**Development of an SPE-UPLC-MS/MS method for the determination of 13 cytostatics in water**

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Anticancer drugs are classified into various categories, mainly including cytotoxic, cytostatic and hormonally active compounds. Even though cytostatics have an increased pharmacological effectiveness, they also present secondary side effects and considerable health risks mainly cytotoxic, genotoxic (mutagenic), carcinogenic, embryotoxic and/or teratogenic effects. Cytostatics and their human metabolites enter the environment through discharge of untreated or partly degraded wastewater/hospital effluents, eventually reaching surface or groundwater resources. Since they can induce hazardous side effects, these compounds are placed among the upcoming emerging issues in water system, deserving immediate attention.

In this study, a fast, sensitive and robust analytical method was developed and optimized, for the simultaneous determination of 13 cytostatics with various physicochemical properties, in aqueous matrix, using LC-ESI-MS/MS. The selected compounds included 5-fluorouracil, methotrexate, paclitaxel, irinotecan, doxorubicin, epirubicin, etoposide, oxaliplatin, temozolomide, capecitabine, gemcitabine, cyclophosphamide and docetaxel.

Chromatographic separation is performed on an Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) by Waters maintained at 30 ◦C. The mobile phase composition consisted of binary mixtures with 0.1% of formic acid in ultrapure water (A) and 0.1% formic acid in methanol (B) using a gradient elution. The flow rate was set at 0.3 mL min−1 and 10 μL were injected. Tandem MS operating parameters (ESI mode) were selected for optimum and unambiguous identification of the analytes, using selected reaction monitoring (SRM) transitions per compound, by a triple quadrupole detector (TqDetector, Acquity Waters, USA). All compounds were identified in positive ESI mode except for 5-fluoracil which was detected in negative mode. For isolation and preconcentration of the selected compounds, several SPE materials (MCX, C18, graphitized carbon and HLB) were tested in an automated off-line solid-phase extraction assembly, in order to achieve maximum recovery and sensitivity.

The efficiency of SPE methods was tested for a number of parameters, such as cartridge material, sample volume, pH of initial sample and different elution solvents. Chromatographic separation in positive mode was achieved in 23 min. The two selected ion transitions as well as the retention time for each compound are given as follows: 5-fluorouracil (negative mode) 129>42, 86; 1.3 min, gemcitabine 264>122, 95; 1.6 min, temozolomide 195>138; 2.3 min, methotrexate 455>308, 175; 6.1 min, cyclophosphamide 261>140, 106; 8.8 min, irinotecan 587>124, 167; 9.2 min, etoposide 589>229, 185; 9.9 min, doxorubicin 544>130, 361; 10.6 min, capecitabine 360>244, 174; 10.7 min, epirubicin 544>361, 397; 10.8 min, paclitaxel 854>105, 286; 13.4 min, docetaxel 808>226/808>282; 13.8 min, oxaliplatin 396>134, 162; 18.7 min. HLB and MCX cartridges have achieved increased recoveries for most of the compounds, with adequate repeatability. The developed chromatographic methods were proved reliable and highly sensitive, able to be used for a routine analysis of the 13 cytostatics in drinking and surface waters at low detection limits.

Keywords: LC-MS/MS, SPE, cytostatics

Acknowledgements: The authors would like to thank the S. Niarchos Foundation for the provision of the analytical instrument LC-MS/MS.