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Analysis of Corticosteroids by HPLC-UV and HPLC-MS by Particle Beam Interface

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The Antidoping Lab of Rome is studying a method for the detection and the identification of corticosteroids in human urine.
Since the analysis in GC-MS, very often, require previous derivatization of the samples, with side effects, we prefer to use the HPLC for these kinds of compounds.

For the screening analysis we use the HPLC-UV with diode array detector, on the other side for the confirmations the HPLC-MS by particle beam interface (PB).

The system used by us is shown in figure 1. The switch valve in position 2-1 helps to avoid that a part of the more polar compounds, eluted very quickly, goes into the analytical column. That is very important because it makes the life of the column longer.

For our compounds the RP deactivated column gives better separations compared to the CN and C18 columns. The pre-column (4.6 cm x 2 cm) has the same packing as the analytical column. The detector can be a UV diode array or a mass spectrometer.

To test the sensitivity of the diode array detector we used a mixture of two natural corticosteroids, cortisone and hydrocortisone, by injecting 50 ng of each (figure 2).

In the same analytical conditions we also studied the behaviour of other natural and synthetic corticosteroids. In figure 3 a separation of eight of them is shown.

We also made excretion studies of some drugs containing corticosteroids administering them to non athlete volunteers.
25 mg of cortisone, 25 mg of prednisone and 25 mg of deflazacort were given in a single dose.

Blank urine were collected before the administration.
After administration of prednisone and deflazacort the urine were pooled for 12 hours.

For the cortisone, instead, scheduled sampling were made.
The urine samples were prepared according to the scheme shown in figure 4.

The hydrolysis of the sample did not give other information, compared to the free fraction, and at the same time the background is more disturbed. In figure 5 the free fraction of a male blank urine is shown. Two peaks with the same retention time of cortisone and hydrocortisone are evident at the same instrument sensitivity.

In the same figure the chromatogram of the free fraction of female blank urine is also reported.

In figure 6 the excretion study of cortisone is shown. After three hours it is possible to notice that both the peak of cortisone and hydrocortisone are increased compared to the blank urine while after six hours differences are not seen. The excretion study of prednisone showed that the main metabolite is prednisolone, Rt=19.2 min. The unchanged prednisone is evident at Rt= 14.4 min. There are also three other peaks, probable metabolites, at Rt=16.6, 27.2, 32.1 min respectively of the chromatogram of figure 7.

In figure 8 the conjugated fraction is also reported.
In the case of the administration of deflazacort we can notice, in figure 9, that most of it is excreted unchanged with other two probable metabolites.

The use of HPLC-MS by PB permitted us to identify some of the peaks of the previous chromatograms.

One of the disadvantages of PB is the sensitivity, lower than the GC-MS, but at the same time it is a very simple system and gives the possibility to work in EI and in CI.

The temperature and the helium pressure of the solvatation chamber were set at 50° C and 40-45 psi respectively, the source at 200° C.
To test the efficiency of the system we used a standard mixture (1 μg of each). The TIC in full scan of it is reported in figure 10.
To test the sensitivity of the system we injected 50 ng of cortisone in SIM mode (figure 11).

The mass spectra of the investigated synthetic and natural standards are in figure 12.

The mass spectra in full scan of the peak at Rt= 14.4 and 19.2 of figure 7 and of the peak at Rt = 17.6 of figure 9 are shown in figure 13. They confirmed the results, reported before, in HPLC-UV.

Figure 14 shows the aquisition in SIM mode for the same sample of figure 7.

The sensitivity of the system is incresed at least by ten times working in Chemical Ionization.

Good results at the moment have been obtained for cortisone and hydrocortisone using ammonia or methane as reactant gas, working at a source temperature of 130° C (figure 15).

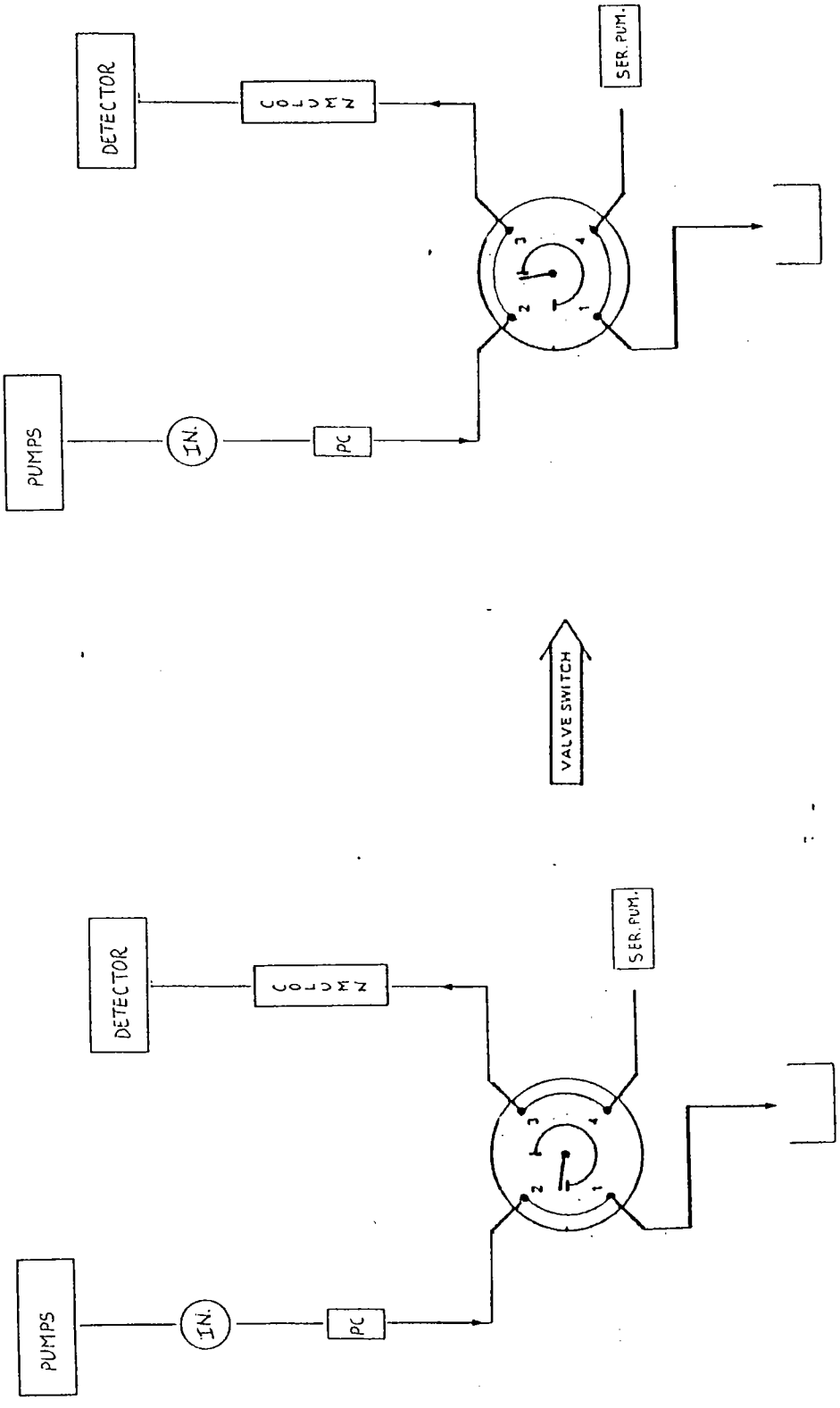

DETECTOR
COLUMN
SER. PUM.
PUMPS
IN.
PC
VALVE SWITCH
DETECTOR
COLUMN
SER. PUM.
PUMPS
IN.
PC

FIG. 1

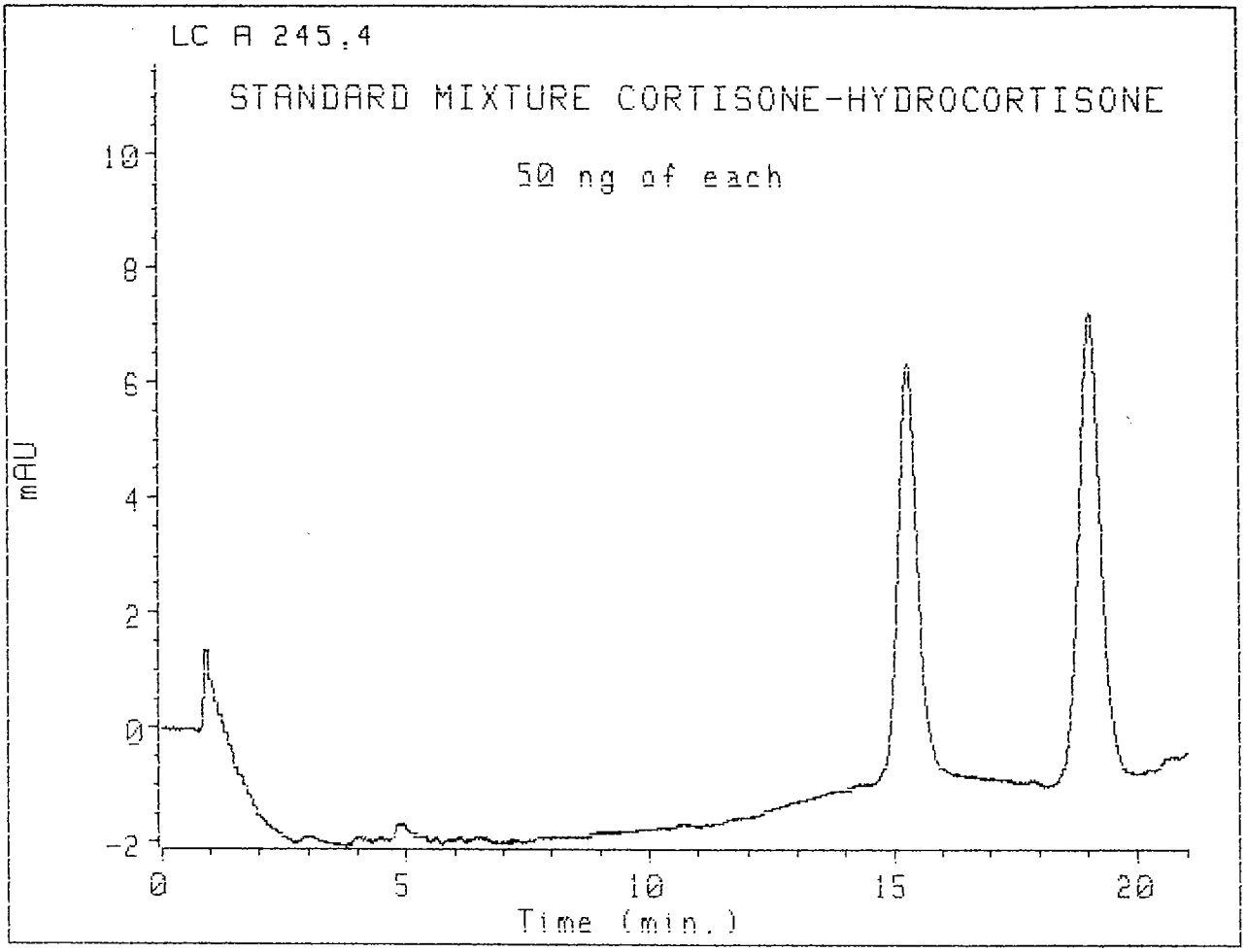


FIG. 2

Column: Supelcosil ABZ 150 mm x 4.6 mm 5 um spherical Flow 0.4 ml/min. UV= 245 nm

Time (min)						
0.01	Solvent	A :	50.0 %	B :	50.0 %	
0.01	Contact	4 :	on			
0.01	Column	:	0			
0.80	Column	:	1			
13.00	Solvent	A :	50.0 %	B :	50.0 %	
25.00	Solvent	A :	10.0 %	B :	90.0 %	
35.00	Solvent	A :	10.0 %	B :	90.0 %	
40.00	Solvent	A :	50.0 %	B :	50.0 %	
45.00	Solvent	A :	50.0 %	B :	50.0 %	

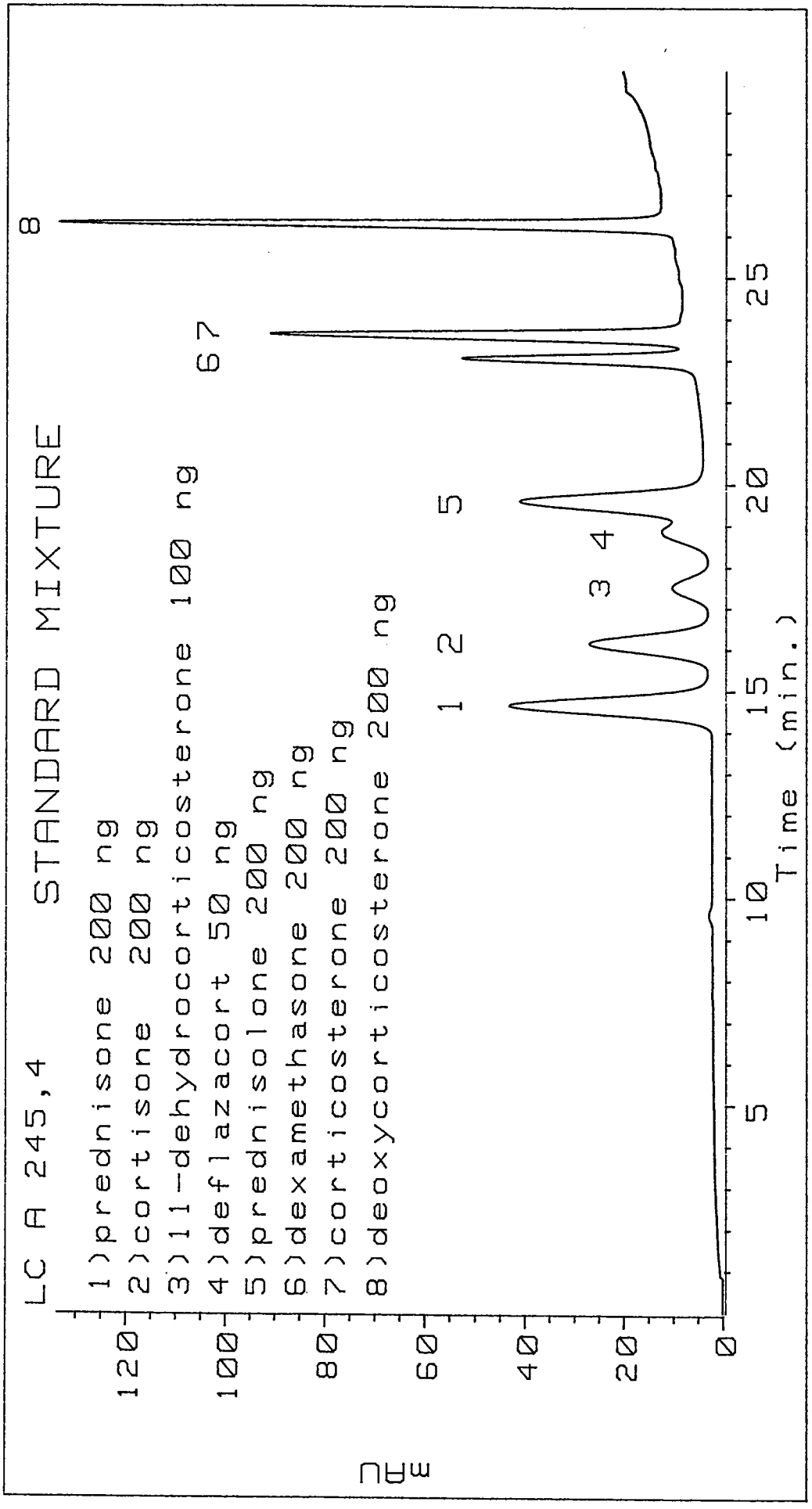

LC A 245,4
STANDARD MIXTURE
1)prednisone 200 ng
2)cortisone 200 ng
3)11-dehydrocorticosterone 100 ng
4)deflazacort 50 ng
5)prednisolone 200 ng
6)dexamethasone 200 ng
7)corticosterone 200 ng
8)deoxycorticosterone 200 ng
1 2
3 4
5
67
8
mAU
120
100
80
60
40
20
0
5
10
15
20
25
Time (min.)

FIG. 3

SAMPLE PREPARATION

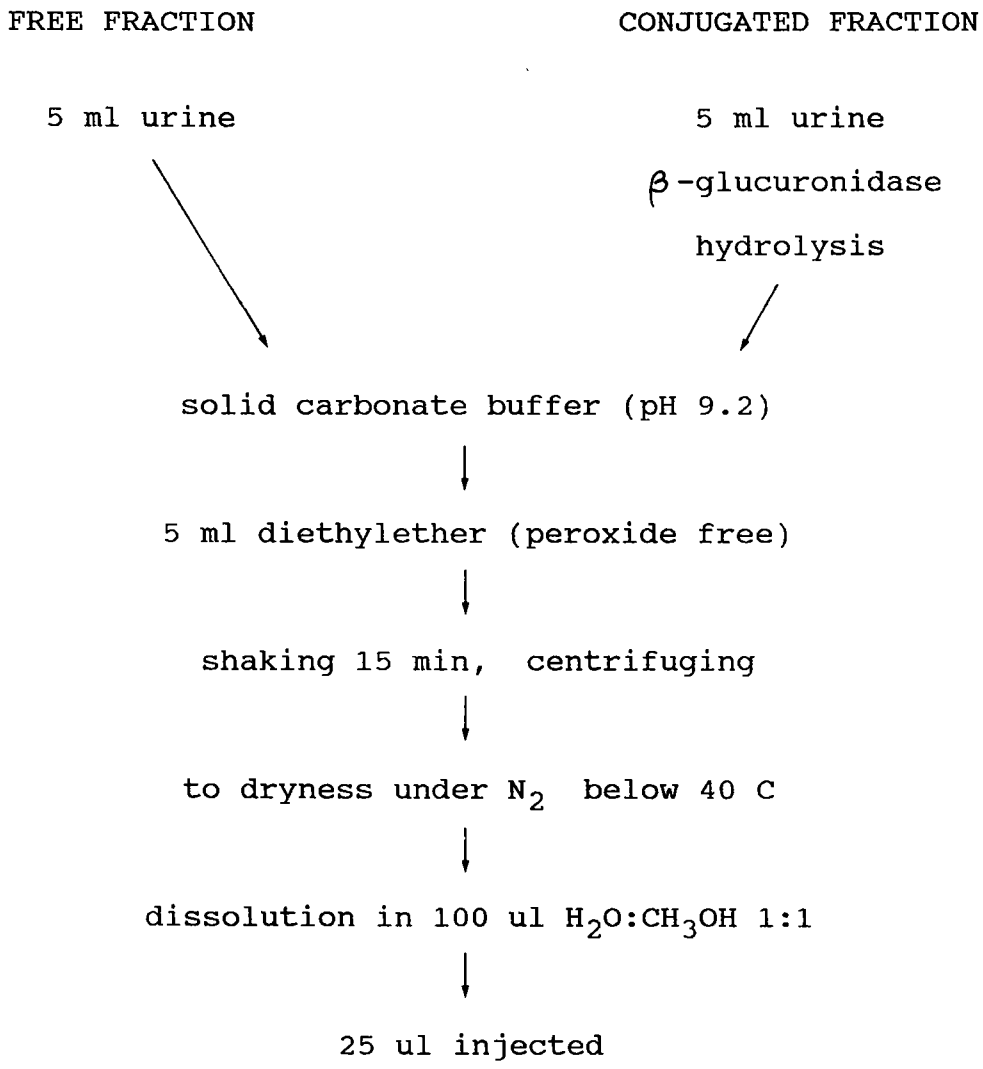
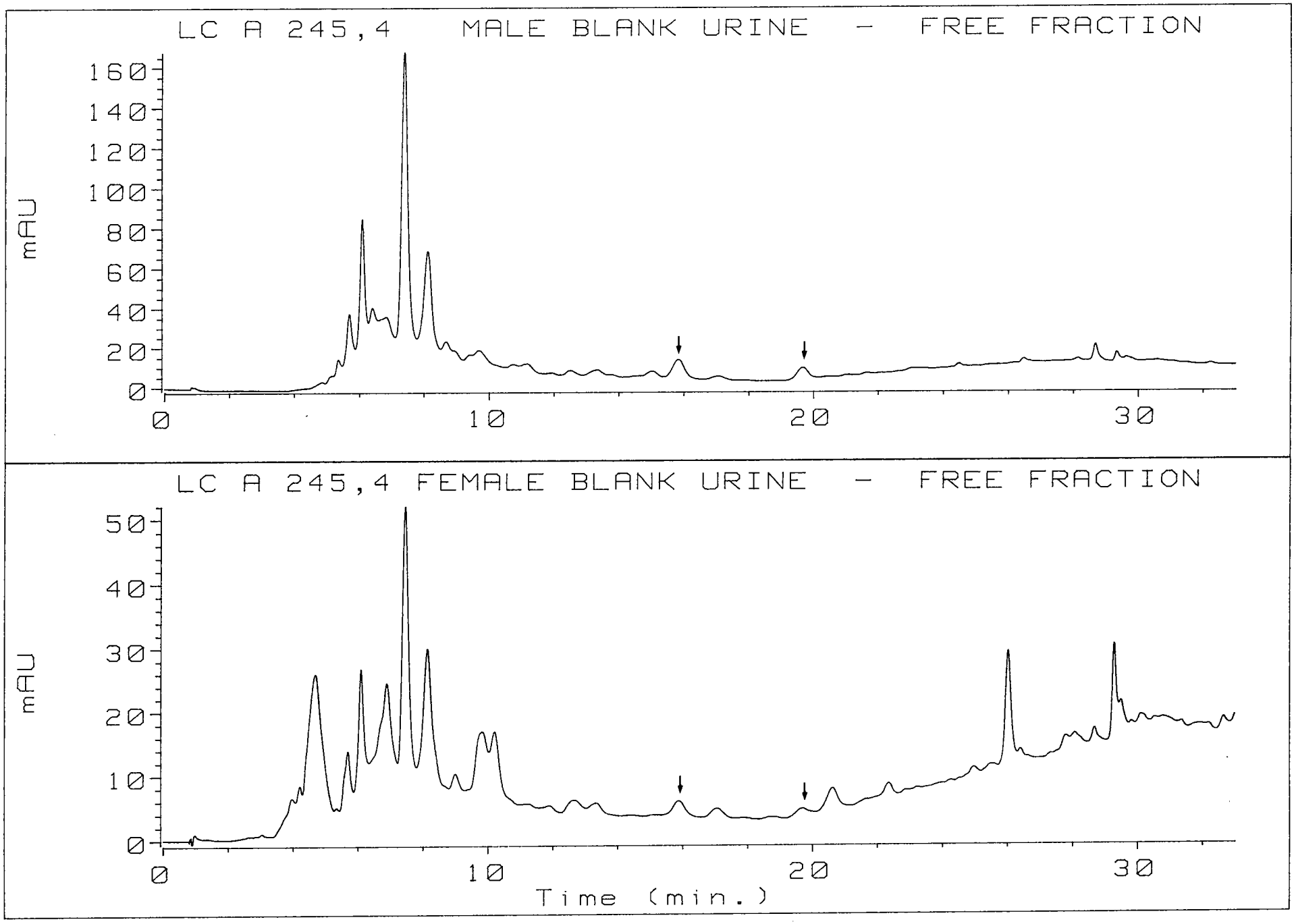


FIG. 4


LC A 245,4 MALE BLANK URINE - FREE FRACTION
mAU
160
140
120
100
80
60
40
20
0
0
10
20
30
LC A 245,4 FEMALE BLANK URINE - FREE FRACTION
mAU
50
40
30
20
10
0
0
10
20
30
Time (min.)

256

FIG.5

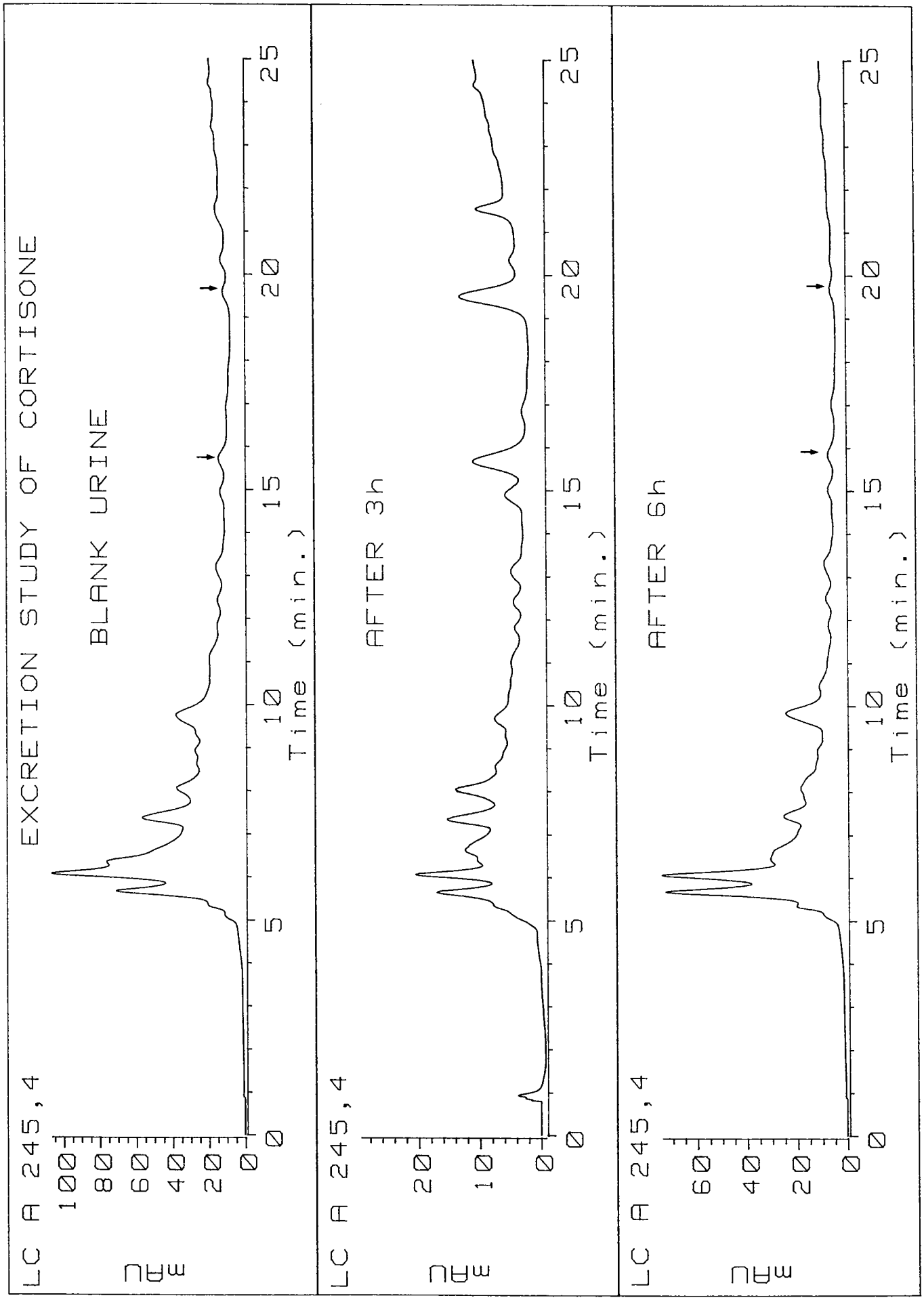

EXCRETION STUDY OF CORTISONE
BLANK URINE
AFTER 3h
AFTER 6h
LC A 245,4
mAU
Time (min.)
0
5
10
15
20
25
100
80
60
40
20

FIG.6

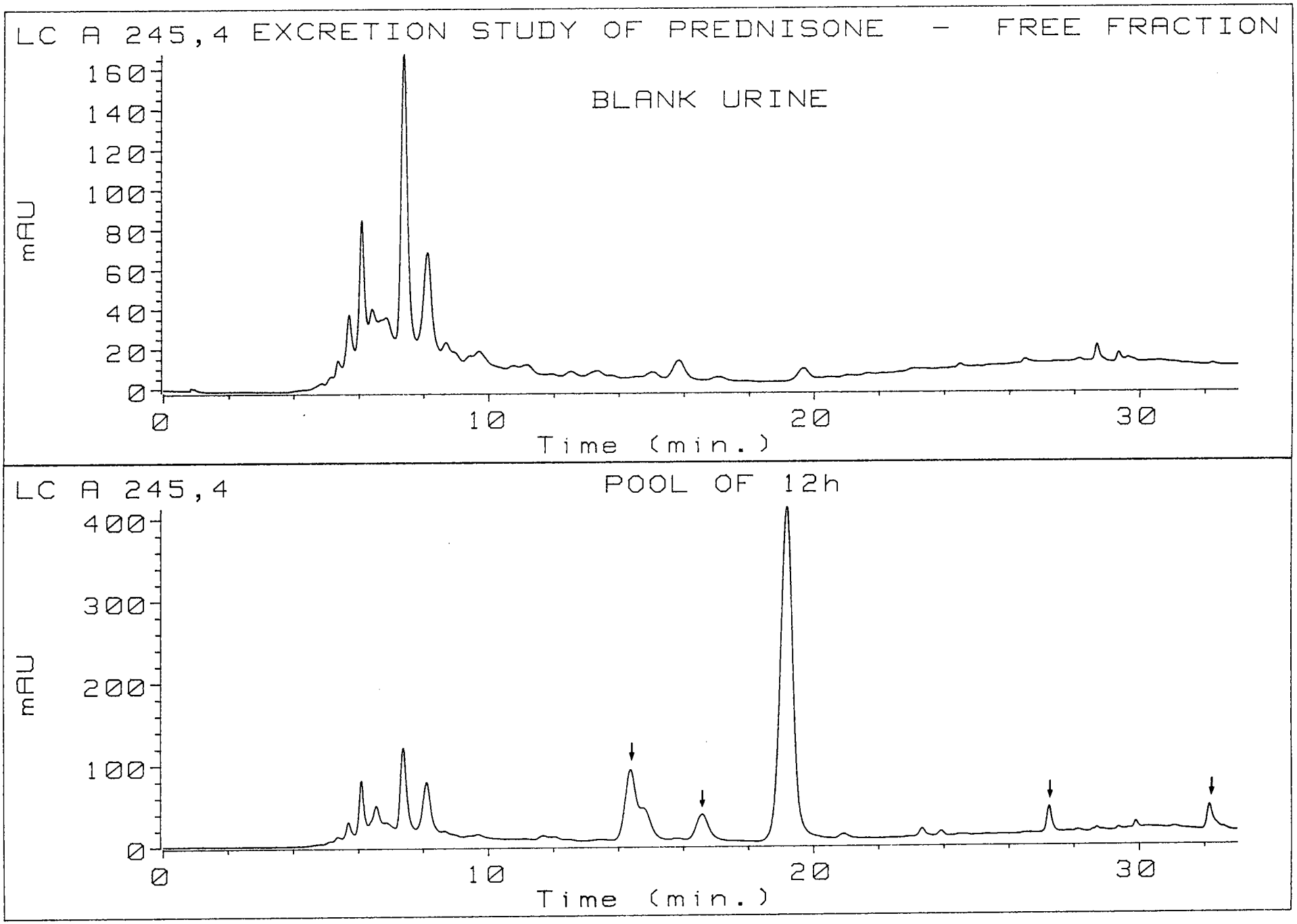
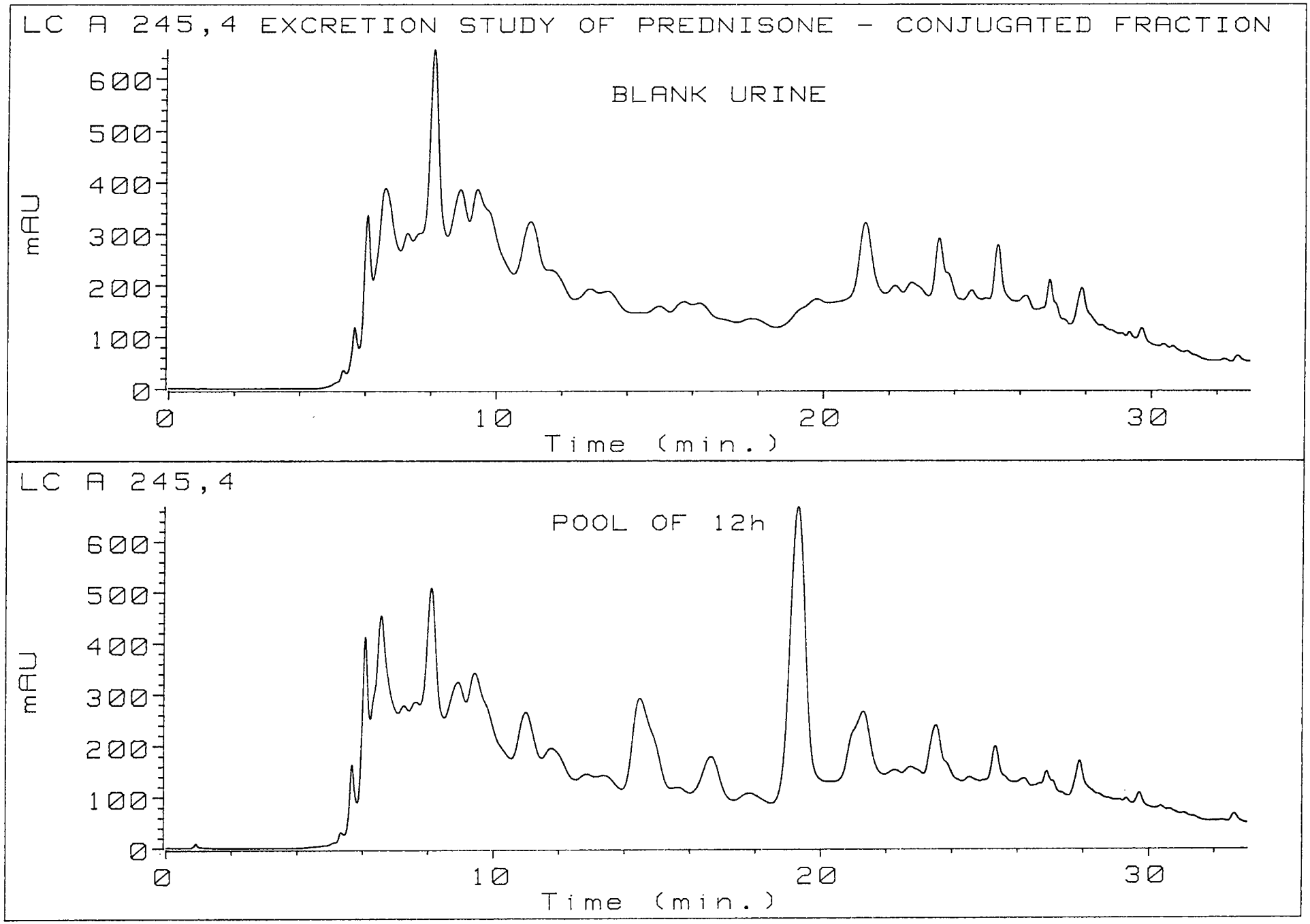
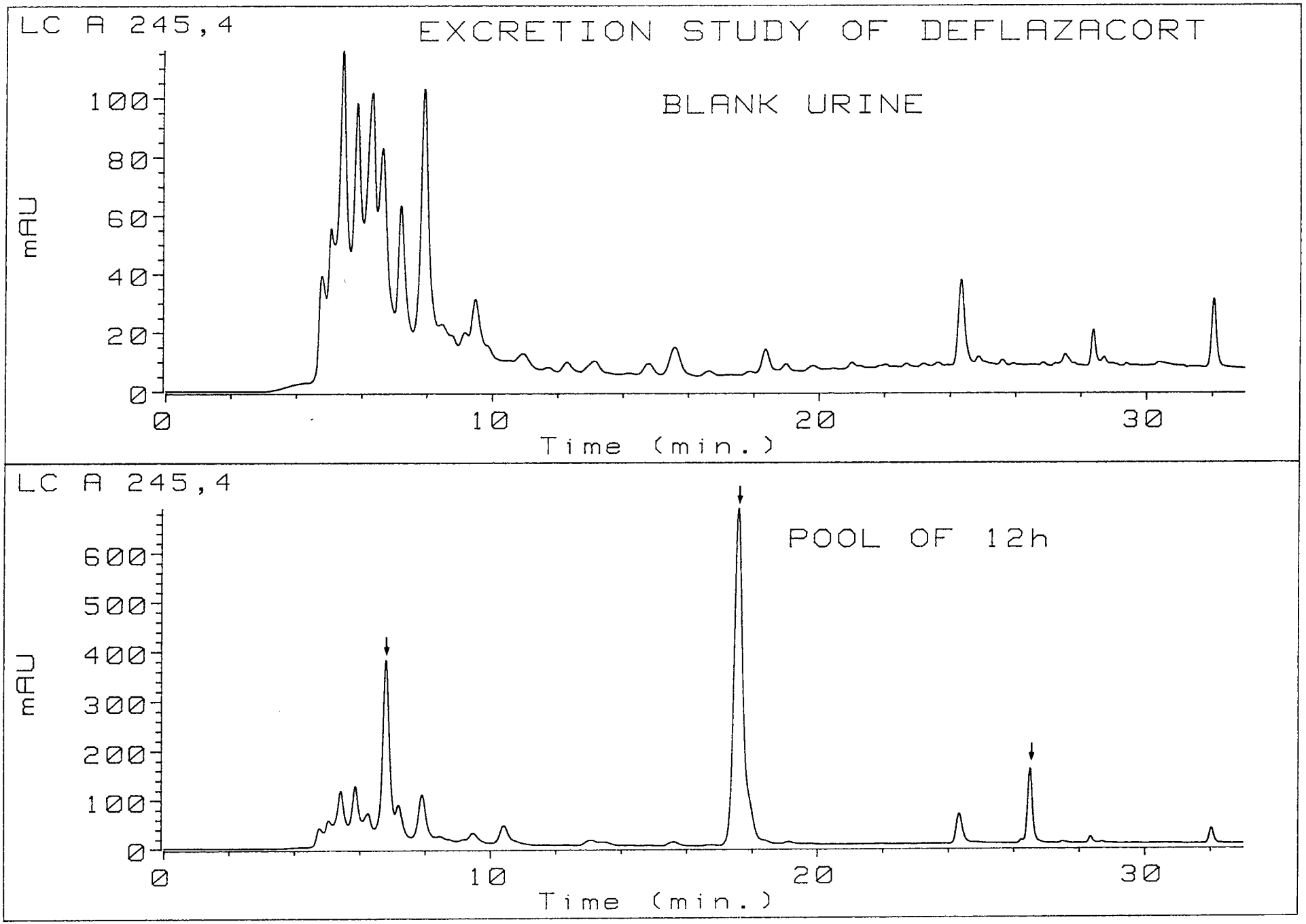

LC A 245,4 EXCRETION STUDY OF PREDNISONE - FREE FRACTION
BLANK URINE
mAU
160
140
120
100
80
60
40
20
0
0
10
20
30
Time (min.)
LC A 245,4
POOL OF 12h
mAU
400
300
200
100
0
0
10
20
30
Time (min.)

FIG.7


LC A 245,4 EXCRETION STUDY OF PREDNISONE - CONJUGATED FRACTION
BLANK URINE
mAU
600
500
400
300
200
100
0
0
10
20
30
Time (min.)
LC A 245,4
POOL OF 12h
mAU
600
500
400
300
200
100
0
0
10
20
30
Time (min.)

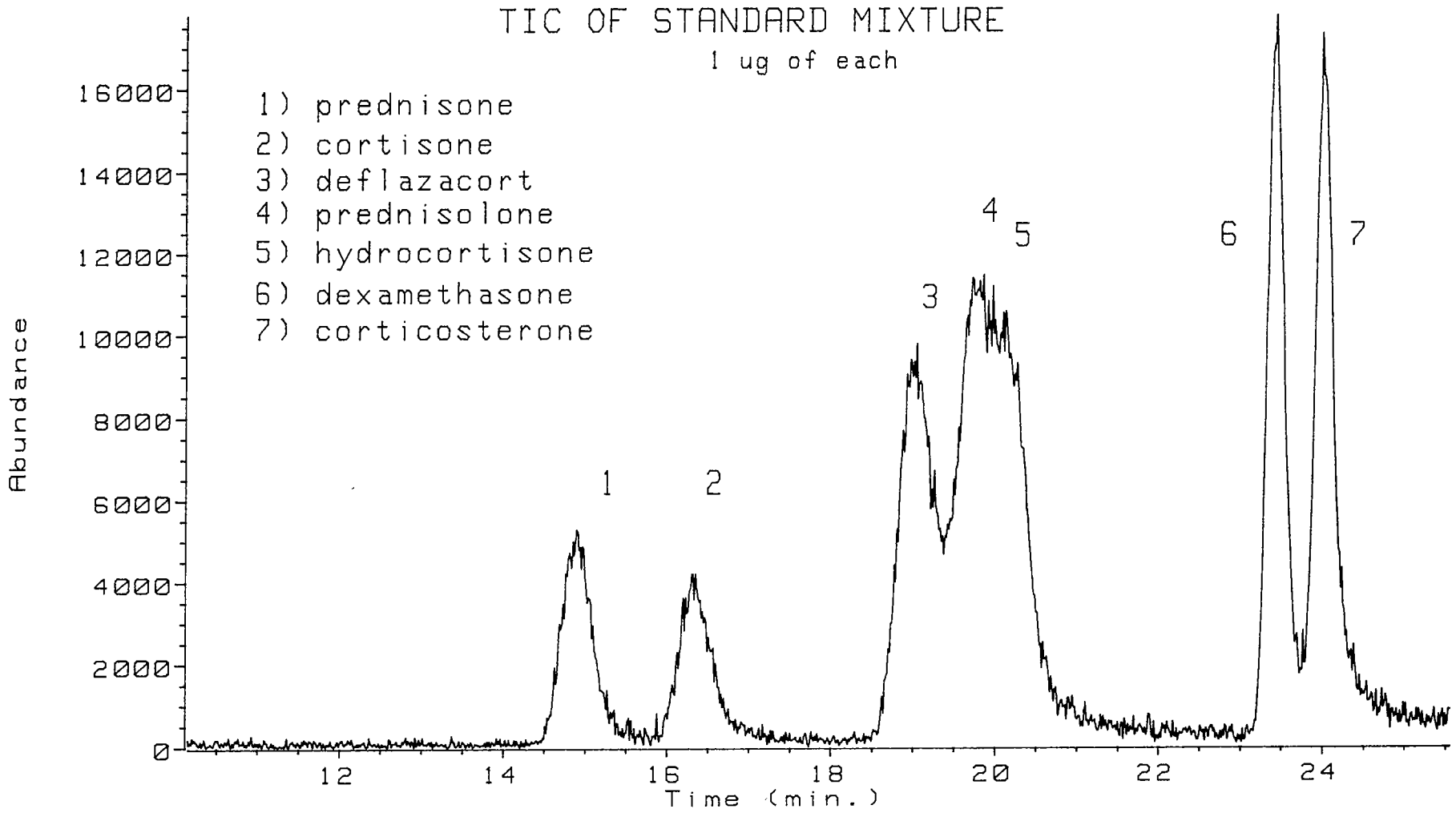
259

FIG.8


LC A 245,4
EXCRETION STUDY OF DEFLAZACORT
BLANK URINE
mAU
100
80
60
40
20
0
0
10
20
30
Time (min.)
LC A 245,4
POOL OF 12h
mAU
600
500
400
300
200
100
0
0
10
20
30
Time (min.)

260

FIG.9


TIC OF STANDARD MIXTURE
1 ug of each
1) prednisone
2) cortisone
3) deflazacort
4) prednisolone
5) hydrocortisone
6) dexamethasone
7) corticosterone
Abundance
16000
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
12
14
16
18
20
22
24
Time (min.)

261

FIG.10

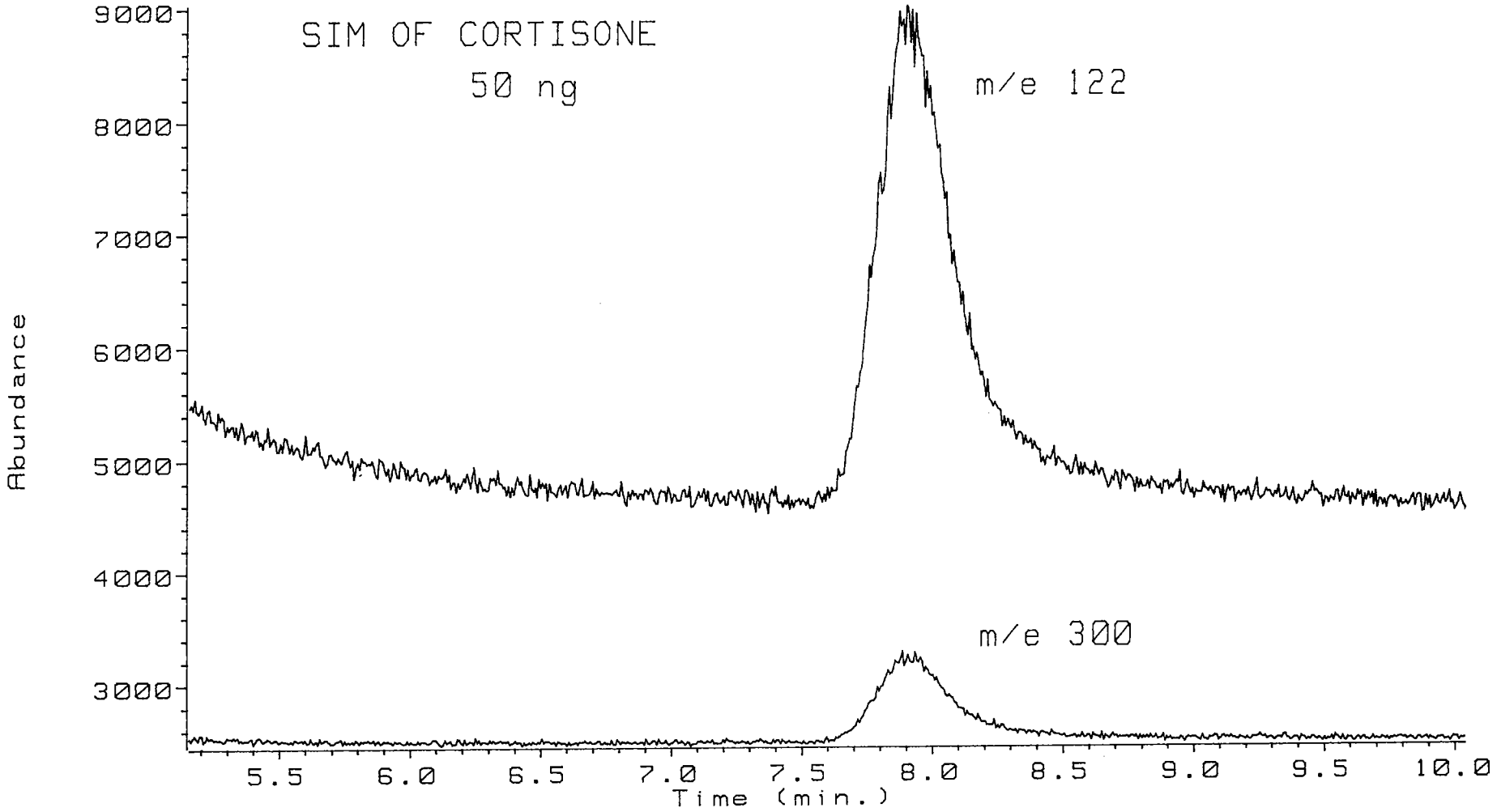

SIM OF CORTISONE
50 ng
m/e 122
m/e 300
Abundance
9000
8000
7000
6000
5000
4000
3000
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
Time (min.)

FIG.11

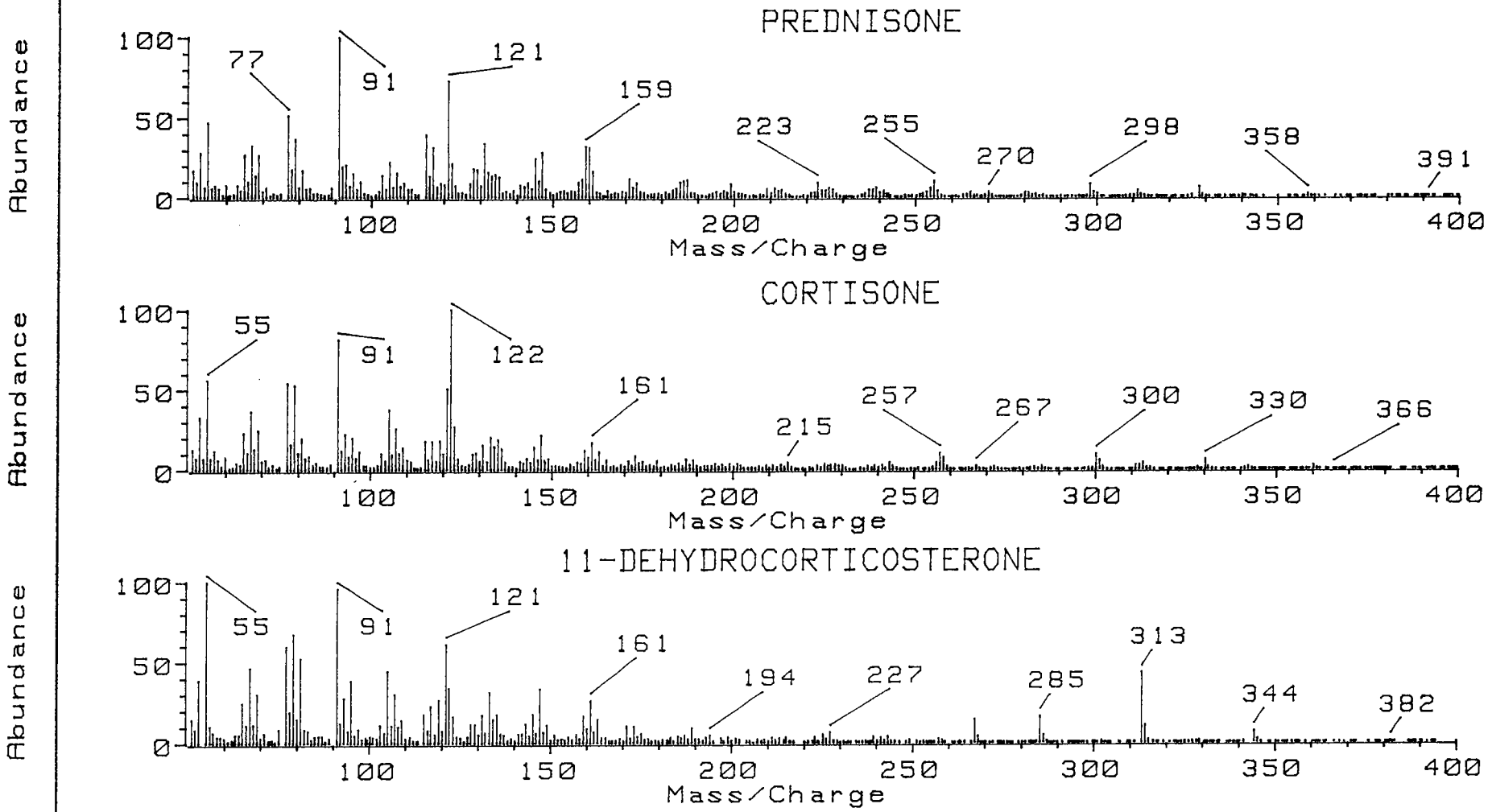


FIG.12 a

Mass Spectra of corticosteroids . Full scan EI

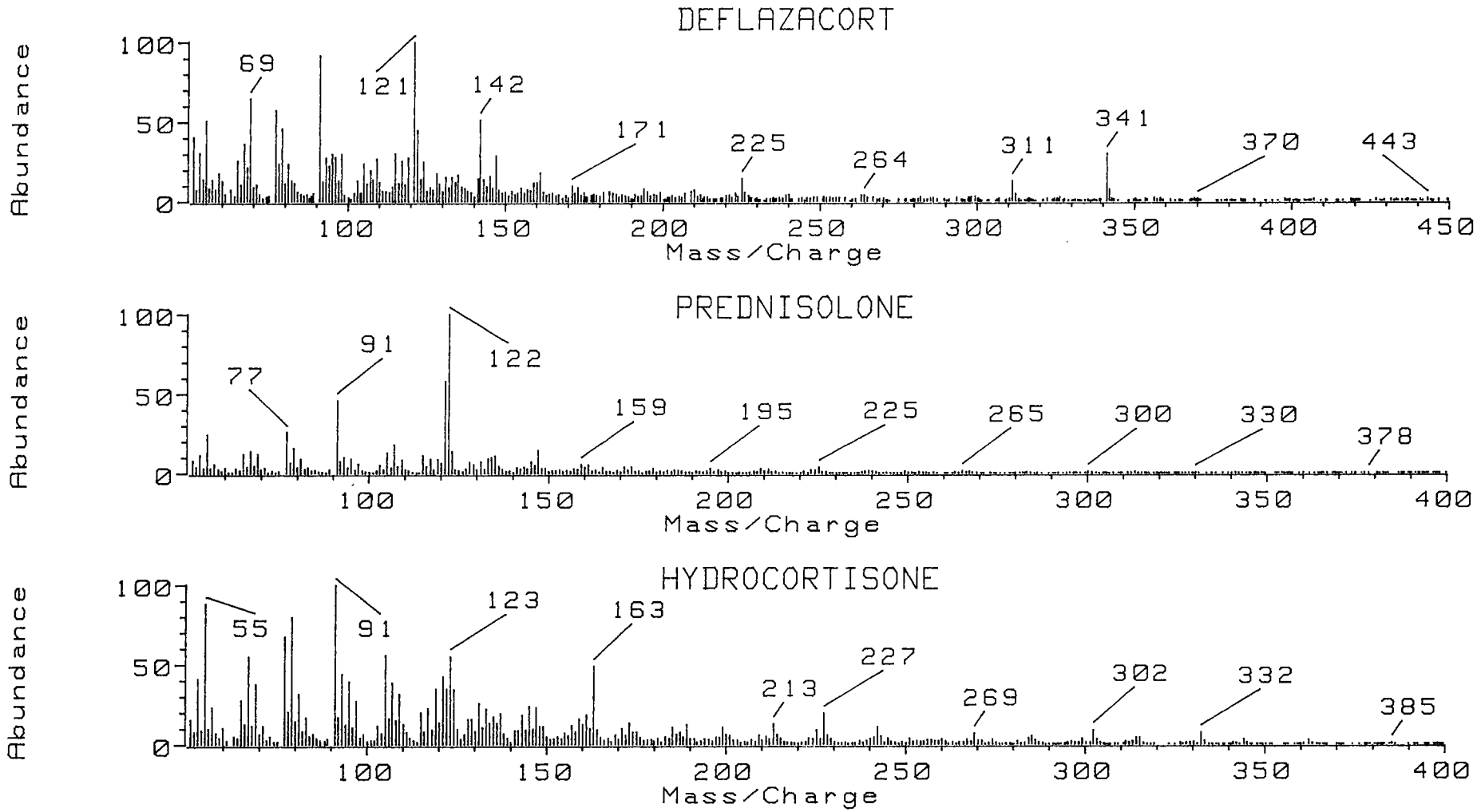


FIG. 12 b

Mass Spectra of corticosteroids. Full scan EI

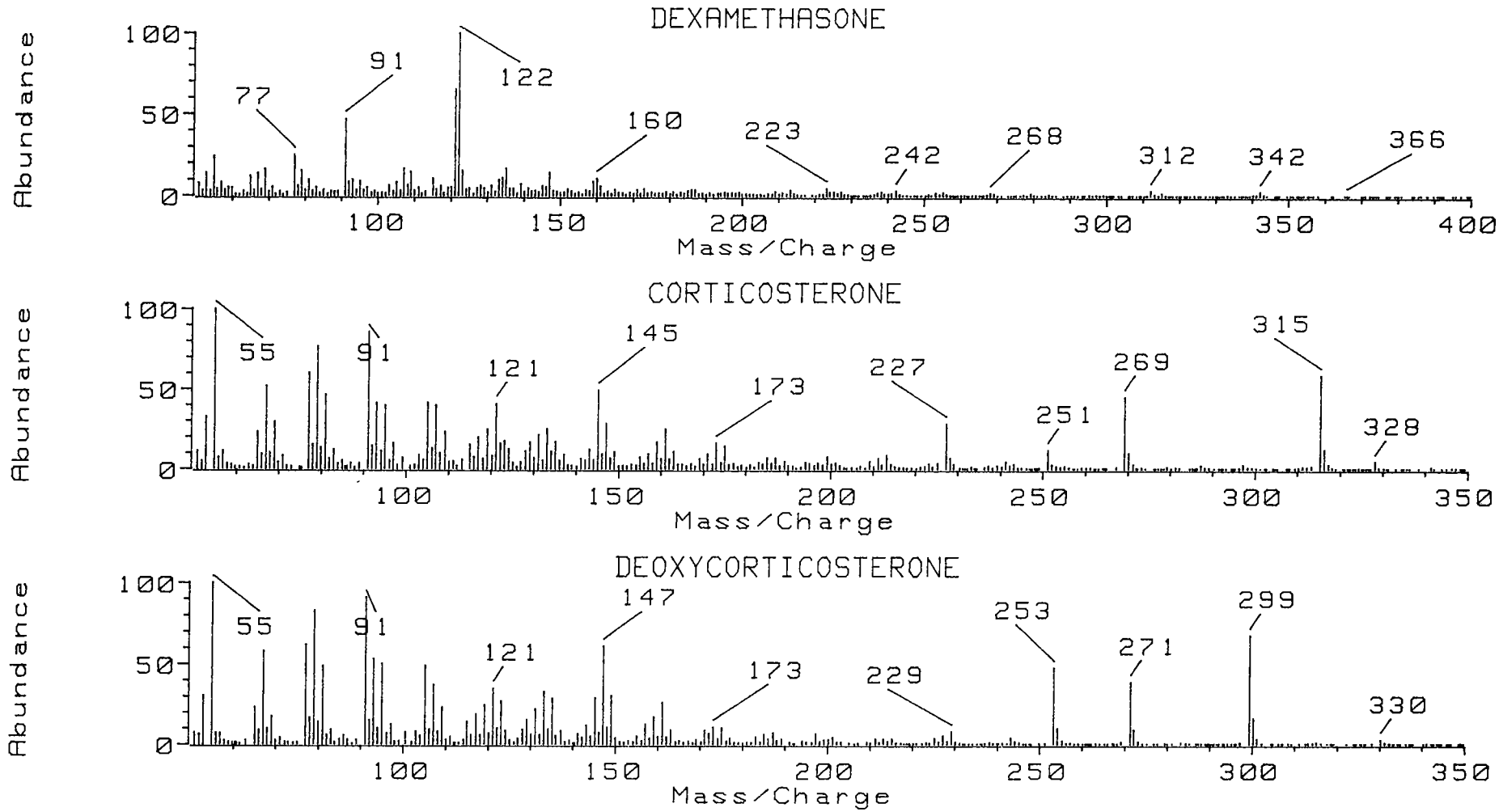


Fig. 12 c

Mass Spectra of corticosteroids. Full scan EI

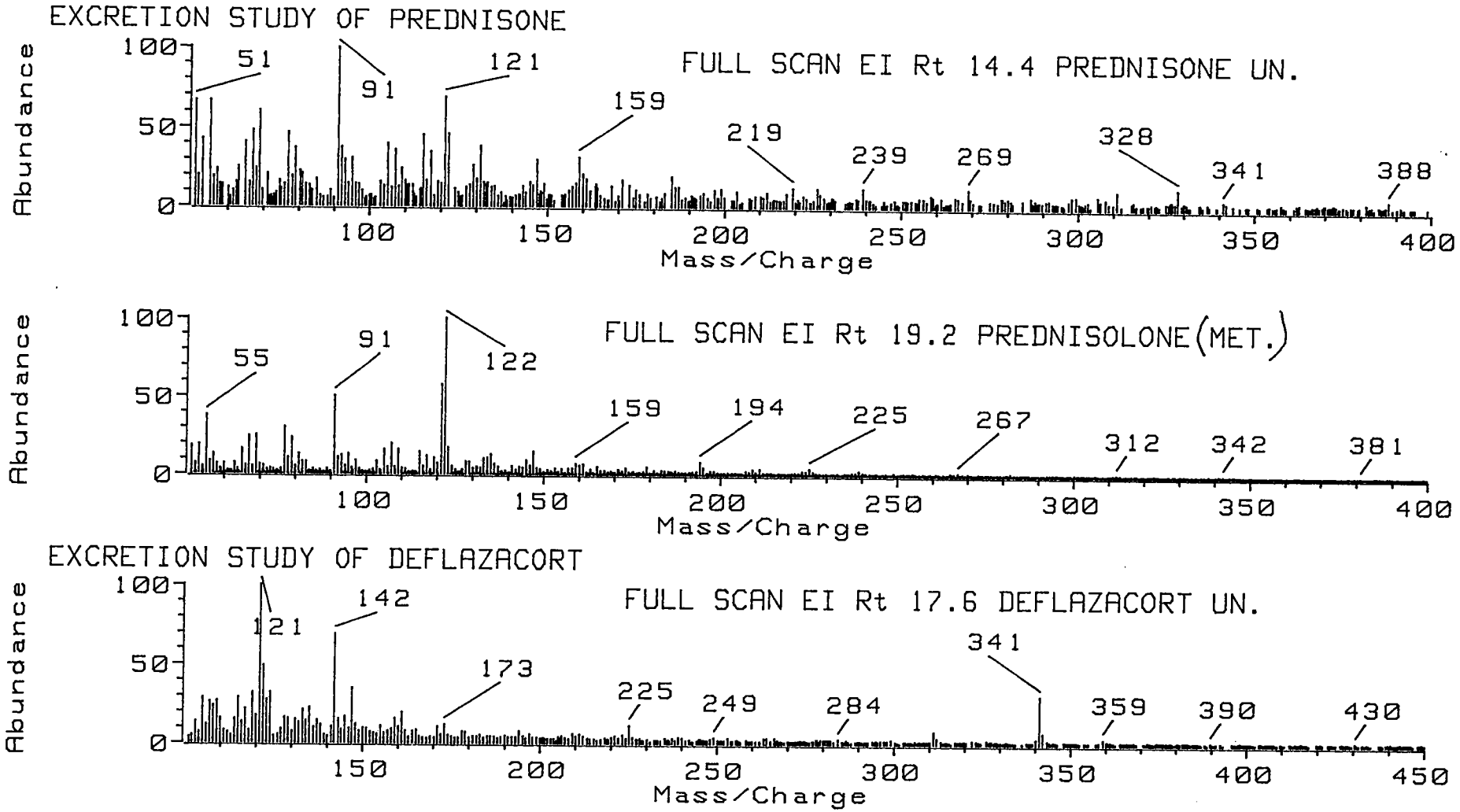

EXCRETION STUDY OF PREDNISONE
FULL SCAN EI Rt 14.4 PREDNISONE UN.
Abundance
100
50
0
51
91
121
159
219
239
269
328
341
388
100
150
200
250
300
350
400
Mass/Charge
FULL SCAN EI Rt 19.2 PREDNISOLONE(MET.)
Abundance
100
50
0
55
91
122
159
194
225
267
312
342
381
100
150
200
250
300
350
400
Mass/Charge
EXCRETION STUDY OF DEFLAZACORT
FULL SCAN EI Rt 17.6 DEFLAZACORT UN.
Abundance
100
50
0
121
142
173
225
249
284
341
359
390
430
150
200
250
300
350
400
450
Mass/Charge

FIG.13

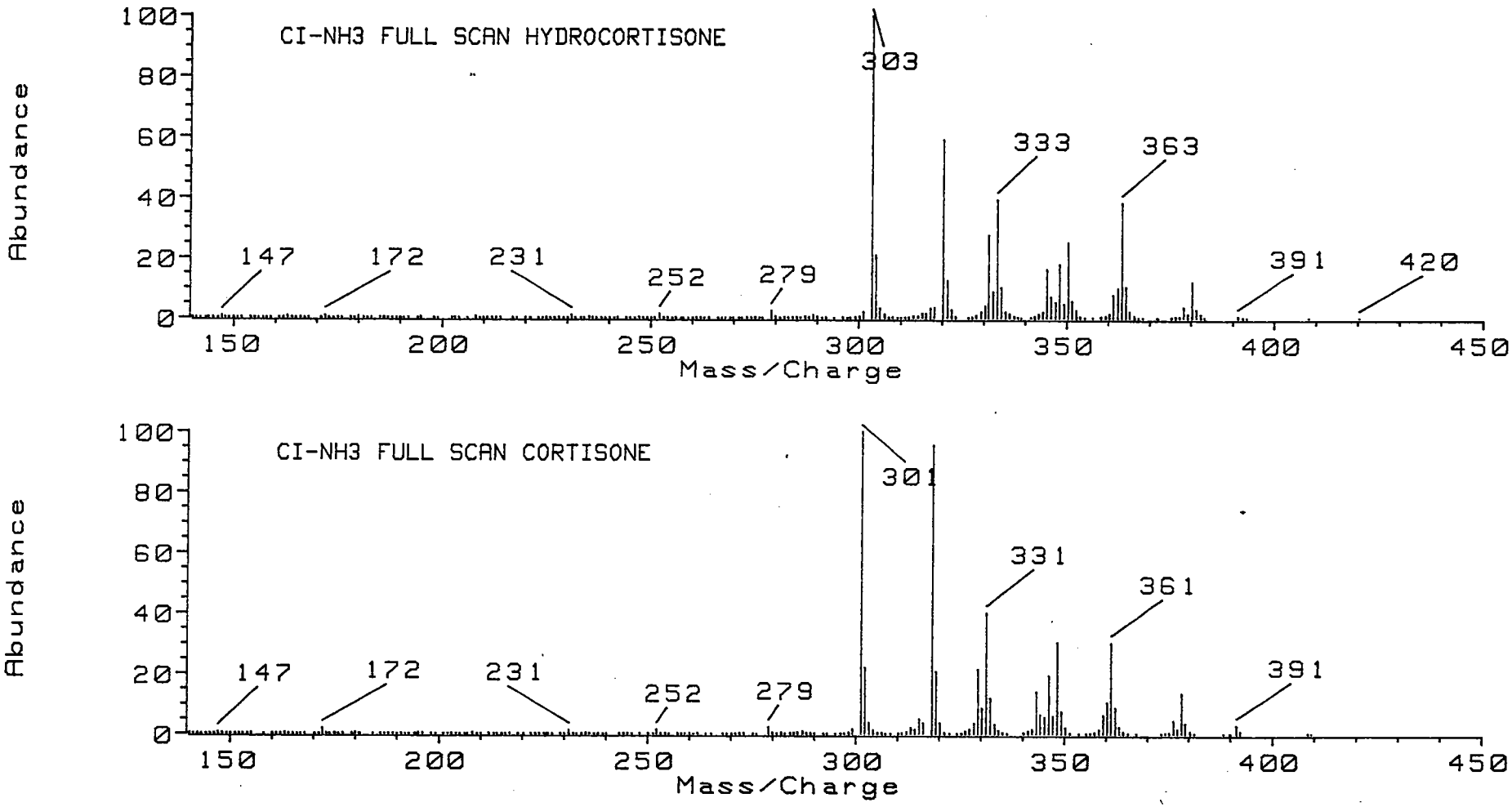

CI-NH3 FULL SCAN HYDROCORTISONE
Abundance
100
80
60
40
20
0
147
172
231
252
279
303
333
363
391
420
150
200
250
300
350
400
450
Mass/Charge
CI-NH3 FULL SCAN CORTISONE
Abundance
100
80
60
40
20
0
147
172
231
252
279
301
331
361
391
150
200
250
300
350
400
450
Mass/Charge

FIG.15