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Application News

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Spectrophotometric Analysis

Low-Concentration Measurement of Duloxetine Hydrochloride by RF-6000 Spectrofluorophotometer

Because pharmaceutical compounds express efficacy and side-effects even in trace amounts, control of the contents of all ingredients contained in pharmaceutical products is critical for ensuring the quality and safety of those products. When the content of a target ingredient is a trace amount, analytical techniques utilizing high-sensitivity and high-selectivity fluorescence detection can be applied.

Here, the antidepressant duloxetine hydrochloride was measured, and its lower limit of detection and lower limit of quantitation were evaluated.

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Low-Concentration Measurement by RF

Analytical techniques using light include absorption photometry and fluorescence spectroscopy. In absorption photometry, light is irradiated on a substance and the light transmitted through the substance is measured. In fluorescence spectroscopy, light is irradiated on the target substance and the fluorescence emitted by the substance is measured.

In absorption photometry, Lambert-Beer's law holds, as shown in Eq. (1). Assuming a constant optical path length, absorbance is proportional to concentration. On the other hand, in fluorescence spectroscopy, fluorescence intensity is expressed by the product of the quantity of light absorbed by the sample and the quantum yield, which shows the luminous efficiency of fluorescence, as shown in Eq. (2). For low-concentration samples, Eq. (3) is derived from Eq. (1) and Eq. (2). Because the wavelength is fixed and the optical path length is also constant in quantitative analysis, fluorescence intensity is proportional to concentration, in the same way as absorbance in absorption photometry.

The sensitivity of absorption photometry is decided by the detection limit of the difference in the quantity of light of the reference luminous flux and the sample luminous flux, whereas in fluorescence spectroscopy, the light emission intensity is detected from the zero state. As a result, the sensitivity of this method is 100 to 1,000 times higher than that of absorption photometry, making this method suitable for measurement of low-concentration samples.

$$\mathrm{Abs} = \mathrm{Log}_{10}\,(I_0/I) = \varepsilon cL \qquad (1)$$

Abs: absorbance, I_0: intensity of irradiated light,
I: intensity of transmitted light, ε: molar absorption coefficient,
c: concentration of sample, L: optical path length

$$F = (I_0 - I)\Phi \qquad (2)$$

F: fluorescence intensity, I_0: irradiation intensity of excitation light,
I: intensity of transmitted light, Φ: quantum yield

$$F = 2.303\ \varepsilon cL\Phi\ I_0 \qquad (3)$$

Instrument Used

Fig. 1 shows the appearance of the Shimadzu RF-6000 spectrofluorophotometer used in this experiment. The RF-6000 realizes the highest level SN ratio in its class, SN ratio = 1,000 or more (RMS), supporting measurement of low-concentration samples.

Fig. 1 RF-6000 Spectrofluorophotometer

Duloxetine Hydrochloride

Duloxetine hydrochloride displays efficacy and effectiveness in treatment of depression and depressive states, diabetic neuropathy, fibromyalgia, chronic low back pain, and pain associated with osteoarthrosis. Table 1 shows the characteristics of duloxetine hydrochloride [1], and Fig. 2 shows its chemical structural formula.

Table 1 Characteristics of Duloxetine Hydrochloride

Chemical name	:	(+)-(S)-N-Methyl-3-(1-naphthyloxy)-3-(2-thienyl) propylamine monohydrochloride
Molecular formula	:	$C_{18}H_{19}NOS \cdot HCl$
Molecular weight	:	333.88
Properties	:	White powder or lumpy form. Easily soluble in methanol and dimethyl sulfoxide (DMSO), slightly soluble in ethanol (99.5), and sparingly soluble in water.
Melting point	:	165 °C

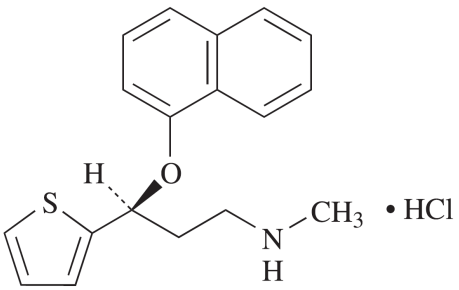

Fig. 2 Chemical Structural Formula of Duloxetine Hydrochloride

Low-Concentration Measurement of Duloxetine Hydrochloride

Duloxetine hydrochloride was dissolved in a 0.05 mol/L acetic acid solution and adjusted to standard solutions with concentrations of 0.01 to 0.80 μg/mL. After confirming the optimum excitation wavelength by measuring the 3-dimensional fluorescence spectrum, the lower limit of detection and lower limit of quantitation were evaluated under the measurement conditions shown in Table 2.

Table 2 Measurement Conditions

Instrument	: Shimadzu RF-6000 spectrofluorophotometer
Excitation wavelength	: 237 nm
Fluorescence wavelength range	: 290 to 400 nm
Fluorescence wavelength	: 337 nm
Scan speed	: 600 nm/min
Sampling pitch	: 1.0 nm
Bandwidth	: Ex 10 nm, Em 10 nm
Sensitivity	: High

Fig. 3 shows the fluorescence spectra of the standard solutions of duloxetine hydrochloride, and Fig. 4 shows the calibration curve prepared with the fluorescence intensity of 337 nm. A satisfactory result was obtained, as the square of the correlation coefficient (R2) was 0.99987.

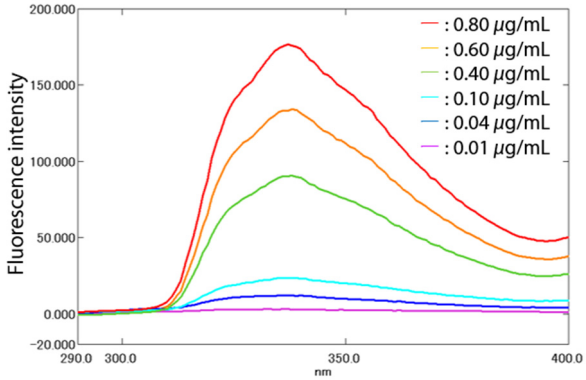

Fig. 3 Fluorescence Spectra of Standard Solutions of Duloxetine Hydrochloride

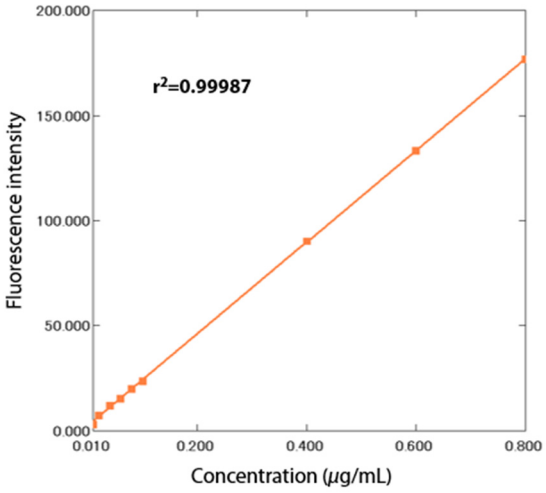

Fig. 4 Calibration Curve

Lower Limit of Detection and Lower Limit of Quantitation

The lower limit of detection is the minimum concentration at which it can be said that the target substance cannot be detected in the blank signal distribution (blank: value obtained in blank determination test). The most frequently used lower limit of detection is a value (concentration) separated +3 σ from the average blank value, where σ represents the standard deviation. The lower limit of quantitation is the minimum concentration value at which the results of a quantitative analysis can have adequately reliability. In this case, the most commonly used lower limit is a value (concentration) separated +10 σ from the average blank value [2].

Both of these values can be calculated by Eq. (4). For the lower limit of detection, k = 3, and for the lower limit of quantitation, k = 10.

$$A = \frac{k\sigma}{a} \quad (4)$$

A: concentration, σ: standard deviation of blank,
a: slope of calibration curve

The calibration curve equation in Fig. 4 is as follows:

y (fluorescence intensity) = 218.371 × (concentration) + 2.34753

Table 3 shows the lower limit of detection and the lower limit of quantitation of duloxetine hydrochloride calculated by substituting the respective values into Eq. (4).

Table 3 Lower Limit of Detection and Lower Limit of Quantitation of Duloxetine Hydrochloride

Lower limit of detection	Lower limit of quantitation
0.0002 μg/mL (0.2 ppb)	0.0007 μg/mL (0.7 ppb)

Spectra with little noise were obtained by fluorescence spectroscopy using the RF-6000. The lower limit of detection is 0.0002 μg/mL, and the lower limit of quantitation is 0.0007 μg/mL, showing that measurement is possible down to extremely low concentrations.

Conclusion

The antidepressant drug duloxetine hydrochloride was measured using a Shimadzu RF-6000 spectrofluorophotometer, and its lower limit of detection and lower limit of quantitation were evaluated. This experiment showed satisfactory results in both cases, demonstrating the applicability of fluorescence spectroscopy to low-concentration measurement. This technique is effective for analysis when the sample quantity is limited, for example, in the development of pharmaceutical products.

References
(1) https://pins.japic.or.jp/pdf/newPINS/00058451.pdf (as of Sept. 18, 2019)
(2) Michihisa Uemoto, Fundamental Knowledge for Reliable Analysis - Concepts and Definitions of the Limit of Detection and the Limit of Quantitation, Bunseki, 2010, 5

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