Potential of high-performance liquid chromatography with photodiode array detection in forensic toxicology

BY

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- The application of LC for Systematic toxicological analysis (STA)
- HPLC – system
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Purpose

- The potentials and limitations of high-performance liquid chromatography-photodiode array detector.

1. Introduction

- Biological matrices -toxic compounds (e.g., blood, urine, stomach contents, tissues)
- Rational chemical-analytical called *systematic toxicological analysis (STA)*

1. Introduction (Continued)

- ***Systematic toxicological analysis*** *(STA)*

  (1) To detect if the specimen contains any harmful substance(s);

  (2) To identify the substance(s) involved;

  (3) to determine the quantity of the substance(s) involved and to interpret the outcomes in regard to the reason for carrying out the analysis

1. Introduction (Continued)

- *STA* -depends on the quality of the analytical system

  - Thin-layer chromatography (TLC),
  - Gas chromatography(GC),
  - Mass spectrometers (MS)
  - High-performance liquid chromatography (HPLC)

HPLC – system

- **Mobile phase**
  - --Isocratic eluting
  - --Gradient eluting
- **Pump**
- **Injection port**
  - --Automatic
  - --Manual
- **Column**

HPLC – system (continued)

- **Detector**

  --UV detector

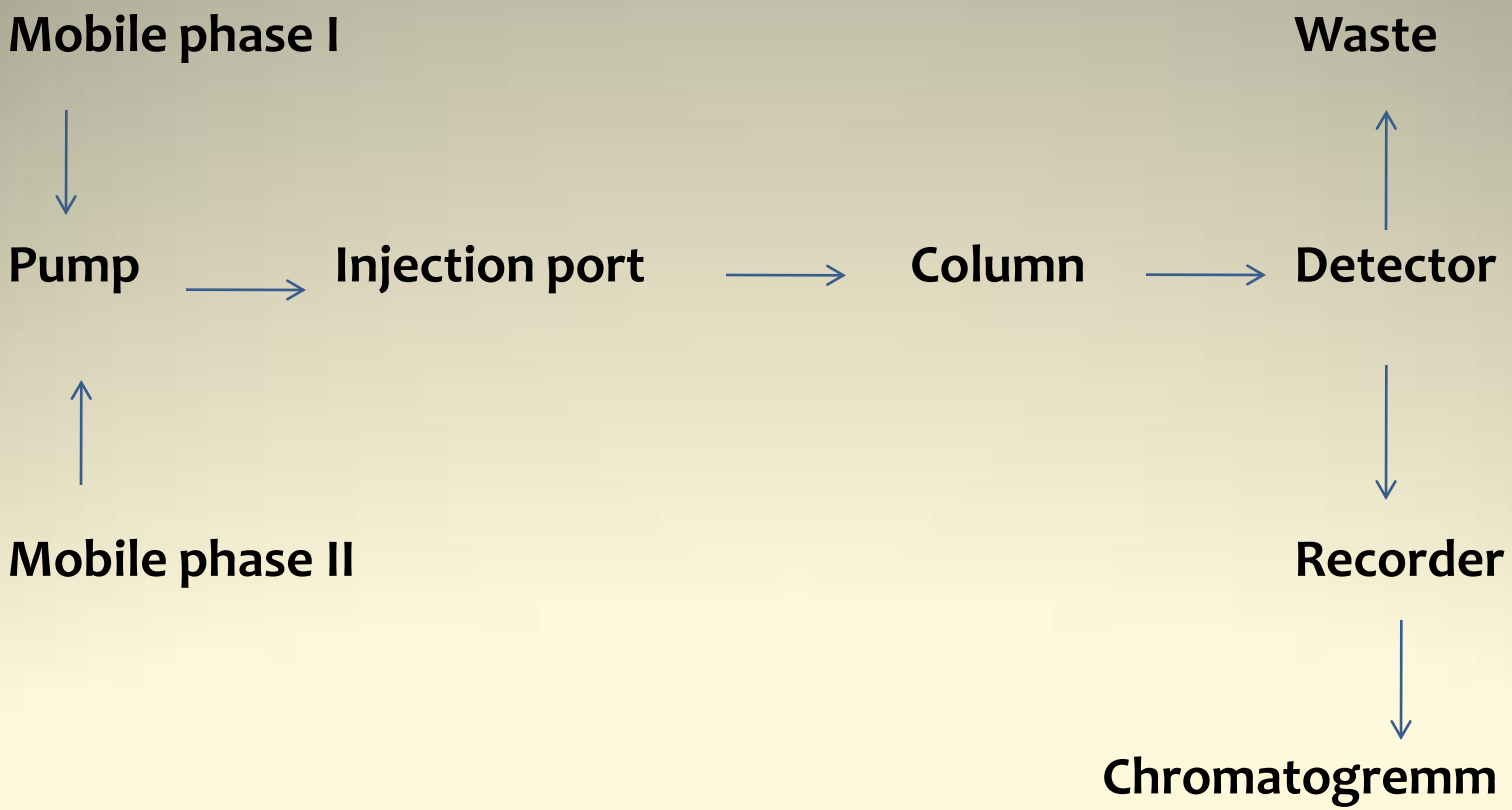
  --Fluoresence detector

  --RI detector

  --Electrochemical detector

  --Conductivity detector

HPLC – principle



2. The application of LC for STA

- *2.1 Column packing materials*
  - *2.1.1 Underivatized silica*
  - *2.1.2 Bonded-phase packing material*

- *2.2 Photodiode array detection*

2. The application of LC for STA (continued)

2.1 Column packing materials

2.1.1 Underivatized silica

- Different brands
- Different batches
- Chromatographic conditions should be exactly defined and strictly followed
- different column systems (and different extraction procedures) in the analysis

2. The application of LC for STA (continued)

- *2.1 Column packing materials*

  2.1.2 Bonded-phase packing material

  - The most popular technique used in STA
  - Became clear that in reversed-phase chromatography
  - Developed a HPLC separation of more than two hundred toxicologically

2. The application of LC for STA (continued)

2.2 *Photodiode array detection*

- Useful in view of applications to STA.
- Reliability of the HPLC
- UV spectrum of a known compound
- Before running a library search

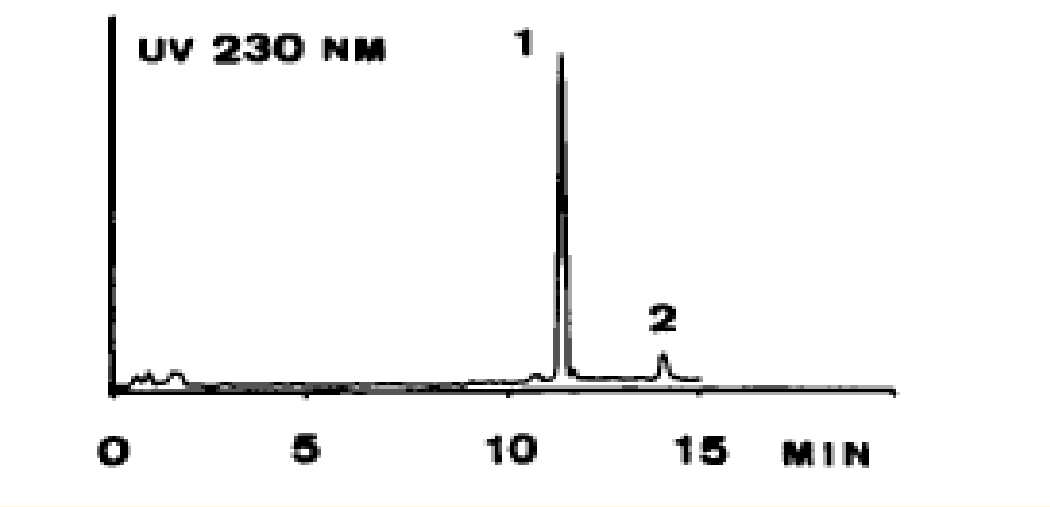
3. Applications of HPLC to STA

- *3.1. Case 1: a fatal case of trazodone and dothiepin poisoning*
- *3.2. Case 2: a fatality involving azide*
- *3.3. Case 3: unexpected suicide by chloroquine*
- *3.4 Case 4: cocaine, polydrug abuse and forensic evidence*

3. Applications of HPLC to STA (continued)

Case studies

- *3.1. Case 1: a fatal case of trazodone and dothiepin poison*

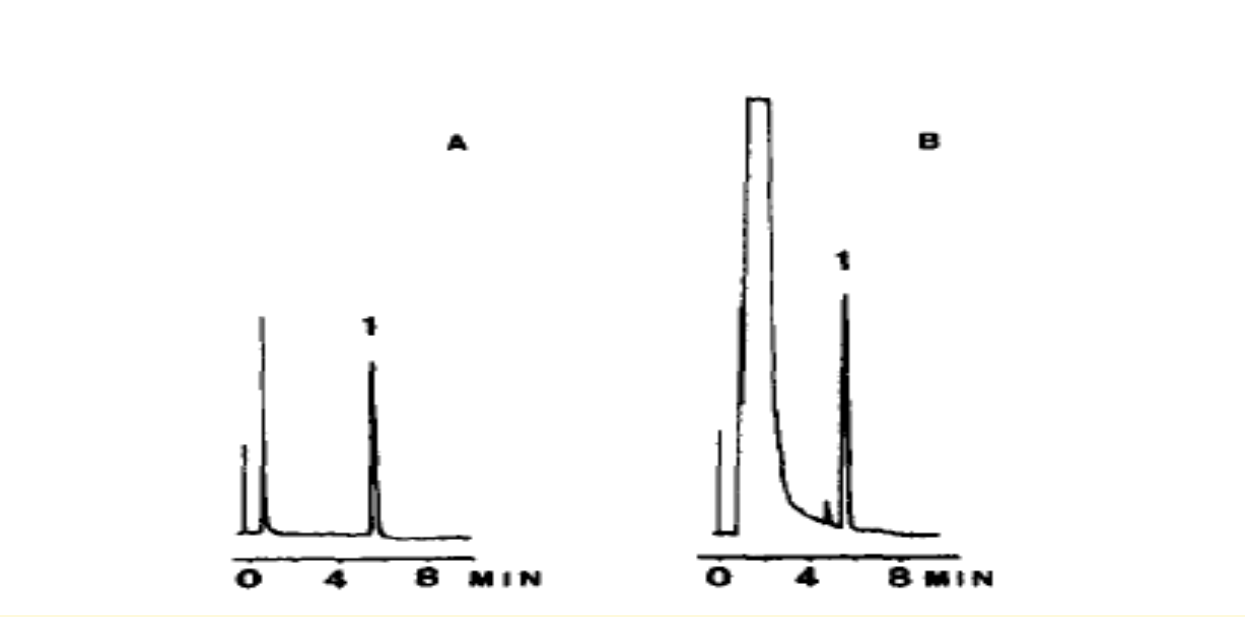


HPLC-DAD trace (displayed at 230 nm) of a blood sample extract. Peak identification: (1) trazodone and (2) dothiepin.

3. Applications of HPLC to STA (continued)

Case studies

- *3.2. Case 2: a fatality involving azide*



HPLC-DAD trace (displayed at 230 nm) of a hydrolyzed urine sample extract. Peak identification: (1) dothiepin sulfoxide, (2) trazodone, (3) metabolites of nordiazepam and lorazepam (hydrolyzed), (4) metabolite of diazepam (hydrolyzed),aod (5) dothiepin

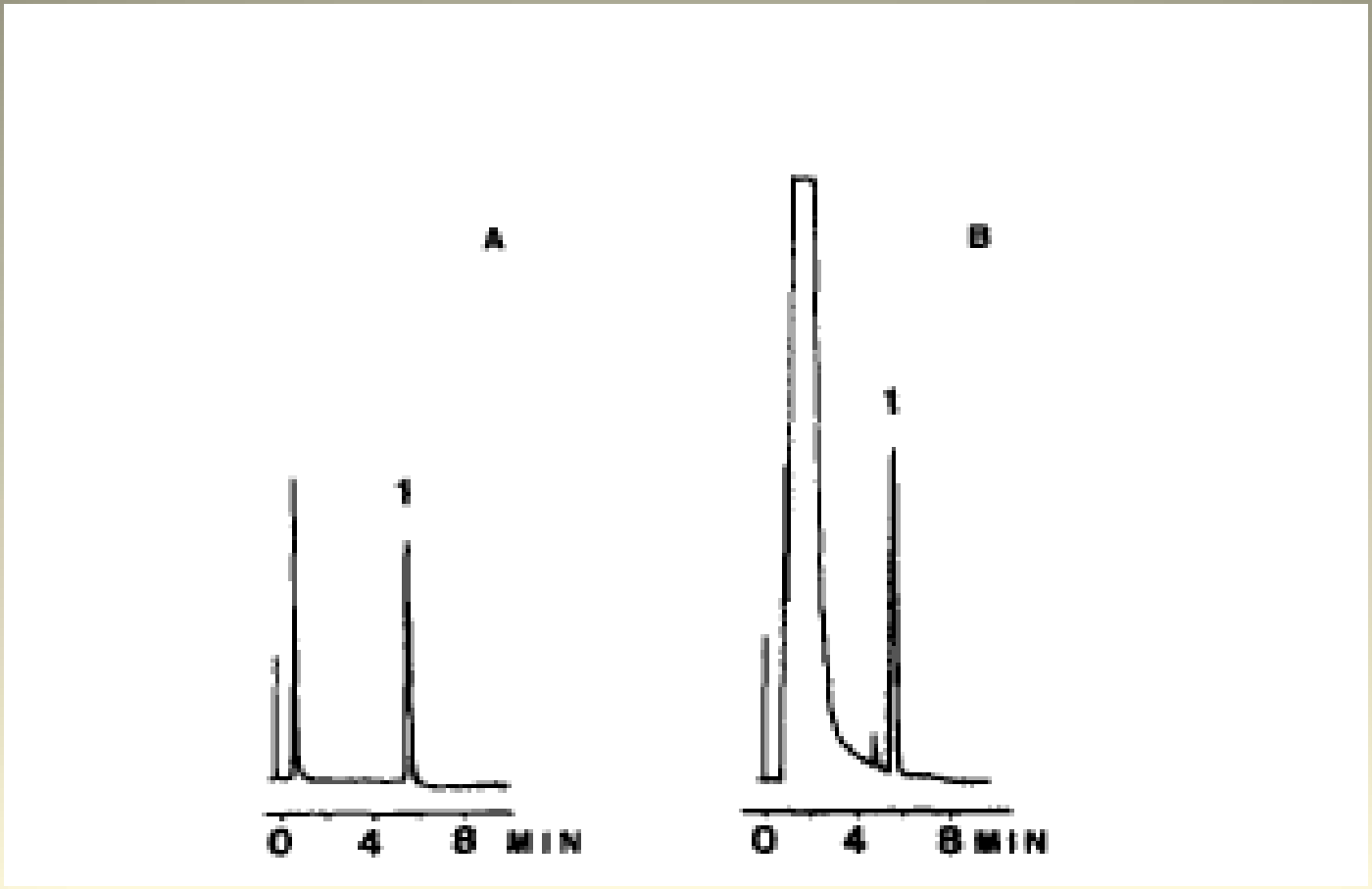
3. Applications of HPLC to STA (continued)

Case studies

- *3.3. Case 3: unexpected suicide by chloroquine*

- Chloroquine ; malaria infections

- HPLC-DAD screening method promptly led us to solve this forensic problem.

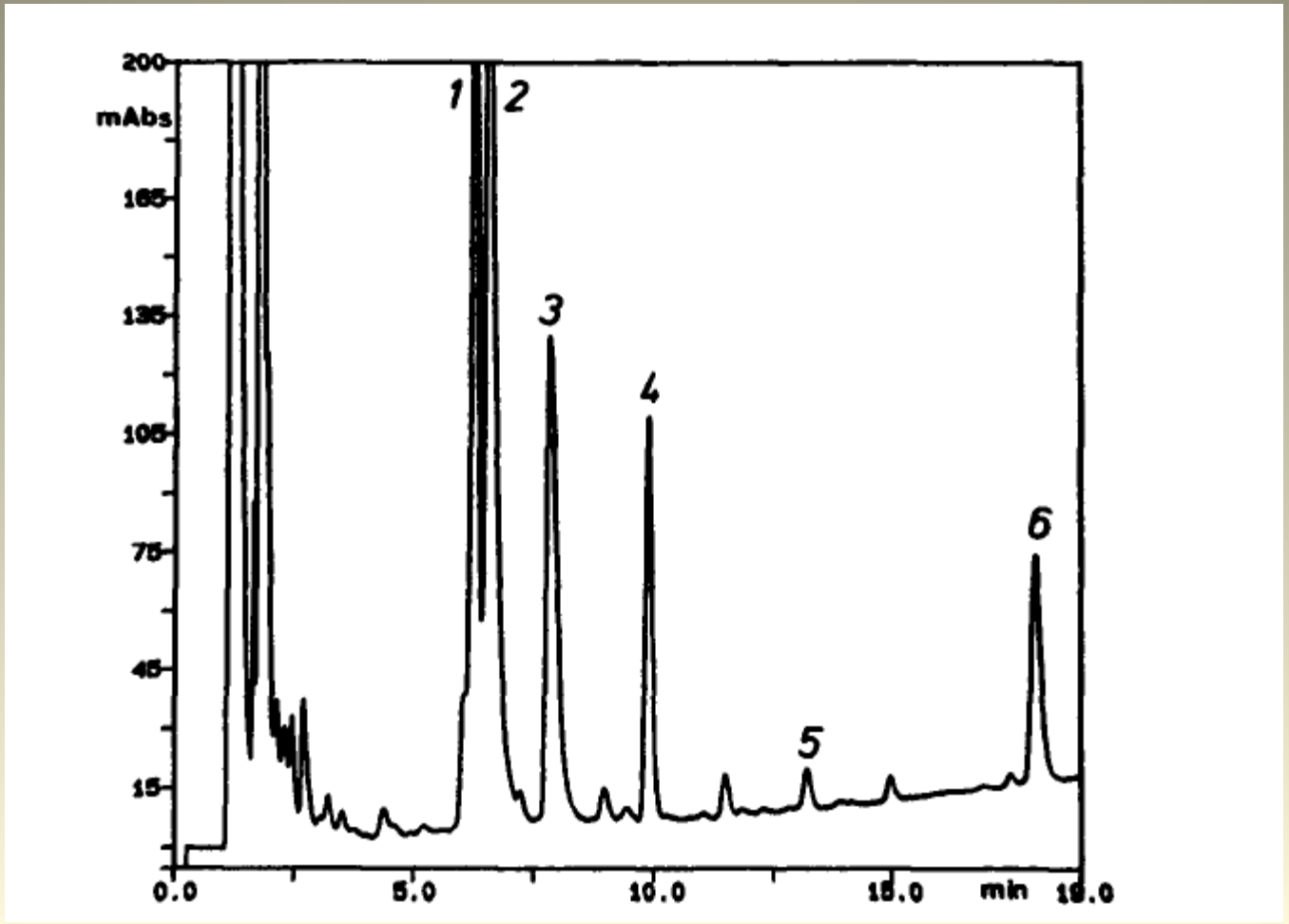


HPLC-DAD chromatograms (displayed at 240 nm) of (A) the deceased's appropriately diluted bile; peak 1 (tr: 5.70 min): 3,5-dinitrobenzoyl azide; azide level, 1283 p,g/ml. (B) An undiluted 5-txg/ml sodium azide standard; peak 1 (tr: 5.65 rain): 3,5-dinitrobenzoyl azide

3. Applications of HPLC to STA (continued)

Case studies

- *3.4 Case 4: cocaine, polydrug abuse and forensic evidence*



HPLC-DAD trace (displayed at 230 nm) of the urine extract. Peak identification: (1) benzoylecgonine; (2) 3,4-methylenedioxy methamphetamine (XTC); (3) 3,4-methylenedioxy ethylamphetamine (EVA); (4) (I.S. j) 2'-methylbenzoylecgonine; (5) cocaine; (6) (I.S.2) 2'-methylcocaine.

Conclusion

- HPLC-DAD offers many advantages in terms of specificity, sensitivity, speed and ruggedness.

- Application fields and excellent quantitative potential

THANK YOU