TOLUENE IN BLOOD

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BACKUP DATA REPORT

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TOLUENE in blood: Backup Data Report

INTRODUCTION AND BACKGROUND

Toluene is a clear, colorless liquid with a distinctive smell. It occurs naturally in crude oil

and in the tolu tree. It is produced in the making of coke from coal and is a by-product in the

manufacture of styrene.

Toluene is utilized in a wide variety of industrial processes. It is present as a solvent in

many commercial products such as paints, thinner, adhesives, cleaning agents, pesticides, and

explosives; and as a cleaner and degreaser of metal [1][2]. The ubiquitous use of these chemicals

in industry generates a large potential for excessive occupational exposure.

Toluene has for a number of years been abused for its narcotic effect on the central

nervous system. There are over 1,400 inhalable products available on the market, which fit into

the categories of solvents, aerosols, gases, and nitrites. The growth in inhalant users is especially

prevalent among the youth and in the military [3].

Symptoms of high toluene exposure are similar to alcohol abuse. Lower concentrations

cause irritation of mucous membranes. Higher levels non-specifically depress the central nervous

system and are associated with headaches, drowsiness, dizziness, confusion, and even

unconsciousness and death. The onset of the effects varies broadly from person to person and

seems to be influenced by prior exposure.

After toluene is taken into the body, more than 75% of it is removed within 12 hours. It

may leave the body unchanged during respiration or in the urine [2]. Urinary excretion of

unchanged toluene is generally less than 5% [4]. During a 5-hour inhalation exposure to toluene,

15% to 20% of the dose absorbed is eliminated unchanged in expired air [5]. Following a 7-hour

exposure, about 80% of the absorbed amount of toluene is excreted as metabolites. The major

elimination pathway is through the urinary metabolite hippuric acid. O-cresol, which is also a

metabolite, accounts for less than 1% of the absorbed toluene. Metabolites are not accumulative

over the workweek, but toluene may be, depending on the level of obesity of the exposed person

[6].

The Biological Exposure Indices (BEI) Committee recommends a BEI in blood of 0.05

mg/L for toluene [7].

This report evaluates a method for quantifying toluene in blood samples utilizing a gas

chromatograph equipped with a flame ionization detector and headspace automatic sampler. The

headspace technique is a method in which the air above a sample is injected into the analytical

system rather than injecting the actual sample or an extract of the sample. A method based on a

headspace technique seems ideal in this situation because the volatile nature of the analyte allows

it to easily enter the headspace upon heating of the sample. It also eliminates the need for extra

sample extraction steps and prevents injecting relatively dirty biological matrices onto the

instrument where contamination and wear may occur.

NOTE: Proper safety precautions should always be taken when dealing with any chemical

but especially when working with biological fluids such as blood. Manipulating biological

samples poses a serious health risk because of the potential transmittance of infectious diseases

including hepatitis and HIV. Lab coats, goggles, and gloves must be worn at all times. Work

should be performed in an isolated hood where possible. All waste is required by law to be

disposed of in a properly labeled, autoclavable container.

REAGENTS AND MATERIALS

Presented in Table 1 is the list of reagents and solvents used for this method and its

evaluation. Isobutanol was used as the internal standard to normalize the values

TABLE 1. LIST OF CHEMICALS

Chemical	Vendor	CAS #	Purity	Lot #
Toluene	Aldrich	108-88-3	99.8%	BO 08980AO
Isobutanol	Fisher	78-83-1	99.8%	85540Z
Ethylene Glycol	Aldrich	107-21-1	99+%	JA06146HA
Water	DataChem Laboratories	7732-18-5	ASTM Type II	--

of toluene determined in the blood samples. Ethylene glycol was used as the solvent for preparing the primary stock solution. The internal standard solution was made up in water. Blood used in the study for standards and test samples was whole blood obtained from donation centers. The stock blood was stored at 4 °C in the plastic storage bag that it was originally collected in and sodium citrate was added at donation center to control clotting.

In the original write up of method 8002 the primary standard solution was made up in acetone and the secondary standards or stock standards were made up in water. From the stock standards were made the working or blood standards. Because of the high instability of the water based stock standards a more suitable solvent was sought. It was felt that the solvent needed would have to be soluble in both water and toluene. Acetone seemed to be ideal but when used to make up the secondary standards, produced numerous artifact peaks, especially at the higher temperature of 99 °C. Other solvents such as ethanol and methanol were tested and were also found to be unacceptable for the same reasons. It was found that ethylene glycol was ideal as a standard solvent because of its high boiling point and low vapor pressure. Ethylene glycol, when used as a standard solvent, produced few artifact peaks even at 99 °C. Under the right storage conditions standards made up in ethylene glycol are stable for over a week (Figure 1, Table 2). These standards need to be stored at 4 °C in airtight glass containers having non-toluene permeable lids and very little headspace.

TABLE 2. COMPARISON OF OLD AND NEW STANDARDS MADE UP IN ETHYLENE GLYCOL

Standard ID	Old Standards		New Standards		Concentration (µg/mL)	Peak Area % Difference
	Date Made Up	mL Headspace*	Date Made Up	mL Headspace*		
PRS	10/30/2002	6	12/31/2002	0	5.76	10.5
IS1	10/30/2002	5	12/31/2002	0	0.576	13.9
IS2	10/30/2002	4	12/31/2002	0	0.0576	14.1
IS3	11/8/2002	2	12/31/2002	0	0.00576	8.5
* As of 12/31/2002 in 20-mL vials.						

Repeated opening of the standard containers and removal of their contents ultimately results in the loss toluene as can be seen in Table 2 and in Figure l.

FIGURE 1
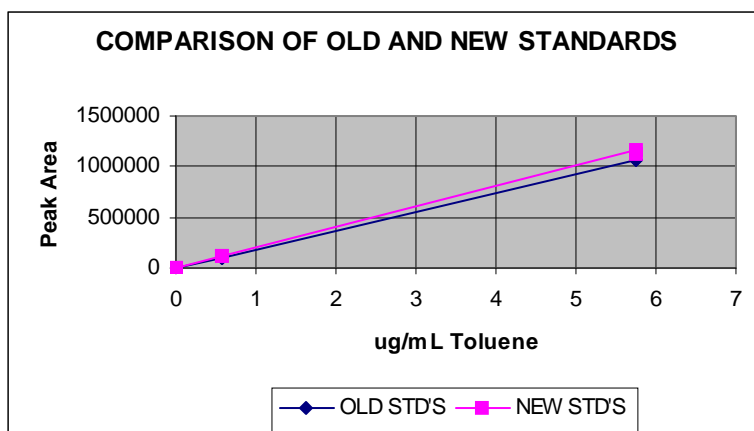
Originally the method called for the addition of citric acid dextrose solution (ACD). To evaluate the usefulness of ACD in the method, differing levels of ACD was added to headspace vials containing 1.5 mL of blood spiked at 1.845 ug/mL toluene and 1.5 mL of internal standard solution. Each level was made up in triplicate and analyzed on the headspace gas chromatograph. From the results there was no evidence that the ACD solution benefited the method (Figure 2). A literature search shed little light on the use of ACD for the determination of toluene in blood. Some researchers add it to blood to act as a preservative [8] and others to act as an anticoagulant

[9]. Perhaps for long-term storage of samples for future analysis it might be useful, but this is beyond the scope of this study. As a result of these findings the addition of ACD in the method, was discontinued.

FIGURE 2. THE EFFECT OF ACD ADDITION SOLUTION
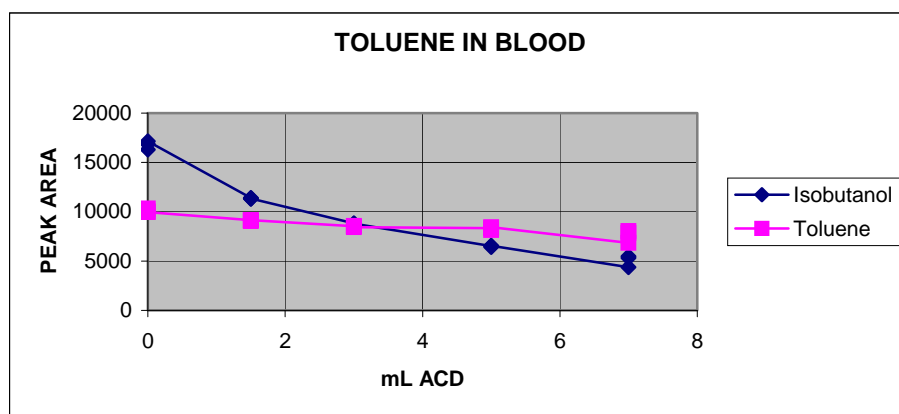
Preparation of Primary Stock and Internal Standard Solutions

To make the primary stock solution, a 50-mL volumetric flask was filled partially with ethylene glycol. With a micro liter syringe, a specific, measured volume of toluene was added to the flask. The 50-mL volumetric flask was brought to volume with ethylene glycol, mixed, and the solution was transferred to a 13 X 100 mm glass culture tube and capped with a Teflon-lined cap. The solution was stored at 4 °C in the dark until needed. The concentration of the analyte in the stock solution was calculated using the volume of analyte added, its density, its purity factor, and the total dilution volume. For example:

$$20 \mu L \cdot TOLUENE \times \left(\frac{0.8669 \cdot mg}{\mu L} \right) \times (0.998) \times \left(\frac{1}{50 \cdot mL} \right) = 0.346 mg / mL$$

The original method called for the use of isobutanol as an internal standard. Because no complaints were registered as to the use of isobutanol it was felt that there was no need to change to an internal standard that was more like toluene. For this study the main function of an internal

standard has been to help correct for partial injections, which take place when the needle in the headspace sampler becomes restricted. This has been a rare occurrence, but when it has happened the drop in the isobutanol GC peak area has been a good indication of a restricted needle. Other than this the internal standard could have been eliminated from the method.

The internal standard solution was prepared by diluting 25 µL of isobutanol to 1-liter with de-ionized water. This was done using a 1-liter volumetric flask. The original level of internal standard was 40 mg/L water, but as the sensitivity of the method was improved the peak area of isobutanol became too great and needed to be reduced. For the method evaluation the internal standard concentration was 20 mg/L calculated as follows:

$$25\mu L \cdot ISOBUTANOL \times \left(\frac{0.8018 \cdot mg}{\mu L} \right) \times (0.998) \times \left(\frac{1}{1 \cdot L} \right) = 20 mg / L$$

This concentration of internal standard was employed because it provided good peak shape, peak area, and reproducibility for the internal standard.

SAMPLE PREPARATION AND INSTRUMENT CONDITIONS

Sample Preparation

The samples are prepared by first adding 1.5 mL of the internal standard solution to a 20-mL Perkin Elmer HS-40 headspace vial. Then a graduated Luer-Lok syringe with a twenty gauge needle is used to transfer 1.5 mL of the sample from its shipping vial to the headspace vial. The vial is then quickly sealed with an aluminum crimp-cap and PTFE/Butyl septa. The vials contents are then mixed and analyzed. If blood samples are not going to be transferred to headspace vials and analyzed immediately, they should be stored at 4 °C in their original shipping vials. If possible no headspace should be present in the sample container. Any headspace will result in toluene loss depending on the headspace volume [10] Samples shipped

from the field should also be sent in completely filled containers where possible. To prevent

toluene from escaping, the samples and standards should be opened to the air as little as possible.

One of three approaches can be taken in sampling a vial. First, if the sample vial is

equipped with a septum an aliquot of blood can be removed with a graduated syringe. Two

twenty gauge needles can be used, one for the syringe and the other to allow air to displace the

sample when being withdrawn. Second, using one needle, air can be injected into the vial with a

syringe before the sample is withdrawn. Third, the lid is removed and using a macro pipette an

aliquot of blood is removed. Glass beads can then be added to eliminate the headspace and the

container is recapped. Once a headspace is created after an aliquot is removed a second sampling

may result in lower values. This depends on the length of time between samplings, the volume of

the headspace, and the amount of sample remaining in the vial.

Instrument Conditions

All of the samples and standards were run on the same system with the same set of

conditions. The system consisted of a Perkin Elmer Autosystem gas chromatograph equipped

with a FID detector and a Perkin Elmer HS-40 headspace auto sampler. The column is a fused

silica capillary column (DB-624, 75m X 0.53mm I.D., 3.0 µm film). Figure 3 shows the

temperature program that was utilized. The head pressure was maintained at 21 psi. The

FIGURE 3. TEMPERATURE PROGRAM
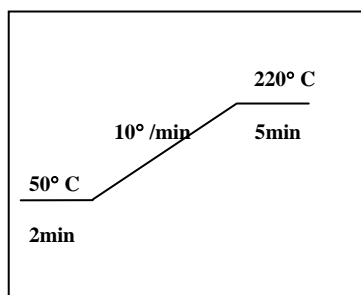
carrier gas consisted of pre-purified helium and the FID detector was supplied with pre-purified

hydrogen and filtered air. The injector and detector temperatures were 129 °C and 250 °C

respectively. The injection conditions were split-less or direct on column injection for one

minute then the splitter was turned on with a flow of 130 mL/min. Failure to use the splitter in

this method, results in heavy carry over for both the internal standard and toluene. The headspace

auto-sampler was set to the following conditions:

Transfer Temp:	129 °C	Withdrawal:	0.80 min
Thermostat Time:	30 min	Needle:	120 °C
GC Cycle Time:	26 min	Sample:	99 °C
Pressurize:	4.0 min	Inject:	0.4 min

Table 3 shows the typical retention times of the analytes given these conditions. The final

TABLE 3. ANALYTE RETENTION TIMES

Analyte	Retention Time (min)
Isobutanol	6.53
Toluene	9.11

temperature (220 °C) was maintained until the column appeared to be clean in order to help

avoid any carryover into the next sample injection. To insure that there was no carry over from

the high concentration standards to other standards or samples, headspace vials containing only

water were inserted in the run after the high standard. A visual examination of the blank water

runs ensured that there was no carry over of ether toluene or isobutanol.

**Optimization of Instrument Conditions**

Sample Heating Test. The conditions listed above were determined by running a battery

of tests. In these test water was used in place of blood, because government regulations have

made blood difficult to obtain. Headspace vials containing 1.5 mL of water and 1.5 mL of

internal standard solution were spiked with toluene and run in sets of four. After each set was
analyzed the conditions were changed and the next set was run.

Heating time of 30 minutes and head pressure or 21 psi was chosen for the first test. The
first set in this test was analyzed at 72 °C, after which the temperature was raised approximately
10 degrees and the next set was run. This was repeated until 99 °C had been reached (Figure 4).
The results showed that the levels of both toluene and isobutanol increased in the headspace as
temperature increases. The effect of the higher temperatures on isobutanol eliminates the need
for the addition of salt to the samples. For compounds that are highly water soluble, the addition
of salt shifts their solubility from the aqueous phase to gaseous phase [11], thus improving peak
response. The recommended head pressure listed in the operations manual for the HS-40 is 21
psi for a sample temperature of 99 °C. This pressure was chosen because the highest temperature
of the test was 99 °C. Because of concern for running the headspace sampler at this temperature
the boiling points of blood and water were checked. This was done using an oil bath, hot plate,
and thermometer. At the lab elevation of 4000 ft it was found that on the day of the test, water
boiled at 97 °C and blood showed weak signs of boiling at 105 °C. For the headspace vials used
in this test there were no indications of pressure problems for either blood or water at 99 °C*.
***NOTE:** For water to boil at 100 °C the atmospheric pressure would have to be 14.7 psi. The
method uses a head pressure of 21 psi. At this pressure water would have to be heated to 111 °C
before boiling would occur, this temperature is well above the temperature of 99 °C used in the
method.

FIGURE 4
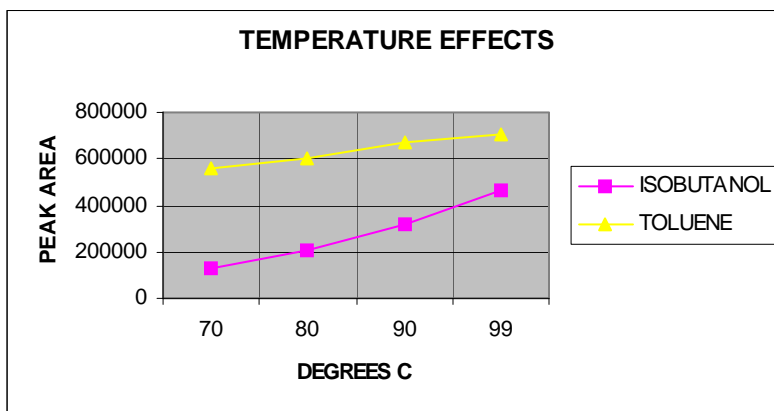
Heating Time Test. Having established the operating temperature and head pressure, the next step was to establish the heating time needed for the samples. Spiked water samples again were run in sets of four varying only the time the samples were heated (Figure 5). Both toluene and isobutanol increase in the headspace with increased time up to 30 minutes. It was felt that the test did not need to go beyond 30 minutes.

FIGURE 5
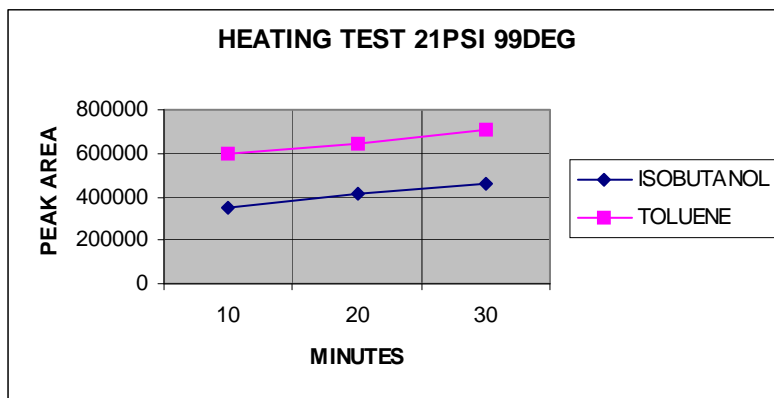
Injection and Pressurization Test. The Perkin Elmer headspace system does not use sampling loops. In this case the amount of sample that is transferred to the column is time and pressure controlled. The sample vial is first pressurized, for a set time, through the injection

needle. Then the carrier gas is interrupted and the pressurized gas in the sample vial is allowed to

backflow out of the vial and onto the column for a set amount of time. Having determined the

head pressure the optimum time of injection and pressurization was needed.

The pressurization test was set up so that sets of four spiked water samples were run at

four different times from one minute to four minutes (Figure 6). Although there was not a great

increase in peak area from one minute to four minutes, four minutes was selected at the

recommendation of Perkin Elmer based upon the belief that the longer time would result in

improved precision.

The injection test was run from 0.08 to 0.6 minutes again using spiked set of water

samples (Figure 7). As can be seen in this injection test, time has a significant impact on peak

response. The injection time of 0.4 minutes was chosen because times greater than this showed

indications of column overload at higher concentrations of toluene and isobutanol.
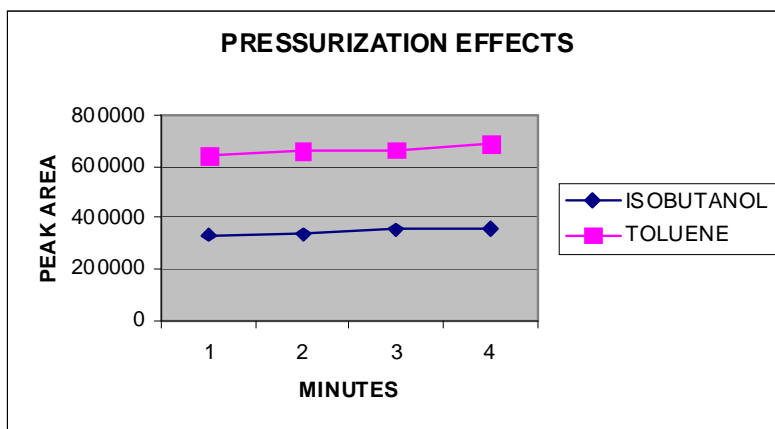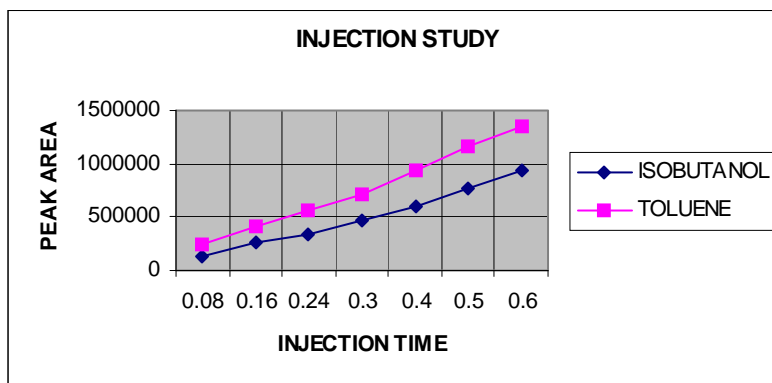
FIGURE 6

FIGURE 7

Calibration

To quantify the amount of toluene present in samples, a calibration curve should be constructed daily with at least five standards made up in blood, run in duplicate and covering the anticipated concentration range of the samples. The blood standards are prepared by adding 1.5 mL of the internal standard solution and 1.5 mL of the stock blood to a 20-mL headspace vial and then spiking known amounts of the toluene/ethylene glycol working stock solution into each using a graduated micro-syringe. To avoid loss of toluene the spike should be made either under the solution or after the vial has been capped through the septum. Along with the working standards at least two blood blanks need to be prepared by following the same procedure only omitting the toluene spike. The same stock blood used for making the blood standards should be used for the blood blanks.

After analyzing the samples and standards, a calibration graph is generated for toluene by plotting the ratio of isobutanol and the blank corrected toluene (peak area of toluene minus the blank divided by the peak area of the internal standard on the same chromatogram) on the y-axis vs. µg of toluene/mL of blood on the x-axis. A linear or quadratic model is then generated using this data depending on which model provides the best fit. The concentration of toluene in the samples is then calculated from the ratio of its toluene peak area and its internal standard peak

area. Of the stocks of blood tested and used there has been a blank peak response in the range of 0.005 µg/mL toluene for each.

Chromatographic Conditions

Figure 8 displays a typical chromatogram when the previously described conditions are employed. Isobutanol was chosen as the internal standard because of its availability and stability in water. It also elutes in a relatively interference-free region of the chromatogram. It may appear that the initial temperature program ramp begins too slowly and that higher initial temperatures and ramps would decrease the analysis time. Although this may be the case, it is not recommended at very low levels of toluene. A temperature program that has a steeper gradient may cause overlapping and interfering peaks in some samples.

FIGURE 8. TYPICAL CHROMATOGRAM
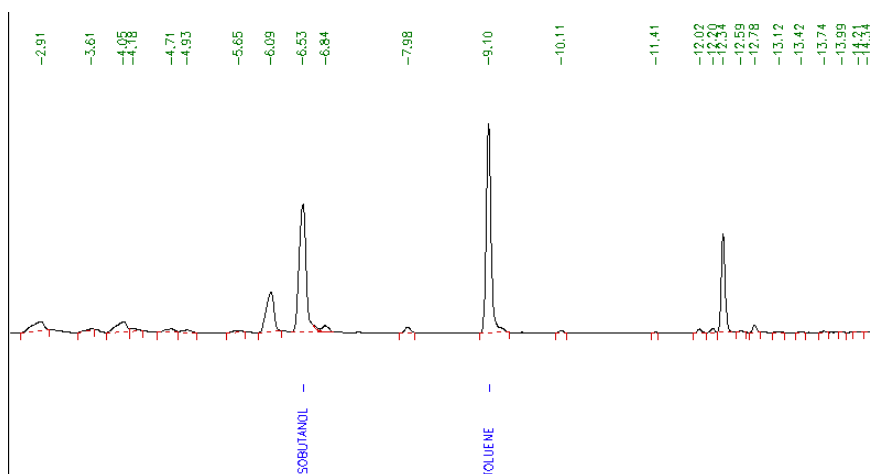
MATRIX EFFECTS

When toluene standards made up in water are compared to toluene standards made up in blood a large bias can be seen. Compared to water, toluene in blood is approximately 40% lower in peak area. To see if this bias was due to a component of blood, an aliquot of whole blood was spun down and the two fractions were separated (plasma only and red blood cells). Each fraction

was sampled four times and each sample was spiked and analyzed. The average results show a

significant bias between the fractions (Table 4). The results indicate that the red blood cells may

have an affinity for both toluene and isobutanol.

By adding fibrous material to the headspace vials containing blood, recoveries equaling

that of water standards can be achieved (Figure 9). When heated the blood samples and standards

quickly gel. This gelatinous phase may act to inhibit the diffusion of toluene into the headspace.

The addition of fibrous material, such as glass fiber filters, increases the surface area of the

blood, thus improving diffusion to the headspace and effectively diminishing the matrix effect.

Other fibrous materials such as Whatman filters and cotton gauze were tried but these

contributed significant background contamination.

TABLE 4. COMPARISON OF TOLUENE AND ISOBUTANOL RECOVERY FROM BLOOD AND PLASMA

	Isobutanol		**Toluene**	
	Plasma	**Blood**	**Plasma**	**Blood**
Peak Area	429920	370209	425723	372735
	420984	363803	440821	367175
	433598	370128	467014	386693
	435936	361061	*459521*	396225
Average	430110	366300	448270	380707
% Difference	17.4		17.7	

FIGURE 9. MATRIX EFFECTS ON TOLUENE IN BLOOD AND WATER
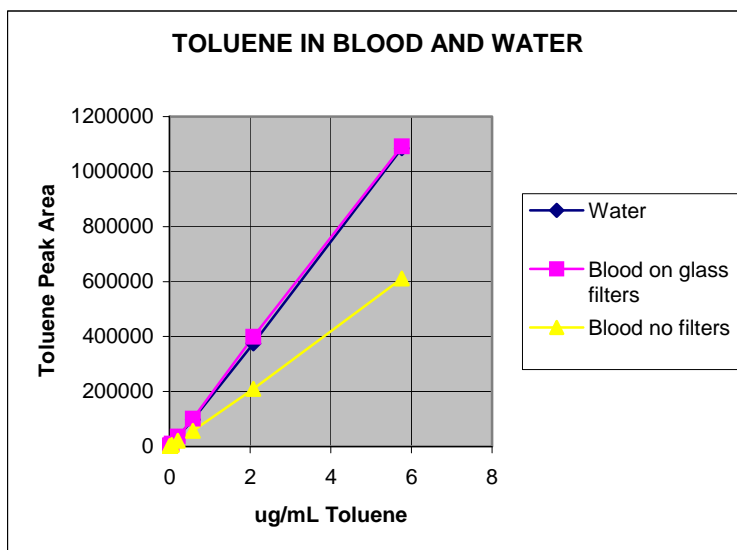
LIMIT OF DETECTION AND QUANTITATION STUDY

This study was to determine the limit of detection (LOD) of the method based upon

blood spiked with toluene. The limit of quantitation (LOQ) for toluene was then calculated as

10/3 times the LOD. The resulting LOQ was then used to determine the target concentrations for

all subsequent steps in the method evaluation.

Blood standards were prepared in duplicate by spiking headspace vials containing 1.5 mL

of blood and 1.5 mL of internal standard solution with aliquots of the stock standards (described

in Reagents and Materials section). Table 5 lists the range of concentrations used during the

study. The samples were then prepared and analyzed as described in the Sample Preparation and

TABLE 5. LOD/LOQ STANDARDS

Standard	Toluene (µg/mL)
$1	5.76
$2	2.08
$3	0.576
$4	0.208
$5	0.0576
$6	0.0208
$7	0.00576
$8	0.00208

Instrument Conditions section. The LOD and LOQ were estimated by fitting the data to a

quadratic curve followed by applying Burkart's Method to the data [12]. The correlation

coefficient for toluene was 0.999 for the quadratic curve. The data provided an LOD estimate of

0.006 µg/mL for toluene. This equates to a LOQ of 0.02 µg/mL.

This experiment was repeated three different times on three different days to determine if

the LOD was consistently achievable. The LODs ranged from 0.003 to 0.008 µg/mL. Although

lower LODs were occasionally obtained, 0.006 µg/mL was chosen as the method's LOD because

it was consistently obtainable.

LONG-TERM STABILITY STUDY

This experiment is designed to assess the stability of toluene in blood under various

conditions of storage. The study covers a period of 30 days with samples stored both at room

temperature (24 °C) and under refrigeration (4 °C). The study was performed using whole blood

for both samples and standards. For samples to be considered stable, they must have a loss of

less than 10% over a period of seven days [13]. Once at the laboratory, the analysis might be

further delayed for various reasons; therefore, the study was extended for 30 days.

More than one attempt at running the stability study was made. It was found that blood could not be spiked with toluene and then transferred to storage vials without experiencing substantial loss. An open vial or any headspace can result in the escape of toluene from the sample. To resolve the problem 2-mL GC vials were filled with 1.5 mL of blood, and the vials were sealed with standard crimp on aluminum septum caps. Next the blood in the vials was spiked with toluene by injecting the blood through the septum using a syringe. To stop any loss of toluene through the puncture hole a dab of silicon rubber was then applied to the top of the septum. These vials were then stored at either 24 °C or 4 °C. At the time of analysis the 2-mL vials were first covered on the bottom end with a one inch square piece of aluminum foil. This was done, by centering the vial on the aluminum foil square. Using the thumb and forefinger the foil was smoothed and wrapped over the bottom and around the sides of the vial. The vials were then placed upside down inside a 20-mL headspace vials to which 1.5 mL of internal standard solution was then added. Using a center punch and small hammer the bottom of the 2-mL vials was then shattered and the headspace vials was quickly capped and sealed. The purpose for the aluminum foil was to stop the blood inside of the 2-mL vial from splashing out when the vial was shattered. The headspace vial was next shaken and rotated till the contents were fully mixed and the aluminum foil was at the bottom of the headspace vial. Because of extra surface area created by the addition of the 2-mL vials and aluminum foil the results would tend to be biased high (see section on Matrix Effects), therefore the standards were also prepared, at the time of the analysis, in the same manor. The spike level was set at 0.161 ug/mL (just below 10 x LOQ). Thirty samples and seven blanks were individually made up. On each scheduled day the number of samples needed plus one blank were removed from storage and analyzed. With each sample set, a fresh series of standards was prepared and analyzed to produce a calibration curve. Table 6

shows the schedule for the analysis of the samples stored at room temperature (24 °C), the length

of storage and the results for each set of samples.

TABLE 6. LONG TERM STABILITY STUDY AT ROOM TEMPERATURE (24 °C)

Storage Time	Sample #	Storage Temperature	Target Value µg/mL (Toluene)	Amount Recovered µg/mL (Toluene)	% Recovered (Toluene)
1 Day	1	24 °C	0.161	0.158	98.1
	2	24 °C	0.161	0.158	98.1
	3	24 °C	0.161	0.155	96.3
	4	24 °C	0.161	0.141	87.6
	5	24 °C	0.161	0.156	96.9
	6	24 °C	0.161	0.152	94.4
7 Days	1	24 °C	0.161	0.157	97.5
	2	24 °C	0.161	0.155	96.3
	3	24 °C	0.161	0.157	97.5
	4	24 °C	0.161	0.151	93.8
	5	24 °C	0.161	0.147	91.3
	6	24 °C	0.161	0.165	102.5
Average				0.1544	95.9
Std. Dev.				0.006	3.8

The samples are stable for seven days at room temperature as long as they do not clot.

Blood used in this study was citrate treated to prevent clotting (see Reagent and Background

section). Field samples if collected in untreated vacuum blood collection vials will clot in a

matter of hours even if they are refrigerated. For this reason blood collection vials need to be

treated with an anti-clotting agent.

To determine the stability of the blood samples for longer terms of storage, up to 30 days,

an experiment was performed as per the recommendations from the "NIOSH Guide for Air

Sampling and Method Development and Evaluation" [13]. The 2-mL vial blood samples were

divided into one group of six, and four groups of three. Since the data from the one-day storage

at room temperature was already performed in the previous experiment, it was not deemed

necessary to repeat the experiment a second time. The eighteen samples were refrigerated at 4

°C. The group of six samples was analyzed after seven days. The remaining four groups of three

samples each were analyzed after 10, 14, 21, and 30 days. Results from the refrigeration study

are presented in Table 7.

TABLE 7. LONG TERM STABILITY STUDY AT 4 °C

Storage Time	Sample #	Storage Temperature	Target Value µg/mL (Toluene)	Amount Recovered µg/mL (Toluene)	% Recovered (Toluene)
7 Days	1	4 °C	0.161	0.165	102.5
	2	4 °C	0.161	0.156	96.9
	3	4 °C	0.161	0.154	95.7
	4	4 °C	0.161	0.171	106.2
	5	4 °C	0.161	0.162	100.6
	6	4 °C	0.161	0.168	104.3
10 Days	1	4 °C	0.161	0.158	98.1
	2	4 °C	0.161	0.162	100.6
	3	4 °C	0.161	0.156	96.9
14 Days	1	4 °C	0.161	0.162	100.6
	2	4 °C	0.161	0.153	95.0
	3	4 °C	0.161	0.148	91.9
21 Days	1	4 °C	0.161	0.158	98.1
	2	4 °C	0.161	0.158	98.1
	3	4 °C	0.161	0.158	98.1
30 Days	1	4 °C	0.161	0.162	100.6
	2	4 °C	0.161	0.159	92.5
	3	4 °C	0.161	0.157	97.5
Average				0.159	98.6
Std. Dev.				0.005	3.6

The results from the long-term stability study show that toluene is stable in blood for at least 30 days as long as they are kept at 4 °C with no headspace in the sample container and the lid is well sealed.

PRECISION, ACCURACY, AND BIAS STUDY

A study was performed to determine the precision, accuracy, and bias of the method using whole blood. The bias for a method must be less than or equal to 10%. The coefficient of variation (CV) must be less than or equal to 0.1. When these conditions are met the method is deemed unbiased and accurate with 95% probability to within ±25% of the actual concentrations [13].

During the study, six concentrations ranging from 1 x LOQ (0.02 µg/mL) to 300 x LOQ (6.0 µg/mL) were studied. Whole blood (1.5 mL) was used for each sample. Six samples at each level (a total of 36 samples) were spiked with toluene and isobutanol solutions (described in Reagents and Materials section) to produce the desired concentrations. The samples were then prepared and analyzed according to the method. Table 8 displays the average recoveries of the analytes over the ranges studied.

TABLE 8. AVERAGE RECOVERIES OVER VARIOUS LEVELS

Level (xLOQ)	Rough Target (µg/mL)	Actual Spike (µg/mL)	Average % Recovered	CV
		Toluene	Toluene	Toluene
1	0.02	0.02076	105.9	0.044
3	0.06	0.0576	97.0	0.037
10	0.2	0.2076	98.7	0.008
30	0.6	0.576	100.9	0.025
100	2	2.076	100.1	0.014
300	6	5.76	100.6	0.010

The data were processed to determine the coefficient of variation (CV) at each

concentration level and whether these levels could be pooled using Barlett's test of homogeneity

[13]. The pooled coefficients of variation of those that passed the 95% test were then used for

calculating the overall precision and accuracy of the method as presented in Table 7. The 1 x

LOQ level was not included in the calculations presented in Table 9.

TABLE 9. PRECISION, BIAS, AND ACCURACY

Analyte	Range (µg/mL)	Accuracy (%)	Bias		Precision
			Average	Range	Overall ($\hat{S}_{rT}$)
Toluene	0.0208 to 5.76	<5	0.0006	-0.013 to 0.0085	0.0157

SUMMARY

All data derived during the method development met all NIOSH criteria for precision,

bias, and accuracy in all studies performed [13]. The method proved to be rugged and adaptable

to human blood samples.

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