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Simultaneous Determination of Tasimelteon, M9, M11, M12, M13, and M14 in Human Plasma by UPLC-MS/MS and its Applications

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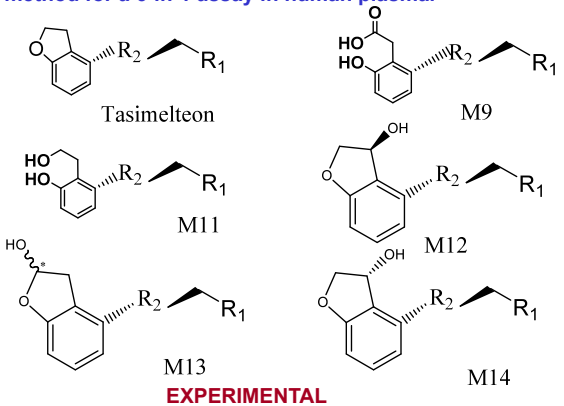
INTRODUCTION

Tasimelteon is a specific and potent agonist of melatonin receptors. It is in development for the treatment of mood and circadian rhythm disorders and currently in clinical trials. To obtain the plasma concentrations and the pharmacokinetic parameters of tasimelteon and five of its metabolites, having a reliable bioanalytical method for simultaneous determination of the six analytes is highly desirable in terms of throughput and cost saving. However, the combination of having three isomers and the diverse polarities of analytes presents analytical challenge. The chromatographic separation power of UPLC and its benefit of enhance sensitivity seems a perfect solution at hand. Here, we report the development and validation of a reliable UPLC-MS/MS method for a 6-in-1 assay in human plasma.



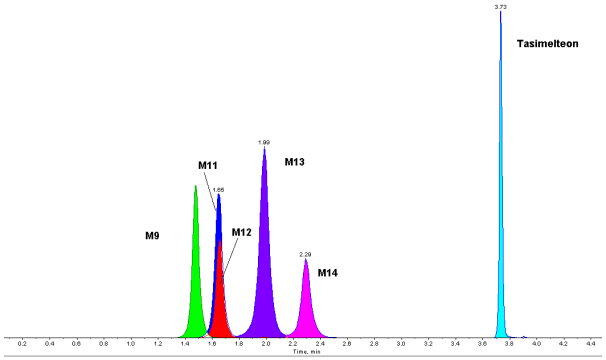
EXPERIMENTAL

Tasimelteon, M9, M11, and M13 have their corresponding stable isotope labeled internal standards. M13-d3 was chosen as the internal standard for M12 and M14 due to their high degree of structural similarity. Tasimelteon was de-tuned to maintain linearity within the assay range. Water-loss product ions of M12, M13, and M14 were chosen as parent ions to achieve the needed sensitivities. A liquid-liquid extraction method with optimized pH buffer was developed to obtain consistent and sufficient extraction recovery for the six analytes. The UPLC-MS/MS total run time is 6 minutes.

SAMPLE PREPARATION

200 µL of human plasma was spiked with cocktail internal standards with pH 3 buffer and immediately processed by liquid-liquid extraction with 3 mL of MtBE. The supernatant was evaporated and reconstituted with 200 µL of reconstitution solvent for LC-MS/MS analysis.

Mass Spectrometry: Sciex, API 4000/API5000. TIS Positive
UPLC: Waters Acquity UPLC
Mobile Phase: A: H2O:FA/ 100:0.1; B: MeOH:FA/ 100:0.1
Program: Gradient. Starting @15%B; ramping to 100%B.
Flow rate: 0.7 mL/min
Column: Waters CSH Fluoro-Phenyl (2.1 x 50 mm, 1.7 µm)



REPRESENTATIVE CHROMATOGRAMS

APPLICATION and OBSERVATION

- The validated method shows adequate selectivity, sensitivity, specificity, accuracy, and reproducibility for analysis of Tasimelteon, M9, M11, M12, M13, and M14 in the human plasma and was applied for the first clinical study.
- Incurred sample reanalysis passed for Tasimelteon, M9, M11 and M13, but failed for M12 and M14 in the first sample analysis study.
- Investigation was initiated. It was found that M13-d3 did not work well as the internal standard for M12 and M14 in the study samples. Interestingly, Tasimelteon-d3 was tested and found to serve better as the internal standard for both M12 and M14 in the standards/QCs as well as in the study samples as indicated in the ISR result comparison table below.

M13-d3 as IS for M12 and M14	Tasimelteo-d3 as IS for M12 and M14
40% Passed	100% Passed

- Validation runs were reprocessed by changing the IS for M12 and M14 from M13-d3 to tasimelteon-d3. It was also confirmed that the change of IS did not impact any tests performed or the validity of the method.

CONCLUSIONS

- A "6-in-1" UPLC-MS/MS method was developed and validated for the measurement of tasimelteon, M9, M11, M12, M13, and M14 in human plasma
- Six analytes have been shown to be stable in human plasma under all conditions tested.

-- Freeze/Thaw stability: 5 cycles at -70°C
-- Bench-top stability at room temperature: 19 hours
-- Reinjection reproducibility : 263 hours at 4°C
-- Long-term frozen stability: 108 days at -70°C

- The modified method has been successfully applied to several clinical studies. Incurred sample reanalysis demonstrated excellent reproducibility for all analytes.
- The method also demonstrated the following:

-- Structural similarity should not be the only criteria for choosing analogue IS;
-- Advantage of having stable isotope labeled internal standard for an LC-MS/MS assay;
-- Benefit of performing incurred sample reanalysis to check assay reliability.