

ACCURACY (CLSI EP9-P)⁽¹⁰⁾

The performance of this method (y) was compared with the performance of a similar acetaminophen method (x) on a Hitachi 717. Fifty seven patient serum samples ranging from 0.6-34.0 mg/dL (41-2250 µmol/L) gave a correlation coefficient of 0.9976. Linear regression analysis gave the following equation:








$$\text{This method} = 1.01 (\text{reference method}) - 0.38 \text{ mg/dL } (25.2 \text{ } \mu\text{mol/L}).$$

The performance of this method with plasma (y) was compared to the performance of this method with serum (x) on an Advia 1650. Twenty-five serum and plasma samples spiked with acetaminophen ranging from 0.04-19.5 mg/dL (2.7-1319 µmol/L) gave a correlation coefficient of 0.9996. Linear regression analysis gave the following equation:

$$\text{This method (plasma)} = 1.01 [\text{This method (serum)}] - 0.01 \text{ mg/dL } (0.7 \text{ } \mu\text{mol/L}).$$

REFERENCES

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Definitions for Symbols	
 Batch code	 Use by YYYY-MM-DD or YYYY-MM
 Manufacturer	 Catalog number
 Consult instructions for use	 Temperature limitation
 <i>In vitro</i> diagnostic medical device	

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IN50510-11
August 16, 2011



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ACETAMINOPHEN-SL ASSAY

CATALOGUE NUMBER: 505-10 **SIZE:** R1: 1 x 10 mL, R2: 2 x 10 mL
505-30 R1: 3 x 10 mL, R2: 6 x 10 mL

INTENDED USE

For the IN VITRO quantitative measurement of acetaminophen in serum and plasma.

TEST SUMMARY

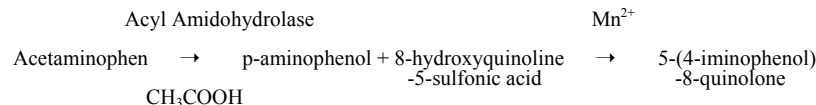
Acetaminophen (paracetamol) is used as an analgesic in many different formulations.⁽¹⁾ While therapeutic doses rarely cause adverse side effects, the effect of long term treatment with acetaminophen is unclear. Cases have been reported where chronic excessive use of acetaminophen has led to hepatotoxicity and nephrotoxicity.^(2,3) In cases of acute overdosage, acetaminophen can cause severe hepatic damage leading to hepatic failure if untreated.^(4,5,6)

The management of acetaminophen overdose requires early recognition of the drug in the bloodstream. Toxicity is generally reported at concentrations over 20 mg/dL (1324 µmol/L). N-acetylcysteine has been used as an antidote in conjunction with intensive support care. Early diagnosis of acetaminophen-induced hepatotoxicity is important since initiation of therapy within 8 hours of ingestion lessens the potential for hepatic injury, and decreases the mortality rate.⁽⁷⁾

The majority of methods for measuring acetaminophen are based on spectrophotometric or chromatographic principles. Chromatographic methods are specific for the parent compound, however, they are not well suited to emergency laboratories. Spectrophotometric methods are simpler and more rapid, but do not always offer the desired specificity.

This spectrophotometric method is rapid, reliable, convenient, and specific for acetaminophen.

TEST PRINCIPLE



The enzyme, acyl amidohydrolase, cleaves the amide bond of the acetaminophen molecule, leaving p-aminophenol and acetate. The p-aminophenol is reacted with 8-hydroxyquinoline in the presence of manganese ions to form a colored compound, 5-(4-aminophenyl)-8-quinolone. The increased absorbance at 615 nm due to the formation of 5-(4-aminophenyl)-8-quinolone is directly proportional to the concentration of acetaminophen in the sample.

REAGENTS

Acetaminophen Enzyme Reagent (R1): A solution containing buffer (pH 8.6 at 25°C), 0.2 mmol/L MnCl₂•4H₂O, ≥0.9 KU/L Acyl Amidohydrolase (microbial), surfactant, preservatives.

Acetaminophen Color Reagent (R2): A solution containing 0.1 mol/L sodium carbonate buffer (pH 12.2 at 25°C), 30 mmol/L 8-hydroxyquinoline-5-sulfonic acid, surfactant, preservatives.

Acetaminophen Calibrator: A 5 mL solution containing buffer (pH 5.0 at 25°C), 15.1 mg/dL (1000 µmol/L) acetaminophen, preservatives.

WARNINGS AND PRECAUTIONS FOR USE

R43: May cause sensitization by skin contact.
S24/25: Avoid contact with skin and eyes.
Avoid ingestion.
See Material Safety Data Sheet for additional information.

REAGENT PREPARATION, STORAGE AND STABILITY

Reagents are ready for use.

Supplied reagents are stable at 2-8°C until expiry date.

Stability claims are based on real time studies.

REAGENT DETERIORATION

The reagents should be clear. Turbidity would indicate deterioration.

DISPOSAL

Dilute with large volumes of water; dispose into sewer system in accordance with all Federal, Provincial, State, and local regulations.

SPECIMEN

Fresh, clear, unhemolysed serum or lithium heparinized plasma. Specimens should be assayed promptly.

SAMPLE STORAGE

Samples should be stored at 2-8°C.

ANALYTICAL SPECIFICITY (CLSI EP7)⁽¹⁰⁾

Interference from N-acetylcysteine (NAC) was evaluated on a Beckman SYNCHRON CX[®] analyzer. Using a significance criterion of >10% variance from control, acceptable results were obtained to a concentration of 800 mg/L N-acetylcysteine (NAC) in a 10.4 mg/dL (688 µmol/L) acetaminophen sample; this invitro analysis was performed approximately two hours after the addition of NAC to a serum pool. Note: Significantly reduced Acetaminophen recovery has been demonstrated in situations where testing has been performed immediately after the introduction of NAC. It is recommended that laboratories review NAC treatment and monitoring protocols to determine the extent of the potential interference.

This method does not measure the common metabolites of acetaminophen (glucuronide, cysteine, and mercapturate).

Cross Contamination studies have not been performed on automated instruments. Certain reagent/instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Interferences from icterus, lipemia, hemolysis were evaluated for this Acetaminophen method on the Hitachi 717 analyzer using a significance criterion of >10%. Interference data was collected in serum. Plasma data is expected to be similar.

Concentration of Acetaminophen		Substance Tested	Concentration of Substance Where No Significant Interference was Observed	
2 mg/dL	154 µmol/L	Hemoglobin	200 mg/dL	31 µmol/L
2 mg/dL	154 µmol/L	Intralipid	400 mg/dL	1200 mg/dL (13.55 mmol/L) Simulated Triglycerides
2 mg/dL	154 µmol/L	Bilirubin*	24 mg/dL	410 µmol/L

The information presented above is based on results from Sekisui Diagnostics studies and is current at the date of publication.

*Acetaminophen concentrations in the therapeutic range have been observed with hyperbilirubinemic patient samples when no acetaminophen had been administered. At a bilirubin concentration of 32 mg/dL (547 µmol/L), an apparent acetaminophen concentration of 1.0 mg/dL (63 µmol/L) was observed in a sample that contained no acetaminophen.

Morris et al.⁽⁸⁾ performed interference studies on 71 drugs at a concentration of 1 g/L using a similar acetaminophen method and found no significant interference.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S.⁽⁹⁾

ANALYTICAL PROCEDURE

MATERIAL PROVIDED

Sekisui Diagnostics' Acetaminophen reagents and calibrator.

MATERIALS REQUIRED (BUT NOT PROVIDED)

1. Automated analyzer capable of accurately measuring absorbance at appropriate wavelength as per instrument application.
2. Quality Control materials.

TEST CONDITION

For the data presented in this insert, studies using this reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:56, and a primary wavelength reading of 600 nm and a secondary wavelength reading of 700 nm. For assistance with applications on automated analyzers within Canada and the U.S., please contact Sekisui Diagnostics Technical Services at (800)565-0265. Outside Canada and the U.S., please contact your local distributor.

CALIBRATION

Calibration material should be used to calibrate the procedure. The frequency of calibration on automated systems is dependent on the system and the parameters used.

QUALITY CONTROL

A normal and abnormal control should be analyzed as required in accordance with local, state and federal guidelines. The results should fall within the acceptable range as established by the laboratory.

CALCULATIONS

The analyzer calculates the acetaminophen concentration of each sample.

TEST LIMITATIONS

A sample with an acetaminophen concentration exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

REFERENCE INTERVALS⁽⁷⁾

Therapeutic concentration: < 3 mg/dL (199 µmol/L)

Toxic concentration: > 20 mg/dL (1324 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish its own expected range.

PERFORMANCE CHARACTERISTICS

Data presented was collected on a Roche/Hitachi[®] 717 analyzer unless otherwise stated.

REPORTABLE RANGE (CLSI EP6)⁽¹⁰⁾

Reportable range is dependent on the sample to reagent ratio and the incubation temperature. The linearity of the procedure described is 38 mg/dL (2500 µmol/L). This data results in a reportable range of 0.3 mg/dL to 38 mg/dL (20 µmol/L to 2500 µmol/L).

PRECISION STUDIES (CLSI EP5)⁽¹⁰⁾

Data was collected on two concentrations of control sera using a single lot of reagent in 40 runs conducted over 20 days.

Concentration		Total SD		Total CV %	Within Run SD		Within Run CV %
mg/dL	µmol/L	mg/dL	µmol/L		mg/dL	µmol/L	
2	115	0.02	1.6	1.4	0.02	1.2	1.0
14	950	0.26	17.5	1.8	0.08	5.2	0.6