APPENDIX A3.2.1-22

SCIENTIFIC REPORT – CARBONYLS

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SCIENTIFIC REPORT

METHOD COMPARISON OLD VS NEW: QUANTIFICATION OF CARBONYLS COMPOUNDS

Program Name	Analytical capability
Project Name	Tobacco and Aerosol analyses (WP Method performance)
Type of document	Scientific report

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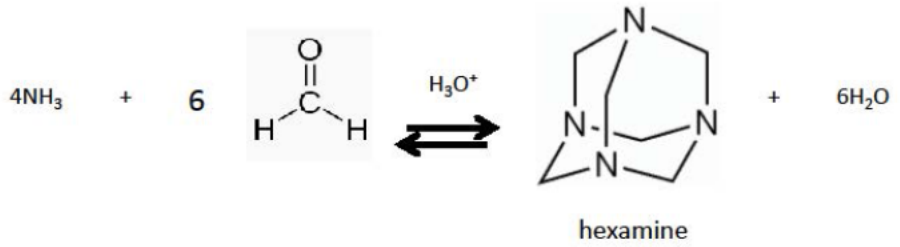
Summary

In the context of the transfer of the method, Analysis of eight carbonyls in aerosol, on a new High Performance Liquid Chromatography - Tandem Mass Spectrometry (HPLC-MS/MS) system, PMI ID 9907, the method was reviewed as follow:.

- Aerosol collection: the method was aligned with Coresta recommended method CRM74
- Analytical method: HPLC solvent and the MS conditions were modified to improve the baseline, especially for formaldehyde, as well as peak shape and peak resolution of carbonyl compounds.

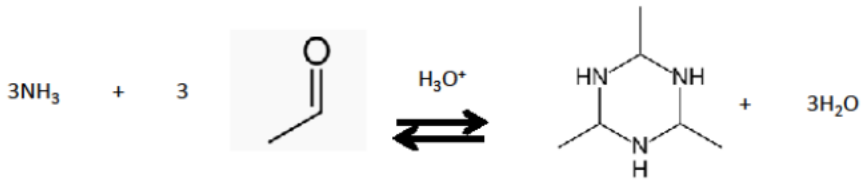
1 Introduction

In the past, inter-laboratory studies on conventional cigarettes had shown that with the current method used in the Analytical Labs (LA), provides lower results for formaldehyde and acetaldehyde than the recommended Coresta method. In fact, the major difference comes from the trapping solution where DNPH is dissolved in pure acetonitrile using the AL method instead of DNPH dissolved in a mixture of water/acetonitrile (50:50) using the Coresta method. The addition of 50% of water to an acetonitrile/DNPH solution is thought to prevent a loss of carbonyl compounds. This loss of carbonyl compounds could be due to a formation of amines following the addition of ammonia to the carbonyl group of aldehydes or ketones. Typical examples of amination of formaldehyde and acetaldehyde with ammonia are shown in Figure 1 and 2 generating hexamine respectively acetaldehyde ammonia trimer.



hexamine

Figure 1: Formaldehyde and ammoniac generating hexamine



Acetaldehyde ammonia trimer

Figure 2: Acetaldehyde and ammoniac generating acetaldehyde ammonia trimer

The transfer of the method, Analysis of eight carbonyls in aerosol, on the HPLC-MS/MS system, PMI ID 9907, allowed the adjustment and the improvement of the method, especially for the baseline and quantification of formaldehyde in NGPs (refer to Figure 3), the peak shape and peak resolution (see Figure 4) by modifying the HPLC solvent and MS parameters. In fact, in the current method a mixture of acetonitrile: water: tetrahydofuran (THF): isopropanol (30:59:10:1 % (v/v)) is used as HPLC solvent whereas THF is not really suitable in the LC-MS technique due to a THF tendency to polymerize in APCI mode leading to possible ion suppression. In addition, it was necessary to replace the internal standard acetone-D_6 which demonstrated a poor stability in the trapping solution containing 50% water by acetaldehyde-$^{13}C_2$ (refer to Figure 5).

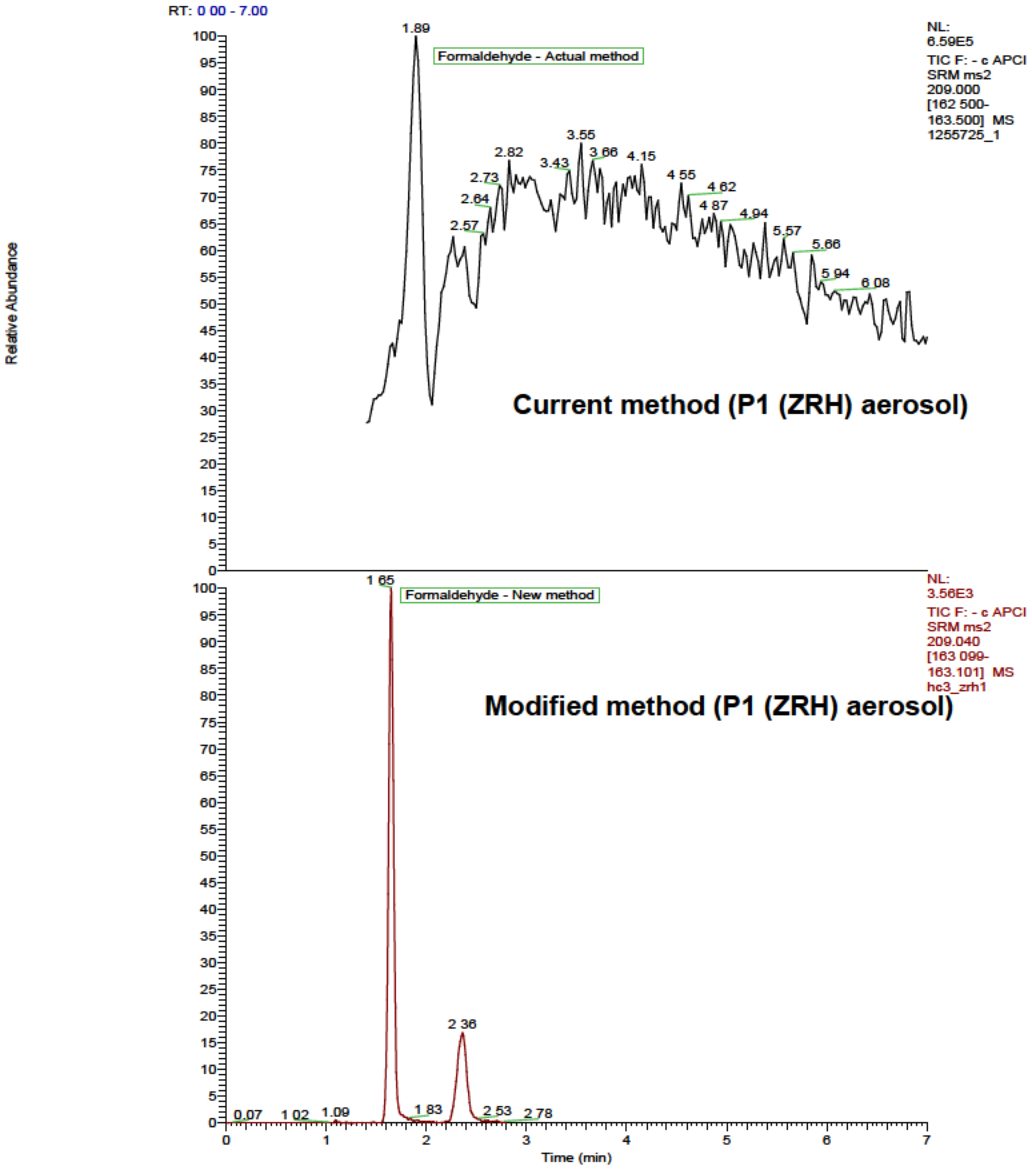


Figure 3: comparison of formaldehyde of a P1 (ZRH) aerosol analyzed with the current versus new method

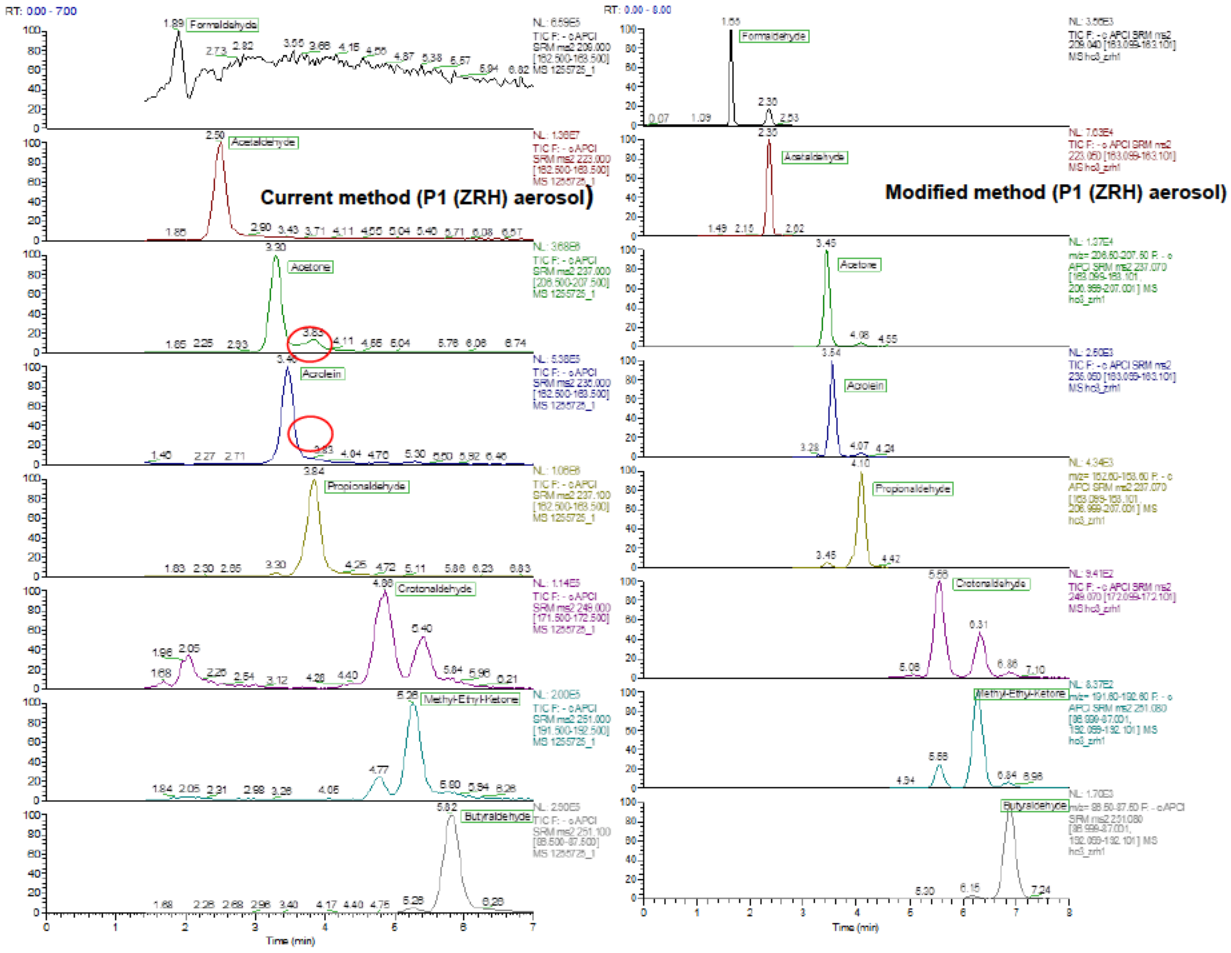


Figure 4: Comparison between the current versus modified method for the peak shape and the peak resolution

Remark:

Due to an improved peak shape and a better separation of carbonyls, interfering peaks are disappeared for acetone, acrolein, crotonaldehyde and methyl-ethyl-ketone. Depending how peaks were integrated with the current method, this improvement could slight impact the amount of carbonyls in the aerosol.

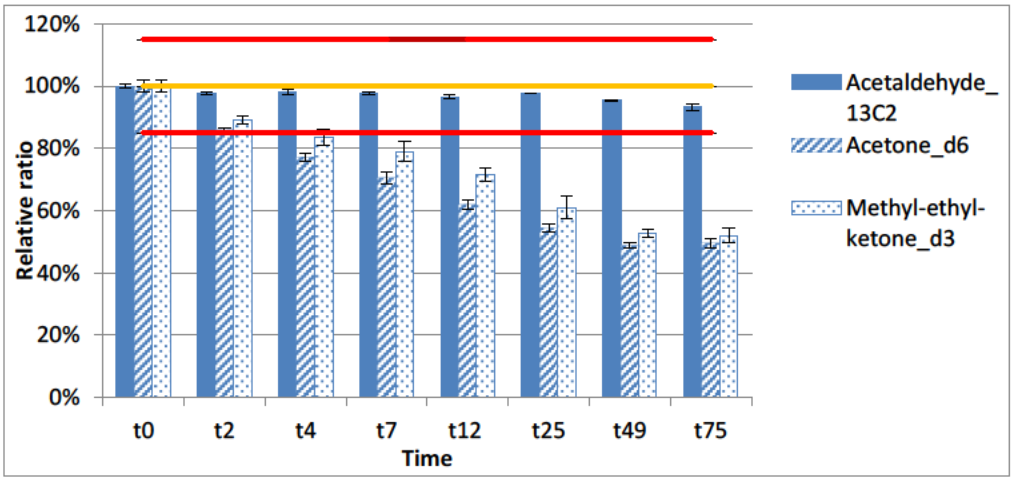


Figure 5: Comparison of the stability of different internal standard stock solution kept at room temperature

2 Objective

The purpose of this document is to compare the current method with the updated method and to highlight the impact of the analytical modifications of the method on the quantification of carbonyl compounds in the aerosol.

3 Material & Methods

3.1 Samples

The following samples were used for the comparison study:

- 3R4F (Reference item)
- ZRH_MNT (P1) (Batch n°: B-08544/08545)

3.2 Analytical method(s)

The aerosol generation was performed following the trapping method. Carbonyl were collected by trapping the aerosol in three consecutive impingers containing 30 mL each of a 15 mmol/L DNPH solution for 3R4F and 10 mL for P1 (ZRH_MNT). After collection, the impinger solutions were mixed together and an aliquot of 1 mL was transferred into 2 mL amber LC vial. 50 µL of pyridine were added around 15 minutes after the end of the smoking (maximum 30 min) to stop the derivatization. Preceding the analysis, 0.5 mL of the ISTD solution (acetaldehyde-$^{13}C_2$-DNPH) was mixed with the derivatized solution and was then analyzed by LC-MS/MS using.

The following parameters were modified, compared to the current methods, (Analysis of eight carbonyls in aerosol):

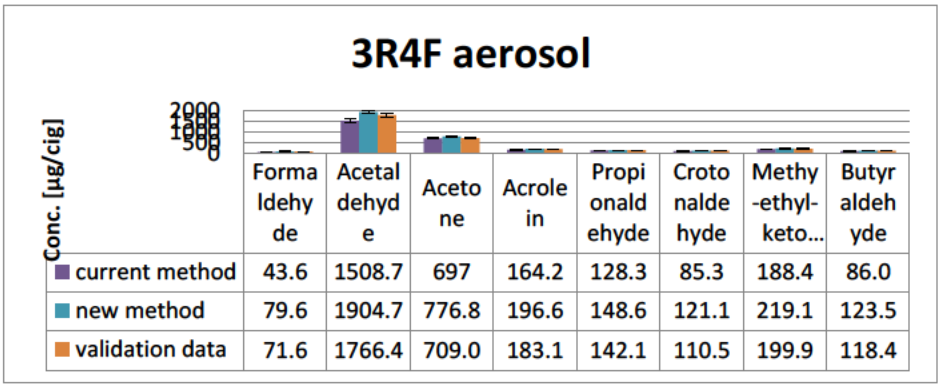
Table 1: Modified parameters applied on updated methods

Modification	Current method	New method
Trapping solution	25 mM DNPH in acetonitrile solution containing 27 mM of perchloric acid	15 mM DNPH in acetonitrile:water 50:50 % (V/V) containing 27 mM of perchloric acid
Volume of trapping solution for conventional cigarettes (3R4F)	2 impingers containing 40 mL each	3 impingers containing 30 mL each
Internal Standard	Acetone-D_6	acetaldehyde-$^{13}C_2$
HPLC mobile phase	acetonitrile: water: tetrahydrofuran (THF): isopropanol *30:59:10:1 % (v/v)*	Acetonitrile: Water 48:52 % (v/v)
Injection volume	10 µL	5 µL
Temperature of auto-sampler	20°C	5°C
Solvent of standard solutions	Acetonitrile (different to the sample solution)	15 mM DNPH in acetonitrile:water 50:50 % (V/V) containing 27 mM of perchloric acid (same solution for the samples)

4 Results & Discussion

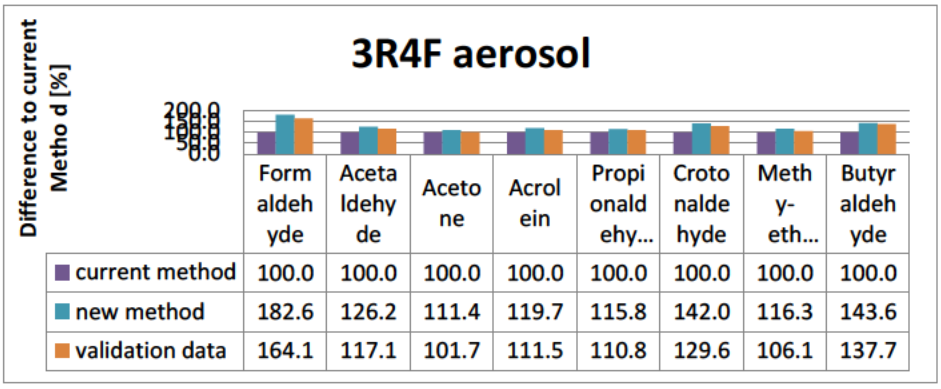
Four analytical replicates of 3R4F cigarettes and 16 replicates of P1 (ZRH_MNT) were generated on a linear smoking machine and analyzed following the current methods on the HPLC_MS/MS system PMI ID 6176 Thermo TSQ Quantum. Further 4 replicates of 3R4F cigarettes and 16 replicates of P1 (ZRH_MNT) were generated on another smoking day and analyzed following the new methods on the HPLC_MS/MS system PMI ID 9907 Thermo Vantage. Results are compared and reported in the table below. It must be pointed out; the collection of the aerosol was done on different days for both methods.

Analytical results for carbonyls are summarized in Table 2 and depicted from Figure 6 to 9.



	Formaldehyde	Acetaldehyde	Acetone	Acrolein	Propionaldehyde	Crotonaldehyde	Methy-ethyl-keto...	Butyraldehyde
current method	43.6	1508.7	697	164.2	128.3	85.3	188.4	86.0
new method	79.6	1904.7	776.8	196.6	148.6	121.1	219.1	123.5
validation data	71.6	1766.4	709.0	183.1	142.1	110.5	199.9	118.4

Figure 6: Comparison of carbonyl amounts obtained for 3R4F aerosol obtained with the current and the new method.



	Formaldehyde	Acetaldehyde	Acetone	Acrolein	Propionaldehy...	Crotonaldehyde	Methy-eth...	Butyraldehyde
current method	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
new method	182.6	126.2	111.4	119.7	115.8	142.0	116.3	143.6
validation data	164.1	117.1	101.7	111.5	110.8	129.6	106.1	137.7

Figure 7: Normalized values of carbonyls for 3R4F aerosol for the comparison of the current and the new method.

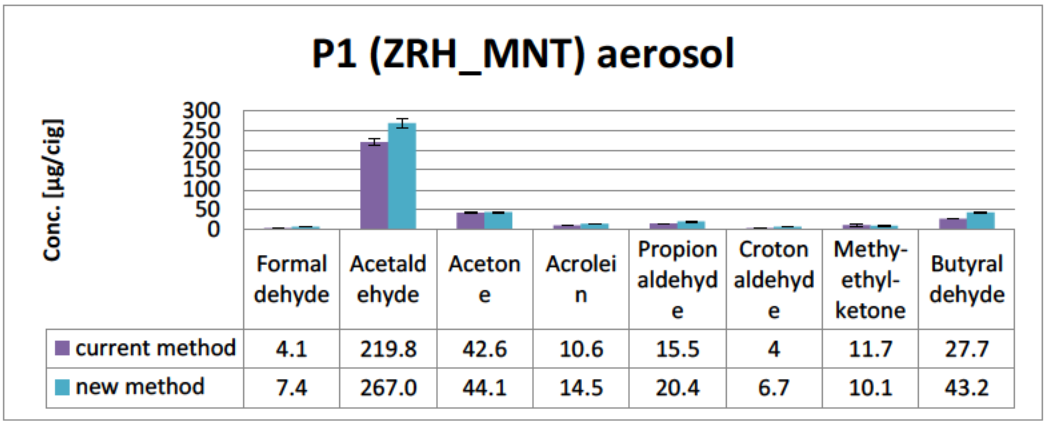


Figure 8: Comparison of carbonyl amounts obtained for P1 (ZRH_MNT) aerosol obtained with the current and the new method.

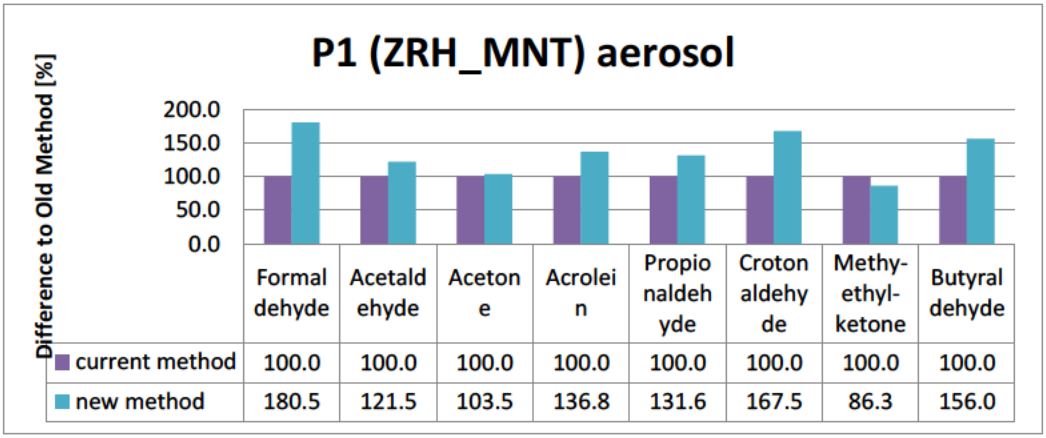


Figure 9: Normalized values of carbonyls for P1 (ZRH_MNT) aerosol for the comparison of the current and the new method.

Table 2: Carbonyls results - Comparison current method versus new method

	3R4F					P1 (ZRH_MNT)			Reduction to 3R4F [%]	
	CORESTA reference values [µg/cig]	Mean values of the validation (n=16) [µg/cig]	current method [µg/cig]	new method [µg/cig]	Diff. [%]	current method [µg/cig]	new method [µg/cig]	Diff. [%]	Current method	New method
Formaldehyde	76.0±37.7	71.6±7.5	43.6	79.6	***83***	4.1	7.4	***79***	***90.6***	***90.7***
Acetaldehyde	1605.8±388.9	1766.4±119.4	1508.7	1904.7	***26***	219.8	267	***21***	***85.4***	***86.0***
Acetone	596.8±179.7	709±44.8	697	776.8	***11***	42.6	44.1	***3***	***93.9***	***94.3***
Acrolein	155.4±41.2	183.1±11.8	164.2	196.6	***20***	10.6	14.5	***38***	***93.5***	***92.6***
Propionaldehyde	122.5±38.7	142.1±8.7	128.3	148.6	***16***	15.5	20.4	***32***	***87.9***	***86.3***
Crotonaldehyde	49.9±22.1	110.5±10.7	85.3	121.1	***42***	4	6.7	***69***	***95.3***	***94.5***
Methy-ethyl-ketone	147.0±78.5	199.9±17.0	188.4	219.1	***16***	11.7	10.1	***-14***	***93.8***	***95.4***
Butyraldehyde	71.8±31.4	118.4±9.0	86	123.5	***44***	27.7	43.2	***56***	***67.8***	***65.0***

Results:

- An important increase of carbonyl compounds can be noticed with the new method mainly for formaldehyde in 3R4F and P1 (ZRH_MNT) aerosol then for crotonaldehyde and butyraldehyde and finally for acetaldehyde and acrolein. The same trend can be observed for both 3R4F and P1 (ZRH_MNT) test items meaning that the reduction P1 (ZRH) versus 3R4F remain at the same level than with the current methodology.
- Overall, results obtained for the 3R4F aerosol are within the range of the reference values from Coresta but situated on the upper limit. Particularly crotonaldehyde shows outlying values for the new as well as for the current method which cannot be explained yet.

5 Conclusions

- The method was adapted to assure a complete and efficient collection of carbonyl compounds. The modification of the trapping solution, containing 50% water to avoid the amination of carbonyl groups, involved some major adjustments due to a lower trapping efficiency and the poor stability of the internal standard in this solution:
  - Increase of the number of impingers,
  - the internal standard acetone-D_6 was replaced by acetaldehyde-$^{13}C_2$,
  - standards are prepared in the same trapping solution as the samples to escape a bias in the quantification due to the different slope when standards are prepared only in acetonitrile or in a mix of acetonitrile/water
  - the auto-sampler was cooled down to 5°C to extend the stability of carbonyl compounds.
- The documentation was improved regarding the baseline noise in the chromatographic region of formaldehyde, peak shape and peak resolution for all carbonyl compounds. Furthermore, due to the enhanced peak resolution, no overlapping peaks can be observed anymore for acetone, acrolein

crotonaldehyde and methyl-ethyl-ketone. In fact, this improvement could have a direct impact on the quantification of carbonyls.

Overall, obtained values are within the range of the the reference values from Coresta but situated on the upper limit with the exception of crotonaldehyde and are somewhat higher than the current method. The same trend for crotonaldhyde can also be observed in the current method but cannot be explained yet. The slight higher values obtained for the modified methods compared to the current methods can be explained by the addition of all improvements.

6 Revision history

Version N°	Author	Description of change (including reason for change)	Type of change
1.0	Hofer, Iris	Original issue	1
1.0 SV	Garcia, Eva	Submission Version	2

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

7 Approvals

	Name	Role	Signature	Date
Author	Eva Garcia	Manager Central Analytical Labs		14-Jul-2016
Owner	Serge Maeder	Director PMI Testing Labs Governance		14.7.1.2016
QA Review	Florence Kapp	QA Officer		14 Jul 2016