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™
- Data extracted automatically from UNIFI™ using a Library Generation 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 generation[1].

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 µg/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.

RESULTS

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 sub-structures 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.

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.

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.

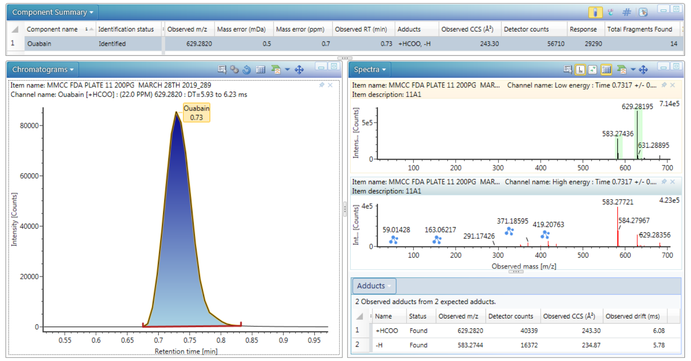

Figure 1. The UNIFI™ analysis centre

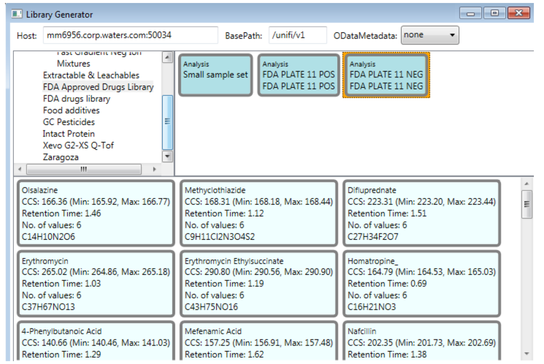

Figure 2. The Library Generation Application

Item Name	Formula	Structure	Adduct	CCS	CCS Max %Diff	Retention Time	RT Min	RT Max	RT Diff	Intensity	Number	Fragment 1	Fragment 2	Fra
4-Aminosalicylic Acid	C7H7NO3	4-Aminosalicylic Acid.mol	-H	124.75	0.11	0.79	0.79	0.79	0	1550	6	108.0455		
4-Phenylbutanoic Acid	C10H12O2	4-Phenylbutanoic Acid.mol	-H	140.66	0.26	1.29	1.29	1.3	0	104	6			
Avanafil	C23H26ClN7O3	Avanafil.mol	-H	221.03	0.34	1	1	1	0	2012	6	374.1025		
Carprofen	C15H12ClNO2	Carprofen.mol	-H	158.78	0.21	1.48	1.48	1.48	0	1249	6	228.0586	226.0429	19
Closantel	C22H14Cl2I2N2O2	Closantel.mol	-H	226.01	0.07	1.68	1.68	1.68	0	6872	5	126.905	344.8279	31
Dexlansoprazole	C16H14F3N3O2S	Dexlansoprazole.mol	-H	171.27	0.07	1.08	1.08	1.08	0	1040	3	164.005		
Dicloxacillin	C19H17Cl2N3O5S	Dicloxacillin.mol	-H	200.59	0.28	1.42	1.42	1.42	0	2371	6	326.9767	329.9746	
Difluprednate	C27H34F2O7	Diflueprednate.mol	+HCOO	223.31	0.06	1.51	1.51	1.51	0	62361	6	399.1613	357.1508	48
Diflupredna te	C27H34F2O7	Diflueprednate.mol	-H	214.48	0.37	1.51	1.51	1.51	0	1108	6	399.1613	357.1508	48
Droperidol	C22H22FN3O2	Droperidol.mol	-H	189.92	0.2	0.94	0.94	0.94	0	2281	6	185.072	133.0407	14
Dydrogesterone	C21H28O2	Dydrogesterone.mol	-H	186.41	0.26	1.79	1.79	1.79	0	7515	6	149.0972	133.0659	
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
Erythromycin	C37H67NO13	Erythromycin.mol	-H	269.97	0.13	1.03	1.02	1.03	0	237	5	498.3072	249.1496	32
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
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
Flumethasone	C22H28F2O5	Flumethasone.mol	+HCOO	192.59	0.21	1.23	1.22	1.23	0	39586	6	379.1726	325.1245	32
Halcinonide	C24H32ClFO5	Halcinonide.mol	+HCOO	208.79	0.27	1.55	1.55	1.55	0	37557	6	433.1787	397.202	39
Halcinonide	C24H32ClFO5	Halcinonide.mol	-H	202.13	0.26	1.55	1.55	1.55	0	19248	6	433.1787	397.202	39
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
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
Homatropine_	C16H21NO3	Homatropine_.mol	-H	164.79	0.16	0.69	0.69	0.69	0	164	6			
Ifenprodil	C21H27NO2	Ifenprodil.mol	-H	184.28	0.09	0.98	0.98	0.99	0	370	6	306.1863	133.0659	
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
Methazolamide	C5H8N4O3S2	Methazolamide.mol	-H	145.98	0.17	0.84	0.84	0.84	0	15621	6	77.9655	57.9757	
Methyclothiazide	C9H11ClF3N3O4S2	Methyclothiazide.mol	-H	168.31	0.08	1.12	1.12	1.13	0	29325	6	321.9728	246.9853	18
Nafcillin	C21H22N2O5S	Nafcillin.mol	-H	202.35	0.3	1.38	1.38	1.38	0	1729	6	272.0751	243.0445	26

Figure 3. Output from the Library Generation Application

Figure 4. The library creation workflow

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.

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