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Highly sensitive detection of pharmaceutical compounds in biological fluids using capillary electrophoresis coupled with laser-induced native fluorescence

Julie Schappler, Aline Staub, Jean-Luc Veuthey, Serge Rudaz*

Laboratory of Pharmaceutical Analytical Chemistry, School of Pharmaceutical Sciences - EPGL, University of Geneva, University of Lausanne, Bd d'Yvoy 20, 1211 Geneva 4, Switzerland

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ABSTRACT

Due to its selectivity and sensitivity, capillary electrophoresis coupled to laser-induced fluorescence has evolved as a useful analytical tool for determining drugs in biological samples. This paper describes a simple, sensitive, efficient, and rapid method for analyzing propranolol without derivatization in plasma by CE-LIF using a diode solid-state laser at 266 nm. An experimental design methodology was assessed for the investigation of electrophoretic parameters and a Box-Behnken design was selected to optimize both sensitivity and efficiency. The CE-LIF method was linear over the concentration range of 50–850 ng mL^{-1} and successfully applied to a real forensic sample.

1. Introduction

Capillary electrophoresis (CE) is a powerful separation technique that has found numerous applications for a wide range of compounds in various analytical fields. This technique has several advantages, such as a high efficiency, rapid method development, simple instrumentation and low solvent and sample consumption; these features are the main reasons for its success. UV–vis spectrophotometry is likely the most widely used detection technique in CE because of its on-line configuration simplicity and quasi-universality, particularly in the pharmaceutical field. However, its sensitivity, which is directly related to the optical pathlength afforded by the capillary I.D. (in the μm range), remains the major bottleneck of this technique. UV detection requires relatively high analyte concentrations and is often unsuitable for bioanalytical applications, since many drugs present a high volume of distribution, resulting in low concentration levels [1]. To overcome this sensitivity issue, appropriate sample preparation procedures for analyte preconcentration and/or highly sensitive detectors [2], such as fluorescence techniques, should be considered. Indeed, the latter are generally more sensitive than absorption-based methods, since fluorescence signals are directly proportional to excitation power. Lamp-based fluorescence provides detection limits below 10^{-6} M, although focusing inside the capillary could be difficult. Because lasers are easily focused and allow high efficient excitation, laser-induced fluorescence (LIF) is particularly adaptable for CE [3]; furthermore, the high irradiance (I) provided by a laser leads to better signal-to-noise ratio (S/N), since signal intensity and noise are proportional to I and $I^{1/2}$, respectively. LIF detection provides very low limits of detection (LOD), with an improvement up to 10^5 compared to conventional UV detection [4]. While LOD down to 10^{-12} M have been reported [5], this sensitivity level is rather difficult to achieve for numerous reasons. Chemical derivatization is generally required, particularly for non-fluorescent analytes and fluorophores that cannot be excited at the available laser wavelength [6–8], and the kinetics of derivatization reactions often contribute to detection limitations (sometimes already at the 10^{-7} M level). Furthermore, co-migrating interferences may also be labelled and provide fluorescence properties comparable to those of the analyte [9]. LIF detection is only advantageous if the background noise is not increased by the same factor as the analyte signal. Some lasers exhibit power instability and increase the background noise, leading to a low increase in the overall sensitivity. However, interesting improvements have been recently implemented to overcome this issue. Diode lasers that feature very low output power fluctuations have been introduced, which limit intensity fluctuations, background noise amplification and spatial instability. Furthermore, their reduced size combines a moderate price with a long life span. Most diode lasers were first developed for the infrared and

* Corresponding author. Tel.: +41 22 379 65 72; fax: +41 22 379 68 08.
E-mail address: serge.rudaz@pharm.unige.ch (S. Rudaz).

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visible wavelengths but UV diode lasers are now commercially available.

As already mentioned, because chemical structures do not always possess a strong chromophore or fluorophore, derivatization procedures with a suitable fluorescent label, which matches the laser excitation wavelength, are often mandatory for sensitive detection [10]. A major feature of UV diode lasers is their ability to directly implement CE-LIF methods for natively fluorescent analytes, which is particularly advantageous because a variety of drugs contain a fluorophore that can be excited in the UV region. Numerous lasers have been developed for generating excitation wavelengths in the UV region. Initially, continuous wave argon-ion lasers, which presumably were widespread over various laboratories, were modified to provide UV excitation wavelength. For instance, frequency-doubled Ar lasers were tuned to obtain excitation wavelengths at 244 nm [11], 257 nm [12,13] or 275 nm [14]. Other materials were also shown to provide UV excitation wavelengths with comparable performance, *e.g.* pulsed KrF [15–17] or pulsed NeCu [18] lasers both emit at 248 nm, and XeCl laser coupled to a frequency-doubled dye laser allows generation of a 280 nm excitation wavelength [19]. Nevertheless, the aforementioned gas lasers present several previously listed restrictions, such as power instability, which ultimately lead to sensitivity limitations. Diode solid-state lasers, such as the pulsed Nd:YAG laser emitting at 266 nm [20], have emerged to address this constraint. Numerous publications were dedicated to experiments achieved on a conventional CE-LIF system with a 266 nm laser (CE-LIF 266). Most of them considered the determination of neurotransmitters in urine [21,22] or brain microdialysis samples [23], while few papers were dedicated to pharmaceutical drug analysis [24]. Furthermore, only univariate optimization was carried out for developing CE-LIF 266 procedures, without considering potential interactions that could occur between experimental factors.

In this paper, a multivariate optimization approach was applied to CE-LIF 266 analysis of propranolol as a model pharmaceutical compound present in biological matrices. The chemometric methodology involves the simultaneous investigation of the selected experimental factors, attributable to an adapted design of experiments (DOE), allowing high quality information from a relatively limited number of trials [25–28]. To our knowledge, this is the first application of this methodology to CE-LIF 266 optimization. The quantitative performance of the CE-LIF 266 method was assessed with an appropriate procedure and performed in agreement with regular guidelines and validation protocols [29–32]. The performance criteria evaluated were selectivity, response function, trueness, precision (repeatability and intermediate precision), accuracy, linearity and limit of quantitation (LOQ). The methodology was finally applied to the quantitation of propranolol in a real plasma sample issued from a forensic case.

2. Materials and methods

2.1. Chemicals

Propranolol (PROP) hydrochloride was obtained from Aldrich (Schnelldorf, Germany) and 3,4-methylenedioxyamphetamine (MDA) was purchased from Lipomed AG (Arlesheim, Switzerland). Chemical structures are reported in Fig. 1. Acetonitrile (ACN) and methanol (MeOH) were of analytical reagent grade from Panreac (Barcelona, Spain). Ethanol (EtOH) was of analytical reagent grade from Fisher Scientific (Leicestershire, UK). Phosphoric acid, formic acid, acetic acid, 2-(*N*-morpholino)ethanesulfonic acid (MES), 3-morpholinopropanesulfonic acid (MOPS), sodium

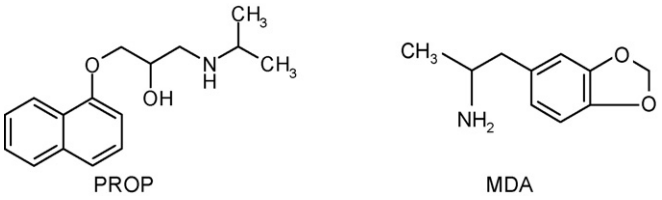

Fig. 1. Chemical structures of investigated compounds.

hydroxide (NaOH), formamide (FA) and *N*-methylformamide (NMF) were of analytical reagent grade from Fluka (Buchs, Switzerland). Tris(hydroxymethyl)aminomethane (TRIS) was of analytical reagent grade from Riedel-de-Haën (Buchs, Switzerland). α-, β- and γ-cyclodextrins (CD) were purchased from Sigma (Buchs, Switzerland), while hydroxypropyl β-CD (HP β-CD) and carboxymethyl β-CD (CM β-CD) were obtained from Roquette (Lestrem, France) and Cyclolab (Kötlichen, Switzerland), respectively. Ultrapure water was supplied by a Milli-Q RG purification unit from Millipore (Bedford, MA, USA). Blank plasma was obtained from the Blood Center of the Geneva Hospital (Geneva, Switzerland). Actual blood samples were obtained from the Institut Universitaire de Médecine Légale (IUML, Geneva, Switzerland).

2.2. Background electrolyte (BGE) and sample preparation

The BGE consisted of a 25 mM (ionic strength) TRIS-phosphate buffer at pH 2.8. The pH value was measured with a SevenMulti pH meter (Mettler-Toledo, Schwerzenbach, Switzerland), calibrated daily with four aqueous solutions at pH 2.00, 4.00, 7.00 and 10.00 from Riedel-de-Haën (Buchs, Switzerland). Stock standard solutions of analytes were prepared by dissolving each compound in MeOH to obtain a concentration of 1 mg mL^{-1}, which were stored at 4 °C until use. Standard solutions of analytes at the desired concentrations were prepared daily by appropriately diluting stock solutions in water. Sample solutions were stable for more than two days at 4 °C, and no degradation was observed for the tested analytes during analysis.

2.2.1. Solutions used for calibration

The calibration standards (CS) were samples in the matrix containing known concentrations of analytes, which were prepared in an independent way. Three levels ($k = 3$) were selected, corresponding to low (estimated limit of quantitation, LOQ), medium and high concentrations of the investigated range. CS were replicated twice ($n = 2$) and independently prepared in three different series ($j = 3$). Human blank plasma was spiked with the drug mixture at varied concentrations: 50, 500 and 1000 ng mL^{-1} of PROP and 500 ng mL^{-1} of MDA as I.S.

2.2.2. Solutions used for validation

Validation standards or quality control samples (QC) consisted of reconstituted samples in the matrix containing known concentrations of analytes and independently prepared from the CS. According to the international conference on harmonization (ICH) and FDA guidelines as well as recommendations from the "société française des sciences et techniques pharmaceutiques" (SFSTP), three series ($j = 3$) of three independent replicates ($n = 3$) were prepared at each concentration level ($k = 4$). Human blank plasma was spiked with the drug mixture at following concentrations levels: 50, 100, 500 and 850 ng mL^{-1} of PROP and 500 ng mL^{-1} of MDA as I.S.