

TRACE ANALYSIS UTILIZING PAPER CHROMATOGRAPHY AND RADIOACTIVE PRECIPITANTS

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The technique¹ we are describing employs radioisotopes to determine trace quantities of elements at the submicrogram level. It consists of paper chromatography as the analytical means for the separation of the elements and radiometric precipitation for the quantitative determination of the inorganic ions.

Paper chromatography, as a useful analytical tool for the separation of inorganic materials^{2,3,4}, has shown rapid advancement in recent years. The literature indicates that by correct selection of the solvent, utilization of complexing agents, and knowledge of the chromatographic process, quantitative isolation of most elements may be accomplished.

Radiometric precipitation is a process that utilizes radioactive labeled precipitants for the quantitative determination of the elements. For example, calcium in an unknown may be determined by precipitating with the phosphate ion labeled with ³²P; and the activity level will be proportional to the weight of the calcium in the precipitate. Examples of this method appear in the determination of 0.01 micrograms of calcium as ¹⁴C labeled oxalate by CHIRIBOGA⁵, and as ¹⁸F labeled fluoride by KUDAHL⁶.

The two methods were first combined by VAN ERKELENS⁷ in which he determined lead as the phosphate by spraying the separated lead with ³²P labeled sodium hydrogen phosphate. VAN ERKELENS⁸ also published a method for the determination of metal sulfides by precipitation with ³⁵S labeled hydrogen sulfide. We have eliminated interferences by careful selection of the separation solvent; introduced the use of tracers or radioautographs for the location of the exact area to cut from the paper for precise radiometric measurement; and extended the method to other elements that precipitate as sulfates, phosphates, and sulfides.

The overall technique requires that aliquots of the sample be spotted adjacent to each other on a paper strip. Tracer amounts of the elements to be analyzed are added to one of the aliquots and the chromatogram developed. The desired elements are located by a chromatographic scanner which detects the added tracer activity. When several tracers are used the areas are identified by absorption techniques. The labeled precipitant is spotted on the paper and migrated chromatographically over the identified areas to effect precipitation of the element. The areas of interest are washed free from excess precipitant, then cut from the paper and analyzed radiometrically. The activity level is compared to an analytical curve prepared by the same procedure with known amounts of the element.

EXPERIMENTAL

Reagents and tracers

All reagents used were of reagent grade.

$H_2^{35}S$. 0.1 μ c/mM of sulfide. Available in lecture bottles containing 150 l of H_2S gas from the Matheson Company, East Rutherford, New Jersey.

$H_2^{35}SO_4$. 0.3 N H_2SO_4 —1.6 μ c/mM of sulfate. Available from Oak Ridge National Laboratory as $H_2^{35}SO_4$ in a small volume of HCl; 5 mc is diluted to 5 ml with 0.3 N H_2SO_4 .

$H_3^{32}PO_4$. 0.3 N H_3PO_4 —30 μ c/mM of phosphate. Available from Oak Ridge National Laboratory as $H_2^{32}PO_4$ in a small volume of HCl; 5 mc is diluted with 0.3 N H_3PO_4 .

$(NH_4)_2H^{32}PO_4$. 1.0 N $(NH_4)_2HPO_4$ —10 μ c/mM of phosphate. Available from Oak Ridge National Laboratory as $H_3^{32}PO_4$. 5 mc is diluted to 5 ml with a solution containing 50 mg of $(NH_4)_2HPO_4$ per ml of H_2O previously adjusted to pH of 8.

Liquid scintillator solution. 500 mg POPOP [1,4-bis-2-(5-phenyloxazolyl)benzene], 10 g PPO (2,5-diphenyloxazole), 50.0 g naphthalene, 750 ml dioxane, 125 ml methyl alcohol, 125 ml ethylene glycol monoethyl ether.

Alizarin Red S. 0.1% (w/v) alcoholic solution of sodium alizarin sulfonate.

Cadmium chloride solution. 20 g of cadmium chloride is dissolved in 400 ml of water; 600 ml of ammonium hydroxide is added with mechanical stirring.

Ammonium sulfide reagent. Take 300 ml of a 10% NH_4Cl solution and adjust the pH to 8 with NH_4OH . Bubble labeled $H_2^{35}S$ through the solution for 1 h at a flow rate of 80 cc/min. The solution should be made fresh for each run.

Tracers. ^{133}Ba , ^{89}Sr , ^{45}Ca , ^{95}Zr , ^{54}Mn , 7Be , ^{151}Hf .

Apparatus

Chromatographic tanks. All glass apparatus including frames and troughs available from chromatographic suppliers. The tank dimensions are 12 in. \times 12 in. \times 24 in. and are constructed for descending chromatography.

Chromato-Beta Analyzer. Geiger, gas flow, and scintillation detectors available from nuclear detector equipment suppliers.

Radioautographic equipment. Kodak Blue Brand X-ray Film, 14 in. \times 17 in., Type III, Class C. This film requires an overnight exposure when ^{32}P precipitants having activities of 100 d.p.m. of ^{32}P per square centimeter are used. A 48–72 h exposure is necessary when ^{35}S precipitants are exposed. The cassette used is a Kodak Exposure Holder 14 in. \times 17 in. with the thin lead sheet removed to minimize backscatter. The radioautographs are developed with X-ray developer for 3.5 min at 58°F and fixed with X-ray fixer for 8–10 min.

Polyethylene dispensing bottles. Polyethylene plastic baby bottles, No. 38133, Scientific Furnishings, Westhampnett Road, Chichester, Sussex, England⁹. All radioactive tracers and precipitants are stored and dispensed by weight from these small bottles.

PROCEDURES

Spot aliquots of the sample 15 cm from one end of the paper 2 cm apart on a 5 \times 56 cm Whatman No. 42. Add tracer activities of the elements (A)* to be analyzed

to one of the aliquots and develop by descending chromatography in the tank overnight using the separating solvent (B). Remove and air-dry. Scan and locate the added tracers with the Chromato-Beta Analyzer. Add 5–10 mg of the labeled precipitants (C) 5 cm above the sample origin and 5–10 mg of the non-radioactive precipitant (D) 5 cm above the aliquot where the tracers were added. Develop by descending chromatography in a second tank with the second solvent (E) overnight. Remove from the tank and air-dry. Cut from the paper strip areas adjacent to those where tracers were previously located. Assay the radioactive precipitate.

Follow this procedure when elements are to be analyzed as sulfates or phosphates. Make specific additions of tracers and reagents as indicated by the alphabetical letters.

Sulfates—Sr, Ba, Ca, Pb

A. Tracers: ^{133}Ba , ^{89}Sr , ^{45}Ca .

B. Separating solvent: 40 % ethanol + 40 % methanol + 20 % 2 N HCl by volume.

C. Labeled precipitant: 0.3 N $\text{H}_2^{35}\text{SO}_4$.

D. Non-radioactive precipitant: 0.3 N H_2SO_4 reagent.

E. Second solvent: 90 % water + 10 % ethanol by volume.

Place paper, cut from the strip, in a vial, add 15 ml of the liquid scintillator solution, and assay for ^{35}S content using a liquid scintillation spectrometer.

Phosphates—Zr, Hf

A. Tracers: ^{95}Zr –(^{95}Nb), ^{151}Hf .

B. Separating solvent: 87.5 % ether + 12.5 % HNO_3 (v/w).

C. Labeled precipitant: 0.3 N $\text{H}_3^{32}\text{PO}_4$.

D. Non-radioactive precipitant: 0.3 N cold H_3PO_4 .

E. Second solvent: 74 % isopropanol + 25 % water + 1 % conc. NH_4OH + trichloroacetic acid (5 g/100 ml of solvent).

Mount the paper, cut from the strip, on a planchet with a beta phosphor and assay for ^{32}P using a beta-phosphor scintillation counter.

Phosphates—Th, Be, Al, Mn, Ba, Sr, Ca

A. Tracers: ^{54}Mn , ^{133}Ba , ^{89}Sr , ^{45}Ca , ^7Be .

B. Separating solvent: Antipyrine-EDTA-dioxane (2). Mix 2 ml water and 2 ml conc. HNO_3 , add 0.05 g EDTA (free acid), and warm the mixture gently until solution is complete. Allow solution to cool, add 100 ml dioxane and 1 g antipyrine which dissolves in the cold. The mixture is stable for 3 days after which it becomes yellow in color.

C. Labeled precipitant: 0.3 N $(\text{NH}_4)_2\text{H}^{32}\text{PO}_4$.

D. Non-radioactive precipitant: 0.3 N $(\text{NH}_4)_2\text{HPO}_4$.

E. Second solvent: 5 % ethanol + 95 % water by volume in ammonia atmosphere.

Mount the paper, cut from the strip, on a planchet with a beta phosphor and assay for ^{32}P using a beta-phosphor scintillation counter.

* Alphabetical letter notations refer to specific tracers and reagents.

Sulfides—Ni, Mn, Co, Pb, Zn, Fe

The procedure is to spot aliquots of the sample 15 cm from one end on 1 × 56 cm Whatman No. 42 paper strips. Prepare a similar strip and spot 10 mμg of Ni, Mn, Co, Pb, Zn and Fe as chlorides at a single origin. Designate this as your reference strip. Develop the strips in 87 % acetone, 8 % HCl, 5 % H₂O (w/v) for 8 h. Remove and air-dry. Spray the reference strip with Alizarin Red S and identify the elements which have migrated from the origin in the following order: Ni, Mn, Co, Pb, Zn and Fe. Use the reference strip as a guide and mark the element areas on the sample strips. Expose in a tank saturated with NH₄OH for one hour. Develop by descending chromatography in the prepared ammonium sulfide solution for three hours. Remove, air-dry, and radioautograph overnight. The radioautograph will confirm the area obtained from the reference strip.

Cut out the identified element areas and place in vials. Add 15 ml of liquid scintillator solution and assay for ³⁵S content using a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Tracers

Tracers, radioactive isotopes of the elements to be analyzed, are used as references to locate the elements on the paper strip. Where tracers are not available separate strips containing amounts of the element that are detectable by color reactions to sprays are substituted. When several tracers are used the Chromato-Beta Analyzer with its absorption equipment is capable of resolving qualitatively the mixture of activities. We have found the migration rates for the individual element to be reproducible if the apparatus, solvent, and developing time are constant and the sample is from the same matrix. It is necessary to find out the original location of a specific element in the same system and occasionally confirm it by using the reference.

Separation solvent

There are a large number of factors involved in the choice of a solvent for the separation of inorganic ions. Texts^{2,3,4} are available however that describe these solvents for most of the elements. We have used these sources for choices of solvents and have experimentally checked listed migration rates as well as possible interfering elements. This technique is adaptable to elements not listed as long as a separation solvent is chosen that prevents interference from elements that have similar migration speeds and precipitate with the labeled precipitant.

Labeled precipitant

The choice of the labeled precipitant depends on the solubility of the compound formed in the second solvent and the amount of the precipitant. A time study to determine the mechanism of precipitation was performed in which ten and one microgram quantities of barium were placed on eight strips spotted with labeled sulfate and developed. The strips were removed from the chromatographic tanks at 2-h intervals and radioautographed. The following facts are apparent from Fig. 1:

1. Precipitation occurs as soon as the precipitant contacts the elements.
2. The higher the concentration of the element on the paper the larger the area that must be cut for radiometric assay.

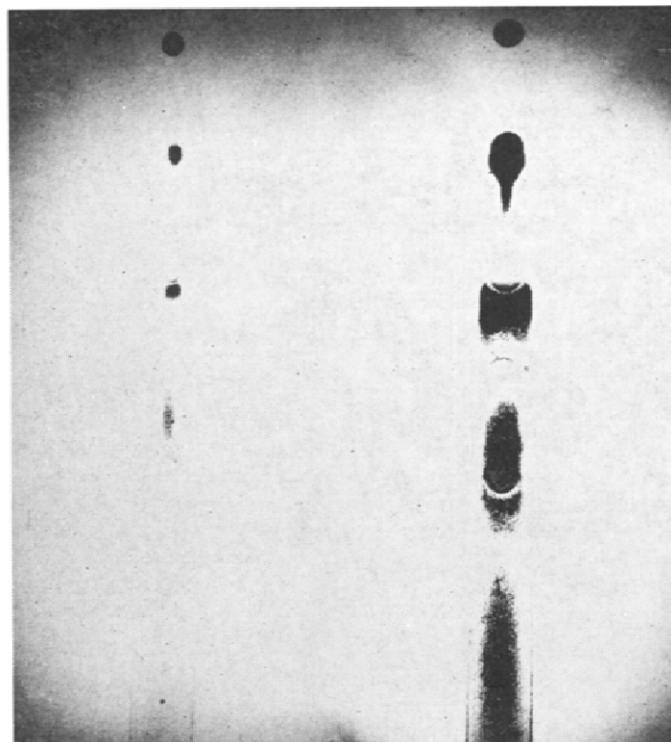
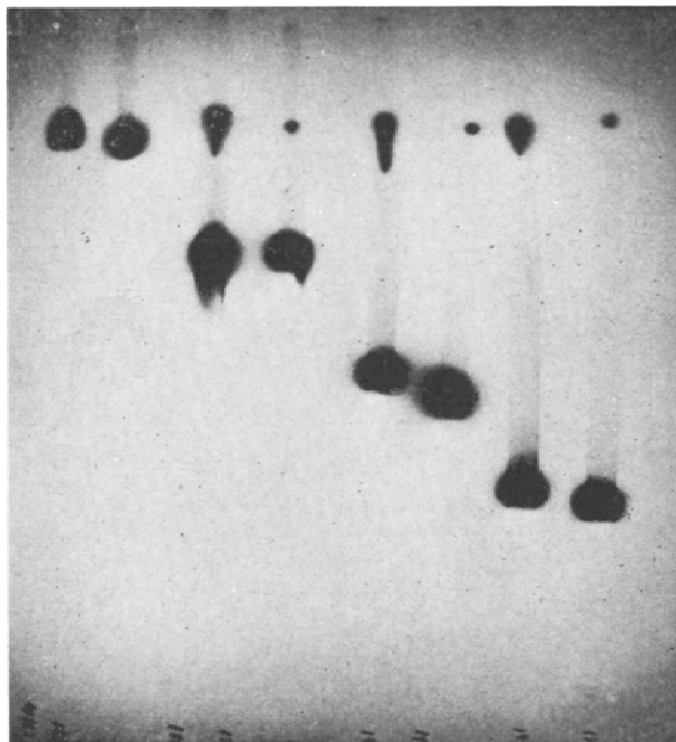


Fig. 1. Time study of the action of the labeled precipitant.

Fig. 2. Effect of the amount of precipitant on precipitation of ten and one micrograms of thorium.

3. The greater the area occupied by the precipitant the more wash is required to remove excess precipitant.

The effect of the amount of precipitant is shown in Fig. 2 where ten and one microgram amounts of thorium are spotted at intervals along the chromatogram. This shows that in order to obtain concentrated, well-defined precipitates, it is necessary to have sufficient precipitant available when contact is made with the element. With insufficient precipitant, precipitation occurs in diffuse bands.

Non-radioactive precipitant

The non-radioactive precipitant converts the reference, containing the tracer, to the same salt as the sample. If movement occurs in the second solvent the tracer will still locate the area containing the element of interest.

Second solvent

The solvents used to migrate the labeled precipitant must meet the following requirements:

1. *Media for quantitative precipitation.* That is, the solvent used to migrate the labeled precipitant must not dissolve the precipitated salt of the element. The most efficient migrating solvent we have found so far is water and, at submicrogram levels, salts of elements are soluble. This is why we presently have completed methods for salts that are sparingly soluble in water, such as barium as the sulfate and basic phosphate, thorium as the basic phosphate, strontium and calcium as basic phosphates, manganese and aluminum as basic phosphates, zirconium and hafnium as the acid phosphates, and the transition elements as sulfides. Precipitates of solubility may be

analyzed by this technique, at a loss of sensitivity providing calibration curves are obtained using the same technique. The more insoluble the precipitate in the second solvent, the closer the gross activity is to the calculated theoretical activity.

2. *Movement of excess precipitant.* The solvent must be capable of separating and moving excess precipitant from the area to be cut out for radiometric assay. If this is not efficiently done the activity level will not represent accurately the weight of the unknown element in the precipitate. Fig. 3 shows the efficiency of water on the migration of diammonium phosphate labeled with ^{32}P from ten, five, and one microgram amounts of thorium, while Fig. 4 shows the inefficiency of isopropanol as a second solvent.

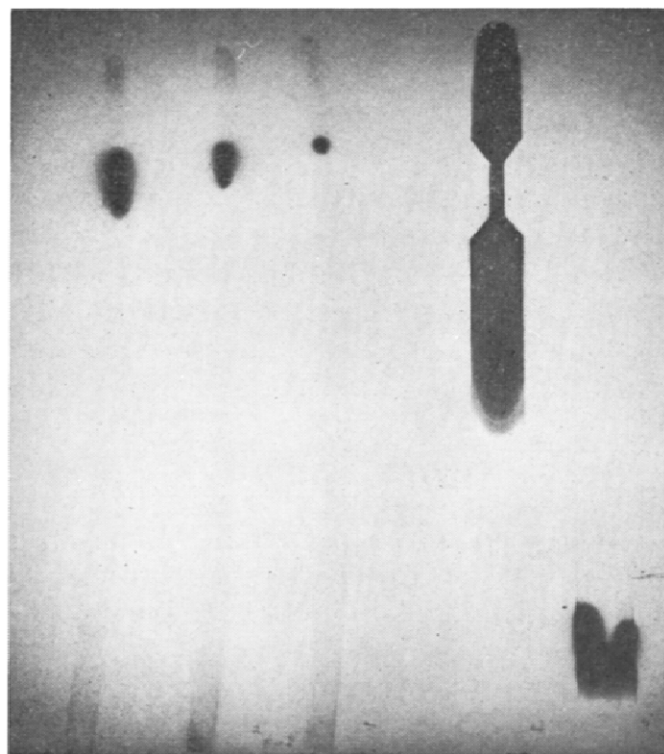
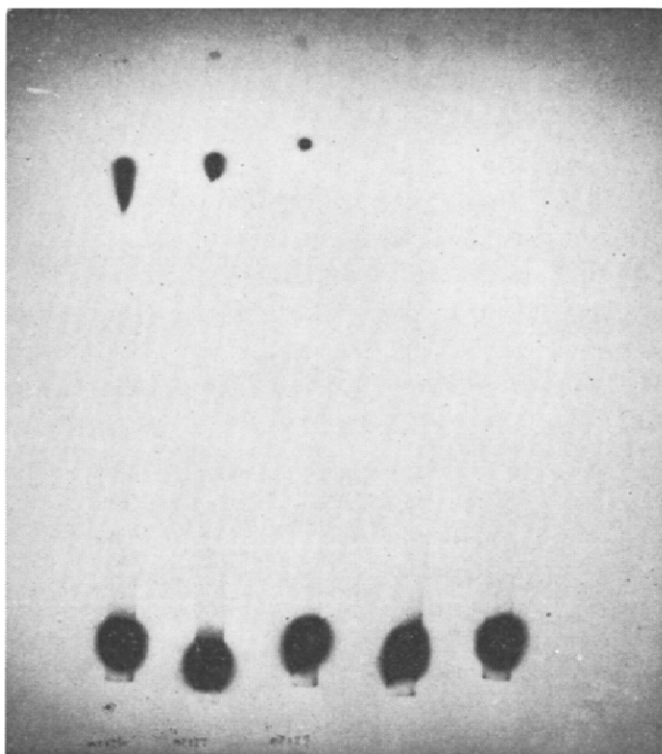


Fig. 3. Efficiency of water on the migration of diammonium phosphate.

Fig. 4. Comparison of water and isopropanol for the migration of diammonium phosphate.

3. *Migration of precipitate after formation.* There must be ample separation between the front of the precipitated element and the tail of the precipitant. Milligram quantities of precipitant are used for each radiometric precipitation and such quantities have a tendency to tail when developed chromatographically. This is illustrated in a time study shown in Fig. 1 where water migrates the phosphate ion and leaves the precipitated thorium phosphate.

Recovery of element

The orientation of elements on a paper strip after use of the second solvent is shown in Fig. 5. The unlabeled circle is where the sample aliquots were originally placed. The reference aliquot has had the tracer activities added and the numbers are the results obtained after scanning. The areas, indicated in Fig. 5 as blocks, are

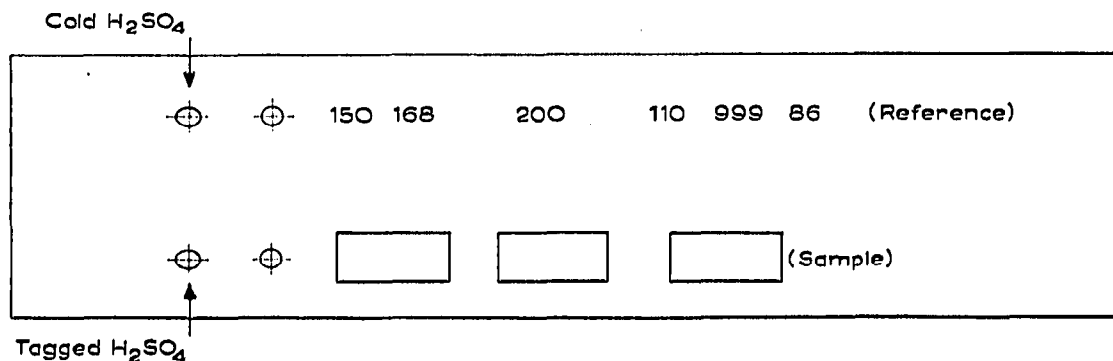


Fig. 5. Orientation of elements on a paper strip after development.

1.5 by 3 cm and are cut from the paper for radiometric assay. These areas contain the elements quantitatively.

Sensitivity

The sensitivity limitations of the technique have not been fully established but all elements we have analyzed may be determined at the 0.01 microgram level. Some elements such as barium as the sulfate, thorium as the phosphate, and zirconium as the phosphate have been determined at the 0.001 microgram level. The sensitivity is dependent on the counting equipment. The Chromato-Beta Scanner is sensitive enough to determine levels of the elements above one microgram on the paper. Below this level it is recommended that liquid scintillation counting be used for ^{35}S and thin plastic phosphor counting for ^{32}P .

Another factor in sensitivity is the specific activity of the labeled precipitant. It has been our experience that specific activities higher than those recommended are of limited value with the present solvents available for migration. The increase in background is higher than the corresponding sensitivity increase.

Accuracy and precision

The accuracy and precision of the technique have been evaluated for the sulfates, phosphates and sulfides listed in the procedures.

An aliquot of the solution containing known amounts of barium and 2 to 5 mg of calcium and strontium was developed in the separating solvent and precipitated as the sulfate. Table I lists the average counting rate obtained from the precipitation

TABLE I
DETERMINATION OF BARIUM AS THE SULFATE

Barium (μg)	Observed activity (c.p.m.)
10	404,200 \pm 1.2% *
1.0	41,400 \pm 3.4%
0.1	4,200 \pm 8.0%
0.01	452 \pm 12%
0.001	44 \pm 22%

* The standard deviation of the mean of ten determinations at the 90% confidence level.

of barium as the labeled sulfate for levels of barium on the paper of from 0.001 to 10 μg . This average counting rate was obtained for ten determinations at each level and is listed in column 2. The standard deviation, expressed in percent, is that of the mean at the 90 % confidence level.

TABLE II

DETERMINATION OF ZIRCONIUM AS THE ACID PHOSPHATE

<i>Zirconium</i> (μg)	<i>Observed activity</i> (c.p.m.)
0.08	100 \pm 10%
0.8	1,000 \pm 3.2%
4.0	5,100 \pm 1.3%
8.0	10,000 \pm 1.0%

Quantitative data for the determination of zirconium as the acid phosphate are presented in Table II. Zirconium was separated from hafnium, its only interference, and precipitated with ^{32}P labeled phosphoric acid. The average counting rate expressed in column 2 is for a minimum of six determinations at each level of zirconium added.

Preliminary data listed in Table III show the accurate precipitation of the rare earth elements as phosphates. The accuracy and precision are excellent for the rare earth elements tested.

TABLE III

DETERMINATION OF RARE EARTHS AS THE BASIC PHOSPHATE

<i>Rare earth element</i>	<i>Amount added</i> (μg)	<i>Amount found</i> (μg)
Nd	1.15	1.12 \pm 5.9%
Eu	1.18	1.18 \pm 2.8%
Gd	1.19	1.20 \pm 2.8%
Tb	1.20	1.19 \pm 1.6%
Dy	1.21	1.17 \pm 7.1%
Ho	1.22	1.19 \pm 8.2%
Er	0.88	0.88 \pm 1.8%
Lu	1.24	1.24 \pm 5.3%

An aliquot of a solution containing known amounts of nickel, 1 to 2 mg of iron and 0.5 to 1.0 mg of manganese and copper was carried through the sulfide procedure. The percent recovery and standard deviation of triplicate analyses for nickel are listed in Table IV.

TABLE IV

DETERMINATION OF NICKEL AS THE SULFIDE

<i>Nickel added (μg)</i>	<i>Nickel found (μg)</i>	<i>Percent recovered</i>
0.038	0.036 0.036 <u>0.035</u> Av. 0.036	94
0.097	0.094 0.094 <u>0.092</u> Av. 0.093	96
0.45	0.45 0.44 <u>0.44</u> Av. 0.44	98
0.95	0.90 0.92 <u>0.93</u> Av. 0.92	97
3.18	2.95 3.10 <u>3.08</u> Av. 3.04	96
7.18	6.90 7.00 <u>6.90</u> Av. 6.93	97

SUMMARY

The technique described is a sensitive analytical tool developed for the determination of trace elements. It combines paper chromatography, as the separation process, with radiometric precipitation for quantitative determination. Procedures are described that are capable of determining submicrogram quantities of elements. Besides illustrating the use of radioisotopes in analytical chemistry, it is accurate, reproducible, inexpensive, and does not require a high degree of skill to perform the analysis.

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