應用液相層析串聯式質譜技術於台灣產蕨類植物特定蕨素成分之分析研究

Applying LC-MS/MS to the Analysis of Pterosins in Pteridophytic Floras of Taiwan

中文摘要

蕨素成分（pterosin）爲一群結構相近之倍半萜類化合物（sesquiterpene），以 1-indanone 爲基本骨架，種類繁多甚爲複雜，廣泛存在於蕨類植物。爲了解蕨素成分（pterosin）於蕨類不同品種間之分布情形，本研究選擇二十五件台灣產蕨類植物，應用液相層析串聯式質譜技術（liquid chromatography-tandem mass spectrometry, LC-MS/MS），針對蕨類三種特定成分-蕨素 A（pterosin A）、蕨素 I（pterosin I）、蕨素 Z（pterosin Z），建立其快速分析方法，並應用於二十五件蕨類植物之低、中、高極性溶媒分配萃取層之分析，以追蹤該類成分之分布情形。實驗部分使用矽膠鍵結十八碳烷基、粒徑 3 μm 之層析管柱，以 0.25%甲酸-水-氰甲烷混合液爲移動相，線性梯度沖提，利用正離子電灑法（positive ion electrospray ionization）之離子化方式。定性分析方面，擇定質子加成離子之母離子（parent ion），進行子代離子掃描分析，所得質譜裂解碎片訊息，建入質譜圖庫。本分析法在多重離子裂解監控（multiple reaction monitoring, MRM）分析模式下，pterosin A、I 及 Z 成分之最低檢出極限可達 9.97 ng/mL、7.47 ng/mL 及 3.60 ng/mL；同日內、異日間測試，其 RSD 分別小於 7.52%及 11.95%；檢量線之線性相關係數達 0.995 以上。各萃取層之成分含量，pterosin A 介於 1.15 至 9615.4 μg/g 之間，以蕨之二氯甲烷萃取層含量最高；pterosin I 介於 6.3 至 10845.5 μg/g 之間，以熱帶鱗蓋蕨之正己烷萃取層含量最高；pterosin Z 介於 11.0 至 15480.2 μg/g 之間，以熱帶鱗蓋蕨之正己烷萃取層含量最高。此外，也建立了蕨類具有 pterosin 化合物光譜特徵之特定成分的質荷比訊號分布資料表，可作爲研究蕨類 pterosin 成分之重要參考。本研究利用高效液相層析裝置，結合光二極體陣列檢測器及串聯式質譜儀二種檢測器，應用於蕨類植物結構相近之複雜成分群檢測，具備快速、簡便、靈敏、高專一度之特性。

英文摘要

This study aimed to establish an analytical method for screening certain types of pterosins and studies the distribution of those in ferns. Pterosins are a large group of naturally occurring sesquiterpenes with indanone skeleton. Widely distributed in the Pteridophyte, having been found to have cytotoxicity and smooth muscle relaxant activity, there are still new findings related to pterosins in the ongoing pharmacological study. Here we introduced a newly developed method of applying LC-MS/MS to the analysis of pterosins in ferns and conducted a survey towards 25 species of ferns from Taiwan.

The optimized LC-MS/MS condition was as follows. Separation was carried out using a reverse phase C18 column under gradient elution. Mobile phase comprised a mixed solvent system of 0.25% formic acid-water-acetonitrile and was set at a flow rate of 0.5 mL/min with a split ratio of 1:1 into PDA and tandem mass spectrometer. The electrospray ionization source was operated in the positive mode. The mass

parameters including cone voltage, capillary voltage, collision energy, ion source temperature, desolvation temperature and desolvation gas flow were subsequently optimized. Proton adduct ions of each analyte were fairly detectable under current analytical condition and were selected as parent ions for further daughter ion scan. Qualitative analysis was achieved by daughter ion analysis. The characteristic daughter ion mass spectra of pterosins were generated for library search. Quantitative analysis was accomplished by MRM (multiple reaction monitoring) analysis. Limits of detection using MRM analysis were defined as 9.97 ng/mL of pterosin A, 7.47 ng/mL of pterosin I, and 3.60 ng/mL of pterosin Z. R.S.D. of intraday and interday repeated analysis were within 7.52% and 11.95%, respectively.

The result shows that pterosins were detected in 10 species out of 25, mainly found in the families of Dennstaedtiaceae and Pteridaceae, mostly in the non-polar or less polar partition layer such as n-hexane, dichloromethane or ethyl acetate. Levels of pterosin A, pterosin I and pterosin Z detected in the 10 species of ferns ranged from 1.15 to 9615.4 μg/g, from 6.3 to 10845.5 μg/g and from 11.0 to 15480.2 μg/g, respectively. Amounts of pterosin A were found to be highest in P. aquilinum var. latiusculum while amounts of pterosin I and Z were highest in M. speluncae. Mass information of those signals with pterosin-like UV spectrum in the chromatographic system was recorded. A fast, sensitive and highly specific method coupling both a photodiode array (PDA) detector and tandem mass spectrometer was developed in this study.