Effect of Time, pH, Alcohol and Sugar Content on Nicotine Re-lease from Pouches Available on Slovene Market

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Abstract:

Tobacco-free nicotine pouches, first introduced in Sweden, are made as a less hazardous product for cigarette consumers. Instead of tobacco leaves, they consist of nicotine-containing powder and other ingredients such as water, salts, natural aromatic oils and others, that will boost the flavor and effect and make it last longer. This research aimed to investigate the extraction efficiency of nicotine from poaches of different brands. The effects of alcohol and sugars present in saliva at different pHs and consumption times were investigated as well. We optimized a High performance liquid chromatography with diode-array detection method (HPLC-DAD) for nicotine determination and its quantification. We used reversed-phase chromatography (RP-HPLC), and a mixture of sodium hydrogen carbonate and acetonitrile as a mobile phase (85:15, *v/v*). Different brands of nicotine pouches were used. Nicotine extraction rate showed positive linear dependence on time. The percentage of extraction was measured at different intervals, up to two hours, without getting a plateau. Nicotine extraction lowers with the increasing volume of saliva. The obtained results have shown that the increasing ethanol concentration in saliva (from 5-40 %) leads to a higher extraction rate, coming up to 85 %. In the case of sugar addition, the results were variable. Experiments with *White fox* pouches have shown that extraction of nicotine decreases with a higher concentration. On contrary, *Siberia* pouches do not exhibit that correlation. When it comes to pH, we used pH range that usually varies in saliva and the results have shown no significant differences.

Keywords: Nicotine, Pouches, HPLC-DAD, Extraction

1. Introduction

As people's awareness regarding the hazard of cigarettes grows, many companies have come up with alternative/potentially less harmful products to replace their use. One of these products, nicotine pouches, also known as tobacco-free snus, hailing from Sweden. These pouches are weighing up to 1g, do not contain tobacco, but have nicotine powder, sweeteners, flavorings, salts, and plant-based fibers. They are placed between the lip (usually the upper lip) and the gums, and the holding time can be up to an hour, although it is usually 10-15 minutes. The long-term health impact of nicotine pouches is still unknown. Nicotine pouches contain nicotine which is harmful to young people in any form. Due to the absence of tobacco leaf, the Food and Drug Administration does not classify oral nicotine pouches as a smokeless tobacco product (Robichaud et al., 2020). Side effects of use can include irritation of the gums, sore mouth, hiccups, nausea, nicotine addiction and more. Only 10-20 % of nicotine reaches the systemic circulation. This means that only 1-2 mg of nicotine is absorbed into the blood from a one-gram pouch containing around 16 mg of nicotine (Miller-Holt et al., 2022). Nicotine is absorbed quite rapidly from Swedish snus. Studies on Swedish snus users have shown that the plasma steady-state levels of nicotine and its main metabolite, cotinine, are like Swedish snus users and cigarette smokers (Scherer et al., 2022, Miller-Holt et al., 2022). Various brands of tobacco-free snus are available on the Slovenian market, and for the purposes of this research, we chose only some of them and compared the results obtained. We investigated how much nicotine is released from the bag into the saliva, trying to simulate people's daily habits. As you can eat and drink prior to using snus, in addition to varying the pH and the time of use, we investigated the effect of alcohol and sweeteners on nicotine release, wanting to simulate people's everyday life this way (Bishop et al., 2022).

Photo 1. Nicotine pouches; *White fox* and *Siberia.*

2. Materials and Methods

2.1. Materials

Different brands of nicotine pouches were used: *White fox*, *Siberia* (*GN Tobacco*, Sweden) (**Photo 1**), *Velo* (R.J. Reynolds Vapor Company, USA), *and Thunder* (*V2 Tobacco*, Denmark), but the first two expressed better extraction rate, so we continued our research on them. We used three types of *White fox* pouches: The original (blue) and Double-mint (green), both with 12 mg of nicotine per poach (0,75 grams), and Full-charge (red) with 16 mg of nicotine per poach (1 gram). Siberia pouches were *Regular* (big) with 16.4 mg of nicotine per pouch and *Slim,* (small) with 10.66 mg of nicotine per pouch. To stay in accordance with scientific ethics, the results will be presented by the alternative names previously stated in brackets (blue, green, red, big and small). In Slovenia, nicotine pouches are available in every tobacco shop and gas stations, so we obtained them from one of these.

The artificial saliva solution was prepared by dissolving 0.69 g of Sodium phosphate monobasic monohydrate (NaH_2PO_4), 0.4 g of sodium chloride (NaCl), 0.795 g of calcium chloride ($CaCl_2$), 0.4 g potassium chloride (KCl), 0.005 g sodium sulfide (Na_2S) and 1 g of urea, in distilled water in final volume of 1 liter.

2.2. Methods

For examining dependence of:

- volume of saliva, we used vials filled with 2, 4, 6, 8 and 10 milliliters of saliva
- pH of saliva, we used 1 M HCl and 1 M of NaCl for establishing wanted pH (5, 6, 7 and 8)
- alcohol concentration, we used different percentage (5, 10, 15, 20, 30 and 40 %) of ethanol mixed with saliva
- sugar (saccharose) concentration, we used 5, 10 and 15 % of sugar dissolved in saliva; for each of these experiments, we put 2 ml of prepared solution in the vials (except for investigating the impact of volume of saliva, where we put 2, 4, 6, 8 and 10 ml), placed one pouch inside, and left it in water bath on 37 degrees.

After 15 minutes, 1 ml from each vial was taken and placed in the vials for further detection on HPLC-DAD. For measuring time dependence, we used big vial filled with 10 ml of saliva. Then we placed pouch in vial and put it in the water bath on 37 degrees. We had 2 repeats per each type of nicotine pouches. 1 ml of saliva was taken after 5, 10, 15, 30, 45, 60, 90 and 120 minutes and placed into the vials for HPLC-DAD. Experiments are done in duplicate (for volume, time, and alcohol), or in triplicate (for pH and sugar dependence). Calibration curve was constructed for nicotine measurement from six standard solutions specifically: 50, 100, 150, 200, 250 and 500 mg/ml. The standard solutions were prepared by serial dilution of proper amount from stock standard solutions with saliva.

Chromatography Conditions: we used HPLC equipped with a diode array detector (DAD) for nicotine detection. C18 Agilent HP-5MS (19091S-433) Capillary Column (30M x 0.25mm x 0.25µm) was used. Mobile phase was consisting of acetonitrile (ACN) and sodium hydrogen carbonate buffer (pH 9.2, 2 M) (15:85, *v*/*v*), at a flow rate of 1,2 ml/min, running time 15 min and a UV detection achieved at 259 nm. For buffer preparation, we used 20 ml of sodium carbonate solution (2,2 g/100 ml, 0.2M), and 230 ml of sodium bicarbonate (1.68 g/100 ml, 0.2M). Filled to 1 liter with distilled water (Bansal et al., 2018).

3. Results

The results are shown in the **Tables 1-6.** We measured areas under the signals in chromatograms and calculated the percentage of nicotine extraction based on the known concentration of nicotine in pouches and in solutions diluted from the stock.

Table 1. Nicotine extraction rate (%) depending on the volume of saliva (White fox - green).

volume [ml]	green		
	Area	mg of nicotine	%
2	23935.35	5.08	42.36
4	16611.5	3.53	29.40
6	9343.6	1.98	16.54
8	6892.6	1.46	12.20
10	6027.65	1.28	10.67

Table 2. Nicotine extraction rate (%) depending on the alcohol percentage found in saliva (White fox - blue, red)

	blue			red		
Alcohol %	average	mg of nicotine	%	average	mg of nicotine	%
5	1037.2	0.22	18.35	20419.45	4.34	27.10
10	1347.2	0.29	23.84	19915.6	4.23	26.43
15	1373.4	1.29	24.30	21842.25	4.64	28.99
20	1641.45	0.35	29.04	53908.7	11.45	71.55
30	1853.05	0.39	32.79	52031.2	11.05	69.06
40	2104.7	0.45	37.25	56161.45	11.93	74.54

Table 3. Nicotine extraction rate (%) depending on the sugar percentage found in saliva White fox - (blue, red)

	blue			red		
Sugar %	average	mg of nicotine	%	average	mg of nicotine	%
0	4073.4	0.87	72.09	2891.55	0.61	51.17
5	2928.93	0.62	51.83	2800.6	0.59	49.56
10	2675.8	0.57	47.35	2554.63	0.54	45.21
15	2194.33	0.47	38.83	2311.37	0.49	40.90

Table 4. Nicotine extraction rate (%) depending on the sugar percentage found in saliva (Siberia - small, big)

	small			big		
Sugar %	average	mg of nicotine	%	average	mg of nicotine	%
0	1658.40	0.35	33.04	2088.4	0.44	27.04
5	1462.73	0.31	29.14	1163.43	0.25	15.06
10	1070.83	0.23	21.33	1362.83	0.29	17.65
15	1443.93	0.31	28.76	1717.8	0.36	22.24

Table 5. Nicotine extraction rate (%) depending on the pH of the saliva (White fox - green, Siberia - small)

	green			small		
pH	average	mg of nicotine	%	average	mg of nicotine	%
5	20703.47	4.40	36.64	19902.80	4.23	39.65
6	23041.60	4.89	40.78	19617.23	4.17	39.08
7	21986.43	4.67	38.91	20003.13	4.25	39.85
8	19621.23	4.17	34.72	15342.93	3.26	30.56

Table 6. Nicotine extraction rate (%) depending on the time pouches spent in saliva (White fox - green, Siberia - small, big)

	green			small			big		
time [min]	average	mg of nicotine	%	average	mg of nicotine	%	average	mg of nicotine	%
5	2797	0.59	4.95	943.75	0.20	1.88	2521.95	0.54	3.27
10	3597.5	0.76	6.37	1886.85	0.40	3.76	4650.25	0.99	6.02
15	4308.15	0.91	7.62	2241	0.48	4.64	4565.45	0.97	5.91
30	6565.05	1.39	11.62	2747.55	0.58	5.47	4925.1	1.05	6.38
45	8649.35	1.84	15.31	4326.95	0.92	8.62	6223.2	1.32	8.06
90	10683.05	2.27	18.91	7564	1.61	15.07	12780.35	2.71	16.55
120	14238.75	3.02	25.20	7791.35	1.65	15.52	14246.85	3.03	18.45

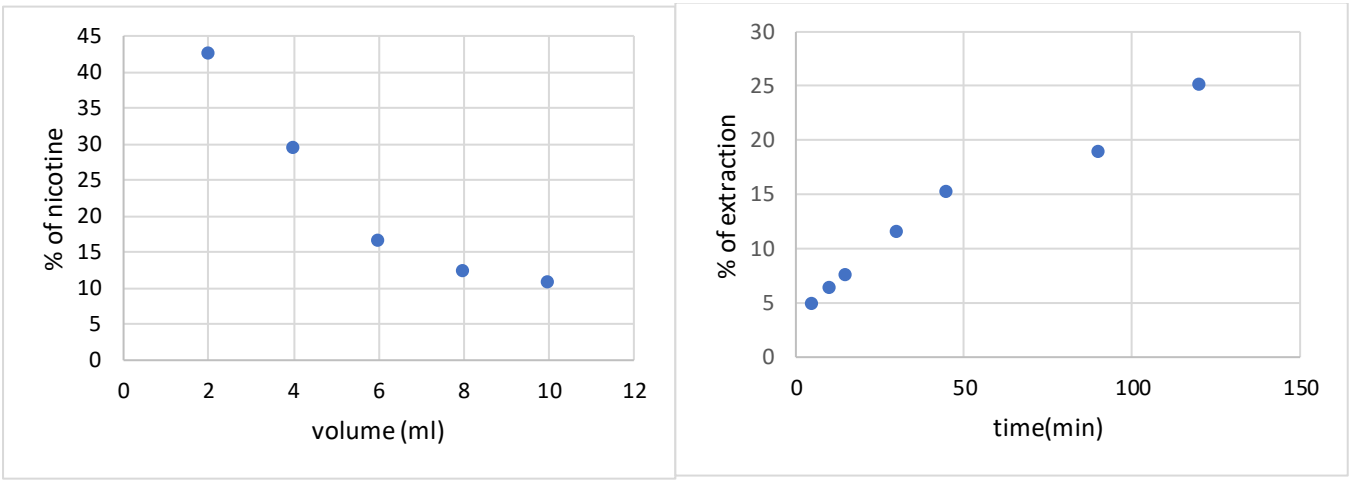

Figure 1. Nicotine extraction rate (%) depending on the volume of saliva (left) and time pouches spent in the saliva (right); White fox - green pouches.

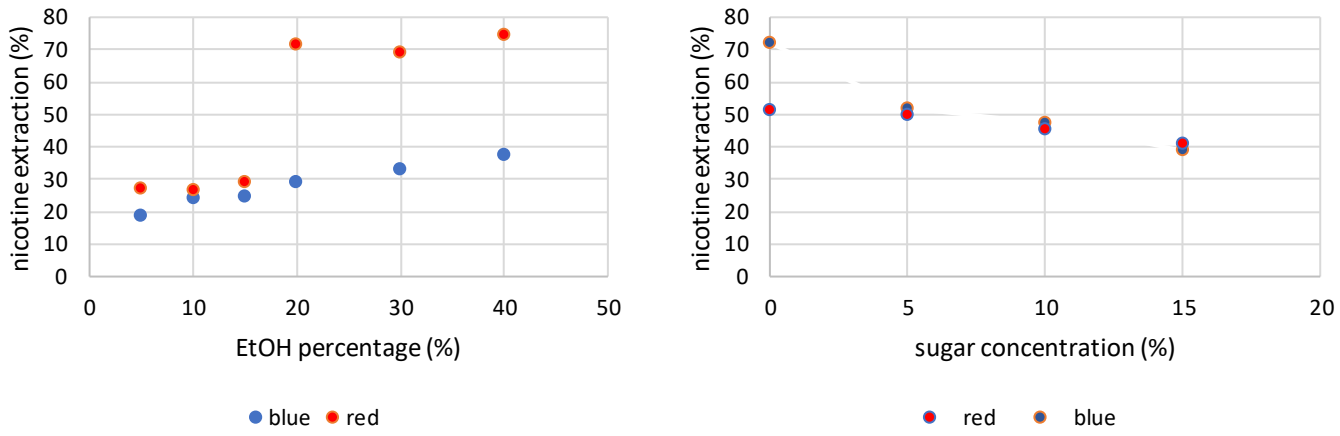

Figure 2. Nicotine extraction rate (%) depending on the percentage of alcohol (left) or sugar (right) found in saliva (White fox – blue, red pouches).

4. Discussion

Time dependence: With all pouches, nicotine extraction rate showed positive linear dependence with time. The percentage of extraction was measured at different intervals, up to two hours, without getting a plateau. At the beginning, we have a linear increase of the extraction % with the time, but after 45 minutes, the increase is slower, but it continues to rise. Maximum of extraction was achieved after 120 minutes, and it was 25 % with the green pouches. Nicotine extraction lowered with the increasing volume of saliva. For 2 ml of saliva (which was the minimal volume enough to cover pouches completely), we found an extraction rate 42 % in some brands of pouches. Ethanol dependence: The obtained results have shown that the increasing ethanol concentration in saliva (from 5-40% EtOH) leads to a higher extraction rate, coming up to 85% with some pouches. We had a second peak following the first one, possibly as a product of nicotine degradation, or the difference occurred because of the protonated groups. Sugar dependence: In the case of sugar addition, the results were variable. Experiments with red and blue pouches have shown that extraction of nicotine decreases with a higher concentration (5, 10 and 15 %) of sugar. On

the contrary, big and small pouches do not exhibit that correlation, potentially because of the difference in pouch material or ingredients in them. We observed that the sacks are less tight and nicotine could be distributed unevenly. When it comes to pH, we used a pH range that usually varies in saliva (from 5 to 8), and the results have shown no significant differences. In some cases, we had very big areas measured under the peaks, so we had to dilute them 10 times to make it calculable.

5. Conclusion

We standardized the existing HPLC-DAD method for nicotine extraction and used it for nicotine quantification in saliva under specific conditions of extraction. As shown in the results, some of the substances, such as alcohol or sugar, can modulate nicotine extraction rate from pouches. Consumers could potentially adjust nicotine consumption by using products that contain these substances.

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Conflicts of Interest: The authors declare no conflict of interest.

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