Spectroscopy Letters: An International Journal for Rapid Communication

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lstl20

Differentiation of Isomeric Polyphenolic Glycosides That Possess Regioisomeric Acylated Monosaccharide Residues by Electrospray Ionization-Tandem Mass Spectrometry

Shang-Gao Liao [a], Zhen Wang [a], Fa Sun [b], Li-Juan Zhang [a], Xun He [a], Lin Zheng [a], Ai-Min Wang [a], Yong-Jun Li [a], Yong Huang [a], Yan-Yu Lan [a] & Yong-Lin Wang [a]

[a] Provincial Key Laboratory of Pharmaceutics in Guizhou Province, School of Pharmacy, Guiyang Medical College , Guiyang , Guizhou , PR China

[b] Department of Urinary Surgery , Affiliated Hospital of Guiyang Medical College , Guiyang , Guizhou , PR China

Accepted author version posted online: 27 Feb 2013.Published online: 21 Oct 2013.

To cite this article: Shang-Gao Liao , Zhen Wang , Fa Sun , Li-Juan Zhang , Xun He , Lin Zheng , Ai-Min Wang , Yong-Jun Li , Yong Huang , Yan-Yu Lan & Yong-Lin Wang (2014) Differentiation of Isomeric Polyphenolic Glycosides That Possess Regioisomeric Acylated Monosaccharide Residues by Electrospray Ionization-Tandem Mass Spectrometry, Spectroscopy Letters: An International Journal for Rapid Communication, 47:1, 19-23, DOI: 10.1080/00387010.2013.775464

To link to this article: http://dx.doi.org/10.1080/00387010.2013.775464

Spectroscopy Letters, 47:19–23, 2014

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010.2013.775464

Taylor & Francis
Taylor & Francis Group

Differentiation of Isomeric Polyphenolic Glycosides That Possess Regioisomeric Acylated Monosaccharide Residues by Electrospray Ionization–Tandem Mass Spectrometry

Shang-Gao Liao[1], Zhen Wang[1], Fa Sun[2], Li-Juan Zhang[1], Xun He[1], Lin Zheng[1], Ai-Min Wang[1], Yong-Jun Li[1], Yong Huang[1], Yan-Yu Lan[1], and Yong-Lin Wang[1]

[1]Provincial Key Laboratory of Pharmaceutics in Guizhou Province, School of Pharmacy, Guiyang Medical College, Guiyang, Guizhou, PR China
[2]Department of Urinary Surgery, Affiliated Hospital of Guiyang Medical College, Guiyang, Guizhou, PR China

ABSTRACT Isomeric polyphenolic glycosides that possess regioisomeric *O*-acylated saccharide residues can be found in many medicinal plants and their preparations as well as some synthetic intermediates. The need for a pure and sufficient quantity of compounds for nuclear magnetic resonance measurements calls for a microanalytical method for their rapid differ entiation. In the current investigation, an electrospray ionization–tandem mass spectrometric method was developed for rapid differentiation of two pairs of regioisomeric acylated monosaccharides, quercetin-3-*O*-(3″-*O*-galloyl)-α-L-rhamnopyranoside and quercetin-3-*O*-(2″-*O*-galloyl)-α-L-rhamnopyranoside as well as quercetin-3-*O*-(3″-*O*-galloyl)-*β*-D-glucopyranoside and quercetin-3-*O*-(2″-*O*-galloyl)-*β*-D-glucopyranoside; the mechanisms governing their similar or differential fragmentations are also discussed. The work may facilitate the structural determination of these regioisomers and their analogs in pure form or in complex matrix.

KEYWORDS electrospray ionization, polyphenolic glycosides, regioisomeric *O*-acylated monosaccharide, structural differentiation, tandem mass spectrometry

INTRODUCTION

Isomeric polyphenolic glycosides that possess regioisomeric *O*-acylated saccharide residues can be found in many medicinal plants and their preparations[1–4] as well as some synthetic intermediates.[5–7] The present approaches for their structural determination mainly involve nuclear magnetic resonance (NMR) spectroscopic techniques, but the requirement of a pure and high quantity of compounds for NMR measurements suggested that these compounds could be structurally determined only when they were obtained in sufficient quantity.[1–7] In view of the complexity and trace amounts of chemical constituents in crude synthetic products and medicinal

Received 24 December 2012; accepted 8 February 2013.

Address correspondence to Shang-Gao Liao and Yong-Lin Wang, Provincial Key Laboratory of Pharmaceutics in Guizhou Province, School of Pharmacy, Guiyang Medical College, 9 Beijing Road, Guiyang, Guizhou, 550004, PR China. E-mail: lshangg@163.com (S.-G. Liao); ylwang_gmc@163.com (Y.-L. Wang)

plants and their preparations, a rapid, reliable, and microanalytical method is needed to determine their structures in complex systems (e.g., a plant extract or a crude synthetic product with various reactants and by-products). In addition, since these isomeric polyphenolic glycosides contained almost the same structural subunits, the general analytical methods (e.g., UV and IR) could not be used alone to discriminate the structures of these isomers even when they were obtained in pure form and with sufficient amounts. The developed analytical method should therefore take into consideration the easy discrimination of these isomeric glycosides. As coupling of liquid chromatography with tandem mass spectrometry has become a routine method for the identification and structural determination of various phenolic glycosides in crude plant extracts[8,9] and electrospray ionization–tandem mass spectrometry (ESI-MS/MS) has gained success in differentiating quite a number of positional isomers of various structural types,[1,10–21] the use of ESI-MS/MS for differentiation of these positionally isomeric polyphenolic glycosides may possibly facilitate the rapid structural determination of these compounds.

In the previous report,[1] the fragmentation patterns of various *O*-acylated monosaccharide residues were investigated and some fragmentation mechanisms were proposed. However, direct differentiation of the regioisomeric acylated monosaccharides by their product ions has not yet been discussed. Besides, fragmentations were also not discussed in detail, which leaves some confusing data for the readers. In this report, an MS/MS method has been developed for the differentiation of the two pairs of isomeric polyphenolic glycosides that possess regioisomeric *O*-acylated monosaccharide residues, quercetin-3-*O*-(3″-*O*-galloyl)-α-L-rhamnopyranoside (**1**) and quercetin-3-*O*-(2″-*O*-galloyl)-α-L-rhamnopyranoside (**2**) as well as quercetin-3-*O*-(3″-*O*-galloyl)-*β*-D-glucopyranoside (**3**) and quercetin-3-*O*-(2″-*O*-galloyl)-*β*-D-glucopyranoside (**4**) (Fig. 1). The mechanisms underlying their differential fragmentations were also discussed.

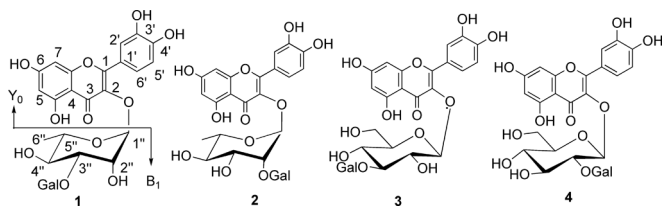


FIGURE 1 **Structures and mass fragment nomenclature for the four polyphenolic glycosides.**

EXPERIMENTAL

Reagents and Chemicals

Quercetin-3-*O*-(3″-*O*-galloyl)-α-L-rhamnopyranoside (**1**), quercetin-3-*O*-(2″-*O*-galloyl)-α-L-rhamnopyranoside (**2**), quercetin-3-*O*-(3″-*O*-galloyl)-*β*-D-glucopyranoside (**3**), and quercetin-3-*O*-(2″-*O*-galloyl)-*β*-D-glucopyranoside (**4**) were obtained in our laboratory as natural products. Their structures were confirmed by MS, ^{1}H-NMR, and ^{13}C-NMR.[22]

Mass Spectrometry

All the mass experiments were carried out using a Waters ACQUITY TQD (triple quadrupole mass spectrometer, Waters, Milford, Massachusetts, USA) equipped with a Z-spray ESI source. The acquisition parameters were collision gas, helium (He); nebulizing and drying gas, nitrogen (N_2); source temperature, 120°C; desolvation temperature, 350°C; cone gas flow, 50 L/hr; desolvation gas flow, 650 L/hr; collision gas flow, 0.18 mL/min; capillary voltage, 3.0 kV; extractor voltage, 3.0 V; and RF lens voltage, 0.50 V. MS/MS of all the components was performed at a cone voltage of 20 V and a collision energy of 14 eV. Each standard solution (*ca.* 20 μmol/L) was prepared by dissolving the standard in 50% CH_3CN/0.1% formic acid in water. MS spectra were acquired by direct injection of the standard solutions into the MS system.

RESULTS AND DISCUSSION

ESI-MS/MS study of quercetin-3-*O*-(3″-*O*-galloyl)-α-L-rhamnopyranoside (**1**) (Fig. 2(A)) and

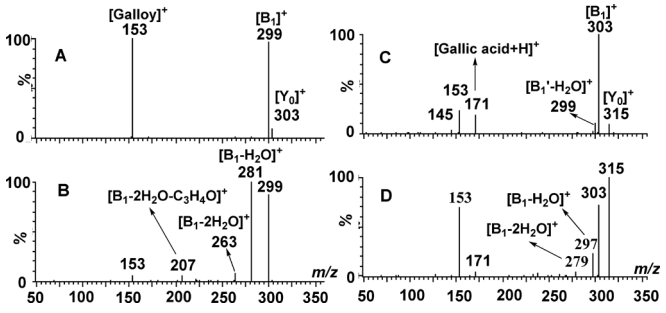


FIGURE 2 **MS^2 spectra (*m/z* 50–350) of the $[M+H]^+$ (*m/z* 601 for 1 and 2; *m/z* 617 for 3 and 4) of the four polyphenolic glycosides (A: 1; B: 2; C: 3; D: 4).**

quercetin-3-*O*-(2″-*O*-galloyl)-α-L-rhamnopyranoside (**2**) (Fig. 2(B)) showed that, at the collision energy of 14 eV, both $[M+H]^+$ (m/z 601) of compounds **1** and **2** could be fragmented to give a galloyl rhamnosy cation $[B_1]^+$ (m/z 299) (Fig. 1) and a $[Galloyl]^+$ (m/z 153). However, a protonated aglycone $[Y_0]^+$ (m/z 303) (Fig. 1) was only observed for the $[M+H]^+$ (m/z 601) of **1**, and the relative abundances of galloyl cation $[Galloyl]^+$ (m/z 153) differ significantly between the two isomers. The cation was detected as a base peak in the product ion spectrum of the $[M+H]^+$ (m/z 601) of **1** but only as a negligible ion in that of **2**. Besides, successive losses of two water molecules (to yield m/z 281 for $[B_1\text{-}H_2O]^+$ and m/z 263 for $[B_1\text{-}2H_2O]^+$) from the product ion $[B_1]^+$ were only observed for the $[M+H]^+$ (m/z 601) of quercetin-3-*O*-(2″-*O*-galloyl)-α-L-rhamnopyranoside. The two regioisomers could therefore be easily discriminated.

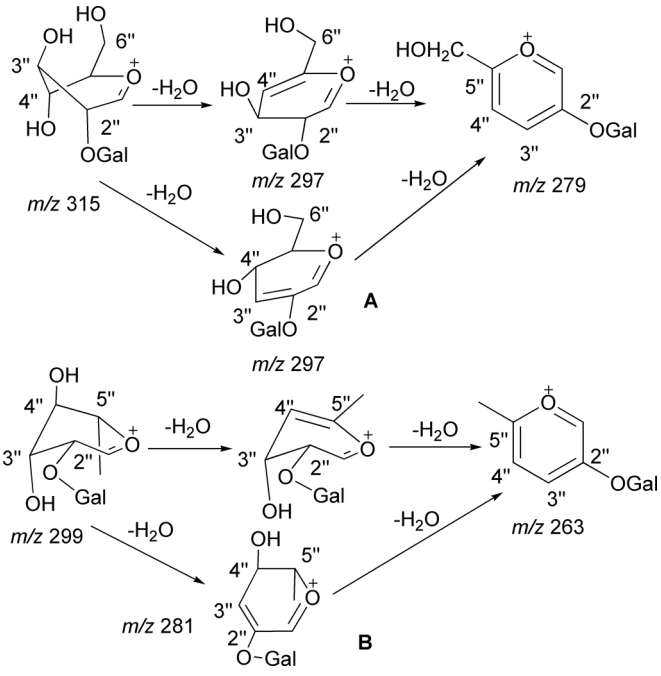
It should be noted that almost no ions with m/z larger than that of $[B_1]^+$ (m/z larger than 350 were not shown in Fig. 2; see supplementary and associated data) were observed. $[Galloyl]^+$ and other fragment ions seemed to be derived from the product ion $[B_1]^+$.

As in the cases of isomeric rhamnosides (**1** and **2**), successive losses of two water molecules (to yield m/z 297 for $[B_1\text{-}H_2O]^+$ and m/z 279 for $[B_1\text{-}2H_2O]^+$) were also only observed for the $[M+H]^+$ (m/z 617) of 2″-*O*-galloyl isomer (**4**) (Fig. 2(D)). However, different fragmentation patterns were also observed for the $[M+H]^+$ (m/z 617) of the two isomeric glucosides (**3** and **4**) (Fig. 2(C) and 2(D)). Although only very weak $[Y_0]^+$ (m/z 303) was detected in the product ion spectrum of the $[M+H]^+$ (m/z 601) of 3″-*O*-galloyl isomeric rhamnoside (**1**), intense $[Y_0]^+$ (m/z 303) was observed for both of the two isomeric glucosides (**3** and **4**). Besides, obvious protonated gallic acid (m/z 171) was only observed for the $[M+H]^+$ (m/z 617) of the two isomeric glucosides (**3** and **4**), with the one from that of **3** being more intense. Further discrimination of the two regioisomeric glucosides could be accomplished by analysis of their $[B_1]^+$, $[B_1\text{-Gallic acid}]^+$, and $[Galloyl]^+$. The galloyl glucosyl cation $[B_1]^+$ (m/z 315) was rather intense (and as the base peak) for **4** but was relatively weak for **3**, while the galloyl cation $[Galloyl]^+$ (m/z 153) was rather weak for **4** but relatively intense for **3**. A $[B_1\text{-Gallic acid}]^+$ (m/z 145), however, was only observed in the product ion spectrum of **3**.

In the previous report,[1] we suggested an intramolecular hydrogen bonding between the 6″-OH and the 3″-*O*-galloyl for the loss of a gallic acid (and hence formation of a protonated gallic acid at m/z 171) for the fragmentation of the 3″-*O*-galloyl glucosyl ion $[B_1]^+$, yet the fragmentation patterns of its regioisomeric $[B_1]^+$ ions and their corresponding polyphenolic glycosides were far from being completely understood. Efforts were therefore made in the current investigation to compare their fragmentations and to find out the possible mechanisms that lead to their similar or differential fragmentations between the two pairs of isomers.

Fragmentation of the glycosidic bond would generate a $[Y_0]^+$ or a $[B_1]^+$ (Fig. 1), and replacement of a 5″-methyl (as in the rhamnosides) by a 5″-hydroxymethyl (as in the glucosides) might possibly reduce more or less the stability of the $[B_1]^+$ and hence observation of higher product ion intensity ratios of $[Y_0]^+/[B_1]^+$ for the two glucosides (**3** and **4**) as compared to those for the rhamnosides (**1** and **2**) (Fig. 2).

Dehydration of the $[B_1]^+$ of 2″-*O*-galloyl isomers at the C4″-C5″ (Scheme 1(A) and 1(B)) or the C3″-C2″ (Scheme 1(B)) bond initiated by the 4″- or 3″-axial-OH lost one water molecule from $[B_1]^+$, while



SCHEME 1 **Successive dehydration scheme proposed for the $[B_1]^+$ (m/z 299 for 2 and m/z 315 for 4) of 2″-*O*-galloyl isomers (A: 2; B: 4).**

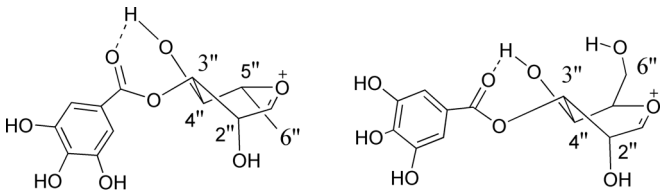
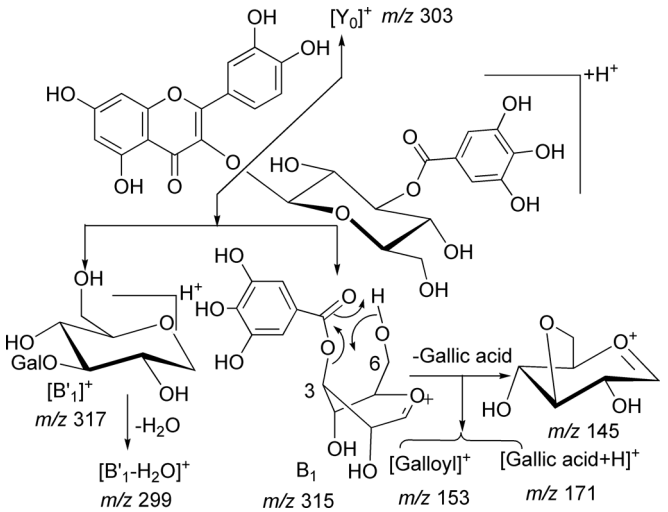


FIGURE 3 **Intramolecular hydrogen bonding between the 4″-OH and the 3″-*O*-galloyl in the 3″-*O*-galloyl isomers.**



SCHEME 2 **Fragmentation scheme proposed for the $[M+H]^+$ (m/z 617) of glucoside 3.**

aromatization of the newly generated ions by dehydration would lead to the loss of the second water molecule. The presence of an intramolecular hydrogen bonding between the 4″-OH and the 3″-*O*-galloyl in the 3″-*O*-galloyl isomers (Fig. 3) retarded not only the loss of a water molecule but also that of a gallic acid ($[B_1\text{-}170]^+$) from $[B_1]^+$. Although the latter (m/z 145, $[B_1$-gallic acid$]^+$) could be observed in the product ion spectrum of **3**, its formation might result from an intramolecular hydrogen bonding between the 3″-OH and the 6″-*O*-galloyl of their $[B_1]^+$ (Scheme 2).[1]

The peculiar $[B'_1]^+$ ion at m/z 299 observed only in the product ion spectrum of **3** might involve the generation of a protonated 3″-galloyl 1″-deoxyglucoside (m/z 317) and its subsequent dehydration (Scheme 2).

CONCLUSION

ESI-MS/MS has been proved to be one of the most powerful structural techniques for the discrimination of various regioisomers in a single experiment.[1,10–21] In the current investigation, we have shown that the two pairs of isomeric polyphenolic glycosides that possess regioisomeric *O*-acylated monosaccharide residues could be discriminated simply by their product ions and their relative intensity. The mechanisms proposed for their close or different fragmentations might facilitate the structural determination of these regioisomers and their analogs in pure form or in complex matrix.

ACKNOWLEDGMENTS

This research was supported by projects supported by the National Natural Science Foundation of China (No. 81160516), Governor of Guizhou Province (No. 2010-43), Guizhou Science and Technology Department (2012-7040), and Special Program of GSTD for Construction of the Technological Innovation Team for Development and Application of TCMs.

REFERENCES

1. Liao, S.-G.; Zhang, L.-J.; Wang, Z.; Sun, F.; Li, Y.-J.; Wang, A.-M.; Huang, Y.; Lan, Y.-Y.; Wang, Y.-L. Electrospray ionization and collision-induced dissociation tandem mass spectrometric discrimination of polyphenolic glycosides: exact acylation site determination of the *O*-acylated monosaccharide residues. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2483–2492.
2. Ahmad, V. U.; Bader, S.; Arshad, S.; Ahmed, A.; Khan, A.; Iqbal, S.; Rasheed, M.; Tareen, R. B. Brauhenefloroside E and F; acylated flavonol glycosides from *Stocksia brauhica* Linn. *Magn. Reson. Chem.* **2011**, *48*, 304–308.
3. Şöhretoğlu, D.; Sakar, M. K.; Sterner, O. New galloylated flavonoid glycosides from *Geranium stepporumdavis*. *Helv. Chim. Acta* **2009**, *92*, 520–524.
4. Moharram, F. A.; Marzouk, M. S. A.; Ibrahim, M. T.; Mabry, T. J. Antioxidant galloylated flavonol glycosides from *Calliandra haematocephala*. *Nat. Prod. Res.* **2006**, *20*, 927–934.
5. De Oliveira, E.; Humeau, C.; Chebil, L.; Maia, E.; Dehez, F.; Maigret, B.; Ghoul, M.; Engasser, J.-M. A molecular modelling study to rationalize the regioselectivity in acylation of flavonoid glycosides catalyzed by *Candida antarctica* lipase b. *J. Mol. Catal. B: Enzym.* **2009**, *59*, 96–105.
6. Liu, C.; Richards, M. R.; Lowary, T. L. Probing the effect of acylation on arabinofuranose ring conformation in di- and trisaccharide fragments of mycobacterial arabinogalactan. *J. Org. Chem.* **2010**, *75*, 4992–5007.
7. Simerská, P.; Kuzma, M.; Pišvejcová, A.; Weignerová, L.; Macková, M.; Riva, S.; Křen, V. Application of selectively acylated glycosides for the α-galactosidase-catalyzed synthesis of disaccharides. *Folia Microbiol.* **2003**, *48*, 329–337.
8. Yang, M.; Sun, J.; Lu, Z.; Chen, G.; Guan, S.; Liu, X.; Jiang, B.; Ye, M.; Guo, D.-A. Phytochemical analysis of Traditional Chinese Medicine using liquid chromatography coupled with mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 2045–2062.
9. Wu, H.; Guo, J.; Chen, S.; Liu, X.; Zhou, Y.; Zhang, X.; Xu, X. Recent developments in qualitative and quantitative analysis of phytochemical constituents and their metabolites using liquid chromatography mass spectrometry. *J. Pharm. Biomed. Anal.* **2013**, *72*, 267–291.

10. Suomela, J.-P.; Leskinen, H.; Kallio, H. Analysis of isomeric forms of oxidized triacylglycerols using ultra-high-performance liquid chromatography and tandem mass spectrometry. *J. Agric. Food Chem.* **2011**, *59*, 8095–8100.
11. Raju, B.; Ramesh, M.; Srinivas, R.; Chandrasekhar, S.; Kiranmai, N.; Sarma, V. Differentiation of positional isomers of hybrid peptides containing repeats of β-nucleoside derived amino acid (β-Nda-) and L-amino acids by positive and negative ion electrospray ionization tandem mass spectrometry (ESI-MS^n). *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 703–717.
12. Dinesh Kumar, C.; Naresh Chary, V.; Dinesh, A.; Reddy, P. S.; Srinivas, K.; Gayatri, G.; Sastry, G. N.; Prabhakar, S. Differentiation of isomeric 2-aryldimethyltetrahydro-5-quinolinones by electron ionization and electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2815–2827.
13. Liu, G.-Q.; Dong, J.; Wang, H.; Hashi, Y.; Chen, S.-Z. Differentiation of four pairs of furocoumarin isomers by electrospray ionization tandem mass spectrometry. *Eur. J. Mass Spectrom.* **2010**, *16*, 215–220.
14. Liao, S.-G.; Zhang, L.-J.; Li, C.-B.; Lan, Y.-Y.; Wang, A.-M.; Huang, Y.; Zhen, L.; Fu, X.-Z.; Zhou, W.; Qi, X.-L.; Guan, Z.-Z.; Wang, Y.-L. Rapid screening and identification of caffeic acid and its esters in *Erigeron breviscapus* by ultra-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2533–2541.
15. Gonçalves, A.; Ducatti, D.; Grindley, T.; Duarte, M.; Noseda, M. ESI-MS differential fragmentation of positional isomers of sulfated oligosaccharides derived from carrageenans and agarans. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1404–1416.
16. Ramesh, V.; Reddy, P. N.; Srinivas, R.; Srinivasulu, G.; Kunwar, A. C. Differentiation of three pairs of positional isomers of hybrid peptides with repeats of phenylalanine-β^3-h-valine/β^3-h-valine-phenylalanine by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1401–1408.
17. Li, R.; Wu, Z.; Zhang, F.; Ding, L. Differentiation of three pairs of aconite alkaloid isomers from *Aconitum nagarum* var. *lasiandrum by electrospray ionization tandem mass spectrometry. Rapid Commun. Mass Spectrom.* **2006**, *20*, 157–170.
18. Bhaskar, G.; Prabhakar, S.; Venkat Narsaiah, A.; Nagaiah, K.; Ramanjaneyulu, G. S.; Vairamani, M. Differentiation of isomeric substituted diaryl ethers by electron ionization and chemical ionization mass spectrometry. *Eur. J. Mass Spectrom.* **2006**, *12*, 161–170.
19. Clifford, M. N.; Johnston, K. L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900–2911.
20. Bateman, K. P.; Locke, S. J.; Volmer, D. A. Characterization of isomeric sulfonamides using capillary zone electrophoresis coupled with nano-electrospray quasi-MS/MS/MS. *J. Mass Spectrom.* **1997**, *32*, 297–304.
21. Leskinen, H.; Suomela, J.-P.; Pinta, J.; Kallio, H. Regioisomeric structure determination of α- and γ-linolenoyldilinoleoylglycerol in blackcurrant seed oil by silver ion high-performance liquid chromatography and mass spectrometry. *Anal. Chem.* **2008**, *80*, 5788–5793.
22. Zhang, L.-J.; Wang, Y.-L.; Wang, Z.; Li, Y.-J.; Huang, Y.; Long, Q.-D.; Liao, S.-G. Study on the chemical constituents of the active fraction of *Polygonum capitatum*. *Zhongyaocai (J. Chin. Med. Mat.)* **2012**, *35*, 1425–1428.