Quantitative Determination of Phosphatidylcholine by an HPLC-RI System

Toshihiko Yamagishi*, Hiroshi Akiyama, Shinichi Kimura and **Masashi Toyoda**
Nisshin Flour Milling Co., Ltd., Research Center, 5-3-1 Tsurugaoka, Oi-machi, Iruma, Saitama, 354 Japan

The following describes the quantitative determining method for phosphatidylcholine (PC) using the HPLC-RI system which we have developed. It uses Lichrosorb, Si 60 (10 μm), 4.6 mm × 250 mm as the column and a mobile phase consisting of n-hexane/isopropanol/water = 1:4:1. In this report, we compared data from selected high-purity (60–100 wt%) samples using the HPLC-RI, HPLC-UV and conventional TLC-P methods.

Under the conditions we described, the HPLC-UV method was somewhat affected by fatty acid compositions. As a result, there were some inconsistencies in the measured values. However, the HPLC-RI method we propose was applicable to PC from both egg yolk and soybeans. In addition, the HPLC-RI method produced data which correlated well with data from the TLC-P method, and this data was highly accurate and exhibited satisfactory reproducibility.

Phospholipids are widespread in biology and widely used as emulsifiers in the food industry. Known for being the major component of biomembranes, phospholipids have been studied for their various functions (1–3).

The most widely accepted method for quantitatively determining phospholipids is the conventional, thin-layer chromatography (TLC) method. This method consists of collecting each phospholipid after separation and determining the phosphorus content. A more simplified procedure for quantitatively determining phosphorus by two-dimensional TLC was recently published as the standard method to be newly adopted in Japan by the Japan Oil Chemists' Association in December, 1986 (4).

Although it is considerably better than the conventional method, the new procedure still requires many steps as well as a skilled analyst. In addition, if there are many samples, quantitative determination requires many hours, and accurate values can be difficult to obtain.

The compound "lecithin" is the generic term for phospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), lysophosphatidylcholine (LPC), and the like, indicating PC in the chemical sense of the word.

Many reports on the HPLC method for PC analysis have been released. However, since most of the reports use UV as a detector, measured values are affected by differences in fatty acid composition, particularly if unsaturated fatty acids are present in the composition of PC (5,6). On the other hand, the values obtained by the RI-detector are hardly affected by differences in fatty acid composition.

Although there have been some reports relating to the HPLC-RI system for quantitative determination of PC, these reports revealed certain restrictions and problems. For example, in some reports, the samples required similar fatty acid compositions, the standard product used in calibration. In others, it was difficult to separate PC from other phospholipids (7).

*To whom correspondence should be addressed.

We examined a quantitative determination method using the HPLC-RI system for high-purity PC (60–100 wt%) from both soybeans, which contain a more highly unsaturated fatty acid composition, and egg yolk, which contains many saturated fatty acids. We also compared the measured values for selected high-purity PC using the HPLC-RI, HPLC-UV, and TLC-P methods.

Since the procedure for our HPLC-RI method was extremely simplified, the measured values from this method were very accurate, exhibited satisfactory reproducibility, and were correlated very well with the values from the phosphorus measurement method, which is commonly referred to as the "TLC-P method" (4).

We had already established an industrial refining process for obtaining high-purity PC which is equal to or higher in purity than the reagent (Sigma P6263).

To evaluate the refining process we had to measure the PC purity of the intermediate or final products. But since the conventional quantitative determination method for PC purity could not be done rapidly, accurately or simply, the method was unacceptable for our purposes. Therefore, we studied how to rapidly and accurately measure the purity of PC, and this has led to the development of the HPLC-RI system, which is described below.

EXPERIMENTAL

Materials. Selected high purity PC were: sample 1, reagent, PC from soybeans (Sigma P6263, Sigma Chemical Co., St. Louis, MO); sample 2, working standard (Nisshin St-03, Wako Pure Chemical, Osaka, Japan); samples 3–6 and 9–10, refined PC by Nisshin from crude soy lecithin; sample 7, Epikuron 200 (Lucas Meyer, Hamburg, West Germany); sample 8, soyaphosphatide NC95 (Nattermann Phospholipid, Köln, West Germany); sample 11, reagent, PC from egg yolk (Sigma P5388); sample 12, PC from a mixture of no. 11 and no. 13; sample 13, reagent, PC from egg yolk (Sigma, P8640); sample 14, reagent, PC from egg yolk (Wako, No. 128-02511); and sample A, one of No. 11.

HPLC method. The equipment and conditions used are as follows: A pump: Model 600 (Waters, Milford, MA); column: Lichrosorb, Si-60 (10 μm), 4.6 mm × 250 mm; solvent: n-Hexane/IPA/Water = 1/4/1 (v/v); flow rate: 1 ml/min; detector: UV, Model-481 (UV-210 nm) (Waters); RI, Shodex RI SE-51 (Showadenko, Japan); integrator: Model 7000A (System Instruments, Tokyo, Japan); autosampler: WISP 710B (Waters); and injection: 20 μl.

The solvents for the HPLC grade (Kanto Chemical, Tokyo, Japan) were mixed and deaerated (with an aspirator or supersonic waves) and then used as the solvent for the mobile phase of the present experiment. Twenty μl of the sample solution was injected through the autosampler. Both UV and RI area values were measured by the integrator (7000A, System Instruments).

Quantitative determination. To use the calibration curve, about 250 mg of our working standard PC (Lot No. St-03), which was refined by Nisshin Flour Milling Co. Ltd., was weighed accurately and dissolved in eluent to

produce a 50 ml solution. Assume the solution to be Standard-100 (St-100). By injecting 20 μl of this solution, the quantity of PC became 250 mg/50 ml × 20 × 10^{-3} ml = 100 μg. A 9 ml sample from St-100 was pipetted and mixed with eluent to produce a 10 ml solution (St-90). An 8 ml sample from St-100 was pipetted and mixed with eluent to produce a 10 ml solution (St-80). A 7 ml sample from St-100 was pipetted and mixed with eluent to produce a 10 ml solution (St-70). A 6 ml sample from St-100 was pipetted and mixed with eluent to produce a 10 ml solution (St-60).

Each of these samples was injected three times, and the three measured values were then averaged.

Replacing the injection quantity of each standard PC with Y and the measured area value for Y with X, a regression equation was obtained. By using the method of least squares, a calibration curve was produced whenever the eluent was renewed.

Sample preparation and quantitative determination. About 50 mg of each sample was accurately weighed, dissolved, and then mixed with eluent to produce a 10 ml test sample. Two test samples were taken from each sample, and each test sample was injected twice.

The observed values of each test sample were derived by averaging the two measured values of each test sample. The purity of each sample was derived by averaging the observed values of the two test samples.

In order to generalize the working standard, which was purified by Nisshin Flour Milling Co., Ltd., we calculated the purity of each sample based on the reagent of Sigma Co., Ltd.

The following equation yielded the purity value used:

$$\text{PC purity (wt\%)} = \frac{\text{aX + b (μg)}}{\text{Sample injection quantity (μg)}} \times 100 \times \text{f} \qquad \text{[Eq. 1]}$$

TLC-P method. The method suggested by the Japan Oil Chemists' Association in 1986 (4) was used for quantitative determination of PC purity.

RESULTS

Example of quantitative determination by the HPLC method. Table 1 shows an example of quantitative determination by the HPLC method.

Calibration curve. Table 1 shows the steps for weighing and dissolving of working standard PC (Nisshin, Lot No. St-03). Table 2 shows the results of measurements of standard solution (area values in the case of RI). Figure 1 and

TABLE 1

Weighing and Dissolving of Standard

Weighing and dissolving	Concentration of standard solution (mg/ml)	PC content of 20 μl injected sample (μg) (= Y-axis)	
Standard (St-03) 250.42 mg → HPLC solvent ↓ 50 ml	5.0084	100.17	St-100
9 ml → HPLC solvent ↓ 10 ml	4.5076	90.151	St-90
8 ml → HPLC solvent ↓ 10 ml	4.0067	80.134	St-80
7 ml → HPLC solvent ↓ 10 ml	3.5059	70.118	St-70
6 ml → HPLC solvent ↓ 10 ml	3.0050	60.101	St-60

TABLE 2

Peak Area Measurements of Standard Solution (in case of RI)

	St-100	St-90	St-80	St-70	St-60	
1st time	2705660	2421788	2103573	1855523	1575337	
2nd time	2651468	2397342	2119700	1870471	1594923	
3rd time	2707076	2418411	2122464	1882832	1579040	
Average	2688070	2412510	2115250	1869610	1583100	= X-axis
C.V. (%)	1.1795	0.5491	0.4823	0.7314	0.6573	

C.V. = coefficient of variation.

Table 3 show the calibration curve and regression equation obtained from the results in Tables 1 and 2.

Quantitative determination of test samples. Tables 4 and 5 show examples of quantitatively determining sample A. Table 4 shows sample weighing and dissolving of the sample A. Table 5 shows sample measurement results of the sample A.

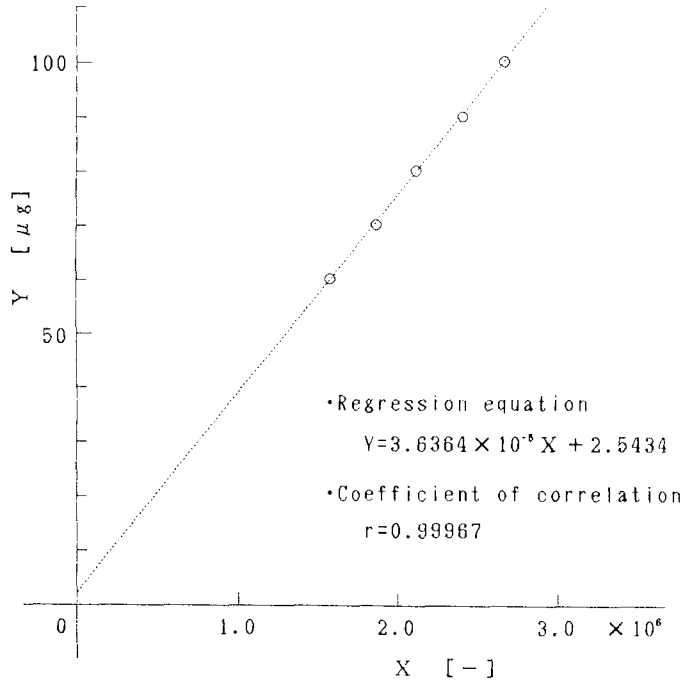

FIG. 1. Calibration curve for Tables 1 and 2.

TABLE 3

Calibration Curve Data and Regression Equation

Calibration curve data after treated by statistical method		Regression equation
X	Y	$Y = 3.6364 \times 10^{-5} X + 2.5434$ [Eq. 2]
2.68807×10^6	100.17	
2.41251×10^6	90.151	
2.11525×10^6	80.134	Coefficient of correlation
1.86961×10^6	70.118	
1.58310×10^6	60.101	r = 0.99967

Calculation of PC purity. By using the values in Tables 4 and 5 for Eq. 1 and for Eq. 2 of Table 3, the PC purity for both A-1 and A-2 was calculated as follows: The value of "f" in Eq. 1 was 1.013.

$$\text{A-1} = \frac{3.6364 \times 10^{-5} \times 2580080 + 2.5434}{100.72} \times 100 \times 1.013 = 96.92\%$$

$$\text{A-2} = \frac{3.6364 \times 10^{-5} \times 2561030 + 2.5434}{100.32} \times 100 \times 1.013 = 96.61\%$$

By averaging the results of A-1 and A-2, the PC purity (relative purity on the assumption that the purity of Sigma P6263 is 99%) of sample A became: (96.92 + 96.61)/2 = 96.77%.

Calculating the conversion factor (f-value) of the working standard PC (Nisshin, Lot no. St-03) vs Sigma PC (Product No. P6263). We compared two PC samples. One was the working standard which was used for the measurement and was purified by Nisshin. The other was the high-purity reagent (Sigma P6263), which was indicated to be refined from soybeans. The Sigma reagent had a designated purity of approximately 99%. The purity of the Sigma reagent, which was regarded as an unknown sample, was obtained by the method described above. In this case, Eq. 1 was used, assuming that f = 1. Three samples from Sigma P6263 were weighed, dissolved, and injected twice, respectively. Each averaged value of two measured values was corrected by weight, and the corrected values were averaged to obtain the purity with each detector as follows. Measured values by RI: Sigma-1 = 97.73%; Sigma-2 = 97.46%; and Sigma-3 = 98.08%.

The purity of the Sigma sample was obtained by averaging the above three values, i.e., 97.76% (RI). Conversely, setting the purity of Sigma P6263 at 99%, as described previously, the purity of our working standard (Nisshin, Lot no. St-03) was calculated as follows: (99/97.76) × 100 = 101.27%. Therefore, f(RI) = 1.013.

Measured value by UV. Sigma-1 = 96.97%; Sigma-2 = 96.17%; and Sigma-3 = 96.86%. The purity of the Sigma reagent was obtained by averaging the above three values, i.e., 96.67% (UV). Conversely, setting the purity

TABLE 4

Weighing and Dissolving of Unknown Sample A

Sample no.	Amount of weighing	Dissolving	Concentration of sample solution (mg/ml)	Amount of injected sample by 20 μl (μg)
A-1	50.36 mg	HPLC solvent ↓ → 10 ml	5.036	100.72
A-2	50.16 mg	HPLC solvent ↓ → 10 ml	5.016	100.32

TABLE 5

Peak Area Measurements of Unknown Sample

	A-1	A-2	
1st time	2569327	2554681	
2nd time	2590832	2567376	
Average	2580080	2561030	= X
C.V. (%)	0.5894	0.3505	

C.V. = coefficient of variation.

of the Sigma P6263 at 99%, the purity of our working standard (Nisshin PC, Lot no. St-03) was calculated as follows: (99/96.67) × 100 = 102.41%. Therefore, f(UV) = 1.024.

Comparison of measured data by the TLC-P method with those by the HPLC-RI method and the HPLC-UV method. The observed data for each sample are shown in the column PC-Rel. of Table 6. A comparison was made by using the relative purity based on sample no. 1 (Sigma P6263). Sample no. 1 shows Sigma P6263 as the reference with purity at 99%, as described earlier. The data marked with an asterisk in Table 6 indicate the observed values when the decomposition time of the sample was doubled in the TLC-P method.

Comparison of the HPLC-RI and HPLC-UV methods vs the TLC-P method. Table 6 shows that the measured values by the UV method for high-purity PC from soybeans (nos. 2–10) are higher than those by the RI method. In addition, sample no. 6, which was prepared by mixing sample no. 5 with a small amount of a low-purity sample (about 55% prutiy) should have indicated a lower purity than that of sample no. 5. However, the value of sample no. 6 obtained by the UV method indicated a higher purity than that of sample no. 5. This was obviously inconsistent. When the RI method was used, no inconsistency resulted.

TLC-P method. The samples were analyzed according to the predetermined TLC-P method (4). When each decomposition time was increased (doubled) the measured values were increased only for low-purity samples from both soybeans and egg yolk.

The analytical process was very long, causing large variations. To obtain values with a low coefficient of variation (C.V.) a large number of data were needed. The report (4) shows a C.V. of about 7%.

Comparison of the HPLC-RI method with the TLC-P method. Figures 2 and 3 show Table 6 graphed according to the TLC-P method. The measured values by the HPLC-RI method are highly correlated with those by the TLC-P method. Also, the HPLC-RI method shows a much smaller coefficient of variation (C.V.) than the TLC-P method (Table 6), thus showing more accuracy. Table 7 shows that the various fatty acid compositions of egg yolk PC are rather different from those of the standard product (Nisshin, St-03), which was refined from

TABLE 6

Comparison of Measured Relative Purity of PC by Three Methods

Sample no.	HPLC-UV PC-Rel. (%)	HPLC-UV C.V. (%)	HPLC-RI PC-Rel. (%)	HPLC-RI C.V. (%)	TLC-P PC-Cal. (%)	TLC-P C.V. (%)	TLC-P PC-Rel. (%)
(Soybean PC)							
1	99.0		99.0		86.12	3.24	99.0
2	102.45	0.44	101.28	0.31	89.46	3.22	102.84
					89.17*	3.00	102.77*
3	106.03	0.39	100.72	0.88	89.37	2.05	102.73
4	105.67	0.95	99.42	1.67			
5	107.21	0.12	100.45	0.89	89.12	2.06	102.45
6	107.97	1.36	94.21	0.63	83.36	2.05	95.82
7	104.37	0.62	94.50	0.17	81.39	2.78	93.56
8	97.83	0.70	90.98	0.53	75.74	0.79	87.07
					78.93*	0.65	90.73*
9	85.70	0.84	78.18	0.60	65.05	1.74	74.77
					69.01*	2.55	79.33*
10	67.94	0.15	63.34	0.63	52.87	1.90	60.78
					54.17*	0.78	62.27*
(Egg yolk PC)							
11	74.03	1.00	95.51	1.74	83.45	1.33	95.92
12	67.15	0.32	82.50	0.54	71.60	1.60	82.31
13	54.41	1.96	62.46	1.39	56.26	1.03	64.68
					56.87*	0.75	65.37*
14	74.35	0.56	67.71	0.27	56.86	1.24	65.36

Asterisks indicate the observed values when the decomposition time of each sample was doubled in the TLC-P method. PC-Rel. = relative purity based on No. 1 (Sigma P6263). PC-Cal. = calculated purity based on phosphorus contents by the TLC-P method. C.V. = coefficient of variation.

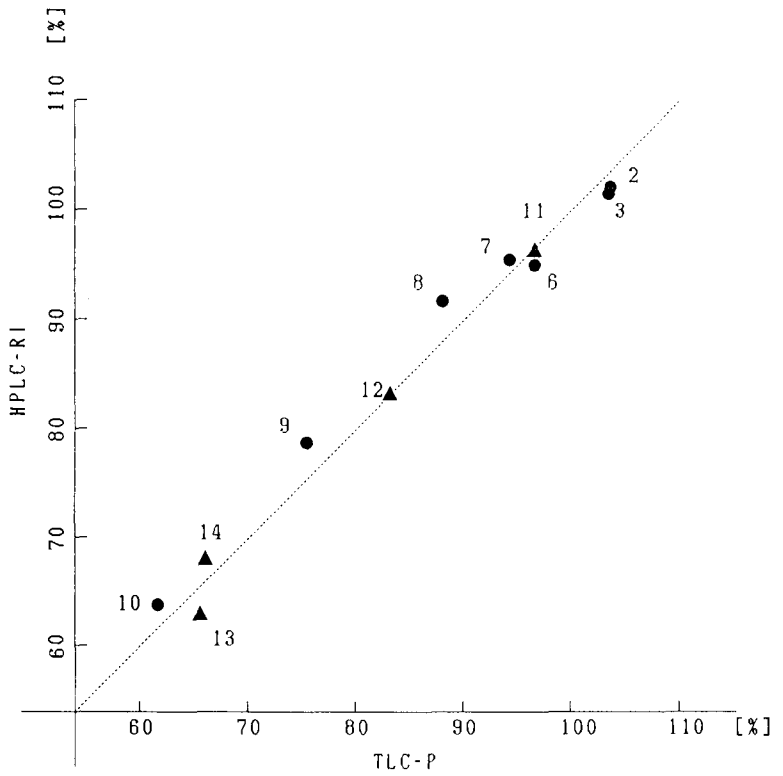

FIG. 2. Relationship between TLC-P and HPLC-RI. Soybean: r = 0.99416; egg yolk: r = 0.99948. ●, Soybean; ▲, egg yolk.

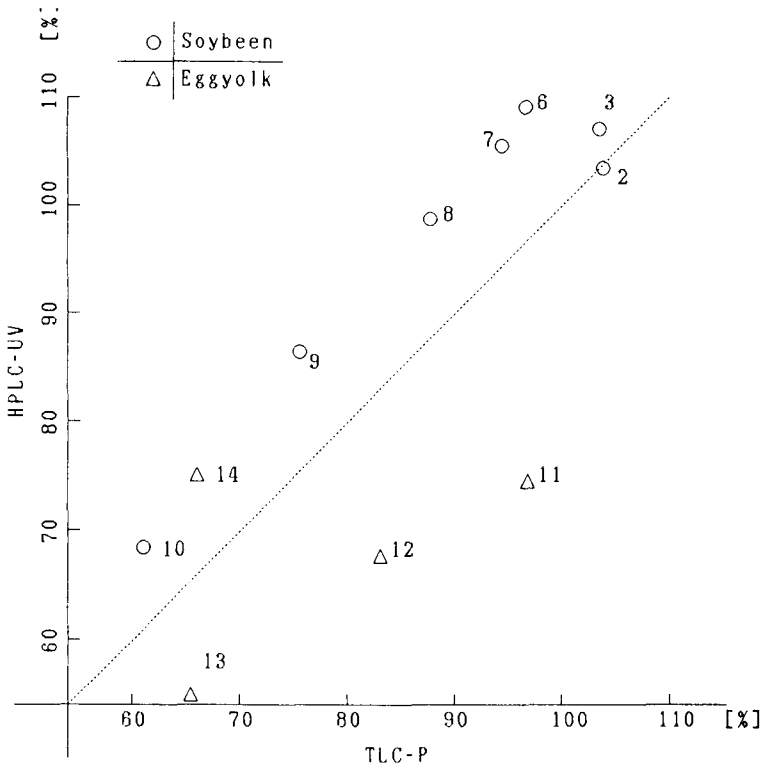

FIG. 3. Relationship between TLC-P and HPLC-UV.

TABLE 7

Fatty Acid Composition of Different Batches of Soybean Lecithin and Egg Yolk Lecithin (GLC area method [%])[a]

Sample no.	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4
(Soybean PC)						
1	14.6	4.0	13.1	62.8	5.4	
2	14.5	4.5	12.3	62.7	5.4	
3	13.1	3.6	11.6	64.9	6.1	
6	14.2	3.9	11.4	64.0	5.8	
7	13.3	3.1	9.7	66.3	7.0	
8	13.4	3.4	9.8	66.1	6.9	
Ref. 2	17 ~ 21	4 ~ 6	12 ~ 15	53 ~ 57	6 ~ 7	
Ref. 8	10 ~ 15	1 ~ 3.5	6 ~ 13	61 ~ 71	4 ~ 7	
(Egg yolk PC)						
11	32.8	13.0	31.0	15.2	0.3	3.1
13	28.4	15.6	27.7	14.3	0.3	6.5
14	29.6	14.2	26.8	13.3	0.5	4.8
Ref. 2	35 ~ 37	9 ~ 15	33 ~ 37	12 ~ 17	0.5	3.7

[a]Samples are the same as in Table 6.

soybeans. The measured values for the egg yolk PC by the HPLC-RI method, even if based on the standard from soybeans, are also highly correlated with the values by the TLC-P method.

DISCUSSION

Many reports have been released regarding the method for quantitatively determining PC by HPLC, but most of them use low wavelength UV detectors. However, at a wavelength of about 210 nm, which is generally used and corresponds to the edge of maximum absorption of double bonds, measured values are easily affected by differences in fatty acid composition and slight deviations in wavelength. Therefore, it seems to be difficult to obtain accurate values. On the other hand, the RI method is hardly affected by differences in fatty acid composition. However, the detector sensitivity of RI is very low as compared with the UV method. Moreover, the RI method is easily affected by temperature, and the stability of base-line is poor. But, as mentioned previously, the RI method is hardly affected by differences in fatty acid composition. In other words, the RI method is hardly affected by the purity and origin of PC, so it can be applicable for various PC samples. What is considered to be the greatest advantage for the study of this method is the fact that the measured values of low-purity PC and high-purity PC are more consistent, and thus, more reliable.

Reproducibility (accuracy) of observed values by HPLC and rejection limit for data. Tables 2, 5 and 6 show that the HPLC method previously described yields very few variations (i.e., the coefficient of variation is very small) and has high reproducibility. For example, the data from the calibration curve is derived by measuring one sample three times at each point. The coefficient of variation for each group is only a few percent (mostly less than 1.5%), with very high reproducibility. In addition, the calibration curve thus obtained is extremely linear, in fact, the coefficient of correlation is more than 0.999 and all points are located on a straight line. This result is evidenced in both the UV and the RI methods.

Therefore, if there is no problem with either the HPLC equipment or the sample preparation, i.e., if the sample is measured correctly, the coefficient of variation for each measurement group must be smaller than a few percent. This means that if the coefficient of variation for each data group is high, there must be errors in the sample preparation or in the equipment, and the data should therefore be discarded.

To check this hypothesis, five samples were injected HPLC more than 10 times, with a range of 20% to 100% of PC (equivalent to 20 μg to 100 μg), and checked for variations in data. The coefficient of variation for measured data group was 2% or less (Table 8).

Therefore, based on the conditions of this method, it can be concluded that since the measurement of PC purity by HPLC is very accurate for each measured value,

TABLE 8

Repeatability of Peak Area (HPLC-RI)

PC content (μg)	100	80	60	40	20
Peak area value of HPLC-RI	2693556	2160814	1587688	1072904	546294
	2680189	2119226	1622430	1062003	532160
	2681407	2127814	1639332	1080225	559614
	2667039	2130263	1605498	1064089	556784
	2676540	2134127	1612935	1087720	544968
	2724147	2135324	1605884	1093360	540902
	2673807	2152746	1599955	1103036	542172
	2682978	2127268	1590043	1086392	540916
	2675156	2185537	1640255	1084924	541035
	2662302	2153472	1634672	1099258	542628
	2656139	2148984	1619435	1086627	553509
	2655561	2149122	1654259	1090228	541669
Mean	2677400	2143720	1617700	1084230	545221
Std. dev.	18547.7	18442.9	21145.4	12670.4	7773.5
C.V. (%)	0.6927	0.8603	1.3071	1.1686	1.4257

Std. dev. = standard deviation. C.V. = coefficient of variation.

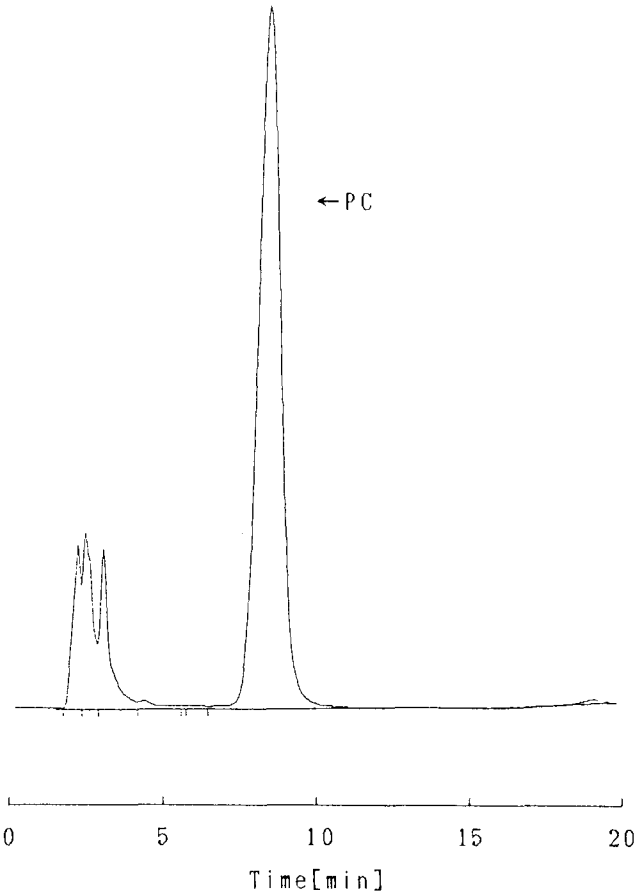

FIG. 4. HPLC-UV chromatogram of no. 9.

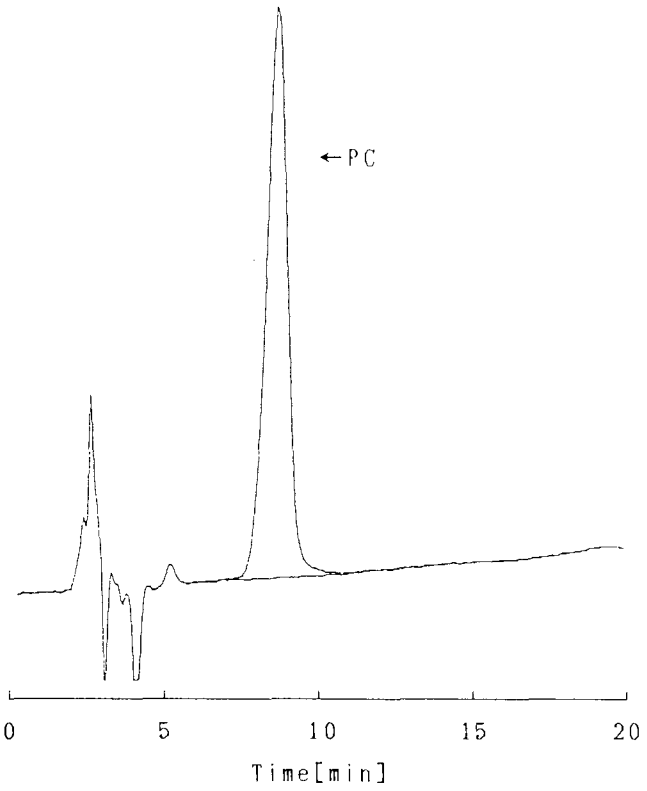

FIG. 5. HPLC-RI chromatogram of no. 9.

that if the coefficient of variation (C.V.) for a group of measured data is higher than a few percent, then the data is due to some abnormality in the equipment and should be rejected.

Comparison of HPLC chromatograms. Soybean PC is shown in Figures 4 and 5. When the UV method is used, a very sharp peak is obtained, as shown in Figure 4. However, the measured values are slightly higher (Fig. 3). Compared with the UV method, the RI method exhibits an inclined base-line but the reproducibility of area values was high, as can be seen in Table 8.

Egg yolk PC can be found in Figures 6 and 7. The UV method yielded area values that do not correlate with the TLC-P method as shown in Figure 3. Moreover, the retention time was short, and the egg yolk PC peak was round (Fig. 6, for samples no. 13 in Table 6).

The RI method yielded the same peak-shape and retention time for both egg yolk and soybean PC, even if high-purity soybean PC is used as the standard as already described. In addition, the measured values for egg yolk PC by HPLC-RI are also as good as those by the TLC-P method. Therefore, the RI method enables measurement of PC purity irrespective of fatty acid composition.

Effective range of calibration curve. In the calibration cruve PC contents were plotted as the ordinate and peak

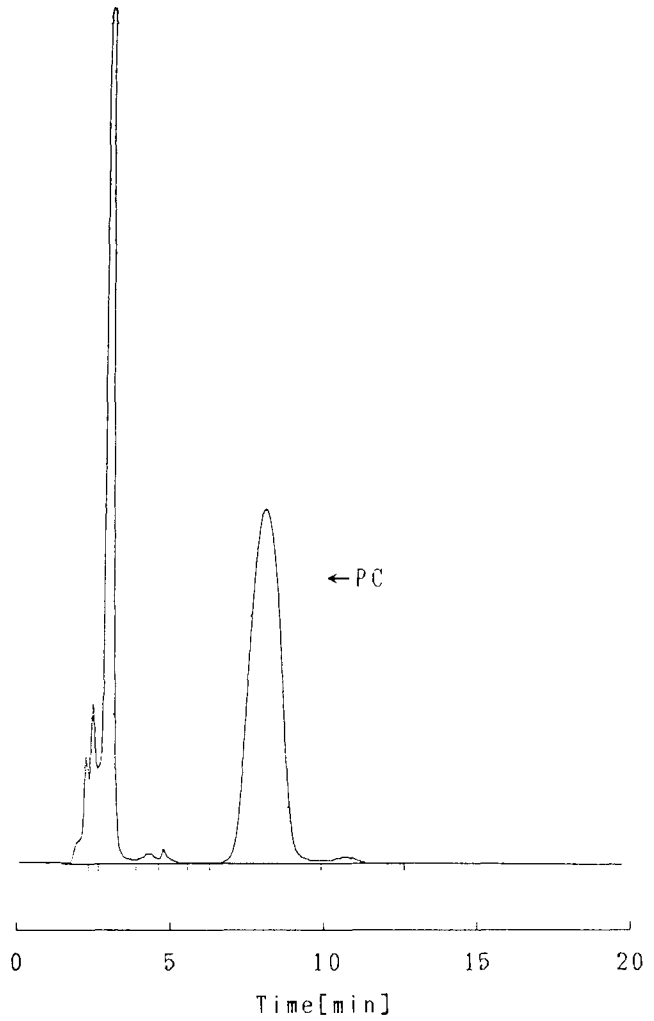

FIG. 6. HPLC-UV chromatogram of no. 13.

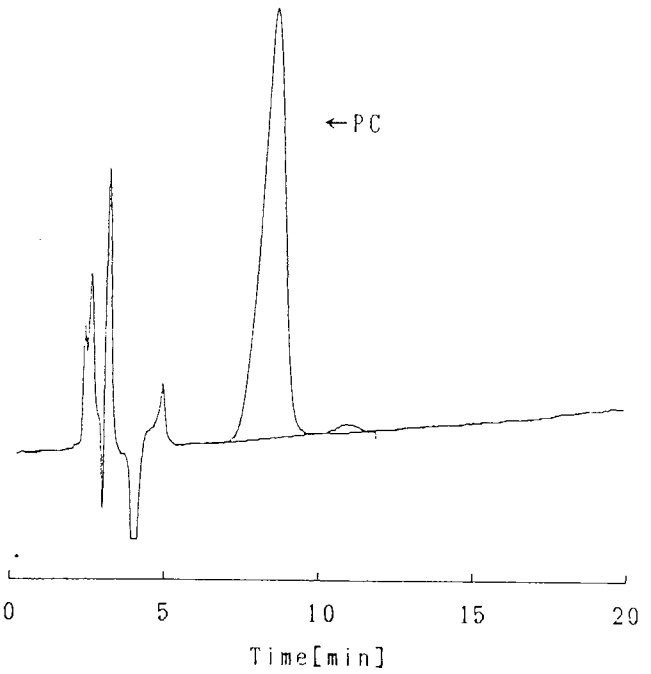

FIG. 7. HPLC-RI chromatogram of no. 13.

area values as the abscissa. The result of our investigation shows that the calibration curve tends to be downwardly convex for both the UV and RI methods. Thus, in order to obtain a calibration curve with a high coefficient of correlation (i.e., a straight line), we considered restricting the range of the calibration curve. For instance, we considered dividing the graph into two regions according to PC-injection quantity, one being in the range of 50–100 μg (corresponding to a PC purity of from 50 to 100 wt%), and the other in the range of 0–50 μg (corresponding to a PC purity of from 0 to 50 wt%), and drawing separately high and low purity calibration curves for determination. It was expected that the above procedure would cause an increased coefficient of correlation (i.e., linearity) and a larger gradient in the high-purity region of the calibration curve than in the low-purity region. It has been proven in the above procedure that if the available range of the calibration cruve is roughly divided into a high-purity region and a low-purity region, this leads to a coefficient of correlation for the calibration curve of about 0.999. Accordingly, highly accurate measured values can be obtained.

Advantages of HPLC method. The advantages of the developed HPLC-RI method over the conventional measuring method (the TLC-P method) are given as follows: Measurement is easy and simple. The actual operations involve only weighing and dissolving samples and then setting the prepared sample solution on the HPLC-RI auto-sampler. In contrast to the TLC-P method, the HPLC-RI method provides reliable measured values (a lower coefficient of variation), requires no skilled operator, and can be applied routinely.

Many test samples which can be placed in the auto-sampler, can be measured sequentially. About four or five hours are required for setting new eluent for base-line stabilization, and one set of analysis can be completed in about 24 hours (night-time unmanned operation is possible with an auto-sampler).

Effect of decomposition time on the TLC-P method. As mentioned above, measured values fluctuate depending on decomposition time in the case of low-purity PC sample in the TLC-P method. In the case of new procedure set forth by the Japan Oil Chemists' Association in 1986 (4), the PC amount, which is scraped from the silica gel in the low-purity sample, is the equal to that in the high-purity PC sample, because the injected amount of PC to TLC-plate is kept almost constant by changing the concentration of the sample solution.

Therefore, there seems to be no error in measuring the phosphorus content in the PC. Since the decomposition time in the high-purity sample hardly affects measured values, some substances contained in low-purity PC, which cannot be separated from PC on TLC, may have caused fluctuation in measured values.

Contamination by phospholipids other than PC. In the HPLC-RI method each retention time, which is measured under the conditions for the injected high-purity samples of PE, PI, LPC, or PS is substantially different from that of PC. It was confirmed, therefore, that PC and other phospholipids are not eluted simultaneously under these conditions (i.e., no other phospholipid is mixed in the PC peak under the above condition).

In the TLC-P method, according to the report (4), it is clear that PC is separated from other phospholipids in the photograph of TLC. This substantiates the findings of our analysis.

Based on the above results, it is our opinion that this method is routinely applicable for quantitative determination for high-purity PC, and we propose that it replace the conventional TLC-P method.

As stated earlier, during the investigation of the refining method for high-purity PC we sought to measure PC purity rapidly and accurately in order to evaluate our refining method. As a result of our efforts, we have developed an efficient and highly reliable analytical method in addition to an industrial process for producing high-purity PC which is equal to or higher in purity than the reagent (Sigma P-6263).

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