

Instructions for Sample Testing

General instructions:

The test evaluation consists of two different sections. The tests focus on accuracy, repeatability as well as evaluation of the library matching function and peak purity of the specified LC system in this tender. Exact method conditions, column and in-line filter are found in Appendix A. Tenderers are required to obtain the specified column and in-line filter as listed.

Tenderers are required to fill up **Annex E Summary Sheet for Sample Testing Results** (please download from Gebiz) and submit hardcopies* of results for ALL sections **within 2 weeks from tender closing date.**

Material Package

- 1) Instructions for sample testing
- 2) Report layout documents consisting of the following:
Sequence printout, Calibration report, Sample report, Peak Purity and Library matching report

*Hardcopies of results shall have all information displayed and printed in a format reflected in the report layout documents handed over. Terminology used in peak purity reports may varied depending on manufacturers. However, Tenderer must demonstrate that the relevant information is still reflected in the hardcopy.

Section A: Accuracy and Repeatability studies

For this section, the materials in Set A will be used for the evaluation.

Set A comprises of 7 vials, labelled Calib-A, ACA, ACB, Sample A1, A2, A3 and A4. Testing should be carried out using the TEST method specified in Appendix A.

Analysis will be carried out on 4 samples- **Samples A1, A2, A3 and A4** which are sample mixtures containing peak of interest (POI) and internal standard (ISTD).

Tenderers are required to demonstrate on their tendered LC system that the following criteria can be met:

- (i) Accurate and repeatable results with good intra-day reproducibility;
- (iii) Peak purity of both POI and ISTD passes for Calib-A, ACA, ACB and **Samples A1-4**.

In the event that the peak purity software reflects that the peaks are non-homogenous (i.e. peak purity fail) in the standards solution (i.e. Calib-A, ACA, ACB), Tenderers must apply and demonstrate how to incorporate correction factors to ensure acceptable peak purities (i.e. peak purities passed) are obtained for the standards solutions. The exact correction factors determined will need to be applied to the samples to obtain its peak purity results. All results (pre and post peak purity failure) are to be submitted.

The decision to incorporate the rinsing method is based on the Tenderer's discretion.

Tenderers are to analyse **Samples A1-4** in the **exact** sequence as listed below. The sequence is to be completed within the day.

Refer to Appendix B for information required.

Sequence order for **Section A:**

Type of sample	Sample Name to be keyed	Number of injections	Data file name
Purified Water	Purified water	1	Blank1
Calib-A	Calib-A	2	CalibA-1,CalibA-2
ACA	ACA	1	ACA-1
ACB	ACB	1	ACB-1
Sample A1 x 3	Sample A1	3	A1-1R, A1-2R, A1-3R
Purified Water	Purified water	1	Blank2
Sample A2 x 3	Sample A2	3	A2-1R, A2-2R, A2-3R
Purified Water	Purified water	1	Blank3
Sample A3 x 3	Sample A3	3	A3-1R, A3-2R, A3-3R
Purified Water	Purified water	1	Blank4
Sample A4 x 3	Sample A4	3	A4-1R, A4-2R, A4-3R
Purified Water	Purified water	1	Blank5
ACA	ACA	1	ACA-2
ACB	ACB	1	ACB-2

Section B: Evaluation of library matching function

For this section, the materials in Set B will be used for the evaluation.

Set B comprises of 9 vials, with 5 standards labelled **S1-5**, and 4 samples labelled **T1-4**. Where possible, testing should be carried out using the GEN method listed in Appendix A. If alternative methods are used, the conditions should be indicated in Appendix B.

Tenderers are required to demonstrate on their tendered LC system that the following criteria can be met:

- (i) Correct identification of the analytes through UV library matching. Criteria of a positive match should be indicated by the Tenderer in Appendix B, and supported by published literature.
- (ii) Satisfactory peak purity of identified analytes in **Standard solutions S1-S5** and **Samples T1-T4**.

In the event that the peak purity software reflects that the peaks are non-homogenous (i.e. peak purity fail) in the standards solution and samples, Tenderers must apply and demonstrate how to incorporate correction factors to ensure acceptable peak purities are obtained for the standards solutions. The exact correction factors determined will need to be applied to the samples to obtain its peak purity results. All results (pre and post peak purity failure) are to be submitted.

Standard Solutions

This set of 5 standard solutions should be analysed using GEN method as stated in Appendix A. The identity of the standard solutions are as follows:

S1: POI1
S2: POI2
S3: POI3
S4: POI4
S5: POI5

Each of the standard should be saved into the following 6 libraries:

L-Gen: Library containing all 5 standards
L-POI1: Library containing only POI1
L-POI2: Library containing only POI2
L-POI3: Library containing only POI3
L-POI4: Library containing only POI4
L-POI5: Library containing only POI5

Note 1: The library building and analysis of the samples is to be completed within the day.

Note 2: Where possible, the retention time of S1 to S5 should not be saved to the libraries.

Sample Solutions

This set of sample solutions should be analysed using the method as stated above, where possible. In the event that an alternative method is used, the conditions of the method must be provided.

Instructions:

Analyse **Sample T1-T4** using GEN method in Appendix A.

Match the peaks in each sample solution to the libraries as specified in each table listed in Appendix B. Where the software is able to generate a quantitative value indicating the closeness of the spectra match (i.e. quality match factor or whichever term it is used), it should be indicated in the tables in Appendix B.

Refer to Appendix B for information required.

Appendix A

Mobile Phase Preparation

First, measure appropriate volumes of the respective solvents and mix well. Using the proportions below, add the appropriate amount of diethylamine, and the corresponding amount of conc. phosphoric acid. Shake well. Measure the pH of the resultant solution. Adjust the pH accordingly using diethylamine, if the pH is not within the range of 3 ± 0.05 . Refer to table below for detailed breakdown.

<i>Solvent / Reagent</i>	<i>For 1 litre of mobile phase</i>	
	Composition: 20:80	Composition: 10:90
HPLC Methanol	200 ml	100 ml
Purified Water	800 ml	900 ml
Diethylamine (DEA)	5 ml	
Conc Phosphoric Acid	4 ml, adjust to pH 3 gradually using DEA	

Method conditions for Section A

Column: ZORBAX SB C8, 50 x 4.6mm i.d., 1.8 μ m (Agilent Part No. 827975-906-SB-C8)

In-line Filter: Fast LC In-line Filter, 4.6mm, 0.2 μ m filter (Agilent Part No. HP 5067-1553)

Mobile Phase: 20% MeOH: 80% H₂O, 0.5% diethylamine (pH=3)

Method Name	Mobile Phase MeOH:Buffer (%)	Flow Rate (ml/min)	Column Temp (°C)	Injector Volume (μ l)	Wavelength λ (nm)	Detector Spectrum range (nm)	Approx. Run Time (min)
TEST	20:80	1.5	35	0.5	215	205 – 350	5

Appendix A (continued)

Method conditions for Section B

Column: ZORBAX SB-C8, 50mm x 4.6mm i.d., 1.8 µm (Agilent Part No. 827975-906-SB-C8)

In-line Filter: Fast LC In-line Filter, 4.6mm, 0.2µm filter (Agilent Part No. HP 5067-1553)

Mobile Phase: 10% MeOH: 90% H₂O, 0.5% diethylamine (pH=3)

Method Name	Mobile Phase MeOH:Buffer (%)	Flow Rate (ml/min)	Column Temp (°C)	Injector Volume (µl)	Wavelength λ (nm)	Detector Spectrum range (nm)	Approx. Run Time (min)
GEN	10:90	2.0	45	0.5	215	205 – 350	6

Sample information (Sample A1-A4)

Sample amount: 1 mg

Volume: 1 ml

Dilution: 1

Calib-A

Refer to Table below for order of elution of analytes in calibration standard for Section A.

Eluting order	Compound
1	ISTD
2	POI

Appendix B

General Instructions:

The documentations of the test results should be placed in the following order:

SECTION A:

- 1) Summary Sheet of Section A
- 2) Sequence Printout for Section A
- 3) Results of Section A according to order in the sequence table
- 4) Results of Calib, Controls and Samples must have their peak purity printouts directly behind the chromatogram. Failed peak purity printouts will be placed before corrected peak purity reports.

SECTION B:

- 1) Summary Sheet of Section B
- 2) Sequence Printout for Section B
- 3) Printout of UV library saved to the respective libraries
- 4) Results of Section B according to order in the sequence table.
- 5) Peak purity results of Samples must have their peak purity printout directly behind the chromatogram. Failed peak purity printouts will be placed before corrected peak purity reports.
- 6) Library match results in the order of match to L-Gen and the subsequent libraries as listed in Appendix B.