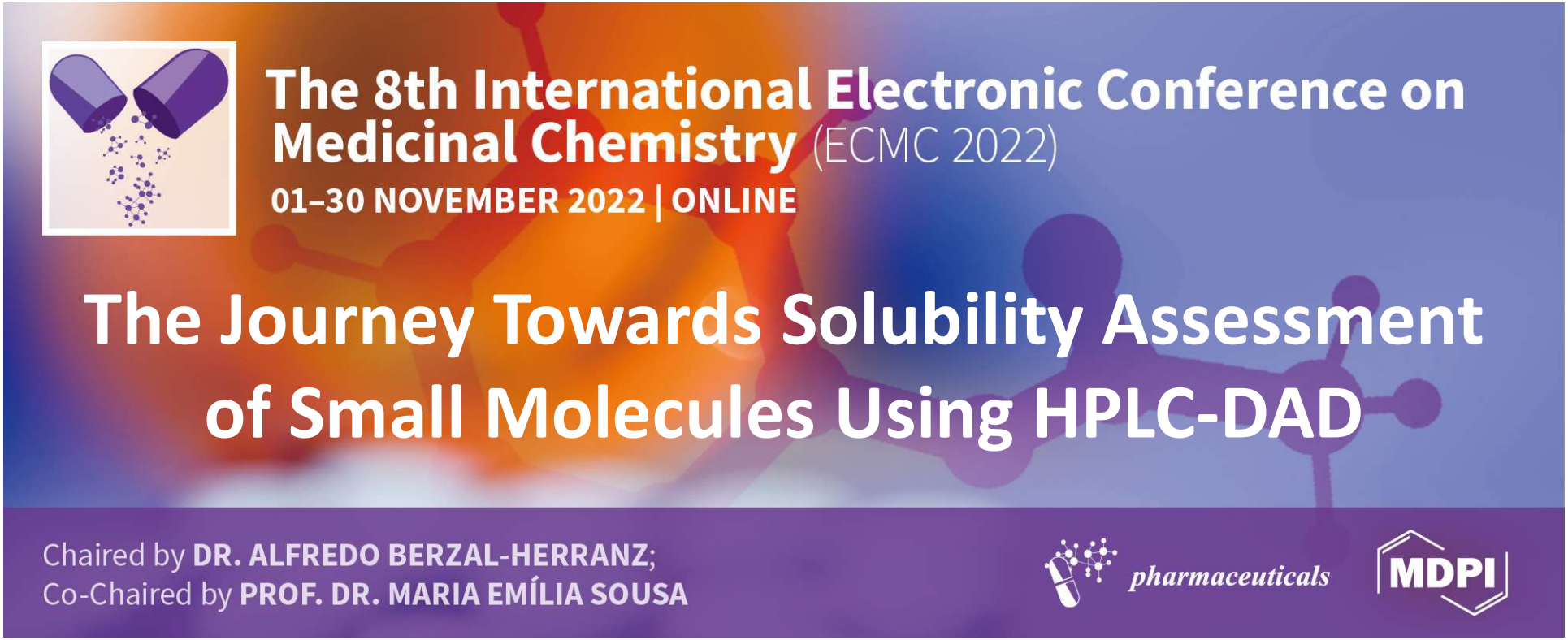

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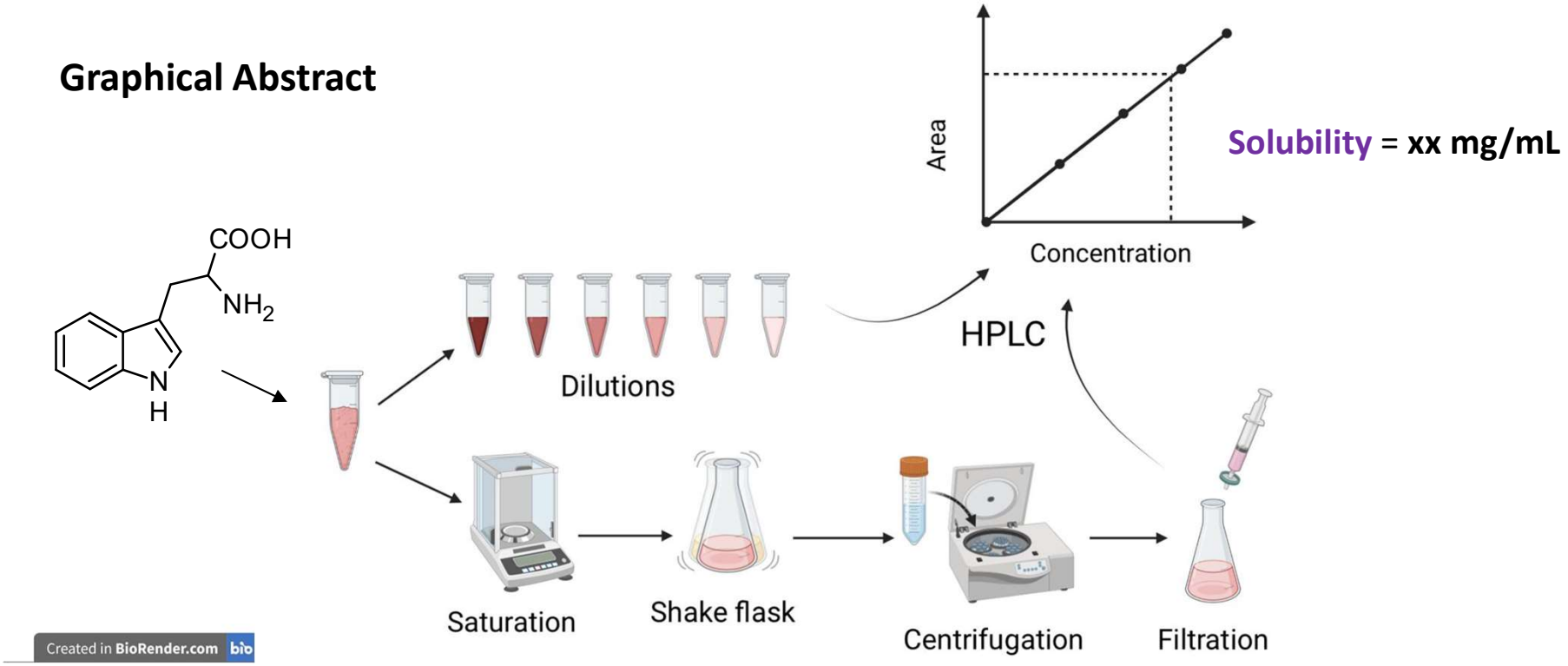
The journey towards solubility assessment of small molecules using HPLC-DAD

Graphical Abstract



Abstract:

Through the process of developing bioactive small molecules, solubility determination is a crucial step. Many research papers treating problems related to solubility are published, but none of them fully describes the methods and steps for solubility assessment. In addition, in silico prediction tools and databases, such as SwissADME, ACD/Percepta, DrugBank and many others offer the possibility to have approximative solubility values based on the structure of the molecule. Although, significant differences can be observed depending on the database and the conditions of the experiment: solvent, pH, temperature… etc. The lack of data can be a barrier to obtaining details on solubility measurement methods. This presentation aims to describe, step by step, some attempts of solubility determination using HPLC-DAD.

Keywords: HPLC; in silico prediction; shake-flask; small molecules; solubility

Introduction – Solubility, a key property for small molecules

- Among all physico-chemical properties of small molecules (e.g. MW, °C, LogP, H-bond donors H-bond acceptors, TPSA), **solubility** is one of those to be well controlled.

- **Low solubility** lead compounds: variable intestinal absorption, precipitation at higher concentrations, time-consuming experiments…

- During all drug discovery process, it is crucial **to know the solubility of all lead compounds** before new structural optimizations.

Introduction – What solubility are we talking about?

○ Kinetic solubility

= Apparent solubility

"… kinetic solubility estimation involves solubilization of drugs in an organic solvent, generally DMSO which is most frequently used in biological assays"

As a screening assay, by pipetting test compound into a stock solution of 10 mM DMSO…

Shah RP et al. Thermodynamic solubility determination of khellin in eight mono-solvents at the range of 298.15 to 323.15 K. J Mol Liq 2022;351:11863

○ Thermodynamic solubility

= Equilibrium solubility

"The equilibrium solubility of a compound is defined as the maximum quantity of that substance which can be completely dissolved at a given temperature and pressure in a given amount of solvent, and is thermo-dynamically valid as long as a solid phase exists which is in equilibrium with the solution phase"

Brittain H.G. Thermodynamic vs. kinetic solubility: Knowing which is which. Amer. Pharm. Rev. 2014;17:10-15

Introduction – Thermodynamic solubility

- **HPLC-UV**

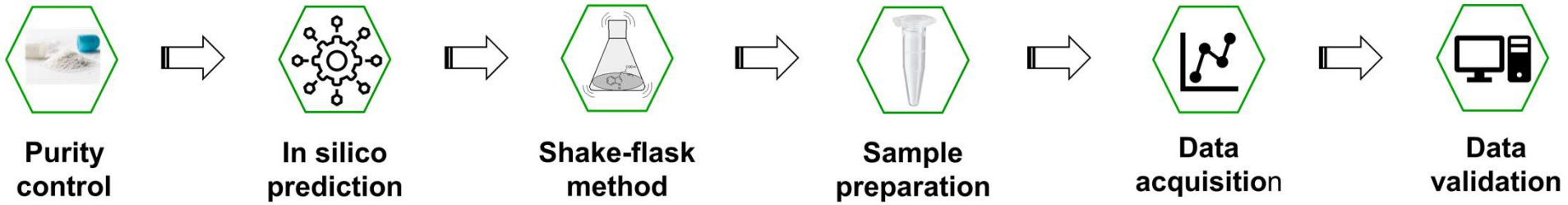
Kajita Y et al. Discovery of novel 3-piperidinyl pyridine derivatives as highly potent and selective cholesterol 24-hydroxylase (CH24H) inhibitors. J Med Chem 2022;65:3343-3358

Gold standard solubility experimental method must integrate parameters to control/check/adjust:

pH
T °C
ionic strength
salt/buffer effects
phase separation

Barrett JA et al. Discovery solubility measurement and assessment of small molecules with drug development in mind. Drug Discov Today 2022;27(5):1315-1325

Introduction – A 6-step method



and beyond UV detection?

Estimated solubility (Se)
SwissADME, PubChem…

24-hrs, T °C, pH

dilution scale
calibration curves

HPLC-DAD

ICH Q2(R2)

accuracy, specificity, LoD, LoQ, linearity, range, robustness

Pantaleão SQ et al. Recent advances in the prediction of pharmacokinetics properties in drug design studies: A review. ChemMedChem 2021;17:e202100542

Results and discussion – Step 1

Purity control

- Select a molecule (tryptophan/TRP)
- Check its purity by uHPLC-MS (minimum 98%, TLC)
- If not pure, use a ***flash purification system*** (for original small molecules)
- Select a control (= small molecule with similar Se)

Model puriFlash® XS 520 Plus

Columns

IR-50SI-F0012
IR-50SI-F0025

IR-20SI-F0012
IR-20SI-F0025

PF-15SIHP-F0012
PF-15SIHP-F0025

Results and discussion – Step 1

Purity control

and beyond

- Select an LC and a column
- Use a relevant solvent system
- Choose the right wavelength for analysis

Run analysis

ChemStation for LC 3D System - Rev. B.04.03 [54]

Agilent 1100 series

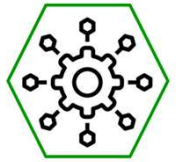
TRP detection by UV

Column: **Eclipse XDB-C18** (5 µm, 4.6 x 150 mm)

Wavelength: **280 nm**
Mobile phase:
75% water/25% ACN

t_R : **5 min**
1.2 mL/min
120 bars

Results and discussion – Step 2



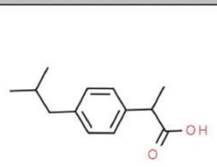
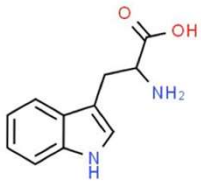
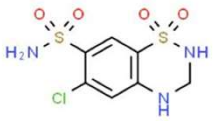
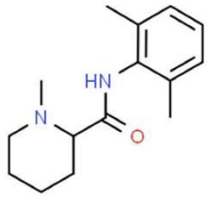
In silico prediction

- **Determine Se**

By using databases (e.g. PubChem), webservers (e.g. Osiris Property Explorer, SwissADME), platforms (e.g. PhysChem suite)...

Table with four classical SMs

a = Yalkowsky *et al*. 1992;
b = Yalkowsky *et al*. 2010;
c = Delaney *et al.* 2004;
d = Ali *et al.* 2012;
*SILICOS-IT

	Ibuprofen	Tryptophan	Hydrochlorothiazide	Mepivacaine
Structure				
MW (g/mol)	206.28082	204.22900	297.73912	246.34798
cLogP (ACD Chemsketch 2021.2.1)	3.72 +/- 0.23	1.04 +/- 0.031	- 0.07 +/- 0.28	2.04 +/- 0.27
miLogP (Molinspiration)	3.46	- 1.08	- 0.06	1.91
Solubility (mg/mL) via DrugBank	0.0210[a]	13.4[a]	0.722[a]	7.00[a]
Solubility (mg/mL) via ChemSpider	0.0580[a]	1.05[a]	111[b]	1.56[a]
Solubility (mg/mL) via PubChem	0.0210[a]	13.4[a]	0.722[a]	0.621[a]
Solubility (mg/mL) via SwissADME	0.0909[c] 0.0230[d] 0.0749*	42.2[c] 158[d] 0.401*	4.33[c] 1.44[d] 0.401*	0.559[c] 1.37[d] 0.0215*

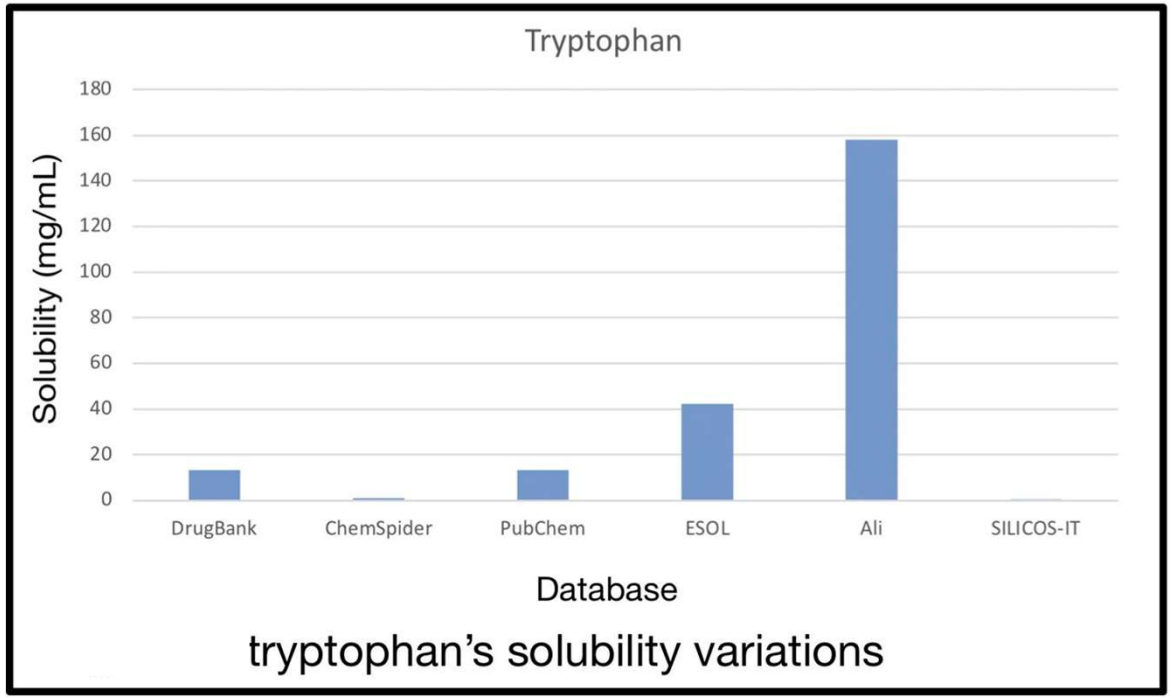
Results and discussion – Step 2

In silico prediction

- **Determine Se**

For TRP:
Se = 13.4 mg/mL

Important point:
Saturate the media with the tested molecule/control (**weigh 20% more**)



tryptophan's solubility variations

Results and discussion – Steps 3 and 4

Shake-flask method

- **Shake samples** (test, control)

16 mg TRP in **1 mL of aqueous media** (water, PBS 7.4)

Shake them **24 hrs** (orbital shaker)

Centrifugate and filtrate (remove compound excess)

Sample preparation

- **Prepare a calibration range**

Choose the concentration range (**0.01 – 0.3 mg/mL** for TRP)

Calculate the dilutions and volumes to be sampled (Vss = 10 mL; Fd = 65): (Vss*Se)/Fd = **2.062 mg TRP in 10 mL**

Vss: volume of stock solution; Fd: dilution factor

Results and discussion – Steps 4

Sample preparation

○ Prepare a calibration range

Stock solution used to prepare 7 concentrations (100%, 85%...)

Tube No	Vss to take (mL)	Vsolvent to add (mL)
1	0.500	0
2	0.425	0.075
3	0.350	0.150
4	0.275	0.225
5	0.200	0.300
6	0.125	0.375
7	0.050	0.450

○ Prepare calibration ranges

And **three** identical calibration ranges are prepared = 21 tubes for one experiment

For **test sample** (in duplicate/triplicate): after filtration, apply a **Fd = 130** (final conc. of test sample 0.10 mg/mL)

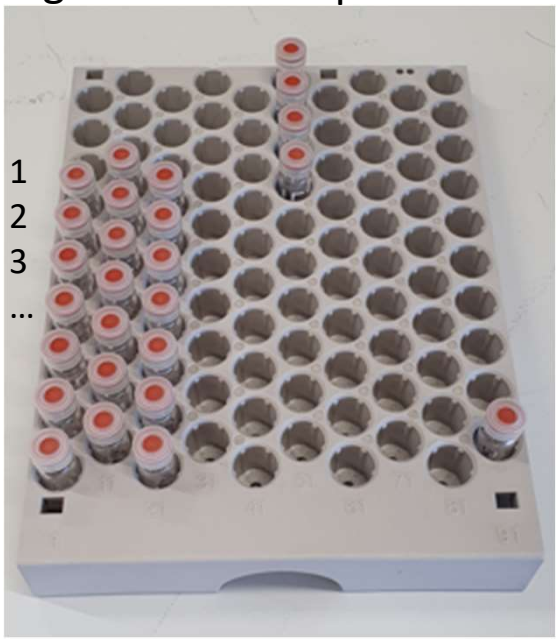
Results and discussion – Step 5

Data acquisition

Agilent 1100 series

3 identical ranges

samples for analysing



7 conc.

solvent to wash

Results and discussion – Step 5

Data acquisition

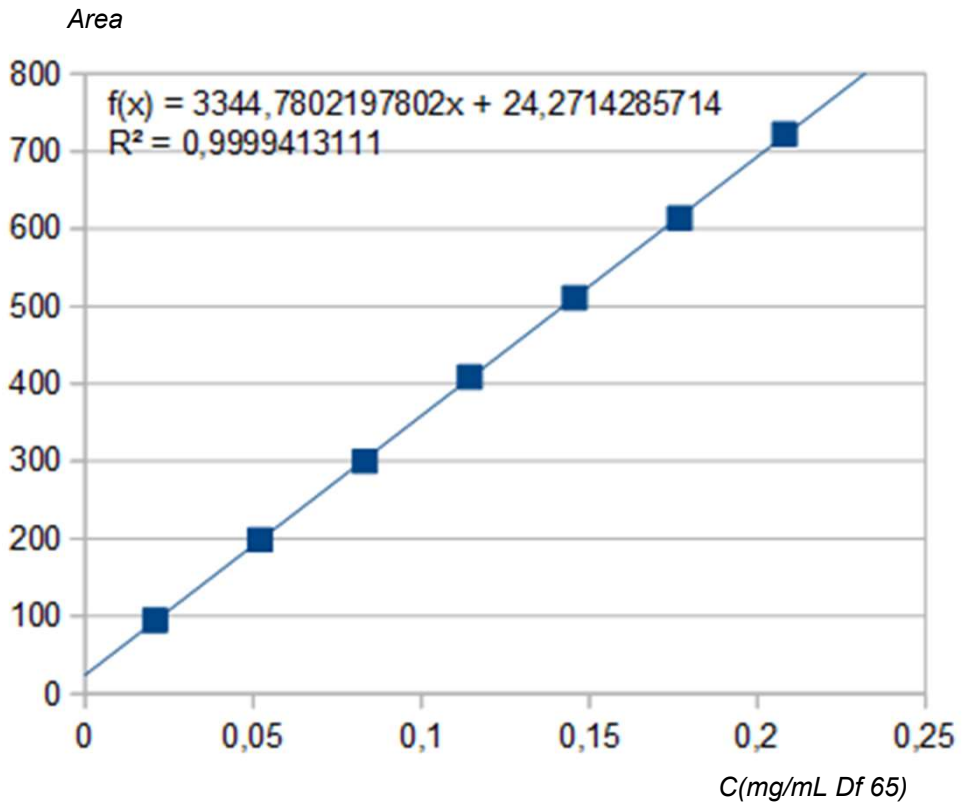
- **Select a calibration curve (according to R^2)**

and **analyse your samples**
(test, control **in water**)

For TRP:

Se = 13.4 mg/mL (target value)

S_{exp} = **12.6 mg/mL**
at 18 °C, pH 5.8



Results and discussion – Step 5

Data acquisition

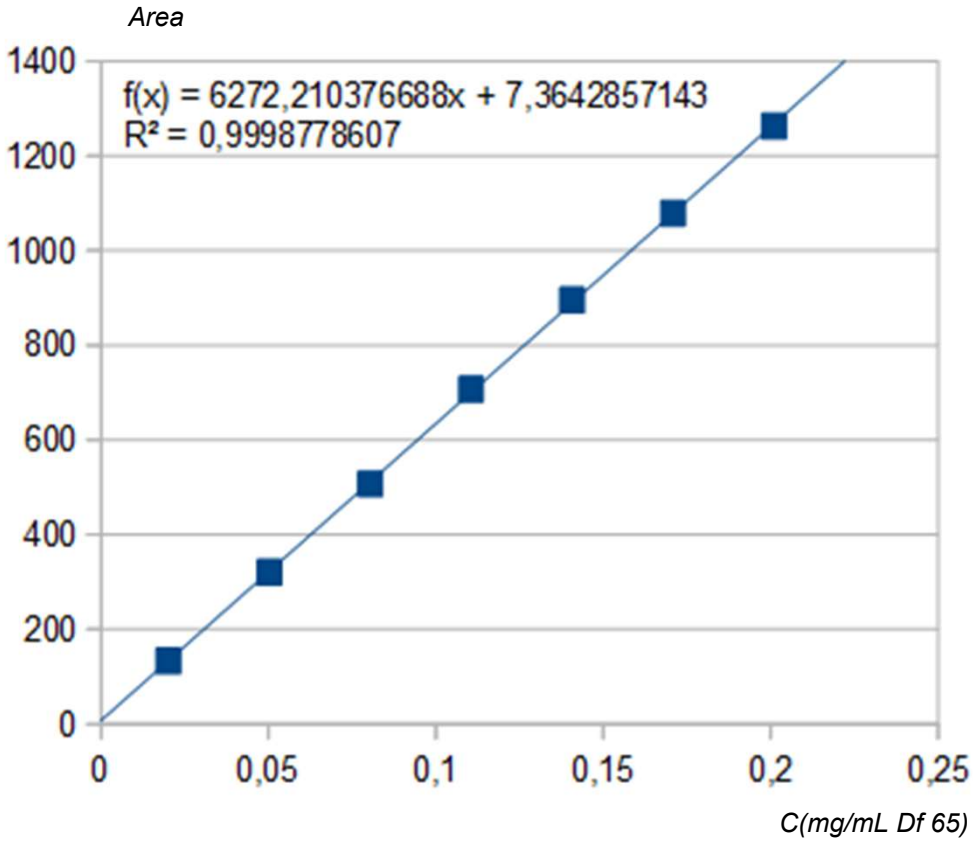
- **Select a calibration curve (according to R^2)**

and **analyse your samples**
(test, control **in PBS 7.4**)

For TRP:

Se = 13.4 mg/mL (target value)

Day 1: S_{exp} = **15.4 mg/mL**
(19 °C; pH 7.31)



Results and discussion – Step 5

- **Select a calibration curve (according to R^2)**

and **analyse your samples**
(test, control **in PBS 7.4**)

For TRP:

Se = 13.4 mg/mL (target value)

Day 1: S_{exp} = 15.4 mg/mL (19 °C; pH 7.31)

Day 2: S_{exp} = **15.2 mg/mL** (19 °C; pH 7.31)

Day 3: S_{exp} = **22.0 mg/mL** (23 °C; pH adjusted to 7.4 with NaOH)

Day 4: S_{exp} = **15.9 mg/mL** (23 °C; pH 7.31)

Results and discussion – Step 5

Data acquisition

○ Problems/Explanations

Why did we observe a difference in Se between water and PBS?

S_{exp} = **12.6 mg/mL**
at 18 °C, pH 5.8 **in water**

S_{exp} = **15.4 mg/mL**
at 19 °C, pH 7.31 **in PBS**

The solubility of amino acids is higher in alkaline media

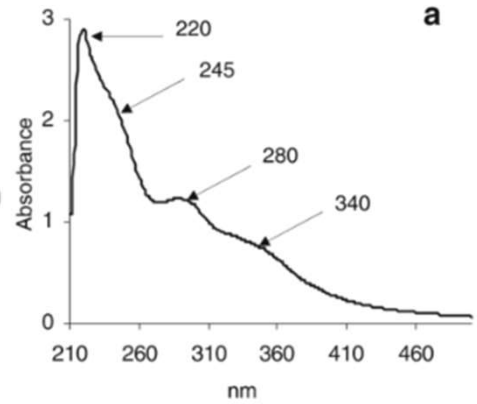
Seve. Les acides aminés : Propriétés physicochimiques Chapter 2, **2011**

Why did the concentration increase after pH adjustment?

S_{exp} = **22.0 mg/mL** (23 °C; pH adjusted to 7.4 with NaOH)

NaOH is detected at 280 nm

⇒ **Increased** peak area



Gonzalez et al. Appl Microbiol Biotechnol **2006**;73:141–150

Conclusions

Data validation

- **According to Se, HPLC-DAD may or may be not enough**

✓ *Se > 10 mg/mL* (e.g. tryptophan, theophyllin)	**HPLC-DAD +++**
✓ *Se < 0.1 mg/mL* (e.g. ibuprofen)	**HPLC-DAD --**
✓ *1 < Se < 10 mg/mL* (e.g. mepivacaine)	***in progress***
✓ *0.1 < Se < 1 mg/mL* (e.g. hydrochlorothiazide)	***in progress***

- **For smaller values of Se, other options may be interesting to use…**

Conclusions

Data validation

✓ **HPLC-DAD-MS, HPLC-DAD-MS(n)**

Detection with Se < < 10^{-3} mg/mL

Huang HS et al. HPLC-DAD-ESI-MS analysis for simultaneous quantitation of phenolics in Taiwan elderberry and its anti-glycation activity. Molecules 2019;24(21):3861

✓ **Step 6** : Method validation according to ICH Q2(R2)

accuracy, specificity, limit of detection (LoD)
limit of quantitation (LoQ), linearity, range

ICH Q2. https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures, accessed April 6, 2022

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