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Short communication

# Novel quartz flow-cell as a post-column photochemical reactor for high-performance liquid chromatography

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**Abstract**

The construction of a new post-column photochemical reactor with quartz flow cells in series for high-performance liquid chromatography (HPLC) is described. The performance of the new reactor was compared with a conventional open tubular PTFE coil reactor. The sensitivity, accuracy and precision obtained with both reactors are comparable. The new reactor has the obvious advantages of smaller cell volume as well as inertness and resistance to not only light and heat produced by the UV lamp, but also to organic solvents in the mobile phases, which results in greatly improved durability, reduced peak broadening and shorter chromatographic run times. Application of the new reactor to the fluorescence detection of DU-6859a, a new fluoroquinolone antimicrobial agent, in human serum is reported. © 1998 Elsevier Science B.V.

*Keywords:* Quartz flow cell; Post-column reactors; Detection, LC; DU-6859

## 1. Introduction

Photochemical reaction detection is a variation of on-line post-column derivatization, which has found particular usefulness in biomedical, pharmaceutical and forensic analyses. It is employed to determine highly diverse types of compounds by high-performance liquid chromatography (HPLC) with UV, fluorescence and electrochemical detection [1–6].

Several workers [7–11] have described the construction of post-column photochemical reactors, most of which employed PTFE tubing as the reaction coils. Attempts to use quartz capillaries as reaction coils, to assure good transparency to the incident UV light, were unsuccessful because quartz capillaries are expensive and fragile and tight connections were not easily made.

This paper describes the development of a new photochemical reactor that consisted of two quartz flow cells connected in series (total cell volume, 290 μl) and a 20-W U tube UV-lamp. The performance of this new reactor was compared to that of a photochemical reactor with a coiled PTFE tube. As an application, the determination of DU-6859a, a

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new fluoroquinolone antimicrobial agent [12], is reported.

## 2. Experimental

### 2.1. Chemicals and reagents

7-[(7*S*)-7-Amino-5-azaspiro[2,4]heptan-5-yl]-8-chloro-6-fluoro- 1-[(1*R*, 2*S*)-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid sesquihydrate (DU-6859a; purity, 100.2%) and the internal standard (I.S.) used for the serum assay (DX-9484; purity, 98.6%) were synthesized in Daiichi Pharmaceuticals (Tokyo, Japan). The chemical structures of DU-6859a and DX-9484 are shown in Fig. 1. DU-6859 represents the anhydrate of DU-6859a. Tetrahydrofuran (THF) was a HPLC-grade solvent (Kanto Chemicals, Tokyo, Japan). All other chemicals were of analytical-reagent grade and were used without further purification. Purified water (Milli-Q system, Waters, Millipore, Milford, MA, USA) was used in all aqueous solutions. Consera (Nissui Seiyaku, Tokyo, Japan) was used as control human serum.

### 2.2. HPLC instruments

The chromatographic system consisted of a high-performance liquid chromatograph (Model LC-10AD, Shimadzu, Kyoto, Japan), a fluorescence detector (Model F-1080, Hitachi, Tokyo, Japan), operated at an excitation wavelength of 280 nm and at an emission wavelength of 430 nm, and a reversed-phase Inertsil ODS-2 (150×4.6 mm I.D., 5 μm particle size) column (GL Sciences, Tokyo, Japan). A precolumn filter (Sumika Chemical Analysis Services, Osaka, Japan) was attached in front of the analytical column. Samples of 50 μl were injected automatically to the HPLC column by an autosampler (Model AS-8000, Tosoh, Tokyo, Japan).

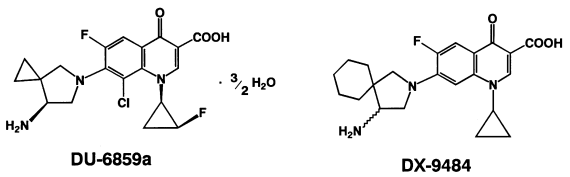


Fig. 1. Chemical structures of DU-6859a and DX-9484.

### 2.3. Photochemical reactors

Two photochemical reactors (reactor 1 and reactor 2) were used in this study. Reactor 1 was constructed by preparing a 20-m open tubular reactor using a PTFE tube (0.3 mm I.D. and 0.5 mm O.D.; Chukoh, Tokyo, Japan). This coil was placed around a 10-W UV lamp (330×25.5 mm diameter; Irica, Kyoto, Japan). The lamp and coil assembly was located inside a reactor housing and air cooling was by means of a fan. The volume of the reaction coil was 1410 μl.

Reactor 2 (Model Σ-9842, Irica) had a completely different design. It consisted of two quartz flow cells connected in series (total cell volume, 290 μl), and was placed at a distance of 24 mm from the center of a 20-W U tube UV lamp (Irica). The quartz flow cell was a large rectangular quartz plate (10×10×121 mm) with a channel (6×0.2×121 mm). Two quartz flow cells were placed in a stainless cell holder and connected by a set of PEEK side holders and a connecting tube. The design of reactor 2 is shown in Fig. 2.

### 2.4. Analytical methods

The concentrations of DU-6859 in human serum were determined using a HPLC method as previously reported [13]. In brief, DU-6859 and the I.S. were extracted from serum using a Bond Elut $C_8$ LRC column. The extracts were chromatographed on a reversed-phase column using tetrahydrofuran–50 m*M* $KH_2PO_4$ (adjusted to pH 2 by adding phosphoric acid)–1 *M* ammonium acetate (19:81:1, v/v) as the mobile phase at a flow-rate of 1.0 ml/min (method 1). The analytes eluted from the analytical column were irradiated with UV light in reactor 1.

All analytical conditions were the same as those for method 1, except that reactor 2 was used instead of reactor 1 for post-column photolysis of DU-6859.

## 3. Results and discussion

DU-6859a, a new fluoroquinolone (Fig. 1), has a

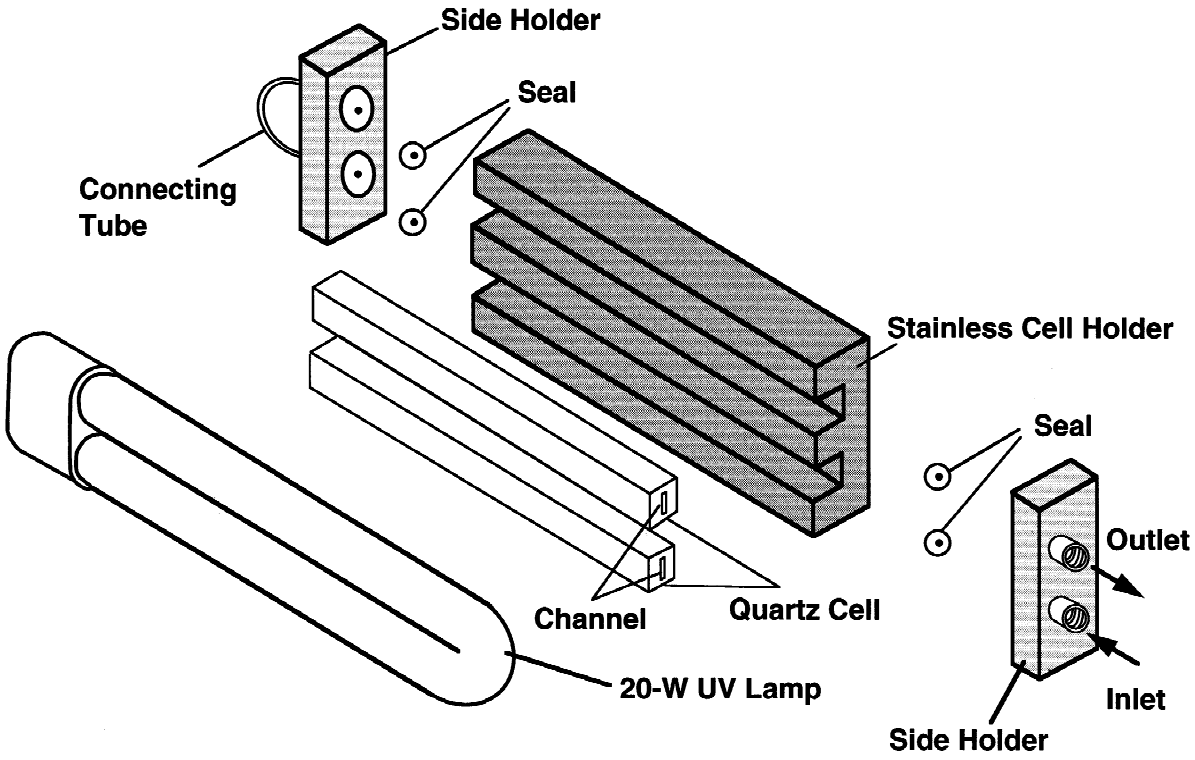


Fig. 2. Schematic diagram showing how two quartz flow cells were positioned with respect to the U-tube UV lamp.

chlorine atom at the 8-position of the quinolone ring [12]. It was found that the photo-decomposition product(s) of DU-6859a showed much stronger fluorescence properties compared to DU-6859a. This fact was used to develop a highly sensitive and selective HPLC method for the determination of DU-6859 in human serum and urine [13], where we found that post-column photolysis improved the sensitivity for the detection of DU-6859 by a factor of approximately 43. We had previously used reactor 1, which consisted of a reaction coil made of a PTFE capillary (20 m) wound around a 10-W UV-lamp. It was reported that when a PTFE capillary was used in a photochemical apparatus, fluoride was liberated from the tubing during irradiation with UV light [14] and that this caused the tubing to turn brittle and, eventually, to rupture. In addition, it has been our experience that tetrahydrofuran, which is used as an organic modifier in the mobile phase, would also reduce the durability of the reaction coil. Selavka et al. [11] reported that the use of organic solvents other than methanol, ethanol or acetonitrile would cause swelling, leakage or rupture of the PTFE tubing. Therefore, in the previous study, the reaction coil had to be replaced due to leakage of the PTFE tubing after about one month of use.

It is obvious that for photochemical reactions the optical and physicochemical properties of the material used for the reactor are of great importance. Compared to the PTFE tubes, quartz assures not only much better transparency to UV light but also inertness and resistance to light, heat and organic solvents.

In the present study, we developed a new photochemical reactor (reactor 2). The improved durability of the quartz cell allows the use of a 20-W UV-lamp instead of the 10-W UV-lamp that was used in reactor 1.

In order to compare the performance of the reactors, the precision and accuracy of methods 1 and 2 were evaluated by analyzing human serum spiked with 0.00745–0.947 μg/ml of DU-6859 ($n$=

Table 1
Precision and accuracy for the determination of DU-6859 in human serum by methods 1 and 2

| Theoretical concentration (μg/ml) | Method 1 | | | Method 2 | | |
|---|---|---|---|---|---|---|
| | Mean found concentration (μg/ml) | C.V.[a] (%) | R.E.[b] (%) | Mean found concentration (μg/ml) | C.V.[a] (%) | R.E.[b] (%) |
| 0.00745 | 0.00812 | 5.3 | 9.0 | 0.00746 | 14.1 | 0.1 |
| 0.00149 | 0.0154 | 2.2 | 3.5 | 0.0148 | 2.8 | −0.8 |
| 0.0298 | 0.0299 | 3.0 | 0.3 | 0.0294 | 9.6 | −1.5 |
| 0.0595 | 0.0576 | 3.3 | −3.1 | 0.0577 | 2.9 | −3.0 |
| 0.119 | 0.117 | 2.2 | −1.7 | 0.117 | 1.0 | −1.3 |
| 0.237 | 0.231 | 4.4 | −2.7 | 0.235 | 2.9 | −0.9 |
| 0.474 | 0.446 | 3.0 | −5.9 | 0.459 | 2.7 | −3.1 |
| 0.947 | 0.959 | 3.7 | 1.2 | 0.955 | 1.9 | 0.8 |

[a]C.V.=coefficient of variation, $n=5$.[b]R.E.=relative error.

5) (Table 1). The drug concentrations determined by methods 1 and 2 correlated well with each other. The accuracy and precision obtained with both methods are acceptable for clinical pharmacokinetic studies. The C.V.s were 2.2–4.4% for method 1 and 1.0–9.6% for method 2; the relative errors ranged from −5.9 to 3.5%, and from −3.1 to 0.8%, respectively, at concentrations above 0.0149 μg/ml.

Representative chromatograms of control serum spiked with DU-6859 (0.237 μg/ml) and the I.S., obtained with methods 1 and 2, are shown in Fig. 3. Peaks for DU-6859 and the I.S. were well resolved from each other.

The volume of the quartz flow cell is approximately one fifth of that of the reaction coil, which resulted in improved peak shape and shorter chromatographic run times, as can be seen in Fig. 3. The smaller cell volume also results in shorter residence time of analytes in the photochemical reactor, which would reduce the yields of photochemical reactions. The residence time of DU-6859 in reactors 1 and 2 were approximately 1.4 and 0.15 min, respectively. The intensity of the fluorescence signal obtained with reactor 2, however, was comparable to that observed with reactor 1, possibly due to the excellent transparency of the quartz flow cell towards UV light and also because of the stronger UV light used in reactor 2. For both methods 1 and 2, fluorescence detection at an excitation wavelength of 280 nm and at an emission wavelength of 430 nm resulted in a limit of quantitation of 0.01 μg/ml for serum. Furthermore, the crystal flow cell is very stable in the presence not

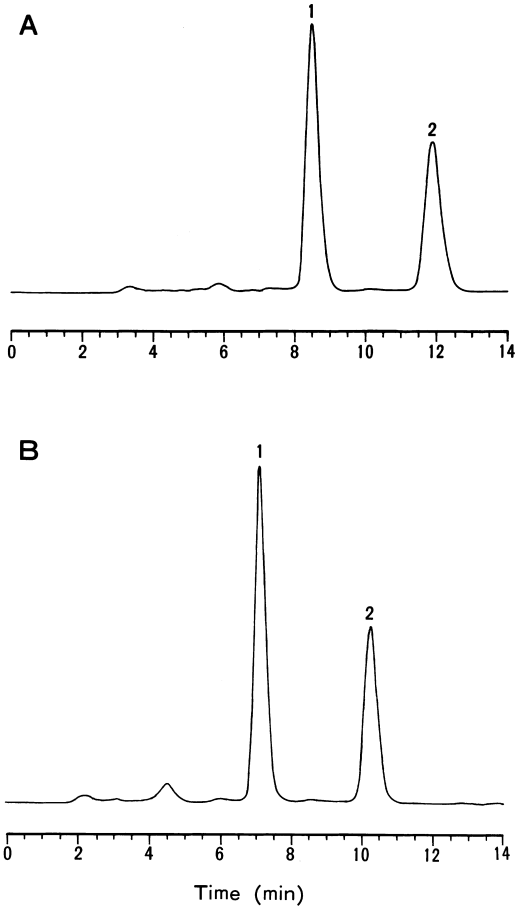


Fig. 3. Representative chromatograms of extracts of control human serum spiked with I (0.237 μg/ml) and II. (A) Obtained with method 1 and (B) with method 2. The chromatograms are shown with a full scale of 360 mV.

only of light and heat but also of organic solvents. Reactor 2 has significantly improved durability and permits the use of various kinds of organic solvents as modifiers in the mobile phases, which could not be used in PTFE photochemical reactors.

## 4. Conclusion

The newly developed quartz flow cell is much more suitable for use in photochemical reactors than conventional PTFE tubing. Its effective energy transfer ensures rapid photochemical reactions and, therefore, intense signals after relatively short residence times. The improved durability of the cell adds to the advantages of the quartz flow cell over PTFE coils. The new quartz flow cell reactor will extend the applicability of post-column photochemical derivatization in HPLC.

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