Method Article

Cheap and easy human breath collection system for trace volatile organic compounds screening using thermal desorption – gas chromatography mass spectrometry

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Abstract

• By analyzing the VOCs presents in our breath, we could identify if some components should not be present in our bodies, or their concentration is higher or lower than normal.
• To collect breath samples for VOC analysis, we looked into the current available methodologies and, due to their high prices, tried to develop our own easy and cheap device. A simple single use Minigrip LDPE plastic bag was used in this work and its efficiency and performance were tested.
• After breath collection, samples were analyzed using Thermal Desorption (TD) system, coupled with Gas Chromatography Mass Spectrometer (GC-MS).

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Fig. 1. Tedlar® Bag (Left) and developed Minigrip plastic bag collection system (Right).

Fig. 2. Vacuum Manifold stopcock valve and PVC Tubing.

Method details

The system we developed is similar to using Tedlar® bags (Fig. 1), which are widely used for breath collection for its simplicity, reproducibility, easy to transport and relatively cheap (around 10€ / 3L bag). Those single-use bags are made out of polymer polyvinyl fluoride, which was patented by Dupont™. Another polymer bags have been studied as good candidates for gas analysis, as Nalophan®, Cali-5-Bond™, FlexFoil® and Teflon® polymers. Even though those materials prove to have very good performance and little contamination coming from the materials, they need to be conditioned before collecting the sample. Furthermore, reusability of the bags generates many possible problems for most of the polymers, making them single-use bags. Similar to those polymer bags, we employed a simple Minigrip LDPE plastic bag to collect breath samples (Fig. 1). Several bag sizes were compared obtaining similar results. A small plastic vacuum manifold valve was added to the center of the bag, connecting it with small PVC tubing through the inside, creating a sealed connection from where to fill in the bag (Fig. 2). Even though Tedlar® bags are relatively cheap, using Minigrip LDPE plastic bags is much cheaper (around 0.03€ / 3L bag) an easier to purchase. Quantification has been tested with promising results, but fast diffusion of VOCs to outside the system has been observed. Therefore, our system is recommended only for single-use, fast collection and identification of VOCs in place for screening purposes. VOCs shall be collected into a proper sorbent as fast as possible after breath collection using our system.
Table 1

Thermal desorption system (ATD 400) parameters.

| Carrier Gas          | Helium 5.0 | Oven Temp. | 220°C |
|----------------------|------------|------------|-------|
| Desorption Flow       | 60mL/min   | Oven Hold  | 5 min |
| Outlet Split          | 6mL/min    | Trap Temp  | 220°C |
| Inlet Split           | No         | Trap Hold  | 3 min |

Materials needed

- Minigrip LDPE bag 200×250 mm (it works also for any other size);
- Vacuum Manifold plastic stopcock valve;
- PVC tubing Cristall Extra (5mm inner Ø / 7mm outer Ø) 15mm long;
- PVC tubing Cristall Extra (2mm inner Ø / 4mm outer Ø) 30mm long.

Device construction

1. We open the Minigrip LPDE plastic bag and introduced the 15mm long PVC tube (5mm inner Ø). We properly seal the bag and place the small PVC tubing in the center of the bag;
2. Holding the PVC tubing, we pierce it with the stopcock valve until fully connected. This will create a tight connection with the inside of the bag;
3. We connect the nitrogen gas system to the stopcock and open the valve to allow the flow of nitrogen fill the bag slowly;
4. Once the bag is around 80% full we place it inside the oven at 50°C for at least 2h;
5. After 2 hours we take the bag out of the oven, empty the gas content, and repeat the filling process;
6. Once we repeat the process at least twice, we empty the content of the bag and add the long PVC tubing (4mm outer Ø) at the end of the stopcock for better filling;
7. The bag is now conditioned and ready for sample collection;
8. After usage, the plastic bag and PVC tubing are disposed; meanwhile the stopcock valve is cleaned and disinfected using iso-propanol to be reused for another bag.

Once our collection system is ready, we tried collecting breath samples from volunteers. They were asked to fill the 3L LDPE plastic bags (already conditioned) twice in order to collect a total of 6L of breath sample. Collected breath samples are pumped through a thermal desorption tube at a flow of 1000mL/min. After collecting and analyzing the breath samples from 23 different volunteers during different times of the day (Fig. 4), we could detect at least 123 different VOCs (Table 3).

In order to analyze the VOCs collected inside the bag, a thermal desorption (TD) system was employed. The main element in a TD system for breath collection is a stainless steel sorbent tube (3.5" long, 0.25" outer diameter) filled with Tenax®TA (Markes International Ltd., Llantrisant, UK). Collected breath samples are pumped through the TD tubes using an air pump (SKC Inc. Aircheck Sampler 224-44XRMM model). When breath samples are passing through the sorbent, the main components get trapped inside. TD tubes are loaded into the TD system (ATD 400; Perkin Elmer) where the tubes are heated, releasing the trapped compounds into the system using the parameters from Table 1. Compounds are then separated and identified using a GC-MS system (Agilent 6890N coupled to Mass Spectrometer Waters Micromass AutoSpec Premier). The DB-5MS, 30m, 0.25 mmID, 0.25 mm (Agilent Technologies) column temperature was held at 40°C for 3 min and was then increased by 5°C/min to 120°C, followed by ramping at 10°C/min to 220°C. The MS analyses were performed in full-scan mode, using a scanning range of 50–200 amu. The ion source was maintained at 250°C, and ionization energy (EI+) of 70eV was used for each measurement. Compound identification was performed by library match using NIST mass spectral library.

Supplementary material and/or Additional information

We are exposed to a vast amount of volatile organic compounds (VOCs) [1] and most of them are coming from anthropogenic sources: car pollution, household products like cleaning agents, cosmetics, fragrances, solvents from paints, food products that we could find also in our working places among
Fig. 3. Device construction steps: Step 1 (top left); Step 2 (top middle and right); Step 3 (bottom left); Step 4 (bottom middle); Step 6 (bottom right).
Fig. 4. Breath chromatogram from a volunteer collected in the morning.
Table 2

|          | Day1          | Day2          | Day3          |
|----------|---------------|---------------|---------------|
|          | Toluenes     | Xylenes       | Limonenes     | Menthol       | Toluenes     | Xylenes       | Limonenes     | Menthol       | Toluenes     | Xylenes       | Limonenes     | Menthol       |
| Tube 01  | 3153         | 6196          | 1125          | 1061          | 4147         | 18340         | 25040         | 5140          | 4047         | 11784         | 11046         | 5213          |
| Tube 02  | 3819         | 7340          | 1686          | 1045          | 6927         | 21912         | 35898         | 4754          | 9813         | 21715         | 21715         | 7877          |
| Tube 03  | 6835         | 10956         | 1249          | 1038          | 5879         | 30853         | 56652         | 8769          | 5367         | 20859         | 19733         | 6489          |
| Tube 04  | 956          | 1482          | 0 (N.D.)      | 0 (N.D.)      | 9624         | 31862         | 37554         | 8771          | 8131         | 20153         | 16789         | 5099          |
| Average  | 3691         | 6494          | 1015          | 786           | 6644         | 25742         | 38786         | 6859          | 6840         | 21691         | 17321         | 6170          |
| RSD (%)  | 66%          | 60%           | 71%           | 67%           | 35%          | 26%           | 34%           | 32%           | 38%          | 42%           | 27%           | 21%           |

plenty of many more processes [2]. Thousands of VOCs are emitted from the human body daily [3]. Many VOCs or deviations in their levels have been linked to specific diseases. Hence, the VOCs are considered biomarkers and are used for disease diagnose and monitoring [4].

Several techniques are currently available for breath VOC collection and monitoring. Some of them focus on breath collection: Breath samplers like ReCIVA® (Owlstone Medical) or Bio-VOC™ (Markes) [5,6] or sampling bags like Tedlar® (DuPont™), Nalophan®, Cali-5-Bond™, FlexFoil® and Teflon® polymers [7–10]. Other systems analyze breath directly (PTR-MS, GC-IMS, e-noses). However, most of those techniques might be very complex and/or expensive. The aim of the study is to find the easiest, fastest and cheapest method for breath collection, in order to facilitate the sampling from patients that need testing for disease monitoring. As well as find the optimal parameters for screening the whole spectra of VOCs presents in breath, specially the one presents in trace levels with the minimum background interferences.

Another 3 different systems were previously tested before obtaining the last successful version.

1st system for breath sample collection: Face Mask 4 tubes collection system

The first system is basically a face mask (Intersurgical EcoLite™) connected to 4 thermal desorption tubes through a self-made connector and a T-piece directional valve (Intersurgical) (Fig. 5).

The flow of air from the pump was set to 1000mL/min in order to avoid breakthrough of the TD tubes, as the flow would be too fast for the sorbent to capture all the VOCs. However, as the average person breathing is 5L of air/minute, the collected breath passing through the tubes was not fast enough. The excess breath produced had to be collected into an adjacent 2L reservoir bag (Intersurgical) so that the person could keep breathing normally. (Fig. 5)

This first system was tried out for several volunteers from whom their breath samples were collected. Breathing through the mask was uncomfortable for all of them, difficulty of breathing. The quantities of VOCs collected into the 4 tubes were not reproducible (Table 2). This suggests that the flow that goes through each tube is different, probably because the flow is not individually controlled.
Fig. 5. Face Mask 4 tubes collection system (left); self-made TD tube connector (middle); T-piece directional valve (right).
After several minutes of breath collection the adjacent reservoir bag was filled out, making even more difficult the breathing process as the pumping system couldn't handle the high flow rate.

Several blanks were measured by leaving the system connected left on the table, and many different compounds were collected coming from the background, which would interfere with the breath VOCs, many plasticizers and aromatics were coming from the plastics and tubing, as well as from the adjacent bag.
All the tubing, mask, and bag should be either cleansed and conditioned, or new components shall be used for each new participant, making the process even longer and/or expensive.

4 vs 1 TD tubes

Using 4 tubes for breath collection would allow us to take a bigger amount of sample in a faster way. Because the maximum recommended flow for each TD tube is around 250mL/min, using 4 tubes at the same time would allow us to collect 1000mL/min of breath sample. However, as we can control only the total flow from the pump, we cannot know for sure if the flow is divided equally between the 4 tubes, as we could prove in Table 2. That will make the sampling inaccurate, as some tubes will be collecting smaller amounts of sample while others will take a flow higher than the recommended. In addition to that, the sample will be divided into 4 different tubes, needing an extra volume of sample in order to increase the concentration of possible trace compounds. Because of that we considered the option of using only 1 TD tube instead. Even though the time of sampling will be higher, all the compounds will be trapped in a single TD tube, allowing us to detect many more compounds of interest.

2nd system for breath collection system: Face Mask 1 tube collection system

In order to facilitate the breathing collection process for the patients and the reproducibility of the samples, the volunteers were asked to breath until adjacent bag was filled, which is connected to a bypass system in which all the air coming from the volunteer will go straight into the thermal desorption tube. All the air collected into the bag will pass through the TD tube as well, because it can only flow forward thanks to a Anti-pollution T-piece directional valve (Intersurgical).

The breathing system was still very uncomfortable for volunteers, as it was hard to breathe. Contamination coming from bag was not solved as blanks will show different compounds every time. Because it is not a stable background, it cannot be subtracted from the sample. System should be still cleaned and conditioned, or discarded for each person.

3rd system for breath sample collection: Mouth tip 1 tube breathing system

To make the breathing even easier and more comfortable, the mask was exchanged with a mouth tip. Volunteers were asked to inhale through their nose and exhale through their mouth this way filling the bag with exhaled air.

This breathing system is more comfortable for the volunteers. But many compounds were still interfering from the bag, which has to be cleaned or discarded afterwards.

Last system developed: LDPE Minigrip bags

As we solved the problem of comfort, we had still the problem of contamination coming from the system. After the sample collection, the adjacent bag should be thoroughly cleaned or simply discarded, making the process very long or expensive. In order to fix that, we decided to exchange the adjacent bag, and all the tubing for a simpler system. When using the Minigrip plastic bags we could discard the bags after each sample due to its cheap price. However, we discovered that many compounds are coming from it [Fig. 8]. And because of that, we had to condition the bags. To do that, we filled the bag with an inert gas and place them in the oven for 2 hours. After that, we collected the content into TD tubes and analyze it. The inert gases used were argon and nitrogen. Both of them showed similar results, therefore we kept using nitrogen due to its lower price.

After conditioning the bags we could see how the amount of compounds decrease dramatically, obtaining a clean enough background (Fig. 9).

Nevertheless, we conditioned the bag a second time to see if we could improve the background. We could see on Fig. 10 that we need to condition at least twice to get the lowest interference from bag contaminants.
Fig. 8. Chromatogram from Minigrip LDPE bag non-conditioned.
Fig. 9. Chromatogram from Minigrip LDPE bag after 1st conditioning.
Fig. 10. Chromatogram from Minigrip LDPE bag after 2nd conditioning. (Retention times shifted from previous results due to cutting the column).
Fig. 11. Chromatograms of LDPE bags filled with Nitrogen after 24 - 48 - 72h (Green – Red – Purple respectively).
Background study

In order to study the background emissions from the LDPE plastic bag after conditioning and collection of samples, bags were filled with nitrogen gas and left for several hours. The content was collected in order to determine any components coming from the bag.

The LDPE bags, once conditioned, can be used within at least 72h without any new interference coming from the bag materials.

After considering the option of only 1 TD tube, we should then check the collection flow of the sample. Thermal desorption tubes are supposed to handle a maximum flow of 250mL/min. If the flow is higher some compounds might not get trapped inside. For a given volume of 3L of breath sample, we studied the difference of using a flow of 250mL/min and 1000mL/min.

As we can see from Fig. 12, the quantity of compounds trapped in the sorbent decreases when we increase the flow rate, especially for lighter compounds. However, we loss only around 40% for very volatile compounds, around 20% for heavier compounds, and only a 4% of the heaviest, compared to 4 times shorter time to collect the samples. So we could say that it is worth collecting the sample at 1000ml/min which will shorten the sampling time and we will still collect the majority of the compounds present in breath.
And because we are saving time in collecting the sample we could then try to collect a bigger amount of breath sample. Bag sizes of 1 and 3 liters were compared. Refilling the sample again would increase the amount of sample, increasing as well the amount of trace compounds. Refilling the sample up to 6 times was studied, which would allow collecting up to 18 liter of breath, concentrated into the TD tube.

After seeing the results in Fig. 13 we decided that the most time effective volume is 6000mL. Because we obtained enough amount of all range of compounds without saturating the sorbent. Furthermore, we will need to fill the 3L bag for 2 times in order to get the most compounds in the fastest time.

**Stability of VOCs**

Three different 2-liter conditioned bags were injected with 5 μl of Alkane mix standard solution each. The content from the bag was collected after 0, 2, 4 and 6 h.

As we could see from the results, more volatile compounds (C7) will leave the system much faster than less volatiles. It will also depend on the concentration of those compounds.

In addition to that, breath samples were collected from the same person at the same time. The content from the first bag was analyzed just after collection, the rest of the bags was analyzed after 4 and 24 h. The most volatile and abundant compound (Isoprene) was compared.
Fig. 16. Chromatogram from breath samples. Different breath collections: Single breath nose inhale (Red), several breath mouth inhale (green), several breaths after first volume discarded (purple).
The quantity of isoprene decreases more than 50% after keeping the breath sample for 4 hours in the sampling bag. Therefore, we do not recommend using our system for holding the gas content for longer than a few minutes, as very volatile compounds will leak out of the bag.

Breathing mode influences

As bags shall be filled by the volunteers, different breath collections might be obtained. 3 different ways of collecting the breath was studied. As we can see in Fig. 16, first breathing mode was a big inhale through the nose and filling the bag in a single breath (red color chromatogram). The second mode studied was small inhale through the nose and filling the bag in several breaths (green color chromatogram). The final mode (purple color chromatogram) was big inhale through the nose, then discard half of the breath, and collect the final part, repeating until the bag was filled.

Similar number of compounds are obtained using the 3 different methods. However, when discarding the first half of the breath a higher intensity of heavier (less volatile) compounds are detected. As our main objective is to obtain the highest number of compounds possible to be able to study the full spectra of compounds coming from the volunteers, the last method might be preferred.

Diffusion of water vapour from LDPE bag outside the system was observed to be within 30 min after breath collection.

Calibration

We studied the possibility of performing a calibration for VOC quantification, using our self-constructed bags. A standard solution of Pyridine and Furfuryl alcohol mixture was prepared. A calibration from 5 to 100ug/l was performed by injecting the standard through the stopcock valve and filling the bag with nitrogen gas. The content was then collected through the TD tube and analysed obtaining the results showed in Fig. 17.

There seems to be very good linearity when performing a calibration of Pyridine and furfuryl alcohol from 5 to 100 ug/l. However, as we discussed previously, the fast diffusion of the compounds out of the system makes it very inaccurate (Figs. 3, 6, 7, 11, 14 and 15). We recommend using our system for a fast, cheap and easy collection of breath samples, when trapped into a TD tube just after the collection of the sample, and with qualitative purposes (VOC screening), not quantitative.

The list of all the compounds that we were able to identify after collecting the breath of 23 different volunteers at different times of the day is listed is Table 3.
### Table 3

List of VOCs.

| Nr | $T_R$ (min) | Compound                                |
|----|-------------|-----------------------------------------|
| 1  | 0.86        | Acetone                                 |
| 2  | 0.86        | Isoprene                                |
| 3  | 0.94        | Hexane                                  |
| 4  | 1.11        | Octane                                  |
| 5  | 1.21        | Isopropyl acetate                       |
| 6  | 1.13        | Benzene                                 |
| 7  | 1.30        | Allyl methyl sulfide                    |
| 8  | 1.38        | Methyl Propyl sulfide                   |
| 9  | 1.44        | Carbonic acid                           |
| 10 | 1.52        | 1-(methylthio)-1-Propane                |
| 11 | 1.59        | Unknown                                 |
| 12 | 1.73        | Pyridine                                |
| 13 | 1.80        | Toluene                                 |
| 14 | 2.16        | Hexanal                                 |
| 15 | 2.46        | Octanal                                 |
| 16 | 2.27        | Tetrachloroethylene                     |
| 17 | 2.35        | Dihydro-2-methyl-3-furanone             |
| 18 | 2.41        | Butyl Acetate                           |
| 19 | 2.47        | Methyl Formate                          |
| 20 | 2.65        | Methylpyrazine                          |
| 21 | 2.66        | CNHn                                    |
| 22 | 2.78        | Furfural                                |
| 23 | 2.93        | Propanoic acid                          |
| 24 | 3.30        | Furfuryl alcohol                        |
| 25 | 3.20        | Xylene (o,m,p)                          |
| 26 | 3.38        | Hexanoic acid                           |
| 27 | 3.90        | $C_nH_{2n+2}$                           |
| 28 | 4.15        | Aldehyde                                |
| 29 | 4.26        | Butyl Glycol                            |
| 30 | 4.45        | Methyl Formate                          |
| 31 | 4.52        | Acetylfuran                             |
| 32 | 4.56        | Dimethyl Pyrimidine                     |
| 33 | 4.82        | Unknown                                 |
| 34 | 4.91        | $\alpha$-Pinene                         |
| 35 | 5.05        | $C_nH_{2n+2}$                           |
| 36 | 5.20        | Hexadecanol                             |
| 37 | 5.23        | Hexylene Glycol                         |
| 38 | 5.26        | Camphene                                |
| 39 | 5.52        | Propyl benzene                          |
| 40 | 5.76        | m-Ethyl methylbenzene                   |
| 41 | 5.97        | psi-cumene                              |
| 42 | 6.06        | $\beta$-Pinene                          |
| 43 | 6.26        | Ethyl Toluene                           |
| 44 | 6.39        | Unknown                                 |
| 45 | 6.58        | $C_nH_{2n+2}$                           |
| 46 | 6.69        | Mesitylene                              |
| 47 | 6.72        | $\beta$-Myrcene                         |
| 48 | 6.83        | VinylFuran                              |
| 49 | 6.98        | Decane                                  |
| 50 | 7.11        | 3-Carene                                |
| 51 | 7.37        | Dipropylene glycol monomethyl ether     |
| 52 | 7.47        | Unknown                                 |
| 53 | 7.49        | Dipropylene glycol monomethyl ether     |
| 54 | 7.59        | m-Cymene                                |
| 55 | 7.73        | Limonene                                |
| 56 | 7.87        | Eucalyptol                              |
| 57 | 7.91        | $C_nH_{2n+2}$                           |
| 58 | 7.94        | Propanoic acid                          |
| 59 | 7.98        | Decanal                                 |

(continued on next page)
| Nr  | $T_R$ (min) | Compound                                      |
|-----|------------|-----------------------------------------------|
| 62  | 8.01       | Ethylhexanol                                  |
| 63  | 8.09       | $C_{n}H_{2n+2}$                               |
| 64  | 8.13       | 1-Phenyl-1,2-butanediol                       |
| 65  | 8.50       | $C_{n}H_{2n+2}$                               |
| 66  | 8.64       | 4-Carene                                      |
| 67  | 8.71       | (Methyl tridecane) $C_{n}H_{2n+2}$            |
| 68  | 8.87       | $C_{n}H_{2n+2}$                               |
| 69  | 8.93       | Ethyl Methyl Benzene                          |
| 70  | 9.10       | (Methyl decane) $C_{n}H_{2n+2}$               |
| 71  | 9.23       | $C_{n}H_{2n+2}$                               |
| 72  | 9.31       | Dihydro myrcenol                              |
| 73  | 9.41       | Hexahydronerolidol                            |
| 74  | 9.51       | 4-Caranol                                     |
| 75  | 9.59       | $\alpha$-Cumyl alcohol                        |
| 76  | 9.90       | Dihydrocarveol                                |
| 77  | 9.99       | 2-Butyl-1-octanol                             |
| 78  | 10.12      | Linalyl anthranilate                          |
| 79  | 10.08      | Undecane                                      |
| 80  | 10.15      | Nonanal                                       |
| 81  | 10.16      | Bergamioi                                     |
| 82  | 10.46      | Menthadienol                                  |
| 83  | 10.62      | Unknown                                       |
| 84  | 10.83      | Unknown                                       |
| 85  | 11.18      | camphor                                       |
| 86  | 11.52      | Isomenthone                                   |
| 87  | 11.71      | Unknown                                       |
| 88  | 11.86      | Isomenthol                                    |
| 89  | 11.96      | Unknown                                       |
| 90  | 12.16      | Menthol                                       |
| 91  | 12.20      | Unknown                                       |
| 92  | 12.18      | Naphthalene                                   |
| 93  | 12.52      | Unknown                                       |
| 94  | 12.68      | Benzene carboxylic acid                       |
| 95  | 12.87      | Unknown                                       |
| 96  | 13.00      | $C_{n}H_{2n+2}$                               |
| 97  | 13.16      | Decanal                                       |
| 98  | 13.64      | 2-Phenoxy ethanol                             |
| 99  | 14.25      | D-Carvone                                     |
| 100 | 14.63      | Linalyl isobutyrate                           |
| 101 | 14.66      | Oxalic acid, bis(trimethylsilyl) ester         |
| 102 | 15.35      | Isobornyl acetate                             |
| 103 | 15.60      | 4-tert-Butylcyclohexyl acetate                |
| 104 | 15.86      | Tridecane                                     |
| 105 | 16.25      | $C_{n}H_{2n+2}$                               |
| 106 | 16.85      | Oxalic Acid                                   |
| 107 | 17.12      | $\alpha$-Terpinyl butyrate                   |
| 108 | 17.68      | $\beta$-Vinyl napththalene                    |
| 109 | 17.89      | $C_{n}H_{2n+2}$                               |
| 110 | 18.48      | $C_{n}H_{2n+2}$                               |
| 111 | 18.74      | Caryophyllene                                 |
| 112 | 19.12      | Verdyl Acetate                                |
| 113 | 19.27      | Oxalic acid                                   |
| 114 | 20.33      | $\alpha$-Cetone                               |
| 115 | 20.76      | $C_{n}H_{2n+2}$                               |
| 116 | 20.87      | 3-Biphenylol                                  |
| 117 | 22.32      | Oxalic acid                                   |
| 118 | 22.43      | Phthalic acid                                 |
| 119 | 22.78      | Benzophenone                                  |
| 120 | 23.03      | $C_{n}H_{2n+2}$                               |
| 121 | 23.16      | $C_{n}H_{2n+2}$                               |
| 122 | 23.23      | Unknown                                       |
| 123 | 25.36      | Isopropyl myristate                           |
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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