Analysis of Tobacco Constituent Extraction by Snus Users

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Introduction:

- A better understanding of exposure and intake of tobacco constituents during snus use can provide valuable insights into its potential effects.
- A variety of techniques have been employed to assess exposure, from biomarkers for NNK and nicotine, to the use of in vitro extraction systems to examine toxic metals.
- These approaches are restricted by either the limited range of available biomarkers, or difficulties in ensuring that in vitro systems mimic real-world use.
- A versatile alternative approach is chemical analysis of the snus portion before and after use to determine the level of constituent extraction.

Analytical approach:

- The chemical content of used snus was measured and compared with levels of constituents in unused “control” samples; the amount extracted from the snus portion by the user was calculated as follows:
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  \text{Amount extracted} = \frac{\text{Quantity in unused snus} - \text{Quantity in used snus}}{\text{Sample weight}} \times 100\%
  \]
- A number of “multi-analyte” methods were developed to examine multiple constituents from the same snus portion, as shown in Figure 1.
- Snus samples were solvent extracted; the choice of solvent was dictated by the target analytes, as shown in Figure 1.

Basic techniques:

- Water extraction: Snus was extracted in 40ml of water in a 50ml plastic centrifuge tube, shaken for 30 minutes, centrifuged, and analysed
- Methanol extraction: Snus was extracted in 20ml of methanol in a 20ml plastic centrifuge tube, shaken for 30 minutes, centrifuged, and analysed
- Ethanol extraction: Snus was extracted in 20ml of ethanol in a 20ml glass tube, shaken for 30 minutes, centrifuged, and analysed

Five Stage Method Validation Process:

- Equivalence of results from new method against existing pre-validated individual analyte methodology
- No perturbation of analysis through presence of saliva
- No influence of combined extraction and sub-sampling
- Stability under transport and storage conditions
- Execution of a small-scale pilot study

Trial Protocol:

- Trials conducted in Stockholm and Lund, Sweden by an established consumer research agency, GfK
- Recruited existing snus users, aged 18-72, who use at least 8 samples per day for 1 hour per sample
- Each volunteer provided informed consent
- Paid for involvement

Recruitment of Panellists:

- Volunteer snus users
- Contacted and invited to participate by telephone

Screening steps:

- Loose users invited pre-trial to try product to evaluate acceptability
- Loose snus sample sizes: Users prepared 10 samples in their normal way; samples weighed and consistency checked (results are shown in Figure 2); panellists selected from near the mean of the sample set
- Pouched users were interviewed about product preferences to assess compatibility with trial products

Results from pilot studies on pouched snus (n=20):

- Pouch weight gain: 27%  Nicotine extracted: 31%
- Pouch moisture pre-use: 48%  Pouch pH pre-use: 8.18
- Pouch moisture post-use: 78%  Pouch pH post-use: 7.98

Conclusions:

The methodology provides a flexible and robust approach for measurement of constituent extraction from snus by users.