

PHARMANET GC-MS/MS

Overcome your LC-MS/MS Challenges

PharmaNet offers GC-MS/MS services for companies conducting clinical trials in therapeutic areas such as oncology, women's health, and endocrinology, where monitoring of hormone levels is critical for evaluating the efficacy of treatments. At PharmaNet, we excel at determining the therapeutic agent (LC-MS/MS) and the affected hormones (GC-MS/MS) through parallel analyses of a single sample.

GC-MS/MS analysis of hormones such as estrogens and androgens offers distinct advantages in specificity, sensitivity, precision, and less interference when compared with LC-MS/MS or immunoassay techniques.⁽¹⁻³⁾

TESTOSTERONE ANALYSIS

PharmaNet has experience in steroid analysis and (Laboratory 8 in Tables 1 and 2) recently participated with seven other laboratories to evaluate the performance of the total testosterone assay in each lab.⁽²⁾ Laboratory 1 used the NIST reference method, and the results for all labs are shown below.

	Detection Method	Overall mean bias	Mean bias at TT > 3.47 nmol/L (95%CI) [normal adrogen levels]	nmol/L (95%CI) [androgen deficient levels]
Laboratory 2	LC/MS/MS	-14.1	-10.5	-17.8
Laboratory 3	LC/MS/MS	9.9	1.7	18.2
Laboratory 4	LC/MS/MS	10.1	9.6	10.7
Laboratory 5	LC/MS/MS	-7.4	-2.3	-12.6
Laboratory 6	LC/MS/MS	19.2	16.8	21.6
Laboratory 7	LC/MS/MS	17.2	9.1	25.3
Laboratory 8	GC/MS/MS	-2.1	-2.2	-2.0

Table 1: Observed bias (accuracy) among 30 serum samples tested at each laboratory compared to performance

Detection Method	Interval precision (%RSD) at androgen deficient levels (0.29 nmol/L)	Interval precision (%RSD) at normal androgen levels (10.30 nmol/L)	
Laboratory 2	13.13	3.38	
Laboratory 3	17.75	9.62	
Laboratory 4	25.58	11.36	
Laboratory 5	2.52	1.74	
Laboratory 6	17.65	2.55	
Laboratory 7	BR	1.40	
Laboratory 8	GC/MS/MS	2.67	2.19

BR: below reportable range of method

Table 2: Interval precision at multiple levels. Results are from analysis of five replicates of each level on two separate days.

The PharmaNet GC-MS/MS testosterone method outperformed the LC-MS/MS methods by maintaining single-digit accuracy and precision at all concentrations. The strength of the assay performance across a range of testosterone concentrations make the GC-MS/MS assay ideal to support studies with hypogonadal males where levels below the 10.30 nmol/L considered normal⁽²⁾ are expected.

PharmaNet GC-MS/MS

- PharmaNet has the largest number of operational GC-MS/MS systems.
- More than 60 years of collective experience in GC-MS/MS method development.
- PharmaNet has analyzed 210,000+ samples over the past 5 years by GC-MS/MS.
- PharmaNet knows how to leverage GC-MS/MS to solve problems where LC-MS/MS has been ineffective.

Benefits of GC-MS/MS

- Advantageous for small, polar molecules (e.g., metabolites, biomarkers, RNA-type molecules) which do not ionize well by LC-MS/MS.
- Improved signal to noise ratio and lower detection limits. These compounds are derivatized which increase their molecular weight and pulls them away from the high background noise observed in the low mass range.
- Highly efficient and effective separation and sharp, intense peaks (helps lower detection limit).
- Unlike LC-MS/MS, does not encounter matrix effects (e.g., ion suppression / enhancement).

References

1. Santen, R., Memers, L., Ohorodnik, S., Settlage, J., Langecker, P., Blanchett, D., Gross, P. E., and Wang S. Steroids (2007) 72, 666
2. Vesper, H.W., Bhasin, S., Wang, C., Tai, S., Dodge, L. A., Singh, R. J., Nelson, J., Ohorodnik, S., Clarke, N. J., Salameh, W. A., Parker, R. C. Jr., Razdan, R., Monsell, E. A., and Myers, G. L. Steroids (2009) 74, 498.
3. Thakur, R. A., Williard, C., Rajasekaran, A. Chromatography Today (2010) 3, 22.
4. Ingle, J. N., Buzdar, A. U., Schaid, D. J., Goetz, M. P., Batzler, A., Robson, M. E., Northfelt, D. W., Olson, J. E., Perez, E. A., Desta, Z., Weinstraub, R. A., Williard, C. V., Flockhart, D. A., Weinshiboum, R. M. Cancer Res. (2010), 70, OF1-9.

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ESTRADIOL ANALYSIS

Estradiol concentrations are a critical factor in evaluating aromatase inhibitors used for breast cancer treatment. As breast tissue is stimulated by estrogens, decreasing their production is a way of suppressing recurrence of the breast tumor tissue. Maximum suppression of estradiol concentration is targeted as a secondary endpoint, which in turn necessitates the use of the most specific and sensitive quantitative assay available.

PharmaNet, using a validated GC-MS/MS analytical method, participated in a multicenter evaluation of anastrozole. The results from 191 subjects evaluated prior to and during anastrozole treatment are shown in the graph below.⁽⁴⁾ The method used was easily able to distinguish the changes in estrogens and again supports the conclusion that the enhanced specificity of the GC-based assay is key to achieving the lowest possible detection levels.

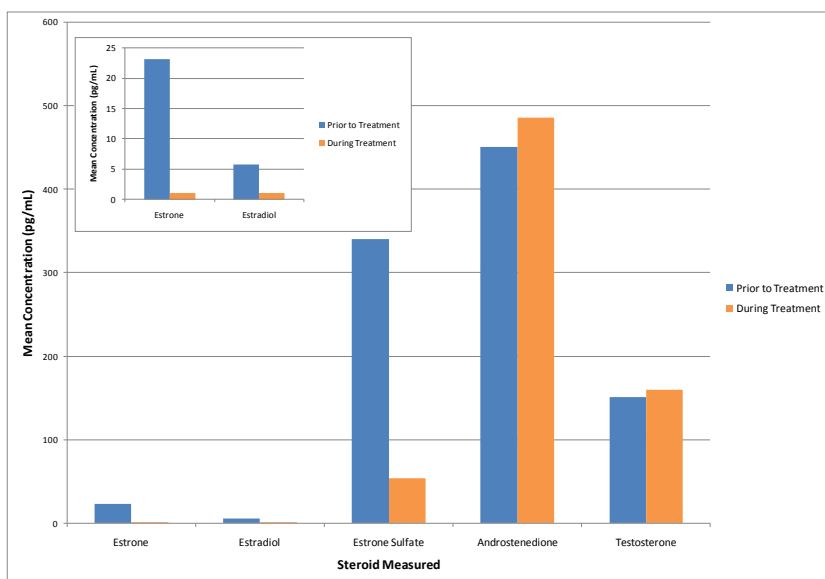


Figure 1. Changes in steroid levels of postmenopausal women before and during treatment with anastrozole.

PharmaNet's estradiol assay has been used successfully for the analysis of thousands of samples with a lower limit of quantitation (LLOQ) of 0.625 pg/mL. In order to support investigation of more advanced therapeutics agents, the LLOQ is being revalidated to 0.3 pg/mL in 2010.

CONCLUSION

The examples of GC-MS/MS assay performance shown here are superior to competing assays and techniques. While these are only two examples, the utility of GC-MS/MS has been successfully extended to the quantitation of other endogenous biomarkers, such as prostaglandins and neurotransmitters, and to a number of therapeutic agents.

About PharmaNet Development Group, Inc

PharmaNet Development Group, Inc. a global drug development services company, provides a comprehensive range of services to the pharmaceutical, biotechnology, generic drug and medical device industries. The Company offers early and late stage consulting, Phase I clinical studies and bioanalytical analyses, and Phase II, III and IV clinical development programs. For more information please visit www.pharmanet.com.

