Separation and Quantification of Polyethylene Glycol–conjugated Liposome Components by Reversed-phase HPLC Analysis with UV and Evaporative Light Scattering Detection
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Purpose
Liposomes incorporating polyethylene glycol (PEG)-conjugated lipids (PEGylated liposomes) are of great interest as drug delivery carriers. The lipid composition is one of important factors to ensure the quality/safety/efficacy of liposomal products. The lipid hydrolysis is also considered a critical parameter for the chemical stability of liposomal products. Thus, in this study, we attempted to develop a simple reversed-phase HPLC method with an evaporative light scattering detector (ELSD) for simultaneous determination of lipid components and hydrolysis products in PEGylated liposomes.

Methods
Hydrogenated soy phosphatidylcholine, cholesterol, PEG-conjugated lipid, lysophosphatidylcholine, and free fatty acids were separated using a C18 column with a gradient mobile phase consisting of 4 mM ammonium acetate buffer (pH 4.0) and 4 mM ammonium acetate in methanol at a flow rate of 1.0 ml/min. Then the lipids were sequentially monitored using a UV detector (205 nm) and an ELSD. The ELSD conditions were as follows: the drift tube temperature was set at 45°C, the nitrogen gas-pressure was set at 350 kPa, and the gain was set to 6. PEGylated liposomes were prepared by the modified ethanol injection method.

Results
The HPLC-UV/ELSD method provided sufficient repeatability (< 0.33 %), linearity (> 0.997%), and recovery rate (>97.62–103.80%) for all lipids. This validated method was used for the assessment of the composition change during the preparation process of liposomes, and it was found that after the dialysis for external solution exchange, the percentages of HSPC and DSPE-PEG slightly decreased, whereas the percentage of Chol was slightly increased. In addition, the increase of hydrolysis products of phospholipids under a heat-accelerated or low-pH condition could be observed.

Conclusion
This HPLC-UV/ELSD method will be useful for quantifying the hydrolysis products in liposomal products as well as the lipid composition.