Validation of the utilities and equipment involved in OMV drug substance manufacturing process was undertaken prior to the start of the validation studies in accordance with the relevant validation master plans (VMP). Validated analytical methods were used for in-process and release testing for each of the studies performed.

Table 2. Summary of Validation Master Plans for OMV

VMP	OMV B40 (suite A)	OMV B11
Qualification	Atlas 274109	Atlas 271498
Cleaning	Atlas 277809	Atlas 272300
Process	Atlas 279237	
Analytical	Atlas 268286	

3. RISULTATI (Results)

Each study is outlined below with a summary of the study, results and conclusions.

3.1 Full process validation (Building 11) – OMVZ/028/11/PVR/00

3.1.1 Summary

During the process validation study for OMV manufacturing in B11 (Siena), the concentration and inactivation phase was validated according to defined process parameters, and confirmed with the related quality attributes. The study demonstrated that over 10 batches performed, the inactivation step was performed in a consistent manner, and effectively inactivated the *N. meningitidis*.

3.1.2 Results

Validation results of the concentration and inactivation step from the building 11 process validation are provided in Table 3 below.

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Table 3 Validation Results for Concentration and Inactivation Step in Building 11

Parameter	Acceptance Criteria	Lot 02-001	Lot 02-002	Lot 02-003	Lot 02-004	Lot 02-007	Lot 02-008	Lot 02-009	Lot 02-010	Lot 02-011	Lot 02-012
Process Parameters											
Culture temperature [1]	< 15°C	7-12	8-12	8-12	9-11	6-8	8-10	6-8	7-11	8-9	6-14
Mass of concentrate in the feed tank	40 ± 2 Kg	40	40	40	40	40	40	40	40	40	40
Agitation setting in feed tank	4	4	4	4	4	4	4	4	4	4	4
Feed pressure	0.5± 0.1 bar	0.5	0.5	0.5	0.5	1.0 [2]	0.5	0.5	0.5	0.5	0.5
Mass of concentrate after second concentration step	17 ± 1 Kg [3]	17	17	17	17	17	17	17	17	17	17
pH of concentrate after buffer A addition	8.6 ± 0.2	8.5 [4]	8.5 [4]	8.4 [4]	8.4 [4]	8.4 [4]	8.6	8.6	8.6	8.6	8.6
Buffer A recirculation time	20 min [5]	20	20	20	20	20	20	20	20	20	20
Calculation of quantity (L) of Buffer A1 to be added	(Volume of concentrate + Volume of Buffer A)/20	Done	Done	Done	Done	Done	Done	Done	Done	Done	Done
Feed pressure during inactivation	1.0 bar [6]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Buffer A1 recirculation time	30 min [7]	30	30	30	30	30	30	30	30	30	30
Quantity of Buffer A for final washing	21 Kg [8]	21	21	21	21	21	21	21	21	21	21
Washing time	NLT 10 min	10	10	10	10	10	10	10	10	10	10

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Parameter	Acceptance Criteria	Lot 02-001	Lot 02-002	Lot 02-003	Lot 02-004	Lot 02-007	Lot 02-008	Lot 02-009	Lot 02-010	Lot 02-011	Lot 02-012
Quality Attributes											
Bioburden of rinse water from ultrafilter prior to use [9]	< 10 CFU/mL	0	0	0	0	0	No data [10]	No data [10]	No data [10]	0	0
Endotoxin content of rinse water from ultrafilter prior to use [9]	< 0.25 IU/mL	<0.050	<0.050	<0.050	<0.050	<0.050	No data [10]	No data [10]	No data [10]	<0.050	0.55 [11]
Pressure in the retentate line [12]	0.1-0.2 bar	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Pressure in the permeate line [12]	0.1-0.2 bar	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Quantity of Buffer A added	2.905 – 10.135 L	16 [13]	15 [13]	19 [13]	18.1 [13]	17 [13]	10.5 [13]	10	8	10	10
Quantity of Buffer A1 added	0.71 – 1.79 L	1.7	1.6	1.8 [14]	1.8 [14]	1.7	1.4	1.4	1.3	1.4	1.4
pH at the end of inactivation	7.84-8.54	7.99	8.09	7.96	8.12	7.90	8.15	8.26	8.10	8.22	8.04
Weight of inactivated material collected including wash	35.219 – 53.501 Kg	55 [15]	54 [15]	58 [15]	57 [16]	54 [15]	49	47	44	48	48
Inactivation control	absence of *N.meningitidis* serogroup B	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence
Total time for concentration and inactivation	2.5 – 4.0 hrs	2h30min	2h35min	2h35min	2h35min	2h40min	2h35min	2h40min	2h30min	2h30min	2h28min [16]

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[1] Minimum and maximum value of temperature during concentration phase are reported
[2] Feed pressure for Lot 02-007 out of range during concentration phase. This event was deemed to have no impact on the integrity of the ultrafiltrationmembrane and no impact on product quality.
[3] The mass of concentrate after second concentration step has been redefined as the volumetric concentration factor (VFC) in a QRA post-validation. Process development data supports a range of 9-25 VFC and therefore the range has been reassessed as NMT 25 and implemented in production.
[4] Results for pH in lots 02-001, 02-002, 02-003, 02-004 and 02-007 are reported for completeness of information, however, they are considered not reliable due to an issue with the pH probe. This deviation was deemed to have no impact on the quality of the drug substance based on the maximum theoretical pH reached (Buffer A has a pH of 9) and all affected lots met specification at the final phase of production.
[5] The Buffer A recirculation time as been reassessed in a QRA post-validation with an acceptance criteria of NLT 20 min. Data from process development show that a minimum of 10 min are required to adequately mix the suspension.
[6] A range has been introduced around the target feed pressure during inactivation (1 bar ± 0.2 bar) following a QRA post-validation.
[7] The acceptance criterion for Buffer A1 recirculation time has been redefined as NLT 30 minutes in a QRA post-validation.
[8] The range for the process parameter of quantity of Buffer A used for final washing has been redefined in a QRA post-validation. The range is now defined as NLT 2.5 Kg of buffer A per m2 of membrane and has been implemented in production.
[9] Bioburden and endotoxin controls on rinse water from ultrafilter were additional controls performed only to support process validation at the time the validation was performed. Currently the bioburden test is performed routinely during manufacturing.
[10] After execution of lot 02-007, the process validation study was considered complete in terms of execution, pending outcomes of QC testing. For this reason, the additional PV samples for bioburden and endotoxin in rinse water from ultrafilter were not taken for lots 02-008, 02-009 and 02-010. The sampling was resumed for lots 02-011 and 02-012 when it was determined that additional data for the overall PV study was required. Considering the positive outcome of the samples executed for the other lots with the exception of lot 02-012 and the increased number of lots the absence of sampling was not considered to have a real impact on the process validation.
[11] The result for endotoxin in rinse water from the ultrafilter was above the acceptance limit for lot 02-012. An investigation concluded that there was no impact on the quality of the OMV bulk based on a comparison of the endotoxin data in the final OMV sterile bulk concentate for lot 02-012 and the previous lots where this out-of-range was not seen.
[12] The data reported for pressure in the retentate and permeate line are the minimum values registered during the process of concentration of the culture broth.
[13] Out-of-range data for quantity of Buffer A added in lots 02-001, 02-002, 02-003, 02-004, 02-007 and 02-008. Please refer to issue with pH probe.
[14] Out-of-range data for quantity of Buffer A1 added in lots 02-003 and 02-004. Please refer to issue with pH probe.
[15] Out-of-range data for quantity of weight of inactivated material collected after washing for lots 02-001, 02-002, 02-003, 02-004 and 02-007. Please refer to issue with pH probe.
[16] Out-of-range datum for total time for inactivation and concentration process for lot 02-012. This parameter is not critical to the quality of the product and is used only to monitor the repeatability of this phase of the process. The time for recirculation of the Buffer A1 is critical and met the acceptance criteria for all lots. Therefore, the slight reduction in the total process time for this phase of the process (2 minutes less than foreseen) is not considered to impact the consistency of the process.

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3.1.3 Conclusions from process validation Building 11

Pre-determined acceptance criteria for process parameters were met for the 10 lots included in the study with the exception of pH values of the concentrate after Buffer A addition (pH values for lots 02-001, 02-002, 02-003, 02-004 and 02-007 were not reliable) and the feed pressure during the concentration step for lot 02-007 (out of acceptance range). Pre-determined acceptance criteria for all critical quality attributes and release specifications were met for the 10 lots included in the study with the exception of the extra sampling for bioburden and endotoxin of the rinse water from the ultrafilter prior to use (samples were not taken for lots 02-008, 02-009 and 02-010 and lot 02-012 yielded an out of range result). All events were investigated and closed out without impact on the product quality or the process validation study. Process consistency has been demonstrated for this step with regard to the inactivation.

3.2 Inactivation Kinetics Validation (B11) – OMVZ/028/11/PVR/02-01

3.2.1 Summary

The aim of this study was to verify the kinetics of the inactivation in the full scale manufacturing process following the addition of Buffer A1 (Tris-EDTA buffer containing 10% sodium deoxycholate (DOC) as inactivating agent). For this study, samples were taken from the process at defined timepoints during the inactivation phase and were analyzed for specific bioburden (in Quality Control Laboratories) and for inactivation control (in Production area). The study demonstrated that the inactivation agent was able to inactivate the culture at Time 0 (zero) with an initial bacterial load of 10^9 CFU/mL of *N. meningitides*.

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3.2.2 Results

Validation results of the inactivation kinetics study in building 11 are provided in Table 4 below.

Table 4: Validation Results for Kinetics of Inactivation (B11)

Parameter	Acceptance Criteria	Time (minutes)	Lot 02-010	Lot 02-011	Lot 02-012
Prior to addition of Buffer A1					
Specific Bioburden (according to SOP 202292)	Not applicable	End of Fermentation	TNTC	7.6×10^9 CFU/mL	7.6×10^9 CFU/mL
After addition of Buffer A1					
Specific Bioburden (according to SOP 202292)	Not applicable	0	0	0	0
		5	0	NP [1]	NP [1]
		10	0	NP [1]	NP [1]
		15	0	NP [1]	NP [1]
		30	0	NP [1]	NP [1]
Inactivation Control (according to SOP 202298)	Absence of *N. meningitides* serogroup B after 15 and 30 min	0	Conform	Conform	Conform
		5	Conform	Conform	Conform
		10	Conform	NP [2]	NP [2]
		15	Conform	Conform	Conform
		30 [3]	Conform	Conform	Conform

TNTC = Too numerous to count; NP = not performed

[1] The validation protocol required testing for the specific bioburden for all lots at Time 0, 5, 10, 15, and 30 minutes. However it was decided to reduce the resting for lots 02-011 and 02-012 to Time 0 only. This reduction was necessary as the high number of samples foreseen did not permit the analysis of the samples in real time, preventing the correct monitoring of the inactivation kinetics. In practice, samples were analyzed with a progressively increasing delay with respect to the actual sampling time (as was observed when the full sampling was performed in the first lot) and the samples were not representative of the real time of contact with the deoxycholate.

[2] The validation protocol required inactivation control for all lots at Time 0, 5, 10, 15, and 30 minutes. However it was decided to eliminate the 10 min timepoint for lots 02-011 and 02-012. The reduction was necessary for the same reason as given above.

[3] The result of the inactivation control at the 30 minutes timepoint was extrapolated from the routine test for inactivation performed in this phase.

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3.2.3 Conclusions from inactivation kinetics validation B11

Pre-determined acceptance criteria for the study were met for the 3 lots, and various timepoints analyzed. Although the overall number of samples was reduced during the study, the data reliably demonstrate the complete inactivation of *N. meningitides* at Time 0 using deoxycholate at the concentration used in the process. Process consistency has been demonstrated for this step with regard to the inactivation.

3.3 Inactivation Kinetics Robustness Study– TR 250708-01

3.3.1 Summary

An additional inactivation kinetics study was performed to demonstrate the robustness of the inactivation process. The materials used in the study were provided by the production department (full scale test run material), and the study itself was performed in the Quality Control Laboratories. Samples of the fermentation material were plated and treated with various concentrations of Buffer A1, and the contact time was varied from 0 up to 20 minutes.

Inactivation tests were carried out in accordance with the company procedure on plates containing Mueller Hinton medium. At each timepoint (and Buffer A1 concentration) five (5) plates were inoculated with 0.1 mL and five with 0.01 mL of fermentation broth. The inactivation agent at various concentrations was added in the same volumetric ratio as during production (1:20).

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3.3.2 Results

Study results of the inactivation kinetics robustness study in QC laboratories are provided in Tables 5 and 6 below.

Table 5: Study Results for Inactivation Kinetics Robustness Study (0.1mL inoculation)

Product Batch	Initial cell density	Buffer A1 strength	Results at various time of contact (according to SOP 202298)		
			Mean CFU/plate at T_0	Mean CFU/plate at $T_{10\ min}$	Mean CFU/plate at $T_{20\ min}$
MEB-TRUN02	6.1×10^9 CFU/mL (0.1 mL inoculation)	Normal	0	0	0
		1:2 dilution	0	0	0
		1:4 dilution	0.6	0	0
		1:8 dilution	TNTC	TNTC	TNTC
MEB-TRUN03	1.2×10^9 CFU/mL (0.1 mL inoculation)	Normal	0	0	0
		1:2 dilution	0	0	0
		1:4 dilution	2.4	0.2	0
		1:8 dilution	TNTC	0	0

TNTC = Too numerous to count;

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Table 6: Study Results for Inactivation Kinetics Robustness Study (0.01mL inoculation)

Product Batch	Initial cell density	Buffer A1 strength	Results at various time of contact (according to SOP 202298)		
			Mean CFU/plate at T_0	Mean CFU/plate at $T_{10\ min}$	Mean CFU/plate at $T_{20\ min}$
MEB-TRUN02	6.1×10^9 CFU/mL (0.01 mL inoculation)	Normal	0	0	0
		1:2 dilution	0	0	0
		1:4 dilution	0	0	0
		1:8 dilution	TNTC	TNTC	TNTC
MEB-TRUN03	1.2×10^9 CFU/mL (0.01 mL inoculation)	Normal	0	0	0
		1:2 dilution	0	0	0
		1:4 dilution	0.6	0	0
		1:8 dilution	TNTC	0	0

TNTC = Too numerous to count

3.3.3 Conclusions from inactivation kinetics robustness study

The results demonstrate that the concentration of Buffer A1 used during the process with a contact time of 30 minutes is sufficient to achieve the complete inactivation of the bacterial culture. Moreover, a concentration of Buffer A1 corresponding to 25% of that normally used during the inactivation step leads to complete inactivation after only 20 minutes of contact. Based on the results obtained, the process is sufficiently robust to tolerate a potential error in the volume of Buffer A1 added up to 25% of the value required in the procedure.

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3.4 Full process validation (B40 Suite A) – OMVZ/028/40/PVR/03-01

3.4.1 Summary

During the process validation study for OMV manufacturing in B40 (Rosia Suite A), the concentration and inactivation phase was validated according to defined process parameters, and confirmed with the related quality attributes. The study demonstrated that over 5 batches performed, the inactivation step was performed in a consistent manner, and effectively inactivated the *N. meningitidis*.

3.4.2 Results

Validation results of the concentration and inactivation step from the building 40 process validation are provided in Table 7 below.

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Table 7 Validation results for Concentration and Inactivation from Building 40

Parameter	Acceptance Criteria	Lot 01-1201	Lot 01-1202	Lot 01-1203	Lot 01-1204	Lot 01-1205
Process Parameters						
Culture temperature during concentration phase	≤ 15°C	≤15	≤15	≤15	≤15	≤15
Feed pressure during concentration phase	0.5 ± 0.1 bar	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Final concentration volume	22 ± 1 Kg [1]	22	22	22	22	22
pH after Buffer A addition	8.6 ± 0.2	8.6	8.6	8.6	8.5	8.6
Recirculation time after Buffer A addition	20 min [2]	20	20	20	20	20
Feed pressure during inactivation	1 bar [3]	1	1	1	1	1
Recirculation time after Buffer A1 addition	30 min [4]	30	30	30	30	30
Washing buffer A quantity	NLT 20 Kg [6]	20	20	20	20	20
Washing time with Buffer A	NLT 10 min	10	10	10	10	10
Quality Attributes						
Retentate pressure	0.1–0.2 bar	0.170-0.192	0.170-0.180	0.168–0.191	0.170-0.190	0.172–0.195
pH at the end of the inactivation phase	7.84 – 8.54	8.06	8.06	8.06	8.07	8.04
Inactivation control	*N.meningitidis* absence	Absence	Absence	Absence	Absence	Absence

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NLT = not less than

[1] The mass of concentrate after second concentration step has been redefined as the volumetric concentration factor (VFC) in a QRA post-validation and implemented in production.

[2] The Buffer A recirculation time as been reassessed in a QRA post-validation with an acceptance criterion of NLT 20 min.

[3] A range has been introduced around the target feed pressure during inactivation (1 bar ± 0.2 bar) following a QRA post-validation.

[4] The acceptance criterion for Buffer A1 recirculation time has been redefined as NLT30 minutes in a QRA post-validation.

[5] The range for the process parameter of quantity of Buffer A used for final washing has been redefined in a QRA post-validation. The range is now defined as NLT 2.5 Kg of buffer A per m^2 of membrane and has been implemented in production

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3.4.3 Conclusions from process validation B40 Suite A

Pre-determined acceptance criteria for process parameters were met for the 5 lots executed. Process consistency was demonstrated for this step in Building 40 Suite A with regard to the inactivation.

4. DISCUSSIONE & CONCLUSIONI (Discussion & Conclusion)

This document summarizes data from historical OMV process validation studies, as well as a laboratory scale study executed as shown below.

Table 8. Summary of Inactivation Studies for OMV

Document reference	Period of Execution	Full scale or lab scale
Full process validation (B11) OMVZ/028/11/PVR/00	March to June 2007	Full scale (350L Fermentation)
Inactivation Kinetics Validation (B11) OMVZ/028/11/PVR/02-01	May to June 2007	Full scale (350L Fermentation)
Inactivation Kinetics Robustness Study TR 250708-01	February 2008	Lab scale (culture plates)
Full process validation (B40 Suite A) OMVZ/028/40/PVR/03-01	April to May 2008	Full scale (450L Fermentation)

These studies demonstrate the inactivation of *N.meningitidis* Type B Strain New Zealand, following the addition of Buffer A1, takes place in a consistent, robust and reproducible manner and that the process is capable of manufacturing product which is consistently in accordance to the release specification.

Furthermore these studies demonstrate that when the inactivation agent is added at the predetermined concentration, the inactivation is essentially instantaneous. For this reason, the data provided in these various studies is considered sufficient to support the effectiveness of the inactivation step, and a full scale study of the inactivation

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