# ISOLATING DRUGS FROM BIOLOGICAL FLUIDS USING "SOLVENT FIRST" PROTEIN PRECIPITATION

## INTRODUCTION

Protein precipitation is a routine, high throughput bioanalytical sample preparation technique. It is used to remove proteins from biological fluid samples prior to the analysis of drugs and their metabolites by LC-MS/MS. The technique has wide applicability in bioanalysis from discovery support through to clinical studies.

Historically, protein precipitation has been carried out in vials or collection plates, followed by centrifugation. Protein precipitation by 96-well filtration has more recently been used as a high throughput, easy to automate alternative to the traditional approach. However, most filterplates require the plasma sample to be dispensed before the precipitating solvent is added — the "plasma first" method.[1] This approach can lead to filtrate breakthrough before precipitation is complete, resulting in cloudy extracts and blocked wells.

ISOLUTE® PPT+ Protein Precipitation Plates have been designed to overcome the problems of the first generation 96-well filterplates. The functionalized bottom frit holds up organic solvents, allowing the precipitating solvent to be dispensed into each well prior to sample addition. This "solvent first"[2] approach has a number of benefits to the subsequent analytical technique. This Application Note reviews the use of ISOLUTE PPT+ plates for the isolation of drugs from biological fluids, including the method, recovery data and the impact of the filtrate's cleanliness on analyte quantitation.

## EXPERIMENTAL

ISOLUTE PPT+ plates were processed using the following "solvent first" procedure.

| | |
|---|---|
| Plate: | ISOLUTE PPT+ Protein Precipitation Plate |
| Pipette: | 300 µL acetonitrile into each well |
| Pipette: | 100 µL plasma into each well |
| Pause: | 2 minutes |
| Apply Vacuum: | –15 "Hg for 3 minutes and collect the filtrate |

Once dispensed, acetonitrile remained in the bottom of each well, forming a "pool" of precipitating solvent, prior to sample addition. Direct application of the sample into the precipitating solvent initiated precipitation. The filterplate was then left to stand for 2 minutes, to allow complete precipitation; no vortex mixing was required.* The filtrate did not pass into the collection plate until the vacuum was applied.

**The effect of vortex mixing on analyte recovery and amount of co-extracted material in the final filtrate was investigated. No significant difference in analyte recovery or reduction in co-extracted material was observed.*

Biotage

### *Analyte Recovery*

An ISOLUTE PPT+ plate was used to isolate two analytes, propranolol and nadolol (both 10 ng/µL) from human plasma samples using the "solvent first" method.

### *Analytical Conditions*

**LC-MS/MS Conditions (nadolol):**

| | |
|---|---|
| Column: | Polaris 3 µm C18-A 50 x 2.0 mm |
| Mobile Phase: | 70:25:5 (v/v) water/0.1% (v/v) formic acid:MeCN:MeOH |
| HPLC System: | Varian ProStar binary pump |
| MS System: | Varian 1200L Triple quadrupole |
| Ionization Mode: | ESI+ |
| SRM Transition: | 310 > 254 (-16 eV) |

**HPLC Conditions (propranolol):**

| | |
|---|---|
| Column: | Genesis 4 µm C18 150 x 4.6 mm |
| Mobile Phase: | 60:40 (v/v) 0.2% (v/v) phosphoric acid pH 2.5:MeOH + 200 µL DEA |
| HPLC System: | Agilent HP1100 |
| Flow Rate: | 1.4 mL/min. |
| Wavelength: | 220 nm |

### *Results*

| Analyte | % Recovery | % RSD |
|---|---|---|
| Propranolol | 93.6 | 7.6 |
| Nadolol | 75.4 | 6.1 |

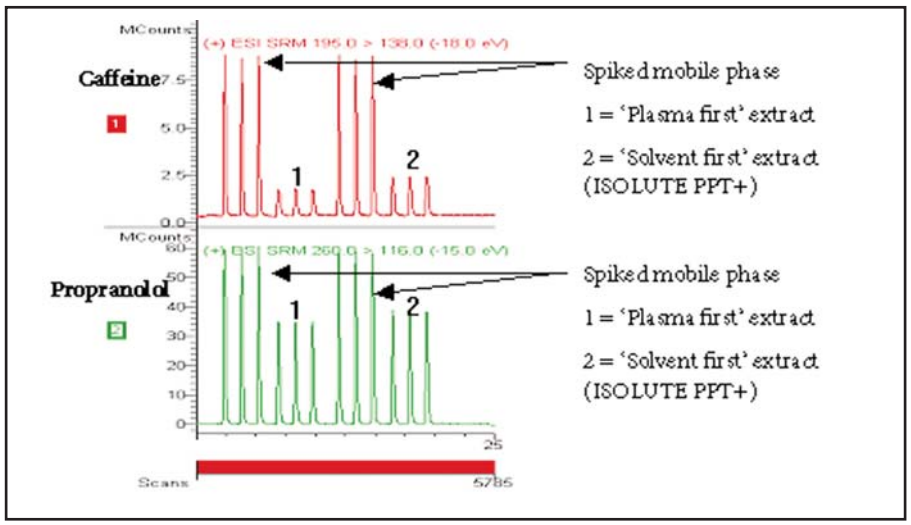
### *Effect of Plasma Extractables on Ion Suppression in LC-MS/MS*

The problems of ion suppression and matrix effects, and the challenges endogenous materials can present to analyte quantitation in LC-MS/MS are well known. The effect of protein precipitation extractables on analyte signal using electrospray LC-MS/MS was investigated. Flow injection analysis was used to investigate ion suppression, as reported by Bonfiglio et al .[3]

Blank human plasma samples (100 µL) were processed using the "solvent first" and "plasma first" methods. Blank plasma samples were evaporated and reconstituted in mobile phase containing caffeine and propranolol at 1 µg/mL. Aliquots (5 µL) of the extracts were injected directly into the ES interface without an LC column in place. Three replicate injections were analyzed in the SRM mode and compared with mobile phase containing caffeine and propranolol respectively.

**LC-MS/MS Conditions:**

| | |
|---|---|
| Mobile Phase: | 50:50 (v/v) MeCN:water containing 0.1% (v/v) formic acid |
| HPLC System: | Varian ProStar binary pump / well plate sampler |
| MS System: | Varian 1200L Triple quadrupole |
| Ionization Mode: | ESI+ |
| SRM Transitions: | Caffeine 195>138, Propranolol 260>116 |




*Mass chromatograms showing effect of residual endogenous material from 'plasma first' and 'solvent first' filtrates on signal response of caffeine and propranolol, compared to spiked mobile phase.*

## SUMMARY

ISOLUTE PPT+ plates have an optimized frit material that allows the isolation of drugs from biological fluids using a "solvent first" protein precipitation procedure. The procedure affords high reproducible recoveries for a wide range of analytes from biological fluids, typically plasma.

ISOLUTE PPT+ plates allow the precipitation of additional plasma proteins compared with first generation 96-well filterplates. The procedure presents a cleaner filtrate for the subsequent analysis. The removal of these additional endogenous materials from the filtrate reduces possible ion suppression or matrix effects.

## REFERENCES

1. TN120 Sample Preparation by Protein Precipitation using the ISOLUTE Protein Precipitation Plates, Biotage.
2. TN130 Sample Preparation using ISOLUTE PPT+ Protein Precipitation Plates, Biotage.
3. R. Bonfiglio, R.C. King, T.V. Olah, K. Mwerkle; Rapid Commun. Mass Spectom., (1999) 13 1175 – 1185


138-702

North America
Main Office: +1 434 979 2319
Toll Free: +1 800 446 4752
Fax: +1 434 979 4743
Order Tel: +1 434 220 2687
Order Fax: +1 434 296 8217
ordermailbox@biotage.com

Europe
Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@eu.biotage.com

Japan
Tel: +81 422 28 1233
Fax: +81 422 28 1236
jp_order@biotage.com
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