Quantitative analysis of mitragynine in human urine by high performance liquid chromatography-tandem mass spectrometry

Shijun Lua, Buu N. Trana, Jamie L. Nelsenb, Kenneth M. Aldousa.
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By

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Advisor

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Introduction

- **Mitragynine** :

  9-Methoxy-corynantheidine

- **The primary active alkaloid in the plant**

  Mitragyna speciosa Korth

Introduction

- **Leaves of *Mitragyna speciosa***

  - Mitragynine 66.2 %
  - Speciogynine 6.6 %
  - Speciociliatine 0.8 %
  - Paynantheine 8.6 %

Introduction

- **A new alkaloid :**

  -7 α -hydroxy-7H-mitragynine 2.0 %

Takayama H., "Chemistry and Pharmacology of Analgesic Indole Alkaloids from the Rubiaceous Plant, *Mitragyna speciosa*," *Chem.Pharm. Bull.* **52**(8) 916-928 (2004)

Introduction

- **In Thailand : *Kratom***

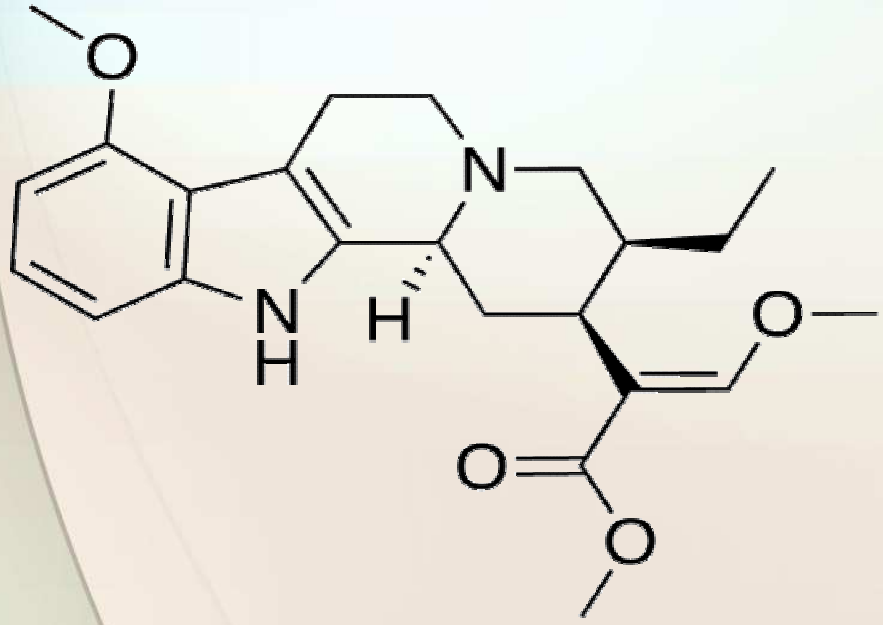
Introduction

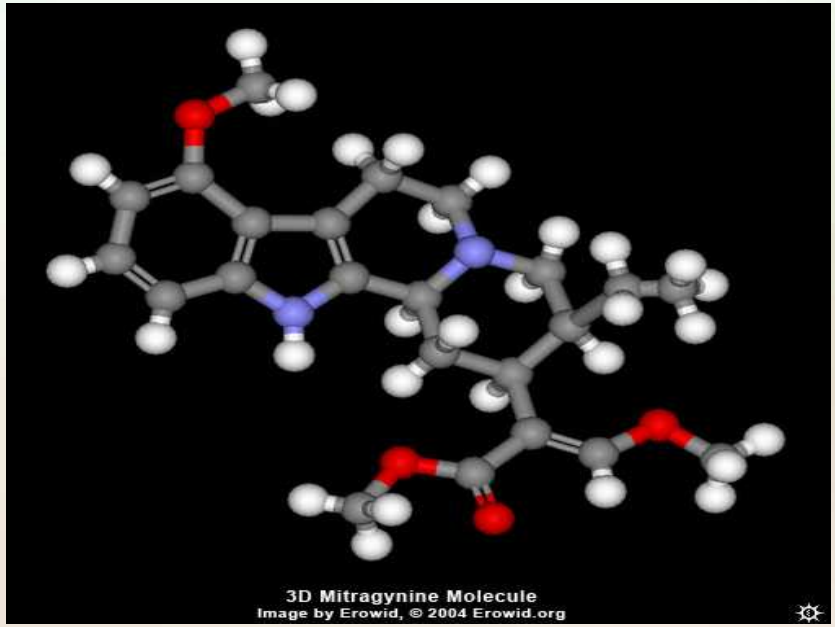
Introduction


THAI
MITRAGYNA-SPECIOSA.COM

Introduction

- **The chemical structures**
- **Pharmacological properties of mitragynine**

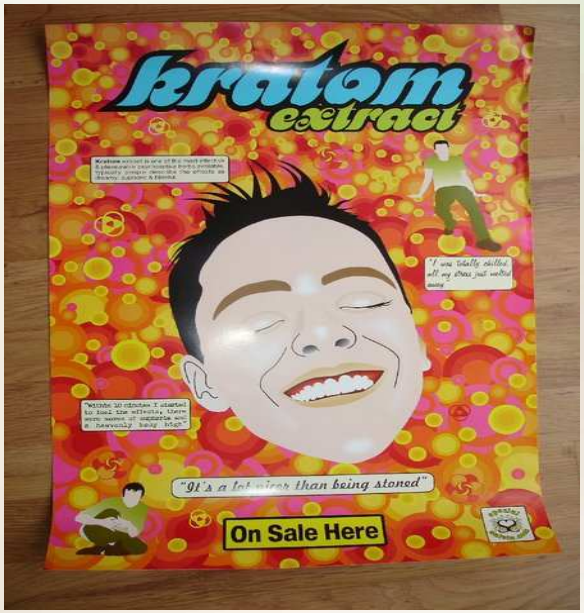




$CH_{23}H_{30}N_2O_4$: MW 398.5

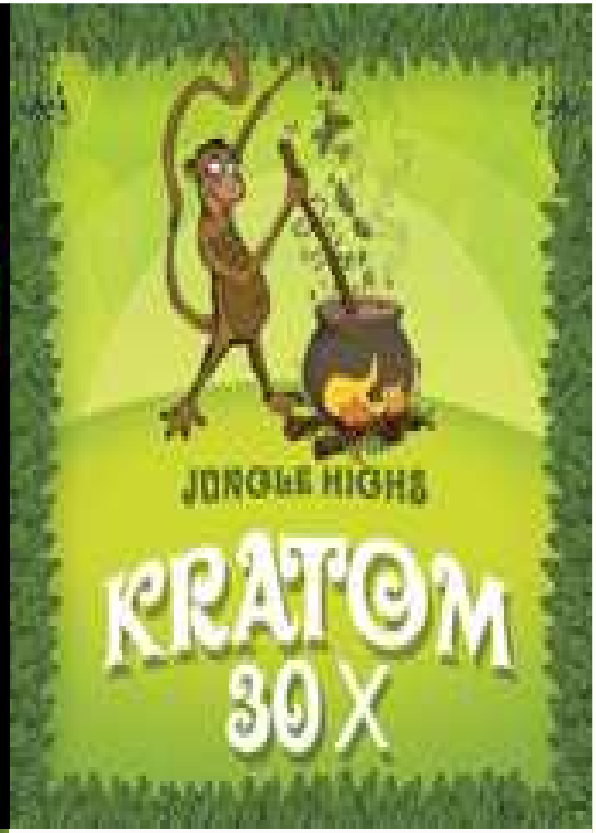
Introduction

- The current widespread availability of kratom on the Internet.




TRIPPY HERBS
KRATOM
10X
MITRAGYNA SPECIOSA


15 " x " Kratom


JUNGLE HIGHS
KRATOM
30X


FST

Introduction

- **HPLC-ESI/MS/MS**

Experimental

2.1 Reagent

- The raw kratom leaves powder

2.1 Reagent (to…)

- Ajmalicine ($C_{21}H_{24}N_2O_3$; purity 99%)
- Anhydrous di-sodium hydrogen orthophosphate
- Acetic acid 99.8%
- Ammonium acetate 99.99%

2.1 Reagent (to…)

- Ammonium hydroxide
- All solvents used were HPLC grade or better
- SilicAR 60 Å silicagel
- Purified water ; with a Nanopure Diamond water system

2.2 LC-MS/MS instrument

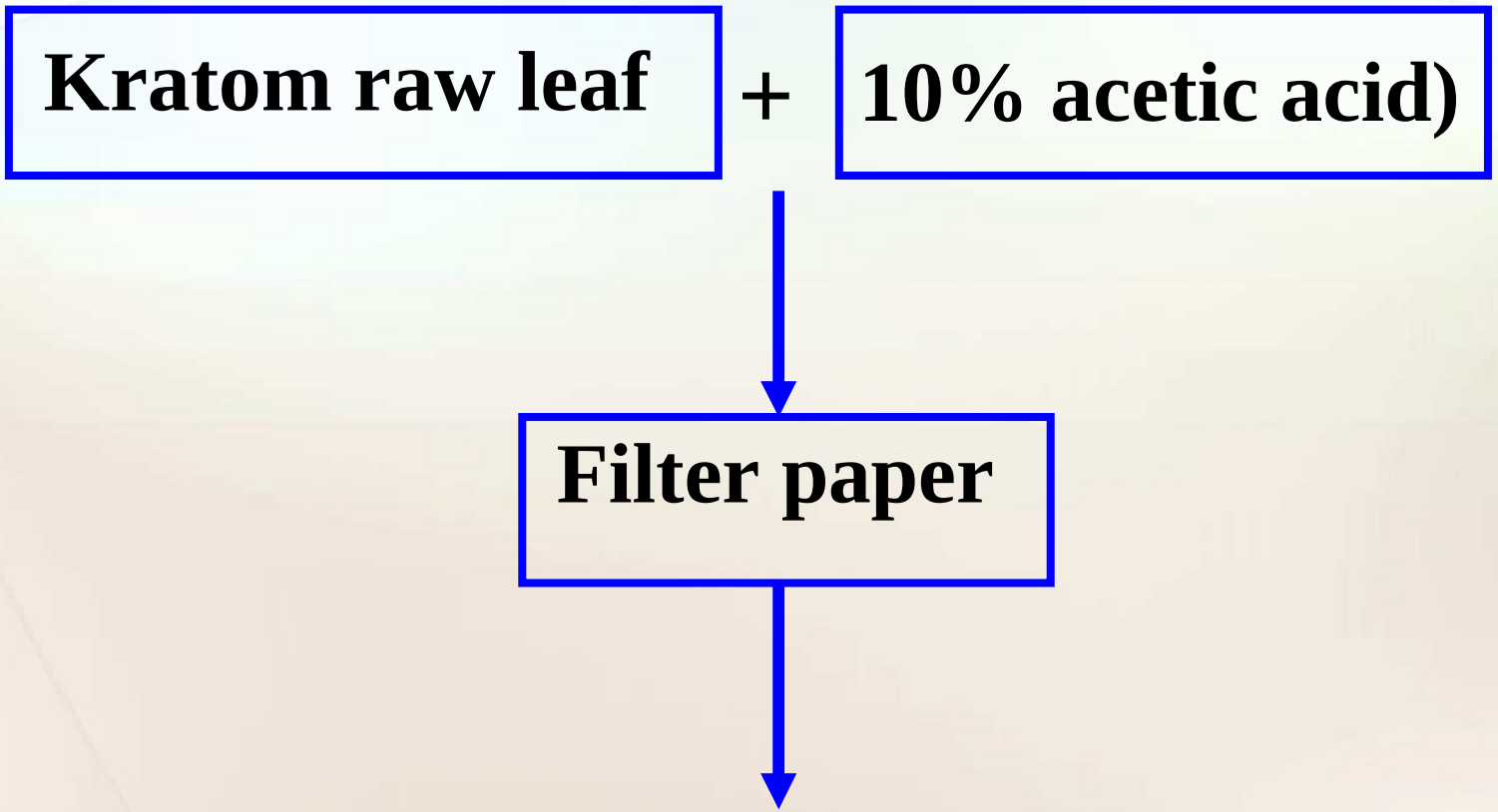
- **HPLC-MS/MS system**

  - Agilent Technologies1200 Series HPLC.

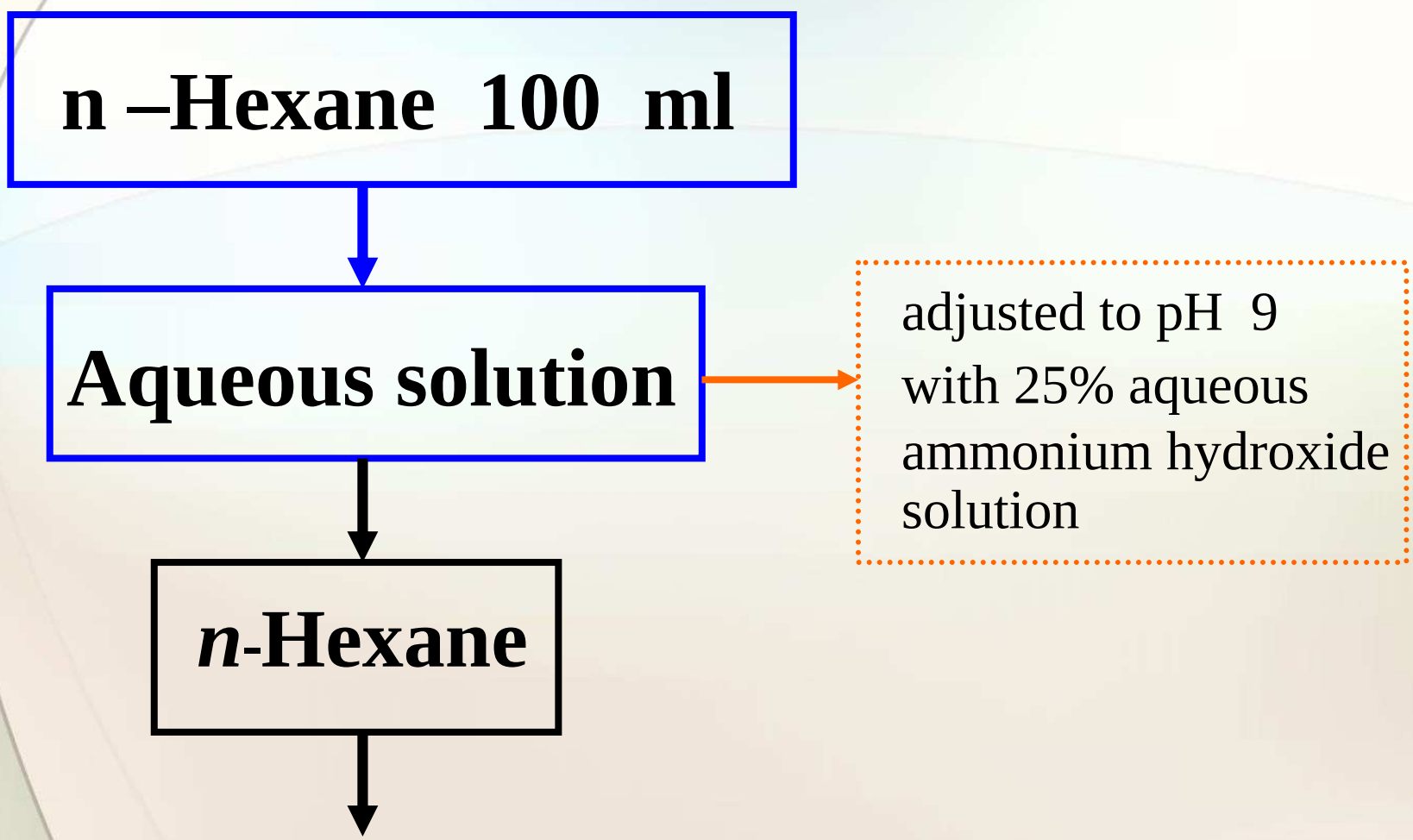
  - API- 2000 triple quadrupole mass spectrometer with a turbo electrospray ionization (ESI) source.

2.3 Purification of mitragynine

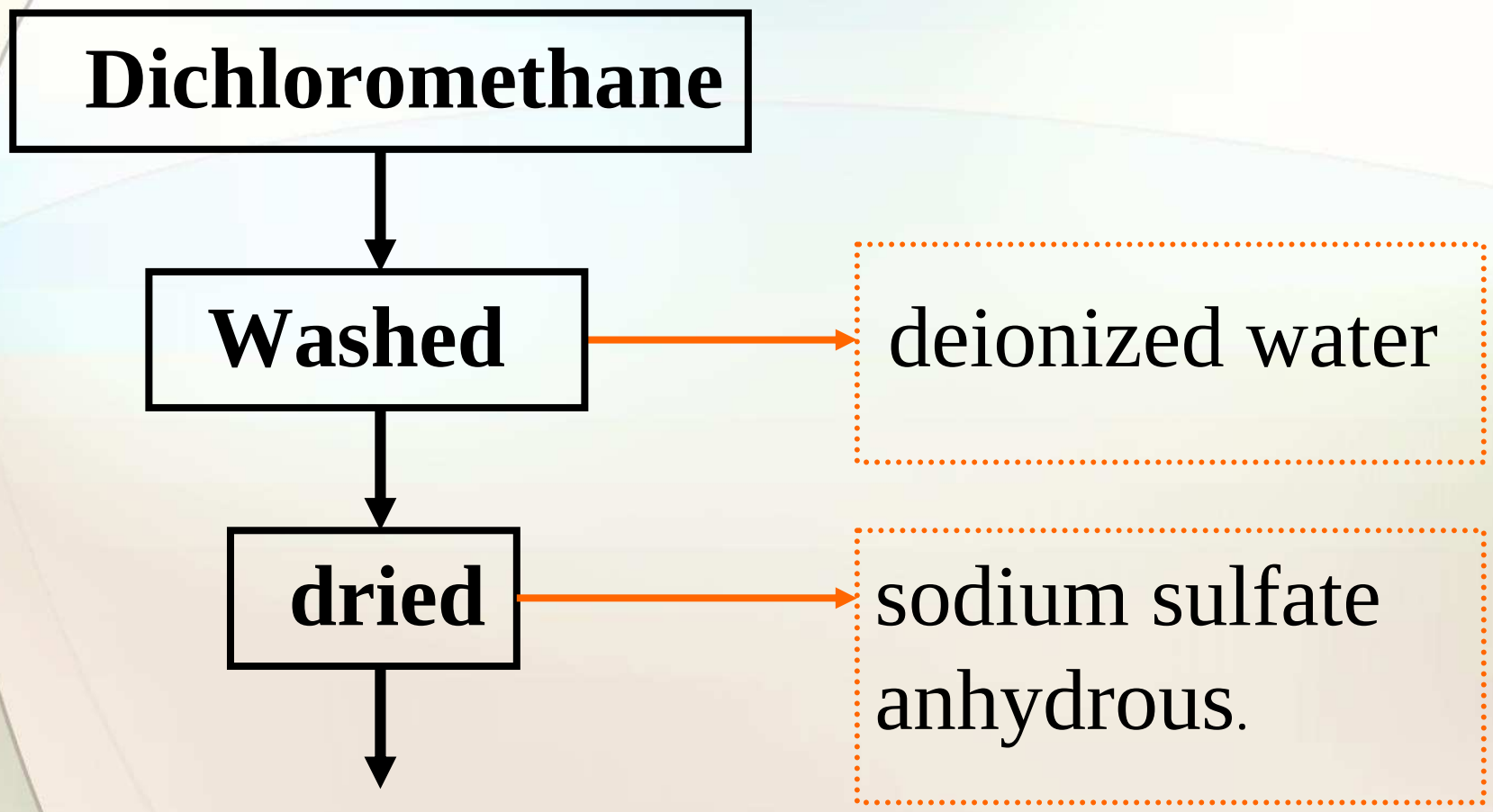
(Houghton et al., Ponglux et al. and Janchawee et al.)



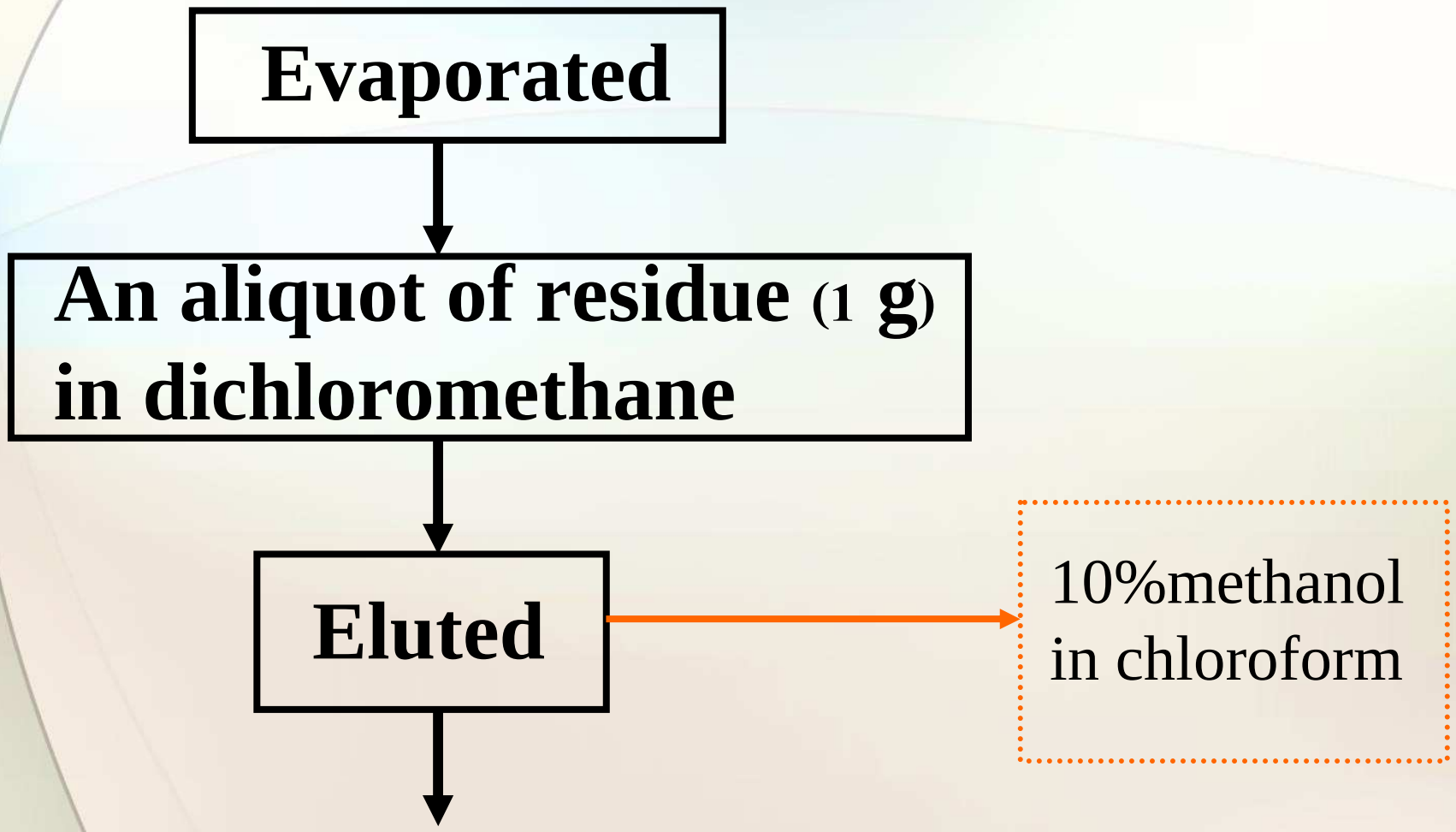
2.3 Purification of mitragynine (to…)



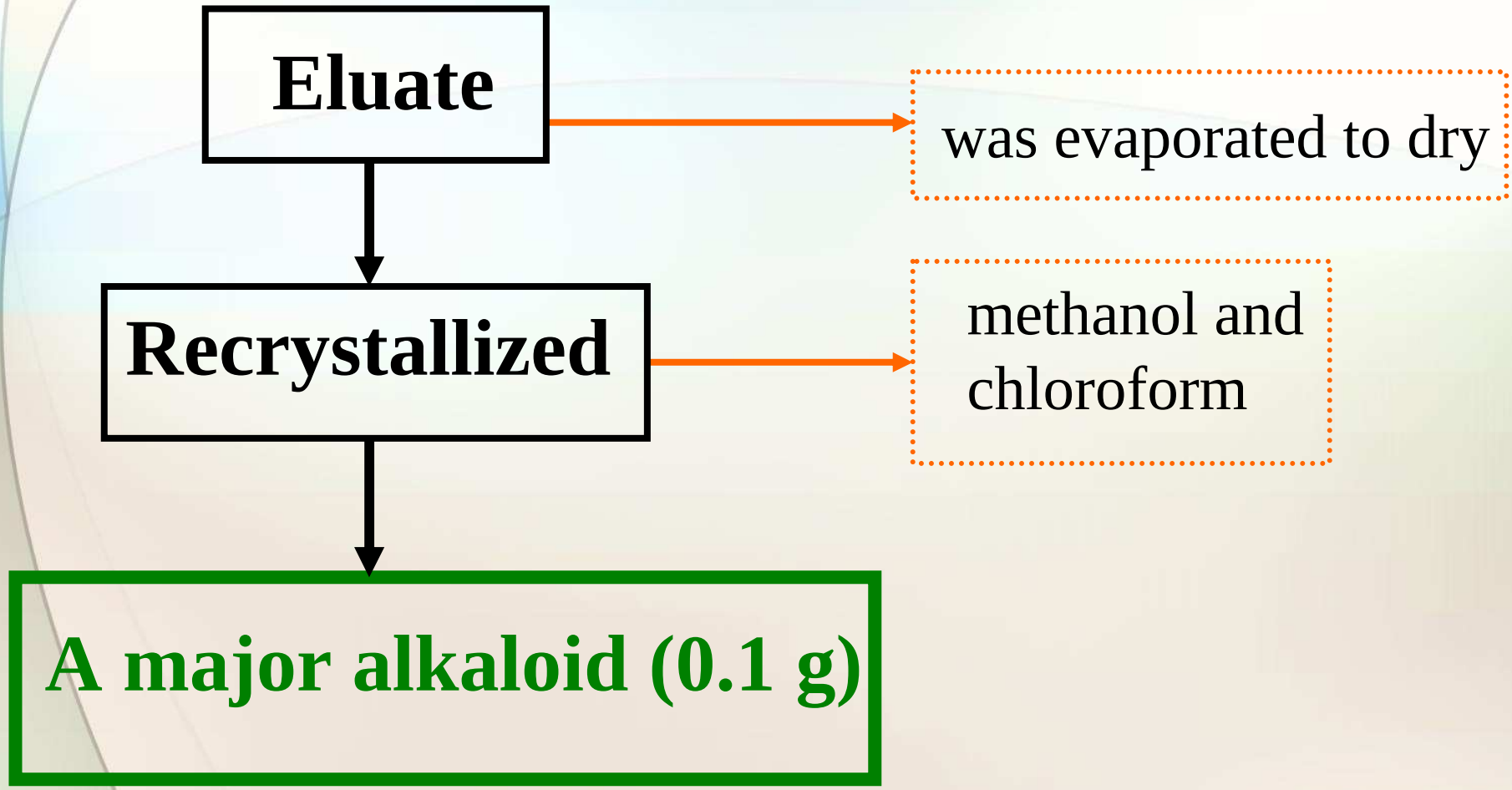
2.3 Purification of mitragynine (to…)



2.3 Purification of mitragynine (to…)



2.3 Purification of mitragynine (to…)



2.3 Purification of mitragynine (to…)

- Purified mitragynine was found :

  - A predominant single chromatographic peak by (GCMS)

2.3 Purification of mitragynine (to…)

- The spectrum of purified mitragynine

($C_{23}H_{30}N_2O_4$; exact molecular mass=398.2207)

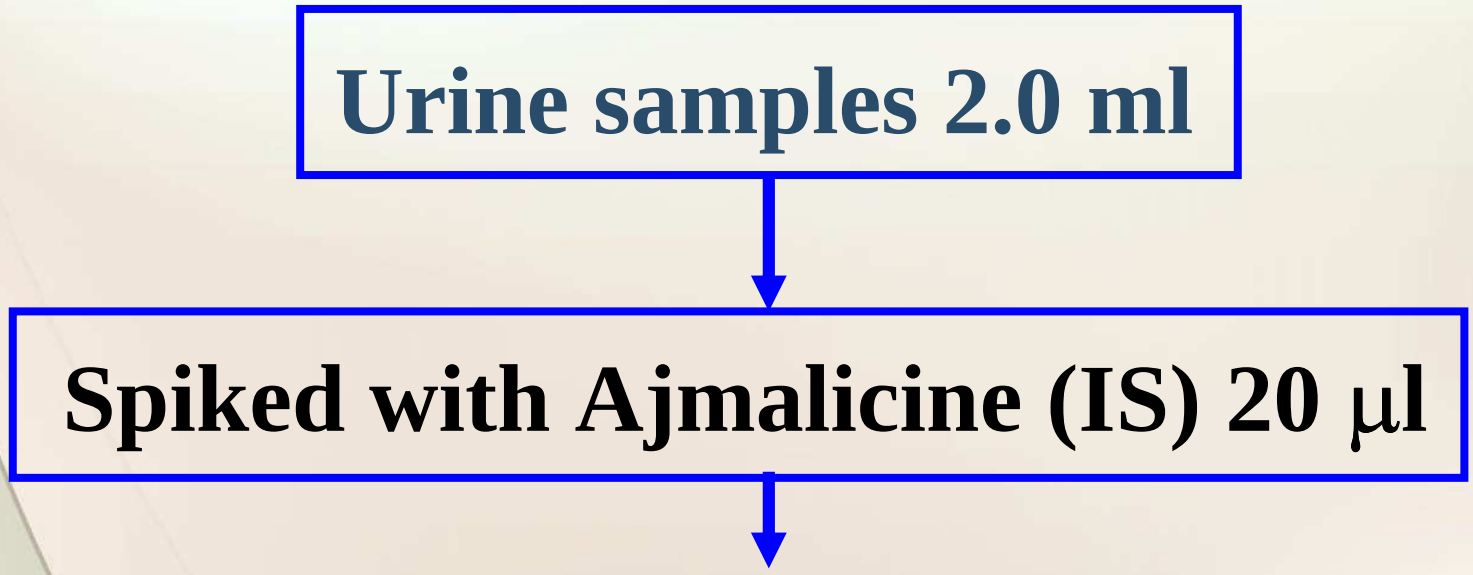
- Confirmed by comparison to the NIST 98 mass spectral library

2.4 Preparation of standard solutions

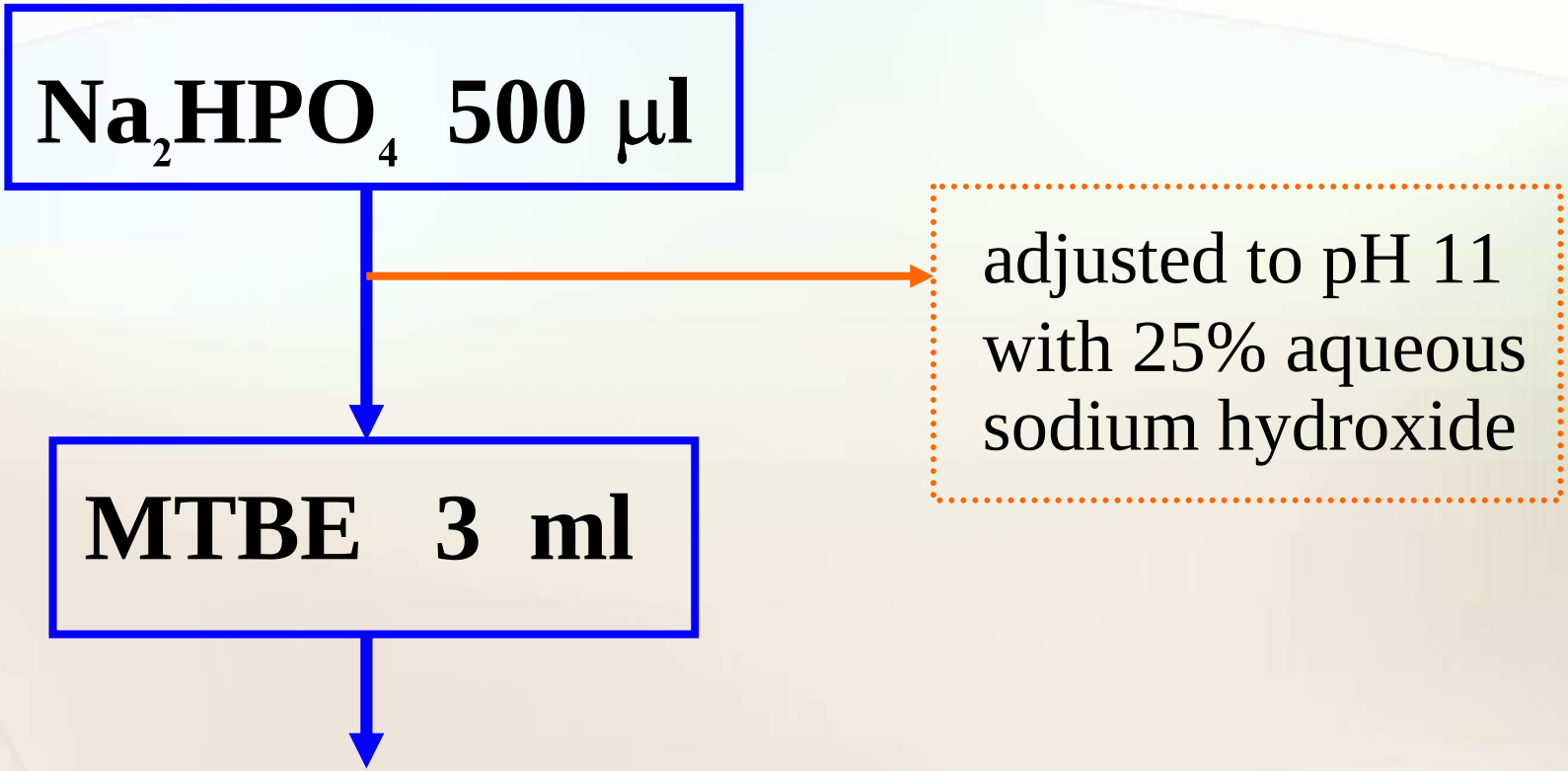
- Stock solutions : 500g/ml of mitragynine
  100g/ml of ajmalicine (IS)
- Stored at −20 °C
- Stable : 60 days

2.5 Sample extraction

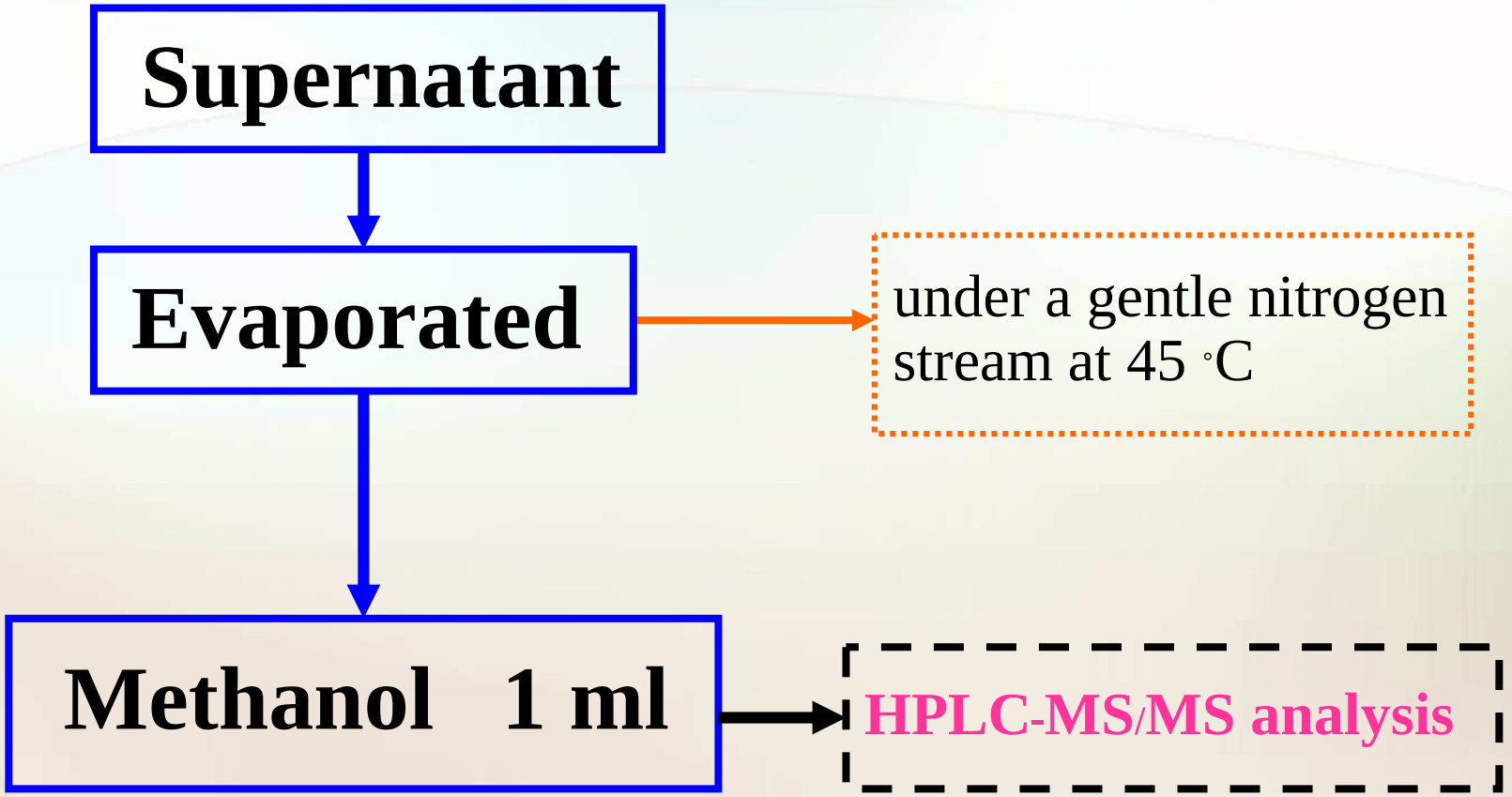
- Both pooled blank and patient urine samples were stored at − 80 ◦C until analysis



2.5 Sample extraction (to…)



2.5 Sample extraction (to…)



2.6 Calibration

- **IS technique** : Using Analyst software
- **Regression analysis** : Calibration equation and correlation coefficient (*r*)
- **Linearity** : Seven standard concentrations at 0.01,0.025, 0.05, 0.2, and 5.0 ng/ml

2.6 Calibration (to…)

- Limits for calibration curve of mitragynine :

  ± 20% for relative standard deviation (RSD)

- Correlation coefficient of 0.99 or greater

2.7 Method development and quality control

- **QC** : Three matrix samples

  -Spiked with mitragynine at 0.1, 1.0 and 5.0 ng/ml

- **The precision** : within-day , Inter-day

  -Inter-day precision

  (when fresh, after 1 day, 7 days and 28 days)

2.7 Method development and quality control (to…)

- **The acceptance criterion**

- Accuracy : recovery was within ± 30 %

- Precision : RSD value within ± 20 %.

2.7 Method development and quality control (to…)

- The lower limit of quantification (LLOQ) of mitragynine
- Set at five times of the method detection limit (MDL)

3. Results and discussion

3.1. MS/MS optimization

- The operating parameters for the ESI source
- The best mass spectrometric :

  - Mitragynine

  - Ajmalicine.

3.1. MS/MS optimization(to…)

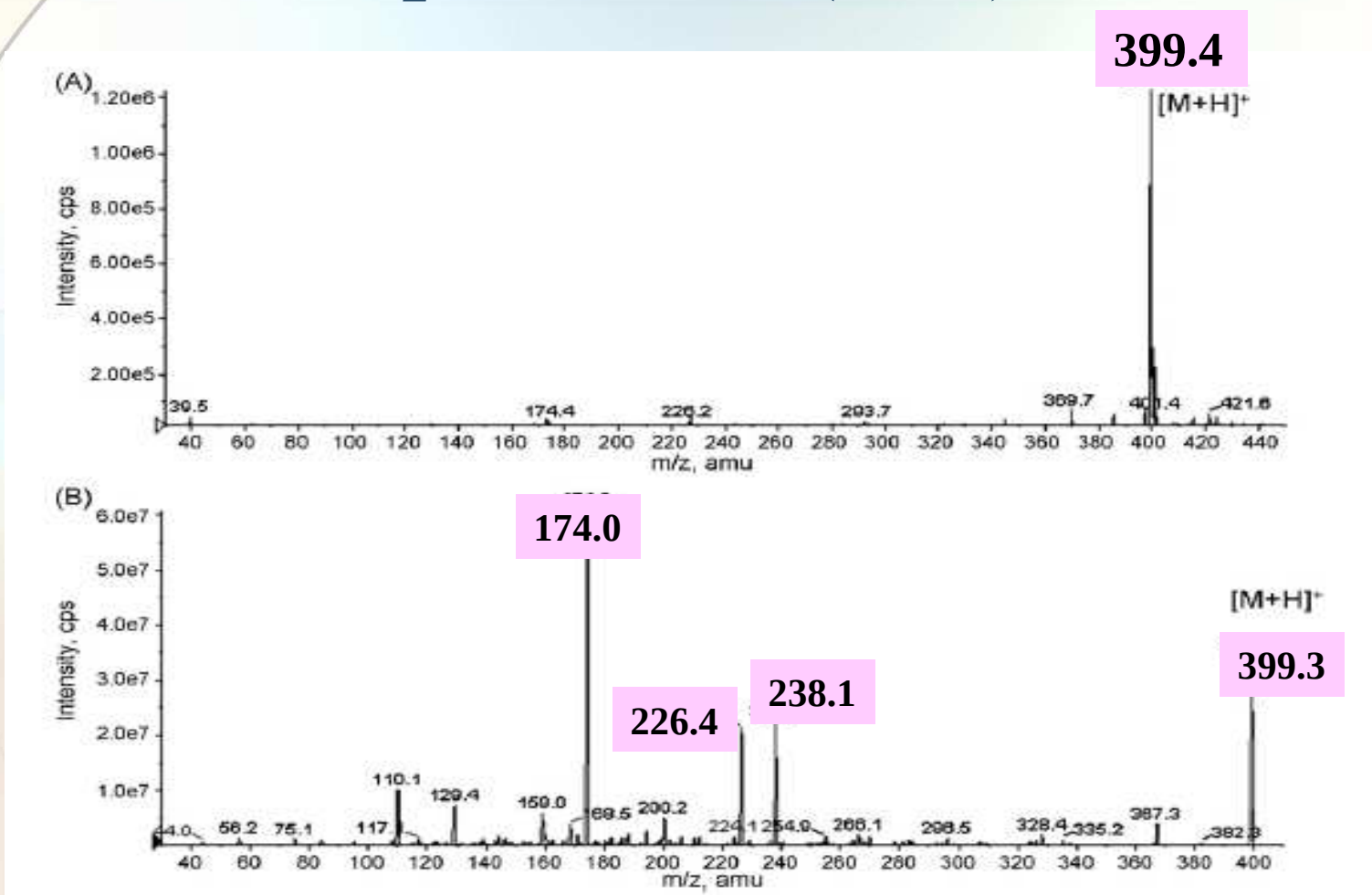


Fig. 1. Mass spectra of mitragynine. (A) Positive ESI in full-scan mode, and (B) in transaction of [M+H]+ *m/z* 399 product-ion scan mode acquired at collision energy of 40 eV.

3.1. MS/MS optimization (to…)

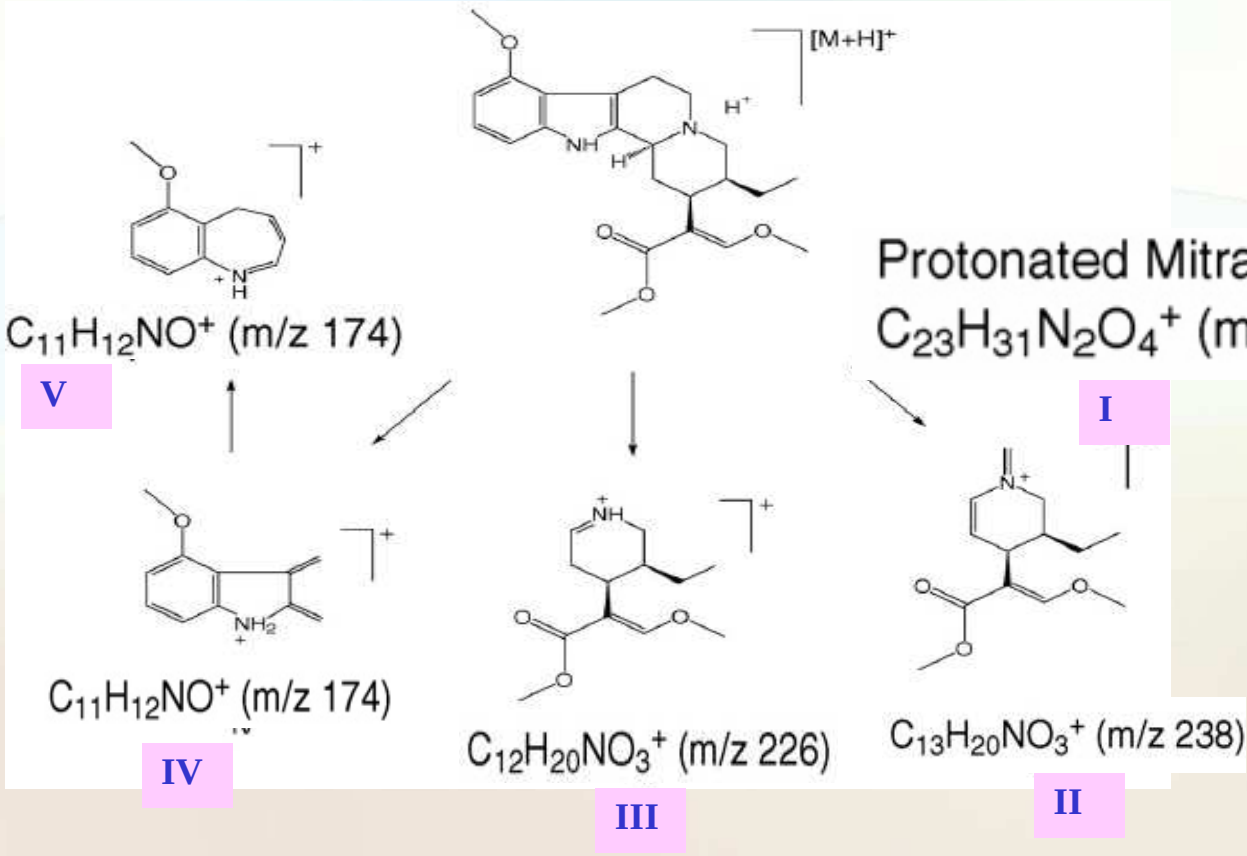


Fig. 2. Chemical structure of protonated mitragynine (I) and tentative identification of its fragment patterns (II, III, IV, and V) under CAD conditions. The structure analog to V was suggested by Khmel'nitskii

3.1. MS/MS optimization (to…)

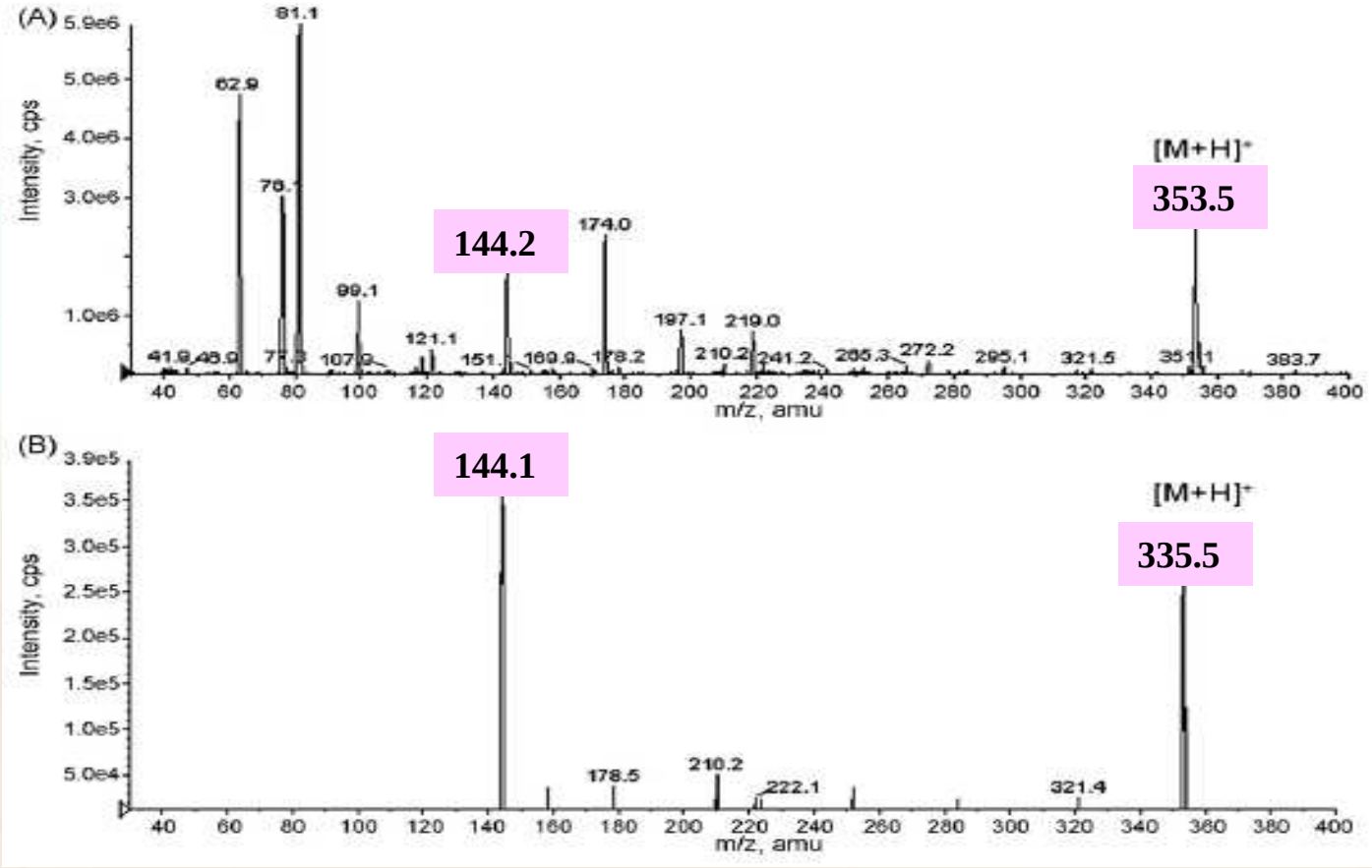


Fig. 3. Mass spectra of ajmalicine. (A) Positive ESI in full-scan mode. (B) in transaction of $[M+H]^+$ *m/z* 353 product-ion scan mode acquired at collision energy of 30 eV (B).

3.1. MS/MS optimization (to…)

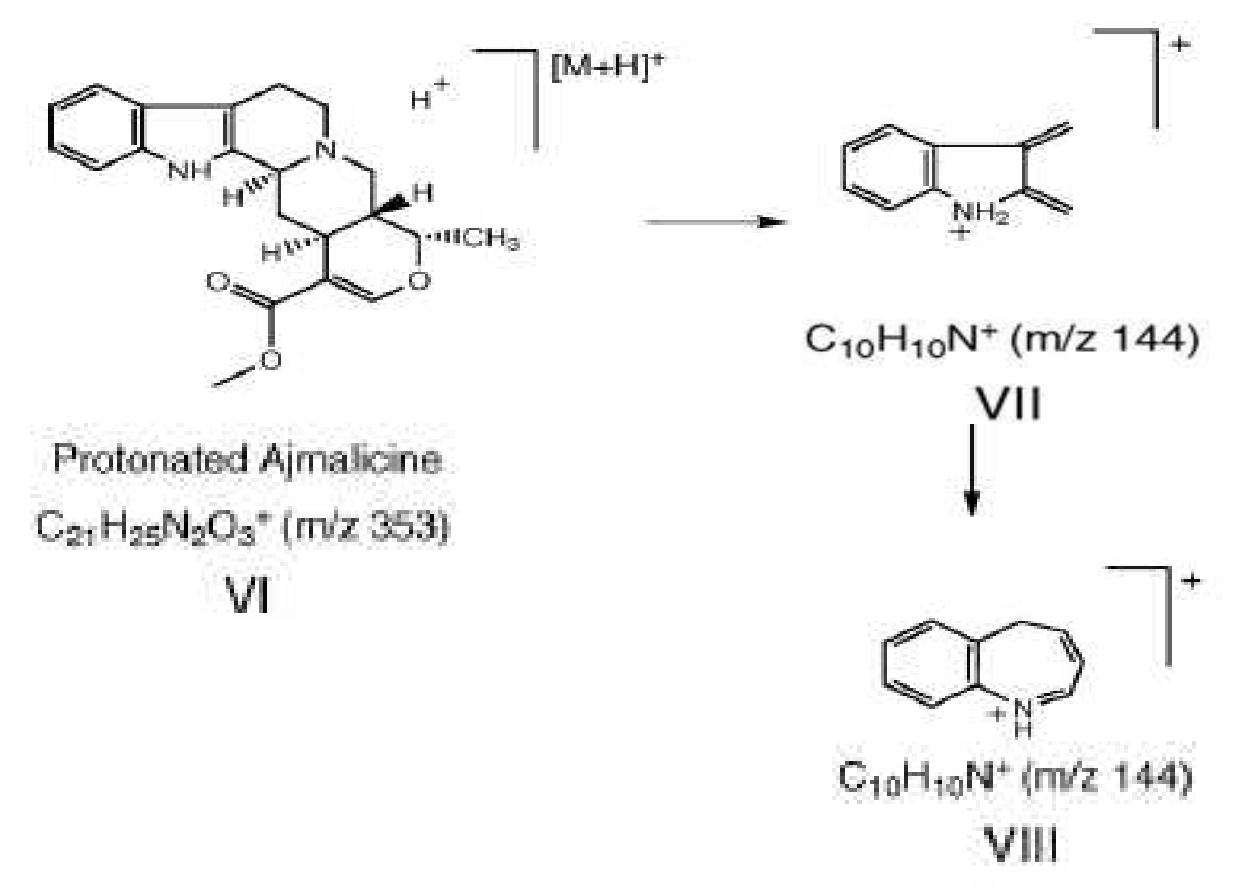


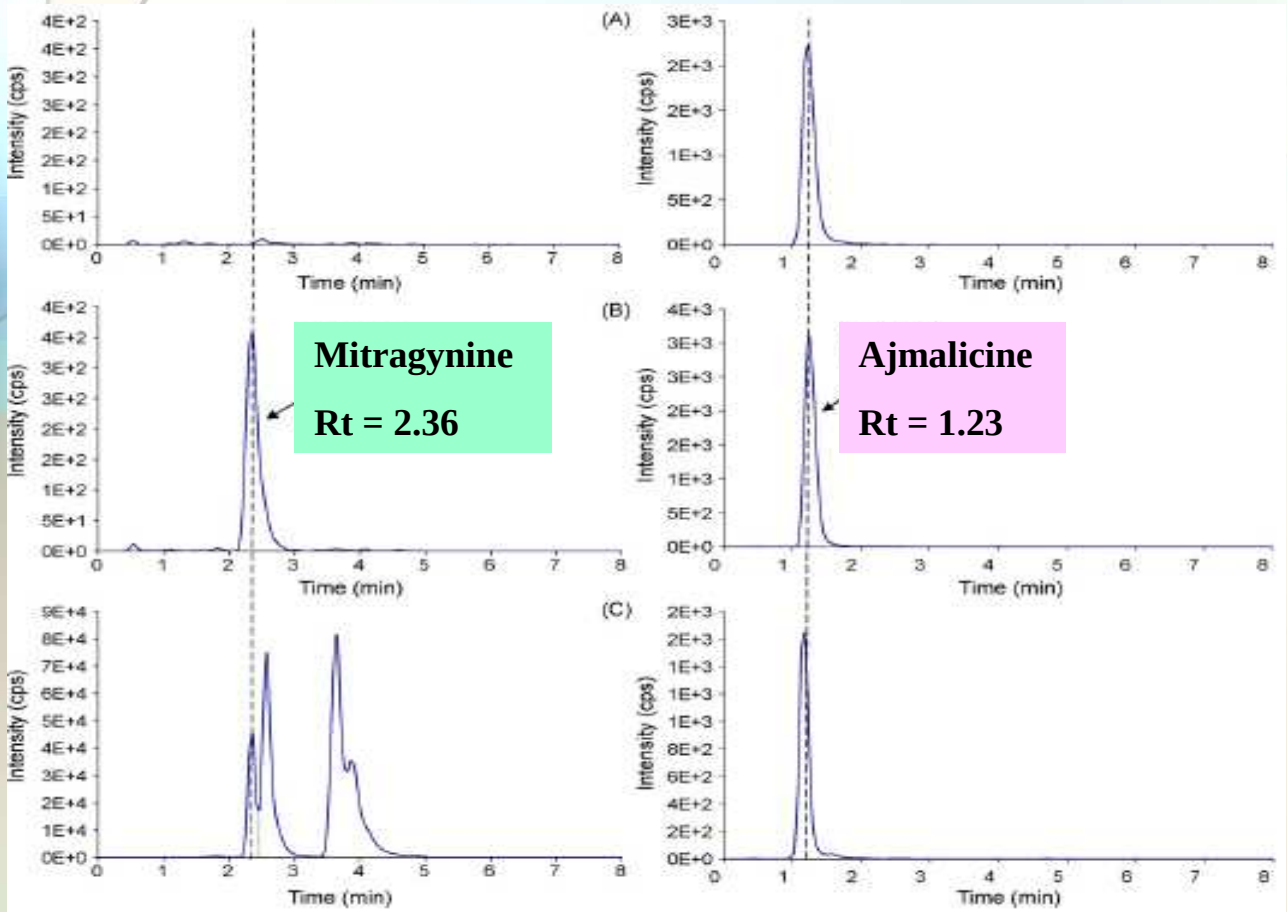
Fig. 4. Chemical structure of protonated ajmalicine (VI) and tentative identification of its fragment patterns (VII, and VIII) under CAD conditions. The structure of VIII was suggested by Khmel'nitskii

3.1. MS/MS optimization (to…)

Table 1 Optimized MS/MS operating parameters for mitragynine and ajmalicine obtained from API 2000 tandem mass spectrometry.

MS/MS parameter	Mitragynine	Ajmalicine
Polarity	Positive	Positive
Precursor ion (m/z)	399	353
Product ion (m/z)	174, 226, 238	144
Collision energy (eV)	45	40
Declustering potential (V)	50	50
Ionspray voltage (V)	4500	4500
Ion source temperature (°C)	550	550

3.2. LC analysis



(A) Blank urine

(B) 5 ng/ml standard solution

(C) urine extract from a kratom user

Fig. 5. HPLC-MS/MS extracted chromatograms of mitragynine (left) and ajmalicine (right). The transitions of *m/z* 399 > 174, 399 > 226, and 399 > 238 were used to monitor mitragynine, and the transition of *m/z* 353 > 144 was used for ajmalicine.

3.3. Evaluation of liquid extraction

Table 2 Mean extraction recoveries of mitragynine (analyte) and ajmalicine (IS) at level of 1 ng/ml in different solvents (five replicates each).

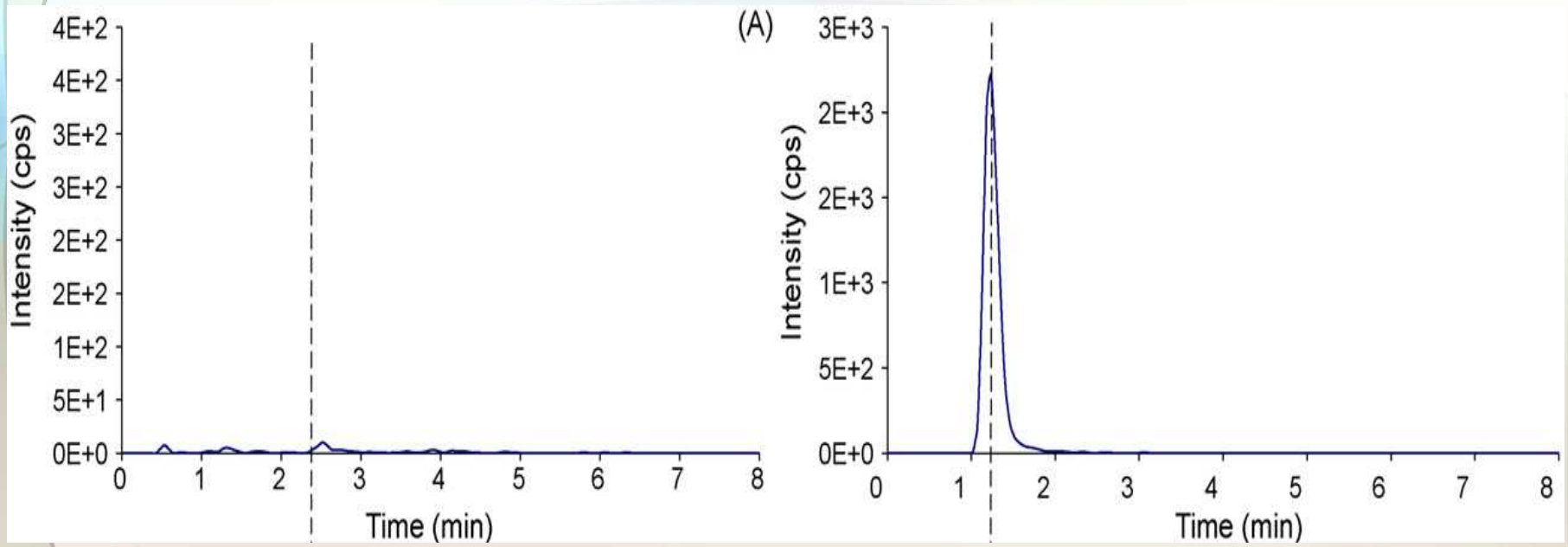
	Mitragynine		**Ajmalicine**	
Solvent	**Mean recovery, %**	**RSD**	**Mean recovery, %**	**RSD**
Ethyl acetate	49	13	60	15
Ethyl ether	82	12	90	10
MTBE	**81**	**8**	**92**	**8**

3.4. Quality control and method validation

- **The linear regression** :

  - Indicated an accuracy of 90-115%
  - Correlation factor $r > 0.995$

3.4. Quality control and method validation (to…)



(A) Blank urine

Fig. 5. HPLC-MS/MS extracted chromatograms of mitragynine (left) and ajmalicine (right).

3.4. Quality control and method validation (to…)

Table 3 Intra-day assay precision formitragynine determination in human urine in triplicate for each level.

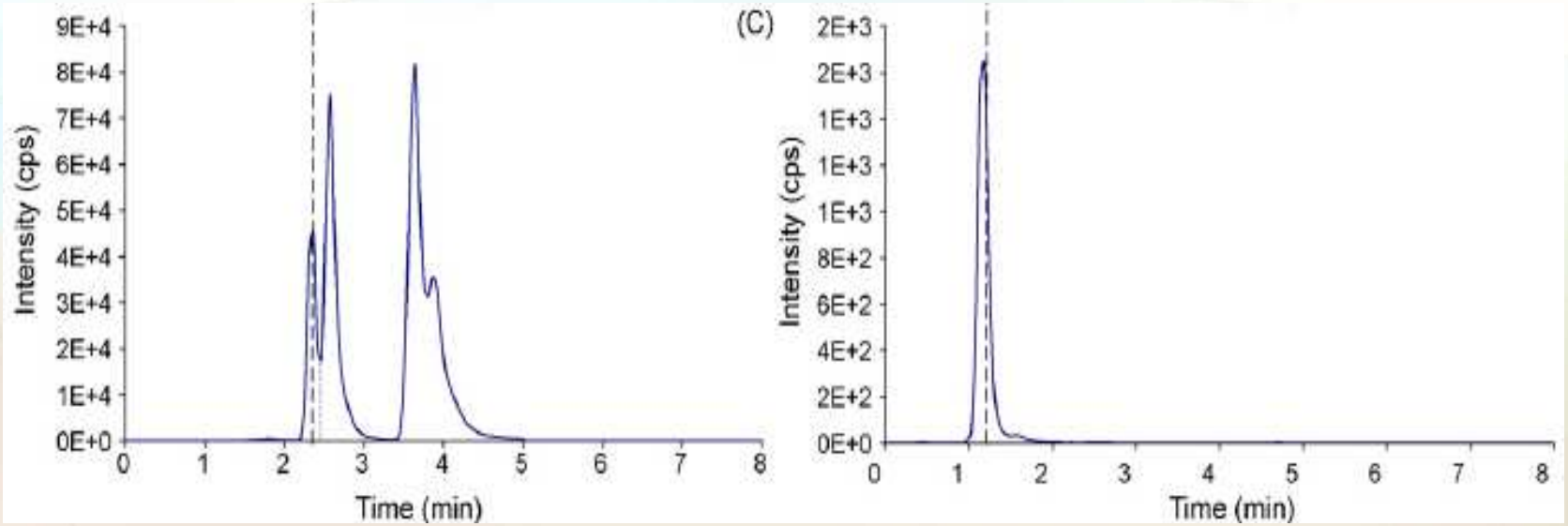
Nominal concentration (ng/ml)	**Measured concentration (ng/ml)**	**RSD**
0.1	**0.1**	**22**
1	**1.1**	**12**
5	**4.9**	**16**

3.4. Quality control and method validation (to…)

Table 4 Inter-day assay precision for mitragynine in urine measured in triplicate for each level.

Analysis time (age of sample)	0.1 ng/ml		1 ng/ml		5 ng/ml	
	Mean recovery, %	RSD	Mean recovery, %	RSD	Mean recovery, %	RSD
Fresh	90	22	109	12	98	16
1 day	80	33	93	16	94	5
7days	90	11	102	7	96	10
28 days	110	9	115	13	103	8

3.4. Quality control and method validation (to…)



(C) urine extract from a kratom user

Fig. 5. HPLC-MS/MS extracted chromatograms of mitragynine (left) and ajmalicine (right).

4.Conclusion

- Consumption of kratom can lead to a detectable content of mitragynine residue and its metabolite in urine.

4.Conclusion

- Mitragynine residue in urine sample was extracted using MTBE and analyzed on HILIC column coupled to a tandem mass spectrometry.

4.Conclusion

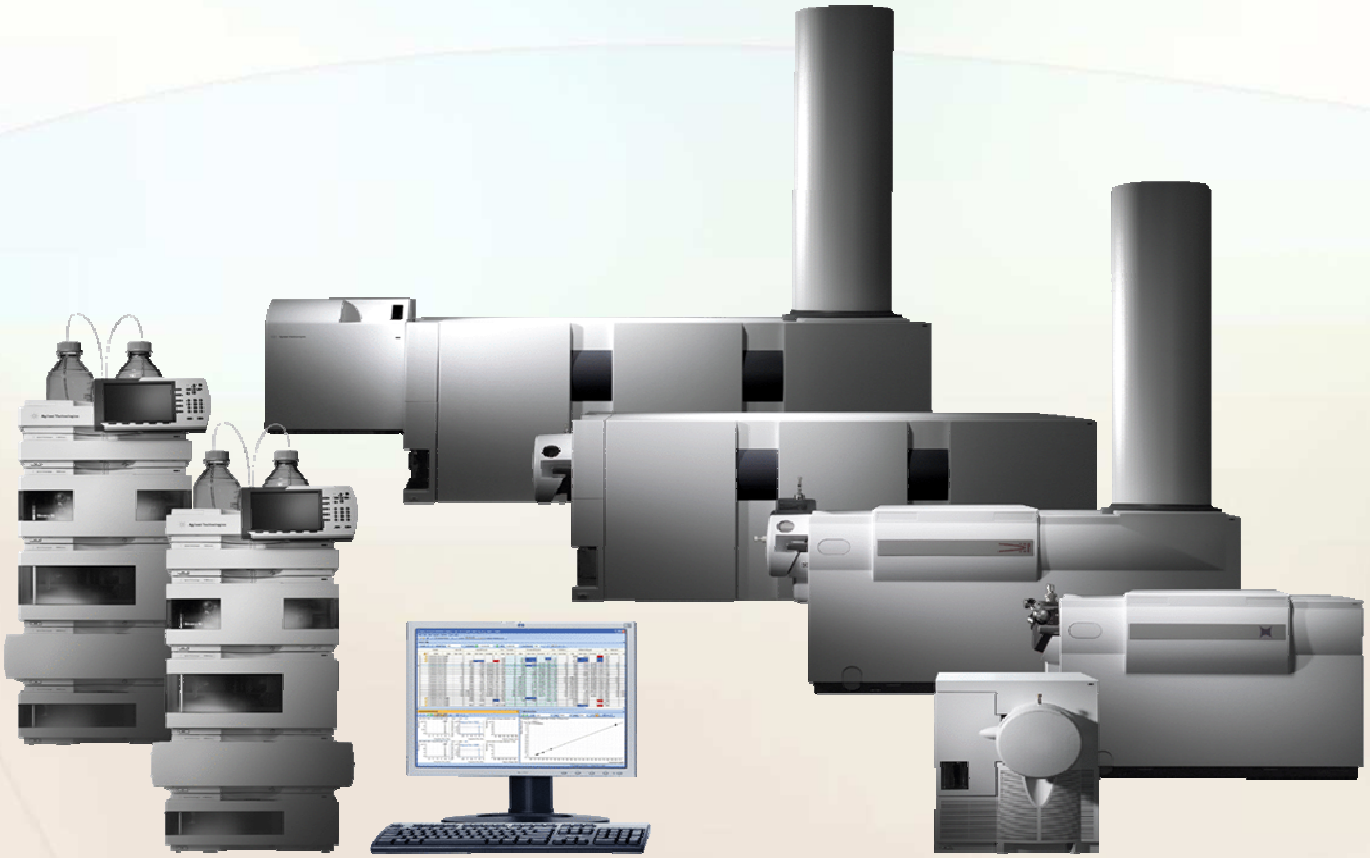
- Ajmalicine was found to be a suitable IS both for the extraction and the HPLC-MS/MS analysis of mitragynine.

4.Conclusion

- High accuracy, precision, and sensitivity were demonstrated for HPLC-MS/MS analysis of mitragynine in urine matrix, with detection and quantitation limits of 0.02 and 0.1 ng/ml, respectively.

Discussion

LC-MS/MS Tof,Q-trap, Q-tof



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CHIANG MAI DRUG DEPENDENCE TREATMENT CENTER

UDONTHANI DRUG DEPENDENCE TREATMENT CENTER

THANYARAK INSTITUTE OF DRUG ABUSE

KHAN KAEN DRUG DEPENDENCE TREATMENT CENTER

SONGKHLA DRUG DEPENDENCE TREATMENT CENTER

PATTANI DRUG DEPENDENCE TREATMENT CENTER

King's Mother Make a speech
"Is drug addict a human? If he is a human...

Can we support him? If we can support him, we can bring him a new life ...We should do"


THANK YOU FOR ATTENTION