Simultaneous Metabolite Identification and Quantitation with UV Data Integration Using LightSight® Software v2.2

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Overview

Integration of UV and MS data is highly desirable for biotransformation studies. For example, in vitro metabolic stability studies can provide qualitative metabolism data and kinetics for the parent compound. Relative quantitation of metabolites detected can also be performed and integration of UV data is very useful for this workflow. Manual processing and correlation of both data streams is time consuming. Here, we show qualitative and quantitative data collected by acquiring both mass spectrometric and UV data in the same experiment, and then processing and correlating both data types automatically in LightSight® software v2.2.

Introduction

Drug metabolism studies often require estimation of relative abundances of metabolites detected in addition to structure elucidation. While MS based detection is a powerful technique for detection and structure elucidation, small changes in the structure and functional groups present in a metabolite can often lead to major differences in ionization efficiency and fragmentation. Therefore, it is often difficult to estimate metabolite concentrations based on the MS signal alone due to the absence of a reference standard for quantitation. By comparison, UV absorbance is considered to provide a more uniform response which allows for estimation of relative abundances of metabolites.

Integration of MS and analog data processing is highly desirable as it allows the drug metabolism scientist to perform structure elucidation based on the MS/MS data, estimate concentration based on the UV data, and generate one integrated report.

In this technical note, we describe the use of LightSight® software version 2.2 to study the in vitro metabolism of bromocriptine using QTRAP® LC/MS/MS system in combination with integrated UV detection, data processing and reporting.

Figure 1. Correlation of UV and MS data in the Processing Workspace of LightSight® software version 2.2.

Experimental Conditions

Bromocriptine was incubated at 50 µM in rabbit liver microsomes using an NADPH regenerating system under oxidative conditions at 37°C. After 1 hour, the incubation was quenched with an equal volume of acetonitrile. The supernatant was then diluted 1:5 with water for injection. This gave a final concentration of 5 µM for bromocriptine in the T_0 control.

The LC gradient was designed such that bromocriptine eluted at approximately 60% of the gradient, thus ensuring that all metabolites, whether of increased or decreased polarity, could be adequately chromatographed. A 5 µL volume of sample was injected onto a Phenonomenex HPLC column (50 x 2 mm). Prior to the mass spectrometric analysis, the LC eluent was fed through a Shimadzu SDP20AV dual wavelength detector, set to 302 nm. Predictive multiple reaction monitoring with information dependant acquisition (pMRM-IDA) methods were created using LightSight® software v2.2 and run on a 4000 QTRAP® hybrid triple quadrupole linear ion trap mass spectrometer. With prior knowledge of the parent drug and its fragmentation pathway, theoretical MRM transitions for metabolites can be determined based on a particular biotransformation set. The pMRM experiments were built using the Phase I comprehensive biotransformation set provided by the LightSight® software. A total of 166 MRM transitions were included in the pMRM survey scan linked to a single enhanced product ion (EPI) dependent scan. The EPI experiment was acquired at a scan rate of 4,000 Da/sec with dynamic fill time turned on. Structural elucidation of the MS/MS fragments was achieved with the assistance of the ACD\Labs MS Fragmenter software package.

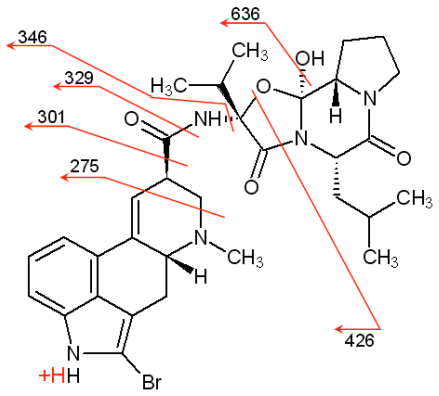


Figure 2. The MS/MS fragmentation of bromocriptine

Results and Discussion

Figure 2 shows the molecular structure of bromocriptine, with sites of fragmentation that occur during MS/MS analysis indicated along with the nominal mass of the fragment. The m/z 346 fragment ion was chosen as the Q3 mass for the pMRM method. Compounds with higher lambda maximum, above the typical solvent noise that exists from 100 to 150 nm, will yield excellent data quality that correlates nicely with MRM data. Bromocriptine, with a lambda max of 302 nm, works exceptionally well.

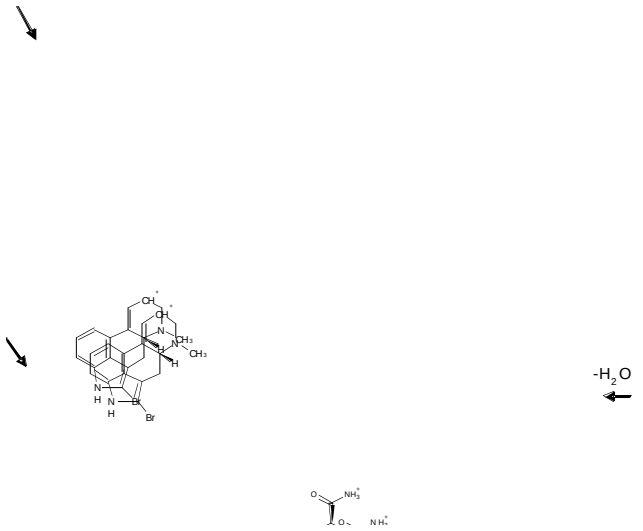
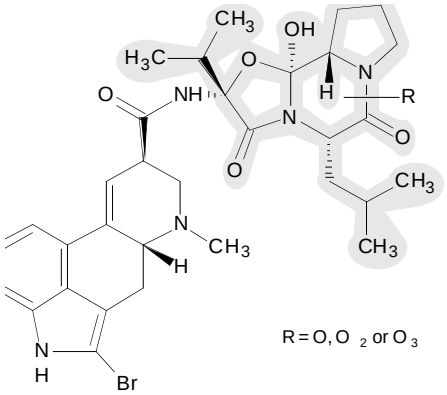
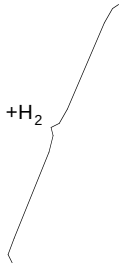


Figure 3. The comparison between bromocriptine (bottom panel) and a di-oxidation metabolite reveals that all the fragments at or below m/z 426.1 are common between both spectra. Therefore, the sites of metabolism do not occur on these portions of the parent drug.





R = O, O 2 or O 3

M9, M12, M13, M14: Oxidation
M4: Tri -Oxidation
M5, M6, M8, M10: Di -Oxidation

M11: Oxidation + Hydrogenation
M6: Di -Oxidation + Hydrogenation
M7: Tri -Oxidation + Hydrogenation

Figure 4. Proposed structures for metabolites of bromocriptine detected in the microsomal incubation.

Peak ID	Biotransformation	Mass Shift	Expected m/z	Q1 / Q3	R.T. (min)	Peak Area	% Area	Peak Height	R.T. (min.) - Analog	Peak Area - Analog	% Area - Analog
M1	Demethylation	-14	640.2	640.2 / 299.9	6.84	6.39E+04	0.5	7.00E+03			
M2	Demethylation	-14	640.2	640.2 / 299.9	7.19	3.47E+05	2.9	3.12E+04			
M3	Loss of 30	-30	624.2	624.2 / 299.9	7.5	3.62E+05	3.1	3.36E+04			
M4	Tri-Oxidation	48	702.2	702.2 / 345.9	7.83	5.33E+04	0.4	7.40E+03			
M5	Di-Oxidation	32	686.2	686.2 / 345.9	8.45	4.28E+05	3.6	5.61E+04	8.48	2.53E+01	2.9
M6	Di-Oxidation + Hydrogenation	34	688.2	688.2 / 347.9	8.63	2.61E+05	2.2	3.40E+04	8.65	1.65E+01	1.9
M7	Tri-Oxidation + Hydrogenation	50	704.2	704.2 / 347.9	8.74	5.39E+05	4.5	3.61E+04	8.65	1.65E+01	1.9
M8	Di-Oxidation	32	686.2	686.2 / 345.9	8.84	3.52E+06	29.7	4.05E+05	8.86	1.88E+02	21.3
M9	Oxidation	16	670.2	670.2 / 345.9	9	9.20E+05	7.8	1.10E+05	9.02	7.61E+01	8.6
M11	Oxidation + Hydrogenation	18	672.2	672.2 / 347.9	9.23	1.69E+06	14.3	1.74E+05	9.25	1.70E+02	19.2
M10	Di-Oxidation	32	686.2	686.2 / 345.9	9.23	7.46E+05	6.3	8.06E+04	9.25	1.70E+02	19.2
M12	Oxidation	16	670.2	670.2 / 345.9	9.26	1.78E+06	15	1.87E+05	9.25	1.70E+02	19.2
	Parent	0	654.2	654.2 / 345.9	9.73	5.25E+05	4.4	5.94E+04	9.7	1.57E+01	1.8
M13	Oxidation	16	670.2	670.2 / 345.9	9.83	2.52E+05	2.1	3.04E+04			
M14	Oxidation	16	670.2	670.2 / 345.9	10.19	3.19E+05	2.7	4.51E+04	10.2	3.57E+01	4
	Parent	0	654.2	654.2 / 345.9	10.71	4.46E+04	0.4	5.80E+03			

Table 1. The metabolites identified using LightSight® software sorted by retention time.

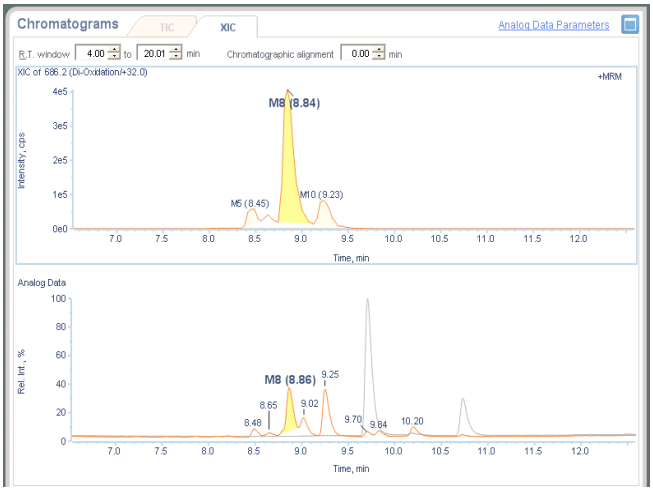


Figure 5. The selected ion chromatogram for the di-oxidation metabolites of bromocriptine (top) and the UV chromatogram (bottom).

If there is complementary analog data (i.e. PDA, UV, ADC data) available, LightSight® software will correlate the MS data to the analog data and vice versa. The potential metabolite table in the software would contain metabolites found and both data types as well as ensure that any potential metabolite that is only detected in either the analog data or the MS data are found. Using the LightSight® software, a total of 14 metabolites were detected and identified and of those 14, 11 were oxidative metabolites of bromocriptine; a summary of the MS and UV data for these metabolites are given Table 1. Figure 1 shows the correlation between the MS data and the UV trace performed in the LightSight® software

The metabolites included single, di-, and tri-oxidations of bromocriptine. The proposed structures of some of the metabolites are given in Figure 4. Figure 3 shows the comparison between a tri-oxidation metabolite of bromocriptine and parent bromocriptine MS/MS spectra. The pMRM transition that corresponds to the triple-oxidation metabolite shows 4 clear peaks in the chromatography and the UV trace further confirms that these peaks are potential metabolites (Figure 5).

Conclusions

- LightSight® software version 2.2 efficiently identifies, confirms, and quantitates metabolites using both MS and analog data.
- Allows for both allows structure elucidation based on the MS/MS data and estimated concentration based on UV in a single workspace.

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