Investigation of Carcinogenic Compounds in Tobacco Smoke

Hanti Jiang
Beijing World Youth Academy, Beijing, China
Email: Evelynjiang_ht@163.com

***Abstract*—Tobacco specific nitrosamines are one of the most carcinogenic compounds present in the tobacco smoke. Studies have been conducted and multiple methods have been developed to detect and analyze the tobacco specific nitrosamines in order to understand their effects on human body. In recent years, researchers have developed and brought varies methods in drug analysis. The focus of this paper is the current status of Liquid Chromatography (LC), Capillary Electrophoresis (CE), Gas Chromatography (GC) coupled with Mass Spectrometry (MS) in the analysis of carcinogenic compounds in tobacco smoke. We have discussed the advantages and disadvantages of each method, as well as their applications in qualification and quantification of tobacco specific nitrosamines. By combining the conventional methods of LC-MS, CE and GC-MS, together with the newly-developed fields of nanomaterials and metabolomics, scientists are able to effectively quantify and analyze different carcinogens in tobacco smoke.**

***Index Terms*—tobacco specific nitrosamines, carcinogenic compounds, chromatography- mass spectrometry, nanomaterials, metabolomics**

I. Introduction

Around the world, almost 1 billion men and 250 million women are daily smokers. It is estimated that, among all the people alive today, 500 million of them will eventually get killed by tobacco use. Cigarette smoking causes 90% of lung cancer cases [1]. Among the more than 60 types of carcinogens in cigarette smoke, tobacco specific nitrosamines have a dominant role in causing cancer. The presence of these tobacco specific nitrosamines may result in DNA damage which can lead to mutations, causing cell-growth dysregulation and cancer. In order to acquire a deeper understanding of the roles that carcinogenic compounds in tobacco smoke played in cancer development, identification and quantification of tobacco specific nitrosamines are highly desired and commonly investigated.

Various detection methods have been developed and applied in the measurement of carcinogenic compounds in tobacco smoke, including Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS), Gas Chromatography-Mass Spectrometry (GC-MS), and so on [2]. LC-MS, one of the most popular approaches, involves the using of high-performance LC and MS to first ionize the sample, then separate, and finally detect the ions. While the CE method depends on the electric field applied to separate samples and analyze chemicals. As for the GC-MS, it is an alternative that uses gas chromatography instead. Applicable for volatile and thermal stable analytes, GC-MS is also widely used in the field of chemical analysis.

In recent years, applications of nanomaterials are across the fields of medicine, biology, and energy technology, etc. Researchers have also applied nanomaterials in the analysis of carcinogenic compounds in tobacco smoke, and have obtained satisfied results. Three kinds of nanomaterials – nanoparticles, frameworks, and monolith – are employed for such investigations. The usage of nanomaterials in this analysis is mainly based on the nano-size pores formed in the materials, which can be modified into suitable size for the extraction of tobacco specific nitrosamines and get rid of the complicated matrix at the same time.

Besides the approaches mentioned above, metabolomics [3] offers a different perspective for the investigation of carcinogenic compounds in tobacco smoke. Metabolomics is defined as the comprehensive analysis of metabolites in a biological system. The two types of metabolomics used in tobacco analysis – targeted and untargeted, with slightly different methodology in practice – can be applied depending on the nature and the goal of the analysis. By comparing the sample groups with and without tobacco treatment, significantly changed metabolites can be identified, and the pathway analysis using MetaboAnalyst could provide additional information about the perturbed pathways.

The purpose of this paper is to provide a snapshot of the investigation of carcinogenic compounds present in tobacco smoke, which includes the application of LC-MS, CE, as well as GC-MS in the analysis of carcinogenic compounds in tobacco smoke. Besides, the employment of nanomaterials and metabolomics in the study of tobacco specific nitrosamines is also discussed. Further research could be conducted via the combination of targeted metabolomics with untargeted metabolomics for deeper understanding of the effects that tobacco specific nitrosamines potentially have on human body.

Manuscript received February 24, 2020; revised May 29, 2020.

doi: 10.18178/ijpmbs.9.3.140-143

II. Application of LC-MS in the Analysis of Carcinogenic Compounds in Tobacco Smoke

LC-MS, which stands for Liquid Chromatography - Mass Spectrometry, is widely used in the fields of medicine, food testing, and metabolomics, etc. By incorporating the chromatographic separation with the qualitative analysis of the MS, complex chemicals such as carcinogenic compounds in tobacco smoke can be separated and analyzed effectively. LC-MS is mainly composed of sample introduction, ionization source, mass analyzer, and a detector. After the sample chemical is introduced, the analytes will be ionized for further mass analysis, by which the chemicals will be separated based on the mass-to-charge ratio determined. One of the most famous ionization sources is Electrospray Ionization (ESI). It was first invented by John B. Fenn from the Virginia Commonwealth University, who won the Nobel Prize of Chemistry in 2002. During ESI analysis, the sample is passed through a spray needle capillary when the electrical energy is applied, to disperse the small solvent droplets. Subsequently, the solvent will evaporate and the analyzed ions will be ejected from the tube, following by mass analyzer analysis [4]. Triple Quadrupole (QqQ) and Time of Flight (ToF) are the most widely used mass analyzer, because QqQ is suitable for quantitative analysis while ToF is an ideal tool for qualitative studies. As an example, Clayton *et al.* used the LC-MS method for the quantification of Tobacco-specific N-nitrosamines (TSNA), more specifically, for the determination of the four most abundant compounds of TSNA: N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N-nitrosonornicotine (NNN) [5]. According to their research, TSNA play an important role in tobacco smoke carcinogenesis. By using the MRM mode of QqQ-MS, the limits of detection were only 0.44-0.91 ng/mL for the four TSNA. Furthermore, the developed method was applied in the real tobacco filter analysis, effective and reproducible results were obtained.

III. Application of CE in the Analysis of Carcinogenic Compounds in Tobacco Smoke

Besides LC-MS, another productive tool used for the analysis of tobacco carcinogens is CE-MS, which stands for Capillary Electrophoresis - Mass Spectrometry. In order to conduct the separation and determination of chemical substances, CE method involves the using of the quartz capillary, buffer, an UV detector, and an electric field. When the sample chemicals pass through the thin capillary tube, the analytes can be separated based on their different mobility under the high voltage that has applied, following by UV detection [6]. Due to the electroosmotic flow (EOF) phenomenon appears inside the capillary tube, the anions and cations will be separated during this process.

Compared with LC-MS, CE-MS requires smaller volume of sample and takes shorter analysis time. CE method also exhibits higher accuracy than other electrophoresis methods, such as the 2D gel electrophoresis. Owing to these advantages, CE has also been applied in the measurement of N-nitrosamines. Determination of six TSNAs using the technique of CE-MS was conducted by Chen Li *et al.*, they successfully analyzed the TSNAs present in rabbit serum and compared the results of CE-MS with that of CZE method in the process, showing that CE-MS has a higher sensitivity and more suitable for the analysis of chemical substances with more complex structures [7]. This study demonstrates the high development potential of CE technique in the field of toxicity, such as but not limited to tobacco carcinogens quantitative analysis.

IV. Application of GC-MS in the Analysis of Carcinogenic Compounds in Tobacco Smoke

Similar to LC-MS, Gas Chromatography - mass spectrometry (GC-MS) is another type of analytical chromatography used in analyzing chemical components. It is specifically designed for the analytes to be volatile and thermal stable, with the boiling point generally lower than 500℃. GC-MS normally consists of a compressed gas cylinder, where the sample is injected in the form of liquid; a valve and a filter, where the sample is vaporized; a column oven, in which the sample flows and separated (Fig. 1). After that, the sample will flow through a heated surface and then go to the ion source for electron impact. Following by the uses of mass analyzer and detector, same to the LC-MS process. Different from LC-MS, GC-MS uses inert gas as the carrier gas instead of liquid. Another aspect that sets these two techniques apart is the structure of the chromatographic column, for that of LC-MS is packed, whereas the open-tube column is normally employed by GC-MS, resembling a hollow capillary.

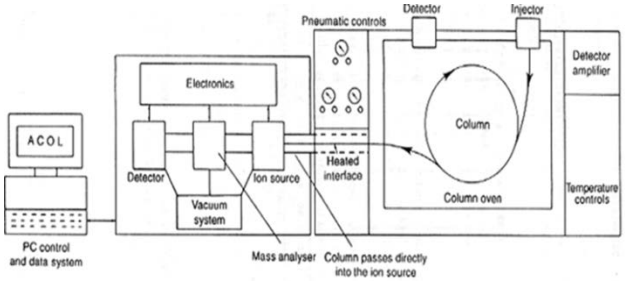

Figure 1. Gas chromatography – mass spectrometry (GC-MS)

Owing to the special characteristics of GC-MS as it can be used to test the presence of specific substances, GC-MS is widely used in analyzing tobacco carcinogens in tobacco smoke. For example, a research conducted by Monica Culea *et al.* in analyzing the polycyclic aromatic hydrocarbons (PAHs) using GC-MS method demonstrated the application of GC-MS for the determination of tobacco carcinogens. In their experiments, the urine sample was tested to determine the levels of naphthalene, phenanthrene, and anthracene quantitatively using the ThermoFinnigan Trace Gas Chromatography along with the Trace DSQ Mass

Spectrometry [8]. The chromatograms are shown in the form of the SIM mode, and the results demonstrated the accuracy and precision of the GC-MS method.

V. Nanomaterials in the Analysis of Carcinogenic Compounds in Tobacco Smoke

A newly-developed field in the analysis of tobacco carcinogen is the application of nanomaterials. Three kinds of common nanomaterials: nanoparticles, Frameworks, and monolith have been employed for the investigation of carcinogenic compounds in tobacco smoke. Firstly, the nanoparticles are particles between 1 and 100 nanometers (nm) in size. One type of nanoparticle that is used for carcinogen analysis is the activated carbon sorbent. Because of the correspondence between the molecular structure of the TSNA and the pore size of the nanoparticles, the activated carbon sorbent is able to trap approximately 60% of the TSNA, according to the research done by Chun Ling Shi and coauthors [9]. This allows the extraction of the specific target TSNA, which means the higher the selectivity of the nanoparticle that matches to the TSNA, the higher the percentage of removal, resulting in less TSNAs remain in the test sample. By adding ferric oxide, the pore size of the carbon sorbent is able to be modified and become more suitable for the target analyte, thus improving the precision of the tobacco carcinogen extraction (Fig. 2).

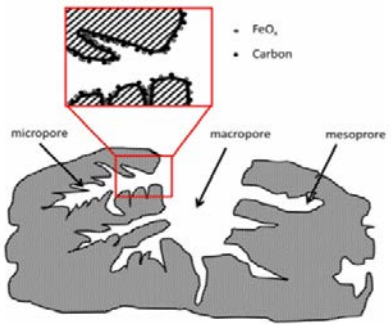

Figure 2. Ferric oxide is added to the carbon sorbent in order to modify pore size to improve tobacco carcinogen extraction [9].

Besides nanoparticles, the metal framework of 3D copper (II) hydroxide is also used in similar TSNAs analysis. With the ability of self-assemble, the metal particle is able to grow continuously, during which the framework can be optimized by controlling the time to match with the target TSNA [10].

Monolith refers to a separation medium comparable to a single large particle that the shape and volume fill with the interior of the separation column entirely without interarticular voids [11]. Monolith is known to be a multifunctional nanomaterial, for it can also be used in the chromatographic column of GC-MS technique. In a research done by O. Chienthavorn and his colleagues, the application of the monolith capillary trap in the field of nitrosamine extraction was investigated. The results demonstrated significant differences between the sensitivity with and without the usage of monolith, and has confirmed the feasibility of this specific method [12].

VI. Metabolomics Study

Metabolomics refers to the identification and quantification of the metabolic chemicals and products in a biological system. For the study of metabolomics profiling, two most commonly used analytical platforms are MS-based ionization and Nuclear Magnetic Resonance (NMR)-based spectra. The overall basic experimental workflow of metabolomic analysis is listed as follows (Fig. 3). Firstly, the metabolites are extracted, with one commonly-used method of methanol extraction. This is followed by the analytical methods including LC-MS, GC-MS, and NMR. During this stage, the sample data is being filtered and analyzed. The data collected are then searched in the database for the identification of metabolites. Metlin database, a platform for searching and identifying metabolites, is commonly used in the study [13]. Finally, the metabolism pathway or function analysis is conducted, marking the end of the metabolomic analysis of this round.

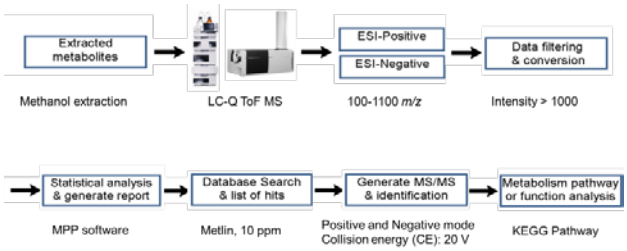

Figure 3. Experimental workflow of metabolomic analysis.

The analytic method differs based on the type of metabolomic study: targeted and untargeted [14]. As an example, Romel Dator *et al.* have studied the NNK-metabolites profiling by the approach of untargeted metabolomics using the MS-based metabolic profiling method. They conducted a crossover experiment using the targeted and untargeted approaches towards analyzing and screening the known and unknown 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolites in rats, a potent lung carcinogen present in all tobacco products and one of the most powerful carcinogens in tobacco smoke (Fig. 4) [15]. With the unknown metabolites identified, the rat characterized urine from NNK-treated rats can be used as an internal standard for the future relative quantitative determination of NNK metabolites in human body.

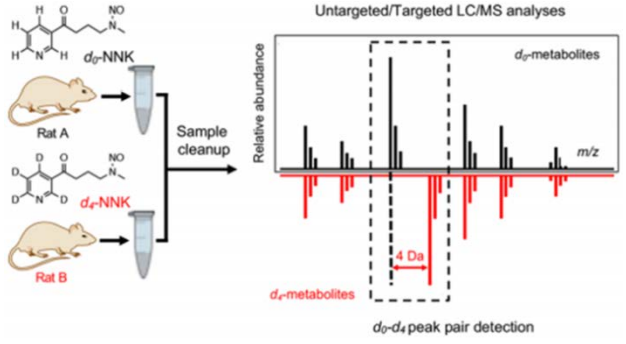

Figure 4. Metabolites profiling by the approach of untargeted metabolomics using the MS-based metabolic profiling method [15].

VII. Conclusion

As we can see, various methods and technologies (e.g. LC-MS, CE, GC-MS) have been used in analyzing tobacco carcinogens, each method has advantages and flaws. Hence, we need to select the best way for different purposes considering their different uses and properties. For future study, metabolomics and nanomaterials have showed great potentials in analyzing tobacco carcinogens, for that they are the emerging areas that can potentially be further developed for even more functions and effects in resolving complex problems and expanding the capability of chemical analysis and sensing. Due to the special properties of nanomaterials, the precision of their performances is ensured. Along with the combination of targeted and untargeted metabolomics study, advances in the equipment and method for chemical analysis are waiting for new discoveries.

Conflict of Interest

The author declares no conflict of interest.

Author Contributions

Hanti Jiang researched and wrote the paper.

Acknowledgment

The author wishes to thank Ms. Linda Zhang for reviewing the manuscript.

References

[1] S. S. Hecht, "Tobacco carcinogens, their biomarkers and tobacco-induced cancer," *Nature Reviews Cancer*, vol. 3, pp. 733-744, 2003.

[2] K. A. Shah and H. T. Karnes, "A review of the analysis of tobacco-specific nitrosamines in biological matrices," *Critical Reviews in Toxicology*, vol. 40, pp. 305-327, 2010.

[3] B. B. Misra and S. Mohapatra, "Tools and resources for metabolomics research community: A 2017-2018 update," *Electrophoresis*, vol. 40, pp. 227-246, 2019.

[4] C. S. Ho, C. Lam, M. Chan, R. Cheung, L. Law, L. Lit, K. Ng, M. Suen, and H. Tai, "Electrospray ionisation mass spectrometry: Principles and clinical applications," *The Clinical Biochemist Reviews*, vol. 24, pp. 3, 2003.

[5] P. M. Clayton, A. Cunningham, and J. D. H. Heemst, "Quantification of four tobacco-specific nitrosamines in cigarette filter tips using liquid chromatography-tandem mass spectrometry," *Analytical Methods*, vol. 2, pp. 1085-1094, 2010.

[6] B. C. Durney, C. L. Crihfield, and L. A. Holland, "Capillary electrophoresis applied to DNA: Determining and harnessing sequence and structure to advance bioanalyses (2009-2014)," *Analytical and Bioanalytical Chemistry*, vol. 407, pp. 6923-6938, 2015.

[7] C. C. Li, Z. Chen, D. W. Wen, J. X. Zhang, W. H. Cong, B. Yu, Y. P. Liao, and H. W. Liu, "Determination of tobacco-specific N-nitrosamines in rabbit serum by capillary zone electrophoresis and capillary electrophoresis-electrospray ionization-mass spectrometry with solid-phase extraction," *Electrophoresis*, vol. 27, pp. 2152-2163, 2006.

[8] M. Culea, O. Cozar, and E. Culea, "PAHs in cigarette smoke by gas chromatography-mass spectrometry," *Indoor and Built Environment*, vol. 14, pp. 283-292, 2005.

[9] C. L. Shi, S. H. Li, and X. D. Sun, "New activated carbon sorbent with the zeolite-like selectivity to capture tobacco-specific nitrosamines in solution," *Chemical Engineering Journal*, vol. 339, pp. 170-179, 2018.

[10] H. W. Hou, Y. Zhu, and Q. Y. Hu, "3D Cu(OH)(2) hierarchical frameworks: Self-assembly, growth, and application for the removal of TSNAs," *Journal of Nanomaterials*, 2013.

[11] K. Stulik, V. Pacakova, J. Suchankova, and P. Coufal, "Monolithic organic polymeric columns for capillary liquid chromatography and electrochromatography," *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*, vol. 841, pp. 79-87, 2006.

[12] O. Chienthavorn, N. Ramnut, P. Subprasert, A. Sasook, and W. Insuan, "Effective and reusable monolith capillary trap of nitrosamine extraction by superheated water from frankfurter sausage," *Journal of Agricultural and Food Chemistry*, vol. 62, pp. 1240-1246, 2014.

[13] C. Guijas, J. R. Burke, and X. D. Almenara, "METLIN: A technology platform for identifying knowns and unknowns," *Analytical Chemistry*, vol. 90, pp. 3156-3164, 2018.

[14] E. Gorrochategui, J. Jaumot, S. Lacorte, and R. Tauler, "Data analysis strategies for targeted and untargeted LC-MS metabolomic studies: Overview and workflow," *Trac-Trends in Analytical Chemistry*, vol. 82, pp. 425-442, 2016.

[15] R. Dator, L. B. V. Weymarn, P. W. Villalta, C. J. Hooyman, L. A. Maertens, P. Upadhyaya, S. E. Murphy, and S. Balbo, "In vivo stable-isotope labeling and mass-spectrometry-based metabolic profiling of a potent tobacco-specific carcinogen in rats," *Analytical Chemistry*, vol. 90, pp. 11863-11872, 2018.

Hanti Jiang is a student from Beijing World Youth Academy, was born in Qingdao, China, May 26th 2003. She is enthusiastic about Biology and wishes to gain deeper understanding and exploration into the field of Life Sciences. She has had internship experience in Beijing Genomics Institute, and has studied in Johns Hopkins Unviersity summer program in the course of Introductin to Laboratory Research. She has experiences in operations of genetic editing of zebra fish and many other biotechnologies.