

Quality assessment of Cranberry (*Vaccinium macrocarpon*) based food supplements: HPTLC, HPLC and PAC quantification as effective tools for quality management

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Abstract

Cranberry-based food supplements with extracts from the fruits of *Vaccinium macrocarpon* are widely marketed for urinary tract health. As food supplements containing plant extracts, the so-called botanicals, are known to have quality problems the present study assembled efficient analytical protocols for evaluation of cranberry products for unambiguous identity testing and quantitation of procyanidins. Twelve cranberry-containing food supplements available in the D-A-CH region were included into the study. The analytical investigation comprised uniformity of mass testing of single-dosed capsule preparations, identity fingerprinting by HPTLC and HPLC and photometric quantification of the procyanidin content. Major deficiencies were detected such as labelling inconsistencies and deviations in capsule mass which indicated poor manufacturing control. The chromatographic analysis revealed that three products did not show specific anthocyanin fingerprints being typical for *V. macrocarpon*. In addition, deficiency was obvious concerning batch-to-batch conformity of certain brands. The results highlight the urgent need for specific analytical methods in quality control to verify botanical authenticity. Results again underscore critical regulatory gaps between the pharmaceutical-like presentation of such food supplements and their actual quality. Harmonized quality standards and stricter regulatory oversights are urgently needed to ensure transparency, consumer safety, and product integrity in the botanical supplement market.

1. Introduction

Cranberries, the fruits from *Vaccinium macrocarpon* Ait. (Ericaceae), originating from the high moors of North America are used for its preventative effect against uncomplicated urinary tract infections (UTI). The positive effects of cranberries for UTI are due to the inhibition of early host-pathogen interaction, especially in regard to the adhesion of FimH-dominated uropathogenic *Escherichia coli* (UPEC) to bladder and kidney cells of the human host (Scharf et al. 2017; Gbinigie et al. 2020). These antiadhesive properties have been discussed to be due to a direct interaction of oligomeric proanthocyanidins (PAC) with the mannose-binding side of UPEC. Due to the low bioavailability of PAC after oral intake this mode of action might not contribute to a high extend to the positive effects of cranberry extract for UTI (Scharf et al. 2017; Scharf et al. 2020). In contrast, recent studies indicated an indirect antiadhesive effect, as cranberries stimulate the secretion of the highly mannosylated glycoprotein Tamm-Horsfall Protein (syn. THP, uromodulin) in the distal part of the kidney, which again interacts with the mannose-binding side of UPEC, preventing the adhesion of UPEC to uroplakin-receptors on the bladder epithelium (Scharf et al. 2019). For this, cranberry extracts are assessed nowadays as inductors of the innate immune defense within the urinary tract.

For medical use, the European Medicines Agency (EMA) has published two monographs for liquid cranberry preparations within the traditional use and distinguishes between two indications, namely for the treatment of acute, uncomplicated UTI with mild symptoms, and additionally for prevention of recurrent uncomplicated UTI (European Medicines Agency - Herbal Medicinal Products Committee 2022).

The phytochemical composition of cranberries is mainly characterized by the presence of oligomeric A-type PAC, anthocyanins, flavonoids, mainly flavonols, organic acids, and xyloglucan-like polysaccharides; for review see (Scharf et al. 2020; Xu et al. 2025). Cranberries contain also a high molecular weight fraction, known as non-dialyzable material (NDM) that has been reported to exert antiadhesive activity against different bacteria, as e.g. *Helicobacter pylori* strains to gastric epithelial cells (Burger et al. 2000; Burger et al. 2002) and *S. sobrinus* to saliva-coated hydroxyapatite beads (Yamanaka et al. 2004). The nature of NDM is not clear but it is discussed to be a mixture of polymeric A-type PAC in combination with denatured proteins and xyloglucan (Neto et al. 2017).

In regard to positive effects against bacterial pathogens in the urinary tract and the THP-stimulation in the kidney, especially A-type PAC are in the focus (Howell et al. 2005). Cranberry A-type PAC differ structurally

from PAC found in other tannin-rich plants, such as green tea, grapes, or cocoa, dominated by B-type PAC, which do not exert antiadhesive activity (Howell et al. 2005).

Cranberries typically contain six major anthocyanins, specifically the 3-*O*-glycosides of the anthocyanidins cyanidin and peonidin, which are linked to β -D-glucose, β -D-galactose, or α -L-arabinose (Foo et al. 2000). This specific anthocyanin-pattern (structural features see Fig. 1) can be used for identity testing of cranberry extracts, as it differs clearly from other fruits from the Ericaceae plant family and other anthocyanin containing plant material.

Even though cranberry extracts are widely used for medical applications and a variety of clinical studies with positive outcome for UTI treatment have been reported, no cranberry-containing herbal medicinal products (HMP) have been registered in Germany. Instead, a steadily growing market for cranberry-based dietary supplements and food products has emerged. However, the European Food and Safety Authority (EFSA) has not approved any health claims for cranberry products until now (European Commission 2025). This has led to the marketing of cranberry-based food supplements with vague indications for use, most of which are promoted to consumers as providing benefits for the urinary tract system. In many cases it is not clear for the consumer if such a product is a registered drug-like HMP or a food supplement. This again leads to the problem of product quality. While HMP have controlled and reproducible quality due to legislation, registration and the underlying GMP guidelines, food supplements are covered by the national food laws and are therefore not subject to the strict requirements for efficacy, safety and quality that apply to authorized medicinal products. This lack of approval in combination with a presentation of food supplements similar to authorized drugs makes it difficult for consumers and health professionals to assess the quality of food supplements.

Recent analytical investigations of food supplements containing plant extracts, the so-called botanicals, indicated strong quality deficiencies in many cases, ranging from food fraud without any active ingredients to strongly underdosed products and to products containing serious falsifications or even toxic adulteration (Lechtenberg and Hensel 2019; Gaspar et al. 2021; Sendker et al. 2022; Brouns et al. 2025). Therefore, many international societies and governmental institutions repeatedly ask for stringent analytical batch-to-batch control of food supplements by specific and validated analytical protocols, establishment of a nutravigilance system and governmental reactions against manufacturer in case of quality violence.

Cranberry food supplements are the top-selling products in the segment of food supplements. Differences between products are due to the respective PAC content. The dried press cake, obtained from the fruits after the juice has been squeezed out, contains about 0.8 to 1.5% PAC (American Botanical Council 2017). Many of the food supplements use this pre-extracted bulk material as “active ingredient”. On the other side cranberry drinks are characterized by PAC content of 3 to 5%, while pure cranberry juice should contain 12 to 24% PAC (American Botanical Council 2017). Falsification of cranberry products with other lower-cost PAC-enriched extracts have been described (e.g., peanuts from *Arachis hypogaea*, grape seeds from *Vitis vinifera*) (American Botanical Council 2017)). Also, adulterants with anthocyanin-enriched extracts are known (e.g. mulberry from *Morus* spp., hibiscus from *Hibiscus sabdariffa*, black beans from *Phaseolus vulgaris*, black rice from *Oryza sativa*) (American Botanical Council 2017). Additionally, anthocyanin-enriched extracts from other *Vaccinium* species (e.g. *V. corymbosum*, *V. oxycoccos*, *V. vitis-idaea*) might be suitable to simulate cranberry, but can clearly be differentiated by the respective anthocyanin fingerprint (Brown et al. 2012).

As until now no monograph for cranberries has been published in the European Pharmacopoeia, the aim of this analytical project was to compile relevant analytical methods for the authentication of cranberry extracts, evaluating the typical anthocyanin pattern of the fruits by HPTLC and HPLC and colorimetric quantification of total PAC content. This collection of methods was used to assess the quality of food supplements obtained from different sources.

2. Materials and Methods

2.1 Materials and reference standards

If not stated otherwise, solvents, reagents, and consumables were obtained from VWR International (Darmstadt, Germany). All solvents and reagents were of analytical quality. Water was produced by a Millipore Simplicity 185 system (Merck, Darmstadt, Germany). Procyanidin A2 (lot 16753) was obtained in reference standard quality from Phytolab (Vestenbergsgreuth, Germany). Cy3Gal (lot 18), Cy3Glc (lot 48) and Peo3Gal (lot 03) were obtained in reference standard quality from ExtraSynthese (Genay, France). *V. vitis-idea* was obtained as dried lingonberries from Galke (Bad Grund, Germany), lot 47545 (IPBP No. 917).

Authenticated plant material from *Vaccinium macrocarpon* was obtained from the medicinal plant garden of University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Münster, Germany. Authentication was performed by AH and AB. Voucher samples are stored at the archives of the institute (IPBP 916). Authenticated plant material from *Vaccinium oxycoccos* was obtained from a controlled garden area in Münster, Germany (GPS positioning 51.9942, 7.5937). Authentication was done by Mr. André Niermann, voucher sample IPBP 918).

2.2. Cranberries, cranberry juices, cranberry containing food supplements

Twelve different cranberry products were obtained on a commercial base for internet shops, pharmacy stores and herbal trade shops (Table 1). Product #01 represents a concentrated cranberry juice (puree), #11 are dried cranberries, the other products represent food supplements in capsule form. Declaration of supplement facts and specific claims of the respective products are displayed in Table 1.

Table 1
Overview of the analyzed cranberry-based food supplements

No.	Supplier or distributor	name	Shelf life	LOT/ batch number	Nutrition facts, content, serving size	Further labeling (translated into English)
#01	Anton Hübner GmbH & Co KG	Donath Vollfrucht Cranberry ungesüßt	19.04.2023	---	Cranberry puree	
#02	Bergland Pharma GmbH & Co KG	Bergland Cranberry Lutschtabletten	06/2025	L2172/1	Cranberry juice powder (36%) ^a 15 mg ^b 3 capsules ^c	Contains additionally Acerola powder with vitamin C (10%),
#03	Avitale GmbH	Avitale Cranberry Kapseln	10/2025	L2291/3	Crannberrypowder AV36 400 mg ^a 18 mg ^b 2×1 capsule per day ^c	Contains additionally excipients
#31	Avitale GmbH	Avitale Cranberry Kapseln	09/2026	L3249/1	Crannberrypowder AV36 400mg ^a 18mg ^b 2×1 capsule per day ^c	Contains additionally excipients
#04	Dm- drogerie markt GmbH + Co. KG	Mivolis Cranberry + Vitamin C	10/2025	393001	Cranberry-Extract 300 mg ^a 18 mg ^b 2×1 capsule ^c	Contains additionally excipients and vitamin C
#41	DM Drogerie Markt GmbH + Co. KG	Mivolis Cranberry + Vitamin C	03/2026	39009	Cranberry-Extract 300 mg ^a 18 mg ^b 2×1 capsule ^c	Contains additionally excipients and vitamin C
#05	Pro medico Handels GmbH	pure encapsulations – Cranberry Extrakt	05/2025	2260666	Cranberry extract (CranMax): 500 mg ^a 1 capsule per day ^c	Contains additionally cranberry fibers and cranberry seed oil, excipients
#06	11A Nutrithek GmbH	Cranberry 36	12/2025	25122	Cranberry extract: 53 mg ^a 36 mg ^b	Contains additionally excipients
^a mg Cranberry extract or concentrate per capsule/unit; ^b total [mg] procyanidin per capsule/unit; ^c serving size; * specified in the enclosed product leaflet, but not on the primary packaging box						

No.	Supplier or distributor	name	Shelf life	LOT/ batch number	Nutrition facts, content, serving size	Further labeling (translated into English)
					1 capsule per day ^c	
#07	Hecht Pharma GmbH	Cranberry 400mg GPH-Kapseln	08/2027	405.77	Cranberry Concentrate (400 mg = 10 g Cranberrys) ^a 3×1 capsule ^c	Contains additionally excipients
#08	Naturafit GmbH	Cranberry 400	12/2024	41221	Cranberry Powder: 400 mg (= 10 g pure Cranberry) ^a 4 capsules per day after food ^c	Contains additionally excipients
#09	Quiris Healthcare	Cystorenal plus	09/2024	0090894	Cranberry extract: 300 mg (25:1) ^a 12 mg ^b 3×1 capsule ^c	Contains additionally pumpkin seed extract 4:1 (18%) vitamin C, vitamin B2, excipients
#10	NeoElementum OÜ	CardioBalance	06/2024	1042023	Cranberry extract 225 mg ^a 2×1 capsule per day ^c	Contains additionally chokeberry extract: 200 mg (20:1), Calcium butyrate 200 mg*, magnesium 150 mg, Gymnema Sylvestre 100 mg*, L-Arginine 150mg, extract from hawthorn leaves and flowers 150 mg (6:1), Hibiscus flower powder 50 mg, Olive leaf extract 50 mg (10:1), Grape seed extract 50 mg (8:1), Blackcurrant fruit extract 25 mg (4:1), Coenzyme Q10 20 mg, Vitamin B6 1.4 mg,
^a mg Cranberry extract or concentrate per capsule/unit; ^b total [mg] procyanidin per capsule/unit; ^c serving size; * specified in the enclosed product leaflet, but not on the primary packaging box						

No.	Supplier or distributor	name	Shelf life	LOT/ batch number	Nutrition facts, content, serving size	Further labeling (translated into English)
						Folic acid 100 µg
#11	Adler Vital	Cranberries	29.10.2023	NOV2322W3		
#12	Vitarmony Laboratoires	Cranberry	06/2025	1637YAA	Cranberry extract: 200 mg ^a 6 mg ^b 2×1 capsules per day ^c	Contains additionally excipients
^a mg Cranberry extract or concentrate per capsule/unit; ^b total [mg] procyanidin per capsule/unit; ^c serving size; * specified in the enclosed product leaflet, but not on the primary packaging box						

2.3. Cranberry dry reference extract (CDE)

Authenticated fruits from *V. macrocarpon* were harvested and frozen immediately. Fifty grams of the frozen fruits were pulverized crushed in an ice-cold mortar and extracted with 500 mL of ethanol 96% (v/v) for 30 min by use of a rotor-stator extractor (Ultra Turrax, IKA, Germany). The suspension was filtered (VWR filter paper 413) and the clear solution was evaporated to dryness under *vacuo*, followed by lyophilization to yield 5% (w/w, related to the fresh plant material) of the cranberry dry reference extract, which is termed as CDE in the following.

2.4. Fingerprinting by HPTLC (PAC, anthocyanins, chlorogenic acid, hyperoside)

For identity testing of cranberry extract HPTLC analysis was used based on a protocol of the USP (United States Pharmacopeia Convention 2023, 2024). Procyanidin A2 (1 mg/mL in MeOH), Cy3Gal, Cy3Glc, Peo3Gal, chlorogenic acid and hyperoside (0.2 mg/mL MeOH) served as reference standards. Cranberry dry extract (CDE) was used as reference extract.

Test samples, for which a defined PAC content was specified by the manufacturer (#02, #03, #31, #04, #41, #06, #08, #12) were investigated by preparing solutions as follows: an aliquot of the contents of the capsules, equivalent to 8.5 mg of total PAC, is transferred to a 10 mL centrifuge tube. Seven milliliters of water are added. For #05, #07, #08, #10 and CDE 50 mg sample material is mixed with 7 mL of water. The respective suspensions are sonicated for 10 min and subsequently centrifuged for 5 min at 3600 × g. The clear supernatant is used for further processing. A solid-phase extraction cartridge (C18, 500 mg/3 mL, Macherey-Nagel, Germany) is conditioned with 3 mL of MeOH. Subsequently, 3 mL of water are loaded on the cartridge and sucked into until the solvent level is 1 to 2 mm above the stationary phase. 1.5 mL of the test solution is loaded onto the cartridge and sucked into the column. The eluate is discarded. The stationary phase is rinsed with 1 mL of water-MeOH (8:2, v/v). The eluate is discarded. Finally, 1 mL of MeOH is used to elute the retained compounds. This eluate is collected as the test solution for subsequent HPTLC.

Mobile phase for HPTLC: ethylformiate, formic acid, water, toluene (60:8:6:3, v/v/v/v). Spotting volume: 5µL for references and 15 µL for samples, 8 mm. Development: 70 mm. Drying: 5 min by air stream at RT. Detection 1: 100 to 105°C for 5 min, followed by spraying with a solution of diphenylboryloxyethylamine (10 g/L) in MeOH, evaluation in day light and λ = 366 nm. Detection 2: spraying with a mixture of 170 mL ice-cooled MeOH in 20 mL of glacial acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde, followed by heating of the TLC plate to 100° C for 5 min. Evaluation in day light.

2.5. Fingerprinting of anthocyanins by HPLC

HPLC system: Shimadzu LC10A with system controller (SCL10A), autosampler (SIL10A), pumps (LC10AT), degasser (JASCO DG-2080-53), oven (CTO10A) and UV-VIS detector SPD10A. Stationary phase: GeminiNX 110 Å, 5 µm, 250 mm (Phenomenex, USA), (alternatively with same selectivity Kromasil 100 Å, 5 µm, 250 mm, Phenomenex, USA). Mobile phase: (A) water, formic acid (91.5:8.5, v/v); (B) water, MeOH, acetonitrile, formic acid (41.5:22.5:22.5:8.5, v/v/v/v); gradient: $t_{0\text{min}}$: 80% (A), $t_{35\text{min}}$: 75% (A), $t_{46\text{min}}$: 35% (A), $t_{57\text{min}}$: 0% (A). Flow rate: 1.0 mL/min. Injection volume: 10 µL for reference solutions, 30 µL for test solutions. Oven temperature: 30°C. Detection: $\lambda = 535$ nm.

To recognize possible adulteration with the small cranberry (*V. oxycoccos*), the respective fruits from authenticated plants were harvested and treated as described for preparation of CDE, using 10 g fruits extracted with 100 mL EtOH 96% (v/v).

For adulteration with lingonberry (*V. vitis idaea*), the dried fruits were frozen using liquid nitrogen and subsequently pulverized. One gram of the pulverized material was mixed with 10.0 mL of water and extracted in an ultrasonic bath for 15 min. The extract was centrifuged ($3600 \times g$, 5 min) and the clear supernatant collected for solid-phase extraction (SPE) [SPE was employed to concentrate the anthocyanins, since a direct extraction of the fruits with MeOH yielded insufficient anthocyanin concentrations]. An SPE cartridge (C18, 500 mg, 3 mL; Macherey-Nagel, Germany) was conditioned with 3 mL MeOH, followed by 3 mL water. Subsequently, 4 mL of the aqueous extract were loaded onto the cartridge and eluted. Subsequently, 1.0 mL of a mixture of water/MeOH (80:20, v/v) was loaded onto the cartridge and eluted. Finally, the anthocyanins were eluted with 1.0 mL of MeOH. The eluate served as the test solution for HPLC analysis.

Test solutions from cranberry food supplements: The equivalent of an average mass from one capsule of the test products was weighted exactly into a flask and was extracted with 25.0 mL of a mixture of MeOH and hydrochloric acid (98: 2 v/v). After centrifugation ($3600 \times g$, 5 min), 5.0 mL of the clear supernatant were diluted with phosphoric acid in water (10% w/w) to 20.0 mL to serve as test solution for HPLC.

Test solution from cranberry dried fruits: 1 g of dried fruits were extracted with 10.0 mL of MeOH: water (1:1 v/v) by ultrasonic treatment for 15 min. Subsequently, the suspension was centrifuged ($3600 \times g$, 5 min). The clear supernatant serves as test solution.

Test solutions from cranberry liquid preparations: 10 mL of the liquid samples were transferred to a sample tube and were centrifuged ($3600 \times g$, 5 min). The clear supernatant served as test solution for HPLC.

The HPLC protocol was validated for the parameters system precision ($\pm 0.54\%$), linearity in the range of 5 to 100 µg/mL and recovery rate $100.7 \pm 1.7\%$ by using Pn3Gal (Supplementary Data File, Figure S1).

2.6. Quantification of PAC (United States Pharmacopeia Convention 2023, 2024)

Reagent solution: 4-(Dimethylamino)-cinnamaldehyd (DMAC, 1 mg/mL) in 0.4 mol/L sulfuric acid. The solution must be prepared fresh every day. Standard stock solution: procyanidin A2, 90 µg/mL in MeOH. Standard solutions: A minimum of five calibration solutions, ranging from 10 to 30 µg/mL procyanidin A2 in MeOH, were prepared from the standard stock solution by dilution with MeOH.

Test solution from cranberry capsule preparations: A portion of the capsule content, equivalent to 36 mg of total PAC, was transferred to a 100 mL flask, suspended in MeOH, sonicated for 10 min, followed by shaking for 10 min. 10 mL of the resulting suspension were centrifuged for 5 min at $1950 \times g$. The clear supernatant is diluted with MeOH to a test solution being nominally equivalent to a PAC content of 0.02 mg/mL.

For the samples without a PAC declaration 2.0 g of sample material were transferred to a 100 mL flask, suspended in 100.0 mL MeOH and sonicated for 10 min. The suspension was centrifuged ($1950 \times g$, 5 min). The clear supernatant was used for further analysis.

The reagent solution (250 µL) was mixed with 50 µL of the test sample solutions in a 96-well plate. A PAC-free solvent control, containing MeOH, is used as a blank. The resulting absorbance was determined constantly for 5 min at $\lambda = 640$ nm immediately after mixing the reagents. The highest absorbance is recorded. Absorbance values obtained from the standard solutions are used for calibration ($R^2 > 0.98$) to calculate total PAC content as procyanidin A2. The total PAC content per capsule is calculated as follows:

$$PAC\ content\ (\% \text{ of specified content}) = c \times V_U \times (W_{AF}/W) \times (V_F/V_A)$$

c: concentration (mg/mL) of sample solution, calculated as procyanidin A2 from the calibration; V_U : volume (mL) of sample stock solution; W_{AF} : average filling (mg) per capsule; W: weight of the capsules content (mg) for preparation of the sample stock solution; V_F : final volume (mL) of the sample solution; V_A : volume (mL) of the aliquot of the sample stock solution.

For validation of the protocol, the recovery rate (> 99%) was determined by spiking test solutions with procyanidin A2 reference standard (n = 3).

All assays were performed in two independent investigations with n = 3 technical replicates. For final evaluation the respective mean values were used.

Validation of the protocol was performed concerning linearity of detection signals in the range of 10 to 30 µg/mL of procyanidin A2 (Supplementary Data File, Figure S2).

2.7. Uniformity of mass for single-dose preparations (Bundesinstitut für Arzneimittel und Medizinprodukte 2024)

Twenty randomly selected capsules are weighted. The content of each capsule is removed by a gentle air stream. The capsule shells are weighed, and the net filling mass is determined. The test meets the requirements of if not more than two capsules deviate +/- 7.5% from the average filling mass. None of the capsules may deviate more than +/- 15% from the mean value. In cases of average filling mass of < 300 mg the respective limits are +/- 10% and +/-20%.

3. Results and discussion

Analytical investigations of a series of twelve cranberry containing food supplements (Table 1, #01 to #12), representing the cranberry market in the D-A-CH region, was performed. Beside cranberry puree (#01), dried cranberry fruits (#11), and lozenges (#02) most of the investigated products were capsule preparations. Table 1 displays a detailed overview on the characteristics of the different products. The test samples were obtained from internet trading as well as from community pharmacies and herbal shops. Freshly harvested, authenticated and air-dried plant material from *V. macrocarpon* served as reference.

Table 1: Overview of the analyzed cranberry-based food supplements

No.	Supplier or distributor	name	Shelf life	LOT/ batch number	Nutrition facts, content, serving size	Further labeling (translated into English)
#01	Anton Hübner GmbH & Co KG	Donath Vollfrucht Cranberry ungesüßt	19.04.2023	---	Cranberry puree	
#02	Bergland Pharma GmbH & Co KG	Bergland Cranberry Lutschtabletten	06/2025	L2172/1	Cranberry juice powder (36%) ^a 15 mg ^b 3 capsules ^c	Contains additionally Acerola powder with vitamin C (10%),
#03	Avitale GmbH	Avitale Cranberry Kapseln	10/2025	L2291/3	Crannberrypowder AV36 400 mg ^a 18 mg ^b 2x1 capsule per day ^c	Contains additionally excipients
#31	Avitale GmbH	Avitale Cranberry Kapseln	09/2026	L3249/1	Crannberrypowder AV36 400mg ^a 18mg ^b 2x1 capsule per day ^c	Contains additionally excipients
#04	Dm- drogerie markt GmbH + Co. KG	Mivolis Cranberry + Vitamin C	10/2025	393001	Cranberry-Extract 300 mg ^a 18 mg ^b 2x1 capsule ^c	Contains additionally excipients and vitamin C
#41	DM Drogerie Markt GmbH + Co. KG	Mivolis Cranberry + Vitamin C	03/2026	39009	Cranberry-Extract 300 mg ^a 18 mg ^b 2x1 capsule ^c	Contains additionally excipients and vitamin C
#05	Pro medico Handels GmbH	pure encapsulations - Cranberry Extrakt	05/2025	2260666	Cranberry extract (CranMax): 500 mg ^a 1 capsule per day ^c	Contains additionally cranberry fibers and cranberry seed oil, excipients
#06	11A Nutrithek GmbH	Cranberry 36	12/2025	25122	Cranberry extract: 53 mg ^a 36 mg ^b 1 capsule per day ^c	Contains additionally excipients
#07	Hecht Pharma GmbH	Cranberry 400mg GPH-	08/2027	405.77	Cranberry Concentrate (400	Contains additionally

Kapseln					mg = 10 g Cranberrys) ^a	excipients
					3×1 capsule ^c	
#08	Naturafit GmbH	Cranberry 400	12/2024	41221	Cranberry Powder: 400 mg (=10 g pure Cranberry) ^a 4 capsules per day after food ^c	Contains additionally excipients
#09	Quiris Healthcare	Cystorenal plus	09/2024	0090894	Cranberry extract: 300 mg (25:1) ^a 12 mg ^b 3×1 capsule ^c	Contains additionally pumpkin seed extract 4:1 (18%) vitamin C, vitamin B2, excipients
#10	NeoElementum OU	CardioBalance	06/2024	1042023	Cranberry extract 225 mg ^a 2×1 capsule per day ^c	Contains additionally chokeberry extract: 200 mg (20:1), Calcium butyrate 200 mg *, magnesium 150 mg, Gymnema Sylvestre 100 mg *, L-Arginine 150mg, extract from hawthorn leaves and flowers 150 mg (6:1), Hibiscus flower powder 50 mg, Olive leaf extract 50 mg (10:1), Grape seed extract 50 mg (8:1), Blackcurrant fruit extract 25 mg (4:1), Coenzyme Q10 20 mg, Vitamin B6 1.4 mg, Folic acid 100 µg
#11	Adler Vital	Cranberries	29.10.2023	NOV2322W3		
#12	Vitarmonyl Laboratoires	Cranberry	06/2025	1637YAA	Cranberry extract: 200 mg ^a 6 mg ^b	Contains additionally excipients

2×1 capsules per
day^c

^a mg Cranberry extract or concentrate per capsule/unit; ^b total [mg] procyanidin per capsule/unit; ^c serving size; * specified in the enclosed product leaflet, but not on the primary packaging box

Deficiency in product declaration of cranberry food supplements

At a first glance the test sample #10, which represents a combination of different plants extracts revealed strong differences in the declaration of the product concerning the information given on the primary packaging material and the information leaflet (Supplementary Data, Figure S3). Two ingredients, specified on the product leaflet, have not been specified on the capsules box. From this point of view product #10 does not meet the legal requirements concerning information of the consumer.

Uniformity of mass of single dose cranberry products

As the presentation of the food supplements is similar to that of medicinal products, test on uniformity of mass for single-dose preparations according to the European Pharmacopoeia (Bundesinstitut für Arzneimittel und Medizinprodukte 2024) was performed for the solid oral dosage forms. One sample (#08) did not meet the requirements, as one capsule deviated more than 15% from the mean. This result indicates the need for improvement of the technical production of capsules and tablets in the field of food supplements, as similar investigations a few years ago have also shown serious shortcomings for solid formulations concerning uniformity of mass (Gaspar et al. 2021).

Identity testing by HPTLC

HPTLC fingerprinting according to the methodology of USP-NF 2024 (United States Pharmacopeia Convention 2023, 2024) was employed for identity testing of the samples, using chlorogenic acid, hyperoside, procyanidin A2, cyanidin-3-*O*-galactosid (Cy3Gal), cyanidin-3-*O*-glucosid (Cy3Glc) and peonidin-3-*O*-galactosid (Peo3Gal) as reference compounds (Fig. 1). Detection was performed after spraying with diphenylboryloxyethylamine and monitoring at day light at $\lambda = 366$ nm, and after derivatization with anisaldehyde-sulfuric acid (Fig. 2A to C).

After derivatization with diphenylboryloxyethylamine the reference standards hyperoside (hyp), Cy3Gal, Cy3Glc and Peo3Gal showed characteristic-colored bands at R_f -values between 0.2 and 0.6. The cranberry dry extract exhibited bands matching Cy3Gal, Cy3Glc and Peo3Gal, as well as hyperoside. At $\lambda = 366$ nm the fluorescent bands for chlorogenic acid (chl.ac) ($R_f = 0.65$) and hyperoside ($R_f = 0.6$) were clearly visible, while Cy3Gal, Cy3Glc and Peo3Gal are not visible under these conditions. The CDE lane revealed multiple bright fluorescent zones in the middle to upper R_f region. The evaluation of the test samples was based on the presence, intensity and distribution of these characteristic zones in the chromatograms. Sample #02 corresponds to the reference profile, although the zones appeared weaker. Sample #03 showed partial similarity to the reference. In contrast, sample #04 did not correspond and for #05 and #08 no detectable zones were observed. A clear match with the reference profile was observed for sample #06. Sample #09 and #10 revealed partial correspondence, but as these products contain combinations of different plant derived ingredients a clear verification is not possible by HPTLC.

After derivatization with anisaldehyde / sulfuric acid reagent, procyanidin A2 (PA2) gets visible and appears as an intense red zone at $R_f = 0.9$, which appears prominently in CDE. The presence of a matching band at $R_f = 0.9$ in samples #02, #03, #31, #04, #41, #06, #07, #09, #12 indicates the presence of PA2. PA2 is not detectable in test samples #05, #08 and #10 (Fig. 2C).

At this point it can be stated that this HPTLC protocol provides valuable qualitative and semiquantitative insights and allows efficient comparison of the different chromatographic profiles of test samples. However, for certain samples the interpretation of the band pattern remained inconclusive due to weak or overlapping zones. To strengthen the analytical reliability further confirmation using HPLC is recommended. The complementary use of HPTLC and HPLC provides higher analytical confidence and ensures the unambiguous authentication of sample identity.

Fingerprinting of anthocyanins by HPLC

For further analysis, HPLC of the test samples was performed with special regard to the cranberry-specific anthocyanin pattern, based on a protocol of the United States Pharmacopeia (United States Pharmacopeia Convention 2023). The method was validated for precision, linearity and recovery rate (Supplementary Data File, Figure S1). Cranberry dry extract CDE was used as reference for qualitative investigation. The six anthocyanins **1** to **6** (Fig. 1) being typical for *V. macrocarpum* fruits were used for product authentication. Assignment of the peaks was performed by the respective reference standards. An exemplary chromatogram of *V. macrocarpon* is displayed in Fig. 3. Cy3Gal (**1**), Cy3Ara (**3**), Pn3Gal (**4**) and Pn3Ara (**6**) represent the four main peaks, Cy3Glc (**2**) and Pn3Glc (**5**) elute as minor peaks at t_R 9 to 24 min. The respective aglycons appear as minor peaks at a later retention (40 to 50 min) or are not present at all (Fig. 3).

For unambiguous identification of *V. macrocarpon* USP requires the presence of anthocyanins **1** to **6**. Additionally, it is required that no other peak than those already mentioned between Cy3Gal (**1**) and Pn3Ara (**6**) has a higher intensity than the peak for Cy3Glc (**2**). Furthermore, the ratio of the major peonidin-glycosides (**4** and **6**) to the major cyanidin-glycosides (**1** and **3**) must be between 0.5 and 1.9. Figure 3 shows a typical anthocyanin chromatogram of *V. macrocarpon*. To identify potential adulterations, comparative analysis of the anthocyanin profile of lingonberries (*V. vitis-idaea*) and the small cranberry (*V. oxycoccos*) was performed (Fig. 3). Both species belong to the Ericaceae family and the same genus and exhibit similar morphological characteristics to *V. macrocarpon*, suggesting the possibility of adulteration (Brown et al. 2012). As displayed in Fig. 3 both extracts exert different anthocyanin pattern and can be differentiated easily by monitoring the six anthocyanins characterizing the cranberry extracts. Lingonberries test solution elutes several peaks with only one dominant peak, which was identified as Cy3Glc (**1**). *V. oxycoccos* elutes a chromatogram that is more comparable to that of cranberries. However, the presence of a greater number of peaks, arranged in a distinct ratio to each other, allows also a clear differentiation between the different species (Fig. 3).

Subsequently, the test samples were analyzed by the validated HPLC protocol (Fig. 4).

Three of the twelve test samples (#03, #04, #10) exhibited anthocyanin profiles which are different from that of *V. macrocarpon*. Test sample #03 revealed a complex anthocyanin fingerprint, not typical for cranberry. As this sample did not conform to the specification, a second batch of the same product (#31) was obtained from the same supplier on a commercial basis. Analysis of this second batch again revealed no typical cranberry anthocyanin pattern. Surprisingly, the two batches (#03 and #31) from the same product brand revealed completely different anthocyanin composition (Fig. 5). It seems astonishing that this product not only contains none of the typical cranberry natural products, but also lacks batch-to-batch conformity.

Similar results were also obtained for different batches from test sample #04. Product #04 had an anthocyanin pattern which does not conform to that specified for cranberries (Fig. 4). This result is also in accordance with the data obtained from the TLC testing (Fig. 2). The respective adulteration of #04 could not be assigned to a distinct plant species. Surprisingly, analysis of a second batch of the same product (#41) revealed an anthocyanin pattern, which again did not meet the cranberry requirements, but was also different from the elution pattern observed for #04 (Fig. 5). This again means, that no batch-to-batch conformity is given for the product.

Test sample #10 is a combination product with different botanical ingredients (chokeberries, hibiscus flowers, olive leaves, grape seed, blackcurrant extracts), a clear statement on identity of cranberry in this product is not possible. The presence of cranberry is possible, but due to the combination with other plant extracts which also contain anthocyanins a clear statement cannot be made for this product.

For the other test products (#01, 02, 05, 06, 07, 08, 09, 11 and 12) identity of cranberry fruits was confirmed by the HPLC testing.

Quantification of PAC (United States Pharmacopeia Convention 2023)

The quantification of PAC is based on a colorimetric reaction of 4-(dimethylamino)cinnamaldehyde (DMAC) in an acidic medium with the analytes. The assay exploits the reaction of DMAC with flavanols, leading to the formation of a chromophore, which can specifically be monitored at $\lambda = 620$ nm. The intensity of the resulting absorption is directly proportional to the concentration of the PACs in the solution. For quantitative evaluation of PAC content standard calibration was used and the respective results were expressed as procyanidin A2 equivalents (Prior et al. 2010; Cardellina II and Gafner 2018; Vidal-Casanella et al. 2021).

Evaluation of the test samples indicated relevant quality deficiencies concerning the PAC content. In none of the samples for which a defined PAC content has been specified, this amount of PAC could be quantified (Table 2).

Table 2

Summary of quality investigation of cranberry-based food supplements with regard to identity, uniformity of mass of single dosed preparations and total PAC-content

No.	Identity [TLC, HPLC]	Uniformity of mass [Ph. Eur.]	Content PAC				
			PAC specification [mg/ unit]	Total PAC content per unit [mg], calc. as procyanidin A2; mean (range), n = 2	Recommended daily dose, corresponding to PAC content [mg] per day	Total PAC content per day [mg], calc. as procyanidin A2 According analysis	
#01	+	n.a.	no declaration	n.a	---	---	---
#02	+	n.a.	15	0.95 (0.35– 1.55)	3 capsules	45	2.9
#03/31	- / -	+	18	5.41 (5.34– 5.49)	2 capsules	36	10.8
#04/41	- / -	+	18	7.35 (5.67– 9.03)	2 capsules	36	14.7
#05	+	+	no declaration	1.27 (1.17– 1.36)	1 capsule	---	1.3
#06	+	+	36	11.26 (10.38– 12.13)	1 capsule	36	11.3
#07	+	+	no declaration	3.02 (2.91– 3.12)	3 capsules	---	9.1
#08	+	-	no declaration	1.16 (0.45– 1.86)	4 capsules	---	4.6
#09	+	+	12	1.29 (0.55– 2.04)	3 capsules	36	3.9
#10	(+)	+	no declaration	6.29 (4.87– 7.71)	2 capsules	---	12.6
#11	+	n.a.	no declaration	n.a	---	---	---
#12	+	+	6	0.65 (0.24– 0.88)	2 capsules	12	1.3
n.a.: not analyzed; -: negative result; + positive result							

Samples for which no specific PAC content was provided by the supplier had been evaluated according the corresponding USP monograph for that product type (e.g. Cranberry fruit juice concentrate). Among all samples, only sample #07 complies with the USP specification, which requires a minimum PAC content of 0.6% (w/w), calculated as procyanidin A2. Although this product meets the official quality criteria, the actual PAC dosage provides by the serving size is substantially lower than the intake levels typically regarded as effective. Therefore, only a limited recommendation can be made. Table 2 summarizes the respective results and compares manufacturers declaration with the results found in our investigations.

However, some limitations of the DMAC method must be noticed. The assay only quantifies soluble PACs, while insoluble PACs which are bound to fiber material, pomace or proteins remain undetected. As a result, the PAC content of products derived from cranberry press cake, where a considerable amount of PACs are bound to insoluble matrix constituents, may be underestimated (Brendler and Gafner 2017). Additionally, another limitation of the DMAC assay relates to the choice of the calibration standard. Procyanidin A2 is used as a reference standard for calibration. As cranberry products contain a heterogenous mixtures of PACs with

varying degrees of polymerization, the use of procyanidin A2 reduces accuracy with respect to absolute PAC quantification. For this reason, DMAC-derived PAC concentrations should be interpreted as relative rather than absolute values (Brendler and Gafner 2017).

Cranberry-based food supplements are widely marketed for urinary tract health, although their regulatory classification as food supplements rather than herbal medicinal products results in less stringent quality requirements. Substantial quality deficiencies have been detected in the present study. One product failed to meet legal labeling obligations due to inconsistencies between packaging and leaflet information. Testing of uniformity of mass revealed deviations exceeding governmental limits for one test sample, which shows insufficient manufacturing protocols. Identity testing by HPTLC seems to be a highly efficient way to have a close insight into the overall composition of the extracts and HPLC can specifically analyze the anthocyanin pattern as complex fingerprint authentication demonstrated a variability in anthocyanin profile. These findings underscore the lack of standardized raw materials and show again the critical gap between the pharmaceutical-like presentation of food supplements and their actual analytical and regulatory quality. In contrast to medicinal products, food supplements are not subject to the strict requirements and legislation of drug legislation, for this the national governmental institutions strictly controls manufacturers on the different levels of raw materials, products, production technology, quality control, quality assurance, pharmacovigilance and organizational structures. Food supplements are regulated by the national food laws, which shift responsibility for the marketed products towards the manufacturer (European Union 2025). For food supplements registration only online notification of the governmental institution before placing the product on the market is required; no assessment, testing or further authorization is carried out. As a result, a non-transparent and low-quality market for food supplements is obvious, especially for botanicals. While vitamins and minerals substances are listed and regulated in Directive 2002/46 (European Parliament and the Council 2002), the botanicals are still on hold and therefore unregulated. Although the European Food and Safety Agency (EFSA) is responsible for the evaluation of health claims, food supplements are frequently advertised and marketed with health claims in practice. This makes it even more challenging for consumers to differentiate between medicinal products and food supplements. Previous studies have already demonstrated that the quality of botanicals is unacceptable (Gaspar et al. 2021; Brouns et al. 2025). This assumption was confirmed by the study presented here, which also highlights the urgent need for harmonized quality standards, transparent labeling and improved regulatory standards to ensure consumer safety.

Abbreviations

CDE

cranberry dry extract

Cy3Gal

Cyanidin-3-*O*-galactoside

Cy3Glc

Cyanidin-3-*O*-glucoside

EMA

European Medicines Agency

HPLC

High pressure liquid chromatography

HPTLC

High performance thin layer chromatography

PAC

proanthocyanidin

Pn3Gal

paeonidin-3-*O*-galactoside

Ph. Eur.

European Pharmacopoeia
TLC
thin layer chromatography
UPEC
uropathogenic *Escherichia coli*
UTI
uncomplicated urinary tract infections.

Declarations

Author Contribution

AB: data curation, formal analysis, investigation, methodology, writing; ML review and editing, supervision; AH: conceptualization, funding, writing. All authors reviewed the manuscript

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

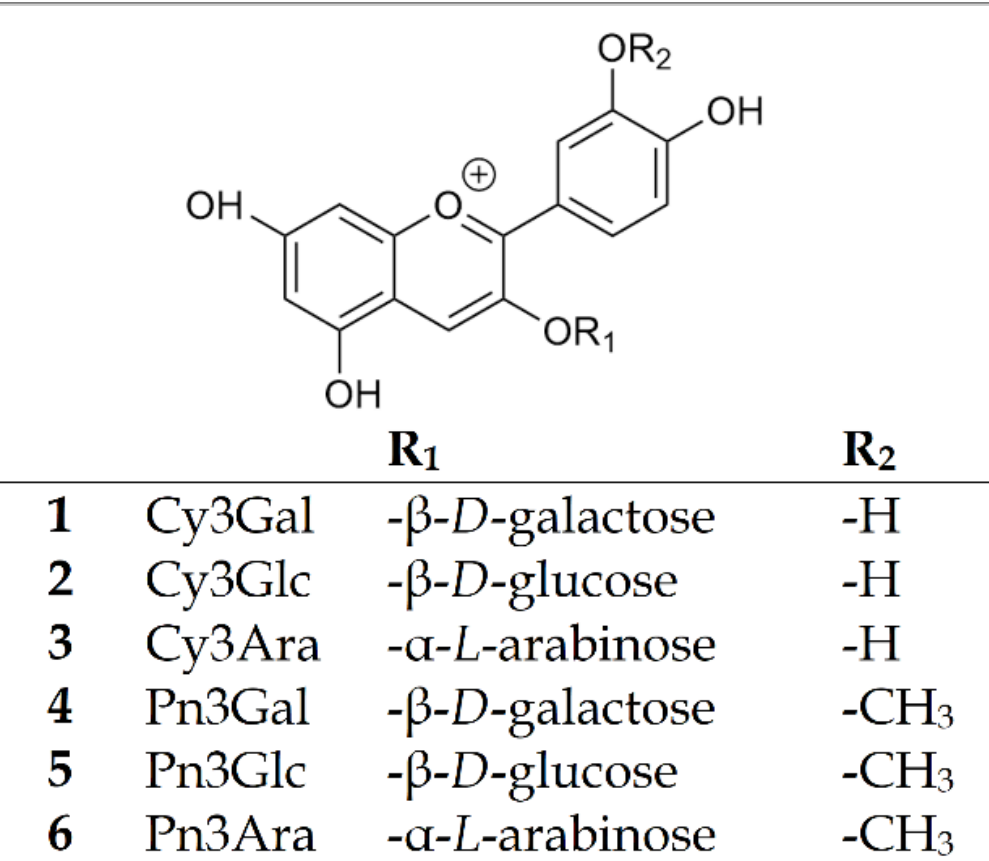


Figure 1

Structural features of anthocyanins **1** to **6** to be used identification of cranberry fruits from *Vaccinium macrocarpon*. Cy3Gal/Glc/Ara: Cyanidin-3-*O*-galactoside/glucoside/arabinoside; Peo3Gal/Glc/Ara: paeonidin-3-*O*-galactoside/glucoside/arabinoside.

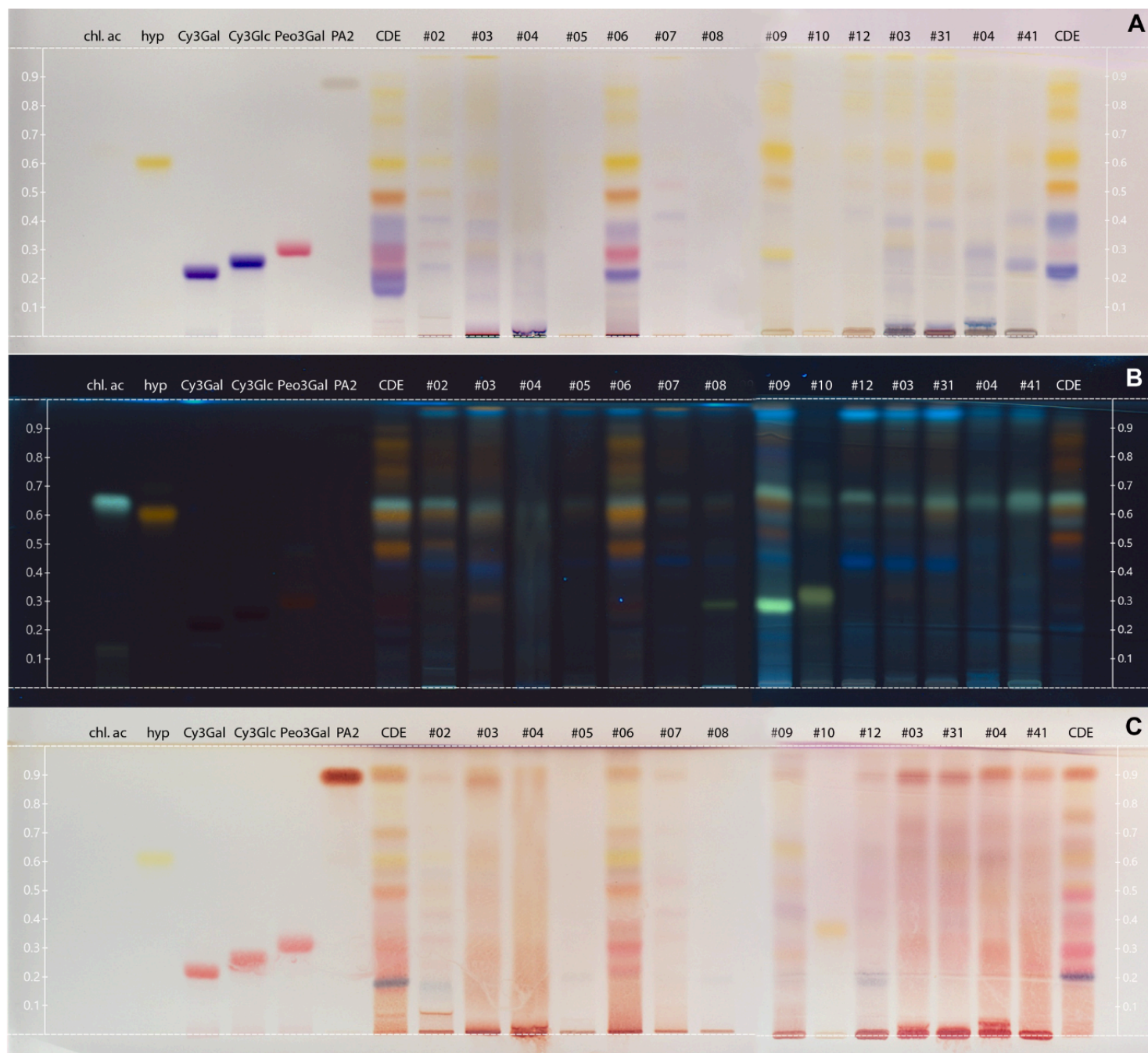


Figure 2

Structural features of anthocyanins **1** to **6** to be used identification of cranberry fruits from *Vaccinium macrocarpon*. Cy3Gal/Glc/Ara: Cyanidin-3-*O*-galactoside/glucoside/arabinoside; Peo3Gal/Glc/Ara: paeonidin-3-*O*-galactoside/glucoside/arabinoside.

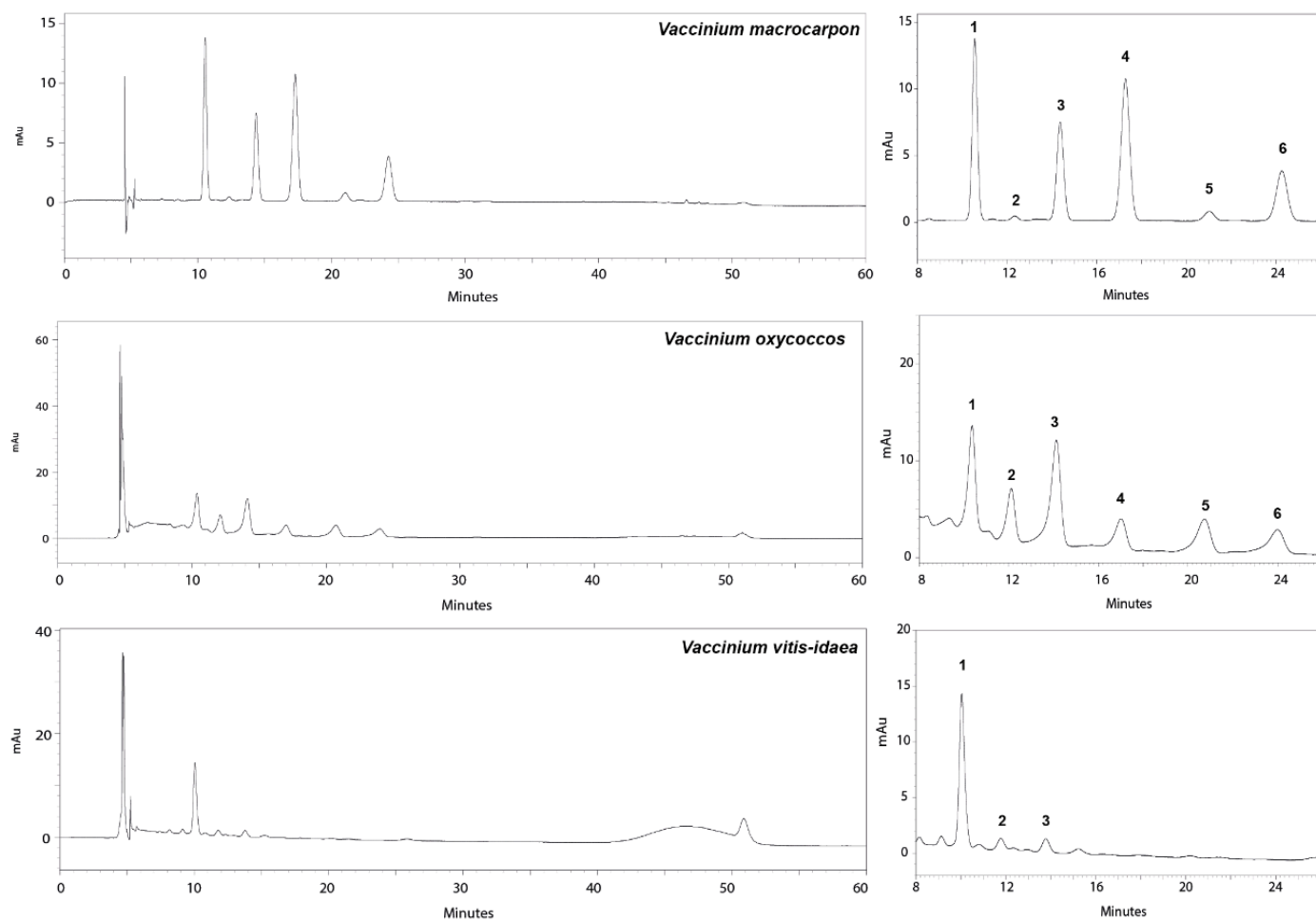


Figure 3

HPLC fingerprinting of anthocyanins from different *Vaccinium* species: Cranberries from *Vaccinium macrocarpon*, fruits from *V. oxycoccus*, and lingonberries from *V. vitis-idaea*. Left side: overview chromatograms, right side: enlargement of the relevant elution time of anthocyanins **1** to **6** between tR 8 to 24 min.

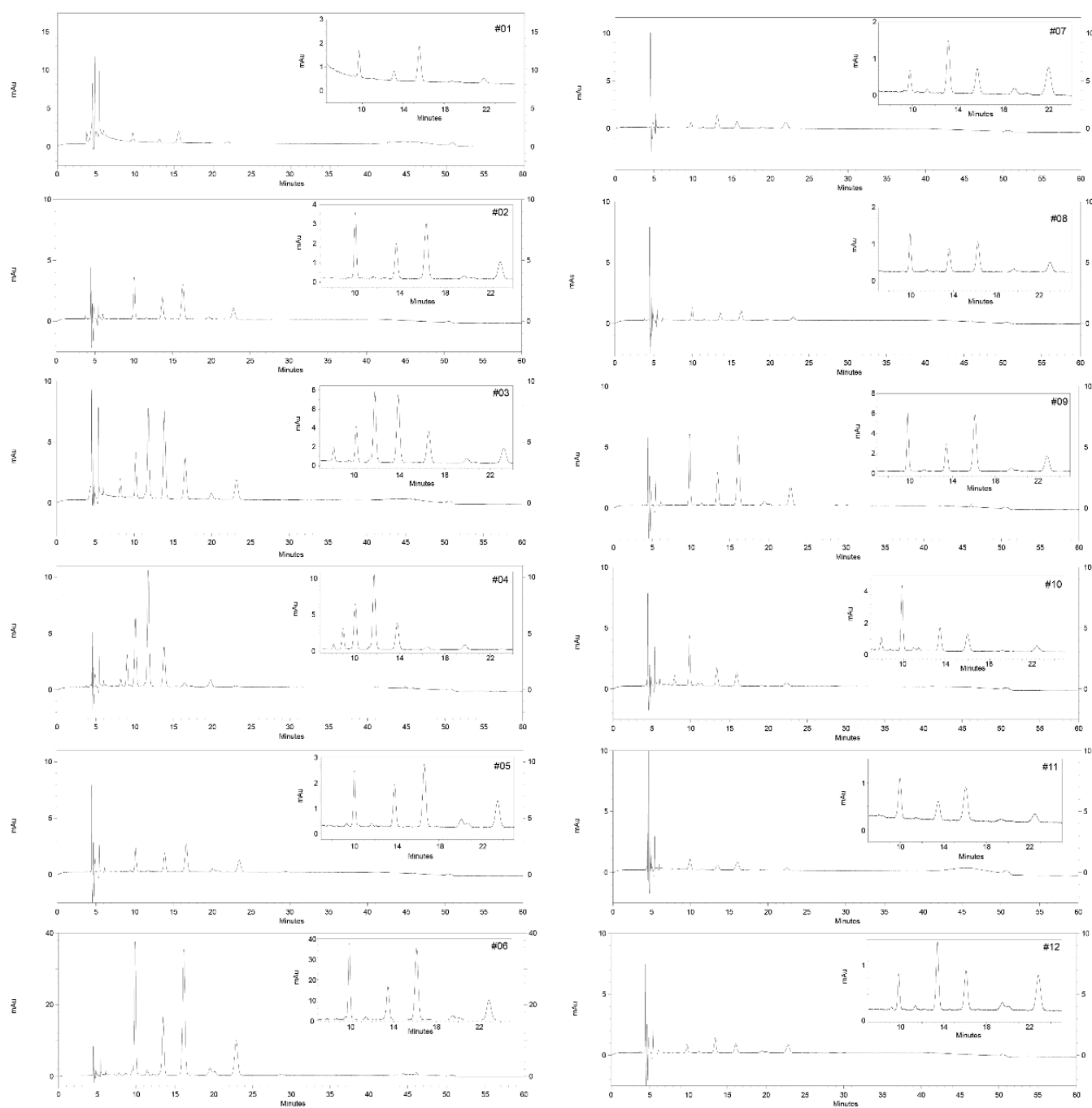


Figure 4

HPLC fingerprinting of the anthocyanin pattern of test samples #01 to #12. Test samples #03 and #04 do not conform to the declaration of the products.

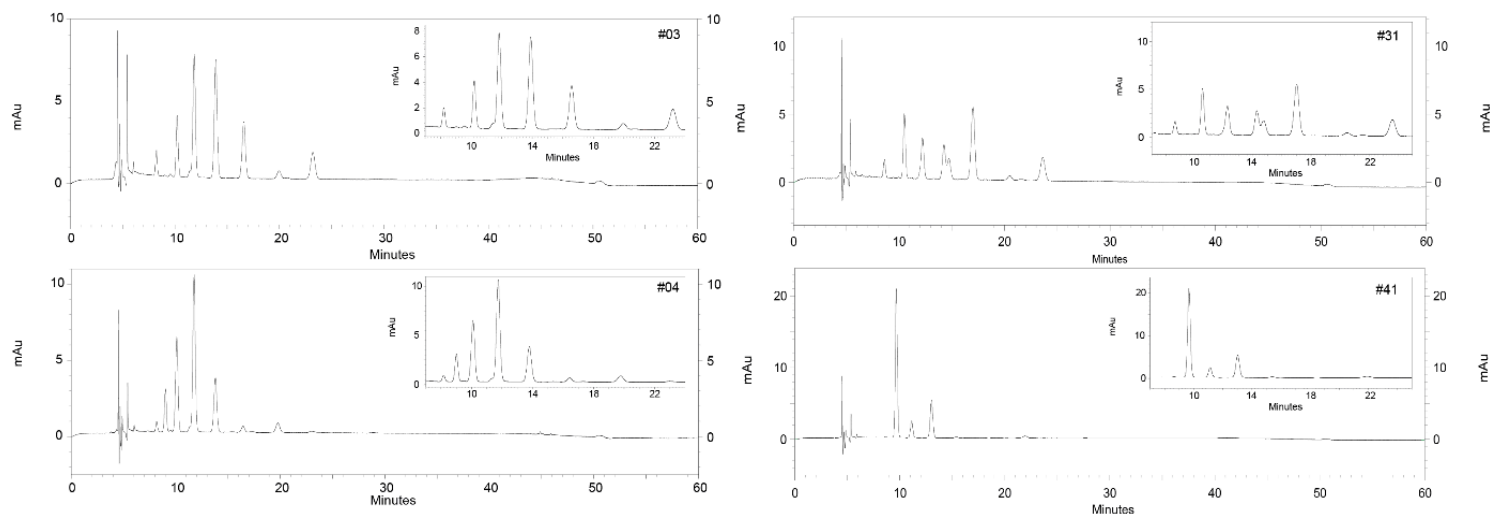


Figure 5

HPLC fingerprinting of anthocyanins pattern from two different batches of the same test samples #03 (03 and #31) and #04 (#04 and #41) obtained from the same supplier at different times. Data indicate different anthocyanin patterns from batch to batch, and identity of extract from cranberry fruit is not given.

Supplementary Files

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