Development of Enhanced Collection and Extraction Techniques for *In Vitro* Toxicology Testing of Next Generation Nicotine Products

P. Kosachevsky[1,2], *B. Coffa*[3,2], *K. Aleksa*[2], and D. Iorgulescu[2].
[1]Vitronix LLC, Richmond, VA; [2]Labstat International Inc., Kitchener, ON, Canada; and [3]ToxPharm LLC, Mechanicsville, VA.

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Overview

Testing aerosol-collected condensate from Next Generation Nicotine Products (NGNPs) such as Electronic Nicotine Delivery System (ENDS) or Electrically Heated Tobacco Products (eHTP), in *in vitro* toxicology assays is limited by OECD guidelines limiting the maximum allowable solvent levels. Higher dose testing requires more concentrated condensates but generating these in sufficient volume for testing in *in vitro* assays can be challenging and time consuming. This study presents the development and characterization of an optimized aerosol collection method using a rotary smoke machine and enhanced extraction methodology for NGPNs.

Study Goal

Develop and validate an optimized collection and extraction method for NGNPs by:

- Modifying the rotary smoke machine for ENDS and HTP products, enabling non-horizontal device orientation.
- Reducing aerosol losses and collection time by using multiple pads in custom holders.
- Enhancing extraction recovery through improved shaking and filtration techniques.

Method

A commercially available puff-activated ENDS pod system and an eHTP product were used to generate aerosol. The particulate phase or aerosol collected mass (ACM) and gas vapor phase (GVP) fractions were collected by passing the aerosol through a Cambridge Filter Pad (CFP), followed in series by a fritted impinger containing a trapping solution (20 mL of CMF-PBS or ethanol (EtOH)), chilled in an ice bath (~0° C). The collected aerosol fractions, ACM on the CFP, and GVP in impinger can be tested separately in *in vitro* toxicology assays by extracting ACM from the pad with DMSO. Alternatively, they can be tested together as whole aerosol (WA) condensate by extracting the CFP with ACM in the impinger content (EtOH), which captures GVP. Aerosol was generated using a Borgwaldt RM20D rotary smoking machine by sequentially puffing on five ENDS devices or ten eHTP devices. The particulate phase (ACM) was captured by using two systems:

1. **Reference (standard) collection:** A single 92 mm CFP for both ENDS and eHTP devices. (**Figure 2 and Figure 4**).
2. **Optimized collection: (a)** ENDS - five 44 mm CFPs in custom-made holders, fitted next to ENDS device mouthpiece and connected to the rotary ring (**Figure 3**). **(b) eHTP -** ten 30 mm CFPs in custom-made holders, fitted next to tobacco heated sticks (THS) and connected to the rotary ring (**Figure 5**).

The trapping capacity of these different CFP configurations was assumed to be comparable based on total filter pad surface area (five (5) 44 mm CFPs = 76.0 cm², ten (10) 30 mm CFPs = 70.7 cm², and one 92 mm CFP = 66.5 cm²). Device weight loss (DWL) and ACM were measured gravimetrically.

Method - Electronic Nicotine Delivery System (ENDS)

The ENDS device connector on the rotary machine was modified to allow ENDS devices to be loaded at an ~30° downward angle from horizontal, equivalent to the 4 o'clock position (**Figure 1**).
Puffing was conducted under 'Standard' regime as specified in ISO 20768:2018 (CRM81), with a 55.0 mL puff volume, 3.0-second puff duration, 30.0-second puff frequency (**Figure 2 and Figure 3** - simplified schematic of the rotary smoking machine setups for ENDS).
To prevent device overheating, puffing was performed in blocks of 50 puffs per device. Puffing continued until a target ACM of at least 3000 mg ACM was accumulated (~300 puffs or ~60 minutes per collection), using either a single 92 mm CFP or a combination of five 44 mm CFPs.

Figure 1. ENDS smoking rotary machine setup

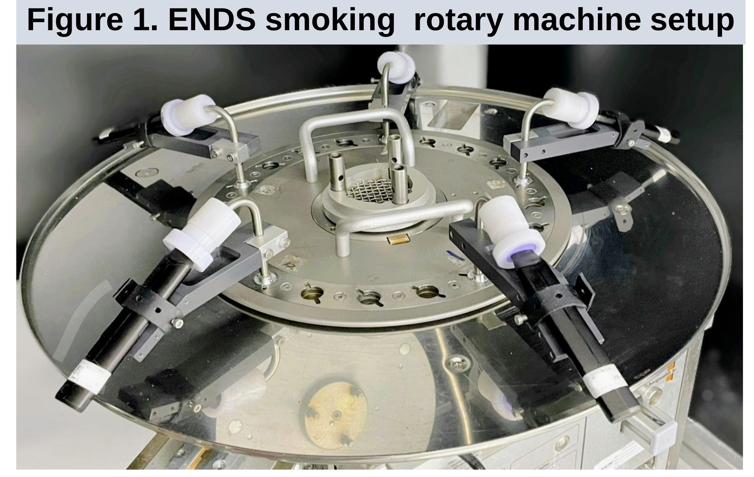


Figure 2. Reference Collection (ENDS)

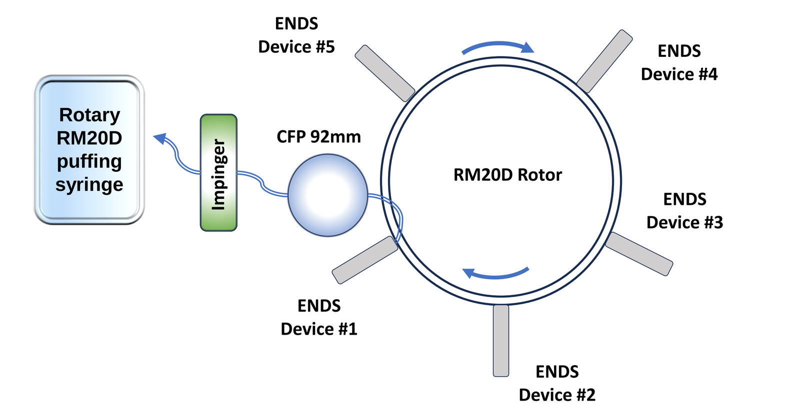
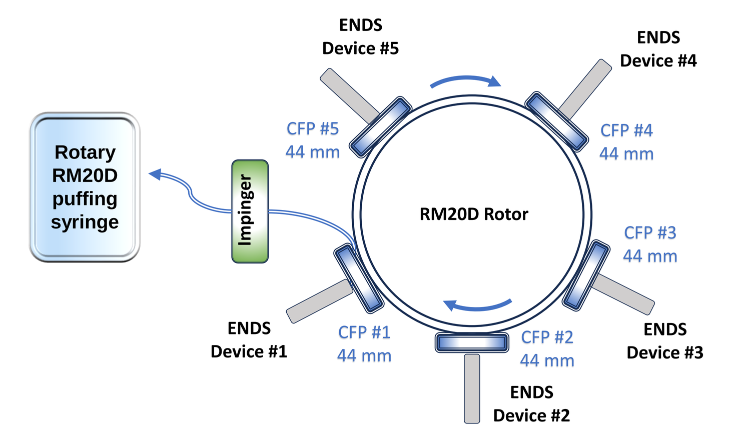


Figure 3. Optimized Collection (ENDS)



Method - Electrically Heated Tobacco Products

A total of 80 eHTP sticks were vaped in a horizontal orientation, with a predefined number of puffs per tobacco heated stick (THS). (**Figure 4 and Figure 5** - simplified schematic of the rotary smoking machine setups for eHTP). HTP conditioning and testing were performed under 'Standard' conditions, following a puffing regime with a 2.0-second puff duration, 30.0-second puff frequency, and a wave puff profile as specified in ISO 5501-1.

Figure 4. Reference Collection (eHTP)

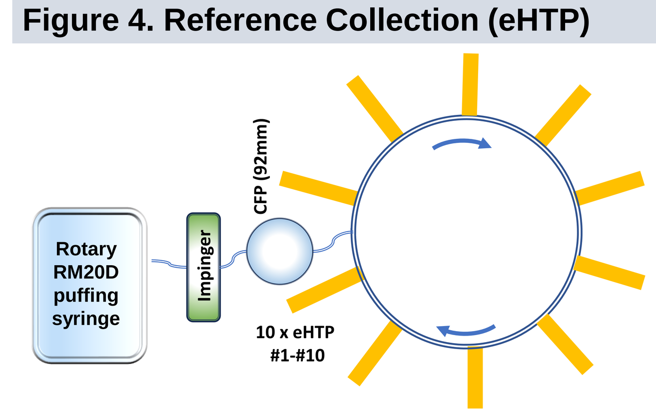
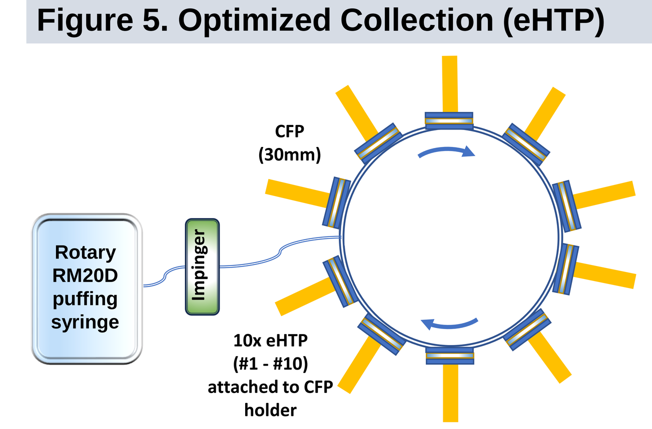


Figure 5. Optimized Collection (eHTP)



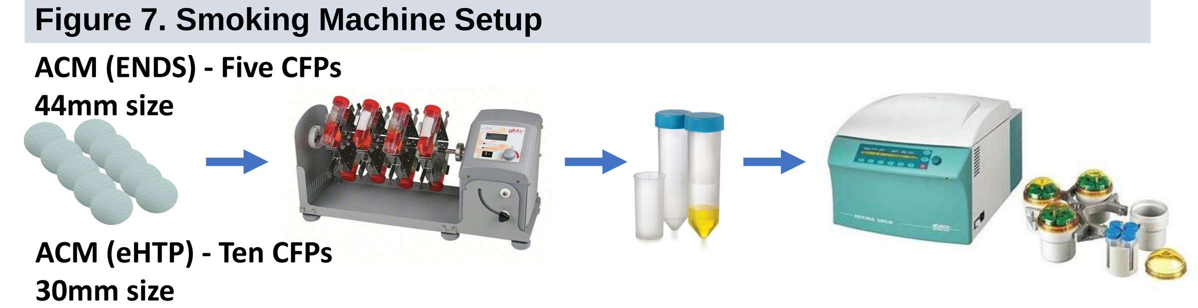
Method - Extraction

The extraction was performed using two methods:

1. **Reference (standard) extraction** procedure as per Health Canada Official Method T-501 (**Figure 6**), which involves an Erlenmeyer flask with a wrist shaker followed by cheesecloth filtration.
2. **Optimized extraction** procedure (**Figure 7**), utilizing a disposable sterile Falcon tube with rotary shaker and centrifuge filtration of the pad remains.

Figure 6. Smoking Machine Setup

Figure 7. Smoking Machine Setup



The ACM collected on either a single 92 mm pad or a combination of five 44mm pads for ENDS and ten 30mm pads for HTP was extracted in an adjusted amount of DMSO (~15 mL for ENDS / 18-24 mL for HTP) to achieve target concentration of 200 mg ACM/mL for ENDS and 100 mg/ACM for HTP. For whole aerosol condensate generation in ENDS, the CFP was extracted in the impinger content, which contained 20 mL of ethanol used to trap the GVP, to reach a target concentration of at least 150 mg ACM/mL.

Results ENDS

Aliquots of the ACM extract in DMSO and EtOH were used to determine **nicotine and glycerol** by gas chromatography (GC) with flame ionization detector (FID).
The **nicotine and glycerol** results from DMSO and EtOH extracts, collected and extracted using standard Reference and Optimized methods, demonstrate that the modifications do not affect the ACM extract formulation. Both collections and extraction methods produced comparable nicotine and glycerol levels, with percent differences up to 2%. (**Figure 8 and Figure 9**).

Figure 8. Results of nicotine (ENDS)

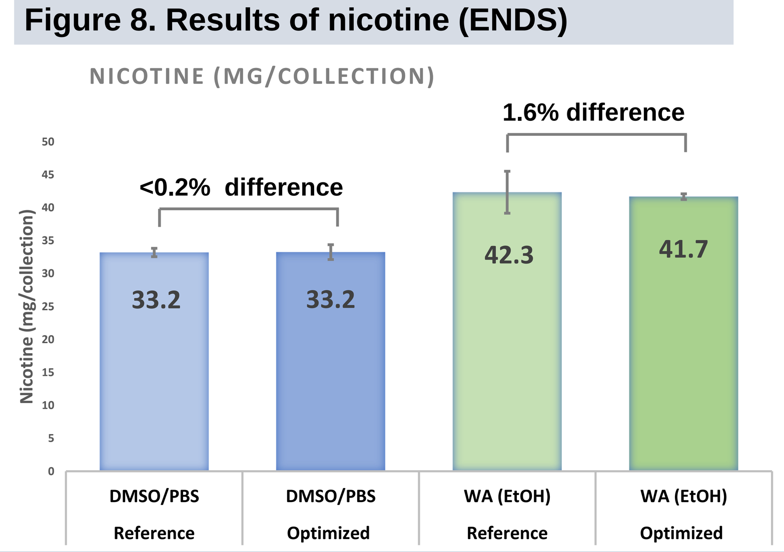
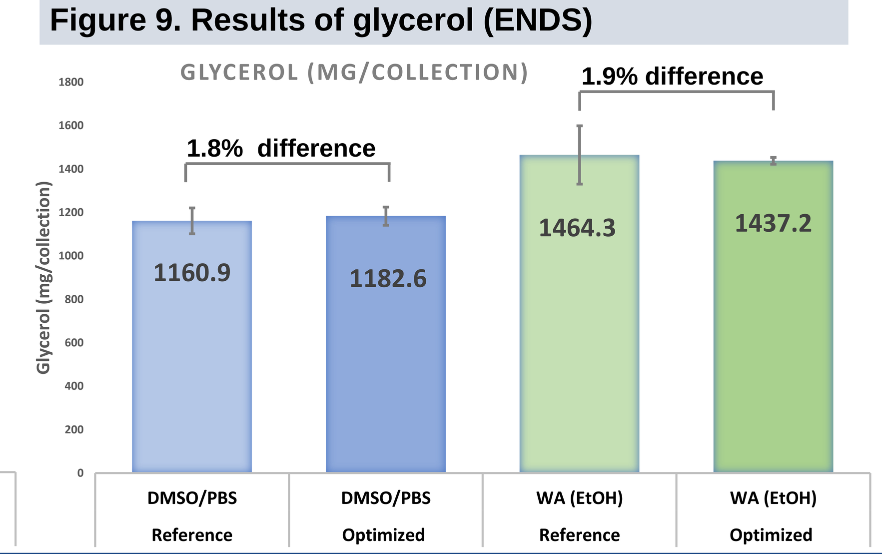
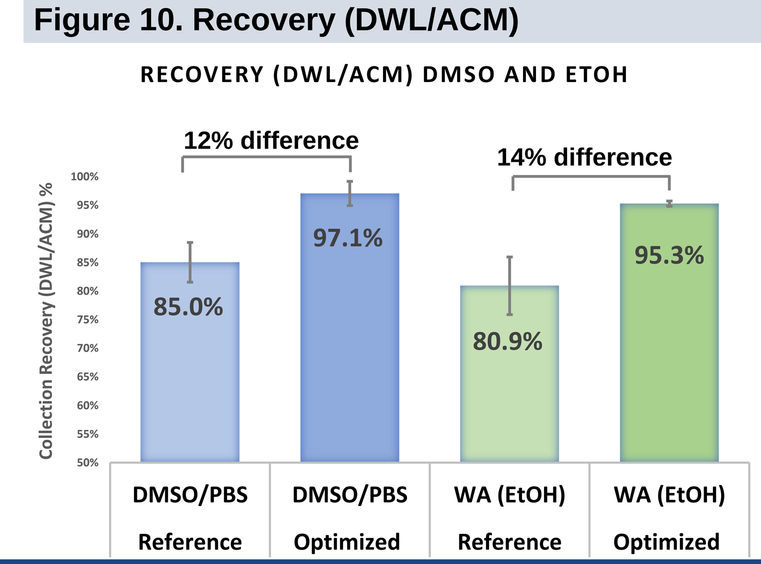


Figure 9. Results of glycerol (ENDS)



The optimized collection method demonstrated improved ACM collection efficiency and reduced aerosol losses. DWL/ACM recovery showed a 12% improvement for the DMSO-PBS matrix and a 14% improvement for the whole aerosol (EtOH) compared to the reference method (**Figure 10**).

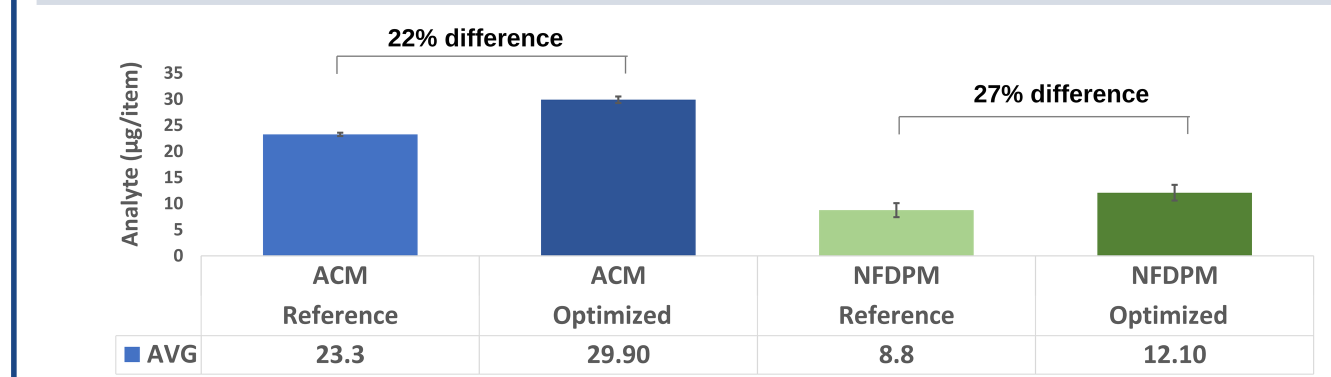
Figure 10. Recovery (DWL/ACM)



Results eHTP

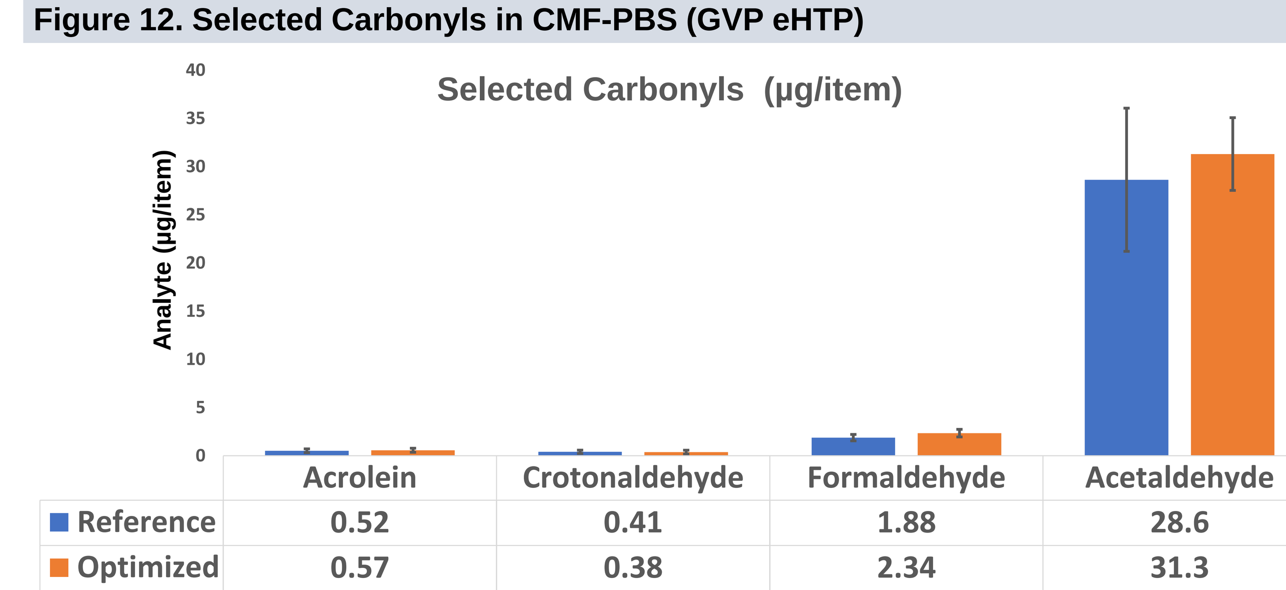
A subset of pads was extracted with IPA solution and analyzed for nicotine, glycerol, propylene glycol, and menthol using GC-FID and GC-TCD.
The optimized collection method resulted in a 22% increase in ACM and a 28% increase in nicotine-free dry particulate matter (NFDPM) while preventing water condensation in the aerosol train compared to the reference method (**Figure 11**).

Figure 11. Results ACM and NFDPM – Reference and Optimized collection (eHTP)



An aliquot of the CMF-PBS (GVP) was extracted, derivatized, and analyzed for selected **carbonyls** using GC/MS (SIM mode) with an RTX-5ms column. Carbonyl results from both collection methods were comparable, with percent differences of 8-20%, depending on the analyte (**Figure 12**).

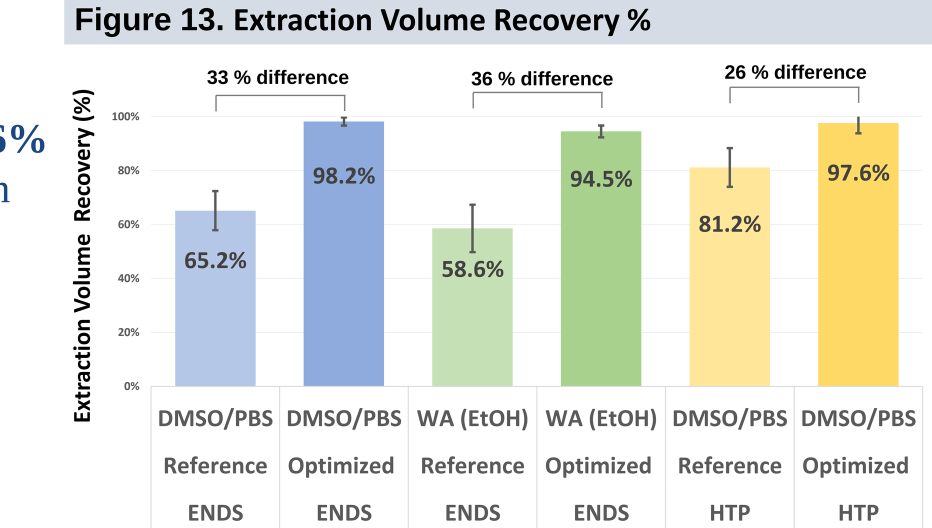
Figure 12. Selected Carbonyls in CMF-PBS (GVP eHTP)



Optimized Extraction

The optimized extraction method demonstrated a **26-36% increase** in extraction recovery volume compared to the reference method (**Figure 13**).

Figure 13. Extraction Volume Recovery %



Summary & Conclusions

1. The optimized collection process improved efficiency, reduced collection time, and enabled rapid collection of high ACM.
2. Centrifuge filtration increases extraction recovery volume by up to 36%, allowing for faster condensate generation.
3. Using a rotary shaker with disposable sterile Falcon tubes enhanced sterility.
4. The rotary smoke machine is a benchmark for toxicology collection and the only commercially available GLP-compliant solution. While originally designed for cigarettes, simple modifications allow its use for ENDS and eHTP devices, avoiding the need for extensive validation required for custom aerosol generation instruments.