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Determination of Cholesterol in Milk and Dairy Products by High-Performance Liquid Chromatography

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A sensitive high-performance liquid chromatographic method with Nova-Pak C_{18} column for the determination of cholesterol in milk products was studied. To optimize separation of cholesterol, various mobile phases such as hexane/2-propanol, hexane/tetrahydrofuran, hexane/ethyl acetate, acetonitril/methol, acetonitril/methol/2-propanol was compared. Acetonitril/methol/2-propanol was superior to other mobile phase systems in separation. A liquid-liquid extraction(LLE) for cholesterol was simplified with non-polar solvent, hexane to remove interfering compounds and had a recovery(100±1.0) of cholesterol. A solid phase extraction(SPE) method using C_{18} Sep-pak was developed and compared with LLE. The saponified sample was directly transferred into SPE tube and eluted fat-soluble compounds with acetic acid/ethanol. The cholesterol in SPE tube was collected by eluting ethyl acetate/hexane. The SPE method was rapid and highly reproducible. However, the SPE method is relatively expensive. The both extraction methods are useful in combination with saponification of esterified cholesterol to facilitate total cholesterol determination. The detection limit at 205nm was up to 0.01μg. The newly developed HPLC method was simple, accurate and has the advantage over the many methods commonly used.