



Evaluating Methods Under FDA CTP Validation Guidance: Total Error Probability in Practice for HPHC Methods in Nicotine Pouches

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Introduction



Method validation is a process to confirm that the **analytical test method is suitable and reliable for its intended purpose**

Analytical methods for HPHCs were historically **validated according to ICH and/or FDA bioanalytical guidance** ^{1,2}

FDA CTP guidance for tobacco products introduces total error probability as an acceptance criterion and new validation recommendations³

Analytical Measurement Procedure (AMP)

describes sample preparation from a solution to instrument measurement

Analytical Sampling Procedure (ASP)

describes sampling of the tobacco product for the analytes of interest

Total Error Probability (TEP)

combining the accuracy and precision error into a probability function, not required in traditional ICH or FDA bioanalytical guidance

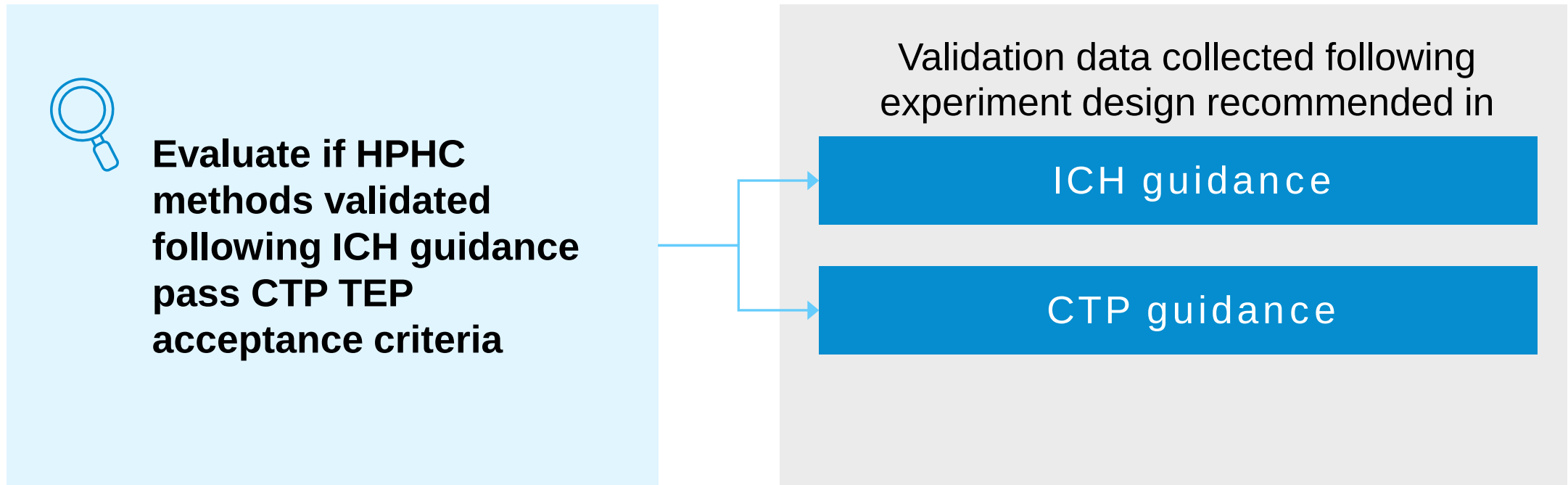
1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonised Guideline, Validation of Analytical Procedures, Q2(R2), November 2023.

2. M10 Bioanalytical Method Validation And Study Sample Analysis Guidance For Industry, November 2022.

3. FDA CTP, Validation And Verification Of Analytical Testing Methods Used For Tobacco Products, January 2025.



Objective



Difference Between ICH and FDA CTP Guidance

Validation Elements	ICH Q2 (R2)/FDA Bioanalytical	FDA CTP 2025
Linearity/Calibration	Three runs, one injection per run % Dev $\pm 15\%$ (cal1 $\pm 20\%$), $R^2 \geq 0.95$	5 injections for each level on one day TEP ≤ 0.05
Accuracy	≥ 3 independent replicates Accuracy: $\pm 15\%$ ($\pm 20\%$ at LLOQ)	≥ 7 replicates from pooled solution TEP ≤ 0.05
Intermediate Precision	≥ 3 runs %RSD $\leq 15\%$ (20% at LLOQ) or analytes specific	≥ 6 scenarios TEP ≤ 0.05



FDA CTP guidance recommends **more replicates, different experimental designs, TEP criteria** for all elements



Total Error Probability (TEP)

A Total Error Probability (TEP) combines accuracy and precision error into a probability function and demonstrates the likelihood that an analyte's true value can be determined within a user-defined confidence level (i.e. 95%)

$$\text{Total Error Probability} = 1 - (t(qU, df) - t(qL, df))$$

- $t(q, df)$ = cumulative Student's t distribution
- qU = (Upper-Mean) / ($RSD \cdot \sqrt{1 + 1/n}$)
- qL = (Lower-Mean) / ($RSD \cdot \sqrt{1 + 1/n}$)
- df = $n - 1$, n = number of replicates
- **Upper** = upper acceptance of recovery (%)
- **Lower** = lower acceptance of recovery (%)
- **Mean** = average % recovery
- **RSD** = reported as %

TEP $\leq$ 0.05 indicates the precision and accuracy are suitable for their intended purposes

Source: FDA CTP, Validation and Verification of Analytical Testing Methods Used for Tobacco Products, January 2025



Acceptance Limits are Concentration Dependent Based on Horwitz-Thompson Equation

Concentration (w/w)	%RSD (AMP*) (%)	AMP* Acceptance Limit at 95% Prediction Interval (%)	%RSD (ASP^) (%)	ASP^ Acceptance Limit at 95% Prediction Interval (%)
10 mg/g	2	100 ± 5.2	4	100 ± 10.5
1 mg/g	2.8	100 ± 7.3	5.7	100 ± 14.9
100 µg/g	4	100 ± 10.5	8	100 ± 20.9
10 µg/g	6	100 ± 15.7	11	100 ± 28.8
1 µg/g	8	100 ± 20.9	16	100 ± 41.9
0.1 µg/g	11	100 ± 28.8	22	100 ± 57.5
<0.1 µg/g	22	100 ± 57.5	22	100 ± 57.5

*AMP- Analytical measurement procedure ^ASP- Analytical sampling procedure

Limits calculation: $100\% \pm t(0.975, n-1) \times \text{RSD} \times \sqrt{(1 + 1/n)}$, $n = 7$, using Horwitz RSD values from FDA CTP 2025 guidance Table 3

$t_{0.975, n-1}$ is the two-tailed t-distribution at a 5% significance level (95% confidence) with n-1 degrees of freedom



Calibration Standard/Linearity - Anatabine

Calibration Level	Nominal Conc. (ug/mL)	ICH/Bioanalytical Guidance (n=3 days)			FDA CTP Guidance (n=5 injection on 1 day)		
		Accuracy (%)	%RSD (%)	TEP ^a	Accuracy (%)	%RSD (%)	TEP ^a
Cal1	0.025	95.2	6.76	0.092	97.5	6.99	0.0328
Cal2	0.100	103.0	0.83	0.002	101.8	2.84	0.0014
Cal3	0.250	101.5	1.86	0.007	101.5	3.62	0.0033
Cal4	0.500	100.0	6.68	0.084	99.2	2.00	0.0003
Cal5	0.999	100.9	4.40	0.039	100.2	2.93	0.0015
Cal6	2.499	98.9	5.13	0.052	99.8	2.15	0.0004
Cal7	4.997	100.2	1.08	0.002	100.0	1.26	0.0001



The **three-day design failed** TEP while the **five-injection design passed**, driven by day-to-day variability and more replicates rather than method performance

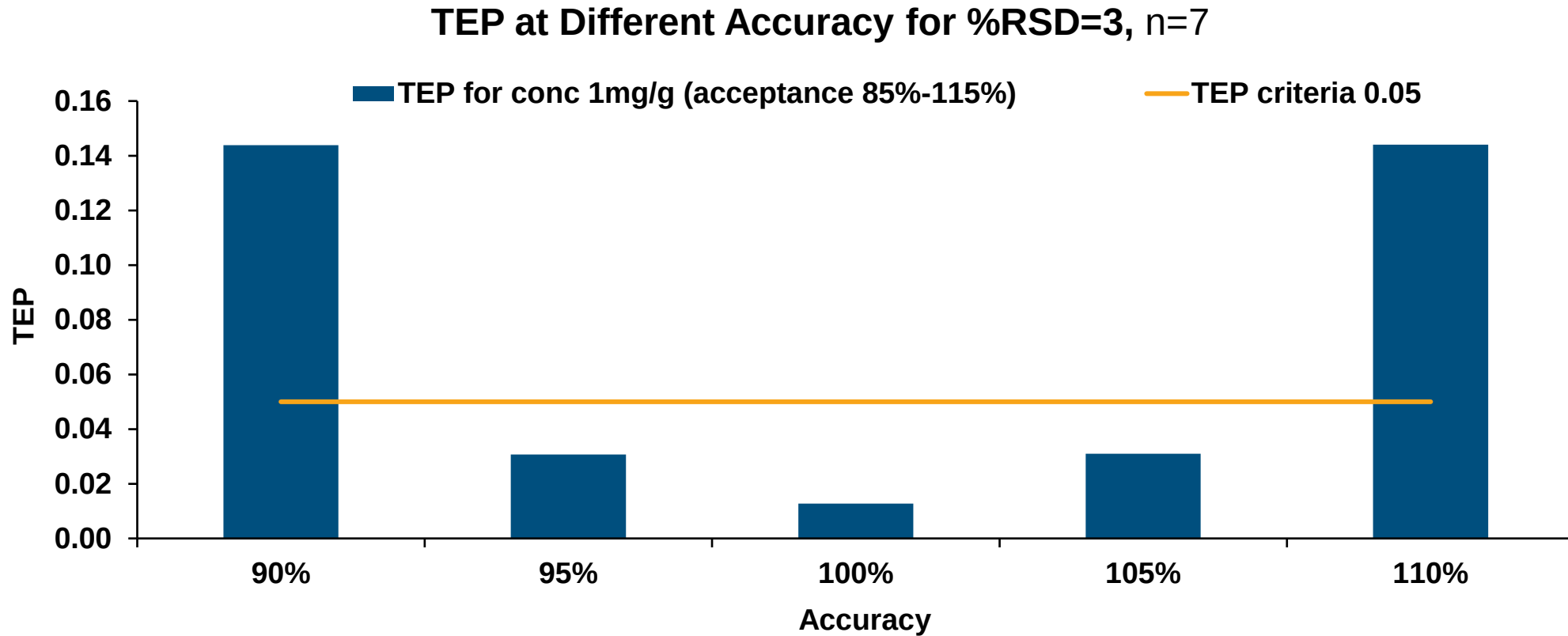
^aAcceptance 75%-125% was used for TEP calculation.



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Impact of Accuracy on TEP



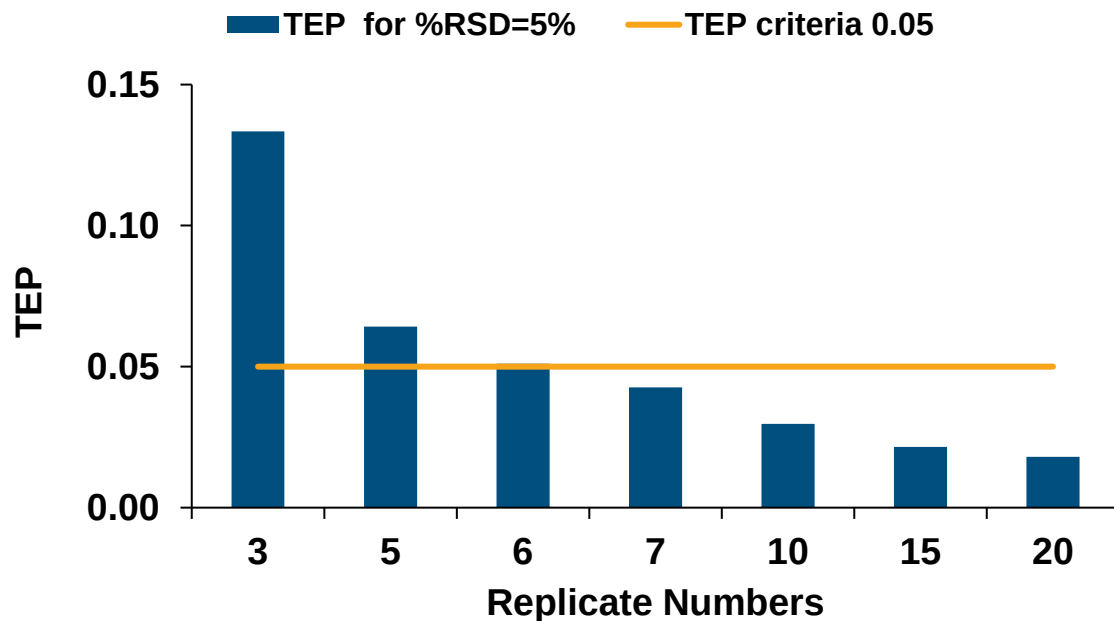
Accuracy/recovery has an impact on passing TEP and should be close to target of 100% as much as possible



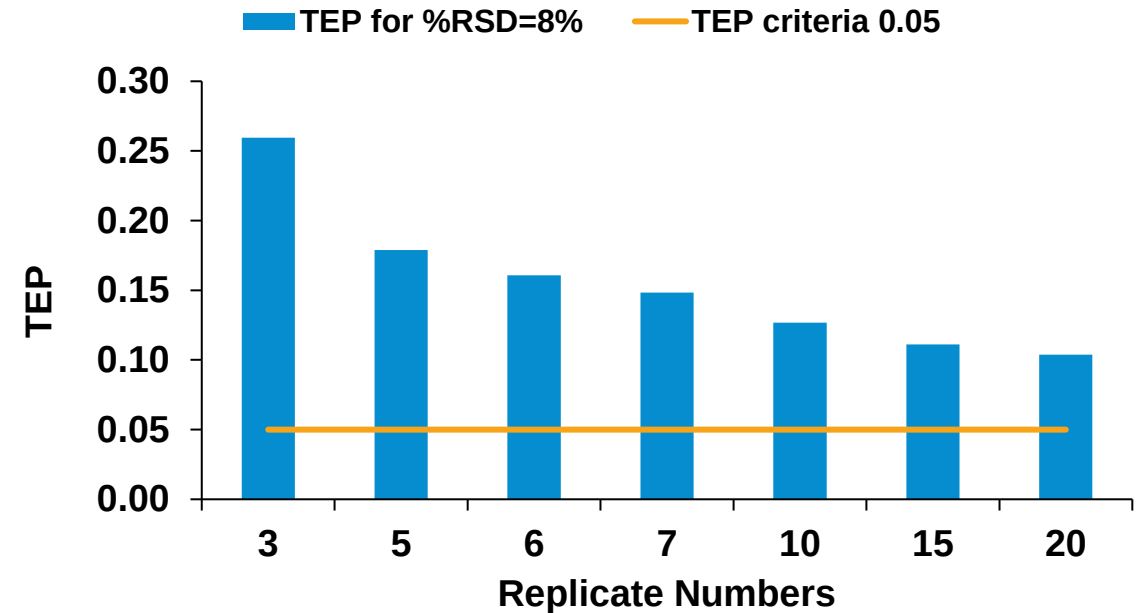
Impact of Replicate Numbers on TEP

TEP based on acceptance limit 85% to 115% and mean 103.3%

TEP for %RSD=5% With Different Replicate



TEP for %RSD=8% With Different Replicate



More replicates improve the chance of passing TEP, but a high-variability method still fails at 20 replicates



Example - Impact of Replicate Numbers on Nicotine Accuracy TEP

Accuracy for Nicotine in Pouches

Replicates	Samples	Target (mg/g)	%RSD (%)	Recovery (%)	TEP ^a (≤ 0.05)
n=3	Low fortification	7.1	1.15	94.7	0.011
	High fortification	14.1	1.64	94.1	0.074
n=6	Low fortification	7.1	1.25	94.8	0.00042
	High fortification	14.1	1.18	94.4	0.0066



Running more replicates improves likelihood of passing TEP

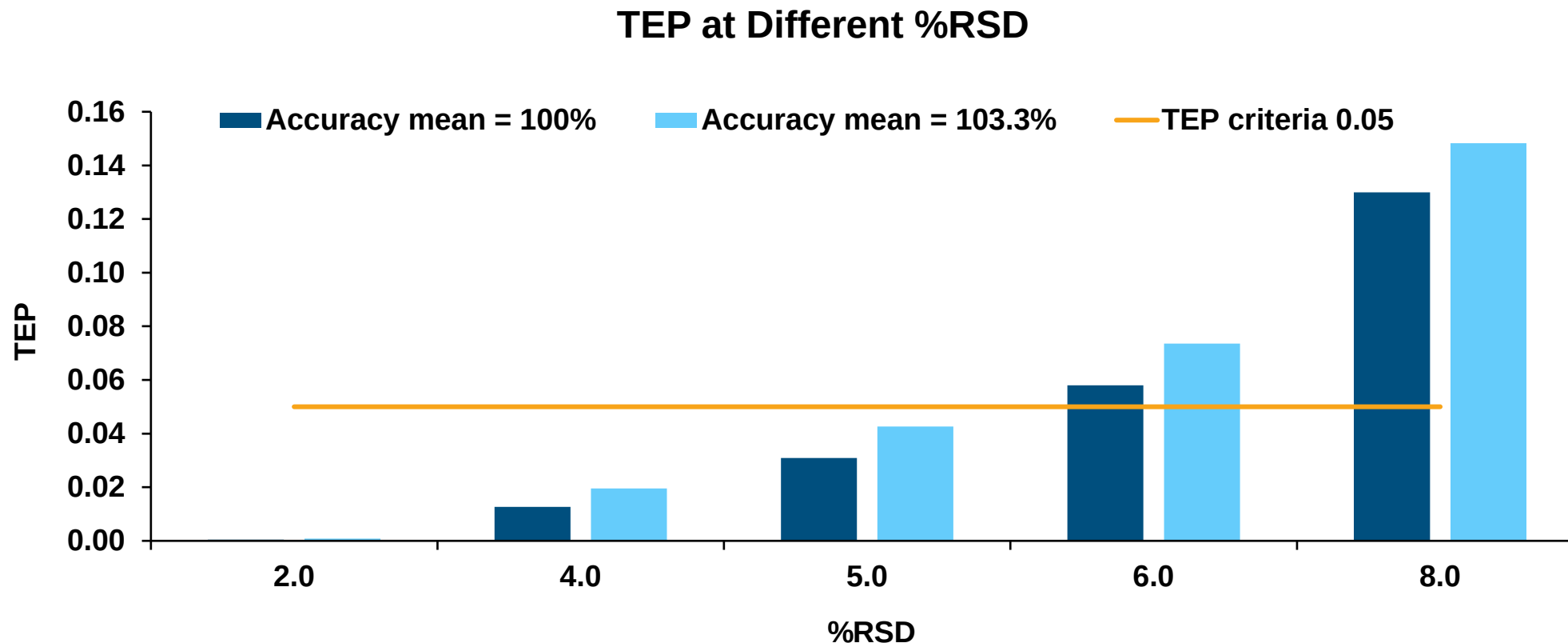
^aNicotine pouch placebo samples fortified with nicotine individually. TEP calculation based on acceptance limit 90%-110%



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Impact of Precision (%RSD) on TEP



Precision drives TEP: a shift from 5% to 6% RSD moves the result from pass to fail

TEP calculation based on acceptance limit 85%-115%, n=7

Example - Impact of Precision on Nicotine Accuracy TEP

Nicotine Accuracy for 6mg Nicotine Pouch Fortified with 1X Concentration

Sample Code	Experimental Design	Target Fortified Conc (µg/mL)	Recovery (%)	%RSD (%)	TEP ^a (≤ 0.05)
A	7 independent replicates	15.0	103.6	3.12	0.055
B	7 independent replicates	15.0	101.4	3.82	0.056
A	7 replicates from a pooled extract	15.0	102.5	2.07	0.008
B	7 replicates from a pooled extract	15.0	102.8	1.87	0.006



Independently fortified replicates failed TEP due to high %RSD from between-sample variability

^aTEP calculation based on acceptance limit 90%-110%, n=7



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Key Takeaway

FDA CTP guidance introduces new recommendations for experiment design and Total Error Probability (TEP) when compared to ICH guidance

More replicates for accuracy
(7 replicates)

More scenarios for intermediate precision
(6 scenarios)

Pooled sample solutions are specified for accuracy and precision

TEP is a new and distinct acceptance criterion which combines accuracy and precision

Methods validated following ICH guidance may not pass FDA TEP acceptance criteria because the experimental designs specified by ICH and FDA CTP are notably different





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