HPTLC for the analysis of API-cleaning samples

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Ties Raijmakers

Schering-Plough

About Schering-Plough

- Global health care company with three integrated businesses:
  - Pharmaceutical
  - Animal Health
  - Consumer Health Care
- Business, research, manufacturing and sales operations in more than 140 countries
- Approximately 55,000 colleagues worldwide, about 5,000 in Oss

Chemical production

Analytical methods used for cleaning validation in chemical production:

- Residue on evaporation
- TOC
- HPTLC
- Conductivity

Overview

- Equipment/materials
- Validation
- Reagents
- Mobile phase selection
- Sample pretreatment
- Standard series
- Evaluation

HPTLC/TLC: equipment and materials

- HPTLC / TLC plates
- Development chamber
- Application device (automatic sampler, glass capillaries, disposable micro pipettes)
- Detection device (e.g. spraying, immersion, heating)
- Evaluation device (viewing box with ultraviolet light 254/366 nm)
- Digistore / Wincats

Validation

The validation of the HPTLC method for cleaning validation consisted of:

- Investigation of accuracy, precision, linearity and range on a model substance.

- Specificity can be derived from the data base containing Rf values in 4 different mobile phases.

- Robustness investigation on each individual product (= determination of the effect of evaporation a sample solution at a temperature of 50 °C).

Reagents

- Suitable solvents (water or organic solvents)
- Mobile phase (e.g. mixture of organic solvents)
- Detection reagent (e.g. iodine, sulphuric acid solution in alcohol)

Mobile phase selection

- Database available with Rf-values of each product in 4 mobile phases.

- Each mobile phase has a mobile phase reference standard.

- Mobile phase with most suitable Rf-value and relative Rf-value (R_{st}= Rf value of product / Rf value of mobile phase reference standard) will be selected.

- Database includes all products, intermediates, degradation products, starting materials, finished products.

Example database

			T.Et 9.1		T.Et 1.1		M.Amme 7.3		M.Me 9.1		
Max deviation Rf value											
Max deviation Rst			Rf	Rst	Rf	Rst	Rf	Rst	Rf	Rst	
Measured Rf-value											
Rf-value standards											
Standard number											
			T.Et 9.1		**T.Et 1.1**		**M.Amme 7.3**		**M. Me 9.1**		
Product name	**Detection**	**Std nr**	**Rf 1**	**Rst 1**	**Rf 2**	**Rst 2**	**Rf 3**	**Rst 3**	**Rf 4**	**Rst 4**	
Product 1	HS	1501	0.13	0.36	0.54	1.2	0.85	1.18	0.55	1.68	

Sample pretreatment

Swabs
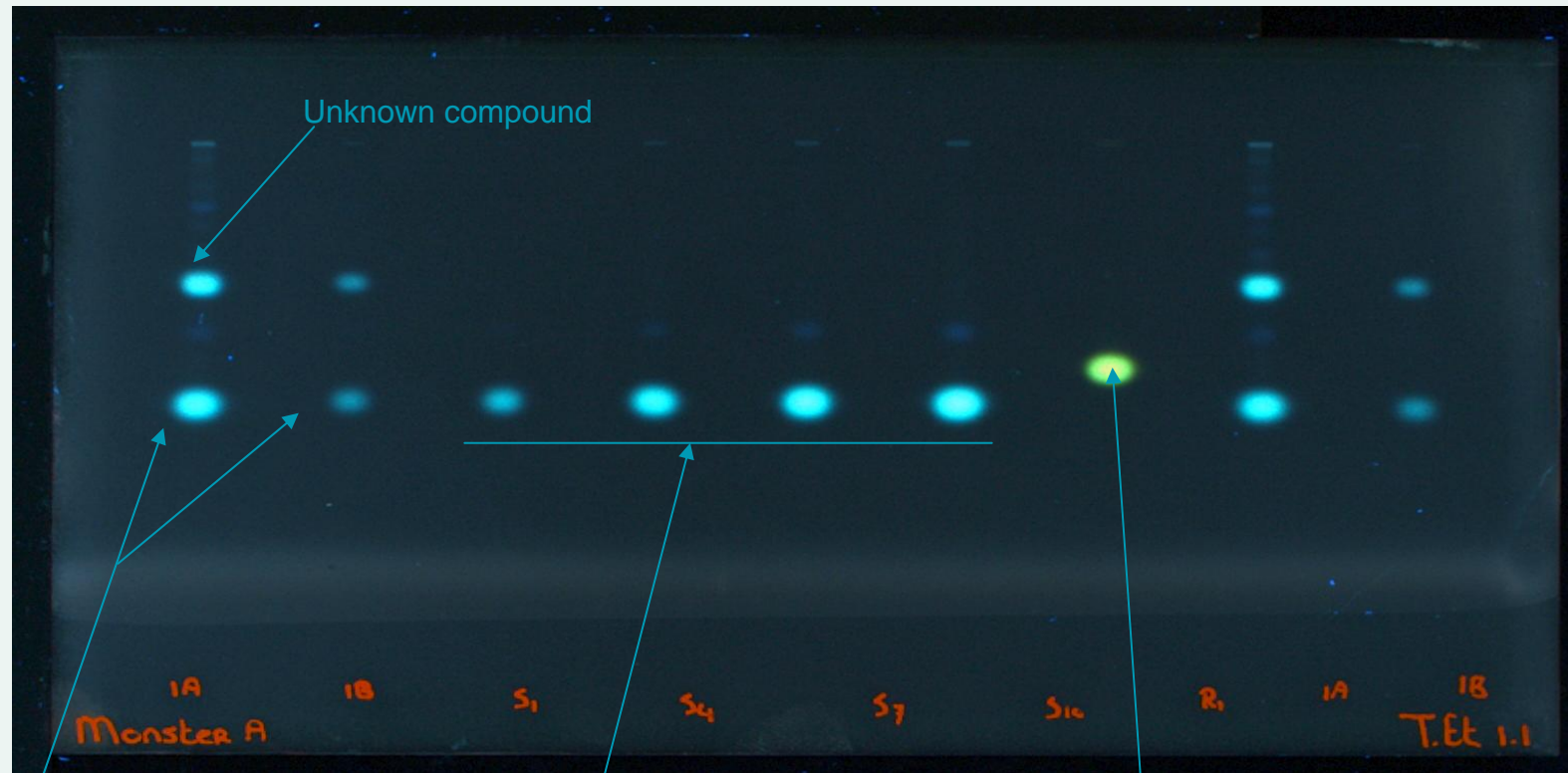Extraction of product with suitable solvent

Rinse solution
Concentration of sample by evaporation
(only in case of low specification)

Standard series

- Determination of product content in swabs/rinse solutions against standard series of last product which has been in contact with the equipment.

- Concentrations of standard series depend on type of analysis (rinse or swab) and type of equipment:

  - Rinse solution   : fixed concentrations
  - Swabs   : fixed concentrations
  - Final purification   : limit calculated on therapeutic day doses of last and next product

HPTLC for the analysis of API-cleaning samples

- Mobile phase : Toluene : Ethyl Acetate 1:1

Evaluation

- Intensity of the spots (both the "known" contaminant and unknown compounds) in the sample chromatogram will be compared to the intensity of the standard solutions.

- The reported result is the concentrations of the "known" contaminant and the unknown compounds.

- Investigation of the unknown compound, in the other three mobile phases.

- Determine Rf–value and the relative Rf-value (= Rf value / Rf value of mobile phase reference standard).
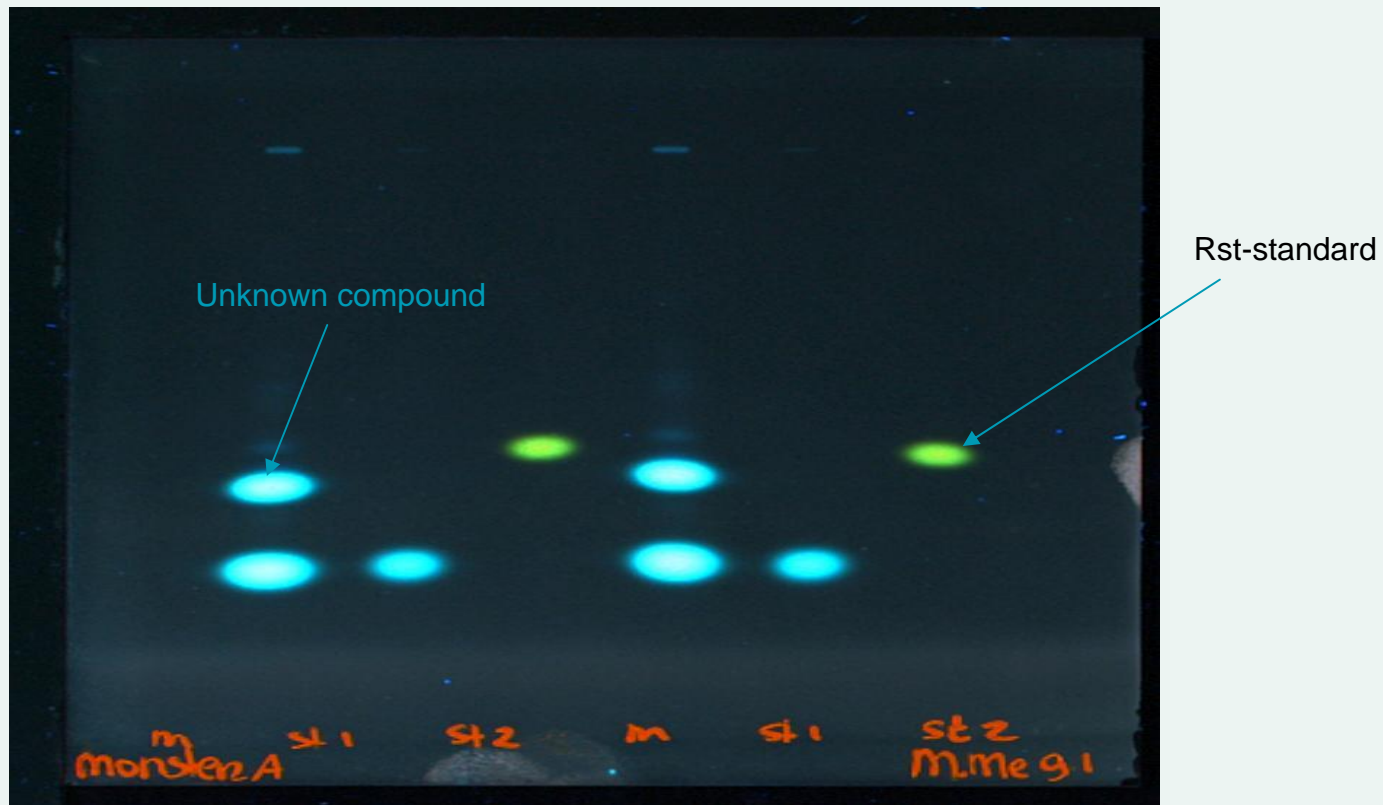
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HPTLC for the analysis of API-cleaning samples

- Mobile phase: Toluene : Ethyl Acetate 9:1
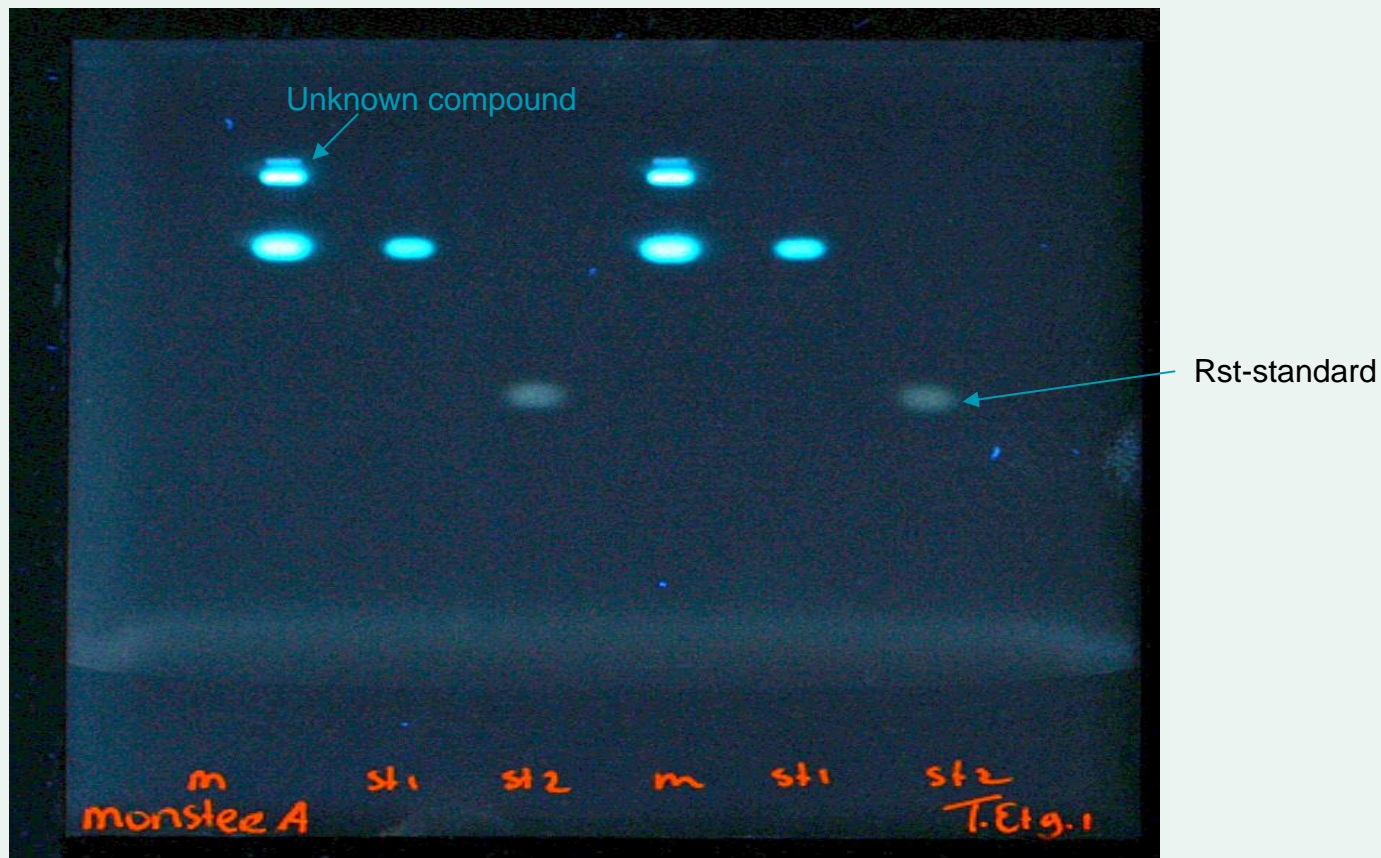
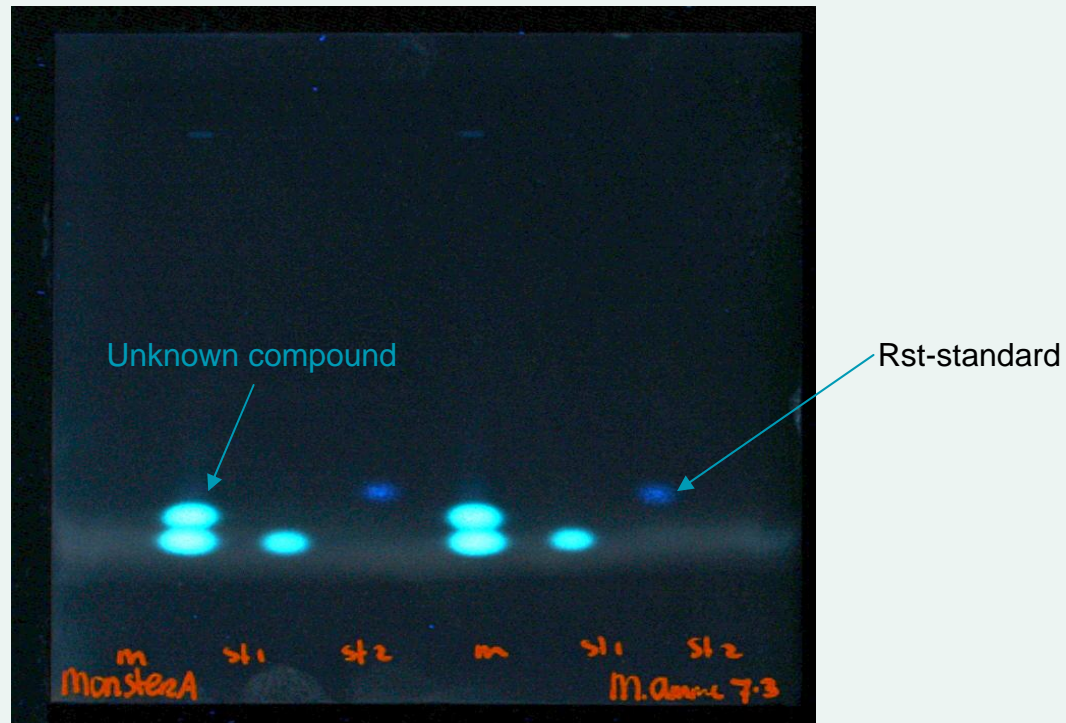# HPTLC for the analysis of API-cleaning samples

- Mobile phase Dichloromethane : Methanol 9:1

HPTLC for the analysis of API-cleaning samples

- Mobile phase: Dichloromethane : Ammoniacal methanol 7:3

Example database

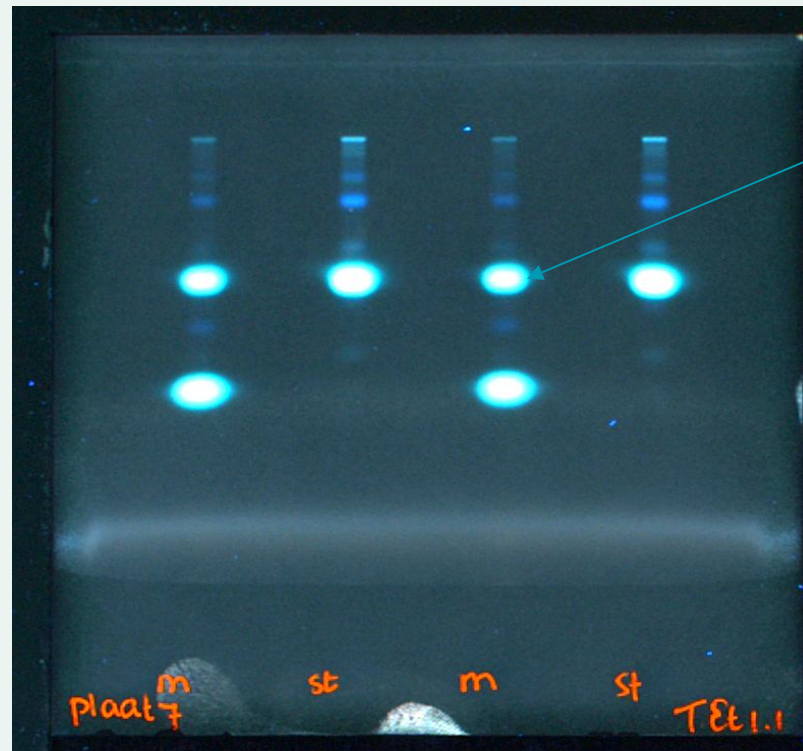|			T.Et 9.1		T.Et 1.1		M.Amme 7.3		M.Me 9.1		
Max deviation Rf value	0.14										
Max deviation Rst	0.24	Rf	Rst	Rf	Rst	Rf	Rst	Rf	Rst	
Measured Rf-value		0.03	0.07	0.3	0.64	0.85	1.06	0.64	1.07	
Rf-value standards		0.43		0.47		0.8		0.6		
Standard number										
			T.Et 9.1		T.Et 1.1		M.Amme 7.3		M. Me 9.1		
Product name	**Detection**	**Std nr**	**Rf 1**	**Rst 1**	**Rf 2**	**Rst 2**	**Rf 3**	**Rst 3**	**Rf 4**	**Rst 4**
Product 1	HS	1245	0.03	0.08	0.35	0.78	0.84	1.18	0.56	1.27
Product 2	HS	861	0.01	0.03	0.37	0.79	0.86	1.22	0.57	1.24
Product 3	UV	1006	0.06	0.17	0.26	0.57	0.83	1.25	0.56	1.08
Product 4	HS	502	0.04	0.12	0.39	0.76	0.89	1.27	0.51	1.13
Product A	CI-TMB	8490	0.02	0.04	0.3	0.81	0.8	1.09	0.5	1.04
Product Z	HS	1233	0.06	0.15	0.27	0.57	0.85	1.24	0.61	1.31

HPTLC for the analysis of API-cleaning samples
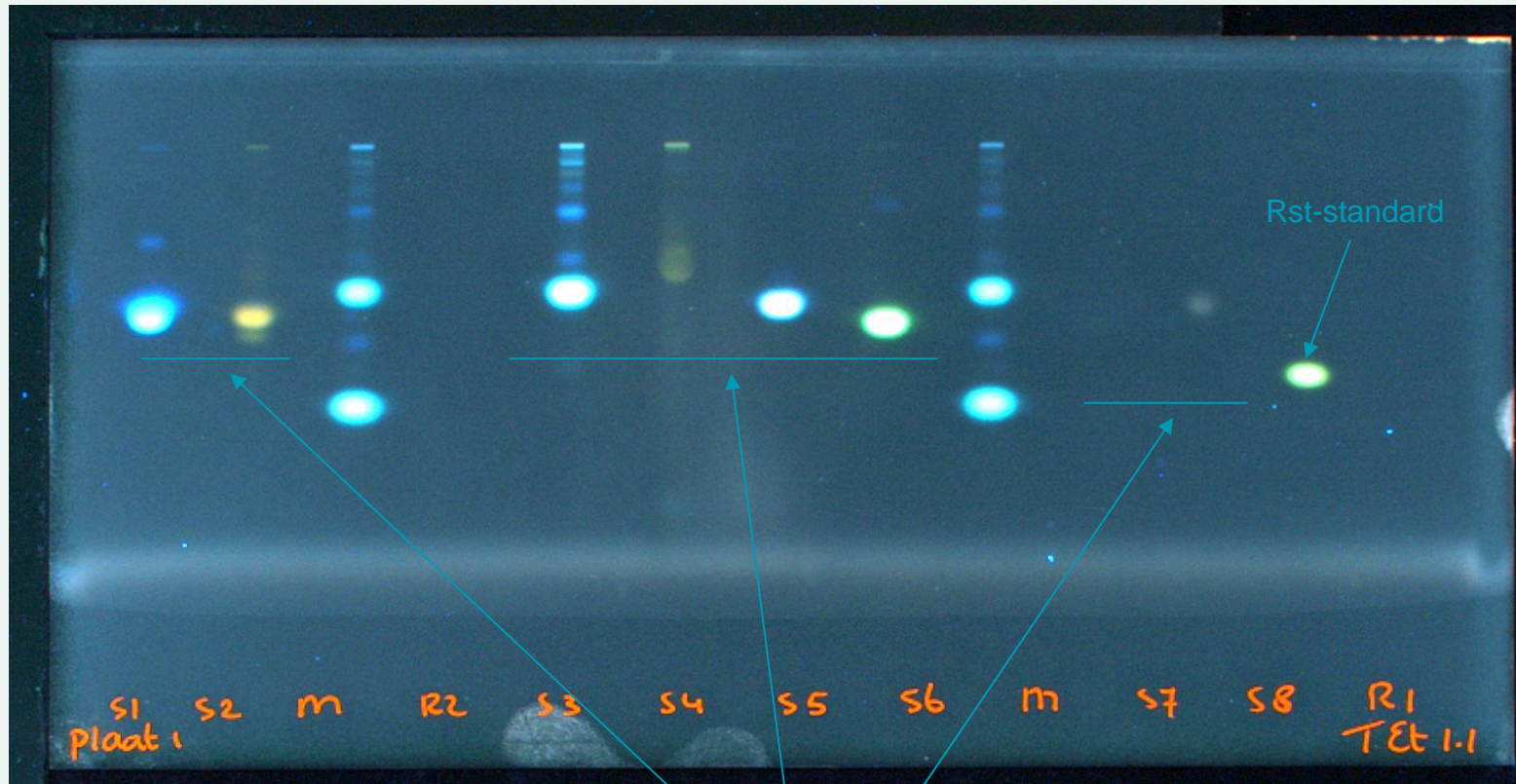
Selected standards from database

Rst-standard

HPTLC for the analysis of API-cleaning samples

- Final identification unknown compound

Unknown compound