

AROMATISCHE AMINES

Analyse met GC

Werkgroep organische analyses – 10/10/2019

Aromatische amines	Indelingscriterium GS (Vlaamse bijlage 2.3.1) (µg/L)	30% norm - LOQ (µg/L)
monochlooranilines		
2-chloroaniline	Σ1	Σ0.3
3-chloroaniline		
4-chloroaniline		
dichlooranilines		
2,3-dichloroaniline	Σ0.2	Σ0.06
2,4-dichloroaniline		
2,5-dichloroaniline		
2,6-dichloroaniline		
3,5-dichloroaniline	Σ20 (3,4-dichlooraniline+1,2- dichloorbenzeen)	Σ6 (3,4-dichlooraniline+1,2- dichloorbenzeen)
3,4-dichloroaniline		
chloortoluidines		
2-chloro-4-methylaniline	Σ8	Σ2.4
3-chloro-2-methylaniline		
4-chloro-2-methylaniline		
2-chloro-5-methylaniline		
2-chloro-6-methylaniline		
4-chloro-3-methylaniline		
dichloorbenzidines		
3,3-dichlorobenzidine	Σ0.5	Σ0.15
4,4-diamino-2,2-dichlorobiphenyl		
andere		
2-amino-4-chlorophenol	10	3
4-chloor-2-nitroaniline	2	0,6
benzidine	0,6	0,18
4,4-diaminodifenylmethaan	REACH kandidaatslijst van zeer zorgwekkende stoffen voor autorisatie	0,1
4,4'-Methylene-bis(2-chloroaniline)	idem	0,1
2,4-diaminotoluene	idem	0,1

Gewenste LOQ (µg/l)

0.1

0.01

0.4

0.07

3

0.6

0.18

0.1

0.1

0.1

METHODE ONTWIKKELING

UPLC-MS – directe injectie

- LOQ dichlooranilines is te hoog (LOQ = 1.1 µg/l; LOQ-eis = 0.06 µg/l)
- Terugvindingen te laag en %RSD te hoog
- met SPE: wisselende recoveries op Oasis HLB

GC/MS na derivatisering

- directe derivatisering met azijnzuuranhydride bij pH 7 -> niet alles gederivatiseerd
- directe derivatisering met benzoylchloride bij pH 7 -> niet alles gederivatiseerd
- derivatisering na extractie met heptafluorobutyric anhydride (HFBA)
 - alle componenten worden terug gevonden behalve 2-amino-4-chlorophenol
 - LOQ met GC/MS is te hoog, overstappen op GC-MS/MS

DERIVATISERING MET HFBA

- 500 ml waterstaal doperen met IS
- pH op >9 brengen met NaOH 1N
- extraheren met 2 keer 25 ml DCM (telkens 5 min krachtig schudden)
- gecombineerde DCM-fase drogen over Na_2SO_4 en indampen tot 3 ml
- 50 μl HFBA toevoegen, vial afsluiten en derivatiseren bij 70°C gedurende 10 min
- vial laten afkoelen, openen en wassen met 2 ml K_2HPO_4 buffer 1M
- buffer (bovenste fase) verwijderen, DCM-fase drogen met Na_2SO_4
- indampen tot 1 ml en analyseren GC-MS/MS in SRM

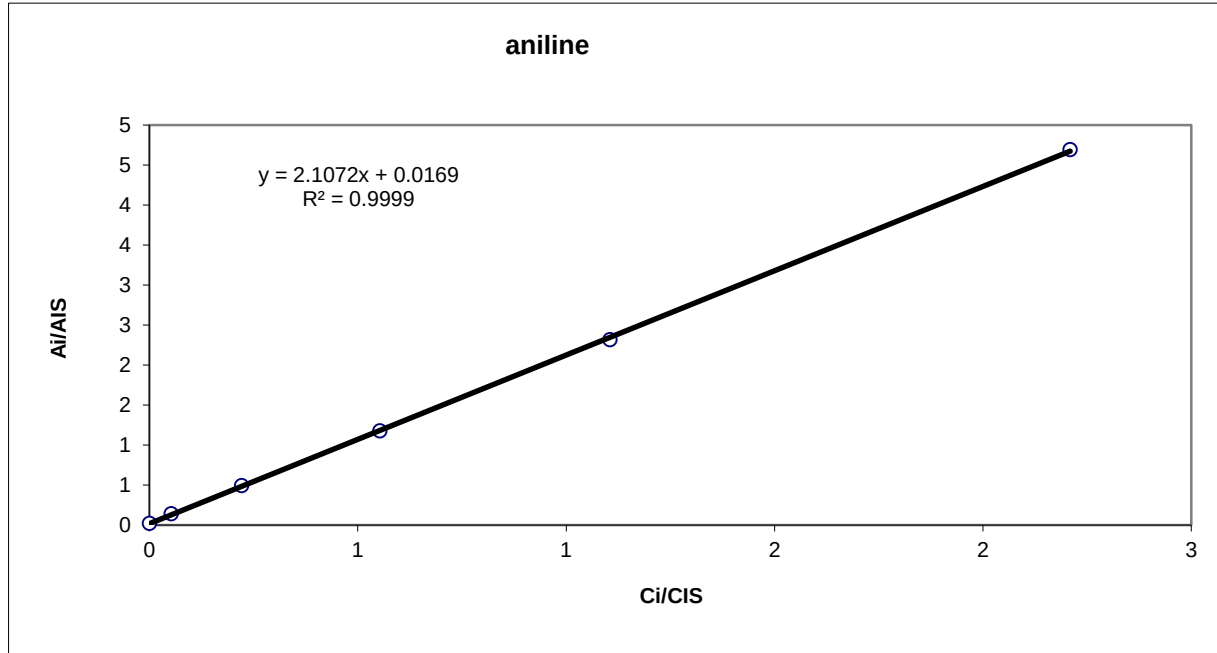
Retentietijd min.	Component	Moederion m/z	Dochterion m/z	Energie eV
11.78	D4-4-chloroaniline (IS1)	327	103	2
		327	130	2
12.01	D3-2,5-dichloroaniline (IS2)	325	151	2
		325	206	2
14.84	13C-3,4-dichloroaniline (IS3)	363	138	2
		363	166	2
16.36	D3-2,4-diaminotoluene (IS4)	348	320	10
		517	348	2
25.87	D8-benzidine (IS5)	584	387	2
		584	584	0
26.38	D8-4,'4-methylenedianiline (IS6)	594	594	0
		594	397	2
26.93	D6-3,'3-dichlorobenzidine (IS7)	650	453	15
		650	615	15

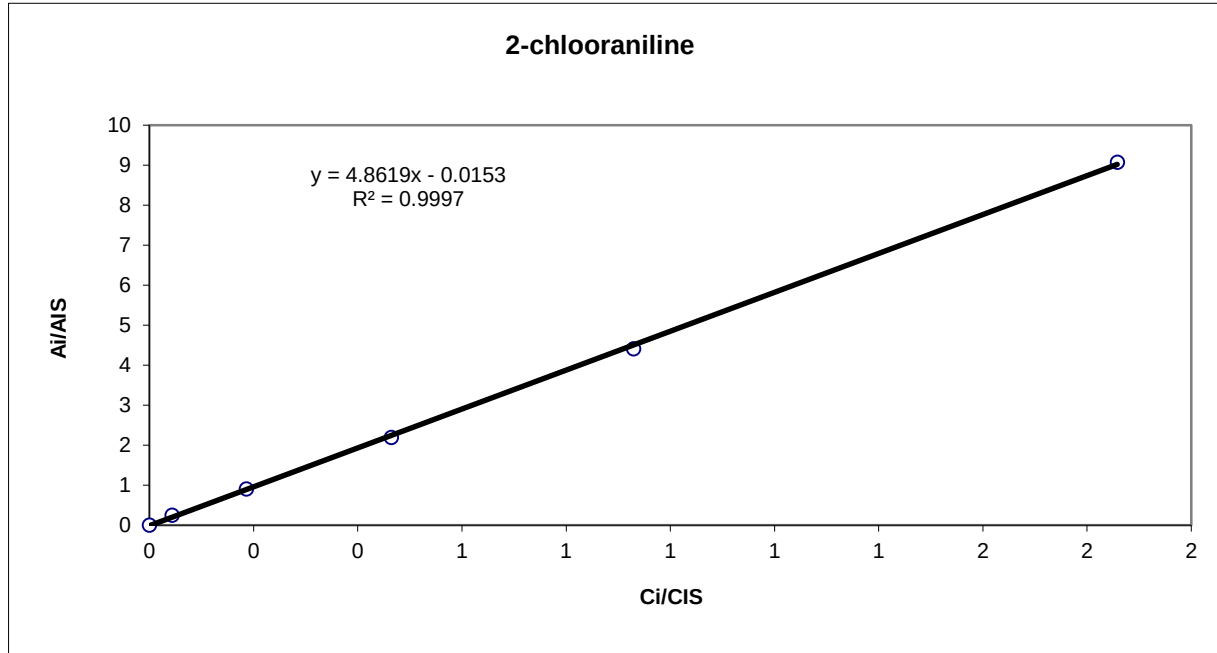
Retentietijd min.	Component	Moederion m/z	Dochterion m/z	Energie eV	Interne standaard
8.16	aniline	289	92	2	IS1
		289	120	2	
9.11	2-chloroaniline	288	169	2	IS1
		323	288	5	
10.85	2-chloro-5-methylaniline	302	183	2	IS1
		337	302	5	
11.25	2-chloro-4-methylaniline	302	183	2	IS1
		337	302	5	
11.58	3-chloroaniline	154	111	2	IS1
		323	154	2	
11.79	2-chloro-6-methylaniline	302	183	2	IS1
		337	302	5	
11.83	4-chloroaniline	154	99	2	IS1
		323	154	2	
12.01	2,4-dichloroaniline	322	203	2	IS2
		357	322	5	
12.05	2,5-dichloroaniline	322	203	2	IS2
		357	322	5	
12.21	2,3-dichloroaniline	322	203	2	IS2
		357	322	5	
12.59	3-chloro-2-methylaniline	337	168	2	IS1
		337	302	5	
12.89	4-chloro-2-methylaniline	337	168	2	IS1
		337	302	5	

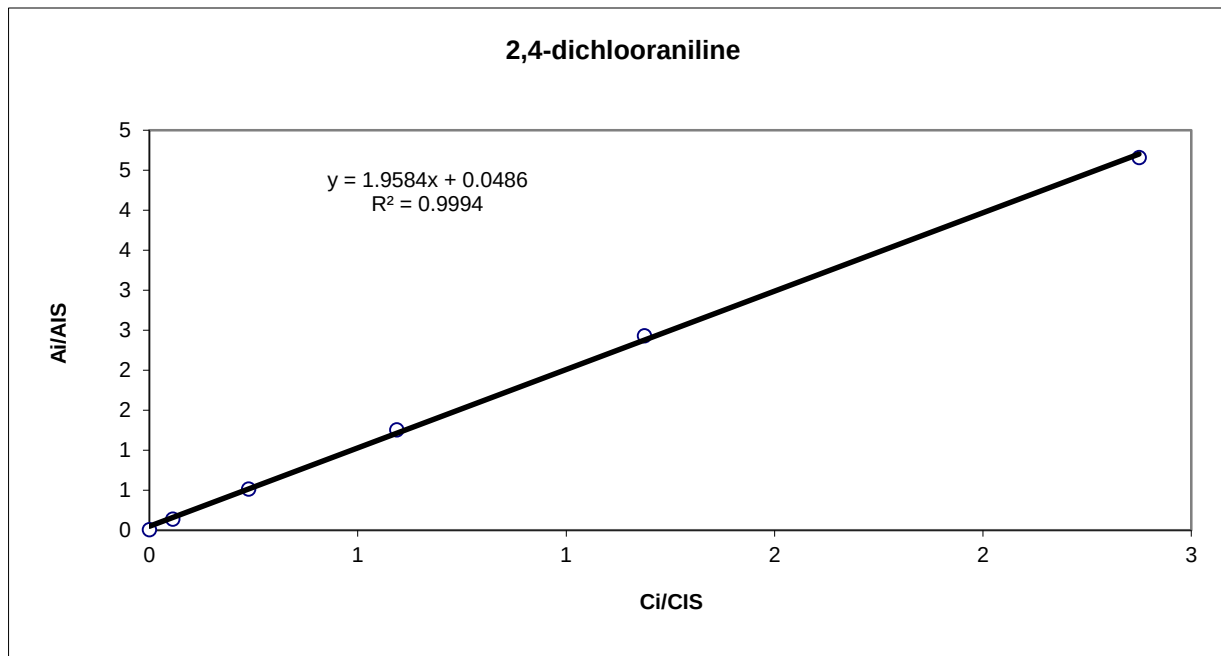
Retentietijd min.	Component	Moederion m/z	Dochterion m/z	Energie eV	Interne standaard
13.34	4-chloro-3-methylaniline	337	140	2	IS1
		337	168	2	
13.45	2,6-dichloroaniline	322	203	2	IS2
		322	233	2	
14.11	3,5-dichloroaniline	357	160	2	IS3
		357	188	2	
14.86	3,4-dichloroaniline	357	160	2	IS3
		357	188	2	
14.97	4-chloro-2-nitroaniline	322	203	2	EXT
		322	302	2	
16.42	2,4-diaminotoluene	345	317	5	IS4
		514	345	2	
19.19	2,6-dichloro-4-nitroaniline	206	176	2	EXT
		206	206	0	
25.94	benzidine	576	379	2	IS5
		576	576	0	
26.42	4,'4-methylenedianiline	590	590	0	IS6
		590	132	5	
26.99	3,'3-dichlorobenzidine	644	609	5	IS7
		644	644	0	
27.31	2,2'-dichlorobenzidine	644	644	0	IS7
		644	447	2	
27.56	4,4'-methylene-bis(2-chloroaniline)	623	300	2	EXT
		658	623	2	

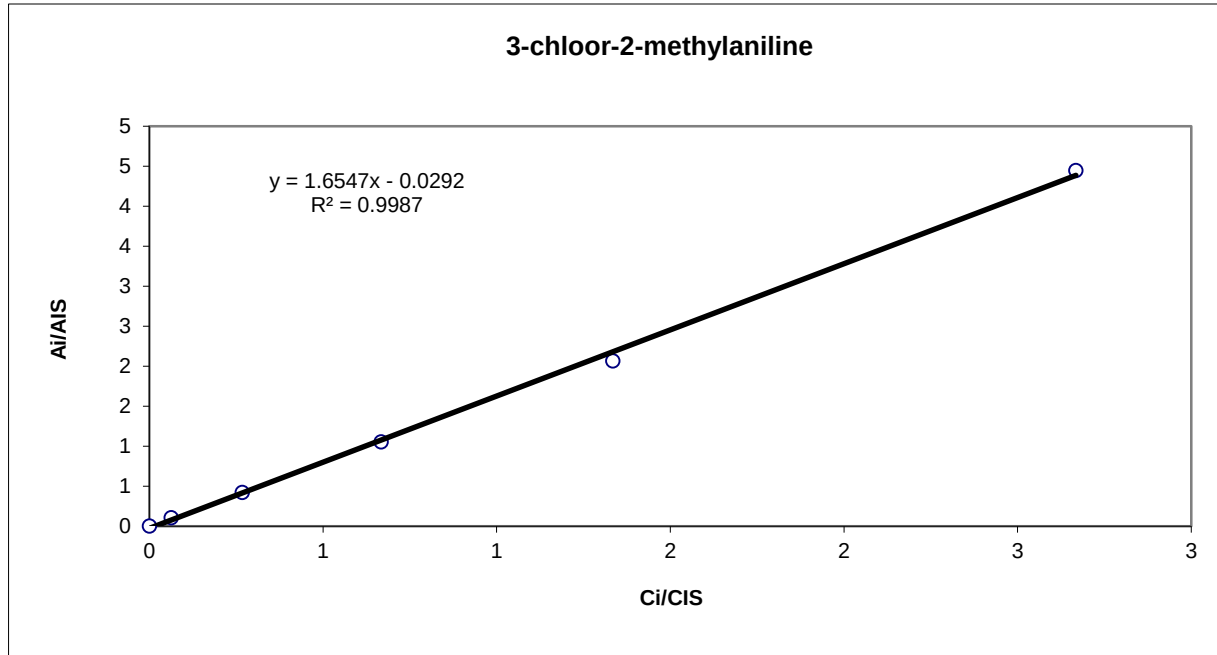


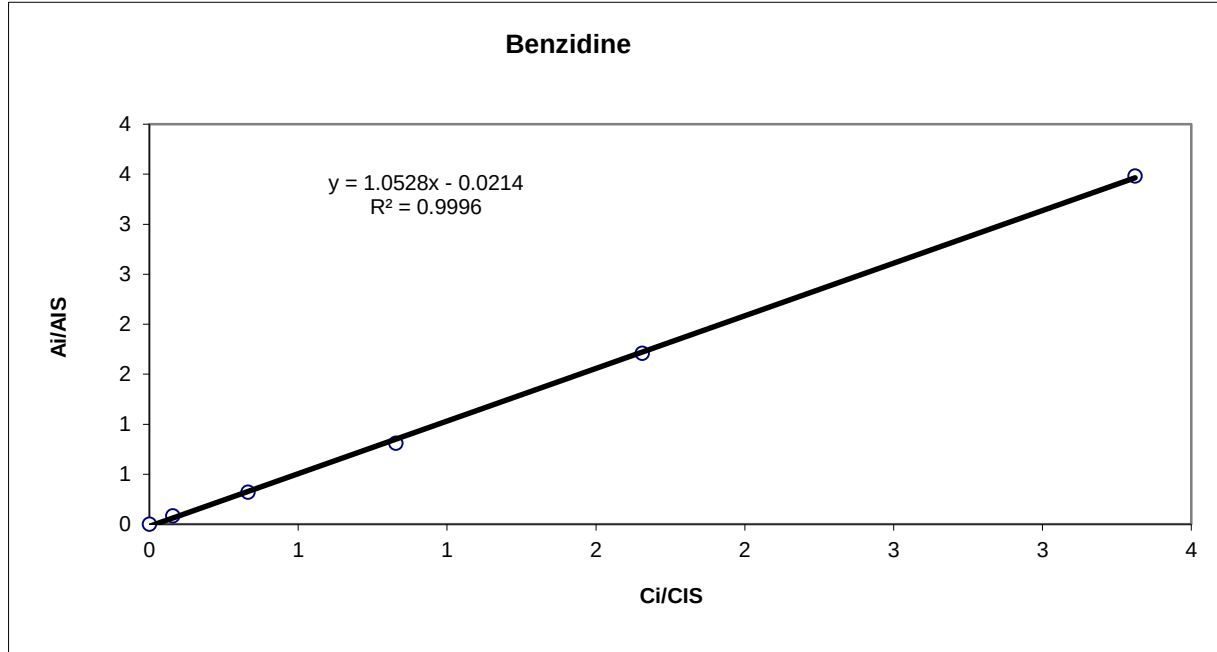
Lineariteitsreeks: 0.005 – 0.2 mg/l in meetextract
(=0.01 – 0.4 µg/l waterstaal)

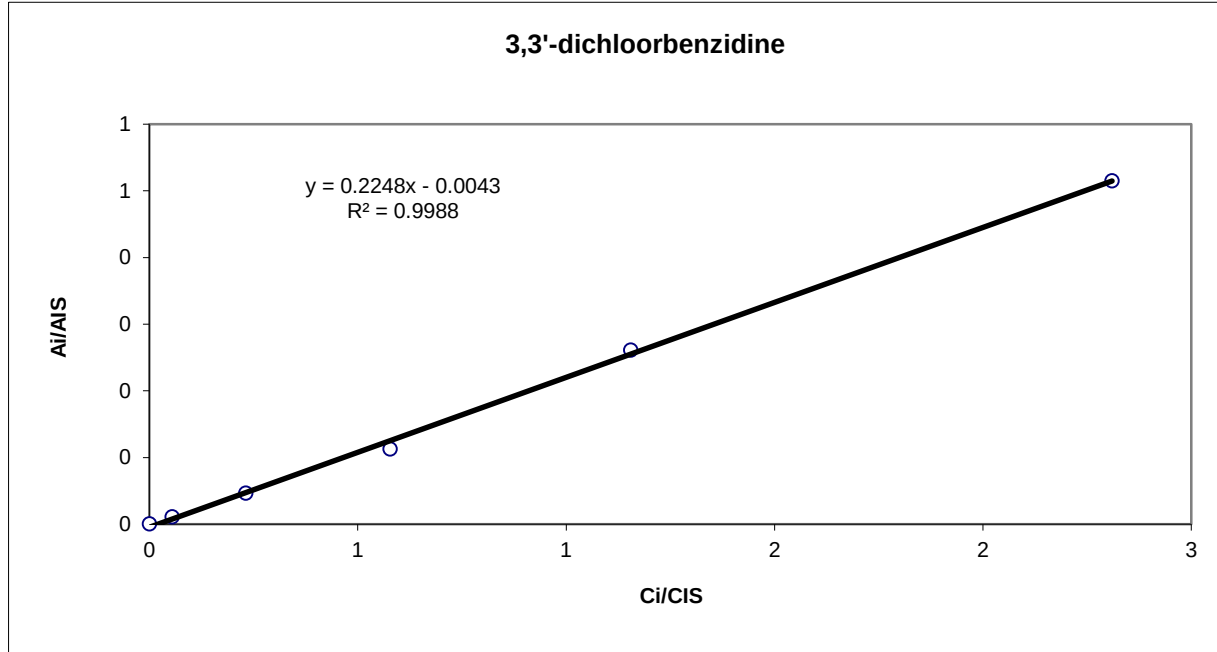






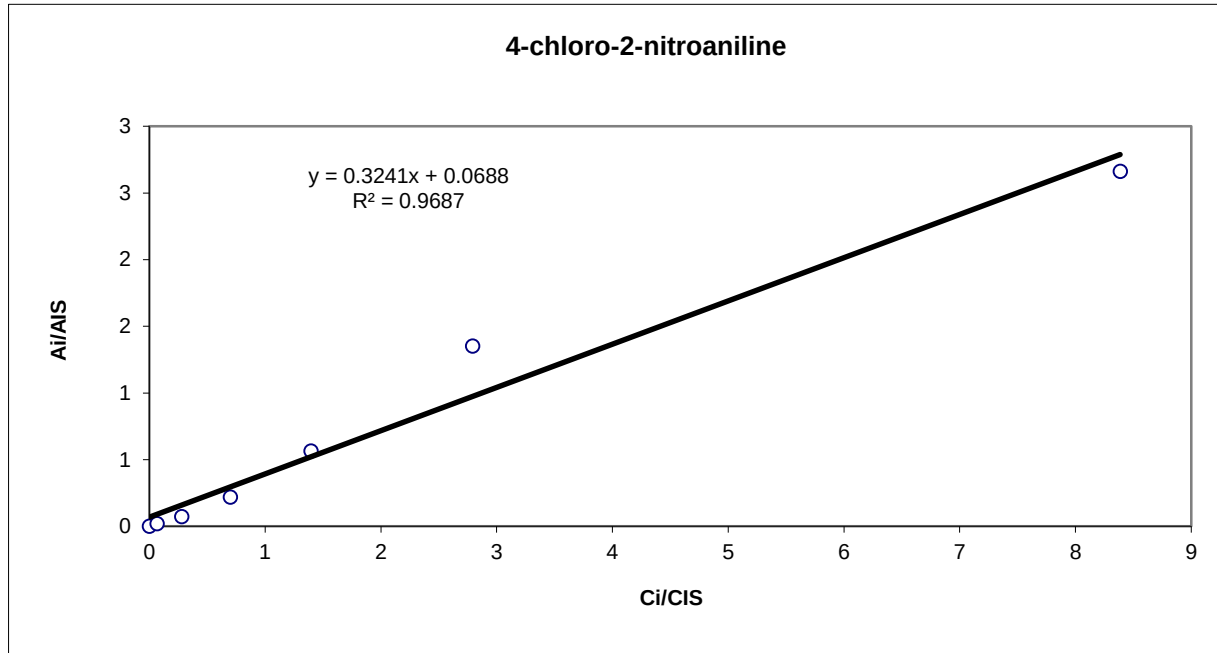




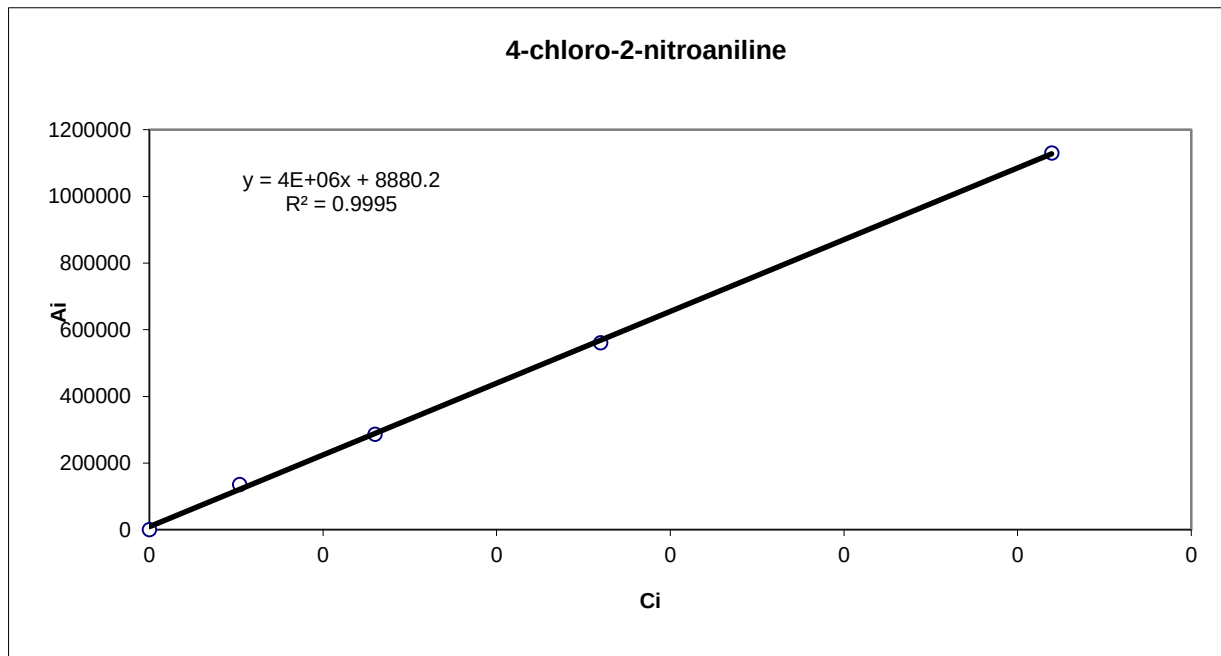


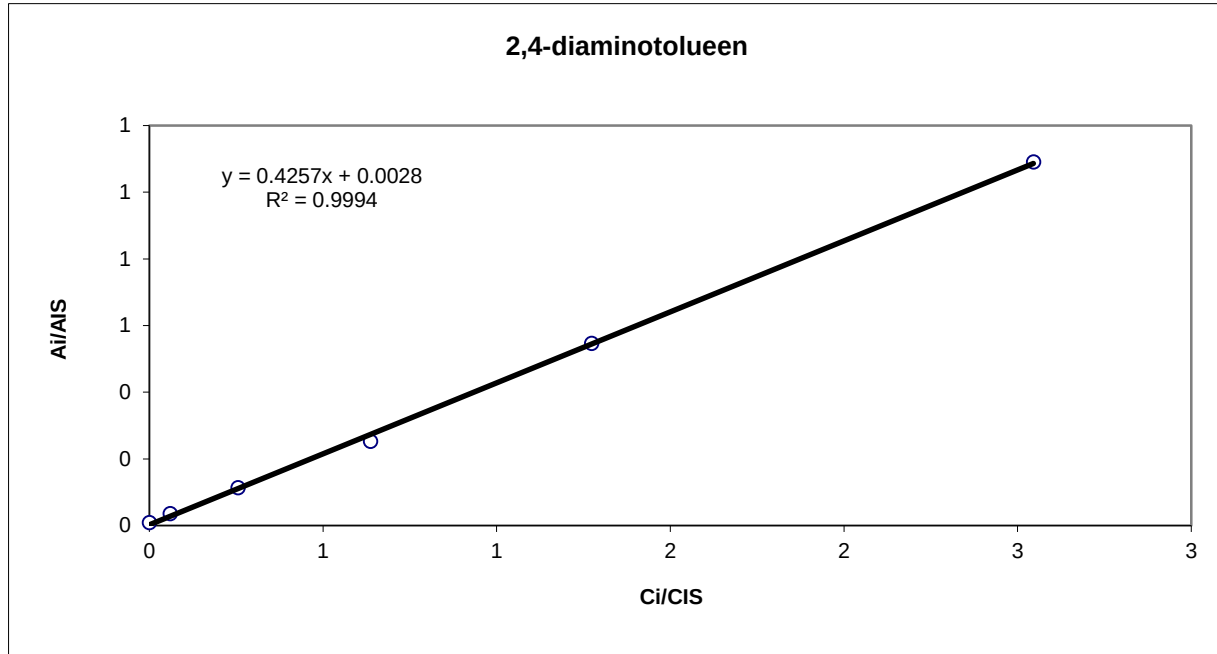


t.o.v. D3-2,4-diaminotolueen: niet lineair

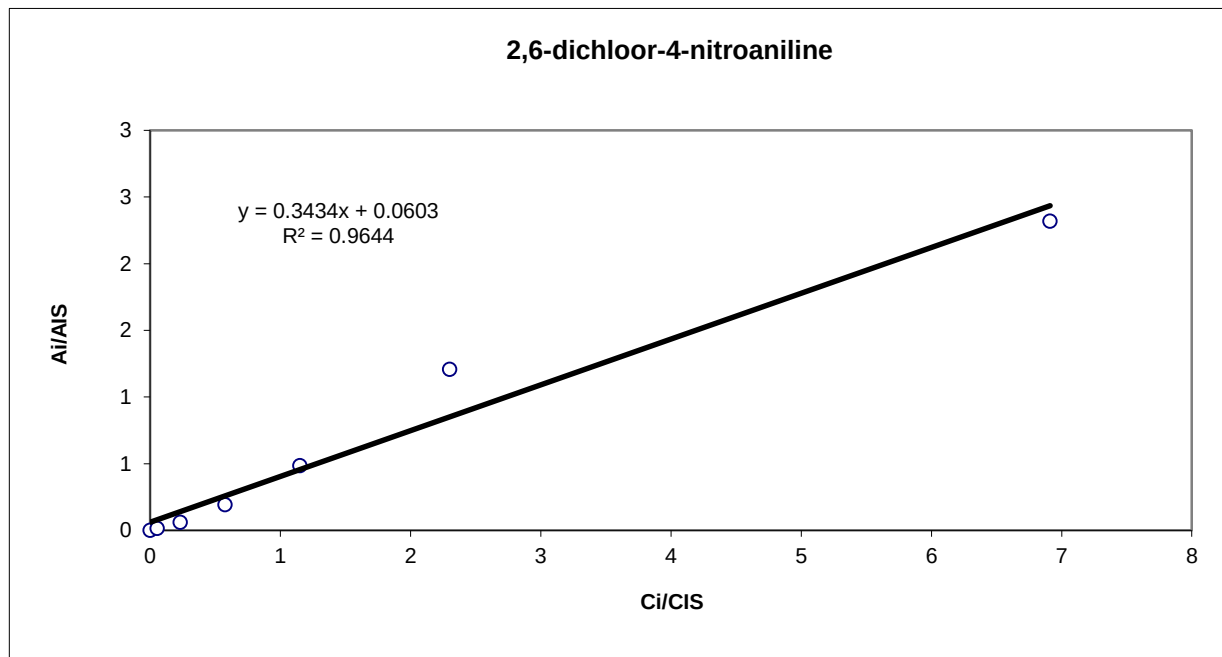


Met externe kalibratie: betere lineariteit

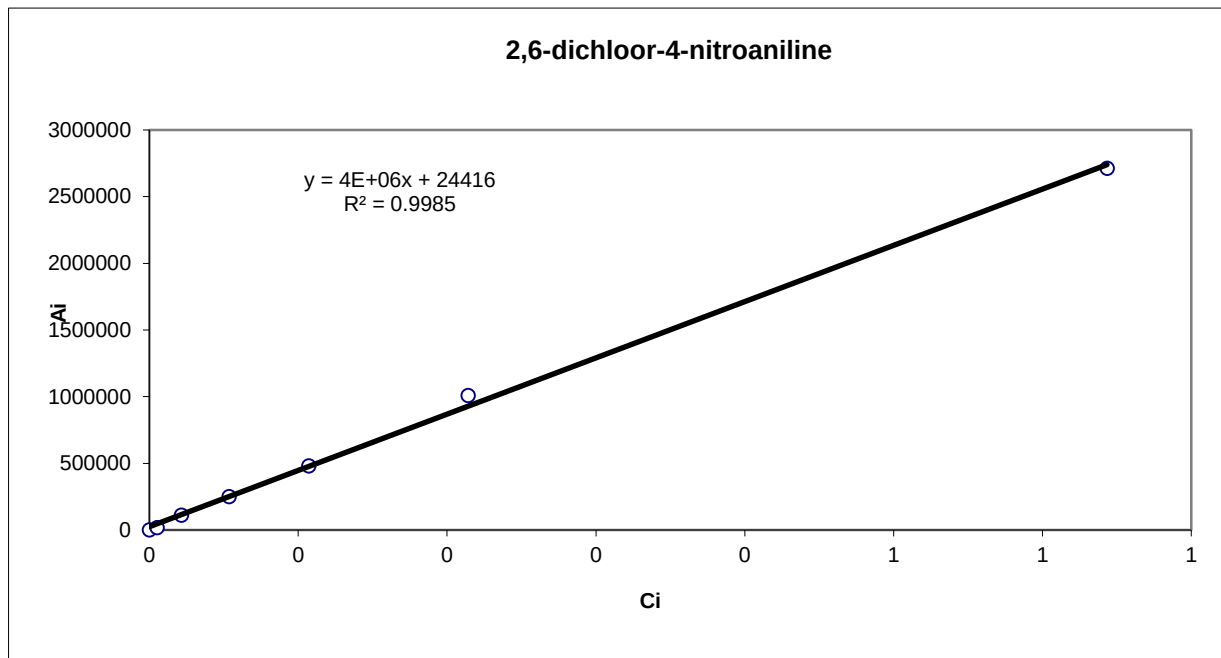


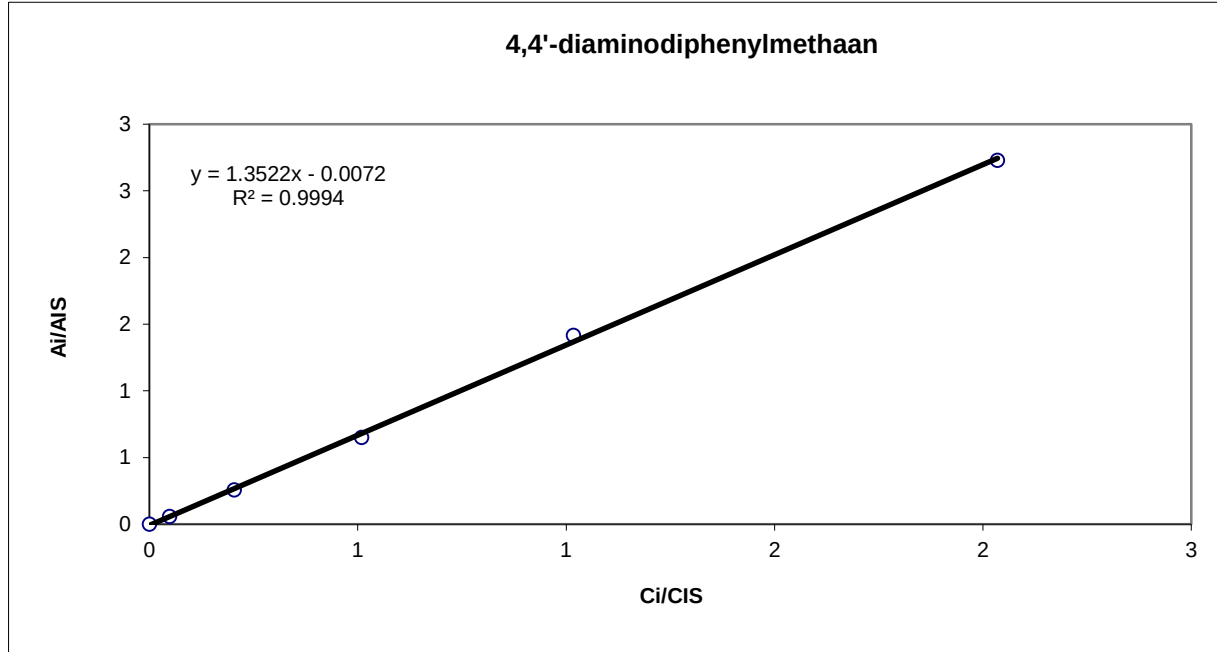


t.o.v. D3-2,4-diaminotolueen: niet lineair

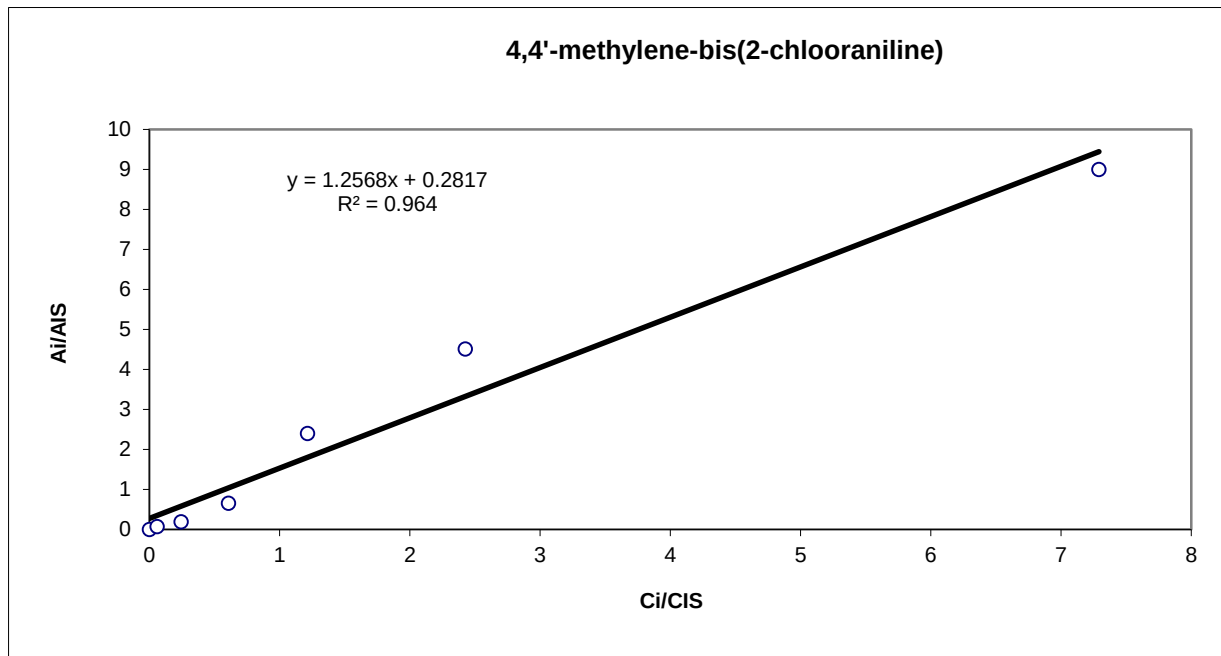


Met externe kalibratie: betere lineariteit

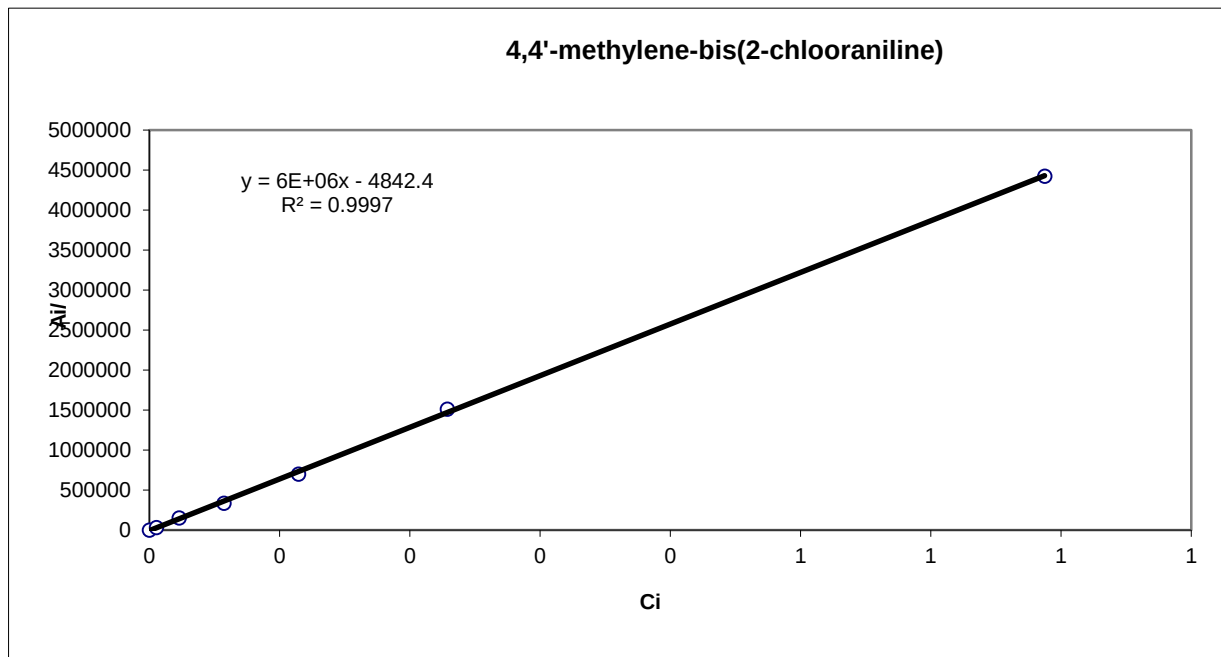




t.o.v. D3-2,4-diaminotolueen: niet lineair



Met externe kalibratie: betere lineariteit





Component	Meetconcentratie		Afwijking	Staalconcentratie		LOQ-eis
	Cmin mg/l	Cmax mg/l		Cmin µg/l	Cmax µg/l	
aniline	0.005	0.20	18	0.009	0.40	
2-chloroaniline	0.004	0.17	13	0.008	0.34	0.1
3-chloroaniline	0.005	0.20	5	0.010	0.41	0.1
4-chloroaniline	0.006	0.23	3	0.012	0.47	0.1
2,3-dichloroaniline	0.006	0.26	14	0.012	0.52	0.01
2,4-dichloroaniline	0.005	0.22	15	0.010	0.44	0.01
2,5-dichloroaniline	0.005	0.21	25	0.010	0.42	0.01
2,6-dichloroaniline	0.006	0.26	16	0.012	0.51	0.01
3,5-dichlooraniline	0.005	0.23	9	0.010	0.46	0.01
3,4-dichlooraniline	0.005	0.20	15	0.010	0.41	0.01
2-chloro-4-methylaniline	0.006	0.24	10	0.012	0.48	0.4
3-chloro-2-methylaniline	0.006	0.24	5	0.012	0.48	0.4
4-chloro-2-methylaniline	0.005	0.23	5	0.010	0.46	0.4
2-chloro-5-methylaniline	0.005	0.21	11	0.010	0.43	0.4
2-chloro-6-methylaniline	0.004	0.17	16	0.008	0.34	0.4
4-chloro-3-methylaniline	0.005	0.22	4	0.010	0.43	0.4
3,3'-dichloorbenzidine	0.005	0.22	4	0.010	0.43	0.07
2,2'-dichloorbenzidine	0.006	0.24	1	0.012	0.48	0.07
benzidine	0.007	0.31	4	0.014	0.61	0.18
4-chloro-2-nitroaniline *	0.026	0.26	14	0.052	0.52	0.6
2,4-diaminotolueen	0.006	0.24	29	0.012	0.47	0.1
2,6-dichloor-4-nitroaniline *	0.005	0.64	24	0.010	1.29	
4,4'-diaminodiphenylmethaan	0.005	0.19	8	0.010	0.38	0.1
4,4'-methylene-bis(2-chlooraniline) *	0.005	0.69	2	0.010	1.37	0.1

* Kalibratie dmv externe standaard



DW/GW en OW (resp. Spa water en Kanaalwater)

- analyse van 1 gedopeerd staal in zesvoud
- onder reproduceerbaarheid (aparte meetreeksen)
- laag niveau: 0.04-0.06 $\mu\text{g/l}$; hoog niveau: 0.2 – 0.3 $\mu\text{g/l}$
- kalibratiestandaarden doorlopen de ganse procedure

AW

- analyse van 5 gedopeerde reële afvalwaters
- analyses in duplo onder reproduceerbaarheid
- laag niveau: 0.04-0.06 $\mu\text{g/l}$; hoog niveau: 0.2 – 0.3 $\mu\text{g/l}$
- kalibratiestandaarden doorlopen de ganse procedure



Component	LAAG (0.04 - 0.06 µg/l)		HOOG (0.2 - 0.3 µg/l)	
	% Recovery	% RSD	% Recovery	% RSD
aniline	109	2	103	4
2-chloroaniline	102	2	98	2
3-chloroaniline	97	3	98	2
4-chloroaniline	100	2	99	2
2,4-dichloroaniline	105	2	101	2
2,5-dichloroaniline	108	3	100	4
2,3-dichloroaniline	106	2	100	3
2,6-dichloroaniline	104	3	102	2
3,5-dichloroaniline	100	2	98	2
3,4-dichloroaniline	102	3	99	2
2-chloro-5-methylaniline	99	2	97	1
2-chloro-4-methylaniline	99	2	98	1
2-chloro-6-methylaniline	99	6	96	2
3-chloro-2-methylaniline	97	2	98	2
4-chloro-2-methylaniline	96	1	97	2
4-chloro-3-methylaniline	95	3	97	2
4-chloro-2-nitroaniline *	54	8	70	9
2,4-diaminotoluene	97	3	98	1
2,6-dichloro-4-nitroaniline *	48	9	66	11
benzidine	98	2	98	1
4,4-diaminodifenylmethaan	98	3	99	2
3,3-dichlorobenzidine	96	3	100	2
2,2'-dichlorobenzidine	91	3	99	3
4,4-methylene-bis(2-chloroaniline) *	44	9	71	14

* Kalibratie dmv externe standaard



Component	LAAG (0.04 - 0.06 µg/l)		HOOG (0.2 - 0.3 µg/l)	
	% Recovery	% RSD	% Recovery	% RSD
aniline	104	7	106	1
2-chloroaniline	100	2	103	2
3-chloroaniline	98	3	101	2
4-chloroaniline	101	3	101	2
2,4-dichloroaniline	106	2	99	3
2,5-dichloroaniline	108	3	101	1
2,3-dichloroaniline	107	2	100	2
2,6-dichloroaniline	107	1	103	4
3,5-dichloroaniline	103	2	97	2
3,4-dichloroaniline	105	3	97	2
2-chloro-5-methylaniline	101	3	102	2
2-chloro-4-methylaniline	100	3	102	2
2-chloro-6-methylaniline	98	4	104	3
3-chloro-2-methylaniline	99	3	101	2
4-chloro-2-methylaniline	98	4	100	3
4-chloro-3-methylaniline	96	5	100	2
4-chloro-2-nitroaniline *	65	10	75	5
2,4-diaminotoluene	100	5	105	14
2,6-dichloro-4-nitroaniline *	65	10	78	5
benzidine	96	2	98	1
4,4-diaminodifenylmethaan	100	9	98	2
3,3-dichlorobenzidine	98	2	99	2
2,2'-dichlorobenzidine	91	4	99	2
4,4-methylene-bis(2-chloroaniline) *	61	11	77	3

* Kalibratie dmv externe standaard

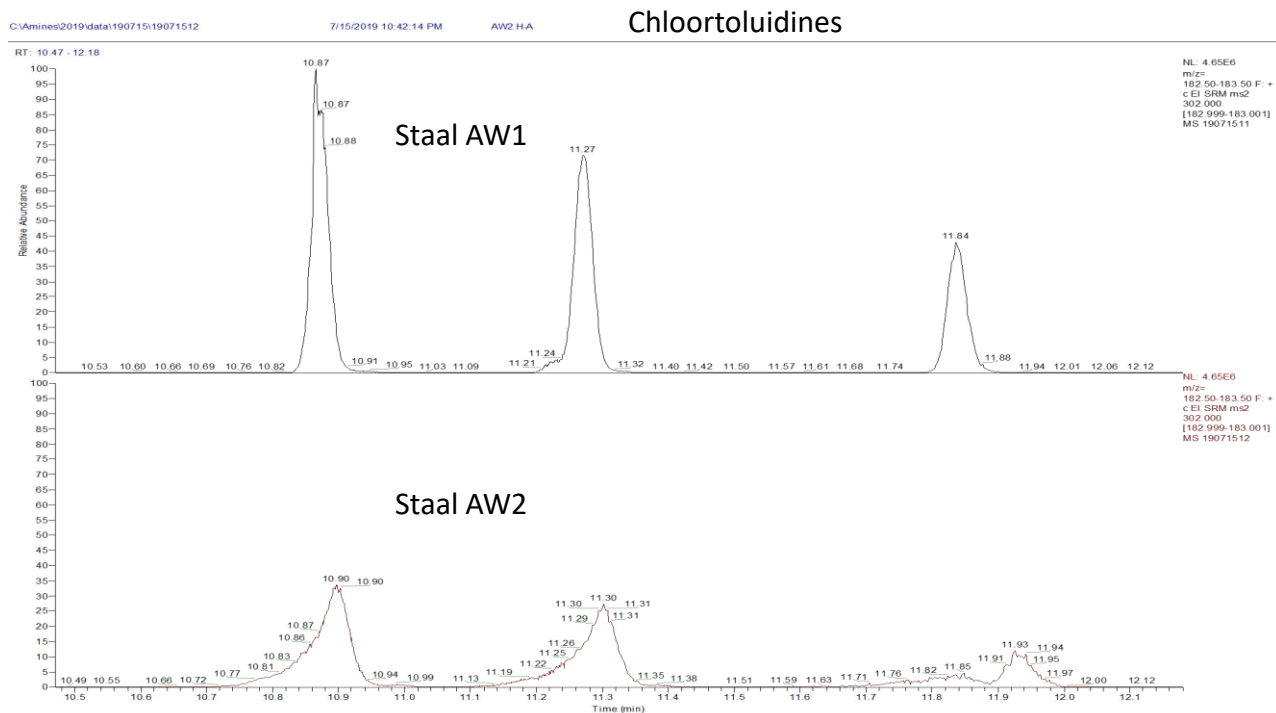


% Recov IS	DW/GW Laag					DW/GW Hoog					Min	Max	Gemidd
	1	2	3	4	5	1	2	3	4	5	%	%	%
d4-4-chlooraniline	65	67	55	60	59	65	79	76	83	74	55	83	68
d3-2,5-dichloroaniline	58	63	50	55	58	63	79	72	78	72	50	79	65
13C-3,4-dichloroaniline	61	66	52	58	60	64	78	74	80	72	52	80	67
d3-2,4-diaminotoluene	68	64	57	67	70	77	86	67	84	67	57	86	71
d8-benzidine	56	59	46	55	57	63	79	79	85	70	46	85	65
d8-4,4-methylenedianiline	55	54	42	53	53	63	75	75	81	58	42	81	61
d6-3,3-diClbenzidine	53	56	43	52	52	61	72	76	79	66	43	79	61

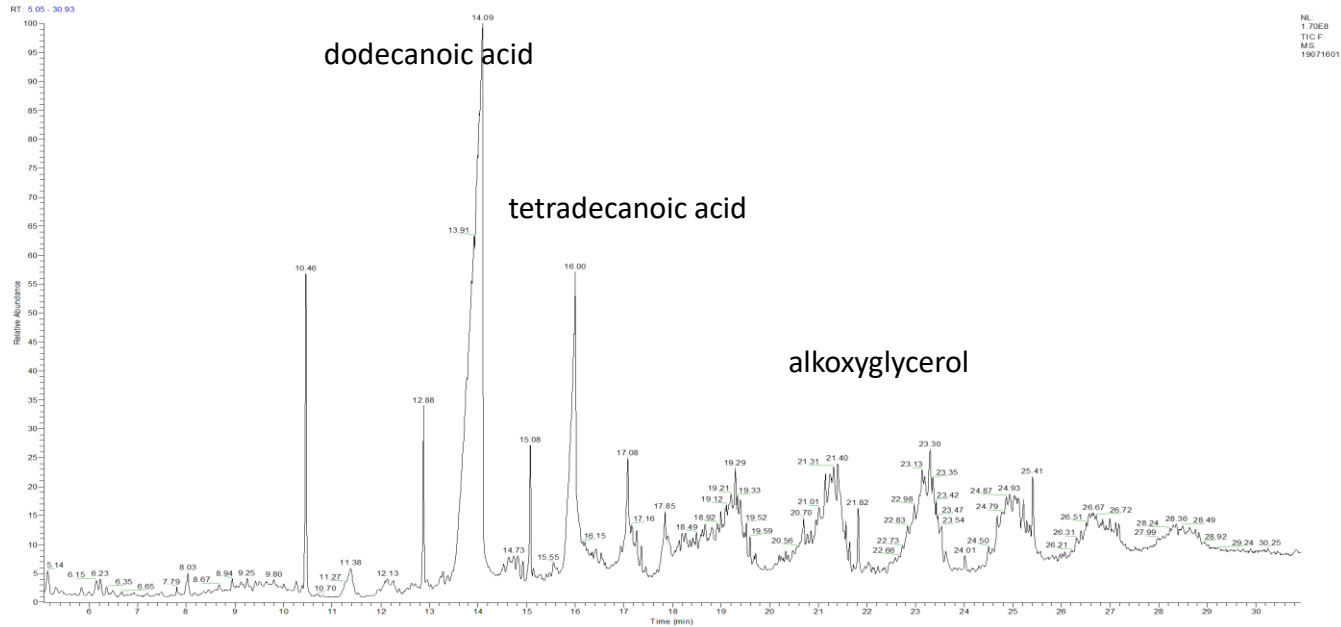
% Recov IS	OW Laag						OW Hoog						Min	Max	Gemidd
	1	2	3	4	5	6	1	2	3	4	5	6	%	%	%
d4-4-chlooraniline	63	73	80	69	68	65	79	77	78	84	76	70	63	84	74
d3-2,5-dichloroaniline	64	69	74	61	62	61	81	77	79	82	76	74	61	82	72
13C-3,4-dichloroaniline	67	71	79	66	66	65	79	79	77	82	75	73	65	82	73
d3-2,4-diaminotoluene	69	82	77	87	58	59	89	86	95	91	95	72	58	95	80
d8-Benzidine	64	75	78	67	60	61	88	80	84	86	82	68	60	88	74
d8-4,4-methylenedianiline	61	69	76	61	56	54	76	73	72	70	77	68	54	77	68
d6-3,3-diClbenzidine	67	73	75	62	62	63	81	77	78	81	77	75	62	81	73



Staal AW2: slechte piekvormen omwille van interferenties



Staal AW2: full scan screening



- Interferenten zijn apolaire en zure componenten; clean-up dmv zure extractie vooraf?
- Staal niet duplo geïnjecteerd, dus reproduceerbaarheid berekend ahv 4 duplostalen



Component	LAAG (0.04 - 0.06 µg/l)		HOOG (0.2 - 0.3 µg/l)	
	% Recovery	% RSD	% Recovery	% RSD
aniline	168	8	121	6
2-chloroaniline	125	4	102	5
3-chloroaniline	100	4	99	4
4-chloroaniline	102	4	98	2
2,4-dichloroaniline	114	2	103	2
2,5-dichloroaniline	100	2	98	2
2,3-dichloroaniline	99	2	100	1
2,6-dichloroaniline	106	6	100	2
3,5-dichloroaniline	97	2	98	3
3,4-dichloroaniline	164	4	114	6
2-chloro-5-methylaniline	93	6	96	7
2-chloro-4-methylaniline	94	7	96	7
2-chloro-6-methylaniline	93	9	94	7
3-chloro-2-methylaniline	98	7	99	7
4-chloro-2-methylaniline	100	7	97	8
4-chloro-3-methylaniline	103	4	93	4
4-chloro-2-nitroaniline *	148	6	84	42
2,4-diaminotoluene	90	14	95	2
2,6-dichloro-4-nitroaniline *	143	10	83	34
benzidine	101	3	97	2
4,4-diaminodifenylmethaan	102	6	97	3
3,3-dichlorobenzidine	99	3	101	2
2,2'-dichlorobenzidine	111	6	90	8
4,4-methylene-bis(2-chloroaniline) *	116	7	74	30

* Kalibratie dmv externe standaard



% Recov IS	AW Laag								
	1A	1B	2A	3A	3B	4A	4B	5A	5B
d4-4-chlooraniline	135	143	91	113	104	122	105	120	98
d3-2,5-dichloroaniline	119	133	109	108	103	121	120	112	117
13C-3,4-dichloroaniline	129	137	104	112	105	115	108	116	118
d3-2,4-diaminotoluene	206	204	5	48	52	90	59	120	49
d8-benzidine	127	154	7	111	107	88	63	125	59
d8-4,4-methylenedianiline	144	140	12	138	133	103	73	156	59
d6-3,3-diClbenzidine	106	125	29	107	108	89	85	103	108

% Recov IS	AW Hoog									Min	Max	Gemidd
	1A	1B	2A	3A	3B	4A	4B	5A	5B	%	%	%
d4-4-chlooraniline	161	141	84	114	58	51	45	80	84	45	161	105
d3-2,5-dichloroaniline	150	134	110	112	46	48	46	71	76	46	150	101
13C-3,4-dichloroaniline	153	135	51	111	49	44	40	73	77	40	153	101
d3-2,4-diaminotoluene	389	353	18	130	99	91	68	125	125	48	206	105
d8-benzidine	196	167	8	122	72	37	32	88	93	32	196	103
d8-4,4-methylenedianiline	193	206	19	128	61	42	37	86	91	37	206	112
d6-3,3-diClbenzidine	150	124	25	111	46	37	37	65	70	37	150	92



% Recov IS	DW/GW			OW			AW		
	Min	Max	Gemidd	Min	Max	Gemidd	Min	Max	Gemidd
d4-4-chlooraniline	55	83	68	63	84	74	45	161	105
d3-2,5-dichloroaniline	50	79	65	61	82	72	46	150	101
13C-3,4-dichloroaniline	52	80	67	65	82	73	40	153	101
d3-2,4-diaminotoluene	57	86	71	58	95	80	48	206	105
d8-benzidine	46	85	65	60	88	74	32	196	103
d8-4,4-methylenedianiline	42	81	61	54	77	68	37	206	112
d6-3,3-diClbenzidine	43	79	61	62	81	73	37	150	92



- Met HFBA derivatisering na extractie worden alle componenten teruggevonden, behalve 2-amino-4-chloorfenol
- Met GC-MS/MS meting is de gewenste LOQ haalbaar
- De reproduceerbaarheid en terugvinding zijn goed in alle watertypes behalve voor 4-chloro-2-nitroaniline, 2,6-dichloro-4-nitroaniline en 4,4'-methylenebis(2-chloroaniline); deze drie componenten kunnen slechts indicatief bepaald worden in afwachting van geschikte interne standaarden
- Recovery IS in DW/GW en OW: 40-100%; in AW: 30-200%
- Voor zwaar beladen AW kan er interferentie optreden, VITO zal nagaan of opzuivering dmv zure wassing van het staal mogelijk is