Mahidol University
Wisdom of the Land

An overview of toxicology testing: current methods and routine services

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Outlines

- Overview of toxicology testing
- Current methods
  - Therapeutic drug monitoring and drug intoxication
  - Alcohol and toxic alcohols
  - Drug of Abuse
  - Broad-spectrum testing
  - Cyanide
  - Solvents and other Volatile Substances
  - Trace Elements and Toxic Metals

Definition

- Analytical toxicology is the detection, identification and often also the measurement of drugs and other foreign compounds (xenobiotics) in biological and related specimens to help in the diagnosis, treatment, prognosis, and prevention of poisoning.

Flanagan RJ. Developing an analytical toxicology service: principles and guidance. Toxicol Rev. 2004;23(4):251-63.

Applications of analytical toxicology

- Clinical toxicology
- Forensic toxicology
- Therapeutic drug monitoring
- Occupational and environmental toxicology

Toxicology Laboratory Services in Ramathibodi Hospital

- 133 Tests
- Therapeutic drug monitoring
- Emergency toxicology testing
- Drugs of Abuse: single targeted and multiple targeted DOA screening
- General Toxicology Screen: screening for drugs and drug of abuses, drugs and pesticides screening
- Toxic Alcohols (Ethylene Glycol, Methanol)
- Acetylcholinesterase and Cholinesterase
- Paraquat
- Cyanide
- Solvents and other Volatile Substances
- Trace Elements and Toxic Metals

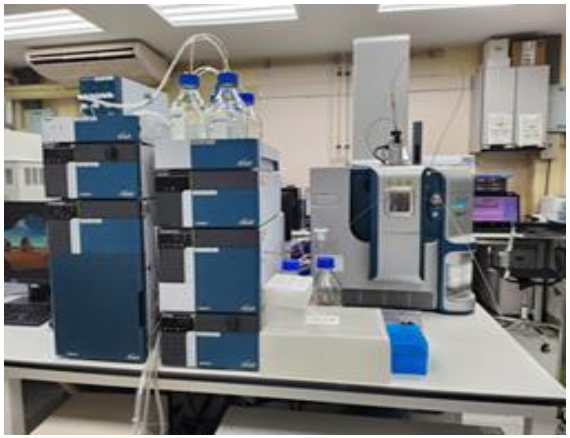
Laboratory Facilities

- 2 Automation
- 1 Double-beam spectrophotometer
- 2 HPLC (DAD, FLD)
- 2 HS-GC-FID
- 2 GC-MS
- 1 LC-MS
- 4 LC-MS/MS (2Q-TOF, QTRAP, QQQ)
- 1 F-AAS and 1 GF-AAS
- 1 LC-ICP-MS

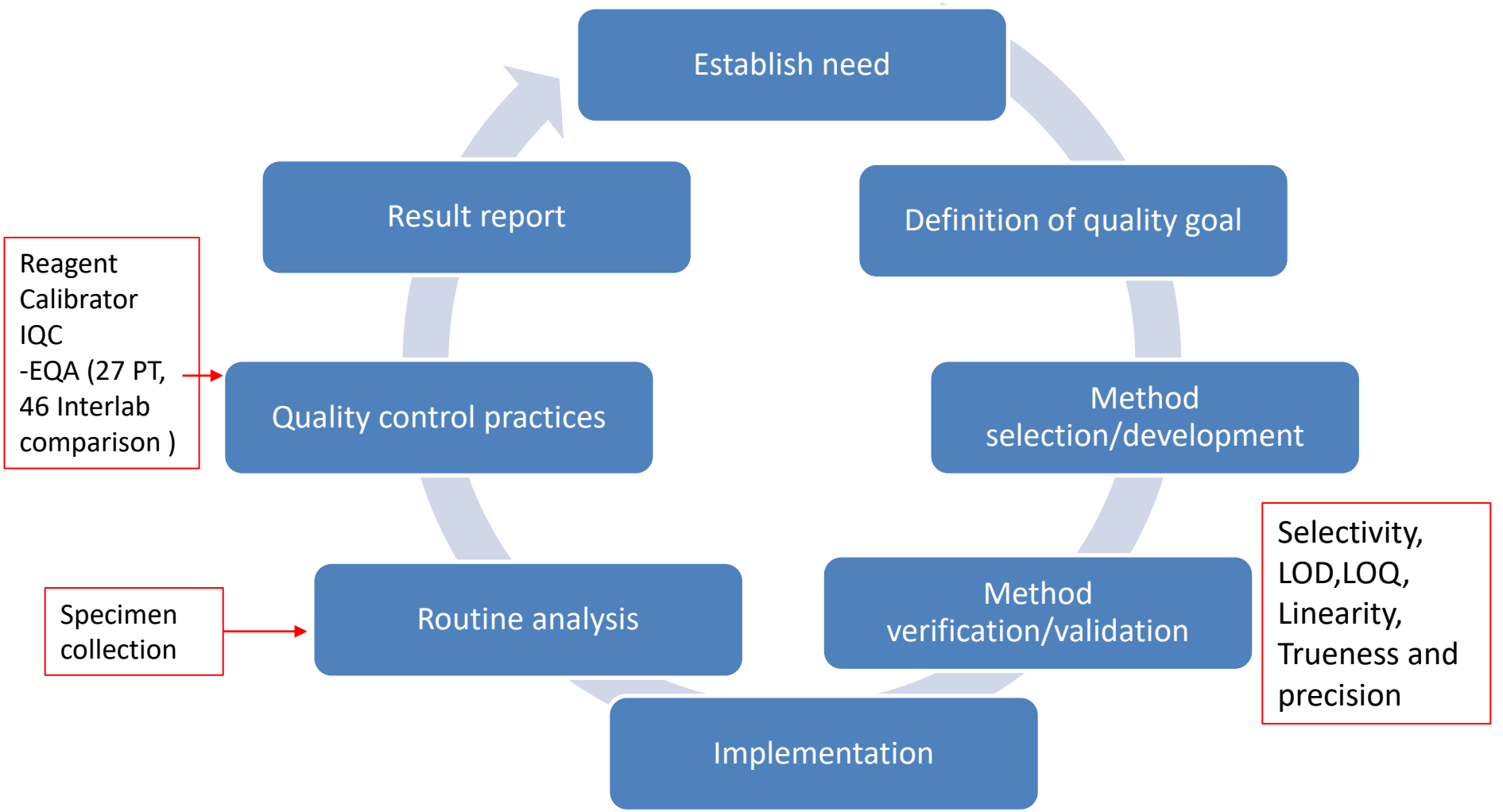
New Instruments

Sensitivity ↑
Sample volume ↓
TAT ↓

- X500R QTOF (SCIEX)
- QTRAP 6500+ system (SCIEX)
- Xevo TQ-S micro Triple Quadrupole Mass Spectrometry (Waters)



Implementation of new method



Adapted from Burtis, C.A. and Bruns, D.E. (2015) Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7th Edition, Elsevier Missouri, Saunders.

CLIA proficiency testing criteria for acceptable analytical performance

Toxicology CLIA 2024		
Analyte or Test	**NEW Criteria for AP**	**OLD AP**
Acetaminophen	**TV ± 15% or or ± 3 mcg/mL (greater)**	None
Alcohol, blood	TV ± 20%	TV ± 25%
Blood lead	TV ± 10% or 2mcg/dL (greater)	TV ± 10% or ±4 mcg/dL (greater)
Carbamazepine	**TV ± 20% or ± 1.0 mcg/mL (greater)**	TV ± 25%
Digoxin	TV ± 15% or ± 0.2 ng/mL (greater)	None
Gentamicin	TV ± 25%	Same
Lithium	**TV ± 15% or ± 0.3 mmol/L (greater)**	TV ± 0.3 mmol/L or 20% (greater)
Phenobarbital	**TV ± 15% or ± 2 mcg/mL (greater)**	TV ± 20%
Phenytoin	TV ± 15% or ± 2 mcg/mL (greater)	TV ± 25%
Salicylate	**TV ± 15% or ± 2 mcg/mL (greater)**	None
Theophylline	TV ± 20%	TV ± 25%
Tobramycin	TV ± 20%	TV ± 25%
Valproic acid	TV ± 20%	TV ± 25%
Vancomycin	TV ± 15% or ± 2 mcg/mL (greater)	None

https://www.westgard.com/clia-a-quality/quality-requirements/1002-2024-clia-requirements.html

Specimen

Antemortem

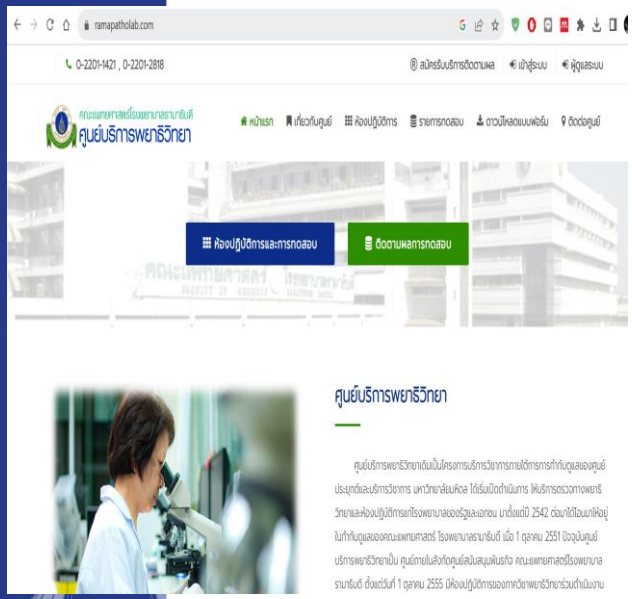
- Blood
- Urine
- Gastric contents

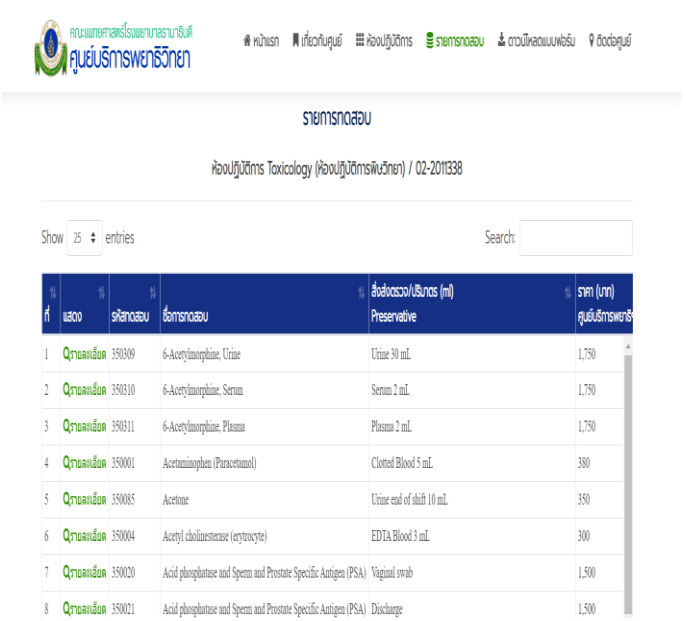
Postmortem

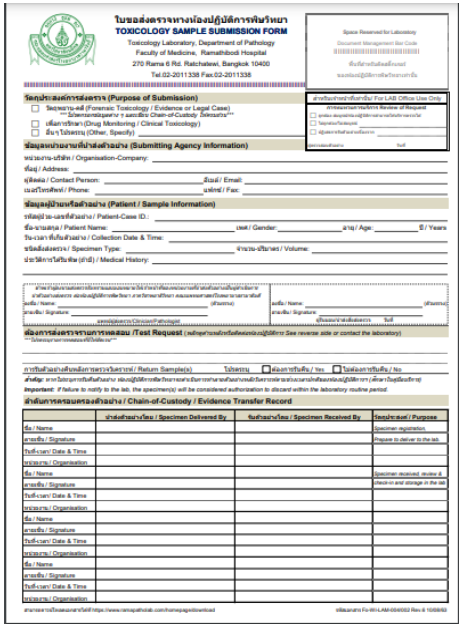
- Urine
- Blood
- Vitreous Humor
- Gastric contents
- Bile
- CSF

Specimen collections

- Information is available online at https://www.ramapatholab.com
  - SOP for specimen collection and transportation
  - Request form with chain of custody
  - Test catalog 292 items







Current methods

- Therapeutic drug monitoring and drug intoxication
- Alcohol and toxic alcohols
- Drug of Abuse
- Broad-spectrum testing
- Cyanide
- Solvents and other Volatile Substances
- Trace Elements and Toxic Metals

Therapeutic drug monitoring and drug intoxication

Immunoassay and enzymatic assay

- Available 24 hrs, TAT 1 hr
- ISO 15189 accredited
- 12 drugs
- Carbamazepine · Carbamazepine · Digoxin · Gentamicin · Methotrexate · Paracetamol · Phenobarbital · Phenytoin · Salicylic acid · Theophylline · Valproic acid · Vancomycin

LC-MS/MS

- Voriconazole
- Itraconazole
- Fluconazole
- Posaconazole
- Lamotrigine
- Levetiracetam

Urine Immunoassay (qualitative)

- Cocaine
- Opiates
- Barbiturates
- Amphetamines
- Phencyclidine

Therapeutic drug monitoring and drug intoxication

QTRAP 6500+ (TAT 5 days)

- Drug quantitative
- Clozapine and metabolite
- Nicotine and metabolite Pregabalin
- Gabapentin
- Lamotrigine
- Acyclovir

Xevo TQ-S micro (TAT 3 days)

- Topiramate
- Voriconazole
- Levetiracetam
- Itraconazole
- Fluconazole
- Posaconazole

Alcohol and Toxic alcohols

- Ethanol
  - Forensic cases
- Analytical Method: Headspace-GC-FID
- Sample types: WB (NaF), serum, urine, CSF, vitreous humors, other
- Analytical measurement range: 5-400 mg/dL
- %RSD: ±5
- 2 GC column, duplicate
- ISO 17025 accredited

- Methanol, Isopropanol
- Analytical Method: Headspace-GC-FID
- Sample types: WB(NaF), urine
- Analytical measurement range: 1-50 mg/L

Drug Of Abuse

- Screening: urine immunoassay
- Confirmation: GC-MS or LC-MS/MS (Quantitative)
  - Single targeted: Gamma-Hydroxybutyric Acid (GHB), ketamine, mitragynine
  - Drugs panel
    - 5-Panel: Amphetamines, Cocaine metabolite, Cannabinoid metabolite, Opiates Phencyclidine
    - 9-Panel: Amphetamines, Barbiturates, Benzodiazepines, Cannabinoid metabolite Cocaine metabolite, Methadone, Opiates, Phencyclidine, Propoxyphene
    - Drugs of abuse panel: Amphetamines, Barbiturates, Benzodiazepines, Buprenorphine, Cannabinoid metabolite, Cocaine metabolite, Fentanyl, Ketamine, Methadone, Methaqualone, Opiates, Phencyclidine, Propoxyphene
- Broad-spectrum testing

Example

Targeted DOA Screening

	Scope of Testing	LOD
Amphetamines	Amphetamine, ephedrine, MDA, MDEA MDMA, pseudoephedrine, Methamphetamine, Phentermine	10 ng/mL
Cannabinoids	THC, Hydroxy-THC, Carboxy-THC	2.0 ng/mL
Cocaine	Cocaine, Benzoylecgonine, Ecgonine Methylester Cocaethylene	10 ng/mL
Opiates	6-Acetylmorphine, Codeine, Hydrocodone, Hydromorphone, Morphine, Oxycodone, Oxymorphone	5.0 ng/mL for 6-MAM, 10 for other

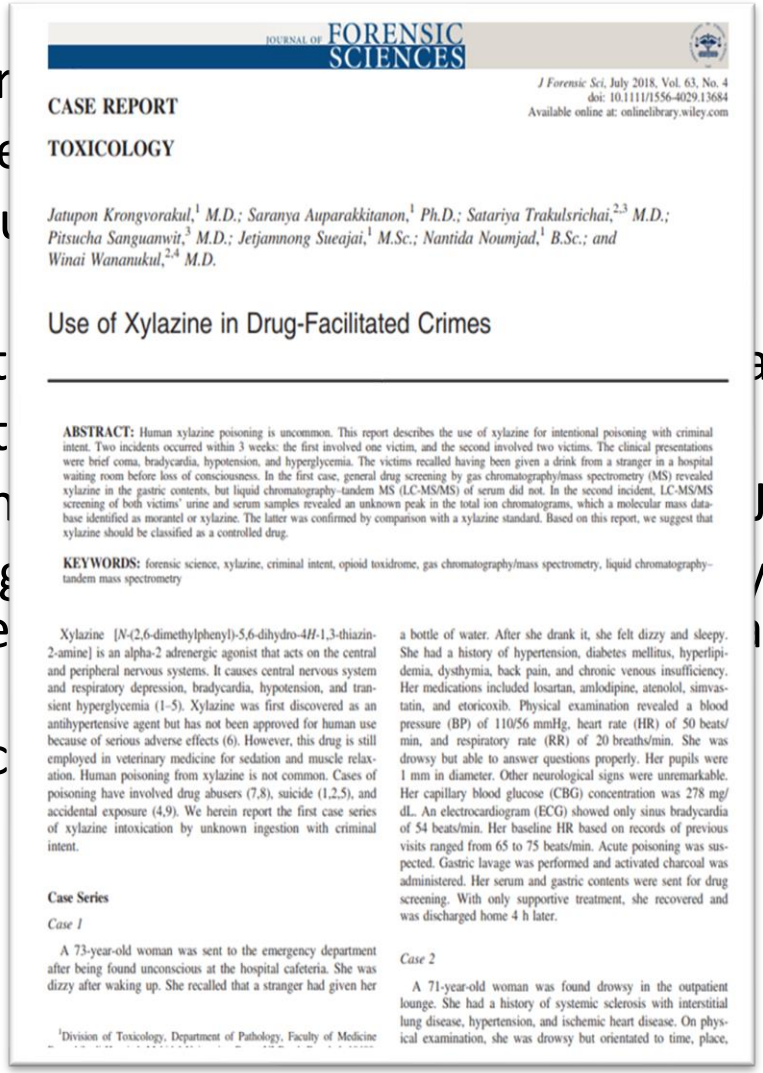
Comprehensive or broad-spectrum testing

- Broad-spectrum testing is for patients admitted to the hospital who remain intoxicated, obtunded, or comatose, where a broad-spectrum ("comprehensive") screening panel is necessary to cover drugs and substances that may have clinical significance and would not be identified based on the findings of the first tier of laboratory tests.
- Results of these tests might be used for more long-term management and/or counseling of patients.

Wu AH, McKay C, Broussard LA, Hoffman RS, Kwong TC, Moyer TP, Otten EM, Welch SL, Wax P;NACB. National academy of clinical biochemistry laboratory medicine practice guidelines: recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department. Clin Chem. 2003 Mar;49(3):357-79

Comprehensive or broad-spectrum testing

- Screening for
- Analytical Me
- MS modes: fu
- LOD: various
- Scope of test ances and metabolites
- Drug-facilitat
- Synthetic can JWH-073, JWH-250
- Other: Mitrag ysergic acid diethylamide cid GHB, ketamine, cathinone
- ISO 17025 ac



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CASE REPORT

TOXICOLOGY

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Use of Xylazine in Drug-Facilitated Crimes

ABSTRACT: Human xylazine poisoning is uncommon. This report describes the use of xylazine for intentional poisoning with criminal intent. Two incidents occurred within 3 weeks: the first involved one victim, and the second involved two victims. The clinical presentations were brief coma, bradycardia, hypotension, and hyperglycemia. The victims recalled having been given a drink from a stranger in a hospital waiting room before loss of consciousness. In the first case, general drug screening by gas chromatography/mass spectrometry (MS) revealed xylazine in the gastric contents, but liquid chromatography–tandem MS (LC-MS/MS) of serum did not. In the second incident, LC-MS/MS screening of both victims' urine and serum samples revealed an unknown peak in the total ion chromatograms, which a molecular mass database identified as morantel or xylazine. The latter was confirmed by comparison with a xylazine standard. Based on this report, we suggest that xylazine should be classified as a controlled drug.

KEYWORDS: forensic science, xylazine, criminal intent, opioid toxidrome, gas chromatography/mass spectrometry, liquid chromatography–tandem mass spectrometry

Xylazine [*N*-(2,6-dimethylphenyl)-5,6-dihydro-4*H*-1,3-thiazin-2-amine] is an alpha-2 adrenergic agonist that acts on the central and peripheral nervous systems. It causes central nervous system and respiratory depression, bradycardia, hypotension, and transient hyperglycemia (1–5). Xylazine was first discovered as an antihypertensive agent but has not been approved for human use because of serious adverse effects (6). However, this drug is still employed in veterinary medicine for sedation and muscle relaxation. Human poisoning from xylazine is not common. Cases of poisoning have involved drug abusers (7,8), suicide (1,2,5), and accidental exposure (4,9). We herein report the first case series of xylazine intoxication by unknown ingestion with criminal intent.

Case Series

Case 1

A 73-year-old woman was sent to the emergency department after being found unconscious at the hospital cafeteria. She was dizzy after waking up. She recalled that a stranger had given her a bottle of water. After she drank it, she felt dizzy and sleepy. She had a history of hypertension, diabetes mellitus, hyperlipidemia, dysthymia, back pain, and chronic venous insufficiency. Her medications included losartan, amlodipine, atenolol, simvastatin, and etoricoxib. Physical examination revealed a blood pressure (BP) of 110/56 mmHg, heart rate (HR) of 50 beats/min, and respiratory rate (RR) of 20 breaths/min. She was drowsy but able to answer questions properly. Her pupils were 1 mm in diameter. Other neurological signs were unremarkable. Her capillary blood glucose (CBG) concentration was 278 mg/dL. An electrocardiogram (ECG) showed only sinus bradycardia of 54 beats/min. Her baseline HR based on records of previous visits ranged from 65 to 75 beats/min. Acute poisoning was suspected. Gastric lavage was performed and activated charcoal was administered. Her serum and gastric contents were sent for drug screening. With only supportive treatment, she recovered and was discharged home 4 h later.

Case 2

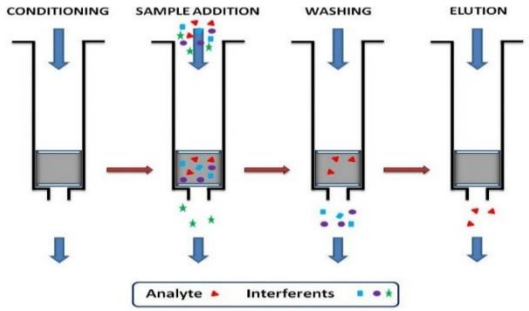
A 71-year-old woman was found drowsy in the outpatient lounge. She had a history of systemic sclerosis with interstitial lung disease, hypertension, and ischemic heart disease. On physical examination, she was drowsy but orientated to time, place,

[1]Division of Toxicology, Department of Pathology, Faculty of Medicine

Comprehensive or broad-spectrum testing

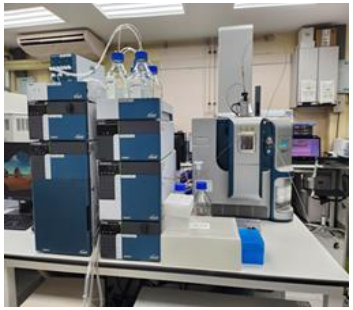
Urine 1 mL
Add IS, β-Glucuronidase
Mix, incubate 48±2°C 2 hrs
Add Na_3PO_4

Serum 0.2 mL, add IS, Na_3PO_4,
Mix and centrifuge

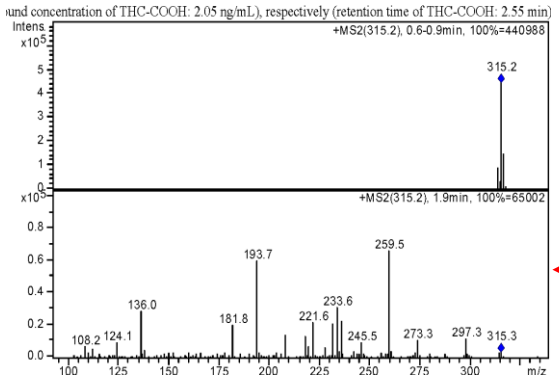


Mixed-mode Cation exchange SPE

Inject



LC-QTOF
C18 column
Full scan mode (screening) and
MRM mode (confirmation)



Data analysis

Comprehensive or broad-spectrum testing

- Drug and pesticides screening
  - Sample: gastric contents
  - GC/MS : Qualitative
  - Organophosphate, Carbamate, Organochlorine, Pyrethroid
- Drugs of Abuse Panel
  - Sample: urine
  - LC-HRMS, LC-MS/MS or GC/MS Quantitative Analysis/Confirmation
- Screening for Drugs and Drugs of Abuse

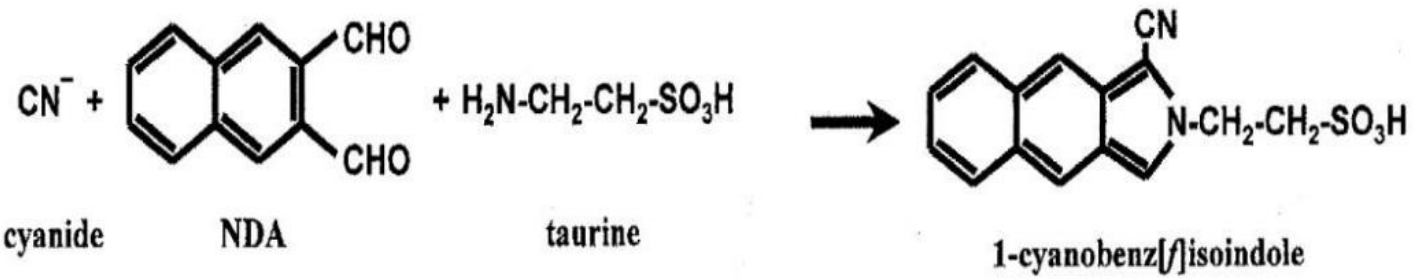
Cyanide

- Colorimetry
  - Prussian blue test
- HPLC-DAD (LOD 0.1 ug/mL, LOQ 0.5 ug/mL)
- HPLC-FLD
  - LOD 0.01 µg/mL, LOQ 0.025 µg/mL
  - Reportable range 0.025-8 µg/mL

HPLC-FLD

Adapted from

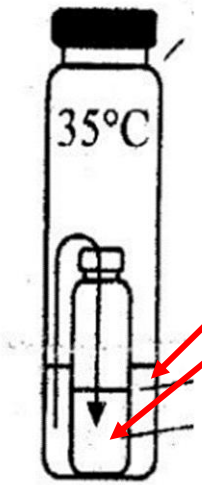
- Felscher D, Wulfmeyer M. A new specific method to detect cyanide in body fluids, especially whole blood, by fluorimetry. J Anal Toxicol. 1998 Sep;22(5):363-6.
- Sano A, Takimoto N, Takitani S. High-performance liquid chromatographic determination of cyanide in human red blood cells by pre-column fluorescence derivatization. J Chromatogr. 1992 Nov 6;582(1-2):131-5.



NDA = 2,3-naphthalenedialdehyde

Sample preparation: micro-diffusion

Hydrocyanic acid



WB 200 µL + 10% sulfuric acid 500 µL

5 mM Taurine 200 µL + 0.1 mM NDA 200 µL

Rest in Fume hood for 60 minutes

Table IV. Reaction of Several Anions with 2,3-Naphthalenedialdehyde and Taurine

Anion	Concentration (μg/mL)	Retention time (min)	Relative peak area	Fluorescending substance
Cyanide	0.1	1.764	1	1-cyano-2-benzoiso-indole derivate
Thiocyanate	50	–	–	
	100	–	–	
Thiosulfate	50	–	–	
	100	–	–	
Sulfide	50	–	–	
	100	2.0*	0.3	Sulfide derivate*
Sulfite	50	–	–	
	100	1.9*	0.2	Sulfite derivate*
Hexacyanoferrate-(II)	50	–	–	
	100	–	–	
Hexacyanoferrate-(III)	50	–	–	
	100	–	–	

* Detected only after direct addition to NDA and taurine solution.

Felscher D, Wulfmeyer M. A new specific method to detect cyanide in body fluids, especially whole blood, by fluorimetry. J Anal Toxicol. 1998 Sep;22(5):363-6.

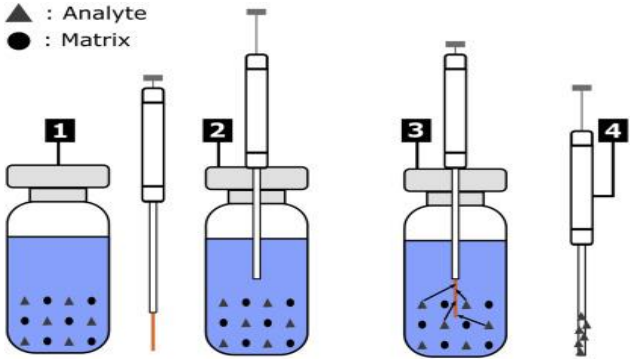
Cyanide: Specimen

- Whole Blood :1 – 3 mL of EDTA (quantitative), NaF (qualitative), seal with paraffin (stability 72 h.-1 wk (Clarkes) at 4 °C)
- Urine (qualitative) 15 mL
- Gastric content (qualitative) 50 mL
- Forensic
  - Whole Blood : 10 mL of EDTA, NaF or lithium heparin, stable for 1 wk at 4 °C but is unstable if frozen or kept at room temperature(Clarkes)

Solvents and other Volatile Substances

- Single Targeted and metabolites
  - HPLC (DAD,FLD)
  - SPME-GC/MS: 9 Compounds
  - LC-MS/MS

- Volatile Organic Compounds panels
  - SPME-GC/MS -> HS-GC/MS
  - Scope: Benzene, Ethyl benzene, Toluene, Styrene, Xylenes



B.A. Weggler, B. Gruber, P. Teehan, R. Jaramillo, L. Dorman, Chapter 5 - Inlets and sampling, Sep. Sci. Technol. 12 (2020) 141–203.

Example

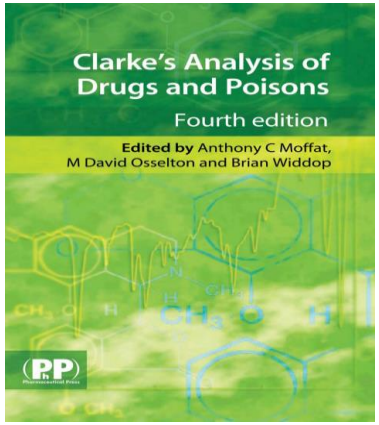
Volatile substances and metabolites	Method
Benzene - ttMA, - S-Phenylmercapturic acid	SPME-GC/MS HPLC-DAD LC-MS/MS
Toluene - Hippuric acid - O-cresol	SPME-GC/MS HPLC-DAD HPLC-FLD
Styrene - Mandelic acid - Phenylglyoxylic acid	- HPLC-DAD HPLC-DAD
Xylene - Methyl hippuric acid	SPME-GC/MS HPLC-DAD

Trace Elements and Toxic Metals

- F-AAS
- GF-AAS
- LC-ICP-MS
  - Toxic metal profiles: Total Arsenic, Cadmium, Lead, Mercury
  - Arsenic Speciation: inorganic arsenic (As3+, As5+) plus methylated metabolites (MMA and DMA)

Challenges in developing analytical toxicology

- The high capital cost of equipment
- Requirements for a skilled labor force
- Lack of automation
- Most are laboratory-developed tests, few FDA-approved kits (IVD)
- Required certified reference materials, QC and proficiency testing programs
- Lack of interfacing with LIS


Clarke's Analysis of Drugs and Poisons
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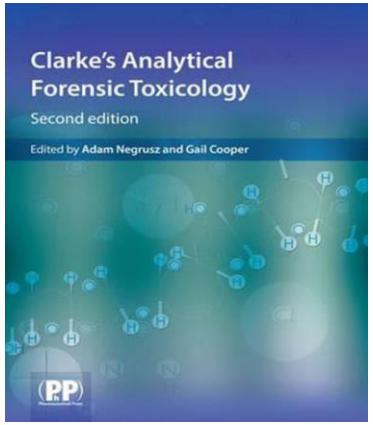
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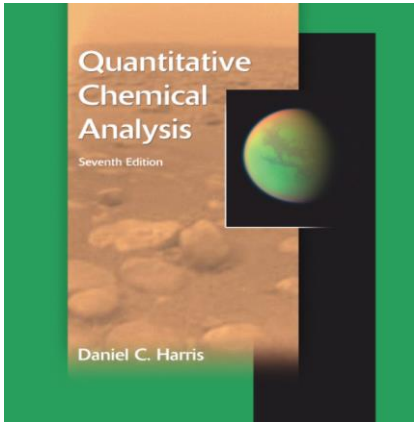
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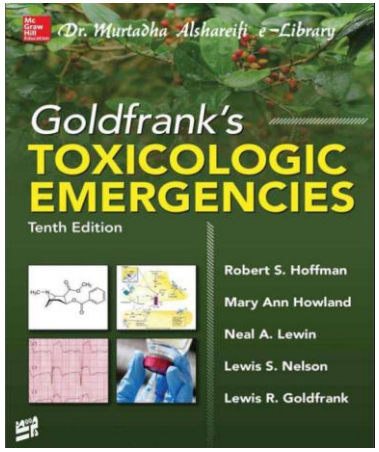
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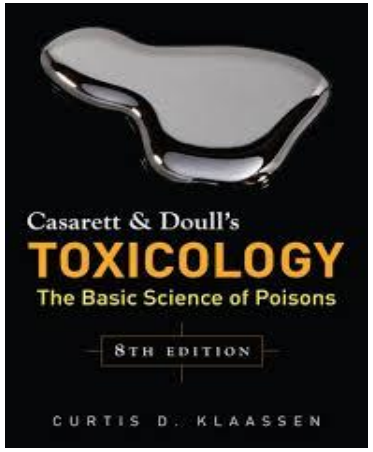
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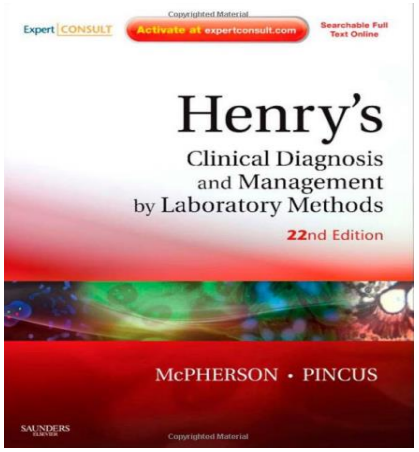
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SAUNDERS

The End

Table 1

Limit of detection (LOD) and limit of quantitation (LOQ) of spiked plasma and urine paraquat by micellar liquid chromatography

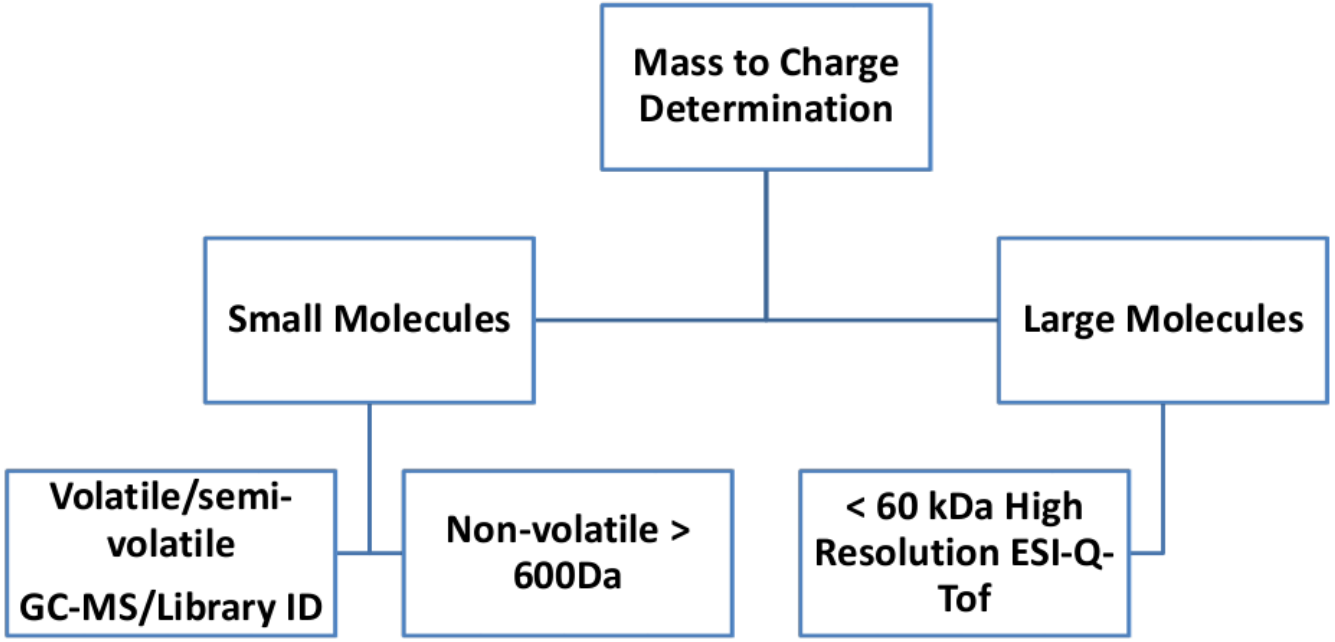
Parameter	Plasma paraquat[a] (µg/ml)	Urine paraquat[b] (µg/ml)
Mean concentration of paraquat detected	0.20	0.20
SD (n = 10)	0.005	0.020
LOD (3 x SD)	0.01	0.06
LOQ (10 x SD)	0.05	0.20

[a]Plasma spiked with 0.20 µg/ml paraquat; [b]Urine spiked with 0.20 µg/ml paraquat

µg/ml: microgram per milliliter; SD: standard deviation

Mass Spectrometry Flow Chart

How to choose your technique based on the type of analyte



https://www.uoguelph.ca/aac/facilities/mass-spectrometry