#56 Determination of Organic Acids by GC-FID: On Cartridge Derivatization by Silylating Reagent (MTBSTFA)

Alexander S.C. Pennington
Enthalpy Analytical, LLC, Durham, NC, 27713
74th Tobacco Science Research Conference, August 29 – September 1, 2021, Boston, MA, USA

Introduction

Today, ENDS devices are largely split between freebase nicotine formulations and nicotine salt formulations. Nicotine salt formulations are used in order to alter the taste and mouth-feel of the delivered aerosol and can be made from a variety of salts like nicotine-lactate or nicotine-benzoate. Along with the phenomenon of altering the perceived taste of nicotine, organic acids can also be components in flavored formulation for ENDS products. Due to the prevalence of organic acids in ENDS products, the US Food and Drug Administration (FDA) have started to add organic acids to the list of harmful and potentially harmful constituents (HPHCs). The levels of organic acids in ENDS liquids and aerosols, in some cases, can be very high and there are a wide range of organic acid compounds that can be used to create a variety of desired effects in regards to nicotine taste. Accurate and efficient methods that are able to detect a wide range of these organic acid compounds will be important in determining the risk involved when these acids are added to the ENDS formulations. Ideally, this would involve a method that can provide analysis of organic acids both at the low and high detection limits simultaneously while maintaining accurate response and resolution of all compounds. Developed here is a method that does just that, with the ability to provide accurate analysis for 15 or more organic acid compounds over a total calibration range of 0.4 µg/mL to 1000 µg/mL and complete resolution of all commonly utilized organic acid compounds.

Method Details

Traditionally, organic acid analysis is performed by IC using an aqueous mobile phase and separation with an anion exchange column. This allows for low levels of detection; however, many of the common organic acid compounds tend to coelute, and the average run time for the analysis is 70 minutes. As an alternative, we took the concept of anion exchange and applied it to a preparatory method that could generate a sample matrix that was appropriate for GC analysis. Being able to perform the analysis by GC, we were able to both reduce the overall run time of the analysis to 25 minutes and improve the observed resolution between common coeluting compounds. This included a derivatization step to transform the acid compounds into more volatile derivatives that would also give a stronger signal by FID and allow for the same low detection limit, as observed by IC, to be maintained. The concept of anion exchange was applied in order to aid in the trapping of the organic acid components as a silylation derivatization scheme was found to be the most useful in the derivatization of carboxylic acid compounds. Weak anion exchange cartridges are used in combination with a vacuum manifold to trap the carboxylic acid compounds. Then a silylation reagent, n-tert-butyldimethylsilyl-n-methyltrifluoroacetamide (MTBSTFA), is added in order to derivatize the acids on the cartridge and allow for elution by an aprotic organic solvent in the form of acetonitrile.

Analyte	IC Method RT (min)	GC Method RT (min)	IC Method LOQ (µg/mL)	GC Method LOQ (µg/mL)
Acetic Acid	12.3	5.7	0.5	0.4
Propionic Acid	14.1	6.7	0.5	0.4
Levulinic Acid	NA	9.7	NA	0.4
Lactic Acid	11.8	10.9	0.5	0.4
Benzoic Acid	40.3	11.1	0.5	0.3
Oxalic Acid	48.1	11.7	0.5	0.4
Malic Acid	41.4	18.0	0.5	0.4
Citric Acid	54.6	20.4	0.5	0.4

Table 1 – Comparison of retention time (RT) and method quantitation limits (LOQ) between the standard IC method for organic acid analysis and the GC-FID method for organic acid analysis.

"On Cartridge" Derivatization Preparation

Calibration standards and samples are both diluted and/or trapped into acetonitrile before being extracted with the anion exchange cartridge. The cartridges used are Phenomenex Strata X-AW polymeric weak anion exchange cartridges. They are pre-washed with acetonitrile to wet the cartridges and an aliquot of the standard/sample is added (usually 1 mL) and allowed to elute through the anion exchange cartridge. The cartridges are then washed twice with 2 mL of acetonitrile to ensure that only the carboxylic acid compounds are retained. The next step is critical to the success of the derivatization; a vacuum is applies to the cartridges to remove all excess liquid that has also been retained during the washing step. It is important to only pull vacuum until the excess liquid is removed and then the vacuum must be released. This step has the chance to condense water into the cartridges if the vacuum is pulled for an extended period of time and this condensed water can negatively impact the derivatization of the carboxylic acids. After excess liquid is removed the silylation reagent (MTBSTFA) is added to each cartridge in two aliquots of 100 µL and left to derivatize for at least 1 hr. Once derivatization has been complete, 800 µL of acetonitrile and vacuum being pulled on the cartridges is used to elute the acid compounds. The extracts are analyzed by GC-FID using a Restek 5sil-MS column. Each calibration compounds regression is quadratic with a coefficient of determination of at least 0.995.

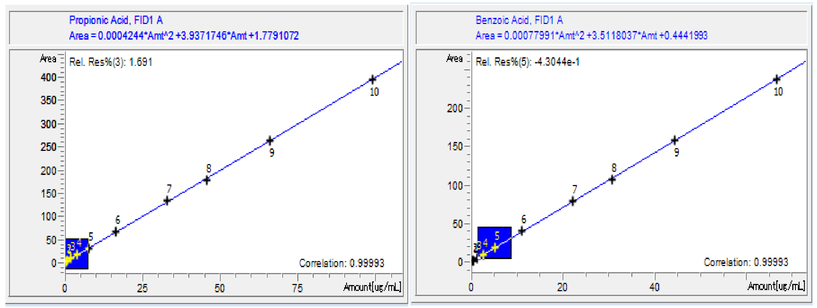

Figure 1 – Example calibration curve of Propionic Acid and Benzoic Acid.

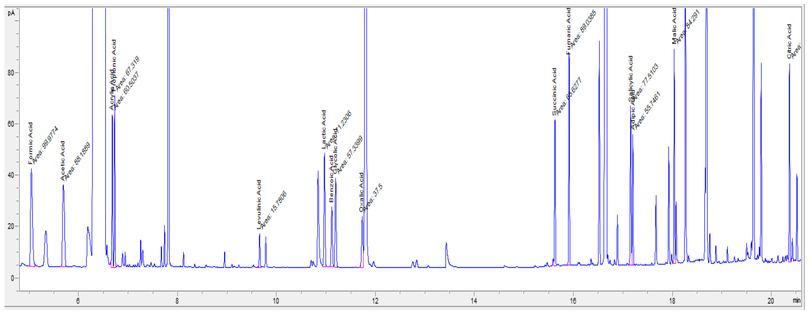

Figure 2 – Chromatogram of Standard 6 of Organic Acids.

Analyte Recovery

Commercial samples were not available at the required concentrations, for all organic acids tested, for use as validation samples. The samples used in the validation were prepared from spiking an E-liquid base (50:50 PG:VG, 6% Nic) with the required concentrations of all validated organic acids. Analyte recovery was determined using five samples prepared on reach of three analysis days. The average percent recoveries are listed in the table below. The average percent recovery across all replicates was 80%-120%. ENDS devices were also used to produce aerosol samples for validation of the organic acids. These were prepared by spiking the trapped aerosol matrix of an e-liquid base (50:50 PG:VG, 6% Nic) with the required concentrations of all validated organic acids. The same criteria were used for aerosol validation as for e-liquid validation, and the percent recovery across all replicates was also 80%-120%.

Analyte	Day 1 Ave %Rec.	Day 2 Ave %Rec.	Day 3 Ave %Rec.	Cumulative Ave %Rec.
Formic Acid	100	104	105	103
Acetic Acid	102	105	106	105
Propionic Acid	101	103	104	103
Levulinic Acid	82	112	99	98
Lactic Acid	101	103	104	103
Benzoic Acid	101	103	104	103
Glycolic Acid	101	103	105	103
Oxalic Acid	74	80	83	79
Succinic Acid	99	101	104	101
Salicylic Acid	98	95	97	97
Malic Acid	101	103	104	103
Citric Acid	98	99	101	100

Table 2 – ENDS E-liquid analyte average percent recovery.

Figure 3 – Vacuum manifold, Strata X-AW anion exchange cartridges, MTBSTFA reagent, and extract.

Method and Instrument Precision

Method precision for e-liquid analysis was determined using five replicate samples prepared on three analysis days. Intraday precision was within 5% for all analytes. Interday precision was within 6% for all analytes except for Levulinic acid which was within 13%. Levulinic acid is a reactive acid and has a short stability window leading to the variability seen in the validation. Method precision for aerosol analysis was also determined using five replicate samples. Aerosol was generated using an in-lab prepared e-liquid sample spiked with the organic acids. This showed much more variation in the intraday and interday precision which can be attributed variability in the devices that were used to generate the aerosol. Variability in aerosol generation may also be attributed to the poor aerosol transfer of organic acids which is about 47% on average. Instrument precision was determined using five mid-level calibration points. A level six standard was injected five times over a period of three days for a total of fifteen data points. Intraday precision was within 1% for all analytes. Interday precision was within 3% for all analytes except for Levulinic acid which was within 13%.

Conclusion

The purpose of this development was to extend the list of organic acid analytes that are able to be quantitatively analyzed, and to significantly reduce the run time compared to the current IC analysis. Since organic acids are comprised of a carboxylic acid functional group, typically they would not be a good candidate for GC-FID analysis. This was overcome by the use of a silylation reagent (MTBSTFA) that allows for the increase in signal response of the organic acid derivatives, and also allows for the analytes to become more volatile making them more suitable for GC analysis. The silylation reagent that is being used for this analysis does not specifically react with only carboxylic acid groups. It targets any acidic hydrogen that is bound to an oxygen, which includes any –OH group that is found on any molecule. This is overcome by the use of the anion exchange cartridges to trap the organic acids and to use the cartridge as a "surface" to facilitate the derivatization of the organic acid analytes by the silylation reagent. By using this derivatization scheme, the organic acids are able to be derivatized without having any reaction interferences caused by other compounds that readily react with carboxylic acids. With the improved resolution and shortened run time by using GC instrumentation, the list of organic acid compounds was expanded to include 15 compounds while the total run time was cut from 70 minutes per injection to 25 minutes per injection. The validation criteria set for the calibration curve linearity, instrument precision, and analyte recovery were within all acceptance criteria bounds. Furthermore, a direct comparison was made between the method described and the industry standard method of IC. The comparison showed that the organic acid by GC-FID method generated data that was no more than 5% different from the IC method. Having met all the acceptance criteria set with this validation, this method is fit for purpose.

References

1. "Trace Analysis of Volatile Organic Acids with the Agilent J&W DB-624UI GC Column" Agilent.com. Agilent Technologies. Web. July 31, 2012.
2. "Strata-X Polymeric SPE" testing.portlab.ru/sites/default/files/spe_strata-x_ordering_information.pdf. Phenomenex. Web.
3. "Gradient Separation of Organic Acids and Inorganic Anions Using the IonPac AS-11 Column." Dionex.com. Dionex Corporation. Web. Feb.-Mar. 2011.