

A Selective and Sensitive LC-MS/MS Method for the Determination of Beclomethasone Dipropionate and Beclomethasone-17-Propionate in Human Plasma



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Overview

A sensitive and specific liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method capable of quantifying beclomethasone dipropionate (BDP) and a metabolite, beclomethasone 17-propionate (B-17-P), in human plasma is described.

The analytes were extracted from 0.5 mL plasma by a reverse phase (C18) solid phase extract method. The separation was achieved on a reverse phase (C18) HPLC column, and analytes, along with their respective internal standards, were monitored using an AB SCIEX API-5000 tandem mass spectrometer, employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation (LLOQ) attained for BDP and B-17-P were 10 and 20 pg/mL, respectively.

The method has been successfully applied to a concentration assay of human plasma samples as part of bioequivalence studies.

Introduction

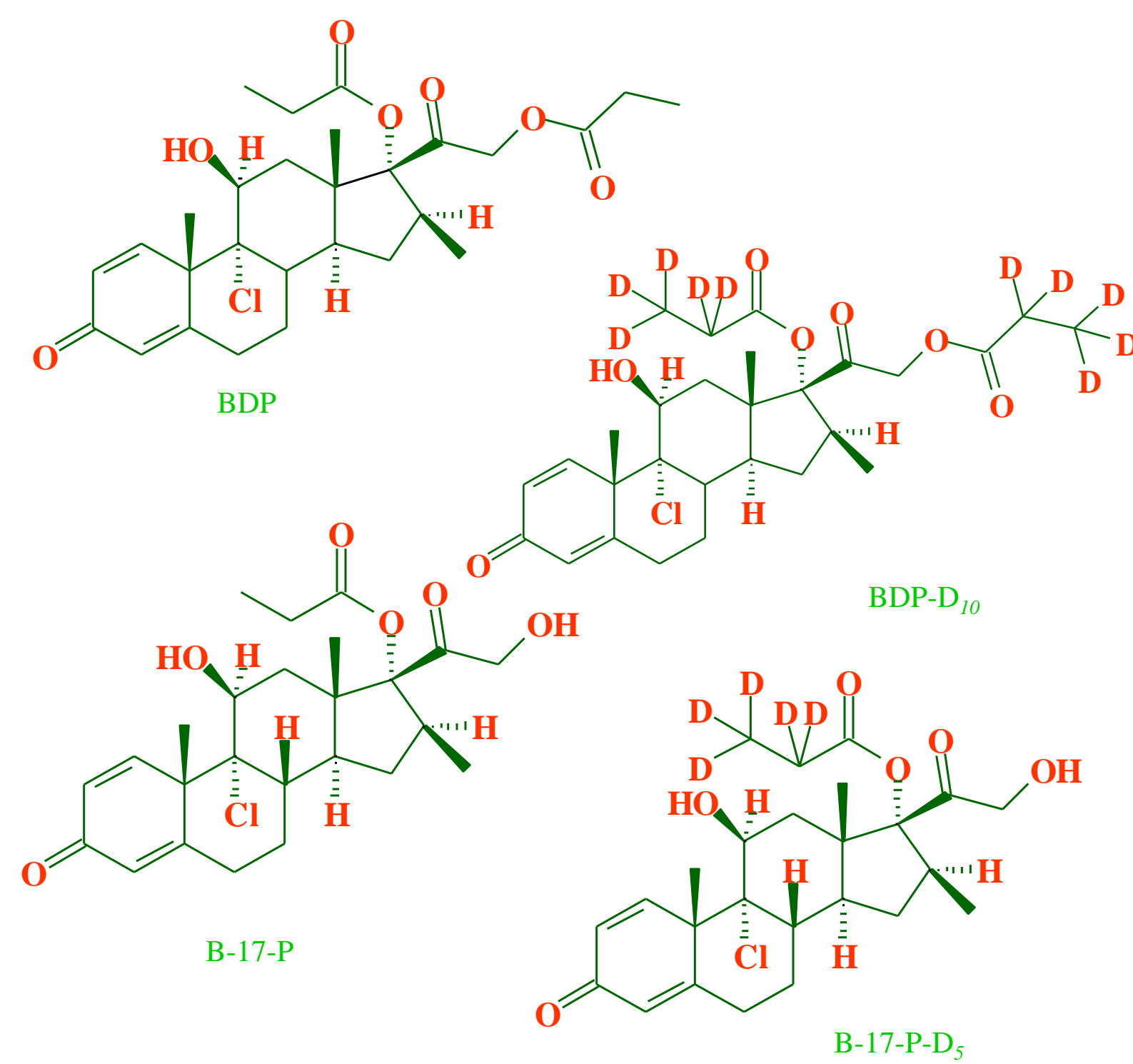
Beclomethasone dipropionate (BDP) is a topically active corticosteroid used in the treatment of asthma and rhinitis. In recent years, several bioanalytical methods have been developed to support pharmacokinetic (PK) analysis in clinical trials, with the most sensitive method having a lower limit of quantification (LLOQ) of 50 pg/mL for both BDP and B-17-P in human plasma. BDP is extensively metabolized in the liver after oral administration, resulting in very low plasma levels. To improve PK evaluation in clinical trials, we have developed an LC-MS/MS method with increased sensitivity compared with existing methods. The LLOQs were lowered to 10 and 20 pg/mL for BDP and B-17-P, respectively.

Experimental

Sample Preparation

BDP, B-17-P, and the internal standards (BDP-D₁₀ and B-17-P-D₅) were transferred onto the preconditioned Strata C18-E 96-well plate (Phenomenex, precondition with 1 mL of methanol and 1 mL of water). The SPE plate was washed with 1.6 mL of water, 0.8 mL of 20% methanol in water, and 0.8 mL of Hexane. Analytes and internal standards were then eluted using a mixed solution of ethyl acetate and hexane.

Structures



LC-MS/MS Conditions

Liquid Chromatography:

HPLC System: Shimadzu LC-10AD
Analytical Column: C18 column, 2.0 x 30 mm, 2.5 μm
Mobile Phase A) 0.4% acetic acid in water
Mobile Phase B) Acetonitrile
Mobile Phase C) Methanol (for column back wash)
Gradient
Flow rate: 0.5 mL/min
Column Temperature: 50 °C
Injection Volume: 25 μL

Mass Spectrometry

MS System: AB Sciex API-4000
Condition: LC/(+)ESI-MS/MS (MRM)
MRM Transition:
BDP: 521.3 → 301.2
B-17-P: 465.2 → 279.3
BDP-D₁₀: 531.3 → 301.2
B-17-P-D₅: 470.2 → 279.2

Results and Discussion

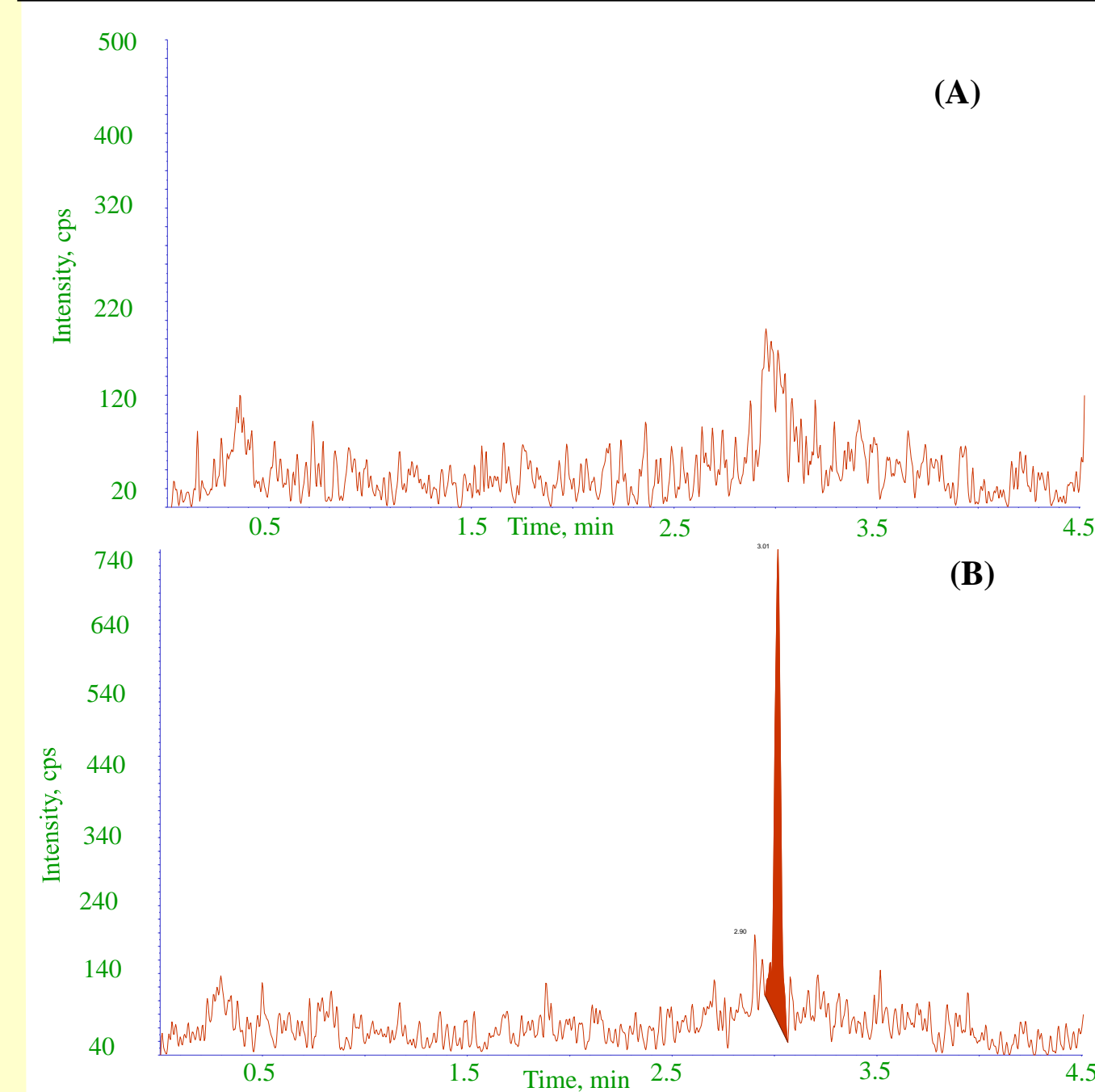


Figure 1. Ion chromatograms of blank plasma (A), and 10 pg/mL BDP extracted from plasma (B)

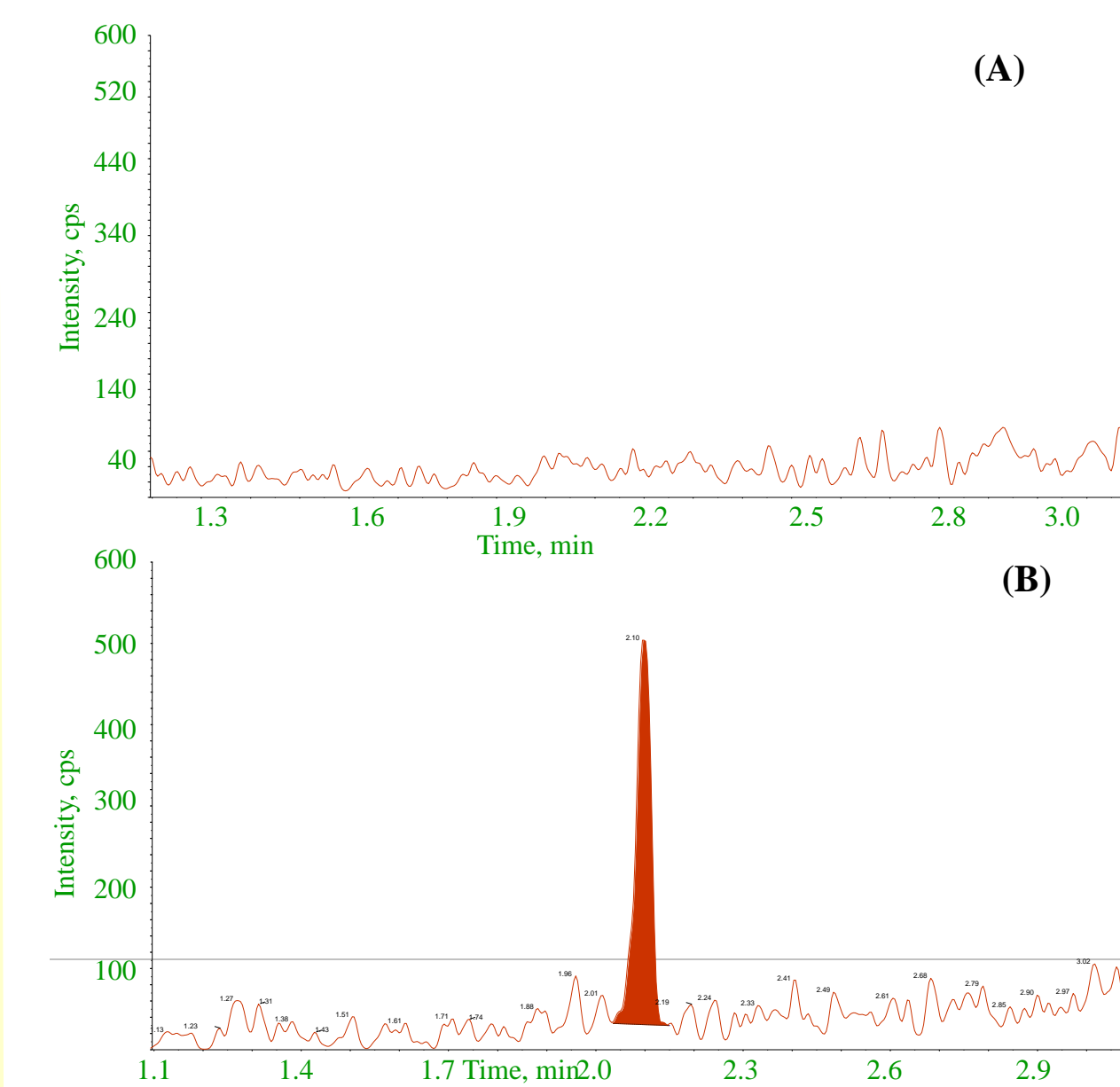


Figure 2. Ion chromatograms of blank plasma (A), and 20 pg/mL B-17-P extracted from plasma (B)

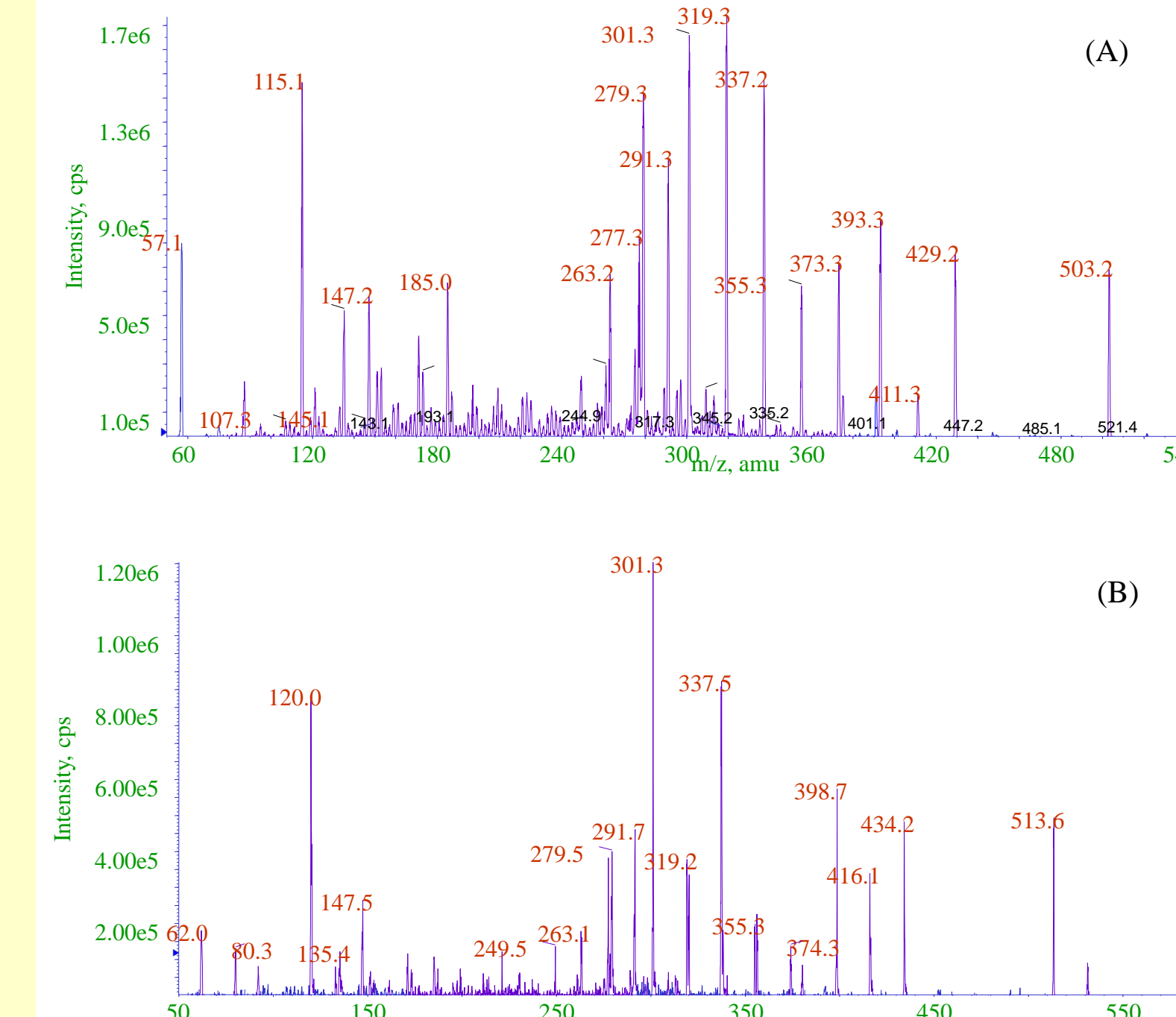


Figure 3. Product ion scan of BDP at 521.3 Da (A), and BDP-D₁₀ at 531.3 Da (B).

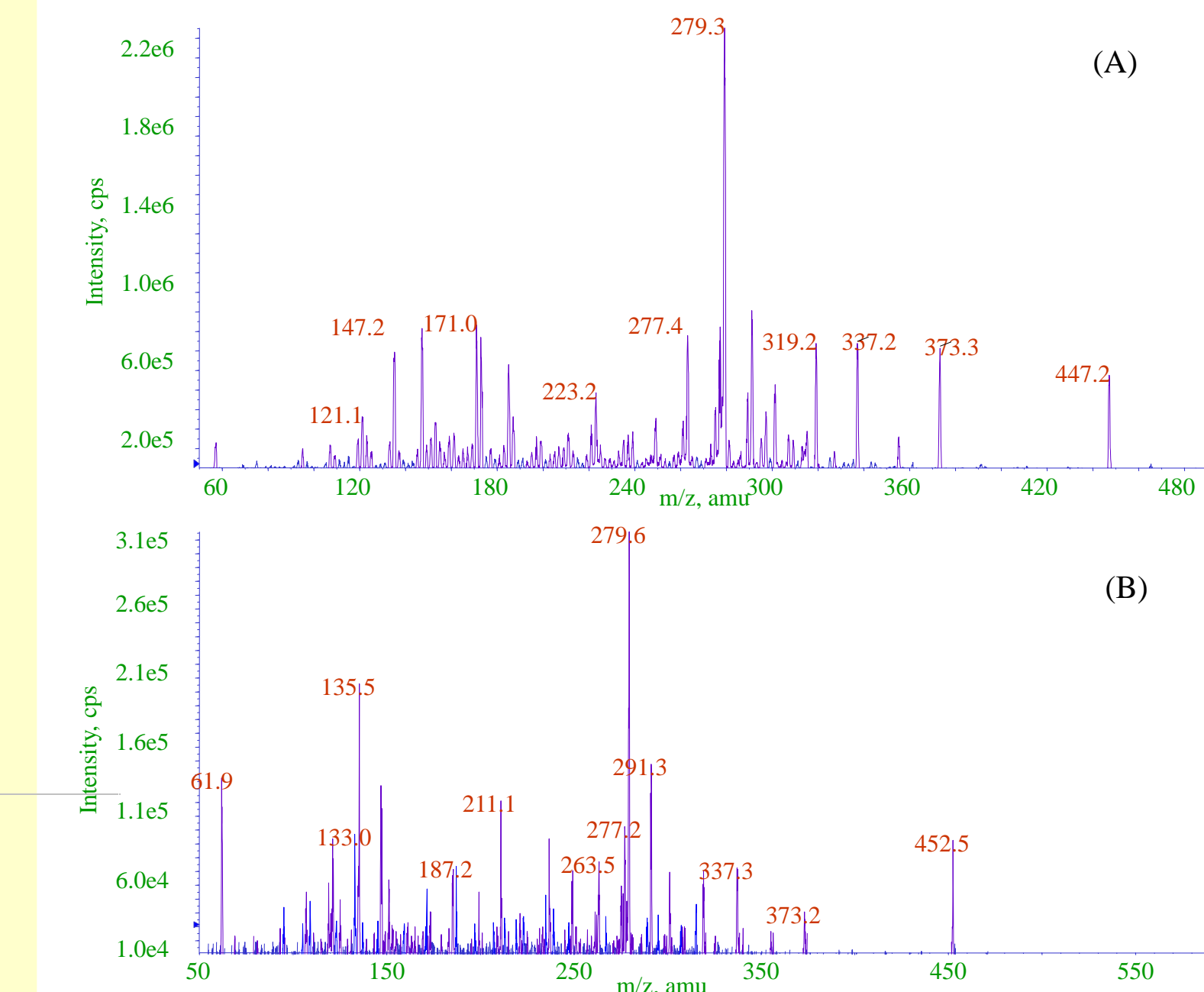


Figure 4. Product ion scan of B-17-P at 465.2 Da (A), and B-17-P-D₅ at 470.2 Da (B).

Table I. Validation Data Summary of BDP in Human Plasma

Calibration Range		10 to 2,500 pg/mL		
Correlation coefficient (3 batches)		0.991 to 0.998		
Accuracy & Precision		Accuracy	Precision	
Inter-Batch (n=15)	QC	RE%	CV%	
	LLOQ	10	-0.70	12.9
	Low	30	-5.33	9.23
	Medium	1000	-7.20	2.88
High	2000	-6.50	4.30	
Method Recovery		Compared with Nominal Value (%)		
		62.40		
		Condition	Accuracy	
			RE%	
Freeze/Thaw	3 Cycles, -70 °C	-10.5	-1.33	
Bench-To	4 hrs, Room Temperature	-8.50	-5.33	
Autosampler Extract Stability	3 Days, Room Temperature	-8.50	-4.67	
Long-Term Storage Stability	61 Days, -70 °C	-6.80	-11.33	

Table II. Validation Data Summary of B-17-P in Human Plasma

Calibration Range		20 to 5,000 pg/mL		
Correlation coefficient (3 batches)		0.9970 to 0.9971		
Accuracy & Precision		Accuracy	Precision	
Inter-Batch (n=15)	QC	RE%	CV%	
	LLOQ	20	4.00	13.6
	Low	60	1.83	7.89
	Medium	2000	-6.00	7.23
High	4000	-4.50	3.32	
Method Recovery		Compared with Nominal Value (%)		
		62.56		
		Condition	Accuracy	
			RE%	
Freeze/Thaw	3 Cycles, -70 °C	-7.25	-8.23	
Bench-To	4 hrs, Room Temperature	-5.75	-5.67	
Autosampler Extract Stability	4 Days, Room Temperature	-5.75	-3.54	
Long-Term Storage Stability	61 Days, -70 °C	-0.25	-2.11	

- In previous methods, two factors affected the detection limit: low extraction recovery and high matrix suppression. In addition, larger sample volumes (1-2 mL) were needed in order to achieve the desired LLOQ. Such conditions would require the use of individual extraction cartridges, which not only make sample preparation much longer, but could also introduce more error in the sample preparation procedure.

- A unique SPE method eluting with ethyl acetate and hexane dramatically increases the extraction recovery as well as decreases matrix interference, and the required LLOQs were achieved using only 0.5 mL of human plasma. Thus, sample clean-up can be done using a 96-well SPE plate, which makes sample preparation automation possible.

- Excellent linearity was obtained with a coefficient of determination (R²) greater than 0.991 for BDP and 0.997 for B-17-P. The inter-day (n=15) precision (CV%) and accuracy (RE%) for all QC plasma samples, including LLOQ were 2.88—12.9% and -7.20—-0.74%, respectively. (Table I) for BDP, and 3.32—13.6% and -6.00—4.00%, respectively (Table II) for B-17-P. Three freeze/thaw cycles, 4-h ambient temperature storage, and 2 month long-term storage at -70 °C QC appeared to have little effect on the quantitation.