Stanbio Salicylate Liqui-UV® Proc. No. 2410

For the *In Vitro* Quantitative Determination of Salicylate in Serum

Summary and Principle

Salicylate is a common drug used in many formulations due to its analgesic and anti-inflammatory properties. Salicylate intoxication occurs often in children due to its accessibility, in chronic patients with regular need of medication or in patients who are taking combination prescription and non-prescription dosages. Overdosage of salicylate is also associated with suicide attempts in adolescents and adults.[1] Serum concentrations exceeding 600 mg/L are usually lethal. [2,3,4] Salicylate overdose can cause metabolic acidosis with a high anion gap, gastrointestinal and central nervous system disturbances, as well as encephalopathy and renal failure.[5] Immediate detection and treatment of this medical emergency is essential. Therefore, a method for the rapid and accurate determination of salicylate is needed. Enzymatic methods[6] used for the quantitation of salicylate, as opposed to traditional chemical tests[7,8] provide specificity and simplicity. In addition, the Stanbio Salicylate Reagent offers a rapid and convenient method for salicylate quantitation.

The present enzymatic assay for the determination of salicylate depends upon the conversion of salicylate in the presence of NADH by Salicylate hydroxylase (SH) to catechol and NAD.[6]

$$\text{Salicylate} + \text{NADH} + O_2 + 2H^+ \xrightarrow{\text{Salicylate hydroxylase}} \text{Catechol} + NAD^+ + H_20 + CO_2$$

The conversion of NADH to NAD is measured by the decrease in absorbance at 340 nm. The decrease is proportional to the concentration of salicylate present in the sample.

Reagents

Reagent (R1), Ref. No. 2411

Contains 0.3 mM NADH

Reagent (R2), Ref. No. 2412

Contains 20,000 U/L Salicylate Hydroxylase

Standard - 300 mg/L, Ref. No. 2413

Contains Salicylate in an aqueous buffer

Precautions: For *in vitro* diagnostic use only. No special precautions are needed with these reagents. However, general care in reagent handling is recommended.
Handle serum and blood specimens as potentially infectious samples and follow the guidelines established by the Centers for Disease Control (CDC), Atlanta, Georgia, for blood collection and handling.

Reagent Preparation: Reagents are ready to use.

Reagent Storage and Stability: The reagents are stable for 24 months when stored at 2-8 °C when kept separated. The aqueous Salicylate Standard is stable at 2-8 °C for 24 months after manufacture. Do Not Freeze.

Specimen Collection and Preparation

Serum is the recommended specimen.

Sample Stability: Serum containing salicylate can be stored at 2-8 °C for one week.

Interfering Substances: The following substances, when added at a concentration of 500 mg/L to a serum containing 280 mg/L of salicylate, showed no interference.

Substance	% Recovery
Acetaminophen	103
Acetylsalicylic acid	105
Bilirubin	103
EDTA disodium	102
Ibuprofen	100
α–Ketobutyric acid	100
Methyl salicylate	100
Phenol	103
Salicylamide	102
Sodium benzoate	104
Sodium oxalate	100
Theophylline	99
Uric acid	104

Ethanol was tested at a concentration of 5% and found not to interfere. Heparin at a concentration of 14.3 u/mL and hemoglobin at OD540 of 2.0 were tested and found not to interfere. Additionally, when sodium citrate, sodium fluoride and oxalic acid at concentrations of 3.8 mg/mL, 2.5 mg/mL and 2.0 mg/mL respectively, were added to the same serum pool containing 280 mg/L of salicylate, no interference was observed.

However, in serum containing 280 mg/L salicylate, 500 mg/L, concentrations of the drugs p-aminosalicylic acid, 2,5-dihydroxybenzoic acid and 2,5-dihydroxyphenylacetil gave biased values of +115%, +23% and +19% respectively.

Procedure

The Salicylate Liqui-UV® Test can be used in either 5 minute procedures below. Any spectrophotometer that reads absorbance to the third decimal place may be used.

Conditions:
Wavelength.....340 nm
Temperature.....37°C
Mode.....End Point
Sample Volume.....50 µL
Reagent Volume.....2.04 mL
Sample to reagent ratio.....1/42

Procedure #1

Procedure:
1) Into four 3 mL cuvettes add 2.0 mL of Reagent A at 37 °C.
2) Add 50 µL of serum to cuvettes 1 and 2.
3) Add 50 µL of Salicylate Standard to cuvettes 3 and 4.
4) Add 40 µL of water to cuvette 1 (serum blank) and cuvette 3 (std. blank).
5) Add 40 µL of Reagent B to cuvettes 2 and 4.
6) Measure the absorbance of all cuvettes 5 minutes after the addition of Reagent B.
7) Subtract the absorbance of cuvette 2(OD_{serum}) from the absorbance of cuvette 1 ($OD_{serum\ blank}$).
8) Subtract the absorbance of cuvette 4 (OD_{std}) from the absorbance of cuvette 3 ($OD_{std\ blank}$).

Calculations:

$$\frac{OD_{serum\ blk} - OD_{serum}}{OD_{std\ blk} - OD_{std}} \times 300\ mg/L \times dil\ of\ serum = Salicylate\ mg/L$$

Procedure #2

Procedure:
1) Into two 3 mL cuvettes add 2.0 mL of Reagent A at 37 °C.
2) To cuvette 1 add 50µL of serum and measure the initial OD_{340} (T_o).
3) To cuvette 2, add 50 µL of Salicylate Standard and measure the initial OD_{340} ($T_o$std).
4) To cuvette 1, add 40 µL of Reagent B and measure the final OD_{340} (T_F) at 5 minutes.
5) To cuvette 2, add 40 µL of Reagent B and measure the final OD_{340} (T_F std) at 5 minutes.
6) Calculate the ΔOD_5: (OD T_o x 0.981) - OD T_F

Calculations:

$$\frac{\Delta OD_{5\ serum}}{\Delta OD_{5\ std}} \times 300\ mg/L \times dil\ of\ serum = Salicylate\ mg/L$$

Quality Control: In order to assure consistent performance, it is recommended that both a normal and an abnormal serum control be assayed with each run.

Expected Values[2]

The quantitation of salicylate is important in cases of drug overdose. Serum concentrations above 300 mg/L are generally toxic and concentrations exceeding 600 mg/L are usually lethal.

Performance Characteristics

Linearity: The procedure described is linear to 1000 mg/L salicylate. For higher concentrations, dilute the samples with distilled water. Repeat the assay and multiply the results by the dilution factor.

Sensitivity: Concentrations of salicylate of about 80, 90 and 100 mg/L can be clearly distinguished at the 99.9% confidence limit with this method.

Accuracy: Analytical recovery of salicylate added to human serum pools gave a mean recovery of 96% from the following individual values.

	% Recovery
Pool 1 (100 mg/L)	99
Pool 2 (200 mg/L)	95
Pool 3 (300 mg/L)	97
Pool 4 (400 mg/L)	94

Comparison of 30 serum samples between the Stanbio Salicylate Liqui-UV® and the DCL Enzymatic Salicylate Assay Reagent Kit (Diagnostic Chemicals Limited) showed a correlation coefficient of r=0.9939. The comparison of the two methods produced the following correlation equation: Stanbio=0.914DCL + 4.047 mg/L, Std. Error: S_{xy}=13.8 mg/L.

Precision: Studies were conducted using 3 serum pools containing 50, 150 and 300 mg/L of salicylate. The following results are an average of 18 determinations.

Within Run	Mean	SD	CV(%)
Pool I	51.9	1.57	3.0
Pool II	151.8	5.18	3.4
Pool III	293.7	10.24	3.5
Run-to-Run			
Pool I	50.1	2.72	5.4
Pool II	147.8	8.56	4.4
Pool III	290.0	7.66	2.6

References

1. Longenecker RM, Tafton JE and Edwards RB, Clin Chem 30(8), 1369 (1984).
2. Tietz NW, "Fundamentals of Clinical Chemistry"' pp 1728, 1856 (1976).
3. Don AK, Pediatrics 26, 800 (1960).
4. Natelson S, "Microtechniques of Clinical Chemistry for the Routine Laboratory", pp 332 (1957).
5. Hammond PM, "A Simple Colorimetric Assay to Determine Salicylate Ingestion Utilizing Salicylate Monooxygenase," VIIIth International Conference on Enzyme Engineering (1985).
6. You K and Bittikofer JA, Clin Chem 30(9), 1549 (1984).
7. Trinder P, Biochem J 57, 301 (1954).
8. Keller WJ, Am J Clin Pathol 17, 415 (1947).

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