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Flow injection photochemical spectrofluorimetry for the determination of carbamazepine in pharmaceutical preparations

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Abstract

Upon on-line photochemical reaction carbamazepine (CBZ) can be converted to a strong fluorescent compound which has a maximum emission wavelength of 478 nm and maximum excitation wavelength of 254 nm. Acidity of reaction medium and the acid type were found to be critical for the on-line photochemically induced fluorescence, dilute hydrochloric acid being the most suitable. Based on these observations, a flow injection photochemical spectrofluorimetric approach for determination of the drug was developed. At optimized conditions, a detection limit of 0.08 ng ml^{-1} CBZ was achieved at the sampling rate of 80 h^{-1}. Eleven determinations of a 100 ng ml^{-1} CBZ standard solution gave a RSD of 0.45%. A linear calibration curve was obtained in the CBZ concentration range of 2–250 ng ml^{-1}. The developed method was successfully applied to assay the CBZ contents in pharmaceutical tablets.

Keywords: Carbamazepine; Pharmaceutical formulations; Flow injection; Photochemical spectrofluorimetry

1. Introduction

Carbamazepine (5*H*-dibenz[*b*,*f*]azepine-5-carboxamide, CBZ) is a type of tricyclic drug which is frequently used in the treatment of trigeminal neuralgia and as an anticonvulsant. High performance liquid chromatography (HPLC) and UV-spectrophotometry is regulated, respectively, by U.S. Pharmacopeia [1] and China Pharmacopeia [2] as the official method for assay of the drug in tablets.

CBZ has little native fluorescence. Oxidized by cerium(IV) it turns to a fluorescent compound. Based on this chemical oxidation reaction, Kuhn et al. [3] developed a spectrofluorimetric method and De la Pena et al. [4] established a kinetic fluorimetric approach for the determination of the drug in serum. However, the by-product Ce(III) of the derivation also emits strong fluorescence with a maximum excitation wavelength of 260 nm, which is close to the most favorable excitation wavelength (254 nm) of the derived fluorescent compound of CBZ. As a result, an

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unfavorable excitation wavelength (410 nm) had to be used in the Ce(IV)-oxidation based fluorescence methods to eliminate the interference of Ce(III), leading to a relatively poor detection limit (0.01 μg ml^{-1}) [4]. Recently, Pan and Yao [5] reported an electrolysis-based fluorescence method for determination of the drug in tablets. In their method CBZ was oxidized to a fluorescent compound by permanganate that was formed by electrolysis of Mn^{2+} in acidic medium. Neither Mn^{2+} nor permanganate emits fluorescence in the condition of determination of CZB. Thus the most favorable wavelength of the derived fluorescent compound of CZB was used, leading to a low detection limit of 8.4×10^{-10} mol l^{-1} (i.e. ~ 0.2 ng ml^{-1}). However, a 40–60 min period of electrolysis-based derivation was required to obtain a stable and maximum fluorescence intensity.

Owing to their inherent advantages over ordinary chemical reactions such as quicker reaction rate, smaller dilution factor and less chemicals involved, photochemical reactions have been applied in flow injection (FI) systems coupled to spectrofluorimetry for determination of some drugs [6–9] in pharmaceutical preparations. To our best knowledge, no such photochemical spectrofluorimetry (PCF) has been reported for CBZ.

In the present work, the photochemical reaction of CBZ was studied. It was found that upon on-line photochemical reaction in a medium of dilute hydrochloric acid, CBZ was converted to a strongly fluorescent compound. Based on the observation, a sensitive, fast and reliable FI on-line photochemically spectrofluorimetric approach was developed for the determination of CBZ in tablets.

2. Experimental

2.1. Apparatus

A Model RF-540 spectrofluorimeter (Shimadzu, Kyoto, Japan) equipped with a xenon lamp was used to measure fluorescence intensity. A Model LZ 1010 peristaltic pump (Zhaofa Institute for Lab. Automation, Shenyang, China), a Rheodyne 5020 six-port PTFE injection valve (Cotati, CA, USA) with a 200 μl sampling loop, and a 120 μl rectangular quartz flow-through cell (Shimadzu) were employed to set-up the FIA manifold as illustrated in Fig. 1. The photochemical reactor was constructed by freely coiling a knotted reactor, which was home-made from 200 cm × 0.5 mm i.d. PTFE tubing as described in Ref. [9], around a 6-W low-pressure mercury lamp. PTFE tubings of 0.5 mm i.d. were used for all connections, and PVC pump tubing of appropriate diameter was used to drive the carrier, reagent and sample solutions.

2.2. Reagents

The stock solution of CBZ (500 μg ml^{-1}) was prepared by dissolution of 50 mg the drug (purchased from National Institute for the Control of Pharmaceutical and Biological Product, Beijing, China) in 40 ml ethanol and dilution to 100 ml with dually distilled water. A 0.01 mol l^{-1} hydrochloric acid solution was prepared as usual. All the used chemicals, except for CBZ standard, were analytical grade, and dually distilled water was used throughout the work.

2.3. Sample preparation

An appropriate amount (equivalent to 50 mg CBZ) of previously powdered sample was accurately weighed into a 50 ml volumetric flask. Ethanol was then added to the flask until reaching the mark. After sonication for 15 min, the remain-

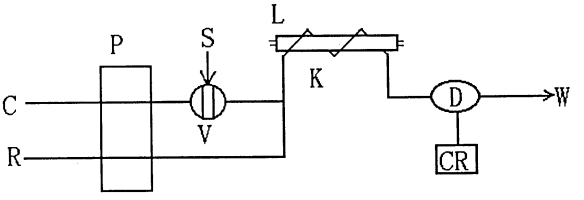

Fig. 1. The manifold for FI on-line PCF. C, carrier (H_2O, 1.5 ml min^{-1}); CR, chart recorder; D, flow-through detector (120 μl dead volume); K, three dimensionally disoriented photochemical reactor made of a 200 cm × 0.5 mm i.d. PTFE tubing; L, 6-W low pressure mercury lamp; P, peristaltic pump; R, 0.01 mol l^{-1} HCl (1.5 ml min^{-1}); S, sample solution; V, six-port injection valve with 200 μl sampling loop; W, waste.

ing insoluble residue was filtrated off. The filtrate was step-wise diluted to about 10 μg ml^{-1} (this intermediate solution was used for UV determination of the analyte) with a diluted HCl (1:180) solution, then step-wise diluted to about 100 ng ml^{-1} with water.

2.4. Procedure

The excitation and emission wavelengths of the spectrofluorimeter were set at 254 and 478 nm, respectively, both with a bandpass of 10 nm. Before measurement, the mercury lamp and the peristaltic pump were turned on, allowing the carrier and reagent solutions to be continuously driven through the photochemical reactor and the flow-through cell until a stable baseline was established. A test solution was fulfilled into the sampling loop by switching the injection valve into the sampling position for 30 s. Then the loaded test solution was injected into the carrier by switching valve into the injection position, and the fluorescence signal was recorded. Fifteen seconds later, the valve was switching back to the sampling position for next cycle. Three replicates were run for each test solution, and the mean peak-height was used for quantification.

3. Results and discussion

3.1. The medium for photochemical reaction

CBZ is a tricyclic compound which shows little native fluorescence. When chemically oxidized by Ce(IV) [3,4] or electrolysis based chemically oxidized in the presence of Mn^{2+} [5], it became intensively fluorescent. In the present work, an attempt was made into the photochemical conversion of the drug into fluorescent compound. As expected, upon exposure to UV-radiation CBZ in aqueous solution was quickly derived to a fluorescent compound that had a maximum emission wavelength of 478 nm. It was found that acidity of reaction medium and the type of acids used to adjust the acidity had critical effect on the on-line photochemically induced fluorescence. As shown in Fig. 2, when hydrochloric and sulfuric acid

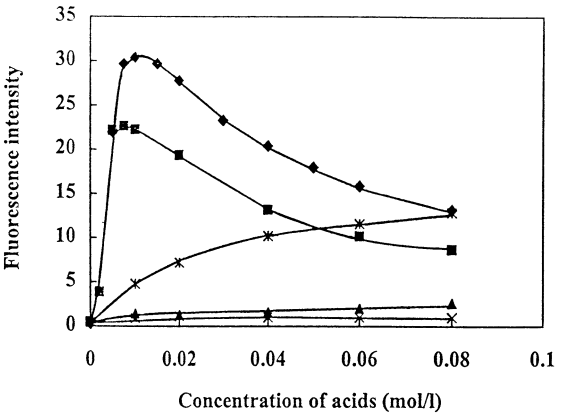

Fig. 2. The influence of acid concentrations in the reagent stream on the photochemically induced fluorescence intensity. CBZ concentration, 100 ng ml^{-1}; other experimental conditions were the same as Fig. 1 with the exception of the reagent stream. ◆, hydrochloric acid; ■, sulfuric acid; ▲, nitric acid; ※ , perchloric acid; × , acetic acid.

solutions were used as the reagent stream (see Fig. 1), the PCF signals first increased then decreased with the increase of the acid concentration. The optimum acid concentrations were found approximately in the same range of 0.005–0.015 mol l^{-1} for both acids although use of 0.01 mol l^{-1} hydrochloric acid solution produced the signal that was about 40% higher than that produced in sulfuric acid with the same concentration. When perchloric acid was used, however, the PCF signals were gradually increased with the increase of the acid concentrations up to the highest concentration tested. Very weak PCF signals were observed in both nitric and acetic acids media despite that the signals also trended to slowly increase with the increase of the acid concentration. Since 0.01 mol l^{-1} HCl solution produced the highest PCF signals, it was used as the reagent stream in the following studies.

It has been reported that organic solvents such as acetone [10], alcohol [11], and surfactants [12] could significantly sensitize photochemical reaction of some compounds. An investigation was made into the effect of these reagents on the photochemical reaction of CBZ. In the tests, dilute organic solvent or surfactant solutions prepared with 0.01 mol l^{-1} HCl were used as the reagent solutions, and the PCF signals observed

with the these reagent solutions were compared to that observed with the reagent solution containing only 0.01 mol l^{-1} HCl. It was found that alcohols (methanol and ethanol) and surfactants (sodium lauryl sulfate, sodium dodecyl benzene sulonate, cetyl trimethylammonium bromide, cetyl pyridinium chloride, Triton X-100 and cyclodextrin) at the concentrations of less than 0.1% severely depressed the PCF signals, and that acetone and acetonitrile at the same concentration levels only slightly reduced the signals. It was noted that about half intensity of the PCF signal remained when acetonitrile concentration in the reagent-stream increased up to 20%. This feature could be further exploited for use in post-column fluorescence derivation in HPLC. Based on these observations, the optimum medium for the on-line photochemical reaction of CBZ was 0.01 mol l^{-1} HCl solution which was used to be on-line merged with the sample zone at the same flow rate (as illustrated in Fig. 1).

The excitation and emission spectra of the photochemical product of CBZ were illustrated in Fig. 3. It is interesting to note that the spectra of the photochemical product of CBZ are much the same as those obtained by electrolysis-produced permanganate oxidation [5]. According to these spectra shown in Fig. 3, excitation wavelength of 254 nm and emission wavelength of 478 nm were, respectively selected for the photochemically spectrofluorimetric determination of CBZ.

3.2. Optimization of physical parameters

In our previous work on acetone sensitized PCF of reserpine [10], it was found that the optimum tubing length of photochemical reactor, which is in direct proportion to the irradiation time for the analyte, was dependent on the analyte concentration: longer reactor tubing was required by analyte of the higher concentrations. As a result, the PCF signals, measured with a given reactor, of different analyte-concentrations were not strictly in direct proportion to the analyte concentrations, and the linear dynamic range of calibration curve became another major concern in addition to sensitivity. With this experience taken in mind, the optimization of the physical

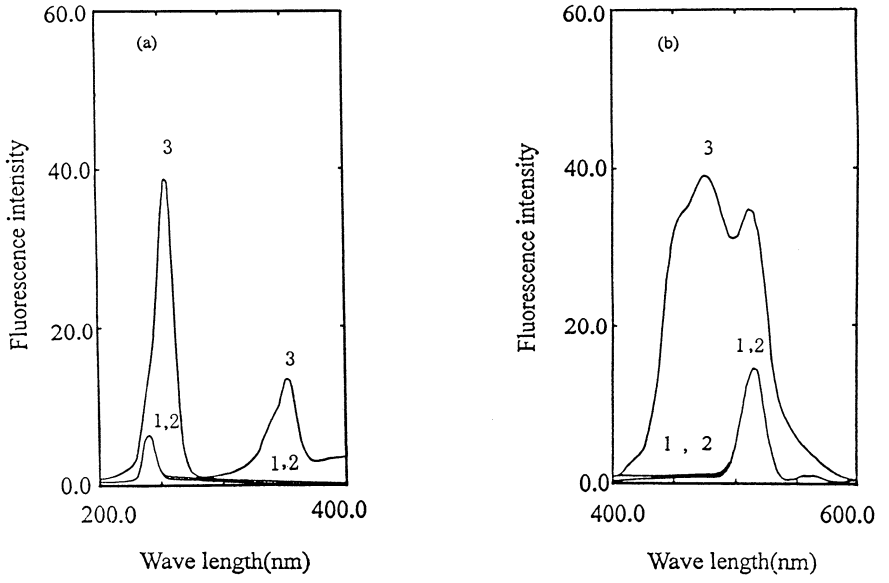

Fig. 3. Excitation (a) and emission (b) spectra of CBZ and its photochemical product. A stream of test solution was on-line merged with a stream of 0.01 mol l^{-1} HCl (both streams were at the flow rate of 1.5 ml min^{-1}) and delivered to pass the photochemical reactor and flow-through cell while the spectrofluorimeter was scanning: (1) 100 ng ml^{-1} CBZ without irradiation; (2) solvent blank with irradiation; (3) 100 ng ml^{-1} CBZ with irradiation.

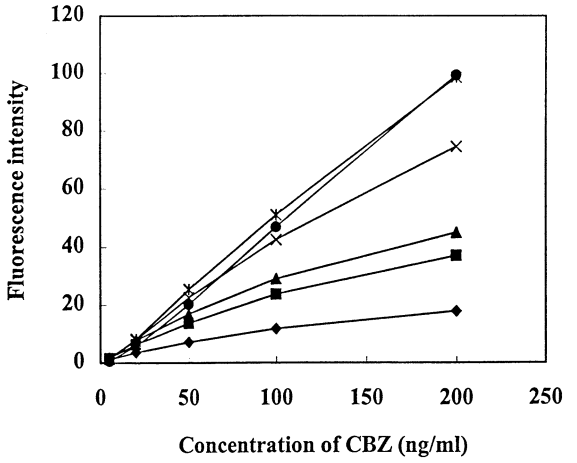

Fig. 4. The influence of reactor tubing length on the sensitivity and linearity. Standard solution series containing 5, 20, 50, 100 and 200 ng ml^{-1} CBZ were used to construct calibration curves; other experimental conditions were the same as Fig. 1 with exception of reactor tubing length. ◆, 50 cm; ■, 90 cm; ▲, 150 cm; ×, 180 cm; ※, 200 cm; ●, 250 cm.

parameters was aimed at both sensitivity and linearity. Using a series of standard solutions containing 5, 20, 50, 100 and 200 ng ml^{-1} CBZ, a set of calibration curves were constructed with photochemical reactors of various tubing length at a constant flow rate of 1.5 ml min^{-1} for both carrier water and reagent streams. As illustrated in Fig. 4, calibration curves of convex shape were observed by use of reactors made of short tubing, and both the sensitivity and linearity of the calibration curves were gradually improved with the increase of the tubing length up to 200 cm. This indicates that the yields of the photochemically induced fluorescent compound are concentration-dependent. For the tested concentration range, the reactor made of 200 cm tubing produced the best calibration curve with respect to both sensitivity and linearity. Thus, the reactor of 200 cm tubing was selected. No attempt to optimize the flow rate of the carrier and reagent streams was made because adjustment of flow rate was difficult for the invariable-speed peristaltic pump employed in the work.

Using the optimized reactor, the effect of injected sample volume on the PCF signals was also studied. It was found the PCF signals were rapidly increased with the increase of sampling volume up to 200 μl, after which the signals were gradually leveled off. As a result, a sampling loop of 200 μl was used. The relative larger sampling volume required by the present FIA system may be ascribed to the relatively larger dead volume of the rectangular flow-through cell (120 μl).

3.3. Interference studies

Dihydrocarbamazepine is an intermediate compound that is formed in the synthesis of CBZ and frequently coexists in the CBZ tablet. Tests showed that dihydrocarbamazepine emitted neither native fluorescence nor PCF under the conditions for PCF of CBZ, and that for the determination of 100 ng ml^{-1} CBZ at least the same concentration level of coexisting dihydrocarbamazepine could be tolerated.

As mentioned before, ethanol depressed the PCF signal of CBZ. According to the pharmacopeia regulation, nevertheless, ethanol was used as solvent to dissolve the analyte due to the low solubility of CBZ in water. Thus the effect of ethanol concentration in the test solution on the PCF signal was examined in detail. As illustrated in Fig. 5, constant PCF signals of CBZ could be obtained in the ethanol concentration range of 0.008–0.020%. Based on this observation, we designed the sample preparation method (see Sec-

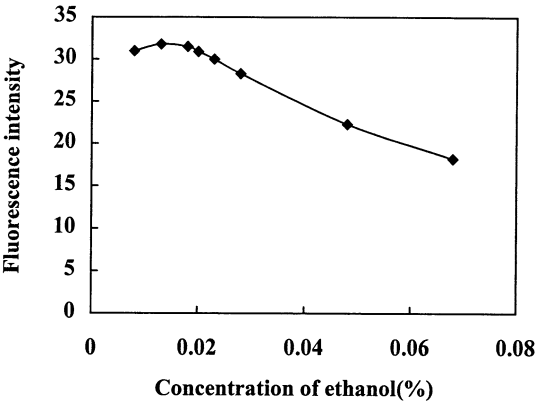

Fig. 5. The influence of ethanol concentration on photochemically induced fluorescence intensity. Test solution contained 100 ng ml^{-1} CBZ and various concentrations of ethanol; other experimental conditions were the same as Fig. 1.

Table 1
Analytical performances of the FI on-line PCF for the determination of CBZ

Detection limit (ng ml^{-1})	0.08 (3σ)
Relative standard deviation (%)	0.45 (100 ng ml^{-1}, $n=11$)
Sampling rate (h^{-1})	80
Regression equation	$F=-2.354+0.692C$[a]
Linear dynamic range (ng ml^{-1})	2–250
Determination coefficient (R^2)	0.9991
Sample consumption (ml per run)	1 (including to waste)

[a] F, fluorescence intensity in arbitrary units; C, CBZ concentration in ng ml^{-1}.

tion 2), which led to test solutions containing approximately 0.01% ethanol and 100 ng ml^{-1} analyte for PCF measurement.

3.4. Analytical performances

The analytical performances of the FI on-line photochemical spectrofluorimetric approach are listed in Table 1. Compared to the spectrofluorimetric methods based on chemical oxidation [4] and electrolysis-based chemical oxidation [5] not only the sample throughput rate but also the detection limit were significantly improved. The improvement in the detection limit may be ascribed to the most favorable excitation wavelength (254 nm) of the derived CBZ compound being used (in comparison to Ref. [4]), and might be due to the small dilution factor being applied by on-line photochemical reaction (in comparison to Ref. [5]).

3.5. Application

The developed FI on-line photochemical spectrofluorimetric approach was applied to assay CBZ content in tablets. Two brands of CBZ tablet were analyzed. The accuracy of the approach was first checked by spike-recovery test on the solutions prepared from a brand of tablet. From real sample solutions containing 50 ng ml^{-1} analyte, the recoveries of spiked 25, 50 and 75 ng ml^{-1} standard were 101 ± 2.2 (mean $\pm$ S.D., $n=3$), 101 ± 1.7 and 99.7 ± 1.2%, respectively. Table 2 lists the analytical results obtained by both the present and the official methods (UV-spectrophotometry) regulated by China Pharmacopeia [2]. The results of the present method are in good agreement with those of official method.

Table 2
Determination of CBZ in pharmaceutical preparations

Sample	Labeled[a]	Found[a] (mean $\pm$ S.D., $n=4$)	
		Present method	Official method [2]
Tablet 1	0.1	0.0938 ± 0.0006	0.0952 ± 0.0008
Tablet 2	0.1	0.0959 ± 0.0007	0.0964 ± 0.0008

[a] Expressed in g per tablet.

4. Conclusions

CBZ can be derived to a strongly fluorescent compound by on-line photochemical reaction. Acidity of the reaction medium and the acid type used to control the acidity significantly affect the photochemically induced fluorescence. Dilute hydrochloric acid is the most suitable medium for the reaction. The developed FI photochemical spectrofluorimetric method, featuring high throughput rate, low detection limit and excellent precision can be applied to assay the CBZ content in pharmaceutical tablet. Since fairly strong PCF signals could be obtained in the acetonitrile-containing medium, it is potentially promising for the on-line photochemical reaction to be applied to HPLC systems with post-column derivation and fluorescent detection for biomedical analysis of the drug.

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