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Scientific Resource

Advanced Instruments OsmoTECH® HT Automated Micro-Osmometer: An Introduction

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Introduction

In recent years, the Biopharmaceutical industry has experienced rapid growth and innovation through many different therapeutic modalities. One of the challenges the industry faces is finding efficient ways to monitor critical quality attributes (CQAs) throughout the bioprocess, such as pH, metabolites, endotoxin levels, product concentration, and osmolality. While the industry moves toward more automated processes, it is of utmost importance to maintain confidence in decision making early in the bioprocess to ensure a safe and effective drug product further down the line.

For many different drug product technologies, osmolality is a common CQA monitored throughout the bioprocess. Osmolality is commonly tested in raw material QC, solution preparation, cell culture monitoring, buffer exchange monitoring, stability testing, final product QC and release, and much more. To follow industry trends of automation and higher throughput, Advanced Instruments has developed the first 96 well plate-based osmometer available to the market, called the OsmoTECH® HT. Automated high throughput testing is valuable to many labs as it provides significant time and cost savings, while reducing human error. High throughput testing of osmolality is especially critical for formulation development workflows, in which many different solutions must be evaluated in a short period of time. Notably, formulations involving lipid nanoparticles (LNPs) containing genetic material must be carefully monitored, as LNPs are osmotically sensitive and require precise isotonic conditions to prevent premature rupture (1).

When automating any process certain attributes are critical to any process, these include:

- Ability to test multiple samples in a continuous and consistent way
- Minimizing sample volume: this can vary depending on the technology and assay, but sample waste is always a concern
- Minimal carry-over of sample
- Inter and intra-plate variability is minimized
- The potential for edge effect and evaporation are minimized

One area that has shown interest in automating the osmolality measurement is in downstream buffer and formulation development. Sample volume is a critical consideration and one that can have large consequences when deciding on which PAT analysis to choose. Those in formulation development run multiple analyses on their formulations, so minimizing sample volume is of utmost importance. The OsmoTECH HT aims to maintain the characteristic accuracy of that found with the Advanced Instrument's intelligent freezing point depression technology which enables precise measurements of osmolality and strong inter and intra-run repeatability. The following study will demonstrate that the OsmoTECH HT can run with high accuracy and precision a variety of sample types, with no-carry over or edge effect.

Method and Materials:

Scope of Testing

The scope of testing involved the evaluation of two OsmoTECH HT osmometers.

For the salt standards, the number of tests per instrument ranged from 16 tests per instrument for the 0 mOsm/kg standard, to 96 tests per instrument for the 2000 mOsm/kg standard. For the protein-based standards, the number of tests per instrument was 48 tests. Each test was performed as one replicate per well of the 96 well plate.

For the protein controls, 48 samples were tested per level of protein control on each instrument. Each test was performed as one replicate per well of the 96 well plate.

For carryover examination, testing was column-based and used Protinol™ 320 and the 50 mOsm/kg salt standard. Each test was performed as one replicate per well of the 96 well plate.

For the protein and sugar solutions, ten solutions containing a range of bovine serum albumin (BSA) and sucrose were analyzed in 8 tests per sample on one OsmoTECH HT. Each test was performed as one replicate per well of the 96 well plate.

For evaporation examination, one full plate of Clinitrol 290™ and one full plate of the 400 mOsm/kg standard were tested on each OsmoTECH HT. Each test was performed as one replicate per well of the 96 well plate.

Material/Equipment used in all the studies are shown below

Material/Equipment	AI Part number
OsmoTECH HT	OsmoTECH HT
OsmoTECH HT Micro-sample tubes	HT1000
Calibration and Verification Standard Set	3MA635
Pipette capable of delivering 50 µL (calibrated)	NA
Pipette tips	240821
OsmoTECH HT Calibration Tray	635951
Sealing Roller for plate evaporation cover	807006
OsmoTECH HT 96 Well Kit	635920
OsmoTECH HT System Fluid	635934
OsmoTECH HT Cleaning Reel	635910

All runs were made following the protocol provided in the OsmoTECH HT user guide.

For the precision & accuracy testing:

The solutions shown below were tested for the number of replicate tests indicated.

Solutions	N Reps	AI Part number
0 mOsm	16	3MA000
50 mOsm	16	3MA005
100 mOsm	32	3LA011
Clinitrol™ 290	48	3MA029
300 mOsm	48	3MA003
500 mOsm	48	3LA051
850 mOsm	48	3MA085
900 mOsm	48	3LA091
1500 mOsm	64	3LA151
2000 mOsm	96	3MA200
Protinol™ Protein-Based Controls (3-levels)	48	3MA028

Carryover Analysis:

To determine if sample carryover from well to well is present, twelve independent runs were made on two OsmoTECH HT osmometers using two sample types, the protein containing Protinol 320 (Part # 3MA028) reagent as well a 50 mOsm salt standard (Part #3MA005). The samples were run in the following sequence:

- 3 replicates of high-level sample (Protinol 320)
- 5 replicates of low-level sample (50 mOsm/kg standard)

Evaporation Analysis

To determine the level of evaporation or edge-effect, 96 well microplates were filled with Clinitrol 290 and 400 mOsm/kg salt standard and run on two separate OsmoTECH HT instruments using the OsmoTECH HT 96 Well Kit, and roller which includes the plate cover to eliminate evaporation.

Complex Sample Analysis

To assess the OsmoTECH HT's ability to run more complex samples, cocktails of different concentrations of BSA (Proliant Cat# 68100-100 g) and Sucrose (MilliporeSigma Cat# S0389-500 g) were made at the following concentrations and run on two OsmoTECH HT instruments following standard operating procedures.

100 mg/ml BSA, 100 mM Sucrose
100 mg/ml BSA, 200 mM Sucrose
100 mg/ml BSA, 300 mM Sucrose
100 mg/ml BSA, 350 mM Sucrose
150 mg/ml BSA. 100 mM Sucrose
150 mg/ml BSA, 200 mM Sucrose
150 mg/ml BSA, 300 mM Sucrose
200 mg/ml BSA, 100 mM Sucrose
200 mg/ml BSA, 200 mM Sucrose
200 mg/ml BSA, 300 mM Sucrose

Results

Accuracy and Precision with Salt Standards:

Salt standards ranging from 0-300mOsm/kg were tested on two OsmoTECH HT instruments. Single replicates per well were performed, and the number of wells tested per plate ranged from 16 wells with the 0 mOsm/kg sample to 48 wells with the 300 mOsm/kg sample. All results met the acceptance criteria for accuracy and precision, with no outliers observed.

The highest standard deviation (SD) observed was 1.1 mOsm/kg with the 300 mOsm/kg sample, which is well within the maximum accepted SD of 3 mOsm/kg.

							Acceptance Criteria	
Sample	**Instrument**	**N**	**Mean (mOsm/kg)**	**SD**	**Min (mOsm/kg)**	**Max (mOsm/kg)**	**Mean Accuracy (± 3)**	**SD**
0 mOsm/kg	OsmoTECH HT #1	16	0.1	0.3	0	1	0 - 3	≤ 3
	OsmoTECH HT #2	16	0.1	0.3	0	1	0 - 3	≤ 3
50 mOsm/kg	OsmoTECH HT #1	16	51.0	0.0	51	51	47 - 53	≤ 3
	OsmoTECH HT #2	16	51.0	0.0	51	51	47 - 53	≤ 3
100 mOsm/kg	OsmoTECH HT #1	32	100.3	0.5	100	102	97 - 103	≤ 3
	OsmoTECH HT #2	32	100.3	0.5	100	101	97 - 103	≤ 3
Clinitrol™ 290	OsmoTECH HT #1	48	291.4	0.7	290	294	287 -293	≤ 3
	OsmoTECH HT #2	48	291.2	0.7	290	292	287 -293	≤ 3
300 mOsm/kg	OsmoTECH HT #1	48	300.5	1.0	299	304	297 - 303	≤ 3
	OsmoTECH HT #2	48	300.9	1.1	299	303	297 - 303	≤ 3

Table 1. Testing of salt standards from 0-300 mOsm/kg

Salt standards ranging from 500-2000 mOsm/kg were tested on two OsmoTECH HT instruments. Single replicates per well were performed, and the number of wells tested per plate ranged from 48 wells with the 500 mOsm/kg sample to 96 wells with the 2000 mOsm/kg sample. All results met the acceptance criteria for accuracy and precision, with no outliers observed. The highest coefficient of variance (%CV) observed was 0.5, which is well within the maximum accepted %CV of 1.0.

							Acceptance Criteria	
Sample	**Instrument**	**N**	**Mean (mOsm/kg)**	**%CV**	**Min (mOsm/kg)**	**Max (mOsm/kg)**	**Mean Accuracy (± 1%)**	**%CV**
500 mOsm/kg	OsmoTECH HT #1	47	501.4	0.2	500	505	495 - 505	≤ 1
	OsmoTECH HT #2	48	504.1	0.2	502	506	495 - 505	≤ 1
850 mOsm/kg	OsmoTECH HT #1	48	850.9	0.2	847	856	842.5 - 858.5	≤ 1
	OsmoTECH HT #2	48	850.5	0.3	846	859	842.5 - 858.5	≤ 1
900 mOsm/kg	OsmoTECH HT #1	48	901.7	0.3	895	911	891 - 909	≤ 1
	OsmoTECH HT #2	48	904.3	0.2	898	908	891 - 909	≤ 1
1500 mOsm/kg	OsmoTECH HT #1	64	1492.7	0.5	1480	1508	1485 -1515	≤ 1
	OsmoTECH HT #2	64	1502.2	0.5	1483	1521	1485 -1515	≤ 1
2000 mOsm/kg	OsmoTECH HT #1	96	2004.1	0.5	1973	2028	1980 - 2020	≤ 1
	OsmoTECH HT #2	96	2003.4	0.4	1985	2033	1980 - 2020	≤ 1

Table 2. Testing of salt standards from 500-2000 mOsm/kg

Accuracy and Precision with Protein Controls:

The Protinol™ protein-based controls (Bovine Serum Albumin), ranging from ~240 to ~320 mOsm/kg were tested on two OsmoTECH HT instruments. A single replicate test was performed per well, and 48 wells were tested per sample. All results met the acceptance criteria for accuracy, precision, and repeatability. The highest SD observed was 1.8 mOsm/kg for the Protinol 320 sample, which is well below the maximum acceptable SD of 3 mOsm/kg.

							Acceptance Criteria	
Sample	**Instrument**	**N**	**Mean (mOsm/kg)**	**SD**	**Min (mOsm/kg)**	**Max (mOsm/kg)**	**Accuracy**	**SD**
Protinol 240	OsmoTECH HT #1	48	**237.4**	**0.9**	**236**	**239**	233 – 247	≤ 3
	OsmoTECH HT #2	48	**236.5**	**1.7**	**233**	**240**	233 – 247	≤ 3
Protinol 280	OsmoTECH HT #1	48	**277.1**	**1.1**	**275**	**279**	273 287	≤ 3
	OsmoTECH HT #2	48	**278.7**	**1.5**	**276**	**282**	273 287	≤ 3
Protinol 320	OsmoTECH HT #1	48	**317.1**	**1.8**	**313**	**321**	313 327	≤ 3
	OsmoTECH HT #2	48	**317.2**	**1.8**	**313**	**321**	313 327	≤ 3

Carryover Results:

Carryover testing was performed on two OsmoTECH HT instruments using Protinol™ 320 as the high-level sample and the 50 mOsm/kg salt standard as the low-level sample. Testing on the 96 well plate was column-based and began at the top of the plate as indicated by the arrow in **Figure 1**'s heatmap. Carryover would be indicated by the low-level controls showing results higher than the expected value. Therefore, no evidence of carryover was detected from the high-level sample into the low-level sample.

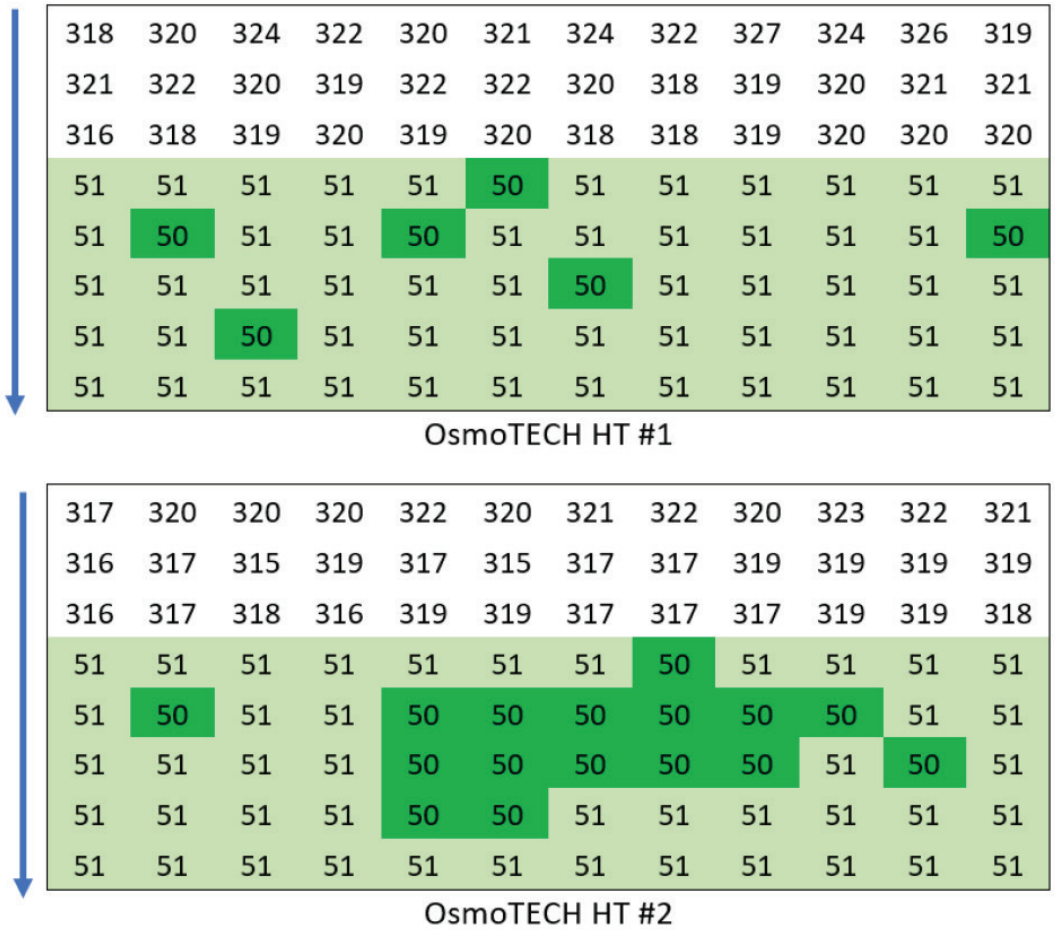


Figure 1. Osmolality results from column-based carryover examination on two plates

Protein and Sugar Solution Results

Ten solutions containing a range of BSA and sucrose were analyzed on one OsmoTECH HT. One replicate was tested per well, and 8 wells were tested per sample so a total of 8 replicate tests were performed. No testing errors occurred during the testing of these samples. All samples except for the highest concentration of BSA and sucrose (200mg/mL, 300mM) had %CVs less than 1.5, demonstrating good repeatability with these samples.

BSA (mg/mL)	Sucrose (mM)	Mean (mOsm/kg)	SD	%CV
100	100	150.9	1.6	1.1
	200	292.0	3.5	1.2
	300	446.8	3.2	0.7
	350	541.1	5.0	0.9
150	100	176.0	1.5	0.9
	200	329.0	4.3	1.3
	300	523.1	4.6	0.9
200	100	215.6	2.4	1.1
	200	403.3	5.5	1.4
	300	613.6	13.8	2.2

Table 4. Testing of BSA and sucrose ranging from 100mg/ML BSA 100mM sucrose to 200mg/ml BSA 300 mM sucrose

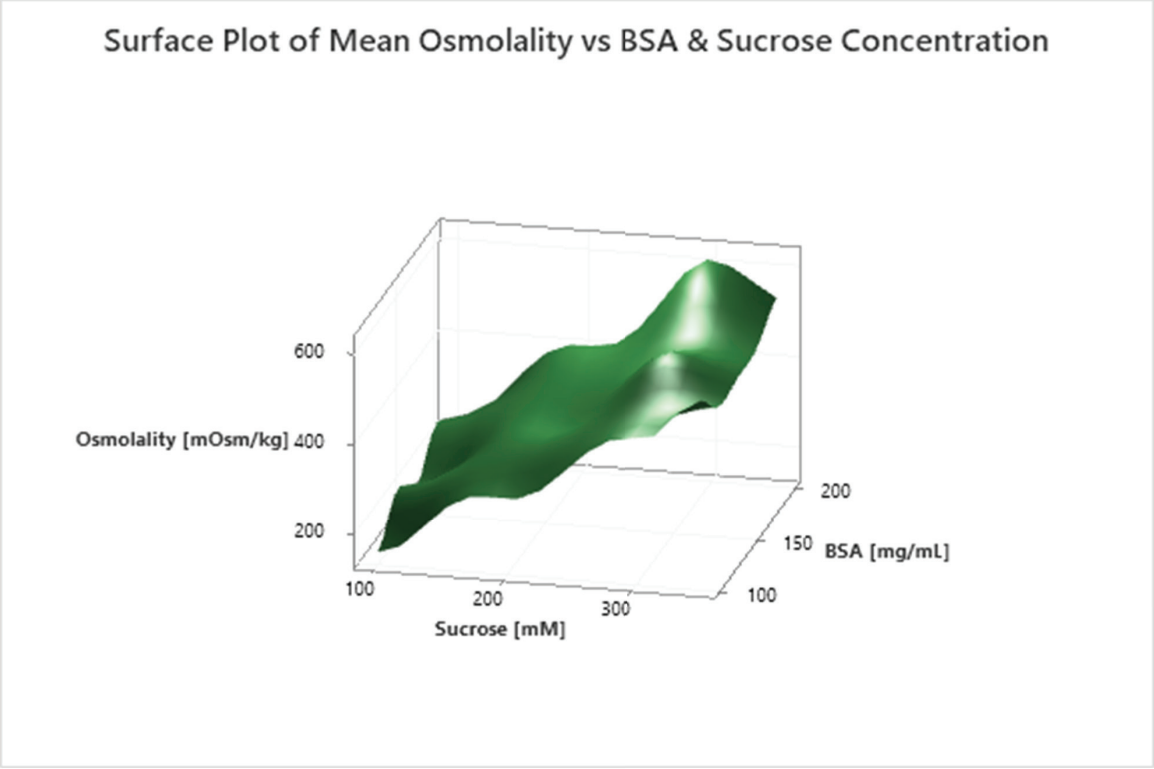


Figure 2. The osmolality results from the BSA and sucrose samples are shown above. As the BSA and sucrose concentrations increase, osmolality increases.

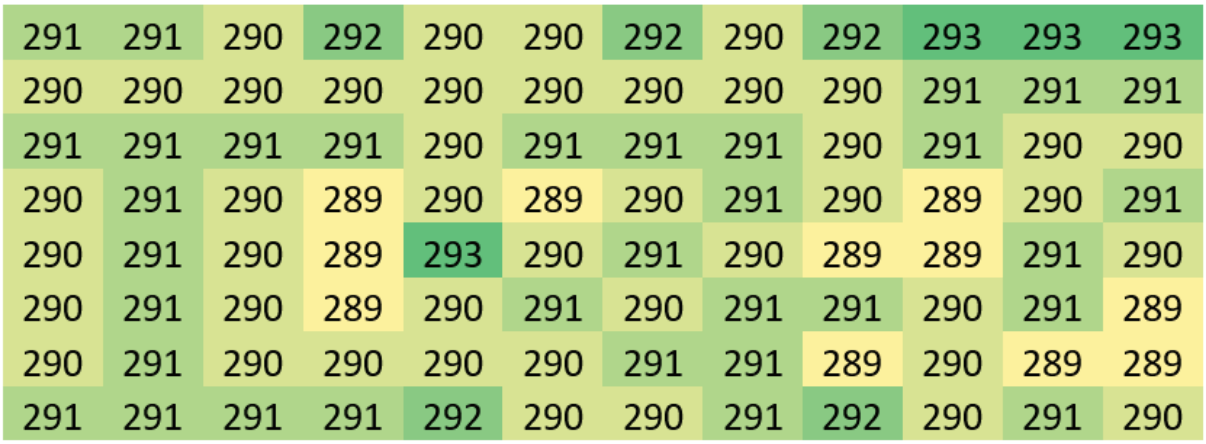
Evaporation Results

Evaporation is indicated by osmolality results that are significantly higher than expected, due to the removal of water from the solution, which increases the total solute concentration of the solution.

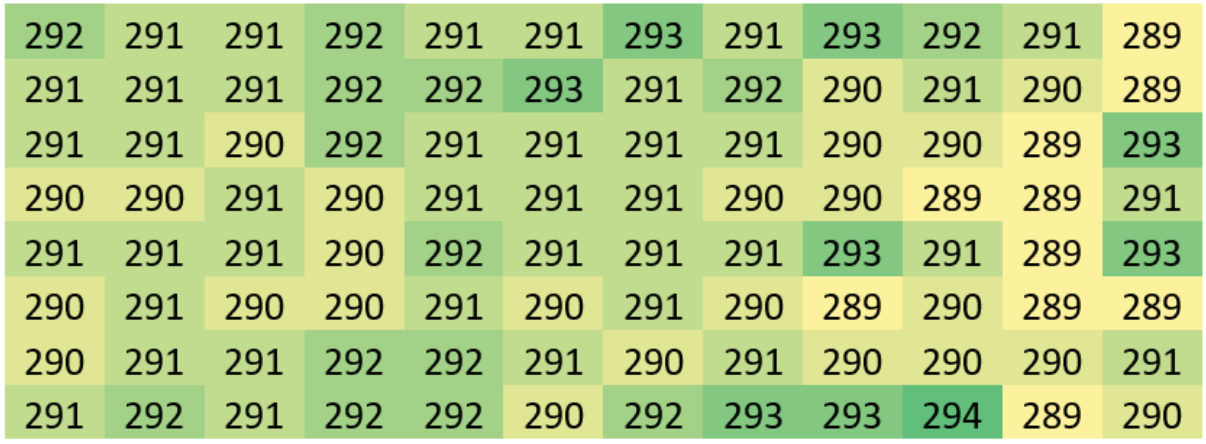
Osmolality results were analyzed on two OsmoTECH HT instruments with a full 96 well plate of Clinitrol 290™. All individual results were within 290 ± 4 mOsm/kg. The mean accuracy results for each plate were within the allowable specification of 290 ± 3 mOsm/kg. The precision results for the plates are within the allowable specification of 1 SD (<3 mOsm/kg). There is no evidence of evaporation based on the osmolality results.

	OsmoTECH HT #1			OsmoTECH HT #2		
	Mean (mOsm/kg)	Standard Deviation	%CV	Mean (mOsm/kg)	Standard Deviation	%CV
Clinitrol 290™	290.5	0.92	0.30%	290.9	1.11	0.40%
400 mOsm/kg standard	401.8	1.09	0.30%	401.2	1.17	0.30%

Table 5: Averages taken from two 96 well microplates each run on a different OsmoTECH HT with different Solutions.



OsmoTECH HT #1



OsmoTECH HT #2

	OsmoTECH HT #1	OsmoTECH HT #2
Mean	290.5 mOsm/kg	290.9 mOsm/kg
SD	0.92	1.11
%CV	0.3%	0.4%

Figure 3: Heat map of two microplates run to determine impact of evaporation on osmolality using the Clinitrol 290™ standard.

Osmolality results from plates analyzed on two OsmoTECH HT osmometers with a full plate of the 400 mOsm/kg Calibration Standard. All individual results were within 400 ± 4 mOsm/kg. The mean accuracy results for the plates are within the allowable specification of 400 ± 3 mOsm/kg. The precision results for the plates are within the allowable specification of 1SD (≤ 3 mOsm/kg). There is no evidence of evaporation based on the osmolality results.

	1	2	3	4	5	6	7	8	9	10	11	12
A	402	402	401	401	400	400	401	402	404	403	404	401
B	400	400	401	401	402	400	400	401	402	401	401	402
C	401	401	400	401	400	400	401	401	401	402	401	399
D	401	403	401	402	402	402	403	403	403	403	402	402
E	401	402	401	402	401	401	403	403	402	403	403	402
F	402	402	401	401	402	401	402	404	402	403	402	401
G	401	401	402	402	402	401	402	403	402	403	402	400
H	401	403	402	403	403	402	403	404	404	403	403	402

OsmoTECH HT #1

	1	2	3	4	5	6	7	8	9	10	11	12
A	402	401	402	402	402	400	402	401	404	404	399	400
B	402	401	402	400	402	402	401	401	400	400	400	401
C	400	400	401	403	401	401	402	400	401	401	399	401
D	403	401	401	401	401	400	401	400	401	400	399	401
E	402	401	401	402	400	401	401	401	400	398	400	399
F	401	401	401	401	400	401	400	402	401	401	400	402
G	403	402	402	401	400	401	401	402	404	400	402	400
H	402	402	402	401	401	403	403	404	403	402	403	401

OsmoTECH HT #2

	OsmoTECH HT #1	OsmoTECH HT #2
Mean	401.8 mOsm/kg	401.2 mOsm/kg
SD	1.09	1.17
%CV	0.3%	0.3%

Figure 4: Heat map of two microplates run to determine impact of evaporation on osmolality of a 400 mOsm/Kg salt standard.

	Clinitrol 290			400 mOsm/kg standard		
	Mean (mOsm/kg)	Standard Deviation	%CV	Mean (mOsm/kg)	Standard Deviation	%CV
Outer Edge wells	291.01	0.91	0.31	401.76	1.03	0.26%
Inner Wells	290.47	0.61	0.21	401.28	0.65	0.16%

Table 6: Averages of the outer wells and inner wells of two 96 well microplates run on two different OsmoTECH HT instruments

Discussion

The OsmoTECH HT, is designed for performing freezing point depression osmolality analysis in a high throughput fashion using 96 well microplates. Based on the results of this study, we observed no testing errors (such as freezing errors or outliers) and the results were all within the stated specification of the instrument.

As each sample was tested as a single replicate from each well, the minimal sample volume of 50 µL, proved to be sufficient for accurate measurement of osmolality, which ultimately reduces the opportunity for sample waste. The results within each microplate also show that there is minimal intra-plate variability.

The carryover analysis, which is of critical importance to anyone running multiple sample types within a microplate, demonstrates that the sample probe used in the instrument did not cross-contaminate subsequent wells. This is accomplished thorough cleaning of the pipette and sample probe in between each sample. This eliminates any concern of carryover for the sample types tested within this experiment. We do recommend that with viscous or samples with sticky compositions, that a similar carry-over test be done to verify any carry-over concerns.

Minimal variability between instruments was shown consistently throughout the study by testing most samples on two OsmoTECH HT instruments.

To assess the OsmoTECH HT's ability to read more complex solutions, we focused on different concentrations of BSA and sucrose as a model. Complex solutions require more time to stabilize at their freezing temperature due to the more complex nature of their molecular interactions. This molecular complexity lends a more complex analysis when measuring osmolality. The data collected using the OsmoTECH HT shows that it is capable pf accurately measuring osmolality at concentrations of BSA and sucrose up to 200mg/mL BSA and 200mM sucrose (Table 2 and Figure 4).It is interesting to note that a solution containing 200mg/mL BSA and 300mM sucrose showed a result out of specification, while all other solutions in Table 4 showed excellent reproducibility. The molecular complexity of the 200mg/mL BSA/300mM sucrose solution may hamper reaching a consistent freezing point depression, resulting in a readout that is out of specification.

Evaporation concerns stem from the fact that the instrument tests one sample at a time, so some samples are left in the plate for some time before testing, especially if a full 96 well plate is being tested. Each sample test takes about 90 seconds, and a full plate of 96 samples takes approximately 3 hours and 40 minutes, which would allow for evaporation under the correct conditions This concern is addressed with the adhesive evaporation cover that is placed on the plate. In this experiment, the results demonstrate that the samples do not evaporate while in queue to be tested, eliminating the concern of edge-effect or evaporation on the OsmoTECH HT.

In summary, this study has shown that the OsmoTECH HT is capable of automated high-throughput osmolality testing within

acceptable limits for precision and accuracy. Compared to single-sample and lower-throughput multi-sample osmometers, the OsmoTECH HT has the added benefit of more walk-away time for completion of other lab duties. This can be especially valuable in settings where lots of samples must be processed, such as cell culture labs, solution preparation labs, and formulation development labs. Samples that are tested in these lab settings are generally simple (e.g., low concentration solutions, simple salt and/or ionic solutions), and can be reliably tested on the OsmoTECH HT as demonstrated by the data from this experiment.

For it's intended applications, the OsmoTECH HT can produce reliable, precise, and accurate osmolality results with minimal technician interaction with the instrument. The OsmoTECH HT provides the ability to set up a run with sample locations on the 96 well plate as well as sample IDs for each position and will automatically test each specified sample within the plate once the run has started. Sample locations and IDs can be pre-loaded to the system as well, so commonly used plate layouts can be stored on the instrument for future use. In addition, the OsmoTECH HT boasts the same flexible data management features, robust data integrity, and cGMP compliant software as previous TECH osmometer models, and can therefore be applied in labs with different compliance needs, from R&D to process development to GMP bioprocessing.

References

(1) Karmacharya, P., Patil, B.R. & Kim, J.O. Recent advancements in lipid–mRNA nanoparticles as a treatment option for cancer immunotherapy. J. Pharm. Investig. (2022). https://doi.org/10.1007/s40005-022-00569-9

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