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Validation of an LC–MS/MS method for the determination of epirubicin in human serum of patients undergoing Drug Eluting Microsphere-Transarterial Chemoembolization (DEM-TACE)

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ABSTRACT

Drug Eluting Microsphere-Transarterial Chemoembolization (DEM-TACE) is a new delivery system to administrate drugs in a controlled manner useful for application in the chemoembolization of colorectal cancer metastases to the liver. DEM-TACE is focused to obtain higher concentrations of the drug to the tumor with lower systemic concentrations than traditional cancer chemotherapy. Therefore a specific, precise and sensitive LC–ESI-MS/MS assay procedure was properly designed to detect and quantify epirubicin at the concentrations expected from a transarterial chemoembolization with microspheres. Serum samples were kept acidic (pH approximately of 3.5) and sample preparation consisted of a solid phase extraction (SPE) procedure with HLB OASIS® cartridges using a methylene chloride/2-propanol/methanol mixture solution to recover epirubicin. The analyses consisted of reversed-phase high-performance liquid chromatography (rp-HPLC) coupled with tandem mass spectrometry (MS/MS). Accuracy, precision and matrix effect of this procedure were carried out by analyzing four quality control samples (QCs) on five separate days. The validation parameters were assessed by recovery studies of spiked serum samples. Recoveries were found to vary between 92 and 98% at the QC levels (5, 40, 80 and 150 μg/L) with relative standard deviation (RSD) always less than 3.7%. The limit of detection (LOD) was set at 1 μg/L. The developed procedure has been also applied to investigate the different capability of two types of commercially available microspheres to release epirubicin into the human circulatory system.

1. Introduction

Epirubicin belongs to the anthracycline anti-tumor antibiotics, a class of chemotherapy drugs with a broad activity against a wide range of tumors, including breast and lung ones [1]. Numerous methods have been developed using high-performance liquid chromatography (HPLC) with various detectors. Traditionally, HPLC in combination with ultraviolet detection [2–7] and capillary zone electrophoresis (CZE) coupled with an amperometric detector [8] were employed to determine anthracyclines in biological fluids. However, because of these techniques suffer from low sensitivity and specificity only few quantitative methods were developed. Ricciarello et al. [9] carried out an alternative system of detection based on electrochemical detector composed of an amperometric electrode coupled with a coulometric electrode for the simultaneous determination of doxorubicin and epirubicin in human plasma. Nevertheless, to date the best sensitivities have been obtained with fluorescence detection (FL) and mass spectrometry detection (MS or MS/MS). Therefore, HPLC in combination with both detectors has been used to develop quantitative methods for a number of anthracyclines and their metabolites for several years [10–22]. However, while assays based on LC–MS techniques showed long runtimes [11] or complex sample preparation processes [10,12–15], FL-based methods were developed on laborious protein denaturing procedures (e.g. with zinc sulphate) where plasma samples were separated by centrifugation [17]. A labor-intensive sample pretreatment was also carried out by Rudolphi et al. [22] to detect epirubucin in human plasma and liver homogenate. The authors coupled a sample processing precolumn to an analytical reversed-phase (RP) column with fluorescence detection. In this procedure they avoided the separation of the proteins from the sample. Others

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sample preparation procedures have been extensively studied to quantify anthracyclines from biological matrices and cell samples by buffering plasma samples to basic conditions with pH values that varied from 8.4 to 9 [12,16,19]. Liquid-liquid extraction (LLE) has been also employed, in particular to detect anthracyclines in cell samples where these molecules are highly bound to DNA [12], but the total recovery of epirubicin was in the range 64–75%. Recently, an LC–MS assay for the detection of doxorubicin has been carried out [13] using solid phase extraction (SPE). However, the recovery ranged from 68 to 71%.

The aim of the present study was to obtain a rapid, sensitive and specific method based on the successful hyphenation of LC and mass spectrometry. This was achieved and the validated assay is based on a very simple and fast SPE procedure that permits high recovery percentages of epirubicin to be obtained. The procedure allows the separation of epirubicin from serum samples of patients treated with a new pharmaceutical preparation that leads to the reduction of blood flow. Thus, this method was designed to cover serum concentrations expected in patients under chemotherapy treatments based on transarterial chemoembolization (TACE) with drug-eluting beads. In the field of oncology, this new drug delivering system is focused to obtain higher concentrations of the drug to the tumor and lower systemic concentrations than traditional cancer chemotherapy.

In order to display the suitability of the developed procedure as demonstrated by quantification of serum samples, the concentration–time curves of epirubicin are also reported as a function of different types of drug-eluting beads.

2. Materials and methods

2.1. Chemicals and reagents

Epirubicin hydrochloride (EPI) was kindly supplied by Pfizer S.p.A. (Research & Development Center, 20014 Nerviano-MI, Italy). The internal standard (IS), trofosfamide (TR, 99.7% purity), was obtained from Baxter Oncology GmbH (Frankfurt am Main, Germany). Acetonitrile, methanol, 2-propanol, methylene chloride, formic acid and hydrochloric acid, all of LC gradient grade, were purchased from VWR International Ltd. (Merck House, Poole, UK). All other reagents used were of analytical grade and are regularly available in a laboratory. OASIS® hydrophilic–lipophilic balance (HLB) cartridges (10 mg; 1 mL) were purchased from Waters Associates Milford, MA, USA. DC-Beads™ (Biocompatible UK Limited, Surrey, UK) and HepaSphere™ microspheres were purchased from Biosphere Medical, France.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a Series 200 LC quaternary pump (Perkin-Elmer, Norwalk, CT, USA). The analytical column was a 150 mm × 4.6 mm × 5 μm Hypersil BDS C8 (Thermo Electron Corporation, Bellefonte, PA, USA) protected with a guard column of the same type. Mobile phase consisted of a mixture of 0.1% formic acid in water and acetonitrile. Chromatographic separation was achieved using mobile phases A and B. Mobile phases A and B were 0.1% formic acid and acetonitrile in the ratios of 80/20 and 30/70 (v/v), respectively.

The flow rate was 1.0 mL/min and splitting ratio was set at 1:20. The injection volume was 20 μL. Linear gradient elution was employed with the following steps: at start of run 1 min isocratic with 100% phase A, then 10 min to 100% phase B and finally 5 min isocratic with 100% phase B. An additional time of 5 min with 100% phase A was enough to equilibrate the system and return it to the initial condition for subsequent analysis. The total run time was 16 min.

Mass chromatograms were recorded using an API 300 triple quadrupole mass-spectrometer (Perkin-Elmer-Sciex, Toronto, Canada) equipped with an electrospray (ESI) ionization source operating in positive ion mode with an ionspray voltage set at +5800 V. The instrument was used to obtain both the mass spectra (MS1) and the product ion spectra (MS2). Data were acquired and calculated using a power Macintosh 7500/100 with an Apple Macintosh System 7.5.3 (PE Sciex). Polypropylene glycol was used to calibrate the instrument in positive mode and to adjust the peak width (FWHM) to 0.7 Da over the m/z 100–800 range for HPLC-ESI-MS/MS spectral acquisition. Tuning and MS/MS spectra were performed for the target analyte and the IS by direct infusion of a 1 μg/mL standard solution mobile phase A at a flow rate of 20 μL/min with a Harvard syringe pump (Bonaduz AG, Switzerland) without split. The protonated molecules were fragmented by collision-activated dissociation (CAD). Nitrogen was used as the collision gas at a setting of 8 (arbitrary units). MS/MS detections were carried out in selected reaction monitoring (MRM) mode. Mass transitions of m/z 544 → 397 and 323 → 154 were optimized for epirubicin and trofosfamide, respectively. The ESI-MS/MS operating parameters were set at +5 eV and 1 V as collision energy (R02–R03) and ion energy voltage, respectively.

2.3. Standard sample preparation

Drug free human sera from healthy volunteers were obtained from various sources. Control serum samples or analyte fortified serum were treated identically. To a 1.0 mL portion of the serum sample a 0.10 mL aliquot of a 200 ng/mL trofosfamide internal standard solution was added. The sample solution was vortexed. Then, 2.0 mL of 0.1N hydrochloric acid (pH 1.5) was added. Because of the extensive binding of epirubicin to serum protein [23–24], we decided to buffer matrix at an acidic pH value over the other conditions described in literature. Afterwards, serum specimens were spiked with the working standard solutions of epirubicin. Fortified sera were loaded onto the OASIS® HLB cartridges for the solid phase extraction (SPE). The HLB cartridge was previously equilibrated with 0.5 mL of methanol and purified water. To carry out a conditioning step, 0.5 mL of methylene chloride was added.

3.0 mL of the spiked sample were loaded on the cartridge, then washed at a flow rate of 0.05 mL/s with 0.5 mL of 5% methanol in water. The cartridges were dried by passing air for 20 min by means of a vacuum pump. The analytes were eluted (flow rate 0.05 mL/s) using a methylene chloride/2-propanol/methanol mixture in a rate of 50:25:25 (v/v), into 15.0 mL Eppendorf tubes.

The eluted solutions were dried under a stream of nitrogen and the residue was reconstituted in 200 μL of mobile phase A. These samples were vortexed for 1 min and filtered on 0.45 μm filters into 1.5 mL microtubes. Aliquots of 20 μL were injected for HPLC-ESI-MS/MS assay.

2.4. Collection of patients' sera

The development of this procedure is suitable for use in our clinical studies and to quantify EPI in serum samples obtained from patients treated with microsphere eluting beads-transarterial chemoembolization of 100 mg epirubicin dose. Informed written consent was obtained from all patients. To compare EPI concentration–time curves of patients who were treated with different embolization agents, i.e. DC-Beads and HepaSphere, nine serum samples between time-zero and 5, 10, 20, 40, 60, 120, 180, 360 min were collected after the therapy was initiated. Whole blood collected into tubes was processed at room temperature to yield serum by centrifugation at 4000 × g for 5 min. Serum for this assay was aliquoted (1.0 mL) and immediately frozen at −20 °C before the analysis.

2.5. Validation study

A full validation according to FDA guidelines was performed for the assay in human serum [28,29] by means of linearity, accuracy and precision, matrix effect determination and carry over.

Eight non-zero calibration standards were prepared freshly every day for the concentrations of 3.0, 4.0, 10.0, 20.0, 30.0, 50.0, 100.0 and 200.0 μg/L. Each standard sample was obtained by placing 100 μL of the appropriate dilutions in 1.0 mL of human serum. Each calibration standard was analysed in duplicate ($n = 2$) in five analytical runs. The internal standard method was used for serum concentration quantification: the area ratios of EPI to trofosfamide were plotted against the spiked concentrations. To fit the calibration curve the simplest relationship that provided acceptable back-calculated concentrations for the standards was used. Therefore, two replicates of calibration curves were analysed and estimated by linear regression procedure. The standard curve was considered acceptable if the calculated accuracies of >75% of standards were within 15% of the nominal value and no systematic deviations over the linear range were observed.

The lower limit of quantification (LLOQ) was determined by analyzing spiked samples ($n = 5$) at the concentration of 3.0 μg/L. Quality control samples (QCs) were prepared in human serum at four concentrations (5.0, 40.0, 80.0 and 150.0 μg/L). All QCs were prepared freshly for each run.

Matrix effect (ion suppression or ion enhancement), extraction recovery and overall method recovery were assessed at four concentrations as QCs by comparing the peak areas of standards prepared in mobile phase (set A), standards prepared in human serum when the complete SPE procedure was carried out (post-spike serum samples; set B) and standards spiked in human serum (pre-spike serum samples; set C). Based on this protocol [27], matrix effect (ME), extraction recovery (ER), and overall process recovery (PR) mean values for epirubicin were evaluated and converted to percentages. Similar method was used to assess ME, ER and PR mean values for trofosfamide. Matrix effect, extraction recovery and overall process recovery were assessed at the concentration of 20 μg/L by comparing the peak areas of IS prepared in mobile phase (set A), in human serum when the complete SPE procedure was carried out (set B) and peak areas of IS spiked in human serum (set C) over the 5 days of validation study. The loss of signal represents the ion suppression.

The precision and accuracy of the method were assessed by intra- and inter-day validation over 5 non-consecutive days. The intra-day accuracy and precision were evaluated by processing QCs in 5 replicates ($n = 5$) at four concentration levels. The concentration of the QCs was calculated *vs.* the daily calibration curves. The inter-day accuracy and precision were determined by analyzing quality control samples in 25 replicates ($n = 25$) of each concentration. Accuracy was determined as the ratio between the back-calculated concentration and the nominal value and expressed as a percentage. The relative standard deviation (RSD) was used as a measure of precision. The intra- and inter-assay accuracies should be within the recommended ranges and reported in the international guidelines [28,29]. The lower limit of detection (LOD) level was obtained as three times the SD of the LC–MS/MS peak areas detected at the retention times of the analyte of interest.

2.6. Stability

Stability at ambient temperature was assessed at two concentration levels (5.0–80.0 μg/L) of the quality control samples.

Freeze–thaw stability was studied by analysing the low and high QCs that were frozen overnight, at normal storage temperature (−20 °C) and thawed unassisted at room temperature. When completely thawed, the samples were frozen again at the same temperature for 24 h and thawed. This freeze–thaw cycle was repeated two more times. After the third cycle, the samples were analysed. Then, sera were refrozen at least 24 h between the third and fourth cycle. The concentration of analytes in the samples under these storage conditions was calculated *vs.* the daily calibration curves, and the percentage deviation of the average of results from the corresponding nominal value was obtained. The analytes were considered stable when ±20% of the initial concentration was found. The found sample concentrations are compared with their nominal values.

Table 1
Summary of matrix measurement data for the internal standard (trofosfamide).

Nominal conc. (20.0 μg/L)	Matrix measurement	Recovery (%)	
	Suppression (%)		
Day 1			
Mean	92.7	87.4	81.1
SD	2.4	0.3	0.7
RSD%	2.6	0.4	0.9
Day 2			
Mean	93.2	86.1	79.7
SD	2.5	0.7	1.8
RSD%	2.7	0.9	2.3
Day 3			
Mean	93.9	90.6	85.1
SD	2.6	0.3	0.8
RSD%	2.8	0.3	1.0
Day 4			
Mean	104.2	98.4	102.5
SD	3.4	0.1	0.2
RSD%	3.2	0.1	0.2
Day 5			
Mean	90.3	89.3	80.6
SD	2.3	0.3	0.8
RSD%	2.6	0.4	0.9
	ME%	ER%	PR%
($n = 20$/day)	B/A	C/B	C/A

3. Results and discussion

3.1. Chromatography and detection

Separation of epirubicin and internal standard was performed under acidic conditions. The analyte was eluted with a gradient starting at 80% formic acid (pH of approximately 3) and 20% acetonitrile. This condition was chosen to give epirubicin retention and to minimize ion suppression on its response. The optimized chromatographic conditions developed for this procedure proved to be specific and have no major interferences. All non-fortified serum samples chromatographed showed no interfering peaks. The blank samples and the time-zero samples obtained from patients showed no interferences for EPI or the internal standard, trofosfamide, at the selected mass transition used for quantification. Trofosfamide was chosen for this procedure because daunorubicin, commonly used as internal standard for measuring anthracyclines [10–16], showed to have different ionization properties at the sample preparation conditions. Furthermore, daunorubicin gave a very broad and asymmetric peak shape in the MRM chromatographic profiles and that was likely due to the feature of this IS, a molecule extremely "sticky" to most surfaces [12]. In conclusion, to reduce the potential for analytical error we found that the most appropriate IS was trofosfamide. TR showed more stability than daunorubicin and exhibited an excellent peak shape with a retention time very close to the target analyte. Furthermore,

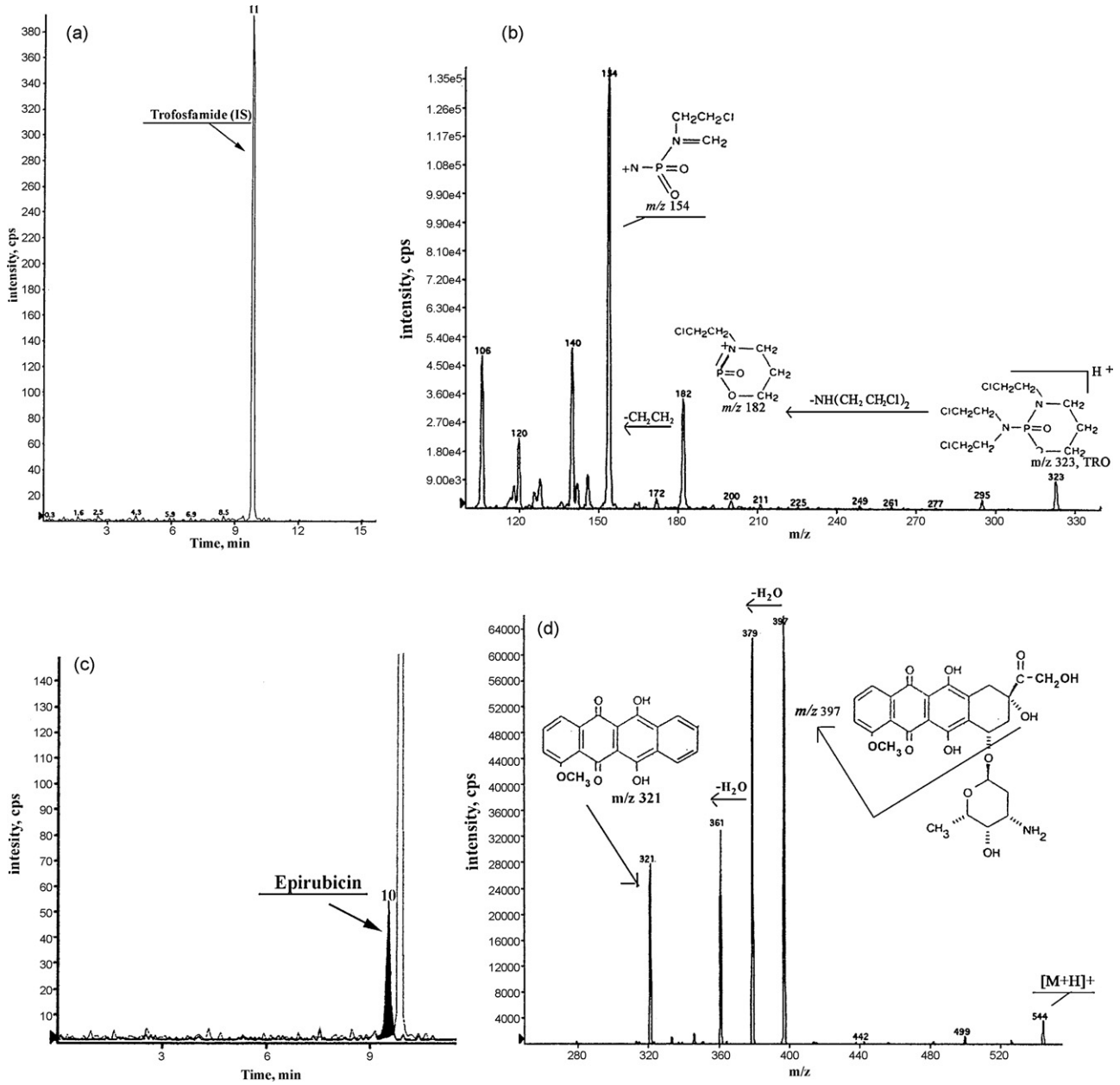

Fig. 1. (a) XIC of +MRM chromatogram of a zero blank serum sample fortified to contain trofosfamide (IS) at the concentration of 20 μg L^{-1} and its MS/MS product ion scan (b). (c) XIC of +MRM chromatogram of a pre-spiked serum samples prepared to contain epirubicin at the concentration of 5.0 μg L^{-1} and its MS/MS product ion scan (d).

to demonstrate that trofosfamide had a very positive influence in reducing the variability of this MS-based assay, the summary data relative to matrix effect, extraction and overall recovery of trofosfamide are shown in Table 1. Due to extraction efficiency similar to that of epirubicin over all the validation days, trofosfamide was therefore selected as internal standard of the first choice.

Fig. 1a shows a typical extracted ion chromatogram (XIC) fortified to contain 20 μg/L of IS. Similarly, XIC chromatogram of a serum standard sample at the concentration of 5 μg/L is reported in Fig. 1c. The chromatographic baseline displayed a very small background and the peaks at the retention time of the analyte did not interfere with the EPI itself. Sample carry over was minimized by the use of a 80/20 (v/v) 0.1%formic acid/acetonitrile rinse of the injector needle and loop when the first blank sample was injected after the highest level calibration standard.

Since this procedure was designed for use as a test to compare the feasibility of two different embolization agents, high specificity was required. Thus, mass transition for EPI, m/z 544 → 397, was chosen because it was unique for this compound and produced the greatest response (see Fig. 1d). The fragmentation pattern, discussed in detail elsewhere [10,14], is reported in the frame of this Fig. 1d. Similarly, mass transition for TR, m/z 323 → 154, was chosen because the fragment ion exhibited the highest detector response and the fragmentation pattern is displayed in Fig. 1b.

3.2. Extraction conditions and matrix effect study

Solid phase extraction (SPE) procedure by the use of 50/25/25 (v/v) methylene chloride/isopropanol/methanol as eluting solvent was ultimately chosen for this procedure due to its higher extraction efficiency than methanol as previously reported [13]. Various SPE procedures were tried during the early development stage of this assay including Phenomenex Strata-X® Polymeric Sorbent (Phenomenex, USA) and Varian Bond Elut® C18 (Lake Forest, USA)

Table 2
Summary of matrix measurement data for epirubicin.

Nominal conc. (μg/L)	Matrix measurement Suppression (%)	Recovery (%)	
5.0	92.7 ± 0.4	92.2 ± 1.3	85.5 ± 0.5
40.0	97.6 ± 2.2	90.8 ± 1.5	88.6 ± 3.2
80.0	95.1 ± 0.5	98.8 ± 0.9	93.9 ± 0.2
150.0	95.6 ± 0.9	97.2 ± 0.3	93.0 ± 0.2
	ME% (mean ± SD)	ER% (mean ± SD)	PR% (mean ± SD)
(n = 25)	B/A	C/B	C/A

Table 3
Summary of assay performance data.

Dynamic range of epirubicin		
Nominal conc. (μg/L)	Back-calculated conc.[a] (μg/L)	Accuracy%
3.0	3.24 (±0.15)	108.0
4.0	3.87 (±0.32)	96.8
10.0	9.00 (±0.11)	90.0
20.0	18.87 (±0.75)	94.3
30.0	27.86 (±1.47)	92.9
50.0	50.08 (±1.01)	100.1
100.0	100.60 (±0.80)	100.6
200.0	203.69 (±0.84)	101.9

[a] The calibration curves were fitted to the concentration *vs.* the peak-area ratio (EPI peak area)/(IS peak area). Each value is the mean (±SD) of replicates analysed in five analytical runs.

as well as liquid-liquid extraction (LLE) by the use of chloroform. The above mentioned sorbents gave lower percentages of recovery than the OASIS® HLB cartridges. Extraction efficiency (ER%) was decreased by 15% also with LLE.

To improve overall process recovery, it was also important to keep the pH value of serum samples at least two units lower than pK_a of epirubicin [25,26] that is a weak base (pK_a of ∼7.7). All the fortified samples and human serum from patients were kept roughly acidic (pH approximately of 3.5) by adding 2 mL of 0.1N HCl to 1 mL aliquot of matrix. Several buffers were investigated but only the acidic condition was found necessary to give the high extraction efficiencies reported. This acidic condition was also suitable to dissociate serum protein binding of epirubicin.

Recovery studies were based on the peak area ratios of the fortified and extracted samples (set C) to control samples spiked after the SPE procedure was completed (set B). Recoveries from five independent matrices on every day of validation were found to vary between 92 and 98% at the QC levels (n = 25 on 5 days). The overall process recovery displayed the same range characteristics, i.e. 80–93% (Table 2). Similarly, the overall process recovery of trofosfamide extracted under similar conditions to the target analyte was found to vary between 80.6% (±0.8) and 102.5% (±0.2), as shown in Table 1.

For the assessment of matrix effect, the peak area ratios of epirubicin fortified in SPE extracted matrices (post-spiked serum samples from independent sources) and peak areas of standard solutions (set A) were used. Epirubicin exhibited a moderate matrix suppression effect, with ESI responses ranging from 92 to 97%. In all cases, the observed results were found to be within acceptable limits [28–29]. Table 2 summarizes all the matrix data. Also trofosfamide evidenced the same extent of matrix effect. ME% was found to vary between 90% (±2.3) and 104.2% (±3.4) with a precision ranging from 2.6% and 3.2% (Table 1).

3.3. Validation study

3.3.1. Linearity

The assay was linear over the validated concentration range of 3.0–200.0 μg/L. The best fit for the calibration curve was obtained by using a linear regression with correlation coefficients always better than 0.998.

For epirubicin, calibration curves were analysed over all the days of the validation study and the calibration curve was $y = 0.0392 \pm (0.005)x - 0.0555 \pm (0.021)$; with $y = (\text{mean} \pm \text{SD})x - (\text{mean} \pm \text{SD})$. The found concentrations (±SD) for each calibration standard are shown in Table 3. Calibration curves were fitted to the nominal concentrations *vs.* the peak area ratio between EPI peak area and IS peak area. In all cases, the back-calculated concentrations showed accuracies of 96.8–108.0%, which are in agreement with the international guidelines [28–29].

3.3.2. Precision and accuracy

Intra-assay precision was determined as the standard deviation of an analytical run divided by the mean of the run at each level of the quality control sample. Inter-assay precision was obtained as the ratio between the standard deviation of the five analytical runs and the overall mean. To summarize intra-assay RSDs%, the lowest and the highest value, obtained over the 5 days of validation, are reported in Table 4. Intra-assay precision was shown to vary from 1.4 to 9.0% and the inter-assay RSD was never greater than 5.2%. The measured LLOQ for epirubicin was set at 3.0 μg/L with an inter-assay RSD of 8.4% and an accuracy of 104.2%. The LLOQ value was enough to quantify EPI in serum samples of intrahepatic cholangiocarcinoma (ICC) patients treated with DEM-TACE. The LOD value was set at 1.0 μg/L.

3.3.3. Analyte stability

Epirubicin stability in serum samples was assessed over five freeze–thaw cycles at 5.0 and 80.0 μg/L. Results are shown in Table 5. EPI exhibited changes from control (3.9–5.5%) in the three freeze–thaw cycles but overall the change was acceptable. Epirubicin showed instability after the third cycle (day 4) and EPI decreased in concentration by 52–44% at the two levels of the quality control samples (n = 5), respectively.

As has been already reported in the literature for anthracyclines [7,8], drug instability was greater at lower concentrations and a drop (61–59%) was observed after the fourth cycle (day 5). Epiru-

Table 4
Summary of assay performance data.

Lower limit of quantification (LLOQ) intra- and inter-assay data for the LC/ESI-MS/MS determination of epirubicin using quality control samples						
LLOQ n = 5 on 3 days	Nominal conc. (μg/L)	Mean	SD	Inter-assay accuracy %	Precision	
					Intra-assay range (lowest-highest day RDS%) n = 5 on 5 days	Inter-assay RSD% n = 25
LLOQ	3.0	3.13	0.26	104.2		8.4
QC1	5.0	4.07	0.24	94.1	3.6–9.0	5.2
QC2	40.0	41.1	2.07	102.8	1.5–6.9	5.0
QC3	80.0	80.8	2.37	101.0	1.4–3.8	2.9
QC4	150.0	146.7	2.34	97.8	1.6–2.7	1.6

Table 5
Stability data.

		Nominal conc. ($\mu g\,L^{-1}$)	Initial conc. ($\mu g\,L^{-1}$)	Found conc. ($\mu g\,L^{-1}$)	Dev%
Benchtop stability					
24 h (RT) (reconstitution injection solvent)	QC1	5.0	5.05	4.37	−13.5
	QC3	80.0	80.48	74.63	−7.3
Freeze/thaw stability					
Freeze (−20 °C)/thaw cycles repeated three times		5.0	5.07	4.79	−5.5
Freeze (−20 °C)/thaw cycles (4th cycle)	QC1	5.0	4.89	2.31	−52.8
Freeze (−20 °C)/thaw cycles (5th cycle)		5.0	5.09	2.09	−58.9
Freeze (−20 °C)/thaw cycles repeated three times		80.0	80.48	77.36	−3.9
Freeze (−20 °C)/thaw cycles (4th cycle)	QC3	80.0	79.07	44.68	−43.5
Freeze (−20 °C)/thaw cycles (5th cycle)		80.0	80.34	31.18	−61.2

No. of replicates 5 ($n = 5$) for all storage conditions.

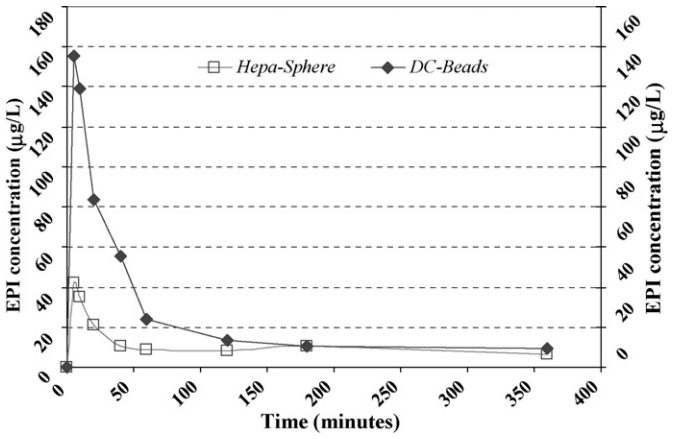

Fig. 2. Concentration–time curves of epirubicin in human serum of two patients receiving a Drug Eluting Microsphere-Transarterial Chemoembolization of 100 mg epirubicin dose.

bicin stability study gave similar results when the dry extracts were reconstituted in the mobile phase. This further investigation proved that within 24 h exposure at room temperature (RT) stability data were acceptable for EPI, as reported in Table 5.

3.4. DEM-TACE study

Serum samples collected from patients who underwent transarterial chemoembolization of therapeutic doses of epirubicin will be analysed and data will be reported in detail elsewhere, this field sample work being part of a larger comprehensive study by this laboratory. The type of data obtained in the present study is exemplified in Fig. 2. Consistent with our observations, it has been reported in the literature that plasma concentrations of a chemotherapeutic agent were minimal in patients treated with DEM-TACE at all time points when compared with conventional treatments [30]. Pharmacokinetic profiles depicted in Fig. 2 show that EPI concentrations ranged between 9.6 and 155.6 μg/L for DC-Beads and 6.6 and 42.2 μg/L for HepaSphere.

In a study of the new microsphere drug delivery system, this is the first time that concentration–time curves are plotted as a function of different types of eluting beads loaded with the same amount of epirubicin.

4. Conclusions

A specific, precise and rapid LC–ESI-MS/MS assay procedure has been developed and validated to quantify epirubicin released by microspheres in serum samples of patients with colorectal cancer metastases to the liver. This assay, designed to perform an important comparison study between two different types of eluting beads, was shown to be accurate enough to process both the fortified standard samples and the actual serum samples buffered to an acid pH value to stabilize epirubicin in matrix. The solid phase extraction procedure was chosen due to its greater reproducibility in comparison to LLE and high extraction efficiencies were also obtained under these sample preparation settings.

This assay procedure will be used by us in an already planned work to aid the clinical decision process in terms of attenuation of dose or even change of regimen.

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