Mercury and Hydroquinone Analysis for Skin Lightening Cosmetics

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Enhancing collaboration and communication among mercury analytical laboratories | 20 February 2025

BACKGROUND

The Regional Resource Centre for Asia and the Pacific (RRC.AP) of the Asian Institute of Technology (AIT), jointly with the Regional Office for Asia and the Pacific (ROAP) of the United Nations Environment Program (UNEP) and collaboration with IDEA consultants Inc., is organizing a Training Program #5 entitled "Laboratory operation for methylmercury analysis in human hair, total mercury analysis in human hair and skin lightening products, and hydroquinone analysis in skin lightening products", to be held on 13 to 17 January 2025 at the IDEA EEM laboratory, AIT, Pathum Thani, Thailand.

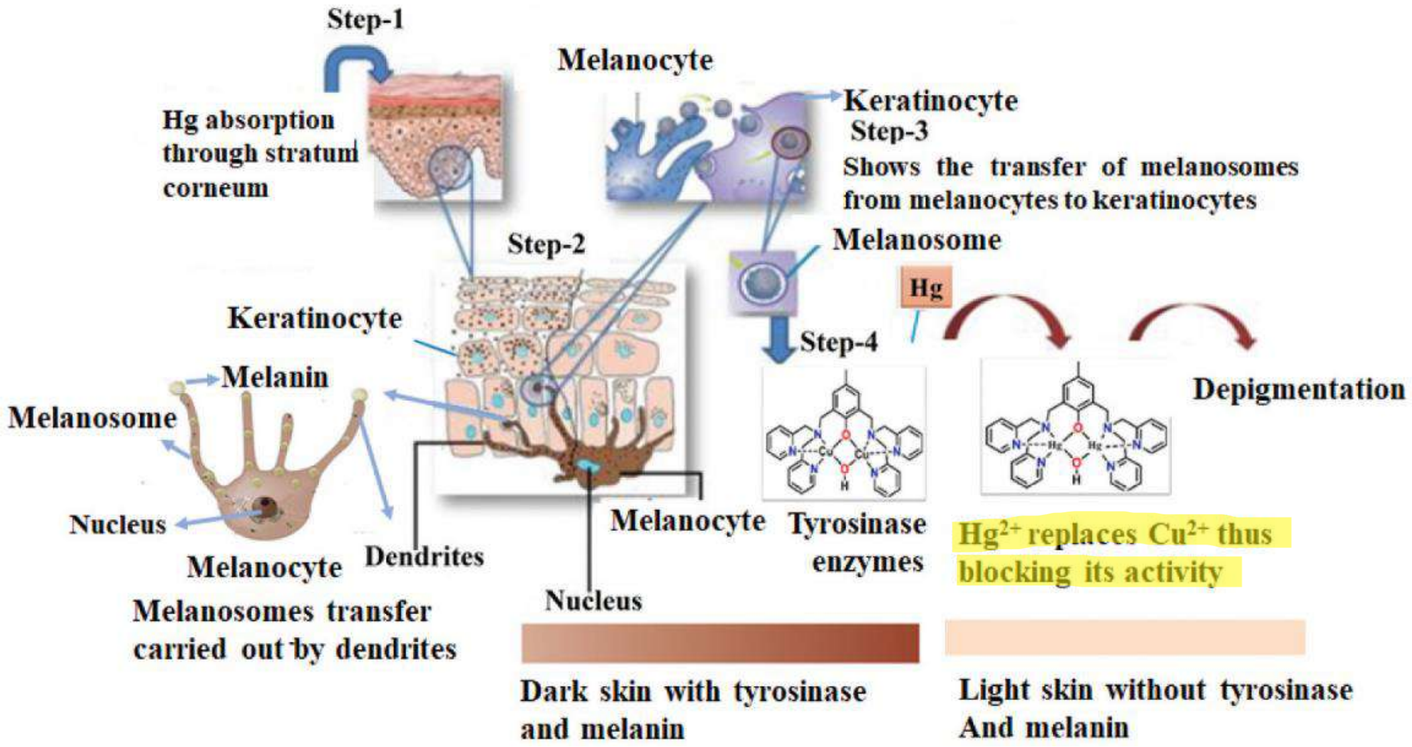
OVERVIEW

- Introduction of total Mercury analysis for skin lightening cosmetics.
- Methodology for pretreatment sample
- Operation procedure of Hg analyzer
- Conclusion

- Introduction of Hydroquinone analysis for skin lightening cosmetics.
- Methodology for pretreatment sample
- Operation procedure of HPLC
- Conclusion

MERCURY ANALYSIS INTRODUCTION

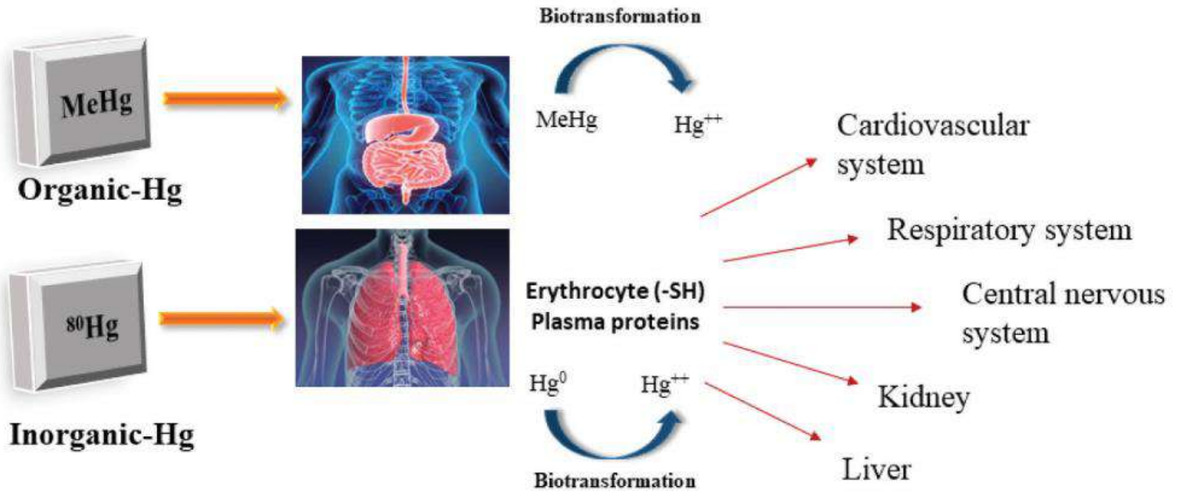
- **Mercury (Hg)** is one of the hazardous substance that mostly illegally add into skin lightening cosmetics while are hazardous and cause major health problems.
- Hg can inhibit the function/activity of Tyrosinase enzymes thus melanin generation which related to skin pigments is blocked.



Raza-Naqvi, Syed Ali, Idrees, Fareeha, Sherazi, Tauqir A., Anjum-Shahzad, Sohail, Ul-Hassan, Sadaf, & Ashraf, Nimra. (2022). TOXICOLOGY OF HEAVY METALS USED IN COSMETICS. *Journal of the Chilean Chemical Society*, *67*(3), 5615-5622. https://dx.doi.org/10.4067/S0717-97072022000305615

MERCURY ANALYSIS INTRODUCTION

- Mercury in skin products (inorganic-Hg) can damage the skin, cause rashes, and discolor the skin. Mercury can also cause more serious health problems, including damage to the nervous system and kidneys.
- Al-Saleh et al. (2005) found elevated levels of Hg in cosmetics from many countries, with samples from Thailand, Lebanon, and England having the highest amounts ranging from 1,281 to 5650 ppm.
- Uram et al. (2010) observed irregular labels and descriptions on the cosmetic products made in Mexico.



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MERCURY ANALYSIS INTRODUCTION

- **MINAMATA CONVENTION ON MERCURY** amended its Annex A text and removed the 1 ppm limitation for skin lightening cosmetics. Each country can set different values depending on the local situation. Such amendment become effective in 2025.
- The maximum permissible limit for Hg set by the US **FDA and WHO in cosmetics** is also 1 ppm (mg/kg).
- This study aims to develop the analysis method of total mercury by NIC Mercury analyzer MA3000 which is convenience, short analysis time and simple sample preparation method.

MERCURY ANALASIS LITERATURE REVIEWS

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Test kit/Strip test/Paper-based devices

- Restriction for screening analysis
- Low sensitivity
- Low precision and accuracy

Cold Vapor Atomic Absorption Spectrometry (CVAAS)

Inductively Coupled Plasma-Mass Spectrophotometry (ICP-MS)

- Requirement of sample digestion/pretreatment
- Consumption of chemicals
- Time-consuming method

MERCURY ANALYZER

Nippon Instrument Cooperation (NIC) Mercury Analyzer WA-5A and MA-3000

1

WA-5A Dual-step Gold Amalgamation, Atomic Spectroscopy Detection

Comply with standard method ; ASTM 5954, ISO 6978, ISO 20552, JLPGA-S-07

Basic features : Innovative dual-cell Tri-Detector with Dual step Gold-Amalgamation

Using for gas/vapor samples

2

MA-3000 Thermal Decomposition (CVAAS)

Comply with standard method ; USEPA 7473, ASTM D6722-01,D6723-10

Operating Principle: Thermal Decomposition, Gold-Amalgamation,

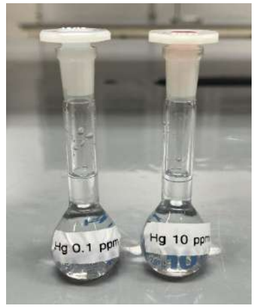
Cold-Vapor Atomic Absorption Spectroscopy (CVAAS)

Using for solid/liquid samples

STANDARD PREPARATION

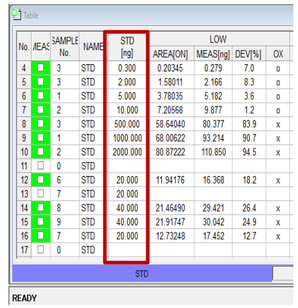
Stock Mercury Standard (10, 0.1 ppm)

- Pipette 100 µL of Mercury standard 1,000 ppm and dilute in 10 mL of 100 mg/L of L-Cysteine in 0.2% HNO_3 for stock Hg 10 ppm
- Pipette 100 µL of the diluted standard 10 ppm and dilute in 10 mL of 100 mg/L of L-Cysteine in 0.2% HNO_3 for stock Hg 0.1 ppm



Dilution series of standard solution (0.1-2,000 ppm)

- Pipette 1, 3, 20, 50, and 100 µL of the stock standard 0.1 ppm in sample holder (boat) with purged 100 mg of additive B for low Hg concentration range (0.1-10 ng)
- Pipette 10, 50, 100, and 200 µL of the stock standard 10 ppm in sample holder (boat)) with purged 100 mg of additive B for high Hg concentration range (100-2,000 ng)



No.	MEAS	SAMPLE No.	NAME	STD [ng]	LOW AREA[ON]	LOW MEAS[ng]	LOW DEV[%]	LOW OK
4	■	3	STD	0.300	0.20345	0.279	7.0	o
5	■	3	STD	2.000	1.58011	2.166	8.3	o
6	■	1	STD	5.000	3.78035	5.182	3.6	o
7	■	2	STD	10.000	7.20568	9.877	1.2	o
8	■	3	STD	500.000	58.64040	80.377	83.9	x
9	■	1	STD	1000.000	68.00622	93.214	90.7	x
10	■	2	STD	2000.000	80.87222	110.850	94.5	x
11	□	0	STD					
12	■	6	STD	20.000	11.94176	16.368	18.2	x
13	□	7	STD	20.000				
14	■	8	STD	40.000	21.46490	29.421	26.4	x
15	■	9	STD	40.000	21.91747	30.042	24.9	x
16	■	7	STD	20.000	12.73248	17.452	12.7	x
17	□	0	STD					

STD

READY

SAMPLE SCREENING AND PREPARATION

Sample screening

Use the toothpick to pick small amount of sample (cream) and then put/tab in sample holder (boat)

Sample preparation

- Low amount of T-Hg sample
  1. Add 100 mg of Additive B into the boat and purge
  2. Weight 50-100 mg of sample into the boat
- High amount of T-Hg sample
  1. Weigh 10 mg of sample and dissolve in 100 mL of 100 mg/L of L-Cysteine in 0.2% HNO_3 (10,000 times of dilution)
  2. Homogeneously mix/sonicate the sample solution
  3. Add 100 mg of Additive B into the boat and purge
  4. Pipette 10 or 100 µL of diluted sample into the boat

CREAM SAMPLE MEASUREMENT

Using method in MA3Win program of NIC MA-3000 Mercury Analyzer that is:

<<Method No.1: ORGANISM(SOLID)>>

Sample heating furnace Hg^{2+}

ATOMIZE-1 = 0 °C/ 0 sec/ 0 L/min

Decomposition furnace Hg^{0}

ATOMIZE-2 = 180 °C/ 120 sec/ 0.4 L/min

Mercury collect Hg^{0}

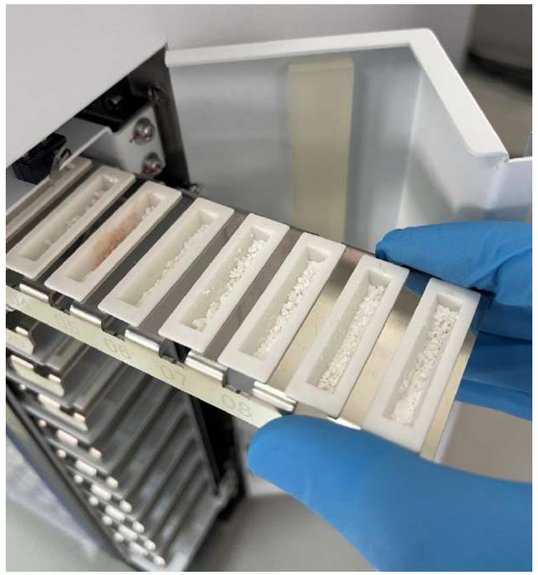
ATOMIZE-3 = 850 °C/ 120 sec/ 0.4 L/min

Mercury purify Hg^{0} for CVAAS

ATOMIZE-4 = 0 °C/ 0 sec/ 0 L/min

Each sample was measured in double.

Measurement time is 5 min.



DILUTED SOLUTION SAMPLE MEASUREMENT

Using method in MA3Win program of NIC MA-3000 Mercury Analyzer that is:

<<Method No.6: WASTEWATER>>

Sample heating furnace Hg^{2+}

ATOMIZE-1 = 150 °C/ 60 sec/ 0.4 L/min

Decomposition furnace Hg^{0}

ATOMIZE-2 = 180 °C/ 120 sec/ 0.4 L/min

Mercury collect Hg^{0}

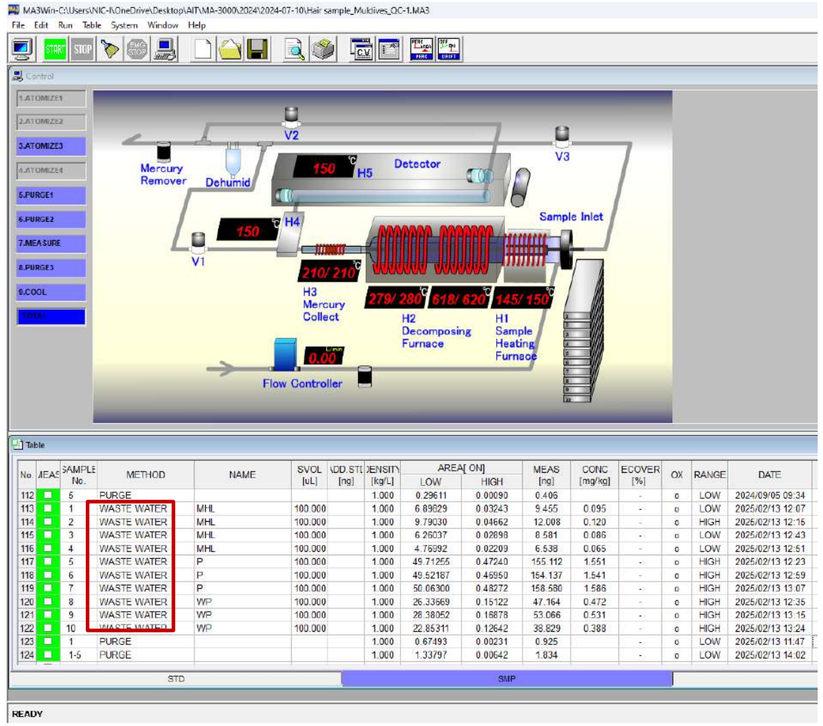
ATOMIZE-3 = 850 °C/ 120 sec/ 0.4 L/min

Mercury purify Hg^{0} for CVAAS

ATOMIZE-4 = 0 °C/ 0 sec/ 0 L/min

Each sample was measured in double.

Measurement time is 7 min.

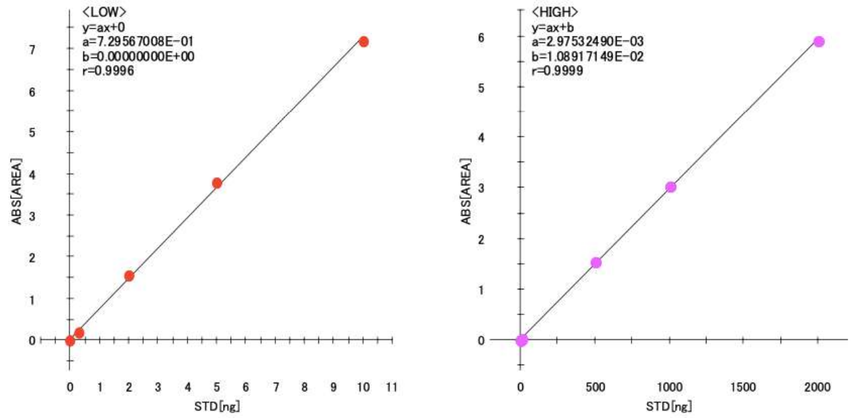


No.	MEAS	SAMPLE No.	METHOD	NAME	SVOL [uL]	ADD STD [ng]	DENSITY [kg/L]	AREA[ON] LOW	AREA[ON] HIGH	MEAS [ng]	CONC [mg/kg]	RECOVER [%]	OX	RANGE	DATE
112		5	PURGE				1.000	0.29611	0.00090	0.406		-	o	LOW	2024/09/05 09:34
113		1	WASTE WATER	MHL	100.000		1.000	6.89029	0.03243	9.455	0.095	-	o	LOW	2025/02/13 12:07
114		2	WASTE WATER	MHL	100.000		1.000	9.79030	0.04662	12.008	0.120	-	o	HIGH	2025/02/13 12:16
115		3	WASTE WATER	MHL	100.000		1.000	6.26037	0.02698	8.581	0.086	-	o	LOW	2025/02/13 12:43
116		4	WASTE WATER	MHL	100.000		1.000	4.76992	0.02209	6.538	0.065	-	o	LOW	2025/02/13 12:51
117		5	WASTE WATER	P	100.000		1.000	49.71255	0.47243	155.112	1.551	-	o	HIGH	2025/02/13 12:23
118		6	WASTE WATER	P	100.000		1.000	49.52187	0.46950	154.137	1.541	-	o	HIGH	2025/02/13 12:59
119		7	WASTE WATER	P	100.000		1.000	50.06300	0.48272	158.580	1.586	-	o	HIGH	2025/02/13 13:07
120		8	WASTE WATER	WP	100.000		1.000	26.33969	0.15122	47.164	0.472	-	o	HIGH	2025/02/13 12:35
121		9	WASTE WATER	WP	100.000		1.000	28.38052	0.16878	53.066	0.531	-	o	HIGH	2025/02/13 13:15
122		10	WASTE WATER	WP	100.000		1.000	22.85311	0.12642	39.829	0.398	-	o	HIGH	2025/02/13 13:24
123		1	PURGE				1.000	0.67493	0.00231	0.925		-	o	LOW	2025/02/13 11:47
124		1-5	PURGE				1.000	1.33797	0.00642	1.834		-	o	LOW	2025/02/13 14:02

CALCULATION AND EVALUATION

Sample calculation

- Perform Hg standard calibration curve from 1-10 ng (low conc. range) and 10-2,000 ng (high conc. range) done automatically by the Mercury Analyzers MA-3000
- Input weight of each sample in mg unit and Hg concentration (ppm) was calculated automatically by the Mercury Analyzers MA-3000



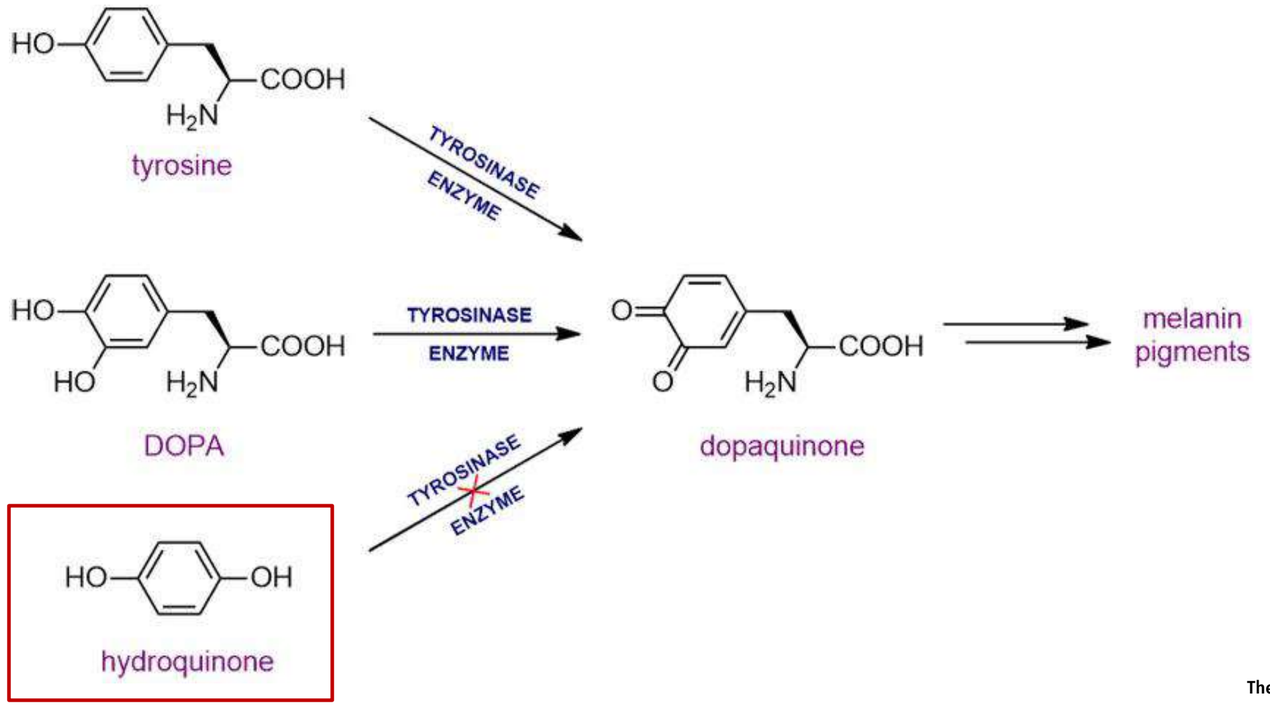
Evaluation method (Quality control)

- Check Limit of Detection (LOD) and calibration curve with CRM NIMD-01 material (Certified standard human hair, 0.794 ±0.05 mg/kg).
- Double check 10% of the total samples (random) to ensure no bias at 90% confidence interval.

Parameter	Value
R^2	>0.999
CRM (3 trials)	0.762 ± 0.002 mg/kg
LOD	0.05 ng
LOQ	0.16 ng
%CV	<10%

HYDROQUINONE INTRODUCTION

- **Hydroquinone (HQ)** is an aromatic organic compound and a potent inhibitor of melanin production used in the treatment of melasma, pigmented acne scars, post-inflammatory hyperpigmentation, and skin discoloration.
- Kojic acid, azelaic acid, glycolic acid, retinoids, salicylic acid, and especially hydroquinone remains the most effective lightening agent for treating common hyperpigmentation disorders.



The mechanism of hydroquinone, labmuffin.com

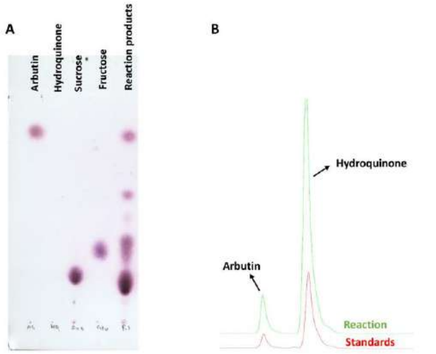
HYDROQUINONE INTRODUCTION

- Hydroquinone is a widely used skin-lightening agent, it is characterized by several adverse effects such as carcinoma, irritative dermatitis, melanocyte destruction, contact dermatitis, and ochronosis. The skin was discolored when consistently applied for a long period of time.
- The hydroquinone metabolites p-benzoquinone and glutathione conjugates are frequently linked to the development of cancer. When hydroquinone-containing cosmetics are used topically over an extended period,
- p-Benzoquinone and hydroquinone conjugates with glutathione may accumulate and cause DNA damage and mutation.

HYDROQUINONE INTRODUCTION

- Concerns over the safety issues surrounding the use of hydroquinone have led many countries around the world, including the UK, EU, US, Australia, Asia, Africa, and others to prohibit the use of hydroquinone in cosmetics.
- Thailand, US, UK, and EU instituted regulations with a 2% limit for cosmetic products and a 4% limit for topical preparations intended for dermatological use.
- Japan still allowed to trade hydroquinone as quasi-drug.
- This study aims to develop the analysis method of hydroquinone by HPLC method which is high sensitivity, high accuracy, and excellent precision.

HYDROQUINONE ANALASIS LITERATURE REVIEWS



Agarwal, N., Rai, A.K. & Singh, S.P. Biotransformation of hydroquinone into α-arbutin by transglucosylation activity of a metagenomic amylosucrase. 3 Biotech 11, 362 (2021). https://doi.org/10.1007/s13205-021-02909-2

Test kit, Thin Layer Chromatography (TLC)

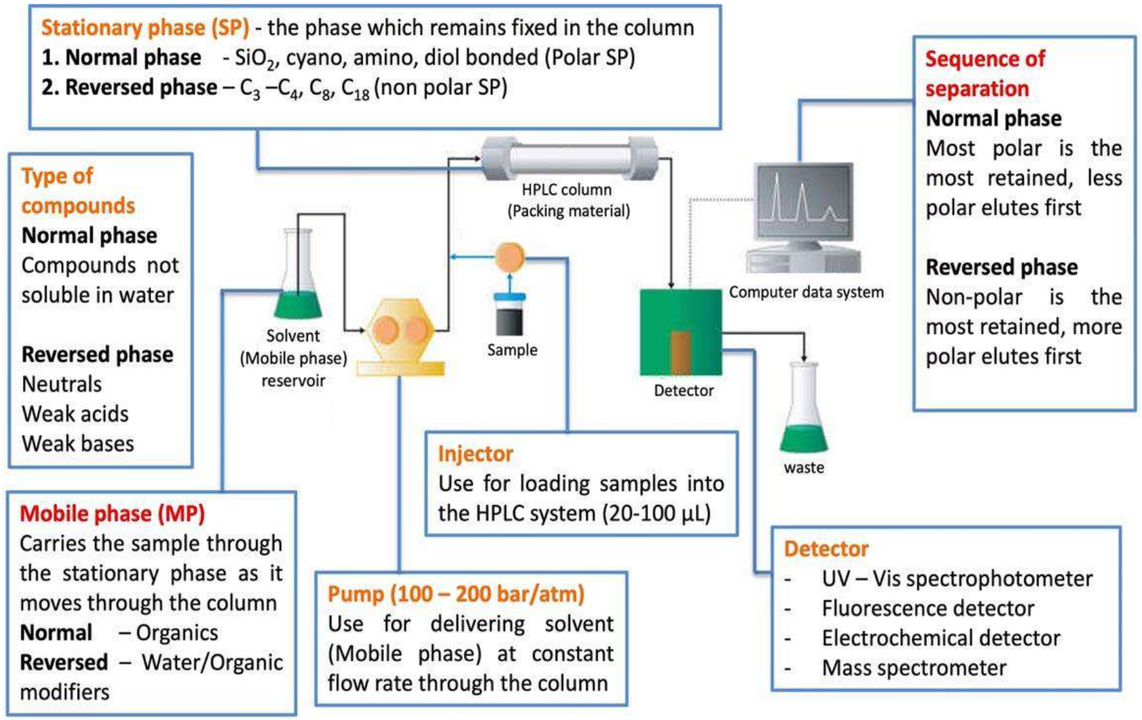
- Restriction for qualitative analysis
- Low sensitivity
- Low precision and accuracy

UV-Vis Spectrophotometry

- Requirement of sample digestion/pretreatment
- High Limit of Detection (LOD)
- Moderate precision and accuracy

High Performance Liquid Chromatography (HPLC)

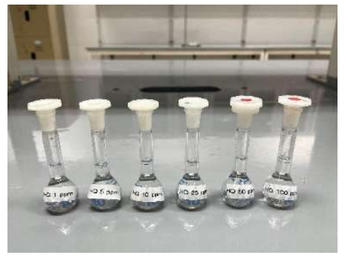
- **High Sensitivity**
- **High Precision and accuracy**
- **High reliable equipment** (acceptable for method validation ISO 17025)



STANDARD PREPARATION

- **Stock hydroquinone solution (1,000 ppm)**

  Weight 25 mg of hydroquinone standard and dilute in 25 mL of Milli-Q water

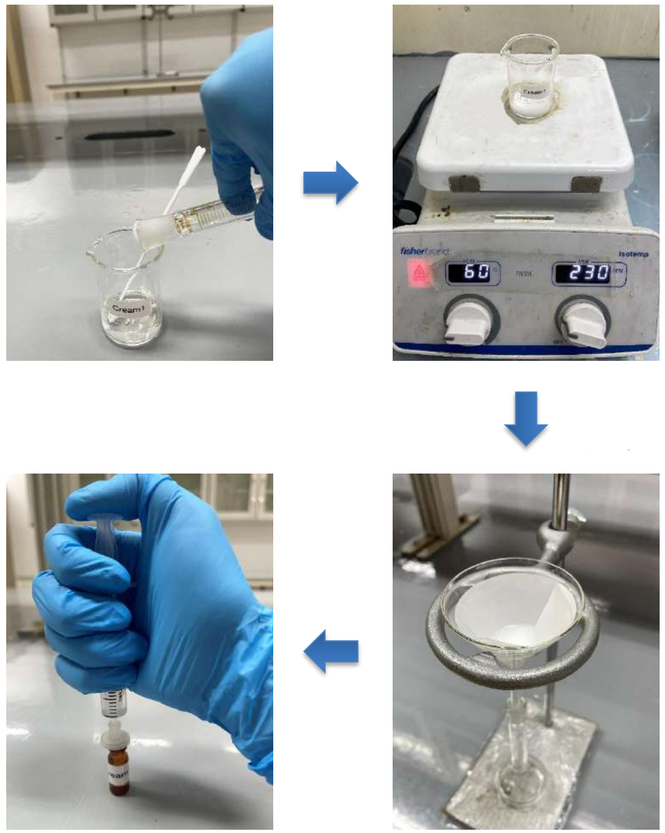


- **Dilution series of standard solution (1-100 ppm)**

Solution	Volume of pipette (μL)	Total volume (mL)	Concentration (ppm)
Stock solution 1,000 ppm	10	10	1
	50	10	5
	100	10	10
	250	10	25
	500	10	50
	1,000	10	100

SAMPLE PREPARATION

1. Weight 0.1-0.2 g of sample in 25 mL- or 50 mL-beakers
2. Add 10 mL of AR grade methanol 99.5% into the beaker
3. Mix and stir at 60 °C for 10 min using hotplate and magnetic bar
4. Filter the sample through 100 mL-volumetric flask by No.1 Whatman filter paper
5. Make volume of the sample to 100 mL by adding Milli-Q water to 100 mL-volumetric flask
6. Filter 1 mL of the sample to HPLC vial by 0.2 µM nylon syringe filter

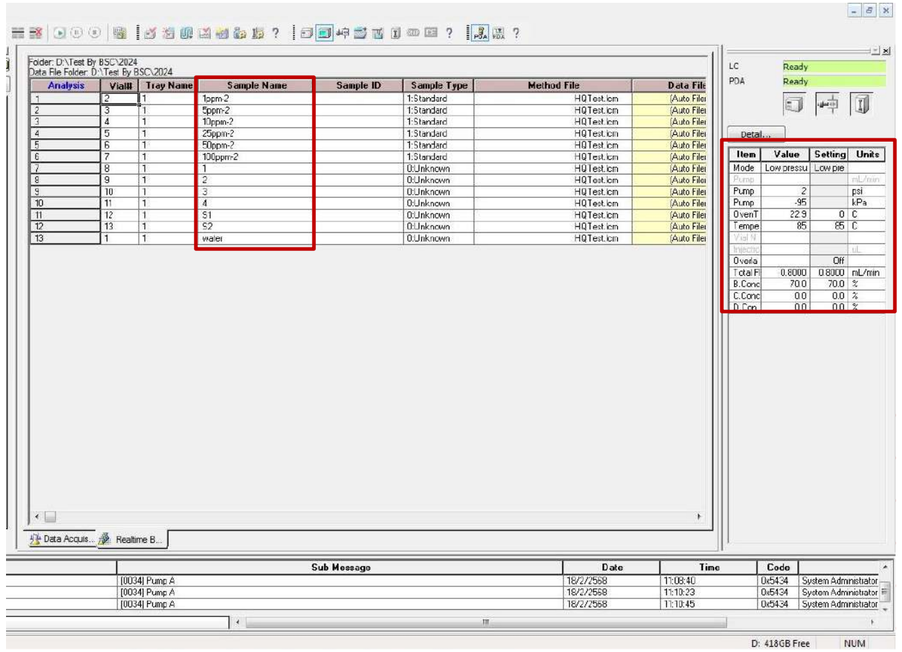


SAMPLE MEASUREMENT

<<Method Reversed phase HPLC)>>
Shimadzu HPLC-20A series LabSolutions LC Program

- *Shim-pack GIS C18 column 5 µm , 150 mm x 4.6 mm*
- *Column temperature : RT, ~25 °C*
- *Mobile phase : water to methanol 70 : 30*
- *Flow rate : 0.8 ml/min, ~ 1,000 psi*
- *Injection Volume : 20 µL*
- *Absorption wavelength : 290 nm*

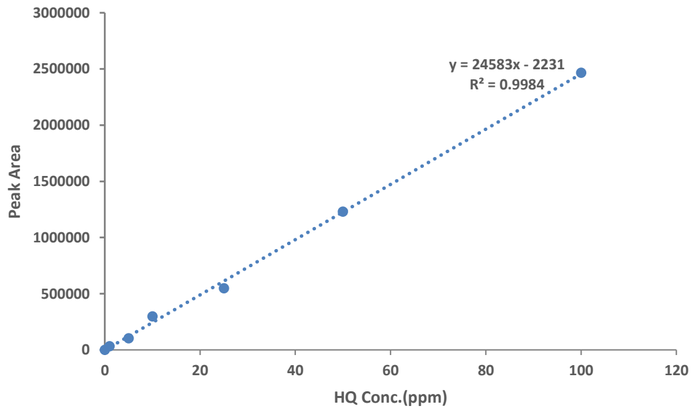
Analysis time is 10 min
Retention time of HQ is around 3.7 min



CALCULATION AND EVALUATION

Sample calculation

- Perform HQ standard calibration curve from 1-100 ppm with peak area
- Calculate HQ concentration in percentage from ppm unit using linear regression with total volume of sample solution and weigh of sample



Evaluation method (Quality control)

- Check Linearity by R^2>0.99

  Linear regression equation

- Check Limit of Detection (LOD) and Limit of Quantification (LOQ)

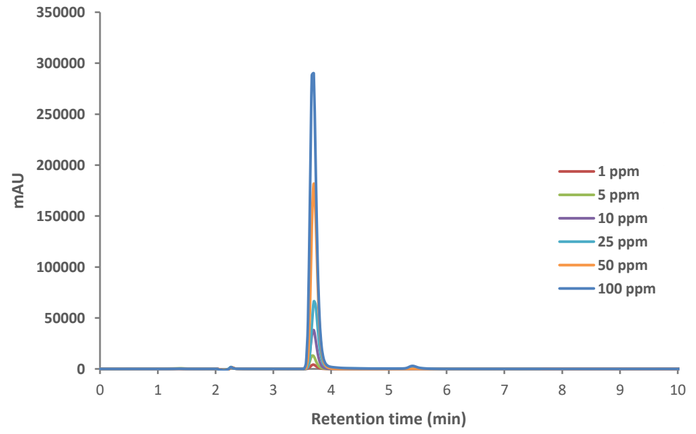
  = 3 SD of blank and 10 SD of blank

- Check precision by %RSD

  (SD / Ave. sample conc.) x 100 % ≤ 10%

- Check accuracy by %Recovery

  80 % ≤ {(Csp – Cs)/Ca} × 100 ≤ 120%



CONCLUSION

- Total mercury analysis for skin lightening cosmetics was determined by specific and direct method of NIC Mercury analyzer MA3000 with simplicity, short analysis time, and high sensitivity.
- Hydroquinone analysis for skin lightening cosmetics was determined by highly sensitive method of reversed phase Shimadzu HPLC with reliability, great precision, and excellent accuracy.

REFERENCES & ACKNOWLEDGEMENT

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IDEA R&D Center | 2025

THANK YOU

Project for promoting Minamata Convention on Mercury

UNEP Networking Webinar #10
Enhancing collaboration and communication among mercury analytical laboratories

Toxicology Laboratory, School of Veterinary Medicine, Hokkaido University, Japan

February 20th 2025

Rio DOYA

毒性学教室
Vetmed Tox Hokkaido University

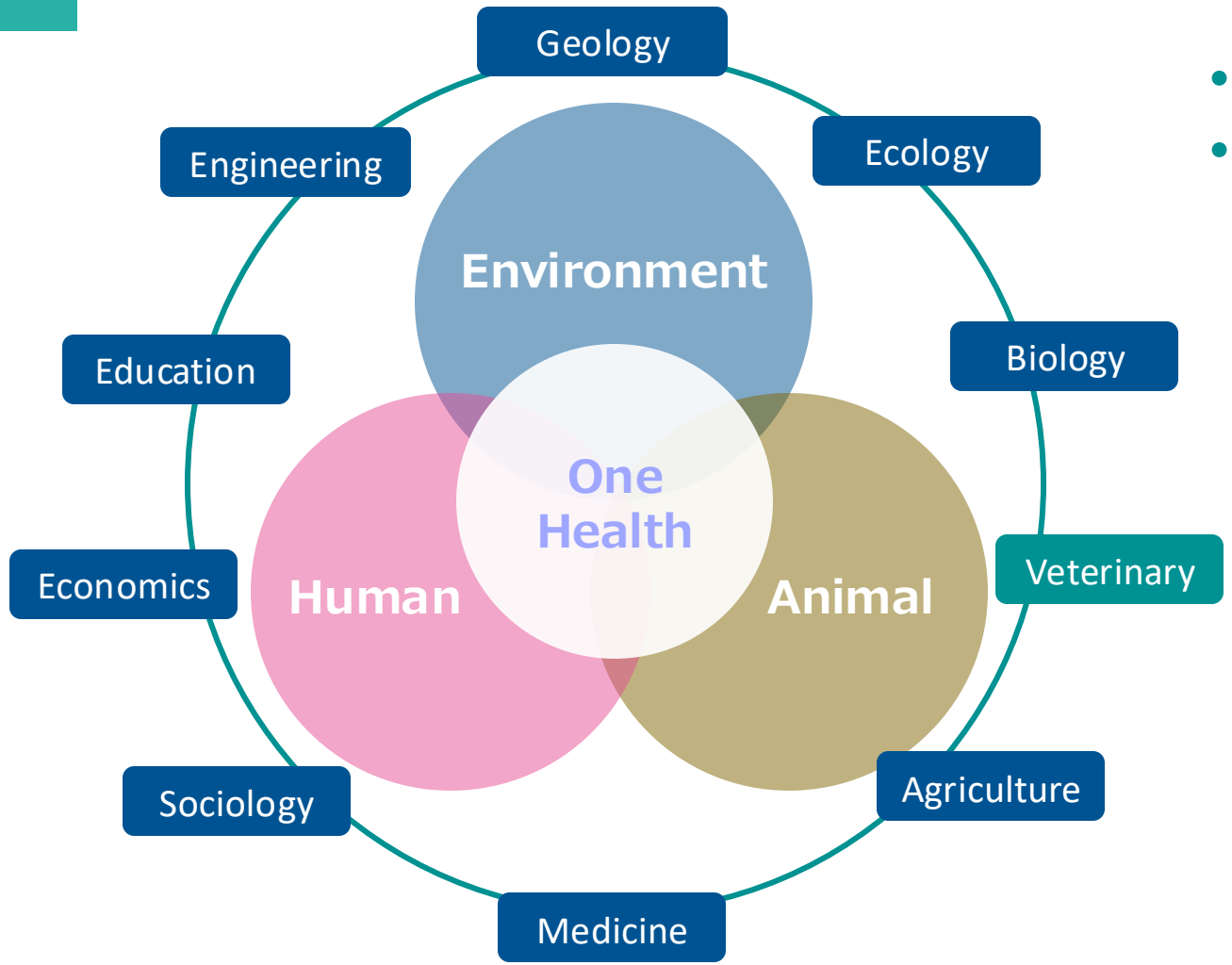
Hokkaido University

Hokkaido University

- One of the oldest National Universities (Since 1876)
- Undergraduate students: 11 thousand
- Postgraduate students: 6.5 thousand

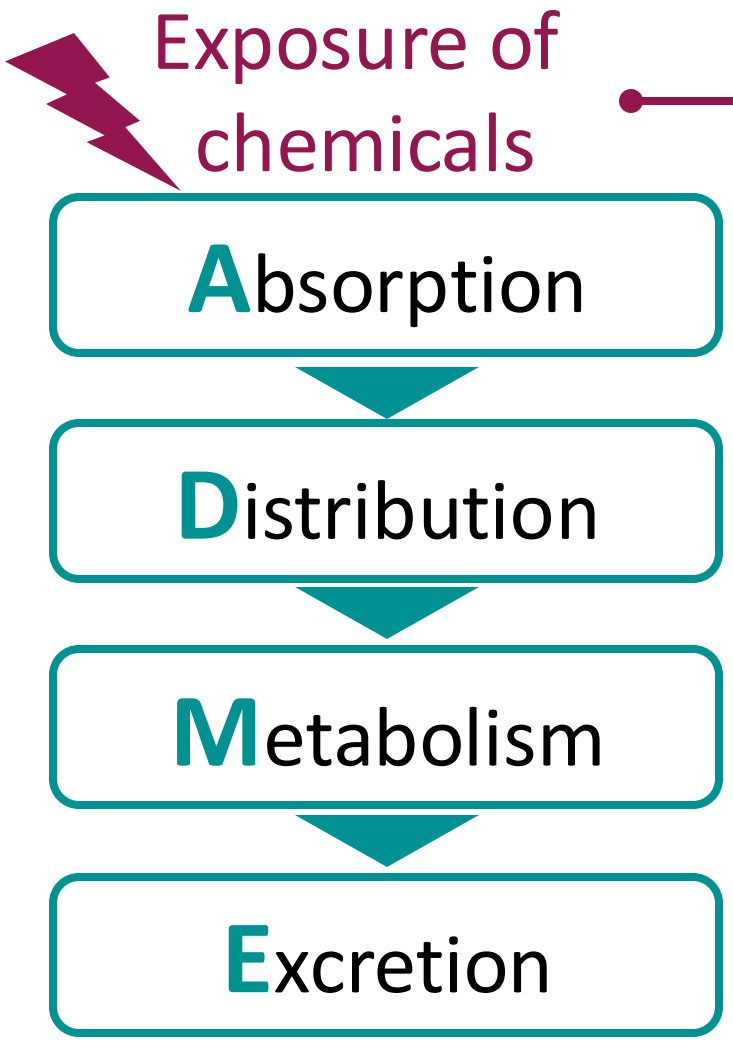



Toxicology in One Health Approach



- School of Veterinary Medicine: Since 1910
- Laboratory of Toxicology: Since 1990
  - Interdisciplinary nature
  - Collaboration with overseas universities
  - Joint program with governmental researchers and international cooperation agencies

Toxicology in One Health Approach



Current research target

- Pesticides
  - Neonicotinoids
  - Pyrethroids
  - Organic phosphorus/Organic chlorides
- Rodenticides
  - 1st & 2nd Generation of anticoagulant
- Micro/Nano plastic
- Heavy metal/Metalloids
  - Lead, Cadmium, Arsenic
  - Mercury

Toxicology in One Health Approach

GLOBAL FACILITY CENTER
北海道大学グローバルファシリティセンター

Exposure of chemicals

- **A**bsorption
- **D**istribution
- **M**etabolism
- **E**xcretion

Chemical analysis : fundamental component

LC-MS, GC-MS

ICP-MS

TDA-AAS

Toxicology in One Health Approach

Exposure of chemicals

- **A**bsorption
- **D**istribution
- **M**etabolism
- **E**xcretion

<u>Biological response/toxicological effects</u>

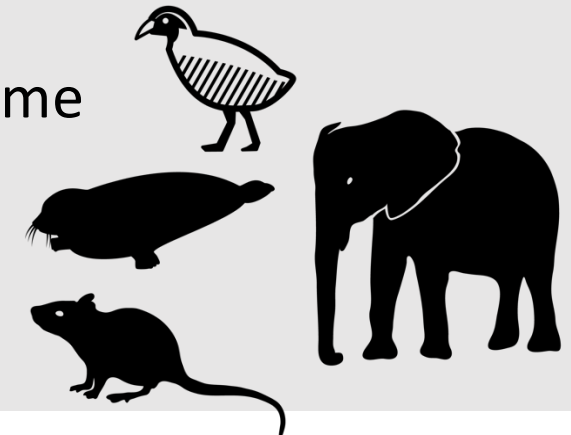
- Biochemical parameter
- RNA expression
- Epigenetic change
- Metabolome analysis
- Behavioral examination
- Socio-economic evaluation

<u>Species Difference</u>

- Phylogenetic analysis of genome
- Metabolic assay

<u>Evolution of Tolerance</u>

- *in-silico* Docking simulation



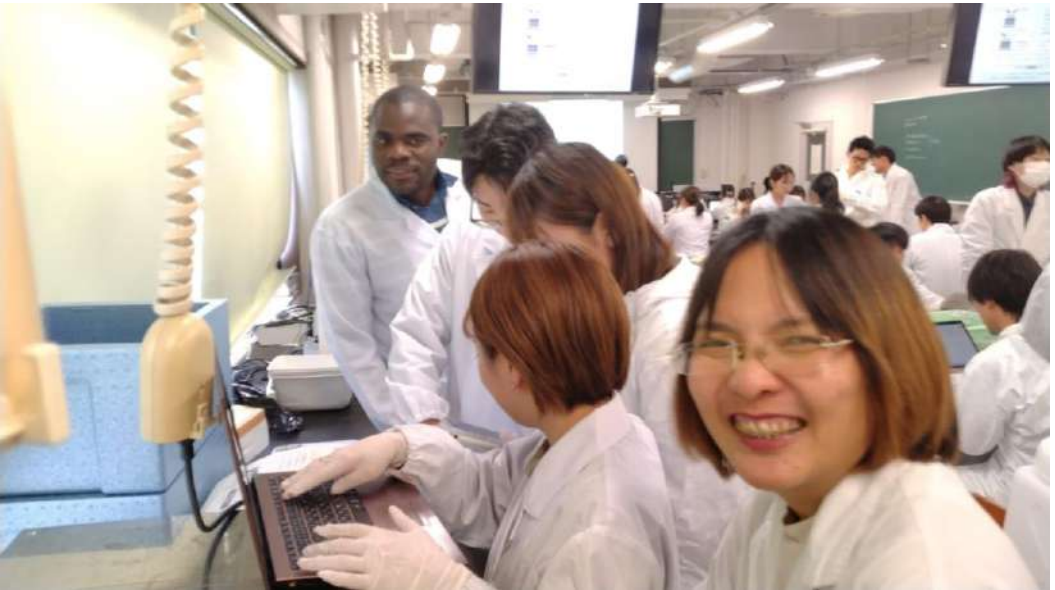
Mercury research

Facility

- MA-3000 [Nippon Instruments]
  - Thermal decomposition amalgamation AAS

Application

- Environmental samples
  - Pollution from human activities
  - Post-disaster assessment
- Wildlife sample
  - Domestic & overseas
  - Combination with C/N stable isotope analysis
- Practical class
- UNEP-PT

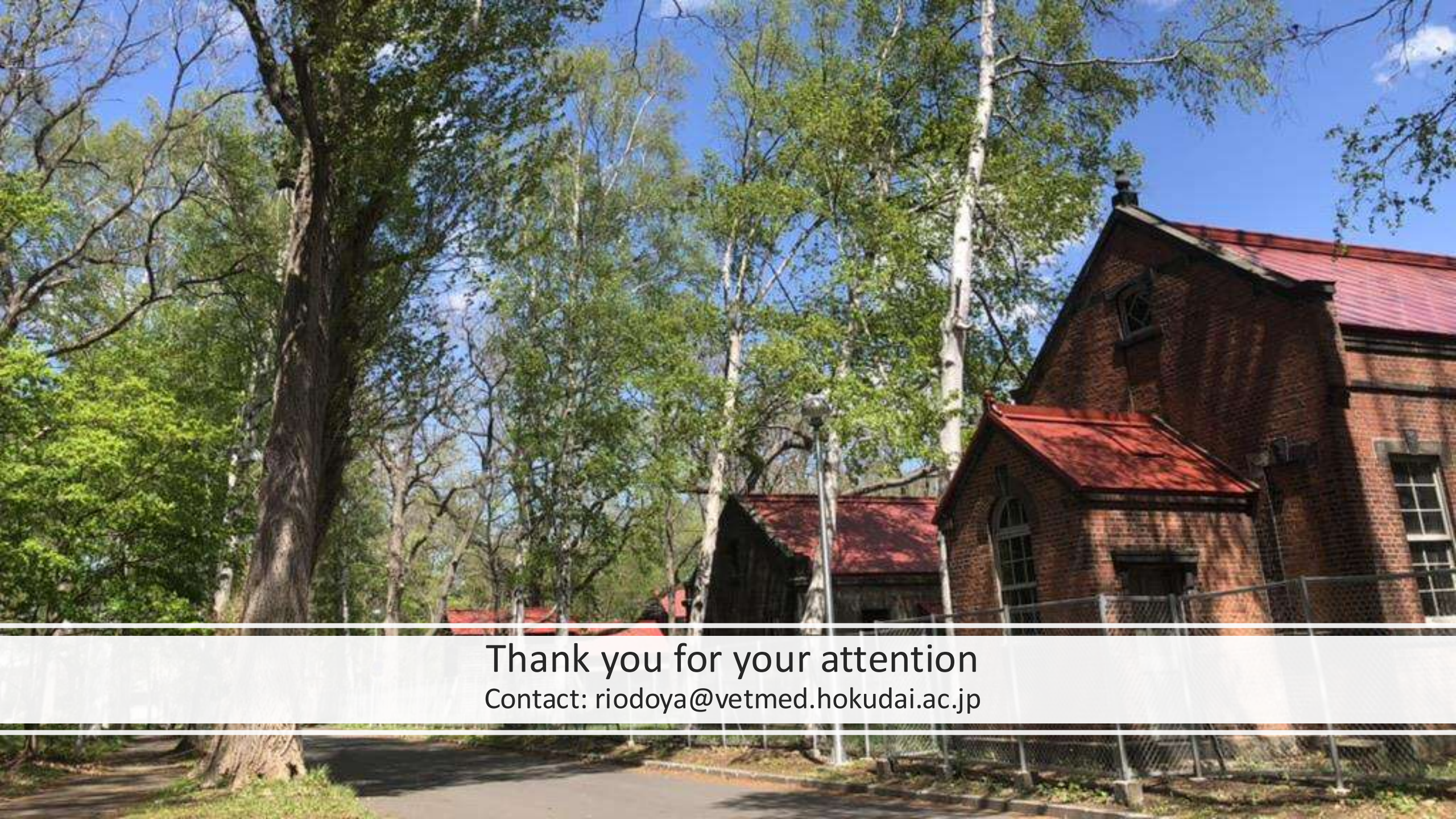



QuEChERS

New Project : ZAZINAMBO

Pre-kickoff Symposium in Botswana
2024 October 8-9th

- ✓Monitoring laboratory
- ✓Network in Southern Africa



Thank you for your attention

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