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Journal of Chromatography A, 1082 (2005) 15–24
JOURNAL OF CHROMATOGRAPHY A
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Quantitative analysis of Δ^9-tetrahydrocannabinol in preserved oral fluid by liquid chromatography–tandem mass spectrometry

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Available online 8 April 2005

Abstract

A rapid and sensitive method for the analysis of Δ^9-tetrahydrocannabinol (THC) in preserved oral fluid was developed and fully validated. Oral fluid was collected with the Intercept, a Food and Drug Administration (FDA) approved sampling device that is used on a large scale in the U.S. for workplace drug testing. The method comprised a simple liquid–liquid extraction with hexane, followed by liquid chromatography–tandem mass spectrometry (LC–MS-MS) analysis. Chromatographic separation was achieved using a XTerra MS C_{18} column, eluted isocratically with 1 mM ammonium formate–methanol (10:90, v/v). Selectivity of the method was achieved by a combination of retention time, and two precursor-product ion transitions. The use of the liquid–liquid extraction was demonstrated to be highly effective and led to significant decreases in the interferences present in the matrix. Validation of the method was performed using both 100 and 500 μL of oral fluid. The method was linear over the range investigated (0.5–100 ng/mL and 0.1–10 ng/mL when 100 and 500 μL, respectively, of oral fluid were used) with an excellent intra-assay and inter-assay precision (relative standard deviations, RSD <6%) for quality control samples spiked at a concentration of 2.5 and 25 ng/mL and 0.5 and 2.5 ng/mL, respectively. Limits of quantification were 0.5 and 0.1 ng/mL when using 100 and 500 μL, respectively. In contrast to existing GC–MS methods, no extensive sample clean-up and time-consuming derivatisation steps were needed. The method was subsequently applied to Intercept samples collected at the roadside and collected during a controlled study with cannabis.

Keywords: LC–MS-MS; Oral fluid; THC

1. Introduction

Currently, there is a strong interest in monitoring drug use through oral fluid testing in the context of driving under the influence, drug treatment, criminal justice, and workplace drug-testing [1–5]. Advantages of this matrix include the ease and non-invasiveness of specimen collection and reduced opportunity for specimen substitution and adulteration. However, two main limitations of oral fluid are apparent: the specimen volume is often small and the analyte concentration is lower than in urine. As such, oral fluid testing is a greater analytical challenge and highly sensitive techniques are required.

Due to the high specificity and the increased signal-to-noise in combination with short chromatographic run times, liquid chromatography–tandem mass spectrometry (LC–MS-MS) allows for specific, selective and sensitive analysis of compounds with a wide polarity range in samples of various nature. It offers the possibility to simplify sample preparation, although this approach should be treated with caution due to the possibility of ion suppression or enhancement as a result of the matrix. Consequently, attention must be paid to the choice of the sampling method and the influence of the collected matrix on the LC–MS-MS analysis. Several meth-

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doi:10.1016/j.chroma.2005.03.031

ods of oral fluid collection have been used. The Intercept is a Food and Drug Administration (FDA) approved sampling device that is used on a large scale in the U.S. for workplace drug testing [6]. It is also used to collect oral fluid samples for confirmation analyses in the joint roadside study between the European Union and the U.S. to detect driving under the influence of drugs [5]. The collection system contains stabilising salts, non-ionic surfactants for surface wetting and antibacterial agents, and guarantees a good stability for most illicit drugs and their metabolites during storage at 4 °C. However, these ingredients can also cause interferences, e.g. ion suppression or enhancement, during LC–MS-MS analysis in the absence of a suitable clean-up method.

Drugs may appear in oral fluid via multiple pathways. Δ^9-tetrahydrocannabinol (THC), the major psychoactive constituent of cannabis, is deposited in the oral cavity during cannabis smoking. This "depot" represents the primary or sole source of THC detected when oral fluid is collected and analysed [7]. Despite the lack of contribution from blood THC to oral fluid concentrations, Huestis and Cone [7] and Niedbala et al. [8] showed that, after dissipation of the initial contamination of oral fluid during smoking (generally within 30 min), THC levels in oral fluid followed a similar time course as plasma THC following smoked cannabis administration under controlled dosing conditions. Within 12 h, both oral fluid and plasma THC concentrations generally declined below 1 ng/mL.

With the exception of a report by Schramm et al. [9], no other studies have revealed evidence of 11-hydroxy-THC or carboxy-THC in oral fluid after smoking of cannabis. However, it appears that in addition to THC, cannabidiol (CBD) and cannabinol (CBN) may be detected in oral fluid after smoking of hashish or marijuana cigarettes [10].

Most laboratories analyse THC in blood and oral fluid by GC–MS(-MS) after extraction and derivatisation [7,8,11–13]. Recently, LC–MS(-MS) has been successfully used to analyse cannabinoids in urine and blood [14–17]. However, only one paper reported on the application of LC–MS to detect THC in oral fluid [18]. These authors reported on the determination of THC in 200 μL of oral fluid, which was obtained by spitting. The limit of detection achieved was 2 ng/mL.

Our aim was to develop a fast and sensitive LC–MS-MS method for the confirmation of THC in preserved oral fluid samples collected with the Intercept. Validation of the method was performed using both 100 and 500 μL of preserved oral fluid. The method was subsequently applied to Intercept samples collected at the roadside and collected during a controlled study.

2. Experimental

2.1. Chemicals

Individual ampoules of THC (at a concentration of 1 mg/mL in methanol) and [$^2H_3$]THC (THC-d_3) (0.1 mg/mL in methanol) were purchased from LGC Promochem (Molsheim, France). Cannabinol and cannabidiol were from Lipomed (Arlesheim, Switzerland). All solvents were HPLC-grade and from Merck (Darmstadt, Germany).

2.2. Specimens

Blank preserved oral fluid, used for the preparation of negative controls, calibrators and quality control (QC) samples was obtained from healthy volunteers and collected with the Intercept collection device (OraSure Technologies, Bethlehem, PA, USA) according to the manufacturer's instructions. Briefly, after gently wiping the collector pad between gum and cheek for approximately 2 min (as a kind of toothbrush), the device is placed in the supplied vial, which contains a stabilising buffer solution, and sealed. After centrifugation, the recovered fluid is transferred in cryotubes and represents a mixture of the collected oral fluid and the buffer in a proportion of approximately 1 to 2. The device collects an average of 0.38 ± 0.19 (SD) mL with a range of 0.05 to 0.8 mL of oral fluid and a dilution factor of 1 in 3 is arbitrarily accepted [6]. The tubes were sealed and stored at −20 °C prior to analysis.

Authentic preserved oral fluid samples were collected by the police at the roadside during roadblocks to intercept drivers under the influence of drugs, using the same procedure as described for the blank samples.

A third series of preserved oral fluid samples was obtained with a similar protocol from nine healthy volunteers with a history of cannabis use. Once a week and for two consecutive weeks, subjects received either a placebo cigarette (containing cannabis where the THC had been previously extracted) or a marijuana cigarette (containing 300 μg THC per kilogram weight). Oral fluid samples were collected 0.5 h before and at various times after drug administration (0.25, 0.5, 1, 1.25 and 1.5 h). Thus, we obtained from each volunteer six oral fluid samples in the placebo condition and six in the THC condition. The study protocol was approved by the ethics committee of the University Hospital of Maastricht in The Netherlands.

2.3. Preparation of standard solutions and sample extraction

An internal standard (IS) working solution of THC-d_3 at a concentration of 10 ng/mL was prepared in methanol. Working solutions of THC at different concentrations (1, 2, 4, 5, 8, 16, 32, 50, 100, 200 ng/mL in methanol) were used for the preparation of calibrators and QC samples. Working solutions were stored at −20 °C, and were prepared monthly.

The extraction procedure was carried out in 10 mL disposable screw top vials of high quality glassware (Chromacol, Herts, UK) with 100 or 500 μL of preserved oral fluid specimen collected with the Intercept device. The pH of the preserved oral fluid samples ranged between 6.0 and 7.0. Fifty microliters of the IS working solution and 4 mL

of hexane were added; when only 100 μL was used, an additional 400 μL of deionised water was added.

After mechanical shaking (30 min) and centrifugation (10 min at 3000 × *g*), the organic phase was transferred to a 5 mL disposable screw top vial (Chromacol) and then evaporated to dryness at 40 °C under nitrogen. The extract was reconstituted in 100 μL of mobile phase and 20 μL was injected into the LC–MS-MS system.

2.4. LC–MS-MS

2.4.1. Chromatography

LC was performed using a Waters Alliance 2690 separation module (Waters, Milford, MA, USA). Analytes were separated on a XTerra MS C_{18} column (150 mm × 2.1 mm, 3.5 μm) (Waters), eluted isocratically with 1 mM ammonium formate–methanol (10:90, v/v), delivered at a flow rate of 0.2 mL/min. The total run time of the method was 8 min. All aspects of system operation and data acquisition were controlled using MassLynx NT 4.0 software.

2.4.2. Mass spectrometry

A Quattro Premier tandem mass spectrometer (Waters) was used for all analyses. Ionisation was achieved using electrospray in positive ionisation mode (ESI+). The optimum conditions were: capillary voltage, 2.0 kV; source block temperature, 120 °C; desolvation gas (nitrogen) heated to 280 °C and delivered at a flow rate of 700 L/h.

In order to establish the appropriate multiple reaction monitoring (MRM) conditions for the individual compounds, solutions of standards [500 ng/mL, in 1 mM ammonium formate–methanol (10:90, v/v)] were infused into the mass spectrometer and the cone voltage (CV) optimised to maximise the intensity of the protonated molecular species $[M + H]^+$. Collision-induced dissociation (CID) of each protonated molecule was performed. The collision gas (argon) pressure was maintained at 0.35 Pa (3.5×10^{-3} mBar) and the collision energy (eV) adjusted to optimise the signal for the most abundant product ions, which were subsequently used for MRM analysis. The transitions were *m*/*z* 315.2 → 193.1 and *m*/*z* 315.2 → 259.3 for THC. The former (and most prominent precursor-product transition) was used for quantification and the latter transition used as a qualifier. The transition for THC-d_3 was *m*/*z* 318.2 → 196.1.

All aspects of data acquisition were controlled using MassLynx NT 4.0 software with automated data processing using the QuanLynx program (Waters).

2.5. LC–MS-MS assay validation

2.5.1. Linearity, limit of quantification (LOQ), precision, accuracy and recovery

Quantification was performed by integration of the area under the specific MRM chromatograms in reference to the integrated area of its respective deuterated analogue.

Linearity was assessed when either 100 or 500 μL of the sample, collected with the Intercept device, was processed and analysed using LC–MS-MS. When 100 μL was used, calibration curves ranged from 0.5 to 100 ng/mL (0.5, 1, 2, 4, 8, 16, 25, 50, 100 ng/mL) and from 0.1 to 10 ng/mL (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 5, 10 ng/mL) when 500 μL of preserved oral fluid was used. Standard response curves were generated daily using a weighted (1/*x*) least-squares linear regression model.

The limit of quantification (LOQ) was defined as the concentration of the lowest calibrator which was calculated to be within ±20% of the nominal value and with a % relative standard deviation (RSD) less than 20% [19,20].

QCs were prepared for every run in blank preserved oral fluid at a concentration of 2.5 and 25 ng/mL for 100 μL of sample and at a concentration of 0.5 and 2.5 ng/mL for 500 μL of preserved oral fluid. Intra-assay precision was evaluated by replicate ($n = 4$) analysis of the two QC samples in one run for each of both volumes of preserved oral fluid. Inter-assay precision was evaluated by replicate analysis of the QC samples in several experiments performed on eight different days by two operators. A comparison of the calculated concentrations of the QC samples to their respective nominal values, was used to assess the accuracy (bias) of the method.

Recovery was estimated by comparing the response of a 5 ng/mL calibrator when the non-deuterated compound was added before the extraction step ($n = 3$) with the response obtained when the non-deuterated analyte was added after sample preparation ($n = 3$). THC-d_3 was added before the extraction step in both conditions.

2.5.2. Stability of unprepared and prepared samples

Stability of THC in preserved oral fluid collected by the Intercept device was monitored in preserved oral fluid samples spiked at the initial concentrations of 1, 10 and 100 ng/mL. THC concentrations in the samples were either determined immediately (control samples, $n = 3$) or following incubation at room temperature or at 4 °C for a period of 24 h ($n = 3$) or 48 h ($n = 3$) after preparation. Stability at each time point was tested against a lower acceptance limit corresponding to 90% of the mean of control samples by a one-sided *t*-test ($P < 0.05$).

For an evaluation of freeze/thaw stability, a calibrator at 5 ng/mL was analysed before (control samples, $n = 3$) and after three freeze/thaw cycles (stability samples, $n = 3$). For each freeze/thaw cycle, the samples were frozen at −20 °C for 24 h, thawed, and then maintained at ambient temperature for 1 h. Stability was tested against a lower acceptance limit corresponding to 90% of the mean of control samples by a one-sided *t*-test ($P < 0.05$).

The stability of THC in the extracted sample (preserved oral fluid initially spiked at 5 ng/mL) was investigated by repeated injections of a mixture of five extracted samples (maintained in the autosampler at 4 °C) over a period of 15 h. Absolute peak areas were plotted as a function of injection

time and the stability of the processed samples tested by regression analysis. Instability of the processed samples would be indicated by a slope that was significantly different from zero ($P < 0.05$).

2.5.3. Assessment of matrix effects

To assess any potential suppression or enhancement of ionisation due to the sample matrix, two types of experiments were performed. In the first experiment, THC (5 ng/mL) was added after extraction of either water or preserved oral fluid i.e. before evaporation, and the peak responses obtained in both conditions were compared. A two-sided *t*-test was used to identify any significant differences ($P < 0.05$).

The second type of experiment involved a continuous post-column infusion of a mixture of THC and THC-d_3 (10 ng/mL at a flow rate of 10 μL/min) to produce a constant elevated response in both MRM channels. The interference of this constant response was monitored following the injection of samples either prior to or after extraction of 100 or 500 μL of preserved oral fluid and compared to the response following the injection of mobile phase only.

3. Results and discussion

The method was validated for linearity, LOQ, precision, accuracy and analytical recovery by the analysis of spiked preserved oral fluid samples, collected using the Intercept device. Two sets of calibration standard samples (in 100 and 500 μL of preserved oral fluid) were prepared for validation of linearity. The linearity data are summarised in Table 1. In each case, a weighted ($1/x$) linear regression line was applied. Linearity with a correlation coefficient $r^2 = 0.999$ was achieved in the range investigated: from 0.5 up to 100 ng/mL when 100 μL of preserved oral fluid was used and from 0.1 up to 10 ng/mL for 500 μL of sample. Fig. 1 shows the MRM chromatograms obtained following the analysis of a sample spiked with THC and THC-d_3 when either 100 and 500 μL of preserved oral fluid was used. For both calibration curves, the lowest calibrators, i.e. 0.5 ng/mL and 0.1 ng/mL when using 100 or 500 μL, respectively, of collected oral fluid, satisfied the criteria for LOQ. It should be noted that the mean resultant specimen volume, following dilution with the preservative solution, varied around 1.2 mL even under controlled conditions [8]. However, in reality, the volume is often reduced in driving under the influence (DUI) cases due to the stimulation of sympathetic nerves which results in the production of a viscous and less abundant oral fluid. This is the case particularly true for regular users of amphetamines [10]. In addition, in these cases the possibility of other drugs should be tested for. This necessitates optimal usage of the minimal amounts of specimen provided. For these cases, the LOQ when using only 100 μL of oral fluid was sufficiently low to meet the requirements of SAMSHA for oral fluid testing (i.e. 2 ng/mL THC in undiluted oral fluid) [21]. However, in pharmacokinetic studies, where the detection of THC over time often necessitates increased sensitivity and a lower LOQ, this can be achieved very simply, by using larger volumes of oral fluid. For example, when using 500 μL of collected oral fluid, the LOQ was determined to be 0.1 ng/mL. Thus, the choice of sample volume will largely depend on the application in addition to the requirements for sensitivity.

These results are comparable with previous GC–MS-MS reports [7,8]. The obtained LOQ for THC was lower than the one reported by Concheiro et al. [18], primarily due to the use of *tandem* MS instead of *single* MS. These authors used undiluted oral fluid collected by spitting. Hence, when using diluted oral fluid, collected with the Intercept device, a lower LOQ is needed to meet the requirements of SAMSHA.

Selectivity of the method was achieved by a combination of retention time, precursor and product ions. Quantification was based on the most prominent product ion (i.e. quantifier); confirmation of THC was evaluated through the presence of the second product (i.e. qualifier). At the LOQ the qualifier had a signal to noise ratio (S:N) > 10:1. The acceptance range for the peak area ratio quantifier/qualifier was 2.36 ± 0.35 for all analyses.

The intra-assay precision (repeatability) and inter-assay precision (reproducibility) were highly satisfactory with all relative standard deviations less than 6% (Table 2). Results indicated that the accuracy of the assay was > 93%. Recovery of the method was $85.6 \pm 0.5\%$.

The stability of spiked samples (1, 10 and 100 ng/mL) was monitored at 24 and 48 h at 4 °C and at room temperature. No statistical significant differences could be observed for the three different concentrations in both conditions. Also no statistical differences could be noted for the stability of spiked samples (5 ng/mL) during three freeze/thaw cycles.

In addition, the potential for any undesired stability of the processed samples was tested. To this end, the stability of THC was monitored by means of repeated injections of extracted samples (5 ng/mL) over a period of 15 h, and by plotting the absolute peak areas as a function of time. The

Table 1
Linearity and sensitivity data for THC in preserved oral fluid

Linearity data					Sensitivity data
Volume oral fluid (μL)	Slope[a]	Intercept[a]	RSD of slope[a]	r^2 (range of five consecutive days)	LOQ (ng/mL)
100	1.0635	0.0209	2.9	0.9993–0.9999	0.5
500	5.3976	−0.0009	4.1	0.9992–0.9999	0.1

Samples were prepared by the liquid–liquid extraction method as described in the text.
[a] Reported values are the mean of five determinations over five consecutive days.

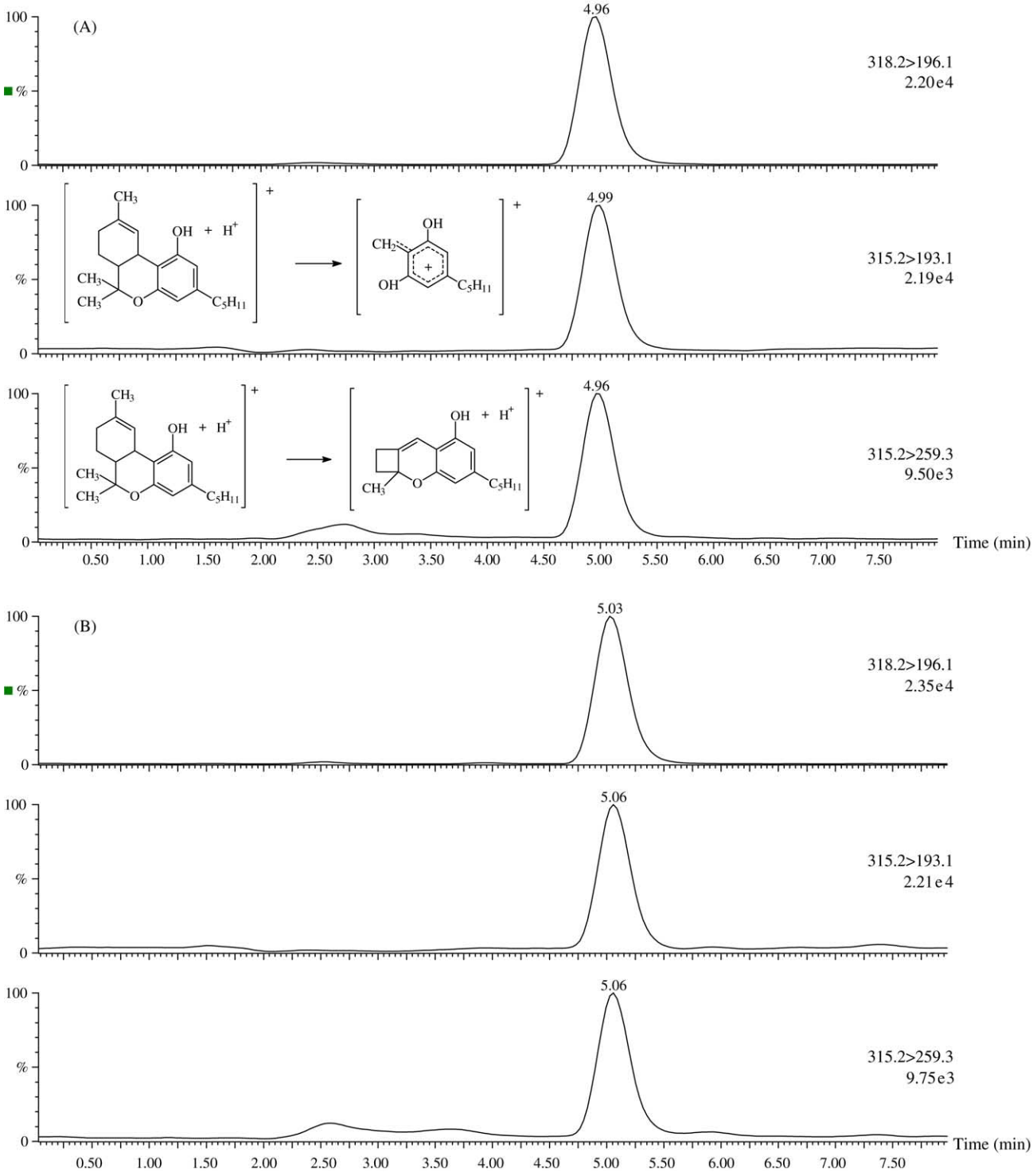

Fig. 1. MRM chromatograms obtained with a single injection of a 100 μL extracted preserved oral fluid sample enriched with 5 ng/mL THC and 5 ng/mL THC-d_3 (A) and of a 500 μL extracted preserved oral fluid sample enriched with 0.8 ng/mL THC and 1 ng/mL THC-d_3 (B). The figure shows the response for THC-d_3 (top trace) and for the two transitions of THC (quantifier and qualifier, middle and bottom traces, respectively). Peak intensity is shown in the top right-hand corner of each trace.

results indicated no significant instability over the course of the experiment.

Insufficient sample clean-up can result in matrix effects, leading to either suppression or enhancement of the analyte response [22–24]. This can lead to variable sensitivities and decreased precision and accuracy. Consequently, in the development of any LC–MS(-MS) method, the potential for any such ion suppression or enhancement should be assessed.

Table 2
Precision[a] and accuracy data for THC for the extraction of 100 and 500 μL of spiked preserved oral fluid samples

Volume oral fluid (μL)	Concentration of QC (ng/mL)	Intra-assay precision			Inter-assay precision		
		Mean concentration found (ng/mL)	RSD (%)	Bias (%)	Mean concentration found (ng/mL)	RSD (%)	Bias (%)
100	2.5	2.5	3.6	−1.0	2.4	2.9	−2.5
	25.0	24.8	5.4	−0.7	24.0	5.4	−4.1
500	0.5	0.5	2.5	−2.4	0.5	4.1	−5.5
	5.0	4.9	0.4	−2.0	4.7	3.8	−6.8

[a] Intra-assay precision was evaluated by the preparation and analysis of four replicates of a low and a high QC in a single assay for both volumes of oral fluid used. Inter-assay precision was evaluated by the preparation and analysis of each QC over eight consecutive days.

The Intercept collector contains a variety of chemicals, i.e. sodium chloride, sodium benzoate, potassium sorbate, bovine gelatin, Tween 20, chlorhexidine digluconate and a blue dye, some of which can interfere with the LC–MS-MS detection signal. To assess this, we compared peak area responses obtained when THC was added after the extraction of blank preserved oral fluid with the responses obtained when THC was added to an extract where the preserved oral fluid was substituted with water. No statistically significant different peak areas were observed.

Post-column infusion experiments (based on the method described by Bonfiglio et al. [22]) were performed to provide information of the effect of matrix throughout the course of the whole chromatographic run and not just at the elution time for the analytes. The effect on THC response obtained following the injection of a mobile phase control is shown in Fig. 2A. As expected, no changes in response were observed. The effects on THC response obtained following the injection of a sample prior extraction and after extraction of 100 and 500 μL of preserved oral fluid are given in Fig. 2B, C and D, respectively. The results confirm the usefulness of the liquid–liquid extraction as a sample clean-up before chromatography: a decrease of 100% in response starting from ∼1.7 min was observed when no sample clean-up was performed. A reduction of 50% was still noted at the moment of elution of THC, probably due to the elution of endogenous components. When injecting extracted samples, this suppression was still apparent but restored by the elution time of THC. In addition to THC, cannabidiol and cannabinol are two components that are also present in the

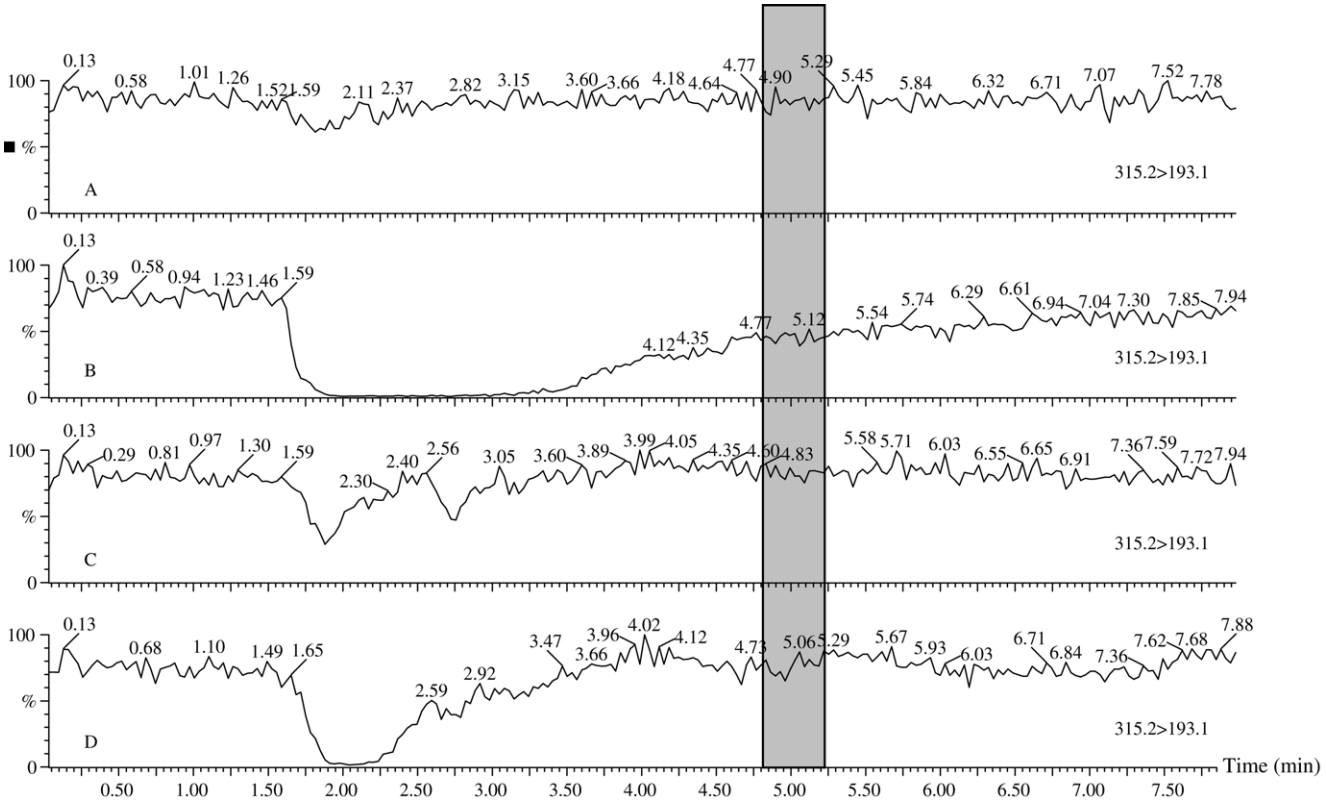

Fig. 2. Evaluation of the effect on THC response of an injection of a mobile phase control (A), a blank sample prior to extraction (B) and the same sample following the extraction of 100 and 500 μL of preserved oral fluid (C and D, respectively). The shaded area indicates the elution position of THC.

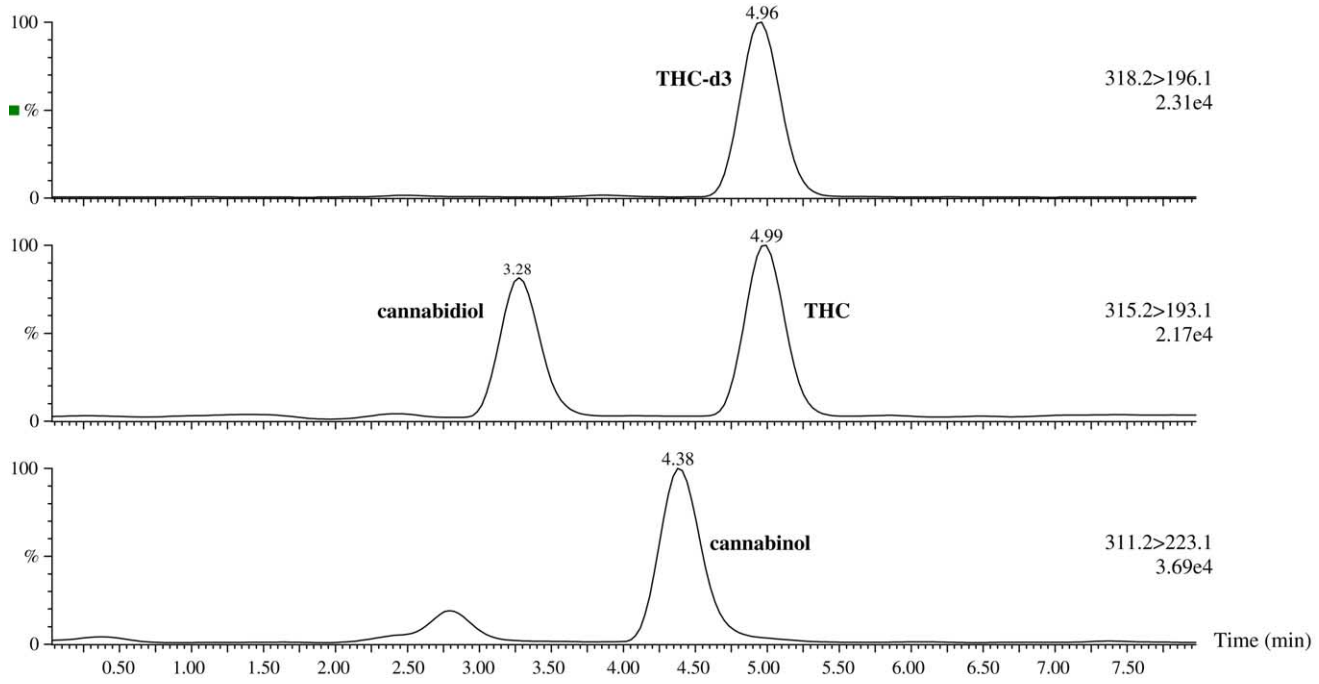

Fig. 3. LC–MS-MS analysis of an extracted 100 μL blank oral fluid sample enriched with 5 ng/mL THC-d_3 (top trace), THC and cannabidiol (middle trace) and cannabinol (bottom trace). Peak intensity is shown in the top right-hand corner of each trace.

Cannabis sativa plant and may also be detected in oral fluid. To evaluate their potential for interference, standards were analysed using the developed LC–MS-MS method. This is particularly important in the case of cannabidiol since this component has the same molecular mass (and thus the same protonated species) as THC and shows the same product ions after CID. Cannabidiol eluted at 3.28 min and was chromatographically resolved from THC. In contrast, cannabinol did not produce any response in the monitored MRM channel due to a different molecular mass. The appropriate MRM transition for this component was m/z 311.2 → 223.1, as determined by direct infusion experiments. Cannabinol was demonstrated to elute at 4.38 min. Fig. 3 shows the MRM chromatograms obtained following LC–MS-MS analysis of an extracted 100 μL blank oral fluid sample enriched with 5 ng/mL THC-d_3, THC, cannabidiol and cannabinol.

The validated LC–MS-MS method was applied to the analysis of 102 oral fluid samples collected with the Intercept from volunteers who had received either a placebo cigarette or a marijuana cigarette. THC concentrations obtained after smoking a single marijuana cigarette are shown in Fig. 4. For these cases only the presence of THC had to

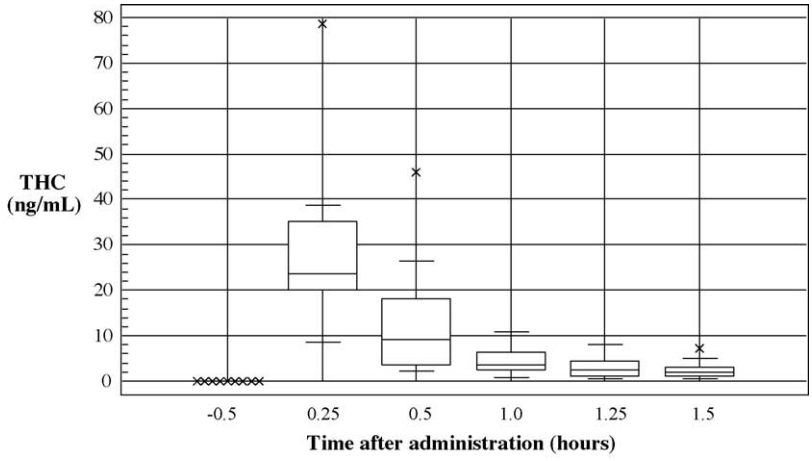

Fig. 4. Box- and whisker plots of THC levels in preserved oral fluid samples from nine healthy volunteers following smoking of a single marijuana cigarette. Oral fluid samples were taken 0.5 h prior to smoking and at 0.25, 0.5, 1, 1.25 and 1.5 h after smoking. Concentrations plotted on the *Y*-axis are expressed as ng/mL. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum value, excluding "outside" (not present) and "far out" values (cross marker) which are displayed as separate points.

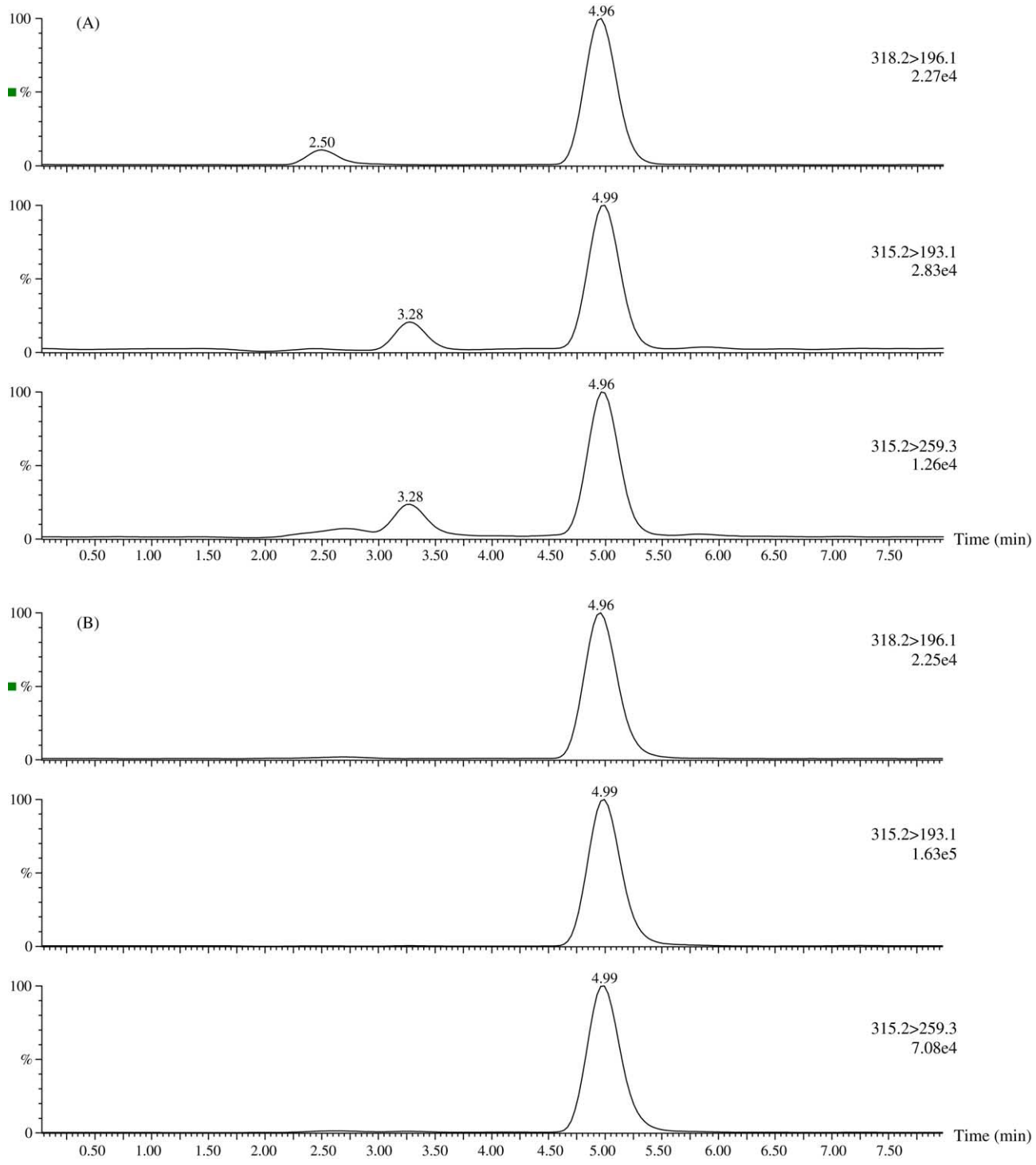

Fig. 5. Typical MRM chromatograms obtained following the analysis of two authentic preserved oral fluid specimens obtained from drivers in a roadside setting. Concentrations were 5.7 ng/mL (A) and 50.8 ng/mL (B). The figure shows the response for THC-d_3 (top trace) and for the two transitions of THC (quantifier and qualifier; middle and bottom traces respectively). Peak intensity is shown in the top right-hand corner of each trace.

be confirmed, thus 500 μL of oral fluid was used for the analysis. For samples where the response exceeded the upper limit of the standard curve, reanalysis of only 100 μL was performed. At −0.5 h all specimens were negative for THC, except for three subjects in which low concentrations were found (0.2, 0.4 and 2.2 ng/mL). However, it should be noted that in both the placebo and marijuana condition, THC could be detected, probably due to incomplete removal of THC for the preparation of the placebo cigarette. Mean peak (±1 SD) THC concentration in the marijuana condi-

Table 3
Results obtained applying the method to 48 preserved oral fluid samples collected by the police at the roadside

Sample identity	THC (ng/mL)	Sample identity	THC (ng/mL)
1	5.7	25	60.2
2	7.0	26	3.9
3	4.6	27	52.2
4	18.5	28	25.4
5	2.5	29	193.5
6	95.8	30	111.2
7	<LOQ	31	7.3
8	84.7	32	14.6
9	<LOQ	33	1.9
10	0.5	34	4.7
11	4.5	35	100.0
12	3.9	36	23.0
13	31.9	37	57.1
14	50.8	38	88.6
15	34.6	39	3.9
16	56.0	40	375.8
17	81.1	41	3.7
18	11.9	42	4.4
19	107.4	43	4.2
20	92.1	44	4.2
21	10.0	45	4.2
22	17.6	46	4.1
23	94.8	47	4.0
24	37.2	48	4.4

tion occurred at the first specimen collection (0.25 h) and was 30.6 ng/mL (±21.6 ng/mL). Thereafter, THC concentrations declined steadily to mean concentrations of 2.6 ng/mL (±2.3 ng/mL). Overall, concentrations were quite variable; this has also been reported by other authors [8] and may be due to the lack of exact volume measurement of the collection device. The Intercept device is a collection device on which the specimen is absorbed onto a matrix, leading to variable absorbed volumes.

The mean peak concentration is lower than the one reported by Niedbala et al. using the same collection device [8]. This could be due to the fact that the samples were only analysed several months after sampling. During this time the samples were conserved at −20 °C on the pad, i.e. without prior centrifugation. However, no stability studies on this aspect were available from the manufacturer.

During roadside controls for drugged driving, the police collected 48 authentic oral fluid samples for a confirmatory analysis in the laboratory. In these cases only 100 μL of preserved oral fluid was used due to limited sample volume. Fig. 5 shows typical MRM chromatograms of Intercept samples obtained from two marijuana users. In Fig. 5A, the presence of cannabidiol (at a retention time of 3.28 min) was also noted. A summary of the quantitative results for the positive samples is presented in Table 3. In these samples, the median THC concentration was 13.3 ng/mL with a range from 0.5 to 375.8 ng/mL. The measured THC concentrations varied considerably and some samples had to be reanalysed after dilution (one in five dilution with blank oral fluid).

4. Conclusions

A fully validated LC–MS-MS method for the determination of THC in preserved oral fluid, collected with the Intercept device, was developed. The method offers the combination of a very simple liquid–liquid extraction to avoid ion suppression, a high recovery and excellent precision and accuracy, when using either 100 or 500 μL of collected sample. The method was successfully applied to Intercept samples collected at the roadside and collected after a controlled study with cannabis.

Acknowledgement

We would like to acknowledge the Belgian Federal Office for Scientific, Technical and Cultural Affairs.

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