ABSTRACT
Oxytocin (OT) a clinically important nonapeptide was quantitatively determined from blood and pharmaceutical samples using Agilent 6460 LCMSMS. Angiotensin II and D4-Meperidine were used as internal standards. Using soft ionization technique a new transition for OT has been identified and used as a qualifier ion in the quantification.

INTRODUCTION
Introduction: Oxytocin (OT) is a cyclic nonapeptide (CYIQCNPLG-NH₂) with a wide variety of therapeutic applications including stimulating labor, control of post-partum hemorrhage and induction of lactation. Recent studies proved that, when administered unnecessarily or in large doses it acts as antidiuretic, even at therapeutic doses it is responsible for major shifts in blood distribution. It also causes subcutaneous vessel vasodilatation and splanchnic bed, plus coronary vessel vasoconstriction with a resultant drop in mean arterial pressure (MAP) while stimulating myocardial conductivity and heart rate [1]. On the other hand OT also demonstrated an inhibitory and amnestic action on learning and memory in different paradigms. Studies suggest the possible role of this neuropeptide in the regulation of drug abuse. Therefore, OT may act as a neuromodulator on dopaminergic neurotransmission in limbic-basal forebrain structures to regulate adaptive CNS process leading to drug addiction [2]. OT is usually administered as Intravenous (IV) infusion at a range of 0.02 units/mL (40 ng/mL) to 0.12 units/mL (240 ng/mL). Literature review suggests current analytical methods (HPLC-UV/LC-MS) have not established lower detection limits and easily reproducible transitions for the reliable quantitation and confirmation of OT in blood and pharmaceutical formulations (Pharms).

MATERIALS AND METHODS
Calibration standards were prepared in 0.9% NaCl (NS) from 10-200 ng/mL (0.005U to 0.1U/mL) by series of dilutions. Blood and plasma samples containing OT were diluted with NS; D-4 Meperidine for blood and Angiotensin II for plasma preparations were employed as internal standards. For blood sample analysis, negative blood with OT concentration below LLOQ were pooled and spiked with calibrators to create sample matrix. Samples were analyzed on Agilent 6460 quadrupole MSMS equipped with Jest spray ESI connected to Infinity 1260 LC. Separation was achieved on Agilent Zorbax Eclipse C-18 column (4.6X50mmX1.8) using gradient elution. Mobile phases of water:methanol (90:10 v/v) with 5mM ammonium formate (A) and acetonitrile with 0.1% formic acid (B) were used in gradient elution program; 30% B to 70% B over 2.5 min, returning to initial 30% B conditions over 0.5 min and held for 1 min for a total run time of 4 min with a flow rate of 0.4 mL/min. Data was acquired on MRM+ mode and mass transitions (1007.3 - 723.3/202.1/523.5 m/z) were observed for OT. Acquisition parameters were optimized using Agilent Optimizer software. Acquisition parameters and ion transitions were listed in table 1.

RESULTS & DISCUSSION
Surprisingly, the most intense peptide precursor is the singly charged species at 1007.3 m/z and not the expected double protonated ion at 504.2 m/z, which may be due to the fact that OT contains a disulfide bond that restricts peptide protonation [2]. Product ions 723.3 m/z was used as a quant ion and 550.1 m/z was used as qual ion for the quantification of OT. Calibration range of this method was shown to be linear (R² - 0.9969) from 10 ng/mL (0.005 U/mL) to 200 ng/mL (0.1 U/mL). LOD and LOQ were 5 ng/mL (0.0025 U/mL) and 10 ng/mL (0.005 U/mL) respectively. Analytical recovery (82-84%) and % CV for intra & inter assay (n=4) were within the acceptable ranges. No significant carry over (<2%) was observed at 200 ng/mL (0.1 U/mL).

CONCLUSION
We have developed a precise, sensitive and reproducible LCMSMS method for the quantitative determination of Oxytocin in blood and pharmaceutical formulations. The authors believe that this was accomplished with soft ionization of the OT peptide for the formation of reproducible MRM transition ions.

REFERENCES

**Table 1** Acquisition parameters for OT, Angio II and D4-Meperidine

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<th>Compound</th>
<th>Precursor</th>
<th>Product</th>
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<th>Frag</th>
<th>CE</th>
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</tbody>
</table>

ExperTox, Inc., Deer Park, TX

Fig 1 TIC of OT and Angiotensin II

Fig 2: Product ions Oxytocin and Angiotensin II (IS)

Fig 3: Calibration curve for OT (10-200 ng/mL)