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The GC-MS quantitation of urinary 11-nor-delta-9-Tetrahydrocannabinol-9-carboxylic acid after derivatization by direct extractive alkylation.

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INTRODUCTION

THC is the major psychoactive component of cannabis. In humans THC is metabolised and excreted primarily as the glucuronic acid conjugate of THC-COOH. This communication describes the development of an extractive alkylation (EA) method for the GC-MS determination of urinary THC-COOH.

Definitions

THC= Delta-9-tetrahydrocannabinol
THC-COOH= 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid
d_3-THC-COOH= tri-deuterated analog of THC-COOH
THC-COOH-glu= glucuronide conjugate of THC-COOH
SRM 1507= Human urine standard reference material 1507
THA^+= Tetrahexylammonium hydrogen sulphate

EXPERIMENTAL

Instrumentation

Gas Chromatograph	Hewlett-Packard 5890 Series II.
Detector	Hewlett-Packard 5970B MSD operated in SIM mode.
Selected ions for THC-COOH	313, 357, 372
Selected ions for d_3-THC-COOH	316, 360, 375
Column	12m HP Ultra 2
Injector temperature	290°C
Detector temperature	290°C
Initial column temperature	138°C
Final column temperature	300°C
Temperature programme	10°C/min

Preparation of the SM-7 resin columns

The fines were removed from the commercially avaliable 200-400 mesh SM-7 sorbent (Bio-Rad Labs) by suspending it in methanol and decanting the supernatant. This was repeated until the supernatant was clear. The suspended SM-7 sorbent was packed into 2.5-3.0cm columns using disposable glass pipettes (6mm ID) fitted with small plugs of silanised glass wool to act as bed supports. Before use the methanol was removed from each column by conditioning with 2ml of toluene.

Hydrolysis

Aliquots of urine (2ml) were added to 16mm x 150mm teflon-lined screw capped test tubes, fortified with 4ul of 10ug/ml of d_3-THC-COOH (internal standard), made alkaline with 100ul of 6M NaOH and allowed to stand at 25°C for at least 15 min with occasional shaking.

Direct Extractive Alkylation

To each of the hydrolysed samples were added 25ul of 0.2M THA^+ (purchased from Fluka and prepared by dissolving 4.5g of the salt in 50ml of 0.5M NaOH) and 5ml of 0.2M iodomethane in toluene. The urine and toluene phases were mixed at 25°C for 30 min and then centrifuged at 1500g for 5 min. The toluene phases were passed through the pre-prepared SM-7 resin columns, collected in disposable test tubes and evaporated to dryness under a stream of nitrogen at 35°C. The residues were reconstituted in 100ul of toluene before injection of 2ul aliquots into the GC-MS system.

RESULTS AND DISCUSSION

Hydrolysis

The time course for the alkaline hydrolysis of THC-COOH-glu at 25°C and 50°C, illustrated in Fig.1, shows that the reaction is rapid with optimum yields of THC-COOH achieved with an hydrolysis time of 15 mins at 25°C and 5 mins for hydrolysis at 50°C.

Extractive Alkylation Reaction

Fig.2 shows the SIM GC-MS profile obtained after performing alkaline hydrolysis and EA on a urine sample with a THC-COOH concentration of 28ng/ml. The yield of methyl ester-methyl ether derivative of THC-COOH during EA was found to be dependent on the urinary concentration of THA^+, the concentration of iodomethane in the toluene phase and the mixing time of the two phases.

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The time course for the methylation of THC-COOH by EA at 25°C and with urinary THA^+ concentrations of 0.5, 1.0 and 2.5mM is illustrated in Fig.3. The optimum yields were achieved at a THA^+ concentration of 2.5mM and a mixing time of 30min.

Fig.4 illustrates the effect of iodomethane concentration on the yield of the methyl ester-methyl ether derivative of THC-COOH. Optimum yields were obtained for iodomethane concentrations in the range of 0.1-0.4M.

Linearity

The peak area ratios of fragment ions m/z 357 for THC-COOH and m/z 360 for d_3-THC-COOH were subjected to least squares regression analysis. The results in Table I indicate that the inter-day standard curves were reproducible with linear responses for peak area ratios versus concentration.

Accuracy and Precision

The results for the analysis of SRM 1507, shown in Table II, indicate that the method is accurate giving an intra-run coefficient of variation of 4.8%.

The results in Table III for the inter-day analysis of a known positive urine indicate that the method gives an inter-run coefficient of variation of 7.0%.

Recovery and Detection Limit

The method has a relative recovery of 97.5± 1.6% (n=10).The detection limit, defined as the concentration of analyte that gave a signal-to-noise ratio of 3, was 0.25ng/ml.

Conclusions

The advantage of the procedure is that the hydrolysis, extraction and derivatization of the analyte is carried out in the same test tube resulting in: (a) reduced loss of analyte because of fewer transfer steps, (b) reduced sample preparation time and (c) reduced usage of laboratory glassware.

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TABLE I

INTER-RUN STANDARD CURVES OBTAINED BY LINEAR REGRESSION ANALYSIS FOR THE GC-MS QUANTITATION OF URINARY THC-COOH.

Each standard curve was derived from 8 points in the concentration range of 0-300ng/ml and the area ratio of ion 357 : ion 360.

	SLOPE (m)	Y-INTERCEPT (b)	CORRELATION COEFFICIENT (r)
	0.0537	-0.0266	0.9998
	0.0555	-0.0100	0.9999
	0.0513	+0.0852	0.9993
	0.0571	-0.0582	0.9996
MEAN	0.0544	-0.0024	0.9997
S.D.*	0.0025	0.0617	0.0003

* Standard deviation

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TABLE II

ACCURACY OF THE DETERMINATION OF THC-COOH IN HUMAN URINE STANDARD REFERENCE MATERIAL (SRM) 1507.

Certified THC-COOH concentration: 20 ± 2ng/ml

SAMPLE	[THC-COOH] (ng/ml)
1	21.88
2	21.15
3	20.72
4	22.28
5	19.49
6	22.11
7	20.63
8	21.17
9	19.77
10	19.72
MEAN	20.89
S.D.*	1.01
C.V.**	4.8%

* Standard deviation

** Coefficient of variation

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TABLE III

INTER-RUN PRECISION FOR THE DETERMINATION OF URINARY THC-COOH.

Sample volume: 2ml; hydrolysis time: 20min; urinary THA^+ concentration: 2.5mM; organic phase: 0.2M iodomethane in toluene; urine-toluene mixing time:30min.

DAY	[THC-COOH]ng/ml	MEAN [THC-COOH]ng/ml
1	31.20, 30.25, 30.48, 30.75	30.67
2	30.56, 31.34, 28.06, 29.04	29.75
3	26.84, 27.28, 30.01, 29.41	28.39
4	25.67, 25.80, 24.71, 25.24	25.36
5	29.28, 29.42, 27.88, 28.86	28.86
MEAN		28.61
S.D.*		2.01
C.V.**		7.0%

* Standard deviation

** Coefficient of variation

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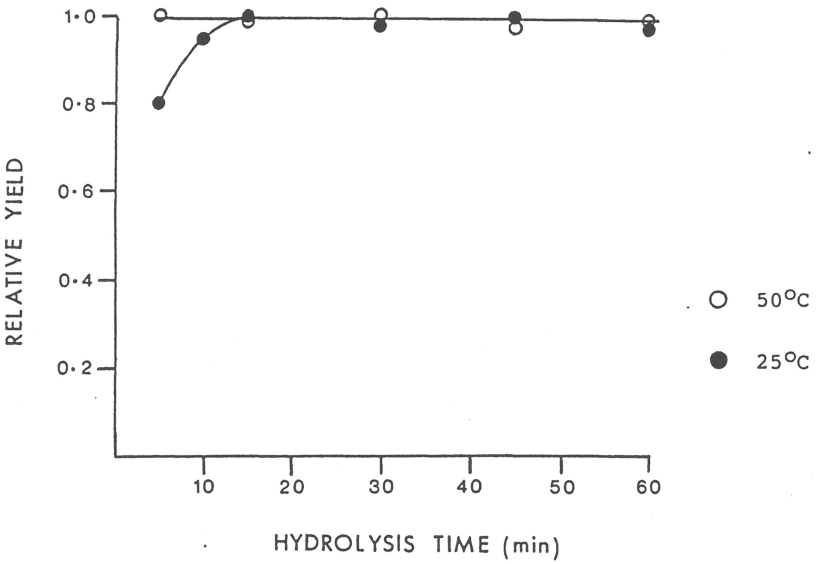

Fig. 1 Time-course for the alkaline hydrolysis of the glucuronic acid conjugate of THC-COOH in urine. Concentration of THC-COOH-glu: 75.8ng/ml (equivalent to a concentration of 50ng/ml of unconjugated THC-COOH).

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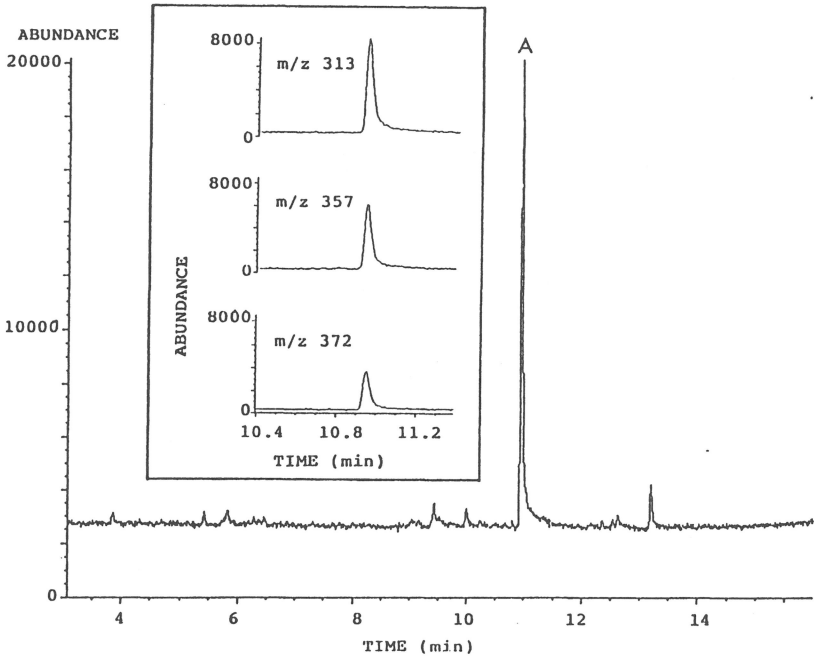

Fig. 2 SIM GC-MS profile of a urine with a THC-COOH concentration of 28ng/ml. The insert displays the m/z 313, 357 and 372 ion traces for the methyl ester-methyl ether derivative of THC-COOH. Peak A= methyl ester- methyl ether derivative of THC-COOH.

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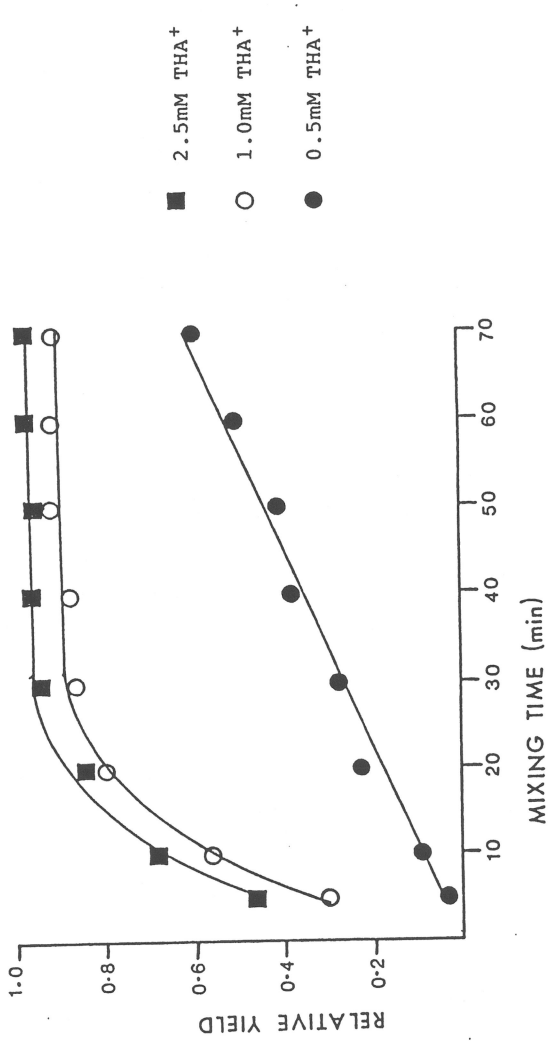

Fig. 3 Time-course for the methylation of THC-COOH by direct EA. Aqueous phase: 2ml of urine; organic phase: 5ml of 0.2M iodomethane in toluene; concentration of THC-COOH: 50ng/ml

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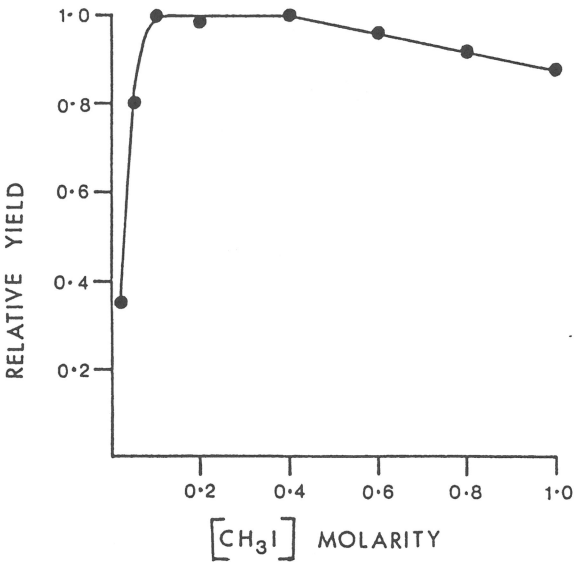

Fig. 4 Effect of iodomethane on the yield of methyl ester-methyl ether derivative of THC-COOH. Aqueous phase: 2ml of urine; concentration of THA^+: 2.5mM; concentration of THC-COOH: 50ng/ml; mixing time: 30min; temperature 25°C.