Determination of Thiamine in Pharmaceutical Preparations by Sequential Injection Renewable Surface Solid-phase Spectrofluorometry

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Fluorometric determination of thiamine requires the conversion of the analyte to fluorescent thiochrome by hexacyanoferrate(III) oxidation in alkaline solution and the isolation of the produced thiochrome from the reaction medium by solvent extraction. It was observed that thiochrome could be concentrated and separated from the reaction medium by solid-phase extraction. The thiochrome sorpted on the surface of octadecyl-alklylated poly-[styrene/divinylbenzene] (C18-PS/DP) microbeads emitted strong fluorescence upon excitation, the maximum excitation and emission wavelengths being 385 nm and 433 nm, respectively. Based on this observation, a sequential injection renewable surface solid-phase spectrofluorometry was developed for the determination of thiamine. A sequential injection system on-line coupled to a chip-based flow-through cell was employed to handle the chemical reaction, bead injection and discharging, and adsorption of thiochrome. Solid-phase fluorometric detection was realized by coupling the chip-based flow-through cell to a spectrofluorometer with a multistrand bifurcated optical fiber. Under the optimized condition, a detection limit of $0.03 \mu\text{g ml}^{-1}$ was achieved at the sample throughput of 30 h^{-1} and consumption of 1 mg C18-PS/DP microbeads for each run. Eleven runs of a $2 \mu\text{g ml}^{-1}$ thiamine standard solution gave a relative standard deviation of 1.0%. The developed approach was successfully applied for the determination of thiamine contents in pharmaceutical preparations.

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Introduction

Thiamine (Vitamin B₁) is an essential nutrient for humans to keep carbohydrate metabolism, to maintain normal neural activity and to prevent beriberi. People usually absorb VB₁ from natural and fortified foods. When needed, the vitamin can be taken in from various pharmaceutical preparations such as multivitamin dosages.

A variety of approaches, such as spectrophotometry,¹ spectrofluorometry,^{2,4} high performance liquid chromatography,⁵ and cathodic stripping voltammetry,⁶ for the determination of thiamine in pharmaceutical preparations have been reported. The most widely used method for the assay of thiamine is the so-called thiochrome method.^{2,4} It involves oxidation of thiamine by hexacyanoferrate(III) in aqueous alkaline solution to fluorescent thiochrome, extraction of thiochrome to an organic phase and measurement of the fluorescence intensity of the organic extract. The extraction of thiochrome is necessary to isolate thiochrome from hexacyanoferrate(III) that may quench the fluorescence. The method suffers intensive and tedious manual operation and poor reproducibility. It also generates a large quantity of toxic wastes. For determination of thiamine in multi-vitamin preparations and foods, HPLC is also

frequently used, because it suffers less interference. HPLC can also be used to analyze the degraded products of thiamine in preparations.⁷ When fluorescence detection was employed in HPLC, precolumn⁷ or postcolumn⁸ derivation of thiamine with alkaline-hexacyanoferrate(III) solution was required.

Flow injection analysis (FIA)^{9,10} has proved to be a powerful technique for automation of chemical analysis, and thus has been widely applied in various fields. Karlberg and Thelander adapted the thiochrome fluorescence method into FIA.¹¹ The developed flow injection (FI) extraction spectrofluorometry featured simple operation, high sample throughput, and good precision. However, this FIA approach still used an inflammable organic solvent and the toxic hexacyanoferrate(III); therefore it inevitably generated toxic wastes. Recently, Chen *et al.*¹² developed an FI on-line photochemical spectrofluorometric method for the determination of thiamine in pharmaceutical preparations and serum.

Solid phase spectrophotometry¹³ and solid phase spectrofluorometry^{14,15} have the merit of combining separation and preconcentration steps with the *in situ* surface absorbency or fluorescence detection, resulting in significant improvements in selectivity and/or sensitivity. When coupling this technique to FI,^{13,14} regeneration of the solid phase after each run is necessary for cyclic use of the solid-phase. Even so, the activity of the solid phase may gradually deteriorate after repetitive use. Furthermore, many chemical reactions taking place on the solid surface are irreversible. Thus they are incompatible with the

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Fig. 1 Schematic diagram of the chip-based flow cell (a) and top view of the PDMS membrane (b). B, microbeads; F, porous filter; G, optical glass; I, inlet port; M, multistrand bifurcated optical fiber; O, outlet port; P, plexiglass; R, PDMS rubber membrane.

requirements of the repetitive use of the solid phase. The advent of FI renewable surface (also referred to as bead injection) solid-phase sensor system¹⁶ avoided the above mentioned troubles for liquid-solid-reaction based assays, and initiated a new methodology for flow/sequential injection analysis.¹⁷ In an FI or sequential injection (SI) renewable surface system, the microbeads were injected in the form of suspension into a jet-ring cell (a specially designed flow-through cell) and trapped therein for adsorbing the analyte in the following zone of sample solution. Fluorescence of the labeled analyte was *in situ* determined in the jet-ring cell with a fluorescence microscope equipped with a photomultiplier tube. After each measurement the spent beads were discharged to waste, and the jet-ring cell was ready for the next cycle of determination. Thus, regeneration of the bead surface was not required. The operation mode of the renewable surface techniques allows one to consider irreversible chemistry methods for separations and sensing, with concomitant improvements in binding specificity that may not be available with conventional, reversible surface interactions. When different solid supports and functional groups and various detection modes are adopted, the renewable surface technique has been applied for immunoassay^{16,18,19} with fluorescence detection, for enzyme^{20,21} determination with spectrophotometric detection, and for nucleic acid purification.²² Recently, Wang and Fang designed a sandwiched, chip-based flow-through cell, which was coupled to a spectrophotometer *via* a bifurcated optical fiber, for SI renewable solid-phase spectrophotometric determination of trace chromium.²³ Compared to the jet-ring cell, the chip-based flow-through cell contained no moving parts, and thus had the advantages of simplicity in fabrication and operation.

We recently adapted the SI renewable-surface technique to solid-phase spectrofluorometry for thiamine determination. The environment-friendly and automated method developed had been successfully applied to the determination of thiamine in pharmaceutical preparations.

Experimental

Apparatus

The sequential injection system was composed of a syringe pump (P/N 50300-0.9 Kloehn Co., Ltd., Las Vegas, USA)

Fig. 2 Experimental set-up of sequential injection renewable surface system. B, microbead suspension; C, H₂O carrier; F, chip-based flow-through cell; H, holding coil (0.7 mm i.d. $\times$ 200 cm); M, multistrand bifurcated optical fiber; P, syringe pump; R, mixed NaOH and K₃Fe(CN)₆ oxidizing solution; S, spectrofluorometer; V₁, six-position valve; V₂, two-position valve; W, waste; CR, chart recorder.

equipped with a 2.5 ml syringe, a two-port non-distribution valve, and a six-port distribution valve (P/N 50120 Kloehn Co., Ltd., Las Vegas, USA). Both the syringe pump and the distribution valve were controlled by a personal computer using software written in Visual Basic. A Model RF-540 spectrofluorometer (Shimadzu, Kyoto, Japan) was used for recording spectra and measuring fluorescence intensity. A chart recorder (Dahua Instrument Co., Shanghai, China) was used to record the peak traces.

The chip-based flow-through cell was a specific modification mode of the sandwich design described in Ref. 23. It was a four-layer structure as shown in Fig. 1a. The top and bottom Plexiglass wafers (60 mm $\times$ 50 mm $\times$ 3 mm) were used to accommodate inlet and outlet ports at the top and to fix the fiber-optical probe at the bottom, respectively. Between the two Plexiglass wafers, a PDMS membrane (30 mm $\times$ 20 mm $\times$ 0.7 mm) with a cut-through channel (10 mm $\times$ 1.8 mm) and a porous filter (pore diameter 20 μ m), and an optical glass wafer (30 mm $\times$ 20 mm $\times$ 1 mm) were held in place (Fig. 1b). Four screws were applied to tighten the four-layer structure so that no leakage would happen when solution or beads suspension was delivered to pass through the cell. With this modification, the flow-through cell was coupled to the fiber-optical probe *via* the optical glass window, resulting in less stray light and background fluorescence.

A multistrand bifurcated optical fiber (Chunhui High Tech. Co., Nanjing, China), composed of 32 fibers (core diameter of 80 μ m) for incident light and 32 fibers for emission light, was used to couple the chip-based flow-through cell to a spectrofluorometer, as described in detail in the section of *Method development*. Flow manifold is shown in Fig. 2; PTFE tubing with 0.7 mm i.d. was used to fabricate the holding coil and to make connections.

Reagents

Thiamine hydrochloride stock solution (1000 mg l⁻¹) was prepared by dissolving 0.1 g of thiamine hydrochloride (Sigma) in 100 ml of 0.01 mol l⁻¹ HCl; it was stored in a refrigerator. Working standard solutions were prepared daily by appropriate dilution of the stock solution with water.

Alkaline oxidizing solution {0.003% (w/v) in K₃Fe(CN)₆ and 4% (w/v) in NaOH} was daily prepared by mixing 1 ml of 0.15% (w/v) K₃Fe(CN)₆ solution with 50 ml of 4% (w/v) NaOH solution.

The suspension of C18-PS/DP microbeads was prepared by mixing 0.24 g octadecyl-alkylated poly(styrene/divinylbenzene)

Table 1 The procedure of operation of SI system^a

Step No.	Two-port valve position	Distribution valve position	Volume processed / μ l	Time duration /s	Function
1	Left	1	200	1	H ₂ O aspiration
2	Right	6	420	4	C18 beads suspension aspiration
3	Right	1	620	5	C18 beads loading
4	Left	1	300	2	H ₂ O aspiration
5	Right	3	300	2	Oxidant solution aspiration
6	Right	4	200	1	VB ₁ solution aspiration
7	Right	3	300	2	Oxidant solution aspiration
8	Right	4	200	1	VB ₁ solution aspiration
9	Right	5	400	2	H ₂ O aspiration
10	Right	5	300	2	H ₂ O injection
11	Right	5	300	2	H ₂ O aspiration
12	Right	5	300	2	H ₂ O injection
13	Right	5	300	2	H ₂ O aspiration
14	Right	5	300	2	H ₂ O injection
15	Right	5	300	2	H ₂ O aspiration
16	Right	5	300	2	H ₂ O injection
17	Right	5	300	2	H ₂ O aspiration
18	Right	5	300	2	H ₂ O injection
19	Right	1	1200	72	Flow cell washing, VB ₁ loading
20	Right	1	600	6	Wasted beads backsuction
21	Right	2	800	8	Waste and holding coil washing

a. Total time of one cycle: 120 s.

microbeads, which were taken from commercial solid-phase-extraction cartridges (Transgenomic, Omaha, USA), in 100 ml 2% (v/v) ethanol solution.

All the used reagents except thiamine hydrochloride were of analytical grade or better, and double-distilled water was used throughout.

Sample preparation

Tablet: An appropriate amount (equivalent to 20 mg thiamine for vitamin B₁ tablet and 6 mg thiamine for multiple vitamin B tablet) of previously powdered sample was accurately weighed into a 100 ml calibrated flask, and 10 ml of 0.1 mol l⁻¹ HCl solution was added, then diluted to the mark with water. After sonication for 20 min, the insoluble residue was filtered off. The filtrate was diluted step-wise with water to a concentration of about 4 μ g ml⁻¹ thiamine for determinations.

Syrup: An aliquot of 3 ml (equivalent to 0.3 mg thiamine for multiple vitamin B syrup) was measured into a 100 ml calibration flask, and 10 ml of 0.1 mol l⁻¹ HCl solution was added; then diluted to the mark with water.

Operation procedure

The excitation and emission wavelengths of the spectrofluorometer were set at 385 nm and 433 nm, respectively. The procedure of operation is listed in Table 1. Typically, the suspension of C18-PS/DP microbeads was transported into the flow-through cell (steps 1 – 3). The water, oxidant and sample were respectively aspirated through the six-

port selection valve into the holding coil (steps 4 – 8), where the sample zones and oxidant zones were mixed by repetitive flow reversals (steps 9 – 18). Then the mixture of sample and oxidant was delivered to pass the C18-PS/DP-bed in the flow-through cell while the fluorescence signal peak was recorded (step 19). After the fluorescence signal reached its maximum, the used C18-PS/DP microbeads were flushed to waste, and the connecting tubing and the holding coil were cleaned with water (steps 20 – 21).

Results and Discussion

Method development

In an innovative work on flow injection renewable surface technique, Ruzicka *et al.*¹⁶ employed a fluorescence microscope equipped with a photomultiplier tube and optical filters as the fluorescence detector. A specially designed jet-ring cell was directly positioned underneath the objective lens of the microscope, and aligned to the optical system of the microscope. In the present work, however, a general spectrofluorometer was used as the detector for sequential injection renewable surface solid-phase spectrofluorometry (SI-RSSF). Thus, the coupling of a flow cell to the spectrofluorometry was not straightforward. The spectrofluorometry-equipped flow through cells for HPLC or flow injection analysis were unsuitable for detection of the bead-surface fluorescence because the geometry of the flow-through cell was designed for liquid-flow only. The home-fabricated chip-based flow-through cell that has a large ratio of area to depth (favorable for detection of the surface fluorescence produced in the renewable bead) could not be directly coupled to the optical system of the spectrofluorometer. Thus, a multistrand bifurcated optical fiber was used for coupling the chip-based flow-through cell to the spectrofluorometer. The channel width of the flow-through cell was designed to be close to the optical diameter of the common terminal of the incident and reflectant multistrand fibers, so that as much as possible of the excitation light from the fibers could be introduced into the fibers, and as much as possible of the emission light from the bead surface could be collected. In addition, it was observed that the excitation beam of the spectrofluorometer was focused to form a light spot of about 2 $\times$ 10 mm, which was much bigger than the optical diameter of the incidental terminal of the multistrand fibers. In order to collect as much as possible excitation radiation, an additional lens of 5 mm o.d. was fixed in front of the incident terminal of the fibers. With the additional lens, a 60% enhancement in the sensitivity of the coupled system was obtained. In order that the spectrofluorimeter could be easily transformed between routine use mode and the SI-RSSF mode, a rectangular jig for fixing and alignment of the incidental and reflectional fiber terminals was used. It was made of a piece of hard rubber with the same size as a usual optical silica cell (10 mm $\times$ 10 mm $\times$ 40 mm). Two holes, perpendicular to each other, of internal diameter approximately 0.05 mm smaller than the external diameter of the terminal cylinders, were used to fix the two terminals. When the spectrofluorometer was used for SI-RSSF, the rubber jig was accommodated in the metal cell-holder in the cell-compartment, and the two terminals were inserted into the two holes, respectively. When used for routine fluorescence measurement with general quartz cell, the rubber holder was simply replaced by a general optical quartz cell. Thus, the transformation of the two detection modes may be finished in 5 min.

Using fluorescein as a fluorescent chromophore, the

Fig. 3 Influence of bead amount on SI-RSSF signals. Thiamine concentration in reaction medium, $2 \mu\text{g ml}^{-1}$; sampling volume, 1.5 ml; bead-concentration in suspension, 2.4 mg ml^{-1} ; flow rate, 1.0 ml min^{-1} .

effectiveness of the FI-RSSF system was checked. It was found that fluorescence intensity of 142 was measured for a $40 \text{ mg } \mu\text{l}^{-1}$ fluorescein solution in the chip-based flow-through cell and double fluorescence intensity (285) was observed for $400 \mu\text{l}$ of $0.5 \text{ mg } \mu\text{l}^{-1}$ fluorescein in aqueous solution that had been driven to pass the chip-based flow-through cell where C18-PS/DP microbeads had been retained beforehand. In other words, a 160-fold increase in the sensitivity was obtained due to the concentration effect of the C18-PS/DP microbeads.

Preliminary tests for the determination of thiamine

As mentioned in the introduction, fluorometric determination of thiamine involves the conversion of the non-fluorescent analyte to a fluorescent compound, thiochrome, by hexacyanoferrate(III) oxidation, isolation of the less water-soluble thiochrome from the oxidizing reagent by solvent extraction, and measurement of the fluorescence intensity in the organic phase. It seems potentially promising to substitute solvent extraction by solid-phase extraction and to detect the fluorescence signal of thiochrome absorbed in the microbead surface. If this idea is realized, the inflammable and toxic solvent could be avoided, and the method for fluorometric determination of thiamine might be more environment-friendly. In the preliminary tests, the oxidation of thiamine by hexacyanoferrate(III) was carried out batch-wise in a volumetric flask, while an appropriate amount of C18-PS/DP microbead suspension was injected into the chip-based flow-through cell. As expected, a fluorescent signal was observed upon injection of the mixed solution of the analyte and reagent into the C18-PS/DP bead-retained flow-through cell, and the signal was gradually increased with the increase in the injected volume. In contrast, application of either the analyte solution alone (e.g. in absence of the oxidizing reagent) or the reagent blank into the bead-retained cell produced no fluorescence signal at all. Thus it is verified by the tests that thiochrome could be collected by the C18-PS/DP microbeads and that the surface-adsorbed thiochrome could emit fluorescence upon excitation. Spectral studies showed that the C18-PS/DP sorbed thiochrome had a maximum wavelength at 385 nm for excitation spectrum and a maximum wavelength at 433 nm for emission spectrum. Compared to the spectra observed in homogeneous solution,¹¹ the spectra on the C18-PS/DP microbeads were found to have a 15 nm red shift for maximum excitation wavelength and a 7 nm blue shift for maximum emission wavelength.

Since the C18-PS/DP microbeads acted as both the sorbent for

Table 2 Effect of various sample loading patterns

Test No.	Sample loading pattern ^a	Fluorescence intensity
1	400 μl sample and 600 μl oxidant	54.2
2	600 μl oxidant and 400 μl sample	46.7
3	300 μl oxidant, 400 μl sample and 300 μl oxidant	60.0
4	200 μl sample, 300 μl oxidant, 200 μl sample and 300 μl oxidant	66.8
5	200 μl oxidant, 200 μl sample, 200 μl oxidant, 200 μl sample and 200 μl oxidant	65.8

a. Thiamine 4.0 mg l^{-1} , oxidant 0.1% $\text{K}_3\text{Fe}(\text{CN})_6$ and 4% NaOH, the flow rate 1.0 ml min^{-1} , C18 1.0 mg .

collection of the thiochrome and the medium for fluorescence detection, the amount of the retained beads in the cell may be critical for the sensitivity of the SI-RSSF system. Using the procedure described in the above section (e.g. after batch-wise oxidation of thiamine, the mixed analyte and reagent solution was applied to the flow-through cell where a certain amount of beads had been injected and retained), the influence of the bead amount on the SI-RSSF signals was investigated. As can be seen in Fig. 3, the maximum SI-RSSF signal was observed when 1.0 mg beads were injected into the cell. The decline in the signals observed for bead amounts less than 1.0 mg may be ascribed to the fact that the beads were not enough to cover the detection window of the cell. The decrease in the signals for the bead amount more than 1.0 mg may be attributed to decrease in the average concentration of the thiochrome molecules sorbed on the bead surface, due to an excess of beads being retained in the part of the cell that was out of the detection window.

It should be pointed out that discharge, rather than regeneration, of the used beads after each determination was based on two considerations. Firstly, elution of the thiochrome from the beads may lead to generation of waste containing organic solvent. Secondly, the beads may not withstand the repeated attack of the strong alkaline solution since the beads, according to the manufacturer's regulation, can only be used in the pH range 1–14. Thus the SI-RSSF operation mode was not only feasible but also necessary for the solid phase fluorometric determination of thiamine. Based on these observations and considerations, an SI-RSSF approach for the determination of thiamine in pharmaceutical preparations was developed as described in the following sections.

Optimization of experimental conditions for the SI-RSSF

The mode of sample loading. The fluorescence signals observed with five different sample-oxidant sequential injection configurations, e.g. sample-oxidant, oxidant-sample, sample-oxidant-sample-oxidant and oxidant-sample-oxidant-sample-oxidant, were evaluated. The results are listed in Table 2. The best response characteristic was obtained for the configuration of sample-oxidant-sample-oxidant. Therefore, this configuration was adopted, as described in the operation procedure.

The concentrations of $\text{K}_3\text{Fe}(\text{CN})_6$ and sodium hydroxide. Experiments revealed that the concentration of oxidizing species $\text{K}_3\text{Fe}(\text{CN})_6$ in the reagent solution had a critical influence on the SI-RSSF signals. As shown in Fig. 4, the signals of SI-RSSF sharply increased with the increase of $\text{K}_3\text{Fe}(\text{CN})_6$ concentration from zero to 0.003% (w/v), after which the signals gradually declined. The decline in signals

Fig. 4 Influence of $K_3Fe(CN)_6$ concentration on SI-RSSF signals. Thiamine concentration, $2 \mu g ml^{-1}$; sample volume, $400 \mu l$; NaOH concentration in the oxidizing reagent: 4% NaOH; oxidizing-reagent volume, $600 \mu l$; flow rate, $1.0 ml min^{-1}$; bead amount, 1.0 mg.

Fig. 5 Influence of NaOH concentration on SI-RSSF signals. Concentration in the oxidizing reagent: 0.003% $K_3Fe(CN)_6$. Other experimental conditions were the same as those described in Fig. 4.

Fig. 6 Influence of sample volume on SI-RSSF signals. Oxidizing reagent solution: 0.003% $K_3Fe(CN)_6$ + 4% NaOH; Other experimental conditions were the same as those described in Fig. 4, with the exception of sample volume.

might be due to a quenching effect caused by the $K_3Fe(CN)_6$ molecules that remained in the sorbent beads. In the following experiment, a reagent solution containing 0.003% (w/v) $K_3Fe(CN)_6$ was used.

Oxidation of thiamine to thiochrome by $K_3Fe(CN)_6$ requires a strong alkaline medium. For the conventional batch-wise approach, the optimized sodium hydroxide concentration in the

Table 3 Tolerance levels of concomitants (thiamine concentration, $2.0 mg l^{-1}$)

Concomitant	Tolerance limit/ $mg l^{-1}$
Pyridoxine	500 ^a
Nicotinamide	200 ^a
Ascorbic acid	10
Riboflavin	1.0
Cyanocobalamine	0.8

a. The highest levels tested.

oxidizing reagent solution was reported to be $3.5 mol l^{-1}$.⁴ It was found in the present study that the SI-RSSF signals were rapidly increased with the increase of sodium hydroxide concentration in the range 0 – 1% (w/v), after which the increase rate was gradually slowed down, and the signal finally leveled off at the 12% (w/v) concentration (Fig. 5). To prevent the flow-through cell, especially the filter plug mounted in the cell, from being seriously damaged by the corrosive, concentrated sodium hydroxide solution, a compromise concentration (4% w/v) for sodium hydroxide in the reagent solution was adopted.

The flow rate of sample loading and the sample volume. The influence of flow rate for sample loading on the SI-RSSF signals was studied at different sampling volumes. Test showed that the signals were almost linearly increased with the decrease in the flow rate. Although lower flow-rates may produce higher signals, the time required for each run was enhanced in direct proportion. As a compromise between the sensitivity and the sample throughput, a flow rate of $1.0 ml min^{-1}$ was selected.

At the flow rate of $1.0 ml min^{-1}$, the effect of the sampling volume on the SI-RSSF signals is as shown in Fig. 6. For the test solution containing $2 \mu g ml^{-1}$ thiamine the SI-RSSF signals were linearly increased with the increase of the sampling volume up to $500 \mu l$, after which the curve gradually leveled off, indicating that the bead surface was gradually saturated by the thiochrome. To leave a relatively wide safety margin for any potential interfering species that may be also adsorbed by the beads, a sampling volume of $200 \mu l$ was selected.

Interference study

The possible interference from the concomitant species commonly existing in the pharmaceutical preparations on the determination of thiamine was investigated. A substance was considered not to interfere with the determination of thiamine if the variation in the fluorescence intensity due to the presence of concomitant was less than 5%. The results are shown in Table 3.

Analytical performances

The analytical performances of the SI-RSSF approach under the optimized experimental conditions are listed in Table 4. Compared with conventional manually operated thiochrome method,⁴ the present automated method has the merits of higher analytical speed, better reproducibility, less manual operation and avoidance of flammable and toxic organic solvent use. Compared with HPLC method, the present method is superior in simplicity of instrumentation, analytical speed, operation cost, and avoidance of organic solvent, although the former is more suitable for samples with a complicated matrix.

Application

The proposed method was applied for the assay of thiamine in three preparations containing thiamine: vitamin B₁ tablet,

Table 4 Analytical performance

The sample volume (μl)	200
Detection limit (ng ml ⁻¹)	31 (3σ)
Relative standard deviation (%)	1.0 (2.0 μg ml ⁻¹ , <i>n</i> = 11)
Regression equation	$F = 10.949 \times C + 12.774^a$
Linear dynamic range (μg ml ⁻¹)	0.06 – 8.0
Determination coefficient (<i>R</i>)	0.9992
The sample frequency (h ⁻¹)	30

a. *F*, fluorescence intensity; *C*, thiamine concentration in μg ml⁻¹; the interception is the reagent (including the beads themselves) blank.

multiple vitamin B tablet and multiple vitamin syrup. The analytical results, together with those obtained by the pharmacopoeia-regulated method (thiochrome method), are listed in Table 5. With significant improved precision, the results obtained by the present method were in good agreement with those obtained by the regulated method.

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Table 5 Determination of thiamine in pharmaceutical preparations

Sample	Thiamine content/mg	Thiamine found (mean ± SD, <i>n</i> = 3, mg)	
		Present method	Regulated method ^a
Vitamin B ₁ tablet	10.0	10.17±0.16	10.11±0.19
Multiple vitamin B tablet ^a	3.0	3.01±0.04	2.98±0.10
Multiple vitamin B syrup ^b	100	97.5±1.60	95.1±5.74

a. Other species: VB₂ 1.5 mg, VB₆ 0.2 mg, nicotinamide 10 mg, and calcium pantothenate 1 mg.

b. Other species in 1000 ml syrup: VB₂ 20 mg, VB₆ 30 mg, nicotinamide 100 mg, calcium pantothenate 10 mg, limonene 5 ml, ethanol 70 ml, and sucrose 200 g.