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Application News

SSI-LCMS-015

Liquid Chromatography Mass Spectrometry

Analysis of Digoxin, Nadolol and Metoprolol Using a Fast Polarity Switching DUIS Method

LCMS-2020

Summary

Qualitative analysis of three marker and nine unknown compounds in were tested using the LCMS 2020 single quadruple mass spectrometer using a dual ion source (DUIS). In addition, quantitation of the marker compound digoxin was also tested. An LCMS method was developed and analyzed for all compounds in these samples.

Method

The three marker compounds were digoxin, nadolol and metoprolol (shown in **Figure 1**). A standard for digoxin was prepared in house at 10 ppm (µg/mL). The digoxin standard was further diluted in methanol for calibration levels for LCMS analysis. Standards for nadolol and metoprolol were not available. The unknown samples were injected directly onto the LCMS system.

Both scan and selection ion monitoring (SIM) methods were used for MS analysis. The details of these events are described in **Table 1**. The dual ion source (DUIS) was used for ionization of all compounds to be analyzed. This ionization source is capable of both APCI and ESI ionization simultaneously. Both the scan range and SIM values are included in **Table 1**.

For quantitative analysis, dilutions of the digoxin standard were made as shown in **Table 2**. A calibration curve was prepared and the unknown digoxin samples were analyzed for digoxin content.

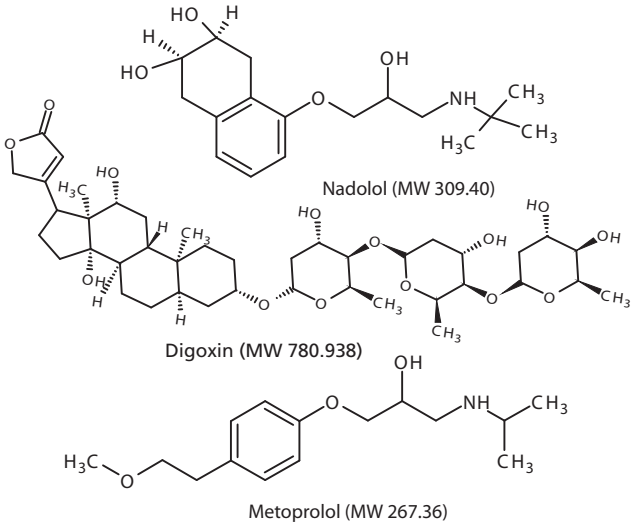


Figure 1: Structures of digoxin, nadolol and metoprolol

Results and Discussion

The chromatogram for the separation of the 10 ppm standard of digoxin using both scan and SIM modes is shown in **Figure 2**. The left inset below the chromatogram, shows the MS spectrum for digoxin with a 779.0 m/z which corresponds to the $[M-H]^-$ peak and 825.0 m/z which corresponds to the $[M+Formate]^-$ peak. The right inset below the chromatogram, shows the MS spectrum for the scan event. **Figure 3a** shows both the scan and SIM chromatograms for the compound nadolol and **Figure 3b** shows both the scan and SIM chromatograms for the compound metoprolol. **Figure 4** shows both the scan and SIM chromatograms for the nine different unknown compounds. Both the marker compounds and unknown samples gave an intense response under these chromatography and ionization conditions. The chromatography could be shortened further but it was not done because of potential unknown interferences with real samples.

The calibration curve was linear in the tested range for digoxin (r^2 = 0.9998). Nine calibration points were used for quantitative analysis. The calibration curve for digoxin is shown in **Figure 5.** The limit of detection (LOD, S/N>3) for digoxin (825.0 m/z) was 4.5 ppb (ng/mL), which was the lowest concentration examined. Selecting a m/z of 779.0 gave an LOD of approximately 50 ppb (ng/mL) using these method conditions which was a tenfold loss in sensitivity. Therefore, a m/z of 825.0 was used for the quantitation of digoxin in

Event time (sec)			0.1
Mode			DUIS
Drying Gas (L/min)			15
Nebulizing Flow (L/min)			1.5
Interface Temp (°C)			350
DL (°C)			300
Heat Block (°C)			500
			Channels
Event 1	SIM	(-)	779, 825
Event 2	SIM	(+)	310; 268; 271; 481; 443; 500; 445; 476; 502; 456; 445
Event 3	Scan	(+)	250 - 850 m/z
Event 4	Scan	(-)	250 - 850 m/z

Table 1: MS Parameters for the LCMS 2020

Level	Conc. (ppm)
1	10
2	3.33
3	1.11
4	0.37
5	0.123
6	0.041
7	0.0137
8	0.00685
9	0.00457

Table 2: Calibration Levels

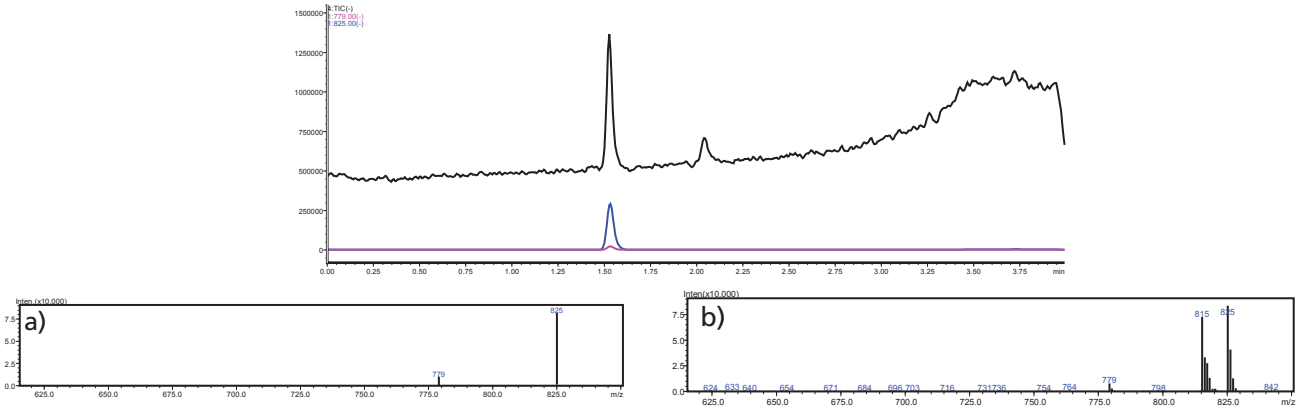


Figure 2: Scan (black) and selected ion monitoring (SIM) of 10 ppm standard of digoxin. Both 779.0 m/z [M-H](pink) and 825.0 m/z [M+Formate-H](blue) were monitored. Below: MS spectra for (a) SIM and (b) scan for digoxin.

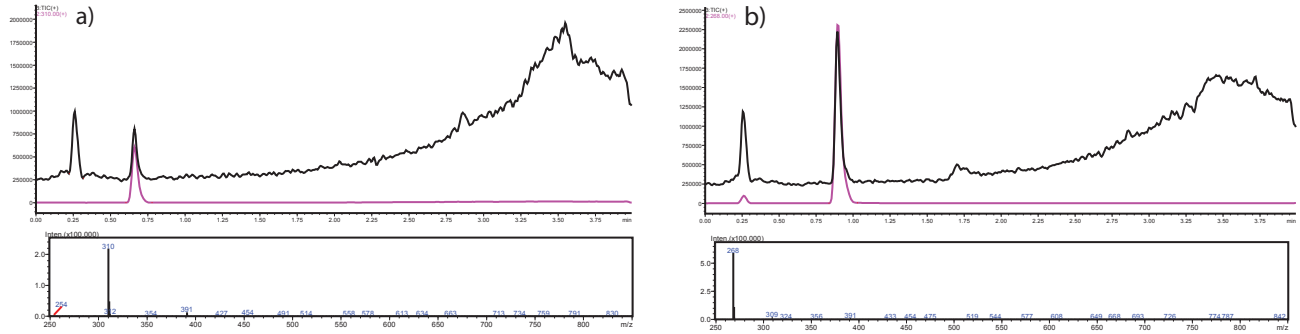


Figure 3: a). Scan (black) and SIM chromatogram for nadolol (pink). Below: MS spectrum for nadolol. b). Scan (black) and SIM chromatogram for metoprolol (pink). Below: MS spectrum for metoprolol.

the unknown samples. The MS chromatograms for digoxin at three concentrations, 3.33 ppm, 0.041 ppm and 0.00457 ppm are shown in **Figure 6**. The SIM chromatograms for digoxin found in the assay (T1, D1, S1) are shown in **Figure 7**. The MS chromatograms for the other two marker compounds, nadolol and metoprolol, found in the assay are shown in **Figure 8**. The MS chromatograms for the nine unknown compounds (A-I) in the assay are shown in **Figure 9**. Each MS chromatogram is from a well which corresponds to the lowest values for the compound. As shown in **Figure 9**, each compound was easily detected using these method conditions.

The amount of digoxin present in the assay samples S1 – S3 was much lower than in the other samples but the MS was still able to detect the compound at low levels. The quantitative level of digoxin present in the real samples is shown in **Table 3**. There were a total of 80 different digoxin samples in the Digoxin-plate provided. Each sample was run one time and the concentration (both ppm and ppb) and peak area for each well is displayed in the table. The values for the receiver wells (S1-S3) were approximately 2-9% of the initial concentration and donor wells after 2 hours (D1-D2) were approximately 85% of the original concentration.

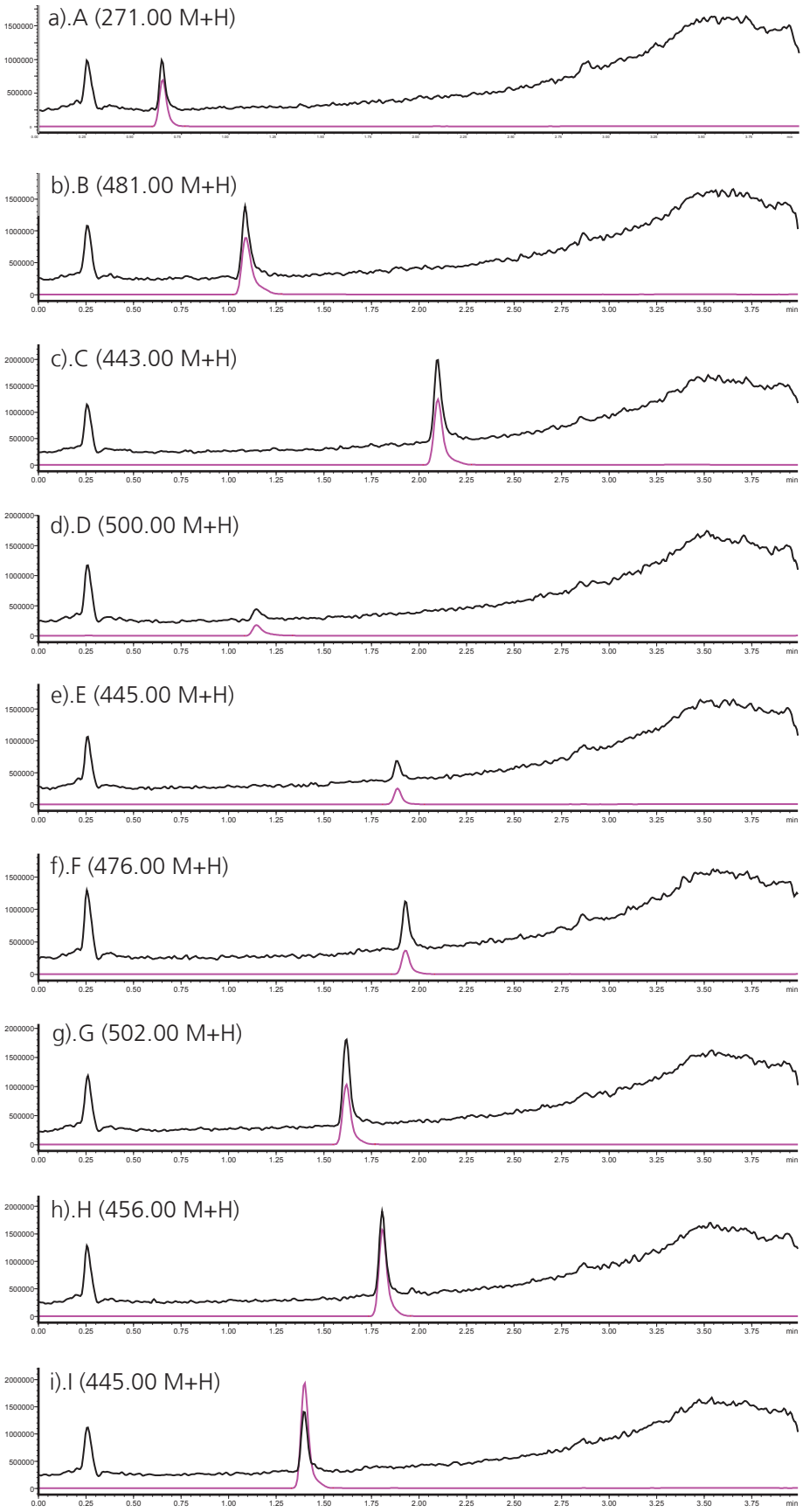


Figure 4: Scan and SIM chromatograms for 9 unknown compounds.

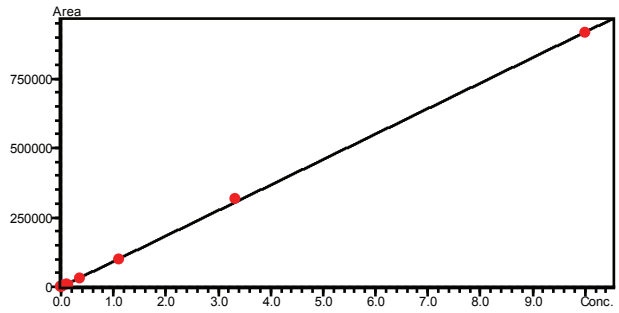


Figure 5: Calibration curve for digoxin.

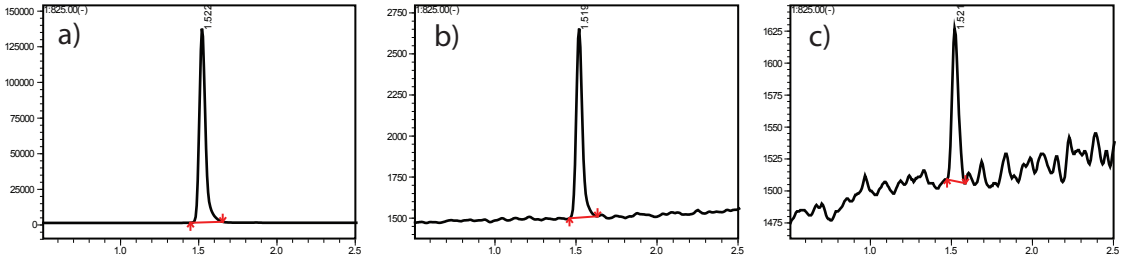


Figure 6: MS chromatograms for digoxin standard at (a) 3.33 ppm, (b) 0.041 ppm, and (c) 0.00457 ppm. SIM monitored is 825 m/z.

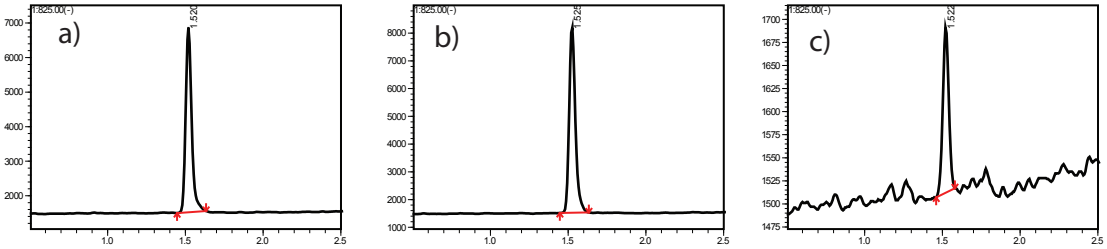


Figure 7: MS chromatograms for digoxin in sample plate (a) T2 (b) D1 (c) S1. SIM monitored is 825 m/z.

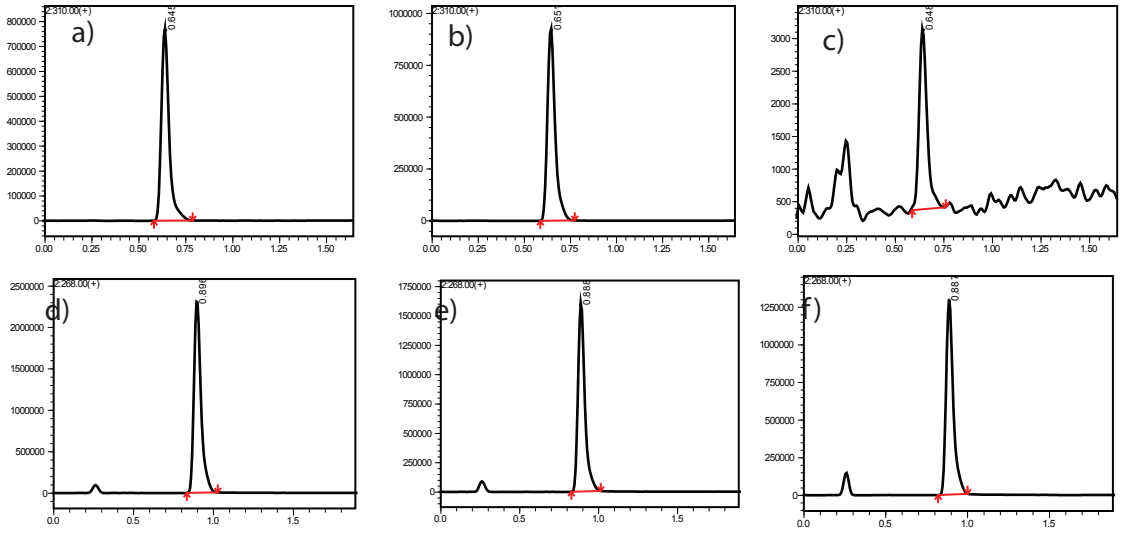


Figure 8: MS chromagrams for nadolol (above) and metoprolol (below) in sample plate. Top Nadolol: (a) T2, (b) D2, (c) S2 Bottom Metoprolol: (d) T1, (e) D1, (f) S1

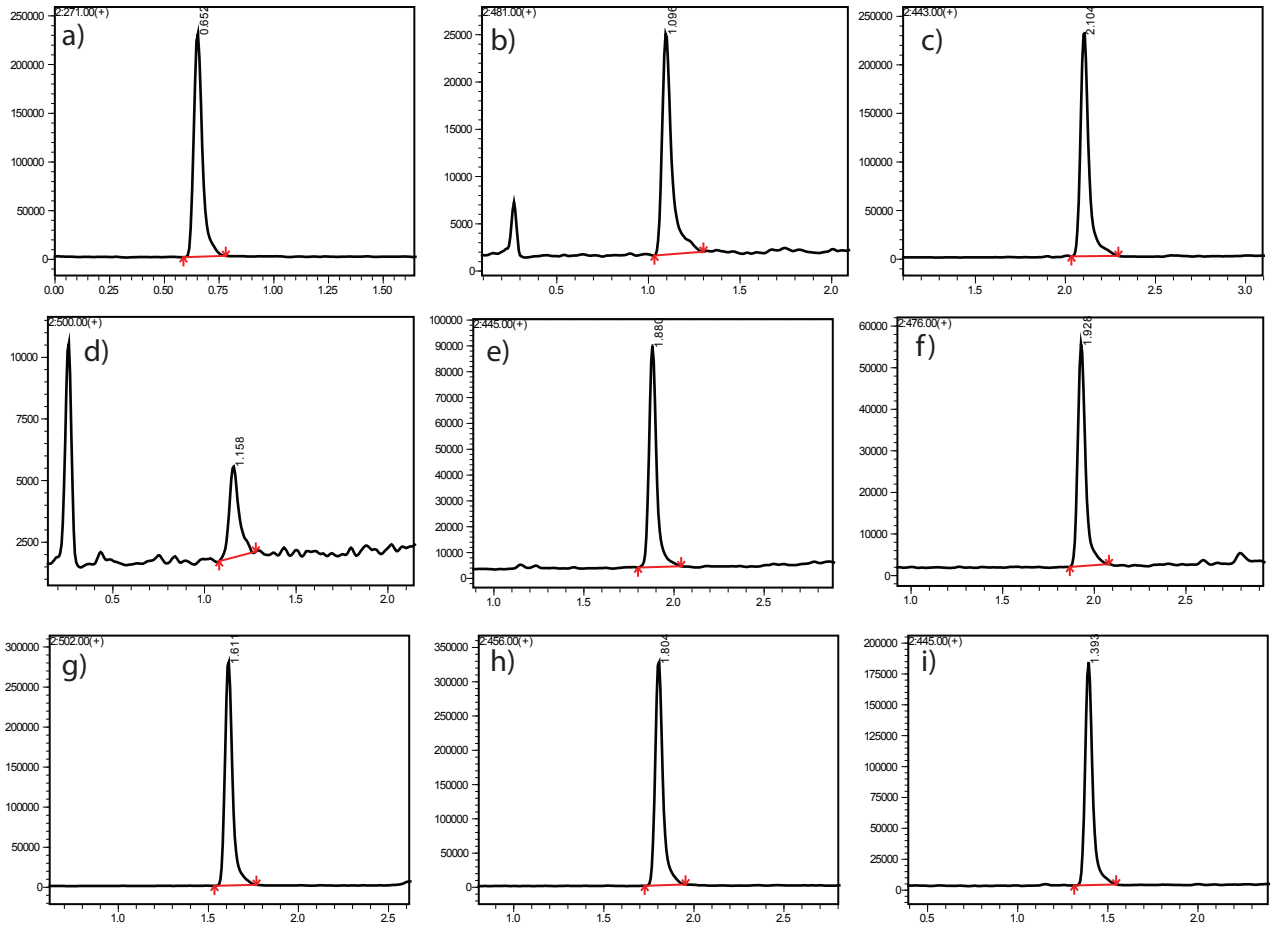


Figure 9: MS chromagrams for the nine unknown samples from S wells. Top: (a) A,(b) B, (c) C Middle: (d) D, (e) E, (f) F Bottom: (g) G, (h) H, (i) I

There are a few outliers with this data but with replicate runs and simplifying the method to only using SIM mode, the data could be improved.

Table 4 shows the retention time and peak areas for both the nadolol and metoprolol for both samples in the Digoxin-plate and Plate 1. The receiver response for nadolol (S1-S3) in Plate 1 has a lower intensity than in the Digoxin plate, however, it is still able to be easily detected. The peak areas for the metoprolol samples were very intense. **Table 5** shows the peak areas for the nine unknown compounds. The receiver samples are approximately 2-17% of the initial conditions and the donor samples after 2 hours are approximately 75-90% of the initial conditions. These determined values are comperable to the expected values for these compounds in this assay.

DIGOXIN										
Digoxin-Plate	T1_01	T1_02	T1_04	T1_05	T1_06	T1_07	T1_08	T1_10	T1_11	T1_12
Pk area	8314	6519	9507	10171	7111	8304	9112	5547	7156	10482
Conc (ppm)	0.096	0.076	0.109	0.116	0.083	0.096	0.104	0.066	0.083	0.119
Conc. (ppb)	96.0	76.0	109.0	116.0	83.0	96.0	104.0	66.0	83.0	119.0
	T2_01	T2_02	T2_04	T2_05	T2_06	T2_07	T2_08	T2_10	T2_11	T2_12
Pk area	9197	5850	6393	10296	10282	6514	6613	7152	10144	7735
Conc (ppm)	0.105	0.069	0.075	0.117	0.117	0.076	0.077	0.083	0.116	0.089
Conc. (ppb)	105.0	69.0	75.0	117.0	117.0	76.0	77.0	83.0	116.0	89.0
	T3_01	T3_02	T3_04	T3_05	T3_06	T3_07	T3_08	T3_10	T3_11	T3_12
Pk area	6384	6758	6392	10746	7494	6641	7953	7165	10579	11031
Conc (ppm)	0.075	0.079	0.075	0.122	0.087	0.077	0.092	0.083	0.120	0.125
Conc. (ppb)	75.0	79.0	75.0	122.0	87.0	77.0	92.0	83.0	120.0	125.0
	S1_01	S1_02	S1_04	S1_05	S1_06	S1_07	S1_08	S1_10	S1_11	S1_12
Pk area	99	1223	115	268	652	14498	7524	8268	15343	11451
Conc (ppm)	0.006	0.018	0.006	0.008	0.012	0.163	0.087	0.095	0.172	0.130
Conc. (ppb)	6.0	18.0	6.0	8.0	12.0	163.0	87.0	95.0	172.0	130.0
	S2_01	S2_02	S2_04	S2_05	S2_06	S2_07	S2_08	S2_10	S2_11	S2_12
Pk area	141	1299	176	514	609	18589	7909	8837	9331	13592
Conc (ppm)	0.007	0.019	0.007	0.011	0.012	0.208	0.091	0.101	0.107	0.153
Conc. (ppb)	7.0	19.0	7.0	11.0	12.0	208.0	91.0	101.0	107.0	153.0
	S3_01	S3_02	S3_04	S3_05	S3_06	S3_07	S3_08	S3_10	S3_11	S3_12
Pk area	138	1391	167	563	565	16442	12427	14530	14324	11745
Conc (ppm)	0.007	0.020	0.007	0.011	0.011	0.184	0.140	0.163	0.161	0.133
Conc. (ppb)	7.0	20.0	7.0	11.0	11.0	184.0	140.0	163.0	161.0	133.0
	D1_01	D1_02	D1_04	D1_05	D1_06	D1_07	D1_08	D1_10	D1_11	D1_12
Pk area	6050	5833	6903	8823	5782	5411	5676	3033	3706	3936
Conc (ppm)	0.071	0.069	0.080	0.101	0.068	0.064	0.067	0.038	0.045	0.048
Conc. (ppb)	71.0	69.0	80.0	101.0	68.0	64.0	67.0	38.0	45.0	48.0
	D2_01	D2_02	D2_04	D2_05	D2_06	D2_07	D2_08	D2_10	D2_11	D2_12
Pk area	5798	6462	4641	5513	7718	4127	6975	4510	5254	8344
Conc (ppm)	0.068	0.076	0.056	0.065	0.089	0.050	0.081	0.054	0.062	0.096
Conc. (ppb)	68.0	76.0	56.0	65.0	89.0	50.0	81.0	54.0	62.0	96.0

Plate 1	T1_01	T2_01	T3_01	S1_01	S2_01	S3_01	D1_01	D2_01
Pk area	18,697	11,910	18,366	487	679	455	14,467	21,230
Conc (ppm)	0.209	0.135	0.205	0.010	0.012	0.010	0.163	0.236
Conc. (ppb)	209.0	135.0	205.0	10.0	12.0	10.0	163.0	236.0

Table 3: Quanitative data for digoxin in the assay.

Nadolol (Digoxin Plate)	T1	T2	T3	S1	S2	S3	D1	D2
Ret. Time	0.661	0.659	0.657	0.658	0.653	0.659	0.655	0.652
Peak Area	1,728,794	1,670,357	2,079,558	11,362	15,000	13,890	2,207,411	1,971,636
Nadolol (Plate 1)								
Ret. Time	0.653	0.651	0.648	0.649	0.648	0.645	0.645	0.645
Peak Area	1,990,610	2,709,802	2,202,684	1,738	7,975	10,021	2,140,682	2,211,167

Metoprolol (Digoxin Plate)	T1	T2	T3	S1	S2	S3	D1	D2
Ret. Time	0.896	0.892	0.893	0.887	0.889	0.888	0.888	0.885
Peak Area	7,144,249	5,000,989	6,434,597	3,964,004	3,650,326	3,747,897	4,877,013	4,752,324
Metoprolol (Plate 1)								
Ret. Time	0.882	0.881	0.877	0.877	0.876	0.876	0.876	0.874
Peak Area	5,520,513	5,490,836	7,030,378	3,885,968	2,463,537	2,713,610	5,325,596	5,642,236

Table 4: Retention times and peak areas for nadolol and metoprolol in the assay.

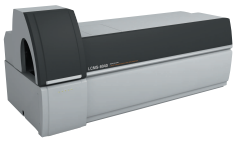
Unknown (Pk areas)	T1	T2	T3	S1	S2	S3	D1	D2
A	1,982,965	2,513,578	2,177,557	635,140	663,211	711,134	1,809,674	2,401,184
B	3,523,652	2,472,463	3,372,554	86,843	123,744	131,998	2,053,798	2,335,651
C	4,390,874	4,369,047	4,805,541	803,380	749,141	928,353	3,028,895	4,051,761
D	728,146	535,482	718,328	10,529	19,441	14,040	149,637	121,393
E	750,131	1,110,631	1,084,640	336,471	251,757	415,088	612,633	868,336
F	1,157,083	998,508	1,205,125	213,456	189,754	159,502	955,618	990,475
G	3,332,140	3,883,812	4,221,444	828,410	946,600	1,071,547	2,983,811	2,899,834
H	5,092,405	5,207,564	4,024,199	1,201,002	977,408	1,129,551	3,945,148	3,004,201
I	5,998,149	6,100,037	4,659,995	788,199	527,713	1,062,409	4,246,820	4,551,932

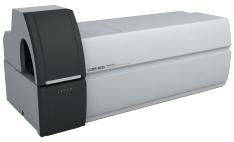
Table 5: Peak areas for the nine unknown compounds in the assay.

Conclusion

The LCMS-2020 single quadruple instrument was able to demonstrate both scanning and selected ion monitoring methods using fast scanning, polarity switching and dual ionization for the identification and quantitation of multiple compounds in the assay.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8030


LCMS-8080


LCMS-2020


LCMS-IT-TOF

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