POSTER SUMMARY

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A summary of "Uromics: Metabolomics in Urine for Seroquel®, Latuda®, and Haldol®"

Overview:

Identification of some drug metabolites is typically done using blood samples, however urine analysis is also a viable way to determine metabolites from a given drug. Reports indicate that there is poor compliance with antipsychotic drug prescriptions, and three antipsychotic drugs, Seroquel®, Latuda® and Haldol®, were analyzed for alternative metabolites that could be monitored for improved patient positivity. Both known and unknown metabolites were identified using LC-QTOF in combination with hydrolysis. Seroquel® (Quetiapine) usage is currently tested by monitoring N-desalkyl quetiapine or 7-hydroxy quetiapine, only 12% of the radioactive dose. This study shows that the carboxylic acid and sulfoxide metabolites are vital in establishing patient positivity. Smaller fragments of lurasidone are reported to be the most prevalent metabolites in Latuda® (Lurasidone). This study revealed that lurasidone and hydroxylurasidone, along with the unconfirmed metabolites M-21 and M-22, are actually the most abundant metabolites of Latuda® in urine. Haldol® (Haloperidol) was reported as not being conjugated during metabolism, with reduced haloperidol and two small acid fragments as the main metabolites. However, this study discovered that haloperidol is substantially glucuronidated and hydrolysis significantly improves detection.

Material and Methods:

To establish the metabolite distribution, patient samples which tested positive for or were prescribed Seroquel®, Latuda®, and/or Haldol® were tested using an Agilent 6530 LC-QTOF and analyzed against a comprehensive library containing known and possible metabolites of the three antipsychotic drugs. Samples were diluted five-fold with 250 ng/mL of hydrocodone-D6 in water. To confirm the conjugation of compounds, hydrolysis was performed with about 3,000 U of IMCSzyme®, a purified genetically modified β-glucuronidase, at 60˚C for 60 minutes. Samples were analyzed on a Waters Acquity UPLC® Xevo TQ-MS system using a Waters Acquity UPLC® CSH Phenyl-Hexyl 2.1 x 50 mm, 1.7 µm column with an Acquity inline filter. Mobile phase A, 2 mM ammonium acetate + 0.1% formic acid in water, started at 98% and mobile phase B was ramped to 90% , 100% methanol, over 2.8 minutes with a flow rate of 0.8 mL/min.

For hydrolysis, Seroquel® and Haldol® samples were diluted five-fold with 400 µL of a master mix containing 0.2 µg/mL Quetiapine-D8 or 0.2 µg/mL Haloperidol-D4 plus 0.2M phosphate buffer pH 7.5 and ~7,500 U/mL IMCSzyme®. For testing unhydrolyzed samples, Seroquel®, Latuda®, and Haldol® samples were diluted five-fold with 400 uL of internal standard in 0.1% formic acid (0.2 µg/mL Quetiapine-D8, 0.2 µg/mL Haloperidol-D4, or 0.9 µg/mL Lurasidone-D8).

Results

Table 1. shows the average analyte responses of quetiapine metabolites in 12 patient urine samples. This distribution reveals that quetiapine carboxylic acid is in fact the most abundant metabolite followed by N-desalkyl quetiapine, 7-hydroxy desalkyl quetiapine, and quetiapine sulfoxide.

Table 1. Quetiapine Metabolite Distribution in Human Urine

Analyte -> Patient	7-Hydroxy Desalkyl Quetiapine	7-Hydroxy Quetiapine	Quetiapine Caroboyxlic Acid	N-Desalkyl Quetiapine	O-Desalkyl Quetiapine	Quetiapine	Quetiapine Glucuronide	Quetiapine Sulfoxide
1	7.27	0.65	6.71	8.48	0.00	0.19	0.40	1.81
2	23.21	7.76	43.61	16.66	0.73	2.08	6.23	10.81
3	5.22	0.00	27.86	11.15	1.08	8.21	0.02	2.02
4	10.36	6.86	26.14	4.18	0.23	1.87	5.23	12.90
5	0.39	0.08	5.36	0.80	0.00	0.08	0.52	0.23
6	5.16	1.23	34.19	2.94	0.00	0.58	3.16	4.10
7	2.25	0.86	10.95	4.09	0.04	0.85	1.06	2.22
8	7.24	0.54	51.31	13.44	0.00	0.31	4.74	1.79
9	7.95	3.73	13.07	8.81	0.30	0.98	1.86	6.54
10	5.22	0.35	3.60	5.20	0.00	0.12	0.29	1.09
11	3.21	0.15	8.23	2.09	0.00	0.01	1.18	0.58
12	0.04	0.04	0.75	0.08	0.00	0.06	0.09	0.12
Average	**6.46**	**1.86**	**19.32**	**6.49**	**0.20**	**1.28**	**2.07**	**3.68**

**Values are average analyte responses relative to an internal standard, Hydrocodone-D6, which was present in every sample. This is meant to demonstrate relative abundance only. Patients samples were prescribed and tested positive for quetiapine in a current validated confirmation method. Quetiapine carboxylic acid is the most abundant metabolite followed by 7-Hydroxy Desalkyl Quetiapine, N-Desalkyl Quetiapine, and Quetiapine Sulfoxide. The overall identification scores of the quetiapine carboxylic acid and quetiapine sulfoxide were higher than the 7-hydroxy desalkyl quetiapine and N-desalkyl quetiapine, giving higher confidence in the identification.*

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Results Continued

Table 2. depicts results from unhydrolyzed and hydrolyzed samples from 114 patients prescribed Seroquel®. These results establish the importance of performing hydrolysis in testing for Seroquel®, with 95% and 96% increases in detection of quetiapine and 7-hydroxy quetiapine after hydrolysis, respectively. Quetiapine carboxylic acid was the most abundant metabolite, demonstrating its significance in Seroquel® positivity. The greatest overall positivity rates, 48% and 50%, are in agreement with the reported noncompliance rates of ~60% in schizophrenia and 48.1% to 69% in bipolar disease.

Table 2. Results from Patient Samples Prescribed Seroquel® Before and After Hydrolysis

Statistics	Pre-Hydrolysis Results				Post-Hydrolysis Results			
	Quetiapine	7-Hydroxy Quetiapine	Quetiapine Carboxylic Acid	Quetiapine Sulfoxide	Quetiapine	7-Hydroxy Quetiapine	Quetiapine Carboxylic Acid	Quetiapine Sulfoxide
Average Concentration*	16	37	2105	84	284	84	1722	98
Standard Deviation	14	37	4129	84	403	124	2937	107
Maximum Concentration*	60	161	23950	289	1939	592	11404	466
Median Concentration*	10	20	374	53	109	40	347	56
Minimum Concentration*	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
% Positivity Rate	18%	31%	47%	35%	39%	37%	50%	34%
	31%		47%		41%		50%	
	48%				50%			
Average % Increase Post-Hydrolysis	N/A	N/A	N/A	N/A	95%	96%	-2%	-19%

These results are from 114 random patients who were prescribed Seroquel® and run on a method testing for quetiapine and the three metabolites 7-hydroxyquetiapine, quetiapine carboxylic acid, and quetiapine sulfoxide with and without hydrolysis. There is a significant increase, ~95% and ~96%, in the concentration of quetiapine and 7-hydroxyquetiapine, respectively post hydrolysis. This also shows the immediate impact of the quetiapine carboxylic acid on positivity of patients. The overall greatest positivity rates achieved, 48 & 50%, align with the reported noncompliance rates of ~60% in schizophrenia and 48.1% to 69% in bipolar disease.

**All Concentrations are in ng/mL*

Samples from 12 patients prescribed Latuda® were tested on LC-QTOF and lurasidone as well as eight of its metabolites were identified. Lurasidone and hydroxylurasidone were recognized as the best analytes for monitoring lurasidone usage. Metabolites M-21 and M-22 do not have standards available so a method for monitoring lurasidone was developed after further investigation of hydroxylurasidone.

Twenty patients which were administered the lowest chronically administered dose of Latuda® (20 mg/day) were analyzed in triplicate over three days to test a method developed for monitoring lurasidone usage. Lurasidone, hydroxyl lurasidone, S-methyl lurasidone (M21), and S-methyl hydroxy lurasidone (M22) were monitored. The results are consistent with data from patients using higher doses of Latuda®.

Haldol® studies showed that while haloperidol is reported to not be conjugated, significant haloperidol was liberated after hydrolysis. Refer to the study "Enzyme Hydrolysis of Haloperidol Glucuronide; A Major Urine Metabolite of Haldol®" for more information.

Conclusions:

Uromics studies using LC-QTOF can yield more accurate metabolite profiles than metabolomics studies using GC/MS. While Seroquel® compliance is normally measured by monitoring N-desalkyl quetiapine and 7-hydroxy quetiapine, this study showed that monitoring quetiapine carboxylic acid and quetiapine sulfoxide in urine gives accurate results. By monitoring these additional metabolites and performing hydrolysis, the percent of quetiapine positivity is significantly increased. Testing Seroquel® samples with GC/MS can be problematic since the carboxylic acid and sulfoxide metabolites are not compatible with the high temperatures and extraction techniques, leading to the choice of N-desalkyl quetiapine or 7-hydroxy quetiapine as the ideal analytes for monitoring.

The most abundant Latuda® metabolites are historically reported to be smaller fragments, but this study demonstrated that lurasidone itself is present in high enough quantities to indicate positivity in most patient samples, with the additional monitoring of hydroxylurasidone improving the detection of lurasidone usage. The metabolites M-21 and M-22 would increase detection further, but standards are not yet available for these compounds.

This information was summarized by IMCS from the poster "Uromics: Metabolomics in Urine for Seroquel®, Latuda®, and Haldol®" presented by Erin C. Strickland - Amerityx, Ltd. at MSACL 2016