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# DETERMINATION OF $\Delta^9$-TETRAHYDROCANNABINOL IN HUMAN BLOOD AND SALIVA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

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## SUMMARY

A rapid, sensitive and selective determination of $\Delta^9$-tetrahydrocannabinol (THC) in human plasma, serum and saliva was developed with high-performance liquid chromatography with electrochemical detection. Initially, samples were deproteinized, followed by a one step liquid–liquid extraction. Samples were measured by high-performance liquid chromatography with electrochemical detection with 4-dodecylresorcinol as the internal standard. The minimal detectable limit for THC in biological samples was ca. 1 ng/ml with a signal-to-noise ratio $> 3$, corresponding to an on-column sensitivity for THC of ca. 0.5 ng. The detector was operated at $+0.90$ V vs. Ag/AgCl and exhibited linearity over a concentration range of 1–150 ng/ml with correlation coefficients of the standard curves $> 0.99$.

## INTRODUCTION

A variety of analytical methodologies have been applied to the detection of $\Delta^9$-tetrahydrocannabinol (THC) in biofluids. In particular, THC has been determined by gas chromatography (GC) [1,2], gas chromatography–mass spectrometry (GC–MS) [3–5], and radioimmunoassay (RIA) [6–9]. Assays employing GC and GC–MS are both highly sensitive and selective, but these techniques require rigorous sample preparation, concentration and derivatization and are time-consuming. RIA assays can be highly sensitive but may not be selective, and may react with other structurally related derivatives. As an alternative, high-performance liquid chromatography (HPLC) can be used as a separation technique which is capable of purifying THC from biofluids without elaborate sample work-up. Unfortunately, ultraviolet detectors are not sufficiently sensitive for the quantitation of THC at human physiological concentrations. As a result, post-column derivatization [10,11], GC [12], GC–MS [13] and RIA [14,15] have all been employed to detect THC after an HPLC separation process.

Recently, an HPLC method was reported which employed electrochemical detection (ED) for the simultaneous determination of free cannabinoids and acidic cannabinoids in plant material, tar and marijuana ash [16]. This methodology demonstrated a potential for quantitation at expected THC physiological concentrations. A method for the detection of nantrodol, a synthetic analogue of THC, has also been developed with HPLC–ED [17]. We have investigated the use of HPLC–ED as an analytical methodology for the routine assay of THC in human biofluids (plasma, serum and saliva). The initial goal was the development of an assay with the required sensitivity (1–150 ng/ml) for determination of THC in blood and saliva.

This report details the results of these studies. The described assay by HPLC–ED is sensitive to 1 ng/ml and the extraction is rapid and reliable. The method is capable of 100 analyses per day by a single analyst. The assay was used for the analysis of THC in plasma, serum and saliva of human subjects who had smoked marijuana.

## EXPERIMENTAL

### *Standards and reagents*

Marijuana cigarettes, THC, 11-hydroxy-THC, 9-carboxy-11-nor-THC, cannabidiol, cannabinol, hexahydrocannabinol and cannabichromene were provided by the Research Technology Branch, Division of Research, National Institute on Drug Abuse ( Rockville, MD, U.S.A.). The marijuana cigarettes contained 2.8% by weight of total THC. Analysis of the cannabinoid derivatives indicated that purity was greater than 95% by GC and 100% by thin-layer chromatography. Other standards and reagents were of reagent-grade quality and were obtained from the following sources: 4-dodecylresorcinol, sodium monochloroacetate (Aldrich, Milwaukee, WI, U.S.A.); monochloroacetic acid (Mallinckrodt, Paris, KY, U.S.A.); methanol, toluene (Burdick & Jackson Labs., Muskegon, MI, U.S.A.), sodium chloride (Fisher, Fair Lawn, NJ, U.S.A.); 70% perchloric acid (J.T. Baker, Phillipsburg, NJ, U.S.A.).

### *Extraction*

THC was extracted from 1.0 ml of biofluid (plasma, serum or saliva) by addition of 0.05 ml of methanol containing 52.5 ng of 4-dodecylresorcinol (internal standard). Deproteinization was carried out by the addition of 2.0 ml of methanol and 0.20 ml of 70% perchloric acid. This mixture was vortexed for 30 s and centrifuged at 2000 *g* for 2 min. The precipitate was discarded and the supernatant was pipetted into a tube containing 1.0 ml of saturated sodium chloride and 0.15 ml of toluene. The mixture was vortexed for 30 s and centrifuged at 2000 *g* for 2 min. The organic phase was removed for HPLC–ED analysis.

### *Chromatographic system*

The HPLC system (Waters Assoc., Milford, MA, U.S.A.) consisted of the following components: computer (DEC Professional Operating System Model 350)

control of pumps, automatic injector and measurement of the analogue signal from the detector; two dual-piston pumps (Model 510), one which delivered the mobile phase and the other was devoted to washing the column with methanol; automatic injector (WISP Model 710B); a $C_{18}$ analytical column (Waters, μBondapak $C_{18}$, 15 cm × 3.9 mm I.D.). The operating system was designed to perform a set of twelve chromatographic analyses followed by rinsing the column with methanol (3 ml/min for 15 min). The column was re-equilibrated with mobile phase (3 ml/min for 15 min) before another set of twelve samples was analyzed. The procedure was designed to perform rapid analysis with periodic washing of the column in order to eliminate retained substances.

Compound detection was achieved with an amperometric detector system (Model LC-4B, BAS, West Lafayette, IN, U.S.A.) employing a glassy carbon working electrode and an Ag/AgCl reference electrode. The electrochemical potential of the working electrode was set at +0.90 V vs. Ag/AgCl. All potentials are referenced versus the Ag/AgCl reference electrode.

The mobile phase was prepared by solution of sodium monochloroacetate and monochloroacetic acid in distilled water followed by addition of methanol (water–methanol, 77.5:22.5) to provide final concentrations of 0.10 *M* sodium monochloroacetate and 0.025 *M* monochloroacetic acid. Chromatography was performed at room temperature at a constant flow-rate of 3.0 ml/min with an upper limit of 172.4 bar. Under these conditions the retention times for THC and 4-dodecylresorcinol were 5.67 and 7.05 min, respectively.

*Calibration curve, reproducibility and recovery*

Calibration curves for THC were obtained by plotting peak-height ratios of THC to internal standard versus THC concentration over the range of 1–150 ng/ml. Samples containing THC concentrations which exceeded this range were diluted and redetermined. Precision and accuracy were examined over the course of the clinical study by assaying plasma samples containing five different concentrations of THC. During assay development, control plasma was collected for use in quality control studies. Known concentrations of THC (Table II) were added and mutiple aliquots were frozen for later use with each assay run. The recovery of THC was determined with plasma samples containing known amounts of THC. Peak-height ratios were compared with those of unextracted standards.

*Subjects, dosing and sample collection*

Healthy drug-free volunteers with a history of marijuana abuse participated in the study. The subjects resided on a closed research ward with medical surveillance. The study was conducted under the guidelines for the protection of the human subjects [18].

Blood and mixed saliva samples were collected prior to administration of the marijuana dosages and at selected time intervals after marijuana administration. Saliva collection was facilitated by providing a small piece of sour candy prior to each collection. All samples were stored at −20°C until analysis.

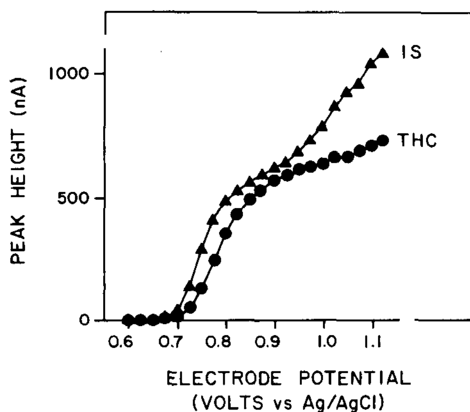


Fig. 1. Hydrodynamic voltammogram of THC and 4-dodecylresorcinol (internal standard, IS). Chromatographic conditions as described in text.

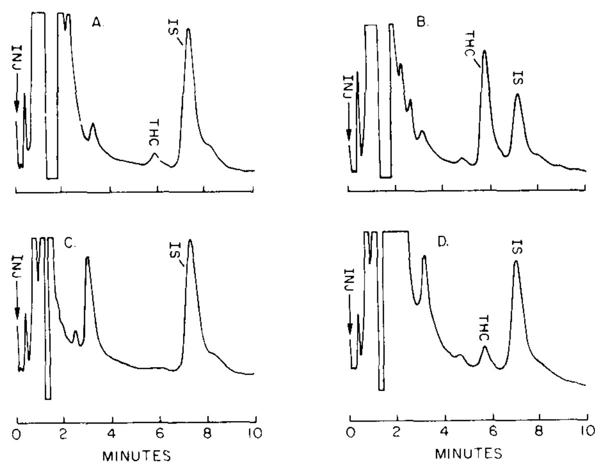


Fig. 2. Chromatograms of human saliva extracts. (A) Control saliva with added THC (10 ng) and internal standard (IS) (52.5 ng); (B) control saliva with added THC (200 ng) and IS (52.5 ng); (C) control saliva with added IS (52.5 ng); (D) saliva from subject collected 120 min after smoking one marijuana cigarette (2.8% THC content) with added IS (52.5 ng). THC content in (D) was calculated as 26.8 ng/ml.

## RESULTS AND DISCUSSION

For THC, an increase in the applied potential in the range of + 0.60 to + 0.90 V resulted in a substantial increase in the oxidative current. Above + 0.90 V, the oxidative current exhibited a plateau on the hydrodynamic voltammogram (see Fig. 1). The electrochemical behavior of the internal standard was similar to THC with the exception of a monotonically increasing current as a function of potential above + 0.95 V. Therefore, a working potential of + 0.90 V was chosen as an optimum working voltage which could maximize the THC signal while maintaining a relatively selective electrochemical process.

The extraction scheme which was developed for THC in human biofluids

TABLE I

HPLC-ED RETENTION DATA

Relative retention times (RRT) and capacity factors ($k'$) of marijuana constituents and THC metabolites with respect to THC (retention time 5.67 min).

| Compound | RRT | $k'$ |
|---|---|---|
| 11-Hydroxytetrahydrocannabinol | 0.469 | 2.91 |
| Cannabidiol | 0.511 | 3.28 |
| 9-Carboxy-11-nor-$\Delta^9$-tetrahydrocannabinol | 0.526 | 3.39 |
| Cannabigerol | 0.543 | 3.54 |
| Cannabinol | 0.829 | 5.93 |
| THC | 1.00 | 7.36 |
| Hexahydrocannabinol | 1.18 | 8.87 |
| 4-Dodecylresorcinol (internal standard) | 1.24 | 9.37 |
| Cannabichromene | 1.34 | 10.19 |

(plasma, serum and saliva) employed a deproteinization step followed by a liquid–liquid extraction. This procedure yielded a high extraction efficiency for THC (84.0%, range 83–96%) with rapid sample purification. With automatic HPLC injection a single analyst could perform over 100 assays per day.

The water–methanol (77.5:22.5) mobile phase yielded baseline resolution ($R_s > 2.5$) and minimized the chromatographic analysis time ($\leq 14$ min). Under these conditions, THC and internal standard were eluted at 5.67 and 7.05 min, respectively. Fig. 2A and B shows typical chromatograms for control saliva containing THC and internal standard. No interfering peaks were noted at the retention times for THC and internal standard when control biofluids (saliva, plasma, serum) were analyzed.

Under the experimental conditions, two chromatographic peaks were eluted approximately 4 to 5 h after injection of biofluid extracts. Owing to these long retention times, it was possible to assay a number of samples (at least twelve injections) before interferences occurred. After ten or twelve chromatographic assays were completed (14 min per assay), a methanol wash (3 ml/min for 15 min) removed these long retained peaks from the column, followed by re-equilibration of the column with mobile phase.

The assay was initiated to demonstrate the feasibility of employing HPLC–ED techniques for pharmacokinetic studies of THC in various biofluids at the expected physiological concentrations. Other marijuana constituents, as well as THC metabolites, were chromatographed to evaluate the selectivity of this HPLC–ED methodology. Their retention times, relative to THC are tabulated (see Table I) and it can be seen that none of these substances interfere with the assay of THC.

The linearity of the calibration curves (peak-height ratios of THC to internal standard versus THC concentrations) was evaluated in all biofluids in concentrations up to 150 ng/ml. The least-squares fit to a typical calibration curve yielded a regression equation of $Y = -0.00244 + 0.0105 \cdot X$, with a typical correlation coefficient $> 0.99$.

TABLE II

ACCURACY AND PRECISION

Quality control study of THC in plasma at various concentrations.

| THC added (ng/ml) | THC found (ng/ml) | Recovery (%) | Coefficient of variation (%) | | *n* | Days |
|---|---|---|---|---|---|---|
| | | | Within-day | Between-day | | |
| 2 | 1.89 | 94.6 | 8.9 | 11.7 | 9 | 3 |
| 4 | 3.84 | 96.2 | 10.7 | 5.5 | 9 | 3 |
| 5 | 5.09 | 101.8 | 4.9 | 7.3 | 18 | 6 |
| 10 | 10.05 | 100.5 | 2.2 | 7.8 | 21 | 7 |
| 55 | 53.5 | 97.3 | 3.1 | 7.1 | 16 | 4 |

Reproducibility of the assay was determined from blank plasma spiked with five different THC concentrations ranging from 2 to 50 ng/ml. The results are shown in Table II, where the accuracy and precision data are presented as a function of THC concentration. Within-day precision ranged from 3.1 to 10.7% and between-day precision ranged from 7.1 to 11.7%. Table II also shows that there was a higher degree of uncertainty at the lower concentrations than at the higher levels of THC.

In order to support ongoing pharmacokinetic and psychological studies and to demonstrate the usefulness of this HPLC–ED procedure, a variety of serum and saliva samples from subjects who received known amounts of marijuana were assayed for THC. Chromatograms of control saliva and saliva after marijuana are shown in Fig. 2C and D, respectively. Typical pharmacokinetic profiles of THC concentrations as a function of time in serum and saliva of one subject after smoking are illustrated in Fig. 3. It is evident that initial saliva levels of THC substantially exceed plasma levels. A likely explanation is that THC is deposited as a depot in the oral cavity during smoking [19]. The initial high concentration of THC disappeared rapidly followed by a slower release of THC with continued appearance in saliva through 7 h. The pattern of THC in saliva observed in this

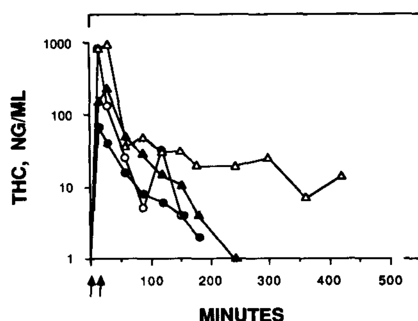


Fig. 3. Time course of THC in serum and saliva of a human subject after smoking one or two marijuana cigarettes (2.8% THC content). Arrows indicate smoking times for marijuana cigarettes. △-△, Saliva, two marijuana cigarettes; ▲-▲, serum, two marijuana cigarettes;○-○, saliva, one marijuana cigarette; ●-●, serum, one marijuana cigarette.

study is somewhat similar to those observed in other studies of THC in saliva [1,19] but differ in the magnitude of the initial concentrations. The observed concentrations of THC in excess of 500 ng/ml are somewhat remarkable, particularly in view of the fact that subjects in the present study were allowed free access to liquids throughout the course of the study and frequently drank substantial amounts of liquids during the smoking process. Fluid intake was allowed in order to simulate normal social settings in which marijuana is used. These initial results further suggest that detection of THC in saliva might serve as an indication of recent marijuana use.

In conclusion, HPLC–ED detection of THC has been demonstrated to be a rapid, reliable and sensitive analytical tool for the analysis of THC at the physiological concentrations of THC in blood and saliva obtained from human subjects after smoking marijuana.

REFERENCES

1 G. Ray, M. Crook, N. West, M. Kwoka, G. Rehagen, J. Cox, E. Murrill and K. Flora, J. Chromatogr., 317 (1984) 455.
2 N.K. McCallum and S.M. Shaw, J. Anal. Toxicol., 5 (1981) 148.
3 V.W. Hanson, M.H. Buonarati, R.C. Baselt, N.A. Wade, C. Yep, A.A. Biasotti, V.C. Reeve, A.S. Wong and M.W. Orbanowsky, J. Anal. Toxicol., 7 (1983) 96.
4 R.L. Foltz, K.M. McGinnis and D.M. Chinn, Biomed. Mass Spectrom., 10 (1983) 316.
5 D.J. Harvey, J.T.A. Leuschner, and W.D.M. Paton, J. Chromatogr., 202 (1980) 83.
6 C.E. Cook, H.H. Seltzman,, V.H. Schindler, C.R. Tallent, K.M. Chin and C.G. Pitt, Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.), 42 (1982) 19.
7 B. Law, P.A. Mason, A.C. Moffat and L.J. King, J. Anal. Toxicol., 8 (1984) 14.
8 S.M. Owens, A.J. McBay, H.M. Reisner and M. Perez-Reyes, Clin. Chem., 27 (1981) 619.
9 S.J. Gross, T.E. Worthy, L. Nerder, E.G. Zimmerman, J.R. Soares and P. Lomax, J. Anal. Toxicol., 9 (1985) 1.
10 H.K. Borys and R. Karler, J. Chromatogr., 205 (1981) 303.
11 P.J. Twitchett, P.L. Williams and A.C. Moffat, J. Chromatogr., 149 (1978) 683.
12 E.R. Garrett and C.A. Hunt, J. Pharm. Sci., 66 (1977) 20.
13 A. Ohlsson, J-E. Lindgren, A. Wahlen, S. Agurell, L.E. Hollister and H.K. Gillespie, Biomed Mass Spectrom., 9 (1982) 6.
14 P.L. Williams, A.C. Moffat and L.J. King, J. Chromatogr., 186 (1979) 595.
15 Y. Nakahara and H. Sekine, J. Anal. Toxicol., 9 (1985) 121.
16 M.A. Peat, M.E. Deyman, and J.R. Johnson, J. Forensic Sci., 29 (1984) 110.
17 R.M. Shepard and G.M. Milne, Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.), 42 (1982) 7.
18 Protection of Human Subjects, Code of Federal Regulations, 45 CFR 46, Federal Register, Vol. 40, August 8, 1975, revised as of January 26, 1981, U.S. Government Printing Office, Washington, DC, pp. 1-16.
19 R.L. Hawks, Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.), 42 (1982) 125.