



Original article

Quantitative determination of doxorubicin in the exosomes of A549/MCF-7 cancer cells and human plasma using ultra performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

In cancer therapy, exosomes efflux enhances resistance of cancer cells toward anticancer agents through mediating the transport of anticancer drugs outside the cells. In this study, a rapid, simple and highly sensitive ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed and validated for the determination of Doxorubicin (DOX) in exosomes of cancer cells and human plasma using Ketotifen as an internal standard (IS). Plasma samples spiked with DOX and two cancer cell lines (A549 & MCF-7) were incubated with different concentrations of DOX and IS. The analytes were then extracted with methanol after protein precipitation and the chromatographic separation was carried out using a C18 column, with a mixture of acetonitrile–water–formic acid (85:15:0.1%, v/v/v) as mobile phase. Multiple reaction monitoring (MRM) was utilized to monitor the protonated precursor to product ion transitions of m/z 544.25 > 397.16 and m/z 310.08 > 96.97 for the quantification of DOX and IS, respectively. The method was linear over ranges of 1–1000 ng/mL for DOX in plasma and 2–1000 ng/mL for DOX in exosome samples. The lower limit of quantification of this method was 1 ng/mL, 2 ng/mL and 2 ng/mL in human plasma, A549 & MCF-7 cells respectively. Intra- and inter day precision of all quality control concentrations were less than 10.33% and the accuracy values ranged from –4.82 to 12.60%. The optimized UPLC-MS/MS method proved to be fast, specific, simple and highly sensitive and was successfully applied for the estimation of DOX in the exosomes of cancer cells and plasma. © 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Doxorubicin (DOX) is an anthracycline antineoplastic agent frequently used in the treatment of various types of tumors such as leukemia, breast, bone, lung, bladder, thyroid and stomach cancers (Duggan and Keating, 2011; Xiong et al., 2010; Czyz and Jakubowska, 2008). DOX binds to DNA topoisomerase enzymes

and intercalates between DNA base pairs causing irreversible DNA damage which inhibits DNA replication, transcription and topoisomerase activity leading to cell death (Laroche-Clary et al., 2000; Tacar et al., 2013). Chemotherapy resistance is one of the biggest challenges in cancer treatment. Exosomes can enhance the development of cancer resistance by promoting the drug efflux from tumor cells (Corcoran et al., 2012). Exosomes are nano-sized (30–90 nm) vesicles released from different types of mammalian cells (Filipazzi et al., 2012; Bobrie et al., 2011) and they were previously considered as garbage bags to dispose cellular waste. However, recent reports have shown that exosomes play a very important role in cellular communication and attracted a great interest after the discovery that they contain biological materials such as DNA, RNA, Proteins and lipids (Ekström et al., 2014). These biological materials can modify the function of other recipient cells. The exosomes released from the cancer cells, may result in

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proliferation of the recipient cells, angiogenesis, immunosuppression and resistance to chemotherapy (Filipazzi et al., 2012). Establishment of a rapid and sensitive method for the quantification of drugs effluxed in exosomes may increase our understanding of exosomes role in chemotherapy resistance and may open the avenues for finding agents that reduce exosome release from cancer cells and hence decrease their resistance to anticancer agents.

A range of analytical methods were previously developed to extract, separate, and quantify DOX and other compounds in different biological matrices using UPLC-MS/MS (Liu et al., 2008; Ahmed et al., 2009; Arnold, 2004; Kakkar et al., 2010; Ma et al., 2015a, 2015b; Dong and Xiao, 2017; Xian et al., 2016; Dong et al., 2018), however, none of the reported methods in the literature determined doxorubicin in the exosomes of cancer cells. Moreover, the majority of these methods had several drawbacks such as long analysis time, laborious extraction procedure, low sensitivity and high limit of quantifications (LOQ). Liu and co-workers developed LC-MS/MS method for the determination of doxorubicin in rat plasma with LOQ of 20 ng/mL (Liu et al., 2008). Although some methods were successfully developed and applied to quantify DOX in plasma with LOQ of 1 ng/mL [12], the sample preparation procedure was laborious, time-consuming and the retention time of doxorubicin was relatively long (Liu et al., 2008; Ma et al., 2015a, 2015b). Some published analytical methods employed liquid chromatography with fluorescence detection for quantification of DOX in mice plasma (Han et al., 2016) and DNA of tumor cells using liquid-liquid extraction (Lucas et al., 2016) with LOD of 5 and 10 ng/mL respectively. Other studies reported the determination of DOX in MCF-7/Adr cells using UPLC-MS/MS method (Ma et al., 2015a, 2015b); however, to the best of our knowledge, there are no published methods for the estimation of DOX in the exosomes of cancer cells. The aim of the present study was therefore to develop and validate a simple, sensitive and quick UPLC-MS/MS method to quantify DOX in both plasma and exosome samples.

2. Experimental

2.1. Materials and reagents

High purity (>99%) doxorubicin, 10-[(3-Amino-2,3,6-trideoxy- α -L-lyxohexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-5,12-naphthacenedione, Ketotifen fumarate, 4-(1-Methyl-4-piperidinylidene)-4,9-dihydro-10H-benzo[4,5]cyclohepta [1,2-b] thiophen-10-one, LC-MS-grade acetonitrile and formic acid were purchased from Sigma- Aldrich (St. Louis, MO, USA).

All aqueous solutions used in this study were prepared using Millipore purified water (Milli-QR Gradient A10R, 0.22 μ m, Millipore, Mosheim Cedex, France).

The human non-small cell lung cancer cell line (A549) & the human breast cancer cell line (MCF-7) were purchased from the American Type Culture Collection (ATCC) and were maintained in Roswell Park Memorial Institute Medium (RPMI-1640 Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA).

2.2. Cell culture and drug addition

The cancer cells were incubated at 37 °C in a humidified atmosphere of 5% CO₂. Mycoplasma was tested at 3 months intervals. Before the exosomes isolation, cells were incubated in medium supplemented with exosome-free serum before the experiment and during the whole procedure. Doxorubicin was added to the cells in different concentration (0.1 μ M, 1 μ M, 10 μ M and 100 μ M) for 2 h. The cells were washed with PBS and again incubated in medium supplemented with exosomes-free serum for 48 h.

2.3. Exosomes isolation

The exosomes isolation was done according to the method described previously by Zaborowski et al. (2015). The conditioned media was collected from the cells into the falcon tubes and was centrifuged at 2000g for 15 min at 4 °C. The pellet at the bottom was discarded and the supernatant was shifted to ultracentrifuge tubes by using syringe and centrifuged at 20,000g at 4 °C for 35 min by using Ti70 Beckman coulter's rotor. The supernatant was filtered after the centrifugation, by using filter of suitable pore size (0.2 μ m). The filtered media was transferred to ultracentrifuge tube with the help of syringe. The remaining pellet that contains debris or apoptotic bodies was again discarded. Finally, the heat sealed ultracentrifuge tubes were again centrifuged at 100,000g at 4 °C for 70 min. The pellet containing exosomes was marked and the tubes were kept on the ice after removing the supernatant.

The pellet was dissolved in 1 \times PBS (phosphate buffer saline) and stored at -70 C for further experiments.

2.4. Chromatographic conditions

The chromatographic analysis was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) equipped with a binary pump, online degasser, quaternary solvent manager, and auto-sampler with an injection loop of 10 μ l. The chromatographic separation was performed on Acquity UPLC BEH™ C18 column (50 \times 2.1 mm, i.d., 1.7 μ m, Waters, USA) by isocratic elution using mobile phase composed of acetonitrile: water: formic acid (85:15:0.1%, v/v/v) at a flow rate of 0.2 mL/min. The injection volume was 10 μ l and the column temperature was kept at room temperature.

2.5. Mass spectrometric conditions

A triple-quadrupole tandem mass spectrometer (MicromassW Quattro micro™ Waters Corp., Milford, MA, USA) connected with UPLC system through an electrospray ionization (ESI) interface operated in positive ionization mode was used for mass spectrometric analysis. MS analysis was carried out using multiple reaction monitoring (MRM) to monitor the protonated precursor to product ion transitions of m/z 544.25 > 397.16 and m/z 310.08 > 96.97 for the quantification of Doxorubicin and Ketotifen, respectively.

The dwell time was 0.2 s and nitrogen was used as a desolvating gas at a flow rate of 600 L/h. The ionization source conditions were as follows: desolvation temperature 350 °C; source temperature 150 °C; the collision gas (argon) flow 0.1 mL/min; and capillary voltage 1.45 kV. Compound dependent parameters like cone voltage and collision energy were set at 50 V and 15 eV for Dox. and 50 V and 21 eV for IS respectively. The parameters of mass analyzer were set as follows: LM1 and HM1 resolution 15 and 15 respectively; ion energy 1; LM2 and HM2 resolution 15 and 15 respectively, ion energy 2.

The UPLC-MS/MS system control was performed by Lynx software (Version 4.1, SCN 882) and data was processed and analyzed using TargetLynx™ program.

2.6. Preparation of standards, IS and quality control solutions

Standard stock solutions of Dox and IS were prepared separately by dissolving an accurately weighed amount of each compound in methanol to give a final concentration of 1.2 mg/mL. Stock solution of Dox was used for both calibration standards and quality control (QC) samples. Working solutions of Dox were prepared by serial dilution of the stock solution using methanol to obtain calibration curve standards in the range of 1–20000 ng/

mL. The calibration standards of plasma were prepared by spiking 50 μ l aliquot of each working solution and 50 μ l of the 100 μ g/mL internal standard to blank human plasma yielding calibration standards in the range of 1–1000 ng/mL. The calibration standards of exosomes were prepared by spiking 50 μ l aliquot of each working solution and 50 μ l of the 100 μ g/mL internal standard to 50 μ l aliquot of cancer cells (A549 & MCF-7) yielding calibration standards in the range of 2–1000 ng/mL.

QC samples at three different concentrations (150, 500 and 800 ng/mL) were prepared in the same way as described above and were treated as LQC, MQC and HQC respectively. All stock and working standard solutions were kept at -80°C until analysis.

2.7. Sample preparation

Frozen plasma samples (stored at -80°C) were thawed to room temperature and vortex for one minute to ensure homogeneity before extraction. A volume of 50 μ l of working standard and 50 μ l (10 μ g/mL) of IS were added to 100 μ l of plasma sample. 800 μ l of methanol was then added for protein precipitation after vortexing for 1 min followed by centrifugation at 12,000 rpm for 10 min. 400 μ l of supernatant was then separated and transferred to HPLC vial, and aliquots of 10 μ l of the sample were injected into the UPLC –MS/M system.

100 μ l of exosome samples (dissolved in PBS) were spiked with known concentration of IS, the samples were then extracted with 1 mL ethyl acetate (twice). The ethyl acetate layer was separated and evaporated under reduced pressure. The obtained sample was then reconstituted in 1 mL methanol and transferred to HPLC vial, and aliquots of 10 μ l of the sample were injected into the UPLC –MS/M system.

2.8. UPLC MS/MS method validation

Method validation was carried out according to the guidelines of the United States Food and Drug Administration (US-FDA) and European Medicines Agency (EMA) (Smith, 2010, 2012). The validation of this method aimed to evaluate its performance in terms of selectivity, linearity, precision, accuracy, recovery, dilution integrity and stability of analytes during both short-term sample processing and long-term storage.

2.8.1. Selectivity and specificity

To investigate the selectivity of the method towards possible interferences from plasma matrices, the chromatograms of blank plasma were compared with those of QC plasma samples. There was no interference from endogenous peaks at the retention times of analytes and internal standard under the established chromatographic condition. Analysis was performed using the proposed extraction protocol spiked with standard DOX at LOQ level (1 ng/mL) and IS at 10 ng/mL level.

The selectivity of the method in exosomes analysis was assessed by analyzing blank cancer cells, cancer cells spiked with DOX and IS.

2.8.2. Linearity and standard curve

The linearity of the method was evaluated by analysis of six standard samples with different concentrations (1–1000 ng/mL). Linearity was established by fitting the calibration curves from accepted five precision and accuracy batches by least-square regression model, $y = mx + b$, weighted by $1/x^2$, in which y is the peak area ratio of analytes to IS, m is slope of the calibration curve, b is the y -axis intercept of the calibration curve and x is the analyte concentration (DOX). The regression parameters from the calibration curves were used to calculate the concentrations of analyte in the quality control samples and test samples. The lowest stan-

dard on the calibration curve was considered as the limit of quantification (LOQ), if the analyte response was at least five times more than that of blank plasma.

2.8.3. Precision and accuracy

Intra-day accuracy and precision were determined by analyzing 15 replicates of QC samples at three concentration levels (150, 500, and 800 ng/mL). Accuracy was expressed as a percentage of deviation from the corresponding nominal value (relative error, RE). The precision of the assay was expressed as relative standard deviation (RSD) and evaluated by the determination of QC samples at three concentrations levels. The inter-day precision and accuracy were determined by analyzing three replicates of QC samples at three different concentration levels on five consecutive days using three replicates.

2.8.4. Extraction recovery and matrix effect

To evaluate the matrix effect, standard quality control stock solutions of DOX at three concentration levels were spiked in plasma, cancer cells, and Millipore-grade water, the later considered the un-extracted quality control samples. Three replicates of each standard QC solutions and the un-extracted standard QC samples were analyzed and processed as usual. Extraction recoveries were assessed by comparing mean peak of the extracted QC samples with those spiked into blank matrix (water). Nine replicates of aqueous Ketotifen were also run for the recovery of Ketotifen and the matrix effect of the internal standard was evaluated in the same manner.

2.8.5. Stability evaluation

Three replicates of QC samples at low and high concentrations were used to evaluate the stability of analytes under a variety of storage and processing conditions such as the bench top stability (samples were stored at room temperature for ~ 6 h, short-term stability), freeze thaw stability (three cycles, from -80 to room temperature), auto-sampler storage stability (samples were stored for ~ 48 h under auto-sampler condition), and long-term stability (samples were stored at -80°C for 30 days). The working solutions and stock solutions of DOX and the IS were also evaluated for stability at room temperature for 12 h and at refrigerator temperature (below 10°C) for 15 days. The samples are considered stable in plasma if the deviation from the calculated concentration of stability quality control samples was within $\pm 15\%$.

3. Result and discussion

Exosomes play an important role in the pathogenesis of many diseases. Recent studies highlighted their role in chemotherapy resistance by mediating the transport of anticancer drugs outside the cells (Corcoran et al., 2012; Khan et al., 2017). In this study, we developed and validated a rapid and sensitive method for the quantification of DOX in exosomes, which may increase our understanding of exosomes role in chemotherapy resistance and help in finding agents that reduce exosome release from cancer cells.

3.1. Optimization of chromatographic condition

The chromatographic parameters in terms of peak intensity, resolution and retention time were optimized by trying different chromatographic conditions. For instance, several mobile phase compositions including mixture(s) of organic solvents such as acetonitrile and methanol along with pure water; with the addition of 0.1% formic acid as proton promotor were evaluated. Different flow-rates in the range of 0.2–0.5 mL/min using isocratic or gradient elution were also tested to find out the best chromatographic

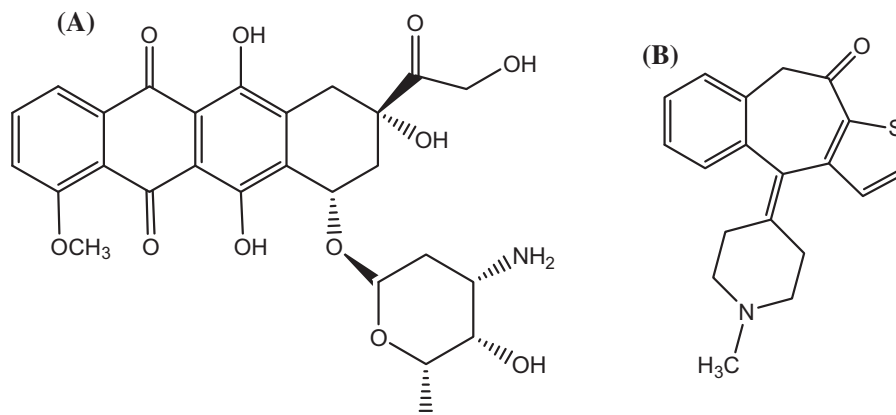


Fig. 1. Chemical structure of Doxorubicin [A] and Ketotifen (IS) [B].

separation conditions of DOX and IS (structures are shown in Fig. 1). Chromatographic peaks of both DOX and IS were sharp and baseline separation was achieved with no interferences. The retention time of DOX and IS was 0.45 and 0.49 min, respectively. The best chromatographic conditions for separation were obtained using an isocratic elution of a mobile phase composed of acetonitrile: water: formic acid (85:15:0.1%, v/v/v) and a flow rate of 0.2 mL/min, on Acquity UPLC BEH™ C18 column (50 × 2.1 mm, i.d. 1.7 μm) as a stationary phase.

UPLC-MS/MS instrumental parameters were optimized for the determination of both DOX and IS. Both analytes were detected by tandem mass spectrometry using multiple reaction monitoring (MRM) to monitor the protonated precursor to product ion transitions of m/z 544.25 > 397.16 and m/z 310.08 > 96.97 for Doxorubicin and Ketotifen, respectively (Fig. 2). The selection of ketotifen as an internal standard was based on its physicochemical properties, which are similar to DOX and thus generated comparable retention times. Analysis showed that the signal intensity of positive ion detection mode was much higher than that of negative ion detection for DOX and IS. MS parameters such as ESI source temperature, desolvation temperature, capillary voltage, cone voltage, flow rate of cone gas and desolvation gas were carefully optimized to achieve the optimal intensity for both DOX and IS.

By increasing the cone voltage gradually, intensity of analytes ions increased significantly. The strongest ion signals were achieved when the cone voltage was set up at 25 V and the collision energy at 17 eV for Dox. (m/z 397). For IS the most abundant fragment ion was obtained at m/z 96.97 with an optimal collision energy of 34. The MS spectra of DOX and IS are shown in Fig. 2.

3.2. Selectivity

Selectivity of the method was determined by comparing the chromatograms of blank plasma and blank cancer cells with those from samples spiked with the analyte at the limit of quantitation (LOQ). No interference from endogenous peaks were seen at the retention times of the analytes under the established chromatographic method. Thus, the method is selective for quantification of DOX and Ketotifen in plasma and exosomes of cancer cells as shown in Fig. 3.

The linearity of the method was determined by plotting the peak area ratio of analyte to IS versus concentrations of DOX using the least-squares regression analysis of six data points (Table 1). This analysis gave a typical regression line for DOX in plasma ($y = 4.36 \times 10^{-5} x + 2.40 \times 10^{-3}$, $r^2 = 0.9974$), A549 cancer cell line

($y = 5.38 \times 10^{-2} x + 1.1 \times 10^{-2}$, $r^2 = 0.9993$) & MCF-7 cancer cell line ($y = 5.32 \times 10^{-2} x + 5.55 \times 10^{-2}$, $r^2 = 0.9995$). Good linearity was observed for DOX over the concentration range 1 to 1000 ng/mL in human plasma and 2 to 1000 ng/mL in the exosomes of both A549 & MCF-7 cell lines. The lower limit of quantification of this method was 1 ng/mL, 2 ng/mL and 2 ng/mL in human plasma, A549 cells & MCF-7 cells respectively, which make it sensitive enough to conduct pharmacokinetic studies of DOX in humans.

3.3. Assay precision and accuracy

The precision of intra-day and inter-day results of DOX (expressed as %RSD) in the QC samples were below 7.38%, 9.30% and 10.33% for plasma, A549, and MCF-7 cells respectively (as shown in Table 2). The accuracy (expressed as %RE) of intra-day and inter-day for the investigated QC samples were in the range of -4.82 to 8.94%, -6.68 to 12.51% and -5.94 to 12.60% for plasma, A549, and MCF-7 cells respectively. These results show that the established method is precise and accurate and the assay values are within the acceptance limit of <15% and \pm 15% for precision and accuracy respectively.

3.4. Recovery and matrix effect

The percentage recoveries (mean \pm SD) of DOX (Table 3) obtained from plasma at three different QC concentrations (150, 500 and 800 ng/mL) were 99.16 ± 0.45 , 96.85 ± 2.86 and 99.79 ± 3.95 , respectively, & % recoveries from the exosomes of A549 cells were 91.43 ± 3.6 , 93.44 ± 5.7 , and 95.75 ± 8.1 respectively, whereas the % recoveries from the exosomes of MCF-7 cells were 90.82 ± 5.9 , 90.79 ± 7.6 and 93.48 ± 9.13 respectively. The % recovery achieved for IS at the concentrations employed were 100.52 ± 3.3 %, 96.98 ± 4.41 and 97.36 ± 5.6 respectively. These results show that the extraction method of DOX was efficient and concentration independent with no significant matrix effect.

3.5. Stability

All stability tests for DOX were summarized in Table 4. The stabilities of DOX solutions were assessed at two different concentrations of QC samples (low and high concentrations). The relative standard deviation of all samples was within $\pm 10.49\%$ ($< \pm 15$), indicating that DOX spiked in plasma and cancer cells was stable and exhibited no significant degradation under the storage or the handling conditions assessed. Moreover, the stock solutions and working standard of DOX and IS were also stable for 15 days at

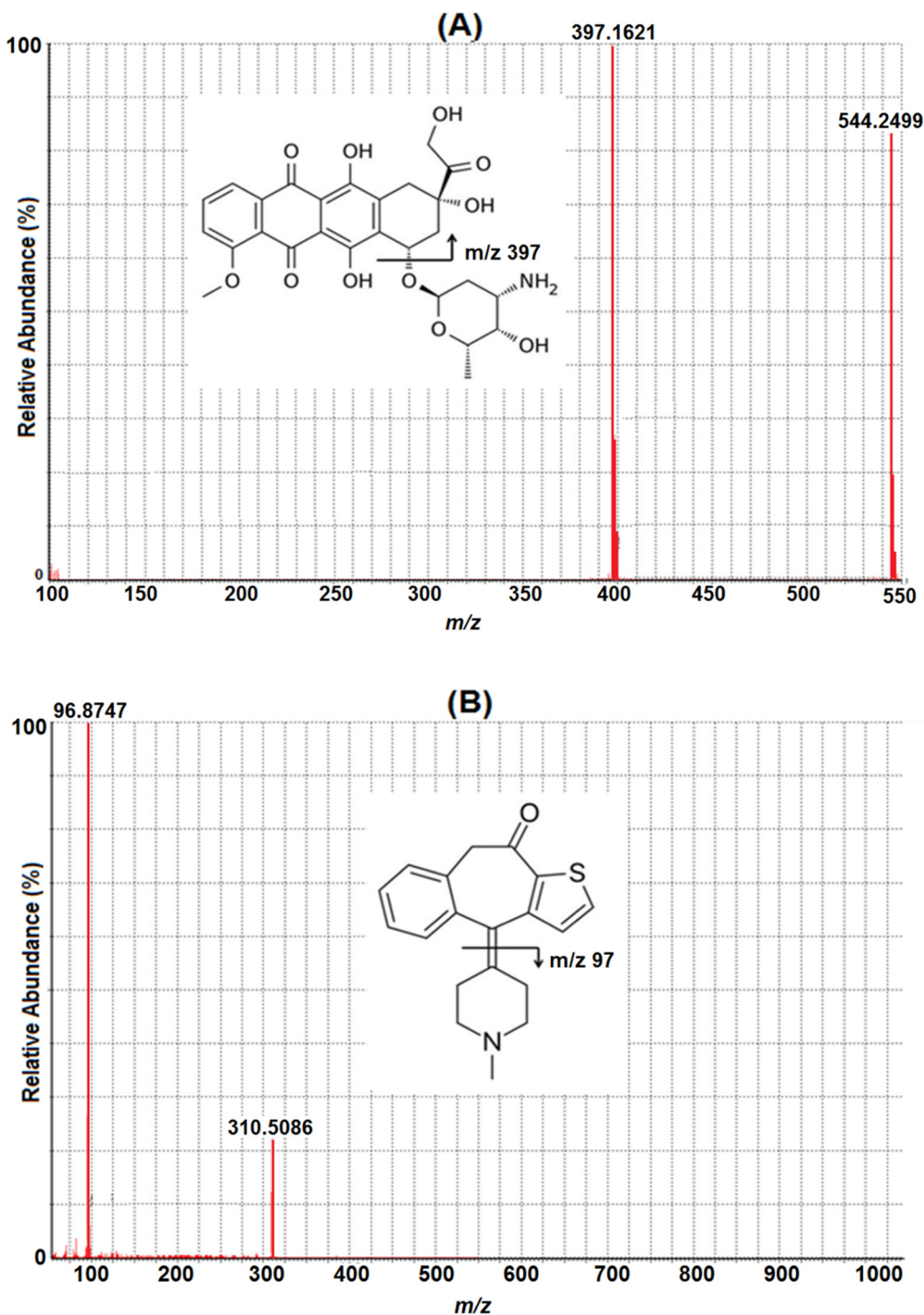


Fig. 2. The product ion spectra of Doxorubicin (A) and Ketotifen (IS) (B).

refrigerator temperature (below 8 °C). These results indicate that DOX was stable up-to 30 days at –80 °C in spiked plasma and up to 15 days in aqueous solution in refrigerator, as reported previously.

3.6. Advantages of the proposed method over the reported methods

This method was developed and validated for the determination of DOX in human plasma and exosomes extracted from cancer cells by UPLC-MS/MS. The optimized method has significant advantages

over to the previously reported LC-MS/MS methods in terms of simplicity and sensitivity. The method reported in this study involves simple one-step protein precipitation for sample preparation and isocratic elution containing acetonitrile: water: formic acid (85:15:0.1%, v/v/v) at a flow rate of 0.2 mL/min. The retention time was only 0.49 min, which is suitable for high throughput analysis. More importantly, the findings of this study may shed more insight into the role of exosomes in mediating the efflux of anticancer agents outside the cancer cells leading to chemotherapy resistance.

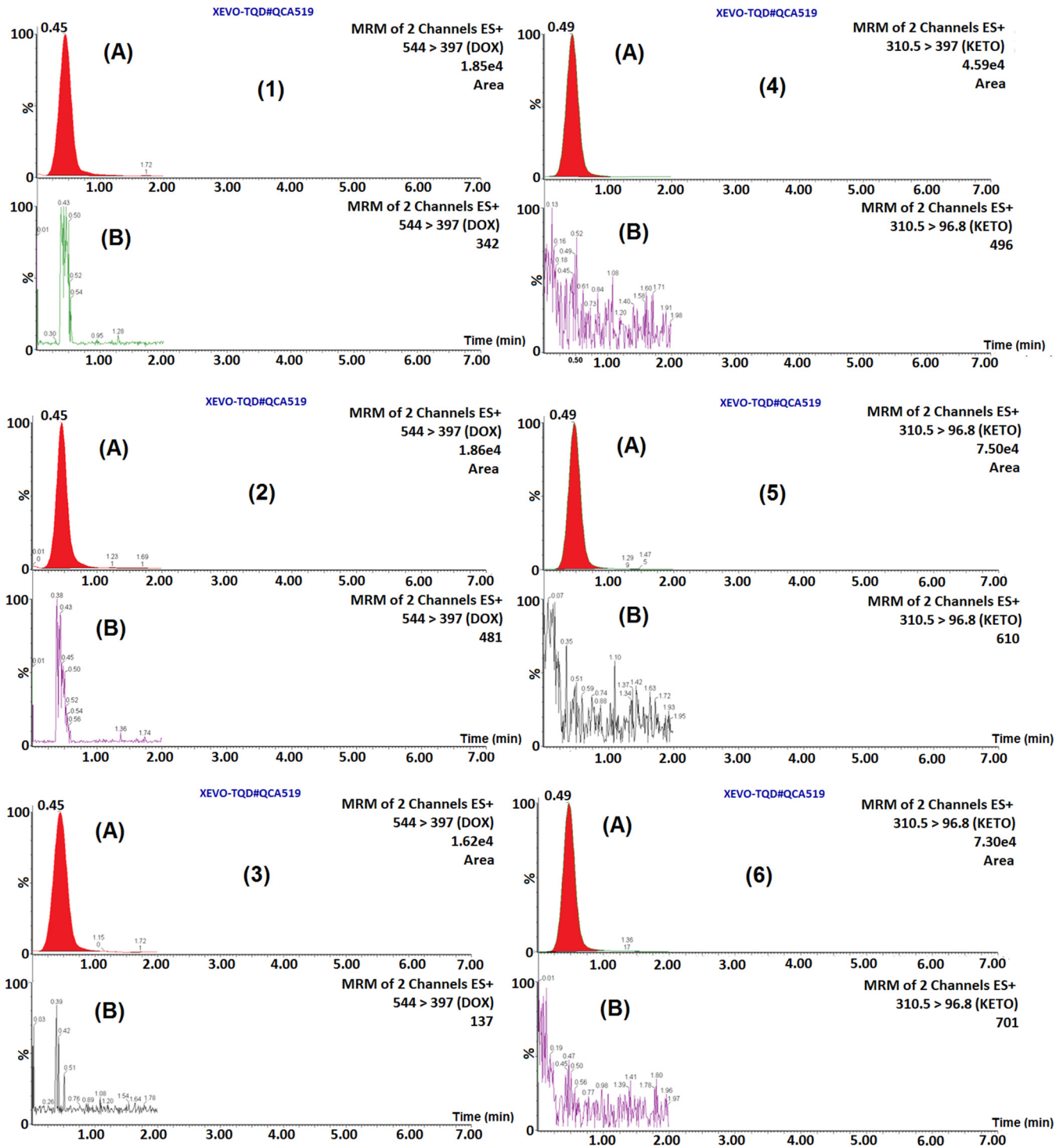


Fig. 3. Representative chromatograms of LLOQ [A panels] and blanks [B panels] for DOX and IS: (1) DOX in plasma (2) DOX in A549 (3) DOX in MCF-7 (4) IS in plasma (5) IS in A549 (6) IS in MCF-7.

Table 1
The linearity & LOQ of the assay for Doxorubicin.

Doxorubicin media	Linear range (ng/mL)	Linear equation (n = 5)	Correlation coefficient	LOQ (ng/mL)
Plasma	1–1000	$y = 4.36 \times 10^{-5} x + 2.40 \times 10^{-3}$	0.9974	1
A549 cell line	2–1000	$y = 5.38 \times 10^{-2} x + 1.1 \times 10^{-2}$	0.9993	2
MCF-7 line-2	2–1000	$y = 5.32 \times 10^{-2} x + 5.55 \times 10^{-2}$	0.9995	2

Table 2

The inter and intraday precision and accuracy values for QC samples (n = 5 days, 3 replicates [inter-day], n = 15 [intra-day] for each QC level).

Analyte matrix		Nominal conc. (ng/mL) – (Inter run)			Nominal conc. (ng/mL) – (Intra run)		
		150	500	800	150	500	800
Plasma	*Conc. (ng/mL) ± SD	136.63 ± 9.116	493.81 ± 21.33	838.56 ± 38.23	136.63 ± 10.08	477.2 ± 18.5	818.63 ± 22.06
	RSD (%)	6.596	4.29	4.55	7.38	3.88	2.69
	Accuracy (% RE)	8.911	1.24	-4.82	8.91	4.56	-2.33
A549 cell line	*Conc. (ng/mL) ± SD	133 ± 12.3	485.32 ± 25.3	853.45 ± 45.67	131.23 ± 11.4	482.14 ± 27.5	855.45 ± 37.75
	RSD (%)	9.300	5.27	5.41	8.700	5.72	4.42
	Accuracy (% RE)	12.400	2.94	-6.68	12.510	3.57	-6.93
MCF-7 cell line	*Conc. (ng/mL) ± SD	131.1 ± 13.4	482.41 ± 27.2	847.52 ± 42.78	129.93 ± 12.3	479.54 ± 19.2	842.65 ± 41.25
	RSD (%)	10.330	5.27	5.05	9.500	4.00	4.90
	Accuracy (% RE)	12.600	3.518	-5.94	13.380	4.09	-5.33

* Measured concentration.

Table 3

The percentage recovery for the analysis of DOX and IS in plasma, and exosomes extracted from A549 and MCF-7 cell lines (n = 9).

Analyte	Nominal concentration (ng/mL)	Plasma	A549	MCF-7
		Mean % recovery ± RSD (%)		
DOX	150	99.16 ± 0.45	91.43 ± 3.6	90.82 ± 5.9
	500	96.85 ± 2.86	93.44 ± 5.7	90.79 ± 7.6
	800	99.79 ± 3.95	95.75 ± 8.1	93.48 ± 9.13
IS	5000	100.52 ± 3.37	96.98 ± 4.41	97.36 ± 5.6

Table 4

Stability of DOX under different storage conditions (n = 3).

Analyte matrix		Freeze-thaw stability		Bench-top		Long term		Auto sampler	
		Nominal conc. (ng/mL)		Nominal conc. (ng/mL)		Nominal conc. (ng/mL)		Nominal conc. (ng/mL)	
		150	800	150	800	150	800	150	800
Plasma	Measured conc. (ng/mL)	145.03 ±	860.17 ±	142.633 ±	886.07 ±	147.43 ±	880.57 ±	143.17 ±	866.6 ±
	± SD	15.21	28.45	8.24	22.63	4.15	31.71	13.26	58.03
	RSD (%)	10.4	3.31	5.78	2.55	2.81	3.60	9.26	6.69
A549	Measured conc. (ng/mL)	138.23 ±	856.46 ±	140.53 ±	881.98 ±	145.34 ±	885.67 ±	141.78 ±	871.5 ±
	± SD	16.71	30.56	9.52	24.53	6.26	28.12	15.47	43.13
	RSD (%)	12.09	3.57	6.77	2.78	4.30	3.17	10.91	4.95
MCF-7	Measured conc. (ng/mL)	137.73 ±	859.24 ±	139.62 ±	882.05 ±	142.23 ±	883.81 ±	144.28 ±	869.6 ±
	± SD	17.2	29.53	9.15	21.43	8.26	32.82	10.34	57.65
	RSD (%)	12.49	3.44	6.55	2.43	5.80	3.71	7.16	6.63

4. Conclusions

In summary, a simple, sensitive, rapid, and high-throughput UPLC-MS/MS method was developed and validated to determine the concentration of DOX in human plasma and exosomes extracted from cancer cells. Establishment of such a rapid, selective and sensitive method for the quantification of Dox in exosomes can be used to study chemotherapy resistance, and develop novel agents to reduce exosome release from cancer cells and hence decrease resistance to anticancer agents. The presence of DOX in exosomes of A549 & MCF-7 cancer cells, which was confirmed by this study, highlight their possible role in chemotherapy resistance. Moreover, the proposed method could be used for pharmacokinetic and toxicokinetic studies of DOX in human plasma. This method involved simple one-step protein precipitation with short retention time (0.49 min), and to the best of our knowledge, this is the first validated UPLC-MS/MS method for the determination of doxorubicin in exosomes.

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Conflict of interest

There are no conflicts to declare.

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