Quantitation of Four Chiral Drug Compounds Simultaneously Using Normal Phase LC-MS/MS Equipped With an APPI Ion 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 µL plasma and 0.5 ng lanoconazole as internal standard.

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

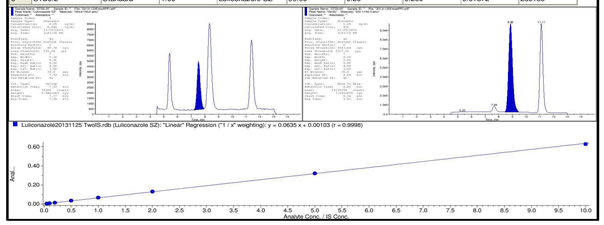
Extract is dried down at 40 °C and reconstituted in 100 uL ethanol for injection.

HPLC Conditions	Description
Mobile Phase A	Ethanol
Mobile Phase B	Hexane
Autosampler Temperature	Ambient
Column	Phenomenex Lux, Cellulose-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_2EDTA

Time (Minute)	Flow Rate, mL/min	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	

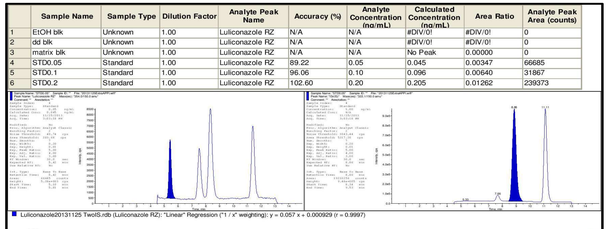
Chromatogram and Standard Curve for SZ

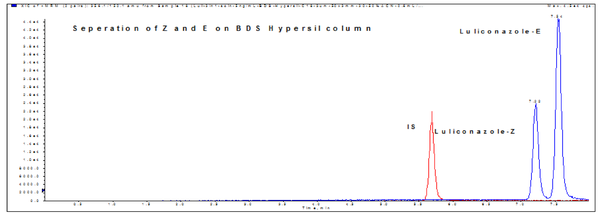
	Sample Name	Sample Type	Dilution Factor	Analyte Peak Name	Accuracy (%)	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	Area Ratio	Analyte Peak Area (counts)
1	EtOH blk	Unknown	1.00	Luliconazole SZ	N/A	N/A	#DIV/0!	#DIV/0!	0
2	dd blk	Unknown	1.00	Luliconazole SZ	N/A	N/A	#DIV/0!	#DIV/0!	0
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



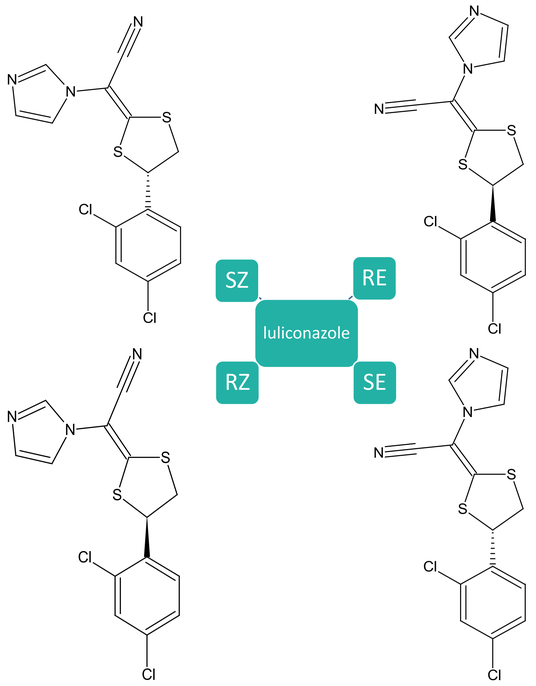
Chromatogram and Standard Curve for RZ

	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 RZ	N/A	N/A	#DIV/0!	#DIV/0!	0
2	dd blk	Unknown	1.00	Luliconazole RZ	N/A	N/A	#DIV/0!	#DIV/0!	0
3	matrix blk	Unknown	1.00	Luliconazole RZ	N/A	N/A	No Peak	0.00000	0
4	STD0.05	Standard	1.00	Luliconazole RZ	89.22	0.05	0.045	0.00347	66685
5	STD0.1	Standard	1.00	Luliconazole RZ	96.06	0.10	0.096	0.00640	31867
6	STD0.2	Standard	1.00	Luliconazole RZ	102.60	0.20	0.205	0.01262	239373





Compound Structure



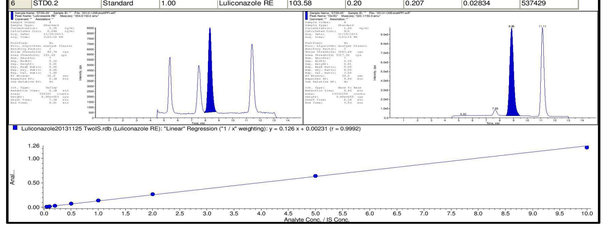
RESULTS

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.**

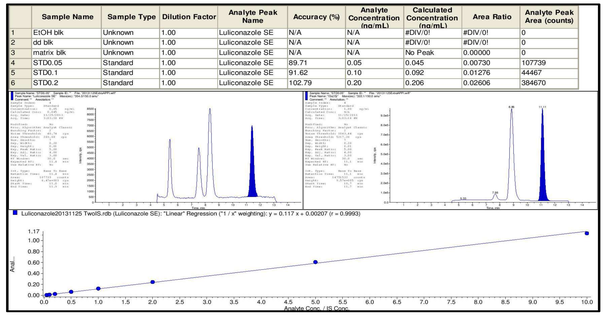
Chromatogram and Standard Curve for RE

	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 RE	N/A	N/A	#DIV/0!	#DIV/0!	0
2	dd blk	Unknown	1.00	Luliconazole RE	N/A	N/A	#DIV/0!	#DIV/0!	0
3	matrix blk	Unknown	1.00	Luliconazole RE	N/A	N/A	No Peak	0.00000	0
4	STD0.05	Standard	1.00	Luliconazole RE	87.66	0.05	0.044	0.00781	150101
5	STD0.1	Standard	1.00	Luliconazole RE	87.70	0.10	0.088	0.01333	66319
6	STD0.2	Standard	1.00	Luliconazole RE	103.58	0.20	0.207	0.02834	537429



Chromatogram and Standard Curve for SE

	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 SE	N/A	N/A	#DIV/0!	#DIV/0!	0
2	dd blk	Unknown	1.00	Luliconazole SE	N/A	N/A	#DIV/0!	#DIV/0!	0
3	matrix blk	Unknown	1.00	Luliconazole SE	N/A	N/A	No Peak	0.00000	0
4	STD0.05	Standard	1.00	Luliconazole SE	89.71	0.05	0.045	0.00730	107739
5	STD0.1	Standard	1.00	Luliconazole SE	91.62	0.10	0.092	0.01276	44467
6	STD0.2	Standard	1.00	Luliconazole SE	102.79	0.20	0.206	0.02606	384670



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.