Method validation for methanol quantification present in working places

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Abstract. Given the widespread use of methanol by different industry sectors and high toxicity associated with this substance, it is necessary to use an analytical method able to determine in a sensitive, precise and accurate levels of methanol in the air of working environments. Based on the methodology established by the National Institute for Occupational Safety and Health (NIOSH), it was validated a methodology for determination of methanol in silica gel tubes which had demonstrated its effectiveness based on the participation of the international collaborative program sponsored by the American Industrial Hygiene Association (AIHA).

1. Introduction
Methanol is widely used as an industrial solvent in the production of other chemicals, such as paint and varnish removers and in automotive antifreeze and defrosting solutions as well as liquid windshield wipers. Many other products such as plastics, paints and explosives are derived from the main product of methanol, formaldehyde [1] [2] [3].

Once absorbed, methanol is slowly eliminated by human body, therefore it should be regarded as a cumulative poison. Although short exposures to vapors should not cause adverse effects when they become daily may result in methanol accumulation sufficient to cause health damage [5] [7]. Chronic exposure to methanol vapor has been reported to produce symptoms such as headache, dizziness, blurred vision, eye irritation, nausea and gastrointestinal upset [2].

A review of data on environmental levels and human exposure notes that most emissions to the environment arise from the production and use of methanol as a solvent in industrial processes and, to a lesser extent, from a variety of other industrial processes and consumer applications [2].

Regarding the toxicological effects of methanol, a regulatory standard enacted by the Ministry of Labor and Employment of Brazil established the methanol exposure limit that a worker may be submitted in production environment. For working hours of 48 hours per week, the tolerance limit specified for methanol is 156 ppm [4]. There is an obvious need to develop an inexpensive, sensitive, rapid, and reliable alternative GC (gas chromatography) method to determine methanol in low level presented in working environments.

Based on NIOSH 2000 method [6], a method was optimized and validated using gas chromatography with flame ionization detection and headspace injection in order to quantify methanol presented into silica gel samplers.
2. Materials and Methods

2.1. Materials
Reverse-osmosis type quality water (purified with a Milli-RO plus Milli-Q station from Millipore, Milford, MA) and HPLC quality were used throughout.

2.2. Extraction step
Methanol is collected form the air by a silica gel tube, containing two sections of 20/40 mesh silica gel (front = 100 mg, back = 50 mg) separated by a 2-mm urethane foam plug. After the forced passage of air through the tube using of a vacuum pump, the adsorbent is transferred to a vial headspace with 2 mL of ultrapure water. The sealed vial is placed in an ultrasound bath for 30 minutes before the injection.

2.3. Chromatographic conditions
A headspace injection system (Perkin Elmer Turbo Matrix 40 Trap) coupled to gas chromatograph with flame ionization detector (Perkin Elmer Clarus 500) were used to quantify methanol extracted from silica gel adsorbent. The analyses were performed under the following chromatographic conditions: Column, RTX-1301 (Crossbond® cyanopropylphenyl dimethyl polysiloxane), 15 m × 0.32 mm i.d., DF = 1 mm, from Restek (Bellafonte, PA). The temperature of the FID was 240ºC, and the injector temperature was 200ºC. The oven temperature was programmed to 40ºC (for 6 min), Helium was the carrier gas with a flow of 0.5 mL/min for two minutes followed by an increase of 100mL/min to 1mL/min. The parameters of headspace injection were: incubation time of 30 minutes, temperature of 90 ºC, needle temperature 200 ºC and time of pressurization of 1 minute. The retention time for methanol in the chromatographic system was observed at 2.567 minutes.

![Figure 1. Chromatogram of an aqueous solution with 50 mg/L methanol (2.567 minutes).](image-url)
2.4. Method Validation

Validation of the method was made in terms of selectivity, linearity, recovery, repeatability and inter-day precision, quantification and detection limits, according to the guidelines of INMETRO (Brazil Metrological Institute) [8].

3. Results and Discussion

3.1. Method Validation

3.1.1. Selectivity

The t test was used to compare the slope of the analytical curves of the analytes in solution and with fortified matrix (silica gel tubes), which presented $t_{\text{calculated}} < t_{\text{table}}$ (Table 1), showing that there was no matrix effect for this analyte.

**Table 1.** Comparison of analytical curves of methanol in solution and extracted from fortified matrix

| Concentration (mg/L) | Analytical curve in solution | Analytical curve extracted from fortified matrix |
|----------------------|------------------------------|-------------------------------------------------|
|                      | Area                         | Area                                            |
| 50                   | 30544                        | 30760                                          |
|                      | 29649                        | 29068                                          |
|                      | 29029                        | 29068                                          |
|                      | 55522                        | 56494                                          |
| 100                  | 55925                        | 54393                                          |
|                      | 57357                        | 56306                                          |
|                      | 11386                        | 113114                                         |
| 200                  | 119023                       | 110210                                         |
|                      | 112304                       | 113773                                         |
|                      | 239995                       | 221744                                         |
| 400                  | 225010                       | 231476                                         |
|                      | 234666                       | 229870                                         |
|                      | 336433                       | 339304                                         |
| 600                  | 346917                       | 340823                                         |
|                      | 338986                       | 352482                                         |
|                      | 460882                       | 472138                                         |
| 800                  | 467082                       | 477241                                         |
|                      | 460962                       | 475651                                         |
|                      | 552981                       | 575848                                         |
| 1000                 | 558220                       | 566656                                         |
|                      | 567105                       | 584156                                         |

Angular Coefficient 568 580
Linear Coefficient 1067 -855
$R^2$ 0.999240 0.999203
$t_{\text{calc}}$ 0.419072
$t_{\text{tab}}$ 2.024394
3.1.2. Linearity
Method linearity was determined by evaluating the regression curve and is indicated by the square correlation coefficient ($R^2$). The line of best fit for the relationship between the ratio of peak area and internal standard area and concentration of analytes in the samples was determined by linear regression performing calibration curves in the considered concentration ranges (50-1000 mg/L), depicted in Figure 2. The slope was calculated taking into account calibration curves in triplicate prepared in methanol aqueous solution. Linearity was achieved with a minimal $R^2$ of 0.99. The residue graph (Figure 3) showed randomly distributed around the center line, without a trend, confirming the absence of random errors. Cochran test showed (Table 2) that the residues are homocedastic ($C_{calculated} < C_{table}$). Regression analysis of calibration data achieved satisfactory linearity over the considered concentration range (50-1000 mg/L). Grubbs test was applied in the results of analytical curve and no outlier value was observed (Table 3).

**Figure 2.** Analytical curve of methanol in aqueous solution.  
**Figure 3.** Residuals graphic of analytical curve.

### Table 2. Cochran test for metanol analytical curve

| Cochran values  |       |
|-----------------|-------|
| $C_{calculated}$ | 0.349 |
| $C_{table}$     | 0.561 |

3.1.3. Limit of Detection (LOD) and quantification (LOQ)
In order to determine the limits of detection and quantification it was performed an evaluation of 10 fortified matrix at low concentration value of LOD was obtained by multiplying the standard deviation by $t_{table}$ (n= five samples, IC = 95%). LOQ value was based on multiplying LOD value by 3.3. The obtained LOD was 2.6 mg/L methanol, while LOQ presented a calculated result of 8.5 mg/L methanol (Table 4). Fortified matrices were tested at the calculated concentrations of LOQ and the results of signal / noise ratio were greater than five and the relative standard deviation of less than 5%.
Table 3. Grubbs test for outlier evaluation

| Nominal Methanol Concentration (mg/L) | Experimental Methanol Concentration (mg/L) | Average Conc. (mg/L) | Standard Deviation | Variance | G calculated | G table |
|--------------------------------------|--------------------------------------------|----------------------|--------------------|----------|--------------|----------|
| 50                                   | 51.9                                       | 50.5                 | 1.340              | 1.796    | 1.055        | 0.934    |
| 100                                  | 96.5                                       | 97.1                 | 1.697              | 2.880    | 1.129        | 0.774    |
| 200                                  | 207.6                                      | 200.61               | 6.187              | 38.283   | 1.126        | 0.785    |
| 400                                  | 394.1                                      | 408.54               | 13.367             | 178.666  | 0.891        | 1.081    |
| 600                                  | 608.6                                      | 597.81               | 9.621              | 92.556   | 1.123        | 0.795    |
| 800                                  | 820.1                                      | 812.85               | 6.259              | 39.170   | 1.155        | 0.589    |
| 1000                                 | 980.4                                      | 982.59               | 12.564             | 157.866  | 1.074        | 0.904    |

Table 4. Limit of Detection (LOD) and quantification (LOQ) of Methanol

| Parameters                     | Experimental concentrations of methanol (mg/L) |
|--------------------------------|-----------------------------------------------|
| 50 mg/L (nominal concentration)| 50.8                                          |
|                                | 49.8                                          |
|                                | 49.7                                          |
|                                | 49.6                                          |
| Average Standard Deviation     | 1.1                                           |
| $t_{table}$                    | 2.2622                                        |
| LOD                             | 2.6 mg/L                                      |
| LOQ                             | 8.5 mg/L                                      |
3.1.4. Recovery
Fortified matrices were prepared in three known concentrations (low, medium and high) over the working range as shown in table 5. The recovery of this method was determined by analysis of nine replicates of samples spiked with methanol at three different concentrations and the results were quite satisfactory the average recovery of 99.9% was calculated.

| Nominal Concentration (mg/L) | Recovery (%) |
|-----------------------------|--------------|
| 50                          | 99.2         |
| 50                          | 99.5         |
| 50                          | 99.5         |
| 400                         | 101.2        |
| 400                         | 98.0         |
| 400                         | 101.3        |
| 1000                        | 99.6         |
| 1000                        | 100.6        |
| 1000                        | 100.2        |
| **Average**                | **99.9**     |

3.1.5. Repeatability
Nine determinations were carried along the working range at three concentration levels (triplicates per concentration level) in matrix fortified. Then calculated the standard deviation per concentration level studied and all values were below 2% (Table 6).

| Level | Concentration (mg/L) | Average | Standard deviation | Repeatability limit | RSD% |
|-------|-----------------------|---------|--------------------|---------------------|------|
| 1     | 49.7                  | 49.7    | 0.0856             | 0.2398              | 0.2  |
| 2     | 392.0                 | 400.6   | 7.4309             | 20.8065             | 1.8  |
| 3     | 1005.8                | 1001.5  | 4.9287             | 13.8004             | 0.5  |

3.1.6. Inter-day Precision
Different analysts analyzed samples under the same sample preparation and equipment conditions (Table 7). After comparison of variances (F test) and means (t test) it was not observed statistically significant difference between the two assessments by different analysts. On tables 8 are depicted the results of F test and t test.
3.1.7. Reproducibility
Given the lack of certified reference material, the participation in an interlaboratory program (IP) was crucial to evaluate the effectiveness of the methodology validated, which had as provider of the study the American Industrial Hygiene Association (AIHA). All results were satisfactory in PI, as shown on Table 6, with z-scores below 2.0.

Table 7. Values obtained from the two analysts.

| Nominal Concentration (mg/L) | Experimental concentration (mg/L) |
|-----------------------------|----------------------------------|
|                             | Analyst 1 | Analyst 2 |
|                             |           |           |
| 50                          | 51.9      | 50.7      |
|                             | 50.3      | 50.9      |
|                             | 49.2      | 51.4      |
|                             | 198.5     | 202.8     |
| 200                         | 207.6     | 200.7     |
|                             | 195.7     | 209.7     |
|                             | 590.2     | 599.6     |
| 600                         | 608.6     | 600.9     |
|                             | 594.6     | 605.7     |
|                             | 1049.8    | 1009.6    |
| 1000                        | 971.2     | 1011.0    |
|                             | 980.4     | 997.2     |

Tabela 8. Result of F and t test for the two analysts

| Parameters                  | Analyst 1 | Analyst 2 |
|-----------------------------|-----------|-----------|
| Average                     | 465.8     | 462.3     |
| Variance                    | 150212.5  | 149297.5  |
| Evaluations number          | 12        | 12        |
| Freedom level               | 11        | 11        |
| F                           | 1.00613   |           |
| P(F<=f)                     | 0.496048  |           |
| F critical                  | 2.817930  |           |
| Stat t                      | 0.022236  |           |
| P(T<=t)                     | 0.49123   |           |
| t critical                  | 1.717144  |           |

Table 9. Interlaboratory Program results (AIHA)

| Samples | Laboratory Results | Designated value | z-Score |
|---------|--------------------|------------------|---------|
| 1       | 0.617 mg           | 0.618 mg         | 0.0     |
| 2       | 1.683 mg           | 1.523 mg         | 1.5     |
| 3       | 0.068 mg           | 0.081 mg         | -1.9    |
4. Conclusions
In conclusion, the headspace injection coupled with GC–FID method using capillary column presented here is a highly sensitive, rapid, and reliable procedure to determine methanol in tubes with silica gel adsorbent.

The limit of quantitation obtained by this method (15ppm) is below more than ten times of the defined exposure limit (156 ppm), considering 5 L of collected air. Therefore, the validated methodology meets the specifications of regulatory standard number 15 of Ministry of Labour and Employment of Brazil.

5. References
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