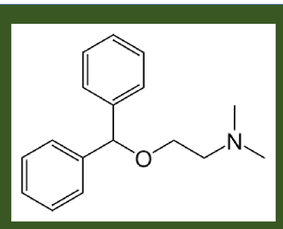
Quantitative Analysis of Diphenhydramine (DPH) Using Dispersive Liquid-Liquid Microextraction (DLLME) Paired with Gas Chromatography-Mass Spectrometry (GC-MS)

Ryan Kearns, Shokouh Haddadi

Department of Chemistry, SUNY Oswego, Oswego, NY 13126



Introduction

Diphenhydramine (DPH) is a peripheral H1 receptor antagonist drug with antihistaminic properties. Benadryl is a common over-the-counter antihistamine, which contains DPH as it's active ingredient. DPH possesses sedative properties and has been reported as being used in drug facilitated crimes (DFC) and has been classified as a "date-rape-drug" (Carter J. *et* al. 2000). Many perpetrators have been using DPH to spike alcoholic drinks at bars and clubs due to its synergistic properties with alcohol and ease of access. Normally, sedation/drowsiness occurs after a concentration of 30-40 ng/mL is within the blood stream (1-2 pills) and mental impairment at ≥60 ng/mL (2+ pills) (Couper F. *et*. al). However, when paired with the sedative affects of alcohol it takes a smaller dosage for these symptoms to occur due to their synergism. DPH has a short half-life of 4-8 hours making it difficult to detect based on the already low concentration of its initial dosage being rapidly metabolized. This is a common challenge in forensic toxicology with improved/cost effective methods being sought after for the detection and quantification of various drugs and compounds such as DPH.

Purpose

The goal of this work is to develop a dispersive liquid-liquid microextraction (DLLME) method to exhaustively extract DPH from aqueous solutions before separation, detection, identification, and quantification by gas chromatography coupled to mass spectrometry (GC-MS). The method was first tested with gas chromatography with flame ionization detector (GC-FID).

Methods

The proposed and tested DLLME method for extraction of DPH from standard aqueous solutions of DPH:

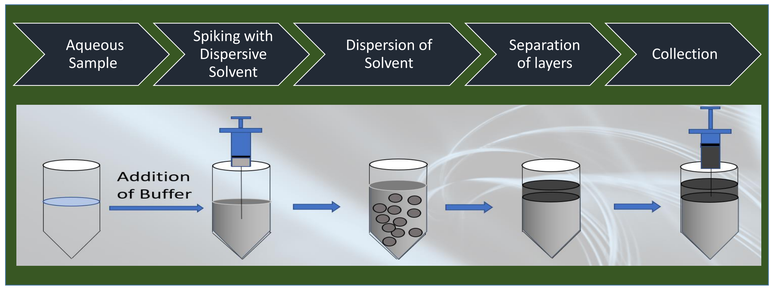


***Figure 1: D**iagram **of** DLLME procedure.*

Preparing Aqueous DPH Sample (5 ppm):

- 50 µL of n-Butanol was added around the glass of six 15 mL glass centrifuge vials using a micropipette.
- 5 mL Ultra Pure water was pipetted into the centrifuge vials.
- 25 µL of 1000 ppm stock DPH added to vials using a micropipette.
- Samples were sonicated for 15 minutes and stored at 4 °C inside a fridge until extractions were performed.

Preparing Standard Methanolic Solution (250 ppm):

- 75 µL of methanol was transferred into 2 mL brown-stained glass vial using a micropipette.
- 25 µL 1000 ppm DPH stock solution was added to the vial using a micropipette.
- The solution was sonicated and stored at 4 °C inside a fridge until GC analysis was performed.

Methods Continued

DLLME Extraction of DPH from Aqueous Samples:

- 20 µL of phosphate buffer and 50 µL of 2 M NaOH were added to each aqueous solution to adjust the pH at 12.
- Samples were shaken for 10 minutes and left to settle.
- Samples were then spiked with 1 mL of DLLME solvent mixture (toluene: acetonitrile, 13:40), and centrifuged for 10 minutes.
- Samples were then placed into an ethyl acetate bath in a holding tray and liquid nitrogen was added to freeze the aqueous layer (5 minutes).
- Unfrozen toluene layer (top layer) was collected form the samples using a micropipette and added into a 2 mL brown-stained glass vial.
- A double extraction was performed on three of the six aqueous samples.

Evaporation and Reconstitution of Aqueous Samples:

- The 2 mL brown stained-glass vials containing the collected toluene layer were connected to a Schlenk line in order to evaporate the solvent.
- Using nitrogen gas, the vials were dried after evaporation.
- Reconstitution was performed by adding 100 µL methanol around the inside of the vial.
- The samples were sonicated and allowed to homogenized at 4 °C.

***Figure 2:** Freezing the aqueous layer in ethyl acetate bath and evaporation of toluene.*

Instrumental analysis

- Thermo Scientific Trace 1310 GC
- Injection temperature: 250 °C
- Temperature program:
  - ➢ Held at 150 °C for 9 min
  - ➢ Increased to 250 °C at 25 °C /min
  - ➢ Held for 2 min at 250 °C
  - ➢ Total run time: 15 min
- Detector temperature: 300 °C

Results

Utilizing GC-FID the extraction recoveries were calculated using the following equation:

$$Extraction\ Recovery\ (ER) = \frac{Peak\ Area\ (Collected)}{Peak\ Area\ (Standard)} \times 100$$

***Table 1**. Extraction recoveries of single and double extraction*

Sample	Retention Time (min)	Peak Area	Extraction Recovery	Average Extraction Recovery	Standard Deviation (s)	%RSD
5 ppm (aq)(1)	13.192	0.6574	105	92	18	19
5 ppm (aq)(2)	13.192	0.4534	72			
5 ppm (aq) (3)	13.196	0.6270	100			
5ppm 2x (aq) (1)	13.194	0.5381	86	96	11	11
5ppm 2x (aq) (2)	13.196	0.6741	108			
5ppm 2x (aq) (3)	13.196	0.5979	95			
Direct 250 ppm	13.196	0.6266				

Results Continued

Identification of DPH

The identification of the extraction of DPH was confirmed by the retention times in Table 1. The comparison of the effects of a double extraction on extraction recovery can be seen in Figure 3.

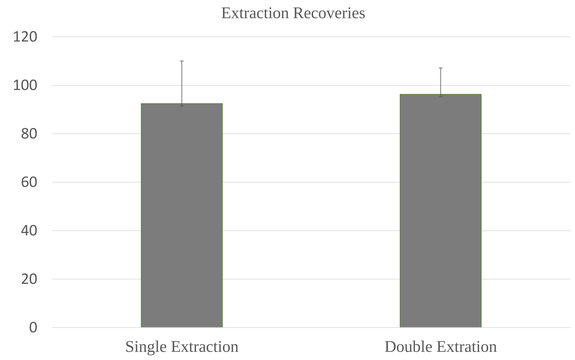


***Figure 3**: Comparison of extraction recoveries with standard deviation error*

Conclusion

The results suggest that the DLLME procedure is successful in the extraction of DPH from aqueous solutions based on the retention times found for the standard and aqueous samples. Based on the extraction recoveries, a double extraction may not prove necessary; however, it seems to have better reproducibility compared to a single extraction and does not take too long to perform. The results were promising in the success of this project, but further work is needed with the use of synthetic urine samples and the creation of a calibration graph. Also, GC-MS should be used to achieve lower detection limits.

Future Goals

- Prepare aqueous solutions containing DPH in a 5 ng/mL (ppb) to 2 µg/mL (ppm) range, and perform a double extraction using DLLME to test detection at ppb levels.
- Prepare direct calibration solutions containing DPH in a 250 ng/mL (ppb)to 100 µg/mL (ppm) range and run all samples in GC-FID or GC-MS for analysis.
- Explore the extraction of a mixture of antihistamines (DPH, Cetirizine, and Hydroxyzine) using DLLME.

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