

Demethylated Metabolites of Oxycodone and Hydrocodone in Human Hair and Nail Specimens

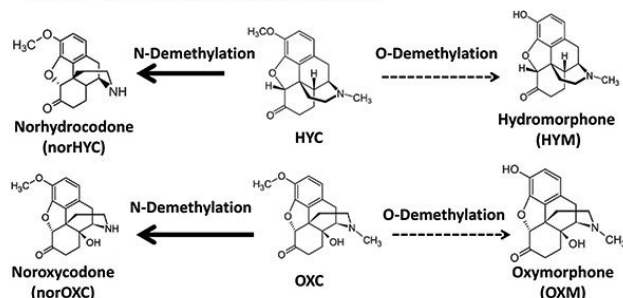
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OBJECTIVE

Evaluate demethylated metabolites in keratinized matrix as indicator suggesting long-term hydrocodone (HYC) and oxycodone (OXC) ingestion.

INTRODUCTION

- One difficulty of interpreting hair and nail drug testing is to distinguish environmental route of exposure from ingestion.
- N- and O-Demethylated metabolites of parent drugs are critical to make such differentiation.



METHOD

Sample Preparation

- 10-50 mg Hair and nail powder.
- Add 0.1M HCl; overnight incubation
- Single-point calibrator: 100 pg/mg

SPE

- Use UCT (CSDAU) clean screen extraction SPE column.
- Condition; load sample; wash; elute
- Evaporate sample; reconstitute for LC-MS/MS

LC-MS/MS

- Agilent 1100 HPLC system.
- Sciex 3200 Q-trap mass spectrometer (MRM)
- Phenomenex Hydro RP column 50x2 mm 2.5 μ m; 9.25-minute run time.

Definition of Positive Results

- HYC and OXC (quantitative measure): ≥ 100 pg/mg.
- Metabolites (qualitative measure): 1) S/N $\geq 3:1$; 2) mass ratio within acceptable range; 3) acceptable peak shape; 4) relative retention time to its internal standard within acceptable range.

Ion transitions

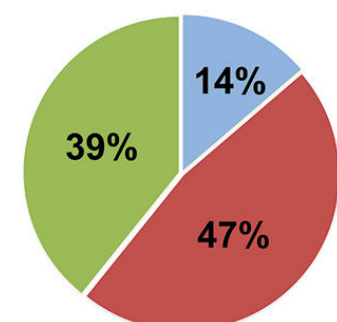
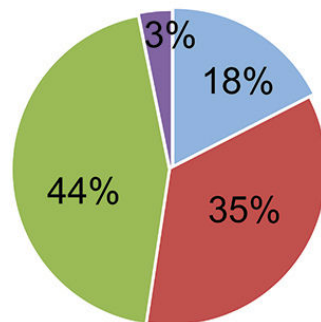
Analytes	Precursor Ion (Q1)	Product Ion (Q3)
D3 HYC	303.3	199.0
HYC*	300.2	171.1
HYC	300.2	199.4
NORHYC*	286.1	199.1
NORHYC	286.1	187.2
D3 HYM	289.3	185.2
HYM*	286.4	157.3
HYM	286.4	185.1
D3 OXC	319.2	301.2
OXC*	316.3	298.3
OXC	316.3	241.2
NOROXC*	302.1	248.2
NOROXC	302.1	227.1
D3 OXM	305.4	230.2
OXM*	302.4	227.3
OXM	302.4	198.1

* Used as quantifying ion transition.

DISTRIBUTION OF METABOLITE PROFILES IN HYC AND OXC POSITIVE CASES

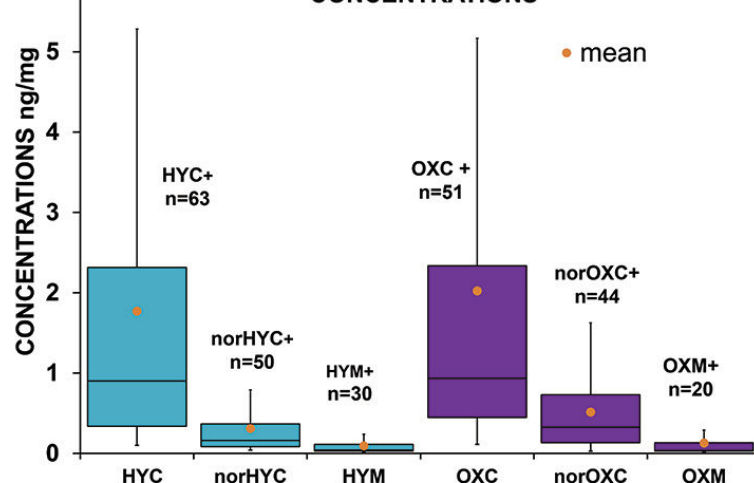
HYC POSITIVE CASES (n = 63)

OXC POSITIVE CASES (n = 51)



- HYC only
- HYC+ norHYC
- HYC+norHYC+HYM
- HYC+HYM
- OXC only
- OXC+ norOXC
- OXC+ norOXC +OXM

DISTRIBUTION OF PARENT DRUG & METABOLITE CONCENTRATIONS



RESULTS

- 63 Positive HYC positive keratinized specimens (≥ 100 pg/mg):**
 - 28 (44%) had both norHYC and HYM detected.
 - 22 (35%) had norHYC detected without HYM.
 - 11 (18%) had neither demethylated metabolites detected (HYC only).
 - 2 (3%) had HYM detected.
- 51 Positive OXC positive keratinized specimens (≥ 100 pg/mg):**
 - 20 (39%) had both norOXC and OXM detected.
 - 24 (47%) had norOXC detected without OXM.
 - 7 (14%) had neither demethylated metabolites detected (OXC only).

DISCUSSION & CONCLUSION

- Current HYC and OXC confirmatory method only included HYM and OXM for analyses.
- Including norHYC to the method increased the percentage of HYC positive cases suggesting long-term drug ingestion from 47% to 82%.
- Including norOXC to the method increased the percentage of OXC positive cases suggesting long-term drug ingestion from 39% to 86%.
- Future work is full validation of norHYC and norOXC quantitation to improve the current HYC and OXC confirmatory method.

REFERENCES

- E.J. Cone, Y.H. Caplan, D.L. Black, T. Robert, and F. Moser. Urine drug testing of chronic pain patients: licit and illicit drug patterns. J. Anal. Toxicol. 32: 530-543 (2008).
- S.H. Weinstein and J.C. Gaylord. Determination of oxycodone in plasma and identification of a major metabolite. J. Pharm. Sci. 68: 527-528 (1979).