Electronic Supplementary Information (ESI)

Eight-Port Splitter Validation

A sodium chloride solution (22 g in 1000 mL of demineralized water) was nebulized into the generation system described in this study with a disposable inhalotherapy nebulizer (Glenwood Laboratories GK 1800, Canada). Several samples were collected using a pre-weight 37 mm polyvinylchloride membrane, porosity of 5 µm, installed on a cellulose pad and mounted in a 37 mm closed-face cassette. Air sampling was conducted at 1.5 L/min using the same methodology described in the article (Materials and Methods). The gravimetric analysis (pre-weight and post-weight) was performed using a Mettler-Toledo MX-5 microbalance (Fisher Scientific, Canada) equipped with a Polonium-210 static eliminator installed in a room where humidity and temperature were controlled. Samples were kept in a desiccator for a minimum of 16 hours before the analysis. The system provided an NaCl concentration of between 20 and 28 mg/m³ from test to test. The average within-test variability (n = 4 to 8 per test, for 3 tests), expressed as relative standard deviation, was less than 5%.

Analytical Methods

Chemicals

The MDI (98%); N-methyl-amino-methyl-9 anthracene (MAMA) (98%); dibutylamine; DBA (> 99%); acetone (HPLC grade), and acetic anhydride, AA, (98%) were purchased from Sigma-Aldrich (USA) and were used without further purification. MDI-DBA and the MDI-DBA-d9 standard kits were supplied by Supelco (USA). MAMA-MDI was prepared by Nuchem Therapeutics (Canada). Acetonitrile (CAN), water (H_2O), formic acid (FA) (optima grade), and phosphoric acid (H3PO4) (> 99%) were obtained from Fisher Scientific (Canada). Toluene (> 99%) was purchased from EMD Millipore (USA) and dimethylformamide from the J.T. Baker Company (Fisher Scientific, Canada). The glacial acetic and sulfuric (96%) acids, dimethylformamide, triethylamine (HPLC grade), DMF (HPLC grade), and methanol, MeOH (HPLC grade) were obtained from J.T. Baker (Fisher Scientific, Canada).

Instrumentation

Lab preparation involved the use of an Eberbach shaker (Michigan, USA), a Branson B-52 ultrasonic bath (Danbury, USA), and a Sorvall ST 40R centrifuge (Thermo Scientific, Canada). A Zymark Turbovap LV-ZW700 evaporator was used (Biotage, USA). MAMA method samples were analyzed using an ultra-high-performance liquid chromatographic (UPLC)–photodiode array (PDA) system consisting of an LC1290 from Agilent (USA) with a Zorbax Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm (Agilent, USA). The software used to operate this system and analyze the data was OpenLab CDS, also from Agilent. Asset samples were analyzed using an ultra-high-performance liquid chromatographic–mass spectrometry (UPLC-MS/MS) system consisting of a Waters Acquity UPLC coupled with a Waters Xevo TQ triple quadrupole MS (USA) equipped with an electrospray source and an autosampler having a partial loop and a needle overfill feature (10 µL). The column was an Acquity UPLC BEH C18, 1.7 µm, 2.1 mm x 100 mm, from Waters

(Santry, Ireland). The software used to operate the system and analyze the data was Masslynx, V4.1, from Waters (USA).

Analysis

MAMA Method

Calibration standards with MAMA-MDI derivative were prepared in DMF to cover a concentration range of 0.015 to 0.700 µg of MDI per 2 mL. The mobile phase consisted of trimethylamine (TEA) buffer (30 mL TEA + 940 mL H2O, pH 3 with H3PO4) (eluant A) and ACN (eluant B), respectively. Each sample was desorbed in 2 mL of desorption solution (60 mL TEA buffer + 140 mL ACN and 400 mL DMF). The LC method used an isocratic elution program, 25% A and 75% B, at 1 mL/min, 8 µL sample injection and column kept at 30°C. PDA detection was performed at λ = 254 nm. Run time for each analysis was 8 minutes.

Asset Method

Asset standards and samples were prepared according to Halpenny et al.(1) The prepared solutions were injected (10 µL) into the UPLC-MS via its autosampler, set at 15°C. The mobile phase was composed of ACN + 0.1% FA (eluant A) and water + 0.1% FA (eluant B). The run started with a gradient of 70% eluant A (0.5 min.), ramped to 90% eluant A (2.5 min.), held at 90% eluant A (2 min.), ramped to 100% eluant A (3 min.), and finally equilibrated at 70% eluant A (2 min.). The flow rate in the column was 0.6 mL/min. and the temperature was maintained at 50°C. The Xevo TQ was used in positive mode with the capillary voltage set at 2 kV and the source temperature at 150°C. The desolvation temperature and flow were 500°C and 1,000 L/h, respectively, while the collision gas flow was set at 0.15 mL/min. The [M+H]+ for MDI-DBA is m/z 509.3 and that of the internal standard, MDI-DBA-d18, is m/z 527.4. The data were acquired in multiple reaction monitoring (MRM) mode (Table S1). Manual adjustments were made on the integrations to ensure that the entire peak was covered before the data were recorded. The results were used to create a regression calibration curve with linear fit.

Table S1. MRM species calculated and measured.

Substances	Calculated [M+H]+	MRM transitions	Cone (V)	Collision energy (eV)
MDI-DBA	509	509.3–130.2	35	20
MDI-DBA-d18	527	451.2–139.1	45	30

Quality Control

For each method, all analytical sequences incorporated quality control samples (QCS). For each sequence, the calibration standards were analyzed, followed by a reagent blank (RB), a QC spiked at the limit of quantification (QCLOQ), a QC at a value representing 50% of the dynamic range (QC50%) and then followed by the samples. Every 10 samples, the QC50% was analyzed to verify the calibration of the analytical run. At the end of the sequence, the RB, QCLOQ, and QC50% were analyzed again, followed by the calibration standards. Each result was reported considering the fact that the QCS were in the acceptable range, which were ± 20% and ± 50% for QC50 and QCLOQ respectively.

References

1. Halpenny M, Brown J. ASSET™ EZ4-NCO Dry Sampler Extraction Procedure: Sigma-Aldrich; 2013 [Available from: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/Instructions/1/ASSET_EZ4-NCO_Extraction.pdf.