Analysis of buphedrone and Phase I metabolites in mice urine

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Introduction

Synthetic cathinones, such as buphedrone (BUPH) or mephedrone, are members of a heterogenous family of new psychoactive substances (NPS) that exhibit unique neuropharmacological effects, analogous to amphetamine, cocaine and methamphetamine. Compounds from this class comprise over 130 substances and were first detected in Europe in 2004. Since then, they have rapidly and extensively appeared in the illicit market, surpassing controlled substance legislation and being responsible for many intoxications and overdose deaths worldwide [1]. This situation has fostered the attention of the clinical, forensic and scientific community in order to better understand cathinone metabolism, and find out potential new markers to estimate drug consumption and confirmation of drug use.

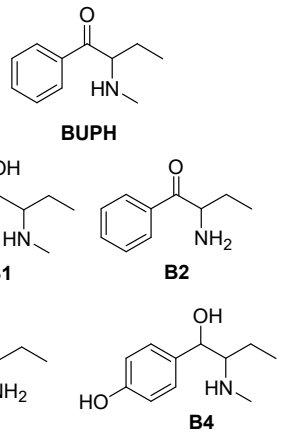
Objectives

- Synthesis and characterization of BUPH and metabolites derived from its *N*-dealkylation, *β*-keto reduction and/or 4-aryl hydroxylation
- Preliminary screening of mice urine, using high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) in full scan mode, to search for precursor ions corresponding to parent drugs and expected metabolites
- Identification and quantification of BUPH and corresponding Phase I metabolites, using tandem HPLC-MS (HPLC-MS/MS) in multiple reaction monitoring (MRM) mode, to achieve high selectivity and sensitivity

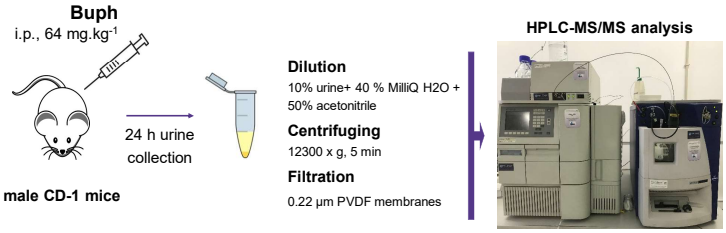
Experimental

Synthesis

- BUPH was synthesized by bromination of starting butyrophenone and subsequent reaction with methylamine
- Metabolites B1, B2 and B3 were chemically obtained following the synthetic procedures used in the synthesis of the parent drug for introduction of the amine group and using sodium borohydride for carbonyl reduction
- B4 was obtained following acetylation of 4'-hydroxybutyrophenone and subsequent basic hydrolysis during reaction with methylamine and sodium borohydride



***In vivo* assay**



Results

Characterization and optimization of MS/MS parameters

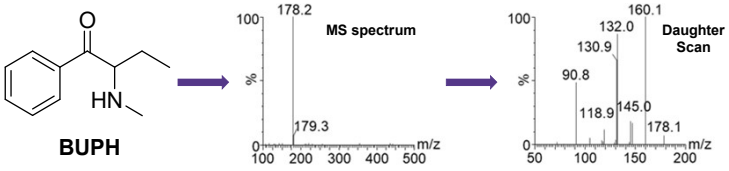


Table 1. Optimized MS/MS parameters for BUPH and metabolites. $[M+H]^+$: precursor ion; MRM1: quantification transition; C.E.: collision energy; MRM2: confirmation transition.

Compound	$[M+H]^+$	Cone Voltage/ V	Product ions	MRM1 (C.E./ eV)	MRM2 (C.E./ eV)
BUPH	178	12	91, 119, 131, 132, 145, 147, 160	178 > 160 (15)	178 > 132 (15)
B1	180	12	91, 107, 131, 133, 162	180 > 162 (12)	180 > 133 (15)
B2	164	12	91, 117, 118, 119, 146, 147	164 > 118 (12)	164 > 91 (12)
B3	166	12	91, 106, 131, 148	166 > 148 (10)	166 > 131 (10)
B4	196	12	107, 147, 149, 178	196 > 178 (9)	196 > 147 (12)

Quantification of BUPH and metabolites in mice urine

- The most excreted metabolite, B2, results from *N*-dealkylation of BUPH (80.3±11.4 µg.mL-1)
- B3, the *N*-dealkylated alcohol (59.6±4.7 µg.mL^{-1}), and B1, *N*-alkylated alcohol (5.65±0.48 µg.mL^{-1}) were also detected

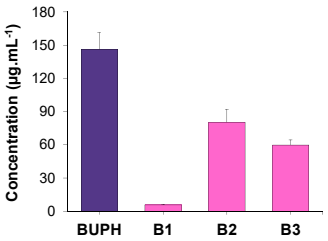


Figure 1. Quantification of BUPH and metabolites in 24 h urine samples from mice single-administered with BUPH. Results are presented as mean concentration ± SEM (µg.mL^{-1})

Conclusion

- BUPH and metabolites derived from its N-dealkylation and β-keto reduction were detected and quantified in 24 h urine samples from mice administered with a single drug dose of 64 mg.kg^{-1} (i.p)
- To the best of our knowledge, this is the most complete study on the metabolism of buphedrone regarding the quantification of metabolites
- Future studies should focus on the study of Phase II metabolites in order to find out if some of these compounds are excreted as glucuronide or sulfate conjugates

Acknowledgements

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Disclosure

The authors declare that they have no conflict of interest