Technical Overview

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Agilent 6546 LC/Q-TOF: Gaining Higher Confidence and Throughput in Metabolite Analysis

Authors

Karen E. Yannell, PhD
Agilent Technologies, Inc.
Santa Clara, CA, USA

Christian Klein, PhD
Agilent Technologies, Inc.
Santa Clara, CA, USA

Nicola Zamboni, PhD
ETH Zurich
Zurich, Switzerland

Introduction

Metabolomics is constantly pushing the boundaries of complex analysis by mass spectrometry (MS). For high-throughput profiling of large sample collections, the trend is to accelerate chromatographic separation, and compensate the loss of temporal resolution with increased mass resolution and accuracy. In the most extreme form, chromatography is completely omitted to achieve thousands of injections per day with flow injection. Albeit powerful, high-throughput analysis of complex biological extracts is analytically challenging because of the wide dynamic range in concentration of metabolites in biological extracts. Whether or not chromatography is used, mass spectrometers must provide high resolution and mass accuracy over the full mass range, across many orders of dynamic range within the same scan, and at high acquisition rates.

This Technical Overview describes the capabilities of the Agilent 6546 LC/Q-TOF. The improved features of this instrument permit the analysis of a complex biological matrix with no prior separation. *E. coli* metabolome samples were prepared by ethanol extraction of bacteria growing exponentially on glucose minimal medium. These samples were analyzed by flow injection (1.5 µL) using an Infinity II pump equipped with restrictive capillary. The 6546 LC/Q-TOF was tuned in negative mode, and data were acquired at 1.5 Hz over a *m/z* 50 to 1,000 mass range. The results show resolution >30,000 for analytes greater than *m/z* 118, improved mass accuracy, large spectral dynamic range, high sensitivity, and isotopic fidelity. Ultimately, this increased instrument performance allows more features to be found, profiled, and annotated in the *E. coli* sample.

Resolution and mass accuracy

The 6546 LC/Q-TOF achieves a resolution of at least 30,000 for ions above *m/z* 118, and greater than 60,000 for larger ions (*m/z* 1,521, R = 60,000). These values were reached in complex *E. coli* extracts regardless of the peak intensity and acquisition rate (Figure 1). Mass accuracy was largely within 0.3 mDa throughout the mass range, independent of peak intensity (Figure 2). This performance allows identification of molecular formulas without prior chromatographic separation and, therefore, increased throughput.

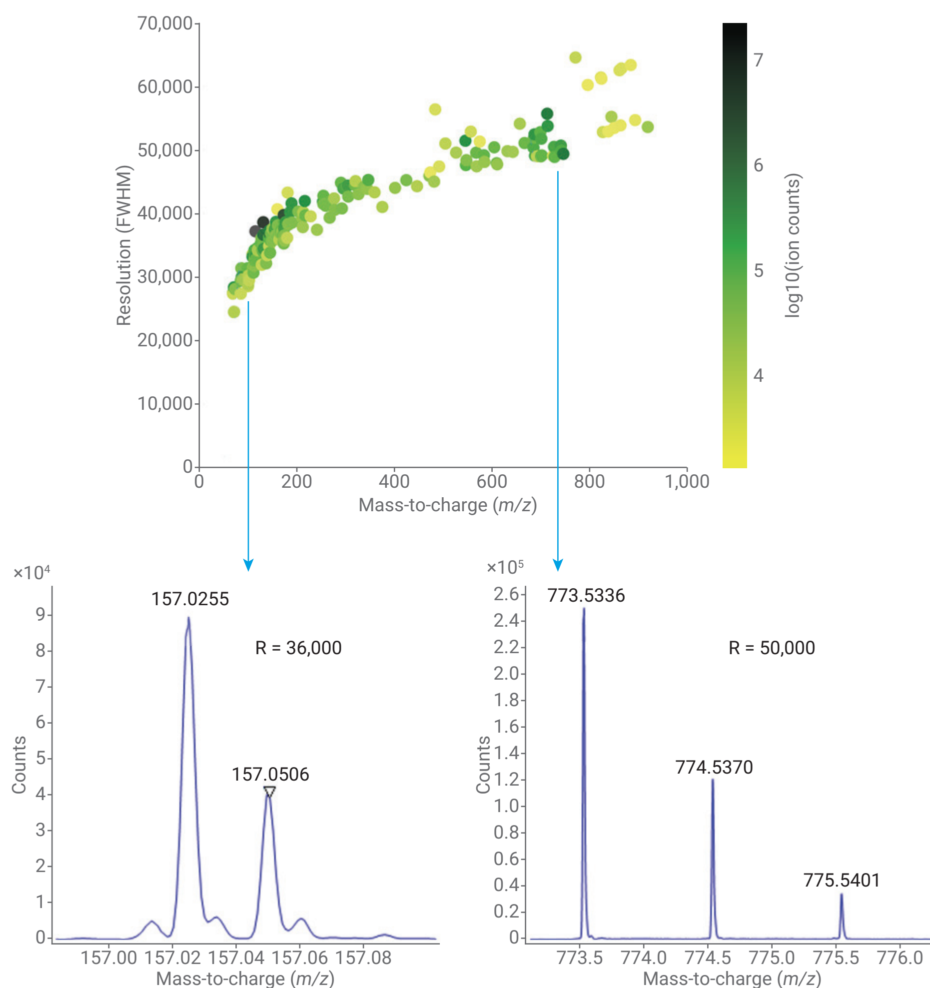

Figure 1. Resolution is plotted over the mass range for analytes in an *E. coli* sample analyzed by flow injection coupled with the 6546 LC/Q-TOF. Ion intensity is noted by color. Insets show the peak shapes for low and high mass ions.

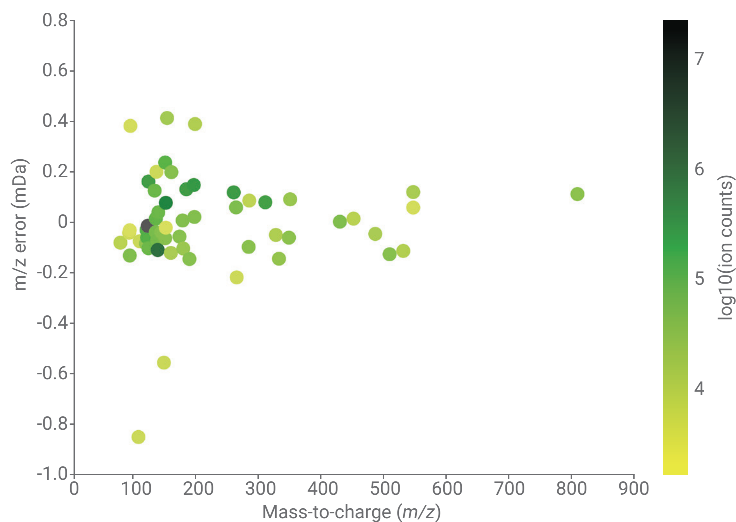

Figure 2. Mass error (mDa) is plotted over the mass range for extracted analytes in an *E. coli* sample analyzed by flow injection with the 6546 LC/Q-TOF. Ion intensity is noted by color.

Sensitivity and intrascan dynamic range

A dilution series of the *E. coli* extract was prepared in water, ranging from a 9X concentration to an 81-fold dilution. A concentration of 1X corresponds to the concentration routinely used on previous generations of Q-TOF instruments. Each sample was combined with the same amount (1X) of uniformly ^{13}C-labeled *E. coli* extract to mitigate diverging matrix effects in the recorded intensities. Triplicate injections were performed.

Analytes across the mass range were extracted, and their intensities were plotted to show the sensitivity of the 6546 LC/Q-TOF at low concentrations (or dilutions, Figure 3). The signal intensity was very stable at lower concentrations, and error only began to increase around the 81-fold dilution. In the analysis of *E. coli* extracts at the standard 1X concentration, the sensitivity of the 6546 LC/Q-TOF enabled the detection of several metabolites similar to those obtained with the Agilent 6550 iFunnel Q-TOF LC/MS (Figure 4).

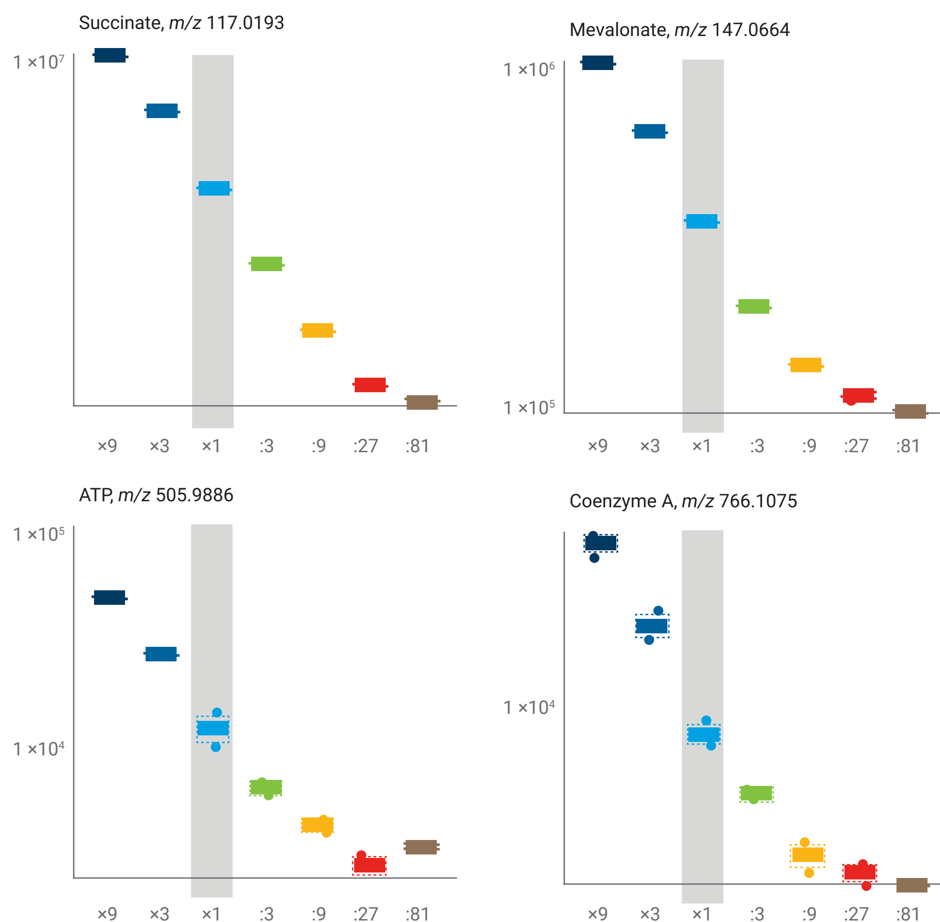

Figure 3. Ion intensity of four extracted analytes was plotted against the *E. coli* sample dilution. Error bars represent standard deviation when n = 3. The grey highlighted sample, 1X, is the standard concentration of *E. coli* samples used with earlier Q-TOF generations.

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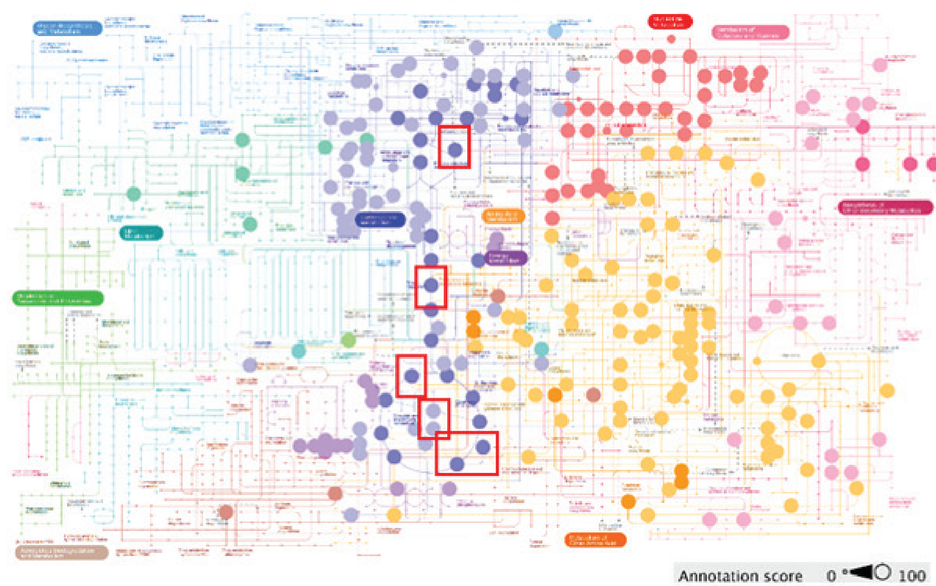

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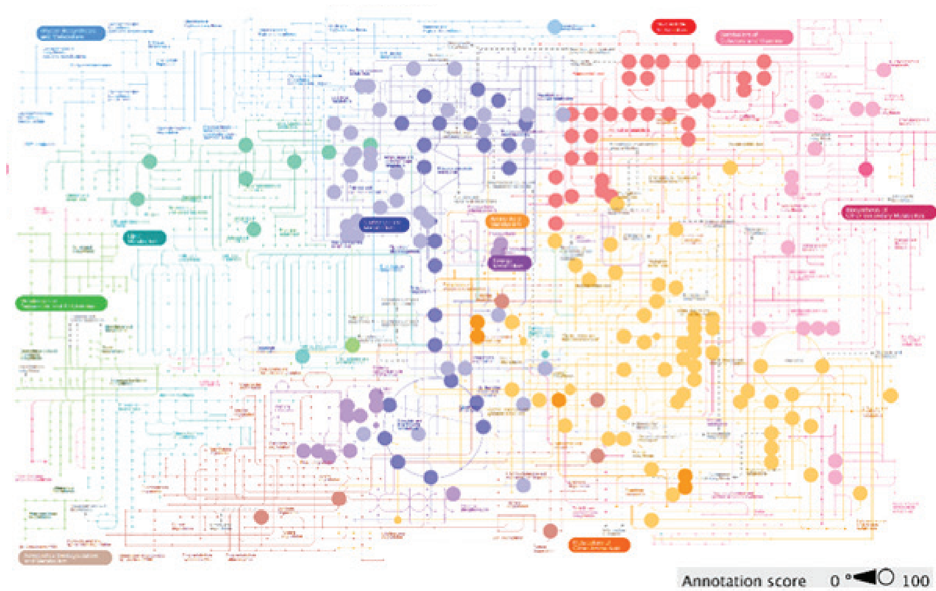

Figure 4. Metabolite map for features in the 1X *E. coli* sample when it was analyzed by the 6546 LC/Q-TOF and the 6550 iFunnel Q-TOF LC/MS. The colored circles represent features (or metabolites) detected in the sample. The 6546 LC/Q-TOF was able to identify more features (red squares) in several different metabolite classes compared to the 6550 Q-TOF LC/MS.

Additionally, the 6546 LC/Q-TOF has five orders of in spectrum dynamic range. This is more than could be explored using pure *E. coli* extracts, so the sample was spiked with $^{13}C_2$-succinate. The novel 10 GHz analog to digital (ADC) acquisition system of the 6546 LC/Q-TOF enables precise detection of peaks in a complex sample spanning nearly four orders of magnitude within the same scan (Figure 5). This allows for quantification and identification of metabolites over the full dynamic range of complex extracts. This aspect is especially important when measuring complex matrices at high throughputs with little to no sample preparation or chromatography, since these samples often have both low and high intensity analytes of interest. In summary, the 6546 LC/Q-TOF provides continuous access to a full intrascan dynamic range without compromises in resolution, accuracy, sensitivity, or scan speed.

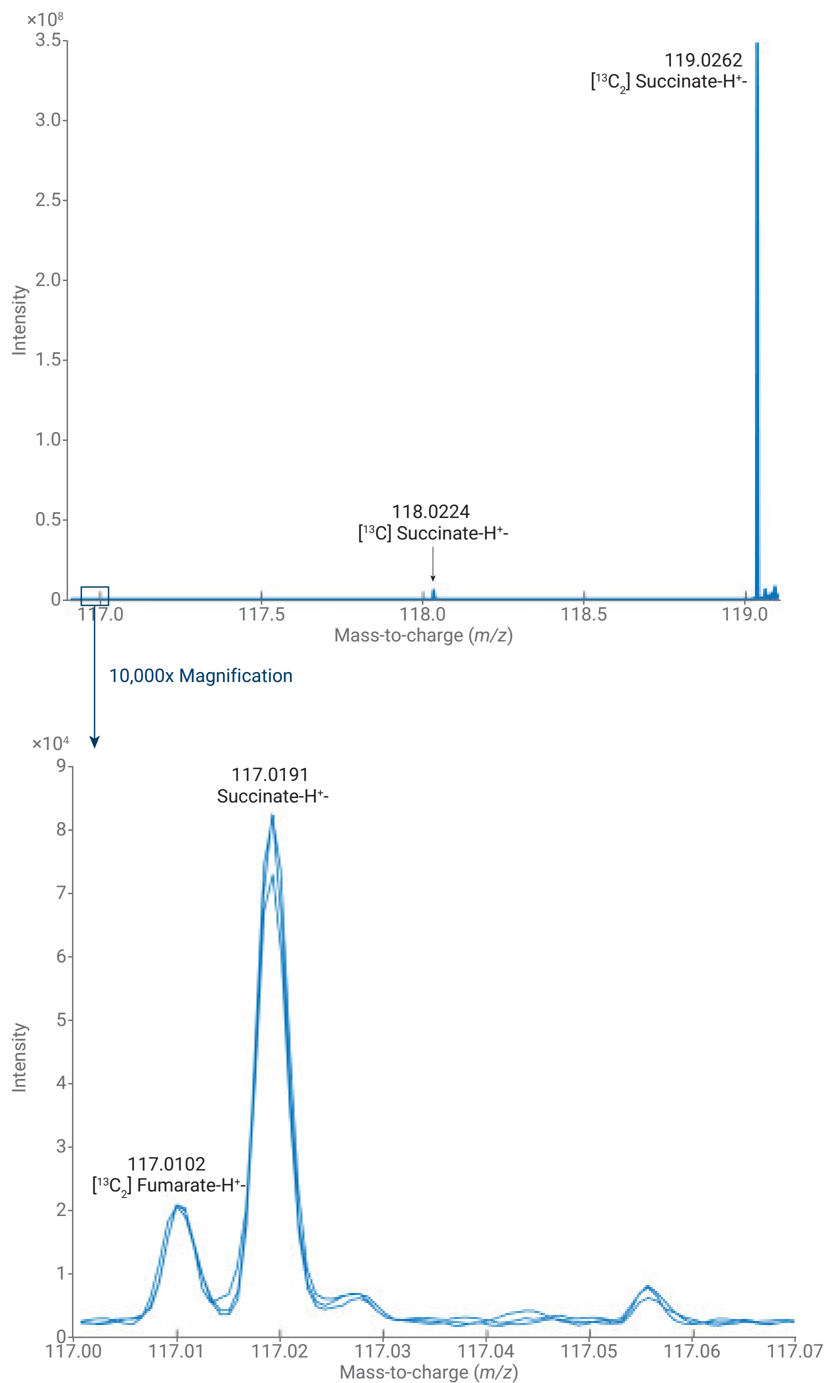

Figure 5. Intrascan dynamic range of the 6546 LC/Q-TOF. A 1X ^{13}C-enriched *E. coli* extract was spiked with $^{13}C_2$-succinate. Three technical replicates are superimposed. Despite the vast difference in intensities (10^4 to 10^8 counts), accuracy, and resolution are preserved and allow precise determination of both abundant and rare peaks within the same spectrum.

Isotopic resolution and fidelity

Further testing evaluated isotopic labeling performance of the 6546 Q-TOF LC/MS. In principle, accurate isotopic analysis depends on spectral resolution and linear dynamic range. Even in the absence of chromatographic separation, we found that the high resolution of the 6546 LC/Q-TOF enabled clear separation of most ^{13}C and ^{2}H isotopologues in crowded regions. Figure 6 illustrates this for the mass range with lowest resolution (*m/z* 100). The mass accuracy of <0.3 mDa ppm error across the whole range of masses and abundances enables prediction of molecular formulae with great confidence. For dozens of representative metabolites, the relative isotopic accuracy error (RIAE) was calculated. For most features, the RIAE was below 20 % (that is, in the range obtained with triple quadrupole detectors). Larger error was only observed for analytes with monoisotopic peaks nearing 10^4 intensity. These analytes have isotopes at or below 10^3, and therefore in the baseline. The 6546 LC/Q-TOF robustly combines high resolution and isotopic fidelity by maintaining high accuracy in quantification.

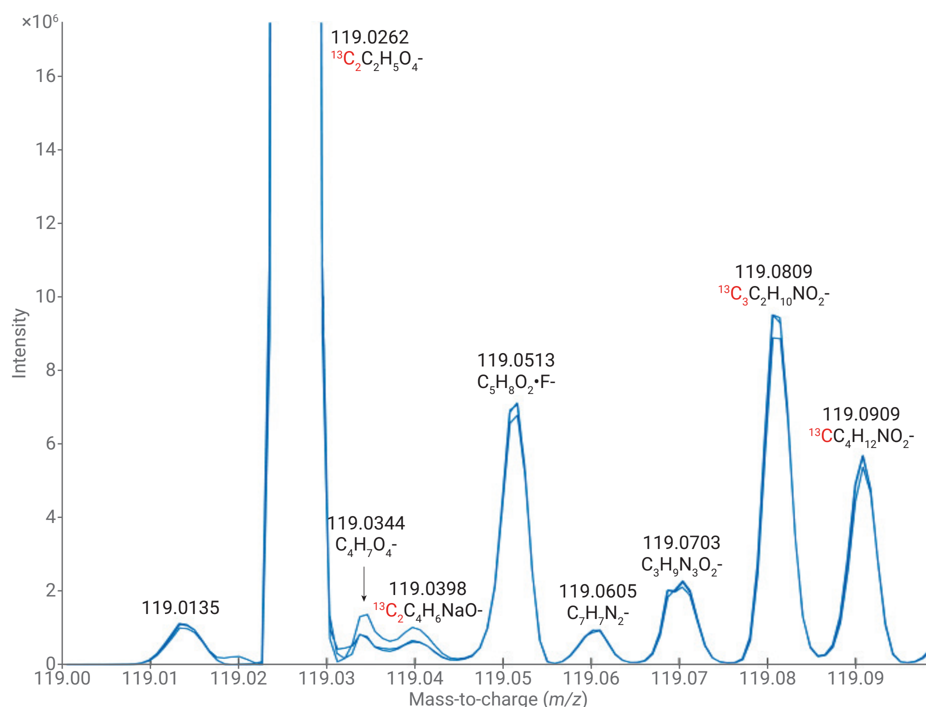

Figure 6. Spectral resolution of proximal ^{13}C and ^{2}H peaks in the low *m/z* range of an enriched *E. coli* extract measured by the 6546 LC/Q-TOF using flow injection.

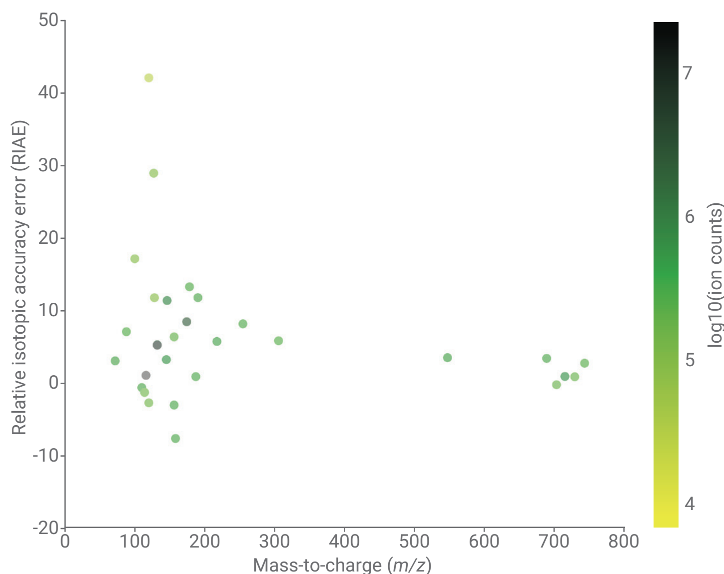

Figure 7. Accuracy of isotopic measurements. The RIAE reports the percent error for the ratio between theoretical and measured M+1/M+0. RIAE values <20 are considered excellent. Log of the sum of ion intensity over 10 scans is noted by color. Worse RIAE values are observed only for peak ions of low intensity, with M+1 ion counts of about 5 to 10 % of the monoisotopic peaks and falling in the range of the baseline.

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