HPLC PURITY ASSAY AND RELATED SUBSTANCE ASSAY FOR MANNITOL

	Name	Position	Signature	Date
Author:	D. Ainslie	QC Supervisor	DAinslie	29 NOV 21
Reviewed by:	A. Jakupovic	QC Laboratory Coordinator	AJakupovic	29 Nov 21
Authorised by:	D. Ainslie	QC Supervisor	DAinslie	29 NOV 21

Table of Contents

1. PURPOSE 1
2. SCOPE 1
3. ASSOCIATED DOCUMENTS 2
4. REFERENCED DOCUMENTS 2
5. DEFINITIONS 2
6. SAFETY 3
7. EQUIPMENT AND REAGENTS 3
8. PROCEDURE 4
9. CALCULATIONS 10
10. REPORTING 11
11. TRENDING 13
12. ACCEPTANCE CRITERIA: MANNITOL CHECK STANDARD 14
13. QUALITY RECORDS 15
14. CHANGE HISTORY 16

1. PURPOSE

To describe the procedure for the determination of the assay of Mannitol and related substances of Mannitol in Mannitol raw material and spray dried Mannitol by HPLC using an RID detector.

2. SCOPE

This Test Method applies when determining the Mannitol assay and Mannitol related substances on the Mannitol raw material and spray dried Mannitol samples. The test method was based on the Ph. Eur. method from the Mannitol monograph and has been reviewed for compliance with the current edition (Ph. Eur. 10.5) at the time this test method is made effective.

This method also details the procedure for the mannitol identity check by HPLC retention time when required for specific markets.

3. ASSOCIATED DOCUMENTS

- SRP-004 Reference Solutions A & B for Mannitol Assay
- SRP-039 Resolution Solution for Mannitol Assay
- SRP-040 Maltitol & Isomalt Solution for Mannitol Assay
- FM-092 Assay/Related Substances of Mannitol HPLC Raw Data Sheet
- FM-415 Mannitol Identity by Assay
- FM-427 Mannitol Assay and Related Substances Test Record
- MES-001 Mannitol Assay & Related Substances Results Sheet

4. REFERENCED DOCUMENTS

- SOP-021 Document Control Procedure
- SOP-068 Operation and Maintenance of the Shimadzu HPLC
- SOP-084 Procedure for the Creation, Validation, Use and Maintenance of Master Excel Spreadsheets (MES)
- SOP-087 General Laboratory Procedure
- SOP-136 Handling of Quality Control Laboratory Standards
- SOP-202 HPLC and LC-MS Column Use Procedure
- SOP-208 QC Laboratory Trending Procedure
- SOP-221 Annual Product Quality Reviews
- SOP-245 Operation and Maintenance of the Milli-Q Water System in the QC Laboratory
- SOP-261 Operation and Maintenance of Filtration Apparatus for HPLC and LC-MS Mobile Phase Filtration
- SOP-311 Procedure for Storage and Collection of Pharmaxis WFI for Use in QC Lab
- SOP-324 Operation of the Shimadzu LabSolutions CS Software
- TM-008 Method for Determination of Water Content of Mannitol by Karl-Fischer
- TM-015 Loss On Drying
- ELB-074 Issue of Laboratory Copy Forms (QA Issue)
- FM-186 Approved QC Laboratory Chemicals & Consumables List
- LB-017 HPLC Column Logbook
- LB-240 HPLC Single Injections Logbook
- RN-13-010 Report for Verification of Pharmaxis WFI and Merck Lichrosolv Water for Use in TM-005, TM-006, TM-010

5. DEFINITIONS

Term	Definition
BP	British Pharmacopoeia
DAB	German Pharmacopoeia
HPLC	High Performance Liquid Chromatography
Ph. Eur.	European Pharmacopoeia

Term	Definition
RF	Response Factor
RID	Refractive Index Detector
RRT	Relative Retention Time
% RSD	Percent Relative Standard Deviation
Pharmaxis WFI	Pharmaxis Water for Injection. Water manufactured at Pharmaxis' 20, Rodborough Road site in accordance with SP-571

6. SAFETY

6.1. Refer to SOP-087 for general information for safety in the QC laboratory and refer to SOP-068 for information about safe operating of the HPLC.

6.2. Safety glasses and laboratory coats should be worn when using the HPLCs.

6.3. Care should be taken when handling glassware.

6.4. Care should be taken when handling liquids near electrical equipment.

6.5. Be careful of high temperature HPLC operating conditions used in the method.

7. EQUIPMENT AND REAGENTS

7.1. REAGENTS AND CONSUMABLES

7.1.1. Refer to FM-186 for details of approved chemicals and consumables.

7.2. HPLC CONDITIONS

7.2.1. The HPLC instrument, detector and method parameters are detailed in the following tables. The acceptance criteria for System Suitability, Standard Suitability, Check Standard and other criteria (refer to sections 8.4, 8.5, 8.6 and 8.7 and FM-092) must be met in order for each run on the HPLC to be considered acceptable.

Instrument Parameters	
Mobile Phase	100% HPLC Grade Water, Water for Injection, Milli-Q Water (refer to SOP-245 for use of Milli-Q system), Pharmaxis WFI (refer to SOP-311 for storage and collection of Pharmaxis WFI). Following SOP-261, degas mobile phase by filtering under vacuum using 0.2µm membrane filter. The mobile phase has an expiry of five days from the date of

Instrument Parameters	
	degassing.
Flow Rate	0.5mL/min
Injection Volume	20µL
Run Time	40 minutes
Needle Wash	As per mobile phase
Approx. Retention Time (Mannitol)	18 minutes
Column	Benson Carbohydrate Column BP-100 Ca++ 300mm x 7.8mm, 10µm
Guard Cartridge	Carbo BC-100 Ca++ 10µm Part No. 96274
Column Temperature	85°C ± 1°C
Detector	Refractive Index

Refractive Index Detector Parameters	
Mode	Analytical
Polarity	Positive
Cell Temperature	40°C ± 2°C

Method Parameters	
Quantitation	Area

8. PROCEDURE

8.1. TEST SOLUTION AND CHECK STANDARD PREPARATION AND STORAGE

8.1.1. Mannitol Assay and Related Substances testing is to be documented using FM-427 Mannitol Assay and Related Substances Test Record. The form is to be issued a copy number from ELB-074 Issue of QC Laboratory Forms (QA Issue) prior to testing.

8.1.2. Preparation of the reference solutions is performed in accordance with the following procedures:

- SRP-004 for the preparation of Reference Solution A & B for Mannitol assay
- SRP-039 for the preparation of Resolution Solution
- SRP-040 for Maltitol and Isomalt Solution

8.1.3. Test Solutions and Reference Solutions A and B are to be used within 24 hours of preparation. Resolution and Maltitol & Isomalt Solutions have an expiry date of six months from the date of preparation when stored at -16°C ± 2°C, otherwise are to be used the same day as prepared.

8.1.4. A check standard is to be prepared using FM-427 as Sample1 and Sample2 each time a sequence is run.

8.1.5. The Check Standard is stored in the stability chambers at the stability conditions of 25°C/60%RH, and allowed to equilibrate to the QC lab temperature and relative humidity (at least one hour) before testing.

8.1.6. Use an approved 0.45µm Nylon syringe filter to filter, into a HPLC vial, blank, sample and reference solutions to be injected into the HPLC, discarding the first 2mL of the filtrate.

8.2. HPLC SYSTEM START UP AND SHUTDOWN

8.2.1. Follow SOP-068 and SOP-324 for setting up of instrument and refer to section 7.2 for HPLC instrument and method parameters.

8.3. SEQUENCE SET UP, SYSTEM SUITABILITY AND STANDARD SUITABILITY

8.3.1. The analyst is to record sequence information on FM-092. The information recorded is to include instrument, column and mobile phase details, and sample information.

8.3.2. Sequences are named according to the following format <*Date*>*XYYYY*.

Where:

- <Date> is in the format according to SOP-087
- X is used for first sequence of the day for the instrument. If there is more than one sequence on the instrument per day use Y, Z etc.
- YYYY are the last four digits of the HPLC tag number

An example sequence name is: 25Jan14X0111

8.3.3. A blank (water used in the preparation of test solutions) injection should be performed at the beginning and end of the run. The baseline must be stable and without any interfering peaks. Consideration must be given to both the size and position of all peaks to determine if they are interfering peaks.

Note: Running of additional blank injections at the beginning of the sequence for system or column equilibration purposes with the associated instrument equilibration method is acceptable.

8.3.4. Perform a single injection of the Maltitol and Isomalt Reference Solution. This is for identifying known impurities.

8.3.5. Perform a single injection of the Resolution Solution and ensure the resolution criteria is met as per section 8.4.4.

8.3.6. For system suitability (repeatability), one vial of the first Reference Solution A preparation, and one vial of the first Reference Solution B preparation are to be injected 6 times. System suitability injections are performed prior to the injections of the bracketing standards and sample preparations.

8.3.7. Note that if system suitability was performed previously on the system and the same mobile phase and system parameters were used and unaltered (i.e. the flow was not stopped) then the repeatability can be taken from the initial sequence.

8.3.8. If repeatability is not performed as per section 8.3.7, then perform single injections of the second Reference Solution A and second Reference B preparation.

8.3.9. For Standard Suitability, compare the last injection of the repeatability injections (if repeatability is performed) with the second Reference Solution A (for assay) and second Reference Solution B (for related substances) preparations. If the repeatability is not required, compare the injection of the first Reference Solution A with the injection of the second Reference Solution A (A(a) and A(b)) and compare the injection of the first Reference Solution B preparation with the second Reference Solution B (B(a) and B(b)).

8.3.10. Perform a single injection of the first Reference Solution A preparation and a single injection of the first Reference Solution B preparation to start the standard bracket.

8.3.11. Inject the check standard preparations followed by the sample solution preparations (one injection per sample preparation). Not more than 6 sample injections should be made between bracketing standards.

8.3.12. Perform a single injection of the first Reference Solution A preparation, and a single injection of the first Reference Solution B preparation, to complete the bracket.

8.3.13. Ensure that a blank injection is performed (section 8.3.3) to end the sequence.

8.4. ACCEPTANCE CRITERIA FOR SYSTEM SUITABILITY

8.4.1. The acceptance criteria for system suitability injections is:

Reference Solution A: %RSD $\leq$ 0.85%

Reference Solution B: %RSD $\leq$ 5.0%

The sequence is considered invalid if the system suitability criterion fails.

8.4.2. For tailing, the average of the six injections for both Reference Solution A and Reference Solution B is to be between 0.8 and 1.5 or if repeatability is not performed tailing is obtained from average of two standard suitability injections for the Mannitol peak.

8.4.3. The plate count on the first injection of Reference Solution A and Reference Solution B is to be not less than 4000.

8.4.4. The resolution between the Mannitol and Sorbitol peaks must be not less than 2.0.

8.4.5. Resolution Calculation (BP/EP calculation):

$$R = 1.18 (t_B - t_A) / (W_{B\,0.5} + W_{A\,0.5})$$

Where:

R	=	resolution
t_A	=	retention time of peak A
t_B	=	retention time of peak B
$W_{A\,0.5}$	=	Peak width at half height of peak A
$W_{B\,0.5}$	=	Peak width at half height of peak B

Note that the Shimadzu LabSolutions software calculates this and is referred to as Resolution (DAB).

8.5. ACCEPTANCE CRITERIA FOR STANDARD SUITABILITY

8.5.1. The standard suitability passes when the following criterion is met:

$$\frac{RF_{highest}}{RF_{lowest}} \leq 1 + \frac{(2 \text{ x } \%RSD)}{100}$$

Where RF is calculated by the formulae below:

$$RF = \frac{\text{Mannitol Area}}{\text{Standard Concentration (mg/mL)}}$$

%RSD is taken from the six repeatability injections. If the assay %RSD is <0.5%, then 0.5% is to be entered into the equation. For the Related Substances (Reference Solution B), if the %RSD is <2.0% then enter 2.0% into the equation.

8.6. PROCESSING

8.6.1. Process data with Shimadzu software in accordance with the current version of SOP-324.

8.6.2. If related substances Unknown 7 peak elutes so close to Mannitol peak manual integration may be performed with QC Supervisor or QC Laboratory Coordinator approval.

8.6.3. Asymmetry is to be within acceptance criteria for system suitability (if repeatability is performed) or standard suitability if repeatability is not performed.

8.6.4. Print the following and attach to the initiated Assay/Related Substances HPLC Raw Data Sheet (FM-092):

- System Suitability Reports for Assay (Reference Solution A) and Related Substances (Reference Solution B).
- Batch Summary Report for assay and related substances brackets.

- The related substances chromatograms of the bracketing standard B and sample solutions. Note that for sample solutions, the first preparation chromatogram has to be printed in duplicate.
- Batch Table Report
- Method Report
- Resolution Solution Chromatogram Report
- Maltitol & Isomalt Solution Chromatogram Report
- Blank (water used for test solution preparation) Injections Chromatogram Reports
- Any manually integrated chromatograms.

Note: All chromatograms/reports are printed by the 2nd Level Approver.

8.6.5. Check the results of the replicate standard injections. The difference between the two areas must be less than or equal to 1.0% for Reference Solution A, or less than or equal to 5.0% for Reference Solution B for the results to be acceptable when calculated as follows:

$$\% \text{ Difference} = \frac{(\text{Highest Area - Lowest Area})}{\text{Lowest Area}} \text{x} 100$$

8.6.6. Determine the results using the current authorised Microsoft Excel Spreadsheet MES-001, as per SOP-084.

8.6.7. Check the plate count on the last injection of Reference Solution A and Reference Solution B from the Chromatogram printout. If it is below 4100, the column must be reconditioned or disposed.

8.6.8. Check end bracket asymmetry for assay and related substance Reference Solutions during the processing of the sequence. The asymmetry is to be within 0.8-1.5 for the Mannitol peak. If asymmetry is above 1.5 but less than or equal to 1.9 the sequence is acceptable and column should be reconditioned before further use. If the asymmetry is above 1.9 the sequence is invalidated and column must be reconditioned or disposed of.

8.7. MANNITOL IDENTITY CHECK

8.7.1. The identity check is required for spray dried mannitol used for Bronchitol for the Russian market only.

8.7.2. Identity testing by assay is documented using FM-415 Mannitol Identity by Assay. The form is to be issued a copy number from ELB-074 Issue of QC Laboratory Forms (QA Issue) prior to testing. One copy is issued for each pooled sample tested.

8.7.3. Perform identity test by comparing Mannitol peak retention time of start of bracket standard, replicate one and replicate two samples and end of bracket

standard. An assay sample solution chromatogram and assay standard solution chromatogram is to be printed and attached to FM-415 form. The identity test complies when retention time of Mannitol peak of the sample solutions is comparable with retention time of Mannitol peak of the standard solutions.

8.8. RETENTION TIMES

8.8.1. Approximate retention times and relative retention times (RRT) are as follows:

Peak	Retention Time (Minutes)	Relative Retention Time
Lecithin	5.6	0.31
Unknown 6	7.2	0.40
Isomalt 1	12.7	0.71
Unknown 2	13.3	0.74
Maltitol	13.5	0.75
Isomalt 2	13.6	0.76
Unknown 10	14.0	0.78
Unknown 3	14.7	0.82
Unknown 9	15.5	0.87
Unknown 4	16.2	0.91
Unknown 8	16.8	0.94
Unknown 7	17.1	0.96
Mannitol	17.9	1.00
Sorbitol	20.4	1.14

Notes:

- Isomalt consists of a mixture of 6-O-α-D-glucopyranosyl-D-glucitol (ie. Isomaltitol) and 1-O-α-D-glucopyranosyl-D-mannitol and thus elutes as two peaks. The Maltitol and Isomalt reference solution will indicate the retention times for these peaks.
- Isomalt 1 was previously identified as Unknown 1 so earlier work refers to this labelling of the peak.
- Unknown 5 was identified as Lecithin.
- Any new unidentified impurity peaks will be successively labelled after Unknown 10.

8.9. EXAMPLE CHROMATOGRAMS

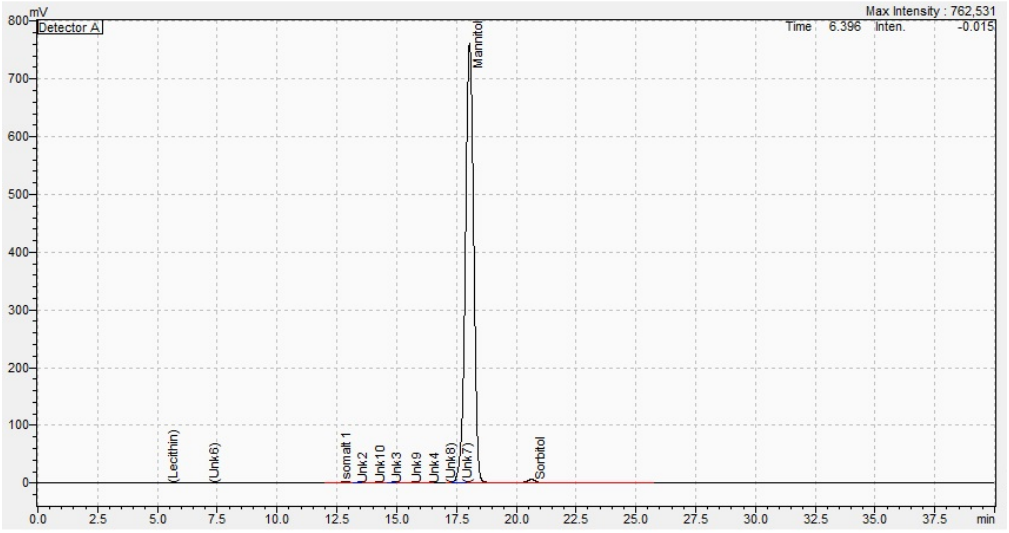

Figure 1: Mannitol Assay and Related Substance Sample Full Chromatogram

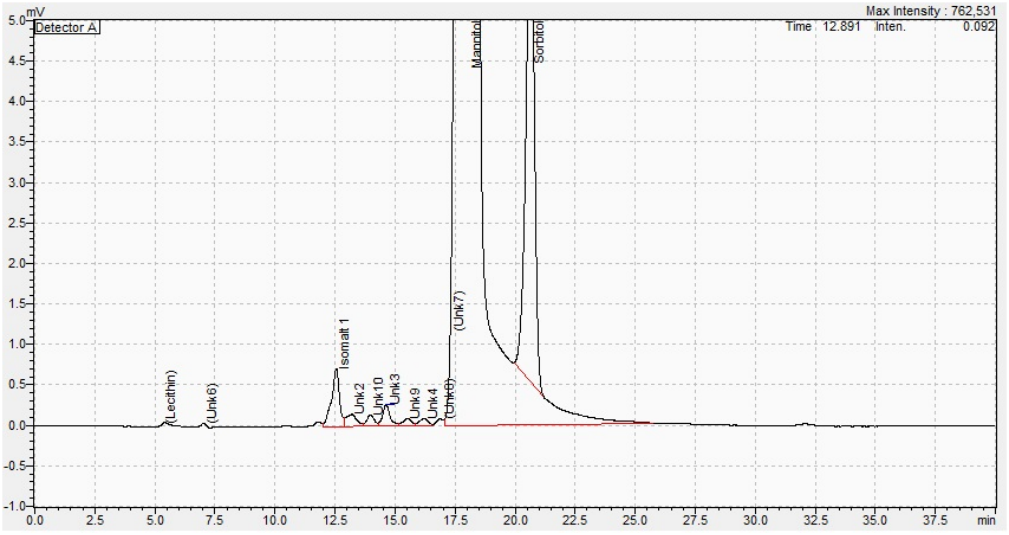

Figure 2: Mannitol Assay and Related Substance Sample Zoomed Chromatogram

9. CALCULATIONS

9.1. MANNITOL ASSAY

9.1.1. Quantitate the Mannitol peak in the Test Solutions with reference to the Reference Solution A bracketing solution.

$$\%\ \text{w/w Mannitol (as is)} = \frac{\text{Area}_{\text{Sample}} \times \text{Weight}_{\text{Std}}(\text{mg}) \times \text{P}(\%) \times 100(\text{mL})}{\text{Area}_{\text{Std}} \times \text{Weight}_{\text{Sample}}(\text{g}) \times 10(\text{mL}) \times 1000(\text{mg/g})}$$

$$\%\ \text{w/w Mannitol (anhydrous)} = \frac{\text{Area}_{\text{Sample}} \times \text{Weight}_{\text{Std}}(\text{mg}) \times \text{P}(\%) \times 100(\text{mL})}{\text{Area}_{\text{Std}} \times \text{Weight}_{\text{Sample}}(\text{g}) \times 10(\text{mL}) \times 1000(\text{mg/g})} \times \frac{100\%}{(100\text{-W})\%}$$

Where:

- $Area_{Sample}$ – is the area of the Mannitol peak in the Test Solution.
- $Area_{Std}$ – is the average area of the Mannitol peak in Reference Solution A.
- $Weight_{Sample}$ – is the weight of Mannitol (in g) used in the Test Solution.
- $Weight_{Std}$ – is the weight of Mannitol Secondary Standard (in mg) used to prepare Reference Solution A (replicate used for bracketing of samples).
- P – is the purity (as a percentage) of the Mannitol Secondary Standard.
- W – is the %w/w water content.

Note: Water content may be determined by Karl Fischer Titration (TM-008, anhydrous result) and/or by Loss on Drying (TM-015, dried result), refer to the relevant specification for reporting requirements.

For the Mannitol assay to be acceptable, the % difference between duplicate sample preparation results must be ≤1.0% as follows:

$$\% \text{ Difference} = \frac{(\text{Highest Result - Lowest Result})}{\text{Lowest Result}} \text{x}100$$

9.2. RELATED SUBSTANCES

9.2.1. Integrate any secondary peaks in the Test Solution and quantitate them with reference to the peak areas of the primary (Mannitol) peak in Reference Solution B bracketing solution. Calculate the total impurities and the total unidentified impurities.

$$\% \text{ w/w Mannitol (as is)} = \frac{Area_{Sample} \text{ x } Weight_{Std}(mg) \text{ x } P(\%) \text{ x } 100(mL) \text{ x } 2(mL) \text{ x } 100(\%)}{Area_{Std} \text{ x } Weight_{Sample}(g) \text{ x } 10(mL) \text{ x } 100(mL) \text{ x } 1000(mg/g) \text{ x } 100(\%)}$$

Where:

- Rel Subs $Area_{Sample}$ – is the area of the related substances peak in the Test Solution.
- Rel Subs $Area_{Std}$ – is the average area of the Mannitol peak in Reference Solution B.
- $Weight_{Sample}$ – is the weight of Mannitol (in g) used in the Test Solution.
- $Weight_{Std}$ – is the weight of Mannitol Secondary Standard (in mg) used to prepare Reference Solution A (replicate used for bracketing of samples).
- P – is the purity (as a percentage) of the Mannitol Secondary Standard.

10. REPORTING

10.1. MANNITOL ASSAY REPORTING

10.1.1. Note that for an assay result to be valid the Mannitol Check Standard result must

be within the acceptance criteria (see section 12).

10.1.2. Mannitol Assay will be reported according to the decimal points as stated in the specifications of Mannitol. For trending purposes, see Section 11.

10.1.3. The results will be reported as '% w/w Mannitol (as is)', '% w/w Mannitol (anhydrous)' and '% w/w Mannitol (dried substance)'.

10.2. MANNITOL RELATED SUBSTANCES REPORTING

10.2.1. The following criteria are to be used for the related substances reporting when quantified against a 2.0% Reference Standard B Solution:

- Note that any related substances < 1.0% will be reported to two decimal points.
- For any unidentified peaks that are present in the sample chromatograms at the same retention time as in the blank injection, and of relatively the same area and height, it is not to be reported but stated as 'not detected'. However, sample peak area is to be subtracted from the blank peak area at the same retention times if the area in the sample chromatogram is greater than the blank peak. Reporting will then be done as mentioned below for unidentified impurities.
- For unidentified peaks in the sample, report any peaks ≥ 0.05%. Peaks below 0.05% will be reported as '< 0.05%'. Note: 0.05% is the limit of quantification for this test method.
- For the identified impurities Sorbitol, Lecithin, Maltitol, Isomalt 1 and Isomalt 2, report any peaks ≥ 0.05%. Peaks that are detected but are below 0.05% will be reported as '< 0.05%'. Any identified impurity peaks that are not detected will be reported as 'Not Detected'.
- For total impurities, all peaks ≥ 0.05% are to be included. Peaks stated as '<0.05%' will be excluded from the total.

10.2.2. The following control limits will be followed when reporting Isomalt, Unknown 2, Unknown 3, Unknown 10 and Maltitol peaks:

- The peak eluting in the window of RRT 0.74 and RRT 0.76 will still be labelled as 'Unknown 2', peak eluting at RRT 0.78 labelled as 'Unknown 10' and peak eluting at RRT 0.82 is 'Unknown 3'.
- The total peak area of Isomalt 1, 'Unknown 2', 'Unknown 10' and 'Unknown 3' will be controlled to ≤0.2%. This will ensure that the two Isomalt peaks and the Maltitol peak will not exceed 0.2%. If this area exceeds 0.2%, an investigation into the identity of the peak(s) labelled 'Unknown 2', 'Unknown 10' and/or 'Unknown 3' needs to be conducted to decide whether the increased peak area is due to 'Unknown 2', 'Unknown 10', 'Unknown 3', Isomalt 2 or Maltitol.

10.2.3. For trending of the related substances refer to section 11 below.

11. TRENDING

11.1. MANNITOL ASSAY AND RELATED SUBSTANCES TRENDING

11.1.1. The trending of Mannitol assay and related substances by HPLC is to be performed in accordance with the current version of SOP-208 QC Laboratory Trending Procedure.

11.1.2. The current Mannitol assay and related substances trending data file will be saved at the following file path:

11.1.3. P:\Manufacturing\QC Trending\Assay\Assay Results Trending\XXXX Assay Results Data. The file name for Mannitol assay is to be "Assay Trending XXXX – Data Entry File". Note: XXXX in the file path and in the filenames is for the year the testing was performed.

11.1.4. The trending data files are protected Microsoft Excel files. The Excel function 'Allow Users to Edit Ranges' will be used to allow entry of new data. The data entry cells will be reviewed periodically (at least monthly) by the QC Laboratory Coordinator or delegate to ensure security of data.

11.1.5. Notes:

- Assay results are to be entered with 2 decimal places in the "Assay Result %w/w" column for trending purposes. The result type selected for this entry should be "Replicate". The Average assay result, reported to 2 decimal places is entered in the same column, but the result type selected now should be "average".
- Individual related substances results are to be entered to three decimal places and the average to two decimal places.

11.1.6. Data entry of the test details and results for the Mannitol Check Standard and all samples tested is to be performed by the analyst performing the test. Each replicate and average result is to be entered as a separate row.

11.1.7. Data entered is to be checked by the QC staff member performing the checking of the Mannitol assay testing data.

11.1.8. Refer to SOP-221 for requirements for review of assay and related substances data for QC test samples and refer to relevant Test Protocols for requirements for review of assay and related substances data for development and stability samples.

11.2. MANNITOL ASSAY/RELATED SUBSTANCES SYSTEM SUITABILITY TRENDING

11.2.1. The current HPLC System Suitability file for Mannitol assay and related substances trending data file will be saved at the following file path:

11.2.2. P:\Manufacturing\QC Trending\Assay\System Suitability Trending\XXXX Assay SSB Data

11.2.3. The file name for Mannitol assay is to be "Assay SSB Trending XXXX – Data Entry File".

11.2.4. Note: XXXX in the file path and in the filenames is for the year the testing was performed.

11.2.5. Data entry for the trending of the HPLC System Suitability Performance is to be performed by the analyst performing the test. Ensure that all the required fields are entered in the data file, including plate count (DAB) and %RSD area for assay and related substances.

11.2.6. Data entered is to be checked by the QC staff member performing the checking of the Mannitol assay testing data.

12. ACCEPTANCE CRITERIA: MANNITOL CHECK STANDARD

12.1. For the Mannitol assay and related substances results to be valid, the Mannitol Check Standard must be within the required acceptance criteria as determined below.

12.2. The preparation and use of the Mannitol Check Standard is described in the current version of SOP-136. The check standard for assay is to be of Spray Dried Mannitol Blend.

12.3. When preparing a new check standard, a sufficient amount of Spray Dried Mannitol Blend is to be obtained so that approximately 36 months' worth of material is available.

12.4. The Check Standard for Mannitol assay and related substances testing is given a 36 month expiry from the date it was first used for routine testing. An expiry label is to be attached in accordance with SOP-136 at the time of the first use.

12.5. Review of the "Assay Trending XXXX – Data Entry File" is to be performed monthly for Mannitol check standard data. Trend analysis must be performed before any assay testing is performed in a calendar month; otherwise the trending is required to be completed within the first week of the month. The procedure for trend analysis of the Mannitol Check Standard is stated as follows.

12.6. The acceptance criterion for the assay of the Mannitol Check Standard is determined monthly. It is to be determined within the first week of the month. The acceptance criteria is derived as follows:

12.6.1. The data for the Mannitol Check Standard is to be copied from the "Assay Trending XXXX – Data Entry File" (see section 11.1.3) into the Check Standard yy.MPX file located in the P:\Manufacturing\QC Trending\Check Standards\Check Standard yy (Assay) folder. Note yy indicates the relevant check standard number of the check standard used.

12.6.2. The minimum number of replicates to establish the acceptance criteria for the initial setup of the new Mannitol Check Standard should be 6. Every container of the check standard must have been tested as a replicate for the setup of the check

standard acceptance criteria.

12.6.3. The initial acceptance criteria use the mean of the initial replicates and the final standard deviation result obtained for all data of the previous check standard

$$(\text{Average Result}_{[\text{New Check Standard}]} - 3\sigma_{[\text{Previous Check Standard}]}) \text{ to}$$
$$(\text{Average Result}_{[\text{New Check Standard}]} + 3\sigma_{[\text{Previous Check Standard}]})$$

12.6.4. The initial acceptance criteria is valid for one month. The QC Supervisor may extend the use of the standard deviation of the previous check standard for calculation of the acceptance criteria if insufficient data has been obtained after one month for satisfactory calculation of the range.

12.6.5. The first week of every month a control chart is to be prepared for the assay result. The control chart is located at: P:\Manufacturing\QC Trending\Check Standards\Check Standard 1 – Assay\folder.

12.6.6. The "Assay Result %w/w" data should be used for control chart purposes. The control chart is to show the average and ± 3 standard deviations (i.e. 3σ) for all data generated for the check standard. The acceptance criteria for the check standard are then stated on the control chart as being within the range of

$$(\text{Average Result} - 3\sigma) \text{ to } (\text{Average Result} + 3\sigma)$$

Note: The standard deviation is obtained from the mean assay results from each check standard analysis. The acceptance criteria range is derived from ALL data obtained for the check standard currently being used.

12.6.7. The printed control chart, with the acceptance criteria for the check standard for next month stated, is to be signed and dated by the QC staff member who performed the analysis.

12.6.8. The control chart is then checked and signed by either the QC Supervisor, or a QC Laboratory Coordinator.

12.6.9. The printed control chart is then filed in the 'Current' tab of the Assay by HPLC Trending Folder. Any superseded charts must be moved to the appropriate superseded tab.

13. QUALITY RECORDS

13.1. Complete FM-092 and attach HPLC printouts to the form. Store the raw data in the HPLC raw data file.

13.2. Attach the following documentation to FM-427 and file in batch release records file:

- One of the sample chromatograms
- Signed and dated MES-001 print outs.

13.3. Completed copies of SRP-004, SRP-039 and SRP-040 are filed in folders in the QC Laboratory.

13.4. All documentation will be kept in accordance with the current version of SOP-021.

14. CHANGE HISTORY

Version	Date Effective	Section	Description and Rationale
18	30Nov21	2	Ph. Eur. reference updated to refer to current edition at time of review.
		4	FM-183 removed and SOP-221 added as form was made obsolete due to trending of QC data being performed in accordance with Annual Product Quality Review procedures. SOP-110 removed and SOP-324 added as the operating software for the Shimadzu HPLCs has been upgraded to use LabSolutions software in accordance with CR 833.
		8.2 and 8.6.1	Reference to SOP-110 updated to SOP-324 in line with upgrade of operating software as per CR 833.
		8.3.9	Description of check for standard suitability when repeatability is not required updated to align with sequence structure.
		8.3.13	Cross reference for preparation of blank corrected from 8.3.4 to 8.3.3.
		8.4.5	Operating software reference updated from Class VP to LabSolutions in accordance with software update as per CR 833.
		8.6.4	Reports to be printed and names of reports updated in accordance with requirements for sequence processing using the LabSolutions software.
		8.6.7	Procedure for determining plate count updated for use of LabSolutions software.
		8.9	Example chromatograms updated to show appearance as displayed in LabSolutions software.
		11.1.8	Reference to FM-183 replaced with SOP-221 as trending of QC data is performed in accordance with Annual Product Quality Review procedure.
		12.3 and 12.4	New information added to detail the amount of material required when preparing a new check standard and the expiry period for the check standard as required by CAR 876.
		12.6.1	Minitab file extension updated from .MPJ to .MPX in line with current file extension after update of Minitab version.

Version	Date Effective	Section	Description and Rationale
17	11May20	3	Associated Document SRP-004 the name is changed to Reference Solution A & B for Mannitol Assay. to align with the changes required for CAR 772
		3	Added new documents FM-427, SRP-039 and SRP-040 to align with the changes required for CAR 772.
		3	Updated name for FM-092 to match current document title.
		4	Added new document LB-240 as per the Change Request CR 836
		4	Shimadzu HPLC Class VP Software versions 7.4 is deleted from the name of the document SOP-110 as per CR 737
			Updated name for ELB-074 to match current title.
		8.1.1 to 8.1.3	Use of the required Associated Documents for the preparation of various solutions for the Mannitol Assay and related Substances updated as per CAR 772.
		8.1.4	Check Standard Sample1 and Sample 2 are the first Test Solutions to run in the Bracket, for clarity. Information updated to use FM-427 for preparation in line with change to documentation as per CAR 772.
		8.3.3	Replaced the requirement of Trial Injection with the acceptability of additional blank injection for the equilibration of the System and the Column used, asper CR 836
		8.7.2	Section reworded to match wording in other test method documents and detail requirement for form to be issued prior to use.
		13.2 and 13.3	Section updated/added to detail requirements for filing of test records due to the creation of new documents FM-427, SRP-039 and SRP-040.
16	28Nov18	All	Document updated to use current version of controlled template (TE-006-03) in accordance with document control procedure requirements.
		2	Updated Ph. Eur. version to current version. Added information regarding mannitol identity check as required to be included in accordance with CR 802.
		3	FM-415 added to Associated Documents new form created in accordance with CR 802.
		4	ELB-074 added to Referenced Documents as this log is referenced in the method.
		8.7	New section created to define identity test as required by CR 802 for Russian market.

Version	Date Effective	Section	Description and Rationale
15	11Sep17	8.4.3 & 8.6.7	Theoretical Plate (TP) count limit is changed to not less than 4000 and an alert limit of 4100 for both Assay and Related Substances as per the change proposed in Change Request # CR540.
		8.6.2	Added section on manual integration of Unknown 7 in accordance with CM-12-005-02.
		8.7.1	Approximate retention times (RT) and relative retention times (RRT) table updated in accordance with CM-12-005-02 and CAR 539. Related substances Unknown 7, Unknown 8, Unknown 9 and Unknown 10 included in table. Unknown 6 updated to Unknown 10 in the last bullet point paragraph as this is latest labelled unidentified impurity peak.
		8.8	Updated example chromatograms to the current for Assay and Related Substances as per CM-12-005-02 and CAR 539.
		10.2.2	Unknown 10 added to the section as it falls within RRT window range 0.74 to 0.82 and therefore is a part of in house requirement that sum of Isomalt 1 & 2 + Unknown 2 + Unknown 10 + Unknown 3 is ≤0.2% (Ref. CAR 539). Deleted last bullet point "The Maltitol peak area will be controlled at ≤0.2%" as this is already mentioned in above bullet point two. Added Unknown 3 to 10.2.2 paragraph as it was missed in previous versions update.
		11.1.4 & 12.4.8	Instrumentation Team Leader and QC Laboratory Team Leader changed to QC Laboratory Coordinator due to restructure of the QC Laboratory.
14	12Aug15	All	Referenced Pharmaxis WFI water as an alternative option for HPLC Grade Water. Refer to RN-013-010 for Report & CR544
		4	Added SOP-311, TM-015 & RN-13-010 as referenced documents.
		5	Added Term Pharmaxis WFI
		9.1 & 10.1.3	Testing Requirements and Specifications changed in accordance with CR564 requirements.
13	04Nov14	2	European Pharmacopoeia reference updated to 8.2 to the most recent version at time of document review.
		3	Associated document MES-001 title updated.
		4	SOP-021 added to referenced documents as it is referenced in Test Method. Reference documents SOP-110, SOP-084, SOP-202 & LB-017 title updated.

Version	Date Effective	Section	Description and Rationale
13	04Nov14	7.2.1	Information regarding varying of HPLC conditions after consultation with QC Supervisor removed as this is not routinely performed. Any changes to HPLC conditions are assessed for impact on quality though a Deviation Report. Specified critical parameter for RID detector and method calibration parameters as required by CAR 492. Deleted reference to Table 1 renamed table header to Instrument Parameters and rearranged rows to improved table flow. Added information for HPLC conditions variability.
		8.2	Deleted reference to Table 1 and reference update to section 7.2
		8.3.2	Sequence name information updated to capture current routine.
		8.3.6	Deleted reference to Section 6.5 and reference update to section 8.4.4
12	31Jan13	2.	European Pharmacopoeia reference updated to most recent version at time of document update.
		8.1.1	Test and Reference Solutions expiration date clarified.
		8.4.2, 8.6.2 & 8.6.7	Asymmetry acceptance criteria revised in accordance with CR 422. Note 8.6.7 new section created.
		12.4	Corrected subsections numbering to 12.4 section to correct values.
11	29-Feb-12	All	Update to current version of controlled template (TE-006-02) in accordance with SOP-021 requirements.
		2.	European Pharmacopoeia reference updated to most recent version at time of document review.
		3. & 4.	SRP-004 moved from Reference Documents to Associated Documents as this is the correct location.
		4.	FM-186 and SOP-245 added to Referenced Documents as these procedures are referenced in the method.
		5.	Definition of RRT added as this abbreviation is used in the method.
		6.	New section ‘Safety’ added in accordance with TE-006-02 requirements.
		7.	Milli-Q Water added as a suitable reagent in accordance with CR 273 and RN-10-051. Expiry period for mobile phase added.
		8.1.4.	Solutions filtration requirements updated in accordance with CR 390.
		8.3.	Sequence name information added and requirement to complete FM-092 detailed.
		8.6.2.	Section added to include missing processing step.

Version	Date Effective	Section	Description and Rationale
11	29-Feb-12	9.	$Weight_{Std}$ description for calculation of related substances corrected to reference the weight used for the standard preparation used for the preparation of the replicate used as the bracketing standard rather than the average of the duplicate preparations. This was incorrect in the previous version however was correct in MES-001. Additional information added to the $Weight_{Std}$ description for calculation of mannitol assay was added to for clarification. Assay and related substances formulae modified to match formulae used in MES-001. This includes changes to units used but is not a change to the methods of calculation.
		10.2.	Update internal criteria for reporting of related substances Unknown 2, Unknown 3, Isomalt and Maltitol in accordance with amendment to CR 207.
10	05-Jan-10	2.	Update Ph. Eur reference to 6.6.
		7.1	Correct calculation for % mannitol (anhydrous). Typographical error in previous version only.
		7.2	Correct calculation for % related substances. Typographical error in previous version only.
9	15-Oct-09	All	Update format in accordance with SOP-021-05 and TE-006-01.
		6.0	Added 'Procedure' as a subtitle to include test solution preparation, HPLC conditions, system suitability, measurement, processing, retention times and example chromatograms under one heading.
		6.1	The part number of Benson BP-100 Ca Analytical Calcium column has been changed from # 800 to # 1000-0 in accordance with CR#222.
		6.2	Included check standard preparation method & storage, sample solution storage details and added filtration of blank and samples as per Change Request CR205.
		6.4	Changed subtitle to 'Sequence Setup, System Suitability and Standard Suitability' as this section now combines section 9.5 (Standard Suitability) & 9.6 (Measurement). This was done to clarify the procedure and identify the sequence of events carried out for HPLC injections.
		6.5 & 6.6	New sub-section added: Acceptance Criteria for System Suitability and Standard Suitability, to collate all acceptance criteria for system performance checks.
		6.5.4	Resolution criteria changed from not less than 2 to not less than 2.0 in accordance with CR#199.
		6.7	Added extra information on what other reports need to be printed as this is routinely performed.

Version	Date Effective	Section	Description and Rationale
9	15-Oct-09	6.8	Updated Retention Time table to reflect changes as proposed in Change Request No. CR207.
		6.9	New Example Chromatograms included for Assay and Related Substances.
		7.0	Separate heading for Calculations for assay and related substances.
		7.1	Added calculations for assay on 'as is' basis for Mannitol and changed purity to be as decimal, not as a percentage.
		7.2	Changed purity to be as decimal, not as a percentage
		8.0	New section to read as 'Reporting'. Added to clarify how to report assay and related substances for Mannitol as per Change Request No. CR207.
		9.0	Complete review and update of trending section as per the requirements of SOP-208.
		10.0	New section to include 'Acceptance Criteria' for Mannitol check standard data.

END OF DOCUMENT