# CHIRAL PURIFICATION OF IRIDIUM (III) COMPLEXES BY SFC

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## INTRODUCTION

**Iridium (III) complexes are phosphorescent materials used in organic light-emitting diode (OLED) applications and as intracellular fluorescent probes in biological applications.[1] Octahedral iridium complexes bearing at least two bidentate ligands exhibit intrinsic metal-centered stereochemistry. During synthesis of Iridium (III) complexes, stereoisomers are formed as a racemic mixture. Interestingly, different photophysical properties exist between enantiopure and racemic emitters, which can affect device performance. In order to obtain enantiopure complexes, chiral resolution is required to isolate the pure enantiomers.[2]**

**The chiral separation of Iridium (III) complexes has been previously accomplished by normal phase chiral HPLC.[3,4] SFC, however, has many advantages over normal phase HPLC, especially for chiral separation and purification. Here, two example Iridium (III) complexes, Bis[2-(4,6-difluorophenyl)pyridinato-C$^2$,*N](picolinato) iridium(III)* (Flrpic) and Fac-Tris[2-phenylpyridinato-C$^2$,*N]iridium(III*) ($Ir(ppy)_3$) will be used to demonstrate the separation and purification of the enantiomers of Iridium (III) complexes using SFC (structures are shown in Figure 1). Method screening and development using the Waters ACQUITY UPC$^2$ system will be discussed and purification will be accomplished using the Waters Prep SFC 150 Mgm System.**

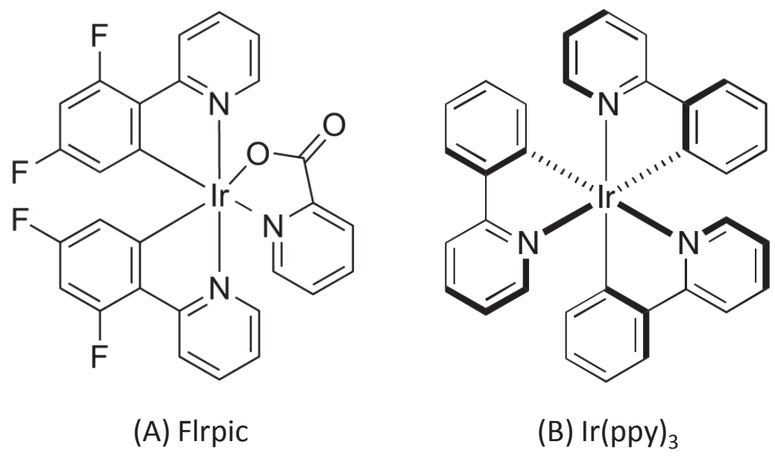


*Figure 1: Structures for (A) Bis[2-(4,6-difluorophenyl)pyridinato-C$^2$,N](picolinato)iridium(III) (Flrpic) and (B) Fac-Tris[2-phenylpyridinato-C$^2$,N]iridium(III) ($Ir(ppy)_3$)*

## METHODS

### Sample Preparation

The Flrpic sample (A) was prepared by dissolving 10 mg in 10 mL of 50:50 acetonitrile:isopropanol, for a 1 mg/mL concentration. The $Ir(ppy)_3$ sample (B) had very limited solubility and was prepared by sonicating 5 mg in 10 mL of acetonitrile for 5 mins, followed by filtration through a 0.45 µm syringe filter.

### Analytical Method Screening Conditions

System: Waters ACQUITY UPC$^2$ System, plumbed for modifier-stream injections

Software: MassLynx 4.1 Software

Flow rate: 3 mL/min

Gradient:

| Time (min) | % $CO_2$ | %Co-Solvent |
|---|---|---|
| 0 | 70 | 30 |
| 5 | 50 | 50 |
| 6 | 50 | 50 |
| 7 | 70 | 30 |
| 9 | 70 | 30 |

Pressure: 124 bar (1800 psi)

Temperature: 40°C

ACQUITY PDA Channel 1: 251 nm

Column: 4.6 x 150mm, 5 µm, Chiralpak IA

Injection volume: 2 µL

Co-solvents: Screening will be discussed

### Preparative method conditions:

System: Waters Prep SFC 150 Mgm System

Software: Chromscope 2.0

Column: 21x150mm, 5 µm, Chiralpak IA

Temperature: 40°C

Pressure: 124 Bar

2489 UV Detector: 251nm

Scaled-up flow and solvent conditions will be discussed.

### Fraction analysis:

System: Waters ACQUITY UPC$^2$ System plumbed for modifier-stream injections.

Software: MassLynx 4.1 Software

Methods: as noted in figures

## RESULTS & DISCUSSION

Initial screening using standard 5 to 50% gradients showed either no elution of the compounds, or very late elution. As a result, the screening gradients were adjusted to start at a higher 30% co-solvent condition. Also, based on sample solubility, acetonitrile (ACN) and isopropanol (IPA) were screened as probable co-solvents that would work well for eluting these compounds. Preparative column availability was limited to a 21 x 150, 5 µm, Chiralpak IA for chiral separations, so a matching 4.6 x 150 mm, 5 µm Chiralpak IA column was used for method development on the Waters ACQUITY UPC$^2$ System. The results of the co-solvent screening are shown in Figure 2. The Flrpic sample showed good separation using the 50:50 mix of acetonitrile and isopropanol, while the $Ir(ppy)_3$ sample separated well using isopropanol as the co-solvent.

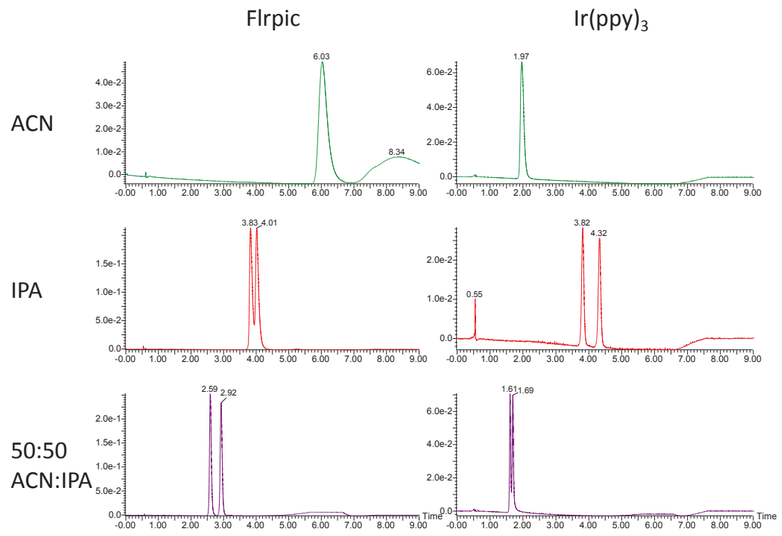


*Figure 2: Co-solvent screen of Flrpic and $Ir(ppy)_3$ on the 4.6 mm Chiralpak IA column. Method conditions: 3 mL/min total flow, 30-50% co-solvent in 5 minutes, 40°C, 124 bar, 2 µL injection volume.*

Isocratic separation conditions were determined so that stacked injections could be performed at the prep scale. The elution co-solvent percentages were calculated, based on the retention times and gradient slope, to be 38% for Flrpic and 43% for $Ir(ppy)_3$. In SFC, 5-10% is subtracted from the elution percentage to determine a good starting point for isocratic separation conditions. Using this strategy, the optimal flow conditions were determined to be 30% 50:50 acetonitrile:isopropanol for the Flrpic sample (Figure 3, (A)) and 38% isopropanol for the $Ir(ppy)_3$ sample (Figure 3, (B)). It was observed that the Flrpic separation was greatly affected by the slightest change in the ACN:IPA ratio of the co-solvent, including the make up of the sample diluent. Both methods were optimized using 20 µL injection volumes on the UPC$^2$ System before scale-up to the Prep SFC 150 Mgm System.

In order to scale the flow conditions, the $CO_2$ flow rates on the UPC$^2$ were converted from the volumetric (mL/min) to mass (g/min) flow rates before scaling geometrically. This was done to account for the differences in $CO_2$ pump control between the UPC$^2$ System (volumetric flow) and the Prep SFC 150 Mgm System (mass flow).[5] The scaled flow conditions were determined to be 60.4 mL/min at 31% for the Flrpic sample and 60.7 mL/min at 39% for the If$(ppy)_3$ sample. The injection volumes were scaled geometrically from 20 µL on the 4.6 mm ID column to 420 µL on the 21 mm ID column. The resulting scaled chromatography, shown in Figure 3, (C) and (D) provided good resolution and peak shape.

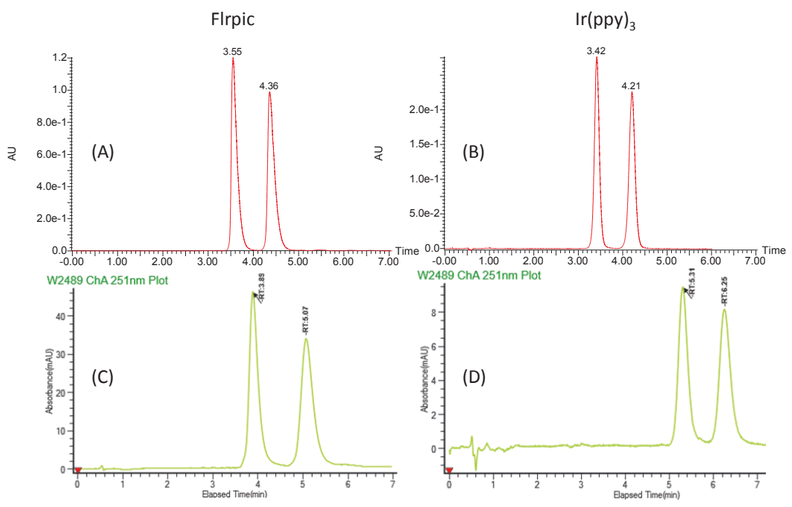


*Figure 3: Optimized isocratic and scaled-up separations of Flrpic and Ir $(ppy)_3$ samples on the 4.6 mm and 21 mm Chiralpak IA columns. Method conditions (A): 3 mL/min total flow, 30% 50:50 ACN:IPA, 40°C, 124 bar, 20 µL injection volume, (B): 3 mL/min total flow, 38% IPA, 40°C, 124 bar, 20 µL injection volume, (C): 60.4 mL/min total flow, 31% 50:50 ACN:IPA, 40°C, 124 bar, 420 µL injection volume, (D): 60.7 mL/min total flow, 39% IPA, 40°C, 124 bar, 420 µL injection volume*

The $Ir(ppy)_3$ sample was separated using isopropanol as the co-solvent, but was significantly more soluble in acetonitrile, which was consequently used as the diluent. Due to the limited solubility, the injection volume was increased to 500 µL. Despite using modifier-stream injection mode for sample introduction, this resulted in the diluent affecting the chromatography by decreasing retention time and resolution, .

During stacked injections, the effect was more significant because the sample diluent was on-column with previously injected peaks. As a result, and in order to maintain resolution during stacked injections of the Ir(ppy)$_3$ sample, the co-solvent percentage was lowered to 38% isopropanol.

For both Iridium(III) complex samples, five stacked injections were performed and the separated enantiomers were collected. The resulting chromatography is shown in Figure 4.

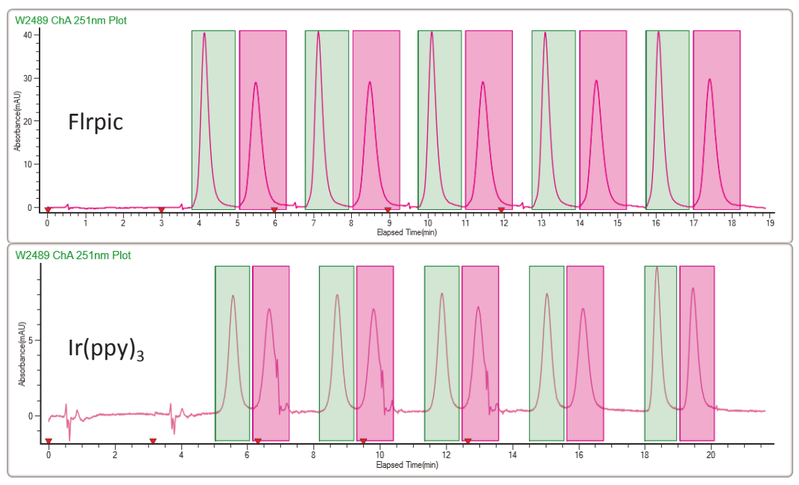


*Figure 4: Stacked injections with collection of Flrpic and $Ir(ppy)_3$ samples using the 21 mm Chiralpak IA column on the Prep SFC 150 Mgm System. Method conditions (Flrpic): 60.4 mL/min total flow, 31% 50:50 ACN:IPA, 40°C, 124 bar, 420 µL injection volume, ($Ir(ppy)_3$): 60.7 mL/min total flow, 38% IPA, 40°C, 124 bar, 500 µL injection volume*

The Flrpic fractions were transferred to 100 mL flasks and diluted to volume with acetonitrile. A standard was prepared by transferring 2.1 mL (the total volume injected) of the Flrpic sample to a 100 mL flask and diluting to volume with acetonitrile. Recovery was determined by injecting 10 µL each of the standard and the two fractions.

The $Ir(ppy)_3$ fractions were rotovapped down, transferred to 25 mL flasks and brought to volume with acetonitrile. The standard was prepared by transferring 2.5 mL (the total injected volume) of the $Ir(ppy)_3$ sample to a 25 mL flask and diluting to volume with acetonitrile. Recovery was determined by injecting 20 µL each of the standard and the two fractions.

Fraction analysis was performed on the ACQUITY UPC$^2$ System using the optimized isocratic method conditions (Figure 5). Recovery was calculated by dividing the peak area of the fraction by the area of the corresponding standard peak and multiplying by 100. For the Flrpic sample, fraction 1 recovery was 95% and fraction 2 recovery was 97%. For the Ir $(ppy)_3$ sample, fraction 1 recovery was 87% and fraction 2 recovery was 89%. The lower recovery was likely due to the lower solubility and sample transfer during the dry down and reconstituting process. No impurities were detected in the Flrpic fractions or fraction 1 of the $Ir(ppy)^3$ sample. Fraction 2 of the $Ir(ppy)_3$ sample contained a small amount (~1.5%) of peak 1.

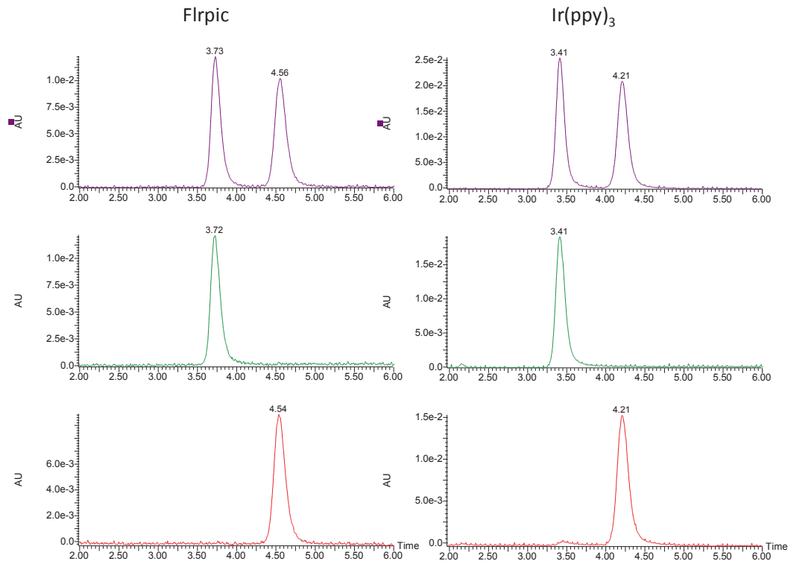


*Figure 5: Fraction analysis of the Flrpic and $Ir(ppy)_3$ fractions on the 4.6 mm Chiralpak IA column on the ACQUITY UPC$^2$ System. Method conditions (Flripic): 3 mL/min total flow, 30% 50:50 ACN:IPA, 40°C, 124 bar, 10 µL injection volume, (B): 3 mL/min total flow, 38% IPA, 40°C, 124 bar, 20 µL injection volume*

## CONCLUSIONS

- **Isocratic methods were developed to successfully separate the enantiomers of two iridium (III) complexes on the ACQUITY UPC$^2$ System, showing that SFC is a good technique for the chiral separation of these complexes.**
- **Successful scaling of the chiral separations was demonstrated from the ACQUITY UPC$^2$ System to the Prep SFC 150 Mgm System.**
- **Purification of the enantiomers of the iridium (III) complexes was accomplished using stacked injections, resulting in enantiomerically pure fractions and good sample recovery.**
- **SFC purification of the enantiomers of iridium (III) complexes would allow for better control over the photophysical properties and applications affected by these complexes.**

### References

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