



Validation and application of the UPLC-PDA method for authenticity control and fingerprinting of anthocyanins in *Vaccinium* L. berries

Gabriele Vilkickyte^{1,*}, Lina Raudone^{1,2}

¹ Laboratory of Biopharmaceutical Research, Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162 Kaunas, Lithuania

² Department of Pharmacognosy, Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162 Kaunas, Lithuania

* Corresponding author: gabriele.vilkickyte@lsmu.lt



Background

Public interest in beneficial effects of anthocyanins considerably enhanced the demand for commercially available anthocyanins rich food products and dietary supplements. On account of the prevention of mislabelled origin of *Vaccinium* L. berry's derived natural products, it is critically important to analyze the accumulation of anthocyanins among these berries and implement a valuable tool for authentication purposes [1, 2].

Materials and methods

The UPLC-PDA method was developed and validated using bilberries, cranberries, and lingonberries matrixes. The best resolution and sensitivity values, within the shortest time, were achieved by using 10% formic acid (A) and acetonitrile (B) as mobile phase components, with the flow rate of 0.5 mL/min, gradient elution (0–2 min, 95%; 2–7 min, 91%; 7–9 min, 88%; 9–10 min, 75%; 10–10.5 min, 20%; 10.5–11 min, 20%; 11–12 min, 95% of A), injection volume of 1 μ L, and column (ACE C18; ACT, Aberdeen, UK; 100 \times 2.1 mm, 1.7 μ m) temperature maintained at 30 $^{\circ}$ C.

Results

Satisfactory UPLC-PDA method system suitability, linearity, precision, trueness, and specificity for the analysis of anthocyanins in tested berries extracts were achieved after the development of the experimental conditions. The proposed method ensured the separation of 20 anthocyanins in bilberries, 15 in cranberries, and 9 in lingonberries, during 12 min analysis (Fig.1). In comparison with other techniques, our validated method has the advantage of rapidness, accuracy, and detection of aglycones—anthocyanidins or minor anthocyanins. To the best of our knowledge some of them—cyanidin, peonidin, malvidin in cranberries, cyanidin, and 3-glucosides of malvidin and petunidin in lingonberries, have been reported for the first time.

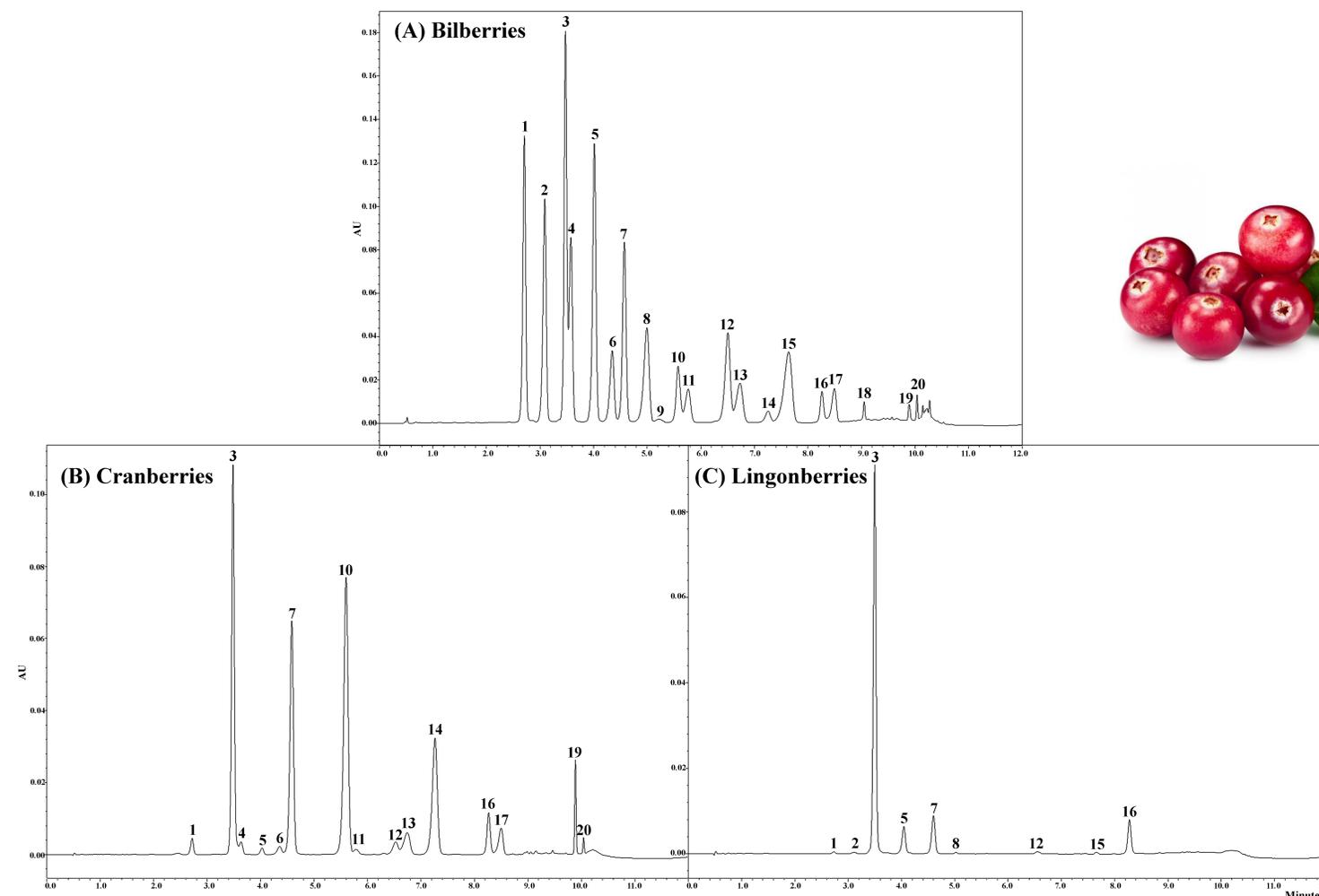


Fig. 1. UPLC-PDA profiles at 520 nm, showing the anthocyanins separation in (A) bilberries, (B) cranberries, and (C) lingonberries. Peak assignments: (1) delphinidin 3-galactoside, (2) delphinidin 3-glucoside, (3) cyanidin 3-galactoside, (4) delphinidin 3-arabinoside, (5) cyanidin 3-glucoside, (6) petunidin 3-galactoside, (7) cyanidin 3-arabinoside, (8) petunidin 3-glucoside, (9) delphinidin, (10) peonidin 3-galactoside, (11) petunidin 3-arabinoside, (12) peonidin 3-glucoside, (13) malvidin 3-galactoside, (14) peonidin 3-arabinoside, (15) malvidin 3-glucoside, (16) cyanidin, (17) malvidin 3-arabinoside, (18) petunidin, (19) peonidin, (20) malvidin.



Conclusions

This reliable method is suitable for the routine screening and quantification of anthocyanins in berries of *Vaccinium* genus or related food products to assess their diversity or assure their quality and authenticity.

References

- [1] Hurkova, K.; Uttl, L.; Rubert, J. et al. Food Chem 2019, 284, 162–170
- [2] Pojer, E.; Mattivi, F.; Johnson, D. et al. Compr Rev Food Sci Food Saf 2013, 12, 483–508