SHIMADZU

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Enhanced screening for Active Pharmaceutical Ingredients (APIs) through the integration of a single quadrupole mass spectrometer

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1. Overview

- A single quadrupole mass spectrometer was integrated into an API screening workflow to enhance impurity characterization.
- The *m/z* information on the APIs were displayed onto the UV chromatogram for additional confirmation of the compound.

2. Introduction

The pharmaceutical industry analyzes active pharmaceutical ingredients (APIs) in clinical samples, formulation prototypes, and/or commercial fabrications for API identification, quantitation, and impurity screening. The industry has relied on liquid chromatography coupled with a photodiode array detector to perform these analyses; however, it can only measure compounds that absorb in the UV-Vis portion of the electromagnetic spectrum. Single quadrupole mass spectrometers (MSs) can measure compounds that ionize, which covers a wider range of analytes for impurity or unknown identification. Despite the advantages of MS analysis, it can be difficult to integrate MSs into active systems due to the operational complexity and larger footprint. This work will demonstrate the benefits of adding a compact MS into an existing workflow for API analysis.

3. Methods

Sixteen APIs common to the pharmaceutical industry were selected as analytes of interest. Stock standard solutions (1 – 4 mg/mL) of each standard were prepared in methanol and further diluted with a 50:50 methanol:water (v/v) solution. The analysis was performed on a Shimadzu Nexera HPLC system coupled with a photodiode array detector (PDA) and a LCMS-2050 single quadrupole mass spectrometer. The method utilized a C18 column (3.0 × 50 mm, 5 µm) using water containing 0.1% formic acid and methanol as the mobile phases with a gradient elution scheme. Positive and negative mode scans from m/z 100 – 2000 and Selected Ion Monitoring (SIM) were used to analyze the 16 analytes. The PDA was set to scan from wavelength 190 – 700 nm.

Table 1. Summary of the LCMS method parameters

LCMS-2050	Parameters	LC-40	Parameters
Ion source	DUIS	**Column**	Shimadzu Nexcol C18 3.0 × 50 mm, 5 µm
Nebulizing gas	2.0 L/min	**Flow rate**	1 mL/min
Desolvation temperature	450 ˚C	**Mobile phase A**	0.1% formic acid in water
DL temperature	200 ˚C	**Mobile phase B**	Methanol
Heating gas	7.0 L/min	**Injection volume**	1 µL
Drying gas	5.0 L/min	**Column oven temperature**	40 ˚C
Polarity	Positive and Negative	**PDA scan range**	190-700 nm

4. Results and Discussion

4-1. Integrating UV and *m/z* information

The LCMS-2050 was used to demonstrate the analytical processes involved in product characterization and impurity screening. Sixteen APIs were used to explore the benefit of integrating a single quadrupole mass spectrometer into an existing HPLC-PDA method. **Figure 1** shows an UV chromatogram of the API mixture (1 to 1000 µg/mL) measured with a PDA detector under the LC conditions described above. Eight distinct peaks were identified in the UV chromatogram at 237 nm. By using the LCMS-2050, the *m/z* of the analyte precursor ions in these peaks were identified. Previously, side-by-side comparisons of UV and MS chromatograms were only possible by opening separate windows, but now this information can be viewed together using the new LabSolutions function, Mass-It™. This function allowed quick comparison of the UV and MS data by visualizing the detected API *m/z* in an UV chromatogram for more efficient data interpretation..

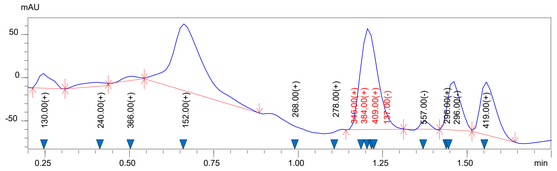

Figure 1. UV chromatogram at 237 nm of the 16 APIs（1 to 1000 µg/mL).

4-2. Monitoring APIs using a Mass spectrometer

With the additional mass-to-charge information, these APIs can be monitored by using selected-ion mode (SIM) on the LCMS-2050. This added selectivity allowed sensitively screening more analytes with a short run time for higher throughput. By using the more specific *m/z* generated by the LCMS-2050 in comparison to the UV peaks obtained by HPLC-PDA, chromatogram with better resolution were obtained. **Figure 2** shows MS chromatograms of a standard mixture (1 to 1000 µg/mL) of the sixteen APIs analyzed using SIM.

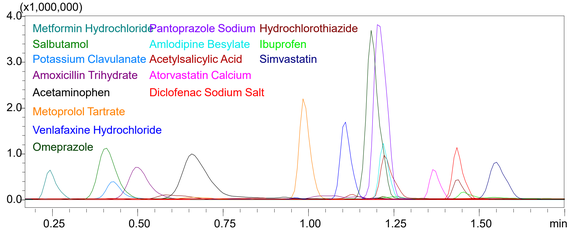

Figure 2. MS chromatograms (SIM) of API standard mixture at concentrations ranging from 1 to 1000 µg/mL.

4-3. Targeted quantitation

Polarity, *m/z,* limit of quantitation (LOQ), and reproducibility (%RSD) for each compound are summarized in **Table 2**. The %RSD at the LOQ was less than or equal to 5% with LOQ as low as 0.05 µg/mL for metformin. These results showcase the high precision and sensitivity available when utilizing the LCMS-2050. The low LOQ were achieved by low background signal acquired using the MS under SIM. **Figure 3** shows the chromatograms for two of the sixteen APIs (metformin and venlafaxine) at the LOQ and their corresponding blanks.

Table 2. Quantitation summary of the APIs measured using selected-ion monitoring (SIM) (n = 5).

APIs	Polarity	*m/z*	LOQ (µg/mL)	%RSD at LOQ (Area)
Metformin Hydrochloride	+	130	0.05	3.5
Salbutamol	+	240	0.05	4.6
Amoxicillin Trihydrate	+	366	0.25	1.2
Acetaminophen	+	152	0.50	1.6
Metoprolol	+	268	0.05	4.3
Venlafaxine Hydrochloride	+	278	0.05	4.9
Omeprazole	+	346	0.50	4.4
Pantoprazole Sodium	+	384	1.00	2.7
Amlodipine Besylate	+	409	0.10	3.3
Diclofenac	+	296	0.25	3.6
Simvastatin	+	419	0.50	4.0
Potassium Clavulanate	-	198	4.00	4.7
Hydrochlorothiazide	-	296	0.50	3.2
Acetylsalicylic Acid	-	137	0.50	4.2
Atorvastatin Calcium	-	557	0.50	4.3
Ibuprofen	-	205	50.0	5.0

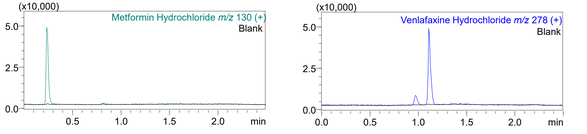

Figure 3. Chromatograms for two APIs and their corresponding blank at the LOQ (0.05 µg/mL).

5. Conclusions

Integrating a single quadrupole mass spectrometer into the current API workflow enhanced the capability of routine API screening. The LCMS-2050 provided a highly efficient and selective solution for API analysis using mass detection. HPLC-PDA was able to identify 8 of the 16 APIs, with the m/z of the analytes visualized in the same window using the comprehensive Mass-It function. The LCMS-2050 was able to distinguish between four co-eluting APIs due to the selectivity gained by using Shimadzu's powerful, compact single quadrupole mass spectrometer. The LCMS-2050 demonstrated low noise and remarkable reproducibility for all sixteen APIs. A compact single quadrupole mass spectrometer system integrated into a current API workflow unlocked a wider range of APIs and impurities analyses.