Introduction:
Biopharmaceutical companies run thousands of samples each week through assays designed to assess structural properties of newly synthesized molecular entities. Advancements in LC/MS and related technologies continually drive development of new assay types aimed at differentiating the impact of minor structural changes on drug binding. Bioanalytical staff are charged with implementing new methodologies while managing a broadening range of studies that are considered routine and are applied in screening context. Here we describe a ‘plug and play’ approach featuring dual-channel gradient LC/MS/MS with 30 sec/sample throughput. Methods were automatically downloaded in a blinded fashion at remote location to demonstrate ease of use by non-expert and suitability for ‘routine’ sample analysis. Results were compared with traditional multiplexed LC methods.

Methods:
• The sample delivery system consisted of an LS-I autosampler with 10-plate capacity, 8-2 position UHPLC valves (and 4 injection ports).
• The 2-position valves are accessible under the plate deck and across the front of sampling deck for optimal LC plumbing relative to the ion source inlet.
• The system was configured with two Shimadzu Nexera binary gradient pumps and Sciex 5500 mass spectrometer.
• Halo2x20mm C18, 2.7µ. LC columns were plumbed in dual-stream mode, 0.7 min linear gradient on each channel.
• The system was controlled by LeadScape software (SoundAnalytics). LeadScape handled batch creation, valve scheduling, gradient LC control, MS signal acquisition and data review.

DiscoveryQuant/LeadScape Automation

2. Bioanalytical Automation
- ‘Global’ MRM LC/MS/MS conditions are stored in DiscoveryQuant Microsoft SQL Server® database.
- LeadScape can bind to global database or a smaller subset MS-Access database.
- The MS-Access database has identical architecture to parent but contains a limited and specific subset of information relevant for a specific analyses.

System Performance (Results):

3. Remote Lab/ CRO
- Uses LeadScape to bind client MS-Access database.
- Imported text files contain plate/well IDs to guide sample delivery on LS-I. MS/MS conditions are downloaded.

4. LS/I- LeadScape WorkStation
5500 Qtrap plumbed for ‘Duality’ dual-stream LC/MS/MS Dual Binary Gradient Nexera pumps (Shimadzu)

5. MDCK 4 in 1
Nadolol, labetalol, metoprolol and amprenavir MRM conditions were combined during ‘import with vial positions’ approach (Fig. 3). 18 injections per file. Propranolol ISTD.

6. Duality binary gradient LC (30 sec/sample)
Dual-Stream LC/MS/MS, 1 min cycle time per channel CH1/CH2 Injections are offset by 30 sec. Flowrate was 0.85 mL/min. Halo 2x20mm, C18, 2.7µ.

7. Saquinavir (efflux control substrate)
Study was run in duplicate at 90, 120 and 180 min timepts. Signal response pattern shows strong B-A Efflux. Multiply-injected file peak response graphic speeds data review and quality

Conclusions
An MDCK permeability and MDR1 transporter study was run at Genentech Inc. (GNE, South San Francisco, CA) using standard cell-based ADME-Screening protocol.

Initial sample analysis was completed at GNE using 2-channel multiplexed method on Aria LX-2 system. Sample plates and Aria batch files and GNE DQ database, were sent to Sciex (Framingham, MA) post analysis.

The Study was rerun at Framingham demo lab using LS-I/LeadScape system integrated with Sciex 5500 MS. MRM methods and injection sequences were imported directly from GNE DQ database using LeadScape (plug and play). Results obtained in Framingham were identical to those obtained at GNE. Throughput was increased 4-fold in comparison to Aria LX-2 dual-stream approach.