

**Spectral Service**



Laboratorium  
für Auftragsanalytik GmbH

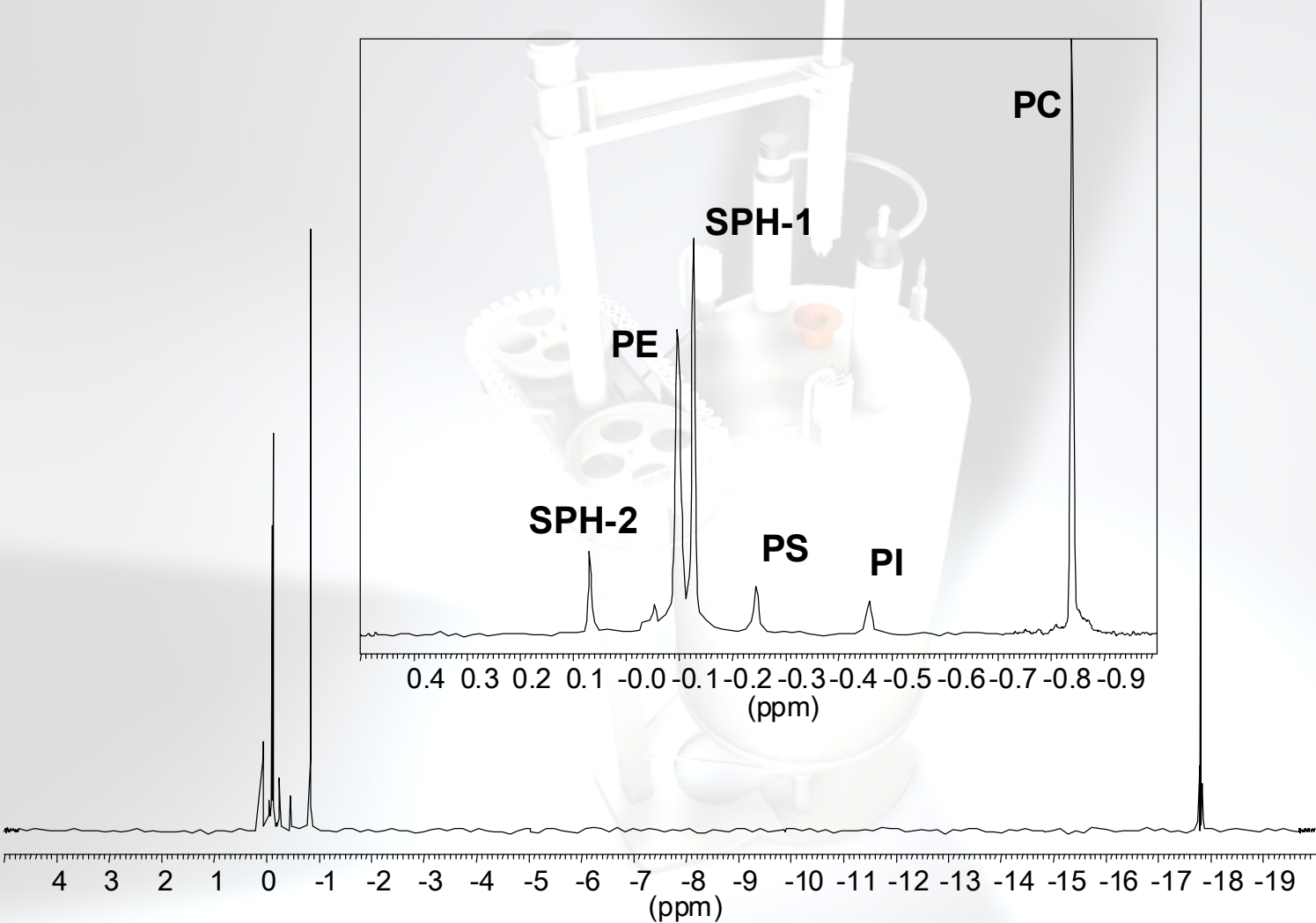


***Characterisation of milk lecithin  
by NMR and LC-MS***

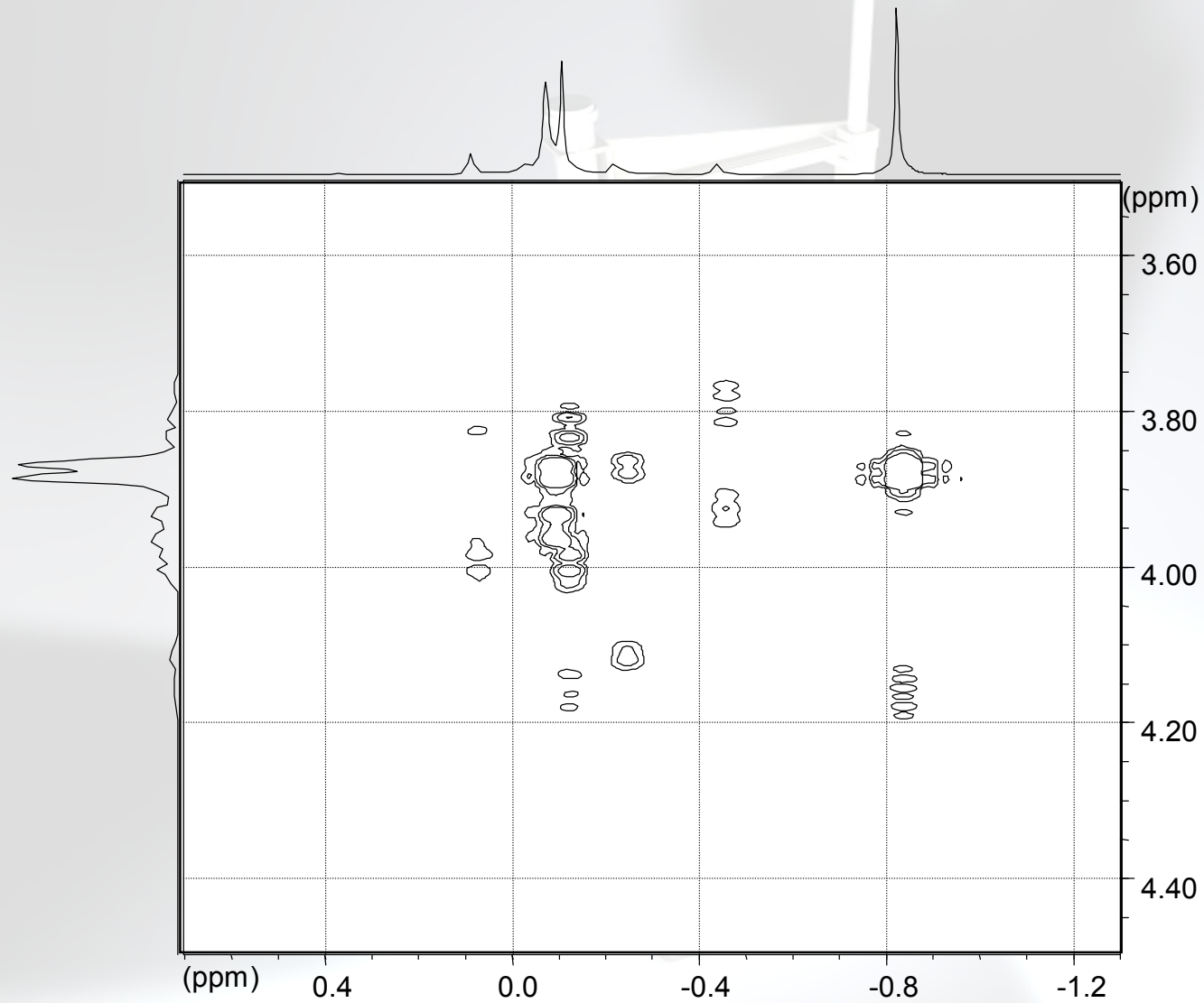
Dr. Bernd Diehl  
Spectral Service GmbH

7<sup>th</sup> May 2008

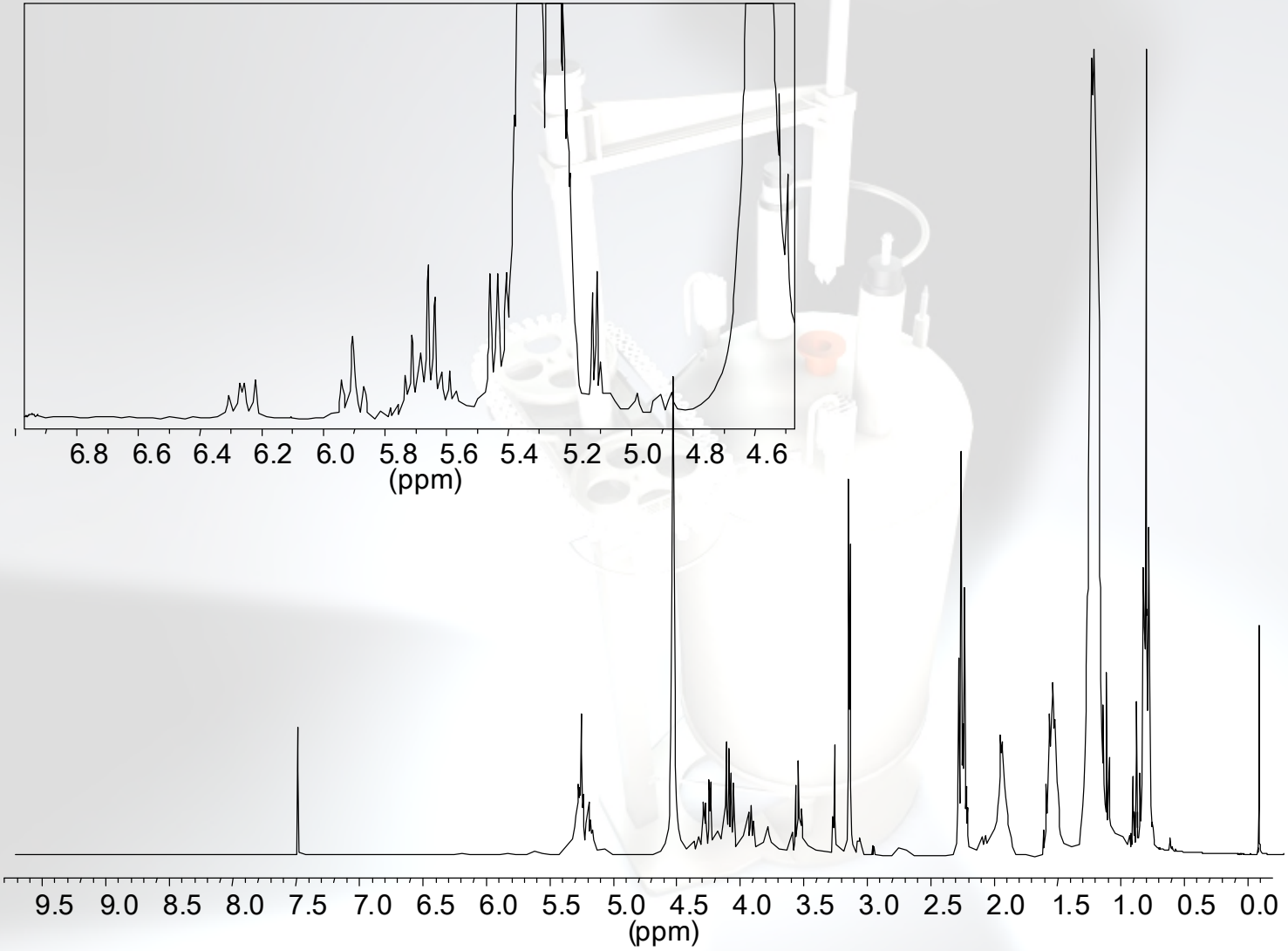
# $^{31}\text{P}$ -NMR spectrum of milk lecithin



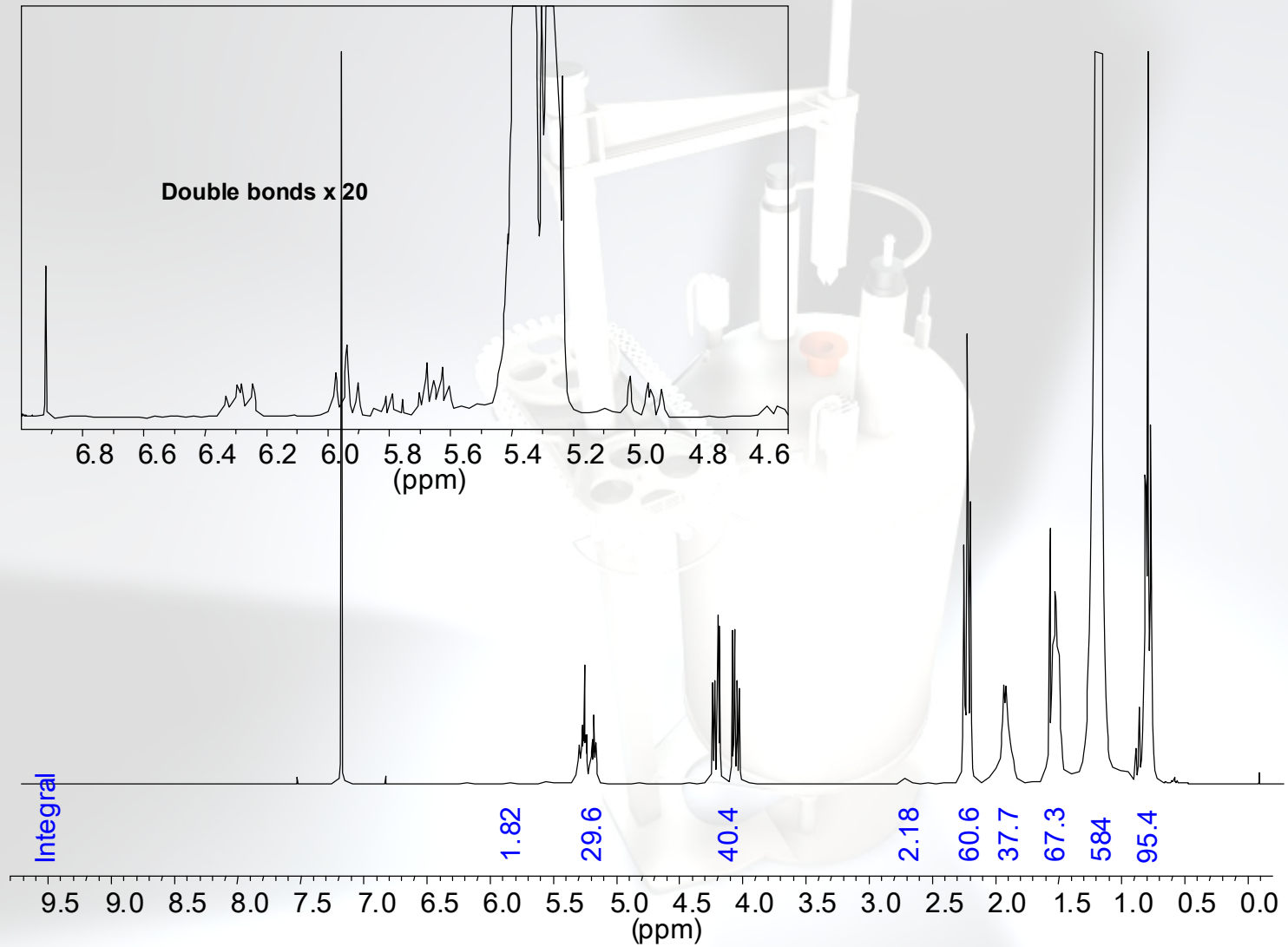
# P,H COSY-NMR spectrum of milk lecithin



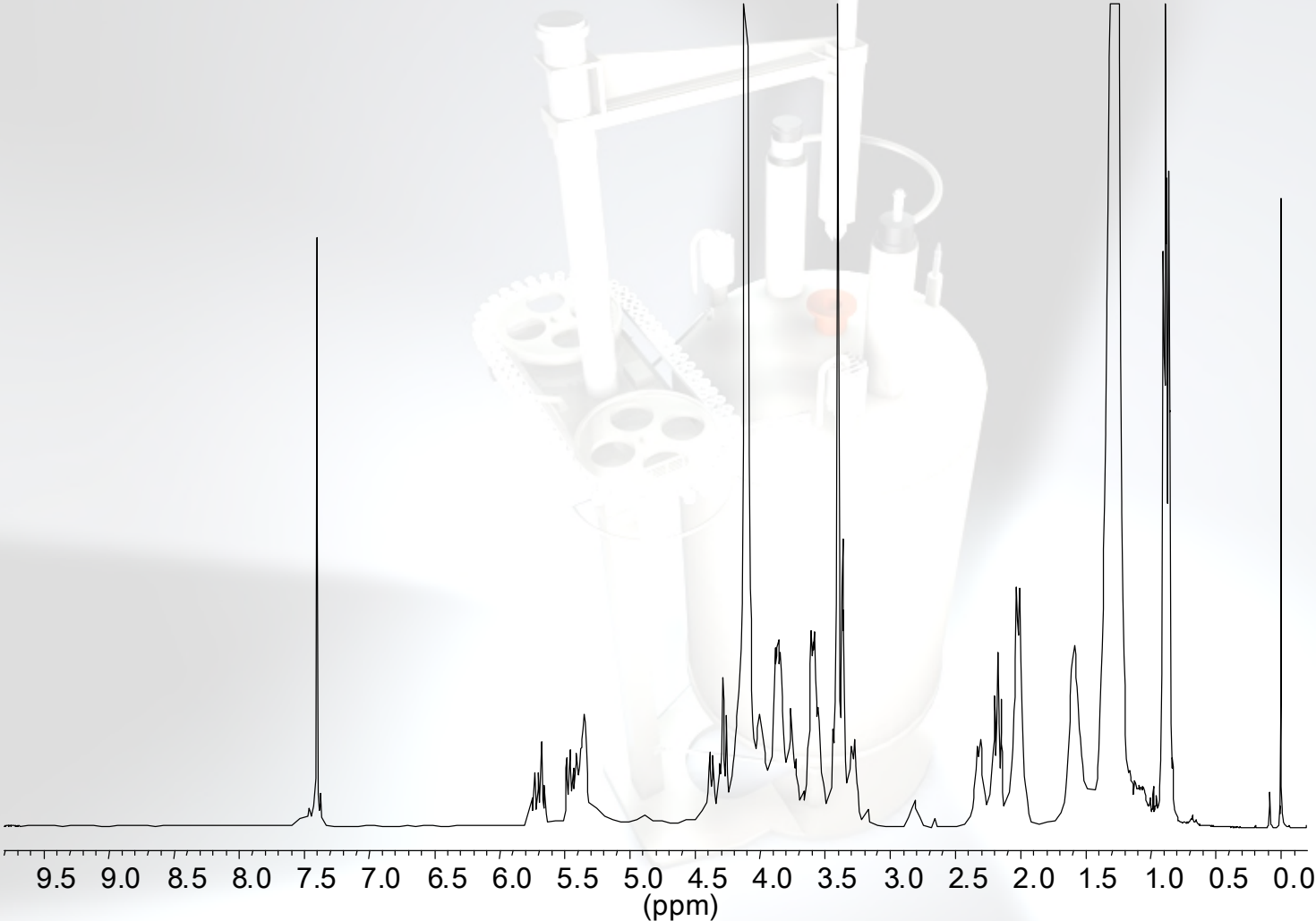
# $^1\text{H}$ -NMR spectrum of milk lecithin



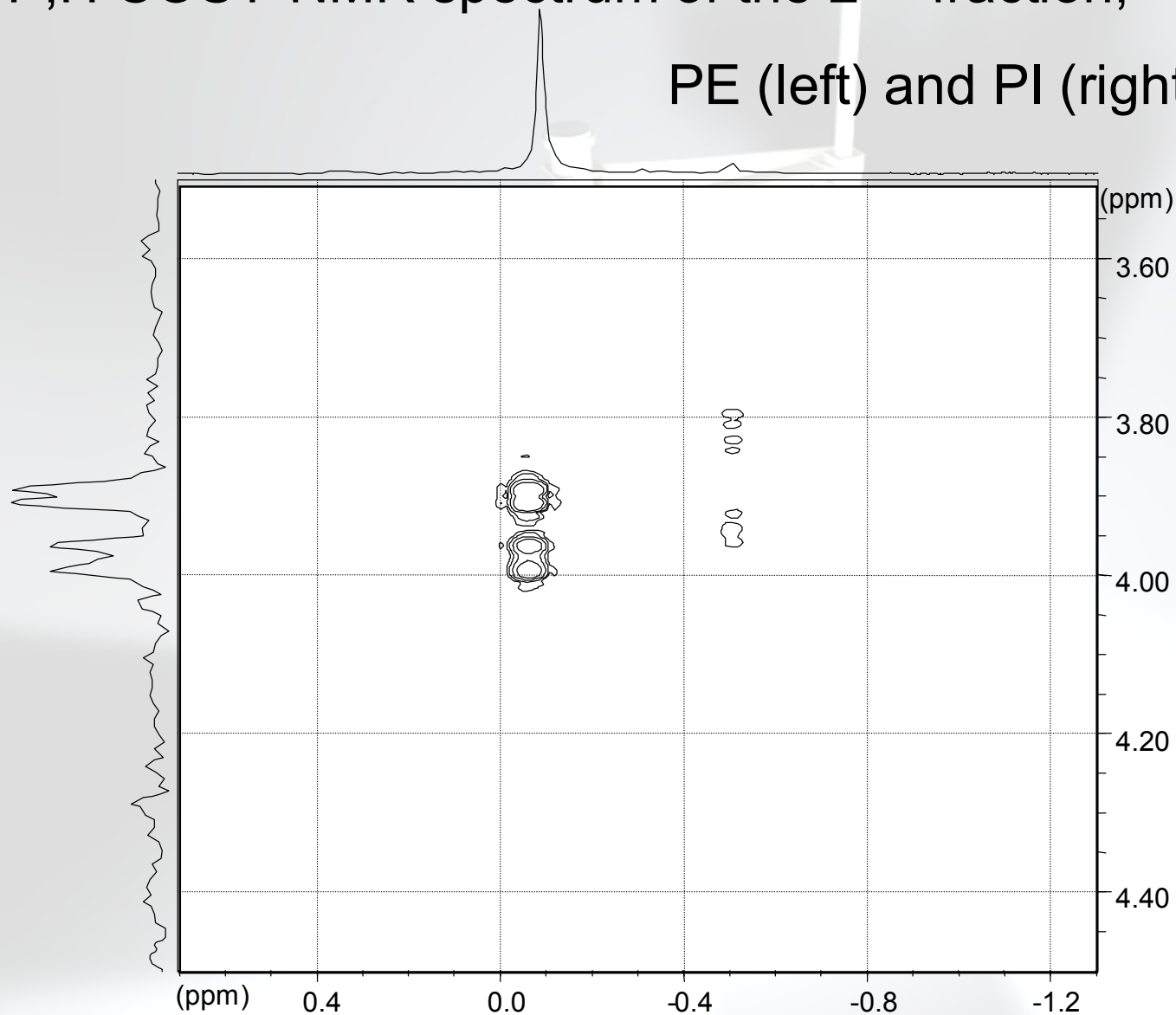
# $^1\text{H}$ -NMR spectrum of the 1<sup>st</sup> Fraction, triglycerides



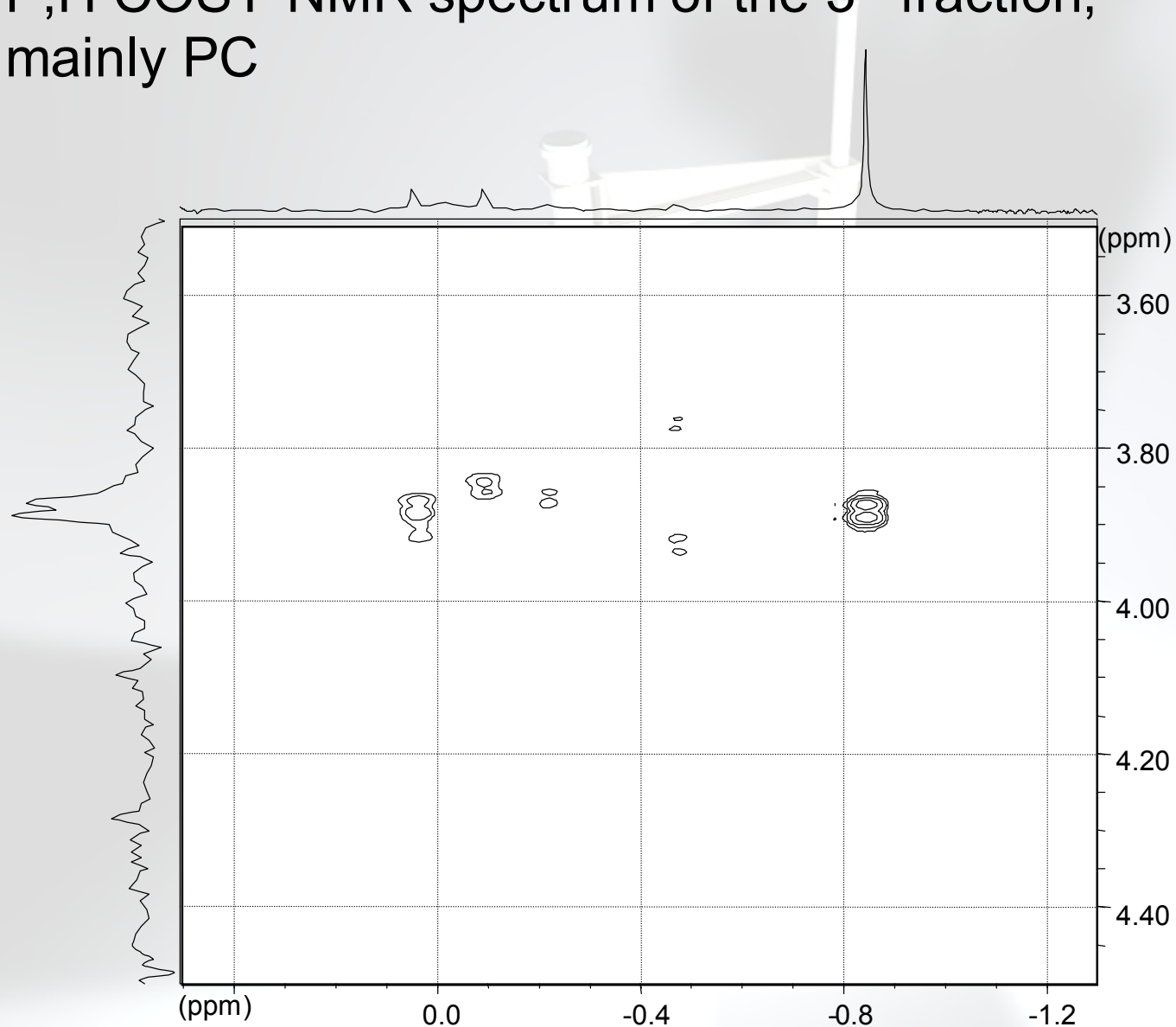
# $^1\text{H}$ -NMR of Sphingolipids of milk (cerebroside)



# P,H COSY-NMR spectrum of the 2<sup>nd</sup> fraction, PE (left) and PI (right)

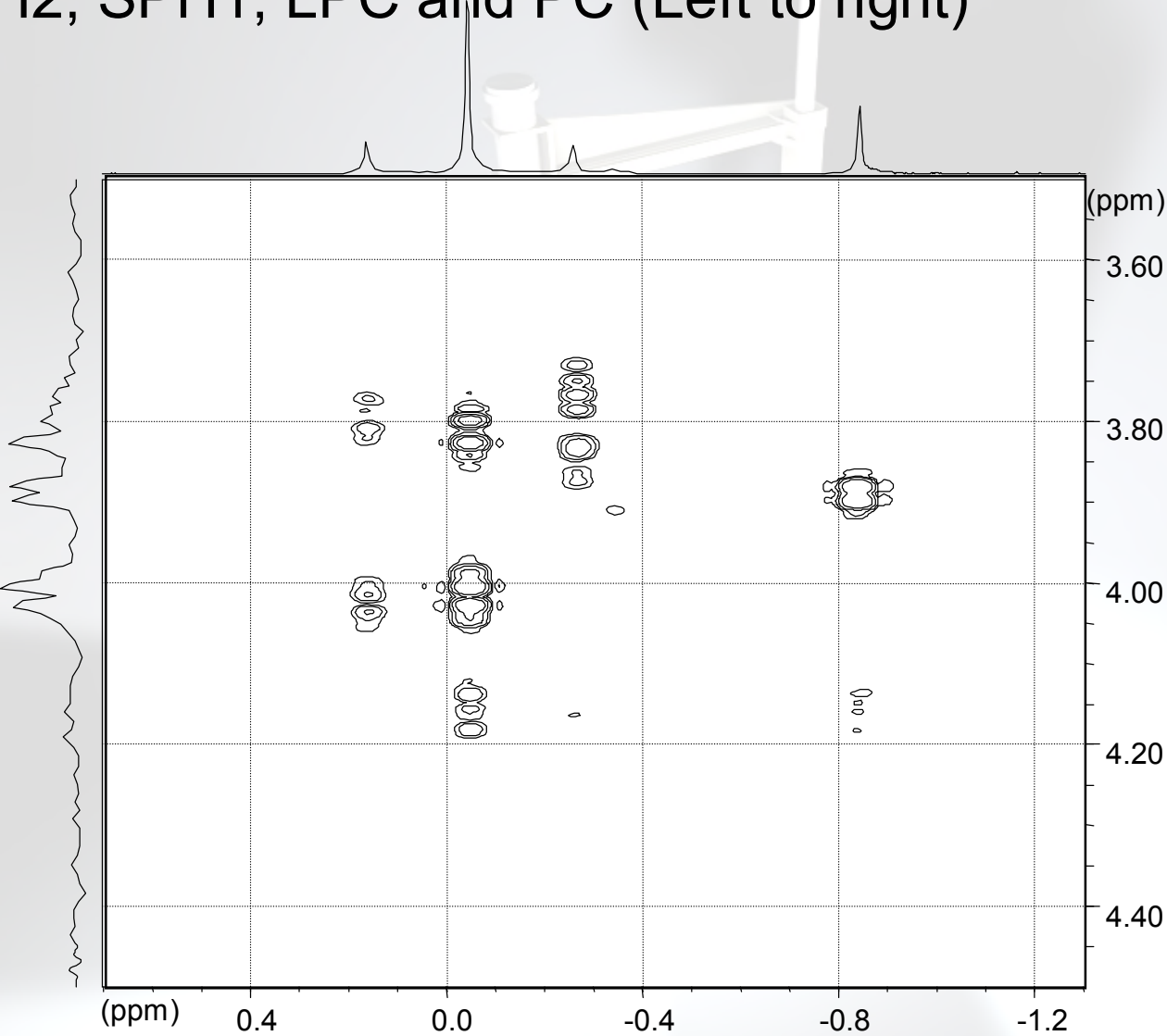


# P,H COSY-NMR spectrum of the 3<sup>rd</sup> fraction, mainly PC

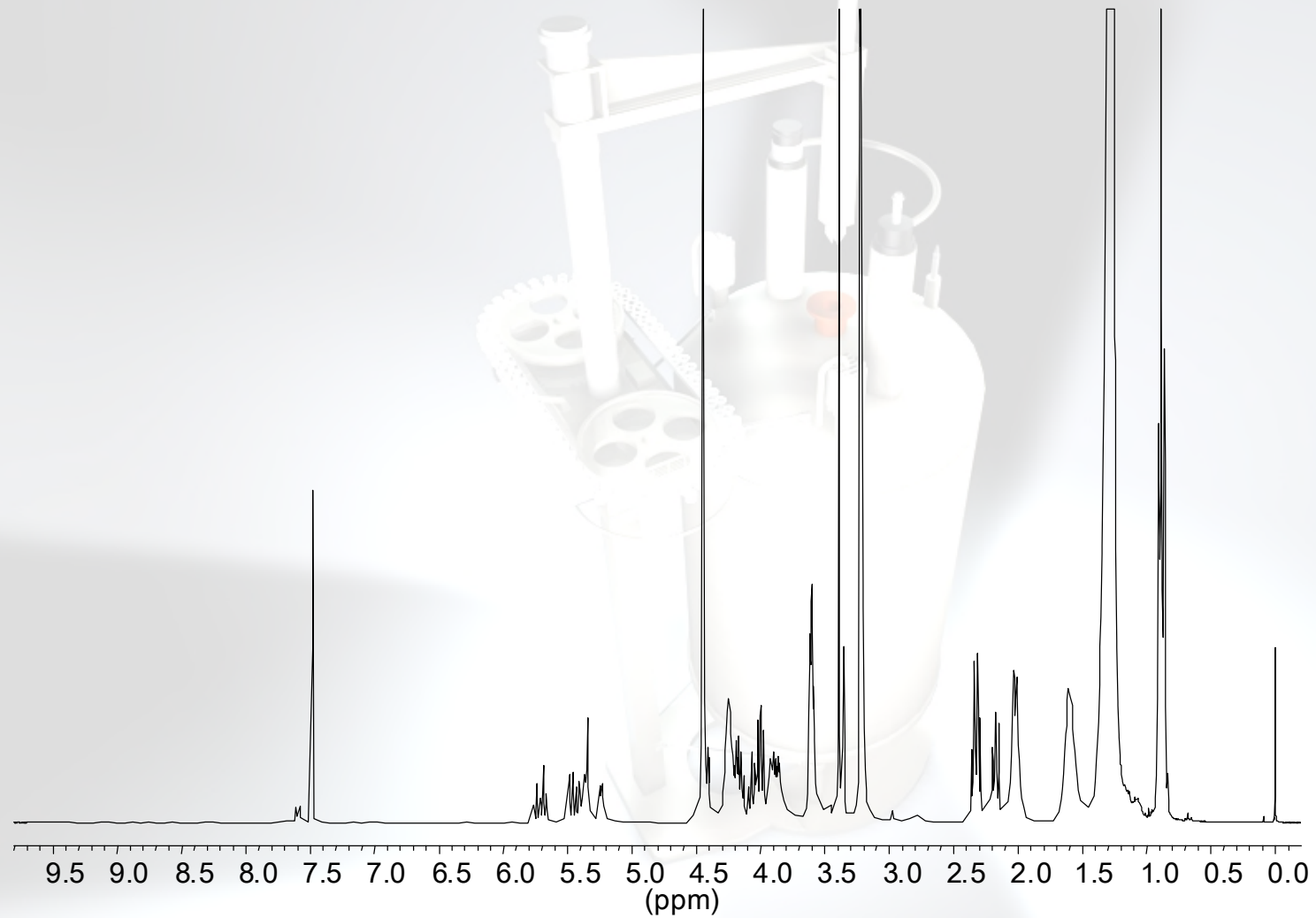




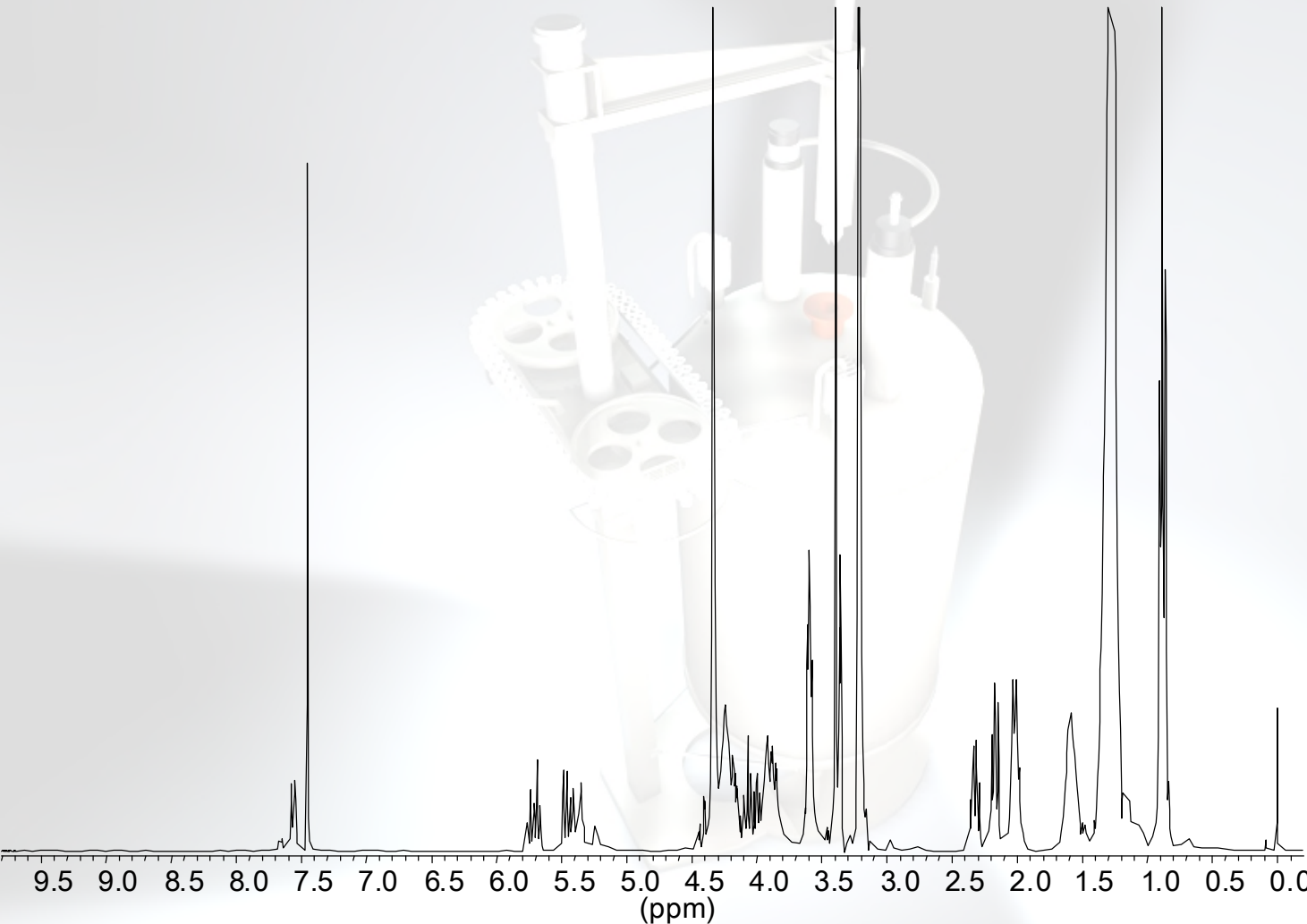
# P,H COSY-NMR spectrum of the 4<sup>th</sup> fraction, SPH2, SPH1, LPC and PC (Left to right)



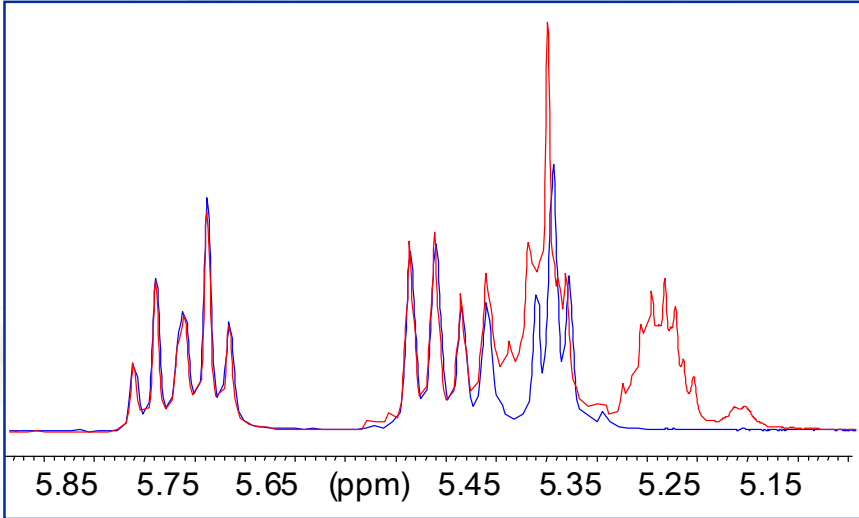
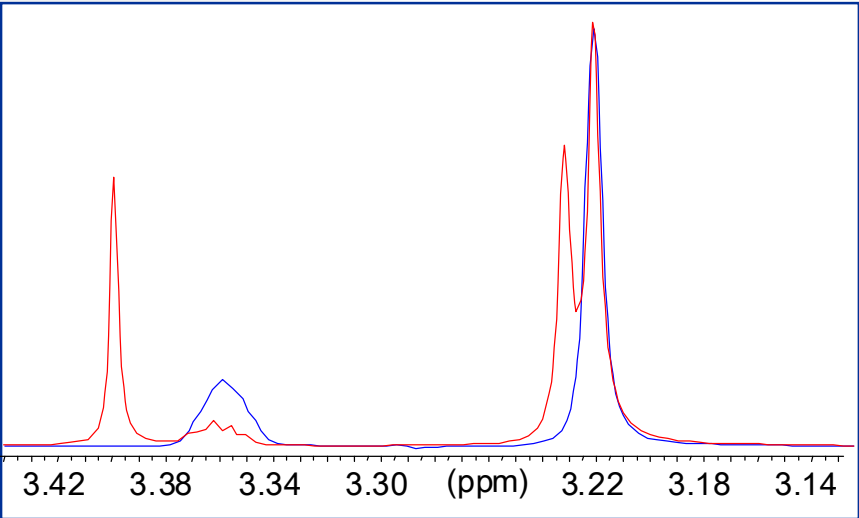
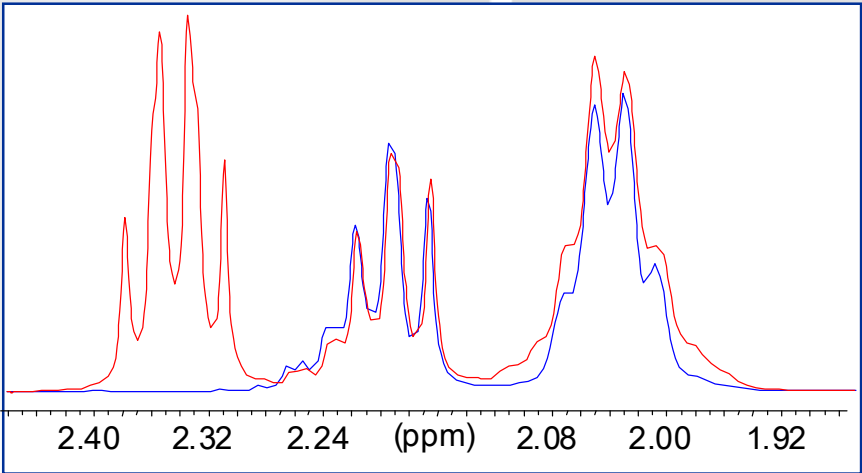
# $^1\text{H}$ -NMR of Sphingomyelin from milk containing PC



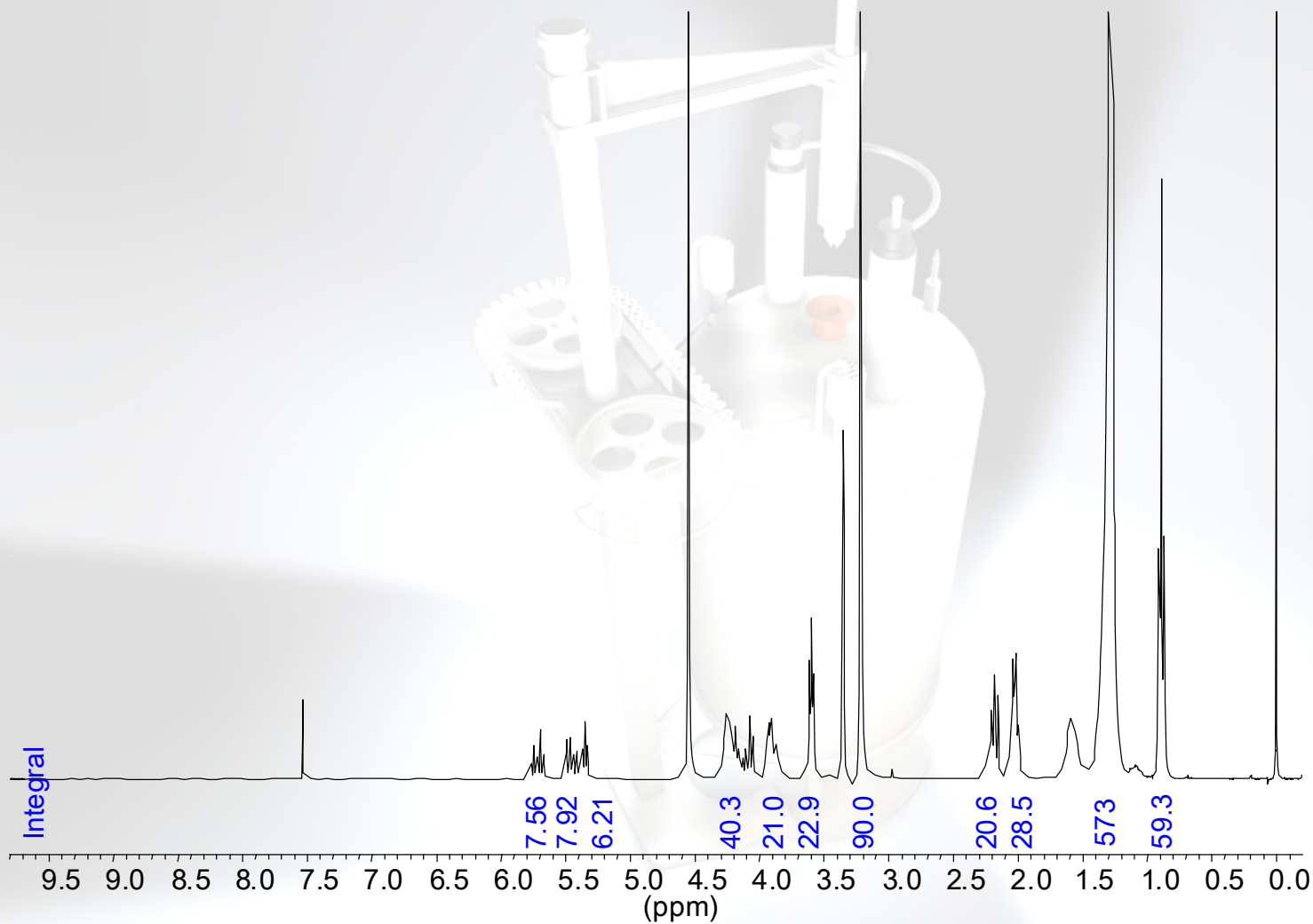
# $^1\text{H}$ -NMR of Sphingomyelin from milk containing less PC



Red: pure Sphingomyelin  
Blue: Sphingomyelin + PC



# $^1\text{H}$ -NMR of Sphingomyelin from bovine brain



# $^{31}\text{P}$ -NMR of Sphingomyelin fraction from milk, containing PC

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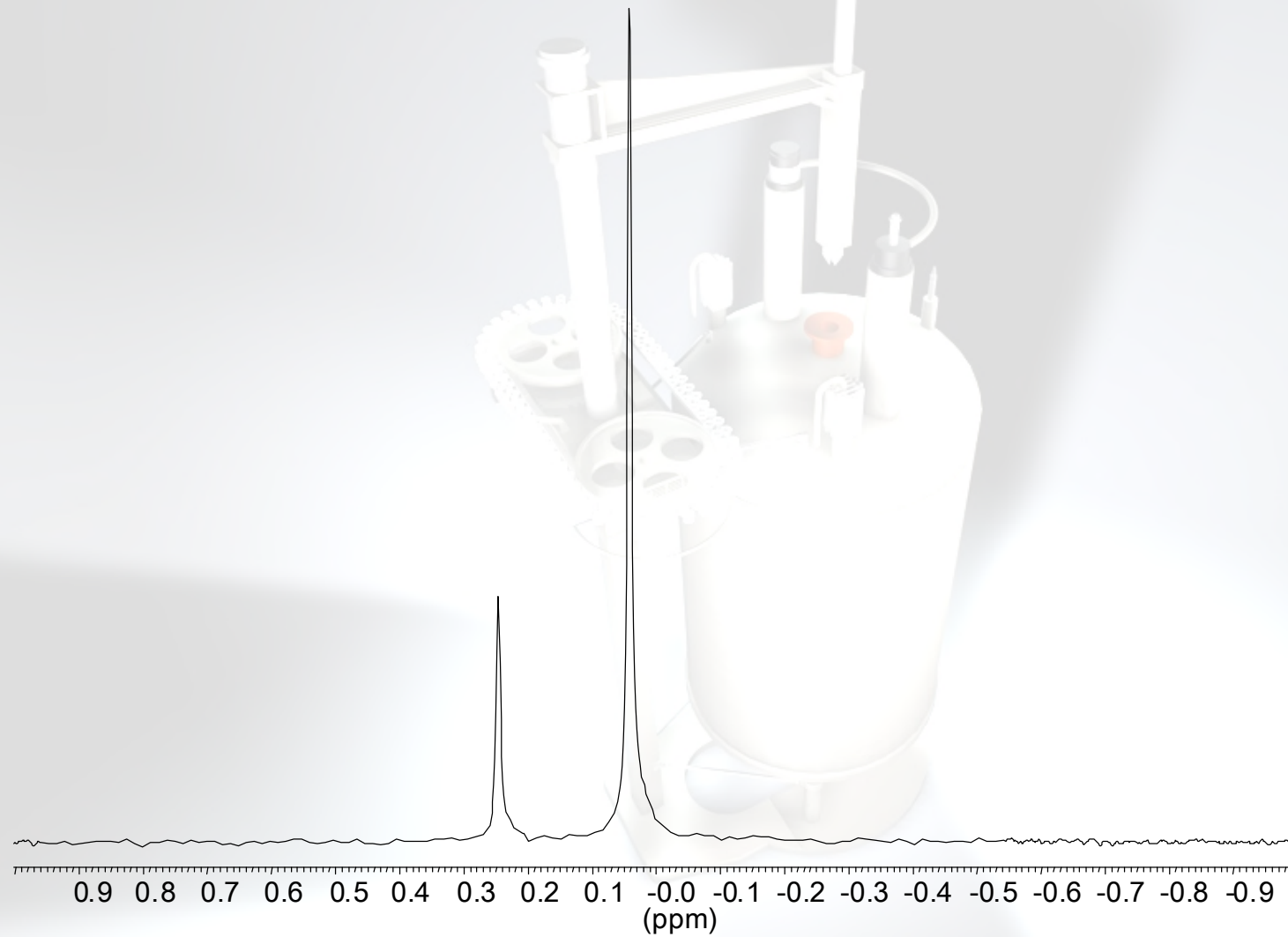


# $^{31}\text{P}$ -NMR of Sphingomyelin from bovine brain

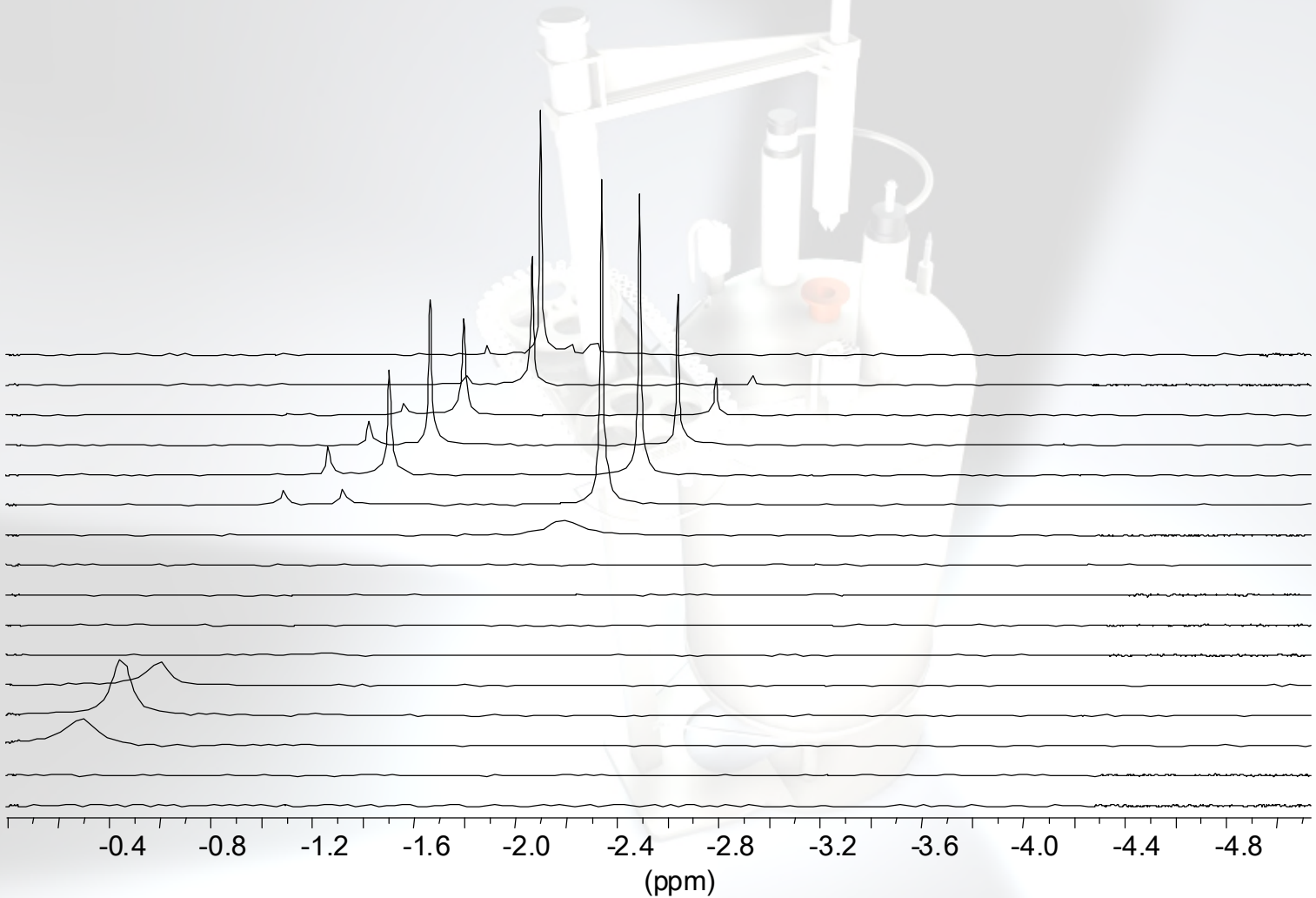
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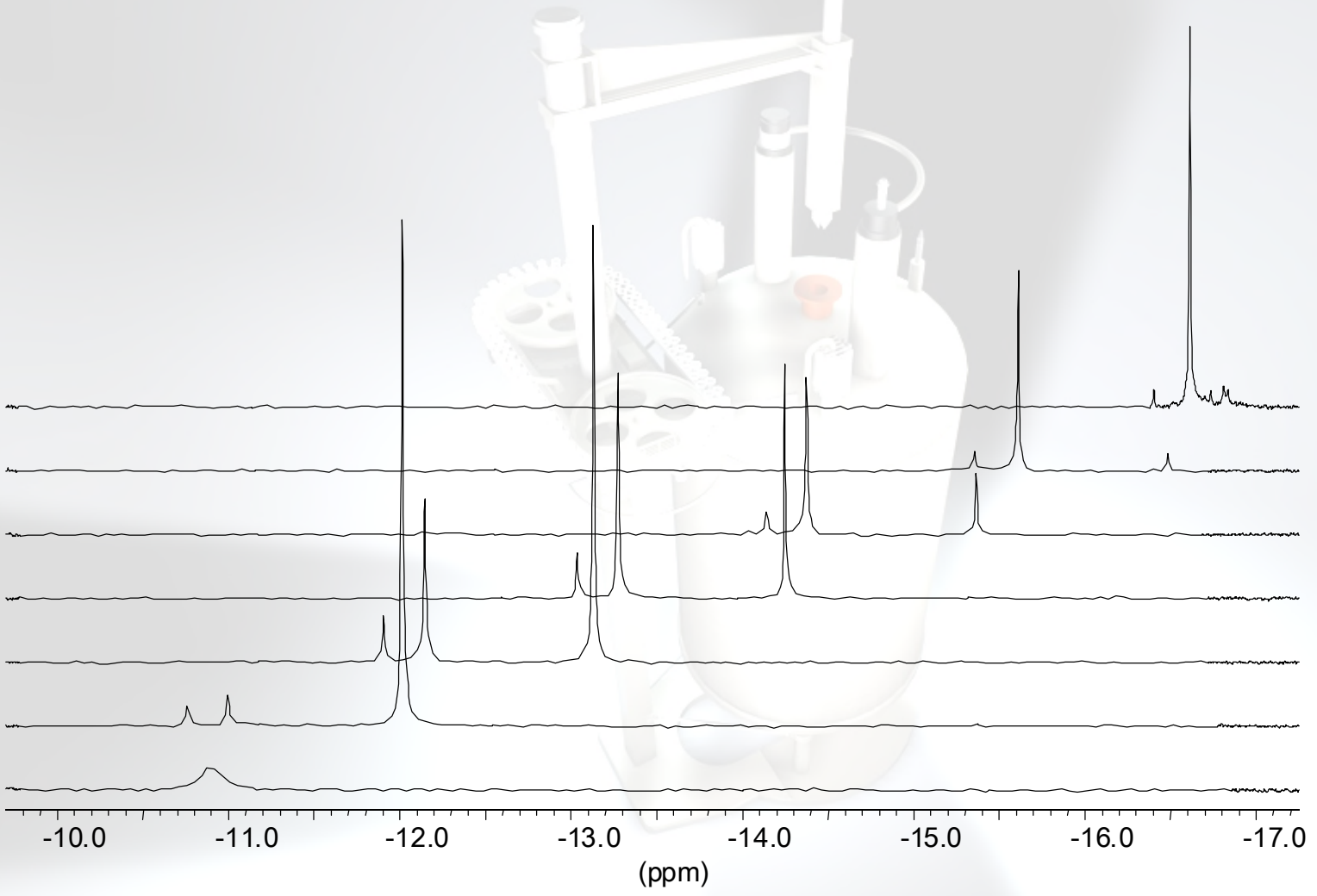


# Offline Column Chromatography $^{31}\text{P}$ -NMR coupling of Milk lecithin, Fraction 9 - 24

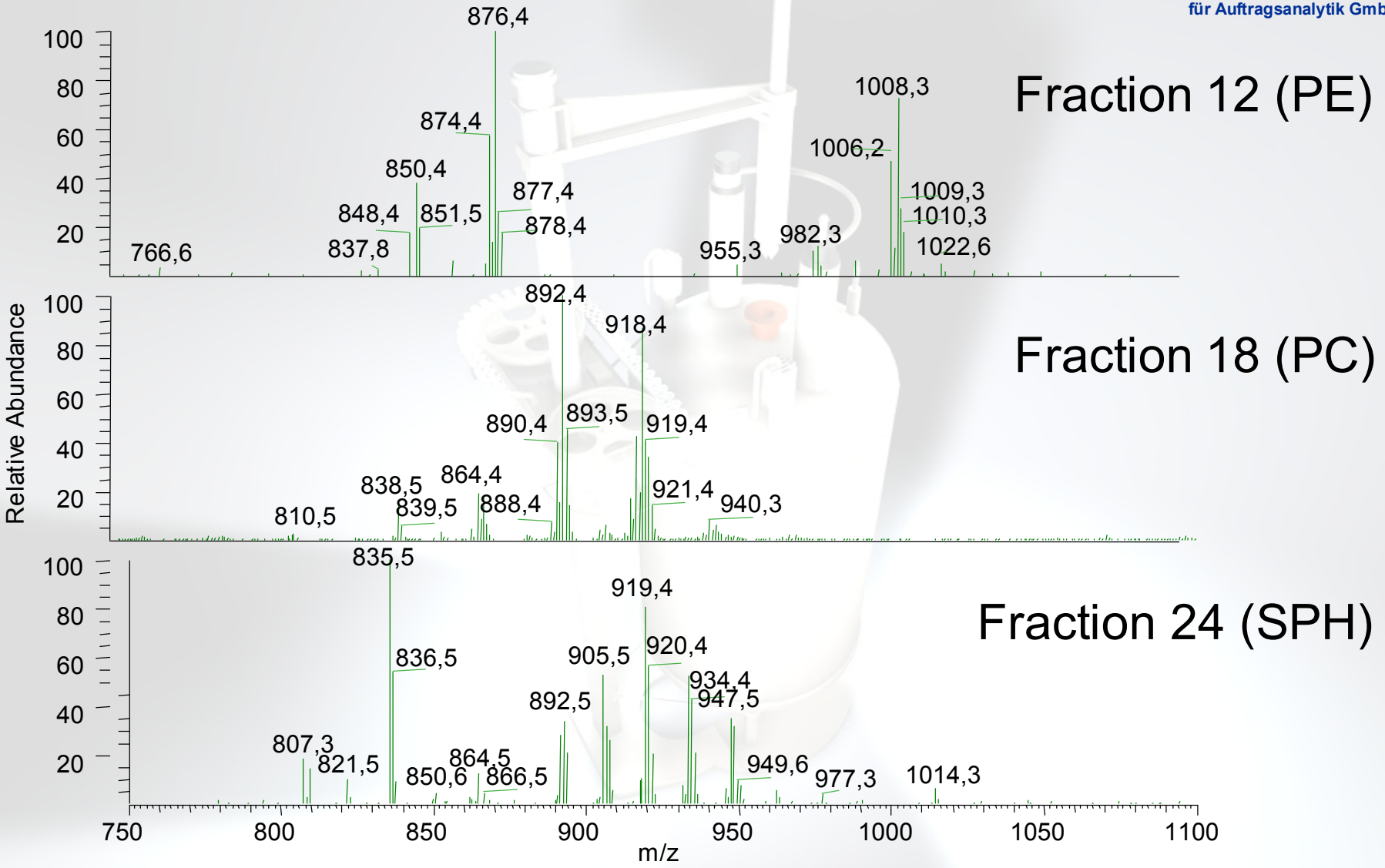




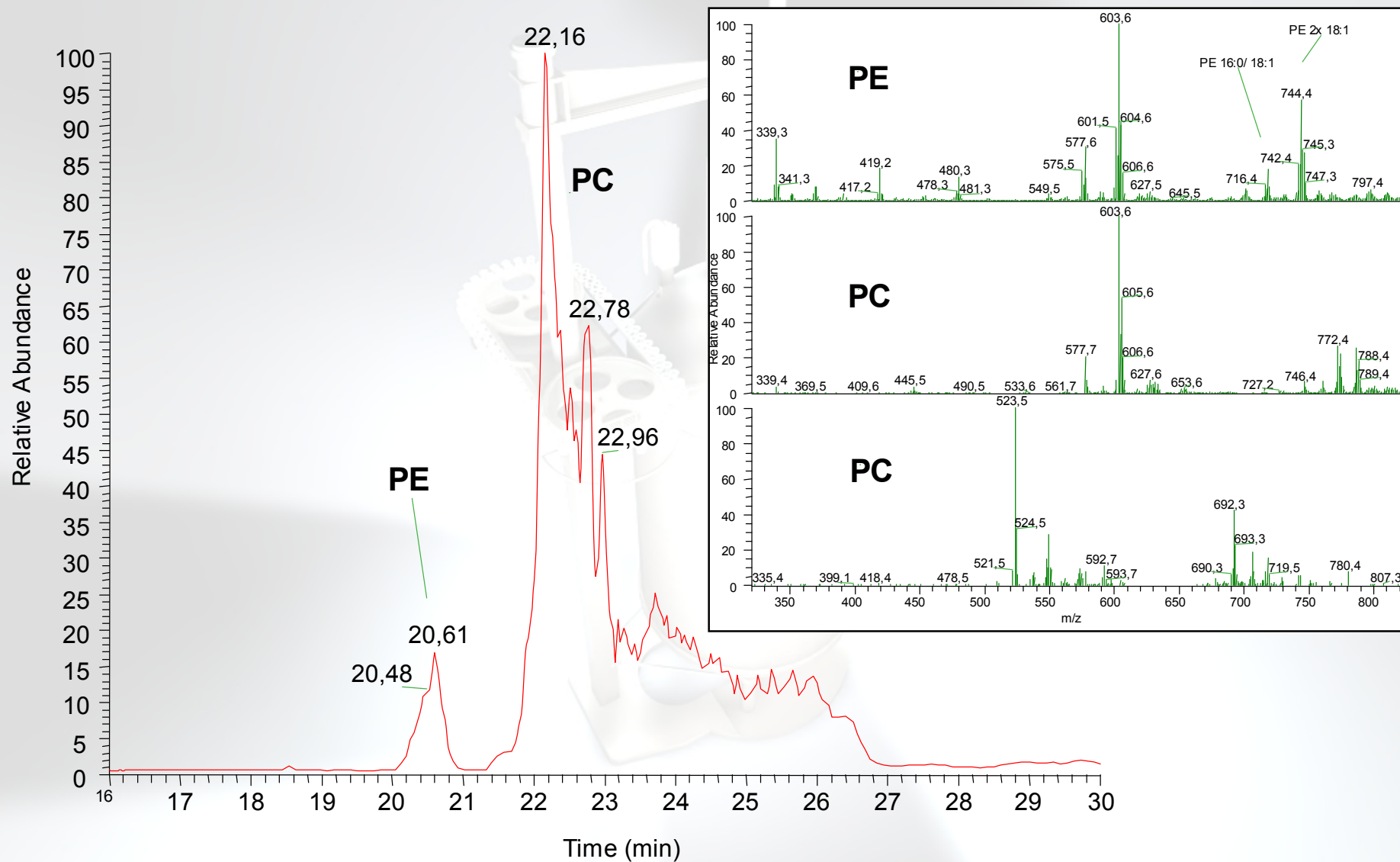
# Offline Column Chromatography $^{31}\text{P}$ -NMR coupling of Milk lecithin, Fraction 18 - 24



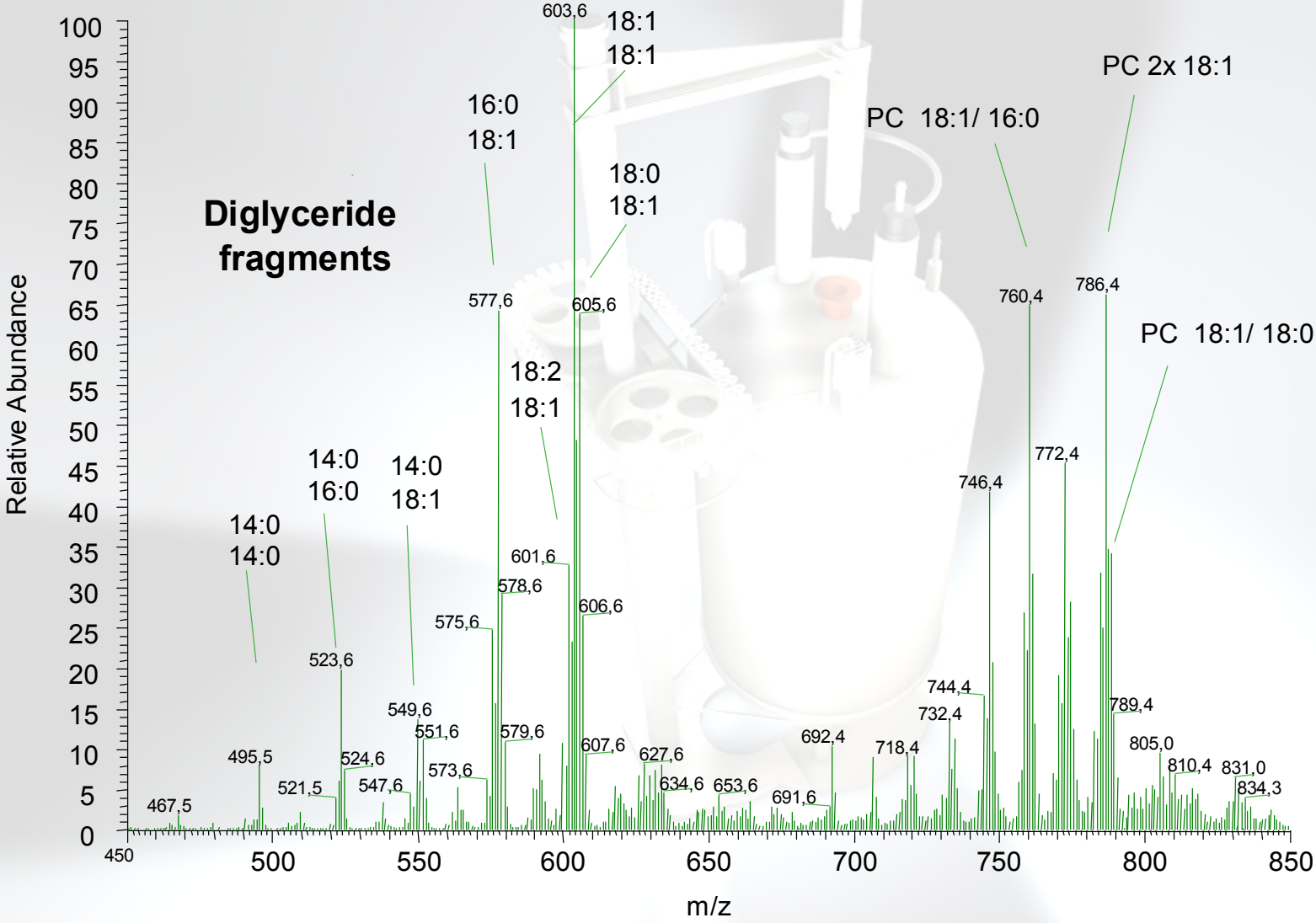
# +ESI of Milk fractions, [M+Cs]<sup>+</sup>



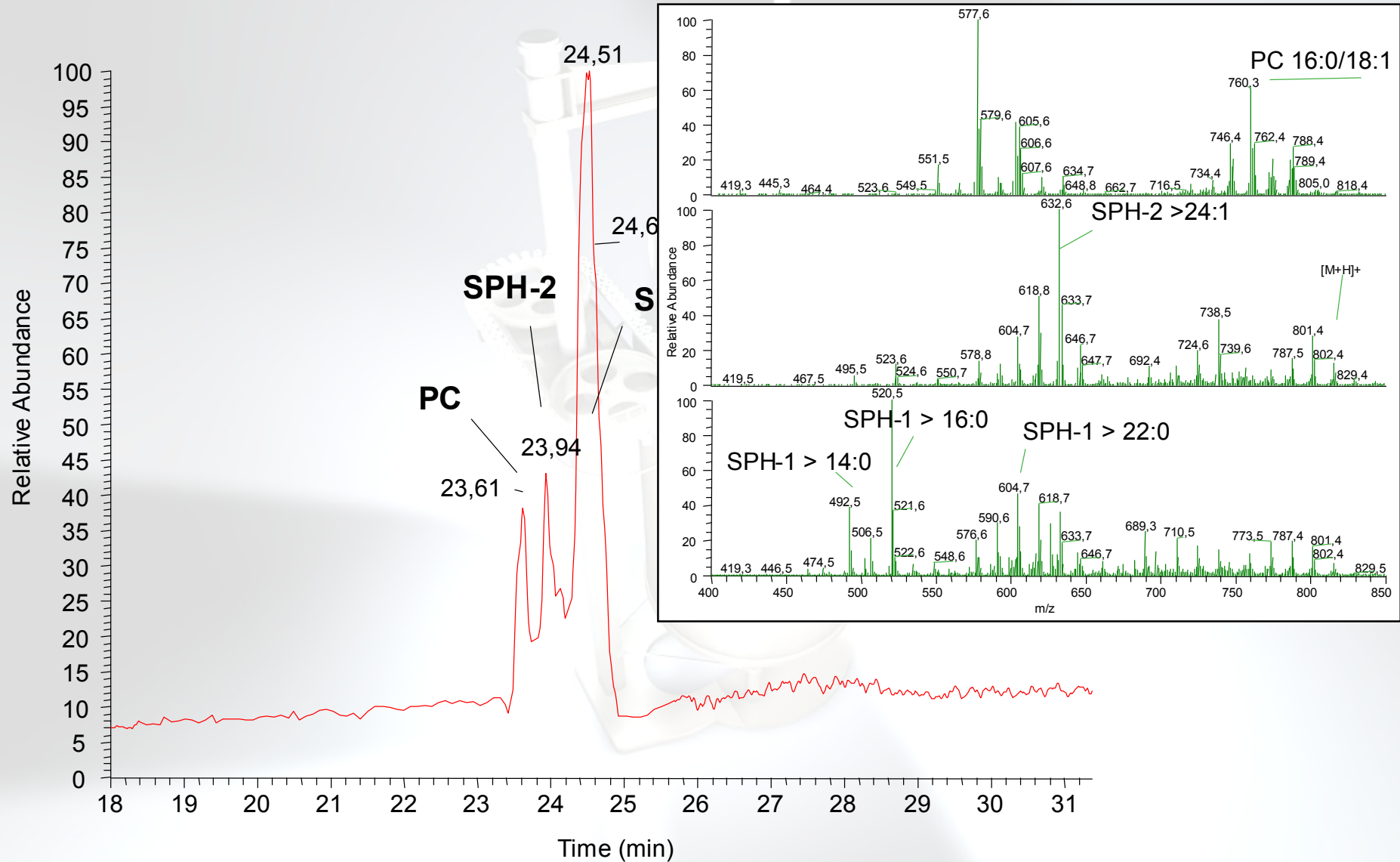
## LC-MS of PC and PE, Fraction 18



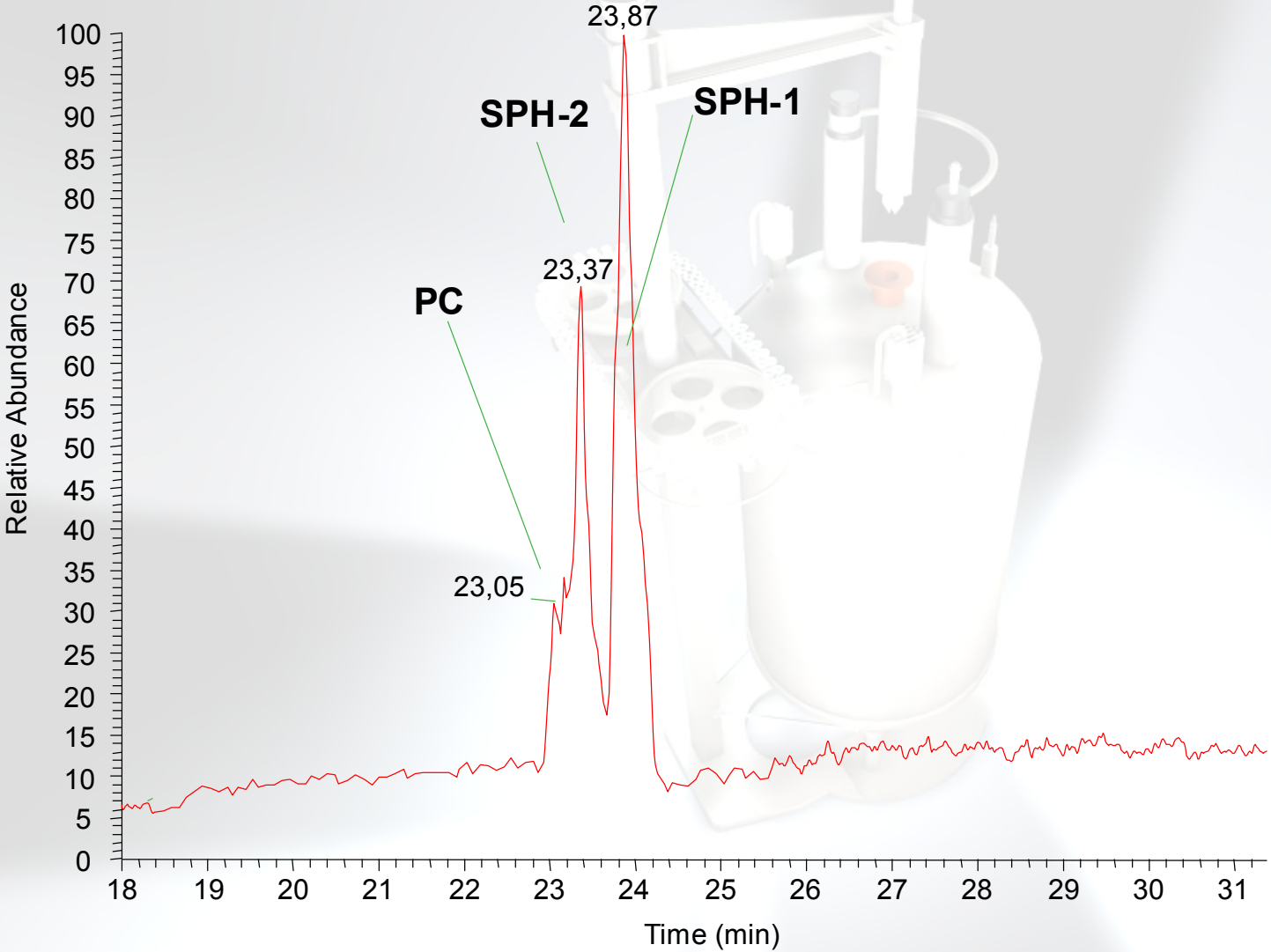
# LC-MS +APCI of PC from Milk



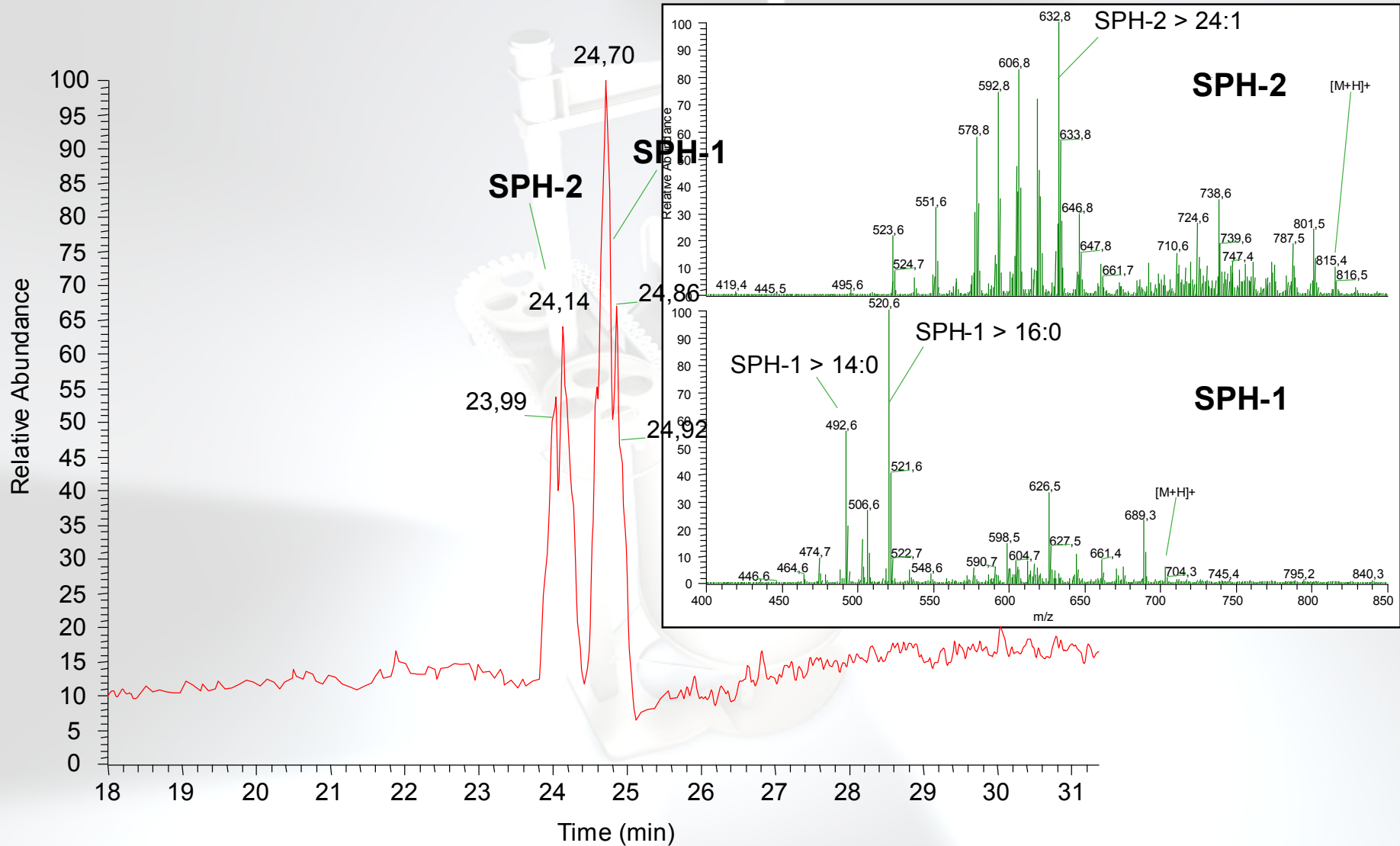
## LC-MS of SPH with PC, Fraction 22



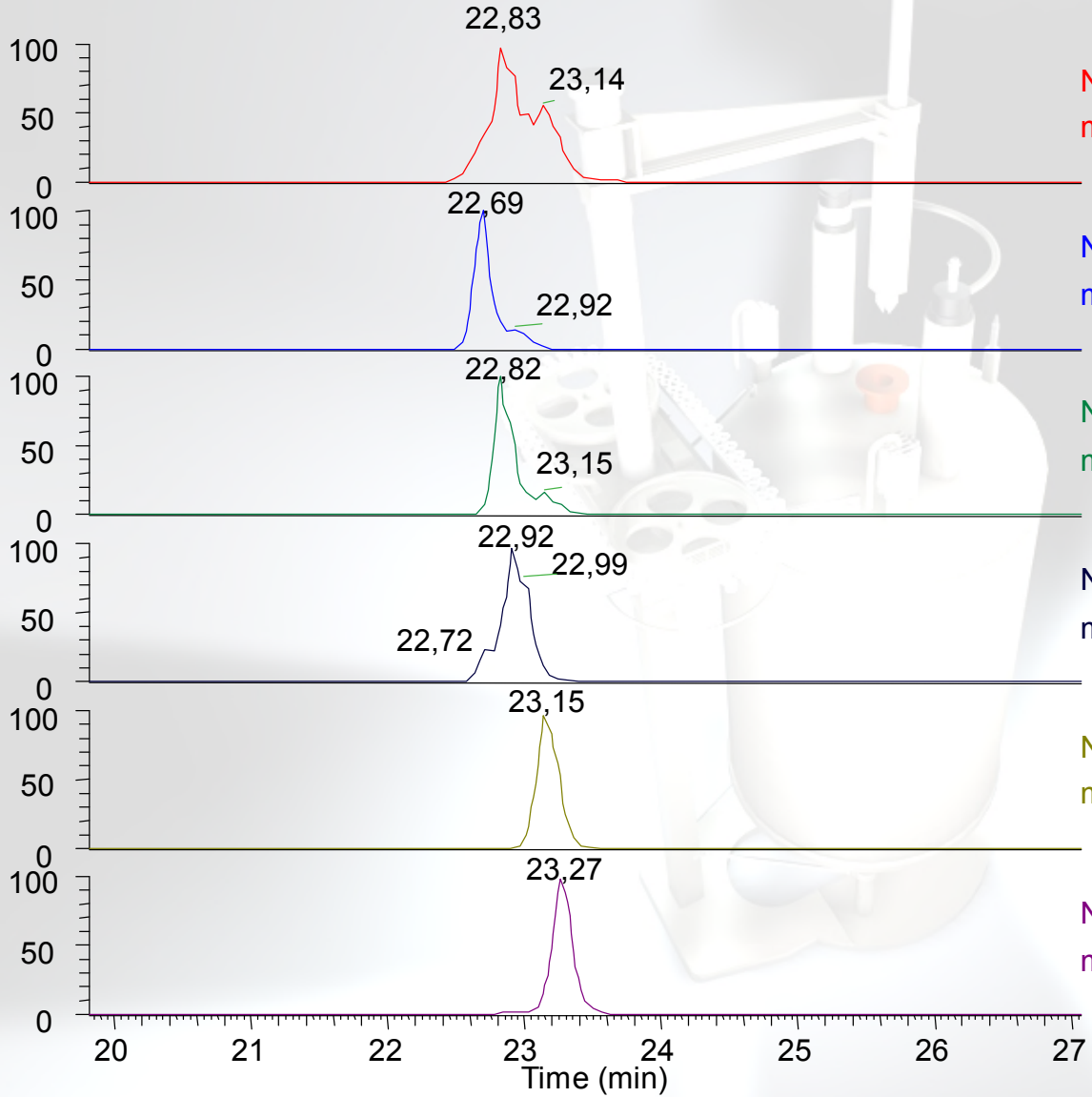
# LC-MS of SPH, Fraction 23



## LC-MS of SPH, Fraction 24



# LC-MS of SPH from Bovine Brain



NL3.60E7  
m/z = 400-1000 MS

NL: 1.33E6  
m/z = 607 MS

NL: 3.24E6  
m/z = 551 MS

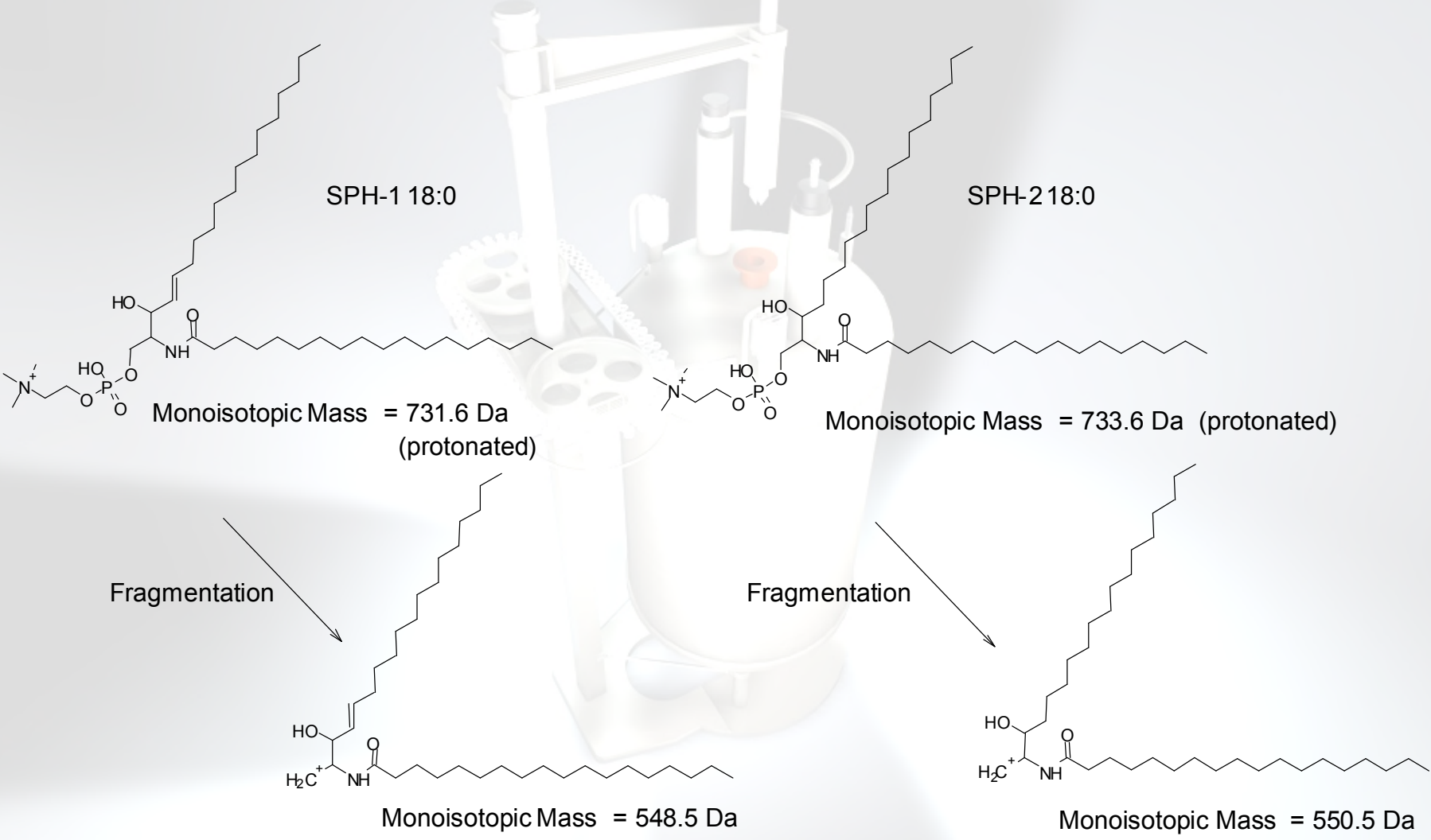
NL: 1.74E6  
m/z = 605 MS

NL: 6.19E6  
m/z = 549 MS

NL: 5.09E5  
m/z = 521 MS



# Sphingomyelin type 1 and 2, main fragmentations



## Average Molecular Weight of Milk-PC

m/z	%	MW	Part. MW
523,6	3,72	705,6	26,3
549,6	3,15	731,6	23,1
551,6	2,33	733,6	17,1
575,6	6,19	757,6	46,9
577,6	14,52	759,6	110,3
579,6	1,13	761,6	8,6
591,6	2,82	773,6	21,8
599,6	2,90	781,6	22,7
601,6	10,08	783,6	79,0
603,6	30,65	785,6	240,8
605,6	21,58	787,6	169,9
607,6	0,93	789,6	7,4
	100,00		<b>773,7</b>

# Average Molecular Weight of Milk-SPH

<b>SPH-1</b>			
m/z	%	MW	Part. MW
464,5	0,98	646,5	6,3
478,6	1,37	660,6	9,0
492,6	26,37	674,6	177,9
506,6	12,43	688,6	85,6
520,6	48,79	702,6	342,8
534,6	2,21	716,6	15,8
548,6	1,98	730,6	14,5
576,7	2,51	758,7	19,1
590,7	1,57	772,7	12,1
604,7	0,91	786,7	7,1
606,6	0,89	788,6	7,0
	100,00		<b>697,2</b>

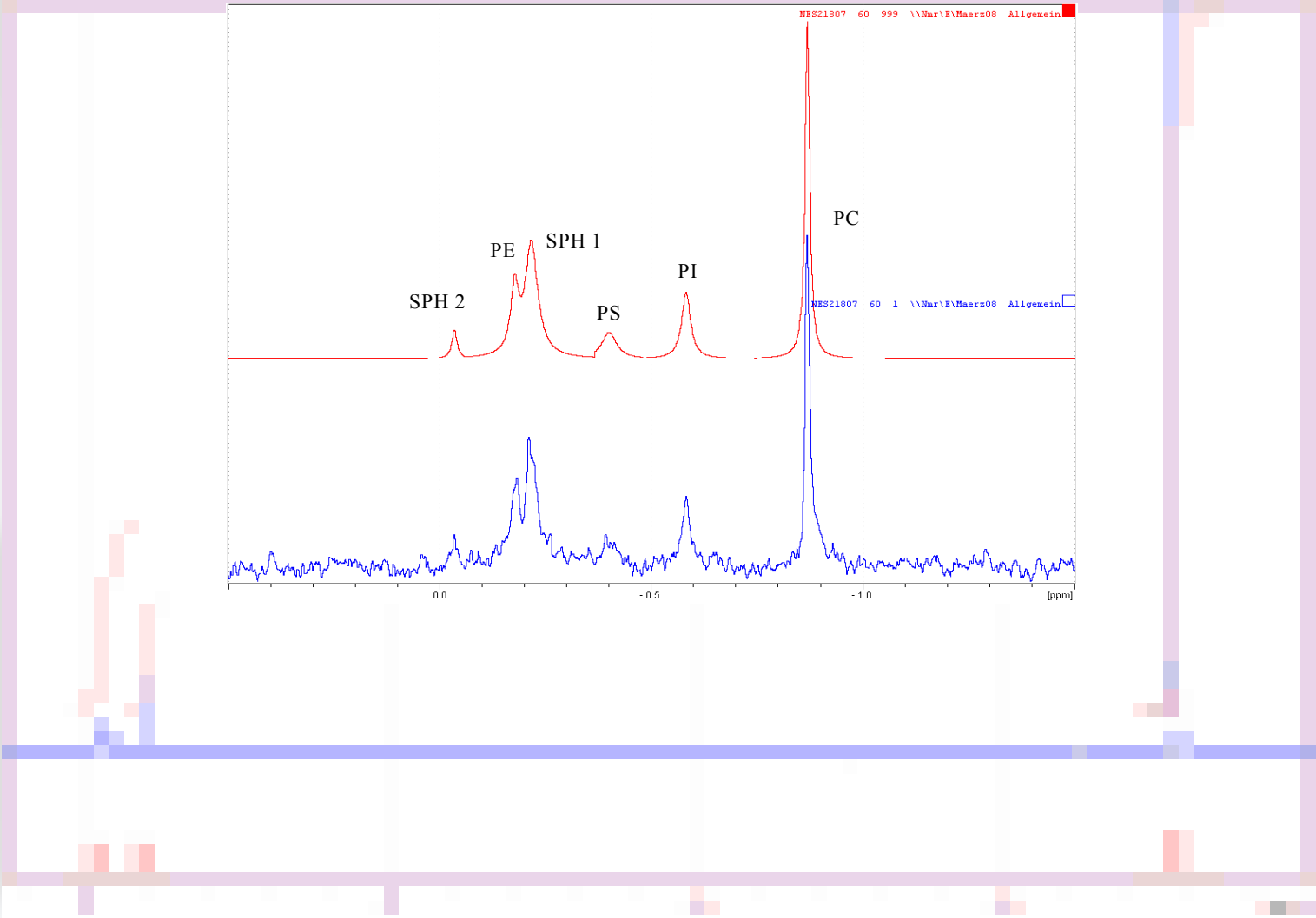
## Average Molecular Weight of Milk-SPH

<b>SPH-2</b>			
<b>m/z</b>	<b>%</b>	<b>MW</b>	<b>Part. MW</b>
578,7	11,74	760,7	89,28
592,8	14,19	774,8	109,92
604,7	8,91	786,7	70,06
606,7	15,59	788,7	122,97
618,8	14,97	800,8	119,89
632,7	26,88	814,7	219,01
646,8	5,86	828,8	48,58
660,7	1,87	842,7	15,72
	100,00		<b>795,42</b>

## Average Molecular Weight of Milk-SPH

<b>SPH-1 + SPH-2</b>			
m/z	%	MW	Part. MW
464,5	0,54	646,5	3,47
478,6	0,75	660,6	4,95
492,6	14,43	674,6	97,33
506,6	6,80	688,6	46,82
520,6	26,69	702,6	187,56
534,6	1,21	716,6	8,66
548,6	1,08	730,6	7,91
576,7	1,37	758,7	10,43
590,7	0,86	772,7	6,63
604,7	0,50	786,7	3,90
606,6	0,49	788,6	3,83
578,7	5,32	760,7	40,43
592,8	6,42	774,8	49,78
604,7	4,03	786,7	31,73
606,7	7,06	788,7	55,69
618,8	6,78	800,8	54,30
632,7	12,17	814,7	99,19
646,8	2,65	828,8	22,00
660,7	0,84	842,7	7,12
	100,00		<b>741,71</b>

# NES21807 <sup>31</sup>P-NMR quant, Deconvolution



## Data Sheet Phospholipid content in NES20726

**Sample Ident.:** Milk Lecithin reference material, Ch.-B.:  
P0080720858/20

Lab. No:

**Spectral Service Code: NES20726-A**

Results from: 14.09.2007

Phospholipid	Weight-%	Mol-%	MW [g/mol]
PC	10,25	31,30	770,0
1-LPC	---*)	---*)	515,0
2-LPC	---*)	---*)	515,0
PI	1,36	3,84	835,0
LPI	---*)	---*)	570,0
PS-Na	1,67	4,92	797,2
LPS	---*)	---*)	517,0
SPH	9,00	27,49	770,0
PE	10,01	32,46	725,0
LPE	---*)	---*)	470,0
APE	---*)	---*)	990,0
PG	---*)	---*)	758,0
DPG	---*)	---*)	682,5
PA	---*)	---*)	685,0
LPA	---*)	---*)	430,0
Other	---*)	---*)	770,0
Sum	32,29	100,00	
Phosphorus	1,32		