

# Quantitative determination of the metabolite Ac-Tβ<sub>1-14</sub> in in-vitro and urine of rats treated with Thymosin β<sub>4</sub> by LC Orbitrap HR-MS/MS



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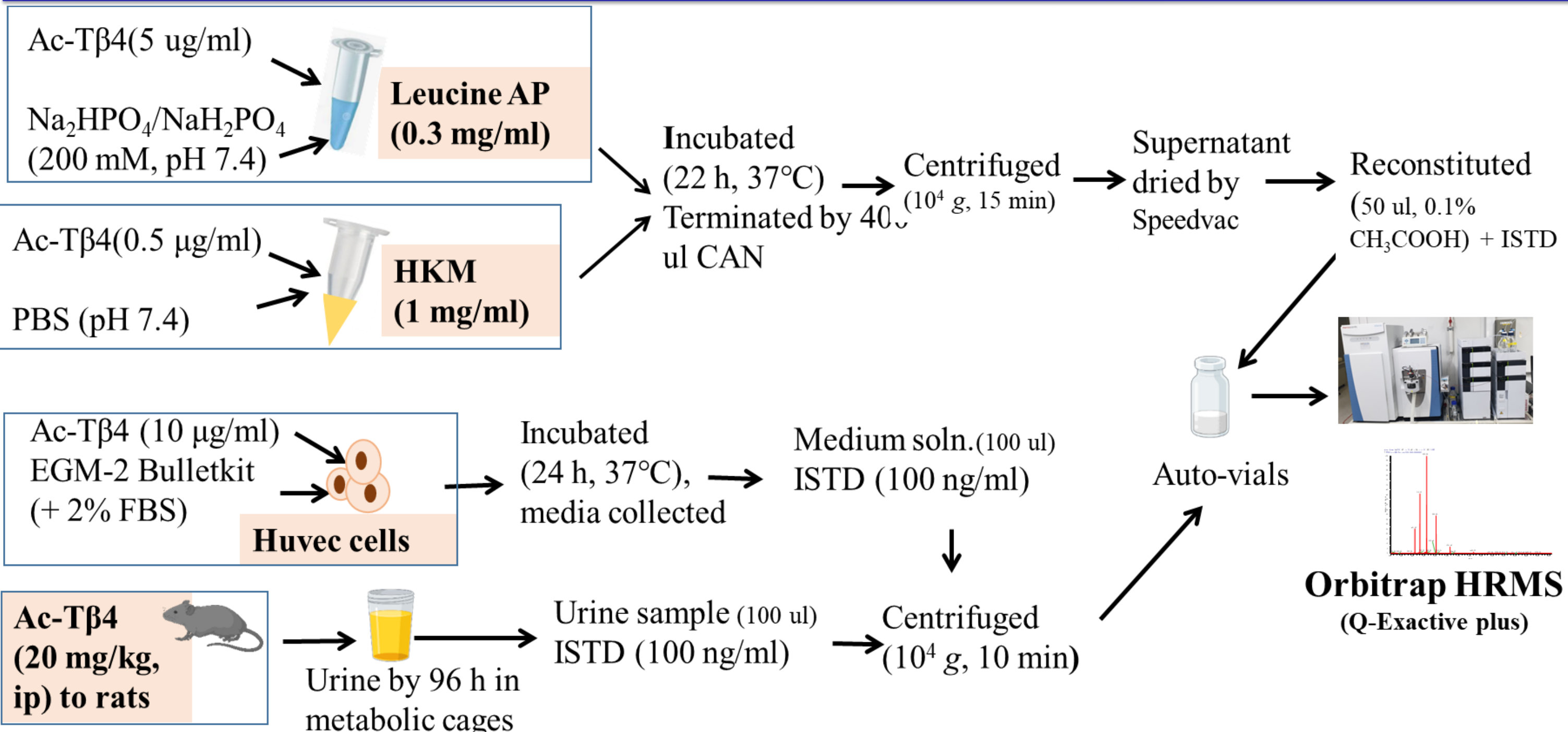
## Introduction

Thymosin β<sub>4</sub> (Tβ<sub>4</sub>) was reported to exert beneficial bioactivities such as tissue repair, anti-inflammation, and reduced scar formation. It is listed as a prohibited substance in sports by the World Anti-Doping Agency. However, no metabolism studies of Tβ<sub>4</sub> were reported yet. Previously, our lab reported in an in-vitro experiment that a total of 13 metabolites were found by using multiple enzymes, and six metabolites (Ac-Tβ<sub>31-43</sub>, Ac-Tβ<sub>1-11</sub>, Ac-Tβ<sub>17-43</sub>, Ac-Tβ<sub>1-14</sub>, Ac-Tβ<sub>1-15</sub>, and Ac-Tβ<sub>1-17</sub>) were confirmed by comparing with their synthetic standards.

## Objective

This study was aimed at validating the metabolite analysis in rats urine and to develop a method for quantifying the metabolites.

## Materials and Methods



## Validation data for quantification

Table 1 Method validation for metabolite Ac-Tβ<sub>1-14</sub> quantification in urine samples

Conc. (ng/ml)	Intra-day		Inter-day	
	Reproducibility <sup>b</sup> (RSD%)	Accuracy <sup>a</sup> (RE%)	Reproducibility (RSD%)	Accuracy (RE%)
1	5.24	10.2	6.35	14.07
10	5.86	0.21	3.32	6.05
20	3.64	4.99	3.23	1.84

Table 2 Method validation for metabolite Ac-Tβ<sub>1-14</sub> quantification in urine samples

Compound name	Matrix effect (10 ng/ml)	<sup>a</sup> LOD (ng/ml)	<sup>b</sup> LOQ (ng/ml)	Equation	r <sup>2</sup>
AcTB <sub>1-14</sub>	8.41	0.19	0.58	Y=0.0016x-0.0003	0.9995

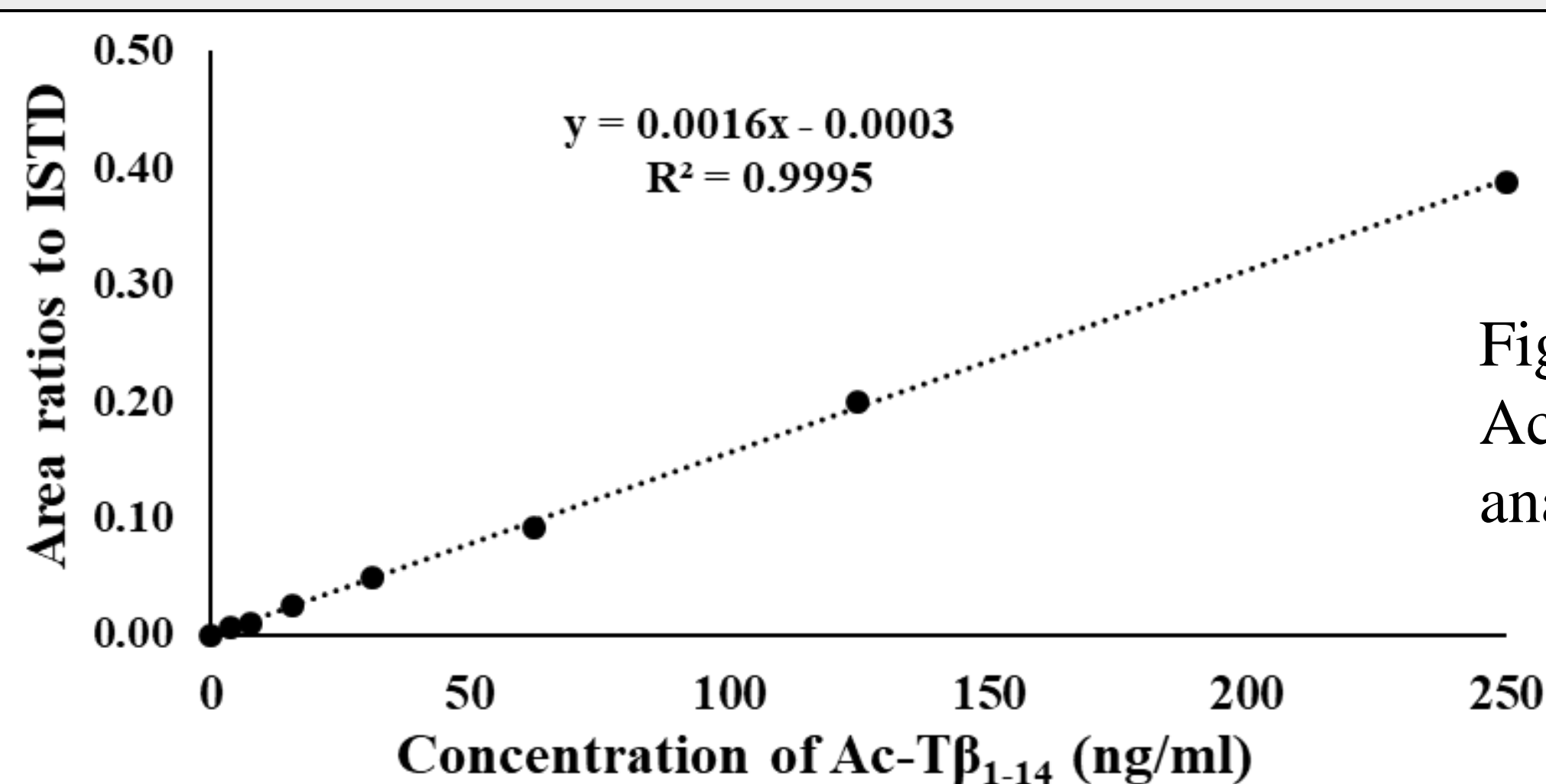


Fig. 1 Calibration curve for Ac-Tβ<sub>1-14</sub> quantitative analysis

## Results

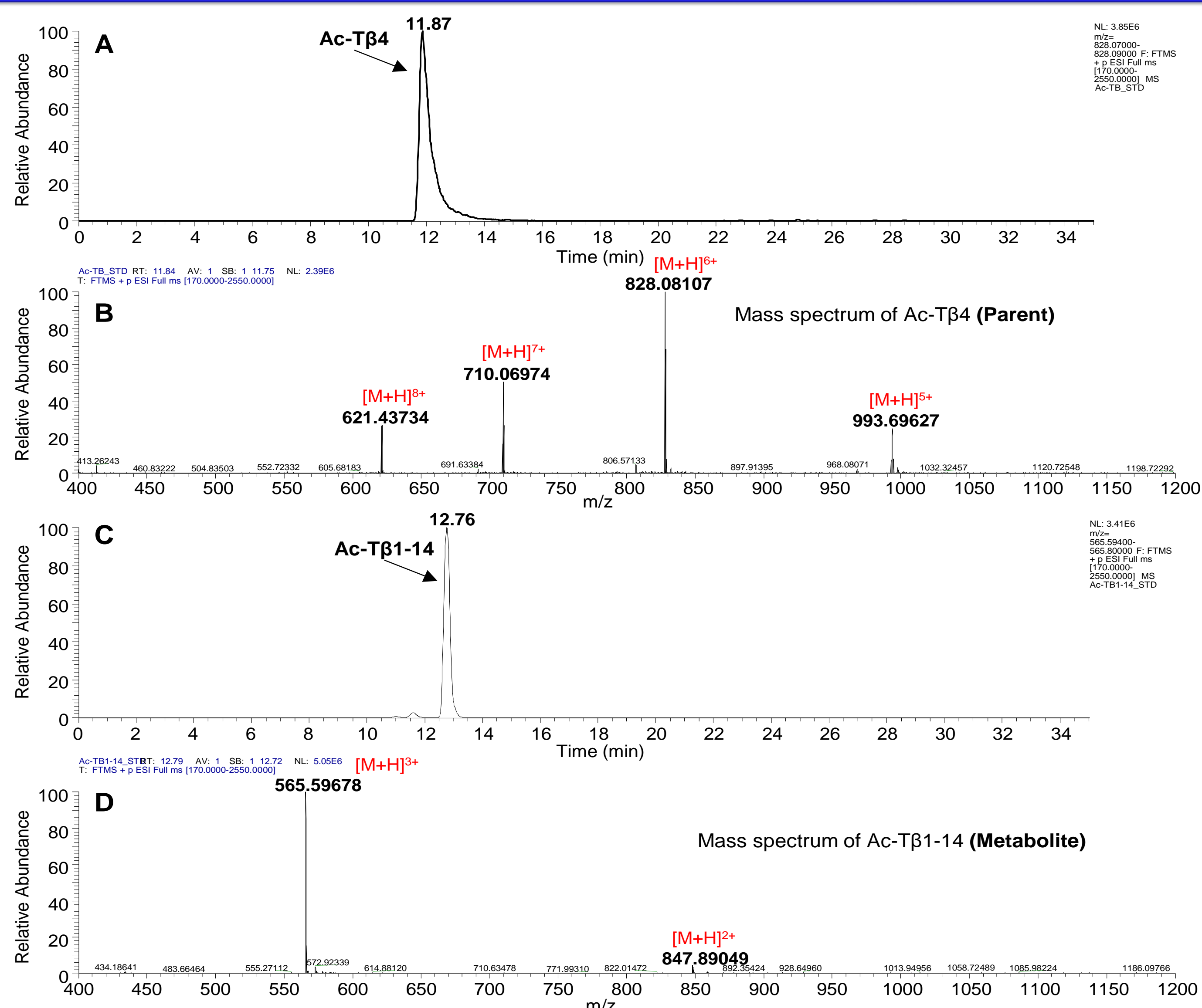


Fig. 2 The extracted respective ion chromatograms and mass spectra of parent Ac-Tβ<sub>4</sub> (A, B) and its metabolite Ac-Tβ<sub>1-14</sub> (C, D)

## Results

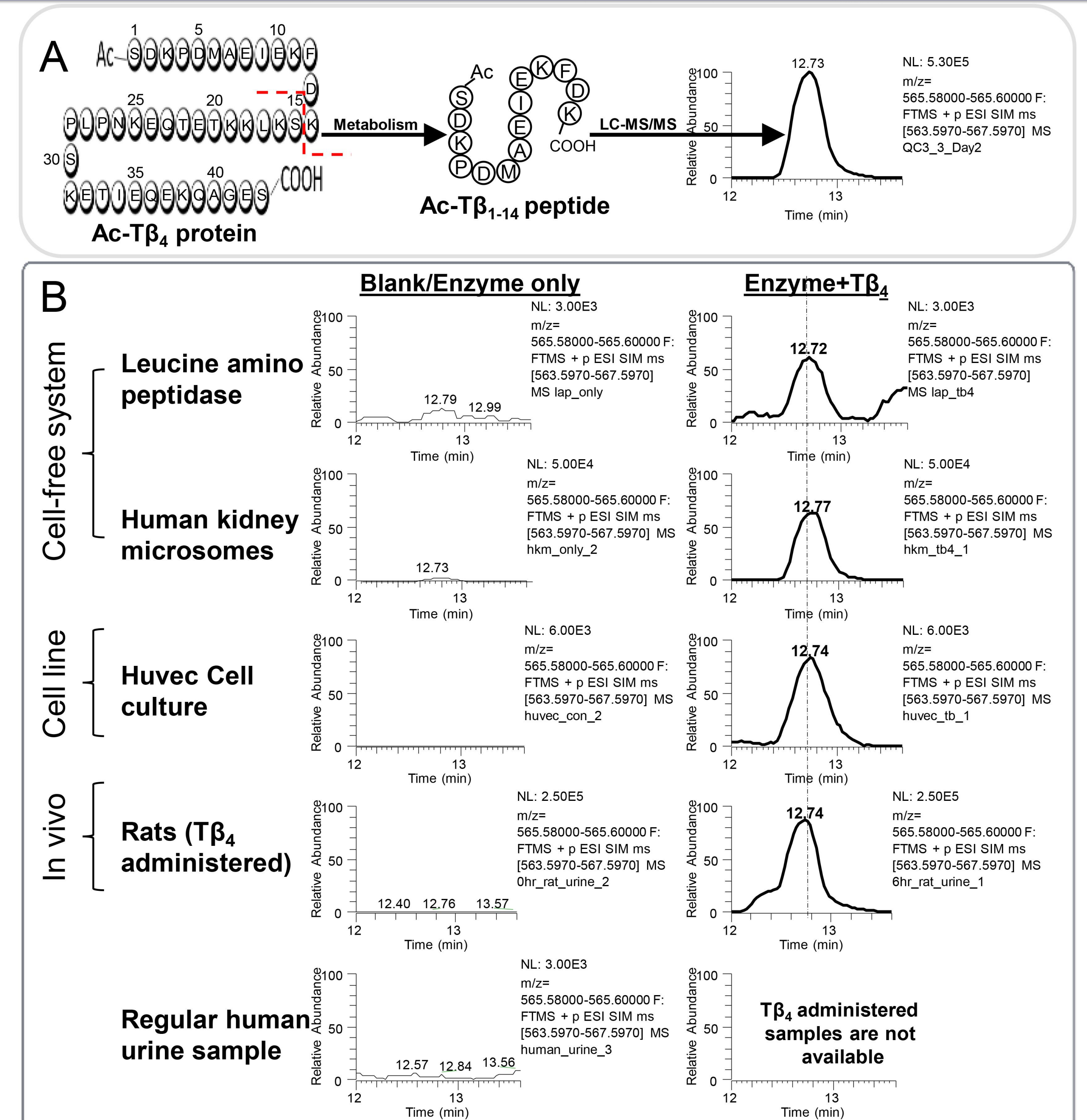


Fig. 3 Schematic diagrams of Ac-Tβ<sub>4</sub> metabolism (A). Ac-Tβ<sub>4</sub> was metabolized to Ac-Tβ<sub>1-14</sub> in different systems such as enzymes, huvec cells, and rats. The metabolite was not present on its control or blank samples including human urine samples (B)

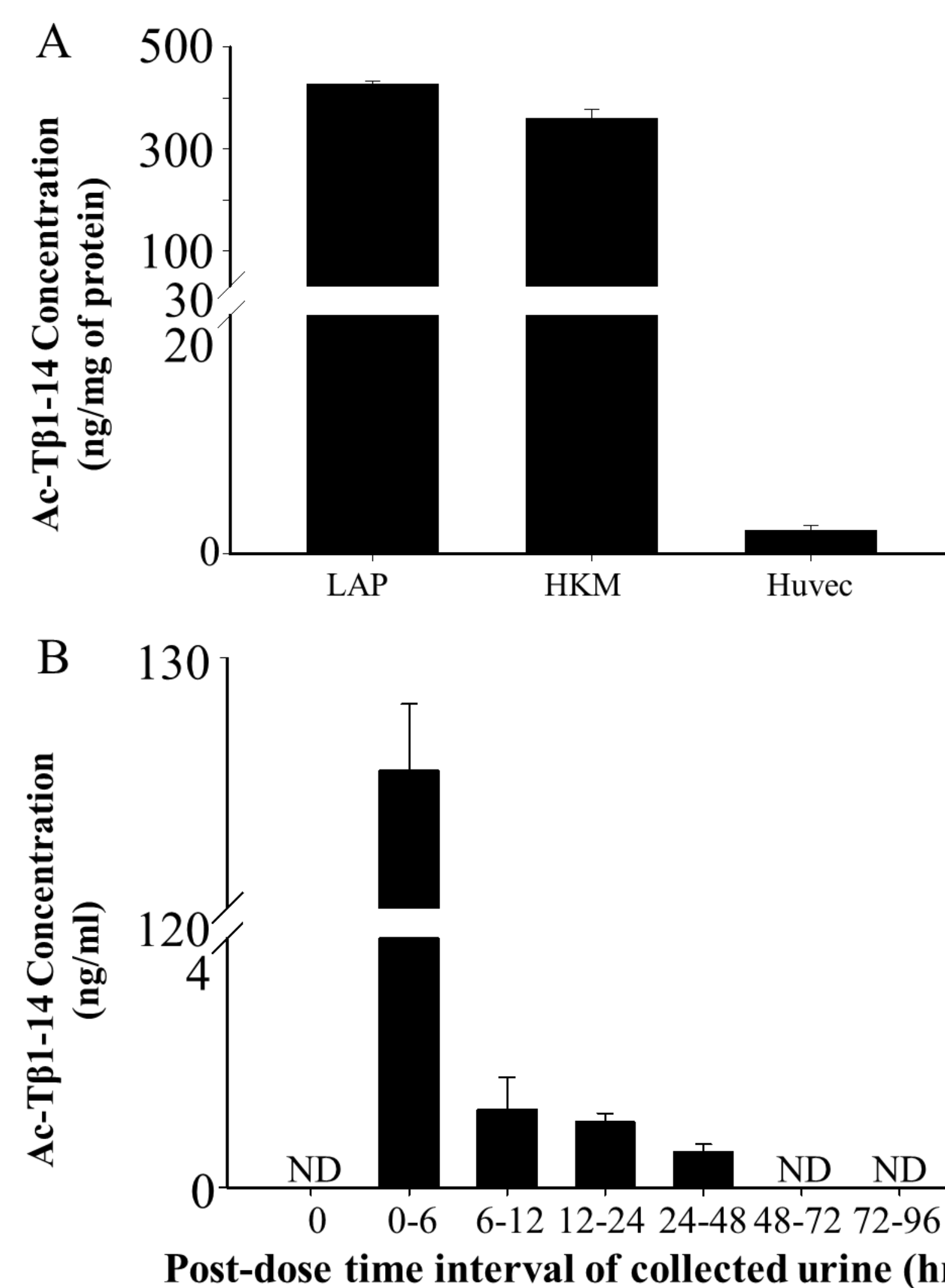


Fig. 4 Quantification of the metabolite Ac-Tβ<sub>1-14</sub> in leucine amino peptidase-buffer system (LAP), cultured huvec cells, and human kidney microsomes (HKM) after Ac-Tβ<sub>4</sub> treatment (A), and in urine of rats after Ac-Tβ<sub>4</sub> administration (B)

## Summary

- Ac-Tβ<sub>1-14</sub> is commonly detected in all different systems such as enzymes, huvec cells, and rats.
- The metabolite of Ac-Tβ<sub>4</sub>, Ac-Tβ<sub>1-14</sub>, is the only detectable in rats (without extraction step).
- Ac-Tβ<sub>1-14</sub> was not detected in non-treated rats and human blank urine (n = 8 individuals).

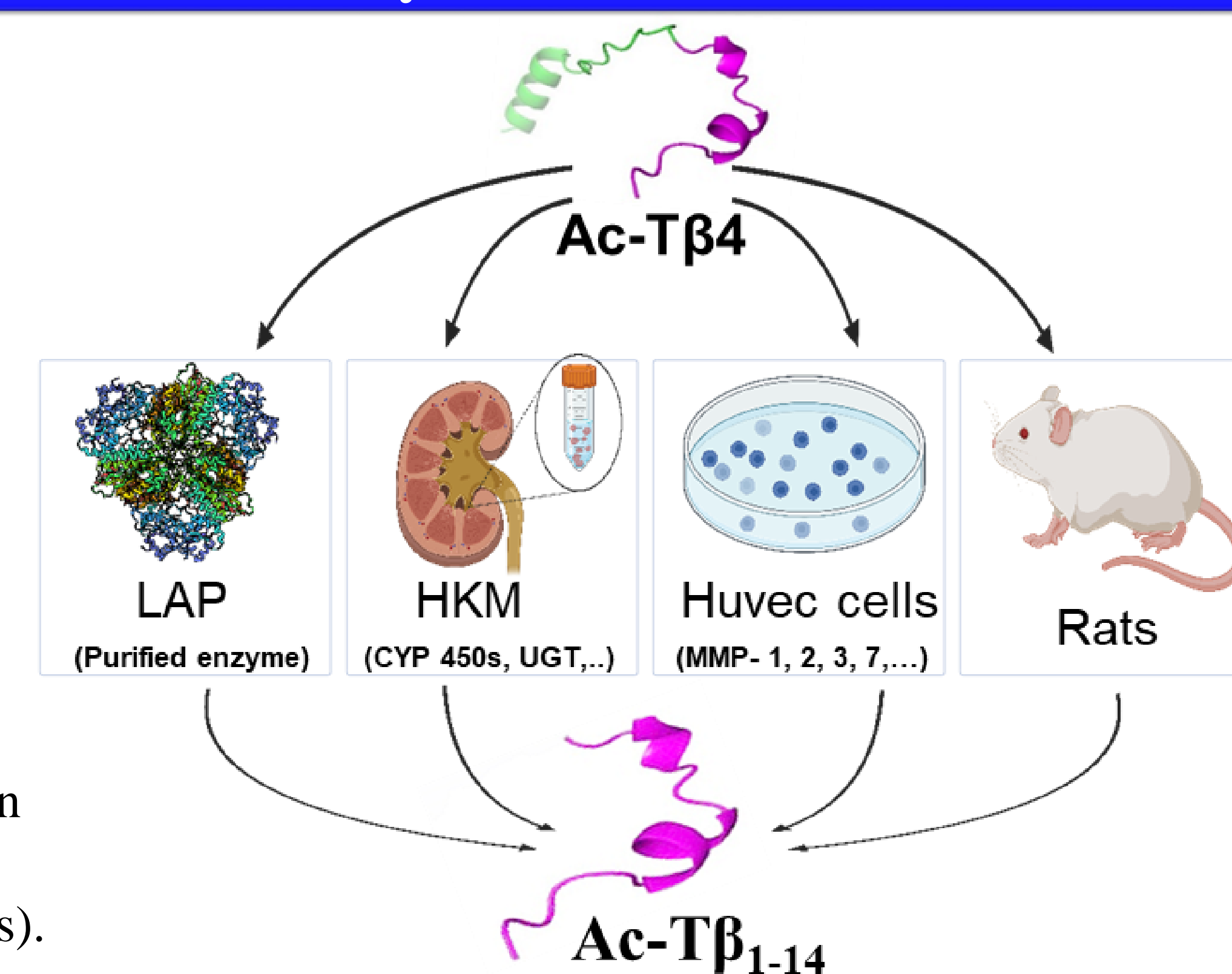


Fig. 5 A summary of the generation of Ac-Tβ<sub>1-14</sub> metabolite from the parent protein Ac-Tβ<sub>4</sub>

This data suggest that urinary Ac-Tβ<sub>1-14</sub> metabolite is a potential biomarker for screening the parent Ac-Tβ<sub>4</sub> in sports, requiring further study in Ac-Tβ<sub>4</sub>-positive human urine samples.