

# QUANTIFYING UNCERTAINTY FOR THE DETERMINATION OF FORMALDEHYDE IN LEATHER BY COLORIMETRIC METHOD

by

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## ABSTRACT

The uncertainty in the analysis of formaldehyde in leather by colorimetric method was investigated and estimated according to ISO 17025 requirements. A total of sixteen various components contributing to the uncertainty of analysis result were identified and quantified, with separate uncertainty budgets. Among them, seven were related to volumetric measurements whereas the other nine were non volumetric. The study suggests that repeatability holds the largest uncertainty followed by formaldehyde recovery and standard calibration curve, while molecular weight and purity of potassium dichromate held the lowest uncertainty. Under carefully controlled experimental conditions the expanded uncertainty of the result is found to be plus or minus 0.676 mg/kg for a mass fraction of 30.73 mg/kg of formaldehyde in leather. Furthermore, the affect of uncertainty contribution from standard calibration curve and extraction temperature on the expanded uncertainty is explored to formulate valid suggestions for regulating the combined standard uncertainty.

## RESUMEN

La incertidumbre en el análisis de formaldehído en el cuero por el método colorimétrico fue investigado y estimado de acuerdo con los requerimiento de la ISO 17025. Un total de dieciséis diferentes componentes que contribuyen a la incertidumbre en los resultados del análisis fueron identificados y cuantificados, con presupuestos inciertos por separado. Entre ellos, siete estaban relacionados con las mediciones volumétricas, mientras que los otros nueve fueron no volumétricos. El estudio sugiere que la repetibilidad posee la mayor incertidumbre seguido por la recuperación de formaldehído y la curva de calibración estándar, mientras que el peso molecular y la pureza del dicromato de potasio conservan la menor incertidumbre. Bajo condiciones experimentales cuidadosamente controladas la incertidumbre expandida del resultado se encontró  $\pm 0,676$  mg/kg de una fracción de masa de 30,73 mg/kg de formaldehído en cuero. Además, el efecto de la contribución de la incertidumbre en la curva de calibración estándar y de la temperatura de extracción en la incertidumbre expandida es explorada para formular sugerencias válidas para la regulación de la incertidumbre estándar combinada.

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## INTRODUCTION

Statistical evaluation and assessment has greatly changed the way analysis was treated in earlier days. With the advancement in modern analytical chemistry, the quality of a method is thoroughly analyzed on the basis of many parameters including measurement uncertainty. Presentation of analytical results along with a combined uncertainty value is a pre-requisite for method validation and quality assurance as per requirement of the Clause 5.4.6 of the IEC/ISO 17025 standard: 2005.<sup>1</sup> Many articles and research papers have been published on uncertainty so far and the list is growing rapidly.<sup>2</sup> This trend is also penetrating into newer avenues such as the analysis in Clinical Chemistry<sup>3</sup> and to analysis based on chromatographic techniques.<sup>4</sup> Many analytical methods have been critically analyzed with respect to uncertainty.<sup>5,6</sup>

The analysis of formaldehyde in leather and textile articles has gained much interest because of the stringent regulations put up by international regulatory bodies due to the extremely toxic effects of formaldehyde on human health<sup>7,8</sup> as well as on the environment.<sup>9</sup> Formaldehyde has been graded as 'Carcinogenic to humans' (Group I), by International Agency for Research on Cancer (IARC),<sup>7,10-12</sup> as there is evidence that formaldehyde causes nasopharyngeal cancer.<sup>7</sup> The findings by IARC have necessitated manufacturers and exporters of all commodities to strictly monitor the levels of formaldehyde on priority basis.

The specific test method (IUC 19-2) chosen for this investigation is the determination of formaldehyde in leather by colorimetry, by International union of leather technologists and chemists societies (IULTCS). The test method complies with both ISO (ISO 17226-2: 2008) and EN (EN ISO 17226-2) standards.

There are three main reasons for carrying out this exercise. First, to calculate the uncertainty associated with the analysis result; secondly, to formulate a generally acceptable method for the estimation of uncertainty budget for the chemical analytical method that can be employed easily with convenient adjustments each time, and thirdly, to identify the critical steps of the experiments which contribute the most to the uncertainty of measurement and could be subjected to improvement or careful precautionary measures. There are many factors contributing to the overall uncertainty of the result. These factors have been identified, analyzed and an uncertainty budget has been allocated for them in accordance with the ISO<sup>1</sup> approach, also presented in various guides like EURACHEM and CITAC etc.<sup>13-15</sup>

## ANALYTICAL PROCEDURE

Experimental details of the method can be obtained from the standard method (IULTCS/ IUC 19-2, ISO 17226-2: 2008). The method is based on colorimetric measurement of a colored complex formed by the reaction of formaldehyde and Acetyl acetone under controlled environmental conditions (40°C). First of all formaldehyde solution is standardized against standard potassium dichromate solution, serving as a primary standard. A standard calibration curve is plotted by measuring absorbance of different working standard solutions of formaldehyde at 412 nm. The weighed leather sample is ground, homogenized and extracted with water, for 1 hour at 40°C temperature in a shaking water bath. The extract is then treated with acetyl acetone to give a yellow colored compound, the absorbance of which is measured and used to determine the concentration of formaldehyde in the leather sample, with the help of the standard calibration curve. These steps are shown diagrammatically in Fig. 1.

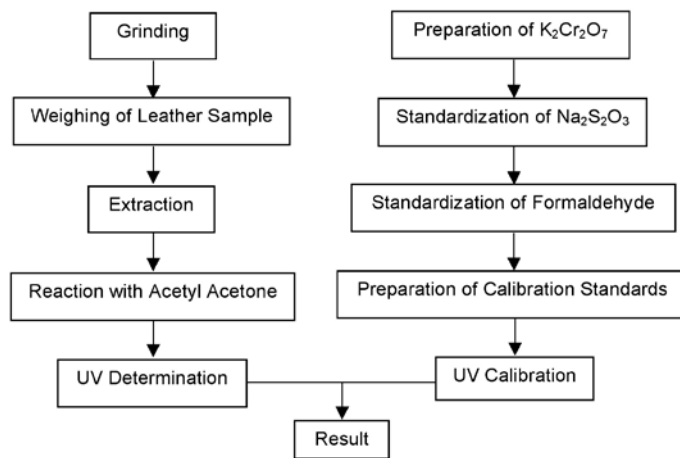


Figure 1. Flow diagram for determination of Formaldehyde in leather.

### Definition of the Measurand

The measurand in this analysis is the mass fraction of formaldehyde in leather sample,  $C_f$ , estimated by colorimetry. The influence quantities for the measurand are the mass of potassium dichromate, its purity, its molecular weight, mass of leather sample taken, extraction time and temperature for formaldehyde extraction, slope of the calibration curve, formaldehyde recovery and various other volumetric sources (glassware) used in the experiment.

### Identification of Uncertainty Sources

The test method comprise of the following two main parts:

- a. Standardization of formaldehyde solution.
- b. Determination of formaldehyde content in leather.

The calculation formulae are mentioned in Appendix B. The experiment has been subjected to critical analysis for the identification and estimation of uncertainty sources associated with different influence quantities. The identified uncertainty sources are shown in the cause and effect diagram (Fig. II.), also called as a 'fish bone' diagram. The detail of the components is presented separately in Tables II and I. Separate budget is also allocated to each of these components. The uncertainty sources are divided into two broad categories for the sake of convenience, i.e. non-volumetric and volumetric sources, and the uncertainty contribution due to volumetric sources is further divided into the following four heads (see figure II and table II):

- Preparation of standard  $K_2Cr_2O_7$  solution
- Standardization of  $Na_2S_2O_3$  solution
- Standardization of formaldehyde solution
- Sample extrac

#### Non-volumetric Sources

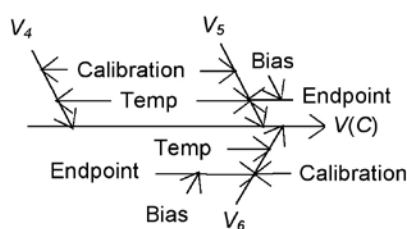
1. Molar Mass,  $M_w$ : In this experiment potassium dichromate serves as a primary standard. After combining the uncertainty of the atomic weights,<sup>16</sup> the standard uncertainty in the molecular mass  $u(M_w)$ , of potassium dichromate is obtained using equation 1 and 2, through the established approach.<sup>13</sup>

$$\text{standard uncertainty} = \frac{\text{Quoted uncertainty}}{\sqrt{3}} \quad (1)$$

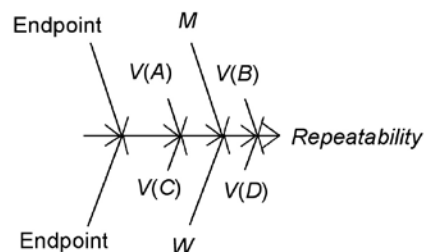
$$\text{Total Standard uncertainty} = \text{standard uncertainty} \times \text{No. of atoms} \quad (2)$$

The quoted uncertainties follow a rectangular distribution. Although the uncertainty budget for molecular weight is negligible but it is included here for the sake of completeness. The molecular weight of potassium dichromate and its associated standard uncertainty is presented in Table I.

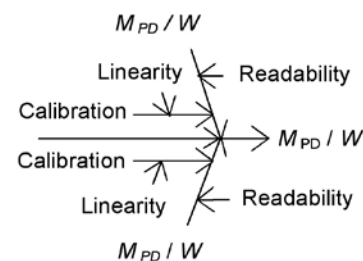
- Purity,  $P$ : The supplier's certificate quotes a purity of  $99.9954\% \pm 4.6 \times 10^{-5}$  for Potassium dichromate (Oxidimetric Standard, traceable to NIST, SRM 136f). The standard uncertainty  $u(P)$ , (table I) is calculated by considering a rectangular distribution.<sup>13</sup>
- Mass of Potassium Dichromate,  $M_{PD}$ : The weighing procedure is a weight by difference, therefore the contribution due to gross and tare is counted separately as shown in the cause and effect diagram (Fig. II.). The calibration itself has two possible uncertainty sources: the sensitivity and the linearity of the calibration function. As the weighing is done on the same scale and over a small range of weight so the sensitivity contribution can be neglected. The linearity value is the maximum difference between the actual mass on the pan and the reading of the scale.



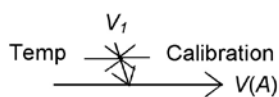
C & E diagram for V(C)



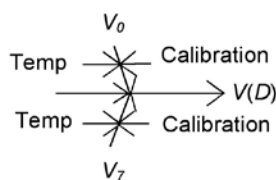
C & E diagram for repeatability



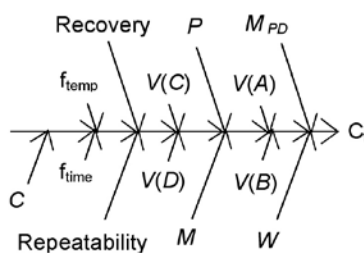
C & E diagram for masses ( $M_{PD}$  &  $M_{PD} / W$ )



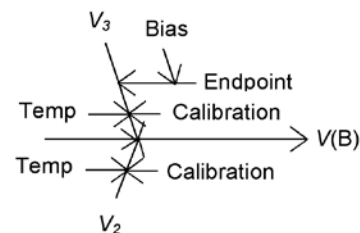
C & E diagram for V(A)



C & E diagram for V(D)



Final C & E diagram



C & E diagram for V(B)

Figure 2. Cause and effect (C & E) diagram.

The linearity of the balance is reflected by calibration which quotes a value of  $\pm 0.15$  mg. The balance manufacturers own uncertainty evaluation recommends the use of a rectangular distribution to convert the linearity contribution to a standard uncertainty.<sup>13</sup> The contribution due to repeatability of weighing is included in the combined repeatability term. The mass of  $K_2Cr_2O_7$  weighed and the associated standard uncertainty  $u(M_{PD})$  is shown in table I.

4. Mass of Leather Sample,  $W$ : The uncertainty sources in sample weighing are similar to those discussed in mass of potassium dichromate. The contribution due to repeatability of sample weighing is included in the combined repeatability term. Standard uncertainty due to weighing  $u(W)$  is shown in table I.

5. Formaldehyde Concentration: The standard calibration curve was drawn with the help of least square fit calculation,<sup>17</sup> which assumes that the uncertainties of the abscissa values are considerably smaller than the uncertainties of the ordinate values. Therefore, the usual uncertainty calculation procedures for concentration of formaldehyde in the extract only reflect the uncertainty in the absorbance and not the uncertainty of the calibration standards. In this case, however, the uncertainty of the calibration standards is sufficiently small to be neglected.<sup>13</sup> The standard uncertainty  $u(C)$  associated with concentration

of formaldehyde in leather extract, through least square fit, is treated as explained by Taylor,<sup>18</sup> using equations 3, 4, and 5.

$$\sigma_y^2 = \frac{1}{N-2} \sum (y_i - b - mx_i)^2 \tag{3}$$

where  $\sigma_y^2$  gives the uncertainties in the ordinate values, whereas;

$N$ : number of data points

$y_i$ : absorbance of the  $i^{\text{th}}$  calibration standard

$b$ : intercept

$m$ : slope

$x_i$ : concentration of the  $i^{\text{th}}$  calibration standard

$$u(C) = \sqrt{\frac{N\sigma_y^2}{\Delta}} \tag{4}$$

where;

$$\Delta = N(\sum x_i^2) - (\sum x_i)^2 / N \tag{5}$$

and  $u(C)$  is the standard uncertainty in the slope

**TABLE I**  
**Non-volumetric uncertainty budgets for formaldehyde estimation in leather.**

Influence Quantities	Description	Value $x$	Standard uncertainty $u$	Relative Standard uncertainty $u(x)/x$	Percent Uncertainty Budget %
$Rep$	Repeatability	1.0	0.008800	0.008800	32.54
$P$	Purity of $K_2Cr_2O_7$	0.999954	0.000027	0.000027	0.10
$M_{PD}$	Mass of $K_2Cr_2O_7$	0.5884 g	0.000132 g	0.000225	0.83
$W$	Mass of leather Sample	2.0628 g	0.000132 g	0.000064	0.24
$M_W$	Molecular mass of $K_2Cr_2O_7$	294.1846 g.mol <sup>-1</sup>	0.001382 g.mol <sup>-1</sup>	0.000005	0.02
$C$	Slope from linear least square fit graph	0.266471 ml/ $\mu$ g	0.001050 ml/ $\mu$ g	0.000022	14.52
$f_{temp}$	fractional change in formaldehyde recovery as a function of extraction temperature	1.0	0.002370	0.002370	8.76
$f_{time}$	fractional change in formaldehyde recovery as a function of extraction time	1.0	0.001460	0.001460	5.40
$Rec$	Recovery	0.96	0.004270	0.004450	16.38
	<b>Total non-volumetric contribution</b>				<b>78.79</b>

6. Effect of Temperature on Formaldehyde Extraction,  $f_{temp}$ : A number of studies to determine the effect of temperature on formaldehyde release from leather were carried out. The change in formaldehyde release at temperature near 40°C is approximately linear, with a gradient of approximately 0.82%°C<sup>-1</sup>, determined experimentally. (The detail is covered in the experimental section).
7. Effect of time on Formaldehyde Extraction,  $f_{time}$ : Experimental data has revealed that the rate of change of formaldehyde recovery is 0.1265%.min<sup>-1</sup>. (The detail is covered in the experimental section).
8. Repeatability,  $rep$ : It is not a practical approach to accommodate the repeatability contributions of various individual operations therefore many sets of duplicate determinations were carried out for the estimation of the contribution due to repeatability, with different types of leather samples. The difference (termed as the normalized difference) within each set was determined and divided by the mean value. The standard deviation of the normalized difference was obtained and divided by the square root of two in order to correct from standard deviation for pairwise

differences to standard uncertainty for single values.<sup>13</sup> This value,  $u(rep)$  represents the different repeatability terms as shown in the cause and effect diagram (Fig. II).

9. Recovery,  $Rec$ : A validation study was carried out with twenty homogenized samples split into two parts, from which one part was spiked. The mean recovery of these samples was found to be 96% with a standard deviation of 1.91%. The standard uncertainty,  $u(Rec)$  was calculated as the standard deviation of the mean<sup>13</sup> (see equation 6).

$$u(Rec) = \frac{S_r(\%) \sqrt{R}}{100} \quad (6)$$

where;

$S_r(\%)$ : Percent standard deviation

R: Percent recovery

#### Volumetric Sources

10. Volume: Volumetric measurements have three major influences; calibration, repeatability and temperature

**TABLE II**  
**Volumetric uncertainty budgets for formaldehyde estimation in leather.**

Volume categories	Influence Quantities	Description	Value x	Standard uncertainty u	Relative Standard uncertainty $u(x)/x$	Percent Uncertainty Budget %
V(A)	$V_1$	Volume of K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> solution prepared	100 ml	0.048560 ml	0.000486	1.79
V(B)	$V_2$	Volume of K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> used in standardization of Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	10.0 ml	0.005030 ml	0.000503	1.86
	$V_3$	Titer of Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> for K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	12.1 ml	0.005990 ml	0.000495	1.83
V(C)	$V_4$	Volume of formaldehyde solution used for Standardization	5.0 ml	0.003480 ml	0.000696	2.57
	$V_5-V_6$	Titer difference of Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> volume used for blank and standardization of formaldehyde solution	6.4 ml	0.015210 ml	0.002453	8.79
V(D)	$V_7$	Volume of leather sample extract	5.0 ml	0.003480 ml	0.001313	2.57
	$V_0$	Elution volume in ml	50 ml	0.024320 ml	0.000486	1.80
		<b>Total volumetric contribution</b>				<b>21.21</b>

Note:

1. The individual uncertainties are calculated up to 6 decimal places to highlight minor changes in uncertainty introduced due to experimental modifications or changes in individual budgets.
2. The percent uncertainty budget depends on different factors such as equipment, handling and calibration of glassware, therefore the figures could vary slightly on case to case basis.

effects. The quoted uncertainties from calibration of glassware follow a triangular distribution (equation 7).<sup>13</sup>

$$u_1 = u_q / \sqrt{6} \quad (7)$$

where;

$u_1$  : uncertainty contribution from calibration

$u_q$  : quoted uncertainty for glassware calibration

According to the manufacturer the glassware has been calibrated at a temperature of 20°C, whereas the laboratory temperature varies between the limits of ± 4°C. The uncertainty from this effect is calculated from the estimate of the temperature range and the coefficient of the volume expansion. The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation (equation 8).<sup>13</sup>

$$u_2 = v_1 \times k \times \Delta t / \sqrt{3} \quad (8)$$

where;

$\Delta t$  : temperature variation in the lab

$k$  : coefficient of expansion of water

$v_1$  :  $i^{\text{th}}$  volume measurement

Finally  $u_1$  and  $u_2$  contributions are combined according to equation 9.

$$u = (u_1)^2 + (u_2)^2 \quad (9)$$

The description, standard uncertainties  $u(V_0)$ ,  $u(V_1)$ ,  $u(V_2)$ ,  $u(V_3)$ ,  $u(V_7)$  from calibration and the relative standard uncertainties associated with different volumes;  $V_0$ ,  $V_1$ ,  $V_2$ ,  $V_3$  and  $V_7$ , respectively are given in Table II. The standard uncertainty,  $u(V_5 - V_6)$ , after combination of  $u(V_5)$  and  $u(V_6)$  according to the rule of propagation of uncertainty for addition and subtraction<sup>17,18</sup> is given in Table II. The contribution due to volumetric repeatability is included in the combined repeatability term  $u(rep)$ .

The method also has provision for samples with high formaldehyde content, which can be analyzed by appropriately diluting the sample extract. In that case, an additional volumetric uncertainty term  $u(V_8)$  may be included in the combined standard uncertainty equation 10.

11. End-Point Detection: The problem with visual detection is that some excess titrant has to be added to get the end point of titration. This excess titrant corresponds to the difference between equivalence point and the end point. The bias has to account for the two titrations involved. As a precaution, starch

is always added just before the end point otherwise some iodine remains complexed with starch, deviating away from the equivalence point of the titration.<sup>19</sup> This difference is corrected through a blank determination therefore a separate budget has not been allocated.

$$\frac{u_c(C_f)}{C_f} = \sqrt{\left[\frac{(rep)}{rep}\right]^2 + \left[\frac{(uP)}{P}\right]^2 + \left[\frac{u(MPD)}{MPD}\right]^2 + \left[\frac{u(V_0)}{V_0}\right]^2 + \left[\frac{u(V_1)}{V_1}\right]^2 + \left[\frac{u(V_2)}{V_2}\right]^2 + \left[\frac{u(V_3)}{V_3}\right]^2 + \left[\frac{u(V_4)}{V_4}\right]^2 + \left[\frac{u(V_5-V_6)}{V_5-V_6}\right]^2 + \left[\frac{u(V_7)}{V_7}\right]^2 + \left[\frac{u(W)}{W}\right]^2 + \left[\frac{u(MW)}{MW}\right]^2 + \left[\frac{u(C)}{C}\right]^2 + \left[\frac{u(f_{temp})}{f_{temp}}\right]^2 + \left[\frac{u(f_{time})}{f_{time}}\right]^2 + \left[\frac{u(Rec)}{Rec}\right]^2} \quad (10)$$

### Combined Standard Uncertainty

The different influence quantities in formaldehyde analysis and the associated uncertainties are presented in Table I and II. For the multiplicative expression given in section (b) of Appendix B, the uncertainties associated with each influence quantity is combined as shown in equation 10.

## EXPERIMENTAL

### Standards and Reagents

All the chemicals used were A.R grade chemicals. Formaldehyde solution, extra pure grade (37%, USP) was purchased from Scharlau. Sodium thiosulfate was purchased from BioM Laboratories, USA. Potassium dichromate, SRM 136f (Oxidimetric Standard, traceable to NIST), was purchased from USA. Potassium iodide was obtained from BDH chemicals Ltd., Poole, England. Ammonium acetate was arranged from Riedel-de Haën.

### Calibration

The UV-visible spectrophotometer (double beam), model Nicolet Evolution 100 from Thermo Electron Corporation, shaking water bath, and all the glassware were calibrated from National Physical and Standards Laboratory, (PCSIR), Karachi. The shaking water bath was arranged from Lab companion, BS – 11, having a temperature resolution of 0.1°C, and a maximum shaking speed of up to 185 RPM.

The experiment was performed according to the standard method (IUC 19-2), considering all the necessary precautions. The selected sample was chrome tanned goat leather prepared in the tannery of Leather Research Center, PCSIR, Karachi. The leather sample was ground according to the grinding procedure EN ISO 4044 and thoroughly homogenized. The temperature was maintained at 40°C ± 0.5°C for both the extraction and complexation reaction of formaldehyde with acetylacetone, at a shaking speed of 65 RPM. The affect of extraction temperature was empirically determined yielding a

value of  $0.82\% \cdot ^\circ\text{C}^{-1}$ . The  $\pm 0.5^\circ\text{C}$  tolerance allowed by the standard method leads to a factor  $(0.82^\circ\text{C}^{-1} \times 0.5) / 100$ , which is equal to  $\pm 0.0041$ .

The affect of time on formaldehyde extraction was experimentally determined to be  $0.1265\% \text{ min}^{-1}$ . The allowed tolerance in extraction time is  $\pm 2 \text{ min}$ , which causes a variation of  $(0.1265 \times 2) / 100$  equal to  $\pm 0.00253$ .

Both uncertainties follow a rectangular distribution. The standard uncertainties in extraction time  $u(f_{\text{time}})$  and extraction temperature  $u(f_{\text{temp}})$  are presented in Table I. The absorbance measurements were taken up to three decimal places. The extract was quickly filtered in Witt's Pot to avoid losses.

### Application Example

All the uncertainty components were evaluated for their respective budgets for a practical example using the derived uncertainty equation 10. The value of slope (m) from the least square fit (forced through zero) calculation was found to be  $0.266217 \text{ kg/mg}$ . The combined standard uncertainty  $u(C_p)$  finally comes out to be  $0.011 \text{ mg/kg}$ . For expanded uncertainty  $U(C_p)$ , a coverage factor of 2 ( $k = 2$ ) is incorporated resulting in a confidence interval of approximately 95%. Hence the formaldehyde content in the examined leather sample, should be reported as;  $30.73 \pm 0.676 \text{ mg/kg}$  ( $k = 2$ , 95% confidence level). (The emphasis is not on figures, so this value is a nearest approximation only). To avoid tedious calculation, this could be done on Excel 2007, so that the result can be easily obtained by just changing a few figures each time. The same software was programmed to generate a graphical presentation of the uncertainty budget as shown in Figure III.

### Limitations:

Although most of the uncertainty sources are discussed in this study but still there are some sources that are very difficult to estimate accurately, such as the uncertainty in absorbance measurement due to a highly colored sample and sample homogenization errors etc.

### Bias Study

The standardization of sodium thiosulphate and formaldehyde, both involve redox titrations. Both titrations were performed after gently purging nitrogen gas through the solution and under nitrogen layer to avoid the interference due to oxygen in iodometry. This approach follows the principle that it is better to prevent any bias than to correct for it.

### Additional Aspects

The evaluation and estimation procedure does not incorporate any allowance for repeatability of the spectrophotometer readings. This is because the modern instruments are fairly stable over the duration of the experiment while long term variations are compensated through the calibration performed before the measurement of the unknowns.<sup>14</sup>

## RESULTS AND DISCUSSION

The experiment has been studied from sample grinding to final result for all possible contributions to uncertainty. The individual uncertainty budgets are presented in Fig. III.

The contributions are easy to visualize and understand using a histogram. Fig. III is obtained from a tedious calculation on an Excel worksheet, the details of which are not mentioned here for the sake of simplicity.

As mentioned in the introduction, the uncertainty budget is split into two parts;

a. Volumetric uncertainty

b. Non-volumetric uncertainty

There is a good agreement between the combined standard uncertainty obtained from manual calculation and that obtained from spreadsheet calculation corroborating the validity of the whole exercise. The percent contributions of the individual uncertainty components are presented in table I and II (last columns). Though, a large number of glassware is used in the experiment but the total uncertainty contribution is only 21.21% of the overall expanded uncertainty. On the other hand the non-volumetric contribution accounts for the remaining 78.79%, among which repeatability (32.54%), formaldehyde recovery (16.38%) and the standard calibration curve (14.52%) constitute the major budget portion that imposes marked influence on the uncertainty of the results. These parameters also highlight the potential areas in the experiment, which can be improved. The uncertainty contribution of time and temperature set for extraction emphasizes on their strict control. The contribution from the uncertainty of molecular weight of the primary standard (potassium dichromate) is negligibly small and in the current situation it can be omitted,

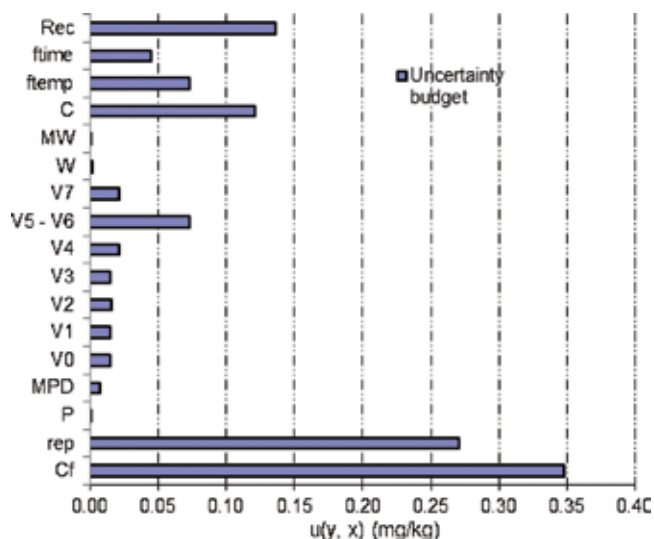


Figure 3. Uncertainty contributions in analysis of formaldehyde in leather.

but it is included for the sake of completeness. The contributions from the weighing procedures are also comparatively small.

**POSSIBLE IMPROVEMENTS**

Though, the vast number of steps in the experiment introduces a large number of uncertainty sources but a useful study of their impact on the overall uncertainty suggests possibilities of certain improvements. For this reason the affect of some of the major shareholders of uncertainty budget on the expanded uncertainty has been explored.

**Calibration Curve:**

The influence of uncertainty associated with the slope of the calibration curve,  $u(C)$  on expanded uncertainty is shown in table III and figure IV, which suggest that the quality of the standard calibration curve is very crucial in determining the overall uncertainty. This relation was explored by calculating the Pearson correlation coefficient ‘r’<sup>20</sup> and exploring its impact on the expanded uncertainty with parallel monitoring of its percent budget contribution.

The slope of the calibration curve has a significant uncertainty budget, emphasizing the importance of the calibration step in the experiment. It is evident from the graph (figure IV) that the correlation coefficient ‘r’ must be above 0.990 in order to have an acceptable uncertainty contribution. But a good calibration curve should have an ‘r’ value within a narrow range of 0.998 to 1.000, as it is this region of the graph in which the overall uncertainty is most drastically affected and the percent contribution is also comparatively low (figure 4).

**$f_{temp}$ :**

The uncertainty budget for temperature variation in extraction is estimated on the basis of allowed tolerance by the method. However, if we restrict our temperature variation within a narrower limit, for e.g. +/- 0.1°C (easily achievable on modern digital instruments) the uncertainty contribution could be reduced further. In order to get an empirical estimate of the overall uncertainty, a practical temperature tolerance (+/- 0.2°C) has been used rather than the tolerance in the method (+/- 0.5°C), which reduced its contribution in the overall uncertainty to almost half of its initial value at +/- 0.5°C. The complete pattern of impact in the range of interest is explored in figure V and table III. The extraction temperature tolerance is in direct relation with its percent contribution (as can be expected), while the expanded uncertainty increases exponentially with it. The graph depicts that by limiting the temperature tolerance from +/- 0.5°C to +/- 0.2°C, the expanded uncertainty can be reduced by more than 5%.

**Recovery:**

The contribution from recovery is the second largest among all the other sources. Although improvement in this component

**TABLE III**  
**The affect of correlation between concentrations of formaldehyde standards and absorbance and extraction temperature tolerance on expanded uncertainty.**

Pearson Correlation Coefficient “r”	Percent Contribution %	Expanded Uncertainty U
1.000	14.23	0.6952
0.999	43.12	1.2802
0.998	54.63	1.8676
0.997	59.95	2.2712
0.996	64.10	2.6759
0.995	67.43	3.0791
0.994	68.86	3.2796
0.993	70.93	3.6053
0.992	72.05	3.8023
0.991	73.08	3.9985
0.990	74.37	4.2675
Temperature Tolerance		
± 0.10	1.84	0.6804
± 0.20	3.62	0.6823
± 0.30	5.33	0.6853
± 0.40	6.98	0.6896
± 0.50	8.59	0.6952

Note: The above data has been obtained through an Excel program by keeping rest of the contributions as constant, especially a constant slope and hence a constant mass fraction of formaldehyde in leather.

is possible with slight modification in the experiment, which is actually a diversion towards method development and does not fit in the scope of this study, but it does point out towards space for improvement. Although the extraction of formaldehyde imposes some limitations on the efficiency of recovery but there is space for some modification that could improve the percent recovery. For instance if the temperature

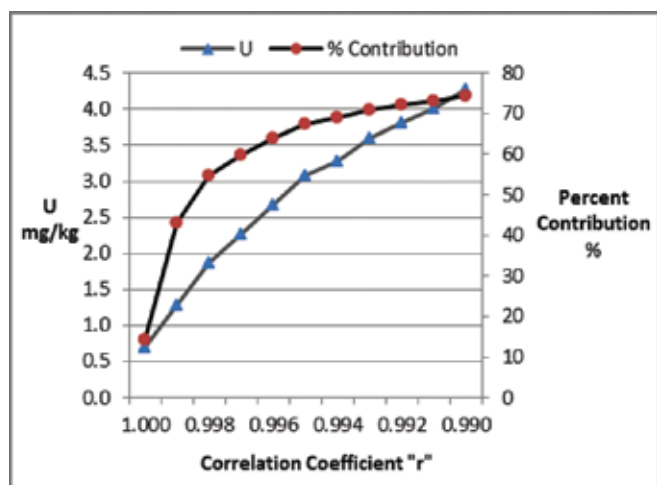


Figure 4. Impact of correlation between concentrations of formaldehyde standards and their absorbance on its percent contribution (budget) and expanded uncertainty.

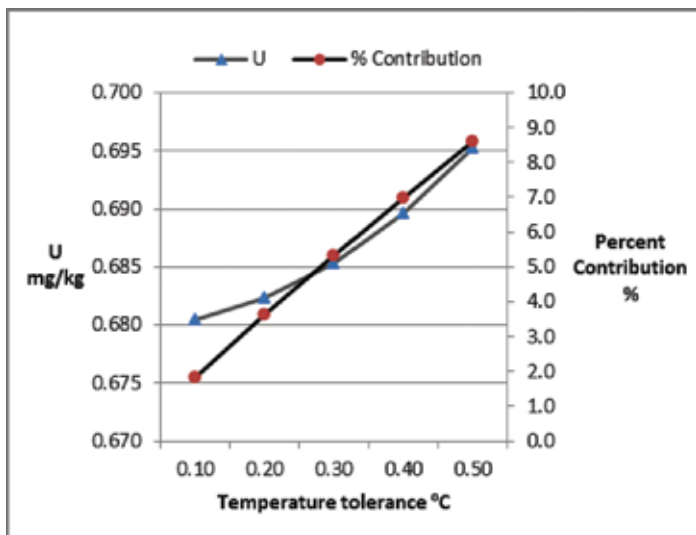


Figure 5. Effect of extraction temperature tolerance on its percent contribution (budget) and on expanded uncertainty.

is regulated by means of a microwave source which instigates heating by targeting polar molecules<sup>21</sup> then higher recoveries and lower uncertainty budget for recovery is possible. Generally speaking, temperature control during extraction and quick filtration can minimize the uncertainty contribution from recovery, while instrument calibration, proper warm up time, stable power supply (free from fluctuation) and other appropriate precautionary measures can reduce the contribution due to graph slope and absorbance measurements.

## CONCLUSION

The findings present a good overview of the experiment for formaldehyde determination in leather, with reference to the requirements of quality assurance and uncertainty estimation.

It is evident that the number of individual steps in the experiment gives rise to various uncertainty sources but the estimation of their contribution also suggests that these uncertainties can be minimized under controlled experimental conditions if not eliminated by following the necessary precautions. A total of sixteen different uncertainty sources are identified among which six have volumetric origin while ten are non-volumetric. Major emphasis should be placed on the non-volumetric uncertainty sources, the largest contributors among which are slope of the calibration curve, formaldehyde recovery factor, extraction temperature etc. An attempt was made to identify the uncertainty sources in this experiment but chances of improvements always exist which can be brought in through the different ways discussed or through new approaches.

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## APPENDIX A

### Abbreviations

HCHO: Formaldehyde

PCSIR: Pakistan Council for Scientific and Industrial Research

## APPENDIX B

a. Standardization of formaldehyde solution.

$$C_{HCHO} = \frac{(V_6 - V_5) \times C_{\text{thiosulfate}} \times M_{HCHO}}{2 \times V_4 \times (1/1000)} \text{ mg/kg} \quad (1)$$

where;

$C_{HCHO}$ : concentration of formaldehyde stock-solution, in mg/10 ml

$V_4$ : volume of formaldehyde solution used for standardization, in ml

$V_6$ : titer of thiosulfate solution for the blank, in ml

$V_5$ : titer of thiosulfate solution for the sample solution, in ml

$M_{\text{HCHO}}$ : molecular weight of formaldehyde, 30.02 g/mol

$C_{\text{thiosulfate}}$ : concentration of sodium thiosulfate solution, M

The values 2 and 1000 are conversion factors

b. Determination of formaldehyde content in leather.

$$C_f = \frac{(E_p - E_e) \times V_0 \times V_f \times f_{\text{time}} \times f_{\text{temp}} \times \text{rep}}{F \times W \times V_7 \times \text{Rec}} \text{mg/kg} \quad (2)$$

where;

$C_f$ : mass fraction of formaldehyde in mg/kg of leather sample

$E_p$ : absorbance of filtrate after reaction with acetyl acetone

$E_e$ : absorbance of filtrate (initial color)

$V_0$ : volume of elution in ml (standard conditions: 50 ml)

$V_7$ : aliquot taken from filtrate in ml (standard conditions: 5 ml)

$V_f$ : volume of final solution obtained after reaction, in ml (standard conditions: 10 ml)

$F$ : gradient of calibration curve (dy/dx) in ml/ $\mu$ g

$W$ : mass of leather in g

$f_{\text{time}}$ : fractional change in formaldehyde recovery as a function of extraction time

$f_{\text{temp}}$ : fractional change in formaldehyde recovery as a function of extraction temperature

$\text{rep}$ : repeatability effect

$\text{Rec}$ : Recovery

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