# The Determination of Diacetyl and Acetylpropionyl in Aerosols From Electronic Smoking Devices Using Gas Chromatography Triple Quad Mass Spectrometry *

*by*

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## SUMMARY

A reliable and sensitive method for the measurement of the level of diacetyl (2,3-butanedione) and acetylpropionyl (2,3-pentanedione) in the aerosol (both the particles and the suspending gas) of electronic smoking devices (e-cigarettes) has been developed. The method uses a gas chromatographic separation on a Carbowax type column with the measurement of the analytes on a triplequadrupole mass spectrometer working in positive MRM mode. The method has been validated using standard requirements regarding selectivity, sensitivity, recovery, accuracy, and repeatability. The limit of quantitation (LOQ) for the method was determined to be 0.41 ng/mL for diacetyl and 0.21 ng/mL for acetylpropionyl as measured for standards. These values translate to an LOQ of 0.082 ng/puff for diacetyl and 0.042 ng/puff for acetylpropionyl as measured for an e-cigarette with 50 puffs placed in 10 mL acetone. The samples analyzed included collected aerosols from several e-cigarettes, and a number of liquids used in electronic cigarettes (e-liquids). 3R4F Kentucky reference cigarette was also analyzed for evaluating the accuracy of the procedure, with good agreement with data from the literature. Diacetyl and acetylpropionyl were distributed in both particulate phase and also in vapor phase. The levels of diacetyl and acetylpropionyl in particulate phase collected from 3R4F cigarettes were found to represent only about 22% for diacetyl and only 31% for acetylpropionyl, while the vapor phase for diacetyl represented 78% and for acetylpropionyl 69% of the total analyte. The levels of diacetyl and acetylpropionyl in the aerosols of most electronic smoking devices were found to be very low, with a few exceptions. The analysis of the two analytes in several e-liquids available on the market showed a very large range of levels. Some of the e-liquids from the market are likely to have diacetyl and/or acetylpropionyl intentionally added. [Beitr. Tabakforsch. Int. 27 (2017) 145–153]

## ZUSAMMENFASSUNG

Es wurde eine zuverlässige und empfindliche Methode für die Messung der Konzentrationen von Diacetyl (2,3-Butandion) und Acetylpropionyl (2,3-Pentandion) im Aerosol (sowohl in den Partikeln als auch dem Suspensionsgas) von elektronischen Rauchgeräten (E-Zigaretten) entwickelt. Die Methode umfasst eine gaschromatographische Trennung auf einer Carbowax-Säule mit der Messung der Analyten in einem Triple-Quadrupol-Massenspektrometer in positivem MRM-Modus. Die Methode wurde mittels Standardanforderungen hinsichtlich der Selektivität, Sensitivität, Wiederfindung, Genauigkeit und Wiederholbarkeit validiert. Die Bestimmungsgrenze (LOQ) für die Methode beträgt 0.41 ng/mL für Diacetyl und 0.21 ng/mL

für Acetylpropionyl, wie für Standards gemessen wurde. Diese Werte ergeben eine LOQ von 0.082 ng/Zug für Diacetyl und 0.042 ng/Zug für Acetylpropionyl, wie für eine E-Zigarette mit 50 Zügen in 10 mL Aceton ermittelt wurde. Zu den analysierten Proben gehörten Aerosole von mehreren E-Zigaretten sowie eine Anzahl von Flüssigkeiten, die in elektronischen Zigaretten verwendet werden (E-Liquids). Auch die Kentucky-Referenzzigarette 3R4F wurde zur Bewertung der Genauigkeit des Verfahrens analysiert; dabei wurde eine gute Übereinstimmung mit den Daten in der Literatur festgestellt. Diacetyl und Acetylpropionyl waren sowohl in der Partikelphase als auch in der Dampfphase verteilt. Dabei wurden in der Partikelphase von 3R4F-Zigaretten lediglich circa 22% Diacetyl und nur 31% Acetylpropionyl festgestellt, während in der Dampfphase 78% des Diacetyl und 69% des Acetylpropionyl gefunden werden konnten. Die in den Aerosolen von den meisten elektronischen Rauchgeräten gemessenen Konzentrationen von Diacetyl und Acetylpropionyl waren mit einigen wenigen Ausnahmen sehr niedrig. Die Analyse der zwei Analyten in verschiedenen im Handel erhältlichen E-Liquids ergab eine sehr große Bandbreite an Konzentrationen. Bei einigen E-Liquids im Handel ist es wahrscheinlich, dass Diacetyl und/oder Acetylpropionyl absichtlich hinzugefügt wurden. [Beitr. Tabakforsch. Int. 27 (2017) 145–153]

RESUME

Une méthode sensible et fiable fut mise au point afin de mesurer le niveau de diacétyle (butane-2,3-dione) et d'acétylpropionyle (pentane-2,3-dione) présent dans l'aérosol (tant parmi les particules que dans le gaz de suspension) contenu dans les cigarettes électroniques (vapoteuses). La méthode s'appuya sur une séparation par chromatographie gazeuse sur une colonne de type Carbowax, accompagnée de la mesure des analytes sur un spectromètre de masse de type triple quadripôle en mode MRM positif. La méthode fut validée sur la base des normes habituelles concernant la sélectivité, la sensibilité, la récupération, la précision et la répétabilité. La limite de quantification (LOQ) de la méthode fut définie à 0.4 ng/mL pour le diacétyle et à 0.21 ng/mL pour l'acétylpropionyle, telle que mesurée pour les étalons. Ces valeurs se traduisirent par une LOQ de 0.082 ng/bouffée de diacétyle et 0.042 ng/bouffée pour l'acétylpropionyle, telles que mesurées pour une cigarette électronique de 50 bouffées placées dans 10 mL d'acétone. Les échantillons analysés inclurent des aérosols prélevés sur plusieurs vapoteuses et un certain nombre de liquides utilisés dans les cigarettes électroniques (liquides à vapoter). La cigarette de référence Kentucky 3R4F fut également analysée dans le souci d'évaluer la précision du procédé, faisant ainsi écho aux données répertoriées dans la littérature. Le diacétyle et l'acétylpropionyle se répartirent tant dans la phase particulaire que dans la phase gazeuse. Les niveaux de diacétyle et d'acétylpropionyle dans la phase particulaire relevés sur les cigarettes 3R4F ne représentèrent, selon les observations, qu'environ 22% pour le diacétyle et seulement 31% pour l'acétylpropionyle tandis que la phase gazeuse correspondit, pour le diacétyle, à 78% et pour l'acétylpropionyle, à 69% de l'analyte total. Les niveaux de diacétyle et d'acétylpropionyle dans les aérosols de la plupart des cigarettes électroniques s'avérèrent très bas, à quelques exceptions près. L'analyse des deux analytes de plusieurs liquides à vapoter disponibles sur le marché mit en exergue une très large plage de niveaux. Certains des liquides à vapoter disponibles sur le marché sont susceptibles d'avoir subi une adjonction volontaire de diacétyle et/ou d'acétylpropionyle. [Beitr. Tabakforsch. Int. 27 (2017) 145–153]

ABBREVIATIONS

| | |
|---|---|
| GC | Gas chromatography |
| HCI | Health Canada Intense |
| HPLC | High performance liquid chromatography |
| I.S. | Internal standard |
| ISO | International Standard Organization |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MRM | Multiple reaction monitoring |
| MS | Mass spectrometry |
| SD | Standard deviation |
| RSD | Relative standard deviation |
| TPM | Total particulate matter |

INTRODUCTION

2,3-Butanedione (diacetyl) is used as a flavoring agent (food additive), and as a component of fragrances. Also the compound occurs naturally in certain alcoholic and non-alcoholic beverages (1) and in several types of tobacco (2). Concerns have been raised about exposure to diacetyl (3) and it has been indicated that the compound may contribute or may even cause severe respiratory disorders at the workplace (4). For this reason, Occupational Safety and Health Administration (OSHA) issued a guidance regarding the use of diacetyl as a food flavoring (5). The National Institute for Occupational Safety and Health (NIOSH) also issued a number of reports regarding the exposure to diacetyl and 2,3-pentanedione (acetylpropionyl) at the working place (6). Because of the presence of diacetyl and acetylpropionyl in mainstream cigarette smoke (burn-down products) the measurement of these compounds in smoke has been of interest and several studies on this subject are reported in the literature (7, 8). In one of these procedures (8), a method of the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) used for other carbonyl compounds analysis (9) has been expanded to include diacetyl and acetylpropionyl. More recently, the evaluation of diacetyl and acetylpropionyl in e-vapor products became of interest (10, 11), and several presentations were made at Tobacco Science Research Conferences (TSRC) (12, 13) regarding this topic.

The present study describes a new very sensitive method for the analysis of diacetyl and acetylpropionyl in e-vapor products including several commercially available e-liquids (refill products) as well as the collected aerosols (both the particles and the suspending gas) from a number of electronic smoking devices (e-cigarettes).

The measurements for the levels of diacetyl and acetylpropionyl have been performed by different analytical procedures. The measurements (for diacetyl) were performed on various matrices such as wine (14), beer (15), food (16), urine (17), and environmental air (18). Related to the analysis of diacetyl in e-liquids and tobacco products, the procedures for analysis can be based on gas chromatography followed by mass spectrometry (MS) (11–13), or on high performance liquid chromatography (HPLC) (9). The HPLC procedure recommended by CORESTA is based on derivatization with dinitrophenylhydrazine and UV detection (8, 9).

## MATERIALS AND METHODS

### Materials

Several standard compounds including diacetyl, acetylpropionyl, as well as acetone, were purchased from Sigma-Aldrich (St. Louis, MO, USA). $d_7$-Quinoline was purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). For the filtration of extracts, 0.45 µm PVDF filters were used (Whatman Autovial, GE Healthcare, Little Chalfont, UK). The GC vials were 2-mL with screw top caps with septa.

### Instrumentation

The smoking machine was a Cerulean SM 450 (Molins Cerulean, Milton Keynes, UK). A platform shaker, InnOva 2100 (New Brunswick Scientific, Enfield, CT, USA), was used for the extraction of the samples. The analysis was performed on a GC-MS/MS 7890B-7000C from Agilent (Agilent Technologies Inc., Wilmington, DE, USA). The GC separation was performed on an Agilent J&W DB-WaxEtr chromatographic column, 30 m × 0.25 mm i.d. with 0.25 µm film. The data were processed using Mass-Hunter workstation software B.06.00 for qualitative analysis and B.07.00 for quantitative analysis, both from Agilent.

### Standard preparation

An initial standard stock solution containing 105 µg/mL diacetyl and 123 µg/mL acetylpropionyl was prepared in acetone and an internal standard (I.S.) stock solution of $d_7$-quinoline was prepared in acetone, containing 2.55 µg/mL. Also, a diluting solution containing 1% glycerin and 1% propylene glycol in acetone was prepared. From the initial standard stock solution, 200 µL was placed in a 10-mL flask which was brought to the volume using the diluting solution. This way, the highest standard containing 2100 ng/mL diacetyl and 2460 ng/mL acetylpropionyl was obtained. From the initial solution, working standards were prepared by successive dilutions as indicated in Table 1.

From each standard solution, 1.00 mL (precisely) was placed in a 2-mL vial, and 20 µL of I.S. stock solution was added (to generate a concentration of 50.00 ng/mL I.S.).

### Sample collection and extraction

Some of the analyzed e-liquids were available in bottles. Other e-liquids were extracted from cartridges. For removing the e-liquid from the cartridge, the cartridge was placed in a centrifuge tube and subjected to 3000 rpm for 3 min. For the e-liquid samples, about 500 mg e-liquid (precisely weighed) was dissolved in 10 mL acetone. From this solution, 1 mL was precisely measured in a 2-mL GC vial and 20 µL of $d_7$-quinoline I.S. stock solution was added. These solutions were analyzed by GC-MS/MS.

The aerosol sample collection from the electronic smoking devices was conducted with a Cerulean SM 450 linear smoke machine using a square wave profile. The particulate phase of each sample was collected on a 44-mm Cambridge pad. Each port was further connected with a 30 mL midget impinger filled with 10 mL acetone. The target aerosolizing regimen was 55 mL puff volume, 3 sec puff duration, and 30 sec puff interval. The puff volume offset was varied for each impinger to deliver a measured 55 mL volume. The samples were collected from individual ports to a final number of puffs per sample that depended on the smoking device (platform). For one type of platform, puffs 1–50, 101–150, 201–250, and 401–450 were collected on separate pads and impingers, and for a different platform puffs 1–100 and 101–200 were collected. The power units were fully charged prior to testing. Batteries were exchanged such that a fully-charged battery was used at the beginning of aerosol collection and starting at puff 201 (if these were taken). Three shams (from ports with no samples) were collected to ensure that the compounds identified were from the products and not part of the sample collection process. The room conditions were maintained at 60% ± 2% relative humidity, 22 °C ± 2.0 °C, and barometric pressure 715–745 mm Hg. The total particulate matter (TPM) collected on the pads was weighed (as a difference showed between initial and final weight of the pad + pad holder). The vapor phase of each sample was collected in the

**Table 1. The list of standards in ng/mL.**

| Compound | Standard (ng/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Level 7 | Level 6 | Level 5 | Level 4 | Level 3 | Level 2 | Level 1 |
| Diacetyl | 2100 | 525 | 105 | 52.5 | 21.0 | 5.25 | 1.05 |
| Acetylpropionyl | 2460 | 615 | 123 | 62.1 | 24.6 | 6.15 | 1.23 |

**Table 2. GC operating parameters.**

| Parameter | Description | Parameter | Description |
|---|---|---|---|
| Initial oven temperature | 35 °C | Inlet initial temperature | 120 °C |
| Initial time | 7.0 min | Initial time | 7.0 min |
| Oven ramp rate | 2.5 °C/min | Heating rate | 600 °C/min |
| Oven final first ramp | 50 °C | Final inlet temperature | 290 °C |
| Final time first ramp | 0 min | Injection volume | 3.0 µL |
| Oven ramp rate | 30 °C/mm | Carrier gas | Helium |
| Oven final second ramp | 260 °C | Flow mode | Constant flow |
| Final time | 5.0 min | Flow rate | 1.1 mL/min |
| Total run time | 25 min | Nominal initial pressure | 12.2 psi |
| Inlet type | Multi mode | Average flow velocity | 26.23 cm/sec |
| Inlet mode | Pulse splitless | Total flow | 19.1 mL/min |
| Pulse pressure | 20 psi | Holdup time | 1.88 min |
| Purge flow time | 0.75 min | Outlet pressure | Vacuum |
| Purge flow | 15 mL/min | Transfer line heater | 280 °C |

impingers containing 10 mL acetone. The impingers were weighed before and after sample collection to determine the remaining volume of acetone (some acetone evaporates during smoking). After the samples were collected, the particulate phase pads were placed in the impinger containing the corresponding vapor phase to create combined whole aerosol samples. The samples were shaken on a platform shaker for 15 min at 200 rpm. A 1.0 mL aliquot of each sample was transferred to GC vials and 20 µL of stock I.S. solution was added to each vial. This solution was used for the GC-MS/MS analysis. For the evaluation of the accuracy of the present method, 3R4F cigarettes were also evaluated for the levels of diacetyl and acetylpropionyl. The 3R4F Kentucky reference cigarettes were smoked using a Cerulean SM 450 using a bell wave profile under two different conditions, International Standard Organization (ISO) (19) and Health Canada Intense (HCI) (20). The ISO regimen used a puff volume of 35 mL with a puff frequency of 60 sec, 2 sec puff duration, and no ventilation blocking of the cigarettes. The HCI regimen used a puff volume of 55 mL with a puff frequency of 30 sec, 2 sec puff duration, and all ventilation holes of the cigarette blocked. The particulate matter was collected on 44 mm Cambridge pads. The vapor phase was collected for the 3R4F cigarette in two impingers with glass beads each containing 50 mL acetone. For each smoking regimen, the TPM from three cigarettes was collected on a 44 mm Cambridge pad. The impingers were weighed before and after sample collection to determine the remaining volume of acetone. After the samples were collected, the particulate phase pads were placed in 50 mL acetone and the samples were shaken on a platform shaker for 15 min at 200 rpm. A 0.2 mL aliquot of each sample was further diluted with another 10 mL acetone. From this diluted solution, a 1.0 mL aliquot was transferred to a GC vial and 20 µL of stock I.S. solution was added. These solutions were subject to GC-MS/MS analysis. The weight of the remaining acetone with aerosols from e-cigarettes or with smoke from 3R4F cigarette was used for re-calculating the levels of analytes in the analyzed samples.

*Gas chromatographic separation*

The separation of the components of the samples in acetone

solution was performed using the conditions described in Table 2 (on the DB-WaxEtr chromatographic column).

Besides the temperature program that assures good separation of the analytes of interest and the elution of all main components from the analyzed samples (such as propylene glycol, nicotine, and glycerin), the GC conditions were designed to assure a relatively low initial injection temperature (120 °C). This temperature was selected slightly above the boiling point of acetylpropionyl (110–112 °C), but low enough to avoid potential generation of the analytes from the decomposition of certain flavors that may be present in the analyzed sample. Also, a relatively large injection volume was selected in order to enhance as much as possible the method sensitivity.

*MS/MS detection*

The conditions for the tandem mass spectrometer detection are given in Table 3.

Under the previously described conditions, a typical chromatogram for a standard containing about 50 ng/mL analytes (standard Level 4) is displayed in Figure 1.

**Table 3. Conditions for the MS/MS detection.**

| Parameter | Description |
|---|---|
| Ion source | EI |
| Acquisition mode | MRM (positive) |
| Source temperature | 230 °C |
| Collision cell He | 2.25 mL/min |
| $N_2$ collision gas | 1.5 mL/min |
| Gain factor | 10 |
| Diacetyl transition | 86.0 → 43.0 |
| CE energy for diacetyl | 2 V |
| Acetylpropionyl transition | 100.0 → 57.0 |
| CE energy for acetylpropionyl | 1 V |
| $d_7$-Quinoline transition | 136.0 → 108.0 |
| CE energy for $d_7$-quinoline I | 5 V |
| MS1 resolution | Unit |
| MS2 resolution | Unit |
| Dwell time for each compound | 150 ms |
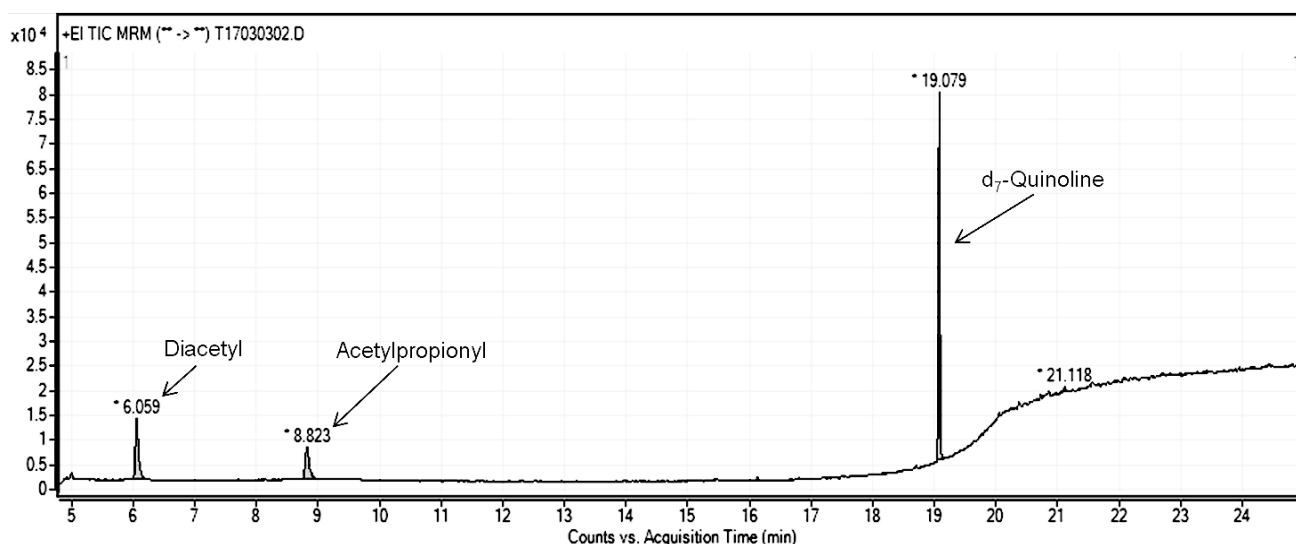
**Figure 1. Chromatogram of Standard Level 4 (about 50 ng/mL of the analytes) with I.S. (at 50 ng/mL $d_7$-quinoline).**

**Table 4. Coefficients for the calibration curves $Y = a X^2 + b X + c$ used in quantitation.**

| Compound | a | b | c | $R^2$ |
|---|---|---|---|---|
| Diacetyl | −1.13988 | 159.816 | −0.70536 | 0.9996 |
| Acetylpropionyl | −4.22341 | 348.6078 | 0.261438 | 1.0000 |

*Quantitation*

The quantitation for the two analytes was performed using calibrations of the concentration of the analyte plotted as a function of *(peak area analyte)/(peak area I.S.)*. For this purpose, the standards indicated in Table 1 were used. Better fits of the data were obtained using quadratic calibration curves, although the deviation from linearity is very small. The coefficients a, b, and c for the calibration curves of the form $Y = a X^2 + b X + c$, where $Y$ is the *concentration of the analyte* and $X$ is *(peak area analyte)/(peak area I.S.)* are given in Table 4.

*Method validation*

This method for diacetyl and acetylpropionyl analysis can be considered as having very good specificity, being based on MS/MS detection in multiple reaction monitoring (MRM) mode. Also, chromatographic separation offers excellent separation of the analytes from other compounds potentially present in vapors or e-liquids. Positive identification of each analyte was based on retention time, as well as precursor and product ions, but additional confirming ions were not utilized since the molecules were small and other fragmentations were not truly diagnostic and in addition difficult to obtain (in particular for diacetyl). The validation was further performed regarding limits of detection (LOD) and limits of quantitation (LOQ), linearity range, precision and intermediate precision, spike/recovery, accuracy, as well as solutions stability (see e.g., (21)).

The LOD and LOQ were established using only standards with 1% glycerin and 1% propylene glycol (no other residual matrix components were used in the determinations to establish a practical LOD or LOQ (21)). For establishing LOD and LOQ, the Level 1 standard (see Table 1) was repeatedly injected ten times. The LOD was taken as 3 SD (SD = standard deviation) and LOQ was taken as 10 SD. The results are indicated in Table 5. As indicated in Table 5, very low LOD and LOQ values were obtained for both analytes. The corresponding LOD and LOQ calculated per puff for an electronic smoking device with the collection of 50 puffs per pad and extracting the pad with 10 mL acetone are indicated in Table 6.

**Table 5. The values for LOD and LOQ for diacetyl and acetylpropionyl in ng/mL standard solution.**

| Compound | LOD (ng/mL) | LOQ (ng/mL) |
|---|---|---|
| Diacetyl | 0.12 | 0.40 |
| Acetylpropionyl | 0.08 | 0.25 |

**Table 6. The values for LOD and LOQ for diacetyl and acetylpropionyl in ng/puff as calculated for 50 puffs placed in 10 mL acetone.**

| Compound | LOD (ng/puff) | LOQ (ng/puff) |
|---|---|---|
| Diacetyl | 0.024 | 0.081 |
| Acetylpropionyl | 0.016 | 0.050 |

**Table 7. Relative standard deviation (RSD%) for Level 1 and Level 3 standards.**

| Compound | Level 1 | Level 3 |
|---|---|---|
| Diacetyl | 3.91% (n = 5) | 4.89% (n = 10) |
| Acetylpropionyl | 1.48% (n = 5) | 3.36% (n = 10) |

Regarding linearity, the best fit for the calibration data generated curves that were not linear. In order to cover a large range from about 1 ng/mL to about 2500 ng/mL a quadratic calibration curve has been utilized.

The precision of the method was verified by injecting five times the standard Level 1 and ten times the standard Level 3. The results as relative standard deviation (RSD%) for these measurements are indicated in Table 7.

The recovery of the method has been evaluated by smoking a single type of e-cigarettes and collecting puffs 1–100 and puffs 101–200. To these samples were added specific volumes of stock solution (similar to standard Level 3 and

equivalent with standard Level 4 from Table 1, but slightly different since a new set of standard was prepared). The results for the recovery study are presented in Table 8.

As indicated in Table 8, the recovery ranged from 99.06% to 105.55% for diacetyl and from 99.3% to 107.74% for acetylpropionyl, indicating excellent recovery.

In addition to sample spiking, the efficiency of sample collection using a single impinger has been evaluated. For this purpose, sample collection from the electronic smoking devices was also performed on several samples using two impingers in series, each with 10 mL acetone. The results of this collection are shown in Table 9.

The results from Table 9 indicate that one impinger is sufficient for collecting the diacetyl and acetylpropionyl generated by electronic cigarettes.

Accuracy of the procedure was verified using smoke from 3R4F cigarettes with results reported in the literature (8). The results obtained for diacetyl from the smoke of 3R4F cigarettes are indicated in Table 10. The collection of smoke was performed using two impingers although one impinger was proven sufficient for collecting aerosols from

**Table 8. Results for the recovery study in ng/mL.**

| Sample | Added diacetyl (ng/mL) | Diacetyl (ng/mL) | RSD% | Recovery (%) | Added acetyl-propionyl (ng/mL) | Acetyl-propionyl (ng/mL) | RSD% | Recovery (%) |
|---|---|---|---|---|---|---|---|---|
| E-cig X 1–100 | — | 8.56 | 0.10 | — | — | 7.83 | 4.86 | — |
| | 21.8 | 31.31 | 4.24 | 104.37 | 19.2 | 26.90 | 1.59 | 99.32 |
| | 54.5 | 63.88 | 0.68 | 101.51 | 48.0 | 59.55 | 2.11 | 107.74 |
| E-cig X 101–200 | — | 6.65 | 1.56 | — | — | 4.19 | 5.08 | — |
| | 21.8 | 29.66 | 7.28 | 105.55 | 19.2 | 24.71 | 4.49 | 106.88 |
| | 54.5 | 60.64 | 1.67 | 99.06 | 48.0 | 53.33 | 4.22 | 102.38 |

**Table 9. Results in ng/puff for diacetyl and acetylpropionyl from two different electronic smoking devices and two impinger collections.**

| Electronic device/flavor | Diacetyl | | | | Acetylpropionyl | | | |
|---|---|---|---|---|---|---|---|---|
| | Impinger 1 + Pad (ng/puff) | RSD% | Impinger 2 (ng/puff) | RSD% | Impinger 1 + Pad (ng/puff) | RSD% | Impinger 2 (ng/puff) | RSD% |
| Device 1 | 32.42 | 0.89 | 0.3 | 0.4 | 22.66 | 0.68 | 0.8 | 12.0 |
| Device 2 | 9.00 | 14.76 | 0.0 | — | 4.19 | 13.38 | 0.2 | 5.2 |

**Table 10. Results in µg/cigarette for diacetyl in 3R4F cigarettes.**

| Smoking protocol | Pad (µg/cig) | RSD% | Impinger 1 (µg/cig) | RSD% | Impinger 2 (µg/cig) | RSD% | Total (µg/cig) |
|---|---|---|---|---|---|---|---|
| ISO average | 24.41 | 7.91 | 89.82 | 7.55 | 0.55 | 70.25 | 114.78 |
| HCI average | 86.78 | 3.76 | 249.48 | 1.21 | 0.67 | 31.87 | 336.93 |

**Table 11. Results in µg/cigarette for acetylpropionyl in 3R4F cigarettes.**

| Smoking protocol | Pad (µg/cig) | RSD% | Impinger 1 (µg/cig) | RSD% | Impinger 2 (µg/cig) | RSD% | Total (µg/cig) |
|---|---|---|---|---|---|---|---|
| ISO average | 9.07 | 17.64 | 19.75 | 9.11 | 0.70 | 23.31 | 29.52 |
| HCI average | 35.09 | 4.25 | 56.19 | 7.36 | 0.58 | 15.40 | 91.87 |

electronic smoking devices. However, a burn-down cigarette generates significantly more diacetyl and acetylpropionyl compared to electronic cigarettes, and two impingers were used for smoke collection. The results for acetylpropionyl from smoke of 3R4F cigarettes are indicated in Table 11. The results from Tables 10 and 11 show very good agreement with previously published results for 3R4F cigarette (Ref. (8) page 422: average of 107.5 μg/cig diacetyl and 15.4 μg/cig acetyl-propionyl for ISO smoking). The results also show that diacetyl as well as acetylpropionyl are present in both, particulate phase and vapor phase, with a predominant composition in vapor phase (about 78% diacetyl in vapor phase and 22% in particu-late phase, and about 69% acetylpropionyl in vapor phase and 31% in particulate phase for the 3R4F cigarette).

Based on the good precision, recovery and stability of the analytical method, it was expected that the new procedure is an accurate and precise method for diacetyl and acetylpropionyl analysis. Other aspects related to the validation of this method were verified. This included the stability of primary stock solutions of standards, of working standard solutions, of the concentrated internal standard solutions, and that of extracted samples when stored at 2 °C to 8 °C in a refrigerator. It was found that the primary stock solutions of standards and of concentrated internal standard solutions are very good for 12 days (% difference from Day 0 analysis less than 6%). The stability of working standards was verified for 25 days, and also was found to be very good (differences from Day 0 results less than 5%). The same good results (less than 10% diffe-rence) were found for the extracted sample solutions for 4 days.

## RESULTS AND DISCUSSION

Two types of samples were evaluated in this study. The first type were aerosols from a number of electronic smoking devices. The second type were liquids used in electronic smoking devices (e-liquids).

*Results for aerosols from several electronic smoking devices*

The results for aerosols from various electronic smoking devices are indicated in Table 12.

**Table 12. Results for aerosols from various smoking devices expressed in ng/puff.**

| Sample description | | Diacetyl | | Acetylpropionyl | |
|---|---|---|---|---|---|
| Brand | Puff range | (ng/puff) | RSD% | (ng/puff) | RSD% |
| E-cigarette A | 1–50 | 1.9 | 8.3 | 0.1 | 6.1 |
| | 101–150 | 2.5 | 26.4 | 1.2 | 58.1 |
| | 201–250 | 2.3 | 23.2 | 3.7 | 2.7 |
| | 401–450 | 2.9 | 8.9 | 0.6 | 5.2 |
| E-cigarette B | 1–50 | 9.9 | 21.7 | 3.9 | 11.6 |
| | 101–150 | 13.0 | 24.1 | 4.7 | 13.4 |
| | 201–250 | 41.8 | 8.9 | 13.0 | 3.7 |
| | 401–450 | 43.6 | 9.0 | 14.1 | 15.3 |
| E-cigarette C | 1–50 | 23.8 | 31.2 | 21.1 | 22.0 |
| | 101–150 | 34.2 | 25.1 | 12.7 | 22.6 |
| | 201–250 | 49.9 | 45.9 | 23.1 | 59.7 |
| | 401–450 | 49.7 | 9.5 | 22.9 | 8.1 |
| E-cigarette D | 1–50 | 6.0 | 0.4 | 1.5 | 1.7 |
| | 101–150 | 5.1 | 42.2 | 1.2 | 69.0 |
| | 201–250 | 8.3 | 42.9 | 1.5 | 65.0 |
| | 401–450 | 9.1 | 42.5 | 1.6 | 71.5 |
| E-cigarette E | 1–100 | 6.24 | 21.21 | 4.55 | 19.67 |
| | 101–200 | 3.85 | 23.75 | 3.16 | 20.59 |
| E-cigarette F | 1–100 | 0.72 | 16.94 | 0.51 | 4.88 |
| | 101–200 | 0.50 | 7.26 | 0.23 | 15.16 |
| E-cigarette G | 1–100 | 7.16 | 20.81 | 5.57 | 10.89 |
| | 101–200 | 7.96 | 11.44 | 6.35 | 7.93 |
| E-cigarette H | 1–100 | 3.44 | 38.78 | 3.27 | 32.57 |
| | 101–200 | 4.90 | 15.17 | 4.33 | 22.04 |
| E-cigarette I | 1–100 | 2.18 | 49.84 | 0.23 | 53.82 |
| E-cigarette J | 1–100 | 0.34 | 14.58 | 0.66 | 10.26 |
| E-cigarette K | 1–100 | 1.28 | 12.66 | 0.92 | 93.21 |
| | 101–200 | 2.52 | 85.86 | 0.76 | 42.12 |
| E-cigarette L | 1–100 | 0.96 | 52.72 | 0.25 | 72.92 |
| | 101–200 | 1.96 | 56.17 | 0.40 | 54.12 |

**Table 13. Diacetyl and acetylpropionyl in several e-liquids used in electronic smoking devices.**

| Sample description | Diacetyl | | Acetylpropionyl | |
|---|---|---|---|---|
| | (ng/g) | RSD% | (ng/g) | RSD% |
| E-liquid 1 | 64.8 | 35.7 | 32.0 | 13.1 |
| E-liquid 2 | 73.1 | 25.2 | 35.1 | 3.2 |
| E-liquid 3 | 85.3 | 27.9 | 387.4 | 3.9 |
| E-liquid 4 | 101.7 | 4.3 | 602.5 | 0.6 |
| E-liquid 5 | 109.3 | 19.2 | 518.8 | 2.9 |
| E-liquid 6 | 208.1 | 71 | 203.9 | 26.9 |
| E-liquid 7 | 219.8 | 82.8 | 97.4 | 16.6 |
| E-liquid 8 | 317.1 | 98.6 | 368.3 | 23.1 |
| E-liquid 9 | 502.9 | 2.3 | 111.0 | 9.6 |
| E-liquid 10 | 546.5 | 6.2 | 419.3 | 3.6 |
| E-liquid 11 | 566.1 | 110.5 | 548.7 | 2.9 |
| E-liquid 12 | 597.0 | 9.5 | 26.4 | 14.2 |
| E-liquid 13 | 677.4 | 115.1 | 575.6 | 3.1 |
| E-liquid 14 | 773.8 | 3.8 | 416.5 | 4.8 |
| E-liquid 15 | 786.1 | 1.7 | 490.0 | 2.5 |
| E-liquid 16 | 1042.5 | 129.6 | 897.7 | 2.6 |
| E-liquid 17 | 1648.3 | 1.8 | 429.7 | 3.0 |
| E-liquid 18 | 2078.1 | 3.3 | 465.1 | 2.8 |
| E-liquid 19 | 2084.3 | 3.9 | 248.4 | 2.0 |
| E-liquid 20 | 2824.8 | 6.5 | 8132.8 | 1.9 |
| E-liquid 21 | 3157.3 | 1.8 | 687.5 | 1.2 |
| E-liquid 22 | 3717.4 | 5.1 | 229.7 | 14.9 |
| E-liquid 23 | 6164.8 | 5.0 | 725.3 | 1.2 |
| E-liquid 24 | 6682.1 | 1.5 | 1540.8 | 1.7 |
| E-liquid 25 | 11504.6 | 0.7 | 20982.6 | 2.0 |
| E-liquid 26 | 16886.7 | 2.4 | 234.7 | 11.3 |
| E-liquid 27 | 17532.4 | 3.3 | 151856.9 [a] | 2.5 |
| E-liquid 28 | 18639.9 | 3.4 | 543.1 | 42.9 |
| E-liquid 29 | 25226.6 | 0.4 | 8737.2 | 2.0 |
| E-liquid 30 | 26113.5 | 2.5 | 303.7 | 4.9 |
| E-liquid 31 | 27433.0 | 1.0 | 6846.3 | 0.7 |
| E-liquid 32 | 39509.2 | 1.9 | 3419.2 | 1.8 |
| E-liquid 33 | 54965.1 [a] | 4.5 | 21514.2 | 3.8 |
| E-liquid 34 | 73196.7 [a] | 1.9 | 10778.0 | 0.5 |
| E-liquid 35 | 74256.3 [a] | 3.6 | 156356.4 [a] | 3.0 |
| E-liquid 36 | 105498.9 [a] | 2.2 | 1022.9 | 1.7 |
| E-liquid 37 | 452567.0 [a] | 2.4 | 261.5 | 8.9 |

[a] These values were outside the calibration range and were calculated based on a linear dependence $Y\ (ng/mL) = 144.13\ X + 8.6368$ (for diacetyl) and $Y\ (ng/mL) = 315.47\ X + 10.466$ (for acetylpropionyl).

For aerosol collection, a number of sham runs were performed. The average result for diacetyl was 0.09 ng/puff, and for acetylpropionyl was 0.04 ng/puff. All aerosol samples were analyzed in triplicate (including aerosol generation).

*Results for several e-liquids from the market*

The results in ng/g e-liquid for diacetyl and acetylpropionyl in several e-liquids from the market are listed in Table 13. All e-liquid samples were analyzed in duplicate.

As indicated in Table 13, the levels of diacetyl and acetylpropionyl vary significantly in different e-liquids. Some e-liquids seem to have diacetyl and/or acetylpropionyl added by purpose. Assuming an average weight per puff of 10 mg the corresponding level of ng/puff contribution from transfer from the e-liquid to each puff can be estimated by dividing by 100 the values from Table 13 that are expressed in ng/g of e-liquid.

CONCLUSIONS

An original method for the analysis of diacetyl and acetylpropionyl has been developed and partially validated. The method has excellent LOQ values and has been successfully utilized for the measurement of the two analytes in the particulate and vapor phase generated by several electronic smoking devices. In addition, the analytes of interest were also measured in a number of e-liquids available on the market.

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