Automated Sample Preparation for Mercury Analysis in Wood Materials

Ellen Vorberg*, Kerstin Thurow*, Heidi Fleischer†, Steffen Junginger†and Norbert Stoll†,
*CELISCA - Center for Life Science Automation, University Rostock, Rostock, Germany
†Institute of Automation, University Rostock, Rostock, Germany
Email: Ellen.Vorberg@celisca.de

***Abstract*—Laboratories providing environmental measurements will gain on improving analysis´ efficiency, robustness, and reliability by automating sample preparation for the analysis of toxic elements, such as arsenic, cadmium, mercury, and lead. Nevertheless, automating analytical sample preparation is still an unsolved challenge due to the fact that commercially available, automated platforms are not suitable for environmental applications. However, using the developed system for automated mercury analysis results in less cost and time consuming steps. The developed system enables precise and reliable sample preparation. Besides, results are in excellent agreement with the true value corresponding to a level of confidence of approximately 95 % (P = 95%).**

***Index Terms*—mercury determination, sample preparation, life science automation, analytical analysis.**

I. Introduction

Entering the environment, toxic elements, such as mercury and cadmium, can lead to adverse health effects and potentially death. Nevertheless, recognizing and minimizing common sources of toxic elements, dangerous exposures can be prevented [1]. Accordingly, laboratory testing that is capable of detecting and managing toxic element exposure is required. For determining total mercury content, laboratory testing has to provide precise, fast and sensitive methods. Thus, laboratories, that provide environmental measurements are constantly faced with the demands for improved quality, better economical results, and shorter sample turnaround times [2].

By automating sample preparation, laboratories will gain on all of these demands [3] since sample preparation techniques are currently the rate limiting step in testing processes [4], [5]. Thereby, automated systems reduce the variability in sample preparation [6], [7], improve the accuracy of the experiment, and allow for rapid analysis. Furthermore, automated systems ensure the safety of the analyst by removing risk-involving procedures, such as handling of highly active substances including toxic elements, from the operator. Moreover, based on the high costs of many reagents, the trend in laboratory automation is toward increasing miniaturization [8]. Thereby, automated systems enable less consumption of solvents [9] and decrease the costs for waste disposal [10].

Biological applications are the prime candidates for laboratory automation [11] due to the fact that they use the Multi-Titer-Plate (MTP)-format, which is provided by commercially available automated platforms. In contrast automating analytical sample preparation for environmental measurements is still an unsolved challenge due to the fact that commercially available platforms are not suitable for the requirements of analytical sample preparation. In detail, analytical sample preparation necessitates higher volume ranges and can require the utilization of highly active (aqueous) solutions, such as concentrated acids or toxic organic solvents with different viscosities. Besides, for performing analytical sample preparation, non-standard temperatures and pressures are needed demanding the utilization of inert materials and various sample preparation vessels.

Automating the most complicated analytical procedure is possible using current automation technology. Due to the fact that the problem is solely economic automating analytical sample preparation is still an unsolved challenge. In detail, instruments used for repetitive analytical analysis are frequently equipped with automatic sample processors [12], [13]. These plant components are capable of doing serial pipetting and dilution conveniently. However, they automate only one or two steps of a full analysis scheme. Besides, they merely enable pipetting of lower volume ranges. In addition they are not capable of handling the wide range of vessels needed for performing analytical sample preparation.

Thus, automating analytical sample preparation calls for various workstations, which have to be capable of handling a wide range of vessels. These workstations have to provide liquid handling options allowing automated pipetting of both, small and higher volume ranges. Besides, workstations have to enable sample handling options fulfilling capping and storage of the samples. Furthermore, for automating analytical sample preparation, a system integrator moving vessels between the peripheral devices is needed.

II. Method Development

Fleischer and Thurow [14] developed and described an analytical method that allows the sensitive determination of total mercury content in wood materials. In a first step the method for the analysis of mercury was enhanced and miniaturized.

In detail, after adding the ICP MS Rhenium Standard to the acid (Aqua Regia), 2ml of the prepared acid were added to 62,5mg of the sample. The samples were predigested for a period of 20 minutes. One blank sample was included at

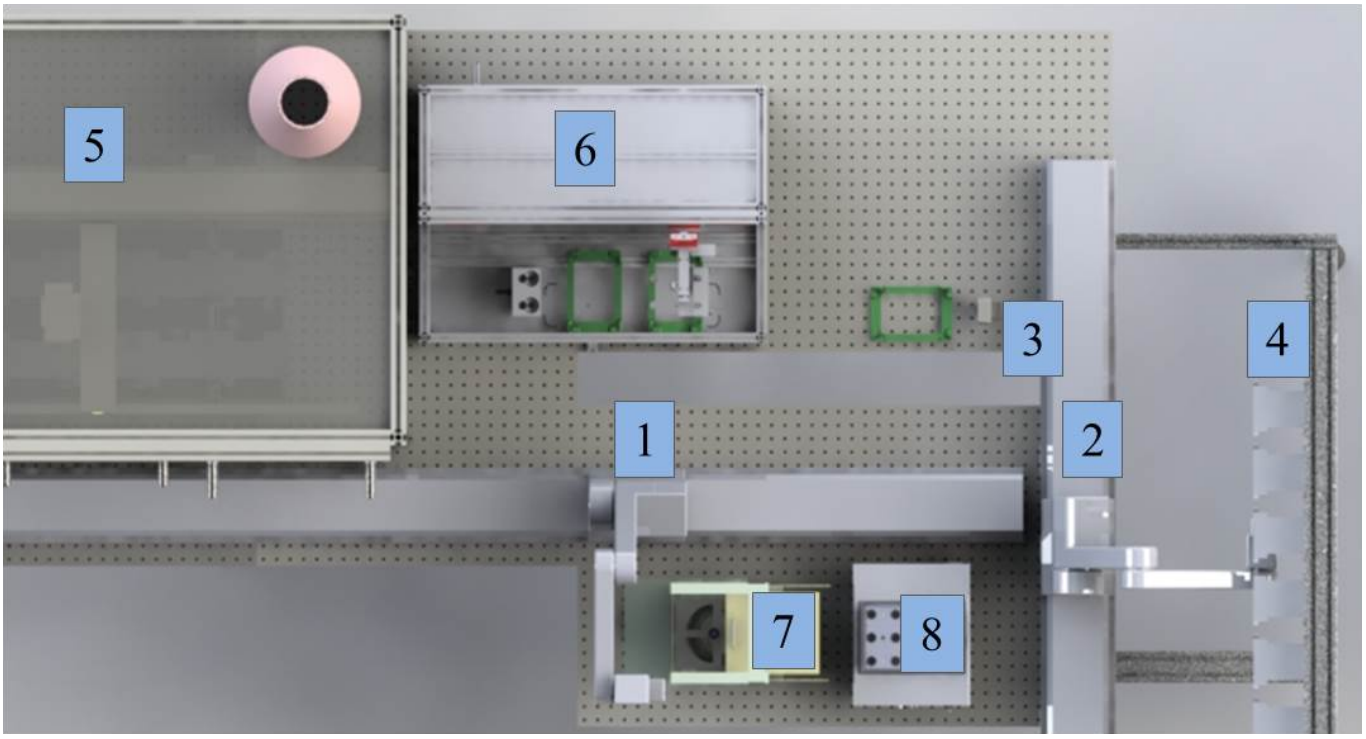


Fig. 1. Concept for the suitable automation system for automated analytical sample preparation for mercury analysis including two ORCA®robots mounted on a linear rail enhancing the accessible workspace (1 +2), the re-grip station for handing over the samples including bar-coding options for sample identification (3), the ORCA®hotel with sample storage options (4), the Biomek®2000 liquid handler with housing and exhausting system enabling dealing with evaporating samples and fluids (5), the self-developed liquid handler for pipetting of higher volume ranges up to 10ml (6), the weighing station (7) – not used in the presented application, the thermoshaker (8) – not used in the presented application

every digestion run. The basic microwave digestion was the same procedure for both, the original and the miniaturized sample preparation. After cooling down, the Xpress microwave digestion vessels were uncapped. Finally 1ml of the clear sample solution was transferred to an analysis vessels filled with 11.5ml of ultra pure water. The rhenium standard allows appraisal of potential evaporation during the microwave digestion run, and thus, provides amendment of the calculated results of measurement. If there is no evaporation at all, rhenium concentration has to be 100ppb.

Consequently, the original manual procedure was changed to smaller volumes. Thus, merely one quarter of the original sample mass and acid volume was used providing less consumption of solvents and decreased waste disposal. Furthermore, using merely the half of the digested sample after the microwave digestion step enabled the utilization of only 12.5% of the solvent used in the original method. The enhanced sample preparation process was used for both, validation of the automated process and the manual procedure. Both were compared using ICP MS for analyzing the results.

III. Automation Concept

The method includes the digestion of grounded wood samples with nitric acid under microwave conditons followed by an ICP-MS determination of the mercury content in the resulting liquid samples.

In general, all steps (=laboratory operation unit; LUO) in this method are subjected to automation. A concept for a suitable automation system has been developed (see Fig. 1). The samples are stored in the hotel until they are processed within the system. A classical laboratory robot (ORCA®, Beckman Coulter, USA) is the central element and acts as a system integrator and transport system. Liquid delivery and dilution steps are performed on the integrated Biomek® 2000 (for volumens up to 1 ml) and a self developed diluting station (for volumes up to 10 ml). Since nitric acid is used in the process, the system has been covered with an housing and can be exhausted. The complete process includes also a microwave digestion step. Due to safety reasons, this step has to be performed under a separate hood. Thus, a mobile robot system has been integrated for the sample transport between the automated sample preparation system and the microwave hood.

A. System Integrator

Working as system integrator, two laboratory robots (ORCAs®) were implemented into the developed automated system. The ORCAs® were mounted on a linear rail enhancing the accessible workspace. Furthermore, a re-grip station for handing over the samples and an ORCA®hotel with 196 positions for different kinds of MTPs have been integrated providing up to 96 wells per position. The ORCA®is able to deal with the MTP-format, and moreover, capable of

handling individual vials and tubes. However, avoiding individual transport of samples, the utilization of MTPs provides accomplishing of 96 reactions simultaneously, and therefore, less cost and time consuming steps.

Due to the fact that MTPs are not sufficient for analytical sample preparation because of the mentioned requirements, such as higher volume ranges, the utilization of chemically highly active aqueous solutions, and non-standard temperatures and pressures, the required sample preparation vessels were embedded into the MTP-footprint. Embedding the vessels ensures both, handling the MTP-format, and therefore, transport of up to 24 vessels simultaneously, and moreover, allowing the utilization of the applicable sample preparation vessels for the analytical procedure.

B. Liquid Handling

Liquid transfer steps, such as diluting, aliquoting, and adding of the internal standard(s) are essential for automating standard operation procedures in multistep analytical sample preparation. Actually, liquid handling equipment constitutes the largest segment of the laboratory automation market. However, commercially available automated platforms are usually configured for handling the MTP-format. Therefore, embedding the required sample preparation vessels into the MTP-footprint is a suitable solution ensuring the availability of liquid handling options for multistep analytical sample preparation.

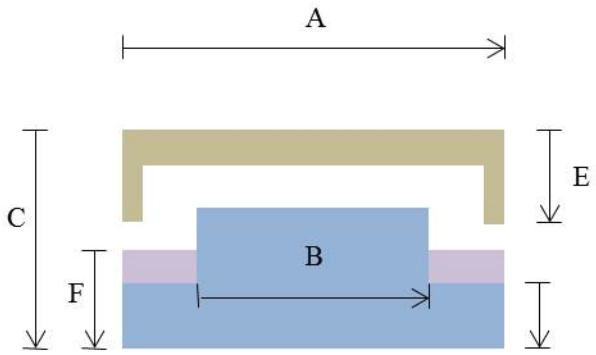
To ensure the entire task performance, the embedded vessels have to meet the specifications of the liquid handler software (as shown in Fig. 2). Seeing that the predetermined parameters are based on the MTP-contours, the sample preparation vessels have to be smaller than 115mm and thinner than 120mm. Therefore, a wide range of vessels can be used. The microwave vessels used in the original manually operated sample preparation procedure did not comply with these requirements. Nevertheless, using miniaturization for mercury analysis allows utilization of smaller microwave vessels, which were in agreement with the specifications of the automation devices´ software.

The Biomek®2000 enables pipetting volume ranges from 1µl up to 1ml with an inaccuracy of 0.6%. For handling higher volume ranges, a further self-developed liquid handler has been integrated providing volumes up to 10ml with an inaccuracy of 1% using a Hamilton® dispenser with an incorporated 10ml syringe. Due to its Cartesian configuration and an additional labware holder, this liquid handler is capable of handling the conceived trays.

Enabling dealing with evaporating samples and fluids, the housing with an included exhaust system was built for the Biomek®2000.

C. Sample handling

Screwing the vessels is an underestimated challenge due to the fact that the vessels have to be transferred to a screw-tightening robot one by one. Furthermore, the screw heads are solely suitable for a low range of port diameters. Therefore, to provide a wide range of various diameters, screw heads have to be replaced automatically. To avoid these time-consuming and cost intensive steps, the presented concept comprises the idea of capping the whole MTP-footprint with all the implemented vessels with just one lid.



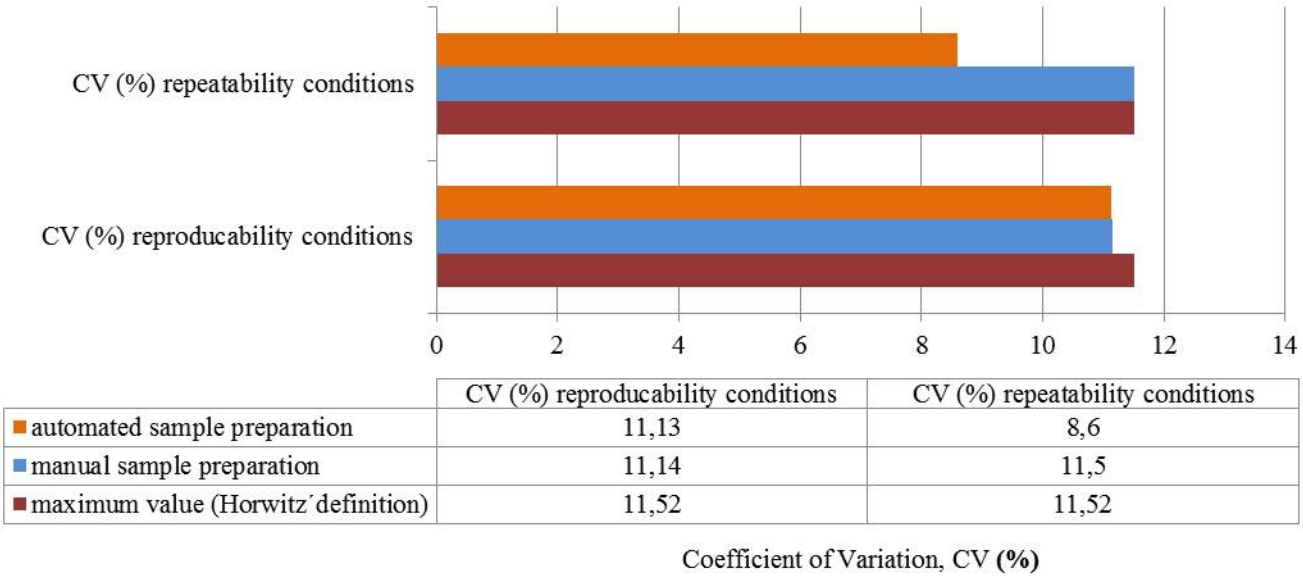
Lid length	≤ 130mm
(A) Lid width	≤ 100mm
(B) Top width	≤ 120mm
(C) Lid on height	≤ 120mm
(D) Lip height	≤ 60mm
(E) Lid height	≤ 60mm
(F) Lifter height	≤ 60mm
Top height	≤ 115mm

Fig. 2. Labware configuration software of the commercially available liquid handler.

The Biomek®2000 gripper tool is capable of gripping this lid irrespective of the port diameter in one single step allowing opening of up to 24 vessels simultaneously. For gripping this lid, the conceived trays have to be smaller than 120mm. Moreover, for gripping the whole tray, a gripper lip is necessary. Depending on the labware configuration, the highest feasible level of this lip amounts to 60mm. Furthermore, to avoid collisions between the vessels and the gripper, ensuring especially this level is necessary. A second lip has been attached providing the tight fit of the vessels.

IV. Results and Discussion

The developed system had to fulfill automated sample preparation for mercury analysis in organic samples. Both, the automated and the miniaturized manual sample preparation procedure were compared while using one quarter of the original sample mass and acid volume. ICP MS was used for analyzing the results.



	CV (%) reproducability conditions	CV (%) repeatability conditions
automated sample preparation	11,13	8,6
manual sample preparation	11,14	11,5
maximum value (Horwitz´definition)	11,52	11,52

Fig. 3. Repeatability and Reproducibility Coefficient of Variation – calculated for the automated and the manual sample preparation procedure while using ICP MS for analyzing the results

A. Bias Error, Discrimination Threshold and Calibration

Bias error of the measuring instrument (ICP MS) was 0.026µg/l, which corresponds to a Coefficient of Variation (CV) of 1.6%. Bias error was calculated while performing ten measurements of the same sample. Discrimination threshold was 6.1ng/l and calculated from the mean value of ten blank measurements and the threefold of the value´s standard deviation (SD). Determining 5 calibration points in the range of 0.1 up to 10µg/l, the coefficient of determination for both mercury calibration curves was $\geq$ 0.9999 while using Blank offset and linear curve fit.

B. Repeatability Standard Deviation

Preparing 25 samples per day, repeatability SD was calculated while using repeatability conditions for the automated and the manual sample preparation procedure. Repeatability SD of the automated process was 0.051mg/kg (CV = 8.6%), and therefore, 25% lower than the repeatability SD of the manual procedure, which was 0.067mg/kg (CV = 11.5%). Furthermore, repeatability SD of both procedures did not exceed the maximum value (CV = 11.52%) according to the Horwitz´ definition [15].

C. Reproducibility Standard Deviation

Preparing 10 samples per day on five consecutive days, reproducibility SD was measured under reproducibility conditions. Using the automated system, reproducibility SD was 0.064mg/kg (CV = 11.13%). Thus, the reproducibility SD was similar to the reproducibility SD of the manual procedure, which was 0.063mg/kg (CV = 11.14%). Moreover, results did not exceed the maximum value (CV = 11.52%) according to the Horwitz´ definition. Results are shown in Fig. 3.

D. Results of Measurement

Using reference material for validating the automated system, the true value of mercury was 0.60±0.14mg/kg according to the Certificate of Analysis, ERM®-CD 100 (BAM, Berlin, Germany). Results of the measurement for the automated sample preparation process were 0.574±0.018mg/kg (n = 50), which corresponds to an amount of 95.7% of the true value of mercury. Consequently, results of measurement were in excellent agreement with the true value of mercury. Verified by the David test, all measured values followed normal distribution including a level of confidence of 95 % (P = 95%). Results of the measurement for the manual procedure were 0.566±0.018mg/kg, which corresponds to an amount of 94.3% of the true value of mercury. Consequently, results of measurement were in excellent agreement with the true value of mercury. Verified by the David test, all measured values followed normal distribution including a level of confidence of 95 % (P = 95%).

V. Conclusion

The system´s functionality has been confirmed in the validation sequence. Using the developed system enables precise and reliable multistep analytical sample preparation for mercury analysis in wood materials. Results are in excellent agreement with the true value corresponding to a level of confidence of approximately 95% as defined in the Guide to the expression of uncertainty in measurement, ISO, 1993. Moreover, using automated sample preparation enables mercury analysis in wood materials for up to 480 samples per day.

VI. Acknowledgment

The authors wish to thank the State Ministry of Education and Research (Ministerium für Bildung, Wissenschaft und Kultur MV, Germany) for the financial support of this project (FKZ: V-630-S-105-2010/352 and V-630-F-105-2010/353). The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] D. Keil, J. Berger-Ritchie and G. McMillin, *Testing for toxic elements: A focus on arsenic, cadmium, lead, and mercury*, Lab. Med., vol. 42, No. 12, pp. 735-742, 2011.

[2] W. De Jong,E.G. De Vries and I. Kema, *Current status and future developments of LC-MS/MS in clinical chemistry for quantification of biogenic amines*, Clin. Biochem., vol. 44, No. 1, pp. 95-103,2011.

[3] T. Knudsen, *Fully automated sample preparation and analysis in the mining industry*, IFAC P. Vol. (IFAC-PapersOnline) 2007, pp. 153-158, 2007.

[4] D. Teitz, S. Khan, M. Powell and M. Jemal, *An automated method of sample preparation of biofluids using pierceable caps to eliminate the uncapping of the sample tubes during sample transfer*, J. Biochem. Bioph. Meth., vol. 45, No. 2, pp. 193-204, 2000.

[5] J. Sims, A. Smith, D. Patel, R. Batchelor and J. Carreira, *Automated sample preparation for icp analysis of active pharmaceutical ingredients and intermediates*, J. Lab. Autom., vol. 16, No. 5, pp. 377-380, 2011.

[6] F. Raymond, S. Metairon, R. Borner, M. Hofmann and M. Kussmann, *Automated target preparation for microarray-based gene expression analysis*, Anal. Chem., vol. 78, No. 18, pp. 6299-6305, 2006.

[7] E. Plise, D. Tran and L. Salphati, *Semi-automated protein binding methodology using equilibrium dialysis and a novel mixed-matrix cassette approach*, J. Pharm. Sci., vol. 99, No. 12, pp. 5070-5078, 2010.

[8] T. Chapman, *Lab automation and robotics: Automation on the move*, Nature, vol. 421, No. 6923, pp. 661, 663, 665-666, 2003.

[9] J. Moreda-Pineiro, E. Alonso-Rodriguez, P. Lopez-Mahia, S. Muniategui-Lorenzo, D. Prada-Rodriguez, A. Moreda-Pineiro and P. Bermejo-Barrera, *Determination of major and trace elements in human scalp hair by pressurized-liquid extraction with acetic acid and inductively coupled plasma-optical-emission spectrometry*, Anal. Bioanal. Chem., vol. 388, No. 2, pp. 441-449, 2007.

[10] J. Quintana, M. Miro, J. Estela and V. Cerda, *Automated on-line renewable solid-phase extraction-liquid chromatography exploiting multisyringe flow injection-bead injection lab-on-valve analysis*, Anal. Chem., vol. 78, No. 8, pp. 2832-2840, 2006

[11] P. Mirsky, A. Chatterjee, A. Sauer-Budge and A. Sharon, *An automated, parallel processing approach to biomolecular sample preparation*, J. Lab. Autom., vol. 17, No. 2, pp. 116-124, 2012.

[12] S. Lopez, M. Gomez, M. Hernando and A. Fernandez-Alba, *Automated dynamic headspace followed by a comprehensive two-dimensional gas chromatography full scan time-of-flight mass spectrometry method for screening of volatile organic compounds (VOCs) in water* , Anal. Methods, vol. 5, No. 5, pp. 1165-1177, 2013.

[13] A. Aberl and M. Coelhan, *Determination of sulfur dioxide in wine using headspace gas chromatography and electron capture detection*, Food Addit. Contam. A, vol. 30, No. 2, pp. 226-233, 2013.

[14] H. Fleischer and K. Thurow, *Determination of total mercury content in wood materials, part 2: Icp-ms-a multielement method,* Am. Lab., vol. 45, No. 8, pp. 6-11, 2013.

[15] W. Horwitz, *Evaluation of Analytical Methods for Regulation of Foods and Drugs,* Anal. Chem., vol. 54, No. 1, pp. 67A-76A, 1982.