Enzymatic Assay of α-CHYMOTRYPSIN (Insoluble)  
(EC 3.4.21.1)

PRINCIPLE:

ATEE + H₂O → α-Chymotrypsin > N-Acetyl-L-Tyrosine + Ethanol

Abbreviation used:
ATEE = N-Acetyl-L-Tyrosine Ethyl Ester

CONDITIONS:  T = 30°C, pH 8.0

METHOD:  Titrimetric

REAGENTS:

A. 50% (w/w) Methanol Solution (MeOH)  
(Prepare 25 ml in deionized water using Methanol, Absolute.)

B. 50 mM N-Acetyl-L-Tyrosine Ethyl Ester (ATEE)  
(Prepare 20 ml in Reagent A using N-Acetyl-L-Tyrosine Ethyl Ester.)

C. 500 mM Calcium Chloride Solution (CaCl₂)  
(Prepare 25 ml in deionized water using Calcium Chloride, Dihydrate.)

D. 100 mM Sodium Hydroxide Solution Standardized (NaOH)  
(Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous. Standardize according to the ACS Reagent Procedure.)

E. α-Chymotrypsin Enzyme, Insoluble (Enzyme)  
(Use α-Chymotrypsin, Insoluble, 5 – 10 mg.)

PROCEDURE:

Weigh (in milligrams) the following reagents into a suitable container:

Test
Reagent E (Enzyme) 5.00 – 10.00
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PROCEDURE:  (continued)

Then pipette (in milliliters) the following reagents:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
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<tbody>
<tr>
<td>Deionized Water</td>
<td>6.00</td>
</tr>
<tr>
<td>Reagent C (CaCl₂)</td>
<td>2.00</td>
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</tbody>
</table>

Mix by swirling and adjust the pH to 8.4 with Reagent D.  Equilibrate to 30°C and then add:

<table>
<thead>
<tr>
<th></th>
<th>2.00</th>
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<tbody>
<tr>
<td>Reagent B (ATEE)</td>
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Mix by swirling and incubate at 30°C.  When the pH reaches 8.0 begin timing the reaction.  Run the reaction for 1 - 5 minutes.  Maintain the pH of the reaction mix at 8.0 by the addition of small volumes (0.10 ml) of Reagent D.  Record the volume of Reagent D used to maintain the pH at 8.0 and the time required.

CALCULATIONS:

\[
\text{Units/mg solid} = \frac{(\text{Molarity of NaOH})(\text{NaOH})(1000)}{(\text{mg solid})(T)}
\]

NaOH = Volume (in milliliters) of Reagent D used in the assay  
1000 = Conversion factor to μmoles from millimoles  
mg solid = mg solid of α-chymotrypsin, insoluble, used in the assay  
T = Time of assay (in minutes) required to maintain the pH at 8.0

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of ATEE per minute at pH 8.0 at 30°C.

INITIAL ASSAY CONCENTRATIONS:

In a 10.00 ml reaction mix, the initial concentrations are 100 mM calcium chloride, 10 mM N-acetyl-L-tyrosine ethyl ester, and 5 - 10 mg of α-chymotrypsin, insoluble.
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REFERENCES:


NOTES:


2. This assay is based on the cited references.

This procedure is for informational purposes.