Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2016, **14**(4): 0278–0285
Chinese Journal of Natural Medicines

Appropriate choice of collision-induced dissociation energy for qualitative analysis of notoginsenosides based on liquid chromatography hybrid ion trap time-of-flight mass spectrometry

WANG Guang-Ji [Δ], FU Han-Xu [Δ], XIAO Jing-Cheng, YE Wei, RAO Tai, SHAO Yu-Hao, KANG Dian, XIE Lin, LIANG Yan [*]

Key Lab of Drug Metabolism & Pharmacokinetics, State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

Available online 20 Apr., 2016

[ABSTRACT] Liquid chromatography hybrid ion trap/time-of-flight mass spectrometry possesses both the MS^n ability of ion trap and the excellent resolution of a time-of-flight and has been widely used to identify drug metabolites and determine trace multi-components in natural products. Collision energy, one of the most important factors in acquiring MS^n information, could be set freely in the range of 10%–400%. Herein, notoginsenosides were chosen as model compounds to build a novel methodology for the collision energy optimization. Firstly, the fragmental patterns of the representatives for the authentic standards of protopanaxadiol-type and protopanaxatriol-type notoginsenosides were obtained based on accurate MS^2 and MS^3 measurements via liquid chromatography hybrid ion trap/time-of-flight mass spectrometry. The extracted ion chromatograms of characteristic product ions of notoginsenosides in *Panax Notoginseng* Extract were produced under a series of collision energies and compared to screen the optimum collision energies values for MS^2 and MS^3. The results demonstrated that the qualitative capability of liquid chromatography hybrid ion trap/time-of-flight mass spectrometry was greatly influenced by collision energies, and 50% of MS^2 collision energy was found to produce the highest collision-induced dissociation efficiency for notoginsenosides. Addtionally, the highest collision-induced dissociation efficiency appeared when the collision energy was set at 75% in the MS^3 stage.

[KEY WORDS] Collision energy; Collision-induced dissociation; Notoginsenosides; Qualitative analysis

[CLC Number] R969.1 **[Document code]** A **[Article ID]** 2095-6975(2016)04-0278-08

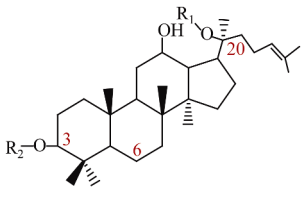
Introduction

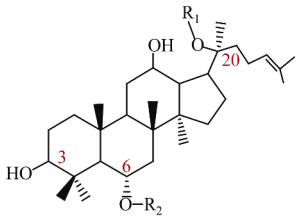
Over the past two decades, mass spectrometry (MS) has played an important role in the drug discovery and development process since it could provide excellent selectivity, sensitivity, and speed. Especially, the continuous development of high resolution and multiple-stage MS has increased the potentials of MS for detailed structural elucidation and broadened its applicability in the analysis of complicated constituents in Chinese herbal medicine [1-2]. Liquid chromatography hybrid ion trap/time-of-flight mass spectrometry (LC-IT-TOF MS), a uniquely designed mass spectrometer which combins an ion trap with a TOF analyzer, could provide high-precision MS^n information with high sensitivity, high mass resolution and stable mass accuracy over seven days of acquisition [3-6]. Recently, LC-IT-TOF MS has been successfully applied to the identification of major constituents in *Artemisia capillaries* [7], *Antimigraine* [8], *Danshen decoction* [9], *Danggui Buxue Tang* [10], *Millettiae Pulchrae Radix* [11], and *blueberry anthocyanins* [12]. Collision energy (CE), a crucial parameter of multi-stage MS, plays a vital

[Received on] 10-Sep.-2015
[Research funding] This work was supported by the National Nature Science Foundation of China (Nos. 81273589, 81374054), the Nature Science Foundation of Jiangsu Province (BK20131311), the fundamental research special fund of China Pharmaceutical University (PT2014 YK0081), Jiangsu Provincial Promotion Foundation for the Key Laboratory of Drug Metabolism and Pharmacokinetics (BM2012012), and Jiangsu Key Laboratory of Drug Design and Optimization.
[*Corresponding author] Tel: 86-25-83271060, Fax: 86-25-83271060, E-mail: liangyan0679@hotmail.com
[Δ]Co-first authors.
These authors have no conflict of interest to declare.

role in using LC-IT-TOF MS for qualitative analysis [13]. In 2013, LC-IT-TOF MS was used to differentiate isobaric steroid hormone metabolites by Tedmon *et al*. [6]. However, the adjustment process for the CE was not detailed although the CE during collision induced dissociation was adjusted at each tandem MS stage. To our best knowledge, there is no report about the influence of CE on the qualitative analysis of complex components in herbal medicine based on LC-IT-TOF MS.

Panax notoginseng, an important traditional Chinese medicine mainly produced in Yunnan, Guangxi and Szechwan provinces in China, possesses a wide range of pharmacological actions, such as dilating vessels, decreasing myocardial oxygen consumption, inhibiting platelet aggregation, reducing blood fat, removing free radical, and anti-inflammation and antioxidant effects [14-17]. Notoginsenosides are the most important biologically active ingredients in *Panax notoginseng* and the structures of several major constituents (ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, Re, R1, Rf, Rg2, Rg1, and Rh1) are shown in Fig. 1 [18-21]. Most of those notoginsenosides can be divided into two major groups, including protopanaxadiols (PPD) with sugar moieties attached to the C-3 and/or C-20 and the protopanaxatriols (PPT) with sugar moieties at C-6 and/or at C-20. In recent years, more and more notoginsenosides have been discovered with the development of analytical instruments, and LC-IT-TOF MS has played an important role in the identification and classification of notoginsenosides. In 2008, Zheng *et al*. applied LC-IT-TOF MS in identifying constituents in *Shengmai* injection based on diagnostic fragment-ion-based extension strategy, with 30 ginsenosides being rapidly detected and identified [22]. In 2013, LC-IT-TOF MS was used to identify the main ingredients in a well-known herb prescription *Yunnan Baiyao*, with 17 notoginsenosides being structurally identified [23].





PPD-Type Notoginsenoside	R_2	R_1
Rb1	Glc^{1}-^{2}Glc-	Glc^{1}-^{6}Glc-
Rb2	Glc^{1}-^{2}Glc-	$Ara(p)^{1}$-^{6}Glc-
Rd	Glc^{1}-^{2}Glc-	Glc-
Rg3	Glc^{1}-^{2}Glc-	H

PPT-Type Ginsenoside	R_2	R_1
R1	Xyl^{1}-^{2}Glc-	Glc-
Re	Rha^{1}-^{2}Glc-	Glc-
Rg1	Glc-	Glc-
Rg2	Rha^{1}-^{2}Glc-	H

Fig. 1 Structures of representative PPD-type and PPT-type notoginsenosides

In the current study, notoginsenosides were chosen as model compounds to build a systematic and rapid methodology based on LC-IT-TOF MS for the CE optimization for qualitative analysis of complex components. The fragmentation behavior study under different stages of MS^n and variation of CE allowed us to recognize the importance of CE and provide a convenient method to optimize CE values for the analysis of complex components in herbal medicine.

Experimental

Reagents and chemicals

Four PPD-type notoginsenosides (Rb1, Rb2, Rd, and Rg3), four PPT-type notoginsenosides (R1, Re, Rg1, and Rg2) and a *Panax Notoginseng* extract (PNE) were all purchased from the Department of Nature Medical Chemistry, Jilin University (Jilin, China). The structures of notoginsenosides are shown in Fig. 1. HPLC-grade acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was purified using a Milli-Q Ultrapure water system with the water outlet operating at 18.2 MΩ (Millipore, Bedford, Maryland, USA). Other chemicals and solvents used in the present study were all of analytical grade.

Sample preparation

The primary stock solution of 1.0 mg·mL^{-1} of PNE was prepared by dissolving 10.0 mg of PNE in HPLC grade acetonitrile in a 10-mL volumetric flask filled up to the mark with the same solvent. The working solution at a 5 μg·mL^{-1} concentration was prepared by diluting the primary stock solution (50 μL) in 10 mL acetonitrile.

Chromatographic and mass spectrometric conditions

The LC experiments were conducted on a Shimadzu (Kyoto, Japan) HPLC system consisting of an LC-20AD binary pump, a SIL-20AC autosampler, and a CTO-20AC column oven. Chromatographic separation was performed on a C_{18} reversed phase LC column (Luna, Phenomenex, 5 μm particles, 2.1 mm × 150 mm). The mobile phase (delivered at 0.2 mL·min^{-1}) consisted of solvent A, H_2O containing 0.02% acetic acid (*V*/*V*), and solvent B, acetonitrile containing 0.02% acetic acid (*V*/*V*). A binary gradient elution was employed for the separation, and the consecutive program was as follows: an isocratic elution of 25% solvent B for the initial 5 min,