**Supplementary materials**

# Development and validation of a green chromatography for the determination of anthocyanins in haskap berry, mulberry and blackberry

Jun Sang, Qun Ma, Cui-qin Li*

*Key Laboratory of the Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry, National Engineering Laborat*

*College of Life Sciences, Shaanxi Normal University, Xi'an, Shaanxi 710062, China*

**Supplementary Table 1** Mobile phases of liquid chromatography for analysing anthocyanins from various samples in literatures.

| Samples | Mobile phase composition | Reference [a] |
|---|---|---|

* Corresponding author. Tel.: +86 29 85310266; fax: +86 29 85310546.

*E-mail address*: licuiqin16@snnu.edu.cn (C.-Q. Li)

| | A phase (v/v) | B phase (v/v) | |
|---|---|---|---|
| Grape skin | 10% formic acid in water | 10% formic acid in methanol | [24] |
| Grape seed and skin | 0.2% TFA in water | 0.2% TFA in acetonitrile | [25] |
| Lotus petal | TFA/formic acid/water (0.1 : 2 : 97.9) | TFA/formic acid/acetonitrile/water (0.1 : 2 : 35 : 62.9) | [26] |
| Sweet cherry | 0.1% TFA in water | 0.1% TFA in methanol | [27] |
| Andean potato | 4.5% formic acid in water | acetonitrile | [28] |
| Red cabbage | 4.5% formic acid in water | acetonitrile | [29] |
| Aronia melanocarpa | 0.5% TFA in water | 0.5% TFA in acetonitrile | [30] |
| *Lycium ruthenicum* Murray | 5% formic acid in water | acetonitrile | [31] |
| Purple sweet potato | 0.5% TFA in water | acetonitrile | [32] |
| Bluebarry | Formic acid/water/ acetonitrile (10 : 87 : 3) | Formic acid/water/ acetonitrile (10 : 40 : 50) | [17] |
| Purple tomato | 5% formic acid in water | Formic acid/acetonitrile/water (5 : 47.5 : 47.5) | [33] |

[a] References are as follows:


24 M. O. Downey and S. Rochfort, Simultaneous separation by reversed-phase high-performance liquid chromatography and mass spectral identification of anthocyanins and flavonols in Shiraz grape skin, *J. Chromatogr. A*, 2008, **1201**, 43-47.

25 S. Munoz, M. Mestres, O. Busto and J. Guasch, Determination of some flavan-3-ols and anthocyanins in red grape seed and skin extracts by HPLC-DAD: Validation study and response comparison of different standards, *Anal. Chim. Acta*, 2008, **628**, 104-110.

26 Z. Yang, X. Wei, F. Gao, L. Wang, H. Zhang, Y. Xu, L. Li, Y. Ge, J. Zhang and J. Zhang, Simultaneous analysis of anthocyanins and flavonols in petals of lotus (*Nelumbo*) cultivars by high-performance liquid chromatography-photodiode array detection/electrospray ionization mass spectrometry, *J. Chromatogr. A*, 2009, **1216**, 106-112.

27 C. G. Grigorasa, D. Destandau, S. Zubrzycki and C. Elfakir, Sweet cherries anthocyanins: An environmental friendly extraction and purification method, *Sep. Purif. Technol.*, 2012, **100**, 51-58.

28 M. M. Giusti, M. F. Polit, H. Ayvaz, D. Tay and I. Manrique, Characterization and quantitation of anthocyanins and other phenolics in native Andean Potatoes, *J. Agric. Food Chem.*, 2014, **62**, 4408-4416.

29 N. Ahmadiani, R. J. Robbins, T. M. Collins and M. M. Giusti, Anthocyanins Contents, Profiles, and Color Characteristics of Red Cabbage Extracts from Different Cultivars and Maturity Stages, *J. Agric. Food Chem.*, 2014, **62**, 7524-7531.

30 H. Wangensteen, M. Braunlich, V. Nikolic, K. E. Malterud, R. Slimestad and H. Barsett, Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: Antioxidant and enzyme inhibitory effects, *J. Funct. Foods*, 2014, **7**, 746-752.

31 H. Jin, Y. Liu, F. Yang, J. Wang, D. Fu, X. Zhang, X. Peng and X. Liang, Characterization of anthocyanins in wild *Lycium ruthenicum* Murray by HPLC-DAD/QTOF-MS/MS, *Anal. Methods*, 2015, **7**, 4947-4956.

32 Y, Hu, L. Deng, J. Chen, S. Zhou, S. Liu, Y. Fu, C. Yang, Z. Liao and M. Chen, An analytical pipeline to compare and characterise the anthocyanin antioxidant activities of purple sweet potato cultivars, *Food Chem.*, 2016, **194**, 46-54.

33 X. Su, J. Xu, D. Rhodes, Y. Shen, W. Song, B. Katz, J. Tomich, W. Wang, Identification and quantification of anthocyanins in transgenic purple tomato, *Food Chem.*, 2016, **202**, 184-188.

**Supplementary Table** 2 The original data of the determination of intra- and inter-day precision, repeatability, stability and recovery of cyanidin-3-*O*-glucoside in haskap berry, mulberry and blackberry. [a]

| Samples | Precision [b] Intra-day precision | Inter-day precision | Repeatability [b] | Stability [b] | Recovery Berry sample solution [b] | Cyanidin-3-*O*-glucoside solution [c] | Mixture solution [b] |
|---|---|---|---|---|---|---|---|
| Haskap berry [d] | 22064.2 | 22084.8 | 22064.2<br>21796.9 | 22064.2<br>22170.9 | 11261.2 | 3566.7 | 13535.4 |
| | 22133.4 | 21802.0 | 22258.3<br>22300.9 | 21952.5<br>21968.0 | | | 13401.1 |
| | 21770.6 | 22091.7 | 22271.6<br>22276.5 | 22301.9<br>21816.3 | | | 13458.6 |
| Mulberry [e] | 15189.8 | 15263.3 | 15189.8<br>15495.9 | 15189.8<br>15432.6 | 7998.4 | 3566.7 | 8859.4 |
| | 14995.9 | 15336.0 | 15581.4<br>14869.4 | 14896.7<br>15603.1 | | | 8898.1 |
| | 15620.4 | 14996.1 | 14909.5<br>15446.8 | 15068.4<br>15001.2 | | | 8912.9 |
| Blackberry [e] | 2241.5 | 2261.9 | 2241.5<br>2245.3 | 2241.5<br>2232.9 | 2025.5 | 3566.7 | 3469.0 |
| | 2243.4 | 2244.5 | 2171.6<br>2255.9 | 2262.0<br>2183.3 | | | 3461.7 |
| | 2250.8 | 2260.7 | 2328.2<br>2300.7 | 2261.9<br>2227.5 | | | 3422.4 |

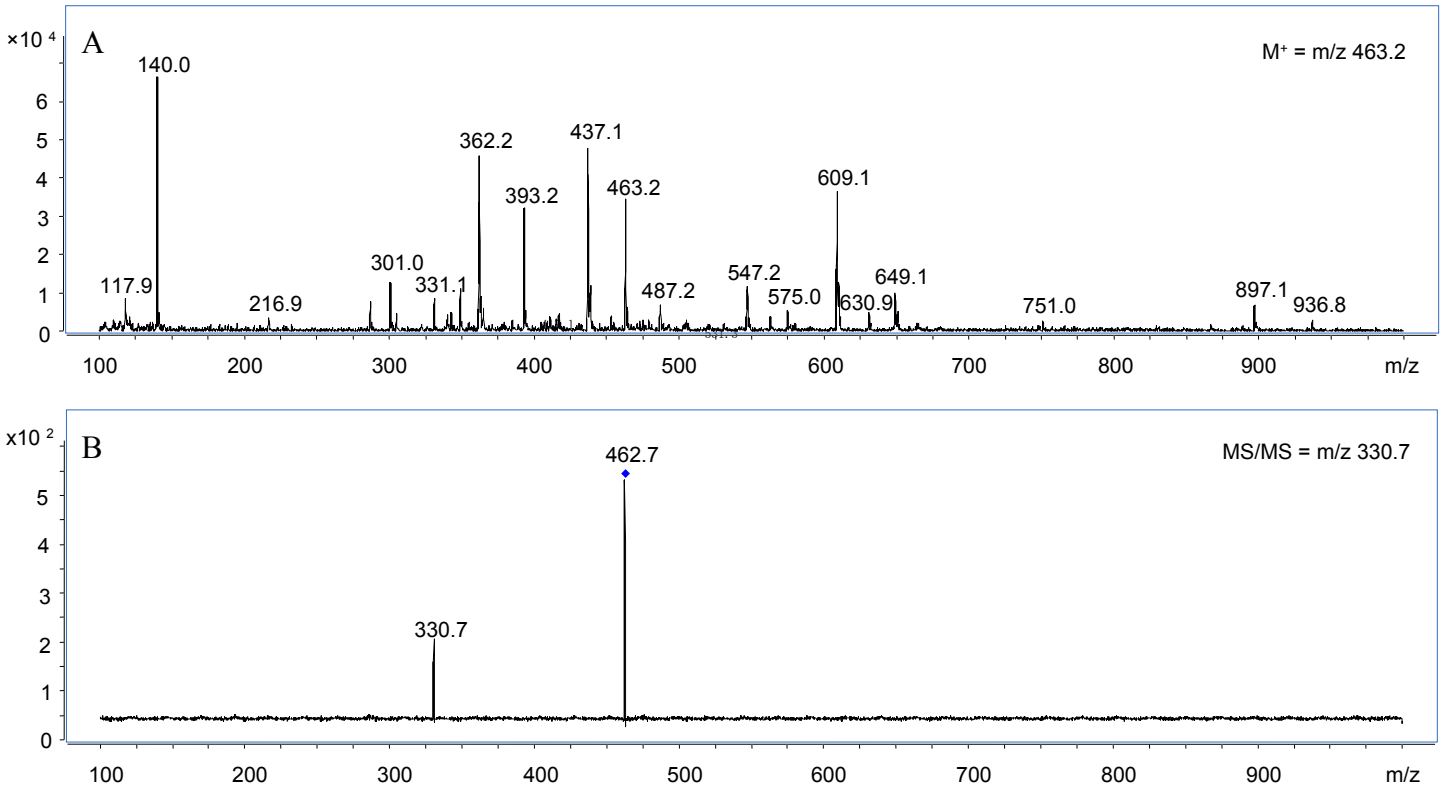
[a] The data are the values of peak area, and the precision, repeatability and stability were directly evaluated by the peak area of analyte.

[b] The injection volume is 5 μL for HPLC analysis.

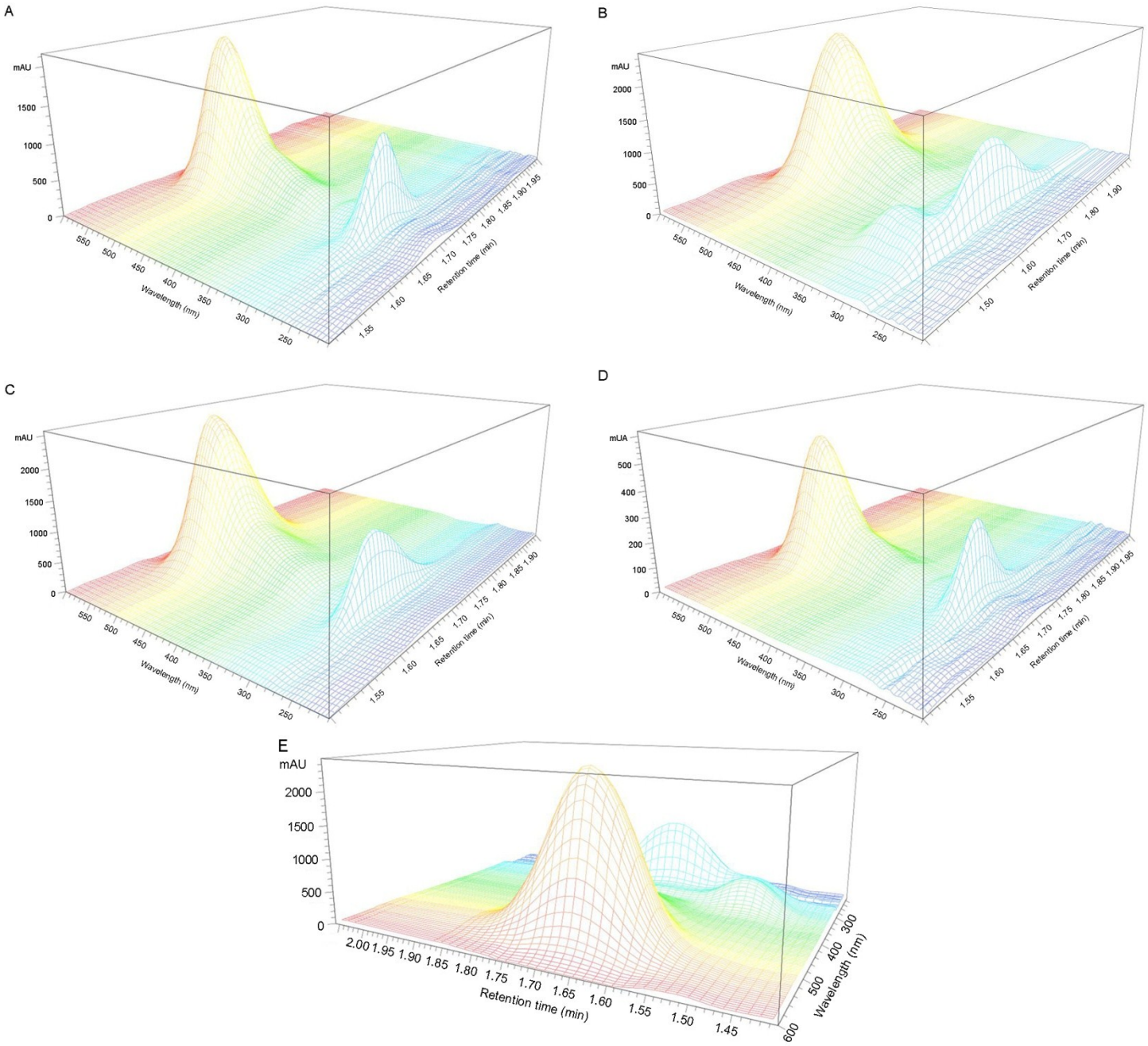
[c] The injection volume is 1 μL for HPLC analysis.

[d] Means 200 μL of cyanidin-3-*O*-glucoside solution was added into 400 μL of berry sample solution for recovery determination.

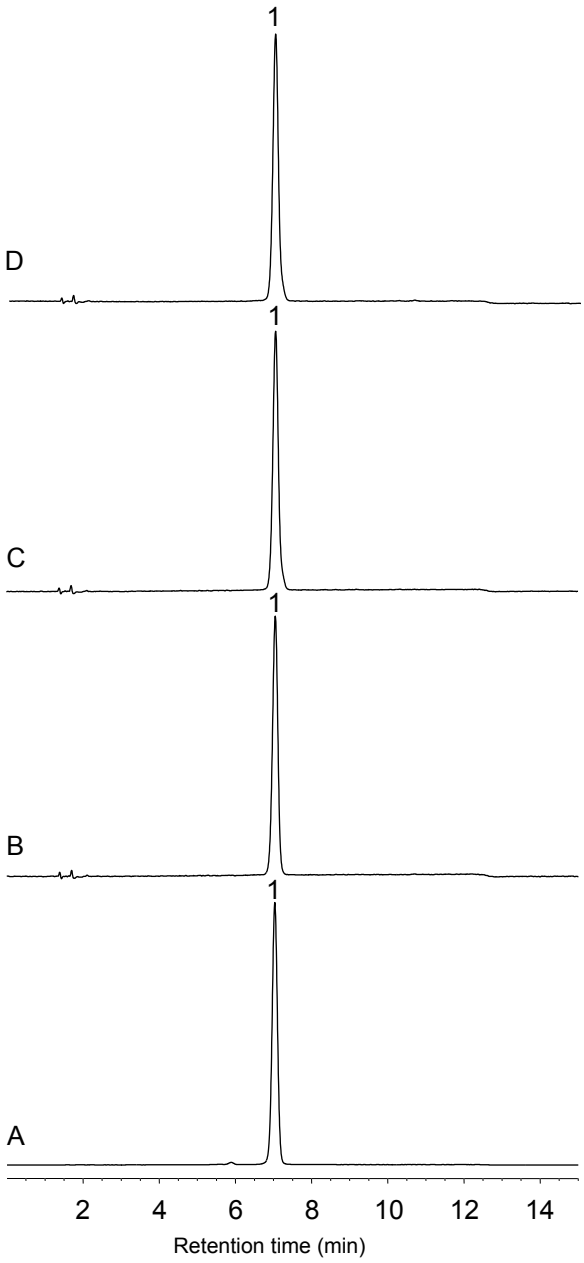
[e] Means 50 μL of cyanidin-3-*O*-glucoside solution was added into 500 μL of berry sample solution for recovery determination.



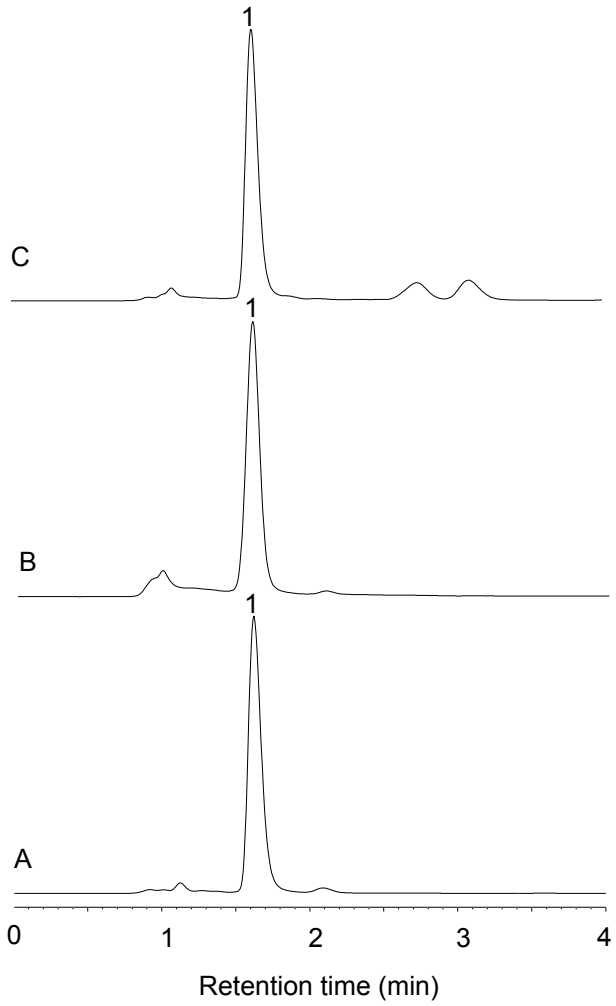

**Supplementary Fig. 1** Ms spectrum (A) and MS/MS spectrum (B) of anthocyanin 8.




**Supplementary** Fig. 2 Three-dimensional chromatograms of cyanidin-3-*O*-glucoside in standard solution (A), haskap berry sample (B and E), mulberry sample (C) and blackberry sample (D).




**Supplementary Fig. 3** The second-dimension HPLC chromatograms of cyanidin-3-*O*-glucoside (peak 1) from standard solution (A), blackberry sample (B), mulberry sample (C) and haskap berry sample (D). The chromatographic conditions were as follows: the analysis column was a ZORBAX Eclipse Plus C18 column (150 mm × 4.5 mm, 5 μm particle, Agilent Technologies Co., Ltd., USA); the mobile phase consisted of a trifluoroacetic acid aqueous solution (0.1%, v/v) and acetonitrile; gradient elution program was 10-25% of acetonitrile from 0 to 15 min; and the other parameters were same as the developed method.




**Supplementary Fig. 4** HPLC chromatograms of recovery determination of cyanidin-3-*O*-glucoside (peak 1) from haskap berry sample (A), mulberry sample (B) and blackberry sample (C).