Evaluation of Acetylsalicylic Acid (acidic) Drug in Serum Samples with the RubyPro Protein Precipitation Plates from Orochem Technologies Inc.

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Analysis of serum samples for endogenous substances and drugs presents special problems because of the high and variable components of proteins and other ions. Deproteinization of serum samples with acetonitrile is a useful and rapid technique in analysis of drugs in serum samples.

Acetylsalicylic acid (acidic drug) was analyzed in Serum by an Automated Protein Crash Methodology using the Orochem Protein Crash Filtration device and the OroFlex-96 Personal Pipettor. The Orochem Protein Precipitation 96-well plate is designed to hold acetonitrile or methanol in the filter well without a cap mat or seal. The ability to interface the 96-well Protein Crash plate with robotic workstation, allows high throughput solvent dispensing, followed by sample transfer and mixing for complete precipitation of proteins in high throughput format. Following protein precipitation in these "reactor" wells, sample filtration is accomplished with the Orochem Positive pressure processor (ORPSP-96).

The resulting extract was analyzed by HPLC. The samples were prepared in duplicate, along with 2 controls (that contained only the solvent mixture).

High Performance Liquid Chromatography A Symmetry C18 column (3.5 µ, 4.6 × HPLC150 mm) was used. The mobile phase (Water/Methanol/Acetonitrile:20/40/40) was pumped at 0.5mL/min. A 20-µL injections were made, and the peaks were monitored at Abs220.

Reagents

- Organic Solvent - 100% aqueous acetonitrile.
- Serum, FBS Fetal Bovine Serum
  Acetylsalicylic acid and Acetaminophen drugs

Materials

- Orochem Protein Precipitation Plate, OC21PPT20
- Deep Well collection plate Orochem OT-850356
- HPLC ,C18 column equipment
- OroFlex-96 Personal Pipettor
- Orochem Positive pressure processor (ORPSP-96)
- Sheeva Orochem Plate shaker

Protocol Overview

Serum spiking-

For Acetylsalicylic acid drug, 0.1 ml of the concentrated drug solution (0.1mg/ml) was mixed with 0.9 ml bovine serum to obtain a final concentration of 0.01mg/ml of drug in serum.

Serum Deproteinization

1) 800 mL of 100 % acetonitrile was added to all the wells of the plate.
2) 200 mL of spiked (FBS-Fetal Bovine Serum) drop wise was added to all wells of the plate.
3) This was followed by mixing for 5 min on a plate shaker and then filtered at max leaving the Positive pressure on for an additional 45-60 s upon complete filtration.

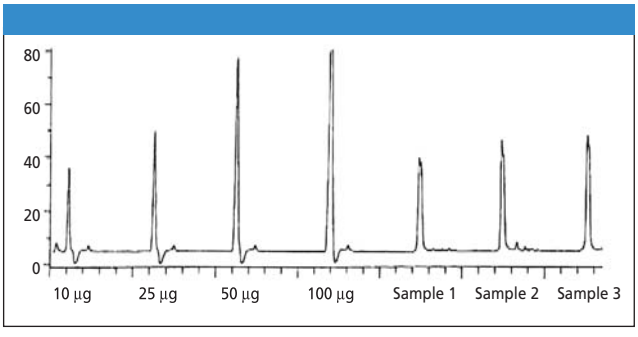

Figure 1: Chromatogram for analyte Acetylsalicylic acid following protein crash.

Results and Conclusions

The Protein Crash method is fast and simple. Recovery of both acidic and basic drugs from spiked serum is satisfactory (100% for Acetylsalicylic acid). The total sample preparation time was only 20 min.

Orochem Protein Crash Plate along with Positive Processor offers a rapid and accurate method for analysis of drugs in Serum or Plasma samples. The ability to interface the 96-well Protein Crash plate with robotic workstation, allows high throughput solvent dispensing, followed by sample transfer and mixing for complete precipitation of proteins in high throughput format.

Reference:

(1) Iranian J of Pharmaceutical Research 2002, 1:43-46 , Evaluation of Acetonitrile Deproteinisation of the Serum samples for the analysis of Drugs in Serum using Capillary Zone Electrophoresis.
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(3) J Chromatogr. 1992 Nov 27; 583(1): 131-6.

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