**Shifting the Linear Range in Electrospray Ionization by In-Source Collision-Induced Dissociation**

Hideaki Ishii, Hiroaki Yamaguchi, and Nariyasu Mano

**Abstract**

The goal of this study was to demonstrate the utility of in-source collision-induced dissociation for shifting the linear range in liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS). The linear ranges for uracil, deoxyuridine, and uridine were shifted from 0.3–300, 1–100, and 10–1000 ng/mL to 10–1000, 30–3000, and 100–10000 ng/mL, respectively, by changing the declustering potential controlling in-source collision-induced dissociation. This technique should be considered for control of linear range in simultaneous quantitative measurements of extremely different amounts of compounds, drugs, and metabolites using LC/ESI-MS/MS.

**Key words** in-source collision-induced dissociation; linear range; electrospray ionization

In mass spectrometry, the linear ranges of responses are relatively narrow due to saturation of ionization and/or detection by an electron multiplier. Ionization saturation is particularly common in electrospray ionization (ESI), especially in the case of compounds that are more likely to ionize. When linearity cannot cover the quantitative range due to saturation, the experimentalist must reduce the injection volume or dilute the samples. Consequently, multiple analyses are required when analyzing more than two analytes with extremely different responses, such as a drug and its metabolites.

The isotopologue transitions method is one effective means to avoid such problems. This method prevents saturation by restricting ion detection on the electron multiplier. As a result, a large number of ions produced during ESI are introduced into mass analyzer. Inversely, confining the amount of ions entering the mass analyzer while maintaining linearity represents another potentially effective approach to overcoming linearity saturation.

In this study, we investigated the utility of controlling the amount of ions entering the mass analyzer by in-source collision-induced dissociation (CID) to control the linear range by analyte. Uracil (U), deoxyuridine (dUrd), and uridine (Urd) were used as model compounds.

**Experimental**

**Chemicals** U, dUrd, and Urd were purchased from Sigma-Aldrich Chemical (St. Louis, MO, U.S.A.). 5-Fluorouracil (5-FU), used as an internal standard (IS), was acquired from Tokyo Chemical Industry (Tokyo, Japan). Stock solutions (1 mg/mL) were prepared independently in water. Standard working solutions (0.3, 1, 3, 10, 30, 100, 300, 1000, 3000, 10000 ng/mL) were prepared by diluting the stock solutions in acetonitrile. IS working solution (1 µg/mL) was prepared by diluting stock solution with water. To investigate linearity in the concentration range 0.3–30000 ng/mL, 500 µL aliquots of standard working solution and 100 µL IS working solution were transferred into a vial and mixed. LC/MS-grade acetonitrile was purchased from Kanto Chemical (Tokyo, Japan). High-purity water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, U.S.A.).

**Instrumentation** A Shimadzu Nexera series liquid chromatography consisting of binary pumps, an online degasser, an autosampler, and a column oven (Shimadzu, Kyoto, Japan) was linked to a linear ion trap quadrupole mass spectrometer equipped with turbo ionspray interface (QTRAP 4500, AB SCIEX, Framingham, MA, U.S.A.). Analytes were separated using a SeQuant® ZIC®-cHILIC (2.1 mm i.d.×100 mm, 3 µm, Merck Millipore, Darmstadt, Germany), with a guard column (2.1 mm i.d.×20 mm) whose temperature was maintained at 40°C. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile), with gradient elution as follows: 0–1 min, 3%; 1–2 min, 3–15%; 2–6 min, 15–50%; 6–6.01 min, 50–3%; and 6.01–11 min, 3% solvent A at a flow rate of 0.3 mL/min. Eluate was mixed with water, at a flow rate of 0.05 mL/min, after LC separation.

All analytes were monitored in selective reaction monitoring (SRM) mode. Instrument settings were as follows: heater temperature, 400°C; curtain gas, 10 psi; nebulizer gas, 50 psi; heater gas, 80 psi; collisionally activated dissociation gas, 9 arbitrary units; ionspray voltage, −4500 V; dwell, 30 ms. For U, dUrd, Urd, and IS, respectively, declustering potential (DP), −45, −55, −60, and −55 V; collision energy, −28, −12, −16, and −30 V; and collision cell exit potential, −11, −5, −9, and −7 V. All peak integrations were performed using Analyst i6.2 software. Linearity of the peak area ratios of each analyte vs. nominal analyte concentrations were fitted by least-squares linear regression using 1/y^2 weighting factors.

**Linearity of U, dUrd, and Urd** Linearities of U, dUrd, and Urd were investigated under various MS conditions. To determine whether saturation occurred at the electron multiplier or under ESI, product ions with different relative intensities observed in product ion spectra of dUrd and Urd (Figs. 1B, C) were monitored in SRM mode. Next, 10DPs were used, increasing in 10 V steps from the values listed above in
order to monitor the transitions from \( m/z \) 110.8 to 41.8, \( m/z \) 226.7 to 110.7, and \( m/z \) 242.7 to 110.9 for U, dUrd, and Urd, respectively; the linear range was compared for each condition. 5-FU used as an IS was monitored using the transition from \( m/z \) 128.8 to 41.8 to normalize responses of analytes.

Criteria for linearity were set as follows: (1) Regression lines should be composed of at least four successive points in triplicate. (2) Accuracy (mean back-calculated concentration, expressed as a percentage of nominal concentration) must be within ±15% (or within ±20% at the lower limit of quantitation (LLOQ)). (3) Precision, expressed as the percentage of the coefficient of variance (CV), cannot exceed 15% (or 20% at the LLOQ).

**Results and Discussion**

The product ion mass spectra obtained by infusion of each standard solution at a concentration of 1 \( \mu \)g/mL are shown in Fig. 1. U yielded a simple product ion spectrum with one product ion at \( m/z \) 42, derived from a precursor ion at \( m/z \) 111; by contrast, dUrd and Urd produced multiple product ions, the most abundant of which was a common product ion at \( m/z \) 111 corresponding to the base. In the product ion spectrum of dUrd, four product ions were observed: \( m/z \) 41.9, with a relative intensity of 5%; \( m/z \) 110.7, 100%; \( m/z \) 135.8, 3%; and \( m/z \) 183.8, 25%. The product ion spectrum of Urd contained six product ions: \( m/z \) 41.7, with a relative intensity of 3%; \( m/z \) 109.8, 35%; \( m/z \) 110.9, 100%; \( m/z \) 139.7, 5%; \( m/z \) 151.9, 15%, and \( m/z \) 199.8, 50%. The structure of 5-FU (the IS) is similar to that of U, but its product ion spectrum was quite different, possibly due to the electronegativity of fluorine.

Typical SRM chromatograms are shown in Fig. 2. U, dUrd, and Urd were detected at retention times of 3.55, 4.06, and
4.43 min, respectively. Both dUrd and Urd produced a fragment ion derived from the base at m/z 111 by in-source CID; therefore, two extra peaks appeared in the SRM chromatogram of U (Fig. 2A).

When the initial conditions were used, U, dUrd, and Urd exhibited good linearity in the ranges 0.3–300, 1–100, and 10–1000 ng/mL, respectively (Fig. 3A). For dUrd, the use of the transition from m/z 226.7 to 183.8, with a relative intensity of about 25%, shifted the linear range to 3–300 ng/mL. Although the upper limit of quantitation (ULOQ) increased to 300 ng/mL using the transitions from m/z 226.7 to 41.9 and m/z 226.7 to 135.8, the LLOQ also increased to 10 ng/mL, resulting in a narrowing of the linear range. On the other hand, the linear range of Urd remained constant despite the change of ion content reaching the electron multiplier.

The effect of DP on linearity range was investigated with the transitions from m/z 226.7 to 110.8, m/z 226.7 to 110.7, and m/z 242.7 to 110.9 for U, dUrd, and Urd, respectively, with SRM kept constant (Fig. 3B). When DP was set to −75 V for U, LLOQ increased to 10 ng/mL (a 30-fold increase relative
to 0.3 ng/mL), whereas ULOQ increased 3-fold relative to the initial value. Thus, the 30 V change in DP changed the linear range from 0.3–300 to 10–1000 ng/mL for U. For dUrd, the linear range changed from 1–100 to 30–3000 ng/mL when DP was changed from −55 to −85 V, and a DP change greater than 50 V made it impossible to obtain a regression line with four successive points. The 30 V change in DP shifted the linear range from 10–1000 to 100–10000 ng/mL for Urd, and this shift was associated with a reduction in the slope of regression lines (Fig. 4). Although peak responses in SRM mode decreased with in-source CID, it was possible to shift the linear range. Therefore, this is an effective technique for simultaneous measurement of compounds present in extremely different concentrations using SRM analysis.

**Conflict of Interest** The authors declare no conflict of interest.

**References**