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Validation of method for the determination of mercury in the auxiliary substances azorubine 21% and azorubine 85% using cold-vapor atomic absorption spectrometry

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Abstract: Heavy metals, such as mercury (Hg), sometimes can be found in auxiliary substances intended for pharmaceuticals use. Although the concentration of those elements is very low, their control is very important because of its toxicity. Permissible concentration of mercury (Hg) in Azorubine 21% and Azorubine 85% is prescribed by the Directive of the European Commission concerning the specific purity criteria on food coloring. The focus of this paper is on validating reliable methods of Hg determination in auxiliary substances mentioned above, by Cold-vapor Atomic Absorption Spectrometry after microwave acid digestion of solid samples. To obtain possibly present Hg in Azorubine by conversion to Hg^{2+} ions, samples were treated with a mixture of 1 mL MQ water + 1 mL 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 and heated by microwave for 30 min. on 1000 W in sealed TFM^{TM} – PTFE tubes. The resulting solutions are diluted and analyzed for Hg using cold vapor atomic absorption spectrometry with sodium borohydride as a reducing agent. The method was successfully validated and can be applied for the determination of Hg in solid samples of Azorubine 21% and Azorubine 85%, with value of recovery factor of 95% to 104% and 96% to 105%, respectively.

INTRODUCTION

Azorubine is synthesized red color belonging to mono azo class from the group of organic azo dyes and is well soluble in water. It is used in the pharmaceutical industry and for the the food coloring, especially for the food that has to be heat treated after fermentation. (EFSA (2009).

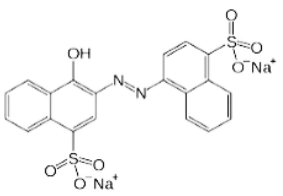

Figure 1: Azorubine (Structural formula)

Heavy metals, such as Mercury (Hg) can be found sometimes in excipients Azorubine 21% and Azorubine 85%, as a result of manufacturing process.

Like other heavy metals, mercury performs no function in the body. Mercury in its different forms is poisonous, and can be inhaled, ingested and even absorbed through unbroken skin. It is bio-accumulated and bio-magnified. Some organometallic mercury species, such as monomethyl- and dimethyl mercury are known to be extremely toxic. Although the concentration of those elements in azorubine mixture is very low, testing for the mercury is very important because of its toxicity.

Pharmaceutical companies use Azorubine in production process as base color for some syrups and solutions. The quality of these auxiliary substances is controlled prior to usage.

Permissible concentration of mercury (Hg) in Azorubine 21% and Azorubine 85% is prescribed by the Directive of the European Commission concerning the specific purity criteria on food coloring and is less than 1 part per million (0.0001 %) (EU Commission, 1999; WHO, 2008).

For the determination of trace element quantities in natural and synthesized products, the material has to be digested in a first step

Commonly used methods for digestion of complex samples involve heating samples with strong oxidation acid such is HNO_3, $HClO_4$, and H_2SO_4.

Usual methods for analyzing carbon based raw material such as coal, petroleum distillates and refined carbon based products have employed microwave assisted digest practices with various detection techniques.

The use of microwave ovens as heat sources not only drastically reduces the heating times of organic reactions, but the reactions often proceed more efficiently and selectively than when conduction heating methods are used. This is because microwave energy is transferred uniformly and almost simultaneously to the entire sample, thus eliminating any hot spots that may result in side reactions (Gilbert, Martin, 2011).

From experimental and literature source we find that best results in measuring concentration of mercury were achieved after using microwave acid digestion by mixing the test samples with 1 mL MQ water + 1 mL 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 and digesting it for 30 min. on 1000 W.

The most common analytical technique for Hg determination is cold vapor atomic absorption spectrometry (CVAAS). The CVAAS was adopted as a standard method for analysis of Hg in foodstuffs. This technique is based on the chemical reduction of mercury, usually by Sn^{2+} or BH_4^- ions to elemental Hg which is swept from the solution by a carrier gas to a quartz cell placed in the optical path of an atomic absorption spectrophotometer where the absorption of Hg is measured (Silva, Toth, Rangel, 2006).

However, no acceptable validated method for determining the mercury content in Azorubine used in pharmaceutical industry has been published so far in relevant pharmaceutical regulations, therefore there is a need for this method's validation.

The aim of the present study was to validate methods of Hg determination in Azorubine 21% and Azorubine 85%, by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion of solid samples.

For validation process following parameters will be considered: specificity, linearity, range, accuracy, precision, detection limit, quantitation limit and stability.

EXPERIMENTAL

Material, chemicals and reagents

Following materials chemicals, and reagents were used: Azorubin – Carmoisin general formula $C_{20}H_{12}N_2Na_2O_7S_2$, known also as E122, (Frutarom Etol d.o.o. - Azorubin 21%; BTC Europe GmbH (BASF SE) – Azorubin 85%;); Mercury standard solution 1000 mg L^{-1} (Merck KgaA, Germany); 65% HNO_3 (Merck KgaA, Germany); 70% $HClO_4$ (Merck KgaA, Germany); 96% H_2SO_4 (Sigma Aldrich, England); 30% HCl (Sigma Aldrich, England); NaOH (Merck KgaA, Germany); $NaBH_4$ (Merck KgaA, Germany); 99,999 % Argon (Messer, BiH); Water quality of Milli-Q (MQ) was used in the preparation of all solutions.

The calibration curves (6-12 µg/L) for Hg were established with solutions prepared from a 1000 g/L certified stock solution.

The reducing reagent was prepared dissolving 7.5 g $NaBH_4$ and 2.5 g NaOH in 250 mL of MQ water.

As a carrier solution used 5 mol/L HCl prepared diluting 264 mL of 30% HCl to 500 mL with MQ water.

Apparatus and equipment

Microwave- assisted digestion was carried out on an instrument Microwave PRO from Anton Paar manufacturer. Mercury was determined by cold vapor technique using VGA 77 chemical vapor generation system (Varian, USA), coupled to the AA spectrometer model AA240FS (AAS Varian, USA). An Hg hollow cathode lamp (Varian, USA) operated at 6mA was used.

Procedure

A microwave assisted digestion procedure was carried out to obtain total Hg from Azorubine samples. Two replicates of Azorubine 21% and 85%, each were directly weighed (~0.50 g) into TFM™ – PTFE tubes. In each tube 1 mL of MQ water, dissolved sample and 1 mL solution of concentrated 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 was mixed together Microwave digestion was performed using a three- step program: 5 min at 1000 W power ramp, 30 min at 1000 W power hold followed by 15 min of cooling.

The resulting solution was diluted to 50 mL with MQ water. An aliquot of solution was reduced with a 3% $NaBH_4$ (w/v) in 1% NaOH and 5 mol/L HCl (as a carrier solution) and then passed to a gas-liquid separator where the evolving Hg^0 was swept in a stream of argon to the flow-through mercury cell and the atomic absorbance signal was recorded.

Measurements were carried out at the wavelength of 253.7 nm.

Concentration of mercury in Azorubine 21% and Azorubine 85% was calculated according to the following exation:

$$\frac{C \cdot 50}{m} = ppm\ Hg$$

C - measured concentration of Hg (ppb)

m- weight (mg)

RESULTS AND DISCUSSION

The values of the following parameters were determined during the analytical validation procedure: selectivity, linearity, *LOD*, *LOQ*, range, repeatability (precision), accuracy and stability (Ermer, Miller, 2005; Brentall, Clarke, 2001).

Selectivity

In the cold vapor technique, mercury is released from the sample, and then, after reduced to atomic mercury carried in a stream carrier gas to the absorption cell, where the absorption of the radiation (λ=253.7 nm) emitted by a hollow mercury cathode lamp, is measured. This measurement method guarantees high selectivity of mercury determination for two reasons: gas-liquid separator separates evolving Hg^0 gas from liquid and absorption is measured using a characteristic wavelength for mercury.

Linearity

A series of 5 standard solutions and 5 spiked sample solutions was prepared with a mercury content of 4, 6, 8, 10 and 12 ng/mL. For each of the solutions, three measurements were obtained. The calibration was carried out as a function of instrument signal and mercury content. Peak height of <1 ng Hg concentration was used as the instrument signal. Based on the results, regression parameters were found and the calibration curve determined. A high regression coefficient r (0.9987), demonstrates a high linear procedure.

Limit of Detection (*LOD*) and Quantification (*LOQ*)

The detection and quantitation limits were calculated by ratio SE/S: LOD = 3x (SE/S) and LOQ = 10x (SE/S). SE is standard error of the intercept deviation for 10 measurements of the spiked sample solutions with mercury concentration in expected LOQ range and S is the slope of the calibration graph correspond to Hg concentration.

LOD was taken to be 0.9075 ng/mL. Calculated for a mass of a sample of 500 mg, this corresponds to Hg concentration in Azorubine samples of 90.7 ng/g. However, LOQ was determined to be LOQ=3.3·LOD, i.e. 2.99 ng/mL (299 ng/g).

Range

The measurement range is a concentration range from the LOQ section to the maximum standard solution concentration used for calibration; it is therefore equal to 3.00-12.00 ng. Calculating for the mass of the sample determined to be 500 mg, this corresponds to a mercury concentration range of 300 ng/g-1200 ng/g.

Repeatability (precision)

Repeatability was determined from a series of six independent measurements of standard solutions of mercury in target concentration (10 ppb) and real samples with added mercury in same target levels. It was determined as the CV value for the series.

Obtained results (Table 1. and Table 2.) demonstrate a high level of repeatability (precision).

Accuracy

Repeatability was determined from a series of three independent measurements for each of three spiked sample solutions with mercury concentration of 80%, 100% and 120% from target concentration (10 ppb). It was determined as the Recovery value for the series.

From obtained results (Table 1. and Table 2.) it can be concluded that used method is very precise.

Stability

Stability test was conducted by measuring three standard solutions in 3 different concentrations (80%, 100% and 120%) and real samples with added mercury of the same concentration, freshly prepared and after 24 hours at ambient conditions.

As an indication of the stability of the solution, % Difference (%D) was calculated according to the equation:

$$\%D = \frac{[c(found\ (fresh)) - c(found\ (after\ 24\ hours))] \cdot 100}{c(found(fresh))}$$

Obtained results (Table 1. and Table 2.) demonstrate that solutions of standard and samples were stable for 24 hours at ambient conditions.

Table 1: Results of validating methods of Hg determination in auxiliary substances Azorubine 21% by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion

Tested parameters	Results	Acceptance criteria
Specificity	No interference	No interference
Accuracy (Recovery)	95.07% ÷ 104.30%	80% ÷ 120%
Detection limit	0.9075 μg mL^{-1}	-
Quantification limit	2.9947 μg mL^{-1}	≤ 0,5x limit
Repeatability (CV)	0,2489%	≤ ± 5%
Intermediate precision (CV)	3.01%	≤ ± 20%
Linearity	y = 0.015 + 0.0033x	
Correlation coefficient	R = 0.9987	0.99
Stability (%D)	0,52%	< 20,0%

Table 2: Results of validating methods of Hg determination in auxiliary substances Azorubine 85% by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion

Tested parameters	Results	Acceptance criteria
Specificity	No interference	No interference
Accuracy (Recovery)	96.46% -105.50%	80% - 120%
Detection limit	0.9075 μg mL-1	-
Quantification limit	2.9947 μg mL-1	≤ 0,5x limit
Repeatability (CV)	0,9942%	≤ ± 5%
Intermediate precision (CV)	2.16%	≤ ± 20%
Linearity	y = 0.015 + 0.0033x	
Correlation coefficient	R = 0.9987	0.99
Stability (%D)	-0,30%	< 20,0%

CONCLUSION

Based on the results, obtained from validating methods of Hg determination by Cold-Vapor Atomic Absorption Spectrometry after microwave acid digestion, it can be concluded:

1. All the validation results are in accordance with acceptance criteria.

2. The method was successfully validated and can be applied for the determination of Hg in solid samples of Azorubine 21% and Azorubine 85% with recovery factor value of 95% to 104% and 96% to 105%, respectively.

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REFERENCES

Brentall, A.E., Clarke, G.S. (2001) Validation of analytical methods. In Ahuja, S., Scypinski, S. (Ed.), *Handbook of Modern Pharmaceutical Analysis.* (p.p. 429-457). Academic Pres.

EFSA (European Food Safety Authority) (2009). *Scientific Opinion on the re-evaluation of Azorubine/Carmoisine (E 122) as a food additive.* Parma, Italy.

Ermer, J., Miller, J.H.M. (2005). *Method Validation in Pharmaceutical Analysis*. (1st Ed.) WILEY-VCH Verlag GmbH & Co. KGaA.

EU Commission (1999). *Specific purity criteria concerning colours for use in foodstuffs*. Commission Directive 1999/75/EC.

Gilbert, J., Martin F.S. (2011*). Experimental Organic Chemistry- A Miniscale and Microscale Approach*. (5th Ed.) BROOKS/COLE Cengage Learning.

Silva, M.F., Toth, I.V., Rangel, A.O. (2006). Determination of mercury in fish by cold vapor atomic absorption spectrophotometry using a multicommuted flow injection analysis system. *Analytical Sciences*, *22 (6)*, 861–864.

WHO - Word Health Organization (2008). *Development of Paediatric Medicines:Pharmaceutucal Development*. WHO QAS/08.257.

Summary/Sažetak

Teški metali, kao što je živa (Hg), ponekad se mogu naći u polaznim supstancama za farmaceutsku upotrebu. Iako je njihova koncentracijaveoma niska, praćenje istih je veoma važno zbog njihove toksičnosti. Dozvoljena koncentracija Hg u Azorubinu 21% i Azorubinu 85% je propisana Direktivom Europske komisije u vezi s posebnim kriterijima čistoće boja za hranu. Fokus ovog rada je na validaciji precizne i tačne metode određivanja Hg u Azorubinu 21% i Azorubinu 85%, koristeći tehniku hladnih para atomske apsorpcione spektrometrije nakon mikrotalasne kiselinske digestije čvrstih uzoraka. Da bi eventalno prisutnu Hg u Azorubin 21% i Azorubinu 85% preveli u obliku Hg^{2+} iona uzorci su tretirani smjesom koja sadrži 1 mL MQ vode + 1 mL 65% HNO_3 + 1 mL 70% $HClO_4$ + 5 mL 96% H_2SO_4 i zagrijavani mikrovalnim zračenjem u periodu od 30 min. na 1000 W. Koncentracija Hg u uzorku je nakon toga određena tehnikom hladnih para atomske apsorpcione spektrometrije uz natrij borohidrid kao rededucens. Metoda je uspješno validirana i može se primjenjivati za određivanje Hg u čvrstom uzorku Azorubina 21% i Azorubina 85%, uz vrijednost *recovery* faktora od 95% -104% i 96-105%, redom.