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High-performance capillary electrophoresis measurement of dolastatin-10

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Abstract

A high-performance capillary electrophoresis (HPCE) assay was used to determine the concentration of a potent cytotoxic agent, dolastatin-10, in human plasma. Following extraction from plasma, using a solid-phase C_{18} cartridge, capillary zone electrophoresis was used to separate, detect and quantitate dolastatin-10 using the structurally related compound dolastatin-15 as the internal standard. Migration times for both dolastatins are less than 20 min. The recovery of the drug was approximately 90% and was quantified over the assay range of 39 to 5000 ng/ml with good precision and accuracy. The method is linear up to 5000 ng/ml with a lower limit of detection of 25 ng/ml. Data resulting from the use of the assay for the in vitro metabolism of the drug are presented. This is the first report of a validated HPCE assay for determining dolastatin-10 levels in human plasma.

Keywords: Dolastatin-10

1. Introduction

Dolastatin-10 (DOL-10, Fig. 1) is a unique linear pentapeptide (785 Da) isolated and fully characterized from the sea hare *Dolabella auricularia* as originally described by Pettit and coworkers [1,2]. This lipophilic peptide has been shown to be a very potent antineoplastic and antimitotic agent with efficacy against several murine leukemia tumor models and human tumor cell lines [3–7]. It is the first reported antimitotic compound derived from an animal source. DOL-10, one of several isolated dolastatins including the depsipeptide dolastatin-15 (DOL-15 with a molecular mass of 837, Fig. 1) from this marine animal [8–10], is composed of unusual amino acids unique to this species which are derived metabolically from more common essential amino acids. Structurally, DOL-10 consists of two α-amino acids (dolavaline and valine) and two γ-amino acids (dolaisoleucine and dolaproine) linked to an unusual primary amine (dolaphenine) at its carboxyl terminus. The three amino acids of the amino terminus of DOL-10 (Dov, Val and Dil) are reported to be required for tubulin binding while the remaining two residues (Dap and Doe) are needed for the interference with the vinca alkaloid binding and GTP binding to tubulin and in the uptake of DOL-10 by cells in culture [11,12].

Due to its potent cytotoxic potential and activity against a variety of tumor cell lines, DOL-10 was chosen by the National Cancer Institute for further evaluation as a potential antitumor agent. In anticipa-

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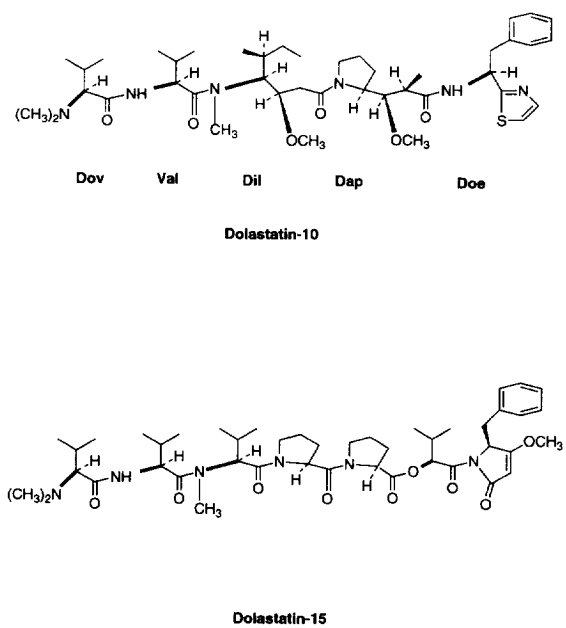

Fig. 1. Molecular structures of dolastatin-10 and dolastatin-15 (internal standard).

tion of clinical testing of this compound, an analytical assay was developed which could be used with additional enhancement of sensitivity for the evaluation of pharmacodynamic and toxicity parameters of DOL-10. This is the first report of an analytical method for determining DOL-10 concentrations in a biological plasma matrix. Pettit et al. [13] have reported a reversed-phase HPLC assay for both DOL-10 and DOL-15 in a synthetic mixture matrix; however, this method was developed to determine the purity of both drugs without consideration to biological samples and the necessity for sufficient assay sensitivity. A radioimmunoassay for DOL-10 has been reported by Aherne et al. [14] with a limit of detection (LOD) in mouse plasma of 2.5 ng/ml. Noteworthy in their pharmacokinetic studies with mice, the rabbit antiserum used in their assay was cross-reactive with DOL-15 to the extent of 65%. A nonspecific bioassay for determination of DOL-10, with growth inhibitory activity against the L1210 murine leukemia cell, has been developed for use in human and murine plasma [15]. Besides DOL-10, all cytotoxic species produced in this in vivo assay are quantified. This method has an LOD of 0.5 ng/ml in plasma. These investigators do not detail their HPLC method for DOL-10 as noted in their abstract. We have previously reported the preclinical pharmacokinetics of DOL-10 in mice using a tritiated form of the drug and measuring radioactivity in urine and plasma [16]. In this report we also describe a reversed-phase HPLC method, albeit not fully optimized, for separating the parent DOL-10 from metabolites generated using an activated S9 rat liver preparation. Herein, we report a validated HPCE method for the determination of DOL-10 in human plasma and present the application of this method towards the preliminary determination of metabolites generated in vitro from an activated S9 rat liver preparation.

2. Experimental

2.1. Reagents and chemicals

Both DOL-10 and DOL-15 were provided as dry powders from the National Cancer Institute (Bethesda, MD, USA). Methanol (HPLC grade) and hexane (ACS-certified grade) were obtained from EM Science (Gibbstown, NJ, USA). Butyl chloride (HPLC grade, 99.5+%) was obtained from Aldrich (Milwaukee, WI, USA). Water was obtained daily from a Milli-Q System (Millipore, Bedford, MA, USA). Sodium dihydrogen phosphate (monohydrate, ACS-certified) and potassium monohydrogen phosphate (anhydrous, ACS-certified) were obtained from Fisher Scientific (Houston, TX, USA). Magnesium chloride (hexahydrate), D-glucose-6-phosphate (98%, disodium salt hydrate), glucose-6-phosphate dehydrogenase (1000 units as suspension in 3.2 *M* ammonium sulfate) and β-nicotinamide adenine dinucleotide phosphate (NADP, 98%, sodium salt) were purchased from Sigma (St. Louis, MO, USA). The phosphoric acid (99.999%, 85% in water) and sodium hydroxide (99.99%, semiconductor grade) used in the HPCE assays were obtained from Aldrich. Buffers used in the HPCE assays were adjusted to the indicated pH using 1 *M* phosphoric acid and were filtered before use with a 0.2 μm nylon acrodisc syringe filter from Gelman Sciences (Ann Arbor, MI, USA). Outdated human plasma was obtained from the M.D. Anderson Cancer Center Blood Bank (Houston, TX, USA) and was cen-

trifuged to remove clotted material before use. The S9 fraction of rat liver homogenate (Aroclor 1254-activated) was obtained from Microbiological Associates (Rockville, MD, USA).

Stock solutions of DOL-10 and DOL-15 (100 μg/ml) were prepared in 100% methanol and stored at −20°C. Working solutions of each (25 μg/ml in methanol) were prepared from the stock solutions and kept at −20°C. Under these conditions the solutions were stable for at least 3 months. Sample aliquots were dried under nitrogen at ≤40°C before addition of plasma or sample buffer.

2.2. Extraction procedure

For method validation, plasma (1.0 ml) was added to dried samples (in glass) of DOL-10 (39–5000 ng) and the internal standard DOL-15 (1250 ng) to be analyzed. After vortex-mixing, the sample was diluted with water (4.0 ml), mixed, loaded onto a conditioned trifunctional t-C_{18} solid-phase extraction cartridge (Waters Sep-Pak, 100 mg/1 ml capacity) and vacuum-eluted. Cartridge conditioning involved rinsing with methanol (2 ml) followed by water (2 ml). Following plasma elution, each cartridge was washed with water (1 ml), vacuum dried for 2 min, then washed with hexane (1 ml) and the eluants discarded. The dolastatins were eluted with butyl chloride (3 ml) and the eluants dried under nitrogen at ≤40°C. Samples were stored dried at −20°C until analysis. Immediately before HPCE analysis, the samples were reconstituted in 50 μl of sample buffer (below) and transferred to microvial cups (50 μl capacity).

In the metabolism studies, a liquid–liquid extraction was used for the drug and metabolites. Each sample (1 ml) was shaken once with butyl chloride (5 ml) at high speed for 1 h using a rocker shaker (Eberbach Ann Arbor, MI, USA). The extractants were dried under nitrogen at room temperature and stored dried at −20°C until analysis.

2.3. Capillary electrophoresis

The high-performance capillary electrophoresis (HPCE) assay of DOL-10 was performed using a Beckman P/ACE System 2200 equipped with Gold 8.1 software for instrument control and computer data acquisition and integration and a Beckman P/ACE System 2100 equipped with P/ACE 2000 Series software. Both systems were coupled to IBM PS/2 computers. Each instrument was equipped with fixed-wavelength UV detection. Analysis of DOL-10 was achieved with an untreated, fused-silica capillary (Beckman) of dimensions 75 μm×57 cm (50 cm to detector window). The capillary cartridge contained an aperture plate of dimensions 100×200 μm. The run buffer was 50 m*M* NaH_2PO_4 (pH 2.9) and the sample buffer was 5 m*M* NaH_2PO_4 (pH 2.9)–methanol (9:1). On-line UV detection of components was monitored at 200 nm. A constant separation potential of 16.0 kV was used which produced a current of approximately 70 μA in this system. The sample was pressure-injected (0.5 p.s.i.) for 6 s (from the positive electrode) onto the capillary before the separation. The capillary temperature was maintained at 25°C during the 20 min separation. Data were collected at a rate of 5 Hz and a rise time of 1 s. Integration was done by peak area using the area ratio of DOL-10 to DOL-15 to correct for variations in extraction, sample injection, etc.. At the beginning of each day, the capillary was conditioned with the following solutions in the sequence: 100% methanol (10 min), 1 *M* NaOH (5 min), 0.1 *M* NaOH (5 min), water (5 min) and run buffer (15 min). An initial run of a neat sample of DOL-10 was necessary to allow the capillary to achieve proper equilibration before the running of actual samples. Following each separation, the capillary was rinsed with the following solutions in the sequence: 0.1 *M* NaOH (3 min), water (2 min), run buffer (2 min) and run buffer equilibration (0.5 min).

2.4. UV spectra

The UV absorption spectra for the dolastatins were obtained using a scanning Cary 1E UV–Vis spectrophotometer (Varian Instruments) at room temperature. Spectra of each were obtained in 10 and 100% methanol.

2.5. Linearity

The linearity of the method was assessed by adding human plasma (1.0 ml) to increasing amounts of DOL-10 (dried sample) for the extraction samples.

For neat samples, sample buffer (50 μl) was added to increasing amounts of DOL-10 (dried sample). Concentrations of DOL-10 ranged from 39 to 5000 ng/ml (DOL-15 internal standard at 1250 ng/ml). The ratio of the peak areas for DOL-10 to DOL-15 were plotted against the DOL-10 concentration to check for linearity, and the correlation coefficient and the estimate of the error about the line were calculated. Analyses were performed in duplicate or triplicate and the average values were reported. The linearity curve was used to calculate the concentration of DOL-10 in the plasma samples of the validation study.

2.6. % Recovery

The extraction efficiency was assessed by comparing the analyzed DOL-10 levels of the extracted samples to those of the neat samples at each individual level performed in duplicate or triplicate. DOL-10 concentrations were 39, 78, 156, 313, 625, 1250, 2500 and 5000 ng/ml. Negative controls were also analyzed.

2.7. Precision and accuracy

Both within-day and between-day assay precision were determined for extracted samples. Two concentrations of DOL-10 (500 and 3000 ng/ml) were included in these studies. For within-day precision, 10 replicates of each sample were tested on the same day. For between-day precision, each sample was tested in triplicate on four separate days. In each precision study, the mean concentration, standard deviation and percentage coefficient of variation were calculated. The reproducibility in the migration times for DOL-10 and DOL-15 was also assessed for these samples. For accuracy, comparison of the expected levels of DOL-10 to the measured levels was used and the percentage relative error determined.

2.8. Sensitivity

The limit of detection or sensitivity for DOL-10 was defined as the lowest concentration of the drug resulting in a signal-to-noise ratio of 4:1 for a blank plasma sample. The limit of quantitation was determined to be that level of drug with a coefficient of variation (C.V.) and a percentage deviation of the nominal concentration of less than 20%.

2.9. DOL-10 metabolism in vitro

A mixture of S9 fraction (0.5 ml, 10 mg protein) and NADPH-generating system (0.5 ml, final concentrations: 1 m*M* NADP, 10 m*M* glucose-6-phosphate, 0.25 units/ml of glucose-6-phosphate dehydrogenase, 4 m*M* magnesium chloride and 100 m*M* potassium phosphate buffer; pH 7.4) was added to 10 μg DOL-10 (dried). Samples (in duplicate) were incubated at 37°C for 1, 15 and 30 min. A "0" time control was prepared by immersing the sample in ice immediately after adding reagents. Upon completion of metabolism, samples were quenched in ice for 1 min, centrifuged to remove debris and then extracted with butyl chloride (above) prior to HPCE analysis.

3. Results and discussion

Of the two possible modes available in HPCE for the separation of drug/metabolite compounds, CZE (capillary zone electrophoresis) [17] and MECC (micellar electrokinetic capillary chromatography) [18], we chose CZE for the analysis of DOL-10. Although DOL-10 is lipophilic, it possesses one ionizable group (a tertiary amine at the amino terminus of the peptide structure) that led us to consider CZE initially in the method development. It is likely that the MECC technique could have been employed since it finds wide use in the analyses of both neutral, hydrophobic compounds and relatively polar, ionizable solutes. In the CZE technique, we chose a conventional acidic, phosphate-buffer system that is typically used in the HPCE analysis of peptides [19]. The sample buffer concentration was one-tenth the concentration of the run buffer (5 m*M* versus 50 m*M*) in order to optimize the separation and sensitivity in the system. It was necessary to have 10% methanol in the sample buffer for solubilization of DOL-10. Under these acidic conditions (pH 2.9), the tertiary amine is expected to be fully protonated (positively charged). Both dolastatins have a relatively low charge density at pH 2.9 (only one positively charged site in each molecule) and

therefore migrate relatively slowly (electrophoretically) towards the cathode in HPCE in comparison to the other components in the extracted sample. Additionally, the electroosmotic flow provides migration of all components towards the cathode. We used a bare fused-silica capillary with dimensions 75 μm× 57 cm for our separations. No attempt was made to use specially-designed coated or chemically-modified capillaries for these separations. The longer capillary served to give good separations while the larger diameter provided better sensitivity of DOL-10. From the electropherograms, we observed a degradation of DOL-10 and DOL-15 in the sample buffer when left at room temperature for a few hours but remained stable for about 3 days when stored at −20°C (data not shown). DOL-15 showed a more rapid degradation compared to DOL-10 due likely to the relative ease in acid hydrolysis of the one ester bond in DOL-15 (DOL-10 is devoid of ester linkages; amide bonds are much more difficult to hydrolyze compared to ester bonds). Consequently, we elected to keep the samples dry at −20°C until HPCE analysis.

With the limited availability of fixed-wavelength UV detection on the HPCE instrument, we chose to monitor at low UV (200 nm) in order to obtain the greatest sensitivity for DOL-10 in the extracted plasma samples. As expected at this wavelength, many signals would be predicted to be present in the electropherograms due to the non-specificity of this detection. However, this did not present problems in our plasma analyses due to the use of the t-C_{18} cartridge in combination with butyl chloride elution for sample preparation giving remarkably clean electropherograms (Fig. 2). Monitoring at 214 nm gave significantly smaller signals in the electropherograms. The UV absorption spectrum of DOL-10 in water–methanol (9:1) gave λ_{max}=206 nm (ε=25 700 M/cm) and 238 nm (ε=7400 M/cm). For DOL-15, λ_{max}=207 nm (ε=29 250 M/cm) and 247 nm (ε=12 000 M/cm). The UV spectra in 100% methanol showed only slight absorption shifts from those run in 10% methanol with higher extinction coefficients at the lower wavelengths observed in 10% methanol.

Solid-phase extractions (SPE) of DOL-10 were used for the drug-spiked human plasma samples. Shown in Fig. 2 are typical electropherograms from analyses of DOL-10 using DOL-15 as the internal standard. Clearly, both dolastatins are well-resolved from each other and from endogenous peaks. Average migration times (as defined from the precision studies performed during method validation) were 15.7 min for DOL-1.0 and 16.5 min for DOL-15, a separation of 0.8 min and an average migration time of 1.3 min for the combined compounds. The Waters t-C_{18} solid-phase cartridge provided superior extraction results for the dolastatins compared to other SPE cartridges from various manufacturers (C_{18}, C_8, C_2, cyanopropyl, cyclohexyl and phenyl bonded phases), which failed to sufficiently retain either dolastatin. Butyl choride was shown to be a selective elution solvent for the dolastatin compounds using the specific t-C_{18} cartridge. Following a variety of wash steps, the use of other more conventional elution solvents (methanol, acetonitrile and ethyl acetate) for the dolastatins resulted in several other components, including co-eluting interferents, being removed from the solid-phase support (data not shown). Elution with dichloromethane gave broad signals prior to the relatively clean migration region of the dolastatins. For monitoring the dolastatin metabolites using HPCE, the butyl chloride elution is superior to other elution solvents. In our procedure, the hexane wash was required to remove highly lipophilic components in the plasma, many migrating prior to the migration of the dolastatins and some in major amounts that co-migrated with the dolastatins (data not shown). Hexane did not affect the recovery of the dolastatins. Liquid–liquid extractions (LLE) using either ethyl acetate or butyl chloride gave clean electropherograms and excellent recoveries, albeit at the higher concentration range for DOL-10. Very poor recoveries were observed at the lower concentration range. Diethyl ether LLE gave very broad, ill-defined electropherograms using the HPCE conditions. It is important to note that the plasma sample must be diluted at least 1:4 with water prior to the SPE procedure in order to get adequate recovery of the drug and internal standard. Failure to dilute the sample or diluting less than the above amount results in a much poorer recovery of DOL-10 (typically 40–60% recovery). Losses were shown to be due to column overloading. The Waters t-C_{18} cartridge has been applied to the pre-treatment of aqueous environmental samples for trace organic analysis [20]

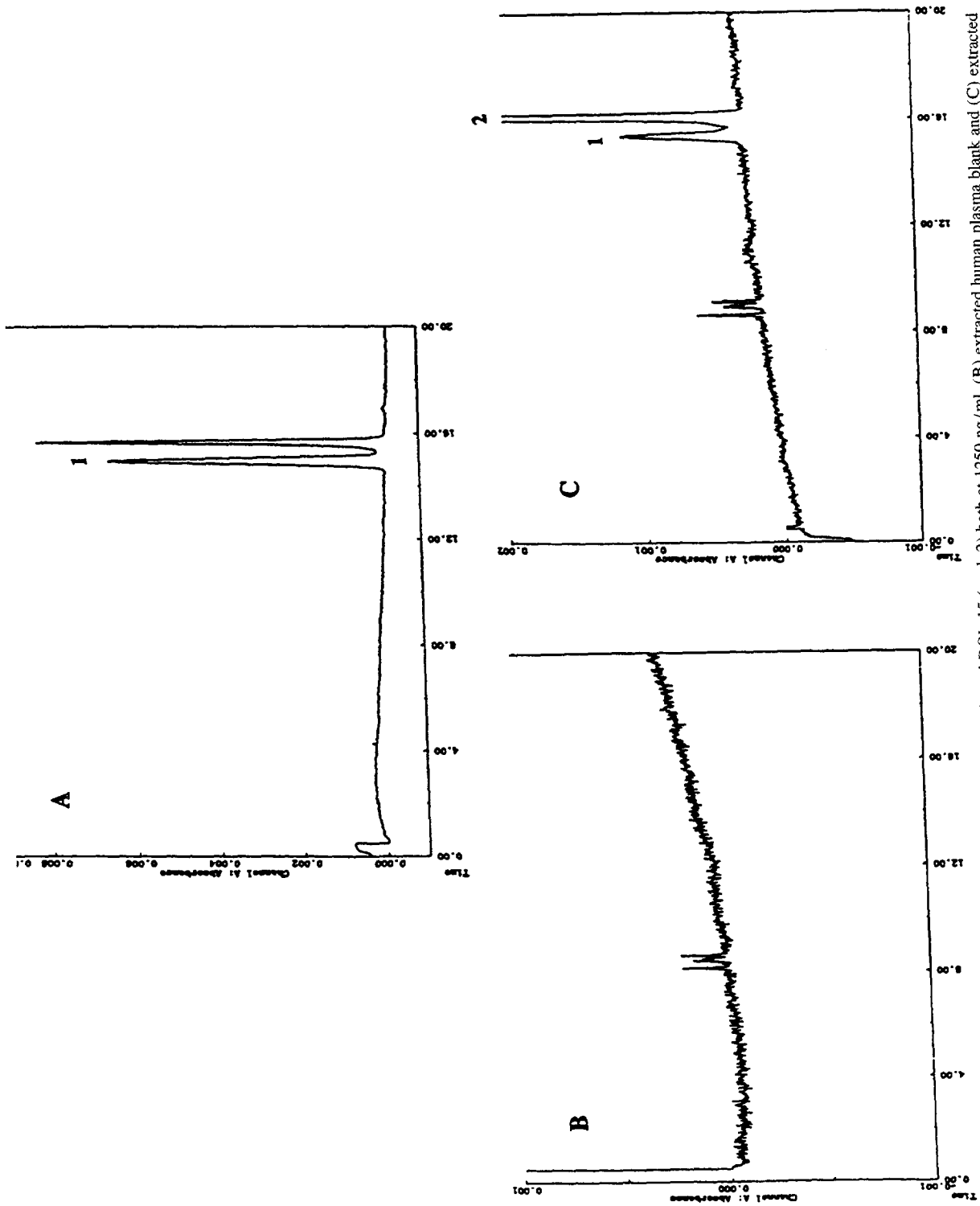

Fig. 2. HPCE electropherograms of (A) neat sample of DOL-10 (peak 1) and DOL-15 (peak 2) both at 1250 ng/ml, (B) extracted human plasma blank and (C) extracted human plasma containing DOL-10 (peak 1: 313 ng/ml) and DOL-15 internal standard (peak 2: 1250 ng/ml).

and for drugs and endogenous components in relatively large volumes of biological matrices [21–23]. That concept led us to consider a large dilution of the plasma sample prior to loading onto the cartridge. The larger size Waters t-C_{18} cartridge (200 mg/3 ml capacity) was shown to be useful for more rapid processing of plasma samples of at least 1 ml amounts.

This HPCE assay represents one of many rapidly-emerging HPCE techniques used to measure drug and metabolite concentrations in biological matrices [24,25]. DOL-15, the internal standard used in the present assay, may also be developed as an anti-cancer agent [26]. In that case, replacing DOL-15 with DOL-10 as the internal standard would provide a suitable method for quantitation of DOL-15 in plasma. Although the levels of DOL-10 expected to be encountered in upcoming clinical trials will be significantly lower than the limit of detection (LOD) for this HPCE analytical assay, enhancement of sensitivity can be accomplished by on-line coupling of the HPCE instrument to a mass spectral (MS) detector. Reports on this technique are quickly proceeding from the analytical field [27,28]. The MS detector would provide the necessary high sensitivity and specificity needed for analyzing DOL-10 drug levels in the low ng/ml to pg/ml therapeutic concentration range in plasma. Additionally, in-depth metabolite studies of DOL-10 could be undertaken. Fluorescence detection of DOL-10 is not possible without labelling the molecule first with a fluorescent tag. This would require some novel chemistry since the molecule does not possess any identifiable sites for conventional fluorescent labelling. Electrochemical detection would not be expected to provide the sensitivity necessary for the analyses in patient samples. Currently, the electrochemical detector for the HPCE system is not commercially available. There are other means available to enhance the sensitivity in HPCE (comparable to or even better than HPLC) using conventional IJV detection [29,30]. These methods involve novel designs in the capillary structure at the detector window and on-line concentration techniques of the sample following injection (stacking and isotachophoresis). Again, although these methods promise to enhance the LOD to possibly low ng/ml for DOL-10, they would not offer the sensitivity for clinically-relevant samples. For our assay a significant amount of stacking is probably occurring with the dolastatins, although additional enhancement of sensitivity may be possible by using buffered-pH gradients and higher separation temperatures. Nonetheless, the excellent separation efficiencies, rapid and reproducible analysis times, minimal sample volumes and cost effectiveness afforded by HPCE in combination with better sensitivity detectors (i.e. electrochemical [31], fluorescence [32] and mass spectral [28]) are expected to make for a more ideal instrument for drug/metabolite separation and structural identification in clinical pharmacology studies.

3.1. Assay precision and accuracy

Within-day and between-day reproducibilities for DOL-10 extracted from human plasma are shown in Table 1. Two different drug levels were tested (500 and 3000 ng/ml). For precision, the coefficients of variation (C.V.) ranged from 6.2 to 12.8%. For accuracy, the relative errors ranged from 1.7 to 15.2%. During these studies the precision was also determined for the migration times in the electropherograms. For the intra-assay studies, the mean migration times for DOL-10 and DOL-15 were 15.69±0.63 min (C.V.=4.17%) and 16.47±0.66 min (C.V.=4.0%), respectively, at 500 ng/ml. At 3000 ng/ml, the mean migration times were 15.78±0.89 min (C.V.=5.6%) and 16.61±0.95 min (C.V.=5.7%) for DOL-10 and DOL-15, respectively. For the inter-assay studies, the mean migration times for DOL-10 and DOL-15 were 15.71±0.78 min (C.V.=5.0%) and 16.47±0.83 min (C.V.=5.0%), respectively, at 500 ng/ml. At 3000 ng/ml, the mean migration times were 15.69±0.44 min (C.V.=2.8%) and 16.49±0.47 min (C.V.=2.9%) for DOL-10 and DOL-15, respectively. The reproducibility results in the drug migration times (3–6% C.V.) are typical for HPCE assays [33]. Improvements are possible by frequent replenishment of run buffers with fresh ones during continuous runs.

3.2. Linearity and sensitivity

The HPCE assay for DOL-10 was linear over the concentration range 39–5000 ng/ml in human plasma. The equation resulting from the analysis of the

Table 1
DOL-10 precision/accuracy validation

Nominal conc. (ng/ml)	Mean calculated conc.±S.D. (ng/ml)	% C.V.[a]	% Relative error[b]
Within-day precision and accuracy (n=10 at each level)			
500	424±36 (high=479, low=364)	8.6	15.2
3000	3160±190 (high=3660, low=2930)	6.2	5.3
Between-day precision and accuracy (n=12 at each level)			
500	479±61 (high=598, low=386)	12.8	4.2
3000	2950±230 (high=3150, low=2450)	7.8	1.7

[a] % C.V.=(S.D./mean conc)×100.
[b] % Relative error=[(calculated mean−nominal value)/nominal value]×100.

regression plot for DOL-10 was $y=0.846x-0.016$, where y is the peak-area ratio (DOL-10/DOL-15) and x is the concentration of DOL-10 (in μg/ml). The coefficient of correlation (r) was 0.999 (r^2= 0.998) and the standard deviation of residuals from the line ($S_{y/x}$) was 0.070. This equation was used to determine concentrations of DOL-10 in the plasma samples of the validation study. For comparison, the neat samples of DOL-10 yielded a line with the equation $y=0.930x-0.043$ ($r=1.00$, $r^2=0.999$ and $S_{y/x}=0.048$).

The sensitivity for this DOL-10 assay was determined to be 25 ng/ml in human plasma at a signal-to-noise ratio of 4:1. No endogenous components interfered with this determination from a blank sample. The limit of quantitation for this assay was established at 39 ng/ml DOL-10. At this level the C.V. was 19.8% (n=7) with a percentage deviation of the nominal concentration of 17.9% (mean conc.=32±6 ng/ml). It is interesting to note that a very small peak (as a shoulder) immediately precedes the appearance of the DOL-15 peak in the electropherograms. This peak did not interfere with the quantitation of DOL-10 and is apparently an intrinsic contaminant of the standard sample of the internal standard.

3.3. Extraction recoveries

The extraction recovery for each of the different DOL-10 concentrations (n=2 or 3) between 156–5000 ng/ml varied between 89 and 126% based on comparison of the peak-area ratio from the extracted samples to the peak-area ratio from the neat samples. Lower recoveries, however, were observed at the lowest concentrations analyzed (78 and 39 ng/ml DOL-10). The variance in recoveries observed for the different concentrations of DOL-10 was controlled by use of DOL-15 as the internal standard.

3.4. DOL-10 metabolism in vitro

The in vitro metabolism of DOL-10 using an S9 rat liver preparation revealed that three metabolites are produced, a major and two minor ones in approximately equal abundance. Shown in Fig. 3 are the electropherograms of the extracted metabolite mixture incubated at 37°C for 30 min and the control. An LLE method was used for sample pretreatment rather than the SPE method because of the large amount of drug used in this study. The use of butyl chloride in the extractions resulted in exceptionally clean electropherograms throughout the 20 min run. Baseline separation of all species was observed. The major metabolite (M1) migrated prior to and very near the migration of the parent drug. The average migration time of M1 was 13.0 min for all the samples tested (DOL-10 averaged 13.9 min). The two minor metabolites (M2 and M3), with nearly identical peak areas, migrated later (average migration times of 16.9 and 18.7 min, respectively). No additional signals were observed with up to 30 min of run time. There was a time dependent increase (0 to 30 min) in the peak area of M1 with concomitant decrease in DOL-10 over the 30 min

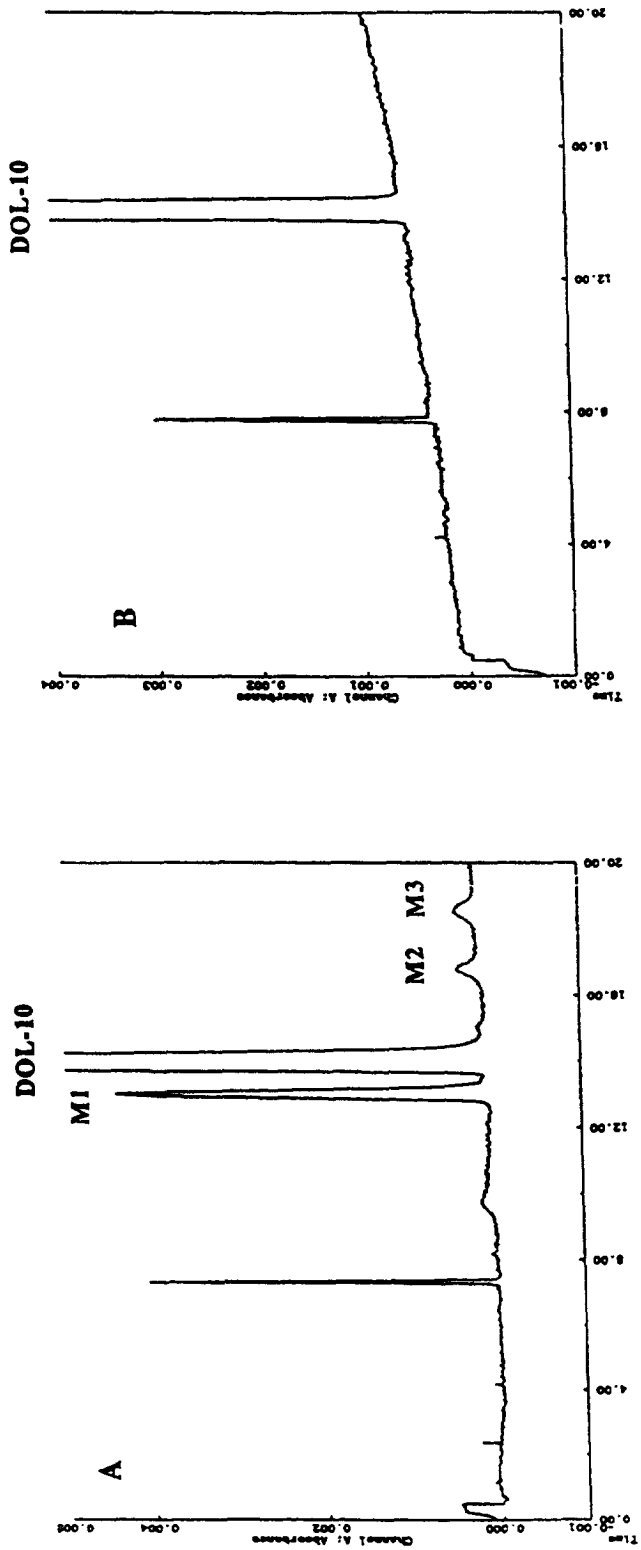

Fig. 3. HPCE electropherograms of DOL-10 metabolism mixture using S9 rat liver preparation (A) 30 min incubation at 37°C and (B) control.

incubation period. The time-dependent formation of M2 and M3 is less clear.

We are preparing to perform a semi-preparative scale separation of these metabolites on the HPCE system in order to fully characterize the structures of these species using an off-line mass spectral analyzer. Comparison of these results to those reported from our pre-clinical studies of DOL-10 [16], showing a dihydroxylated species as being produced in the same S9 preparation, will be made. These efforts will be of great benefit to the clinical phase trial studies.

4. Conclusions

We have developed a HPCE method for DOL-10 in human plasma that is quantified over the assay range of 39 to 5000 ng/ml with a sensitivity of 25 ng/ml using 1 ml of sample. The recovery of the drug averaged approximately 90% over this range of concentrations. Use of the specific t-C_{18} solid-phase extraction cartridge coupled with butyl chloride elution gave very clean electropherograms. Run times were less than 20 min for the dolastatins. Full performance evaluation of this method gave good precision and accuracy and excellent linearity over the assay range. This method was applied to the in vitro metabolism of the drug using an S9 rat liver preparation. This study revealed that up to three metabolites are produced, a major and two minor species. Continuing experiments in our laboratories will establish the identity of these metabolites in an effort to understand more fully the pharmacokinetics and dynamics of this current, clinical phase trial drug. Also, a more sensitive HPCE assay for DOL-10 using conventional UV detection or mass spectral detection will be helpful for the analysis of clinical samples.

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