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Development of a simple, rapid and high-throughput fluorescence polarization immunoassay for glycocholic acid in human urine

Qiyi He[a,1], Xiping Cui[a,1], Ding Shen[a], Yingshan Chen[a], Zhengyun Jiang[a], Rui Lv[a], Sergei A. Eremin[b,c], Suqing Zhao[a,*]

[a] Department of Pharmaceutical Engineering, Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou, 510006, People's Republic of China
[b] Faculty of Chemistry, M. V. Lomonosov Moscow State University, 119991, Moscow, Russia
[c] National Research Technical University MISiS, 119049, Moscow, Russia

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ABSTRACT

In this paper, a simple, rapid and high-throughput fluorescence polarization immunoassay (FPIA) based on polyclonal antibodies (PAb) is described for the determination of glycocholic acid (GCA) in human urine. Three fluorescein-labeled GCA (tracers) with different structures and spacer bridges were synthesized and purified by thin-layer chromatography (TLC). The structure effect of tracers on the assay was investigated and the sensitivity of best tracer in the optimized FPIA demonstrated an IC_{50} value of 306 ng/mL. The working range of FPIA was 36 ~ 2 600 ng/mL and the limit of detection (LOD) was 9 ng/mL. The developed FPIA was time-saving that could be completed within 10 min. Human urine samples spiked with GCA were analyzed by this method, followed by confirmation with commercial enzyme immunoassay analysis (EIA). Excellent recoveries and correlation between these two methods were observed (R^2 = 0.996), suggesting the developed FPIA could be applied to screening of GCA in human urine samples without complicated cleanup.

1. Introduction

Bile acids are produced only in the liver as the end products of cholesterol catabolism in humans and, after conjugation with glycine or taurine, are transported across the canalicular membrane of the hepatocytes via bile into the intestine, where they are reabsorbed into portal circulation and returned to the liver [1–3]. Almost all the metabolic reactions in the formation and conjugation of bile acids take place within the hepatic cells. In the absence of hepatobiliary disease, the vast majority of the bile acids are cleared from the portal blood by hepatic uptake, while the remainder spills into the peripheral circulation [4,5].Thus, the serum bile acid concentration is a sensitive indicator of liver function [6]. The greatly increased bile acid concentrations are seen in sera of patients with hepatobiliary disease [7,8]. Many reports have shown that glycocholic acid (GCA), one kind of conjugation bile acid, is a newly identified specific and sensitive biomarker for hepatocellular carcinoma (HCC) [8,9]. The concentration of GCA in the blood of healthy people is below 3 μg/mL (http://www.hmdb.ca/). In addition, the urine has been shown to contain a wealth of metabolic information that may be altered due to diseases, and do satisfy the criteria of minimal invasiveness, reasonable cost, or minimal time demand [10]. Therefore, routine urinalysis would be of significant advantage and useful for primary diagnosis, surveillance and early detection of HCC.

Currently, some approaches have been established for determination of GCA in serum and urine, such as high-performance liquid chromatography (HPLC); the limit of detection (LOD) reported as 5.6 μg/mL [11], ultra performance liquid chromatography-mass spectrometry (UPLC-MS); LOD 0.5 ng/mL [12], liquid chromatography-electrospray tandem mass spectrometry (LC–MS-MS); LOD 10 ng/mL [13], matrix-assisted laser desorption/ionization time-of-flight-mass spectrometry (MALDI-TOF-MS); LOD 4.2 μg/mL [14]. Despite the advantage of being highly sensitive and selective, instrumental methods require extensive sample preparation and cleanup procedures that become laborious and time-consuming, advanced infrastructure

* Corresponding author.
E-mail addresses: chesto36@163.com (Q. He), cuixiping1989@163.com (X. Cui), shending92@163.com (D. Shen), Chenysgdut@163.com (Y. Chen), jkk928@163.com (Z. Jiang), dro1125439@163.com (R. Lv), eremin_sergei@hotmail.com (S.A. Eremin), sqzhao@gdut.edu.cn (S. Zhao).
[1] Qiyi He and Xiping Cui contributed equally to this work and should be considered co-first authors.

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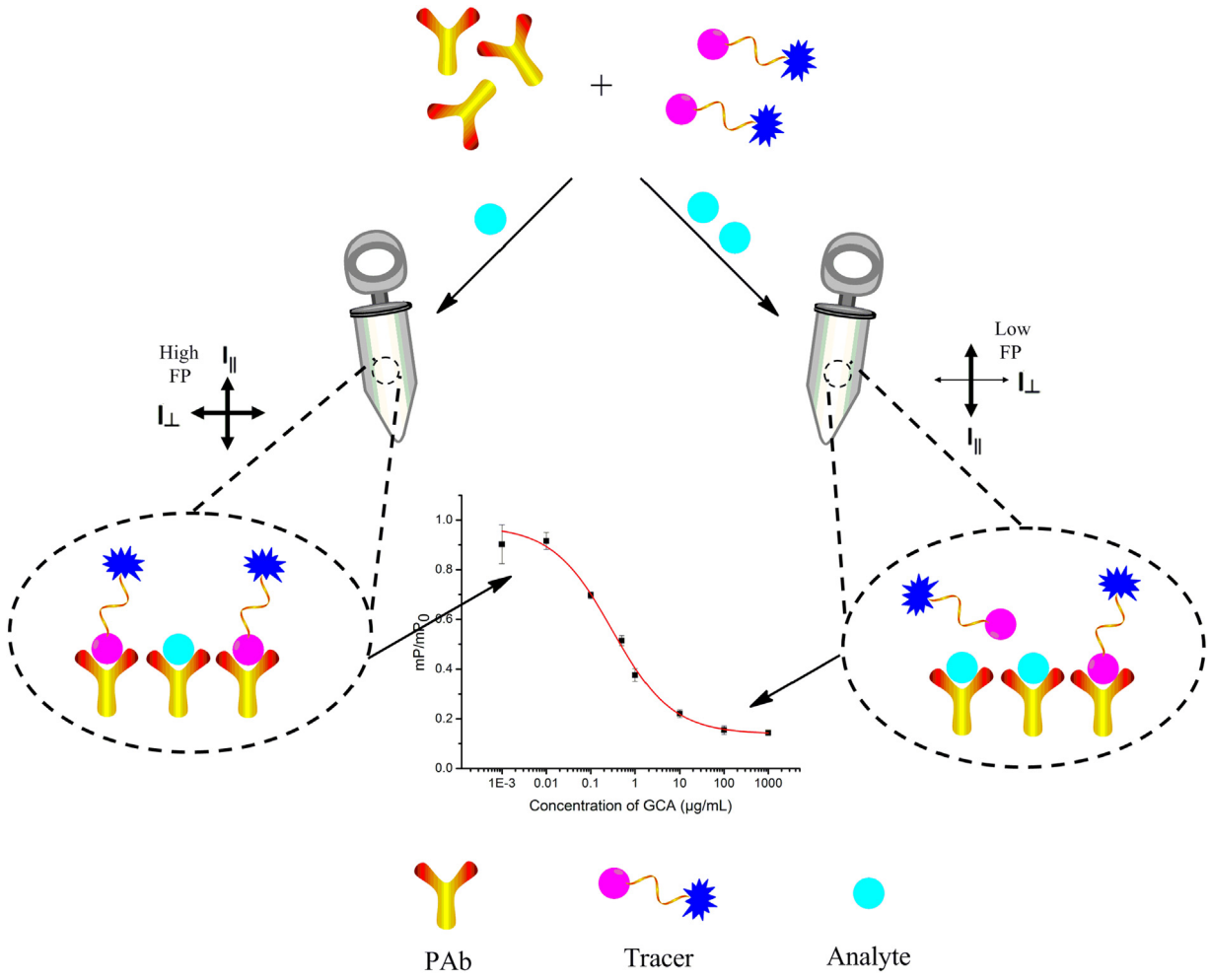

Fig. 1. The basic principle of FPIA for small molecule.

to support complex instrumentation, and specific expertise and well-trained operator. On the other hand, immunoassay methods have been proven to be the choice for easy, relatively inexpensive, and high-throughput screening of environmental contaminants [15,16], food additives [17,18], and biological metabolites [19,20]. In the previous literature, polyclonal antibodies (PAb) based radioimmunoassays have been reported to monitor GCA, with a half-maximum signal inhibition concentration (IC_{50}) of 2.2 μg/mL [21]. Despite its high sensitivity, this radioimmunoassay displays several disadvantages, including the exposition risk of radiation hazards and reagents, the necessity for specially trained staff, and management of radioactive wastes [22]. As an alternative, enzyme-linked immunosorbent assay (ELISA) provides simple, sensitive, specific and inexpensive tool for analysis of various targeted analytes. However, ELISA is a heterogeneous method, which involves multiple washing steps, and long reaction time (1–2 h). Fluorescence polarization immunoassay (FPIA) is a homogeneous technique (no separation or washing steps), which allows the determination of analytes within a short period of time. FPIA is a competitive immunoassay method based on the increase in fluorescence polarization (FP) of the fluorescent-labeled hapten (tracer) when bound by the specific antibody [23–25]. If the concentration of the target analyte is high in the sample, it will compete with the tracer for binding with the antibody. So there will be more unbound tracer in the solution. The light emitted by the unbound tracer, which rotates quickly in solution, is highly depolarized and, therefore, the FP is low. On the contrary, if the concentration of the target analyte is low, more tracers will combine with the antibody. The light emitted by the tracer-antibody complex, which rotates slower in solution, remains polarized and, the FP is high. The basic principle of FPIA is depicted in Fig. 1.

The main objective of the present research was to investigate the use of FPIA for high-throughput detection of GCA in human urine samples based on PAb. Three fluorescein-labeled GCA (tracers) with different chemical structures and spacer bridges were synthesized. The effects of tracer structure, tracer concentration and antibody dilution on FPIA performance were investigated to achieve the best sensitivity and reproducibility of the method. Under the optimal conditions, the developed FPIA was successfully applied to detect GCA in human urine samples. Furthermore, the results obtained by FPIA were validated by commercial enzyme immunoassay (EIA). Therefore, the developed FPIA is a promising method to rapidly and high-throughput detect GCA in urine samples.

2. Materials and methods

2.1. Materials and reagents

All reagents were of analytical grade unless otherwise specified. Glycocholic acid (GCA) was obtained from TRC (Toronto, Canada). Cholic acid (CA), ursodeoxycholic acid (UCA), deoxycholic acid (DCA), glycylursodeoxycholic acid (GLDCA), lithocholic acid (LCA), chenodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA) were purchased