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Direct and fast determination of paclitaxel, morphine and codeine in urine by micellar electrokinetic chromatography

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ABSTRACT

A micellar electrokinetic chromatography (MEKC) method was developed for the determination of paclitaxel, morphine and codeine in human urine from patients under cancer treatment. The background electrolyte consisted of a borate buffer (pH 9.2; 20 mM) with sodium dodecyl sulfate (60 mM) and 5% MeOH. The applied voltage was 25 kV, temperature was 20 °C and the sample injection was performed in the hydrodynamic mode. All analyses were carried out in a fused silica capillary with an internal diameter of 75 μm and a total length of 57 cm. The detection of target compounds was performed at 212 nm. Under these conditions, a complete separation of paclitaxel, morphine and codeine was achieved in less than 15 min. According to the validation study, the developed method was proved to be accurate, precise, sensitive, specific, rugged and robust. This method was applied to the analysis of six urines samples from different cancer patients undergoing treatment with paclitaxel or/and codeine. In all the urine paclitaxel determination were done.

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1. Introduction

Patients with cancer are at considerate risk of drug–drug interactions. Typically, such patients will receive a large number of drugs during their treatment, including several different cytotoxic agents in multi-drug chemotherapy regimens, hormonal agents, and also supportive care with antiemetics, analgesics, and anti-infective agents, among others. Drug interactions in oncology are of particular importance owing to the narrow therapeutic index and the inherent toxicity of anticancer agents. Interactions with other medications can cause small changes in the pharmacokinetics or pharmacodynamics of a chemotherapy agent that could significantly alter its efficacy or toxicity.

Chronic pain is extremely prevalent among patients with cancer. Approximately one-third of patients have pain while undergoing active therapy for the disease, and more than three-quarters have pain during the last stages of illness [1,2]. Fortunately, experience suggests that cancer pain can be relieved in more than 70% of patients using a simple opioid-based regimen [3–5].

Opioids remain the cornerstone of pharmacotherapy for cancer treatment. The three-step analgesic ladder developed by the World Health Organization (WHO, 1986) under the leadership of Dr. Kathy Foley is the first widely accepted model in the treatment of cancer pain [6] (Fig. 1).

Paclitaxel is a diterpene amide that was initially isolated from the bark of the Pacific yew (*Taxus brevifolia*) which shows unique antitumor and antileukemic activities [7,8]. It has been shown to produce responses in patients with different types of cancer, such as ovarian [9], breast [10], lung [11], head and neck region [12] and malignant melanoma [13]. The antineoplastic activity of paclitaxel is known to be mediated by building to tubulin, stabilizing microtubules and blocking the transit of cell cycling from G2-phase to the M-phase [14]. Paclitaxel is given as a chemotherapy infusion and it is administered in a hospital or clinic by a specially trained health care professional. Paclitaxel is highly lipophilic and practically insoluble in water, the drug is currently formulated in 1:1 (v/v) Cremophor EL®/ethanol mixture, available as Taxol®. Although paclitaxel is a frontline antineoplastic agent for treatment of solid tumors, the paclitaxel-evoked pain syndrome is a serious problem for patients. For this reason, the pain treatment must be considered in the paclitaxel medical use. Codeine is an opium alkaloid that is available as single agent or in combination with other analgesics. Codeine is metabolized to active drug by P450 (CYP) 2D6 [15]. The analgesic effect of codeine is largely attributed to the production of its active metabolite morphine. Codeine's potency is 1/10 of morphine. Some patients with low or undetectable CYP2D, derive no analgesic effect from codeine and however other individuals may have two or more copies of the CYP2D6 gene, resulting in rapid metabolism of the target drug. CYP2D6 metabolizes

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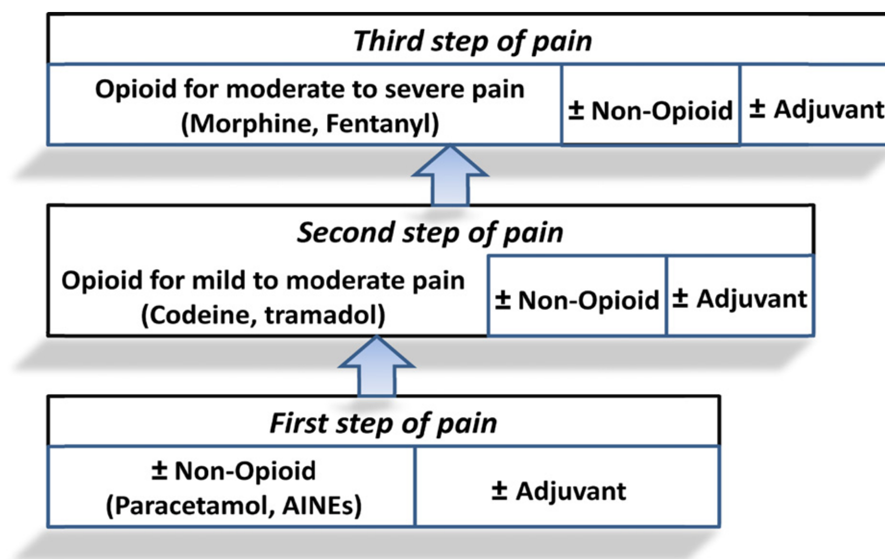

Fig. 1. The World Health Organization (WHO) cancer pain treatment step ladder.

and activates codeine into morphine, which then undergoes glucuronidation.

Morphine is considered to be a prototype opioid agent. The WHO expert committee considers morphine a major pain-relieving compound and advocates for its wide global availability for treatment of cancer-related pain [6]. Unlike many other opioids, morphine is an opiate and a natural product. Morphine has a high potential for addiction; tolerance and psychological dependence develop rapidly, although physiological dependence may take several months to develop. The liver is the principal site of morphine metabolism. The elimination half-life of morphine is approximately 2 h.

A number of assay methods have been published for the determination of paclitaxel in biological fluids, including micellar electrokinetic chromatography [16,17], liquid chromatography–mass spectrometry [18–29], HPLC-UV [30–34] and immunoassay [35,36]. These methods utilize either solid-phase extraction (SPE) [21], on-line SPE [20], protein precipitation and SPE [18], liquid–liquid extraction (LLE) [22], LLE using micro sample volumes, solvent extraction followed by column-switching, or solvent extraction and SPE [23].

Numerous methods are available for the quantification of common pain-relieving opioid drugs. These methods typically determined one or two compounds with their metabolites. Only recently have sensitive multi-opioid quantification methods using GC–MS [37–40] and LC–MS [41–47] techniques been described for these opioids in biological fluids. The limits of quantification, for example for morphine and codeine, by GC–MS have been at the level of 10 ng/mL [38], and by LC–MS as low as 1 ng/mL [46].

The aim was to study the involvement of opioid on established paclitaxel-induced pain, testing two common analgesics drugs as codeine and morphine and to propose a method for determining the three compounds in urine.

To our knowledge, this is the first method reported for the simultaneous assay of the two analgesic and a taxane drugs (codeine, morphine paclitaxel,) until now. This mixture of chemotherapy compounds as the paclitaxel and two different analgesic medications as codeine and morphine has been necessary to the different treatment based on the severity of the cancer pain (moderate to severe pain). In the current research, we have developed and validated a robust, fast and sensitive capillary electrophoresis method for the determination of paclitaxel, codeine and morphine in urine samples.

2. Materials and methods

2.1. Instrumentation

Capillary electrophoresis experiments were performed using a Beckman P/ACE 5510 (Beckman instruments, Palo Alto, CA, USA) equipped with a DAD and P/ACE station software was used. Fused-Silica capillaries of 75 μm id and 375 μm od with total and effective lengths of 57 and 50 cm, respectively, were used. UV detection was performed at 212 nm for all analytes.

pH measurements were performed using a Crison model 2002 pH meter with a combined glass electrode (Alella, Barcelona). Urine centrifugation was carried out with a Selecta apparatus (Abrera, Barcelona).

Previously, all samples were filtered through of 0.45 μm nylon membrane filters (Millipore).

2.2. Reagents and solutions

Paclitaxel was supplied by Tocris, and morphine and codeine were supplied by Sigma–Aldrich (St. Louis, USA). All other chemical were commercially available and of analytical grade.

Several buffer solutions with different pH values were prepared using the following reagents: sodium dihydrogenphosphate, disodium hydrogenphosphate, borid acid, ammonium acetate, sodium hydroxide, hydrochloric acid, acetic acid and ammonium hydroxide. All of these compounds were obtained from Panreac (Barcelona, Spain).

The running buffer in all CE experiment was 20 mM borate, pH 9.2, containing 60 mM SDS and 5% v:v of methanol. The buffer was prepared by dissolving an appropriate amount of boric acid in water adjusting the pH with NaOH.

2.3. Operating conditions

Prior to the first use, the capillary was conditioned by consecutive flushing with 0.5 M NaOH for 2 min and the separation buffer for 2 min in order to avoid adsorption processes.

Different vials of electrolytes were used for rising and separating operations in order to maintain a constant electrolyte level on the anodic side. The set of separation vials was changed after every six separation runs. Injection of the samples was performed by hydrodynamic mode at 0.5 psi for 7 s. Optimized separations were carried

out at 25 kV for 15 min at 20 °C. Under these conditions the current was 45.5 μA. Duplicate injections of the solutions were performed and average peak areas were used for the quantitation.

2.4. Sample preparation

Fresh human urine samples were obtained from different healthy volunteers, whereas clinical urine samples were provided by different patients, who have been submitted to paclitaxel treatment for her cancer disease with or without codeine. The determination of paclitaxel, codeine and morphine from the biological samples was quickly performed with the next procedure after a centrifugation step (5000 rpm, 5 min, 20 °C), filtration and they were subsequently introduced into the CE equipment.

2.5. Preparation of standard and quality control samples

Stock solution of paclitaxel, codeine and morphine were prepared in methanol at the concentration of 500 mg/L and stored at 4 °C. Calibration curves were prepared by spiking the appropriate standard solution in 0.5 mL of blank urine. The quality control (QC) samples were separately prepared in blank urine at the concentration 0.2, 0.4, 0.5, 0.6, 0.8, 2, 8 and 10 mg/L for morphine, codeine and paclitaxel.

3. Results and discussion

3.1. Preliminary experiments

As mentioned before, there are no previous works concerning the simultaneous determination of morphine, codeine and paclitaxel by capillary electrophoresis. Therefore, a preliminary study was carried out using standards of these compounds dissolved in water at a concentration 5 mg/L for each compound with hydrodynamic injection at 0.5 psi for 6 s. The selected groups of compounds have pK_a values: codeine pK_a 8.2, morphine pK_a 8.0 and paclitaxel pK_a 12. Therefore, in order to achieve a suitable separation, first, the effect of background electrolyte pH (in the range between 2 and 12) on resolution between peaks and analysis time was investigated.

Paclitaxel, as was expected, was in a non-ionic form for all the studied values buffers pH. Due to the impossibility to achieve the complete separation of the selected compounds, we tried this separation by MEKC technique. Usually, MEKC is carried out with buffers containing some surfactant; SDS is the most widely used.

3.2. Optimization of the separation conditions by MEKC

In the optimization of the separation conditions for the codeine, morphine and paclitaxel, the effects of pH, running buffer concentration, micellar concentration, organic modifiers, applied voltage, temperature and injection time were studied.

After previous experiments a SDS concentration of 50 mM was considered as a high enough concentration value to add to the background electrolyte. This concentration was chosen as a compromise between the generated current intensity and the necessary concentration of the pseudo-stationary phase. As consequence the starting conditions selected were: 50 mM SDS, 30 kV and 20 °C as separation voltage and cartridge temperature, respectively

In order to evaluate the influence of pH and composition of electrolyte on the separation, resolution and migration times of the three compounds, several 20 mM buffers solutions at different pH were prepared: phosphate buffer solutions, whose pH was adjusted in the range (2–3.5) and (6.5–8.5), acetate buffers solutions that were adjusted between 4 and 6 pH units and borate buffer solutions that were adjusted between 8.5 and 10 pH values. The best separation of the studied compounds was achieved at pH 9.2, which is provided by borate buffer solution therefore this pH value was selected for the following studies. The concentration of borate buffer solution was varied between 10 and 60 mM in order to study the influence of this parameter on the resolution between peaks of the analytes and analysis time. The best results were obtained when 20 mM was used as concentration of buffer solution.

The effect of SDS concentration on migration times and resolution between peaks was researched at the values 50, 60, 70, 80, 90 and 100 mM using 20 mM borate buffer pH 9.2 as separation electrolyte. The separation of all the analytes in the least time possible with a good resolution was achieved at 60 mM; thus, this value was selected as optimum.

The addition of several organic solvents to the buffer electrolyte to improve selectivity and separation efficiency was also evaluated. Different organic solvents such as methanol, acetonitrile and 2-propanol were tested and in all the cases their concentration was varied between 0% and 15% in the separation electrolyte. It was found that using ACN and 2-propanol not allow the separation of all the studied compounds, however when using methanol a significant improvement in the separation was observed. So this solvent was chosen as electrolyte separation additive and was studied to 2, 5, 8, 10, 12 and 15% percentage and the best resolution, analysis time and peak shapes were obtained when a percentage of 5% (v/v) of this solvent in the separation electrolyte was fixed.

The effect of voltage applied was investigated from 15 to 30 kV in steps of 5 kV. As expected, increasing the applied voltage increases EOF, leading to shorter analysis time and higher efficiencies. A 25 kV voltage value was selected as the best compromise in terms of run time, generated current, and efficiency in the separation.

Possible changes in efficiency, migration times and injection volumes caused for the capillary temperatures were tested. In this sense, several electropherograms were recorded varing this parameter (18, 20, 25, 30 and 35 °C) As expected, an increase of temperature resulted in an increase of the EOF that allows a decrease of migration times due to the electrolyte viscosity decreasing. The selected temperature was 20 °C because it provided the best resolution, run time not too long and the generated electric current was always lower than 47 μA.

Finally, in order to improve the detection and quantification limits, different injection times were tested (between 3 and 8 s) at a constant pressure of 0.5 psi. From this study 7 s of injection time was chosen as the optimum value.

Representative electropherograms are shown in Fig. 2 for drug-free human urine and urine spiked with morphine, codeine and paclitaxel. As can be seen the compounds were well separated from co-extracted endogenous components and no interferences were observed at the migration times of our compounds.

3.3. Validation of method

3.3.1. Stability of solutions

In all cases the stability was evaluated by repetitive injections in the CE equipment over a period of time range between 60 min (urine samples) and daily (stock and dilute solutions). The parameters considered were the migration time and the peak areas of the three compounds.

The stock and diluted solutions of codeine, morphine and paclitaxel were stored at 4 °C and they were found to be stable for at least 1 month and 7 days respectively.

The stability of urine samples spiked with the studied analytes was evaluated and it was found that these samples are stable for at least 7 h.

Also, stability of the urine samples after three freeze/thaw cycles was evaluated in duplicate. The signal for each compound in the samples subjected to the freeze/thaw cycles were compared to

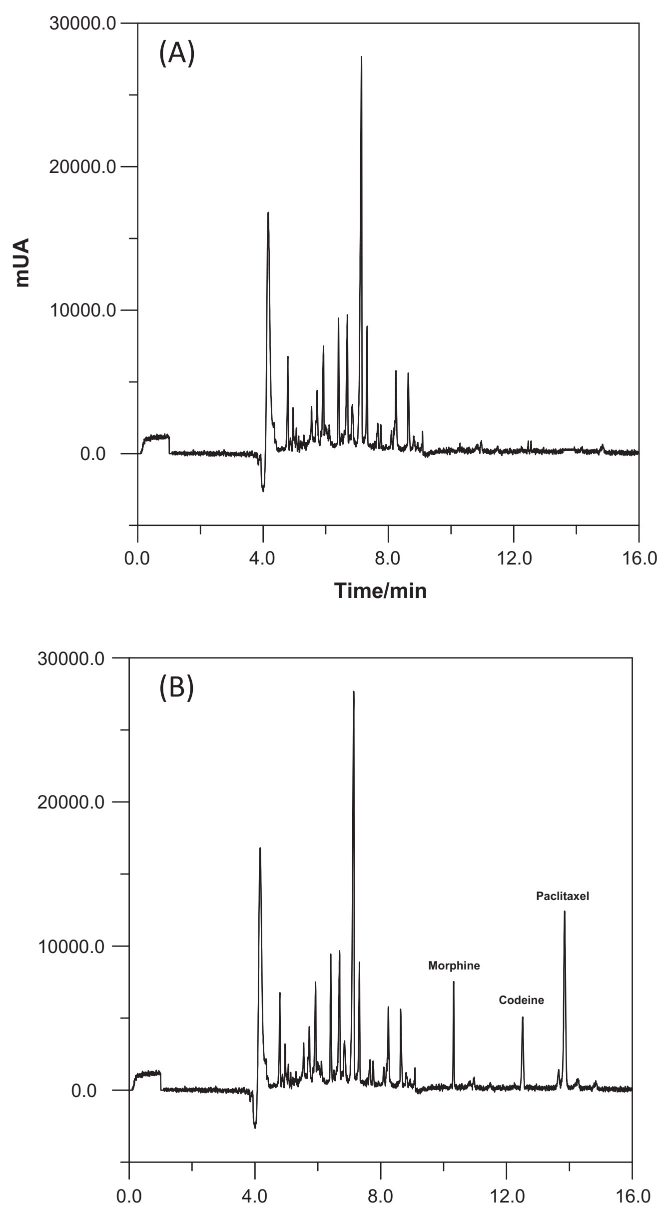

Fig. 2. (A) Urine blank, (B) MEKC electropherogram of a urine samples spiked with 3 mg/L of morphine, codeine and paclitaxel. *Operating conditions*: 20 mM borate buffer (pH 9.2), 60 mM SDS and 5% MeOH, 25 kV, 20 °C, hydrodynamic injection (7 s, 3.45 kPa).

those obtained in freshly prepared samples. It was observed that the freeze/thaw cycles did not affect.

3.3.2. Precision

The precision of the proposed method for determining morphine, codeine and paclitaxel was investigated in repeatability and intermediate precision terms for migration times and peak area of three compounds in accordance with the International Conference on Harmonization (ICH) criteria.

In order to test the electrophoretic procedure suitability, nine injections of urine samples spiked with codeine, morphine and paclitaxel were made. The precision of the migration times and peak areas were satisfactory with RSDs between 0.62% and 1.12% for migration times and between 2.70% and 3.39% for peak areas for all the studied compounds. With regard to intermediate precision, this operation was repeated on different days and RSD values less than 1.39% were obtained for migration times and less than 6.92% for peak areas. Comparison of the two sets of data with the aim of detecting random errors was carried out by applying the Snedecor *F*-test on these RSD values. Significant differences were not found in any case at a confidence level of 95%.

3.3.3. Linearity

The linearity of the proposed method was checked by injecting urine solutions spiked with the drugs at concentration ranging from 0.1 to 10 mg/L for all compounds. The calibration was determined from duplicate injection at seven different concentration levels for every compound. The calibration curves for the three drugs were computed using the peak area of each analyte by using a least-squares linear regression analysis. The satisfactory linear regression equations and their regression coefficients (Table 1) could indicate the linearity of codeine, morphine and paclitaxel responses over the studied concentration ranges.

The lack of fit test was carried out by plotting the residuals (distances of the experimental points from the fitted regression lines) against concentration. If there is no lack of fit (that is, the calibration is inherently linear) the plot will look like a random sample from a normal distribution with zero mean. This was the situation observed on applying this test to our calibration graphs and the linear nature of the relationship was thus confirmed.

3.3.4. Accuracy

The accuracy expresses the closeness or agreement between the value found and the value that it is accepted as a reference value. In order to test the accuracy of the proposed method, several aliquots of codeine, morphine and paclitaxel solution were added into human urine samples. These samples were analyzed using the proposed electrophoretic procedure. Recoveries were calculated versus external standards with lower and upper concentrations for

Table 1
Statistical parameters of the MEKC method.

	Calibration equation	R^2	Linearity (mg/L)	LOD (mg/L)	LOQ (mg/L)
Morphine	$Y = (1911.9 \pm 88.8)x + (183.3 \pm 130.6)$	0.9996	0.30–10	0.09	0.30
Codeine	$Y = (3628.0 \pm 21.1)x - (66.7 \pm 100.6)$	0.9999	0.22–10	0.07	0.22
Paclitaxel	$Y = (15,352.0 \pm 244.7)x + (800.8 \pm 594.1)$	0.9990	0.07–10	0.03	0.07

Table 2
Accuracy of the proposed MEKC method.

Sample compounds	Sample 1		Sample 2		Sample 3		Sample 4	
	Added (mg/L)	Recoveries (%)	Added (mg/L)	Recoveries (%)	Added (mg/L)	Recoveries (%)	Added (mg/L)	Recoveries (%)
Morphine	0.7	93.3	1.0	98.0	5.0	102.3	10.0	107.1
Codeine	0.4	94.9	0.7	100.1	1.0	101.7	10.0	104.3
Paclitaxel	0.3	101.1	1.0	92.8	3.0	98.3	5.0	100.4

each sample. As it can be observed in Table 2, recoveries between 92.8 and 107.1% were obtained in all cases.

3.3.5. LODs and LOQs

The LOD was obtained as the concentration of the drug corresponding to a peak area three times higher than baseline noise level and the LOQ was calculated as three times the LOD. The reached LOD and LOQ values for three compounds are shown in Table 1. The LOQs were subsequently validated by the analysis of four different blank urine samples spiked with amounts of each compound corresponding to their respective LOQs. The relative errors obtained in this verification were lower than 10% in all cases.

3.3.6. Integral robustness-ruggedness evaluation

The United States Pharmacopeia (USP) defines ruggedness [48] as "the degree of reproducibility of the test results obtained by the analysis of the same samples under a variety of normal test conditions such as different days, several reagent lots, different lots, different instruments, various laboratories, different elapsed assay times,. . ." where all of these factors are external to the written analytical method. The robustness of a method is defined by both the USP and ICH "Tripartite guidelines as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal use. Ruggedness can therefore be regarded as a measure of the absence of external influences on the test results, whereas robustness measures the lack of internal influences on these results. In the work described here we tested the influence of variations in both internal and external parameters of the method (e.g. pH and ionic strength of buffer, SDS concentration, voltage, capillary temperature, etc.), the influence of which has been studied at different levels. The Plackett–Burman fractional factorial model, which is based on balanced incomplete blocks, was employed to evaluate this aspect of the method. For statistical reasons (concerning effects on interpretation), designs with fewer than eight experiments are not used, while those with more than 24 experiments were considered unpractical [49]. To date, this model has been satisfactorily applied only to the evaluation of robustness. In this case was utilized a novel Plackett–Burman design that involves the evaluation of both robustness and ruggedness effects (eleven factors and twelve experiments, $N = 12$). The choice of variables (factors) and the levels at which they are tested is very important for a reliable robustness/ruggedness test. Variables must be significant in practice and levels must reflect the variation that can usually be observed. The external (ruggedness) and internal (robustness) factor (A–K) selected for our model are presented in Table 3, which also shows the (+) and (−) levels for every factor, these are, respectively, upper and lower values with regard to the optimal one in the procedure. The effects of varying the levels of the most critical electrophoretic responses of the method were investigated. The ranked effects of every factor for a selected electrophoretic response were calculated by simple addition of its (−) and (+) assay test results,

Table 3
Variables selected as factors and values chosen as levels.

Factors	External/internal	Optimum	Level (−)	Level (+)
A. Different days	External	–	1	2
B. Different buffers	External	–	1	2
C. Different patiens	External	–	1	2
D. [Buffer] (mM)	Internal	20	18	22
E. [SDS] (mM)	Internal	60	58	62
F. % MeOH	Internal	5	4	6
G. Voltage (kV)	Internal	25	24	26
H. t_{iny} (s)	Internal	7	6	8
I. Temperature (°C)	Internal	20	19	21
J. λ Detection (nm)	Internal	212	211	213
K. Lavado NaOH (min)	Internal	2	1.8	2.2

Table 4
Paclitaxel determination in urines of different patients.

	Cancer type	Treatment	Cycle number	Cycle dose (mg)	Total dose (mg)	pH	Conductivity (mS)	Dilution	$[\text{Paclitaxel}]_{\text{direct}}$ (mg/L)	$[\text{Paclitaxel}]_{\text{STD ADDITIONS}}$ (mg/L)
Patient 1	Head and neck	-Salmeterol -Ipratropio -Rabeprazole -Gliclazide -Paracetamol -Sulfametoxazol -Prednisone	6	101	606	6.03	18.32	2:8	2.41	2.49
Patient 2	Breast	-Paracetamol	7	137	959	5.03	15.88	Direct	1.11	1.08
Patient 3	Breast	-Sulfametoxazol -Trimetoprim -Naproxen -Bemiparine -Trimetazidine -Simvastatin -Omeprazole -Ocular coliri	9	152	1368	5.66	14.69	2:8	4.32	3.90
Patient 4	Gastric	-Simvastatin -Rabeprazole -Deflazacort -Betahistine -Acenocoumarin -Ramipril -Ezetimibe -Trimetazidine	6	170	1077	7.34	16.14	2:8	2.34	2.17
Patient 5	Breast	-Ferroglycine	1	131	131	5.23	24.5	Direct	1.21	1.32
Patient 6	Cervix	-Dexamethasone -Codeine -Cloperastine -Tolteradine -Almagate -Pantoprazole	1	180	180	–	–	Direct	1.67	1.60

with the total divided by half the number of runs. The *M* values are constant for any given design and are actually the means of the order statistics [50] for a sample size of eleven. Finally, the ranked effects of the 11 factors (on the *x*-axis) were plotted against the *M* values (on the *y*-axis) for each critical electrophoretic response. The results from this plot must be near to a straight line. If a value lies outside this straight line, it can be concluded that the method is not rugged/or robust (as classified by its corresponding factor). However, if the results from the plot form a (nearly) straight line, it can be concluded that the analytical method is rugged and robust over the conditions tested in the run design. The robustness/ruggedness evaluation was performed in our case by carrying out duplicate injections of spiked urine samples containing 1.0 mg/L of all compounds. The results of the levels variations effects for the 11 factors on migration times, peak areas and peak high were calculated for morphine, codeine and paclitaxel. As an example, Fig. 3 shows the plot corresponding to the ranked effects of the 11 selected factors versus *M* values for the peak area for morphine. It can be seen from this plot that all the points lie on a straight line and, therefore, our analytical method can be considerate robust and rugged with regard to this electrophoretic response.

In general terms, the described robustness/ruggedness test showed our electrophoretic method is both robust and rugged enough for the critical electrophoretic responses; being assessed for all the variation tested in this study.

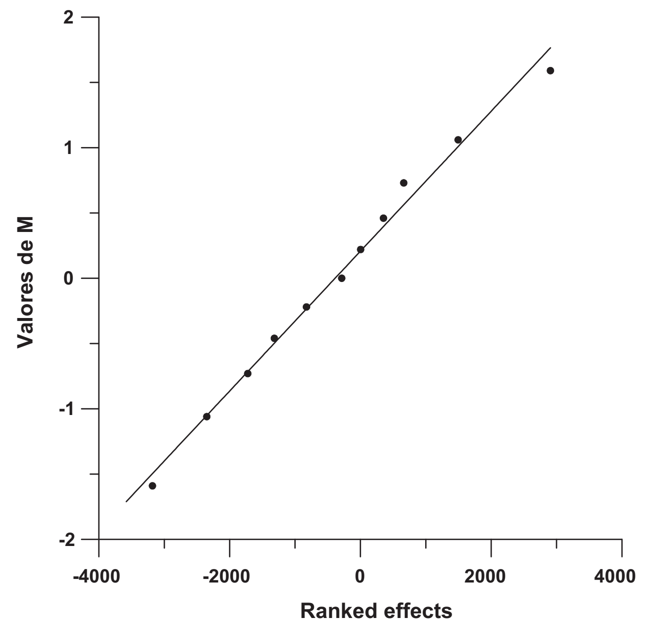

Fig. 3. Plot corresponding to *M* values for the peak area for morphine vs. ranked effects of the 11 selected factors.

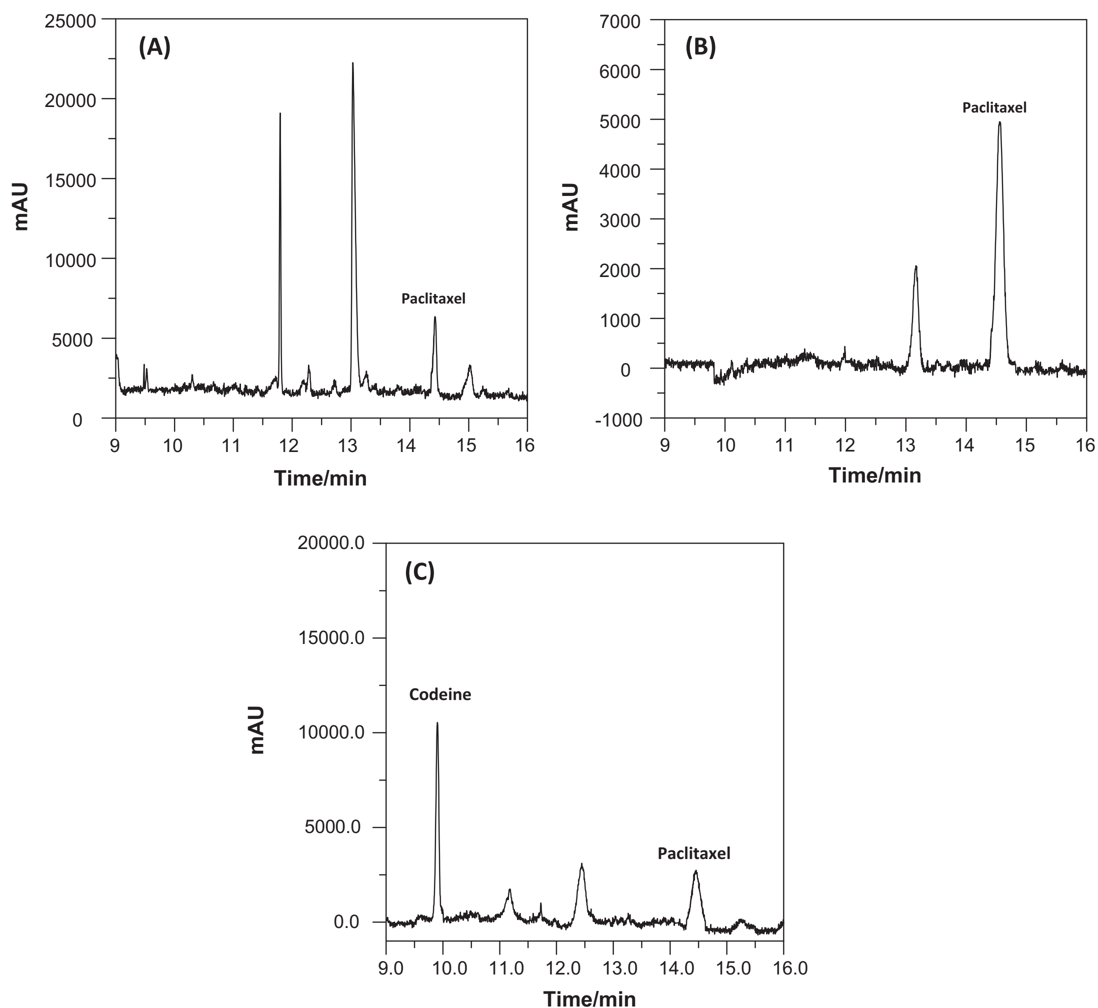

Fig. 4. Electropherograms corresponding to urine samples analyzed after the paclitaxel infusion corresponding to their chemotherapy treatment. (A) Patient with head and neck cancer, (B) Patient with gastric cancer, (C) Patient with cervix cancer. *Operating conditions*: 20 mM borate buffer (pH 9.2), 60 mM SDS and 5% MeOH, 25 kV, 20 °C, hydrodynamic injection (7 s, 0.5 psi).

3.4. Applications

To demonstrate the applicability of this method, it was used to quantify concentrations of paclitaxel in urines samples of patients with different cancer types (three breast cancer patients, a head and neck cancer, a cervix cancer and a gastric cancer patient) who received dose weekly of paclitaxel between 101–180 mg/m^2. Prior of the paclitaxel infusion, the patients received treatment with dexamethasone 20 mg, ondansetron 8 mg, dexchlorpheniramine 5 mg and ranitidine 10 mg. Paclitaxel was administered as a 3-h infusion. Urine was taken after paclitaxel infusion. Human urine samples were submitted to a centrifugation step (5000 rpm, 5 min, 20 °C) and filtration. In order to evaluate the possible matrix effect, the method of standard addition was used for the determinations of paclitaxel in human urine. Concentrations found using direct measured and the standard additions are shown in Table 4 and as can be seen they coincide with those obtained by direct measurement by the proposed method. Also, the cervix cancer patient (Patient 6 in Table 4) was treated with codeine (27.8 mg), so, in this patient codeine could also be quantified. Direct urine from this volunteer undergoing treatment with paclitaxel and codeine was analyzed at 3 h after its administration. The determination was carried out in duplicate and the concentration of codeine found was 5.14 mg/L.

Fig. 4 shows the electropherograms from the urine samples of three patients under treatment with paclitaxel with or without codeine under conditions optimized in this paper.

4. Conclusions

In this paper a rapid, easy, robust and sensitive method electrophoretic-UV detection method for the simultaneous determination of morphine, codeine and paclitaxel in human urine samples is developed. Until now, all the MEKC methods publied for determining these analytes require long time of sample preparation however the propose method does not need sample treatment and provides adequate limits of quantification for the application to the determination of the studied compounds at clinically relevant concentrations. The method is satisfactorily optimized and the validation step is adequately carried out. The method is applied to the determination of these compounds in urine samples of patients with different cancer types who received dose of these compounds weekly.

Also, others conclusions were established:

- No interferences were observed in the samples analyzed, including the co-administration of different drugs (analgesic, antibiotic, anticoagulants, diuretic).
- There was an increasing amount of paclitaxel in urine with the number of cycles of chemotherapy. It could be related to the accumulative toxicity of the paclitaxel in the body.

This method could be used to know if the co-administrations of other pharmaceutical compounds have a possible influence in the paclitaxel metabolism and if it is possible to propose an individualized chemotherapy depending on the particular health condition, although the number of patients is scarce.

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