

Validation and High Throughput Analysis of Ranolazine in Human Plasma Using Automated Liquid Handling Robotics

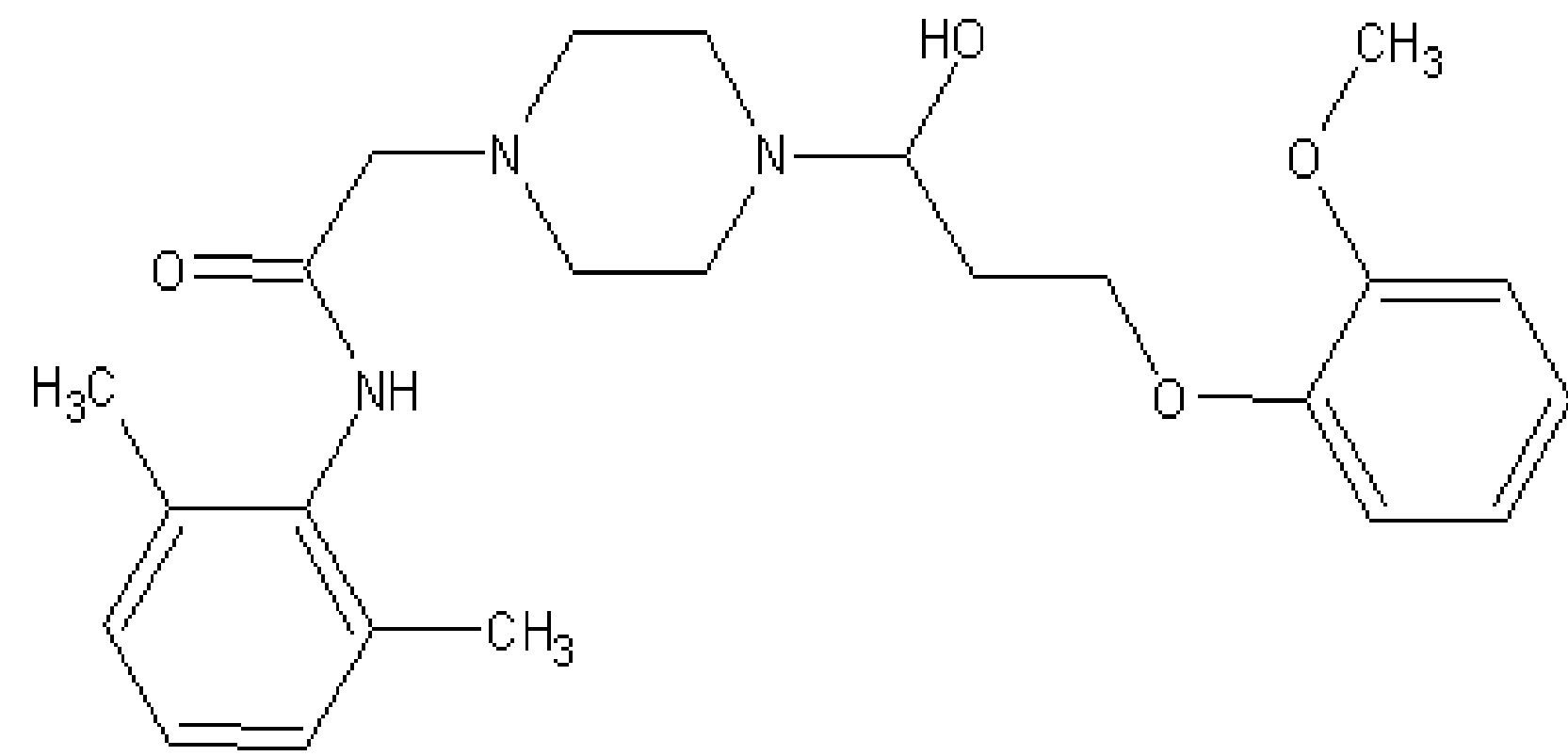
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INTRODUCTION

The purpose of this study was to automate an extraction methodology approach for high throughput analysis of large-scale bioequivalency and other bioanalytical studies, while maintaining the high accuracy and precision requirements of a GLP method. The extraction of ranolazine (Figure 1) from human plasma was automated for a large bioequivalency study. Liquid handling robotics, such as the Hamilton Microlab® STAR^{LET} and the Tomtec Quadra 96™ were used to automate the sample processing and protein precipitation extraction procedure. The desired outcome was to increase sample output while maintaining high accuracy and precision.

The STAR^{LET} method was evaluated for clot detection in plasma samples and reagent dripping. The method (using the STAR^{LET} and Quadra 96™) was then validated for the determination of ranolazine in human plasma. Intra-assay, inter-assay and inter-channel accuracy and precision were tested.

Figure 1. Structure of Ranolazine



CHROMATOGRAPHY AND MASS SPECTROMETRIC CONDITIONS

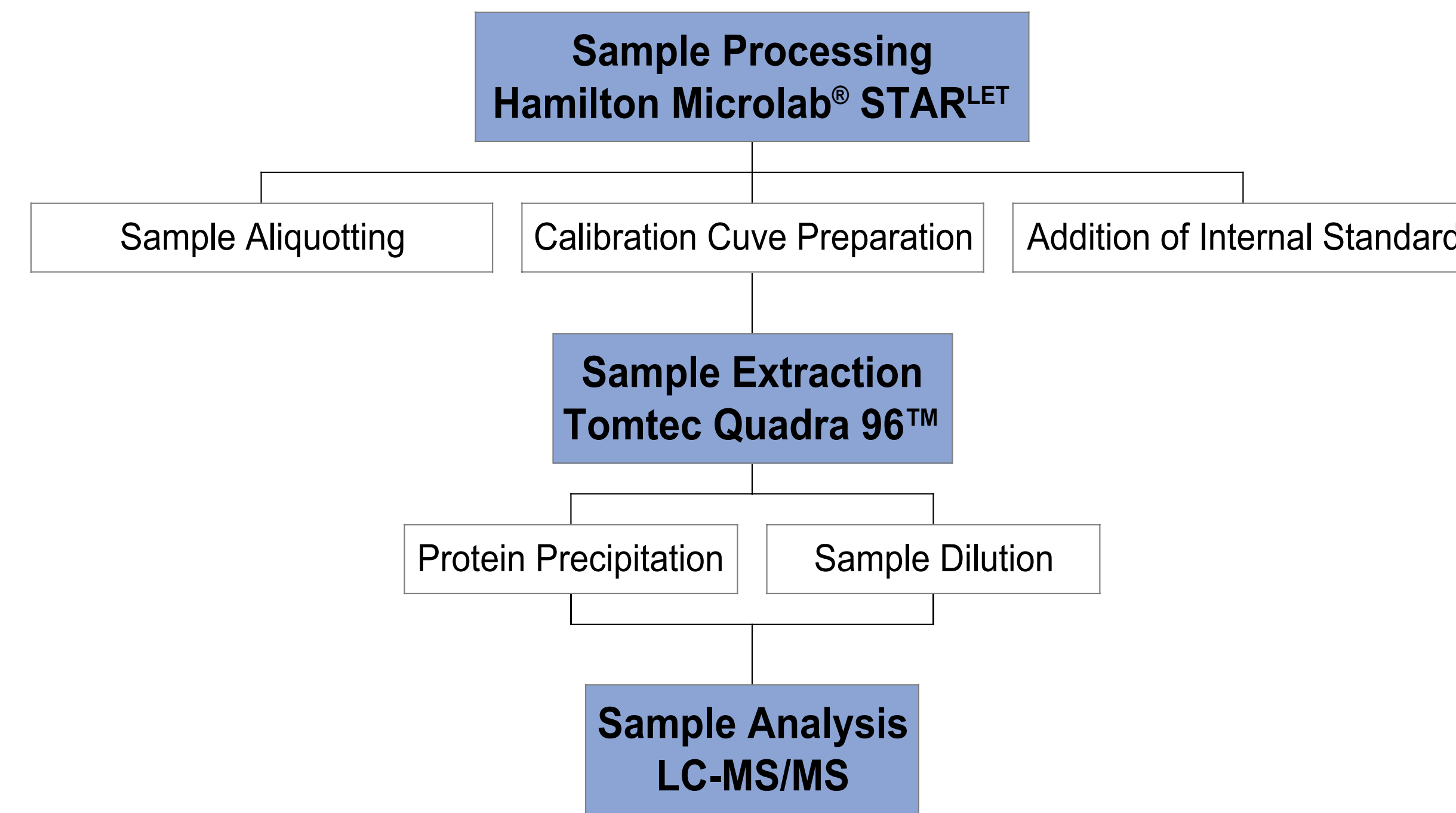
Chromatography:
Column: ACE 5 Phenyl, 100 x 2.1 mm (Advanced Chromatography Technologies)
Guard: ACE 5 Phenyl, 10 x 2.1 mm (Advanced Chromatography Technologies)
Column Temp.: Ambient
Injection Vol.: 10 µL
Flow Rate: 0.4 mL/min (1:3 split)
Mobile Phase A: 0.1% formic acid
Mobile Phase B: ACN with 0.1% formic acid
Mobile Phase Composition: Isocratic, 60:40 (v/v) Mobile Phase A: Mobile Phase B

Mass Spectrometric Detection:
Analyte Transition: Ranolazine m/z 428.1 → 279.1
Internal Standard Transition: D3-Ranolazine m/z 431.1 → 282.0
Mass Spectrometer: Sciex API 365™/EP 10+ Mass Spectrometer

METHODS

Ranolazine and an internal standard (D3-Ranolazine) were extracted from 100 µL of human plasma (sodium heparin) by protein precipitation using 9:1 acetonitrile:methanol. The supernatant was then diluted and analyzed by LC-API/MS/MS. Run times were approximately 2.5 minutes.

The Hamilton Microlab® STAR^{LET} pipetting system was used to aliquot quality control samples (QCs) into a 96-well format, prepare the calibration curve, and add internal standard. The Tomtec Quadra 96™ was then used for the protein precipitation extraction and dilution of the extracts prior to analysis by LC-API/MS/MS.



METHOD VALIDATION

Two validation runs for Ranolazine in human plasma were analyzed using N=3 QCs per pipetting channel (N=24 total QCs, using eight pipetting channels) at three concentrations spanning the calibration curve range. A third run was included with N=1 QC replicate per pipetting channel (N=8 total QCs) at each concentration. Single aliquots from individual vials were used to mimic the pipetting of study samples. These single aliquot QC samples were used to determine intra-channel, intra-assay, and inter-assay accuracy and precision. In addition, multiple aliquots were taken from single QC tubes in each run, and were used to mimic analytical QCs. Calibration curve and QC samples were extracted using one and two plate methods.

In the event of STAR^{LET} pipetting failure due to clotted samples, etc. (in which case, samples would require manual pipetting), QCs were manually pipetted and extracted along with the STAR^{LET} pipetted samples.

In addition, clotted plasma samples were evaluated for clot detection by Hamilton Vector Software, and reagents were tested for dripping.

The Tomtec Quadra 96™ was used for non-critical pipetting steps, and therefore, specific validation studies with this instrument were not conducted.

Table 1. Intra-Assay Quality Control Statistics for Ranolazine in Human Plasma

Run 1		Quality Control Concentrations (ng/mL)		
		100	2000	8000
Channel 1	Mean	106	2030	8060
	%CV	1.96	2.43	0.755
	%Acc.	106	102	101
Channel 2	Mean	105	2000	8220
	%CV	3.98	3.32	1.82
	%Acc.	105	100	103
Channel 3	Mean	103	2010	8100
	%CV	3.24	4.72	0.445
	%Acc.	103	101	101
Channel 4	Mean	105	2020	8120
	%CV	1.98	3.30	1.03
	%Acc.	105	101	102
Channel 5	Mean	108	2010	8220
	%CV	NC	3.66	0.253
	%Acc.	108	101	103
Channel 6	Mean	105	2020	8150
	%CV	3.36	4.50	1.04
	%Acc.	105	101	102
Channel 7	Mean	105	2010	8050
	%CV	2.52	4.90	1.00
	%Acc.	105	101	101
Channel 8	Mean	103	2010	8200
	%CV	1.94	4.90	0.735
	%Acc.	103	101	103
Intra-Assay	Mean	105	2010	8140
	%CV	2.7	3.4	1.16
	%Acc.	105	101	102

Table 2. Inter-Assay Quality Control Statistics for Ranolazine in Human Plasma

Run #		Quality Control Concentrations (ng/mL)		
		100	2000	8000
1	Mean	105	2010	8140
	sd	2.83	68.5	94.7
	%CV	2.70	3.40	1.16
	%Acc.	105	101	102
2	Mean	104	2010	8220
	sd	2.53	58.8	99.9
	%CV	2.43	2.92	1.22
	%Acc.	104	101	103
3	Mean	105	2030	7990
	sd	2.26	29.8	136
	%CV	2.15	1.47	1.71
	%Acc.	105	102	99.9
Inter-Assay	Mean	104	2020	8150
	sd	2.64	59.6	125
	%CV	2.52	2.96	1.53
	%Acc.	104	101	102

Table 3. Manually Pipetted Quality Control Results and Statistics for Ranolazine in Human Plasma

Run #	Quality Control Concentration 2000 ng/mL	
	Amount Found (ng/mL)	Acc. (%)
3	1950	97.5
	1960	98.0
	1900	95.0
Mean	1940	
sd	32.1	
%CV	1.66	
%Acc.	97.0	

RESULTS

Clotted human plasma was pipetted by the STAR^{LET}. In clotted plasma experiments, two of eight samples were successfully pipetted (verified gravimetrically), and the remaining six samples were flagged by clot detection software (Figure 2A). In centrifuged human plasma (free from clots), all samples were successfully pipetted (Figure 2B). During the actual method validation, two samples were flagged for clot interference during pipetting, and were recorded by the instrument software (data not shown).

Human plasma and solvents used during the extraction were evaluated for dripping. Dripping was not observed when an aspirated human plasma sample was held for at least 30 seconds prior to dispense (which exceeds the time required for a sample to be dispensed). Solvents used during the extraction were also evaluated for dripping. Dripping was not observed when solvents were held for at least 90 seconds prior to dispense (data not shown).

The calibration curve range was 50.0-10000 ng/mL. A chromatogram of the analyte at the LLOQ and internal standard is shown in Figure 3. The linear regression of the curves for peak area ratios versus concentration was weighted by 1/x² (reciprocal of the square of the analyte concentration). Calculated coefficient of determination (r²) values were used to evaluate linearity of the curves. The assay produced linear calibration curves over the concentration range (Figure 4).

Intra-channel pipetting accuracy (in each of the eight channels) ranged from 103-108%, 100-102% and 101-103% for the low, mid and high concentrations, respectively. Intra-channel precision was ~ 4.90%. Intra-assay accuracy values were 105%, 101%, and 102% and intra-assay precision values were 2.70%, 3.40% and 1.16%, for the low, mid, and high QCs, respectively (Table 1).

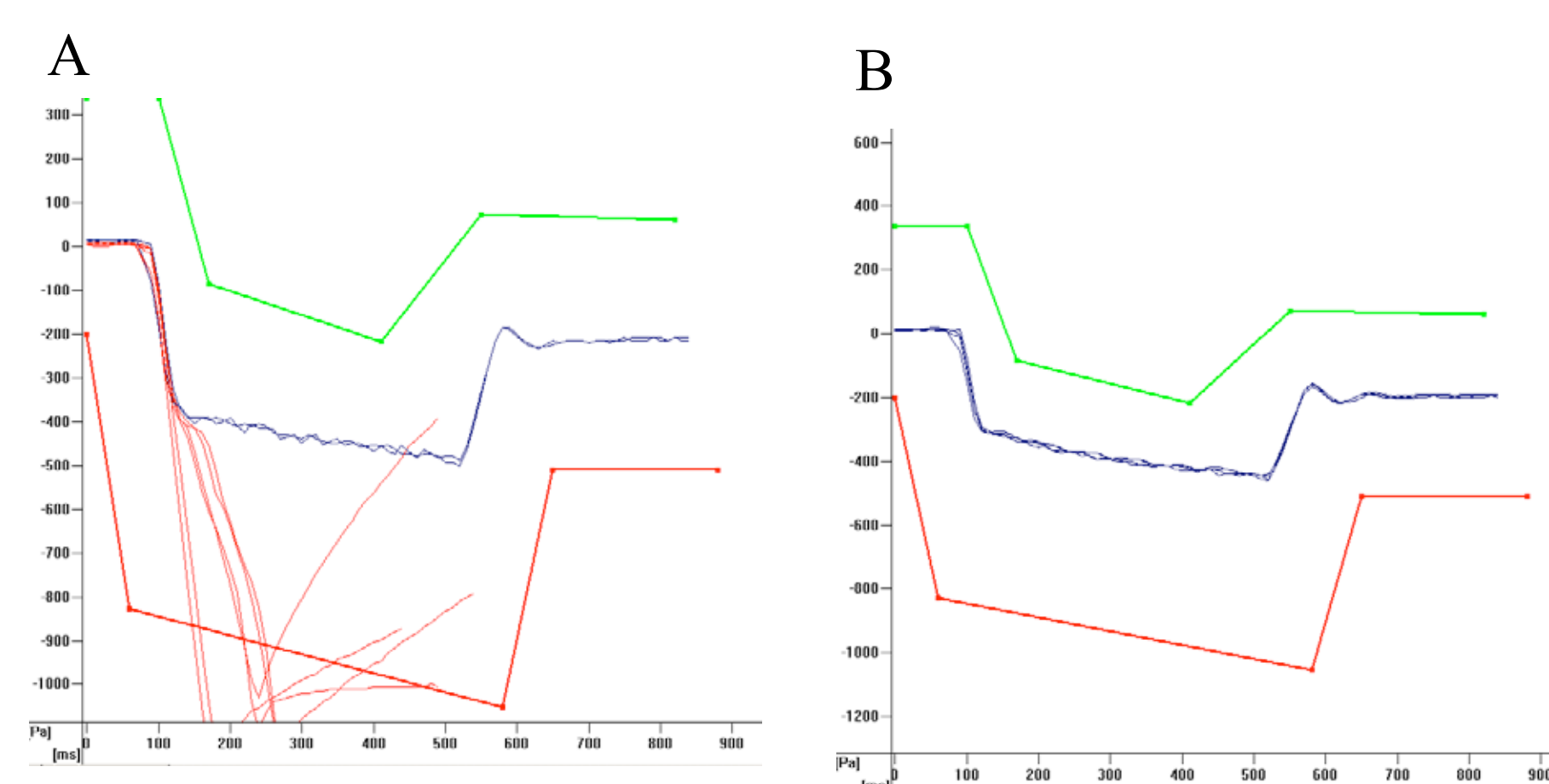
Inter-assay accuracy values were 104%, 101%, and 102% and inter-assay precision values were 2.52%, 2.96% and 1.53%, for the low, mid, and high QCs, respectively (Table 2).

In the event of a STAR^{LET} pipetting failure due to clotted samples, etc, QC samples were manually pipetted and quantified against the calibration curve prepared by the STAR^{LET}. Mean accuracy was 97.0% and precision was 1.66% (Table 3).

CONCLUSIONS

- The automated method was successfully validated and provided high accuracy and precision.
- The method was not subject to dripping, and clots were successfully detected by the instrument software.
- The automated method increased sample throughput at least two-fold compared to manual extraction, and has been successfully applied to large-scale bioequivalency studies.

Figure 2. Clot Detection



Hamilton Vector Software monitors the pressure inside the pipetting tip to accurately detect clots in samples. The green band represents the upper pressure limit and the bottom red band represents the lower pressure limit. The blue traces represent successful pipettings (within the limit of the pressure curve), while the red traces are of samples with clot detect errors. A) Pressure curves for clotted human plasma samples. B) Pressure curves for human plasma samples without clots.

Figure 3. Representative Chromatogram of Ranolazine at the LLOQ

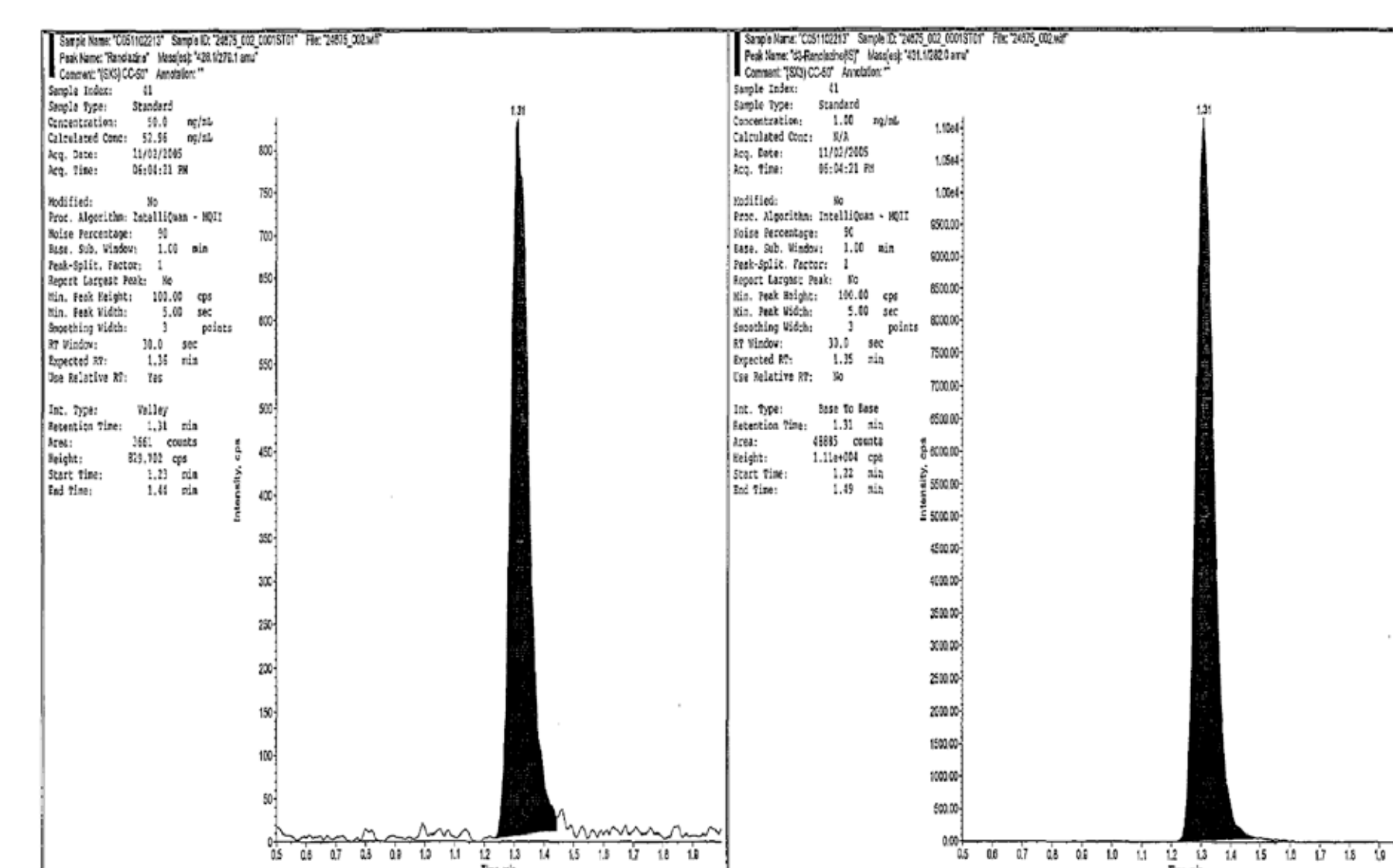


Figure 4. Representative Calibration Curve Plot for Ranolazine in Human Plasma

