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Development of a Standardized Procedure for Cleaning Glass Apparatus in Analytical Laboratories

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ABSTRACT

Adequate cleaning of analytical glassware is an essential procedure that determines the reliability of assays and tests carried out in laboratories, keeping the glassware free of interference from residues left by previous tests. In the present paper, standard cleaning procedures are proposed for laboratory glassware, which were tested on cyanocobalamin as a marker contaminant. A spectrophotometric method was used for quantitative determination of both residual marker and cleaning product. Beakers, volumetric flasks and volumetric pipettes were successfully cleaned with a 2% detergent solution, with several rinses in water. Vials were cleaned adequately in an ultrasonic bath. These procedures utilize non-toxic and cheap reagents, factors of paramount importance for their application in routine laboratory analysis.
Keywords: Validation Studies. Detergents. Laboratory Techniques and Procedures. Glassware Cleaning.

The cleaning of analytical glassware is an essential procedure for the successful carrying out of laboratory assays and tests without interference from the residues of previous analyses. It is necessary to assure the quality of future products handled in the equipment, to prevent cross-contamination and as a Good Laboratory Practices (GLP) requirement. When the laboratory uses glass equipment for optical measurements, even greater care is needed, because minute residues of products may interfere with the final outcome of the analysis that follows, when the same glassware is used without a standardized and proved cleaning method (USP, 2009).

The cleaning validation falls within the scope of the major regulatory agencies worldwide as a requirement for industries and laboratories. However, publications concerned with this topic refer, in general, to validation of the cleaning of equipment and surfaces, not of glassware (Jenkis & Vanderwielen, 1994; Klinkenberg et al., 2003; Coutinho et al., 2009; Peres, 2009). The USA *Food and Drug Administration* (FDA, 1993) and the Brazilian *National Agency of Sanitary Surveillance* (Brasil, 2006) also direct their guidelines to the cleaning of equipment. Similarly, the *United States Pharmacopoeia* discusses the need for validation of glassware cleaning processes, but cites no methods or procedures to be adopted (USP, 2009).

To ensure the utmost cleanliness of the glassware, the residues from previous analyses and of the cleaning material itself must be carefully quantitated. Cleaning agents can leave traces of their components on the glass surface, despite their ability to eliminate contaminants. For example, it is well known that detergent surfactants require many rinses with water after the cleaning procedure for the glass to be suitable for use. It follows that cleaning agents should be carefully chosen and the methods of washing should also be tested by validated methods for the detection of their residues (Zayas et al., 2006; USP, 2009).

The most traditional procedures used to clean glassware employ a saturated solution of potassium (or sodium) dichromate in concentrated sulfuric acid (called "chromic acid" or "sulfochromic solution"), highly alkaline sodium hydroxide solution (Health Sciences Authority, 2008) or nitric acid. The sulfochromic mixtures contain hexavalent chromium, considered one of the 129 most critical pollutants by the U.S. Environmental Protection Agency. These products are powerful reagents that can cause serious environmental problems when discarded and they are also expensive for analytical laboratories with large daily volumes of analytical solutions (Dias & Satte, 2003).

Other strategies have been developed recently, such as the cool hydrogen flame that removes particles from the glass surface (Liang et al., 2009) and self-cleaning glass (2010) with super-hydrophobic or photocatalytic coatings, the latter generating reactive species on the surface that degrade organic matter deposited on the glass. The main limitation of these approaches is the large initial investment required.

In light of these considerations, the aim of this study was to develop a standard cleaning method that does not utilize strongly acidic or alkaline chemicals, to provide clean glassware that can be safely used in routine laboratory tests.

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All reagents used were analytical reagent grade and purchased from Vetec (Rio de Janeiro, Brazil), except for the analytical grade cyanocobalamin (marker contaminant), supplied by Deg (São Paulo, Brazil). The cyanocobalamin standard was purchased from United States Pharmacopeia (Rockville, USA). Prolab Neutro detergent was supplied by Labnews (São Paulo, Brazil). Ultra-pure water was produced in an aquaMAX™ *Ultra* 370 water purifier from Young Lin Instrument Co Ltd (Anyang, Korea). All glassware, except for vials and syringes, was previously calibrated to the Brazilian Calibration Network standard (RBC – *Rede Brasileira de Calibração*). A Shimadzu AY220 analytical balance (Kyoto, Japan) and a Varian Cary 50 Probe UV-Vis spectrophotometer (Mulgrave, Australia) with a reading probe, properly calibrated and qualified, were used for analysis. An Ultron Cristófoli model 2 ultrasound bath (Campo Mourão, Brazil) was used in a cleaning procedure.

Several procedures were tested to find out which were best suited to the purposes of the study. Cleaning agents tested were: water, detergent and sodium hydroxide solution. A 1.000 mg mL^{-1} cyanocobalamin aqueous solution was transferred to three units each of the following glassware: 50 mL beaker, 10 mL volumetric flask, 5 mL volumetric pipette and vials and syringes used for injection into HPLC. The solution was left in contact with the glass apparatus for 30 min, to impregnate it with the contaminant. The solution was then disposed of and the various washing procedures described below were carried out. After that, the glass was dried in air at room temperature and then filled with water and shaken for two minutes to dissolve residual contaminant. The contents of syringes and vials, whose volume and internal diameter are small, were transferred to test tubes before the readings. The absorbance of the final aqueous solution was read in the spectrophotometer at 361 nm, to measure residual cyanocobalamin, with water as a blank, as recommended in the British Pharmacopoeia (2010). Cyanocobalamin was chosen as cleaning test residue because of its high molar absorptivity and successful use in a previous study (Negrão et al., 2007).

Cleaning procedures:

Detergent: Volumetric pipettes, beakers and flasks were washed with 2% detergent (soaking of glassware in detergent, followed by brushing), followed by 10 rinses with tap water, three with ultra-pure water and one with 77 °GL ethanol (i.e. 77% by volume in Europe and USA).

Water and ethanol: For vials, three groups were created, each one with three items (Group 1: 2 rinses with 77 ºGL ethanol, 5 with ultra-pure water and another rinse with 77 ºGL ethanol; Group 2: two rinses with 77 ºGL ethanol, 10 rinses with ultra-pure water and finally another rinse with 77 °GL ethanol; Group 3: 2 rinses with 77 ºGL ethanol, 15 rinses with ultra-pure water and finally another rinse with 77 °GL ethanol). For syringes, two groups were created, with three items each (Group 1: 2 rinses with 77 ºGL ethanol, 10 with ultra-pure water and one with 77 º GL ethanol; Group 2: 2 rinses with 77 ºGL ethanol, 5 with ultra-pure water and one more with 77 ° GL ethanol).

NaOH (1N): After the impregnation with cyanocobalamin solution, some vials were initially cleaned with 2 rinses with 77 ºGL ethanol and 5 rinses with ultra-pure water. Next, they were left to soak in 1N NaOH for 15 hours (overnight). Finally, they were rinsed 3 times with ultra-pure water and 77 °GL ethanol.

Ultrasonic bath: Vials and syringes, after impregnation with cyanocobalamin solution, were precleaned by rinsing them once with 77 ºGL ethanol and then once with ultra-pure water. Subsequently, they were sonicated for 15 minutes. They were then divided into two groups. One group was rinsed 5 times with ultra-pure water and once with 77 ºGL ethanol and the second group 10 times with ultra-pure water and once with 77 ºGL ethanol.

The glass apparatus cleaned with detergent was tested to check whether the latter leaves residues after the wash. After this cleaning procedure, the items of glass were filled with water and stirred for two minutes. This rinsing water was tested for residual detergent, by the method described in *Standard Methods for the Examination of Water & Wastewater* (Eaton et al., 2005).

The linearity of the quantitative methods was tested on an analytical curve of the absorbance versus the concentration of the analyte (x, mg mL^{-1}). The calibration curves were linear for cyanocobalamin (slope = 0.0298; slope standard deviation = 0.005; intercept = 0.004; intercept standard deviation = 0.0006; n = 3) and for sodium lauryl ether sulfate (SLES), the active component of the detergent (slope = 0.1983; slope standard deviation = 0.003; intercept = 0.031; intercept standard deviation = 0.0298; n = 3), with correlation coefficients $r^2 > 0.99$ (r^2 = 0.9915 for cyanocobalamin over the range of 0.02–0.5 mg mL^{-1} and r^2 = 0.9954 for SLES over the range of 1.25–3.75 mg mL^{-1}). Data for each concentration level (triplicate) were treated statistically by analysis of variance (ANOVA), which returned values $F_{calculated} < F_{critical}$ (F = 0.91; $F_{critical}$ = 4.83 for cyanocobalamin and F = 0.16; $F_{critical}$ = 4.83 for SLES), thus verifying the linear model and the validity of the linear regression for both cyanocobalamin and SLES. On the basis of these data, the null hypothesis could be rejected, indicating that the linearity was satisfactory.

For cyanocobalamin, the limit of detection (LOD) was 0.0558 μg mL^{-1} and the limit of quantitation (LOQ) was 0.1859 μg mL^{-1}. For SLES, the LOD was 0.4509 μg mL^{-1} and the LOQ was 1.5028 μg mL^{-1}. The LOD was used as a guide to define the residue limit acceptance criteria. Brazilian legislation (Brasil, 2006) recommends a maximum limit of 10 ppm (= 10 μg mL^{-1}) of the contaminant in the subsequent product. In this study, the criterion "not detectable" was used to ascertain the efficiency of cleaning procedures, since it is more stringent than the limit of 10 ppm.

Table 1 shows that washing with 2% detergent was satisfactory for the cleaning of beakers, flasks and pipettes. The residual concentrations of cyanocobalamin on all items were below the detection limit of the method, so they were all considered free of residue, according to the criterion used.

Vials are more difficult to clean, because of their small internal capacity. The use of disposable vials and other glassware is widespread, especially in industry. In analytical laboratories, costs hinder this practice, and alternatives to disposal are constantly proposed. Another factor encouraging the reuse of vials is concern for the environment, because glass takes many years to decompose and many units are used daily in HPLC analysis. Thus, various methods that might enable vials to be cleaned and reused in routine analysis were investigated.

Table 1. Results of marker contaminant residue quantitation.

Cleaning procedure	Glassware	Abs (λ=361 nm)	Residue (μg mL^{-1})
2% detergent	Beaker 50 mL	0.0001	nd
		0.0002	nd
		0.0004	nd
		0.0013	nd
	VF 10 mL	0.0014	nd
		0.0014	nd
	VP 5 mL	0.0003	nd
		0.0002	nd
		0.0001	nd
Water and 77º GL ethanol	Vial – 5 rinses	0.0016	0.13
		0.0012	0.13
		0.0027	0.09
	Vial – 10 rinses	0.0036	0.07
		0.0037	0.06
		0.0024	0.08
	Vial – 15 rinses	0.0017	0.06
		0.0014	nd
		0.0020	0.10
	Syringe – 5 rinses	0.0003	nd
		0.0046	0.17
		0.0009	nd
	Syringe – 10 rinses	0.0001	nd
		0.0010	nd
		0.0005	nd
1N NaOH	Vial	0.0078	0.27
		0.0050	0.18
		0.0059	0.21
	Vial – 5 rinses	0.0007	0.03
		0.0013	0.06
		0.0015	0.06
	Vial – 10 rinses	0.0000	nd
		0.0002	nd
Ultrasonic bath		0.0006	nd
	Syringe – 5 rinses	0.0007	nd
		0.0013	0.06
		0.0015	0.06
	Syringe – 10 rinses	00000	nd
		0.0002	nd
		0.0006	nd

nd = not detectable; VF = volumetric flask; VP = volumetric pipette.

Owing to their small internal volume, the use of detergent was not considered for vials and syringes, because it would be extremely difficult to remove its residue. It was decided to use only purified water and 77 °GL ethanol, but it was found that this procedure did not achieve the expected result in vials (Table 1).

The use of an alkaline solution (1N NaOH) was also unsatisfactory. In fact, it increased the amount of detectable residues. Alkaline solutions, generally made from sodium hydroxide (or potassium hydroxide) dissolved in water (pH > 9.0), are designed to etch lightly the glass surface. This etching of the glass should ensure the removal of surface residues, resulting in a clean surface (Birch, 2009), but in this specific test, the reaction with the inner surface of the glass was prejudicial to the cleaning procedure.

Finally, the use of ultrasonic bath was tested for physical removal of the residues from vials and syringes. The results show that this procedure was more effective than the others, for vials. Therefore, 15 min treatment in the ultrasonic bath, followed by 10 rinses with ultra-pure water, was chosen as a standard method for cleaning the vials. Syringes were also cleaned without detergent, just one rinse with alcohol and 10 rinses with ultra-pure water being sufficient to remove the marker contaminant.

To ensure completely the efficiency of the cleaning procedure, the removal of all the detergent during the rinsing was also checked. The results are shown in Table 2.

Table 2. Results of detergent residue quantitation.

Glassware	Abs (λ=361 nm)	Residue (μg mL-1)
Beaker 50 mL	0.0011	nd
	0.0001	nd
	0.0003	nd
VF 10 mL	0.0314	nd
	0.0217	nd
	0.0477	nd
VP 5 mL	0.0038	nd
	0.0445	nd
	0.0313	nd

nd = not detectable; VF = volumetric flask; VP = volumetric pipette.

No item of the tested glassware showed detectable residues of detergent after the chosen cleaning method. The number of rinses used was essential to this result, since surfactants are generally known to require many rinses for their complete removal.

The rinse with 77 ºGL ethanol after each procedure was used in order to decrease the drying time, thus optimizing the time taken to reuse the glasses. In routine analysis, it is essential that the glassware dry in a short time, to expedite the start of the next tests.

It was evident, therefore, that the cleaning of glassware requires a more comprehensive study than is usually done. Each item and type of glass has its peculiarities and the best method for each case should be established.

In this study, an attempt was made to use "clean procedures" that do not generate significant residues or environmental contamination. Thus, the procedures proved here to be effective do not affect the environment and require no special treatment of the residues generated by them. It is noteworthy that the adoption of "green policies" is highly relevant to society nowadays. Sustainable and responsible business practices are essential in the present global scenario (Pinto et al., 2009).

The washing procedures for glass apparatus presented here were proven to be effective. Thus, they will ensure the adequate cleaning of glassware and the accuracy and reliability of tests carried out in them.

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RESUMO

Desenvolvimento de procedimento padronizado para a lavagem de vidraria em laboratórios analíticos

A lavagem da vidraria analítica é um procedimento essencial e determinante na confiabilidade dos resultados de testes e ensaios, a despeito da interferência dos resíduos de análises anteriores. Neste trabalho, foram propostos procedimentos de limpeza de vidrarias utilizando cianocobalamina como um marcador da eficiência de limpeza. Foi utilizado método espectrofotométrico para determinação dos resíduos do marcador e também do agente de limpeza. Béqueres, balões volumétricos e pipetas volumétricas foram comprovadamente limpos com detergente a 2% e múltiplos enxágues. Vials e seringas foram apropriadamente limpos utilizando-se banho ultrassônico. Esses procedimentos de limpeza fazem uso de reagentes baratos e não tóxicos, parâmetros de suma importância para sua aplicação em rotina laboratorial de análises físico-químicas.
Palavras-chave: Estudos de Validação. Detergentes. Técnicas e Procedimentos de Laboratório. Limpeza de Vidraria.

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