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METHODS OF CARBONYL COMPOUNDS DETERMINATION IN AEROSOL GENERATED FROM ELECTRONIC CIGARETTES

METODY OZNACZANIA ZWIĄZKÓW KARBONYLOWYCH W AEROZOLU GENEROWANYM Z ELEKTRONICZNYCH PAPIEROSÓW

Abstract: An electronic cigarette, known as an electronic nicotine delivery system or commonly as an e-cigarette, has become a popular alternative to traditional tobacco products since its introduction on the market. It is currently used in many countries around the world and has a wide circle of followers. However, research on the composition of the aerosol inhaled by users of electronic cigarettes is still underway. The results of these studies allow estimating exposure to toxic chemicals. They can become a tool to assess the harmfulness of these devices use. One of the major groups of compounds whose content is determined in the aerosol is carbonyl compounds. They are formed by the partial decomposition of propylene glycol and glycerin (used as base substances) and flavourings at the aerosol generation temperature. Prolonged exposure to carbonyl compounds such as formaldehyde or acetaldehyde is a known factor to increase the risk of asthma and cancer. Hence, precise measurements of these compounds content in the aerosol are important to assess the effect of electronic cigarettes on the users health. This article provides a brief overview of the methods used to determine the carbonyl compounds content in an aerosol generated from electronic cigarettes.

Keywords: electronic cigarette, aerosol, carbonyl compounds, analytical methods

Introduction

An electronic cigarette, known as an electronic nicotine delivery system or commonly as an e-cigarette, has become a popular alternative to traditional tobacco products since its introduction on the market. It is currently used in many countries around the world and has a wide circle of followers. A potential less harmfulness of an electronic cigarette aerosol containing nicotine inhaled by users makes an important merit of these devices compared to smoke of traditional tobacco products. An argument in support of that premise is a different chemical composition of the aerosol. In replacement of burning tobacco leaves, generating harmful and toxic compounds [1], an e-liquid with nicotine is heated. E-liquids mainly consists of nicotine (available also "free of nicotine" e-liquids), flavours and base substances: glycerol and propylene glycol. Due to the fact that nicotine is provided to a user organism without the toxic compounds present in tobacco smoke, electronic cigarettes can be considered as a useful tool for nicotine replacement therapy. So that the electronic cigarette could assist in the overcome smoking addiction, comprehensive scientific studies concerning consequences of its use on the user health are required. Research assessing the harmfulness of the electronic cigarette use include both a long-term observation of the effects of exposure to the aerosol generated from described devices and detailed composition analysis of the inhaled aerosol. The results of these studies allow us to estimate

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exposure to toxic chemicals. To date, the research has shown that harmful carbonyl compounds are formed by the partial decomposition of base components (propylene glycol and glycerin) [2-4] and flavourings at aerosol generation temperature [5]. This group of chemical compounds is the most responsible for the carcinogenic effects of tobacco smoke [6]. Low molecular carbonyl compounds (acetaldehyde, acetone, acrolein, crotonaldehyde, formaldehyde, methylethylketone) are on the list of chemicals and chemical compounds identified by the FDA as harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke [7]. All belong to the respiratory toxicant group. Acetaldehyde, crotonaldehyde and formaldehyde are carcinogens. In addition, acrolein is cardiovascular toxicant, and acetaldehyde has addictive properties. Hence, accurate measurements of these compounds concentration in the aerosol, and consequently the estimation of the electronic cigarettes impact on health are important for users, especially adolescents fascinated and experimented with these devices.

This paper provides a brief overview of the five methods used to determine the carbonyl compounds content in the aerosol generated from electronic cigarettes.

2,4-dinitrophenylhydrazine derivatives

The most commonly used approach for the determination of carbonyl compounds in the aerosol generated from electronic cigarettes is the derivatives formation of aldehydes and ketones with 2,4-dinitrophenylhydrazine (DNPH) - coloured hydrazones. This coupling agent is used with a variety of sorption techniques: silica gel or glass filters deposition and solution preparation as well. To separate obtained DNPH-adducts (coupled aldehyde or ketone with DNPH) is usually used high-performance liquid chromatography (HPLC) and to detect - UV spectrometry at 360 nm (depending on the maximum absorption of hydrazones). The greatest advantage of the presented approach is the ability to simultaneous analyse many different aldehydes and ketones in a complex mixture [8]. On the other hand, some researchers point out some limitations: time-consuming extraction or desorption of the resulting compounds, high specificity for the target analyte, and inapplicable for low concentrations. Hereunder, there are described five methods for the determination of carbonyl compounds in the electronic cigarette aerosol using DNPH as a derivatising reagent.

Tubes packed with silica adsorbent

A determination approach of the carbonyls content in the electronic cigarettes aerosol based on the use of silica sorbent tubes includes direct substance extraction from the aerosol to the solid phase. The carbonyl compounds from the aerosol are trapped in silica gel tubes saturated with 2,4-dinitrophenylhydrazine (DNPH) (Fig. 1). For the aerosol chemisorption, the tube presented in Figure 1, is placed between the electronic cigarette mouthpiece and a smoking machine or another device for puffing performing according to the set parameters.

The tube is placed directly behind the electronic cigarette mouthpiece to minimise the potential loss of the analysed compounds. Aldehyde and ketone-DNPH derivatives are usually desorbed using acetonitrile. It might be useful to mention that the obtained samples should be analysed on the day of collection. That may prevent the possible formation of multiple derivative acrolein-DNPH peaks and consequently avoid underestimation of the real carbonyl compound concentration [9-11]. The obtained solution is analysed utilising high-performance liquid chromatography with a diode array detector (HPLC-DAD).

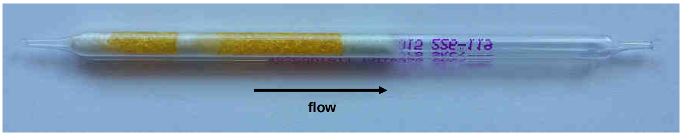

Fig. 1. Tubes filled with 2,4-dinitrophenylhydrazine (DNPH) coated silica gel adsorbent in two separate sections (150/300 mg) from SKC

The aerosol generated from the electronic cigarette consists of a gaseous phase and a particulate phase. The chemical compounds formed as a result of the device use are present in both phases. According to Kosmider et al. [12], the described DNPH coated silica gel tubes are designed to capture carbonyl compounds only from the gaseous phase. Probably part of formaldehyde is in the particle phase so that the measurement results may be understated. It should be noted that this method is recommended by the US Environmental Protection Agency as a standard method for carbonyl compounds determination in ambient air (TO-11A method) [13].

Quartz filters

McAuley et al. [14] investigated the effect of using electronic cigarettes on indoor air quality. With the aim of analysing electronic cigarette aerosols for carbonyl compounds, they used two methods. Apart from the described in the previous subdivision tubes filled with DNPH saturated silica gel, they used quartz filters as well. The aerosol was diluted with commercial zero air to a volume of 110 dm^3, following the aldehyde collection on glass fibre filters treated with 2,4-dinitrophenylhydrazine and phosphoric acid. The sample filters, similarly as the tubes, were extracted with acetonitrile and the obtained solution of DNPH-adducts was subjected to HPLC analysis using a PDA detector.

Dual-cartridge method

For determination of carbonyl compounds in electronic cigarette aerosol Uchiyama et al. [15] developed a new method using coupled two cartridges. An approach is based on the cartridges contained reagent-impregnated silica particles. The first cartridge contains silica filling coated with hydroquinone (HQ) to collect carbonyl compounds. In the second one, silica is impregnated with 2,4-dinitrophenylhydrazine (DNPH) for carbonyls derivatisation. Coupled cartridges are connected to an electronic cigarette, so the aerosol generated by a smoking machine drawn into the HQ-cartridge, and then the DNPH-cartridge (HQ-DNPH method). The use of hydroquinone has two functions. Hydroquinone is a radical trapping agent, thus inhibits the decomposition of acrolein and its polymerisation. Secondly, acrolein-DNPH adducts may be unstable, reacting with each other and with next DNPH molecules [8]. Therefore, the aerosol is collected on the HQ-coated sorbent, followed by DNPH derivatisation, and such procedure prevents these side reactions and potential underestimation of acrolein content. After collecting the aerosols, the coupled cartridges are extracted using acetonitrile containing 1 % phosphoric acid. It is important to maintain the proper sequence of compound extraction in the direction of HQ-cartridge by the DNPH-cartridge. As a result, the reaction with DNPH occurs in the acetonitrile eluate. Adding acid to acetonitrile accelerates the reaction of acrolein with DNPH. A portion of ethanol is added to the collected samples. The next step is the HPLC-UV analysis. The authors state that this method allows analysis of carbonyl compounds containing from 1 to 10 carbon atoms. Moreover, it has a good reproducibility (RSD less than 2.1%) [8].

2,4-dinitrophenylhydrazine trapping solution

Chemisorption of the electronic cigarettes aerosol in DNPH solution is another approach for determining the content of carbonyl compounds in the aerosol using a specific coupling reaction of aldehydes and ketones with DNPH. For this purpose, DNPH solutions in acetonitrile [16] or a mixture of acetonitrile and water [4, 17] with phosphoric acid [4] or perchloric acid [16] are prepared. These solutions are placed in glass impingers, and then electronic cigarette aerosol is passed through them. Figure 2 shows an outline of the laboratory set used in this method. The smoking machine enables puffing the aerosol from electronic cigarette according to predetermined parameters. The direction of the aerosol flow is also marked.

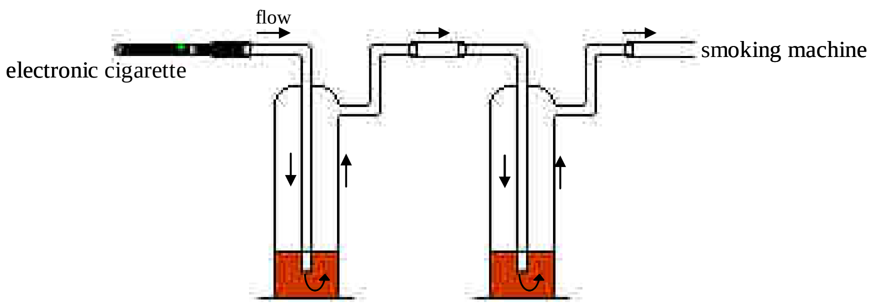

Fig. 2. Outline of the laboratory set to generate and collect electronic cigarette aerosols using DNPH solutions for derivatisation

After the aerosol settle, the contents of the impingers are intensively mixed. Solutions are left for the derivatisation reaction. A portion of the thus obtained solution is taken, and the reaction is quenched with a solution of base - Trisma^{TM} (Tris) [17, 18] or pyridine [4]. After thorough mixing, an aliquot of the prepared samples is transferred to a chromatography vial and analysed. The analysis is performed by high-performance liquid chromatography (HPLC). Carbonyl compounds are detected via a diode array detector (DAD) [17] or UV [4, 16]. Researchers adopted this method of analysis for electronic cigarette aerosol from Coresta No 74. "Determination of selected carbonyls in mainstream cigarette smoke by HPLC" [18].

Cambridge filter pad and Carboxen-572 cartridge

Uchiyama et al. [2] proposed a method that enables simultaneous determination of carbonyl compounds in aerosol as gaseous compounds (gaseous phase) and particulate matter (particulate phase). That is a valuable approach since some carbonyl compounds, as a highly harmful formaldehyde [12], may be present in the both phases. Therefore, the determination of its contents only in one of the phases take effect in underestimation of the analyse results. This method uses a solid sorbent cartridge, packed with Carboxen-572, for gaseous compounds collection and glass fibre - Cambridge filter pad for particulate phase. Figure 3 shows a schematic diagram of an electronic cigarette aerosol collection system. The smoking machine is capable of generating the aerosol according to the specified parameters and drawing it into the system for sorption.

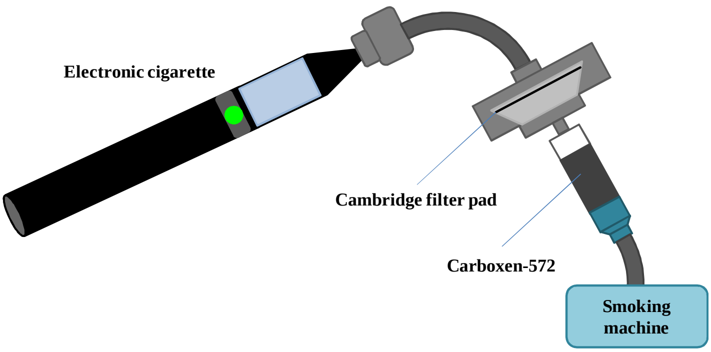

Fig. 3. Schematic diagram of an electronic cigarette aerosol collection system (taken from [2])

Aldehydes and ketones adsorbed in a solid sorbent cartridge are eluted with carbon disulphide and methanol. An aliquot of methanol solution is taken. The Cambridge filter pad after collecting the e-cigarette aerosol is extracted with only methanol, and a portion of the extract is also taken. The samples of the analysed substances from gaseous and particulate phases in methanol solutions are transferred to volumetric flasks. Then an enriched DNPH solution is added. After the derivatisation reaction, the solutions are diluted with ethanol, followed by HPLC analysis and detection using DAD detector. A flowchart of the aerosol collection and analysis procedure for the simultaneous determination of carbonyl compounds in the gaseous and particulate phase is shown in Figure 4.

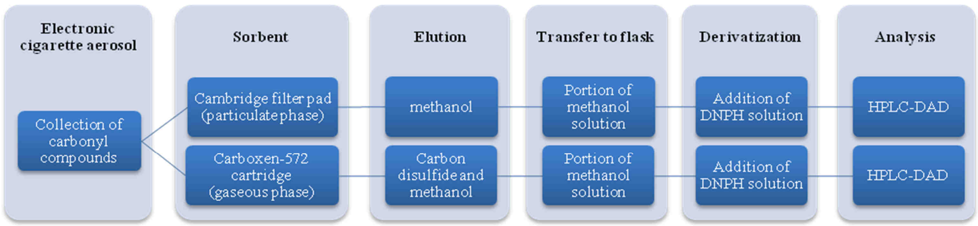

Fig. 4. Flowchart of the procedure for determination of carbonyl compounds in aerosol generated from electronic cigarette using Cambridge filter pad and solid sorbent cartridge

As the authors report, this method can be used at high concentrations of carbonyl compounds. Moreover, it also enables parallel analysis of carbonyl compounds and volatile organic compounds.

Bromination

The method also based on the formation of carbonyl compounds derivatives to determine them in the electronic cigarette aerosol was used by Papousek et al. [19]. The researchers used a system consisting of an electronic cigarette combined with a water-filled impinger and then with a vacuum source equipped with a flowmeter. Such laboratory set enabled adsorption of carbonyl compounds from aerosol in water. Research was limited to acrylamide and acrolein - carcinogenic, mutagenic and neurotoxic compounds. Concerning chemical terms, these compounds are nonionic and highly polar, with a low boiling point. Thus, for a very good separation in gas chromatography and accurate determination by mass spectrometry, a suitable derivatisation method was required. A portion of the solution was taken from the impinger, and the analysed compounds were brominated with elemental bromine in an aqueous medium in the presence of $KBrO_3$ and KBr (Fig. 5). Darkness and cool reaction conditions eliminated radical substitution and significantly inhibited the electrophilic substitution reaction. The resulting dibromo derivatives were extracted with organic solvent (ethyl acetate), and the extract was dried (over $MgSO_4$) to avoid the undesirable reversible nucleophilic addition of water. Before analysis with gas chromatography-mass spectrometry technique, samples were preconcentrated, and n-propanol (n-PrOH) was added. The alcohol reacted with acrolein in an acidic reaction environment to form the acetal, what protected the aldehyde group from undesired reactions. Also, the high temperature of the injector provided for the elimination of the alcohol and the possibility of quantitative determination of the aldehyde. Also to ensure quantitative dehydrobromination (Fig. 5), especially acrylamide, triethylamine (TEA) was added to the sample prior to analysis. Then, gas chromatography-mass spectrometry (GC-MS) analysis was performed.

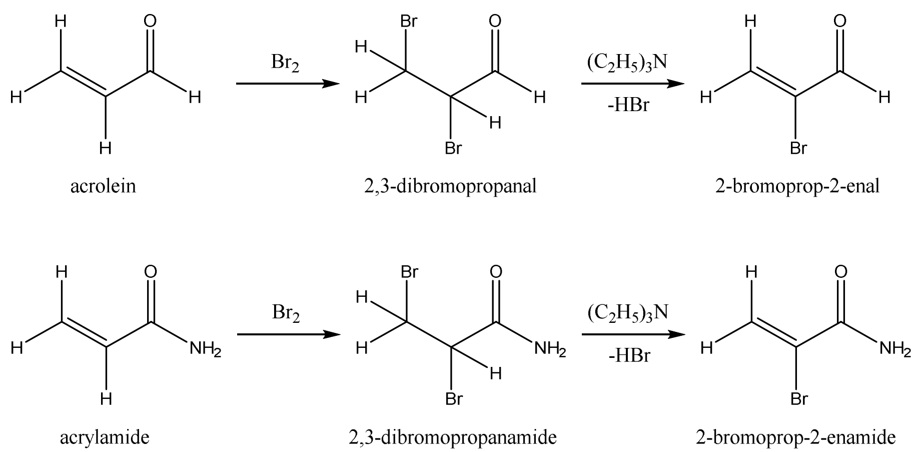

Fig. 5. Scheme of chemical transformations of acrolein and acrylamide

The advantages of this method as a result of bromination include decreasing these compounds polarity, increasing their extractability, improving selectivity and increasing the molecular weight [19]. In addition, brominated compounds have a specific isotopic pattern and more symmetric peaks, which results in improved selectivity, yield and lower detection levels in the mass spectrometry method. A short time of the bromination reaction (30 min) is an attractive merit of the presented method. The authors point out that the methodology developed by them can also be used for the determination of similar gaseous products in smoke generated by the conventional cigarette or cigar smoking.

Multi-sorbent thermal desorption tubes

Herrington et al. [20] proposed a method for the determination of carbonyl compounds in electronic cigarette aerosols, including the most harmful formaldehyde, acetaldehyde and acrolein, based on multi-sorbent thermal desorption tubes (TD tubes). Tubes are packed with Tenax TA, Carbograph 1 TD and Carboxen 1003 (Restec Corporation, Bellefonte, PA, USA). Figure 6 shows an aerosol adsorption system. With the use of a gas tight syringe, an aerosol generated from an electronic cigarette was drawn into TD tube.

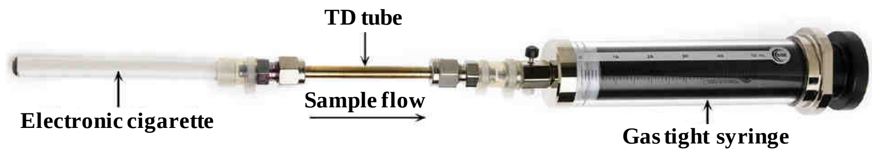

Fig. 6. Sampling set for drawing and collection electronic cigarette aerosol into a thermal desorption tube (taken from [20])

After an aerosol collection, TD tubes are transferred to the thermal desorption instrument (Markes UNITY TM) connected to the GC-MS for thermal extraction, separation and detection of analysed compounds (TD-GC-MS method). The method provides simultaneous analysis of many different groups of chemicals contained in the aerosol generated from the electronic cigarettes (alkanes, alkenes, aromatic compounds or halogenated compounds), but the hydrocarbons of not more than C_2-C_{32}. Moreover, only one puff (40 cm^3) is collected for analysis, thus minimising potential interferences or overloading of propylene glycol and glycerin, as well as preventing a "dry puff" [21]. The authors claim that the advantages of the proposed method are its reproducible results, sensitivity, and the ability to a quick and easy analysis of the aerosol generated from electronic cigarettes. However, researchers using this method should consider that elevated temperature conditions during TD-GC-MS analysis may potentially cause pyrolysis of adsorbed chemical compounds. Therefore, it isimportant to choose the suitable thermal desorption parameters.

Microreactor functionalized by aminooxy reagent

The method to determine even trace amounts of aldehydes and ketones using the preconcentration approach has been proposed by Ogunwale et al. [22]. This method uses silicon microreactor with a coating phase. Microreactors are fabricated from silicon wafers with standard microelectromechanical system fabrication technique (MEMS technology). Each microreactor has thousands of micropillars, providing a large surface for capturing the aerosol components passed through the microreactor. The micropillars surface was functionalized with a tertiary ammonium salt 4-(2-aminooxyethyl)-morpholin-4-ium chloride (AMAH) infusing a solution AMAH in methanol. The solution was introduced into the microreactor, and then the solvent was evaporated using a vacuum oven. The use of such prepared microreactor was based on collecting an electronic cigarette aerosol into a Tedlar bag using a cigarette-smoking robot and then passing it through a microreactor with a vacuum pump (flow of 3.5 cm^3/min). In this way, the carbonyl compounds present in the aerosol could be adsorbed onto the micropillars surface via the oximation reaction (Fig. 7).

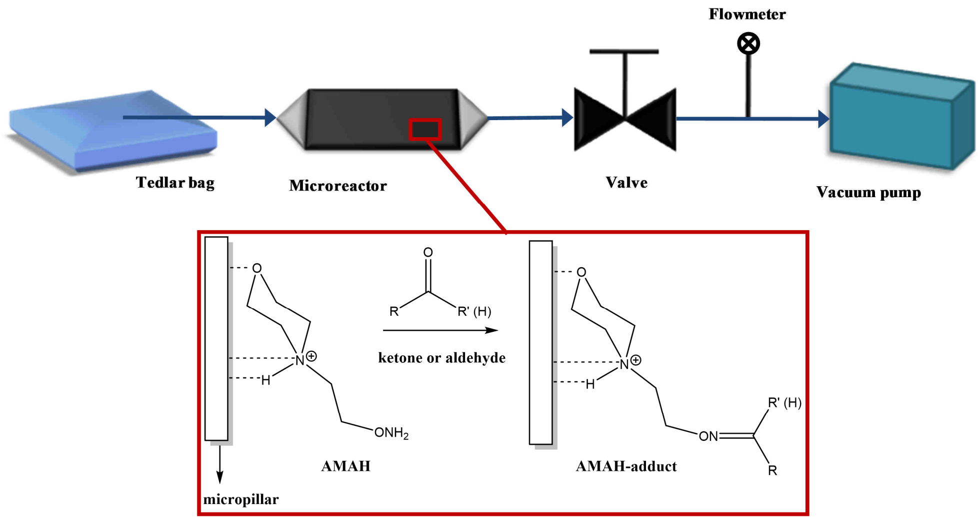

Fig. 7. Scheme of chemical reactions of carbonyl compounds in microreactor (taken from [23])

The next step was the elution of obtained AMAH-adducts with methanol contained an internal standard (AMAH-cyclohexanone). Poly-4-vinylpyridine (PVP) was added to the solution to neutralise the positive charge AMAH-adducts (Fig. 8). In this way, AMA-adducts (4-(2-aminooxyethyl) morpholine) were obtained.

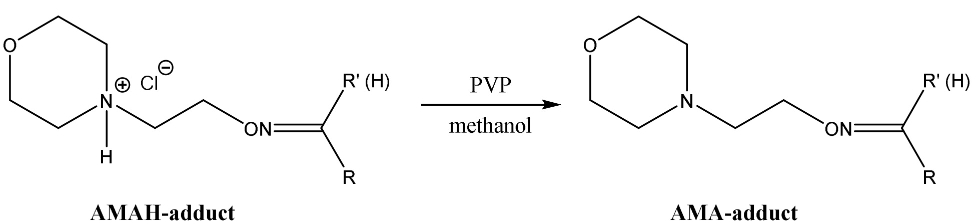

Fig. 8. Basification of AMAH-adducts with PVP (taken from [23])

Samples containing AMA-adduct were analysed by GC-MS technique. The advantage of this method is the superior lower limit of detection, an efficient and quantitative method for capturing traces of carbonyl compounds in air and exhaled air [23, 24].

Solid-phase microextraction-gas chromatography mass spectrometry method

The modern analytical method of solid-phase microextraction (SPME) technique for the qualitative and quantitative determination of carbonyl compounds in aerosol generated from electronic cigarettes was used by Sala et al. [25]. The procedure is based on fibre derivatisation. The fibre (triphasicdivinylbenzene/carboxen/polydimethylsiloxane) is saturated with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) as derivatisation agent. To generate and collect an electronic cigarette aerosol for analysis, the smoking machine is used, and its output is directed to a 60 cm^3 glass vial. Then the functionalized SPME device is exposed to electronic cigarette aerosol. The derivatisation reaction to form the corresponding oximes undergoes on the fibre (Fig. 9).

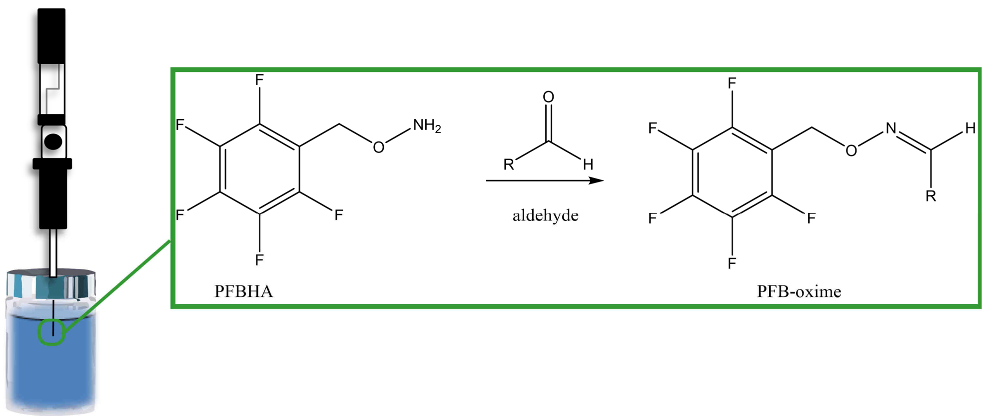

Fig. 9. Aerosol sampling by SPME technique and derivatisation reaction undergoes on fibre

Thus prepared sample was analysed for formaldehyde, acetaldehyde and acrolein as PFB-oximes using headspace/GC method and detected by means of mass spectrometry. Before starting to generate and collect aerosol, a deuterated internal standard was added to the e-liquids. Internal standard minimised a potential loss or variation during the sample preparation procedure. Moreover, that provided a direct comparison between the various analytes and experiments and the quantitative assessment of the electronic cigarette users exposure to harmful carbonyl compounds. The advantage of the presented method is no need to extract the determined substances.

Summary and conclusions

The paper presents various approaches for determining the content of carbonyl compounds in an aerosol generated from electronic cigarettes. These methods use a variety of aerosol collecting techniques as well as different ways of binding the substance on the surface or in solution. Each of them has advantages but also limitations in use. Below all the discussed methods are gathered together along with references to the publications in which they were described (Table 1).

Table 1

Overview table with the methods presented in this paper along with references to the publications in which they were described

Methodology for carbonyl compounds trapping 2,4-dinitrophenylhydrazine derivatives	**Study**
Tubes packed with silica adsorbent	McAuley et al. (2012) [14], Goniewicz et al. (2013) [26], Kosmider et al. (2014) [12], Geiss et al. (2016) [27], Jo et al. (2016) [28], Khlystov et al. (2016) [5], Kosmider et al. (2016) [29]
Quartz filters	McAuley et al. (2012) [14]
Dual-cartridge method	Uchiyama et al. (2013) [15], Bekki et al. (2014) [3]
2,4-dinitrophenylhydrazine trapping solution	Hutzler et al. (2014) [17], Flora et al. (2016) [16], Gillman et al. (2016) [4]
Cambridge filter pad and Carboxen-572 cartridge	Uchiyama et al. (2016) [2]
Bromination	Papousek et al. (2014) [19]
Multi-sorbent thermal desorption tubes	Herrington et al. (2015) [20]
Microreactor functionalized by aminooxy reagent	Ogunwale et al. (2017) [22]
Solid-phase microextraction-gas chromatography mass spectrometry method (SPME-GC/MS)	Sala et al. (2017) [25]

Electronic cigarettes are popular as a substitute for conventional cigarettes, as well as among adolescents. They are rapidly evolving by introducing new constructional solutions that affect the chemical composition of electronic cigarette aerosols. Therefore, it is important to have fast, cheap and reliable methods at disposal for determining the content of not only carbonyl compounds in aerosol but also other toxic substances. That will allow for an effective safety estimation of the electronic cigarettes use.

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METODY OZNACZANIA ZWIĄZKÓW KARBONYLOWYCH W AEROZOLU GENEROWANYM Z ELEKTRONICZNYCH PAPIEROSÓW

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Abstrakt: Elektroniczny papieros, zwany systemem dozującym nikotynę lub potocznie e-papierosem, od chwili wprowadzenia na rynek stawał się popularną alternatywą tradycyjnych wyrobów tytoniowych. Obecnie stosowany jest w wielu krajach całego świata i ma szerokie grono zwolenników. Jednakże wciąż trwają badania nad składem aerozolu wdychanego przez użytkowników elektronicznych papierosów. Wyniki tych badań pozwalają na oszacowanie narażenia na toksyczne związki chemiczne. Mogą one stać się narzędziem służącym ocenie szkodliwości stosowania tych urządzeń. Jedną z głównych grup związków, których zawartość oznacza się w aerozolu, są związki karbonylowe. Związki te tworzą się w wyniku częściowej dekompozycji substancji bazowych (glikolu propylenowego i gliceryny) oraz dodatków smakowo-zapachowych w temperaturze generowania aerozolu. Długotrwała ekspozycja na związki karbonylowe, takie jak formaldehyd czy acetaldehyd, jest znanym czynnikiem podwyższającym ryzyko astmy i chorób nowotworowych. Stąd dokładne pomiary zawartości tych związków w aerozolu są istotne ze względu na badania wpływu na zdrowie stosujących elektroniczne papierosy. Niniejszy artykuł stanowi zwięzły przegląd stosowanych metod oznaczania zawartości związków karbonylowych w aerozolu generowanym z elektronicznych papierosów.

Słowa kluczowe: elektroniczny papieros, aerozol, związki karbonylowe, metody analityczne