

An Automated Parallel Analytical Scouting System for Chiral Separations

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Introduction

- Chirality has become vitally important in the pharmaceutical, chemical, and agricultural industries.
- The asymmetry property that make compounds chiral can produce critically different pharmacological effects in biological systems.
- Demand for stereoselective separation techniques and analytical assays to evaluate the enantiomeric purity of chiral compounds has increased.
- Chiral chromatography has become a necessary tool for the analytical determination of enantiomeric purity and the determination for preparative chiral purification .
- Off-site screening of chiral compounds on specific stationary phases is available, but this option is not practical if large numbers of compounds are to be screened in a minimal time frame.

- The automated system presented offers a simple in-house solution for the screening of chiral compounds.
- The system is capable of screening a chiral compound on 4 separate stereoselective columns simultaneously, increasing the throughput by 75%, that is, 4 analytical separations with chiral detection in 15 minutes versus > 60 minutes for chiral analysis in series.
- Once the system has determined the optimum chiral column for separation, the system can then run a preparative chiral purification/separation on the same phase and the same system with fraction collection
- The systems capabilities and throughput were evaluated and will be presented.

Evaluation of the Concept

- Samples employed to evaluate the system
- Columns incorporated into the system
- Verifying robustness, consistency, and time savings

Parallel Chiral System

- GX-281 Liquid Handler, with (1) direct inject valve, 20 μ L injection loop, (1) direct inject valve, 5 mL loop, FC valve
- Preparative HPLC, 30 mL/min, Solvent selector multiposition 6 or 8 solvents (0.06" ID)
- (4) UV/VIS detectors, analytical flow cell, 5 mm, 12 μ L, inlet 0.01" ID, 16 mm, outlet 0.01" ID 54 mm, 254 nm, 0.01 AUFS
- (1) UV/VIS detector, preparative flow cell, 0.2 mm, 0.7 μ L, 254, nm, 1.0 AUFS
- (1) PDR-Chiral detector

- (2) Manifolds, P-154, 5-port, PEEK, 0.04" ID (1.00 mm), 1/16", 22.3 uL swept volume
- (4) 18 mm, 0.01" ID SS tubing, (1) 24 mm, 0.01" ID SS tubing, (1) 12 mm, 0.01" ID, TEF tubing
- Columns: Chiral Technologies: Chiralpak AD-H, AS-H, OD-H, OJ-H, 4.6 x 250 mm
- Mobile Phase: 10:90 Isopropanol:Hexane, column flow rate 1.0 mL/min, system flow rate 4.0 mL/min
- Sample: Trans-Stilbene oxide (1 mg/mL, analytical); 20mg/mL (preparative)
Nicotine (1 mg/mL, analytical); 20 mg/mL (preparative)

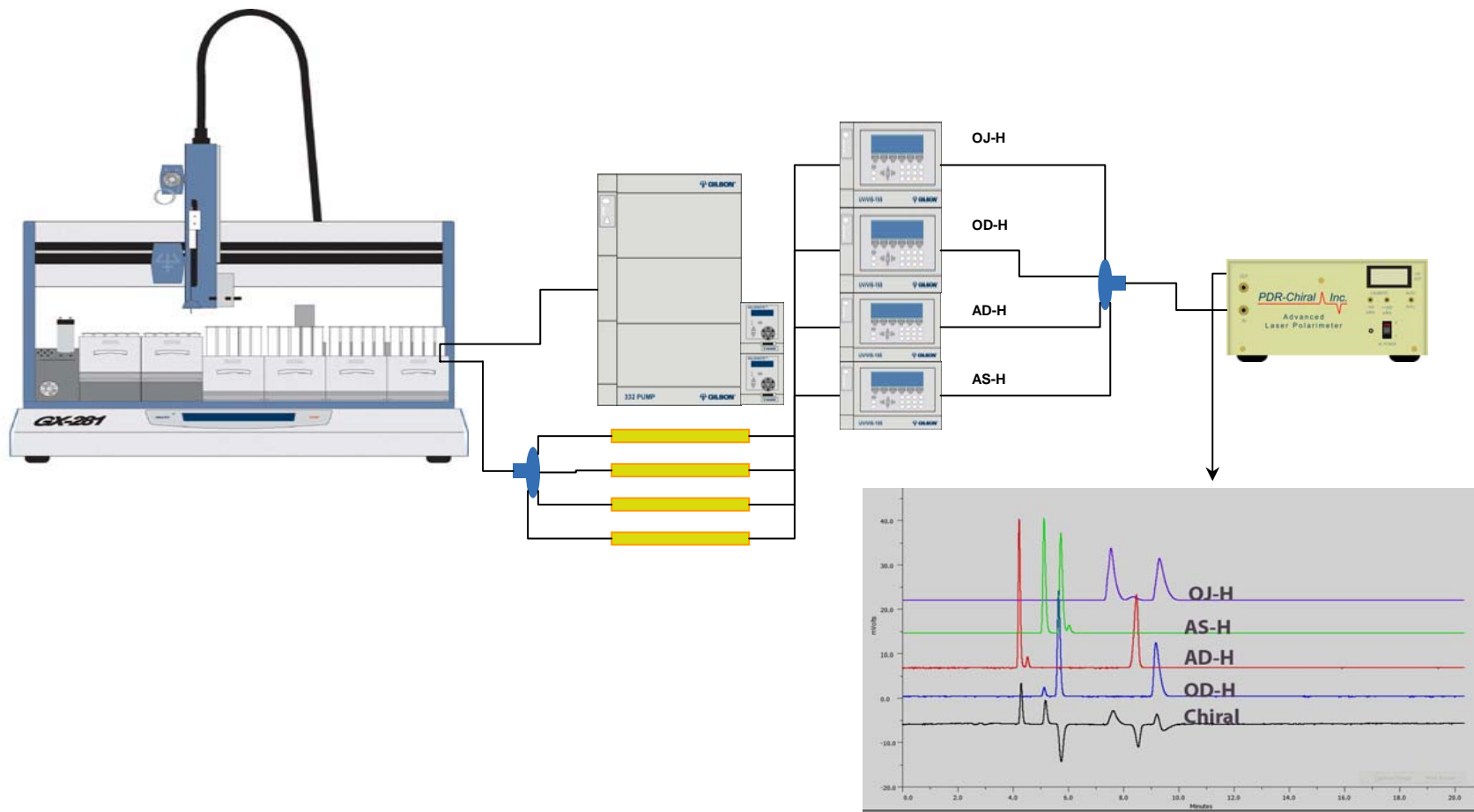
Options for Analytical Chiral Separation:

- **Basic Approach In-House:** A series of chiral columns are evaluated for optimum separation of a stereoselective compound under analytical HPLC conditions. High pressure switching valves are employed to switch through columns, at least 4 columns are assessed, 15-20 min runs time, at least 1 hour to process (1) compound. Attempting this approach on individual HPLC systems or changing chiral selective column within a system is impractical, adding substantial amounts of analysis, and manual intervention time.
- **Basic Approach Out-Source:** The same as the in-house approach; however, another company off-site is employed to run the samples and report the chiral data with a substantial increase in analysis time and cost per compound.

Options for Analytical Chiral Separation:

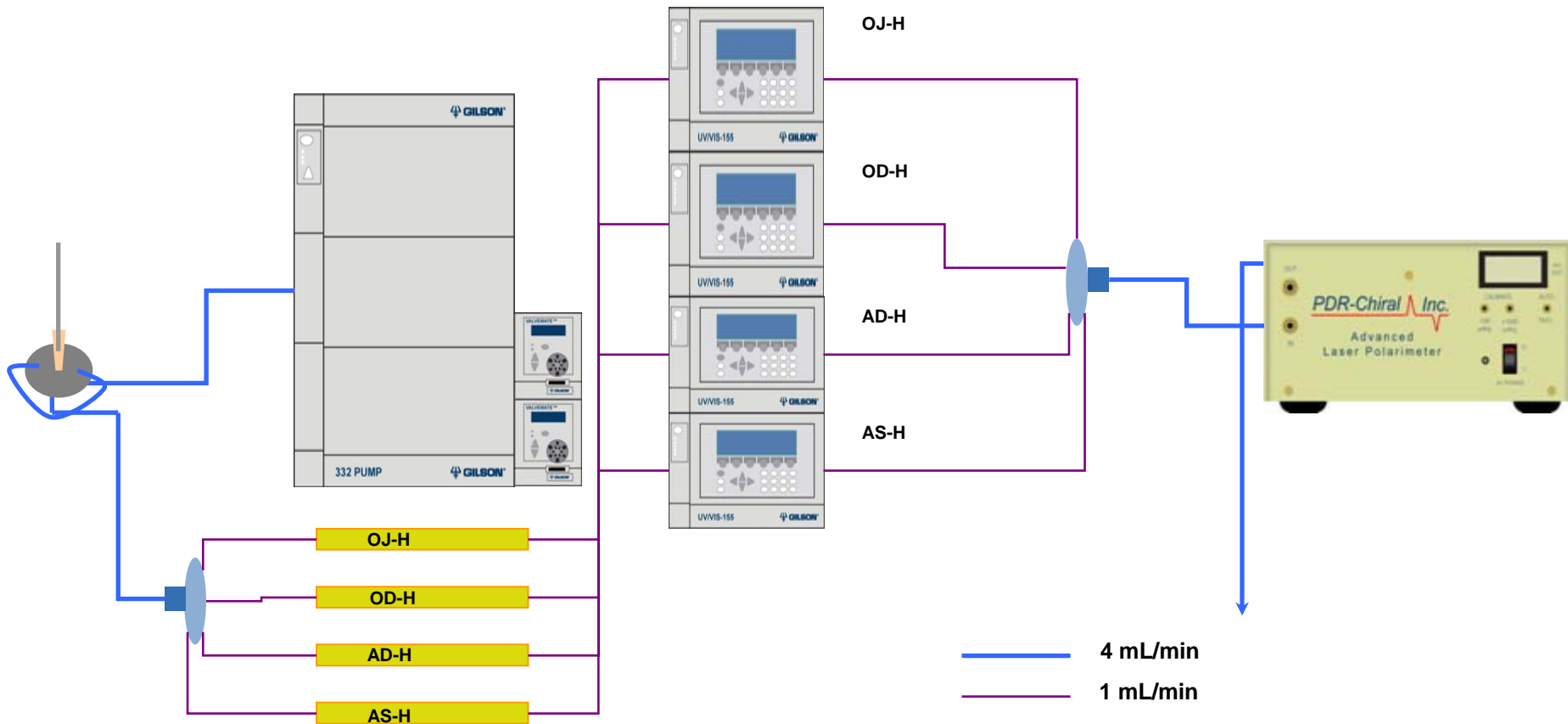
- **Parallel Chiral Analysis:** All four chiral columns are run simultaneously under the same mobile phase conditions. The injected sample and flow is split by the manifold into each chiral column. Each column is attached to a UV/VIS detector and the outlet flow from each UV/VIS detector is recombined by a manifold before entering the chiral detector, automatically offering the optimum analytical separation with chirality. The system is automatically capable of changing the polarity of the solvent system, allowing for an entire chiral screen to be accomplished without manual intervention. The system can also advance the compound through the preparative purification phase with fraction collection.

Chiral Parallel System:



Trans-Stilbene Oxide

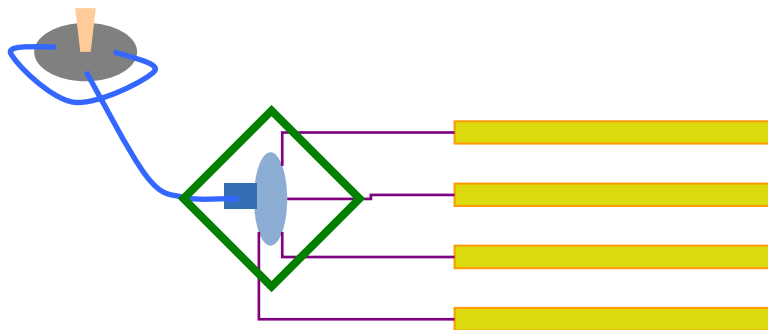
Chiral Parallel Flow Path:



System Pressure = 795 psi

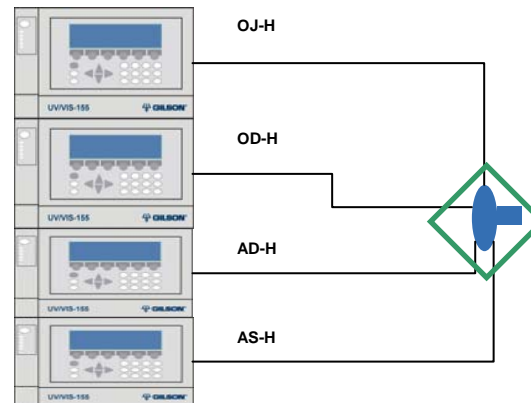
Evaluation of Parallel Chiral Flow Path:

- 1ST) Each chiral column's integrity was evaluated:
 - System pressure from each column: all mobile phases, if solvent selection is used, must be evaluated individually and recorded (log file)
- 2nd) Flow rate parameters are set based on the data from 1st
- 3rd) Parallel chiral manifold placed **BEFORE** the columns are added to the system



Evaluation of Parallel Chiral Flow Path:

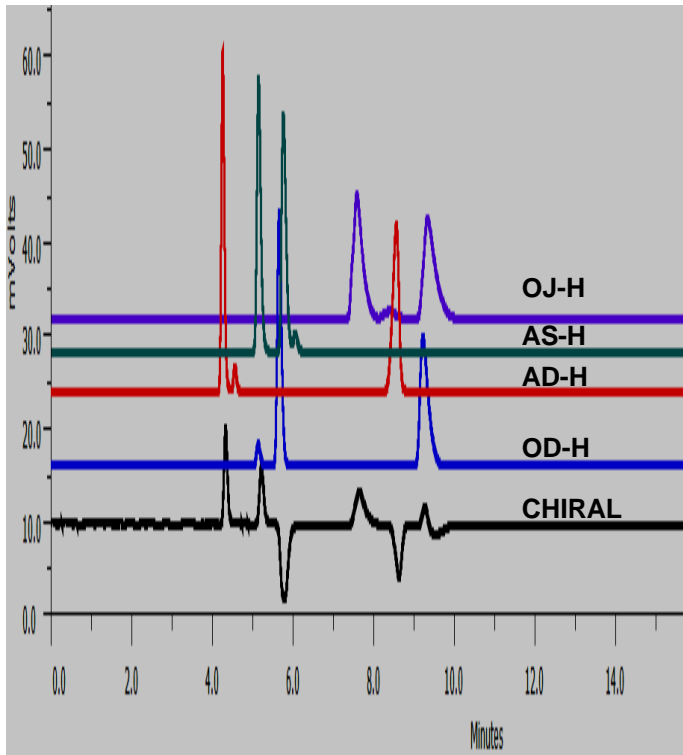
- 4th) Pre-detectors parallel chiral system evaluation: System pressure and individual column flow rates are documented (in-line flow rate meters not used) based on mL/min observed - 10 readings documented/column
- 5th) Post UV/Vis detector parallel manifold placed **AFTER** the (4) UV/VIS detectors prior to the chiral (polarimeter) detector



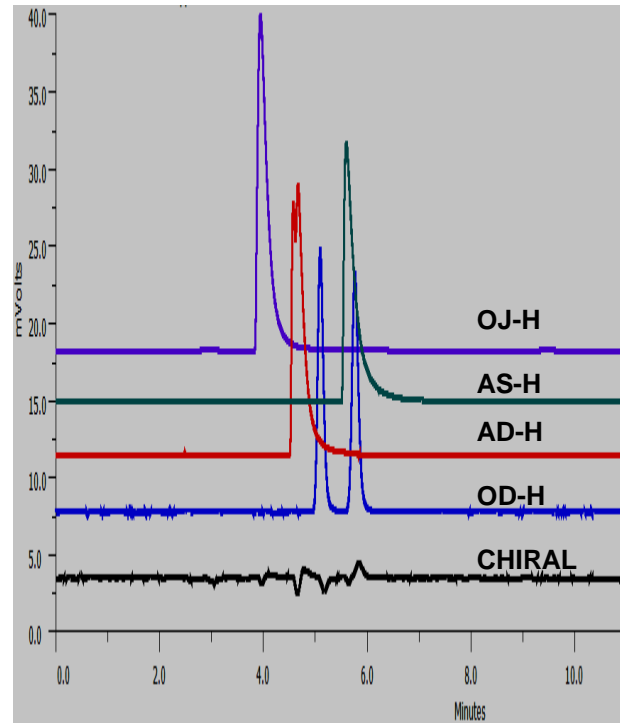
Analysis from the Parallel Chiral System:

- Trans-Stilbene oxide (TSO) and Nicotine (1 mg/mL) were injected into the system, 20 uL
- The chromatogram depicts the separation observed from each chiral column and the overall optical rotation
- Evaluation for a given compound is achieved within a quarter of the usual analysis time, 75% time savings

Analysis from the Parallel Chiral System:

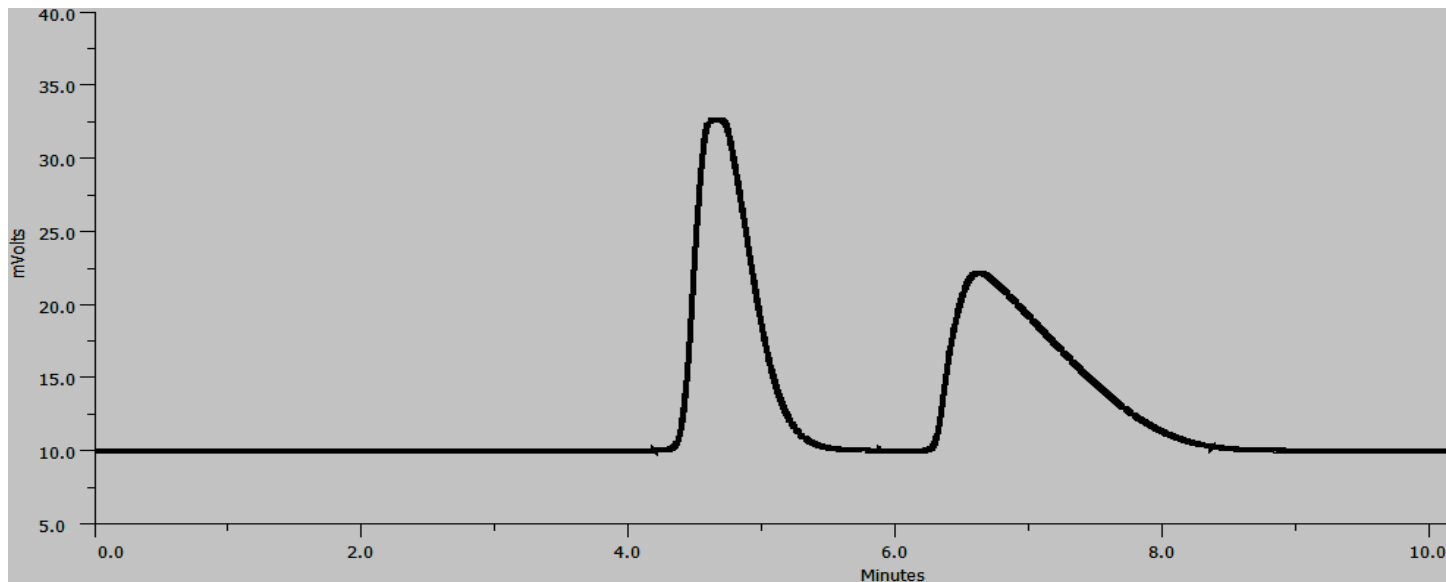


TSO



NICOTINE

Semi-Preparative Chiral Scale Up



Semi-preparative purification of TSO on two 21 x 50 mm, 20 micron, Chiral Technologies OD (columns in series), 50 mgs on column, 3,000 uL injection, 0.05 mm flow cell path length

Analysis from the Parallel Chiral System:

- Consistency and robustness of the system was evaluated by analyzing both TSO (AD-H) and Nicotine (OD-H) over several days
- System pressure remained constant, < 5% variance
- Peak area for TSO and Nicotine, < 3% variance
- Retention Time for TSO and Nicotine, < 0.4% variance

Conclusion:

- The parallel chiral system was shown to be a robust and efficient approach to analytical chiral scouting
- Basic HPLC system, with mobile phase selection capabilities, does not require complicated column switching valves
- Increased sample throughput by 75%