23rd – 24th April 2015

Alternatives for Skin sensitization testing and assessment:
a joint Cefic-LRI / Cosmetics Europe / EPAA workshop

Exceptions to the Rule – Conflicting Hazard Data and Applicability Limits

Winfried Steiling – Henkel AG & Co KGaA, Helsinki / FIN, 23rd April 2015

VERBAND DER CHEMISCHEN INDUSTRIE e.V.
WIR GESTALTEN ZUKUNFT.
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Point of Departure – Comparison of 32 Chemicals *in vitro/in vivo*

structure	DPRA	h-CLAT	KeratinoSens	LuSens	MUSST	LLNA
dihydroxy naphthaline	./.		-		-	
amino chloro phenole			-		-	
dihydroxy pyridine	./.		-		-	
amino pyridine			-		-	
diamino toluene			-		-	
tetraamino pyrimidine	./.	-	-	-	-	
amino chloro cresol		-	-	-	-	
dihydroxy pyridine	./.	-	-	-	-	
hydroxy pyridine	./.	-	-	-	-	
pyrimidine triamine	./.	./.		-	-	-
phenylpyrimidine triamine		./.		-	-	-
pyrimidine triamine		./.		-	-	inconclusive
bis(aminophenyl) dimethyl azanium	./.	./.		-	-	inconclusive
phenyl imidazol propyl amine	./.	./.		-	-	
aminofunctl. polysiloxane, methoxy & ethoxy term		./.		-		
N-(3-(trimethoxysilyl) propyl)ethylene-diamine				-		
propylpipyridyl functionalised silicone		./.	./.	-	./.	
propylpipyridyl functionalised silicone	./.	./.	./.	-	./.	
aminoethylamino-iso butylmethyl dimethoxy silane				-		
aminosil-sesquioxane, methoxy-terminated				-		-
methylamino-siloxane with glycidyl trimethyl-ammonium chloride		./.		-	./.	
reaction product of vinyltriacetoxy-silane, glycidoxypropyltrimethoxysilane				-		
flavanoid	./.			-	-	
cyclohexyl derivative, ester				-	-	
ascorbic acid conjugate				-	-	
chromone	./.			-	-	
cyclohexyl derivative, ether				-	-	
pyrimidine structure				-	-	
cyclohexyl derivative, ether				-	-	
cyclohexyl derivative, ester				-	-	
small sugar				-	-	
ascorbic acid conjugate				-	-	

Consistent *in vitro/ in vivo* test results only 5/32 (16%)

	indication for a non-sensitizer
	indication for a sensitizer
-	not tested
./.	not applicable/testable

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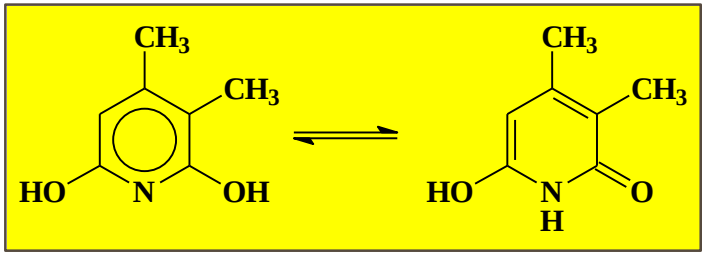
Critical Solubility

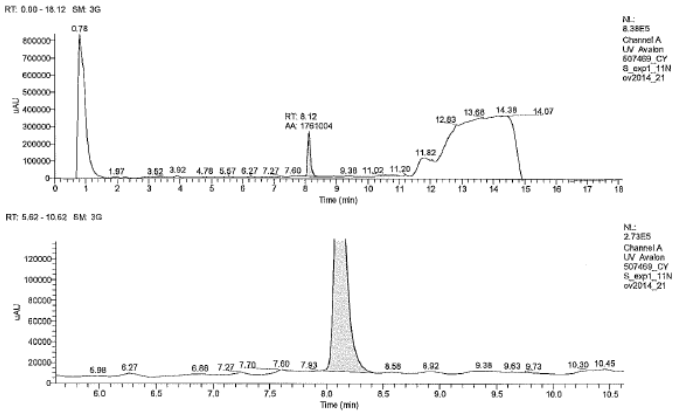


Solvents:

- *Non-soluble in:*
  ACN, water, IPA, acetone, ACN/water, DMSO/ACN (1:9)
- *but soluble in:*
  DMSO/ACN (1:1)

Sample Name: RCcysCDMSO/acn-1
Data File: 507469_CYS_exp1_11Nov2014_21
Operator: HEA
Original Data Path: D:\PROJECTS\507469\CYSTEINE
Acquisition Date: 11/12/14 09:15:18 PM
Instrument Method: D:\Projects\SOP Methoden\DPRA\cysteine and lysine determination
Injection Volume(µl): 4.00
Run Time(min): 20.05

Channel A = 220 nm
Channel B = 258 nm



Suitability for the HPLC

Sample code	Peak area at 220 nm (µAU)	Concentration (mM)
RCcysA-1	2664149	0.512
RCcysA-2	2565672	0.493
RCcysA-3	2688381	0.516
	Mean	0.507
	SD	0.013

Stability of the reference

Sample code	Peak area at 220 nm (µAU)	Concentration (mM)
RCcysC-1	2644480	0.508
RCcysC-2	2663053	0.511
RCcysC-3	2629268	0.505
	Mean	0.508
	SD	0.003

Impact on the depletion

Sample code	Peak area at 220 nm (µAU)	Concentration (mM)
$RCcysC_{DMSO/ACN}$-1	1761004	0.337
$RCcysC_{DMSO/ACN}$-2	1937311	0.371
$RCcysC_{DMSO/ACN}$-3	1714760	0.328
	Mean	0.345
	SD	0.023

SD = Standard Deviation

Conclusion: → List of appropriate solvents should be extended

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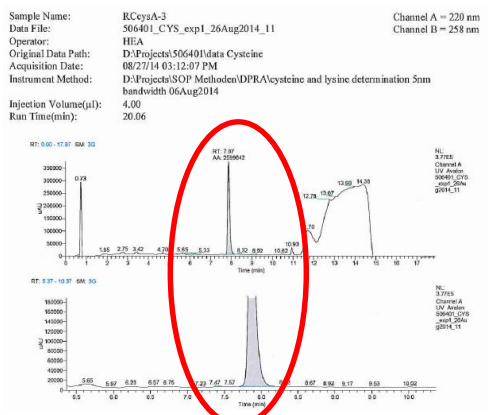
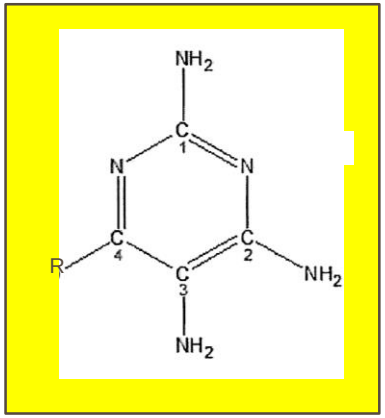
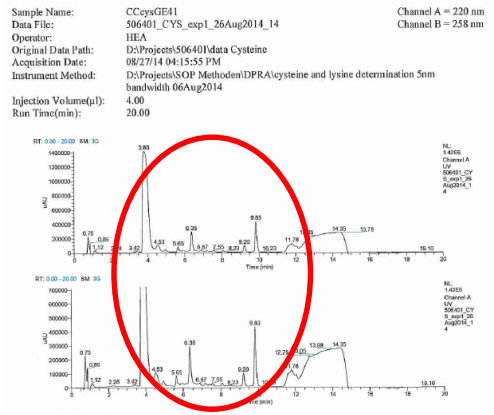
Instability of a Test Substance with the Used Solvent

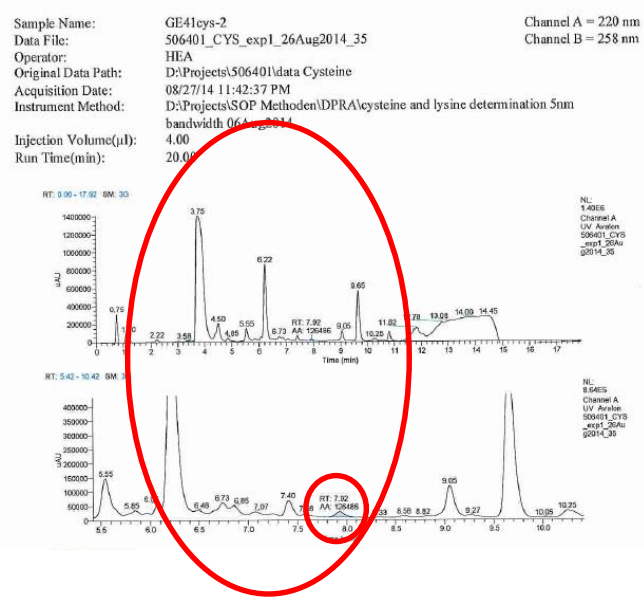


Component Name	Area	RT
Cysteine_220nm	2599642.39	7.87
Cysteine_258nm	149863.31	7.87





→ No co-elution with the peptide

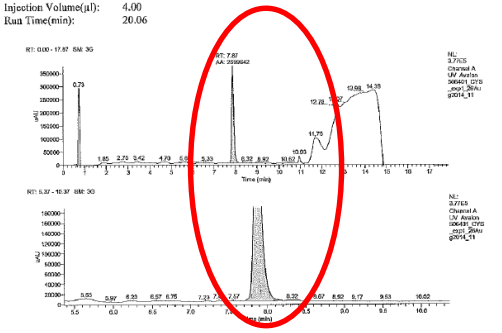


Purity:
99.0 area% (HPLC)
96.7 wt% (quant. ^{1}H-NMR)

Conclusion: → Impact on peptide depletion by solvent/test item reactivity

Co-elution of the Test Substance with Cys-Peptide

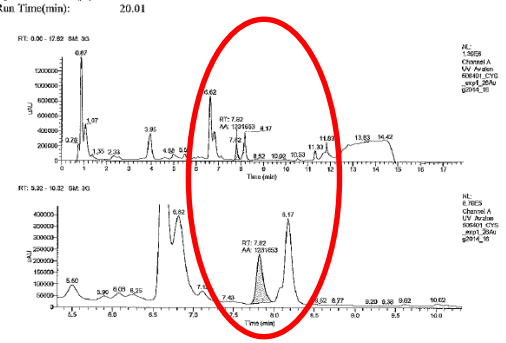
Sample Name: RCcysA-3
Data File: 506401_CYS_exp1_26Aug2014_11
Operator: HEA
Original Data Path: D:\Projects\506401\data Cysteine
Acquisition Date: 08/27/14 03:12:07 PM
Instrument Method: D:\Projects\SOP Methoden\DPRA\cysteine and lysine determination 5nm bandwidth 06Aug2014
Injection Volume(µl): 4.00
Run Time(min): 20.06
Channel A = 220 nm
Channel B = 258 nm



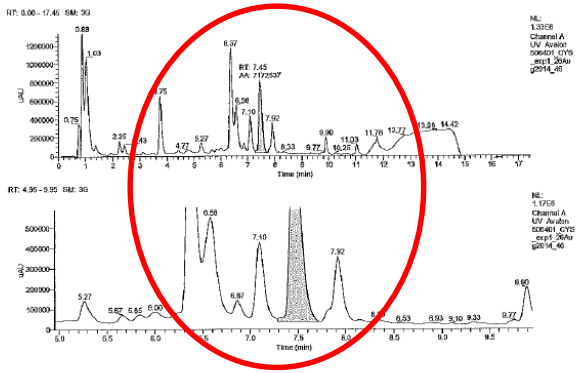
Component Name	Area	RT
Cysteine_220nm	2599642.39	7.87
Cysteine_258nm	149863.31	7.87

Phenyl imidazole propyl amine

Sample Name: CCcysKN172
Data File: 506401_CYS_exp1_26Aug2014_16
Operator: HEA
Original Data Path: D:\Projects\506401\data Cysteine
Acquisition Date: 08/27/14 04:58:28 PM
Instrument Method: D:\Projects\SOP Methoden\DPRA\cysteine and lysine determination 5nm bandwidth 06Aug2014
Injection Volume(µl): 4.00
Run Time(min): 20.01
Channel A = 220 nm
Channel B = 258 nm



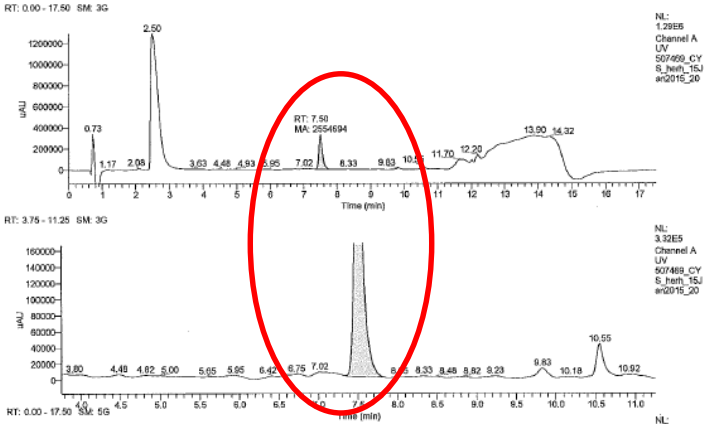
Sample Name: KN172cys-3
Data File: 506401_CYS_exp1_26Aug2014_46
Operator: HEA
Original Data Path: D:\Projects\506401\data Cysteine
Acquisition Date: 08/28/14 03:36:35 AM
Instrument Method: D:\Projects\SOP Methoden\DPRA\cysteine and lysine determination 5nm bandwidth 06Aug2014
Injection Volume(µl): 4.00
Run Time(min): 20.04
Channel A = 220 nm
Channel B = 258 nm

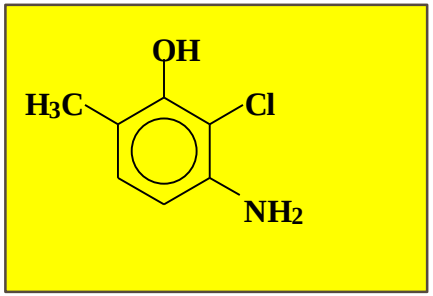


Conclusion: → Co-elution

Conflicting Hazard Data

Sample Name:	SAT140080cys-1	Channel A = 220 nm
Data File:	507469_CYS_herh_15Jan2015_20	Channel B = 258 nm
Operator:	HEA	
Original Data Path:	D:\PROJECTS\507469\CYSTEINE	
Acquisition Date:	01/16/15 03:43:56 PM	
Instrument Method:	D:\Projects\SOP Methoden\DPRA\cysteine and lysine determination	
Injection Volume(µl):	4.00	
Run Time(min):	20.04	





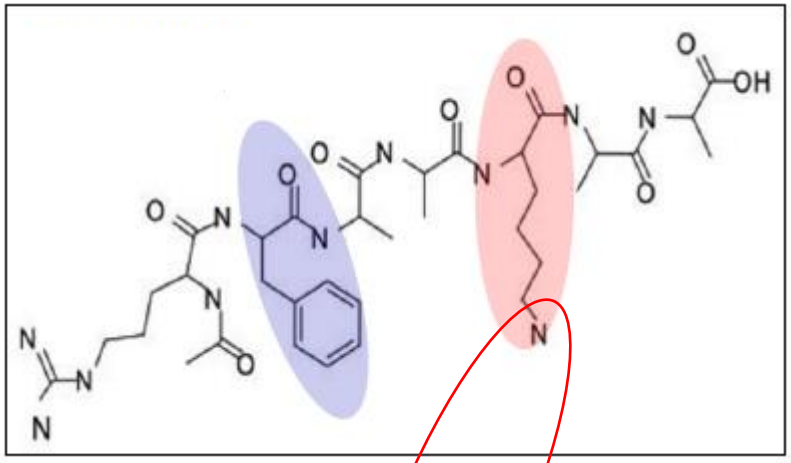
Test substance	SPCC depletion		SPCL depletion		Mean of SPCC and SPCL depletion	Reactivity class	
	Mean	± SD	Mean	± SD		Cysteine 1:10 / Lysine 1:50 prediction model	Cysteine 1:10 prediction model
SAT 140080	8.2%	± 4.4%	9.5%	± 5.2%	8.8%	Low reactivity	NA

SD = Standard Deviation; NA = Not Applicable

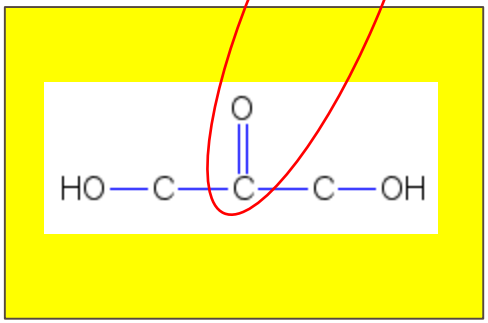
Conclusion: → low sensitization potential *versus* neg. LLNA

Peptide Reactivity

Lys-peptide



Schiff's base



REACH conclusion:

1,3-dihydroxyacetone

General Information

Not classified	✓
Implementation	EU

Peptide Reactivity associated with Skin Sensitization: A Comparison of the DPRA with Computational Programs

Urbisch D[1], Honarvar N[1], Mehling A[2], Kolle SN[1], Becker M[1], Teubner W[3], Landsiedel R[1]

81. Jahrestagung DGPT 2015, Kiel, P349

Conclusion: → Predicted for sensitization *versus* neg. LLNA

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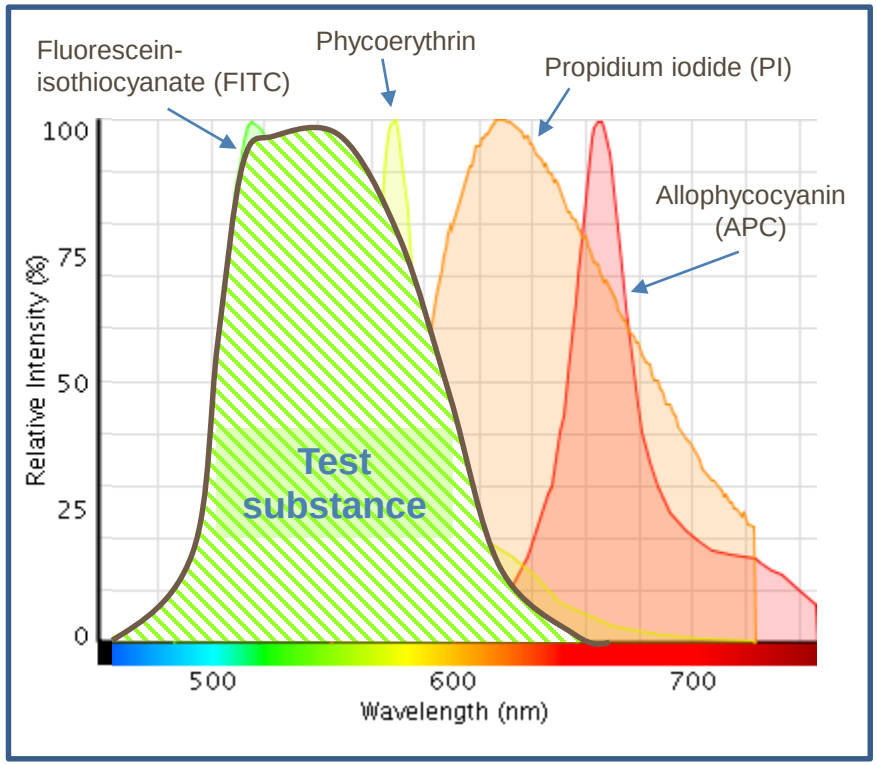
Auto-Fluorescence in Flow Cytometry



Antibody labelling of CD 86 and CD54 should not be limited to FITC!

Cytotoxicity measurement should not be limited to PI (CV75 request)!

Conclusion: → More flexibility in fluorescence markers for the h-CLAT

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Summary and Conclusion

Scientific expertise is needed to correctly read such *in vitro* test results.

- The possible impact of solvents, chemical reactivity and stability of test systems have to be taken in to account
- To better cover the diversity of chemical/physical parameter of chemicals the flexibility of standard protocols should be increased

Conclusion:

The premise of using individual *in vitro* test results for the decision on skin sensitization (ITS, IATA) is the applicability of the test method and the reliability of the resulting data.

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