Identification of Impurities in an Expired Standard Drug Mixture by Using Multiple Ionization Methods and msFineAnalysis

INTRODUCTION

Gas chromatography combined with high-resolution time-of-flight mass spectrometry (GC-HRTOFMS) is a powerful tool for the analysis of complex mixtures. The AccuTOF GC-Alpha (JMS-T2000GC) mass spectrometer is fast, accurate and sensitive with high mass-resolving power and high mass accuracy.

Data analysis based on database matching alone does not necessarily provide reliable assignments for compounds that have similar electron ionization (EI) mass spectra or compounds that do not have entries in mass spectral databases. JEOL *msFineAnalysis* software provides automated analysis that makes use of all of the information available from a GC-HRTOFMS analysis: elemental composition determination from soft and hard ionization with exact mass measurements and accurate isotope data, database searching, fragment ion coverage, and retention index data.

In this work, GC-MS analysis of a 12-year-old standard drug mixture was carried out with the AccuTOF GC-Alpha (JEOL JMS-T2000GC) mass spectrometer using the combination electron ionization/photoionization (EI/PI) ion source and the chemical ionization (CI) ion source. Data analysis using JEOL *msFineAnalysis* software carried out chromatographic deconvolution and identified the drugs and impurities by combining and integrating all of the information from EI and PI analysis.

Name	Composition	*m/z*
Caffeine	$C_8H_{10}N_4O_2$	194.07982
Glutethimide	$C_{13}H_{15}NO_2$	217.109727
Phenobarbital	$C_{12}H_{12}N_2O_3$	232.084245
Lidocaine	$C_{14}H_{22}N_2O$	234.17267
Carbamazepine	$C_{15}H_{12}N_2O$	236.094407
Methaqualone	$C_{16}H_{14}N_2O$	250.110063
Desipramine	$C_{18}H_{22}N_2$	266.177736
EDDP	$C_{20}H_{23}N$	277.182496
Cocaine	$C_{17}H_{21}NO_4$	303.146516
Methadone	$C_{21}H_{27}NO$	309.208711

Table 1. Compounds in Capillary Drug Mix #1. The standard was diluted in methanol to a concentration of 10 ng mL^{-1} and analyzed using the conditions in Table 2.

EXPERIMENTAL

The labeled contents of the drug standard mixture (DEA-Exempt Capillary Drug Mix #1, 100 ng mL^{-1} in methanol, Alltech PN 01464) are shown in Table 1. Although this standard was kept in the laboratory freezer, it was purchased in 2009 and was frequently used for experiments. The sample is well past its expiration date, so many of the compounds have partially decomposed or reacted, resulting in the loss of potency and the formation of impurities.

The mass spectrometer was auto-tuned and mass-calibrated with perfluorotributylamine (PFTBA) one week prior to the measurements. The sample was measured in EI mode and in PI mode with the combination EI/PI ion source and in CI mode. A mixture of 5% ammonia in methane was used as the CI reagent gas. Although the mass spectrometer was retuned in CI mode with octamethylcyclotetrasiloxane, it was not necessary to repeat the mass calibration. Multiple drift correction was applied using m/z 281.05114 from octamethylcyclotetrasiloxane introduced by opening the reference inlet automatically for 0.25 min at 3.5 minutes and 12.5 minutes. Centroided mass spectra for the drug mixture were processed with chromatographic deconvolution by using the JEOL *msFineAnalysis* software.

An alkane retention index standard (Qualitative Retention Time Index Standard, Restek Catalog # 31080) was measured using a 1/100 split injection and the GC oven program given in Table 2. The alkane retention index standard was measured in EI mode only.

RESULTS

JEOL *msFineAnalysis* software was used to create a calibration using the data for the retention time index standard (Figure 1). Clicking on each peak in the total ion current chromatogram displayed a mass spectrum and database search for that peak, making it easy to assign the alkane standards despite the presence of heavy silicone interferences that had been introduced into the vial by previous repeated injections through the silicone seal. The retention index information for the standard was saved to a named file and used to calculate the retention indices for the components in the chromatograms for the drug mixture.

GC	
Autosampler	Agilent 7693A
Column	DB5-MS, 30 m, 0.25 mm ID, 0.25 m film
Flow rate	1 ml/min constant flow
Injection	1.5 μL, splitless, 280°C
Initial Temperature	100˚C (1 min.)
Rate:	35˚C / min
Final temperature	280˚C
Hold time	5 min.
Total time	13.1 min.
MS	
Resolving power	>30,000 (at *m/z* 614)
Ionization	EI (70 eV), PI (deuterium lamp), and CI (5% ammonia in methane)
m/z range acquired	50-500
Spectral acquisition rate	10 Hz
External mass calibration	PFTPA
Drift correction standard	Octamethylcyclotetrasiloxane
Reference valve opened at	3.5 and 12.5 minutes for 0.25 min each

Table 2. Measurement Conditions

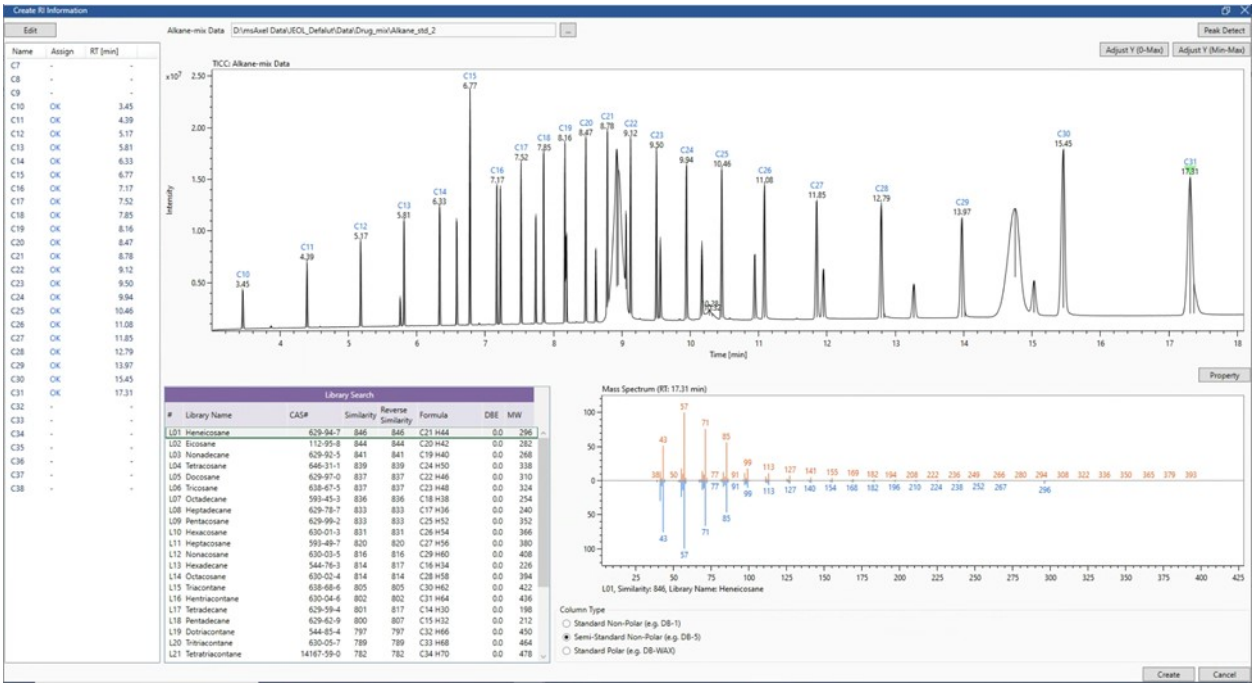

Figure 1. Creating a retention index calibration with the alkane standard. Clicking on any peak instantly displays the mass spectrum and database search result. This makes it easy to distinguish alkane peaks from silicone interferences resulting from repeated injections through the silicone vial cap.

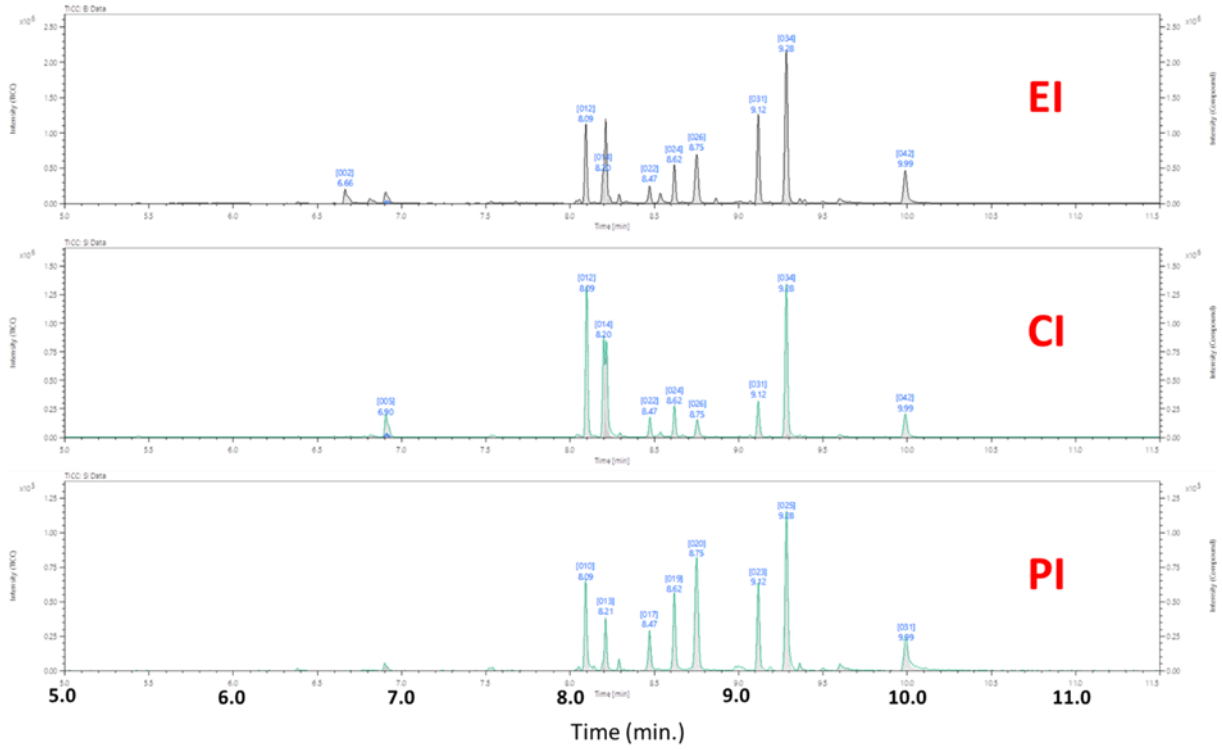

Figure 2. Total ion current chromatograms for analysis of the drug mixture obtained by electron ionization (EI), chemical ionization (CI) and photoionization (PI). Retention times are automatically aligned by *msFineAnalysis*, but compound-dependent response factors can differ for the different ionization modes.

Data analysis was carried out automatically by specifying the names of the hard ionization (EI) and soft ionization (PI or CI) data files. The total ion current chromatograms for each ionization method are shown in Figure 2. The results of the EI/CI analysis are shown in Figure 3 with the chromatograms and chromatographic deconvolution results for the EI data (top) and CI data (bottom) and a tabular summary of the information for each compound. Compound assignments with a high degree of confidence are highlighted in blue in the summary table. Compounds that do not have matching entries in the selected databases are highlighted in white, and compounds with multiple isomers are highlighted in yellow.

The table summarizes results for molecular ion elemental composition determination from exact mass and isotope data from both EI and CI data, database searching, fragment ion coverage, and retention index matching from the EI data. Clicking on the entry for any peak brings up additional windows to view more details of the analysis of that compound. The analyst can review each assignment and approve or interactively interpret the target compound assignments while inspecting the detailed analysis. Decisions about the assignment for isomers with indistinguishable EI mass spectra can be made by examining the retention index data. The analysis repeated by using the EI and PI data gave similar results (not shown).

Chromatographic deconvolution with *msFineAnalysis* is highly effective in separating chromatographically unresolved components as shown in Figure 4. The deconvolved mass spectra show excellent database matches with no cross-contamination from the overlapping peaks. The database search results are shown in Figures 5 and 6 for glutethimide (similarity = 910) and lidocaine (similarity = 895), respectively.

Despite long-term storage, all of the labeled components (Table 1) could still be detected. Many of the impurities related to specific components in the mixture were also detected. For example, methyl ecgonidine and methyl ecgonine are impurities that can be present in cocaine. Iminodibenzyl and iminostilbene are impurities that may be present in carbamazepine. Desimpramine and imipramine only differ by the presence of an N-methyl group. Methyl palmitate and methyl stearate have many sources, and could have been introduced from a variety of sources over the 12-year period in which the sample was stored. Table 3 summarizes the results for 19 compounds that are identified with high certainty and 4 low-level compounds with fair-to-poor database similarities, but with good agreement for elemental compositions and retention indices for the database matches.

Group analysis can also be carried out with *msFineAnalysis* to identify compounds having similar structures that result in common fragment ions or common neutral losses. Figure 7 shows the identification of the tricyclic antidepressants (imipramine, desipramine and carbamazepine) and a characteristic impurity (iminostilbene).

Figure 3. Results of the EI/CI data analysis by *msFineAnalysis*

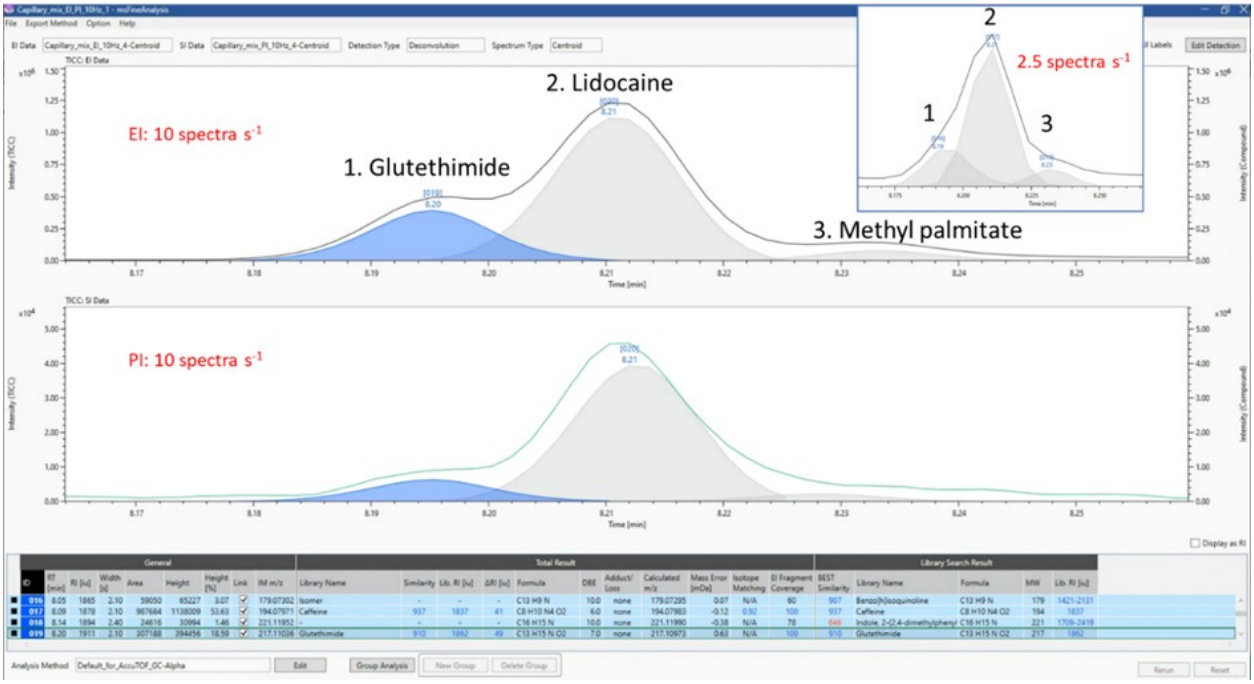

Figure 4. Chromatographic deconvolution of data acquired at 10 spectra s^{-1} showing separation of glutethimide (8.20 min), lidocaine (8.21 min) and methyl stearate impurity (8.23 min). The inset shows that deconvolution is still effective at separating these components even with a slower spectral acquisition rate (0.25 spectra s^{-1}) and poor GC separation.

Figure 5. Database search results for the chromatographically deconvolved mass spectrum of glutethimide.

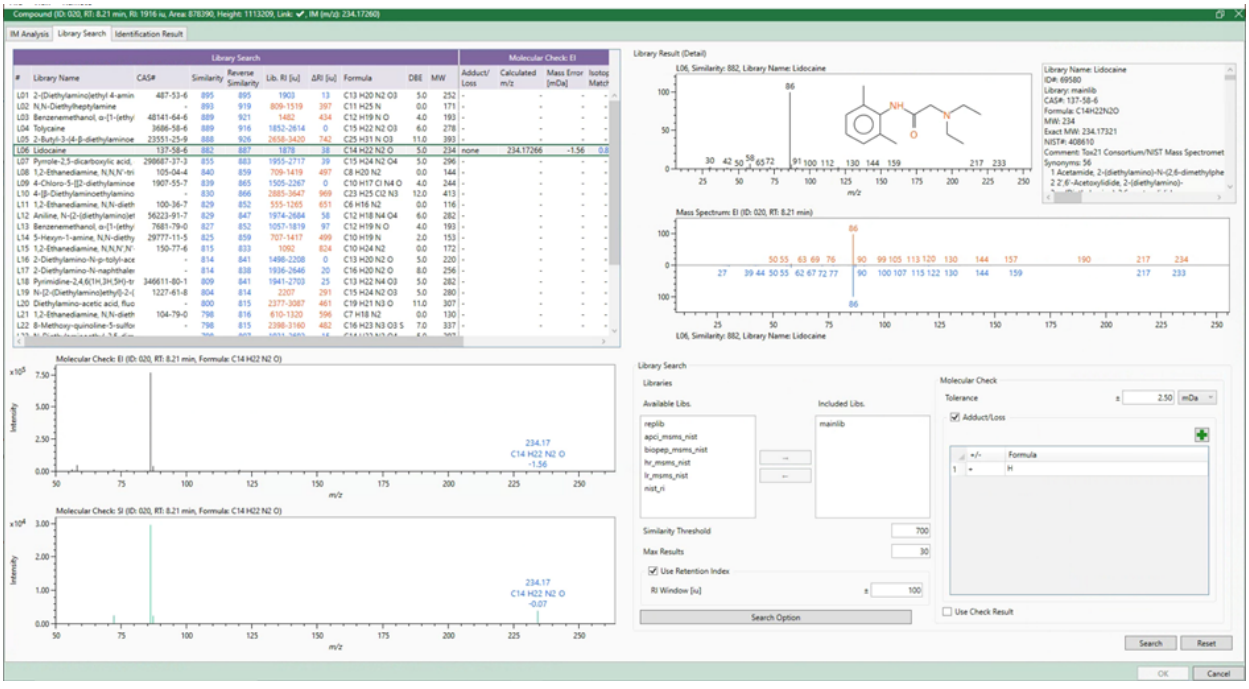

Figure 6. Database search results for the chromatographically deconvolved mass spectrum of lidocaine.

RT [min]	Area	Library Name	Similarity	Formula	Adduct/Loss	Mass Error [mDa]	Note
6.38	19274	Methyl ecgonidine	804	C10 H16 N O2	+H	0.15	Cocaine impurity
6.81	89326	Methyl ecgonine	647	C10 H17 N O3	+H	0.10	Cocaine impurity
6.82	42678	1,4-Benzenedicarboxylic acid, dimethyl ester	849	C10 H10 O4	none (EI)	-1.40	
6.90	193228	Benzoic acid, 4-ethoxy-, ethyl ester	907	C11 H15 O3	+H	0.13	
8.09	**1054298**	**Caffeine**	**928**	**C8 H11 N4 O2**	**+H**	**0.08**	**Stimulant**
8.14	22345	Indole, 2-(2,4-dimethylphenyl)-	639	C16 H15 N	+H	-0.02	
8.20	**385272**	**Glutethimide**	**930**	**C13 H16 N O2**	**+H**	**0.06**	**Barbiturate**
8.21	943020	**Lidocaine**	916	C14 H23 N2 O	+H	0.00	Local anesthetic
8.23	72565	Methyl palmitate	732	C17 H38 N O2	+NH4	-0.11	
8.29	122700	Benzenamine, N,N-diethyl-4-(1-phenylethenyl)-	467	C18 H21 N	+H	0.08	
8.45	22186	EMDP	758	C19 H22 N	+H	0.05	Opiate
8.47	245177	Iminodibenzyl	915	C14 H14 N	+H	0.09	Tricyclic
8.53	**199582**	**Phenobarbital**	**826**	**C12 H13 N2 O3**	**+H**	**0.06**	**Barbiturate**
8.62	527459	Iminostilbene	920	C14 H12 N	+H	0.05	Carbamazepine
8.75	**942815**	**EDDP**	**929**	**C20 H24 N**	**+H**	**-0.17**	**Opiate**
8.86	68646	Methyl stearate	812	C19 H39 O2	+H	-0.30	
9.12	**1232363**	**Methadone**	**945**	**C21 H28 N O**	**+H**	**-0.34**	**Opiate**
9.28	**2305038**	**Methaqualone**	**957**	**C16 H15 N2 O**	**+H**	**-0.10**	**Hypnotic**
9.39	**66183**	**Cocaine**	**797**	**C17 H22 N O4**	**+H**	**-0.36**	**Cocaine**
9.50	49806	Imipramine	794	C19 H24 N2	none (PI)	0.18	Tricyclic
9.60	**139382**	**Desipramine**	**781**	**C18 H23 N2**	**+H**	**-0.31**	**Tricyclic**
9.87	15276	Octadecanoic acid, butyl ester	511	C22 H44 O2	+H	-0.60	
9.99	**786912**	**Carbamazepine**	**901**	**C15 H13 N2 O**	**+H**	**0.01**	**Tricyclic**

Table 3. A summary extracted from the *msFineAnalysis* reports. The 10 drugs in Table 1 are highlighted in bold. The root-mean-square mass accuracy for all 23 compounds is 0.36 mDa.

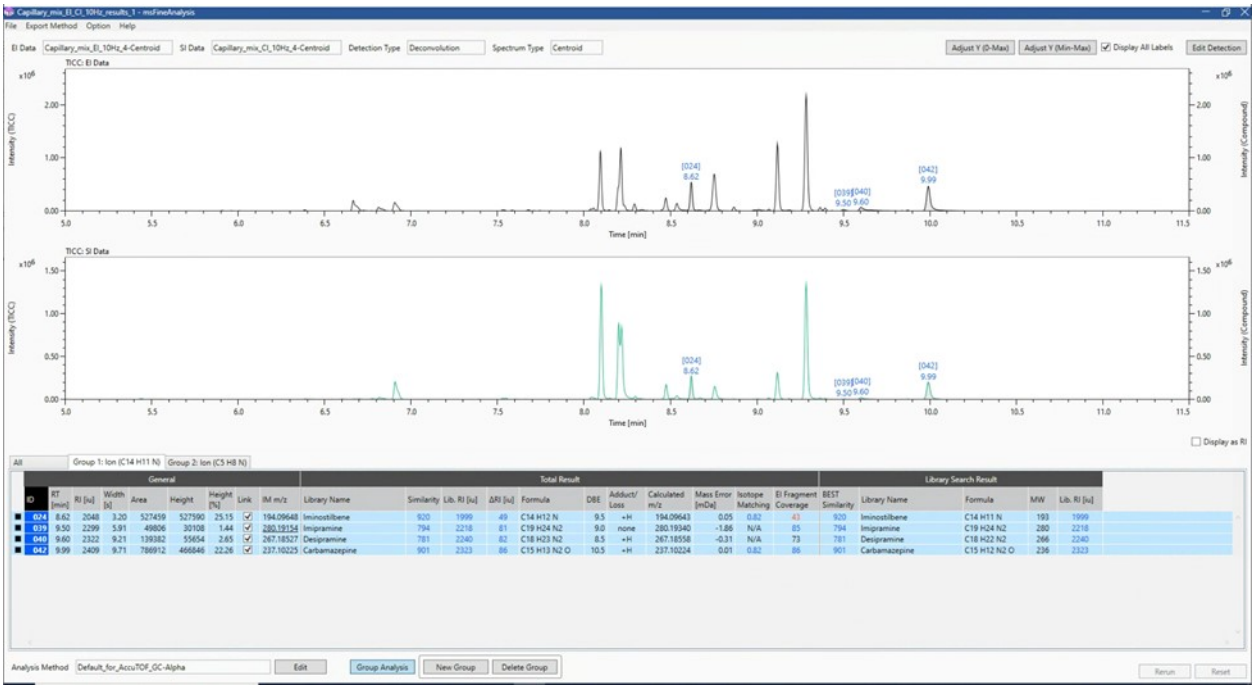

Figure 7. Group analysis for the common fragment ion $C_4H_{11}N^+$ identifies related tricyclic compounds.

CONCLUSION

The AccuTOF-GC Alpha provided high-resolution accurate-mass data for all detected compounds in both EI, CI, and PI modes. The root-mean-square mass accuracy for the identified components was 0.00036 u (0.36 mDa) for 24 components that exhibited molecular ions in the EI, CI, or PI mass spectra. Two low-level contaminants did not show clear peaks for the protonated molecules in the CI mass spectra, but molecular ions were observed in their EI or PI mass spectra (see Table 3).

Although some low-level components did not have entries in the mass spectral database, all ten of the listed drugs were detected, together with several impurities or degradation products. Assignments were confirmed by *msFineAnalysis software* using a combination of exact-mass and accurate-isotope data from both soft and hard ionization, database searching, fragment ion coverage and retention index matching.

JMS-T2000GC AccuTOF™ GC-Alpha GC-MS System