



Specifications Methods

GX-241 Liquid Handler

Part Number 26150004

This document contains information about the methods used to obtain the specifications listed in the Technical Specifications in the GX-241 Liquid Handler User's Guide (LT350025-02).

Table of Contents

Injection Repeatability.....	2
Preparative System	2
Preparative Method – GX Direct Injection Module	2
Analytical System.....	5
Analytical Method – GX Direct Injection Module.....	5
Injection Repeatability Calculation	7
Injection Carryover.....	8
Analytical System.....	8
Analytical Method – GX Direct Injection Module.....	8
Injection Carryover Calculation	10
GX Syringe Pump Volumetrics.....	11
Volumetric Accuracy System	11
Volumetric Accuracy Method.....	11
Volumetric Accuracy Calculation	12





Injection Repeatability

Preparative System

GX-241 Liquid Handler

- 125 mm Z arm clamped at 125 mm
- Constricted beveled-tip probe (part number 27067374), constricted beveled tip, stainless steel; 221 x 1.5 x 1.1 mm ID
- Rinse station (part number 26054000)

GX Syringe Pump

- 1 mL syringe (part number 25025343)
- Transfer tubing (part number 499471112) 5.5 mL, 1.5 mm ID

GX Direct Injection Module

- GX Direct Injection Module, 1/16", prep (part number 261354)
- Continuous flow Valco® valve with a vertical direct connection for the injection port for 1/16" OD sample loops
- 1.5 mm stainless steel injection port (part number 26035413)
- 1 mL stainless steel sample loop (part number 49440001)
- GX Direct Injection Module plumbing package (part number 26035470)

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Preparative Method – GX Direct Injection Module

HPLC Pump

Gilson 322 Pump

- H2 Pump Heads

Mobile Phase / Rinse Solvent

- Isocratic 23% Methanol / 77% Water
- Rinse Solvent 100% Water

Flow Rate

- 30 mL/min

Detector

Gilson 152 UV/Vis Detector

- 0.2 mm (Prep) Flow Cell (part number 104441)
- Wavelength: 270 nm
- Sensitivity: 0.01
- Peak Width: 0 sec

Column

- Phenomenex Luna® 5u C18(2) 100A, 50 x 21.2mm, 5 micron

Luna® is a registered trademark of Phenomenex, Inc.



Rinse Volume

- The injection port was rinsed with 500 μL from reservoir at 4 mL/min
- The outside of the probe was rinsed with 500 μL from reservoir at 10 mL/min

Injections

- Partial Loop injection - 500 μL was injected into a 1 mL sample loop
- Ten injections of 1 mg/mL 4-Acetamidophenol in water from one sample tube

Injection Method

Partial Loop Injection

1. Move Z to Z Safe Height
2. Set the injection valve to Inject
3. Move to Drain/Rinse Station GX-241
4. Move Z to Z Option and Z Offset
5. Home dilutor
6. Move Z to Z Safe Height
7. Aspirate air gap 6 μL at 0.3 mL/min
8. Move to Sample Well
9. Move Z to Z Option and Z Offset
10. Aspirate injection volume (Injection Volume + Extra Volume) at 0.5 mL/min
11. Move Z to Z Safe Height
12. Move to Injection Well
13. Move Z to Z Option and Z Offset
14. Dispense the Extra Volume at 0.5 mL/min
15. Switch injection valve to Load
16. Wait 1.2 sec for the valve to switch
17. Dispense the injection volume at 0.5 mL/min
18. Wait Injection Equilibration Time
19. Synchronize
20. Switch injection valve to Inject
21. Home dilutor
22. Rinse injection port: Aspirate 500 μL from reservoir and Dispense from probe at 4 mL/min
23. Move Arm to Rinse Station GX-241
24. Move Z to Z Option and Z Offset
25. Home dilutor
26. Rinse probe: Aspirate 500 μL from reservoir and Dispense from probe at 10 mL/min



TRILUTION LC Method - Preparative

Time (min)	Task	Parameters
0	151 152 Detector Settings	Wavelength = 270 nm, Peak Width = 0, Sensitivity = 0.01
0.03	Detector Autozero Channel	N/A
0.10	Partial Loop Injection	Default values, except for Extra Volume (20 µL) and Injection Volume (500 µL)
0.15	Sync	To Synchronize 1 of Partial Loop Injection Task
0.2	Start Data Collection	N/A
1.6	Stop Data Collection	N/A
0-1.80	Mobile Phase	23% Methanol / 77% Water Flow Rate 30 mL/min
Analysis Settings		
Front Slope = 25		
Back Slope = 25		
Peak Width = 0.2 min		
Default Baseline		



Analytical System

GX-241 Liquid Handler

- 125 mm Z arm clamped at 125 mm
- Beveled-tip probe (part number 2507256), Grooved, septum-piercing; beveled tip, stainless steel; 221 x 1.5 x 0.4 mm ID
- Rinse station (part number 26054000)

GX Syringe Pump

- 250 mL syringe (part number 25025342)
- Transfer tubing (part number 499424013) 1.1 mL, 0.8 mm ID

GX Direct Injection Module

- GX Direct Injection Module, 1/16", analytical, stainless steel (part number 261356)
- Continuous flow Valco® valve with a vertical direct connection for the injection port for 1/16" OD sample loops
- 1.5 mm stainless steel injection port (part number 26035413)
- 20 µL stainless steel sample loop (part number 49440007)
- GX Direct Injection Module plumbing package (part number 26035470)

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Analytical Method – GX Direct Injection Module

HPLC Pump

Gilson 306 Mobile Phase Pump (2), 811D Mixer, 805 Manometric Module

- 5SC Pump Heads
- 0.7 mL mixing chamber

Mobile Phase / Rinse Solvent

- Isocratic 30% Acetonitrile / 70% Water
- Rinse Solvent 50% Methanol / 50% Water

Flow Rate

- 1 mL/min

Detector

Gilson 156 UV/Vis Detector

- 5 mm (Analytical) Flow Cell (part number 104445)
- Wavelength: 254 nm
- Sensitivity: 0.01
- Peak Width: 0 sec

Column

- Phenomenex Luna® 5u C18(2) 100A, 75 x 4.6mm, 5 micron

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Rinse Volume

- The injection port was rinsed with 500 μ L from reservoir at 4 mL/min
- The outside of the probe was rinsed with 500 μ L from reservoir at 5 mL/min

Injections

- Total Loop injection (Overfill) - 5 times overfill of a 20 μ L sample loop was injected
- Ten injections of 0.1 mg/mL Caffeine in water from one sample tube

Injection Method

Total Loop Injection Overfill

1. Move Z to Z Safe Height
2. Set the injection valve to Inject
3. Move to Drain/Rinse Station GX-241
4. Move Z to Z Option and Z Offset
5. Home dilutor
6. Move Z to Z Safe Height
7. Aspirate air gap 3 μ L at 0.3 mL/min
8. Move to Sample Well
9. Move Z to Z Option and Z Offset
10. Aspirate injection volume ((Loop Overfill x Loop Volume) + Extra Volume) at 0.5 mL/min
11. Move Z to Z Safe Height
12. Move to Injection Well
13. Move Z to Z Option and Z Offset
14. Switch injection valve to Load
15. Wait 1.2 sec for the valve to switch
16. Dispense the injection volume (Loop Overfill x Loop Volume) at 0.5 mL/min
17. Wait Injection Equilibration Time
18. Synchronize
19. Switch injection valve to Inject
20. Home dilutor
21. Rinse injection port: Aspirate 500 μ L from reservoir and Dispense from probe at 4 mL/min
22. Move Arm to Rinse Station GX-241
23. Move Z to Z Option and Z Offset
24. Home dilutor
25. Rinse probe: Aspirate 500 μ L from reservoir and Dispense from probe at 5 mL/min



TRILUTION LC Method – Analytical

Time (min)	Task	Parameters
0.00	155 156 Detector Settings	Wavelength = 254 nm, Peak Width = 0, Sensitivity = 0.01
0.03	Detector Autozero Channel	N/A
0.05	Total Loop Injection Overfill	Defaults values except for Loop Volume Overfill (5), Loop Volume (20 µL), Injection Z Offset (-1 mm), Outside Rinse Flow Rate (5 mL/min)
0.10	Sync	To Synchronize 1 of Total Loop Injection Overfill
0.15	Start Data Collection	N/A
2.00	Stop Data Collection	N/A
0–2.00	Mobile Phase	30% Acetonitrile / 70% Water Flow Rate 1 mL/min
Analysis Settings		
Front Slope = 25		
Back Slope = 25		
Peak Width = 0.2 min		
Default Baseline		

Injection Repeatability Calculation

CV = (Standard deviation for injection set / Average area for injection set) * 100



Injection Carryover

Analytical System

GX-241 Liquid Handler

- 125 mm Z arm clamped at 125 mm
- Constricted beveled-tip probe (part number 27067374), constricted beveled tip, stainless steel; 221 x 1.5 x 1.1 mm ID
- Rinse station (part number 26054000)

GX Syringe Pump

- 1 mL syringe (part number 25025343)
- Transfer tubing (part number 499424013) 1.1 mL, 0.8 mm ID

GX Direct Injection Module

- GX Direct Injection Module, 1/16", Analytical, Stainless Steel (part number 261356)
- Continuous flow Valco® valve with a vertical direct connection for the injection port for 1/16" OD sample loops
- 1.5 mm stainless steel injection port (part number 26035413)
- 20 µL stainless steel sample loop (part number 49440007)
- GX Direct Injection Module plumbing package (part number 26035470)

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Analytical Method – GX Direct Injection Module

HPLC Pump

Gilson 306 Mobile Phase Pump (2), 811D Mixer, 805 Manometric Module

- 5SC Pump Heads
- 0.7 mL mixing chamber

Mobile Phase / Rinse Solvent

- Isocratic 20% Acetonitrile / 80% Water
- Rinse Solvent 60% Methanol / 40% Water

Flow Rate

- 1 mL/min

Detector

Gilson 156 UV/Vis Detector

- 5 mm (Analytical) Flow Cell (part number 104445)
- Wavelength: 254 nm
- Sensitivity: 0.01
- Peak Width: 0 sec

Column

- Phenomenex Luna® 5u C18(2) 100A, 75 x 4.6mm, 5 micron

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Rinse Volume

- The injection port was rinsed with 1500 μL from reservoir at 4 mL/min
- The outside of the probe was rinsed with 1000 μL from reservoir at 10 mL/min

Injections

- Total Loop injection (Overfill) - 5 times overfill of a 20 μL sample loop was injected
- Ten injections of 0.5 mg/mL Caffeine in water from four sample tubes. Each sample injection was followed by two blank (water) injections.

Injection Method

Total Loop Injection Overfill

1. Move Z to Z Safe Height
2. Set the injection valve to Inject
3. Move to Drain/Rinse Station GX-241
4. Move Z to Z Option and Z Offset
5. Home dilutor
6. Move Z to Z Safe Height
7. Aspirate air gap 20 μL at 0.3 mL/min
8. Move to Sample Well
9. Move Z to Z Option and Z Offset
10. Aspirate injection volume ((Loop Overfill x Loop Volume) + Extra Volume) at 0.5 mL/min
11. Move Z to Z Safe Height
12. Move to Injection Well
13. Move Z to Z Option and Z Offset
14. Switch injection valve to Load
15. Wait 1.2 sec for the valve to switch
16. Dispense the injection volume (Loop Overfill x Loop Volume) at 0.5 mL/min
17. Wait Injection Equilibration Time
18. Synchronize
19. Switch injection valve to Inject
20. Home dilutor
21. Rinse injection port: Aspirate 1500 μL from reservoir and Dispense from probe at 4 mL/min
22. Move Arm to Rinse Station GX-241
23. Move Z to Z Option and Z Offset
24. Home dilutor
25. Rinse probe: Aspirate 1000 μL from reservoir and Dispense from probe at 10 mL/min



TRILUTION LC Method - Analytical

Time (min)	Task	Parameters
0.00	155 156 Detector Settings	Wavelength = 254 nm, Peak Width = 0, Sensitivity = 0.01
0.03	Detector Autozero Channel	N/A
0.05	Total Loop Injection Overfill	Sample Zone and Sample Well = Variables set in sample list (to Blank or Sample zone/well) Defaults except for Air Gap (20 µL), Loop Volume Overfill (5), Loop Volume (20 µL), Injection Z Offset (-1mm), Injection Rinse Volume (1500 µL), Outside Rinse Volume (1000 µL), Outside Rinse Flow Rate (10 mL/min)
0.10	Sync	To Synchronize 1 of Total Loop Injection Overfill
0.15	Start Data Collection	N/A
2.50	Stop Data Collection	N/A
0-2.50	Mobile Phase	20% Acetonitrile / 80% Water Flow Rate 1 mL/min
Analysis Settings Front Slope = 5 Back Slope = 5 Peak Width = 0.2 min Horizontal Baseline		

Injection Carryover Calculation

$\% \text{Carryover} = (\text{Area of the Blank Peak} / \text{Area of the Sample Peak}) * 100$



GX Syringe Pump Volumetrics

Volumetric Accuracy System

GX-241 Liquid Handler

- 125 mm Z arm clamped at 125 mm
- Probe:
 - Grooved, septum piercing probe (part number 27067383), beveled tip, stainless steel; 221 x 1.5 x 0.4 mm ID [1 mL Syringe Tests]
 - Grooved, septum piercing probe (part number 27067382), beveled tip, stainless steel; 221 x 2 x 0.8 mm ID [10 mL Syringe Tests]
- Rinse station (part number 36054000)

GX Syringe Pump

Syringe Size	1 mL	10 mL
Syringe	1 mL (part number 25025343)	10 mL (part number 25025345)
Inlet Tubing	Inlet tubing (part number 499484021) 1000 x 3 x 2 mm ID, FEP	Inlet tubing (part number 499484021) 1000 x 3 x 2 mm ID, FEP
Transfer Tubing	<u>10% and 50% test</u> Transfer tubing (part number 499424013) 1.1 mL, 0.8 mm ID <u>90% test</u> Transfer tubing (part number 499471112) 5.5 mL, 1.5 mm ID	Transfer tubing (part number 499474103) 10.5 mL, 1.5 mm ID

Volumetric Accuracy Method

Solvent

- Deionized Water, degassed

Transfer

- Ten transfers of deionized, degassed water into empty result tubes for each test
- For each syringe, the following syringe capacities were tested: 10%, 50%, 90% of syringe capacity



Volumetric Accuracy Method

Transfer

1. Move Z to Z Safe Height
2. Aspirate Air Gap Volume at Air Gap Flow Rate
3. Move probe to Source Well
4. Move Z to Z Option and Z Offset
5. Aspirate Volume at Flow Rate
6. Move Z to Z Safe Height
7. Move probe to Result Well
8. Move Z to Z Option and Z Offset
9. Dispense Volume at Flow Rate
10. Move Z to Z Safe Height
11. Move to Inside Rinse Station
12. Move Z to Z Option and Z Offset
13. Rinse probe: Aspirate 250 µL from reservoir and Dispense from probe at 10 mL/min
14. Move Z to Z Safe Height
15. Move to Outside Rinse Station
16. Move Z to Z Option and Z Offset
17. Rinse probe: Aspirate 500 µL from reservoir and Dispense from probe at 10 mL/min

Refer to table below for Air Gap Volume, Air Gap Flow Rate, Volume, and Flow Rate used for each test. Each syringe size (1 mL and 10 mL) was tested at 10%, 50%, and 90% of syringe capacity.

Syringe Size	1 mL	10 mL
Air Gap at Air Gap Flow Rate (10%, 50%, 90%)	20 µL at 1 mL/min	50 µL at 1 mL/min
10% Volume at Flow Rate	100 µL at 1 mL/min	1000 µL at 5 mL/min
50% Volume at Flow Rate	500 µL at 5 mL/min	5000 µL at 10 mL/min
90% Volume at Flow Rate	900 µL at 10 mL/min	9000 µL at 25 mL/min

Volumetric Accuracy Calculation

$$\text{Accuracy (\%)} = (\text{Actual Volume delivered} / \text{Expected Volume}) * 100$$

Volumetric accuracy data was obtained gravimetrically. Each result tube was pre-weighed. A volume of deionized, degassed water was dispensed into each result tube. The result tube was weighed to determine the actual volume delivered.

All gravimetric data was obtained using a Sartorius AC 210P four-place balance.