High-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) based on collision-activated dissociation is a widely used technique to obtain structural information for unknown compounds in complex mixtures. However, this technique sometimes does not produce useful structural information as many isomeric ions fragment in a similar manner. Gas-phase ion-molecule reactions provide a promising approach for the structural characterization of isomeric compounds. The identification of functionalities in analytes based on the measured data and the selection of neutral reagents for new types of analytes can be time-consuming and challenging. We introduced an automated machine-learning guided with HPLC MS/MS system to facilitate the identification of functional groups and prediction of most probable neutral reagents in new types of unknown analytes. Experiments were performed using modified thermo scientific linear quadrupole ion trap mass spectrometer coupled with atmospheric pressure chemical ionization source. Three neutral reagents were introduced into a nine pulsed-valve reagent inlet that quickly released them into the ion trap in an automated manner. HPLC/MS/MS experiments were carried out using data-dependent scan method to isolate the three most abundant ions generated upon the ion-molecule reactions in order to isolate the unknown protonated analyte. Diagnostic product ions formed were used for machine learning-based models to predict functionalities in unknown protonated analytes and selection of suitable neutral reagents for new analytes. Machine learning-based models predicted reaction outcomes and interpreted experimental results to predict functionalities in unknown compounds by obtaining the elemental composition of the protonated analytes and their ring double bond equivalent value via high-resolution measurements. A chemical graph-based interpretable machine learning approach enabled the automation of the identification of functionalities in previously unknown protonated analytes and selection of suitable neutral reagents for previously untested analyte types. Collectively, this study will ultimately aid in improving analytical techniques for potentially mutagenic drug Impurities and metabolites.