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COMBINED HIGH-PRESSURE LIQUID CHROMATOGRAPHY AND RADIOIMMUNOASSAY METHOD FOR THE QUANTITATION OF Δ^9-TETRAHYDROCANNABINOL AND SOME OF ITS METABOLITES IN HUMAN PLASMA

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SUMMARY

A high-pressure liquid chromatography–radioimmunoassay (HPLC–RIA) method for the measurement of cannabinoid levels in plasma is described. The method is capable of quantifying 0.1 ng of a cannabinoid in 1 ml of plasma. The experimental procedure consists of an initial separation of cannabinoids in a plasma extract by HPLC followed by collection of the HPLC eluate and RIA. A chromatogram consisting of the cross-reacting cannabinoids in plasma may then be constructed. The plasma concentrations of cannabinoids with retention volumes equivalent to those of Δ^9-tetrahydrocannabinol, cannabinol and mono-hydroxylated metabolites have been measured by this technique.

INTRODUCTION

Over the last decade there has been increasing interest from a research and forensic science viewpoint in qualitative and quantitative analyses of cannabinoids in blood. There is now a wide variety of methods available, most of which are based upon some form of chromatography.

The most popular techniques for the analysis of tetrahydrocannabinol (THC) in plasma have been chromatographic methods. Thin-layer chromatography has been used[1] and gas chromatography (GC) following derivatisation to a species suitable for electron-capture or flame photometric detection[2–4]. Linked GC–mass spectrometric (MS) methods have been described[5–7] as well as HPLC[8]. Methods for quantitation of 11-hydroxy-Δ^9-THC and Δ^9-THC-11-oic acid using GC–MS have been reported[9,10] and applied to the analysis of human plasma[11].

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A major problem encountered with these chromatographic methods has been the presence of endogenous interfering substances in plasma which usually required the use of an extensive extraction procedure for their exclusion. Immunoassay methods, because of the specific nature of the immune-reaction involved, avoid many of these difficulties and the simplicity of the immunoassay method results in shorter analyses times. Cannabinoid RIAs have been described[12–15] as well as a free-radical immunoassay[16]. These immunoassay methods, unlike the chromatographic methods, usually give a combined result obtained from several cannabinoids present in a body fluid and this lack of specificity makes interpretation of RIA levels difficult. A more detailed examination of cannabinoid levels could be made either by using a series of RIA procedures specific for individual cannabinoids similar to those specific for Δ^9-THC and Δ^9-THC-11-oic acid[17] or by combining a chromatographic separation stage with the normal cannabinoid RIA. For the latter method a plasma extract may be chromatographed by HPLC and the eluate monitored by RIA to measure the quantities and elution volume of cross-reacting cannabinoids in the sample.

This report describes such an HPLC–RIA method. The analysis time for the method is comparable with other chromatographic techniques but will quantitate THC and some of its metabolites in one analysis at levels down to 0.1 ng/ml.

EXPERIMENTAL

Radioimmunoassay

Materials and equipment. Antiserum (133Y/22/5) for the assay was obtained from Dr. J. D. Teale, Department of Biochemistry, University of Surrey, Great Britain. Δ^9-THC and other cannabinoid compounds were generously provided by the National Institute on Drug Abuse, Rockville, Md., U.S.A. Δ^9-(G-^{3}H)-THC was purchased from the Radiochemical Centre, Amersham, Great Britain, polyvinylpyrrolidone-40 and charcoal (Norit A) from Sigma, St. Louis, Mo., U.S.A., Dextran T70 from Pharmacia, Uppsala, Sweden, and methanol (AnalaR) and all other chemicals and solvents were obtained from BDH, Poole, Great Britain. An Intertechnique SL30 was used for liquid scintillation counting.

Method. Three volunteers smoked tobacco cigarettes impregnated with 10 mg (subject 1 and 2) and 8 mg (subject 3) of Δ^9-THC over a 10-min period. Blood samples (10 ml) were taken at timed intervals after the subject had finished smoking. Anticoagulant (K_2EDTA, 10 mg) was added to the blood which was centrifuged to separate the plasma. This was stored at −20° until analysis.

The RIA method was based upon that described by Teale *et al.*[18] but was modified by the replacement of the THC solubilising agent Triton X-405 with methanol and with the replacement of bovine γ-globulin in the assay buffer with polyvinylpyrrolidone-40. Antiserum (133Y/22/5) was stored as aliquots in buffer (0.1 *M* phosphate buffer pH 7.5, containing 0.2% polyvinylpyrrolidone-40) at −20° and diluted to 1:300 before use. Solutions of Δ^9-THC used to calibrate the assay were made up in aqueous methanol (50%, v/v, pH 7.5) at concentrations ranging from 500 pg/ml to 50 ng/ml and stored at −20°. Δ^9-(^{3}H)-THC was also stored at −20° at a concentration of 0.25 μCi/ml (12 Ci/mmole) in aqueous methanol (50%, v/v, pH 7.5) ready for use.

The plasma sample to be assayed was mixed with three volumes of methanol,

vortexed and allowed to stand for 30 min. It was then centrifuged and the supernatant added directly to the assay tubes. Normal human plasma similarly treated was used in the total, non-specific binding and zero tubes and for assay dilutions. Using the protocol in Table I, reagents were added to a series of assay tubes (duplicated), the antiserum was added last. These were then allowed to stand at room temperature for 1 h. Dextran-coated charcoal (pH 9.5)[18] was added, the tubes were centrifuged and after 2 min contact time, 500 μl of the supernatant from each tube were counted.

TABLE I

RADIOIMMUNOASSAY PROTOCOL

Reagent	*Volume (μl)*				
	Total counts tube	*Non-specific binding tube*	*Zero tube*	*Standard tube*	*Sample tube*
^{3}H-THC (50% MeOH)	50	50	50	50	50
THC standard (50% MeOH)	—	—	—	100	—
50% MeOH	100	100	100	—	100
Plasma sample extract	—	—	—	—	100
Normal plasma extract	100	100	100	100	—
Diluent buffer	250	350	250	250	250
Antiserum	100	—	100	100	100
	Incubated for 1 h at room temperature				
Diluent buffer	200	—	—	—	—
Dextran-coated charcoal (2.5%)	—	200	200	200	200
	Centrifuged and 500 μl of supernatant counted				

High-pressure liquid chromatography–radioimmunoassay

Materials and equipment. A constant-flow pump (M-6000A Waters Assoc., Milford, Mass., U.S.A.) was used to deliver a methanol–water eluent to a stainless-steel HPLC column (10 cm × 4.6 mm I.D.) slurry-packed with Spherisorb-5-ODS (Phase Separations, Flintshire, Great Britain). Samples were introduced onto the HPLC column with a six-port injection valve (Spectroscopy Accessory, Sideup, Great Britain) fitted with a 10-ml injection loop. Column eluate was either monitored with an ultraviolet (UV) detector at 280 nm (Cecil CE212) or collected with a Struers Samplomat fraction collector (Camlab, Cambridge, Great Britain). A freezedrier (Model SB4, Chemlab. Instruments, Ilford, Great Britain) was used to remove solvents.

Method. Plasma (0.2–1 ml) was mixed with three volumes of methanol, vortexed, left to stand for 30 min and centrifuged. The supernatant was removed, the residue mixed with methanol and the sample again centrifuged. Water was added to the combined supernatants to give a methanol concentration of 50% (v/v). The HPLC pump, sample loop and column were flushed with aqueous methanol (50%, v/v) and the sample solution was injected onto the column. A stepped solvent elution programme was used: 10 ml of a mixture of methanol–water (50:50), 10 ml of methanol–water (62.5:37.5), 20 ml of methanol–water (72.5:27.5, 1 ml/min), and eluent fractions were taken every 30 sec. Solutions containing the appropriate THC standards and the HPLC fractions were freezedried. The freezedrier was flushed with argon before and after drying to prevent atmospheric oxidation of sensitive metab-

olites. A solution of Δ^9-(^{3}H)-THC (0.025 μCi in 500 μl of a mixture of methanol–diluent buffer, 30:70) was added to each of the dried tubes with a solution of antiserum in diluent buffer (100 μl of 1:300 antiserum solution). The non-specific binding tubes received diluent buffer in place of antiserum solution. The subsequent RIA procedure was the same as that described above.

Cannabinoid retention volumes were determined by monitoring the eluate with either RIA for cross-reacting compounds, or UV absorption (280 nm) for non-cross-reacting compounds. UV detection required μg quantities compared with the ng quantities used with RIA detection.

High-pressure liquid chromatography–gas chromatography–mass spectrometry

Materials and equipment. HPLC equipment was the same as that described in the previous section. GC–MS was conducted using a Pye 104 GC equipped with an OV-17 column (0.3 m × 2 mm I.D., Gas-Chrom Q, 80–100 mesh) interfaced to a VG Micromass 16F Mass Spectrometer. The internal standard (5′-$^2H_3$)-Δ^9-THC was kindly supplied by Dr. S. Agurell, Stockholm.

Method. The GC–MS method used was similar to that described by Agurell *et al.*[5] (5′-$^2H_3$)-Δ^9-THC (10 ng) in methanol was added to the plasma sample (1 ml) and the mixture extracted with methanol as described previously. The plasma extract was chromatographed using a 10-cm ODS column (4.6 mm I.D.) and methanol–water (67.5:32.5) as the eluent. The THC fraction (at a retention volume of approximately 20 ml) was collected, freezedried and the residue dissolved in hexane. This was gas chromatographed with a mass spectrometer in the multiple ion-detection mode tuned to ions of *m/e* 299 and 314 for THC and 302 and 317 for tri-deuterated THC. The THC retention time was 4 min with a helium flow-rate of 20 ml/min at 190°. The THC plasma concentration was calculated from the ratio of ion intensities for THC and deuterated THC using a previously prepared calibration graph.

RESULTS AND DISCUSSION

The assay method was similar to that described by Teale *et al.*[18] except that methanol replaced Triton X-405 as the THC solubilising agent. This modification increased the solubility of THC and reduced non-specific binding in the assay. A reduction of blank levels was obtained with normal plasma samples together with improved assay sensitivity.

Teale *et al.*[18] investigated antiserum specificity and found no cross-reaction with 24 non-cannabinoid drugs. In our study 44 non-cannabinoid drugs were examined (Table II) at a concentration equivalent to 400 μg/ml plasma and again no cross-reaction was observed with any of the compounds. Antiserum specificity to cannabinoid compounds using the modified RIA procedure was examined (Table III). Results were similar to those obtained by Teale *et al.*[18] in that the antiserum cross-reacted with the same avidity to THC as it did to some of the closely related THC metabolites.

The levels of cross-reaction in plasma samples obtained from three subjects who smoked THC impregnated cigarettes are given in Table IV. Levels of cross-reaction were highest for those samples obtained immediately after smoking while values obtained for subsequent samples fell to a constant level for the remainder of

TABLE II

MISCELLANEOUS DRUGS TESTED FOR CROSS-REACTIVITY IN THE RADIOIMMUNOASSAY

Adrenaline	Methaqualone hydrochloride
Amitriptyline hydrochloride	Morphine sulphate
Amphetamine sulphate	Nicotine hydrogen tartrate
Ascorbic acid	Nitrazepam
Aspirin	Oestradiol
Barbitone	Papaverine hydrochloride
Bromodiphenhydramine hydrochloride	Paracetamol
	Pentobarbitone
Caffeine	Phenobarbitone
Codeine phosphate	Phenmetrazine theoclate
Cocaine hydrochloride	Phenylbutazone
Chlorodiazepoxide	Progesterone
Chloropromazine hydrochloride	Promazine hydrochloride
N,N-Dimethyltryptamine	Spironolactone
Diphenhydramine hydrochloride	Stilboestrol
Doxepin	Sulphadimidine
Ephedrine hydrochloride	Sulphamethoxypyridazine
Ergometrine maleate	Tetracycline hydrochloride
Ethylenediamine tetraacetic acid	Thiopentone sodium
Imipramine	Trifluoperazine
Lignocaine	Tryptamine hydrochloride
Mescaline sulphate	Tyramine hydrochloride
Methadone hydrochloride	

TABLE III

CROSS-REACTIVITY OF Δ^9-THC METABOLITES AND RELATED COMPOUNDS IN THE RADIOIMMUNOASSAY

THC = Δ^9-tetrahydrocannabinol, CBN = cannabinol, CBD = cannabidiol.

Compounds	*Amount required for 50% depression of binding (ng)*
Δ^9-THC	0.6
Δ^8-THC	0.6
CBN	0.6
11-Hydroxy-Δ^9-THC	0.6
Δ^9-THC-11-oic acid	0.6
11-Hydroxy-CBN	0.6
CBN-11-oic acid	0.6
Hexahydro-CBN	0.6
$\Delta^{9,11}$-THC (Exo-cyclic compound)	0.6
8α-Hydroxy-Δ^9-THC	0.6
8β-Hydroxy-Δ^9-THC	3.0
8α,11-Dihydroxy-Δ^9-THC	2.0
8β,11-Dihydroxy-Δ^9-THC	9.0
1′-Oxo-CBN	3.0
1′-Hydroxy-CBN	10.0
5′-Hydroxy-Δ^9-THC	30.0
CBD	>50.0
Cannabicyclol	>50.0
Cannabichromene	>50.0
Cannabigerol	>50.0

TABLE IV

THE PLASMA CONCENTRATION OF Δ^9-THC IN VOLUNTEERS WHO HAD SMOKED Δ^9-THC DETERMINED BY RIA, HPLC AND GC–MS

ND = Not determined.

Subject	*Time after smoking (min)*	*Plasma concentration of Δ^9-THC (ng/ml)*		
		*Direct RIA**	*RIA after separation by HPLC*	*GC–MS*
1	Control	23**	0	0
	2	67	47	55
	12	48	15	18
	24	47	7	9
	34	48	5	8
	64	47	3	5
	126	48	1	2
2	Control***	ND**	ND	ND
	3	63	45	58
	13	44	20	30
	24	53	9	16
	34	47	6	9
	64	58	4	5
	124	50	2	1
3	Control	0	0	0
	2	37	26	26
	22	7	4	3
	31	5	1.1	ND
	60	9	0.9	ND
	120	6	0.8	ND

* These values include a contribution from THC metabolites.

** Subject 1 and 2 were both cannabis users and cannabinoid material may have been present in the plasma before the experiment.

*** A control sample was taken for subject 2 but this was lost during centrifugation.

the 2-h experiment. The absence of a continuing decline of plasma cross-reacting cannabinoids over the course of the experiment could not be fully explained without a greater understanding of the changes of plasma concentrations of the THC metabolites. These data were obtained by using an HPLC separation stage prior to RIA.

Chromatograms representing the elution of cross-reacting compounds from an HPLC column were constructed for each plasma sample by plotting the RIA results obtained for consecutive HPLC eluent fractions against their retention volumes. Two of the chromatograms produced by this method are represented in Figs. 1 and 2. Individual components were quantified by summing the data points for each area of cross-reaction and relating these to the original volume of plasma used. Plasma THC levels for the three subjects were measured by this method and compared with THC levels obtained by the GC–MS method (Table IV). These results showed a biphasic elimination pattern for THC (*e.g.*, Fig. 3), but values obtained by the HPLC–RIA method were consistently lower than those obtained by GC–MS (Table IV). This discrepancy could not be fully explained by losses that occurred during the plasma extraction and chromatographic stages of the HPLC–RIA procedure which were found to be approximately 10%. The HPLC–RIA results for subjects 1 and 2 were obtained approximately six months after the GC–MS analyses were performed. It it possible that decomposition of the THC had occurred during this period.

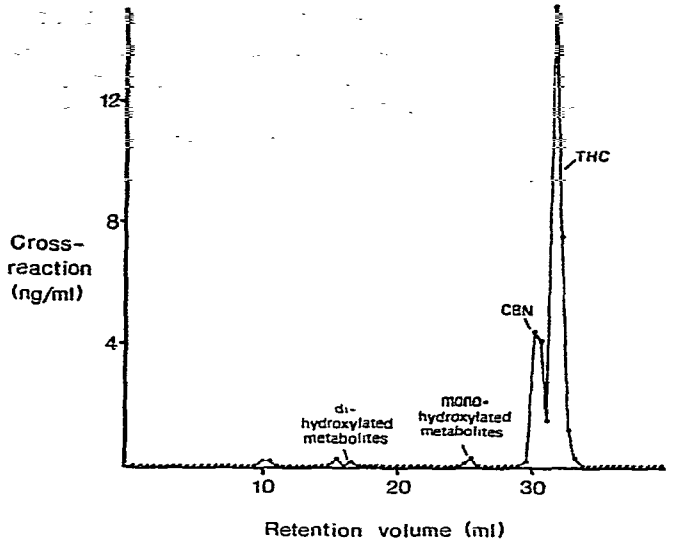

Fig. 1. HPLC–RIA chromatogram of a plasma sample from subject 3 taken 2 min after smoking Δ^9-THC (8 mg). The retention volumes of THC, CBN, the mono-hydroxylated metabolites and di-hydroxylated metabolites are marked.

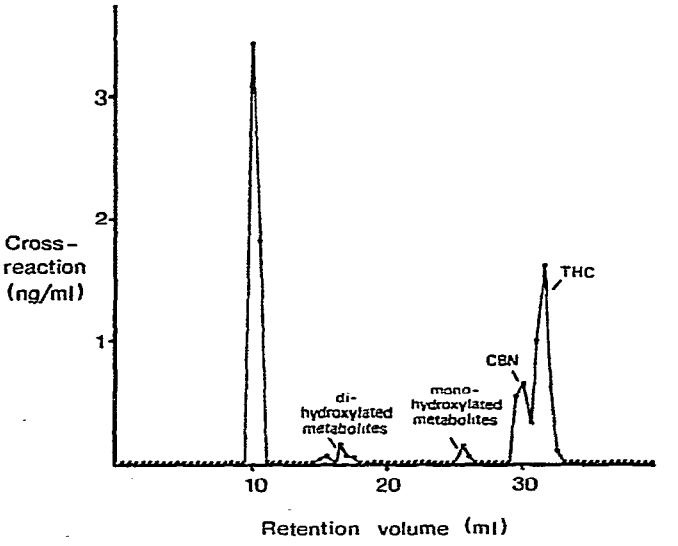

Fig. 2. HPLC–RIA chromatogram of a plasma sample from subject 3 taken 22 min after smoking Δ^9-THC (8 mg). The retention volumes of THC, CBN, the mono-hydroxylated metabolites and di-hydroxylated metabolites are marked.

The presence of cross-reacting compounds in the plasma samples other than THC was also indicated by the HPLC–RIA data. A comparison of cannabinoid retention volumes (Table V) with the HPLC–RIA data, showed the presence of cross-reacting compounds in the plasma samples with retention volumes corresponding to

those of cannabinol (CBN), mono-hydroxylated metabolites and di-hydroxylated metabolites (Figs. 1 and 2). Control plasma samples contained no indication of the presence of these cannabinoids. Subject 1 was a regular user of cannabis and the control sample contained cross-reacting material which had a retention volume of 10 ml. This was probably due to the consumption of cannabinoid material at some time before the experiment. Subject 3 was not a cannabis-user and his control sample contained no indication of cross-reacting material.

TABLE V

CANNABINOID HPLC RETENTION VOLUMES

Compounds	*Retention volume (ml)* *
Δ^9-THC	31.5
Δ^8-THC	31.0
CBN	30.0
11-Hydroxy-Δ^9-THC	25.5
Δ^9-THC-11-oic acid	10.0
11-Hydroxy-CBN	25.5
CBN-11-oic acid	10.0
8α-Hydroxy-Δ^9-THC	25.5
8β-Hydroxy-Δ^9-THC	20.5
8α,11-Dihydroxy-Δ^9-THC	17.0
8β,11-Dihydroxy-Δ^9-THC	16.5
1′-Oxo-CBN	29.0
1′-Hydroxy-CBN	22.5

* For the system described in the text.

CBN has been reported as present in plasma samples after THC administration[19] which suggested that CBN was a metabolic product of THC. However, Wall *et al.*[11] found almost no CBN in plasma after the intravenous administration of THC to human subjects and concluded that CBN was not a THC metabolite. Plasma concentrations of the compound eluting with the retention volume of CBN found in this study (Table VI) appeared to follow a biphasic elimination pattern similar to that of THC (Fig. 3). This suggests that a metabolic explanation for the presence of CBN in these samples is unlikely.

One of the other areas of cross-reaction observed with the HPLC–RIA data had a retention volume of 25.5 ml. The metabolites 11-hydroxy-Δ^9-THC, 8α-hydroxy-Δ^9-THC and 11-hydroxy-CBN all have this retention volume with the HPLC system used and it is possible that the observed level of cross-reaction may represent a contribution from each of these metabolites. Levels of cross-reaction at this retention volume reached a maximum shortly after Δ^9-THC smoking for the three subjects, falling slowly over the remainder of the experiment (Fig. 3). The concentrations and elimination curves for this area of cross-reaction are similar to those of 11-hydroxy-Δ^9-THC reported in plasma samples obtained from subjects receiving intravenously administered Δ^9-THC[11].

An area of cross-reaction at the retention volumes corresponding to the dihydroxylated metabolites 8α,11-dihydroxy-Δ^9-THC and 8β,11-dihydroxy-Δ^9-THC was also observed with the HPLC–RIA data (Figs. 1 and 2). These metabolites were

TABLE VI

THE PLASMA CONCENTRATIONS OF CROSS-REACTING CANNABINOIDS OTHER THAN Δ^9-THC IN VOLUNTEERS WHO HAD SMOKED Δ^9-THC DETERMINED BY HPLC–RIA

Subject	*Time after smoking (min)*	*Plasma concentration as determined by RIA and expressed as ng/ml of cross-reacting cannabinoid*	
		Peak elution at the position of mono-hydroxylated metabolites	*Peak elution at the position of CBN*
1	Control	0	0
	2	0.6	9.0
	12	1.2	2.1
	24	0.6	1.4
	34	0.5	0.5
	64	0.2	0.2
	126	0.1	0.1
2	3	1.0	5.0
	13	1.0	2.5
	24	1.4	1.7
	34	0.5	0.7
	64	0.3	0.2
	124	0.4	0.1
3	Control	0	0
	2	0.3	9.0
	22	0.3	1.2
	31	0.1	0.4
	60	0.1	0.3
	120	0.1	0.2

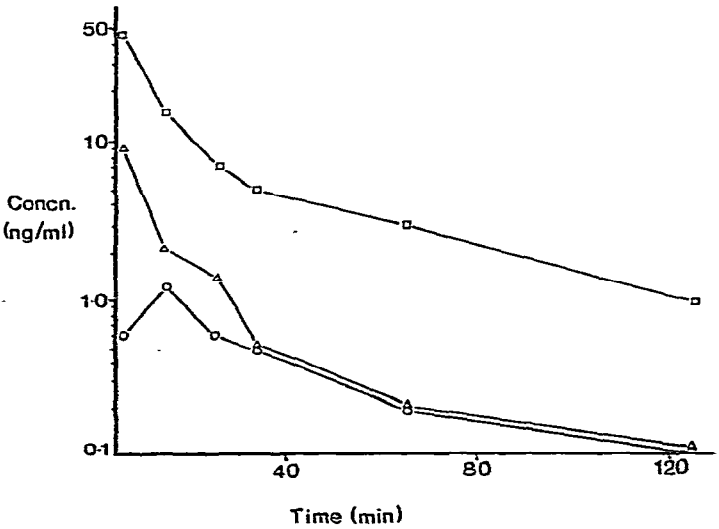

Fig. 3. Elimination from plasma of cannabinoids with retention volumes equivalent to Δ^9-THC (□), CBN (△) mono-hydroxylated metabolites (○) for subject 1.

not well resolved with the chromatographic system used (Table V) and do not cross-react in the RIA to the same extent as each other or to Δ^9-THC (Table III). Quantitative results for these compounds were not therefore, obtained from the HPLC–RIA data.

A further feature of the HPLC–RIA data was the presence of an area of cross-reaction eluting from the HPLC column at a retention volume of 10 ml (Figs. 1 and 2). This corresponds to the retention volume of metabolite conjugates observed in human urine samples obtained after Δ^9-THC administration as well as to the mono-carboxylated metabolites Δ^9-THC-11-oic acid and CBN-11-oic acid (Table V). This area of cross reaction may therefore represent a mixture of cross-reacting metabolite conjugates and the mono-carboxylated metabolites. These cross-reacting components may be separated by acidifying the HPLC eluate. The contribution of this area of cross-reaction to the total RIA result increased as the THC concentration decreased so that the total RIA result remained approximately constant for the 2-h period after THC administration (Table IV). The absolute identification of the structure of cross-reacting compounds will require the isolation of sufficient quantities of material to obtain MS data.

HPLC–RIA provides a convenient method for separating, presumptively identifying and quantifying THC and some of its metabolites in plasma. It has the advantage over previously reported methods of using a small volume of sample to quantify simultaneously THC and some of its metabolites. The application of this method to samples submitted for forensic examination has been reported[20].

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REFERENCES

1 J. A. Vinson, D. D. Patel and A. H. Patel, *Anal. Chem.*, 49 (1977) 163.
2 D. C. Fenimore, R. R. Freeman and P. R. Loy, *Anal. Chem.*, 45 (1973) 2331.
3 E. R. Garrett and C. A. Hunt, *J. Pharm. Sci.*, 62 (1973) 1211.
4 N. K. McCallum, *J. Chromatogr. Sci.*, 11 (1973) 509.
5 S. Agurell, B. Gustafsson, B. Holmstedt, K. Leander, J. E. Lindgren, I. Nilsson, F. Sandberg and M. Asberg, *J. Pharm. Pharmacol.*, 25 (1973) 554.
6 J. Rosenfeld, B. Bowins, J. Roberts, J. Perkins and A. S. Macpherson, *Anal. Chem.*, 46 (1974) 2232.
7 R. Detrich and R. L. Foltz, *Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.)*, 7 (1976) 88.
8 J. L. Valentine, P. J. Bryant, P. L. Gutshall, O. H. M. Gan, E. D. Thompson and H. C. Niu, *Res. Monogr. Ser., Nat. Inst. Drug Abuse. (U.S.A.)*, 7 (1976) 96.
9 J. Rosenfeld and V. Y. Taguchi, *Anal. Chem.*, 48 (1976) 726.
10 M. Nordqvist, J. E. Lindgren and S. Agurell, *Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.)*, 7 (1976) 64.
11 M. E. Wall, T. M. Harvey, J. T. Bursey, D. R. Brine and D. Rosenthal, *Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.)*, 7 (1976) 107.

12 J. D. Teale, E. J. Forman, L. J. King and V. Marks, *Nature (London)*, 249 (1974) 154.
13 J. D. Teale, E. J. Forman, L. J. King and V. Marks, *Lancet*, ii (1974) 553.
14 S. J. Gross, J. R. Soares, J.-R. R. Wong and R. E. Schuster, *Nature (London)*, 252 (1974) 581.
15 C. E. Cook, M. L. Howes, E. W. Amerson, C. G. Pitt and D. Williams, *Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.)*, 7 (1976) 15.
16 M. Cais, S. Dani, Y. Josephy, A. Modiano, H. Gershon and R. Mechoulam, *Febs. Lett.*, 55 (1975) 257.
17 S. J. Gross and J. R. Soares, *Res. Monogr. Ser., Nat. Inst. Drug Abuse (U.S.A.)*, 7 (1976) 10.
18 J. D. Teale, E. J. Forman, L. J. King, E. M. Piall and V. Marks, *J. Pharm. Pharmacol.*, 27 (1975) 465.
19 N. K. McCallum, B. Yagen, S. Levy and R. Mechoulam, *Experientia*, 31 (1975) 520.
20 J. D. Teale, J. M. Clough, L. J. King, V. Marks, P. L. Williams and A. C. Moffat, *J. Forensic Sci. Soc.*, 17 (1977 177).