Syva Nitrite Validity Test: Periodic Reverification on The Hitachi 717 Chemistry Analyzer

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Contents

1 Purpose

An annual reverification of the Syva Nitrite Validity Test is performed to establish that the analytical methodology remains valid.

2 Instrumentation and Parameters

A Hitachi 717 running System FD version 7176000-04-07 with Data FD version 7176001-00-01 was used to analyze study samples. Data processing and calculations were performed with the R software environment version 2.8.1[1] using results produced by the instument. The instrument was set to use the following parameters:

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CHEMISTRY PARAMETERS

TEST              [NT   ]
ASSAY CODE        [1POINT  ]:[36]-[0]
SAMPLE VOLUME     [ 3][ 3]
R1 VOLUME         [150][ 50][NO ]
R2 VOLUME         [150][ 50][NO ]
WAVELENGTH        [800][415]
CALIB. METHOD     [LINEAR   ][0][0]
STD.(1) CONC.-POS.[  0.0]-[16]
STD.(2) CONC.-POS.[  200]-[23]
STD.(3) CONC.-POS.[     ]-[ 0]
STD.(4) CONC.-POS.[     ]-[ 0]
STD.(5) CONC.-POS.[     ]-[ 0]
STD.(6) CONC.-POS.[     ]-[ 0]
SD LIMIT          [  999]
DUPLICATE LIMIT   [ 1000]
SENSITIVITY LIMIT [    0]
ABS.LIMIT(INC/DEC)[32000][INCREASE]
PROZONE LIMIT     [     0][LOWER]
EXPECTED VALUE    [     0]-[   199]
TECH. LIMIT       [     1]-[  3500]
INSTRUMENT FACTOR [ 1.0]
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3 LOD/ULOL

3.1 Description of Methods

The limit of quantitation (LOQ) and limit of linearity (ULOL) are reverified at the 25 and 2000 µg/mL levels, respectively. Quintuple analyses are performed at each point, and the resulting data are used to calculate the mean and sample standard deviation. The criteria for reverification are results within ±20% of the target values.

3.2 Summary of Statistical Data

	Control point	
	25 µg/mL	2000 µg/mL
Mean	23.2	2281.2
SD	0.45	9.96
CV%	1.9	0.4

3.3 Discussion

All results were within ±20% of the target values.

4 Carryover

The extent of carryover from samples at an extreme out-of-range concentration (X) of 2000 µg/mL to samples at the 200 µg/mL (D) decision point is evaluated annually.

4.1 Description of Methods

Carryover studies are performed using the method of Armbruster et al. [2] with the sequence

$$\mathrm{D_1\,D_2\,D_3\,X_1\,X_2\,D_4\,X_3\,X_4\,D_5\,D_6\,D_7\,D_8\,X_5\,X_6\,D_9\,X_7\,X_8\,D_{10}\,X_9\,X_{10}\,D_{11}}.$$

Percent carryover is evaluated as the percent difference in response of carryover candidate samples vs normal samples

$$100 \times \frac{[\mathrm{D_4 + D_5 + D_9 + D_{10} + D_{11}}] - [\mathrm{D_2 + D_3 + D_6 + D_7 + D_8}]}{[\mathrm{D_2 + D_3 + D_6 + D_7 + D_8}]}.$$

A two-sample t test using pooled variances are used to compare the means between carryover candidates and the decision point,

$$H_0\colon \quad \mu_\mathrm{X} - \mu_\mathrm{D} = 40,$$
$$H_a\colon \quad \mu_\mathrm{X} - \mu_\mathrm{D} < 40,$$

The 40 µg/mL level is chosen for comparison as it constitutes 20% of the decision point value. Carryover is expected to bias results in the direction of the carryover concentration and the result is evaluated at a significance level $\alpha = 0.01$.

Analytical Results

	Carryover level
	2000 mg/dL
Carryover %	-0.1
p-value	3.4×10^{-10}

Discussion

Carryover from 2000 µg/mL samples was determined to be a relatively insignificant factor. The t test supports (i.e., $p \leq \alpha$) the conclusion that the difference in means between carryover candidates and decision point samples is less than 20% of the decision point cutoff. In addition, the apparent carryover followed a gradient opposite to the hypothetical bias that would be expected.

4.2 Specificity/Interference

Specificity studies are performed to assess the ability of the assay to discriminate the following substances from nitrite at the levels indicated:

Potassium permanganate	1000, 2500, and 5000 μg/mL
Pyridinium chlorochromate	200, 500, and 1000 μg/mL
Sodium dichromate	200, 500, and 1000 μg/mL
Iodine	8, 9, and 10 mg/mL
Sodium hypochlorite	0.5, 1.0, and 2.0% (w/v)
Hydrogen peroxide	30% (w/v)
Potassium nitrate	10 mg/mL

Description of Methods

All salt concentrations are based on the mass of the anion, and units of concentration are converted to micrograms per milliliter for statistical calculations. Linear regression of the response, R, on concentration, C,

$$\mathrm{E}(R/C) = \beta_0 + \beta_1 C,$$

is performed for analytes that are tested at multiple concentrations. Percent cross-reactivity is calculated as

$$100 \times \frac{C_d}{C_a} = 100 \times \frac{200\beta_1}{200 - \beta_0},$$

where C_d/C_a is the ratio of the nitrite decision-point cutoff concentration, C_d, to the concentration of interfering analyte that is necessary to elicit an equivalent response, C_a.

4.3 Analytical Results

Potassium nitrate (10 mg/mL) and hydrogen peroxide (30%) produce responses of 0 and 2 μg/mL, respectively. See Figure 1 for a graphical summary of the cross-reactivity data for the remaining oxidants.

	Linear Regression Coefficients		
Analyte	β_0	β_1	*Cross-reactivity, %*
Potassium permanganate	42.53	5.72×10^{-2}	7.3
Pyridinium chlorochromate	-8.57	1.66×10^{-1}	15.9
Sodium dichromate	-8.12	2.06×10^{-1}	19.7
Iodine	-24.83	9.50×10^{-3}	0.8
Sodium hypochlorite	-256.00	2.63×10^{-1}	11.5

4.4 Discussion

Potassium permanganate, pyridinium chlorochromate, sodium dichromate and sodium hypochlorite were all cross-reactive with the Syva Nitrite Validity Test. Iodine had a very low cross-reactivity. Hydrogen peroxide and potassium nitrite did not exhibit cross-reactivity at levels of 300 000 and 10 000 μg/mL, respectively.

5 Conclusions

1. The LOQ and ULOL were reverified at the 0.5 and 300 mg/dL levels, respectively.
2. No evidence for carryover was found for candidate samples at the 2.0 mg/dL decision point following blank or 1000 mg/dL samples.

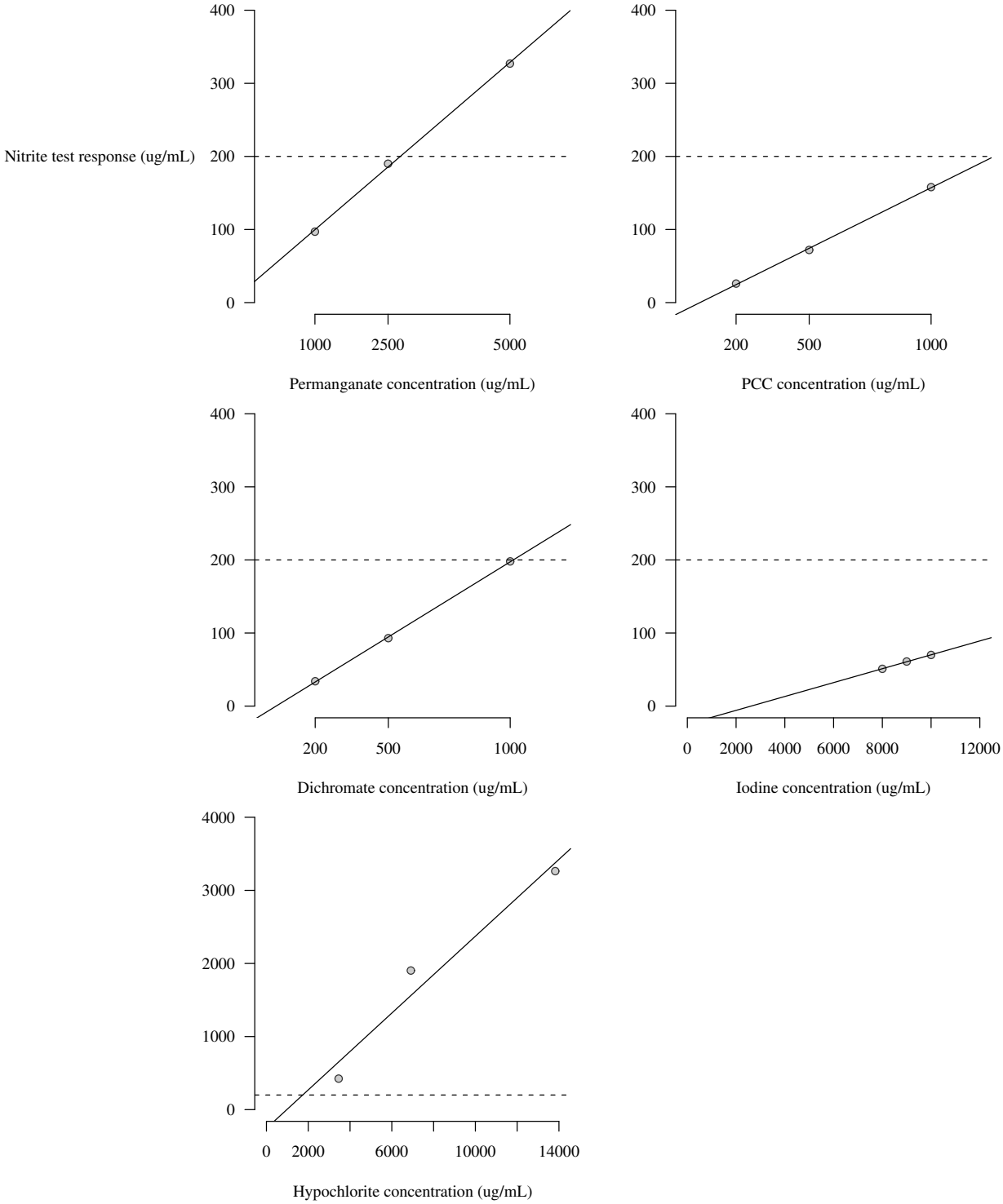

Figure 1: Cross-reactivities of selected oxidants. The decision point is indicated by a dashed line.

3. The cross-reactivities of oxidants including chromium(VI) species, iodine, potassium permanganate and sodium hypochlorite was characterized; samples containing these substances will produce an elevated resonse.

This method meets requirements for reverification, and is valid for analysis of forensic urine samples under the Federal guidelines for a drug-free workplace.

Bibliography

(1) R Development Core Team, *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2008, ISBN 3-900051-07-0.

(2) Armbruster, D. A.; Schwarzhoff, R. H.; Hubster, E. C.; Liserio, M. K. Enzyme immunoassay, kinetic microparticle immunoassay, radioimmunoassay, and fluorescence polarization immunoassay compared for drugs-of-abuse screening. *Clin Chem* **1993 Oct**, *39*, 2137–2146.