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Short communication

Ion chromatography combined with online electrochemical derivatization and fluorescence detection for the determination of carbamazepine in human plasma

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ABSTRACT

This paper describes the determination of carbamazepine (CBZ) in human plasma using ion chromatography combined with online electrochemical derivatization and fluorescence detection. Separation of CBZ with anion exchange column was demonstrated to be feasible using either basic (10 mM NaOH) or acidic (0.1 M H_3PO_4) reagent with a small amount of acetonitrile (ACN) added as eluent. Electrochemical derivatization of CBZ into a strongly fluorescent product, which could be carried out only under the acidic condition, was investigated via the previously reported electrolytic cell (EC), as well as two modes of acidification. The linear range of CBZ for human plasma was between 10–2000 $\mu g\,L^{-1}$ under the optimized experimental conditions. The limit of detection (LOD, S/N=3) was 1.3 $\mu g\,L^{-1}$ and the relative standard deviation (RSD, $n=7$) was 2.6%. Better sensitivity and selectivity of the present method were demonstrated in comparison with ion chromatography with ultraviolet detection (IC-UV). The spiked recoveries of CBZ in 2 human plasma samples were 78.5 and 114%, respectively.

1. Introduction

Carbamazepine (5H-dibenz [b, f] azepine-5-carboxamide, CBZ) is a tricyclic drug for the treatment of epilepsy, trigeminal neuralgia and schizophrenia [1–3]. It has been the first-choice antiepileptic drug for the wide range of seizure disorders in both adults and children due to its efficacy and acceptable safety profile. Monitoring the concentration of CBZ in human plasma is of great importance to clinical analysis and disease treatment. Hence, several publications deal with the determination of CBZ in human plasma. High performance liquid chromatography (HPLC) in conjunction with different detectors, such as UV [4], electrochemical [5], and mass spectroscopy (MS) [6–8], is considered as a universally accepted method. Fluorescence detection (FD) possesses the features of good sensitivity and selectivity, thus it is an attractive method in analytical science. CBZ is a weakly fluorescent substance and cannot be directly detected fluorometrically [9]. Therefore, derivatization techniques, such as chemical [10] and photochemical [9], were developed to oxidize CBZ into strongly fluorescent product which was then detected fluorometrically in flow injection analysis (FIA). However, chemical oxidation which used lead dioxide (PbO_2) [10,11] as an oxidizing reagent required toxic chemicals and cumbersome sample preparations. Chen and co-authors [9] determined the CBZ in pharmaceutical preparations with flow injection photochemical spectrofluorimetry using 200 cm of PTFE tubing for the reactor. The authors pointed out that it would be potentially promising for this online photochemical reaction to be applied to HPLC system with post-column derivatization and fluorescence detection. Unfortunately, no such method has been reported until now.

Ion chromatography combined with post-column electrochemical derivatization and fluorescence detector (IC/ED/FD) for the determination of phenolic compounds has been reported in our previous work [12]. The IC mode showed more advantage relative to the HPLC mode in some specific chromatographic separation aspects since the polymer-based IC column can be used in the wide pH range (0–14). This paper describes, for the first time, the separation of CBZ with IC technique using different ion exchange columns as well as eluents. Online electrochemical derivatization was also studied using two kinds of acidification modes (see Section 3.3). The present method was sensitive and selective, and was applied for the determination of CBZ in biological fluids.

2. Experimental

2.1. Material and chemicals

CBZ and oxcarbazepine (OXC) were of analytical grade and were purchased from Aladdin Co. (Shanghai, China). HPLC grade

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acetonitrile (ACN), methanol and all other chemicals were from Huipu Co. (Hangzhou, China). Water was purified with Milli-Q (Millipore, Molsheim, France) system to a specific resistivity of 18.2 MΩ cm.

2.2. Solutions

Stock solution of CBZ was prepared by dissolving 10 mg of CBZ into 50 mL of ACN to form a concentration of 200.0 mg L^{-1} (0.85 mM). To prepare standard CBZ working solution, an aliquot of 0.5 mL of the stock solution was diluted to 10.0 mL in a volumetric flask, and 0.005–2 mL of this solution was further diluted to 10.0 mL with a mixture of water-ACN (90:10) reagent; the concentration of standard working solutions ranged from 5.0 to 2000 μg L^{-1}. The stock solution was stored in the refrigerator at −4 °C and the working standard solutions were freshly prepared every day.

2.3. Analysis procedure

The schematic diagram of the electrolytic cell (EC) was described in previous report [12]. It comprised mainly two porous titanium electrodes and a cation-exchange membrane. Fig. 1 describes the scheme of the present analysis procedure. After separation with the IC column, CBZ was detected with UV detector, and was then delivered to the anodic porous electrode of the EC where electrochemical oxidation occurred. The oxidation product was delivered to the fluorescence detector. The UV and fluorescence signals were recorded via Dionex UCI 100 universal chromatography interface. Data collection and analysis were performed via a personal computer equipped with Dionex Chromeleon 6.8 software.

2.4. IC conditions

Isocratic elution was carried out with IC system consisting of a P580 pump (Dionex, Sunnyvale, CA, USA), IonPac AG11(Dionex, 4 mm × 50 mm) and AS11 column (Dionex, 4 mm × 250 mm), a UVD340U diode array detector, and a RF-535 fluorescence detector (Shimadzu Co., Japan) with the bandwidth of 20 nm. The mobile phase was 100.0 mM H_3PO_4 containing 10% (v/v) of ACN with the flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL.

2.5. Preparation of human plasma

Blood plasma samples were obtained from epilepsy patients in the First Affiliated Hospital of Medical College, Zhejiang University, China. Both were treated with sodium citrate as anticoagulant and were stored at −20 °C before they were analyzed.

Preparation of human plasma was carried out by protein precipitation. For the isolation of the analytes from human plasma, 0.5 mL acetonitrile and 0.25 mL purified water were added to a 0.25 mL plasma sample. The mixture was centrifuged at 4000*g* and 25 °C for 30 min. The supernatant was filtered through a 13 mm membrane syringe filter (Xiboshi, pore size 0.45 μm, Tianjin Fuji Tech Co. Tianjin, China) prior to the injection into the system.

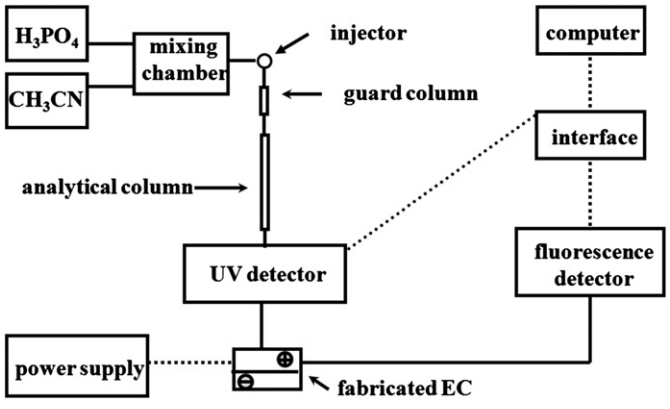


Fig. 1. Schematic diagram of instrumental setup.

2.6. Recovery

The absolute recovery of the drug from plasma was measured by analyzing 1 mL of drug free plasma samples spiked with CBZ at 25, 75 and 150 μg L^{-1} concentrations. After chromatography and electrochemical derivatization processes, the peak areas were compared with those of standard aqueous solutions of CBZ at the same concentrations.

2.7. Validation of the method

Repeatability (within-day precision) was evaluated by replicate analysis of spiked plasma containing CBZ at the same concentrations as those of the recovery study. The analytes were repeated six different times during one day. Reproducibility (between-day precision) was defined by analyzing the same plasma samples spiked as above on six different days.

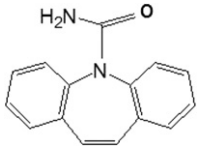
3. Result and discussion

3.1. Choice of separation system

The molecular structure and physicochemical properties of CBZ are summarized in Table 1. CBZ was usually eluted via reversed-phase high performance liquid chromatography (RP-HPLC), with aqueous buffer and organic modifier (methanol or acetonitrile) as mobile phase [13–15]. In the current study, the separation of CBZ was attempted using cation or anion exchange columns. Dionex cation-exchange columns, such as CS12A, CS16 and CS17, were tested, with methanelsulfonic acid plus ACN as the eluent. However, CBZ was found to be eluted at dead time, indicating that it was retained on none of these cation exchange columns. Therefore, cation exchange columns are not suitable for the separation of the analyte.

Anion exchange column (Dionex IonPac AS11 column) was tested for the separation of CBZ, with 10 mM NaOH plus 10% ACN as the eluent. The separation efficiency was evaluated by both the UV and fluorescence detectors, as shown in the Supporting Information (SI-Fig. 1). CBZ could be eluted in 4 min, which was much shorter than the previous report using chiral HPLC column (38 min) [4]. Moreover, the sharp and the asymmetric factor could be obtained (the peak width of CBZ at half height was 0.85 min and the asymmetric factor was 1.6), as shown in the UV

Table 1
Molecular structure and physicochemical properties of CBZ.

Compound	Structure	pK_a	Water solubility
CBZ		13.90	112

chromatogram. However, no signal was observed in the fluorescence chromatogram after electrochemical oxidation, indicating that the basic condition might have hampered the electrochemical reaction. This is consistent with the previous report pointing out that the oxidation of CBZ to form a strong fluorescent compound could be carried out only in the acidic condition [9]. Therefore, choice of eluent should be re-considered. However, from the UV chromatogram it could be found that NaOH plus ACN was still suitable to be used as the eluent for the separation of CBZ on the AS11 column. Determination of CBZ using IC-UV method is feasible.

Since anion exchange column could be used with a wide pH range (0–14), phosphoric acid (H_3PO_4) was chosen as eluent, and ACN or methanol was used as organic modifier. The fluorescence signal was observed after electrochemical derivatization, revealing that acidic supporting electrolyte has critical effect on the formation of fluorescent substance after electrochemical oxidation [9]. Consistent with reference [9], methanol greatly depressed the fluorescence signal in the photochemical process, while ACN had only slight influence on the fluorescence signal of CBZ. This observation was also demonstrated in the present study showing that the fluorescence signal (peak area mV min) of 1.0 mg L^{-1} CBZ with ACN as organic modifier was about 4.8 times larger than that of methanol with the same concentration. Furthermore, ACN has stronger eluting capability than that of methanol, for the retention time of 1.0 mg L^{-1} CBZ using 10% ACN as the organic modifier was 4.3 min, which was 5.0 min shorter than that using 10% methanol. As a result, H_3PO_4 and ACN were selected as the eluent. The maximum fluorescence signal of CBZ after electrochemical derivatization was obtained with the eluent of 100 mM H_3PO_4 and 10% ACN. The UV and fluorescence chromatograms of CBZ are shown in Fig. 2. There is a delay (0.45 min) in the fluorescence chromatogram in comparison with UV chromatogram as the UV detector was followed by the self constructed EC and fluorescence detector. Based on the above-mentioned experimental phenomena, as well as the structure and the physicochemical properties of CBZ, it could be speculated that CBZ might be eluted under a mixed-mode retention mechanism involving both reversed-phase interactions of the analyte with the unfunctionalised areas of the polymeric ion-exchange resin,

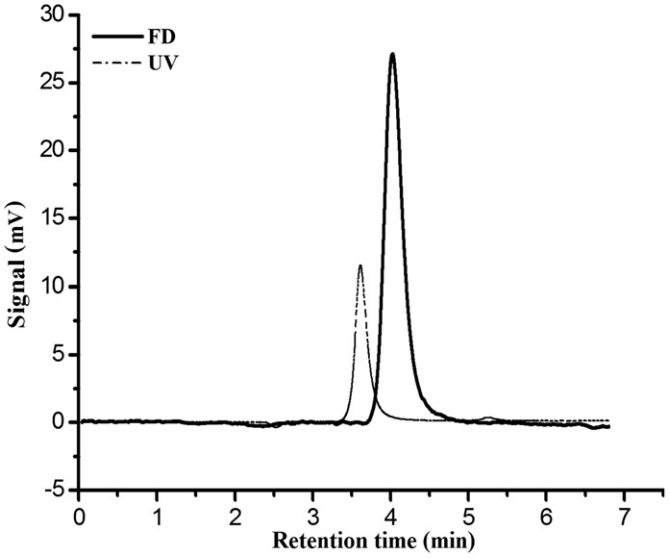


Fig. 2. The UV and fluorescence chromatograms of CBZ (1.0 mg L^{-1}) with acidic eluent. Column: IonPac AG 11 and AS 11 column. Eluent: 100 mM H_3PO_4+10% (v/v) ACN at the flow rate of 1.0 mL min^{-1}. UV detection: λ=225 nm. Fluorescence detection: $\lambda_{ex}/\lambda_{em}$=254/478 nm. Potential: 1.6 V.

and coulombic interactions of the analyte with the charged functional groups on the stationary phase (anion exchange mode) [16]. Furthermore, acidic eluent was specifically helpful to the production of fluorescence signal after electrochemical oxidation.

3.2. Choice of detection wavelengths

The experiment for the optimization of excitation and emission wavelength was carried out according to the previous report [9]. The potential of EC was set at 1.6 V in the whole process and the concentration of CBZ was 1.0 mg L^{-1}. The optimal excitation and emission wavelength for electro-oxidized CBZ was obtained at 254 and 478 nm, which was consisted with literature ……..[9].

3.3. Electrochemical derivatization of CBZ

Supporting electrolyte, which is simultaneously used as eluent in the current study, is very important for the electrochemical derivatization of CBZ. When 10 mM NaOH plus 10% ACN was initially employed as eluent/supporting electrolyte, no fluorescence signal was found (the potential was varied from 0 to ~4.0 V). Therefore, two types of post-column acidification were carried out to investigate the effect between the pH of the supporting electrolyte and the electrochemical oxidation behavior of CBZ. Namely, high concentration of H_2SO_4 (2.5 M) at the flow rate of 0.2 mL min^{-1} was added to the eluent using a mixing tee before and after EC, respectively. This is necessary because there might be two possible reaction ways for CBZ to emit fluorescence: one is that the electrochemical oxidation and the sequential fluorescence production of CBZ could only be carried out in the acidic solution. Therefore, H_2SO_4 was added before EC; the other is that CBZ could be electrochemically oxidized in the basic medium (10 mM NaOH), while the oxidation product might further react with H_2SO_4 which was added after EC. This final acidified product might have fluorescence property and give signal when detected by the fluorescence detector. The experimental results turned out that large fluorescence signal of 1.0 mg L^{-1} CBZ was observed as H_2SO_4 was put before EC. On the contrary, no fluorescence signal was produced as the addition of H_2SO_4 after EC. Those phenomena indicated that electrochemical oxidation of CBZ might not occur in basic medium but under acidic conditions [15] (pH=3 in reference).

3.4. Optimization of potential for electrochemical derivatization

The relationship between the potential of the EC and the fluorescence signal of CBZ was investigated using 100 mM H_3PO_4 and 10% ACN as the supporting electrolyte, as shown in Fig. 3. The fluorescence signal rose dramatically from the potential of 1.0 V and reached its maximum at the potential of 1.6 V. This observation might have resulted from the formation of dimer of CBZ [17], the connection of two benzene rings enlarged the covalent structure, which subsequently emitted much stronger fluorescence signal. However, the signal decreased remarkably when the potential exceeded 1.6 V. This phenomenon might be because of the oxidation of water at the anode due to the predominant electrode reactions, or the oxidative destruction of the primary analyte. As a result, the optimal potential was selected as 1.6 V.

3.5. Chromatographic performance and validation of procedure

The analytical data were obtained under the optimal experimental conditions which were described above. The linear equation of CBZ in aqueous solution was $y=18.81x-1.06$ with the

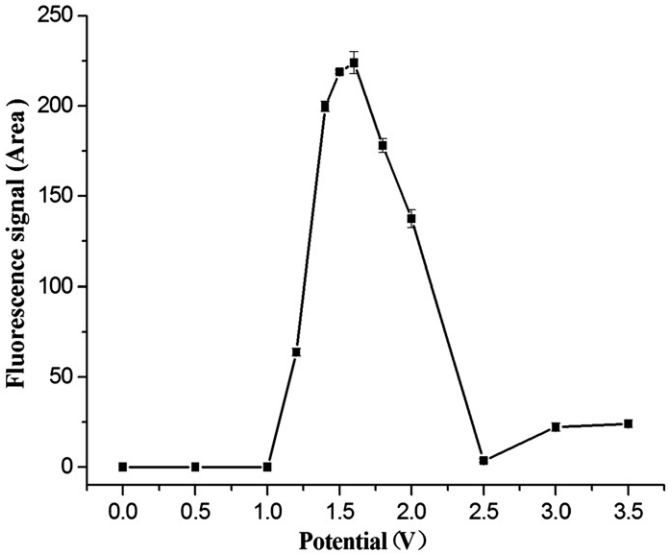


Fig. 3. Effect of electrochemical derivatization potential. The fluorescence signal of 10.0 mg L^{-1} CBZ at every potential was the mean of three determinations, and the standard deviations were from 0 to 3.2%.

Table 2
Spiked recoveries of CBZ in control blood sample procedure.

Spiked level (μg L^{-1})	Recovery (%)	R.S.D. ($n=3$) (%)
Within day		
25	84.3	3.7
75	102	1.7
150	112	0.9
Day-to-day		
25	76.2	6.2
75	82.5	4.7
150	102	3.6

linear range of 5–2000 μg L^{-1} and correlation coefficient (R^2) of 0.9999. The limit of qualification (LOQ, based on signal-to-noise ratio of 10, $S/N=10$) was 2.0 μg L^{-1} and limit of detection (LOD, $S/N=3$) was 0.6 μg L^{-1}. The linear range for CBZ in human plasma was 10–200 μg L^{-1} with the correlation coefficient (R^2) of 0.9992, and the LOQ and LOD for plasma were 3.5 and 1.3 μg L^{-1}, respectively. Intra-day and inter-day relative standard deviation (R.S.D.) values were found to be within 3.7% and 6.2% for plasma, respectively. The statistical parameters are given in Table 2.

3.6. Interference effect

Oxcarbazepine is the derivative of CBZ and may interfere with the determination of CBZ in real sample analysis, such as wastewater [18]. Its potential interference effect on CBZ in human plasma was tested using UV and FD, respectively. The chromatogram is shown in the Supporting Information (SI-Fig. 2). No fluorescence signal was observed for OXC, indicating that OXC could not emit fluorescence after electrochemical oxidation. Furthermore, the resolution of OXC was determined as 2.21 via European Pharmacopeia (EP) formula which used the next main peak (CBZ) in the chromatogram as reference peak. However, our experiment revealed that the resolution in the UV chromatogram decreased dramatically with the increasing concentration of OXC. Furthermore, when the concentration of OXC was 20 mg L^{-1}, the resolution was 0.5, indicating that OXC had interference effect on the determination of CBZ. From the UV chromatogram in SI-Fig. 2 it could be concluded that OXC, which was usually separated on the silica-based RP-HPLC column [4], could also be eluted on the AS11 column with the present experimental conditions. Therefore, determination of OXC using IC technique would be quite possible, and this work is under way in our lab.

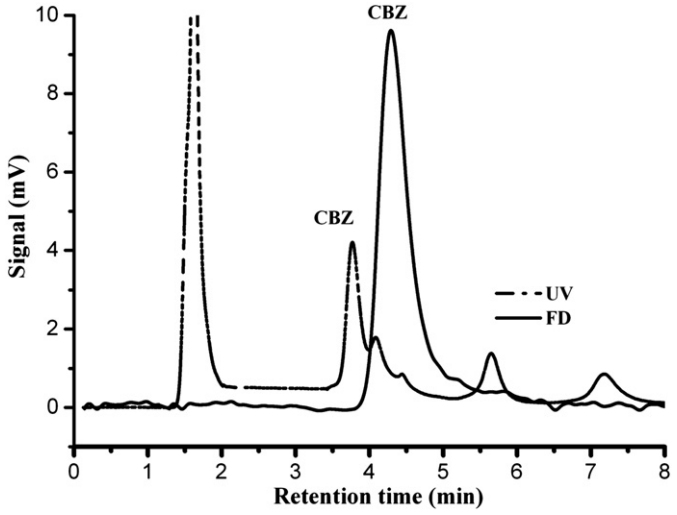


Fig. 4. Chromatograms of CBZ in sample no. 2. The experimental conditions are the same as in Fig. 2.

3.7. Real sample analysis

Determination of CBZ in human plasma was performed under the optimized experimental conditions described above. The chromatogram of sample no. 1 with 100-fold dilution is shown in the Supporting Information (SI-Fig. 3). CBZ could be detected only with IC-ED-FD method other than IC-UV, which demonstrated that the sensitivity of the fluorescence detection after electrochemical derivatization was much higher than that of IC-UV. Relatively higher concentration of CBZ in sample no. 2 was detectable with both UV and FD, as shown in Fig. 4. From the fluorescence chromatograms (SI-Fig. 3 and Fig. 4) of these two samples it could be found that CBZ had a wider peak bandwidth than that of the earlier reported figures, such as Fig. 2 and SI-Fig. 2. This is because the real samples had much more complex matrix than that of standard solutions. Some unknown compounds which coexisted with CBZ would affect the peak shape of the target analyte. For example, these unknown substances were obviously observed during the elution time of CBZ in UV chromatogram, as shown in Fig. 4. However, the fluorescence chromatogram of CBZ is much cleaner than that of UV, indicating that the present method has the great potential to decrease the chance for possible interference from complex matrix. From this part it could be concluded that the present method had much better sensitivity and selectivity than that of IC-UV. The analytical results are shown in Table 3. The spiked recoveries ranged from 78.5 to 114%.

4. Conclusion

In this paper, determination of CBZ in human plasma was performed using ion chromatography combined with online electrochemical derivatization and fluorescence detection. CBZ could be well separated on the anion exchange column using either acidic or basic eluent. Moreover, CBZ was specifically sensitive to fluorescence detection after electrochemical derivatization under acidic condition. A good selectivity could be obtained by the combination of IC separation, electrochemical

Table 3
Analytical results of samples with the present method.

Sample no.	[a]Found ($\mu g\ L^{-1}$)	Spiked ($\mu g\ L^{-1}$)	[b]Reclaimed ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%) ($n=3$)
[c]1	18.2 ± 0.12	20.0	33.9 ± 0.09	78.5	4.8
2	420 ± 0.40	500	990 ± 0.06	114	1.3

[a] Each represents the value (mean $\pm$ SD) of three determinations.
[b] "Reclaimed" means the concentration value of the CBZ in the real sample which was spiked with the standard solution.
[c] Sample no. 1 with 100-fold dilution.

derivatization and fluorescence detection. The present work demonstrated again that some organic compounds, which have been conventionally separated with silica-based HPLC column, can also be well determined using IC technique [12]. The current study further broadens the application of IC/ED/FD.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.09.039.

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