Ira S. Lurie,[1] *M.S.; Albert R. Sperling,*[1] *Ph.D.; and Richard P. Meyers,*[1] *B.S.*

The Determination of Anabolic Steroids by MECC, Gradient HPLC, and Capillary GC

REFERENCE: Lurie, I. S., Sperling, A. R., and Meyers, R. P., "**The Determination of Anabolic Steroids by MECC, Gradient HPLC, and Capillary GC,**" *Journal of Forensic Sciences*, JFSCA, Vol. 39, No. 1, January 1994, pp. 74–85.

ABSTRACT: Methodologies are presented for the qualitative and quantitative determinations of anabolic steroids in forensic exhibits using micellar electrokinetic capillary chromatography (MECC), high performance liquid chromatography (HPLC) and capillary gas chromatography (GC). Analyses of representative pharmaceutical dosage forms (including a commercial aqueous suspension, a commercial tablet, several commercial oil samples and various simulated dosage units) were performed using a simple, one step quantitative extraction procedure with methanol. Good agreement was obtained between all three techniques. Retention, migration and linearity data are presented and compared for over twenty anabolic steroids commonly encountered in forensic exhibits. A principal component analysis study confirmed the orthogonality of the three techniques.

KEYWORDS: forensic science, anabolic steroids, chromatographic analysis

The extensive and increasing abuse of anabolic steroids has resulted in world wide controls, both legal and through the regulations of various sports governing bodies. In the United States, 24 of the most commonly abused steroids (along with their various esters and salts) were placed in Schedule III of the Controlled Substances Act in February of 1991 [*1*]. This action expanded the analyses of steroids from the toxicological (blood and urine) to the forensic arena (seized exhibits), and mandated the development of definitive qualitative and quantitative methodologies for typical pharmacological dosage forms (tablets, aqueous suspensions and oil solutions).

The analysis of steroids is complicated by the sheer number of known steroids (over 7000), the close structural similarities common to the entire class, and (in some cases) their poor volatility, thermal lability and low UV extinction coefficients. Not surprisingly, there is little definitive work to date specifically concerning forensic analyses of anabolic steroids. MECC has been reported for the separation of a few esters of anabolic steroids [*2*]. HPLC has been used for both qualitative [*3–5*] and quantitative determination [*5,6*] of various anabolic steroids. GC has been used for the analysis of limited numbers of anabolic steroids [*6–9*]; however, most of the work has been qualitative rather than quantitative. In the first two studies, dual wavelength (254 and 280 nm) detection was used; in the latter studies, diode-array UV detection [*5*] and UV and light scattering

Received for publication 29 March 1993; revised manuscript received 1 July 1993; accepted for publication 3 July 1993.

[1]Senior Research Chemists and Forensic Chemist, respectively, Special Testing and Research Laboratory, Drug Enforcement Administration, McLean, VA.

detection were employed [6]. In the first three studies, however, two distinct isocratic systems were required for comprehensive analyses, one for the parent anabolic steroids and another for their corresponding esters. For the final study, an isocratic system was used for determination of a commercial preparation containing four testosterone esters [6]. In addition, no linearity studies were conducted for the previous reported studies. Walters et al. [5] used an extraction solvent containing 90% methanol and 10% water which required multiple extraction for esters. Sandra and David reported on the quantitative analysis of a commercial preparation containing testosterone esters using capillary GC and capillary supercritical fluid chromatography [6]. For the HPLC procedure, methanol was the extraction solvent; however, no experimental details were given as to sample preparation.

Herein, we report the simultaneous qualitative and quantitative analyses of the most commonly encountered controlled anabolic steroids and steroid esters via MECC, HPLC and GC using a single step extraction procedure.

Experimental Procedure

Instrumentation

All MECC analyses were run on an Applied Biosystems Model 270A-HT capillary electrophoresis system (San Jose, CA) interfaced with a PE Nelson Turbochrom-3 chromatographic data system (Cupertino, CA). The fused-silica capillary column used in this study was a 72 cm × 50 μ I.D. (50 cm length to detector) (Polymicro Technologies, Scottsdale, AZ) and was conditioned by successively aspirating with 1 M sodium hydroxide for 10 minutes, followed by water for 10 minutes, and finally by the run buffer for 10 minutes.

All HPLC analyses were run on a Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT) fitted with an ISS 100 autosampler (Perkin-Elmer), a Zorbax ODS 5 μ column (25 cm × 4.6 mm I.D.) (MAC-MOD Analytical, Chadds Ford, PA), and a 1040 M diode array detection system (Hewlett-Packard, Waldbronn, Germany).

All GC analyses were run on a Perkin Elmer Sigma 2000 gas chromatograph equipped with a flame ionization detector (FID) and interfaced with a Perkin Elmer 7700 data system. The capillary column used was a fused silica, cross linked, and bonded DB-1 30 m × 0.25 mm I.D. with a 0.25 μ film thickness (J & W Scientific, Folsom, CA). The carrier gas was hydrogen (zero grade) with an average gas velocity of 41 cm/s.

The principal component analysis study was run using the "Einsight" program with autoscaled data (Infometrix, Seattle, WA).

Materials

All chemicals and solvents used in the study were reagent grade or better. Deionized water used to prepare all mobile phases and the run buffer was obtained from a Millipore Milli-Q system (Bedford, MA). All steroids were obtained from the Reference Standards Collection of the Drug Enforcement Administration's Special Testing and Research Laboratory and were pharmaceutical grade or better.

MECC Analysis

The MECC run buffer was acetonitrile/sodium dodecyl sulfate (SDS) solution 2:3; the SDS solution contained 75 mM SDS in 10 mM phosphate/10 mM borate buffer at pH 9.0. A voltage of 25 kV and a temperature of 30°C was used for all runs.

For linearity and precision studies, a standard mixture of methyltestosterone, testos-

terone propionate and testosterone enanthate was dissolved in acetonitrile/methanol 3:1 and diluted with 75 mM SDS to a final solution containing 60% SDS. All serial dilutions were made using this solution.

For the methyltestosterone tablet assay, the tablets were crushed and ground up, and an amount equivalent to 10 mg of steroid was weighed into a 10 mL volumetric flask and diluted to volume with methanol. The resultant solution was sonicated for 15 minutes and a 1.0 mL aliquot of the filtered sample solution plus 3.0 mL of acetonitrile were pipetted into a 10 mL volumetric flask and diluted to volume with 75 mM SDS. The sample solution was filtered prior to a 1 second injection onto the instrument.

A 1.0 mL aliquot of a well shaken testosterone aqueous suspension sample equivalent to 50 mg of steroid was pipetted into a 100 mL volumetric flask and approximately 50 mL of methanol was added. The solution was vortexed for 2 minutes and diluted to volume with methanol. A 1.0 mL aliquot of the resulting methanolic solution was removed and treated as above.

For the analysis of oil samples, 1.0 or 2.0 mL of sample equivalent to 100 mg of steroid ester was pipetted into a 100 mL volumetric flask. Approximately 50 mL of methanol was added. The solution was vortexed for 3 minutes, diluted to volume with methanol and allowed to sit for several hours. The solution was then filtered and a 1.0 mL aliquot plus 5.0 mL of acetonitrile was pipetted into a 10 mL volumetric flask and diluted to volume with 75 mM SDS. The resultant solution was sonicated for 15 minutes, filtered and analyzed as above.

HPLC Analysis

All gradient HPLC analyses were performed using a 1.0 mL/min flow rate and a water/methanol solvent mixture. A linear gradient from 70% to 100% methanol over 15 minutes followed by a 15 minute hold was used for all runs.

For linearity and precision studies, a standard mixture of methyltestosterone, testosterone propionate and testosterone enanthate was dissolved in methanol and diluted to 70% with water; all serial dilutions were made using this solution.

For the analysis of tablets and aqueous suspension, sample preparations were handled exactly as detailed in the MECC section except that the 1.0 mL methanolic aliquot was diluted to 10 mL with 6.0 mL of methanol and 4.0 mL water prior to analysis; 25 μL injections were used for all runs. Similarly for oil samples a 1.0 mL methanolic aliquot was diluted to 10.0 mL with 7.0 mL of methanol and 3.0 mL water prior to analysis. 25 μL injections were used for all runs.

Capillary GC Analysis

For qualitative and quantitative analyses, the following temperature programming was used: initial temperature, 180°C (1 minute hold); then ramped at 10°C/minute to 230°C, then at 1°C/minute to 245°C and finally at 30°C/minute to 295°C (final hold 15 minutes). The detector and injector temperatures were maintained at 285°C. A split ratio of 30:1 was used for all injections.

Two additional sets of GC oven temperature parameters were found to be extremely useful for the rapid quantitative analyses of samples containing only one or a small number of steroids (generally the most common type of forensic exhibit that occurs). The first was an isothermal run at 290°C; the second, an alternate temperature program: initial temperature, 200°C (1 minute hold); then ramped at 12°C/minute to 280°C (final hold sufficient to elute all components). For all quantitative work, a Perkin-Elmer programmed temperature vaporizer (PTV) injector port was used, with an initial temperature

of 85°C (held for 0.1 minute) then raised immediately to 285°C. A 30:1 split ratio was maintained for all injections.

Linearity studies on 27 steroids were conducted using the latter two sets of oven parameters. The steroids were run either separately or as mixtures in chloroform, from which serial dilutions were made. Since the listed upper and lower injection limits will vary somewhat for each individual column, the analyses should ideally be performed using mid-range concentrations.

The internal standard solutions were prepared to contain 1 mg/mL of either tetracosane (C-24) or triacontane (C-30). For all Group 1 steroids, (Table 2) the standard solutions contained approximately 0.2 mg/mL of the respective steroid and 0.08 mg/mL tetracosane (C-24) internal standard in chloroform. For group 2 steroids, (Table 2) the standard solutions contained approximately 0.4 mg/mL of the respective steroid and 0.4 mg/mL triacontane (C-30) internal standard in chloroform.

The extraction treatment of the dosage form preparations was similar to that detailed in the MECC section. A tablet amount equivalent to 10 mg was weighed into a 25 mL flask and extracted by shaking with methanol. Chloroform and the appropriate internal standard solution were added prior to analysis.

An aliquot of an aqueous suspension equivalent to 10 mg of steroid was pipetted into a flask and extracted by shaking with methanol. Chloroform and the appropriate internal standard solution were added prior to analysis.

The analysis of dosage forms in oil necessitates a prior extraction with methanol in order to prevent large quantities of oil from being injected onto the column, which would not only rapidly degrade the column but also foul the detector. One or two mL of the steroid in oil equivalent to 100 mg of steroid was pipetted into a 100 mL volumetric flask. After adding 50 mL of methanol, vortexing for 3 minutes, and bringing the flask to volume, it was allowed to sit for several hours as previously described. An aliquot of the methanolic solution equivalent to 10 mg of steroid was pipetted into a 25 mL flask. Chloroform and the appropriate internal standard solution were added prior to analysis.

Results

Qualitative Determination

Retention and migration data are presented in Table 1; chromatograms are presented in Figs. 1 to 3. A review of the results indicates that HPLC and GC analyses give essentially equivalent resolution, with MECC having slightly lower resolving power. All three techniques however, suffer from coincident retention/migration data for various steroid pairs. Methandriol/stanolone are not separated by any of the three presented methodologies. Similarly, nandrolone/methandrostenolone are not resolved by HPLC and MECC. Testosterone cypionate/testosterone enanthate are not separated by HPLC. Methandriol dipropionate/stanozolol and boldenone/methyltestosterone are pairs not resolved by GC. Stanozolol/testosterone acetate, danazol/nandrolone propionate, and danazol/clostebol acetate are pairs not separated by MECC. It should be noted that clostebol, clostebol acetate, stanozolol, danazol, and dromostanolone are not amenable to quantitation by GC due to thermal decomposition. As was previously reported, multi-wavelength detection results in increased selectivity and/or sensitivity in HPLC analyses [5] (Fig. 2); MECC exhibits similar enhancements (Fig. 3). In the latter study, because the MECC instrument used possessed only a single wavelength detector, three separate injections were run in order to simulate multiwavelength detection. It was of interest to note that 200 nm detection gave a twofold increase in sensitivity vs 210 nm for stanalone and methandriol, that is, the two compounds with weak extinction coefficients. The use of methanol in the mobile phase precluded the use of 200 nm detection for HPLC analyses.

TABLE 1—*Retention of anabolic steroids relative to testosterone.*

Compound	MECC	HPLC	GC
Fluoxymesterone	0.925	0.78	1.50
Boldenone	0.964	0.74	1.05
Nandrolone	0.979	0.84	0.91
Methandrostenolone	0.985	0.86	1.12
Testosterone	1.00[a]	1.00[b]	1.00[c]
Methyltestosterone	1.02	1.17	1.05
Methandriol	1.06	1.25	0.89
Stanolone	1.06	1.25	0.89
Boldenone acetate	1.12	1.46	1.27
Stanozolol	1.16	1.69	1.68
Testosterone acetate	1.17	1.76	1.21
Nandrolone propionate	1.22	1.88	1.29
Danazol	1.23	1.52	[d]
Clostebol acetate	1.24	1.90	[d]
Testosterone propionate	1.26	2.01	1.43
Methandriol 3 acetate	1.26	2.13	1.10
Testosterone isobutyrate	1.35	2.17	1.54
Nandrolone phenylpropionate	1.44	2.25	2.28
Testosterone cypionate	1.64	2.63	2.19
Testosterone enanthate	1.69	2.60	1.92
Methandriol dipropionate	1.81	2.98	1.70
Nandrolone decanoate	2.06	2.87	2.26
Boldenone undecylenate	2.20	2.73	2.62
Testosterone undecanoate	2.36	3.18	2.56
Oxymetholone	[e]	[f]	1.28
Oxandrolone	—	—	1.17
Testosterone isocaproate	—	—	1.77
Testosterone decanoate	—	—	2.36

[a]retention time 13.9 minutes
[b]retention time 9.2 minutes
[c]retention time 13.8 minutes
[d]breaks down GC
[e]doesn't chromatograph MECC
[f]breaks down HPLC

A principal component analysis study was undertaken for 17 steroids in order to compare the three techniques; two HPLC isocratic runs were also included as a control. This technique uses a combination of mathematical and interpretative methods to create a set of axes to visualize the data vectors and the variance in experimental data. In addition, it gives some insight into the underlying factors influencing the data vectors. The scores plot in Fig. 4 represents data sets with 17 variables each. The individual data sets are chromatographic techniques with variables representing the retention of individual anabolic steroids relative to testosterone. All three methodologies appear to be orthogonal (Fig. 4). This is manifested by the result that all three techniques are well separated from each other in two dimensional space (present in different quadrants). Chromatographic runs a and b (Fig. 4) represent the published data [5] and a repeat of the experimental conditions in our laboratory for isocratic HPLC. As expected, the scores plots for these latter runs are highly similar. The loadings plot (Fig. 5) describes the importance of an individual steroid in defining the principal component. Highest loaded variables are toward the outer edges of a plot while variables having little significance are more toward the cross hairs. All steroids studied contribute significantly to the variance. In addition, certain groups of steroids give similar contributions to the loadings plot; that is, there are some correlations of the groupings with structure. All of the

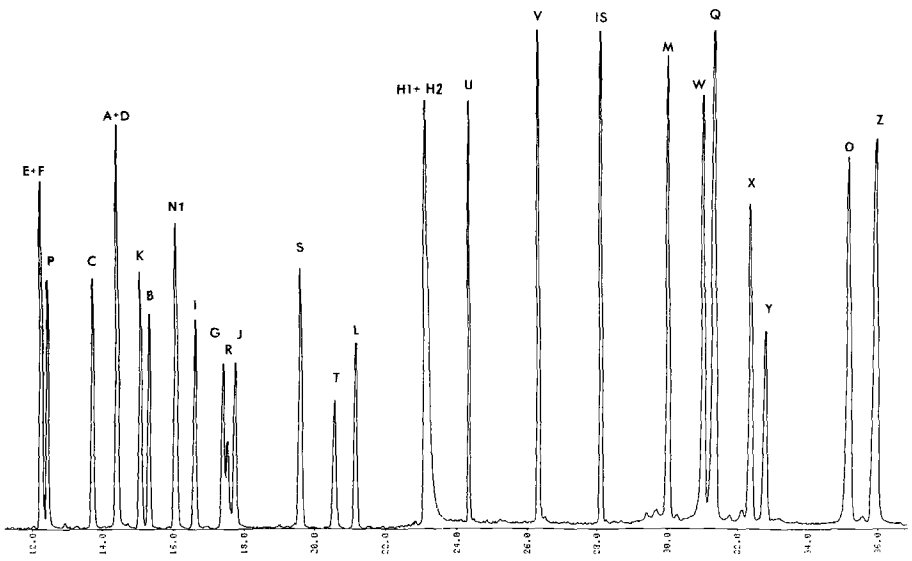

FIG. 1—*Capillary GC chromatogram of an anabolic steroid mixture. See Experimental Section for GC conditions. Peaks:*

A = Boldenone
B = Methandrostenolone
C = Testosterone
D = Methyltestosterone
E = Methandriol
F = Stanolone
G = Boldenone acetate
H = Danazol
H1 = Methandriol dipropionate
H2 = Stanozolol
I = Testosterone acetate
J = Nandrolone propionate
K = Methandriol-3-acetate
L = Testosterone isobutyrate
M = Testosterone cypionate
N = Uncontrolled steroid
N1 = Oxandrolone
O = Testosterone undecanoate
P = Nandrolone
Q = Nandrolone phenylpropionate
R = Oxymetholone
S = Testosterone propionate
T = Fluoxymestrone
U = Testosterone isocaproate
V = Testosterone enanthate
W = Nandrolone decanoate
X = Testosterone decanoate
Y = Testosterone phenylpropionate
Z = Boldenone undecylenate
IS = Internal standard, tetratriacantane (C-34)

nonesterified steroids (except for methyltestosterone) which contain either an enone or dienone moiety cluster in the left quadrants in the loadings plot. The two steroids that have unsaturated ester moieties (boldenone undecylenate and nandrolone phenylpropionate) cluster in the upper left quadrant. Finally, four steroids containing aliphatic esters cluster in the upper right hand corner.

The analyses of typical forensic exhibits are occasionally complicated by the presence of a wide variety of both controlled and non-controlled steroids as well as additional adulterants and diluents; in certain geographical regions in the United States, up to 50% of all illicitly marketed steroids are in fact counterfeits [*10*]. The analyses of such exhibits require screening techniques of high discriminatory power. Based on the present study, HPLC or capillary GC represent the best choices for a single chromatographic system. If a diode-array UV detector is utilized, the discriminatory power of HPLC is further (and significantly) enhanced, as there are salient differences between the UV spectra of many of the parent compounds [*11*]. In the CND study, individual UV spectra were obtained in methanol. A comparison of UV spectra obtained in this study using the diode-

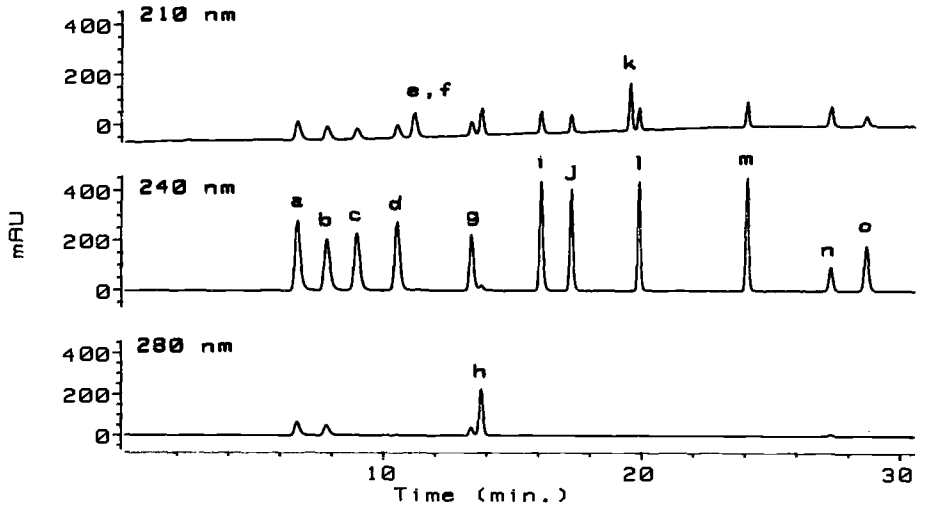

FIG. 2—*HPLC gradient chromatogram of an anabolic steroid mixture. See Fig. 1 for identity of peaks and experimental section for HPLC conditions.*

array UV detector, as shown in Figs. 6 to 8, indicate that, although significant differences exist, various steroids have identical spectra. Testosterone, methyltestosterone, fluoxymesterone and nandrolone, which all contain an enone moiety, give virtually identical spectra with UV maxima at 242.5 nm. Boldenone and methandrostenolone, both of which contain a dienone moiety, again give virtually identical spectra with UV maxima at 246.5 nm. Stanozolol, which contains a pyrazole ring, has a UV maxima at 224.5 nm, while clostebol, which contains a 4-chloro enone moiety, has a UV maxima of 255.5 nm. For

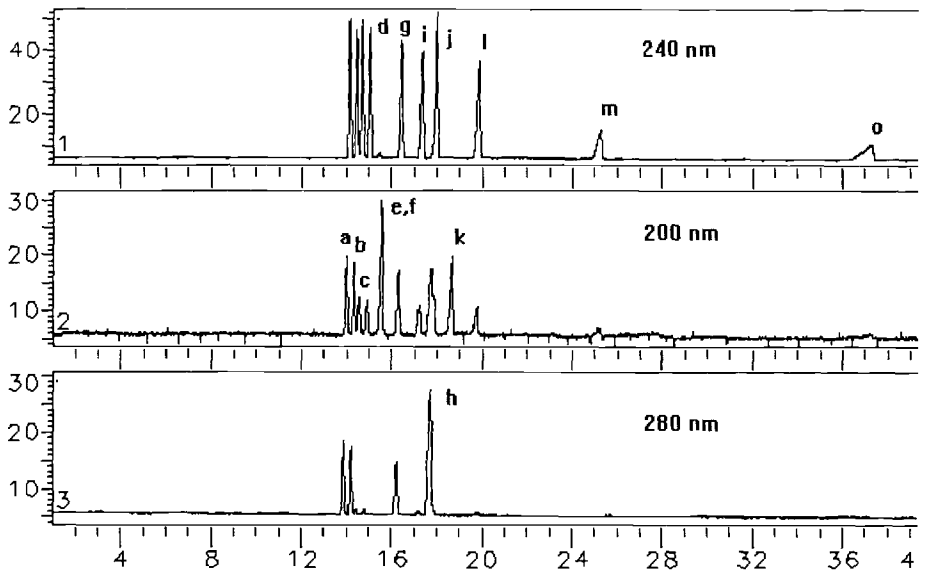

FIG. 3—*MECC electropherogram of an anabolic steroid mixture. See Fig. 1 for identity of peaks and experimental section for MECC conditions.*

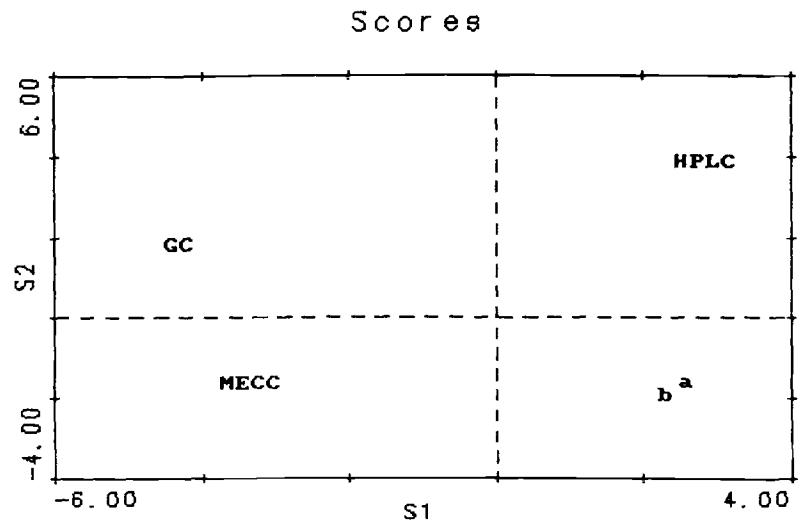

FIG. 4—*Principal component analysis scores plot using 17 anabolic steroids for capillary GC, MECC, Gradient HPLC and isocratic HPLC (a and b).*

danazol the interaction of the isoxazoline ring with the A-ring double bond gives a UV maxima of 284.5 nm. Methandriol, which contains only an isolated double bond, has no discernible absorbance maxima, while stanolone, which contains only a ketone, has a UV maxima at 280 nm. Not unexpectedly, homologous esters (that is, same parent steroid with different alkyl ester groups) have highly similar UV spectra (Figs. 7 and 8); the lone exception is nandralone phenylpropionate, which has an additional maxima at 206.5 nm due to the phenyl substituent. These results also suggest that the use of a diode-array UV detector for MECC would similarly increase specificity.

As a multi-technique approach to screening, principal component analysis indicates

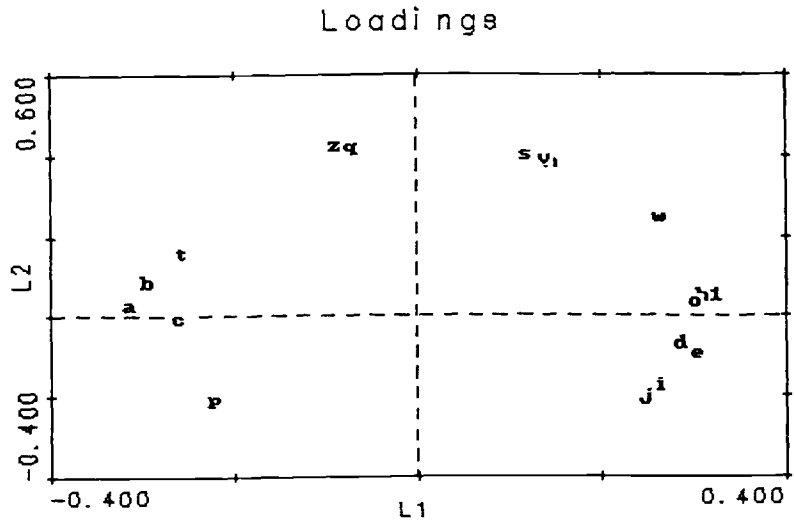

FIG. 5—*Principal component analysis loadings plot using 17 anabolic steroids for capillary GC, MECC, Gradient HPLC and isocratic HPLC. See Fig. 1 for identity of compounds.*

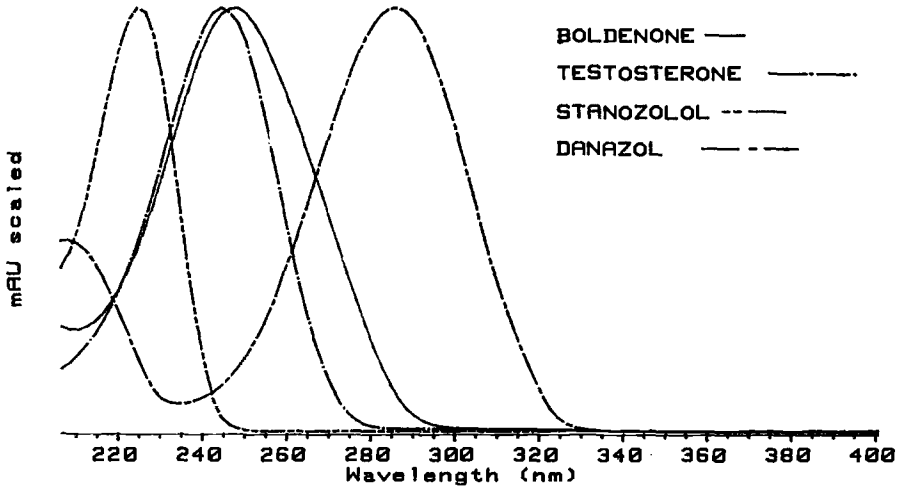

FIG. 6—*UV spectra of anabolic steroids.*

that the combined use of MECC, HPLC and GC have high discriminatory power. This arises because of the orthogonality of the three techniques as well as the fact that all anabolic steroids examined contribute to the variance.

Quantitative Determination

Peak area linearity is observed for all steroids examined by GC (Table 2). Peak area linearity was similarly observed for methyltestosterone, testosterone propionate and testosterone enanthate on HPLC and MECC (Table 3). In a related study, peak height linearity was also observed for methyltestosterone, testosterone propionate and testosterone enanthate on HPLC; however, only methyltestosterone displayed peak height linearity via MECC. The non-linearity for testosterone propionate and testosterone enan-

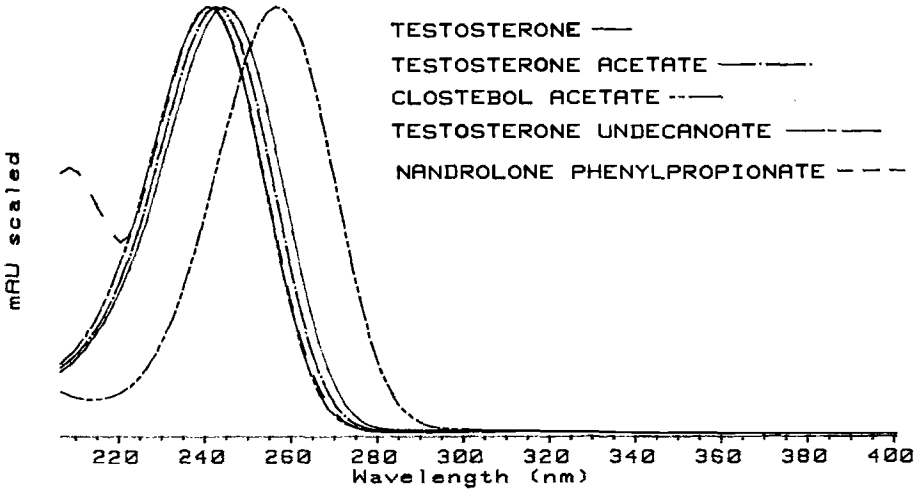

FIG. 7—*UV spectra of anabolic steroids.*

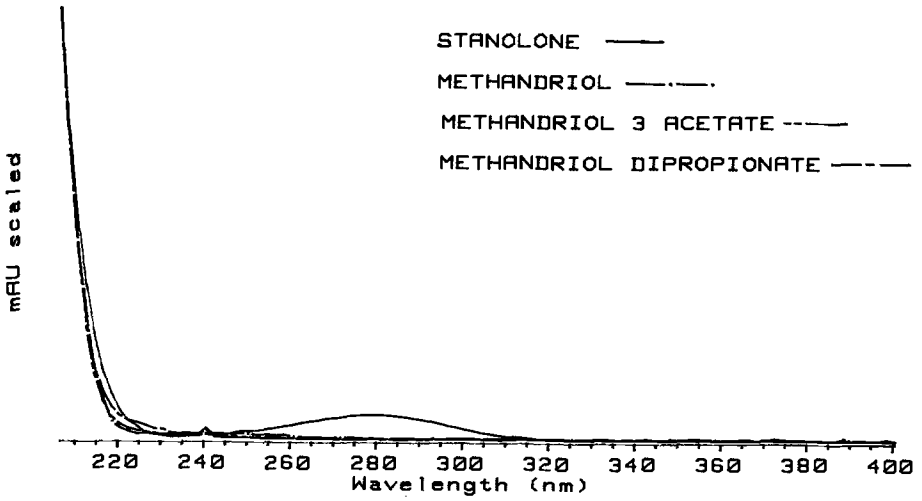

FIG. 8—*UV spectra of anabolic steroids.*

TABLE 2—*Results of GC linearity study.*

Steroid	Linearity range micrograms injected on column	Correlation coefficient	Temperature
	Group 1		
Methyltestosterone	.0010–0.27	1.000	200–280
Testosterone	.0010–0.17	1.000	200–280
Testosterone acetate	.0017–0.10	1.000	200–280
Testosterone isobutyrate	.0003–0.27	1.000	200–280
Testesterone propionate	.0003–0.27	1.000	200–280
Boldenone	.0017–0.27	1.000	200–280
Nandrolone	.0017–0.33	1.000	200–280
Fluoxymestrone	.0017–0.33	1.000	200–280
Testosterone isocaproate	.0017–0.33	1.000	200–280
Methandriol	.0010–0.27	.998	200–280
Methandriol 3-acetate	.0010–0.27	1.000	200–280
Oxymetholone	.0010–0.17	.996	200–280
Stanolone	.0010–0.33	1.000	200–280
Nandrolone propionate	.0010–0.33	1.000	200–280
Methandrostenolone	.0017–0.17	1.000	200–280
Boldenone acetate	.0017–0.17	1.000	200–280
Methandriol dipropionate	.0017–0.17	1.000	200–280
Oxandrolone	.0030–0.27	1.000	200–280
	Group 2		
Nandrolone decanoate	.0017–0.050	.999	290
Testosterone cypionate	.0017–0.27	1.000	290
Testosterone enanthate	.0017–0.17	1.000	290
Testosterone undecanoate	.0017–0.17	1.000	290
Testosterone decanoate	.0026–0.27	1.000	290
Boldenone undecylenate	.0026–0.27	.999	290
Nandrolone phenylpropionate	.0030–0.17	.996	290
Testosterone Phenypropionate	.0030–0.17	1.000	290
Boldenone benzoate	.0030–0.27	1.000	290

TABLE 3—*Results for linearity study.*

Drug	Linearity range (mg/mL)	Correlation coefficient
MECC (peak area)		
Methyltestosterone	0.0389–1.0	0.9993
Testosterone propionate	0.0389–1.0	0.9990
Testosterone enanthate	0.0389–1.0	0.9992
MECC (peak height)		
Methytestosterone	0.0389–1.0	0.9995
HPLC (peak area)		
Methyltestosterone	0.01024–1.0	0.9990
Testosterone propionate	0.01024–1.0	1.000
Testosterone enanthate	0.01024–1.0	0.998
HPLC (peak height)		
Methyltestosterone	0.01024–0.4	1.000
Testosterone propionate	0.01024–0.4	1.000
Testosterone enanthate	0.01024–1.0	1.00

thate is possibly due to limited solubility in the micelle, since as the concentration of ester was increased the amount of heading was also observed to increase. This arises since solutes at the leading edge of the sample band (which are insoluble in the micelles) enter the run buffer and are propelled by osmotic flow ahead of the sample band. Solutes at the tailing edge of the sample band are propelled back into the sample zone and thereby result in a sharpening of the left boundary of the zone. For reasons that are unclear, the migration times become greater with an increase in concentration. Weinberger and Albin [*12*] reported similar observations for peak shape and retention time when studying non-steroidal anti-inflammatory drugs via MECC; however, they were analyzing negatively charged compounds where electrophoretic effects could explain the observed results.

The use of methanol as a quantitative extraction solvent was investigated using GC. In contrast to a previous study using methanol/water 9:1 [*4*], straight methanol gave good recoveries using a single extraction, even for the most difficult matrices, that is, highly lipophilic esters in oils. Recovery efficiency was determined via standard addition using a commercial exhibit of testosterone propionate in oil. The sample was analyzed and then a known amount of steroid was added. The reanalysis gave a 99.5% recovery of the added amount. In addition, two simulated oil solutions (testosterone propionate, 100 mg/mL in sesame oil and nandrolone decanoate, 100 mg/mL in sesame oil) and one simulated tablet (10 mg of methyltestosterone in standard tablet exipients) were prepared and analyzed. The recoveries obtained were 99.7, 97.0 and 99.0%, respectively.

Quantitations of commercial preparations, including methyltestosterone tablets, a testosterone aqueous suspension and testosterone propionate, testosterone enanthate and

TABLE 4—*Quantitative analysis of anabolic steroids.*

Commercial preparation	% Recovery MECC	HPLC	GC
Methyltestosterone tablets	99.6	98.9	97.0
Testosterone aqueous suspension	83.5	86.5	82.7
Testosterone propionate in oil	96.5	97.2	96.3
Testonsterone enanthate in oil	98.1	101.6	98.8
Nandrolone decanoate in oil	93.4	93.7	92.0

TABLE 5—*Relative standard deviations for chromatographic precision.*[1]

Compound	Concentration (mg/mL)	mt[2]	R.S.D (%) area	height
MECC				
Methyltestosterone	0.130	0.77	1.51	1.51
Testosterone propionate	0.130	0.32	1.52	
Tesotsterone enanthate	0.130	0.67	1.86	
HPLC				
Methyltestosterone	0.160	0.52	1.35	2.59
Testosterone propionate	0.160	0.52	1.68	3.26
Tesotsterone enanthate	0.160	0.41	1.35	2.97

[1] $n = 5$.
[2] Migration time for MECC and retention time for HPLC.

nandrolone decanoate oil solutions were performed using peak area and internal standard. Excellent agreement was achieved using all three presented methodologies (Table 4).

Finally, a comparison study of the chromatographic precision of MECC vs HPLC at the typical working concentration for anabolic steroids (approximately 0.1 mg/mL) was undertaken (Table 5). The precision obtained for MECC migration times (less than 0.78%) was only slightly poorer than that observed for HPLC retention times (less than 0.53%). In addition, comparable peak area precisions of less than 1.9% were obtained for both techniques. The MECC peak height precision for methyltestosterone (1.52%) was significantly better than that obtained using HPLC (2.59%).

References

[1] Code of Federal Regulations, Title 21, part 1300 to end, February 13, 1991, p. 5754.
[2] Vindevogel, J. and Sandra, P., "Resolution Optimization in Micellar Electrokinetic Chromatography: Use of Plackett-Burman Statistical Design for the Analysis of Testosterone Esters," *Analytical Chemistry*, Vol. 63, 1991, pp. 1530–1541.
[3] Noggle, F. T., Clark, C. R., and DeRuiter, J., "Liquid Chromatographic and Spectral Analysis of the 17-Hydroxy Steroids," *Journal of Chromatographic Sciences*, Vol. 28, 1990, pp. 162–166.
[4] Noggle, F. T., Clark, C. R., and DeRuiter, J., "Liquid Chromatographic and Mass Spectral Analysis of the Anabolic 17-Hydroxy Steroid Esters," *Journal of Chromatographic Sciences*, Vol. 28, 1990, pp. 263–268.
[5] Walters, M. J., Ayers, R. J., and Brown, D. J., "Analysis of Illegally Distributed Anabolic Steroid Products by Liquid Chromatography with Identity Confirmation by Mass Spectrometry or Infrared Spectrophotometry," *Journal of Association of Official Analytical Chemists*, Vol. 73, 1990, pp. 904–926.
[6] Sandra, P. J. and David, F., "A Case Study in Selecting a Chromatographic Technique," *LC-GC*, Vol. 7, 1989, pp. 746–748.
[7] Coleman, P. D., A'Hearn, E., Taylor, R. W., and Le, S. D., "Anabolic Steroids—Analysis of Dosage Forms from Selected Studies from the Los Angeles County Sheriff's Scientific Services Bureau," *Journal of Forensic Sciences*, Vol. 36, No. 4, July 1991, pp. 1079–1088.
[8] Chiong, D. M., Consuegra-Rodriquez, E., and Almirall, J. R., "The Analysis and Identification of Steroids," *Journal of Forensic Sciences*, Vol. 37, No. 2, March 1992, pp. 488–502.
[9] Georgakopoulos, C. G., Tsika, O., Kiburis, J. C., and Jurs, P. C., "Prediction of Gas Chromatographic Relative Retention Times of Anabolic Steroids," *Analytical Chemistry*, Vol. 63, 1991, pp. 2025–2028.
[10] Drug Enforcement Administration, Office of Diversion Control, personal communications, 1991 and 1992.
[11] Analytical Profiles of the Anabolic Steroids (1989), CND Analytical, Auburn, AL.
[12] Weinberger, R. and Albin, M., "Quantitative Micellar Electrokinetic Capillary Chromatography: Linear Dynamic Range," *Journal of Liquid Chromatography*, Vol. 14, 1991, pp. 953–972.

Address requests for reprints or additional information to
Ira S. Lurie
Special Testing and Research Lab, DEA
7704 Old Springhouse Rd.
McLean, VA 22102