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# Sensitive ergotamine determination in pharmaceuticals and biological samples using cloud point preconcentration and spectrofluorimetric detection

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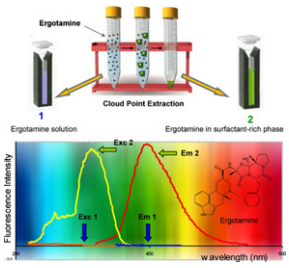
## HIGHLIGHTS

- A highly efficient cloud point extraction method was developed for ergotamine.
- Direct gel-state fluorescence determination was performed after extraction.
- Emission advantages of undiluted surfactant rich phase were explored for the first time.
- A total enhancement factor of 1325 was achieved for ergotamine determination.
- The simple, low cost, non-toxic methodology was successfully applied to real samples.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A new cloud point extraction (CPE) method for ergotamine analysis using fluorimetric detection is described. Ergotamine from an aqueous solution was preconcentrated into a smaller surfactant-rich phase using nonionic surfactant polyoxyethylene(7.5)nonylphenylether (PONPE 7.5). Differently from the conventional CPE procedure in which the resulting surfactant-rich phase is diluted by a fluidificant before its analysis, in this method the fluorescence measurements were carried out directly onto the undiluted surfactant-rich phase. The high viscosity provided by the undiluted surfactant rich phase greatly improved the fluorescence emission of ergotamine, leading to a total enhancement factor of 1325. This spectral advantage plus the preconcentration factor achieved, contributed to the method sensitivity allowing the ergotamine determination at trace level concentration. Under optimal experimental conditions, a linear calibration curve was obtained from $3.81 \times 10^{-7}$ to 1.10 μg mL$^{-1}$, with detection and quantification limits of 0.11 and 0.38 pg mL$^{-1}$, respectively. The accuracy and versatility of the present methodology were proved by analyzing ergotamine in real samples of different natures such as pharmaceuticals, urine and saliva.



## 1. Introduction

Ergotamine belongs to the ergot family of alkaloids, an amide derivate closely related to the tetracyclic compound ergoline. The ergot alkaloids are bio-synthesized by *Claviceps purpurea*, a fungus which mainly affects to rye grain and other plants, being

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considered contaminants in harvest. Ergotamine was first isolated in 1918 and has been used for therapeutic purposes since the 1950s [1], commonly to treat vascular headache. It has antiserotonin effects, α-adrenergic blocking activity and a direct stimulating action on smooth muscle, especially of blood vessels and uterus (oxytocic effect) [2,3]. This drug is highly toxic and in large, repeated doses can produce symptoms of ergot poisoning, known as ergotism, which can be roughly divided into convulsive symptoms and gangrenous symptoms [4,5]. Convulsive symptoms include painful seizures and spasms, diarrhea, parenthesias itching, psychosis, headache, nausea and vomiting. The hallucinations can resemble those produced by LSD (lysergic acid diethylamide, to which the ergotamine is an immediate precursor and therefore shares some structural similarities). The marked vasoconstriction induced by ergotamine can cause gangrene symptoms, affecting the distal structures more poorly vascularized such as the fingers and toes, leading to the loss of affected tissues [6,7].

Ergotamine can also pass from mother to child through the placenta or during lactation, causing ergotism in infants. Due to its high potency and toxicity, ergotamine must be carefully employed at low concentrations; the most frequently recommended dosage to prevent or abort migraine attacks is 1 mg [5].

After an oral dose, ergotamine is extensively metabolized in the liver; the unmetabolized drug is erratically secreted by saliva and only traces are excreted in urine and feces. The elimination half-life of ergotamine from plasma is about 2 h, but the drug may be stored in some tissues and be released later, manifesting its therapeutic or toxic effects during this process [7].

In the last years, several methodologies have been developed for ergotamine determination in diverse samples including Flow-Injection (FI) Chemioluminiscence [8], FI-Fluorimetry [9], Gas-chromatography [10], Spectrofluorimetry [11], Liquid-chromatography [12] and Radioinmunoassay [13]. The high native fluorescence of ergotamine in aqueous solution has led to the development of several fluorimetric methodologies for its quantification or applied as detection system. However, for most of them, exhaustive sample treatment and successive extraction steps are necessary in order to minimize matrix interferences; in addition, in some cases, sensitivity results inadequate for its application to real biological samples [14]. Therefore, determination of ergotamine in biological samples still requires the development of simple, sensitive, precise, selective and inexpensive analytical methodologies.

Organized surfactant media have found increasing application in many areas of separation science, due to their singular properties and unquestionable advantage with respect to conventional phase separation techniques. The crucial factor in successful application of micellar systems is associated with the ability to selectively solubilizing and interacting with solute molecules [15,16]. Cloud point extraction (CPE) has proved to be efficient for concentrating several analytes from aqueous samples [15–19], including organic pollutants such as pesticides, aromatic hydrocarbons [20,21] and to monitoring drugs level in biological samples [22,23]. Additional advantages are related with the employment of small amounts of non-toxic reagents and the generated wastes are environmentally friendly.

As result of CPE, the analyte initially present in the large bulk solution is extracted into the micelles of surfactants with clouding behavior. For this purpose several surfactants can be used, mostly nonionic but also anionic ones [18]. After phase separation provoked by altering some experimental conditions, the analyte is preconcentrated into the smaller surfactant-rich phase. By proper dilution with a fluidificant, the analyte concentrated in the viscous surfactant rich-phase is determined by an adequate detection method. This dilution process of the surfactant-rich phase unfailingly leads to minor preconcentration factors [24].

In this paper, the high extraction efficiency of CPE procedure has been combined with the inherent sensitivity of molecular fluorescence for the analysis of ergotamine. In contrast to the conventional CPE procedure in which the surfactant-rich phase is fluidified, emission advantages of undiluted surfactant rich-phase in rigid gel state have been explored for the first time. This additional spectral advantage improved greatly the sensitivity leading to a low detection and quantification limits, and therefore, allowed the determination of trace levels of ergotamine in real samples.

## 2. Experimental

### 2.1. Instrumentals

A Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp and quartz cells of 0.4 mL (optical path length: 10 mm; internal width: 1 mm × 10 mm; Spectrosil® Quartz model 52/Q/1, Starna Cells Inc., UK) were used for molecular fluorescence measurements.

A Beckman CE P/ACE system MDQ (California, USA) equipped with UV–vis detection system was employed for validation of the proposed methodology. Untreated bared fused silica capillary (Simplus Capillaries™, length of 60 cm with 75 μm ID) was purchased from MicroSolve™, NJ, USA.

A pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940 with combined glass electrode was used for monitoring pH adjustment.

A Rolco™ centrifugal (Buenos Aires, Argentine) was employed to accelerate the phase-separation process.

### 2.2. Reagents and assay solutions

All standard and chemicals used throughout the experiment were of analytical reagent grade. Solvents were of analytical reagent grade or HPLC grade when in contact with the CE system.

#### 2.2.1. Standard solutions

Ergotamine tartrate was kindly provided by Andrómaco S.A. (Buenos Aires., Argentine). Standard solution of ergotamine tartrate containing 1.0 mg $mL^{-1}$ was prepared dissolving the reagent in ultra pure water and it was stored at 5 °C in a 50 mL amber color flask. The standard working solution was prepared daily by adequate dilution with ultra pure water.

#### 2.2.2. Extracting solution

Anionic surfactant PONPE 7.5 (polyoxyethylene(7.5)nonylphenylether) from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan) was employed as the extracting agent. Due to its high viscosity, direct application of the pure reagent is not experimentally convenient. Thus, the surfactant reagent needs to be fluidified as follows: 10 g PONPE 7.5 were mixed with 40 mL ethanol (Merk Darmstadt, Germany), and made up to 100 mL with ultra pure water.

#### 2.2.3. Buffer solution

A buffer solution of $1 \times 10^{-2}$ mol $L^{-1}$ sodium tetraborate (Mallinckrodt Chemical Woks, New York, Los Angeles, St. Louis, USA) was prepared dissolving the reagent in ultra pure water. The desired pH was obtained by adding a diluted solution of HCl (Merk Darmstadt, Germany) or NaOH (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA).

### 2.3. Samples

#### 2.3.1. Pharmaceuticals

Sample solutions were obtained by dissolving tablets of Tetralgin™ (CRAVERI, Buenos Aires, Argentine) and Ibupirac Migra™ (PFIZER, Buenos Aires, Argentine), labeled as containing 1 mg ergotamine tartrate in association with caffeine, methochlopramide and dipyrone in Tetralgin™, and with caffeine and ibuprofen in Ibupirac Migra™. Four tablets of each trademark were weighed and finely powdered. Portions of powder equivalent to 1 mg of ergotamine were accurately weighed and dissolved in 25 mL of ultra pure water acidified with 1 mL HCl ($1 \times 10^{-4}$ mol $L^{-1}$). Solutions were filtered, received in 50 mL volumetric flasks and made up with ultra pure water.

#### 2.3.2. Biological fluids

Aliquots of 10 mL of fresh human urine were centrifuged for 5 min at 3500 rpm ($1350 \times g$). 3 mL of supernatants were collected from each tube and transferred into new test tubes and stored in refrigerator until assaying. To avoid the inner filter effect from its highly fluorescent matrix of urine, a final dilution factor of 5:100 was chosen as optimum for the ergotamine determination.

For saliva samples, aliquots of 5 mL of fresh human saliva were made up to 10 mL with water in order to diminish the viscosity. After mixing well, samples were placed in centrifuge tubes and were centrifuged for 10 min. 2 mL of supernatants were collected and transferred into new test tubes and stored at 0 °C until assays.

### 2.4. General analytical procedure

Aliquots of standard working/sample solution ($5 \times 10^{-3}$ to 1 μg of ergotamine) were mixed with 1 mL of a buffer solution (borax solution of $1 \times 10^{-2}$ mol $L^{-1}$, pH 8.5) and 300 μL of extracting solution into a set of graduated centrifuge tubes. The whole mixtures were taken up to 10 mL with ultra pure water and then centrifuged for 15 min at 3500 rpm ($1350 \times g$). After being cooled in an ice bath for 5 min, the separated surfactant-rich phase became a transparent viscous gel at the bottom of the tubes and the aqueous phase could be poured off by simple inversion of tubes. The remaining viscous surfactant-rich phase (approximately 200 μL) was then carefully transferred by micropipette into the 400 μL quartz cell and the fluorescent emissions were measured at $\lambda_{em}$ = 425 nm using $\lambda_{ex}$ = 313 nm.

#### 2.4.1. Determination of ergotamine in spiked urine and saliva sample

Aliquots of urine sample solution (0.5 mL) or saliva sample solution (2 mL) processed as described in Section 2.3.2 were spiked with different concentrations of ergotamine (0.0–0.5 μg) and analyzed as described before in the general procedure.

#### 2.4.2. Capillary electrophoresis

After proceeding as described in general procedure, the viscous surfactant-rich phase is diluted with 200 μL of methanol and determined by CE.

The carrier electrolyte consisted of an aqueous solution of 0.02 mol $L^{-1}$ sodium dihydrogenphosphate, adjusted to pH 4.5 with phosphoric acid (0.05 mol $L^{-1}$). The working conditions of CE were as follows:during the analysis, the temperature of the capillary was kept at 30 °C and the voltage applied was 25 kV. Sample injection was performed by hydrodynamic mode (0.5 Psi during 5 s). The studied analyte were detected by UV absorbance at 200 nm, in less than 9 min.

## 3. Results and discussion

### 3.1. Surfactant selection

Depending upon the nature of the solute and the organized surfactant system, a solute can bind to different regions of the aggregate. Typically, a polar molecule can bind to the surface of micelles via electrostatic interactions while non-polar molecule is solubilized/partitioned into the hydrophobic micelle core. The grade which an uncharged molecule is partitioned into the hydrophobic micellar core relies on the magnitude of its solubilization or affinity to the micelle core, the molecule polarity and the solution composition. Once organic molecules are partitioned into micelles core, the extraction or separation of those molecules from the matrix can be achieved by phase separation as occurs in CPE.

Cloud point phenomena have been achieved typically for aqueous solutions of some nonionic surfactants. Even though some authors have proposed the use of ionic surfactant solutions as an alternative to the conventional nonionic surfactants for the preconcentration of charged substances [25], the use of the sodium dodecyl sulphate (SDS) for CPE of ergotamine was inadequate. Unlike nonionic surfactants systems, phase separation of SDS solution is induced by the presence of high levels of inert salts. Moreover, the exact salt concentration that induces the cloudy phenomenon is greatly influenced by several experimental conditions, such as temperature, ionic strength, presence of additives and analyte concentration. Therefore, CPE of ergotamine using SDS surfactant has not shown enough operative robustness.

Non-ionic surfactant PONPE 7.5 was chosen as the extractant for several reasons. First of all, the aqueous solution of PONPE 7.5 at a concentration of 0.3% (w/v) becomes instantaneously cloudy at room temperature, without the need for modifying experimental conditions. Secondly, after the centrifugation phase separation takes place, leading to a small volume and transparent viscous surfactant-rich phase at the bottom of the tubes. Moreover, compared to other nonionic surfactants, PONPE 7.5 shows a low fluorescence background signal. Studied extraction parameters and their optimal conditions are presented in Table 1.

After phase separation in traditional CPE procedure, the surfactant-rich phase is fluidified by the addition of an adequate diluting agent which is necessary for its transference to the measurement cell. Therefore, it is inevitable that a decrease in the analytical sensitivity in this step occurs.

In order to reach a higher preconcentration factor, the undiluted surfactant-rich phase was directly transferred to the 0.4 mL micro quartz cell. To obtain reproducibility in results, this operation was carefully achieved using a micropipette. The rapid charging and/or discharging of surfactant-rich phase produces bubbles or turbidity in the gel mass and consequently, lose of reproducibility.

As well as improving the preconcentration factor, the microenvironment provided by the surfactant-rich phase enhanced the fluorescence emission of ergotamine; the high viscosity of the medium promoted the transition of excited analyte to ground state by fluorescent emission instead of non-radiation relaxation. Consequently, the native fluorescence emission is largely improved (Fig. 1). As a result, a great sensitivity enhancement was achieved

**Table 1**
Ergotamine CPE experimental conditions.

| Parameter | Studied range | Optimal condition |
|---|---|---|
| $C_{PONPE\ 7.5}$ (w/v) | 0.05–0.50% | 0.20% |
| pH | 3.5–13.0 | 8.5 |
| $C_{buffer}$ (mol $L^{-1}$) | $5 \times 10^{-4}$–$5 \times 10^{-3}$ | $2 \times 10^{-3}$ |
| Centrifugation time (min) | 5–20 | 15 |

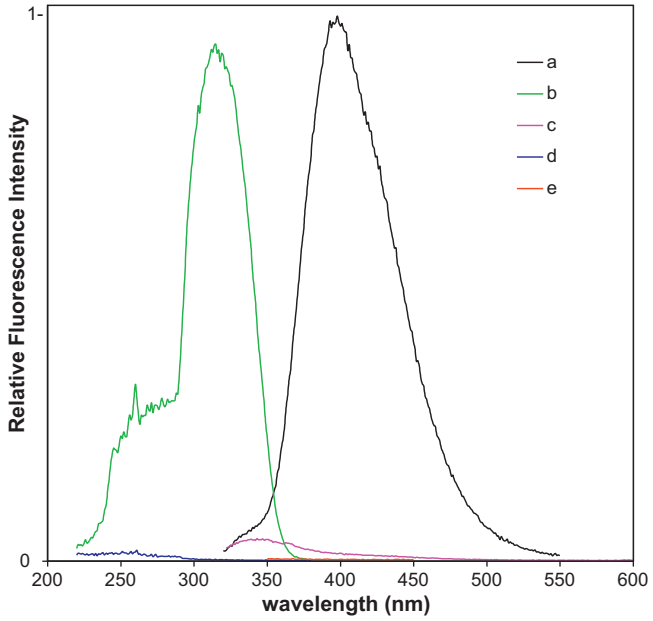


**Fig. 1.** Excitation and emission spectra of ergotamine in surfactant-rich phase. (a and b) Ergotamine 0.05 μg mL$^{-1}$ diluted surfactant-rich phase; (c) surfactant-rich phase without ergotamine; (d and e) Emission spectra of ergotamine 0.05 μg mL$^{-1}$ in ethanol. $\lambda_{ex}$ 325 nm; $\lambda_{em}$ 425 nm.

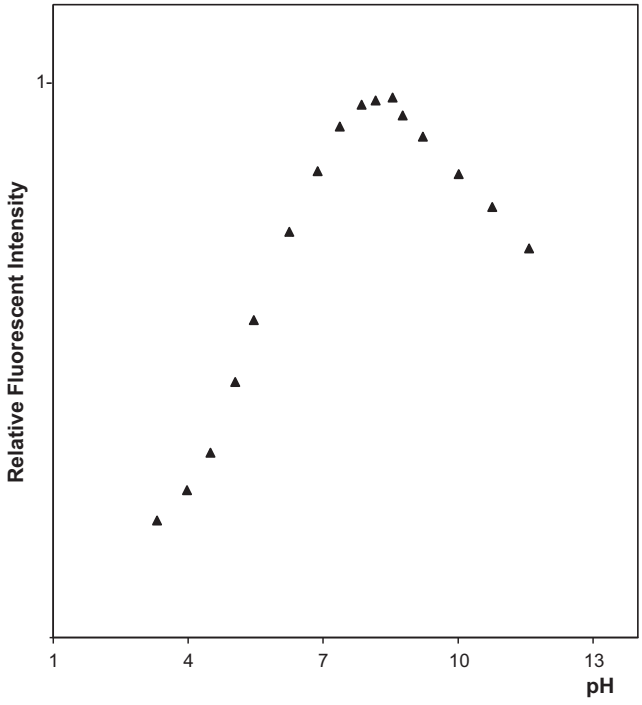


**Fig. 2.** Effect of equilibration pH on ergotamine CPE.

not only due to the improvement of the pre-concentration factor, but also by the enhancement of the fluorescence emission due to the viscous microenvironment provided by the surfactant-rich phase leading to a enhancement factor > 1000. The low detection and quantification limits obtained were lower and the linearity range broader than other methodologies [9–14].

### 3.2. Effect of pH on ergotamine CPE

Organic ionizable molecules show maximum extraction at pH values where the uncharged form prevails and therefore, the partitioning into the hydrophobic micellar core is facilitated. In order to determinate the optimal range of pH for ergotamine extraction, trials were carried out at different values of pH. Each desired pH value was obtained by the addition of buffers: acetic acid/acetate for pH between 3.5 and 6.0; phosphoric acid/phosphate for pH between 6.0 and 8.0; borax for pH above 8.0.

The best pH condition for ergotamine extraction was shown at alkaline pH, where the non-charged form prevails; the maximum was reached to pH 8.5 (Fig. 2). Thus, pH of 8.5 was chosen as optimal for ergotamine CPE. For pH higher than 9.0, a decrease of the extraction efficiency was observed probably due to a deprotonation of pyrrole group on ergoline nucleus (p*K*a of 9.76 [26]).

### 3.3. Figures of merit

Under optimal conditions, calibration curve for ergotamine CPE were performed according to general procedure. The detection and quantification limit were calculated using the relation $k(SD)/m$ where $k = 3$ for LOD and 10 for LOQ; SD is the standard deviation from 6 replicate blank responses (0.011) and $m$ is the slope of the calibration curve (288,223, $R^2 = 0.997$). Detection and quantification limits calculated were $1.15 \times 10^{-7}$ and $3.81 \times 10^{-7}$ μg mL$^{-1}$, respectively, with linearity range of $3.81 \times 10^{-7}$ to 1.10 μg mL$^{-1}$. According to IUPAC definition, the slope of calibration graph ($m$) represents the sensitivity of calibration. These obtained results confirmed the high sensitivity of the proposed methodology.

A comparison was made between calibration curves of ergotamine obtained with this methodology and ergotamine ethanolic solution with the same instrumental. The enhancement factor was calculated as the improvement in sensitivity with respect to the fluorescence signal of ethanolic solution. As a result, the present methodology showed an enhancement factor of 1325 fold with respect to ergotamine ethanolic solution.

### 3.4. Validation and application

In order to study the accuracy of the developed methodology, recovery studies were carried out by standard addition method. Known amounts of analyte at four different concentration levels (0.01–0.04 μg mL$^{-1}$) were added to two commercial trademarks (Tetralgin$^{TM}$ and Ibupirac Migra$^{TM}$) and determined applying the general procedure (Table 2). The results obtained showed quantitative recoveries for ergotamine, indicating good accuracy of the proposed method. These results were statistically compared against those obtained with the official method UV–vis spectrophotometry [27], applying student's test, giving *t*calculated of 0.456. As calculated *t*-test was lower than its tabulated value ($t_{(0.05,5)} = 2015$), it can be concluded that there is an insignificant difference between obtained results applying spectrophotometry and the developed methodology, with 95% probability level (Table 3).

#### 3.4.1. Determination of ergotamine in pharmaceuticals

The proposed methodology was applied to determine ergotamine in tablets for two commercial formulations; the results are presented in Table 4. It is noteworthy that in the tested formulations ergotamine is combined with other active drugs, such as caffeine, dipyrone, ibuprofen and metoclopramide. The most advantageous aspect of this procedure for the analysis of ergotamine in these formulations is the fact that has allowed selective fluorimetric quantification.

The average ergotamine concentration determined in the sample without the addition of ergotamine was taken as a base value. Then, known quantities of ergotamine were added to the others aliquots, and their concentrations were determined following the

**Table 2**
Recovery study and validation by CPE-CE[a] of proposed methodology applied to pharmaceutical samples.

| Sample | Added ($\mu g\,mL^{-1}$) | CPE-spectrofluorimetry | | Sample | Added ($\mu g\,mL^{-1}$) | CPE-CE | |
|---|---|---|---|---|---|---|---|
| | | Found ($\mu g\,mL^{-1}$)[d] | Recovery (%) ± RSD (%)[e] | | | Found ($\mu g\,mL^{-1}$)[d] | Recovery ± RSD (%)[e] |
| | – | 0.099 ± 0.004 | 99.8 ± 1.0 | | – | 19.23 ± 1.1 | 96.17 ± 5 |
| | 0.01 | 0.110 ± 0.003 | 100 ± 2.7 | | 10.0 | 31.16 ± 1.2 | 103.8 ± 4.5 |
| A[b] | 0.02 | 0.119 ± 0.003 | 99.1 ± 2.5 | A[c] | 20.0 | 39.01 ± 0.9 | 97.52 ± 2.2 |
| | 0.03 | 0.129 ± 0.002 | 99.2 ± 1.5 | | 30.0 | 50.10 ± 1.3 | 100.2 ± 2.6 |
| | 0.04 | 0.141 ± 0.002 | 100.7 ± 1.4 | | | | |
| | Ergotamine content = 0.9976 mg ± 1.82% | | | | Ergotamine content = 0.9937 mg ± 3.57% | | |
| | – | 0.101 ± 0.002 | 101 ± 2.0 | | | | |
| | 0.01 | 0.110 ± 0.002 | 100 ± 1.8 | | | | |
| B[b] | 0.02 | 0.121 ± 0.003 | 100.8 ± 2.5 | | | | |
| | 0.03 | 0.130 ± 0.001 | 100 ± 0.8 | | | | |
| | 0.04 | 0.141 ± 0.001 | 100.7 ± 0.7 | | | | |
| | Ergotamine content = 1.006 mg ± 1.56% | | | | | | |

[a] CE experimental conditions: buffer phosphate 0.02 mol $L^{-1}$, pH 4.5, applied voltage 25 kV, capillary temperature 30 °C; sample injection hydrodynamic mode (0.5 Psi during 5 s); spectrophotometric detection at 200 nm. Surfactant rich phases obtained from samples were diluted with 200 μL of methanol.
A: Tetralgin; B: Ibupirac Migra.
[b] A and B equivalent to 0.1 $\mu g\,mL^{-1}$.
[c] A equivalent 20 $\mu g\,mL^{-1}$.
[d] Mean value, $n = 3$.
[e] Average of all tested concentration levels.

**Table 3**
Statistical analysis of assayed results obtained from Tetralgin™ (1 mg) and Ibupirac Migra™ by proposed methods respect to the UV–vis spectrophotometric method [21].

| Sample | CPE-spectrofluorimetry Mean value[a] (mg) ± RSD (%) | UV–vis spectrophotometry [21] Mean value[b] (mg) ± RSD (%) | $t_{calculated}$ |
|---|---|---|---|
| A | 0.997 ± 0.85 | 0.992 ± 1.1 | 0.456 |
| B | 1.005 ± 0.76 | 0.998 ± 1.05 | 0.449 |

$t_{(0.05,\,5)}$ = 2015 tabulated value for 95% level of confidence (two-tailed test).
Samples A and B: Tablet of Tetralgin™ and Ibupirac Migra™, respectively.
[a] Average of 6 replicates using final concentration of 0.1 $\mu g\,mL^{-1}$.
[b] Average of 6 replicates using final concentration of 50 $\mu g\,mL^{-1}$.

**Table 4**
Recovery test for biological samples.

| Sample | Added ($\mu g\,mL^{-1}$) | Found[b] ($\mu g\,mL^{-1}$) | Recovery[a] (%) ± RSD (%) |
|---|---|---|---|
| Urine[b] | 0.025 | 0.026 ± 0.001 | 104.0 ± 4.0 |
| | 0.050 | 0.051 ± 0.002 | 102.0 ± 4.0 |
| | 0.075 | 0.074 ± 0.001 | 98.6 ± 1.3 |
| | 0.100 | 0.100 ± 0.002 | 100.0 ± 2.0 |
| | 0.150 | 0.149 ± 0.001 | 99.3 ± 0.6 |
| | 0.250 | 0.253 ± 0.002 | 101.2 ± 0.8 |
| Saliva[c] | 0.025 | 0.026 ± 0.001 | 104.0 ± 4.0 |
| | 0.050 | 0.052 ± 0.001 | 104.0 ± 2.0 |
| | 0.075 | 0.076 ± 0.002 | 101.3 ± 2.6 |
| | 0.100 | 0.101 ± 0.002 | 101.0 ± 2.0 |
| | 0.150 | 0.149 ± 0.001 | 99.3 ± 0.6 |

[a] Mean value, $n = 3$.
[b] 500 μL of urine.
[c] 2 mL of 1:1 diluted saliva.

developed procedure. Moreover, the surfactant-rich phases were analyzed by Capillary Zone Electrophoresis (CZE), in order to confirm the ergotamine by a separation method (Table 2). The obtained results were in concordance with ergotamine nominal contents of the analyzed pharmaceutical formulation (0.998 ± 1.82% and 0.994 ± 3.57% mg of ergotamine was found by the present methodology and by CZE, respectively, for Tetralgin™ tablet, labeled as contend 1.0 mg per tablet).

### 3.4.2. Determination of ergotamine in urine and saliva sample

Regarded to confirm the accuracy and applicability of this methodology, recovery studies using spiked urine and saliva samples were made. The working interval of $1 \times 10^{-4}$ to $1 \times 10^{-2}$ $\mu g\,mL^{-1}$ was chosen for estimating the ergotamine value in real samples from patients under treatment. The results presented in Table 4 show satisfactory recovery data with relative deviation standard between 0.6 and 4.0 (mean value of 3 repetitions) demonstrating the feasibility of applying the developed methodology in a complex biological matrix.

Emission spectra of urine sample with and without CPE were compared (Fig. 3), demonstrating the efficacy of the developed

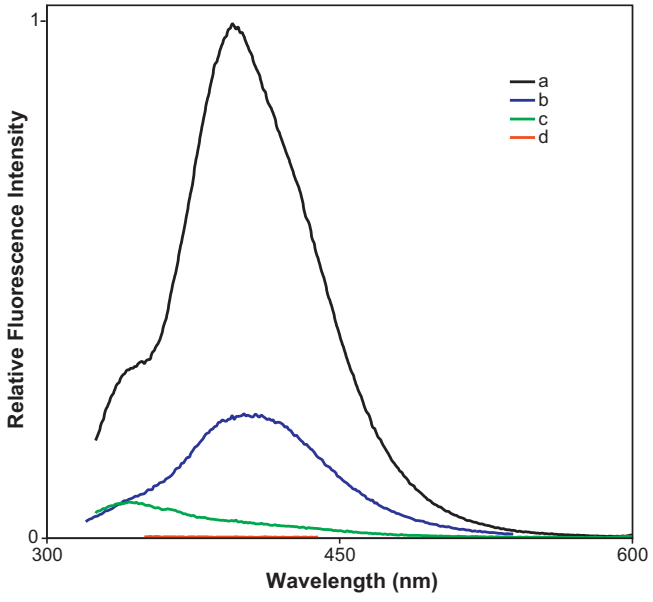


**Fig. 3.** Emission spectra of ergotamine in urine sample. (a) Urine sample spiked with 1.0 $\mu g\,mL^{-1}$ ergotamine, treated with CPE general procedure. (b) Urine sample emission. (c) Urine after CPE, without ergotamine. (d) Ergotamine 1.0 $\mu g\,mL^{-1}$ in ethanol.

methodology to eliminate potential spectral interference from urine, which is a highly fluorescent matrix. Urine sample was spiked with ergotamine tartrate leading to a final concentration of 1.0 $\mu g\,mL^{-1}$ and then, a dilution factor of 5:100 was selected as a compromise between internal filter effect and the linear analytical range.

The analysis of ergotamine in urine and saliva by the presented methodology has the advantage of simple sample pretreatment, without the need of successive extraction-washing steps or separation instrumentation, which in most cases is necessary. Moreover, the calibration curve obtained from spiked samples, has the same calibration slope to that for aqueous medium, indicating the absence of matrix effect. Thus, the determination of ergotamine in urine could be made by direct comparison with the aqueous standard solution at the same instrumental conditions.

## 4. Conclusions

The developed methodology for ergotamine determination combines all known advantages of CPE with the inherent sensitivity of spectrofluorimetry. In comparison to traditional separation techniques, it is a simple operating procedure using non-toxic and environmentally friendly reagents.

The distinctive characteristic of this proposal is the use of undiluted surfactant-rich phase in the determinative step with an enhancement factor of 1325 respect to the ethanol solution of ergotamine. The methodology has been validated by performing recovery studies and the results have been compared against the spectrophotometric official method and CE. The present methodology was successfully applied to the quality control of ergotamine in commercial pharmaceutical formulations. What can be seen is the potentiality and versatility of this methodology for the quality control of ergotamine in pharmaceutical formulations and in biological fluids drug monitoring.

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