Degradation Products Profiling of Mycophenolate Mofetil using UHPLC and High Resolution Benchtop Mass Spectrometer

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Introduction

Stress testing provides information about drug substances and drug products under the influence of various environmental factors. It can provide drug safety information, determine recommended storage conditions, shelf life, and the specificity of analytical methods. Information on degradation products can lead to the development of improved compounds or formulations.

Mycophenolate Mofetil (MMF), brand name CellCept, is an immunosuppressant and prodrug of Mycophenolate Acid (MPA). MMF is used extensively in transplant medicine for treatment of organ transplant rejection and autoimmune diseases. MMF was used as model compound in this study.

A comprehensive workflow for rapid and confident degradation profiling of Mycophenolate Mofetil has been developed by employing Thermo Fisher Scientific Accela™ UHPLC system and bench-top Orbitrap Q Exactive™ mass spectrometer, with data processing software Mass Frontier™. Thermal and peroxide-catalyzed degradation study of Mycophenolate Mofetil API at pH 2.0, 3.5, 6.0, and 8.2 were carried out. Rapid degradation product profiling was achieved by utilizing HRAM full scan and data dependant MS/MS at high resolution with polarity switching. The HRAM MS and MS/MS spectra, as well as positive/negative switching, enable confident degradation product identification and structure elucidation.

Experimental

Material and Reagent

Mycophenolate Mofetil API CAS# 128794-94-5 was obtained from 2A PharmaChem (Lisle, IL) and qualified purity as >98%.

Mycophenolate Mofetil degradants Standards

Mycophenolic Acid, CAS# 24280-93-1 Sigma-Aldrich.

Mycophenolate Mofetil N-Oxide, CAS# 224052-51-1 Molcan Corporation Toronto, Canada

O-Desmethyl Mycophenolic Mofetil, CAS# 1322681-36-6 Molcan Corporation Toronto, Canada

Hydrochloric acid, ammonium acetate, sodium hydroxide, and hydrogen peroxide were obtained from Sigma-Aldrich.

Sample Preparation

- Mycophenolate Mofetil stock solution, 2.5 mg/ml in Acetonitrile, was prepared by dissolving 25 mg in 10 mL Acetonitrile.
- Hydrochloric acid (0.01 N, pH 2), ammonium acetate (5 mM, pH 6), sodium hydroxide (0.01 N pH 8), and hydrogen peroxide (3%, pH 8.2. commercial hydrogen peroxide 30% was diluted 10-fold with water) were used for acid, base and oxidation studies. The stress solution pH was adjusted with Ammonium Hydroxide or Acetic acid.
- 0.25 mg/mL stress solutions were prepared by 10-fold dilution of 2.5 mg/mL Mycophenolate Mofetil stock solution with pH 2, pH 6, pH 8.2 and hydrogen peroxide (3%, pH 8.2). The stress solutions placed in oven at 60 ºC. Samples were taken at 1, 20, 48 and 72 hours and directly analyzed by LCMS.

HPLC Method

HPLC system: Thermo Accela 1250 pump, Open Accela Autosampler and Accela PDA
Column: Thermo Dionex Acclaim 2.1x 150 columns, 2.2 µm,
Column heater temp: 35 °C
Mobile phases: A - H_2O with 0.1% formic acid
B - Acetonitrile with 0.1% formic acid
C - H_2O with 100 mM Ammonium Format at pH 5
Gradient : 20% B from 0 to 0.5 min, then a linear gradient to 40%B in the next 15 min.
Flow rate: 500 µL/min
Injection volume: 1 µL.

Mass Spectrometry Method

Mass spectrometry: Q Exactive Benchtop HR MS equipped with HESI-II source.
Ionization: ESI Positive\Negative switching
Full Scan acquisition (FMHW at m/z 200) : m/z120-1000 amu at Resolution of 70,000
MS/MS acquisition:
data dependent by intensity at resolution of 35,000
HCD Normalized collision energy:
35% with 25% ramping

Results and Discussion

I. UHPLC-High Resolution MS for Rapid Degradation Profiling

Rapid degradation profiles were achieved by UHPLC-high resolution MS analyses. The high quality HRMS full scan spectra provide important information for determining elemental compositions of degradants, and to obtain HR fragmentation information of molecular ions from subsequent data dependent MS/MS.

Figure 1. Total Ion Chromatograms of MMF at pH 2.0, 60 ºC in 1, 20, 48 and 72 hours reaction time.

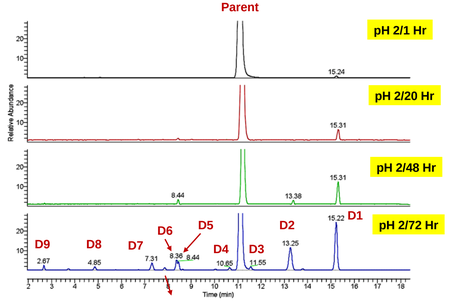

Figure 2. Total Ion Chromatogram of MMF at pH 2.0, 3.5, 6.0 and 8.2 in the Presence of 3% Hydrogen Peroxide in One Hour Reaction Time

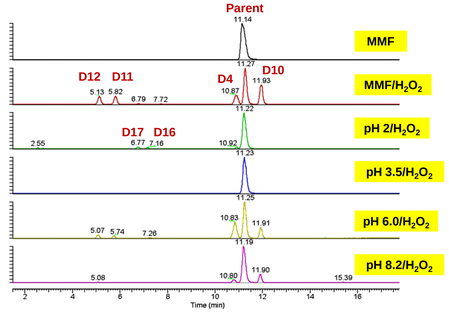

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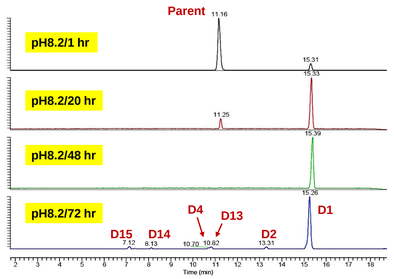
Figure 3 .Chromatograms of MMF at pH 8.2, 60 ºC at of 1, 20, 48 and 72 hours.



II. High Resolution MS/MS for Degradant Structure Elucidation

HRHCD fragmentation with collision energy ramping functionality generates information-rich fragments, which make it easier for degradant structure elucidation.

Figure 4. Full Scan and Data Dependant MS/MS Spectra of Degradant D11

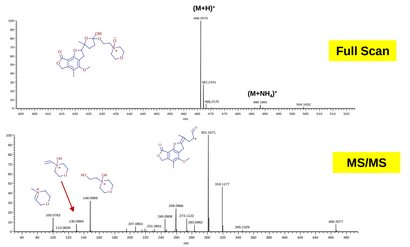


Figure 5. Full Scan and Data Dependant MS^2 Spectra of Degradant D5

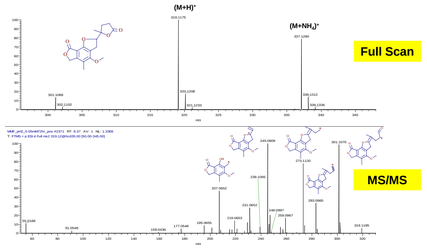


Table1. Degradants Identified Using HRAM Full Scan and Data Dependant MS/MS from pH 2.0 solution and Hydrogen Peroxide

Compound	Peak ID	Molecular Formula	Calculated (M+H)	Measured (M+H)	Error (ppm)
Parent	Parent	$C_{23}H_{31}NO_7$	434.2173	434. 2178	1.0
MF Acid	D1	$C_{17}H_{20}O_6$	321.1333	321.1331	- 0.4
Lactone	D2	$C_{17}H_{20}O_6$	321.1333	321.1331	- 0.4
Ester	D3	$C_{17}H_{20}O_6$	321.1333	321.1331	- 0.4
	D4	$C_{17}H_{18}O_6$	319.1176	319.1175	-0.47
**	D5&D15	$C_{17}H_{18}O_6$	319.1176	319.1175	-0.47
cis MMF	D6	$C_{23}H_{31}NO_7$	434.2173	434. 2175	0.40
	D7	$C_{17}H_{17}O_8$	349.0918	349.0915	-0.79
Aldehyde	D8	$C_{11}H_{10}O_5$	223.0601	223.0602	0.14
	D9	$C_{23}H_{31}NO_8$	450.2122	450. 2123	0.8
N-Oxide	*D10	$C_{23}H_{31}NO_8$	450.2122	450. 2123	0.8
**N-Oxide	*D12	$C_{23}H_{31}NO_8$	450.2122	450. 2123	0.8
	*D11	$C_{23}H_{31}NO_9$	466.2072	466.2074	0.52
	*D13	$C_{14}H_{12}O_5$	261.0758	261.0757	-0.4
**	D14	$C_{17}H_{18}O_6$	319.1176	319.1175	-0.47
**	D16	$C_{23}H_{32}NO_8Cl$	486.1889	486.1896	1.47
**N-Oxide	D17	$C_{23}H_{31}NO_8$	450.2122	450. 2126	0.83

* Observed from Hydrogen Peroxide only. ** Structures need to be confirmed by NMR

III. Polarities Switching for Comprehensive Degradant Identification

Rapid positive/negative switching experiments were carried out by acquiring a full scan in positive mode followed by data dependant MS^2 HCD fragmentation. This was followed by switching to negative mode full scan and by data dependant MS^2 HCD fragmentation. Polarities switching on the fly provides different, but complementary fragmentations for degradants. These polarity-unique fragments can aid in confident structure identification (Figure 6).

Figure 6. HRAM Full Scan and MS/MS spectra of Degradant D3

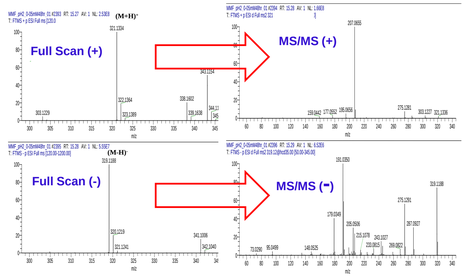
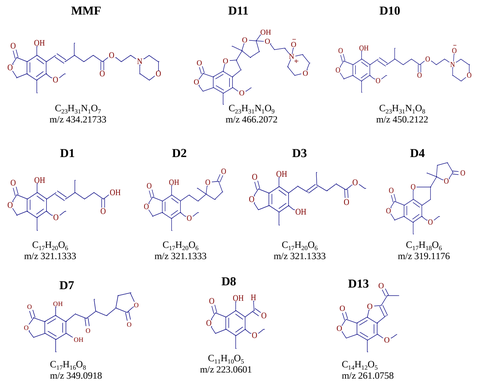


Figure 7. Proposed Structures of Degradation Products Identified by UHPLC- HRAM



Conclusion

Thermal and hydrogen peroxide degradation study of Mycophenolate Mofetil API at pH 2.0, 3.5, 6.0, and 8.2 were carried out. Rapid, confident degradation product profiling were achieved by utilizing benchtop high resolution Mass spectrometer Q Exactive coupled with UHPLC system, see figure 1,2 and 3.

➢The high resolution accurate mass measurement (HRAM) full scan spectra allow quick profiling – obtaining critical information of elemental composition of degradants.

➢ Information-rich higher energy collision dissociation (HCD) MS/MS spectra facilitate confident structural elucidation of degradants.

➢ Comprehensive degradation product identification was enabled by positive/negative switching in full scan and MS/MS modes, see figure 4 and 5.

➢ Data analysis software Mass Frontier great improved the speed and confidence of structural elucidation of degradants.

The functionalities of Q Exactive bench-top Orbitrap MS enable fast and efficient degradants profiling in an all-in-one UHPLC/HR-MS/MS platform, which can significantly increase the throughput of degradant identification in drug discovery and development.

References:

1. M. Brandl, Degradation Products of Mycophenolate Mofetile in Aqueous Solution Drug Development and Industrial Pharmacy, 25(3), 361-365 (1999)
2. FDA Guidance for Industry. Analytical Procedures and Methods Validation (draft guidance), August 2000.
3. ICH guidelines Q1A (R2). Stability Testing of New Drug Substances and Products (revision 2), November 2003.