Determination of tetracyclines in sewage sludge using high performance liquid chromatography combined with time of flight mass spectrometry

Christian Dye[1], Mikael Remberger[2], Lennart Kaj[2], Martin Schlabach[1]
1: Norwegian Institute for Air Research (NILU)
2: IVL Swedish Environmental Research Institute

Introduction

Tetracyclines are used as human infection medicine, veterinary medicine and as husbandry growth promoters. Generally, the human and animal body poorly absorbs the tetracyclines and 25-75% of the compound leaves the organisms by excretion. The use of sewage sludge and manure as fertilizers makes a potential for tetracyclines to reach soil and aquatic environments, and chemical analytical methodology is needed to monitor the environmental fate of the tetracyclines. Sewage sludge is a highly complex matrix with hundreds of organic compounds at high concentration levels, and the tetracyclines are present at relatively low concentrations. This is a challenging situation for the analytical chemist, and in this work a suitable analytical method is suggested.

Methodology

Extraction:

The solid samples were stored in polyethylene containers and frozen immediately after sampling. The frozen samples were thawed and spiked with meclocycline as internal standard. The sample extraction of tetracycline was performed by means of aqueous ion pair extraction ($MgCl_2$(aq)).

Instrumental analysis:

Liquid chromatography was performed with an Agilent 1100 liquid chromatography system (Agilent Technologies, Waldbronn, Germany), equipped with an autosampler, a quaternary pump, an on-line degassing system and a diode array detector (UV). The compound separation was performed with a reversed phase C18 column and gradient elution was performed with 0.075% formic acid acetonitrile and water. The analytical detector was a Micromass LCT orthogonal-acceleration time-of-flight (TOF) mass spectrometer (MS) equipped with a Z-spray electrospray ion source and a 4 GHz time to digital converter (TDC) (Micromass Ltd., Wythenshawe, Manchester, UK). The instrument was operated in positive electrospray. The data processing and instrument (HPLC/HRMS) control were performed by the MassLynx software, and quantitation was performed with signal extraction of a peak width of 90 mDa (typical).

Table 1: Molecular Ion Adduct and confirming ions.

Compound	MW	$\{M+H\}^+$	Confirming ion
Oxytetracycline	460	461	444
Tetracycline	444	445	427
Chlorotetracycline	478	479	481
Doxycycline	444	445	428
Demeclocycline	463	464	466
Meclocycline-ISTD	476	477	460

Results

The analytical method has been validated for sewage sludge, manure and sediments. The tetracycline recovery from spiked samples is in the range 70-105%. The methodological detection limits is in the range 0.1-1 ng/g (wet weight). The high resolution mass spectrometry is well suited for complex matrixes as sewage sludge, manure and sediments.

Acknowledgements:

Swedish Environmental Protection Agency.
Norwegian Research Council.

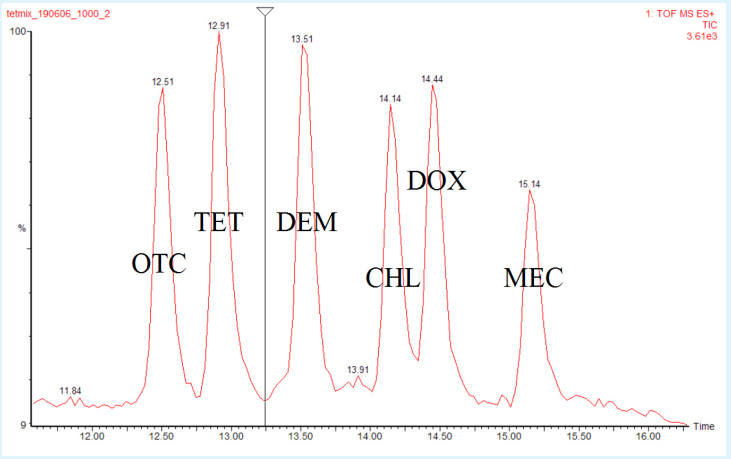

Figure 1: Total ion current chromatogram of a standard solution containing 1 mg/L of the individual tetracyclines Oxytetracycline (OTC), Tetracycline (TET), Demeclocycline (DEM), Chlorotetracycline (CHL), Doxycycline (DOX) and Meclocycline (MEC).

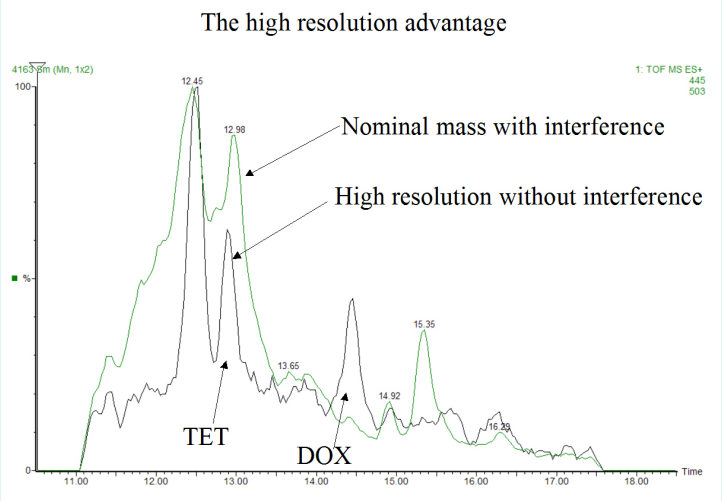

Figure 2: The high resolution mass spectrometry provides chromatograms which is significant cleaner than obtained by nominal mass spectrometry. This makes high resolution mass spectrometry well suited for complex matrixes as sewage sludge.

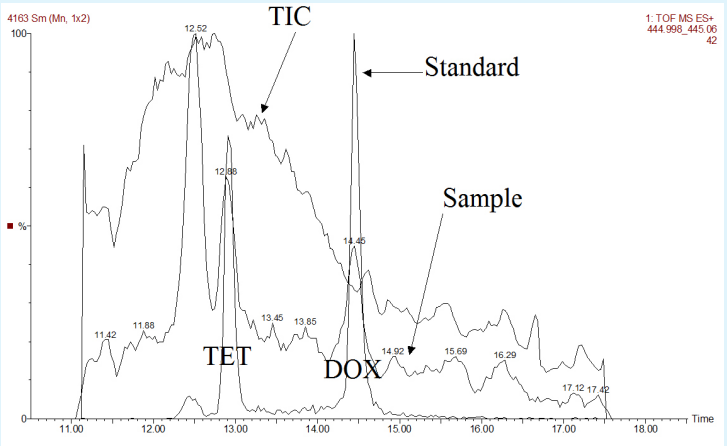

Figure 3: Chemical analysis of a STP sludge sample containing 487 ng/g (ww) Tetracycline (TET) and 242 ng/g (ww) Doxycycline (DOX).