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Simultaneous micellar electrokinetic chromatography and liquid chromatography of adriblastina and tarabine PFS Their application to some biological fluids

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Abstract

The current work presents analytical procedures for simultaneous determination of tarabine PFS and adriblastina by micellar electrokinetic chromatography (MEKC) and liquid chromatography (LC). For MEKC analysis, separations and identifications were accomplished using uncoated fused-silica capillary with hydrodynamic injections in the presence of 50 mM borate/phophate pH 8.7 and 100 mM SDS. The migration times of tarabine PFS and adriblastina were found to be 2.70 and 4.40 min, respectively. Calibration curves were established for 10–300 ng/mL ($r = 0.998$) tarabine PFS and for 8–120 μg/mL ($r = 0.999$) adriblastina. For LC analysis, separations were performed on teicoplanin stationary phase with reversed mobile phase containing methanol–buffer pH 4.05 (20:80%, v/v) at 285 nm. The retention times of tarabine PFS and adriblastina were 5.18 and 7.20 min, respectively. Calibration curves were established for 3–90 μg/mL ($r = 0.998$) tarabine PFS and for 10–120 μg/mL ($r = 0.999$) adriblastina. Finally, MEKC and LC methods were applied for the simultaneous determination of analytes in urine samples.

Keywords: Tarabine PFS; Adriblastina; MEKC; LC; Simultaneous determinations; Urine samples

1. Introduction

Adult acute myeloid leukemia (AML) or acute non-lymphocytic leukemia (ANLL) is a disease in which cancer (malignant) cells are found in the blood and bone marrow. The bone marrow is the spongy tissue inside the large bones in the body that makes red blood cells, white blood cells and platelets. There are several treatments for all patients with AML; the primary treatment is chemotherapy. Advances in the treatment have resulted in substantially improved complete remission rates. Perhaps the most common drug treatment plan of AML is the induction regimen consisting of combination chemotherapy of tarabine PFS plus daunorubicin or adriblastina [1–3]. For the treatment courses that consisted of 7 days continuous infusion with tarabine PFS and 3 days of adriablastina, 78% of patients went into remission [3]. Tarabine PFS was also used in combination chemotherapy with adriblastina for acute limphoblastic leukemia (ALL) resulting in cure rates of 30–70%. Unfortunately, some of fatal side effects can be arisen with this kind of chemotherapy, e.g. cardiotoxicity (heart damage), gastrointestinal effects (nausea, vomiting, diarrhea), bleeding and fever. Therefore, it is necessary to evaluate simple, fast and reliable analytical methods for the simultaneous determination of tarabine PFS and adriablastina in biological fluids.

Macrocyclic antibiotics represent a relatively new class of selectors in liquid chromatography (LC), which have been introduced by Armstrong and co-workers [4,5] and showed high selectivity and sensitivity for several achiral and chiral compound classes [6]. It was found that only eight antibiotics

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have been used as selectors; one of the recent selectors is teicoplanin phase. This column is packed with a unique chromatographic stationary phase produced by chemically bonding the macrocyclic glycopeptide teicoplanin to a high purity 5 μm silica gel through spacer arm of optimum chain length, which yields several stable ether linkages that are non-hydrolytic. The teicoplanin is considerably more surface active having three sugar moieties surrounding four cavities and a methyl dodecyl side chain. All of the defined mechanisms for this kind of columns include complexation, hydrogen bonding, dipole stacking and steric interactions as well as the inclusion mechanism.

In literature, there are several reports for the determination of tarabine PFS individually or with its metabolites or synthetic analogs by capillary electrophoresis (CE) [7,8] or LC [9–11]. Also, adriblastina was determined by CE [12–15] or LC [16–20] individually or with its major metabolites and/or synthetic analogs. These methods suffer from long analysis times or pretreatment of biological samples or low sensitivity. Unfortunately, there are few reports for the simultaneous determination of pharmaceutical compounds that are used recently in combination chemotherapy. At the best of our knowledge, none of these reports was devoted for the simultaneous quantification of tarabine PFS and adriblastina by CE or LC.

The aim of the present work is studying and optimizing reliable analytical methods for the simultaneous separation of tarabine PFS and adriblastina using micellar electrokinetic chromatography (MEKC) and liquid chromatography. Furthermore, estimating of their quantities in biological fluids like urine samples was achieved.

2. Experimental

2.1. Materials and solutions

Fresh solutions of tarabine PFS (cytarabine, 99%) and adriblastina (doxorubicin hydrochloride, 98%) were prepared daily in double distilled water. Solutions were diluted with the same solvent as required for standard solutions. Tarabine PFS was provided by Upjohn s.a., Puurs, Belgium. Adriblastina was provided by Pharmacia Upjohn S.P.A. (Milan, Italy). Sodium dodecyl sulphate (SDS) and sulfadimethazine (used as internal standard) were purchased from Fluka (Milan, Italy). Sodium hydroxide, phosphoric acid, di-sodium hydrogen phosphate, boric acid, *N*-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropan-sulfonic acid (AMPSO), methanol (ultrapure for HPLC) and acetonitrile 200 (for UV) were obtained from Carlo Erba (Milan, Italy). Triethylamine (99%) was purchased from Sigma and acetic acid (99.7%) was obtained from Aldrich (Germany). All organic solvents were used without further purifications. All other chemicals were of analytical reagent grade and were used without further purification. Water used for the preparations of solutions and mobile phases was purified by a Milli-Rx apparatus (Millipore, Milford, MA, USA).

The running buffer in MEKC analysis was prepared by mixing the adequate volume of 50 mM aqueous solution of boric acid and phosphoric acid, then adjusting pH with 1 M NaOH. An accurately weighed amount of SDS was then added and then filling up the volume with water.

The mobile phases used for Chirobiotic T™ LC column using a polar organic phase were composed of different percentages (0.1, 0.2, 1.0, 2.0%) of acetic acid and triethylamine in the presence of 100% methanol. The mobile phases for reversed phase were composed of either water or 1.0% triethylamine acetate (TEAA) buffer with different amounts of methanol or acetonitrile as organic modifiers. The buffer was prepared by dissolving 1.0 mL triethylamine in 200 mL water and then adding glacial acetic acid to adjust the appropriate pH value prior to the addition of buffer and an organic modifier. The mobile phase used for normal phase mode was composed of 20% isopropanol and 80% *n*-hexane.

The preparation of urine samples for drug determination was done as mentioned in previous work [21].

2.2. Apparatus

2.2.1. For MEKC analysis

All separations were carried out using a ^{3D}CE capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany), equipped with a diode array detector. The data were collected on a personal computer using the ^{3D}CE-ChemStation software, Version A09. Uncoated fused-silica capillary (Agilent Technologies) of 48.5 cm (40 cm effective length) × 50 μm i.d. was used.

2.2.2. For LC analysis

Separations on Chirobiotic T™ LC (Astec, Whippany, NJ, USA) column 250 × 4.6 mm i.d. (teicoplanin) were carried out with Beckman 110B Solvent Delivery System, injector Reodyne and Beckman System Gold 166 Detector. Data were collected with Gold Nouveau Chromatography Data System, Version 1.6 (Beckman Instruments Inc.). The pH values of mobile phases were adjusted using pH-meter (basic 20, Crison) at 20 ± 2.0 °C. This instrument was calibrated by using standard universal buffer solutions at different pHs.

2.3. Analytical separation conditions

2.3.1. For MEKC analysis

Running buffer: One hundred millimolar SDS in 50 mM phosphate/borate buffer (pH 8.7), applied voltage; 15 kV with anode at the inlet and cathode at the outlet, current; 57.5 μA, temperature; 25 °C, detection; UV–vis absorbance at λ = 260 nm or 300 nm, capillary; uncoated fused-silica 50 μm (i.d.) × 48.5 cm (effective length 40 cm) with a detection window built-in by burning off the polyimide coating on the capillary, hydrodynamic injection; pressure 50 mbar

for 5 s. Before the injection into the CE system, each solution (running buffer and sample solutions) was subjected to filtration through a 0.22 μm membrane.

Washing procedure: Prior the first use, the capillary was conditioned by flushing 1 M NaOH at 50 °C for 20 min followed by a rinse with 0.1 M NaOH for 10 min and finally washed with water at 25 °C for 10 min. At the beginning of the day, the capillary washed briefly with 0.1 M NaOH for 2 min, water for 3 min and then equilibrated with the running buffer for 5 min. The repeatability of migration times was found to be strongly dependent on the rinse procedure; the highest reproducibility was obtained by flushing the capillary between runs as following: 1 min with 0.1 M HCl, 1 min with water and 2 min with running buffer. Vials of buffer were replaced every five injections to keep the same reservoirs level of the buffer and to avoid changes of electroosmotic flow (EOF) due to the electrolysis of the solutions. At the end of the day, the capillary washed carefully with 0.1 M NaOH for 5 min and finally with water for 10 min.

2.3.2. For LC analysis

The mobile phase: Methanol:buffer (1% TEA, acetic acid) (20:80%, v/v, pH 4.05), temperature; 25 °C, flow rate; 0.8 mL/min, detection; UV–vis absorbance at $\lambda = 285$ nm, injected amount; 0.1 mg/mL of sample solution using loop injections (5 μL). Mobile phase was filtered through 0.45 μm membrane filter and degassed before using for LC analysis.

Washing procedure: For the first use, the column was washed with 20 mL water at 0.8 mL/min and then conditioned with desired mobile phase for 1 h until equilibrium was established when a stable baseline was achieved. At the end of the day the column was washed with methanol or acetonitrile:water (50:50%, v/v). For long-term storage, the column was conditioned with isopropanol.

2.4. Calibration curves

2.4.1. Simultaneous standard addition of tarabine PFS and adriblastina

Standard stock solutions of tarabine PFS, adriblastina (1500 μg/mL) and sulfadimethazine (500 μg/mL) were prepared in water. Working solutions were prepared by diluting with the same solvent. For MEKC analysis, five mixed standard solutions were prepared by mixing 250 μg/mL of internal standard solution (sulfadimethazine) with diluted working solutions to give concentrations 50–1000 and 8–200 μg/mL of tarabine PFS and adriblastina, respectively. Triplicate injections were measured at 300 nm for each solution and the ratios of the corrected peak area (peak area/migration time) of drug to that of internal standard were plotted against the drug concentration to obtain the calibration graphs. For LC analysis, five mixed standard solutions were prepared containing tarabine PFS concentrations 3–100 μg/mL and adriblastina concentrations 12–200 μg/mL. The calibration graphs were plotted between the peak heights (average value of triplicate injections at 285 nm for each solution) against the analyte concentration.

2.4.2. Standard addition of tarabine PFS or adriblastina in the presence of a high constant amount of another

Standard stock solutions of tarabine PFS and adriblastina (4000 μg/mL) were prepared in water. Working solutions were prepared by diluting with the same solvent. For MEKC analysis, five standard solutions have been prepared by mixing 190 μL of compound wanted to be investigated with 10 μL of another compound giving concentrations ranged from 0.1 to 3.0% (m/m). Triplicate injections were made for each standard solution. Ultra-trace measurements of tarabine PFS were obtained at 260 nm while adriblastina was measured at 300 nm. The calibration graphs were constructed by plotting corrected peak area (corrected peak area of analyte/corrected peak area of the external compound) against concentration of analyte. For LC analysis, the same procedure has been made at 285 nm, pH 4.05. The calibration graphs were plotted between peak heights against concentrations of analyte.

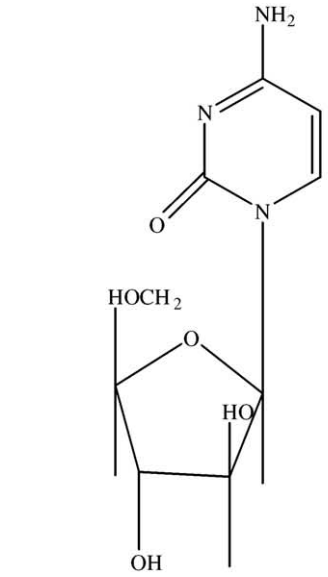
3. Results and discussion

3.1. Method optimization

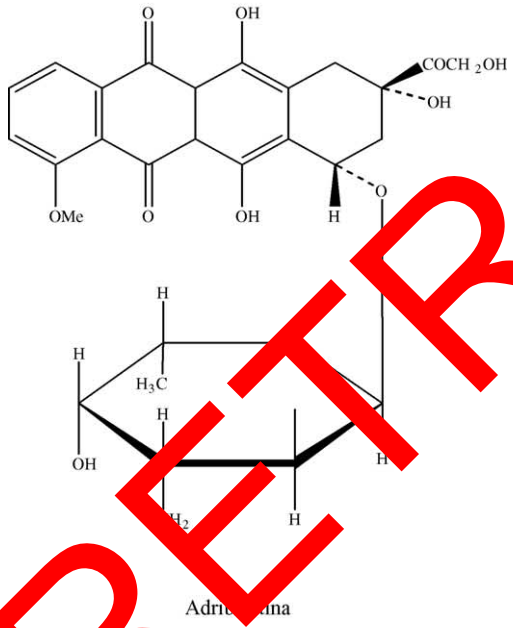
3.1.1. MEKC analysis

The structures of both tarabine PFS and adriblastina are depicted in Fig. 1. From the analytical ionization point of view for the distinct functional groups in both compounds, it was predicted that these compounds exist in protonated form in highly acidic media and in deionized form in slightly acidic and alkaline media. In highly alkaline media, adriblastina ($pK_a = 9.36$) should be ionized giving its anionic form. Therefore, in the pH range (7.5–10), the simultaneous assay of neutral tarabine PFS and adriblastina required the use of MEKC to give simple, selective, reproducible and rapid analysis. In this operational mode, micelles added to the buffer solution form with the neutral compound a charged complex with an effective electrophoretic mobility [22]. The differential partition of the neutral species in the retentive phase obtained gives rise to the separation due to their differential migration rates.

Nature and concentration of buffer and surfactant, buffer pH and instrumental parameters such as temperature and applied voltage can all significantly influence MEKC analysis and should be incorporated in the method development strategy. Preliminary studies showed that sodium dodecyl sulphate was a good choice as anionic surfactant in the desired pH range. The most familiar alkaline buffers (pH 7.5–10) like Britton–Robinson, borate, phosphate, borate/phosphate and AMPSO buffers were tested and the most promising results were obtained with borate/phosphate buffer. This latter offered the best selectivity and in general had a good buffering capacity in a quite large pH interval, making it is possible to increase the buffer concentration and to add the desired SDS



Tarabine PFS



Adri[illegible]ina

Fig. 1. Structure of drugs investigated.

amount without an excessive rise in the measured current. The buffer concentrations were tested in the range (20–80 mM) with varying the pH values (7.5–10) on the separation of tarabine PFS and adriblastina considering the analysis time, sensitivity and selectivity. The best results with acceptable generated current ($\leq$70 μA) were achieved in the presence of 50 mM borate/phosphate buffer at pH 8.7. During the optimization, one problem related to the morphology of tarabine PFS peak (the first peak) was noticed due to its closeness to the system peak. Therefore, several trials with high surfactant concentrations starting from 60 up to 120 mM (Fig. 2A and B) were tested keeping into consideration the analysis time required for complete separation of both analytes. A further increase of SDS concentration was not considered of practical use because of the high current generated. The best rapid, sensitive with high resolution and acceptable morphology of the first peak were achieved in the presence of 100 mM SDS. The other operational parameters, e.g. temperature and applied voltage, were also optimized. The applied voltages were tested in the range (5–20 kV); the best analysis time with preferable current value and se[illegible] was achieved by applying voltage 15 kV at cont[illegible]led temp[illegible]ature 25 °C. The migration order of both ana[illegible]es was checke[illegible] by injecting each individually; it w[illegible] fou[illegible] that tarabi[illegible] PFS migrated first and then adrib[illegible]stina. The [illegible]avelen[illegible] was set at 300 nm for all followin[illegible] simultan[illegible]ous det[illegible]ations of tarabine PFS and adribla[illegible]na in [illegible]ach micrograms of both analytes were achie[illegible]d. Ho[illegible]r, wavele[illegible]th 260 nm was used for nanogram [illegible]determinatio[illegible] of tar[illegible]ine PFS in the presence of a high c[illegible]nst[illegible]t amount of [illegible]lastina as described in the experimental part.

3.[illegible].2. LC analysis

LC was also applied for comparison purposes and was offered an alternative approach for CE. Teicoplanin stationary p[illegible]se was use[illegible] as it is known to possess good selectivity for achi[illegible] and [illegible]iral pharmaceuticals. Also, teicoplanin phases allow a sensitive simultaneous determination of both analy[illegible]with rapid analysis time compared to other columns [9–11,16–20]. This behavior may be attributed to its characteristic surface activity due to the presence of four fused macrocyclic rings, which form "semirigid basket", four ionizible phenolic moieties and three carbohydrate moieties consisting from D-glucosamine and D-mannose as well as its rapid kinetic interactions. Furthermore, teicoplanin column appears to be a multimodal stationary phase in that it can be used in the reversed phase mode, normal phase mode and polar-organic mode with great stability and capacity. This character is very useful to visualize full picture for the separation of two drugs and the separation mechanism.

3.1.2.1. Polar organic phase mode. This is a modification of the polar organic mode described for cyclodextrin bonded phases due to the presence of stronger polar groups in the macrocyclic peptides. The key factor in obtaining complete separation is the acid/base ratio. The concentrations of acid and base can be varied from 2.0 to 0.001%. Above 2%, the analyte becomes too polar and the system assumes typical reversed phase behavior, whereas below 0.001% it behaves as in normal phase chromatography [23]. Therefore, the variation of acid and base concentrations between 2 and 0.1% in the presence of 100% methanol has been studied. It was found that the separation of adriblastina with 1/2 (acid/base ratio) was not achieved. By raising the acid content, the simultaneous separation of both analytes was evaluated but with long analysis time up to about 40 min due to the increase of solvent polarity. By checking the elution order of

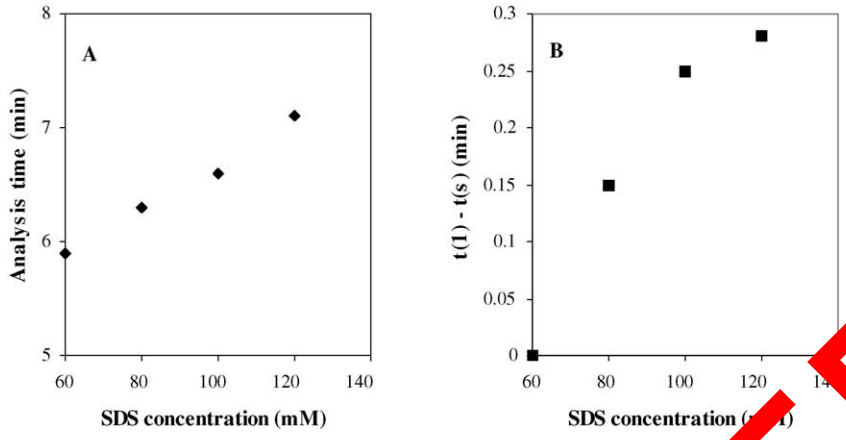


Fig. 2. Window diagrams for the effect of sodium dodecyl sulfate concentration on the analysis time of tarabine PFS and adriblastina (A) and the separation of tarabine PFS (t_1) from system peak (t_s) (B). Electrophoretic conditions: 50 mM phosphate/borate running buffer pH 8.7; voltage 15 kV; temperature 25 °C; hydrodynamic injection 50 mbar; detection at 300 nm. Fused silica capillary 50 μm i.d. (effective length 35 cm).

both analytes, it was observed that tarabine PFS was eluted first and then adriblastina. The possible mechanism of their interactions with teicoplanin phase may be due to the formation of hydrogen bonding and/or inclusion complexation and steric interactions.

3.1.2.2. Normal phase mode. The mobile phase composed of 20% isopropanol and 80% *n*-hexane was used for normal phase studies. All experiments revealed poor separation of both drugs with long analysis time up to 50 min. Therefore this kind of separation modes cannot be applied for further investigations.

3.1.2.3. Reversed phase mode. In reversed phase mode, there are small amounts of organic modifiers in the presence of water or buffers. The presence of organic modifiers affects the selectivity of separation and the presence of buffers increases the efficiency of resolution. Acetonitrile, methanol, ethanol and tetrahydrofuran afford good selectivity for a variety of analytes. Typical concentration in the presence of alcohol was 20% and 10% in the presence of acetonitrile or tetrahydrofuran [23]. Therefore, the simultaneous separation of tarabine PFS and adriblastina in 20% methanol or 10% acetonitrile in the presence of 80% or 90% water or TEAA buffer (pH 4.05) has been evaluated. The separation of both

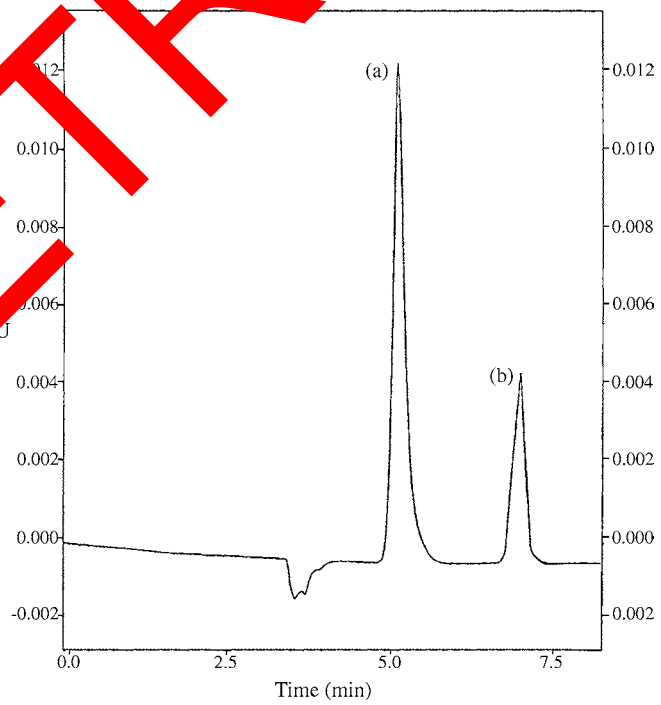


Fig. 3. Simultaneous reversed phase LC of (a) tarabine PFS and (b) adriblastina. Chromatographic conditions: mobile phase methanol:buffer (1% triethylamine, acetic acid) (20:80%, v/v, pH 4.05). Other conditions as in the text.

analytes (0.1 mg/mL) using 20% methanol and 80% water was achieved with retention times at 7.43 and 11.86 min for adriblastina and tarabine PFS, respectively. By replacing methanol with 10% acetonitrile under the same conditions, it was found that the analysis time was reduced to 8.0 min but with poor resolution. The influence of TEAA buffer on the simultaneous separation of both analytes was tested. The best separation was achieved in the presence of 80% buffer and 20% methanol, pH 4.05 with analysis time up to 7.5 min (Fig. 3). From the above studies in different media, it was observed that tarabine PFS was less retained than adriblastina in slightly acidic media and the opposite was observed in neutral media due to changing of the ionic nature of analytes and teicoplanin stationary phase. The suggested mechanism for the simultaneous separation recognition is the same as discussed in polar organic phase with the tendency to form hydrophobic interactions. Therefore, simple, rapid, sensitive and selective simultaneous separation of tarabine PFS and adriblastina was achieved by reversed phase LC in the presence of 20% methanol and 80% TEAA buffer, pH 4.05 with flow rate 0.8 mL/min at 25 °C. These optimal conditions were operated for all further LC experiments.

3.2. Method validation

The validation principles that usually apply in chromatographic analysis [22], also apply to CE methods. Thus, the procedure under optimized conditions for CE and LC required the assessment of selectivity, robustness, linearity, etc.

3.2.1. Precision

Multiple injections (10 times) from a single mixed standard solution were performed to demonstrate the repeatability of the migration time and the peak area or height under optimal conditions. For MEKC analysis, 10 replicate runs of a reconstituted mixture of 16.5 μg/mL tarabine PFS and adriblastina in the presence of 18 μg/mL sulfadimethazine as an internal standard were carried out in three different days. The obtained R.S.D. (intra-days) were 4.0 and 4.4% for tarabine PFS and 3.2 and 3.6% for adriblastina regarding migration time and corrected peak area, respectively at 300 nm with correlation coefficient ≥0.9991. For LC analysis, 10 replicate runs of a reconstituted mixture of 6.0 μg/mL tarabine PFS and 6.0 μg/mL adriblastina were carried out at 285 nm, pH 4.05 in three different days. The obtained R.S.D. (intra-days) were 2.8 and 3.0% for tarabine PFS and 3.2 and 3.7% for adriblastina regarding migration time and peak height, respectively with correlation coefficient ≥0.9991. The above results with acceptable R.S.D. values for both MEKC and LC approaches give an indication of the applicability of both procedures for analytical estimations.

3.2.2. Linearity

3.2.2.1. Simultaneous standard addition of tarabine PFS and adriblastina. For evaluating the quantitative applicability of MEKC method, five mixed different concentrations in the range of 50–1000 μg/mL tarabine PFS and 8–200 μg/mL adriblastina in the presence of 250 μg/mL sulfadimethazine (I.S.) solution were analyzed. These concentrations are suitable to determine both analytes in each biological matrix. The linearity between corrected peak area (Y) against variant concentrations of analyte (X, mg/mL) was investigated at 300 nm, pH 8.7 giving the following equations: $Y = -0.218X + 0.009$ with $r^2 \approx 0.9999$ (tarabine PFS) and $Y = 1.312X + 0.003$ with $r^2 \approx 0.9998$ (adriblastina). For the quantitative applicability of LC method, different concentrations in the range of 3–100 μg/mL and 12–200 μg/mL for tarabine PFS and adriblastina, respectively, were investigated at 285 nm, pH 4.05. The calibration graphs ($n = 6$) were plotted between the peak heights (each point is an average value of triplicate injections, mAU) against the analyte concentration (mg/mL) giving the following equations: $Y = 1.134X + 0.034$ with $r^2 \approx 0.9985$ (tarabine PFS) and $Y = 0.388X + 0.021$ with $r^2 \approx 0.9993$ (adriblastina).

3.2.2.2. Standard addition of tarabine PFS or adriblastina in the presence of a high constant concentration of another. In order to check also the applicability of our systems for the simultaneous determination of each analyte down to 0.1% in the presence of another compound, the following procedures were constructed. For MEKC analysis, five working solutions of concentrations ranged from 10 to 300 ng/mL of tarabine PFS corresponding to 0.1–3.0% (m/m) have been measured in the presence of 10 μg/mL adriblastine at 260 nm. The same procedure was repeated for five concentrations of adriblastine ranged from 8 to 120 μg/mL in the presence of 4000 μg/mL tarabine PFS at 300 nm. Table 1 indicates data collected by applying above procedures. Each value is an average of triplicate determinations per three consecutive days. For LC analysis, five reconstituted mixtures of tarabine PFS (3–90 μg/mL) in the presence of 3000 μg/mL adriblastine corresponding to 0.1–3.0% (m/m) and 10–120 μg/mL adriblastine in the presence of 4000 μg/mL tarabine PFS have been measured at 285 nm, pH 4.05. All obtainable results are reported in Table 1.

3.2.3. Accuracy

The accuracy was determined by applying the optimized analytical approaches with three injection replicates to reconstituted mixtures of both drugs at three concentration levels covering the linearity range (50, 100, 200 μg/mL for MEKC and 10, 50, 100 μg/mL for LC). The obtained mean recoveries of data collected by replicating the procedure three consecutive days ranged from 99.4 to 100.4%. As a further confirmation of both accuracy and linearity, the response of tarabine PFS or adriblastine as a function of its concentration was evaluated in the presence of a high and constant amount of another drug. These experiments were carried out by means of the standard addition method as described in the linearity section. The accuracy was also evaluated by recovery studies; urine solutions were spiked with known quantities of the related substances in the presence of internal standard (in case

Table 1
Calibration graph data of tarabine PFS and adriblastina: $y = mX + q$; where slope (m) and intercept (q) ($n = 5$)

Analyte	Method	m (S.D.)	q (S.D.)	r	Concentration range (μg/mL)
Tarabine PFS[a]	MEKC	7.232 (0.156)	−0.005 (0.002)	0.998	0.01–0.30
Adriblastina[b]	MEKC	6.481 (0.077)	0.007 (0.012)	0.999	8.00–120.00
Tarabine PFS[c]	LC	6.132 (0.098)	0.015 (0.022)	0.998	3.00–90.00
Adriblastina[d]	LC	5.552 (0.045)	0.009 (0.008)	0.999	10.00–120.00

[a] In the presence of 10 μg/mL adriblastina at 260 nm.
[b] In the presence of 4000 μg/mL tarabine PFS at 300 nm.
[c] In the presence of 3000 μg/mL adriblastina at 285 nm.
[d] In the presence of 4000 μg/mL tarabine PFS at 285 nm.

of MEKC method). The fortified solutions were analysed and the obtained recoveries ranged from 99.1 to 101.2%. Therefore, our analytical methods give sufficient accuracy.

3.2.4. Selectivity and sensitivity

The selectivity of the analytical methods was demonstrated by the use of certified commercially available reference compounds for each considered analyte. The peak identity was attributed on the basis of the migration or retention times and it was also confirmed by the UV spectra recorded with a diode-array detector. It should be pointed out that the optimized conditions offer a baseline resolution without interference by other excipients or essential ions or organic compounds existing in urine medium.

The sensitivity of the CE method was evaluated with UV detection for simultaneous tarabine PFS and adriblastina at 300 nm. However, the sensitivity of LC method was tested at constant UV detection 285 nm. Limit of detection (LOD) was estimated at the lowest analyte concentration able to provide a signal-to-noise ratio of 3; these data were 2.5 ng/mL and 0.95 μg/mL of tarabine PFS and 3.0 and 3.13 μg/mL of adriblastina for MEKC and LC, respectively. Limit of quantitation (LOQ) for studied drugs were similarly evaluated considering a typical signal-to-noise ratio of 10; these data were 9.00 ng/mL and 2.05 μg/mL of tarabine PFS and 8.0 & 9.25 μg/mL of adriblastina for MEKC and LC, respectively.

3.2.5. System robustness and stability

During robustness testing, a method must prove to be able to remain unaffected by small variations in method parameters, thus showing its own reliability during normal usage [24–26]. In the case of MEKC approach, the most effective five parameters were the same considered in the optimized step and their experimental domain are reported in Table 2.

For LC analysis, there are four parameters that were varied in small ranges to check the system suitability in analytical applications. Table 3 summarizes the obtained experimental data.

The system stability was checked by injecting the concentration corresponding to the quantitation limit of both analytes during 48 h and comparing the collected results with data collected from freshly prepared reconstituted standard mixtures. The R.S.D. was found to be 3.6–4.3% indicating that our systems were stable for a long analysis time.

3.3. Application

The described methods were suitable for the drugs quantitation in biological fluids like urine samples. The most recent drug treatment plan of AML and ANLL is the induction regimen consisting of combination chemotherapy of tarabine PFS plus adriablastine [1–3]. For the treatment courses, 7 days continuous infusion with tarabine PFS and 3 days of adriablastine were realized. After oral administration, only about 20 and 25% of tarabine PFS and adriblastine, respectively reach the circulation [27]. Less than 10 and 15% of the injected dose of tarabine PFS and adriblastine, respectively, is excreted unchanged in the urine. By applying our proposed approaches, it was found that 8–10% of the injected tarabine PFS dose and 13–15% of the injected adriblastine dose were determined in urine samples with recoveries ranged from 99.5 to 101.5%. Fig. 4 indicates a representative electropherogram for the simultaneous determination of tarabine PFS and adriblastine in urine (curve a) and with addition of 38 μg/mL of both analytes (curve b). The recovery studies were carried out by means of comparison with a reference solution (single calibration point method), which

Table 2
Robustness test of micellar electrokinetic chromatography method (injecting of 75 μg/mL tarabine PFS and 40 μg/mL adriblastina at 300 nm)

Parameter	Tarabine PFS (time, min)	Adriblastina (time, min)	Recovery for time (%)	Tarabine PFS (corrected peak area[a])	Adriblastina (corrected peak area[a])	Recovery for area (%)
SDS concentration (99–101 mM)	2.70–2.71	6.39–6.40	99.92–100.12	0.075–0.076	0.079–0.081	99.49–100.01
Buffer concentration (49–51 mM)	2.69–2.70	6.39–6.40	99.75–100.05	0.074–0.075	0.078–0.080	99.51–100.00
Buffer pH (8.65–8.75)	2.68–2.70	6.38–6.40	99.63–99.84	0.074–0.076	0.078–0.079	99.55–99.85
Temperature (24–26 °C)	2.70–2.72	6.37–6.39	99.84–100.37	0.075–0.077	0.077–0.079	99.73–100.04
Applied volt (14–16)	2.69–2.70	6.38–6.39	99.75–100.07	0.074–0.075	0.078–0.080	99.78–100.05

[a] Corrected peak area was calculated by the ratio of corrected peak area of analyte to corrected peak area of internal standard.

Table 3
Robustness test of reversed phase LC method (injecting of 12 μg/mL tarabine PFS and 35 μg/mL adriblastina at 285 nm)

Parameter	Tarabine PFS (time, min)	Adriblastina (time, min)	Recovery for time (%)	Tarabine PFS (peak height, AU)	Adriblastina (peak height, AU)	Recovery for height (%)
Methanol concentration (19.5–20.5%)	5.17–5.19	7.19–7.21	99.82–100.10	0.014–0.016	0.011–0.012	99.64–100.10
Buffer pH (4.00–4.10)	5.17–5.18	7.20–7.22	99.83–100.25	0.013–0.014	0.011–0.012	99.85–99.95
Temperature (24–26 °C)	5.18–5.19	7.20–7.21	99.74–99.95	0.014–0.016	0.010–0.011	99.63–100.04
Flow rate (0.7–0.9)	5.17–5.19	7.19–7.20	99.95–100.17	0.014–0.015	0.011–0.012	99.88–100.05

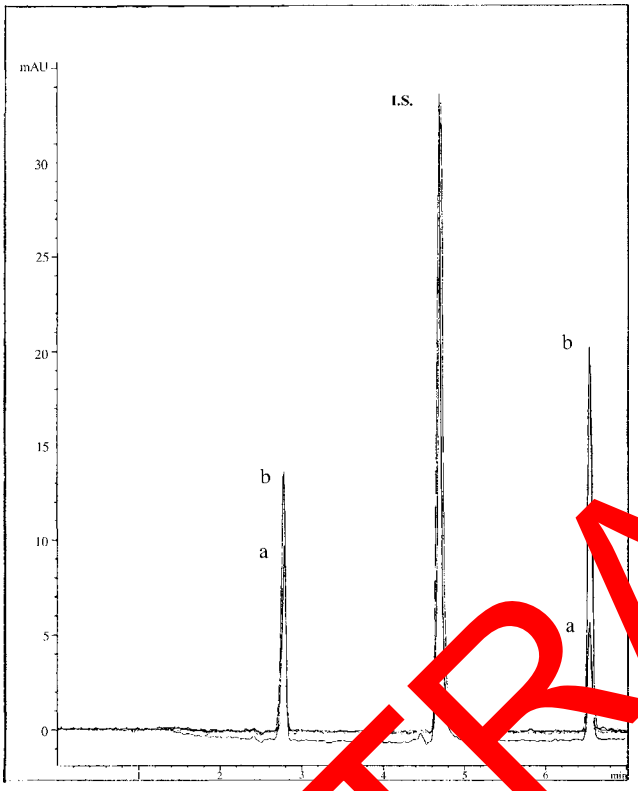


Fig. 4. Electropherogram for the simultaneous determination of tarabine PFS and adriblastine in urine (curve a) and with the addition of 38 μg/mL of both analytes (curve b). Other conditions as in the text.

was not significantly different from the value obtained by the standard addition method. Therefore, our MEKC and LC approaches gave very close results to the recommended ones [27] with good recoveries, which indicate the applicability of both systems for the simultaneous evaluation of drugs used in combination chemotherapy. In addition, all the analytical values fall within the labeled amount of 90–110% with R.S.D. values less than 4.0% required by USP XXV [28].

In the present work, a simple, specific, sensitive, stable, rapid and reliable MEKC and LC methods have been successfully demonstrated for the simultaneous determination of tarabine PFS and adriblastina. Both the optimization and validation of the systems were performed and the obtained results make the proposed analytical approaches useful for the application to urine samples.

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