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Development and validation of a high-performance liquid chromatographic method for quantification of anthocyanidins.

Maria Rosana Ramirez[1(PG)*], Ana Lucia Aboy[1(TC)], Amélia Teresinha Henriques[1(PQ)], Maria Do Carmo bassols Raseira[2(PQ)].

mariarosanar@yahoo.com.br

[1] *Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752, CEP 90.610-000, Porto Alegre (RS).*
[2] *Estação EMBRAPA/ CLIMA TEMPERADO, Pelotas, RS, Brazil.*

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Introdução

In fruits and plants, anthocyanidins occur as glycosylated forms, anthocyanins, and the complex anthocyanidins glycoside pattern can be reduced to six major anthocyanidins by acid hydrolisis, these common aglycon forms, are cyanidin, delphinidin, peonidin, petunidin, malvidin, and pelargonidin. There are many papers on analysis of anthocyanins in fruit and vegetables but, only a few papers reported the quantification of anthocyanidins aglycones[1]. In the present study, we developed and acid hydrolisis HPLC method for quantification of individual anthocyanidins in *Vaccinium* extract. Fruit were produced by EMBRAPA, Pelotas, RS. *Acid hydrolysis of anthocyanins:* The pigments were hydrolized for 1 h at 100 °C, in water/methanol solution containing 2 N HCL. Samples were immediately cooled to room temperature for HPLC analysis.

Identification and quantification of each compound was based on retention time and UV spectra in HPLC-DAD (Waters 2690), by comparison with pure commercial standards of known concentrations, using a Symmetry C_{18} reverse-phase column (Waters). Gradient of mobile phase (A) water 0.1% TFA, (B) acetonitrile 0.1% TFA with a flow rate of 0.7 mL/min, column temperature, 26º C. Linear gradient, initial percentage of B (15%) to 50 minutes (100%); column temperature, 26 C; 10 µL injection. Ultraviolet visible absorption of anthocyanidins were registred at 520 nm.

The method being examined for specificity, linearity, accuracy, precision, LOD and LOQ[7-2]. For specificity validation, a volume of 10 µl of standard, sample.

Resultados e Discussão

This HPLC method has been validated revealing good specificity for the analysis of the cyanidin contained in berries. As demonstrated by the results shown in Fig. 1 a, b good separation effect for five anthocyanidins (delphinidin, cyanidin, peonidin, petunidin, and malvidin) from *Vaccinium* was obtained with our methodology.

The linear equation and the determination coefficient (r^2) were respectively: Y = 40000000 + 769058, 0.9981. The result shows that an excellent correlation existed between the peak area and concentration of the analyte. The results of the LOD and LOQ analyses for the cyanidin ranged from 0.026 to 0.079 µg/ml, respectively, indicating that the analytical method for the quantification of the compound exhibited good sensitivity. Good related recovery rates for cyanidin were obtained: 93.69, 100.01, 101.70 ± 0.62%.The results of precision indicated that the analytical method for quantification of the cyanidin revealed good precision Intra-day precision (n=3) ± RSD% 2.87 ± 1.51. Inter-day precision ± RSD% 2.86 ± 1.35; Repetibility ± RSD% 2.94 ± 1.58 (n=6).

Analysis of berries samples:The peak area was determined for each condition and the method showed to be robust. After acid hydrolysis fefteen anthocyanins contained in blueberry extract became 5 anthocyanidin peaks (fig. 1), they were delphinidin, cyanidin, petunidin, peonidin, and malvidin.

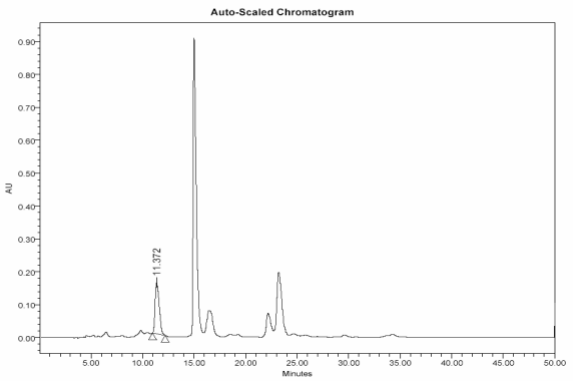
Conclusões

Identities of major anthocyanidins were confirmed. Acid hydrolisis greatly simplifies the anthocyanidin separation. Such a technique seems convenient, which is very applicable for the simultaneous accurate quantification of anthocyanidins found in various fruits and vegetables.

Agradecimentos

CNPq, PROPESQ-UFRGS and FAPERGS.

Figure 1. HPLC cromatograms of *Vaccinium* sample



Typical HPLC cromatograms of blueberry sample showing delphinidin (11 min), cyanidin (co-injected with the standard), petunidin (16,5 min), peonidin (22 min) and malvidin (23.5 min).

[1] Zhang, Z.; Kou, X.; Fugal, K.; Mc Laughlin J. J. *Agric. Food Chem*. **2004**, *52*, 688.
[2] Guide for Validation of Analytical and Bioanalytical Methods, **2003**. Resolution RE no. 899, *Brazilian Sanitary Surveillance Agency*, Brasilia, DF, Brazil.