Printed in the Republic of Korea

ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 34 No. 2, 68-77, 2021

https://doi.org/10.5806/AST.2021.34.2.68

Development and validation of a qualitative GC-MS method for THCCOOH in urine using injection-port derivatization

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(Received March 4, 2021; Revised March 30, 2021; Accepted March 31, 2021)

Abstract: Cannabis is one of the most abused drugs in Korea. The main psychoactive component in cannabis, Δ^9-tetrahydrocannabinol, is metabolized to 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) and THCCOOH-glucuronide (THCCOOH-glu) in the human liver, whereby the amount of THCCOOH-glu found in urine is twice as high as that of THCCOOH. The analytical process adapted by the majority of urine drug-testing programs involves a two-step method consisting of an initial immunoassay-based screening test followed by a confirmatory test if the screening test result is positive. In this study, a qualitative gas chromatography-mass spectrometry (GC-MS) method was developed and validated for the detection of THCCOOH in human urine, where THCCOOH-glu was converted into THCCOOH by alkaline hydrolysis. For purification of the urine extract prior to instrumental analysis, high-speed centrifugation was used to minimize interference. In addition, an injection-port derivatization method using ethyl acetate and N,O-bis(trimethylsilyl)-trifluoroacetamide containing 1 % trimethylchlorosilane was employed to reduce the time required for derivatization, and an aliquot of the final solution was injected into the GC-MS. The method was validated by measuring the selectivity, limit of detection (LOD), and repeatability. The sensitivity, specificity, precision, accuracy, Kappa, F-measure, false positive, and false negative rate were determined by comparing the GC-MS results with those obtained using the immunoassay. The LOD was determined to be 0.32 ng/mL, while the repeatability was within 9.1 % for THCCOOH. Furthermore, a comparison study was carried out, whereby the screening immunoassay exhibited a sensitivity of 86.4 % and a specificity of 100 % compared to GC-MS. The applicability of the developed method was examined by analyzing spiked urine and forensic urine samples obtained from suspected cannabis abusers (n = 221).

Key words: qualitative analysis, validation, injection-port derivatization, urinary THCCOOH, GC-MS

1. Introduction

Recently, many countries, including the USA, Canada, Australia, Colombia and the Republic of South Africa, have allowed the use of cannabis as a medicine or as an item of personal preference. In the

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USA, while the federal government has legislated cannabis as a regulated drug and prohibits the use of cannabis, there is a trend towards cannabis legalization. Indeed, the medical use of cannabis is allowed in 30 states, and it is legal to sell cannabis as an item of personal preference in nine states and District of Columbia.[1] In 2018, 244 domestic cases of cannabis smuggling from USA and Canada were reported, with 33.6 kg of cannabis being seized.[2] The increase in cannabis production and trafficking to Korea can be attributed to a ripple effect from the legalization of cannabis for medical and recreational use in most states in the USA. Cases of large-scale cannabis cultivation have also been reported in residential studio apartments and commercial buildings in downtown Seoul, Korea, where cannabis cultivation skills had been learned online. According to the White Paper on Narcotics Crime published by the Supreme Prosecutors' Office of Korea, the annual number of cannabis offenders in Korea was 1,139 in 2015, 1,435 in 2016, 1,727 in 2017, 1,533 in 2018, and 2,629 in 2019.[3] Although there may be variations depending on the degree and frequency of crackdowns on illicit drugs, the distribution of illicit drugs is consistently increasing. Furthermore, there is an increasing number of cases where drugs are purchased through online distribution channels based at home or abroad, thereby augmenting the number of domestic cannabis offenders.[4]

One of the main psychoactive components in cannabis, Δ^9-tetrahydrocananabinol, is metabolized to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) and THCCOOH-glucuronide (THCCOOH-glu) in the liver, and the amount of THCCOOH-glu found in human urine is reportedly twice as high as that of THCCOOH.[5] The current method employed for the analysis of THCCOOH-glu includes the conversion and analysis of THCCOOH-glu in the form of conjugates to THCCOOH, whereby acid/base hydrolysis and enzymes are often employed, or the THCCOOH-glu itself can be directly analyzed.[6-9]

When analyzing urine samples using an immunoassay to determine if cannabis usage has taken place, the accuracy and reliability of the analysis may be reduced due to cross-reactivity or specificity/sensitivity issues. In order to overcome this problem, a two-step method was adopted, based on an initial screening test followed by a more specific confirmatory test if the specimen screened positive. Immunoassays are mainly applied to screening tests due to their facile use and automation, as well as the relatively fast acquisition of analytical results.[10,11] For this reason, immunoassays are widely used in many countries for screening tests, and in particular for initial screening. For confirmation of the results, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-MS (LC-MS) are generally employed.[12,13] Although GC-MS has been considered the gold standard in confirmatory analytical instruments for abused drugs in urine, GC-MS cannot be used for the direct analysis of polar drugs and their metabolites. Therefore, the use of derivatization to improve the volatility of an active compounds is required prior to GC separation. In addition, since GC-MS can apply both the selected ion mode (SIM) and the full-scan mode simultaneously, it is also useful for identifying unknown substances by searching the mass spectral library in full-scan mode. Compared to LC-MS, it has an additional advantage of being less affected by the matrix.[14,15]

Due to the complexity of the urine matrix and the presence of endogenous compounds in the urine samples, the selection of an appropriate sample preparation technique is of particular importance for sample clean-up to improve sensitivity of the method. Sample preparation enables the isolation of target compounds from the interfering components, and their subsequent concentration for accurate identification.

To minimize the analytical run time, we herein report the use of vortex-assisted extraction instead of conventional solid-phase and liquid-liquid extraction techniques to reduce sample preparation time. In addition, as a means to shorten the slow derivatization process associated with GC-MS, injection-port derivatization (IPD) is employed, whereby the derivatization reaction is performed in the hot GC injection port prior to sample injection. Indeed, this technique has previously been reported to be superior to off-line

derivatization owing to its simplicity, reaction efficiency, and low consumption of potential toxic reagents.[16] To date, IPD has been applied in the analysis of herbicides, polyphenols, patulin, fluoxetine and cocaine, although no study has reported the application of IPD in the analysis of THCCOOH.[17-21] The developed qualitative GC-MS method is validated by determination of the limit of detection (LOD), sensitivity, specificity, selectivity, and repeatability.[22-24] To confirm the effectiveness of this GC-MS method for qualitative purposes, the selectivity and reproducibility are further confirmed by its application to spiked urine samples and urine samples obtained from cannabis offenders. Finally, the usefulness of the developed method is confirmed by comparison with the results of immunoassay tests.

2. Experimental

2.1. Chemicals and reagents

THCCOOH (100 μg/mL in methanol) and THCCOOH-glu (100 μg/mL in methanol) were purchased from Cerilliant (Austin, TX, USA). THCCOOH-d_9 (100 μg/mL in methanol) was used as an internal standard (IS), and was also purchased from Cerilliant. The ONLINE DAT Cannabinoids II reagent used in the immunoassay was purchased from Roche Diagnostics (Mannheim, Germany). N,O-Bis(trimethylsilyl)-trifluoroacetamide containing 1 % trimethylchlorosilane (BSTFA+1 % TMCS) as a derivatization reagent was supplied by Acros Organics (Morris Plains, NJ, USA). The organic solvents, ethyl acetate (HPLC grade) and hexane (HPLC grade), were purchased from J.T. Baker/Avantor (Center Valley, PA, USA). The acetic acid used in the pretreatment process was purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan), and the 5 M KOH (potassium hydroxide) solution was purchased from Daejung Chemicals and Metals Co. Ltd. (Gyeonggi-do, Korea). All other reagents used were of ACS grade or higher.

Standard solutions of THCCOOH and THCCOOH-glu were prepared by serial dilution as required, and the IS solution was prepared by dilution in methanol to a final concentration of 1 μg/mL. The standard solutions were stored at -20 °C until required for use.

2.2. Instrumental conditions

The immunoassay instrument used for initial screening was a Cobas c311 analyzer (Roche Diagnostics, Germany). The GC-MS instrument used for confirmation was a 7890 Gas Chromatograph/ 5975 Mass Spectrometer (Agilent Technologies, USA), and this was equipped with a DB-5MS UI GC column (30 m × 0.25 mm I.D., 0.25 μm film thickness, J&W Scientific, USA). The GC oven temperature was increased from 200 to 300 °C at a rate of 40 °C/ min and then maintained at 300 °C for 7.5 min. The injection port temperature was set to 280 °C, and the carrier gas (He) flow rate was 1.1 mL/min. The injection method was set to splitless mode with a purge-on time of 1.5 min. Aliquots (2 μL) of the sample were injected into the GC-MS using a 7693 automatic liquid sampler (Agilent Technologies, USA).

The MS source temperature was set to 230 °C and MS analysis was performed using the SIM/scan mode under electron ionization (EI) conditions. In the mass spectrum of the analyte obtained in the scan mode, one of the three characteristic ions was selected as the quantifier ion and the other two were selected as the qualifier ions. Two characteristic ions were selected for IS identification. The ratios of the quantifier ion to the qualifier ions were calculated from the SIM chromatograms. For qualitative GC-MS analysis, the acceptable deviation of the ratio of quantifier ions to qualifier ions was within ±20 %.[25]

2.3. Urine samples

The blank urine sample was prepared by mixing urine from persons who had not consumed cannabis, and was used to examine the method LOD and selectivity. To measure the ratio of the quantitative ion peak to the qualitative ion peak height for the analyte, THCCOOH was added to the blank sample and diluted to concentration of 20, 50, and 100 ng/mL.

The urine samples (n = 221) of suspect cannabis users obtained by district prosecutors' offices and police stations in the Youngnam region were tested.

The samples were refrigerated at 4 °C for 20 days after their receipt, and the samples requiring additional analysis were separated and stored at -20 °C until required for use.

2.4. Sample preparation

Urine samples (2 mL) were mixed with the IS (30 μL, 1 μg/mL) and a 5 M KOH solution (200 μL) in a polypropylene tube (5 mL, Eppendorf). The resulting mixture was then hydrolyzed at 70 °C for 10 min.

After this time, concentrated acetic acid (200 μL) was added to adjust the pH to 4.5, and a mixture of ethyl acetate:hexane (1.5 mL, 1:9 (v/v)) was added prior to vortexing the mixture at 3200 rpm for 1 min. After subsequent high-speed centrifugation at 20000 g for 5 min, an aliquot (1.2 mL) of the supernatant was placed in a test tube for drying. The residue was then re-dissolved in a mixture of ethyl acetate (20 μL) and the BSTFA + 1 % TMCS reagent (20 μL), and an aliquot (2 μL) of the sample was immediately injected into the GC-MS instrument.

2.5. Method validation

To develop and validate the qualitative GC-MS method, its selectivity, LOD, repeatability, sensitivity, specificity, precision, and accuracy were examined.

For the evaluation of the method selectivity, different urine samples were analyzed, and the interference on the retention time (RT) of the analyte was determined using the analyte and IS peaks from the GC-MS SIM chromatogram. In addition, THCCOOH-glu, a phase II metabolite of THC and the most likely cause of cross-reaction in the actual urine sample, was added to the blank sample to examine whether interference took place.[26]

The LOD was determined using the standard deviation between the signals (S) from the results of 12 urine samples containing the analyte at the same concentration and the noise (N) obtained from 12 blank samples. The result with an S/N ratio of ≥3 was selected.

To examine the reproducibility, the proximity of the measured values obtained by repeatedly analyzing the aliquots collected from the homogenized sample was determined. For this purpose, homogenized QC samples of three concentrations (i.e., 20, 50, and 100 ng/mL) were prepared, and each sample was divided into seven aliquots for repeated testing. The measured value should not exceed 15 % of the relative standard deviation (RSD %).

The results of the immunoassays and the GC-MS analyses were compared to evaluate the method sensitivity, specificity, precision, and accuracy.[27] The immunoassay used for the screening test was a Cobas c311 analyzer combined with an ONLINE DAT Cannabinoids II, with a cut-off value of 20 ng/mL. In the case where the result of the immunoassay screening analysis was equal to or higher than the cut-off value, it was deemed positive. In the case where the result of the screening test was positive and THCCOOH was detected by GC-MS, it was deemed to be a true positive (TP) result, while if the results of both analyses were negative, it was it was deemed to be a true negative (TN) result. In the case where the result of the screening test was positive and the result of the GC-MS analysis was negative, this was it was deemed to be a false positive (FP), while if the result of the screening test was negative and the result of the GC-MS analysis was positive, it was it was deemed to be a false negative (FN). The sensitivity and specificity were calculated using the equations TP/(TP + FN)×100 and TN/(TN + FP)×100, respectively, while the method precision and accuracy were calculated using the equations TP/(TP+FP)×100 and (TP+TN)/(TP+TN+FN+FP)×100, respectively.

3. Results and Discussion

3.1. Optimization of GC-MS parameters

To optimize the chromatographic separation and improve the peak shape of the analyte, the GC temperature program was examined. In addition, since the hydroxyl and carboxyl groups of the analyte could be adsorbed on the inner wall of the non-polar capillary tube, thereby hindering separation, derivatization to the non-polar THCCOOH-2TMS was carried out, as described above. A full scan mass spectrum

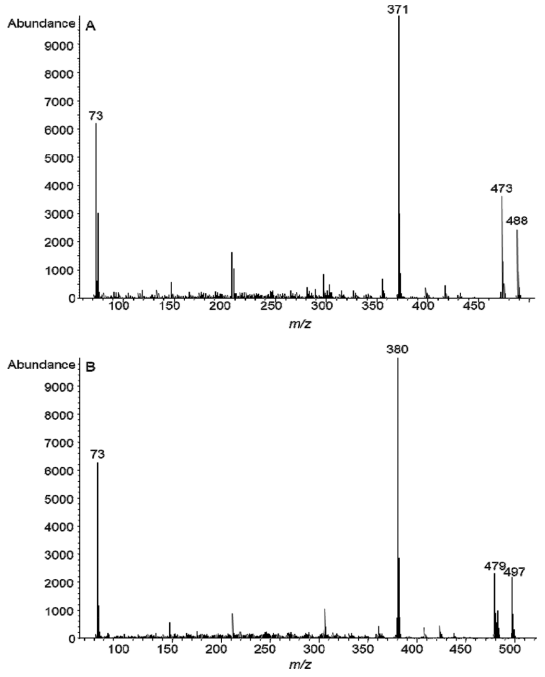

Fig. 1. GC-MS mass spectra for trimethylsilyl (TMS)-derivatives of (A) the analyte and (B) the internal standard.

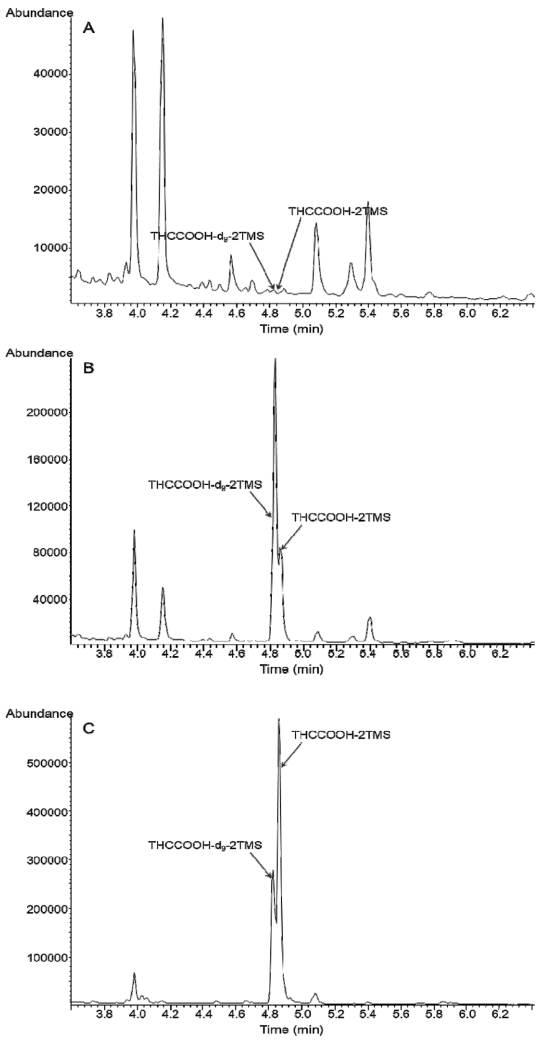

Fig. 2. Representative GC-MS extracted ion chromatograms of (A) blank urine without the IS, (B) spiked urine containing 20 ng/mL of THCCOOH and (C) cannabis positive urine sample.

was obtained to determine the quantifier and qualifier ions and to specify the retention times of the target compounds. Thus, *Fig.* 1 shows the mass spectra of THCCOOH-2TMS and the IS, while the RTs, quantifier ions, and qualifier ions of the derivatized analyte and the IS are shown in *Table* 1.

Representative GC-MS chromatograms obtained from the blank urine and spiked urine samples are shown in *Fig.* 2A and 2B. To determine any effects caused by exogenous interferences, the target analyte spiked at concentrations of 20 ng/mL in blank urine, whereby no significant interferences were observed.

For qualitative analysis using GC-MS, the acceptable ion ratio range was set within ±20 % of the average ratio of the quantifier ion peak height to the qualifier ion peak height obtained using aliquots of THCCOOH mixed with blank samples at concentrations of 20,

Table 1. Retention times, molecular weights, and ions selected for GC-MS analysis of the trimethylsilyl-derivatives of THCCOOH and its internal standard (IS)

Compound (TMS)	Retention time (min)	Molecular weight	Quantifier ion (*m/z*)	Qualifier ions (*m/z*)
THCCOOH-d_9 (IS)	4.84	497	380	497
THCCOOH	4.88	488	371	473, 488

50, and 100 ng/mL, and using the cannabis positive urine samples (n = 57). The ratios of the quantifier ion peak height to the qualifier ion peak heights (n = 2) for THCCOOH-2TMS were 3.04 and 4.65, respectively, and the tolerance intervals were 2.43-3.64 and 3.72-5.58, respectively.

3.2. Sample preparation

Ideally, extraction techniques should be facile, with new techniques allowing shorter extraction times and reduced solvent consumption. The selection of the extraction solvent is particularly important in liquid-liquid extractions. Thus, to evaluate the influence of the organic solvent volume on the extraction yield, a series of experiments were performed using different ratios of hexane to ethyl acetate, i.e., 9:1 (v/v), 8:1, 7:1, 6:1, 5:1, and ethyl acetate alone. Among the tested solvent mixtures, hexane:ethyl acetate (v/v, 9:1) resulted in the greatest extraction efficiency and provided a comparatively high analytical signal. The experimental results showed that the peak areas of the target compounds increased upon increasing the hexane content. Subsequently, a reciprocal shaker (65 rpm, 20 min) and vortex-assisted extraction (3200 rpm, 1 min) were compared for their effects on the liquid-liquid extraction, although no differences were observed. Vortex-assisted extraction was therefore employed to reduce the extraction time.[28]

When an analyte undergoes phase II metabolism, it is excreted in the urine as a glucuronide conjugate, for example, and this conjugate can release its free form by hydrolysis using acids, bases, and enzymes. In our case, since THCCOOH-glu was converted to THCCOOH by hydrolysis using a strong base, the remaining THCCOOH present in the urine combined with the THCCOOH derived from THCCOOH-glu could lower the LOD during instrumental analysis. The optimal hydrolysis condition was found as follows. First, while the hydrolysis temperature was fixed at 60 °C, the amount of THCCOOH-glu converted to THCCOOH was measured at intervals of 5 min over a 20 min period. The amount of THCCOOH detected after 5 min of hydrolysis was similar. Next, after fixing the hydrolysis time to 10 min, the amount of detection was measured at room temperature and up to 60-80 °C at 10 °C intervals. It was finally set to 60 °C and 10 min as there was no significant difference at other temperatures except for the room temperature.

The extraction was therefore carried out by addition of the extraction solvent to the urine sample and subsequent vortex-assisted extraction at 3200 rpm for 1 min. To remove any interfering substances, high-speed centrifugation was performed at 20000 g for 5 min to obtain the supernatant.

As reported previously, GC-MS method can be carried out in combination with a solid phase extraction and trimethylsilylation (TMS) derivatization reaction, which requires a total sample processing time (i.e., sample preparation plus instrumental analysis) of an hour. The LC-MS method can be used as a liquid phase extraction-based analysis method that does not include a hydrolysis step, requiring the sample processing time of half hour.[9,28] However, our developed method required a sample pretreatment time within 25 min, and the total sample preparation time was 30 min, thereby rendering it faster than the LC-MS method. Use of the IPD process shortened the analysis time, and was more economical than existing GC-MS and LC-MS methods.

3.3. Chemical derivatization

To shorten the derivatization time required prior to GC-MS analysis, derivatization of the target compound was achieved using IPD. Thus, BSTFA+1 % TMCS, which is suitable for the derivatization of a compound containing a hydroxyl group and a carboxyl group, was used as the derivatization reagent for the purpose of this study.[29] The optimal conditions for this process were determined by initially examining a range of ethyl acetate to BSTFA+1 % TMCS ratios, i.e., 1:0 (v/v), 1:1 (v/v), and 1:4 (v/v), whereby a ratio of 1:1 was found to provide the best efficiency. When the content of the derivatization reagent was higher than that of ethyl acetate, the baseline increased. On the other hand, when the ethyl acetate content was increased above the optimal value, the reaction efficiency decreased. Upon examination of the baseline levels and the peak areas of the target compounds

after the derivatization process, it was apparent that the optimal peak shape and maximum area were obtained at a ratio of 1:1(v/v).

The GC injector temperature is also an important parameter in terms of the IPD efficiency, since the derivatization reaction takes place at the high-temperature GC injection port. We therefore examined injection port temperatures of 260-300 °C at 20 °C intervals, and found that the peak area of the analyte increased as the temperature of the injection port increased. The highest peak area was detected at 300 °C; however, a value of 280 °C was employed upon considering manufacturer recommendations and the durability of the septum. Moreover, the purge flow and purge-on time were also optimized, with values of 75 mL/min and 1.5 min being selected for further experiments (*Fig.* 3).

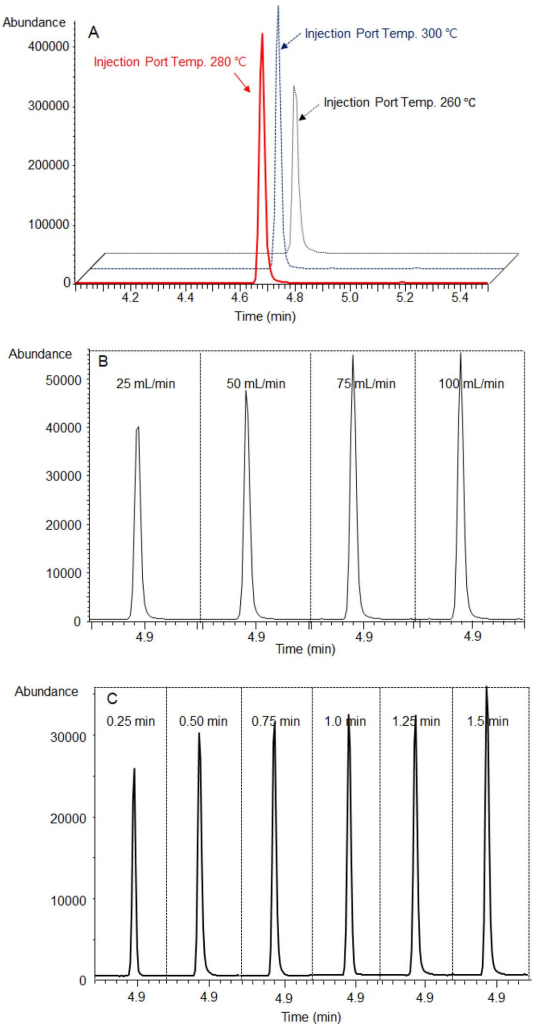

Fig. 3. Optimal conditions of (A) injection-port temperature, (B) purge flow and (C) purge-on time for injection-port derivatization GC-MS analysis.

As a result of chemical derivatization, THCCOOOH-2TMS was obtained by the attachment of two trimethylsilyl (TMS) functional groups. To confirm the stability of this compound, stability tests were performed at room temperature over 12 h (testing at 1 h intervals) using a GC-MS instrument equipped with an automatic liquid sampler. THCCOOOH-2TMS was found to be stable at room temperature for up to 12 h, with a relative standard deviation of 3.3 %.

3.4. Method validation

The selectivity, LOD, repeatability, sensitivity, specificity, precision, and accuracy of the developed method were then evaluated. Blank urine samples (n = 12) were tested to examine the selectivity, where no interfering substances were present to affect analysis of the target compound. Importantly, following the addition of THCCOOH-glu as a potentially interfering compound that could be present in authentic urine samples, no interference was observed.

The LOD was determined to be 0.32 ng/mL, which is considerably lower than the cut-off value (20 ng/mL) of the immunoassay. *Table* 2 shows the repeatability for the QC samples of three different concentrations, and this was found to be within 9.1 %.

The specificity and sensitivity were calculated by comparing the immunoassay and GC-MS results, as outlined in *Table* 3. More specifically, the sensitivity

Table 2. Repeatability of the measurements (n = 7)

Compound	Nominal concentration (ng/mL)	Repeatability[a] (% RSD)
THCCOOH	20	5.3
	50	7.2
	100	9.1

[a]Repeatability expressed as RSD (relative standard deviation) of the peak area ratios of the analyte to the internal standard.

Table 3. Test results and performance of the Cobas c311 analyzer vs GC-MS

			GC-MS		
			Positive	Negative	Sum
Test results	Cobas c311 Analyzer	Positive	57 (TP[a])	0 (FP[b])	57
		Negative	9 (FN[c])	155 (TN[d])	164
		Sum	66	155	221
Performance	Sensitivity (%)	86.4	TP/(TP+FN)×100		
	Precision (%)	100.0	TP/(TP+FP)×100		
	F-measure	0.93	2(Sensitivity×Precision)/(Sensitivity+Precision)		
	Specificity (%)	100.0	TN/(TN+FP)×100		
	Accuracy (%)	95.9	(TP+TN)/(TP+TN+FN+FP)×100		
	FP rate (%)	0.0	FP/(TP+TN+FN+FP)×100		
	FN rate (%)	4.1	FN/(TP+TN+FN+FP)×100		

[a]TP: true positives, [b]FP: false positives, [c]FN: false negatives, [d]TN: true negatives

and specificity were determined to be 86.4 and 100 %, while the precision and accuracy were 100 and 95.9 %, respectively. The kappa value, which is a coefficient between 0.8 and 1.0, represents the degree of agreement between the immunoassay and GC-MS. In our case, an excellent degree of agreement was evident, with a kappa value of 0.8988 being obtained. In addition, the F-measure value, a classification evaluation value considering both sensitivity and precision expressed as a harmonic average, was calculated as 0.93. This means that the assay was not biased toward either sensitivity or precision. To evaluate the possibilities of cross-reactivity, the false positive rate (FP rate, %) was calculated. Importantly, no cross-reaction was found in the forensic urine samples (n = 221). The false negative rate (FN rate, %) was 4.1 %. As the confirmatory test, GC-MS analysis was able to detect up to 0.32 ng/mL, which was a much lower concentration than the cut-off value at 20 ng/mL, resulting in the nine false negative samples.

Finally, the confusion matrix was calculated using the R-3.6.3 caret package (www.r-project.org), and its code is depicted in *Fig*. 4. *Table* 3 present the classification table and statistics obtained from the combined immunoassay and GC-MS results.

```
Confusion Matrix and Statistics

          Reference
Prediction Negative Positive
  Negative      155        9
  Positive        0       57

               Accuracy : 0.9593
                 95% CI : (0.9241, 0.9812)
    No Information Rate : 0.7014
    P-Value [Acc > NIR] : < 2.2e-16

                  Kappa : 0.8988

 Mcnemar's Test P-Value : 0.007661

            Sensitivity : 0.8636
            Specificity : 1.0000
         Pos Pred Value : 1.0000
         Neg Pred Value : 0.9451
             Prevalence : 0.2986
         Detection Rate : 0.2579
   Detection Prevalence : 0.2579
      Balanced Accuracy : 0.9318

       'Positive' Class : Positive
------------
# confusion matrix for THCCOOH

library(caret)
library(ggplot2)

cd1 <- read.csv("C:/Rwork/ca_data_2020.csv", header = T)
predicted <- factor(cd1$y_pred,levels=c(0,1),
                    labels=c("Negative","Positive"))
actual <- factor(cd1$y_true,levels=c(0,1),
                 labels=c("Negative","Positive"))
cm1 <- confusionMatrix(data = predicted, reference = actual,
                       positive = "Positive")
show(cm1)
```

Fig. 4. Statistical analysis of the confusion matrix.

3.5. Forensic applications

The detection of THCCOOH by immunoassay screening alone is not considered reliable or accurate due to the possibility of cross-reactivity and an insufficient specificity/sensitivity. Therefore, a two-step method was employed consisting of an initial screening test followed by a more specific confirmatory test if the specimen screened positive. After immunoassay screening, forensic urine samples (n =

221) were tested using the developed method as the confirmatory test, and representative GC-MS chromatograms of the urine samples of cannabis abusers are shown in *Fig.* 2C. The obtained results therefore indicate that the methodology developed in this study allows for the successful detection of THCCOOH in forensic urine samples.

4. Conclusions

We herein reported the development and validation of a qualitative gas chromatography-mass spectrometry method for the detection of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH), a metabolite of Δ^9-tetrahydrocannabinol, in human urine. Essentially, this method was developed for confirmation following an immunoassay screening, and following its validation, it was applied in the analysis of forensic urine samples (n = 221). The use of vortex-assisted extraction and high-speed centrifugation enabled a reduction in the sample extraction time to <1 min, and successfully removed interfering substances to achieve sample purification. In addition, an injection-port derivatization method was effectively employed to shorten the derivatization time. Upon application of our method to forensic urine samples of potential cannabis users, the test results for THCCOOH were obtained quickly and accurately without further analysis being required. These results are of importance due to the issues related to increased cannabis usage and the associated demands on testing facilities.

Acknowledgements

The authors declare no competing financial interest.

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