Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1011–1016
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Development and validation of a method for the quantitation of Δ^9 tetrahydrocannabinol in human plasma by high performance liquid chromatography after solid-phase extraction

Chadi Abbara [a,*], Romain Galy [a], Amine Benyamina [b], Michel Reynaud [b], Laurence Bonhomme-Faivre [a]

[a] *Laboratoire de Pharmacologie, Service de Pharmacie, Hôpital Paul BROUSSE (AP-HP), 12, Avenue Paul Vaillant Couturier, 94800 Villejuif, France*
[b] *Service d'addictologie, Hôpital Paul BROUSSE (AP-HP), 12, Avenue Paul Vaillant Couturier, 94800 Villejuif, France*

Received 25 October 2005; received in revised form 27 December 2005; accepted 7 January 2006
Available online 24 February 2006

Abstract

A high performance liquid chromatography (HPLC) procedure for the determination of Δ^9 tetrahydrocannabinol (THC) in human plasma is described. A two-step solid-phase extraction on CN cartridges was coupled with a reversed phase HPLC system. THC was eluted using a mobile phase composed of methanol, acetonitrile and tetrabutylammonium perchlorate solution (0.005 M, pH 3.2), through a C_{18} Nucleosil column and detected at a wavelength of 215 nm. Calibration curve was linear over the range 5–100 ng/ml with a lower limit of quantification validated at 5 ng/ml. Extraction recovery using the developed extraction procedure was higher than 85%. This method is presently used for the quantification of THC in plasma samples from regular cannabis smokers.

Keywords: Tetrahydrocannabinol; HPLC; Analytical method validation

1. Introduction

Δ^9 Tetrahydrocannabinol (THC) is the main psychoactive constituent of Cannabis Sativa L., the more frequently abused drug after alcohol and nicotine [1]. During marijuana smoking, THC is rapidly absorbed in larger amounts than when taken orally, and due to its strong lypophilic nature, it rapidly spreads throughout the body. The efficiency of the smoking process depends on multiple factors, including cigarette potency (∼5–50 mg), depth and length of inhalation, and past experience of the smoker leading to an important variability in the amount of THC delivered to the blood stream [2].

THC is rapidly metabolized primarily by cytochrome P450 enzymes in the liver and other tissues to 11-hydroxy-THC, an equipotent active metabolite, to 11-nor-9carboxy Δ^9 tetrahydrocannabinol (THC-COOH), an inactive metabolite found to be the primary cannabinoid metabolite excreted in the urine, and to numerous other cannabinoids [3].

Several methods for the quantification of THC and THC-COOH are already published [1,4–23].

Usually gas chromatography/mass spectrometry (GC/MS) technique was used for the routine screening of THC and THC-COOH [4–12]. However, this procedure is demanding and time consuming, since it requires derivatization of the analytes.

Among the most recent developments, liquid chromatography (LC) combined with mass spectrometry (MS) has formed the basis of improved methods for THC quantification [1,13–16]. Although the specificity (transition MRM) and the sensibility (1–5 ng/ml) of these methods are good, the equipment necessary to carry out the analysis is expensive, and usually, not available in most of the hospital laboratories.

The use of more commonly available detectors was reserved to the electrochemical detectors (ED) [17–21]. Ultra violet detectors are used only in methods developed for the quantification of THC-COOH in urine [22,23]. However, no method is yet published to quantify THC and/or THC-COOH in plasma by HPLC–UV.

* Corresponding author. Tel.: +33 145593838; fax: +33 145593728.
E-mail address: chadi.abbara@pbr.ap-hop-paris.fr (C. Abbara).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.01.017

This paper describes a rapid method for the quantification of THC in human plasma using a HPLC–UV method following a solid-phase extraction procedure. This method was used for the determination of THC in plasma samples from regular cannabis smokers.

2. Experimental

2.1. Chemicals

Acetonitrile and methanol (for HPLC grade) were obtained from VWR (Fontenay sous Bois, France). Tetrabutylammonium perchlorate, 1-octanosulfonic acid sodium salt and Δ^9 tetrahydrocannabinol (100 μg/ml in methanol) were obtained from Sigma Aldrich (Saint Quentin Fallavier, France).

Lopinavir, used as internal standard, was a kind gift from ABBOTT Labs (North Chicago IL, USA). For injection quality water was bought from C.D.M. Lavoisier (Paris, France).

2.2. Preparation of the spiked plasma samples

Calibration standards of THC were prepared in drug free human plasma by spiking with concentrated standards in order to obtain a concentration range between 5 and 100 ng/ml.

Quality control (QC) samples for THC were prepared in drug free human plasma by spiking with concentrated standards. Three levels of QC concentrations were prepared, a low level at 15 ng/ml, a medium level at 45 ng/ml, and a high level at 90 ng/ml. These QC samples were used for the bioanalytical method validation and for the in run validation.

The QC samples were prepared in batches simultaneously, stored at −20 °C as 1600 μl aliquots and thawed on the day of analysis at room temperature.

2.3. Sample extraction procedure

On the day of analysis, calibration, QC and patients samples were thawed on the bench and allowed to equilibrate at room temperature.

Briefly, 500 μl plasma sample (calibration, QC, patient) were diluted with 700 μl of internal standard solution (at 25 μg/ml in acetonitrile). After vortex mixing during 30 s, the sample was ultra centrifuged for 8 min at 10,500 rpm. The supernatant was mixed in a 5 ml glass tube, with 800 μl of 1-octanosulfonic acid sodium salt solution at 0.2 M during 30 s.

The extraction procedure of the obtained solution was preformed on solid-phase extraction (SPE) cartridges using a 12 tubes vacuum manifold. Three milliliter per hundred milligrams Upti-Clean CN-S (INTERCHIM, Asnière sur seine, France) cartridges were used.

The mixture was loaded under vacuum on a CN extraction cartridge previously activated with 2 ml of methanol and 2 ml of water. The cartridge was then washed with 2 ml of water and dried under pressure for 5 min. Elution was carried out with 2 × 0.4 ml of methanol. The methanolic solution was evaporated under a stream of nitrogen in water bath at 40 °C. The residue was reconstituted in 200 μl aliquot of water–methanol (1:3, v/v). A 50 μl aliquot was then analyzed by the chromatographic system.

2.4. Chromatographic conditions

The chromatographic system consisted of a Shimadzu LC6A isocratic pump, SIL20A autosampler set at an injection volume of 50 μl and a SPD6A UV detector set at 215 nm. Chromatography was carried out at room temperature using a Nucleosil C_{18} column (125 mm × 4.6 mm, 3 μm i.d.) (INTERCHIM), equipped with a guard column filled with the same material. The mobile phase composed of methanol, acetonitrile, and tetrabutylammonium perchlorate solution (0.005 M) at pH 3.2 (50:25:25, v/v/v), was delivered at a flow rate of 1.2 ml/min.

The Shimadzu LC-Solution® software was used to pilot the chromatographic instrument and to process the data (area integration, calculation and plotting of chromatograms).

2.5. Analytical method validation

The validation of the method was based on the guidelines published on line by the United States Food and Drug Administration [24].

The QC samples already prepared were used for precision and accuracy determination, the three QC levels were chosen to cover the calibration curve range.

Precision was calculated as the coefficient of variation (C.V.%) within a single run (intra-assay) and accuracy, as the percentage of deviation between nominal and measured concentrations.

The lower limit of quantification (LLOQ) was experimentally chosen as the minimal concentration in plasma samples which could be confidently determined. Food and Drug Administration guidelines recommend that the deviation between measured and nominal concentration at LLOQ should not deviate more than ±20% with a precision <20%.

The specificity, which is the ability of analytical method to differentiate and quantify the analyte in the presence of other components, was studied by applying the extraction procedure to plasma samples from six non drug consuming subjects, according to Shah et al. [25].

The extraction recovery was established with QC samples at the three levels with $n = 3$ for each level. The absolute recovery was calculated by comparing the peak area responses of the processed samples to those obtained after direct injection of the same amount of the analyte.

2.6. Applications

This method is currently used to quantify THC in plasma samples from regular cannabis consumers before the entrance in a withdrawal protocol. It is also used to check out the time of cannabis consuming in patients of whose urine test is positive.

3. Results

3.1. Selectivity

The proposed HPLC method enables the quantification of THC in plasma with UV detection at 215 nm. Using the chromatographic conditions mentioned above, the retention times for I.S. and THC are 4.7 and 13.5 min, respectively. Fig. 1, shows a chromatogram of zero point plasma. Figs. 2 and 3 show the chromatographic profiles of a calibration and a QC sample, respectively. Fig. 4 shows a chromatogram of a real plasma sample.

The peaks of the I.S. and THC were well separated and the blank plasma shows no peak interfering on the retention time of the studied molecule. The selectivity was confirmed by analyzing the most frequent over the counter drugs (Parcetamol, Aspirin, INAS), antibiotics, antidepressors and benzodiazepines. All the tested drugs appeared to be eluted at different times and did not interfere with THC analysis.

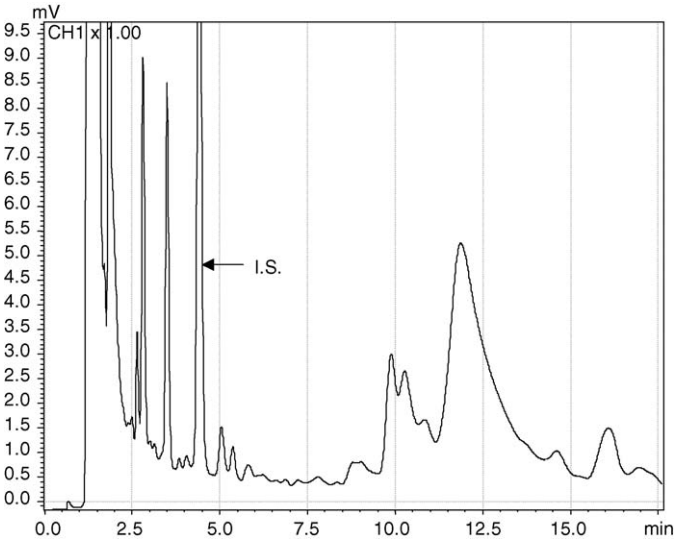

Fig. 1. Chromatographic profile of a zero point plasma.

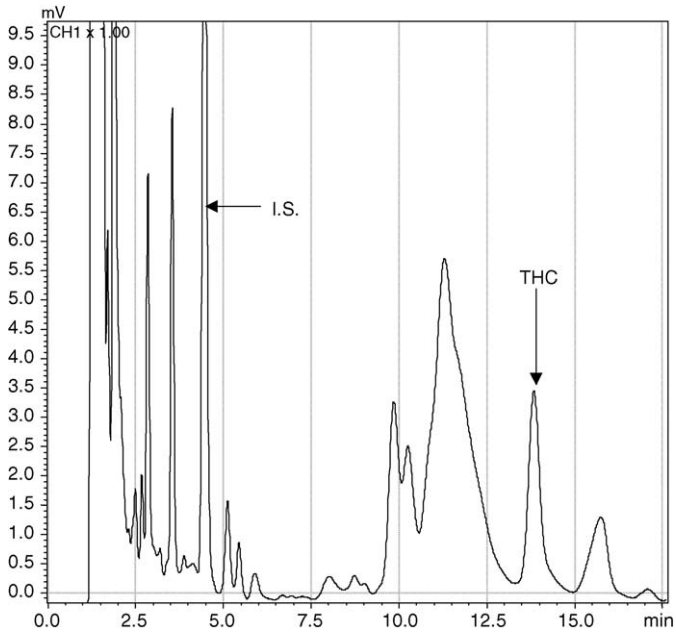

Fig. 3. Chromatographic profile of a plasma quality control of THC (90 ng/ml) spiked with I.S.

To improve selectivity and resolution, a tetrabutylammonium perchlorate solution had been used in the mobile phase. The use of this counter ion allowed the separation of THC from endogenous interfering peak. The use of 1-octanosulfonic acid sodium salt as an ion counter or the absence of a counter ion (a

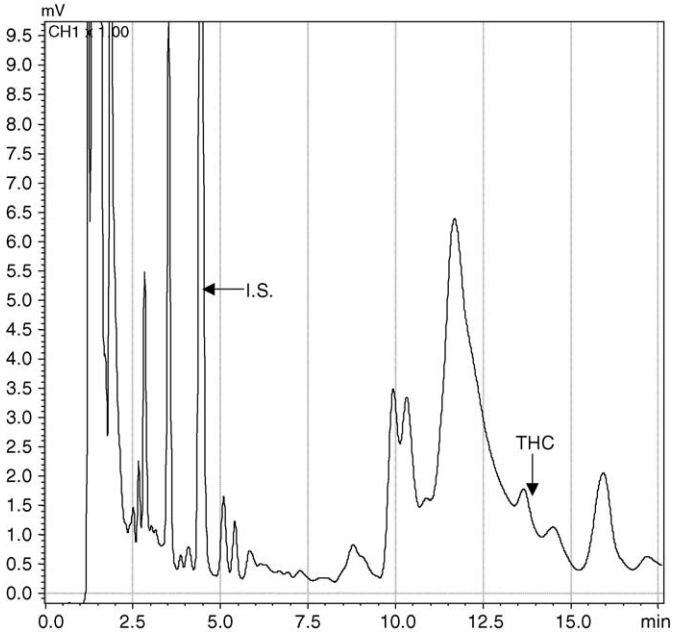

Fig. 2. Chromatographic profile of a plasma calibration sample of THC (15 ng/ml) spiked with I.S.

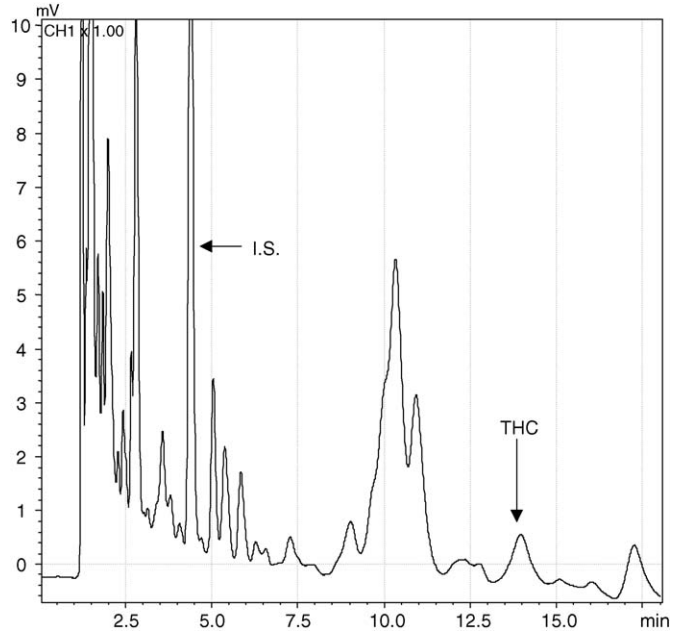

Fig. 4. Chromatographic profile of a real plasma sample containing THC (calculated concentration = 28 ng/ml) and spiked with I.S.

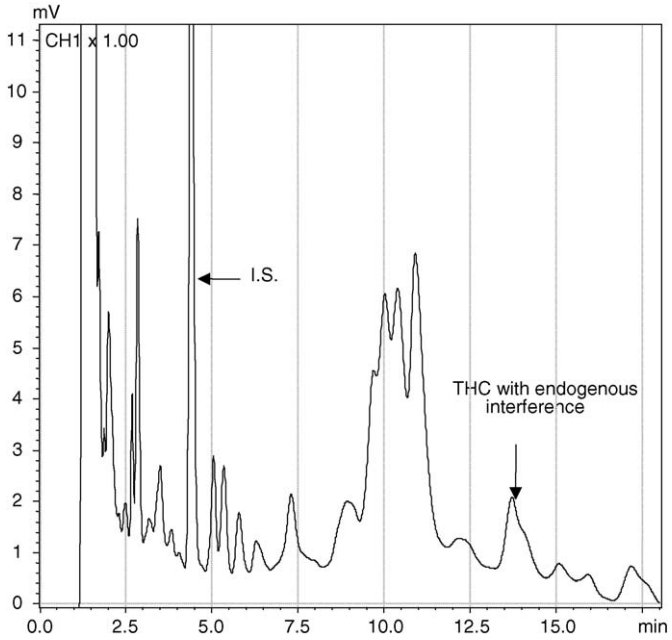

Fig. 5. Chromatographic profile of a spiked plasma extract analyzed using a mobile phase composed of methanol, acetonitrile and 1-octanosulfonic acid sodium salt solution (0.005 M) (50:25:25, v/v/v).

phosphate buffer at pH 3.2), did not allow a good separation of THC from endogenous interferences (Fig. 5).

To diminish the run time, methanol was added to the mobile phase.

With a mobile phase containing methanol, acetonitrile and tetrabutylammonium perchlorate solution (25:50:25, v/v/v), the chromatographic run time was around 30 min, THC retention time being approximately 28 min. The use of a mobile phase composed of methanol, acetonitrile and tetrabutylammonium perchlorate solution (50:25:25, v/v/v), allowed a good separation of THC from endogenous interferences (with a retention time of ∼14 min) and a reasonable run time (16 min).

3.2. Extraction procedure and recovery

The recovery was calculated by comparing peak area of spiked plasma extracts to those obtained after direct injection of the same amount of THC.

The recoveries at three QC levels [mean (R.S.D.); $n = 3$] were 94% (5.5%), 91% (8.1%) and 89% (3.2%) for QC low, QC medium and QC high, respectively.

The solid-phase extraction was carried out after protein precipitation of the plasma sample using acetonitrile, which permitted a pre purification of the sample and, after mixing the acetonitrile with 1-octanosulfonic acid sodium salt solution (pH 3.2, 0.2 M), a good retention of THC on the extraction cartridges. In order to allow THC to be retained on the cyano cartridges protein precipitation was an essential step since with non protein precipitated plasma samples, diluted with 1-octanosulfonic acid sodium salt solution (pH 3.2, 0.2 M), the THC extraction recovery was <5%.

Nevertheless, extraction recoveries were <10% when methanol was used to precipitate the proteins, in spite of the application of the same solid-phase extraction procedure described above.

3.3. Calibration curves

A 7 point calibration curve in human plasma ranging from 5 to 100 ng/ml for THC was analyzed in singlicate for each run.

THC standard curve was satisfactorily described by $1/x$ weighted (x, concentration) least squares linear regression. The slope of the calibration curves obtained throughout method validation was stable with values averaging 0.0042 (±10%) ($n = 4$). Over the concentration range 5–100 ng/ml the correlation coefficient r of the calibration curve remained excellent and >0.99.

3.4. Validation of the HPLC method: precision, accuracy and LLOQ

Precision and accuracy of the measure of the quality control samples were given in Table 1. The concentration levels of THC QC samples (15, 30 and 90 ng/ml) were selected to cover the range of concentrations expected in smoker plasma samples.

Throughout these concentration ranges, the mean intra-assay precision was similar, always lower than 13.5%. Over all, the mean inter-assay precision for THC was good with mean C.V.s within 11.1%. The intra-assay deviation (bias) from the nominal concentrations of THC was between 3.2 and 9.1% and the range of inter-assay deviation was always <9.3%.

By analyzing plasma from outdated transfusion bags spiked with decreasing concentrations of THC, the lower limit of quantification was experimentally found to be 5 ng/ml. For this concentration, the mean C.V. and deviation were 9.9 and 10.7%, respectively.

3.5. Sample stability

3.5.1. Stability of plasma samples kept frozen at −20 °C

No evidence of THC decomposition was found during plasma samples storage in the freezer at −20 °C for at least 2 months. Calibration and QC samples were prepared in batches, and distributed in 5 ml borosilicate glass tubes. The concentrations of QC samples remained stable at least up to 2 months. Plasma collected from patients was stored in the same type of tubes at −20 °C prior to the analysis.

3.5.2. Stability of plasma samples after one, two and three freeze–thaw cycles

The variation on THC concentrations when submitting QC samples to successive three freeze–thaw cycles was checked. The results obtained indicate that no significant loss of THC is to be expected after up to three freeze–thaw cycles.

3.5.3. Stability of plasma samples left at room temperature

The stability of plasma samples left at room temperature for 24 h was checked. The variations of the levels of THC at concentrations of 100 ng/ml was lower than 8.8%, a value compromised

Table 1
Precision and accuracy for THC quantification in plasma (15, 30 and 90 ng/ml)

Theoretical concentration (ng/ml)	Calculated concentration (ng/ml)	S.D.	Precision (C.V. (%))	Accuracy (%)
Intra-assay ($n=6$)				
15	16.4	2.21	13.5	9.05
30	31.0	0.55	1.77	3.24
90	94.0	4.38	4.66	4.40
Inter-assay ($n=6$)				
15	16.4	1.82	11.1	9.30
30	29.8	2.34	7.85	−0.78
90	87.6	4.21	4.81	−2.70

within the assay variability, indicating that at room temperature, the plasma samples appear to be stable.

3.5.4. Stability of extracts samples in HPLC vials (i.e. ready for HPLC analysis) at +4 °C

The stability of plasma extracts (i.e. reconstituted in methanol–water, 3/1, v/v) submitted to HPLC analysis was checked at +4 °C for 24 h.

The variations over time for THC, expressed in percentage of the starting levels (i.e. after immediate analysis), were within the 4–12% in samples left in the autosampler at +4 °C. These results indicate therefore that, taking account of the analytical variability, the processed sample stability is acceptable throughout HPLC analysis performed within 1 day (about 60 samples).

3.6. Clinical application

This method is presently used in our laboratory for the determination of THC plasma levels in regular cannabis consumers. Plasma samples were taken from these patients before their hospitalization for the application of a withdrawal procedure. THC plasmatic concentrations were interestingly higher than 20 ng/ml, while, in occasional cannabis consumers, THC concentrations were most of the time lower than 10 ng/ml.

These results seemed to be in agreement with those already published. THC plasma concentrations varied between 300–30 ng/ml (0.2 h after THC smoking) and 4–1 ng/ml (4 h after THC smoking) [26–28].

4. Discussion and conclusion

A fully validated method for the determination of THC, the main psychoactive cannabinoid in cannabis, by HPLC–UV has been presented. This method provides a robust procedure for the quantification of THC in human plasma samples after two-step treatment.

To the best of our knowledge, this is the first report describing an assay of Δ^9 tetrahydrocannabinol by HPLC–UV after solid-phase extraction in plasma.

The development of a relatively complex extraction procedure was necessary in order to obtain a high specificity since a UV detector particularly at 215 nm was used. This wavelength was chosen to provide enough sensibility for the quantification of low THC plasmatic concentration. Although selectivity and sensibility issues could be more easily handled by the use of LC–MS/MS technology, the latter is not available in the most of hospital laboratories.

Our method was relatively time consuming and of lower sensibility than LC–MS methods, but it required instruments available in conventional hospital laboratories. Furthermore, it could be used for simultaneous determination of THC and THC-COOH in plasma (data not shown). In addition, this method could be used, after minor adjustments, for the determination for THC and/or THC-COOH in other biological matrix such as oral fluid or urine.

In summary, we have developed a HPLC–UV method for THC quantification in human plasma samples. The procedure is enough sensitive and specific and involves a two-step extraction method. It has been validated and applied to about 20 real samples.

References

[1] B. Maralikova, W. Weinmann, J. Mass Spectrom. 39 (2004) 526–531.
[2] C.A. Hunt, R.T. Jones, J. Pharmacol. Exp. Ther. 512 (1980) 35–44.
[3] M.A. Peat, Advances in Analytical Toxicology II.R.C. Baselt Edition, Book Medical Publishers, Chicago, 1989.
[4] C. Giroud, A. Menetrey, M. Augsburger, T. Buclin, P. Sanchez-Mazas, P. Mangin, Forensic Sci. Int. 123 (2001) 159–164.
[5] S. Steinmeyer, D. Bergel, S. Warth, T. Kramer, M.R. Moeller, J. Chromatogr. B 772 (2002) 239–248.
[6] P.R. Stout, C.K. Hom, K.L. Klette, J. Anal. Toxicol. 25 (2001) 550–554.
[7] M. Yonamine, N. Tawil, R.L.M. Moreau, O. Alves Silva, J. Chormatogr. B 789 (2003) 73–78.
[8] E.J. Cone, L. Presley, M. Lehrer, W. Seiter, M. Smith, K.W. Kardos, D. Fritch, S. Salamone, R.S. Niedbala, J. Anal. Toxicol. 26 (2002) 541–546.
[9] R.S. Niedbala, K.W. Kardos, D.F. Fritch, S. Kardos, T. Fries, J. Waga, J. Robb, E.J. Cone, J. Anal. Toxicol. 25 (2001) 289–303.
[10] N. Samyn, A.C. Van Haeren, Int. J. Legal. Med. 113 (2000) 150–154.
[11] P. Kintz, V. Cirimele, B. Ludes, J. Anal. Toxicol. 24 (2000) 557–561.
[12] S. Feng, M.A. Elsohly, S. Salamone, M.Y. Salem, J. Anal. Toxicol. 24 (2000) 395–402.
[13] S. Valiveti, A.L. Stinchcomb, J. Chromatogr. B 803 (2004) 243–248.
[14] M. Concheiro, A. De Castro, O. Quintela, A. Cruz, M. Lopez-Rivadulla, J. Chromatogr. B 810 (2004) 319–324.
[15] H. Teixeira, P. Proenca, A. Verstraete, F. Corte-Real, D.N. Vieira, Forensic Sci. Int. 150 (2005) 205–211.
[16] M. Laloup, M. Ramirez Fernandez Mdel, M. Wood, G. De Boeck, C. Henquet, V. Maes, N. Samyn, J. Chromatogr. A 803 (2005) 15–24.
[17] E.C. Nyoni, B.R. Sitaram, D.A. Taylor, J. Chromatogr. B 679 (1996) 79–84.
[18] Y. Nakahara, H. Sekine, E. Cook, J. Anal. Toxicol. 13 (1989) 22–24.
[19] Y. Nakahara, H. Sekine, J. Anal. Toxicol. 9 (1985) 121–124.

[20] L.K. Thompson, J.E. Cone, J. Chromatogr. 421 (1987) 91–97.
[21] D.S. Isenschmid, Y.H. Caplan, J. Anal. Toxicol. 10 (1986) 170–174.
[22] V. Bianchi, G. Donzelli, J. Chromatogr. B. 675 (1996) 162–167.
[23] S.D. Ferrara, L. Tedeschi, G. Frison, F. Castagna, J. Anal. Toxicol. 16 (1992) 217–222.
[24] Guidance for industry, Bioanalytical method validation. www.fda.gov/cder/guidance/4252fnl.pdf, 2001.
[25] V.P. Shah, K.K. Midha, S.V. Dighe, et al., Pharm. Res. 9 (1992) 588–593.
[26] M.A. Huestis, J.E. Henningfizld, E.J. Cone, J. Anal. Toxicol. 16 (1992) 276–282.
[27] M.A. Huestis, E.J. Cone, J. Anal. Toxicol. 28 (2004) 394–399.
[28] Pharmacokinetic, Evaluation of Published Studies on Controlled Smoking of Marijuana, www.druglibrary.org/schaffer/Misc/driving/s16p1.htm.