results of recovery test were 96.9~102.4% for retinol and 98.1~104.3% for retinylpalmitate. We also investigated on stability of retinol and retinylpalmitate products after opening according to variation of temperature. The contents of retinol and retinylpalmitate products were decreased in the range of 78.4~95.1% and 40.4~85.7% at cold storage condition, 60.2~85.6% and 20.2~79.7% at room temperature, 12.5~35.2% and 10.0~39.3% at acceleration condition(40℃) after five months. Therefore it is better to store at cold temperature in use.

[PD4-12] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Development of the Diagnostic Method for Galactosemia

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A new reversed-phase HPLC method has been developed for the determination of galactose in blood spot (50μL) on Guthrie filter paper using 8-Amino-2-naphthalenesulfonic acid (8,2-ANS) as derivatization reagent. In a metabolic pathway from galactose to glucose-6-phosphate, there are three enzymes involved such as galactokinase, transferase, and epimerase. The deficiency of one of these enzymes causes accumulation of galactose in blood, which provides a pathgnomonic marker. Galactose was extracted from blood spot on filter paper and derivatized with 8,2-ANS to produce Schiff bases, and reduced under sodium cyanoborohydride. This is ready for the HPLC analysis for a diagnosis of Galactosemia. The linear range was between 80 nmol and 20 pmol and the limit of detection (S/N=3) of this method was 0.9 ng/mL. The mean recovery of galactose was 104.29 % with a SD of 3.62 %. with correlation coefficiency of 0.9999. The control range of galactose in blood of Korean newborn (specimen collected with 7 days after birth) were below 6mg/dL for male and female (n=5 for each gender) without any difference. We applied 11 anonymous blood spots in which diagnosis has already made by enzyme assay as one of the galactokinase, transferase, or epimerase deficiencies. All the patients blood spots showed abnormal elevation of galactose. These results suggest that the new method is a valuable tool for the diagnosis of metabolic disorder, Galctosemia.

[PD4-13] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Rapid Monitoring of PEGylation Process by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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The covalent attachment of polyethylene glycol (PEG) has received the increasing attention as a well-established technique that has the capacity to overcome several problems of protein and peptide for therapeutic needs. One of the great challenges of PEG chemistry is characterization of PEGylated conjugates. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was investigated as a method for the rapid control and optimization for PEGylation of peptide and protein. RC160 and ricin A chain (RTA) were used as the model systems of peptide and protein, respectively. MALDI-TOF MS was useful for not only determining the true molecular mass of PEGylated species, but also identifying the individual species contained in a preparation. The PEG-RC160s were characterized by a bell shaped distribution of equally spaced molecular ions 44 Da apart, while PEG-RTA having higher molecular weight showed a unresolved peak of the individual oxyethylene unit. MALDI-TOF MS was demonstrated to be also useful for optimizing the PEGylation conditions through the control of the pH and stoichiometry of the components in the reaction. Moreover, it provides the merits of speed, high resolution, small sample requirements, ease of determination, and simple data manipulations over other analytical tools.