DEVELOPMENT OF A BIOLOGICALLY-REPRESENTATIVE LABORATORY ANALYSIS SYSTEM FOR THE MEASUREMENT OF EMISSONS FROM SNUS DURING USE

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INTRODUCTION

The 2014 revision of the European Commission Tobacco Products Directive refers to the reporting of emissions from tobacco products, including substances released during the process of using smokeless tobacco products[1]. We have previously reported one approach to determining extraction of a range of constituents from different snus products during use by consumers and data from studies employing this approach[2-6]. However, such studies can be costly and time-consuming, and as such the availability of a laboratory-based analysis system to model the extraction of constituents from snus to the same extent as that seen in human studies would be beneficial.

The development of the laboratory approach we present here has been guided by human extraction data for a number of constituents, obtained over a typical 60-minute usage period (previously shown to be the median usage duration of a snus pouch). The method was developed to be biologically representative. The importance of flow rate as well as amount and composition of the material used to represent the buccal mucosa was assessed.

MATERIALS AND METHODS

A flow system capable of holding a snus pouch in position and allowing the ingress of fresh extracting media (to represent human saliva) into the pouch, and egress of used media from the system, was constructed. The system was housed in a transparent incubator (Bibby Scientific, model SI60D) maintained at body temperature (37°C) and fed with artificial saliva[7] via a peristaltic pump (Cole Parmer, model 07523-90 with pump head (07519-20) and cartridge (07519-85)). An extraction period of 60 minutes was employed, with the snus pouch held between two layers of borosilicate glass beads (10g per layer = 96 beads per layer).

Levels of constituent extraction from Lucky Strike Original (brown) Swedish snus pouches were determined by analysing each pouch for the analyte of interest after extraction and comparing with the analyte content of an unused pouch, using previously-reported methods[2]. The levels of constituent extraction obtained were compared to a set of extraction values obtained with snus users during real world use of the same product[2]. These values are shown in Table 1.

Table 1: Reference extraction data (human use) used to guide development of the laboratory method

Constituent	Amount in Product	% Extracted
Nicotine	9.6 ± 0.90 mg/pouch	33.3 ± 9.86
NNN*	344.4 ± 29.79 ng/pouch	35.6 ± 8.46
NNK*	191.8 ± 19.66 ng/pouch	37.8 ± 8.10
Linalyl Acetate	150.1 ± 12.30 µg/pouch	19.2 ± 13.49
Linalool	148.8 ± 12.87 µg/pouch	30.4 ± 9.65

*NNN = N'-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

EFFECT OF FLOW RATE

Initial testing with artificial saliva at flow rates between 0.1 and 1 mL/min clearly demonstrated significant over-extraction of nicotine in comparison to the reference data. Lower flow rates were subsequently examined (Figure 1).

It was found that nicotine extraction was controlled at lower flow rates. A flow rate between 0.01 and 0.03 mL/min would be expected to represent the reference data for nicotine most closely. However, it was also clear that linalyl acetate and linalool remained under-extracted at these flow rates compared to the reference data.

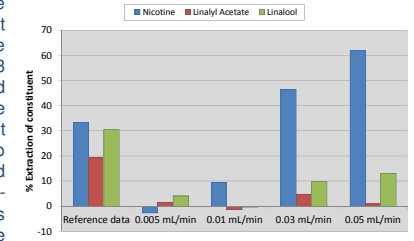

Figure 1: Effect of flow rates on constituent extraction

EFFECT OF BEAD MATERIAL

Given the findings above, it was considered that the lipophilic nature of the buccal mucosa (likely to be a key driver for extraction of sparingly-soluble constituents such as linalyl acetate and linalool) may need to be integrated into our extraction system. The effect of replacing the glass beads with beads manufactured from three different lipophilic materials was evaluated. It was found that the most promising of the materials tested was nitrile rubber (Figure 2).

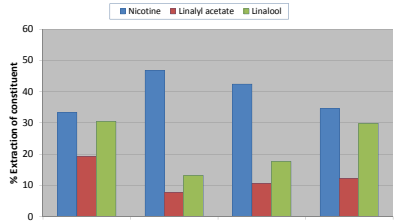

Figure 2: Effect of bead material on constituent extraction

REPEATED TESTING

Employing a flow rate of 0.02 mL/min and using nitrile rubber beads (The Precision Plastic Ball Company, part # BN0156070A) to represent the human gums, thirty pouches were extracted for 60 minutes each using the flow system and analysed for the extent of extraction of nicotine, linalyl acetate, linalool, NNK and NNN. The results were compared to the reference data (Figure 3).

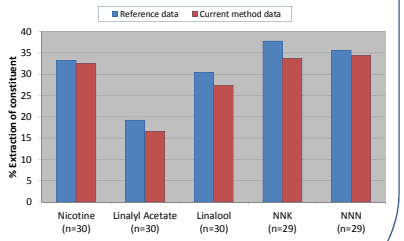

Figure 3: Mean values from repeated testing compared to reference data

SUMMARY OF CURRENT METHODOLOGY

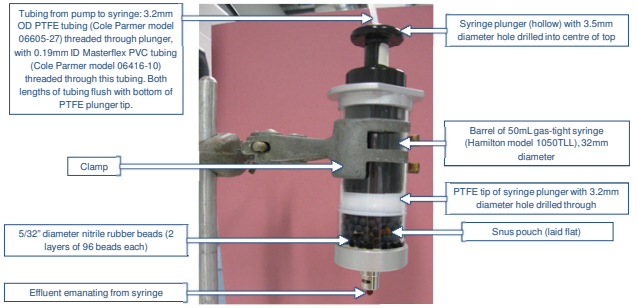

Figure 4: Snus extraction system

The system (Figure 4) is maintained at 37°C in a transparent incubator. The snus pouch is laid flat between the two layers of nitrile rubber beads (96 beads per layer) and extracted with artificial saliva (0.02 mL/min) for 60 minutes, before being removed and analysed for extent of extraction.

NEXT STEPS

Recommended next steps include determining whether similar extraction is obtained using a simpler, less costly extraction media (such as an amylase solution) in place of artificial saliva; the identification of a research grade material to represent the human gums in place of nitrile rubber; assessment of the performance of the method against a wider range of analytes and snus products; and to fully assess repeatability and reproducibility.

CONCLUSIONS

The methodology was shown to achieve levels of extraction of nicotine (33%), NNN (34%) and NNK (34%) from snus pouches similar to that observed with consumers (33%, 36% and 38%, respectively). Additionally the extraction of two sparingly-soluble constituents, linalool and linalyl acetate, was shown to represent human extraction data. The technique appears to be of value from both product development and regulatory perspectives, and validation against further human data for other products and constituents may confirm its potential utility in the reporting of emissions from this product category representative of everyday consumer use.

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2014 CORESTA Congress – Québec City, Canada: 12-16th October 2014 **Poster Number** STPOST26