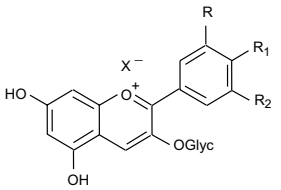
Anthocyanins
vs. anthocyanidins

The blueberry or bilberry (Vaccinium myrtillus L.) fruits are well-known source of **anthocyanins** and their extracts are widely used in dietary botanicals and pharmaceutical market for the treatment of vascular and vision disorders.



Flavylium cation, the basic structure of anthocyanins.

The term anthocyanin, initially coined to designate the substance responsible for the color of cornflower (from the Greek *anthos*, flower and *kuanos*, blue), applies to a group of water-soluble pigments responsible for red, pink, mauve, purple, blue, or violet color of most flowers and fruits.
These pigments (the anthocyanins) occur as **glycosides**, and their aglycones (the anthocyanidins) are derived from the 2-phenybenzopyrylium cation, more commonly referred to as flavylium cation, a name that emphasizes the fact that these molecules belong to the vast group of flavonoids in the broad sense of the term.

The drugs containing anthocyanins are used for the preparation of galenicals designed to treat the symptoms linked to capillary and venous fragility.

A brief
glossary

Bilberry fruits represent one of the main anthocyanins-containing drugs.

The bilberry anthocyanins are C-3 glucosides, galactosides, and arabinosides of cyanidin, peonidin, delphinidin, malvidin and petunidin.

In the bilberry extracts **the anthocyanidins (aglycones) are considered degradation products**: for this reason an analytical method suitable to identify and quantify anthocyanins and anthocyanidins is a key tool for evaluating the bilberry plant material and extracts quality.

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Analytical methods: UV vs **HPLC**

Different analytical methods used for standardization of the Bilberry extracts and preparations are available from Pharmacopoeias and from the literature. However, **most of them do not satisfy modern analytical requirements** and are not convenient for reproducing measurements.

The most common analytical methods use **UV-visible spectrophotometry** that allows the quantification of anthocyanins by detection in the visible region. In spite of the fact that these methods are very popular, they lack in specificity and do not allow the identification of each anthocyanin. As a consequence, these methods are not suitable for the identification of the anthocyanins extracts produced with different plant materials (raspberry, blackberry, black currant, elderberry, etc.).

High-performance Liquid Chromatography seems to be the best technique for standardization of anthocyanin extracts allowing the evaluation of the individual anthocyanin. Unfortunately, most of these methods are not reproducible and do not allow the complete separation of all the constituents.

Sometimes in order to simplify the UV-visible and the HPLC procedures the original extract is modified by **acidic hydrolysis** followed by the measurement of this way formed aglycones (anthocyanidins) abundance.

These kinds of analytical methods are **far from satisfactory** since, if some proanthocyanidins are present in the extracts, they too form anthocyanidins by hydrolysis and provide an overestimation of anthocyanin content.

Beside, this procedure does not allow the quantification of the **real content of degradation products** (anthocyanidins).

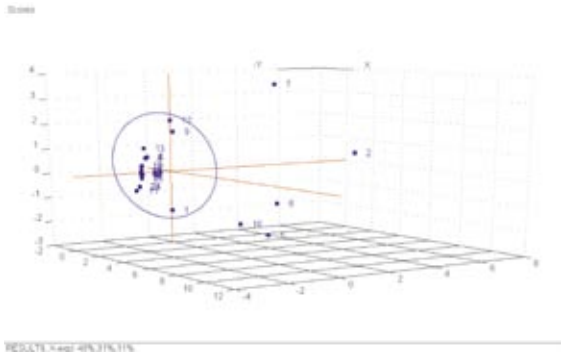
Overcoming the analytical issues

In order to overcome the previous discussed analytical issues Indena has **developed and validated an HPLC method** that allows the identification and the **direct quantification** of all the bilberry anthocyanins both in plant material and in extracts.

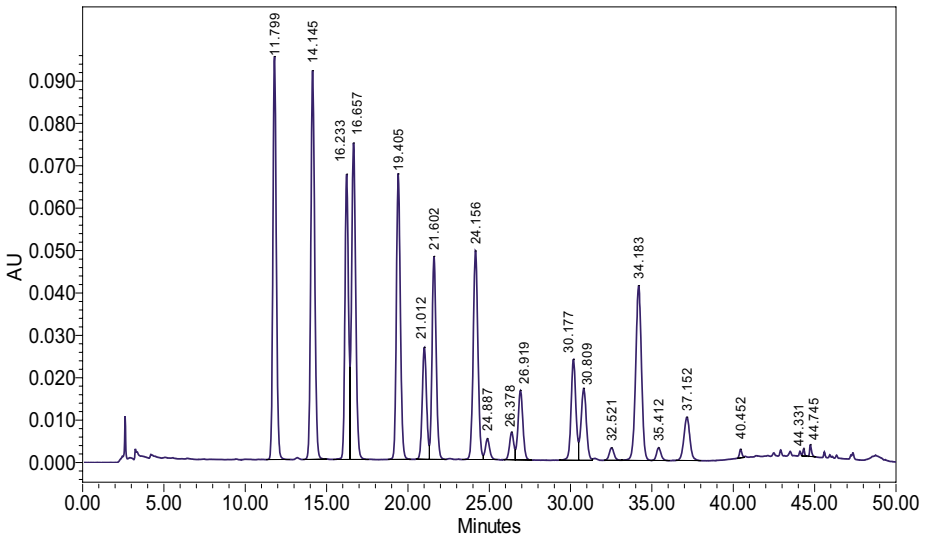
The quantification procedure foresees cyanidin-3-glucoside as external standard and the content of **each individual anthocyanin** is evaluated making use of a molecular-weight-correction factor: as a matter of fact according to the literature there is a direct correlation between molecular weights and responses (absorbance/concentration) between anthocyanins containing similar aglycons (anthocyanidins).

The method is endowed with a good reproducibility and due to its **high specificity** is suitable to identify unequivocally the botanical raw materials used for manufacturing and to evaluate the extracts composition providing a high degree of product **consistency and quality**.

Additionally, the results obtained with the above HPLC methodology submitted to a multivariate statistical evaluation as multivariate analysis (Principal Component Analysis, PCA) foresee the evaluation of minor composition differences between the extracts increasing the discrimination power of this methodology.
A graphical representation of the chemometric evaluation, in which several bilberry extracts with similar HPLC anthocyanins content are compared, is reported.



The typical bilberry extracts are confined in the ellipse. The extracts outside the ellipse have overall different anthocyanins composition undetectable by simple comparison of HPLC results.



A typical HPLC profile obtained with this method.

COMPOUND	RETENTION TIME
Delphinidin-3-O-galactoside	11.8
Delphinidin-3-O-glucoside	14.15
Cyanidin-3-O-galactoside	16.23
Delphinidin-3-O-arabinoside	16.66
Cyanidin-3-O-glucoside	19.41
Petunidin-3-O-galactoside	21.01
Cyanidin-3-O-arabinoside	21.6
Petunidin-3-O-glucoside	24.16
Delphinidin	24.89
Peonidin-3-O-galactoside	26.38
Petunidin-3-O-arabinoside	26.92
Peonidin-3-O-glucoside	30.18
Malvidin-3-O-glucoside	30.81
Peonidin-3-O-arabinoside	32.52
Malvidin-3-O-galactoside	34.18
Cyanidin	35.41
Malvidin-3-O-arabinoside	37.15
Petunidin	40.45
Peonidin	44.33
Malvidin	44.75

Source: Dr. Roberto Pace - Analytical Research Director