POSTER SUMMARY

integrated micro-chromatography systems

A summary of " Development of an LC/MS-MS Naloxone Hydrolysis Assay Using a Recombinant Beta-Glucuronidase for Urinalysis"

Overview:

Naloxone is a synthetic narcotic that acts as an opioid antagonist countering the effect of opioid overdose. It is also co-formulated with buprenorphine (Suboxone®, Zubsolv®, Bunavail™ buccal film) to prevent misuse and to produce withdrawal symptoms if the combination formulation is altered or naloxone is administered parenterally. A pain medication monitoring laboratory evaluated IMCSzyme® recombinant β-glucuronidase hydrolysis efficiency for the naloxone assay, which deconjugates the major urinary metabolites of naloxone, nornaloxone (noroxymorphone), and 6β-naloxol. The study reported ~70% hydrolysis efficiency at 65 °C for 60 minutes, with satisfactory precision and reproducibility.

Material and Methods:

Drug free urine was fortified with naloxone-3-glucuronide at 3,000 ng/mL to liberate 1,952.6 ng/mL of naloxone upon complete hydrolysis. A total of ≥ 1,631 units of IMCSzyme® recombinant β-glucuronidase was present in each sample. Hydrolysis efficiency was assessed in ten replicates across 3 days at 55 °C and 65 °C with incubation times of 0, 15, 30, 45, and 60 minutes. The optimal hydrolysis method at 65 °C for 60 minutes was validated with from patients who were prescribed Suboxone. Analysis was completed on a Waters Acquity TQD UPLC®-MS/MS system. Analytes were separated chromatographically at 50 °C in a 2.1-minute gradient using a Waters Acquity UPLC® BEH Phenyl, 1.7 µm, 2.1 x 50 mm, 100 Å column. Data was processed using Waters MassLynx software.

Results

Limits and linearity were assessed in five replicates of each of eleven concentrations, ranging from 5 ng/mL to 10,000 ng/mL. The LOQs and LODs for naloxone and 6β-naloxol were 5 ng/mL. Precision and accuracy were also tested in 10 replicates of each of three concentrations (75, 750, and 7,500 ng/mL) for inter- and intra-day reproducibility. Data was acceptable with accuracies within ± 12.61% and %CVs less than 12.59%.

Table 1: Hydrolysis Efficiency & Correction Factor Results

Compound Name	Replicates	Day 1	Day 2	Day 3	Intraday
		1953 ng/mL			
Naloxone-3-glucuronide (3000 ng/mL)	1	1461.7	1416.6	1442.1	1359.0
	2	1354.6	1301.2	1367.9	1392.3
	3	1300.3	1426.7	1412.0	1402.6
	4	1276.1	1393.3	1428.0	
	5	1422.7	1430.1	1422.3	
	6	1376.8	1383.9	1427.8	
	7	1336.0	1376.1	1446.8	
	8	1284.4	1404.9	1261.6	
	9	1368.8	1400.3	1399.6	
	10	1408.7	1389.5	1417.5	
	Mean	1359.0	1392.3	1402.6	1384.6
	%CV	4.29%	2.49%	3.68%	1.34%
	%Target	69.60%	71.30%	71.83%	70.91%
Correction Factor	Calculated/ Mean	1.437	1.402	1.392	1.410
	%CV				1.35%

The hydrolysis efficiency was performed similar to a precision & accuracy experiment where ten replicates of the hydrolysis control (3,000 ng/mL Naloxone-3-glucuronide – 1,953 ng/mL target concentration of naloxone) were run over the course of three days. A correction factor was applied to ensure that the calculated concentrations reflected 100% hydrolysis efficiency since the actual efficiency remained consistently ~70% hydrolysis. To calculate the correction factor the target concentration was divided by the mean for each day. The average of the correction factor for each day, 1.410, was used to ensure that the concentration for each sample reflected 100% hydrolysis efficiency.

The hydrolysis efficiency was performed similar to a precision and accuracy experiment where ten replicates of the hydrolysis control (3,000 ng/mL naloxone-3- glucuronide – 1,953 ng/mL target concentration of naloxone) were run over the course of three days (Table 1). A correction factor of 1.41 was applied to ensure that the calculated concentrations reflected 100% hydrolysis efficiency since the actual efficiency remained consistently at ~70% hydrolysis. The application of hydrolysis method on thirty six patient samples showed that all were positive for naloxone (concentration range 8.1 – 9,623.5 ng/mL) and 19 were positive for 6β-naloxol (concentration range < 5.0 – 186.6 ng/mL).

Conclusions

IMCSzyme® was a satisfactory choice for naloxone hydrolysis assay. Naloxone glucuronide is the most prevalent metabolite in urine relative to the other metabolites monitored. Application to patient sample analysis was 100% successful in detecting positivity of naloxone in all of the Suboxone patient samples tested.

References

1. R. C. Baselt. Disposition of toxic drugs and chemicals in man, 10th edition. Biomedical Publications, Seal Beach, CA, 2014.
2. W. B. Fang, Y. Chang, E. F. McCance-Katz, and D. E. Moody, Determination of naloxone and Nornaloxone (Noroxymorphone) by High Performance Liquid Chromatography-Electrospray Ionization, J. Anal. Tox. 33, 2009, 409-417.
3. P. Heikman, M. Haddinen, M. Gergo, and I. Ojanpera, Urine Naloxone Concentration at Different Phases of Buprenorphine Maintenance Treatment., Drug Treatment and analysis, 6, 2014, 220-225.

This information was summarized by IMCS from the poster "Development of an LC/MS-MS Naloxone Hydrolysis Assay Using a Recombinant Beta-Glucuronidase for Urinalysis" by Gregory L McIntire - Ameritox, Ltd. at SOFT 2015