

Planar chromatography and digital autoradiography analysis of radiolabelled metabolites from microdialysis fractions

M. Haaparanta-Solin¹, T. Grönroos¹, P. Marjamäki¹, O. Eskola¹, J. Bergman¹ and O. Solin^{1,2}



¹Turku PET Centre, Turku, Finland

**²Radiochemistry Laboratory, University of Helsinki
Finland**

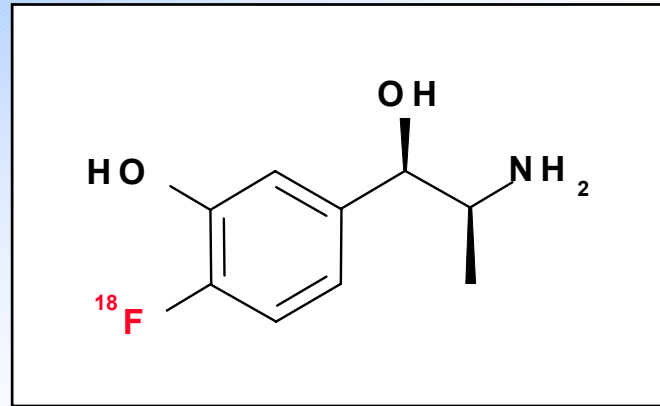
Background

Sampling and analysis of radiolabelled metabolites in blood *in vivo* from small animals is needed:

- **when developing new radiolabelled tracers**
- **for metabolite correction for input function with animal PET-camera (dynamic imaging)**

PET = Positron Emission Tomography

^{18}F ; $T_{1/2} = 109.8 \text{ min}$, $\beta^+_{\text{max}} = 650 \text{ keV}$



(1R,2S)-4-[^{18}F]fluoro-metaraminol

- **Specific radioactivity ~ 10 GBq/ μmol**
- **1 Bq corresponds to ~ 0.1 fmol**

Background cont.

By using microdialysis it is possible:

- to collect samples from blood with good time resolution**
- to analyse samples without sample handling i.e samples are ready for chromatographic analysis**

Background cont.

Analysis of microdialysis fractions is difficult because of:

- **low volume**
- **high specific radioactivity (i.e. low mass)**
- **low amounts of radioactivity**

Aim of the Study

Combination of

- **Microdialysis (MD)**
- **Planar chromatography**
- **Digital autoradiography with imaging plate**

as a method for measuring

- **blood radioactivity concentration**
- **radiolabelled metabolite formation**

***in vivo* as a function of time in small animals**

Methods

Microdialysis (MD)

- **The microdialysis probe operates as an artificial blood vessel**
- **Continuous blood sampling with high temporal resolution and without any sample handling**

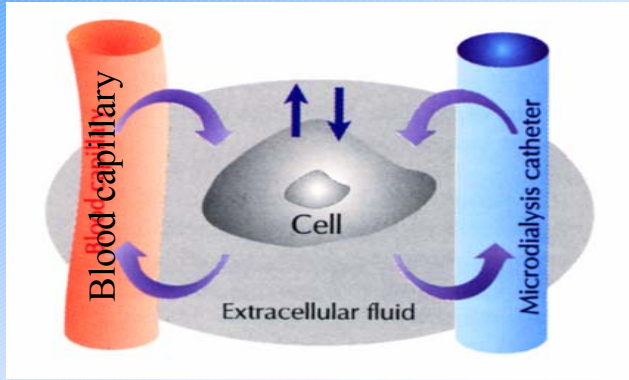
Planar chromatography

- **Combines instrumental and high performance TLC (HPTLC)**
- **All sample components are observed in the same chromatogram**

Digital Autoradiography (DAR) with imaging plate

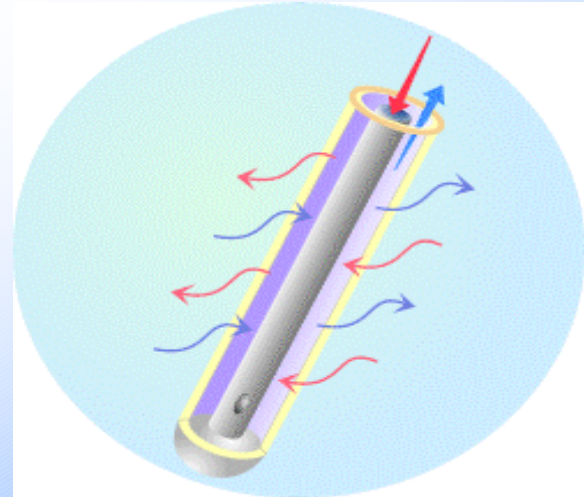
- **Sensitive, high resolution**
- **Wide dynamic range of linear response for beta-particles**

Principle of microdialysis

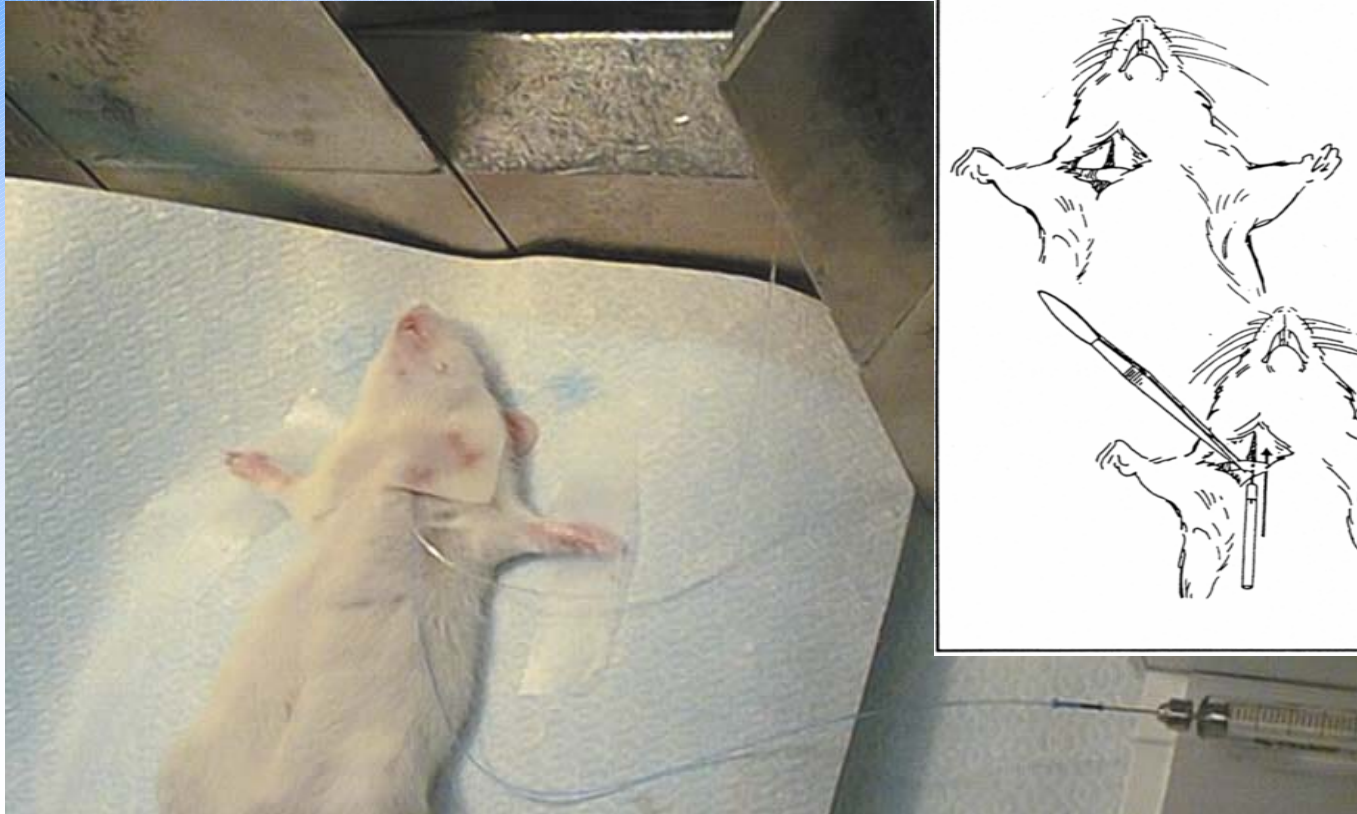


- on-line sampling in extracellular fluid *in vivo*
- semipermeable membrane (cutoff 30 kD)

- probe perfused with physiological buffer
- protein free sample



Microdialysis probe in vena jugularis

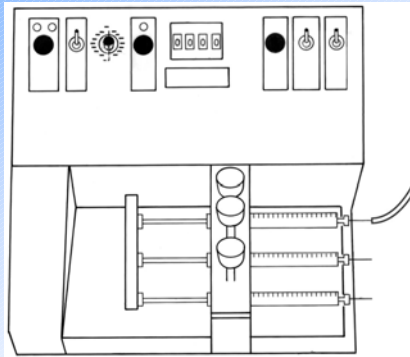


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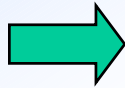
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Microdialysis

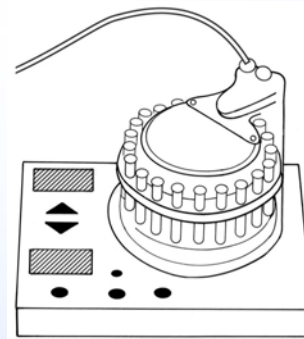
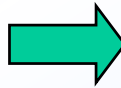
➤ **tracer injected in tail vein**



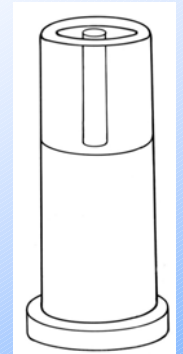
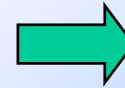
MD pump



Rat

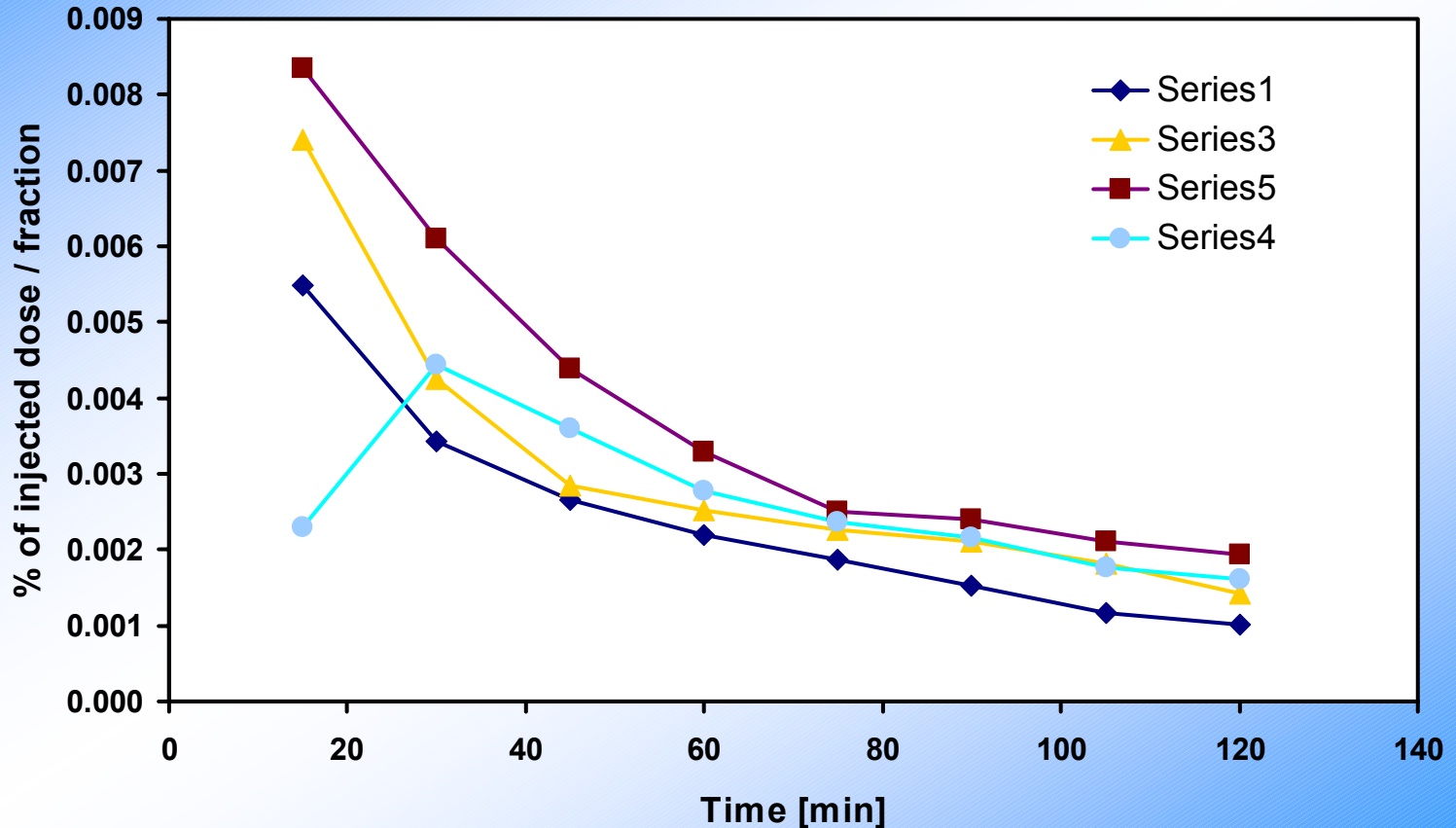


Fraction collector

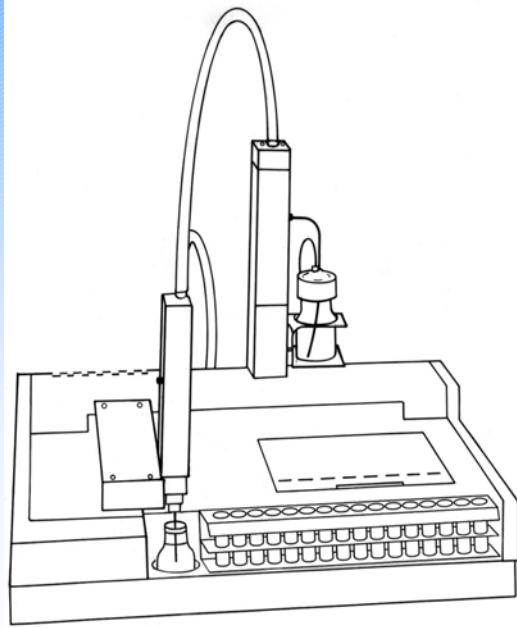


**Radioactivity
determination**

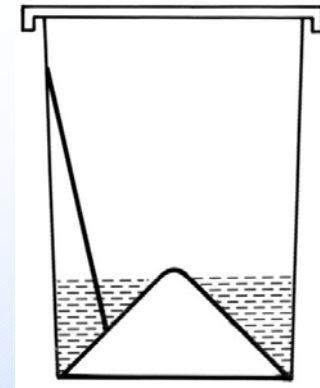
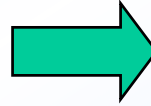
Time-activity curves of microdialysates



Planar chromatography

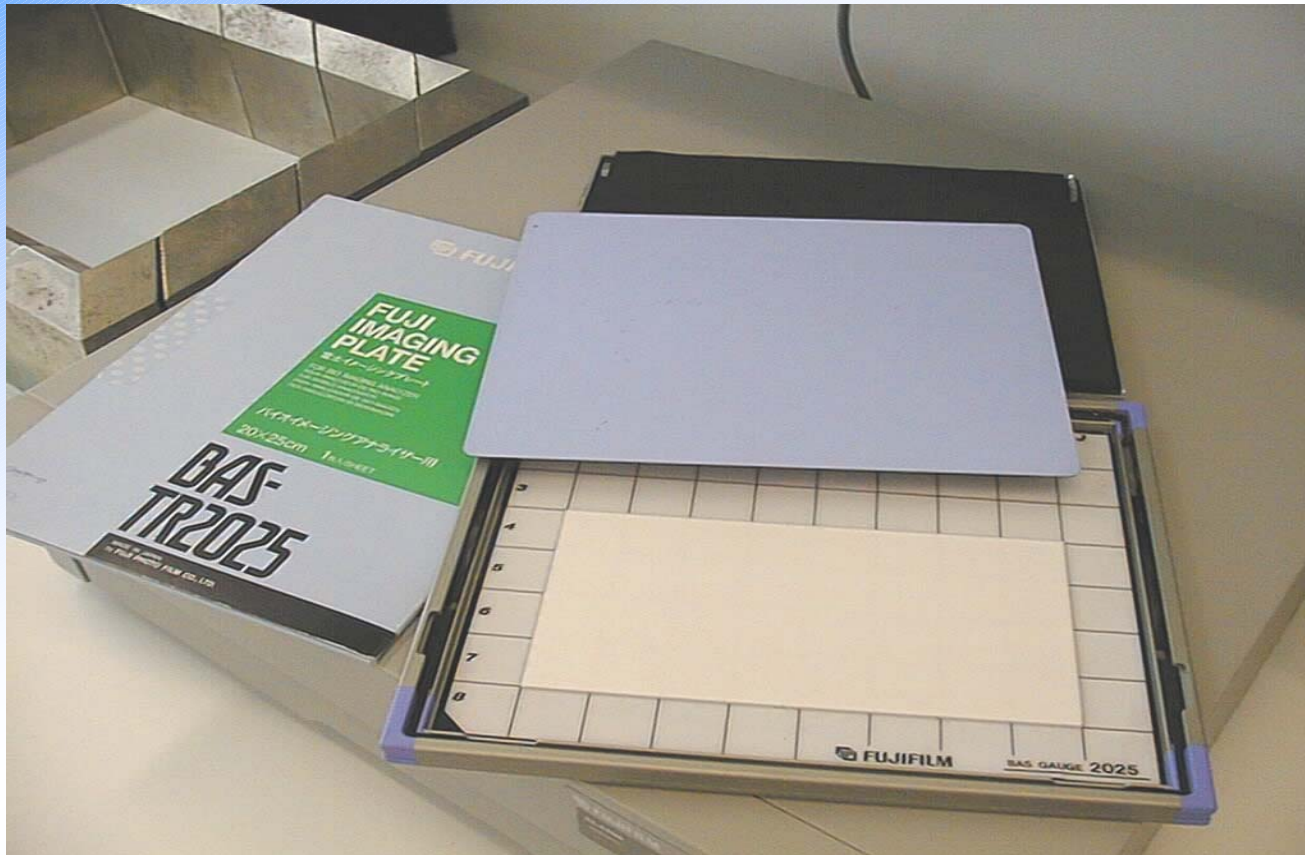


Sample application



TLC development

Imaging plate for DAR



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Imaging plate

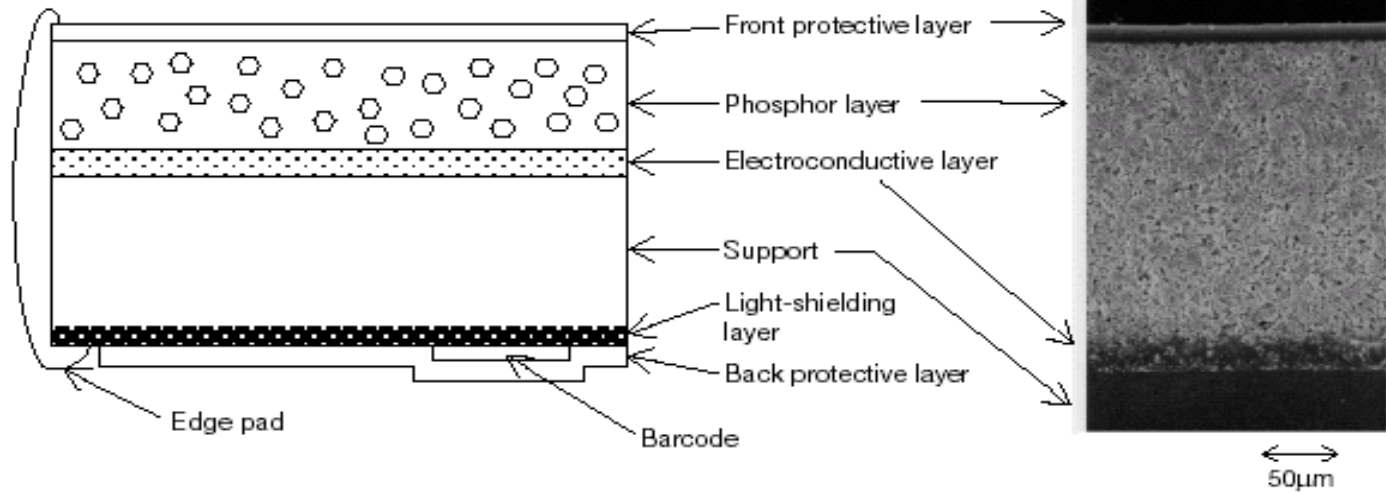
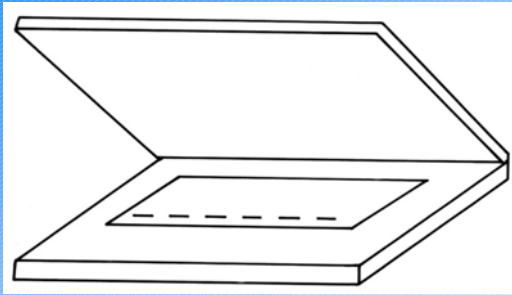


Figure 4 IP's typical cross-sectional diagram and a SEM photograph

Source: Fuji Home page

Digital autoradiography



PSL autoradiography

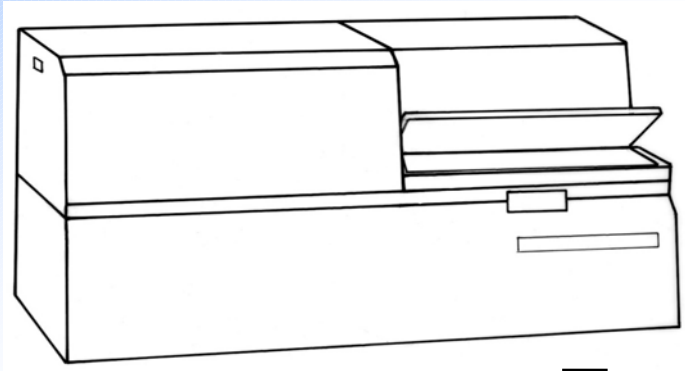
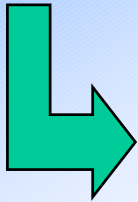
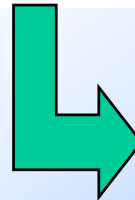
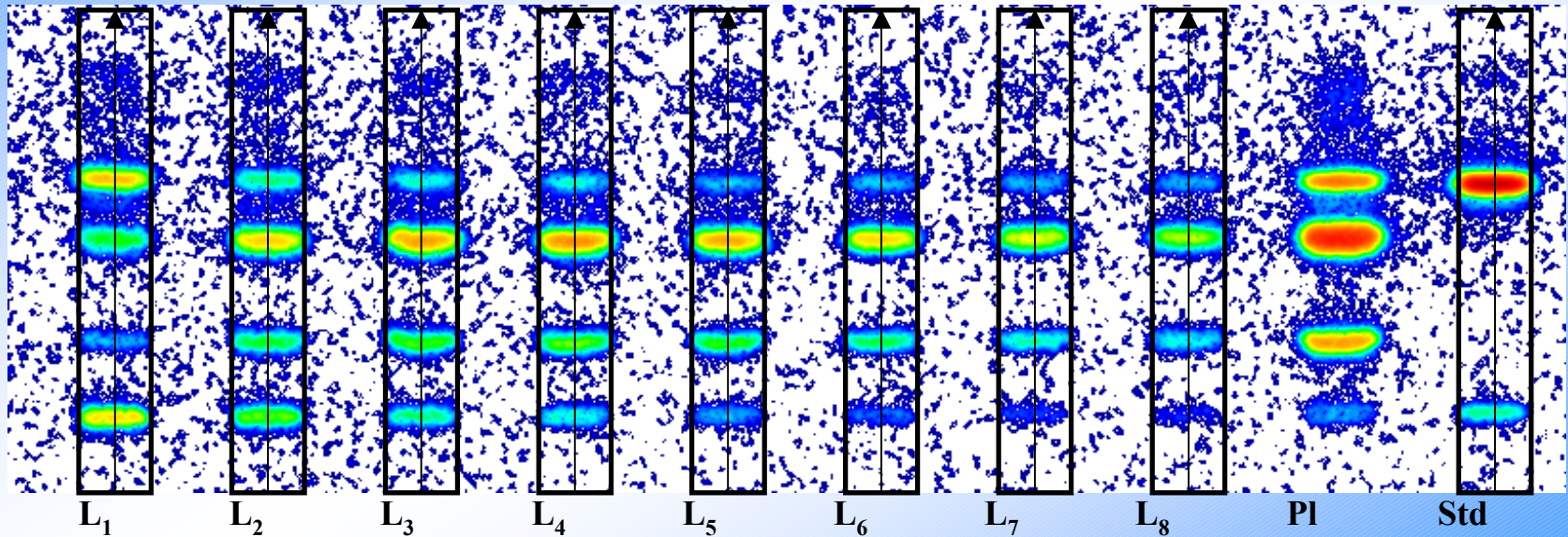


Image reader



Data storage and analysis

Image of radioactivity distribution on TLC plate

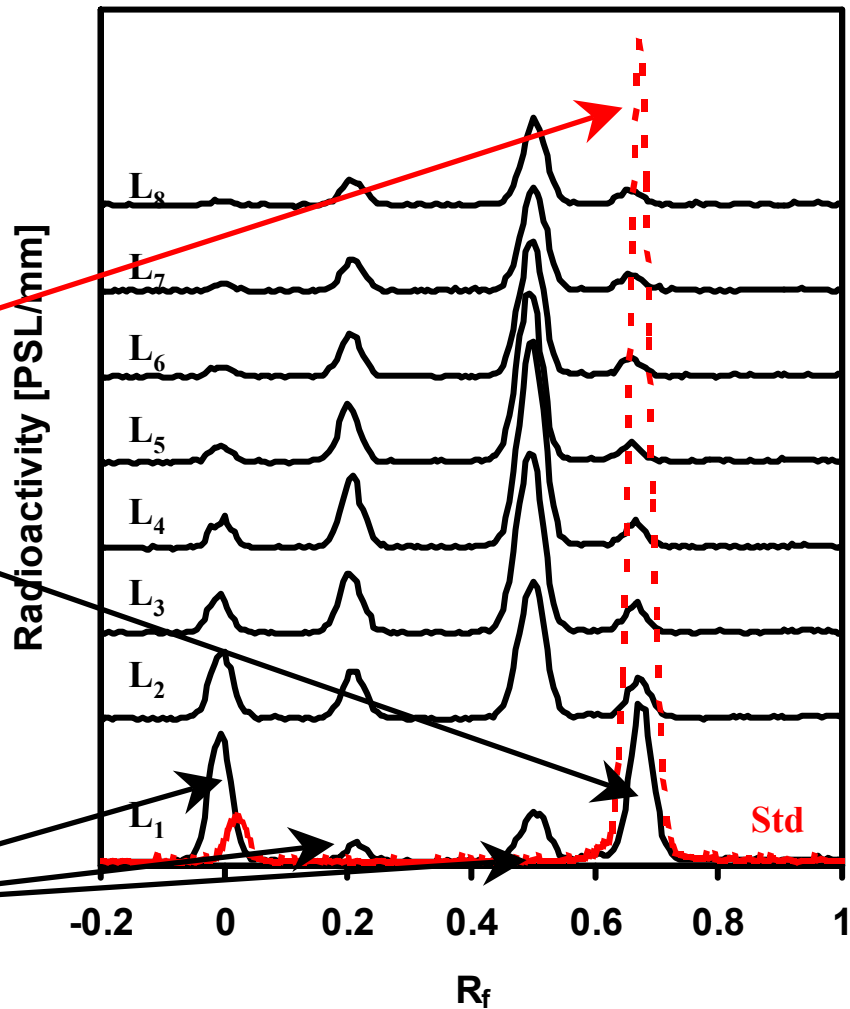


Analysis of samples

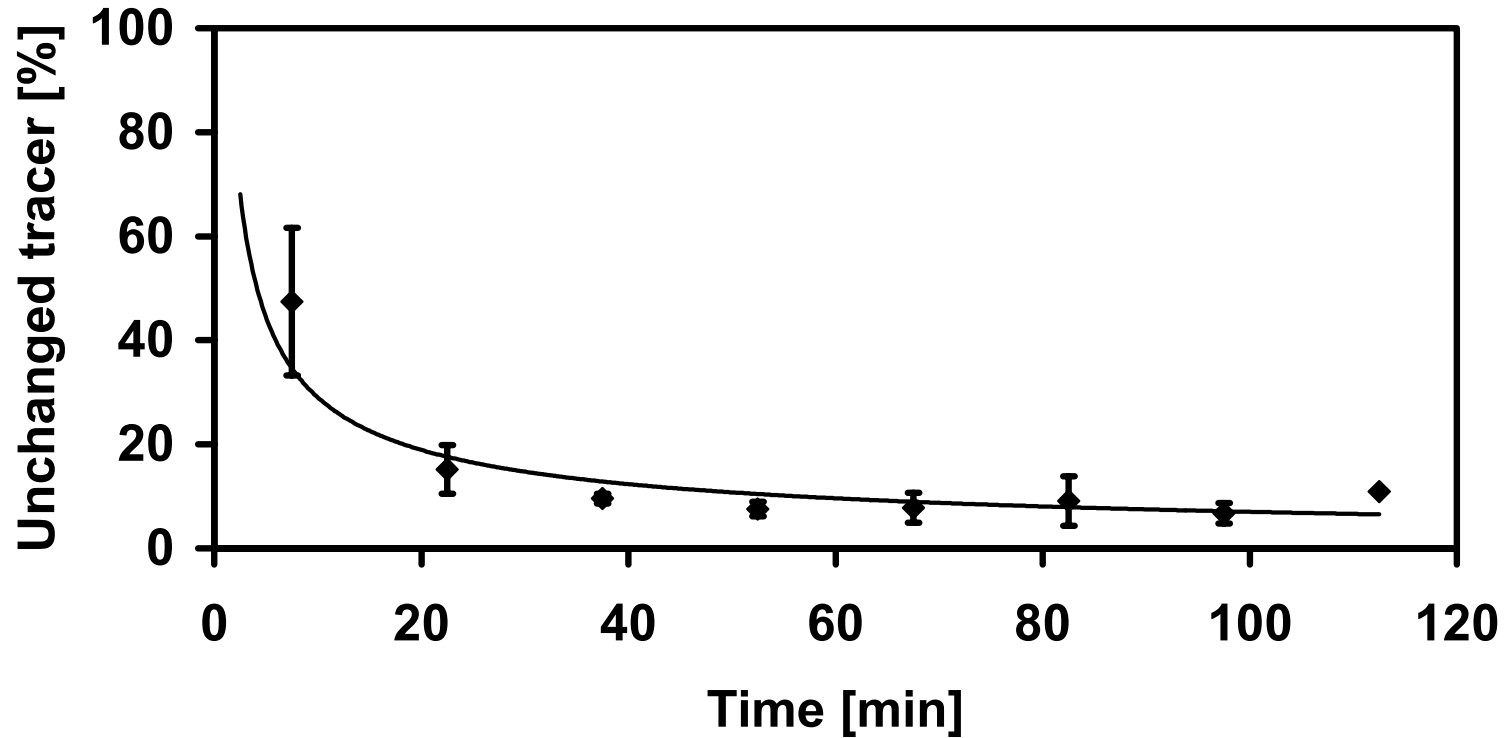
Standard

Unchanged tracer

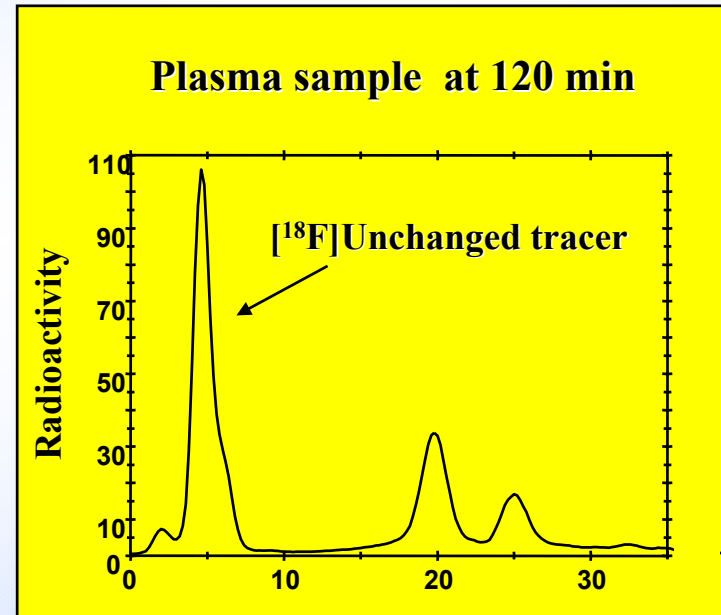
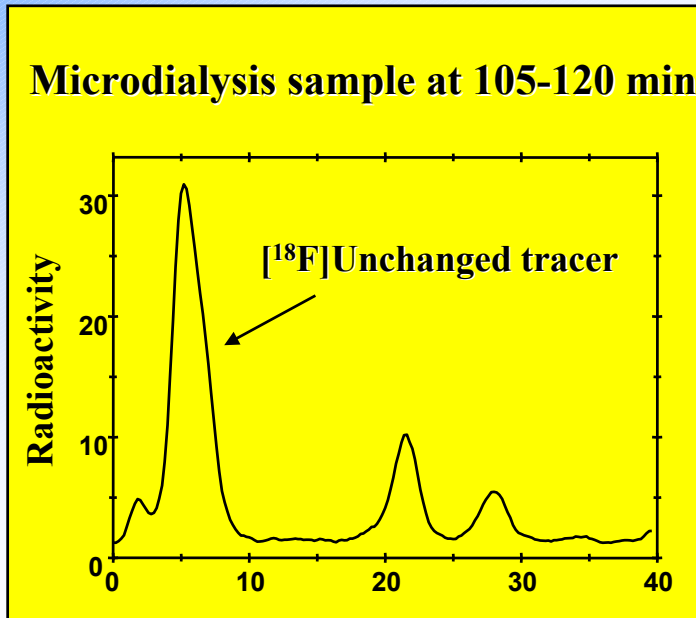
Metabolites



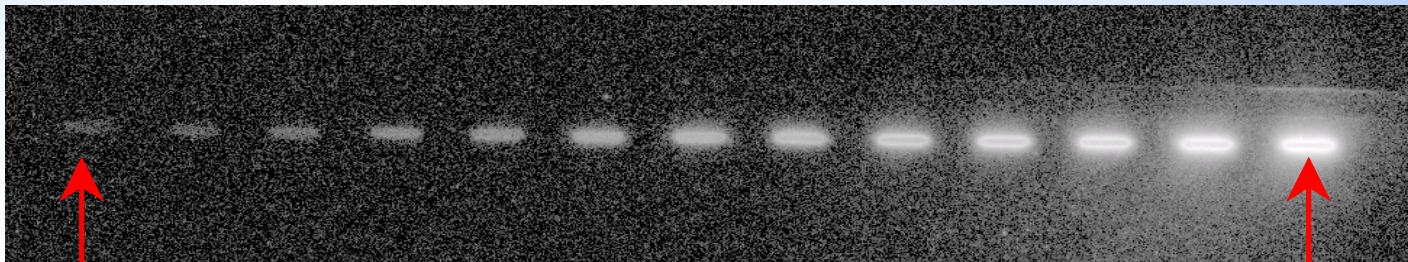
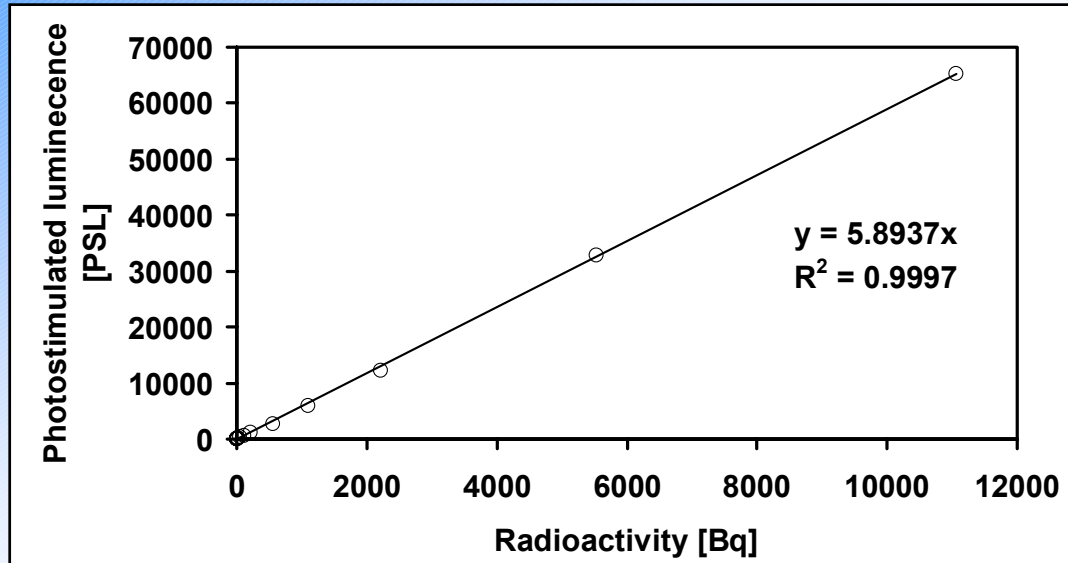
Unchanged [¹⁸F]FMR in MD jugularis fractions



Comparison of radiolabelled metabolite analysis by MD and blood sampling



Linearity and sensitivity of imaging plate with ^{18}F



1.1 Bq

At time of start of exposure

11 kBq

Exposure for 1 h

Summary

Planar chromatography with DAR as an analysing method for radiolabelled metabolites from MD fractions:

Pros

Sensitive, high resolution

Wide dynamic range of linear response for beta-particles

All sample components are observed in the same chromatogram

Cons

Best suitable for β -emitting nuclides (both β^- and β^+)

Special equipments are required (TLC applicator, phosphoimager)