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Fast gas chromatography and negative-ion chemical ionization tandem mass spectrometry for forensic analysis of cannabinoids in whole blood

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Abstract

The present work describes a fast gas chromatography/negative-ion chemical ionization tandem mass spectrometric assay (Fast GC/NICI-MS/MS) for analysis of tetrahydrocannabinol (THC), 11-hydroxy-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) in whole blood. The cannabinoids were extracted from 500 μL of whole blood by a simple liquid–liquid extraction (LLE) and then derivatized by using trifluoroacetic anhydride (TFAA) and hexafluoro-2-propanol (HFIP) as fluorinated agents. Mass spectrometric detection of the analytes was performed in the selected reaction-monitoring mode on a triple quadrupole instrument after negative-ion chemical ionization. The assay was found to be linear in the concentration range of 0.5–20 ng/mL for THC and THC-OH, and of 2.5–100 ng/mL for THC-COOH. Repeatability and intermediate precision were found less than 12% for all concentrations tested. Under standard chromatographic conditions, the run cycle time would have been 15 min. By using fast conditions of separation, the assay analysis time has been reduced to 5 min, without compromising the chromatographic resolution. Finally, a simple approach for estimating the uncertainty measurement is presented.
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Keywords: Fast GC; NICI-MS/MS; Cannabinoids; Whole blood; Validation; Forensic toxicology

1. Introduction

Cannabis is considered to be the most widely abused illicit drug in Europe. Indeed, statistical information shows that 30% of the under-forties age group has already consumed this drug. Such consumption levels necessitate fast, sensitive, and reliable methods of analysis to be employed by forensic laboratories to assist police investigations.

In humans, tetrahydrocannabinol (THC) is extensively metabolized in its two main metabolites: 11-hydroxy-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH). All three compounds are detected in blood and the quantification of THC is absolutely necessary in cases involving drivers under the influence of drugs [1]. Indeed, a person is considered unable to drive when THC level in blood is higher than 0.5–2 ng/mL according to the countries. Knowledge of the two metabolite concentrations becomes interesting with the application of mathematical models, which can predict the time when marijuana was consumed [2] and also in estimating the user's driving capacity [3].

Several reviews [4–6] summarize the techniques used to analyze cannabinoids. Gas chromatography combined with mass spectrometry (GC/MS) and electron impact ionization (EI) remains the major analytical tool for determination of THC and its metabolites in blood, serum, and plasma. Only a few papers report the application of liquid chromatography (LC) and particularly LC/MS [7]. Analyses are usually performed in the simple EI mode that provides scope for library search [8]. However, negative-ion chemical ionization (NICI) is a good alternative because the soft ionization observed can give a better sensitivity and selectivity for compounds having high electronegativity [9,10]. Nevertheless, this ionization mode often provides only

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one ion which is not enough for toxicological requirements. The use of tandem mass spectrometry (MS/MS) may allow reaching enough transitions for identification and quantification.

Moreover, an interesting way to improve cannabinoid analysis is to increase sample throughput. Indeed, the use of fast GC will provide higher capacity of the laboratory, and therefore, a significant coast reduction per unit of sample.

The purpose of this work was first, to show the power of NICI coupled with tandem mass spectrometry (NICI-MS/MS) in fast analyses of toxicological standard compounds, and second, to develop and establish the validity of a routinely applicable method that allows quantification of THC, THC-OH, and THC-COOH by decreasing analysis time. This article describes a useful method for analysis of cannabinoids in whole blood, which has the advantage of small specimen size, rapid sample preparation, accelerated chromatography, and the use of selective NICI-MS/MS.

2. Experimental

2.1. Chemicals and reagents

THC, THC-OH, THC-COOH, THC d_3, THC-OH d_3, and THC-COOH d_3 at 1000 or 100 μg/mL in methanol were purchased from Cambridge Isotope Laboratory (Andover, USA).

Working standard solutions were prepared by dilution in methanol of stock solutions to reach concentrations of interest ranging from 0.01 to 1 μg/mL. After use, stock and working solutions were stored at −20 °C.

Trifluoroacetic anhydride (TFAA) and hexafluoro-2-propanol (HFIP) were obtained from Supelco (Bellefonte, USA) and Fluka (Buchs, Switzerland), respectively. All solvents used such as hexane, methanol, chloroform, and ethyl acetate were of high-performance chromatographic grade from Merck (Darmstadt, Germany). Human blood was supplied by the University Hospital of Geneva (Geneva, Switzerland). Commercial controls were purchased from Medichem (Steinenbronn, Germany).

2.2. Preparation of solutions

Working standard solutions were used to spike 500 μL of whole blood to reach concentrations of interest. Thus, a final concentration of 50 ng/mL of THC d_3 and THC-OH d_3, and 100 ng/mL of THC-COOH d_3 was obtained by adding 25 μL of a solution at 1 μg/mL of THC d_3 and THC-OH d_3, and 50 μL of a solution at 1 μg/mL of THC-COOH d_3 in each sample as internal standard solutions (IS).

Calibration samples were prepared by spiking whole blood at five concentration levels ranging from 0.5 to 20 ng/mL for THC and THC-OH, and from 2.5 to 100 ng/mL for THC-COOH.

2.3. Sample pretreatment

After spiking with IS and adding 100 μL of acetic acid (10%), 500 μL of blood sample was treated by liquid–liquid extraction (LLE) with 2.5 mL of hexane/ethyl acetate (9:1, v/v) by slow horizontal shaking (approximately 200 moves/min) for 10 min followed by centrifugation at 4350 rpm for 10 min. The upper organic phase was then transferred into a conical vial and evaporated to dryness under a gentle stream of nitrogen. Analyte and IS derivatization was achieved by adding 150 μL of chloroform, 150 μL of TFAA, and 75 μL of HFIP. The conical vials were mixed for 15 s, heated at 70 °C for 25 min and then dried under air at 40 °C. The fluorinated derivates were reconstituted with 50 μL of hexane and 2 μL were injected into the GC/MS system. The derivatization method followed is the same as previously reported [11].

2.4. GC/NCI-MS/MS analysis

2.4.1. Equipment

Analyses were performed on a Varian CP 3800 gas chromatograph (Walnut Creek, CA, USA) in combination with a CTC Combi-PAL autosampler (Zwingen, Switzerland), and a Varian 1200 L MS/MS triple quadrupole mass spectrometer (Walnut Creek, CA, USA). Data acquisition and analysis were performed using Varian MS Workstation software (6.8).

2.4.2. Chromatographic conditions

Substances were separated using a fused-silica capillary column (DB-5MS, 15 m × 0.25 mm i.d., film thickness 0.25 μm) and high-purity helium 50 (99.999%) was used as the carrier gas with a constant flow of 1 mL/min. In all analysis, the injector temperature was set at 250 °C and splitless injection was employed with the injection purge valve remaining closed for 1 min.

Under conventional GC/MS/MS, the initial column temperature was set at 70 °C for 1 min, increased to 190 °C at 25 °C/min, and then the rate was slowed to 5 °C/min until it reached 210 °C. Finally, the temperature was increased at 30 °C/min to reach 290 °C and held for 2.5 min for an analysis time of 15 min.

Under Fast GC/MS/MS, the initial column temperature was set at 100 °C for 1 min, increased to 215 °C at 70 °C/min, and then the rate was slowed to 40 °C/min until it reached 240 °C. Finally, temperature was increased at 70 °C/min to reach 290 °C and held for 1 min for an analysis time of 5 min.

2.4.3. MS detection

The NICI mode was used with methane (at a purity of 99.9995%) as reagent gas at a pressure between 8 and 8.5 Torr. Transfer line, manifold, and ion source were operated at 275, 40, and 150 °C, respectively. The MS/MS experiments were based on collisionally induced dissociation (CID) occurring in the collision cell (quadrupole 2), with an argon collision gas pressure of 1.3 mTorr.

All MS/MS parameters are shown in Table 1. The transitions chosen for each compound were based on criteria of abundance and selectivity. The different parameters relative to NICI-MS/MS (reagent gas pressure, CID pressure, collisional energy) were optimized with the use of a statistical approach

Table 1
MS/MS method parameters for cannabinoids

Cannabinoids	Molecular mass (g/mol)		Retention time (min)	FWHM (s)	Dwell time (ms)	MS/MS		
	Compound	Derivative				$Q1 > Q3$ (m/z)	Relative abundance	Collision energy (eV)
THC d_3	317	413	3.677	0.487	25	413.1 > 316.3		20
THC	314	410	3.681	0.485	25	410.3 > 313.3	100	20
						410.3 > 350.2	36	5
						410.3 > 410.2	28	5
THC-COOH d_3	347	593	3.795	0.488	25	425.2 > 364.2		10
THC-COOH	344	590	3.799	0.491	25	422.3 > 361.2	100	10
						422.3 > 309.3	22	25
						422.3 > 402.2	67	5
THC-OH d_3	333	525	3.889	0.499	25	412.0 > 342.2		20
THC-OH	330	522	3.893	0.497	25	409.2 > 339.2	100	20
						409.2 > 151.2	85	15
						409.2 > 409.2	79	5

Quantitation transitions are underlined. The other ones are used to identify the molecule.

(data not presented here). In fast mode GC, duty cycle was decreased to 0.5–0.15 s to obtain a sufficient number of work points to meet quantitative needs (between seven and nine points).

2.5. Validation procedure

Both analytical methods were validated according to a general strategy based on the guidelines of the "Société Française des Sciences et des Techniques Pharmaceutiques" (SFSTP) [12] which was adapted to our specific requirement.

The validation was carried out over three non-consecutive days ($p = 3$). Results allowed establishing a statistical treatment based on variance analysis (ANOVA) to determine the precision and trueness of the method.

For the experiment, a validation day consisted of the preparation of two kinds of whole blood samples, which were calibration samples (Cal) and quality control samples (QC). The Cal were prepared in double ($n = 2$), at five concentration levels ($k = 5$; Cal = 0.5, 1, 5, 10, and 20 ng/mL for both THC and THC-OH, and Cal = 2.5, 5, 10, 50, and 100 ng/mL for THC-COOH).

The analysis of these samples allowed determining the response function of the three analytes for each day. Moreover, the dynamic range of the assay was chosen according to the concentrations of real cases generally observed in routine (see Section 3.5).

The QC were prepared independently in the same way in quadruplicate ($n = 4$), at four concentration levels ($k = 4$; QC = 0.5, 1, 10, and 20 ng/mL for both THC and THC-OH, and QC = 2.5, 10, 50, and 100 ng/mL for THC-COOH) representing the entire range of concentration.

The spiked samples were then treated according to sample pretreatment procedure described earlier. Validation allowed determining specific criteria such as trueness, precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), and extraction recovery, which will be discussed later.

3. Results and discussion

3.1. Sample pretreatment

Sample preparation is an important part of a method development when complex matrices such as whole blood are used. Indeed, the choice of extraction strategy can improve sensitivity and selectivity by a better recovery and by increasing the elimination efficiency of endogenous compounds.

Recovery (extraction efficiency) was determined by comparing the analyte peak area obtained from spiked blood samples having undergone sample pretreatment with corresponding methanolic solutions. Assuming that no suppression occurs during ionization, the results showed that recoveries were of 82, 68, and 55% for THC, THC-OH, and THC-COOH, respectively, with coefficients of variation ($n = 3$) below 5%. The lower recoveries observed for polar compounds were widely compensated with the use of NICI. Indeed, THC-OH and THC-COOH will have, after derivatization with fluorinated agents, a greater number of electronegative atoms than THC, as shown in Fig. 1. In this way, captured properties of thermal energy electrons, arising from the primary ionization reactions of reagent gas, will be more efficient (i.e., more associative owing to a better electroaffinity) for these compounds, and therefore, sensitivity will be greater. Hence, this ionization mode allows using a simpler extraction procedure and so decreasing both reagent and time consumption, with regard to SPE procedures generally used for analysis of cannabinoids [13–15].

Moreover, the overall gain of sensitivity is very interesting since it is then possible to decrease the amount of blood sample at 500 μL instead of the usual 1 mL. This may be important for post-mortem cases where there is often less available blood.

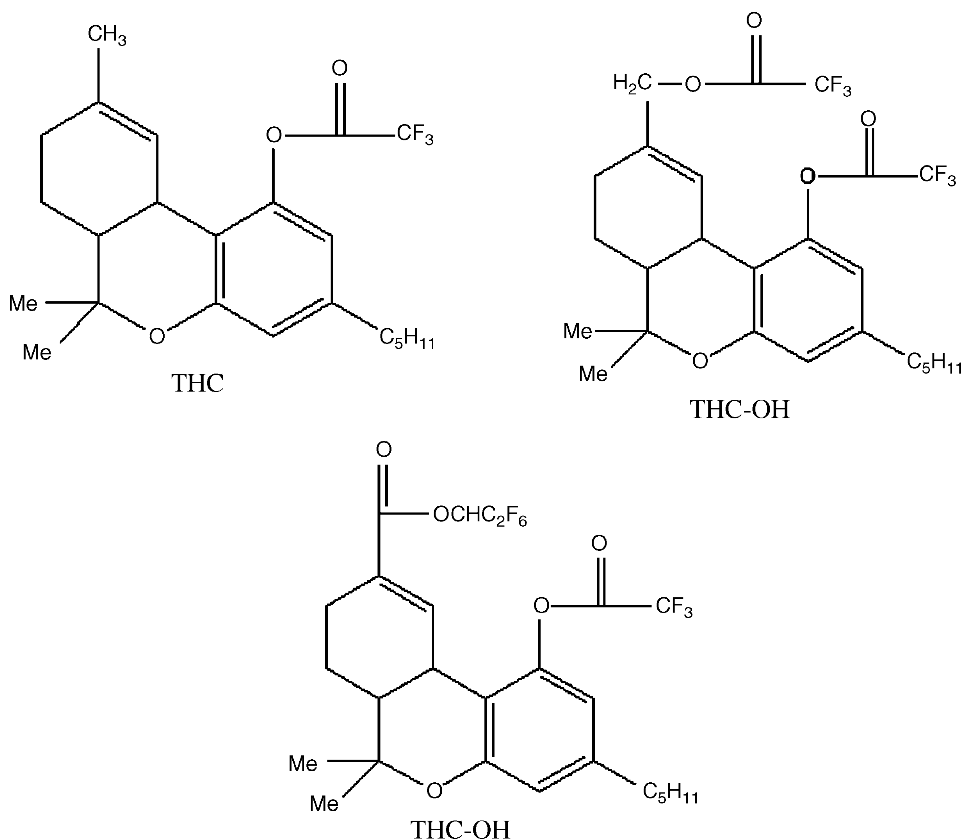

Fig. 1. Chemical structures of the studied cannabinoids after derivatization.

Another advantage of this derivatization is the high mass increment, improving the selectivity by an increase of molecular weight.

3.2. Fast GC

Fast GC clearly has a number of advantages for laboratories. Among them, throughput can be increased significantly, resulting in better productivity and in lower operational costs per sample. Numerous studies have been published regarding theoretical approaches to speed optimization of GC [16,17]. A more accessible equation than those presented in these publications can be used to establish a relation between the retention time and experimental parameters:

$$t_r = \frac{L}{\bar{u}}(k+1) \quad (1)$$

where t_r is the retention time (s), L is the column length (cm), $\bar{u}$ represents the average linear carrier gas velocity (cm/s), and k is the capacity factor.

This equation shows that there are different strategies to decrease analysis time depending on the specific application needs [18]. Indeed, if analysis needs a high resolution, it would be suitable to modify a parameter which does not change resolution, for example, by reducing the diameter of the capillary column. In our case, great separations were observed for all our compounds in conventional GC with an analysis time (t_a) of 15 min, which is reinforced by the selectivity of MS/MS.

An interesting procedure, then, was to trade resolution for time. Among different possibilities such as reducing column length or increasing carrier gas velocity, fast programming temperature was considered to be very attractive. Indeed, the use of a 15 m × 0.25 mm × 0.25 μm column is a good option for the robustness of a method intended for toxicology analysis where blood is a complex matrix, and where frequent maintenances are carried out. Another aspect is that by keeping the same column, it is always possible to lead analysis of other compounds which were carried out on the device without making a new validation.

Nevertheless, according to the Golay–Giddings equation, it could be a good procedure to increase carrier gas velocity slightly above optimal carrier gas velocity (approximately: $\bar{u} = 2\bar{U}_{opt}$). Indeed, this area of the Golay–Giddings curve corresponds to a decrease in retention time for a weak decrease of resolution [19]. Its limitation is that most of the commercial MSs are designed to work optimally at 1–2 mL/min of He flow-rate to ensure a good pumping capability of the device.

Another point to consider is that a modification of both column length and carrier gas velocity implies a diminution of retention times of compounds, but the overall analysis time only depends on column oven program, cool-down, and equilibration time.

By applying fast programming until 70 °C/min, the Fast GC method showed full widths at half-maximum (FWHM) close to 0.5 s (see Table 1) instead of FWHM greater than 2 s by conventional GC. In the same way, time analysis was reduced from 15

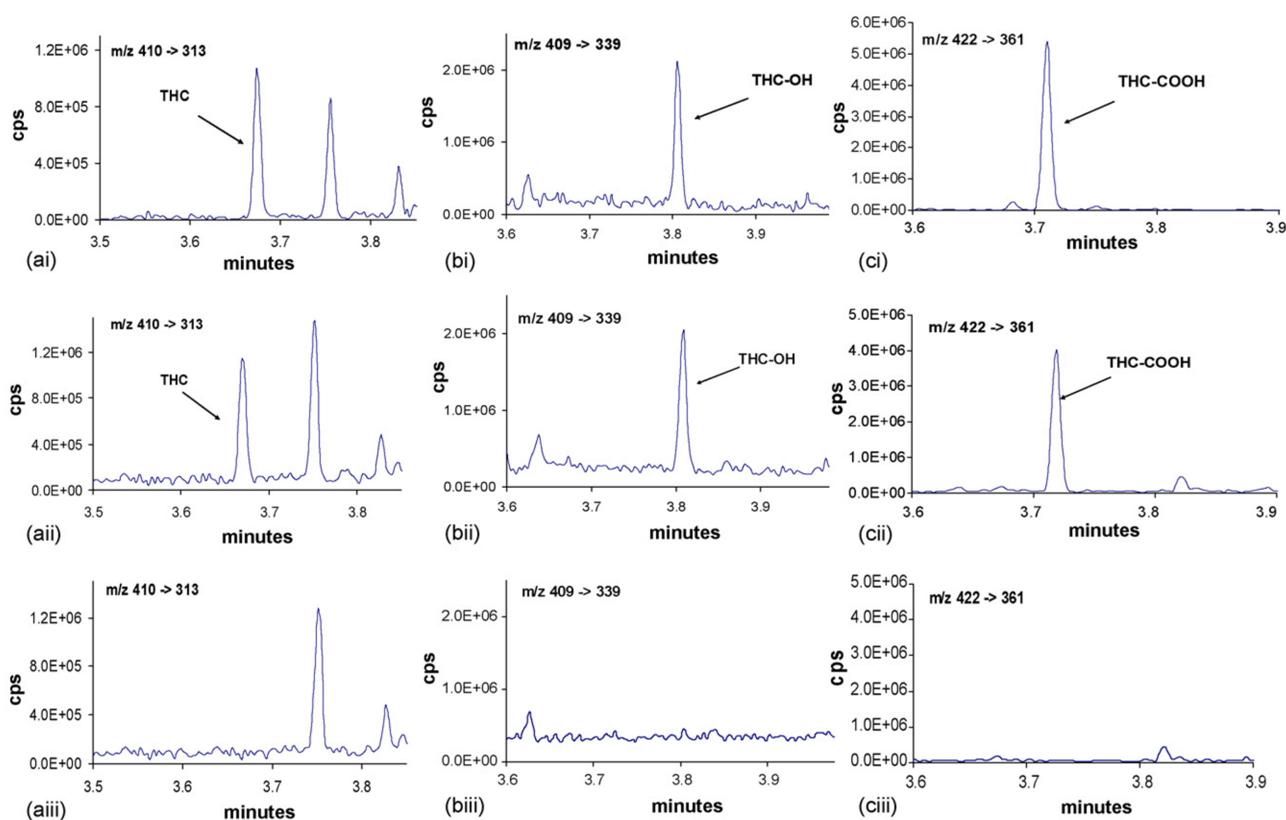

Fig. 2. Representative selected reaction monitoring chromatograms in human whole blood for THC (a), THC-OH (b), and THC-COOH (c) with a real case close to the LOQ (i), a quality control (QC) at LOQ (ii), and a blank blood sample spiked with internal standards (iii).

to 5 min. According to ref. [18], such values are in agreement with fast GC classification.

To gain time on both analysis and recycle times, we have increased the initial oven temperature from 70 to 100 °C. Indeed, the time necessary to cool the oven is longer from 100 to 70 °C than from 200 to 100 °C. In spite of the initial oven temperature being above the boiling point of the solvent, the peak widths of our compounds are all narrow. An explanation may be that in this case, stationary phase focusing predominates. Thus, conditions allowing solvent focusing in splitless mode are unnecessary here, since they lead to longer run times. Fig. 2 illustrates the separation observed for compound of interest by Fast GC.

A usual way to achieve Fast GC is to increase data sampling rate to obtain enough points across a peak. For the experiment, the duty cycle was decreased from 0.5 to 0.15 s. This modification leads to obtaining seven to nine points across a peak, which permitted, as shown in ref. [20], to reach quantitative needs.

3.3. NICI-MS/MS

The NICI ionization mode is well suited for analyzing the xenobiotic with electronegative atoms in whole blood. Indeed, the enhancement of both selectivity and sensitivity observed with NICI is even more advantageous because whole blood contains very few compounds having electronegative moieties. This was checked by testing selectivity of six whole blood samples with NICI and EI ionization modes. The results were in agreement, since chromatograms obtained in NICI mode presented a general decrease in the background interference (see Fig. 3) and also a greater resolution. Thus, as discussed earlier, separation can be strongly accelerated without significant loss of resolution.

In CI mode, the high pressure (8.5 Torr) of the reagent gas implies that formed ions will be thermalized owing to many low-energy collisions. Thus, the precursor ion arrives in front of the CID with a very low internal energy. Hence, the product ion spectra in NICI mode showed a weaker fragmentation than in the EI mode for the same collisional energy and, therefore, fragment ions having a better sensitivity (shown in Fig. 4). Indeed, NICI will influence both degree and kind of observed fragmentation

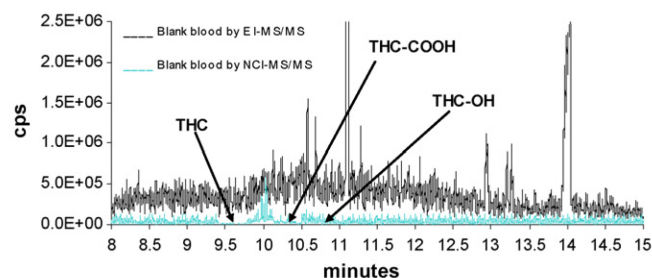

Fig. 3. Comparison of the background interference for injection of blank blood sample by EI-MS/MS (dark line) and NICI-MS/MS (light line). The arrows indicate the retention times of cannabinoids by conventional GC.

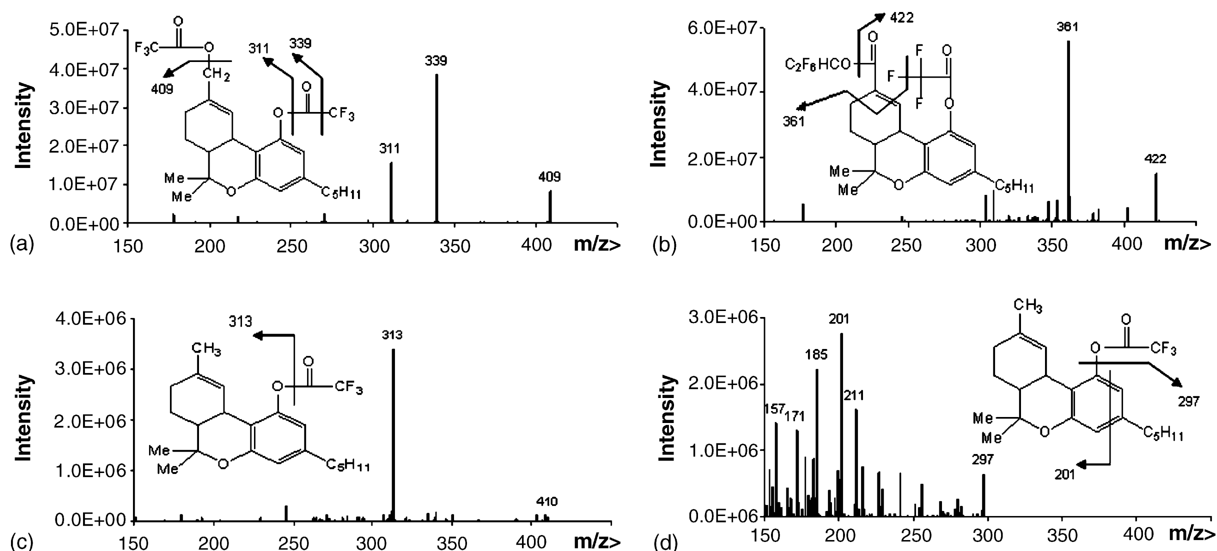

Fig. 4. Product ion spectra of derivative cannabinoids in a triple quadrupole mass spectrometer after collision-induced fragmentation in the second mass spectrometer with a collisional energy of 20 eV for all compounds: (a) THC-OH in NICI mode, (b) THC-COOH in NICI mode, (c) THC in NICI mode and (d) THC in EI+ mode. All spectra were obtained with solutions at 100 ng/mL for each compound.

processes by supporting those having a weak activation energy, which will lead generally to product ions of a relatively high mass. Furthermore, a toxicological limitation with the use of NICI-MS is the lack of obtained ions to carry out quantification and qualification. The use of MS/MS allows making up for this by providing several transitions (see Table 1).

3.4. Validation

In preliminary assays, several regression models were fitted to select the most suitable response function. It was determined by calculating the existing relationship between the response (the drug to internal standard peak area ratio of each compound) and the expected concentration [21,22], and by applying different weighting factors. A comparison was realized between the simple linear regression model based on the least square method and the weighted linear regression model with a weight equal to $1/X$, and also with a weight equal to $1/X^2$. The weighted linear regression model with a weight of $1/X$ was finally chosen, taking into account the relationship between the natural logarithm of the response variance and the natural logarithm of the concentration as described elsewhere [22]. Fig. 5, by comparing the two simplest regression models, shows interest in the use of a weighted regression model to increase the statistical weight of the lower concentrations. Indeed, the simple linear regression model will be generally more influenced by the upper concentrations. However, it can be noted that the use of another weighted regression model ($1/X^2$) did not ameliorate the results (data not shown). The validation data for GC and Fast GC method being very close, only those about Fast GC were presented in Table 2.

3.4.1. Selectivity

As discussed earlier, the selectivity of the method was estimated by analyzing six different blank bloods. Fig. 2 shows the blank blood samples observed for the selected reaction monitoring used for quantification. Thus, it can be noted that no interfering peaks were observed in chromatograms at the retention times of interest compounds.

Table 2
Validation data (k is the number of concentration levels, n the number of repetitions by levels, and p the number of non-consecutive days)

Validation criterion	THC	THC-OH	THC-COOH
Trueness ($k=4$; $n=4$; $p=3$) (%)			
0.5 ng/mL	101.3	107.1	–
1 ng/mL	99.7	100.7	–
2.5 ng/mL	–	–	100.3
10 ng/mL	95.3	91.1	104.7
20 ng/mL	100.9	100.1	–
50 ng/mL	–	–	99.9
100 ng/mL	–	–	102.6
Precision ($k=4$; $n=4$; $p=3$)			
Repeatability/intermediate precision (R.S.D.%)			
0.5 ng/mL	11.3/11.3	7.9/7.9	–
1 ng/mL	6.5/7.8	6.1/7.5	–
2.5 ng/mL	–	–	6.2/10.4
10 ng/mL	4.0/4.0	3.7/3.8	7.4/9.0
20 ng/mL	3.4/3.4	3.1/3.8	–
50 ng/mL	–	–	7.1/7.1
100 ng/mL	–	–	2.3/5.6
Linearity ($k=4$; $n=4$; $p=3$)			
Range (ng/mL)	[0.5–20]	[0.5–20]	[2.5–100]
Slope	1.0049	0.9926	1.023
Intercept	−0.1092	−0.1475	−0.1797
R^2	0.9991	0.9975	0.9998
LOQ (ng/mL)	0.5	0.5	2.5

3.4.2. Trueness and precision

The trueness and the precision of the method were determined with independent QC samples at each concentration level.

Trueness refers to the closeness of agreement between a conventionally accepted value and a mean experimental one [23]. It thus indicates systematic errors. Hence, the trueness was determined by calculating the percentage difference between experimental and theoretical concentration values. For the three compounds studied and for the overall concentration levels, the relative bias of the developed method varied from 0.1 to 8.9%.

Precision indicates random errors. It was assessed by computing the relative standard deviations for the repeatability ($R_{R.S.D.}$) and the between day variability expressed as the intermediate precision ($IP_{R.S.D.}$) [21–23]. Repeatability represents the given precision under conditions of repeatability (the same operator, the same samples, the same reagents, etc.). The intermediate precision represents the precision resulting from repetitions carried out in a laboratory on the same sample but in different conditions (here different days and reagents). As shown in Table 2, both $R_{R.S.D.}$ and $IP_{R.S.D.}$ values were less than 12% for all concentrations tested.

3.4.3. Linearity

Linearity of an analytical method is its capacity inside a concentration range to provide results directly proportional to the concentration contained within the sample [23]. Linearity was calculated by fitting the back-calculated concentrations of the QCs as a function of the introduced concentrations and by applying the linear regression model based on the least square method [22]. The coefficient of determination (R^2) obtained for the three cannabinoids were above 0.9975. In the same way, the slope values were between 0.9926 and 1.023. These founded values show that the method was linear.

3.4.4. Accuracy, uncertainty measurement, LOD and LOQ

Accuracy takes into account the total error, that is, the systematic and random errors, related to the test results [12,23]. It is represented by the accuracy profiles illustrated in Fig. 5. These

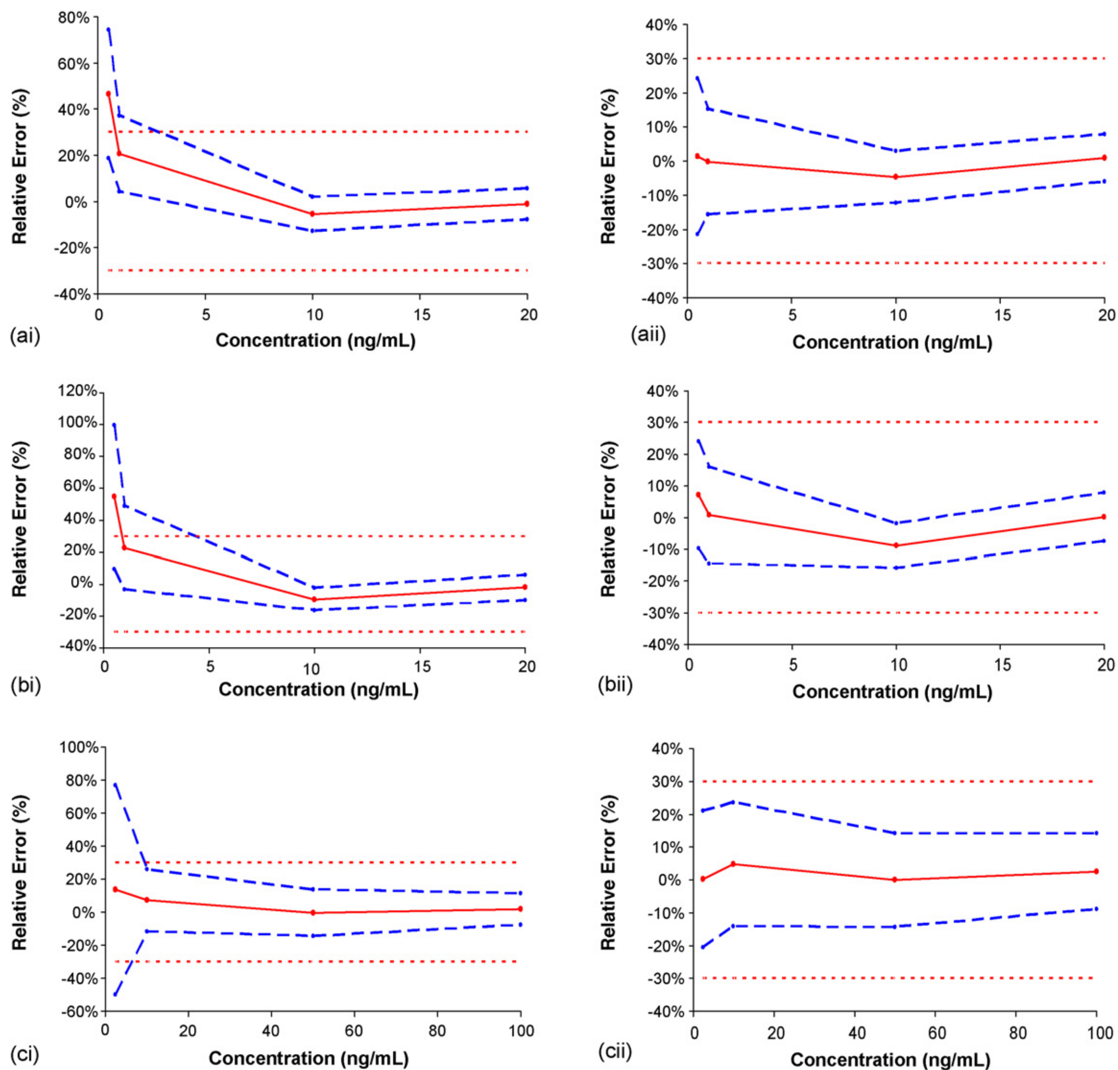

Fig. 5. Accuracy profiles for THC (a), THC-OH (b), THC-COOH (c) with a simple linear regression model (i) and with a weighted linear regression model with a weight equal to 1/*X* (ii). The continuous line represents the trueness, the dashed lines are the upper and lower accuracy limits in relative values and the dotted lines are the upper and lower 30% tolerance limits required in forensic toxicology.

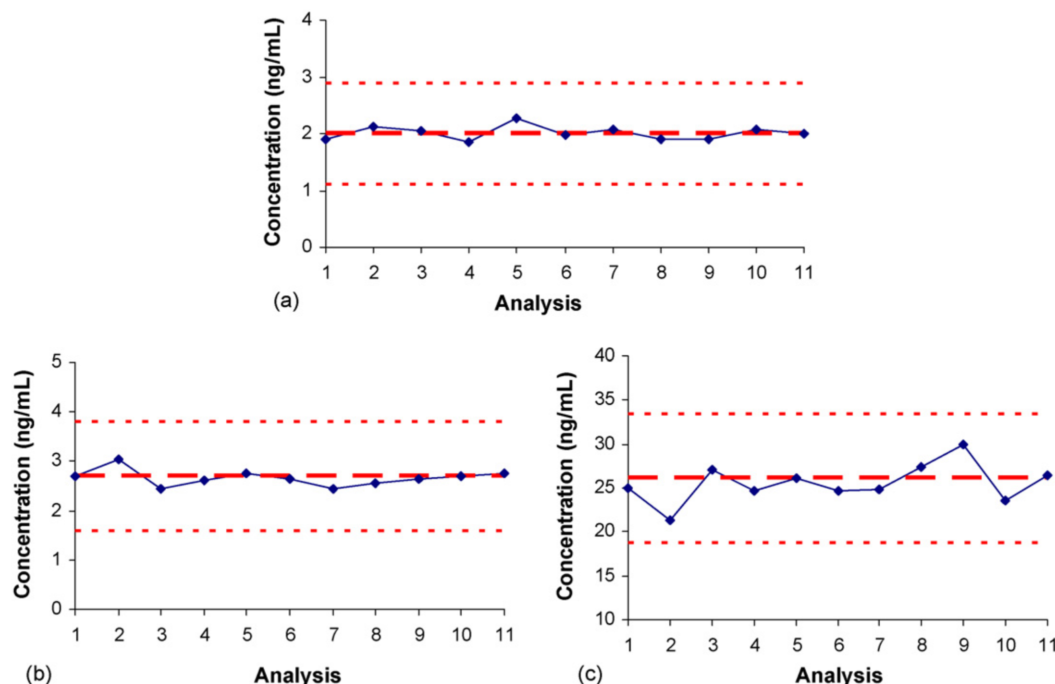

Fig. 6. Results for 11 analyses of commercial controls over a period of 6 months: (a) THC, (b) THC-OH and (c) THC-COOH. The continuous line represents the found concentration, the dashed line represents the attempted concentration and the dotted lines are the upper and lower acceptance limits given by the commercial control.

Table 3
Quantitation of 25 real cases with the calculation of the estimated time between smoking and blood collection and the time interval defined by the lowest and highest 95% confidence limits [2]

Case	[THC] (ng/mL)	[THC-OH] (ng/mL)	[THC-COOH] (ng/mL)	Estimated time (h)	Time interval (h)
1	1.3	0.6	7.9	1.84	[0.70–4.85]
2	7.7	2.6	31.5	1.50	[0.57–3.94]
3	5.1	4.0	35.9	2.06	[0.78–5.42]
4	0.8	0.4	8.5	2.71	[1.03–7.16]
5	1.8	0.5	19.9	2.69	[1.02–7.11]
6	2.1	0.6	6.0	1.23	[0.47–3.24]
7	14.9	9.6	98.6	1.98	[0.75–5.22]
8	6.0	3.2	22.8	1.44	[0.55–3.78]
9	12.6	5.8	34.4	1.19	[0.45–3.13]
10	3.9	1.2	35.4	2.37	[0.90–6.24]
11	7.2	1.5	36.0	1.69	[0.64–4.44]
12	4.4	1.3	6.2	0.82	[0.31–2.15]
13	3.3	1.0	47.3	3.07	[1.16–8.10]
14	17.5	1.1	3.0	0.24	[0.09–0.64]
15	4.8	3.0	19.1	1.48	[0.56–3.89]
16	15.4	9.1	97.4	1.93	[0.73–5.07]
17	13.1	5.9	64.8	1.68	[0.64–4.41]
18	2.5	0.8	15.4	1.84	[0.70–4.83]
19	1.2	0.5	11.8	2.53	[0.96–6.67]
20	6.5	3.6	69.4	2.61	[0.99–6.87]
21	2.7	1.5	21.8	2.24	[0.85–5.90]
22	1.4	0.5	15.0	2.57	[0.97–6.77]
23	0.9	0.3	16.4	3.46	[1.31–9.14]
24	4.0	2.7	73.1	3.54	[1.34–9.35]
25	0.8	1.4	6.0	2.06	[0.78–5.43]

profiles show, in a single graph, all the statistical data and allow providing an estimation of the uncertainty measurement. The confidence limits at 95% of the total measurement error (bias and precision) were then calculated at each concentration level for all three compounds.

The acceptance limits were settled at ±30% according to the requirement generally admitted in forensic toxicology [24]. As the accuracy profiles are comprised within the acceptance limits, the LOQ were fixed at 0.5, 0.5, and 2.5 ng/mL for THC, THC-OH, and THC-COOH, respectively. Furthermore, according to the blood sample origin (driving or autopsy cases), the LOD were found to vary slightly between 0.1 and 0.2 ng/mL for all compounds.

3.5. Application to real cases

The developed procedure was used to determine the concentration levels on more than a hundred real forensic cases. Twenty-five of them are presented in Table 3 with the calculation of the time prediction of marijuana use [2]. Fig. 6 shows the results obtained for 11 analyses of a commercial control over a period of 6 months.

4. Conclusion

This article shows that NICI-MS/MS approach constitutes a true force for the fast toxicological analysis. With its properties of soft ionization, it offered a better selectivity and sensitivity than EI, allowing the use of a simpler sample pretreatment. Validation data showed that NICI-MS/MS allowed reaching a very high degree of trueness, precision, and finally, accuracy. Fur-

thermore, the use of MS/MS generally provided enough ions for forensic and toxicological requirements.

Moreover, this kind of detection is particularly interesting for Fast GC in forensic toxicology. Indeed, as discussed earlier NICI provides a highly specific detection for analytes of interest having electronegative moieties. These kinds of compounds being not abundantly present in blood, NICI-MS/MS chromatograms are characterized by low background interferences, and therefore, by a better resolution than in EI mode, allowing decrease in analysis time. Hence, NICI is the ionization mode of choice for fast and unequivocal analysis in forensic toxicology. From the best of our knowledge, the run time of 5 min is one of the fastest presented in the scientific literature for the analysis of these three cannabinoids in whole blood allowing a higher sample throughput and a reduction in the cost per unit of sample, although a standard column was used. Moreover, with the accuracy profiles, a powerful method for estimating the measurement uncertainty was demonstrated.

Finally, compared with the increased demand for analysis, we think that Fast GC/NICI-MS/MS will play an important role in forensic toxicology in the future.

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