A043 — Comparison of fibrinogen quantification by CIMac™ immunoaffinity chromatography and clotting activity measurement

In diagnostic applications, fibrinogen is measured with clotting activity assay variations, together with other blood clotting factors. In research and preparative applications, larger quantities of anti-coagulants, such as EDTA and citrate, or coagulants such as Ca^{2+} are present in the samples, preventing successful and/or accurate fibrinogen concentration measurements. In such cases an alternative measurement method is needed.

The **CIMac antibody immobilisation platform** provides an elegant solution for immobilisation of antibodies, which are used as immunoaffinity adsorbents. It has been shown that several analytical runs can be performed on a single column. This simplicity is also combined with a fast method, where a chromatographic analysis can be completed in 4 min.

METHOD

Column:	hydrazide-based CIMac™ column with immobilised monoclonal antifibrinogen
Load:	100µL of plasma sample, 10x diluted with PBS
Flow rate:	1.0 mL/min
Mobile phases:	Solvent A: phosphate-buffered saline (PBS)
	Solvent B: 0.1M glycine, pH 2.0
Gradient elution method:	Solvent A (1.5 min), solvent B (1.5min)
Wash:	Solvent A (2min)

> *Figure 1*

A typical chromatographic profile of human plasma on CIMac HDZ-antifibrinogen column. The purity of the fibrinogen in the elution was confirmed by SDS-PAGE analysis.
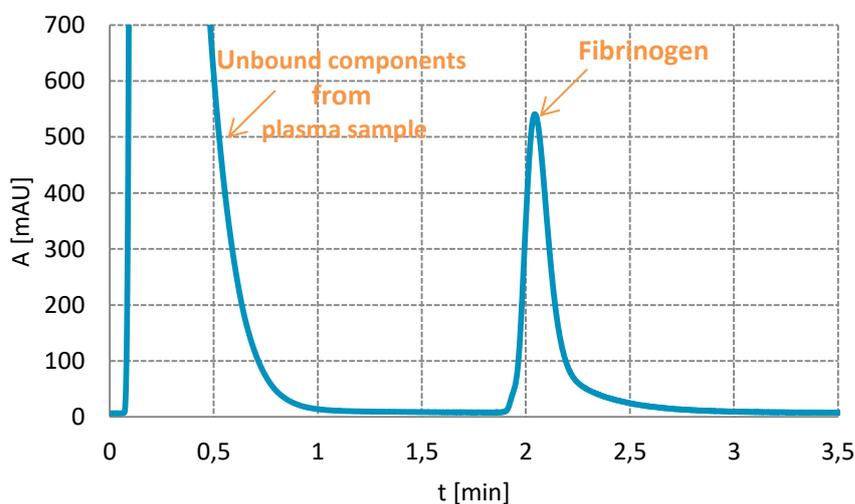
When measuring samples from frozen plasma, the high dynamic range of anti-fibrinogen column is highly beneficial. We obtained only one valid measurement in 3 sample dilutions with the clot method, but were able to measure additional two dilutions with the chromatographic method. An estimated limit of detection for the applied chromatographic method is 0.1 mg/ml, but it could be lowered even more by increasing the injection volume of the sample.

> *Figure 2*

Comparison of the chromatographic method and coagulometric determination for pure fibrinogen sample, dissolved in PBS. The quantification is based on the calibration curve with the calibrated plasma sample with known concentration of fibrinogen (not shown).

The comparison of the coagulometry and chromatographic analysis for a standard fibrinogen shows a good match between both methods. One of the advantages of the chromatographic analysis is the extension of linear range for at least one log. Additionally, no preparation of the sample for the chromatographic run is needed, whereas this is not the case for coagulometric measurement.
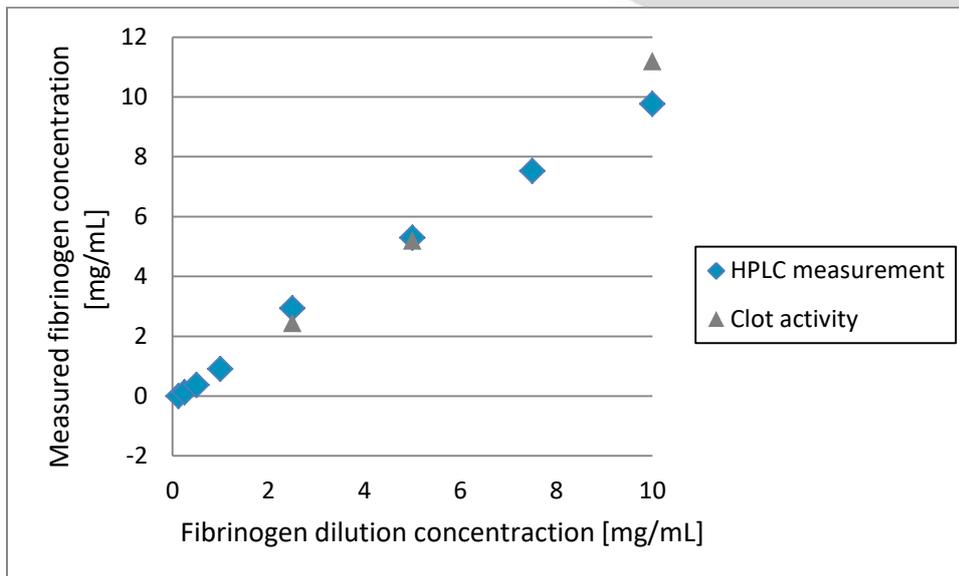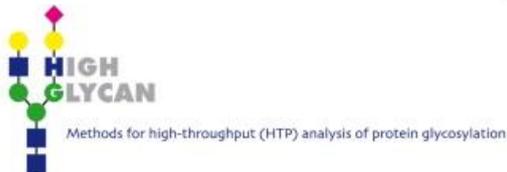

> *Table 1*

Comparison of fibrinogen determination in real plasma sample by two analytical methods

The results obtained for three dilutions of plasma using an HPLC method and that of clotting activity are comparable within the margin of experimental error.

	CIMac™ HDZ-antifibrinogen column	Coagulometer
	c [mg/L]	c [mg/L]
Plasma 10x diluted	2.71	Not Detected
Plasma 5x diluted	2.18	Not Detected
Plasma undiluted	2.16	2.14

CONCLUSION

A direct comparison of the two methods reveals that the affinity method offers a much wider concentration range, especially in the lower concentration range. Furthermore, the method is independent of the use of anticoagulants. Concentration results are comparable, clotting activity measurement offers a slight advantage in speed and automation, but requires a dedicated clotting activity measurement. If a suitable HPLC is already present in the system and an autosampler is available, chromatographic measurements can also be automated, and no additional costs are needed (dedicated cuvettes, expensive reagents).

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Related Products

Catalogue No.	Product description
311.8002-2	CIMmultus™ HDZ-1 Advanced Composite Column (Hydrazide) (Pores 2 µm)
102.8002-2	CIMmic HDZ-0.2 (Hydrazide) (Pores 2 µm)

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