

SpiralTOF™

MALDI for Small Molecule Analysis: A Complex Drug Mixture

Introduction: Matrix-Assisted Laser Desorption Ionization (MALDI) applications to small molecule analysis are often considered to be limited by low-mass matrix ions that can be isobaric with the target analytes. However, with sufficient resolving power, matrix ions and analyte ions can be separated. Additionally, these resolved matrix ions can serve as internal mass reference standards for exact mass measurements.

The SpiralTOF's unique multi-turn ion optics package

a very long (17-meter) flight path within a 1-meter space. Electric sectors and Matsuda plates provide perfect focusing to eliminate ion loss due to beam divergence. Post-source decay fragments occurring in the flight path are eliminated by the ion optics, providing a clean and artifact-free background.

This is shown here for the high-resolution analysis of a mixture of 32 small-molecule drugs analyzed by MALDI with the JEOL SpiralTOF mass spectrometer.

Table 1. Compounds analyzed.

Solution	Compound	Formula
Benzodiazepines Mix #1 (0.25 mg/ml)	Alprazolam	C ₁₇ H ₁₃ ClN ₄
	Clonazepam	C ₁₅ H ₁₀ ClN ₃ O ₃
	Diazepam	C ₁₆ H ₁₃ ClN ₂ O
	Flunitrazepam	C ₁₆ H ₁₂ FN ₃ O ₃
	Lorazepam	C ₁₅ H ₁₀ Cl ₂ N ₂ O ₂
	Nitrazepam	C ₁₅ H ₁₁ N ₃ O ₃
	Oxazepam	C ₁₅ H ₁₁ ClN ₂ O ₂
	Temazepam	C ₁₆ H ₁₃ ClN ₂ O ₂
Capillary Drug Mix #1 (0.1 mg/ml)	Caffeine	C ₈ H ₁₀ N ₄ O ₂
	Carbamazepine	C ₁₅ H ₁₂ N ₂ O
	Cocaine	C ₁₇ H ₂₁ NO ₄
	Despiramine	C ₁₈ H ₂₂ N ₂
	EDDP	C ₂₀ H ₂₄ N
	Glutethimide	C ₁₃ H ₁₅ NO ₂
	Lidocaine	C ₁₄ H ₂₂ N ₂ O
	Methadone	C ₂₁ H ₂₇ NO
	Methaqualone	C ₁₆ H ₁₄ N ₂ O
	Phenobarbital	C ₁₂ H ₁₂ N ₂ O ₃
Stimulants Mix (0.1 mg/ml)	<i>d</i> -Amphetamine	C ₉ H ₁₃ N
	<i>d</i> -Methamphetamine	C ₁₀ H ₁₅ N
	Caffeine	C ₈ H ₁₀ N ₄ O ₂
	Methylphenidate	C ₁₄ H ₁₉ NO ₂
	Cocaine	C ₁₇ H ₂₁ NO ₄
Opiates Mix #1 (0.1 mg/ml)	Codeine	C ₁₈ H ₂₁ NO ₃
	Ethyl morphine	C ₁₉ H ₂₃ NO ₃
	Meperidine	C ₁₅ H ₂₁ NO ₂
	Methadone	C ₂₁ H ₂₇ NO
	Morphine	C ₁₇ H ₁₉ NO ₃
Opiates Mix #2 (0.1 mg/ml)	Codeine	C ₁₈ H ₂₁ NO ₃
	Diacetylmorphine	C ₂₁ H ₂₃ NO ₅
	Hydromorphone	C ₁₇ H ₁₉ NO ₃
	Morphine	C ₁₇ H ₁₉ NO ₃
	Nalorphine	C ₁₉ H ₂₁ NO ₃
Oxycodone	C ₁₈ H ₂₁ NO ₄	

Experimental: Grace Quik-Chek™ DEA-exempt drug standards were used to create the drug mixture. One μl of each of the following drug mixtures was added to a vial: Benzodiazepines Mix #1, Opiates Mix #1, Opiates Mix #2, Stimulants Mix and Capillary Drug Mix. A list of compounds, formulas and concentrations is given in Table 1. 25 μl of a 10 mg/ml solution of the MALDI matrix α -cyano-4-hydroxycinnamic acid (CHCA, formula = $\text{C}_{10}\text{H}_7\text{NO}_3$) in 1:1 water/acetonitrile containing 0.1% trifluoroacetic acid was added to the vial, and a 0.5 μl aliquot was applied to the MALDI plate and allowed to dry.

The SpiralTOF was operated in positive-ion mode using the full 17-meter flight path, a MALDI delayed extraction time of 20 ns and a laser power attenuation of 31%. Matrix peaks were used as internal mass reference standards for calibration with the native MS Tornado software. Mass spectra were exported in plain text format for further processing with Mass Spec Tools II software (version 1.0.5.2). A Microsoft Excel spreadsheet was created with the names and elemental compositions of the target compounds to permit compound searching by the Mass Spec Tools II software based on exact mass measurements.

Results: All but two of the 32 compounds in the target mass list were identified as protonated or sodiated molecules with measured exact masses within 0.001 of the expected m/z (Figure 1, Table II). The two exceptions were phenobarbital and glutethimide. Phenobarbital is a weak acid, and it is possible that it does not protonate under these conditions. Glutethimide has a similar chemical structure to phenobarbital.

High resolving power is required to separate target compounds from interfering isobaric peaks. For example, the ^{35}Cl peak for protonated oxazepam occurs at m/z 287.0587 and the ^{37}Cl peak for protonated diazepam occurs at 287.0770. These peaks are well separated with a resolving power in excess of 40,000 (Figure 2).

Conclusion: The high resolving power available from the SpiralTOF's multi-turn 17-meter flight path permitted the detection of all but two compounds in the 32-component mixture without interference from matrix peaks or post-source decay. The remaining two compounds are apparently not ionized under these conditions. Two isomeric pairs of compounds (morphine/hydromorphone and codeine/hydrocodone) cannot be distinguished solely by high-resolution exact mass measurements. All of the compounds were detected with measured masses within 0.001 of the calculated values.

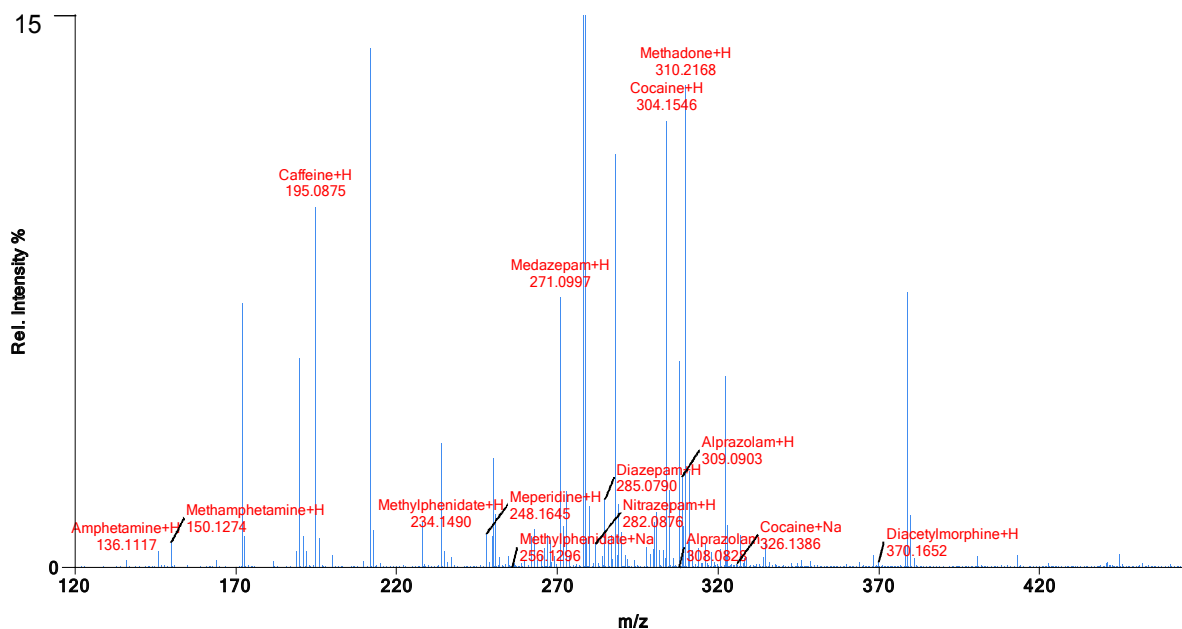


Figure 1. Enlarged view of the mass spectrum of the 32-drug mixture. The base peak is for EDDP at m/z 278.1906.

Table 2. Assignments for compounds detected in the drug mixture.

Name	Composition	Adduct	Measured	Calculated	mmu	Abund.
Alprazolam	C17H13ClN4	+H	309.09033	309.09071	0.38	2.631
Alprazolam	C17H13ClN4	+Na	331.07220	331.07265	0.45	0.089
Alprazolam	C17H13ClN4		308.08279	308.08289	0.09	0.012
Amphetamine	C9H13N	+H	136.11168	136.11262	0.94	0.228
Bromazepam	C14H10BrN3O	+H	316.00836	316.00856	0.20	0.403
Bromazepam	C14H10BrN3O		315.00269	315.00073	-1.95	0.009
Caffeine	C8H10N4O2	+H	195.08751	195.08819	0.68	10.431
Caffeine	C8H10N4O2		409.16174	409.16100	-0.74	0.012
Carbamazepine	C15H12N2O	+H	237.10271	237.10278	0.07	0.299
Carbamazepine	C15H12N2O	+Na	259.08472	259.08472	0.01	0.147
Carbamazepine	C15H12N2O		236.09561	236.09496	-0.66	0.023
Clonazepam	C15H10ClN3O3	+H	316.04871	316.04890	0.20	0.668
Cocaine	C17H21NO4	+H	304.15463	304.15489	0.26	12.930
Cocaine	C17H21NO4	+Na	326.13858	326.13683	-1.75	0.118
Codeine	C18H21NO3	+H	300.15982	300.15996	0.13	1.303
Codeine	C18H21NO3	+Na	322.14267	322.14190	-0.77	0.091
Despiramine	C18H22N2	+H	267.18558	267.18611	0.53	0.927
Diacetylmorphine	C21H23NO5	+H	370.16522	370.16545	0.23	0.160
Diacetylmorphine	C21H23NO5	+Na	392.14761	392.14739	-0.22	0.020
Diazepam	C16H13ClN2O	+H	285.07904	285.07948	0.44	1.975
Diazepam	C16H13ClN2O	+Na	307.06247	307.06142	-1.05	0.031
EDDP	C20H24N		278.19061	278.19089	0.27	100.000
Ethyl morphine	C19H23NO3	+H	314.17581	314.17561	-0.20	0.575
Ethyl morphine	C19H23NO3	+Na	336.15683	336.15755	0.72	0.048
Flunitrazepam	C16H12FN3O3	+H	314.09390	314.09410	0.20	0.572
Hydrocodone	C18H21NO3	+H	300.15982	300.15996	0.13	1.303
Hydrocodone	C18H21NO3	+Na	322.14267	322.14190	-0.77	0.091
Hydromorphone	C17H19NO3	+H	286.14413	286.14433	0.20	0.821
Hydromorphone	C17H19NO3	+Na	308.12628	308.12627	-0.01	0.127
Lidocaine	C14H22N2O	+H	235.18031	235.18104	0.73	0.469
Lidocaine	C14H22N2O	+Na	257.16263	257.16299	0.36	0.066
Lorazepam	C15H10Cl2N2O2	+H	321.01965	321.01976	0.10	0.546
Lorazepam	C15H10Cl2N2O2	+Na	343.00116	343.00170	0.54	0.144
Medazepam	C16H15ClN2	+H	271.09970	271.10020	0.50	7.822
Meperidine	C15H21NO2	+H	248.16452	248.16505	0.53	0.980
Meperidine	C15H21NO2	+Na	270.14542	270.14700	1.58	0.021
Methadone	C21H27NO	+H	310.21683	310.21708	0.26	13.963
Methamphetamine	C10H15N	+H	150.12743	150.12828	0.85	0.736
Methaqualone	C16H14N2O	+H	251.11768	251.11844	0.76	1.553
Methylphenidate	C14H19NO2	+H	234.14902	234.14941	0.39	0.863
Methylphenidate	C14H19NO2	+Na	256.12961	256.13136	1.75	0.008
Morphine	C17H19NO3	+H	286.14413	286.14433	0.20	0.821
Morphine	C17H19NO3	+Na	308.12628	308.12627	-0.01	0.127
Nalorphine	C19H21NO3	+H	312.15970	312.15996	0.26	0.521
Nalorphine	C19H21NO3	+Na	334.14178	334.14190	0.11	0.033
Nitrazepam	C15H11N3O3	+H	282.08759	282.08787	0.29	0.692
Nitrazepam	C15H11N3O3	+Na	304.06805	304.06982	1.76	0.259
Oxazepam	C15H11ClN2O2	+H	287.05865	287.05873	0.07	0.946
Oxazepam	C15H11ClN2O2	+Na	309.04092	309.04067	-0.25	0.147
Oxycodone	C18H21NO4	+H	316.15509	316.15489	-0.20	0.406
Oxycodone	C18H21NO4	+Na	338.13562	338.13683	1.21	0.069
Temazepam	C16H13ClN2O2	+H	301.07379	301.07438	0.59	1.591
Temazepam	C16H13ClN2O2	+Na	323.05618	323.05633	0.14	0.252

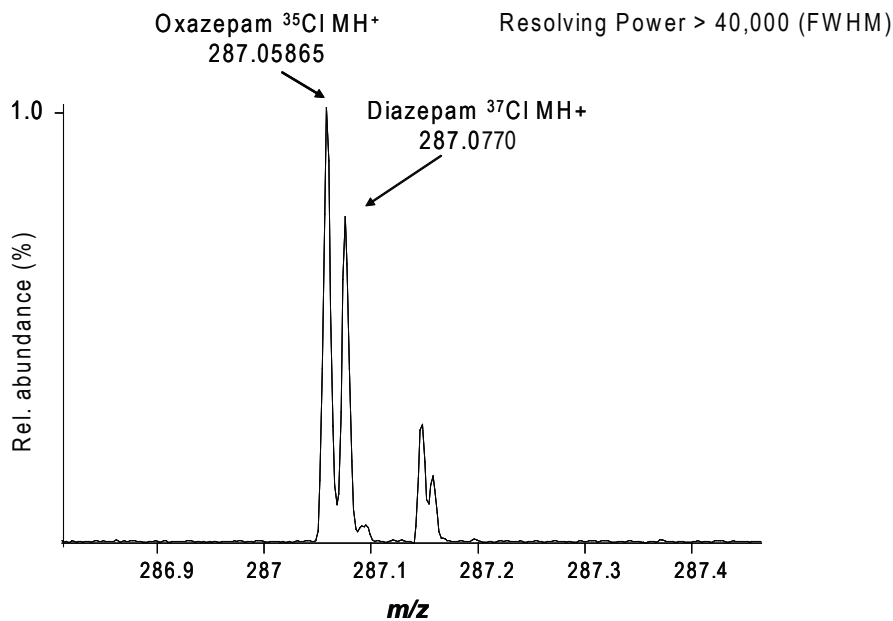


Figure 2. Separation of isotopic peaks for oxazepam and diazepam.