Acetylated methylenediphenyl diisocyanate lysine conjugates in the urine of workers exposed to methylenediphenyl diisocyanate: Supplementary Material, S2

Analytical method for acetyl-MDI-lysine (acMDI-Lys) in urine samples

Chemicals

1M Hydrochloric acid, 2M Sodium hydroxide, glacial acetic acid, ammonium acetate and ammonium hydroxide (for HPLC, 35% solution in water) were purchased from Fisher Scientific (Loughborough, UK). HPLC grade methanol was supplied by Rathburn Chemicals (Walkerburn, UK).

Sample preparation

An analytical standard of acMDI-Lys was made at 2 mmol/L in sodium hydroxide /methanol (using 100 µL of 2M NaOH); from this a working calibration solution of acMDI-Lys was made up in methanol at 2 µmol/L. A calibration range of 10-160 nmol/L was produced by addition of the appropriate amount of calibration solution into 2 mL aliquots of blank urine from a person with no known occupational exposure to MDI or MDA. Deuterated methylene dianiline (100 µL of 50 µmol/L) was added as an internal standard. Quality control (QC) samples were analysed in duplicate before and after every five samples (also analysed in duplicate). Samples then underwent solid phase extraction (SPE). Bond Elut Plexa PCX (60mg, 3 mL cartridges; Agilent, Cheadle, UK) were first washed with 3 mL methanol and then conditioned with 3 mL Milli-Q water before the sample was loaded (2 mL). After the sample load, the cartridge was washed with 3 mL 0.1 M hydrochloric acid in water followed by 3 mL of 0.1 M hydrochloric acid in methanol. SPE cartridges were then dried before elution with two aliquots of 2 mL 5%

ammonium hydroxide solution in methanol. Eluates were then evaporated under a stream of nitrogen and reconstituted in 100 µL of starting mobile phase.

Liquid chromatograph parameters

Instrument: Agilent 1260

Column: Luna C18 150 x 2 mm, 5 µ (Phenomenex, Macclesfield, UK)

Flow: 200 µL/min

Inject: 10 µL

Mobile Phase A: 20 mmol/L ammonium acetate with 0.1% acetic acid

Mobile Phase B: Methanol

Gradient:

Time (min)	%A	%B
0	96	4
3	96	4
15	10	90
21	10	90
21.1	96	4
31	96	4

Mass spectrometer parameters

Instrument: ABSciex 4500 QTrap (AB Sciex, Macclesfield, UK)

Parameter	Positive MRM
CUR: Curtain gas (L/min)	50
CAD: Collision Gas	Medium
Ion Spray Voltage	4500
Temperature (°C)	600
GS1: Ion Gas 1 (L/min)	50
GS2: Ion Gas 2 (L/min)	60

Lens parameters

Analyte	MW	Q1	Q3	DP	EP	CE	CXP
AcMDI-Lys	412	*413.1	241.4	72	8	23	17
		^413.1	106.0	72	8	55	11
d_8-MDA		202.0	107.1	50	7	30	10

*Quantifier transition, ^confirmation transition

MW = Molecular weight, DP = Declustering Potential, EP = Entrance Potential, CE = Collision Energy, CXP = Collision Cell Exit Potential.

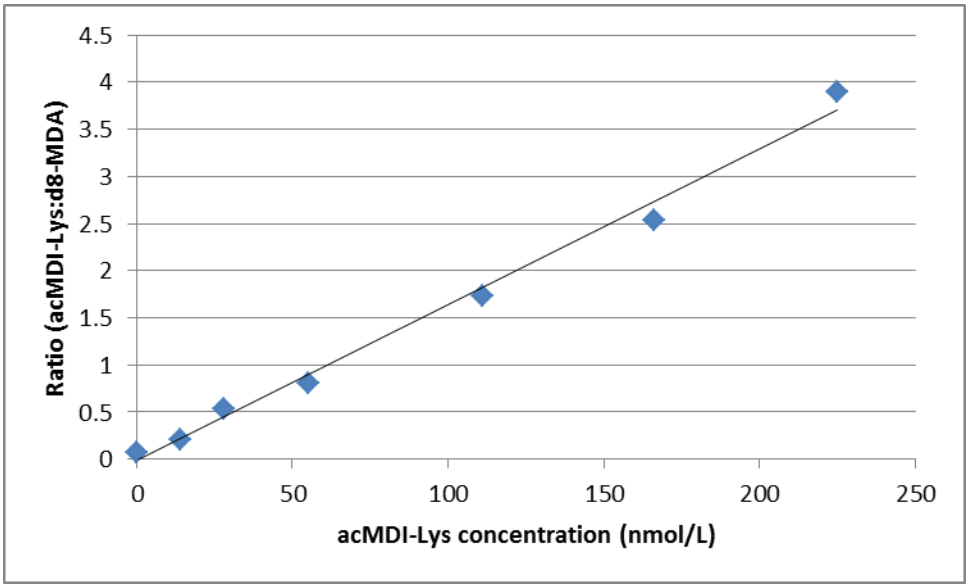

Figure S2.1. Example calibration curve for acMDI-Lys (10 -160 nmol/L) using d_8-MDA as an internal standard. Least squares regression equation: peak ratio = 0.0165 acMDI-Lys (nmol/L) – 0.0176, r^2=0.9914.