Application Note

High-Speed Sensitive Analysis of Sunitinib and Related Metabolites Through UPLC-MS/MS Featuring CORTECS™ Premier C18 Columns With Maxpeak™ High Performance Surfaces (HPS) Technology

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Abstract

Tyrosine Kinase Inhibitors (TKIs) are a class of small molecule drugs used to alter cell growth pathways for the treatment of cancer. Specifically, Sunitinib, a TKI on the market, is available to treat a variety of cancers including pancreatic cancer. Here we create a method to support the therapeutic drug monitoring of Sunitinib and its active metabolite. We accomplish this by utilizing the new CORTECS Premier C_{18} Columns with MaxPeak™ (HPS) Technology combined with MS detection on the Waters™ Xevo™ TQ Absolute System. This method provides sensitive, linear, and reproducible results for the analysis of Sunitinib and its active metabolite.

Benefits

- CORTECS Premier C_{18} Columns with Max Peak offered 5x more height and area signals when compared to traditional stainless-steel systems and columns
- Chromatographic separation is achieved in under two minutes
- Dynamic sensitive linearity was accomplished across the therapeutic dosing range

Introduction

Tyrosine kinase inhibitors (TKIs) are class of small molecule pharmaceuticals that alter biological signaling pathways to modify cell growth. TKIs are applied as cancer treatments, to help prevent the growth and spread of tumors. Sunitinib is one of the many TKIs available for cancer treatment on the market.[1] Sunitinib is approved for variety of cancers, such as pancreatic cancer, in which it has increased the overall survival of patients.[2] As drugs are absorbed by the body, metabolites of the parent drug may form. In the case of Sunitinib, the main active metabolite is *N*-desethyl sunitinib and has been reported to cause toxicity in higher concentrations.[3] Therefore, methods supporting the therapeutic drug monitoring (TDM) of Sunitinib, and its metabolites must be reliable and sensitive to ensure safe and effective cancer treatments.

Given the sensitivity needed for TDM of Sunitinib, we applied the new CORTECS Premier C_{18} Columns featuring new MaxPeak HPS Technology. The MaxPeak HPS Technology has been shown to mitigate some undesirable metal and analyte interactions leading to lower responses in chromatography.[4,5] Further, MaxPeak HPS Technology has been tested on TKIs and provided positive changes in the analysis of these compounds.[6]

Here we develop a method to separate and quantify Sunitinib and its main active metabolite in under two minutes. This method covers therapeutic ranges that are documented in literature, making it applicable to the clinical sector of Sunitinib analysis. Further, we showcase the benefits CORTECS Premier C_{18} Columns featuring MaxPeak HPS Technology has on the chromatography for this method.

Experimental

Stock and standard preparations were designed based on a meta-analysis conducted for bioanalytical assays for TKIs.[7]

Stock Standard Preparation

Sunitinib was purchased from Selleck Chem (Houston, TX). *N*-Desethyl Sunitinib Hydrochloride was purchased from Sigma Aldrich (Milwaukee, WI). Sunitinib-d10 and *N*-Desthyl Sunitinib-d5 from Toronto Research Chemicals (Toronto, ON). Stocks were individually prepared at 10 µg/mL in 4 mL amber vials diluted in 100% Dimethyl-

Sulfoxide (DMSO). Any salt factors were taken into consideration during preparation. Stocks were stored at 2 °C–8 °C. Given the physical properties of DMSO, stocks solidified in cold storage. Vials were wrapped in foil and thawed slowing slowly in an incubator set at 35 °C for approximately 1 hour. Foil and dark preparation conditions were used due to the observed light sensitivity of Sunitinib.[8] Thawed stocks were allowed to equilibrate to ambient room temperature prior to standard preparation.

System Suitability Standards Preparation

Stocks were diluted using 50% Methanol in Deionized Water (diluent). Deuterated standards are individually spiked into plasma at 1% of the total volume at a 1 ng/mL concentration. The use of deuterated standards acted as an internal standard or IS for quantitative purposes for each analyte. A mixed standard containing Sunitinib and *N*-Desethyl Sunitinib in an equal concentration was prepared using concentrated intermediate stocks, that were then spiked into IS containing plasma at 4% of the total volume. Spiked plasma calibration standards underwent protein precipitation. Reactions took place in 1.5 mL centrifuge tubes using 100% Acetonitrile at 3x the volume of plasma. Precipitation reactions were vortexed for approximately 5 seconds. Then, centrifugated at 15,000 rpm and 4 °C for 5 minutes. Finally, 100 µL of the supernatant was combined with 100 µL of diluent in a low volume amber HPLC vial.

Linearity and Limit of Quantitation Standards Preparation

Stocks were diluted using 50% Methanol in Deionized Water (diluent). Deuterated standards are were individually spiked into plasma at 1% of the total volume at a 1 ng/mL concentration. The use of deuterated standards acted as an internal standard or IS for quantitative purposes for each analyte. Individual calibration curve points were prepared using concentrated intermediate stocks, that were then spiked into IS containing plasma at 4% of the total volume. Spiked plasma calibration standards underwent the same protein precipitation as described above.

LC Conditions

System set-up	Premier MaxPeak HPS	Traditional stainless-steel
LC system:	ACQUITY™ Premier LC System	ACQUITY UPLC™ I-Class System
Column(s):	CORTECS™ Premier C_{18} 2.1 × 50 mm, 1.6 µm	CORTECS™ C_{18} 2.1 × 50 mm, 1.6 µm
Vials:	Amber glass HPLC vials – 186000848 and 186002803 (low volume)	
Column temp.:	40 °C	
Sample temp.:	15 °C	
Injection volume:	1 µL	
Flow rate:	0.7 mL/min	
Mobile phase A:	10 mM ammonium formate with 0.1% formic acid	
Mobile phase B:	Acetonitrile with 0.1% formic acid	
Gradient:	*Mobile Phase B (MPB) increases from 3% to 60% over the course of 3 minutes. Then the column is washed by increasing MPB to 95% in 0.1 minutes, then holding for 0.5 minutes. The column is re-equilibrated by reducing MPB to 3% in 0.1 minutes and holding for 1.8 minutes.*	

MS Conditions

MS system:	Waters Xevo TQ Absolute
Ionization mode:	Positive ESI
Acquisition:	MRM mode, described in Table 1
Capillary voltage:	2.00
Collision energy:	5
Source temp.:	150 °C
Desolvation gas flow (L/Hr):	1000
Cone gas flow (L/Hr):	150
Collision gas flow (mL/min):	0.15
Nebulizer gas flow (L/Hr):	300

Data Management

Instrument Control:	Mass Lynx V4.2
Data Processing:	Mass Lynx V4.2

Table 1

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone voltage (V)	Collison energy (eV)	Soft ionization
Sunitinib (SUN)	399.15	283.15	18	30	Yes
Sunitinib-d10 (SUN-d10)	409.21	283.15	16	30	Yes
N-Desethyl Sunitinib (NDSUN)	371.18	283.15	12	26	Yes
N-Desethyl Sunitinib-d5 (NDSUN-d5)	376.05	283.15	10	24	Yes

Results and Discussion

Method of Separation Results

This method was reproducible in the retention and separation of Sunitinib, *N*-Desethyl Sunitinib, and related deuterated standards After 10 injections. The %RSD for area and retention time for IS corrected analytes were ≤ 5% (Table 2 and Table 3). Below, an overlay chromatogram of the 10 injections provides a clear picture of the method's performance (Figures 1a and 1b).

(Area / IS Area) response reproducibility	SUN	NDSUN
Mean	402.6	495.5
Std. dev	19.4	20.1
%RSD	4.8	4.1

Table 2. Table containing the %RSDs for the response from the TKIs mix standard.

Retention time	SUN	NDSUN
Mean	1.7	1.6
Std. dev	0.0	0.0
%RSD	0.3	0.0

Table 3. Table containing the %RSDs for the retention times from the TKIs mix standard.

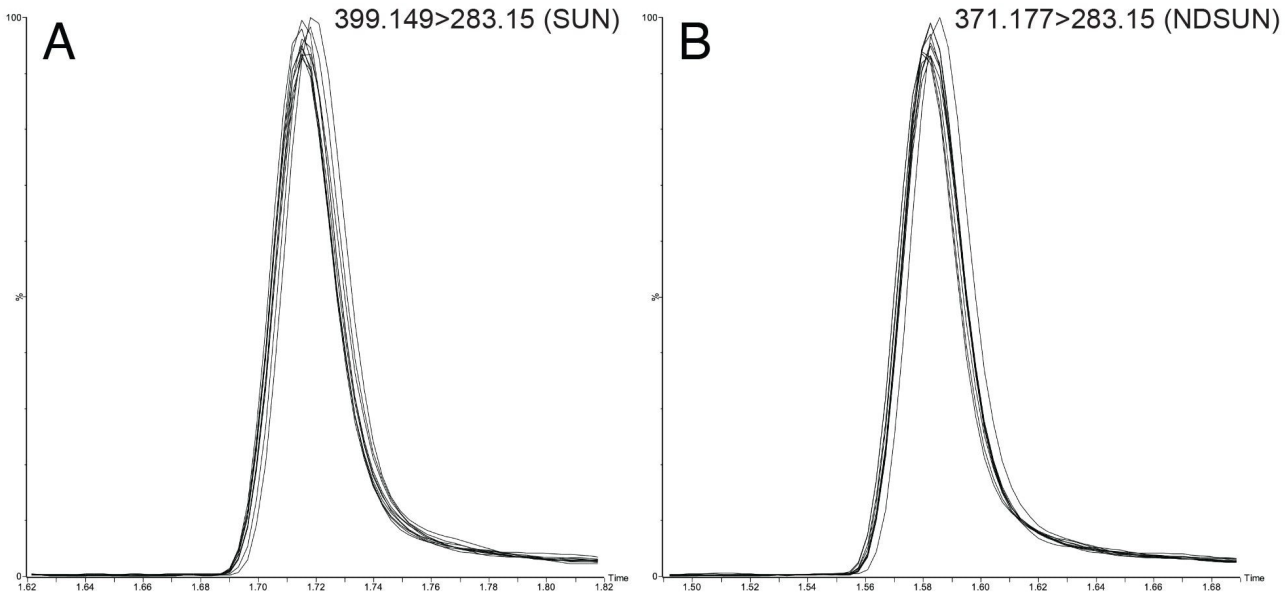

Figure 1a. An overlay chromatogram of the SUN quantitative MRM transition over the course of 10 injections for the TKIs mix standard.

Figure 1b. An overlay chromatogram of the NDSUN quantitative MRM transition over the course of 10 injections for the TKIs mix standard.

This method was compared on a stainless-steel system to highlight the improvements of CORTECS Premier C_{18} Columns with MaxPeak HPS Technology has on TKIs analysis. 10 injections of the system suitability standard were injected onto each of the instrument configurations. Below, results for both systems are compared (Tables 4 and 5, Figures 2a and 2b).

Analyte name	Retention time (min)	Analyte area signal	(Area / IS Area) response	Height signal
SUN	1.7	15757.4	402.6	583724.3
NDSUN	1.6	17571.6	495.5	718068.8

Table 4. Averages for data collected over the course of 10 injections of the TKIs standard using the MaxPeak Premier HPS System set-up.

Analyte name	Retention time (min)	Analyte area signal	(Area / IS Area) response	Height signal
SUN	1.7	3210.7	381.7	122167.7
NDSUN	1.6	3453.1	480.3	135664.2

Table 5. Averages for data collected over the course of 10 injections of the TKIs standard using the Traditional Stainless Steel system set-up.

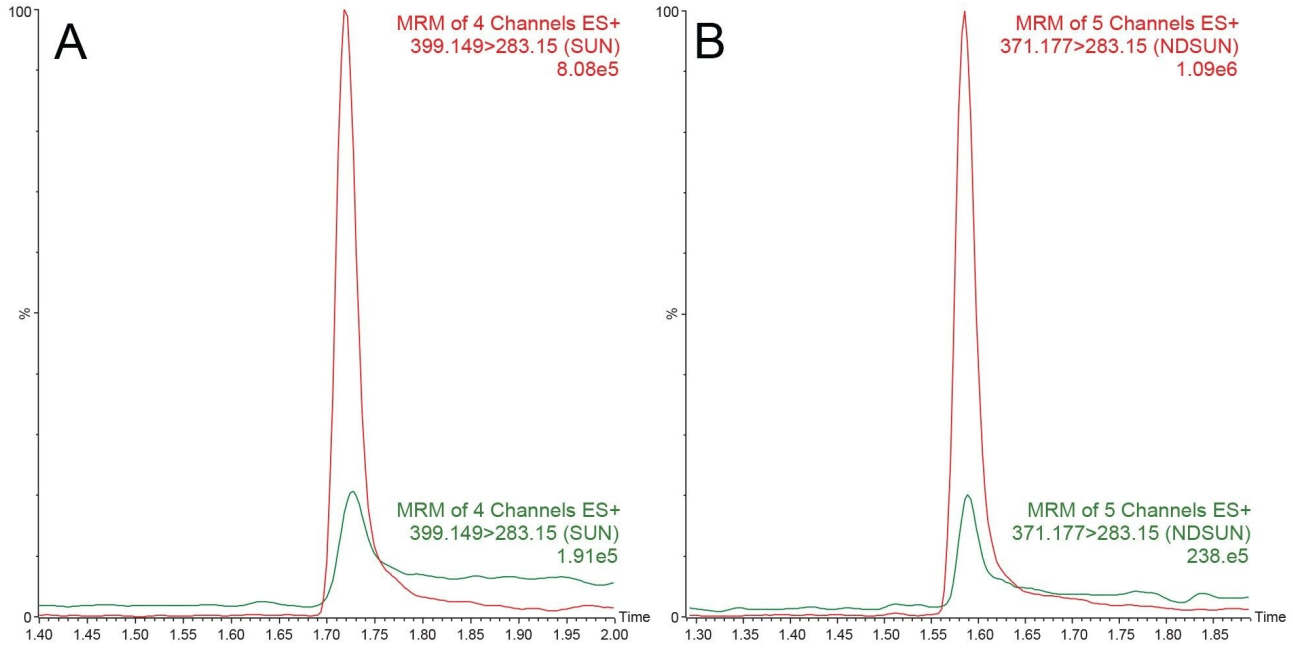

Figure 2a. An example overlay chromatogram of the SUN quantitative MRM transition for the MaxPeak Premier HPS System set-up (red) and Traditional Stainless Steel system set-up (green).

Figure 2b. An example overlay chromatogram of the NDSUN quantitative MRM transition for the MaxPeak Premier HPS System set-up (red) and Traditional Stainless Steel system set-up (green).

The MaxPeak Premier HPS Ssystem set-up offered 5x the height and area response when compared to the Traditional Stainless-Steel system set-up. This will offer advantages for TKIs testing as these increases will lead to more sensitive detection of analytes.

Linearity Results

Linearity was performed on SUN and NDSUN to demonstrate the quantitative suitability for this method. The linear range was established by referencing TDM studies done on Sunitinib. The therapeutic plasma concentration range for Sunitinib was suggested by The British Journal of Clinical Pharmacology to be from "37.5–60 ng/mL for continuous dosing and 50–80 ng/mL for intermittent dosing".[9] Therefore, we covered a plasma concentration range from 0.1 ng/mL to 100 ng/mL for both SUN and NDSUN. These ranges achieved lower quantitation limits compared to previous SUN TDM methods in literature.[10,11] The curves for SUN and NDSUN are below (Figures 3a and 3b).

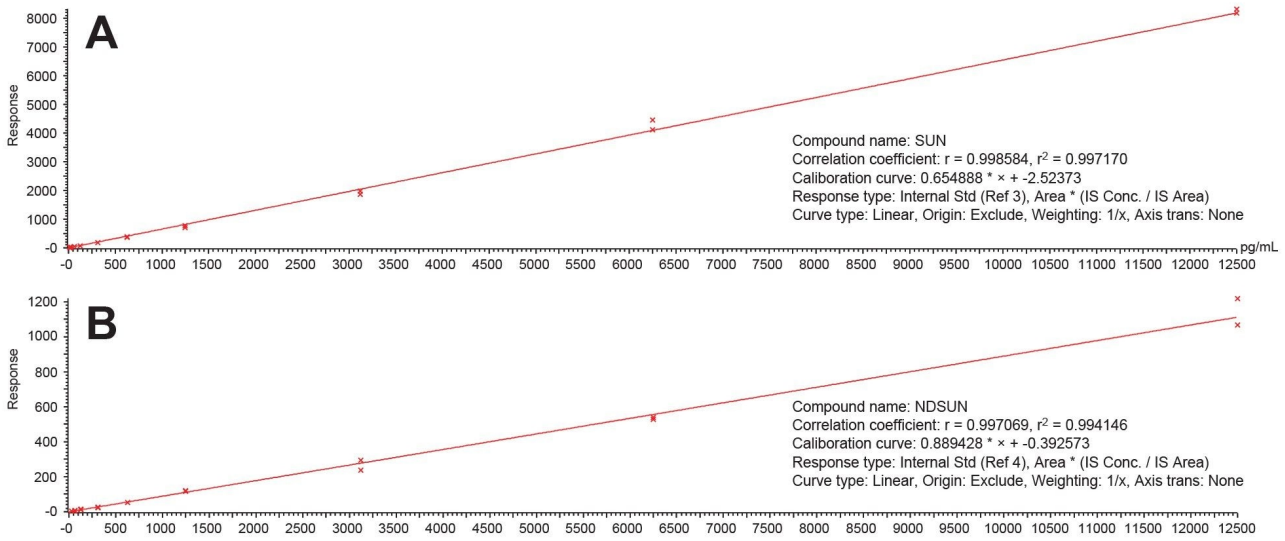

Figure 3a. Ten-point calibration curve for SUN spanning from 0.1 ng/mL to 100 ng/mL in plasma. The R^2 value for the curve was ≥0.997.

Figure 3b. Ten-point calibration curve for NDSUN spanning from 0.1 ng/mL to 100 ng/mL in plasma. The R^2 value for the curve was ≥0.994.

Limit of Quantitation Results

The lowest curve point for both SUN and NDSUN was injected 6 times to establish reproducibility of the lower level quantitative limits (LLOQs). According to the Food and Drug Administration, current bioanalytical assay validation guidelines suggest that the accuracy and precision for the LLOQ should be with in +/- 20% RSD.[12] Tables 6 and 7, and figures 4a and 4b provide a clear picture of the LLOQ results for this method.

Calculated concentration (pg/mL)	SUN	NDSUN
Mean	12.43	12.23
Std. dev	0.55	0.76
%RSD of injections	4.42	6.22
Average percent recovery theoretical concentration 12.5 pg/mL	99.43	97.87

Table 6. The accuracy results for both SUN and NDSUN. Average responses are quantified and measured against the theoretical concentration of the standard. This LLOQ represents 0.01 ng/mL SUN or NDSUN in plasma before protein precipitation and dilution for analysis. The theoretical concentration of the final SUN or NDSUN sample being injected on the column is 12.5 pg/mL, after precipitation and dilution.

(Area / IS Area) response reproducibility	SUN	NDSUN
Mean	0.7	1.0
Std. dev	0.0	0.1
%RSD	4.4	6.3

Table 7. The precision results for both SUN and NDSUN over the course of 6 injections. Both analytes fall with in 20% RSD which meets the recommended bioanalytical assay precision requirements.

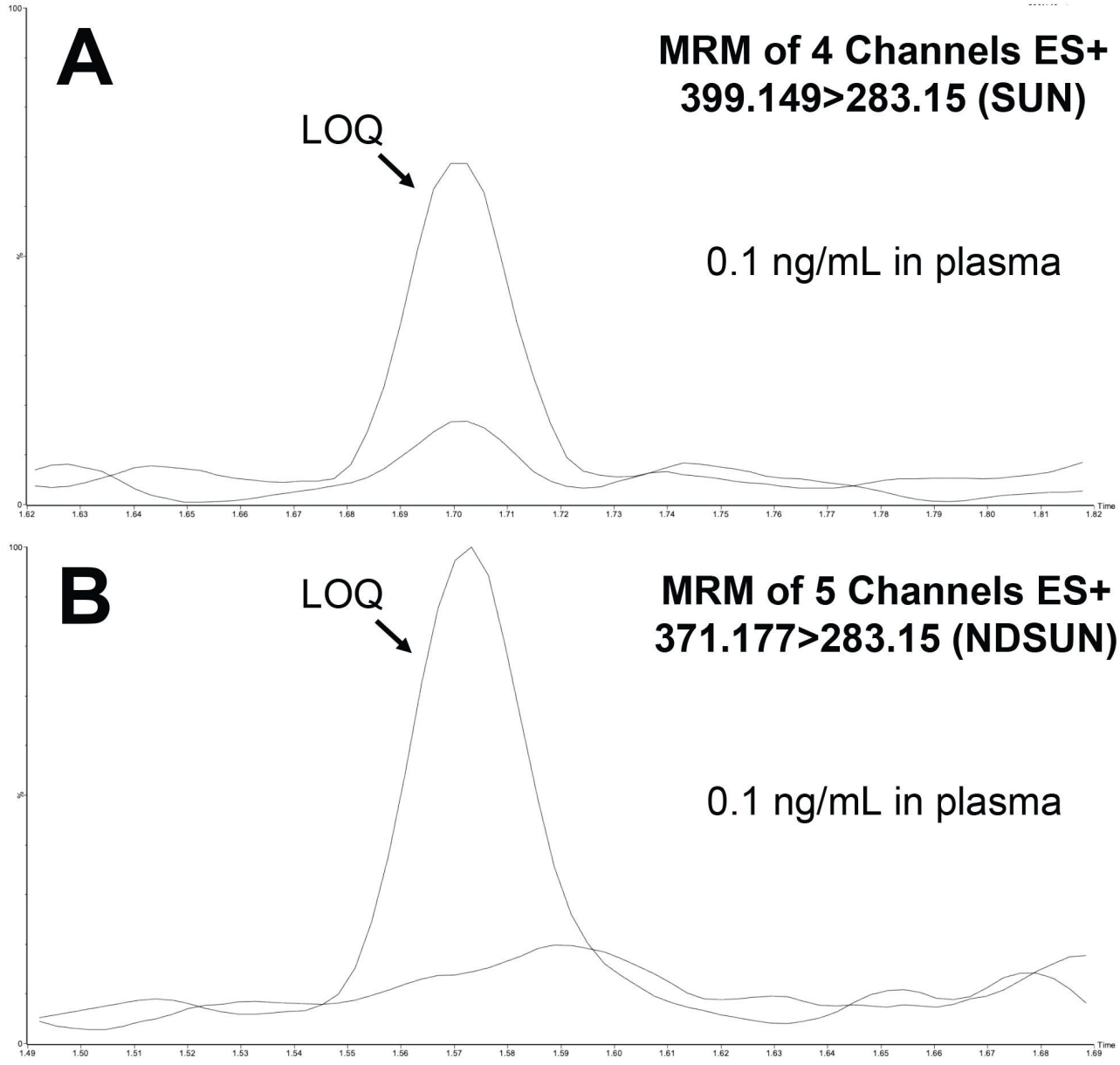

Figure 4a. An example overlay chromatogram of the SUN LOQ MRM transition compared to a blank plasma injection.

Figure 4b. An example overlay chromatogram of the NDSUN LOQ MRM transition compared to a blank plasma injection.

Conclusion

Here, the use of CORTECS Premier C_{18} Columns and Systems with MaxPeak HPS Technology improved the analysis of TKIs when compared to a traditional stainless-steel chromatography set up. Heights and areas of SUN and NDSUN increased 5-fold using MaxPeak HPS Technology. Further, this method enables for TDM across a dynamic linear range while providing reproducible results in under 2 minutes.

References

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