Analytical method and MRM database development using online SPE - LCMSMS for screening anti-cancer drugs and metabolites in hospital's wastewaters and rivers.

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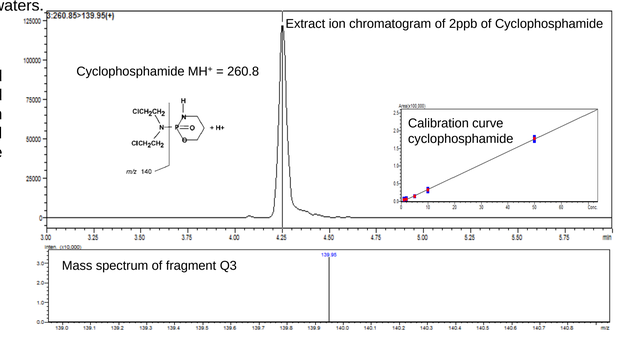
1: I Introduction

Over 50 cytotoxic chemotherapies are used in hospitals. The main anti-cancer drugs used in cancer chemotherapy can be classified into several categories: cytotoxic, the most represented, but also hormones, immune response modifiers and antibodies. Most cytotoxic agents used in cancer chemotherapy interact with DNA or its precursors. Very few studies are evaluating the future of these drugs in wastewater. Potential risks associated with these discharges are poorly understood and require study work and research to better understand the hazards, exposure characterization and assessment risks to human health and the environment. The purpose of the study is to establish an analytical methodology to screen most of the anti-cancer drugs currently used in hospital waste waters.

2: Methods

Water samples are processed by on-line solid phase extraction (SPE) to isolate and concentrate the different cytotoxics. Following extraction, compounds are transferred to an UHPLC column for separation. Detection is performed using Multiple Reaction Monitoring mode on an ultrafast triple quadrupole mass spectrometer. Special attention was given to the orthogonal selectivity and to the working pressure compatibility of the extraction and analytical columns.



3: Analytical Conditions

HPLC (UFLC system Shimadzu)

Column:	Phenomenex Kinetex XB-C18 ; 2.1 x 75 mm
Mobile phase A:	5mM ammonium acetate
Mobile phase B:	acetonitrile
Gradient program:	2%B (0 min) - 90%B (8-9min)
Flow rate:	0.25 mL/min
Column temperature:	40 C

Mass Spectrometer (LCMS-8030 TQ Shimadzu)

Ionization:	ESI
Polarity:	positive
Scan mode:	MRM

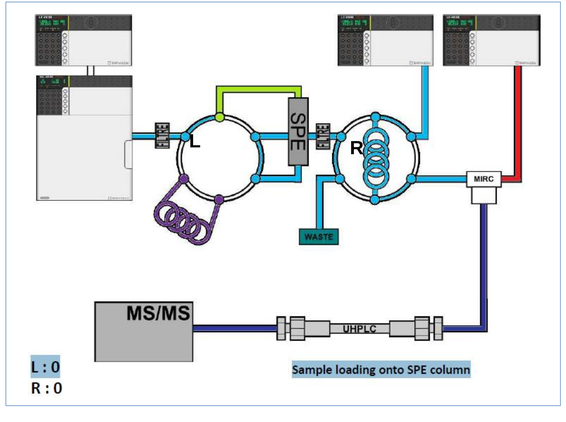
SPE online:

L:0 R:0 Sample loading onto the SPE column SPE column wash for polar interferences removal

L:1 R:0 Backflush of the SPE with the elution loop content

L:1 R:1 Transfert of the SPE content to the Storage loop

L:1 R:0 Transfert of the storage loop to the analytical column

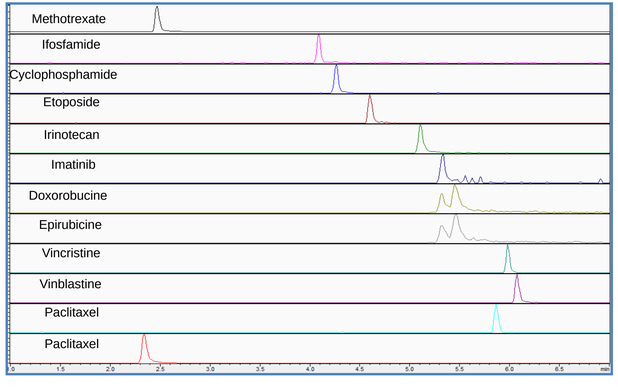


Validation:

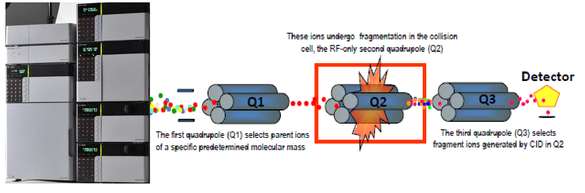
Analysis was validated using linear regression. Each calibration curve was constructed with triplicate of several concentrations rising from 1 à 100 ppb. The stability of stock solutions of the target compounds and the internal standards was evaluated at 4° C after 1 month.

Results:

In a first hand, we have optimized the chromatographic conditions to get enough resolution on the cytotoxics identical MRM transitions. In a second step we are developping the online SPE conditions for optimum recovery of each anticancer drug from spiked samples.



Fast UPLC-MS/MS of 12 anticancer drugs: the chromatogram is obtained after injection of 25 µl of calibration standard (10 ppb). Doxorubicine and epirubicine are quantified by the same MRM transition but identified by two different retention times.



NAME	TRANSITION 1	TRANSITION 2	RT	LOD
Methotrexate	455.00>308.15	455.00>175.10	2.506	
Ifosfamide	260.85>92.00		4.098	
Cyclophosphamide	260.85>139.95		4.271	
Etoposide	606.00>229.05	606.00>2	4.600	
Irinotecan	586.90>124.10	586.90>167.05	5.105	
Imatinib	494.35>394.10	494.35>217.10	5.317	
Doxorobucine	544.00>130.10	544.00>397.00	5.310	
Epirubicine	544.00>130.05	544.00>397.00	5.454	
Vincristine	413.30>392.20	413.30>	5.984	
Vinblastine	811.30>224.00	811.30>335.15	6.078	
Paclitaxel	854.10>286.10		5.989	
Dacarbazine	182.95>166.10	182.95>123.05	2.390	

Conclusion:

This method displays a direct online analysis of wastewaters for their contents in anticancer drugs. The fastness and the sensitivity of the UPLC-MRM approach using the LCMS 8030 triple quadrupole coupled to a small particles Core-Shell column enables an analysis of twelve cytotoxics in less than 10 minutes. This method will be extend to the analysis of the metabolites of these drugs and tested to evaluate the content of anticancer drugs from wastewater samples .

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