

Application Note

No 0002

Research on Betalain Pigments from Red Beetroot (*Beta vulgaris*) Extracts Using LC-DAD-ESI-MS/MS system (LCMS-8030 Shimadzu)

INTRODUCTION

Betalains are water-soluble plant pigments recently emerging as very valuable health-promoting antioxidants (Cai et all. 2003, Gandia-Herrero et all. 2010, Wybraniec et all. 2010). Many betalains, such as betanin **Fig. 1**, are 5-O-glucosides of betanidin (the basic chromophoric aglycone unit). Betanin colorant (E-162) is produced from red beetroots (*Beta vulgaris* L.) and is available as a concentrate produced by vacuum evaporation of beet juice, or as a powder made by spray-drying of the concentrate (Nemzer et all. 2011).

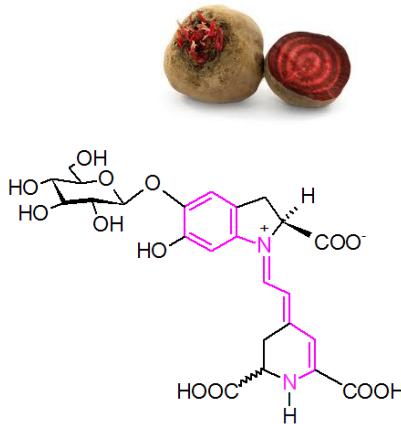


Fig. 1. Chemical structure of betanin, one of the most frequently occurring pigments in *Beta vulgaris* L. root. The long chromophoric unit is marked in purple.

EQUIPMENT AND METHODS

Analysis of different betalain pigments from *Beta vulgaris* L. root extracts was performed by high-performance liquid chromatography equipped with a diode array detection and tandem mass spectrometry with electrospray ionization (LC-DAD-ESI-MS/MS). This work presents a procedure which enables a simultaneous analysis of betalain pigments using negative and positive modes. Among all the available methods for the analysis of betalain pigments, reversed-phase high-



Fig. 2. UHPLC Nexera XR Shimadzu. SIL-20ACXR autosampler equipped with MTP holder (Photo: P. Stalica).

performance liquid chromatography (RP-HPLC) coupled with DAD and MS/MS is the most popular choice.

HPLC CONDITIONS

The chromatographic separation was carried out using an HPLC system (Shimadzu, Japan) consisting of a degasser DGU-20A3R, controller CBM-20A, binary pump Nexera XR LC-20 ACXR, autosampler Nexera XR SIL-20ADX and column oven CTO-20AC. The analytes were separated on a Kinetex C18 column, 100 mm×4.6 mm, 5 µm (Phenomenex). The temperature of the column oven was set to 40 °C, the flow rate was

kept at 0.5 mL/min and the injection volume was set from 1 to 20 μ L. The mobile phase used for the separation was 2% formic acid in water (component A) and MeOH (component B). The chromatographic separation was performed in gradient elution mode: 0 min (5 % B), 12 min (25 % B), 15 min (70 % B). The total time of the chromatographic run was 16 min, while the column equilibration time was set to 5 min. The chromatogram presenting the separation of analytes is shown in **Fig. 4**.



Fig. 3. LCMS-8030 belongs to the series Ultra-Fast Mass Spectrometers from Shimadzu, Japan

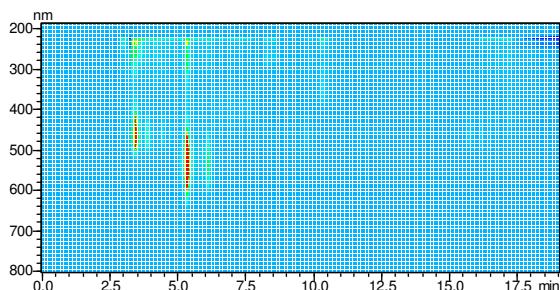


Fig. 4. Contour plot of LC separation of the pigments from the aqueous extract from *Beta vulgaris* L. root.

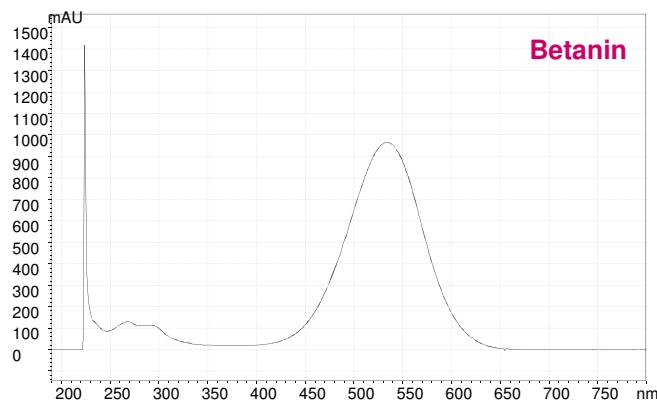


Fig. 5. UV-VIS spectrum of betanin ($\lambda_{\text{max}}=534$ nm) obtained in the eluent during the HPLC run.

MS/MS CONDITIONS

All analyses were done using a Shimadzu LCMS-8030 triple quadrupole mass spectrometer (Shimadzu, Japan) equipped with an ESI source working in the polarity switching MRM mode, scan mode and mainly in the Product Ion Scan mode. Data acquisition and analysis were accomplished with LabSolutions 5.60 SP1 software.

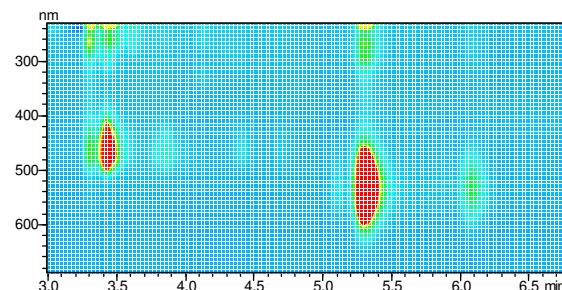


Fig. 6. Contour plot (zoom) of LC separation of the pigments in the aqueous extract from *Beta vulgaris* L. roots. The dominant compounds are betanin ($R_t=5.3$ min), vulgaxanthin I ($R_t=3.4$ min) and isobetanin ($R_t=6.1$ min).

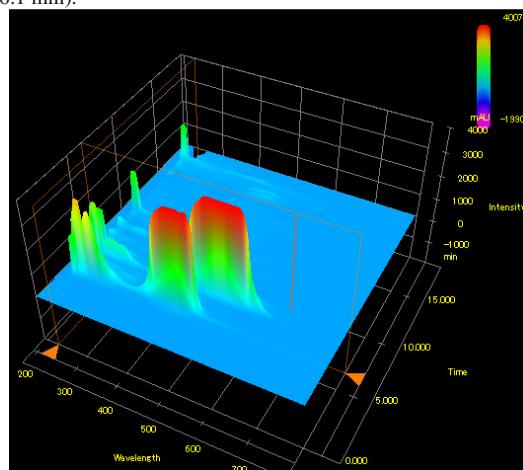


Fig. 7. 3D plot of LC separation of betalains in the aqueous extract from *Beta vulgaris* L. root.

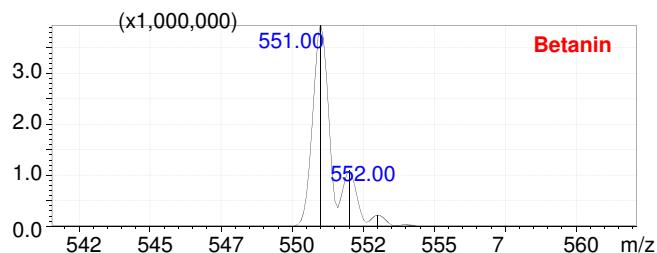


Fig. 8. ESI-MS spectrum of betanin detected by LC-ESI-MS analysis in positive mode ($m/z = 551$)

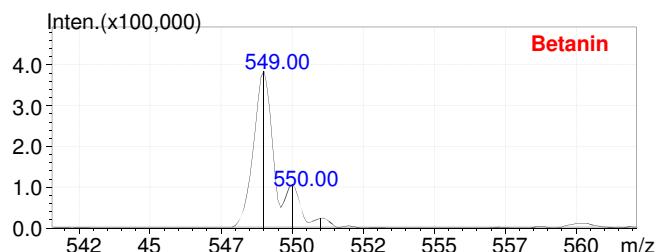


Fig. 9. ESI-MS spectrum of betanin detected by LC-ESI-MS analysis in negative mode ($m/z = 549$)

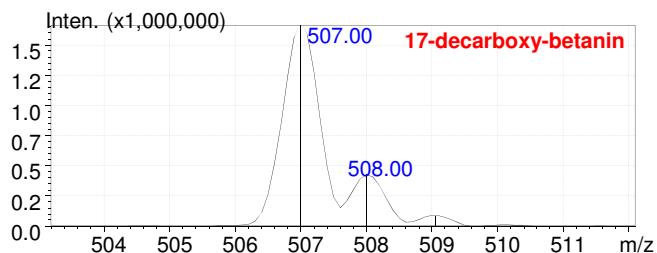


Fig. 10. ESI-MS spectrum of 17-decarboxy-betanin detected by LC-ESI-MS analysis in positive mode ($m/z = 507$).

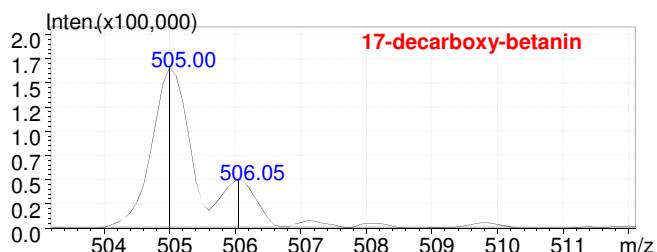


Fig. 11. ESI-MS spectrum of 17-decarboxy-betanin detected by LC-ESI-MS analysis in negative mode ($m/z = 505$).

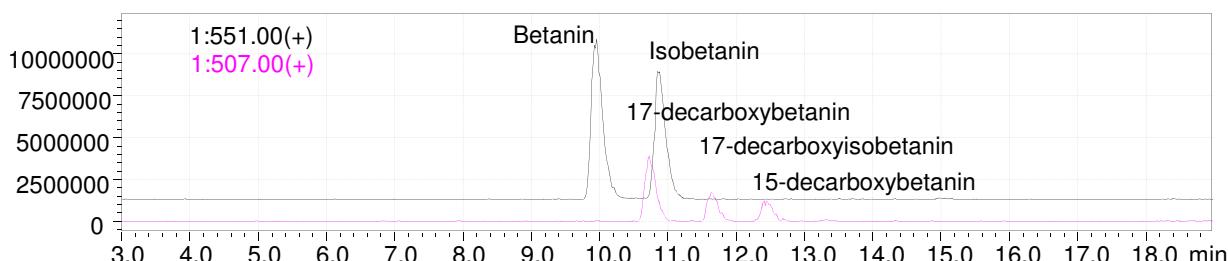
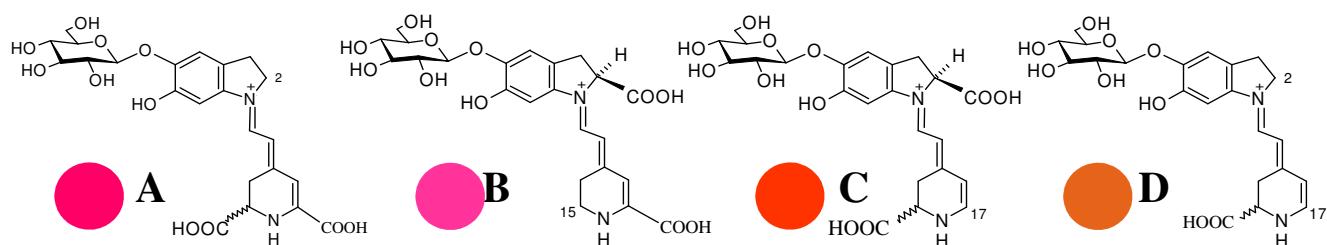


Fig. 12. ESI-MS chromatogram of betanin, isobetanin and its decarboxylated compounds detected in positive mode.



2-decarboxy-
betanin

15-decarboxy-
betanin

17-decarboxy-
betanin

2,17-decarboxy-
betanin

Fig. 13. Structures of decarboxylated derivatives of betanin pigment. Position of decarboxylation has an influence on color and antioxidant activity.

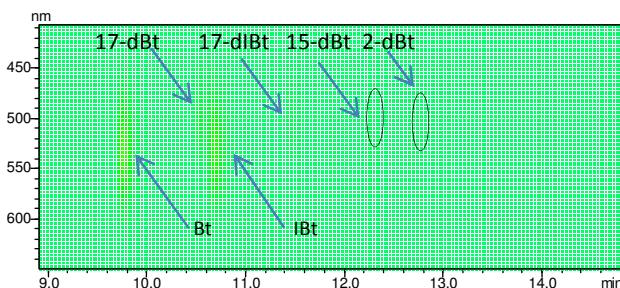


Fig. 14. LC-DAD contour plot of betanine, isobetanin and its decarboxylated compounds

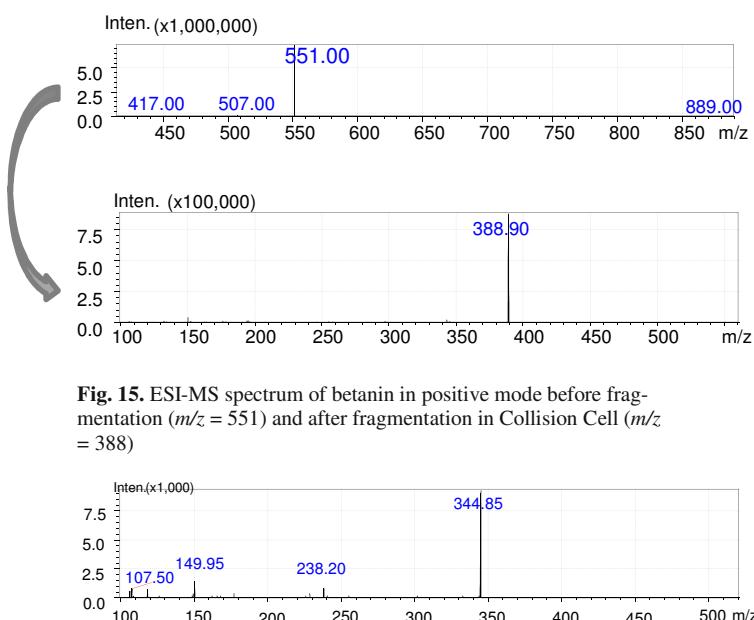


Fig. 15. ESI-MS spectrum of betanin in positive mode before fragmentation ($m/z = 551$) and after fragmentation in Collision Cell ($m/z = 388$)

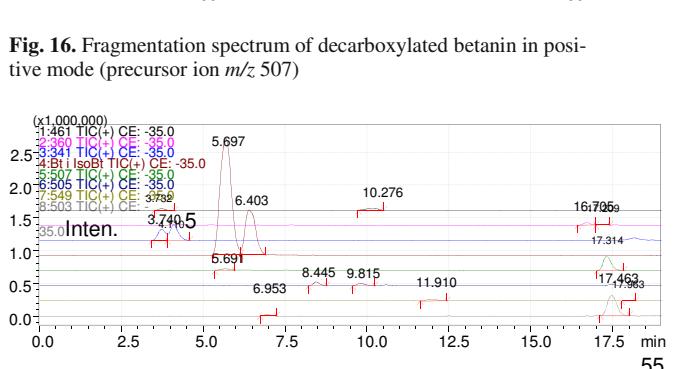


Fig. 16. Fragmentation spectrum of decarboxylated betanin in positive mode (precursor ion m/z 507)

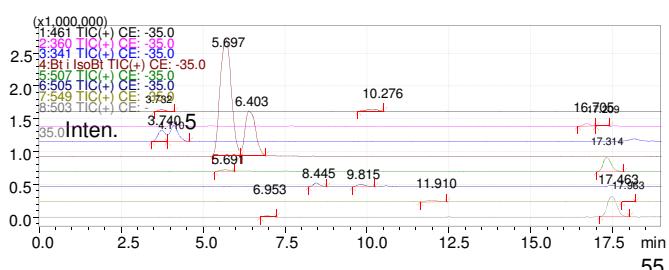


Fig. 17. Fifteen betalain pigments detected in redbeet juice

RESULTS AND DISCUSSION

We obtained interesting results using the LC-DAD-MS/MS system from Shimadzu. **Fig. 4, 5, 6, 7** show DAD chromatograms of *Beta vulgaris* L. root extract. **Fig. 17** additionally shows the MS chromatograms of 15 betalain pigments which are naturally occurring in plants. The analysis of

pigments not only included the naturally occurring pigments, but also the analysis on the thermal stability of the discussed compounds. **Fig. 10, 11, 12** present the spectra of the degradation products of betanin. **Fig. 14** shows the DAD chromatogram of decarboxylated derivatives of betanin. These are the most frequently occurring pigments which are initially found as the first derivatives on the degradation pathway. These include the 17-decarboxylated compounds, 15-decarboxylated compounds and 2-decarboxylated compounds (**Fig. 13**). The fast polarity switching capability of the Shimadzu LCMS-8030 MS/MS detector coupled with the Nexera UHPLC system were crucial in achieving the analytical results and developing this application

LITERATURE

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ACKNOWLEDGMENTS

The autor would like to thank dr. hab. Sławomir Wybraniec from Cracow University of Technology, Institute C-1 for providing the results and scientific consultation.