



APPLICATION NOTES 1

P3 PROTEIN PRECIPITATION MICROPLATE

Testing Site

A drug metabolism department in a leading global pharmaceutical company conducted a trial to evaluate all the various commercial protein precipitation plates currently available with a view of using and recommending it in-house to other departments, the best performing product.

CRITICAL FACTORS

Certain factors in the evaluation were critical to good separations:

Filter leakage

When Acetonitrile is added to denature the sample plasma protein, it is important that this doesn't immediately 'wet out' the filter and cause the filtration plate to leak.

Flow rate

Consistent flow rate of the sample when the vacuum applied is a major advantage.

Non-specific binding of the compound to plate/ filter. The very nature of some filters is to bind analytes, the choice of non-binding filter material is essential to maximise recovery of the analytes/ compounds.

Method

- Add 400µl of Acetonitrile to the well
- Wait 1 to 2 minutes to check for filter leakage
- Rapidly add 100µl plasma sample to the well
- Increase vacuum pressure until sample extraction is visible
- Note:

Recovery of compounds was completed allowing for the effects of ion-suppression on the samples. 100µl spiked plasma was used and compared against spiked standards.

In this experiment the recovery accounts for both the suppression effect on the compound due to the matrix and the recovery of the compound from the matrix.

Ten standard components were tested for in rat, dog and human plasma samples.

RESULTS

Filter leakage

Throughout the investigation the p3 plate showed no signs of leakage. Three of the other

manufacturers plates were ruled out at this point as they had significant leakage.

Flow Rate

The Porvair Sciences p3 plate had consistent flow throughout the plate. Two of the manufacturers plates failed at this point as they had significant blockages.

Non-Specific binding of components

a) Table 1 shows the percentage recovery of the 3 compounds tested, Midazolam (neutral), Diclofenac (acid) and Propranolol (base) in the Porvair Sciences p3 plate.

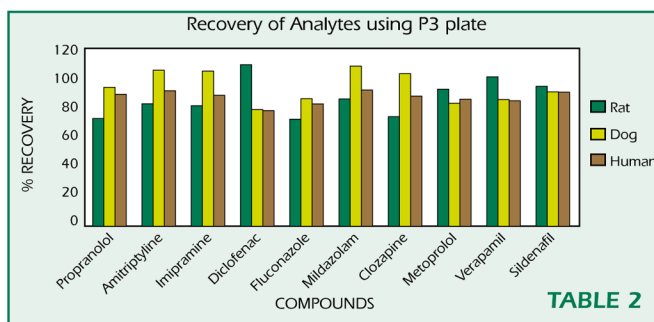
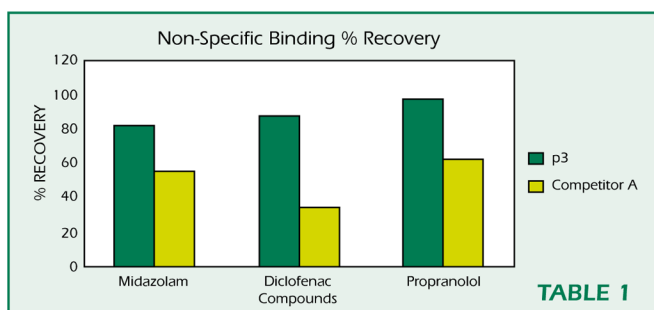
b) Table 1 also shows the best of the other plates tested (Competitor A)

Table 2 shows the recovery of the compounds in various mammalian systems. The p3 plate shows the most consistent recovery of these compounds.

Conclusions

After the evaluation, this department made the decision to use the p3 plates and recommend the plates to other departments for bioanalysis assays featuring protein precipitation. Critical factors for the choice were:

- Clean System – No Leakage
- Good Flow Rate
- Low Non-Specific Binding



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