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Fast LC-MS/MS Screening Method for Antipsychotics with Data Dependent Analysis for Untargeted Analytes

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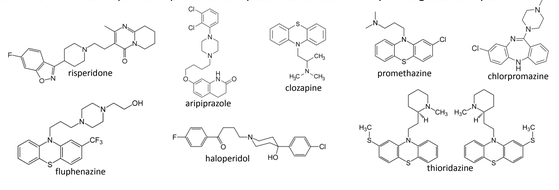
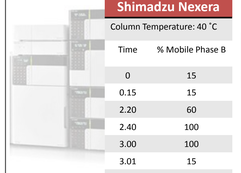
Introduction

Antipsychotic and atypical antipsychotics are used to treat a wide variety of psychiatric conditions such as schizophrenia, acute mania, bipolar disorder, and depression. Recently a high number of children are being treated with antipsychotics for emotional or behavioral disorders. Therapeutic drug monitoring is in demand due to the risk of toxicity, unwanted side effects, and potential pharmacokinetic interactions. Additionally, a co-administered drug can potentially inhibit or induce the metabolism of the antipsychotic drug such as antibiotics, selective serotonin reuptake inhibitors, cigarette smoking, and barbiturates.[1] A fast screening method for a group of typical and atypical antipsychotics has been developed which incorporates the acquisition of full scan MS and data dependent product ion spectra to screen for any untargeted analytes.



Materials and Method

Antipsychotic standards were diluted in 0.1% formic acid at concentrations ranging from 4 ppm-1 ppb. Data was collected on a Shimadzu Nexera UHPLC with a Shimadzu LCMS-8040 mass spectrometer. An Ascentis RP-Amide column was used with a binary gradient of 0.1% formic acid/acetonitrile at 0.4 mL/min. MRM transitions were optimized for the antipsychotics, fluoxetine and phenobarbital. Product ion scans were obtained for each analyte and a spectral library created. Dual polarity data dependent product ion scans were triggered for precursor ions exceeding 1,000,000 cps. Synthetic serum (SeraSub) spiked samples were prepared by protein precipitation using 3:1 cold acetonitrile:serum. The samples were spun at 15,000 rpm for 5 mins, the supernatant dried to completeness and reconstituted with 0.1% formic acid.



LC Parameters Shimadzu Nexera	
Column Temperature: 40 ˚C	
Time	% Mobile Phase B
0	15
0.15	15
2.20	60
2.40	100
3.00	100
3.01	15
4.00	15

MS Parameters Shimadzu LCMS-8040	
Desolvation Line Temperature	200 ˚C
Heat block Temperature	250 ˚C
Nebulizing Gas Flow	2 L/min
Dry Gas Flow	15 L/min
MRM Dwell Time	10 msec
MRM Pause Time	3 msec

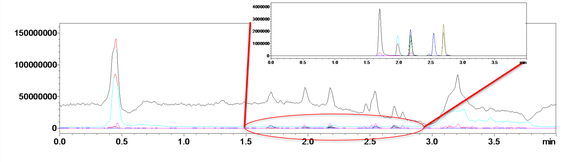
Qualitative Results

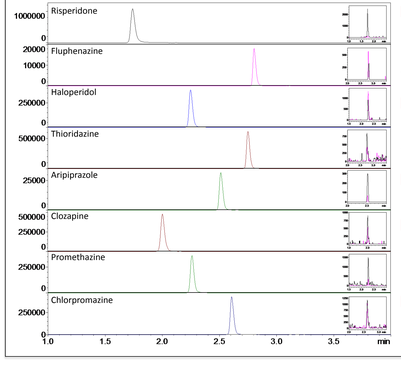

Improved ion optics in the LCMS-8040 for enhanced sensitivity

All antipsychotic standards were purchased from Cerilliant and MRM optimization was performed with CE and transitions (quantifier and qualifier ions) illustrated in the quantification section. Polarity switching was employed to execute data dependent product ions scans embedded within positive and negative Q3 scans to screen for untargeted analytes.

MS Event Cycle Loop Time: 0.438

Type	Event#	+/-	Compound Name m/z	Time (0.000 min - 4.000 min)
MRM	1	+	Risperidone 411.20>191.10, 47	
MRM	2	+	Fluphenazine 439.15>143.20,	
MRM	3	+	Haloperidol 376.35>165.10, 37	
MRM	4	+	Thioridazine 371.10>126.10, 3	
MRM	5	+	Aripiprazole 449.90>287.10, 44	
MRM	6	+	Clozapine 327.30>270.05, 327	
MRM	7	+	Promethazine 285.10>86.10, 2	
MRM	8	+	Clorpromazine 319.00>86.10, 3	
Q3 Scan	9	+	150.00:500.00	
↳ Product Ion Scan	10	+	100.00 > 40.00:500.00	
Q3 Scan	11	-	150.00:500.00	
↳ Product Ion Scan	12	-	100.00 > 40.00:500.00	





- Figure above illustrate TIC chromatograms of the complete MS event cycle: MRMs (insert), Q3 scans, and data dependent triggered product ion scans.
- Figures on left illustrate the individual MRM chromatograms of the standards (quantifier transitions only).
- Small insert is the MRM of the standards at the respective LOD.
- All analytes eluted within 3 minutes with excellent peak shape and retention time stability.
- Signal to noise was ≥ 10 for all analytes.

Quantitative Results

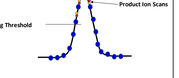
Standards were prepared in spiked synthetic serum and concentrations generated by serial dilution. All analytes produced linear responses with r^2 values between 0.9978 - 0.9999 and limit of detections (LODs) as low as 1.05 ppb.

Calibration Level	Concentration
L1	4 ppm
L2	1.6 ppm
L3	640 ppb
L4	256 ppb
L5	102 ppb
L6	41 ppb
L7	16.4 ppb
L8	6.56 ppb
L9	2.62 ppb
L10	1.05 ppb

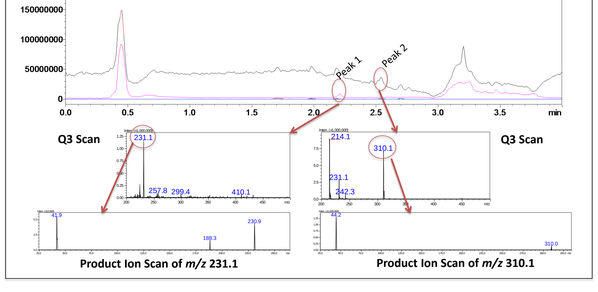
Antipsychotic	Transition	Collision Energies	Dynamic Range	r^2	LOD in synthetic serum extract
Risperidone	411.20 → 191.10 → 110.10	-30 -50	4 ppm – 1.05 ppb	0.9995	0.42 ppb
Fluphenazine	439.15 → 143.20 → 171.20	-35 -25	4 ppm – 41 ppb	0.9989	41 ppb
Haloperidol	376.36 → 165.10 → 123.05	-25 -40	4 ppm – 2.62 ppb	0.9999	2.62 ppb
Thioridazine	371.10 → 126.10 → 98.05	-25 -35	4 ppm – 1.05 ppb	0.9978	1.05 ppb
Aripiprazole	449.90 → 287.10 → 98.10	-30 -40	4 ppm – 41 ppb	0.9988	41ppb
Clozapine	327.30 → 270.05 → 191.95	-25 -45	4 ppm – 2.62 ppb	0.9995	2.62 ppb
Promethazine	285.10 → 86.10 → 71.05	-20 -50	4 ppm – 2.62 ppb	0.9999	2.62 ppb
Chlorpromazine	319.00 → 86.10 → 58.05	-25 -40	4 ppm – 2.62 ppb	0.9995	2.62 ppb

Data Dependent Analysis for Untargeted Analytes

Full mass spectra was collected at a scan rate of 10,000 u/sec throughout the entire run to screen for unknown analytes. Product ion scans were triggered on any ion that exceeded a set threshold level of 500,000 cps at a scan speed of 7,500 u/sec.

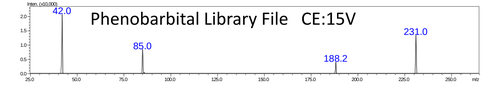


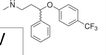
Q3 Scans: black is positive, pink is negative

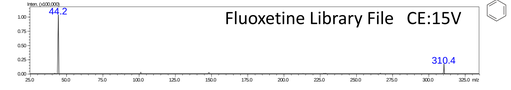


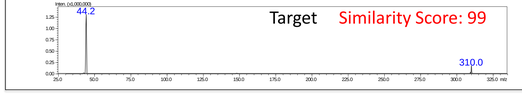
MS/MS Library Searching

The product ion spectra generated from the triggered data dependent events are then automatically compared to a MS/MS library database which contained 157 different analytes. The top hit for Peak A resulted in a similarity score of 89 with the analyte phenobarbital, a known enzyme inducer which ultimately leads to a reduction of certain antipsychotic uptake. The top hit for Peak B resulted in a similarity score of 99 which corresponds to the analyte fluoxetine which is known as an enzyme inhibitor.









Conclusion

A fast, robust, and sensitive LCMS method for the analysis of antipsychotics has been developed using the Shimadzu LCMS-8040 mass spectrometer. Multiple reaction monitoring was used to screen and quantify the presence of known antipsychotic within synthetic serum. In addition, data dependent triggered product ion scans in both polarities enables the detection of unknown analytes, especially those which could interfere with the prescribed antipsychotic. The Shimadzu LCMS-8040 mass spectrometer achieved up to 3x the sensitivity when comparing the same data acquired on the Shimadzu LCMS-8030 mass spectrometer.

References:

1. Sharif, Z.A.: Pharmacokinetics, Metabolism, and Drug-Drug Interactions of Atypical Antipsychotics in Special Populations. *Prim Care Companion J Clin Psychiatry.* **5**, 22-25 (2003)