



Rapid urine-based diagnostics using ATR-FTIR Spectroscopy combined with AI

1st Seminar

Presented by

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Introduction: Why we need to develop diagnostic method

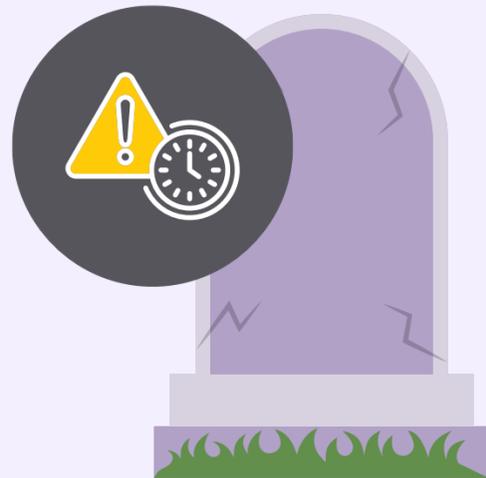
WHO 2023

Conventional laboratory methods

- Require complex sample preparation
- Specialized equipment
- Time-consuming procedures



DELAY clinical decision



IN 2023
3 MILLION
DEATH annually from unsafe healthcare

Need for non-invasive diagnostics

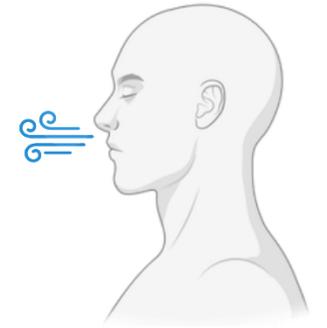
such as:



Urinalysis



Saliva testing



Breath analysis

Analytical technique

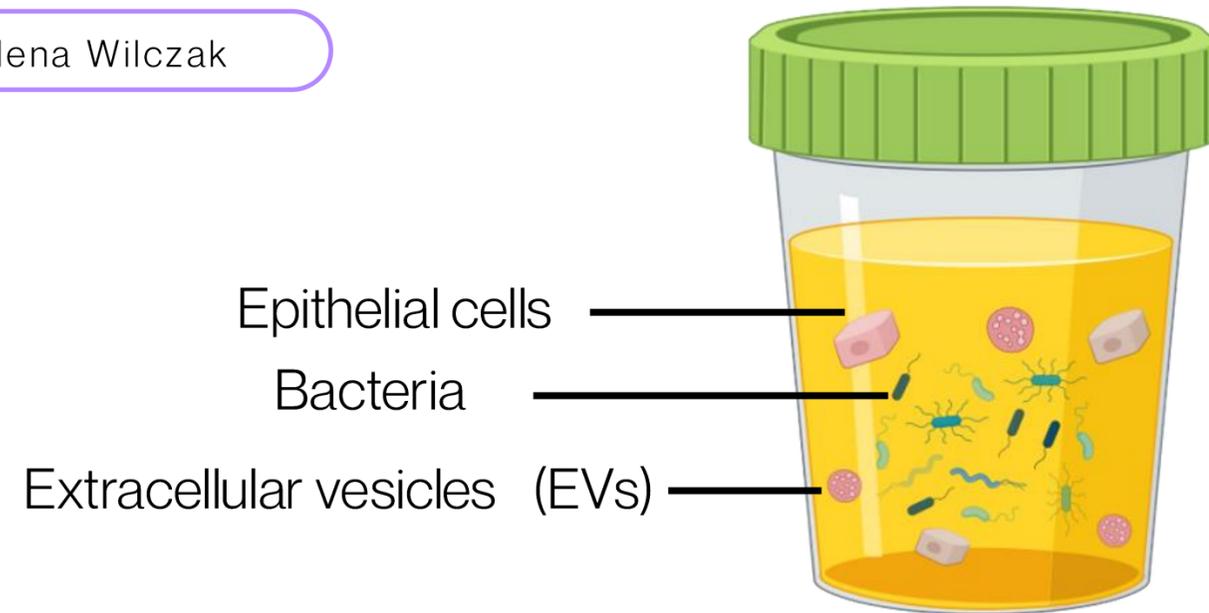
- Rapid biochemical fingerprinting
- Minimal sample preparation
- Suitable for biofluid analysis



**Attenuated Total Reflectance Fourier
Transform Infrared Spectroscopy
(ATR-FTIR)**

Urine: non-invasive clinical sample

Magdalena Wilczak



- Water (~95%)
- Urea (~2%)
- Dissolved salts and ions (~2.8%)
- Creatinine, ammonia, uric acid (~0.2%)

Important routine test for **metabolite detection and diagnostic** purposes

- Reflects systemic metabolic changes
- Serve as a metabolic fingerprint of physiological and pathological conditions.



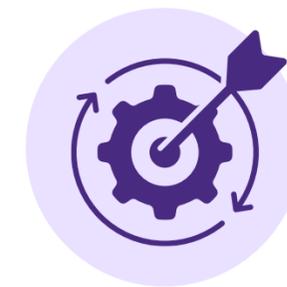
Low-cost



Easy to collect



Non-invasive



**Effective
analysis**



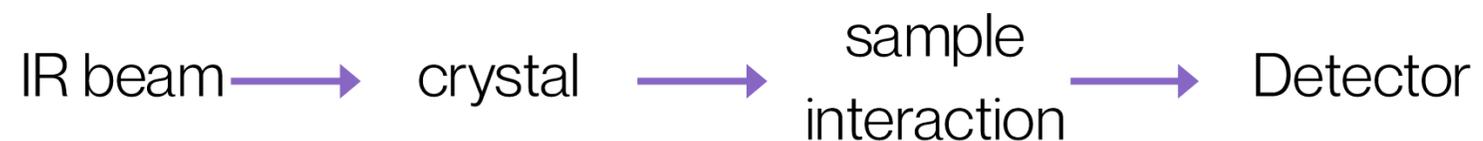
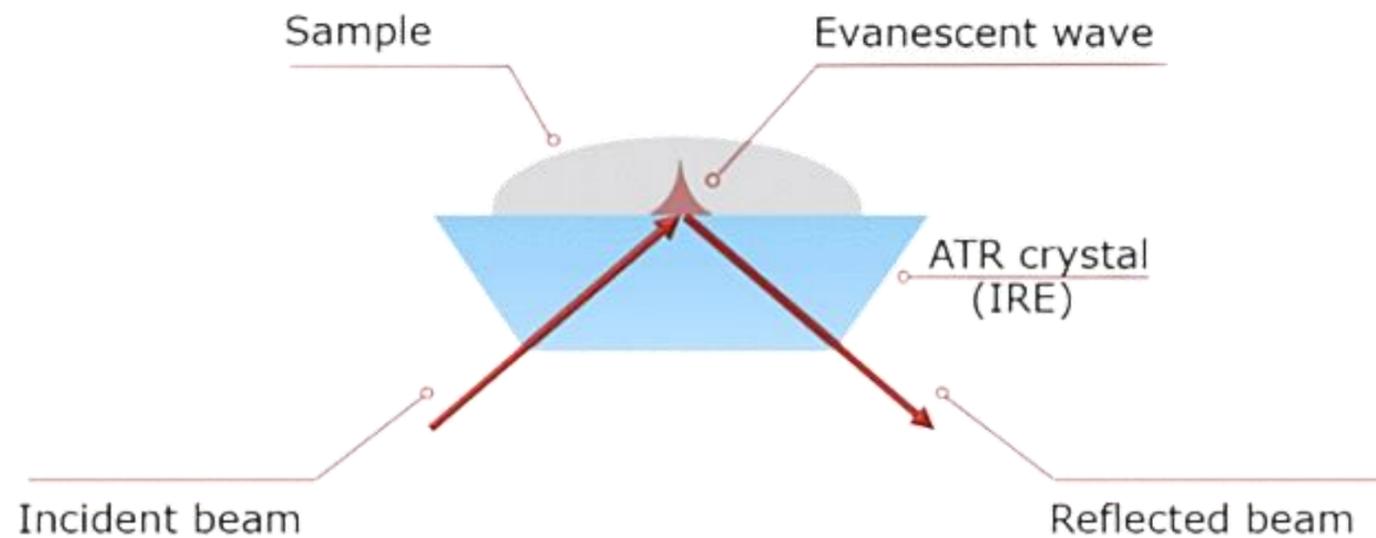
**Health
monitoring**

ATR-FTIR

Magdalena Wilczak

- A vibrational spectroscopic technique
- Obtain molecular fingerprints of samples
- Minimal sample preparation.

Principle of ATR-FTIR



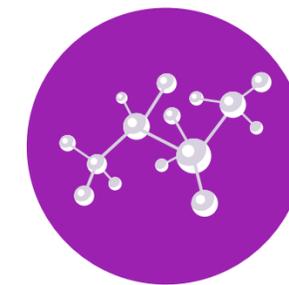
Fingerprint region (~1800-900 cm^{-1})

- Contains complex vibrational bands of molecules
- Unique spectral patterns for compound identification
- Useful for metabolite discrimination in biofluids

Advantage of ATR-FTIR



Rapid



Non-destructive



**Suitable for
biofluid analysis**



High accuracy



Highly sensitive



High Specificity

Seminar paper



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Paper 1st

SJR 2024

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Q1

Discrimination of urine infrared spectral biomarkers for early-stage chronic kidney disease patients using attenuated total reflectance fourier transform infrared spectrometry

Patipat Rachawangmuang^{a,c}, Patutong Chatchawal^{b,c}, Patcharaporn Tippayawat^{b,c,d}, Apinya Jusakul^{c,d}, Ratthapol Kraiklang^e, Worachart Lert-itthiporn^f, Anuchin Najermploy^f, Molin Wongwattanakul^{b,c,d,*}

ORIGINAL ARTICLE



Detection of common drug metabolites in urine using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

Yawen Yu¹ · Tangdong Chen² · Lijuan Yuan^{1,3} · Mao Sun¹ · Yuanming Wu^{1,4}

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Paper 2nd

SJR 2024

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Q2



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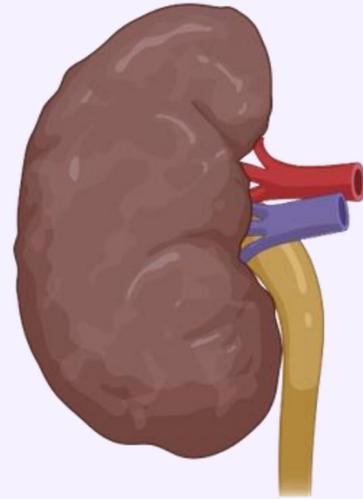
Discrimination of urine infrared spectral biomarkers for early-stage chronic kidney disease patients using attenuated total reflectance fourier transform infrared spectrometry

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Molin Wongwattanakul^{b,c,d,*}

Objective

- Discriminate early-stage CKD and healthy individuals
- Using ATR-FTIR spectroscopy
- Non-invasive random urine samples.

CKD: misdiagnosis early stage



Chronic kidney disease (CKD)

is a non-communicable disorder

- Induced by many factors
- leading to structural and functional
- damage the kidney

Worldwide
400 MILLION
Fell in CKD

Approximately
17.5%
Prevalence in Thailand

Mortality rate
41.5%
1990 - 2017

The definition of CKD

- The estimated glomerular filtration rate (eGFR)

$< 60 \text{ mL/min/1.73 m}^2$

- abnormal renal structure, function
- urinalysis tests over three months

Early-stage CKD is commonly misdiagnosed

Sensitivity
Specificity



Delayed diagnosis **increases** the risk of complications and mortality.

Urine sample



145 leftover

100 healthy, 45 CKD

Criteria	Age	eGFR
Healthy		≥ 90 mL/min/1.73 m ²
i. Early-stage (CKD stage 1–3),	> 17 years old	CKD stage 1: eGFR ≥ 90 mL/min/1.73 m ² CKD stage 2: eGFR 60–89 mL/min/1.73 m ² CKD stage 3: eGFR 30–59 mL/min/1.73 m ²
ii. Late-stage (CKD stage 4–5).		CKD stage 4: eGFR 15–29 mL/min/1.73 m ² CKD stage 5: eGFR < 15 mL/min/1.73 m ²

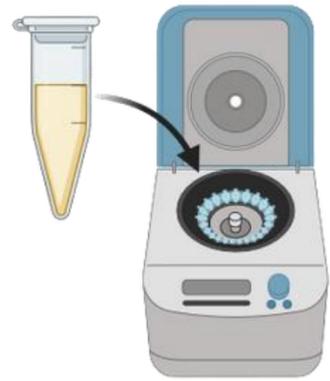
The exclusion criteria

- applied to individuals on kidney transplant recipients and pregnant women.

Result 1

ATR-FTIR spectral comparison in each group

To determine the IR spectrum between healthy, early-stage CKD, and late-stage CKD



10,000 RPM 5 min.

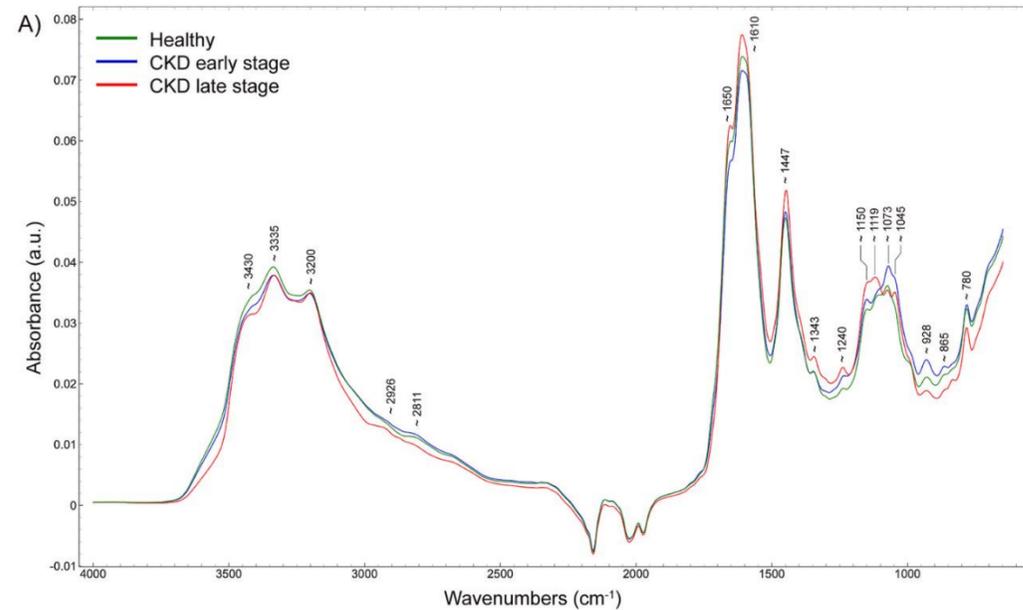


ATR-FTIR

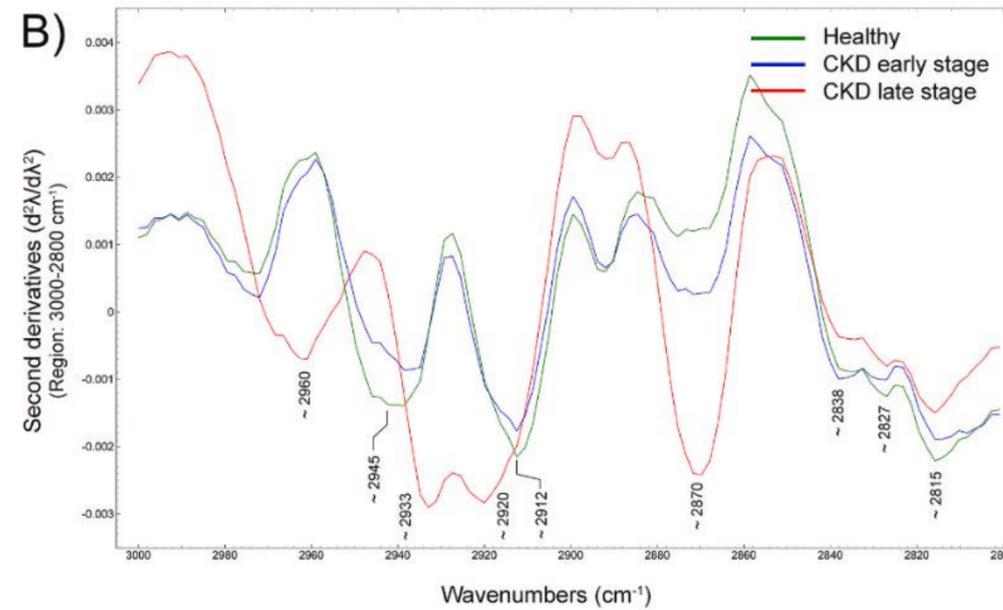
- Spectral range 4000–650 cm^{-1}
- Background scan 64 scan
- Sample scan 64 scan
- Spectral resolution 4 cm^{-1}



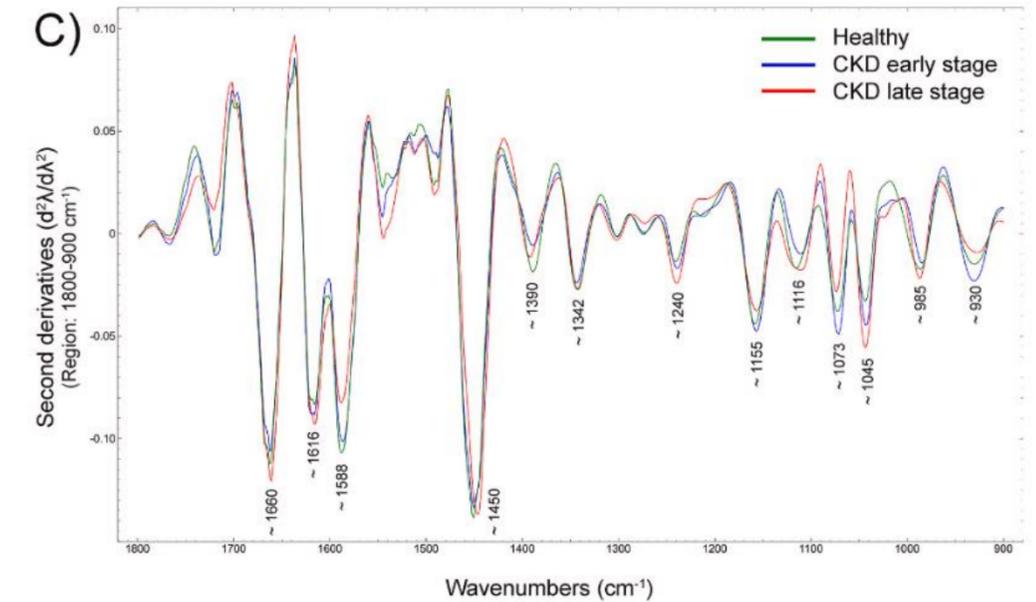
- i. C–H region (3000–2800 cm^{-1})
- ii. Fingerprint region (1800–900 cm^{-1})
- iii. Sub-fingerprint region (1400–1000 cm^{-1})
- iv. Combined region (3000–2800 + 1800–900 cm^{-1})



Full spectral region



C-H region



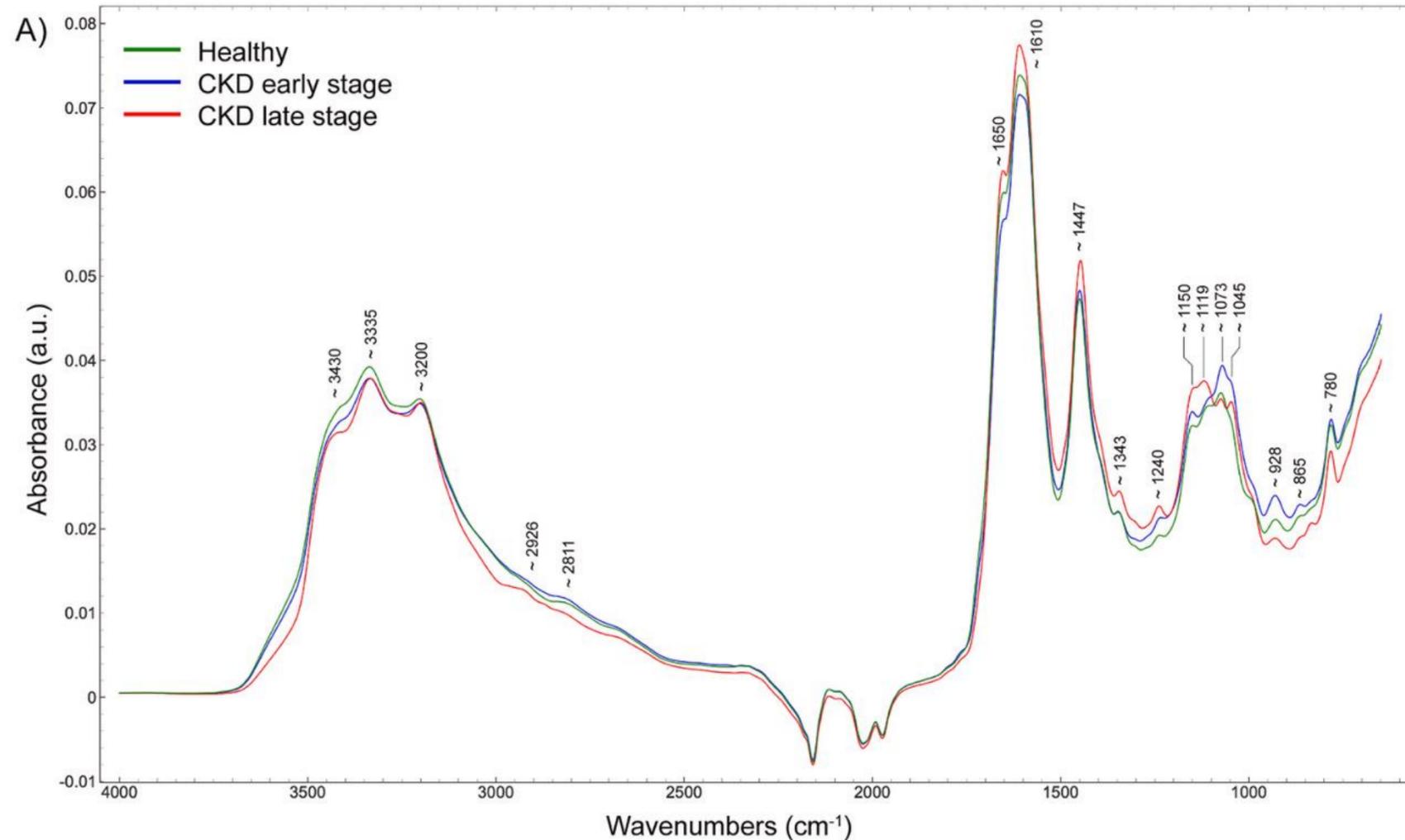
Fingerprint region

Result 1

Obvious clear pattern at 1200–1000 cm^{-1}

Average urine spectra of the three sample groups

— **Healthy**
— **Early-stage CKD**
— **Late-stage CKD**



Healthy

- Stable, balanced pattern

Early-stage CKD

- Minor changes in fingerprint region

Late-stage CKD

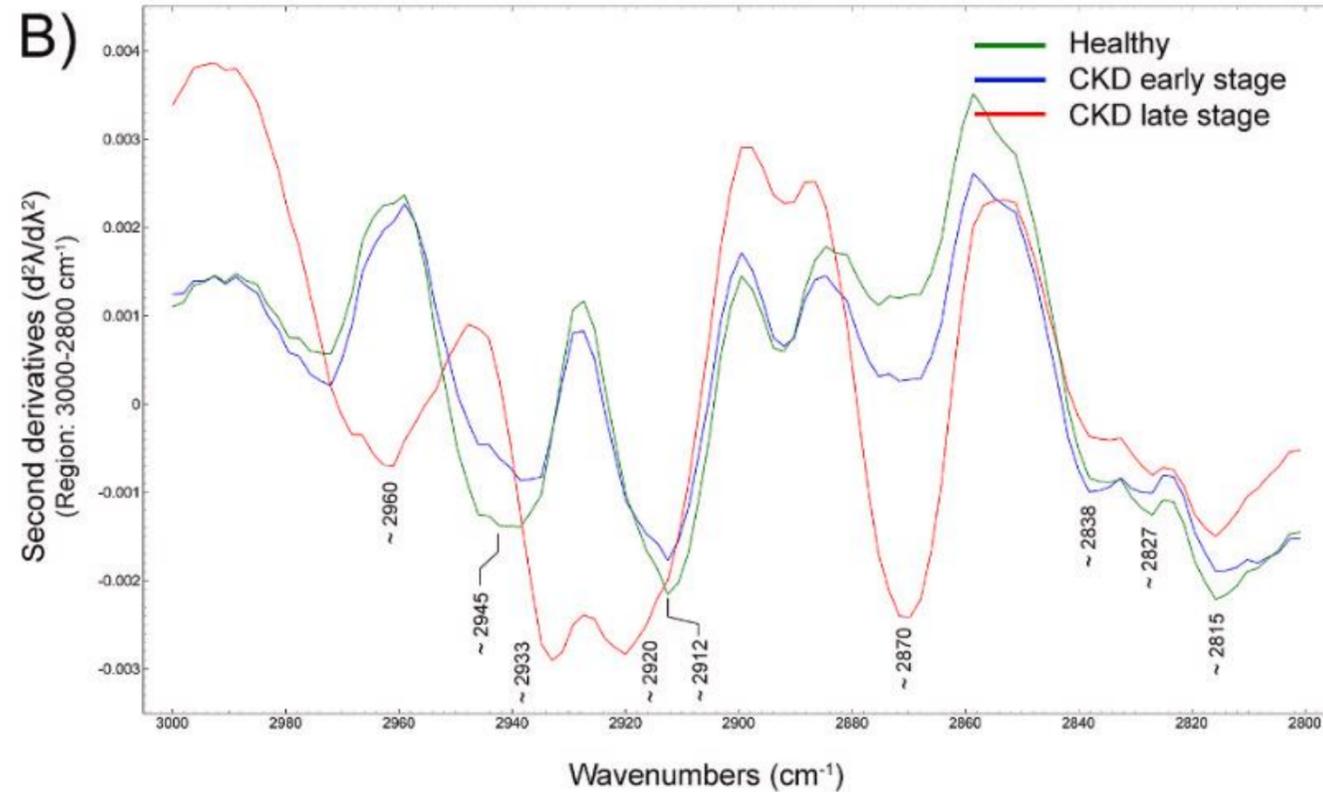
- Clear differences

Result 1

ATR-FTIR spectral comparison in each group

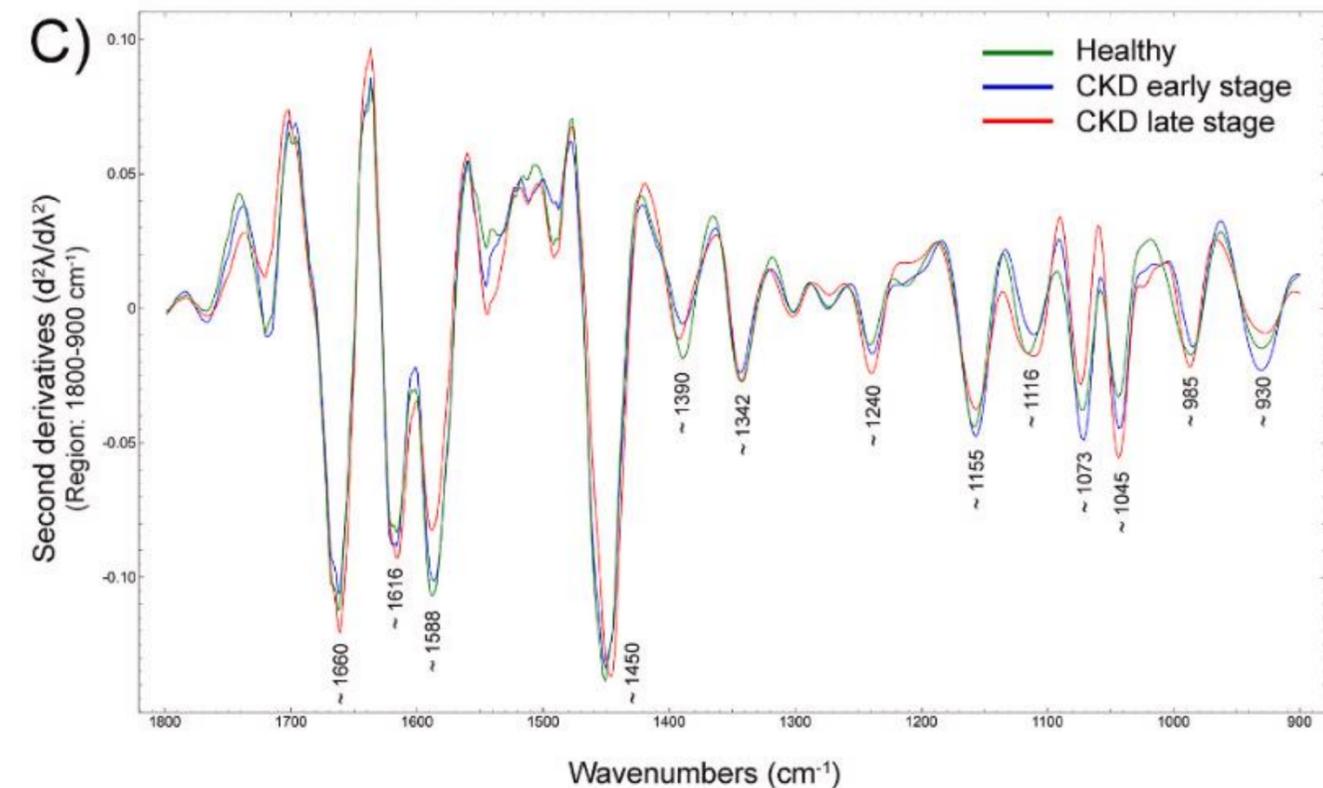
Second derivatives of the C–H region (B) and Fingerprint region (C)

— **Healthy**
— **Early-stage CKD**
— **Late-stage CKD**



3000-2800 cm⁻¹

- Lipid and carbohydrate component
- Early stage **Similar** to Healthy
- Late stage **obviously different**



1400-900 cm⁻¹

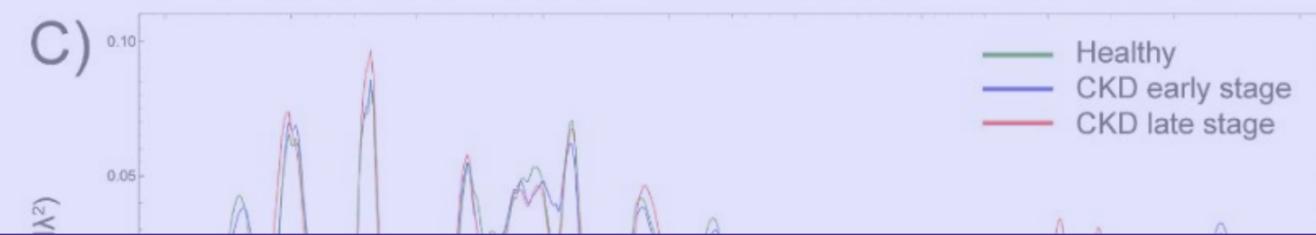
- Proteins, carbohydrates, and nucleic acids.
- **Slightly different**

Result 1

ATR-FTIR spectral comparison in each group

Second derivatives of the C–H region (B) and Fingerprint region (C)

— Healthy
— Early-stage CKD
— Late-stage CKD



So, they consider selecting the C-H region (Lipid and carbohydrate) for further analysis.

3000-2800 cm⁻¹

- Lipid and carbohydrate component
- Early stage **Similar** to Healthy
- Late stage **obviously different**

1400-900 cm⁻¹

- Proteins, carbohydrates, and nucleic acids.
- **Slightly different**

Result 2 PCA results for discrimination

Data analysis

Multivariate

- The C-H region (3000-2800 cm^{-1})
- The Phosphate/carbohydrate region (1400-1000 cm^{-1})



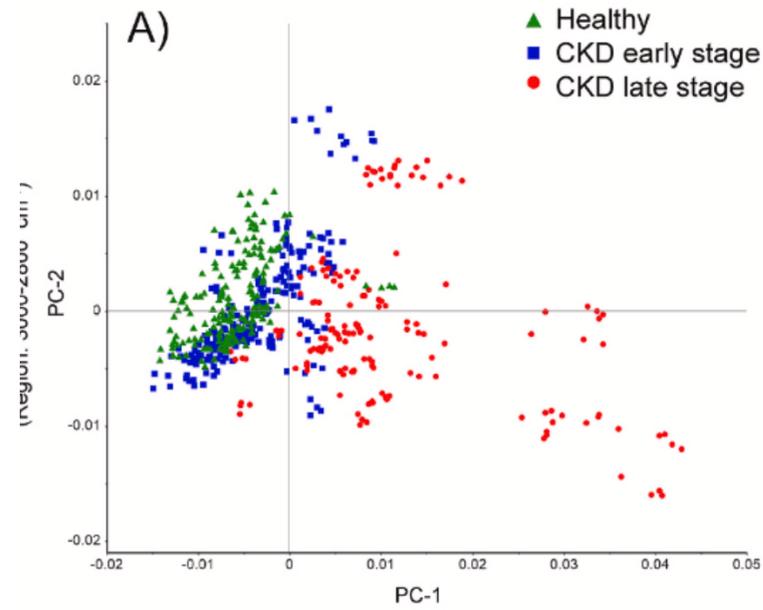
- Principal Component Analysis (PCA)
- Partial Least Square Regression (PLSR)



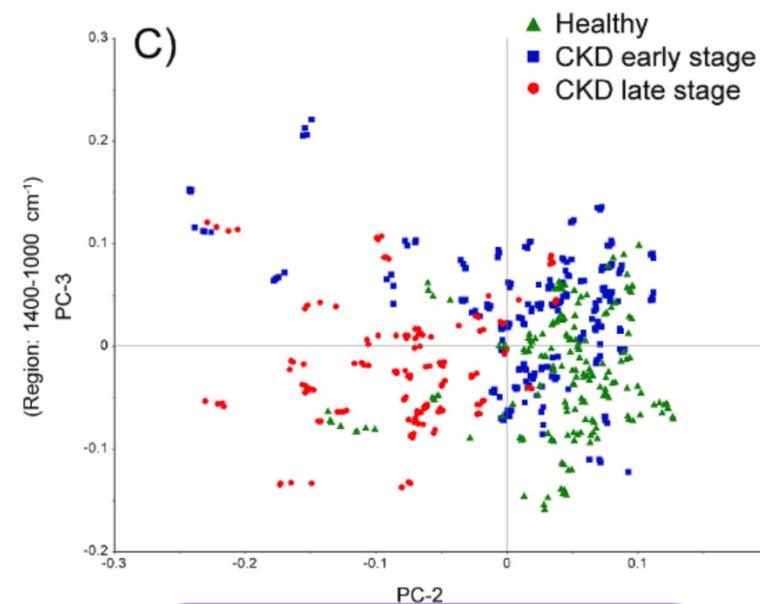
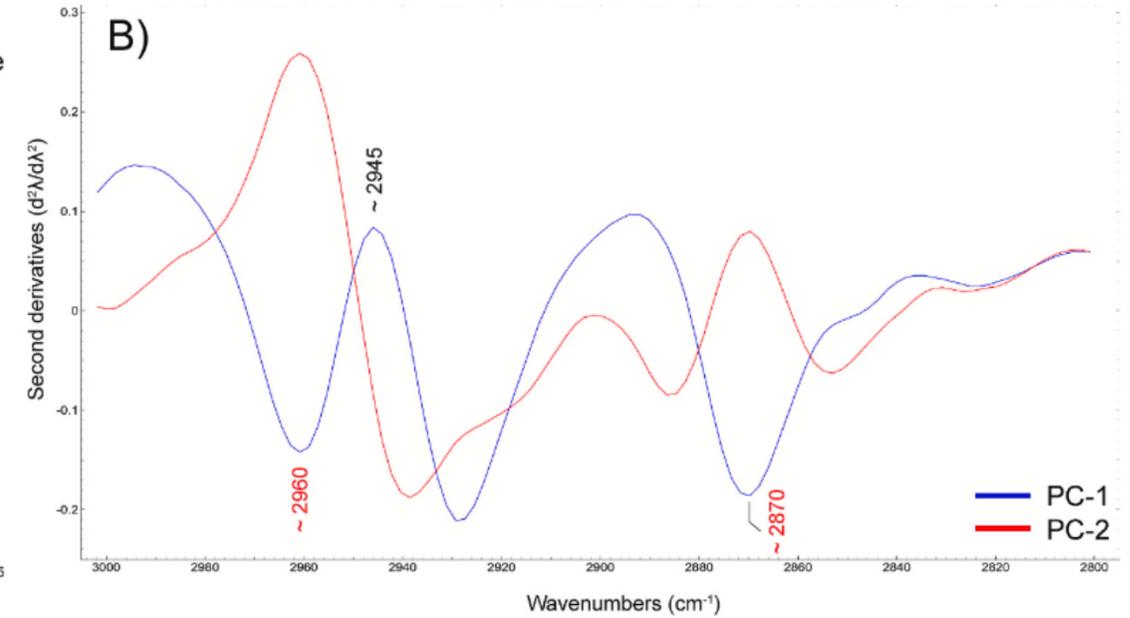
Machine learning

- k-Nearest Neighbor (kNN)
- Decision tree (DT)
- Random Forest (RF)
- Support Vector Machine (SVM)
- Neural Network (NN).

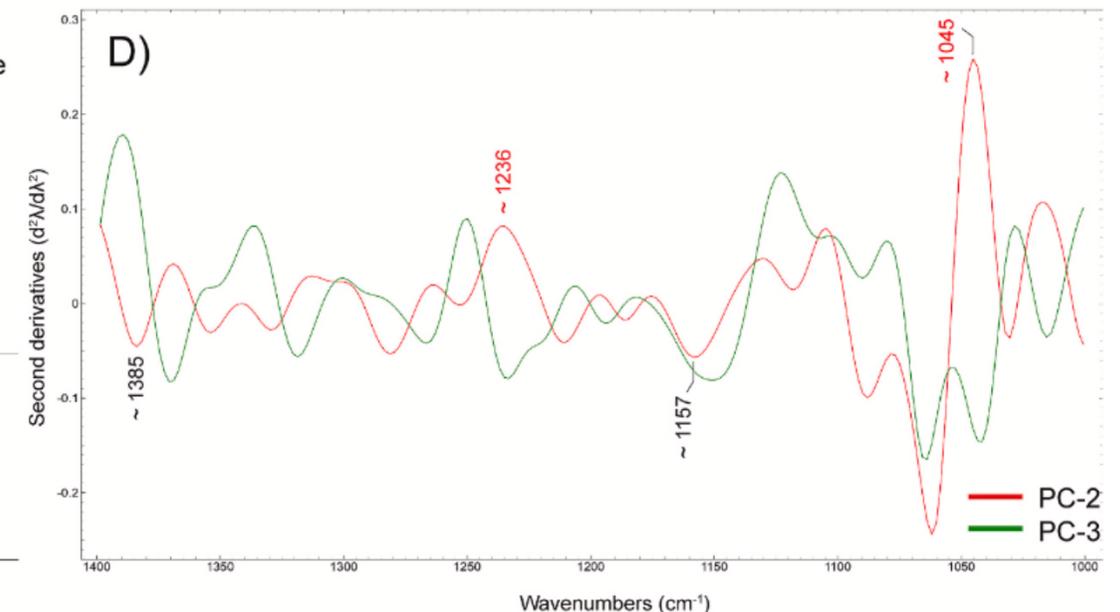
“ **Clearer distinction in the late-stage CKD** ”



3000-2800 cm^{-1}

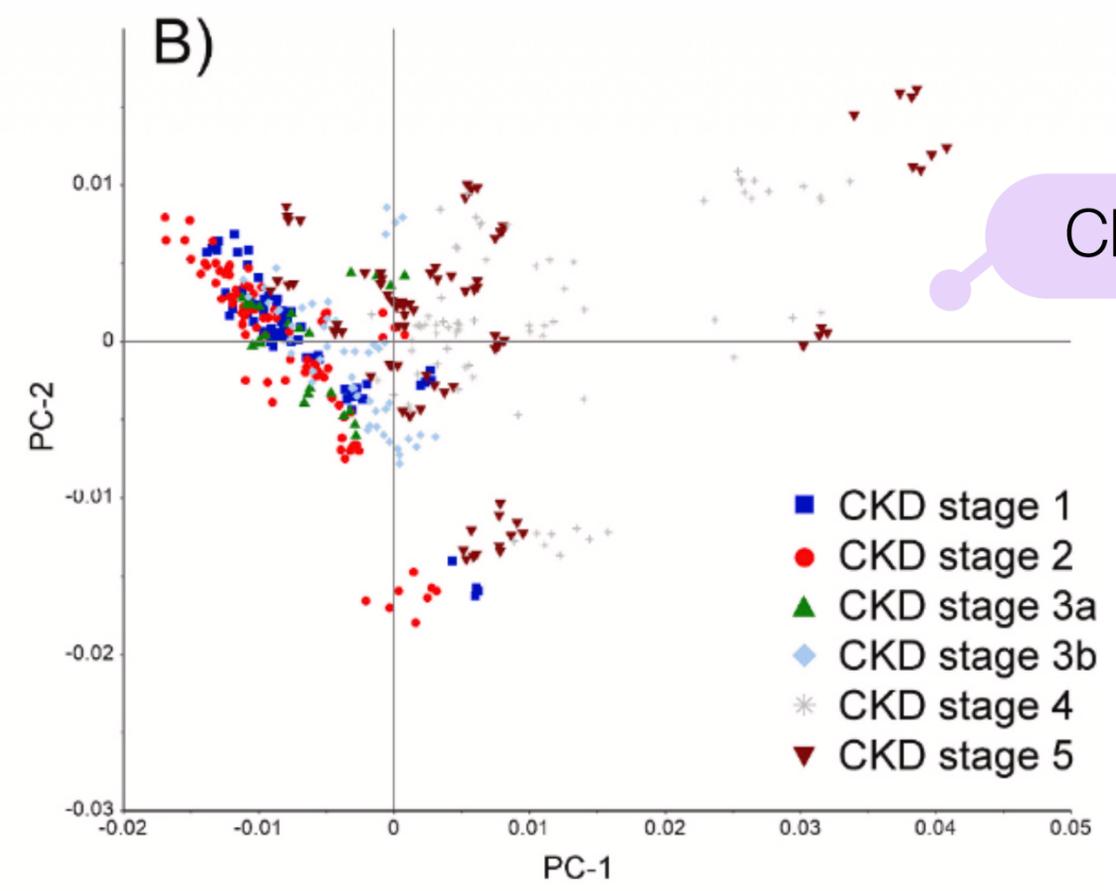
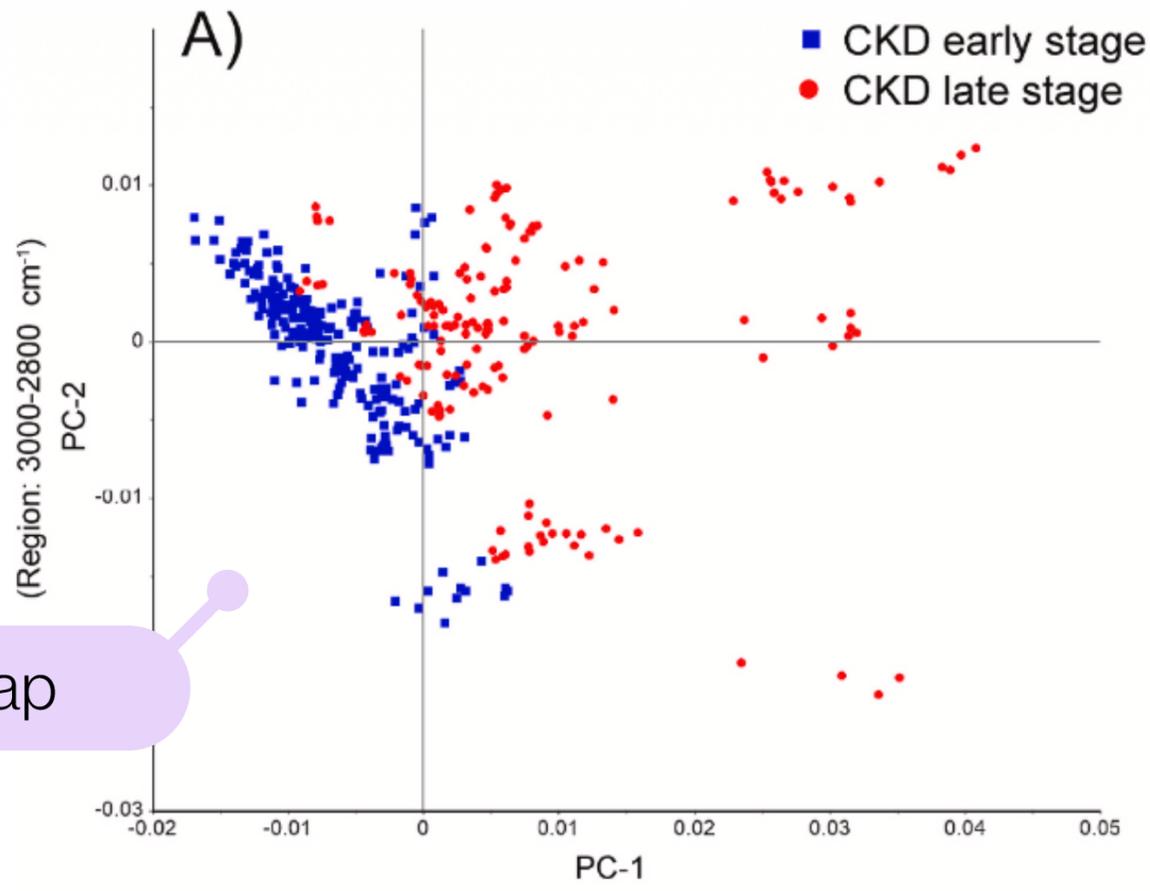


1400-1000 cm^{-1}



Result 3 PCA results for discrimination

To distinguish between early and late-stage CKD

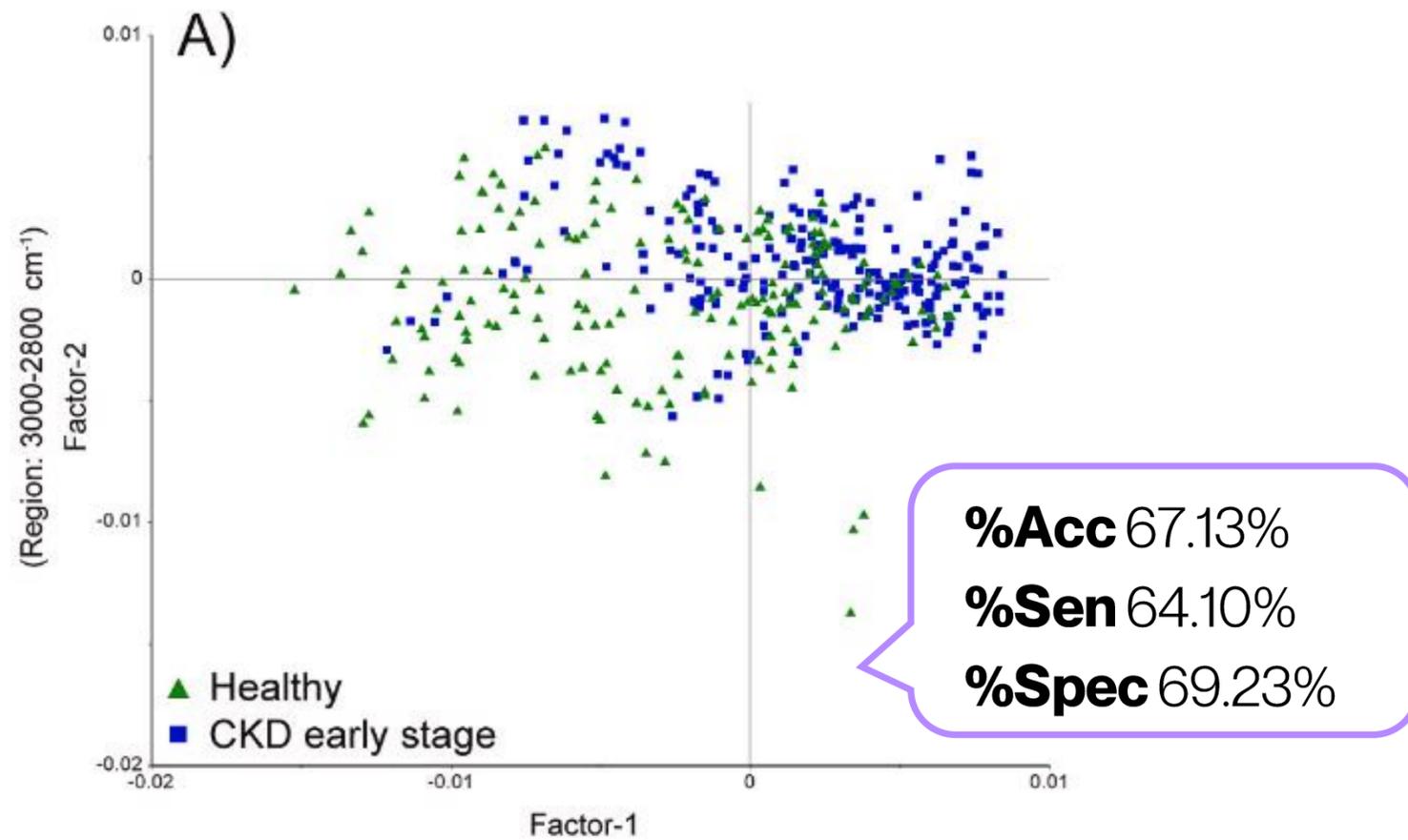


Stage CKD 3b

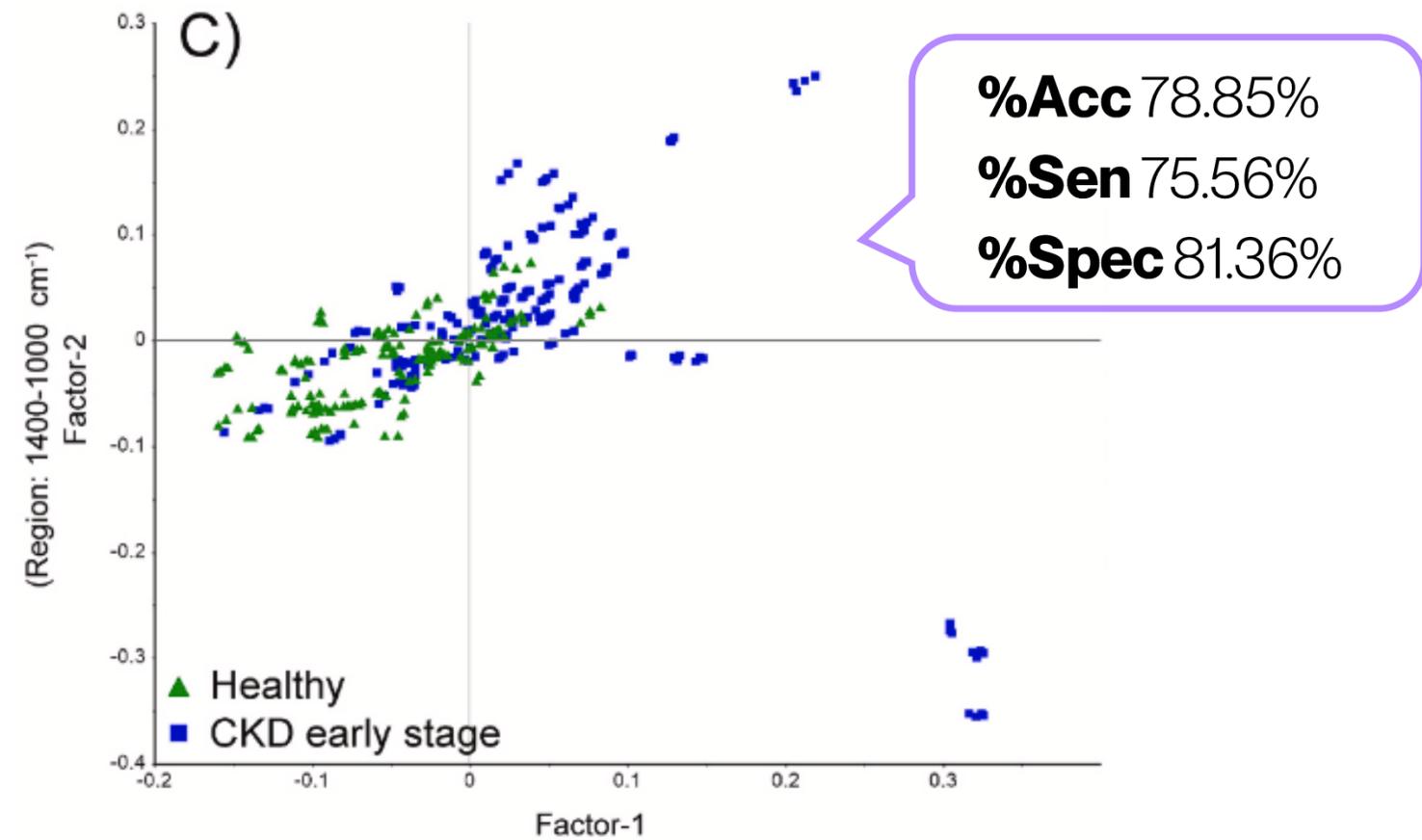
- The boundary between early and late CKD stages.

Result 4 PLS results for discrimination

To show the distinguish between healthy and early-stage CKD



3000-2800 cm^{-1}



1400-900 cm^{-1}

Not clear separated

Result 6 Prediction by trained machine learning models

Classification of early-stage CKD and healthy samples

Neural Network (NN)



Sensitivity: 85%



Specificity: 73%



Accuracy: 77%

Conclusion

Moderate separate early CKD from healthy individuals.

Early CKD stages

- overlapping spectra
- **Stage 3b**  **Transition to late CKD.**

NN using the fingerprint region: **The BEST performance**

- Sensitivity: 85%
- Specificity: 73%
- Accuracy: 77%



Detection of common drug metabolites in urine using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

Yawen Yu¹ · Tangdong Chen² · Lijuan Yuan^{1,3} · Mao Sun¹ · Yuanming Wu^{1,4} 

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Objective

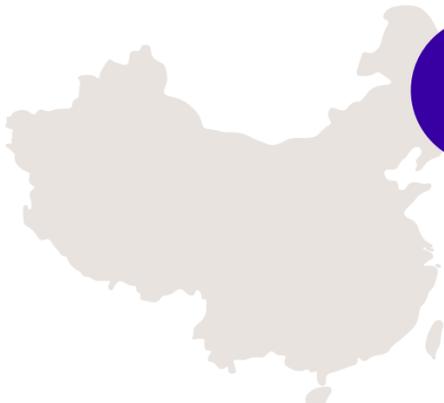
- Establish a rapid, non-invasive methodology
- Detection of drug abuse through the identification of common urinary drug metabolites
- Utilizing ATR-FTIR

Detection of Narcotic drugs



Narcotics

A global problem that many countries are trying to control and suppress.



China

Community **scrutiny** and legal **sanctions**.

Complex Situation

Novel psychoactive substances (NPS)



- They mimic the effects of older medications
- Adolescents are the main risk group, with easy access to NPS through online channels.

Drug use in China has increased by 34%,

- ecstasy and cocaine, popular stimulants among adolescents and working adults.

Need to detect the metabolite of each drug

- Heroin; 6-acetylmorphine (6-AM)
- Cocaine; Benzylecgonine (BE)
- Ecstasy (Methamphetamine); 3,4-methylenedioxyamphetamine (MDA)

Sample and methods

Sample Preparation



Standard solutions

- 1 mg/mL solutions of 6-AM, BE, and MDA



**260
Samples**

- **Calibration set:** 210 samples (blank urine)
- **Validation set:** 50 samples (independent set)
- **Spiked urine with 6-AM, BE, MDA** (1 mg/mL, 5 levels)

ATR-FTIR spectral



2 μ l



ATR-FTIR

- Spectral range 4000–400 cm^{-1}
- Background scan 32 scan
- Sample scan 32 scan
- Spectral resolution 4 cm^{-1}



4 minute
forced-air drying



Collect the IR
spectral

Chemometrics algorithms

Multivariate



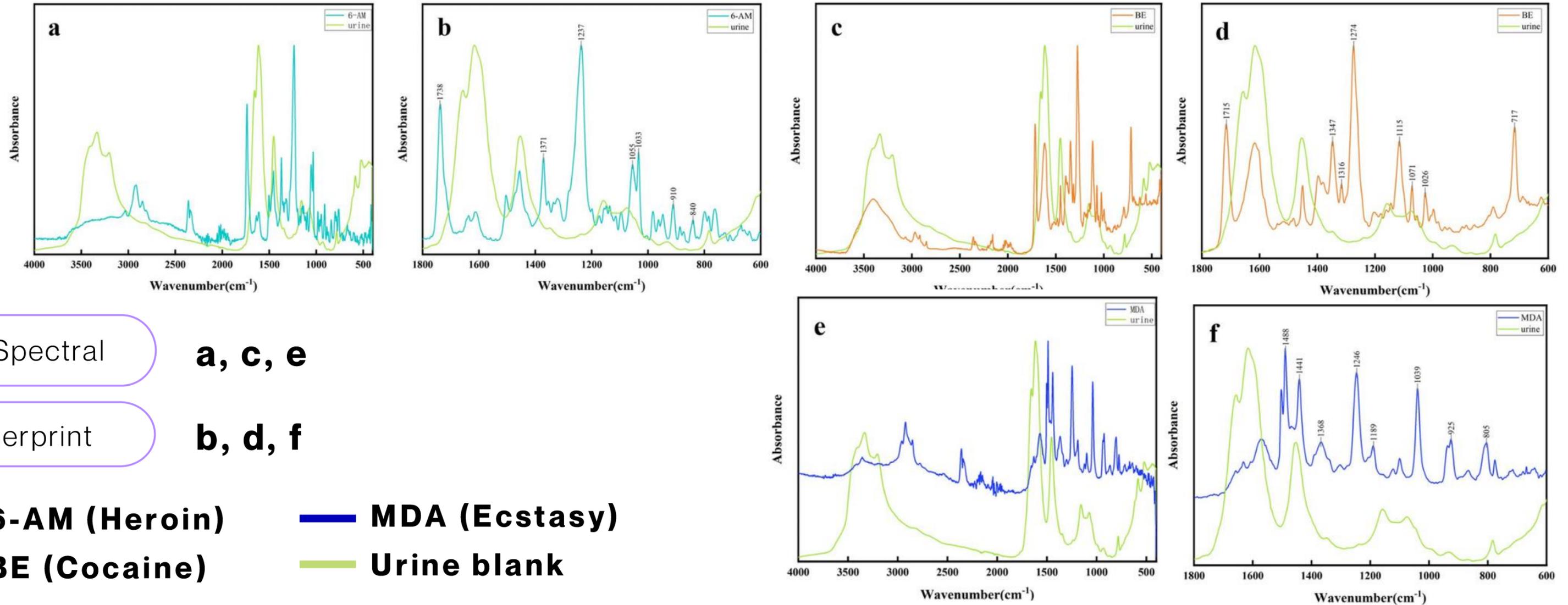
- The full spectral region (3000-2800 cm^{-1})
- The fingerprint region (1800-900 cm^{-1})



PCA, PLS-DA and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA)

Result 1

ATR-FTIR spectral comparison



Clearly separated in every region and every drug

Result 1

ATR-FTIR spectral comparison

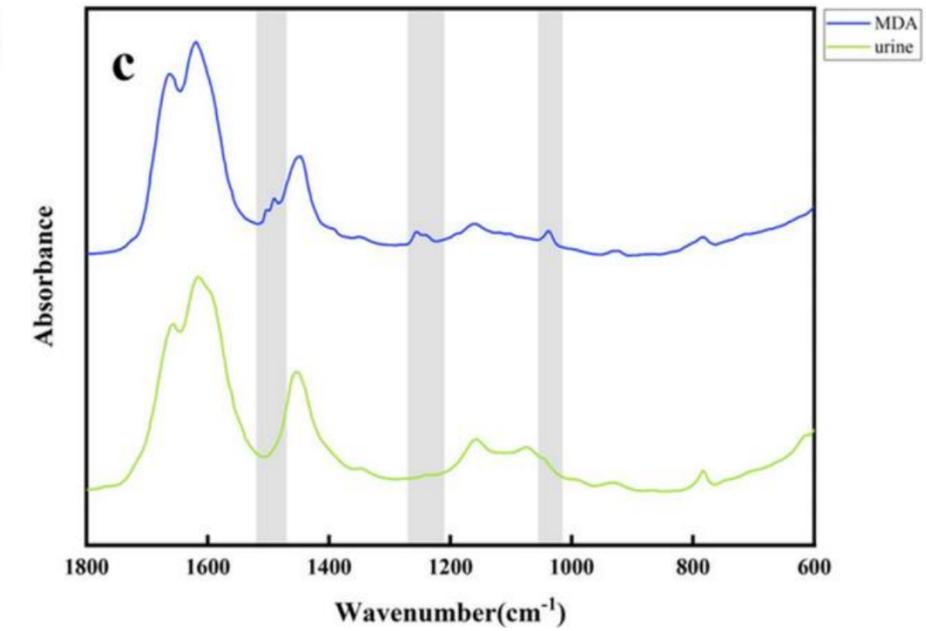
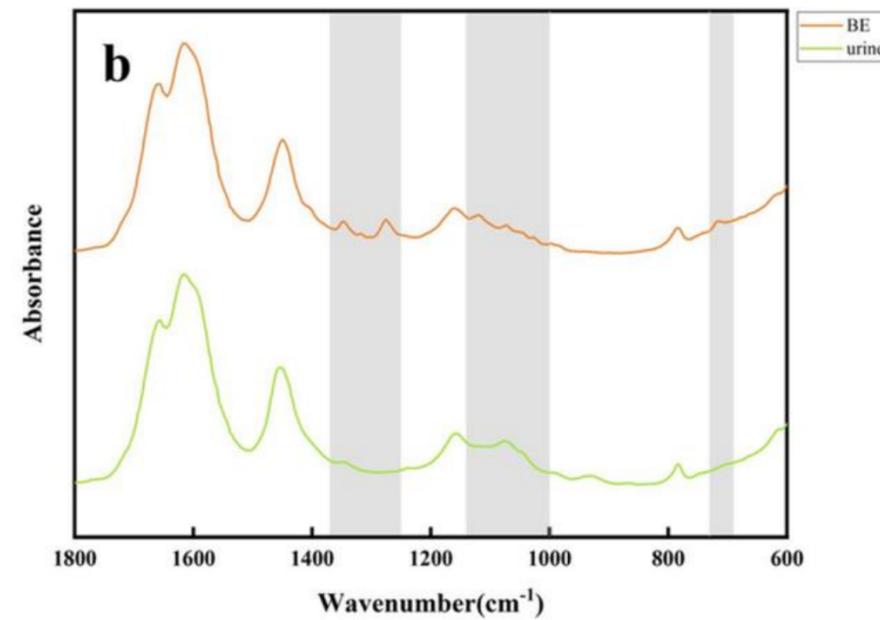
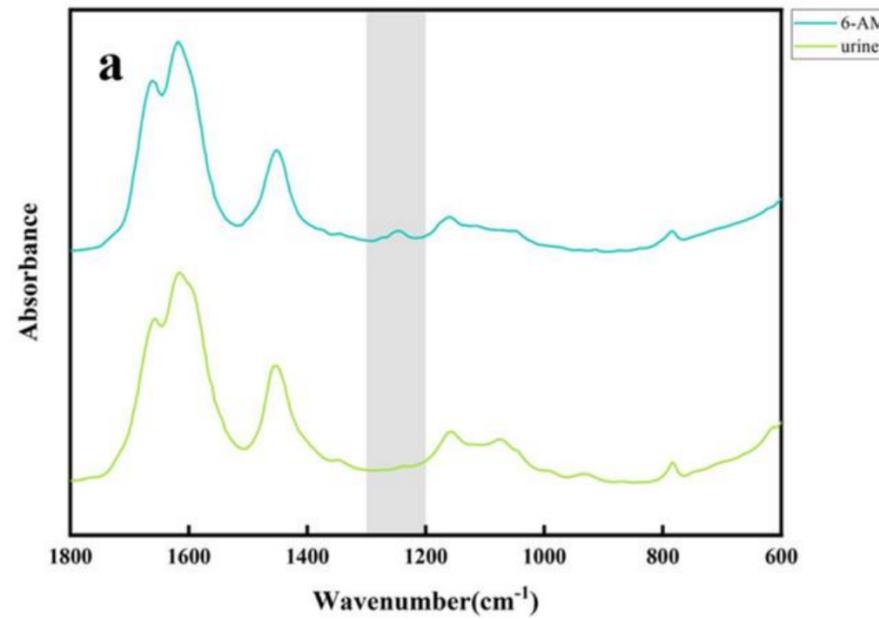
To identified the limit of detections by spike urine

— 6-AM (Heroin)

— MDA (Ecstasy)

— BE (Cocaine)

— Urine blank

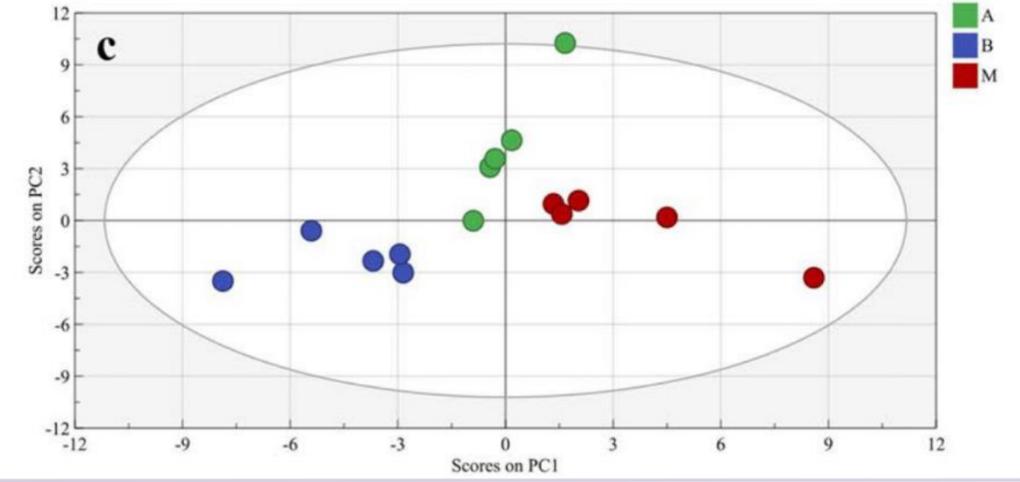
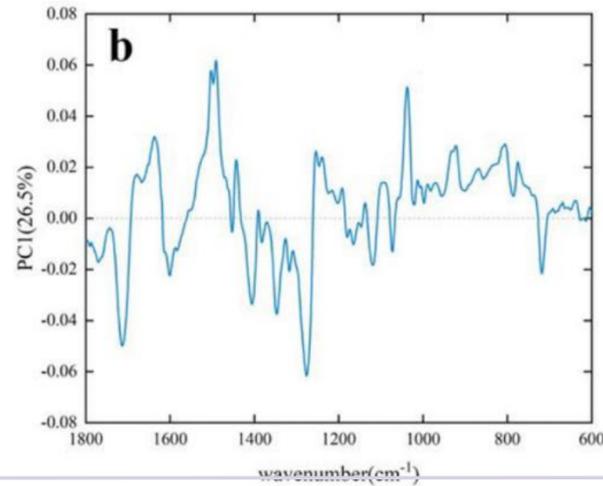
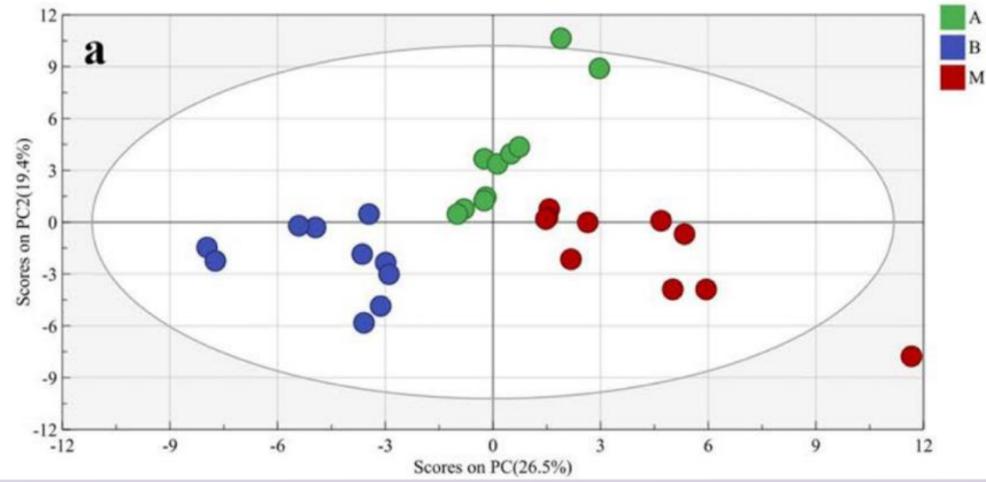


The grey region represent metabolite of drug

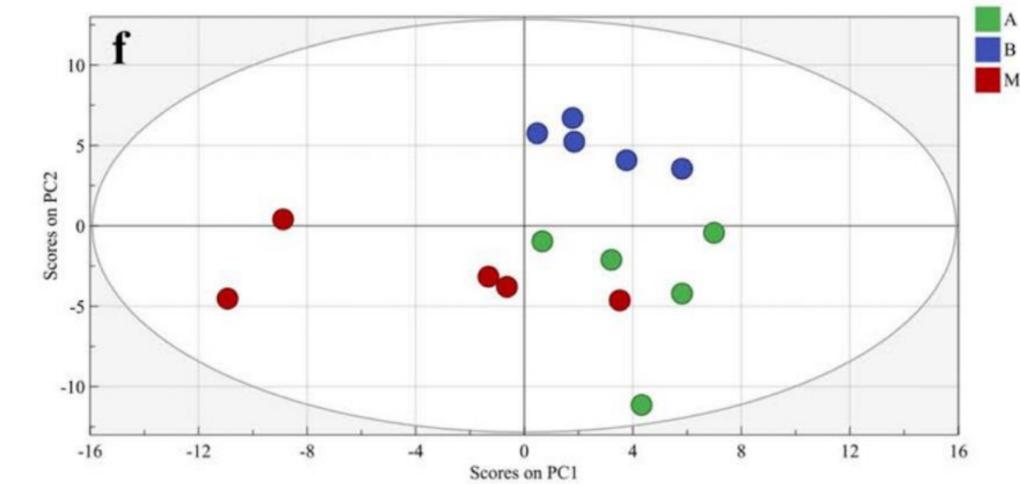
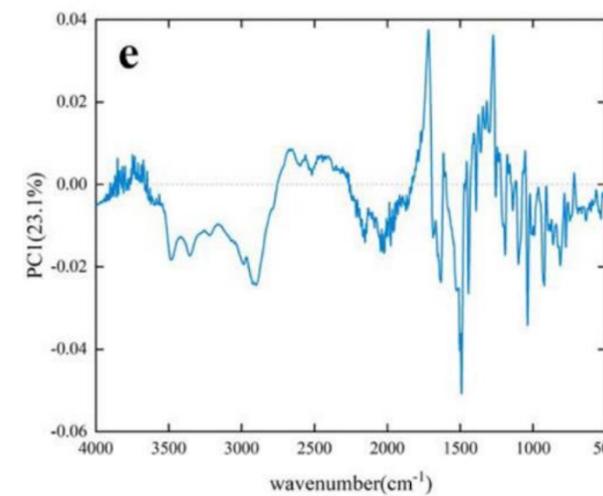
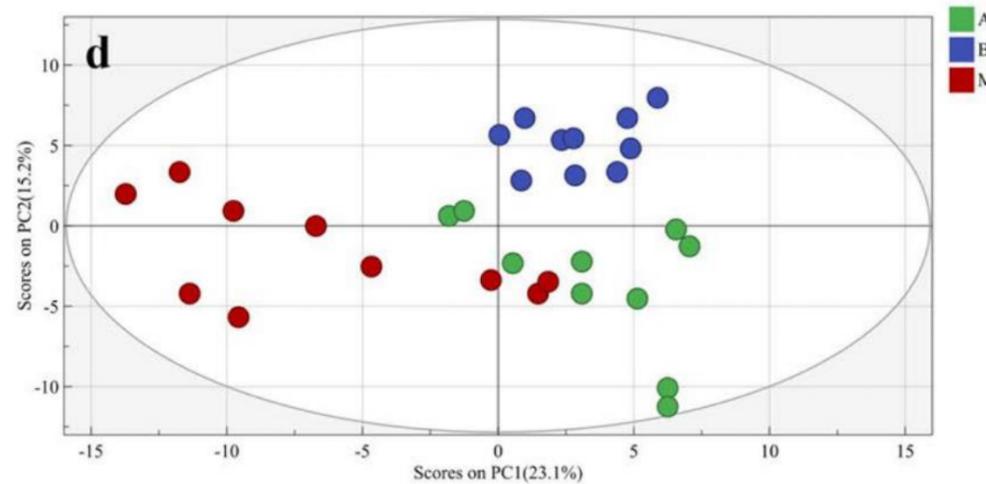
Result 2 PLS-DA score plots

A: 6-AM, B: BE, M: MDA

Fingerprint region



Full spectra region



Calibration set

Loading plot

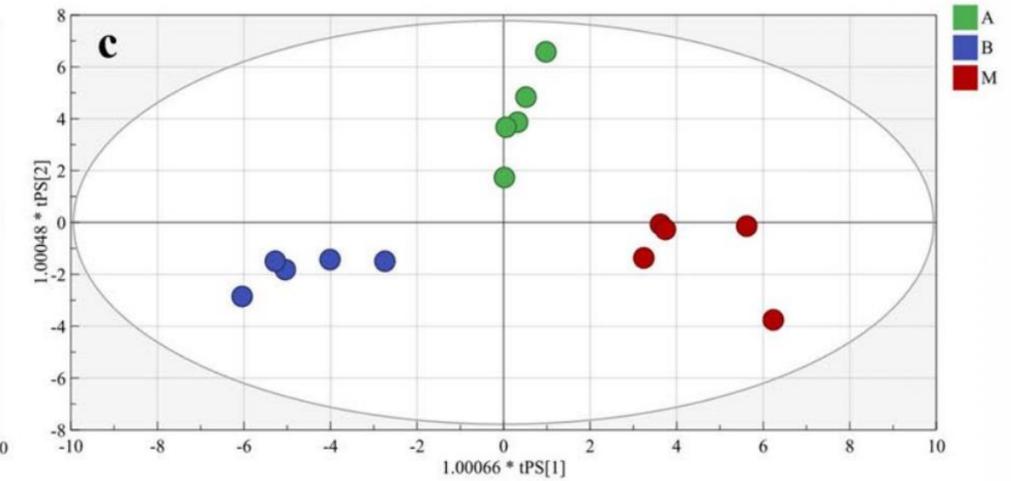
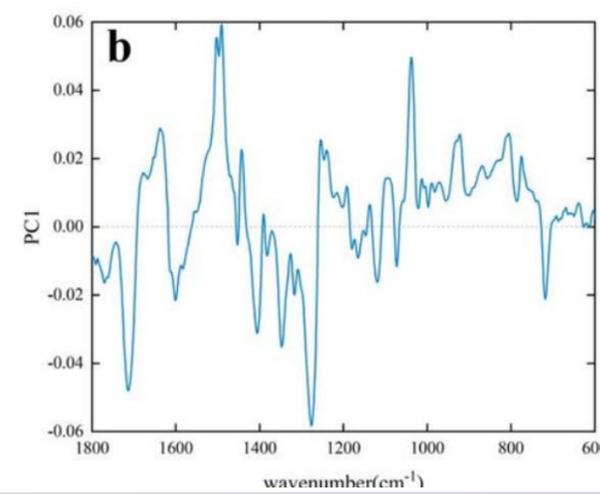
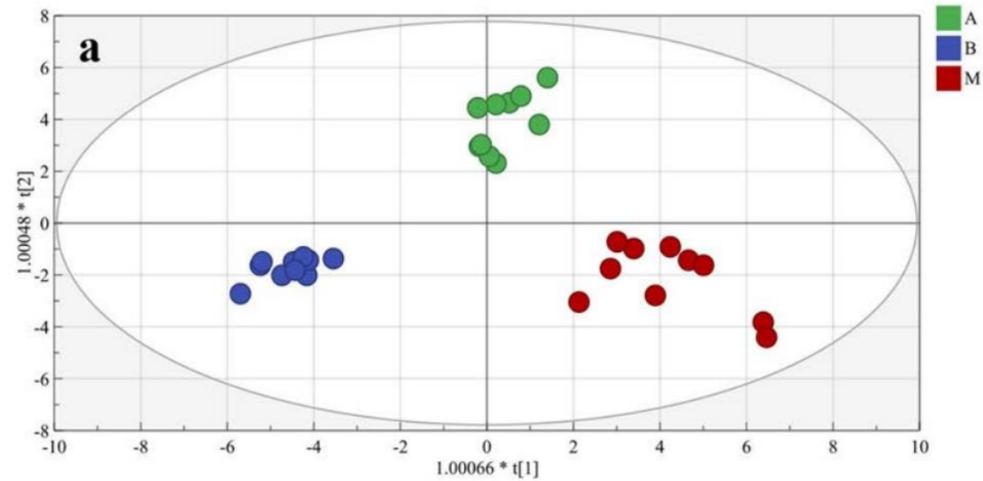
Validation set

Some overlap between the groups.

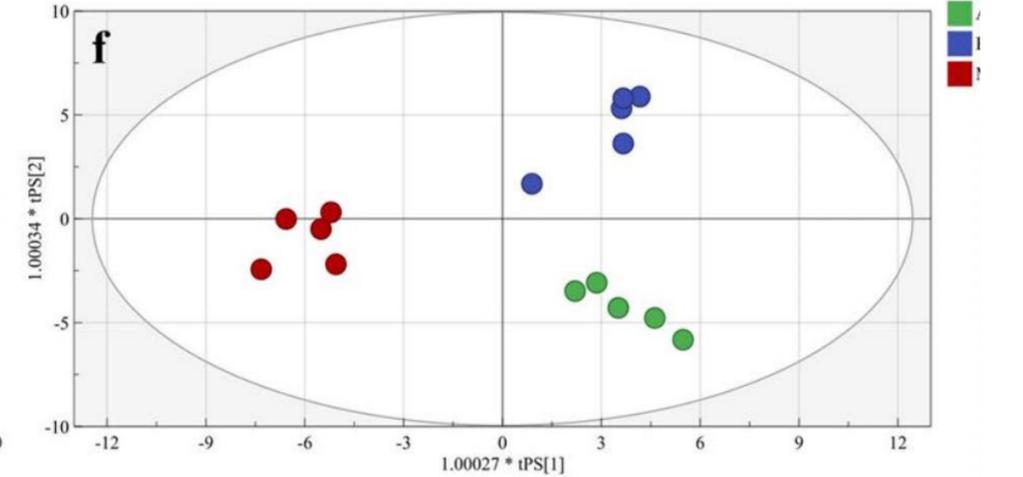
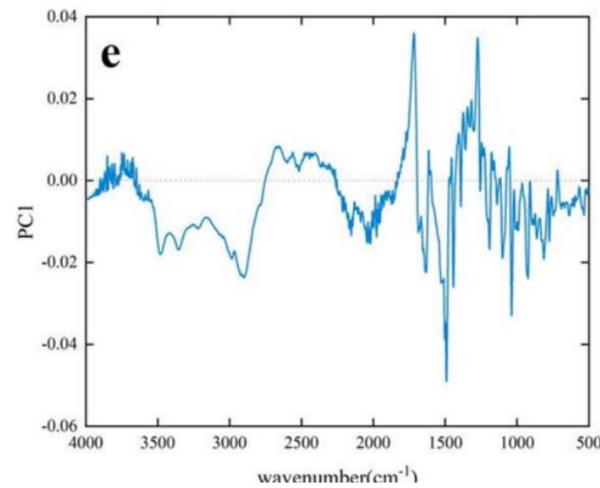
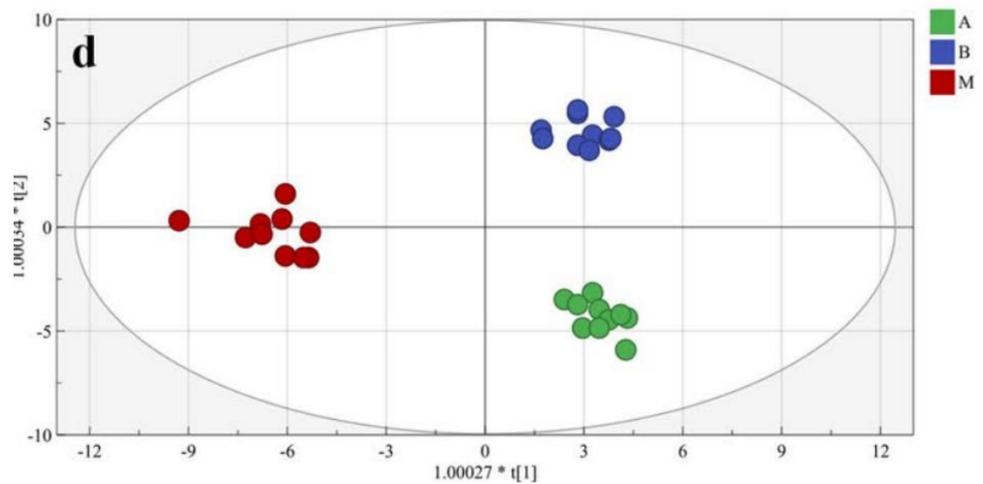
Result 3 OPLS-DA score plots

A: 6-AM, B: BE, M: MDA

Fingerprint region



Full spectra region



Calibration set

Loading plot

Validation set

- **Clearer classification than PLS-DA**
- **Separate all 3 metabolites without group overlap**

Conclusion

ATR-FTIR spectra

- **The fingerprint region enabled differentiation of drug metabolites**

The limit of detection (**LOD**): **0.02 mg/mL**

OPLS-DA: The BEST Performance

- Differentiating heroin (6-AM), cocaine (BE), and ecstasy (MDA) metabolites.

Criticisms

STRONG POINTS

WEAK POINTS

Paper 1st

- Good rationale
- Real patient samples were used
- Clear linkage to CKD clinical stages
- Strong performance outcomes

- Sample size limitations
- Overlap in score plots
- Lacked external validation or cross-population testing.
- Needed before practical clinical application.

Paper 2nd

- Minimal sample preparation and rapid analysis
- Low limit of detection (0.02 mg/mL)
- Use of calibration and validation datasets
- Effective metabolite discrimination using multivariate analysis

- No real drug-user samples
- No diagnostic performance metrics
- Limited PCA separation
- No comparison with LC-MS/MS

ACKNOWLEDGMENT



Asst. Prof. Dr. Wises Namwat

Advisor



Asst. Prof. Dr. Sakawrat Kanthawong

Co-Advisor



Dr. Bhanubong SAIBOONJAN



**Thank you for your
kind attention**