**APPENDIX A3.2.1-25**

**SCIENTIFIC REPORT – PYRIDINE**

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SCIENTIFIC REPORT

# METHOD COMPARISON OLD VS NEW: QUANTIFICATION OF VOLATILE AND SEMI-VOLATILE COMPOUNDS

| | |
|---|---|
| Program Name | Analytical Capability |
| Project Name | Aerosol and tobacco analyses (Work Package (WP) Throughput) |
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## Summary

The method improvement activities for volatiles and semi-volatiles quantification methods lead to a difference on the quantification of some compounds when compared to the current methods used in routine.

The impact of these changes on the final quantifications for 3R4F and P1 (ZRH) aerosol samples was evaluated by the injection of two similar sequences on both instruments (routine and backup) running the two different methods (old: ID6375 and new: ID6357). The results were compared and are presented in this report (refer to paragraph 4 and paragraph 5).

The volatiles and semi-volatiles methods improvement impacted the quantification level reported for 1,3-butadiene and pyridine in 3R4F and P1 (ZRH) aerosol (refer to paragraph 4 and paragraph 5).

## 1 Introduction

In 2013, the volatile and semi-volatile compounds quantification methods were improved and the steps for the validation of the new method were undertaken from August to December 2013 in GC-MS ID6357.

It was observed during method improvement tests that an interference at 1,3-butadiene-d6 retention time (used in routine as internal standard for the quantification of 1,3-butadiene) was present in 3R4F leading to an underestimated quantification. This observation was consistent with the lower quantifications reported by PMI R&D for 1,3-butadiene in 3R4F samples in inter-collaborative tests. This underestimation was corrected by the use of a different internal standard for volatile compounds quantification (use of benzene_d6 as in CORESTA recommended method). For all volatile compounds in the scope of this method (1,3-butadiene, isoprene, benzene, acrylonitrile and toluene) the use of benzene_d6 instead of the corresponding internal standards (1,3-butadiene_d6, benzene_d6, acrylonitrile_d3 and toluene_d8) was validated. For semi-volatile compounds (pyridine, styrene, naphthalene and quinoline) the use of the corresponding labeled internal standards (pyridine_d5, styrene_d8, naphthalene_d8 and quinoline_d7) were maintained.

The standards preparation was impacted by the method improvement and a new range for the calibration curve towards lower concentrations was used. 3R4F samples are now diluted 20x instead of 10x and Reduced Risk Products (RRP) will still be quantified without any dilution. The internal standard (ISTD) solution preparation was also modified: in place of the custom-made Restek internal standard mix solution, a new ISTD_VOC (Volatile Organic Compounds) and ISTD_SVC (Semi Volatile Compounds) solutions are prepared from the pure commercially available labeled compounds and their final concentrations were adjusted to the new calibration curve concentration range.

For 3R4F, and P1 (ZRH) matrices, in an analysis using the current method, it can be observed that there is interference at pyridine_d5 retention time. This is the case for the two referred matrices but not for the standards. This fact leads to an underestimated quantification, due to the lower ratio of compound to internal standard response in the matrices of study when quantified with the calibration curve where this interference is not present. The chromatograms obtained with the improved method show that the interference is now separated and the ISTD responses in samples are in line with those observed in standards.

For styrene_d8, a less significant interference is present when using the current method and is now separated on the new method.

The new concentration of quinoline_d7 in the ISTD mix solution and the changes applied to the new method seem to be beneficial for the internal standard repeatability.

# 2 Objective

The purpose of this document is to report the final quantification and corresponding comparison for volatile and semi-volatile compounds in 3R4F and P1 (ZRH) aerosol samples using the old and new methods. These conclusions will be useful for the evaluation of results released later in routine when the old methods are replaced by the new methods.

# 3 Material & Methods

## 3.1 Samples

On the 29Nov2013, 3R4F and P1 (ZRH) samples (refer to Table 1 for further details) were smoked under Health Canada regimen and injected on routine analysis instrument (GC-MS ID6375) using the current methods (results under RDLIMS project RLS-ZRH-2013-408). Aliquots of the same smoking sample replicates were used to prepare a similar set of vials and these were injected on the backup instrument (GC-MS ID6357) using the improved methods on the same day.

For volatile compounds, it was thought that an error in preparation occurred for 1,3-butadiene standard solution and a contamination was observed for toluene on the 29Nov2013. For this reason, a retest on the same samples kept at -70°C was performed on the 06Dec2013 (7days after the smoking day) only for volatile compounds using a new set of standards preparation. Results are depicted in tables and figures in paragraph 4.

Table 1 – Description of samples used on the VOC&SVC comparison test

| Test Item Name | Sample Description | Number of replicates smoked |
|---|---|---|
| 3R4F | Monitor | 4 |
| P1 (ZRH_MNT) | (b) (4) | 8 |
| | (b) (4) | 8 |

Despite of the different sample numbers attributed to ZRH_MNT (P1) aerosol samples, the batch for the two samples is the same. For this reason, results obtained when applying routine method and new method (2 samples with 8 replicates each) will be combined in one single sample with 16 replicates.

### 3.1.1 Sample Storage

Standard solutions were stored in the freezer at -20°C and samples were stored in the freezer at <-70°C after extraction by stirring and filtration.

## 3.2 Analytical Method(s)

The detailed acquisition method parameters used for volatiles and semi-volatiles quantification with the new method are described in the document *Validation Plan for the quantification of volatile and semi-volatile compounds*.

## 3.3 Data Reprocessing

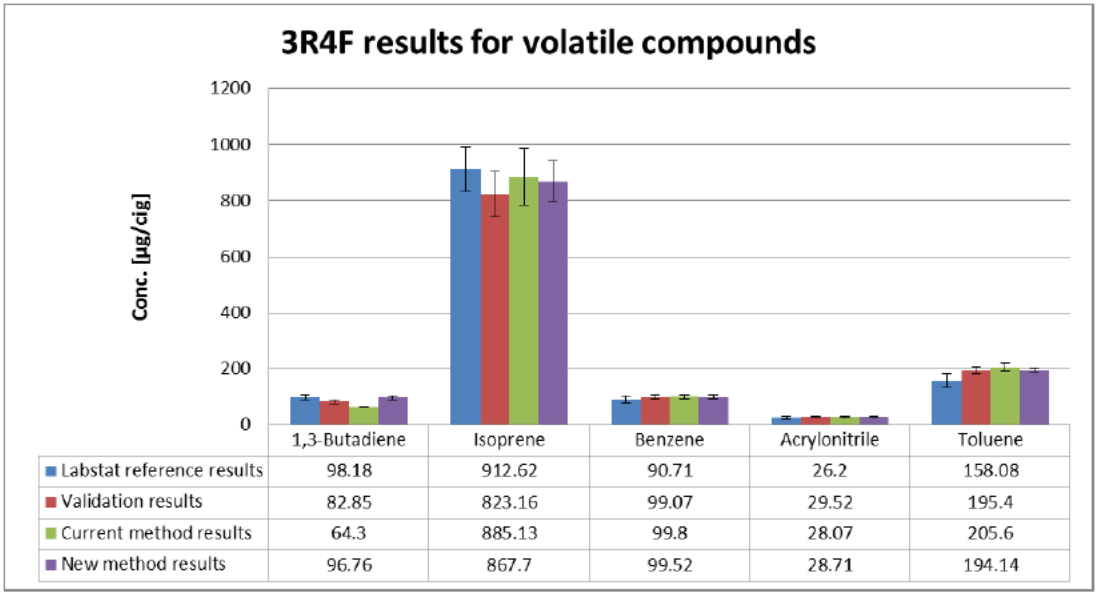
The data acquisition and automatic/manual peak integration were performed with the chromatographic data system Shimadzu GC-MS solution software. Final quantifications for new method were calculated using same

validated excel spreadsheets as for the validation data. For quantification in routine calculations were performed with validated excel spreadsheet "Reprocessing Form for Volatiles and Semi-Volatiles".

## 4 Results & Discussion

In Table 2 to Table 7 and in Table 10 and Table 11 are depicted the results obtained for the analysis of the two batches for all compounds in 3R4F and P1 (ZRH) samples, including the retest for the volatile compounds on the 06Dec2013 (refer to Figure 1 to Figure 6 for graphical representations of the results).

Figure 1 – 3R4F Results Comparison for Volatile Compounds




| | 1,3-Butadiene | Isoprene | Benzene | Acrylonitrile | Toluene |
|---|---|---|---|---|---|
| Labstat reference results | 98.18 | 912.62 | 90.71 | 26.2 | 158.08 |
| Validation results | 82.85 | 823.16 | 99.07 | 29.52 | 195.4 |
| Current method results | 64.3 | 885.13 | 99.8 | 28.07 | 205.6 |
| New method results | 96.76 | 867.7 | 99.52 | 28.71 | 194.14 |

Figure 2 – 3R4F Results Comparison for Semi-Volatile Compounds

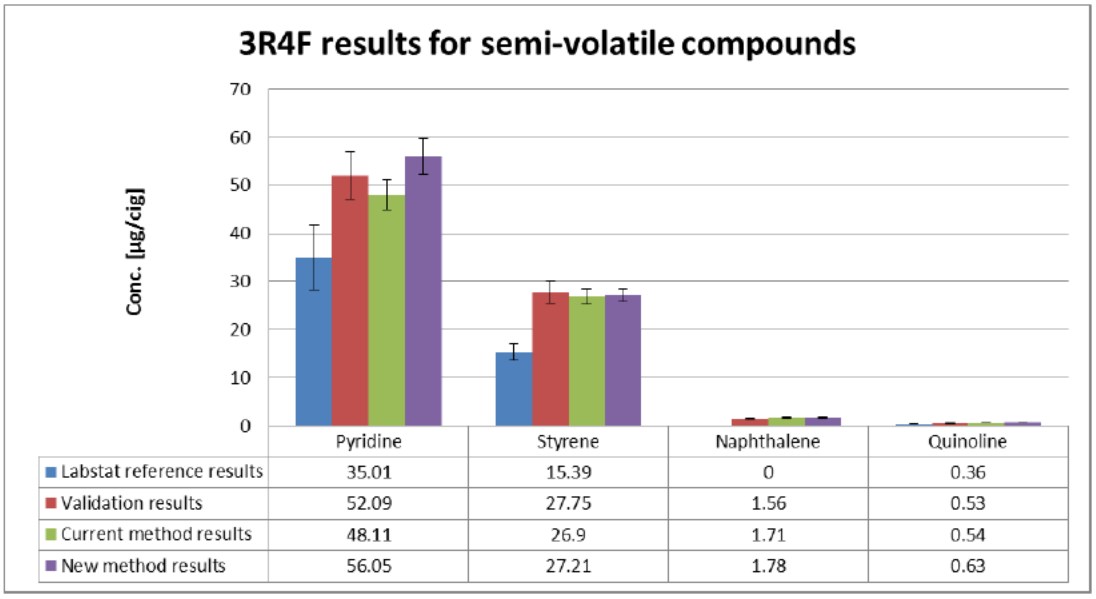



Table 2 – Results Obtained with Current Method for 3R4F (Monitor)

| ***Current Method (29Nov2013)*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Smoke Monitor_1** | 1,3 Butadiene | Isoprene | Benzene | Acrylonitrile | Naphthalene | Pyridine | Toluene | Quinoline | Styrene |
| | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig |
| Relative to Current Method | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| Average | 64.30 | 885.13 | 99.80 | 28.07 | 1.71 | 48.11 | 205.60 | 0.54 | 26.90 |
| Stdev | 1.98 | 101.71 | 5.46 | 2.31 | 0.11 | 3.14 | 12.89 | 0.03 | 1.58 |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Uncertainty | 3.15 | 161.85 | 8.69 | 3.68 | 0.17 | 5.00 | 20.51 | 0.05 | 2.51 |

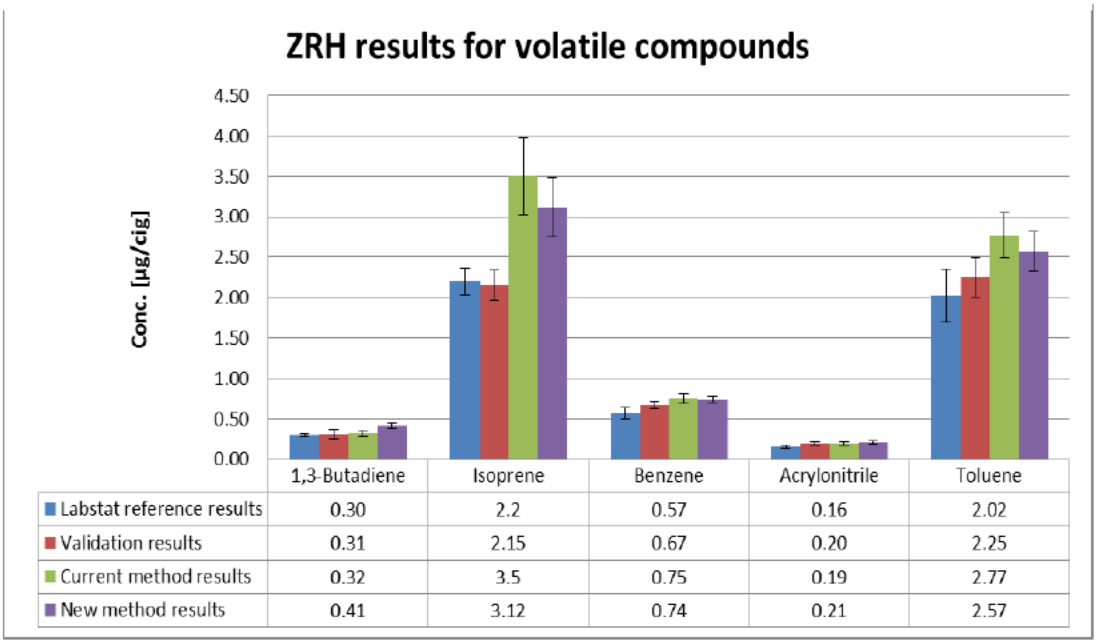
Table 3 – Results obtained with New method for 3R4F (Monitor)

| ***New Method (29Nov2013)*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Smoke Monitor_1** | 1,3 Butadiene | Isoprene | Benzene | Acrylonitrile | Naphthalene | Pyridine | Toluene | Quinoline | Styrene |
| | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig |
| Relative to Current Method | 200.6% | **98.4%** | **99.7%** | **103.2%** | **103.9%** | **116.5%** | **91.7%** | **115.9%** | **101.2%** |
| Average | 128.95 | 871.08 | 99.48 | 28.96 | 1.78 | 56.05 | 188.56 | 0.63 | 27.21 |
| Stdev | 7.33 | 67.14 | 6.11 | 2.50 | 0.11 | 3.71 | 10.76 | 0.07 | 1.35 |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Uncertainty | 11.67 | 106.83 | 9.73 | 3.97 | 0.18 | 5.90 | 17.12 | 0.11 | 2.15 |

Table 4 – Results Obtained with New Method for 3R4F (Monitor): Retest of VOC After 7 Days

| *New Method (Retest VOC, 06Dec2013)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Smoke Monitor_1** | 1,3 Butadiene | Isoprene | Benzene | Acrylonitrile | Naphthalene | Pyridine | Toluene | Quinoline | Styrene |
| | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig | µg/cig |
| Relative to Current Method | **150.5%** | **98.0%** | **99.7%** | **102.3%** | **NA** | **NA** | **94.4%** | **NA** | **NA** |
| Average | 96.76 | 867.70 | 99.52 | 28.71 | NA | NA | 194.14 | NA | NA |
| Stdev | 8.16 | 73.37 | 5.94 | 2.79 | NA | NA | 10.00 | NA | NA |
| N | 4 | 4 | 4 | 4 | NA | NA | 4 | NA | NA |
| Uncertainty | 12.99 | 116.75 | 9.46 | 4.44 | NA | NA | 15.92 | NA | NA |

Figure 3 – P1 (ZRH) Results Comparison for Volatile Compounds




| | 1,3-Butadiene | Isoprene | Benzene | Acrylonitrile | Toluene |
|---|---|---|---|---|---|
| Labstat reference results | 0.30 | 2.2 | 0.57 | 0.16 | 2.02 |
| Validation results | 0.31 | 2.15 | 0.67 | 0.20 | 2.25 |
| Current method results | 0.32 | 3.5 | 0.75 | 0.19 | 2.77 |
| New method results | 0.41 | 3.12 | 0.74 | 0.21 | 2.57 |

Figure 4 – P1 (ZRH) Results Comparison for Semi-Volatile Compounds

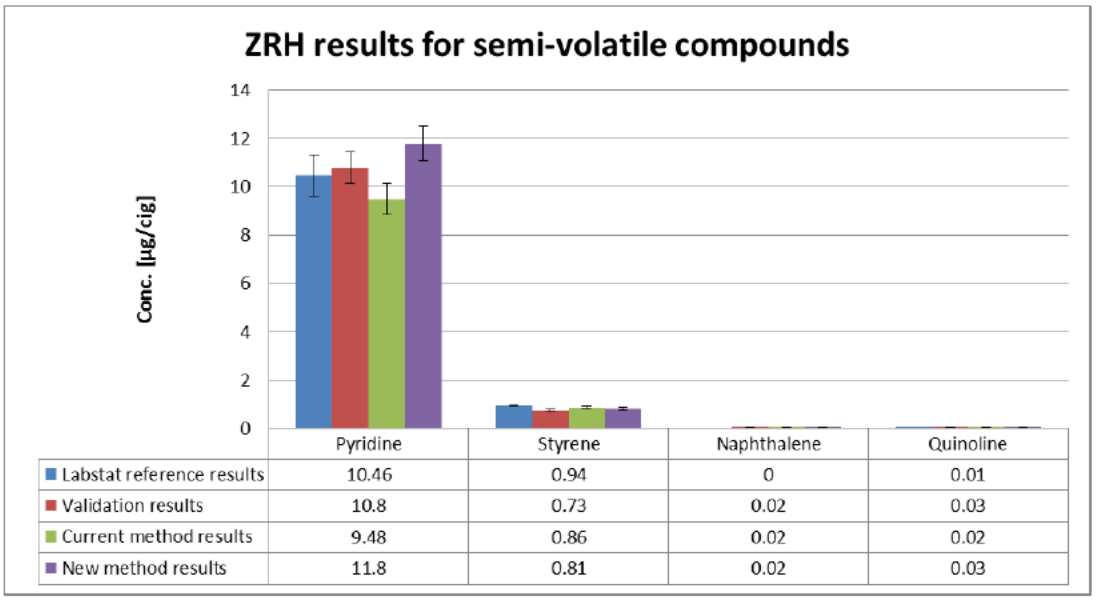



Table 5 – Results Obtained with Current Method for ZRH_MNT Sample (b) (4) 5_1.

| *Current Method (29Nov2013)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (b) (4) 1 | 1,3 Butadiene | Isoprene | Benzene | Acrylonitrile | Naphthalene | Pyridine | Toluene | Quinoline | Styrene |
| | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick |
| Relative to Current Method | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| Average | 0.33 | 3.50 | 0.76 | 0.19 | 0.02 | 9.48 | 2.77 | 0.02 | 0.86 |
| Stdev | 0.04 | 0.48 | 0.06 | 0.02 | 0.00 | 0.64 | 0.28 | 0.00 | 0.07 |
| N | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| Uncertainty | 0.02 | 0.26 | 0.03 | 0.01 | 0.00 | 0.34 | 0.15 | 0.00 | 0.04 |

Table 6 – Results Obtained with New Method for ZRH_MNT (b) (4)

| *New Method (29Nov2013)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (b) (4) | 1,3 Butadiene | Isoprene | Benzene | Acrylonitrile | Naphthalene | Pyridine | Toluene | Quinoline | Styrene |
| | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick |
| Relative to Current Method | 172.7% | **87.0%** | **96.0%** | **113.5%** | **79.8%** | **124.4%** | **90.0%** | **151.7%** | **94.9%** |
| Average | 0.56 | 3.05 | 0.73 | 0.21 | 0.02 | 11.80 | 2.49 | 0.03 | 0.81 |
| Stdev | 0.04 | 0.36 | 0.04 | 0.02 | 0.00 | 0.71 | 0.25 | 0.00 | 0.06 |
| N | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| Uncertainty | 0.02 | 0.19 | 0.02 | 0.01 | 0.00 | 0.38 | 0.14 | 0.00 | 0.03 |

Table 7 – Results Obtained with New Method for P1 (ZRH_MNT) (b) (4) : Retest of VOC after 7days.

| *New Method (Retest VOC, 06Dec2013)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (b) (4) | 1,3 Butadiene | Isoprene | Benzene | Acrylonitrile | Naphthalene | Pyridine | Toluene | Quinoline | Styrene |
| | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick | µg/stick |
| Relative to Current Method | **124.5%** | **89.0%** | **98.2%** | **116.1%** | **NA** | **NA** | **92.9%** | **NA** | **NA** |
| Average | 0.41 | 3.12 | 0.74 | 0.22 | NA | NA | 2.57 | NA | NA |
| Stdev | 0.03 | 0.37 | 0.04 | 0.02 | NA | NA | 0.24 | NA | NA |
| N | 16 | 16 | 16 | 16 | NA | NA | 16 | NA | NA |
| Uncertainty | 0.02 | 0.20 | 0.02 | 0.01 | NA | NA | 0.13 | NA | NA |

Figure 5 – 3R4F Results in Relative Percentage to the Current Method

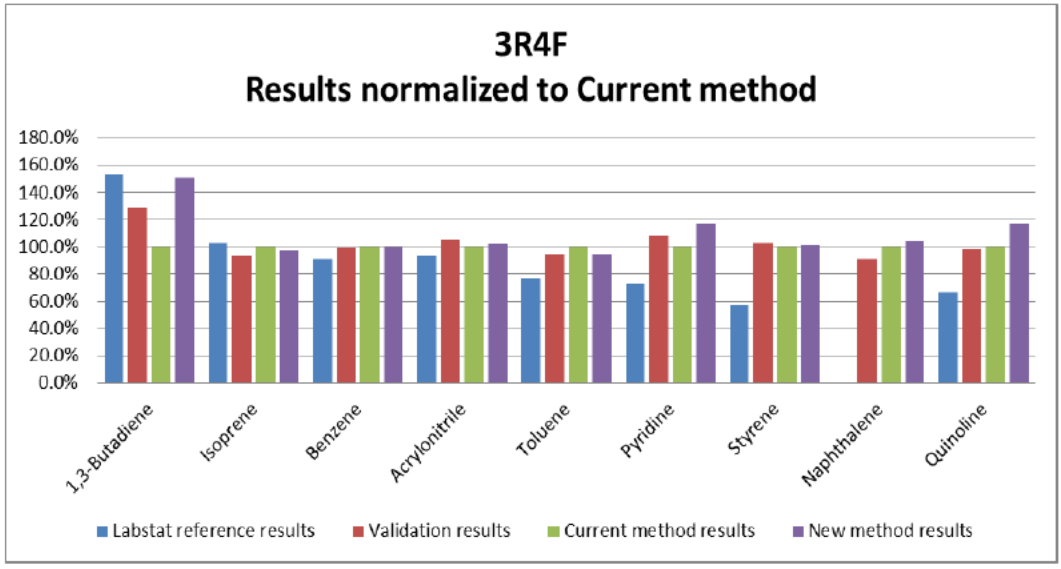


Table 8 – 3R4F Results for Labstat Comparison Test and Obtained with Current and New Method

| 3R4F | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | Labstat reference results | | Validation results | | Current method results | | New method results | | New to Current method |
| | Mean values (n=3) [µg/cig] | Stdev (n=3) [µg/cig] | Mean values (n=20) [µg/cig] | Stdev (n=20) [µg/cig] | Mean values (n=4) [µg/cig] | Stdev (n=4) [µg/cig] | Mean values (n=4) [µg/cig] | Stdev (n=4) [µg/cig] | Diff. [%] |
| 1,3-Butadiene | 98.18 | 8.36 | 82.85 | 7.78 | 64.30 | 1.98 | 96.76 | 8.16 | ***50.5%*** |
| Isoprene | 912.62 | 79.08 | 823.16 | 80.55 | 885.13 | 101.71 | 867.70 | 73.37 | ***-2.0%*** |
| Benzene | 90.71 | 12.52 | 99.07 | 6.01 | 99.80 | 5.46 | 99.52 | 5.94 | ***-0.3%*** |
| Acrylonitrile | 26.20 | 4.29 | 29.52 | 1.73 | 28.07 | 2.31 | 28.71 | 2.79 | ***2.3%*** |
| Toluene | 158.08 | 24.37 | 195.40 | 12.10 | 205.60 | 12.89 | 194.14 | 10.00 | ***-5.6%*** |
| Pyridine | 35.01 | 6.72 | 52.09 | 4.99 | 48.11 | 3.14 | 56.05 | 3.71 | ***16.5%*** |
| Styrene | 15.39 | 1.76 | 27.75 | 2.35 | 26.90 | 1.58 | 27.21 | 1.35 | ***1.2%*** |
| Naphthalene | not measured | NA | 1.56 | 0.14 | 1.71 | 0.11 | 1.78 | 0.11 | ***4.1%*** |
| Quinoline | 0.36 | 0.02 | 0.53 | 0.07 | 0.54 | 0.03 | 0.63 | 0.07 | ***16.7%*** |

Figure 6 – P1 (ZRH) Results in Relative Percentage to the Current Method

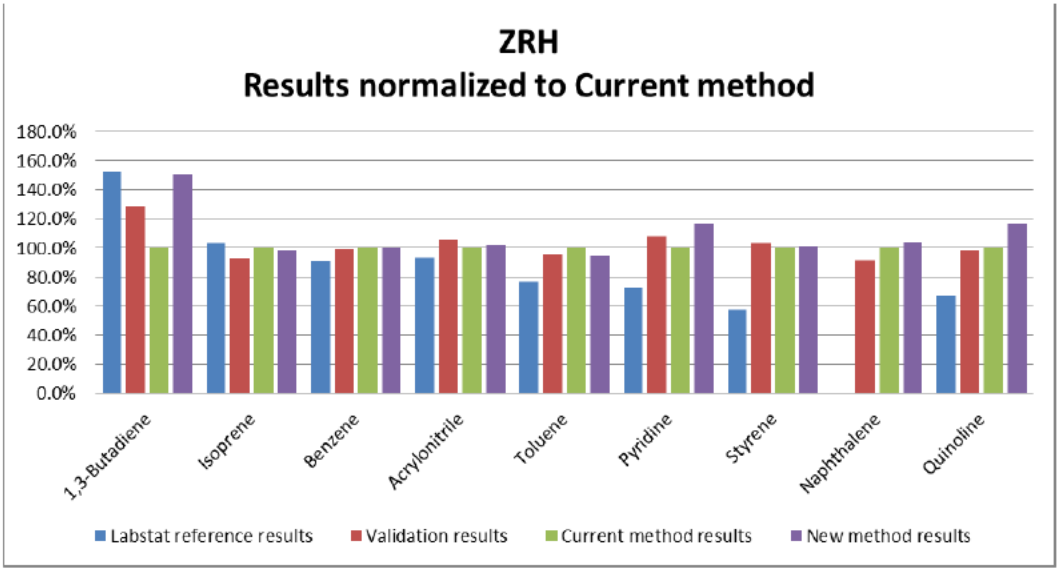


Table 9 – ZRH_MNT Results Comparison with Current and New Method and ZRH_REG Results for Labstat Inter Collaborative Test and Validation Period

| Compound | *P1 (ZRH_REG)* | | | | *P1 (ZRH_MNT)* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Labstat reference results | | Validation results | | Current method results | | New method results | | New to Current Method | Reduction to 3R4F [%] | |
| | Mean values (n=3) [µg/stick] | Stdev (n=3) [µg/stick] | Mean values (n=20) [µg/stick] | Stdev (n=20) [µg/stick] | Mean values (n=16) [µg/stick] | Stdev (n=16) [µg/stick] | Mean values (n=16) [µg/stick] | Stdev (n=16) [µg/stick] | Diff. [%] | Current method | New method |
| 1,3-Butadiene | 0.30 | 0.02 | 0.31 | 0.06 | 0.32 | 0.04 | 0.41 | 0.03 | ***28.1 %*** | ***-99.5 %*** | ***-99.6 %*** |
| Isoprene | 2.20 | 0.17 | 2.15 | 0.19 | 3.50 | 0.48 | 3.12 | 0.37 | ***-10.9 %*** | ***-99.6 %*** | ***-99.6 %*** |
| Benzene | 0.57 | 0.07 | 0.67 | 0.04 | 0.75 | 0.06 | 0.74 | 0.04 | ***-1.3%*** | ***-99.2 %*** | ***-99.3 %*** |
| Acrylonitrile | 0.16 | 0.02 | 0.20 | 0.02 | 0.19 | 0.02 | 0.21 | 0.02 | ***10.5 %*** | ***-99.3 %*** | ***-99.3 %*** |
| Toluene | 2.02 | 0.33 | 2.25 | 0.24 | 2.77 | 0.28 | 2.57 | 0.24 | ***-7.2%*** | ***-98.7 %*** | ***-98.7 %*** |
| Pyridine | 10.46 | 0.86 | 10.80 | 0.66 | 9.48 | 0.64 | 11.80 | 0.71 | ***24.5 %*** | ***-80.3 %*** | ***-78.9 %*** |
| Styrene | 0.94 | 0.03 | 0.73 | 0.05 | 0.86 | 0.07 | 0.81 | 0.06 | ***-5.8%*** | ***-96.8 %*** | ***-97.0 %*** |
| Naphthalene | not measured | NA | 0.02 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | ***0.0%*** | ***-98.8 %*** | ***-98.9 %*** |
| Quinoline | 0.01 | 0.00 | 0.03 | 0.00 | 0.02 | 0.00 | 0.03 | 0.00 | ***50.0 %*** | ***-96.3 %*** | ***-95.2 %*** |

The new method shows similar repeatability for both types of samples, despite of the new dilution factor applied to 3R4F (20x dilution in new method vs. 10x dilution in current method) and lower calibration curve concentrations range for all compounds. For 1,3-butadiene, the quantification in 3R4F in now circa 50% higher with the new method due to the use of a single ISTD used for the quantification of all volatile compounds (benzene_d6). It was shown that this ISTD is suitable for the quantification of all volatiles.

For all compounds the percentage reduction for P1 (ZRH_MNT) concentration relative to 3R4F is maintained when using the new method, although the absolute concentration may vary due to the improvement of the chromatography for some compounds that lead to the correction of the interferences previously observed in the current method as for example 1,3-butadiene, pyridine and styrene. For pyridine, the percentage reduction to 3R4F is slightly lower with the new method (78.9% vs. 80.3% for P1 (ZRH_MNT)). The results obtained during the validation for P1 (ZRH_REG) show that the expected level for pyridine in this matrix is at 10.8±0.66 µg/stick (average of 5 individual smoking replicates smoked and injected on 4 different days). This value is slightly lower than the amount of pyridine found for this specific test on the 29Nov2013 for P1 (ZRH_MNT), which represents 79.3% reduction to 3R4F for data acquired during the validation.

The quinoline percentage reduction to 3R4F with the new method is also lower (~95.2% vs ~96.3%) which can be explained by the low level of quantification for this compound in P1 (ZRH_MNT) samples (0.02 µg/stick to 0.03 µg/stick). In fact, the new dilution for 3R4F leads to lower quantification for quinoline, closer to STD1 concentration. Moreover, during the validation it was observed that the main column state has an impact in the chromatography for quinoline and this point has been addressed in the new update of the WKI, for instrument maintenance. Results obtained for quinoline in 3R4F matrix during the validation are in line with the quantification obtained with the current method for this test.

## 5 Conclusions

The changes applied to the new methods for volatile and semi-volatile compounds resulted on:

- an alignment of 1,3-butadiene quantification in 3R4F with other laboratories' results by the use of benzene_d6 as ISTD;
- a better chromatographic resolution for pyridine_d5 and styrene_d8 peaks in all matrices by means of a new GC column;
- improvement of quinoline_d7 response repeatability in standards and samples;
- the validation of a new dilution for 3R4F (20x diluted) with similar quantification results for all compounds when compared to current method (10x diluted).

As a conclusion, the improved method was shown to be suitable for routine analysis for the quantification of volatile and semi-volatile compounds included in the scope of the methods in 3R4F and P1 (ZRH) matrices. Whenever a comparison of results between averages obtained with the current and new method is needed, differences in absolute quantification (in µg/item) are to be expected as reported in this document, in particular for 1,3-butadiene and pyridine compounds.

Is addition, it was shown that for all compounds the percentage reduction for P1 (ZRH) products to 3R4F is maintained when using the new method.

For more detailed explanation, please refer to Table 10 and Table 11 in paragraph 6.

## 6 Annex

In Table 10 and Table 11 are presented the averages in µg/cig for the quantitation of all volatile and semi-volatile compounds in 3R4F and P1 (ZRH) samples. Highlighted in orange are the results for which the difference between the two averages of results obtained with the two methods is higher than the confidence interval limit at 95%.

Table 10 – Averages and Corresponding Difference Between Results Obtained with Current and New Method for 3R4F Matrix

| | 1,3-Butadiene | | Isoprene | | Benzene | | Acrylonitrile | | Toluene | |
|---|---|---|---|---|---|---|---|---|---|---|
| **3R4F** | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] |
| Current method | 64.30 | 3.15 | 885.13 | 161.85 | 99.80 | 8.69 | 28.07 | 3.68 | 205.60 | 20.51 |
| New method (retest) | 96.76 | 12.99 | 867.70 | 116.75 | 99.52 | 9.46 | 28.71 | 4.44 | 194.14 | 15.92 |
| Averages Difference | 32.46 | µg/cig | -17.43 | µg/cig | -0.28 | µg/cig | 0.64 | µg/cig | -11.46 | µg/cig |

| | Naphthalene | | Pyridine | | Quinoline | | Styrene | |
|---|---|---|---|---|---|---|---|---|
| **3R4F** | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] | Average [µg/cig] | Confidence Interval at 95% [µg/cig] |
| Current method | 1.71 | 0.17 | 48.11 | 5.00 | 0.54 | 0.05 | 26.90 | 2.51 |
| New method (retest) | 1.78 | 0.18 | 56.05 | 5.90 | 0.63 | 0.11 | 27.21 | 2.15 |
| Averages Difference | 0.07 | µg/cig | 7.94 | µg/cig | 0.09 | µg/cig | 0.31 | µg/cig |

For 3R4F sample, this difference is significant for 1,3-butadiene and for pyridine.

1,3-Butadiene quantification was expected to be higher in 3R4F with the new method due to an interference at 1,3-butadiene_d6 retention time, used as ISTD for the quantification of 1,3-butadiene with the current method.

Similar observation was seen for pyridine_d5 for all matrices, with co-elution occurring at this compound's retention time that is now solved by the use of a new column.

Table 11 – Averages and Corresponding Difference Between Results Obtained with Current and New Method for ZRH_MNT Matrix

| | 1,3-Butadiene | | Isoprene | | Benzene | | Acrylonitrile | | Toluene | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ZRH_MNT** | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] |
| Current method | 0.33 | 0.02 | 3.50 | 0.26 | 0.76 | 0.03 | 0.19 | 0.01 | 2.77 | 0.15 |
| New method (retest) | 0.41 | 0.02 | 3.12 | 0.20 | 0.74 | 0.02 | 0.22 | 0.01 | 2.57 | 0.13 |
| Averages Difference | 0.08 | µg/stick | -0.39 | µg/stick | -0.01 | µg/stick | 0.03 | µg/stick | -0.20 | µg/stick |

| | Naphthalene | | Pyridine | | Quinoline | | Styrene | |
|---|---|---|---|---|---|---|---|---|
| **ZRH_MNT** | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] | Average [µg/stick] | Confidence Interval at 95% [µg/stick] |
| Current method | 0.02 | 0.00 | 9.48 | 0.34 | 0.02 | 0.00 | 0.86 | 0.04 |
| New method (retest) | 0.02 | 0.00 | 11.80 | 0.38 | 0.03 | 0.00 | 0.81 | 0.03 |
| Averages Difference | 0.00 | µg/stick | 2.32 | µg/stick | 0.01 | µg/stick | -0.04 | µg/stick |

For P1 (ZRH_MNT) sample, averages difference obtained are higher or lower than the confidence interval limit at 95% for 1,3-butadiene, isoprene, pyridine and quinoline.

1,3-Butadiene and isoprene difference might be explained by the fact the volatile compounds were retested with the new method. 1,3-Butadiene and isoprene are highly volatile compounds and a retest of the sample after 7 days of storage is thought to have an impact on the final quantification. Nevertheless, results from freshly injected individual smoking replicates during the validation with new method for P1 (ZRH_REG) samples (0.31 ± 0.06 µg/stick) are in good agreement with quantitation with current method (0.32 ± 0.04 µg/stick) obtained during this comparison study for 1,3-butadiene. For isoprene this difference is more pronounced in P1 (ZRH_REG) during the validation (2.15 ± 0.19 µg/stick) when compared with results obtained for P1 (ZRH_MNT) for this comparison (3.12 ± 0.37 µg/stick). This difference observed during the validation is in line with results obtained for 3R4F.

For pyridine quantification, averages difference is significantly different from the confidence interval limit at 95% for P1( ZRH_MNT) sample (2.32 µg/stick for the difference vs 0.38µg/stick for the confidence interval limit at 95%). This difference was expected due to the same reasons as for 3R4F described in the paragraph above.

Quinoline quantification with new method (0.02 µg/stick) also differs from current method (0.03 µg/stick) for P1 (ZRH_MNT) sample, although quantification is at a very low level. Control chart limits for P1 reference for current method vary between 0.01 and 0.03 µg/stick, which are also in line with results obtained for this compound's quantification (0.01 to 0.04 µg/stick) throughout the validation tests obtained under intermediate precision conditions (for example, different smoking machine, different analytical lab operator and aerosol collection operator, different days and standards preparation, different instrument maintenance conditions).

## 7 Revision history

| Version N° | Author | Description of change (including reason for change) | Type of change |
|---|---|---|---|
| 1.0 | Correia, Daniela | Original issue | 1 |
| 1.0 SV | Eva Garcia | Submission version (hyperlinks removed) | 2 |

(1. Major change/new version; 2. Minor change; 3 Review without change)

## 8 Approvals

| | Name | Role | Signature | Date |
|---|---|---|---|---|
| Author | Eva Garcia | Manager Central Analytical Labs | | 14-Jul-2016 |
| Owner | Serge Maeder | Director PMI Testing Labs Governance | | 14.Jul.2016 |
| QA Review | Florence Kapp | QA Officer | | 14 Jul 2016 |