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Polymer-coated fibrous extraction medium for sample preparation coupled to microcolumn liquid-phase separations

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

Polymer-coated fibrous material has been introduced as the extraction medium for a miniaturized sample preparation method being coupled with microcolumn liquid chromatography. The preconcentration and the subsequent liquid chromatographic separation of tricyclic antidepressants (TCAs) drugs, amitriptyline, imipramine, nortriptyline and desipramine, was carried out with the hyphenated system. Several basic experimental parameters, such as extraction and separation conditions, were investigated along with the applicability of the method for the analysis of biological fluids. The results clearly showed that the on-line coupled system could be a powerful tool for the analysis of complex mixtures in biological matrix without a large solvent consumption and specially designed instruments. The lowest limit of quantification was quite acceptable for the analysis of TCAs in clinical and forensic situations.

Keywords: Polymer-coating; Fibrous extraction medium; Tricyclic antidepressant drugs; Sample preparation; On-line coupling

1. Introduction

The use of tricyclic antidepressant (TCA) drugs such as amitriptyline, imipramine, nortriptyline and desipramine are becoming increasingly prevalent for the medical treatment of depression, and these drugs are frequently encountered in emergency toxicology screening, drug-abuse testing and forensic examinations. The extraction and isolation of antidepressants in human fluids are very important for the analysis of these drugs in toxicological, pharmaceutical and forensic purposes [1,2], because of their clinical effects through an interaction with noradrenergic or serotonergic systems [3]. For the determination of TCAs in human fluids and tissues, some sample preparation

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methods have been proposed. Liquid–liquid extraction has been widely employed for the sample preparation of TCAs [4,5], but this method possesses some disadvantages, such as the use of a vast amount of organic solvents, the complex multi-step procedures, and it leads to low accuracy and loses the analytes.

Solid-phase microextraction (SPME) has been developed by Pawliszyn et al. as a solvent-free sample preparation method for the analysis of volatile organic compounds by gas chromatography (GC) [6–10]. In the SPME technique, a fused-silica rod with polymeric coatings thereon is inserted into a sample solution or headspace in the sample vial during the extraction process, and then the extracted compounds are thermally desorbed in the GC injection port. Therefore, the technique is quite powerful for the analysis of volatile and thermally stable compounds. However, most of organic compounds cannot be analyzed by GC because they are non- or semi-volatile and some of them are thermally unstable. In order to analyze such compounds, an on-line coupling of SPME with liquid-phase separation techniques, such as liquid chromatography (LC) or capillary electrophoresis should be required. To realize the on-line coupling, some miniaturized sample preparation techniques have been proposed [11–29]. The SPME method has been further developed as the sample preparation for liquid-phase separation techniques with specially designed desorption interfaces [11–21] and with fused-silica GC columns as the extraction device [22–26]. Rasmussen et al. reported on the liquid-phase microextraction technique with a porous polypropylene hollow fiber as the extraction device [27], and a preconcentration method with polymeric membrane materials has been introduced by Tomlinson et al. [28]. Fritz et al. also reported the use of resin disks as a miniaturized solid-phase extraction (SPE) medium [29].

Recently, a specially-designed miniaturized sample preparation method, fiber-in-tube solid-phase extraction (FIT-SPE) [30–34], has been introduced by Saito and Jinno et al. In the technique, the extraction was carried out in the short capillaries packed longitudinally with the fine filaments of solvent-resistant synthetic polymer as the extraction medium. Comparing with conventional particle-packed SPE cartridge, the FIT-SPE device shows a reduced pressure drop during the extraction and desorption. Furthermore, the undesirable plugging effect from insoluble materials in real sample matrices can be minimized in the FIT configuration. Another advantage of the FIT is the possibility for coating polymeric materials on the filaments to enhance the extraction power with an appropriate type of coating onto the fibrous material packed in the extraction tube [34,35].

In this work, a polymer-coated fibrous material has been introduced as the extraction medium for the on-line sample preparation method in micro-column LC (micro-LC). The preconcentration and the subsequent chromatographic separation of four TCA drugs, amitriptyline, imipramine, nortriptyline and desipramine (Fig. 1), was performed in the hyphenated system. Several basic experimental parameters, such as extraction and separation conditions, were investigated along with the applicability for biological fluids.

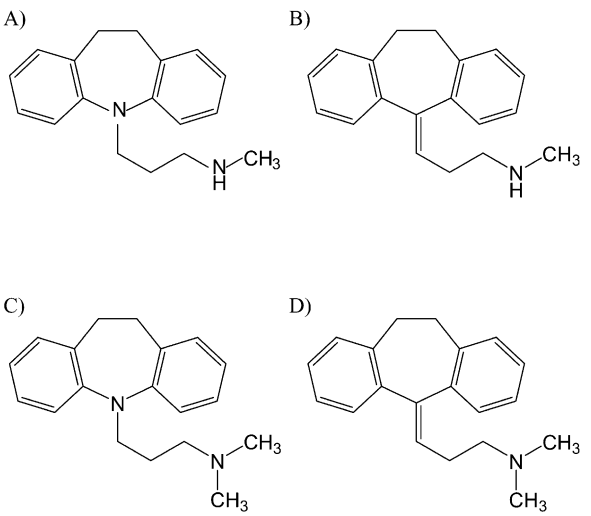

Fig. 1. TCA drugs used in this work. (A) desipramine, (B) nortriptyline, (C) imipramine and (D) amitriptyline.

2. Experimental

2.1. Reagents and materials

Four TCA drugs, amitriptyline (Banyu Pharmaceutical Co., Ltd, Tokyo, Japan), imipramine (Dainippon Pharmaceutical Co., Ltd, Osaka, Japan), and nortriptyline and desipramine (Ciba-Geigy Japan, Tokyo Japan) were kindly donated from each manufacture. All other reagents and solvents were purchased from Tokyo Kasei (Tokyo, Japan) and Kishida Chemical (Osaka, Japan). These reagents and solvents were of analytical reagent grade and used without any purification process. For the preparation of mobile phase and standard sample solutions, water was purified with a Milli-Q water purification system (Millipore, Tokyo, Japan).

Zylon®, poly(*p*-phenylene-2,6-benzobisoxazole), fiber (AS-type) was obtained from Toyobo (Ohtsu, Japan). The chemical structure of Zylon® fiber is shown in Fig. 2.

2.2. Preparation of extraction capillary

The packing of the filaments of Zylon® fibers (11.5 μm o.d.) was made as the following procedure. First, insert the guide fiber into a fused-silica capillary (0.32 mm i.d., 1.0 m length), and then the end of the guide fiber through the capillary is inserted into the tube again, while the guide fiber forms a loop at the outside of the capillary. Next, the filaments of fiber-packing materials are inserted into the loop of the guide fiber, and pull the guide fiber from another end of the capillary to pack these filaments. Then, the length of the packed filaments was adjusted to the same length as the outer capillary by cutting. The suitable number of packed filaments (~ 330 filaments in 0.32 mm i.d. capillary) for the preparation of coated-fiber packed capillary was determined as a half of non-coated one by the preliminary experiments [34].

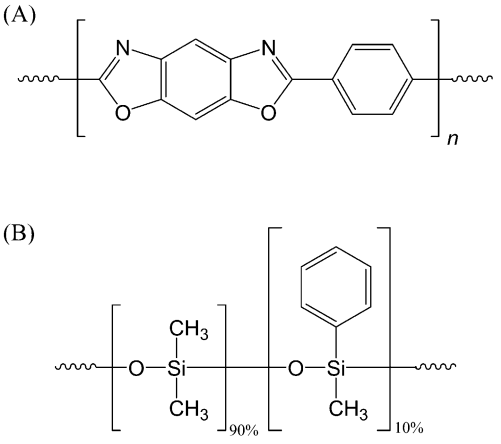

Fig. 2. Chemical structures of (A) Zylon® fiber and (B) HR-52 polymer.

After the fiber-packing process as described above, the polymer coating onto the packed-filaments was carried out just similar to the preparation of open-tubular GC column as described previously [34,35]. As the coating reagent, 5% solution of HR-52 (5%-phenyl-polydimethylsiloxane, Shinwa Chemical) polymer in *n*-hexane/acetone (90/10) was used. The chemical structure of the coating polymer is illustrated in Fig. 2.

The reproducibility for the preparation of the extraction tubing was studied in the preliminary experiments and the results demonstrated that the relative standard deviations (RSDs) for these capillaries, as determined by the extraction power, were less than 3.0% for five extraction capillaries. Although the extraction efficiency should be further investigated for various coated-fiber packed capillaries prepared with different polymer concentration (i.e. film thickness) and structures, the preliminary results [26,32] clearly showed the satisfactory validity of the present extraction capillary as the preconcentration device for TCAs from an aqueous matrix.

2.3. LC measurements

The micro-LC system consisted of a Micro-Tech Scientific Ultra-Plus II Capillary LC Pump (Yamato Scientific, Tokyo, Japan), a UV 2075 Plus UV/Vis detector (Jasco, Tokyo Japan), and two Model 7000 six-port valves (Rheodyne, Cotati, CA). One of these valves was used as the switching valve and the other was employed as the injection valve. Schematic diagram of this system is shown in Fig. 3. For the pumping of sample solution and desorption solvent, two syringe pumps (Microfee-

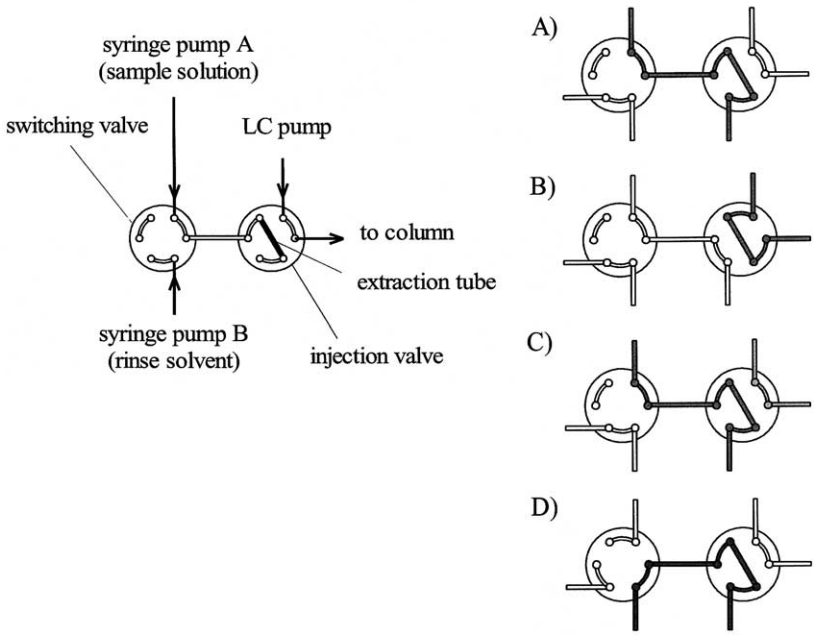

Fig. 3. On-line coupling system of the fiber-packed extraction capillary and micro-LC. (A) extraction, (B) desorption and injection, (C) separation and the next extraction, and (D) re-conditioning processes.

der MF-2, Azumadenki, Tokyo, Japan) were connected to the switching valve. The chromatographic separation was performed with a commercially available octadecylsilica phase, Capcell Pak C18 MG (1.0 mm i.d., 150 mm length, 5 μm particle size, Shiseido, Yokohama, Japan). The mobile phase was prepared with acetonitrile and water.

2.4. Extraction process

For the extraction process (Fig. 3A), a sample solution was pumped through the extraction tube by one of the syringe pumps at a typical flow-rate of 50 μl/min, while the analytes were extracted in the extraction tube installed to the injection valve as the loop. The volume of the sample solution extracted was studied in the range from 125 to 1000 μl on the basis of the preliminary results. As shown in Fig. 3B, the desorption of the extracted analytes was carried out with a flow of mobile phase solvent. The separation and the next extraction can be processed simultaneously by changing the injection valve after a certain period of time for injection (Fig. 3C), which is an advantageous feature for the analysis of multiple samples with an automated system.

The extraction capillaries also exhibited a good stability for repeatable use, typically more than 10 runs without any significant problems, such as a decrease in the extraction power and an increase in the pressure drop through the capillaries. If, in case, the performance was slightly decreased after the consecutive extraction of more than ten times, a simple washing/re-conditioning process with acetonitrile (Fig. 3D), could be employed to make sure the reproducible results in the next 10 extractions. Furthermore, no statistical variation was observed between the replicates of the same extraction tube within the same day and between days.

2.5. Data processing

For data acquisition and processing, V-station chromatography data handling software (GL Sciences, Tokyo, Japan) running on the computer was employed. All measurements were performed

at least three times and the RSDs of retention time and peak area were less than 3.0%.

3. Results and discussion

Fig. 4 shows the effect of the extraction time on the peak areas of four TCAs of 10 ng/ml each in water. A good linear correlation is found for all TCAs over the extraction time range from 2.5 to 20 min. Similar trend was also observed for other standard samples containing 1, 5, 50 and 100 ng/ml of each solute. Although the extraction time more than 20 min could be selected, the extraction time was determined as 10 min for the following experiments taking into account the total analysis time. The linear calibration ranges for these TCAs were also evaluated with several standard solutions and found to be in the range from 1 ng/ml to more than 100 ng/ml with the regression coefficient of more than 0.990 for all TCAs studied.

Typical chromatogram for the separation of TCA drugs is shown in Fig. 5, where an aqueous standard solution of four TCAs was extracted by passing into a polymer-coated fiber-packed capil-

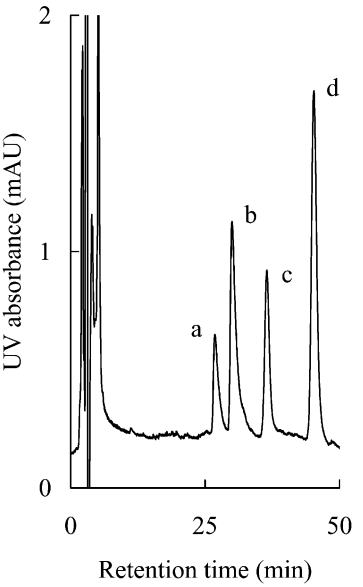

Fig. 5. Chromatogram for the analysis of a standard sample. Sample concentration: 10 ng/ml each. Peaks: (A) desipramine, (B) nortriptyline, (C) imipramine and (D) amitriptyline. Other conditions are in the text.

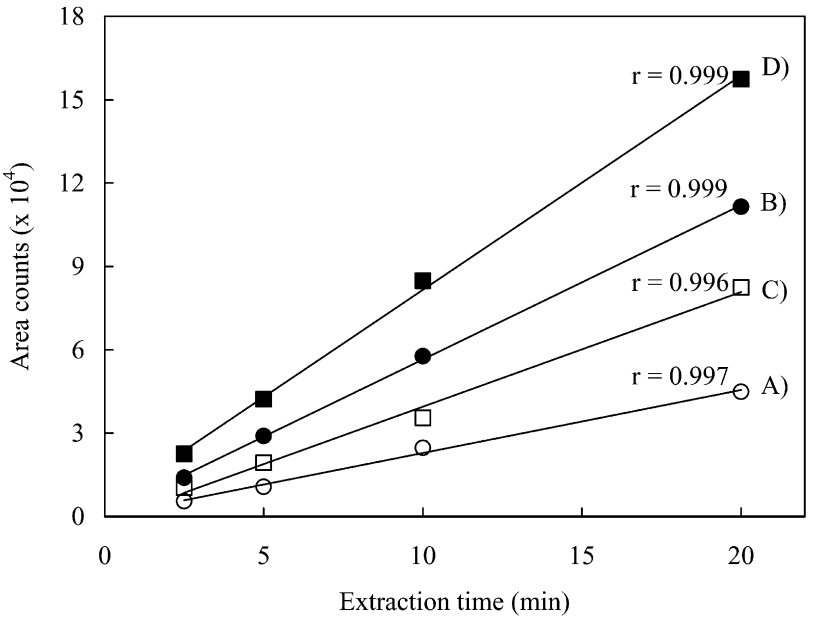

Fig. 4. Effect of the extraction time on the peak areas of TCAs. Extraction flowrate: 50 μl/min. Sample: (A) desipramine, (B) nortriptyline, (C) imipramine and (D) amitriptyline. Other conditions are in the text.

lary for 10 min at the flow-rate of 50 μl/min. From the preliminary experiments on the optimization of chromatographic separation, triethylamine as the mobile phase additive (0.03%) was introduced to minimize an undesirable peak tailing during the separation process [26].

The preconcentration factors for the determination of four TCAs are summarized in Table 1, where the RSDs for the five repeatable determination of the standard sample containing 10 ng/ml each of these drugs were also tabulated. The preconcentration factor of this sample preparation method was calculated by injecting the standard solution in a conventional way, in which the standard sample having a higher concentration (100 ng/ml) was prepared for direct. As described previously [30,31], the injection volume of the direct analysis was adjusted the same as in the internal void volume (5 μl) of the coated-fiber packed capillary. Although the preconcentration factor for these TCAs, especially for polar drugs, should be further improved for the complete extraction by changing the pH value of the sample solution [18,26], it can be said from Table 1 that the present preconcentration method is quite promising as a sample preparation method of these drugs without a large solvent consumption and specially designed instruments. As can be seen, the preconcentration factors in Table 1 also show the extraction yields for these TCAs (as % values). The volume of solvent (acetonitrile) needed, as the mobile phase component, for each analysis was about 1.3 ml.

In order to confirm the applicability of the sample preparation technique for the analysis of real biological sample matrix, the determination of amitriptyline was carried out. Fig. 6 shows a typical chromatogram of a controlled urine sample spiked with amitriptyline of 10 ng/ml. The recovery was determined as about 23% in this particular analysis indicating the same extraction efficiency as the standard water sample. In contrast to the analysis of the standard sample, the desorption and separation conditions should be optimized more for real sample matrix taking into account the adsorption of interferences in the matrix. However, as shown in Table 2, where the lowest limits of quantification (LOQ) for these TCAs were tabulated, the determination range is seemed

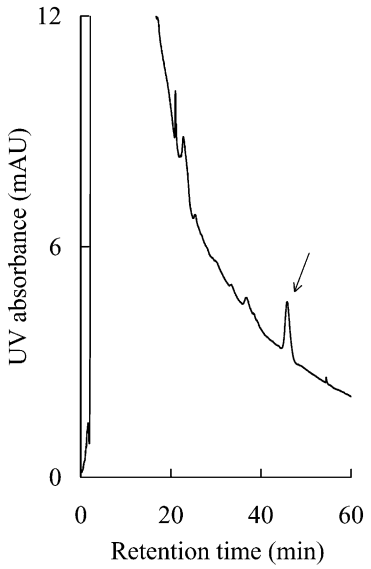

Fig. 6. Typical chromatogram for the analysis of a controlled urine sample spiked with amitriptyline. Concentration of amitriptyline: 10 ng/ml. Other conditions are in the same as in Fig. 5.

Table 1
Preconcentration factors and the RSDs for four TCAs with the polymer-coated fiber-packed capillary as the preconcentration device

	Preconcentration factor	RSD (%)
Desipramine	11.3	5.1
Nortriptyline	13.3	3.5
Imipramine	27.3	5.5
Amitriptyline	23.4	4.1

Calculated for the standard sample containing 10 ng/ml each of TCAs ($n = 5$) with the method described in the text.

Table 2
LOQs for four TCAs with the polymer-coated fiber-packed capillary as the preconcentration device

	LOQ (ng/ml)
Desipramine	1.8
Nortriptyline	0.9
Imipramine	1.1
Amitriptyline	0.5

Determined as S/N = 10.

to be quite acceptable for the rapid analysis of these drugs in clinical and forensic situations [18,26,32].

4. Conclusions

Miniaturized sample preparation method has been developed with polymer-coated fibrous material as the extraction medium for the determination of TCA drugs. The preconcentration and the subsequent chromatographic separation of four typical TCAs, amitriptyline, imipramine, nortriptyline and desipramine, was successfully carried out with the on-line coupled system consisted of the coated-fiber packed-capillary extraction and micro-LC.

Although the extraction and desorption conditions for the analysis of the real biological samples including the type of polymeric coating and fibrous materials [36–38] should be further studied, the results clearly showed that the hyphenated system could be a powerful tool for the rapid determination of TCAs in biological matrix without a large solvent consumption and specially designed instruments.

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