J Fluoresc (2010) 20:1191–1198
DOI 10.1007/s10895-010-0667-4

ORIGINAL PAPER

Simultaneous Determination of Xanthopterin and Isoxanthopterin in Human Urine by Synchronous Fluorescence Spectroscopy

Yi-Qun Wan · Li-Juan Tang · Ting Tan

Received: 27 November 2009 / Accepted: 12 April 2010 / Published online: 24 April 2010

Abstract A simple, rapid, sensitive and selective method for simultaneously determining xanthopterin and isoxanthopterin content in human urine has been developed using synchronous fluorescence spectroscopy based on their intrinsic fluorescence. The synchronous fluorescence spectra were obtained with $\Delta\lambda$=65 nm in a pH 8.5 KH_2PO_4-NaOH buffer solution. The detected wavelengths of quantitative analysis were set at 410 nm for xanthopterin and 325 nm for isoxanthopterin, respectively. Pretreatment of urine samples only was filtrated through a 0.45 μm membrane filter, which was free from the tedious separation procedures. Under optimized conditions, the limits of detection (LOD) were 0.94 ng/mL for xanthopterin and 0.48 ng/mL for isoxanthopterin. The recoveries ranged from 88.0% to 103.8 % for healthy and cancer urine samples, with coefficient of variation between 2.09% and 7.06%. The proposed method has been successfully applied to the simultaneous analysis for xanthopterin and isoxanthopterin in human urine. The results showed that the average level of isoxanthopterin was significantly elevated in urine excreted by stomach cancer patients (P<0.01), while no significant change of xanthopterin level was found between stomach cancer patients and healthy individuals. This potentially indicates that an increase in amounts of isoxanthopterin can be associated with the presence of stomach cancer.

Keywords Xanthopterin · Isoxanthopterin · Human urine · Synchronous fluorescence spectrometry

Y.-Q. Wan (✉)
State key Laboratory of Food Science and Technology,
Nanchang University,
Nanchang, Jiangxi 330047, People's Republic of China
e-mail: yqwanoy@sina.com

Y.-Q. Wan · L.-J. Tang · T. Tan
Center of Analysis and Testing, Nanchang University,
Nanchang, Jiangxi 330047, People's Republic of China

Introduction

Pteridine and its derivatives, which exist in a large number of biological body fluids [1], are a family of organic compounds that play important roles in biochemistry. Xanthopterin, isoxanthopterin, neopterin, biopterin, pterin and pterin-6-carboxylic acid, all belonging to the pteridine family, are the six main types of pterin compounds. These compounds are very important co-factors in the process of cell metabolism, thus their levels are importance parameters in clinical diagnosis. These amounts change when monocyte or macrophages are activated under interferon-γ stimulus by certain diseases such as cancer [2–4], viral infections [5, 6], and renal diseases [7]. Neopterin, whose concentration in serum and urine has been reported to rise in patients suffering from viral infections [8], renal transplant rejections [9] and malignancy [10, 11], has already been considered as a marker for cell-mediated immunity [12]. Furthermore, the investigation has revealed that since different pterins may play various roles in different tumor-related disease [13], each type of tumor shows its own pattern in the terms of changes in the pterins concentrations. Pterins, besides neopterin henceforth, may be associated with the presence of malignant diseases. Therefore, developing a simple, rapid and sensitive assay for simultaneously determining these compounds such as xanthopterin and isoxanthopterin in biological fluids, especially in human urine, becomes very necessary.

Due to the presence of pterin compounds in very trace amounts in biological fluids, their photosensitivity [14], and their similar chemical structures, the quantitative measurement of pterins is difficult. Presently, the determination of pterins mainly focused on high-performance liquid chromatography [15–19], capillary electrophoresis [20], radioimmunoassay [21], enzyme-linked immunosorbent assay [22]. However, most of these methods demand tedious pretreatment and separation procedure, which can be time-consuming and not

cost-effective. Synchronous fluorescence spectroscopy (SFS) is thus an alternative due to its high selectivity and sensitivity as well as its relative low cost. SFS technique has already been successfully used for the simultaneous determination of multi-component systems [23–29], polycyclic aromatic hydrocarbons [26, 27] and analyzing petroleum products in solution [28, 29]. A report on the application of synchronous fluorescence spectroscopy to simultaneous analysis of pterins has been proposed [30], but this method was not used for analysis in real urine samples.

In this work, a simple and quick procedure for the simultaneous determination of xanthopterin and isoxanthopterin in human urine by synchronous fluorescence spectroscopy has been developed. Experimental conditions affecting the fluorescence intensity of the two compounds, such as $\Delta\lambda$, pH value and the dosage of buffer solution, were systematically studied. Under optimized conditions, the interfering factors in urine were eliminated effectively, treated simply, and detected directly. The proposed method has been successfully applied to quantitative evaluation of xanthopterin and isoxanthopterin in human urine with satisfactory results.

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, and the scan speed was 240 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.

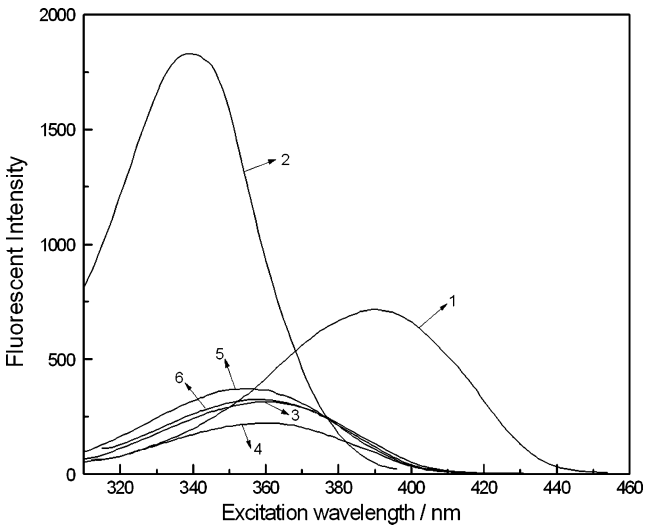

Fig. 1 The exciation spectra of pterins in pH 8.5 KH_2PO_4-NaOH buffer solution. **1**. xanthopterin (0.110 μg/mL); **2**. isoxanthopterin(0.110 μg/mL); **3**. neopterin (0.110 μg/mL); **4**. biopterin (0.110 μg/mL); **5**. pterin (0.110 μg/mL); **6**. pterin-6-carboxylic acid (0.110 μg/mL)

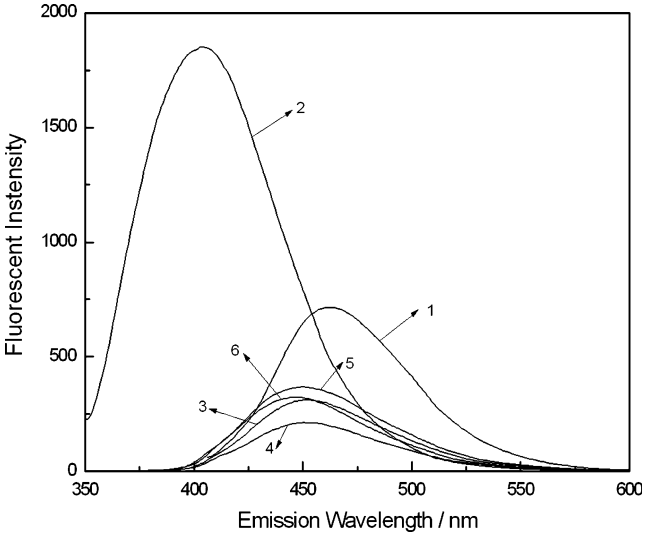

Fig. 2 The emission spectra of pterins in pH 8.5KH_2PO_4-NaOH buffer solution. **1**. xanthopterin (0.110 μg/mL); **2**. isoxanthopterin(0.110 μg/mL); **3**. neopterin (0.110 μg/mL); **4**. biopterin (0.110 μg/mL); **5**. pterin (0.110 μg/mL); **6**. pterin-6-carboxylic acid (0.110 μg/mL)

Reagents

The pteridine standards which include xanthopterin (XAN), isoxanthopterin (ISO), neopterin, biopterin, pterin and pterin-6-carboxylic acid were purchased from Sigma Corp. (St. Louis, MO, USA), and the purities of these standards were all over 97.0%. All the other chemicals or solvents

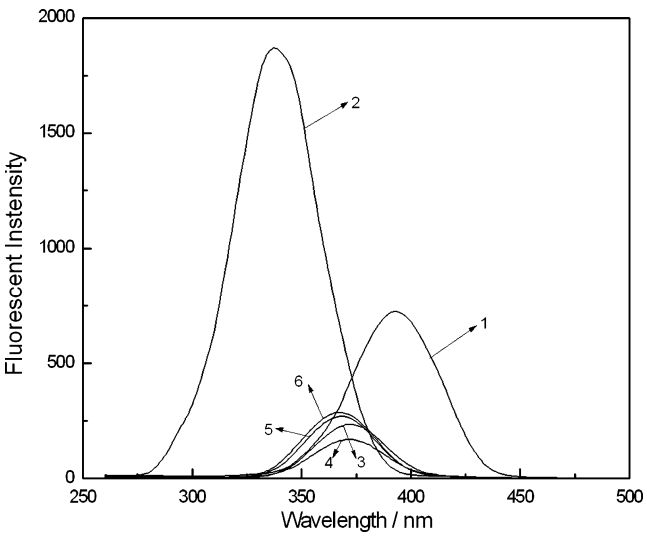

Fig. 3 The synchronous fluorescence spectra of of pterins in pH 8.5 KH_2PO_4-NaOH buffer solution. **1**. xanthopterin (0.110 μg/mL); **2**. isoxanthopterin(0.110 μg/mL); **3**. neopterin (0.110 μg/mL); **4**. biopterin (0.110 μg/mL); **5**. pterin (0.110 μg/mL); **6**. pterin-6-carboxylic acid (0.110 μg/mL)

were of analytical grade. Ultrapure water was prepared by a Milli-Q Water system (Millipore, Bedford, MA, USA). To prepare standard stock solutions, about 2.50 mg for each of the six different pterins 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 mark using ultrapure water. The standard solutions were kept at 0–4°C, and the concentration of each of the stock solutions was calculated to be 0.100 mg/mL. The KH_2PO_4-NaOH buffer solution was prepared by adding different amount 0.200 mol/L potassium dihydrogen phosphate and 1.00 mol/L sodium hydroxide, and the pH value of the buffer was adjusted from 7.0 to 9.0.

Preparation of samples

The urine samples of healthy individuals were collected from student volunteers from Nanchang University (Jiangxi, China). Stomach cancer patient's samples were obtained from local hospitals in Nanchang, P.R. China. The urine samples from healthy subjects and stomach cancer patients were centrifuged at 4000 rpm for 15 min immediately after collection and filtered through a 0.45 μm filter. Treated samples were kept at 0–4°C in the dark until analysis.

Procedure

A suitable amount of each pterin compound, together with 2.0 mL of KH_2PO_4-NaOH buffer solution (pH=8.5), was transferred into a 10-mL amber volumetric flask, diluted to 10 mL with appropriate volumes of ultrapure water. The solution was mixed thoroughly, a portion of each solution was then transferred into a 10-mm quartz cell. The excitation and emission slits were both set at 5 nm. The conventional fluorescent spectra were scanned at excitation wavelength 310–460 nm along with emission wavelength 350–600 nm for six different pterin compounds. Meanwhile, the SFS for samples were measured and recorded at excitation wave-

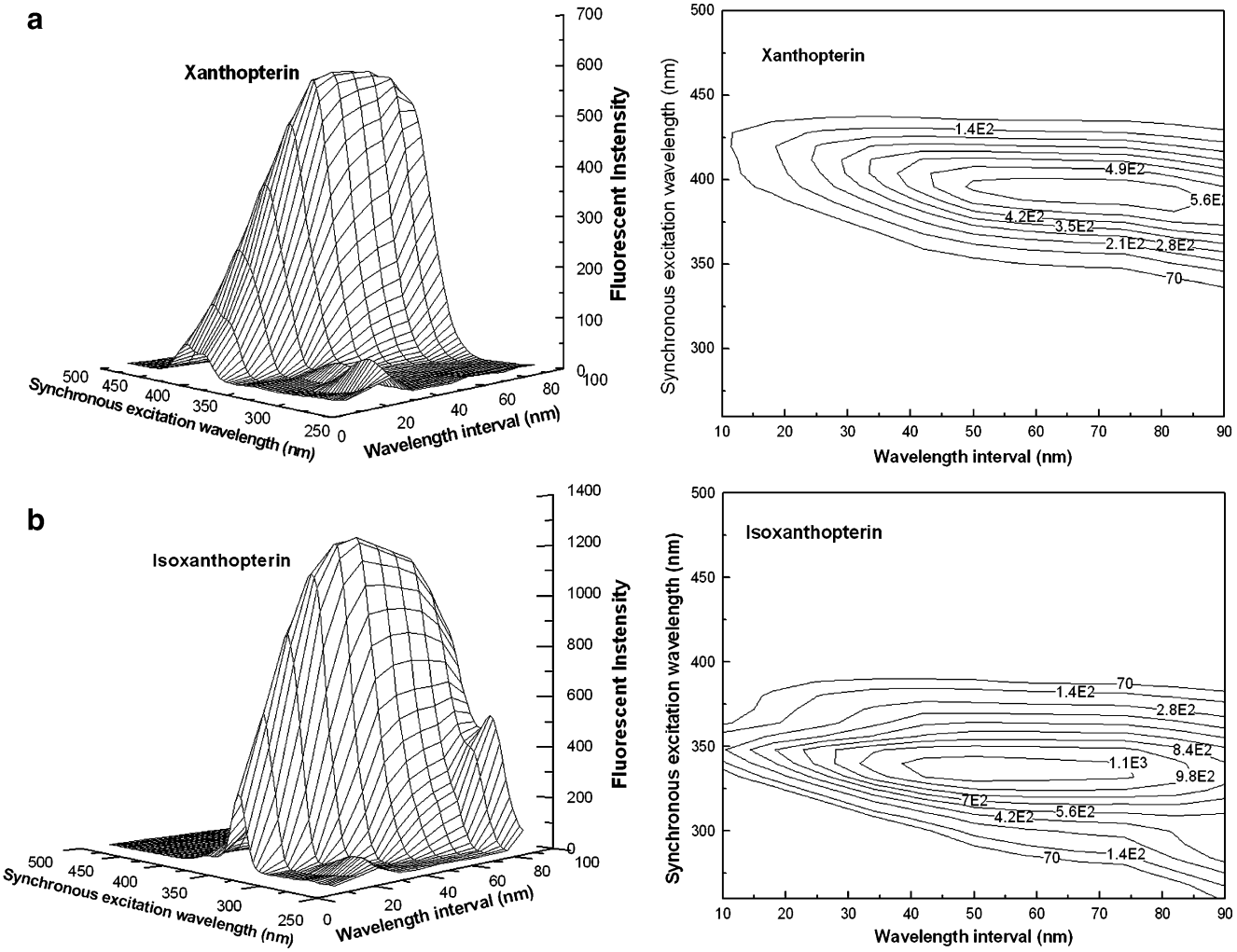

Fig. 4 Three dimensional and two dimensional (contour plots) total synchronous fluorescence spectrain pH 8.5KH_2PO_4-NaOH buffer solution. **1**. xanthopterin (0.110 μg/mL); **2**. isoxanthopterin(0.110 μg/mL)

lengths range of 250–500 nm, with $\Delta\lambda$=65 nm, and the maximum analytical information can be obtained in this region. The data of XAN and ISO were collected at the selected wavelength of 410 and 325 nm, respectively.

Result and discussion

Fluorescence characteristics of pterins

The excitation and emission spectra of each pterin compound in pH 8.5 KH_2PO_4-NaOH buffer solution were presented in Figs. 1 and 2, respectively. The maximum excitation and emission wavelengths were $\lambda_{ex/em}$=338/400 nm for isoxanthopterin, $\lambda_{ex/em}$=390/460 nm for xanthopterin, $\lambda_{ex/em}$=360/450 nm for neopterin, $\lambda_{ex/em}$=360/450 nm for biopterin, $\lambda_{ex/em}$=355/445 nm for pterin and $\lambda_{ex/em}$=358/445 nm for pterin-6-carboxylic acid. It was clear that the spectra of isoxanthopterin and xanthopterin were highly overlapping with neopterin, biopterin, pterin and pterin-6-carboxylic acid. Therefore the simultaneous analysis of XAN and ISO in the pterins mixtures only by conventional spectrofluorimetry is not feasible.

Synchronous fluorescence spectra of pterins

The SFS method, which consists essentially of the simultaneous scanning of both monochromators, maintains a constant wavelength interval ($\Delta\lambda$) between excitation wavelength and emission wavelength [31, 32]. The simplification of the spectral profile, the reduction of band width and contraction of spectral range are the main characteristics of SFS [33, 34]. The synchronous technique allows the stronger peaks to be increased selectively by using a suitable $\Delta\lambda$. Consequently, compared to conventional fluorescence, SFS can provide higher selectivity for identification and estimation which is good for the simultaneous determination of multi-component systems without previous physical separation [23–25].

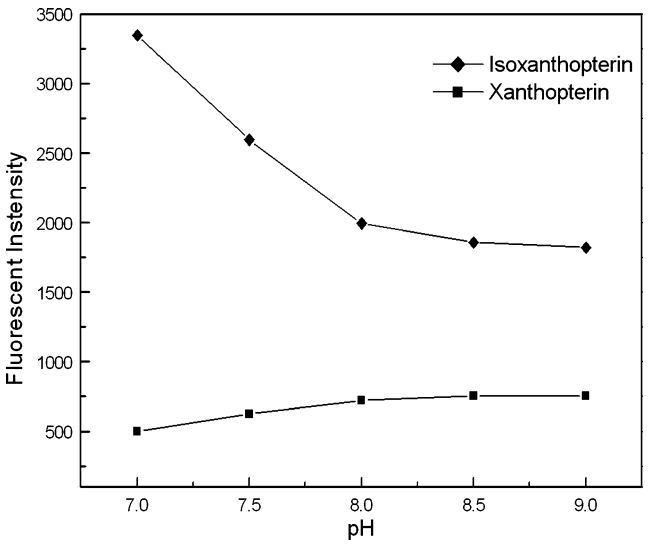

Fig. 5 The effect of pH. **1**. xanthopterin (0.110 μg/mL); **2**. isoxanthopterin(0.110 μg/mL)

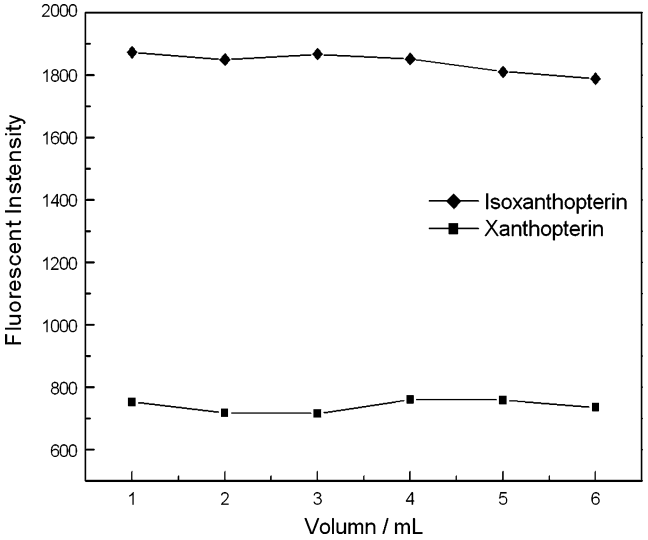

Fig. 6 The dosage of KH_2PO_4-NaOH buffer solution (pH=8.5). **1**. xanthopterin (0.110 μg/mL); **2**. isoxanthopterin(0.110 μg/mL)

The synchronous spectra for the six analytes were obtained with a constant interval 65 nm ($\Delta\lambda_{em}$–$\Delta\lambda_{ex}$)

Table 1 Maximum concentration of main interferents

Interferences	Concentration In ISO (μg/mL)	Concentration In XAN (μg/mL)
Mg^{2+}	61.98	17.4
Ca^{2+}	43.9	27.5
Cu^{2+}	16.5	0.390
Fe^{3+}	3.29	1.10
Mn^{2+}	0.740	0.740
Zn^{2+}	0.320	0.170
L-Leucine	19.7	164
L-Phenylalanine	49.5	57.8
L-Tryptophan	6.12	122
L-Tyrosine	7.59	19.0
Serine	10.7	37.4
Urea	169	169
Uric acid	200	199
Creatinine	1.68	1.14
creatine	12.6	1.51
Glucose	36.7	57.6
Bilirubin	0.580	1.56
Uroporphyrin	1.51	20.1
Coproporphyrin	9.74	19.5

Table 2 Performance data of the SFS method for the determination of ISO and XAN

Parameter	ISO	XAN
Concentration range (μg/mL)	0.010–0.610	0.010–0.640
Standard deviation of the intercept of the regression line, Sa	1.90	1.42
Standard deviation of the slope of the regression line, Sb	0.96	0.58
Limit of detection (LOD) (ng/mL)	0.48	0.94

between the emission and excitation wavelengths and presented in Fig. 3. In the synchronous spectra, the wavelength maxima were located at 390 and 338 nm for XAN and ISO respectively, but the maxima for the other pterins were ranged from 330 to 400 nm. In SFS, the spectral bands of pterins were narrowed compared with conventional fluorescence. Thus, the fluorescent intensity was recorded at 410 nm for XAN and at 325 nm for ISO respectively for simultaneous quantitative determination, where the interference from the other pterins could be eliminated effectively.

Optimization of experiment conditions

Optimum Δλ

The Δλ value is one of the most important parameters for synchronous fluorescent spectra which directly influences spectral shape, band width, peak location and signal intensity [35]. With the purpose of selecting the optimum Δλ value in the synchronous spectra for the six analytes, a wide range of Δλ (10–90 nm) was examined by obtaining the total synchronous fluorescence spectra information of six pterins. The total synchronous fluorescence spectra of XAN and ISO were shown in Fig. 4.

When Δλ was less than 60 nm or more than 70 nm, the synchronous fluorescence intensity of ISO and XAN is weak and the poor separation of the six peaks was obtained. When Δλ was set from 60 nm to 70 nm, the spectral bands of ISO and XAN could be satisfactorily separated from the other four peaks, and the synchronous fluorescence intensity of ISO and XAN were relatively high. Therefore, the wavelength interval of 65 nm was selected as optimum Δλ for SFS measurement and quantitative determination, which was consistent with stokes shift of ISO (65 nm) and approached to stokes shift of XAN (70 nm).

Effect of pH

The influence of pH on fluorescent intensity was studied with the use of a set of KH_2PO_4-NaOH buffer solution (pH range 7.0–9.0) to standard working solutions of XAN and ISO and showed in Fig. 5. As is showed from Fig. 5, when pH value was less than 8.0, the intensity of ISO enhanced greatly with increasing acidity of buffer solution, whereas the intensity of XAN decreased slightly. The results showed the pH range for the determination of XAN and ISO was from 8.0 to 9.0, where the fluorescent intensity remained stable. Therefore, the pH 8.5 was selected as a suitable analytical medium in this work.

Effect of amount of buffer solution

In addition, the dosage of buffer solution was also studied. As can be observed in the Fig. 6, increasing buffer volume from 1.0 mL to 6.0 mL had a little effect on the fluorescent intensity of XAN and ISO. 2.0 mL KH_2PO_4-NaOH buffer solution was thus adopted in subsequent experiments.

Effect of interferents

One challenge for the determination of XAN and ISO is their trace concentrations in urine samples. Furthermore, urine excretions are extraordinary complicated because there are many fluorescent substances and non-fluorescent substances. It is necessary to investigate the effects of main interfering agents present in the urine sample matrix on the determination of ISO and XAN. Upon addition of interfering agents into to standard solutions of ISO (0.510 μg/mL) and XAN (0.100 μg/mL), the tolerable concentration of interfering species was given in Table 1, the change of fluorescent intensity resulted in less than ±5% relative error level. The results suggested that tolerable concentration of interfering species on the determi-

Table 3 Recovery results of healthy people's urine samples (n=5)

Samples	Background (μg/mL)	Added (μg/mL)	Found (μg/mL)					Average Recovery (%)	RSD[a] (%)
Xanthopterin	0.324	0.50	0.821	0.798	0.837	0.811	0.796	97.7	2.09
	0.324	2.00	2.28	2.35	2.50	2.31	2.40	102.2	3.65
Isoxanthopterin	0.501	0.50	0.989	0.992	1.11	1.04	0.968	103.8	5.58
	0.501	2.00	2.47	2.49	2.52	2.47	2.51	99.6	0.92

[a] RSD means relative standard deviation

Table 4 Recovery results of stomach cancer people's urine samples (n=5)

Samples	Background (μg/mL)	Added (μg/mL)	Found (μg/mL)					Average Recovery (%)	RSD[a] (%)
Xanthopterin	0.45	0.50	0.842	0.891	0.923	0.975	0.818	88.0	7.06
	0.45	2.00	2.31	2.46	2.52	2.49	2.39	99.2	3.47
Isoxanthopterin	1.18	0.50	1.61	1.55	1.76	1.72	1.59	93.2	5.44
	1.18	2.00	3.06	3.42	3.23	3.19	3.25	102.5	4.01

[a] RSD means relative standard deviation

nation of ISO and XAN was much higher than the content present in human urine. In other words, either non-fluorescent substances or fluorescent substances including other pterin compounds in urine samples did not interfere with the determination of ISO and XAN.

Table 5 Results of the determination of xanthopterin and isoxanthopterin in urine samples (mean±SD, n=5)

Sample No.[a]	Xanthopterin (μg/mL)[b]	Isoxanthopterin (μg/mL)[b]
1	0.160±0.001	1.300±0.005
2	0.450±0.001	1.180±0.002
3	0.290±0.001	2.730±0.014
4	1.420±0.006	2.030±0.011
5	0.357±0.012	1.120±0.034
6	0.490±0.052	1.010±0.001
7	0.907±0.031	0.968±0.013
8	0.672±0.021	1.310±0.014
9	0.403±0.031	1.280±0.005
10	0.291±0.028	0.897±0.005
11	0.572±0.011	0.911±0.001
12	0.337±0.021	0.941±0.005
13	0.364±0.018	0.748±0.003
14	0.349±0.085	0.744±0.016
15	0.197±0.011	0.338±0.028
16	0.324±0.009	0.501±0.008
17	0.388±0.007	0.877±0.011
18	0.524±0.018	0.716±0.017
19	0.409±0.029	0.818±0.073
20	0.462±0.047	0.902±0.012
21	0.404±0.049	0.831±0.065
22	0.231±0.033	0.751±0.043
23	0.295±0.010	0.599±0.051
24	0.202±0.007	0.618±0.092
25	0.379±0.007	0.864±0.007
26	0.444±0.007	0.727±0.098
27	0.476±0.09	0.932±0.014
28	0.242±0.005	0.554±0.073
29	0.272±0.001	0.882±0.028

[a] Samples 1–13 were stomach cancer patients' urine samples, samples 14–29 were healthy persons' urine samples

[b] Data was shown as mean±standard deviation (n=5)

Analytical application

Calibration curve and detection limits

Under optimized conditions, the calibration curves for ISO and XAN were constructed. The linear relationship between fluorescent intensity and the concentrations of analytes was obtained in the range of 0.010 μg/mL to 0.610 μg/mL for ISO and 0.010 μg/mL to 0.640 μg/mL for XAN. The fluorescent intensity was measured at 325 nm for ISO and at 410 nm for XAN, and the linear regression equations were $I_F = 1.30\times10^4 C_{ISO} + 32.46 (r^2 = 0.9996)$ for ISO and $I_F = 5.00\times10^3 C_{XAN} - 10.94$ (r^2=0.9995) for XAN. The LOD was calculated according to the following equation [36]: LOD= 3.3 Sa/m

Where Sa: the standard deviation of the intercept of the regression line. m: slope of the calibration curve. The analytical results are given in Table 2.

Recovery

With the purpose of testing the accuracy and precision for the proposed method, recovery experiments were performed in urine samples from healthy individuals as well as cancer patients. The recoveries of ISO and XAN were determined by using five replicates of each analytical standard calibration level. As shown in Tables 3 and 4, recoveries of ISO and XAN in healthy subjects were 99.6%–103.8% and 97.7%–102.2%, respectively; in stomach cancer subjects were 93.2%–102.5% and 88.0%–99.2%, respectively. The experimental results suggested that the method could be well applied to simultaneous analysis of ISO and XAN in human urine.

Table 6 Mean urinary excretion levels of pteridines in healthy individuals and in cancer patients

Analyte	Healthy individuals (μgmL^{-1})	Stomach cancer patients (μgmL^{-1})	Significance levels
Xanthopterin	0.332	0.516	Not significant
Isoxanthopterin	0.728	1.26	$P<0.01$

Sample analysis

As cited in the introduction, pterins are excreted in urine by normal healthy humans and cancer patients as a mixture of compounds. We have proposed a simple analytical method to determine XAN and ISO in human urines, with the aim of establishing a SFS method without complicated urine treatment.

Twenty-nine urine samples of healthy individuals and cancer patients were analyzed with results shown in Table 5. As is shown, isoxanthopterin content was in range of 0.338 μg/mL to 0.932 μg/mL for healthy controls while 0.748 μg/mL to 2.730 μg/mL for cancer subjects; xanthopterin content was in range of 0.202 μg/mL to 0.524 μg/mL for healthy controls while from 0.160 μg/mL to 1.42 μg/mL for cancer subjects. Moreover, the average levels of xanthopterin and isoxanthoptein in urine samples of healthy individuals and cancer subjects were studied via T tests, with results shown in Table 6. The theory and methodology for T test are described in detail elsewhere [37]. It was determined that the average level of isoxanthopterin was significantly elevated ($P<0.01$) in urine excreted by stomach cancer patients, while there was no significant change of xanthopterin level between cancer patients and healthy individuals.

Conclusion

In the paper, a simple and fast method based on synchronous fluorescence was proposed for the determination of isoxanthopterin and xanthopterin in urine samples. The interfering agents in urine matrix could be eliminated effectively by filtration through a 0.45 μm membrane filter which was simple and cost-effective. The assay has been successfully applied to the simultaneous determination of these agents in urine samples from healthy individuals and stomach cancer patients. The experimental results exhibited that isoxanthopterin content was in range of 0.338 μg/mL to 0.932 μg/mL for healthy controls while 0.748 μg/mL to 2.730 μg/mL for stomach cancer subjects. The T test results displayed that the average level of isoxanthopterin was significantly elevated in urine samples by stomach cancer patients, and it is probably to be a potential maker for stomach cancers.

Acknowledgements We gratefully acknowledge the financial support provided by Natural Science Foundation of P.R. China (No: 20765002) and the Jiangxi Province National Science Foundation (No: 0620049).

References

1. Grüngreiff K, Reinhold D, Ansorge S (1999) Serum concentrations of sIL-2R, IL-6, TGF-beta1, neopterin, and zinc in chronic hepatitis C patients treated with interferon-alpha. Cytokine 11(12):1076–1080
2. Halpern R, Halpern BC, Stea B, Dunlap A, Conklin K, Clark B, Ashe H, Sperling L, Halpern JA, Hardy D, Smith RA (1977) Pterin-6-aldehyde, a cancer cell catabolite: identification and application in diagnosis and treatment of human cancer. Proc Natl Acad Sci USA 74(2):587–591
3. Weiss G, Kronberger P, Conrad F, Bodner E, Wachter H, Reibnegger G (1993) Neopterin and prognosis in patients with adenocarcinoma of the colon. Cancer Res 53(2):260–265
4. Murr C, Bergant A, Widschwendter M, Heim K, Schröcksnadel H, Fuchs D (1999) Neopterin is an independent prognostic variable in females with breast cancer. Clin Chem 45:1998–2004
5. Fuchs D, Kramer A, Reibnegger G, Werner ER, Dierich MP, Goedert JJ, Wachter H (1991) Neopterin and beta 2-microglobulin as prognostic indices in human immunodeficiency virus type 1 infection. Infection 19(2):98–102
6. Berdowska A, Korczala KZ (2001) Neopterin measurement in clinical diagnosis. J Clin Pharm Ther 26:319–329
7. Reibnegger G, Aichberger C, Fuchs D, Hausen A, Spielberger M, Werner ER, Margreiter R, Wachter H (1991) Posttrans-plant neopterin excretion in renal allograft recipients: reliable diagnostic aid of acute rejection and predictive marker of longterm graft survival. Transplantation 52:58–63
8. Chevillard NA, Dupont HT, Obadia Y, Gallais H, Mege JL, Capo C (2000) Highly active antiretroviral therapy (haart) and circulating markers of immune activation: specific effect of haart on neopterin. Clin Diag Lab Immun 7(5):832–834
9. Kotanko P, Margreiter R, Pfaller W (2000) Urinary N-acetyl-beta-D -glucosaminidase and neopterin aid in the diagnosis of rejection and acute tubular necrosis in initially nonfunctioning kidney grafts. Nephron 84(3):228–235
10. Mohamed KH, Mobasher AA, Yousef AR, Salah A, El-Naggar IZ, Ghoneim AH (2001) A novel marker for cell mediated immunity in patients with PTB and lung cancer. Chest 119(3):776–780
11. Tanaka J, Koshimura K, Tsumori M, Murakami Y, Kato Y (2002) Monitoring of urine nitric oxide (NO) related substrates and immunological competence in hematological malignancy. Acta Biochim Pol 49(1):227–232
12. Turgan N, Habif S, Parildar Z, Ozmen D, Mutaf I, Erdener D, Bayindir O (2001) Association between homocysteine and neopterin in healthy subjects measured by a simple HPLC-fluorometric method. Clin Biochem 34(4):271–275
13. Stea B, Halpern RM, Halpern BC, Smith RA (1981) Urinary excretion levels of unconjugated pterins in cancer patients and normal Individuals. Clin Chim Acta 3(2):231–242
14. Armarego WL, Randles D, Taguchi H (1983) Peroxidase catalysed aerobic degradation of 5, 6, 7, 8-tetrahydrobiopterin at physiological pH. Eur J Biochem 135:393–403
15. De Castro MR, Di Marco GS, Arita DY, Teixeira LC, Pereira AB, Casarini DE (2004) Urinary neopterin quantification by reverse-phase high-performance liquid chromatography with ultraviolet detection. J Biochem Bioph Meth 59(3):275–283
16. Renz BA, Zeitler HJ (1983) Separation of pteridines from blood cells and plasma by reverse-phase high-performance liquid chromatography. Anal Biochem 133(1):68–78
17. Mansilla AE, Merds ID, Salinas F (2001) Analysis of pteridines and creatinine in urine by HPLC with serial fluorimetric and photometric detectors. Chromatographia 53:510–514
18. Montalvo BC, Villar CI, Hornillos RCI, Diez LP (1988) Determination of pterins in urine by high-performance liquid chromatography on C18 columns conditioned with cetyltrimethylammonium bromide. J Chromatogr 458:217–223
19. Merás ID, Mansilla AE, Gómez MJR (2005) Determination of methotrexate, several pteridines, and creatinine in human urine, previous oxidation with potassium permanganate, using HPLC with photometric and Xuorimetric serial detection. Anal Biochem 346:201–209

20. Han F, Bryan H, Shi HL, Lin BC, Ma YF (1999) Pteridine analysis in urine by capillary electrophoresis using laser-induced fluorescence detection. Anal Chem 71(7):1265–1269
21. Mansilla AE, Meras ID, Salinas F (1998) Simultaneous determination of pteridines in multicomponent mixtures using derivative spectrophotometry and partial least-squares calibration. J Pharmaceut Biomed 17:1325–1334
22. Mayersbach P, Augustin R, Schennach H, Schonitzer D (1994) Commercial Enzyme-linked immunosorbant assay for neopterin detection in blood donations compared with RIA and HPLC. Clin Chem 40:265–266
23. Li YQ, Huang XZ, Xu JG (1999) Synchronous fluorescence spectrometric methodology in the wavelength domain. J Fluoresc 9:173–179
24. Seritti A, Nannicini L, Vecchio RD (1996) Use of synchronous scan excitation spectra to detect fluorescent metal organic ligands produced by the marine diatom phaeodactylum tricornutum. Environ Technol 17(1):25–33
25. Li QF, Yao XJ, Chen XG, Liu MC, Zhang RS, Zhang XY, Hu ZD (2000) Application of artificial neural networks for the simultaneous determination of a mixture of fluorescent dyes by synchronous fluorescence. Analyst 125:2049–2053
26. Guiteras J, Ferrer R, Beltran JL (1998) Quantitative multicomponent analysis of polycyclic aromatic hydrocarbons in water samples. Anal Chim Acta 361(3):233–240
27. Patra D, Mishra AK (2000) Multivariate fluorimetric determination of mixture of pyrene, perylene and triphenylene in water samples. Anal Lett 33:2293–2304
28. Patra D, Mishra AK (2000) Effect of sample geometry on synchronous fluorimetric analysis of petrol, diesel, kerosene and their mixtures at higher concentration. Analyst 125(8):1383–1386
29. Patra D, Mishra AK (2001) Concentration dependent red shift: qualitative and quantitative investigation of motor oils by synchronous fluorescence scan. Talanta 53(4):783–790
30. Mansilla EA, Meras ID, Galian R (2001) Simultaneous fluorimetric determination of pteridin derivatives: comparison between synchronous, partial least-squares, and hybrid linear analysis methods. Appl Spectrosc 55(6):701–707
31. Murillo Pulgarín JA, García Bermejo LF (1998) Simultaneous determination of salicylamide and salsalate in serum and urine by first derivative variable-angle synchronous fluorescence spectrometry. Anal Biochem 265:331–339
32. Patra D, Mishra AK (2002) Recent developments in multicomponent synchronous fluorescence scan analysis. Trends Anal Chem 21(12):787–798
33. Lloyd JBF (1971) Nature and evidential value of the luminescence of automobile engine oils and related materials. I. Synchronous excitation of fluorescence emission. J Forensic Sci Soc 11(2): 83–94
34. Lloyd JBF (1971) Synchronized excitation of fluorescence emission spectra. Nature Physical Science 231(20):64–65
35. Ni YN, Lin DQ, Kokot S (2005) Synchronous fluorescence and UV-vis spectrometric study of the competitive interaction of chlorpromazine hydrochloride and Neutral Red with DNA using chemometrics approaches. Talanta 65(5):1295–1302
36. Deepa S, Mishra AK (2005) Fluorescence spectroscopic study of serum albumin-bromadiolone interaction: Fluorimetric determination of bromadiolone. J Pharm Biomed Anal 38:556–563
37. Maugini E, Tronelli D, Bossa F, Pascarella S (2009) Structural adaptation of the subunit interface of oligomeric thermophilicand hyperthermophilic enzymes. Comput Biol Chem 33: 137–148