Determination of Alendronate in Human Urine by High-Performance Liquid Chromatography with fluorescence Detection

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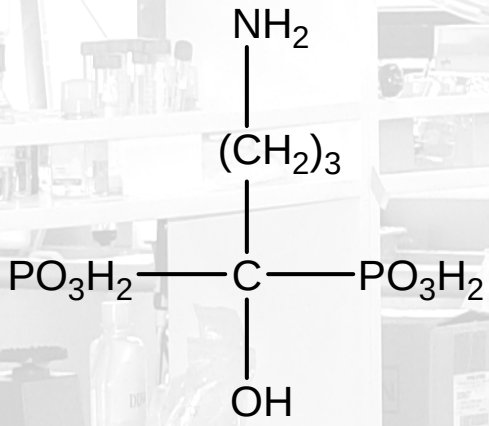
Introduction

A fast and sensitive liquid chromatographic method for the determination of alendronate in human urine was developed and validation. Alendronate and internal standard in human urine were extracted by diethylamine solid-phase extraction, 9-fluorenylmethyl derivative . The column effluent was monitored by fluorescence detection at an excitation 260nm and emission wavelengths 310nm. Analytes were separated on a Onyx C18 (4.6 X 100mm) with 25mM Sodium pyrophosphate in 20 mM citric acid (pH 3.88) / ACN / MeOH = 72.5 / 23.5 / 4 (v/v/v), as mobile phase. The flow rate was 2 ~ 3 mL/min with the total analysis time of 7.5 min. The standard calibration curves were linear over the concentration range 10-2000 ng/mL with correlation coefficient of 0.999. The limit of quantitation (at signal-to-noise ration S/N=10) was 10 ng/mL. This method has good precision (intra-day CV(%) $\leq$ 6.47, inter-day CV(%) $\leq$ 5.65) and accuracy (90.56-105.30%) over a wide dynamic range (10-2000 ng/mL). This method has been successfully applied to the pharmacokinetic study of alendronate in human urine.

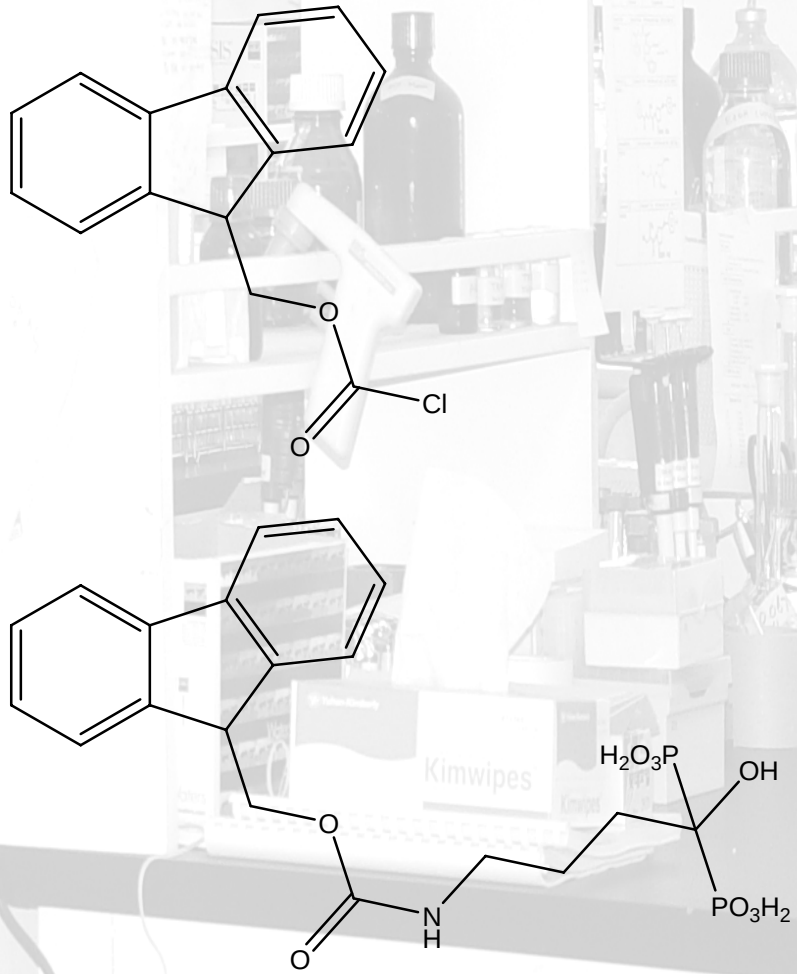
Summary of The Analytical Method

- Matrix : Human urine
- Sample preparation : SPE, 9-fluorenylmethyl derivative
- Concentration range : 10 to 2000 ng/mL
- Chromatography : HPLC
- Detection mode : FLD
- Quantitation method : Internal standard method
- Quantitation by : Peak area ratio

9-Fluorenylmethyl Derivatives



basic condition



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Monolithic HPLC Column

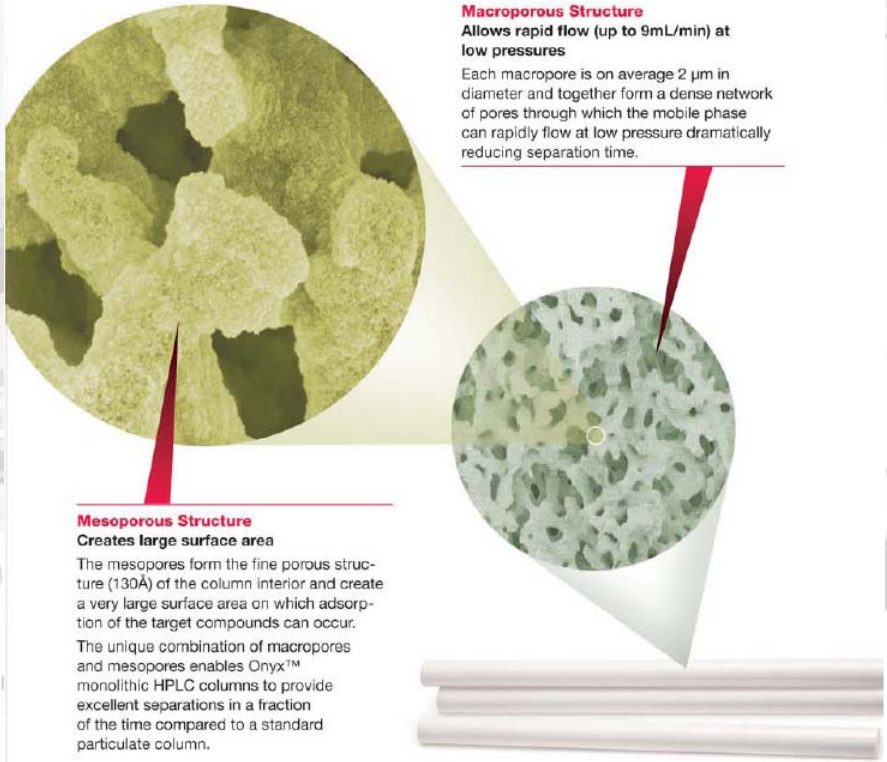
What is Onyx™?

ONYX Finish First

Bimodal Pore Structure

Onyx™ is a silica-based monolithic HPLC column. This technology creates highly porous rods of silica with a revolutionary bimodal pore structure.

The single piece of high-purity polymeric silica gel is then clad in PEEK tubing to make the finished product.



Macroporous Structure

Allows rapid flow (up to 9mL/min) at low pressures

Each macropore is on average 2 µm in diameter and together form a dense network of pores through which the mobile phase can rapidly flow at low pressure dramatically reducing separation time.

Mesoporous Structure

Creates large surface area

The mesopores form the fine porous structure (130Å) of the column interior and create a very large surface area on which adsorption of the target compounds can occur.

The unique combination of macropores and mesopores enables Onyx™ monolithic HPLC columns to provide excellent separations in a fraction of the time compared to a standard particulate column.

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A Comparison between Monolithic and ODS(I)

Performance obtained for alendronate using silica-based reversed-phase column and monolithic columns

	k	A_s	N_{df}	*SIN* [a]
Conventional silica-based reversed-phase column(ODS)	4.91	1.17	10847.31	12
Onyx monolithic column	3.33	1.03	3424.84	38

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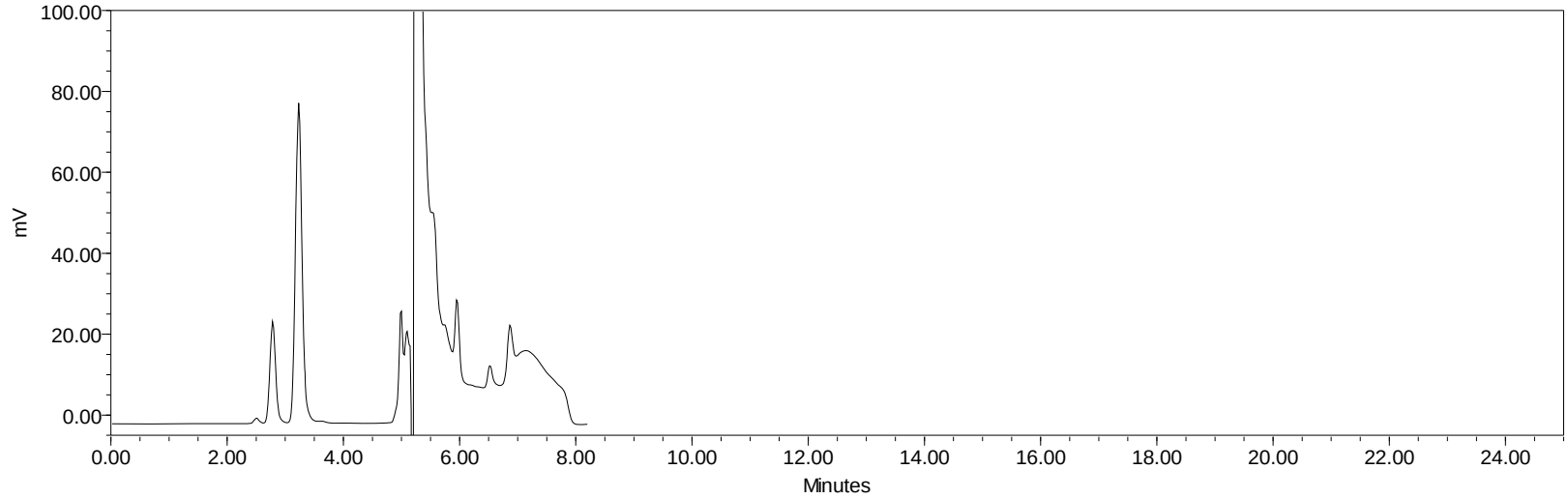
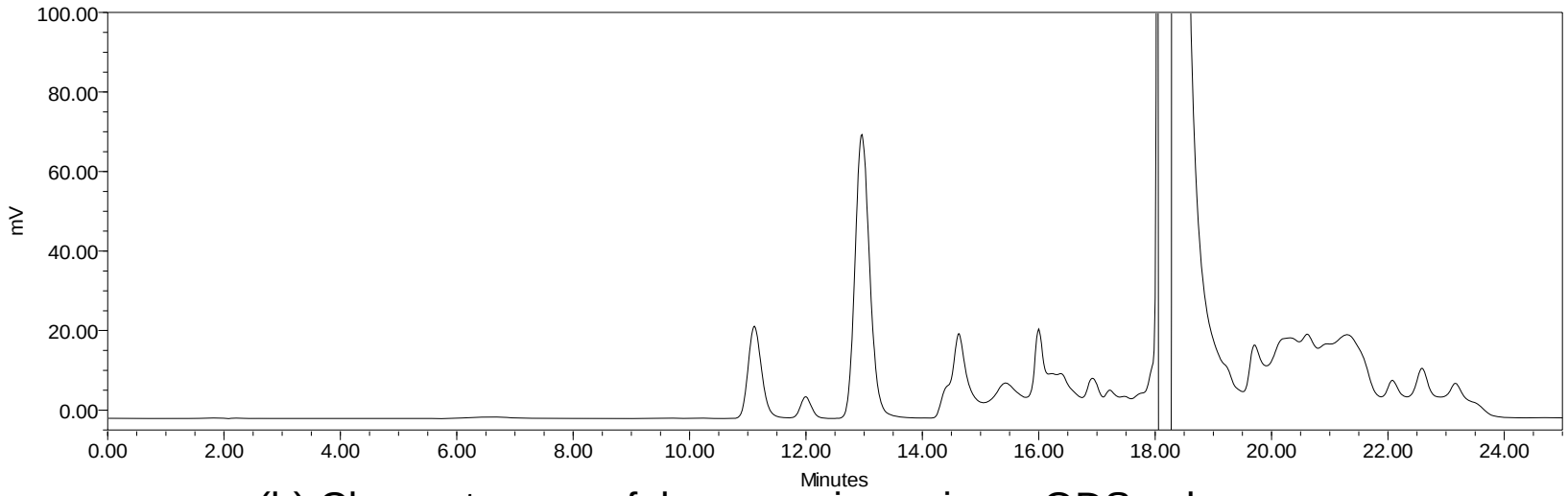
Chromatographic Conditions of HPLC

Mobile phase	25mM Sodium pyrophosphate in 20 mM citric acid (pH 3.88) / ACN / MeOH = 72.5 / 23.5 / 4 (v/v/v)
Flow rate	2 ~ 3 mL/min
Detection	Ex : 260 nm, Em : 310 nm
Injection volume	5 μL
Oven temperature	20 ℃
Column	Onyx C18 (4.6 X 100mm)

A Comparison between Monolithic and ODS(II)

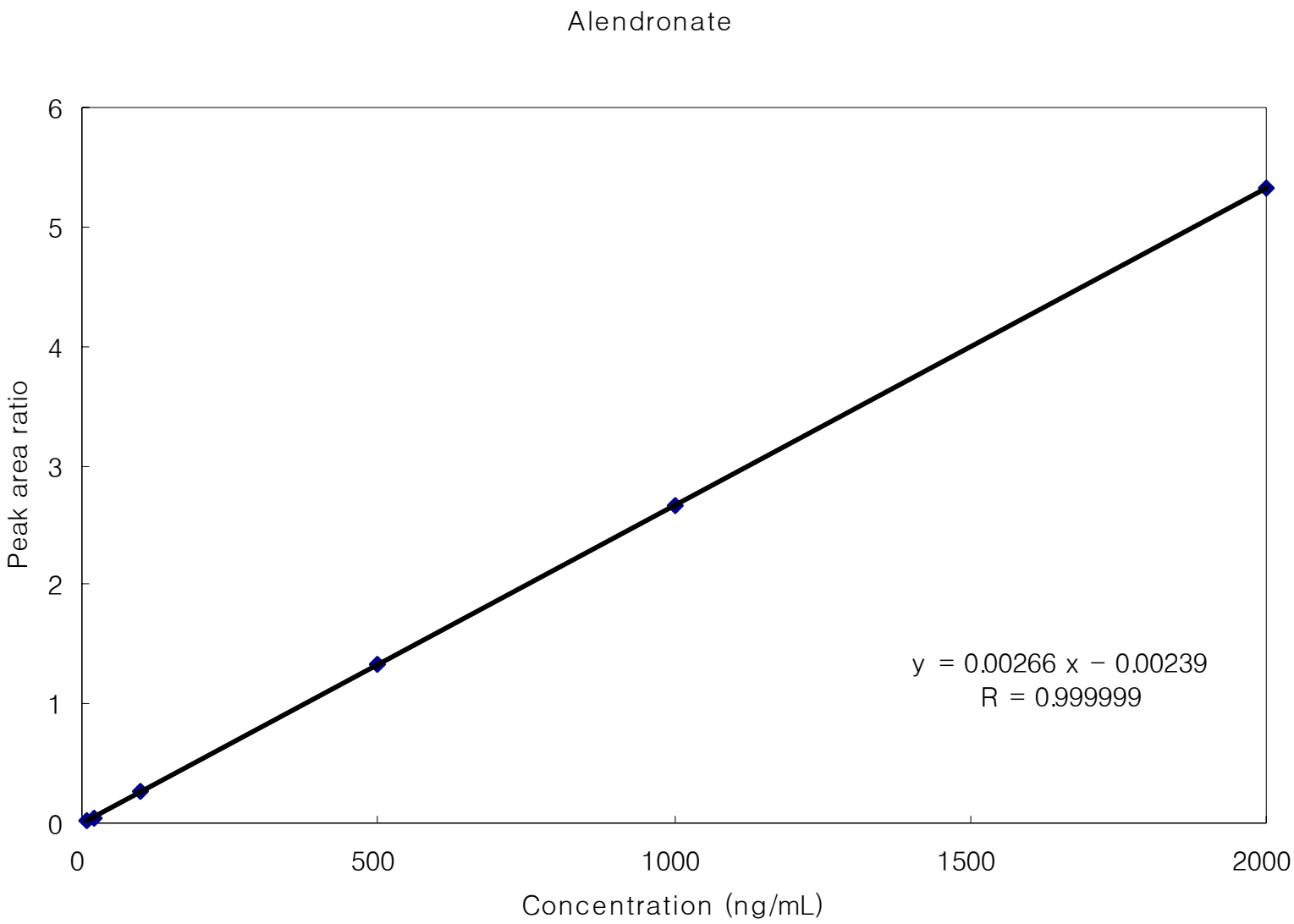


(a) Chromatogram of human urine using a monolithic column

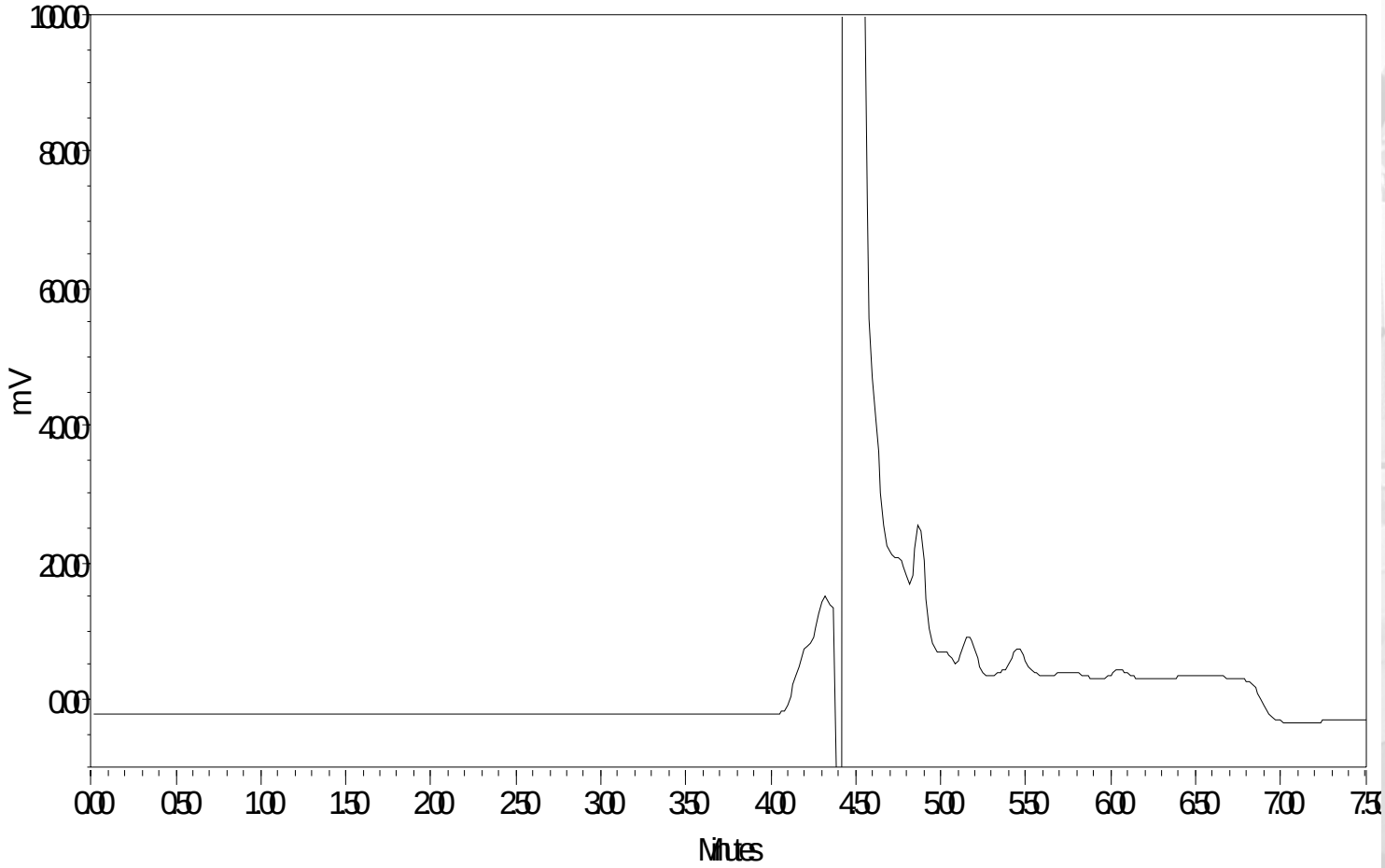


(b) Chromatogram of human urine using a ODS column

Calibration Curve of Alendronate



HPLC Chromatograms of Urine (I)



(a) Chromatogram of blank human urine

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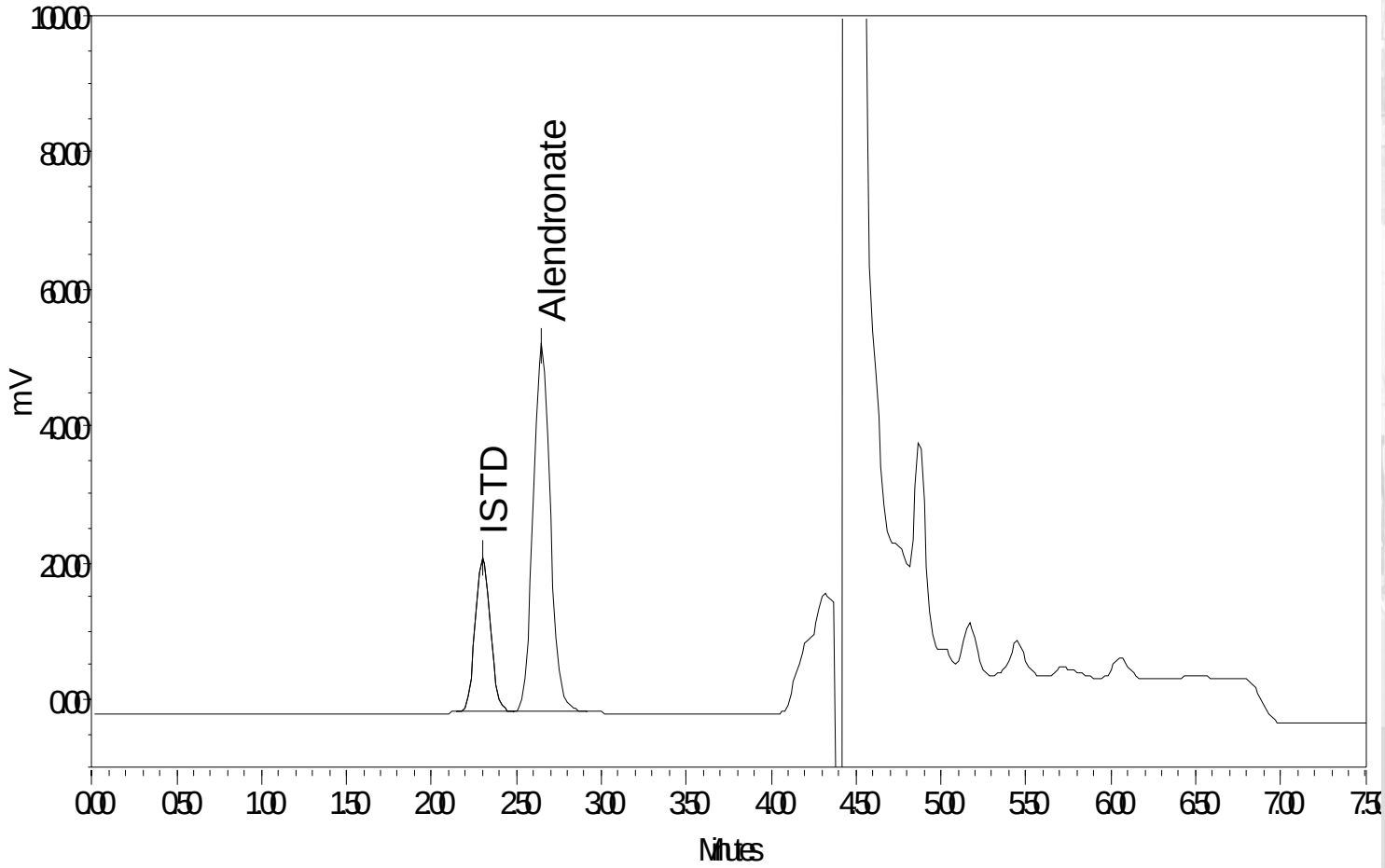
HPLC Chromatograms of Urine (II)



(b) Chromatogram of human urine spike with alendronate (1000ng/mL) and Internal standard

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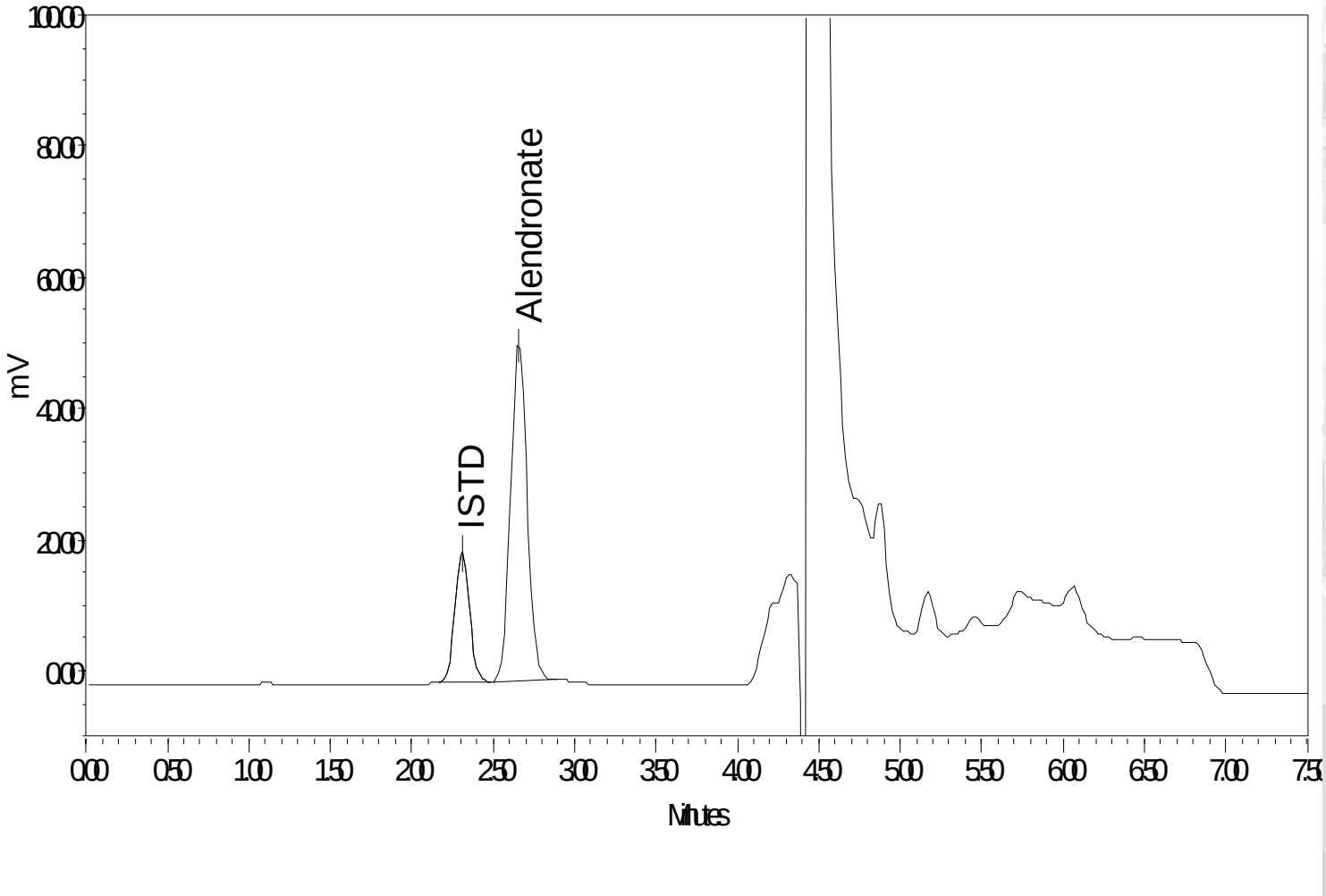
HPLC Chromatograms of Urine (III)



(c) Chromatogram of human urine sample after oral administration of 70mg alendronate capsule

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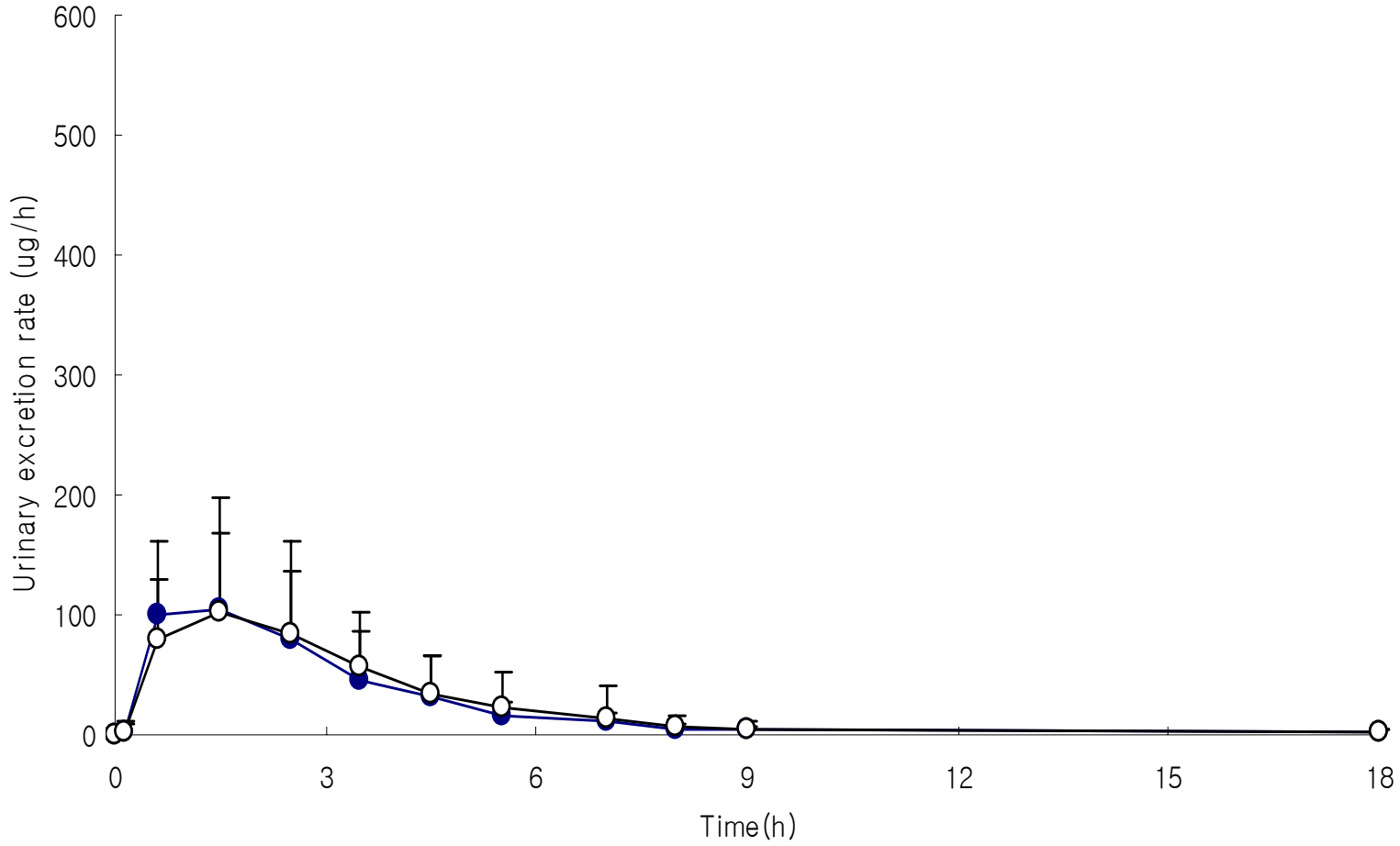
Urinary Excretion Rate of Alendronate in Human Urine- Time Curve



Conclusion

We developed a fast and sensitive HPLC-FLD method for the determination of alendronate in human urine. Validation experiments have shown that the assay has good precision and accuracy over a wide concentration range (10-2000 ng/mL). This method is accurate, reproducible and suitable for the analysis of alendronate in clinical samples.