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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-9030

Highly-Efficient Screening Approach for Toxicological Compounds in Human Blood Samples on LCMS-9030

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User Benefits

- Highly-efficient data analysis workflows for targeted and untargeted screening of toxicological compounds can be performed using the LabSolutions Insight Explore™ – Analyze.
- The workflow was applied to DDA and DIA data of spiked human blood samples acquired on LCMS-9030.

■ Introduction

Efficient multi-target and/or untargeted screening by LC-MS methods have been developed and used in detection and quantitation of drugs of abuse (DoA) in forensic and clinical toxicology [1, 2]. The screening analysis must enable to detect of a wide range of compounds including illicit drugs, narcotics, psychotropics, antipsychotics, pharmaceuticals and other toxic compounds in urine, serum/plasma and whole blood samples. High resolution mass spectrometer (HRMS) coupled with UHPLC has played key roles in such analysis due to the superior mass accuracy (1 ppm) and high MS/MS capacity by Data-Dependent Acquisition (DDA) or Data-Independent Acquisition (DIA) modes on Q-TOF system. The aim of this study is to demonstrate efficient data analysis approaches for screening analysis of toxicological compounds by DDA and DIA modes on LCMS-9030 Q-TOF system. The LabSolutions Insight Explore suite was used as data analysis tools for (1) multi-target screening; (2) HRMS library search for confirmation and (3) untargeted screening and unknown identification. Both DDA and DIA data were processed and analysed. Spiked samples in human blood with mixture of 61 drug standards were used in this work [2].

■ Experimental

Reagents, standards and blood spiked samples

Acetonitrile (LCMS grade) and methanol (LCMS grade) were obtained from commercial suppliers. Ammonium acetate (>99%) of LCMS grade was used as additives in the mobile phase prepared from Milli-Q water. Sixty-one drug compounds were obtained and used to prepare mixed standards and spiked sample in human whole blood [2]. QuEChERS sample preparation procedure was adopted for sample clean-up before LC-MS analysis [1].

LC-Q-TOF analytical conditions

A Q-TOF system, LCMS-9030 (Shimadzu Corporation, Japan) was used in this work. LabSolutions v5.114 and LabSolutions Insight Explore v3.8 SP4 were used for data acquisition in DDA and DIA mode and data processing of MS and MS/MS spectra for efficient detection and identification of targeted and untargeted compounds. The analytical conditions are shown in Table 1.

Table 1 Analytical conditions of on LCMS-9030

LC Conditions	
Column	Kinetex XB-C18 (2.1 mm I.D. x 100 mmL., 2.6 µm)
Flow Rate	0.3 mL/min
Mobile Phase	A: 10 mM ammonium formate, 0.1% formic acid in pure water B: 10 mM ammonium formate, 0.1% formic acid in Methanol
LC gradient	B: 5% (0-1 min) → 40% (2 min) → 100% (12.5-19 min) → 5% (20.5-23 min) stop
Oven Temp.	40°C
Injection Vol.	4 µL
Interface Conditions and MS mode	
Interface	ESI Heated
Interface Temp.	300°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min (N2)
Heating Gas Flow	10 L/min (Air)
Drying Gas Flow	10 L/min (N2)
MS & DDA mode	MS, *m/z* 100~550 (0.1 sec) DDA, *m/z* 50~550; with CE 35V, (+/-) 17V Loop time: 0.2 sec
MS & DIA mode	MS, *m/z* 100~550 (0.1 sec) DIA, *m/z* 50~550; with CE 35V, (+/-) 17V Loop time: 0.55 sec

LabSolutions Insight Explore - Analyze

As shown in Table 1, data acquisition was performed by (a) MS and DDA, (b) MS and DIA. Data analysis was performed using the LabSolutions Insight Explore suite, which include Analyze and Assign etc. The Analyze is for deconvolution of DIA data to generate precursors and provide various functions of in-depth data analysis such as deconvoluted MS/MS spectrum, formula prediction and library search etc. A High-Resolution Accurate Mass Library for Forensic Toxicology [3] was used in library search. The Assign program was used for identification and structural elucidation of unknowns, which links to database searches such as ChemSpider and PubChem. Both Analyze and Assign were highly efficient and flexible in data processing and result displaying.

■ Results and Discussion

Approach 1: Screen and Library Search (DDA)

A quick and straightforward approach for screening of targeted and suspected compounds is illustrated in Figure 1. The Analyze program produced 1043 components from the data file (Mix DOA_DDA_002), which is a pre-spiked whole blood sample [2]. A Screen list in Excel format contained 75 targeted (or suspected) compounds with compound name, formula and accurate mass of protonated ion. The results of Screen are also listed in the same window (Table format), which can be sorted according to compound name, formula, targeted m/z or RT (Figure 2). Next, the found compound can be searched against HRMS library for confirmation or

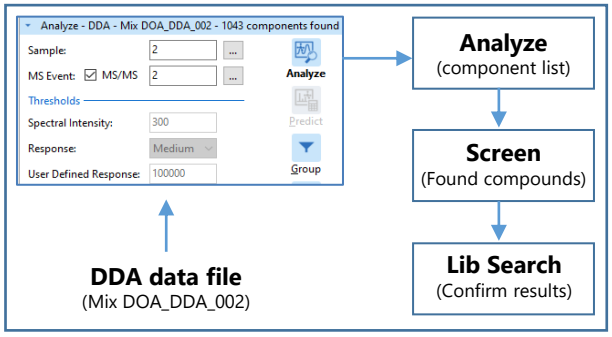

Figure. 1 Data analysis workflow: 1043 components were produced by Analyze, followed by Screen to look for targeted compounds via accurate masses. The detected compound was then confirmed by Lib search.

#	RT	m/z	Response	Precursor Ion	Lib. Compound Name	Lib. SI	Event	Hit #	Target Name	Target Formula	Target m/z	Target RT	Mass Error (ppm)
343	7.062	415.25382	13435	415.25382	----	ND	2	----	----	----	----	----	----
387	7.634	276.15937	171827	276.15937	3,4-methylenedioxypyro...	90	2	1	3,4-Methylene...	C16H21NO3	276.15940	7.469	-0.109
238	5.181	328.15437	207529	328.15437	6-MAM	87	2	1	6-Acethyl Mor...	C19H21NO4	328.15430	5.189	0.213
700	12.736	309.09010	159657	309.09010	Alprazolam	98	2	1	Alprazolam	C17H13ClN4	309.09020	12.363	-0.324
577	11.071	278.19020	133895	278.19020	Amitriptyline	90	2	1	Amitriptyline	C20H23N	278.19030	10.888	-0.359
196	4.633	136.11170	16208	136.11170	Amphetamine	100	2	1	Amphetamine	C9H13N	136.11210	4.544	-2.939
170	4.235	182.11732	269277	182.11732	Anhydroecgonine methy...	86	2	1	Anhydroecgon...	C10H15NO2	182.11760	4.231	-1.537
320	6.530	290.13868	46740	290.13868	Benzoylecgonine	100	2	1	Benzoylecgonine	C16H19NO4	290.13870	6.372	-0.069
304	6.291	195.08749	76888	195.08749	Caffeine	100	2	1	Caffeine	C8H10N4O2	195.08770	6.121	-1.076

Precursor list | Results of Library Search | Results of Screen Search

Figure 2 A screen snapshot of Analyze window which shows all the components (1143) and results of Screen search and Library search

rejection of the compound. Noted that, the library search can be performed for any individual component in the list or the entire table (1043). Figure 2 shows a screen snapshot of the Analyze window. Excluding the replicated results, 56 compounds were found by the Screen search in the sample. It is worth to note that this Screen search is based on only MS spectra and RT, not involved MS/MS spectra. While, the library search uses only MS/MS spectra (DDA). Therefore, the results that are confirmed by both Screen and Library search are reliable.

Approach 2: Screen and Lib Search (DIA data)

When DIA data of the same sample (pre-spiked whole blood) was processed by Analyze, a list of precursors was generated by its deconvolution algorithm. As shown in Figure 3, the Screen search and Library search can also be applied to find and confirm targeted compounds present in the sample. The results can be displayed flexibly in the Precursor window (Fig. 3a) with MS/MS spectrum (Fig. 3b) and XIC (Fig. 3c) of a selected precursor (red square mark). Furthermore, the deconvoluted spectrum can be sent to HRMS library search for confirmation or identification (Fig. 3d).

Approach 3: Untargeted screening (DIA data)

Untargeted screening is also known as general unknown screening (GUS), because pre-selection of analytes is not possible. Although the DDA and DIA modes on LC-Q-TOF provide efficient experimental techniques for untargeted analysis, the subsequent data analysis is often challenge in finding and identifying interested analytes from the huge numbers of MS/MS spectra measured. The **Analyze** program enables to generate precursors from MS/MS spectra of DIA data. These precursors with the respective deconvoluted MS/MS spectra can be submitted to Library search to identify the compounds (Figure 4). However, if

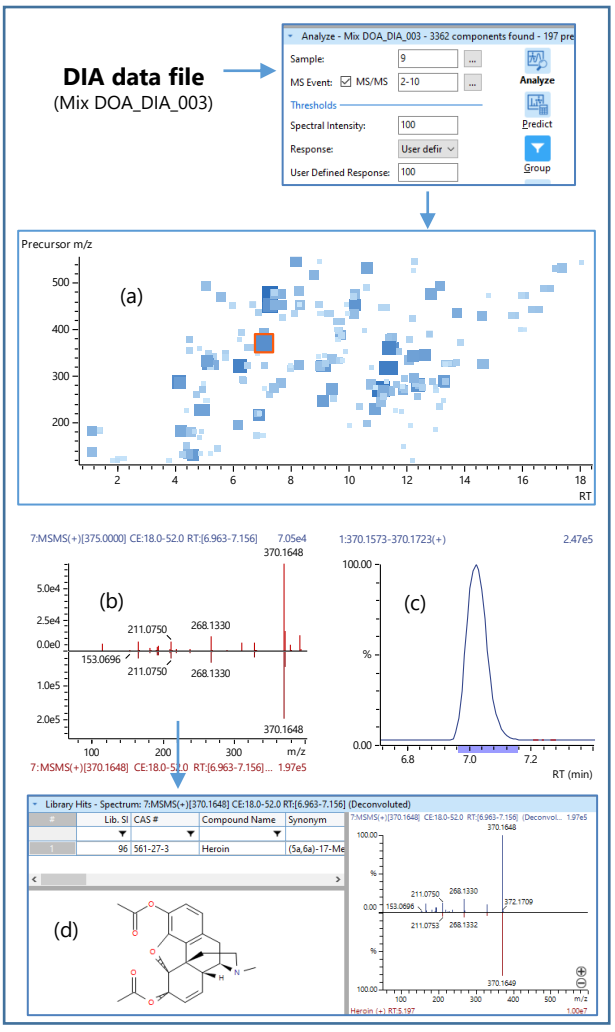

Figure 3 DIA data processing workflow by Analyze: (a) Found P197 precursors, (b) Deconvoluted MS/MS spectrum, (c) XIC of precursor (*m/z* 370.16479) and (d) Lib search result

the MS/MS library used does not include spectrum of the compound, identification will not give a result. In such case, calculating best-matched formula from the accurate mass of a precursor measured may become an important step, because a correct formula provides very critical information for identification of an unknown compound. The Analyze program enables to predict formula of all precursors in a minute.

To predict (simulate) best-matched formula, proper settings must be applied, for example H: 1-150, C: 1-50, N: 0-8, O: 0-8, S: 0-2, P: 0-1; Charge: 1, Error: 1 mDa, DBE: 0-8, and Adduct: H+ etc. As a result, 180 matched formula were generated from 197 precursors that were generated by Analyze (17 without results). However, the results must be carefully reviewed to confirm or reject the prediction. For instance, as shown in Fig. 5 (Top), the predicted formula for precursor *m/z*135.0433 (5.19 min) is $C_2H_7N_4OCl$. However, if reviewing the results in formula predictor window, three matched formula were available as shown in Fig. 5 (Bottom). The #3 formula ($C_8H_6O_2$) actually matches with the library search result, which is phthalaldehyde (CAS#: 643-79-8). This example indicates that formula prediction generate multiple candidates, but only the first one is displayed in the Analyze window. Therefore, it is always needed to review all formula and carefully confirm or reject candidates and further confirmation is often required.

In many cases, library search does not give any result. Identification of an unknown precursor must rely on only the **Assign** program, i.e., database search and MS/MS peak annotation. For example, precursor *m/z*318.3003 (RT=11.14 min) matched a formula of $C_{18}H_{39}NO_3$ (DBE=0), but no result was given in library search. The result of Assign resulted in several structures against ChemSpider database search and fragment annotation. The top candidate obtained was Phytosphingosine (CAS#: 554-62-1). The peak annotation by the Assign program is shown in Figure 6. This structural elucidation by Assign provides user a useful tool in identification of unknown compound. However, it is necessary to note that the results obtained by this approach is only used as reference. Further identification or confirmation by other analytical technique is needed to make a conclusion.

■ Conclusion

In this study, we demonstrate efficient data analysis workflows for targeted and untargeted screening using the LabSolutions Insight Explore – Analyze. With both DDA and DIA data of spiked human blood samples, the approaches 1~3 worked easily and smoothly to generate expected results. However, this study has not involved method validation and quantitation.

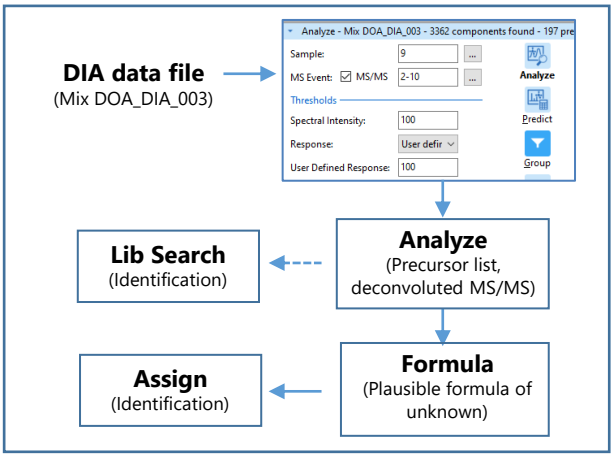

Figure 4 DIA data processing workflow for untargeted screening by Analyze program.

#	RT	Precursor I...	Response	Predicted Formula	Diff. (p...	Score	Lib. Compound ...	Lib. SI	Event
328	4.824	168.10637	10971	----	----	----	----	ND	3
1269	10.913	274.54937	3936	----	----	----	----	ND	5
156	11.882	149.02281	16148	[C2 H5 N4 O2 P+H]+	3.489	54.16	----	ND	2
122	5.190	135.04338	5631	[C3 H7 N4 Cl+H]+	1.333	49.11	Phthalaldehyde	100	2
38	3.797	119.08161	7099	[C4 H10 N2 O2+H]+	0.924	48.94	----	ND	2
44	3.824	118.06798	18563	[C5 H11 N S+H]+	-4.404	33.62	----	ND	2
1382	11.646	282.07934	95050	[C5 H11 N7 O7+H]+	0.248	49.91	----	ND	5
25	1.047	137.04527	126914	[C5 H12 S2+H]+	-0.365	76.86	----	ND	2
33	2.211	123.05481	35507	[C6 H6 N2 O+H]+	-3.901	43.61	----	ND	2
305	4.558	180.07766	90930	[C6 H14 N O3 P+H]+	-4.165	37.57	----	ND	3
1459	11.939	295.07450	81507	[C6 H14 N8 O2 S2+H]+	-3.016	68.25	Estazolam	95	5
498	10.003	194.09565	5364	[C6 H15 N3 O2 S+H]+	-0.618	59.70	----	ND	3
1636	13.058	269.04739	27162	[C7 H9 N8 P S+H]+	-2.750	83.47	----	ND	5
313	4.659	181.07166	13413	[C7 H16 O S2+H]+	0.718	59.61	Theophylline	87	3

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion
1	57.00	134.03575	135.04302	135.04338	0.36	C2 H7 N4 O P	[M+H]+
2	49.11	134.03592	135.04320	135.04338	0.18	C3 H7 N4 Cl	[M+H]+
3	33.49	134.03678	135.04406	135.04338	-0.68	C8 H6 O2	[M+H]+

Figure 5 Formula prediction and library search for 197 precursors (Top); Formula of precursor *m/z* 135.0438 (Bottom).

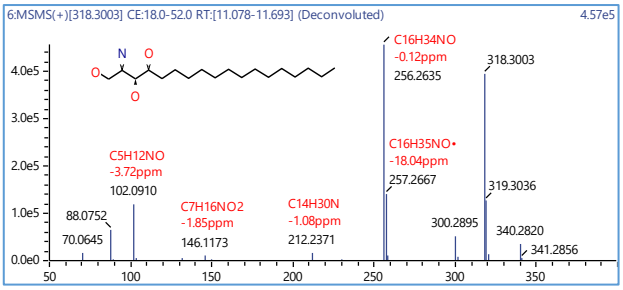

Figure 6 Peak annotation of deconvoluted MS/MS spectrum of precursor *m/z* 318.3003 (RT 11.14) using Assign program.

■ References

1. S. Dulaurent, S. El Balkhi, L. Poncelet, J.-Michel Gaulier, P. Marquet and F. Saint-Marcoux, Anal. Bioanal. Chem. 408: 1467-1474 (2016), DOI 10.1007/s00216-015-9248-3
2. N. Tansrisawad, U. Hoonwijit, A. Tubtimrattana, B. Intawong, S. Tanasarnsopaporn, J. Boonsritan, Z. Sun, C. Comsup, P. Tienprateep, Z. Zhan, ASMS poster MP-572 (2020)
3. High Resolution Accurate Mass Library for Forensic Toxicology, Shimadzu (2022), https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/brochures/16681/c146e459.pdf

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