Application Note No. 012

The measurement of low levels of Cotinine in Saliva.

B E Frost and D J Tuck, Rothmans International Services Ltd, Basildon, Essex

Introduction

Nicotine is metabolised in the body to cotinine and other metabolites such as 3-hydroxycotinine and nicotine N-oxide. After smoking, or exposure to nicotine from Environmental Tobacco Smoke (ETS), cotinine is found in the blood and saliva and, ultimately, it is excreted in the urine. The half life in the body is approximately one hour for nicotine and 18 hours for cotinine.

Cotinine measurements are sometimes used to assess how much nicotine is taken up by smokers and by non-smokers exposed to ETS. Measurements are normally made on urine or saliva, the latter being more convenient, although larger sample volumes can be taken for urine. Typical saliva cotinine levels are 300 ng/ml for regular smokers, 100 ng/ml for occasional smokers and 0-2 ng/ml for non-smokers exposed to ETS.

Studies have shown that a saliva cotinine level of 10 ng/ml can be used as a threshold to distinguish between people who have been exposed to ETS and those who have smoked. Saliva cotinine measurements can, therefor, be used to check on a person's self-reported smoking status, which is not always correctly stated.

Since only about 1 ml of saliva can conveniently be collected from a subject, the measurement of saliva cotinine at less than about 5 ng/ml is rather difficult. Several techniques have been used for the analysis including GC-NPD, HPLC and radioimmunoassay but these methods either lack the sensitivity or selectivity required to measure reliably the low levels of continine that result from exposure to ETS.

A method was developed for measurement of the low levels of cotinine in saliva resulting from ETS exposure. This involved the use of capillary GC-MSD system fitted with an OPTIC injector. The OPTIC enabled large volume injections to be made and, therefore, low detection limits to be achieved, whilst the MSD allowed selective detection and reliable identification of the cotinine. This is important as there are other nitrogen-containing compounds present in saliva at a similar retention time.

Method

After centrifuging the saliva sample, a 0.5 ml aliquot is taken for analysis and N-ethylnorcotinine is added as an internal standard. Then 2 ml of dichloromethane containing 0.01% of triethylamine (modified dichloromethane) is added, followed by 0.5 ml of 660 ammonium hydroxide solution. The purpose of the triethylamine is to prevent adsorption of cotinine by glass surfaces.

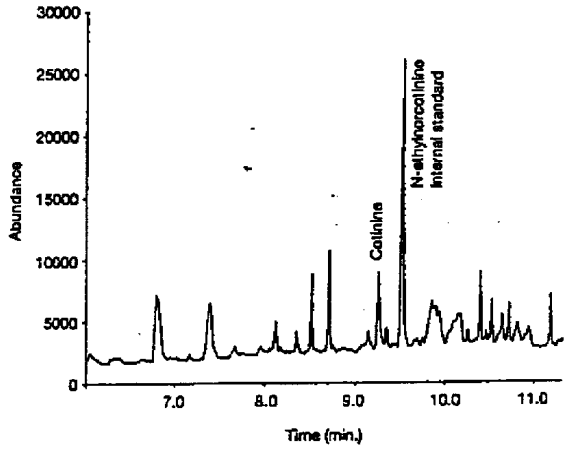

Figure 1. Typical non smokers Saliva containing 0.75 ng/ml Cotinine.

After vortexing and then centrifuging, the aqueous layer is discharged and the dichloromethane is transferred to a 2 ml GC auto sampler vial and evaporated to dryness. The dry residue is transferred to a 0.3 ml GC auto sampler vial using three washes of 100 µL of modified dichloromethane, whilst the liquid in the smaller vial is being evaporated. After evaporation of the small vial to dryness, 100 µL of modified dichloromethane is added and the vial sealed and shaken. With the OPTIC injection port in the solvent purge mode, 50 µL is injected from the auto sampler vial during about five seconds. After one minute, the OPTIC split line is closed and the injection port in heated to 275 ºC.

Operating conditions

OPTIC injector

Initial Temperature	50 ºC
Initial Time	1 minute
Temperature Ramp Rate	16 ºC/min
Final Temperature	275 ºC
Final Time	5 minutes
Split mode	0.0 minutes
Splitless Mode	0.95 minutes

Gas chromatograph

Column: 12 m x 0.2 mm i.d., 0.33 um film thickness HP-1 from Hewlett Packard

Initial Temperature (ºC)	Initial Time (min)	Temperature Ramp Rate (ºC/min)	Final Temperature (ºC)	Final Time (min)
60	0	20	250	3

The MSD was set to monitor masses 176 and 175 for cotinine, 190 and 189 for the internal standard and mass 118 as a confirmation ion for both compounds.
An MSD Macro Programme checked that these masses were in the correct intensity ratios and calculated the amount of cotinine present from its peak area relative to that of the internal standard.
Several different materials were evaluated as ‘packing’ for the OPTIC injection port liner, including quartz wool, silanised glass wool, fused silica beads and Tenax. Teflon wool proved to be the most inert and gave the most consistent results.

Detector Response

The response curve is shown in Figure 2.

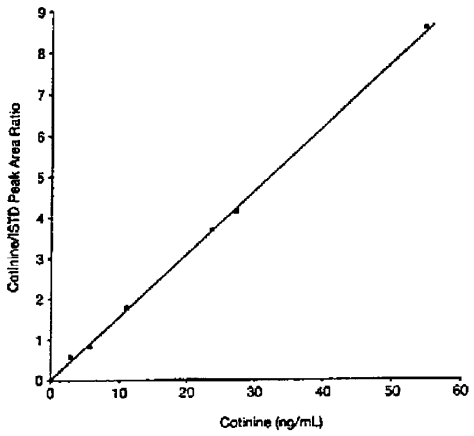

Figure 2. Calibration Curve for Cotinine in Saliva

Conclusion

The use of the OPTIC and large volume injections, in combination with the selectivity and identification capabilities of a mass spectrometer, lead to very low detection limits (approximately 0.1 ng/ml cotinine) and made this a powerful method for the analysis of salivary cotinine.