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# HYDRANAL™ Technical Information Sheet T006 Rev. 1

## Handling of Freeze-dried (Lyophilized) Samples

Medicines for intravenous injections are sometimes supplied as dry substrates in order to prolong their shelf life. The residual water content significantly affects shelf life and can be specified to be even lower than <100 µg $H_2O$ per vial. The determination of water at such low levels can be difficult, mostly because of high hygroscopicity of such material and possible contamination by extraneous moisture once the vial seal has been broken. Thus, the sample needs to be dissolved directly in original vial and transferred to the titration cell without opening of the vial. Addition of formamide can improve the solubility of solid samples.

For the sample transfer from the vial to the titration vessel we recommend two possible procedures.

**Procedure 1 using co-solvent for sample transfer:**
Prepare the titration cell according to standard procedure. For the volumetric cell, use a combination of Hydranal-Composite with Hydranal-Methanol dry or Hydranal-Methanol Rapid or a combination of Hydranal-Titrant with Hydranal-Solvent. For the coulometric cell, use Hydranal-Coulomat AG as anolyte (and Hydranal-Coulomat CG as catholyte in case of the cell with diaphragm).
By means of a syringe with long needle (ideally glass one[1]) inject co-solvent (Hydranal-Methanol dry or Hydranal-Formamide dry or other solvents which suitability is proven) through the lyophilization stopper (septum) into the vial until the sample is fully dissolved (shaking or vibroshaking may be necessary).
Place additional thin needle in the vial septum to release pressure caused by the injection into a closed vial.
Then take the whole solution (co-solvent containing the sample) into a fresh syringe with long needle, inject completely into a pre-titrated volumetric or coulometric cell and determine the water content according to standard procedure.
After blank correction (consider Note 1) this procedure provides the absolute amount of water of the specific vial. If the water content is required in relation to the sample size, the individual vial must be pre-weight, cleaned, dried and back-weight to get the actual sample size for calculation.

**Note 1:** A blank correction for the co-solvent's water content is necessary. Ideally, this blank value is not determined directly from the co-solvent's storage bottle. To obtain a realistic blank value which includes the blank of the vial, the solvent, the syringe and the whole handling, it is rather recommended to simulate the whole dissolution procedure in parallel in pure empty vial without sample. If a series of vials shall be measured, a syringe (ideally glass one) can be rinsed with the co-solvent and such prepared syringe can be used to transfer the co-solvent into all vials: vials with samples and the blank vial. Time interval should always be the same. To allow a perfect calculation, the co-solvent should not be handled by volume but rather by weight.

[1] The recommended glass syringe is described in Technical Information Sheet T007.

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**Procedure 2 using pre-titrated vessel solution for sample transfer:**
Prepare the titration cell according to standard procedure. For the volumetric cell, use a combination of Hydranal-Composite with Hydranal-Methanol dry (or 1:1 v/v mixture of Hydranal-Methanol dry and Hydranal-Formamide dry) or a combination of Hydranal-Titrant with Hydranal-Solvent (or 1:1 v/v mixture of Hydranal-Solvent and Hydranal-Formamide dry). For the coulometric cell, use Hydranal-Coulomat AG as anolyte (or 80 mL Hydranal-Coulomat AG and 20 mL Hydranal-Formamide dry[2]). Cell with diaphragm needs additionally Hydranal-Coulomat CG in the cathodic compartment.
Take approx. 5 mL of the conditioned (dried) vessel solution in a 5 mL long-needle syringe (ideally glass one[1]) and immediately re-inject whole portion to the cell (without taking out the syringe). The titrator indicates the moisture adhering to the syringe and show a certain drift. Repeat purging the syringe until no further drift increase is observed.
Take again 5 mL of dry vessel solution into the pre-dried syringe and inject through the lyophilization stopper (septum) into the sample vial. The sample dissolves/suspends upon shaking. While shaking, the syringe should not be removed from the vial.
Place additional thin needle in the vial septum to release pressure caused by the injection into a closed vial.
Then immediately take the whole sample solution/suspension back into the syringe, inject whole volume into the titration vessel and determine the water content according to standard procedure.
This procedure provides the absolute amount of water of the specific vial (consider Note 2). If the water content is required in relation to the sample size, the individual vial must be pre-weight, cleaned, dried and back-weight to get the actual sample size for calculation.

**Note 2:** In this case the blank value of the vessel solution used to dissolve/disperse the sample and the blank of the syringe can be estimated as nearly zero. However, the blank of the handling and the empty container should be considered. For this purpose, the vessel solution is used in the same way as described above but in pure empty vial without sample (ideally the vial should come from the same lyophilization process but without sample material).

**Direct use in KF Oven**
Newer models of Karl Fischer ovens allow vials to be heated and investigated directly. Such titrations will give as a result the absolute amount of water in the vial, which can be compared with other batches. If a concentration of water is needed, an average mass of the sample needs to be determined separately (by weighing the content of few vials). To work out a proper oven method for individual sample types, further instruction/recommendation is available on request.

Due to inhomogeneity of freeze-dried samples, the water determination should be repeated on several vials. The standard deviation of water results for freeze-dried samples is higher than for normal samples.

Please check our Laboratory Report L725 for an example of detailed protocol.

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[2] Addition of formamide is possible only in cells without diaphragm.