*Chem. Anal. (Warsaw),* **48**, 621 (2003) **SHORT COMMUNICATION**

# HPLC Analysis of Plasminogen and Tissue Plasminogen Activator⊕

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**Key words:** plasminogen, tissue plasminogen activator, HPLC

A chromatographic system for the simultaneous determination of plasminogen and tissue plasminogen activator has been developed. Plasminogen and tissue plasminogen activator could be determined qualitatively and quantitatively within 30 min applying HPLC ($C_{18}$ 5 μm, 250 × 4 mm column) with DAD detector, and gradient elution with the mobile phase comprising 0.1% trifluoroacetic acid (TFA) in water and 0.085% TFA in acetonitrile, at the flow rate of 0.8 mL $min^{-1}$ or 1.0 mL $min^{-1}$. The following retention times for plasminogen and tissue plasminogen activator in human plasma were found: 23.31 min and 23.95 min, respectively.

Opracowano chromatograficzną metodę jednoczesnego oznaczania plazminogenu i tkankowego aktywatora plazminogenu (t-PA). Zastosowanie HPLC z detektorem DAD, kolumną $C_{18}$ (5 μm), 250 × 4 mm, gradientowym przepływem fazy ruchomej, będącej mieszaniną 0.1% kwasu trifluorooctowego (TFA) w wodzie i 0.085% TFA w acetonitrylu, przy natężeniu przepływu 0.8 mL $min^{-1}$ lub 1.0 mL $min^{-1}$, pozwala na jakościowe i ilościowe oznaczenie plazminogenu i t-PA w czasie 30 min. Czasy retencji plazminogenu i tkankowego aktywatora plazminogenu w osoczu ludzkim wynosiły odpowiednio: 23.31 min i 23.95 min.

⊕ Dedicated to the memory of Professor M.S. Tsvett on the occasion of 100th anniversary of the discovery of chromatography

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Plasminogen and tissue plasminogen activator (t-PA) are known to be blood coagulation indicators. They take part in the fibrinolysis process, which regulates clot thawing and protects from thrombosis.

Clot formation is the result of a complex interaction between humoral coagulation factors, platelets and fibrin. Coagulation inhibitors and the fibrinolytic system should counter-balance coagulation and protect the organism from the widespread clot formation. The application of fibrinolytic agents like streptokinase or t-PA to the treatment of pulmonary embolism, coronary heart disease or sepsis significantly improved the recovery process. On the other hand, since unwanted bleeding is the most worrisome potential adverse effect, a substantial number of unsuccessful treatments was due to the lack of control over the fibrinolysis processs. By now most of the treatment regimes have been based on the experimental dosage schemes. The need for the accurate and reliable laboratory monitoring of fibrinolysis during the therapy of the above-mentioned diseases is clearly recognised by doctors these days.

The improvement of the monitoring of fibrynolysis process is accessible to the present-day clinical diagnostics and mostly concerns immunoenzymatic methods [1–4].

Chromatographic methods have been applied either for the analysis of the structure and biochemical transformation of plasminogen [5,6] or tissue plasminogen activator [7–14].

The aim of this work was to design the chromatographic system for the simultaneous determination of plasminogen and t-PA, and to apply it for the real sample (plasma) analysis. Such analysis allows one to monitor the concentration level of the studied compounds as well as the transformation of plasminogen under the influence of tissue plasminogen activator. In addition, the method can be advantageous in controlling the therapeutic level of t-PA (clot thawing).

## EXPERIMENTAL

### HPLC

A Merck–Hitachi L4500A chromatograph was equipped with $RP_{18}$, 5 μm, 250 × 4.6 mm column (J.T. Baker Inc. Phillipsburg, NJ, USA) and diode-array detector (DAD) was used. Experimental data were obtained from the computer integrator with 0.01-min accuracy for the retention time and 0.0001 for the absorbance. 0.1% TFA aqueous solution and 0.085% TFA in acetonitrile were used as the components of the mobile phase of the flow rates of 0.8 mL $min^{-1}$ and 1 mL $min^{-1}$ and the temperature 40°C.

0.1 mg $mL^{-1}$ standard stock solutions of t-PA (Sigma–Aldrich) and plasminogen (Sigma–Aldrich) in 0.1% TFA were prepared. t-PA and plasminogen were loaded onto the chromatographic column in the amount ranging between 0.05 to 5 μg. The spectrophotometric detection was performed at the wavelenght 226 nm.

The analysis of blank plasma samples and plasma samples spiked with the standards of plasminogen and tissue plasminogen activator was carried out.

## RESULTS

At the first stage of the investigation the stability of standard solutions was studied. 0.1 mg $mL^{-1}$ standard solutions of plasminogen and t-PA in 0.1% TFA was stored at +4°C and found to be stable for several weeks. Yet, when TFA was replaced with pure water, the concentration of the studied compounds decreased within two days probably owing to the precipitation, and the peaks of the respective decomposition products were not observed in the chromatogram.

Various chromatographic conditions (columns, mobile phases, gradient elution, flow rates and temperatures) of the separation of plasminogen and t-PA were investigated. The following optimum conditions for the chromatographic separation of the analysed compounds were finally established: column – 250 × 4 mm, $C_{18}$ (5 μm), mobile phase – 0.1% trifluoroacetic acid (TFA) aqueous solution (TFA) (solvent A) and 0.085% TFA in acetonitrile (solvent B), linear gradient elution in the range from 0% to 60% B, analysis time – 30 min, the mobile phase flow rate – 0.8 mL $min^{-1}$ or 1.0 mL $min^{-1}$. The similar gradient elution for the determination of pure t-PA on microbore $C_{18}$ column was used by Mock [11].

The following retention times were found for plasminogen and t-PA at the flow rate of 1 mL $min^{-1}$ $t_R$ = 25.61 and $t_R$ = 26.22 min, respectively (Fig.1.), and at the flow rate of 0.8 mL $min^{-1}$ $t_R$ = 26.84 min and $t_R$ = 27.61, respectively (Fig.2.).

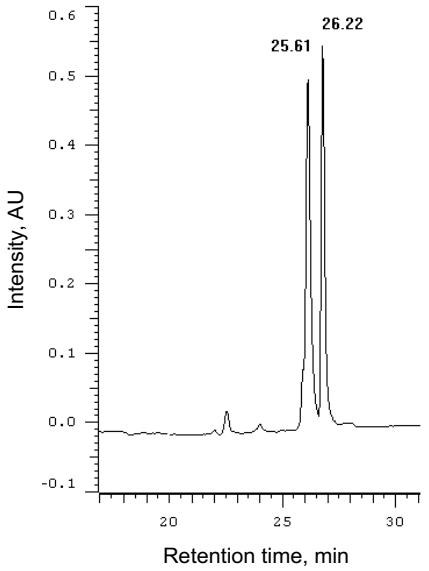


**Figure 1.** Chromatogram of plasminogen ($t_R$ = 25.61 min) and t-PA ($t_R$ = 26.22 min). Experimental conditions: $RP_{18}$ column: 5 μm, flow rate: 1 mL $min^{-1}$

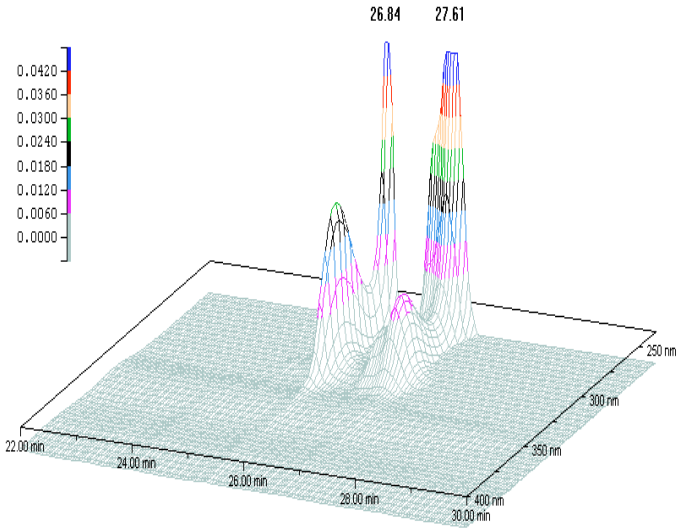


**Figure 2.** Chromatogram of plasminogen ($t_R$ = 26.84 min) and t-PA ($t_R$ = 27.61 min). Experimental conditions: $RP_{18}$ column: 5 µm, flow rate: 0.8 mL $min^{-1}$.

The equations of the calibration plots of the standard solutions obtained with the Merck–Hitachi HPLC software were as follows: y = 13.57(±0.41)x + 0.94 (±0.82), R = 0.9980 for plasminogen, and y = 13.77(±0.31)x + 0.43 (±0.38), R = 0.9982 for t-PA. According to the calibration plots for standards the mass ranged between 0.15–4.00 µg for plasminogen and 0.08–1.50 µg for t-PA. The detection and quantitation limits (LOD and LOQ) were respectively 0.10 µg and 0.13 µg for plasminogen, and 0.05 µg and 0.07 µg for t-PA. Next, the analysis of blank plasma samples and plasma samples spiked with plasminogen and t-PA standards was performed and the following retention times were found for the latter at the flow rate of 1 mL $min^{-1}$: $t_R$ = 23.31 min for plasminogen, and $t_R$ = 23.95 min for t-PA. Figure 3 exemplifies the chromatogram of the plasma sample spiked with standards. The analysis of plasminogen and t-PA in plasma was performed at their concentration exceeding the physiological levels due to the matrix interferences. Thus, at the present stage of research, one may investigate plasminogen transformation in the presence of t-PA, or monitor high levels of t-PA in cardiological therapy. The LOD and LOQ values in plasma were respectively: 42.35 µg and 51.25 µg for plasminogen, and 24.45 µg and 35.25 µg for t-PA. In order to apply the system to the blood analysis for diagnosis and therapy, further research concerning analyte enrichment and plasma matrix removal will be carried out.

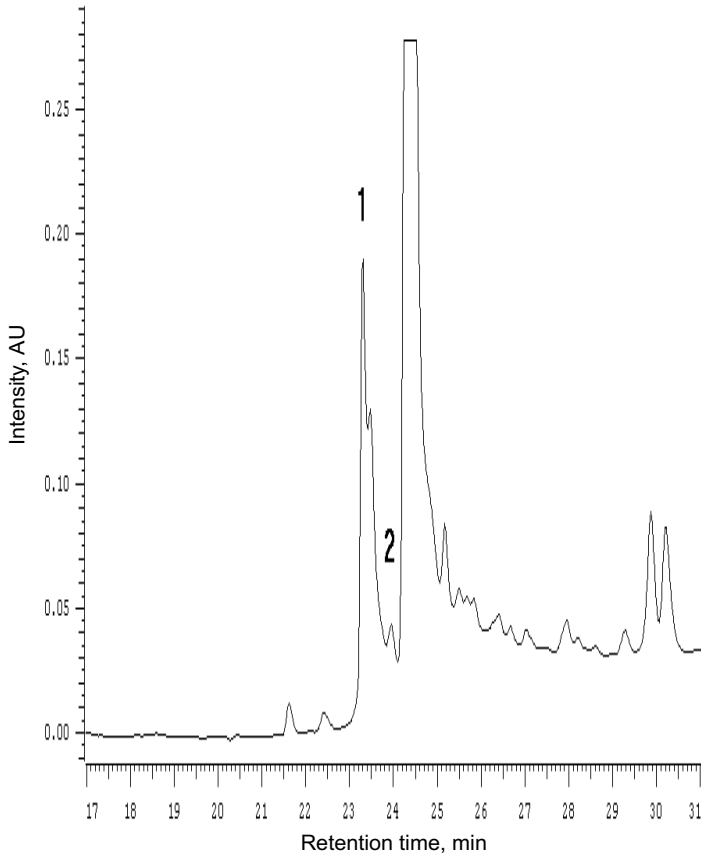


**Figure 3.** Chromatogram of plasma sample spiked with plasminogen (1: $t_R$ = 23.31 min) and t-PA (2: $t_R$ = 23.95 min). Gradient elution at the flow rate of 1 mL $min^{-1}$

REFERENCES

1. Chmielewska J. and Wiman B., *Clin. Chem*., **32**, 482 (1986).
2. Matsuo O., Kato K., Matsuo C. and Matsuo T., *Anal. Biochem*., **135**, 58 (1983).
3. Pietrucha T. and Cierniewski C.S., *Post. Biochem*., **3**, 33 (1991).
4. Kuo B.-S. and Bjornsson T.D., *Anal. Biochem*., **209**, 70 (1993).
5. O'Reilly M.S., Holmgren L., Shing Y., Chen C., Rosenthal R.A., Moses M., Lane W.S., Cao Y., Sage E.H. and Folkman J., *Cell*, **79**, 315 (1994).
6. Wang H., Prorok M., Bretthauer R.K. and Castellino F.J., *Biochem.*, **36**, 8100 (1997).
7. Fleming C.M., Kowalski B.R., Apffel A. and Hancock W.S., *J. Chromatogr. A*, **849**, 71 (1999).
8. Egelund R., Rodenburg K.W., Andreasen P.A., Rasmussen M.S., Guldberg R.E. and Petersen T.E., *Biochem.*, **37**, 6375 (1998).

9. Upshall A., Kumar A.A., Bailey M.C., Parker M.D., Favreau M.A., Lewison K.P., Joseph M.L., Maraganore J.M. and McKnight G.L., *Bio/Technology*, **5**, 1301 (1987).
10. Shikata Y., Kuwada M., Hayashi Y., Hashimoto A., Koide A. and Asakawa N., *Anal. Chim. Acta*, **365**, 241 (1998)
11. Mock K., Hail M., Mylchreest I., Zhou J., Johnson K. and Jardine I., *J. Chromatogr*., **646**, 169 (1993).
12. Apffel A., Chakel J.A., Hancock W.S., Sauders C., Timkulu T.M. and Pungor Jr. E., *J. Chromatogr. A*., **750**, 35 (1996).
13. Taverna M., Baillet A., Werner R. and Bayloco-Ferrier D., *J. Chromatogr*., **558**, 105 (1991).
14. Wu S.L., *Anal. Biochem.*, **253**, 85 (1997).

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