An Alternative Approach to Time-consuming Plasma Stripping for Endogenous Compounds: Application to Progesterone Determination by LC/MS/MS

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Purpose

It is always challenging to obtain an appropriate analyte-free matrix for the preparation of calibration curve for endogenous compounds. One of the most common approaches is charcoal-stripping. Though it is effective in many cases, the process is not only time-consuming, but also difficult to control (the extent of stripping). Moreover, some matrix components could be removed, reducing the similarity of the stripped matrix to a non-stripped one. The purpose of this research was to find a more efficient and easier-to-control-approach and to apply it to the quantitation of progesterone in human plasma by LC/MS/MS.

Methods

Calibration curves were prepared in H_2O-diluted human plasma (up to 10-fold) while QC samples were prepared in both diluted and undiluted plasmas. Supported liquid-liquid extraction (96-well format) with MTBE was used to extract progesterone from 200 µl of human plasma together with its deuterated internal standard. The extracted samples were injected onto an ACE3 C18-AR column (50×2.1 mm) for isocratic separation at a flow rate of 0.25 ml/min and with a run time of 2.8 min. Progesterone was eluted at 1.8 min using a mobile phase of H_2O/CH_3CN (35/65, v/v) and 4 mM ammonium acetate. The detection was carried out with an API 4000 triple quadrupole mass spectrometer with an electrospray source, operating in positive ionization mode with multiple reaction monitoring (m/z 315/109).

Results

Good inter- and intraday precision (≤5.4% CV) and accuracy (≤10.3% diff.) were obtained over the linear range of 0.2-160 ng/ml. The slopes of regression were comparable between normal and diluted matrices (<6% diff.). The mean extraction recovery was 66%. Neither matrix interference nor matrix effect was observed from normal matrix lots, or lipemic and hemolytic plasma, or other common over the counter medications. Progesterone was found to be stable in both diluted and normal plasma after 23 hr at room temperature, or three freeze/thaw cycles, or at least 12 days of storage at -20°C.

Conclusion

A new, fast, convenient, and widely applicable approach to obtain analyte-free matrix for the analysis of endogenous compounds was proposed and it has been successfully applied to the quantitation of progesterone in human plasma by LC/MS/MS.