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# HPLC analysis of four important fern species collected from Udhagamandalam and Coonoor, Nilgiris district, Tamil Nadu, India

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***Abstract:*** *The present study deals with the identification and quantification of phenolic compounds and flavonoids in various fern species like Christella dentata, Cyathea crinita, Dryopteris redactopinnata and Pteris vittata is typically conducted using HPLC analysis. Their analysis is particularly complex as fern species contain number of different phenolic compounds, many of which have similar chemical characteristics such as polarity which makes complete separation of all eluents difficult. The present study method could be used to interpret the results of HPLC analysis of fern species which possess a vast spectrum of phenolic compounds and flavonoids. The compounds identified from these four fern species were Rutin, Quercetin, Kaempferol, chlorogenic acid compounds in Christella dentata, Rutin, Benzoic acid, Quercetin in Cyathea crinita, Rutin, Quercetin, Gallic acid, Luteolin, Kaempferol in Pteris vittata and Rutin, Benzoic acid, Gliricidin 7-o hexoside and Caffeic acid in Dryopteris redactopinnata. These compounds may be used for drug preparation.*

***Key Words:*** *Fern species, Flavonoids, HPLC, Phenolic compounds and UV-Vis absorbance.*

## 1. INTRODUCTION:

Fern species are ancient group of plant kingdom contains so many important secondary metabolites such as alkaloids, flavonoids, terpenoids etc. (Shah *et al*., 2022). Fern species contain several bioactive compounds that could be utilized for therapeutic application (Shah *et al*., 2023). HPLC (high-performance liquid chromatography), sometimes known as high- pressure liquid chromatography, is a technique for separating, identifying and quantifying individual components in a mixture. It depends on pumps to move a pressured liquid solvent containing the sample combination through a solid adsorbent material-filled column. Each component in the sample interacts with the adsorbent material in a slightly different way, resulting in varying flow rates as the components exit the column. HPLC can only evaluate chemicals that are dissolved in solvents. It separates compounds dissolved in a liquid sample and enables for qualitative and quantitative examination of which components are present in the sample, as well as how much of each component is present. HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify, and purify the different components of the mixture. HPLC is a versatile, resilient, and commonly used technology for the isolation of natural products (Piana *et al*., 2013). This methodology is currently gaining favour among many analytical techniques as the preferred method for fingerprinting studies for herbal plant quality control (Fan *et al*., 2006). HPLC's resolving ability is appropriate for the quick analysis and preparation of such multi-component mixtures on both an analytical and preparative scale (Martin and Guiochan, 2005). It is used to identify and quantify secondary metabolites in plant extracts, such as phenolic compounds, steroids, flavonoids, and alkaloids (Barbosa *et al*., 2014). So the HPLC technique were used to identify and quantify phenolic and flavonoid compounds which are present in these fern species. Despite being present in relatively small concentrations, these

compounds are known to impart essential properties to these fern species such as antimicrobial and antioxidant properties (Rashid *et al*., 2022).

## MATERAILS AND METHODS

### Collection and identification of fern species

Extensive field trips were carried out in Udhagamandalam and Coonoor for the collection of ferns species. Udhagamandalam and Coonoor are two sub-districts of Nilgiris district, Tamil Nadu, India. They are situated at the latitude of 11° 24' 0" N and Longitude of 76° 42' 0" E. The specimens (*Christella dentata*, *Cyathea crinita*, *Dryopteris redactopinnata* and *Pteris vitta* leaves) shown in Fig. 1 were then identified with the help of artificial key prepared by Manickam and Irudayaraj, 1992 for *South India* fern species. A complete set of voucher specimens was deposited in the department of Botany, Annamalai University Chidambaram.

### Extraction of the Plant material

The plant materials (*Christella dentata*, *Cyathea crinita*, *Dryopteris redactopinnata* and *Pteris vitta* leaves) utilized for HPLC screening was washed thoroughly under running tap water to remove all debris and soil, and then shade dried for two weeks at room temperature. The air-dried plant material was finely crushed and stored in self-sealing, air-tight polythene bags. Using a Soxhlet apparatus, 30 g of powder was extracted sequentially with 200 mL of methanol, for 7 hours at 50-65 ℃ (not greater than the boiling point of the solvent). Then the extracted material were used for HPLC studies.

### Preparation of standards

Standard solutions were prepared for the nine investigated phenolic compounds and flavonoids, namely Rutin, Quercetin, Kaempferol, chlorogenic acid, Benzoic acid, Gallic acid, Luteolin, Gliricidin 7-o hexoside and Caffeic acid. These solutions were prepared by dissolving the standards in HPLC grade methanol to produce stock solutions of 100 mg/L, which were then used to prepare 50, 40, 30, 20 and 10 mg/L solutions for the standard plots. In addition, a mixture containing 30 mg/L of each phenolic compound in methanol was also prepared. Another similar set of mixtures was also prepared using caffeic acid. We measured the absorbance of the samples of 100 mg/L of each phenolic compound and flavonoid with a UV-Vis spectrophotometer (Shimadzu) at a wavelength between 180-254 nm to find the optimum wavelength for the HPLC measurements.

### High performance liquid chromatography (HPLC) analysis

HPLC analysis of the selected species were performed on a Shimadzu system equipped with a model LC pump, UV-Vis detector, Rheodyne injector fitted with a 20 µl loop and an auto injector. A Hypersil C-18 column (4.6 × 250 mm, 5 µm size) was used. The elution was carried out with gradient solvent systems with a flow rate of 1 ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through 0.45 µ membrane filter and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µl while the wavelength of the UV-Vis detector was set at 254 nm (Mallikharjuna *et al*., 2007; Sharanabasappa *et al*., 2007).

*Christella dentata*

*Cyathea crinita*

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**Fig. 1**

**Result and Discussion**

HPLC spectral data generated by the detector was used to verify the identity of the compounds present in the chromatogram. The qualitative HPLC fingerprint profile of methane extracts of selected species like *Christella dentata*, *Cyathea crinita*, *Dryopteris redactopinnata*, and *Pteris vittata* were detected based on the sharpness of peaks and proper baseline. The result showed different peaks at various retention times with significant percentage peak areas (Fig. 2, 3, 4. and 5 and Table. 1). The HPLC chromatogram of *Christella dentata* confirmed the compounds with the most abundant peaks separated at retention time of 2.985 min, 5.757 min, 9.365 min, 9.746 min, 14.652 min and 24.595 min and peak area 77.759 %, 17.928 %, 1.593 % and 2.431 %, 0.195 % and 0.095 % respectively (Fig. 2). The chromatogram of *Cyathea crinita* also showed that the compound separated at a retention time of 2.967 min, 4.992 min and 5.639 min with the peak area 81.384 %, 8.705 % and 9.911 % (Fig. 3). The compounds present in the *Dryopteris redactopinnata* (Fig. 4) confirmed at the retention time of 3.410 min, 4.630 min, 7.136 min and 17.858 min with peak area 74.541 %, 24.947 %, 0.333 % and 0.179 % respectively. The chromatogram of *Pteris vittata* revealed the compounds at retention time of 2.951 min and 3.260 min, 5.902 min, 10.098 min and 10.538 min with peak area 17.713 %, 65.868 %, 13.383 %, 1.197 % and 1.839 % (Fig. 5). The retention time of selected species matched with the retention time of standards like Rutin, Quercetin, Kaempferol, Gallic acid, Luteolin, Benzoic acid, Caffeic acid, Chlorogen and Glinicidin revealed the presence of Rutin, Quercetin, Kaempferol, chlorogenic acid compounds in *Christella dentata*, Rutin, Benzoic acid, Quercetin in *Cyathea crinita*, Rutin, Quercetin, Gallic acid, Luteolin, Kaempferol in *Pteris vittata* and Rutin, Benzoic acid, Gliricidin 7-o hexoside and Caffeic acid in *Dryopteris redactopinata*.

**Table: 1 HPLC peak values of studied species**

| **Peaks** | ***Christella dentata*** | | ***Cyathea crinita*** | | ***Dryopteris redactopinnata*** | | ***Pterris vittata*** | |
|---|---|---|---|---|---|---|---|---|
| | RT (Min) | Area (%) | RT (Min) | Area (%) | RT (Min) | Area (%) | RT (Min) | Area (%) |
| 1 | 2.985 | 77.759 | 2.967 | 81.384 | 3.410 | 74.541 | 2.951 | 17.713 |
| 2. | 5.757 | 17.928 | 4.992 | 8.705 | 4.630 | 24.947 | 3.260 | 65.868 |
| 3. | 9.365 | 1.593 | 5.639 | 9.911 | 7.136 | 0.333 | 5.902 | 13.383 |
| 4. | 9.746 | 2.431 | | | 17.858 | 0.179 | 10.098 | 1.197 |
| 5. | 14.652 | 0.195 | | | | | 10.538 | 1.839 |
| 6. | 24.595 | 0.095 | | | | | | |

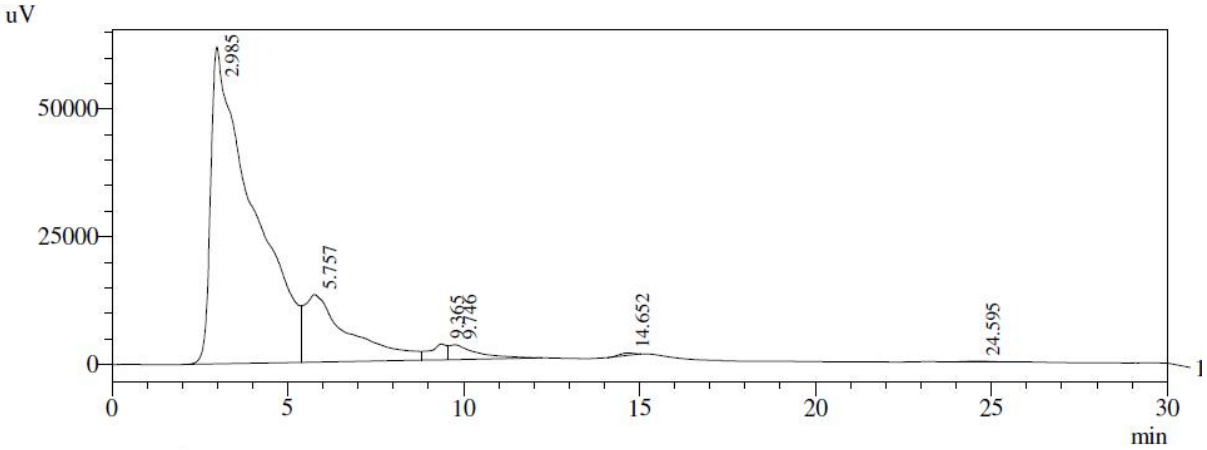


**Fig. 2 HPLC** chromatogram **of Methanolic leaf extract of *Christella dentate***

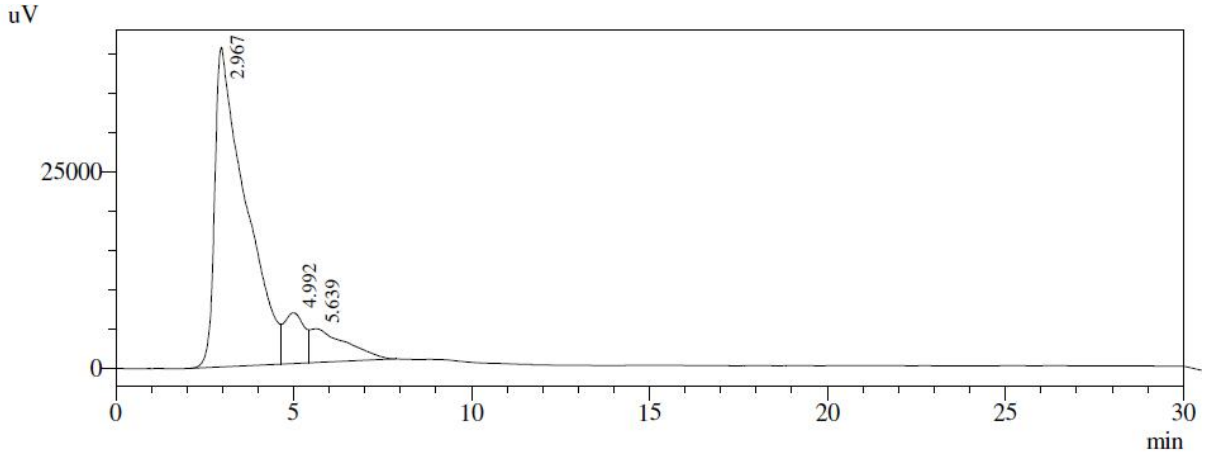


**Fig. 3 HPLC chromatogram of Methanolic leaf extract of *Cyathea crinite***

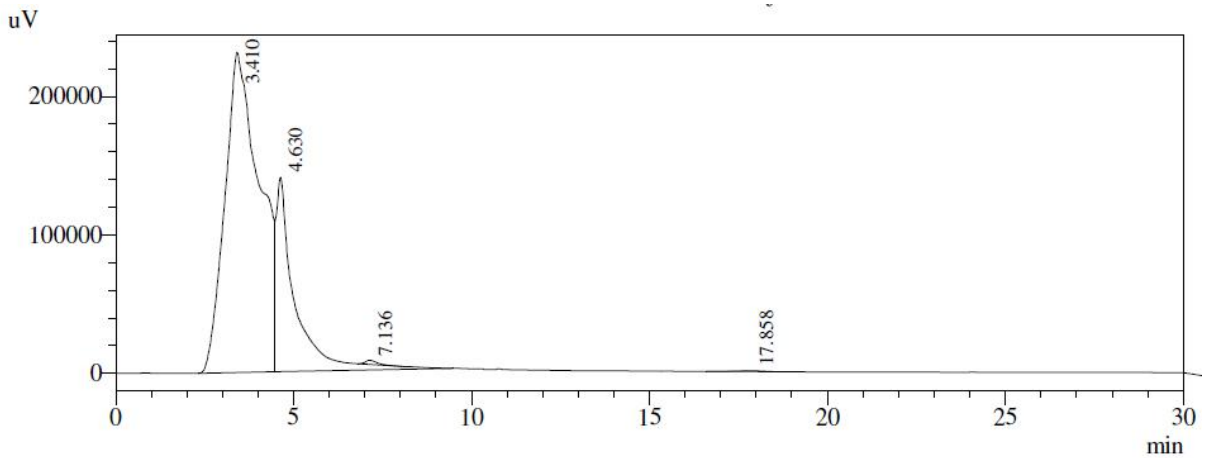


**Fig. 4 HPLC chromatogram of Methanolic leaf extract of *Dryopteris redactopinnata***

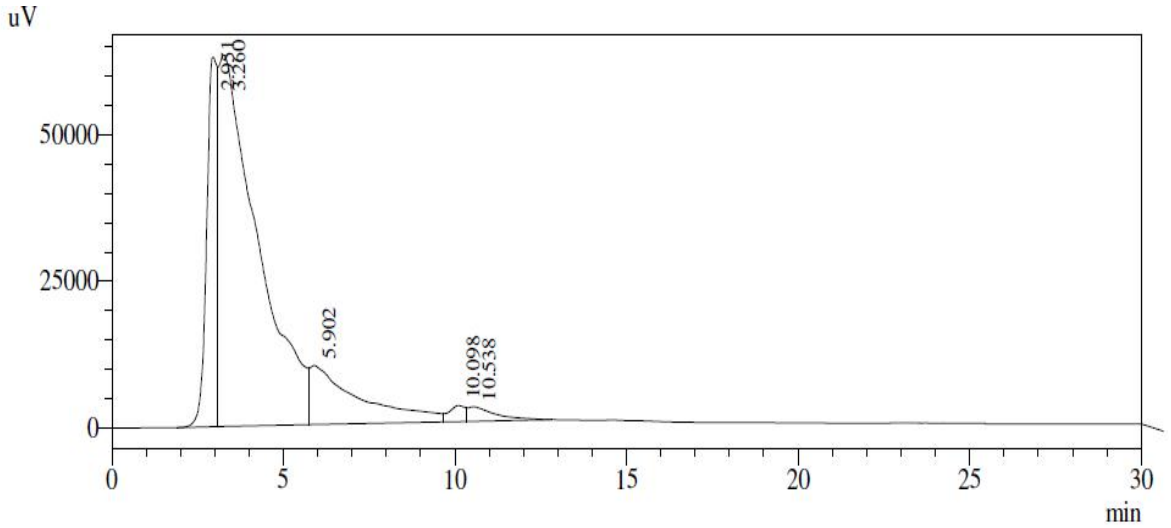


**Fig. 5 HPLC chromatogram of Methanolic leaf extract of *Pteris vittata***

HPLC has several advantages, including the ability to automate and computer processing (Backonja *et al*., 2010), enhanced reproducibility in identifying isolated compounds (Zhang *et al*., 2010), quantitative determination (Douat *et al*., 2011) and the ability to complete the analyses in a relatively short time duration (Douat *et al*., 2011; Reyes *et al*., 2011). It is a highly sensitive method for detection, identification and quantification of any chemical in a particular sample using UV and visible absorbance (Hanachi and Golkho, 2009).

HPLC analysis of *Christella dentata*, *Cyathea crinita*, *Dryopteris redactopinnata* and *Pteris vittata* leaves showed varying patterns in the chromatogram. The results showed various peaks separated at different retention times. The chromatogram also confirmed the presence of a most widely available peak separated at a retention time of 2.985 min with a peak area of 77.759 per cent in *Christella dentata*, 81.384 percent peak area separated at a retention time of 2.967 min in *Cyathea crinita*, 74.541 per cent peak area separated at a retention time of 3.410 min in *Dryopteris redactopinnata* and 65.868 per cent peak area separated at a retention time of 3.260 in *Pteris vittata* respectively. HPLC analysis revealed the presence of Rutin, Quercetin, Kaempferol, chlorogenic acid compounds in *Christella dentata*, Rutin, Benzoic acid, Quercetin in *Cyathea crinita*, Rutin, Quercetin, Gallic acid, Luteolin, Kaempferol in *Pteris vittata* and Rutin, Benzoic acid, Gliricidin 7-o hexoside and Caffeic acid in *Dryopteris redactopinnata*. The results of the HPLC analysis suggested that there may be variances in the chemical contents of the analysed species, requiring a thorough scientific validation of active principles before they may be used medicinally. The information gathered could be valuable in determining chemotaxonomic interrelationships among the species investigated. Plants are screened for potential "biomarkers" using metabolomics techniques (Kooy *et al*., 2009). These techniques play an important role in many aspects of biomedical and phytochemical research including biomarker screening, chemotaxonomy, quality control, bioactivity and toxicity prediction (Liu *et al*., 2010).

## CONCLUSION

HPLC analysis of four fern species namely *Christella dentata, Cyathea crinita, Dryopteris redactopinnata* and *Pteris vittata* leaves in methanol extract revealed the most abundant peak in *Cyathea crinita* at 2.967 min (81.384 %), and it was followed by *Christella dentata* at 2.985 min (77.759 %), *Dryopteris redactopinnata* at 3.410 min (74.541 %) and *Pteris vittata* at 3.260 min (65.868 %). HPLC analysis revealed the presence of Rutin, Quercetin, Kaempferol, chlorogenic acid compounds in *Christella dentata*, Rutin, Benzoic acid, Quercetin in *Cyathea crinita*, Rutin, Quercetin, Gallic acid, Luteolin, Kaempferol in *Pteris vittata* and Rutin, Benzoic acid, Gliricidin 7-o hexoside and Caffeic acid in *Dryopteris redactopinnata*. These identification tests were used to confirm the presence of the active constituents and potential adulterant in Ayurvedic drugs.

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**Conflict of interest**

The author declares that there is no conflict of interest

**REFERENCES:**

1. Backonja M. M., Malan T. P., Van hove G. F., Tobias J. K., (2010). NGX-4010, a high-concentration capsaicin patch, for the treatment of post herpetic neuralgia: A randomized, double-blind, controlled study with an open-label extension. *Pain Medicine*, 11: 600-608.
2. Barbosa Filho V. M., Waczuk E. P., Kamdem J. P., Abolaji A. O., Lacerda S. R., Costa J. G. M., et al. (2014). Phytochemical constituents, antioxidant activity, cytotoxicity andosmotic fragility effects of Caju (*Anacardium microcarpum*). *Industrial Crops and Products*, 55: 280-288.
3. Douat, J., Vachon, P., and Beaudry, F. (2011). Characterization of in vitro metabolism of capsazepine, a vanilloid transient receptor potential channel antagonist, by liquid chromatography quadrupole ion trap mass spectrometry. *Biomedical Chromatography*, 25(4): 479-492.
4. Fan, X. H., Cheng, Y. Y., Ye, Z. L., Lin, R. C., and Qian, Z. Z. (2006). Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Analytica Chimica Acta*, 555(2): 217-224.
5. Hanachi, P., and Golkho, S. H. (2009). Using HPLC to determination the composition and antioxidant activity of *Berberis vulgaris*. *European Journal of Scientific Research*, 29(1): 47-54.
6. Kooy, F., Maltese, F., Choi, Y. H., Kim, H. K., and Verpoorte, R. (2009). Quality control of herbal material and phytopharmaceuticals with MS and NMR based metabolic fingerprinting. *Planta Medica*, 75(07): 763-775.
7. Liu, N. Q., Cao, M., Frédérich, M., Choi, Y. H., Verpoorte, R., and van der Kooy, F. (2010). Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. *Journal of EthnoPharmacology*, 128(1): 230-235.
8. Mallikharjuna, P. B., Rajanna, L. N., Seetharam, Y. N., and Sharanabasappa, G. K. (2007). Phytochemical studies of *Strychnos potatorum* Lf-A medicinal plant. *E-journal of Chemistry*, 4(4): 510-518.
9. Manickam, V. S., and Irudayaraj, V. (1992). Pteridophyte flora of the western ghats, South India. *BI publications*.
10. Martin, M., and Guiochon, G. (2005). Effects of high pressure in liquid chromatography. *Journal of Chromatography A*, 1090 (1-2): 16-38.
11. Piana, M., Zadra, M., de Brum, T. F., Boligon, A. A., Gonçalves, A. F. K., da Cruz, R. C., and Athayde, M. L. (2013). Analysis of rutin in the extract and gel of *Viola tricolor*. *Journal of Chromatographic Science*, 51(5): 406-411.
12. Reyes-Escogido, M. D. L., Gonzalez-Mondragon, E. G., and Vazquez-Tzompantzi, E. (2011). Chemical and pharmacological aspects of capsaicin. *Molecules*, 16(2): 1253-1270.
13. Rashid, S., Ali, S. A., Manikandan. S., and Lakshmanan, A. M. G. (2022). TLC Profiling and Phytochemical evaluation of *Bergenia ligulata* wall. and *Podophyllum hexandrum* Royle-an Endangered medicinal plant. *Indian Journal of Natural Sciences*, 13 (73): 46111- 46118).
14. Shah, A. F., Rashid, and Kumarasamy. D. (2022). Preliminary Phytochemical Studies on Some Medicinally Important Fern Species Collected from Udhagamandalam, Tamil Nadu, India. *Indian Journal of Natural Sciences*, 13 (75): 51361-51368.
15. Shah, A. F., Rashid, S., Mir, S. A., and Kumarasamy. D. (2023). Qualitative Phytochemical Analysis of Some Medicinally Important Fern Species Collected from Coonoor and Udhagamandalam, Tamil Nadu, India. *Indian Journal of Natural Sciences*, 14 (78): 57280-57285.
16. Sharanabasappa, G. K., Santosh, M. K., Shaila, D., Seetharam, Y. N., and Sanjeevarao, I. (2007). Phytochemical studies on *Bauhinia racemosa* lam. *Bauhinia purpurea* Linn. And *Hardwickia binata* roxb. E-Journal of Chemistry, 4(1): 21-31.
17. Zhang, Q., Hu, J., Sheng, L., and Li, Y. (2010). Simultaneous quantification of capsaicin and dihydro capsaicin in rat plasma using HPLC coupled with tandem mass spectrometry. *Journal of Chromatography B*, 878(24): 22922297.