Application Experiment Report of Arginine Enzyme

The purity of arginase was studied by applying reaction experiments, and the presence of creatine or other impurities in the arginase-catalyzed reaction was detected.

I. Experimental Data

Experimental Conditions:

The experiment was conducted using a 400 ml system with an arginine concentration of 20%. The enzyme dosage used was 1400 u/g of substrate. The initial pH of the reaction was maintained between 9.80 - 9.90, and the pH was not controlled during the reaction. The temperature was set at 30°C. Protective agents, including 0.6% anhydrous sodium sulfite and 0.4% cerium chloride tetrahydrate, were added. The entire process was carried out under a closed nitrogen atmosphere. The enzyme batch used was labeled as 20150501, with an activity of 3500 u/g.

Enzyme Batch Number	Reaction Time (min)	Arginine Residual (mg/ml)	Conversion Rate (%)	Ornithine Molar Yield (%)	Other Observations
20150501	90	0.78	99.61	98.24	No impurity peak

II. Liquid-phase Data

1. Liquid-phase Detection Conditions:

(1) Preparation of mobile phase: Weigh 4.08 g of CH3COONa·3H2O, dissolve in 600 ml of ultrapure water, filter through a 0.45 μm water-based filtration membrane, add 400 ml of chromatographic grade methanol, mix well, and degas for about 20 minutes.

(2) Chromatographic column: CenturySIL C8 -BDS 5gm 4.6x250mm

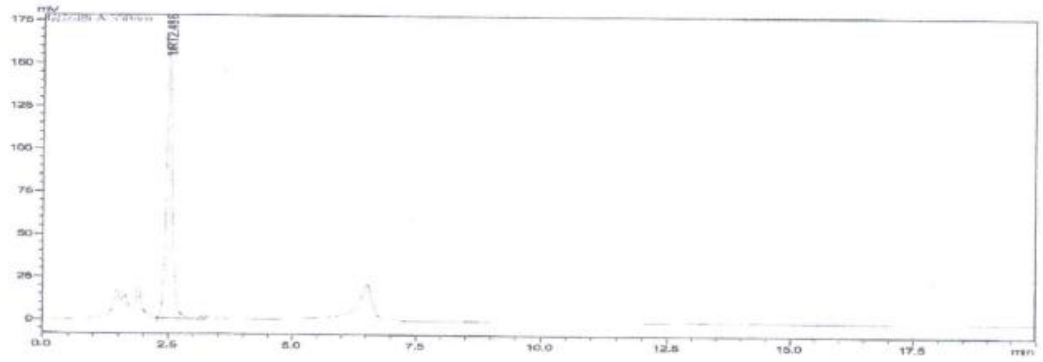
(3) Flow rate: l.00ml/min

(4) Detection: UV detection at 338 nm

(5) HPLC analysis: In a 50 ml volumetric flask containing 0.5 ml of the sample, add 10 ml of 0.4 mol/L borate buffer solution at pH 9.5 and 2 ml of pre-column derivatizing agent. Shake vigorously for 1 min, then add 0.4 mol/L, pH 9.5 borate buffer solution to the mark, mix well, and filter the solution for HPLC analysis.

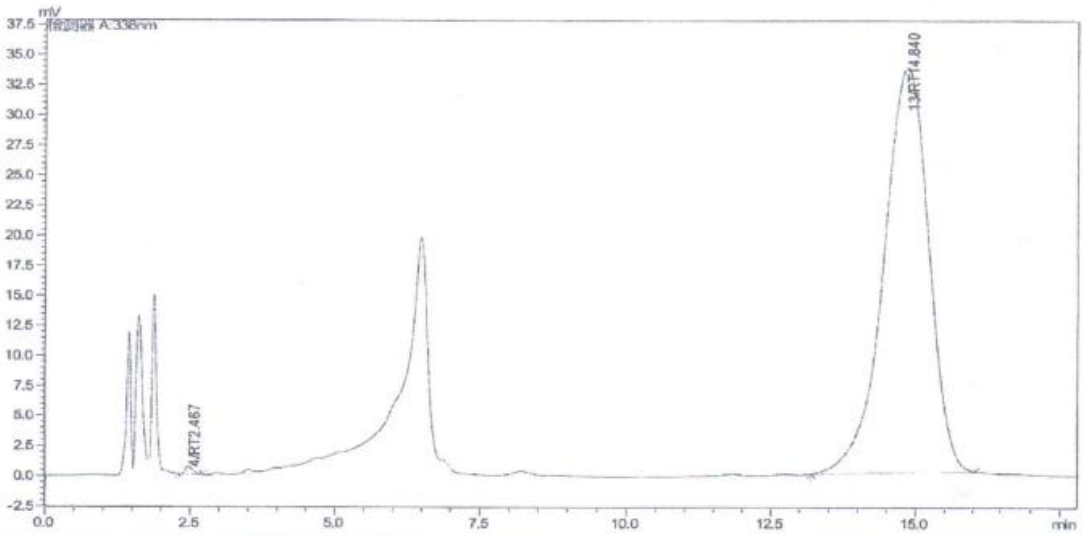
2. Liquid-phase Chromatograms:

Figure 1: Initial Sample



Name	Retention Time (min)	Peak Height	Peak Area	Area Percentage (%)	Separation Efficiency
Arginine	2.486	161957	1328698	100	—

Figure 2: Final Sample



Name	Retention Time (min)	Peak Height	Peak Area	Area Percentage (%)
Arginine	2.467	626	5656	0.3093
Ornithine	14.84	33689	1822643	99.6907

Figure 3: Blank (derivatizing agent)

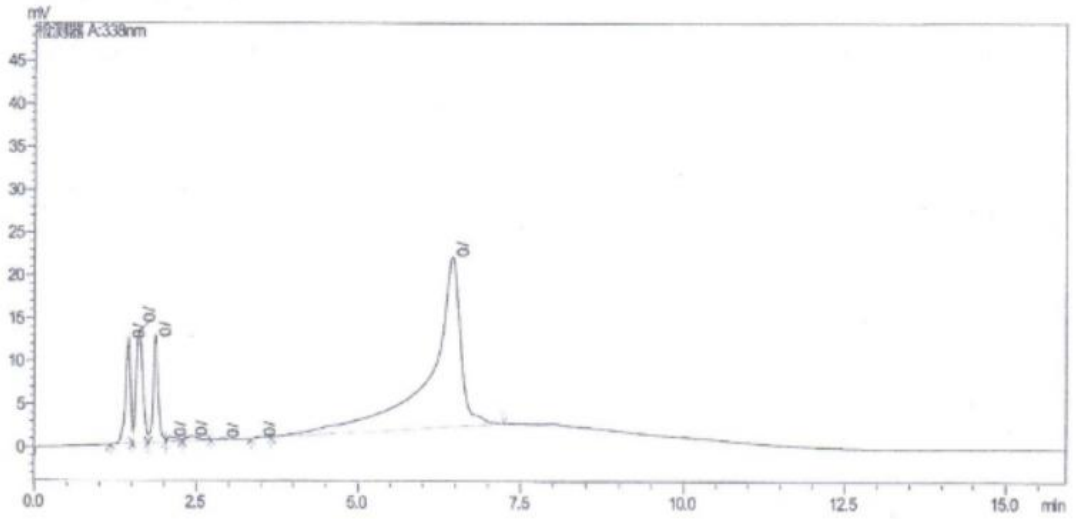
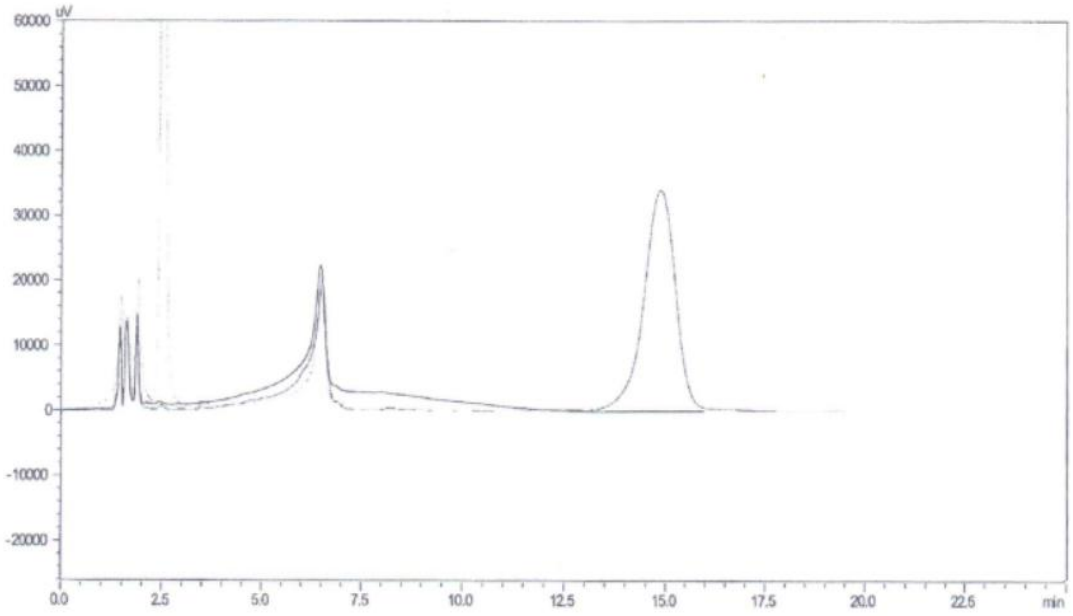


Figure 4: Data Comparison



Note: Black - blank; Red - initial sample; Blue - final sample

III. Summary and Analysis

From the liquid-phase chromatograms, it can be observed that the final sample only shows peaks of the derivatizing agent, substrate (arginine), and product (ornithine), with no appearance of guanidine or unidentified impurity peaks.

Application Research Laboratory
May 18, 2015

精氨酸酶应用实验报告

通过应用反应实验研究精氨酸酶的纯度，检测精氨酸酶促反应中是否有胍氨酸或其他杂质产生。

一、应用实验数据

实验条件：

精氨酸浓度 20%，400ml 体系，投酶量 1400u/g 底物，反应起始 pH9.80~9.90，反应过程不控 pH，温度 30℃，保护剂为 0.6‰的无水亚硫酸钠和 0.4‰的四水氯化锰，全程密闭通氮气保护，酶批号 20150501 批，活力 3500u/g。

酶批号	反应时间（min）	精氨酸残留（mg/ml）	转化率（%）	鸟氨酸摩尔收率（%）	其他情况
20150501 批	90	0.78	99.61	98.24	无杂质峰

二、液相数据

1、液相检测条件

（1）流动相的配制：称取 4.08g$CH_3COONa \cdot 3H_2O$，加 600ml 超纯水溶解后，用孔径 0.45μm 的水系滤膜过滤，加入 400ml 色谱级甲醇，混合均匀后脱气 20min 左右。

（2）色谱柱：CenturySIL C_8 –BDS 5μm 4.6×250mm

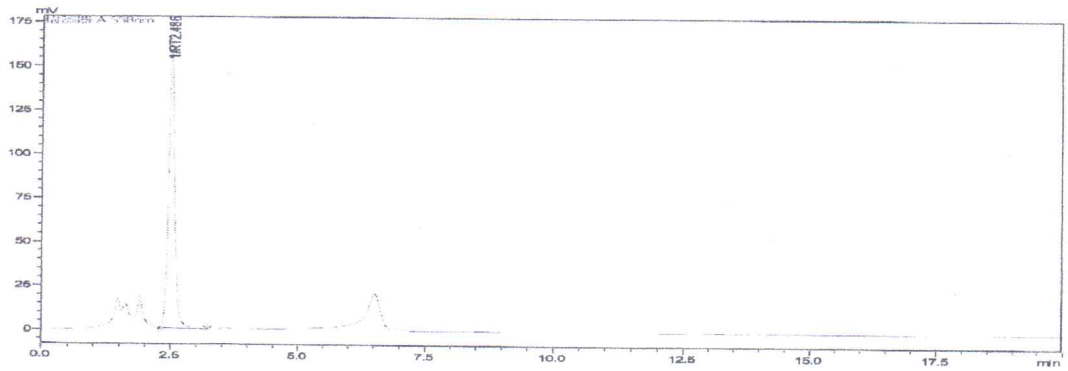
（3）流速：1.00ml/min

（4）检测：UV 检测 338nm

（5）HPLC 分析：取有 0.5mL 样品的 50mL 容量瓶中依次加入 10mL 0.4mol/L、pH9.5 的硼酸缓冲液和 2mL 柱前衍生剂，快速摇动，1min 后加入 0.4mol/L、pH9.5 的硼酸缓冲液定容至刻度，摇匀后过滤进液相进行 HPLC 分析。

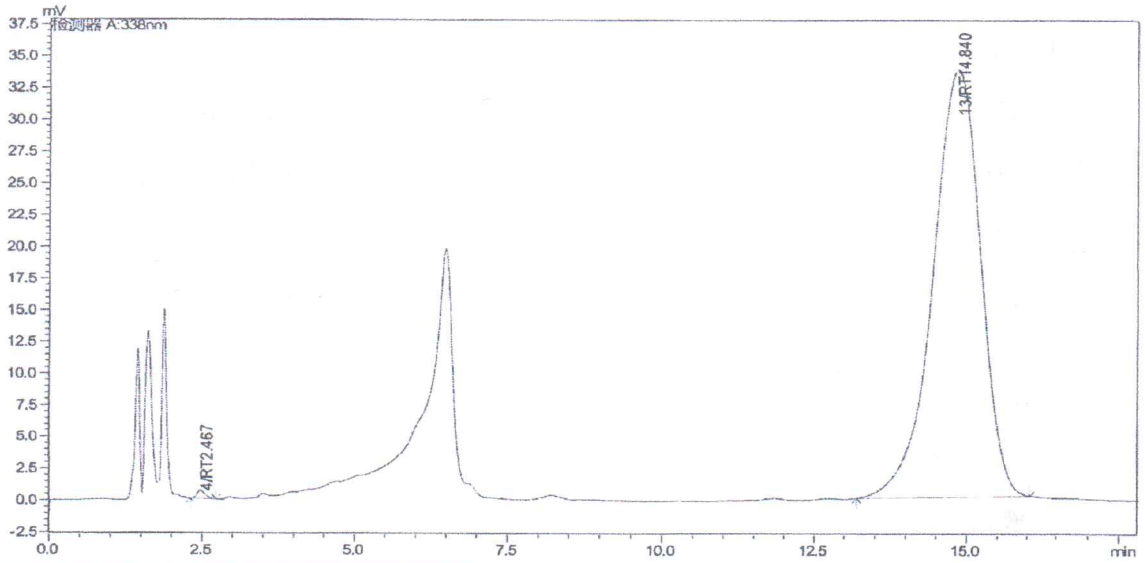
2、液相图谱

图 1：起始样



名称	出峰时间（min）	高度	面积	面积百分比（%）	分离度
精氨酸	2.486	161957	1328698	100	--

图 2：终止样



名称	出峰时间（min）	高度	面积	面积百分比（%）
精氨酸	2.467	626	5656	0.3093
鸟氨酸	14.84	33689	1822643	99.6907

图 3：空白（衍生剂）

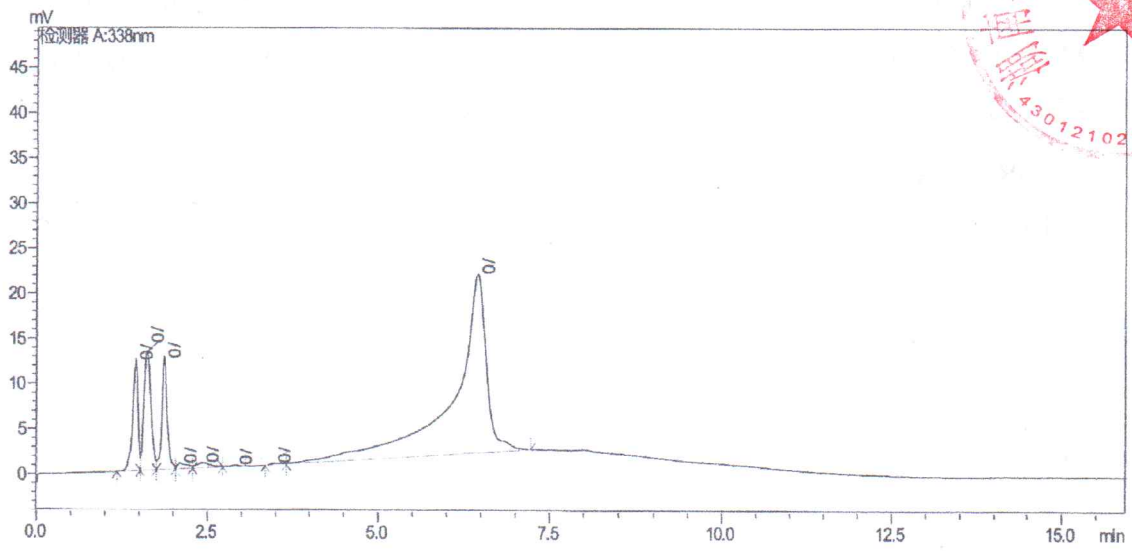
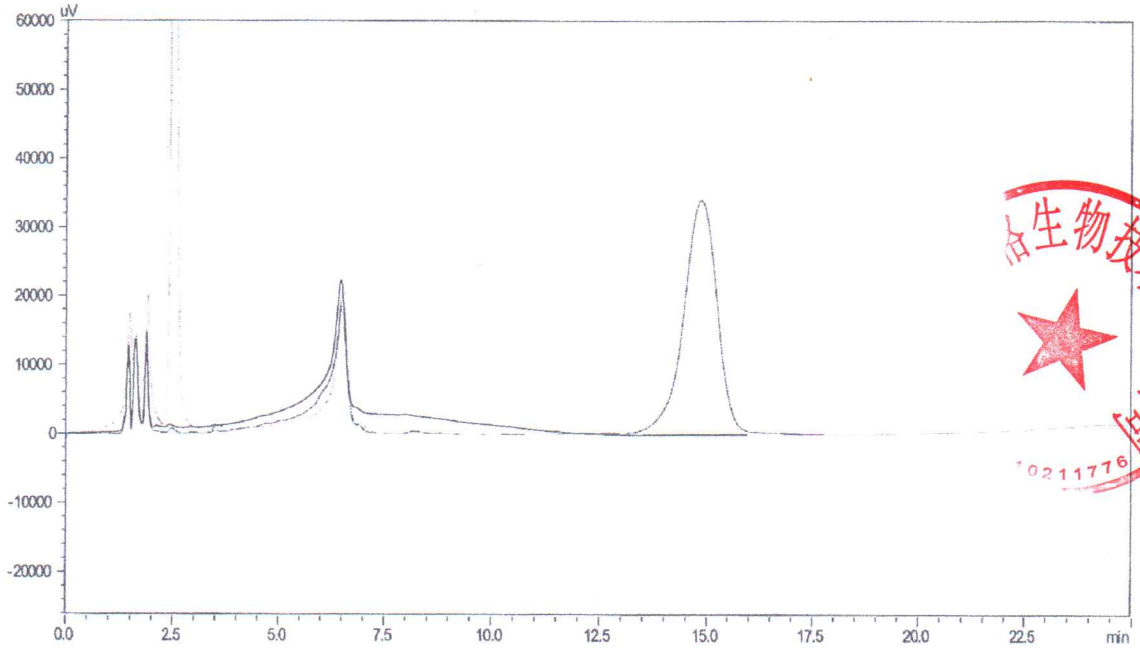


图 4：数据对比



注：黑色：空白；红色：起始样；蓝色：终止样

三、总结分析

由液相图谱可知，转化终止样中只有衍生剂峰、底物（精氨酸）峰和产物（鸟氨酸）峰，未见胍氨酸或不明杂质峰出现。

应用研究室

2015 年 5 月 18 日