# Quantitation of Four Chiral Drug Compounds Simultaneously Using Normal Phase LC-MS/MS Equipped With an APPI lon Source

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## INTRODUCTION

Chiral separation is important but also challenging for bioanalysis of the antifungal drug luliconazole.

#### **Challenges:**

• Measurement of two geometric isomers (E/Z or trans/cis forms) & their chiral (R and S) forms

Chromatographic separation of four enantiomers by LC-MS/MS

• Typical reverse phase chromatographic conditions on non-chiral columns (like BDS Hypersil), baseline separation was only achieved between E and Z forms

• With chiral columns under typical reverse phase conditions, RE, SE, RZ and SZ still cannot be baseline separated and the required sensitivity in pg/mL. **Approach:** 

Since typical reverse phase chromatography conditions used in electrospray or APCI- MS/MS could not achieve the required separation of the 4 enantiomers, we employed APPI (Atmospheric Pressure Photo Ionization) conditions instead.

# METHOD

In this work, APPI (Atmospheric Pressure Photo Ionization) ion source was used to replace the more common ion sources like ESI (Electrospray Ionization) or APCI (Atmospheric Pressure Chemical Ionization).

#### Advantages:

• APPI is well suited to the organic solvents (e.g., hexane), which eliminates the requirement of post column aqueous addition for ESI or APCI. In practice, it was very difficult to make the aqueous addition homogenous therefore, cause signal fluctuation and seriously deteriorated the quantitative measurement.

• The organic mobile phase solvents are good dopants for APPI and lead to significantly enhanced sensitivity of LC-APPI-MS/MS.

#### Sample preparation:

 $200 \ \mu L$  plasma and 0.5 ng lanoconazole as internal standard.

LLE (Liquid-liquid Extraction) using 1.0 mL MTBE.

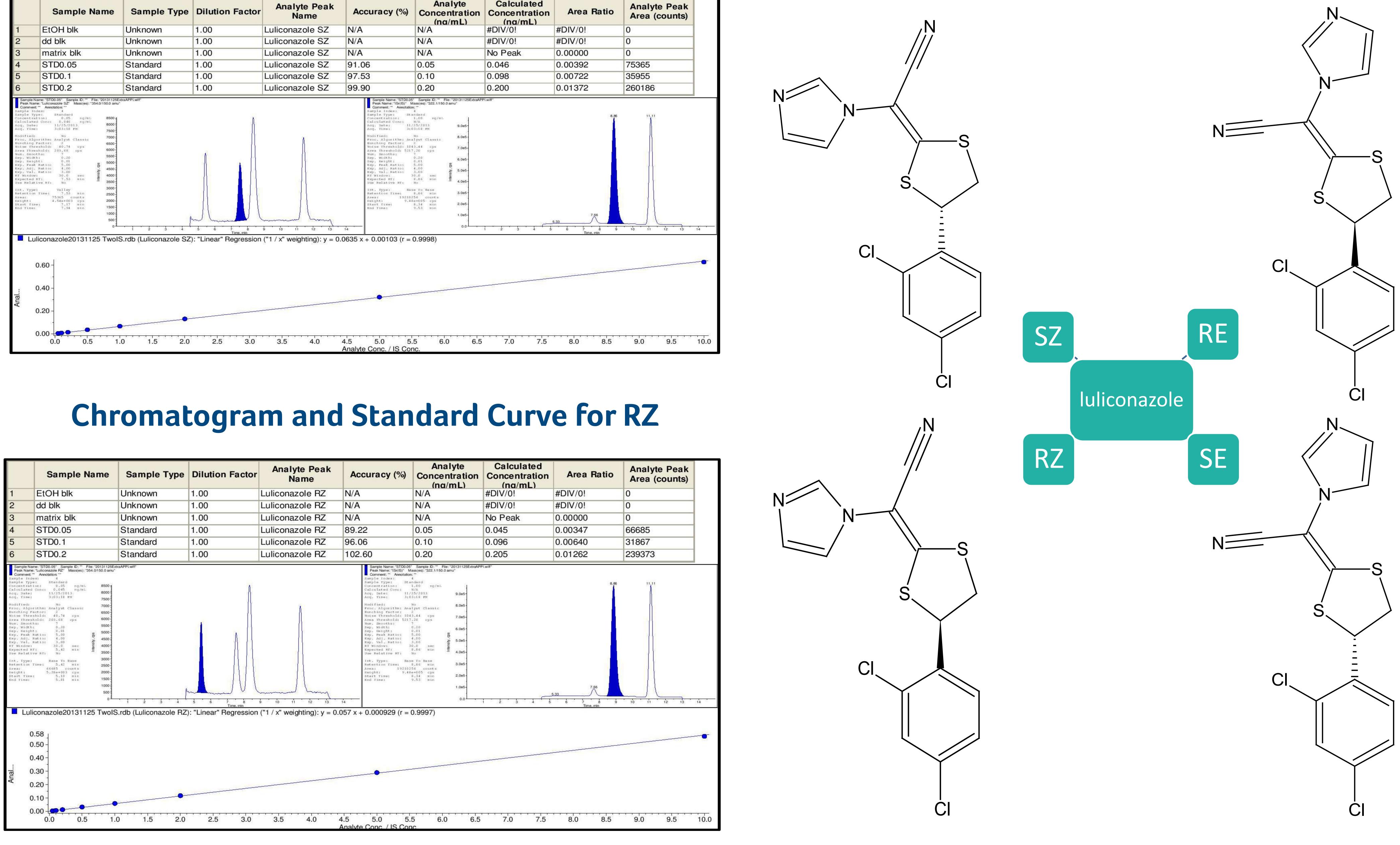
Extract is dried down at 40 °C and reconstituted in 100 uL ethanol for injection.

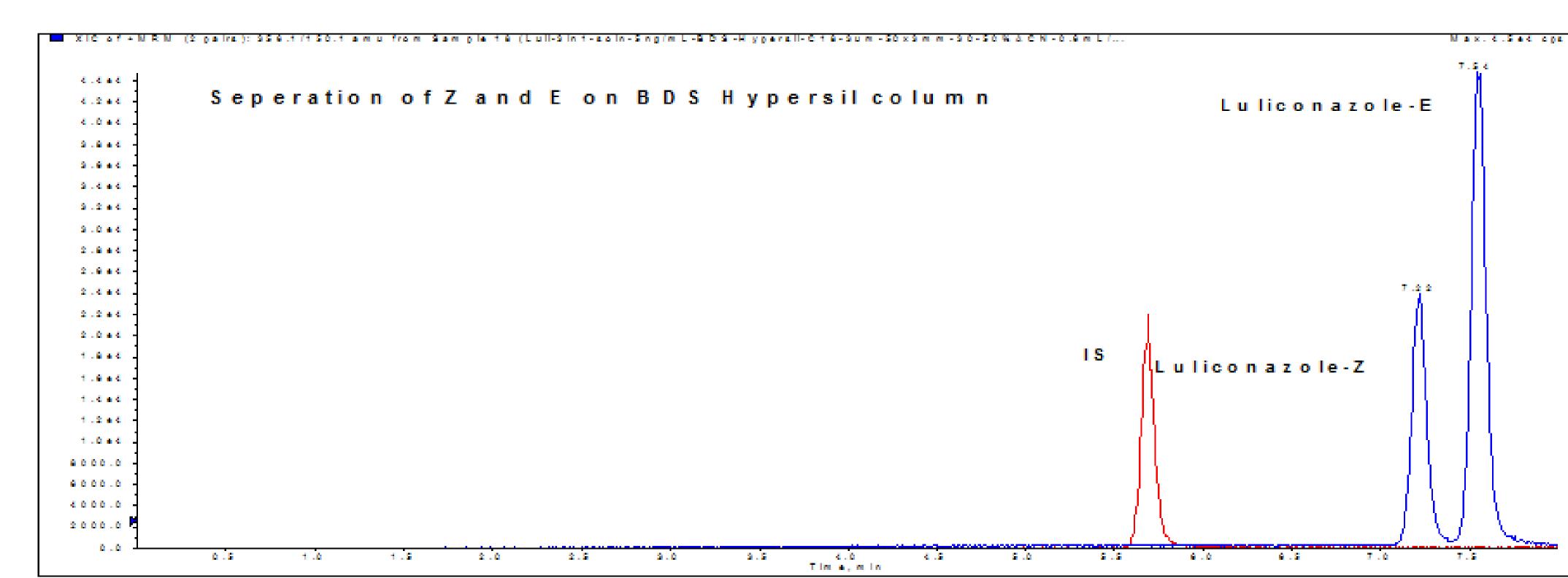
HPLC Conditions	Description		
Mobile Phase A	Ethanol		
Mobile Phase B	Hexane		
Autosampler Temperature	Ambient		
Column	Phenomenex Lux, Cellulose-		
Column	1, 100 x 4.6 mm, 3 μm		
Flow Rate (mL/min)	0.9-1.5		
Injection Volume (µL)	20		
Sciex API 5000 triple quad	APPI		
Ionization Mode	Positive		
Assay Range	50 – 10,000 pg/mL		
Sample Treatment	Liquid-Liquid Extraction		
Anticoagulant	K <sub>2</sub> EDTA		

Time (Minute)	Flow Rate, mL/min	<b>B%</b>	
0	0.9	80	
0.5	0.9	80	
1	0.9	70	
9.3	0.9	66	
9.31	1.5	60	
12.7	1.5	60	
12.71	1.5	80	
14.2	1.5	80	
14.21	0.9	80	
15	Stop		

### **Chromatogram and Standard Curve for SZ**

	Sample Name	Sample Type	Dilution Factor	Analyte Peak Name	Accuracy (%)	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	Area Ratio	Analyte Peak Area (counts)
1	EtOH blk	Unknown	1.00	Luliconazole SZ	N/A	N/A	#DIV/0!	#DIV/0!	0
2	dd blk	Unknown	1.00	Luliconazole SZ	N/A	N/A	#DIV/0!	#DIV/0!	0
3	matrix blk	Unknown	1.00	Luliconazole SZ	N/A	N/A	No Peak	0.00000	0
4	STD0.05	Standard	1.00	Luliconazole SZ	91.06	0.05	0.046	0.00392	75365
5	STD0.1	Standard	1.00	Luliconazole SZ	97.53	0.10	0.098	0.00722	35955
6	STD0.2	Standard	1.00	Luliconazole SZ	99.90	0.20	0.200	0.01372	260186
Comment: Sample Inde Sample Type Concentrati Calculated Acq. Date: Acq. Time: Modified: Proc. Algos Bunching Fa Noise Thres Area Thres Area Thres Num. Smoot) Sep. Width Sep. Width Sep. Width Sep. Width Exp. Peak I Exp. Adj. I Exp. Val. I Exp. Val. I Exp. Val. I Exp. Val. I Expected R Use Relativ Int. Type: Retention Tarea: Height: Start Time:	e: Standard ion: 0.05 ng/mL 8500 Conc: 0.046 ng/mL 8500 11/25/2013 8000 3103:18 PM 7500 No 7000 No 7000 Sold: 40.74 cps 6500 No: 7000 Sold: 203.68 sps 6000 No: 7000 Sold: 203.68 sps 6000 No: 7000 Sold: 5000 Ratio: 5.00 80 Ratio: 5.00 80 Ratio: 5.00 80 Ratio: 3.00 8ec 80 Concer 90 Sold: 30.0 sec 90 Sold: 30.0 sec 90 No 3000 Fime: 7.53 min 90 Valley 3000 Fime: 7.53 min 2500 75365 counts 4.54e+003 cps 2000	rdb (Luliconazole SZ)	): "Linear" Regression		Concentration: Calculated Conc: Acq. Date: 11/ Acq. Time: 3:0 Modified: Proc. Algorithm: Ana Bunching Factor: Noise Threshold: 104 Area Threshold: 5217 Num. Smooths: Sep. Width: Sep. Width: Sep. Height: Exp. Peak Ratio: Exp. Val. Ratio: Exp. Val. Ratio: Exp. Val. Ratio: RT Window: 3 Expected RT: Use Relative RT: Int. Type: Bas Retention Time: Area: 192102 Height: 3.48 Start Time: End Time:	4 ndard 1.00 rg/mL N/A 25/2013 9.0e5- 3:18 PM No 1yst Classic 2 3.44 cps 7.0e5- 7 0.20 cps 7 0.20 6.0e5 5.00 8 4.00 3.00 5.0e5 0.0 sec 9 8.86 min 4.0e5- No 4.0e5- 8.86 min 3.0e5 5.0 cps 7 0.20 0.0 sec 9 8.86 min 1.0e5- 0.0 cps 7 0.00 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.		5.33 6 7 8 9 Time, min	
Anal o	.60 - .40 - .20 - .00	) 1.5 2.0	2.5 3.0		4.5 5.0 5. Analyte Conc. / IS Con		7.0 7.5	8.0 8.5	9.0 9.5







**Compound Structure** 

RESULTS

Under the optimized conditions:

• 4 chiral compounds were baseline separated and eluted at 5.4, 7.5, 8.3 and 11.4 min, respectively.

• internal standard was also separated as two chiral forms, with RT at 9.0 and 11.1 min. It was demonstrated that better accuracy was obtained by applying a second IS for correcting the latest eluting chiral compound, while using the IS at 8.9 min for SZ, RZ, RE and 11.1 min for SE.

• LLOQ was 50 pg/mL for all four chiral compounds.

# (2) FRONTAGE

## **Chromatogram and Standard Curve for RE**

olk 5	Unknown Unknown Unknown Standard Standard Standard	1.00 1.00 1.00 1.00	Luliconazole RE Luliconazole RE Luliconazole RE	N/A	manufacture and the second	(na/mL) #DIV/0! #DIV/0!	#DIV/0! #DIV/0!	0
5 nple ID: "' File: "20131125Extra Mass(es): "354.0/150.0 amu"	Unknown Standard Standard	1.00 1.00	Luliconazole RE		N/A	#DIV/0!	#DIV/01	0
5 nple ID: "' File: "20131125Extra Mass(es): "354.0/150.0 amu"	Standard Standard	1.00		N/A			#DIV/0:	0
nple ID: "" File: "20131125Extra Mass(es): "354.0/150.0 amu"	Standard		I PERSONAL DE	0.0	N/A	No Peak	0.00000	0
nple ID: "" File: "20131125Extra Mass(es): "354.0/150.0 amu"		1 00	Luliconazole RE	87.66	0.05	0.044	0.00781	150101
nple ID: "" File: "20131125Extra Mass(es): "354.0/150.0 amu"	Standard	1.00	Luliconazole RE	87.70	0.10	0.088	0.01333	66319
Mass(es): "354.0/150.0 amu"	Standard	1.00	Luliconazole RE	103.58	0.20	0.207	0.02834	537429
ng/mL 8500 ng/mL 6000 PM 7500 Classic 6500 cps 6500 sec 5500 min 3500 min 2500 min 2500 min 1500 min 1500 0	S.rdb (Luliconazole RE	): "Linear" Regression	("1 / x" weighting): y = 0.1	Calculated Conc: N Acg. Date: 11/2 Acg. Time: 3:03 Modified: N Proc. Algorithm: Anal Bunching Factor: 2 Noise Threshold: 1043 Area Threshold: 5217. Num. Smooths: 7 Sep. Width: 0 Sep. Height: 0 Exp. Peak Ratio: 4 Exp. Val. Ratio: 4 Exp. Val. Ratio: 3 RT Window: 30 Expected RT: 8 Use Relative RT: N Int. Type: Base Retention Time: 8 Area: 1921025 Height: 9.48e Start Time: 9	dard .00 ng/mL /A 5/2013 9.0e5 118 PM o yst Classic .44 cps 20 cps .20 cps .20 6.0e5 .00 8 .00 8 .00 5.0e5 .00 sec .86 min 4.0e5 o To Base .86 min 3.0e5 .34 min 2.0e5 .34 min 1.0e5 .00 0.00	- <u>1</u> - <u>1</u> - <u>5</u>	7.66 5.33 6 7 8 9 Time, min	

### **Chromatogram and Standard Curve for SE**

#### CONCLUSIONS

LC-APPI-MS/MS is a viable alternative to typical electrospray or chemical ionization techniques for bioanalysis of chiral analytes. The APPI source allowed for use of mobile phase which provided both the needed chromatographic separation, as well as enhanced sensitivity needed, in order to quantitate all 4 chiral analytes with a 15 minute run time.