INTRODUCTION

The occurrence of steroids in aquatic environment and their effects on normal endocrine function in aquatic organisms have been subjects of current concern. Several studies have shown that also birds, reptiles and mammals in polluted areas undergo alterations of the endocrine-reproductive system.

At present, a multitude of chemicals have shown to be endocrine disrupters. Among these, natural and synthetic steroids are already effective at the lower ng/L. Their efficient detection in environmental waters is made possible nowadays thanks to numerous analytical approaches available in the literature. This is the case for estradiol (E2), estrone (E1), aldosterone (ALD) and androstenedione (AND) measurement. Estrone-3-sulfate (ES) has also to be considered because of its stability in the environment. But less papers are concerned with androgens, gestagens and their phase I and phase II metabolites (in particular E1).

Two analytical methodologies dealing with 29 steroids at low ppt levels, based on isotopic dilution are presented. The first one is dedicated to free steroids using two SPE preparation steps and a GC-MS/MS detection. The second method is focused on estrone-3-sulfate; identification relies upon 2002/67/EC decision to confirm unambiguously the steroid presence even at ultra-trace levels (<1 ng/L).

Analytical strategy for estrone-3-sulfate

Identification at ppt level (2 transitions monitored)

Blank sample

2 ppt spiked sample

Linearly 0-5 ppt range on spiked samples

CONCLUSION

Two validated analytical methodologies dealing with 29 steroids at low ppt levels, based on isotopic dilution are presented.

The first one is dedicated to free steroids using two SPE preparation steps and a GC-MS/MS detection. The acquisition is carried out in SIM mode on a Quattro Micro instrument (Waters, two transitions followed per compound). In this way, DHEA, 4-androsten-3-one, testosterone and their main metabolites (androstenedione, dihydrotestosterone, androstenedione), estrone, estradiol, ethinylestradiol, progesterone and various contraceptive gestagens are monitored.

The second method is focused on estrone-3-sulfate for which a specific combination between a SPE preparation and a LC-MS/MS detection (Carrimni column, Phenomenex, Agilent 6410 LC-MS/MS system) has been developed. Identification relies upon 2002/67/EC decision to confirm unambiguously the steroid presence even at ultra-trace levels (<1 ng/L).

These methodologies are currently used for the endocrine disruption monitoring in water organism in the field of the quadriennial National French Plan for Health - Environment (PNSE - Plan National Santé Environnement) which will be drawing to a close at the end of 2008.