A comparison of human nicotine dose estimates from filter analysis with nicotine metabolites analysis

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Objective

Compare and correlate three methods of nicotine estimation

- Filter Analysis Method[1]
- Saliva Cotinine Method[2]
- Urinary Nicotine + 5 Metabolites[2]
  - *Nicotine + glucuronide*
  - *Cotinine + glucuronide*
  - *3-Hydroxycotinine + glucuronide*

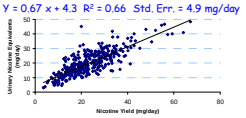
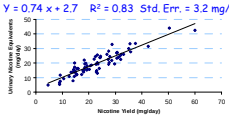
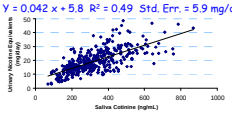
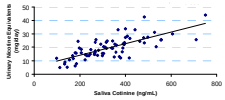
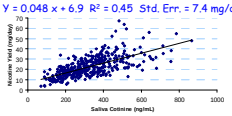
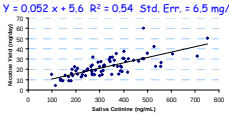
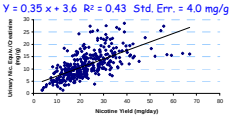
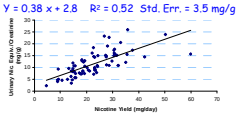
Experimental Design

- 5 day Clinical Study at Covance Clinical Research in Madison, WI to assure compliance
- 74 subjects smoking their own brand
- Allowed to smoke *ad lib.* in a ventilated smoking area
- ~ 10 subjects/week within same tar band
- Wide range of brands to assure robustness of correlations

Sample Collection

- 24-hour urine (Days 1-5) stored @ -70°C
- Saliva at 18:30 (Days 1-5) stored @ -20° C
- All cigarettes smoked (Days 1-5)
  - *Subject returned smoked cigarette to clinician*
  - *Filter removed and stored in jar*
  - *Clinician issued another of the same brand*
  - *Jars shipped overnight to lab each day*
  - *1 cm of mouth end of filter cut day of receipt*
  - *Stored @ -20° C in glass jars*

Correlation Results

	Individual	5 Day Average
Urine vs. Filter	Y = 0.67 x + 4.3 R² = 0.66 Std. Err. = 4.9 mg/day 	Y = 0.74 x + 2.7 R² = 0.83 Std. Err. = 3.2 mg/day (21%) 
Urine vs. Saliva (R^2=0.61 in Ref. 4)	Y = 0.042 x + 5.8 R² = 0.49 Std. Err. = 5.9 mg/day 	Y = 0.043 x + 5.4 R² = 0.55 Std. Err. = 5.3 mg/day (36%) 
Filter vs. Saliva	Y = 0.048 x + 6.9 R² = 0.45 Std. Err. = 7.4 mg/day 	Y = 0.052 x + 5.6 R² = 0.54 Std. Err. = 6.5 mg/day (33%) 
Creatinine Normalized Urine vs. Filter	Y = 0.35 x + 3.6 R² = 0.43 Std. Err. = 4.0 mg/g 	Y = 0.38 x + 2.8 R² = 0.52 Std. Err. = 3.5 mg/g (35%) 

Summary

- ***Each measure correlates significantly with the other two*** *(intercept & slope $p < 0.01$)*
- ***Strongest correlation between 24 hr. urinary metabolites and filter method***
  - *Highest R^2 and lowest standard error*
  - *Significant improvement with 5 day average*
- ***Normalization of urinary metabolites to creatinine degrades correlation***
- ***Urinary nicotine and metabolites are an amalgam of intake over 2 days***
- **Approximately 70% of the cigarette yield appears as urinary nicotine, cotinine, 3-hydroxy cotinine + respective glucuronides**

(Analytes represent 80-90% of total nicotine intake)[3-5]

Source of Urinary Metabolites

- Sequential days of input (filter analysis) and output (urinary analysis)
- Multiple regression of Urinary Metabolites versus Daily Nicotine for Current Day and 2 Previous Days

Metabolite = a + b*Nic (2 days ago) + c*Nic (yesterday) + d*Nic (today)

	Sum of All (R^2=0.73)		Nic. Only		Cot. Only		3-HC Only	
	p-value	Coeff.	p-value	Coeff.	p-value	Coeff.	p-value	Coeff.
Intercept	< 0.01	2.86	0.03	0.77	0.11	0.50	< 0.01	1.59
N - 2	0.73	-0.02	0.16	-0.05	0.82	- 0.01	0.53	0.03
N - 1	< 0.01	0.36	0.31	0.04	< 0.01	0.14	< 0.01	0.18
N	< 0.01	0.36	< 0.01	0.16	< 0.01	0.14	0.23	0.06

- **Total Urinary nicotine + metabolites**
  - *Dependent on input from current & previous day*
  - *Input from 2 days prior not significant*
  - *Dropping N-2 allows inclusion of Day 2 data as well*
  - *Total Metabolites = 3.5 + 0.24 * N-1 + 0.46 * N*
    - **R^2 = 0.71; Standard Error = 4.3 mg/day**
    - **Sum of coefficients ⇒ 70% of Yield in urinary metabolites**
- **Urinary nicotine consistent with literature**
  - *Only depends on current days input*
  - *Allows a closer look at Cotinine and 3-Hydroxycotinine*

Cotinine & 3-Hydroxycotinine

- Assume nicotine in urine is from intake that day (valid from literature & previous regression)
- Residual Nicotine (left for metabolism) = Nicotine Input – Urinary Nicotine
- Multiple regression of Urinary Metabolites versus Daily Residual Nicotine for Current Day and Previous Day

Metabolite = a + b * rNic (yesterday) + c *rNic (today)
= Intercept + b * (rN-1) + c * (rN)

	Cot. + 3-HC		Cot. Only		3-HC Only	
	p-value	Coeff.	p-value	Coeff.	p-value	Coeff.
Intercept	< 0.01	3.78	< 0.01	1.68	< 0.01	2.10
rN - 1	< 0.01	0.36	< 0.01	0.14	< 0.01	0.23
rN	< 0.01	0.26	< 0.01	0.14	< 0.01	0.11
Sum of coefficients				28 %		34 %
Literature[3-5] % from Nic.				25 – 27 %		43 – 53 %

ABSTRACT

Human nicotine intake during smoking has been estimated by either analyzing the metabolites of nicotine in body fluids or by analyzing filters from smoked cigarettes. However, no comparison of the filter analysis method with body fluid analysis methods has been published. Consequently, an in-patient study was conducted with 75 smokers of 1-17mg FTC tar products smoking their own brands. The subjects stayed in a clinic for 5 days and were allowed to smoke in a smoking room whenever they wished. Each smoked cigarette had to be returned to a clinician before another cigarette was issued. The filters were analyzed to estimate the daily mouth intake of nicotine. 24-hour urine samples were collected and analyzed for nicotine, cotinine, 3-OH cotinine and their respective glucuronide conjugates. Saliva samples were collected at 18.30 each day for cotinine analyses. On the fourth day, additional saliva samples were collected 08.30 and at 13.30 to assess any diurnal variations in saliva cotinine levels. Each method correlated significantly ($p<0.01$) with the other two, but the best correlation was between nicotine mouth intake and urinary nicotine and metabolites. Averaging the results over 5 days improved the mouth intake / urinary nicotine correlation even further but had little effect on the saliva cotinine correlations. Multiple regression analysis implies that urinary output is an amalgam of the nicotine input from multiple days.

References

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2. Bentley M (2004) Analysis of human urine and saliva samples from a multi-site study of habitual-smoking male and female volunteers, Covance Laboratories Ltd., Harrogate, North Yorkshire, England
3. Benowitz NL, Jacob III P, Fong I, Gupta S (1994) Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine, J Pharmacol Exp Ther 268:296-303.
4. Byrd GD. Davis RA, Caldwell WS, Robinson JH deBethizy JD (1998) A further study of FTC yield and nicotine absorption in smokers, Psychopharmacology 139:291-299.
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