An improved method for the assay of TiO_2 purity and its determination in drugs using flame atomic absorption spectrometry.

Patricia Mattiazzi[a], Denise Bohrer[a]*, Carine Viana[a], Paulo Cícero do Nascimento[b], Leandro Machado de Carvalho[a,b]

[a] Federal University of Santa Maria, Postgraduate program in Pharmaceutical Sciences, Av. Roraima 1000, 97105-900 Santa Maria, Brazil
[b] Federal University of Santa Maria, Department of Chemistry, Av. Roraima 1000, 97105-900 Santa Maria, Brazil

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ABSTRACT

A quantitative method for determining TiO_2 was developed. The proposed method is based on analysis by flame atomic absorption spectrometry (FAAS) and presents advantages over the current pharmacopeial monographs for TiO_2 assay by dispensing with the use of mercury for sample pretreatment. Solid drugs (tablets and capsules) and TiO_2 were decomposed by a mixture of sulfuric acid/ammonium sulfate, diluted, and analyzed by FAAS. The method was validated according to the USP <1225>Validation of Compendia Procedure and the USP <852> Atomic Absorption Spectroscopy monograph. All parameters were in accordance with the compendium criteria. Analysis of a TiO_2 powder standard sample, which showed purity (99.5%) within the established limits, confirms the adequacy of the proposed method. Six samples were analyzed using the proposed method and consisted of three TiO_2 samples from different suppliers and three different drugs, Cozaar® (Merck), Zocor® (Merck) tablets, and Peprazol® (Libbs) capsules, which were expected to contain TiO_2 as an ingredient. The purity of the assayed TiO_2 samples ranged from 79.2% to 90.5%, and the amount of TiO_2 in the drugs ranged from 2.8 to 3.8 mg/g.

KEY WORDS: Titanium dioxide, excipients, drugs, atomic absorption spectrometry

INTRODUCTION

Titanium dioxide is largely used as a coloring agent in drugs, cosmetics, and foods. Due to its properties, TiO_2 imparts a whiteness to cosmetics and personal care products, which increases opacity and reduces the transparency. This oxide also scatters light, which helps to protect products from deterioration. Additionally, TiO_2 is an important ingredient in sunscreen products (1).

Titanium dioxide, defined as a colorant in CRF Title 21 §73.575 in the US and E171 in Europe, is also widely used as an additive in the food industry to whiten or impart opacity to products. It is commonly found in sweets, chocolate products, biscuits, chewing gum and food supplements (2, 3). For foods that are sensitive to UV light, titanium dioxide is used for safety purposes to prevent spoilage and increase product shelf life (4).

Titanium dioxide is effective at blocking ultraviolet-B and short-wave ultraviolet-A rays, which makes it one of the most effective sunscreen products on the market (5).

In the pharmaceutical industry, it is used as a colorant for ointments, capsules, tablets, lotions, creams, and

*Corresponding address: Postgraduate program in Pharmaceutical Sciences, Federal University of Santa Maria, Av. Roraima, 1000, Santa Maria, RS 97105-900, Brazil, Tel/Fax: +55 55 32208870, e-mail: ndenise@quimica.ufsm.br

various dosage forms. Titanium dioxide as a tablet coating protects ingredients from UV light damage. The titanium dioxide layer also serves as a white base coat, which imparts color (6).

The FDA has assessed the safety of TiO_2 for use in foods, drugs, and cosmetics and has issued regulations approving this ingredient. The amount of food-grade titanium dioxide that is used is small, the FDA has set a limit of 1 percent titanium dioxide for food. Thus, there is currently no indication of a health risk at this level of exposure through its consumption (7).

In the current United States Pharmacopeia monographs for TiO_2 use in food (FFC 9) and drugs, the purity assay is carried out by titration where, after being dissolved in sulfuric acid, titanium is reduced by a zinc amalgam and titrated with potassium permanganate. In these monographs, the method described uses the Jones reductor (Zn-Hg alloy) for Ti reduction. A waste of ca. 0.15 g Hg is produced in each assay (8, 9).

In this study, a method for Ti determination in TiO_2 was developed. The proposed method is based on the use of flame atomic absorption spectrometry (FAAS) for quantification. By replacing the titration procedure with FAAS, the use and waste of mercury is avoided. After dissolution, no reduction is necessary, that is, Ti is measured directly in the solution. The proposed method is less time-consuming and more environmentally friendly than the USP compendial method.

MATERIAL AND METHODS

Parameter optimization for Ti measurement by flame atomic absorption spectrometry

All measurements were performed using an Analytik Jena AG (Jena, Germany) model novAA 300 atomic absorption spectrometer. The instrumental parameters are described in Table 1. Parameters, such as gas flow and burn height, were optimized since they are subject to variations. A titanium hollow cathode lamp (Photron, Australia) was used. A deuterium lamp was used for the measurement of the background signal (Analytik Jena, Jena, Germany). Analyzes were performed using an injection module SFS 6 (Analytik Jena).

Table 1 Flame and equipment parameters for Ti determination

SPECTROMETER PARAMETERS		ATOMIZER PARAMETERS	
Wavelength (nm)	364.3	**Fuel**	Acetylene
Lamp current (mA)	7.0	**Support gas (oxidant)**	Nitrous oxide
Slit width (mn)	0.2	**Gas flow (L/h)**	250
Background correction	Yes	**Burn height (mm)**	8

Reagents

All reagents were of analytical grade and prepared with distilled and deionized water that was further purified using a Milli-Q high purity water device (electrical resistivity of 18.0 MΩ cm) (Millipore, Bedford, USA). Laboratory glassware was stored overnight in 10% (v/v) HNO_3 in ethanol solution, washed with water shortly before use, and dried on a clean bench. The concentrated nitric acid used in this study was supplied by Merck.

A stock Ti standard solution (1000 mg/L) was obtained from the National Institute of Standards and Technology (NIST, USA) and diluted as necessary in order to reach working standard concentrations. Working solutions were prepared in 100 mL volumetric flasks by dilution of the stock solution. Additionally, because Ti ionization can possibly occur during the atomization process, KCl was tested as an ionization buffer (10). For these experiments, working solutions were prepared in a 0.1% KCl solution.

Methodology validation

Validation of the proposed method was performed according to the USP <1225> Validation of Compendial Procedure (11) and the USP <852> Atomic Absorption Spectroscopy monograph (12). Since the procedure is for quantitation of a major component (Category I), the following parameters were assessed: Range, Linearity, Precision, Accuracy, and Specificity. The precision of the procedure was determined through repeatability (intraday precision)

and intermediate precision (inter-day precision). Repeatability was performed with six individual samples of Simvastatin (Zocor, Merck) from the same batch, with the addition of 10 mg/L standard Ti solution, and analyzed on the same day. To determine inter-day precision, the repeatability test was performed on three different days. Measurements were carried out in triplicate. These results totaled 12 independent determinations. Precision was expressed by the relative standard deviations (RSD) of the measurements. Accuracy was determined by analyzing a TiO_2 standard (Sigma Aldrich, product number 14027). Accuracy was also validated by evaluating Ti recovery of Simvastatin spiked samples at three levels of Ti concentration: 15, 30, and 40 mg/L. Spiking was carried out prior to the sample digestion process. Specificity was verified by spiking a Ti sample with appropriate levels of metallic elements. Individual standard solutions (1000 mg/L) (from NIST, USA) of Pb, Hg, Cd, As, Pd, Cr, Mo, Ni, V, and Cu were mixed in a single working solution containing 0.1% (v/v) distilled HNO_3 in order to obtain a 10 mg/L concentration of each element.

TiO_2 assay

In order to decompose the samples, 100 mg of TiO_2 powder was weighed and about 0.2 g of ammonium sulfate added. The mass was transferred to a Kjeldahl flask, 4 mL of concentrated H_2SO_4 (95%) was added and the flask was placed in a heated block (100 ºC). One milliliter of 30% H_2O_2 was added every time the solution turned brown or black. The addition of H_2O_2 was carried out until a clear mixture was obtained. Subsequently, 2.0 mL of HCl was added and the mixture was left for another 24 hours in the heated block. At the end of the digestion, the samples were diluted with ultrapure water to 50 mL in volumetric flasks. Control samples were prepared in a Kjeldahl flask containing all reagents, without the addition of the sample. For tablet and capsule analysis, an entire tablet or capsule was weighed, 0.2 g ammonium sulfate was added, and transferred to the Kjeldahl flask. After the addition of 4 mL of concentrated H_2SO_4, the procedure was followed as described for the TiO_2 samples.

Application using real samples

A total of six samples were assayed: pharmaceutical grade TiO_2 from three different suppliers (acquired in magistral pharmacies with certificate of analysis for TiO_2 content), and three different medications used for common disorders: Lozartan (Cozaar®, Merck), a drug for treating high blood pressure, Simvastatin (Zocor®, Merck), which is a lipid-lowering medication, and Omeprazole (Peprazol®, Libbs), a proton pump inhibitor. All samples were analyzed in triplicate.

RESULTS

Flame AAS measurement of Ti

The stability of Ti solutions is critical. It is necessary to acidify the solutions to reach a pH 1.5, to prevent hydroxide precipitation. It was possible to observe that, after 24 hours, quantitative Ti loss occurred when the pH of the solutions exceeded 3.5 (10). For this reason, samples were tested with, and without, the addition of HCl. Higher absorbance values were obtained in the presence of HCl when compared to the same samples to which no acid was added. Therefore, HCl was added to the samples at the end of the decomposition procedure and analysis carried out immediately.

The measurement by FAAS of certain metals may require the presence of an ionization buffer (10) for the suppression of analyte ionization, mainly if the measurement uses an acetylene-nitrous oxide flame. The addition of 0.1% KCl as an ionization buffer is recommended to determine Ti by FAAS (10). However, there was no increase in absorbance when KCl was added to the samples. Therefore, the samples were only acidified with HCl.

Method validation

Although Chapter <1225> did not require detection and quantification limits (LOD and LOQ) for this type of sample, the limits were determined. The LOD and LOQ obtained by the blank method are presented in

Table 2. Eleven blank samples were analyzed and the standard deviation (SD) of these results applied to LOD = 3 x SD/s and LOQ = 10 x SD/s, where s is the slope of the calibration curve. As the samples were all solids, limits were also calculated in mg/g while considering the use of 100 mg mass of sample.

Linearity and range

Analytical curves were constructed by evaluating the relationship between absorbance (peak area) and concentration. The linear range tested was between 0 and 50 mg/L. Linearity was checked by calculating the regression equation. In all instances, a linear fit was found to be adequate for the purpose, with an r>0.995 (USP <852>) (Table 2).

Precision

Based on the results of linearity and range, the method precision was determined by assaying individual samples of Simvastatin from the same batch, with the addition of 10 mg/L standard Ti solution. Analyte concentration was measured in triplicate and the relative standard deviation (RSD) used for defining the method precision. The acceptance criterion (USP <852>) was RSD <5% (n = 6) for repeatability and RSD <8% (n = 12) for intermediate precision, and the results found were 4.1% and 7.7%, respectively.

Accuracy

Accuracy was tested by determining the TiO_2 content of a sample furnished by Sigma-Aldrich (Product number 14027), which met the purity specifications of BP 2018 and USP 41 - NF 36 (Table 3). The accuracy of the method was further confirmed by analyzing nine samples of the same lot of Simvastatin spiked with15, 30, and 40 mg/L of Ti standard solution. The values found, which ranged from 96.8-103.0%, are within the allowed limits since the acceptance criterion is 95% -105%.

Table 3 Ti recovery (mean ± SD, n=3) in spiked samples and the determination of TiO_2 in a standard material

SAMPLE	SPIKE (mg/L)	FOUND (mg/L ± SD)	RECOVERY (%)
Simvastatin	-	8.9 ± 1.2	-
Simvastatin	15	23.5 ± 0.8	97.3
Simvastatin	30	39.8 ± 0.5	103.0
Simvastatin	40	47.6 ± 0.3	96.8
TiO_2 STANDARD		PRODUCT SPECIFICATION (%)	PURITY FOUND (%)
		99.0 – 100.5	99.5 ± 0.9

Specificity

As AAS techniques are based on radiation absorption of a wavelength, which is characteristic to an element, emitted by an HLC lamp of this element, AAS specificity is very high. Nevertheless, this was verified by spiking a Ti sample with the appropriate levels of metallic elements. The elements selected were those listed in the USP monograph on elemental impurities for drug products, USP <232> (13). The results showed that the assay for Ti was unaffected by the presence of other metallic elements.

Results of real sample analysis

The sample preparation procedure used for medicinal compounds depends on the nature of the substances that will be investigated. The wet decomposition procedure, performed in a Kjeldahl flask with nitric acid, under pressure or microwave assisted, is among one of several techniques used. In this work, sample decomposition by acid digestion using the

Table 2 Calibration curve and regression parameters for Ti determination

RANGE (mg/L)	REGRESSION EQUATION (y=a+bx)/(1+cx)	R^2	LOD (mg/L)	LOQ (mg/L)	LOD* (mg/g)	LOQ* (mg/g)
0-50	y=(0.0134+0.0014x)/(1+0.005909x)	0.9951	2.6	8.7	1.3	4.4

*based on a 100 mg sample

combination of nitric acid and hydrogen peroxide was tested, although samples were not completely digested. A white precipitate was observed at the bottom of the flask in all samples. It is believed that the part of the sample that did not decompose may be TiO_2, which is not soluble in nitric acid. Nevertheless, this precipitate was soluble in a mixture of sulfuric acid and ammonium sulfate as previously described in the USP monograph for TiO_2 assay (8). Therefore, this mixture was tested for the decomposition of the whole sample (tablets and capsules), which resulted in all of them being completely decomposed.

The TiO_2 samples were weighed, transferred to the Kjeldahl flask, and decomposed. For tablet and capsule analysis, the usual practice of combining and grinding a number of units and then weighing a suitable mass for the analysis was not adopted. Because TiO_2 is mainly used for coating unit dose medications, in addition to determining the amount of TiO_2 per unit, the result shows the deviations in TiO_2 mass between the different units of the same lot of the drug. Therefore, we opted to analyze single units and present the results in this manner.

The results of TiO_2 sample analysis, as well as the TiO_2 content of the analyzed drugs are presented in Table 4. Samples of TiO_2 presented purity within the limits in the certificate (or even higher), although these values did not comply with the purity level established by the USP, which is between 99.0% and 100.5%. The amount of TiO_2 found in Lozartan (Cozaar), Simvastatin (Zocor), and Omeprazole (Peprazole) is comparable to the amount found by other authors in other pharmaceutical products (3, 12). The RSDs between 0.2% and 0.5% show that TiO_2 is reproducibly dispensed on the units of the same lot of the drugs.

DISCUSSION

Atomic spectrometric techniques, such as atomic emission spectrometry with inductively coupled argon plasma and flame atomization atomic absorption spectrometry (FAAS), have been used to determine titanium in several matrices (3, 14-17). Nevertheless, the analysis of TiO_2 in pharmaceutical products using one of these techniques is not frequently found in the literature.

Determination by ICP-AES or FAAS have a number of advantages, including rapidity, easy correction of matrix effects and spectral interference, wide linear range, excellent reproducibility and improved versatility. Comparing both techniques, however, shows that the FAAS is less time consuming, easier to perform and less expensive. As the amount of Ti in pharmaceutical products is reasonably high, its evaluation by FAAS is a worthwhile option.

In comparison with the current pharmacopeial method, the proposed method is less time consuming, faster and easier to perform, and friendlier to the environment. If all samples included in this study (standards, TiO_2 and drugs, including replicates) were analyzed following the

Table 4 Concentration levels of TiO_2 in different commercial powder samples and three medicines which contain TiO2 as an inactive ingredient

SAMPLE	SUPPLIER	GRADE	COUNTRY	LOT	PHARMACEUTICAL FORM	PRODUCT SPECIFICATION (%)	Ti (g/kg ± RSD)	TiO_2 (g/kg)	PURITY (%)
TiO_2	Merck	Food*	Germany	K2032211	Powder	74.5 – 81.5	474.9 ± 1.2	791.8	79.2
TiO_2	Eusolex T	Food*	Brazil	K2025211	Powder	74.5 – 81.5	504.9 ± 1.3	841.8	84.2
TiO_2	Nikkol	Food*	Japan	0225	Powder	83.0 – 89.0	542.9 ± 1.6	905.2	90.5
Lozartan	Merck	-	Brazil	OE214	Tablet	–	2.3 ± 0.5	3.8	–
Simvastatin	Merck	-	United Kingdom	049852	Tablet	–	1.7 ± 0.2	2.8	–
Omeprazole	Libbs	-	Brazil	14K0674	Capsule	–	2.0 ± 0.2	3.3	–

*Meets pharmacopeial compendia specifications

pharmacopeial method, approximately 4 g of mercury would have been used and wasted.

CONCLUSIONS

The method proposed for the analysis of food and pharmaceutical grade TiO_2 was performed by flame atomic absorption spectrometry. All parameters of validation were performed according to USP <1225> Validation of Compendia Procedure and were within the range defined by the monograph. This method could satisfactorily replace the current pharmacopoeial method where Ti is determined by titration following a reduction step. Since zinc amalgam is used for Ti reduction, a waste of ca. 0.15 g Hg waste is produced in each assay. By replacing the titration procedure with FAAS, the use and waste of mercury is avoided, thus making the proposed method more attractive, time efficient and environmentally friendly.

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