

# **$^{31}\text{P}$ -NMR Round Robin Test on Phospholipids**

Dr. Bernd W.K. Diehl

Spectral Service

Cologne, Germany

Demonstrating that the results of  $^{31}\text{P}$ -NMR analysis of phospholipid mixtures are independent

- of the NMR laboratory,
  - the NMR instrument supplier,
  - the magnetic field strength
  - and the test item.
- Sample preparation was not part of the Round Robin test.
- Validation of sample preparation had been investigated separately by Spectral Service and published elsewhere;

- The sample preparation was done using the Spectral Service standard operation procedure (SOP-43, according to the method of FOLCH).
- Approx. 100 mg of the phospholipid test item were dissolved in a mixture of 1 ml chloroform-d, 2 ml methanol and 2 ml aqueous Cs-EDTA solution (0.2 mol at pH 7.5).
- The organic layer was separated for  $^{31}\text{P}$ -NMR analysis

# <sup>31</sup>P-NMR Parameter

- Pulse Program: Inverse Gated Decoupling
- Sum of AQ (Acquisition Time) and D1 (Relaxation Delay) had to be constant
- PW 30 , NS = 256, SW 50 ppm (-25 to + 25), O1 = 0 ppm
- AQ at equal TD (Time Domain = digital resolution) depend on field strength

<b>MHz</b>	<b>AQ</b>	<b>D1</b>	<b>Sum</b>	<b>S/N</b>
<b>200</b>	<b>7.37</b>	<b>8.10</b>	<b>15.47</b>	<b>1.00</b>
<b>300</b>	<b>5.47</b>	<b>10.00</b>	<b>15.47</b>	<b>2.12</b>
<b>400</b>	<b>4.04</b>	<b>11.43</b>	<b>15.47</b>	<b>2.83</b>
<b>500</b>	<b>3.22</b>	<b>12.25</b>	<b>15.47</b>	<b>3.54</b>
<b>600</b>	<b>2.69</b>	<b>12.78</b>	<b>15.47</b>	<b>4.24</b>
<b>700</b>	<b>2.32</b>	<b>13.15</b>	<b>15.47</b>	<b>4.95</b>

- 5 different test items were prepared by Spectral Service and sent out to 21 laboratories for analyzing.

Universities	7
Research Institutes	3
Industry	11

- 8 participants were able to run the tests using different equipments/devices (spectrometer or probe)
- NMR spectrometer of 3 different producers were used.

Bruker	25
Jeol	1
Varian	3

# Some more data

➤ 10 different probes were used

➤ 6 different field strengths

200 MHz	1
300 MHz	6
400 MHz	12
500 MHz	6
600 MHz	3
700 MHz	1

➤ 145 different spectra had to be evaluated

# Participants I

Participant	Instrument	Probe	MHz
Universität Hamburg	Bruker AV 400	BBI	400
Universität Hamburg	Varian Gemini 2000		200
Universität Köln	Bruker DPX 300	BBI	300
Universität Köln	Bruker DPX 500	BBI	500
Universität Leipzig	Bruker DRX 600	BBO	600
Universität Marburg	Bruker AV 600	PABBI	600
Universität Marburg	Bruker AV 500	TBI	500
Universität Potsdam	Bruker	BBI	500
Universität Würzburg	Bruker AV 400	BBO	400
Institut für Pflanzenbiochemie	Varian Mercury Plus 400		400
Bundesanstalt für Material- Forschungsinstitut für FMP	Bruker DMX 400	BBI	400
Forschungsinstitut für FMP	Bruker AV 300	QNP	300
Forschungsinstitut für FMP	Bruker DRX 600	BBI	600
Hochschule Merseburg (FH)	Varian Gemini 2000		300

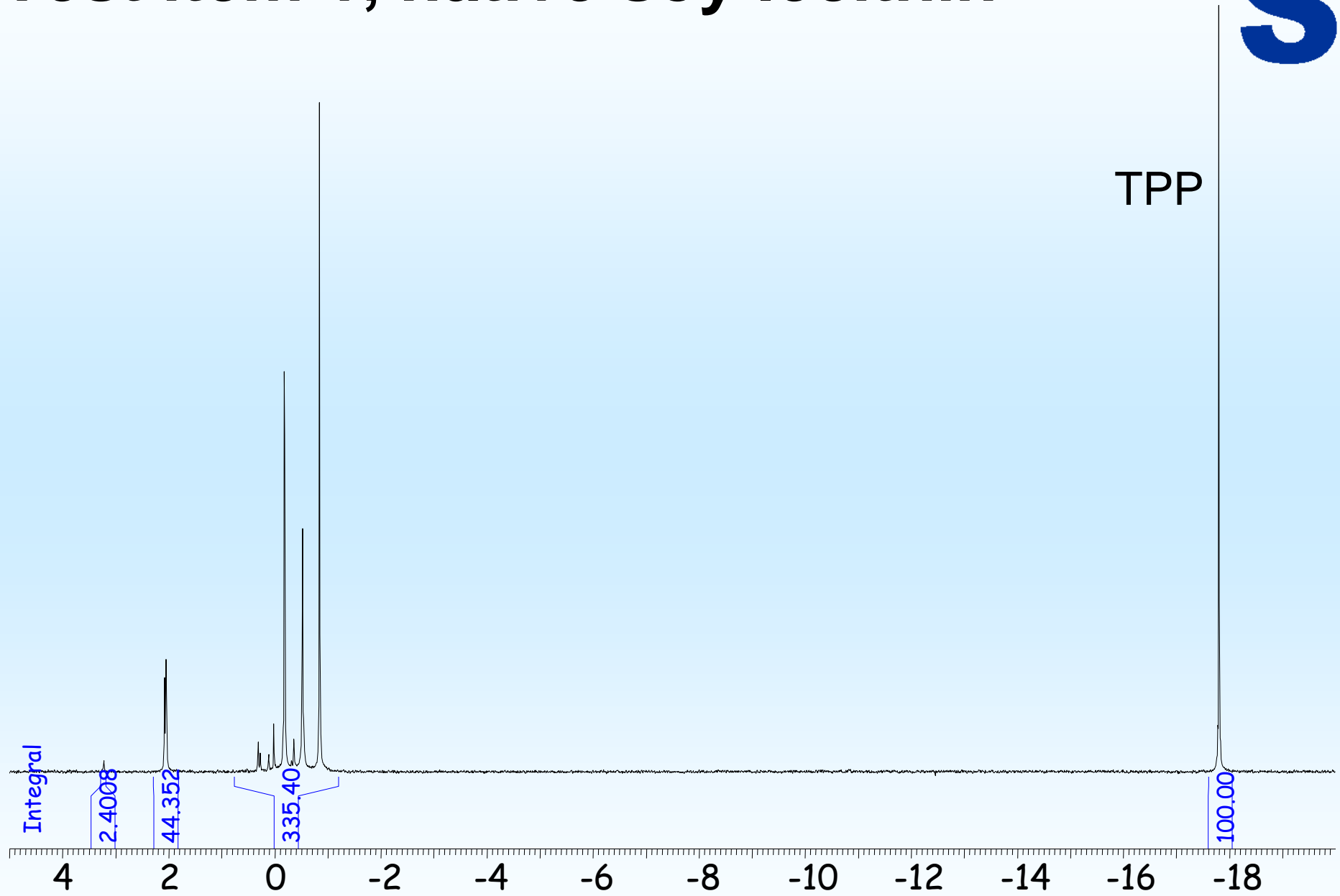
# Participants II

Participant	Instrument	Probe	MHz
ALTANA Pharma AG	Bruker AV 400	PABBI	400
ALTANA Pharma AG	Bruker DRX 400	PABBI	400
Aqura H GmbH	Bruker DRX 500	BBO	500
Aqura M GmbH	Bruker AV 500	BBO	500
BASF-AG	Bruker DPX 400	BBO	400
Bayer CropScience AG	Bruker DPX 400	QNP	400
Bayer Industry Service Krefeld	Bruker DPX 400	QNP	400
Bayer Industry Service Leverkusen	Bruker DPX 400	QNP	400
Bruker BioSpin GmbH	Bruker AV 500	PABBO	500
InfraServ GmbH & Co.	Jeol JNM-EX 400	XX	400
Merck KgaA	Bruker DPX 300	QNP	300
Roche Diagnostics GmbH	Bruker AV 700	BBO	700
Schering AG	Bruker AV 400	PABBO	400
Spectral Service	Bruker AV 300	BBI	300
Spectral Service	Bruker AV 300	QNP	300

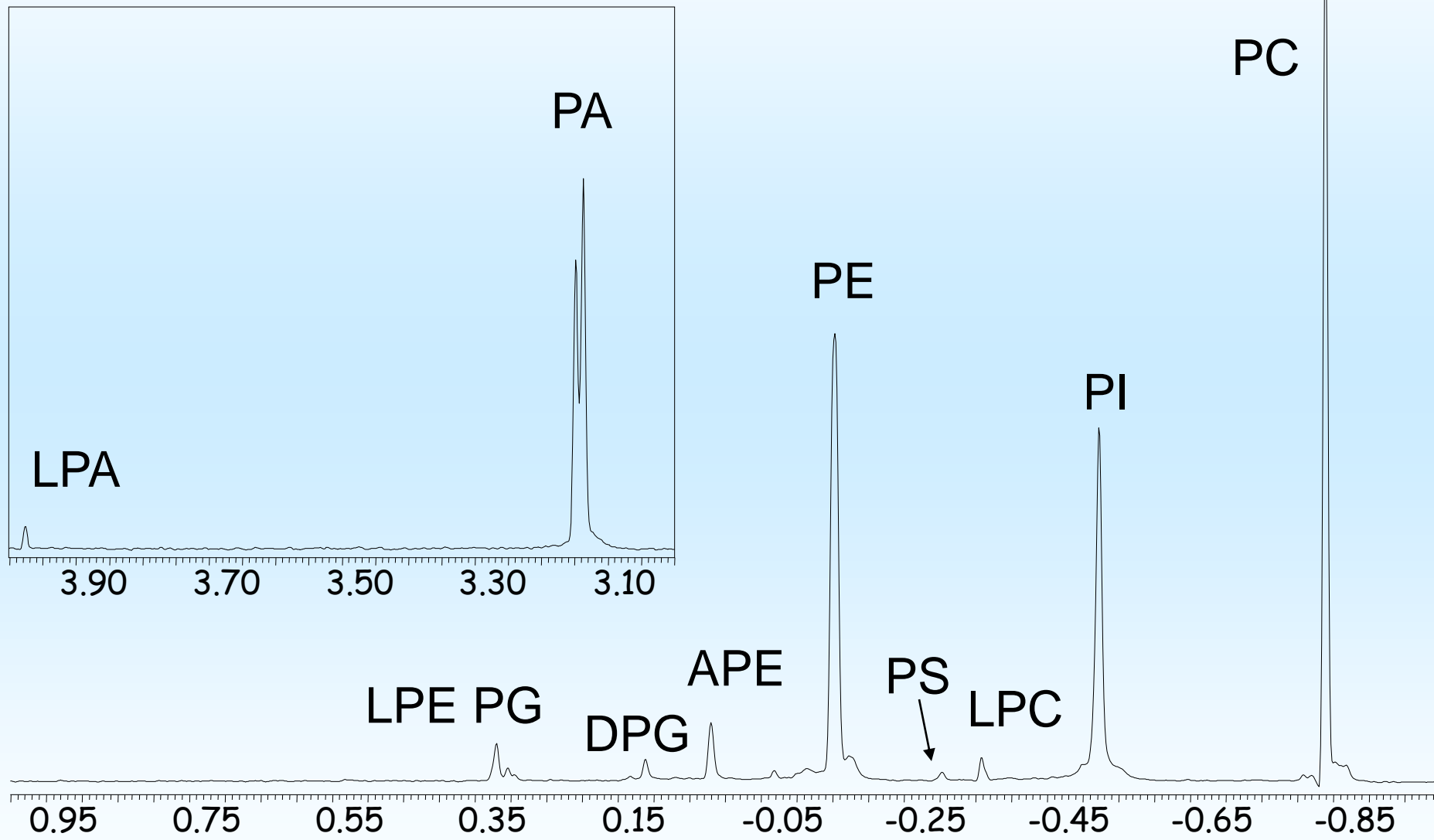


# Test item 1, native soy lecithin

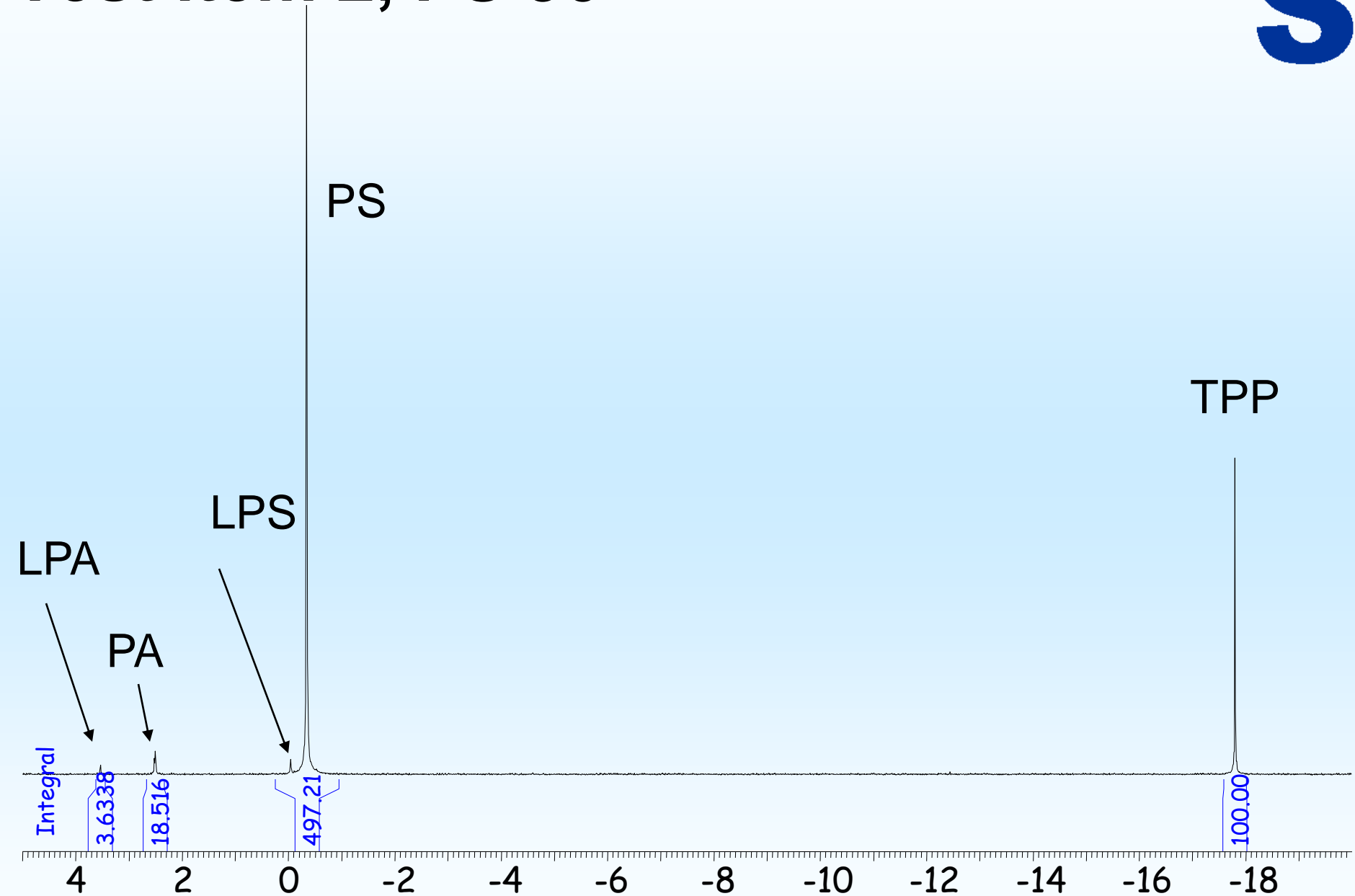
Spectral Service



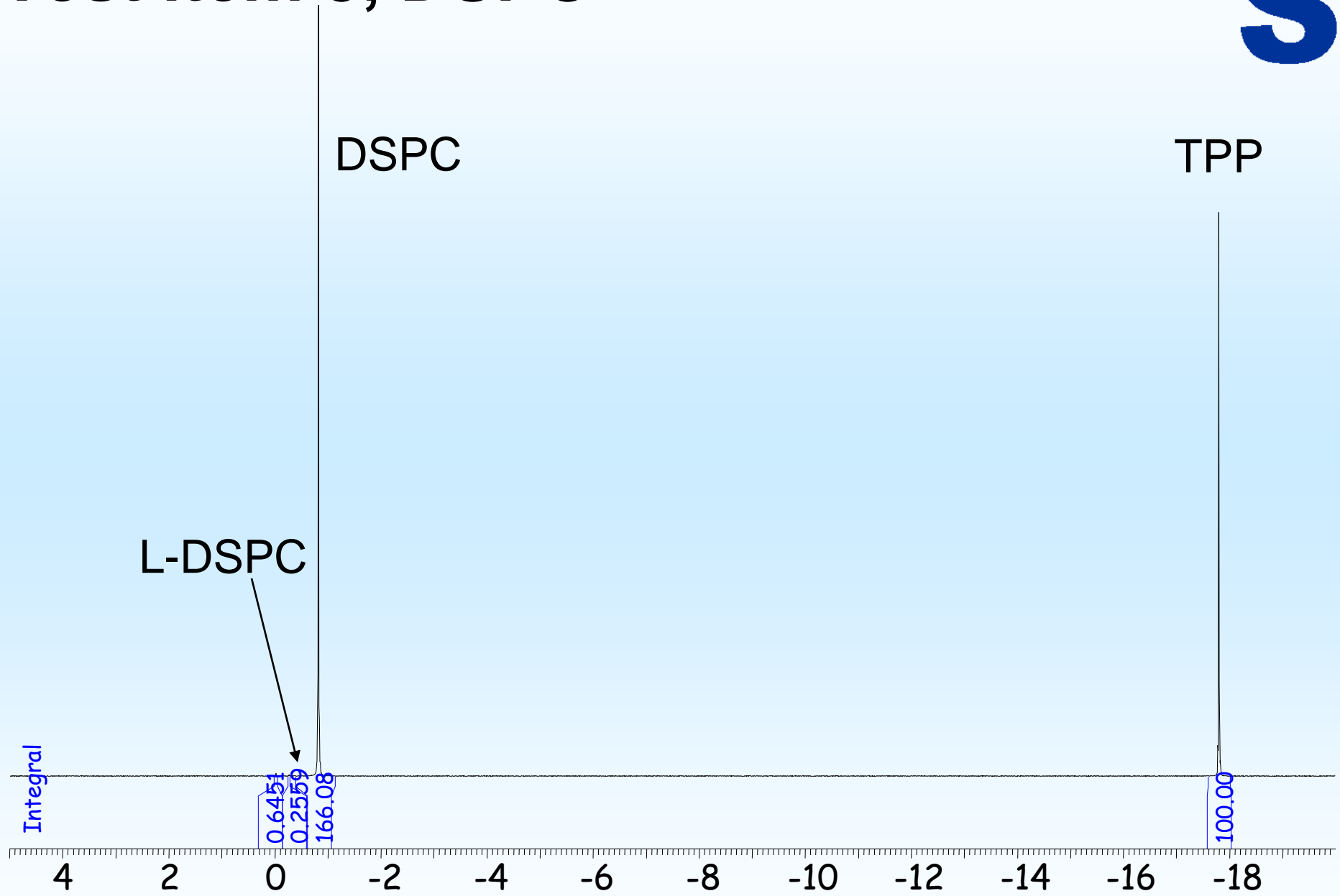
# Test item 1, detail



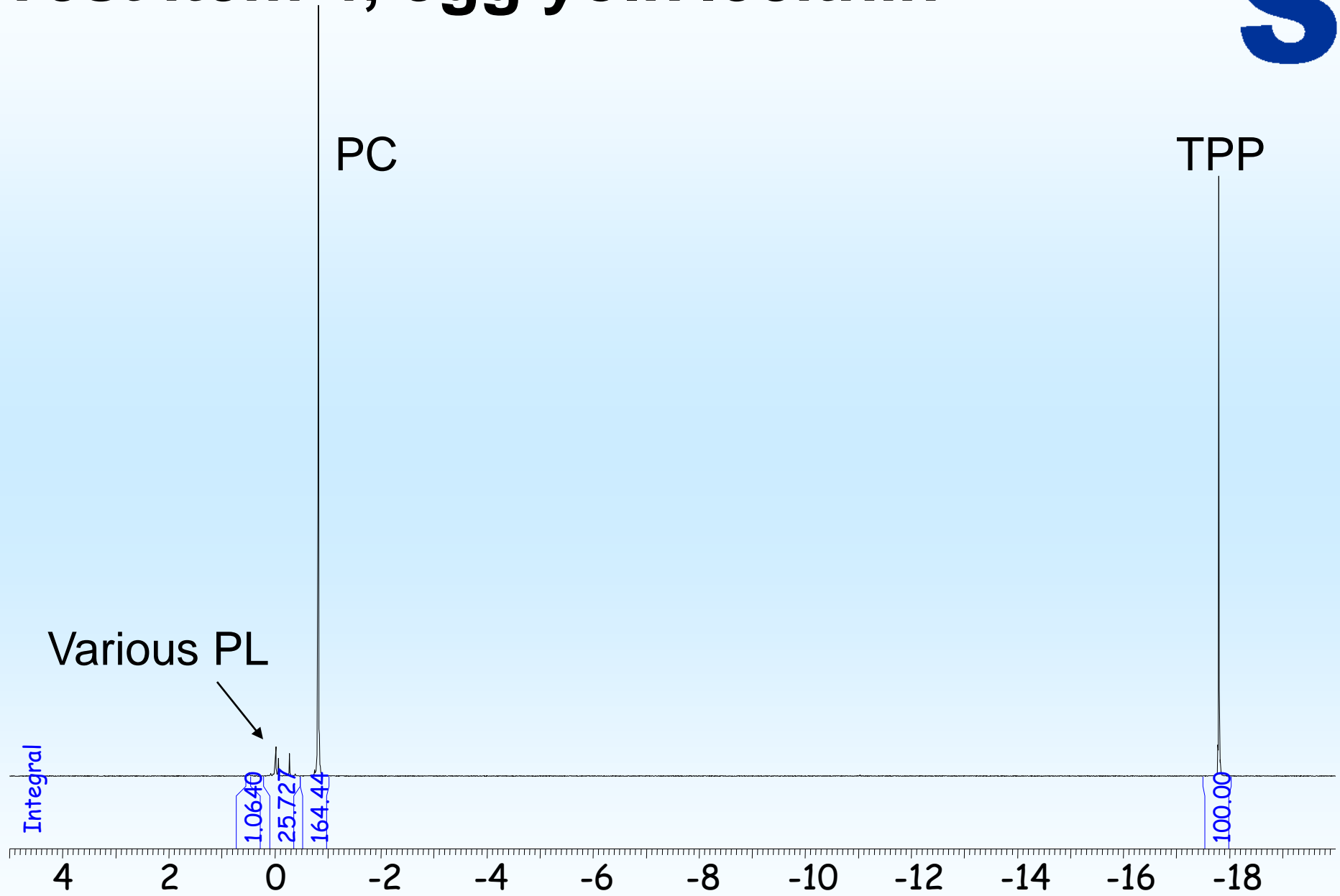
# Test item 2, PS 90



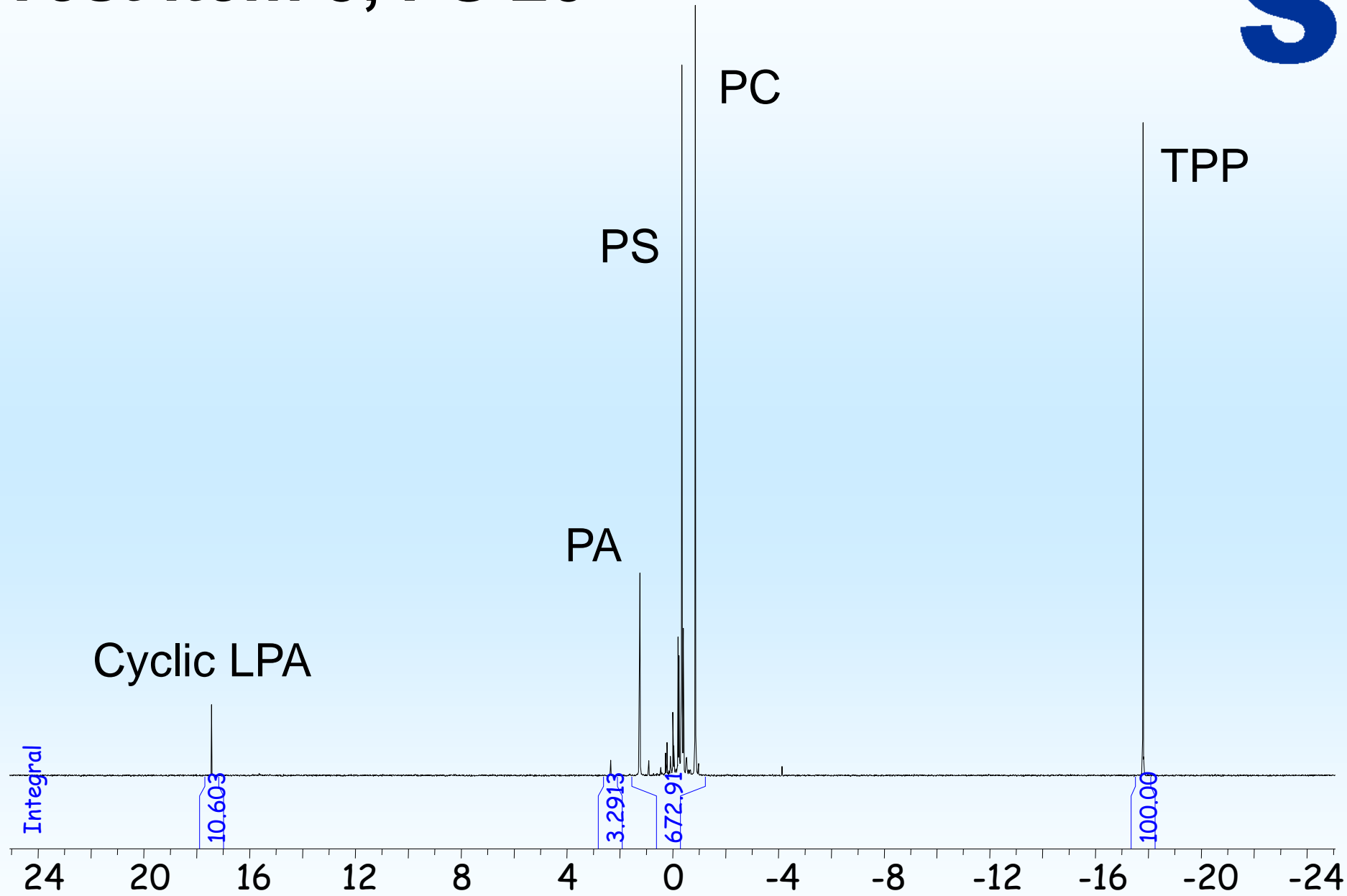
# Test item 3, DSPC



# Test item 4, egg yolk lecithin



# Test item 5, PS 20



# Results I

	Native	PS90	DSPC	Egg (PC)	PS20
Mean	335,47	499,63	166,04	165,29	671,13
STDDEV	6,51	12,93	4,06	3,66	16,70
STDDEV %	1,94	2,59	2,44	2,21	2,49
No.	29	29	29	26	29
1	335,56	499,95	166,16	164,09	667,68
2	325,11	488,22	163,94	163,81	668,62
3	337,20	501,40	165,96	165,35	679,86
4	337,96	501,96	166,96		677,72
5	335,29	498,07	166,03	164,91	675,27
6	336,51	498,65	166,47	165,12	677,24
7	334,31	500,49	164,45		662,75
8	336,43	500,35	165,97	164,95	678,85
9	333,62	494,87	165,02	164,69	667,73
10	334,00	497,41	165,28	164,23	669,88
11	317,75	454,34	153,76	157,26	650,78
12	335,69	507,70	164,56	165,70	678,24
13	334,29	498,29	165,98	164,15	665,12
14	361,45	541,01	180,59	178,32	722,77
15	338,38	500,11	165,70	165,32	672,27
16	334,60	496,58	166,08	164,44	672,91
17	335,40	497,21	166,40	164,32	664,92
18	335,28	495,69	163,84	163,16	674,01
19	335,41	500,43	166,54	163,11	672,98
20	328,43	524,16	174,84	174,89	676,62
21	334,84	497,05	164,80	164,13	664,41
22	336,03	495,53	165,99	165,20	671,96
23	337,37	500,43	165,83	165,71	674,44
24	336,63	497,32	165,46	165,25	607,01
25	336,18	497,77	165,51	164,48	671,73
26	335,74	500,06	165,26	165,02	674,48
27	335,46	503,18	165,39	164,98	671,68
28	337,16	500,78	166,25	164,31	674,75
29	336,57	500,30	166,27	166,02	676,15

# After Grubbs Test

	Native	PS90	DSPC	Egg (PC)	PS20
Mean	335,68	498,94	165,59	164,63	672,24
STDDEV	1,43	2,20	0,80	0,71	4,82
STDDEV %	0,43	0,44	0,48	0,43	0,72
No.	26	25	26	24	26
1	335,56	499,95	166,16	164,09	667,68
2			163,94	163,81	668,62
3	337,20	501,40	165,96	165,35	679,86
4	337,96	501,96	166,96		677,72
5	335,29	498,07	166,03	164,91	675,27
6	336,51	498,65	166,47	165,12	677,24
7	334,31	500,49	164,45		662,75
8	336,43	500,35	165,97	164,95	678,85
9	333,62	494,87	165,02	164,69	667,73
10	334,00	497,41	165,28	164,23	669,88
11					
12	335,69	501,00	164,56	165,70	678,24
13	334,29	498,29	165,98	164,15	665,12
14					
15	338,38	500,11	165,70	165,32	672,27
16	334,60	496,58	166,08	164,44	672,91
17	335,40	497,21	166,40	164,32	664,92
18	335,28	495,69	163,84	163,16	674,01
19	335,41	500,43	166,54	163,11	672,98
20	331,82				676,62
21	334,84	497,05	164,80	164,13	664,41
22	336,03	495,53	165,99	165,20	671,96
23	337,37	500,43	165,83	165,71	674,44
24	336,63	497,32	165,46	165,25	
25	336,18	497,77	165,51	164,48	671,73
26	335,74	500,06	165,26	165,02	674,48
27	335,46	503,18	165,39	164,98	671,68
28	337,16	500,78	166,25	164,31	674,75
29	336,57	500,30	166,27	166,02	676,15



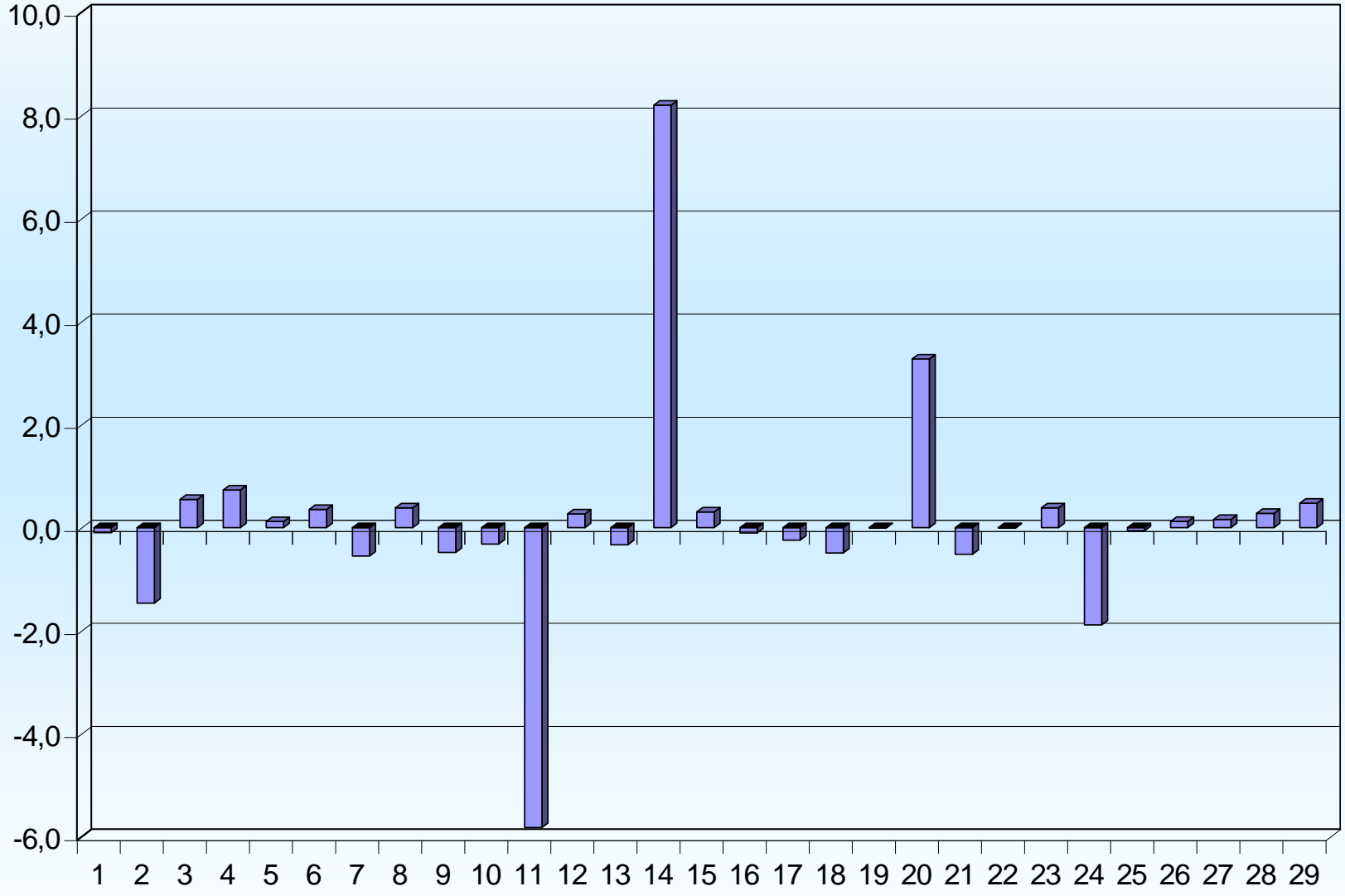
# Diff to Mean

No.	Native	PS90	DSPC	Egg (PC)	PS20
1	-0,04	0,20	0,34	-0,33	-0,68
2	-3,15	-2,15	-1,00	-0,50	-0,54
3	0,45	0,49	0,22	0,44	1,13
4	0,68	0,61	0,83		0,81
5	-0,12	-0,17	0,26	0,17	0,45
6	0,25	-0,06	0,53	0,30	0,74
7	-0,41	0,31	-0,69		-1,41
8	0,22	0,28	0,23	0,20	0,98
9	-0,61	-0,82	-0,35	0,04	-0,67
10	-0,50	-0,31	-0,19	-0,24	-0,35
11	-5,34	-8,94	-7,15	-4,48	-3,19
12	0,00	0,41	-0,62	0,65	0,89
13	-0,41	-0,13	0,23	-0,29	-1,06
14	7,68	8,43	9,06	8,32	7,52
15	0,80	0,23	0,06	0,42	0,00
16	-0,32	-0,47	0,29	-0,11	0,10
17	-0,08	-0,35	0,49	-0,19	-1,09
18	-0,12	-0,65	-1,06	-0,89	0,26
19	-0,08	0,30	0,57	-0,92	0,11
20	-1,15	5,05	5,58	6,23	0,65
21	-0,25	-0,38	-0,48	-0,30	-1,17
22	0,10	-0,68	0,24	0,35	-0,04
23	0,50	0,30	0,14	0,66	0,33
24	0,28	-0,32	-0,08	0,38	-9,70
25	0,15	-0,23	-0,05	-0,09	-0,08
26	0,02	0,22	-0,20	0,24	0,33
27	-0,07	0,85	-0,12	0,21	-0,08
28	0,44	0,37	0,40	-0,19	0,37
29	0,26	0,27	0,41	0,85	0,58

# List of failures

No.	Native	PS90	DSPC	Egg (PC)	PS20	Failure
1	-0,04	0,20	0,34	-0,33	-0,68	
2	????	????	-1,00	-0,50	-0,54	unknown
3	0,45	0,49	0,22	0,44	1,13	
4	0,68	0,61	0,83	no lock	0,81	
5	-0,12	-0,17	0,26	0,17	0,45	
6	0,25	-0,06	0,53	0,30	0,74	
7	-0,41	0,31	-0,69	wrong D1/SW	wrong D1/SW	
8	0,22	0,28	0,23	0,20	0,98	
9	-0,61	-0,82	-0,35	0,04	-0,67	
10	-0,50	-0,31	-0,19	-0,24	-0,35	
11	????	????	????	????	????	unknown
12	0,00	0,41	-0,62	0,65	0,89	
13	-0,41	-0,13	0,23	-0,29	-1,06	
14	????	????	????	????	????	unknown
15	0,80	0,23	0,06	0,42	0,00	
16	-0,32	-0,47	0,29	-0,11	0,10	
17	-0,08	-0,35	0,49	-0,19	-1,09	
18	-0,12	-0,65	-1,06	-0,89	0,26	
19	-0,08	0,30	0,57	-0,92	0,11	
20	-1,15	wrong AQ/TD	wrong AQ/TD	wrong AQ/TD	wrong AQ/TD	
21	-0,25	-0,38	-0,48	-0,30	-1,17	
22	0,10	-0,68	0,24	0,35	-0,04	
23	0,50	0,30	0,14	0,66	0,33	
24	0,28	-0,32	-0,08	0,38	????	Low S/N
25	0,15	-0,23	-0,05	-0,09	-0,08	
26	0,02	0,22	-0,20	0,24	0,33	
27	-0,07	0,85	-0,12	0,21	-0,08	
28	0,44	0,37	0,40	-0,19	0,37	
29	0,26	0,27	0,41	0,85	0,58	

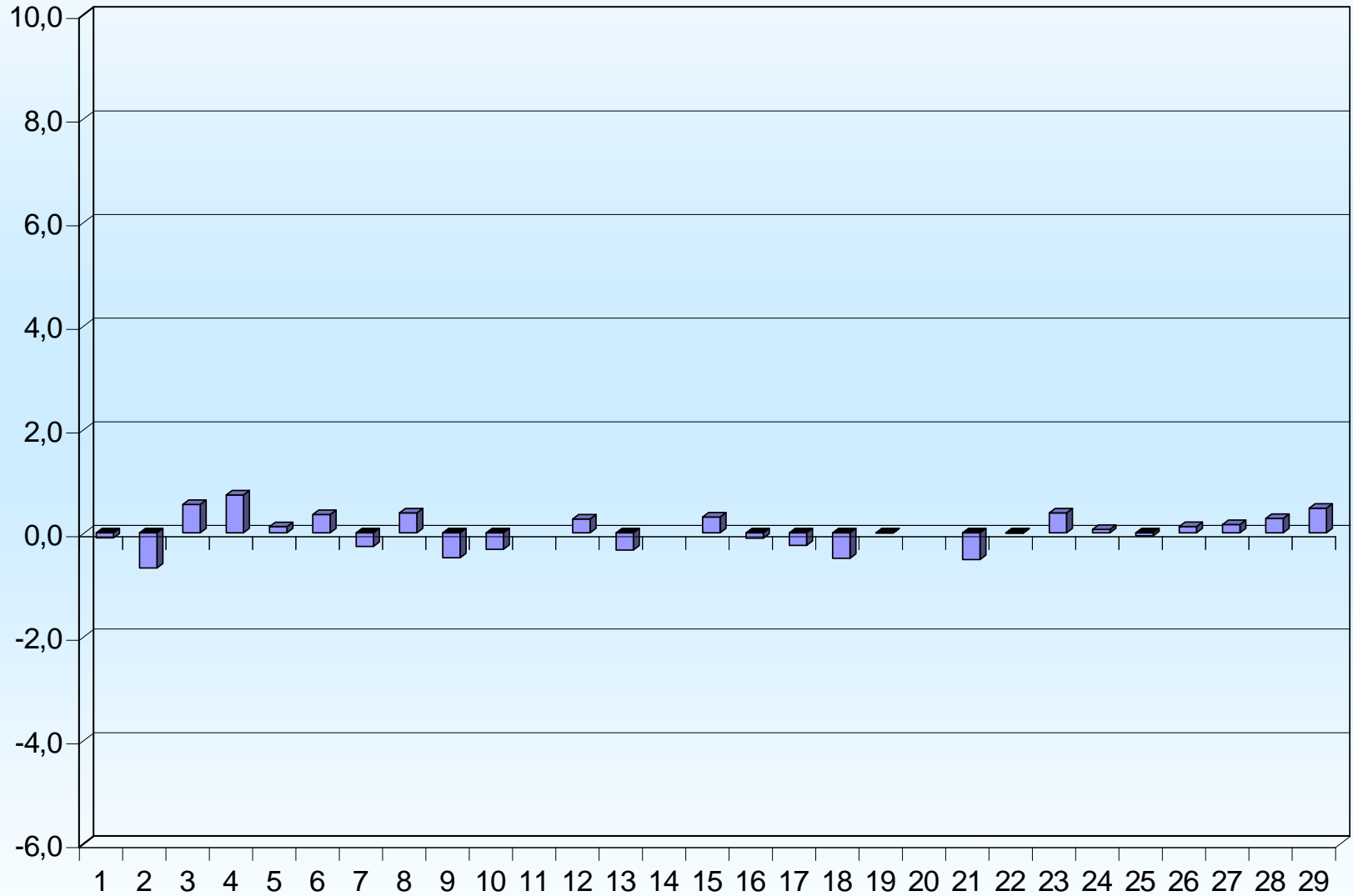
# Mean of differences



# Cumulated differences



# MoD after Grubbs test



- Out of 29 NMR units attended the Round Robin test. 25 fulfilled the requirements on P-NMR analysis of five different phospholipid samples within the variation limit of 1,0 %, 26 fulfilled the test within 2 %.
- Reasons for large deviations layed in wrong parameter settings according to the restrictions, and insufficient tuning and matching of the instruments. Correct decoupler settings may be a problem, too.

- Within the scope of statistical deviations the results of the present Round Robin test are independent
  - of the NMR laboratory,
  - the producer of the NMR instrument (with the restriction of only four non-Bruker devices)
  - the field strength (with restriction of differing S/N ratios)
  - and the test item.