Bestimmung von Weichmachern mit GC-MS und LC-MS/MS in pflanzlichen Ölen

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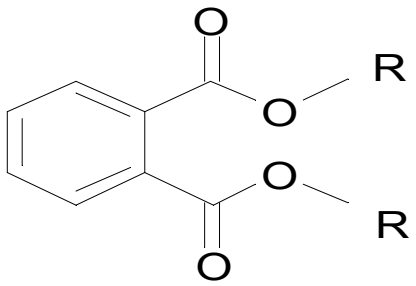
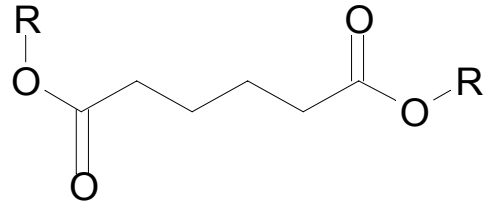
- Phthalsäureester und andere Weichmacher
- Vergleich der Probenvorbereitungen für die GC- bzw. LC-Messung
- Messparameter GC-MS
- Messparameter LC-MS/MS
- Beispielchromatogramme
- Ergebnisse Kalibrierung

Chemische Strukturen



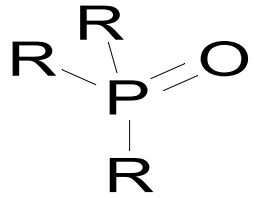
Ester der Phthalsäure

R= Methyl, Ethyl, Butyl, iso-Butyl, 2-Ethylhexyl, n- Octyl, iso-Nonyl, iso-Decyl



Ester der Adipinsäure

R= Methyl, Ethyl, Butyl, iso-Butyl, 2-Ethylhexyl



Ester der Phosphorsäure

R= Butyl, iso-Butyl, Phenyl

Probenvorbereitung

- **Für die GC-MS nach ASU§64 LFBG**
- Extraktion mit Ethylacetat/Cyclohexan (50/50)
- Aufreinigung an Gelchromatographie*
- Einengen
- Splitlose Messung mit GC-MS im SIM mode
- Zeitbedarf : ca 2,5h / Probe

*: kritisch: Abtrennung von DiNP und DiDP

- **Für die LC-MS/MS nach Labormethode**
- Extraktion mit Acetonitril/Wasser (80/20)
- Kalt zentrifugieren
- Messung mit ESI pos am LC-MS/MS im MRM mode
- Zeitbedarf : ca 0,5h / Probe

Parameter GC-MS

Instrument:	GC-MS Agilent 5973 mit GC6890 und CTC Sampler Combi-PAL
Trennsäule:	DB-XLB, 30m x 0,25mm i.d. X 0,25µm Film
Inj.Temp.:	100°C-10°C/min-280°C
Inj.Vol.:	2µl splitless
Ofen-Prgr:	70°C(2min)-5°C/min-230°C-10°C/min-340°C(5min)
Carrier:	He, 0,7ml/min
Transline-Temp.:	300°C
ms dwell time:	30msec/ion
scans/sec:	1,7

Parameter GC-MS ions monitored

Analyt	Quantifier	Identifier	RT
	m/z	m/z	Min
Dimethylphthalat (DMP)	194	163	14.97
Diethylphthalat (DEP)	177	149	16,85
Di-(iso)butylphthalat (D(i)BP	223	149	20,18/21.35
Benzylbutylphthalat (BBP)	206	149	26.63
Diethylhexylphthalat (DEHP)	167	279	28.86
Di-isononylphthalat (DiNP)	293	149	32
Di-isodecylphthalat (DiDP)	307	149	34
Diethyladipat (DEA)	128	157	13.72
Dibutyladipat (DBA)	129	185	18.89
Diethylhexyladipat (DEHA)	259	241	26.83
Tri-(iso)butyl-phosphat (T(i)BP)	211	99	15,51
PCB 209	500		33,71
BBP-D4	210	153	26.63
DEHP-D4	171	283	28.84
DBP-D4	227	153	21,32

Parameter LC-MS/MS

HPLC-MS/MS-Instrument: Quattro Micro (Waters) mit Agilent 1100
Trennsäule: Zorbax SB Aq 2,1 x 100mm, 3,5µ

Eluent/Gradient: A=10mM Ammoniumacetat pH4 HCOOH / B=Acetonitril

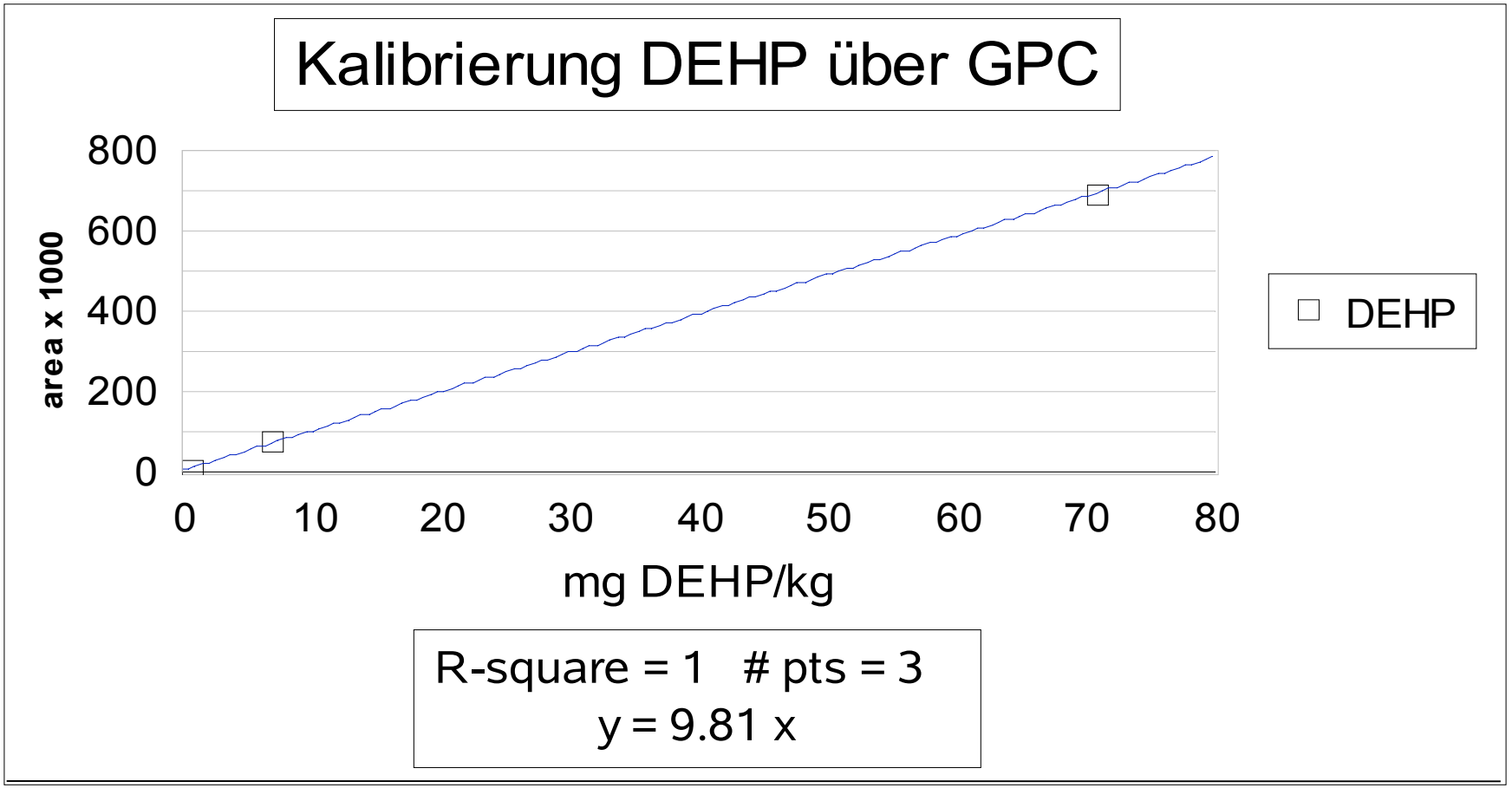
0min	40%A / 60%B	0,20ml/min
3min	40%A / 60%B	0,20ml/min
6min	2%A / 98%B	0,25ml/min
13min	2%A / 98%B	0,25ml/min
5 min	rekonditionieren	0,25ml/min

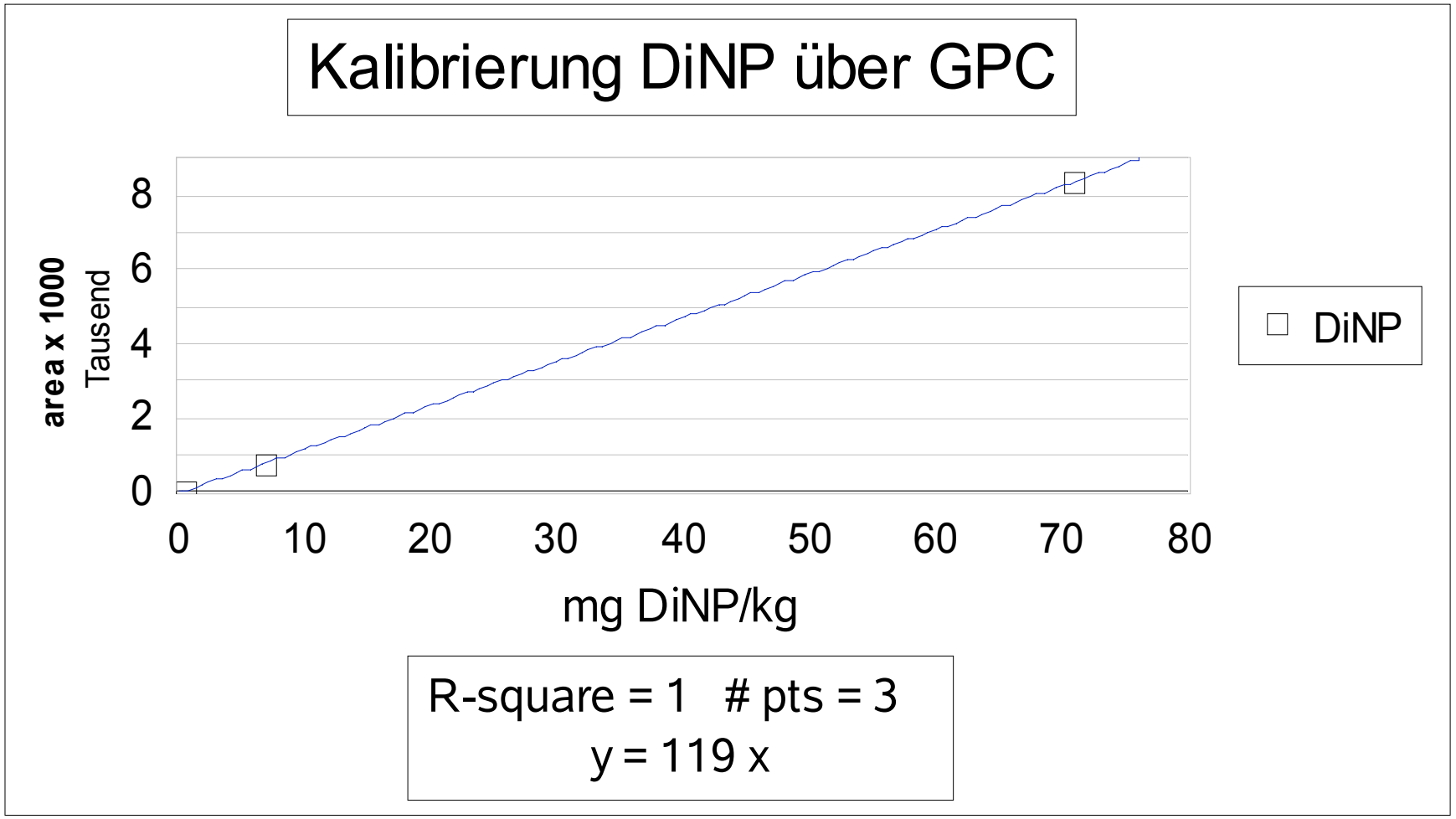
Temperatur: 30°C
Injektionsvolumen: 15µl

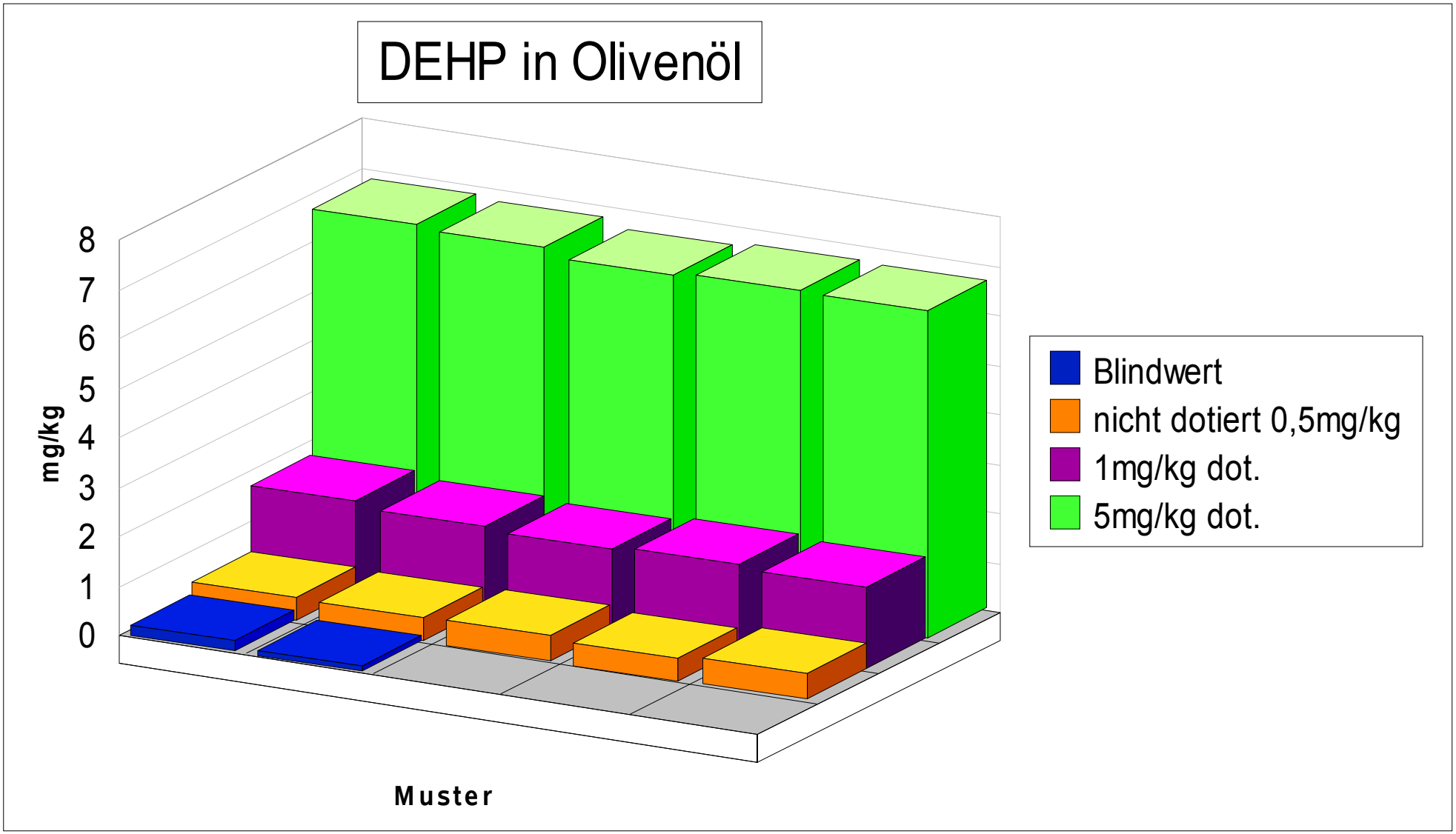
ESI-positiv, Ar als Kollisionsgas, Quellentemperatur: 120°C,
Desolvation Temperature: 350°C

MRM-Parameter (LC-MS/MS)

Analyt	Precursor	Quant-Ion	Ident-Ion	RT
	M/Z 1	M/Z 2a	M/Z 2b	Min
Dimethylphthalat (DMP)	195	163		2,18
Diethylphthalat (DEP)	223	149		2,57
Di-(iso)butylphthalat (D(i)BP	279	149		4,85
Benzylbutylphthalat (BBP)	313	91	149	4,85
Diethylhexylphthalat (DEHP)	391	167	149	11,58
Di-isononylphthalat (DiNP)	419	149	127	12,38
Di-isodecylphthalat (DiDP)	447	149		13,07
Diethyladipat (DEA)	203	128		2,47
Dibutyladipat (DBA)	259	128	147	4,55
Diethylhexyladipat (DEHA)	371	128	259	11,48
Tri-(iso)butyl-phosphat (T(i)BP	267	155	99	3,56
DEHP-D4	395	153		11,88

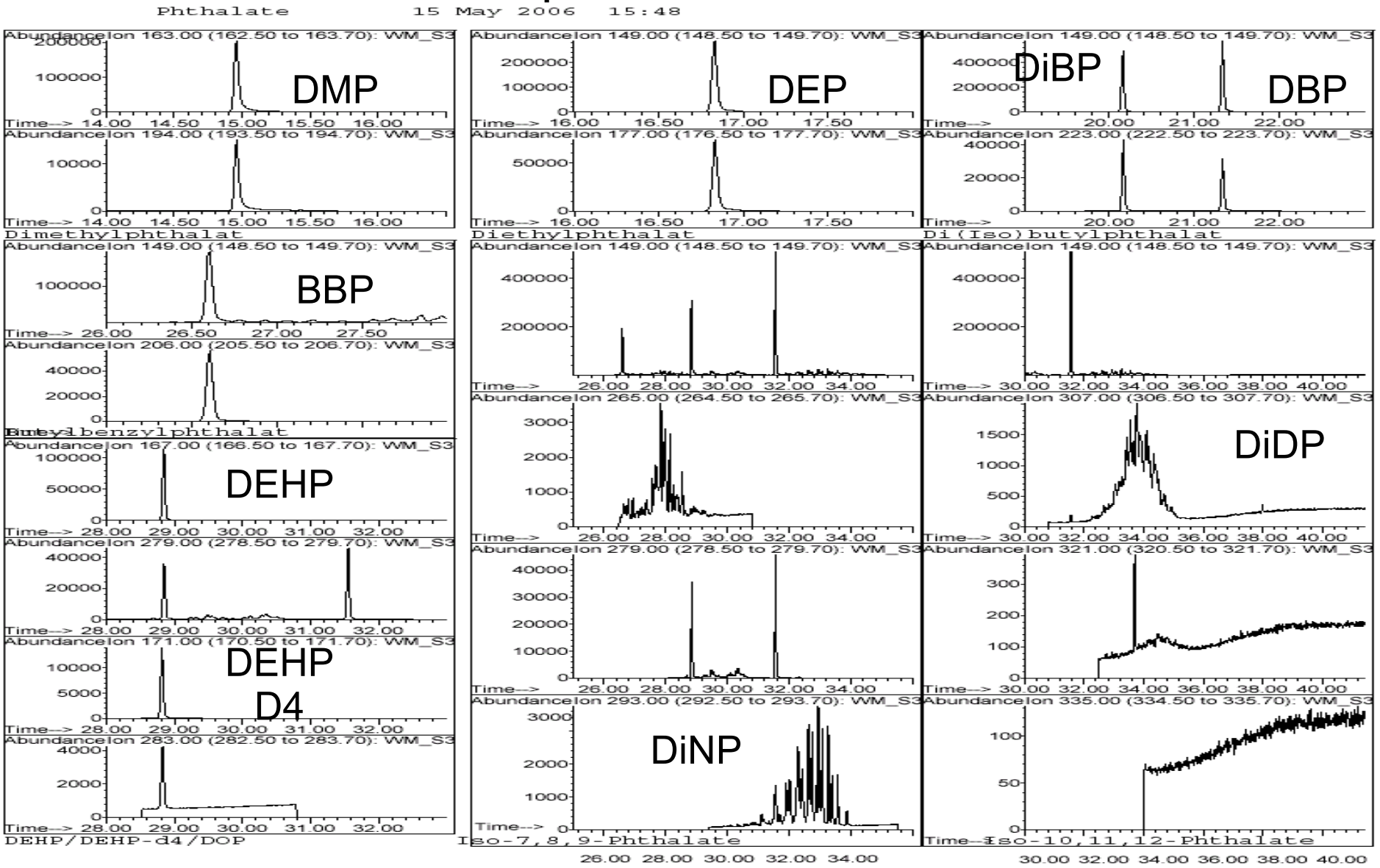

Kalibrierung DEHP über GPC
area x 1000
800
600
400
200
0
0
10
20
30
40
50
60
70
80
mg DEHP/kg
DEHP
R-square = 1 # pts = 3
y = 9.81 x


Kalibrierung DiNP über GPC
area x 1000
Tausend
8
6
4
2
0
0
10
20
30
40
50
60
70
80
mg DiNP/kg
DiNP
R-square = 1 # pts = 3
y = 119 x



	MW mg/kg	StdAbw mg/kg	rel.StdAbw.%	Rec%
Probe	0,51	0,03	6,3	
Probe + 1mg/kg	1,74	0,06	3,2	123
Probe + 5mg/kg	6,7	0,07	1,1	124
Blindwert	0,2			

Auswertung von GC-MS-Chromatogrammen (SIM) über Ionenspuren in Zeitfenstern



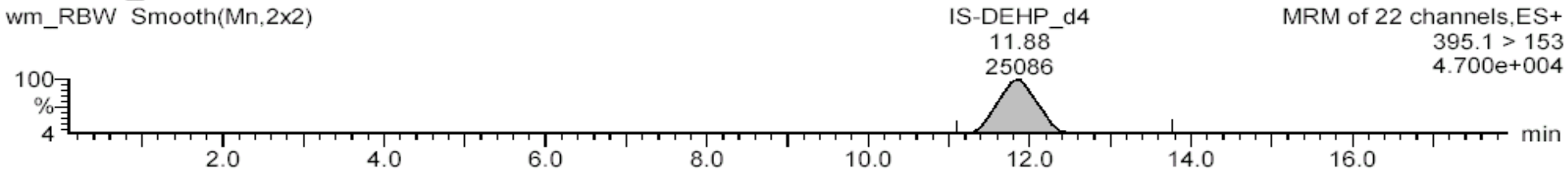
LC-MS/MS Chromatogramm

Reagentienblindwert

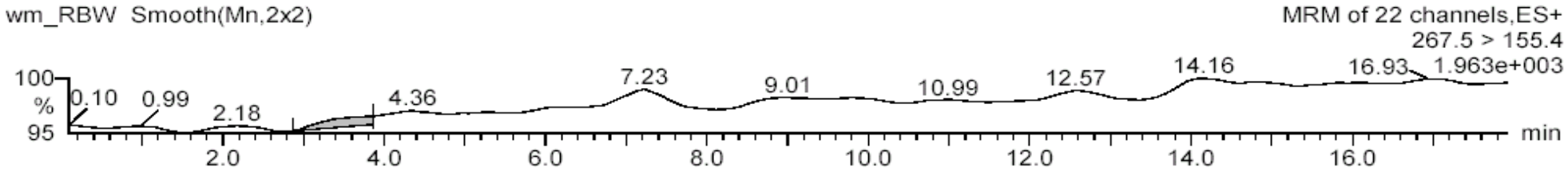
Method: C:\MassLynx\2006_mai\mai_12.PRO\MethDB\WM_06.mdb 01 May 2006 19:35:00
Calibration: 15 May 2006 09:05:31

Name: wm_RBW, Date: 14-May-2006, Time: 18:29:58, Description: , Vial: 1, ID:

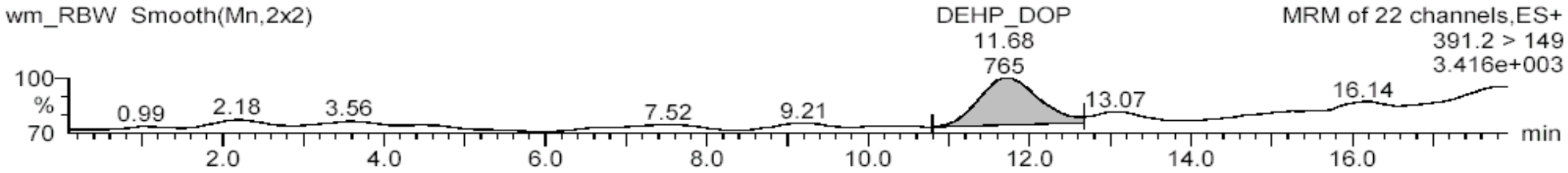
IS-DEHP_d4



TIBPhosphat_a

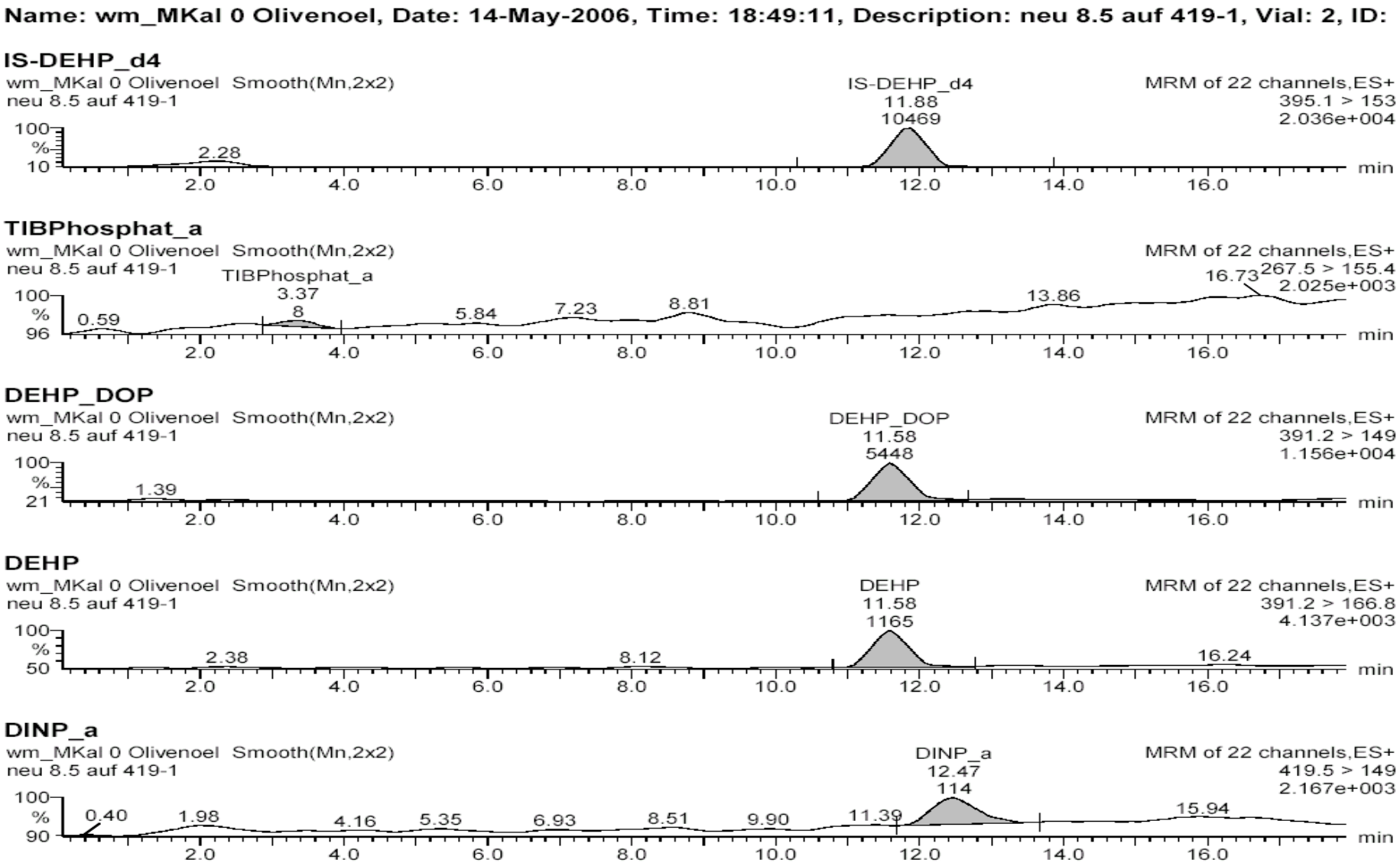


DEHP_DOP



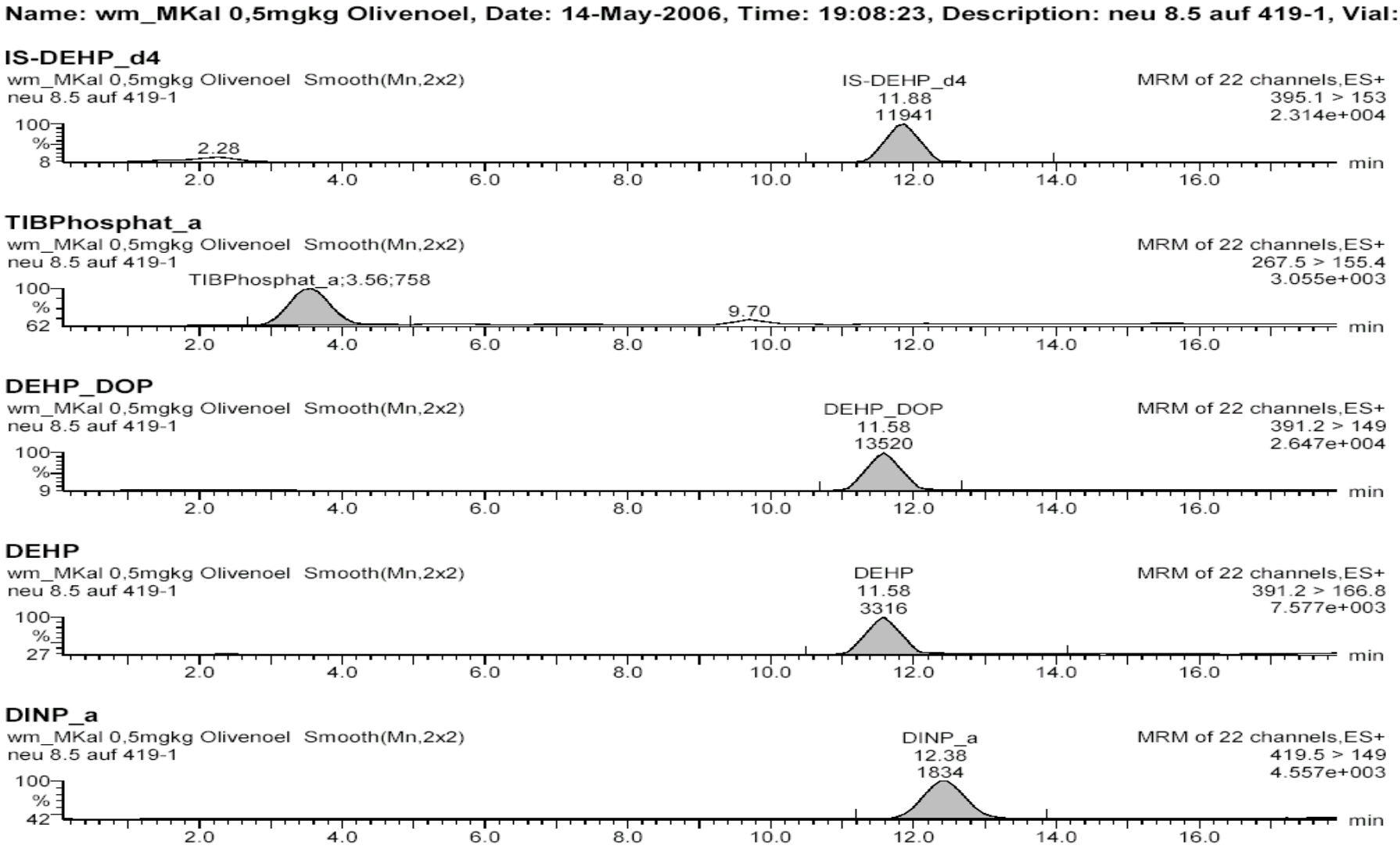
LC-MS/MS Chromatogramm

gering belastetes Olivenöl



LC-MS/MS Chromatogramm

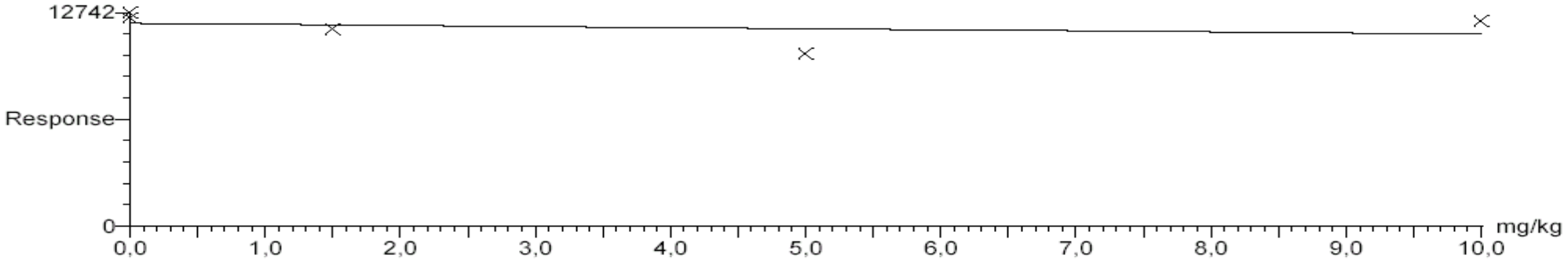
gering belastetes Olivenöl, mit 0,5mg/kg dotiert



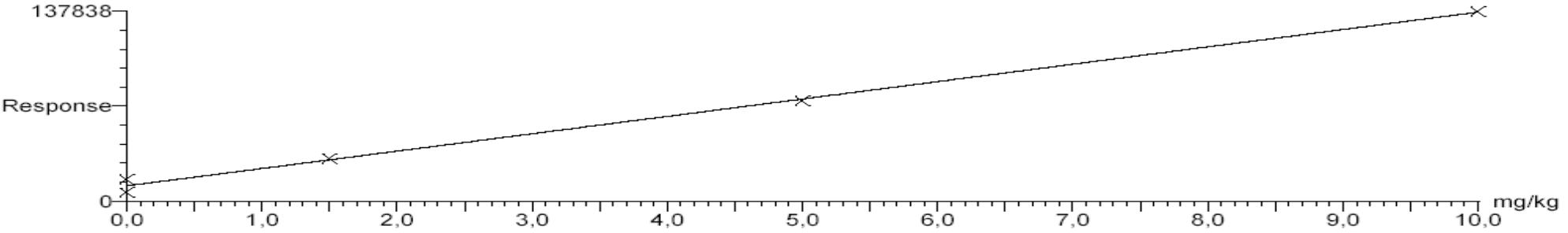
Kalibrierung LC-MS/MS

Method: C:\MassLynx\2006_mai\mai_08.PRO\MethDB\WM_06.mdb 01 Mai 2006 19:35:00
Calibration: 08 Mai 2006 08:10:43

Compound name: IS-DEHP_d4
Correlation coefficient: r = 0,282424, r^2 = 0,079763
Calibration curve: -64,3768 * x + 12097
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

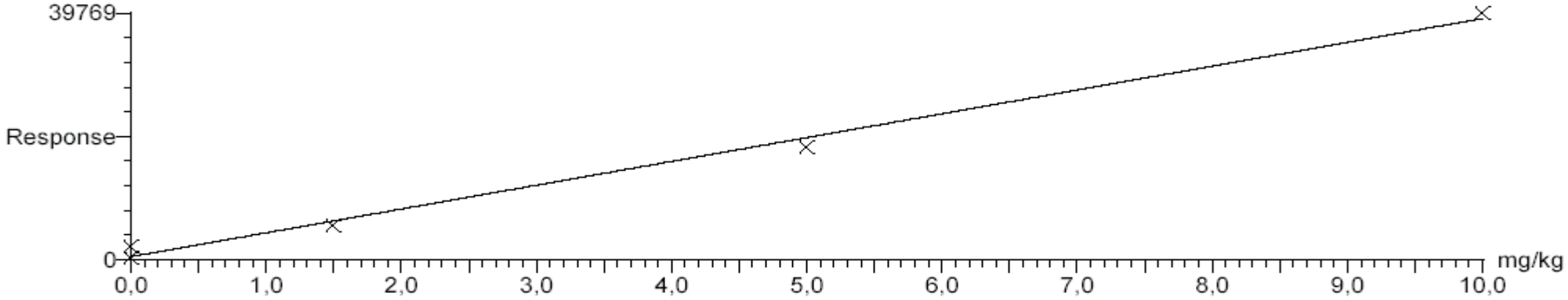


Compound name: DEHP_DOP
Correlation coefficient: r = 0,997744, r^2 = 0,995494
Calibration curve: 12615 * x + 11079,4
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

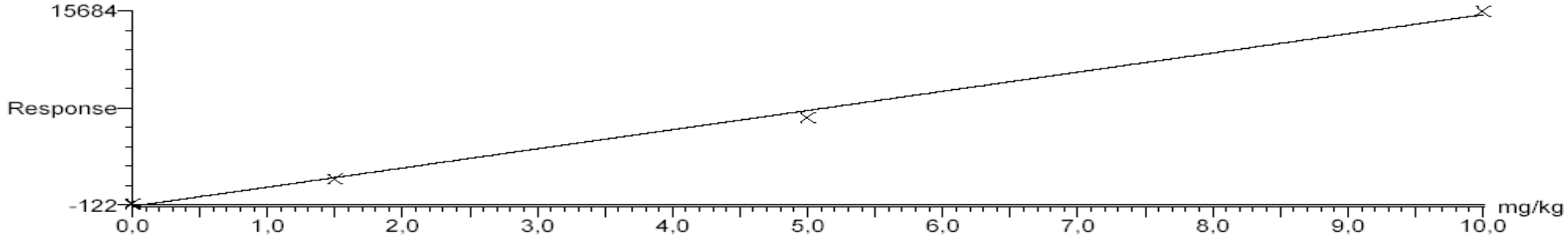


Kalibrierung LC-MS/MS

Compound name: DINP_a
Correlation coefficient: r = 0,996981, r^2 = 0,993972
Calibration curve: 3839,09 * x + 493,664
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None



Compound name: DIDP
Correlation coefficient: r = 0,998533, r^2 = 0,997068
Calibration curve: 1550,06 * x + -121,649
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

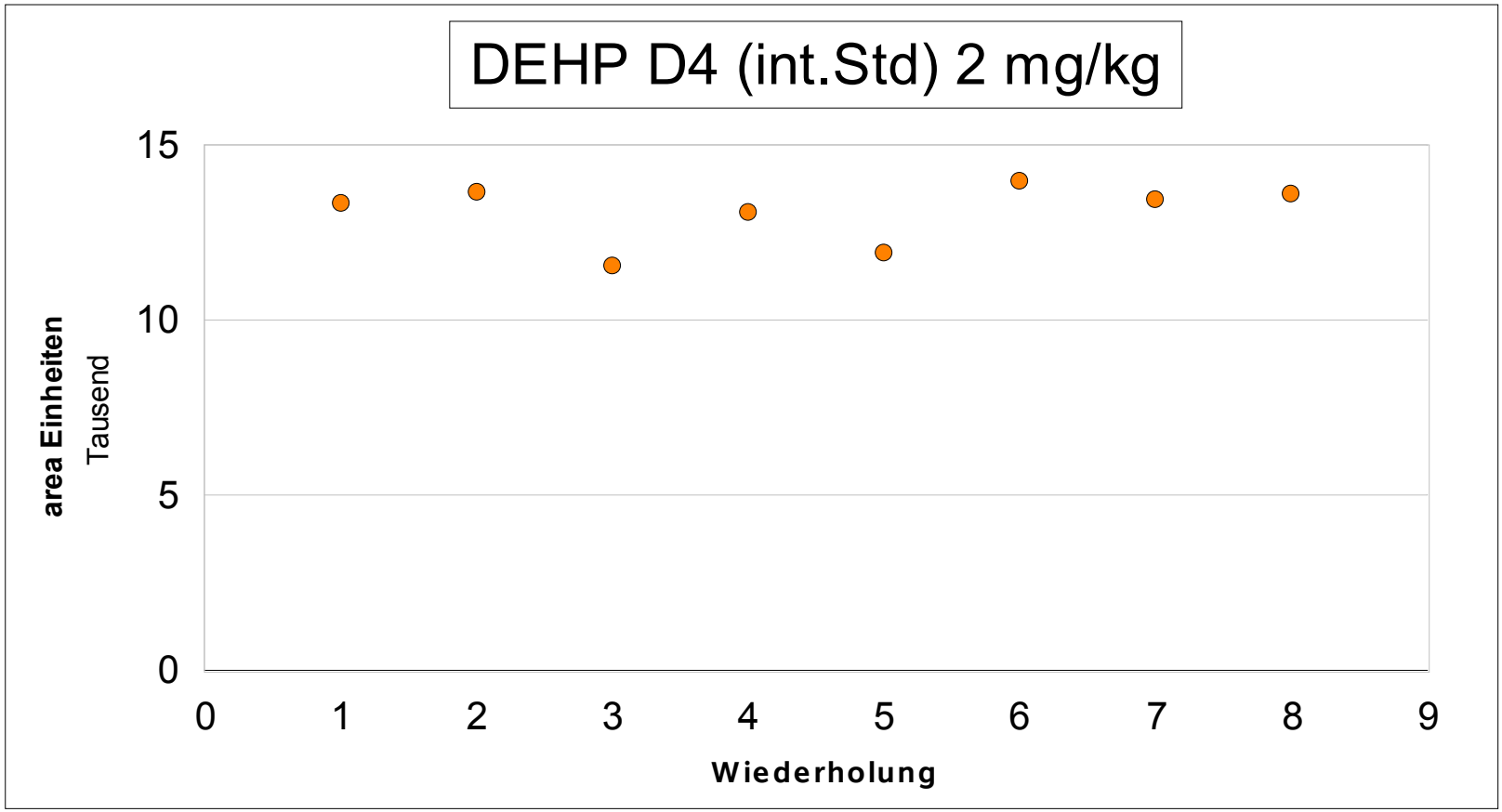


Wiederholungsmessungen DEHP LC-MS/MS

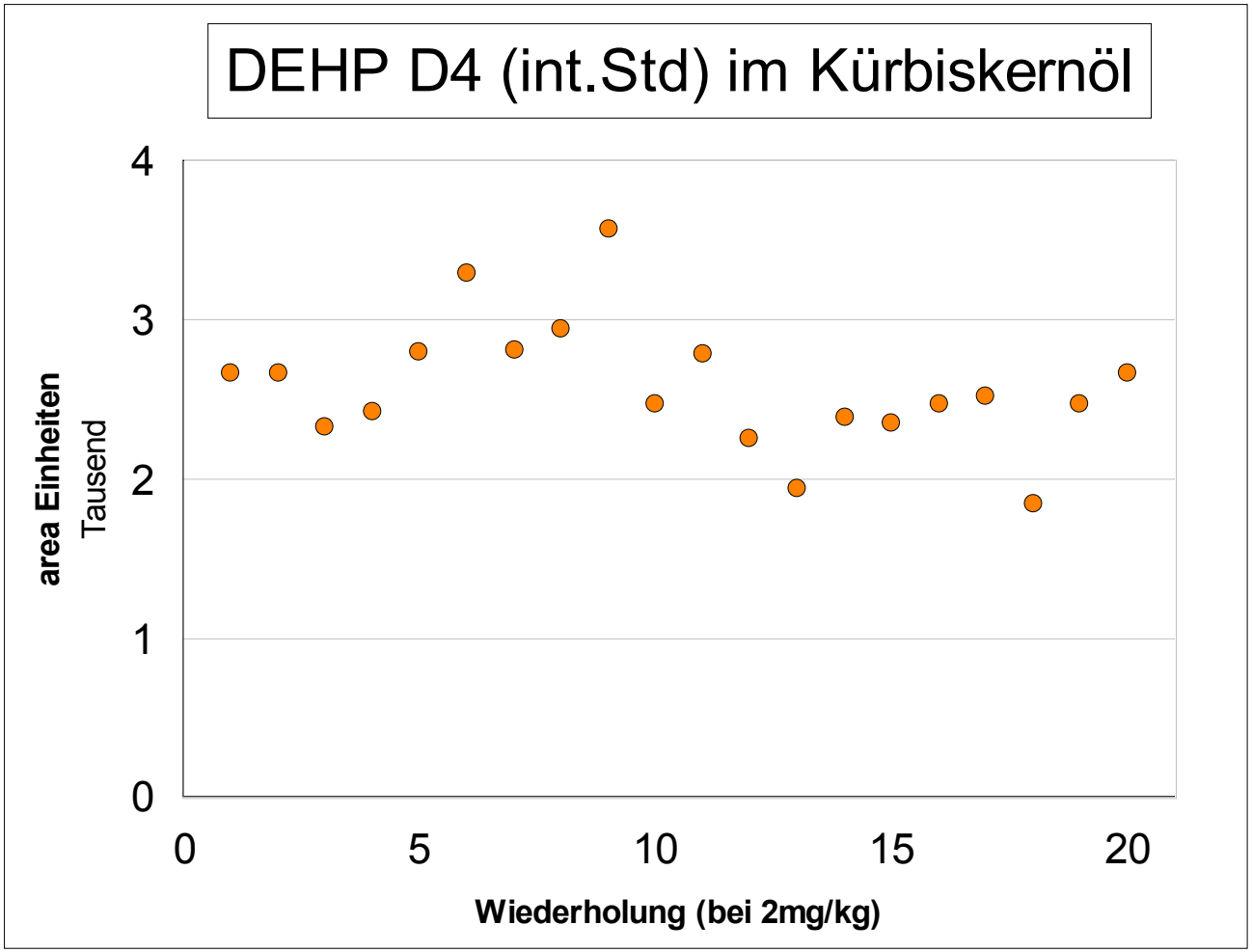
ohne Korrektur durch internen Standard

area Einheiten			int.Std
Wdh Nr.	m/z 149	m/z 167	m/z 153
1	22337	4463	13413
2	19025	3505	13736
3	18487	3647	11610
4	20198	4310	13107
5	16982	3550	11969
6	21113	4400	14024
7	21503	4537	13487
8	20911	3708	13665
Mittelwert	20069,5	4015	13126
Stdabw.	1663,5	420,5	815,4
rel.Stdabw %	8,3	10,5	6,2
Gehaltmg/kg	0,8	0,9	

Interner Standard in Olivenöl



Mittelwert:	13126
Standardabweichung:	815
rel. Standardabweichung:	6,2%



Mittelwert:	25694
Standardabweichung:	391
rel. Standardabweichung:	15,1%

Kürbiskernöl dotiert (n=5)

	DEHP	DINP a	DINP b	soll
Mittelwert	1,24	1,4	1,5	1mg
StdAbw	0,17	0,18	0,24	
RSD %	14,06	12,78	16,33	
Mittelwert	6	6,52	7,12	5mg
StdAbw	0,4	0,31	0,32	
RSD %	6,75	4,79	4,56	
Mittelwert	10,22	9,52	10,5	10mg
StdAbw	0,69	0,33	0,7	
RSD %	6,79	3,48	6,65	