Table VI. Determination of Lipid Concentration in Four Lots of DOXIL®

Lipid	Ingredient amount on labeling (μg/ml)	Calculated concentration (n=3; μg/ml, RSD) Lot#011AFL	Lot#012AGD	Lot#029BJD	Lot#032BKA
Chol[a]	3,190	3,438.13 (0.29)	3,561.10 (0.41)	3,269.43 (0.24)	3,416.59 (0.26)
HSPC[b]	9,580	8,420.41 (1.18)	8,859.23 (0.48)	8,836.49 (1.53)	9,328.49 (1.22)
DSPE-PEG	3,190	2,435.90 (0.76)	2,552.80 (1.18)	2,686.19 (1.09)	2,757.95 (1.22)
Calculated molar ratio Chol/HSPC/DSPE-PEG		43.49/52.17/4.34	43.12/52.53/4.35	40.99/54.27/4.74	40.80/54.56/4.64

[a] Chol was detected by a UV detector
[b] The value of HSPC was calculated from the peak of HSPC-1

starting material. When compared lot-to-lot, while there were almost no differences between the two older lots or the two relatively newer lots, 0.4–2.4% differences were observed between the old and new lots. The ratio of Chol was higher in the old lots and those of HSPC and DSPE-PEG were lower. This may be due to the hydrolysis of phospholipids, which resulted in a reduced amount of HSPC and DSPC-PEG.

Analysis of Lipid Composition After Storage at Different Temperatures

Thus, the developed method was used to assess liposome stability. It is known that the hydrolysis of phospholipid follows a pseudo first-order kinetics model and that the hydrolysis rate correspondingly increases with temperature (7). First, the prepared liposomes were incubated for 3 days at 4, 37, or 57°C and the concentrations of the main hydrolysis products, S-LysoPC and SA, were measured. A significant increase in S-LysoPC and SA was observed with a rise in temperature (Fig. 3). Consistent with this observation, the concentrations of HSPC and DSPE-PEG decreased and that of Chol was stable (data not shown). Next, the concentration of the hydrolysis products in the four lots of DOXIL® were measured (Table VII). The concentrations of S-LysoPC and SA significantly increased in the two older lots (long past the expiration date). It was confirmed that even under storage at 4°C and solution of pH 6.5, *i.e.*, conditions in which the hydrolysis rate was the least (8), the lysophospholipid and free fatty acids were produced by hydrolysis during long-term storage.

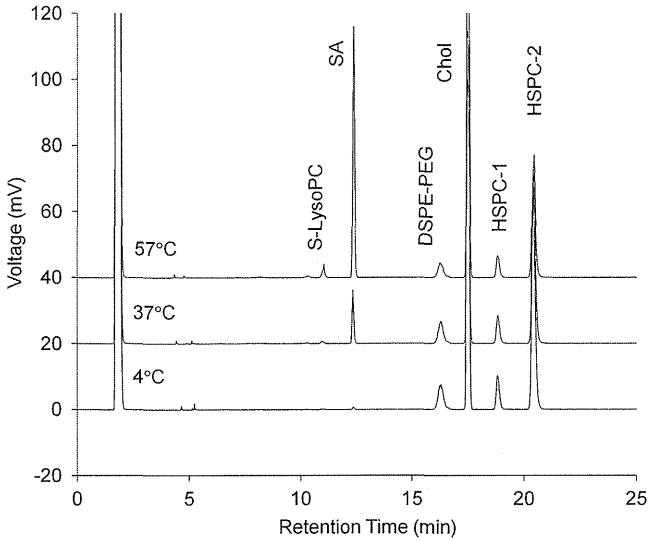

Fig. 3. A stability evaluation of the PEG liposome under each thermal condition (4°C, 37°C, and 57°C). The lipid composition of the PEG liposome, which was dialyzed against 10% sucrose, is presented in Table VI. The PEG liposome was incubated at each temperature for 3 days and diluted with methanol, and 20-μl aliquots of the diluted solution were injected

CONCLUSIONS

A simple reversed-phase HPLC-ELSD method was developed for the quantification of lipids in PEGylated liposomes. Although the linearity and accuracy obtained with UV detection of Chol were better those obtained with ELSD, the HPLC-ELSD method was validated to be linear, precise, accurate, and sensitive. Additionally, the HPLC-UV/ELSD method was found to be suitable for simultaneous determination of HSPC, DSPC-PEG, and Chol as well as their hydrolysis products in PEGylated liposomal products. It was also suggested that accurate quantification of the lipid component enables assessment of changes in lipid composition during the preparation process. In addition, the increase of hydrolysis products of phospholipids under a heat-accelerated condition may be observed. This method will be useful for quantifying the hydrolysis products in liposomal products in a stability test, such as a long-term storage test or an accelerated test, as well as for quantifying the lipid composition of liposomal products.

Table VII. Determination of S-LysoPC and SA Concentration in Four Lots of DOXIL by the HPLC-ELSD

	Calculated concentration (n=3; μg/ml, RSD) Lot#011AFL	Lot#012AGD	Lot#029BJD	Lot#032BKA
	Approximate elapsed time after manufacture			
Lipid	68 months	65 months	29 months	26 months
S - LysoPC	442.83 (3.36)	425.00 (5.56)	238.06 (1.66)	202.61 (2.74)
SA	374.50 (2.10)	376.46 (3.04)	215.19 (2.30)	175.17 (4.59)

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