Hydrophilic Interaction Chromatography (HILIC) Separation of Basic Drugs using MS/MS Detection

Application Note

Drug Analysis

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Abstract

The basic drugs, ranitidine and paroxetine were successfully separated using hydrophilic interaction liquid chromatography (HILIC). The elution order was reversed compared to reversed-phase liquid chromatography (RPLC). Good linearity was shown over the 0.5–100 ppb levels. A spiked serum was cleaned up by protein precipitation and an aliquot was directly injected into the HILIC/ESI-MS system. Recovery was found to be 98% for ranitidine and 79% for paroxetine at the 100 ppb level.

Introduction

Because of its versatility, RPLC is the most widely used technique in all of HPLC. It separates molecules based on the hydrophobic interactions between the nonpolar stationary phase and the organic portions of typical analytes. However, the retention of polar analytes often requires a highly aqueous mobile phase to achieve retention. Highly aqueous systems sometimes lead to problems such as phase collapse (dewetting) [1], decreased sensitivity in mass spectroscopic detection due to poor mobile phase desolvation and ion suppression, and still may not allow retention of very polar analytes. Some specialized packings were developed to allow the use of highly aqueous systems such as polar embedded phases, hydrophilically endcapped reversed phase bonded silicas, wide-pore low-density bonded silicas, short-chain phases, and other special designs [2].

An alternative technique for the separation of highly polar analytes that gets around some of the problems associated with RPLC is HILIC. HILIC requires a high percentage of a nonpolar mobile phase and a polar stationary phase, similar to the requirements in normal phase chromatography (NPC). However, unlike NPC, which uses nonpolar solvents such as hexane and methylene chloride and tries to exclude water from the mobile phase, HILIC requires some water in the mobile phase to maintain a stagnant enriched water layer on the surface into which analytes may selectively partition. In addition, water-miscible organic solvents are used instead of the water-immiscible solvents used in NPC. With HILIC, sorbents such as bare silica, bonded diol, and polyhydroxyethylaspartamide are used. Polar analytes are well retained

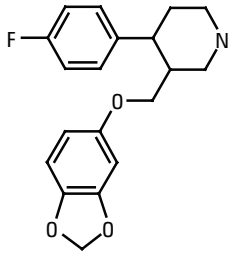
and elute in order of increasing hydrophilicity, just the inverse of RPLC. Sometimes, under HILIC conditions, polar analytes will show a very different selectivity compared to RPLC. It was demonstrated recently that the mechanism of HILIC involves various combinations of hydrophilic interaction, ion exchange, and reversed-phase retention by the siloxane on the silica surface [3].

Basic drugs with amine functionality are often separated by RPLC, and under acidic conditions when protonated may show decreased retention. In the present study, we investigated the separation of basic drugs in serum using RPLC on a C18 column and HILIC on bare silica gel employing electrospray ionization (ESI) and MS/MS detection.

Basic Drugs Studied

The structures of the basic drugs studied are depicted in Figure 1. Paroxetine (Figure 1a) is a psychotropic drug that is administered orally. It has a water solubility of 5.4 mg/mL and in its free base form its molecular weight (MW) is 329.36. Ranitidine (Figure 1b) is an antiulcerative and works by decreasing the amount of acid that the stomach produces. It is freely soluble in water and methanol but sparingly soluble in ethanol. Its MW is 314.41 in its free base form.

a) Paroxetine
Antidepressant
MW 329.36



b) Ranitidine
Antiulcerative
MW 314.41

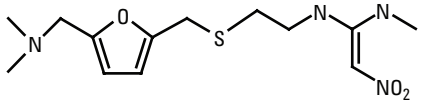


Figure 1. Structures of drug compounds studied.

Chromatographic System

Since mass spectrometry (MS) using ESI was employed for detection, we elected to use 2.1-mm internal diameter (id) columns where the normal flow rate was more compatible.

RPLC

Instrument:	Agilent Series 1100LC
Column:	ZORBAX Eclipse XDB-C18, 2.1 mm × 150 mm, 5 µm)
Mobile phase:	A: 8-mM $HCOONH_4$ in water B: 8-mM $HCOONH_4$ in 95% acetonitrile (MeCN)/5% water
Gradient:	5% B to 90% B in 10 min
Column temperature:	40 °C
Sample volume:	5 µL
Flow rate:	0.3 mL/min

HILIC

Everything same as for RPLC except for column and gradient conditions:

Column:	ZORBAX RX-SIL, 2.1 mm × 150 mm, 5 µm
Gradient:	100% B to 50% B in 10 min

Mass Spectrometry

Instrument:	Series 1100 LC/MSD Trap
Ionization:	Positive ESI
Scan range:	100–500 *m/z*
SIM ions:	*m/z* = 315, 330
Drying gas:	10 L/min at 350 °C
Nebulizer gas:	45 psi
Fragmentor voltage:	0.25 V

Results and Discussion

In order to select the best SIM for online monitoring, the MS spectra of the drug standards were run. Figure 2 shows that an M+1 ion was observed but ions at *m/z* of 192.0 and 175.9 were used for paroxetine and ranitidine, respectively, since they showed stronger signals. These ions were monitored in subsequent runs.

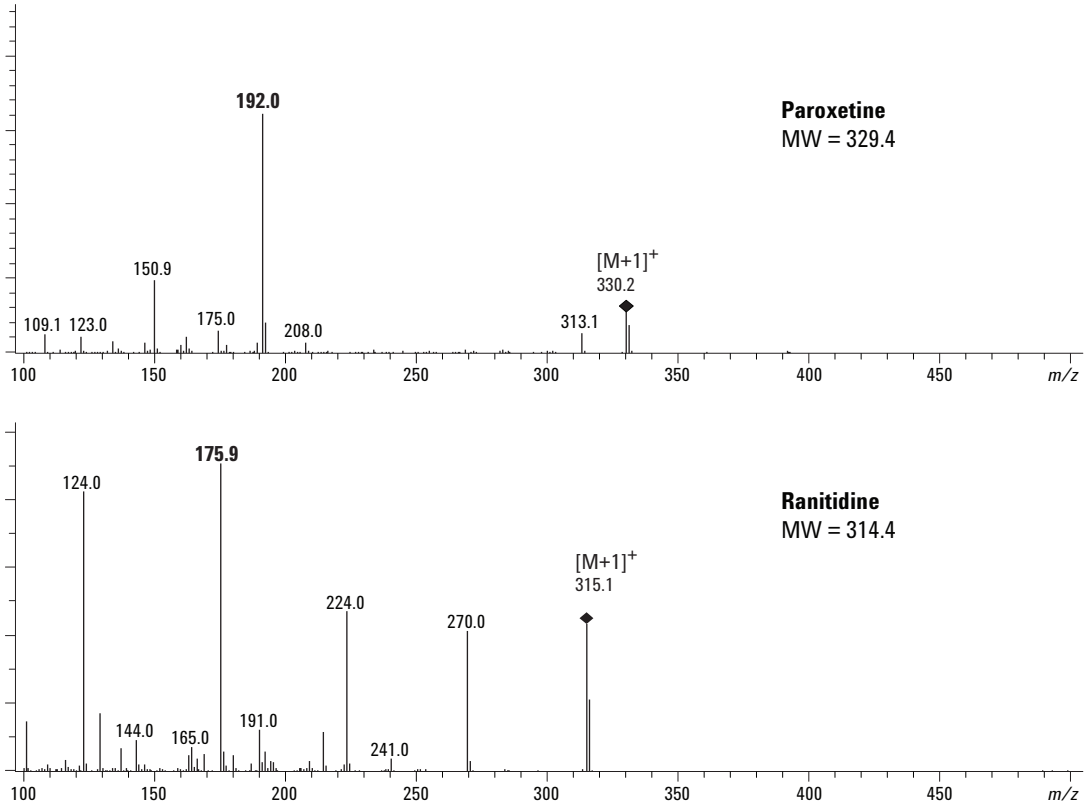


Figure 2. MS/MS spectra of drug standards showing ions selected for subsequent fragmentation.

The RPLC gradient elution total ion chromatogram of standards, shown in Figure 3, gave an excellent and relatively fast separation of the two drugs. However, with ranitidine there was some tailing at the 100 ppb (parts per billion) level. Since we were interested in the measurement of lower levels in serum, this separation was deemed unacceptable so we switched our attention to the HILIC conditions.

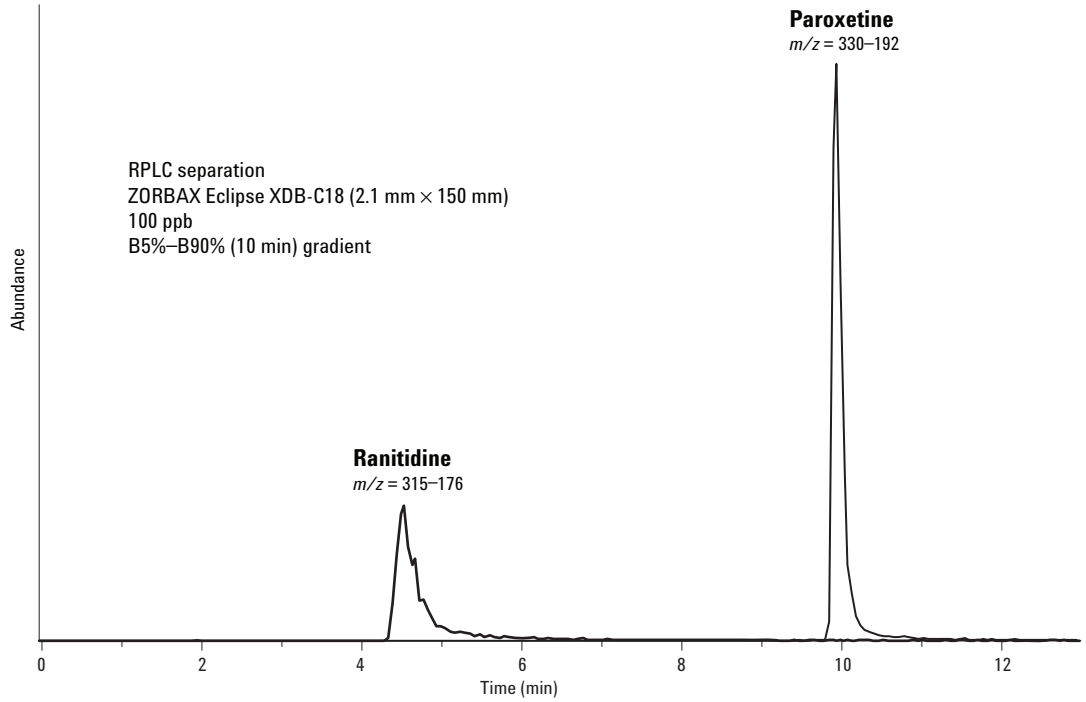


Figure 3. LC/MS/MS separation of paroxetine and ranitidine on ZORBAX Eclipse XDB-C18 column (RPLC mode).

Figure 4 depicts the separation of the two drug standards using HILIC on a silica gel column. Note that the elution order was reversed as might be expected but the peak shape for ranitidine was improved over the RPLC separation. The selectivity was not as good as with RPLC; nevertheless excellent baseline resolution was achieved. In addition, under the conditions employed, the separation was faster than with RPLC.

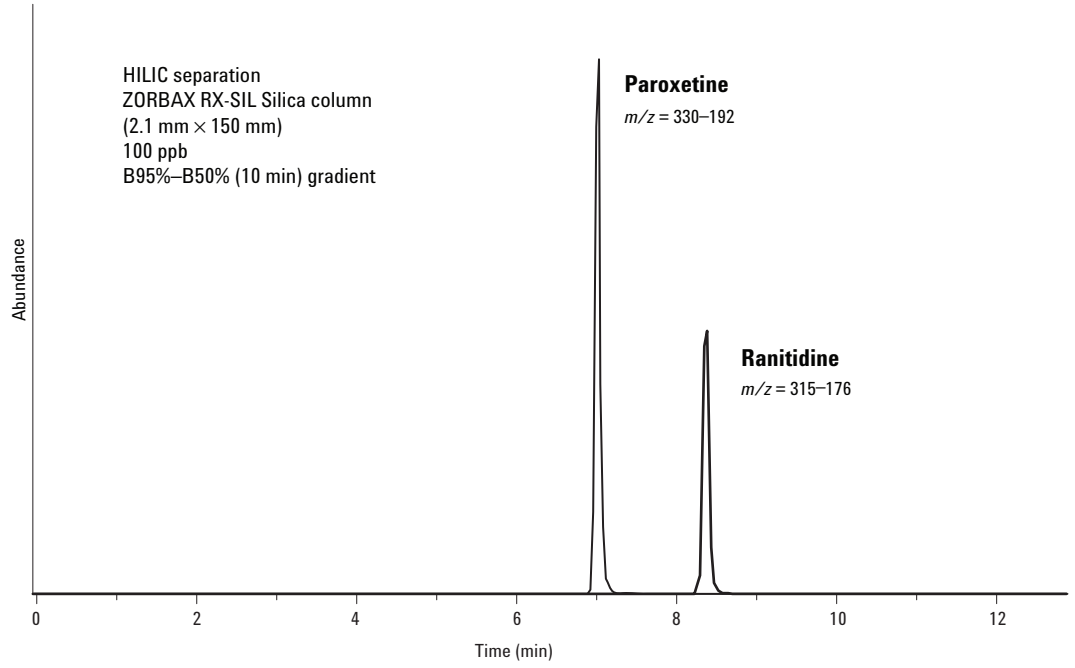


Figure 4. LC/MS/MS Separation of paroxetine and ranitidine on ZORBAX RX-SIL column (HILIC mode) – 100 ppb level.

Figure 5 shows the same separation but now at the 0.5-ppb level. Good sensitivity was noted for both drugs.

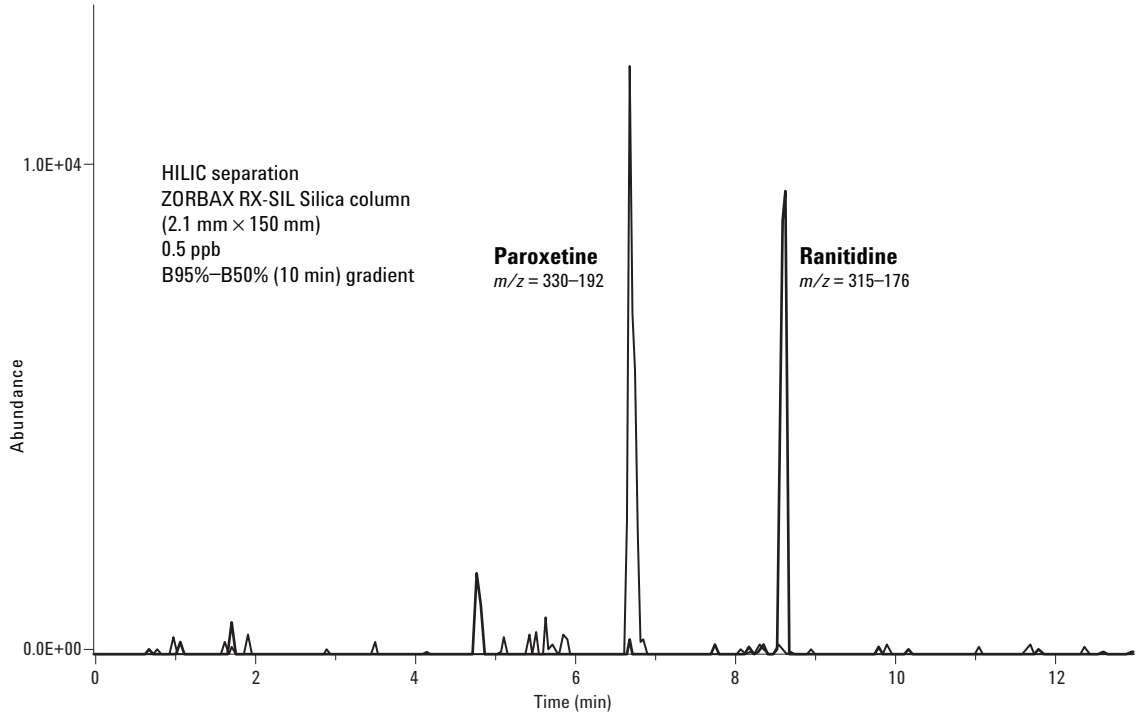


Figure 5. LC/MS/MS Separation of paroxetine and ranitidine on ZORBAX RX-SIL column (HILIC mode) – 0.5 ppb level.

We constructed a calibration curve as shown in Figure 6 and both drug compounds gave good linearity with an acceptable correlation coefficient over the expected concentration range.

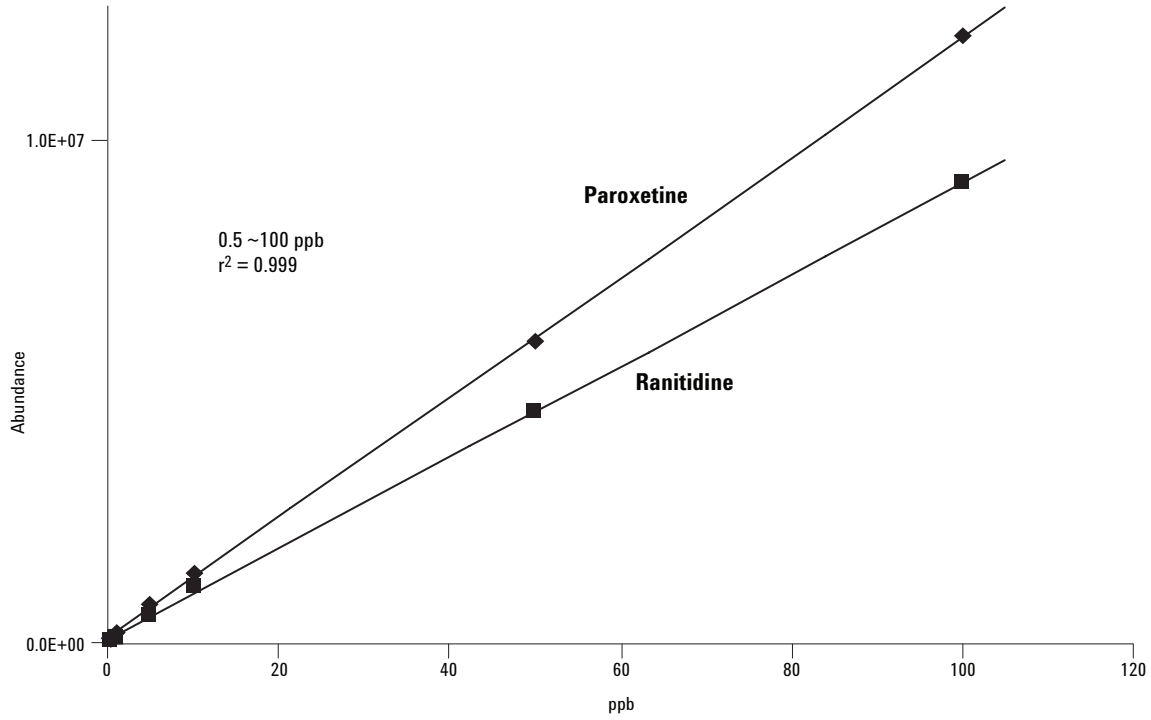


Figure 6. Linearity of paroxetine and ranitidine on ZORBAX RX-SIL Column (HILIC Mode).

Next, a human serum sample was spiked with a 5-µL aliquot of each drug compound (20 ppm) and the sample was prepared according to the sample preparation protocol outlined in Figure 7.

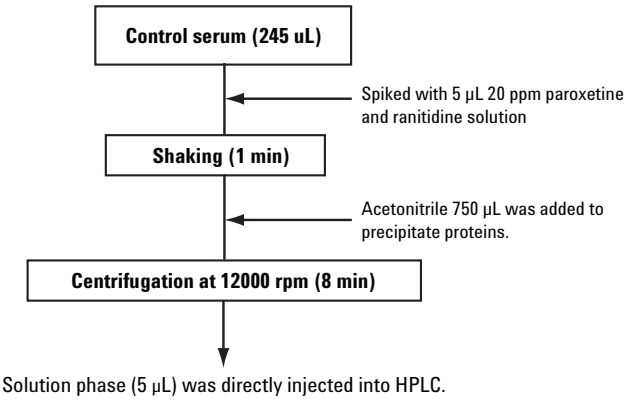


Figure 7. Sample preparation procedure of spiked serum.

A 5-µL aliquot of the cleaned-up serum sample containing the drugs was injected into the HILIC column. The resulting ion chromatograms of Figure 8 (upper trace LC/MS and lower trace LC/MS-MS) show that both compounds were able to be measured at the 100 ppb level serum. Recovery was 79% for paroxetine and 98% for ranitidine, both acceptable at these levels.

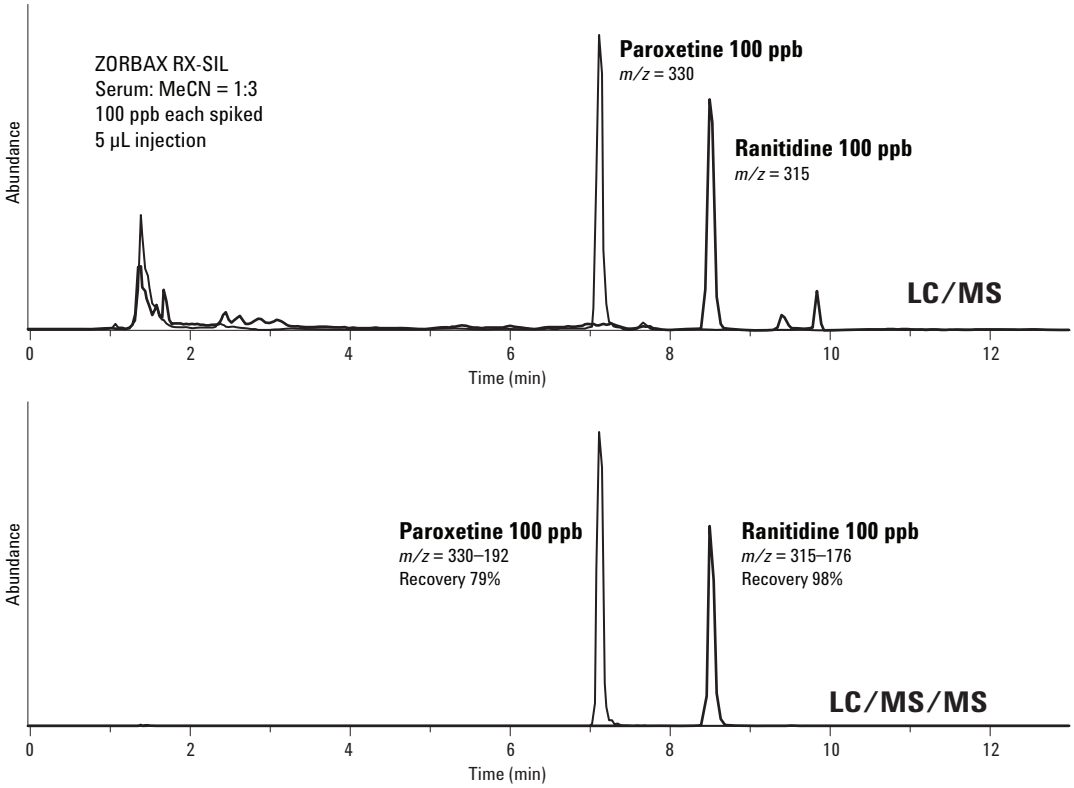


Figure 8. LC/MS and LC/MS/MS analysis of paroxetine and ranitidine spiked into human serum with ZORBAX RX-SIL Column.

Conclusion

Ranitidine and paroxetine were successfully separated using the HILIC mode. The elution order was reversed compared to the RPLC mode. Good linearity was shown over the 0.5–100 ppb levels. A spiked serum was cleaned up by protein precipitation and an aliquot was directly injected into the HILIC/ESI-MS system. Recovery was found to be 98% for ranitidine and 79% for paroxetine at the 100-ppb level.

References

1. M. Przybyciel and R. E. Majors, (2002), *LCGC No. America*, **20** (6), 516–523.
2. R. E. Majors and M. Przybyciel, (2002), *LCGC No. America*, **20** (7), 584–593.
3. W. Naidong, (2003), *J. Chromatogr. B*, **796**, 209–224.

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Printed in the USA
September 8, 2005
5989-3761EN

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