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Review

Current trends in environmental analysis of human metabolite conjugates of pharmaceuticals

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ABSTRACT

Analytical techniques are necessary to facilitate the accurate identification and quantification of human pharmaceutical conjugates, which have the potential to comprise a significant hidden environmental load potentially rivaling those of the parent compounds. This review reflects the current trends in the processing and analysis of human pharmaceutical conjugates. The primary focus was to outline trends in environmental analytical chemistry. However, techniques involved in analysis of bile acid conjugates associated with biological fluids were included, as these provided insight into steroid conjugate analysis that may prove potentially applicable to conjugate analysis in the aquatic environment. Currently, sample collection is typically done by grab samples, and extraction from matrices is mainly achieved by hydrophilic–lipophilic balance cartridges. Reversed-phase liquid chromatography is by far the most common form of separation. The most common column choice was C18, with some inroads being made by the zwitterionic ion chromatography–hydrophilic interaction liquid chromatographic columns to take advantage of the polar moieties of conjugates for separation. The majority of studies used a binary gradient comprised of aqueous buffer and acetonitrile, which afforded good separation and preparation for mass analysis. Quadrupole-time-of-flight mass spectrometry was most commonly used for unknown conjugate identification. There is a noted increase in linear ion traps and high mass resolution mass spectrometers (e.g., Orbitrap™) for the identification and quantification of conjugates, and as such, some hybrid technologies are emerging. However, triple quadrupole instruments remain used for the greatest sensitivity and reproducibility for conjugate quantification. Moreover, the multi-faceted combination of quadrupole-time-of-flight and triple quadrupole will be of great value.

Contents

Abbreviations: E1, estrone; E2, 17β-estradiol; EE2, 17α-ethinylestradiol; E3, estriol; GC, gas chromatography; HLB, hydrophilic–lipophilic balance; HPLC, high performance liquid chromatography; HRMS, high-resolution mass spectrometry; HSS, high strength silica; LC, liquid chromatography; LOD, limit of detection; MCX, mixed-cation exchange; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; Q-LIT, quadrupole-linear ion trap; QqQ-MS, triple quadrupole mass spectrometry; Q-ToF, quadrupole-time-of-flight; QuEChERS, quick, easy, cheap, effective, rugged, safe extraction; RSD, relative standard deviation; SPE, solid phase extraction; UHPLC, ultra-high performance liquid chromatography; WWTP, wastewater treatment plant; ZIC-HILIC, zwitterion-hydrophilic interaction liquid chromatography.

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1. Introduction

Pharmaceuticals are designed to elicit strong biological responses at low doses in their recipient organism. Their presence in aquatic environments globally has led to concerns regarding the potential for adverse toxicological effects by these contaminants on non-target organisms (fish, invertebrates, microbial communities, plants, etc.) [1]. Human pharmaceutical conjugates typically have at least at least one polar moiety that enables the drug to be sufficiently soluble in the intestines and/or blood stream in order to be excreted. Upon human phase I biotransformation, these compounds can be hydroxylated to a more polar and water soluble compound, and be then subject to either phase II conjugation or phase III excretion. As a result, humans excrete both parent and metabolized drug, which enter aquatic environments via wastewater effluent and contribute to the pseudo-persistence of pharmaceuticals. WWTPs typically facilitate processes by which bacterial enzymes can either further biodegrade these parents and metabolites, or possibly deconjugate metabolites back into the parent compound [2,3]. In addition to biotransformation, abiotic processes (e.g., photolysis, hydrolysis) have the potential to transform parent compounds and metabolites into pharmacologically-active TPs that may pose a hazardous threat to aquatic biota found in surface waters receiving wastewater input.

Many pharmacologically active compounds, especially estrogens, have been found in various forms of WWTP: municipal, hospital, pharmaceutical manufacturing, and livestock [4,5]. Estrogen conjugates can comprise up to at least a third of the total estrogen load, in livestock waste lagoons from different concentrated animal feeding lots in the US [6]. High levels of 17α-estradiol are most likely due to transformation processes within the lagoon, given the minimal anthropogenic input of this chemical [6]. Many different forms of pharmaceutically active compounds (e.g., various forms of parent estrogenic compounds, as well as transformation products and conjugates) have been observed from toilet to holding tank to WWTP influent, to receiving waters [7].

Studies have reported the removal rates of personal care products and pharmaceuticals in activated sludge under both nitrifying (aerobic) and denitrifying (anaerobic) conditions [8,9]. After digestion treatment in WWTPs, the remaining sludge can be applied to agricultural fields, and the eventual fate of pharmaceutical conjugates upon such release becomes more complex. Groundwater chemistry and soil chemical reactions further convolute these processes. Sorption to particulates in soils and sediments, and especially in suspended organic matter within effluents and receiving waters can provide another reservoir of pharmaceuticals that could pose a threat to aquatic biota if they desorb and are made bioavailable [10]. This further supports the necessity to create analytical techniques that can accurately account for the conjugate inventory in both aqueous and solid phases.

The environmental significance of pharmaceutical conjugates is important. Conjugates may represent an under-reported reservoir of pharmaceuticals, which can be re-released by microbial deconjugation, and thus expose aquatic biota to toxicological effects of the parent compound. Indeed, transformation of pharmaceuticals can change biological effects markedly, as evidenced by the metabolism of some drugs to a more active form (e.g., demethylation of fluoxetine to norfluoxetine [11]). Moreover, certain TPs could actually be more toxic than the parent compound (e.g., acridine, a photoproduct of carbamazepine [12]). Moreover, metabolism of pharmaceuticals can change the propensity of these chemicals to undergo abiotic or biotic degradation, thus potentially affecting their persistence.

The pharmacological literature is beneficial in providing knowledge of the proportions of pharmaceuticals excreted as parent or metabolites. However, levels of conjugates are more difficult to predict given the potential mixture of conjugates, including multiply-conjugated moieties, in any number of proportions (e.g., singly or doubly glucuronidated or sulfatated or mixed conjugations and so forth). Therefore, robust procedures need to be developed to identify and quantify potentially dynamic amounts of conjugates in the environment.

One of the primary limitations in identifying and quantifying environmentally-relevant TP conjugate is the need for quality standards. There are limited commercially-available standards for analysis of conjugates across all drug classes. These are often costly. Synthesis of conjugates using liver microsomes or Supersomes™ [13,14] for analysis or subsequent use as standards is sometimes necessary.

The primary focus of this review is to critique current analytical techniques for measuring conjugates of human pharmaceuticals in environmental matrices, and to address the needs of identification and quantification of these compounds. The three main features of these techniques outlined in this review are: sample preparation and extraction, separation, and detection. Our aim is to elucidate common trends in the various techniques associated with these categories, to establish those most viable for future directions. Sample preparation and extraction is vital for environmental samples to measure analyte concentrations accurately and precisely (i.e., good recovery). Separation techniques to isolate conjugates from complex matrices are essential for accurate identification and quantification. The choice of instrumentation for mass analysis is especially important for the differentiation of some conjugates from their associated TPs that can arise through biotic and abiotic mechanisms.

2. Methodology for review

This review was compiled and contrasted by searching the literature using Academic Search Premier EBSCO Host, Science Direct CRKN-Elsevier, ProQuest Research Library, Taylor and Francis Library CRKN, CRKN Wiley Online Library, and Web of Science database search engines, for all journal entries published until late 2014, involving the analysis of pharmaceutical conjugates. It became apparent that the analytical techniques used in