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Review Article

Simultaneous determination of three potential cancer biomarkers in rat urine by synchronous fluorescence spectroscopy

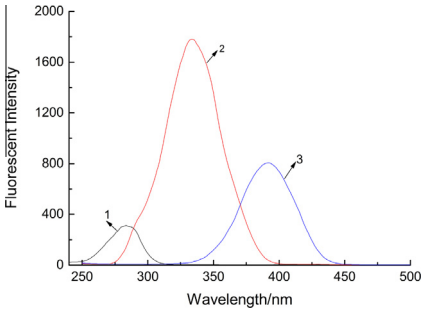
Lingling Huang [a,b], Lan Guo [b,*], Yiqun Wan [a,b,*], Pingping Pan [b], Lei Feng [a]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, Jiangxi 330047, People's Republic of China
[b] Center of Analysis and Testing, Nanchang University, Nanchang, Jiangxi 330047, People's Republic of China

HIGHLIGHTS

- Fluorescence spectrometry was established to determinate TRP, ISO and XAN.
- The pretreatment of sample is free from the tedious separation procedures.
- Investigating the relationship between tumor development and metabolites.

GRAPHICAL ABSTRACT



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ABSTRACT

A rapid, simple, sensitive and accurate method for the simultaneous determination of three potential cancer biomarkers [tryptophan (TRP), isoxanthopterin (ISO) and xanthopterin (XAN)] in rat urine with synchronous fluorescence spectroscopy has been developed. In order to eliminate the interference in urine samples, the synchronous fluorescence spectra were obtained with $\Delta\lambda$ = 70 nm in a KH_2PO_4–NaOH buffer solution (pH = 8.0). The detected wavelengths of quantitative analysis were set at 275 nm for TRP, 325 nm for ISO and 400 nm for XAN, respectively. Under the optimized conditions, the limits of the detection of the three compounds were 2.73 ng/mL, 0.52 ng/mL and 0.94 ng/mL, respectively. The recoveries were in the range of 80.5–98.0%, with the coefficient of variation between 0.62% and 2.48%. The proposed method has been applied to the simultaneous determination of TRP, ISO and XAN in rat urines of bladder cancer group and control group. The determination results showed that the average level of TRP, ISO and XAN had different change trends with the growth of the tumor. The three analytes could be used as potential biomarkers for noninvasive diagnosis of different stage of bladder cancer. However, more data are needed to support this hypothesis.

Contents

* Corresponding authors. Tel.: +86 791 88306047 (L. Guo). Address: State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, Jiangxi 330047, People's Republic of China. Tel./fax: +86 791 88321370 (Y. Wan).
E-mail addresses: guolan@ncu.edu.cn (L. Guo), yqwanoy@sina.com (Y. Wan).

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Introduction

Tryptophan is an essential amino acid in vivo, which is not only very important for protein biosynthesis but also serves as precursor of neurotransmitter serotonin [1]. The catabolism of tryptophan will enhance when inflammation reaction and immune system are activated [2–4]. The immune activation markers interferon-γ triggers expression of the enzyme indoleamine (2, 3)-dioxygenase (IDO) [5], which converts the essential amino acid tryptophan to kynurenine [6,7]. Pterins are derivatives of pteridines, which constitute a large and structurally varied group of natural compounds involved in the biosynthetic pathways of cofactors and vitamins. Xanthopterin, isoxanthopterin, neopterin, biopterin, pterin, pterin-6-carboxylic acid, and 6,7-dimethylpterin are the main types of pterin compounds. These compounds are very important cofactors in the process of cell metabolism, what is crucial for the diagnostic function is the fact that normal somatic cells metabolize pterins differently from cancer cells. When cellular immune system is activated by certain diseases such as cancer [8–10], viral infections [11,12], and organ transplantation reaction [13,14], the amounts of pterins have been found to elevate significantly. Since different pterins may play various roles in different tumor-related disease, each type of tumor may show its special pattern in terms of pterins concentrations [15]. Pterins and amino acids, may be associated with the presence of tumor-related diseases.

Therefore developing a simple, rapid and sensitive assay for simultaneously determining these compounds such as tryptophan, isoxanthopterin and xanthopterin in biological fluids, especially in urine, becomes necessary.

Monitoring the concentrations of biomarkers in urine or serum is the easiest way to observe the clinical significance of a cancer patient's states at regular intervals, and still be capable of predicting tumor formation and relapse. However, Analysising of the subjects in urine or serum is difficult, because of their very trace amounts in biological fluids, their physicochemical characteristics and the complexity of the sample matrix. Presently, the determination of these pterins and amino acids mainly focused on high-performance liquid chromatography [16–19], capillary electrophoresis [20–22], radiommunoassay [23]. However, most of these methods need tedious pretreatment and separation procedure, which are time-consuming and not cost-effective.

Compared with the conventional fluorescence spectra, the synchronous fluorescence technique offers several advantages: spectral simplification, spectral bandwidth reduction and avoiding different perturbing effects [24]. Due to its sharp, narrow spectrum, synchronous fluorescence spectroscopy (SFS) serves as a very simple, effective method of obtaining data without pre-separation for quantitative determination of multi-component systems [25], such as milk [26–28], tea [29], water samples [30] and petroleum products [31,32].

In this paper, a method was developed and optimized for the simultaneous quantification of the TRP, ISO and XAN in rat urine by synchronous fluorescence spectroscopy. Under the optimized conditions, the interfering factors in urine were eliminated effectively, the treated procedure was simple and the detection was direct. Due to the cancer samples were not very easy to get, especially the early stage cancer samples, we established a rat model of bladder cancer and estimated the development stage of cancer with pathologic histology method, then the SFS method was applied to the quantitative evaluation of TRP, ISO and XAN in bladder cancer group and the control group rat urines. Analyzing the relationship between the concentration of the three compounds and the growth of tumor in a rat model of bladder cancer was first reported in this paper, the results exhibited that the average level of TRP, ISO and XAN had different change trends with the growth of tumor.

Experimental

Apparatus

All fluorescence measurements were carried out on a F-4500 spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp source and a 1.0 cm quartz cell, the scan speed was 1200 nm min^{-1}. All pH measurements were detected with a pHS-3 digital pH-meter (Shanghai Rex Device Works, Shanghai, China) with a combined glass calomel electrode.

Reagents

The standards including TRP, ISO and XAN were purchased from Sigma Corp. (St. Louis, MO, USA), and the purities of these standards were all over 97%, all the other chemicals and solvents were of analytical grade. Ultrapure water was prepared by a Milli-Q system (Millipore, Bedford, MA, USA).

To prepare standard stock solutions, approximately 5 mg for each of the three standards was weighed into 25 mL amber volumetric flasks respectively, dissolved with ultrapure water and certain amount of 0.1 mol/L sodium hydroxide, then diluted to the