Determination of Norgestrel and 17-Deacetylnorgestimate in Human Plasma by Methoxyamine Hydrochloride Derivatization Followed by Automated On-Line SPE with LC/MS/MS

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Introduction

Norgestrel (NG) and 17-deacetylnorgestimate (NGMN) are low active progestins. Analysis of NG is challenging due to low concentrations in plasma and medics ionization in LC MS calling either electrospray or APCI. Derivatization of NG with methoxyamine hydrochloride converts the latter group to a 17-methoxime group enhancing ionization in electrospray MS. NGMN, already containing a 2-hydroxy group is unaffected by the derivatization procedure. Ultimately, the derivatization procedure is carried out in a pyridine, considerably the ability to introduce the sample to SPE. Automated on-line SPE provides a simple and highly selective method of sample clean-up, allowing detection at relevant levels (40 pg/mL NG, 20 pg/mL NGMN) in human plasma.

Methods

On-line SPE Conditions: Condition: 1 mL methanol
Sample Extraction Volume: 0.25 mL
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SPE Cartridge: HySphere C2 SE (Spark Holland, The Netherlands)
Mass Transitions: NGMN: 350.05 > 128.45, 342.05 > 137.45
Internal Standards: Norgestrel-d6 and 17-deacetylnorgestimate-d6
Spark Holland Symbiosis SPE-LC system coupled to a Micromass Quattro Micro tandem and determination of Norgestrel and 17-Deacetylnorgestimate in Human Plasma by Methoxyamine Hydrochloride Derivatization Followed by Automated On-Line SPE with LC/MS/MS

Representative Chromatograms for NGMN and the NG - Methoxyamine Complex in Human Plasma

Plasma Blank

NGMN peak (342.05 > 137.45)

NG peak (335.05 > 128.45)

LLOQ

NGMN peak (342.05 > 137.45)

NG peak (335.05 > 128.45)

Conclusions

A robust, specfic and simple assay for the analysis of norgestrel and 17-deacetylnorgestimate in human plasma has been validated.

On-line SPE is an effective clean-up step in achieving sensitivity for methoxyamine hydrochloride derivatized norgestrel and 17-deacetylnorgestimate.

The method is suitable to quantify human plasma in pharmacokinetic studies.

Impact of Matrix Effect

Summary Table 1: Representative Chromatograms for NGMN and the NG - Methoxyamine Complex in Human Plasma

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