IATROSCAN INSTRUMENT APPLICATION

No. 18

Determination of the phosphatidylcholine content in egg yolk lecithin by the IATROSCAN

— Automatic quantitation by the IATROCORDER TC-11—

Determination of the phosphatidylcholine content in egg yolk lecithin by the Iatroscan TH-10 MkIV

- Automatic quantitation by the Iatrocorder TC-11 -

The phosphatidylcholine (PC) content in egg yolk lecithin was determined by the mode of the two-points calibration curve/mocified internal standard method equipped to the Iatrocorder TC-11.

1. Specimens
   (1) yolk lecithin: refined egg yolk lecithin sold on the market
   (2) phosphatidylcholine preparation: Sigma L-α-phosphatidylcholine (egg yolk)

2. Method of analysis
   (1) Preparation of standard solutions

   Following two different solutions were prepared using cholesterol acetate (Cho. A) as an internal standard.

	Cho.A (mg/mL)	PC (mg/mL)
High-concentration standard solution	3.46	5.03
Low-concentration standard solution	3.46	2.012

Method of preparation: The high-concentration standard solution was prepared by dissolving PC in a concentration of 5.03 mg/ml with Cho.A solution (3.46 mg/ml). This standard solution was further diluted with a proper amount of Cho.A solution to give the low-concentration standard solution.

   (2) Preparation of the sample

   A solution of egg yolk lecithin of the following concentration was prepared.

Cho.A (mg/mL)	Egg yolk lecithin (mg/mL)
3.46	5.15

Method of preparation: A sample solution was prepared by dissolving egg yolk lecithin in a concentration of 5.15 mg/ml with Cho.A solution (3.46 mg/ml).

(3) Sample spotting

Ten Chromarod-SIIIs (a rod holder) were used. Four aliquots of the high-concentration standard solution, then three aliquots of the low-concentration standard solution and finally, three aliquots of the sample solution, 1 µl of each, were spotted onto each Chromarod.

(4) development

The sample spot was developed to the distance of 5 cm with a mixture of chloroform, methanol and water (40 : 20 : 2) followed by removal of the solvent by a cold air stream of a dryer and then re-developed to the distance of 10 cm with a mixture of hexane, ether and formic acid (54 : 6 : 0.08). After the development was completed, the solvent was again removed by heating in an oven at 100°C for 1∿2 minutes.

(5) Setting parameters of the Iatrocorder

For quantitation using the two-points calibration curve, it is necessary to input the retention time of the aimed peak to the Iatrocorder previously.

Therefore, the first Chromarod is scanned to obtain the retention times of Cho.A and PC. (After scanning of the first rod is completed, push "pause" key to stop the Iatroscan.) After the retention time of the peak is obtained, input the parameters as given below.

ATTENUATION		32	
CHART	FEED	0.10	
END	TIME	1.000	
MINIMUM	AREA	300	
MINIMUM	HEIGHT	10	
MINIMUM	WIDTH	0.005	
TWICE	TIME	0.000	
CALCULATION	METHOD	13	The two-points calibration curve/ modified internal standard method is specified.

EXTRA DATA

NO.	FUNCTION		
1	PARAMETER A	.515000-01	The amount of the sample in order to express the content in percent
2	PARAMETER B	.346000+01	The amount of the internal standard
3	LSTD PEAK TIME	0.191	The retention time of the peak of the internal standard (Cho.A)
4	ISTD PEAK CO1	.346000+01	The amount of the internal standard
5	ISTD PEAK CO2	.346000+01	The amount of the internal standard

IDENTIFICATION PEAK

NO.	NAME	RT	CO1	CO2
1	I.S.	0.191 The retention time of the peak of the internal standard	3.46000 The amount of the internal standard	3.46000 The amount of the internal standard
2	PC	0.429 The retention time of the peak of PC	5.0300 The amount of the high-concentration standard solution	1.01200 The amount of the low-concentration standard solution

(6) Plotting of the calibration curve

Three rods of the high-concentration standard solution and then three rods of the low-concentration standard solution are scanned, from which the mean value at each concentration is obtained to make the calibration curve.

List the parameters to confirm, then push "STD" key of the Iatrocorder and finally release "pause" of the Iatroscan.

The first sample of the high-concentration standard solution is scanned and the result is printed out.

```
                SAMPLE   1                15:13  JULY 03 1987
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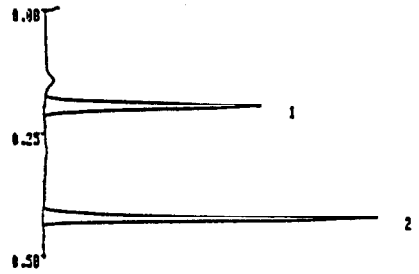



```
CAL. METHOD   13
                SF              PA              PB
       .100000E+01  .515000E-01  .346000E+01

CALIBRATION[1]    1

ISTD PEAK       0.196       11639

NO.     NAME        RT      A OR H  MK      CRT]    CA OR H]

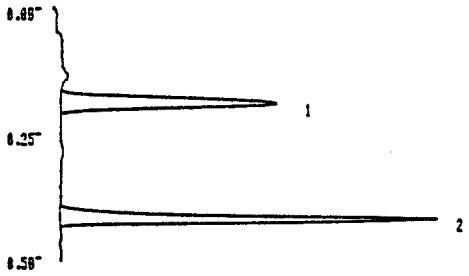
  1      I.S.     0.196       11639          0.192      1.0000
  2        PC     0.430       13705          0.425      1.1774

       TOTAL                  25344                     2.1774
CALIBRATION METHOD  1:END  2:CONTINUE  3:CANCEL-END
4:CANCEL-CONTINUE    1 - 4 KEY PUSH  2
```

Push "2" key to obtain the mean value of 3 samples.

Scan the second sample similarly and then push "2" key.

```
                SAMPLE   3                15:17  JULY 03 1987
```




```
CAL. METHOD   13
                SF              PA              PB
       .100000E+01  .515000E-01  .346000E+01

CALIBRATION[1]    3

ISTD PEAK       0.193       12700

NO.     NAME        RT      A OR H  MK      CRT]    CA OR H]

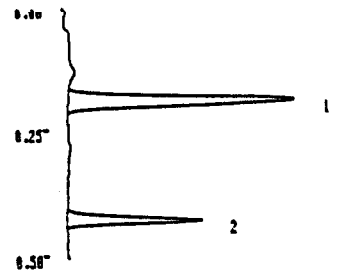
  1      I.S.     0.193       12700          0.192      1.0000
  2        PC     0.429       15552          0.426      1.1721

       TOTAL                  28252                     2.1721
CALIBRATION METHOD  1:END  2:CONTINUE  3:CANCEL-END
4:CANCEL-CONTINUE    1 - 4 KEY PUSH  1
```

After the third sample is scanned, push "1" key.

Next, samples of the low-concentration standard solution are subjected to scanning.

```
SAMPLE   6                15:21  JULY 03 1987
```




```
CAL. METHOD   13
                SF              PA              PB
       .100000E+01  .515000E-01  .346000E+01

CALIBRATION[2]    3

ISTD PEAK       0.191       12753

NO.     NAME        RT      A OR H  MK      [RT]    CA OR HI.

  1     I.S.     0.191       12753          0.191      1.0000
  2       PC     0.432        5250          0.429      0.4169

       TOTAL                 18004                     1.4169
CALIBRATION METHOD   1:END   2:CONTINUE   3:CANCEL-END
4:CANCEL-CONTINUE     1 - 4 KEY PUSH   1
```

After scanning of the third sample is completed, push "1" key in the similar manner to the high-concentration standard solution.

IDENTIFICATION PEAK

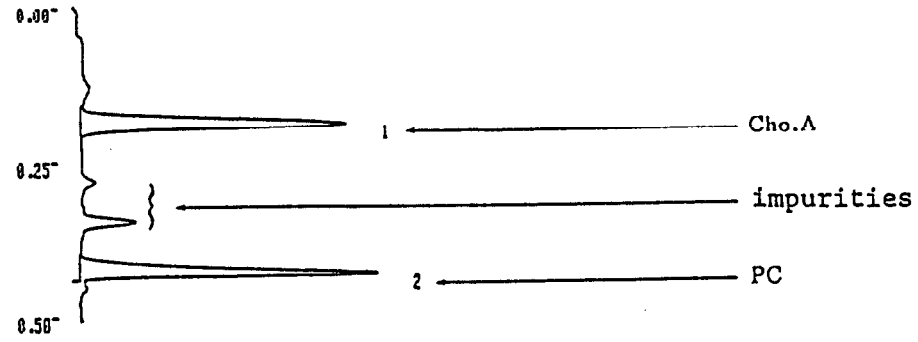
No.	NAME	RT	CO1/CF1	CO2/CF2
1	I.S.	0.191	3.46000	3.46000
			$.000000_{10}+00$	$.100000_{10}+01$
2	PC	0.429	5.03000	2.01200
			$.115505_{10}+01$	$.998844_{10}+01$

Data of the calibration curve is printed out.

(7) Sample analysis

After the chromatogram is recorded, the phosphatidylcholine content is automatically calculated on the basis of data on the calibration curve obtained.

SAMPLE 7 15:22 JULY 03 1987



CAL. METHOD 13

SF	PA	PB
.100000E+01	.515000E-01	.346000E+01

ISTD PEAK 0.188 11459

NO.	NAME	RT	A OR H	MK	CONC
1	I.S	0.188	11459		67.1844
2	PC	0.431	9467	M	70.8236
	TOTAL		20927		138.0081

Indicating the phosphatidylcholine content

The value indicated in CONC column is the result of the following calculation.

{(As/Aist) x slope + intercept} x PB/PA = (weight ratio) x PB/PA

Therefore, the phosphatidylcholine content in the sample is directly calculated if the values for the amount of the sample and the amount of the internal standard are inputted to PA and PB, respectively.

Thus, the phosphatidylcholine content in egg yolk is easily determined with the Iatrocorder TC-11.

(Note) In order to employ the two-points calibration curve, it is necessary to confirm previously that the calibration curve is linear within the range of measurement by the multiple-points calibration curve.