

# Quantification of Select Minerals in Animal Serum and Vitreous Humour by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)




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# Acknowledgements

- DAFM Funding of Academic Fees Scheme
- DAFM Laboratories, Backweston for providing facilities, equipment and materials
- Dr Sean Reidy (AIT), Dr Celine Mannion (Biochemistry / Toxicology Laboratory Senior Research Officer, DAFM)

## Project Background

- Mineral intake and balances are essential in maintaining good animal health and wellbeing.
- Mineral deficiencies and toxicities can have a detrimental impact on animal health.
- Important to diagnose these occurrences quickly particularly in animals of agricultural significance.
- DAFMs strategic plan involves promotion and safeguarding of public health, plant health and animal health and welfare.

# Project Background

- The Biochemistry / Toxicology laboratory provides specialist diagnostic support assisting in the diagnosis of animal clinical diseases or conditions and exposures to environmental pollutants.
- Recent investment in new analytical technologies to enhance the laboratories analytical capabilities
- To provide:
  - ➔ faster analytical turnaround times
  - ➔ greater automation
  - ➔ higher through-put
  - ➔ improved data quality and integrity

# Project Aims

- To develop and optimise a robust multi-element method for the analysis of trace (Zn, Fe) and macro minerals (Mg, Ca, K, P, Na) in animal:
  - ➔ Serum
  - ➔ Vitreous humour
- Animal species include: bovine, ovine, porcine, avian and equine
- Perform a comparative field study on samples of serum Vs vitreous humour.
- Validate developed analytical methods for animal serum and vitreous humour according to ISO 17025.

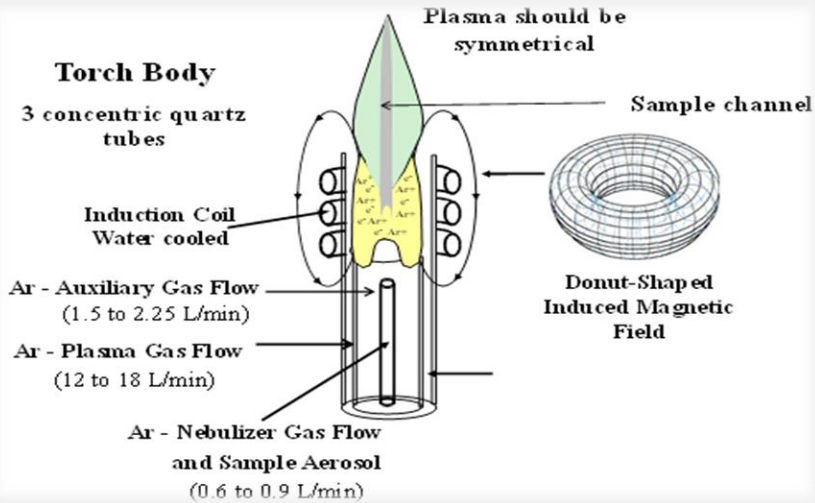
# Equipment Used – Agilent 5100 VDV ICP-OES



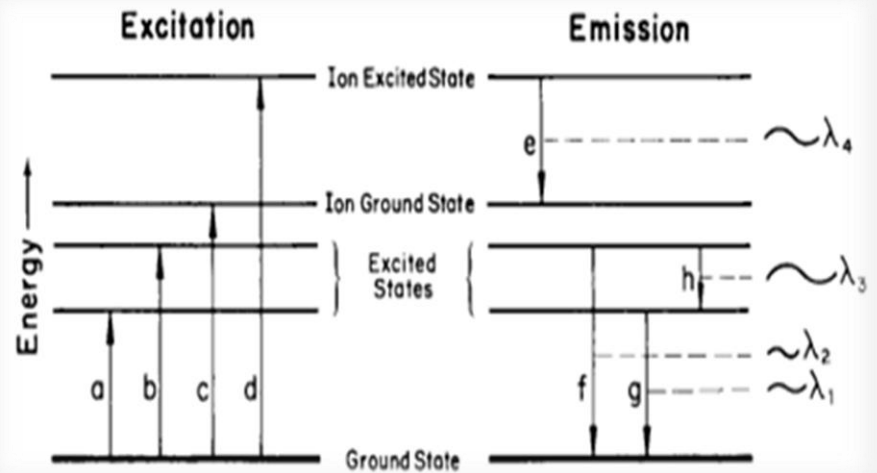
Vertical Dual View Optical Emission Spectrometer coupled with SPS4 Auto-sampling Unit

# Principle of Operation

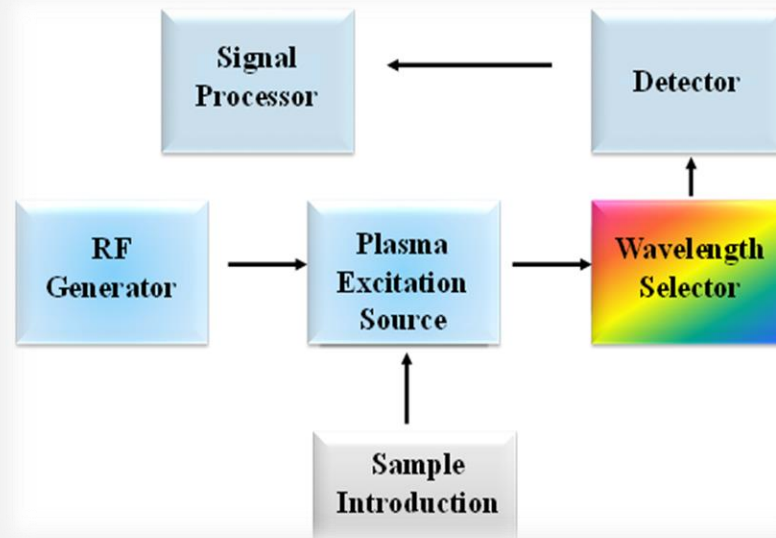
A



B



C



Concentration Track Analysis Sort Results... Hide Columns... Column Properties... Delete Results

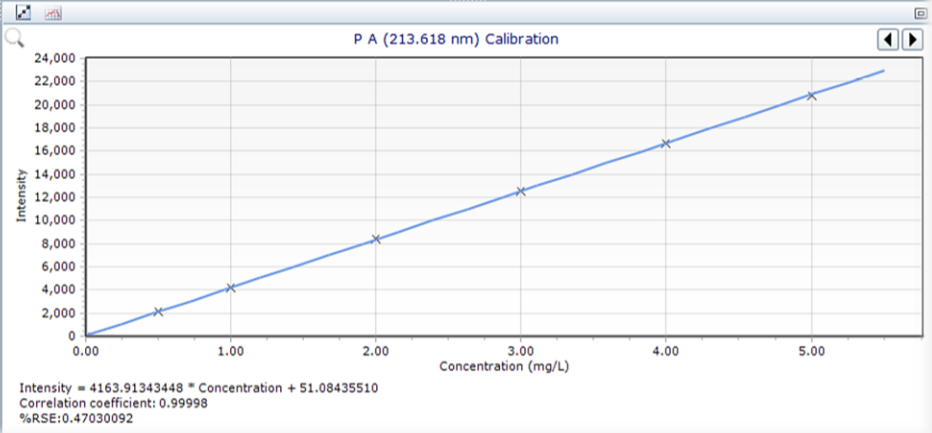
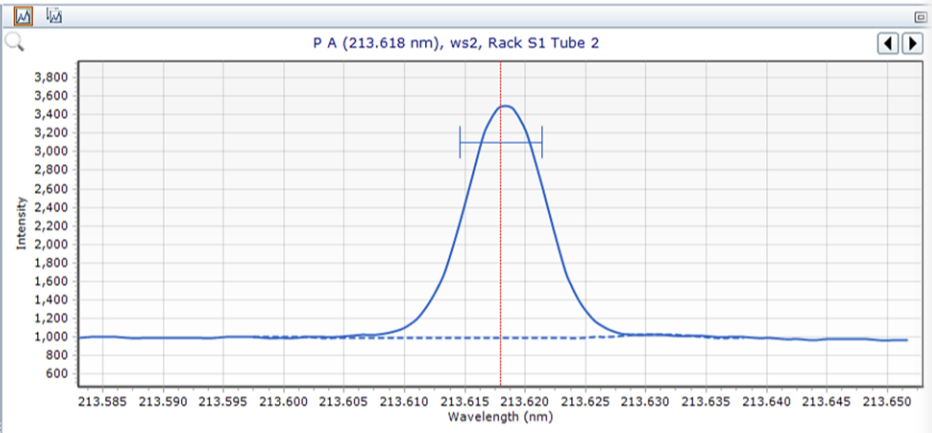
Rack:Tube	Solution Label	Ca R 422.673 nm mg/L	Fe A 238.204 nm mg/L	K R 766.491 nm mg/L	Mg R 280.270 nm mg/L	Na R 589.592 nm mg/L	P A 213.618 nm mg/L	Zn R 213.857 nm mg/L
<input type="checkbox"/> S1:1	ws1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<input checked="" type="checkbox"/> S1:2	ws2	0.500	0.005	0.500	0.050	10.000	0.500	0.005
<input type="checkbox"/> S1:3	ws3	1.000	0.100	1.000	0.100	20.000	1.000	0.100
<input type="checkbox"/> S1:4	ws4	2.000	0.200	2.000	0.200	30.000	2.000	0.200
<input type="checkbox"/> S1:5	ws5	3.000	0.400	3.000	0.400	--	3.000	0.400
<input type="checkbox"/> S1:6	ws6	4.000	0.600	4.000	0.600	50.000	4.000	0.600
<input type="checkbox"/> S1:7	ws7	5.000	0.800	5.000	0.800	--	5.000	0.800
<input type="checkbox"/> 1:1	Sero - Serum Dil Check 1/50	45.605	0.825	76.020	9.802	1652.165	23.145	0.791
<input type="checkbox"/> 1:2	Sero - Serum Dil Check 1/50	46.155	0.819	76.229	9.850	1670.272	22.507	0.792

	Concentration	Intensity
Average:	0.500	2121.437
SD:	N/A	12.439
% RSD:	N/A	0.59
Background:	N/A	983.420

<input checked="" type="checkbox"/>	Replicate	Concentration	Intensity
<input checked="" type="checkbox"/>	1	0.500	2112.420
<input checked="" type="checkbox"/>	2	0.500	2135.627
<input checked="" type="checkbox"/>	3	0.500	2116.263



*Example of literature mineral element reference ranges for animal species*

Elements (Wet Wgt)	Cattle ppm					Pigs ppm					Poultry ppm				
	Deficient	Marginal	Normal	High	Toxic	Deficient	Marginal	Normal	High	Toxic	Deficient	Marginal	Normal	High	Toxic
Calcium	10-60, 0.500-40	70-90, 37-42	80-110, 40-52	14↑,	120-300	<70		90-130			160-200*		200-400*		
Iron	0.15-1.3		1.3-2.5	4-6	18-25	<0.8, 0.15-0.6**		1-15, 0.7-0.8^					1.6-3		
Magnesium	1-11	12-18	18-30	40-75		<14	14-16	18-39	>45		4-10	<16	15-36	31-44.4	30-50
Phosphorus	5-46	40-46	45-60	80-120	>120	35-50	45-55	60-107			9-20	30-50, 9-30	45-60, 60-80	55-70	75-80
Potassium	<98		160-215	>242		94-133***		137-207***	199-250***	>391***			211-356		
Sodium	2299-2851	2989-3219	3104-3449		3449-5748	2851-3104	3150-3219	3219-3449	3449-4138	4138-4598			2805-3747		
Zinc	0.2-0.4	0.5-0.6	0.8-1.4	1.3-16	3-15	0.18-0.25	0.4-0.8	0.7-1.5		1.4-3.3	<0.6	0.8-1.5	1.45-3.4		



# Advantages

- Multi-element analysis capability
- Improved sensitivity, stability and precision
- Improved linear dynamic element ranges
- Reduced interferences
- Excellent screening abilities
- High sample throughput / productivity
- High tolerance to various sample matrix types

# Methodology

- “Dilute and shoot” method:
  - ➔ 1% Nitric Acid
  - ➔ 0.01% Triton X-100
- No acid / microwave digestion
- No Standard Additions
- Benefits:
  1. quick & easy sample preparation step
  2. reduces likelihood of sample contamination
  3. increased sample through-put
  4. reduced reporting times
  5. cost effective

# Research Challenges

- “Dilute & Shoot” sample preparation method – biological interferences from samples.
- External standard curve calibration method – several ranges to be investigated.
- Interferences – physical, chemical & spectral.
- Environmental contamination - Zn, Fe, Mg, P, K, Ca & Na are present in labs.

# Work Completed to Date

- Review of various sample preparation methodologies in scientific literature
  - ➔ Diluent - alkaline Vs acidic
  - ➔ Sample - dilution Vs digestion
- Selection of appropriate sample preparation method
- Identify and procure materials and consumables required paying particular attention to trace metal-grade materials where appropriate.
- Selection of appropriate element wavelengths and measurement parameters.
- Establish the desired calibration range for each element from literature
- Develop robust standard preparation method
- Identification of appropriate internal standards options for use –Y, Sc and Eu



# Future Work

Original Timeline 2019

Revised - Report 2020

Phase	Months (m)											
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24
Literature Review												
* Development of animal serum multi-element Method of Analysis for ICP-OES												
* Development of animal Vitreous Humour multi-element Method of Analysis for ICP-OES												
Comparative field study of animal serum Vs. vitreous humour multi-element composition & concs.												
+ Validation of serum multi-element method												
+ Validation of vitreous humour multi-element method												
Annual progress reporting - AIT												
# Communicate research - presentation, poster, drafting paper publications, etc.												
~ Researcher personal development												
Master's thesis write-up & submission												

## \* Development includes:

- Develop instrument sensitivity check.
- Calibration of instrument semi-quantitative feature.
- Calibration curve development.
- Refine instrument method conditions / wavelengths.
- Internal standard selection & testing.
- Animal serum and vitreous humour matrix investigation.

## + Validation includes:

- Specificity
- Linearity
- Limit of Detection
- Limit of Quantification
- Range
- Accuracy
- Precision
- Robustness
- Stability

## # Research Communication:

- 22<sup>nd</sup> World Congress on Analytical & Bioanalytical Chemistry Aug 2020 – E-Attendee / Poster submission
- 74<sup>th</sup> AVTRW Annual Conference Sep 2020 – Attendee / Poster submission
- Teagasc Knowledge Transfer Conference Sep 2020 – Attendee
- DAFM Backweston R&D Seminar 2020 – Presenter


## ~ Personal Development:

- Agilent spectroscopy Training Webinars
- DAFM – Microsoft PowerPoint Training
- DAFM – Presentation Skills Training
- DAFM – Project Management Foundation Training
- AIT – research training modules
- Scientific conference attendance

# Poster Presentation

- 22<sup>nd</sup> World Congress on Analytical & Bioanalytical Chemistry; UK, London / Aug 2020 – Online Attendee / E-Poster submission
- 74<sup>th</sup> Association of Veterinary Teachers and Research Workers (AVTRW) Annual Conference Glasgow University / Sep 2020 – Attendee / Poster submission

## Quantification of Select Minerals in Animal Serum and Vitreous Humour by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)



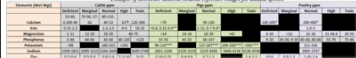
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### Introduction

- Mineral intake and balances are essential in maintaining good animal health and wellbeing
- Deficiencies and toxicities can have a detrimental impact on animal health leading to onset of disease or health conditions.
- Important to diagnose these occurrences quickly particularly in animals of agricultural significance.
- The Biochemistry / Toxicology laboratory provides specialist diagnostic support assisting in the diagnosis of animal clinical diseases or conditions and exposures to environmental pollutants.
- Recent investment in new ICP analytical technologies to enhance the laboratory's analytical capabilities


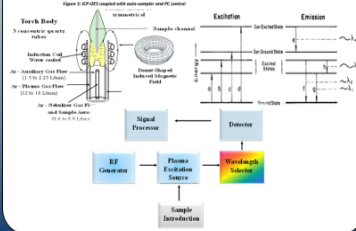


### Objectives

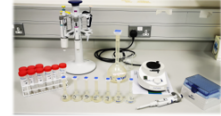
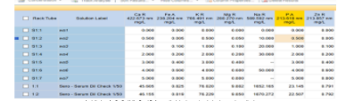
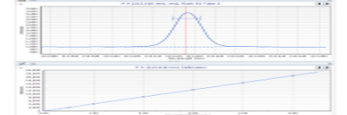
- Development and optimisation of a robust multi-element high-throughput method for the analysis of trace (Zn, Fe) and macro minerals (Mg, Ca, K, P, Na) in animal serum and vitreous humour by ICP-OES.
- Validation of developments to ISO 17025.

### Materials and Methods

- Instrumentation used in this project is a Agilent Technologies 5100 Vertical Dynamic View (VDV) ICP-OES coupled with an SPS 4 high-performance auto-sampling unit (Figure 1). The system is controlled by ICP-Expert V 7.5 software.
- "Dilute and Shoot" sample preparation method

		Conditions	
		Axial	Radial
Read Time (s)		10	10
RF Power (kW)		1.20	1.20
Stabilisation Time (s)		15	1
Viewing Height (mm)		N/A	2
Nebuliser Flow (L/min)		0.6	0.6
Plasma Flow (L/min)		12.00	12.00
Auxiliary Flow (L/min)		1.00	1.00

### Funding

- Research masters funded by the Department of Agriculture, Food and the Marine through the Funding of Academic Fees Scheme.
- Project funding for equipment, training, materials and consumables provided by the Department of Agriculture, Food and the Marine

### Acknowledgements

- Dr. Sean Reidy Athlone Institute of Technology, Ireland.
- Dr. Celine Mannion, DAFM Backweston Laboratory Campus, Ireland
- Department of Agriculture, Food and the Marine, Backweston, Ireland

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2. Puh, R., 1994. Mineral Levels in Animal Health. Diagnostic Data, 2nd ed. Clewbrook, B.C.: Sheep International.

# QUESTIONS

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