Optimal efficiency and in drug tests

Dual Column GCMS-

THCCOOH	35 ng/mL	150 ng/mL	400 ng/mL
Within-day VC %	9.02	7.07	3.21
Between-day VC %	13.75	5.72	12.66
Accuracy (Bias %)	-2.26	-1.56	5.99

Table 1: Overview validation results

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Smoking or chewing the dried leaves of the tobacco plant Nicotiana tabacum, use of nicotine containing gum and nicotine replacement therapies are the most significant sources of nicotine consumption for man. Nicotine intake from food products, including aubergine, potato and tomato, is insignificant and is not expected to be clinically relevant. Cotinine, a major metabolite of nicotine, is employed as a biomarker for assessing exposure to tobacco and to distinguish smokers from non-smokers.

Marijuana continues to be the most commonly used illegal substance in many countries around the world. The primary psychoactive ingredient, delta-9-tetrahydrocannabinol (THC), is deposited in body fat because of its high lipophilicity. The amount of drug stored is a function of the amount, frequency and potency of drug exposure. Over time, it is slowly released from this depot. THC is rapidly metabolized to 11-nor-9-carboxy-THC (THCCOOH). After conjugation with more water-soluble glucuronic acid, it is excreted in urine. THCCOOH is the primary cannabinoid metabolite in urine and is measured to monitor marijuana usage.

GCMS for routine analyses

Quantification of cotinine in serum and of THCCOOH in urine are the most widely used measures to assess smoking practices and in drug-treatment programs, respectively. For both analyses, sensitive validated GCMS methods are required, that can be used for continuous routine analyses. Both components can be chromatographed on a 5-MS capillary column but for THCCOOH-analysis derivatization is required. Silylation yields excellent sensitivities but the limit of detection of cotinine increases when injections are performed on a column used for the analysis of silylated components as well.

These important applications are very good examples of two commonly used methods in clinical laboratories, and due to the silylation two separate columns are needed to maintain quality and reliability. In general, this means either two dedicated expensive GCMS systems or the need to live with several hours required for cool-down, change column and start up again when switching applications. Optimal flexibility in a clinical laboratory, where downtime of a GCMS measuring system is an unwanted but ever present threat, can be obtained when the GCMS is equipped with dual columns, both ending in one shared ion source. A flexible autosampler is able to inject in two injectors, and even with one batch different methods can be run sequentially on different columns.

Double column flow – a "piece of cake"

By using to full advantage the large capacity of the double QP2010 turbo pumps, this feature has been made possible. A total pumping capacity of 325 L/second (260 and 65 L/second) makes a double column flow easily possible. There is practically no loss in sensitivity, due to the design of the ion source and the whole system. As always in practice the users need to be careful with their chromatography and ensure that the peak shape is good. Some considerations should be observed in the choice of columns. Both columns will contribute to the background noise and therefore still influence the sensitivity of the system. However, the latest generation of stationary phases used by capillary column manufactures have such improved stability that bleeding is minimized.

The nicotine metabolism with the cytochrome P450 system is rapid and extensive and involves both oxidation and conjugation. The major pathway of nicotine metabolism is C-oxidation to cotinine. Cotinine can be measured reliably in urine and blood and both matrices are regarded as acceptable for monitoring nicotine exposure in people. The cotinine level in the blood of smokers is approximately 10 times that of nicotine. The mean plasma half-lives of nicotine and cotinine in smokers are about 2 - 3h and 17h respectively. Because of the higher plasma concentrations and

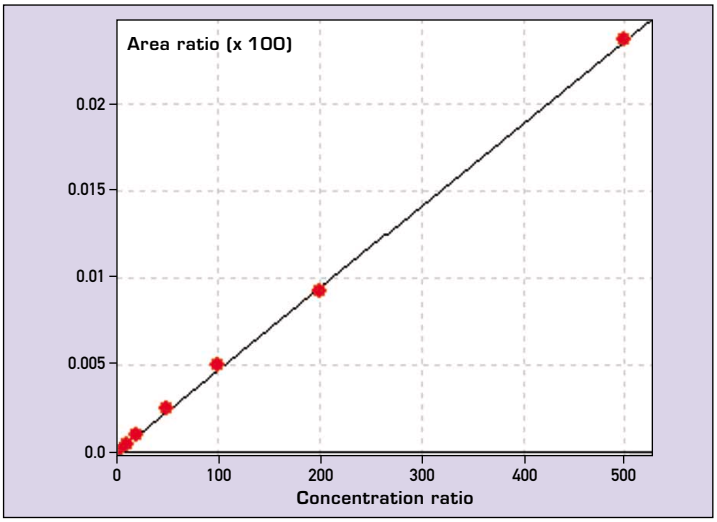


Figure 1: Calibration curve for THCCOOH, R^2 = 0,9995997. Internal Standard: Cannabinol

flexibility

QP2010

the longer half-life of cotinine, measurement of cotinine is less dependent on the exact timing for blood sampling than nicotine measurement. The serum cotinine concentrations in non-smokers should be < 10 ng/mL, while typical plasma cotinine levels for smokers (10 - 30 cigarettes per day) are 150 - 300 ng/mL. The obtained LOQ was 1 ng/mL in serum (S/N = 10).

New drug use or previous marijuana exposures?

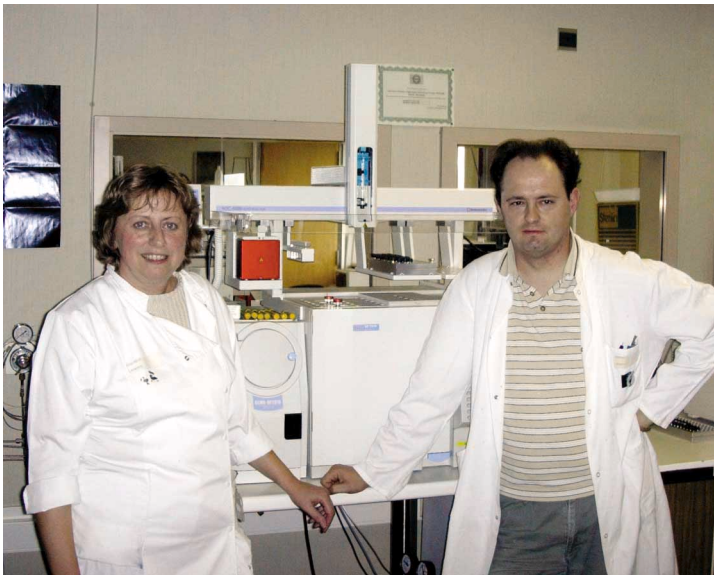
Interpretation of cannabinoid urine drug test results is very important for drug-testing programs. However, it is difficult to determine whether positive results are indicative of new drug use or reflect previous marijuana exposure. Increase in concentration of THCCOOH may be mistakenly interpreted as new drug use during the terminal elimination phase. Specimens may have alternating negative and positive responses around the cutoff concentration of 20 ng/mL. Normalization of drug concentration to creatinine concentration reduces the apparent variability in drug excretion due to urine volume changes. Following marijuana smoking the metabolite/creatinine concentration ratio should ideally decrease till a new episode of drug use, indicated by an abrupt increase of the ratio. A change in the metabolite/creatinine ratios of 1.5 in two positive specimens is usually applied as an indicator of new use.

Both methods were successfully validated on the dual column system (with both columns installed) and as an illustration the data of the THCCOOH-analysis is described in detail. Linearity, precision and accuracy were determined in order to assess the performance of the method. Linear calibration curves were obtained in the concentration ranges from 10 to 500 ng/mL with a mean correlation coefficient (r^2) of 0.9986. A calibration curve is shown in Figure 1.

The minimum quantifiable concentration (LOQ) in urine was determined to be 2 ng/mL based on signal/noise ratios (S/N = 10). The relative standard deviation (RSD) at this concentration level was 14.35 %. The accuracy at the LOQ applied was 9.00 %.
The within-day and day-to-day relative standard deviations were determined at three different concentration levels: 35 ng/mL, 150 ng/mL and 400 ng/mL. The within-day RSDs were < 10 % and between-day RSDs were < 15 % at the three concentration levels.

Accuracy was < 10 % at the three concentration levels. The results are summarized in table 1.

We conclude that with this flexible system the clinical laboratory is optimally served, being able to analyse urgent samples day and night with extremely good performance and reliability for this application.



Dr. Daniëlle Borrey and Dr. Kristof Goderis in front of the GCMS-QP2010

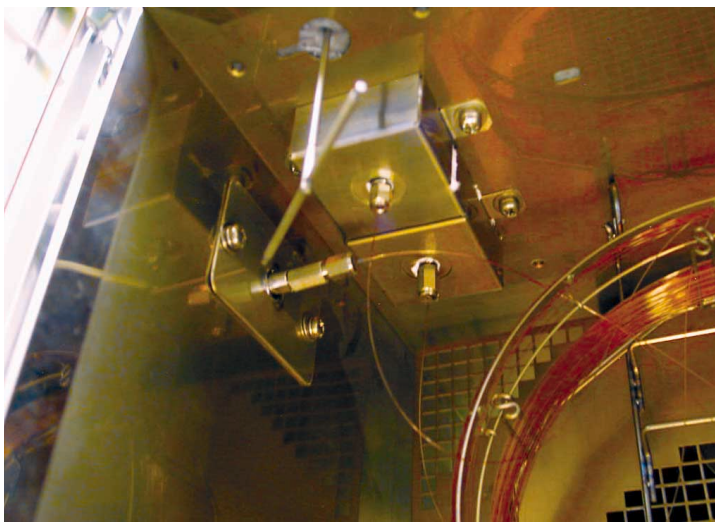


Figure 2: GCMS-QP2010, interior of the GC oven. Two columns connected with two injectors are led into the MS part