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Analysis of nicotinic acid in coffee using the temperature programmable injection method in gas chromatography-mass spectrometry

[1]Aprilia, S.A., [2,3,*]Wonorahardjo, S. and [2]Utomo, Y.

[1]*Graduate Program in Chemistry, Faculty of Mathematics and Natural Sciences, State University of Malang, Malang, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of Malang, Malang, Indonesia*
[3]*Centre of Advanced Materials for Renewable Energy, State University of Malang, Indonesia*

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Abstract

Nicotinic acid is found in various kinds of food, including coffee. The gas chromatography-mass spectrometry (GC-MS) method is currently being developed to analyse the presence of thermal decomposition of the compound during elution. The temperature programmable inlet (TPI) was carried out to optimise the sample recognition and separation, protecting samples from thermal degradation by balancing efficient vaporisation and minimal fragmentation during ionisation. This study aimed to determine whether GC can analyse nicotinic acid from other compounds. The TPI-GC/MS found nicotinic acid, in the form of pyridine-3-carboxylic acid, 1-[bicyclo[4.1.0]heptane-7-carbonyl)amino]-6-oxo-1,6-dihydro-, methyl ester. The compound pyridine-3-carboxylic acid which is another name for nicotinic acid was found at an injection temperature of 240°C.

1. Introduction

Nicotinic acid is included in dissolved vitamins (Arauz *et al*., 2015), which is essential in metabolic processes. It is involved in many vital processes in the body of living organisms and can cause many diseases if lacking, can reduce low-density lipoprotein (LDL) levels in plasma and is used in the treatment of hyperlipidaemia (Mohamed *et al*., 2021). The recommended daily intake is around 15 mg/day.

Nicotinic acid is found in various foods including coffee together with caffeine, chlorogenic acid and some known antioxidants (Wonorahardjo *et al*., 2019). It is a trigonelline derivative compound formed during the roasting process of green coffee beans (Angelino *et al*., 2018; Buckel *et al*., 2019). Trigonelline is also well-known as a good component in coffee.

Several chemical methods have been used for years to analyze nicotinic acid, including the liquid chromatography method with a UV detector. Although sensitivity and selectivity are sometimes a problem, the liquid chromatography method with an MS detector is used as a current method (Angelino *et al*., 2018; Buckel *et al*., 2019; Jeszka-Skowron *et al*., 2020). This study used the liquid chromatography-high resolution mass spectrometry (LC-HRMS) method to determine the presence of nicotinic acid in coffee samples. Many gas chromatography methods have been developed to analyze nicotinic acid in food since it has a low molecular weight, high volatility, and shorter analysis time (Sheppard and Prosser, 1971; Almalki *et al*., 2021). However, the thermal stability of nicotinic acid is still controversial (Moreschi et al., 2009a). Therefore, to overcome these deficiencies, the GC/MS method was developed with an injection temperature setting or temperature programmable inlet, referred to as the TPI-GC/MS method.

TPI is a rather common feature across many GC/MS systems, allowing precise control over the injector temperature (Wei *et al*., 2010). TPI can also help protect samples from thermal degradation during the injection process to ensure that the sample is introduced into the GC column at the optimum temperature. The TPI is programmable to accommodate a wide range of sample types and applications, enabling optimisation of the injector temperature profile for specific samples or analytical purposes. For example, a sample prone to thermal degradation may require a lower initial injector temperature or a slower ramp rate. A more volatile sample may require a higher initial temperature or a

*Corresponding author.
Email: *surjani.wonorahardjo@um.ac.id*

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faster ramp rate. GC/MS systems can achieve increased sensitivity, reproducibility, and accuracy for various applications by optimising the injector temperature profile with TPI. Previously, Japanese researchers had successfully used the TPI-GC/MS method to analyse carbofuran and its derivative compounds in tap water, which are easily hydrolysed and decomposed by heat (Kawamoto and Makihata, 2003).

Coffee has a complex matrix containing various components, including sugars, proteins, and other organic compounds that can interfere with the analysis of nicotinic acid. However, TPI can help optimize the injector temperature profile for the coffee matrix and specific analytes. A lower initial injector temperature is required to minimize the thermal degradation of nicotinic acid and other volatile compounds in the coffee matrix. The injector temperature can then be increased to increase the volatility of nicotinic acid and other target analytes and their derivatives. The thermal properties of the components in coffee are to be described indirectly from the MS profiles. This research aimed to develop the TPI-GC/MS method for analysing nicotinic acid in roasted coffee.

2. Materials and methods

2.1 Materials

Arabica Lemar coffee beans were harvested from the Wonosantri Abadi plantation, Singosari Malang, methanol (optima LC/MS) from Merck, formic acid and acetonitrile (LC/MS). All solvents used were of analytical grade from Merck.

2.2 Equipment

The tools used in this study included a set of coffee roaster machines (NOR coffee roaster machine N5000i), coffee grinders, rotavapors, 50 kg digital sitting scales, analytical balances, a series of Soxhlet tools, LC-HRMS instruments consisting of HPLC (Thermo Scientific Dionex Ultimate 3000) RSLCnano with Microflowmeter with Hypersil Gold aQ column size 50 × 1 mm × 1.9 µm and High Resolution Mass Spectrometer detector (Thermo Scientific Q Exactive), GC/MS instrument (QP2010Plus Shimadzu), 250 mL beaker, beaker measuring 50 mL, watch glass, glass funnel, vial.

2.3 Experimental setting

2.3.1 Sample preparation

Arabica Lemar coffee beans were dried using a conventional drying process, where the coffee beans picked from the tree were spread and dried on an open floor/patio for more than 21 days to dry the juice inside the coffee beans. Then, the coffee beans were sorted based on size. About 1 kg of sorted beans were roasted using the NOR coffee roaster machine N5000i up to 210°C, cooled and ground. About 2 g of ground coffee was extracted by 250 mL of methanol solvent using the soxhletation method. The extract was then concentrated with a rotary evaporator and stored in the refrigerator.

2.3.2 Analysis of nicotinic acid compounds using liquid chromatography-high resolution mass spectrometry method

The sample was diluted with 1500 mL of methanol, then vortexed at 2000 rpm for 2 mins and spun down at 6000 rpm for 2 mins. The supernatant was filtered using a 0.22 µm syringe filter and put in a vial before it was inserted into the autosampler and injected into the LC-HRMS. The mobile phases used were mobile phase A 0.1% formic acid in water, and mobile phase B 0.1% formic acid in acetonitrile with a flow rate of 40 µL/min. The oven temperature was 30°C with an analysis time of 30 mins and detection using the complete scan method with a resolution of 70000 and 17500 on MS2. The spectrum obtained was matched with the Compound Discoverer software with mzCloud MS/MS Library.

2.3.3 Analysis of nicotinic acid compounds using the TPI-GC/MS method

The melting point of nicotinic acid was 235-237°C theoretically, and the injection temperatures chosen were 40°C, 140°C and 240°C, to determine how different compounds can give different MS profiles, depending on the stability of the compound. The injection method was conventional splitless injection. A 1 µL sample was inserted into the GC/MS port with an injection temperature of 40°C. The oven temperature was 40°C for 4 mins and increased to 280°C at a rate of 20°C/min, then held for 13 mins. The ion source and interface temperature were 200°C. The carrier gas was helium, with a 45.3 cm^3/s flow rate. MS mode is a full scan with a mass range of 50-600 m/z. Further analysis was conducted at injection temperatures of 140°C and 240°C with the same procedure as the injection temperature of 40°C.

3. Results and discussion

The extracts were then analysed using the LC/HRMS method to identify the presence of nicotinic acid. Figure 1 is a specific chromatogram from Arabica Lemar coffee samples roasted at 210°C. The total ion chromatogram (TIC) records the total intensity signal of all ions detected on the mass spectrometry at any time point during the analysis. The TIC graph shows the change in intensity of the detected ion signal as a function of elution time. The peaks seen on the TIC represent the discrete compounds detected during the analysis. The

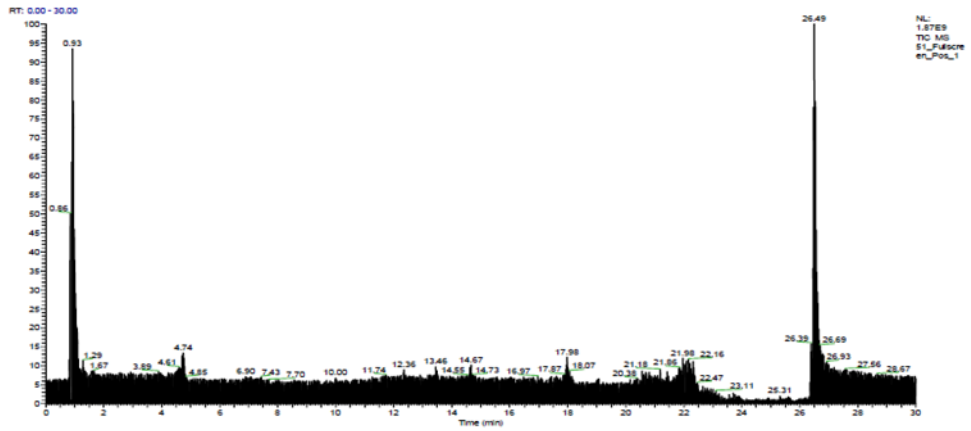

Figure 1. Total ion chromatogram of coffee samples.

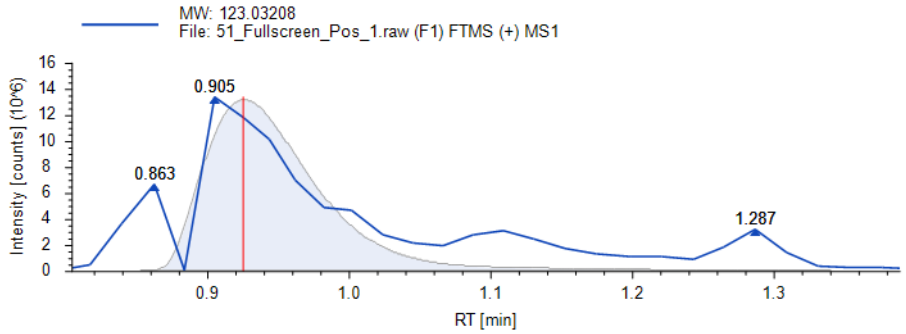

Figure 2. Chromatogram Peak 1 = Phloroglucinol; 2 = 2,2,6,6-tetramethyl-1-piperidinol (TEMPO); 3 = nicotinic acid, and 4 = L-pyroglutamic acid.

peak intensity indicates the number of ions detected from each compound at a particular time (Barea *et al.,* 2022).

Based on the TIC in Figure 1, several single peaks indicate that several compounds were separated. However, the peak indicating nicotinic acid has not been identified in the chromatogram. Figure 2 shows a single peak on the chromatogram indicating nicotinic acid. The peaks are identified by the detector and can be compared to the standards. Peak number 3, with a retention time of 0.926, indicates the presence of nicotinic acid in the sample. Further identification to determine the other compounds in the sample based on Compound Discoverer software with mzCloud MS/MS Library can be seen in Table 1.

The dominant compounds in the coffee sample were the trigonelline compound at 23.76%, followed by the caffeine at 21.97%. It is well known that trigonelline and caffeine are antioxidant compounds in coffee. During the roasting process, trigonelline compounds decompose into volatile compounds such as pyrrole, alkylpyridines, and phenolic compounds, which affect the taste and aroma of coffee, as well as non-volatile compounds such as nicotinic acid (Buckel *et al*., 2019; Konstantinidis *et al*., 2023). The analysis also shows that nicotinic acid is present in the sample with an area coverage of 0.62%. Further identification is seen based on its mass spectra shown in Figure 3(b). The target is nicotinic acid with the selected ion $[M+H]^+$ and its precursor ion at 124 m/z. This is also consistent with the single nicotinic acid mass spectrum data in Figure 3(a) in the PubChem database. Unfortunately in our experiments, no single peak of nicotinic acid was found at the three injection temperatures. Based on mass spectrum data at NIST, the mass spectrum of nicotinic acid is shown in Figure 4 (Cortés-Herrera *et al*., 2019). The mass spectrum of nicotinic acid usually contains a molecular ion peak at m/z 123, corresponding to the molecular weight of nicotinic acid ($C_6H_5NO_2$).

In a gas chromatography-mass spectrometry (GC-MS) analysis, nicotinic acid can undergo various fragmentation pathways, forming different ions and compounds depending on the energy distribution in the molecular body. Most branches will be new fragments or combined with other energetic fragments. Some common fragment ions and potential compound forms that may result from the fragmentation of nicotinic acid include methyl ester of nicotinic acid (Opitz *et al*., 2020). The methyl ester derivative can form due to the loss of a water molecule. The methyl ester form can have a different mass spectrum. Conversely, the pyridine ring in nicotinic acid can also undergo fragmentation, leading to ions corresponding to different parts of the ring (Ziegler *et al*., 2021). Previous research conducted by Opitz (2007) produced mass spectra of nicotinic acid at 70 eV, as shown in Figure 5.

It was found that the mass spectrum was similar to that of nicotinic acid at TPI 240°C with a retention time of 9.942 as shown in Figure 6(a) and similar to that of

Table 1. Mass spectra of roasted coffee samples.

Compound name	Molecular formula	Molecular weight	Retention time [min]	% Area
Trigonelline	$C_7H_7NO_2$	137	937	23.76
Caffeine	$C_8H_{10}N_4O_2$	194	4.723	17.24
Choline	$C_5H_{13}NO$	103	0.95	9.17
4-Aminophenol	C_6H_7NO	109	1006	3.92
Diisobutylphthalate	$C_{16}H_{22}O_4$	278	17.966	3.76
3-Hydroxypyridine	C_5H_5NO	95	1.019	2.94
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	$C_9H_{19}NO$	157	12.367	1.91
6-Methyl-2-pyridinemethanol	C_7H_9NO	123	989	0.84
Nicotinic acid	$C_6H_5NO_2$	123	0.926	0.62
Phloroglucinol	$C_6H_6O_3$	126	0.866	0.52

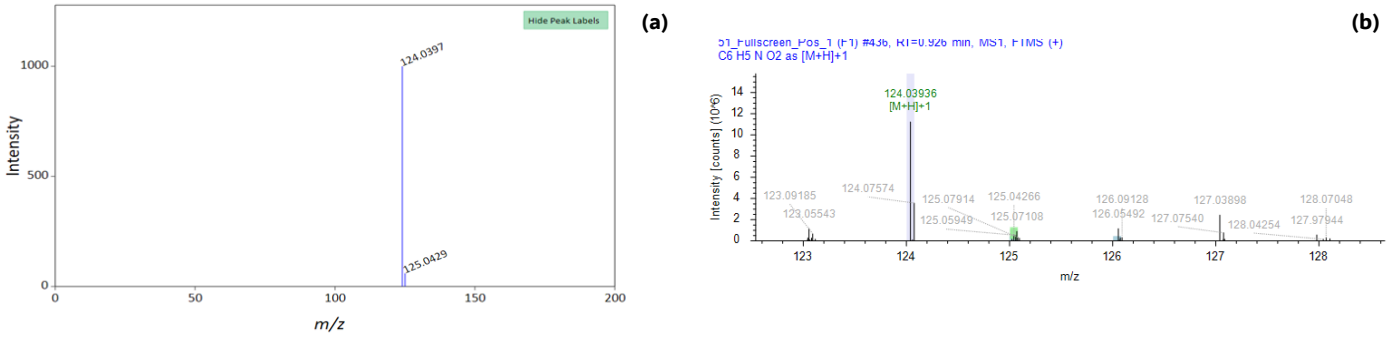

Figure 3. Single mass spectra of nicotinic acid (a) based on PubChem data Click or tap here to enter text.and (b) based on results.

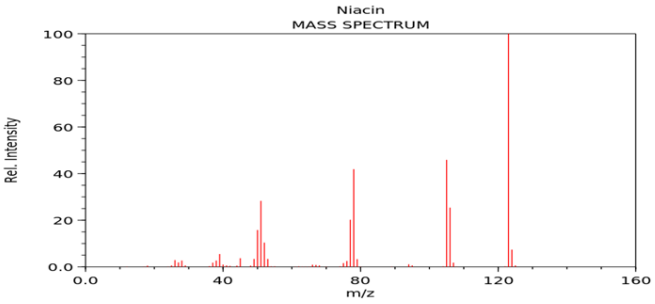

Figure 4. Mass spectra of nicotinic acid. Source: NIST (2003).

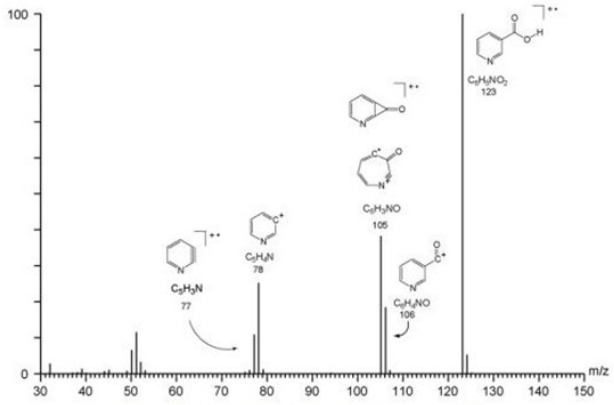

Figure 5. Mass spectra of nicotinic acid based on the experiment. Source: Opitz (2007).

pyridine-3-carboxylic acid. 1-[bicyclo[4.1.0]heptane-7-carbonyl)amino]-6-oxo-1,6-dihydro-, methyl ester in Figure 6(b). Both show similar molecular ion peaks, as produced by the molecular ion peaks at m/z 55, m/z 67, m/z 95 and m/z 123. A molecular ion peak with m/z 55 arises from the loss of the carboxyl group (COOH) from the molecule. This fragment ion corresponds to the molecular ion $[M\text{-}COOH]^+$, which has a mass-to-charge ratio of 55. The molecular ion peak with m/z 67 appears due to the presence of the fragment ion due to the loss of a carbon monoxide (CO) molecule from the molecule. This fragment ion corresponds to the molecular ion $[M\text{-}CO]^+$, which has a mass-to-charge ratio 67.

The fragment ion with m/z 95 frequently appears in the mass spectrum of nicotinic acid because it is the characteristic fragment produced by the loss of a carbon dioxide (CO_2) molecule. The nicotinic acid molecular ion has a mass-to-charge ratio (m/z) of 123, corresponding to an intact molecule with one positive charge ([M+H]+). After ionisation and fragmentation, the molecule can lose a CO_2 from the carboxylic acid group, resulting in a fragment with a mass of 28 atomic mass units resulting in a fragment ion with m/z 95 ($[MH\text{-}CO_2]^+$), which is a prominent peak in the spectrum nicotinic acid mass. The fragmentation of nicotinic acid into 95 m/z fragment ions is a typical process in mass spectrometry and can help identify the presence of nicotinic acid in a sample. The mass spectra of the target are also similar to the mass spectra of nicotinic acid contained in the NIST database in Figure 5, thus strengthening the prediction that the compound was nicotinic acid.

In addition, the compound from the mass spectra in Figure 6(b) was pyridine-3-carboxylic acid, another name for nicotinic acid. Niacin is a derivative of pyridine, the amide form of pyridine-3-carboxylic acid,

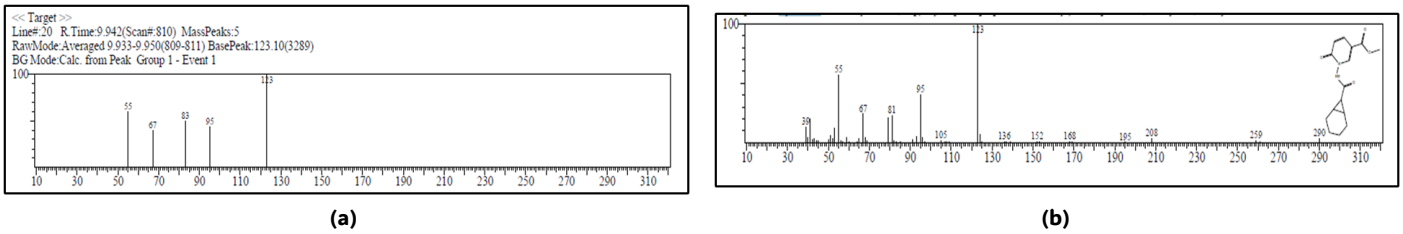

Figure 6(a). Mass spectra of the target compound at retention time 9.942 and (b) Mass spectra of pyridine-3-carboxylic acid, 1-[bicyclo[4.1.0]heptane-7-carbonyl)amino]-6-oxo-1, 6-dihydro-methyl ester based on Willey 8 Library.

also known as nicotinic acid (Cortés-Herrera *et al*., 2019). This indicates that the compound was probably a nicotinic acid derivative. A typical injection temperature range for nicotinic acid analysis in GCMS is between 200-300°C, but the optimal temperature may vary depending on the specific analytical system and sample matrix. In line with this study, it was found that a mass spectrum similar to nicotinic acid was found at TPI 240°C. Whereas at TPI 40°C and 140°C, these compounds were not found.

Sample matrices, such as coffee, undoubtedly contain interfering compounds that suppress the signals of the nicotinic acid analytes and their mass fragments. Sample preparation techniques such as solid-phase extraction or derivatisation may be required to remove interfering compounds (Cortés-Herrera *et al*., 2019). In addition, nicotinic acid may be present at low concentrations in the sample, making it difficult to detect its mass fragments.

4. Conclusion

TPI is programmable for optimization of the injector temperature profile for samples prone to thermal degradation. Nicotinic acid was identified in the roasted coffee samples based on the LC/HRMS analysis results and then verified by programmed injection in GC/MS methods. In the analysis using TPI-GC/MS, nicotinic acid, which was thought to be susceptible to thermal degradation, apparently formed a derivative, namely pyridine -3-carboxylic acid, 1-[bicyclo[4.1.0] heptane-7-carbonyl)amino] -6-oxo- 1,6-dihydro-methyl ester, at an injection temperature of 240°C.

Conflict of interest

The authors declare that there is no conflict of interest.

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