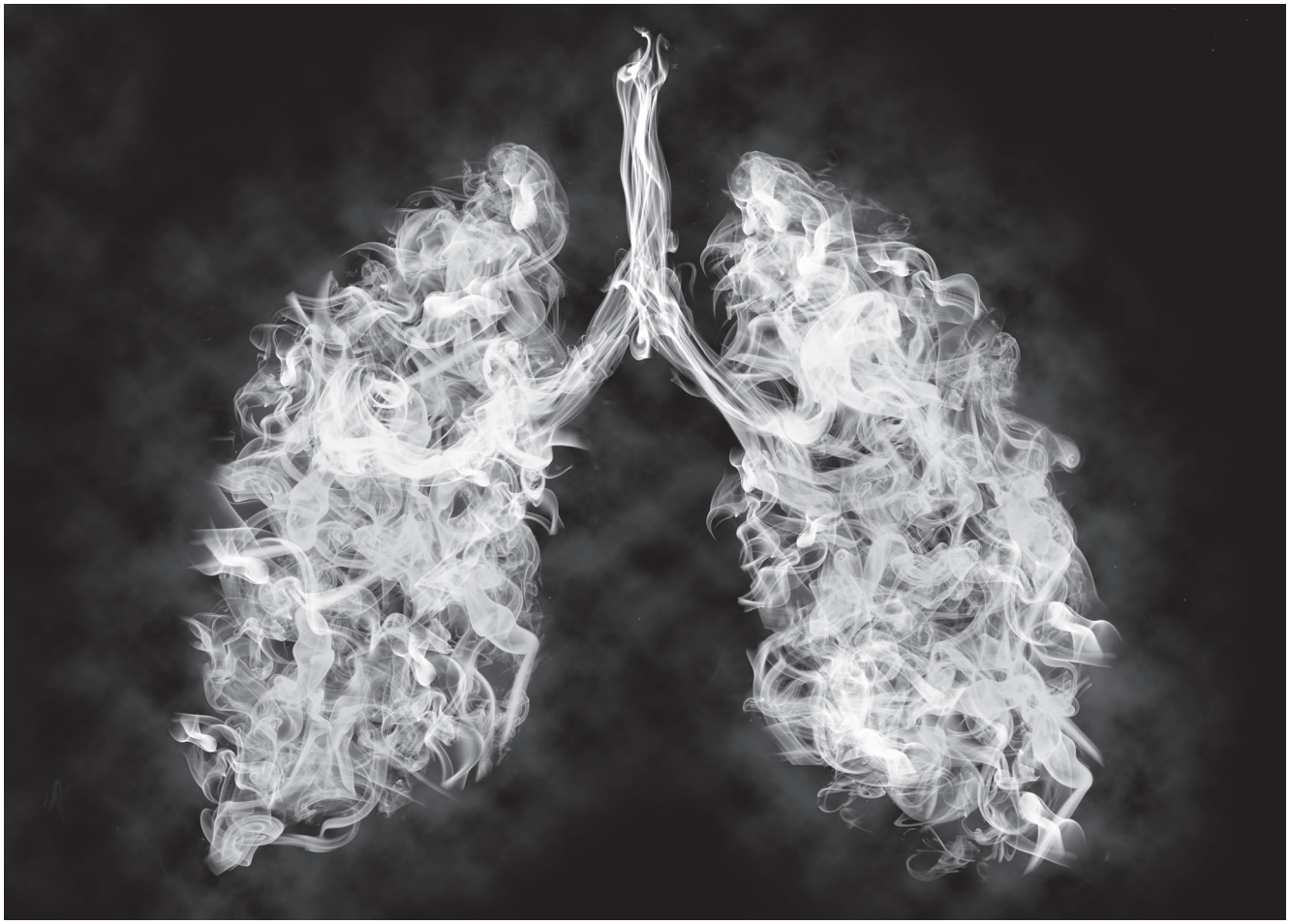
RP vs. HILIC:

Fast UHPLC Analysis of Biomarkers Related to Tobacco Smoke Exposure using **YMC-Triart C18** and **YMC-Triart Diol-HILIC**

In smoking cessation studies, the levels of several biomarkers are considered in order to evaluate the people's exposure to tobacco smoke. The well-known nicotine is typically considered as one of these biomarkers, but it has a half-life of only two hours in the human body. It is therefore not appropriate for the determination of tobacco smoke exposure after several hours or even days. Cotinine is the main metabolite of nicotine and has a half-life of about eighteen hours. Due to its higher stability, it is one of the widely investigated biomarkers related to tobacco products. However, when patients undergo smoking cessation and replace nicotine with nicotine gums and patches, their cotinine levels are the same as smokers.

Therefore, more universal biomarkers for this kind of studies are typically evaluated. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is a metabolite of the lung carcinogen NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and a tobacco-specific nitrosamine. Due to its much longer half-life (10–40 days) compared to nicotine and cotinine, it can still be detected after several days up to a few weeks making it a better marker for long-term studies.



During tobacco smoke consumption human cells are submitted to oxidative stress. As a result, 8-hydroxy-2'-deoxyguanosine (8-OHdG), a specific stress marker related to carcinogenesis, is produced in the human body. In addition to DNA oxidation, the exposure to tobacco smoke can also lead to DNA methylation which is indicated by the biomarker 7-methylguanine (m7Gua). In studies, both markers have been found at higher levels among smokers than non-smokers and are typically used to evaluate the DNA damage.

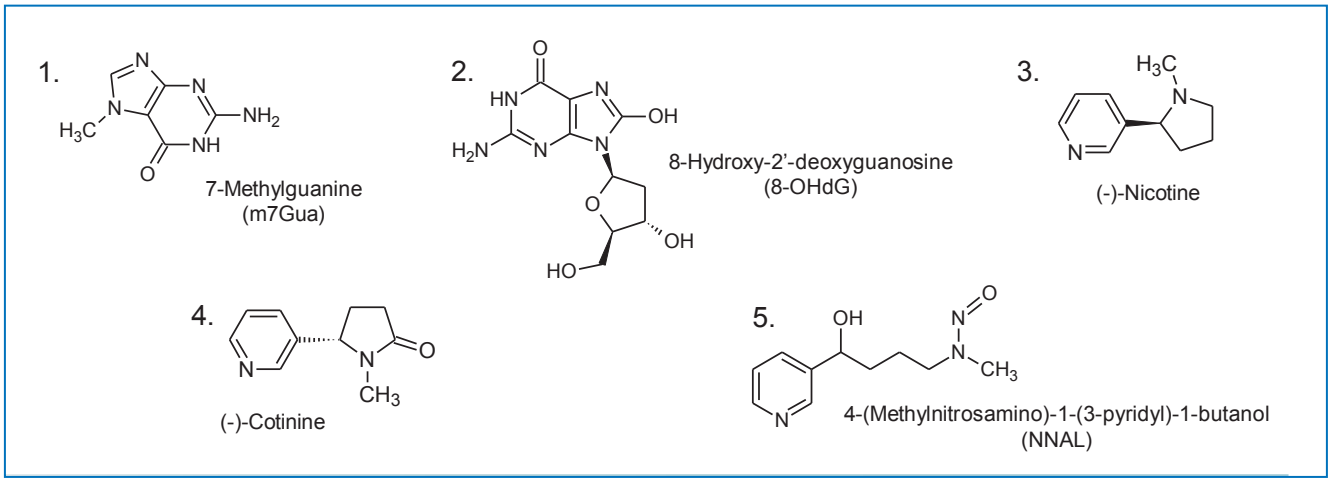
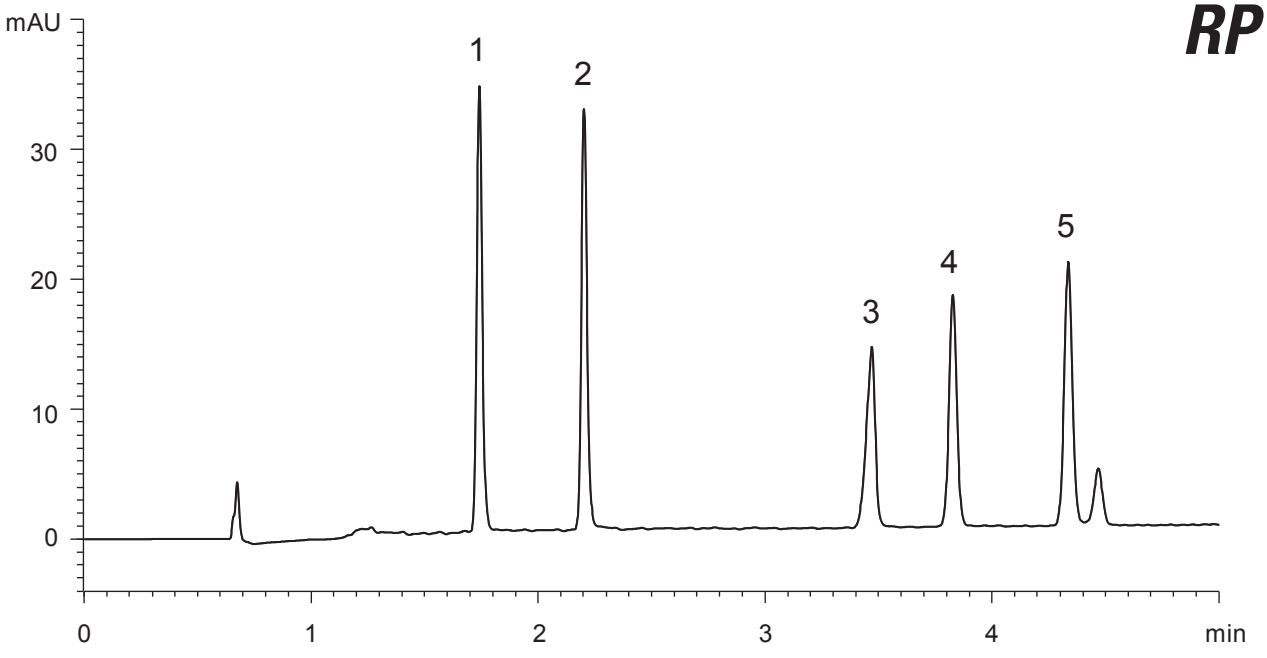


Figure 1: Structures of m7Gua, 8-OHdG, nicotine, cotinine and NNAL.

The five markers mentioned above were analysed using two different separation modes. A YMC-Triart C18 column was used for the analysis in RP mode, whilst a YMC-Triart Diol-HILIC column was chosen for HILIC mode. As UHPLC columns were used, fast separations under 3 and 5 minutes could be obtained.

Even though the analytes' hydrophobicity ranges from quite hydrophobic to hydrophilic, both columns provided highly resolved peaks. Even the hydrophilic DNA damage markers m7Gua and 8-OHdG showed sufficient retention using the YMC-Triart C18 column. Adequate peak shapes could be obtained although the basic analytes were (partly) deprotonated.

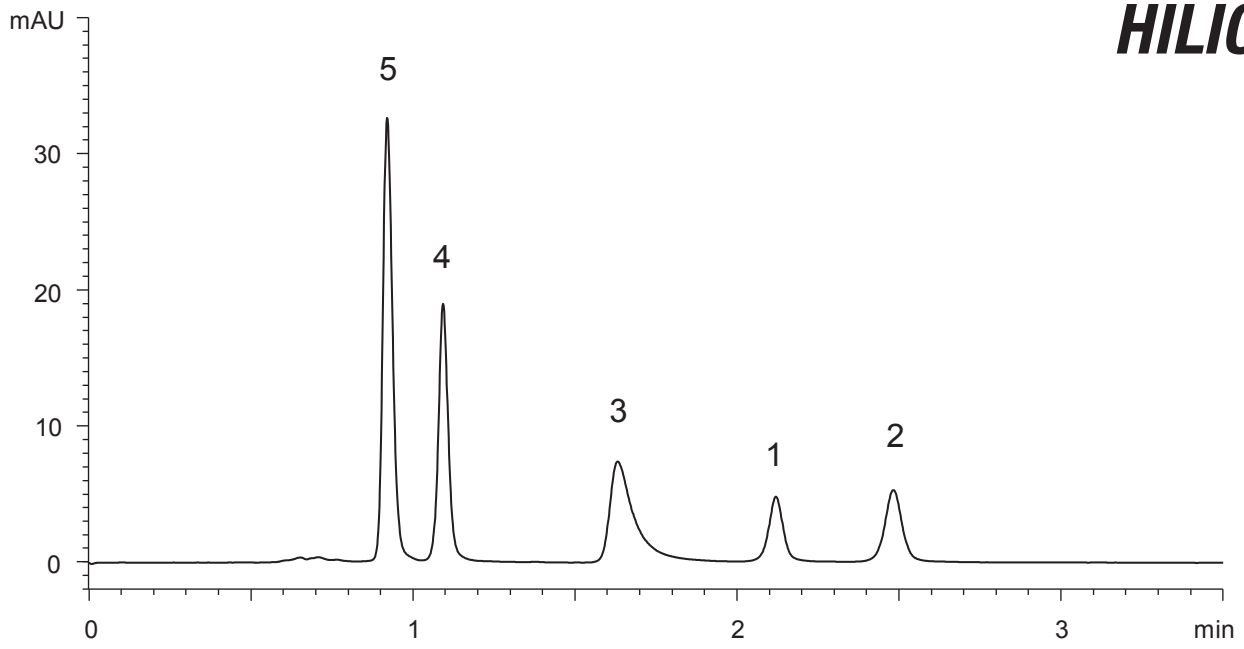


Column:	YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID
Part No.:	TA12SP9-05Q1PT
Eluent:	A) 10 mM CH_3COONH_4
	B) acetonitrile
Gradient:	0–15%B (0–5 min)
Flow rate:	0.4 mL/min
Temperature:	40 °C
Detection:	UV at 254 nm
Injection:	2 µL (0.005~0.01 mg/mL)

Figure 2: Separation of the five markers using a YMC-Triart C18 UHPLC column.

In HILIC mode, the elution order changes compared to RP. Hydrophobic compounds show less interaction with the stationary phase and elute earlier, whereas hydrophilic analytes interact strongly and show greater retention.

As NNAL showed weak retention on YMC-Triart Diol-HILIC due to its hydrophobicity, it could not be resolved in two peaks as in the RP application.



Column:	YMC-Triart Diol-HILIC (1.9 µm, 12 nm) 100 x 2.1 mm ID
Part No.:	TDH12SP9-10Q1PT
Eluent:	100 mM CH_3COONH_4-CH_3COOH (pH 4.5)/acetonitrile (10/90)
Flow rate:	0.4 mL/min
Temperature:	40 °C
Detection:	UV at 254 nm
Injection:	2 µL (0.005~0.0 mg/mL)

Figure 3: Separation of the five markers using a YMC-Triart Diol-HILIC UHPLC column.