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A rapid and sensitive LC/ESI-MS/MS method for quantitative analysis of docetaxel in human plasma and its application to a pharmacokinetic study

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Abstract

Docetaxel is a taxane family antineoplastic agent widely employed in cancer chemotherapy. We developed a liquid chromatography/tandem mass spectrometry method for the determination of docetaxel in human plasma. Plasma samples were deproteinized by acetonitrile containing internal standard paclitaxel. Chromatographic separation was performed on a TSKgel ODS-100V 3 μ m (50 mm \times 2.0 mm i.d.) column using a mobile phase composed of acetonitrile-methanol-water-formic acid (50:5:45:0.1, v/v/v/v). Detection was performed on a triple-quadrupole tandem mass spectrometer with multiple reaction monitoring (MRM) mode via electrospray ionization (ESI) source. This method covered a linearity range of 5-5000 ng/mL with the lower limit of quantification of 5 ng/mL. The intra-day precision and inter-day precision (R.S.D.) of analysis were less than 6.7%, and the accuracy (R.E.) was within \pm 9.0% at the concentrations of 5, 20, 200, and 2000 ng/mL. The total run time was 5.0 min. This method was successfully applied for clinical pharmacokinetic investigation.

Keywords

Docetaxel; LC/ESI-MS/MS; Rapid and sensitive analysis

Abbreviations

LC/MS/MS, liquid chromatography/tandem mass spectrometry; LLOQ, lower limit of quantification; MRM, multiple reaction monitoring

1. Introduction

Docetaxel is an antineoplastic agent separated from plants of *Taxus baccata*.

Docetaxel shows its antineoplastic activity by stabilizing microtubules in dividing cells and is used to treat multiple types of tumor including lung, stomach, prostatic, breast, ovary, endometrial, head and neck cancer. Docetaxel often causes severe adverse effects such as neutropenia, which is a dose-limiting factor of docetaxel. There are some reports, which show the relationship between docetaxel pharmacokinetics and adverse effects [1,2]. The major elimination pathway of docetaxel is hepatic elimination and over 73% of docetaxel is excreted into feces. The drug-metabolizing enzyme cytochrome P450 (CYP) 3A4 plays a major role in docetaxel metabolism [3]. Docetaxel is also a substrate of the drug efflux transporter ABCB1 (MDR1, P-glycoprotein), which is normally expressed in hepatocytes, the intestine, renal tubules, and brain [4,5]. It has also been reported that docetaxel is a good substrate for the apical drug transporter ABCC2 (multidrug resistance-associated protein 2) in vitro [6]. Yet, little in vivo evidence is currently available on how important ABCC2 actually is in the pharmacokinetics of docetaxel. Thus, the pharmacokinetics of docetaxel is complex and highly individual. It has been suggested that pharmacokinetically guided dose individualization might lead to a better treatment outcome, reducing severe side effects and improving efficacy to the maximum extent possible.

Analytical methods have already been developed to evaluate docetaxel concentration in plasma. Earlier methods used liquid chromatography with ultraviolet detection [7,8]. However, these methods have the weakness of limited sensitivity and selectivity. To overcome this weakness, methods using liquid chromatography coupled to mass spectrometry were developed [9-14]. Most assays employ solid-phase

extraction [9] or liquid-liquid extraction [10-13] as a means of sample pretreatment, requiring evaporation steps. The evaporation procedure is time-consuming and has the risk of exposure to the antineoplastic agent. Recently, occupational exposure of medical staff to antineoplastic agents has become a concern [15-17]. Hou et al. reported the quantitative determination of docetaxel in mouse plasma using protein precipitation for sample pretreatment without evaporation step [14]. However, their method was not sensitive enough for pharmacokinetic analysis of cancer patients because lower limit of quantification (LLOQ) was almost 20 ng/mL. In the present study, we developed a rapid and sensitive LC/MS/MS method for docetaxel quantification in human plasma and the method was applied to the pharmacokinetic study in cancer patients.

2. Materials and methods

2.1. Materials

Docetaxel and paclitaxel as an internal standard were purchased from Sigma (St. Louis, MO). HPLC-grade acetonitrile, methanol, and formic acid were purchased from Wako (Osaka, Japan).

2.2. Sample preparation

A stock solution of docetaxel was prepared in methanol at a concentration of 100 µg/mL. A stock solution of internal standard was prepared in acetonitrile at a concentration of 1000 ng/mL. Calibration standards of docetaxel in human plasma were prepared by diluting a plasma working solution at concentrations of 5, 10, 20, 100, 200, 1000, 2000, and 5000 ng/mL. Validation samples of docetaxel in human plasma were prepared by

diluting a plasma working solution at concentrations of 5, 20, 200, and 2000 ng/mL. All solutions were stored at -30°C.

2.3. Sample pretreatment

We performed deproteination by acetonitrile containing the internal standard. Fifty µL of internal standard in acetonitrile (1000 ng/mL) was added to fifty µL of plasma sample. The mixture was vortexed and centrifuged at 10,000×*g* for 10 min at room temperature. The supernatant was filtered with a YMC Duo-Filter (0.2 µm, YMC, Tokyo, Japan) and 3 µL was injected into the HPLC column.

2.4. Chromatographic and mass spectrometric conditions

The HPLC consisted of fully equipped Prominence20A (Shimadzu, Kyoto, Japan). The mobile phase was acetonitrile-methanol-water-formic acid (50:5:45:0.1, v/v/v/v) and was pumped at a flow rate of 0.15 mL/min. From 2.5 to 5 min, the flow was introduced into the mass spectrometer using a switching valve. For the separation column, we used TSKgel ODS-100V (50 × 2.0 mm i.d., 3 µm, TOSOH, Tokyo, Japan). The column temperature was maintained at 40°C.

Mass spectrometry was carried out on an API3200 triple quadrupole mass spectrometer (Applied Biosystems, Carlsbad, CA). Positive ionization electrospray mass spectrometry was performed. The ionspray voltage was set at 4500 V. The turbospray gas (N₂) probe was heated at 300°C. Nitrogen was used as curtain gas, gas 1 and gas 2, and their flows were set to 20, 60, and 30 units, respectively. Unit mass resolution was set in both mass-resolving quadrupoles Q1 and Q3. The multiple reaction monitoring (MRM) transitions monitored were *m/z* 808 to *m/z* 527 for docetaxel

and m/z 854 to m/z 286 for internal standard, respectively. The declustering potential was set at 30V and the values of the collision energy were 15 and 20V for docetaxel and internal standard, respectively. The dwell time was 500 msec. Data were collected and processed using Analyst 1.4.2 data collection and integration software (Applied Biosystems, Carlsbad, CA).

2.5. Method validation

2.5.1. Linearity and lower limit of quantification

For the validation, calibration standards (eight non-zero standards of the analyte, 5, 10, 20, 100, 200, 1000, 2000, and 5000 ng/mL) were prepared in control human plasma and analyzed. Linear regression of ratio of the areas of the analyte and internal standard peaks vs the concentration were weighted by $1/x$ (reciprocal of the concentration). The lower limit of quantification (LLOQ) was defined as the concentration with a signal-to-noise ratio of at least 10 and acceptable precision and accuracy data (R.S.D. and R.E. less than 20%).

2.5.2. Specificity and selectivity

To determine whether endogenous matrix constituents interfered with the assay, six different batches of blank human plasma samples containing neither analyte nor internal standard (double blank) and samples containing the LLOQ of docetaxel (5 ng/mL) and internal standard were prepared and analyzed.

2.5.3. Precision and accuracy

Intra-day (n=6) and inter-day (n=6) precision and accuracy were investigated at four

different levels, 5 (LLOQ), 20, 200, and 2000 ng/mL. Precision was determined on the basis of coefficient of variation (R.S.D.(%)), and the accuracy was calculated as $(\text{observed concentration} - \text{theoretical concentration}) / \text{theoretical concentration} \times 100$ (R.E. (%)).

2.5.4. Extraction recovery and matrix effect

The extraction recovery of docetaxel was determined by comparing the peak areas obtained from blank plasma samples spiked with docetaxel before extraction with those from blank plasma samples to which docetaxel was added after extraction. The matrix effect of docetaxel by plasma matrix components was evaluated by comparing the peak areas of an extract of control plasma to which docetaxel had been added after extraction with the peak areas of mobile phase to which the same amount of docetaxel was added. Experiments were performed at three levels, 20, 200 and 2000 ng/mL, in triplicate. The extraction recovery and matrix effect of the internal standard were also evaluated.

2.5.5. Stability

The stability of docetaxel in human plasma was examined by analyzing three concentrations (20, 200 and 2000 ng/mL) in triplicate. These samples were stored at -30°C for 2 months and at ambient temperature for 4 hours to evaluate long-term and short-term stability, respectively. Freeze-thaw stability was tested following three freeze-thaw cycles (-30°C to ambient temperature). Stability of the processed samples was assessed by reinjecting the samples after 24 hours in an autosampler (4°C).

2.6. Clinical pharmacokinetics investigation

Samples for a pharmacokinetic study were collected from 4 head and neck cancer patients treated with docetaxel at Hokkaido University Hospital. Before treatment, patients gave written informed consent as approved by the institutional review board. Docetaxel was administered at a dose of 75 mg/m² i.v. over 120 min. Whole blood was withdrawn in EDTA tubes at optimal sampling times at the end of infusion, and at 0.5, 1, 2, 3, 6, 9, and 24 hours after the end of docetaxel infusion. Samples were immediately stored at 4°C and centrifuged at 1,700×g for 10 min. The resultant plasma was used for analysis. A non-compartmental model was used to estimate the pharmacokinetic parameters. The maximum plasma concentration was defined as the concentration at the end of infusion. The area under the plasma concentration–time curve (AUC_{0–26 h}) to the last measurable plasma concentration (24 hours after the end of docetaxel infusion) was calculated by the linear trapezoidal rule. Total clearance was calculated from dose divided by AUC_{0–26 h}.

3. Results and discussion

3.1. Mass spectrometry

The positive ion full-scan mass spectra (Q1) of docetaxel and internal standard indicated the presence of the protonated molecular ion [M+H]⁺ as the predominant ion for each compound with *m/z* of 808 and 854, respectively.

The product ion mass spectrum of [M+H]⁺ at *m/z* of 808 is shown in Fig. 1A. Product ions appeared at *m/z* of 527, 509, 327, and 281. The proposed fragmentation pattern for docetaxel is presented in Fig. 1A. The product ion at *m/z* of 527 was the most strongly produced and used for quantitative MRM of docetaxel.

The protonated molecular ion of the internal standard at m/z of 854 was used as a precursor ion to generate the product ion mass spectrum presented in Fig. 1B. The proposed fragmentation pattern for the internal standard is presented in Fig. 1B. For MRM, the fragment at m/z of 286 was used.

3.2. Chromatography

Using the mobile phase described in the *Materials and methods* section, docetaxel and internal standard were perfectly separated. The retention time of docetaxel was 3.6 min and that of internal standard was 4.2 min. Total LC run time was 5 min. From 0 to 2.5 min, LC flow was diverted to waste, we thereby avoided contamination of ion source by impurities such as salts in plasma sample.

3.3. Method validation

3.3.1. Linearity and lower limit of quantification

The present method covered a linearity range of 5 to 5000 ng/mL of concentration in plasma with a weighting by $1/x$. The correlation coefficient (r) was >0.999 . A typical standard curve was $y = 0.00207x + 0.0251$. The LLOQ for docetaxel was 5 ng/mL in plasma. The present method can determine docetaxel concentrations in plasma samples until at least 24 h after 1- to 2-h infusion at a dose of 75-100 mg/m² [11,18]. The method is sensitive enough to evaluate the pharmacokinetics of docetaxel in cancer patients.

3.3.2. Specificity and selectivity

We evaluated the specificity and selectivity of the method. A representative MRM

chromatogram of blank human plasma and chromatograms of LLOQ of docetaxel and the internal standard spiked in human plasma are shown in Fig. 2 (A), (B), and (C), respectively. There was no significant interference from endogenous plasma constituents at retention times of docetaxel and the internal standard.

3.3.3. Precision and accuracy

Intra-day (n=6) and inter-day (n=6) accuracy and precision were investigated at four different levels, 5 (LLOQ), 20, 200, and 2000 ng/mL. The results are summarized in Table 1. The intra- and inter-day accuracy (R.E. (%)) was at most within $\pm 9.0\%$ for all concentrations. The intra- and inter-day precision (R.S.D. (%)) was less than 6.7% for all concentrations. These results suggest that the present method can accurately and reproducibly measure docetaxel in human plasma.

3.3.4. Extraction recovery and matrix effect

The extraction recoveries of docetaxel from human plasma were $82.3 \pm 1.9\%$, $77.6 \pm 9.2\%$ and $89.4 \pm 6.0\%$ at concentrations of 20, 200 and 2000 ng/mL, respectively. The extraction recovery of the internal standard was $91.8 \pm 3.1\%$.

A matrix effect of docetaxel by plasma matrix components was not observed ($97.8 \pm 5.6\%$, $99.0 \pm 7.6\%$ and $95.4 \pm 4.8\%$ at concentrations of 20, 200 and 2000 ng/mL, respectively). The matrix effect of internal standard was $108.1 \pm 4.9\%$ at a concentration of 1000 ng/mL.

3.3.5. Stability

The stabilities of docetaxel in plasma under different conditions were evaluated (Table

2). No significant degradation was observed for 2 months at -30°C (long-term stability), for 4 hr at ambient temperature (short-term stability), and during three freeze/thaw cycles. Post-extraction samples kept in the autosampler at 4°C for 24 h were also stable.

3.4. Clinical pharmacokinetics investigation

The suitability of the developed method for clinical use was demonstrated by the determination of plasma docetaxel levels from 4 head and neck cancer patients treated with docetaxel at a dose of 75 mg/m^2 . The MRM chromatogram of docetaxel in plasma taken from a patient at the end of docetaxel infusion is shown in Fig. 2 (D). No interfering peak was observed, and this analytical method was successfully applied for the determination of docetaxel in patient plasma. The plasma concentration-time profiles of a 2-h intravenous infusion of docetaxel at a dose of 75 mg/m^2 is shown in Fig. 3. The maximum plasma concentration was $2035 \pm 477\text{ ng/mL}$. Plasma concentrations of docetaxel at 24 h after the end of the infusion were above LLOQ in all patients. The $\text{AUC}_{0-26\text{ h}}$ value was $3313 \pm 873\text{ ng h/mL}$, and the total clearance of docetaxel was $23.9 \pm 6.3\text{ L/h/m}^2$. These data indicated in the present study were comparable with previous reports [2,18], and strongly suggest that our method is useful for clinical pharmacokinetic analysis.

4. Conclusion

A rapid and sensitive LC/MS/MS assay was developed for the quantification of docetaxel in human plasma. The sample preparation of the established method is one-step protein precipitation without evaporation step, meaning that not only it needs

simple technique but also the exposure of antineoplastic drugs could be avoidable.

The validation results showed that an accurate, reproducible and selective assay was achieved. This method can be applied for the clinical pharmacokinetics study of docetaxel in cancer patients.

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References

- [1] R. Bruno, D. Hille, A. Riva, N. Vivier, W.W. ten Bokkel Huinnink, A.T. van Oosterom, S.B. Kaye, J. Verweij, F.V. Fossella, V. Valero, J.R. Rigas, A.D. Seidman, B. Chevallier, P. Fumoleau, H.A. Burris, P.M. Ravdin, L.B. Sheiner. *J, Clin. Oncol.* 16 (1998) 187.
- [2] S.D. Baker, A. Sparreboom, J. Verweij, *Clin. Pharmacokinet.* 45 (2006) 235.
- [3] I. Royer, B. Monsarrat, M. Sonnier, M. Wright, T. Cresteil, *Cancer Res.* 56 (1996) 58.
- [4] M.T. Huisman, A.A. Chhatta, O. van Tellingen, J.H. Beijnen, A.H. Schinkel, *Int J Cancer* 116 (2005) 824.
- [5] G. Bradley, V. Ling, *Cancer Metastasis Rev.* 13 (1994) 223.
- [6] R.A. van Waterschoot, J.S. Lagas, E. Wagenaar, H. Rosing, J.H. Beijnen, A.H. Schinkel, *Int. J. Cancer* 127 (2010) 2959.
- [7] L. Zufia López, A. Aldaz Pastor, J.M. Aramendia Beitia, J. Arrobas Velilla, J. Giraldez Deiró, *Ther. Drug Monit.* 28 (2006) 199.
- [8] S. Bermingham, R. O'Connor, F. Regan, G.P. McMahon. *J. Sep. Sci.* **2010**, 33, 1571.

- [9] R.A. Parise, R.K. Ramanathan, W.C. Zamboni, M.J. Egorin, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 783 (2003) 231.
- [10] K.A. Mortier, V. Renard, A.G. Verstraete, A. Van Gussem, S. Van Belle, W.E. Lambert, Anal. Chem. 77 (2005) 4677.
- [11] L.Z. Wang, B.C. Goh, M.E. Grigg, S.C. Lee, Y.M. Khoo, H.S. Lee, Rapid Commun. Mass Spectrom. 17 (2003) 1548.
- [12] J. Guitton, S. Cohen, B. Vjgnal, J.P. Droz, M. Guilaumont, M. Manchon, G. Freyer, Rapid Commun. Mass Spectrom. 19 (2005) 2419.
- [13] Q. Huang, G.J. Wang, J.G. Sun, X.L. Hu, Y.H. Lu, Q. Zhang, Rapid Commun. Mass Spectrom. 21 (2007) 1009.
- [14] W. Hou, J.W. Watters, H.L. McLeod, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 804 (2004) 263.
- [15] T.H. Connor, D.G. DeBord, J.R. Pretty, M.S. Oliver, T.S. Roth, P.S. Lees, E.F. Jr. Krieg, B. Rogers, C.P. Escalante, C.A. Toennis, J.C. Clark, B.C. Johnson, M.A. McDiarmid, J. Occup. Environ. Med. 52 (2010) 1019.
- [16] P.A. Ratner, J.J. Spinelli, K. Beking, M. Lorenzi, Y. Chow, K. Teschke, N.D. Le, R.P. Gallagher, H. Dimich-Ward, BMC Nurs. 9 (2010) 15.
- [17] C. Sottani, B. Porro, M. Comelli, M. Imbriani, C. Minoia, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (2010) 2593.
- [18] S. J. Clarke, L.P. Rivory, Clin. Pharmacokinet. 36 (1999) 99.

Figure legends

Figure 1 Product ion mass spectra of docetaxel (A) and internal standard (B) and proposed fragmentation patterns. Ac, acetyl; Ph, phenyl.

Figure 2 Representative MRM chromatograms of blank plasma (A), LLOQ of docetaxel (5 ng/mL) (B), and internal standard (C) spiked in human plasma, and the chromatogram of docetaxel in plasma taken from a patient at the end of docetaxel infusion (D).

Figure 3 Plasma concentration-time curves of docetaxel in head and neck cancer patients after 2-h infusion at a dose of 75 mg/m².

Table 1 Precision and accuracy of the quantification of docetaxel in human plasma

Analyte	Concentration (ng/mL)	Intra-day (n=6)			Inter-day (n=6)		
		Found (ng/mL)	R.S.D. (%)	R.E. (%)	Found (ng/mL)	R.S.D. (%)	R.E. (%)
Docetaxel	5	4.98 ± 0.29	5.9	-0.3	4.83 ± 0.32	6.7	-3.4
	20	19.72 ± 0.59	3.0	-1.4	20.00 ± 0.78	3.9	0.0
	200	211.8 ± 11.8	5.6	5.9	218.0 ± 5.9	2.7	9.0
	2000	2008 ± 78	3.9	0.4	2033 ± 42	2.1	1.7

Table 2 Stability of docetaxel in human plasma

Stability	Remaining (%)		
	20 ng/mL	200 ng/mL	2000 ng/mL
Long-term stability	101.4 ± 3.8	104.3 ± 2.9	98.9 ± 3.0
Short-term stability	97.8 ± 4.7	94.7 ± 1.2	94.1 ± 1.1
Freeze-thaw stability	100.3 ± 1.1	105.3 ± 5.4	101.4 ± 1.3
Processed sample stability	101.7 ± 4.9	94.7 ± 5.2	101.3 ± 6.0

Figure 1

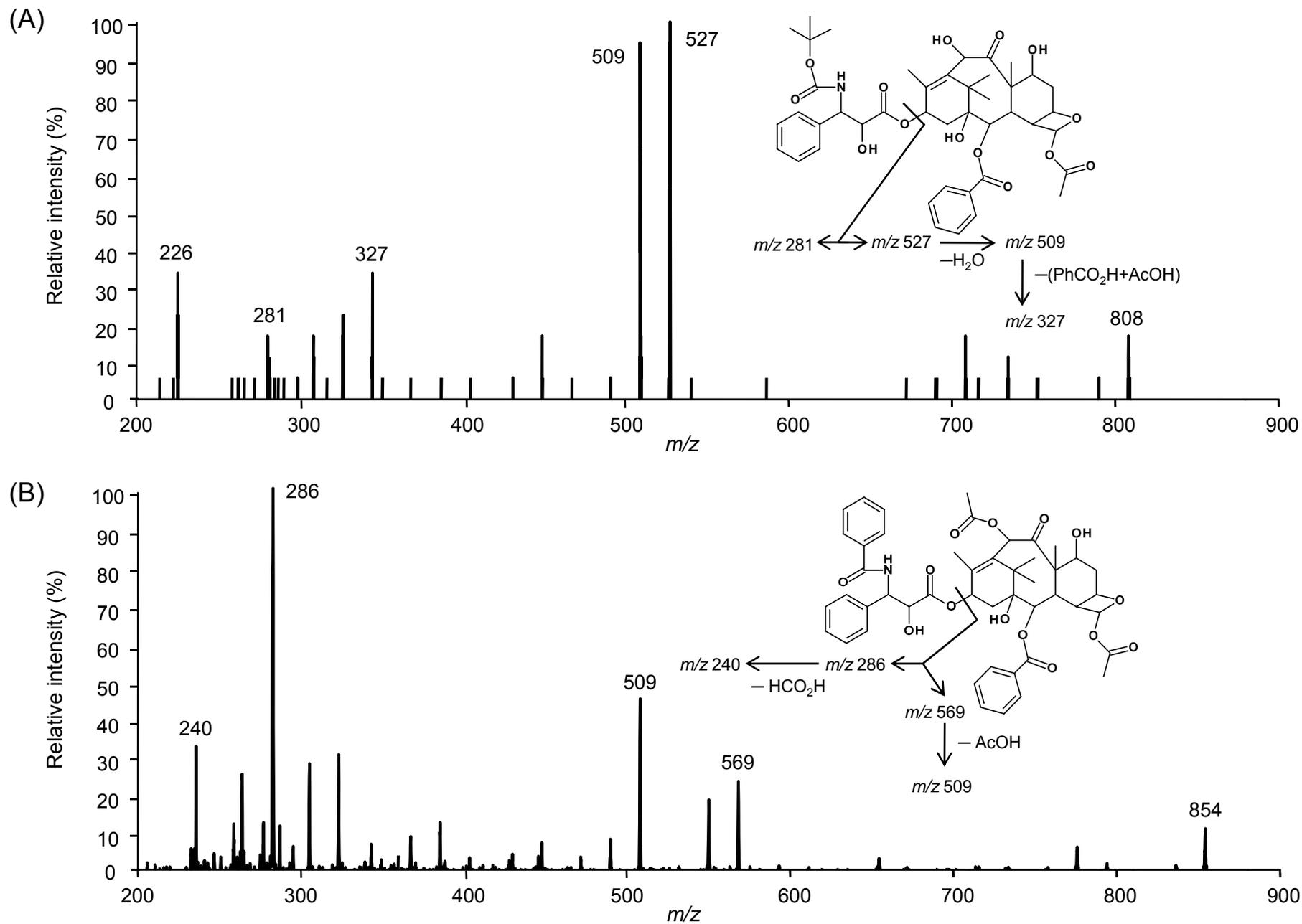


Figure 2

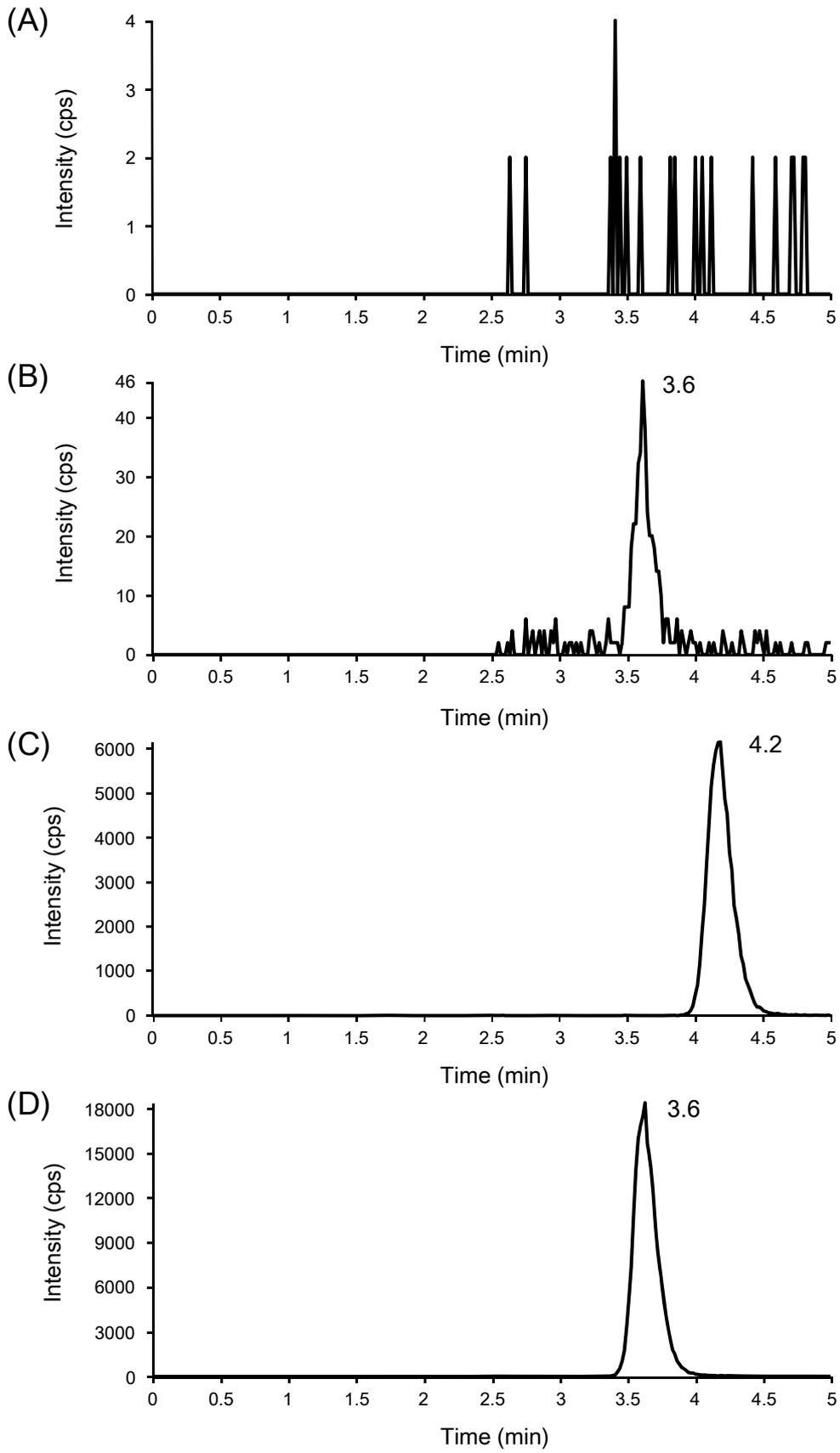


Figure 3

