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QUANTIFICATION OF URINARY 3α,21-DIHYDROXY-5β-PREGNAN-20-ONE AND 5-PREGNENE-3β, 20α-DIOL BY MASS FRAGMENTOGRAPHY

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SUMMARY

A sensitive and accurate method is described for measuring urinary corticosteroids by gas chromatography-mass spectroscopy (GC–MS). Using single peak monitoring (mass fragmentography) and electron impact ionization, the acetates of 3α,21-dihydroxy-5β-pregnan-20-one (tetrahydrodeoxycorticosterone) and 5-pregnene-3β,20α-diol were estimated with deuterio-acetate carriers as recovery markers. With this technique, the coefficient of variation did not exceed 3% for GC–MS analyses of the urinary corticosteroid samples by single peak monitoring. An evaluation of the trimethylsilyl ether derivatives of the two steroids by chemical ionization was also made. Secretion rates determined for deoxycorticosterone derived from specific activities of urinary tetrahydrodeoxycorticosterone and excretion levels of 5-pregnene-3β,20α-diol were slightly lower than those obtained by other methods.

INTRODUCTION

Harris *et al.*[1] and Schambalen *et al.*[2] have reported urinary excretion levels for tetrahydrodeoxycorticosterone (THDOC) of 0·019 mg and 0·024 mg/24 h, respectively, for normal adults. New *et al.*[3], Schambalen *et al.*[2] and Seth *et al.*[4] have determined urinary secretion rates for deoxycorticosterone (DOC) from specific activities of THDOC after administration of radioactive DOC to normal subjects.

We have measured urinary excretion of 20α-hydroxy-5-pregnen-3β-yl sulfate (pregnenediol sulfate) as free 5-pregnene-3β-20α-diol (pregnenediol) after solvolysis [5], and obtained a mean excretion value of 0·04 mg/day, range 0·01 to 0·15 mg/day, for young and elderly men. We have also compared relative rates of production of pregnenolone [5] and pregnenolone sulfate [6] calculated from the respective S.A. of this metabolite, common to both precursors, after administration of [4-^{14}C]-pregnenolone and [7^{3}H]-pregnenolone sulfate to similar subjects.

In an extension of our studies of corticosteroid metabolism in men of various ages, we wished to determine the production of DOC, pregnenolone and pregnenolone sulfate for additional groups of males. This necessitated calculation of the specific activities of THDOC and pregnenediol isolated from urine after administration of tracer doses of the appropriate radioactive precursors. For THDOC these determinations have been made using double isotope methods [2–4]. Since the amounts of the respective urinary metabolites of DOC, pregnenolone and pregnenolone sulfate are excreted in microgram ranges, it appeared that GC–MS, using single peak monitoring (mass fragmentography 7, 8]), would provide an accurate and more rapid method of measurement.

MATERIALS AND METHODS

Isolation and acetylation of urinary THDOC. After i.v. administration of 1 μCi [4-^{14}C]-DOC (S.A.-54·3 mCi/mmol) (New England Nuclear Corporation, Boston, Mass.) urine was collected for 48 h. The urine samples were hydrolyzed with β-glucuronidase (Sigma Company, Saint Louis, Mo.) and extracted with ethyl acetate as previously reported [5]. Preliminary separation of the 2-day neutral extract to eliminate corticosteroids more polar than THDOC was made with paper chromatography using a benzene–formamide system [5] followed by paper chromatography of the overflow in a toluene–propylene glycol system [5]. The overflow of the latter was subjected to further separation in a benzene–formamide system (paper saturated with formamide) for 8 h. The eluate of the radioactive area containing THDOC was applied to a 3 g silica gel column and developed with benzene–ethyl acetate mixtures, predominantly benzene. THDOC was eluted in benzene–ethyl acetate 92:8, v/v. This fraction was chromatographed on paper for 30 h in ligroin–propylene glycol (paper saturated with propylene glycol–methanol 1:1, v/v). The radioactive eluate was acetylated with pyridine and acetic anhydride overnight in the dark at room temperature. After addition of ethyl acetate, the resulting

Abbreviations and trivial names were used as follows: tetrahydrodeoxycorticosterone (THDOC) = 3α-21-dihydroxy-5β-pregnan-20-one; deoxycorticosterone (DOC) = 21-hydroxy-4-pregnene-3,20-dione; pregnenediol sulfate = 20α-hydroxy-5-pregnene-3β-yl sulfate; pregnenediol = 5-pregnene-3β,20α-diol; pregnenolone = 3β-hydroxy-5-pregnen-20-one; pregnenolone sulfate = 5-pregnen-20-one-3β-yl sulfate.

solution was washed with 1N sodium bicarbonate, 1N hydrochloric acid, and water. The concentrated extract of THDOC diacetate was chromatographed on paper in ligroin-propylene glycol (paper saturated with propylene glycol) and eluted. To remove a benzene-soluble, non-radioactive pigmented impurity, the THDOC-diacetate was applied to a 1 g silica gel column, and the impurity eluted with benzene. THDOC diacetate was recovered from the column in benzene–ethyl acetate (99:1, v/v). The ^{14}C-labeled eluate was finally chromatographed on silica gel (GF 254, E. Merck, AG) in a thin-layer system, cyclohexane–ethyl acetate (8:2, v/v). This purified diacetate was admixed with deuterated material for GC–MS analyses.

Isolation and acetylation of urinary pregnenediol. After i.v. administration of 15 μCi of [7α-^{3}H]-pregnenolone sulfate (ammonium salt) (S.A.-20 Ci/mmol) and 1 μCi of [4-^{14}C]-pregnenolone (S.A.-52·8 mCi/mmol) (New England Nuclear Corporation, Boston, Mass.) urine was collected from men for 96 h. The urine samples were hydrolyzed with β-glucuronidase for purposes not relevant to this study and solvolyzed to desulfate and recover the common metabolite of the two precursors, pregnenediol, in unconjugated form. Three-quarters of the resulting neutral extract were subjected to separation in the first two paper chromatogram systems described above for THDOC. The toluene–propylene glycol overflow was concentrated and chromatographed in a ligroin–toluene (1:1, v/v)-propylene glycol system (paper saturated with propylene glycol–methanol 2:1, v/v). The area containing [^{3}H],[^{14}C]-pregnenediol was eluted from the paper and subjected to further separation in a thin-layer system, benzene–ethyl acetate 6:5, v/v (2 times), followed by acetylation of the eluted radioactive peak. The resu'ting diacetate was chromatographed in a thin-layer system, cyclohexane:ethyl acetate 85:15 followed by final separation in a thin-layer system, benzene–ethyl acetate 85:15, v/v. The resulting eluate, when dried, was frequently in crystalline form. This material, diluted with carrier deuterated acetate, was used for GC–MS analysis.

Preparation of THDOC and pregnenediol derivatives. Both THDOC (Mann Research Laboratories, New York, N.Y.) and pregnenediol (Steroids, Pauling, N.Y.) were subjected to t.l.c. on silica gel to determine homogeneity. Then 10 mg amounts of each were acetylated with highly deuterated (d) reagents (99·9% pyridine-d_5 and acetic anhydride-d_6, INC Pharmaceuticals, Cleveland, Ohio) as described above. After extraction of the reaction mixture the acetylated derivatives were crystallized from methanol. The THDOC and pregnenediol d-diacetates were chromatographed in a thin-layer system, cyclohexane–ethyl acetate 80:20, v/v and 85:15, v/v, respectively, to determine the presence of any impurities. Both d-diacetates, when subjected to GC–MS, contained a small percentage of the respective undeuterated (u) diacetate, but there was no evidence of steroid impurities. Diacetates of both steroids were also prepared with undeuterated reagents.

For preparation of trimethylsilyl (TMS) ether derivatives 0·1 mg of steroid was mixed with 0·1 ml BSA (N,O-bis(trimethylsilyl) acetamide (Supelco, Bellefonte, Pa.) and heated for 1 h at 60°C in a closed tube. Portions of the solution were injected directly for GC–MS analysis.

Spectra of derivatives by GC–MS analysis. (a) "Electron impact". Analyses were carried out on a Finnigan Model 1015 mass spectrometer interfaced by a glass, jet-type separator and glass or glass-lined transfer lines to a Finnigan 9500 gas chromatograph MS conditions: analyzer; 120°C, $2–5 \times 10^{-5}$ Torr; source: 70 eV, 500 μA. GC conditions: 0·6–1·5 m U-columns (glass) 2 mm i.d., flow rate about 30 ml/min; other conditions given with individual compounds.

"THDOC diacetate". When injected onto a 0·94 m column packed with 3% OV-1 on Gas Chrom Q (Applied Science, Inc., State College, Pa.) the retention time was 4 min at 230°C. Mass spectrum (% of base peak): 358(M-60, 1·6%) 345(M-73, 4·6%), 284(7·7), 257(38), 175(26), 95(77), 81(base peak, 100%).

"THDOC-diTMS ether". Retention time, 10 min at 240°C; 1·5 m column packed by Finnigan Corp. with 3% OV-1 on Gas Chrom Q. Mass spectrum (% of base peak): 463(M-15, 2%), 375(M-103, 14%), 359(1·9), 285(2·2), 281(5·2), 257(100), 201(17), 175(42), 161(38), 143(52), 107(58).

"Pregnenediol diacetate". The retention time, using the column described above for THDOC diacetate, was 6·5 min at 220°C. Mass spectrum (% of base peak): 342(M-60, 9·9%), 282(M-119, 4·5%), 268(9·9), 254(6·8), 174(24), 161(59), 105(100), 91(100), 81(81).

"Pregnenediol diTMS ether". Retention time, 7 min at 240°C, same 1·5 m column as above. Mass spectrum (% of base peak): 462(M, 0·39%), 447(M-15, 0·24%), 372(M-90, 0·91), 357(0·44), 282(M-180, 1·1%), 267(45), 243(40), 161(74), 159(74), 147(4·3), 129(29), 117(100), 107(2·9), 105(4·3).

(b) "Chemical ionization". This technique was employed using Finnigan Model 1015 in the High Resolution Mass Spectrometry Laboratory of Battelle Memorial Institute, Columbus, Ohio, by Dr. R. Foltz. Conditions: 1·8 m, 2 mm i.d., glass coiled column packed with OV-17 on Gas Chrom Q using methane as carrier gas.

"THDOC diacetate". Retention time 10 min, programmed temperature 250–280°C at 4 C/min. Mass spectrum (% relative abundance): 359 (MH^+-60, 100%): 341(12%), 358(10), 357(10), 299(80), 281(50).

"Pregnenediol diacetate". Mass spectrum (% of base peak): 343(MH^+-60, 2%), 342(3%), 283 (MH^+-120, 100%), 281(3), 149(3), 121(2).

Analysis of urinary samples by mass fragmentography. Analyses were performed on a Finnigan model 1015 mass spectrometer interfaced to the 9500 U-column gas chromatograph and having the "PROMIM" (programmable multiple ion monitor) attach-

ment for monitoring selected peaks (multiple ion detecter). Readout was obtained on a Rikadenki 4-pen strip chart recorder model KA-410. For analyses of mixtures of THDOC diacetate and THDOC diacetate-d_6 one channel of the PROMIM was calibrated to (nominal) mass 345 and another to mass 348 for the M-CH_2OAc ion from the undeuterated and deuterated species respectively. A third channel was calibrated to mass 258 as an additional check of specificity. This peak was due to an undeuterated fragment ion since it showed comparable relative abundance in spectra of the deuterated and undeuterated derivatives. During analysis the mass spectrometer monitored the intensity of ion fragment $m/e = 348$ for 100 ms switched to ion m/e 345 and monitored it for 100 ms, went on to the third ion, back to the first and so on until the compound had passed through the mass spectrometer. The "PROMIM" integrates each reading with the previous one and a smooth peak for total ion intensity with time is obtained on the strip chart recorder. Peak heights are reported here as measures of ion intensities. This method was found to be as accurate as using the area under the curve.

For pregnenediol, peaks 345 and 342 were used to monitor M^+-HOAC for the deuterated and undeuterated species respectively. A third channel was calibrated to mass 282 as an additional check of specificity as described above for THDOC diacetate.

RESULTS AND DISCUSSION

A known quantity of deuterated carrier can be added to a sample as a recovery marker and to improve sensitivity. After passing through the gas chromatography, the mixture is ionized in the mass spectrometer and the relative intensities of the deuterated and undeuterated species of the same fragment ion are measures of the amounts of the respective compounds injected. This is shown in Table 2 and is explained in more detail below.

Acetate and TMS ether derivatives are useful for introducing deuterium into the steroids and for making them more volatile for GC–MS. Use of THDOC and pregnenediol as TMS ethers have been reported in a paper [9] surveying the applicability of this derivative for GC–MS. However, detailed spectra were not reported and we are not aware of the use of this derivative or of the diacetate for quantification of THDOC or pregnenediol from a biological sample by GC–MS.

Before acquiring our own electron impact (E.I.) unit we also considered whether use of E.I. or chemical ionization (C.I.) would be more suitable for the analyses. Access to analytical facilities employing either procedure was available at the time to researchers with federal support. Therefore our preliminary results include an analysis of acetate derivatives using both C.I. and E.I.*

Evaluation of derivatives for analysis. Molecular or fragment ions of pregnenediol and THDOC derivatives suitable for evaluation in the type of analysis outlined above are given in Table 1. As can be seen, both acetate and TMS ether derivatives may be used with either E.I. or C.I. methods. While C.I. is often considered to be a gentler method, the only molecular ion present, albeit in small relative abundance, was observed for the TMS derivative of pregnenediol with E.I. However a mass ion of 100% relative abundance and useful for quantification was obtained using C.I. of the THDOC diacetate. We do not have values on absolute intensity per given mass injected since it was impractical for us to carry out these determinations without direct access to the C.I. instrument.

A potential problem in using diTMS ether derivatives is illustrated for THDOC. The molecular ion was 478 while the highest mass obtained was 463 corresponding to loss of a methyl radical, a common

* C.I. was done at Battelle Memorial Institute; Preliminary E.I. was done at the Massachusetts Institute of Technology.

Table 1. Mass ions suitable for quantification

	C.I.*		E.I.†	
Compound	Mass	Relative abundance, %	Mass	Relative Abundance, %
5-ene-P-diol-diAC‡	743(MH-60)	2	342(M-60)	10
			282(M-119)	5
5-ene-P-diol-diTMS	—		426(M)	0·4
			447(M-15)	0·3
			372(M-90)	1.
			357(M-105)	0·5
THDOC diAC	359(MH-60)	100	358(M-60)	2
			345(M-73)	5
THDOC diTMS	—		463(M-15)	2
			357(M-103)	14

* Compounds were chromatographed on 1·8 m OV-17 columns using methane as carrier gas. Other details in "Materials and Methods."

† Compounds were chromatographed on 0·6–0·9 m OV-1 columns using helium as carrier gas. Other details in "Materials and Methods."

‡ Pregnenediol diacetate.

Table 2. Linearity of ratios of peak heights for combinations of d-THDOC and u-THDOC diacetates

μg THDOC Diacetate							
Ratio	By weight	Corrected	By weight		Corrected	Ratio of $\frac{\text{Corrected d}}{\text{Corrected u}}$	
d:u	d	d-(u in d)[+]	u + u in d		= Total u	Theoretical	Observed*
2·5:1	40	39·68	16·0	0·32	16·32	2·43:1	2·44:1
12·5:1	40	39·68	3·2	0·32	3·52	11·3:1	10·7:1
37·5:1	30	29·76	0·8	0·24	1·04	28·6:1	27·3:1
75·0:1	60	59·52	0·8	0·48	1·28	46·5:1	46·8:1
125:1	100	99·20	0·8	0·80	1·60	62·0:1	65·3:1
250:1	200	198·40	0·8	1·60	2·40	82·7:1	82·9:1

[+] u in d-THDOC diacetate = 0·80%.
* Observed peak height ratio of mass 348/345.
× Mean ratio.

fragmentation. For the deuterated derivative the corresponding mass M^+-CD_3 was 478, the same as that for the molecular ion of the undeuterated material. Therefore it would be unwise to use a molecular ion for analysis if a significant peak at M^+-15, also was obtained.

In the following sections, results of E.I. ionization of the diacetate derivatives are given. These were used in preference to the diTMS ethers because, although the latter appear to be adequate for mass fragmentography, the acetates are more stable for chromatography and storage.

Evaluation of the methods. Analyses of known mixtures of d-(deuterated) and u-(undeuterated) diacetates gave precise and reproducible results. Because the d-diacetates contain small amounts of u-diacetates which contribute to the peak heights of the samples, the % of u-diacetate in the d-diacetate were quantitatively determined for use as a correction factor. Since the peak height ratios varied a small amount when measured on different days, perhaps due to the relative sensitivity of measuring the ions involved, the correction factor was also determined for each series of determinations. In actual practice the % of u-diacetate in the d-diacetate varied on different days from 0·38% to 3·6, mean 1·7% for THDOC, and for pregnenediol, 0·44 to 0·52%, mean 0·47%. Reinjections, after a period of 5 months, of samples taken from the original paired aliquots for 6 subjects resulted in a maximum coefficient of variation of 3·0% for the GC–MS analyses.

Results obtained for combinations of d to u-THDOC diacetate ranging from ratios d:u of 2·5:1 to 250:1 using ions of *m/e* 348 and 345 are given in detail in Table 2. In this series the u in d-THDOC added was 0·80%, which was used as a correction factor to determine: (1) the actual d-THDOC added, i.e. μg d-THDOC diacetate minus μg u-THDOC diacetate contained therein (3rd column), and (2) the actual u-THDOC diacetate present, i.e. μg u-THDOC diacetate added plus u-THDOC-diacetate contributed by the d-THDOC diacetate added (5th column). After these corrections were made, the theoretical ratios were calculated from the known quantities of d + u-THDOC diacetates added (column 7). These are compared in column 8 with the observed peak height ratios of mass 348 to mass 345. The latter values represent the means from duplicate injections which usually agreed within ±5%. The intra-assay coefficient of variation did not exceed 2·7%. The similarity of the calculated and observed ratios for a range of mixtures shows that the monitoring by GC–MS of selected peaks is linear with concentration over a wide range of u + d diacetate combinations. Evaluation of deuterated and undeuterated mixtures of pregnenediol diacetate using ions of *m/e* 345 and 342 showed similar linearity.

Analysis of THDOC and pregnenediol from urine. (1) "THDOC". After i.v. administration of [4-^{14}C]-DOC and collection of urine containing its radioactive metabolite, THDOC was isolated after conjugate hydrolysis and extensive chromatography as detailed in the experimental section. Based on the amounts of radioactivity in the final product after paper and t.l.c., and on the reported secretion rates of DOC and excretion values for THDOC, duplicate aliquots of u-THDOC diacetate were taken for radioactive counting and for determination of selected ion intensities with GC–MS. The amounts of d-diacetate added to the above aliquots, representing 13 young and elderly men, varied from 4 to 10 μg. The total u-THDOC diacetate available for aliquots varied from 1·0 to 14 μg, mean 4·0 μg. The amounts of u-THDOC in the aliquots actually used ranged from 0·25 to 3·4 μg, with most values falling between 0·5 and 1·7 μg. The ratios of ions of *m/e* 348 to 345 corresponding to ratios of d- to u-THDOC-diacetate ranged from 3:1 to 16:1. Examples of results obtained with GC–MS for 2 men are presented in Table 3. The values for the multiple aliquots compare well. From the known quantities of radioactivity and of [^{14}C]-THDOC diacetate calculated to be present after GC–MS, specific activities of THDOC (corrected for the presence of the diacetate moiety) and

Table 3. Examples of calculations and results obtained with GC–MS for aliquots of urinary THDOC and pregnenediol diacetates

Subject	Aliquot ml	d-Diacetate − u in d = d added	PH Ratio*	$\frac{\text{d added}}{\text{PH Ratio}}$ − u in d = Aliquot
		THDOC Diacetate		
		μg added − μg = μg	d:u	μg − μg = μg
S	0·6	10·00 − 0·07 = 9·93	7·56	1·31 − 0·07 = 1·24
	0·3	10·00 − 0·07 = 9·93	14·4	0·69 − 0·07 = 0·62
Sc	0·4	10·12 − 0·16 = 9·96	7·17	1·39 − 0·16 = 1·23
	0·2	10·12 − 0·16 = 9·96	13·1	0·76 − 0·16 = 0·60
		Pregnenediol diacetate		
J	0·2	12·13 − 0·05 = 12·08	3·80	3·18 − 0·05 = 3·13
	0·1	12·13 − 0·05 = 12·08	7·40	1·63 − 0·05 = 1·58
G	0·2	12·13 − 0·05 = 12·08	4·84	2·50 − 0·05 = 2·45
	0·1	12·13 − 0·05 = 12·08	9·67	1·25 − 0·05 = 1·20

* Peak height ratio: *m/e* 348/345 for THDOC diacetate and *m/e* 345/342 for pregnenediol diacetate.

the secretion rate of DOC could be determined. The latter rates varied from 0·027 to 0·153 mg/day for young and elderly men. The mean value for 6 young men was 0·09 mg/day, similar to 0·10 mg/day obtained by New *et al.*[3] for 3 men in the same age range, but less than 0·164 mg and 0·175 mg reported by Schambalen *et al.*[2] and Seth *et al.*[4] respectively. The mean secretion rate for 7 elderly men was 0·057 mg/day. A comparison of the metabolism and secretion of DOC with secretion and excretion of other corticosteroids by the 13 young and elderly men is presented in detail elsewhere [11].

(2) "Pregnenediol diacetate". The derivative of this urinary metabolite was obtained after intravenous administration of a mixture of [7^{3}H]-pregnenolone sulfate and [4^{14}C]-pregnenolone as detailed in the experimental section. Ten mg of deuterated pregnenediol diacetate were added to each of the undeuterated aliquots of the [^{14}C],[^{3}H]-pregnenediol diacetate metabolite. The amounts of the latter in any given aliquot varied from 1·1 to 4·6 μg. Ratios of d- to u-pregnenediol diacetate ranged from 2:1 to 10:1. The u-pregnenediol diacetate recovered after chromatography and available for GC–MS analysis averaged 40 μg for young men. Characteristic results obtained for 2 subjects appear in Table 3. In order to estimate the daily urinary excretion of pregnenediol, a portion of the original 96-h neutral urine extract was subjected to reverse isotope dilution to determine the total radioactivity present as pregnenediol. Calculated from this value and from the specific activity determined for pregnenediol by GC–MS mass analysis, the urinary excretion of pregnenediol for several young men averaged 0·04 mg/day, compared to a mean value of 0·05 mg/day for 12 young men obtained by another method previously described [5, 10].

The secretion and excretion values obtained by the GC–MS method reported here are lower, often to a small degree, than those reported by other methods [2–4]. This probably reflects the excellent specificity GC–MS mass fragmentography methods have, resulting in low background. Indications that spurious background ions are not being mistakenly analyzed are seen in the smooth baselines of the tracings and the symmetry of the peaks for analysis (Fig. 1), after injection of 0·5 μg d-THDOC diacetate and 0·07 μg of urinary THDOC diacetate (A) and 0·9 μg of d-pregnenediol diacetate and 0·11 μg of urinary pregnenediol diacetate.

A low value could be obtained if there was a high background peak in the sample coincident with the deuterium-containing mass peak being analyzed. To

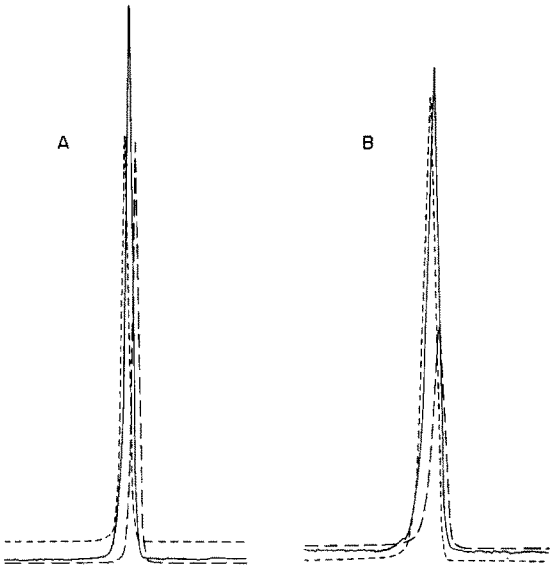

Fig. 1. Tracings of peaks obtained (A) for a combination of urinary and added d-THDOC diacetate: d-diacetate at mass 348 (-----), attenuation 10X; urinary diacetate at 345 (——) attenuation, X; and urinary and d-diacetate at 258 (– –), 10X; and (B) for urinary and added d-pregnenediol diacetate: d-diacetate at mass 345 (-----) attenuation 10X; urinary diacetate at 342 (——), X; and urinary and d-diacetate at 282 (– –), 10X. Differences in peak positions are due to pen offset.

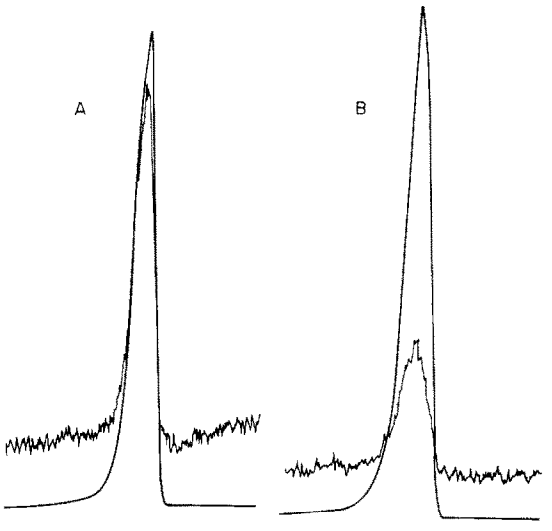

Fig. 2. Tracings of peaks obtained (A) for urinary THDOC at mass 345 (trace 1), attenuation, 20X; and at 348 (trace 2), X; and (B) for urinary pregnenediol at mass 342 (trace 1) attenuation, 20X, and at 345 (trace 2), X.

test for this, samples of urinary THDOC and pregnenediol diacetates, purified in the same fashion as for the analyses detailed in Table 3, were analyzed by GC–MS without being admixed with the deuterated carriers. As can be seen in Fig. 2, after injection of 0·20 μg of urinary THDOC diacetate (A) and 1·2 μg of urinary pregnenediol diacetate (B) there were no significant spurious peaks corresponding to the deuterated ions when signal attenuation was considered. The low background suggests that the analysis could have been carried out at an earlier purification stage thus saving time, improving recovery, and therefore sensitivity. As regards the latter, the instrumentation limit of measuring mass is quite low, less than 0·1% of the amount of deuterated carrier injected, which, in our samples, was 0·5 to 1 μg. However in practice, sensitivity is limited by the isotopic purity of the deuterated carrier. In the cases shown here, the content of undeuterated material was of the order of 1% making the sensitivity about 2–5% of the injected deuterated sample.

In conclusion, we have demonstrated that two urinary corticosteroids may be quantified accurately in the nanogram range by mass fragmentography after conventional sample purification. The method is more sensitive and accurate than colorimetry and is easier to establish than a double-isotope method. Thus it warrants serious consideration as a method of choice especially if a relatively few number of samples are to be analyzed. The data indicate that by employing fewer purification steps and modifying the derivative or method of ionization the method might be simplified and the sensitivity improved significantly.

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