

Cyanide Trapping of Iminium Ion Reactive Intermediates Followed by Detection and Structure Identification Using Liquid Chromatography-Mass Spectrometry

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Introduction: Idiosyncratic or adverse drug reactions typically do not significantly occur until a large population has been studied, mostly during phase III clinical trials. Thus, it has become important to investigate adverse drug reactions in an effort to understand and further prevent such reactions by identifying compounds that have a potential to cause toxicity. Previous methods have employed trapping agents such as glutathione (GSH), potassium cyanide (KCN), and others for the detection and characterization of reactive metabolites in the pre-clinical phase of drug development. Alicyclic amines are widely found in pharmaceuticals and environmental compounds. Metabolic activation of these compounds often involves the formation of an iminium ion that is reactive toward nucleophiles. Extensive studies of nicotine with radiolabeled cyanide have shown the formation of an iminium ion intermediate that binds covalently to cellular macromolecules. We report a relatively high throughput LC-MS/MS method for the detection and characterization of iminium ion reactive intermediates using product ion scanning on a triple quadrupole mass spectrometer.

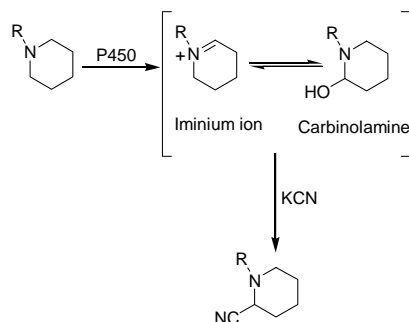


Figure 1. Alicyclic amine metabolism and subsequent trapping via cyanide addition

Methods: Test compounds were individually incubated with human liver or rat liver microsomes in the presence of an NADPH regenerating system and trapping species, either potassium cyanide or GSH to assess iminium ion formation and subsequent detection. All incubations were performed in duplicates. Nicotine incubations were used as positive procedure controls. Two negative controls, incubations without substrate and without NADPH, were associated with each compound. Reactions were terminated at 90 minutes and protein was removed by centrifugation for 20 minutes. Samples were screened using reverse phase liquid chromatography electrospray mass spectrometry. Chromatographic separations were performed on a Waters YMC ODS-AQ C₁₈ column, 2.1 mm id, 50 mm length, 5 μm particle size. Mass spectral analyses were performed on an Applied Biosystems/ MDS Sciex API-4000 triple quadrupole mass spectrometer.

Results and Discussion: Nicotine incubations were initially used to establish method feasibility and subsequently used as a positive procedure control along with two negative controls previously mentioned. LC-MS/MS analyses were optimized for the constant neutral loss (CNL) of 27 or 29, loss of HCN or $H^{13}C^{15}N$ respectively, with microsomal incubations of nicotine with cyanide as the trapping agent. Following detection from CNL scans, MS/MS data for each cyano adduct was collected to determine the most prominent transitions for each adduct detected in CNL experiments. These transitions were subsequently used to simultaneously detect each cyano adduct in all three microsomal species and controls associated with each test compound in LC-MS/MS using selective reaction monitoring. Two isomeric cyano adducts were detected in CNL experiments for nicotine. These isomers reflect the formation of two iminium ion intermediates resulting in cyanide addition to each of the two carbons alpha to the endocyclic nitrogen. This result was consistent with the known metabolic activation of the compound.

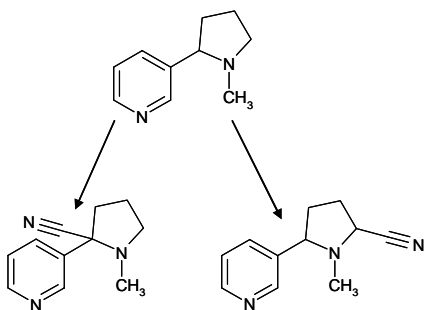


Figure 2. Nicotine cyano adduct isomers

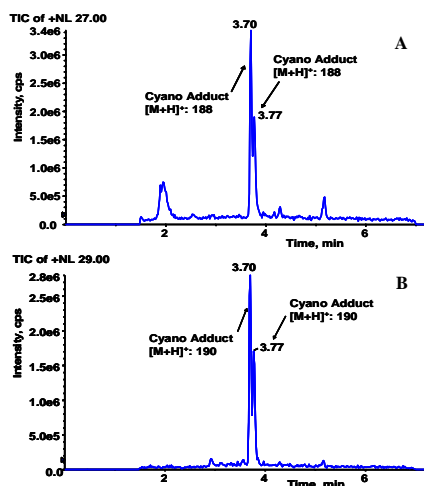


Figure 3. TIC of CNL 27 and 27 for KCN and $K^{13}C^{14}N$

A total of 14 compounds were screened for metabolism dependence and iminium ion formation with the KCN and GSH trapping assay. In general, results for all 14 compounds screened with the KCN were complementary to results obtained with the GSH trapping assay. In a majority of the compounds, few or no GSH adducts with low metabolism dependence were detected, while cyano-adducts with high metabolism dependence for formation were detected in KCN assays.

In some cases, interesting metabolites were trapped with the KCN assay and further characterized in MS/MS analyses. Prochlorperazine metabolism results using the KCN assay detected several metabolism products including an iminium ion, several hydroxylation and/or N-dealkylation metabolites that were formed through an iminium ion intermediate. Also, several metabolism products of nefazodone were trapped with the KCN assay indicating metabolic formation through an iminium ion intermediate.