Altasciences CLINICAL RESEARCH ALGORITHME PHARMA

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Novel In Situ Derivatization Approach for the Sensitive Determination of Menthol in Human Plasma Using Liquid Chromatography Mass Spectrometry

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OVERVIEW

PURPOSE

The determination of menthol in human plasma by LC-MS/MS with liquid-liquid extraction coupled with *in situ* derivatization for improved sensitivity.

METHOD

Menthol was extracted from human plasma using hexane, followed by derivatization with 2-fluoro-1-methylpyridinium-p-toluenesulfonate (FMPTS). The resultant extract was separated on a Zorbax C_{18} column with detection by +ESI/MRM for transitions *m/z* 248.1 > *m/z* 110.2 (menthol) and *m/z* 252.2 > *m/z* 110.2 (menthol-d_4).

RESULTS

Due to the fluorobenzene moiety, FMPTS showed high reactivity towards menthol with product yields > 95%. This reaction also allowed an *in situ* approach by combining derivatization with LLE, which not only simplifies sample preparation, but also avoids recovery loss for derivatized menthol in the evaporation step. The mean extraction recovery from human plasma was > 98%. The incorporation of the permanently charged 4°amine functionality enabled a 200-fold sensitivity gain in positive ESI. Taken together, a rapid, facile and sensitive assay was developed for the quantitation of menthol extracted from human plasma samples.

INTRODUCTION

Menthol is widely used in skin creams, cough medicine, mouth hygiene products and as an additive in cigarettes. While there is a demand for menthol assays in biological matrices, achievable detection limits by LC-ESI-MS/MS are confined by poor ionization efficiency, low recovery in multi-step sample preparation, and loss of volatile menthol in evaporation step. Thus, an *in situ* derivatization approach was employed in this current work to enhance extraction recovery and ESI-MS response in order to achieve a facile and sensitive assay for menthol in biological matrices.

METHOD

Sample Preparation

Plasma samples were extracted with hexane by briefly vortex mixing. Then, the hexane phase was transferred and an *in situ* derivatization approach was employed prior to evaporation, by adding FMPTS and triethanolamine (TEA) as outlined in Figure 1.

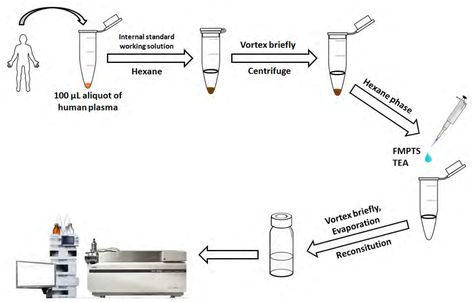
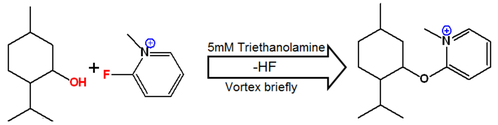


Figure 1. Sample preparation procedure of menthol in human plasma by liquid-liquid extraction coupled with *in situ* derivatization.

Chromatography and MS Detection

- Column: Zorbax SB-C_{18}, (2.1 x 50 mm, 3.5 µm)
- Isocratic elution using heptafluorobutyric acid and ACN
- SCIEX API 3000, MRM acquisition in positive ion ESI:
  - *m/z* 248.1 > *m/z* 110.2 (FMP-derivatized Menthol)
  - *m/z* 252.2 > *m/z* 110.2 (FMP-derivatized Menthol-d_4)



Scheme 1. The derivatization of menthol with FMPTS

RESULTS

DERIVATIZATION

FPMTS is a heterocyclic aromatic comprised of a fluorine atom located *ortho* to a cyclized quaternary ammonium ion, leaving the halogenated carbon susceptible to nucleophilic attack by a primary alcohol. When using TEA as catalyst, menthol rapidly reacts with FMPTS with just a brief vortex mix of the reaction mixture at room temperature (Scheme 1). Due to the presence of the permanently charged quaternary amine functionality in derivatized menthol, a 200-fold gain in sensitivity was achieved under positive ESI conditions (Figure 2).

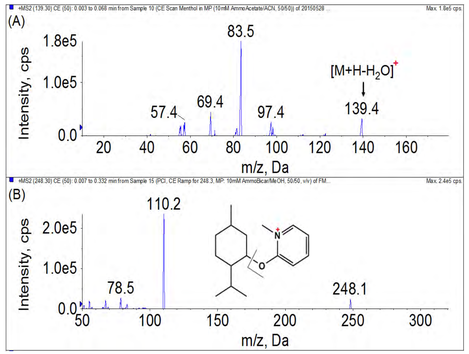


Figure 2. MS/MS spectrum of 250 µg/mL menthol (A) and 1.00 µg/mL FMP-derivatized menthol (B).

IN SITU SAMPLE PREPARATION

Despite the response enhancement of FMP-derivatized menthol, there are several challenges when incorporating such a derivatization scheme into a LLE post-extraction, such as loss of volatile underivatized menthol when evaporating extraction solvent and low recovery due to poor yields of derivatized product as a result of trace aqueous quenching. Thus, an *in situ* derivatization of menthol in hexane extraction solvent with FMPTS catalyzed by TEA was adopted to overcome the complications associated with post-extraction derivatization.

This *in situ* derivatization procedure in hexane (i.e. aqueous-free environment) enabled rapid formation of product with yields > 95%. No loss of derivatized menthol was noted upon evaporation. Extracts reconstituted in mobile phase without further cleanup were free of interference and matrix effect (Figure 3).

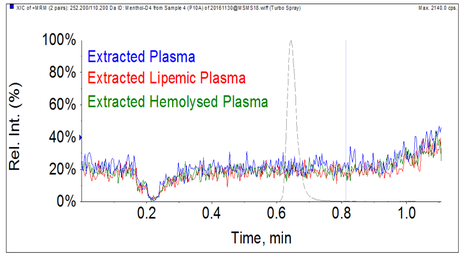


Figure 3. Extracted ion chromatogram of extracted human plasma, lipemic human plasma, and hemolyzed human plasma.

METHOD PERFORMANCE

Application of the *in situ* derivatization method to quantitate menthol was successful over the range 10 - 500 ng/mL when sampling 100 µL of human plasma (Figure 4). All precision and accuracy data met acceptance criteria for a validatable assay (Table 1).

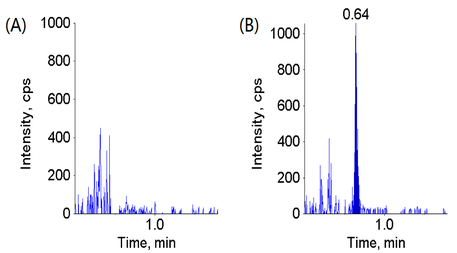


Figure 4. Blank (A) and 10 ng/mL LLOQ extracted from human plasma (B)

Table 1. Between-run precision and accuracy for FMP-derivatized menthol in human plasma.

Curve Code	Concentration (ng/mL)			
	LOQ QC 10.00	QC1 30.00	QC2 330.00	QC3 3750.00
ASM706.01	10.74	30.95	325.98	3698.49
	10.28	33.17	326.50	3401.13
	9.12	32.93	356.21	3353.64
	10.43	30.18	329.44	3392.87
	10.12	26.47	333.93	3979.27
	8.83	27.49	312.21	3748.07
ASM706.02	10.51	26.24	331.05	3779.96
	10.34	29.75	342.57	3699.46
	9.17	29.66	327.87	3651.42
	9.32	29.37	325.55	3355.40
	9.69	26.98	345.78	3295.05
	9.99	28.63	313.74	3714.61
ASM706.03	8.98	29.12	315.20	3504.64
	9.18	29.68	331.43	3630.03
	9.79	33.09	367.41	3743.52
	9.85	31.90	337.06	3535.45
	9.34	30.22	346.31	3836.97
	9.39	30.20	327.33	3474.96
Mean	9.73	29.78	333.09	3599.72
S.D.	0.58	2.13	14.38	193.43
N	18	18	18	18
% C.V.	6.0	7.2	4.3	5.4
% Nominal	97.3	99.3	100.9	96.0

CONCLUSION

The *in situ* derivatization of menthol provided a rapid and robust method with sensitivity gains capable of supporting the targeted LOQ of 10 ng/mL from only 100 µL of plasma.

ACKNOWLEDGMENTS

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