Electrospray Ionization Tandem Mass Spectrometry (esi-ms/ms) Analysis of 1-Bromopropane Mercapturic Acid Metabolites in Urine

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1-Bromopropane (1-BP, CAS 106-95-5), used as an alternative solvent to chlorofluorocarbons and 1,1,1-trichloroethane, has been reported to cause reproductive and neurotoxicity in male rats. The related 2-bromopropane has been shown to cause similar toxicity in rats as well as amenorrhea, oligozoospermia, and anemia induction in workers. Although the mechanism of action of 1-BP has yet to be explained, it is thought that metabolic activation to reactive intermediates may be important. Metabolism of 1-BP is complex and is reported to occur by pathways which include debromination, oxidation by CYP2E1 and glutathione *S*-conjugation. 3-Bromopropionic acid and n-propanol are reported urinary metabolites of 1-BP whereas the glutathione conjugate, *S*-n-propyl-glutathione is further cleaved to *S*-n-propyl-L-cysteine and further to mercapturic acids N-acetyl-*S*-(n-propyl)-L-cysteine (M1), N-acetyl-*S*-(n-propyl)-L-cysteine-S-oxide (M2), N-acetyl-*S*-(2-carboxyethyl)-L-cysteine (M3), and N-acetyl-*S*-(3-hydroxy-n-propyl)-L-cysteine (M4). A potential biomonitoring method was developed to measure urinary levels of (M1), (M2), (M3) and (M4). The mercapturic acid standards as well as the stable isotope-labeled analog of (M1) internal standard were synthesized using the general procedure of van Bladern *et al.* (1980). A BenchMate¤ II robotic workstation was used to automate sample preparation. Bond Elute¤ 500 mg C18 SPE columns were conditioned with acetone, MeOH (5% HCl) and 5% MeOH in H_2O pH 3. Samples were mixed with internal standard and loaded onto columns. A fraction containing >90% of 1-BP metabolites was collected in 3-mL acetone, reduced to dryness under N_2 and dissolved in 1 mL MeOH for HPLC-MS/MS (ThermoQuest Finnegan LCQ tandem mass spectrometer) analysis on a 150 X 2 mm Phenomenex Aqua 3_m C18 300A column. Chromatographic standards were chromatographed using a 10-min linear gradient H_2O 1% acetic acid to MeOH 1% acetic acid at 300 _L/min to elute the compounds of interest within 10 min. During the chromatographic run the mass spectrometer was operated in multiple segments using ESI-MS/MS, in the positive ion mode for detection of protonated (M1), (M2), (M3) and (M4) and Selected Reaction Monitoring of major transition products. Urine samples fortified with a mixture of standards were mixed with 10 _g/mL of internal standard and processed for evaluation of recovery, limits of detection (LOD)

and limits of quantitation (LOQ). Calibration of (M1), (M2), (M3) and (M4) was linear from 30 - 10000 ng/mL (r^2>0.99). The sample preparation and analysis appears to offer significant advantages over typical preconcentration and derivatization procedures that would be required for GC-MS analysis of these compounds. Thus, 1-BP internal exposure levels for various exposure situations can be rapidly determined by analysis of these metabolites in a single assay using a selective automated sample preparation system.

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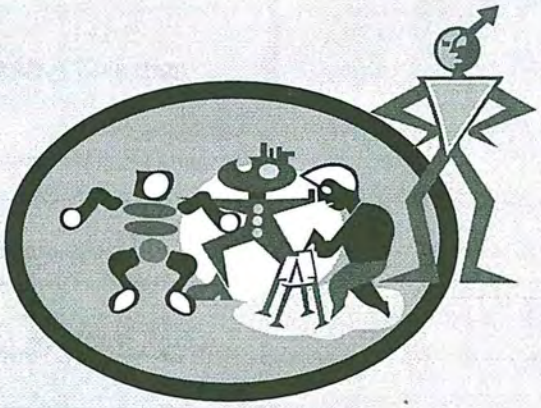

Abstract

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