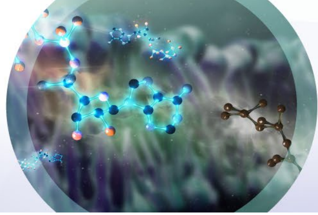

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Untargeted, quantitative metabolomics analysis on the ZenoTOF 8600 system using the novel ZT Scan DIA 2.0 workflow

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This technical note describes the simultaneous untargeted metabolite discovery and quantitative analysis of NIST standard reference material (SRM) 1950 plasma using the novel ZT SCAN DIA 2.0 scan mode on the ZenoTOF 8600 system.

Untargeted metabolomics has traditionally relied on data-dependent acquisition (DDA) scan modes on high-resolution mass spectrometers (HRMS) to provide a metabolic "snapshot" of biological samples. However, the stochastic selection of precursor ions and its reliance on MS-level quantitation limit reproducibility and accuracy. In complex biological matrices, data-independent acquisition (DIA; SWATH) provides comprehensive data sampling by generating MS/MS data of all precursor ions within a specified mass range that enables quantitation at the MS/MS level. However, precursor selection is governed by relatively large windows (>3 Da). Consequently, DIA-derived MS/MS data are frequently affected by chimeric signal contributions and co-isolated interferences, thereby reducing confidence in compound identification and quantitation, particularly for small-molecule omics analysis.

Recently, a novel sliding-window DIA approach—ZT Scan DIA 2.0—was introduced for the ZenoTOF 8600 system (Figure 1) that enables post-acquisition deconvolution to generate a narrower effective precursor ion selection window, greatly improving precursor ion specificity over traditional DIA, and yielding better coverage with accurate MS/MS-based quantitation.

Here, NIST SRM 1950 plasma was analyzed using multiple untargeted metabolomics workflows, including DDA and ZT Scan DIA, on the ZenoTOF 8600 system. The data were processed with MS-DIAL 5.6.200820-alpha to assess how coverage indices for the NIST 1950 plasma metabolome vary across analytical workflows. The results show that ZT Scan DIA delivers both broad coverage of the plasma metabolome and quantitative MS/MS data.

Key features for metabolomics analysis using the ZenoTOF 8600 system

- The high sensitivity and speed of the ZenoTOF 8600 system provide broad metabolic coverage with high confidence using the ZT Scan DIA 2.0 workflow
- ZT Scan DIA 2.0 produces MS/MS-level quantitative data, enabling a higher degree of specificity and accuracy relative to DDA
- The effective Q1 resolution of deconvoluted ZT Scan DIA data is ~ 1.7 Da, which enables post-acquisition data queries with targeted lists of metabolites

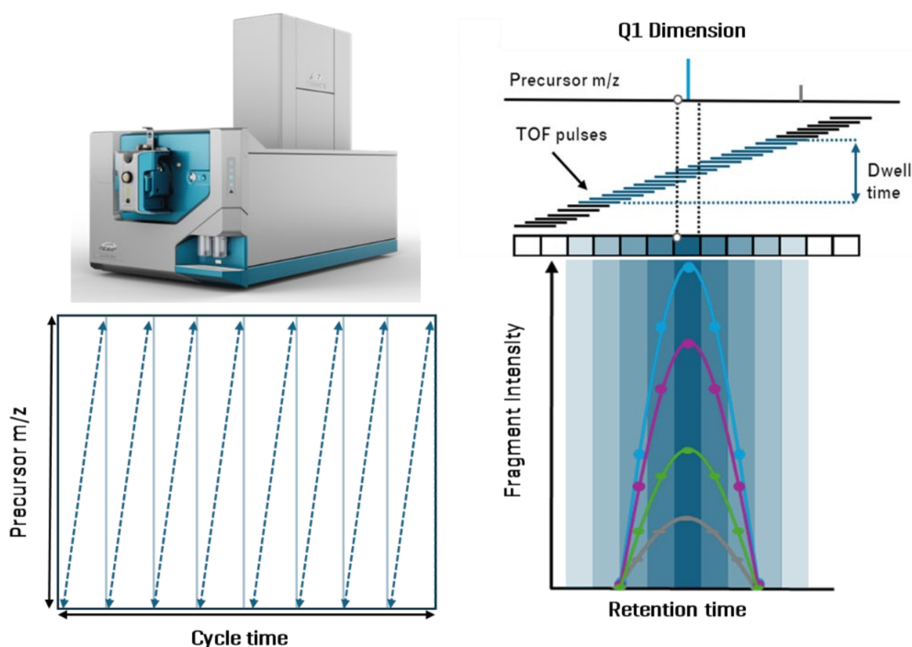

Figure 1. ZT Scan DIA analysis on the ZenoTOF 8600 system. ZT Scan DIA 2.0 is a scan mode that collects DIA omics data using a sliding Q1 window rather than using the discrete windows of traditional SWATH approaches. The data can be deconvoluted post-acquisition using MS-DIAL-5.6.082025-alpha software to extract fragment ions directly correlated with their Q1-dimension-resolved precursor ions. This scan mode yields deeper metabolomics coverage than DDA workflows, and the data are quantitative at the MS/MS level.

Introduction

Metabolomics continues to evolve as a powerful analytical strategy for understanding the chemical state of biological systems [1-3]. By enabling broad, untargeted profiling of endogenous metabolites, mass spectrometry–based metabolomics provides a qualitative and potentially quantitative view of cellular metabolism across diverse sample types. As studies move toward increasingly complex matrices, the demand for greater coverage, higher-quality MS/MS data, and precise quantitation has grown. Modern high-resolution QTOF instruments address many of these needs through improved speed, sensitivity, and linear dynamic range. However, continued advances in data acquisition strategies remain essential for improving data clarity and confidence.

Historically, untargeted metabolomics has relied primarily on DDA-based analyses for metabolite identification. DDA generates selective MS/MS spectra based on precursor ion abundance, enabling compound identification through database searching. However, its stochastic sampling limits reproducibility and MS/MS coverage, especially in complex mixtures where lower-abundance metabolites are often missed. Another drawback of the DDA workflow is that it does not quantify molecules at the MS/MS level, which compromises specificity and quantitative accuracy. These limitations have driven a shift toward the adoption of DIA strategies, which fragment all ions within defined m/z windows, yielding more comprehensive and consistent MS/MS datasets suitable for both qualitative and quantitative analysis.

The development of the Zeno trap [4], an ion accumulation and pulsing device, has ushered in a new era of sensitivity for accurate mass spectrometry (Figure 1). When activated, the Zeno trap boosts MS/MS sensitivity, delivering a reported 4-20x improvement for SWATH DIA workflows [5]. ZT Scan DIA (Zeno Trap–enabled Scanning DIA) builds on this capability by combining a continuously scanning quadrupole with Zeno-trap ion-pulsing to significantly enhance MS/MS sensitivity, thereby improving quantitative accuracy and coverage depth [6]. Unlike conventional DIA approaches, in which fragment ions may originate from multiple co-isolated precursor ions, the ZT Scan DIA workflow assigns fragments to near-unit-resolution precursor bins, thereby substantially reducing chimeric spectra. ZT Scan DIA 2.0 was specifically designed for high-confidence small-molecule analysis. (For simplicity, this workflow will be referred to generically as ZT Scan DIA throughout this report.) Unlike traditional DIA methods that rely on fixed or variable isolation windows, ZT Scan DIA uses a sliding precursor-ion selection window. This approach enables post-acquisition deconvolution to improve precursor specificity, reduce false-positive compound identification, and enhance quantitative robustness—critical advantages for small-molecule omics where iso-baric overlap and matrix interferences are common. ZT Scan DIA has previously demonstrated enhanced quantitative coverage in proteomics [6]; the experiments described in this report focus on small molecules, particularly polar metabolites.

Here, the NIST SRM 1950 plasma extract was analyzed on the ZenoTOF 8600 system to compare untargeted metabolomics using DDA and ZT Scan DIA. The NIST SRM 1950 plasma is a well-characterized biological sample commonly used for quality control in metabolomics analyses [7]. Experiments were also conducted on the ZenoTOF 7600+ system for benchmarking, but these results are not the focus of this report. The quantitative power of ZT Scan DIA is also demonstrated, enabling quantitation of metabolites at the MS/MS level and providing a digital record of the sample from which targeted compound lists can be extracted retrospectively for quantitative analysis.

Materials and methods

Sample preparation: NIST SRM 1950 plasma was extracted with 4 volumes of ice-cold methanol and vortexed for 10 s. A 1:10 volume equivalent of QReSS internal standards to plasma was added, and the sample was vortexed for 10s. The mixture was allowed to rest for 1 hr at 4 °C, vortexed again for 10 s, centrifuged at 15,000 × g for 10 min, and the supernatant was decanted. The supernatant was dried under a stream of nitrogen, and the metabolites were resuspended in water containing 0.1% formic acid to a final concentration of 1 µL of extract per 0.2 µL of plasma equivalents. The solution was directly analyzed by high-performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) using a 0.5 µL plasma-equivalent on-column injection (2.5 µL injection). The QReSS internal standards (Vial 1) were purchased from Cambridge Isotope Laboratories, and the stock solutions were prepared according to the manufacturer's instructions [8].

Chromatography: Samples were analyzed using an Exion LC system with an Acquity Premier CSH Phenyl-Hexyl column (1.7 µm, 2.1 x 100 mm; Waters). Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in methanol. A simple linear gradient from 0 to 99% B was used at a flow rate of 0.4 mL/min. The HPLC rinse solvent was water/methanol/iso-propanol/acetonitrile (1:1:1:1, v/v). The gradient conditions are shown in **Table 1**. A 2.5 µL injection was used (0.5 µL plasma equivalents), and the column temperature was maintained at 50°C. The total runtime was 19 min.

Table 1. Chromatographic gradient conditions

Time (min)	Flow rate (mL/min)	% Mobile pahse B
0	0.4	1
1	0.4	1
13	0.4	99
16	0.4	99
16.1	0.4	1
19	0.4	1

Table 2. Instrument parameter settings for DDA and ZT SCAN DIA analyses using the ZenoTOF 7600+ and 8600 systems

	Parameter	ZenoTOF 7600+ system		ZenoTOF 8600 system	
		POS	NEG	POS	NEG
	Curtain gas (CUR)	35 psi	35 psi	35 psi	35 psi
	Ion source gas 1 (GS1)	60	60	60	60
	Ion source gas 2 (GS2)	60	60	60	60
	CAD gas (CAD)	7	7	7	7
	Source temperature (TEM)	650	650	350	350
	Ion spray voltage (IS)	5500 V	-4500 V	5500 V	-4500 V
	Declustering potential (DP)	80 V	-80 V	-	-
	QJet Declustering potential (DP)	-	-	20 V	-20 V
	Collision energy (CE) TOFMS	12 V	-12 V	12 V	-12 V
	Collision energy (CE) TOFMS/MS	30 V	-30 V	35 V	-35 V
	Collison energy spread (CES)	10 V	10 V	10 V	10 V
	Zeno threshold	200,000 cps	200,000 cps	2,000,000 cps	2,000,000 cps
	TOF and TOFMS/MS start mass	50 Da	50 Da	40 Da	50 Da
	TOF and TOFMS/MS stop mass	1000 Da	1000 Da	1000 Da	1000 Da
	TOFMS accumulation time (Acc)	100 ms	100 ms	100 ms	100 ms
	TOFMS/MS accumulation time (Acc)	10 ms	10 ms	10 ms	10 ms
	Time bins to sum	6	6	6	6
DDA	DDA selection	Top 50	Top 50	Top 50	Top 50
DDA	Exclusion time criteria	6 s after 1 occurance	6 s after 1 occurance	6 s after 1 occurance	6 s after 1 occurance
DDA	Minimum TOF MS intensity	10 cps	10 cps	10 cps	10 cps
ZT Scan	Calculated accumulation time	-	-	6.7 ms	6.7 ms
ZT Scan	Scanning Q1 window	-	-	8.6 Da	8.6 Da
ZT Scan	Deconvoluted Q1 resolution	-	-	1.72 Da	1.72 Da

Mass spectrometry: Metabolomics analysis of NIST SRM 1950 plasma extracts was performed using two instruments: the ZenoTOF 7600+ and 8600 systems. Instrument calibration was maintained using the automated calibrant delivery system (CDS), which calibrated every five samples with an ESI calibration solution specific to the positive- or negative-ion mode (ZenoTOF 7600+ system) or with the new universal calibration solution for both modes (ZenoTOF 8600 system). DDA and ZT Scan DIA experiments were performed using collision-induced dissociation (CID) in the positive- and negative-ion modes. Instrument parameter settings are listed in **Table 2.**

The systems were configured for CID-based DDA experiments to select the top 50 most abundant ions for fragmentation. Dynamic background subtraction (DBS) with a mass tolerance of 50 mDa was applied to both experiments to minimize noise and maximize the MS/MS quality. Once a precursor ion was selected and fragmented, it was dynamically excluded from candidate selection for 6 s. The TOFMS accumulation time was set to 100 ms, and the accumulation time for the dependent TOF MS/MS analysis was 10 ms. A detailed description of the ZenoTOF 7600 system instrument parameters and their relevance to metabolomics DDA experiments has been previously published (9); the parameter descriptions therein apply to the ZenoTOF 7600+ and 8600 systems.

ZT SCAN DIA experiments are designed similarly to SWATH experiments in that a window size is chosen. In the SCIEX OS software, a sliding scale is provided that emphasizes coverage vs. selectivity. Choosing a narrow window size (i.e., high selectivity) improves the deconvoluted Q1 resolution, potentially at the expense of coverage. To calculate the Q1 window size that is achieved after deconvolution, divide the sliding window size by 5. In these experiments, the window was set as narrowly as possible (8.4 Da), corresponding to a Q1 window size of ~1.75 Da. Note that the duty cycle and accumulation times depend on the window size, and the method automatically calculates their effects.

To simplify data analysis in MS-DIAL software, ensure that data for each injection are saved to a separate data file. MS-DIAL software does not parse each experiment within an aggregate data file, so the data are processed as a composite, with a single results file generated. To separate individual experiments from a data file when multiple experiments have been saved under the same data file name, two tools are available to split them into separate results files: ProteoWizard (https://proteowizard.sourceforge.io/) and the SCIEX MS Data Converter 2.0.1, available at SCIEX.com. When converting, the data format output should be centroid.

Table 3. Parameter settings for MSDIAL 5.6.250820-alpha software

Section	Parameter	Setting
Measurement parameters	Ionization type	Soft ionization
	Separation type	Chromatography
	Collision type	CID/HCD
	Data Type	Centroid
	Ion mode	POS
	Target omics	Metabolomics
Data collection	MS1 Tolerance (Da)	0.01
	MS2 Tolerance (Da)	0.025
	RT begin (min)	0
	RT end (min)	18
	MS1 range begin (Da)	50
	MS1 range end (Da)	1000
	MS/MS range begin (Da)	50
	MS/MS range end (Da)	1000
	Max Number Isotopes	5
	Max Charged Number	2
	Number of threads	2
Peak detection	Min Peak Height	200
	Mass Slice Width	0.01
	Smoothing method	Linear weighted moving average
	Smoothing Level	3 (DDA); 2 (ZT Scan DIA)
	Min Peak Width	5 (DDA); 4 (ZT Scan DIA)
Spectrum deconvolution	Sigma Window Value	0.5
	MS/MS abundance cut off (amplitude)	50
	MS/MS relative abundance cut off (%)	0.05
	Exclude after precursor ions	Y
	Keep isotopic ions until: (Da)	5
	Run RT deconvolution	y
Identification	Library	MSMS-Public_all-pos-VS19
	Accurate Mass Tolerance MS1 (Da)	0.01
	Accurate Mass Tolerance MS2 (Da)	0.025
	Retention time tolerance (min)	100
	Spectrum amplitude cutoff (relative)	0
	Spectrum amplitude cutoff (absolute)	0
	Mass range begin	50
	Mass range end	1000
	Dot product score	650
	Weighted dot product score	650
	Reverse dot product score	700
	Matched spectrum percentage	25
	Minmum number of matched spectrum	3
	Use RT for scoring	No
	Use RT for filtering	No
Adduct ion	Adduct	**(+):** [M+H]+; [M+NH4]+; [M+Na]+; [M+K]+ **(-):** [M-H]-; [M-H2O]-; [M+Cl]-; [M+CH3COO]-
Alignment parameters	Reference file	Sample
	RT tolerance (min)	0.1
	MS Tolerance (Da)	0.015
	RT timefactor	0.5
	MS1 factor	0.5
	Peak count filter (%)	0
	N% detected in at least one group (%)	0
	Remove features based on blank information	Yes
	sample max / blank average (fold change)	5

Data processing: Data acquired from DDA and ZT SCAN DIA experiments were processed using MS-DIAL 5.6.082520-alpha software, which will be referred to generically as MS-DIAL 5.6 in this report. This version of MS-DIAL, available at the MS-DIAL website (https://systemsomicslab.github.io/compms/msdial/main.html), was adapted from the MS-DIAL 5.5 versions to process ZT SCAN DIA data.

The software processes DDA and SWATH data analysis as in the 5.5 versions [10,11], but MS-DIAL 5.6 can deconvolute ZT Scan DIA data to resolve the Q1 dimension and improve the correlation between the TOFMS and its corresponding TOF MS/MS spectrum. This is particularly important for small-molecule DIA data analysis, where convolved spectra make it difficult to unambiguously identify metabolites.

Optimal data processing settings for MS-DIAL 5.6 were determined through an iterative process using DDA data (**Table 3**). The same settings were applied to ZT Scan DIA data, except for 2 smoothing parameters, as indicated. The overall optimization process was governed by varying settings to achieve optimal results and maximize the number of high-quality reference-matched metabolites.

In MS-DIAL, quality scoring is governed by an algorithm that combines multiple dot-product variants (i.e., dot product, reverse dot product, and weighted dot product), mass accuracy, and retention time to generate a composite quality score [10]. For this work, we focused on metabolites with quality scores of 1.6 or higher. This value was identified as a suitable threshold for reliable compound identification through manual inspection of the data. Referencing the raw data for confirmation is an approach that should be applied to all metabolomics data, regardless of the processing software used. Here, the different dot-product scores were adjusted so that the number of reference-matched metabolites, which depends heavily on these scores, approximated the number of metabolites with quality scores ≥ 1.6.

Quantitative data processing was performed using the SCIEX OS software Analytics module. DDA data can be used for quantitation at the TOFMS level, and ZT Scan DIA data provide quantitative measurements based on the MS/MS data.

Processed metabolomics results were visualized using MS-DIAL 5.6 software and the Explore and Analytics modules of SCIEX OS, respectively.

Results

DDA analysis of NIST SRM 1950 plasma

The value and strength of untargeted metabolomics come from its ability to identify molecules in complex biological matrices. The quality of an experiment is assessed by metabolite coverage, which depends heavily on the instrument's speed, sensitivity, linear dynamic range, and the precursor ion selection criteria. The ZenoTOF series of instruments features a detector that processes signals at 133 Hz. In practice, this speed corresponds to ~ 100 MS/MS events per second while maintaining a constant mass resolution of >35K. This speed enables DDA experiments to be designed with more dependent scans, which generally translates into better coverage. Here, a Top 50 strategy was used, previously identified as optimal for the ZenoTOF 7600 system [9]. Zeno trap pulsing improves the instrument duty cycle to >90%, thereby dramatically increasing sensitivity. For the ZenoTOF 7600+ system, the instrument sensitivity is ~10X higher than that of previous-generation QTOF instruments, and the ZenoTOF 8600 system is ~ 10X more sensitive than the ZenoTOF 7600 system. Greater sensitivity enables better detection of low-abundance molecules, with the ZenoTOF 8600 system achieving the best coverage.

Table 4. Uncurated MS-DIAL 5.6 results for NIST SRM 1950 plasma data acquired using DDA analysis on the ZenoTOF 8600 system

DDA: ZenoTOF 8600 system, positive ion mode				
	Replicate 1	Replicate 2	Replicate 3	Avg
Features	20247	20720	-	**20484**
MS/MS acquired	5716	5913	-	**5815**
Suggested	4578	4557	-	**4568**
Ref. Matched	180	200	-	**190**
Quality Score > 1.6	160	176	-	**168**

DDA: ZenoTOF 8600 system, negative ion mode				
	Replicate 1	Replicate 2	Replicate 3	Avg
Features	11871	12013	11584	**11823**
MS/MS acquired	4418	4546	4304	**4423**
Suggested	2243	2243	2182	**2223**
Ref. Matched	98	105	93	**99**
Quality Score > 1.6	90	92	88	**90**

Table 5. Curated DDA results. Reference matched compounds with a quality score ≥ 1.6 were manually curated to remove duplicates, complex lipids, and phthalates

	ZenoTOF7600+ system	ZenoTOF 8600 system
Positive ion mode	114	136
Negative ion mode	26	52
Common compounds	3	7
Unique compounds	132	181

NIST SRM 1950 plasma extracts were analyzed by DDA analysis. Using MS-DIAL 5.6 software with the parameter settings listed in **Table 3**, metabolites were identified from DDA data acquired on the ZenoTOF 7600+ and 8600 systems in the positive and negative ion modes. The results are summarized in **Table 4**. The data revealed 258 reference-matched metabolites across the two ionization modes. This number, however, is potentially misleading unless the results are manually curated and confirmed. It is this truism that makes comparative metabolomics challenging, particularly between different instruments. Unless there is clear, open disclosure of how the data are acquired and processed, the absolute numbers of metabolites reported are somewhat meaningless. It is for this reason that the metabolomics and lipidomics communities do not seem to have embraced the total number of analytes identified as the final measure of a small molecule

Table 6. Curated metabolites identified by MS-DIAL 5.6 software from DDA data acquired on the ZenoTOF 8600 system (positive ion mode)

Compound	Quality score	Compound	Quality score	Compound	Quality score
L-Tryptophan	1.98780	Hippuric acid	1.88523	Benzyl-butyl-phthalate	1.73772
Arginine	1.98690	Decanoyl-L-Carnitine	1.88519	Trazodone	1.73747
Carnitine	1.97072	sn-Glycero-3-phosphocholine	1.88337	Glyceryl linolenate	1.73323
Propionylcarnitine	1.96487	Betaine	1.88312	4-Methylphthalic anhydride	1.73296
L-Tyrosine	1.95987	Tris(1-chloro-2-propyl)phosphate	1.88303	N,N-Dimethyltetradecylamine-N-oxide	1.72991
Methionine	1.95864	Theobromine	1.87682	Cortisone	1.72614
Tri(butoxyethyl)phosphate	1.95855	1-Methylnicotinamide	1.87154	N6-Threonylcarbamoyladenosine	1.72528
5-oxo-L-proline	1.95620	Oleoyl-L-Carnitine	1.86871	2-Hydroxycinnamic acid	1.72261
Cotinine	1.95606	Ethylbutanoate	1.86566	Butyryl carnitine	1.72231
Aspartic acid	1.95456	5-Aminovaleric acid betaine	1.86220	p-Octopamine	1.72049
Gabapentin	1.94905	N-Acetylgalactosamine	1.86121	Bilirubin	1.71833
Tris(2-chloroethyl)phosphate	1.94576	2-Naphthylamine	1.86026	Isatin	1.71520
Indole-3-carboxyaldehyde	1.94510	trans-3-Hydroxycotinine	1.85983	Dictamnine	1.71469
Choline	1.94278	Dimethyl sulfoxide	1.85861	Elaidic acid	1.71084
lenticin	1.94118	Cortisol	1.85499	Ergothioneine	1.70706
Creatinine	1.93707	3-Methylxanthine	1.85038	Pentaethylene glycol	1.70615
Piperine	1.93601	DEET	1.84642	Portentol	1.70096
Deoxycarnitine	1.93502	Hexanoyl-L-Carnitine	1.84624	L-Rhamnose	1.69918
Pipecolic acid	1.93421	Acetaminophen glucuronide	1.84430	4-Aminophenol	1.69274
Pantothenic acid	1.93355	3-Hydroxyanthranilic acid	1.83982	C17-Sphinganine	1.69066
Acetylcarnitine	1.93111	Stearoyl-L-Carnitine	1.83922	Biliverdin	1.68971
Kynurenine	1.92889	Prolyvaline anhydride	1.83689	Oleamide	1.68708
L-Proline	1.92883	Indole	1.83514	Brassinolide	1.68547
Isoleucine	1.92824	Octanoyl-L-carnitine	1.82908	Cyromazine	1.68445
Benzoylecgonine	1.92754	Benzyldiphenylphosphine oxide	1.82603	Linoleic acid	1.68376
1,7-Dimethylxanthine	1.92613	N,O-Desmethyltramadol	1.82148	1-Phenanthrenecarboxylic acid	1.68159
L-Phenylalanine	1.92500	epsilon-Decalactone	1.81291	2,2',2''-Nitrilotriethanol	1.68065
3-Indolepropionic acid	1.92359	2,3-dihydroxypropyl hexadecanoate	1.80995	Nicotinamide	1.67943
Trigonelline	1.92099	Cetirizine	1.80994	Acetyl tributyl citrate	1.66773
L-Pyroglutamic acid	1.91845	Indolelactic acid	1.80829	7-Oxocholesterol	1.66514
Caffeine	1.91618	Cyclo(Leu-Pro)	1.80631	gamma-Glutamylglutamine	1.66220
Hypoxanthine	1.91196	Pymetrozine	1.79997	Serine	1.66079
Triphenylphosphine oxide	1.90846	Proline betaine	1.79984	Indole-3-acetaldehyde	1.65932
2-Piperidone	1.90600	Triphenylphosphate	1.79426	Diazepam	1.65889
Naproxen	1.90574	3-Methylhistamine	1.79171	4-Pyridoxate	1.65419
Acetaminophen	1.90488	N-(2-Furoyl)glycine	1.78829	7-hydroxy-2-methyl-3-phenyl-4H-chromen-4-one	1.65303
Quetiapine	1.90399	Histidine	1.78766	1-carbamoylpyrrolidine-2-carboxylic acid	1.64762
Paracetamol	1.90349	Nordiazepam	1.78467	Lauryldiethanolamine	1.64554
Creatine	1.90339	4-Aminophenol	1.78365	17alpha-Hydroxyprogesterone	1.64361
N-(n-octyl)-2-pyrrolidinone	1.90209	Dimethyl sulfoxide	1.78267	Phenethylacetate	1.63304
Uric acid	1.90135	Quinolone	1.78168	Subersic acid	1.62702
gamma-Glutamyltyrosine	1.89975	4-Methylcoumarin	1.77257	Coumaric acid	1.62436
Taurine	1.89951	Minoxidil	1.77246	2-Methylpyrrolidine	1.61764
Indole-3-carbinol	1.89376	Linoleyl Carnitine	1.76000	Androsta-1,4-Dien-3,17-Dione	1.60824
N-Dimethyldodecylamine N-oxide	1.89087	Hexaethylene glycol	1.75428	N,N-Dimethyltetradecylamine	1.60449
Triethylcitrate	1.88677	Diphenylsulfone	1.74356	methyl 3-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-3-phenylpropanoate	1.60397

omics experiment. Rather, it is the quality of the data and the data processing that generate confidence in the experimental results. With high-quality data and transparency into the specifics of data processing, confidence in the results can also be high.

Upon close inspection, the initial DDA results obtained directly from MS-DIAL 5.6 (summarized in **Table** 4) contain duplicate entries due to inconsistent nomenclature in the compound database. The results also include complex lipids (e.g., PC, LPC, and PE) for which the extraction procedure was not optimized and were not the primary targets in these metabolomic studies. Consequently, complex lipids, phthalates, and duplicates were removed from the list of reference-matched compounds. Additionally, metabolites with quality scores below 1.6 were excluded. To further improve confidence, each qualified compound was manually inspected in MS-DIAL 5.6 and/or SCIEX OS. After curation, the initial set of 258 reference-matched compounds was reduced to 181 unique compounds (**Table 5**). These compounds are listed in **Table 6** (positive ion mode) and **Table** 7 (negative ion mode). The compound names with their respective quality scores are provided. In some cases, compound names provided by the processing software were simplified or corrected for spelling.

The same samples were analyzed by DDA analysis using the ZenoTOF 7600+ system. The results generated by these data using MS-DIAL 5.6

Table 7. Curated metabolites identified by MS-DIAL 5.6-alpha software from DDA data acquired using the ZenoTOF 8600 system in the negative ion mode

Compound	Quality score	Compound	Quality score
Indole-3-carboxyaldehyde	1.95138	Glu-Gln	1.78979
Dodecanedioic acid	1.94022	Lactic acid	1.78430
4-Nitrophenol	1.93993	Lauryl sulfate	1.78347
Tridecanedioic acid	1.93437	Bilirubin	1.77006
Glycholic acid	1.91669	2,6-Di-tert-butyl-4-nitrophenol	1.76989
L-Tyrosine	1.91505	Camphanic acid	1.76517
Indole-3-carboxyaldehyde	1.91426	Pseudouridine	1.76236
L-Histidine	1.90386	Indoxyl sulfate	1.76115
Arachidonic acid	1.90025	5-Aminovaleric acid	1.75427
12-Hydroxyoctadecanoic acid	1.89422	Phenylalanine	1.74963
glycoursodeoxycholic acid	1.88995	CMPF	1.74714
Cystine	1.88612	Glycocholic acid	1.74705
Phenylacetylglutamine	1.87371	Creatinine	1.74681
Hexahydronapthalene carboxylic acid	1.87190	Suberic acid	1.74445
Dodecylbenzenesulfonic acid	1.87008	Threonic acid	1.72633
Deoxycholate	1.86286	Taurine	1.72078
Dinoterb	1.85358	Oxypurinol	1.70659
Tetradecylsulfate	1.84984	L-Asparagine	1.69321
Catechol	1.84245	Di-n-butyl phthalate	1.67361
Glutamic acid	1.83909	Uric acid	1.66735
Azelaic acid	1.83658	L-Tryptophan	1.66249
3-Hydroxybutanoic acid	1.83645	Cytidine	1.65698
2-Naphthalenesulfonic acid	1.83422	Arabinofuranosyluracil	1.63371
N-Acetylneuraminate	1.81872	Linolenic acid	1.63368
Theophyline	1.81457	2-Hydroxybutyric acid	1.63312
FA 18:1+1O	1.80973	2-Ketogluconic?acid	1.62869
9-HODE	1.80171		

software were subjected to the same rigid standards as the data acquired on the ZenoTOF 8600 system. There were fewer curated matches (132 vs. 181; **Table 5**), which is attributed to the higher sensitivity of the ZenoTOF 8600 system and has been shown previously with the ZenoTOF 7600 system [12,13]. A comprehensive list of identified compounds using the ZenoTOF 7600+ system is not shown.

Analysis of NIST SRM 1950 plasma using ZT Scan DIA 2.0

ZT Scan DIA offers two significant advantages over the conventional DIA techniques, SWATH and Zeno SWATH. First, SWATH-based DIA requires that the collision cell be emptied between MS/MS acquisitions for each discrete DIA window, resulting in an overhead of 1-2 ms per MS/MS event [6]. As the acquisition speed increases, this cumulative overhead time requirement can reduce the overall duty cycle. In contrast, the ZT Scan DIA approach does not have this limitation, enabling higher duty cycles even at high acquisition speeds. Secondly, in conventional DIA, fragment ions can be assigned to precursors only if their retention times fall within the width of the Q1 transmission window. However, ZT Scan DIA overcomes this limitation through deconvolution, which enables more accurate correlation between the precursor ion and the MS/MS spectrum. During analysis, each fragment becomes visible when the quadrupole transmits the leading edge of the precursor ion *m/z* and disappears when the trailing edge of the quadrupole mass range passes it (**Figure 1**). Thus, fragments can

Table 8. Uncurated MS-DIAL results for NIST SRM 1950 plasma data acquired using ZT Scan DIA analysis on the ZenoTOF 8600 system

ZT Scan DIA: ZenoTOF 8600 system, positive ion mode				
	Replicate 1	Replicate 2	Replicate 3	Avg
Features	23406	23551	23732	**23563**
MS/MS acquired	23331	23457	23633	**23474**
Suggested	6414	6329	6368	**6370**
Ref. Matched	188	199	202	**196**
Quality Score > 1.6	162	172	183	**172**

ZT Scan DIA: ZenoTOF 8600 system, negative ion mode				
	Replicate 1	Replicate 2	Replicate 3	Avg
Features	8747	8798	8729	**8758**
MS/MS acquired	8713	8767	8698	**8726**
Suggested	1993	1984	1965	**1981**
Ref. Matched	107	113	107	**109**
Quality Score > 1.6	102	96	92	**97**

Table 9. Curated metabolites identified by ZT Scan DIA 2.0 analysis of NIST 1950 reference plasma analyzed using the ZenoTOF 8600 system in the positive ion mode

Compound	Quality score	Compound	Quality score	Compound	Quality score
Trimethylamine-N-oxide	1.94342	N,N-Dimethyltetradecylamine	1.81947	1-Methylxanthine	1.72262
Naproxen	1.93873	Pyrrolo[1,2-a]pyrazine-1,4-dione	1.81681	Acenapthylene	1.72059
Piperine	1.93745	Benzaldehyde	1.81537	1,4a-dimethyl-9-oxo-7-propan-2-yl-3,4,10,10a-tetrahydro-2H-phenanthrene-1-carboxylic acid	1.72039
Methionine	1.93555	Dimethylglycine	1.81271	gamma-Glutamylleucine	1.71106
Kynurenine	1.92615	Alanine betaine	1.80434	2-hydroxyquinoline	1.70795
Dimethyl sulfoxide	1.92454	N-Methylhistidine	1.80374	Acetyl tributyl citrate	1.70754
Theobromine	1.92428	Palmitoyl carnitine	1.80293	Theophyline	1.70708
Cotinine	1.92363	Pipecolic acid	1.79976	Digoxin	1.70681
Lathyrol	1.91882	Nordiazepam	1.79902	Glycerophosphocholine	1.70195
Paracetamol	1.91541	Choline	1.79649	Azacyclotridecan-2-one	1.69605
N-Methyl-L-proline	1.91032	Serine	1.79472	4-Hydroxycoumarin	1.69259
Indole-3-carbinol	1.90849	Caffeine	1.79390	Xanthine	1.69196
Indolelactic acid	1.90269	Erucamide	1.79165	Stearamide	1.69158
L-Carnitine	1.89970	Quetiapine	1.79100	Metoclopramide	1.68848
Lysine	1.89815	Propionylcarnitine	1.79061	L-Phenylalanine	1.68832
Trazodone	1.89773	Benzoylecgonine	1.78926	Tris(2-butoxyethyl) phosphate	1.68796
Cystine	1.89527	Naphthalene	1.78856	Triethylcitrate	1.68662
Trigonelline	1.88980	Laurylcarnitine	1.78643	2-Naphthylamine	1.67762
Glyceryl linolenate	1.88666	Benzyl-butyl-phthalate	1.78605	Betaine	1.67641
Cyclo(Leu-Pro)	1.88573	Tyrosine	1.78560	Glutamine	1.67577
Lenticin	1.88369	1-Methylnicotinamide	1.78516	N,N-Dimethyldodecylamine N-oxide	1.67433
4-Aminophenol	1.88331	L-Glutamic acid	1.78257	Tetradecanoyl-L-carnitine	1.67396
Citrulline	1.88147	Acetylcholine	1.78181	Linoleyl carnitine	1.67235
o-Hydroxyhippuric acid	1.87272	Octanoyl-L-Carnitine	1.78175	Enalapril	1.67224
Arginine	1.86953	Cetirizine	1.77774	Tributylamine	1.66920
N-Octyl-2-pyrrolidone	1.86944	H-gamma-glutamyl-glutamine	1.77670	gamma-Glutamyltyrosine	1.66740
Phenylacetylglutamine	1.86517	Biliverdin	1.76841	Acetaminophen glucuronide	1.66570
L-Pyroglutamic acid	1.86390	Ergothioneine	1.76810	Linoleic acid	1.66045
Triphenylphosphine oxide	1.86104	Proline betaine	1.76572	Ursodeoxycholic acid	1.65811
Atenolol	1.85509	Dibutylphthalate	1.76551	N-Acetylneuraminate	1.65524
Aminophylline	1.85314	Tris(2-chloroethyl)phosphate	1.76238	Pseudouridine	1.65383
Tryptophan	1.85296	3-Hydroxyanthranilic acid	1.76203	2-(5-methoxy-4,8,8-trimethyl-2-oxo-2,8,9,10-tetrahydropyrano[2,3-f]chromen-3-yl)-N-(pyridin-2-yl)acetamide	1.65367
Hippuric acid	1.84897	Tri-N-butyl phosphate	1.76149	Dicyclohexylurea	1.64344
Diethyltoluamide	1.84535	Ecgonine-methyl-ester	1.75781	2,3-dihydroxypropyl hexadecanoate	1.64201
Gabapentin	1.84519	Benzalkonium chloride	1.75322	Lauryldiethanolamine	1.64004
CocamidopropylBetaine	1.84194	Benzyldiphenylphosphine oxide	1.75208	Oleoyl-L-carnitine	1.63460
Triphenylphosphate	1.83897	Gentiobiose	1.75152	(3S,6R,9S,15aS)-6-Benzyl-9-isobutyl-3-[6-(2-oxiranyl)-6-oxohexyl]octahydro-2H-pyrido[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10(3H,12H)-tetrone	1.63043
3-Indolepropionic acid	1.83310	Stearoyl-L-Carnitine	1.74593	Homostachydrine	1.63036
Hypoxanthine	1.83240	Uracil	1.74006	Umbelliferone	1.62805
2-Piperidone	1.83232	Bilirubin	1.73833	Pymetrozine	1.62793
Cocaine	1.83052	Hydrocortisone	1.73561	Glycochenodeoxycholic acid	1.62573
3-Formylindole	1.82718	3-Methylxanthine	1.73496	Tris(1,3-dichlorisopropyl)phosphate	1.62345
Ethylbutanoate	1.82586	alpha-Tocopherol	1.73494	Lutein	1.62204
N2,N2-Dimethylguanosine	1.82389	Methyl trans-styryl ketone	1.73210	Glycocholic acid	1.61633
Decanoyl-L-Carnitine	1.82312	Pantothenate	1.73020	2-Aminoacetophenone	1.60941
Acetylcarnitine	1.82247	Sphinganine	1.72645		
Indole-3-acetate	1.82111	N-(9-oxodecyl)acetamide	1.72373		

be mapped to the precursors, which adds the Q1 dimension to the *m/z*, intensity, and RT dimensions. The Q1 dimension from ZT scan DIA data is used to distinguish precursor signal from interferences with a different m/z that can come from internal fragmentation and adducts, or losses occurring at low collision energy. It also helps reduce interference from co-eluting isobaric analytes, contaminants, and high background noise, which adversely affect analyte quantitation, especially at the MS level, even with very high-resolution instruments.

Table 10. Curated metabolites identified by ZT Scan DIA analysis of NIST 1950 reference plasma analyzed using the ZenoTOF 8600 system in the negative ion mode

Compound	Quality score	Compound	Quality score
Tryptophan	1.935731	3-Hydroxybutanoic acid	1.768547
Hexadecanoic acid	1.931	Cystine	1.76832
Salicylic acid	1.928367	Lichesterylic acid	1.764571
Stearic acid	1.916439	2-hydroxyquinoline	1.752601
Trans-Vaccenic acid	1.916322	Citric acid	1.749324
Chlorothalonil-4-hydroxy	1.909376	Phenylalanine	1.743752
Canrenone	1.906096	Indolelactic acid	1.742152
Naphthalene-2-sulfonic acid	1.901118	2-[(1S,2S,4aR,8aS)-1-hydroxy-4a-methyl-8-methylidene-1,2,3,4,5,6,7,8a-octahydronaphthalen-2-yl]prop-2-enoic acid	1.740755
Azelaic acid	1.900582	Aspartic acid	1.73924
Lauryl sulfate	1.887082	Pseudouridine	1.727307
Tridecanedioic acid	1.882689	Indole-3-carboxyaldehyde	1.726854
Perfluorooctanesulfonic acid	1.874859	Tyrosine	1.709689
Alpha-Hydroxydeoxycholic Acid	1.872762	N-[1.3-dihydroxyoctadec-4-en-2-yl]tetracosanamide	1.708123
4-Nitrophenol	1.870408	Taurine	1.707616
5,9-dimethyltetracyclohexadecane-5,14-dicarboxylic acid	1.869023	Dodecylbenzenesulfonic acid	1.706968
Histidine	1.867464	Threonic acid	1.706735
Indole-3-carboxaldehyde	1.865524	dehydroabietic acid	1.706528
Tetradecylsulfate	1.86119	N-[1.3-dihydroxyoctadec-4-en-2-yl]tetracos-15-enamide	1.703189
Napthalene	1.846004	Decanedioic acid	1.702468
Acetaminophen glucuronide	1.844914	Bilirubin	1.699624
Dodecanedioic acid	1.838354	Urinc acid	1.694174
Catechol	1.83698	Oxypurinol	1.692515
Phenylacetylglutamine	1.836841	Lactic acid	1.689006
Palmitic Acid	1.831405	Glycochenodeoxycholic acid	1.67148
Indoxyl sulfate	1.825944	Glycholic acid	1.66626
Dinoterb	1.824187	Triptophenolide	1.665963
12-Hydroxyoctadecanoic acid	1.811896	3-(4-Hydroxyphenyl)lactic acid	1.661717
Cholic Acid	1.809648	Leucine	1.661566
Lupulone	1.805465	Methylmalonate	1.659512
Glu-Gln	1.795578	FA 18:1+3O	1.650745
Glycocholic Acid	1.79484	5-Chloroorsellinic acid	1.646829
Glucose	1.794203	N-[1.3-dihydroxyoctadec-4-en-2-yl]docosanamide	1.645656
4-Hydroxybenzaldehyde	1.792314	2'-Hydroxy-4'-methoxychalcone	1.633795
Glutamine	1.790543	Bezyl alcohol	1.618628
Theophyline	1.786699	2,4-Dihydroxybenzophenone	1.616581
Acetaminophen glucuronide	1.774337	Sulfoursodeoxycholic acid	1.613757
Biliverdin	1.774149		

The same NIST SRM 1950 plasma extracts used for the DDA experiments were analyzed by ZT Scan DIA using the ZenoTOF 8600 system. The acquired data were processed using the same software parameter settings as for DDA, except for 2 of the smoothing parameters, which were slightly lower for ZT Scan data analysis. This change was made to improve peak detection, particularly for low-abundance analytes that may have relatively fewer points across the peak. **Table 8** shows the uncurated results from MS-DIAL 5.6 for the ZT Scan DIA analysis of NIST SRM 1950 plasma on the ZenoTOF 8600 system. The data show a modest increase in the identification of polar metabolites compared to DDA. Curation of the reference-matched compounds (**Tables 9 and 10**) resulted in the identification of 199 unique compounds (139 in the positive ion mode, 73 in the negative ion mode, with 13 common to both). While the increase in curated metabolite coverage with ZT Scan DIA was modest (~10%), the primary

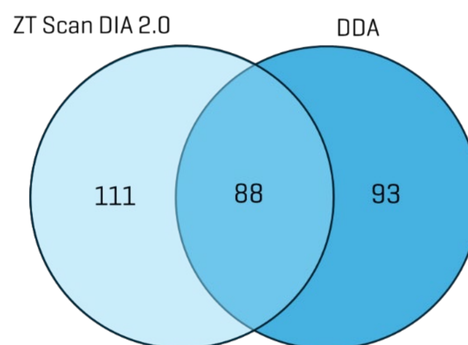

Figure 2. Venn diagram showing the differential coverage of the NIST 1950 metabolome using ZT Scan DIA versus DDA. Of the 199 curated metabolites identified with ZT Scan DIA, 88 are shared with DDA, which represents approximately 56% of the unique compounds. DDA identified about 51% of the unique compounds.

advantage lies in the improved specificity of MS/MS spectra and the ability to perform quantitative analysis directly at the fragment-ion level. Interestingly, a comparison of the curated results obtained with DDA vs. ZT Scan DIA revealed that both methods identified a significant number of unique compounds (**Figure 2**). The two methods identified 88 compounds in common, but ~50% were unique to each method.

Metabolomics research is driven by data quality, which directly affects results obtained from untargeted metabolomics approaches. The challenge with traditional DIA approaches has been the convolution of chimeric spectra that are acquired within each SWATH window. The narrowest window possible on traditional SWATH is 3 Da, which has proven to be too wide for compound specificity in metabolomics analyses. In lipidomics, this relatively large width prevents effective use of SWATH unless a DDA experiment is used to align the data [14]. Using the sliding Q1 window in ZT Scan DIA analysis, the chimeric spectra arising from isobars and other interferences can be removed by the Q1 deconvolution algorithm in MS-DIAL 5.6 (**Figures 3 and 4**). In the positive ion mode (**Figure 3**), two curated molecules were selected: glutamic acid (left panels) and cetirizine (right panels). Listed for each compound are the quality score components, including the accurate mass similarity score and the different variations of the dot product similarity scores, which are summarized by the total quality score (shown as total similarity score). The middle series of panels for each compound shows the measured (left) and deconvoluted (right)

Name:	**Glutamic acid**
Accurate mass:	148.06013
Accurate mass similarity score:	0.9995
Weighted dot product similarity score:	0.8084
Dot product similarity score:	0.8563
Reverse dot product similarity score:	0.9212
Total similarity score:	**1.7826**

Name:	**Cetirizine**
Accurate mass:	389.16212
Accurate mass similarity score:	0.9986
Weighted dot product similarity score:	0.7982
Dot product similarity score:	0.8493
Reverse dot product similarity score:	0.8493
Total similarity score:	**1.7777**

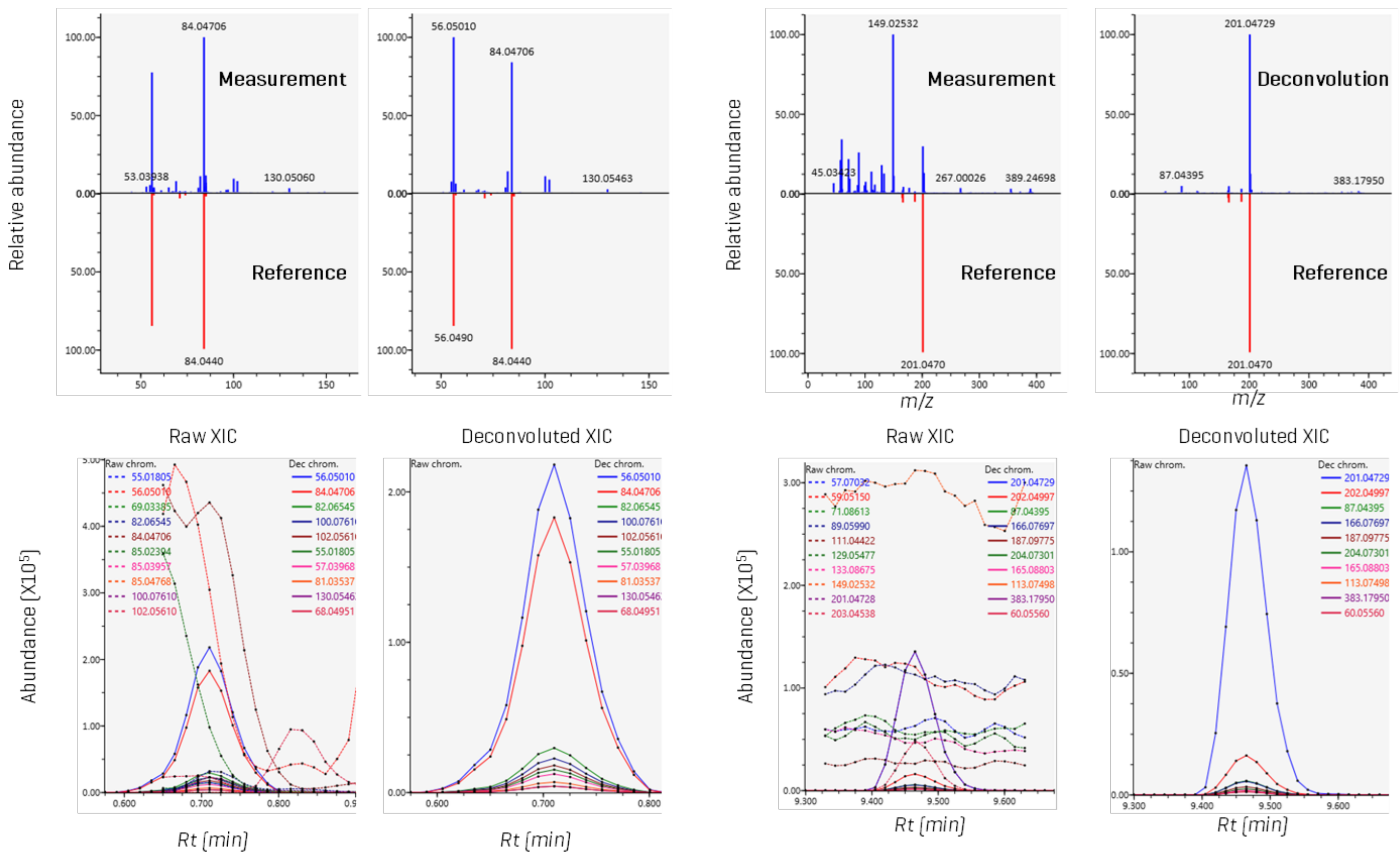

Figure 3. Deconvolution of ZT Scan DIA data (positive ion mode). Glutamic acid (left panels) and cetirizine (right panels) were identified using MS-DIAL 5.6 software. The quality score components and the resulting similarity scores for each analyte indicate a strong correlation between the deconvoluted data and the reference spectra (see the middle-left panel for each analyte). The deconvoluted XICs display clear sets of fragment ion peaks that can be used for quantitation. Data that is not deconvoluted shows clear interferences that could lead to false-positive identification and negatively impact quantitation.

Name:	Biliverdin
Accurate mass [M-H]-:	581.23818
Accurate mass similarity score:	0.9796
Weighted dot product similarity score:	0.8229
Dot product similarity score:	0.8738
Reverse dot product similarity score:	0.8990
Total similarity score:	**1.7741**

Name:	Tyrosine
Accurate mass [M-H]-:	180.06579
Accurate mass similarity score:	0.9966
Weighted dot product similarity score:	0.8709
Dot product similarity score:	0.7341
Reverse dot product similarity score:	0.8516
Total similarity score:	**1.7097**

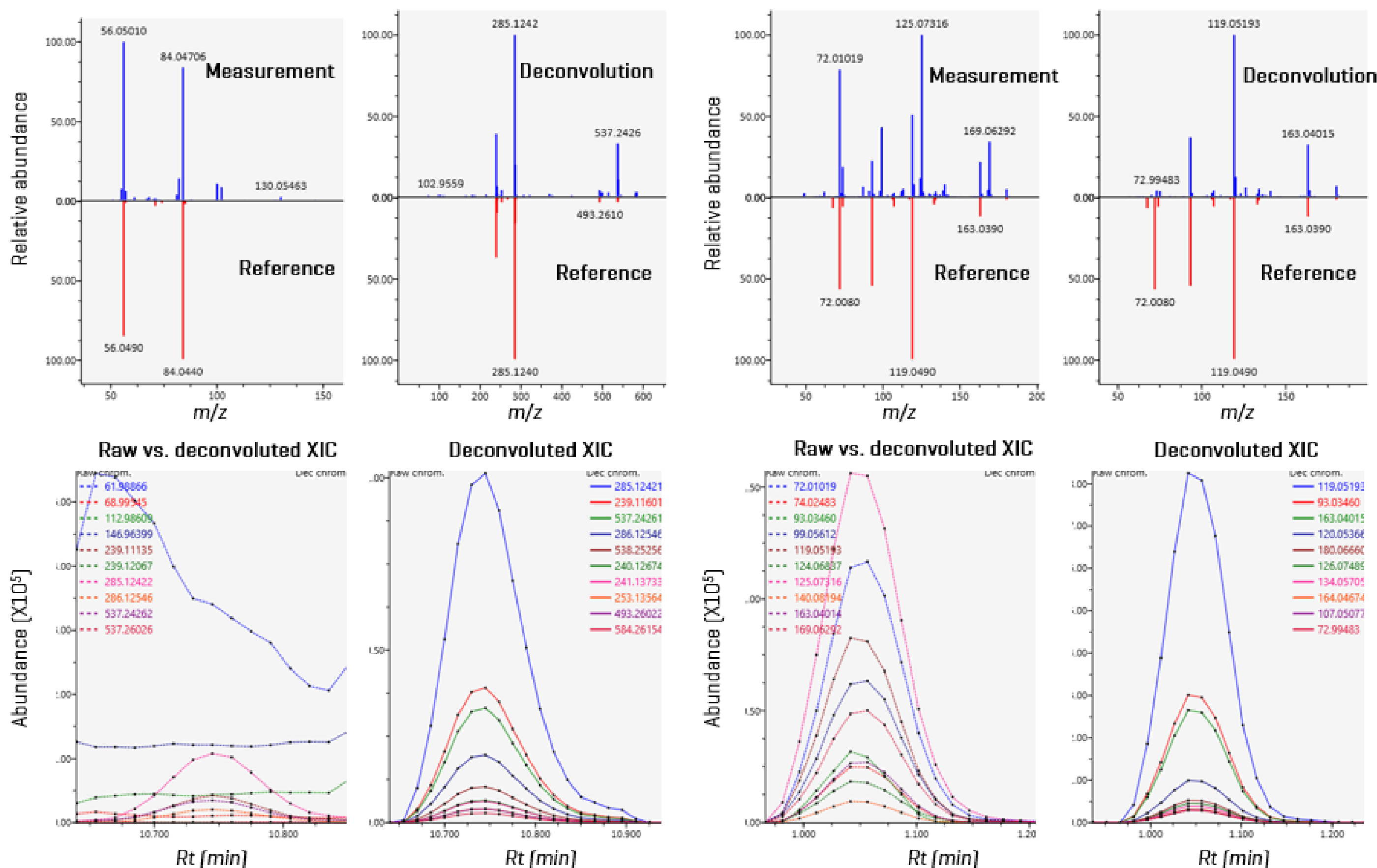

Figure 4. Deconvolution of ZT Scan DIA data (negative ion mode). Biliverdin (left panels) and tyrosine (left panels) were identified by MS-DIAL 5.6 software. The quality score components and the resultant similarity scores for each analyte show a strong correlation between the deconvoluted data and the reference spectra (middle-left panel for each analyte). Deconvoluted XICs show a clear set of fragment ion peaks that can be used for quantitation. The data that is not deconvoluted show clear interferences that could trigger false positive identification and adversely affect quantitation.

spectra. The deconvolution spectra are cleaner, with a significant reduction in chimeric spectral peaks. The reference spectra (red inverted overlay) are shown for comparison. The bottom row of panels shows the raw vs. deconvoluted XIC for each molecule. The chimeric overlap from interfering compounds is more apparent in these panels. The XIC panels for glutamic acid show interference from coeluting species, and the cetirizine XIC panels show a good example of matrix interference (dotted tracings with no apparent peak). In both cases, the Q1 and retention time deconvolution algorithms generate clear spectra that match well to the reference spectra, as indicated by their quality scores,

A similar figure is shown for the ZT Scan DIA analysis in negative ion mode (**Figure 4**), with the compounds biliverdin and tyrosine highlighted. As shown in the positive ion mode, the panels demonstrate the power of Q1 deconvolution to improve the quality of metabolomics data and subsequent identification.

An overall summary of the DDA- and ZT Scan DIA-based analyses is presented in **Table 11.** The greatest difference in the number of curated, reference-matched compounds across the experiments was between the DDA experiments run on the ZenoTOF 7600+ and 8600 systems. The improved coverage of the ZenoTOF 8600 system is attributed to its higher sensitivity [12,13]. The results from DDA and ZT Scan DIA runs on the ZenoTOF 8600 system were similar, with a

Table 11. Overall summary of DDA vs. ZT Scan DIA analyses of NIST 1950 reference plasma using the ZenoTOF 8600 system

	ZenoTOF 7600+ system	ZenoTOF 8600 system	ZenoTOF 8600 system
	DDA		ZT Scan DIA
Positive ion mode	114	136	139
Negative ion mode	26	52	73
Common compounds	3	7	13
Unique compounds	132	181	199

modest improvement for the DIA approach. The greatest difference, however, lies in the ability to directly use ZT Scan DIA data for quantitative analysis at the MS/MS level, a workflow not possible with DDA-based data.

Quantitative analysis of metabolites derived from ZT Scan DIA data

The traditional discovery experimental workflow for metabolomics, DDA, is effective at identification, provided there is an adequate library against which to search, but it falls short in providing highly specific and accurate quantitative information about the molecules. Due to the stochastic nature of DDA precursor targeting, an ideal experiment acquires 1-2 spectra per compound. This is appropriate for compound coverage but does not provide quantitative data at the MS/MS level. In DDA workflows, quantitation is typically performed at the TOFMS level, where co-eluting and isobaric species contribute to elevated baselines and chimeric signals, reducing signal-to-noise and specificity. Quantitation using product ions is more compound-specific than TOF MS data alone, regardless of instrument resolution, because of the high isobaric overlap in small-molecule omics experiments. ZT Scan DIA data provide both types of information: qualitative for compound identification and quantitative at the MS/MS level for specific and accurate quantitation.

A visual comparison of the differences between quantitative analysis of compounds using DDA (TOFMS level) and ZT Scan DIA (TOF MS/MS level) is shown for pseudouridine (Figure 5). The left panel displays the XIC from a survey TOFMS scan, which is typically used for quantitation in DDA experiments. The baseline is high, indicated by the low signal-to-noise ratio (S/N), and several other peaks co-isolate within the 10 mDa mass window of pseudouridine. The right panel shows the XIC from ZT Scan DIA MS/MS data, with only one major peak, exhibiting much higher S/N than the TOFMS XIC.

To test the ability of ZT Scan DIA to quantify compounds, a target list was generated for the QReSS standards added during plasma extraction. For this demonstration, the positive ion mode ZT Scan DIA data were used. In the Analytics module of SCIEX OS software, the accurate precursor and fragment masses were entered into the target list, and the data were processed as usual to generate a results file, summarized in **Table 12**. The compounds extracted from the ZT Scan DIA data were confirmed by precursor and product ion masses and their retention times in a separate MRMHR (high-resolution MRM) experiment with neat standards on the ZenoTOF 7600+ system (data not shown). The average of 3 replicate injections yielded % CV values < 20%. The integrated peaks for each compound are shown in **Figure 6**.

Although this report presents an untargeted metabolomics analysis, the ability to query ZT Scan DIA data post-acquisition makes this workflow targeted as well. The data file collected during analysis is essentially a digital record of the sample, containing all precursor ions within the specified mass range, along with their respective MS/MS spectra. As shown in **Table 8**, the number of features is very close to the number of MS/MS spectra acquired. Due to the sliding Q1 window used in analysis, the effective width of Q1 is 1.75 Da, which is significantly smaller than the widths of the fixed or variable windows used in traditional SWATH. This makes it much easier to correlate a precursor ion with its MS/MS spectrum. As technology evolves, the potential for an even smaller window at or below unit-mass resolution could eliminate the need to run dedicated MRM^{HR} experiments in a targeted assay. That data would already have been acquired during the ZT Scan DIA analysis and would be available for retrospective data

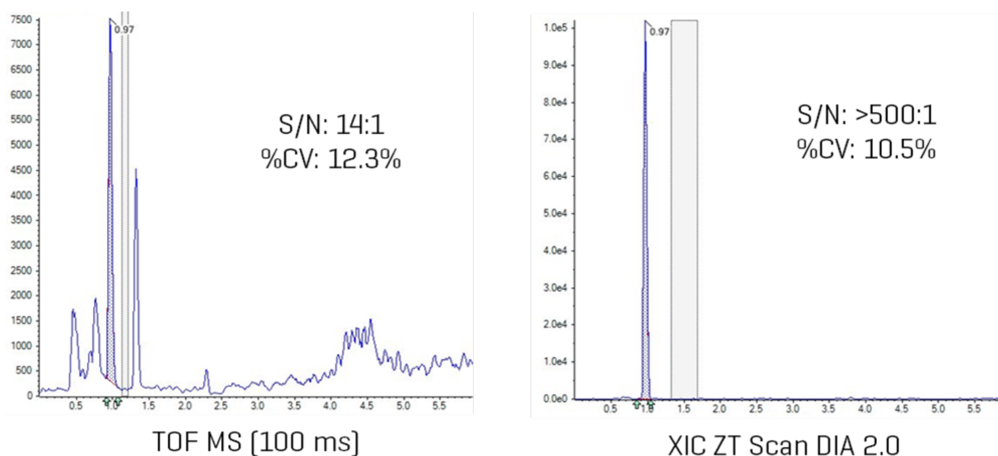

Figure 5. Comparative XIC of pseudouridine from TOFMS vs. ZT Scan DIA data.

Table 12. Targeted extraction of metabolites from ZT Scan DIA data (positive ion mode). The QReSS standards were added to the NIST 1950 reference plasma during the extraction process. Using the Analytics module of SCIEX OS software, quantitative analysis of the standards was performed at the MS/MS level

Component Name	Precursor Mass (Da)	Retention Time (min)	Area 1	Area 2	Area 3	Area Average	%CV
13C3-15N-Alanine	94.062	0.6	3.91E+06	3.81E+06	3.93E+06	3.88E+06	1.6
13C4-1,4Butanediamine	93.121	0.4	8.60E+05	1.02E+06	9.62E+05	9.48E+05	8.6
N-methyl-D3 creatinine	117.085	0.6	1.47E+07	1.48E+07	1.51E+07	1.49E+07	1.2
D4-Ethanolamine	66.085	0.5	1.92E+04	1.99E+04	2.15E+04	2.02E+04	5.8
15N5-Guanosine	289.084	1.8	1.06E+07	9.60E+06	1.22E+07	1.08E+07	12.0
13C5-Hypoxanthine	142.063	1.1	1.01E+07	9.26E+06	1.11E+07	1.02E+07	9.0
13C6-Leucine	138.122	1.1	3.01E+05	3.10E+05	2.78E+05	2.96E+05	5.5
13C6-Phenylalanine	172.106	2.0	1.99E+07	1.94E+07	2.19E+07	2.04E+07	6.6
15N2-Thymine	129.044	1.7	8.60E+06	8.56E+06	9.62E+06	8.92E+06	6.7
13C11-Tryptophan	216.134	3.2	1.66E+07	1.81E+07	1.74E+07	1.73E+07	4.3
13C6-Tyrosine	188.101	1.0	8.19E+06	8.28E+06	8.35E+06	8.28E+06	1.0
13C6-Vitamin B3	129.075	1.1	9.63E+06	9.31E+06	1.04E+07	9.78E+06	5.7
13C4-Fumaric acid	121.032	13.3	2.11E+05	2.01E+05	2.30E+05	2.14E+05	6.7
13C6-Indole-3-acetic acid	182.091	1.0	3.12E+06	2.86E+06	2.99E+06	2.99E+06	4.2
13C4-AKG	151.042	0.9	7.45E+04	9.13E+04	6.92E+04	7.83E+04	14.7
13C3-Pyruvic acid	92.033	3.2	4.17E+04	3.82E+04	3.82E+04	3.93E+04	5.1

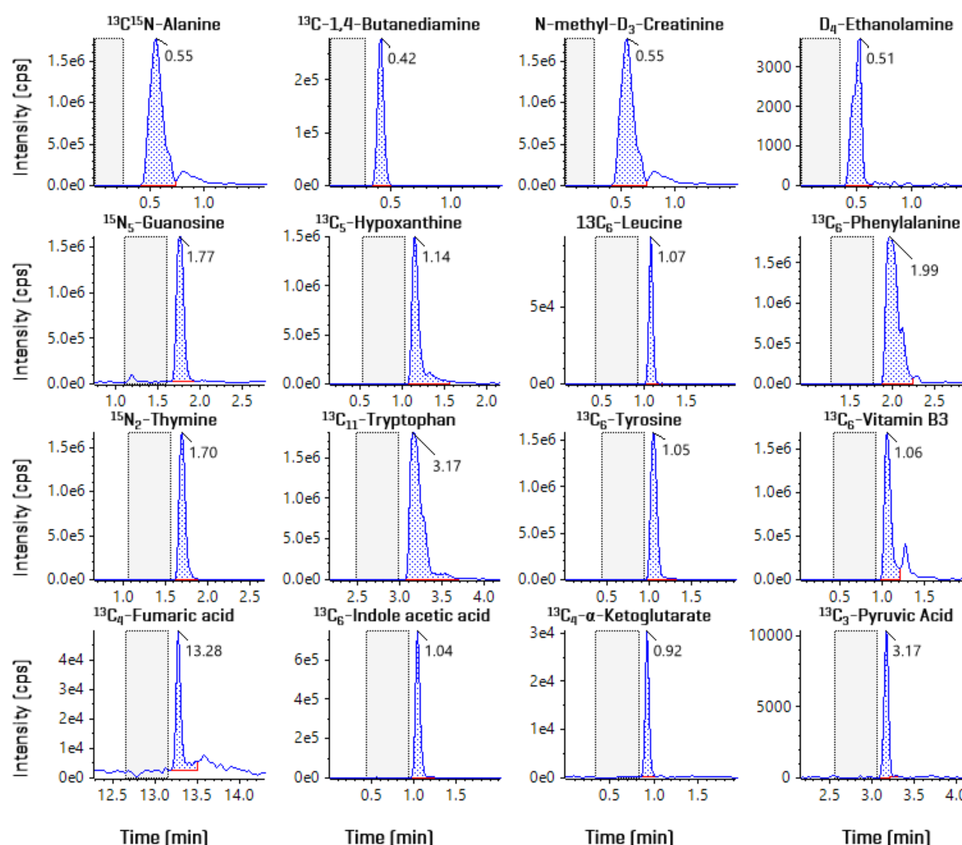

Figure 6. XICs for selected QReSS standards from ZT Scan DIA data acquired using the ZenoTOF 8600 system. NIST SRM 1950 plasma was extracted with QReSS standards. Using the Analytics module in SCIEX OS software, a targeted list of compounds was applied to the data to generate XICs, which were integrated to quantify the standards.

analysis. These same data can, as demonstrated here, be processed with MS-DIAL 5.6 to potentially identify other metabolites of interest.

Conclusions

In summary, the ZenoTOF 8600 system was used to collect untargeted metabolomics data with two methods: DDA and ZT Scan DIA 2.0. The ZenoTOF 8600 identified more curated compounds than the ZenoTOF 7600+ because of its higher sensitivity and ability to detect lower-abundance molecules. ZT Scan DIA resulted in a modest increase in the number of identified compounds and produced data that can be quantified at the MS/MS level. Another goal of this study was to clarify how the data were acquired and processed, and to provide a full report of the results to showcase data quality. Comparing results across different platforms can be challenging, especially in metabolomics, where there is little standardization, and various software platforms and databases are used, making data processing largely opaque. Instead of focusing only on the number of metabolites identified, this work emphasizes the importance of data transparency, precursor specificity, and MS/MS-level quantitation to build confidence in untargeted metabolomics results.

- Optimized parameter settings for MS-DIAL 5.6-alpha software were identified and used in the analysis of DDA and ZT Scan DIA workflows
- The ZT Scan DIA workflow generated a modest 10% increase in metabolite identification compared to DDA, but it also generated highly specific and accurate MS/MS-based quantitative data for the identified compounds
- Deconvolution of ZT Scan DIA data significantly decreased chimeric spectral overlap, improving the correlation between the precursor and product ion spectra
- ZT Scan DIA data can be probed with targeted compound lists to generate MRM^{HR} – like quantitative results

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