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Simultaneous quantification of reparixin and paclitaxel in plasma and urine using ultra performance liquid chromatography-tandem mass spectroscopy (UHPLC–MS/MS): Application to a preclinical pharmacokinetic study in rats

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ABSTRACT

A liquid chromatography-tandem mass spectroscopy (LC–MS/MS) assay was developed and validated to simultaneously quantify anticancer drugs reparixin and paclitaxel in this study. The compounds were extracted from plasma and urine samples by protein precipitation with acetone (supplemented with 0.1% formic acid). Chromatographic separation was achieved using a C18 column, and drug molecules were ionized using dual ion source electrospray and atmospheric pressure chemical ionization (DUIS: ESI-APCI). Reparixin and paclitaxel were quantified using negative and positive multiple reaction monitoring (MRM) mode, respectively. Stable isotope palcitaxel-D5 was used as the internal standard (IS). The assay was validated for specificity, recovery, carryover and sample stability under various storage conditions; it was also successfully applied to measure drug concentrations collected from a pharmacokinetic study in rats. The results confirmed that the assay was accurate and simple in quantifying both reparixin and paclitaxel in plasma and urine with minimal sample pretreatment.

1. Introduction

Substantial evidence has now demonstrated that many cancers, including breast cancer, are regulated by a small population of cells that possess characteristics of stem cells, subsequently also known as cancer stem cells (CSCs). CSCs are responsible for the development, resistance, metastasis and remission of cancers [1,2]. Breast CSCs (BCSCs) are resistant to both radiation therapy and chemotherapy due to protection from specific resistance mechanisms [3]. Conventional therapies are capable of eliminating bulk tumor cells, however, they are often unable to eradicate BCSCs. Gene expression profiling of BCSCs had revealed overexpression of CXCR1, a receptor for proinflammatory chemokine CXCL8 (or IL-8) [4]. CXCL8 has been implicated in the metastasis and poor prognosis of multiple malignancies, including glioma, prostate, breast and ovarian cancers [5].

Reparixin (RPX), [(*R*)(−)-2-(4-isobutylphenyl)propionyl methansulfonamide], is a chemical derivative of phenyl propionic acid developed by Dompe Farmaceutici S.p.a; it is a selective, small organic inhibitor of CXC ligand 8 (CXCL8) [6]. *In vitro* and *in vivo* studies have all indicated that reparixin was capable of eliminating $CXCR1^+$ cells, yet with lower toxicity to bulk tumor cells [7]. This supported the rationale for a combination therapy, where a conventional anticancer compound (e.g., paclitaxel, PTX) is co-administered with reparixin to simultaneously target both bulk tumor cells and cancer stem cells. Paclitaxel and reparixin are both highly lipophilic in nature and possess formulation challenges, for example, poor water solubility, inconsistent stability, short systemic circulation, off-target effect, and undesirable biodistribution. To achieve better chemotherapy efficacy, it is essential to monitor pharmacokinetic and pharmacodynamic characteristics of both drugs in blood and urine after intravenous administration. A convenient and accurate assay to simultaneously quantitate both PTX and RPX in biological matrix would be desirable and helpful.

An analytical method for simultaneous quantification of PTX and RPX in plasma and urine has not been reported. Previously RPX in rat plasma was quantified using an HPLC-UV bioanalytical method [8]. HPLC assays for quantifying PTX in rat plasma possessed higher

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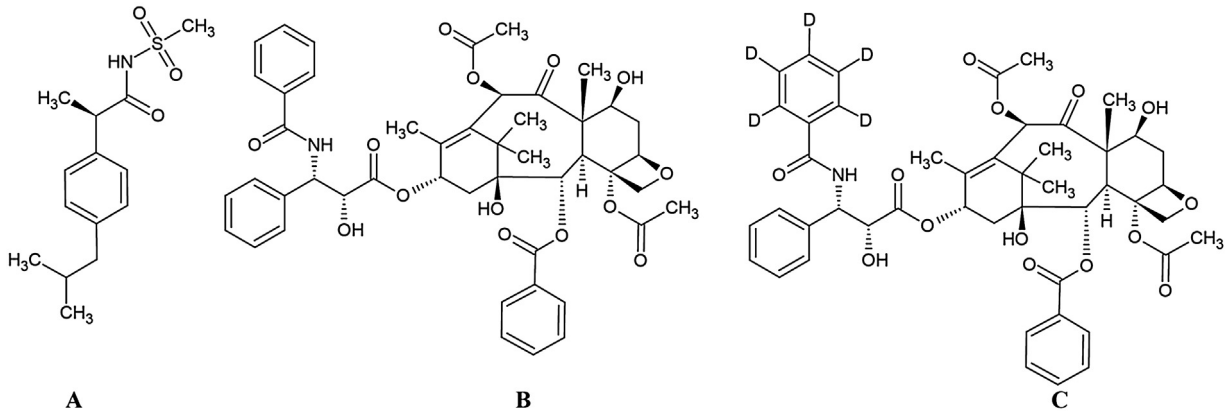

Fig. 1. Chemical Structure of reparixin (A), paclitaxel (B) and paclitaxel-d5 (c).

Table 1
MRM transitions and collision energies used for mass spectrometry analysis.

Molecules	Retention time (min)	Ion mode	MRM Transitions (Da)	Collision energy (eV)
Paclitaxel-D5	1.32	+	859.95 > 291.10	−20
Paclitaxel	1.35	+	854.40 > 286.10	−17
Reparixin	1.68	–	282.20 > 42.20	29

Table 2
Precision and accuracy of method in detecting reparixin and paclitaxel at the LLOQ concentrations.

	Matrix	Spiked Conc. (ng/ml)	Found Conc. (ng/ml)	R.S.D (%)	R.E. (%)
PTX	Plasma	4.88	4.84 ± 0.27	5.54	−0.20
	Urine	1.22	1.30 ± 0.08	6.15	6.56
RPX	Plasma	4.88	4.97 ± 0.17	3.42	1.84
	Urine	9.76	10.35 ± 0.54	5.19	6.05

Table 3
Inter-day precision and accuracy of developed UPLC–MS/MS method for detecting PTX and RPX in rats plasma and urine (Mean ± SD, n = 5).

		Inter-day			
		Spiked Conc. (ng/ml)	Measured Conc. (ng/ml)	R.S.D (%)	R.E. (%)
PTX	Plasma	600	610.46 ± 7.12	1.17	1.74
		300	307.48 ± 4.18	1.36	2.49
		150	152.29 ± 4.55	2.99	1.53
		75	77.60 ± 3.11	4.01	3.46
	Urine	600	588.51 ± 7.86	1.34	−1.92
		300	296.05 ± 6.29	2.12	−1.32
		150	158.97 ± 4.47	2.81	5.98
		75	72.85 ± 2.54	3.48	−2.86
RTX	Plasma	600	600.56 ± 9.40	1.57	0.09
		300	307.58 ± 13.37	4.35	2.53
		150	138.29 ± 8.54	6.18	−7.81
		75	76.69 ± 3.39	4.42	2.26
	Urine	600	589.38 ± 3.71	0.63	−1.77
		300	288.13 ± 4.30	1.49	−3.96
		150	151.79 ± 6.88	4.53	1.19
		75	71.94 ± 2.80	3.89	−4.08

LLOQ values [9,10], which demanded of larger plasma sample volume to achieve precise quantification. More recently LC–MS/MS assays measured the adduct formation (i.e. $[M+ Na]^+$ and $[M+ NH_4]^+$) for quantifying PTX in biological samples [11,12], which in itself was a challenge for acquiring a pronounced and reproducible formation of a single ion. Variations in the ubiquitous presence of sodium in samples, glassware, reagents and solvents make it even more difficult to optimize LC–MS/MS methods to achieve desirable linearity and reproducibility. An analytical method based on LC–MS/MS could certainly benefit drug analysis and monitoring because of higher sensitivity and lower sample volume. Therefore the aim of this study was to develop and validate a rapid and sensitive UHPLC–MS/MS assay to simultaneously quantitate PTX and RPX in plasma and urine samples. The developed assay was validated for accuracy, sensitivity and stability; furthermore the applicability of the method was tested by analyzing drug concentrations collected from a pharmacokinetic study of RPX and PTX co-administered intravenously in rats.