SHIMADZU

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Quantitation of Nicotine, Cotinine and 3-OH-trans-cotinine in urine using LC-MS/MS with a dilute-and-shoot method approach

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1. Introduction

Analyzing cotinine and trans-3-OH-cotinine levels in urine is crucial for assessing tobacco exposure and nicotine metabolism. Cotinine, a metabolite of nicotine, and trans-3-OH-cotinine, its primary metabolite, serve as reliable biomarkers for tobacco use, enabling researchers and clinicians to evaluate smoking prevalence and secondhand exposure. Accurate measurement of these metabolites is essential for epidemiological studies, public health assessments and monitoring smoking cessation programs. The dilute and shoot approach simplifies sample preparation by minimizing the need for extensive extraction procedures, thus reducing analysis time and potential errors and makes automation simple in the future. This method involves diluting the urine sample directly before analysis, allowing for efficient quantification of cotinine and trans-3-OH-cotinine using techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS).

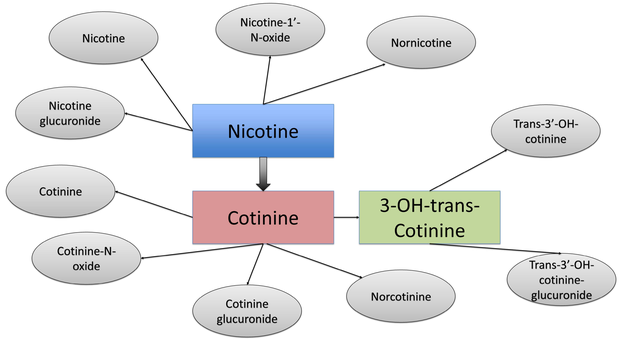

Fig. 1 Nicotine Metabolism

2. Methods

Standards were prepared in fresh urine from non-smokers (nicotine and cotinine free). 20 µL of fresh urine is transferred into a LC-vial, followed by the addition of 20 µL internal standard mix, containing deuterated nicotine and deuterated cotinine.

After addition of 760 µL 70/30 (v/v) water/methanol, the sample was shaken for 1 minute at 2200 rpm. The LC-vial was placed into the autosampler and 5 µL was injected onto a Shimpack Velox Biphenyl column. Gradient analysis was performed with acidified water and methanol at a flowrate of 0.4 mL/min, followed by ESI-MS/MS analysis

Fig. 2 Nexera LC40 – LCMS-8060RX system

UHPLC conditions

Column: Shimpack Velox Biphenyl 50 mm × 3.0 mm, 2.7 µm
Mobile phase A: 0.1% Formic Acid in Water
B: Methanol
Flow rate: 0.4 mL/min
Injection vol.: 10 µL
Column temperature: 30 °C

Time min	%A	%B
0.00	99	1
0.50	99	1
2.50	60	40
3.50	60	40
5.00	5	95
5.50	5	95
5.60	99	1

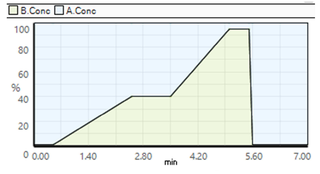

Table 1 Gradient settings Cotinine analysis

MS conditions (LCMS-8060RX)

Ionization: ESI, Positive MRM mode

Compound	Precursor m/z	Product m/z	CE
Trans-3-OH-Cotinine	192.8	80.0/134.0	-26/-20
d4-Nicotine	167.2	121.1/134.1	-25/-21
Nicotine	163.0	117.1/130.0	-25/-20
d3-Cotinine	180.2	80.0/101.2	-23/-22
Cotinine	177.2	80.1/98.1	-30/-21

Table 2 Multiple Reaction Monitoring settings Cotinine analysis

3. Results

- The response ratio of Cotinine and internal standard, Nicotine and internal standard and 3-OH-Cotinine were plotted against the concentration. All compounds showed good linearity (R^2 > 0,99) with linear regression and $1/x^2$ weighing)
- LOD for Cotinine, 3-OH-Cotinine and Nicotine, was 0.003, 0.002 and 0.06 µg/mL.
- Within and between run reproducibility was within 3.5% for all compounds

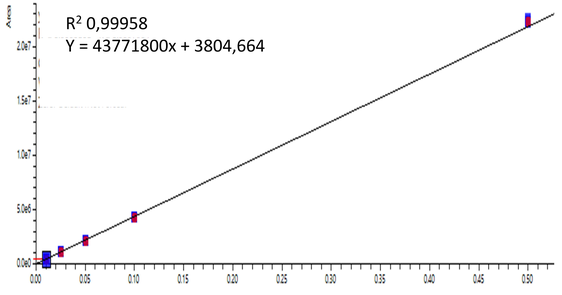

Fig. 3 Linear regression for Cotinine (0,01 – 10,0 µg/mL)

- Carry-over was negligible.
- 6 hour stability exeriments were performed, which showed a maximum deviation of -7.6%.

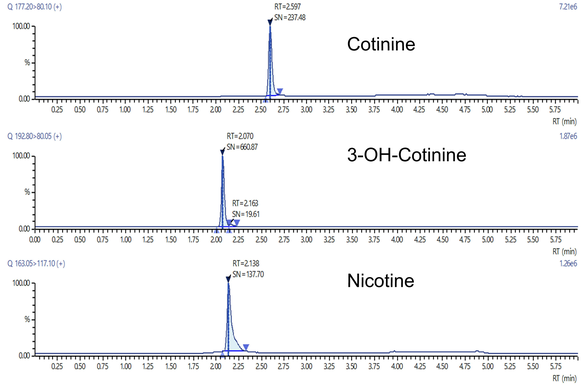

Fig. 4 Example chromatogram of a Urine patient sample, containing Cotinine, 3-OH-Cotinine and Nicotine

4. Conclusion

- Samples were stable for 6 hours in the autosampler at 15 °C.
- No carry-over was observed.
- With this dilute-and-shoot LC-MS/MS method approach, a reliable and cost-effective, time-saving method is developed.
- The method can be easily automated.