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Ultra Fast UHPLC-LCMSMS Method Development in Clinical Drug Monitoring

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1. Introduction

With the development of highly sensitive and fast LC-MS/MS instruments, the triple quadrupole technology has found its way into clinical drug monitoring and is the method of choice for a number of assays. The steadily increasing number of applications in the clinical sector demands fast and efficient development of new LC-MS/MS methods. The foundation for high quality data is made through optimized chromatographic separations. Fully automated optimization of the UHPLC method using Shimadzu's method scouting software (Fig. 1) in combination with automated MS optimization for MRM parameters are the perfect platform for the generation of new triple quad MS methods. Here we report a new and fast procedure for the LC-MS/MS method optimization for clinical drug monitoring.

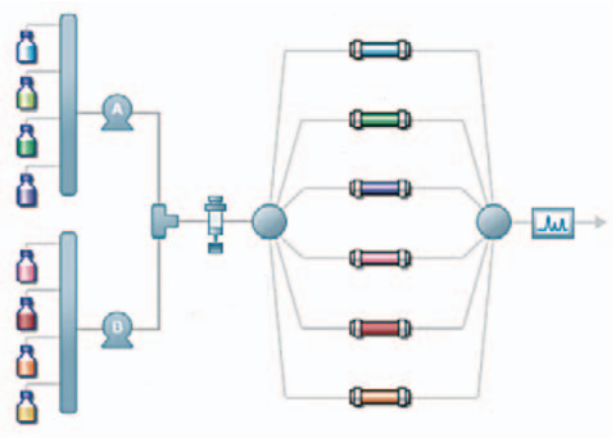

Fig. 1 Combination of columns and solvents during the method scouting process.

LCMS-8040 triple quadrupole mass spectrometer

2. Methods

2-1. LC-MS/MS parameters

One of the first steps during this automated process is the precursor ion selection, followed by the *m/z* adjustment of the precursor. The collision energy is optimized for the most abundant fragments and finally the fragment *m/z* adjustment. Six optimization steps were performed via flow injection analysis, each taking 30 seconds (Fig. 2). The result of these automated steps was the automatic generation of a final MRM method (Table 1).

2-2. UHPLC parameters

Choosing the best HPLC column and composition of eluents are often the most important but time-consuming steps during method development. This can influence sensitivity and separation from potentially interfering matrix effects. Shimadzu Method Scouting was used to determine the best HPLC parameters for the analysis of 14 different drugs. This allowed the combination of 6 HPLC columns with up to 16 different eluents, resulting in the investigation of up to 96 different combinations, requiring only a fraction of the time required by traditional approaches.

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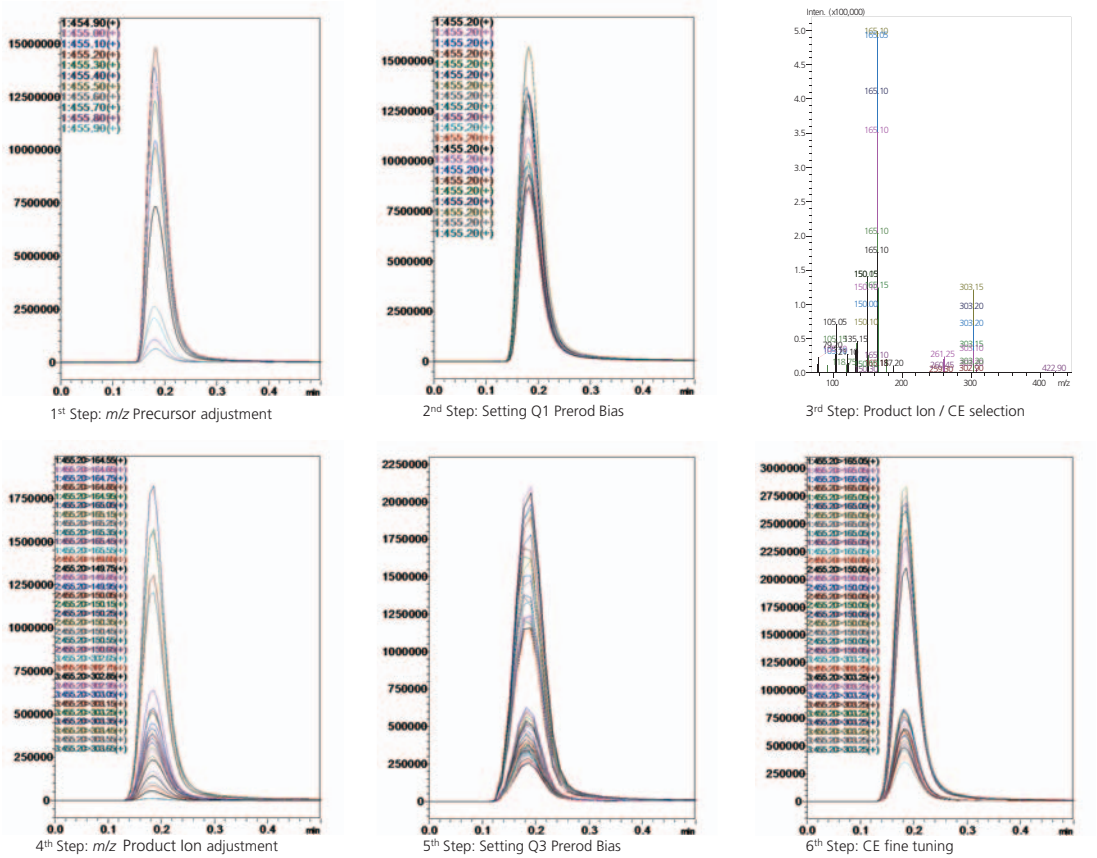


Fig. 2 Automated MRM Optimization of the drug Verapamil on the LCMS 8040

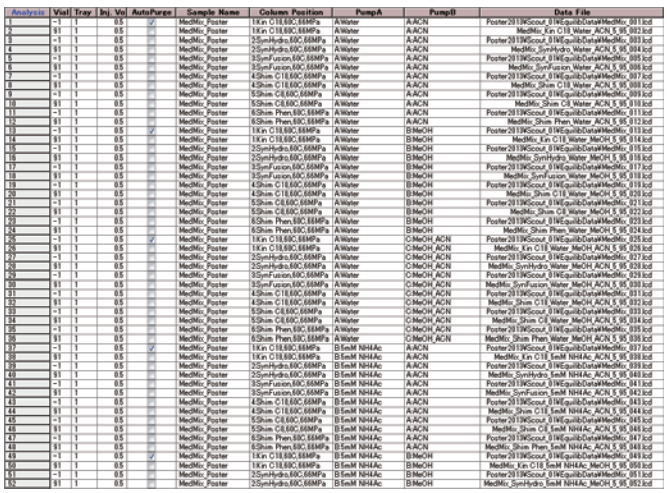
Table 1 Optimized MRM transitions of 14 drugs

Compound	Mode	MRM transitions	Collision energy (kV)
Disopyramide	ESI positive	340.3 > 239.10 / 340.3 > 195.10	-19 / -35
Lidocaine	ESI positive	235.10 > 86.20 / 235.10 > 58.05	-22 / -39
Mexiletine	ESI positive	180.20 > 105.10 / 180.20 > 121.20	-22 / -18
Quinidine	ESI positive	325.30 > 307.10 / 325.30 > 172.05	-26 / -40
Losartan	ESI positive	423.20 > 207.00 / 423.20 > 405.00	-24 / -13
Amiodarone	ESI positive	645.90 > 100.20 / 645.90 > 86.20	-34 / 41
Amitriptyline	ESI positive	278.10 > 105.10 / 278.10 > 233.10	-27 / -18
Chlorpromazine	ESI positive	319.20 > 86.20 / 319.20 > 239.10	-23 / -28
Haloperidol	ESI positive	376.05 > 165.15 / 376.05 > 123.10	-25 / -45
Imipramine	ESI positive	281.25 > 208.00 / 281.25 > 193.10	-27 / -46
Metoprolol	ESI positive	268.25 > 116.15 / 268.25 > 133.00	-20 / -28
Nortriptyline	ESI positive	264.25 > 91.20 / 264.25 > 233.15	-30 / -15
Verapamil	ESI positive	455.20 > 165.05 / 455.20 > 150.05	-34 / -46
Warfarin	ESI negative	307.25 > 160.85 / 307.25 > 249.90	21 / 24

3. Results

3-1. Method development

Traditional method development in HPLC is extremely time consuming. The combination of automated HPLC and MS method development allows the development of complete LC-MS/MS methods within a single day. In this study we show an automated method scouting procedure including the search for optimum column and mobile phase and the gradient conditions. The combination with the fully automated MRM-optimization by flow injection allows a fast development of a final method for the analysis of clinical drugs. Here we show methods automatically generated for the separation, identification and quantification of a mixture of drugs by the use of 7 different solvents and 6 different columns. The primary step to elucidate the best HPLC conditions out of various combinations is the automated batch creation via method scouting software from Shimadzu (Fig. 3).



Analysis	Vial	Tray	Inj. Vo	AutoPurge	Sample Name	Column Position	PumpA	PumpB	Data File
1	-1	1	0.5	✓	MedMix_Poster	1:Kin C18,60C,66MPa	A:Water	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_001.lcd
2	91	1	0.5		MedMix_Poster	1:Kin C18,60C,66MPa	A:Water	A:ACN	MedMix_Kin C18_Water_ACN_5_95_002.lcd
3	-1	1	0.5		MedMix_Poster	3:SynHydro,60C,66MPa	A:Water	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_003.lcd
4	91	1	0.5		MedMix_Poster	3:SynHydro,60C,66MPa	A:Water	A:ACN	MedMix_SynHydro_Water_ACN_5_95_004.lcd
5	-1	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	A:Water	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_005.lcd
6	91	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	A:Water	A:ACN	MedMix_SynFusion_Water_ACN_5_95_006.lcd
7	-1	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	A:Water	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_007.lcd
8	91	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	A:Water	A:ACN	MedMix_Shim C18_Water_ACN_5_95_008.lcd
9	-1	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	A:Water	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_009.lcd
10	91	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	A:Water	A:ACN	MedMix_Shim C8_Water_ACN_5_95_010.lcd
11	-1	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	A:Water	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_011.lcd
12	91	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	A:Water	A:ACN	MedMix_Shim Phen_Water_ACN_5_95_012.lcd
13	-1	1	0.5	✓	MedMix_Poster	1:Kin C18,60C,66MPa	A:Water	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_013.lcd
14	91	1	0.5		MedMix_Poster	1:Kin C18,60C,66MPa	A:Water	B:MeOH	MedMix_Kin C18_Water_MeOH_5_95_014.lcd
15	-1	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	A:Water	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_015.lcd
16	91	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	A:Water	B:MeOH	MedMix_SynHydro_Water_MeOH_5_95_016.lcd
17	-1	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	A:Water	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_017.lcd
18	91	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	A:Water	B:MeOH	MedMix_SynFusion_Water_MeOH_5_95_018.lcd
19	-1	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	A:Water	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_019.lcd
20	91	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	A:Water	B:MeOH	MedMix_Shim C18_Water_MeOH_5_95_020.lcd
21	-1	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	A:Water	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_021.lcd
22	91	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	A:Water	B:MeOH	MedMix_Shim C8_Water_MeOH_5_95_022.lcd
23	-1	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	A:Water	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_023.lcd
24	91	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	A:Water	B:MeOH	MedMix_Shim Phen_Water_MeOH_5_95_024.lcd
25	-1	1	0.5	✓	MedMix_Poster	1:Kin C18,60C,66MPa	A:Water	C:MeOH_ACN	Poster2019\Scout_81\EquilibData\MedMix_025.lcd
26	91	1	0.5		MedMix_Poster	1:Kin C18,60C,66MPa	A:Water	C:MeOH_ACN	MedMix_Kin C18_Water_MeOH_ACN_5_95_026.lcd
27	-1	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	A:Water	C:MeOH_ACN	Poster2019\Scout_81\EquilibData\MedMix_027.lcd
28	91	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	A:Water	C:MeOH_ACN	MedMix_SynHydro_Water_MeOH_ACN_5_95_028.lcd
29	-1	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	A:Water	C:MeOH_ACN	Poster2019\Scout_81\EquilibData\MedMix_029.lcd
30	91	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	A:Water	C:MeOH_ACN	MedMix_SynFusion_Water_MeOH_ACN_5_95_030.lcd
31	-1	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	A:Water	C:MeOH_ACN	Poster2019\Scout_81\EquilibData\MedMix_031.lcd
32	91	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	A:Water	C:MeOH_ACN	MedMix_Shim C18_Water_MeOH_ACN_5_95_032.lcd
33	-1	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	A:Water	C:MeOH_ACN	Poster2019\Scout_81\EquilibData\MedMix_033.lcd
34	91	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	A:Water	C:MeOH_ACN	MedMix_Shim C8_Water_MeOH_ACN_5_95_034.lcd
35	-1	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	A:Water	C:MeOH_ACN	Poster2019\Scout_81\EquilibData\MedMix_035.lcd
36	91	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	A:Water	C:MeOH_ACN	MedMix_Shim Phen_Water_MeOH_ACN_5_95_036.lcd
37	-1	1	0.5	✓	MedMix_Poster	1:Kin C18,60C,66MPa	B:5mM NH4Ac	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_037.lcd
38	91	1	0.5		MedMix_Poster	1:Kin C18,60C,66MPa	B:5mM NH4Ac	A:ACN	MedMix_Kin C18_5mM NH4Ac_ACN_5_95_038.lcd
39	-1	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	B:5mM NH4Ac	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_039.lcd
40	91	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	B:5mM NH4Ac	A:ACN	MedMix_SynHydro_5mM NH4Ac_ACN_5_95_040.lcd
41	-1	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	B:5mM NH4Ac	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_041.lcd
42	91	1	0.5		MedMix_Poster	3:SynFusion,60C,66MPa	B:5mM NH4Ac	A:ACN	MedMix_SynFusion_5mM NH4Ac_ACN_5_95_042.lcd
43	-1	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	B:5mM NH4Ac	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_043.lcd
44	91	1	0.5		MedMix_Poster	4:Shim C18,60C,66MPa	B:5mM NH4Ac	A:ACN	MedMix_Shim C18_5mM NH4Ac_ACN_5_95_044.lcd
45	-1	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	B:5mM NH4Ac	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_045.lcd
46	91	1	0.5		MedMix_Poster	5:Shim C8,60C,66MPa	B:5mM NH4Ac	A:ACN	MedMix_Shim C8_5mM NH4Ac_ACN_5_95_046.lcd
47	-1	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	B:5mM NH4Ac	A:ACN	Poster2019\Scout_81\EquilibData\MedMix_047.lcd
48	91	1	0.5		MedMix_Poster	6:Shim Phen,60C,66MPa	B:5mM NH4Ac	A:ACN	MedMix_Shim Phen_5mM NH4Ac_ACN_5_95_048.lcd
49	-1	1	0.5	✓	MedMix_Poster	1:Kin C18,60C,66MPa	B:5mM NH4Ac	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_049.lcd
50	91	1	0.5		MedMix_Poster	1:Kin C18,60C,66MPa	B:5mM NH4Ac	B:MeOH	MedMix_Kin C18_5mM NH4Ac_MeOH_5_95_050.lcd
51	-1	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	B:5mM NH4Ac	B:MeOH	Poster2019\Scout_81\EquilibData\MedMix_051.lcd
52	91	1	0.5		MedMix_Poster	2:SynHydro,60C,66MPa	B:5mM NH4Ac	B:MeOH	MedMix_SynHydro_5mM NH4Ac_MeOH_5_95_052.lcd

Fig. 3 Batch generated by the method scouting software

Table 2 Solvents and Columns used for method development

Solvent
AA: Water
AB: 5 mM $CH_3COO{\cdot}NH_4^+$; pH 8
AC: 0.1% Formic acid
AD:10 mM $CH_3COO{\cdot}NH_4^+$; pH 4.5
BA: Acetonitrile
BB: Methanol
BC: Acetonitrile / Methanol 50/50 (v/v)

Column
Kinetex 2.6 µ C18 100 × 2.10 mm (Phenomenex)
Synergie 2.5 µ Fusion-RP, 100 × 2.00 mm (Phenomenex)
Synergie 2.5 µ Hydro-RP, 100 × 2.00 mm (Phenomenex)
Shim-pack XR-ODS II 2.2 µ, 100 × 2.00 mm (Shimadzu)
Shim-pack XR-C8 2.2 µ, 100 × 2.00 mm (Shimpack)
Shim-pack XR-Phenyl 2.2 µ, 100 × 2.00 mm (Shimpack)

3-2. Data Recording

The first step is the evaluation of the optimal column / solvent combination using a generic gradient starting with 5% of organic solvent increasing to 95% within a specified time. This initial stage generates a viable method requiring some further optimization. The second step optimizes the slope of the gradient as well as the solvent conditions. Several different conditions were then performed during an overnight analysis. Table 2 shows the used columns and solvents.

3-3. Data comparison

A total number of 162 different combinations were analyzed and evaluated for the best separation and peak intensities (Fig. 4).

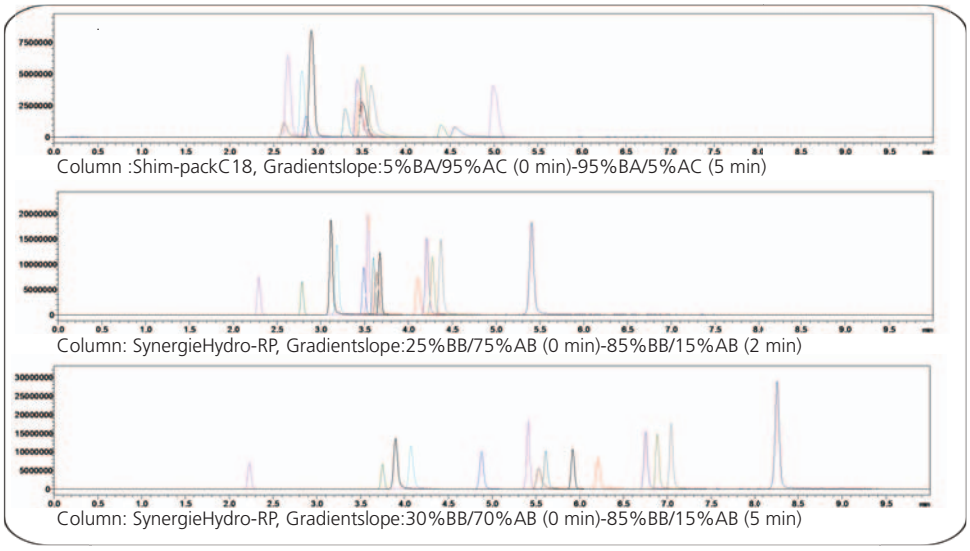


Fig. 4 Examples for poor, medium and good results

3-4. Final method

Flow rate : 0.4 ml / min
Column : Synergie Hydro-RP
Solvent A : 5 mM Ammonium acetate, pH 8
Solvent B : Methanol
Oven temp. : 50°C

Gradient:

0 min : 30% B
5 min : 85% B
5.01 min: 95% B
8 min : 95% B
8.01 min: 30% B
10 min : Stop

4. Conclusion

The Combination of the method scouting software tool coupled Shimadzu's ultrafast LCMS 8040 Triple Quad Mass analyzer is a unique tool for fast and easy method development of LC-MS/MS methods. The chromatographic separation of 14 different drugs as well as their identification and quantification was established successfully within one working day.

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