K6 Validation and Application of the PE TMX 110 Autosystem for Packed Column Analysis of the Confirmation of Volatiles in Death Investigation

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After attending this presentation, the attendee will be knowledgeable in method validation for volatiles by headspace gas chromatography. Documentation of linear dynamic range, precision, and carryover of volatile headspace methods on two instrumental systems will allow the attendee to become familiar with each system. Additionally, the audience member will know how the new TMX HS 110 Autosystem compares to the older HS101 system. Data that supports the validation of the newer TMX HS110 system for use in postmortem work will demonstrate the utility of the automated headspace approach to the audience.

Headspace Gas Chromatography has been widely applied to the determination of alcohol in postmortem specimens. In 2001, the Wayne County Medical Examiner's office (WCMEO) performed 3,572 headspace confirmation analyses on multiple samples from 3175 cases. In order to facilitate this workload automated headspace autosampling is utilized within the postmortem laboratory. The authors report here on the validation of the Perkin Elmer TMX-HS110 system for use in this application.

Validation of the TMX-HS110 system was performed by direct comparison against an existing Perkin Elmer HS101 automated system. All analyses were performed under isothermal conditions on a 6' X 1/8" OD stainless steel Carbopak B 60/80 mesh 5% Carbowax 20-M column at 80°C. The injection needle and transfer line was maintained at 110°C on each instrument. Thermostat temperatures of 60°C were utilized to heat samples before headspace injection. Injection ports and flame ionization detectors (FID) were maintained at 130°C. The chromatographic flow rate of the nitrogen carrier was set at 20 mL/min on each instrument, with the fuel gases hydrogen and compressed air set at 40 and 400 mL/min respectively. Purge flows on these instruments were set to values between 5-12 mL/min.

The headspace autosampler programs were consistent on each system. Thermostat time was 15.0 min, pressurization was 0.5 min, injection time 0.08 min, and withdrawal time 0.20 min. Cycle time was 7.0 minutes and each vial was vented 1 time.

All samples were prepared for analysis by diluting 0.100 mL of specimen, calibrator or control with 1.00 mL of an aqueous n-propanol internal standard solution prepared at 0.160 g/dL concentration. All patient specimens were run in duplicate. Each batch was ran by single point calibration at values of 0.1531, 0.1580, 0.1580, and 0.1562 g/dL for ethanol, methanol, acetone and isopropanol, respectively, on both analysis systems. All standards and controls were prepared in aqueous solutions. In all cases of instrumental comparison studies the same vial set was ran on each instrument in the same sequence. Linearity studies were carried out over a range of 0.007 - 1.5 g/dL for each analyte. Precision studies were performed on both systems n = 5 at up to 5 concentrations over the linear range studied. Carryover was evaluated up to a concentration of 1.531, 1.580, 1.580 and 1.562 g/dL for ethanol, methanol, acetone and isopropanol, respectively.

Turbochrom chromatographic software was validated in parallel on the existing HS 101 analyzer against an existing LCI 100 data acquisition system on the HS 101-headspace analyzer. Data was collected simultaneously on the same sample set by both systems. Subsequent studies and comparisons between the two Headspace/ Chromatographic systems were performed using the Turbochrom chromatographic software.

Finally, three routine batches of patient samples were evaluated on each system. In each case the calibration sequence and sample vial sequence was identical. Analysis of the vial set was first on the HS 101 instrument (reference method), followed by analysis of the same vial set on the TMX HS110 system. In all linearity, carryover, precision and comparison studies, data reduction was performed by the Turbochrom software package using identical integration parameters.

Results of whole blood proficiency studies over an eight-year period demonstrated under the instrumental analysis conditions demonstrated consistency between mean target ranges and results obtained on the HS 101. A correlation coefficient of 0.9979 with a slope of 1.026 for n = 169 was defined. Additionally, each batch analysis includes a reanalysis whole blood sample ran as an in-house control which must meet reporting criteria (within 0.02 g/dL of original result).

Results of the data reduction system comparison between the LCI 100 and Turbochrom software packages on data collected from the HS 101 analyzer demonstrated correlation's of 0.9999 or better for ethanol, methanol, acetone and isopropanol. Standard deviations on this comparison ranged from 0.2 - 5.4 %. From this it was concluded that data reduction from each of these systems resulted in equivalent data.

Correlation between the data on both instruments was 0.999 or better for each analyte**.** Linearity, LOD (relative retention times (RRT) within 2% of expected value and signal to noise >/= 10:1), LOQ (RRT within 2%; concentration within 20% of target value) and ULOL data for both instrument systems was determined to be equivalent and is summarized on Table 1:

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Table 1: Linear ranges for headspace method, LOD, LOQ and ULOL; Ethanol, Methanol, Acetone, Isopropanol (n = 2 at each concentration).

ANALYTE	RANGE g/mL	LOD g/dL	LOQ g/dL	ULOL g/dL
Ethanol	0.0076 - 1.531	0.0076	0.0076	1.531
Methanol	0.0079 - 1.580	0.0079	0.0079	1.580
Acetone	0.0079 - 0.395	0.0079	0.0079	0.395
Isopropanol	0.0156 - 1.562	0.0078	0.0156	1.562

Carryover evaluations of ethanol, methanol, acetone and isopropanol were determined over the linear range defined on Table 1. Carryover is noted to occur at specific concentrations documented on Table 2 at levels of 0.004 g/dL or less for all analytes.

Table 2: Carryover concentration ranges for ethanol, methanol, acetone and isopropanol.

ANALYTE	CONCENTRATION INJECTED g/dL	CARRYOVER g/dL
Ethanol	1.531	0.0018
	0.765	0.0013
	0.382 and below	None
Methanol	1.580	0.0039
	0.790	0.0018
	0.395	0.0012
	0.316 and below	None
Acetone	1.580	0.0002
	0.790 and below	None
Isopropanol	1.562	None

Precision studies performed over the linear range of the assay (n = 5 at each concentration) demonstrated accuracy within 10% of target values. All RT values were within 2% of target values. Within batch CV values over the ranges evaluated were less than 1.00, 3.60, 1.40 and 5.10 % respectively for ethanol, methanol, acetone and isopropanol. Between run, CV values as determined by ethanol controls distributed over the range of 0.050 to 0.500 gm/100 demonstrated values of 2.60 % or less. All values were within 10% of target concentrations.

Direct batch to batch comparisons (batch size n = 73, 97, and 94) between each analytical system was run on each of three separate occasions by 2 analysts. Correlation's between each set of data demonstrated linear relationships with slopes of 0.96 or better and correlation coefficients of 0.9995 or better. Differences between ethanol values on each of the two systems were at mean values of less than 1% with overall standard deviations of the mean value at 6% or less.

The data presented in this study demonstrate that the TMX HS110 system and the HS 101-headspace analyzer produce equivalent data. These instruments under the conditions defined can be used interchangeably. The WCMEO postmortem laboratory currently uses both of these systems in confirmation of alcohol findings.

Headspace Analysis, Ethanol, HS 101, TMX HS110

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