Application Note UV VIS

## Fields of Application/Industry:

- Chemistry/Polymer Industry
- Clinical Chemistry/Medicine/Hygiene/ Health Care
- Cosmetics
- Electronics
- Energy
- Environment/Water/Waste
- Food/Agriculture
- Geology/Mining
- Material Analysis
- Metallurgy/Galvanization
- Pharmacy
- Refineries/Petrochemistry
- Semi-Conductor Technology
- Others

# 8 联池-酶法测定血液中的胆汁酸

**摘要：**

胆汁酸在肝脏中合成并作为消化脂肪的乳化剂。当患有急性病毒性肝炎，肝硬化，肝癌，中毒性肝损害等肝脏疾病时，血浆和血清中的胆汁酸的量会增加。血液中的胆汁酸含量可以用酶方法来测定。因为酶的影响和其特异性可以促进人体器官的新陈代谢，因此进行酶方法的常规分析非常重要。几乎所有的物质都可以被酶化成在紫外区有吸收的辅助酶，通过光度计进行吸光度的变化测量。另外，酶方法分析可以进行快速的检测，几乎不需要样品前处理时间。。

本文主要研究酶方法测定血液中的胆汁酸含量，利用德国耶拿分析仪器股份公司的 SPECORD 210 PLUS 紫外可见分光光度计和带外部热交换装置的帕尔贴冷却八连池装置以及其 WinASPECT PLUS 软件中的特殊工具（含有酶方法测定胆汁酸的分析方法），在 37℃下，对血液中的胆汁酸含量进行测量。带外部热交换装置的帕尔贴冷却八连池装置，温控范围-5℃—105℃之间，控温精度±0.1℃，保证检测在恒温中快速进行。WinASPECT PLUS 软件集成了食品分析中酶方法包，该方法包含相应的测量参数，数据处理等功能，使测量快速，便捷。从而扩展了紫外分光光度计的应用领域。

# Enzymatic Determination of Bile Acids in Blood

## Introduction

Bile acids are synthesized in the liver and act as emulsifiers in the digestion of fats. Increased bile acid levels in plasma or serum occur in case of liver diseases such as acute viral hepatitis, liver sclerosis, liver cancer or toxic hepatic damages.

The concentration of bile acid in blood is determined using enzymatic determination. Because of their effect and substance specificity the enzymes enable a number of simultaneous metabolic processes in the human organism. In the routine analysis enzymatic methods are therefore of great importance. By the measurement of the absorbance change in the UV range of absorbent coenzymes nearly all substances which can be converted by enzymes can be determined photometrically.

## Experimental

In the following application example the concentration of bile acid in blood was determined. For the measurement the SPECORD® 210 PLUS with a Peltier cooled 8 cell changer with external heat exchanger was utilized (Fig. 1 and 2).

Fig. 1: SPECORD® PLUS with Peltier cooled cell holder with external heat exchanger

Fig. 2: Peltier cooled 8 cell changer

### Sample preparation

The determination was performed by using the test combination of DiaSys [1] as well as the bile acid method of the WinASPECT PLUS® software. Because of the low sample volume a semi-micro cell was used.

Determination

The analysis was carried out at a temperature of 37 °C. The fresh serum sample was directly used for measurement. The reference measurement was performed against water.

According to the instruction of the test kit protocol 0.8 µl of distilled water/serum were pipetted into the cells, 600 µl of reagent 1 were added (consisting of diaphorase, $NAD^+$ and NBT), and mixed briefly. After an incubation period of 4 minutes the absorbance $E_1$ was measured. After the addition of 120 µl of reagent 2 and a further incubation period of 5 minutes the second absorbance ($E_2$) was measured. Besides a serum with known bile acid concentration as control standard was measured to check the accuracy of the analysis. The processing of the total method is performed automatically by the WinASPECT PLUS® software.

Results/Evaluation

The absorbance differences for the blank value and the samples are calculated. Afterwards the absorbance difference of the blank value is subtracted from the absorbance differences of the samples. To compensate the decrease of absorbance by addition of reagent 2, the $E_1$ values are multiplied by factor 0.85, which is calculated as follows: (sample volume + volume R1)/total volume.

$$\Delta E = [\,(E_2 - 0.85\,E_1)_{\text{Sample/Standard}}\,] - [\,(E_2 - 0.85\,E_1)_{\text{Blank Value}}\,]$$

The bile acid content is calculated by means of the known concentration of the supplied bile acid standard:

$$\text{Bile acid } c\,[\mu mol/l] = \frac{\Delta E_{\text{Pr}}}{\Delta E_{St}} \cdot c_{St}[\mu mol/l]$$

The calculation of the absorbance differences and the sulfite concentration are performed automatically by the software after the measurement. A bile acid concentration of 7.9 µmol/l blood was determined. The concentration is within the ‘normal’ range (0–10 µmol/l). Pathologically increased bile acid concentrations above 10 µmol/l blood could be an indication of above stated liver diseases or a liver dysfunction.

**Summary**

An enzyme-based analysis is fast and can be performed without time-consuming sample preparation. It is performed under physiological conditions. The reagents are harmless and easy to handle. Furthermore the documentation of individual substances in mixtures is possible. Thanks to an extensive collection of enzymatic methods in the WinASPECT PLUS® software, the determination of the corresponding substance with the relevant measurement setting, sample sequence and evaluation is performed automatically. Furthermore the Peltier cooled cell changer enables enzymatic analyses of multiple samples which require very exact tempering.

**List of references**

[1] Bile acids test-combination for in vitro determination in fresh serum or EDTA plasma on photometric systems, order no. 122129990313, DiaSys

Chemicals provided by Sigma Aldrich® were used.



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