ANALYTICAL METHODS FOR ESTIMATION OF MYCOPHENOLIC ACID IN BULK AND IN PHARMACEUTICAL DOSAGE FORM: A REVIEW

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ABSTRACT
Mycophenolic acid is an anti-metabolite immunosuppressant. It also inhibits the enzyme inosine monophosphate dehydrogenase; essential for purine synthesis. High performance liquid chromatography (HPLC) and the UV are an essential analytical tools in assessing drug product. HPLC methods should be able to isolate, detect, and enumerate the various drugs and drug associated degradants that can form on storage, or manufacturing. It should also detect and enumerate any drugs and drug-related impurities that may be introduced during synthesis. Validation is the process of establishing the performance characteristics and limits of a method and identification of the effects which may change these features and to what extent. This article discusses the current and potential uses of the drug mycophenolic acid as well as the plans and the subjects related to designing UV and HPLC method for development and validation.

Keywords: Mycophenolic acid, Immunosuppressant, HPLC, UV, Validation

Introduction:
Mycophenolic acid is an immunosuppressive drug used for prevention of rejection in solid organ transplantation. It is not only useful in preventing rejection, being even superior to azathioprine, but also seems to cause less adverse effects than other immunosuppressive drugs. [1] It is one of the few drugs, which were discovered more than a century ago and still in active use. The drug is currently used in patients with liver, lung and bone marrow transplantation. [2] Mycophenolic acid has also been used in renal, rheumatological, gastrointestinal, ophthalmological, dermatological and neurological autoimmune diseases. [2] It is a fungal metabolite that was initially discovered by Bartolomeo Gosio in 1893 as an antibiotic against anthrax bacillus, Bacillus anthracis.[3] MPA was not and is not used as an antibiotic because of its side effects profile and as there is availability of safer antibiotics. But, studies are still continued on its antibiotic action.[4]

Though mycophenolic acid remained out of clinical use for decades after discovery in 1983, the interest of researchers in the molecule continued. Fortunately, the efforts of researchers were not futile. MPA was approved in 1995 by USFDA for the prevention of rejection in renal transplant patients. MPA also possess antiviral [5] and antifungal activities. [6] Studies also reported antitumor, and antipsoriasis activities.[7, 8] MPA was therefore found as the broad-spectrum acting drug having antiviral, antifungal, antibacterial, anticancer, and antipsoriasis properties. [9] MPA also has antifibrotic effects.[10]

To advance oral bioavailability, MPA is administered as mycophenolate mofetil. [11] An oral dose of mycophenolate is hydrolyzed quickly during first pass metabolism to mycophenolic acid which is further metabolized to two minor metabolites namely acyl glucuronide (AcMPAG) and phenolic glucoside of MPA.MPA is highly bound to plasma proteins, mainly to human serum albumin(97-99%). [12]

Figure 1: Structure of Mycophenolic Acid
Mycophenolic acid is often used in unification with a calcineurin inhibitor (cyclosporine or tacrolimus) and prednisolone in the primary post-transplant period. [12] MPA hinders inosine monophosphate dehydrogenase, the enzyme that controls the rate
of synthesis of guanine monophosphate in the de novo trail of purine synthesis used in the proliferation of B and T lymphocytes.

“Chromatography” a overall term for a variety of physicochemical separation techniques all of which have in common the circulation of a component between a mobile phase and a stationary phase.

The method of HPLC flourished after it became possible to create columns with filling materials made of very small beads (10μm) and to utilize them under high pressure. The advance of HPLC and the theoretic understanding of the separation process rest on the basic works of Horvath, Knox, Scott, Snyder, Guiochon, Mockel, and others.

### Table 1: Methods for determination of Mycophenolic acid by RP-HPLC and other chromatographic techniques

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug</th>
<th>Method</th>
<th>Description</th>
<th>Reference</th>
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</table>
| 1       | Mycophenolic acid     | RP-HPLC method                              | Column : USP L7 Octylsilane chemically bonded to porous silica C8, (5 m) (4.6 x 250mm)  
Flow rate: 1.5ml/min.  
Mobile phase : Acetonitrile : Buffer (50: 50)  
Buffer: 0.1% v/v solution of Orthophosphoric acid.  
Diluent : Methanol  
Injection volume : 10g/ml  
Detector Wavelength: 254nm  
Temperature : 28ºC  
Retention time : 4.872 min | 13        |
| 2       | Mycophenolic acid     | RP-HPLC method                              | Column:- C18 (size-250 x 4.60 mm, I.D-5 μ) (Phenomenex)  
Flow rate: 1.2 mL/min.  
Detector Wavelength: 216 nm  
Mobile phase : tetra butyl ammonium hydrogen sulphate and methanol(52: 48, v/v)  
Linearity range: 0.5–160 μg/mL (r2 = 0.999)  
LOQ:0.321μg/mL  
LOD:0.102μg/Ml | 14        |
| 3       | Mycophenolic acid     | HPLC assay method                           | Mobile phase: Acetonitrile:  
Sodium acetate buffer (40:60 v/v)  
Column:- C18 (size-250 x 4.60 mm, I.D-5 μ) (Phenomenex).  
Flow Rate:- 1.0 ml/min.  
Detector :-250nm  
Recovery: 99.86–101.54%  
Injection volume: 20 μL. | 15        |
| 4       | Mycophenolic acid and its glucuronide metabolite | HPLC-tandem-MS (HPLC/MS/MS) and an HPLC-UV | Mobile phase: 20 mmol/l NaH2PO4 buffer (pH 3.0, adjusted with 20% phosphoric acid) and methanol (45:55, v/v)  
Column:- Zorbax column (250 mm 4.6 mm i.d, 5 mm)  
Column Temperature- 45ºC,  
Flow rate - 1.2ml/min  
Detector Wavelength: 304 nm  
Linearity- 0.2–50 mg/ml | 16        |
| 5       | Mycophenolic Acid     | LC-MS/MS                                    | Column : Zorbax RP-C18, 2.1=30 mm  
Linearity - 30, 15 and 17  
Mg/L, respectively.  
Imprecision -10%  
flow rate:- 500 mL/min  
Mobile Phase:- 2mmol/L ammonium acetate:water and methanol | 17        |
<p>| 6       | Mycophenolic acid     | HPLC-UV                                     | Mobile phase: 75% methanol and 25% ammonium. | 18        |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Method</th>
<th>Details</th>
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</table>
| 7   | Mycophenolate Mofetil, Tacrolimus with Prednisolone | **Mobile phase:** acetonitrile and 0.35% triethylamine (pH 4.2) with Orthophosphoric acid (70:30)  
**Column:** Kinetex Polar, C18, 5 μm, 4.6 × 250 mm  
**Injection volume:** 20 μL.  
**Linearity:** 10-100 μg/mL  
**Flow rate:** 1.2 mL/min.  
**Detector Wavelength:** 254 nm for Prednisolone and Mycophenolate and 210 nm for Tacrolimus. |
| 8   | HPLC-Ms/Ms | **Column:** Phenomenex Kinetex C18 (30 mm × 4.6 mm, 2.6 μm)  
**Mobile phase:** acetonitrile-water  
**Flow rate:** 0.4 mL/min  
**Spray voltage:** 3250 V  
**Capillary temperature:** 222°C  
**Sheath gas:** 30 arb. unit  
**Sweep gas:** 2 arb. Unit  
**Aux Gas:** 20 arb. Unit  
**Vaporizing temperature:** 324°C  
**Collision gas pressure:** 1.5 m Torr  
**Linear range:** 0.5-30 μg/mL  
**Accuracy and precision range:** 99.76 to 111.38% and from 2.54 to 9.01%, respectively |
| 9   | Mycophenolic Acid | **Mobile phase:** 54:46 (v/v) methanol-0.1% (v/v) aqueous trifluoracetic acid.  
**Flow rate:** 1.2 mL/min.  
**Column:** Kromasil C8  
**Column temperature:** 40°C  
**Detector Wavelength:** 325 nm |
| 10  | Mycophenolic acid | **Mobile phase:** 0.1M triethylammonium phosphate (pH=5.4)-acetonitrile (65:35, v/v)  
**Column:** C8 analytical (250mm 4.6mm, particle size 5μm; Perfectsill, MZ-Analysen technik, Germany)  
**Flow rate:** 1.5 ml/min.  
**Wavelength:** 304 nm  
**LOD:** 0.05 μg/ml  
**LOQ:** 0.2 μg/ml  
**Concentration Range:** 0.2-10 μg/ml |
| 11  | Mycophenolic Acid | **Mobile phase:** 450 ML acetonitrile/550 mL 20 mmol/L phosphate buffer, pH 4.5)  
**Flow rate:** 1.2 mL/min.  
**Run time:** 13 min  
**Retention time:** 5.7 min |
| 12  | Mycophenolate mofetil | **Mobile phase:** toluene, acetone, and methanol 6:2:2(V/V/V/V)  
**Detector Wavelength:** 254 nm.  
**Correlation coefficient:** 0.9998±0.0102  
**LOD:** 20.33 μg/ml  
**LOQ:** 60.72 μg/ml |
Mycophenolate Mofetil Capsule

**RP-HPLC METHOD**

- **Column:** Hypersil BDS C18 (150mm x 4.6 mm, 5μm)
- **Mobile phase:** buffer and Acetonitrile(650:350)V/V
- **Diluent:** Mobile phase
- **Flow Rate:** 2.00mL/min
- **Column Temperature:** 50⁰C
- **Sample temperature:** 20⁰C
- **Injection Volume:** 10µL
- **Wave Length:** 250nm
- **Run time:** 15 min
- **USP Plate Count:** 10348.12
- **Resolution:** 1.658551

References

18. Atcheson B, Taylor PJ, Mudge DW, Johson DW, Pillans PI, Tett SE. Quantification of free mycophenolic acid and its glucuronide metabolite in human plasma by liquid-chromatography using mass spectrometric and


