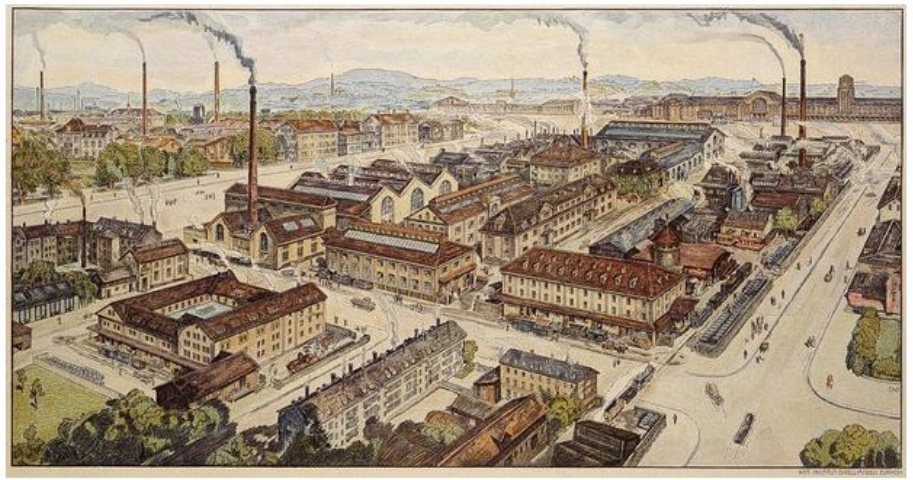


Die Fabrik von Johann Rudolf Geigy in Basel. Von Orell Füssli in Zürich um 1910 gedruckte Lithografie (Schweizerisches Nationalmuseum, Zürich). [...]

SEMI-QUANTITATIVE ANALYSIS OF ORGANIC PIGMENTS IN TATTOO INKS WITH HPLC

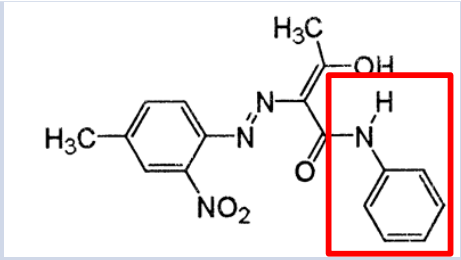
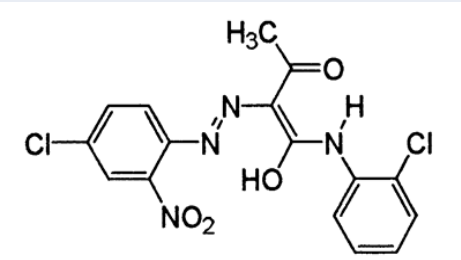
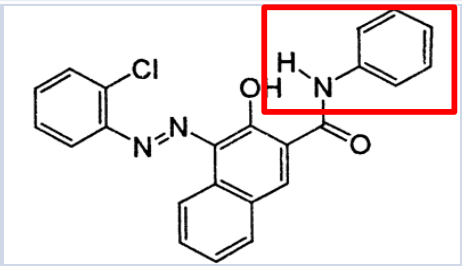
WORK in PROGRESS!

Urs Hauri, Urs Schlegel, Theresa Otz, Christopher Hohl

Regulation of colorants. What is - and what will be soon

Regulation	Res(AP)2008	REACH	Pigments found in Tattoo inks (Basel)
CPR Annex II	Do not contain	0.00005% (0.5 mg/kg)	C.I. 12075 (PO 4) C.I. 15585 (PR 53) C.I. 45170 (PV 10) C.I. 42535 (PV 3) (C.I. 74160 (PB 15) only bureaucratic entry!)
CPR Annex IV restricted	Do not contain	0.00005% (0.5 mg/kg)	C.I. 11680 (PY 1) C.I. 11710 (PY 3) C.I. 21100 (PY 12) C.I. 21108 (PY 83) C.I. 51319 (PV 23) C.I. 73900 (PV 19) C.I. 73915 (PR 122) (C.I. 74260 (PG 7))
Azo colorants	Azo cleavage (without limits and method)	0.1% (1000 mg/kg)	C.I. 11680 (PY 1) C.I. 11767 (PY 97) C.I. 12310 (PR 2) C.I. 12315 (PR 22) C.I. 12477 (PR 210) C.I. 12485 (PR 146) C.I. 21095 (PY 14), C.I. 21108 (PY 83) C.I. 21110 (PO 13)
others / dyes (ECHA table A)	Do not contain	0.1% (1000 mg/kg)	mostly unsuited for tattoo inks – easier to analyze, not covered in this presentation

Inconsistencies & the problem of negative lists

Pigment	Structure	Restricted Amine	Regular inks 2018-2021 (76 samples)	«Cheap inks» 2021 (55 samples)	Restriction
C.I. 11680 (PY 1)		Aniline	0%	0%	CPR Annex IV & ECHA Table A 0.1%
C.I. 11710 (PY 3)		None	0%	16%	CPR Annex IV; 0.00005% (2000 times less)
C.I. 12300 (PR 21)		Aniline	0%	9%	No restriction

Why a selection of colorants? – why not a negative list based on structure?

Yellow and Orange Pigments 2018-2021

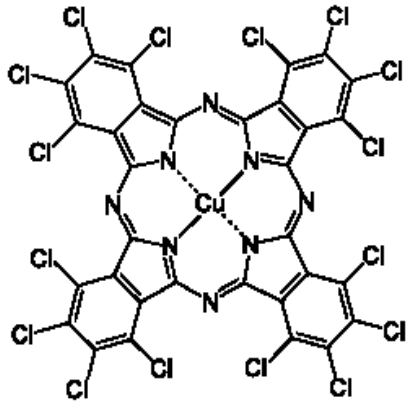
E-Commerce inks			Regular inks		
21090	PY 12	29%	21095	PY 14	37%
11741	PY 74	23%	11741	PY 74	13%
Unidentified		20%	56300	PY 138	11%
21095	PY 14	18%	21110	PO 13	8%
11710	PY 3	16%	11740	PY 65	4%
21110	PO 13	14%	21108	PY 83	4%
Unidentified Isomer of 11738		11%	561170	PO 73	4%
Unidentified		4%	11767	PY 97	3%

Forbidden according to REACH

- Three unidentified yellow pigments with high incidence
- Negative list based on structure could include such substances eventually

Missing Positive List for Pigments – Do we do the right thing? (first tattoo conference in Copenhagen 2013)

Example:



~~C.I. 74260~~

Still used unallowed main green pigment

Chlorinated

Allowed for cosmetics
safe for eye products

→

Not allowed in tattoo inks!

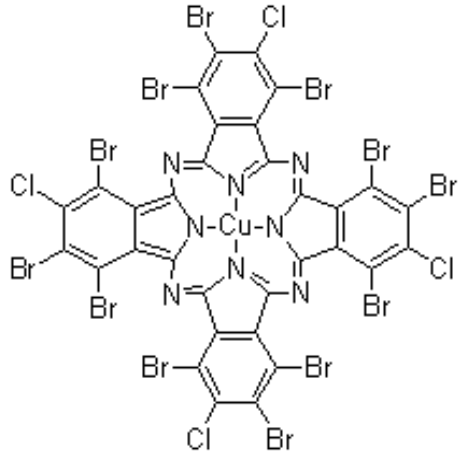
Main replacement pigment

Chlorinated & Brominated

Not allowed in cosmetics

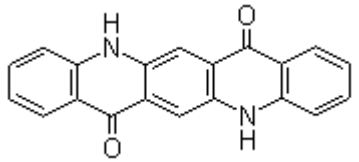
→

Allowed in tattoo inks!
But safer?

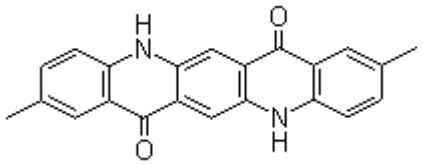


C.I. 74265

Missing Positive List for Pigments – Do we do the right thing?



~~C.I. 73900~~



~~C.I. 73915~~

Main magenta pigments

Not allowed in Leave on Cosmetics

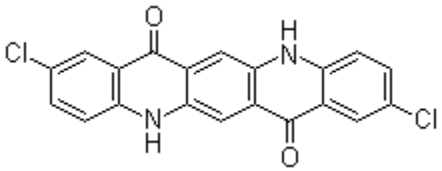
Not allowed in tattoo inks

Main replacement pigment

Chlorinated quinacridone

Not allowed in cosmetics

Allowed! But safer?



C.I. 73907

Colorants – Basel-City Market Surveillance on tattoo inks [1,2)]

Year	Analysed Samples	Samples with banned pigments
2014[1)]	206	24%
2020[2)]	85	22%

- Many producers still don't seem to know their products – ***or do they?***

Reports in english language to be found on
https://www.kantonslabor.bs.ch/berichte/non-food.html

1) https://www.kantonslabor.bs.ch/dam/jcr:d12e5456-c71d-4e59-8f29-4a7d8c38d15d/Tattoo_PMU_2014_EN(UK).pdf

2) https://www.kantonslabor.bs.ch/dam/jcr:d71126f3-85c9-42fb-a16d-8d21f7fc5ee8/2020-Tattootinten.Englisch.pdf

Qualitative Analysis – Going for the main pigments!

Common feature of pigments: **Extremely low solubility**

➢ **Difficult to analyse with chromatographic methods**

In Combination

- Laser Desorption Ionisation -ToF-Mass Spectroscopy*
- Liquid Chromatography coupled to UV/VIS and Mass Spectroscopy
- UV/VIS Spectroscopy

allow for the detection of forbidden or falsely labelled pigments

Quantitative Analysis?

That's where its getting harder still!

* Niederer M, Hauri U, Kroll L and Hohl C. Identification of organic pigments in tattoo inks and permanent make-up using laser desorption ionisation mass spectrometry [version 2; peer review: 2 approved]. *F1000Research* 2018, **6**:2034

HPLC-Method - First step: Screening

1. 1 Drop (10-20 mg) is extracted sequentially with small volumes (2 ml) of

   Dimethylformamide (DMF), N-Methylpyrrolidone (NMP) and Chloronaphthalene (CLN)

   using an ultrasonic homogenizer!
2. Centrifugation -> Colored? -> Filtration -> HPLC
3. Colored residue? -> second solvent

Chromatography

- DMF/NMP-Extracts — Standard RP-HPLC method for colorants
- CLN-Extracts — RP-HPLC method with NMP/Acetonitrile at 80°C

(U)HPLC-DAD

2 Solvent selection valves

- Phosphate buffer pH 6 & NMP/Water
- Methanol/Water & NMP/Acetonitrile

Quaternary UHPLC Pump

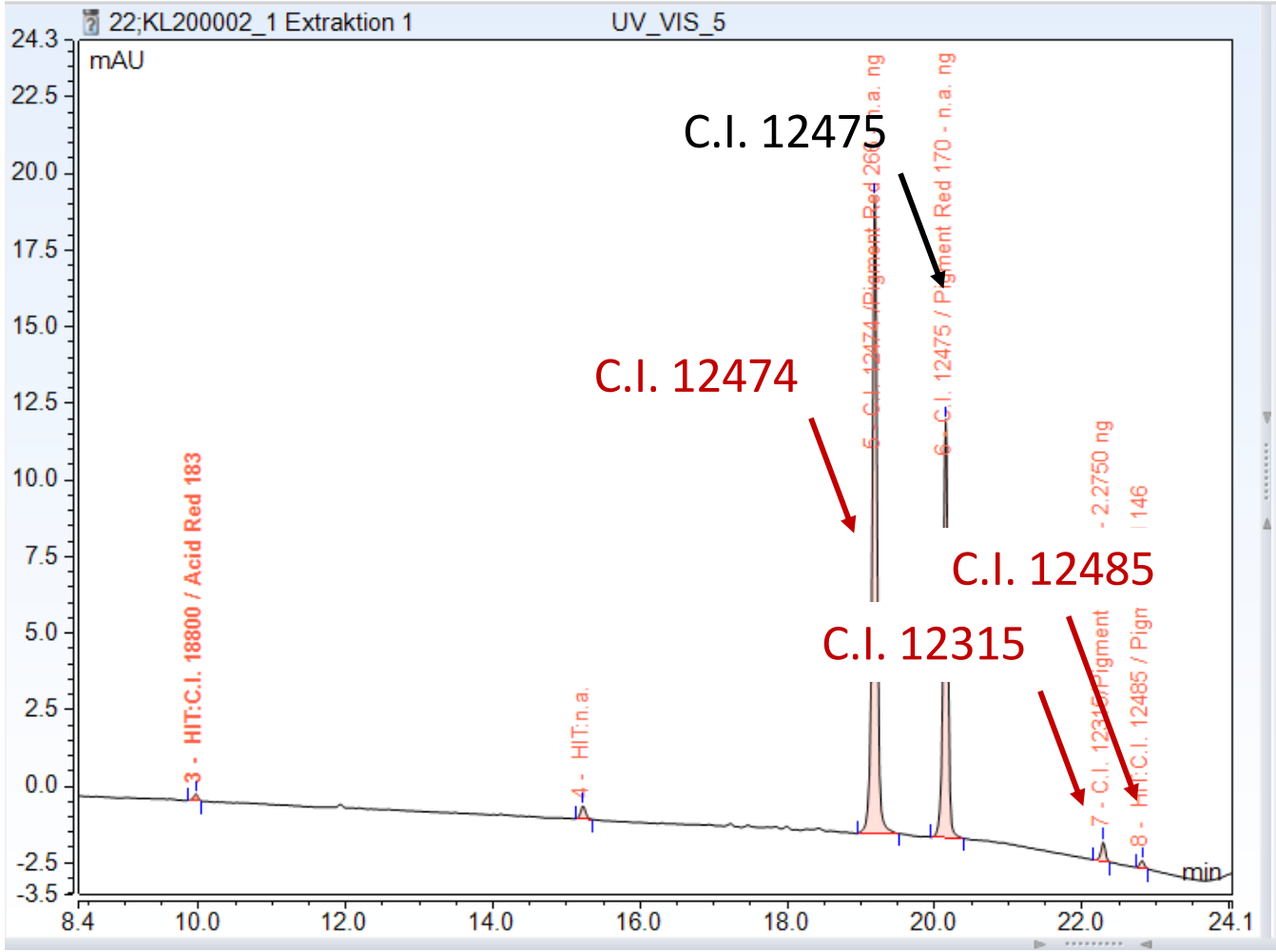
2 Column Selection Valves

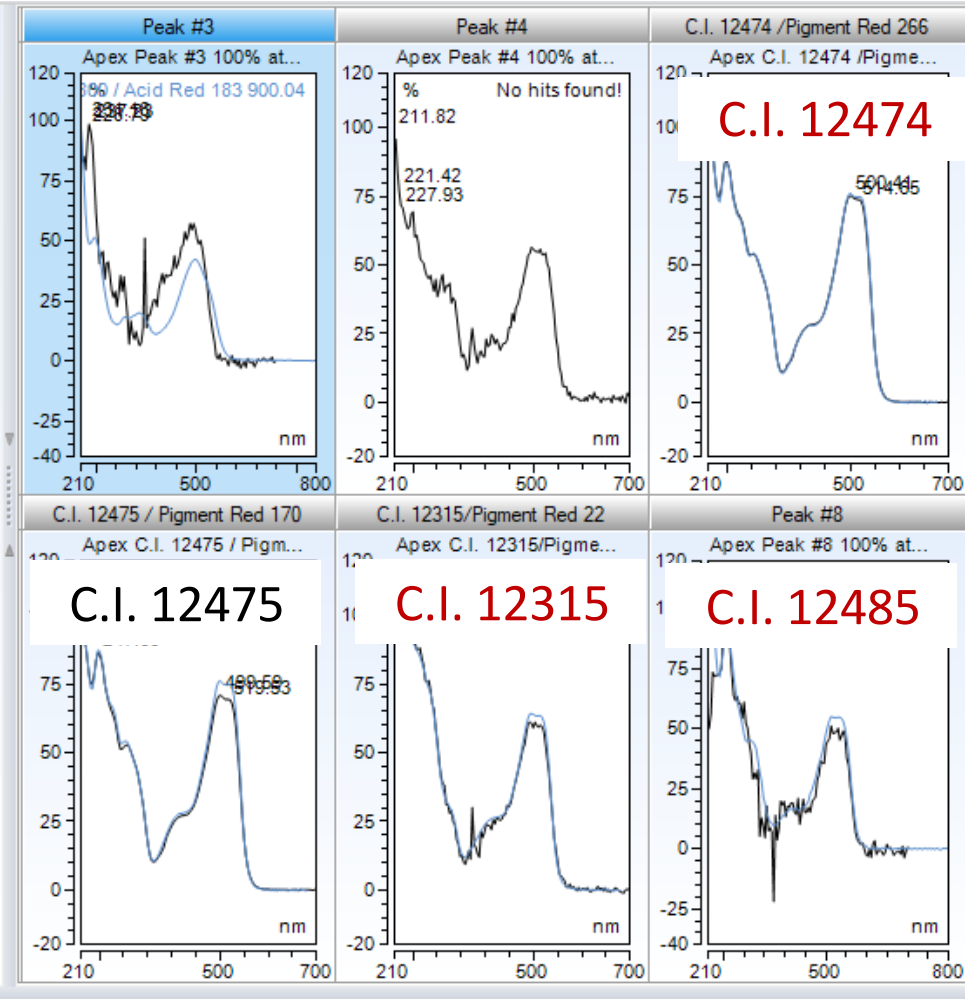
2 Column thermostats

- 40°C & 80°C

Samples of different extraction procedures can be run overnight

Example: Screening of DMF-Extract - 3 restricted candidates





Chromatogram @ 480 to 560 nm

UV/Vis Spectra of colorants against library

Semiquantitative Determination

Screening Result -> Solvent, Chromatography

1. 10-20 mg extracted with 2 ml of solvent (homogeneizer)
   -> take an aliquot for HPLC -> traces
2. Dilute 1:20 -> ultrasonicate with homogeneizer
   -> take an aliquot for HPLC -> tinting colorants, main pigments
3. Dilute 1:20 -> ultrasonicate with homogeneizer
   -> take an aliquot for HPLC -> main pigments

➢ Of course, dilution steps are adapted if necessary!

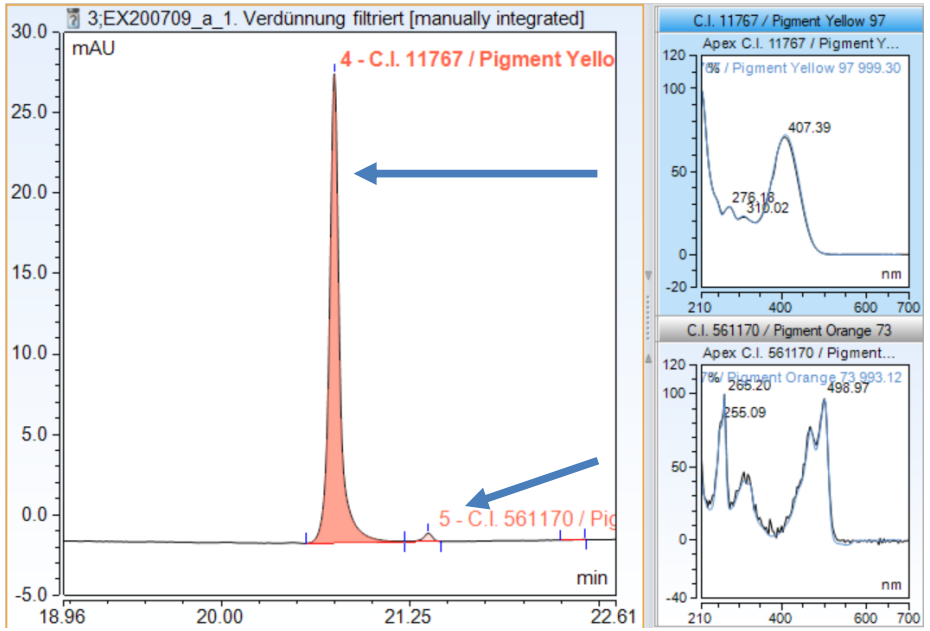
1. 2. (1:20) 3. Extract (1:400)

Mono Azo-pigments

Sample	Pigment	DF	Result HPLC	Result UV/Vis	
EX200709	C.I. 11767 (PY 97)	1:200	21% ± 3%*	21% ± 2%*	✓
EX200708	C.I. 12370 (PR 112)	1:400	37% ± 2%*	35%*	✓

* Assumption: reference substance = 100%

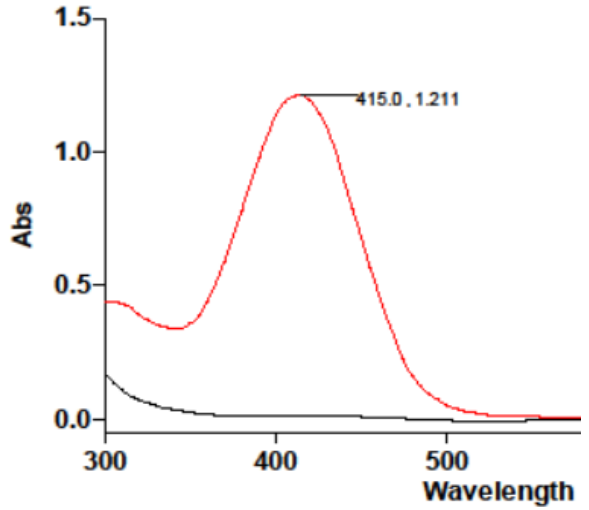
Determination with HPLC



Sample contains mainly C.I. 11767

With only traces of C.I. 561170

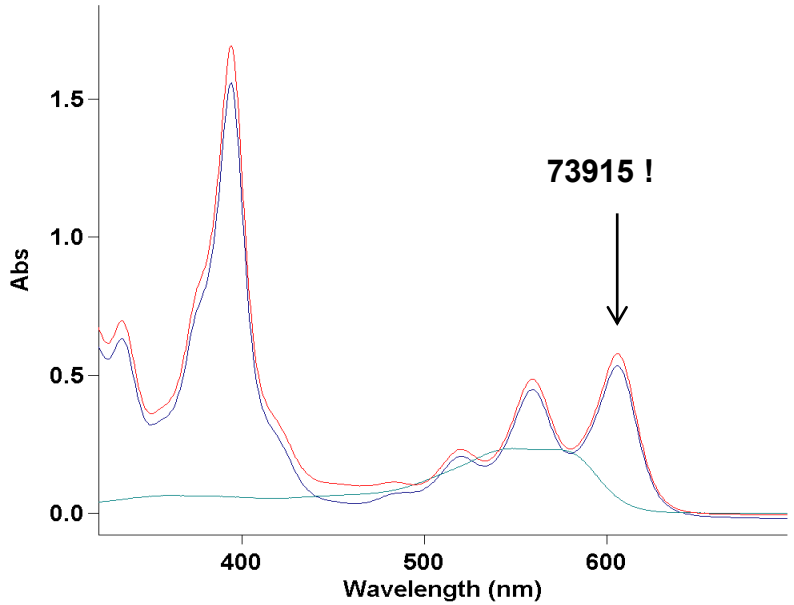
Determination with colorimetry:



Quinacridones

Sample	Color	Pigment	DF	Result HPLC	Result UV/VIS		Disclosed
422	Magenta	C.I. 73900 (PV 19)*	1:40	12.3± 0.2%**	13.1%	✓	C.I. 45170:2
152	Purple	C.I. 73915 (PY 122)*	1:80	26.5%**	27.6%	✓	C.I. 12475
251	Magenta	C.I. 73915 (PR 122)*	1:80	32.1%**	33.2%	✓	C.I. 15865

* undisclosed; ** Assumption: reference substance = 100%

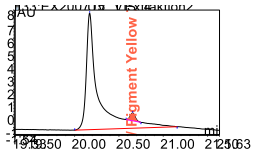


Semi quantitative determination of pigments
Hauri – BfR Berlin 2021

Undisclosed impurities(?) or tinting substances (?)

Sample	Color	Pigment	Ext	Result	RSD	Main Pigment	Disclosed
715	Red	C.I. 11741 (PY 74)*	2	0.220%**	2.8%	C.I. 12477	C.I. 12315
705	Green	C.I. 11741 (PY 74)*	2	0.117%**	16%	C.I. 74260*	C.I. 74160, 21095
148	Orange	C.I. 12315 (PR 22)*	2	1.09%**	2.5%	C.I. 21108*	C.I. 21095, 21160

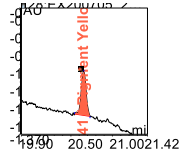
* undisclosed; ** Assumption: reference substance = 100%



← C.I. 12475
(red)

Red sample

Green sample
(only C.I. 74260 visible with the eyes)



↑ C.I. 11741
(yellow)

C.I. 11741
(yellow)

The reference substance – an example

«well definded» cosmetic pigment, sold for cosmetic purpose

INCI	CI 15985
PCPC name	Yellow 6 lake
CAS No.	15790-07-5

Pure dye content / Teneur en colorant pur	15 - 30%
Aluminum hydroxide content / Teneur en alumine	70 - 85%
Soluble chlorides and sulfates (as sodium salts) / Chlorures et sulfates solubles (comme sels de sodium)	2% max
Inorganic matter, insoluble HCl / Matières inorganiques, insolubles HCl	0,5% max
Intermediates, subsidiary colors / Intermédiaires, colorants accessoires	Passes test / Conforme
Fastness to light / Stabilité lumière	Moderate / Moyenne

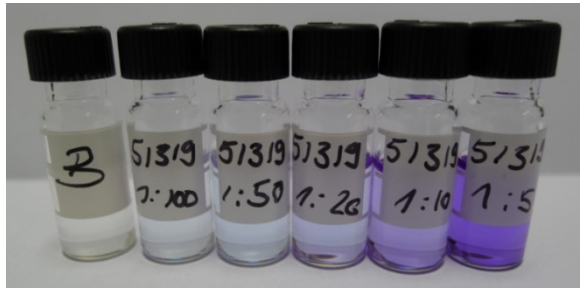
Heavy metals / Métaux lourds [ICP analysis] (ppm)

Lead / Plomb	0 - 2	Zinc / Zinc	0 - 100
Arsenic / Arsenic	0 - 3	Copper / Cuivre	0 - 50
Mercury / Mercure	0 - 1	Barium / Baryum	0 - 50
Chromium / Chrome	0 - 50	Cadmium / Cadmium	0 - 1
Nickel / Nickel	0 - 50	Antimony / Antimony	0 - 10

1. This is a good specification but not a certificate
2. Assuming a content of 22.5% the uncertainty is +/- 33%
   (we could use the lower limit)
3. The content is only 22.5% what about our other references?

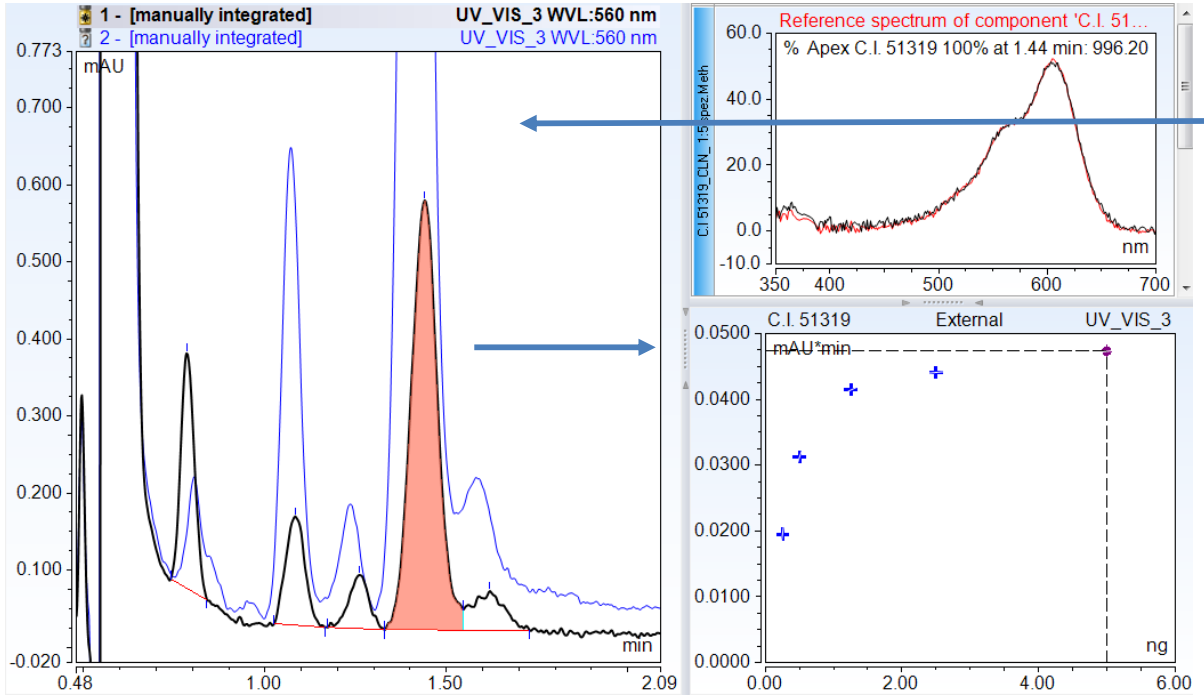
For tattoo pigments:
Often our references are of technical quality only!

The tricky ones – e.g. C.I. 51319 / PV 23



C.I. 51319 / Pigment Violet 23
Serial dilution in Chloronaphthalene (CLN)

CLN-Sample Solutions -
Difficult/Problematic to analyse with HPLC

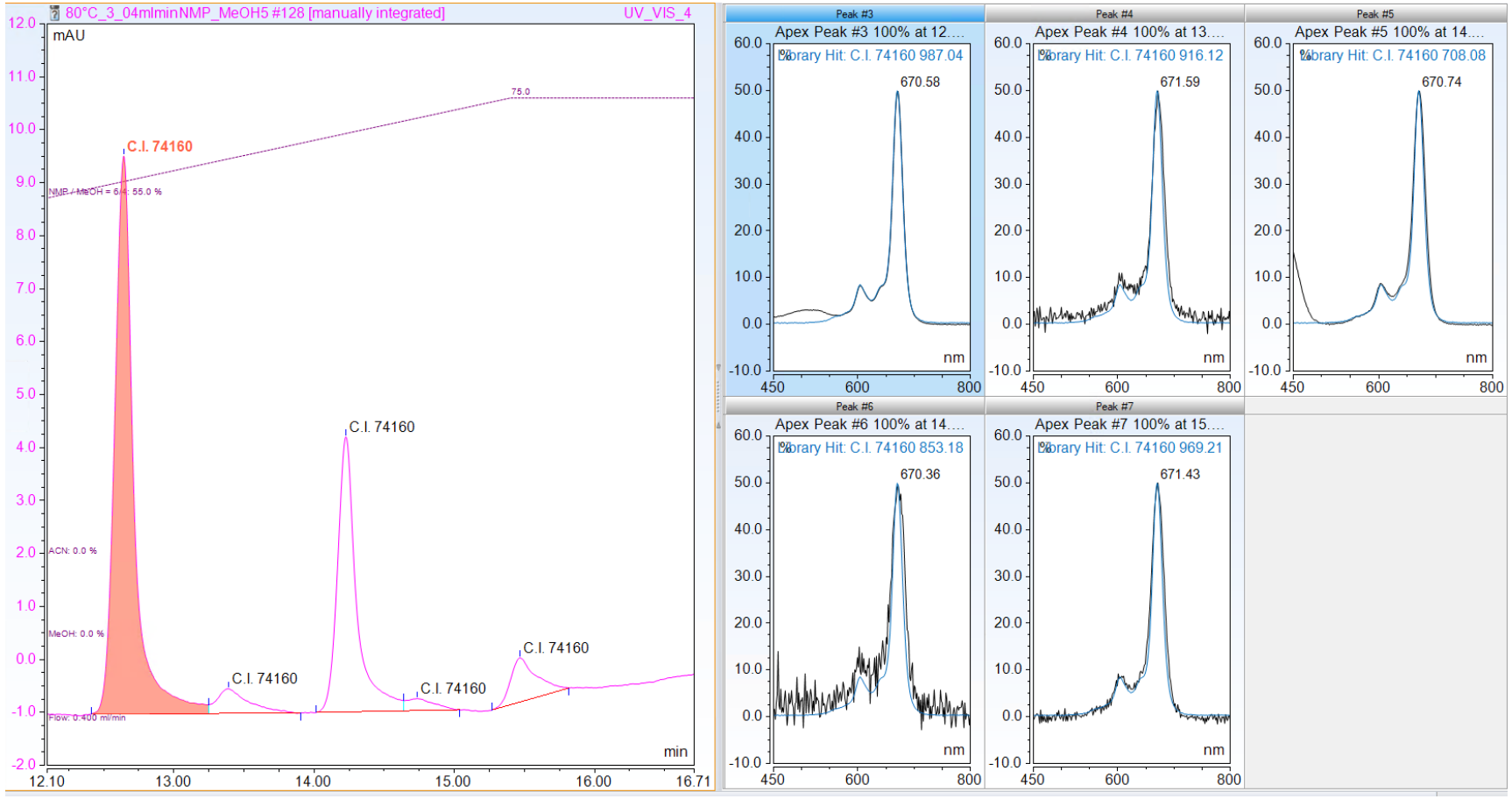


Higher solubility of C.I. 51319 in the sample (blue chromatogram)
Compared to reference (black)

1 Colorant – Many Peaks! **Calibration**

What about Quantitation of C.I. 74160?

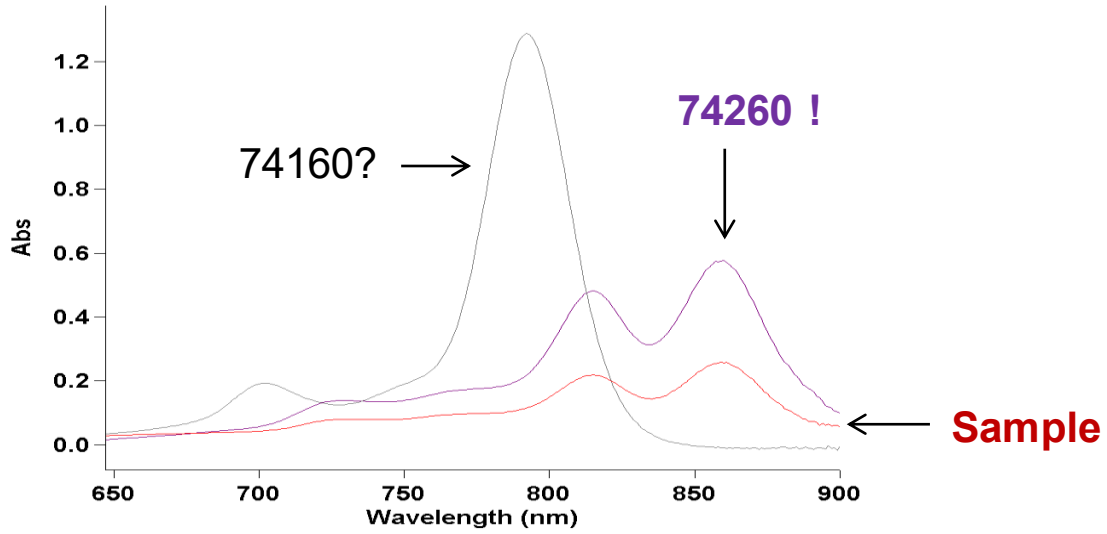
- C.I. 74160 seems feasible with HPLC – but poor solubility, many peaks
- UV/VIS spectroscopy is much easier (for the main pigment)



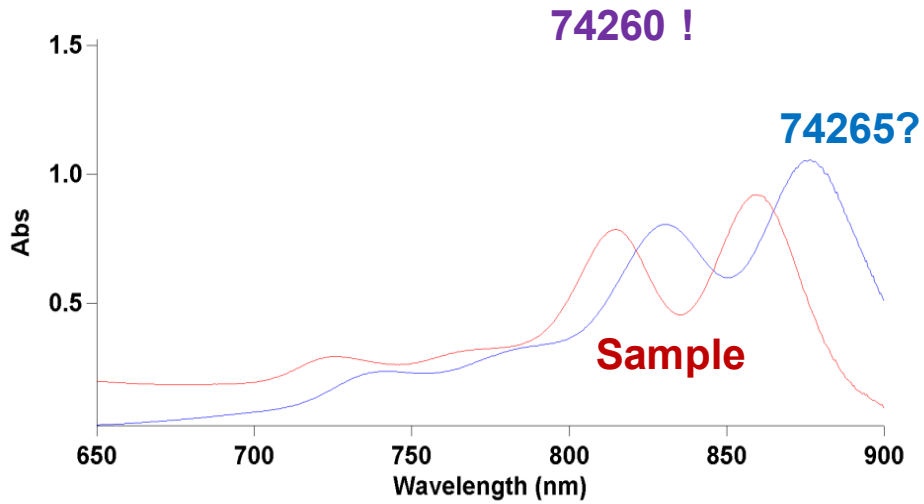
And C.I. 74260?

- There might be options for HPLC we didn't try so far
- But if you don't want to detect/quantify traces **-> (UV-)VIS-Spectroscopy**
- **Until now, only C.I. 74260 was forbidden:**

Green ink –
Disclosed pigments: C.I. 74160 (blue) + 11740 (yellow)



Green ink –
Disclosed pigment: C.I. 74265 (green)



Conclusion

- The determination of mono-azo-pigments with HPLC is established at 0.1%.*
- Quinacridones 73900 and 73915 seem to be feasible too*
- For diazo-pigments, big differences in feasability between

  «easy» 21090 > 21095, 21110, 21160 > 21100, 21108* «very difficult»
- Other tricky restricted pigments are: 51319 and 74160* or a lot of hitherto unrestricted….

  -> UV/VIS spectroscopy is a good option (safe for traces)
- Further: We have no references for: 12390, 12420, 12465, 12466, 21096, 21107

  (which shouldn't be an invitation to producers ;-)

*BUT

Any determination remains semi-quantitative (big measurement uncertainty!) if no quantitative references are available!

Thank you for your attention!

And thanks to my coworkers:

- Christopher Hohl,
- Markus Niederer
- Urs Schlegel,
- Theresa Otz