

Headspace Moisture and Water Activity Applications for Lyophilized Product

Rapid non-destructive moisture determination using headspace analysis

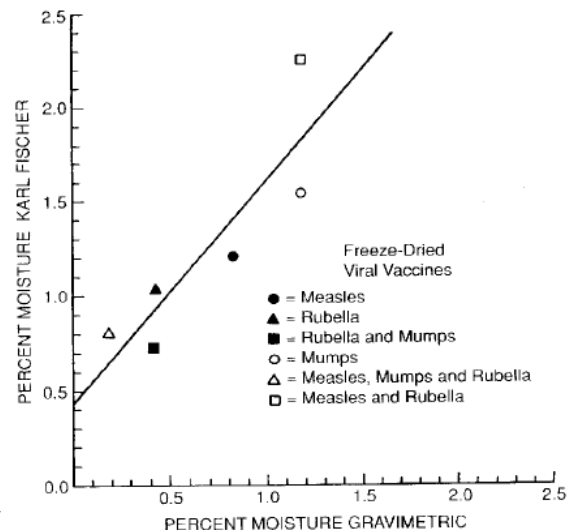


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- Thirty years ago the gravimetric or loss-on-drying method was the standard moisture determination method for freeze-dried pharmaceutical product.
 - Karl-Fischer (KF) titration used more frequently as a new method starting in the early 90's.
 - Total moisture content measured by both methods was different – so people started with correlation studies.

[Ref.] *Measurement of Final Container Residual Moisture in Freeze-Dried Biological Products*, International Symposium on Biological Product Freeze-Drying and Formulation, Bethesda, USA, 1990; *Developm. Biol. Standard.*, Vol. 74, pp. 153-164 (Karger, Basel, 1991)



- Disadvantages of the standard moisture determination methods:
 - Risk of moisture contamination is high - methods dependent on operator skill
 - Methods are time & resource intensive
 - Methods are destructive meaning high value samples are lost



Very difficult (impossible) to analyze statistical sample sets of finished product:

- **Insight into the drying process is limited**
- **Accuracy of moisture stability specifications also limited** due to destructive analysis and assumptions about identical stability samples

Loss on drying → KF Titration → NIR? → Find a better method?

Why? Regulator- understand your process & product, 'statistical confidence in the process'

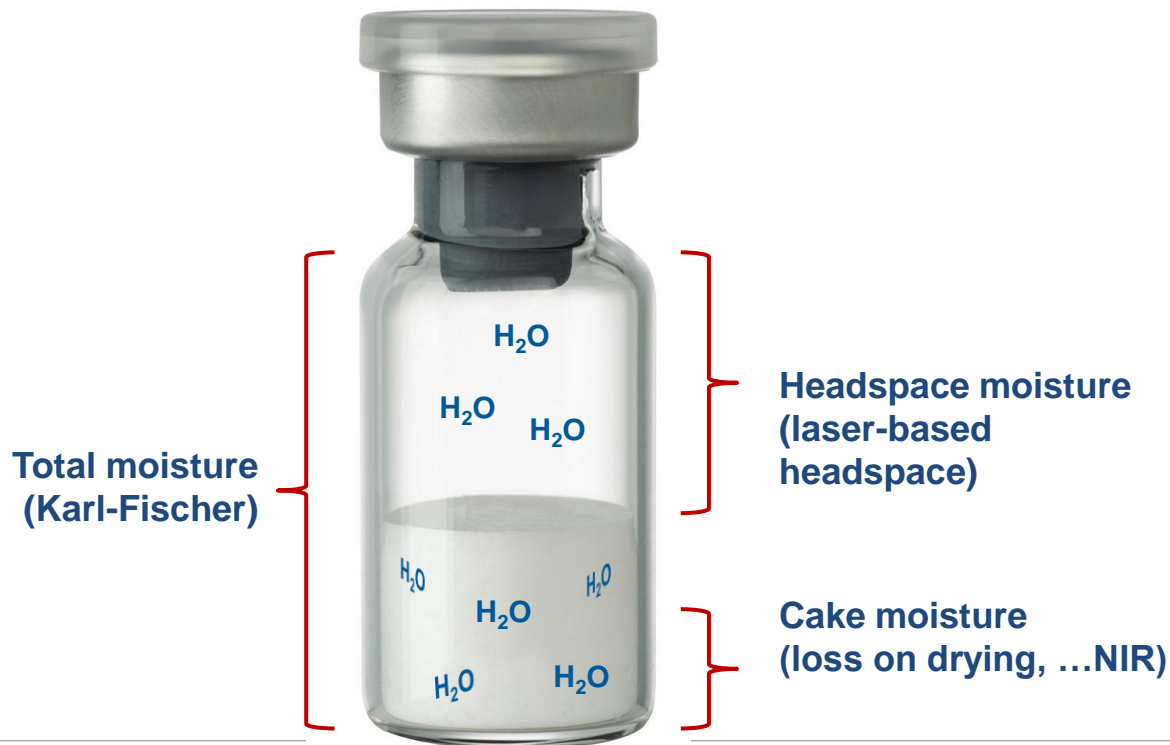
Agenda

- Headspace moisture analysis - Introduction
 - Comparison to standard moisture methods
 - Relation to water activity

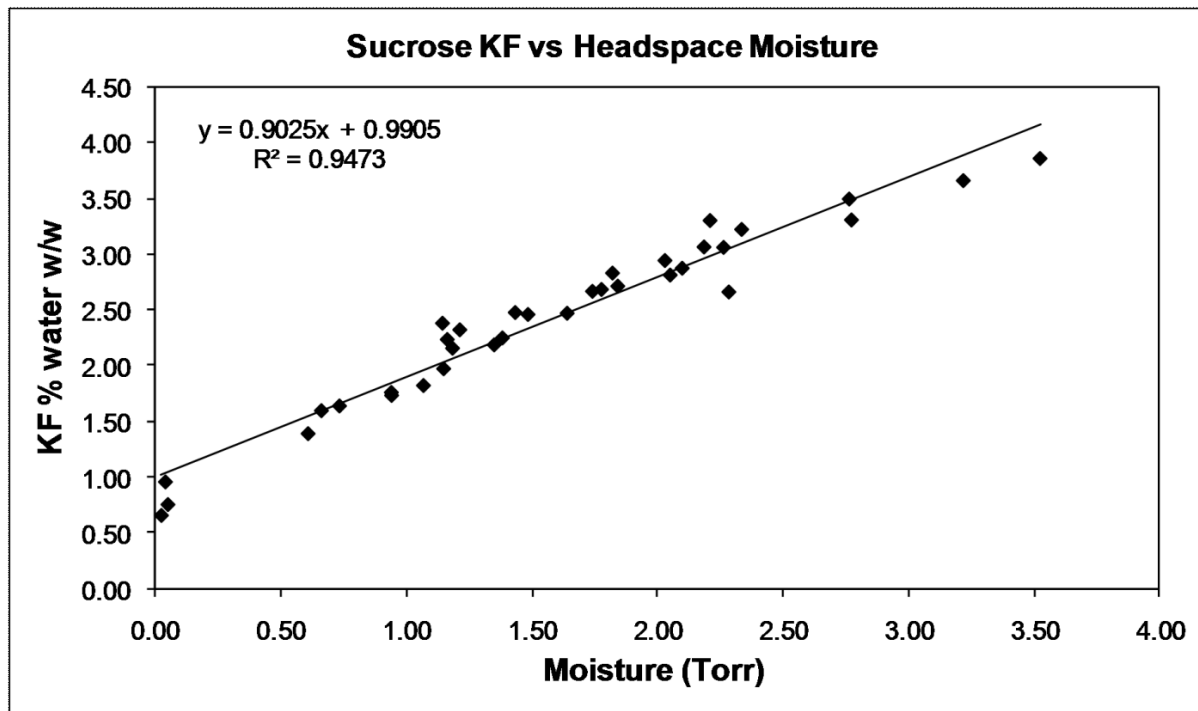
- Application examples / Industry case studies
 - Formulation development
 - Process optimization and scale up
 - Manufacturing & QC

- Q & A

Headspace Moisture



Headspace Moisture – correlation with total moisture content



Water activity definition – thermodynamic property

- **Water activity**, or a_w is the **partial vapor pressure of water** in a substance divided by the standard state partial vapor pressure of water.
 - Indicates how much ‘free’ water is available for chemical reaction
 - A water activity of 0.80 means the vapor pressure is 80% of that of pure water

USP<922> Water Activity implemented in Q1 2021.

- “Total water content is an important quality attribute...However, water may be allocated in more than one compartment within these materials. Some of it may be tightly bound and not available to participate in chemical, biochemical, or physicochemical reactions (e.g., as hydrate salts), whereas some of the water may be more loosely bound and more freely available to participate in reactions... It is important to establish what fraction of the total water is contained in the latter category, and the determination of water activity (a_w) provides this information.”
- “The determination of a_w aids in decisions during ingredient and product processes design, ingredient selection, packaging selection, and product storage. These include:
 - ...
 - Reducing the degradation of active ingredients within product formulations, especially those susceptible to chemical hydrolysis
 - ...
 - Providing a complementary method to the Karl Fischer titration for monitoring changes in water content
 - Controlling and monitoring physical, chemical, and microbial product stability
 - ...”

Laser light matches absorption frequency of water vapor molecule.

Amount of absorbed laser light is dependent on partial water vapor pressure in headspace.



Non-destructive (product not lost, sample can be re-measured)

Rapid (~1 sec)

- Measurement of the water vapor pressure in a sealed container at a defined temperature is a water activity determination.
- Measurement concept can be applied to general containers containing dry material.
- **There will be a trend to replace some total moisture measurements with a water activity determination – USP <922>.**

Example Headspace Platforms for Non-destructive Lyo Moisture Analysis

Benchtop instruments: Laboratory and At-line Instruments & accessories



Inspection Machines: Automated, compact and flexible



For process development and scale-up

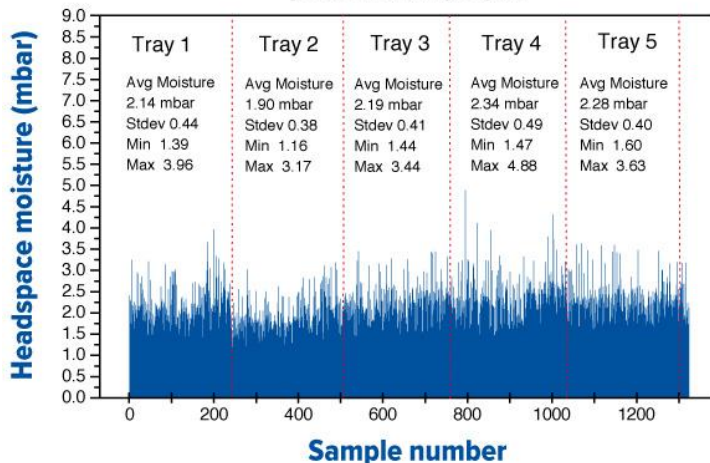
- Driving quality in the pharma industry:
 - Regulators asking for more analytical data (QbD, validation, monitor & control)
 - **FDA** process validation guidance: **‘Statistical confidence in the process’**
 - ‘Collection and evaluation of data, from the process design phase throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products.’
- Analysis of **statistically relevant sample sets of finished product** gives insight enabling decisions that are justified by science-based data
- There is no ‘gold standard’ moisture method – need broader toolbox!



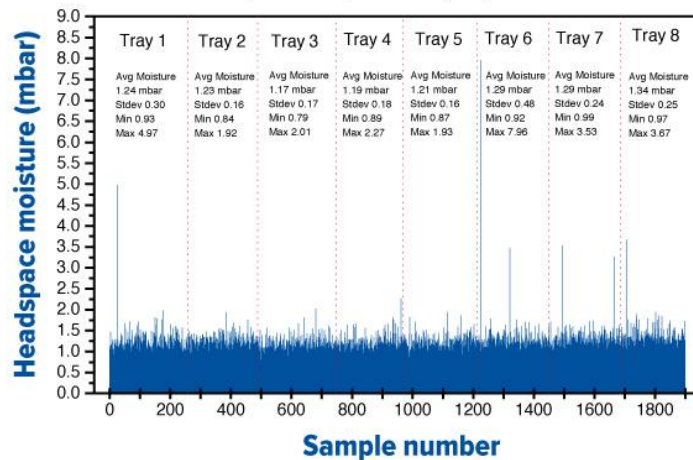
Example: Robust efficient lyo cycle optimization

- Headspace water vapor correlates to product dryness
- 100% headspace moisture determination to characterize and optimize freeze drying cycle

Headspace moisture as a function of tray position Initial lyo cycle



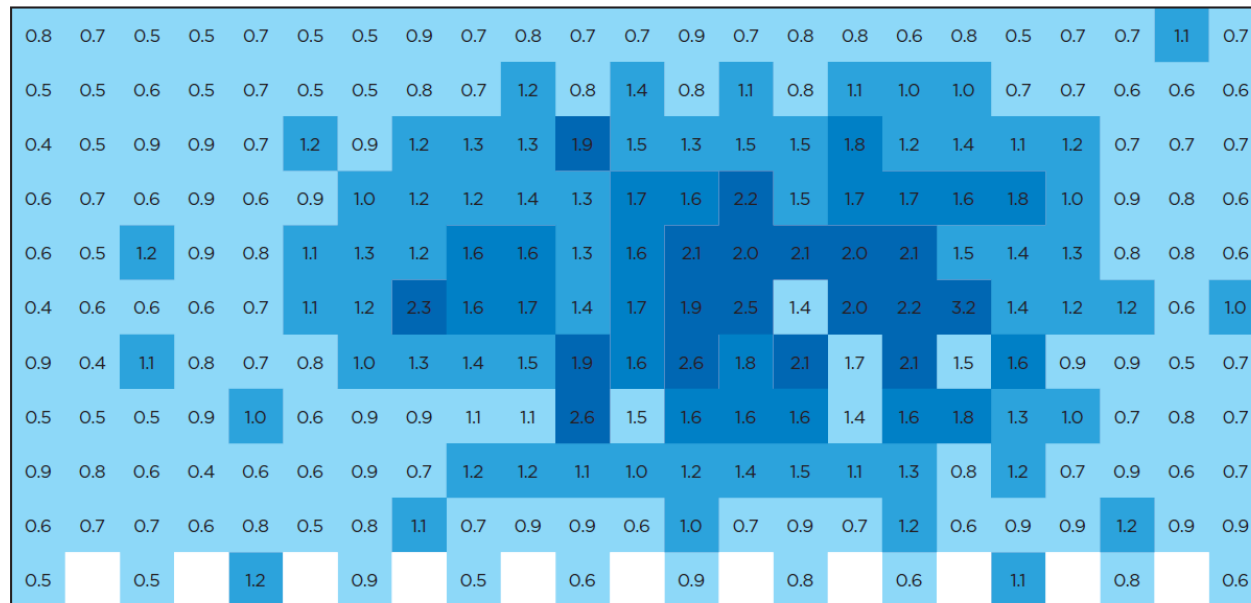
Headspace moisture as a function of tray position Optimized lyo cycle



Total analysis time (both batches): ~ 1 day!

Low value 0.4 torr = 1% KF
 High value 3.2 torr = 4% KF

- Track vial location on the shelf
- Perform 100% headspace moisture analysis to produce shelf moisture map

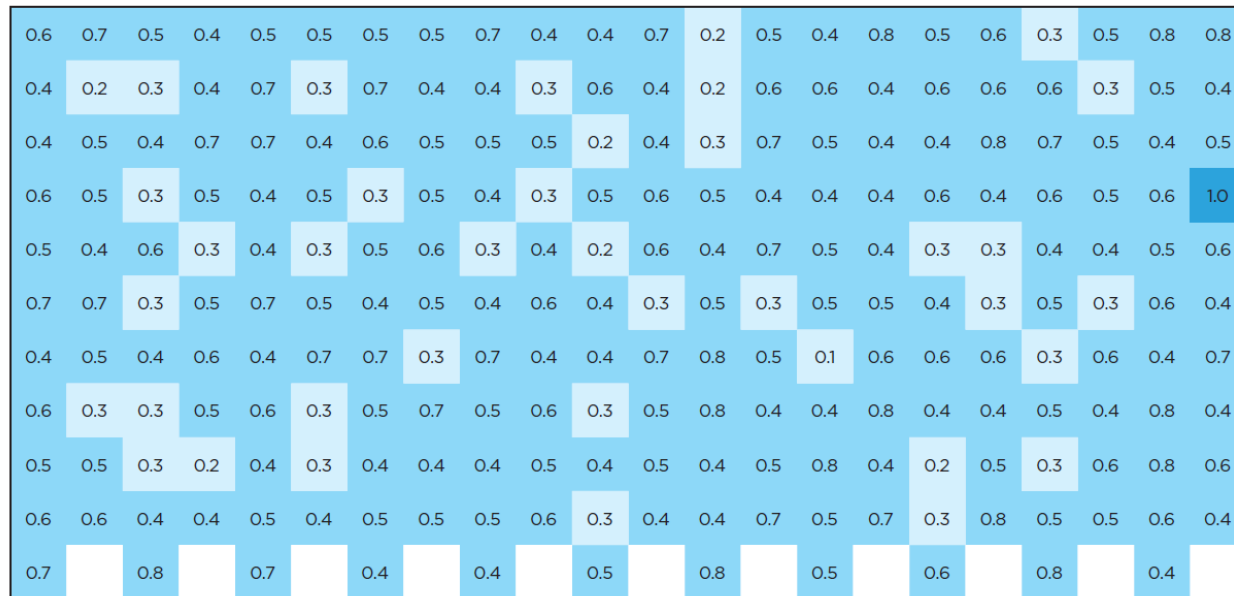


Colour key 0-0.3 0.4-0.9 1.0-1.5 1.6-1.9 2.0-3.2 Data courtesy of Biopharma Technology Ltd

Headspace moisture plot shows 'wet spot' in center of shelf

Low value 0.2 torr = 1% KF
 High value 1.0 torr = 1.8% KF

- Same cycle produced 2nd batch
- Improving heat transfer to the vials resulted in homogenous moisture distribution.



Colour key 0-0.3 0.4-0.9 1.0-1.5

Data courtesy of Biopharma Technology Ltd

Total analysis time per shelf of pilot freeze dryer < 1 hour!

- Model: 5% sucrose solution, 6R vials, 2.5 ml fill
- Full headspace moisture analysis of shelf:
 - Water vapor range of 0.65 to 2.04 mbar correlated to 0.8 to 1.4 % (KF)
 - Headspace results clearly show an inhomogeneous batch
- Edge effects front and side result in lower values at front and side positions (acrylic door, covered with stainless steel plate)

FD02	5% sucrose														
Sh2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0,958	0,811	0,812	0,848	0,852	0,859	0,844	0,876	0,849	0,868	0,831	0,897	0,995	1,032	1,106	
0,843	0,866	0,764	0,911	0,807	0,825	0,832	0,853	0,91	0,96	0,831	0,88	0,918	0,888	1,01	
0,731	0,895	0,865	0,802	1,126	0,837	0,823	1,062	1,061	0,914	1,221	0,83	0,981	0,967	0,945	
0,783	0,825	0,818	1,065	0,808	0,868	1,09	0,93	0,901	1,446	0,837	0,952	0,843	0,929	0,898	
0,719	0,847	1,06	1,132	0,821	1,116	0,954	1,141	0,976	1,137	1,027	0,888	1,087	0,835	0,807	
0,986	0,791	0,845	0,774	1,6	1,43	0,941	1,236	0,966	0,888	1,05	0,899	0,997	0,837	0,771	
0,718	0,766	0,839	0,794	0,817	1,135	0,921	0,767	1,504	0,892	2,035	1,109	0,921	0,77	0,689	
0,71	0,72	0,917	0,845	1,461	0,988	1,238	1,323	1,073	1,373	1,107	0,949	0,75	0,801	0,683	
0,704	0,922	0,946	0,724	1,647	1,015	1,308	1,466	1,213	0,787	0,881	0,797	0,855	0,687	0,752	
0,681	0,709	0,747	0,84	0,705	1,097	1,165	0,982	1,868	1,265	1,213	1,099	1,083	0,742	0,677	
0,76	0,879	0,734	1,399	1,297	0,808	1,351	1,31	1,007	0,93	1,093	1,275	1,014	0,762	0,743	
0,706	0,724	0,823	0,79	0,767	1,087	1,431	1,277	1,081	0,789	1,44	0,965	0,76	0,717	0,657	
0,69	0,725	1,015	1,073	1,471	0,735	1,331	1,126	0,999	1,016	1,155	0,918	0,662	0,785	0,721	
0,649	0,724	0,719	0,755	1	1,226	1,686	0,723	0,991	0,702	1,087	1,017	0,877	0,809	0,859	
0,677	1,165	1,158	0,835	0,832	1,394	0,846	1,432	1,314	0,903	0,774	0,987	0,792	0,747	0,684	
0,657	0,808	0,769	0,878	0,806	1,108	1,27	1,313	0,829	0,714	1,261	0,832	1	0,709	0,703	
0,692	0,902	0,756	0,921	1,156	0,846	0,933	0,928	0,766	0,749	0,835	1,265	0,748	0,685	0,726	
0,654	0,699	0,721	0,886	0,884	0,723	0,832	0,785	1,173	0,775	0,837	0,745	0,745	0,734	0,764	
0,695	0,672	0,783	0,754	0,799	0,876	0,765	0,81	0,986	0,728	0,69	0,771	0,678	0,843	0,813	
0,656	0,711	0,665	0,737	0,853	0,729	0,772	1,037	0,827	0,733	0,764	0,781	0,725	0,758	0,769	
0,707	0,695	0,659	0,698	0,657	0,754	0,746	0,813	0,797	0,67	0,736	0,794	0,791	0,787	0,69	
0,746	0,713	0,844	0,758	0,773	0,7	0,699	0,793	0,814	0,733	0,718	0,728	0,802	0,693	0,755	

- Model: 5% sucrose solution
- Full headspace moisture analysis of shelf:
 - 0.18 to 3.79 mbar, i.e. 0.6% to 2.2% KF
 - More inhomogeneous batch
- Cake appearance acceptable, minor shrinkage in some vials
- Edge effects front, back and side result in lower values (stainless steel door)

FD04 5% sucrose

SH2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.379	0.358	0.434	0.389	0.361	0.378	0.366	0.357	0.184	0.465	0.37	0.366	0.38	0.388	0.399	
0.362	0.342	0.481	0.361	0.724	0.401	0.483	0.433	0.369	0.647	0.369	0.433	0.483	0.469	0.339	
0.321	0.514	0.411	1.765	0.691	0.556	0.942	1.684	1.448	0.867	1.779	0.76	1.103	0.398	0.369	
0.414	1.166	2.279	0.701	0.936	1.52	1.245	1.912	0.644	0.599	0.944	1.194	0.734	0.627	0.351	
0.314	0.434	1.781	2.45	1.222	0.978	1.355	0.609	2.214	1.23	2.338	1.149	1.935	0.746	0.448	
0.435	0.851	1.363	0.657	1.437	0.727	1.364	2.411	1.397	2.607	2.705	1.178	1.808	0.552	0.32	
0.39	0.439	0.515	2.332	1.569	2.863	3.314	0.796	0.815	0.671	2.009	2.124	0.806	0.435	0.356	
0.419	1.044	1.344	0.868	2.009	2.887	2.36	1.791	2.045	2.154	1.073	0.881	0.738	0.548	0.343	
0.324	0.51	2.236	1.741	0.861	1.579	3.786	2.423	0.558	2.298	0.857	3.309	1.881	0.669	0.408	
0.408	0.421	1.537	2.209	2.348	0.604	3.19	1.626	1.831	2.212	0.795	2.845	1.278	0.493	0.325	
0.305	1.504	1.257	1.083	1.698	1.799	2.104	1.63	1.624	2.199	2.284	0.896	2.069	1.529	0.395	
0.488	0.804	0.504	3.424	0.602	3.228	1.459	2.468	2.233	1.008	1.876	0.701	2.491	0.747	0.483	
0.336	0.694	0.935	1.43	1.388	2.623	0.913	1.828	2.009	0.793	1.93	1.001	2.921	1.918	0.443	
0.4	0.515	2.229	1.095	1.452	2.353	2.917	2.318	2.467	2.45	0.804	2.731	1.434	0.53	0.357	
0.437	0.4	0.481	1.381	1.302	2.294	2.495	3.326	3.278	0.658	2.368	1.241	0.785	0.504	0.39	
0.366	0.906	3.334	3.688	1.201	2.144	1.051	1.202	2.837	1.399	2.307	2.965	1.256	0.384	0.363	
0.301	0.369	2.257	1.301	1.004	2.114	1.919	2.571	2.187	2.045	0.63	2.032	0.707	0.751	0.355	
0.335	0.45	0.519	2.507	1.388	1.109	1.045	1.202	0.615	2.912	1.841	2.991	1.494	0.455	0.359	
0.305	0.359	0.782	1.541	1.199	0.578	1.669	0.49	1.303	2.892	0.815	2.355	1.55	0.416	0.424	
0.34	0.378	0.675	0.483	0.584	0.657	0.667	0.705	0.97	0.792	1.29	0.398	0.591	0.417	0.431	
0.338	0.399	0.452	0.34	0.388	0.395	0.36	0.353	0.516	0.473	0.38	0.394	0.378	0.374	0.486	
0.342	0.393	0.356	0.379	0.376	0.351	0.38	0.367	0.361	0.392	0.363	0.401	0.352	0.364	0.358	

5% sucrose, lyophilized with **non-optimized cycle** on two different freeze-dryers

Freeze-dryer Nr 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.9580.8110.8120.8480.8520.8590.8440.8760.8490.8680.8310.8970.9951.0321.106														
0.8430.8660.7640.9110.8070.8250.8320.8530.910.980.8310.880.9180.8681.01														
0.7310.8950.8650.8021.1260.8370.8231.0621.0610.9141.2210.830.9810.9670.945														
0.7830.8250.8181.0650.8080.8681.090.930.9011.4460.8370.9520.8430.9290.898														
0.7190.8471.061.1320.8211.1160.9541.1410.9761.1371.0270.8881.0870.8350.807														
0.9860.7910.8450.7741.61.430.9411.2360.9660.8881.050.8990.9970.8370.771														
0.7180.7660.8390.7940.8171.1350.9210.7671.5040.8922.0351.1090.9210.770.689														
0.710.720.9170.8451.4610.9881.2381.3231.0731.3731.1070.9490.750.8010.683														
0.7040.9220.9460.7241.6471.0151.3081.4661.2130.7870.8810.7970.8550.6870.752														
0.6810.7090.7470.840.7051.0971.1650.9821.8681.2651.2131.0991.0830.7420.677														
0.760.8790.7341.3991.2970.8081.3511.311.0070.931.0931.2751.0140.7620.743														
0.7060.7240.8230.790.7671.0871.4311.2771.0810.7891.440.9650.760.7170.657														
0.690.7251.0151.0731.4710.7351.3311.1260.9991.0161.1550.9180.6620.7850.721														
0.6490.7240.7190.7551.2261.6860.7230.9910.7021.0871.0170.8770.8090.859														
0.6771.1651.1580.8350.8321.3940.8461.4321.3140.9030.7740.9870.7920.7470.684														
0.6570.8080.7690.8780.8061.1081.271.3130.8290.7141.2610.8321.07090.703														
0.6920.9020.7560.9211.1560.8460.9330.9280.7660.7490.8351.2650.7480.6850.726														
0.6540.6990.7210.8860.8840.7230.8320.7851.1730.7750.8370.7450.7450.7340.764														
0.6950.6720.7830.7540.7990.8760.7650.810.9860.7280.690.7710.6780.8430.813														
0.6560.7110.6650.7370.8530.7290.7721.0370.8270.7330.7640.7810.7250.7580.769														
0.7070.6950.6590.6980.6570.7540.7460.8130.7970.670.7360.7940.7910.7870.689														
0.7460.7130.8440.7580.7730.70.6990.7930.8140.7330.7180.7280.8020.6930.755														

Freeze-dryer Nr 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.3790.3580.4340.3890.3610.3780.3660.3570.1840.4650.370.3660.380.3880.399														
0.3620.3420.4810.3610.7240.4010.4830.4330.3690.6470.3690.4330.4830.4690.339														
0.3210.5140.4111.7650.6910.5560.9421.6841.4480.8671.7790.761.1030.3980.369														
0.4141.1662.2790.7010.9361.521.2451.9120.6440.5990.9441.1940.7340.6270.351														
0.3140.4341.7812.451.2220.9781.3550.6092.2141.232.3381.1491.9350.7460.448														
0.4350.8511.3630.6571.4370.7271.3642.4111.3972.6072.7051.1781.8080.5520.32														
0.390.4390.5152.3321.5692.8633.3140.7960.8150.6712.0092.1240.8060.4350.356														
0.4191.0441.3440.8682.0092.8872.361.7912.0452.1541.0730.8810.7380.5480.343														
0.3240.512.2361.7410.8611.5793.7862.4230.5582.2980.8573.3091.8810.6690.408														
0.4080.4211.5372.2092.3480.6043.191.6261.8312.2120.7952.8451.2780.4930.325														
0.3051.5041.2571.0831.6981.7992.1041.631.6242.1992.2840.8962.0691.5290.395														
0.4880.8040.5043.4240.6023.2281.4592.4682.2331.0081.8760.7012.4910.7470.483														
0.3360.6940.9351.431.3882.6230.9131.8282.0090.7931.931.0012.9211.9180.443														
0.40.5152.2291.0951.4522.3532.9172.3182.4672.450.8042.7311.4340.530.357														
0.4370.40.4811.3811.3022.2942.4953.3263.2780.6582.3681.2410.7850.5040.39														
0.3660.9063.3343.6881.2012.1441.0511.2022.8371.3992.3072.9651.2560.3840.363														
0.3010.3692.2571.3011.0042.1141.9192.5712.1872.0450.632.0320.7070.7510.355														
0.3350.450.5192.5071.3881.1091.0451.2020.6152.9121.8412.9911.4940.4550.359														
0.3050.3590.7821.5411.1990.5781.6690.491.3032.8920.8152.3551.550.4160.424														
0.340.3780.6750.4830.5840.6570.6670.7050.970.7921.290.3980.5910.4170.431														
0.3380.3990.4520.340.3880.3950.360.3530.5160.4730.380.3940.3780.3740.486														
0.3420.3930.3560.3790.3760.3510.380.3670.3610.3920.3630.4010.3520.3640.358														

5% sucrose, **2.5% BSA**, lyophilized with an **optimized cycle** on the two different freeze-dryers

Freeze-dryer Nr 1

FD02 5% sucr 25 mg/ml BSA

Sh3

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.32	0.32	0.34	0.35	0.34	0.37	0.35	0.33	0.33	0.40	0.44	0.38	0.39	0.59	0.54	
0.36	0.33	0.33	0.34	0.39	0.35	0.35	0.41	0.39	0.46	0.35	0.39	0.42	0.40	0.31	
0.35	0.40	0.35	0.49	0.37	0.35	0.44	0.37	0.51	0.45	0.36	0.35	0.41	0.43	0.43	
0.41	0.33	0.33	0.43	0.36	0.55	0.40	0.48	0.48	0.41	0.44	0.40	0.40	0.35	0.35	
0.31	0.46	0.35	0.39	0.39	0.43	0.39	0.46	0.53	0.47	0.54	0.40	0.38	0.38	0.34	
0.46	0.34	0.34	0.36	0.35	0.52	0.41	0.39	0.51	0.40	0.47	0.38	0.37	0.37	0.29	
0.35	0.39	0.35	0.41	0.40	0.45	0.37	0.43	0.42	0.50	0.41	0.42	0.38	0.37	0.36	
0.41	0.37	0.38	0.53	0.35	0.38	0.45	0.45	0.57	0.41	0.38	0.37	0.37	0.35	0.33	
0.35	0.38	0.35	0.40	0.51	0.46	0.38	0.37	0.43	0.54	0.38	0.39	0.47	0.31	0.28	
0.31	0.34	0.42	0.35	0.40	0.60	0.38	0.37	0.42	0.40	0.38	0.44	0.33	0.30	0.31	
0.30	0.32	0.33	0.34	0.37	0.39	0.65	0.61	0.42	0.66	0.47	0.45	0.34	0.31	0.27	
0.31	0.46	0.35	0.34	0.38	0.40	0.47	0.37	0.45	0.59	0.42	0.32	0.32	0.26	0.29	
0.30	0.32	0.43	0.51	0.55	0.54	0.35	0.44	0.38	0.39	0.36	0.33	0.42	0.29	0.29	
0.39	0.45	0.31	0.51	0.42	0.37	0.44	0.40	0.62	0.44	0.47	0.33	0.29	0.30	0.31	
0.37	0.33	0.31	0.47	0.36	0.46	0.65	0.37	0.44	0.50	0.42	0.33	0.30	0.30	0.30	
0.37	0.41	0.41	0.34	0.41	0.60	0.43	0.49	0.45	0.55	0.34	0.44	0.34	0.30	0.26	
0.39	0.34	0.34	0.46	0.38	0.48	0.43	0.34	0.44	0.34	0.38	0.33	0.30	0.29	0.30	
0.35	0.45	0.43	0.57	0.38	0.52	0.59	0.38	0.48	0.46	0.49	0.35	0.30	0.29	0.26	
0.34	0.28	0.35	0.34	0.39	0.34	0.41	0.51	0.50	0.43	0.40	0.39	0.34	0.30	0.31	
0.33	0.31	0.41	0.35	0.39	0.38	0.39	0.31	0.36	0.32	0.32	0.38	0.32	0.31	0.25	
0.28	0.33	0.34	0.28	0.31	0.26	0.30	0.32	0.33	0.31	0.29	0.30	0.27	0.28	0.26	
0.27	0.27	0.27	0.30	0.35	0.30	0.28	0.27	0.31	0.29	0.31	0.26	0.36	0.35	0.25	

Freeze-dryer Nr 2

FD04 5% sucrose 25 mg/ml BSA

Sh3

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.201	0.23	0.198	0.285	0.221	0.212	0.246	0.225	0.246	0.242	0.189	0.273	0.233	0.205	0.195	
0.223	0.299	0.238	0.317	0.267	0.335	0.254	0.243	0.354	0.191	0.312	0.237	0.211	0.185	0.204	
0.234	0.244	0.372	0.297	0.402	0.311	0.435	0.512	0.385	0.297	0.255	0.359	0.371	0.243	0.218	
0.323	0.272	0.439	0.5	0.373	0.361	0.398	0.407	0.405	0.376	0.458	0.325	0.377	0.295	0.261	
0.245	0.259	0.384	0.446	0.379	0.51	0.298	0.323	0.387	0.272	0.21	0.382	0.274	0.185	0.265	
0.297	0.271	0.456	0.324	0.331	0.401	0.458	0.469	0.464	0.482	0.323	0.43	0.302	0.279	0.211	
0.212	0.313	0.44	0.367	0.428	0.368	0.248	0.266	0.367	0.397	0.377	0.499	0.355	0.274	0.293	
0.284	0.252	0.368	0.246	0.408	0.353	0.434	0.347	0.433	0.393	0.388	0.285	0.416	0.291	0.266	
0.217	0.316	0.306	0.434	0.363	0.499	0.361	0.32	0.406	0.461	0.303	0.409	0.312	0.429	0.325	
0.27	0.383	0.397	0.355	0.422	0.377	0.466	0.551	0.294	0.429	0.32	0.282	0.38	0.278	0.26	
0.19	0.32	0.239	0.495	0.531	0.359	0.412	0.108	0.478	0.328	0.358	0.306	0.32	0.273	0.336	
0.387	0.305	0.375	0.335	0.455	0.63	0.486	0.381	0.398	0.424	0.418	0.214	0.406	0.369	0.208	
0.346	0.435	0.513	0.371	0.382	0.487	0.484	0.475	0.319	0.337	0.299	0.361	0.284	0.315		
0.209	0.258	0.318	0.583	0.503	0.372	0.495	0.341	0.448	0.451	0.387	0.349	0.287	0.384	0.205	
0.195	0.328	0.463	0.549	0.377	0.581	0.379	0.415	0.563	0.432	0.449	0.334	0.279	0.314	0.252	
0.289	0.279	0.32	0.482	0.476	0.646	0.442	0.317	0.527	0.398	0.404	0.408	0.359	0.301	0.349	
0.23	0.336	0.36	0.372	0.361	0.372	0.369	0.482	0.374	0.357	0.344	0.432	0.205	0.285	0.306	
0.365	0.306	0.403	0.515	0.36	0.438	0.492	0.396	0.484	0.453	0.355	0.377	0.492	0.385	0.21	
0.279	0.339	0.372	0.452	0.319	0.42	0.349	0.763	0.434	0.363	0.421	0.377	0.335	0.295	0.235	
0.32	0.26	0.313	0.514	0.296	0.371	0.481	0.443	0.474	0.389	0.329	0.353	0.329	0.3	0.231	
0.165	0.206	0.231	0.262	0.324	0.287	0.231	0.233	0.308	0.222	0.319	0.238	0.304	0.22	0.225	
0.166	0.18	0.203	0.172	0.17	0.168	0.188	0.172	0.176	0.213	0.225	0.186	0.167	0.199		

Headspace moisture analysis of statistical sample sets enabled efficient cycle optimization and clear demonstration of freeze dryer equivalence

- **Background**

- Substitution of an old freeze dryer into an existing facility during running operations
- Scale-up from 14 m² to 28 m²
- Introduction of improvements such as Point-of-fill filtration
- Determination of intra- and inter-batch variability and systematic QbD approach to comply with recent FDA guidelines

Note: These case study slides were presented at the 2018 PDA Pharmaceutical Freeze Drying Technology conference, Seville, Spain.

- Industry case study: GSK Vaccines Marburg

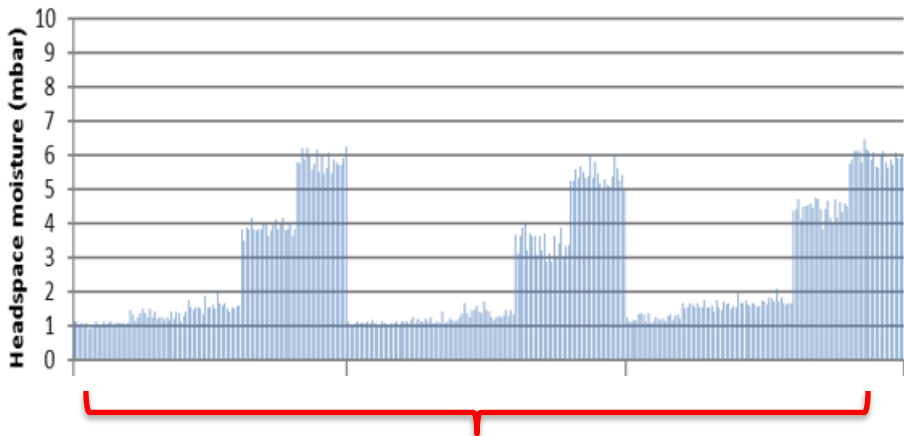
- **Headspace Moisture determination**

- Performance of Design of Experiments in small-scale
- 100% determination of intra-batch variability
- Moisture results were used to analyze the impact and interaction of critical process parameters during freeze drying and process inherent product variability
- Determination of a suitable placebo for full-scale runs based on moisture
- Samples used for moisture analysis were still available for other destructive tests in contrast to Karl-Fischer to allow 1:1 correlation of results

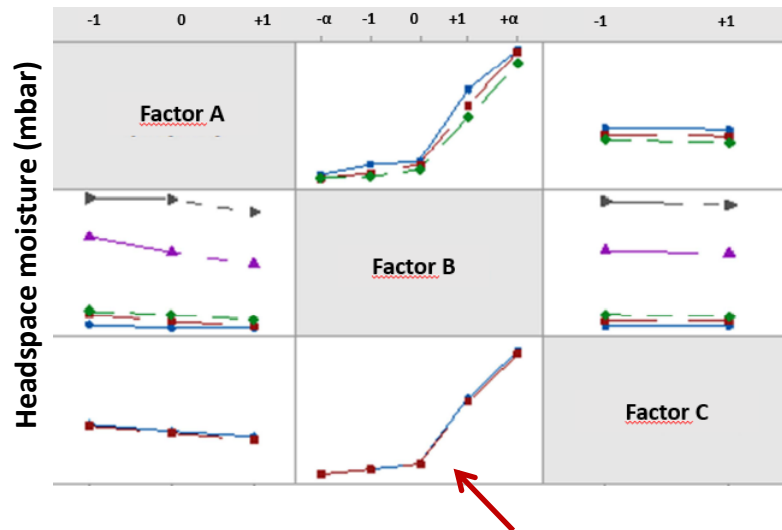
Note: These case study slides were presented at the 2018 PDA Pharmaceutical Freeze Drying Technology conference, Seville, Spain.

Results of DoE small-scale study

Headspace moisture measurements



- Different lyo cycles to verify design space of the process
- Cycles show comparable moisture pattern and interaction with other parameters



- High dependency of product specific attributes with moisture results
- Results were used to define suitable placebo

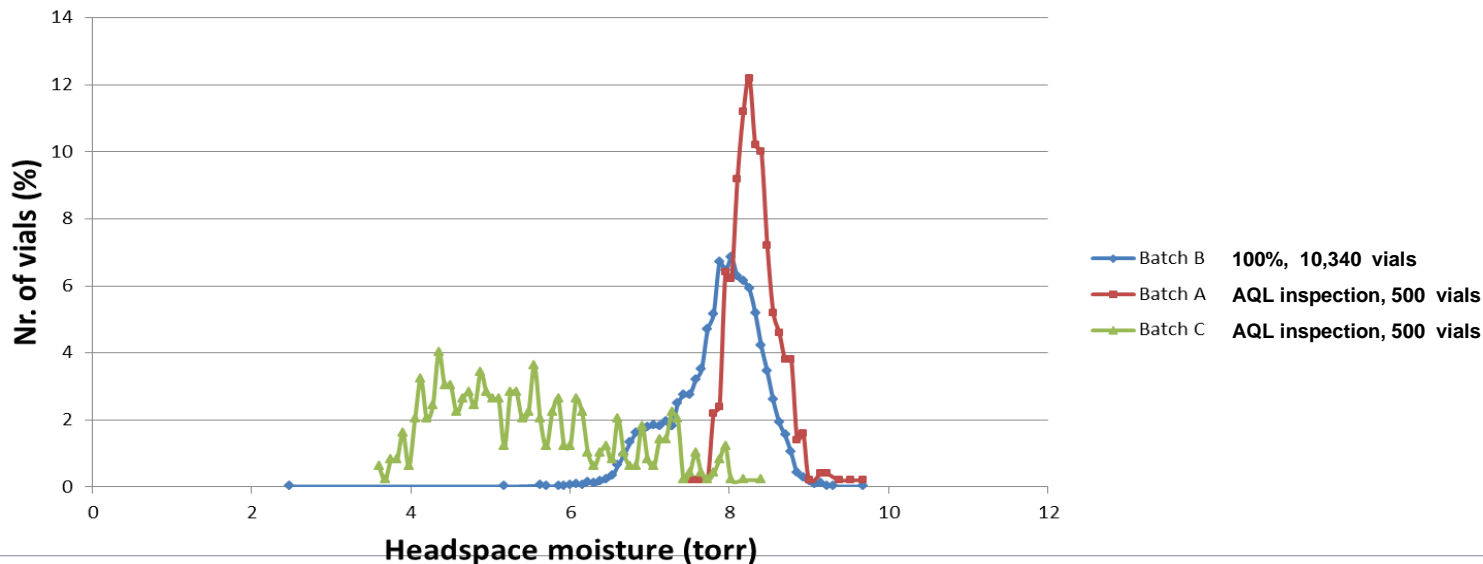
DoE Study Conclusions

- Proof of concept for full-scale runs to minimize project risk and number of full-scale runs
- Determination of suitable Placebo to mitigate lack of drug substance and minimize costs
- Verification of robust design space
- Process knowledge about interaction of critical parameters and their effect in intra- and inter-batch variability

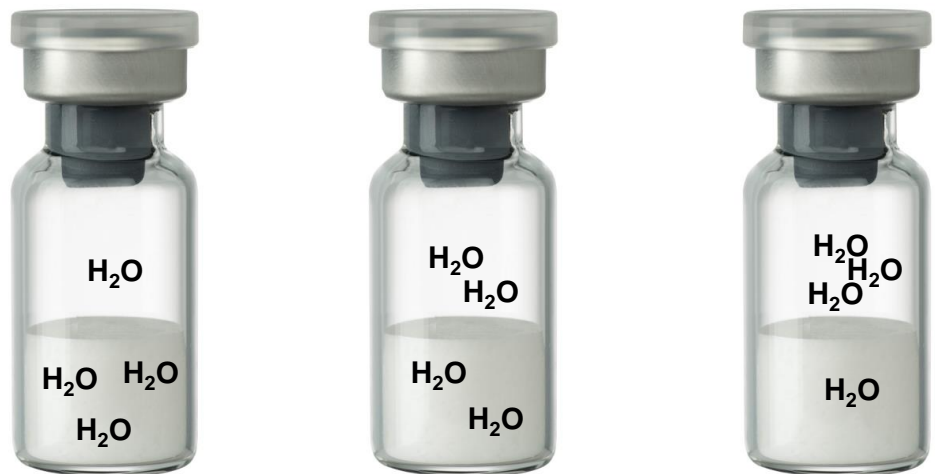
Note: These case study slides were presented at the 2018 PDA Pharmaceutical Freeze Drying Technology conference, Seville, Spain.

- Perform 100% moisture inspection on a ‘typical’ batch (during scale-up or validation, for example) to determine moisture distribution.
- Compare AQL inspections of other batches to the ‘typical batch’.

Moisture distribution 3 consecutive batches



- The previous case studies demonstrated that headspace moisture analysis is a very useful analytical tool for gaining statistical insight into process and product quality.
- USP <922> describes headspace moisture (water activity) as a formal moisture determination method that in many cases is more useful than a total moisture content determination.



Product A

Product B

Product C

INCREASING WATER ACTIVITY 

Total water content is equal in all product vials.
Ratio of bound to unbound water is different.

Water Activity and Water Content are Different

$$K_f \neq a_w$$

- Total Water Content measured by Karl Fischer USP<921> is very different than Water Activity USP<922>
- Each ingredient in a formulation has its own unique water activity versus water concentration. This relationship is typically plotted as a sorption isotherm.
- If you change the formulation or the manufacturing process the water activity of the drug product will change.
- Water activity measures the amount of loosely bound water that is available to participate in undesirable chemical, physical, and microbiological reactions.

- **Motivation:**

- Replace KF titration with rapid non-destructive method for lyo product moisture determination
- Assess correlation between headspace water vapor pressure and degradation of a hydrolytic small molecule API

- **Measurements:**

- Sample sets with range of cake moisture from 0.5-3.0% by weight.
- Measure initial moisture in headspace (water activity at fixed temperature)
- Since measurement is non-destructive, the same samples can be put on stability
- Degradation of the API measured on stability with HPLC
- Look for correlation between water activity and API degradation

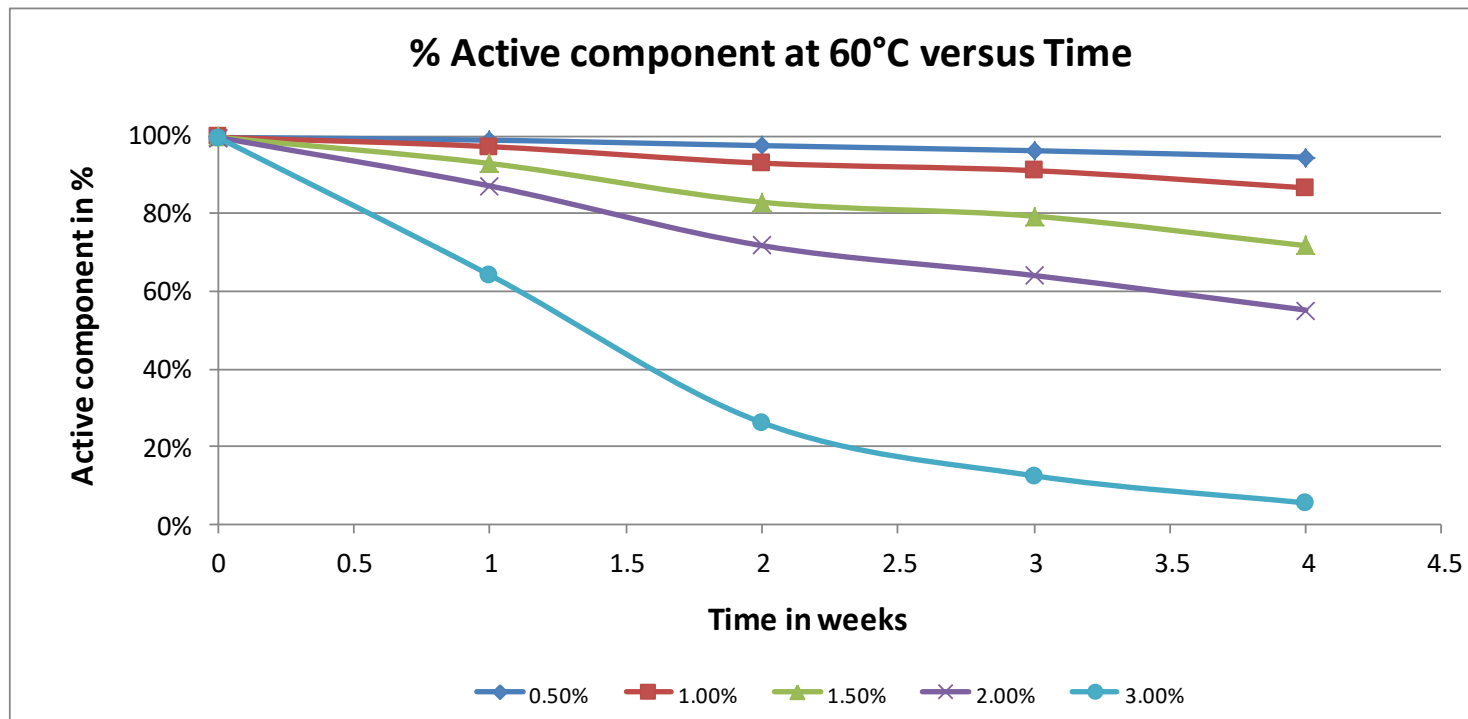
Correlating headspace moisture directly to product degradation

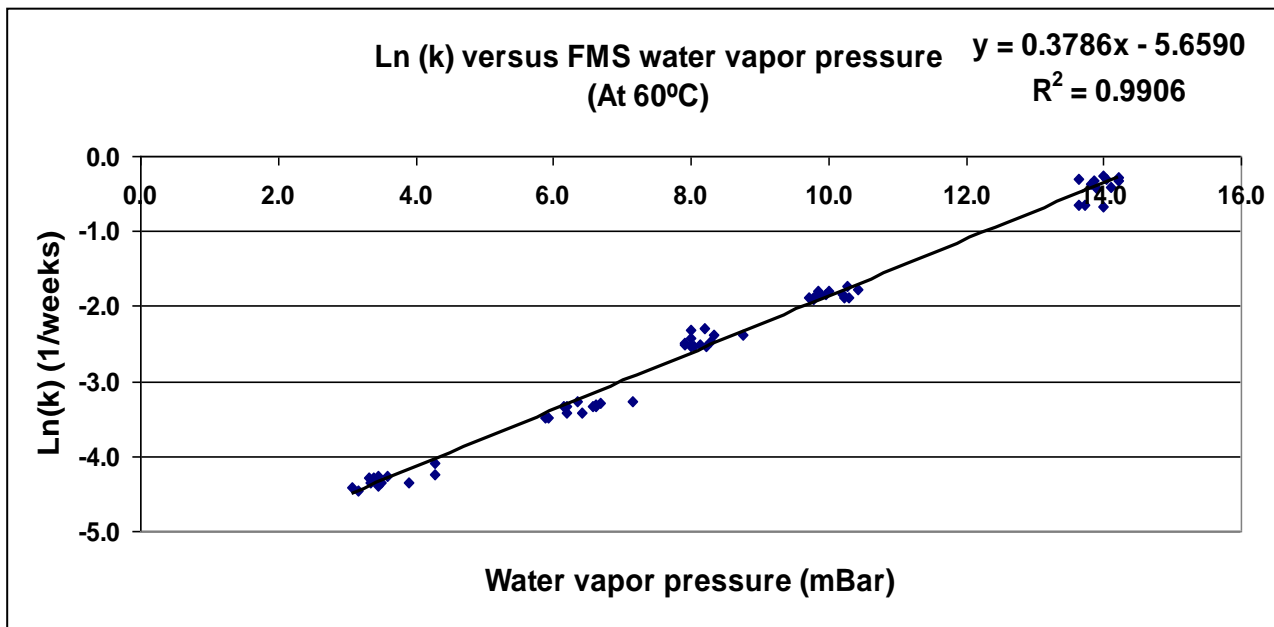
Stability study

Moisture	0.50%	1.00%	1.50%	2.00%	3.00%
25°C	X	X	X	X	X
40°C	X	X	X	X	X
60°C	X	X	X	X	X

Targeted moisture content groups:	0.50 %	1.00 %	1.50 %	2.00 %	3.00%
Corresponding water vapor levels:	4 torr	6 torr	8 torr	10 torr	14 torr

Product degradation results





$$k = \frac{-(\ln(\frac{[At]}{[Ao]}))}{t}$$

- Water vapor (activity) measurement predicts the product stability!
- Potential to replace KF with rapid non-destructive technique for lyo moisture determination

Book title: **“Principles and Practice of Lyophilization Process and Product Development”**

- to be published end of 2022 in memory of Dr. Michael Pikal (Springer)

Chapter on headspace analysis:

- “Laser-Based Headspace Moisture Analysis For Rapid Nondestructive Moisture Determination of Lyophilized products”

- Current moisture determination methods for freeze-dried pharmaceutical product (KF titration, LOD methods) give limited insight into process and product quality.
- Headspace moisture analysis is an analytical tool that can be used for formulation development, process optimization, freeze dryer qualification, and as a formal water activity test method to determine product stability.

Appropriate analytical methods needed for a robust science-based understanding of process and product quality – regulators appreciate and encourage such an approach.

**Thank you
for your attention!**

