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HPLC DETECTION OF ANTITHROMBITIC CALIX[4]ARENE IN BLOOD PLASMA OF ANIMALS

V. A. Didkivskyi[1], *A. O. Pavlenko*[1, 3], *R. Y. Marunych*[1], *A. O. Selikhova*[2], *S. O Cherenok*[2], *T. P. Todosiuk*[4], *S. M. Shevchenko*[4]

[1]Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv
[2]Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Kyiv
[3]ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv,
[4]Bila Tserkva National Agrarian University, Bila Tserkva

E-mail: anastasiia.pavlenko2000@gmail.com

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Previously sodium salt of 5,11,17,23-bis (dihydroxyphosphoryl) methylcalix[4]arene (C-145) was shown to be promising antithrombotic agent and was successfully tested *in vivo* [1, 2].

Aim. This work was focused on the development of the method for the direct detection of this substance in blood plasma and estimation of pharmacokinetics of this compound.

Methods. Wistar rats and outbreed rabbits were kept in the vivarium of Bila Tserkva National Agrarian University on a standard diet. C-145 was injected into the rat's lateral tail vein and into rabbit's marginal vein of the ear (12 mg/kg) or was administrated per-oral. The anticoagulant effects of C-145 in blood plasma were confirmed by activated partial thromboplastin time (APTT) test. HPLC was performed using Agilent 1100 series (Agilent, USA) on the phase cyano ZorbaxCN Column which parameters were L×I.D. 25 cm×4.6 mm.

Results. The maximal antithrombotic effect after the intravenous or per-oral administration of C-145 was observed after 4–6 hours. In particular clotting time in APTT-test in these blood plasma samples was prolonged trice and more (120 s against 46 s in control). Normalization of blood clotting was achieved after 24 hours after the injection.

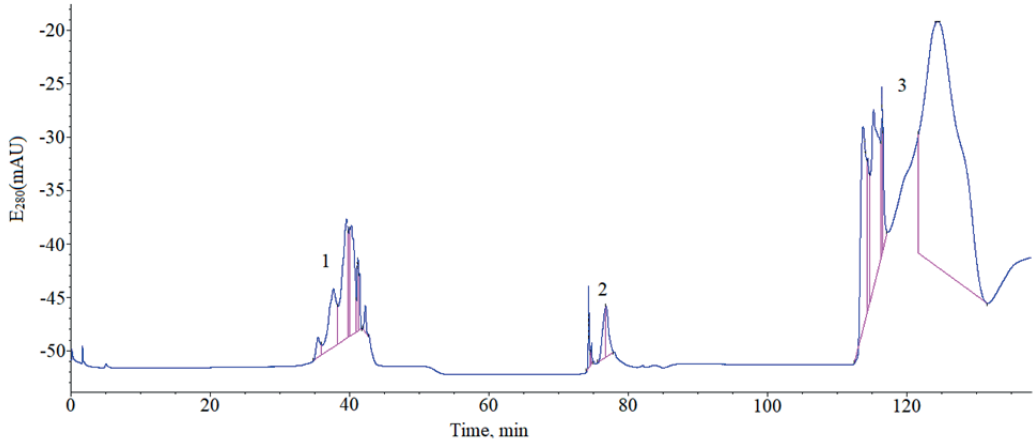

Fig. **HPLC chromatography of detection of calix[4]arene C-145 in rabbit blood plasma after 2 hours of intravenous injection with the use of combined linear gradient**
(acetonitrile 1 % → 100 % — from 30 till 40 minutes, and citrate buffer (0.1 M, pH 6.0) 0 % → 100 % — from 110 till 120 minutes using nitrile column Zorbax CN 25 cm, 4.6 mm, speed of the flow 1 ml/min, t = + 40 °C, UV detection at 280 nm. Fraction 3 — zone of calix[4]arene C-145 elution.

To develop a method for direct C-145 detection in blood plasma we selected samples with maximal prolongation of clotting time. For accurate analysis of blood plasma samples proteins were saturated by 10% trichloroacetic acid. After neutralization by $NaHCO_3$ samples were prepared using 12-port vacuum unit for solid-phase extraction (Agilent, USA) with a Bond-Elut C18 cartridge. The samples that contained C-145 were eluted by 100% methanol for the HPLC analysis performed on the phase cyano ZorbaxCN Column equilibrated with an acetonitrile solution (ddH_2O:AcCN 99:1). Elution was performed using a combined gradient of acetonitrile (100%) and citrate buffer (0.1 M, pH 6.0). The elution zone of C-145 was detected on the 128^{th} minute at 280 nm.

Discussion. C-145 was detected in blood plasma samples of animals after intravenous or per-oral administration of C-145 (Figure). Control blood plasma did not contain the peak of C-145, but it was detected clearly in the buffer solution of C-145 prepared *in vitro*. The presence of C-145 in blood plasma was correlated to the clotting time of the sample.

Conclusion. Application of the developed methods allowed us to confirm the direct antithrombotic effect of calix[4]arene C-145 on blood of experimental animals during intravenous administration. Also HPLC technique enabled to detect this substance in blood plasma and most likely could be applied for other biological solutions and could be modified for the quantitative analysis in the pharmacokinetic studies as well.

Key words: calix[4]arene, blood plasma, thrombosis, fibrinogen, HPLC.

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REFERENCES

1. *Komisarenko, S.V., Kosterin, S.O., Lugovskoy, E.V., Kalchenko, V.I.* Calixarene methylene bisphosphonic acids as promising effectors of biochemical processes. ***Український біохімічний журнал***, 2013, 85 (6), 106–128. http://dx.doi.org/10.15407/ubj85.06.106
2. *Rodik, R. V., Boyko, V. I., Kalchenko, V. I.* Calixarenes in bio-medical researches. *Current Medicinal Chemistry*, 2009, 16 (13), 1630–1655. https://doi.org/10.2174/092986709788186219