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Exploiting sequential injection analysis technique to automate on-line sample treatment and quantitative determination of morphine in human urine

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ABSTRACT

A simple uni-stream sequential injection analysis (SIA) manifold was developed to automate a method for the assay of morphine in human sample. The proposed SIA method includes on-line sample treatment, coupling reaction and spectrophotometric measurement. A rapid algorithm controlled the adopted procedure was critically programmed. For sample treatment, solid-phase extraction (SPE) was carried out into a homemade microcolumn, installed in the SIA manifold. Sufficient sample clean-up, extraction and preconcentration were obtained by SPE. A coupling reaction of morphine with diazonium salt of aniline hydrochloride was adapted to SIA. The product of the reaction, an azo-morphine derivative, was spectrophotometrically detected at 390 nm. Parameters that influenced the efficiency of the proposed method, including solution volumes, diazonium concentration, flow rate and residence time, were optimized. The proposed method was linear in a range of 0.10–2.5 $\mu g\,ml^{-1}$. The limits of detection and quantification were 0.023 and 0.076 $\mu g\,ml^{-1}$, respectively. The detectability of the method was enhanced by the preconcentration and the use of an extended pathlength (50 mm) of a flow cell. The method was validated by an HPLC method. Comparable results with respect to accuracy (recovery 96.3–97.1), repeatability (R.S.D. < 2.4%) and intermediate precision (R.S.D. < 3.1) were gained. The full-automation and miniaturization of the utilized technique offer rapidity, safety in handling urine sample and reagents as well as reduction of reagent and sample volumes. The method is suitable for the application in forensic cases as an initial test and clinical analysis to prevent overdose-induced toxicity.

1. Introduction

Morphine is an analgesic drug used for the treatment of moderate to severe pain, especially for patients undergoing surgical procedures. It is recommended by the World Health Organization for the relief of moderate cancer-related pain [1]. Toxic effects of morphine usage include many serious symptoms. A dose of 120 mg morphine can be fatal. It has been reported that around 90% of orally administrated morphine is excreted in urine within 24 h. Around 10% of the excreted morphine remains un-metabolized [2]. Therefore, to prevent overdose-induced toxicity, the determination of morphine concentration in urine is required for clinical medicine.

Morphine is the primary constituent of opium. It is the most important drug of the opiates group. Commercial opium is usually standardized to contain 10% morphine [3]. In some cases, 6-monoacetylmorphine, the definitive metabolite of heroin, could not be detected in biological fluids for its short half-life of approximately 30 min. In such cases, a detectable amount of morphine and codeine as well as the ratio of morphine-to-codeine of higher than 2 are important criterion to judge the recent use of heroin [4,5]. Therefore, the determination of morphine concentration in urine is also required for forensic cases to prevent the drug of abuse.

In forensic cases, the analytical strategy generally employed for drugs of abuse testing in human urine is a two-stage process, initial and confirmation tests. Initial tests may be immunoassays or chromatographic. The positive result of an initial test is usually confirmed by chromatography–mass spectrometry (MS) [6]. Immunological assay methods are very sensitive and simple. However, they could be impaired by specific (cross-reaction of antiserums) and non-specific (pH and ion strength) interferences. Thin layer chromatography, as another alternative technique for the initial test of morphine, is also simple and inexpensive. Nevertheless, it suffers from a lack of sensitivity and specificity [7]. Recently, other non-separation techniques were also utilized for morphine assay in urine as initial tests including colorimetry [8], flow injection analysis [9] and amperometry [10]. A review manuscript that reported analytical methodologies for morphine assay and its metabolites is available elsewhere [11].

Morphine presents in the urine of patients and addicts in a trace level; thus, a sensitive assay method is desirable. In this

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challenge, many approaches could be applied to improve sensitivity. Detectors with high efficiency such as fluorescence and MS could be used. However, MS is an expensive technique. Fluorescence always requires critical derivatization reactions. The use of extended pathlength in a spectrophotometric detection, which is a simple and inexpensive technique, can improve sensitivity. However, this improvement may not reach the required limit of detection. Solid-phase extraction (SPE), as another approach, can also enhance sensitivity by preconcentration. SPE is also necessary in the analyses of complex samples such as biological fluids to reduce the influence of interferences.

In both forensic and clinical analyses, rapidity and safety in sample handling are of vital interest. The advantages of the sequential injection analysis (SIA) technique, including full-automation, miniaturization and versatility, can meet these requirements. The full-automation offers rapidity and safety in solution handling; and enhances accuracy and precision. The miniaturization also accelerates analysis and drastically reduces consumption of solutions and, consequently, provides better safety to the environment. The versatility empowers analysis with potential manipulations in reagents and sample, i.e. SIA could be used for widely different chemistries simply by changing the flow program. The versatility also allows conducting different on-line analytical processes by installing suitable devices in a SIA manifold. Possible on-line processes include sample treatment (SPE, liquid/liquid extraction, dilution, etc.), developing reactions (chromogenic, fluorescence, chemiluminescence, etc.) and detection (spectrophotometric, potentiometric, etc.). Critical articles that reported the principles, developments and applications of SIA are available in the literature [12–17]. It is noteworthy mentioning that Barnett's research groups utilized the SIA [18,19] and the pulsed flow [20] techniques for adopting methods, with chemiluminescence detection, for the assay of morphine in process samples. The utilized techniques offered high sampling frequency, 120 samples h^{-1}.

In spite of the outstanding advantages of SIA, few on-line SIA methods including sample treatment and developing reactions for drugs assay in biological fluids were proposed [21–24]. The current work proposes constructing a simple and inexpensive SIA manifold to conduct a fully automated method for the assay of morphine in human urine. The proposed procedure includes on-line SPE, coupling reaction and spectrophotometric measurement. The sensitivity of the proposed method was enhanced by applying two approaches, preconcentration and the use of an extended pathlength of a flow cell.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents that used in this study were of analytical grade quality; water was double distilled deionized. Codeine, morphine and 6-monoacetylmorphine were supplied from Lipomed Inc. (Cambridge, MA, USA). Aniline hydrochloride, sodium dihydrogen phosphate, phosphoric acid, hydrochloric acid, sodium nitrite, sodium hydroxide and methanol were supplied from Sigma–Aldrich (Taufkirchen, Germany).

2.2. Instrumentation

The SIA system used in this study is a FIAlab 3500 (Medina, WA, USA). It is composed of a syringe pump (SP), a multi-position valve (MPV), a holding coil (HC), a reaction coil (RC) and a personal computer (Fig. 1). The syringe has a volume of 5.0 ml. The MPV is chemically inert and has eight ports with a maximum pressure of 250 psi (gas)/600 psi (liquid) and a minimal dead volume. 0.03 in. i.d. Teflon tubings and a T-connector, which are supplied from Upchurch Scientific, Inc. (Oak Harbor, WA, USA), were used to connect different units of the SIA manifold and to make both the HC (600-cm long) and the RC (200-cm long). The SIA manifold was controlled by FIAlab for Windows version 5.0.

A C_{18} cartridge (5-cm length, 4.6-mm i.d.), which was supplied from Supelco (Bellefone, PA, USA), was packed in our laboratory with modified silica 45 μm particles.

2.3. Preparation of solutions and samples

To prepare diazonium solution, 0.065 g aniline hydrochloride was dissolved in 1 ml of 1 mmol l^{-1} hydrochloric acid. Then, 0.105 g sodium nitrite was added to the solution. The mixture was stirred for 5 min at 0 °C. The obtained solution is stable for 3 days at 0 °C. Phosphate/phosphoric acid buffer solution adjusted at pH 9.5 was prepared for conditioning the cartridge.

Human urine samples were collected from drug-free volunteers. The samples were adjusted at pH 9.5 by sodium hydroxide. Then, the samples were filtered through a membrane filter (0.45-μm pore size) [25]. The filtrate was spiked with different volumes of morphine to obtain different concentrations ranging from 0.05 to 5.0 μg ml^{-1}.

2.4. SIA procedure

A uni-stream SIA manifold was constructed to perform on-line sample treatment, developing reaction and spectrophotometric measurement. As shown in Fig. 1, water was linked with both the SP and port-1 in the MPV. The buffer solution was linked with port-2. 85 and 100% (v/v) methanol were attached to ports-3 and -4, respectively. Diazonium solution was attached to port-5. A standard solution/sample was attached to port-6. The length of the tubings which connected port-1 with -6 with their respective solutions was 10 cm. The C_{18} cartridge was installed between port-8 and the T-connector. The other side of the T-connector was linked to port-7. The HC was placed between the SP and the MPV while the RC was placed between the T-connector and the Z. The length of tubing which connected port-7 with the RC through the T-connector was 4 cm. The length of tubing which connected port-8 with the RC was 3 cm.

A rapid protocol that performed the proposed SIA procedure was programmed. It is briefly described as follows:

(i) Following the practice of SIA, each solution was first loaded into the HC by aspiration using the SP and then dispensed into the required channel.
(ii) To propel solutions, the syringe was filled with 1000 μl of water. Next, tubes were loaded for the first run with 100 μl of their respective solutions.
(iii) For conditioning the cartridge, 200 μl of each of 100% (v/v) methanol, water and buffer solution were sequentially injected into the cartridge at a flow rate of 20 μl s^{-1}.
(iv) At a flow rate of 10 μl s^{-1}, 200 μl of standard/sample was introduced into the cartridge. Thereafter, standard/sample was flushed with 300 μl of water at a flow rate of 20 μl s^{-1}.
(v) For the elution step, 20 μl of 85% (v/v) methanol was injected at a flow rate of 20 μl s^{-1}.
(vi) At a flow rate of 50 μl s^{-1}, 30 μl of diazonium was injected into the RC directly, i.e. without passing through the cartridge. To allow mixing reagent/eluate, six short reverse strokes were performed with a volume of 10 μl at a flow rate of 50 μl/s.
(vii) For the maximum color development, the flow was stopped for 180 s at 0 °C.