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AUTOMATED HIGH-THROUGHPUT PROTEIN PRECIPITATION

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The push for high-throughput screening has analytical chemists weighing the benefits of cleaner sample preparation methods versus speed and simplicity. According to a recent industry survey (LC-GC July 1999, p.596), protein precipitation is still competitive despite its limitations. Although protein precipitation may not be as clean as other methods, its speed makes it the sample preparation method of choice for a large fraction of researchers assaying biological samples.

In the past, protein precipitation methods have been difficult to automate. There have been two hurdles: centrifuges for use with automation are expensive, and it is difficult for the automation to differentiate between the supernatant and the pellet of crashed proteins. A novel filtration plate (3M Empore, St. Paul, MN) allows automation to bypass the centrifuge. The cleanliness of the filtered sample with 10µm protein particles matches or exceeds that of a centrifuged sample, as demonstrated by a modified Coomassie Blue protein method. We developed an assay which we applied to the filter plate on a positive-pressure robot (Gilson Inc., Middleton, WI) designed to use 96-well filtration plates.

Drug recovery studies were performed on Enalapril and Diltiazem using both manual and automated protein precipitation methods. 100µL of spiked serum was mixed with a four-fold greater volume of acetonitrile. In the manual method, the samples were centrifuged at an RCF of 1550 cm/RPM^2. In the automated method, they were filtered with positive pressure. All samples were assayed via HPLC with UV detection.

Automated and manual methods were contrasted. For samples with concentrations in the middle end of the calibration curve, the inter-assay precision was 5.5% manual and 5.0% automated. The inter-assay accuracy was 102% manual and 98% automated. Throughput was 52 samples/hour manual and 69 samples/hour automated.