TECHNICAL NOTE

NeoBase 2 Non-derivatized MSMS assay validation study on SCIEX Triple Quad 4500MD LC-MS/MS system.

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Introduction

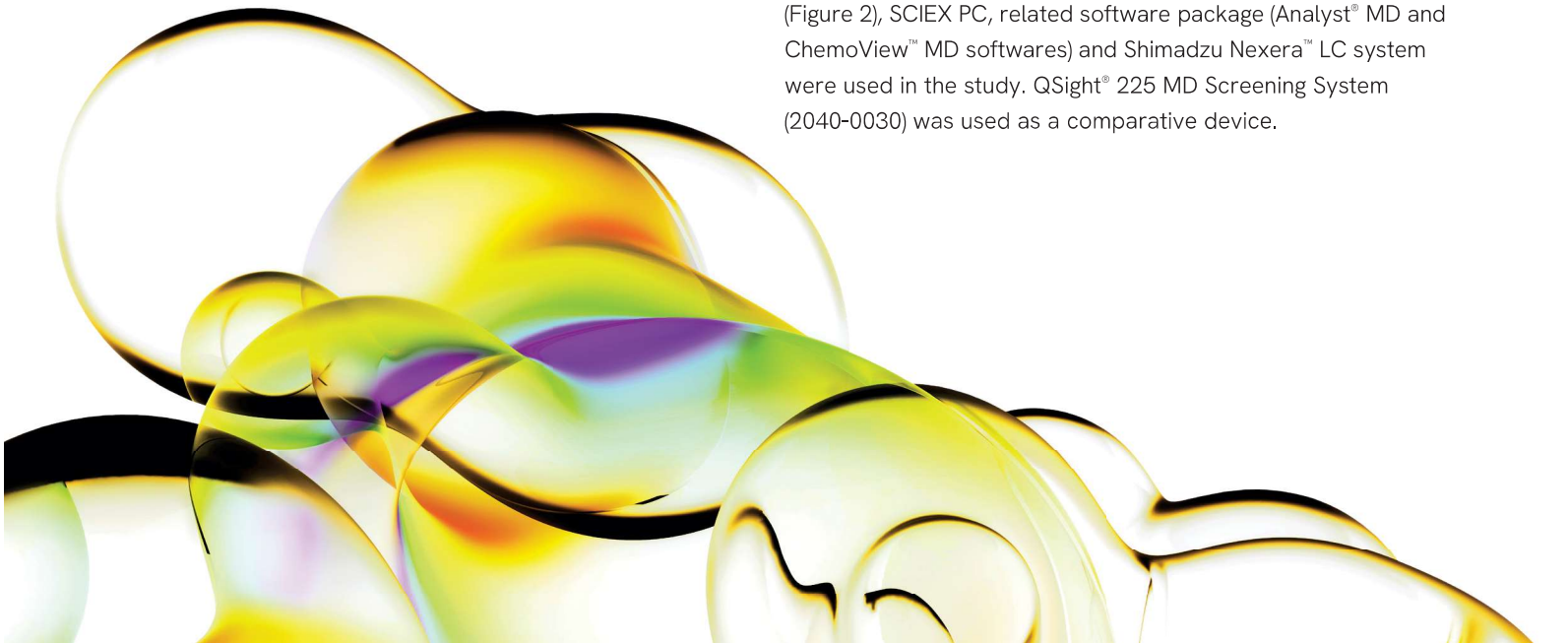
Revvity's NeoBase™ 2 Non-derivatized MSMS kit (3044-0010/ 3044-001U; Figure 1) used together with NeoBase 2 Non-derivatized Assay Solutions (3045-0010) and NeoBase 2 Succinylacetone Assay Solution (3046-0010) is capable of measuring over 50 analytes in a single assay. The kit is intended for the measurement and evaluation of amino acid, succinylacetone, free carnitine, acylcarnitine, nucleoside and lysophospholipid concentration with a tandem mass spectrometer from newborn heel prick blood specimens dried on filter paper. NeoBase 2 is a CE-IVD marked (2017) and FDA cleared (2018) product, and it has been previously validated with multiple MSMS systems, now with SCIEX Triple Quad™ 4500MD LC-MS/MS System (SCIEX 4500MD system).

Figure 1: NeoBase™ 2 Non-derivatized MSMS kit

Figure 2: SCIEX™ 4500MD mass spectrometer

Materials and methods

The purpose of the study was to validate the NeoBase 2 Non-derivatized MSMS assay with SCIEX 4500MD system. Two SCIEX 4500MD systems (2490-1030) consisting of mass spectrometer (Figure 2), SCIEX PC, related software package (Analyst® MD and ChemoView™ MD softwares) and Shimadzu Nexera™ LC system were used in the study. QSight® 225 MD Screening System (2040-0030) was used as a comparative device.



Main steps of the NeoBase 2 Non-derivatized MSMS assay workflow are described in Figure 3.

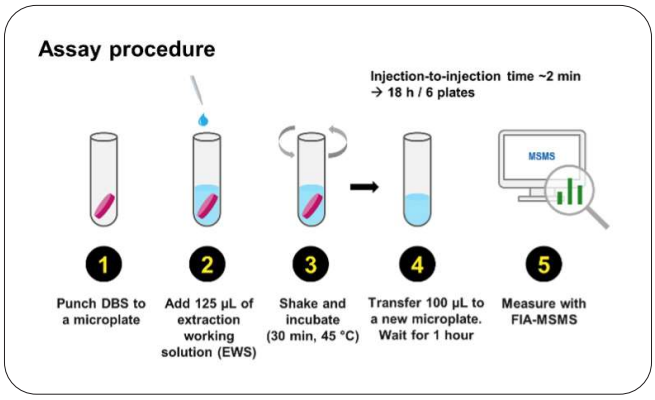


Figure 3: Overview of the NeoBase 2 assay workflow.

Shimadzu Nexera SIL-40C X3 autosampler design in the SCIEX 4500MD system does not have any foil-cutter part protecting the actual sample injection needle, and therefore use of adhesive covers is not recommended to avoid potential adhesive residue clogs in the LC flow path [1]. Due to this recommendation, the NeoBase 2 kit 96-well microplates for the SCIEX 4500MD system sample injections were sealed with separately available Microplate Heat Seals (4185-0010) using the Variable Temperature Heat Sealer (2490-2010 and required adaptor 11206853) instrument.

Most dried blood spot (DBS) samples used in the validation studies were prepared from human whole blood or from human red blood concentrate diluted with heavy charcoal stripped human serum to reach levels below the typical blood endogenous concentration ranges. Briefly, the whole blood bags were centrifuged, pooled and hematocrit was adjusted to typical newborn range 50-55% by plasma removal. For the other sample matrix, the human red blood cell concentrate was washed with 0.9% sodium chloride and the hematocrit value was adjusted accordingly with heavy charcoal stripped human serum. After this, non-enriched endogenous level and multiple different enriched NeoBase 2 control analyte (n = 30) levels were prepared on the filter paper cards (regulated 903 or Ahlström grade 226) and dried. In addition to these study specific samples, also NeoBase 2 Controls (Low, High), Neo Multilevel DBS (L1-L6) cards, Centers for Disease Control and Prevention (CDC) MSMS1QC DBS samples, and leftover newborn DBS samples were used in the study. All the DBS samples were stored with desiccant at -20 °C.

The performed assay validation study covered e.g. precision, linearity, analytical sensitivity, drift (on-board stability) and level of carry-over of the NeoBase 2 analytes determinations on SCIEX 4500MD system. In analytical sensitivity study, the lower limits of assay (i.e. analytical sensitivity) were determined, and in the drift study, it was confirmed that no significant drift occurs between the first and the last plate on the fully loaded autosampler with 12 96-well microplates. In addition, a method comparison study between SCIEX 4500MD and QSight 225 MD systems was done by assaying identical DBS sample sets covering the assay concentration ranges and including leftover newborn specimens (n = 240).

Results and discussion

Performed validation study generally demonstrated good overall NeoBase 2 assay performance and correlation between tested systems.

Linearity, precision, and analytical sensitivity study results for the NeoBase 2 control analytes are summarized in Table 1. With both tested systems, the observed precision profiles were similar, and the precision requirements with all the analytes and concentration levels were met. Results also showed good assay linearity with both systems across the concentration ranges. The lowest measurable analyte concentration (analytical sensitivity limit) was evaluated by serially diluting levels of internal standard and measuring them in non-diluted blood matrix. This analytical sensitivity study demonstrated that the SCIEX 4500MD system sensitivity results were similar in comparison to the QSight 225 MD system.

In the drift study, the overall analyte result variation for all NeoBase 2 control analytes was acceptable over the whole 12-plate run (ca. 36 h run time) with the SCIEX 4500MD system. Therefore, the fully prepared and heat-sealed assay plate can be stored in the SCIEX autosampler for 33 hours before the assay run starts, which is comparable to QSight 225 MD and other previously validated systems.

Carry-over with the SCIEX 4500MD system was evaluated by measuring endogenous level DBS samples right after high concentration DBS sample. Based on this study, no significant carry-over was observed with the SCIEX 4500MD system, even if ASA was expectedly identified as the most carry-over prone analyte similarly to the previously validated QSight 225 MD system.

Method comparison study results between SCIEX 4500MD and QSight 225 MD systems are presented in Table 2. In this study, fitted linear models (Deming regression) were determined for each analyte across wide DBS concentration ranges and including leftover newborn samples. As seen in Table 2, very good overall correlation ($r^2 > 0.99$) between the systems was observed for most of the analytes, whereas slightly lower correlations were observed with three analytes: Ala, Gln\Lys and Gly. Lower correlation of Ala is likely due to lower Creatine to Alanine in-source fragmentation with SCIEX 4500MD system. Gln\Lys and Gly had two outliers among newborn specimen, which contributed to lower correlation coefficient.

Based on the above summarized validation data, NeoBase 2 Non-derivatized MSMS assay performance results were substantially equivalent between the SCIEX 4500MD and QSight 225MD systems.

Table 1: Linearity, precision, and analytical sensitivity study results for the NeoBase 2 control analytes with SCIEX 4500MD and QSight 225 MD.

Analyte	SCIEX 4500MD			QSight 225 MD		
	Linearity (µmol/l)	Precision (µmol/l)	Analytical sensitivity (µmol/l)	Linearity (µmol/l)	Precision (µmol/l)	Analytical sensitivity (µmol/l)
Ala	201 - 1795	259 - 2330	0.45	286 - 1395	287 - 1430	0.91
Arg	5.14 - 410	4.43 - 369	0.05	4.83 - 404	4.43 - 391	0.09
Asa[1]	0.28 - 103	0.15 - 65.8	0.05	0.26 - 122	0.26 - 103	0.05
Cit	13.1 - 1163	12.2 - 1130	0.31	13.4 - 1120	11.3 - 1110	1.25
Gln\Lys	53.5 - 2568	54.3 - 2580	1.53	47.9 - 2585	42.9 - 2330	0.76
Gly	266 - 2563	289 - 2530	4.26	268 - 2458	264 - 2250	2.13
Leu\Ile\Pro-OH	73.9 - 1598	73.0 - 1650	0.39	78.2 - 1598	68.5 - 1550	0.39
Met	1.83 - 925	1.81 - 982	0.38	2.59 - 937	2.49 - 916	0.77
Orn	33.8 - 846	38.4 - 945	0.11	34.6 - 844	31.5 - 835	0.11
Phe	32.5 - 1528	28.9 - 1440	0.28	33.4 - 1528	29.2 - 1510	0.14
Pro	63.1 - 1305	70.7 - 1540	0.17	62.0 - 1313	55.7 - 1310	0.17
Tyr	33.0 - 1540	27.3 - 1350	0.88	37.3 - 1568	31.7 - 1530	3.53
Val	70.8 - 1138	70.2 - 1160	0.11	75.0 - 1193	65.7 - 1150	0.21
C0	14.6 - 443	10.6 - 362	0.01	14.3 - 464	12.6 - 438	0.18
C2	5.48 - 162	4.97 - 154	0.02	5.55 - 162	4.93 - 156	0.01
C3	0.73 - 68.9	0.55 - 56.0	0.02	0.75 - 70.6	0.66 - 68.9	0.02
C4	0.07 - 13.8	0.06 - 12.6	0.01	0.07 - 13.5	0.06 - 13.0	0.01
C5	0.05 - 20.5	0.04 - 20.3	0.01	0.05 - 20.2	0.04 - 19.4	0.01
C5DC\C6OH	0.04 - 8.42	0.04 - 8.21	0.01	0.04 - 8.29	0.03 - 7.75	0.02
C6	0.02 - 9.87	0.02 - 9.79	0.06	0.02 - 9.62	0.01 - 9.62	0.03
C8	0.04 - 46.3	0.03 - 47.5	<0.01	0.06 - 46.7	0.03 - 44.9	0.01
C10	0.02 - 7.90	0.01 - 6.40	0.01	0.03 - 7.55	0.02 - 7.46	<0.01
C12	0.02 - 9.28	0.02 - 9.53	0.02	0.02 - 9.20	0.01 - 9.16	<0.01
C14	0.08 - 9.62	0.07 - 9.37	<0.01	0.08 - 9.25	0.07 - 9.03	<0.01
C16	0.86 - 53.9	0.74 - 45.7	0.01	0.91 - 53.0	0.86 - 52.3	0.01
C18	0.56 - 13.3	0.56 - 12.6	0.01	0.57 - 13.6	0.57 - 13.1	0.01
C26[2]	0.03 - 3.93	0.02 - 3.15	0.01	0.03 - 4.09	0.03 - 3.98	0.01

Analyte	SCIEX 4500MD			QSight 225 MD		
	Linearity (µmol/l)	Precision (µmol/l)	Analytical sensitivity (µmol/l)	Linearity (µmol/l)	Precision (µmol/l)	Analytical sensitivity (µmol/l)
SA	0.14 - 121	0.14 - 136	0.06	0.23 - 117	0.23 - 106	0.11
ADO	0.17 - 43.4	0.14 - 41.7	0.01	0.18 - 43.4	0.14 - 42.4	0.02
C26:0-LPC	0.38 - 6.85	0.37 - 6.04	0.08	0.21 - 7.49	0.20 - 7.55	0.08

[1] Asa is measured as a total concentration of Asa and its anhydrides. [2] Acylcarnitine C26 is not part of 3044-001U kit.

Table 2: Method comparison study result correlation (Deming regression) for the NeoBase 2 control analytes with SCIEX 4500MD and QSight 225 MD including leftover newborn specimens (n = 240).

Analyte	SCIEX 4500MD vs. QSight 225 MD		Analyte	SCIEX 4500MD vs. QSight 225 MD	
	Regression equation	Correlation r^2		Regression equation	Correlation r^2
Ala	y = 1.14x - 293	0.872	C3	y = 1.03x + 0.03	0.998
Arg	y = 0.94x + 0.4	0.999	C4	y = 0.99x + 0.00	0.999
Asa[1]	y = 0.86x - 0.02	0.999	C5	y = 1.04x – 0.00	0.999
Cit	y = 1.03x - 0.13	0.999	C5DC\C6OH	y = 1.07x + 0.00	0.999
Gln\Lys	y = 1.04x + 5.49	0.964	C6	y = 1.05x + 0.00	0.999
Gly	y = 1.09x - 37.85	0.981	C8	y = 1.01x + 0.00	1.000
Leu\Ile\Pro-OH	y = 1.03x - 14.15	0.998	C10	y = 1.05x + 0.00	0.999
Met	y = 0.96x - 0.03	0.999	C12	y = 1.02x + 0.00	0.999
Orn	y = 1.02x + 0.43	0.995	C14	y = 1.01x + 0.00	0.998
Phe	y = 1.02x + 0.22	0.999	C16	y = 1.00x - 0.02	0.999
Pro	y = 1.05x + 4.01	0.996	C18	y = 1.01x + 0.00	0.999
Tyr	y = 1.00x + 0.29	0.997	C26[2]	y = 1.07x + 0.00	1.000
Val	y = 0.99x + 1.43	0.997	SA	y = 0.89x - 0.08	1.000
C0	y = 1.07x - 0.47	0.999	ADO	y = 1.00x + 0.00	1.000
C2	y = 1.03x - 0.34	0.994	C26:0-LPC	y = 1.08x + 0.16	0.993

[1] Asa is measured as a total concentration of Asa and its anhydrides. [2] Acylcarnitine C26 is not part of 3044-001U kit.

Reference

[1] Shimadzu Autosampler SIL-40C X3 (among other models) Instruction Manual. 228-92341E, Jan-2022.

SCIEX validation may not be licensed for Revvity's NeoBase™ 2 Non-Derivatized MSMS kit in accordance with the laws in all countries. Please check with your local representative for availability.

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