Spectrofluorimetric and Spectrophotometric Determination of Aluminium with the Salicyloylhydrazones of Pyridine-2-aldehyde and Pyridoxal

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The analytical applications of the salicyloylhydrazones of pyridine-2-aldehyde (SHPA) and pyridoxal (PSH) are described. PSH reacts with aluminium at pH 2.0 to produce a yellow complex ($\lambda_{max.}$ = 420 nm, ϵ = 2.5×10^4 l mol^{-1} cm^{-1}); another complex can be detected at pH 4.75 ($\lambda_{max.}$ = 425 nm, ϵ = 3.1×10^4 l mol^{-1} cm^{-1}). SHPA reacts with aluminium at pH 5.4 to give a complex having a blue fluorescence ($\lambda_{ex.}$ = 382 nm, $\lambda_{em.}$ = 440 nm) and both reagents are compared. Sensitive and selective methods are proposed for the determination of aluminium ions. The yellow aluminium - PSH complex formed in acid medium (pH = 2.0) has been used for the determination of aluminium in samples of steel and a Portland cement.

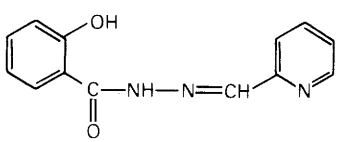
Keywords: *Aluminium determination*; *pyridine-2-aldehyde and pyridoxal salicyloylhydrazones*; *spectrofluorimetry*; *spectrophotometry*

Pyridine aldehyde derivatives have been commonly used as spectrophotometric reagents, but less attention has been devoted to the use of these compounds as spectrofluorimetric reagents.[1] Some pyridoxal derivatives have been employed for biochemical purposes, but little is known about their value in inorganic analysis as spectrofluorimetric and spectrophotometric reagents.

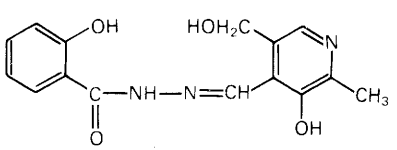
Pyridine-2-aldehyde salicyloylhydrazone has been employed by us as a spectrophotometric reagent for the determinations of nickel and zinc in aqueous solutions,[2] in the solvent extraction - spectrophotometric determinations of vanadium(V)[3] and iron(II),[4] and also in the atomic-absorption - spectrophotometric determination of zinc in analytical reagents, milk and sea water with prior solvent extraction of the metal chelate into pentan-2-one.[5] The reactions of pyridine-2-aldehyde 2-quinoylhydrazone[6] and isonicotinoylhydrazone[7] with metal ions have been investigated.

Pyridoxal salicyloylhydrazone has been used as a spectrophotometric reagent for determining trace amounts of zirconium(IV).[8] The spectrofluorimetric determination of magnesium(II)[9] and zinc(II)[10] by ternary complex formation with pyridoxal nicotinoylhydrazone and amines has been also described.

In this work, the spectrofluorimetric characteristics of salicyloylhydrazones of pyridine-2-aldehyde (SHPA) and pyridoxal (PSH) have been studied. A comparative study of the fundamental analytical data of both reagents is given. Spectrofluorimetric and absorptiometric methods for the determination of trace amounts of aluminium ion using SHPA and PSH, respectively, have been developed.



SHPA



PSH

Experimental

Reagents

The salicyloylhydrazones were prepared[2,8] by condensation of equimolar amounts of the particular aldehyde and salicyloylhydrazone. Pyridoxal salicyloylhydrazone was used as a 0.1 or 0.15% *m/V* solution in ethanol - dimethylformamide (9 + 1 *V/V*). A 0.1% *m/V* solution of the salicyloylhydrazone of pyridine-2-aldehyde in ethanol was also used. An aluminium ion stock solution containing 1 mg ml^{-1} of aluminium was prepared from aluminium(III) nitrate nonahydrate [$Al(NO_3)_3.9H_2O$] and standardised gravimetrically as the oxide.[11] The stock solution was diluted as required immediately before use. Buffer solutions were prepared by conventional methods. Analytical-reagent grade materials were used to prepare all solutions, and distilled water was used throughout.

Apparatus

A Perkin-Elmer MPF-43 spectrofluorimeter was used with 1-cm quartz cells and a xenon-arc source (instrumental sensitivity: 0.1, 0.3, 1, 3, 10, 30 and 100). The instrument was standardised with a 1 μg ml^{-1} solution of quinine ($\lambda_{ex.}$ = 350 nm, $\lambda_{em.}$ = 450 nm). Slit widths were 6 nm for all measurements, and the sensitivity range was 1–3 depending on the concentration of the aluminium solution. All spectra were uncorrected.

Perkin-Elmer 402 and Pye Unicam SP6-500 spectrophotometers equipped with 1-cm glass or quartz cells were used.

Spectrofluorimetric Method for the Determination of Aluminium with SHPA

To a 25-ml calibrated flask, containing a sample or standard solution of 0.5–2.0 μg of aluminium, add 3 ml of 0.1% SHPA solution, 2 ml of ethanol and 5 ml of acetate buffer of pH 5.40; dilute the mixture to the mark with distilled water. Measure the fluorescence intensity ($\lambda_{ex.}$ = 382 nm, $\lambda_{em.}$ = 440 nm; instrumental sensitivity, 1) after 20 min against a reagent blank prepared in a similar way but without aluminium.

A similar procedure can be employed for the determination of 0.1–0.7 μg of aluminium (instrumental sensitivity, 3), but using 1 ml of 0.1% SHPA solution.

Spectrophotometric Method for the Determination of Aluminium with PSH

To the aluminium ion solution (up to 22.5 μg of aluminium) in a 25-ml calibrated flask, add 2.5 ml of 0.5 M potassium nitrate solution, 5 ml of 0.15% PSH solution and 5 ml of

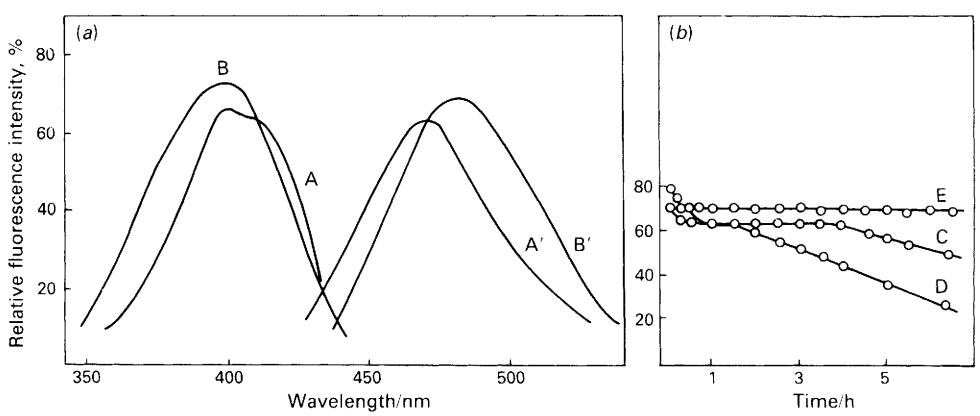


Fig. 1. Fluorescence spectra and stability of both reagents. (*a*) A and A′, excitation and emission spectra of SHPA at pH 5.4 (concentration of SHPA, 4.1 × 10^{-4} M; and instrumental sensitivity, 3). B and B′, analogous spectra for PSH at the same pH value (concentration of PSH, 6.6 × 10^{-5} M; and instrumental sensitivity, 0.1). (*b*) C and D, stability of SHPA in acetate buffer and unbuffered solution, respectively, at the same pH value. E corresponds to the stability of the PSH solution in acetate buffer and in unbuffered solution. Concentrations and instrumental sensitivities as in (*a*).

0.05 M hydrochloric acid; dilute to volume with distilled water. Measure the absorbance at 400 nm against a reagent blank prepared in a similar way but without aluminium, or at 420 nm against distilled water.

Prepare a calibration graph using standard solutions of aluminium(III), treated in the same manner as the samples.

Results and Discussion

Spectrofluorimetric Aspects of the Reagents

SHPA reagent solutions show a green fluorescence in acidic media and exhibit maximum fluorescence intensity at pH 5–6 ($\lambda_{ex.}$ = 400 nm, $\lambda_{em.}$ = 470 nm) [Fig. 1(*a*)]. The fluorescence intensity of SHPA solutions in acetate buffer remained constant for about 3 h, but unbuffered solutions, at the same pH value, are only stable for 50 min [Fig. 1(*b*)].

PSH reagent solutions show an intense green fluorescence in acidic media and exhibit maximum fluorescence intensity at pH 5–6 ($\lambda_{ex.}$ = 397 nm, $\lambda_{em.}$ = 480 nm) [Fig. 1(*a*)]. The fluorescence intensity of PSH solutions remains constant for a longer time (at least 24 h) than that of SHPA solutions in acetate buffer and unbuffered solutions [Fig. 1(*b*)].

PSH and SHPA possess the atomic grouping (HO)C=C–CO–NH–N=CH–C=C(OH) and (HO)C=C–CO–NH–N=CH–C=N–, respectively. The fluorescence of both reagents is in agreement with studies carried out by Taniguchi *et al.*[12] They indicate that hydrazones possessing a hydroxyl group in the *ortho*-position with respect to the imine or carbonyl groups show fluorescent properties. Further, the hydrogen bonds have an important role in the formation of fluorescent species.[13] The greater fluorescence of PSH compared with SHPA may be due to the occurrence of two hydroxyl groups in *ortho*-positions in the former reagent.

A potentiometric - spectrofluorimetric method[14] was used to determine the ionisation constants of SHPA and PSH in 20% and 4% ethanolic solutions, respectively. The average pK values found were pK_1 3.3 and 4.3 and pK_2 6.9 and 7.0, respectively. These pK values are similar to those found spectrophotometrically.

Reaction of SHPA and PSH with Metal Ions

The fluorescent reactions of both reagents with many inorganic ions at various pH values [2.0 (hydrochloric acid), 5.5 (acetate buffer) and 10 (ammonia buffer)] were investigated. The results obtained indicate that only the aluminium ion - SHPA complex has an interesting fluorescent reaction. The fluorescent solutions of PSH showed only quenching effects with the inorganic ions tested.

A comparative study of metal chelates of both reagents carried out spectrophotometrically is given in Table I.

TABLE I

SPECTROPHOTOMETRIC CHARACTERISTICS OF SOLUBLE METAL COMPLEXES WITH SHPA AND PSH IN ACETATE BUFFER

SHPA reagent*				PSH reagent†			
Metal	Colour of the complex	$\lambda_{max.}$/nm	$\varepsilon \times 10^4$/ l mol^{-1} cm^{-1}	Metal	Colour of the complex	$\lambda_{max.}$/nm	$\varepsilon \times 10^4$/ l mol^{-1} cm^{-1}
Ni(II)	Yellow	375	4.40	Cu(II)	Yellow	390	1.74
Cu(II)	Yellow	375	3.00	Fe(II)	Amber	420–490	1.20–0.70
Co(II)	Yellow	365	3.53	U(VI)	Yellow	425	0.60
Zn(II)	Yellow	365	4.90	Ti(IV)	Orange	450	0.18
Ti(IV)	Yellow	380	1.80	Al(III)	Yellow	425	2.97
Bi(III)	Yellow	380	1.83	Ga(III)	Yellow	425	1.04
Pd(II)	Yellow	380	1.32	Ni(II)	Orange	425	2.20
V(V)	Yellow - gold	400	1.78	Co(II)	Orange	420	1.32
Fe(II)	Green	620	0.29	Zn(II)	Yellow	425	0.98

* In a solution containing 20% *V*/*V* of ethanol.
† In a solution containing 20% *V*/*V* of a dimethylformamide - ethanol (1 + 9 *V*/*V*) mixture.

Spectrofluorimetric Study of the Aluminium(III) - SHPA Complex

Aluminium ions form a colourless complex with SHPA, which shows a maximum intensity blue fluorescence ($\lambda_{ex.}$ = 382 nm, $\lambda_{em.}$ = 440 nm) at pH 5.1–5.8 (Fig. 2). The excitation

and emission spectra of the aluminium complex in acetate buffer (pH 5.4) remain stable for at least 4 h, after a reaction time of 20 min. Unbuffered solutions, at the same ionic strength, reach fluorimetric stability after a reaction time of 60 min. With 5 ml of acetate buffer in the mixture good analytical results can be obtained. Changes in the ionic strength (potassium nitrate) have no effect if the solutions are buffered with acetate as indicated above. Temperatures in the range 10–50 °C do not affect the fluorescence intensity, but increasing the temperature above 50 °C causes the fluorescence intensity to decrease by about 3.5% $°C^{-1}$. Increasing the ethanol concentration (from 10 to 40% of ethanol) in the mixture does not have any effect. Therefore, a medium containing 20% of ethanol was chosen for further experimental work.

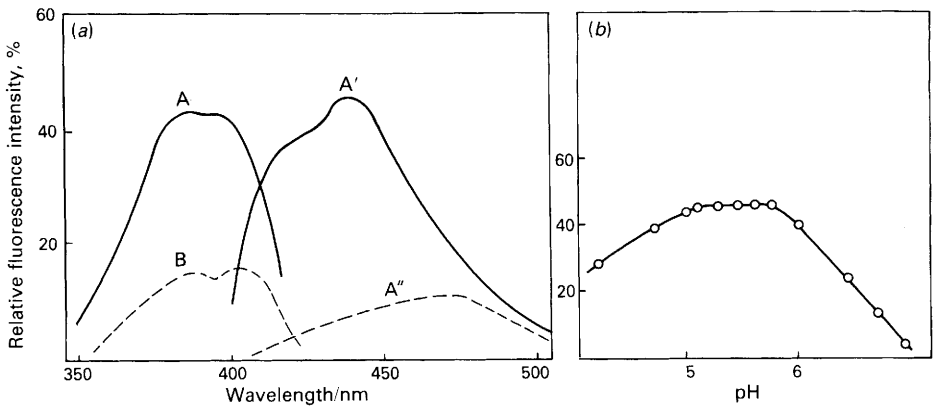


Fig. 2. (*a*) Excitation and emission spectra at pH 5.4 of aluminium - SHPA complex (A and A'), and reagent blank (B and B'). (*b*) Relative fluorescence intensity *versus* pH graph for the aluminium - SHPA complex. In all instances the reaction conditions are: aluminium(III) concentration, 1.8×10^{-6} M; SHPA concentration, 4.1×10^{-4} M; and instrumental sensitivity, 1.

Spectrophotometric Study of the Aluminium(III) - PSH Complex

When dilute aluminium(III) solutions and a 0.1% *m/V* solution of PSH in ethanol are mixed, a yellow complex rapidly forms [Fig. 3(*a*]. The optimum pH ranges for the formation of the yellow complex are 1.8–3.1 and 4.2–6.0 [Fig. 3(*b*)]. The absorption spectra of the aluminium complex remain stable for at least 24 h at pH 2.0 and for 3 d in acetate buffer.

At pH 2.0 (hydrochloric acid), the aluminium complex forms only when the reagents are added in the order aluminium, potassium nitrate, PSH and hydrochloric acid. This may be due to co-ordination in the aluminium chelate occurring by bonding from the oxygen of the carbonyl group, because in moderately acidic media or alkaline solution the hydrogen atom of the –CONH– group can dissociate.[15,16]

Stoicheiometry and Charge of the Aluminium Complexes

The stoicheiometry of the aluminium(III) - salicyloylhydrazones was evaluated in all instances by the continuous variation method and the charge of the complexes was determined by ion exchange using Dowex 50-X8 (sodium form) and 1-X8 (chloride form) resins.

The stoicheiometric ratio found by spectrofluorimetric measurements for the aluminium - SHPA complex at pH 5.4 (with and without acetate buffer) was 1:3. In both instances, the aluminium complex is uncharged.

The stoicheiometry of the aluminium(III) - PSH complex (absorptiometric measurements) at pH 2.0 (hydrochloric acid) was found to be 1:1; the complex has an over-all positive charge. In acetate buffer, the stoicheiometry of the aluminium(III) - PSH complex was found to be 2:3 (stoicheiometric ratio of aluminium:ligand). The same stoicheiometry is obtained by the slope ratio method. In this medium, the aluminium complex is uncharged. If unbuffered solutions, at the same pH value, are used, the stoicheiometric ratio of

aluminium to ligand found is 1:2. The charge of the aluminium complex at pH 4.75 (no buffer) is positive. The acetate ions have an important effect on the aluminium - PSH complex formation, because acetate ions are probably involved in the co-ordination of the complex resulting in neutralisation of the positive charge owing to the formation of a mixed ligand chelate.

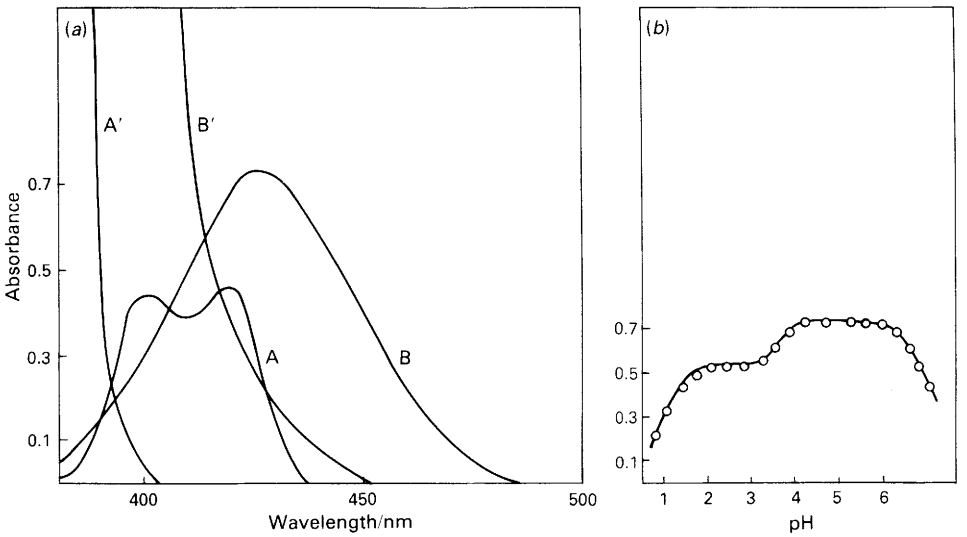


Fig. 3. (*a*) Absorption spectra of the aluminium complexes. A, Aluminium - PSH complex at pH 2.0; B, aluminium - PSH complex at pH 4.75 (acetate buffer); and A′ and B′ reagent blank at the same concentration and for each pH value. (*b*) Effect of pH on the formation of aluminium complexes. In all instances the reaction conditions are: aluminium(III) concentration, 1.8×10^{-5} M; and PSH concentration, 6.6×10^{-4} M.

Determination of Aluminium

Based on the experimental work, two methods are proposed for the determination of trace amounts of aluminium involving the formation of complexes of aluminium wit h SHPA and PSH.

Spectrofluorimetric method

There is a linear relation between the emitted fluorescence intensity and the aluminium ion concentration in the range 20–80 ng ml^{-1} (instrumental sensitivity, 1) or 4–28 ng ml^{-1} (instrumental sensitivity, 3). When the recommended procedures were applied to two series of eleven samples (20–50 ng ml^{-1} of aluminium) the relative standard deviations ($p = 0.05$) of the methods were found to be 1.1% in both instances.

TABLE II

TOLERANCE LIMITS IN THE SPECTROFLUORIMETRIC DETERMINATION OF 0.5 μg OF ALUMINIUM IN 25 ml OF SOLUTION WITH SHPA

Amount of foreign ion added/ μg per 25 ml	Species tolerated
25	Hg(I and II), As(III), La(III), Cr(III), Cd, Pb, W(VI), Li, Na, K, Ca, Mg, Ba, Tl(I), $S_2O_8^{2-}$, BrO_3^-, IO_3^-, SCN^-, $B_4O_7^{2-}$, ClO_4^-, I^-, $S_2O_3^{2-}$, CO_3^{2-}, NO_3^-
12.5	Ni, Th
5	Ce(IV)
2.5	Se(IV), U(VI), Ag, Cu(II), Zn, Mn(II), Pd(II), PO_4^{3-}, SO_3^{2-}, tartrate, EDTA
1.25	As(V), In(III), Bi(III), F^-, Be(II)

Spectrophotometric method

At pH 2.0 (hydrochloric acid), Beer's law is obeyed in the aluminium concentration range 0.2–0.9 μg ml^{-1} and the molar absorptivity at 420 nm is 2.5×10^4 l mol^{-1} cm^{-1}. Ringbom's graph shows that 0.25–0.85 μg ml^{-1} of aluminium is the minimum range of error. The relative standard deviation ($p = 0.05$, $n = 11$) for 0.5 μg ml^{-1} of aluminium is 0.4%.

Effect of diverse ions on both methods

The influence of several ions on the fluorescence intensity of 20 ng ml^{-1} of aluminium has been investigated (Table II). The limiting value of the concentration of foreign ions was taken as that value which caused an error of not more than 1% in the fluorescence intensity. The most serious interferences are from iron(II), iron(III), gallium(III), titanium(IV), tin(II), zirconium(IV), molybdenum(VI), oxalate, citrate and periodate; these interfere when present at concentrations equal to those of aluminium. However, aluminium can be determined without great error, but with reduced sensitivity, in the presence of high concentrations of tin(II), zirconium(IV), molybdenum(VI) and manganese(II). In these instances, samples and standards must be matched for foreign ion concentration (Table III).

TABLE III

EFFECT OF FOREIGN IONS AT HIGH CONCENTRATIONS ON THE FLUORESCENCE INTENSITY OF THE ALUMINIUM - SHPA COMPLEX*

Ion added	Concentration/ ng ml^{-1}	Fluorescence intensity, %	Aluminium recovery/ ng ml^{-1}
Sn(II)	20	15.0	5.5
	100	15.0	5.5
Zr(IV)	20	10.0	3.6
	100	9.9	3.6
Mo(VI)	20	14.6	5.3
	100	15.0	5.5
	200	15.0	5.5
Mn(II)	500	24.5	8.9
	1000	26.0	9.1

* Fluorescence intensity of a 0.5-μg sample of aluminium in 25 ml of solution is 54.9%.

Results of the interference studies in the absorptiometric procedure are given in Table IV. The tolerance towards foreign ions was taken as the largest amount that caused an error of not more than 1% in the absorbance of 0.5 μg ml^{-1} of aluminium. Vanadium(V), copper(II), fluoride and phosphate interfere when present at the same concentration as aluminium.

TABLE IV

TOLERANCE LIMITS* IN THE SPECTROPHOTOMETRIC DETERMINATION OF 0.5 μg OF ALUMINIUM IN 25 ml OF SOLUTION WITH PSH AT pH 2

Amount of foreign ion added/ μg per 25 ml	Species tolerated
2500	Bi, Hg(II), Se(IV), Tl(I), Cd, Ag,† $S_2O_8^{2-}$, SCN^-, $B_4O_7^{2-}$, CO_3^{2-}, $S_2O_3^{2-}$, IO_3^-, I^-, BrO_3^-, NO_3^-, ClO_4^-, alkali and alkaline earth metal ions
1850	Pb
1250	Cr(III)
625	Zn, In(III), Mn(II), As(III)
375	Ce(IV)
250	Pt(IV)
125	Hg(I), As(V), IO_4^-
75	SO_3^{2-}, U(VI), Co(II), Sn(II), Sb(III), Be(II)
25	Fe(II), Zr(IV), Ti(IV), Pd(II), Ga, W(VI)
12.5	Mo(VI), Ni, Th(IV), Fe(III), tartrate, citrate

* The foreign ions were added up to a maximum of 100 mg l^{-1}.
† Sample centrifuged prior to measuring absorbance.

An alternative absorptiometric procedure can also be proposed in acetate buffer, but the interference effects due to foreign ions are greater than when the procedure is carried out at pH 2. This is mainly because few other metal complexes are completely formed at this low pH and many anionic species are not stable.

Comparison of the influence of foreign ions on the spectrofluorimetric and spectrophotometric methods for the determination of aluminium shows that in the spectrofluorimetric procedure the interferences from metal ions are generally greater than those from inorganic anions, the opposite is true for the spectrophotometric method. Therefore, the field of application is wider when both methods are applied.

Application of the Absorptiometric Method to the Determination of Aluminium in Standard Samples

The absorptiometric method using PSH at pH 2.0 (hydrochloric acid) has been applied satisfactorily to the determination of aluminium in two standard samples, a steel and a Portland cement. The steel sample (BCS No. 458) was treated[17] first with hydrochloric acid (1 + 1 *V*/*V*) and then with hydrogen peroxide; the solution was evaporated to dryness. Finally, the residue was lixified with hydrochloric acid. The iron present was removed by extraction with diethyl ether in hydrochloric acid medium. The Portland cement (BCS No. 372) was dissolved[17] in hydrochloric acid (1 + 1 *V*/*V*), then the solution was evaporated to dryness and the residue lixified with dilute hydrochloric acid. The silica was filtered when hot. Six determinations were carried out for each sample (Table V).

TABLE V

SPECTROPHOTOMETRIC DETERMINATION OF ALUMINIUM IN STANDARD SAMPLES BY THE USE OF THE PSH METHOD AT pH 2.0

Standard sample	Aluminium, % Certified	Aluminium, % Found	Standard deviation,* %
Steel (BCS No. 458)	0.14	0.142†	0.70
Portland cement (BCS No. 372)	5.35‡	5.36‡	0.37

* The results obtained were the means of six separate determinations in both instances.

† Ascorbic acid solution (2 ml of 0.1% *m*/*V*) does not interfere in the determination of 0.5 μg ml^{-1} of aluminium and this solution was added to each aliquot of sample solution prior to adding the PSH reagent solution, in order to prevent interferences from iron(III) and vanadium(V). Under these conditions, 2 μg ml^{-1} of either iron or vanadium do not interfere.

‡ Percentage as aluminium(III) oxide.

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