The Development Of A Natural Products Library Using Ion-Mobility Enabled Mass Spectrometry

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OVERVIEW

- Creation of a natural products library containing collision cross section values (CCS) and fragment ion information
- Data acquired on an ion mobility enabled accurate mass instrument and processed using UNIFI™
- automatically from Data extracted UNIFI™ Library Generation using a Application

INTRODUCTION

Libraries derived from mass spectral data are used across a wide range of application areas to target specific sets of compounds within a variety of extracts. Due to the variety of compounds for which screening is employed, it is often necessary to generate application specific library content by acquiring data on standards that are relevant to the area of interest, thereby reducing the number of false detections and shortening the review time for the analyst.

A workflow is presented for the construction of a natural products library incorporating an ion-mobility mass spectrometry collision cross section metric, precursor and mobility aligned product ions. The workflow includes automated extraction of library content from the measured data, improving the efficiency of library generation¹.

METHOD

- The SCREEN-WELL[®] Natural Product Library of 502 compounds was purchased from Enzo BioChem Inc.
- Stock solutions of each compound were prepared at a concentration of 20 μ g/mL in methanol.
- Working concentrations of 200 ng/mL and 20 ng/mL were prepared.
- Single standards were injected on column at 45°C (Waters ACQUITY UPLC BEH C18 (50 mm x 2.1 mm, 1.7 μm)), and a 2.5 min generic ((acetonitrile/water) 0.1% FA) gradient applied, using a Q-TOF IMS platform.
- HDMS^E data from triplicate injections were acquired in both ESI+ and ESI- modes.
- HDMS^E experiments enabled precursor and product ion data to be collected simultaneously.



Figure 1. The UNIFI™ analysis centre



Figure 4. The library creation workflow

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Two sets of data were generated, each containing measurements from 3012 individual experiments (ESI+ and ESI-). The data were processed in UNIFI[™] to generate a set of components each of which is uniquely defined by a combination of m/z, drift-time and retention time (Figure 1). In addition, *in-silico* fragmentation was performed permitting substructures to be assigned to high-energy ions. This yields a rich data set containing multiple adducts, their associated CCS values and product ions for each detected component.

RESULTS

The library content was extracted from the UNIFI[™] analysis using a Library Generation Application (Figure 2). The application averages both the CCS values for precursor ions and additional adducts observed, the measured retention times, over six samples acquired for each standard. Additionally, the application obtains the product ions from each sample automatically and orders them in descending intensity. An output file generated by the application contains metrics on the extracted data which permits review by exception (Figure 3). The library generation workflow is illustrated in Figure 4.

					🐮 # 🛞							
	Observed CCS (A	Å ²) Detect	or counts	Response	Total Fragments	Found I						
H	24	3.30	5671	0 29290		14						
				E; 🖬 🗷 🛛	🔳 🗟 🕶 🛟							
PLATE 11 200PG MAR Channel name: Low energy : Time 0.7317 +/- 0 $\%$ \times												
				:	629.28195 583.27436 631.2	7.14e5 28895						
	200	300	400	500	600	700						
PLA	TE 11 200PG M	AR Chanı	nel name: Hig	h energy : Tin	ne 0.7317 +/- 0.	* ×						
63.	06217 291.174 200	371.1859	95 419.2076: 419.2076: 400 mass [m/z]	3 	583.27721 584.27967 629. 600	4.23e5 28356 700						
		-1-										
m 2	expected addu	cts.										
	Observed m/z	Detector co	unts Observ	ved CCS (Å ²)	Observed drift (n	t (ms)						
	629.2820	4	40339	243.30		6.08						
	583.2744	1	16372	234.87		5.78						
	585.2744		105/2	234.87		5./ð						

Construct

theoretical

library

D Lib	rany Generator			×	Item Name	Formula	Structure	Adduct	ccs	CCS Max %Diff	Retention Time	T Min R	T Max	₹T Diff	Intensity	Number F	ragment 1 F	ragment 2 Fra
	and y ocherator				4-Aminosalicylic Acid	C7H7NO3	4-Aminosalicylic Acid.mol	-H	124.75	0.11	0.79	0.79	0.79	0	1550	6	108.0455	
Host:	mm6956.corp.waters.com:50034	BasePath: /unifi/v1 O	DataMetadata: none 🔻		4-Phenylbutanoic Acid	C10H12O2	4-Phenylbutanoic Acid.mol	-H	140.66	0.26	1.29	1.29	1.3	0	104	6		
					Avanafil	C23H26CIN7O3	Avanafil.mol	-H	221.03	0.34	1	1	1	0	2012	6	374.1025	
	Mixtures	Analysis Analysis	Analysis		Carprofen	C15H12CINO2	Carprofen.mol	-H	158.78	0.21	1.48	1.48	1.48	0	1249	6	228.0586	226.0429 19
	Extractable & Leachables	Small sample set FDA PLATE 11 POS	FDA PLATE 11 NEG		Closantel	C22H14Cl2I2N2O2	Closantel.mol	-H	226.01	0.07	1.68	1.68	1.68	0	6872	5	126.905	344.8279 31
	FDA Approved Drugs Library	FDA PLATE 11 POS	FDA PLATE 11 NEG		Dexlansoprazole	C16H14F3N3O2S	Dexlansoprazole.mol	-H	171.27	0.07	1.08	1.08	1.08	0	1040	3	164.005	
	FDA drugs library				Dicloxacillin	C19H17Cl2N3O5S	Dicloxacillin.mol	-H	200.59	0.28	1.42	1.42	1.42	0	2371	6	326.9767	329.9746
	Food additives				Difluprednate	C27H34F2O7	Difluprednate.mol	+HCOO	223.31	0.06	1.51	1.51	1.51	0	62361	6	399.1613	357.1508 48
	GC Pesticides				Difluprednate	C27H34F2O7	Difluprednate.mol	-H	214.48	0.37	1.51	1.51	1.51	0	1108	6	399.1613	357.1508 48
	Intact Protein				Droperidol	C22H22FN3O2	Droperidol.mol	-H	189.92	0.2	0.94	0.94	0.94	0	2281	6	185.072	133.0407 14
	Xevo G2-XS Q-Tof				Dydrogesterone	C21H28O2	Dydrogesterone.mol	-H	186.41	0.26	1.79	1.79	1.79	0	7515	6	149.0972	133.0659
	Zaragoza 💽				Eprosartan	C23H24N2O4S	Eprosartan.mol	-H	196.97	0.09	0.93	0.93	0.93	0.01	57013	6	379.1486	244.104 33
-	··· · ·				Erythromycin	C37H67NO13	Erythromycin.mol	+HCOO	265.02	0.06	1.03	1.02	1.03	0	2607	6	498.3072	249.1496 32
	lazine	Methyclothiazide	Difluorednate		Erythromycin	C37H67NO13	Erythromycin.mol	-H	269.97	0.13	1.03	1.02	1.03	0	237	5	498.3072	249.1496 32
CCS	: 166 36 (Mip: 165 92 Max: 166 77)	CCS: 168 31 (Min: 168 18 Max: 168 44)	CCS: 223 31 (Min: 223 20 Max: 223 44)	-	Erythromycin Ethylsuccinate	C43H75NO16	Erythromycin Ethylsuccinate.mol	+HCOO	290.8	0.08	1.19	1.19	1.19	0	5080	6	626.3546	684.3964 57
Rete	ention Time: 1.46	Retention Time: 1.12	Retention Time: 1.51	=	Erythromycin Ethylsuccinate	C43H75NO16	Erythromycin Ethylsuccinate.mol	-H	295.15	0.04	1.19	1.19	1.19	0	335	3	626.3546	684.3964 57
No.	of values: 6	No. of values: 6	No. of values: 6		Flumethasone	C22H28F2O5	Flumethasone.mol	+HCOO	192.59	0.21	1.23	1.22	1.23	0	39586	6	379.1726	325.1245 32
C14	H10N2O6	C9H11Cl2N3O4S2	C27H34F2O7		Halcinonide	C24H32CIFO5	Halcinonide.mol	+HCOO	208.79	0.27	1.55	1.55	1.55	0	37557	6	433.1787	397.202 39
					Halcinonide	C24H32CIFO5	Halcinonide.mol	-H	202.13	0.26	1.55	1.55	1.55	0	19248	6	433.1787	397.202 39
Eryth	hromycin	Erythromycin Ethylsuccinate	Homatropine_		Halobetasol Propionate	C25H31ClF2O5	Halobetasol Propionate.mol	+HCOO	215.31	0.09	1.55	1.55	1.55	0	29313	6	447.1989	427.1926 46
CCS	: 265.02 (Min: 264.86, Max: 265.18)	CCS: 290.80 (Min: 290.56, Max: 290.90)	CCS: 164.79 (Min: 164.53, Max: 165.03)		Halobetasol Propionate	C25H31ClF2O5	Halobetasol Propionate.mol	-H	207.23	0.28	1.55	1.55	1.55	0	2760	6	447.1989	427.1926 46
Rete	ention Time: 1.03	Retention Time: 1.19	Retention Time: 0.69		Homatropine_	C16H21NO3	Homatropinemol	-H	164.79	0.16	0.69	0.69	0.69	0	164	6		
No.	of values: 6	No. of values: 6	No. of values: 6		Ifenprodil	C21H27NO2	Ifenprodil.mol	-H	184.28	0.09	0.98	0.98	0.99	0	370	6	306.1863	133.0659
C37I	H67NO13	C43H75NO16	C16H21NO3		Levobupivacaine	C18H28N2O	Levobupivacaine.mol	-H	173.74	0.23	0.96	0.96	0.96	0	33	3		
					Mefenamic Acid	C15H15NO2	Mefenamic Acid.mol	-H	157.25	0.21	1.62	1.62	1.63	0.01	39033	6	196.1132	180.0819 19
4-Ph	nenylbutanoic Acid	Mefenamic Acid	Nafcillin		Methazolamide	C5H8N4O3S2	Methazolamide.mol	-H	145.98	0.17	0.84	0.84	0.84	0	15621	6	77.9655	57.9757
CCS	: 140.66 (Min: 140.46, Max: 141.03)	CCS: 157.25 (Min: 156.91, Max: 157.48)	CCS: 202.35 (Min: 201.73, Max: 202.69)		Methyclothiazide	C9H11Cl2N3O4S2	Methyclothiazide.mol	-H	168.31	0.08	1.12	1.12	1.13	0	29325	6	321.9728	246.9853 18
Kete	ention Time: 1.29	Retention Time: 1.62	Retention Time: 1.38	÷	Nafeillin	C21H22N2O5S	Nafcillin mol	-H	202.35	0.3	1 22	1 22	1 2 2	0	1729	6	272 0751	2/12 0//15 26

Figure 2. The Library Generation Application

Extract Review Acquire and library results process data content

THE SCIENCE OF WHAT'S POSSIBLE.

CONCLUSIONS

- A new library creation procedure has been generated, to automatically incorporate retention time CCS, precursor and product ions.
- Automatic extraction of library content is considerably more efficient and accurate than a manual library creation process.
- A natural products library has been produced with library entries for 399 compounds in positive ion and 299 compounds in negative ion. In combination the library has entries for 456 compounds.

Figure 3. Output from the Library Generation Application



REFERENCES

1. Interfacing Third Party Software Applications To Mass Spectrometry Data Systems: A Library Generation Example, J. Goshawk, M. McCullagh and R.J. Mortishire Smith, Poster Presentation, IMSC 2018, Florence, Italy.