

Technical Report

FlexiFocus™ Slides: Chemically Modified Sample-Focussing MALDI Targets to Enhance Productivity and Throughput

Simona Salivo¹

Abstract:

The FlexiFocus™ slides are chemically modified sample-focussing MALDI targets that have been developed to facilitate high-throughput, automated analysis on the Shimadzu benchtop MALDI-TOF instruments. They consist of a hydrophobic surface surrounding small exposed, hydrophilic regions which facilitate sample containment and focussing in the centre of the spot. They are available with either 48- or 96-sample positions to satisfy different throughput requirements. They are compatible with all common MALDI matrices and solvents, and can be used in various applications. They have also been shown to exhibit enhanced sensitivity performance depending on the sample and application.

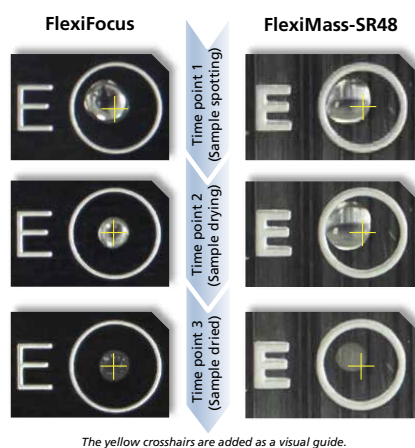
Keywords: FlexiFocus, Focussing, MALDI-TOF, Automated analysis

Introduction

The FlexiFocus™ slide inserts have been developed to facilitate high-throughput, automated analysis using the Shimadzu benchtop MALDI-TOF instruments. These slides are comprised of a metal substrate which has been chemically modified to produce a highly hydrophobic surface. This hydrophobic layer covers the entire slide surface except for small exposed regions (900 µm diameter) in the centre of each sample deposition ring. The combination of the exposed metal centre and the hydrophobic surround helps contain the droplet during spotting (Fig. 1).



Fig. 1 FlexiFocus slide: influence of the chemically modified surface on the containment of the sample droplet.



The yellow crosshairs are added as a visual guide.

Fig. 2 Comparison of sample spotting on a FlexiFocus slide versus a standard stainless steel FlexiMass-SR48 slide. FlexiFocus (left): the sample spotted off-centre migrates and dries in the centre of the well. FlexiMass-SR48 (right): the sample spotted off-centre dries in the location where it was deposited.

Focussing and flexibility

The focussing of the sample spot to the centre of the well is an advantage since it facilitates automated MALDI analysis by preventing off-centre spots, as these tend to produce weaker signals due to misalignment with the acquisition area resulting in only partial sampling of the spot. Fig. 2 shows a comparison of the spotting behaviour between the FlexiFocus and FlexiMass™-SR48 (non-focussing) slides: after spotting on the FlexiFocus slide (time point 1), the sample droplet which has been deposited off-centre promptly migrates and dries to the centre of the well (time points 2 and 3). On the FlexiMass-SR48 slide, the sample dries in the same location where the droplet of solution was deposited, resulting in an off-centre spot. Fig. 3 shows an example of manually deposited samples. Once again, on the FlexiFocus slide the samples dry in the centre of the wells, irrespective of where the droplet was deposited (Fig. 3, left), unlike on the FlexiMass-SR48 slide where there are a number of off-centre spots (Fig. 3, right).

FlexiFocus inserts are available in either 48- or 96- sample position formats (Fig. 4). The FlexiFocus adaptor has been specifically designed to hold a FlexiFocus insert and facilitate analysis in the Shimadzu benchtop MALDI-TOF instruments (Fig. 5). An easy-to-use design with tabs at the corner ensures correct orientation during insertion. Four circular-shape magnets snap the insert into place.

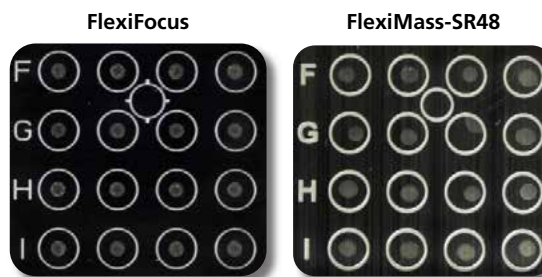


Fig. 3 FlexiFocus (left): all 16 samples dried in the centre of the wells. FlexiMass-SR48 (right): some sample spots are off-centred, reflecting the position of the deposited droplet.



Fig. 4 Left: FlexiMass-SR48 slide.
Middle: FlexiFocus-48 insert and adaptor.
Right: FlexiFocus-96 insert and adaptor.



Fig. 5 FlexiFocus adaptor and insert: four circular-shaped magnets snap insert into place.

Versatility and throughput

The FlexiFocus slides are compatible with commonly used MALDI matrices such as α -Cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 2,5-Dihydroxybenzoic acid (DHB), 3-Hydroxypicolinic acid (3-HPA). They can also be used with most organic solvents such as water, acetonitrile, methanol, ethanol, chloroform, trifluoroacetic acid (<0.1 % (v/v)). They can be used for a wide range of applications, from proteomics, lipidomics, oligonucleotides, to any contexts where high-throughput analysis is required.

Fig. 6 shows the MALDI-MS spectrum of IgA (5 pmol on target) prepared on a FlexiFocus insert. Sample was prepared at 10 pmol/ μ L in 0.1 % TFA, while sinapinic acid MALDI matrix was prepared at 20 mg/mL in 1:1 acetonitrile/0.1 % TFA. The inset (Fig. 6B) shows the dried sample spot: the crystallisation appearance is similar to that obtained on the regular FlexiMass stainless steel target. The expected monomer mass at ~160 kDa is observed along with the doubly-charged species (~80 kDa) (Fig. 6 C). A signal was detected at ~54 kDa, consistent with the mass of the heavy chain (~55 kDa expected).

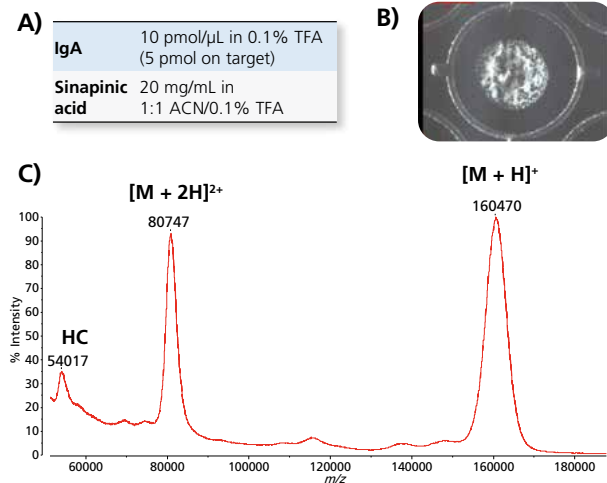


Fig. 6 A) Sample and matrix conditions. B) IgA in SA dried sample spot. C) MALDI-MS spectrum of IgA (5 pmol on target): the monomer mass as well as the doubly-charged and heavy chain (HC) peaks are detected (~160, ~80 and ~55 kDa, respectively). Data acquired on a MALDI-8020.

Fig. 7 shows the MALDI-MS spectrum of triacylglycerols (TAGs) in extra-virgin olive oil (EVOO) prepared on a FlexiFocus insert. EVOO was prepared at 5 mg/mL in chloroform, while DHB matrix was prepared at 1 mg/mL in methanol containing 10 mM sodium trifluoroacetate (NaTFA). Triolein (OOO) dominates the spectrum, followed by OOP, OOL and POL TAGs. This reflects the natural occurrence and abundance of palmitic (P), oleic (O) and linoleic (L) fatty acids in EVOO. It can also be appreciated how the TAG species are isotopically resolved.

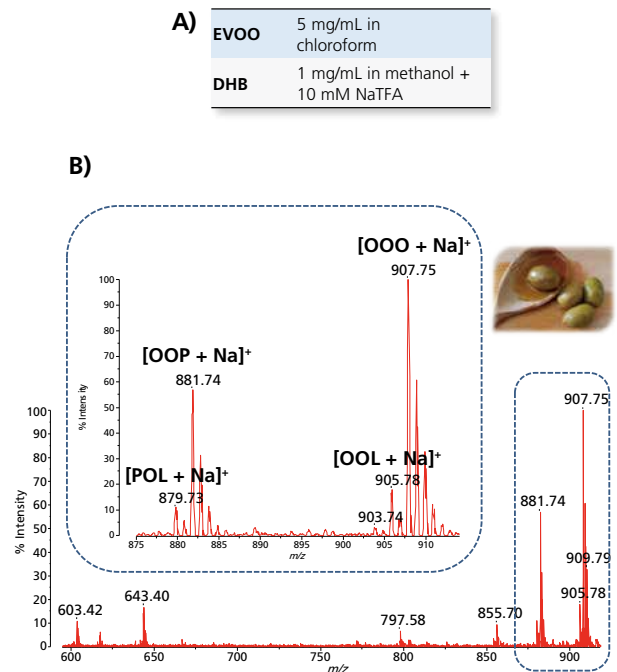


Fig. 7 A) sample and matrix conditions. B) MALDI-MS spectrum of TAGs in EVOO. Top-left inset: expansion on the main TAG region. OOO, OOP, OOL and POL are among the predominant TAGs in EVOO (P: palmitic acid; O: oleic acid; L: linoleic acid). The TAG peaks are isotopically resolved. Data acquired on a MALDI-8020.

Fig. 8 illustrates an example of sensitivity performance using peptides on the FlexiFocus slide. Serial dilutions of Glu1-Fibrinopeptide B (GluFib) peptide were prepared at 500, 200, 100 and 50 amol/ μ L in 30:70 acetonitrile/0.1 % TFA (250, 100, 50 and 25 amol on target). CHCA matrix was prepared at 0.25 mg/mL in 1:1 acetonitrile/0.1 % TFA. The GluFib peak (m/z 1570.7) was reliably detected at all concentrations, even the lowest tested (25 amol on target), with good signal-to-noise ($>3:1$) and isotopic resolution.

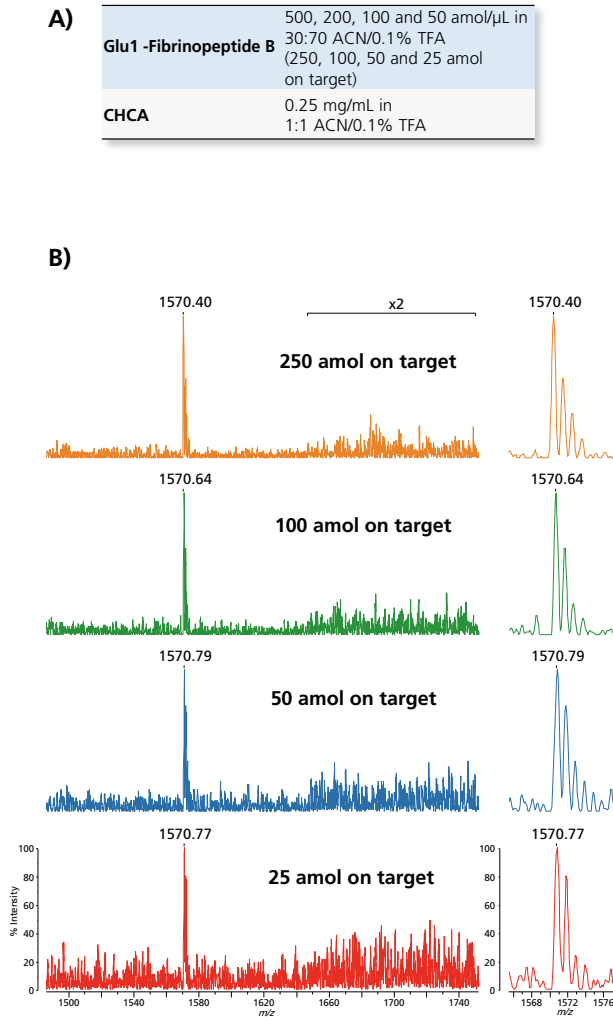


Fig. 8 A) sample and matrix conditions. B) MALDI-MS spectra of GluFib at various concentrations: 250, 100, 50 and 25 amol on target (orange, green, blue and red, respectively). The GluFib peak (m/z 1570.7) is reliably detected with good signal-to-noise ($>3:1$) and isotopic resolution. Data acquired on a MALDI-8020.

Fig. 9 shows an oligonucleotide example (20-mer; ACGTACGTACGTACGTACGT). Sample was prepared at 100 μ M in deionised water and desalted with Dowex[®] ion exchange resin to reduce the salt presence that naturally occurs in oligonucleotides. Ammonium citrate dibasic reagent was prepared at 5 mg/mL in 70:30 acetonitrile/water. 3-HPA matrix was prepared at 10 mg/mL in the ammonium citrate dibasic. Fig. 9C shows the MALDI-MS spectrum obtained for the 20-mer prepared on a FlexiFocus slide. The expected m/z at 6118.05 is observed along with very little amounts of salt adducts. Fig. 9D shows the MALDI-ISD spectrum following in-source decay (ISD) sequence analysis of the 20-mer. The expected ISD fragments were successfully detected with good signal-to-noise allowing 75 % sequence coverage to be achieved.

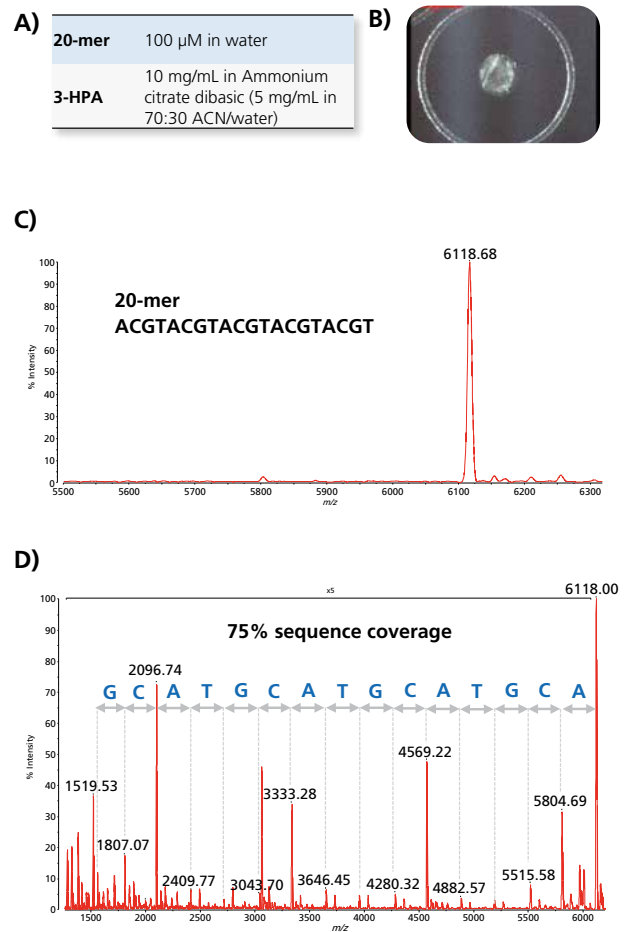


Fig. 9 A) sample and matrix conditions. B) 20-mer oligonucleotide in 3-HPA dried sample spot on a FlexiFocus slide. C) MALDI-MS spectrum of 20-mer. D) MALDI-ISD spectrum of 20-mer: 75 % sequence coverage was achieved. Data acquired on a MALDI-8020.

The FlexiFocus slides are the perfect solution for high-throughput, automated workflows such as those in quality control (QC) settings. They can be used with all the Shimadzu MALDI-TOF platforms and automated analysis software applications, such as QC Reporter™. Fig. 10 shows an example of QC analysis of synthetic peptides carried out using a FlexiFocus-96 slide and the Shimadzu QC Reporter application.

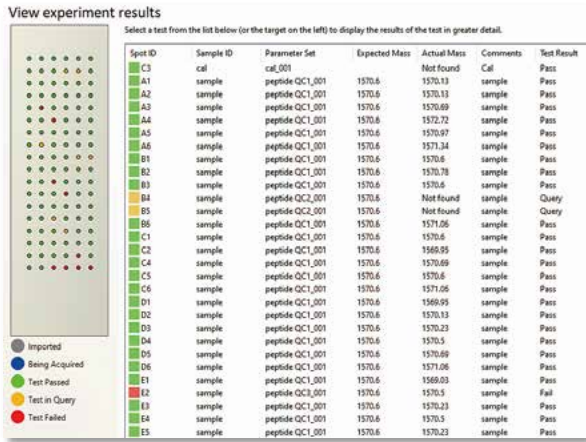


Fig. 10 QC experiment performed with a FlexiFocus-96 slide and the Shimadzu QC Reporter software. The colour-coded slide graphic and summary table provide an intuitive representation of the sample results (green: Pass; orange: Query; red: Fail).

The colour-coded visualization of the results provides an easy reading of the sample outcome (green for Pass, orange for Query, red for Fail), whereas the summary table provides sample details such as spot ID, QC method, expected/detected *m/z* and outcome.

Sensitivity enhancement

Fig. 11 illustrates an example of sensitivity performance comparison between the FlexiFocus and FlexiMass-SR48 slides. Serial dilutions of a commercial BSA digest were prepared at 3 fmol, 1 fmol, 300 amol, 100 amol and 30 amol / μ L in 30:70 acetonitrile/0.1 % TFA (3 fmol, 1 fmol, 300 amol, 100 amol and 30 amol on target). CHCA matrix was prepared at 0.25 mg/mL in 1:1 acetonitrile/0.1 % TFA. With an accumulation rate of 10 shots per profile, a sensitivity enhancement was observed on the FlexiFocus slide compared to the FlexiMass-SR48 (Fig. 11B). Successful protein IDs were obtained through a Mascot PMF search on both slides from the lowest concentrations that produced good quality spectra (100 amol on FlexiFocus (Fig. 11C), and 300 amol on FlexiMass-SR48 (Fig. 11D)).

BSA digest	3 fmol, 1 fmol, 300 amol, 100 amol and 30 amol / μ L in 30:70 ACN/0.1 % TFA (3 fmol, 1 fmol, 300 amol, 100 amol and 30 amol on target)
CHCA	0.25 mg/mL in 1:1 ACN/0.1 % TFA

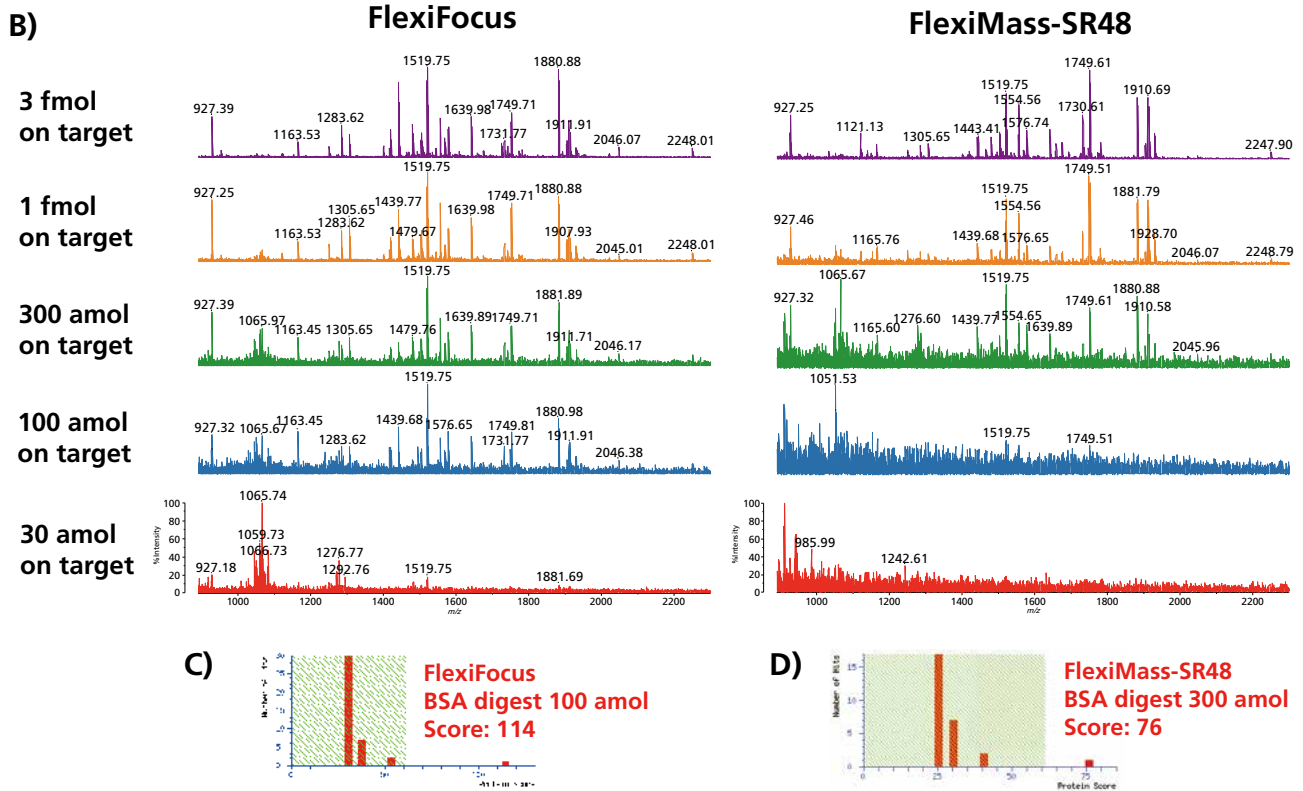


Fig. 11 A) Sample and matrix conditions. B) MALDI-MS spectra of BSA digest at various concentrations on the FlexiFocus (left) and FlexiMass-SR48 (right) slides obtained with an accumulation rate of 10 shots per profile: 3 fmol, 1 fmol, 300 amol, 100 amol and 30 amol on target (purple, orange, green, blue and red, respectively). C) The sample at 100 amol concentration on the FlexiFocus slide was positively identify through a Mascot PMF search. D) The sample at 300 amol concentration on the FlexiFocus-SR48 slide was positively identify through a Mascot PMF search. Data acquired on a MALDI-8020.

Fig. 12 shows an example spectrum of BSA digest 30 amol/μL (30 amol on target) obtained with an accumulation rate of 50 shots per profile on the FlexiFocus slide. Ammonium phosphate monobasic (ADHP) was added at a 10 mM concentration to suppress matrix clusters. A positive ID was obtained through a Mascot PMF search.

A)

BSA digest	30 amol/μL in 30:70 ACN/0.1 % TFA (30 amol on target)
CHCA	0.25 mg/mL in 1:1 ACN/0.1% TFA + 10 mM ADHP

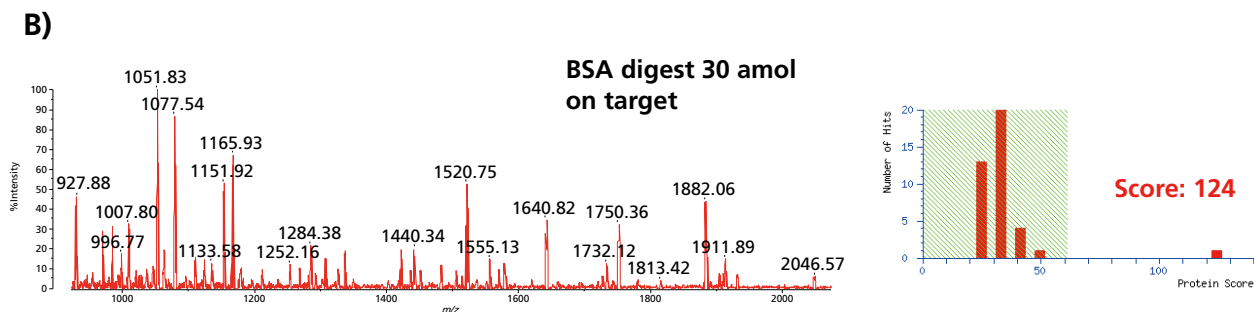


Fig. 12 A) Sample and matrix conditions. B) Left: MALDI-MS spectrum of BSA digest 30 amol on target obtained with an accumulation rate of 50 shots per profile. Right: Mascot PMF protein ID. Data acquired on a MALDI-8020.

Part numbers

Starter kits

Item	P/N
FlexiFocus-48 well starter kit containing adaptor and 20 inserts	224-38626-58
FlexiFocus-96 well starter kit containing adaptor and 20 inserts	224-38623-58

Consumables and spares

Item	P/N
FlexiFocus-48 well insert, pack of 10	224-39906-58
FlexiFocus-96 well insert, pack of 10	224-39907-58
FlexiFocus Adaptor, x 1	224-39909-58

First Edition: October, 2023



Shimadzu Corporation
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, products/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation, its subsidiaries or its affiliates, whether or not they are used with trademark symbol "TM" or "®".

Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".

Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.