



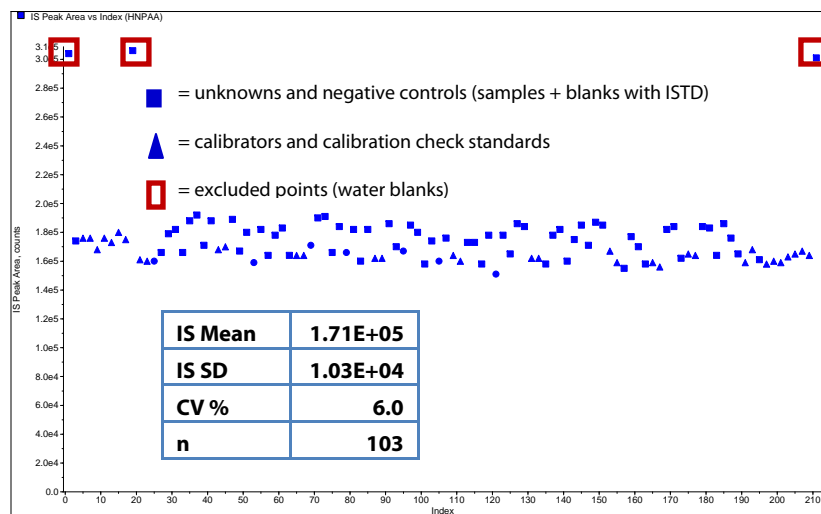
Preparation of Urine Samples for HPLC-MS/MS Detection of HNPAA using the Gilson PIPETMAX® 268

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Table 1: Comparison of HNPAA recovery and CV values from urine samples using the PIPETMAX and manual sample preparation methods.

Method of Aliquot	Mean	CV	n =
% recovery vs. consensus value – PIPETMAX® 268	101%	2.3%	27
% recovery vs. consensus value – MANUAL	100%	4.0%	27

Figure 1: HNPAA internal standard response following PIPETMAX sample preparation.



HNPAA, a metabolite of tetranitromethane, is used to identify exposure to explosives such as Trinitrotoluene (TNT). The Gilson PIPETMAX® 268 was used to prepare urine samples for HPLC-MS/MS analysis of HNPAA to compare results with manual sample preparation. Samples were performed in duplicate, with a 5.3% relative difference between duplicates of n=27. The consistency of internal standard additions for the HNPAA determination was evaluated using the PIPETMAX (Figure 1). Results show a 6% CV for n=103 (106 total injections, with three water blanks excluded). The CV and recovery values obtained were compared with previously run patient sample results performed manually for the original exposure determination. Results show similar accuracy (101% vs. 100%) but improved precision (CV=2.3%) using the PIPETMAX over 27 samples.

Conditions

Sample: HNPAA (4-hydroxy-3-nitrophenylacetic acid) in urine (metabolite from tetranitromethane (TNM) exposure)

Analysis: HPLC-MS/MS

Quantitative MRM Curve r value = 0.9998
 Confirmatory MRM Curve r value = 0.9999
 Blanks = not detected
 Quantitative vs. Confirmatory MRM ratio:
 Mean = 2.2
 SD = 0.058
 CV = 2.6%
 n = 95

Confirmatory result absolute percent difference from quantitative result:
 Mean = 1.7%
 SD = 2.2
 n = 95

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