# Applicability of a Noviplex™ Blood-Plasma Sampling Device for the Analysis of Itraconazole

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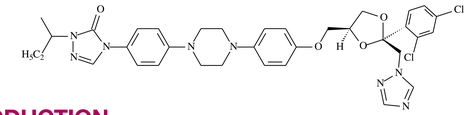
## OBJECTIVE

The objective of this study was to test the performance and applicability of the Noviplex™ blood-sampling device for collection and analysis of bioanalytical samples containing itraconazole, a commonly prescribed antifungal agent.

**Figure 1. Itraconazole**



## INTRODUCTION

The Noviplex technology consists of a series of membranes and filters that remove cells from whole blood through capillary action ending with a simple absorbent disc that collects a fixed volume of plasma. Approximately 2.5 µL of plasma is isolated, when a drop of blood between 25 and 75 µL is applied and plasma separation occurs within 3 minutes of application.[1] Significant advantages associated with this method include a dramatic reduction in the amount of blood needed from participants, elimination of the phlebotomist, venipuncture, and centrifugation necessary for traditional plasma collection, as well as, the possible elimination of dry ice needed for transport.

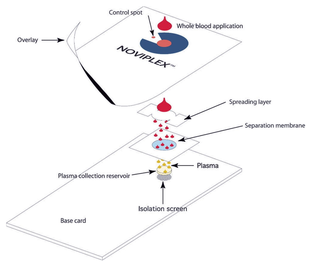
Dried blood spot (DBS) collection on filter paper is an alternative nontraditional sampling method touted as having similar advantages as the Noviplex technology. However, DBS does not remove red blood cells resulting in significant differences in hematocrit levels shown to negatively affect quantification accuracy.[2]

Hematocrit levels can vary between 20% and 70% of blood volume, so impact of this variable may be significant.[1] Noviplex technology, similar to traditional plasma collection, is independent of hematocrit level.

**Figure 2. Illustration of Blood Spotting on the Noviplex Isolation Card.**

A B C

**Figure 3. Illustration of the Migration and Separation of the Red Blood Cells from the Plasma Using the Noviplex Technology.**




## METHODS

Initial testing with neat solutions confirmed minimal adsorption and acceptable recovery of the analyte from the collection discs. Following sample collection as described in Figure 2, the disc loaded with 2.5 µL of plasma was removed from the card with tweezers and placed in a small polypropylene centrifuge tube containing acetonitrile along with internal standard. The sample tube was vortexed for several minutes, centrifuged and the acetonitrile was transferred to a clean 96-well plate, evaporated to dryness, and reconstituted in an appropriate injection solvent prior to analysis by LC-MS/MS.

The traditional extraction method compared used a protein precipitation approach and a small volume of acetonitrile added to a fixed volume of plasma (approximately 40 µL) containing itraconazole, in the presence of the internal standard. This process afforded separation of the compound from a majority of the proteins contained within the plasma; the compound was then transferred to a clean 96-well plate, evaporated to dryness and reconstituted in an appropriate injection solvent prior to analysis by LC-MS/MS.

Finally whole blood samples containing different concentrations of itraconazole and different volumes (30-40 µL) of spotted whole blood were analyzed using the Noviplex technology and the recovery was determined. This evaluation was conducted to assess the effects of variable blood volume application and the reproducibility of the fixed-volume plasma collection discs. It is important to note that whole blood samples were collected and processed using both traditional plasma isolation techniques and the Noviplex plasma isolation cards. Chromatographic separation of all of extracts was performed with an ACE® Phenyl (50 x 3.0 mm) column using gradient elution. Characteristic precursor and product ions were generated and detected using atmospheric pressure chemical ionization (APCI) in positive mode and detected on an AB Sciex API 5000 platform.

## RESULTS

Itraconazole quantitation was initially evaluated for a calibration range of 2.30 1130 ng/mL. However, due to volume restrictions for the plasma isolation from the whole blood samples analyzed with the Noviplex discs, the detectable range was between 22.5 - 1130 ng/mL. Recovery from the Noviplex discs was determined to be 30% to 48% for itraconazole concentrations (22.5 - 1130 ng/mL), while recovery from the protein precipitation was 89 to 93% for itraconazole concentrations (3.00 - 400 ng/mL), refer to Table 1.

**Table 1: Recovery Determination of Itraconazole on Noviplex Cards**

| Standards | | | | | |
|---|---|---|---|---|---|
| Batch | STD | Peak Area EXT | Peak Area REX | Recovery | Ratio of REX/EXT |
| 10 | B | 1737 | 4087 | 43 | 2.35 |
| | C | 3437 | 11859 | 29 | 3.45 |
| | D | 8059 | 19344 | 42 | 2.40 |
| | E | 13545 | 57088 | 24 | 4.21 |
| | F | 33532 | 128503 | 26 | 3.83 |
| | G | 78894 | 230409 | 34 | 2.92 |
| | H | 136450 | 361420 | 38 | 2.65 |
| | I | 319094 | 767637 | 42 | 2.41 |
| | J* | 525107 | 3320390 | 16 | 6.32 |
| | K | 659320 | 1374103 | 48 | 2.08 |

*Rejected

**Figure 4. Representative Chromatograms from the LLOQ Achieved from a Sample Isolated Using Traditional Plasma Extraction (Green), Noviplex Technology (Blue) and a Control Blank Sample (Red).**

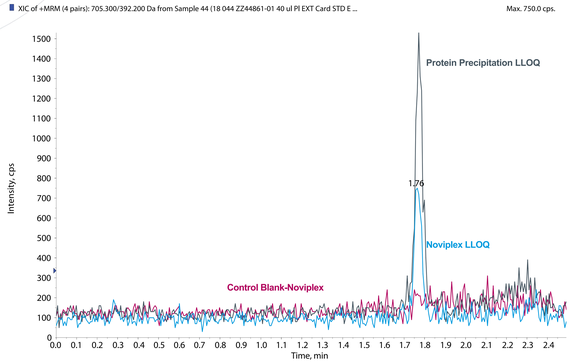
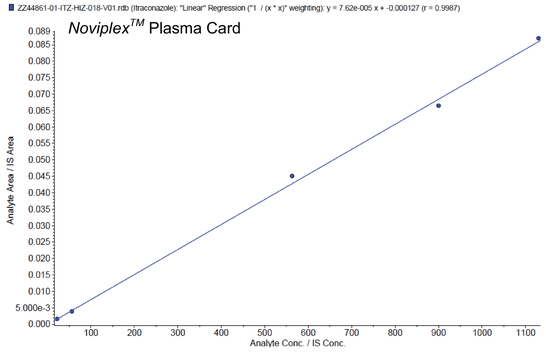
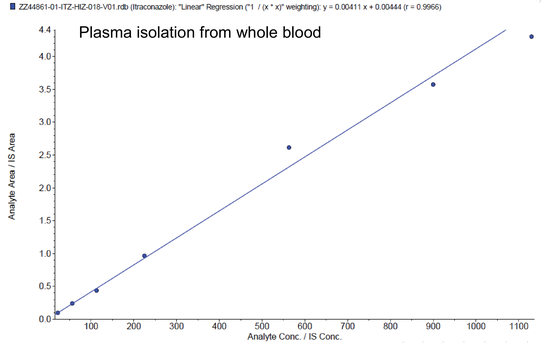



**Figure 5: Whole Blood Analysis of Itraconazole Using Noviplex Technology**




One important benefit in using the Noviplex plasma collection method is the significant reduction in the volume of matrix needed for sample analysis, with the added benefit of minimizing issues related to matrix effect. In this approach the Noviplex technology used approximately 50-fold less sample volume than the traditional plasma method for itraconazole with a loss of only a 10-fold difference in sensitivity. The reduction in sample volume is not only economical, but more importantly reduces the amount of sample needed for collection from study participants. An additional benefit of the reduction in blood volume needed, ease of sample collection, shipping and storage makes it ideal for use in field studies.

## CONCLUSION

The Noviplex plasma collection device was successfully used for the collection and analysis of a pharmaceutically relevant small molecule. There were minimal complications with analyte recovery and adsorption. In traditional dried blood spot analysis a major complication involves the variance associated with differing hematocrit levels from individual patients, however with the removal of the blood cells that occurs using this technology, that factor is minimized. Long term stability of the analyte on the plasma isolation cards and reproducibility of the extraction method are under investigation.

## REFERENCES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- Dewilde, A.; Sadilkova, K.; Sadilek, M.; Vasta, V.; Hahn, S. H., Clin. Chem. 2008, 54, 1961–1968.


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