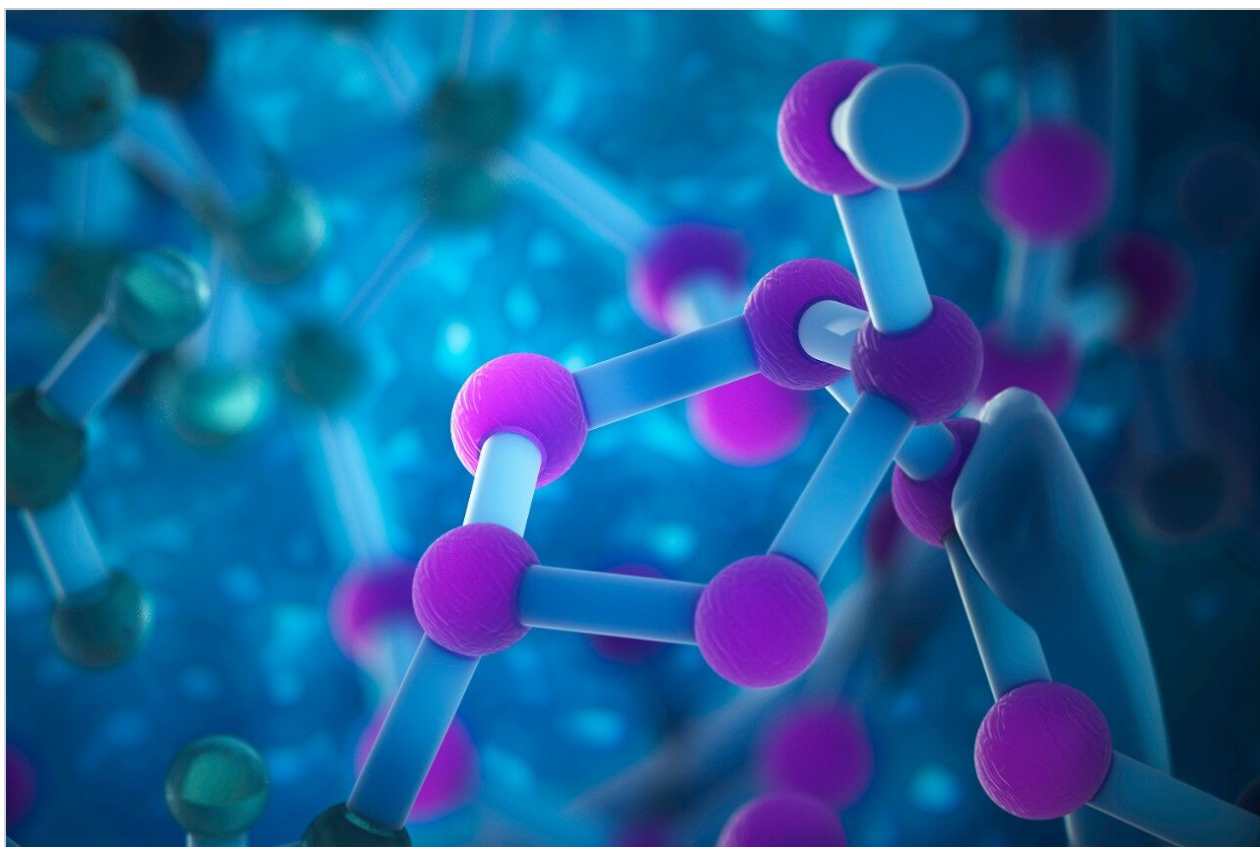


Exact Mass GC-MS Analysis of Amine Monomers Used in Combinatorial Library Production

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Abstract

In this application note, monomer confirmation using the GCT orthogonal Time-of-Flight (TOF) MS detector combined with OpenLynx high throughput software is described. The GCT produces exact mass data for elemental composition calculation and structural elucidation. OpenLynx provides a true 'walk up and use' interface with flexible data browsing and reporting.

Benefits

The combination of GCT and OpenLynx has been shown to be ideal for the high throughput screening of monomers for combinatorial library production.

Introduction

Monomers are the low molecular weight building blocks used in the construction of combinatorial libraries. Confirmation of the identity and purity of these compounds, prior to using them in synthesis, is important in order to ensure that a synthetic route to an identified 'active' can be recreated. In addition, the requirement for post-purification of the final product is significantly reduced if not eliminated.^{1,2,3} In many cases, however, the production of combinatorial libraries has sometimes proceeded with little regard to the purity of starting material. This has more to do with the complexity of analyzing large numbers of compounds than with a lack of desire from the synthetic chemist. While much time has been spent in developing LC-MS techniques and software to analyze the products of a combinatorial synthesis, little has been done to speed the process of analyzing small molecules not easily seen by LC-MS. GC-MS analysis in both electron impact (EI) and chemical ionization (CI) modes has long been used for the analysis of small, volatile compounds.

In this note, monomer confirmation using the GCT orthogonal Time-of-Flight (TOF) MS detector combined with OpenLynx high throughput software is described. The GCT produces exact mass data for elemental composition calculation and structural elucidation.

OpenLynx provides a true 'walk up and use' interface with flexible data browsing and reporting.

Ninety-five (95) primary amines from the Monomers Store facility at GlaxoSmithKline RTP were analyzed by exact mass measurement using the GCT acquiring both EI and CI mass spectra.



Experimental

Experiments were performed using a Micromass GCT oa-TOF mass spectrometer operated in positive ion electron impact (EI+) and positive ion chemical ionisation (CI+) modes with a one second acquisition over a mass range of 35-500Da. The source temperature was set to 180 °C in EI+ and 100 °C in CI+. Ammonia reagent gas at a pressure of 2×10^{-4} mbar was used for the CI analysis.

GC analyses were performed using a HP6890 gas chromatograph split/splitless injector. A J & W Scientific DB5-MS, 15 m x 0.53 mm ID column with a 1 m x 0.1 mm ID fused silica restrictor at the GC transfer line was used in constant flow mode with 1 mL/min Helium flow. The GC temperature program was 150 °C (2 mins) to 250 °C (4 mins) at 50 °C /min. This gave a run time of 8 minutes and injection-to-injection time of 11 minutes.

Results and Discussion

The EI and CI mass spectra of the 95 amines revealed only eight that did not contain the desired compound (vial 74 was broken). The amount of the desired compound present determined from the CI Total Ion

Chromatogram (TIC), ranged from less than a few percent to 100%. This calculation was made using OpenLynx, comparing the peak area of the compound of interest to the total area under the chromatogram. The results shown in Figure 1 were extracted from the OpenLynx Browser file shown in Figure 2.

In addition to this purity estimate, the identity of the impurities can also be determined from the data as shown in the following examples.

Name	Well, Expected Formula	Target	Found	Estimated %
BUTYLAMINE	1, C4H11N	74.0970	YES	70
SEC-BUTYLAMINE	2, C4H11N	74.0970	NO	0
ISOBUTYLAMINE	3, C4H11N	74.0970	NO	0
2-METHOXYETHYLAMINE	4, C3H9NO	76.0762	YES	4
CYCLOPENTYLAMINE	5, C5H11N	86.0970	YES	63
N-AMYLAMINE	6, C5H13N	88.1126	YES	24
3-AMINOPENTANE	7, C5H13N	88.1126	YES	11
2-METHYLBUTYLAMINE	8, C5H13N	88.1126	NO	0
ISODMYLAMINE	9, C5H13N	88.1126	YES	100
NEOPENTYLAMINE	10, C5H13N	88.1126	NO	0
1,2-DIMETHYLPROPYLAMINE	11, C5H13N	88.1126	YES	99
N,N-DIMETHYLETHYLENEDIAMINE	12, C4H12N2	89.1079	YES	39
1,2-DIAMINO-2-METHYLPROPANE	13, C4H12N2	89.1079	YES	19
3-METHOXYPROPYLAMINE	14, C4H11NO	90.0919	YES	74
2-AMINO-1-METHOXYPROPANE	15, C4H11NO	90.0919	YES	15
FURFURYLAMINE	16, C5H7NO	98.0606	NO	0
2,2,2-TRIFLUOROETHYLAMINE	17, C2H4F3N	100.0374	NO	0
CYCLOHEXYLAMINE	18, C6H13N	100.1126	YES	94
3-AMINO-1-PROPANOL VINYL ETHER	19, C5H11NO	102.0919	YES	77
TETRAHYDROFURFURYLAMINE	20, C5H11NO	102.0919	YES	84
HEXYLAMINE	21, C6H15N	102.1283	YES	2
1,3-DIMETHYLBUTYLAMINE	22, C6H15N	102.1283	YES	69
2-AMINO-3,3-DIMETHYLBUTANE	23, C6H15N	102.1283	YES	55
N1,N1-DIMETHYL-1,2-PROPANEDIAMINE	24, C5H14N2	103.1235	YES	43
N,N-DIMETHYL-1,3-PROPANEDIAMINE	25, C5H14N2	103.1235	YES	73
2-AMINO-1-METHOXYBUTANE	26, C5H13NO	104.1075	YES	56
3-ETHOXYPROPYLAMINE	27, C5H13NO	104.1075	YES	74
2-AMINOETHYL ISOPROPYL ETHER	28, C5H13NO	104.1075	YES	90
AMINOACETALDEHYDE DIMETHYL ACETAL	29, C4H11NO2	106.0668	YES	70
3-(METHYLTHIO)PROPYLAMINE	30, C4H11NS	106.0690	NO	0
2-(ETHYLTHIO)ETHYLAMINE	31, C4H11NS	106.0690	YES	79
BENZYLAMINE	32, C7H9N	108.0813	YES	78
4-(AMINOMETHYL)PYRIDINE	33, C6H8N2	109.0766	YES	63
3-(AMINOMETHYL)PYRIDINE	34, C6H8N2	109.0766	YES	76
2-(AMINOMETHYL)PYRIDINE	35, C6H8N2	109.0766	YES	65
5-METHYL-2-FURANMETHANAMINE	36, C6H9NO	112.0762	YES	45
EXO-2-AMINONORBORNANE	37, C7H13N	112.1126	YES	76
2-THIOPHENEMETHYLAMINE	38, C5H7NS	114.0377	YES	58
CYCLOHEPTYLAMINE	39, C7H15N	114.1283	YES	92
3-METHYLCYCLOHEXYLAMINE	40, C7H15N	114.1283	YES	64
2-METHYLCYCLOHEXYLAMINE	41, C7H15N	114.1283	YES	81
4-METHYLCYCLOHEXYLAMINE	42, C7H15N	114.1283	YES	86
1-(2-AMINOETHYL)PYRROLIDINE	43, C6H14N2	115.1235	YES	56
3-AMINOHEPTANE	44, C7H17N	116.1439	YES	71
N,N-DIETHYLETHYLENEDIAMINE	45, C6H16N2	117.1392	YES	63
4-DIMETHYLAMINOBUTYLAMINE	46, C6H16N2	117.1392	YES	58
3-ISOPROPOXYPROPYLAMINE	47, C6H15NO	118.1232	YES	80
4-METHYLBENZYLAMINE	48, C8H11N	122.0970	YES	100

Name	Well, Expected Formula	Target	Found	Estimated %
3-METHYLBENZYLAMINE	49, C8H11N	122.0970	YES	77
2-METHYLBENZYLAMINE	50, C8H11N	122.0970	YES	70
DL-ALPHA-METHYLBENZYLAMINE	51, C8H11N	122.0970	YES	66
(R)-(+)-1-PHENYLETHYLAMINE	52, C8H11N	122.0970	YES	82
L-(-)-ALPHA-METHYLBENZYLAMINE	53, C8H11N	122.0970	YES	84
PHENETHYLAMINE	54, C8H11N	122.0970	YES	54
4-(2-AMINOETHYL)PYRIDINE	55, C7H10N2	123.0922	YES	50
3-(2-AMINOETHYL)PYRIDINE	56, C7H10N2	123.0922	YES	53
2-(2-AMINOETHYL)PYRIDINE	57, C7H10N2	123.0922	YES	66
4-FLUOROBENZYLAMINE	58, C7H8FN	126.0719	YES	31
3-FLUOROBENZYLAMINE	59, C7H8FN	126.0719	YES	27
2-FLUOROBENZYLAMINE	60, C7H8FN	126.0719	YES	15
N-(1-CYCLOHEXYL)IMIDAZOLE	61, C6H11N3	126.1031	YES	79
2-(1-CYCLOHEXYL)ETHYLAMINE	62, C8H15N	126.1283	YES	77
THIOPHENE-2-ETHYLAMINE	63, C6H9NS	128.0534	YES	59
CYCLOOCTYLAMINE	64, C8H17N	128.1439	YES	36
2,3-DIMETHYLCYCLOHEXYLAMINE	65, C8H17N	128.1439	YES	97
(R)-(+)-1-CYCLOHEXYLETHYLAMINE	66, C8H17N	128.1439	YES	95
(S)-(-)-1-CYCLOHEXYLETHYLAMINE	67, C8H17N	128.1439	YES	95
2-(2-AMINOETHYL)-1-METHYLPYRROLIDINE	68, C7H16N2	129.1392	YES	77
2-AMINOETHYL-1-ETHYLPYRROLIDINE	69, C7H16N2	129.1392	YES	54
1-PYRROLIDINEPROPANAMINE	70, C7H16N2	129.1392	YES	88
1-(2-AMINOETHYL)PIPERIDINE	71, C7H16N2	129.1392	YES	62
2-AMINOOCETANE	72, C8H19N	130.1596	YES	80
2-AMINO-6-METHYLHEPTANE	73, C8H19N	130.1596	YES	100
N-(2-AMINOETHYL)MORPHOLINE	75, C6H14N2O	131.1184	YES	88
N,N-DIMETHYLENEPENTANEDIAMINE	76, C7H18N2	131.1548	YES	100
3-DIETHYLAMINOPROPYLAMINE	77, C7H18N2	131.1548	YES	86
ETHYL 3-AMINOBTYRATE	78, C6H13NO2	132.1025	NO	0
3-BUTOXYPROPYLAMINE	79, C7H17NO	132.1388	YES	74
4-AMINOBTYRALDEHYDE DIMETHYL ACETAL	80, C6H15NO2	134.1181	YES	87
AMINOACETALDEHYDE DIETHYL ACETAL	81, C6H15NO2	134.1181	YES	74
1-AMINOINDANE	82, C9H11N	134.0970	YES	67
2-AMINOINDAN	83, C9H11N	134.0970	YES	93
2-(P-TOLYL)ETHYLAMINE	84, C9H13N	136.1126	YES	24
BETA-METHYLPHENETHYLAMINE	85, C9H13N	136.1126	YES	62
ALPHA-ETHYLBENZYLAMINE	86, C9H13N	136.1126	YES	65
3,4-DIMETHYLBENZYLAMINE	87, C9H13N	136.1126	YES	69
3-PHENYLPROPYLAMINE	88, C9H13N	136.1126	YES	26
3-METHOXYBENZYLAMINE	89, C8H11NO	138.0919	YES	69
4-METHOXYBENZYLAMINE	90, C8H11NO	138.0919	YES	46
2-METHOXYBENZYLAMINE	91, C8H11NO	138.0919	YES	70
2-PHENOXYETHYLAMINE	92, C8H11NO	138.0919	YES	18
4-FLUOROPHENETHYLAMINE	93, C8H10FN	140.0876	YES	96
3-FLUOROPHENETHYLAMINE	94, C8H10FN	140.0876	YES	58
2-FLUOROPHENETHYLAMINE	95, C8H10FN	140.0876	YES	43
4-FLUORO-ALPHA-METHYLBENZYLAMINE	96, C8H10FN	140.0876	YES	31

Figure 1. Results of monomer screening.

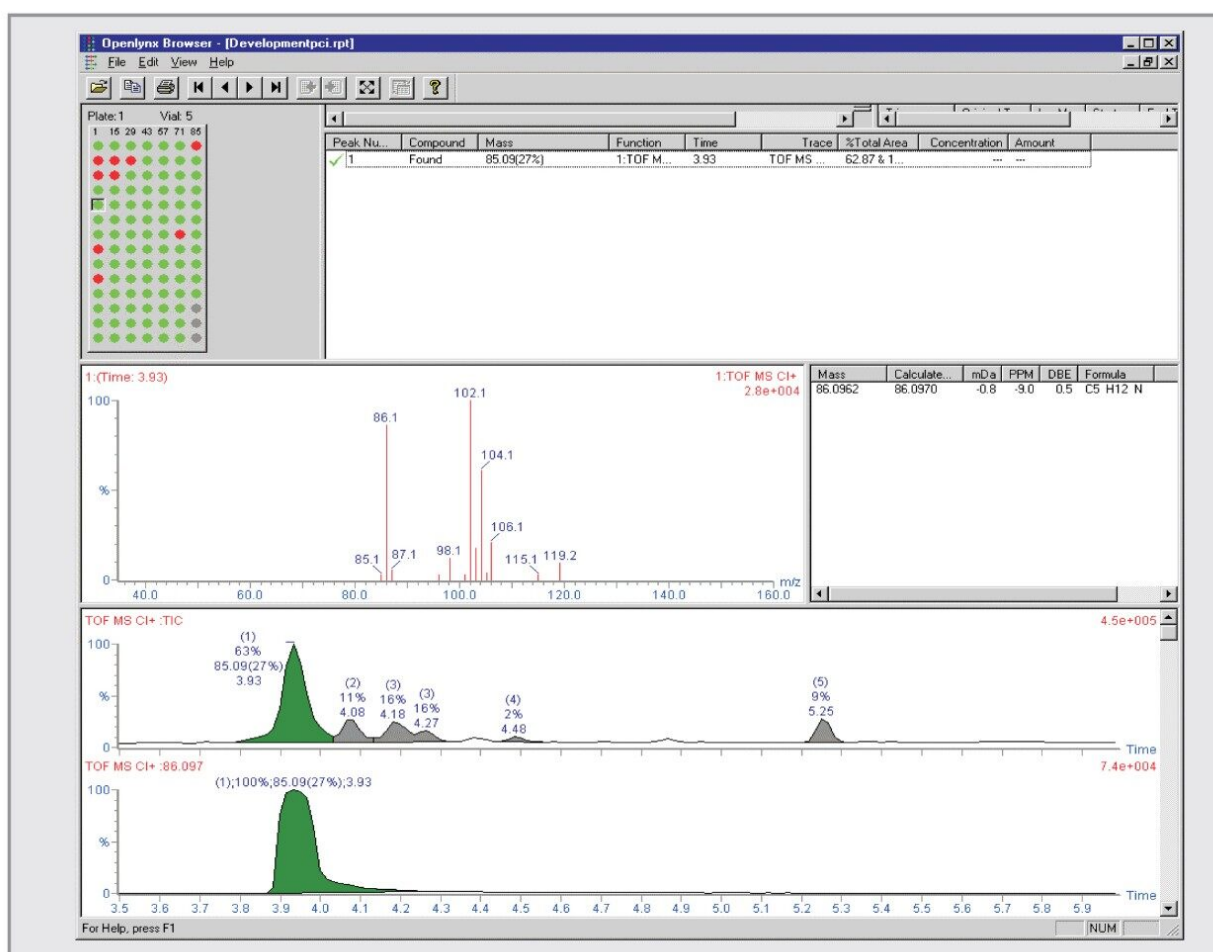


Figure 2. Example of OpenLynx browser results.

Example 1

Cyclopentylamine, m/z 86.097, is present at 63% (Figure 3). Several impurities are present with a nominal mass of 102, but with different elemental compositions. Two of these impurities were identified as saturated oxidized impurities of amines (Figure 4). Impurities at 104 and 106 were also identified as saturated oxidized impurities (Figure 4).

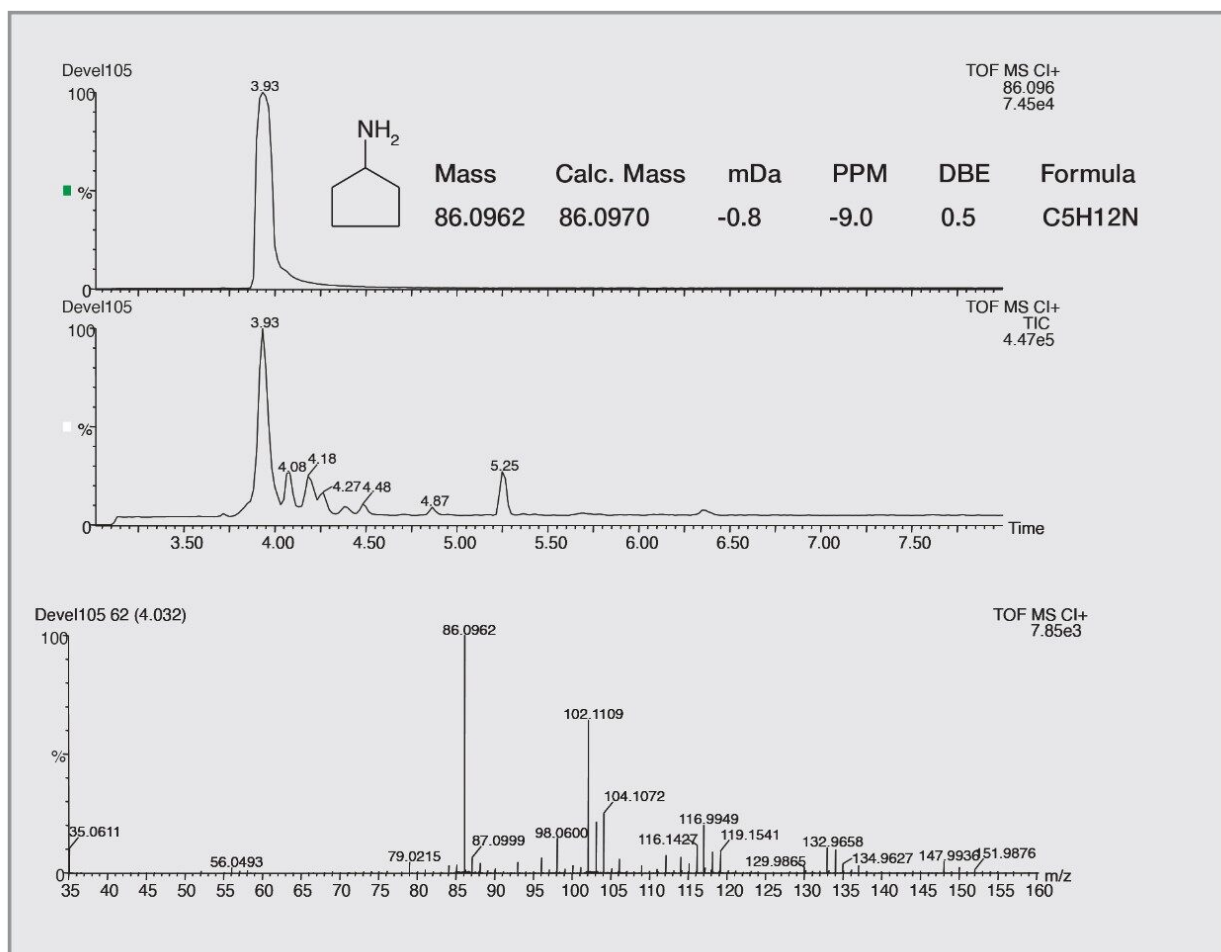


Figure 3. Cyclopentylamine.

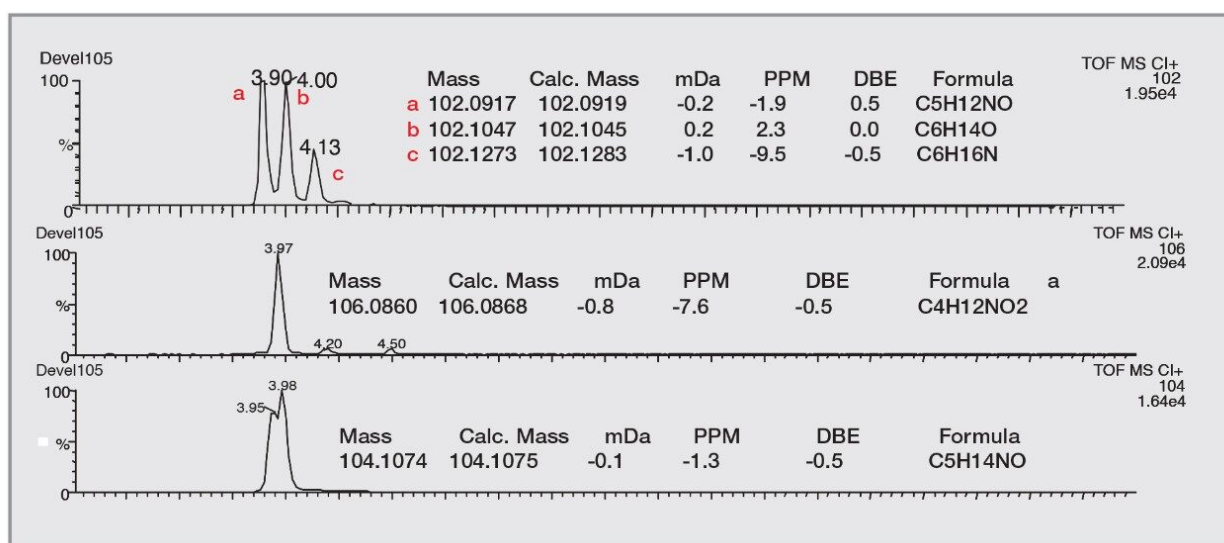


Figure 4. Impurities in cyclopentylamine.

Example 2

Three isomeric fluorobenzylamines were analyzed in vials (4-fluorobenzylamine), (3-fluorobenzylamine), (2-fluorobenzylamine), and showed vastly different impurity profiles. The accurate mass data confirmed that the indicated GC peaks at 4.42 minutes retention time (Figure 5) were due to the desired compounds. The amount of desired compound was 31%, 27%, and 15% respectively. A combination of CI and EI spectra and accurate mass data were used to determine the structure of these impurities. The compounds were from three different vendors and contained one common impurity at approximately 7.5 minutes retention time shown in Figure 5. This compound was determined to be an isomer of the difluorobenzylimine shown in Figure 6. The electron impact spectrum showed only the molecular ion at m/z 231 and a fragment at m/z 109 due to the fluorobenzyl ion. Since these three compounds are from three different vendors, this impurity is probably due to degradation of the amine.

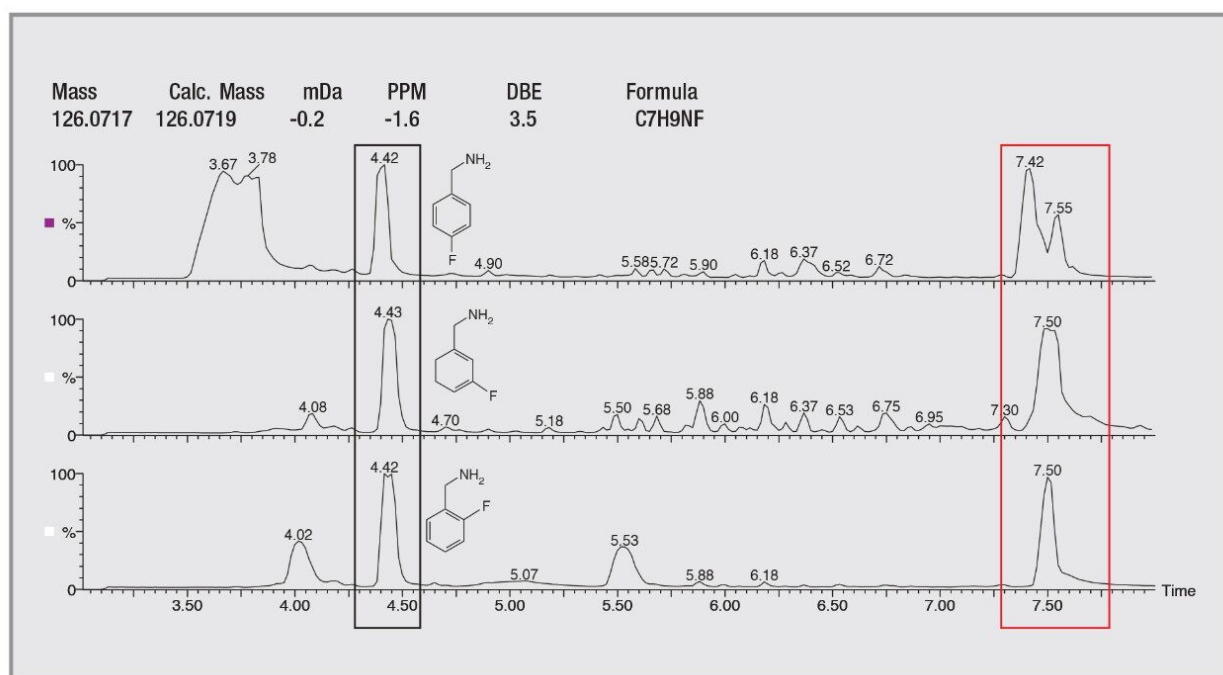


Figure 5. 4-, 3- and 2-fluorobenzylamine and common impurity.

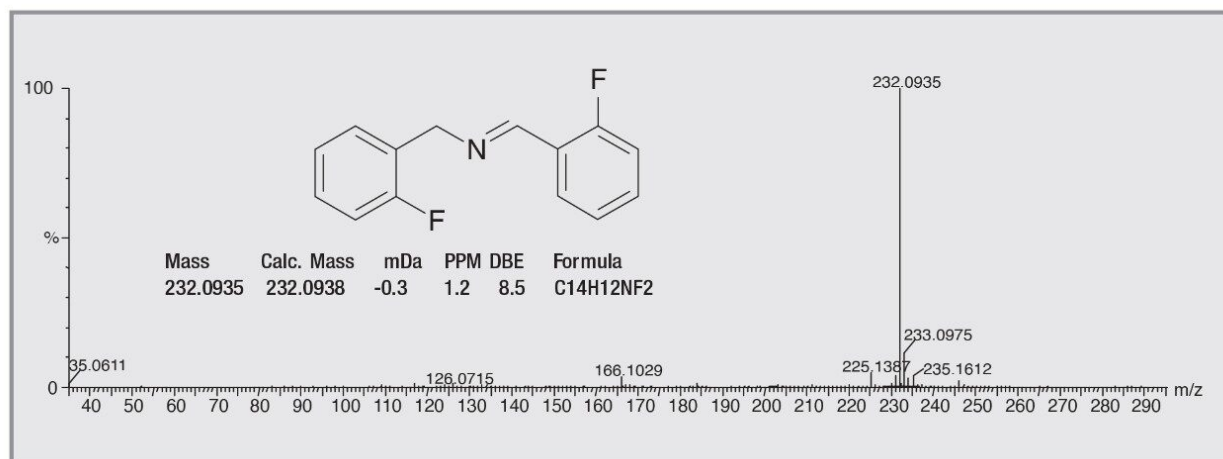


Figure 6. Identification of common impurity.

The major impurity in 4-fluorobenzylamine was determined to be an amide as shown in Figure 7 (CI spectrum) and Figure 8 (EI spectrum). The two impurities present in 2-fluoro-benzylamine (Figure 9) are not related to the desired compound - Figure 10 shows the CI spectra and the elemental composition results. From the exact mass measured fragments in the EI spectra, the structures were determined to be as shown

in Figure 11.

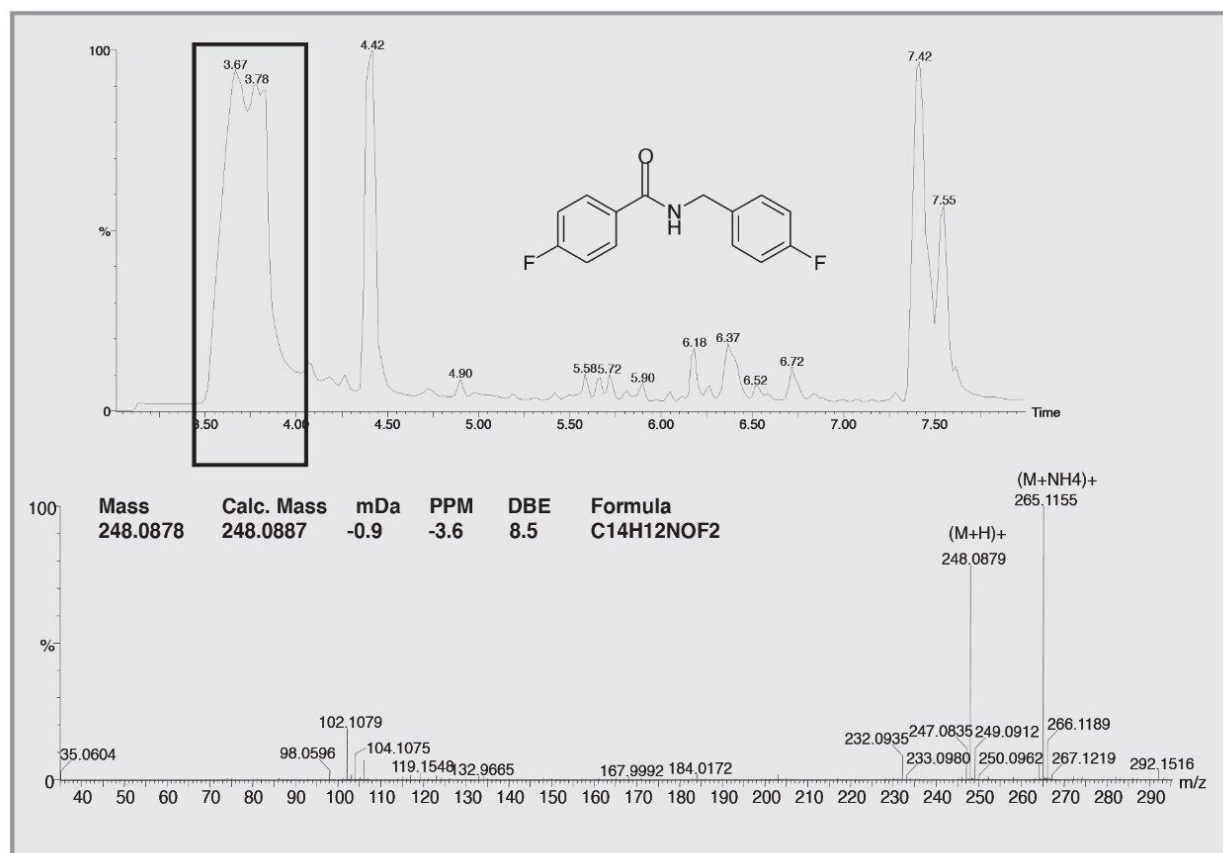


Figure 7. 4-, 3-, and 2-fluorobenzylamine and common impurity.

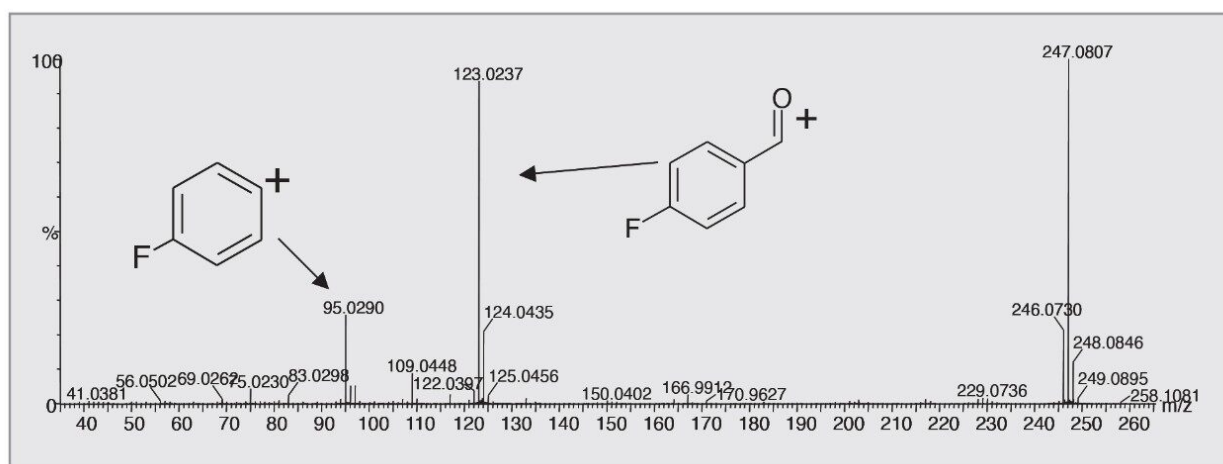


Figure 8. Major impurity in 4-fluorobenzylamine (EI+ spectrum).

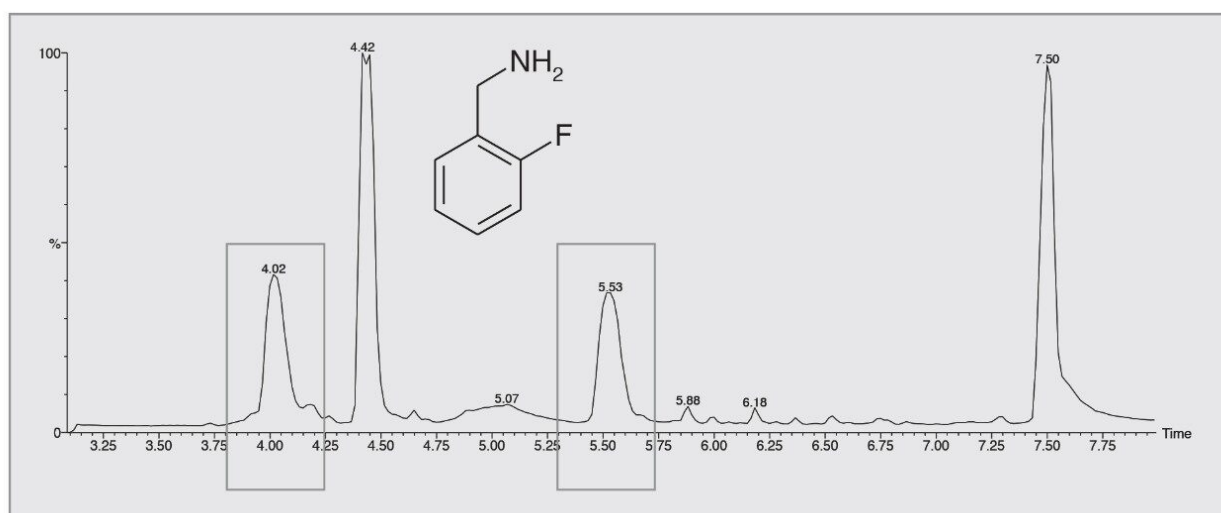


Figure 9. Impurities in 2-fluorobenzylamine.

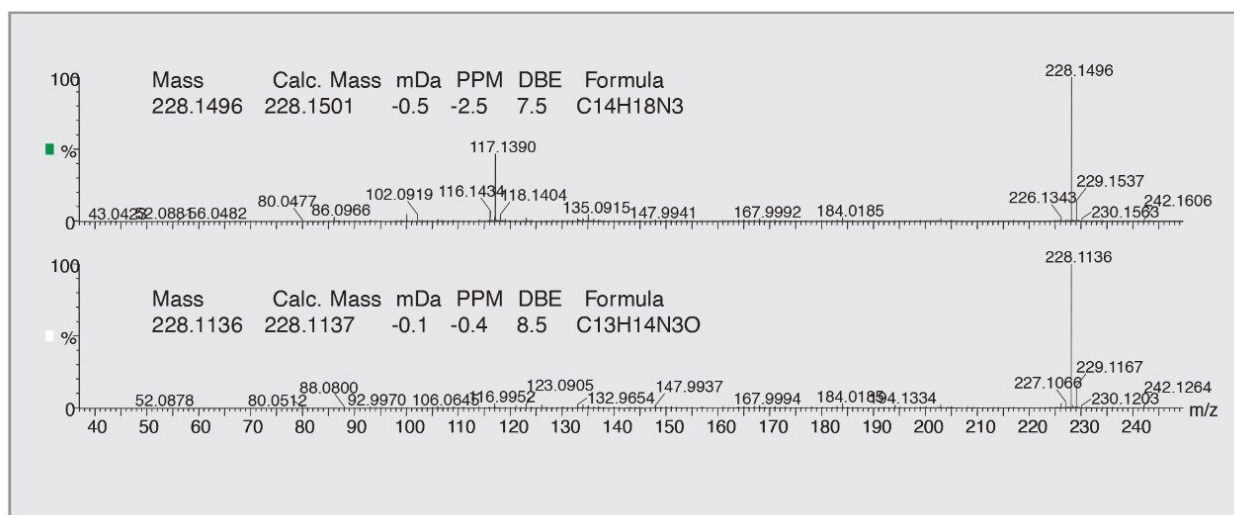


Figure 10. Impurities in 2-fluorobenzylamine (Cl⁺ spectra).

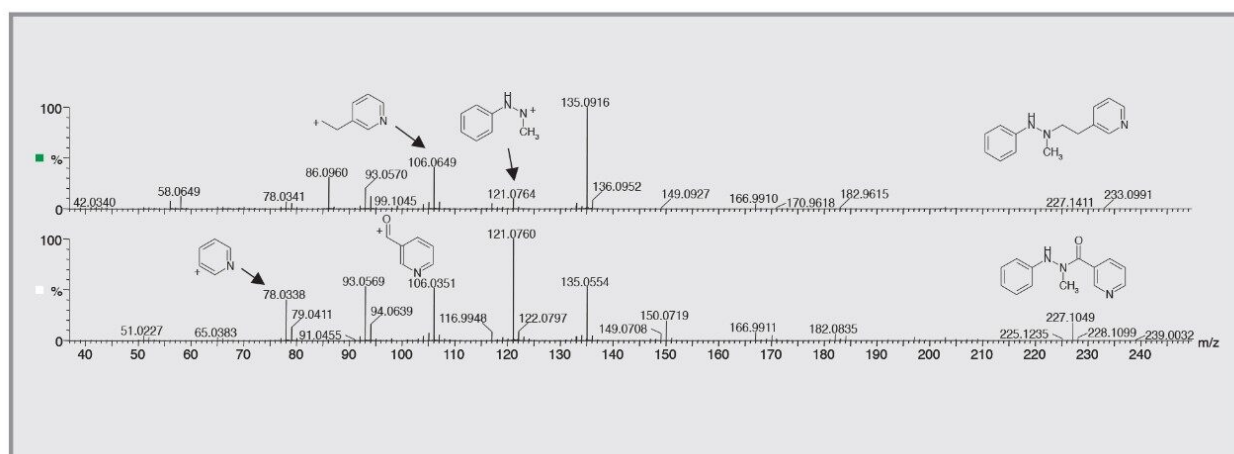


Figure 11. Structures of impurities in 2-fluorobenzylamine (from EI⁺ spectra).

Conclusion

Of the 95 compounds analyzed in this experiment, only eight were found not to contain the desired compound. The accuracy of masses measured for the compounds and impurities presented was calculated. (Figure 12). These masses were measured using a single lock mass in both EI and ammonia CI. The exact

mass data can also be used to calculate elemental composition, thus enabling the identification of impurities in the starting material. The use of purer starting materials can reduce the time necessary for purification and quantitation of final library members. Time can also be saved if it can be demonstrated that the impurities are not likely to lead to impurities in the final product. In the case of the benzylamines, for example, the amine present in all three samples would not present a problem if the primary amine was linked to a solid phase resin. The impurities would not react and could be rinsed from the resin after the coupling reaction had been performed. This type of analysis is easily automated using OpenLynx, a sophisticated batch-processing engine allowing chemists easy access to GCT. This streamlines the analysis of large batches of samples for screening and automatically verifies that a compound of the desired formula is present at each well location and that the purity of each targeted compound exceeds a user-defined threshold.

The combination of GCT and OpenLynx has been shown to be ideal for the high throughput screening of monomers for combinatorial library production.

Compound	Mass	Calc Mass	mDa	PPM	DBE	Formula
Cyclopentylamine	86.0962	86.0970	-0.8	-9.0	0.5	C5H12N
Impurity 1	102.0917	102.0919	-0.2	-1.9	0.5	C5H12NO
Impurity 2	102.1047	102.1045	0.2	2.3	0.0	C6H14O
Impurity 3	102.1273	102.1283	-1.0	-9.5	-0.5	C6H16N
Impurity 4	106.0860	106.0868	-0.8	-7.6	-0.5	C4H12NO2
Impurity 5	104.1074	104.1075	-0.1	-1.3	-0.5	C5H14NO
2-,3-and 4-Fluorobenzylamine	126.0717	126.0719	-0.2	-1.6	3.5	C7H9NF
Common Impurity	232.0935	232.0938	-0.3	-1.2	8.5	C14H12NF2
4- Impurity	248.0878	248.0887	-0.9	-3.6	8.5	C14H12NOF2
2- Impurity 1	228.1496	228.1501	-0.5	-2.5	7.5	C14H18N3
2- Impurity 2	228.1136	228.1137	-0.1	-0.4	8.5	C13H14N3O
Mass Measurement Accuracy RMS			0.57	4.89		

Figure 12. GCT mass accuracy.

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