Quantitative analysis of cholesterol oxidation products and desmosterol in parenteral liposomal drug products

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Cholesterol is one of the major structural components of liposome bilayers. Cholesterol is vulnerable to oxidation, leading to a variety of cholesterol oxidation products (COPs) during liposome preparation and/or storage. The oxidation of cholesterol to COPs could cause the physical properties of liposome bilayers to change, resulting in “leaking” of the drug from the liposome. This altered liposome stability could further affect the safety and efficacy of the liposomal drug, and the presence of bioactive COPs could cause unwanted physiological responses. Herein, we report a liquid chromatography – mass spectroscopy (LC-MS) based analytical method for separating and quantifying COPs present in liposomal parenteral drug formulations from five different vendors. Results show that six COPs and desmosterol (cholesterol precursor) have been detected in liposomal drug products (LDPs). 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-keto-cholesterol, and desmosterol were the major impurities in LDPs. It is worthy to note that none of USP/NF grade cholesterol excipient contains COPs and this suggests that COPs are generated during liposome preparation and/or storage. This method has been validated according to USP validation of compendial procedures, and the validated method can provide potentially referenceable information for the quantification of cholesterol related impurities presented in liposomal drug formulations.