Evaluation of Methylisothiazolinone (MI) Extraction from Sunscreen using Supported Liquid Extraction prior to GC/MS Analysis.

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Introduction

Methylisothiazolinone (MI) is an antimicrobial preservative that is used in a variety of personal care products, such as sunscreens, lotions, cosmetics. MI is a cytotoxin and as a result there is concern because of sensitization and allergic reactions as well as cell and nerve damage. A percentage of the population is at risk from contact dermatitis when exposed to this compound at sufficient concentrations. From July 2015, the European Commission will adopt a ban of MI as an ingredient in leave-on cosmetic products. The structure of MI is displayed in ***Figure 1.***

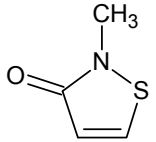


Figure 1. *Structure of MI*

Experimental

Reagents

MI was purchased from LGC Standards (Teddington, UK). Hydrochloric acid (37%), sodium chloride and all solvents (HPLC grade) were purchased from Sigma-Aldrich (Dorset, UK). Milli-Q water (Merck Millipore, Germany) was used throughout. Sun lotion, free from MI, was purchased locally.

Sample Preparation

ISOLUTE® SLE+ Optimized Procedure (*Figure 2.*)

Columns: ISOLUTE® SLE+ 1 mL capacity 'C' columns; p/n 820-0140-C.

Matrix Pre-treatment: Weigh 100 mg of sunscreen into a 2 mL Eppendorf tube. Add 1.5 mL of 50/50 methanol/1M sodium chloride (aq) and vortex mix for at least 10 seconds. Alternatively homogenize the sample with a BeadRuptor®24 instrument without beads for 30 seconds at 5 m/s (1 cycle). The homogenate is centrifuged with 17,000 G for 10 minutes and the supernatant is decanted into a glass tube.

Sample Application: Load 500 **µ**L of the decanted solution onto the column and apply a pulse of vacuum or positive pressure (3-5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Elution: Apply 2 mL of 50/50 hexane/ethyl acetate (v/v) and allow to flow under gravity for 5 minutes. Repeat this procedure a further twice for a total of 6 mL of elution solvent. Apply vacuum or positive pressure (5-10 seconds) to pull through any remaining extraction solvent after the final aliquot.

Post Processing: Prior to evaporation, add 200 mM HCl in isopropanol (100 µL) to each collection tube. This helps stabilize the analyte and minimize losses during the evaporation process. Samples were evaporated without heat and reconstituted in 100 µL EtOAc.

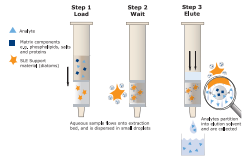


Figure 2. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction procedure.*

GC/MS Conditions

GC: 7890A GC (Agilent Technologies Inc.)

Column: Agilent J&W DB-**5, 30 m x 0.25 mm ID x 0.25 µm**

Carrier Gas: Helium 1.2 mL/min (constant flow)

Inlet: Split, 40:1 ratio

Temperature: 250 °C

Injection volume: **1 µL**

Oven conditions: Initial 60 °C, ramp 10 °C/min to 110 °C, ramp 100 °C/min to 325 °C, hold for 2.0 min

Transfer Line: 280 °C

MS: 5975C MSD (Agilent Technologies Inc.)

Source Temperature: 230 °C

Quadrupole Temperature: 150 °C

Monitored Ions: Ionization was performed using EI. Signals were acquired using selected ion monitoring (SIM): Quantifier ion : 115; Qualifier ions: 87 & 58

Results

MI is extremely volatile and prone to evaporative losses. Initial method development focused on quantifying and reducing the amount of MI lost during the evaporation step. Evaporative losses were investigated by spiking MI into 50/50 hexane/ethyl acetate and comparing to an equivalent concentration not subjected to evaporation. ***Figure 3.*** demonstrates the amount of MI recovered when using hexane or EtOAc reconstitution in the presence and absence of acidic modification (0.2M HCl in IPA) prior to evaporation.

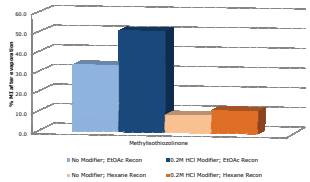


Figure 3. *% recovery of MI following evaporation*

In order to reach 50% recovery of MI as demonstrated above, evaporation was performed without heat, along with careful monitoring of the solvent volumes to prevent over-drying. Even taking into account evaporative losses of 50%, the sensitivity of the assay was sufficient in terms of reaching the required limits of quantitation.

MI is a polar analyte and has a documented logP of 0.23. This polarity combined with the complexity of the matrix required careful method development to partition the analyte into the water-immiscible organic solvent while avoiding co-extraction of fats, oils and other sunscreen interferences. MI is completely soluble in water and mostly soluble in methanol, acetonitrile and hexane.

Prior to analyte recovery work the primary objective was to evaluate extract cleanliness using various extraction protocols. Early sunscreen pre-treatment experiments were performed using acetone, methanol and acetonitrile alongside an aqueous salt solution. These extracts were applied to the ISOLUTE® SLE+ columns and extraction performed using either hexane or ethyl acetate. These experiments demonstrated that the extraction of 250 mg of sunscreen resulted in a significant amount of non-volatile breakthrough in the collection tubes (data not shown).

Further experiments investigated the use of reduced weights of sunscreen prior to pre-treatment, to monitor any improvements to breakthrough. Evaluation of weights down to 100 mg demonstrated improved extract cleanliness. 100 mg of sunscreen was selected for subsequent experiments.

Figure 4. shows a comparison of 2 approaches to matrix homogenization. The two samples on the left suffer from a lack of homogeneity, whereas the samples on the right, mixed using the BeadRuptor®24 are more homogenous.

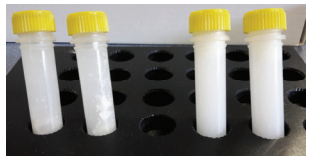


Figure 4. *Sunscreen homogenization after MeOH/NaCl(aq) 50/50. (L) vortex mixed, (R) Beadruptor®24 mixed.*

Further extract cleanliness optimization was performed by investigation of loading volumes on the ISOLUTE® SLE+ column. Sunscreen pre-treatment with 1.5 mL of an aq/organic solvent combination was selected for 100 mg of matrix and loading volumes of 500 µL and 750 µL were evaluated after thorough vortex mixing followed by centrifugation. Superior extract cleanliness was demonstrated using 500 µL loading volumes (data not shown).

Initial recovery work was performed in the absence of matrix to investigate MI partitioning from the aq/organic solutions used for analyte extraction from the sunscreen. Hexane/ethyl acetate 50/50 (2x3 mL) was initially evaluated as the extraction solvent. These extraction protocols resulted in a recovery of up to 70% with corresponding RSDs of 13% (n=7).

Matrix recovery experiments were performed spiking 20 µg of MI into 100 mg of sunscreen, resulting in an inferred product percentage of 0.02% by weight.

100 mg of sunscreen was pre-treated with 1.5 mL of MeCN/H_2O, 500 µL loaded onto the ISOLUTE® SLE+ and extracted with 50/50 hexane/EtOAc. This method demonstrated recoveries of 85% with RSD of 13%. Good chromatographic baseline cleanliness was observed, as shown in ***Figure 5.*** However the visual extract cleanliness was of concern; resulting from the co-extraction of some non-volatile sunscreen components.

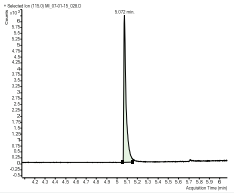


Figure 5. *MI chromatography from extracted sunscreen*

Experiments were then performed using various aq/organic combinations in the pre-treatment. Methanol was used as a replacement for MeCN and the incorporation of an aq salt solution was investigated.

Figure 6. demonstrates recovery data from these experiments. DCM and MTBE returned high recoveries but unfortunately demonstrate substantial cleanliness issues. When pre-treating with salt and methanol, hexane/EtOAc offers the combination of high recoveries and best cleanliness.

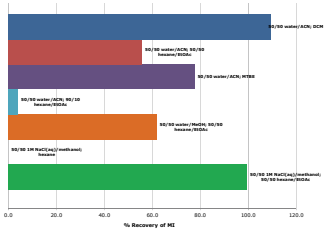
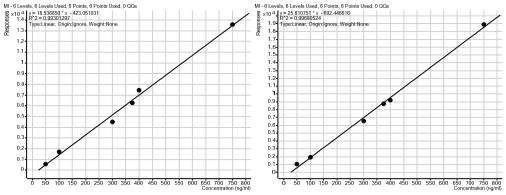


Figure 6. *MI recovery experiments using various pre-treatment and extraction solvent combinations.*

Calibration curves were constructed at concentrations from 50-750 ng/mg with the optimum extraction protocol using two matrix homogenization approaches. ***Figures 7. and 8.*** demonstrate vortex mixed and the BeadRuptor®24 mixed samples respectively. The coefficient of determination (r^2 values) for MI are 0.9930 for the vortex mixing and 0.9968 for the BeadRuptor®24. Both ***Figures 7. and 8.*** also show the ability to quantitate down to 50 ng/mg of sun-lotion.



Figures 7 and 8. *Calibration curves for MI extracted from sun-lotion with the optimized method. Homogenization of sunscreen achieved using vortex and BeadRuptor®24 mixing. Concentration range is 50 ng/mL to 750 ng/mL*

Conclusions

- This poster demonstrates the suitability of ISOLUTE® SLE+ for the rapid and reliable extraction of methylisothiazolinone from sunscreen at a relevant concentration, prior to GC/MS analysis.
- The optimized method offers almost high analyte recoveries with RSDs below 10%, demonstrating excellent chromatographic baseline and visual cleanliness.
- The BeadRuptor®24 offers greater homogeneity when mixing sunscreen matrix compared to manual vortex mixing.

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