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Characterisation of magnesium ascorbyl phosphate, a raw material in cell therapy

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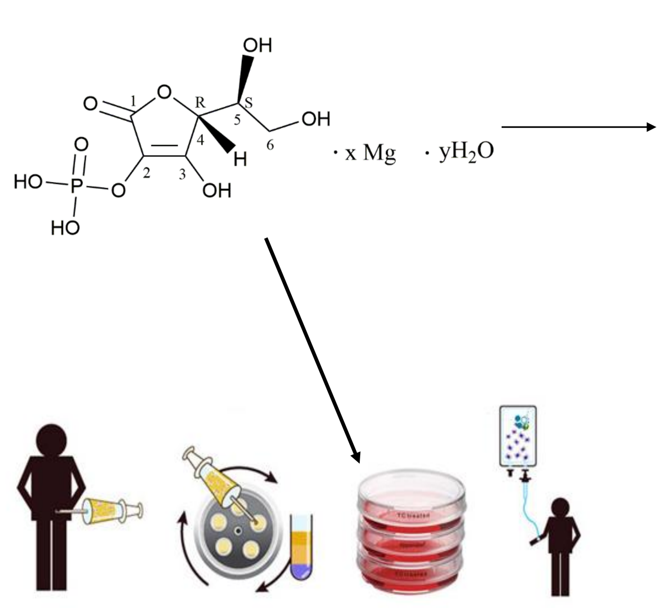
our reference:2018-224b

INTRODUCTION



Entry	CAS	Google hits
1	113170-55-1	10600 (81%)
2	23666-04-8	38
3	135379-37-2	5
4	260064-39-9	5
5	84309-23-9	203
6	185244-85-3	5
7	1486472-69-8	5
8	1713265-25-8	5
9	114040-31-2	2220 (17%)

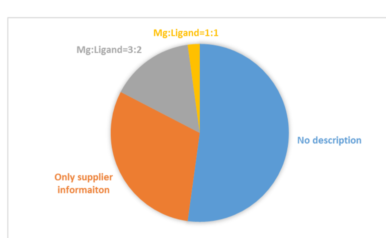


Figure. Scientific awareness to APMg difference: APMg informaiton from literature (sample pool=46)

Advanced therapy medicinal products (ATMPs), encompassing gene, cell and tissue engineering medicinal products, offer completely new perspectives for the treatment of severe diseases. However, the development of this new class of medicines is challenging, *i.a.* due to appropriate quality specification for the raw materials used during their manufacturing. Magnesium ascorbyl phosphate (APMg), with superior biological effects compared to ascorbic acid or sodium ascorbyl phosphate, is frequently used in cell culture as well as in the manufacturing of ATMPs.

PROBLEM: ANALYTICAL-CHEMICAL CHARACTERISATION?

EXPERIMENTAL

- **Stoichiometric composition**: magnesium assay by titration and atomic absorption spectroscopy (AAS), ligand assay by HILIC-HPLC, residual solvent by GC, water content by loss on drying (LOD) and thermogravimetric analysis (TGA)
- **Impurity profiling**: HILIC-HPLC coupled to LC-MS/MS and high resolution mass (HRMS)
- **Aqueous structure investigation**: the APMg structure was studied using potentiometric titration at different ligand-to-magnesium molar ratios

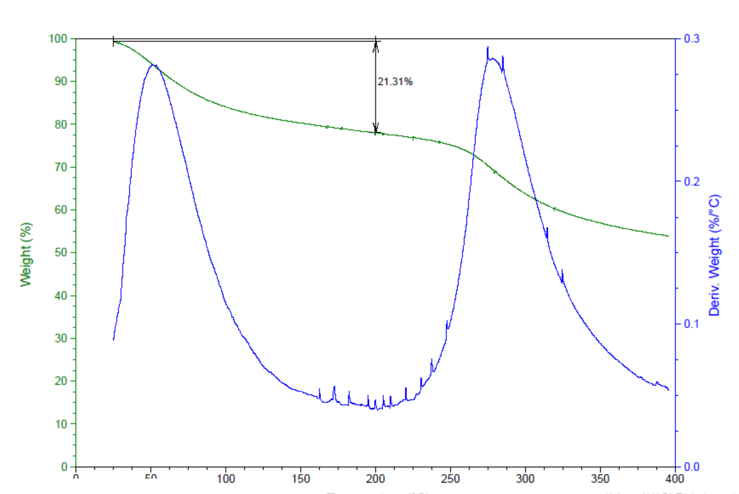
RESULTS and DISCUSSION

1. Stoichiometric composition (2 CAS numbers: Mg/Ligand=3/2 and 1/1)

8 batches of APMg obtained from the market were analyzed using different techniques.

1) Solvent:
   a) residual organic solvent: < RT (GC)
   b) water:
      LOD: 5.8-21.8%
      TGA: 12.7-22.5% →

FT-IR→TGA residue at 200°C: APMg remains intact
TGA residue at 400°C: APMg degraded

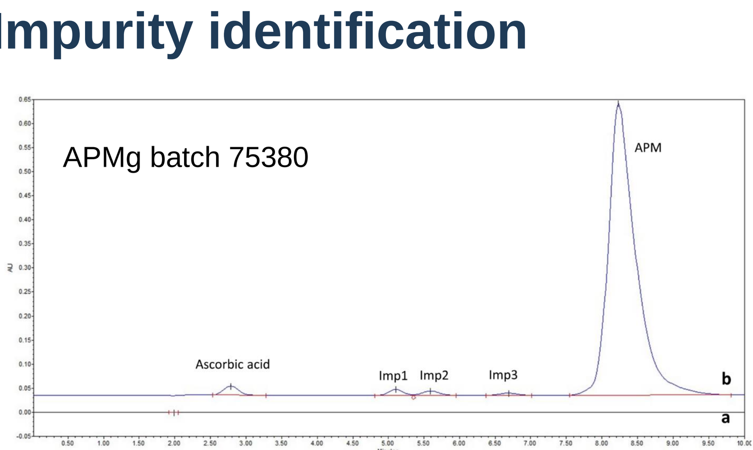


2) Mg content:
   a) AAS: 8.9-10.0%
   b) Titration: 9.2-10.1%

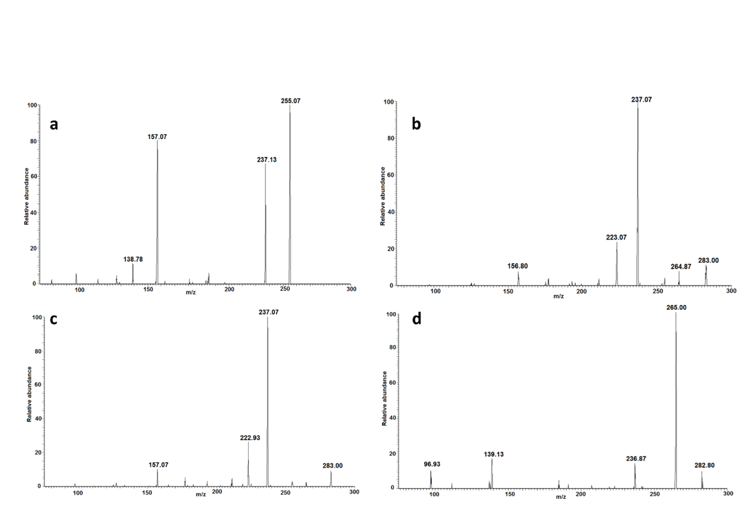
3) Ligand content (HILIC-HPLC): 61.0-75.5%

$(AP)_2Mg_3 \cdot n\ H_2O$ (n:2-5)

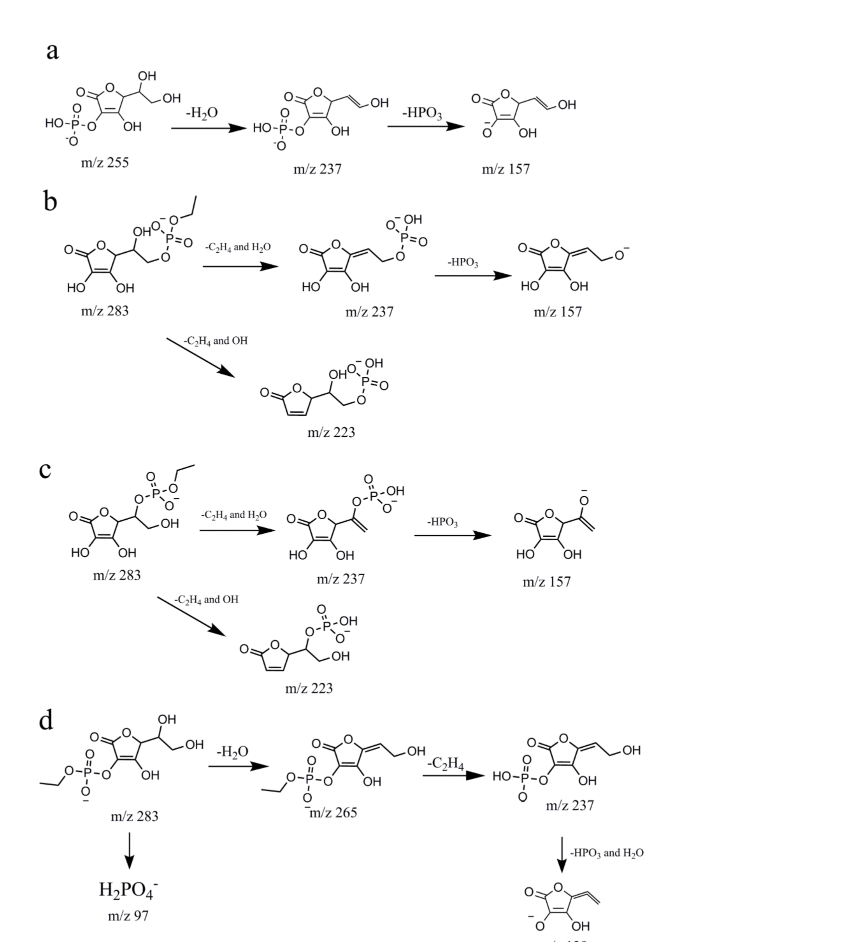
2. Impurity identification



MP: 15 mM **KH_2PO_4 buffer** (pH 2.5 with HCl): acetonitrile (30:70, v/v)
SP: Obelisc N column (3.2 × 150 mm, 5 µm) – 25°C
Flow rate: 0.4 mL/min
UV detection: 240 nm

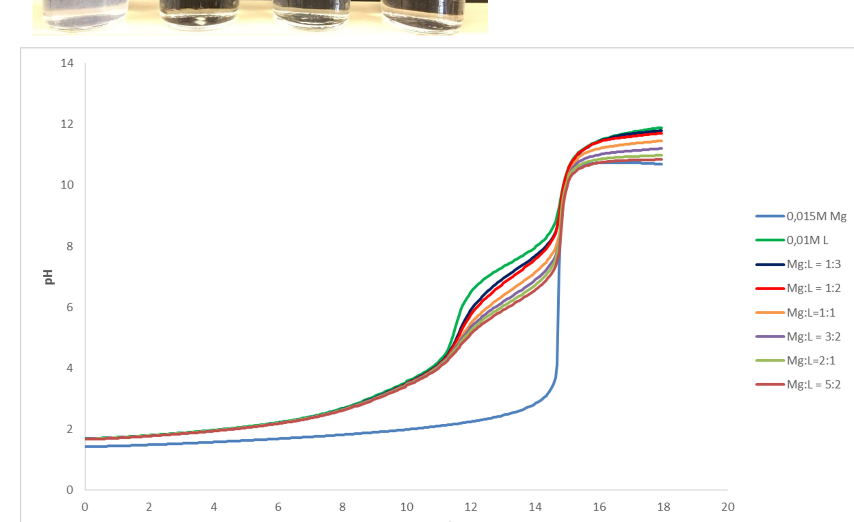


Compound	Ion formula	Found (Da)	Calculated (Da)	Error (mDa)
APM	$C_6H_8O_9P^-$	254.9902	254.9906	-0.4 mDa
Imp1	$C_8H_{12}O_9P^-$	283.0222	283.0219	0.3 mDa
Imp2	$C_8H_{12}O_9P^-$	283.0215	283.0219	-0.4 mDa
Imp3	$C_8H_{12}O_9P^-$	283.0212	283.0219	-0.7 mDa



3. Aqueous structure

Mg complex with ligand→Solubility increased



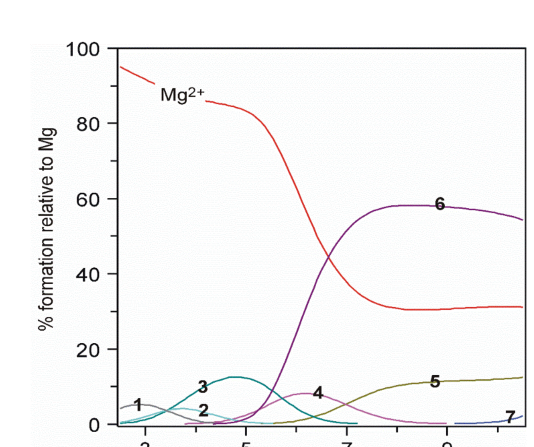


Figure. Species distribution curves as a function of pH for the complexes formed in Mg^{2+}/AP system at 3:2 metal-to-ligand molar ratio, C_{AP} = 0.01M. The numbers correspond to the complexes: 1-$[Mg(AP)_2H_6]^{2-}$, 2-$[Mg(AP)_2H_4]^{3-}$, 3-$[Mg(AP)_2H_3]^{2-}$, 4-$[Mg(AP)_2H]^{3-}$, 5-$[Mg(AP)_2]^{4-}$, 6-$[Mg_3(AP)_2]$, 7-$[Mg(OH)]^{+}$.

CONCLUSION

- APMg: different CAS number but stoichiometry inconsistent
- Different impurity profile: three unknown impurities identified as the ethylation products of APMg
- In the physiological condition: $Mg_3(AP)_2$ complex as main structure in solution

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