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LC-MS/MS for screening and quantifying anti-cancer drugs and metabolites in waste water rejected in Mediterranean sea

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Thérèse Schembri[1]; Mikael Levi[2]; François Gray[1]; Stéphane Moreau[2]; Claude Villard[1]; Pierre Boissery[3]; Daniel Lafitte[1]

[1]Aix Marseille Université, Inserm UMR 911 CRO2, Marseille, France; [2]SHIMADZU France, Noisiel, FRANCE; 3Agence de l'Eau Rhône Méditerranée & Corse, Marseille, France

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1. 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 were separated using off-line solid phase extraction (SPE) to isolate and concentrate different cytotoxic chemicals. Following extraction, compounds were transferred to a UHPLC column for separation. Detection was performed using Multiple Reaction Monitoring (MRM) mode on an ultrafast triple quadrupole mass spectrometer.

2-1. Samples Collection

Waste Water Rejected was collected during a RINBIO* survey campain (July-2012) in the rocky inlet of Cortiou (South Coast of France), where effluent from Marseille wastewater treatment plant are discharged.
Analytes free sea water samples (Blank Matrix) were collected near the shore of Sausset les Pins (Carry le Rouet , France).
Theses samples were filtrated (0.45 µm) and stored at -20°C until the date of analysis.
*Biological Integrators Network

2-2. Samples Pretreatment

SPE cartridge : Phenomenex STRATA-X 33 µ, 200 mg/6 mL
Activation Step : 5 mL of MeOH, then 5 mL of TFA 0,02% in H_2O mQ.
Sample Loading : 500 mL of Water Sample (acidified 0,02% TFA)
Washing Step : 5 mL of TFA 0,02%
Elution Step : 1 mL of MeOH
Sample concentration step: Eluate is dried out under N_2 flow, then reconstituted in 200 µL of MeOH.

Before injection, sample is diluted ten time in 0.02% TFA.

2-3. Analytical conditions

UHPLC (NEXERA System Shimadzu):
Column : Phenomenex Kinetex XB-C18, 2.1 × 75 mm
Solvent A : 10 mM Ammonium Acetate
Solvent B : 0.05% Formic Acid in MeOH (v,v)
Flow rate : 0.6 mL/min
Oven Temperature : 55°C
Gradient program : 5% B (0 min), 35% B (0.01 min) , 70% B (3 min), 90% B (4-5 min)
Injection Volume : 10 µL
Mass Spectrometer (LCMS-8030 TQ Shimadzu):
Ionization : ESI positive
Scan Mode : MRM

3. Validation and Drugs Quantification

Instrumental Detection Limit (IDL) was defined as the lowest amount of cytoxic prepared in standard conditions (id:TFA 0,02%/MeOH, 9:1) detected with S/N > 3 .

Limit of Quantification (LOQ) was defined as the lowest concentration detected after extraction with S/N>10.

For the 13 selected drugs, external calibration curves were constructed with spiked Blank Matrix fortified at 5, 10, 20 and 40 ng/L. For each level, a Recovery Surrogate was prepared by spiking the corresponding level in Blank Matrix after extraction.

Analysis were performed in triplicate and validated with linear regression.

The Fig. 1, shows the experimental curves recorded for cyclophosphamide.

Method Recovery was determined by comparing compounds peak area between spiked samples and Recovery Surrogates.

Matrix Effect was assessed by comparing peak area of the 40 ng/L Recovery Surrogate with a 40 ng/L Surrogate prepared in standard conditions.

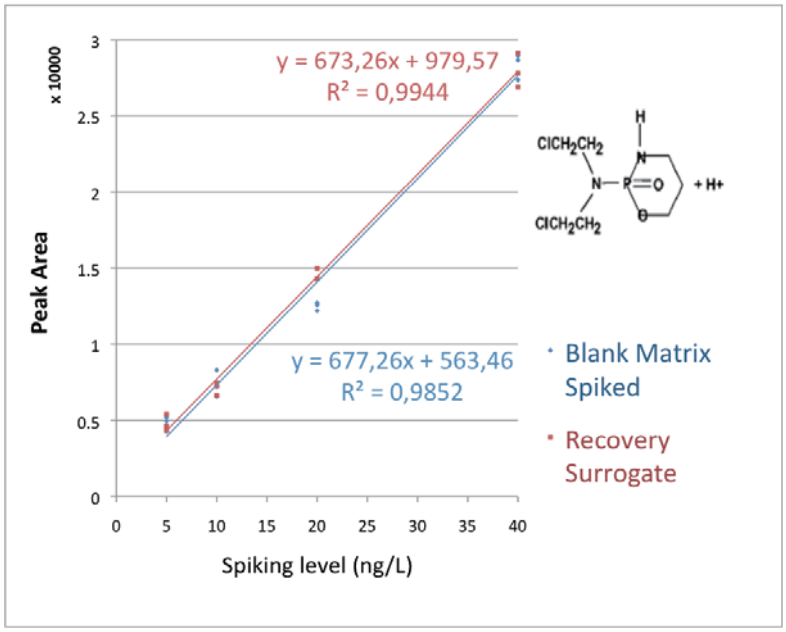

Fig. 1 Calibration curve of Cylophosphamide.

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4. Results

The first stage of the project was dedicated to the MRM transitions optimization. We underline the LCMS-8030 automated process efficiency, that had shortened this time consuming step development.

In a second time, we have optimized the chromatographic conditions to get enough resolution on the cytotoxics with identical MRM transitions (Doxorubicine and Epirubicine) and to schedule MRM. Fig. 2 shows the chromatographic separation of the selected drugs.

Then we developped SPE conditions to reach the maximum recovery for each compound. Those values are shown in Fig. 3.

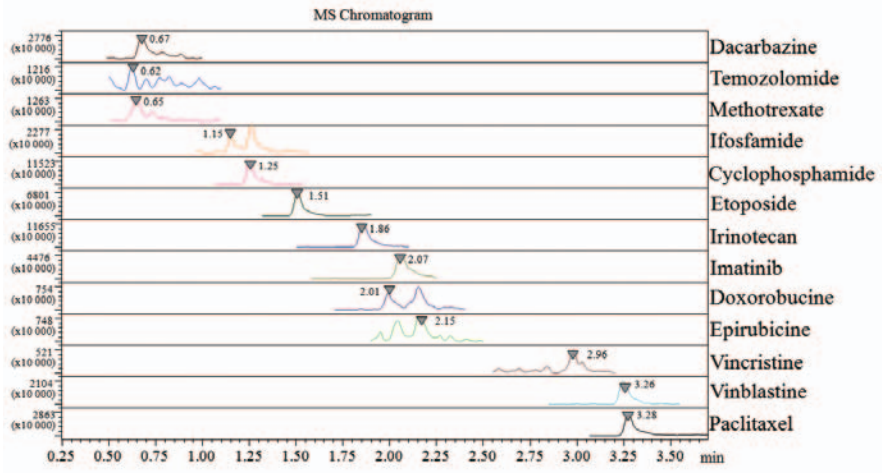

Fig. 2 Typical Chromatogram of spiked Blank Matrix (40 ng/L) after SPE

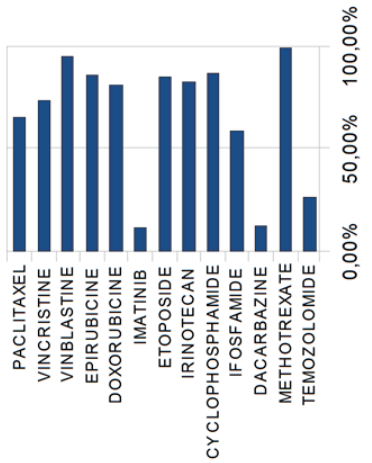

Fig. 3 Absolute Method Recovery

As part of the validation procedure, the described method was applied to the analysis of a wastewater rejected sample.

Fig. 4, displays the experimental chromatogram recorded.

Two of the screening compounds (Ifosfamide and Cyclophosphamide) were positively detected. These two cytoxics present low biodegradability[a] and their occurrence in aquatic environment were reported by other authors[b].

According to RT mismatch and the absence of the ion product (*m/z* 54.95), Temozolomide identification was not validated.

Experimental and Quantitative results are summarized in Table 1.

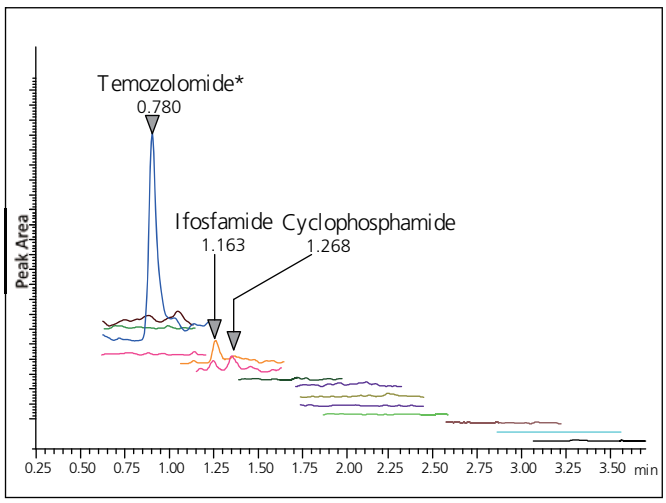

Fig. 4 Real sample Chromatogram

a) Kümmerer K et al, Biodegradability of the anti-tumour agent Ifosfamide and its occurrence in hospital effluents and communal sewage. War.Res. 1997, Vol 31, Issue 11

b) Removal of cytostatic drugs from aquatic environment: A review. J Zhang, Science of the total environment. 2013 Vol:445,-446, pp 281-96

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Table 1 MRM transitions, RT, IDL, LOQ and Real Sample Quantitative Results

Compounds	Transitions	RT (min)	IDL (injected pg)	LOQ (ng/L)	Concentration in Waste Water Rejected
Methotrexate	455.0> 308.15, 175.1	0.65	1	5	<IDL
Dacarbazine	182.95> 166.10, 123.05	0.67	1	5	<IDL
Ifosfamide	260.85> 92.0, 62.95 262.85> 92.05, 94.0	1.15	1	5	13,3 ng.L-1
Cyclophosphamide	260.85> 139.95, 63.0 262.85> 142.0, 120.05	1.25	1	5	<LOQ
Irinotecan	586.9> 124.10, 167.05	1.86	1	5	<IDL
Etoposide	606.0> 229.0 589.0> 229.05	1.51	1	5	<IDL
Imatinib	494.35> 394.1 ,217.1	2.06	2.5	5	<IDL
Doxorubicine	544.0> 130.1, 397.0	2.01	2.5	5	<IDL
Epirubicine	544.0> 130.05, 397.1	2.15	1	5	<IDL
Vinblastine	811.3> 224.0, 355.15	3.26	2.5	5	<IDL
Vincristine	825.4 >765.3 413.4> 353.1, 362.1	2.96	2.5	5	<IDL
Paclitaxel	854.1> 286.1	3.28	1	5	<IDL
Temozolomide*	194.9> 137.9, 54.95	0.62	1	15	not confirmed: RT mismatch transition 194.9>54.95 missing,

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5. Conclusions

This method displays a rapid analysis of wastewaters for their contents in anticancer drugs. The fastness of the UPLC-MRM approach using the LCMS 8030 triple quadrupole enables the simultaneous analysis of thirteen cytotoxics in less than 8 minutes with a ppt level sensitivity.

This method will be extended to the analysis of the metabolites of these drugs and adapted to evaluate the content of anticancer drugs in other matrices like mussels extracts.

1) PIT2, CRO2, Faculté de Pharmacie, Aix-Marseille University, Marseille, France
2) SHIMADZU France, le Luzard 2,Bd Allende, 77448 Marnes la vallée
3) Agence de l'eau Rhône Méditérannée & Corse, 2-4 allée de Lodz 69363 Lyon France
Contact: daniel.lafitte@univ-amu.fr

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