Recommendations for Heated Tobacco and Herbal Product Aqueous Aerosol Extracts (AqE) Generation with Quality Control Measures

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Introduction

Aqueous aerosol extracts (AqEs) of heated tobacco product (HTP) and herbal heated product (HHP) emissions are one of the aerosol collection methods applied for toxicological assessment. Currently, there is a lack of a standardised approach in the generation and chemical characterisation of AqEs. This creates a great challenge for the reproducibility and interpretability of toxicological assessment data. This work offers a standardised approach for the generation, collection, and chemical characterisation of AqEs from HTP and HHP emissions.

Methodology

Test Articles

AqEs test articles were created by generation and collection of aerosol from different variants of HTP and HHP.

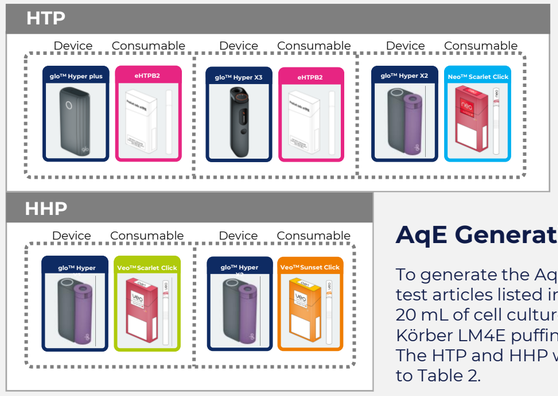


Figure 1: Test products (device and consumable).

A schematic of the HTP/HHP used in this study is shown in Figure 1 with additional details listed in Table 1.

eHTPB2 is an HTP Reference Stick developed by BAT.

Table 1. Products used in this study.

Product Category	Device/Profile	Consumable Name
HTP	glo™ Hyper plus Standard mode	eHTPB2 (HTP Reference)
HTP	glo™ Hyper X3 Standard mode	eHTPB2 (HTP Reference)
HTP	glo™ Hyper X2 Standard mode	Neo™ Scarlet Click
HHP	glo™ Hyper X2 Standard mode	Veo™ Scarlet Click
HHP	glo™ Hyper X2 Standard mode	Veo™ Sunset Click

Parameter	HCIm
Puff volume (mL)	55
Puff frequency (s)	30
Puff durations (s)	2
Vent blocking (%)	0%
Reference(s)	CORESTA [2]

Table 2. Puffing regime used to generate the AqEs from the products used in this study.

AqE Generation Workflow

To generate the AqE, the aerosol from the test articles listed in Table 1 was trapped in 20 mL of cell culture medium using a Körber LM4E puffing machine (Figure 2). [1] The HTP and HHP were puffed according to Table 2.

A step-by-step AqE generation workflow is displayed in Figure 3.

1. Device performance test

Tobacco heating devices (THDs), glo™, were checked for aerosol collected mass (ACM) delivery onto a Cambridge Filter Pad (CFP) to ensure consistent and optimal performance (Figure 2).

2. AqE Control Chart Generation

AqEs were analysed for nicotine via liquid chromatography-mass spectrometry (LC-MS). [3] Individual control charts were generated for each HTP and HHP variant based on the nicotine content of their AqEs.

AqE Generation Workflow (continued)

3. AqE test article generation for *in vitro* testing

AqE samples were generated and validated against the nicotine concentration acceptance ranges as defined by the established control charts.

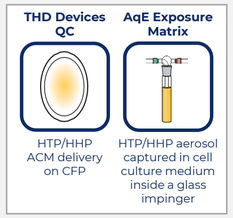


Figure 2: Types of aerosol capture methods. Left: ACM on CFP. Right: Aqueous aerosol extract (AqE).

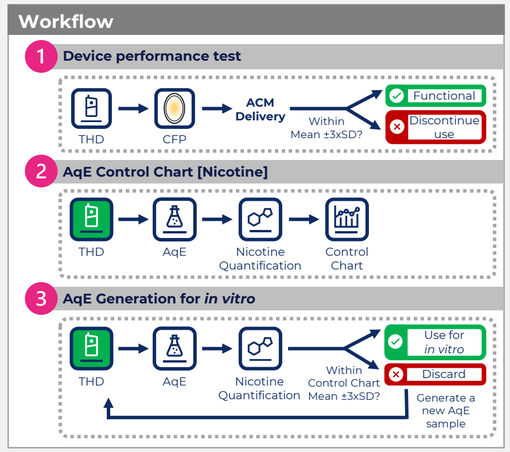


Figure 3: AqE generation workflow. (1) Device performance test pre-AqE generation; (2) AqE control chart creation based on nicotine concentration quantified by LC-MS (Mean ±3xSD); (3) AqE test article generation for *in vitro* testing.

Results

Device Performance Testing

All THDs, glo™, were assessed prior to being used for any AqE generation (control charts and *in vitro* samples). For each individual device (assigned a unique Device ID), ACM delivery onto a CFP was assessed on at least three independent occasions. The CFP Mass Increase (mg) data collected across all devices of a specific type, was used to calculate the mean ± 2 and ±3 standard deviations. This was used as a measure of device performance. Figure 4 illustrates the device performance testing results for the THDs used in this work.

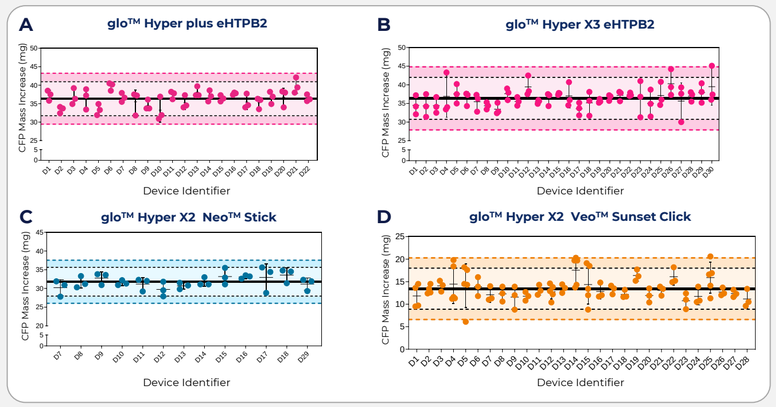
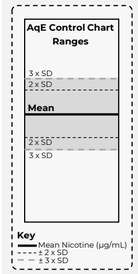


Figure 4: Device performance testing assessed by aerosol collected mass (ACM) delivery onto a Cambridge Filter Pad (CFP). ACM delivery is expressed as CFP Mass Increase (mg). For each THD group, mean CFP Mass Increase (black solid horizontal line) and ± 2 (black dashed horizontal line) and ±3 standard deviations (coloured dashed horizontal line) were calculated. (A) glo™ Hyper plus device with eHTPB2 consumable; (B) glo™ Hyper X3 device with eHTPB2 consumable; (C) glo™ Hyper X2 device with Neo™ consumable; (D) glo™ Hyper X2 device with Veo™ Sunset Click consumable.

AqE Control Charts

Following ensuring that all THDs were performing optimally based on ACM delivery, AqE control charts were prepared to determine acceptable ranges of nicotine concentrations (in µg/mL) for AqE samples generated from the HTP and HHP variants.



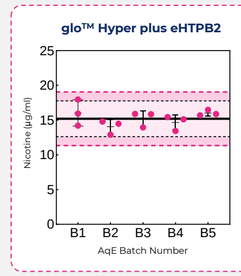


Figure 5. AqE Control Chart on glo™ Hyper plus device with eHTPB2 consumable.

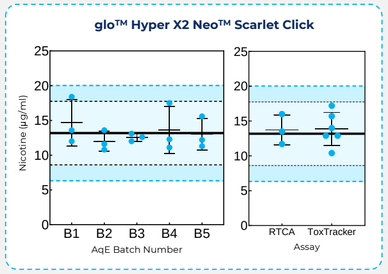


Figure 6. AqE Control Chart on glo™ Hyper X2 device with Neo™ Scarlet Click consumable (left) and AqE samples generated for *in vitro* assay testing (right).

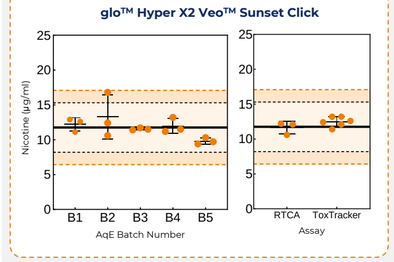


Figure 7. AqE Control Chart on glo™ Hyper X2 device with Veo™ Sunset Click consumable (left) and AqE samples generated for *in vitro* assay testing (right).

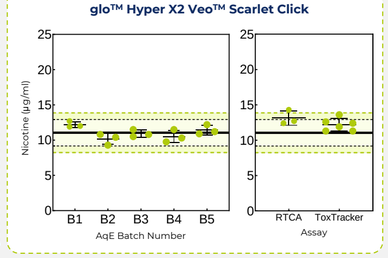


Figure 8. AqE Control Chart on glo™ Hyper X2 device with Veo™ Scarlet Click consumable (left) and AqE samples generated for *in vitro* assay testing (right).

Control charts for each HTP/HHP variant* were comprised of 3 extracts created on 5 independent occasions to calculate the mean ± 2 and ±3 standard deviations based on Nicotine (µg/mL). AqE samples were generated and validated to be in line with the acceptance ranges as defined by the established control charts.

* Each unique combination of device and consumable is considered a different HTP/HHP variant in the context of AqE Control Charts.

Conclusion

The sensitivity of the nicotine quantification was sufficient to distinguish between different aerosols within the HTP and HHP categories. The standardised AqE generation process in combination with the established quality control (QC) measures allowed for the robust and reproducible AqE test article generation and characterisation.

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Contacts

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