

Ultrasound-assisted rapid preparation of water standards for Karl Fischer coulometric titration

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Abstract

Ultrasound-assisted extraction of water from sodium tartrate dihydrate in methanol and DMSO proved to be a reproducible, time-saving method for the preparation of water standards for Karl Fischer titration (KFT). 30 s ultrasonication with a computer-controlled 120W ultrasonic homogenizer (SinapTec NexTgen Lab120) gave comparable extraction performance with samples of 50-800 mg sodium tartrate dihydrate dispersed in 5 mL solvent as 60 min shaking on a horizontal shaker at 300 rpm. Water extraction from sodium tartrate dihydrate, a stable and non-hygroscopic organic salt, which is often used as a secondary standard in volumetric KFT, showed a complex behaviour: The recovery, i.e. the actually extracted amount relative to the total stoichiometric water of a particular sample, varied between ca. 100% and 20%, depending on the amount of tartrate per 5 mL solvent (20-800 mg), the solvent (methanol, DMSO and ethyl acetate), duration and method used for the extraction (ultrasound or shaking). Nearly 100% recovery rates were obtained with the lowest tested amount of sodium tartrate dihydrate (20 mg) in DMSO with ultrasonic extraction, but reproducibility was poor under these conditions. The highest reproducibility of water standards was achieved with 100-400 mg of sodium tartrate dihydrate per 5 mL solvent, methanol as the solvent and with ultrasonic extraction. A typical standard, prepared from 100 ± 0.5 mg sodium tartrate dihydrate in 5 mL dry methanol, produced a water concentration of 1666 ± 28 ppm (N=5). This corresponds to a recovery rate of 42 ± 0.7 %. With ultrasound-assisted extraction, the complete workflow including weighing, extraction and titration can be accomplished within 10-15 min. This results in significant time and cost savings compared to traditional sample preparation workflows based on horizontal shaking.

Introduction

Karl Fischer Titration (KFT) is the common analytical method for quantitative trace water analysis in pharmaceuticals, cosmetics, oil products, various foodstuffs and specialty chemicals. Modern semi- or fully automatic KF-instruments can analyze up to 20 samples per hour, with little hands-on time at low cost. Volumetric KF instruments can quantitate water contents up to 100%, e.g. in foodstuffs. They need precise water standards for the calibration of the titration reagent and the device itself. Coulometric KF instruments are specializing in ultra-low water concentration detection, with lower detection limits on the order of 10 µg water. One of their most salient features is their ability to perform quantitative measurements without calibration. Highly accurate and reproducible results can be obtained. In reality, coulometric as well as volumetric KFT needs to be operated by trained users with extreme care and exactness and regularly controlled with precise water standards, in order to perform quantitatively and accurately. The preparation of precise water standards is a challenge, especially at low concentrations below 10,000 ppm (µg/g). Most users of coulometric KFT rely on commercially available certified reference materials (CRM) for the function control of their KFT instruments. Once opened, these standards have to be used instantly and cannot be stored. This adds considerably to the costs of the analysis, especially when the method is used only once in a while, as is typical of most research labs. Another bottleneck is the limited availability of CRM in different solvents and in a variety of concentrations.

Hence, the motivation of this work was to investigate the rapid in-house preparation of water standards for coulometric KFT. Water was extracted into three dry organic solvents (methanol, DMSO and ethyl acetate) from the stable, non-hygroscopic salt, sodium tartrate dihydrate, using a computer-controlled, laboratory ultrasonic transducer (SinapTec NexTgen Lab120). Ultrasound-assisted extraction was compared to a reference method, extraction by horizontal shaking.

Materials and Methods

A SinapTec NexTgen Lab120 laboratory ultrasonic transducer equipped with a 35 kHz US generator (NS1923-003) and a 6mm titanium probe for volumes between 2 to 50 mL (22D6) was used throughout the study (Fig.1.1). The computer-controlled ultrasonic instrument permits to record data and monitors the amplitude (i.e. the probe displacement), power dissipation, the frequency, the dissipated energy and (optionally) the sample temperature during the ultrasonic treatment in real time. Ultrasonication steps in this study were conducted with a fixed amplitude (70%) and variable times between 10s and 60s, set via a stop condition on the SinapTec software. As an alternative to time control, the software allows the total amount of energy delivered to be preset as a stop condition. This regime is particularly suitable for samples whose volumes and composition vary more than in the present study.

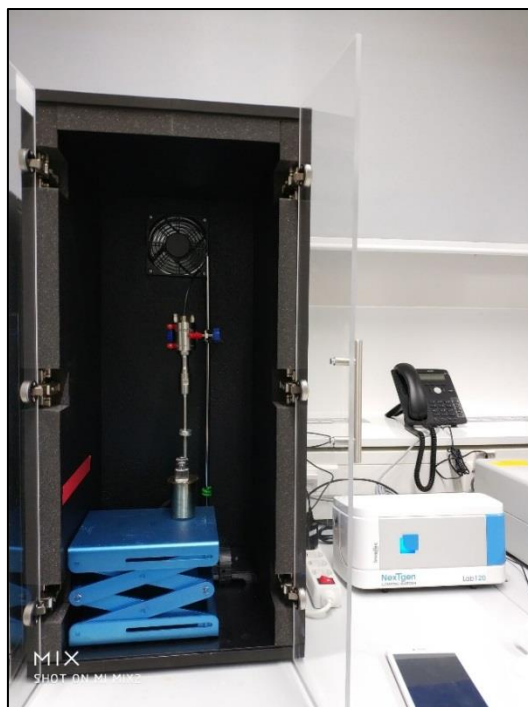


Fig. 1.1: Ultrasonic transducer SinapTec NextGen Lab120

A common horizontal shaker (Edmund Bühler, KL-2) with 300 rpm shaking frequency and up to 60 min shaking time was used as a reference. Shakers like this are commonly used for solubilization and dispersion of reagents in many laboratories (Fig. 1.2).



Fig. 1.2: Horizontal Shaker

Sodium tartrate dihydrate (Honeywell Hydranal, Product no. 34803-100G, Lot I054A, with certified water content 15.69 % (w/w) was used as reference material. Dry methanol (Honeywell Hydranal 34741-1L, Lot J086D, water content < 0,01 % (w/w), dry DMSO (Acros Organics 348441000, Lot1999470) and HPLC grade ethyl acetate (VWR 83621.320, Lot 14Z2631) were used as solvents. A typical water standard was prepared like this: 100 ± 2 mg sodium tartrate dihydrate was weighed into a 6 mL glass vial (Metrohm 62419007, KFT grade), flushed with N₂ and provisionally covered with an aluminum septum cap (Metrohm 61448050, KFT grade). 5 ± 0.1 mL solvent was added with a 5mL glass syringe, and the vial was covered again. The vial was then attached to the ultrasonic probe, in a centered position, 1mm distant from the bottom, and the probe was energized (default ultrasonication mode: fixed amplitude 70%, stop-condition after 30s). These conditions were chosen, to keep the sample temperature below 45°C. The dissipated power was monitored to prove the reproducibility of ultrasonication conditions between experiments. A silicon rubber gasket with a 6mm hole for the probe served to protect the sample from ambient air during ultrasonication. After this, the aluminum septum cap was permanently crimped on the vial, protecting the sample from ambient air and humidity until it was analyzed.

A computer-controlled, coulometric Karl Fischer titration system without diaphragm was used for all KFT experiments (Metrohm Coulometer 831 with KF-cell without diaphragm; software: tiamo 2.5 light). The cell was filled with 100 mL of KF reagent (Honeywell Hydranal "Coulomat AG Oven" 34739-500ML Lot 1260). The reagent was replaced after a total amount of 50 mL of sample has been added (i.e. after ca. 250 sample additions). Titration parameters were selected according to European Pharmacopoeia 2.5.32 (briefly: EP 55 mV; Dynamics 100 mV; Igen 400 mA; Ipol 10 μ A; Startdrift 10 μ g/min; Stopdrift 5 μ g/min; setting time 30s. A detailed protocol can be obtained from the authors).

Liquid samples with a fixed volume of 0.2 mL were injected manually through the injection port with a disposable 1 mL syringe and a 120mm x 0.8 mm hypodermic needle. Fig.2 illustrates the complete workflow from weighing and ultrasonication to KF titration. If not indicated otherwise, identical samples (i.e. same ultrasonication condition, same solvent and sodium tartrate dihydrate amount) were prepared in triplicate (N=3) and analysed with KFT once (n=1). This was justified, because preliminary experiments had shown that the relative standard deviation of KF titration was ca. 1% and thus significantly lower than the cumulated error during sample preparation (3-4%). Samples for the reproducibility and stability study were prepared in five instances (N=5), and each was analysed with KFT in triplicate (n=3), to obtain maximum precision.

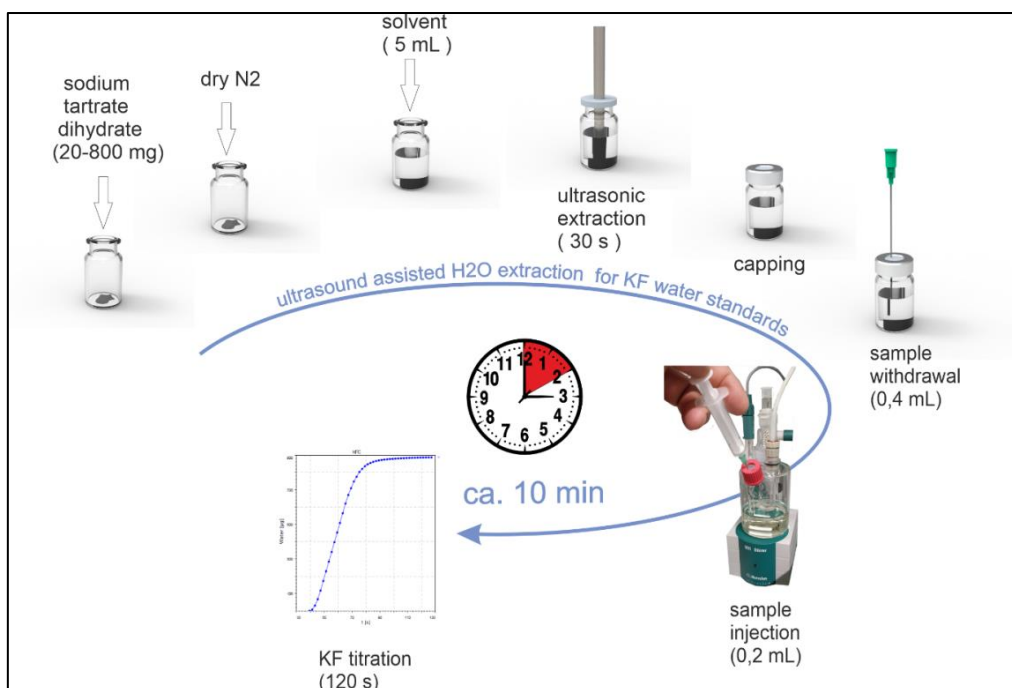


Fig. 2: Ultrasound-assisted preparation of water control standards (complete workflow duration including KF titration: ca. 10-15 min)

Results

Sodium tartrate dihydrate is a non-toxic, stable and non-hygroscopic organic salt with a stoichiometric water content of 15.66% and is commonly used as a secondary standard for titer determination in volumetric Karl Fischer titration. Its main drawback is the poor solubility in dry organic solvents and common KFT reagents (“anolytes”), most of them being methanol-based. Fig. 3 shows water standard preparations with sodium tartrate dihydrate ranging from 50 mg to 800 mg per 5 mL of methanol. As most of the salt is undissolved, it is impossible to assess visually, if and how much of the hydrate water has been extracted by the solvent. On that account, many laboratories have banned the use of sodium tartrate dihydrate and replaced it with costly commercial certified reference materials (CRM) with well defined characteristics. Our motivation was to investigate if the computer-controlled laboratory ultrasonic transducer SinapTec NextGen Lab120 can be used to prepare stable and precise water standards for coulometric KFT. The ultrasonication results were compared to a reference method, horizontal shaking of samples for 60 min.



Fig. 3: Sodium tartrate dihydrate in methanol. The salt is sparsely soluble in methanol, making it impossible to visually assess if water extraction has been completed.

Fig.4 illustrates the time-course of ultrasound-assisted water extraction from 100 mg sodium tartrate dihydrate with 5mL methanol (condition: fixed amplitude 70%, stop-condition at 30s). Nearly complete extraction was obtained after 30 s (solvent temperature = 42°C) (Fig. 5). Further ultrasonication up to the limit of 120s (2 x 60 s, separated by a 2 min break) led to more solvent heating (ca. 52° C), but did not increase the extraction yield. In contrast, it took 30-60 min of horizontal shaking at 300 rpm to reach the same extraction yield. About 40% of the stoichiometric water were extracted with either method under these conditions.

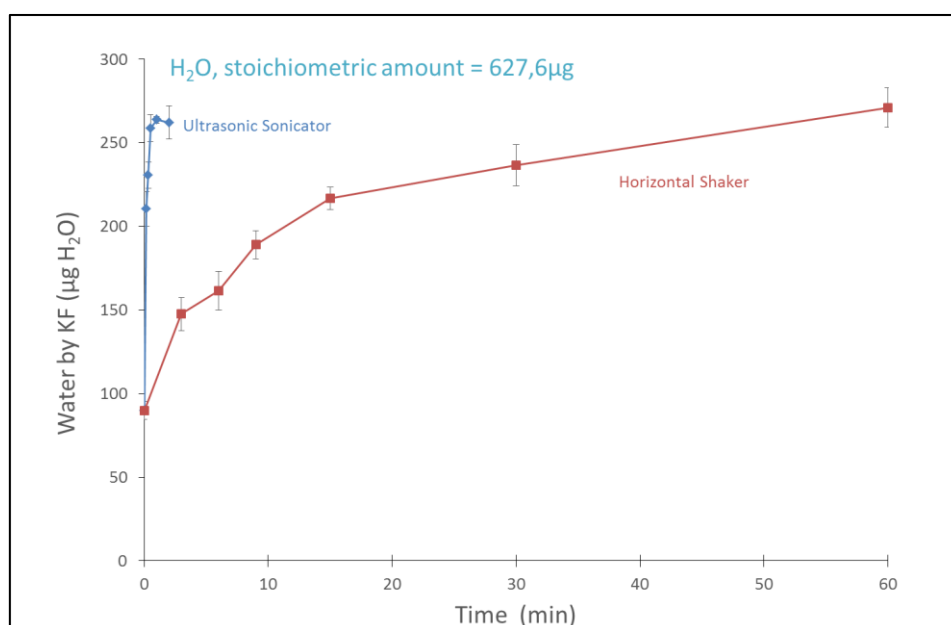


Fig. 4: Time-course of water extraction from 100 mg sodium tartrate dihydrate in 5mL methanol by ultrasonication (blue) vs. horizontal shaking (red). 3 samples per timepoint were prepared (N=3) and analysed by KF once (n=1). Maximum extraction reaches around 40% of the total hydrate water with this ratio of tartrate/solvent.

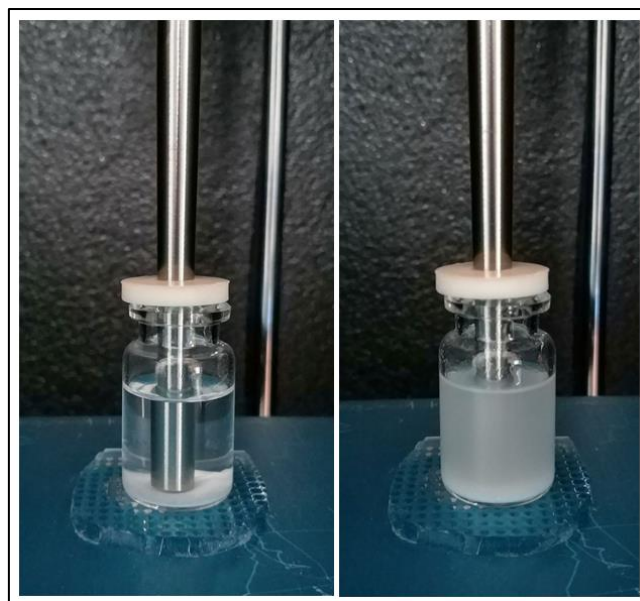


Fig. 5: Sodium tartrate dihydrate (100 mg in 5 mL methanol) before (left) and after (right) 30 s of ultrasonic treatment at 70% fixed amplitude. Temperature rises to 42°C after ultrasonication.

Fig. 6 illustrates the water concentration in ppm, measured in the supernatant by KFT, after preparation of water standards with increasing amounts of sodium tartrate dihydrate per 5 mL methanol. The ultrasonication is performed at 70% fixed amplitude and 30s duration. Shaking is performed at 300 rpm for 60 min. A complex extraction behaviour is observed with a marked difference between ultrasonication and shaking: Below 200 mg sodium tartrate dihydrate per 5 mL methanol, horizontal shaking extracts significantly more water. At higher sodium tartrate dihydrate amounts, the ratio is reversed: Ultrasonication is far more effective.

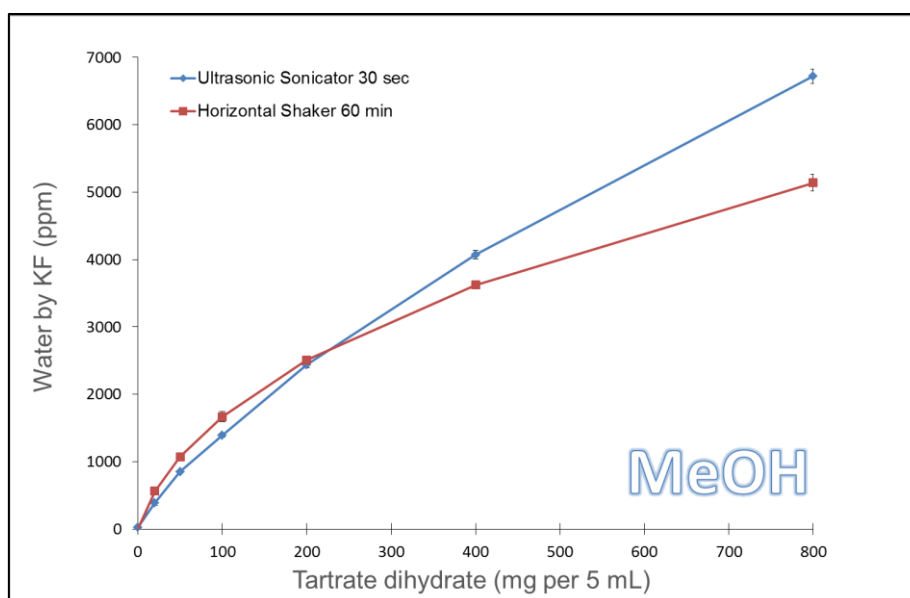


Fig. 6: Water control standards prepared in 5mL methanol (Y-axis: ppm i.e. $\mu\text{g/g}$). 3 samples per concentration were prepared ($N=3$) and analysed by KF once ($n=1$).

To further examine this unexpected result, the same data are replotted with the water recovery (% of stoichiometric water extracted) on the Y-axis (Fig. 7). In this representation, the different effect of ultrasonic energy becomes obvious: Starting from a maximum of 50 % at 20 mg tartrate, the recovery drops to ca. 20% at 800 mg. In contrast, in the shaking experiment, the starting value is 72% at 20 mg tartrate and it drops to 16% at 800 mg. We hypothesized, that due to the short duration of only 30s, ultrasonic treatment would extract only the outer hydration layer (“first water of hydration”) from sodium tartrate dihydrate, corresponding to 7.83% of the sample by weight (May et al. 1989). The first water of hydration represents 50% of the total stoichiometric water of sodium tartrate dihydrate (nominally 15.66%). In contrast, horizontal shaking extracts more than 50% of stoichiometric water at tartrate amounts below 100 mg per 5 mL solvent. The extraction efficiency with horizontal shaking drops quickly, however, when the amount of tartrate exceeds 200 mg per 5 mL methanol. The reproducibility of sample preparation was improving with increasing water concentration, for both extraction methods: At 100-800 mg sodium tartrate dihydrate per 5 mL, the standard deviation was minimal.

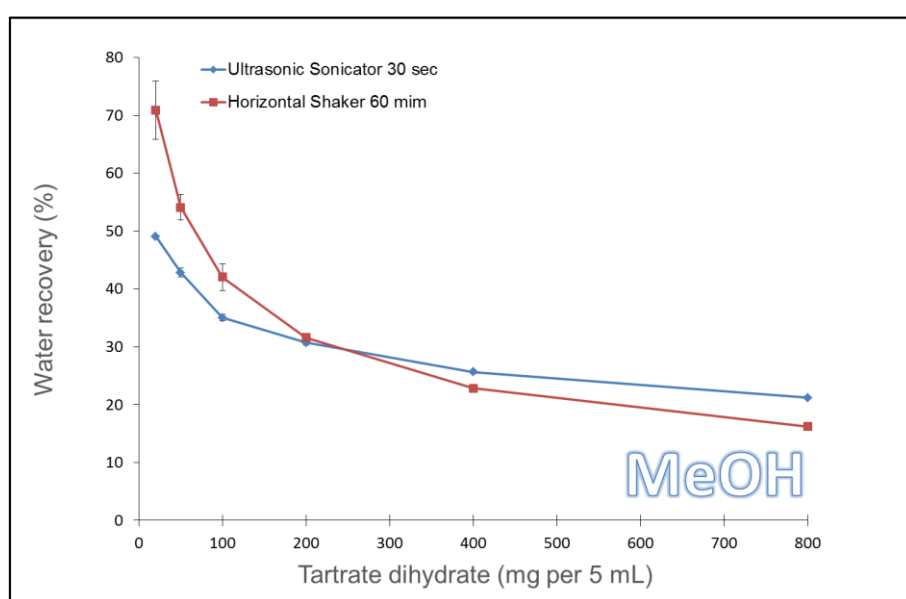


Fig. 7: Water control standards prepared in 5mL methanol (Y-axis: recovery (%) i.e. actual H₂O/ stoichiometric H₂O μ g/ μ g)

We investigated, if the observed difference in water extraction between ultrasonic treatment and shaking was specific to methanol, or was a general effect. To this end, the same experiment (Fig. 6 and Fig.7) was repeated with DMSO and ethyl acetate as the solvent.

Fig. 8 illustrates water extraction in DMSO as the solvent, under the same conditions as Fig.6. DMSO is a non-toxic, polar solvent and could be a less toxic alternative to methanol. In DMSO, ultrasound-assisted extraction performs better than shaking, at all amounts of tartrate tested. If the same data are replotted with the water recovery on the Y-axis (% water extracted, relative to stoichiometric amount), a striking feature can be observed (Fig. 9): At low tartrate per DMSO ratio and ultrasonic treatment, the recovery approaches 100%. This means that nearly all of the stoichiometric water are extracted by DMSO under ultrasonic treatment. It can be visually observed, that sodium tartrate dihydrate swells and assumes a transparent look, when in contact with DMSO. Interestingly, the maximum water recovery with shaking does not exceed 50% with DMSO as the solvent. The different behaviour was not expected. As with methanol, the reproducibility of sample preparation was

improving with increasing water concentration, irrespective of the extraction method: At 100-800 mg sodium tartrate dihydrate per 5 mL, the standard deviation was minimal.

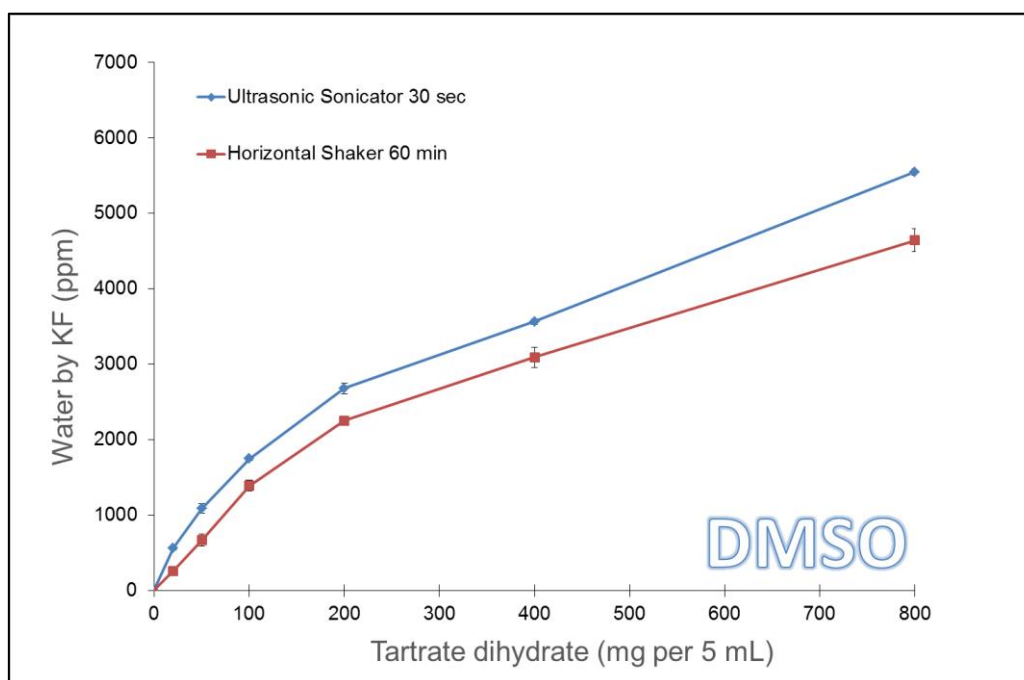


Fig. 8: Water control standards prepared in 5mL DMSO (Y-axis: ppm i.e. $\mu\text{g/g}$). 3 samples per concentration were prepared ($N=3$) and analysed by KF once ($n=1$). DMSO could be a less toxic alternative solvent to methanol. Its water extraction capacity is comparable to methanol.

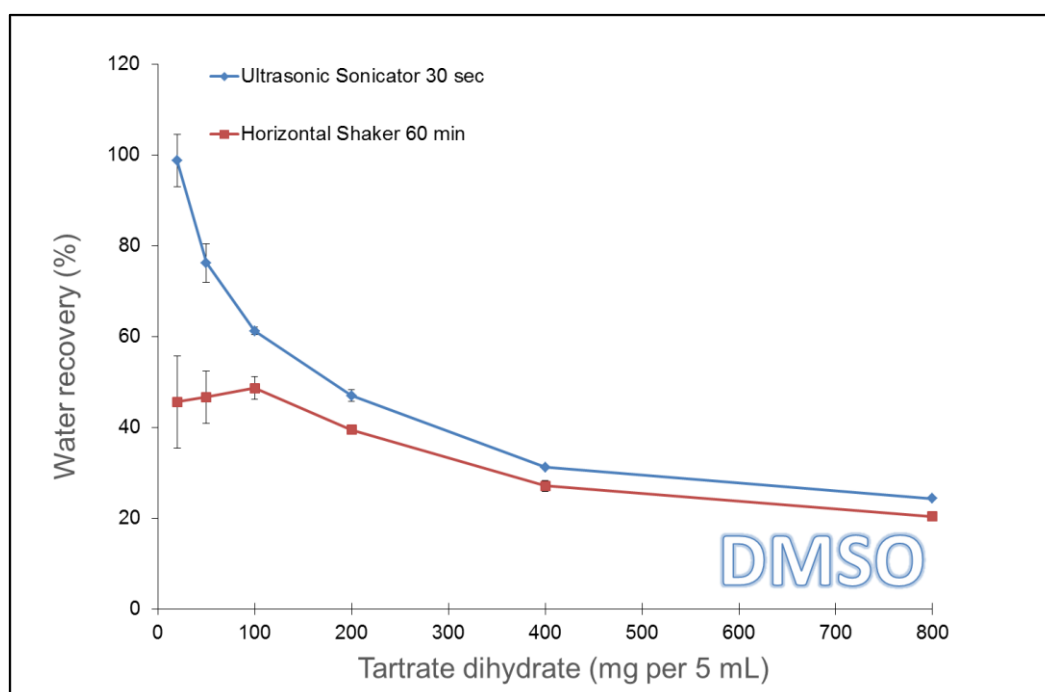


Fig. 9: Water control standards prepared in 5mL DMSO (Y-axis: recovery (%) i.e. actual H_2O / stoichiometric H_2O $\mu\text{g}/\mu\text{g}$)

Fig. 10 illustrates water extraction in ethyl acetate as the solvent, under the same conditions as with Fig. 6. This solvent is less toxic than methanol, and much less polar. However, the extracted amount of water is minute, no matter if ultrasonication or shaking is used. Likewise, the error of KFT determination is high, especially with ultrasonic extraction. Ethyl acetate is not suitable as a solvent for KFT.

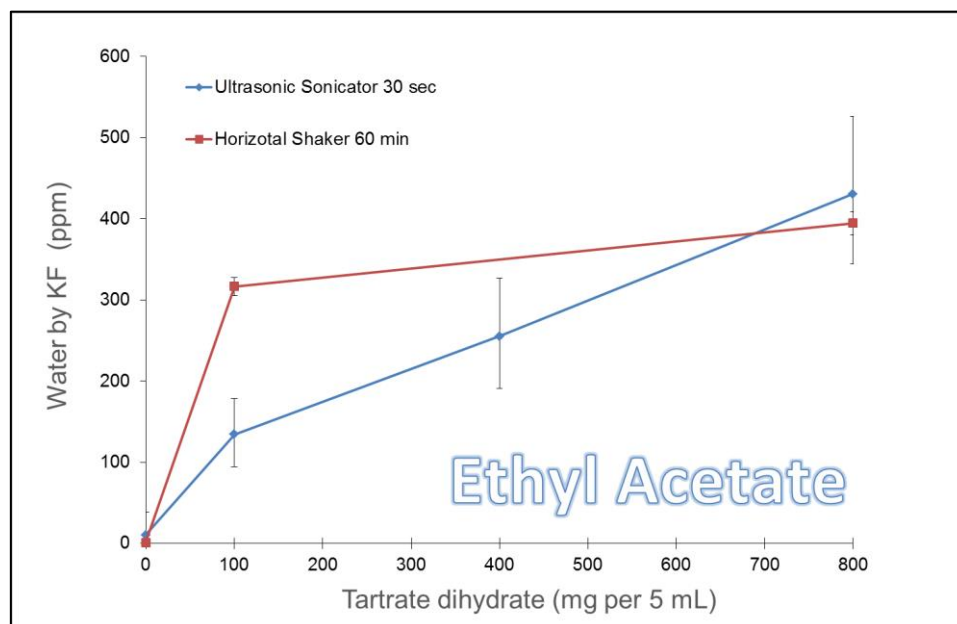


Fig. 10: Water control standards prepared in 5mL ethyl acetate (Y-axis: ppm i.e. $\mu\text{g/g}$). 3 samples per concentration were prepared ($N=3$) and analysed by KF once ($n=1$).

Ethyl acetate is a less toxic and less polar solvent than methanol. Its water extraction capacity is very low, however. It is not suitable for the preparation of water standards.

Up to this point both methanol and DMSO seemed to be equally suitable solvents for the rapid preparation of water standards by ultrasonic treatment. However, the standard deviation of samples prepared in methanol was slightly lower than in DMSO ($7.7 \mu\text{g}$ in MeOH vs. $9.9 \mu\text{g}$ in DMSO, $N=6$), probably because titration of DMSO standards lasted significantly longer and DMSO tended to form bubbles while handled with a 1 mL syringe. Also, DMSO has been described to interfere with some KF reagents in literature. Because of that, we decided to prepare water control standards from 100 mg sodium tartrate dihydrate per 5 mL methanol using an ultrasonic protocol of 70% fixed amplitude for 30 s for fast water extraction. To assess the reproducibility of the protocol, 5 samples were prepared under the same conditions ($N=5$) and analyzed by KFT in triplicate ($n=3$). Fig. 11 illustrates the effective power delivered by the ultrasound system during the reproducibility experiment. Fig. 12 (right) data from the reproducibility experiment. The water concentration was $1666 \pm 28 \text{ ppm}$ ($N=5$, $n=3$).

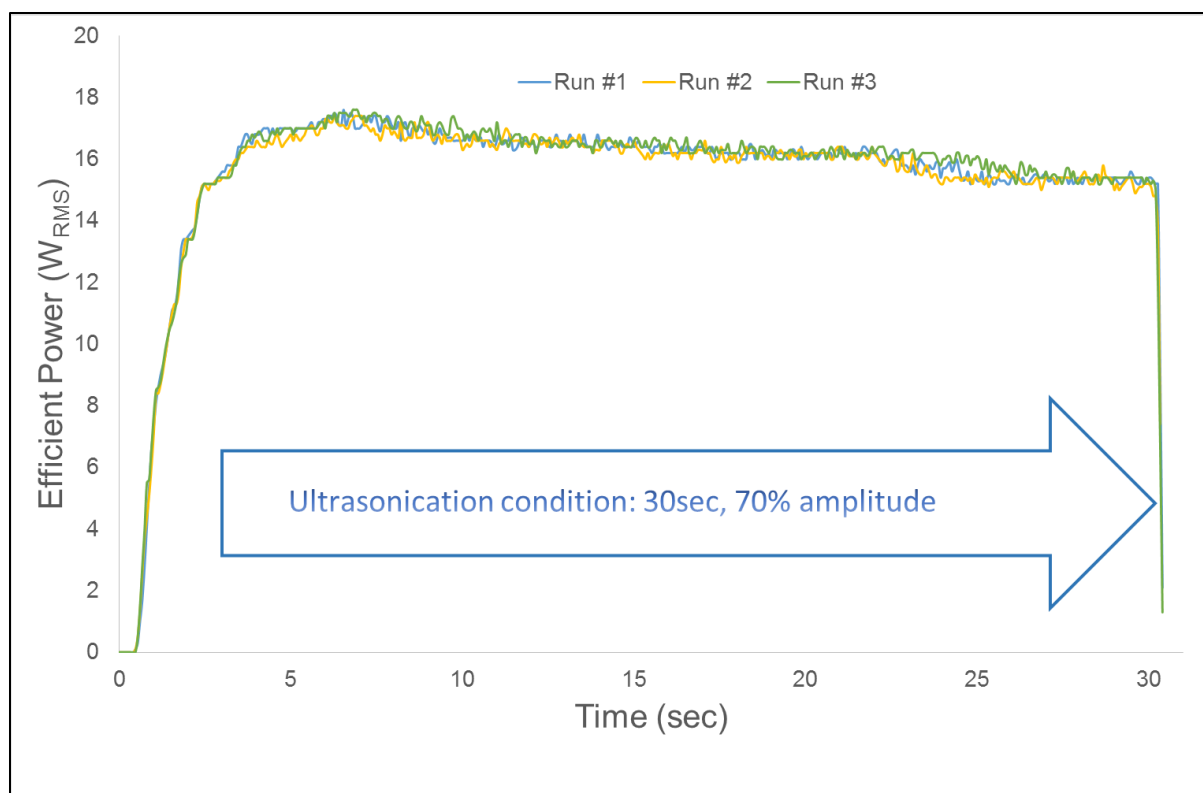


Fig. 11: Ultrasonic power generation with the NexTGen Lab120 homogenizer is highly reproducible: 3 water control standards in methanol are prepared under identical conditions (100 mg sodium tartrate dihydrate per 5 mL methanol. Ultrasonic protocol: fixed amplitude 70%, duration 30 s)

Likewise, the stability of prepared water standards was assessed: 5 samples were prepared ($N=5$) and analyzed by KFT in triplicate ($n=3$) after 0 h, 2h, 4h, 8h and 10 h storage at 25°C. The experiment aimed to check the stability of freshly prepared standards during a working day. Fig. 12 (left) illustrates the data of the stability experiment. No significant change of the water standard was noticed within the tested period of 10 h (water concentration 1698 ± 51 ppm ($N=5$, $n=3$)).

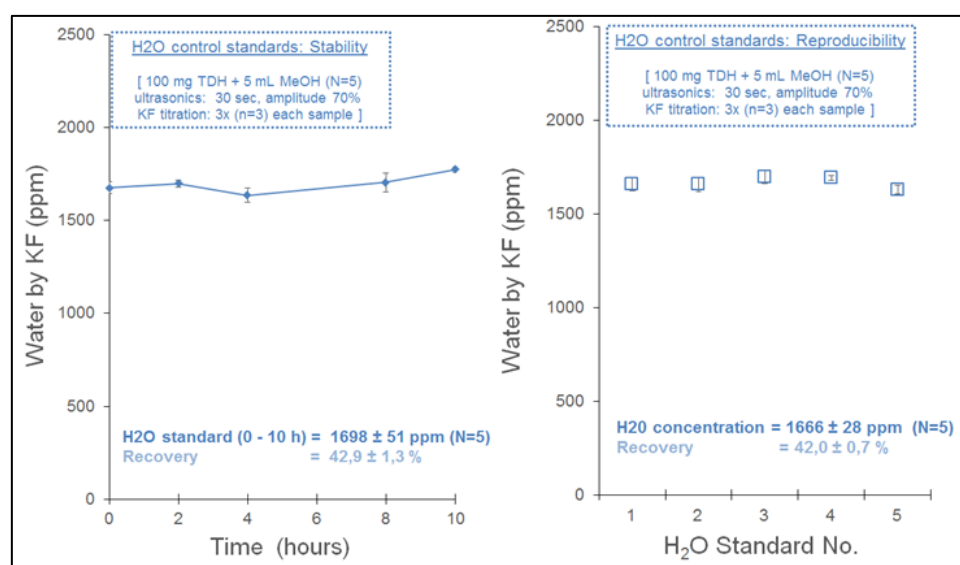


Fig. 12: Reproducibility and stability of 5 water control standards prepared with ultrasonic extraction under identical conditions. 5 samples were prepared ($N=5$) and analysed by KF three times ($n=3$).

Summary

Ultrasound-assisted extraction of water from sodium tartrate dihydrate in methanol and DMSO proved to be an efficient, time-saving alternative to horizontal shaking, a common laboratory method, for the ad-hoc preparation of water standards. A 30 s ultrasonication step with fixed amplitude (70%) gave comparable extraction performance as 60 min horizontal shaking in all three tested solvents. With ultrasound-assisted extraction, the complete workflow including weighing, extraction and titration, can be accomplished within 10-15 min. This is a significant improvement and results in noticeable time and cost savings compared to the classic workflow including horizontal shaking. Interestingly, neither of the methods (or solvents) was able to release 100% of the stoichiometric water from sodium tartrate dihydrate portions between 50 mg and 800 mg (dispersed in 5 mL solvent). The exact recovery depended on the amount of tartrate salt per 5 mL solvent (higher recovery at low amounts), on the solvent and the extraction method (ultrasound or shaking). While it may seem attractive at first sight to use low amounts of 20-50 mg tartrate salt and DMSO as the solvent, to achieve a high recovery, our experiments have clearly shown that this approach is erroneous: The standard deviation of sample preparation is much larger at low tartrate amounts below 100 mg per 5 mL solvent. Weighing errors and, even more importantly, KF titration errors are predominant. This has to be taken into account, if water standards are to be prepared from sodium tartrate dihydrate. We found that stable water standards can be prepared in methanol with high reproducibility and accuracy, if the ultrasonic treatment is short (e.g. 30 s at fixed amplitude 70%) and the sodium tartrate dihydrate amount per 5 mL solvent is in the range between 100 mg and 400 mg. Under these conditions the temperature during sonication rises to max. 42-45°C, while an effective water extraction is achieved (recovery 40-42%). Based on the experimental data, we hypothesize that a 30s ultrasonic treatment in methanol extracts only the “first water of hydration”, similar to isothermal drying under vacuum (May et al. 1989). The “first water of hydration” corresponds to 50% of the stoichiometric amount of 15.66 % or 7.83% of sample weight. If this assumption can be confirmed in further studies, a short but intensive ultrasonic treatment of sodium tartrate dihydrate in methanol may be the best strategy for the preparation of precise water standards for coulometric Karl Fischer titration.

Literature

May JC, Wheeler RM, Grim E; The Gravimetric Method for the Determination of Residual Moisture in Freeze-Dried Biological Products (1989) Cryobiology 26, 277-284



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