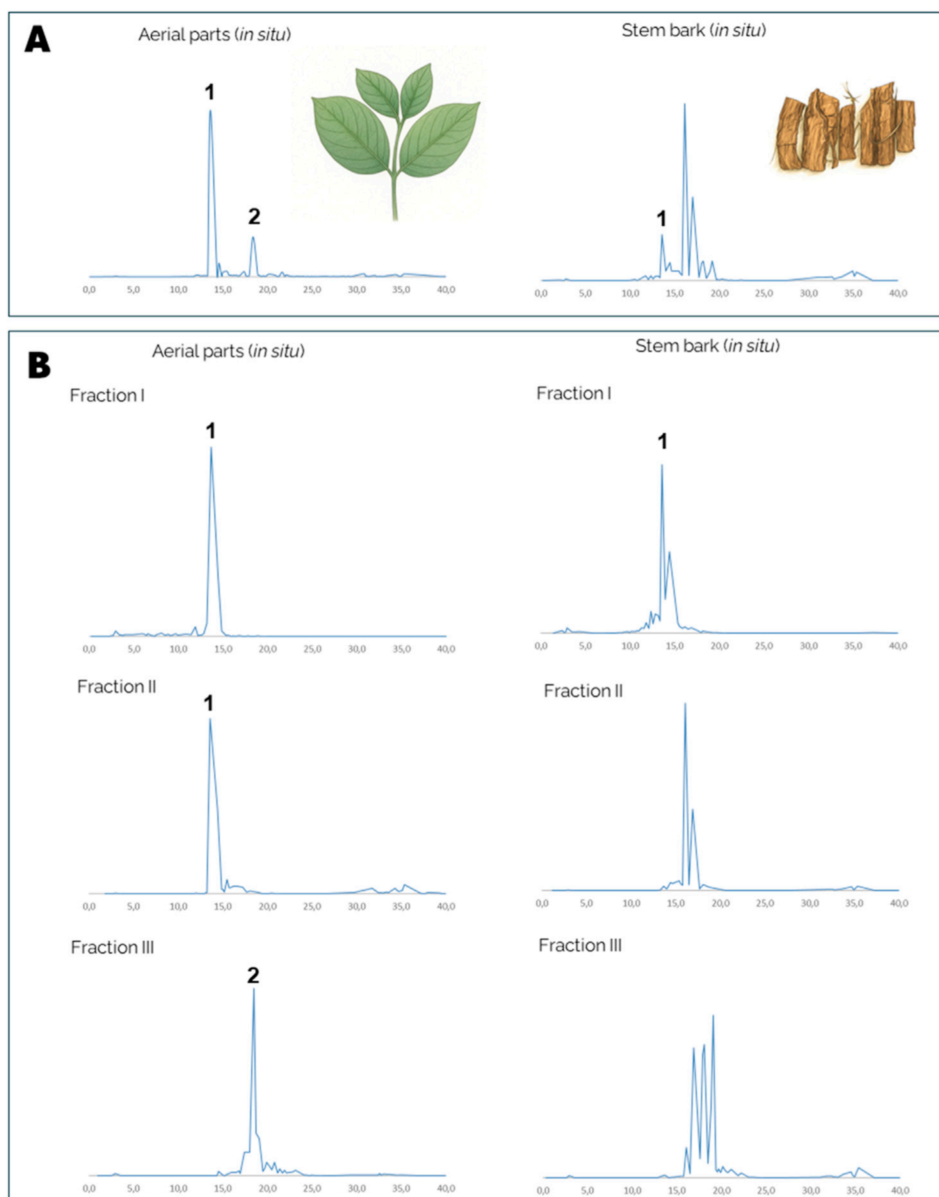


FIGURE 4
LC-UV chromatograms of the crude extracts obtained from of *in situ U. guianensis* **(A)** and the SPE isolated fractions I-III **(B)** under gradient conditions. Fraction I-III were isolated with 35%, 42%, and 59% of MeOH, respectively. The target metabolites, chlorogenic acid (**1**) and 3,4-Di-O-caffeoylquinic acid (**2**) are highlighted in the crude extract and in their respective enriched fractions.

TABLE 1 Mass and isolated yields of fractions I-III.

Samples	Initial mass (g)	Fraction I (g)	Yield (%)	Fraction II (g)	Yield (%)	Fraction III (g)	Yield (%)
In vitro aerial parts	**0.800**	0.4081	**51.0**	0.0650	**8.00**	0.0349	**4.40**
Adventitious roots	**0.800**	0.4030	**50.0**	0.0233	**3.00**	0.0367	**4.60**
In situ aerial parts	**0.800**	0.4234	**53.0**	0.1035	**13.0**	0.1003	**12.5**
In situ stem bark	**0.800**	0.3658	**46.0**	0.1513	**19.0**	0.0479	**6.00**

containing metabolite **2** ranged only from 4.4% to 12.5% (Table 1). It is important to say that fraction III isolated from *in situ* stem bark did not contain metabolite 2 (Figure 4)

Despite the persistent challenges associated with isolating pure NPs from complex mixtures, the protocol described herein demonstrates that the combination of LC with an exploratory

gradient and offline C_{18} SPE fractionation can accelerate the isolation of secondary metabolites. Given that cartridge selectivity is generally lower than that of an LC column, the described workflow is suitable for chromatographic bands that show adequate selectivity, irrespective of the number of peaks in the chromatogram.Target-enriched fractions can subsequently be subjected to isocratic semipreparative runs to obtain compounds of high purity. The herein reported results highlight the efficiency of the protocol for the isolation of target metabolites across all evaluated extracts. It is important to note that 3,4-Di-O-caffeoylquinic acid (**2**) is a high-value quinic acid derivative. Finally, the established LCSPE-*fast* protocol provides a rational and broadly applicable approach for the semipreparative isolation of metabolites from crude NP extracts.

4 Conclusion

The LCSPE-*fast* protocol described herein proved to be fast, efficient, and broadly applicable, enabling the fractionation of crude extracts and the yield of enriched fractions containing the target metabolites. By combining exploratory LC analysis with offline C_{18} SPE cartridges, this approach offers an environmentally friendly alternative for NPs research, characterized by reduced solvent consumption, and operational simplicity. Importantly, the method was effective across all four *Uncaria guianensis* extracts evaluated, both *in vitro* and *in situ*, despite differences in chemical complexity. To the best of our knowledge, this is the first report demonstrating the use of LC-guided offline SPE fractionation for the isolation of secondary metabolites. The rational workflow established here provides a valuable tool for NPs research and can be readily extended to diverse complex extracts from plants, microorganisms, and marine organisms, thereby accelerating the isolation and characterization of bioactive compounds.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

LP: Formal Analysis, Writing – review and editing, Investigation, Methodology. MB: Investigation, Methodology, Formal Analysis, Writing – review and editing. AP: Writing – review and editing, Conceptualization, Methodology, Formal Analysis, Investigation. QC: Formal Analysis, Writing – review and editing, Methodology, Data curation, Investigation, Conceptualization. AL: Writing – review and editing, Methodology, Investigation, Writing – original draft, Conceptualization, Formal Analysis, Data curation.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fntpr.2025.1726849/full#supplementary-material

References

Badawy, M. E. I., El-Nouby, M. A. M., Kimani, P. K., Lim, L. W., and Rabea, E. I. (2022). A review of the modern principles and applications of solid-phase extraction techniques in chromatographic analysis. *Anal. Sci.* 38 (12), 1457–1487. doi:10.1007/s44211-022-00190-8

Beltrame, F. L., Filho, E. R., Barros, F. A., Cortez, D. A., and Cass, Q. B. (2006). A validated higher-performance liquid chromatography method for quantification of cinchonain Ib in bark and phytopharmaceuticals of *Trichilia catigua* used as catuaba. *J. Chromatogr. A* 1119 (1-2), 257–263. doi:10.1016/j.chroma.2005.10.050

Berlinck, R. G. S., Crnkovic, C. M., Gubiani, J. R., Bernardi, D. I., Ióca, L. P., and Quintana-Bulla, J. I. (2022). The isolation of water-soluble natural products - challenges, strategies and perspectives. *Nat. Prod. Rep.* 39 (3), 596–669. doi:10.1039/d1np00037c

Bucar, F., Wube, A., and Schmid, M. (2013). Natural product isolation-how to get from biological material to pure compounds. *Nat. Prod. Rep.* 30 (4), 525–545. doi:10.1039/c3np20106f

Caesar, L. K., Montaser, R., Keller, N. P., and Kelleher, N. L. (2021). Metabolomics and genomics in natural products research: complementary tools for targeting new chemical entities. *Nat. Prod. Rep.* 38 (11), 2041–2065. doi:10.1039/d1np00036e

Dong, M. W. (2019). *HPLC and UHPLC for practicing scientists*. Second edition. Hoboken, NJ: Wiley.

Floros, D. J., Jensen, P. R., Dorrestein, P. C., Koyama, N., and Fenical, W. (2016). A metabolomics guided exploration of marine natural product chemical space. *Metabolomics* 12 (9), 145. doi:10.1007/s11306-016-1087-5

Gu, Y., Feuerstein, M. L., and Warth, B. (2025). High-throughput solid phase extraction for targeted and nontargeted exposomics. *Anal. Chem.* 97 (11), 6075–6082. doi:10.1021/acs.analchem.4c06177

Honório, I. C. G., Bertoni, B. W., and Pereira, A. M. S. (2016). *Uncaria tomentosa* and *Uncaria guianensis* an agronomic history to be written. *Ciência Rural.* 46, 1401–1410. doi:10.1590/0103-8478cr20150138

Huo, C., Nguyen, Q. N., Alishir, A., Ra, M.-J., Jung, S.-M., Yu, J.-N., et al. (2023). Global natural products social (GNPS)-based molecular-networking-guided isolation of phenolic compounds from *Ginkgo biloba* fruits and the identification of estrogenic phenolic glycosides. *Plants (Basel)* 12 (23), 3970. doi:10.3390/plants12233970

Karalija, E., Macanović, A., and Ibragić, S. (2025). Revisiting traditional medicinal plants: integrating multiomics, *in vitro* culture, and elicitation to unlock bioactive potential. *Plants* 14, 2029. doi:10.3390/plants14132029

Khushi, S., Salim, A. A., and Capon, R. J. (2023). Case studies in molecular network-guided marine biodiscovery. *Mar. Drugs* 21 (7), 413. doi:10.3390/md21070413

Lopes, A. A., Chioca, B., Musquiari, B., Crevelin, E. J., França, S. C., Da Silva, M. F. G. F., et al. (2019). Unnatural spirocyclic oxindole alkaloids biosynthesis in *Uncaria guianensis*. *Sci. Rep.* 9 (1), 11349. doi:10.1038/s41598-019-47706-3

McCown, B. H., and Lloyd, G. (1981). Woody Plant Medium (WPM)-A mineral nutrient formulation for microculture of woody plant species. *HortScience* 16, 453.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant* 15 (3), 473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

Ochatt, S., Alan, A. R., Bhattacharya, A., Hano, C., Kiselev, K. V., Marconi, P. L., et al. (2022). Secondary metabolites: a boon from plants, the best chemist in nature: preface from the editors. *Plant Cell. Tiss. Organ Cult.* 149, 1–6. doi:10.1007/s11240-022-02289-2

Pauli, G. F., Chen, S. N., Friesen, J. B., McAlpine, J. B., and Jaki, B. U. (2012). Analysis and purification of bioactive natural products: the AnaPurNa study. *J. Nat. Prod.* 75 (6), 1243–1255. doi:10.1021/np300066q

Queiroz, E. F., Guillarme, D., and Wolfender, J. L. (2024). Advanced high-resolution chromatographic strategies for efficient isolation of natural products from complex biological matrices: from metabolite profiling to pure chemical entities. *Phytochem. Rev.* 23 (5), 1415–1442. doi:10.1007/s11101-024-09928-w

Quinn, R. A., Nothias, L. F., Vining, O., Meehan, M., Esquenazi, E., and Dorrestein, P. C. (2017). Molecular networking as a drug discovery, drug metabolism, and precision medicine strategy. *Trends Pharmacol. Sci.* 38 (2), 143–154. doi:10.1016/j.tips.2016.10.011

Ranušová, P., Matušíková, I., and Nemeček, P. (2021). Optimization of plant extract purification procedure for rapid screening analysis of sixteen phenolics by liquid chromatography. *Separations* 8 (2), 13. doi:10.3390/separations8020013

Salem, M. A., Perez de Souza, L., Serag, A., Fernie, A. R., Alseekh, S., Ezzat, S. M., et al. (2020). Metabolomics in the context of plant natural products research: from sample preparation to metabolite analysis. *Metabolites* 10 (1), 37. doi:10.3390/metabo10010037

Simpson, A. J., Tseng, L. H., Simpson, M. J., Spraul, M., Braumann, U., Kingery, W. L., et al. (2004). The application of LC-NMR and LC-SPE-NMR to compositional studies of natural organic matter. *Analyst* 129 (12), 1216–1222. doi:10.1039/b408064e

Thomasi, S. S., Oliveira, L. M., Venâncio, T., and Ferreira, A. G. (2017). Aplicação de LC-SPE/NMR na Rápida Identificação de Compostos Orgânicos em Fitoterápicos. *Rev. Bras. Ciência, Tecnol. Inovação* 2, 13–22. Available online at: https://seer.uftm.edu.br/revistaeletronica/index.php/rbcti/article/view/776.