AUTOMATED MRM/PRM QUANTITATION · VALIDATION BENCHMARK

Fully automated analysis of MRM/PRM data

Targeted MRM/PRM is the workhorse of biomarker validation and clinical assays. The bottleneck isn’t the mass spec — it’s turning thousands of chromatograms into trustworthy numbers, one manually-reviewed peak at a time.

The problem

Manual peak review is slow, subjective, and doesn’t scale. A single validation run can tie up an experienced analyst for hours to days — and the answer still depends on who did the picking.

What MRMpipe does

From raw vendor files to a validated quant table, fully automated: peak detection, integration, calibration and QC — with light/heavy co-elution enforced at pick time, so the machine doesn’t make the mistakes a rushed reviewer would.

The only question that matters

Does automation actually match an expert’s curated result? We put it to the test on a real **anti-doping panel — 918 samples, 35 peptides —** benchmarking MRMpipe against a technician-curated Skyline result (the accepted gold standard).

THE ANSWER, IN THREE NUMBERS

R^2 0.984
agreement with the curated gold standard (slope 0.998)

~8 min
to process all 918 samples vs >4 h of manual review

≤ CV
equal-or-tighter replicate precision at every level

NEXT PAGE
Agreement on standards & QCs

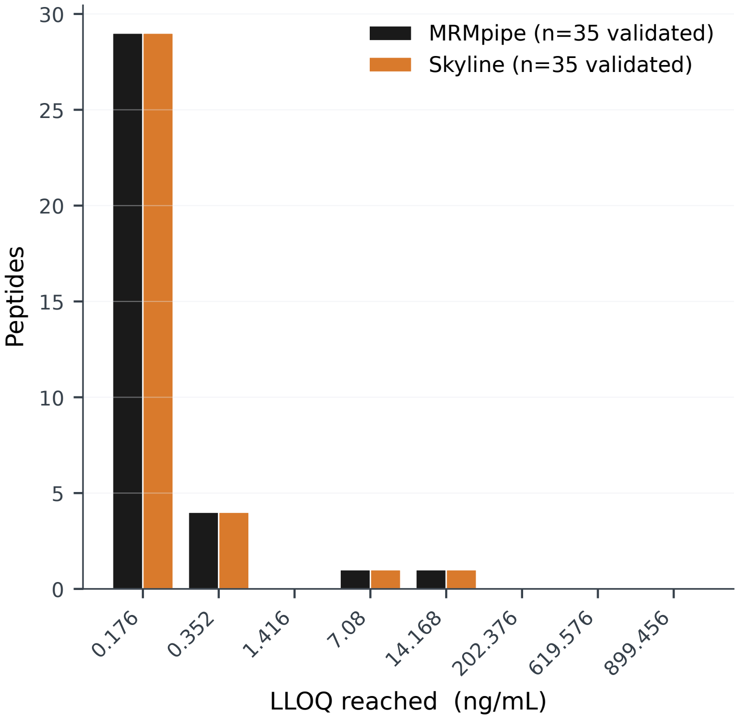
THEN
Calibration slope & replicate CV

FINALLY
Scientific takeaway & bottom line

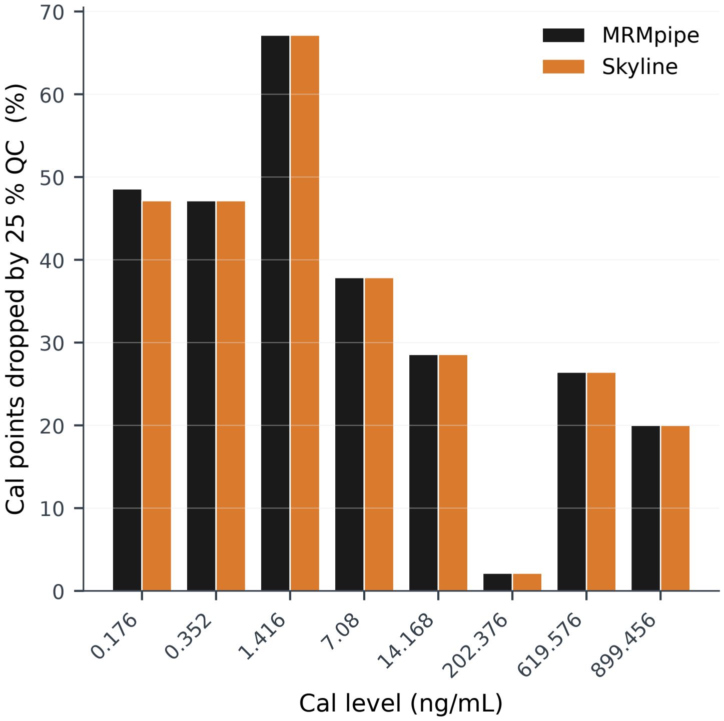
Lower limit of quantitation & cal-replicate survival

For each peptide and pipeline, the LLOQ is the lowest calibrator level whose
≥ 2 replicates survive the 25 % recovery QC. Lower is better.

Distribution of LLOQ across peptides



Per-level QC drop rate



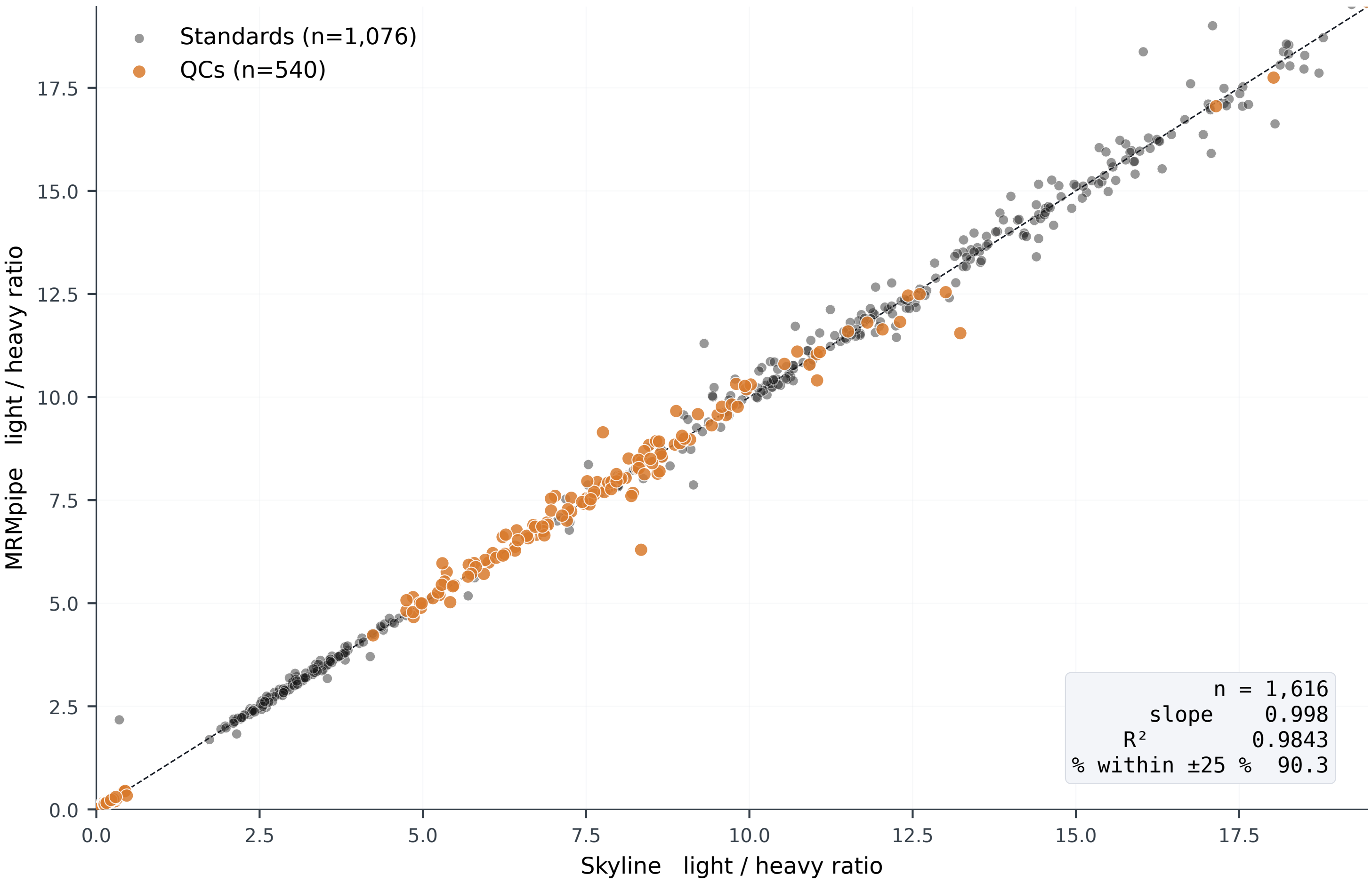
LLOQ overlap

peptide	MRMpipe	Skyline	verdict
EIVPVLVSTR	0.176	0.176	= match
GPFLLGIK	0.176	0.176	= match
VASHTPSLLR	0.176	0.176	= match
ITDTIGPTETSIAPR	0.176	0.176	= match
GGPLDGTYR	0.176	0.176	= match
ATISNDGATILK	0.176	0.176	= match
FLEEHPGGEEVLR	0.176	0.176	= match
AEISGVQGSETQVLR	0.176	0.176	= match
QQASEADAAAATFR	0.176	0.176	= match
ALGPEQLLR	0.176	0.176	= match
AIQDPAFSDGIR	0.176	0.176	= match
VIPSEDNLISEVEK	0.176	0.176	= match
TFITIGDR	0.176	0.176	= match
DLIEAIR	0.176	0.176	= match
ADILQVGLR	0.176	0.176	= match
LSDQFHDILIR	0.176	0.176	= match
EVVEEAENGR	0.176	0.176	= match
LSYSDSDLK	0.176	0.176	= match
VIVDFSSPNIAK	0.176	0.176	= match
LNSAIIYDR	0.176	0.176	= match
GADVWFK	0.176	0.176	= match
FAQNTLGNK	0.176	0.176	= match
SFWSYAFSR	0.176	0.176	= match
LPSGEDYNLK	0.176	0.176	= match
VTEGLTDVILYHQPDDK	0.176	0.176	= match
NELSGALTGLTR	0.176	0.176	= match
LVLDSVK	0.176	0.176	= match
IFINLPR	0.176	0.176	= match
GAEAANVTGPGGVPVQGSK	0.176	0.176	= match
GDGVVLVAPPLR	0.352	0.352	= match
AGVDPLVPLR	0.352	0.352	= match
NLENGALQPSDLDR	0.352	0.352	= match
GAEAANVTGPDGVPVEGSR	0.352	0.352	= match
TLHEVLEK	7.08	7.08	= match
LFTVYPSTK	14.2	14.2	= match

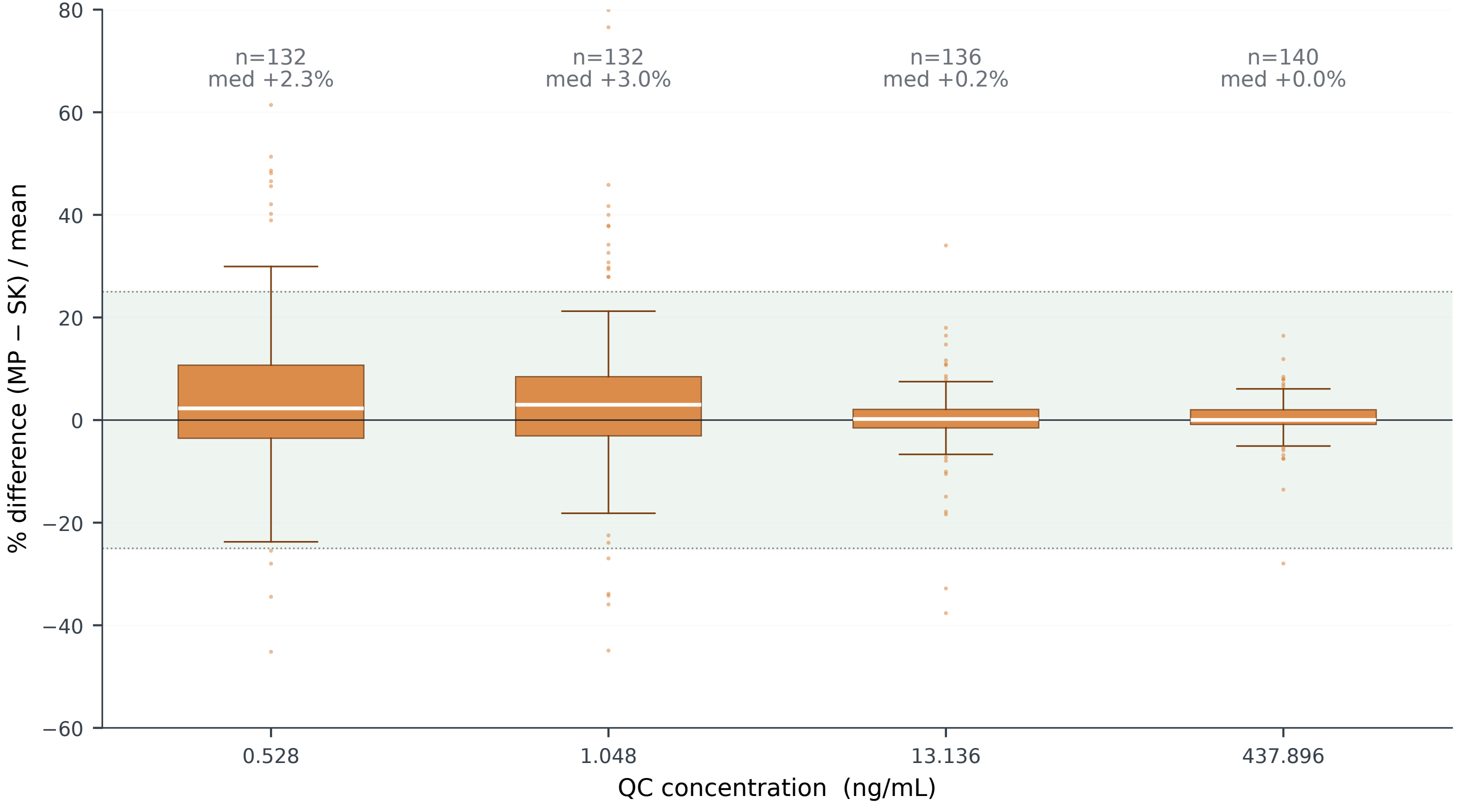
Both pipelines agree on standards + QCs

1,076 standards (black) + 540 QCs (orange), above LLOQ in both pipelines.
Bottom: per-QC-level % difference (standards reserved for calibration).

Identity scatter

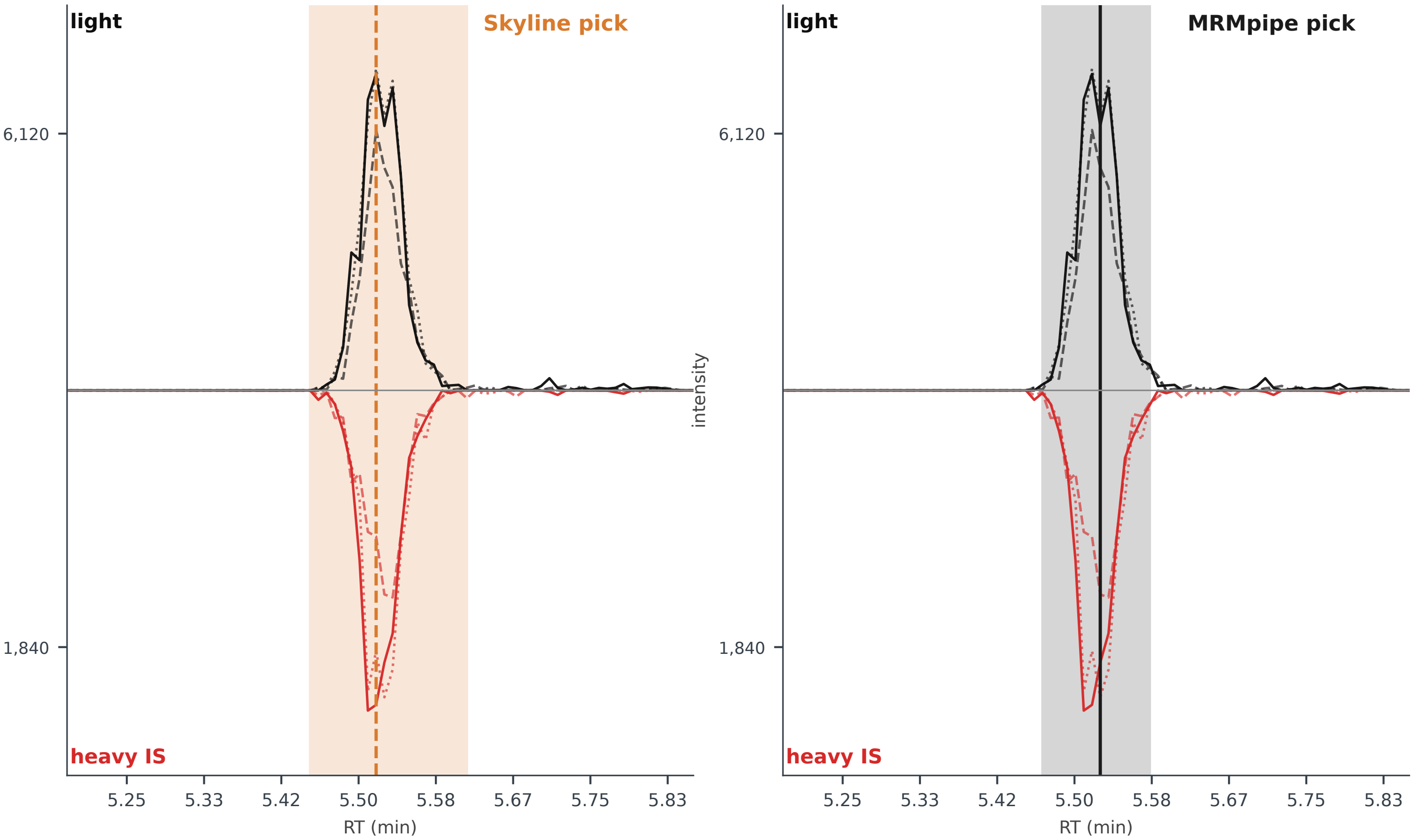


Per-QC-level percent difference · QC samples only

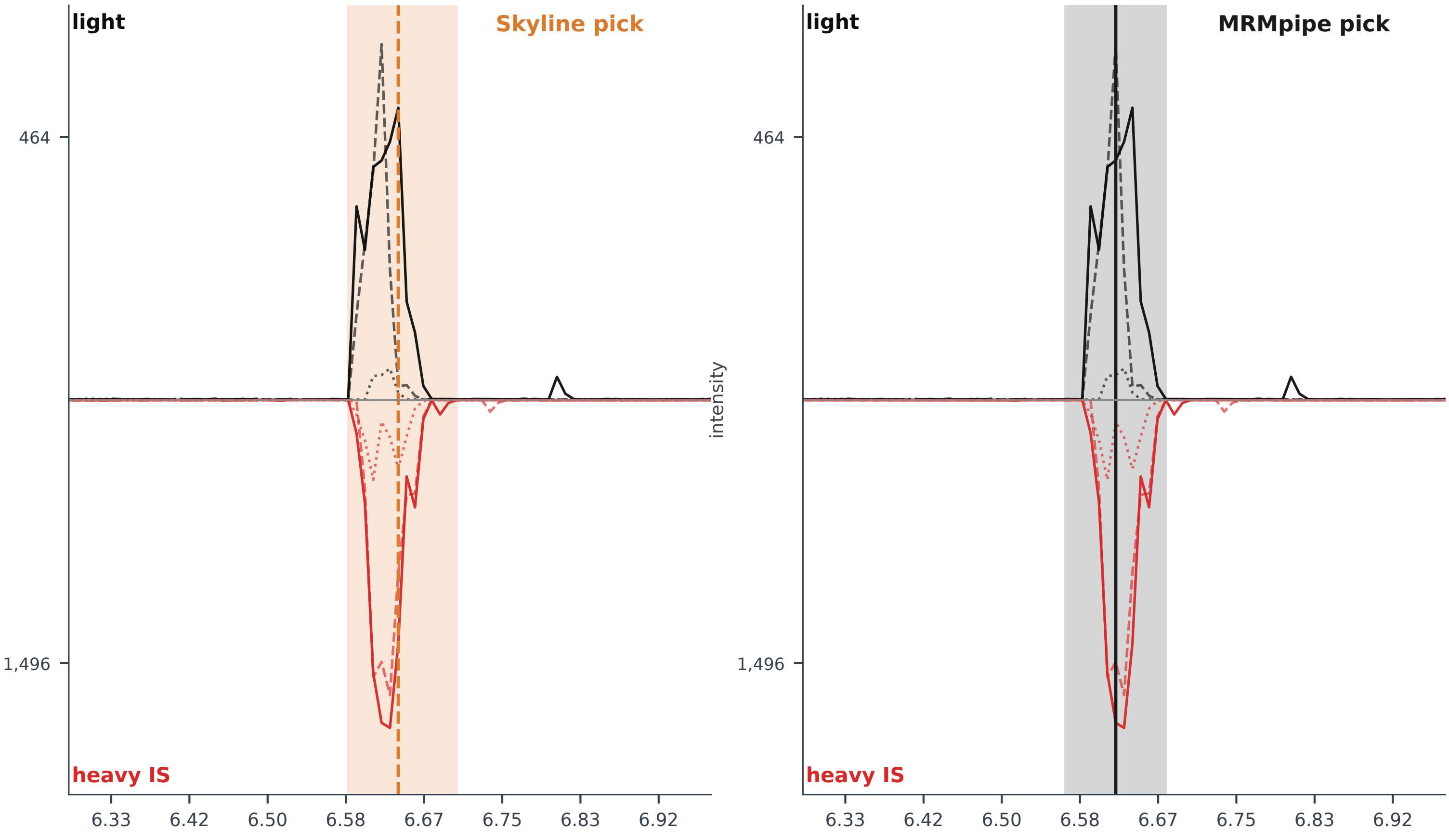


XIC — example picks

Skyline pick (left) vs MRMpipe pick (right) on the same chromatogram.



GAEAANVTGPGGVPVQGSK · STANDARD @ 202.376 ng/mL · L/H ratio SK 3.734 MP 3.729



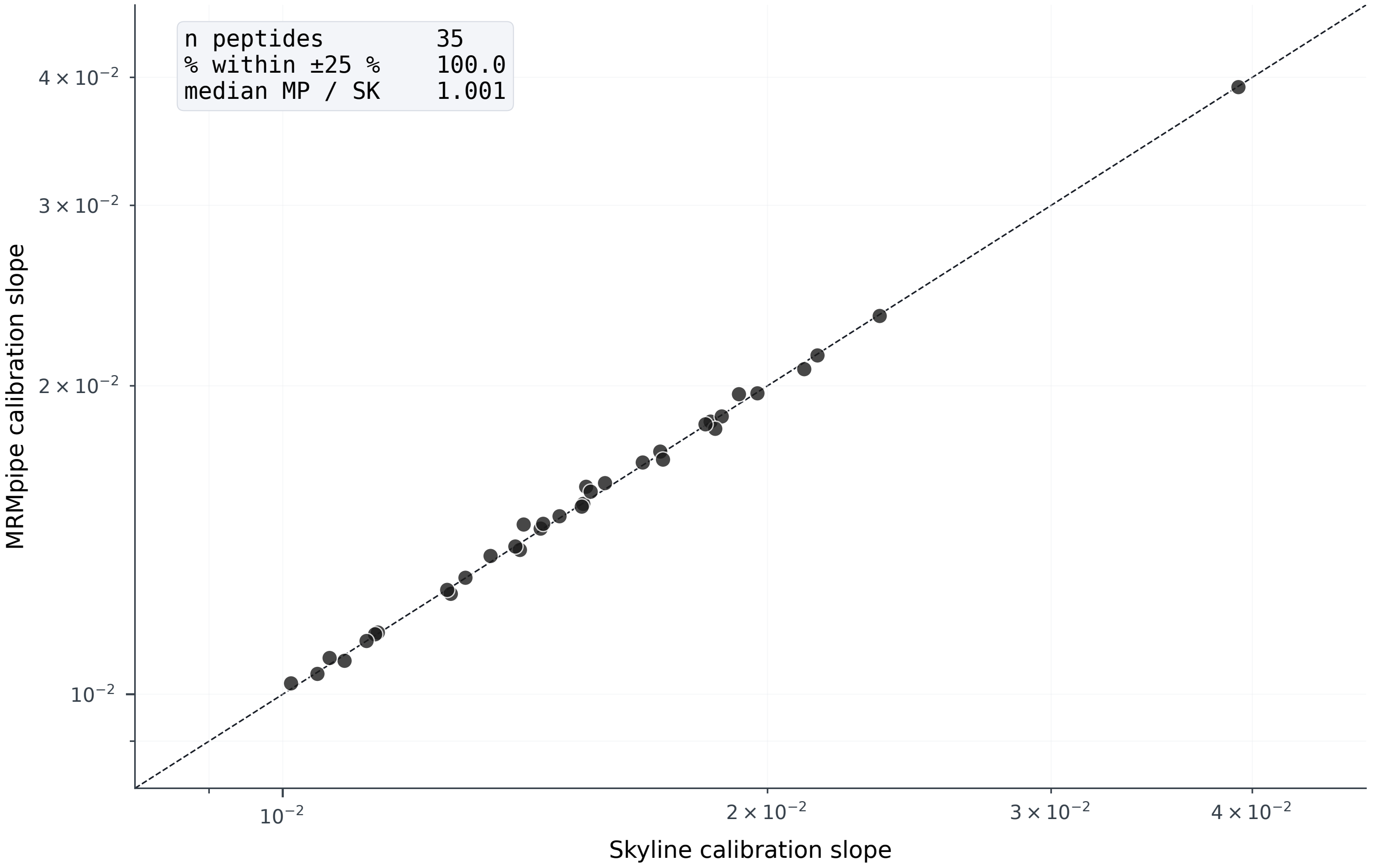
AEISGVQGSETQVLR · QC @ 13.136 ng/mL · L/H ratio SK 0.314 MP 0.262

Light = black, heavy IS = red. Shaded band = integration window.

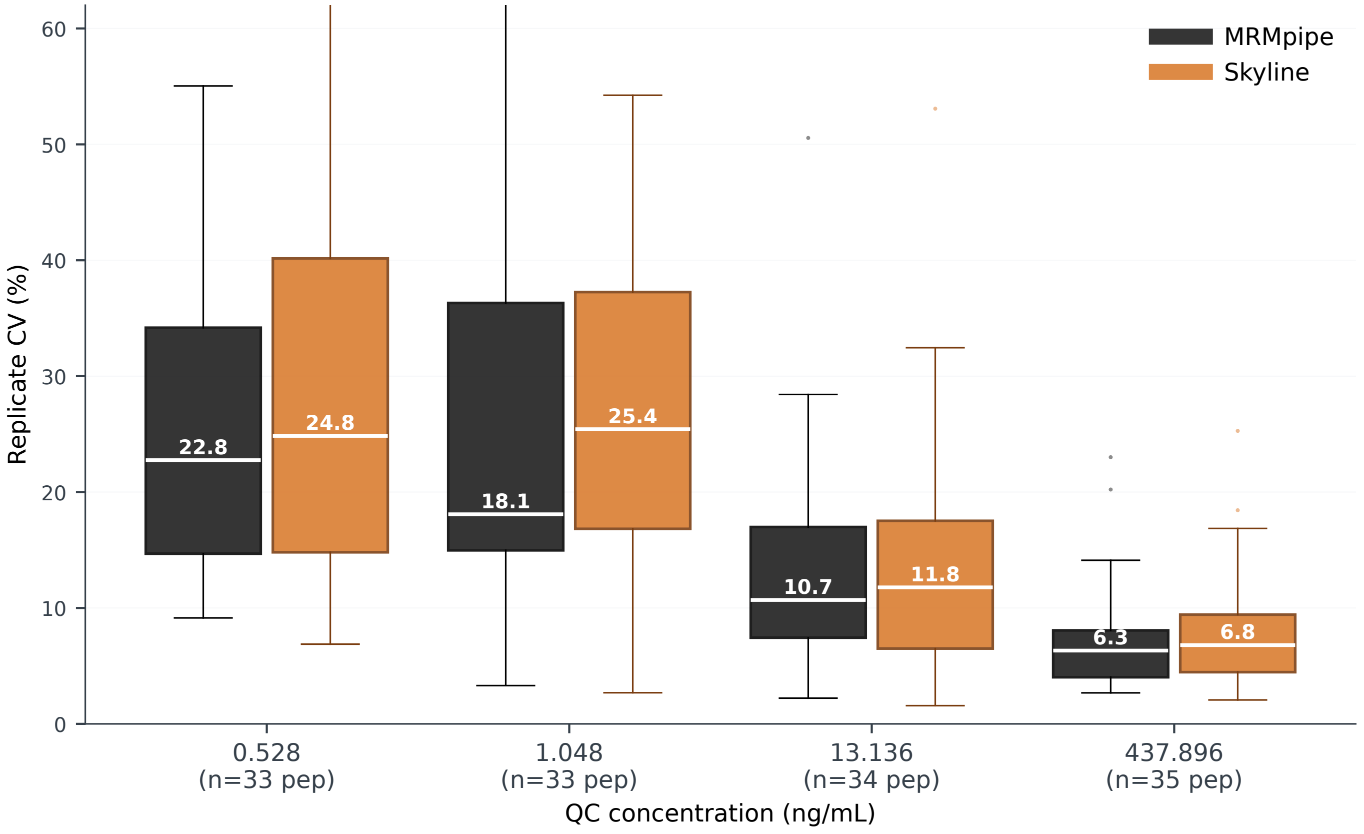
Calibration slope agreement + QC replicate CV

Top: calibration slope MRMpipe vs Skyline across 35 peptides validated in both.
Bottom: replicate CV per QC concentration, all peptides pooled.

Slope vs slope · 35 peptides



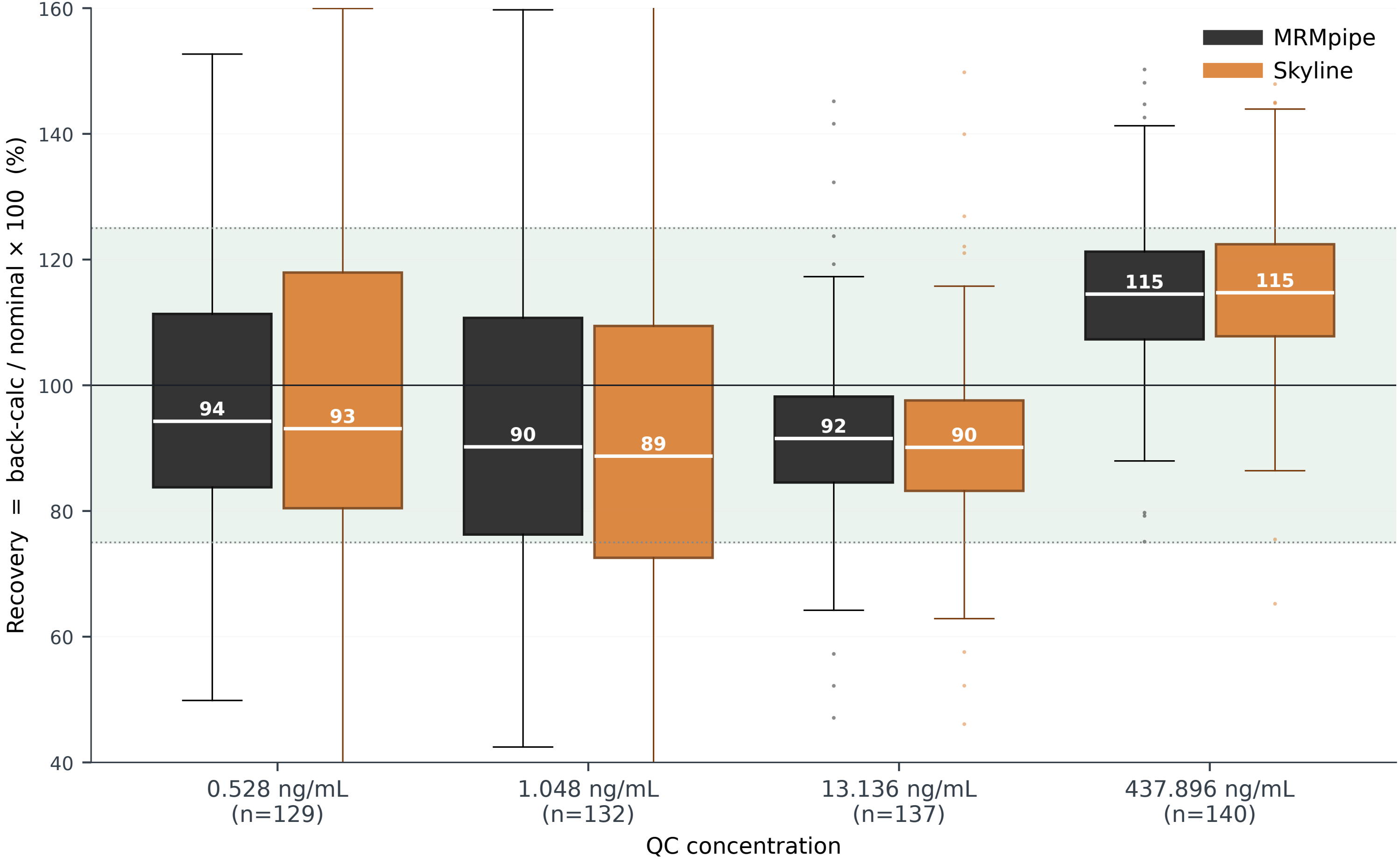
QC replicate CV by concentration · all peptides pooled



Accuracy — distance from the known nominal concentration

Top: recovery box per QC level per pipeline. 100 % = perfect.
Bottom: patient unknowns (no nominal truth) — MRMpipe vs Skyline scatter.

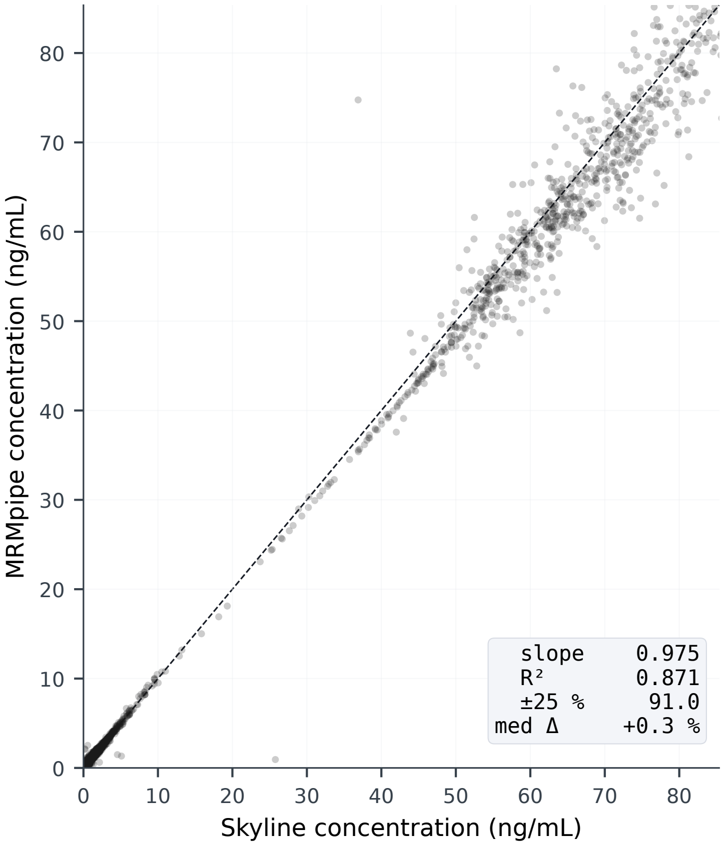
QC recovery — distance from known truth, by pipeline



accuracy metric	MRMpipe	Skyline
median recovery	98.7 %	97.6 %
|bias from 100 %|	1.3	2.4
within ±25 %	75.3 %	68.6 %
P90 |Δ from 100|	37.5	41.7

QC accuracy — pooled across all QC concentrations. Each pipeline's back-calculated QC vs known nominal value. High-conc is prone to fluctuations (heteroskedasticity, 1/x² cal pulled by low-conc points); plus a baseline of pipetting/instrumental error common to both pipelines.

Patient unknowns (n=16,779)



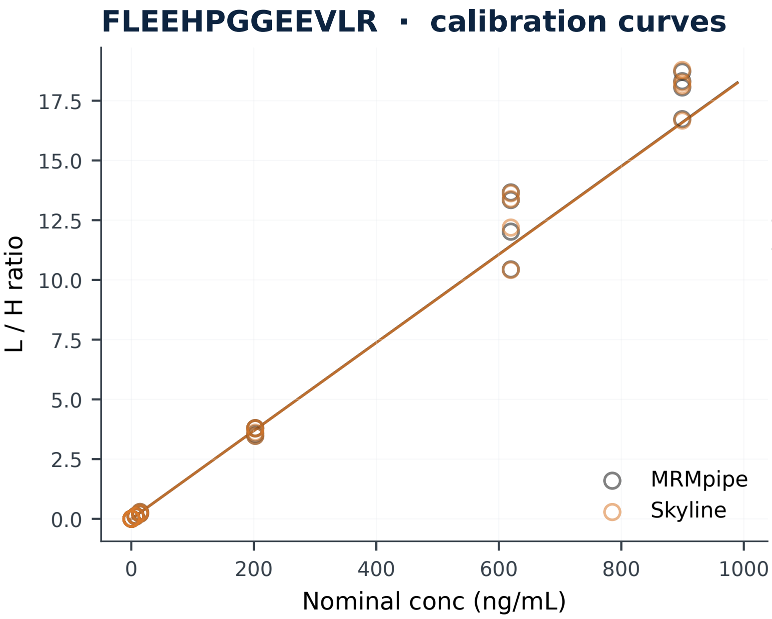
Summary — scientific takeaway

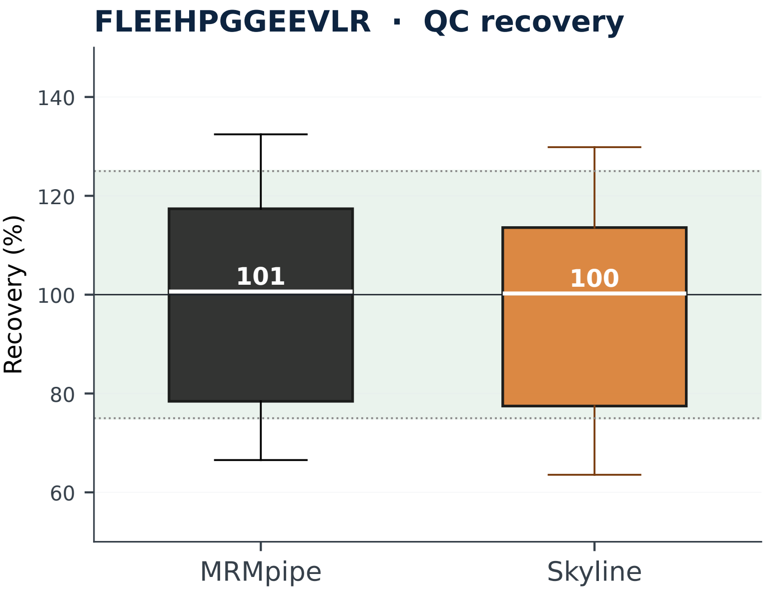
Spotlight peptide: FLEEHPGGEEVLR.

Speed.
MRMpipe processes 918 samples end-to-end in ~8 min wall time (fully automated).

Reproducibility.
MRMpipe replicate CV on QCs is tighter on 79/135 (59 %) of (peptide × level) pairs. Median CV 13.5 % vs 14.7 %.

Accuracy.
MRMpipe |median QC recovery − 100 %| = 1.3 %, Skyline = 2.4 %. MRMpipe slightly closer to nominal.


FLEEHPGGEEVLR · calibration curves


FLEEHPGGEEVLR · QC recovery

metric	MRMpipe	Skyline
analysis time (918 samples)	~8 minutes	manual revision
median QC recovery	98.7 %	97.6 %
median QC replicate CV	13.5 %	14.7 %

Bottom line

MRMpipe reproduces Skyline with slightly tighter CVs and better QC recovery, while being fully automated.