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Fast separation of triterpenoid saponins using supercritical fluid chromatography coupled with single quadrupole mass spectrometry

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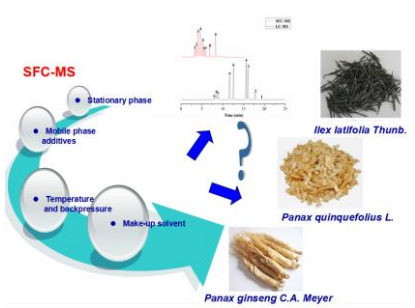
Introduction

Triterpenoid saponins (TSs) are the most important components of some traditional Chinese medicines (TCMs) and have exhibited valuable pharmacological properties[1-2]. Supercritical fluid chromatography (SFC), considered as a green separation technique, is a potential alternative to LC for the analysis of TSs. However, the application of SFC-MS to the analysis of TSs has not yet been reported. In the present study, rapid and efficient SFC-MS methods were developed for the first time for the separation of both TSs standards (kudinosides and ginsenosides) and TSs from natural product extracts. Moreover, a comprehensive comparison between LC-MS and SFC-MS with respect to selectivity and running time was carried out using a mixture of TSs as test sample.

Graphical abstract



Results and discussion

Effect of mobile phase additive

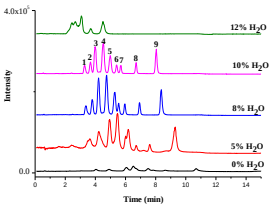


Fig. 1. Effect of water content in the mobile phase.

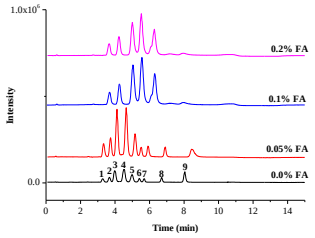


Fig. 2. Effect of the addition of formic acid to the mobile phase.

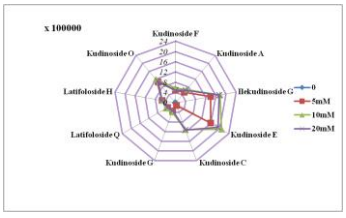


Fig. 3. Effect of the ammonium acetate concentration in the make-up solution on MS response.

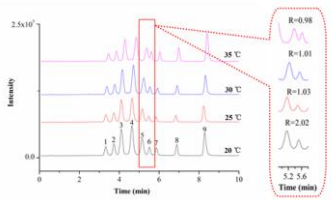
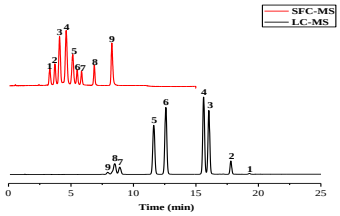


Fig. 4. Effect of temperature on resolution.

Comparison of SFC and LC methods



The SFC-MS method provided separations that were about 2 × faster than LC-MS method.

Fig. 5. Separation of the nine kudinosides in both SFC and LC modes.

Application in TCMs analysis

A series of natural products containing TSs were tested using the developed SFC-MS methods. As shown in **Fig. 6A-E,** kudinosides present in *Ilex latifolia Thunb.* samples could be identified by MS and by comparison with the retention times of the standards. Moreover, the 11 ginsenoside standards and the ginsenosides present in *Panax quinquefolius L.* and *Panax ginseng C.A. Meyer* were also examined under the selected SFC-MS conditions **(Fig. 7).** Several ginsenosides can be identified in *Panax quinquefolius L.* (F2; Rf; Rg_1; Rd; NK; Re; Rc; Rb_2; Rb_1) and *Panax ginseng C.A. Meyer* (CK; Rf; Rg_1; Rd; Re; Rc; Rb_3; Rb_1).

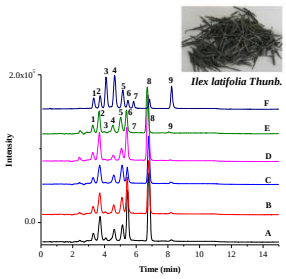


Fig. 6. Total ion chromatograms of the nine kudinosides standards and the extracts of *Ilex latifolia Thunb*.

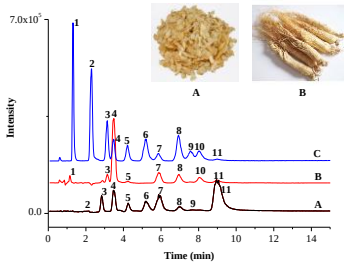


Fig. 7. Total ion chromatograms of the 11 ginsenoside standards (C) and the extracts of *Panax quinquefolius L.* (A), *Panax ginseng C.A. Meyer* (B).

Conclusions

- Rapid and highly efficient SFC-MS methods were developed for the separation of triterpenoid saponins (TSs).
- Water can be considered as a suitable mobile phase additive for SFC-MS operation.
- The SFC-MS method developed for the separation of kudinosides shows higher resolution and shorter running time than LC-MS.
- The SFC-MS approach shows potential for analyzing TSs present in traditional Chinese medicines (TCMs).

Acknowledgements

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References

[1] E. Lesellier, E. Destandau, C. Grigoras, L. Fougere, C. Elfakir, J Chromatogr A 1268 (2012) 157-165.

[2] M. Yoshikawa, T. Murakami, E. Harada, N. Murakami, J. Yamahara, H. Matsuda, Chem. Pharm. Bull 44 (1996) 1923-1927.