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Challenge of high polarity and low concentrations in analysis of cytostatics and metabolites in wastewater by hydrophilic interaction chromatography/tandem mass spectrometry

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ABSTRACT

A method for solid phase extraction and HPLC–MS/MS of the cytostatics 5-fluorouracil, cytarabine, and gemcitabine and human metabolites uracil 1-β-D-arabinofuranoside and 2′,2′-difluorodeoxyuridine in wastewater was established. Wastewater samples from a Swiss hospital were analyzed for 5-fluorouracil, gemcitabine and 2′,2′-difluorodeoxyuridine. The limits of quantification were 5.0, 0.9, and 9.0 ng/L and the maximum concentrations detected were 27, 38, and 840 ng/L, respectively. Along with the method development, retention mechanisms on the hydrophilic interaction chromatography (HILIC) stationary phase were studied. Both partitioning and adsorption play a role in the retention on the tested sulfoalkylbetaine modified silica HILIC column material. The contribution of these two processes is changing over the 1.6–40% range water in the mobile phase. Although the specific break point is difficult to determine, adsorption becomes more significant as the fraction of water in the mobile phase decreases below approximately 16%.

1. Introduction

Ingested pharmaceuticals undergo transformations within the body and are subsequently excreted as some combination of the unchanged compound and its metabolite(s). Many pharmaceuticals have attracted the attention of environmental scientists and are studied for their ecotoxicological effects, removal efficiencies in wastewater treatment processes and occurrence in surface waters, sediment, soil along with ground and drinking water [1,2]. One of several pharmaceutical classes with the potential to have negative environmental effects are cytostatics [3,4]. They are used in cancer treatment as chemotherapeutic drugs designed to damage DNA, inhibit DNA synthesis, and interrupt cell replication. Cytostatics act unselectively on all growing cells and furthermore possess a carcinogenic potency which implies that no threshold values for lowest effect concentrations can be estimated. It is hypothesized that due to their mode of action, practically all eukaryotic organisms are vulnerable to damage, with teratogenicity being the greatest concern at predicted low ng/L levels [4]. Robust environmental occurrence data used for environmental risk assessments will either justify these hypotheses or prove them unfounded.

Cytostatics are divided into five classes according to the Anatomical Therapeutic Chemical Classification System (ATC): L01A alkylating agents; L01B antimetabolites; L01C plant alkaloids and other natural products; L01D cytotoxic antibiotics and related substances; and L01X other antineoplastic agents. Antimetabolites are the most widely used and according to their chemical structure and mechanism of action they are further classified as folic acid analogues, purine analogues and pyrimidine analogues. In this paper we focus on three antimetabolite pyrimidine analogues depicted in Fig. 1: 5-fluorouracil (5-Fu); cytarabine (CytR); and gemcitabine (GemC), as well as the respective human metabolites of cytarabine and gemcitabine: uracil 1-β-D-arabinofuranoside (araU); and 2′,2′-difluorodeoxyuridine (dFdU). The major urinary excreted human metabolite of 5-fluorouracil, α-fluoro-β-alanine (FBAL), suits with respect to its physical–chemical properties more GC–MS, as previously applied [5], than LC–MS analysis. Due to poor sensitivity on the employed instrumentation (over three orders of magnitude lower then required), was this metabolite not included in the developed method. The analytes were selected with regard to human excretion data [6–8], cytostatics consumption in the University Hospital Aachen, Germany (2003–2004), as well as available information on environmental occurrence and fate. While 5-fluorouracil has been previously analyzed in hospital wastewaters [9,10], and some environmentally relevant data for cytarabine and gemcitabine are available [11,12], to our best knowledge,

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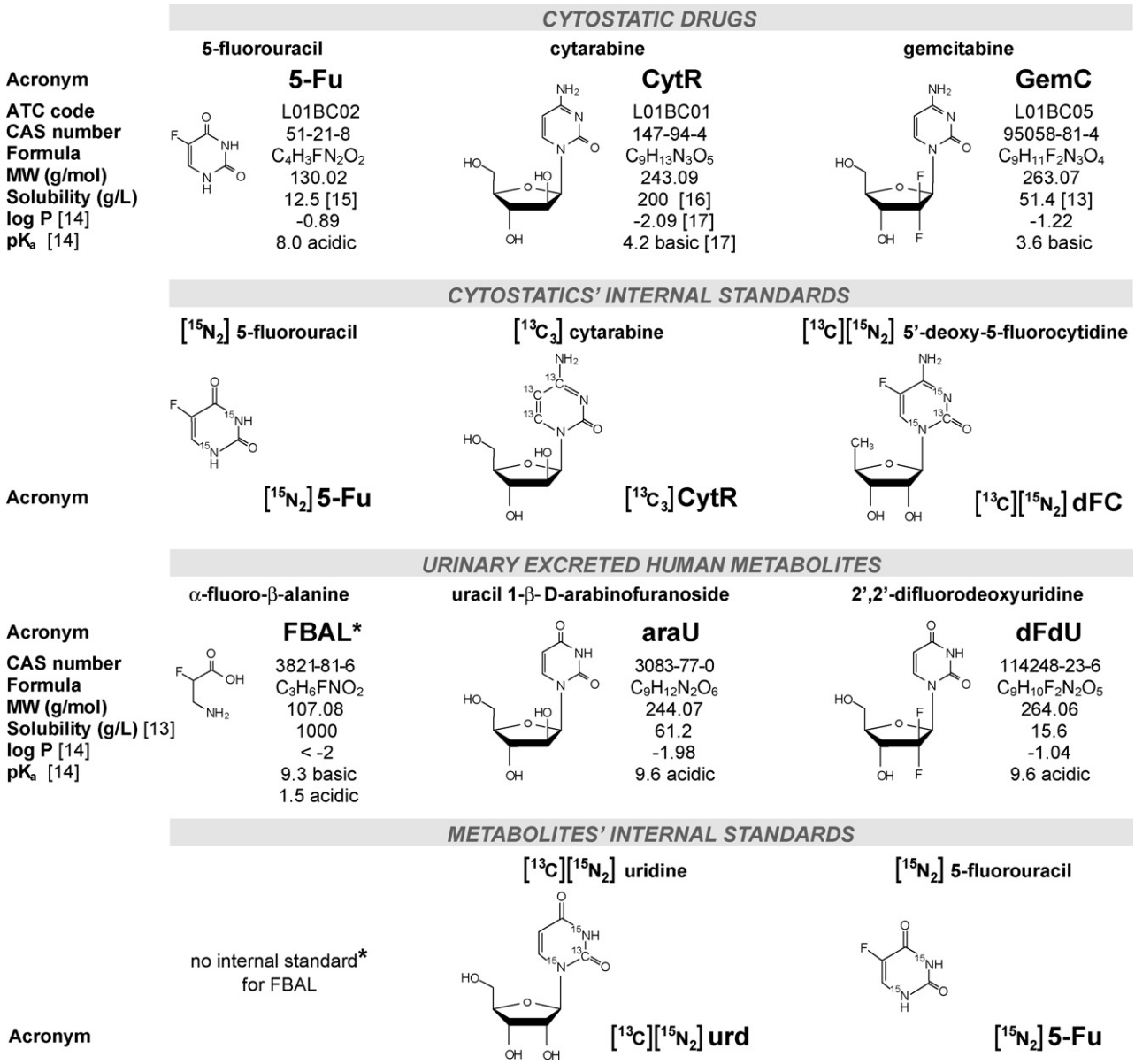

Fig. 1. Physical–chemical properties and labeled internal standards of the selected antimetabolites and their major urinary excreted human metabolites. (*) α-Fluoro-β-alanine was not included in the described analytical method. Data sources: estimated using EPI Suite v 3.20 software from US EPA [13]; estimated using ADME Boxes v 2.5 software from Pharma Algorithms [14]; and experimental values from Refs. [15–17].

the human metabolites of the three cytostatics have not yet been studied in the environment.

The options for possible retention principles for solid phase extraction (SPE) and high performance liquid chromatography (HPLC) are predetermined by retention principle selection and depend on the physical–chemical properties of the analytes, listed in (Fig. 1). The analytes are highly polar, with the logarithm of the octanol/water partition coefficient for un-ionized solute (log P) between −0.89 and −2.09. Further, they are non-volatile and either acidic or basic compounds, but all uncharged at pH around 6.

For SPE it implies that the retention of all target compounds together with a single procedure at pH 6 is possible. This may be achieved using polymeric materials developed specially for retention of polar compounds (polymers with a hydrophilic monomer or chemically modified polymers) as has been previously reported for 5-Fu and CytR [9–11], or carbon-based sorbents. Alternatively, two different procedures with different sorbent materials and sample pHs could be exploited: an anion exchange sorbent for acids at high pH; and a cation exchange sorbent for bases at low pH. The pK_a values of analytes are not compatible with pK_a values of common weak ion exchange sorbents and only strong ion exchange materials can be considered.

HPLC is nowadays a mature analytical technique, nevertheless its development has generally mainly focused on reverse phase (RP) chromatography of non-polar analytes, effectively retained on non-polar stationary phases such as C18. HPLC retention of highly polar molecules, like the analytes we describe in this paper, on reverse phase materials is by definition not effective. If the octanol/water partition coefficient of the molecule (P) is smaller than 1 (or $\log P < 0$), there is not much driving force for partitioning of a polar molecule from the polar aqueous mobile phase to the non-polar stationary phase. As a consequence, polar molecules pass through the non-polar stationary phase with little or no retention, unless they are retained by a secondary interaction, e.g. aromatic interaction. Chromatography on reversed phase materials modified for retention of polar compounds was previously employed