Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A novel chemiluminescence signal amplification strategy based on the capillary electrophoresis platform for highly sensitive competitive immunoassay of biomolecules

Min Shi, Jingjin Zhao, Shuting Li,* Jianniao Tian, Shulin Zhao*

State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, Guangxi Normal University, Guilin, 541004, China

Table of contents

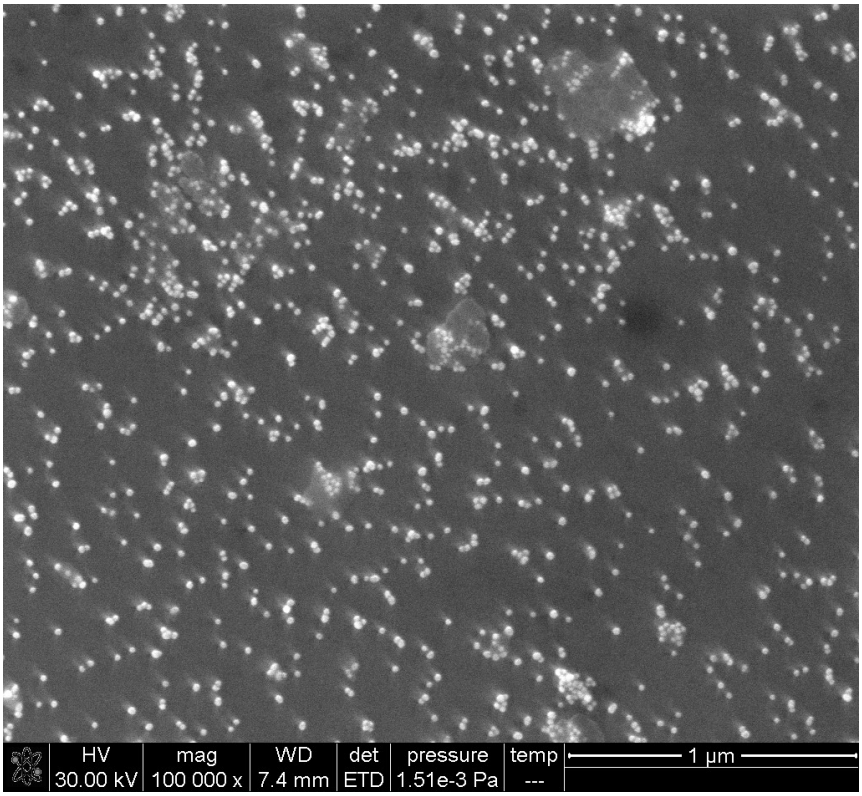

Figure S1. SEM image of AuNPs

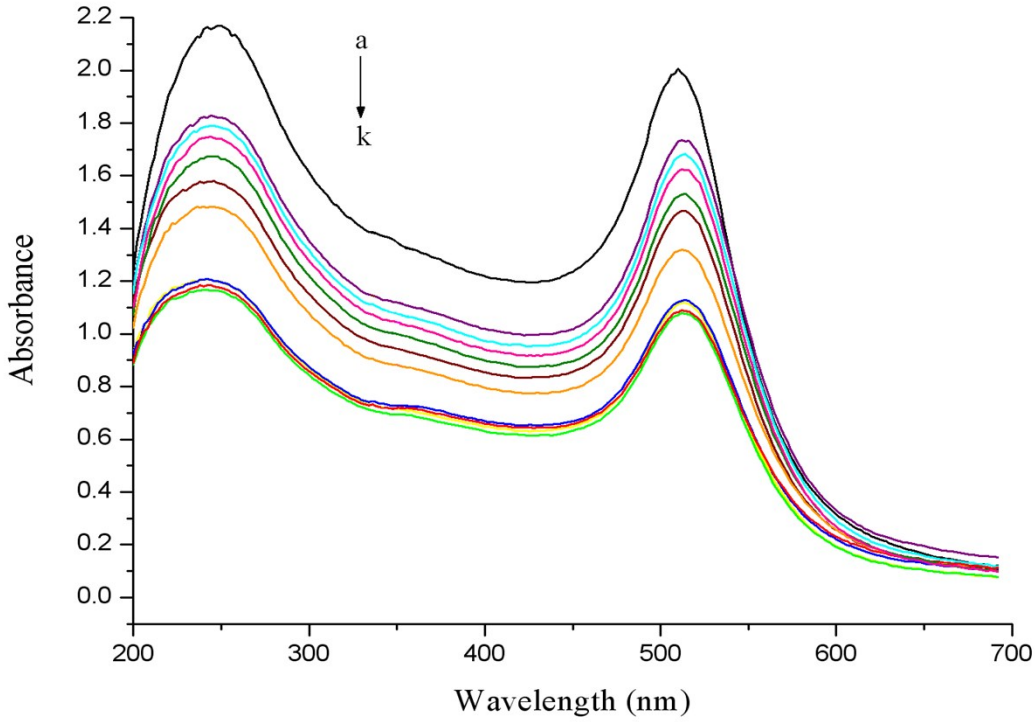

Figure S2. UV-Vis absorption spectra of AuNPs-MPA prepared by 3-MPA of different concentrations. (a, 0; b, 1.0×10^{-9}; c, 1.0×10^{-8}; d, 5.0×10^{-8}; e, 1.0×10^{-7}; f, 5.0×10^{-7}; g, 1.0×10^{-6}; h, 5.0×10^{-6}; i, 1.0×10^{-5}; j, 5.0×10^{-5}; and k, 1.0×10^{-4} M)

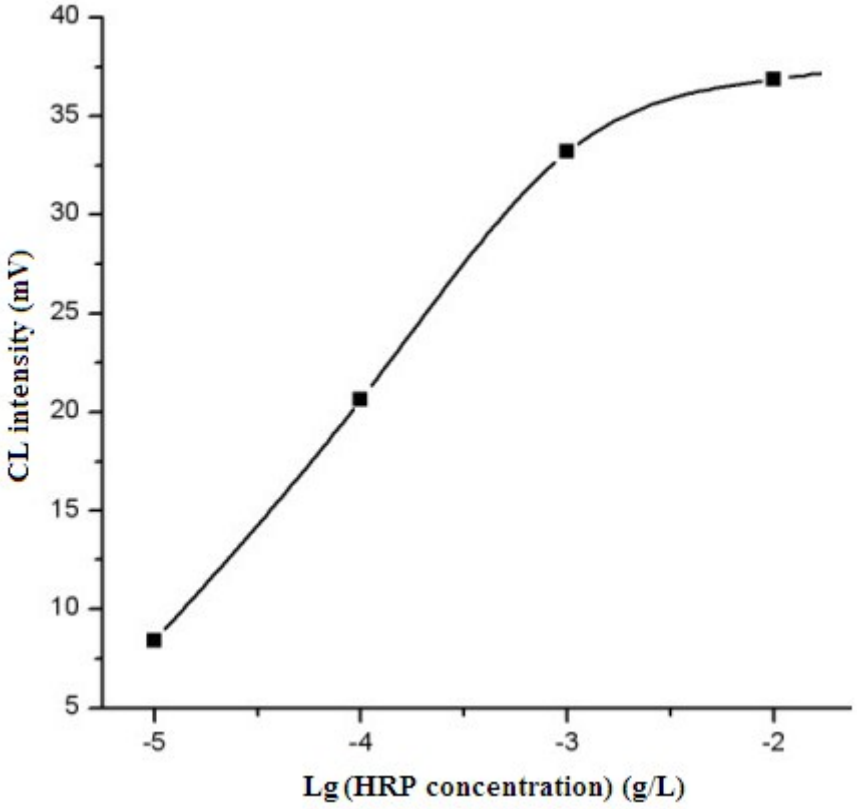

Figure S3. Influence of HRP concentration on the CL intensity.

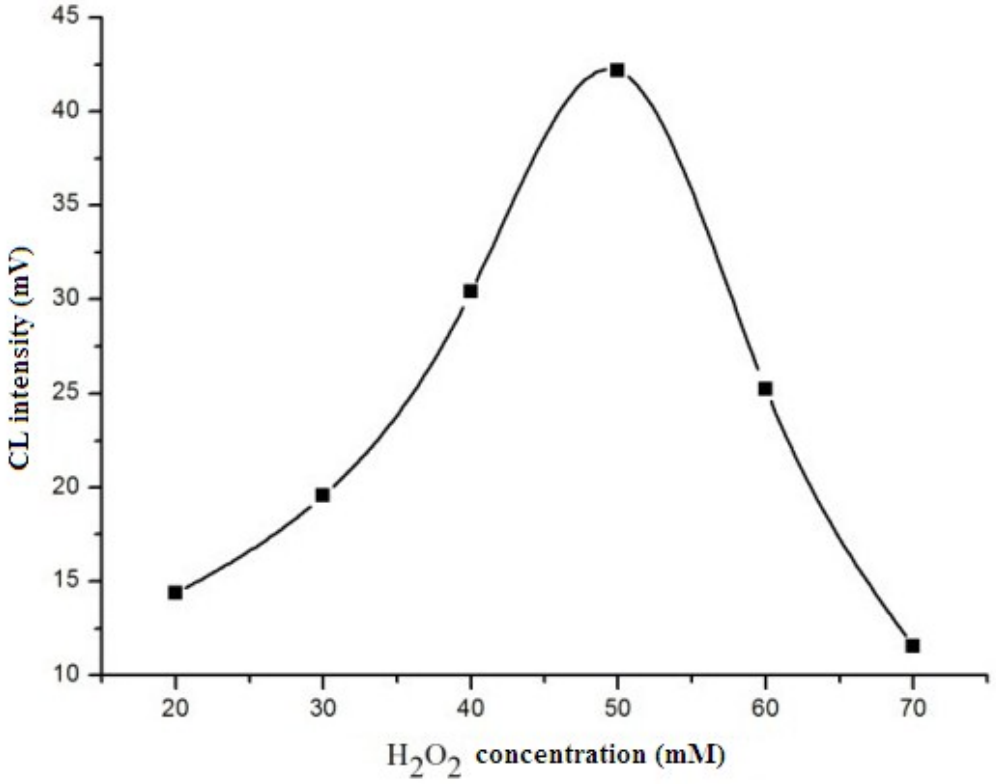

Figure S4. Influence of H_2O_2 concentration on the CL intensity

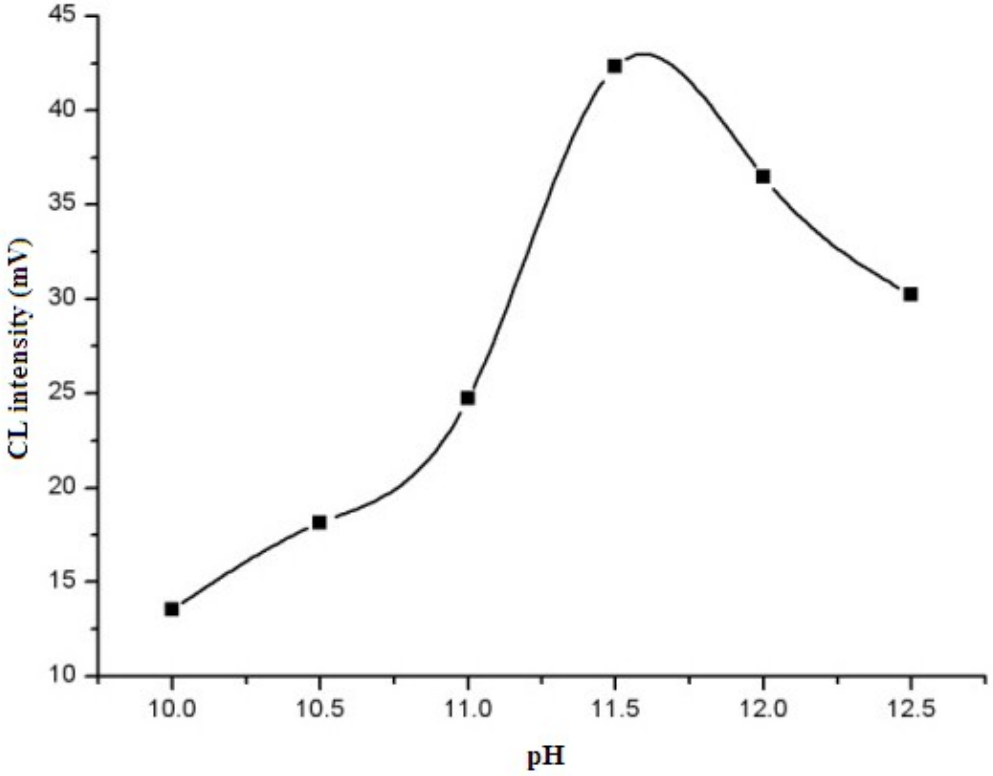

Figure S5. Influence of pH of post-column oxidizing reagent on the CL intensity.

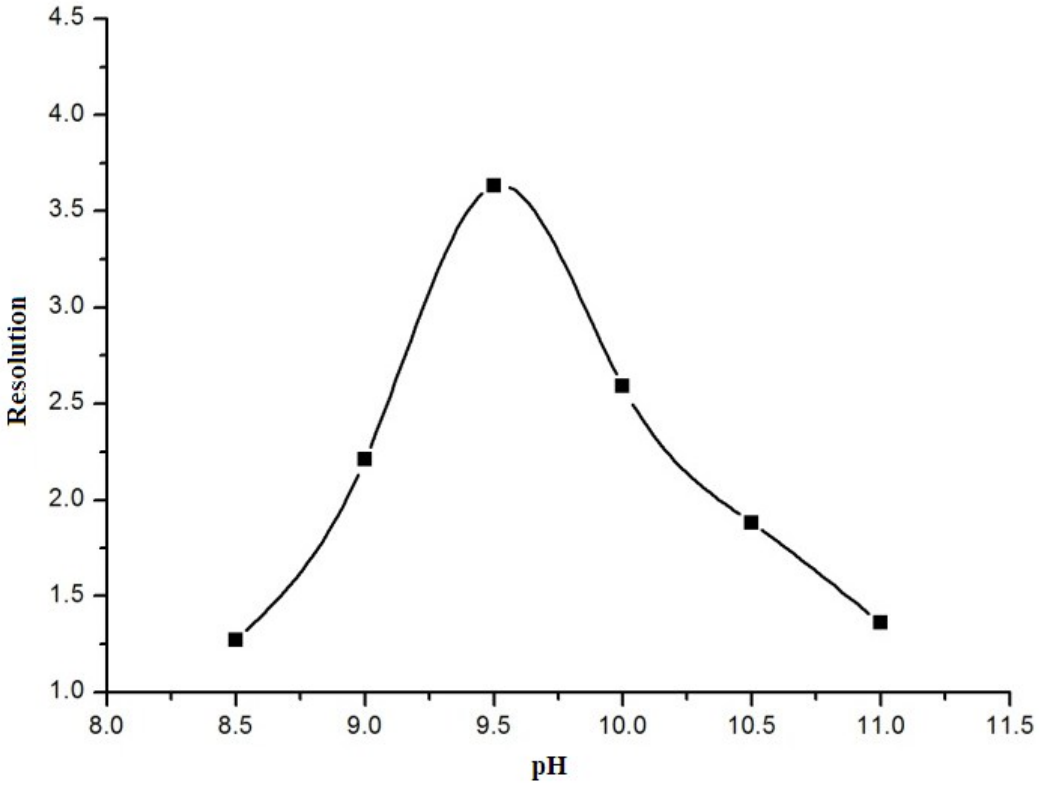

Figure S6. Influence of pH of the electrophoresis buffer on the resolution.

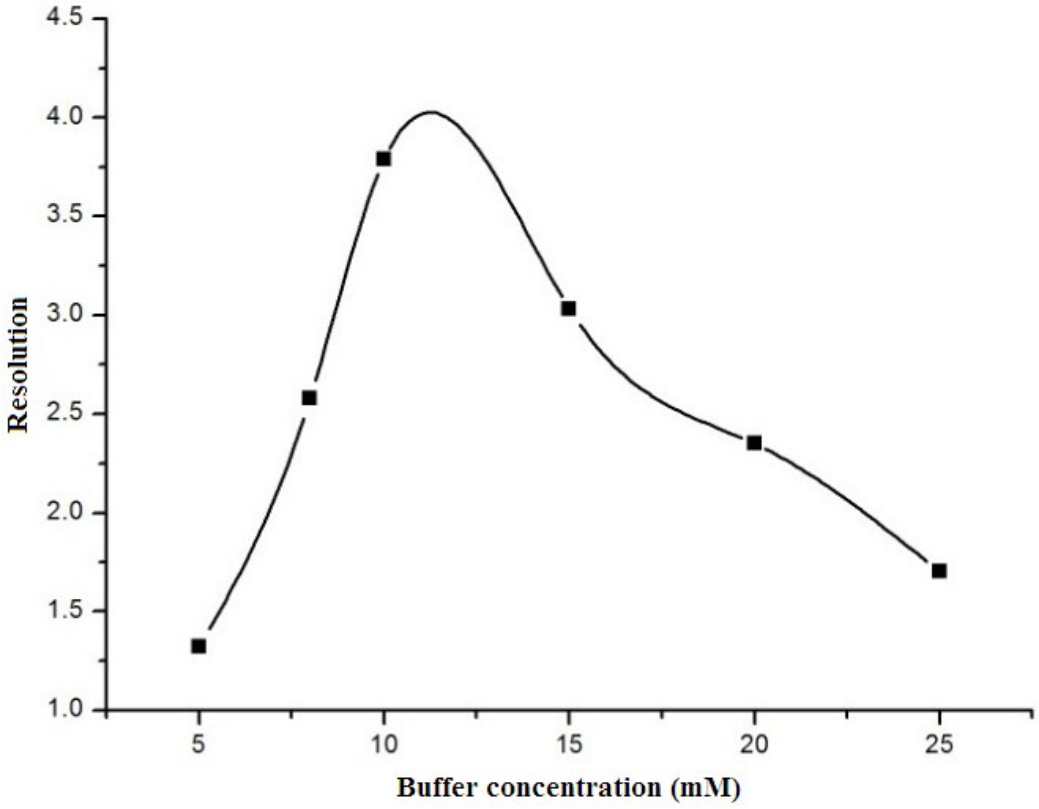

Figure S7. Influence of borax concentration on the resolution.

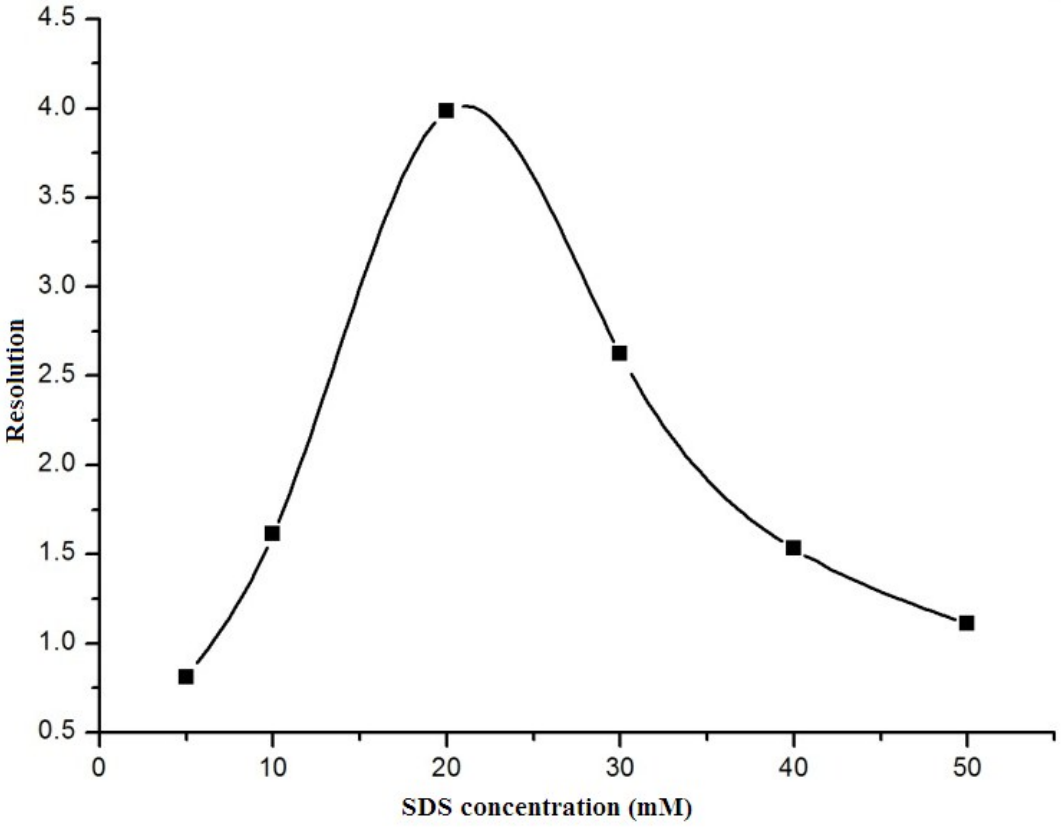

Figure S8. Influence of SDS concentration on the resolution.

Table S1 the sensitivity comparison of different methods for the detection of Te

Analysis method*	Linearity range	Detection limit	Reference
MCE-CL	0.576–57.6 ng/mL	0.288 ng/mL	1
CL-IA	57.6–5760 pg/mL	36 pg/mL	2
CE-LIF	3.70–2000 ng/mL	1.11 ng/mL	3
MIA-FD	2500 pg/ml	12 pg/ml	4
FD	——	1.47 pg/ml	5
ELISA	——	8.9 pg/mL	6
ED	300–40000 pg/mL	90 pg/ml	7
ED	5.0×10^{-3}–50 ng/mL	1.7 pg/mL	8
LC-MS/MS	10–2000 ng/mL	10 ng/mL	9
LC-ESI-MS/MS	1−5000 pg/mL	1 pg/mL	10
CE-CL-IA	0.2−10 pg/mL	0.11 pg/mL	This work

*MCE-CL, microchip electrophoresis chemiluminescence detection; CL-IA, chemiluminescence immunoassay; CE-LIF, capillary electrophoresis laser-induced fluorescence detection; FD, fluorescence detection; MIA-FD, microsphere-based duplex competitive immunoassay fluorescence detection; ELISA, enzyme linked immunosorbent assay; LC-MS; liquid chromatography-mass spectrometry, ED, electrochemical detection.

References

1. Y. Huang, M. Shi, S. Zhao and H. Liang, A sensitive and rapid immunoassay for quantification of testosterone by microchip electrophoresis with enhanced chemiluminescence detection. *Electrophoresis*, 2011, **32**, 3196-3200.
2. P. Luppa, C. Brückner, I. Schwab, S. Hauck, S. Schmidmayr, C. Birkmayer, B. Paulus and H. Hauptmann, Alpha-Biotinylated testosterone derivatives as tracers for a competitive chemiluminescence immunoassay of testosterone in serum. *Clin. Chem.,* 1997, **43**, 2345-2352.
3. H. X. Chen and X. X. Zhang, Antibody development to testosterone and its application in capillary electrophoresis-based immunoassay. *Electrophoresis,* 2008, **29**, 3406-3413.
4. M. Sun, J. Manolopoulou, A. Spyroglou, F. Beuschlein, C. Hantel, Z. Wu, M. Bielohuby, A. Hoeflich, C. Liu and M. Bidlingmaier, A microsphere-based duplex competitive immunoassay for the simultaneous measurements of aldosterone and testosterone in small sample volumes: Validation in human and mouse plasma. *Steroids,* 2010, **75**, 1089-1096.
5. S. H. Bao, Y. F. Peng, S. L. Sheng and O. Lin, Assessment of urinary total testosterone production by a highly sensitive time-resolved fluorescence immunoassay. *J. Clin. Lab. Anal.,* 2008, **22**, 403-408.
6. J. S. Mitchell and T. E. Lowe, Matrix effects on an antigen immobilized format for competitive enzyme immunoassay of salivary testosterone. *J. Immunol. Methods,* 2009, **349**, 61-66.

7. H. Lu, M. P. Kreuzer, K. Takkinen and G. G. Guilbault, A recombinant Fab fragment-based electrochemical immunosensor for the determination of testosterone in bovine urine. *Biosens. Bioelectron.,* 2007, **22**, 1756-1763.

8. M. Eguílaz, M. M. Guzmán, S. Campuzano, A. G. Cortés, P. Y. N. Sedeno and J. M. Pingarrón, An electrochemical immunosensor for testosterone using functionalized magnetic beads and screen-printed carbon electrodes. *Biosens. Bioelectron.,* 2010, **26**, 517-522.

9. D. Borrey, E. Moerman, A. Cockx, V. Engelrelst and M. R. Langlois, Column-switching LC-MS/MS analysis for quantitative determination of testosterone in human serum. *Clin. Chim. Acta,* 2007, **382**, 134-137.

10. M. S. Weinstock, B. L. Williamson, S. Dey, S. Pillai and S. Purkayastha, LC-ESI-MS/MS analysis of testosterone at sub-Picogram levels using a novel derivatization reagent. *Anal. Chem*., 2012, **84**, 9310-9317.