



International Symposium for Thin Layer Chromatography

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HPTLC for the analysis of protein digests and peptides

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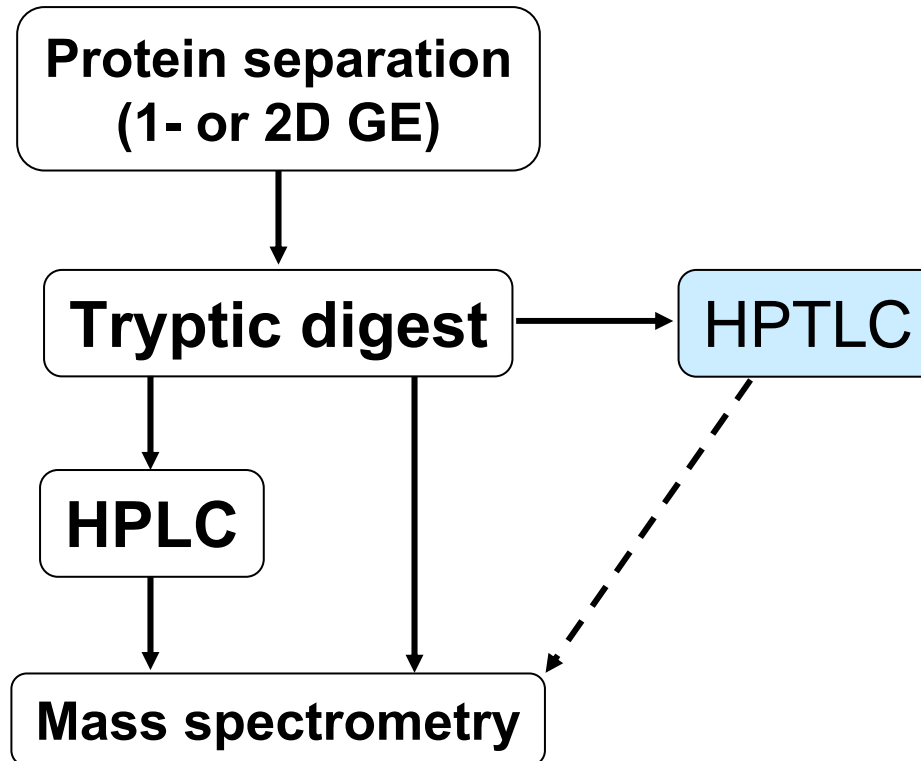
HPTLC for the analysis of protein digests and peptides



- Vision
- Devices
- Stationary- and Mobile phases
- Classical peptide staining
- Multi Colour staining
- Phosphopeptide staining
- Conclusions

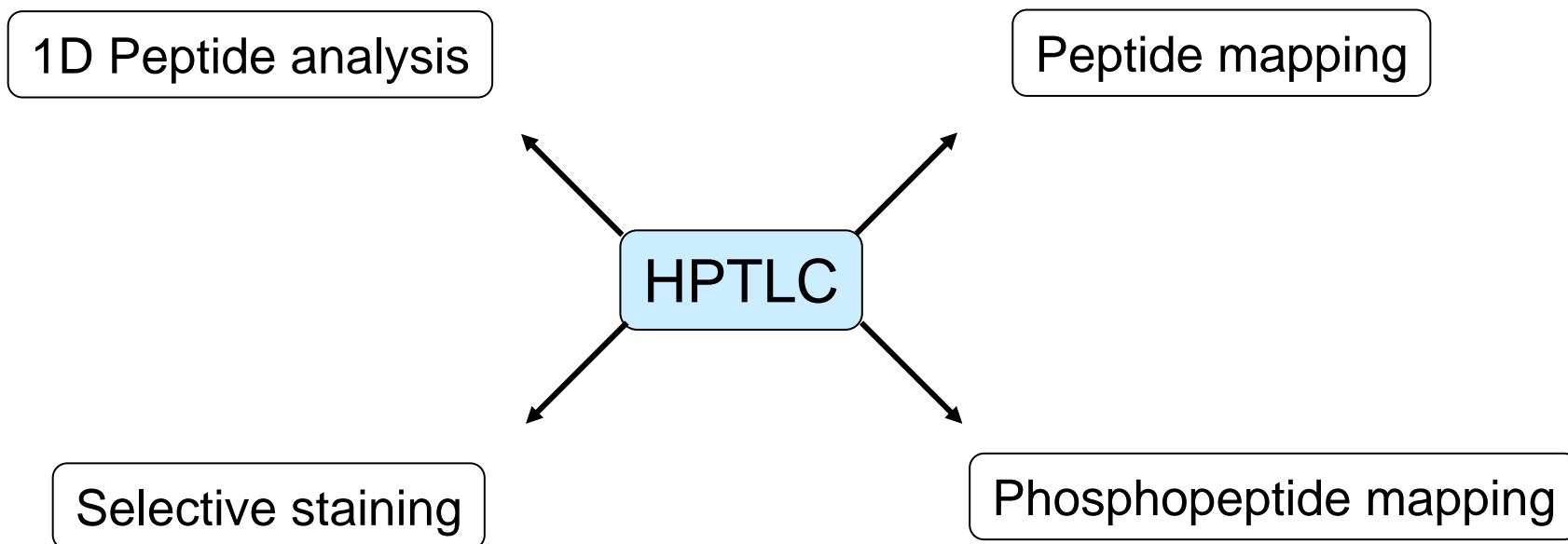
HPTLC for the analysis of protein digests and peptides

➤ Vision



HPTLC for the analysis of protein digests and peptides

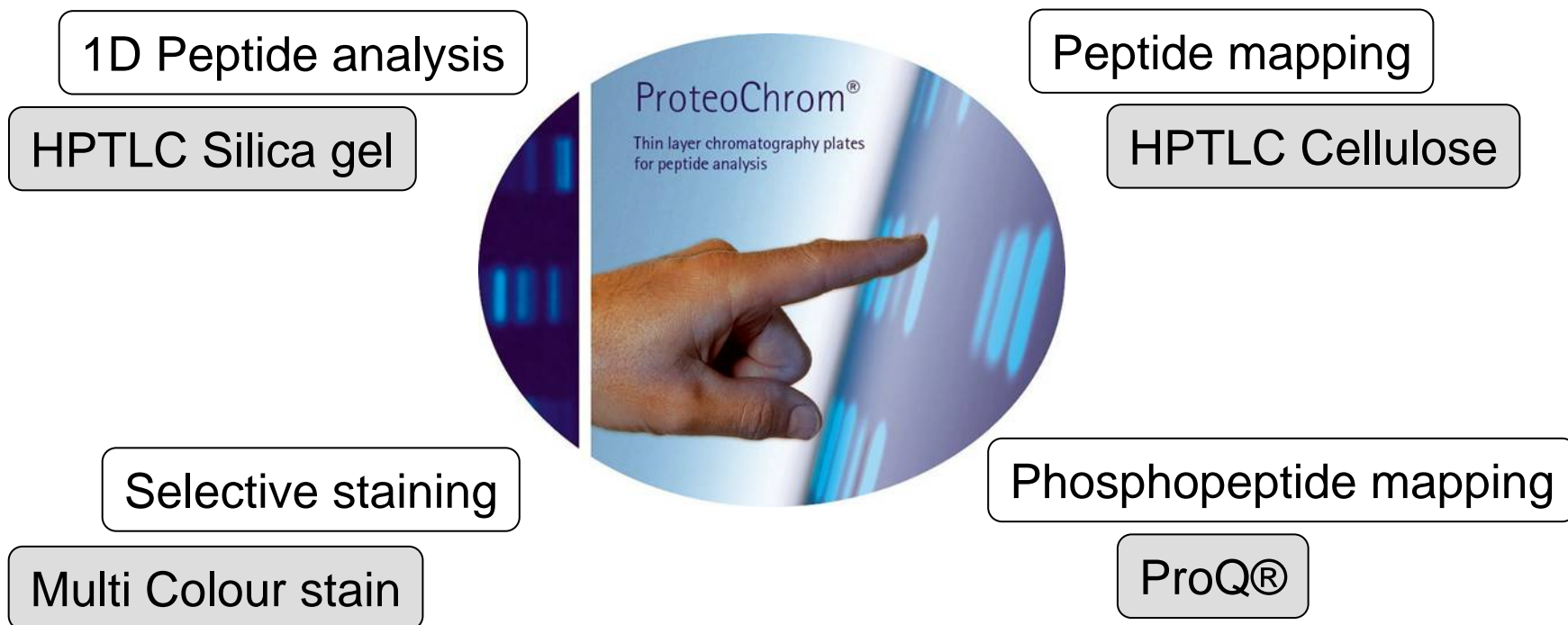
➤ Vision



HPTLC for the analysis of protein digests and peptides



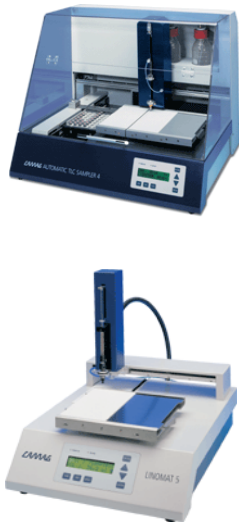



➤ Vision



HPTLC for the analysis of protein digests and peptides



➤ Devices

Application	Development	Derivatisation	Documentation
			

HPTLC for the analysis of protein digests and peptides



➤ Mobile- and stationary phases

One-dimensional	Two-dimensional
ProteoChrom® HPTLC Silica gel 60 F254s 2-butanol/ammonia/pyridine/water (39/10/34/26)	ProteoChrom® HPTLC Cellulose 2-butanol/acetic acid/pyridine/water (30/6/20/24) 2-butanol/ammonia/pyridine/water (39/10/34/26)
ProteoChrom® HPTLC Cellulose 2-butanol/acetic acid/pyridine/water (30/6/20/24)	

HPTLC for the analysis of protein digests and peptides



- Mobile- and stationary phases
Properties of ProteoChrom® layers

ProteoChrom® HPTLC Silica gel 60 F254s	ProteoChrom® HPTLC Cellulose	Benefit
<i>High performance Silica gel</i>	<i>High performance microcrystalline Cellulose</i>	<i>High separation efficiency</i>
<i>Layer thickness 100 µm</i>	<i>Layer thickness 100 µm</i>	<i>High sensitivity</i>
<i>Format 20 x 10 cm on glass</i>	<i>Format 10 x 10 cm on aluminium</i>	<i>Ideal Formats for one – or two dimensional separations</i>
<i>Special binder composition</i>	<i>High layer density</i>	<i>Stability against water / high sample application volume</i>

HPTLC for the analysis of protein digests and peptides

➤ Classical peptide staining

➤ **Ninhydrin - Stain**

Ninhydrin solution
2 minutes 110°C



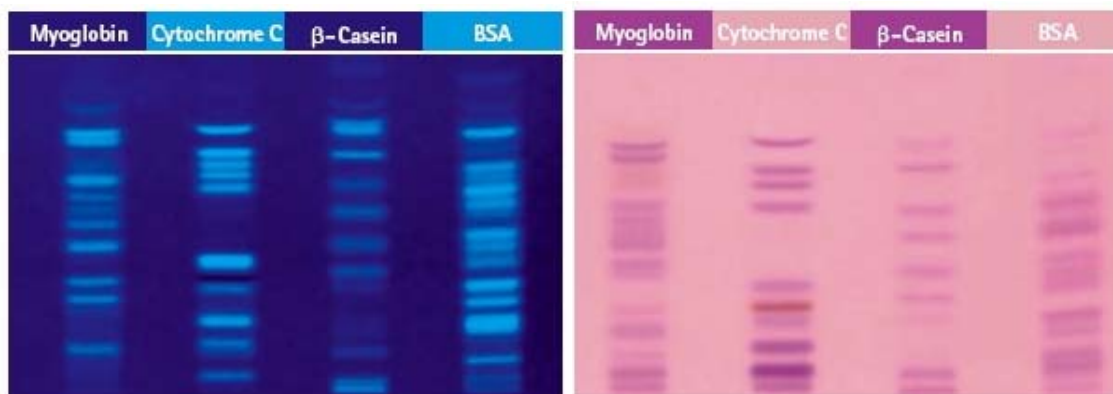
➤ **Fluorescamin - Stain**

Fluorescamin solution
10 minutes at room temperature
Triethylamine solution



HPTLC for the analysis of protein digests and peptides

- Classical peptide staining on ProteoChrom® HPTLC Silica gel 60 F254s plates

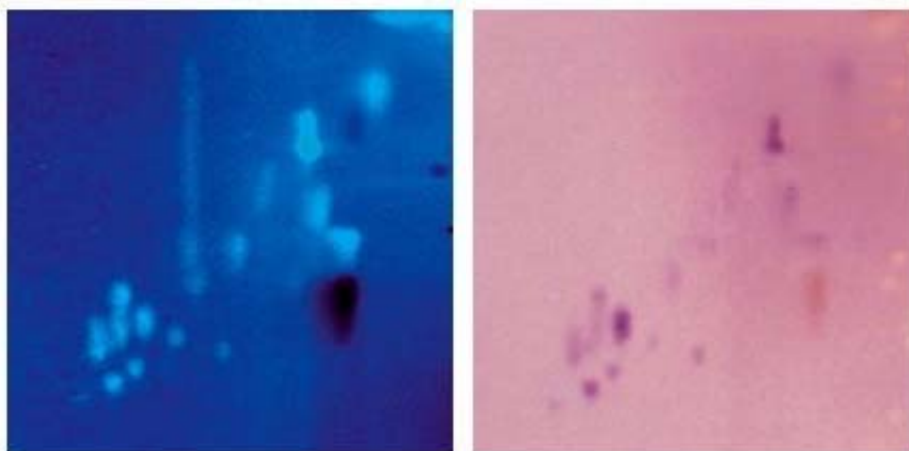


Sample volume: 1,5 μ l, 4 μ l
Concentration: 2 mg/ml
Application system: ATS 4 (CAMAG)
Migration distance: 5 cm
Migration time: 45 min

One- dimensional HPTLC of protein digests followed by Fluorescamin staining (left), or staining with ninhydrin (right)

HPTLC for the analysis of protein digests and peptides

- Classical peptide staining on ProteoChrom® HPTLC Cellulose sheets



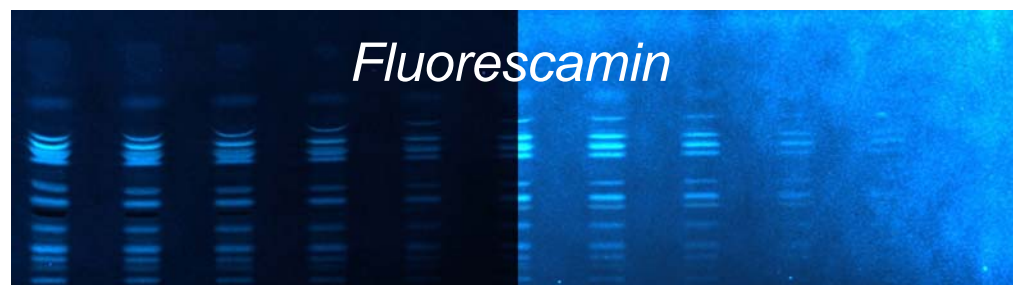
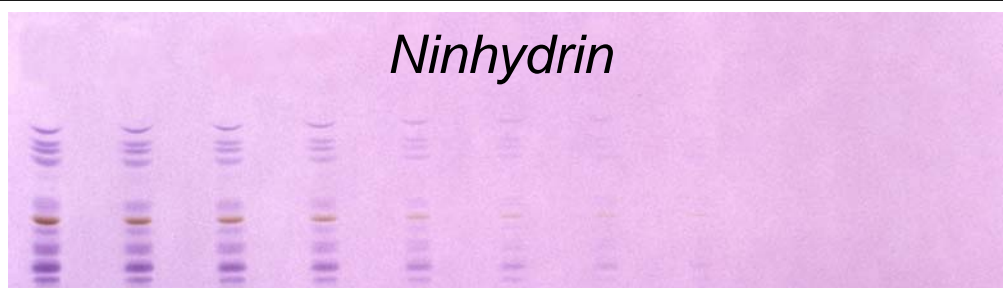
Sample volume:	5 μl
Concentration:	2 mg/ml
Application system:	Linomat V (CAMAG)
Migration distance:	5 cm
Migration time:	1st D: 45 min 2nd D: 50 min

Two-dimensional HPTLC of the tryptic digest of Cytochrome C followed by Fluorescamin staining (left), or staining with ninhydrin (right)

HPTLC for the analysis of protein digests and peptides

➤ Classical peptide staining Sensitivity on ProteoChrom® layers

Concentration [mg / ml]	2	2	2	2	2	2	2	2	2	0,2	0,2
Application volume [μl]	5	4	3	2	1	0,7	0,5	0,3	0,1	0,5	0,1



HPTLC for the analysis of protein digests and peptides

- Multi Colour staining on ProteoChrom® HPTLC Cellulose sheets

- ***Multi Colour - Staining***

Solution A

5 minutes room temperature

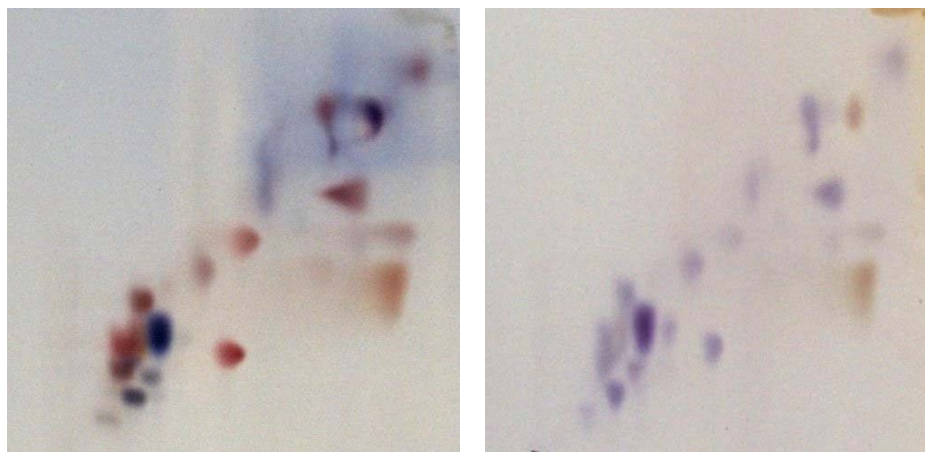
Ninhydrin solution

2 minutes 110°C



HPTLC for the analysis of protein digests and peptides

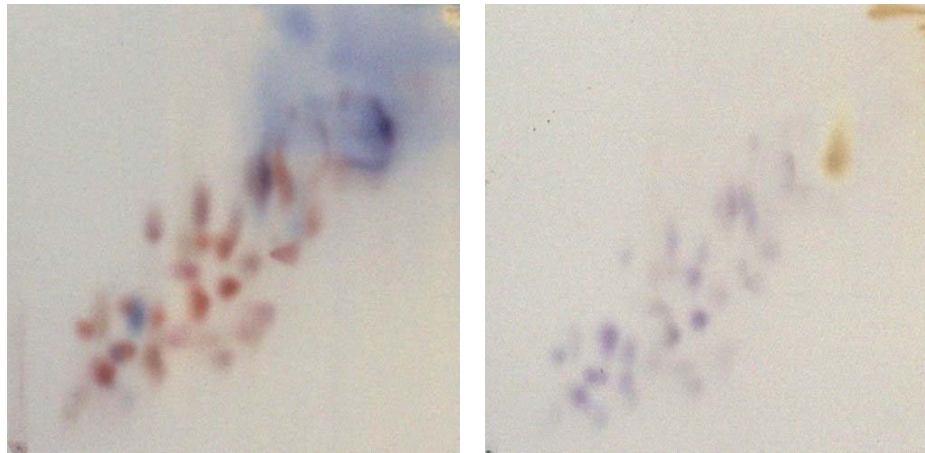
- Multi Colour staining on ProteoChrom® HPTLC Cellulose sheets



Two-dimensional HPTLC of the tryptic digest of Cytochrome C followed by Multi Colour staining (left), and staining with ninhydrin (right)

HPTLC for the analysis of protein digests and peptides

- Multi Colour staining on ProteoChrom® HPTLC Cellulose sheets



***Two-dimensional HPTLC of the tryptic digest of BSA
followed by
Multi Colour staining (left), and staining with ninhydrin (right)***

HPTLC for the analysis of protein digests and peptides



- Phosphopeptide staining on ProteoChrom® HPTLC Cellulose sheets

- **ProQ® - Stain**

- Pro-Q® solution*

- 45 seconds 70°C*

Reagents used from Invitrogen Pro-Q® Phosphoprotein Blot Stain Kit (ProQ® reagent + buffer)

HPTLC for the analysis of protein digests and peptides

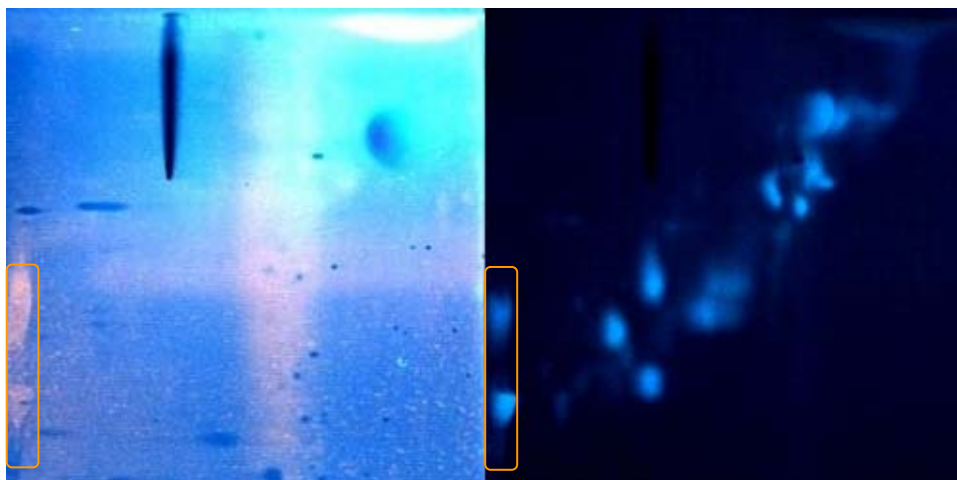
- Phosphopeptide staining on ProteoChrom® HPTLC Cellulose sheets



Two-dimensional HPTLC of a peptide mixture followed by Pro-Q® staining (left), and staining with fluorescamin (right)

HPTLC for the analysis of protein digests and peptides

➤ Phosphopeptide staining on ProteoChrom® HPTLC Cellulose sheets



Sequence of β -Casein

MKVLILACLVALALAR

ELEELNVPGEIVESLSSEESITR

INKKIEK**FQSEEQQQTEDELQDK**

IHPFAQTQSLVYPPFGP IPNSLPQNIP

PLTQTPVVVPPFLQPEVMGVSKVKEA

MAPKHKEMPPFKYPVEPFTESQSLTL

TDVENLHLLPLLQSWMHQPHQPLPPT

VMFPPQSVLSLSQSKVLPVPQKAVPY

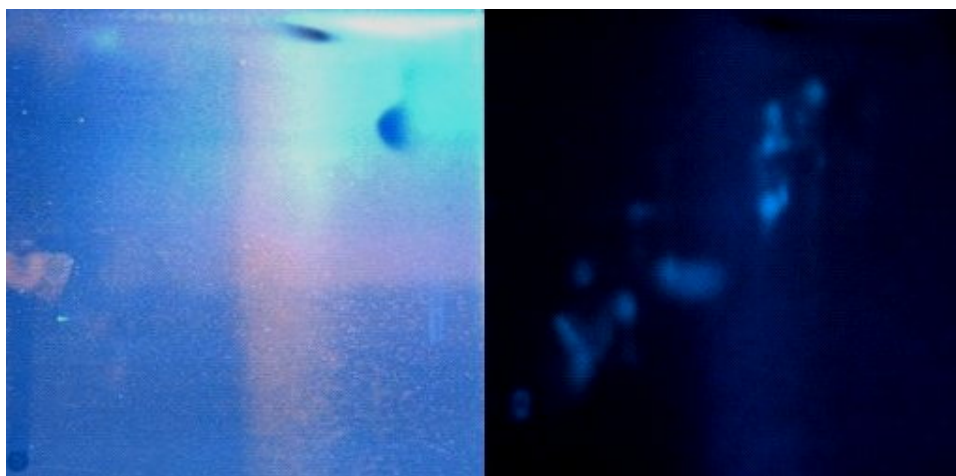
PQRDMPIQAFLLYQE PVLGPVRGPFPIIV

⇒ **Two Phosphopeptides**

Two-dimensional HPTLC of the tryptic digest of β -Casein followed by Pro-Q® staining (left), and staining with fluorescamin (right)

HPTLC for the analysis of protein digests and peptides

➤ Phosphopeptide staining on ProteoChrom® HPTLC Cellulose sheets



Sequence of Myoglobin:
GLSDGEWQQV LNVWGKVEAD
IAGHGQEVLI RLFTGHPETL
EKFDKFKHLK TEAEMKASED
LKKHGTVVLT ALGGILKKKG
HHEAELKPLA QSHATKHKIP
IKYLEFISDA IIVLHSHKHP
GDFGADAQGA MTKALELFRN
DIAAKYKELG FQG

⇒ **No Phosphopeptides**

Two-dimensional HPTLC of the tryptic digest of Myoglobin followed by Pro-Q® staining (left), and staining with fluorescamin (right)

HPTLC for the analysis of protein digests and peptides



➤ Conclusions

- HPTLC can provide a complementary method to GE, HPLC and MS in the field of Proteomics
- HPTLC can close the gap between single amino acids and small peptides determinable in gel electrophoresis
- High sample number per run and high sensitivity on ProteoChrom® HPTLC Silica gel 60 F254 layers
- Two-dimensional peptide mapping on ProteoChrom® HPTLC Cellulose sheets
- Large variety of detection methods on ProteoChrom® layers

