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COMMISSION ON TOXICOLOGY

SUBCOMMITTEE ON ENVIRONMENTAL AND OCCUPATIONAL TOXICOLOGY OF NICKEL*

IUPAC REFERENCE METHOD FOR ANALYSIS OF NICKEL IN SERUM AND URINE BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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for the

Subcommittee on Environmental and Occupational Toxicology of Nickel,
Commission on Toxicology, Division on Clinical Chemistry,
International Union of Pure and Applied Chemistry

Based upon the Work and Recommendations
of the Panel of Evaluators

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INTRODUCTION

The IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel has sponsored the development of this Reference Method for analysis of nickel in serum and urine in order to promote international harmonization of nickel determinations in body fluids (1,2). For background information concerning the analytical biochemistry of nickel, readers may consult a comprehensive review, which has been prepared on behalf of the IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel (3). This Reference Method has been formulated from the work and recommendations of a panel of scientists from thirteen nations who served as evaluators. The Reference Method represents a consensus of observations, suggestions, and opinions of the evaluators. It incorporates attractive features of several techniques that have been published by members of the panel (4-12), and it shares common features with a method published by a Working Party of the British Society for Analytical Chemistry (13). There is no member of the panel of evaluators who has not been obliged, perhaps with lingering regret, to yield personal views or preferences in order to reach this consensus. The evaluators are unanimous about the difficulty of controlling nickel contamination in order to attain the requisite analytical sensitivity, and the importance of (a) a clean, well-equipped laboratory room that is solely devoted to trace metal analysis; (b) an experienced analyst who is personally committed to avoidance of nickel contamination and who has sufficient time and patience to perform the analyses meticulously; and (c) an up-to-date electrothermal atomic absorption spectrometer that is scrupulously maintained in optical and mechanical adjustment. All specimen containers and analytical utensils should be acid-washed, and should be handled with care to prevent nickel contamination from sweat on the analyst's fingers. In the locality of nickel refineries, special precautions may be necessary to overcome nickel contamination, such as use of a laminar-flow hood and surgical attire.

The text of this Reference Method does not mention commercial suppliers of reagents, apparatus, or equipment. Sources of such items are cited in footnotes for the convenience of analysts. Such citations do not imply endorsement of the products by IUPAC or by the evaluators.

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PRINCIPLE OF THE METHOD

Organic constituents of serum or urine are digested with a mixture of nitric, sulfuric, and perchloric acids. The solution is adjusted to pH 7 with ammonium hydroxide, and ammonium tetramethylenedithiocarbamate (ammonium pyrrolidine dithiocarbamate, APDC) solution is added. The bis-(1-pyrrolidinecarbodithioato)nickel[II] complex is extracted into 4-methylpentane-2-one (methyl isobutyl ketone, MIBK). The concentration of nickel in the MIBK extract is measured by electrothermal atomic absorption spectrometry.

REAGENTS, CALIBRATION SOLUTIONS, AND QUALITY CONTROL PREPARATIONS

Acid Digestion Mixture. Into a glass-stoppered borosilicate glass bottle (250 ml capacity) are placed successively 120 ml of nitric acid (650 g/kg; 910 g/litre; relative density 1.40); 40 ml of sulfuric acid (960 g/kg; 1766 g/litre; relative density 1.84); and 40 ml of perchloric acid (760 g/kg; 1169 g/litre; relative density 1.67). Ultra-pure acids are essential.[1] The acids are thoroughly mixed, and the bottle is shielded from dust by a polyethylene outer cap.

Water for Sample Dilution. A screw-cap polyethylene bottle (1 litre capacity) is filled with ultra-pure water.[2] A screw-cap piston-type dispenser[3] is fitted onto the bottle in order to dispense 3 ml volumes of water. The bottle and attached dispenser are shielded from dust by a polyethylene outer cap.

Concentrated Ammonium Hydroxide Solution. Into a screw-cap polyethylene wash bottle (250 ml capacity) is placed 150 ml of ultra-pure ammonium hydroxide solution[1] (250 g/kg; 228 g/litre; relative density 0.91). A screw-cap with fine-tipped delivery tube is fitted onto the bottle to permit dropwise delivery of the contents. The bottle and attached delivery tube are shielded from dust by a polyethylene outer cap.

Dilute Ammonium Hydroxide Solution. Into a screw-cap polyethylene wash bottle (250 ml capacity) are placed 50 ml of ultra-pure concentrated ammonium hydroxide solution[1] and 80 ml of ultra-pure water. A screw-cap with fine-tipped polyethylene delivery tube is fitted onto the bottle in order to permit dropwise delivery of the contents. The bottle and attached delivery tube are shielded from dust by a polyethylene outer cap.

Dilute Nitric Acid Solution. Into a polypropylene volumetric flask (250 ml capacity) is placed 1 ml of concentrated, ultra-pure nitric acid.[1] This is diluted to volume with ultra-pure water and transferred to a polyethylene bottle with utmost precaution against nickel contamination. The bottle is shielded from dust by a polyethylene outer cap. This solution is used for preparation of nickel working calibration solutions.

Bromothymol Blue Indicator Solution. Into a screw-cap polyethylene drop-dispenser bottle (60 ml capacity) are placed 20 mg of bromothymol blue ("certified reagent") and 1 ml of dilute ammonium hydroxide solution. The contents are diluted to 50 ml with ultra-pure water. A screw-cap with fine-tipped polyethylene delivery tube is fitted onto the bottle to permit dropwise delivery of the pH indicator. The bottle and attached delivery tube are shielded from dust by a polyethylene outer cap.

4-Methylpentane-2-one (Methyl Isobutyl Ketone, MIBK). Ultra-pure MIBK[4] is placed in a borosilicate glass bottle (250 ml capacity) with screw-cap piston-displacement dispenser[3] set to deliver 0.7 ml volumes. The bottle and attached dispenser are shielded from dust by a polyethylene outer cap.

Ammonium Tetramethylenedithiocarbamate Solution (Ammonium Pyrrolidine Dithiocarbamate Solution, APDC). Into a screw-capped polypropylene graduated tube (25 ml capacity) is placed 0.5 g of APDC, (1-pyrrolidinecarbodithioic acid, ammonium salt).[5] This is dissolved in 25

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[1] Ultra-pure HNO_3, H_2SO_4, $HClO_4$, and NH_4OH solutions can be obtained from E. Merck Company, Darmstadt, FRG, or J.T. Baker Chemical Company, Phillipsburg, NJ, USA. Each batch should be checked for nickel contamination by electrothermal atomic absorption spectrophotometry. When the concentrated reagent is diluted 1:5 with ultra-pure water and pipetted directly into the graphite cuvet of the electrothermal atomizer, no nickel should be detectable with routine operating parameters.

[2] Ultra-pure water is conveniently prepared by successive use of de-ionization and double distillation from an all-glass or quartz still. The target nickel concentration in the ultra-pure water is <0.2 µg/litre. The nickel concentration is determined by evaporation of 15 ml samples to dryness in digestion tubes, and analysis by the present method.

[3] The piston-type dispenser must be constructed entirely of glass or acid-resistant plastic in order to avoid nickel contamination. A suitable dispenser is supplied by Scientific Industries, Inc., Berkeley, CA, USA.

[4] Ultra-pure MIBK can be obtained from Burdick and Jackson, Inc., Muskegon, MI, USA.

[5] APDC can be obtained from Eastman Chemicals, Inc., Rochester, NY, USA.

ml of ultra-pure water, and the solution is extracted at least three times with 1.4 ml portions of MIBK. The first 2 washings are aspirated and discarded. The last washing is analyzed by electrothermal atomic absorption spectrometry to verify that it contains no detectable nickel. The final aqueous solution of APDC should be colorless and free from precipitate. This solution is prepared immediately before use.

Potassium Phosphate Buffer (1.0 mol/litre, pH 7). Into a 250 ml volumetric flask are transferred 17.0 g of anhydrous KH_2PO_4 and 21.8 g of anhydrous K_2HPO_4. The contents are dissolved in ultra-pure water, and diluted to the volume with ultra-pure water. The solution is transferred to a 250 ml separatory funnel. APDC solution (5 ml) is added, and the mixture is extracted at least 3 times with 10 ml portions of ultra-pure chloroform. The last $CHCl_3$ washing is analyzed by electrothermal atomic absorption spectrometry to verify that it contains no detectable nickel. The buffer solution is transferred to a polyethylene bottle, fitted with fine-tipped polyethylene delivery tube to permit dropwise delivery of the contents. The bottle and attached delivery tube are shielded from dust by a polyethylene outer cap.

Nickel Stock Calibration Solution (100 mg Ni/litre, 1.70 mmol/litre). Into a tared borosilicate glass beaker (25 ml capacity) is weighed 50 mg of nickel powder.[6] Ultra-pure water (5 ml) and ultra-pure concentrated nitric acid (5 ml) are added and the nickel powder is dissolved by cautiously warming the beaker. The cooled solution is quantitatively transferred to a polypropylene volumetric flask (500 ml capacity) and diluted to volume with ultra-pure water. This solution is stored in a screw-capped polyethylene bottle and is stable for at least 1 year.

Nickel Intermediate Calibration Solution (400 µg Ni/litre, 6.81 µmol/litre). Into a polypropylene volumetric flask (500 ml capacity) are pipetted 2 ml of nickel stock standard solution and 2 ml of concentrated ultra-pure nitric acid. The contents are diluted to volume with ultra-pure water and transferred to a screw-capped polyethylene bottle. This solution is prepared every 3 months.

Fe[II]-Cu[II]-Zn[II]-Matrix Solution (10 mg Fe, 10 mg Cu, 10 mg Zn/litre; 0.18 mmol Fe, 0.16 mmol Cu, 0.15 mmol Zn/litre). Into a 200 ml volumetric flask are transferred 17.5 mg of $Fe(NH_4)_2(SO_4)_2.6H_2O$, 5.25 mg of $ZnCl_2$, and 9.75 mg of $CuSO_4$.[7] The contents are dissolved in approximately 150 ml of ultra-pure water. Ultra-pure concentrated HCl (2 ml) is added, and the contents are diluted to the mark with ultra-pure water. This solution is stored in a screw-capped polyethylene bottle and shielded from dust with a polyethylene-outer cap. When this reagent is pipetted directly into the graphite cuvet of the electrothermal atomizer, no nickel should be detectable with routine operating parameters.

Nickel Working Calibration Solutions. Into six polypropylene volumetric flasks (100 ml capacity) are accurately pipetted respectively 0 (blank), 0.5, 1, 2, 3, and 4 ml of nickel intermediate standard solution and 4, 3.5, 3, 2, 1, and 0 ml of the dilute nitric acid solution. Ten ml of Fe-Cu-Zn matrix solution is added to each flask. The contents are diluted to volume with ultra-pure water. These solutions contain 0, 2, 4, 8, 12, and 16 µg Ni/litre, (0, 34, 68, 136, 204, and 273 nmol/litre), respectively, and they are prepared every 2 weeks. The nickel calibration solutions are used for construction of the calibration curve which is prepared with each set of nickel analyses.[8]

Urine Sample for Quality Control. A 24-hour urine specimen (containing 4 to 6 µg Ni/litre) is acidified by addition of 10 ml of concentrated nitric acid per litre. The acidified urine specimen is distributed in 5 ml aliquots in screw-capped polypropylene tubes, which are stored at -20°C. One tube is thawed for inclusion in each set of nickel analyses.

Urine Nickel Recovery Sample for Quality Control. The same 24-hour specimen of urine as used for the quality control sample is also used to determine the recovery of nickel. Into a volumetric flask (250 ml capacity) is transferred 5 ml of nickel intermediate standard solution. The contents are diluted to volume with the acidified urine specimen. The "spiked" urine sample is distributed in 5 ml aliquots in polypropylene tubes, and the tubes are stored at -20°C, One tube is thawed for inclusion in each set of nickel analysis. The nickel concentration obtained by analysis of the "unspiked" urine sample is subtracted from

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[6] Nickel powder (99.99% pure) can be obtained from Ventron Corporation, Beverly, MA, USA.

[7] Reagent grade ferrous ammonium sulfate, zinc chloride, and copper sulfate may be obtained from Fisher Scientific Co., Pittsburgh, PA, USA, or Baker Chemical Co., Phillipsburg, NJ, USA. Each reagent should be tested to ensure that it does not contain detectable nickel.

[8] Depending upon the clinical circumstances, it may be appropriate to delete the low standard (2 µg Ni/litre) and to substitute a higher one (20 µg Ni/litre). The linear working range of the method is 0.5 to 20 µg of nickel per litre of serum or urine. If higher concentrations of nickel are present, the samples should be diluted appropriately with water prior to analysis.

that obtained by analysis of the "spiked" urine sample. The difference obtained is divided by the net concentration of added nickel (8 µg Ni/litre), and the dividend is multiplied by 100 to yield the percent recovery of added nickel.

SPECIAL SUPPLIES, PREPARATIONS, AND APPARATUS

Digestion Tubes. The digestion is performed in borosilicate glass tubes[9] (18 mm outside diameter; 150 mm length; 25 ml capacity).

Blood Collection Apparatus. Teflon-polyethylene intravenous canulae[10] (20 gauge, 5 cm length), polypropylene syringes[11] (10 ml capacity), and polyethylene test-tubes[11] (10 ml capacity) are used for blood collection and serum storage.

Polyethylene Pipettor Tips. Polyethylene tips used to dispense samples, standards, and APDC solution by means of piston-displacement pipettors must be acid-washed before use, as described below.

Polyethylene Tubes. Polyethylene tubes[11] with attached polyethylene stoppers, narrow form (0.75 ml capacity), must be acid-washed as described below.

Washing of Glassware and Plasticware. Before each use, the digestion tubes, polypropylene syringes, polyethylene tubes, and pipettor tips are scrubbed in hot detergent solution[12] and rinsed in tap water. The materials are placed in 2-litre polyethylene canisters and washed in batch fashion by filling and decanting with deionized water, without any contact of the contents with the analyst's hands. After 6 rinses with deionized water, water is completely drained. Fifty ml of concentrated HCl is poured into the canister and the canister is capped tightly. The contents are mixed so that HCl fumes percolate over the surfaces of the contents. The canister is allowed to stand at room temperature for 1 hour. The canister is then filled with deionized water, shaken, allowed to stand for 20 min. The contents are allowed to drain. The contents are rinsed 5 times with deionized water and twice with ultra-pure water. The canister is placed with lid ajar in an oven at 110°C until the contents are dry.

Piston-Displacement Pipettors.[13] Two pipettors are needed, one with dispensing volume of 2 ml to measure samples of urine, serum, and the calibration solutions, and the other of 0.5 ml to pipet the APDC solution.

Digestion Apparatus. The digestion apparatus[14] is electrically heated, with (a) an aluminium block that contains at least 42 holes (20 mm diameter; 80 mm depth) to accomodate digestion tubes, and (b) an continuously variable temperature regulator (to 300°C). Aluminium foil is packed in the bottom of each hole to facilitate heat transfer to the hemispherical base of the digestion tube, and thereby to minimize "bumping". The digestion apparatus, placed in a fume hood, is shielded with a glass or "lucite" canopy. The canopy promotes uniform cooling of the tops of the digestion tubes by the hood air draft, and minimizes contamination of the tubes by dust. The temperature during digestion is monitored by a thermometer suspended in a digestion tube that contains 5 ml of concentrated sulfuric acid.

Atomic Absorption Spectrometer with Graphite-Furnace Atomizer. Electrothermal atomic absorption spectrometers of various models and manufacturers have been found by the panel of evaluators to be satisfactory for analyses of nickel in serum and urine by the present

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9 Suitable "Pyrex" tubes can be obtained from Corning Glass Works, Corning, NY, USA. When new tubes are to be used for the first time, they must be cleaned by addition of 1 ml of acid digestion mixture into each tube. The tubes are placed in the digestion apparatus, and the contents are heated at 300°C for 1 hour. The tubes are then cooled, and residual sulfuric acid is rinsed out with water, and washed according to the routine procedure. The tubes are discarded as soon as they become etched (e.g., after 50-75 analyses).

10 Polyethylene canulae with "Teflon" needle hubs ("IV-Cath", Catalog No. 6745) can be obtained from Becton-Dickinson Co., Rutherford, NJ, USA.

11 Polypropylene syringes ("Monovet" type) and polyethylene tubes can be obtained from Walter Sarstedt, Inc., Princeton, NJ, USA.

12 A laboratory detergent solution (such as "7-X Cleaning Solution", Limbro Chemical Co., New Haven, CT, USA) is satisfactory. The concentrated detergent solution is diluted 30-fold with hot tap water before use. The detergent solution should be tested to ensure that it does not contain detectable nickel.

13 Piston-displacement pipettors should be constructed so that only plastic surfaces are exposed. "Finn-Pipets" (Vangard International, Neptune, NJ, USA) or "Eppendorff Pipets" (Brinkman Instruments, Inc., Westbury, NY, USA) are satisfactory.

14 A suitable digestion apparatus may be obtained from Scientific Products, Inc., Evanston, IL, USA, or it can be assembled by use of a large electric hot plate and an aluminium block in which multiple holes have been drilled.

TABLE 1. Illustrative Analytical Conditions for Electrothermal Atomic Absorption Spectrometry of Nickel in MIBK Extracts of Wet-Digested Serum or Urine Samples

Parameter	Evaluator A[a]	Evaluator B[b]	Evaluator C[c]	Evaluator D[d]	Evaluator E[e]	Evaluator F[f]	Evaluator G[g]
Volume of MIBK extract (µl):	20	20	20	50	50	50	20
Background corrector:	D_2	D_2	D_2	D_2	D_2	none	none
Temperature program:	(a) 25 s ramp 25-120°C (b) 10 s at 120°C (c) 45 s ramp 120-1040°C (d) 10 s at 1040°C (e) 7 s at 2700°C (f) 4 s at 2750°C	(a) 10 s ramp 25-150°C (b) 20 s at 150°C (c) 10 s ramp 150-1000°C (d) 40 s at 1000°C (e) 7 s at 2600°C	(a) 20 s at 100°C (b) 15 s ramp 100-1100°C (c) 25 s at 1100°C (d) 7 s at 2600°C	(a) 20 s at 90°C (b) 50 s ramp 90-1200°C (c) 35 s at 1200°C (d) 12 s at 2700°C	(a) 70 s ramp 25-120°C (b) 10 s at 120°C (c) 45 s ramp 120-1000°C (d) 15 s at 1000°C (e) 7 s at 2700°C	(a) 60 s ramp 25-150°C (b) 5 s at 150°C (c) 55 s ramp 150-1000°C (d) 15 s at 1000°C (e) 7 s at 2600°C	(a) 30 s at 180°C (b) 2 s ramp 180-800°C (c) 13 s at 800°C (d) 2 s at 2200°C
Program for Ar flow (ml/min):	(steps a-d) 300 (step e) 10 (step f) 300	(steps a-d) 40 (step e) 0	(steps a-c) 300 (step d) 50	(steps a-c) 300 (step d) 0	(steps a-d) 300 (step e) 0	(steps a-d) 300 (step e) 0	not relevant

[a]Model AA-5000 spectrometer with HGA-500 electrothermal atomizer and pyrolytic graphite cuvette, (Perkin-Elmer Co., Norwalk, Connecticut, USA). Step (f) is used to clean the cuvette between samples.
[b]Model AA-503 spectrometer with HGA-500 electrothermal atomizer (Perkin-Elmer Co.).
[c]Model 170-50 spectrometer (Hitachi Instrument Co., Tokyo, Japan) with HGA-2200 electrothermal atomizer, and pyrolytic graphite cuvette Perkin-Elmer Co.).
[d]Model AA-306 spectrometer with HGA-74 electrothermal atomizer (Perkin-Elmer Co.).
[e]Model AA-372 spectrometer with HGA-76B electrothermal atomizer (Perkin-Elmer Co.).
[f]Model AA-703 spectrometer with HGA-500 electrothermal atomizer and pyrolytic graphite cuvette (Perkin-Elmer Co.).
[g]Model 775 spectrometer with CRA-90 electrothermal atomizer (Varian Pty., Canberra, Australia).

method. Illustrative operating conditions are listed in Table 1. Accessories for the electrothermal atomic absorption spectrometer may include: (a) automatic sampling system; (b) background correction system, (c) temperature programming system with "ramp" modes; (d) optical pyrometer to monitor the atomization temperature; and (e) strip-chart recorder. Ultra-pure argon (99.99%) is used to flush the electrothermal atomizer.

PROCEDURE

Venepuncture and Serum Separation. The antecubital fossa of the arm is cleansed with ethanol and allowed to dry by evaporation. A tourniquet is applied while a polyethylene intravenous catheter is inserted into an antecubital vein. The stylus of the catheter is removed, and the catheter is flushed with >2 ml of blood, which is discarded. A polypropylene syringe is then used to collect 10 ml of blood. The blood is placed in a polyethylene test tube and capped. The blood is allowed to clot for 45 minutes at room temperature. The test tube is centrifuged at 900 x g for 15 minutes. By use of an acid-washed polyethylene dropper, serum is transferred to a screw-capped polyethylene test tube. The serum specimen is rejected if there is visible hemolysis, lipemia, or turbidity. The serum is stored at 4°C or -20°C until the time of analysis.

Urine Collection. Urine is voided directly into an acid-washed polyethylene bottle with care to avoid contamination from faeces or dust on the clothing. When the urine collection has been completed, ultra-pure hydrochloric acid (6 mol/litre) is added in the proportion of 10 ml of acid solution per litre of urine. The acidified urine is stored in the polyethylene bottle at 4°C or -20°C until the time of analysis.

Digestion Procedure. Samples (2 ml) of serum or urine are transferred to duplicate digestion tubes. Into six more pairs of duplicate digestion tubes are transferred 2 ml of the nickel calibration solutions that contain 0, 2, 4, 8, 12, and 16 µg Ni/litre. Nothing is added to another pair of duplicate digestion tubes.[15] Acid digestion mixture (2 ml) is dispensed into all of the tubes and the tubes are placed in the digestion apparatus at ambient temperature. The tubes are heated initially at 110°C for 1 hour. This is the most critical stage of the digestion, because of the possibility of sample loss by foaming. The digestion then proceeds step-wise as follows: (a) 2 hours at 140°C, (b) 30 min at 190°C, and finally (c) 1 hour at 300°C. At the conclusion of the 4.5 hour digestion period, all nitric acid and perchloric acid should have evaporated. The contents of the tubes should be perfectly clear and colorless, and the sample volume should be approximately 0.2 ml (corresponding to the volume of the residual sulfuric acid).

Extraction of Nickel. After the tubes have cooled to ambient temperature, bromothymol blue indicator solution (3 drops) and ultra-pure water (3 ml) are added to each digestion tube with care to rinse down the wall. Concentrated ammonium hydroxide solution is added dropwise with constant swirling until the colour begins to change to blue. Phosphate buffer (2 drops) is added. Dilute ammonium hydroxide solution is then added dropwise until the colour is light blue-green.[16] APDC solution (0.5 ml) is added to each tube, and the contents are mixed for 10 sec with a vortex-type mixer. The samples are allowed to stand for five min; MIBK (0.7 ml) is added to each tube, and the contents of the tube are mixed for 40 sec with a vortex-type mixer. The aqueous and MIBK phases separate without centrifugation. The MIBK extracts are yellow, owing to the extraction of copper and iron as well as nickel. Approximately 0.5 ml of each supernatant MIBK extract is transferred to a polyethylene tube (0.75 ml capacity) by use of a Pasteur pipette with care to avoid transfer of aqueous phase. The tube is sealed with the attached polyethylene stopper. If necessary, the analysis can be interrupted at this point, and the MIBK extracts can be stored overnight at 4°C.

Atomic Absorption Spectrometry. Samples of the MIBK extracts are pipetted into the graphite cuvette, and atomic absorption is measured at the nickel absorbance line (232.0 nm). Each analyst should determine for the particular instrument (a) the optimal volume of MIBK extract; (b) the optimal instrumental parameters; (c) the optimal temperature program; and (d) whether or not background-correction is advantageous, (see Table 1).

Computations. The heights or areas of peaks on the recorder tracings are measured. The mean reading obtained for duplicate 0 µg Ni/litre samples in the set of calibration solu-

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[15] Despite scrupulous efforts to minimize nickel contamination, the observed nickel concentration that is obtained with 2 ml samples of the "blank" calibration solution (which nominally contains 0 µg Ni/litre) may exceed by 0.1 to 0.4 µg Ni/litre the observed nickel concentration that is obtained with empty digestion tubes. Therefore, it is necessary to run the two sets of "blanks".

[16] The end-point of the pH adjustment (pH 7) should be checked with a pH meter, so that the analyst is familiar with the specific shade of blue-green colour that is sought. A pH meter cannot be used directly to monitor the standard or unknown samples, owing to cross-contamination by the pH electrode.

is subtracted from the mean readings obtained with the other calibration solutions, and the resultant values are plotted to prepare the calibration curve. The mean reading obtained with "blanks" prepared by analysis of duplicate empty digestion tubes is subtracted from the mean readings obtained with the serum or urine samples, and the nickel concentrations in the samples are estimated by reference to the calibration curve.

EVALUATIONS AND DISCUSSION

The IUPAC Reference Method for analysis of nickel in serum and urine by electrothermal atomic absorption spectrometry that is described in this report is based upon four draft protocols that were submitted to evaluators during the period 1976 to 1980. The successive drafts incorporated modifications, refinements, and precautions that were recommended by various evaluators and confirmed by the authors. The penultimate draft protocol, which differed only in minor details from the final Reference Method, was tested by an inter-laboratory survey (14). Ampoules of aqueous nickel solutions, pooled human serum, and pooled human urine were distributed to laboratories in Japan, Canada, USA, UK, France, Norway, and Finland. The seven participating laboratories analyzed these samples in four analytical runs, according to a protocol that assessed within-run precision, run-to-run precision, and recovery of nickel added to urine. The results of these analyses are summarized in Tables 2 to 4.

Table 2. Interlaboratory comparison of nickel analyses[a]

Sample	No. of Labs	Ni Concentration (µg/litre) Mean	±S.D.	Range	Inter-lab C.V. (%)[b]
Std. No. 1	7	3.4	±0.8	1.8-4.9	25
Std. No. 2	7	11.6	±2.8	5.6-14.3	24
Pooled serum	7	12.7	±3.0	7.2-16.2	24
Pooled urine	7	8.3	±1.9	6.6-12.1	23

[a]Kendalls' coefficient of concordance (W) for ranking the 4 samples in order of Ni concentrations = 0.76; $\chi^2 = 16$; $P = < 0.005$.
[b]Coefficient of variation (C.V.) = (SD ÷ mean) x 100.

Table 3. Precision of nickel analyses

Parameter	Sample	No. of Labs	Coefficient of Variation (%) Mean	±S.D.	Range
Within-run precision[a]	Serum	7	10.6	±5.5	2.7-18.1
Run-to-run precision[b]	Serum	6	12.4	±7.0	2.3-21.3

[a]Based upon 4-6 replicate analyses of Ni concentration in a pooled serum, performed within a single run.
[b]Based upon the means of duplicate analyses of Ni concentration in a pooled serum, performed in 3 successive runs.

Table 4. Recovery of nickel added to urine

Sample	No. of Labs	Ni Concentration (µg/litre) Mean	±S.D.	Range
Urine (2 ml) + H_2O (1 ml)	7	5.89	±1.79	4.2-9.7
Urine (2 ml) + Std. No. 1 (1 ml)	7	7.06	±1.89	6.0-10.8
Ni recovered[a] (by difference)	7	1.17	±0.40	0.5-1.8

[a]Recovery of added Ni (1.13 µg/l) = 103 ± 36%; range = 44 to 160%.

Several evaluators provided supplemental data on performance. One evaluator reported that the coefficient of variation of nickel analyses was 7.8%, based upon 21 analyses on consecutive working days of a single urine specimen with mean nickel concentration of 4.2 µg/litre. A second evaluator reported that the coefficient of variation of nickel analyses within a single run was 4.1%, based upon 10 analyses of a single urine specimen within the same day, and that the coefficient of variation from run-to-run was 8.7%, based upon analyses of the same urine specimen on 10 successive working days. The nickel concentration in the urine sample averaged 10.5 µg/litre. A third evaluator reported that recovery of nickel averaged 98% (CV ± 3.4%) based upon additions of nickel in a net concentration of 5 µg/litre to 12 specimens of urine (mean nickel concentration = 3.9 µg/litre). A fourth evaluator reported that recovery of nickel averaged 97% (CV ± 7.0%) based upon additions of nickel in a concentration of 8 µg/litre to 9 specimens of urine (mean nickel concentration = 2.2 µg/ litre). A fifth evaluator used the technique of standard additions to determine the recovery of nickel added in net concentrations of 4, 8, 12, and 16 µg/litre to pooled serum (mean nickel concentration = 1.0 µg/litre). The recovery of nickel averaged 95% when the nickel working standard solutions were prepared without addition of the Fe-Cu-Zn matrix solution; the recovery of nickel averaged 100% when the Fe-Cu-Zn matrix solution was used for preparation of the nickel working standards. This evaluator also found that additions of net final concentrations of Ca (2.5 mmol/litre), Mg (1.0 mmol/litre), Na (140 mmol/litre), and K (80 mmol/litre) to nickel working standard solutions had no detectable effects upon the slope of the calibration curve. Three other evaluators confirmed that use of the Fe-Cu-Zn matrix solution for preparation of nickel working calibration solutions results in strict parallelism between the calibration plot for the nickel solutions and that obtained by additions of nickel to samples of serum and urine in concentrations from 2 to 16 µg/litre.

The Reference Method described in this report should not be employed for analyses of nickel concentrations in whole blood or in tissues (e.g., lung, liver, spleen, and kidney) which are rich in iron. For such analyses, iron interference in electrothermal atomic absorption spectrometry of nickel can be avoided by removing iron following the acid digestion step. Nomoto and Sunderman (7) added HCl to digested samples of whole blood and tissues and extracted iron as $FeCl_3$ into MIBK. Dornemann and Kleist (15) added cupferron (N-nitroso-N-phenylhydroxylamine) to digested samples of tissues and extracted Fe- and Cu-cupferronates into chloroform. The IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel is evaluating these techniques, and is developing a Reference Method for analysis of nickel in whole blood and tissues. The Subcommittee is comparing the results of analyses of nickel in biological materials by electrothermal atomic absorption spectrometry with the results obtained by dimethylglyoxime-sensitized differential pulse polarography at a dropping mercury electrode, as described by Flora and Nieboer (16). The Subcommittee is also investigating freeze-dried preparations of serum and urine that might be suitable reference materials for nickel analyses. The Subcommittee intends eventually to develop a Definitive Method for analysis of nickel in biological materials by stable isotope-dilution mass spectrometry.

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