Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Mueller, J. Robert , Killen, Margrith , Davis, Brian M. and Yannone, Michael E.(1969) 'An Improved Liquid Phase for the Gas-Liquid Chromatography of Plasma Steroids', Separation Science and Technology, 4: 6, 507 — 512
To link to this Article: DOI: 10.1080/01496396908052277
URL: http://dx.doi.org/10.1080/01496396908052277

SEPARATION SCIENCE, 4(6), pp. 507–512, December, 1969

NOTE

An Improved Liquid Phase for the Gas–Liquid Chromatography of Plasma Steroids

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Summary

By using a relatively new liquid phase of moderate polarity (OV-225, Supelco Inc., Bellefonte, Pa.), we have significantly improved the gas–liquid chromatographic analyses of certain steroid hormones from human plasma. This liquid phase has afforded us with nearly complete separation of the free steroids from contaminating organic impurities on the GLC column, thus increasing the accuracy of our analyses due to the Gaussian nature of the recorded chromatographic peaks. Hence, the need for preparing chemical derivatives of these steroids prior to GLC has been eliminated.

INTRODUCTION

Numerous procedures have already been reported for the quantification of steroid hormones in human plasma by gas–liquid chromatography (GLC) (*1–4*). In most instances, however, sufficient organic impurities from the biological fluid remain, even after lengthy purification steps, to necessitate the conversion of the partially purified steroids to various chemical derivatives prior to GLC. Since conventional flame ionization detectors have shown increased sensitivity to these steroid derivatives, the measurement of submicrogram quantities of the various hormones has been greatly facilitated by their use. Unfortunately, the time and technical skill required for the multiple purification steps, derivative formation, and instrument standardization prohibit the use of many of these techniques for routine analyses.

Consequently, we are reporting an important modification to our previously published GLC procedures for the analysis of plasma steroids. This change has brought about a nearly complete separation of the free steroids from contaminating impurities on the GLC column. This increase in chromatographic separation has afforded us increased sensitivity, thereby eliminating the need for preparing steroid derivatives for routine analyses.

MATERIALS AND METHODS

Steroid Purification Procedures

The procedures by which the steroid hormones, testosterone, progesterone, androstenedione and dehydroepiandrosterone, are extracted, purified, and isolated from biological fluids have been previously reported (*5–7*). These respective chemical methods remain unchanged, and the modification in technique presented here is only in regard to the GLC analysis.

Instrumentation

All analyses were carried out on a Packard Analytical Gas–Liquid Chromatograph, Model 7621.

GLC Column Parameters

The ability to effect a desired separation of sample components in GLC is entirely dependent on the type of column employed. When attempting the chromatography of steroids, the choice of an adequate solid phase along with a liquid phase of sufficient polarity and thermal stability to assure a high degree of compound selectivity is most essential. This problem of correct column choice is further complicated when one considers that even after the lengthy chemical procedures normally required to remove and partially purify steroid hormones from plasma, sufficient organic impurities frequently remain which interfere with GLC. Thus, a column must be chosen which will permit adequate separation of the steroids from the biological contaminants. In an effort to achieve this separation, numerous investigators have prepared chemical derivatives of the free steroids. These derivatives were less polar than the native steroids, possessed a higher degree of volatility, and produced a notable increase in analytical specificity.

TABLE 1
Column I—Specifications

Length, 2 ft
I.d., 2 mm
Type, coiled glass
Packing, 1% XE-60 on Gas Chrom CLH (60–70) mesh

GLC operating conditions used for each of the steroids are:

	Testosterone	Andro-stenedione	Dehydro-epiandros-terone	Progesterone
Col. temp., °C	210	210	190	220
Detc. temp., °C	215	215	195	225
Flsh. htr. temp., °C	280	280	280	280
Chrt. speed (cm/min)	1.02	1.02	1.02	1.02
Fl. rt. carrier (He) (ml/min)	90	90	90	90
Fl. rt. oxygen (ml/min)	300	300	300	300
Fl. rt. hydrogen (ml/min)	40	40	40	40

Unfortunately, the necessity of preparing steroid derivatives such as acetates, trifluoroacetates, and trimethylsilyl ethers further complicated the already detailed analytical procedures.

EXPERIMENTAL

In this study we have compared the chromatographic separation of various plasma steroids from contaminating impurities on two separate column packing materials. Table 1 depicts the conditions of chromatography using XE-60 (Applied Science Labs, State College, Pa.), the column packing commonly employed in steroid analyses, while Table 2 shows the chromatographic conditions for the relatively new liquid phase, OV-225 (Supelco Inc., Bellefonte, Pa.).

Varying quantities of the free steroids (0.005 to 0.15 μg) were applied to dry injectors (*8*), air dried, and injected directly onto the GLC column. Peak areas were determined by triangulation and the linearity of detector response established. The recorded retention times for each of the hormones on the respective phases are shown in Table 3.

After standardization of the instrument for the various hormones under the conditions noted, aliquots of purified plasma samples were injected onto the GLC columns. The GLC tracings obtained from

TABLE 2
Column II—Specifications

Length, 2 ft
I.d., 2 mm
Type, coiled glass
Packing, 3% OV-225 on Chromosorb WHP, 100–120 mesh

GLC operating conditions used for each of the steroids are:

	Testosterone	Andro-stenedione	Dehydro-epiandros-terone	Progesterone
Col. temp., °C	235	239	233	237
Dect. temp., °C	242	247	239	242
Flsh. htr. temp., °C	285	282	283	285
Chrt. speed (cm/min)	1.02	1.02	1.02	1.02
Fl. rt. carrier (He) (ml/min)	90	90	90	90
Fl. rt. oxygen (ml/min)	210	300	240	200
Fl. rt. hydrogen (ml/min)	30	40	30	30

plasma testosterone samples injected independently on the two liquid phases are compared in Fig. 1.

By simply comparing the relative retention times of the hormone on the respective columns, one can immediately perceive the advantages of the OV-225 liquid phase. Since the separation of the hormone from all organic impurities is nearly complete, the recorder returns to an effective baseline-zero prior to the elution of the steroid from the GLC column.

Consequently, the geometrical shape of the subsequent steroid peak becomes Gaussian. This improvement has increased the accuracy and

TABLE 3
Retention Times of Steroid Hormones on Two Liquid Phases

Compound	Retention time (min)	
	Column 1[a]	Column 2[a]
Testosterone	.68	6.17
Androstenedione	.68	4.41
Dehydroepiandrosterone	.78	3.62
Progesterone	.68	7.74

[a] Values obtained with GLC operating conditions as shown in Tables 1 and 2.

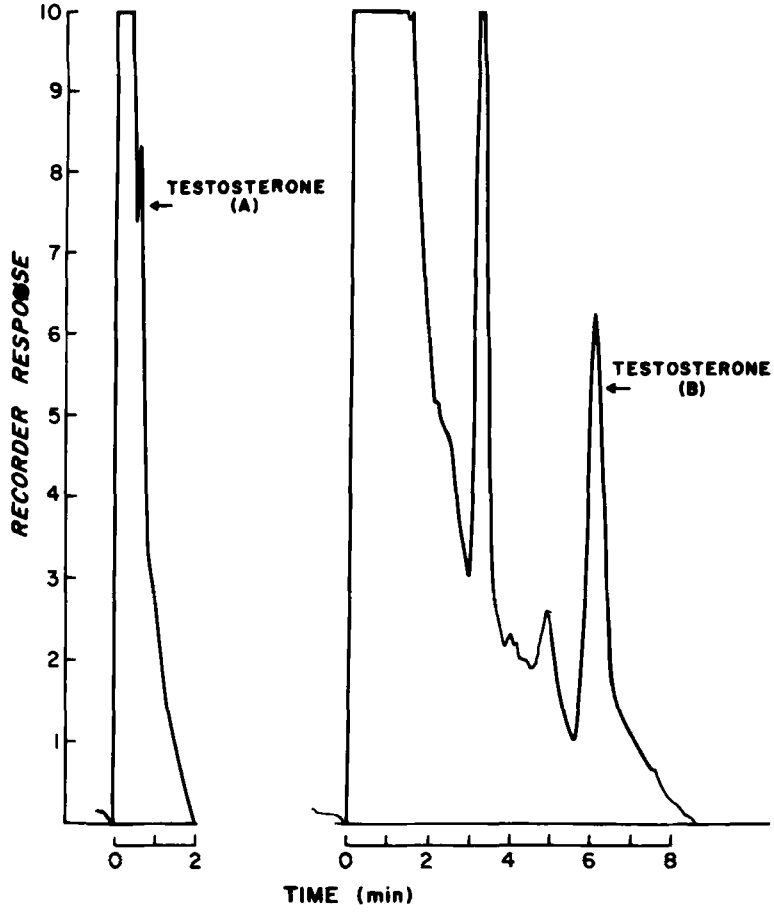

FIG. 1. Plasma testosterone samples chromatographed on Column 1, (A), and on Column 2, (B). Conditions of chromatography are given in Tables 1 and 2.

speed of peak area measurements, since disc and digital integration methods can now be employed.

The GLC analyses of plasma progesterone, androstenedione, and dehydroepiandrosterone gave similar tracings except for the variations in retention times of the individual steroids.

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Received by editor September 11, 1969