Determination of Titanium(IV) as an Additive in Organic Matrices by Reversed-Phase High-Performance Liquid Chromatography with 5,5'-Methylenedisalicylohydroxamic Acid

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Abstract

A reversed-phase liquid chromatographic method for the determination of titanium with 5,5'-methylenedisalicylohydroxamic acid (MEDSHA) is described. The titanium(IV) complex is formed in a MeOH–5×10^{-3}M H_2SO_4 medium and injected onto a Polyspher RP^{18} column for chromatography. The mobile phase is H_2SO_4 (5×10^{-3}M):MeOH (4:96, v/v) and contains 1.5×10^{-3}M MEDSHA. The detection limit for titanium by the proposed method is 18 µg/L. Titanium is determined in two different samples with satisfactory accuracy and precision.

Introduction

Although high-performance liquid chromatography (HPLC) was first applied to the separation of organic compounds, it has since become an attractive method for trace metal determination (1). The sample ions are converted to organic metal chelates that can then be separated by HPLC on reversed-phase or normal-phase (previously extracted in an organic solvent) columns. The most popular chelating reagents include diethyldithiocarbamate (DDC) (2) and 4-(2-pyridylazo)resorcinol (PAR) (3), which form complexes that can be detected spectrophotometrically. The reagent is usually added to the mobile phase to prevent decomposition of the complexes. Most of the reported separations have been made on C_{18} columns, and the pH of the mobile phase has had to be maintained between 2 and 9, except if a styrene–divinylbenzene (polymeric) column is used.

Titanium is added as TiO_2 to some foods and pharmaceutical preparations as a dye or as a thickening agent. This work concerns HPLC determination of titanium using a reagent that belongs to the hydroxamic acid group. The best known reagent of this group is N-benzoylphenylhydroxamic acid, which has been used for the HPLC determination of vanadium and molybdenum (4). This compound and most related hydroxamic acids are sparingly soluble in water and form chelates that are

insoluble. However, we synthesized a complexing reagent (5,5'-methylenedisalicylohydroxamic acid [MEDSHA], Figure 1) that forms several complexes with titanium that are soluble in alcohol–water mixtures (5).

This paper describes the investigation of the optimum chromatographic conditions for the determination of titanium with MEDSHA. The influence of several factors, such as the pH of the medium, the concentration of the reagent, and the composition of the mobile phase, was studied. The method has been applied
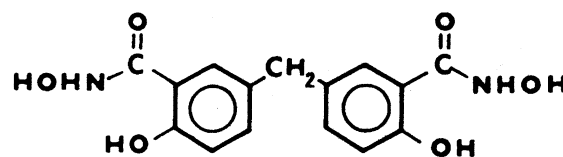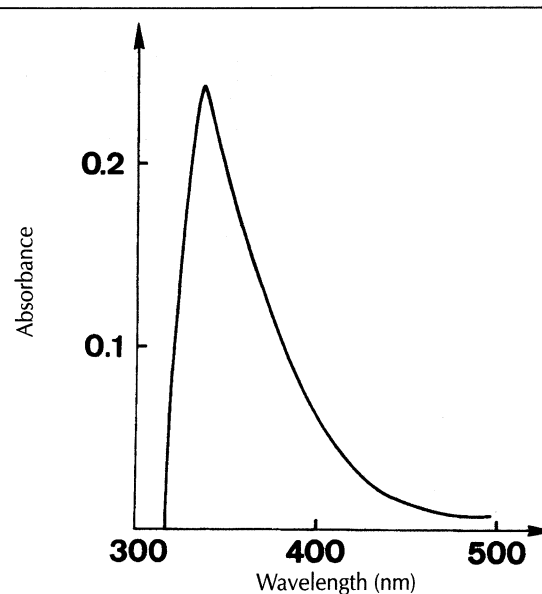

Figure 1. MEDSHA structure.

Figure 2. Absorption spectrum of the Ti(IV)–MEDSHA complex in H_2SO_4 (5×10^{-3}M):MeOH (80:20, v/v). C_{Ti}: 1 µg/mL; C_{MEDSHA}: 1.04×10^{-4}M.

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to the determination of titanium in candy-covered chocolate and hard gelatin capsules; a validation protocol was used to test its reliability.

Experimental

Apparatus

A Hewlett-Packard (Avondale, PA) 1050 series liquid chromatograph equipped with an ultraviolet–visible (UV–vis) variable-wavelength detector, a Rheodyne (Cotati, CA) 7125 loop injector with a 20-μL sample loop, and a 3396-A integrator was used. A Polyspher RP[18] (Merck, Darmstadt, Germany) (150 × 4.6-mm i.d.) column linked to a Polyspher guard column (20 × 4.6-mm i.d.) was used for all separations. A Spectronic 2000 (Bausch & Lomb, Rochester, NY) spectrophotometer with 1-cm quartz cells and a Crison Digit-501 pH-meter was also used.

Reagents

Standard titanium(IV) solution

A stock solution of titanium (400 μg/mL^{-1}) was prepared by heating 0.200 g of pure titanium(IV) oxide (Merck) in a Kjeldahl

Figure 3. Effect of 5 × 10^{-3}M H$_2$SO$_4$ percentage in the mobile phase on the peak height, h (arbitrary units). Mobile phase: H$_2$SO$_4$ (5 × 10^{-3}M):MeOH containing 5.5 × 10^{-5}M of MEDSHA. Flow rate: 0.4 mL/min. Detection wavelength: 340 nm.

Figure 4. Effect of MEDSHA concentration in the mobile phase on the peak height, h (arbitrary units). Mobile phase: H$_2$SO$_4$ (5 × 10^{-3}M):MeOH (4:96, v/v). MEDSHA concentration was variable. Other operating conditions were as in Figure 3.

flask with 8.000 g of ammonium sulphate (Merck) and 25 mL of concentrated sulfuric acid. After cooling, the resulting solution was transferred into a 250-mL calibrated flask and diluted to the mark with a 5% (v/v) sulfuric acid solution. Finally, it was standardized gravimetrically (6). Working solutions were prepared by appropriate dilution.

MEDSHA

The synthesis of MEDSHA was reported previously (7). A 10^{-2}M solution was prepared by dissolving 0.318 g of MEDSHA in 100 mL of methanol (HPLC grade).

All solutions used to prepare the mobile phase were filtered through a 0.45-μm membrane filter and degassed before use. The aqueous solutions were prepared with ultrapure water that was obtained by filtering distilled water through a Millipore (Bedford, MA) Milli-Q RO15 purifier. All other reagents were of analytical reagent grade.

Procedure

A 5 × 10^{-3}M sample solution (0.2 mL) in H$_2$SO$_4$ containing less than 1.5 μg of titanium(IV) was transferred into a 10-mL glass vial, and 4.8 mL of a 10^{-2}M MEDSHA solution in methanol was added. The resulting solution was homogenized and filtered through a disposable syringe filter (0.2 μm), and a 20-μL aliquot was injected onto the chromatographic system. After the polymeric column was equilibrated at 25°C with a mobile phase composed of H$_2$SO$_4$ (5 × 10^{-3}M):methanol (4:96, v/v) containing 1.5 × 10^{-3}M of MEDSHA, the complex was eluted at a flow rate of 0.3 mL/min and detected at 360 nm. The peak heights were measured for quantitative calculations.

Results and Discussion

Spectrophotometric studies

Titanium(IV) reacts with MEDSHA in H$_2$SO$_4$ or hydrochloric acid medium to form several complexes with different stoichiometry (5). These complexes are slightly soluble in an aqueous medium but fairly extractable in organic solvents.

Figure 2 shows the absorbance spectrum that corresponds to a solution of 1 μg/mL of titanium(IV) in H$_2$SO$_4$ (5 × 10^{-3}M):methanol (20:80, v/v) containing 1.04 × 10^{-4}M of MEDSHA. There is an absorbance maximum at 340 nm which does not change when the MeOH percentage is modified, although this percentage must be higher than 70% to avoid forming a precipitate.

Chromatographic studies

The chromatographic behavior of the complex formed in aqueous methanol medium depends on the percentage of the 5 × 10^{-3}M H$_2$SO$_4$ solution in the mobile phase, and, as shown in Figure 3, the maximum peak height was obtained for 4% (v/v).

The addition of MEDSHA to the eluent was necessary in order to prevent the decomposition of the complex. Figure 4 shows that
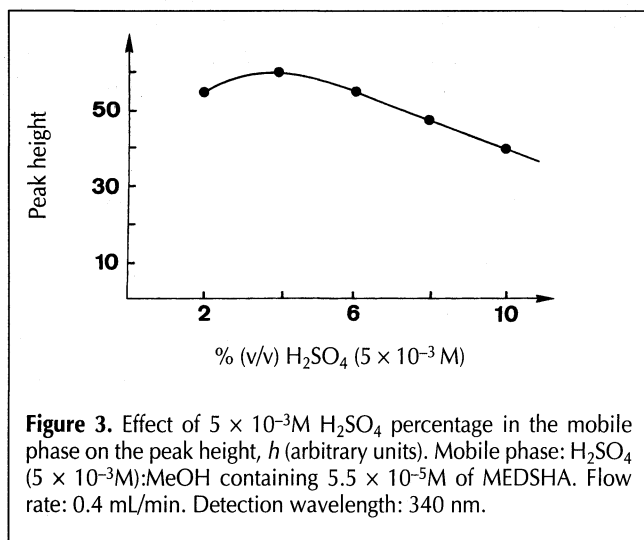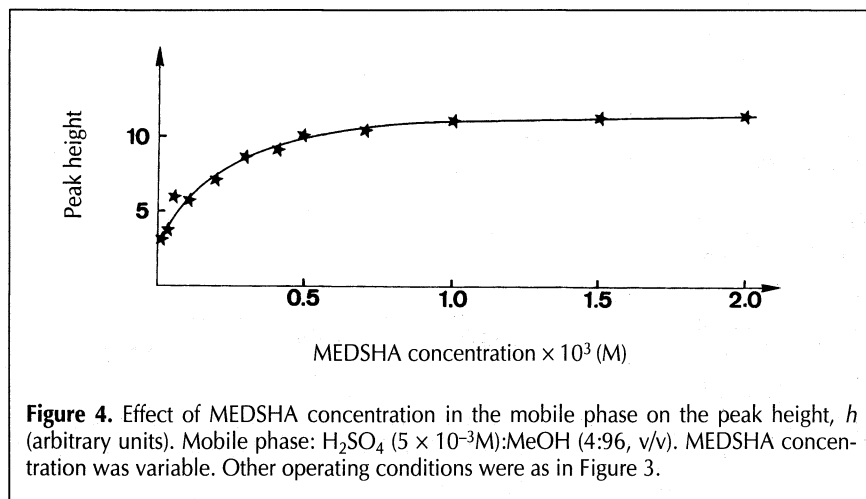
peak height remained constant from a MEDSHA concentration in mobile phase above $1 \times 10^{-3}M$.

The graph of H versus flow rate, where H is the height equivalent to a theoretical plate, indicates that the maximum efficiency (H minimum) was achieved with a flow rate of 0.3 mL/min.

On the other hand, we observed that retention time (t_R) was not affected by MEDSHA concentration in the injected sample, and the maximum peak height was achieved for a concentration above $6.5 \times 10^{-3}M$.

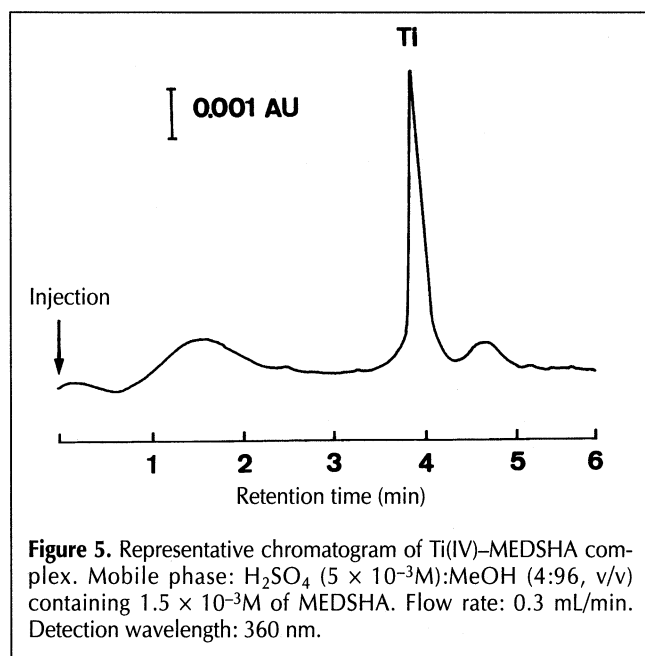We selected a wavelength of 360 nm instead of 340 nm (the absorbance maximum) for the detection of the complex

Figure 5. Representative chromatogram of Ti(IV)–MEDSHA complex. Mobile phase: H_2SO_4 ($5 \times 10^{-3}M$):MeOH (4:96, v/v) containing $1.5 \times 10^{-3}M$ of MEDSHA. Flow rate: 0.3 mL/min. Detection wavelength: 360 nm.

because MEDSHA does not absorb significantly at 360 nm and less baseline fluctuations are observed. Figure 5 shows a typical chromatogram in which the retention time for titanium(IV)–MEDSHA is 3.8 min. This peak assignment was made by injecting a blank sample (without metal ions) and solutions with variable amounts of titanium.

Calibration graph

The data were collected by well-designed calibration experiments (using triplicate responses at each concentration and randomized arrangements). Tests for nonlinearity (for a univariate linear calibration) were based on the analysis of residuals variance from a regression into parts due to "lack of fit" and "pure error"; under these requirements, the dynamic linear range was checked over a 0–300-µg/L range. The repeatability of the method was 2.56%, expressed as the relative standard deviation (RSD) for seven replicate analyses of solutions containing 200 µg/L of titanium.

The detection limit was evaluated in two different ways. Using the equation proposed by Snyder et al. (8) and a signal-to-noise ratio of 2, we obtained a value for the minimum detectable mass of 0.25 ng of titanium, which corresponded to a concentration of 12 µg/L. In another method, we applied a hypothesis test such as the one used by Herrero et al. (9). The null hypothesis (H_0) stated that no analyte was present, so $C_{Ti} = 0$. The alternative hypothesis (H_A) stated that some analyte was present, so $C_{Ti} > 0$. The significance level (α) was the probability (pr) of rejecting H_0 when H_0 is true ($\alpha = pr$ [false positive]). The probability of a type two error (β) was the probability of accepting H_0 when H_0 is false ($\beta = pr$ [false negative]). This procedure involves the assumption of true and false positive values. In our case, their values were assumed as $\alpha = \beta = 0.05$ (according to recommendations of the International Union of Pure and Applied Chemistry). The detection limit found was 18 µg/L.

Effect of foreign ions

A study of interference effects was carried out in two different ways. In one method, cations and anions that are usually present in organic matrices such as foods and pharmaceutical preparations were tested in amounts of up to 5000 µg with 5 µg of titanium. Ions were considered to be noninterfering if they produced an error in the peak height less than 5%. There was no interference from NO_3^-, ClO_4^-, PO_4^{3-}, Li(I), K(I), Na(I), or Mg(II) in at least a 1000-fold mass ratio to titanium(IV); none from Cl^- or Ca(II) at a 750-fold ratio; and none from Cu(II) at a 300-fold ratio.

We also tested those ions that, apart from titanium, had the most sensitive reactions with MEDSHA (i.e., Fe[III], V[V], and Mo[VI]) (7). These species were only studied in amounts up to 15 µg with 5 µg of analyte because, in the analyzed samples, titanium was used as an additive in such a concentra-

Table I. Statistics for the Determination of Titanium in Two Samples*

Sample	Results	AC	SC	YC
Candy-covered chocolate	Slope	153212	143329	108800
	n	8	12	3
	s	806.042	1184.66	390.619

There is no constant error bias (YB). There is no significant difference between the AC and SC slopes. Accuracy test: $t_{cal} = 1.52 < t_{crit} = 2.110$ ($\alpha = 0.05$, df = 17). There is no significant difference.

Sample	Results	AC	SC	YC
Hard gelatin capsules	Slope	158484	143329	632030
	n	8	12	4
	s	1374.97	1184.66	1924.59

There is no constant error bias (YB). There is no significant difference between the AC and SC slopes. Accuracy test: $t_{cal} = 0.255 < t_{crit} = 2.110$ ($\alpha = 0.05$, df = 17). There is no significant difference.

* n = number of measurements in a least-squares fitting set of data, s = standard deviation of the regression-fitted line, YB = Youden blank, df = degree of freedom. t_{cal} and t_{crit} are the calculated and critical values, respectively, of the statistical parameters in the accuracy test.

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Table II. Determination of Titanium in Two Samples

Sample	Proposed method (μg/g)	Proposed method RSD (%)*	Gravimetric method (μg/g)
Candy-covered chocolate	3020	10.22 (n = 4)	2997
Hard gelatin capsules	9825	3.47 (n = 4)	10004

* n = number of replicates used.

matrix effect and that a standard calibration could be used for the determination of this element. However, the accuracy test indicated that the method was accurate, which was confirmed by the reasonably good agreement between the results obtained by the proposed method and those obtained by a gravimetric method (Table II). Finally, the RSD values indicated a satisfactory precision.

tion that the ratio (interferent:titanium) was smaller than 3. At this level, these ions did not interfere.

Sample analysis

To check the reliability of the proposed method, it was applied to the determination of titanium in two samples (candy-covered chocolate and hard gelatin capsules).

In both cases, a measured quantity of sample was put into a beaker and treated with 25 mL of concentrated nitric acid in a sand bath; more acid was added as necessary until foaming ceased. When the organic matter was completely destroyed, 25 mL of perchloric acid (70%, m/m) was added, and the solution was evaporated to dryness twice. The residue was dissolved with 25–30 g of $(NH_4)_2SO_4$ and 100 mL of concentrated H_2SO_4; the resulting solution was concentrated into 25 mL and then diluted to volume (250 mL) with water. Finally, the obtained sample solutions were analyzed according to the described procedure.

Because the analyzed samples were not standard reference materials, a validation study was carried out following the statistic protocol proposed by Cuadros et al. (10), which was based on the analysis of the slope, intercept, and standard deviation of regression obtained on three calibration procedures: standard calibration (SC), standard-additions calibration (AC), and Youden calibration (YC).

First, using the t-test, slopes of the lines obtained by SC and AC calibrations were compared. If the difference between both slopes was not significant, the standard-additions calibration was used to validate the method. Next, it was tested whether the intercepts of the SC and YC regression lines differed significantly from each other, which would have indicated that a systematic error due to matrix components was present. In that case, the true blank of the sample, known as the Youden blank, would have been determined as the difference between both intercepts.

Finally, the accuracy of the results was tested by comparing the analyte contents obtained from the SC and AC graphs; a t-test was used for the comparison of the two means. If the difference was not significant, it was concluded that the method was accurate.

Table I contains the statistics for each analyzed sample. In all cases, there was no significant difference between the slopes of the AC and SC graphs, which indicated that there was no

Acknowledgments

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