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Extraction of oxytocin from human plasma using immunocapture techniques

Recently there has been a lot of talk about oxytocin – also known as the love hormone. With extremely low levels in human plasma of only a few pg/ml (read: finding an A4-sized paper in France) the detection of this hormone is very challenging.

Problems with detecting oxytocin

Oxytocin (OT) is a peptide hormone which is mainly involved during labour and lactation. It has also been found that OT is involved in several social behaviours like bonding and increasing trust and empathy in human. In addition, a possible link between OT and neurological disorders is being investigated (Zhang et al., 2011). This hormone is present in human plasma at extremely low levels of only a few pg/ml. In the pharmaceutical industry the use of lower doses of potent peptide drugs is becoming more and more interesting. Thus, this places higher demands on assays used to measure peptides at low pg/ml and therefore an efficient sample preparation is required. Immunocapture has proven to be an efficient sample clean-up technique for the analysis of proteins and peptides in biological samples using the established tool Liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Winther et al., 2009).

The main aims of this project were to explore different possibilities for the purification of OT from plasma samples using immunocapture techniques and to develop a quantitative assay for the quantification of OT in human plasma samples with high sensitivity using LC-MS/MS.

Proposed Solution

To purify OT from plasma, novel sample preparation methods like immunocapture techniques can be used. In this project, sample preparation with magnetic beads and pre-coated pipette tips were studied. These techniques are both based on antibodies and antibody-antigen interactions. To evaluate the efficiency of the immunocapture sample preparation, the techniques were compared not only to each other but also to solid phase extraction (SPE). SPE was the sample preparation technique used by the company - prior to this project. The magnetic beads used were different types of Dynabeads®. The Dynabeads® are used as a magnetic separation technology providing a consistent surface on which various bioreactive molecules e.g. antibodies can be adsorbed or coupled on. The principle of this method can be seen in figure 1.

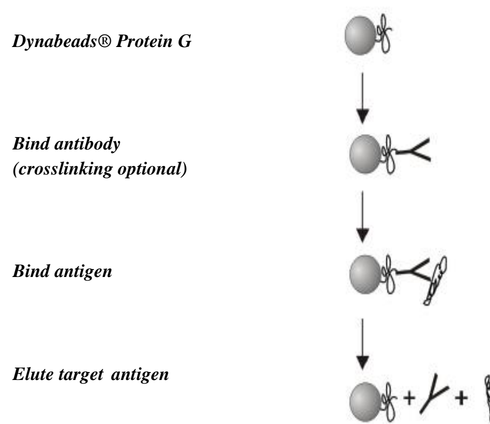

Figure 1. Principle of sample purification using Dynabeads® Protein G (left).

To other immunocapture method investigated was Mass Spectrometric Immunoassay (MSIA™) Pipette Tips. The procedure includes 3 main steps: loading of the affinity ligand, purification and elution of the target analyte. To reduce sample complexity, antibodies are immobilized on a microcolumn surface to purify target analytes. Biological samples, such as plasma, are directed through microfluidic channels to expose the target analyte to the antibody which captures the analyte with high selectivity and specificity. The purified target is then eluted from the microcolumn and ready for MS analysis. The MSIA D.A.R.T's™ were used manually together with the Thermo Scientific Finnpipette Novus i Multichannel Electronic Pipettes. The pipettes employ a repetitive cyclical pipetting motion with up to 999 aspirations in one cycle. This motion allows for coupling of antibody, sample purification and enrichment of the target analyte, elution as well as washing steps where the tips are rinsed. (Thermo Fisher Scientific Inc. , 2014).

All samples were analyzed using two LC-MS/MS systems – one single columned and one coupled columned. To evaluate the developed immunocapture technique, a qualification of the method was performed where the linearity, precision and accuracy were investigated.

Results

The results showed that the Dynabeads® Protein G together with a Millipore antibody worked best. Unfortunately, the MSIA™ method gave inconclusive results and therefore the focus was on improving the Dynabeads® method. To increase the recovery during optimization, parameters like elution, incubation time and sample volume were investigated. It was found that these parameters did not have a great impact on recovery but an increase in sample volume, without changing the eluate volume, did generate approximately a twofold increase in signal, making it possible to obtain a lower limit of quantification of 10 pg/ml. The final immunocapture method showed a good linearity in the concentration range of 10 to 100 pg/ml using seven calibration points. The precision and accuracy were according to international criterions and when comparing the final method with the SPE it was found that a much cleaner extract was obtained with the immunocapture method where endogenous OT was detected whereas no endogenous OT could be seen when using SPE. See figure 2.

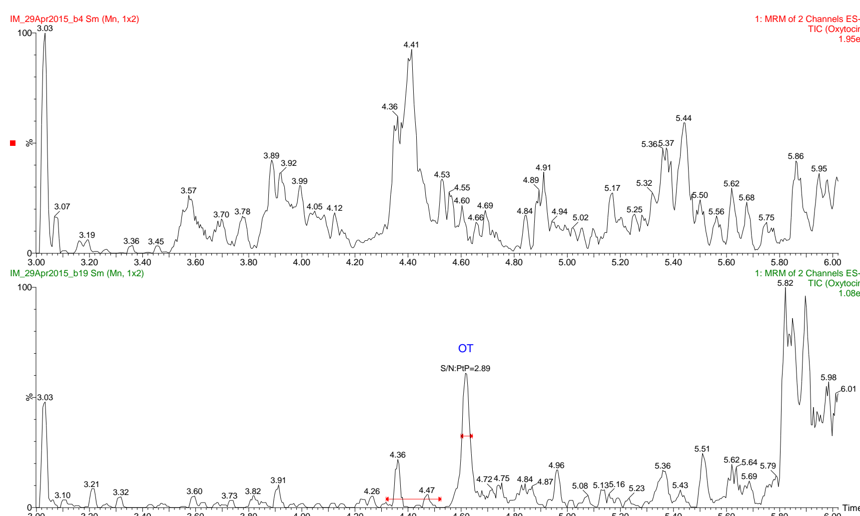

Figure 2. Chromatogram for the plasma blanks of the SPE(upper) - and immunocapture(lower) method respectively.

Conclusion

The approach to use immunocapture techniques for sample preparation prior to LC-MS/MS analysis was shown to have great potential for detecting OT at low concentration levels. The Dynabeads® was shown to be successful whereas the MSIA™ had higher demands on the antibody and the supply of antibodies on the market was unsatisfactory. The immunocapture method showing the most promising results were the Dynabeads® Protein G together with the monoclonal antibody MAB5296. Problems with low recovery was come across but to be able to quantify the method using a LLOQ of 10 pg/ml, the sample volume was increased to generating a two-fold increase in OT response. The qualification of the final method showed a good linearity, precision and accuracy. When comparing the developed method with the SPE method is was very clear that the immunocapture technique enabled a very pure extract where endogenous levels of OT was detected in blank plasma whereas OT levels of 10 pg/ml could not be distinguished from the background noise when using SPE. These observations show that immunocapture is a successful technique for extracting an analyte at very low concentrations in complex matrices such as plasma. The specificity towards the analyte enables a very pure extract to be analysed.

Future Prospects

For future research, a full validation of the final method should be conducted. Also, further optimization of the Dynabeads® would be interesting. A development of a pure presented monoclonal antibody with high specificity would be very satisfying, enabling a biotinylation and also the possibility to do a re-evaluation on the merits of the MSIA™ system. With MSIA™ an automation would be simple to implement whereas it would be more of a challenge, but still interesting to try to automate the Dynabeads® method for future use. Further, it would also be interesting to develop an on-line immunocapture method coupled to a LC-MS/MS system.

References

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