TECHNICAL NOTE

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A summary of "Enzyme Hydrolysis of Haloperidol Glucuronide; A Major Urine Metabolite of Haldol®"

Overview

Haloperidol (Haldol®) is an antipsychotic drug commonly used to treat schizophrenia and other mental health issues. Compliance to haloperidol prescription is poor, with schizophrenia patients reported at 19% adherent and overall patient adherence at about 63%. The current method for monitoring haloperidol usage involves monitoring haloperidol itself. The drug is reported to be present in urine at less than 1%, along with one or more metabolites present in very low levels. Haldol® is also reported to have no glucuronidation of the parent drug. Monitoring Haldol® usage with such low levels of haloperidol and its metabolites heightens the possibility of obtaining false negatives for patients prescribed haloperidol. The attainment of false negatives can misguide a physician to make an unnecessary or unsafe change in a patient's prescription.

There are three main forms in which haloperidol is available: tablets, oral solution, and injectable solutions containing either haloperidol or its decanoate derivative, which is used for longer-lasting effects.

Materials and Methods

Patients prescribed Haldol® or who previously tested positive for haloperidol were screened on an Agilent LC/QTOF to determine the presence of conjugated metabolites. Hydrolysis was performed on the samples at 60° C for 60 minutes using IMCSzyme®, a genetically modified β-glucuronidase, to verify metabolite glucuronidation. Other patient samples were tested with and without hydrolysis. Before hydrolysis, samples were diluted five-fold with 400 µL of a master mix containing internal standard (0.2 µg/mL of haloperidol-D4), 0.2 M phosphate buffer pH 7.5, and ~7,500 U/mL IMCSzyme®. Analysis was carried out 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 ramped to 90 % mobile phase B (100% methanol) over 2.8 minutes at a flow rate of 0.8 mL/min. TQ-MS was acquired by multiple reaction monitoring in positive ESI mode with a source temperature of 150 °C, a desolvation temperature of 600 °C, desolvation gas flow of 1,200 L/hr, and cone gas flow of 100 L/hr.

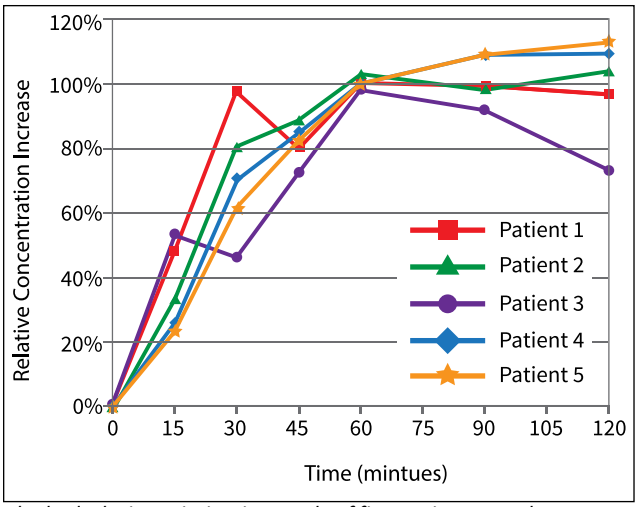
Results

Table 1. Patient samples tested with and without hydrolysis

	Without Hydrolysis			With Hydrolysis		
Dose Type	Negative	Positive	% Positive	Negative	Positive	% Positive
Injectable Haloperidol Decanoate	17/56	36/56	64%	0/20	20/20	100%
Injectable Haloperidol Solution	59/178	119/178	66%	3/35	32/35	94%
Haloperidol Tablets	284/646	362/646	56%	26/181	155/181	85%
Haloperidol Oral Solution	N/A	N/A	N/A	73/106	33/106	31%

There is a significant presence of haloperidol glucuronide. The hydrolysis has immense effects on patient positivity, with a large increase in % positivity of samples with hydrolysis regardless of dose type.

Figure 2. Hydrolysis Time Study



The hydrolysis optimization study of five patient samples. After 60 minutes at 60° C, 100% hydrolysis of haloperidol was achieved.

Conclusions

Though haloperidol has previously been reported to remain unconjugated after metabolism, this study has proven that it is in fact glucuronidated and that performing hydrolysis on patient samples prior to an analysis with LC/QTOF significantly increases true positivity. Performing hydrolysis on samples from patients prescribed Haldol® will monitor their usage more accurately and avoid the acquisition of false negatives.

This information was summarized by IMCS from the technical poster "Enzyme Hydrolysis of Haloperidol Glucuronide; A Major Urine Metabolite of Haldol®" presented by Erin C. Strickland, Amertiox, Ltd. at MSACL 2016