http://dx.doi.org/10.5012/bkcs.2012.33.12.4145

Highly Sensitive Luminescence Assessment of Bile Acid Using a Balofloxacin-Europium(III) Probe in Micellar Medium

Huan Cai, Fang Zhao,* Hailin Si, Shuaishuai Zhang, Chunchun Wang, and Peirong Qi

*School of Chemistry and Chemical Engineering, Shihezi University; Xinjiang Bintuan Key lab of Chemical Engineering for Green Process, Shihezi, Xinjiang 832003, China. *E-mail: nlz837037@163.com*
Received August 5, 2012, Accepted September 28, 2012

A novel and simple method of luminescence enhancement effect for the determination of trace amounts of bile acid was proposed. The procedure was based on the luminescence intensity of the balofloxacin-europium(III) complex that could be strongly enhanced by bile acid in the presence of sodium dodecyl benzene sulfonate (SDBS). Under the optimum conditions, the enhanced luminescence intensity of the system exhibited a good linear relationship with the bile acid concentration in the range 5.0×10^{-9} - 7.0×10^{-7} mol L^{-1} with a detection limit of 1.3×10^{-9} mol L^{-1} (3σ). The relative standard deviation (RSD) was 1.7% (n = 11) for 5.0×10^{-8} mol L^{-1} bile acid. The applicability of the method to the determination of bile acid was demonstrated by investigating the effect of potential interferences and by analyzing human serum and urine samples. The possible enhancement mechanism of luminescence intensity in balofloxacin-europium(III)-bile acid-SDBS system was also discussed briefly.

Key Words : Balofloxacin, Europium(III), Bile acid, Luminescence

Introduction

Bile acids, the major end products of cholesterol catabolism, are a group of amphipathic compounds that belong to the steroid class and are classified as acid sterols. They are a sizable group of biochemically important small molecules, with a variety of structures.[1,2] Bile acids are mainly produced in the liver, secreted in the gallbladder and passed into the small intestine to perform their emulsifying functions. Bile acids serve many important physiological functions, including cholesterol homeostasis, absorption of dietary lipids and fat-soluble vitamins by formation of micelles and both the excretion and recirculation of drugs and toxins.[3] The concentration of bile acid in biological fluids, such as serum or urine, can offer indications regarding an individual's metabolism and potentially useful in formation about hepatobiliary and the diagnosis of diseases such as cholestasis,[4] colon[5-7] and liver cancer.[8] On the other hand, bile acids also have pharmacological activity and some components of bile acids have important therapeutic applications for treating some diseases, such as primary biliary cirrhosis and cholesterol gallstone. Therefore, quantification of bile acid is an important tool for therapy monitoring.

In health, only small quantities of bile acid are found in peripheral circulation and urine. So, the estimation of bile acid in biological fluids has always presented technical difficulty owing to their low concentration and complex structure. Because of the growing interest on bile acid, a number of analytical methods have been developed in the literature for the estimation of bile acid in biological matrices, based on high performance liquid chromatograph (HPLC) with ultraviolet (UV),[9,10] fluorescence,[11] evaporative light scattering[12,13] or electrospray tandem mass spectrometer (MS) detection,[14,15] LC-MS/MS,[16] gas chromatography-mass spectrometry (GC-MS),[17] thin-layer chromatography,[18] nuclear magnetic resonance (NMR),[19] electrochemical sensor,[20-22] chemiluminescence,[23] spectrofluorimetry,[24] ultraviolet-visible spectrophotometry (UV-vis),[25] radioimmunoassay detection and enzymatic-colorimetric method.[26] However, these assay methodologies often suffer from disadvantages of low sensitivity, time consuming, costly reagents and instruments, and complicated pretreatment. The spectrofluorimetric method makes predominant concern owing to its high sensitivity and selectivity. To the best our knowledge, no application of spectrofluorimetric method on determination of bile acid using balofloxacin-europium(III) as luminescence probe has been reported. Table S1 (see Supplementary information) gives a comparison of the analytical methods in terms of linear calibration range and sensitivity for the determination of bile acid. Each of these methods mentioned often offer their own set of advantages and disadvantages.

In recent years, the use of lanthanide-ligand complexes as a novel probe has obtained more attention because of their large Stokes shifts, narrow emission bands, and long luminescence lifetimes. This can be used to reduce potential background fluorescent emission interferences from the biological matrix.[27] Balofloxacin belongs to the fourth generation quinolones containing carboxylic and keto-oxygen atoms (Figure 1). It could form the coordination complex with europium(III), which emitted the characteristic luminescence of europium(III) at 618 nm.[28] But the luminescence intensity of the system was greatly enhanced when bile acid was added in a micellar medium. The enhanced intensity of luminescence is proportional to the concentration of bile acid. On the basis of these observations, a novel method of luminescence enhancement effect of the balofloxacin-europium(III)

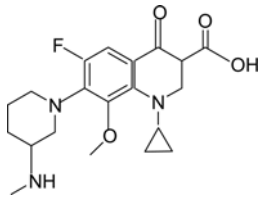

Figure 1. Chemical structure of balofloxacin.

complex for the determination of bile acid has been developed. This method offers potential advantages of simplicity, rapidity, as well as high sensitivity in comparison to the above-mentioned techniques. The method presented has been successfully applied to the determination of bile acid in biological fluids with satisfactory results.

Experimental

Apparatus. The luminescence spectra and intensities were recorded on a Hitachi F-2500 Fluorescence Spectrofluorimeter (Kyoto, Japan) in the range (230-800 nm), using a standard 10 mm path length quartz cell with 10 nm band widths for both the excitation and emission monochromators. All absorption spectra were taken on a UV-2401PC spectrophotometer (Shimadzu, Japan). The pH was measured using a Lei Ci pHs-3C digital pH meter (Shanghai, China).

Chemicals and Reagents. All chemicals were of analytical-reagent grade and used without further purification, and doubly distilled water was used for the preparation of all solutions and during all measurements.

Bile acid was purchased from Beijing Chemical Reagent Plant (Beijing, China). Balofloxacin and tris (hydroxymethyl) amino methane (Tris) were purchased from Sigma Chemical Co. (St. Louis, USA). Eu_2O_3 (purity, 99.99%) was from General Research Institute for Nonferrous Metals (Beijing, China). Sodium dodecyl benzene sulfonate (SDBS) and cetyltrimethylammonium bromide (CTAB) were purchased from Signal Aldrich Corporation (Steinheim, Germany).

Standard stock solution of bile acid (1.0×10^{-3} mol L^{-1}) was prepared by dissolving the required amount of bile acid in 60% acetic acid and then diluted with water. Working standard solutions were freshly prepared by making appropriate dilutions of the stock standard solution with water. A stock solution of balofloxacin (1.0×10^{-3} mol L^{-1}) was made by dissolving appropriate amount of balofloxacin in 0.003 mol L^{-1} NaOH. This solution was stored in a refrigerator at 4 °C and protected from light when not in used. The working standard solutions were freshly prepared by making appropriate dilutions of the stock standard solution with water. A stock standard solution of europium(III) ion (2.0×10^{-2} mol L^{-1}) was prepared by dissolving 0.352 g Eu_2O_3 in 1:1 HCl, evaporating the solution to be almost dry on a water bath, then diluting it to 100 mL with water. The working europium(III) ion solution was obtained by appropriate dilution the stock solution with water. A 0.3 mol L^{-1} Tris-HCl buffer solution (pH 7.5) was prepared by dissolving an appropriate amount of tris (hydroxy methyl) aminomethane in 100 mL water and adjusting the pH using 0.1 mol L^{-1} hydrochloric acid solution. Working solutions were obtained freshly from the stock solution by appropriate dilution before use. Sodium dodecyl benzene sulfonate (SDBS) and cetyltrimethylammonium bromide (CTAB) were freshly prepared by dissolving each reagent in water.

Analytical Procedure. In order to measure the luminescence intensity of balofloxacin-europium(III)-bile acid-SDBS system, certain amounts of balofloxacin, europium(III) ion solution, Tris-HCl buffer solution, bile acid, and SDBS were added into a 10 mL volumetric flask. The resultant mixture was diluted to the mark with water, mixed thoroughly, and allowed to stand for about 20 min at room temperature. The mixed solution was delivered to the quartz cell. The luminescence intensity was measured with the excitation and emission wavelengths of 335 and 618 nm, respectively. Bile acid was found to enhance the luminescence signal of balofloxacin-europium(III)-SDBS system strongly. Determination of bile acid concentration was based on the net luminescence intensity changes from the with and without bile acid sample solution.

Preparation of Samples. Serum samples were obtained from the local hospital. A 1.0 mL serum sample was transferred into a centrifuge tube. The potential effect produced by serum proteins was eliminated with 4 mL of methyl cyanide and centrifuged at 4,000 rpm for 15 min. In order to separate bile acid from both liposoluble and water soluble interfering substances, the supernatant clear solution was collected and mixed with 5 mL of chloroform and 0.5 mL of glacial acetic acid. Liposoluble substances, such as cholesterol and triglyceride, were dissolved in chloroform while bile acid was dissolved in glacial acetic acid. The mixture was sonicated oscillated for at least 10 min to aid dissolution, evaporating the solution to be almost dry on a water bath. Chloroform, methyl cyanide, and water were evaporated with the interference substances dissolved in them.[24] Thus, well worked serum samples were obtained. Urine samples were collected from the volunteers. No further pretreatment was required for urine samples. Further dilutions with water were conducted in order to make the bile acid concentration within the working range.

Results and Discussion

Spectra Characteristics. The luminescence excitation and emission spectra of balofloxacin, europium(III), bile acid, balofloxacin-europium(III), balofloxacin-europium(III)-bile acid, and balofloxacin-europium(III)-bile acid-SDBS systems were drawn at room temperature and are shown in Figure 2. It can be noted, from Figure 2(a), that the maximum excitation peak of the balofloxacin-europium(III)-bile acid-SDBS system is 335 nm and it was used as the excitation wavelength.

It was observed, from Figure 2(b), that neither europium(III) aqueous solution (curve 2') nor balofloxacin aqueous solution (curve 3') exhibits a strong luminescence peak at 618 nm. After the addition of balofloxacin into the europium(III)

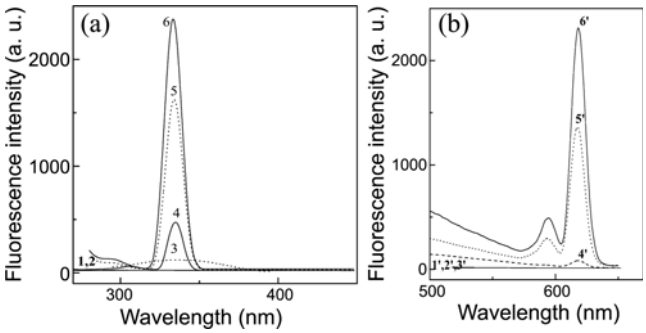

Figure 2. Luminescence excitation (a) and emission (b) spectra: (1and 1') bile acid, (2 and 2') europium(III), (3 and 3') balofloxacin, (4 and 4') balofloxacin-europium(III), (5 and 5') balofloxacin-europium(III)-bile acid, and (6 and 6') balofloxacin-europium(III)-bile acid-SDBS. Conditions: europium(III), 5.0×10^{-5} mol L^{-1}; balofloxacin, 7.0×10^{-6} mol L^{-1}; bile acid, 3.0×10^{-8} mol L^{-1}; Tris-HCl, 0.03 mol L^{-1}; SDBS, 2.5×10^{-4} mol L^{-1}; pH 7.5.

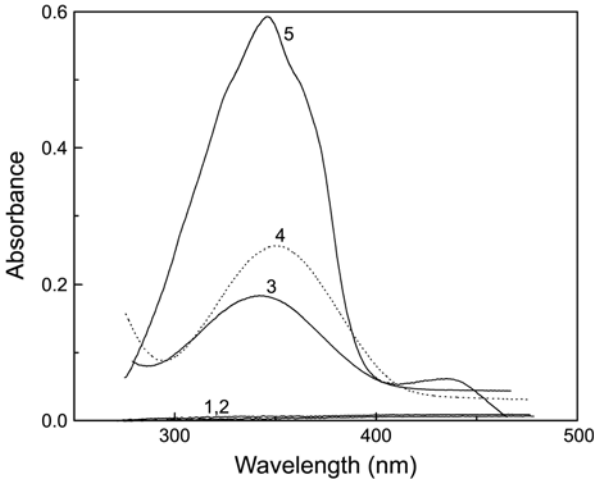

Figure 3. Absorption spectra: (1) bile acid, (2) europium(III), (3) balofloxacin, (4) balofloxacin-europium(III), and (5) balofloxacin-europium(III)-bile acid. Conditions: europium(III), 5.0×10^{-5} mol L^{-1}; balofloxacin, 7.0×10^{-6} mol L^{-1}; bile acid, 3.0×10^{-7} mol L^{-1}; Tris-HCl, 0.03 mol L^{-1}; SDBS, 2.5×10^{-4} mol L^{-1}; pH 7.5.

ion solution, the characteristic luminescence peak of balofloxacin-europium(III) occurred at 594 and 618 nm, which correspond to the $^5D_0$-$^7F_1$ and $^5D_0$-$^7F_2$ transitions of the europium(III) ion, respectively (curve 4'). When the bile acid was added the balofloxacin-europium(III) system, the characteristic peak of europium(III) at 618 nm was enhanced greatly (curve 5'), indicating that bile acid can form a very stable ternary complex with the balofloxacin-europium(III) complex. With the addition of SDBS to the balofloxacin-europium(III)-bile acid system (curve 6'), the luminescence intensity of balofloxacin-europium(III)-bile acid-SDBS system at 618 nm is much larger than that of balofloxacin-europium(III)-bile acid system, indicating that SDBS can go on combining with the balofloxacin-europium(III)-bile acid system to form the balofloxacin-europium(III)-bile acid-SDBS supermolecule complex. So, a wavelength of 618 nm was used as the emission wavelength for the estimation of bile acid.

The absorption spectra of balofloxacin, europium(III), bile acid, balofloxacin-europium(III) and balofloxacin-europium (III)-bile acid were recorded as shown in Figure 3. It was observed that europium(III) ion and bile acid have no absorption peak. An obvious phenomenon was seen that the absorption peak of balofloxacin was increased and the wavelength shifted from 335 to 349 nm when europium(III) ion were mixed with the balofloxacin solution, thus, suggesting the formation of balofloxacin-europium(III) binary complex. However, the absorption of the balofloxacin-europium(III) system was enhanced significantly with the addition of bile acid, which was in accordance with the luminescence increment of the luminescence excitation spectrum of the balofloxacin-europium(III)–bile acid system, and the maximum absorption wavelength showed a slight blue shift from about 349 to 346 nm. This can be explained by the fact that bile acid can form a ternary complex with the balofloxacin-europium(III) system.

Effect of pH Value and Buffers. Balofloxacin is an amphoteric species with a piperazinyl and a quinolone ring; therefore, the pH value and buffer solutions had very significant influences on the formation of the balofloxacin-europium(III) complex and the ability of bile acid to bind to the balofloxacin-europium(III) complex. Thus, the influence of pH value on the luminescence intensity was examined in the range of 6-10. The maximum luminescence intensity of the system was observed in the pH range 7.1-7.7. Thus, we selected pH 7.5 for further research. The effect of buffer solutions, NaAc-HAc, NH_4Cl-HCl, NH_4Ac-HAc, Tris-HCl, glycine-NaOH and KH_2PO_4, on the luminescence intensity was subsequently optimized. The results indicated that 0.03 mol L^{-1} Tris-HCl offered the highest sensitivity.

Effect of Time. The experimental results showed that the luminescence intensity of the balofloxacin-europium(III)-bile acid-SDBS system reached its greatest value in 15 min after all of the reagents had been added and remained stable for at least 180 min. Therefore, all measurements were performed within 180 min for further experiments.

Effect of Reagents Addition Order. The effect of the addition order of the reagents on the luminescence signal was checked. The results showed that an addition order of balofloxacin, Tris-HCl, europium(III), bile acid, and SDBS maximized the luminescence intensity. Thus, this reagent addition order was chosen for the present study.

Effect of Balofloxacin Concentration. The effect of the concentration of balofloxacin on the luminescence intensity of the system was tested in the range from 1.0×10^{-6} to 1.0×10^{-5} mol L^{-1}. The relative luminescence intensity was found to increase with the increasing concentration of balofloxacin and reached a maximum value at 7.0×10^{-6} mol L^{-1}, above which the luminescence intensity decreased slowly. Hence, 7.0×10^{-6} mol L^{-1} balofloxacin was chosen as the optimum concentration for further measurement.

Effect of Europium(III) Ion Concentration. The effect of europium(III) ion concentration on the luminescence emission intensity of the luminescence system was studied at different concentrations from 1.0×10^{-6} to 1.0×10^{-4} mol L^{-1}. The luminescence intensity increased upon increasing

the concentration of europium(III) ion up to 5.0×10^{-5} mol L^{-1}, above which the luminescence intensity became constant. The composition ratio of the balofloxacin to europium(III) ion in the balofloxacin-europium(III)-bile acid-SDBS system is 1:7.1. So, 5.0×10^{-5} mol L^{-1} europium(III) ion was selected in this work.

Effect of Surfactants. Surfactants are often played a vital role to boost up the luminescence intensity of the chelation reaction. Various surfactants, SDS, SDBS, CTAB, TX-100, β-CD, were used to examine the effect of the sensitization behavior on the balofloxacin- europium(III)-bile acid-SDBS system. Results indicated that the maximum luminescence signal was appeared by the use of SDBS as micellar medium. The effect of SDBS concentration on the luminescence signal was investigated. The results showed that luminescence signal reached a maximum value at 2.5×10^{-4} mol L^{-1}, which was so used for the whole experiment.

Analytical Characteristics. Under the aforementioned optimum experimental conditions, the calibration graph of the responses of luminescence intensity (ΔI) versus the concentration of bile acid (c) was linear in the range of 5.0×10^{-9}-7.0×10^{-7} mol L^{-1} (Figure 4). The linear regression equations were given by ΔI = −3.7+7.19C (C being the bile acid concentration, 10^{-9} mol L^{-1}) with a correlation coefficient of 0.9989 during c = 5.0×10^{-9}-5.0×10^{-8} mol L^{-1} and ΔI = 1.97+5.69C (C being the bile acid concentration, 10^{-8} mol L^{-1}) with a correlation coefficient of 0.9994 during c = 5.0×10^{-8}-7.0×10^{-7} mol L^{-1}. The detection limit was 1.3×10^{-9} mol L^{-1} (3σ). The relative standard deviation (RSD) was 1.7% for the 5.0×10^{-8} mol L^{-1} bile acid solution considering to 11 replicate measurements. In comparison with most of the previously reported methods for the determination of bile acid, as shown in Table S1 (Supplementary information), the rapid and simple method presented in this paper shows a comparable linear range and a lower limit of detection.

Interference. To assess the selectivity of the proposed luminescence method for the analysis of bile acid in biological samples, the effect of the potential interferences, such as metal ions, amino acid and the other compounds on the determination of 3.0×10^{-8} mol L^{-1} bile acid was investigated. The experiments were carried out by comparing with the luminescence intensities obtained without and with the potentially interfering substances added. The results, listed in Table S2 (see Supplementary information), shown that these foreign substances had no significant effect on the luminescence intensity of the system for the determination of bile acid.

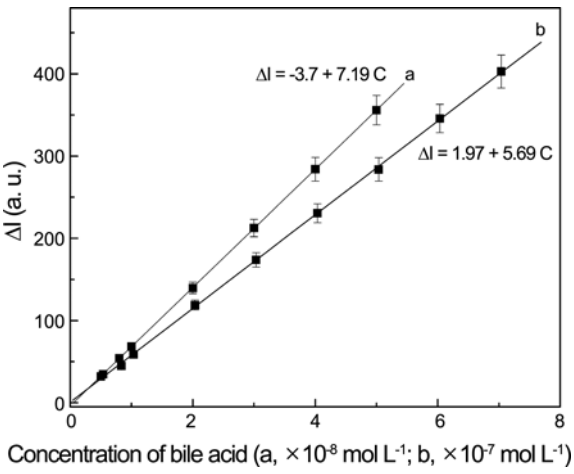

Figure 4. Calibration curves for bile acid obtained by peak height as function of bile acid concentrations.

Table 1. Determination of Bile Acid in Serum and Urine Samples (10^{-6} mol L^{-1}, n = 5)

Sample	Amount in sample ± SD	Added	Found	Recovery ± RSD[a] (%)
Serum1	2.87 ± 0.03	1.0	1.03	103 ± 1.1
Serum2	3.11 ± 0.07	2.0	1.97	98.5 ±1.7
Serum3	2.73 ± 0.09	3.0	3.13	104.3 ± 0.9
Urine1	17.11 ± 0.05	5.0	4.86	97.2 ± 1.3
Urine2	16.73 ± 0.06	10.0	10.24	102.4 ± 1.8
Urine3	18.81 ± 0.08	15.0	14.88	99.2 ± 1.2

[a]Relative standard deviation

Analytical Application. In health, bile acid is normally between 0.48 and 4.8 mmol L^{-1}.[24] The sensitivity and detection limit achieved by the proposed luminescence technique allows the determination of bile acid in biological fluids. Following the procedure detailed in the experimental section, the proposed method was applied to the determination of bile acid in human serum and urine samples. The validity of this method was assessed by applying the standard addition technique and the experimental results are shown in Table 1. Recoveries were found to be 96.7-102%, 97.2-102.4% for human serum and urine samples respectively. Therefore, the presented method can be easily performed and affords good precision and accuracy when applied to analysis bile acid in serum and urine samples.

Possible Reaction Mechanisms. The balofloxacin is an antibiotic of the fluoroquinolone family containing a-carbonyl carboxylic acid configuration. A literature survey revealed that the a-carbonyl carboxylic acid moiety is an ideal ligand for the europium(III) and can strongly combine with europium(III). A balofloxacin molecule is excited from the ground state to the singlet excited state after absorbing ultraviolet light energy, and then transitioned to its triplet state *via* intersystem crossing. Finally, energy is transferred onto one or several excited states of the europium(III) ion by non-radiative transition and a strong characteristic luminescence of the europium(III) ion is emitted.[29] The europium(III) ions possess luminescence characteristics of narrow emission bands, large Stokes shifts, and long luminescence life times. Hence, it avoids potential background luminescence emission interference from the biological matrix. The coordination number of europium(III) ion in its solution is generally 6-10.[30] Based on the experimental results of the molar ratio for balofloxacin to europium(III) ion mentioned above, it is found that the coordination of europium(III) ion is unsaturated. There are still a lot of positive charges and blank orbits in the balofloxacin-europium(III) complex. Bile acid has

hydroxyl group and carboxy group, which exists in an anionic state in water solution. It can bind with balofloxacin-europium(III) complex through coligand and electrostatic interaction. These interactions can direct the combined water molecules in the balofloxacin-europium(III) complex away from the europium(III) ion. Consequently, a very stable ternary complex in close proximity with a high degree of molecular conjugation and a rigid structure can be formed by the electrostatic interactions and coordination between the balofloxacin-europium(III) binary complex and bile acid. Because of the effect of packing and cooperation in the ternary complex, the energy transfer can occur more easily. The nonradiative energy loss through O-H vibration of water molecules in the original balofloxacin-europium(III) binary complex decreased greatly. Hence, the luminescence intensity of the europium(III) ion at 618 nm can be remarkably enhanced after the adding of bile acid and the enhanced luminescence intensity is proportional to the bile acid concentration.

When the SDBS was added into the balofloxacin-europium(III)-bile acid system, owing to the electrostatic attractions and the hydrophobic interactions, SDBS combines with the balofloxacin-europium(III)-bile acid complex to form a supermolecule complex of balofloxacin-europium(III)-bile acid-SDBS. As a result, the coordination microenvironment of balofloxacin and europium(III) was ameliorated to decrease the interaction between the water molecules the ternary complex. The microenvironment created by SDBS micelles can protect the excited species from the collisional quenching of light emission, increase the excited state lifetimes and decrease the rate of radiationless energy transfer processes.[31] Thus, the luminescence intensity of balofloxacin-europium(III)-bile acid-SDBS system at 618 nm can be enhanced strongly by the interactions of balofloxacin, bile acid and SDBS.

Conclusion

In this paper, a novel, sensitive and cost effective luminescence method for the estimation of bile acid based on the solution-based balofloxacin of europium(III) ion complex in the presence of SDBS has been established. In comparison to other published methods in the literature, the proposed method here offers good sensitivity, wide linear range and low detect limit. It has been used for the determination of bile acid in human serum and urine with satisfactory results. The possible luminescence mechanism of the system is also discussed.

Acknowledgments. This project was supported by the National Undergraduate Innovation Program (201210759008) for financial support.

References

1. Russell, D. W. *Annu. Rev. Biochem.* **2003**, *72*, 137.
2. Heubi, J. E.; Setchell, K. D. R.; Bove, K. E. *Semin. Liver Dis.* **2007**, *27*, 282.
3. Vlahcevic, Z. R.; PandaK, W. M.; Stravitz, R. T. *Gastroenterol. Clin. North Am.* **1999**, *28*, 1.
4. Perwaiz,S; Tuchweber, B.; Mignault, D.; Gilat, T.; Yousef, I. M. *J. Lipid Res.* **2001**, *42*, 114.
5. Yousef, I. M.; Tuchweber, B. *Can. J. Gastroenterol.* **1997**, *11*, 541.
6. Ochsenkühn, T.; Bayerdörffer, E.; Meining, A.; Schinkel, M.; Thiede, C.; Nüssler, V.; Sackmann, M.; Hatz, R.; Neubauer, A.; Paumgartner, G. *Cancer* **1999**, *85*, 1664.
7. De Kok, T. M. C. M.; Van Faassen, A.; Glinghammar, B.; Pachen, D. M. F. A.; Rafter, J. J.; Baeten, C. G. M. I.; Engels, L. G. J. B.; Kleinjans, J. C. S. *Dig. Dis. Sci.* **1999**, *44*, 2218.
8. Lee, B. L.; New, A. L.; Ong, C. N. *J. Chromatogr. B* **1997**, *704*, 35.
9. Roda, A.; Piazza, F.; Baraldini, M. *J. Chromatogr. B Biomed. Sci. Appl.* **1998**, *717*, 263.
10. Nobilis, M.; Pour, M.; Kunes, J.; Kopecky, J.; Kvetina, J.; Svoboda, Z.; Sladkova, K.; Vortel, J. *J. Pharm. Biomed. Anal.* **2001**, *24*, 937.
11. You, J. M.; Shi, Y. W.; Ming, Y. F.; Yu, Z. Y.; Yi, Y. J.; Liu, J. Y. *Chromatographia* **2004**, *60*, 527.
12. Kong, W. J.; Jin, C.; Liu, W.; Xiao, X. H.; Zhao, Y. L.; Li, Z. L.; Zhang, P.; Li, X. F. *Food Chemistry* **2010**, *120*, 1193.
13. Kakiyama, G.; Hosoda, A.; Iida, T.; Fujimoto, Y.; Goto, T.; Mano, N.; Goto, J.; Nambara, T. *J. Chromatogr. A* **2006**, *1125*, 112.
14. Bentayeb, K.; Batlle, R.; Sanchez, C.; Nerín, C.; Domeño, C. *J. Chromatogr. B* **2008**, *869*, 1.
15. Xiang, X. Q.; Han, Y.; Neuvonen, M.; Laitila, J.; Neuvonen, P. J.; Niemi, M. *J. Chromatogr. B* **2010**, *878*, 51.
16. Sergi, M.; Montesano, C.; Napoletano, S.; Pizzoni, D.; Manetti, C.; Colistro. F.; Curini, R.; Compagnone, D. *Chromatographia* **2012**, *75*, 479.
17. Batta, A. K.; Salen, G.; Batta, P.; Tint, G. S.; Alberts, D. S.; Earnest, D. L. *J.Chromatogr. B* **2002**, *775*, 153.
18. Fausa, O.; Shalhegg, B. A. *Scand J. Gastroenterol* **1974**, *9*, 249.
19. Gowda, G. A. N.; Ijare, O. B.; Somashekar, B. S.; Sharma, A.; Kapoor, V. K.; Khetrapal, C. L. *Lipids* **2006**, *41*, 591.
20. Li, Y. S.; Liu, W. P.; Gao, X. F.; Chen, D. D.; Li, W. G. *Biosens. Bioelectron.* **2008**, *24*, 538.
21. Bartling, B.; Li, L.; Liu, C. C. *Analyst* **2009**, *134*, 973.
22. Gültekin, A.; Ersöz, A.; Denizli, A.; Say, R. *Sensor. Actuat. B-Chem.* **2012**, *162*, 153.
23. Psarellis, I. M.; Deftereos, N. T.; Sarantonis, E. G.; Calokerinos, A. C. *Anal. Chim. Acta* **1994**, *294*, 27.
24. Wang, T.; Wang, X. L.; Jiang, C. Q. *J. Clin. Lab. Anal.* **2007**, *21*, 207.
25. Collins, B. J.; Watt, P. C. H.; O'Reilly, T.; McFarland, R. J.; Love, A. H. *J. Clin. Pathol.* **1984**, *37*, 313.
26. Schoelmerich, J.; Roda, A.; Deluca, M. *Method. Enzymol.* **1986**, *133*, 215.
27. Yuan, J. L.; Wang, G. L.; Majima, K.; Matsumoto, K. *Anal. Chem.* **2001**, *73*, 1869.
28. Zhao, F.; Qi, Y.; Xiong, W. *Bull. Korean Chem. Soc.* **2012**, *33*, 204.
29. Bunzli, C.; Piguet, C. *Chem. Soc. Rev.* **2005**, *34*, 1048.
30. Kamruzzaman, M.; Alam, Al-M.; Lee, S. H.; Kim, Y. H.; Kim, S. H.; Kim, G. M. *Bull. Korean Chem. Soc.* **2012**, *33*, 105.
31. Wang, L.; Guo, C. C.; Chu, Z. J.; Jiang, W. *J. Lumin.* **2009**, *129*, 90.