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Vapour products — Determination of selected carbonyls in vapour product emissions

Produits de vapotage — Dosage de carbonyles sélectionnés dans les émissions de produits de vapotage

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, Subcommittee SC 3, *Vape and vapour products,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 437, *Electronic cigarettes and e-liquids,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

In many countries, regulation of vapour products requires reporting for carbonyl compounds in emissions. Therefore, there is a necessity to have an International Standard in place to get reliable/comparable data for selected carbonyls in vapour product emissions.

The method in this document is based upon the CORESTA recommended method CRM 96[1] which was written on the basis of the results obtained in an interlaboratory study conducted in 2019 involving 11 laboratories.

Carbonyl compounds are known to be derived from the thermal degradation of the base ingredients of the e-liquid formulations. The experimental design parameters [2],[3] used to collect the aerosolised vapour should be evaluated and documented for each analysis.

Vapour products — Determination of selected carbonyls in vapour product emissions

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices, and determine the applicability of any other restrictions prior to use.

1 Scope

This document specifies a method for the determination of the amount of selected carbonyl compounds (formaldehyde and acetaldehyde) as their 2,4-dinitrophenylhydrazones in vapour product emissions using reversed phase liquid chromatography coupled with ultraviolet or diode array detector (LC-UV or LC-DAD).

This document does not include the analysis of other carbonyl compounds, such as acrolein and crotonaldehyde, due to previous work indicated issues associated with stability of these compounds in the e-liquid solutions that were used to evaluate method performance[4].

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 20768, *Vapour products — Routine analytical vaping machine — Definitions and standard conditions*

ISO 24197:—[1)], *Vapour products — Determination of e-liquid vaporised mass and aerosol collected mass*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
aerosol collected mass
ACM
mass of aerosol collected on a glass fibre filter pad resulting from the operation of a vapour product by a routine analytical vaping machine after a defined number of puffs

Note 1 to entry: Routine analytical vaping machine is covered by ISO 20768.

1) Under preparation. Stage at the date of publication: ISO/FDIS 24197:2022.

3.2
e-liquid vaporised mass
EVM
mass of e-liquid transferred from the vapour product to the aerosol

Note 1 to entry: The term “vapour product mass loss” or “mass loss” refers to the e-liquid vaporised mass.

[SOURCE: ISO 24197:—, 3.3]

3.3
puff block
finite series of sequential puffs as defined by the user or by the test request

EXAMPLE Puff block: 1: puffs 1 to 50, puff block 2: puffs 51 to 100, puff block 3: puffs 101 to 150.

3.4
aerosol trapping system
system for collecting the aerosol from vapour products

Note 1 to entry: For this method the aerosol trapping system consists of a filter trap (pad + holder) and an impinger in series.

3.5
reagent blank
solution that is evaluated to check the level of contamination introduced by the reagents

3.6
aerosol blank
sample from a port that is attached to an *aerosol trapping system* (3.4) that contains no vapour product and is carried through the same collection, preparation and analysis steps as the test samples

4 Principle

The vapour product emissions are generated and collected on a vaping machine according to ISO 20768. The trapping system that is used to trap carbonyls consists of a pad holder containing a glass fibre filter pad according to ISO 24197, in series with an impinger containing an acidified solution of 2,4-dinitrophenylhydrazine (DNPH) in 1:1 acetonitrile:water. Post-vaping, the glass fibre filter pad is combined with the impinger solution and shaken mechanically for 20 min. An aliquot of the sample extract is subsequently neutralised with pyridine and analysed by reversed phase liquid chromatography with ultra violet or diode array detector (RPLC-UV or RPLC-DAD). The carbonyl content in the vapour product emissions is calculated based on an external calibration curve containing the prederivatized DNPH carbonyl compounds. Results are expressed as the weight of carbonyl per puff, per aerosol collected mass (ACM) or per puff block as warranted.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade.

NOTE Alternative reagents can be used provided the suitability and equivalence is verified.

5.1.1 Acetonitrile, **ACN**, HPLC grade.

5.1.2 Ethanol, HPLC grade.

5.1.3 Phosphoric acid, (H_3PO_4, a mass fraction of 85 %, or a volume fraction of 10 % aqueous solution).

5.1.4 Water, grade 1 according to ISO 3696 or equivalent.

5.1.5 Pyridine, minimum purity 99 %.

5.1.6 Formaldehyde-DNPH, minimum purity 99 %.

5.1.7 Acetaldehyde-DNPH, minimum purity 99 %.

5.1.8 2,4-Dinitrophenylhydrazine hydrochloride (DNPH-HCL) or **2,4-dinitrophenylhydrazine (DNPH)**(containing approximately 30 % water).

5.2 Solution preparation

5.2.1 General

Prepare appropriately proportioned amounts of the solutions listed below. All solutions shall be equilibrated to room temperature prior to use. Use graduated cylinders and calibrated pipettes to combine components.

5.2.2 10 % H_3PO_4

Prepare by bringing 118 ml of 85 % H_3PO_4 (5.1.3) to 1 l water (5.1.4). Vendor prepared 10 % H_3PO_4 may be used instead. Store at room temperature.

5.2.3 DNPH trapping solution, prepared with DNPH-HCL (5.1.8)

Dissolve 1,0 g DNPH-HCl (5.1.8) in 500 ml of ACN (5.1.1), combine with 40 ml of 10 % H_3PO_4 (5.2.2) and bring to 1 l with water (5.1.4). Mix solution to ensure all the DNPH-HCl dissolves and no crystals remain. Solution should be prepared fresh weekly, stored at room temperature and protected from light.

NOTE Alternative preparation of trapping solution with DNPH containing 30 % water is provided in Annex A.

5.2.4 Neutralised DNPH trapping solution (if dilutions are required)

Transfer 50 ml of DNPH trapping solution to a suitable size glass bottle and add 2,5 ml of pyridine. Mix solution thoroughly.

5.3 Preparation of standards

5.3.1 General

All solutions shall be equilibrated to room temperature prior to use.

5.3.2 HPLC calibration standards and working solutions

The calibration should cover the concentration range of interest. Annex B provides a suitable concentration range that can be used for the analysis, however, it can be adjusted depending on the level of carbonyls detected in the samples. The user shall ensure the low calibration standard has a sufficient signal to noise ratio for accurate quantitation (≥10:1) and that the calibration curve is linear.

5.3.3 Primary carbonyl standards

Weigh the hydrazones as described in Annex B into individual 25 ml volumetric flasks and dissolve in acetonitrile. Record the concentrations of the free aldehyde equivalents in µg/ml.

5.3.4 Secondary carbonyl standards

Pipette predetermined volumes (see Annex B) of each primary hydrazone standard into a 25 ml volumetric flask and dilute to the mark with acetonitrile.

NOTE Stock solutions of the individual DNPH derivatized carbonyls can be purchased at the required levels.

5.3.5 Carbonyl working standards

Take appropriate volumes (0,05 ml to 5 ml) of the secondary carbonyl standard (5.3.4) and dilute to 10 ml with acetonitrile to prepare calibration standards with approximate carbonyl concentrations (see Annex B).

Transfer to auto-sampler vials and cap.

Stability and storage time should be evaluated by the laboratory.

6 Apparatus

Usual laboratory apparatus and, in particular, the following items.

6.1 HPLC system, equipped with UV and/or DAD detector and a suitable data handling system.

6.2 HPLC columns.

— Disposable guard column: Reversed phase (RP) C18, and

— Analytical column: Reversed phase (RP) C18: 4,6 mm x 15 cm, 1,8 µm, or 2,5 µm or equivalent.

6.3 Vaping machine.

The aerosol is generated on a vaping machine, which follows the specifications specified in ISO 20768.

The standard conditions for puff duration and the puff profile are specified in ISO 20768; other parameters may also be used.

6.4 Aerosol trapping system.

6.4.1 44 mm glass fibre filter pads.

6.4.2 Filter pad holder.

6.4.3 Impingers for trapping emissions from vapour products.

6.4.4 Stem: Bubbler insert.

NOTE A coarse fritted tip impinger has been shown to be fit for purpose, alternative bubble insert can be used if demonstrated that the trapping efficiency is equivalent.

6.5 Analytical balance, minimum of 1 mg, which can be read to the nearest 0,1 mg.

6.6 Syringe filter (PTFE: 0,45 µm) and **disposable syringe**.

6.7 Autosampler vials, caps and PTFE faced septa.

7 Procedure

7.1 Preparation of test samples

All the vapour products to be tested shall be stored according to ISO 20768. Vapour products with rechargeable batteries shall be fully charged before the test. The aerosols are generated on a vaping machine which follows the specifications specified in ISO 20768.

7.2 Glass fibre filter pads handling

Glass fibre filter pads shall be stored in the test atmosphere for a minimum of 24 h prior to determination of pre-testing weights.

For all operations, the operator shall prevent contamination from the fingers by wearing gloves of a proper material (powder free). Between operations, a glass fibre filter pad holder cap, if available, may be installed to prevent water loss or uptake. Glass fibre filter pads shall be processed as quickly after collection as is feasible to prevent uptake or loss of water.

7.3 Aerosol collection and sample preparation

The tested vapour product shall be operated in accordance with the manufacturer recommendations. Samples shall be collected under the conditions specified in ISO 20768. The vaping machine shall be set up to collect aerosol onto the glass fibre filter pad connected in series with an impinger (Figure 1).

Add 35 ml of DNPH trapping solution (5.2.3) to a separate impinger with an insert for each sample to be collected.

Assemble trapping system of the vaping machine in the following order (see Figure 1):

— pre-weighed 44 mm filter pad/holder;

— impinger;

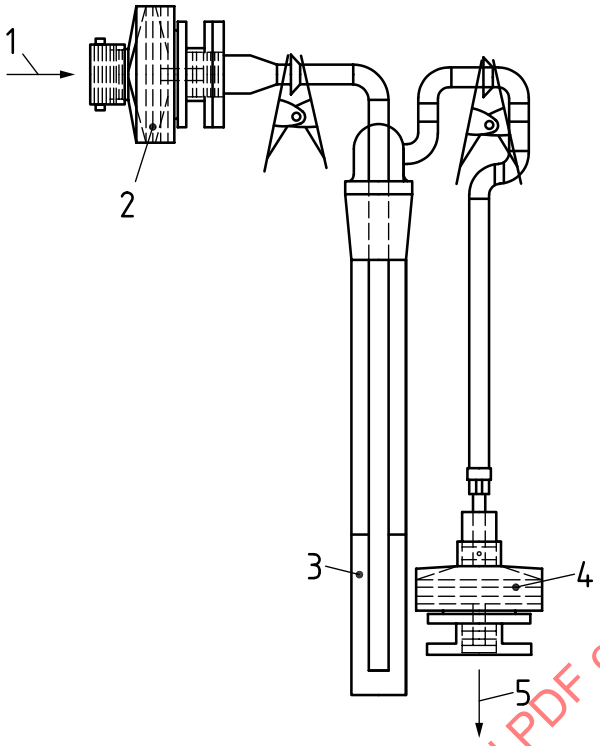
— optional backup filter pad (not included in analysis);

— vaping machine.

Prior to collecting aerosol samples, the trapping system should be evaluated to ensure there are no leaks in the system and to ensure the puff volume is correct.

— Leak check: To determine whether a leak has occurred in the vaping machine impinger setup, use a leak tester.

— Puff volume check: Check and adjust the puff volume drawn by the vaping machine at all channels at the product insertion end of the port with the filter pad/holder and impinger and in line.



Key

1 e-vapour product
2 pre-weighed 44 mm filter pad/holder
3 glass impinger with 35 ml of DNPH
4 optional backup filter pad (not included in analysis)
5 vaping machine

Figure 1 — Aerosol collection setup for carbonyls analysis

Collect aerosol under standard conditions for aerosol generation as specified in ISO 20768.

Since there is no standard impinger design, trapping efficiency shall be verified when validating this method. Ensure the end of the bubbler tube is completely submerged in the trapping solution and that the impinger can adequately hold 35 ml of trapping solution. The trapping system should effectively trap 95 % of the analytes of interest. To check the trapping efficiency of the method, add an additional impinger after the impinger and analyse the contents separately according to the method. If less than 5 % of the compounds are detected in the backup impinger, then only one impinger is required to trap all the carbonyls effectively. Breakthrough or poor trapping efficiency can be due to the size of the impinger, or bubbler tube or due to products with high carbonyl yields.

7.4 Determination of aerosol collected mass (ACM) and e-liquid vaporised mass (EVM)

Aerosol collected mass (ACM) and e-liquid vaporised mass (EVM) shall be determined gravimetrically according to ISO 24197.

7.5 Test portion

Post collection, for each glass fibre filter pad, open the holder and remove glass fibre filter pad with forceps. Fold the pad twice with the aerosol side toward the inside of the folds, being careful to handle only the edge of the glass fibre filter pad. Wipe the inside of the filter holder with the filter pad to ensure all residue is captured. Transfer the glass fibre filter pad to a suitable size glass vial with screw cap. Transfer the entire contents of impinger to the glass vial containing the filter pad and cap.

Extract the filter pad by mechanical shaking for 20 min. Transfer 5 ml of impinger solution into an 8 ml glass vial containing 0,25 ml of pyridine. This solution might become cloudy and can be filtered into an auto-sampler vial using a 0,45 µm PTFE syringe filter and appropriate disposable syringe. Transfer a 1 ml aliquot to an amber auto-sampler vial and seal the vial with a cap. Samples may be diluted in neutralized trapping solution if necessary. Document the dilution factor and dilution preparation. Stability of neutralized samples should be evaluated by the laboratory.

Place impingers and stems in hot soapy water after use. Rinse the pad holders with ethanol and allow them to dry at room temperature.

In instances where the concentration of an analyte determined in samples exceeds the upper limit of calibration, it is recommended to either dilute the samples with the neutralized DNPH trapping solution (5.2.3) or adjust the calibration range appropriately to quantitate the samples. If dilution is performed it is recommended to use the neutralized trapping solution prepared from the same DNPH trapping solution used to collect the samples.

7.6 Setting up the apparatus

Set up the apparatus and operate the HPLC (6.1) in accordance with the manufacturer's instructions. Ensure that the peaks for formaldehyde and acetaldehyde are well resolved from any background peaks found in the reagent blanks.

The chromatographic conditions listed in Table 1 might have to be modified for different instrument configurations and columns. General system suitability requirements should be established by the laboratory and, the elution pattern should be similar to the example chromatogram shown in Annex C.

Suitable operating conditions are as follows:

— Mobile phase A: 100 % deionized water;

— Mobile phase B: 100 % acetonitrile;

— Flow rate: 1,0 ml/min;

— Injection volume: 5 µl;

— Column compartment temperature: 32 °C;

— Run time: 12 min with a 2 min post time;

Table 1 — HPLC Mobile phase gradient

Time min	% Mobile phase A	% Mobile phase B
0,00	35	65
1,49	35	65
1,50	45	55
3,00	45	55
3,01	35	65
3,56	35	65
7,50	25	75
8,00	0	100
9,50	0	100
9,55	35	65
12,00	35	65

— Detector setting;
  — Diode Array Detector (DAD):
    — Signal A: 360 nm, 16 nm slit, reference 510 nm, 100 nm slit;
    — Pre-run and post-run balancing;
    — Margin for negative absorbance: 100 mAU;
  — Variable wavelength detector (VWD):
    — Signal wavelength: 360 nm;
    — Signal peak width: > 0,1 min;
    — Pre-run balancing: Yes;
    — Margin for negative absorbance: 100 mAU;
    — Signal polarity: Positive;
    — Enable analysis when lamp is off: No.

7.7 Calibration of the HPLC system

Inject each calibration standard onto the HPLC system. Record the peak areas (or height) for formaldehyde and acetaldehyde.

Plot the graphs of the carbonyl response versus concentration and calculate the linear regression equation from these data. The graphs should be linear and the regression lines should not be forced through the origin.

A calibration solution should be run as an unknown periodically throughout the analysis sequence to verify the calibration curve remains valid.

7.8 Determination

Inject the test portion (7.5) onto the HPLC system. Record the peak area (or height) for formaldehyde and acetaldehyde.

Acetaldehyde might elute as two peaks because its corresponding hydrazone exists in two isomers. The peak areas should be integrated consistently in both the standards and samples. This may be accomplished by drawing a single baseline tangent across both peaks.

8 Expression of the results

The amount of carbonyls in the test portion is determined in mg/ml using the graph or linear regression equation prepared in 7.7. Ensure that the values lie within the range of the standards prepared according to 5.3. If the test portion concentration exceeds the calibration range, dilute the test portion using neutralized trapping solution or adjust the calibration range appropriately to quantitate the samples.

When determining the amount of carbonyls present in the samples, a background subtraction might be required if the DNPH has inherent levels of carbonyls in the aerosol blank. This is typically conducted prior to doing additional calculations. To determine the true amount of carbonyls in the sample, use Formula (1):

$$A_s = S - B \tag{1}$$

where

A_s is the true concentration of carbonyls in the sample, in µg/ml;

S is the sample concentration, in µg/ml;

B is the blank concentration, in µg/ml.

The test results are expressed in microgram per puff (µg/puff), microgram per mg of ACM (µg/mg), microgram per mg of EVM (µg/mg) or mg/per device as indicated by study design.

Example calculations are given based on Formulae (2) to (4):

$$C_p = [A_s]d\frac{V}{p_n} \tag{2}$$

$$C_{ACM} = [A_s]d\frac{V}{m_{ACM}} \tag{3}$$

$$C_{EVM} = [A_s]d\frac{V}{m_{EVM}} \tag{4}$$

where

C is the content of the analyte, in µg/puff for Formula (2) and µg/mg for Formulae (3) and (4);

$[A_s]$ is the concentration of the analyte obtained from the calibration curve, in mg/ml;

d is the dilution factor (final volume/aliquot volume);

V is the volume of the DNPH trapping solution in the impinger, in ml;

p_n is the number of puffs;

m_{ACM} is the aerosol collected mass, in mg;

m_{EVM} is the e-liquid vaporised mass, in mg.

9 Repeatability and reproducibility

9.1 General

The repeatability and reproducibility data given in this document has been generated with the devices stated below. Other devices with higher performance variances can influence the data variability.

9.2 Results of an interlaboratory study

An international collaborative study was conducted in 2019 involving 11 laboratories[5]. This collaborative study included the analysis of formaldehyde and acetaldehyde in the aerosol of e-vapor product collected according to ISO 20768 puffing conditions. This study evaluated three (3) different e-liquids, provided by Alternative Ingredients, Inc.[2)] specifically for this study, which were aerosolised

2) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO.

using an Aspire Nautilus™[3] tank system with a 1,8 Ω coil and an Evolv™ power unit[4]. Since the levels of these carbonyls were very low in the native aerosol, the e-liquids were evaluated both fortified and unfortified to establish the values for repeatability, *r*, and reproducibility, *R*. The fortified amounts are listed in Tables 2 and 3 in the "Fortified e-liquid content µg/g" column. The statistical evaluation was performed according to ISO 5725-2 and is presented in Tables 2 and 3.

NOTE The collaborative study protocol specifies the use of CRM 81 which was developed into ISO 20768.

Table 2 — Repeatability (*r*) and reproducibility (*R*) limits for acetaldehyde (in µg/g aerosol) according to ISO 20768 puffing conditions

Product	Fortified e-liquid content µg/g	N Labs[a]	Average	*r*	*r* %	*R*	*R* %
Menthol/ Tobacco	0	8	2,33	1,32	56,7	4,80	206
Tobacco	0	9	2,43	1,36	55,9	5,06	208
Unflavoured	0	9	4,03	2,42	60,1	6,98	173
Menthol/ Tobacco	15	8	9,06	3,86	42,6	10,50	116
Tobacco	15	9	11,45	3,51	30,6	15,34	134
Unflavoured	15	9	13,23	4,15	31,4	17,34	131
Menthol/ Tobacco	25	7	15,79	5,31	33,6	18,00	114
Tobacco	25	9	19,24	9,65	50,1	21,19	110
Unflavoured	25	8	17,97	5,45	30,3	19,32	108
Menthol/ Tobacco	35	8	23,20	7,86	33,9	28,72	124
Tobacco	35	9	24,96	12,97	52,0	33,36	134
Unflavoured	35	9	26,59	13,28	49,9	35,64	134

[a] The number of laboratory data sets after removal of outliers.

3) Aspire Nautilus™ tank system is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

4) Evolv™ power unit is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

Table 3 — Repeatability (r) and reproducibility (R) limits for formaldehyde (in µg/g aerosol) according to ISO 20768 puffing conditions

Product	Fortified e-liquid content µg/g	N Labs[a]	Average	r	r %	R	R %
Menthol/ Tobacco	0	7	8,46	3,16	37,4	12,51	148
Tobacco	0	8	8,29	4,33	52,2	11,48	139
Unflavoured	0	8	11,57	3,95	34,1	14,41	124
Menthol/ Tobacco	15	7	17,26	3,27	19,0	17,02	99
Tobacco	15	8	16,72	3,52	21,1	13,67	82
Unflavoured	15	8	20,93	2,68	12,8	12,70	61
Menthol/ Tobacco	25	7	27,98	12,41	44,3	18,56	66
Tobacco	25	8	25,78	6,19	24,0	13,28	52
Unflavoured	25	8	28,55	5,90	20,7	17,31	61
Menthol/ Tobacco	35	7	32,64	10,21	31,3	17,20	53
Tobacco	35	8	30,40	7,39	24,3	19,10	63
Unflavoured	35	8	33,63	6,98	20,7	13,25	39

[a] The number of laboratory data sets after removal of outliers.

10 Test report

The test report shall contain the following:

a) the yield of selected carbonyls in micrograms per puff, micrograms per mg of ACM, micrograms per mg of EVM or micrograms per device;

b) the method used;

c) all conditions not specified in this document or regarded as optional, including puffing regime, number of puffs, device settings;

d) state all tested product(s) each with unique identification;

e) all information should be recorded in fully traceable manner.

Annex A
(informative)

DNPH solution (prepared with DNPH containing approximately 30 % water)

A.1 Preparation

Weigh approximately 6,8 g (24,0 mmol should be achieved) of DNPH (approximately 30 % water) into a 2 l amber volumetric flask and add 1 l of acetonitrile. Dissolve DNPH by alternately gently swirling the flask. Make sure there are no crystals remaining.

When using the recrystalized DNPH (without added water), weigh 4,8 g to achieve the same molarity.

WARNING — Do not sonicate as a precipitation of DNPH can occur.

After the DNPH is dissolved, add 58 ml of the diluted phosphoric acid solution while gently mixing. Dilute to volume with deionized water. The colour of the solution will become bright orange upon addition of the deionized water.

The addition of acid or water will cool the solution and can initiate the precipitation of the DNPH. Add the acid or water slowly. Gentle swirling can be required to maintain the solution at room temperature and to prevent the precipitation of DNPH. If crystals appear, do not sonicate. Solution should be prepared fresh weekly. Store in a cool dry place protected from light.

Evaluate the background levels of carbonyls in the DNPH and discard the solution if the analyte concentration is greater than the LOQ. DNPH (containing 30 % water) can be re-crystallized to reduce the background levels.

A.2 Recrystallization of 2,4-Dinitrophenylhydrazine

The supplied DNPH can contain contaminants or impurities. In this case, recrystallization of DNPH is recommended.

Weigh approximately 35 g of DNPH into a weighing boat. Transfer the DNPH into a clean 2 l Erlenmeyer flask and add a stirrer.

Add 750 ml of anhydrous reagent grade ethanol to the flask. Place the flask on a hot plate equipped with a stirrer. Gently heat the solution with constant stirring.

When the solution is warm, slowly add 1 000 ml of ethyl acetate. Continue to heat and stir (making sure not to boil) until all of the DNPH is completely dissolved. The solution should be clear and a very dark red.

Vacuum filter the hot solution.

Transfer the filtrate to a 2 l Erlenmeyer flask.

If crystallization does not start to occur, scratch the inside of the flask with a glass rod. Cover the Erlenmeyer flask with a watch glass and allow the solution to cool overnight in a cupboard.

Vacuum filter the recrystallized DNPH.

Transfer the crystals into a clean weighing boat that is labelled with the date of recrystallization and the lot of the DNPH. Weigh the recrystallized DNPH. Place the crystals in a desiccator to remove any moisture.

The filtrate can be evaporated down with a rotavapor and vacuum filtered again to recover more crystals.

If recrystallizing a larger quantity of DNPH that will be stored for more than 2 days, it is recommended to add approximately 30 % water. After adding the water, place in an airtight container and label it as containing 30 % water.