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Fluorimetric determination of aminocaproic acid in pharmaceutical formulations using a sequential injection analysis system

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Abstract

A sequential injection analysis (SIA) methodology for the fluorimetric determination of aminocaproic acid in pharmaceutical formulations is proposed. The developed analytical procedure is based on the derivatisation reaction of the aminocaproic primary amine with *o*-phthalaldehyde (OPA) and *N*-acetylcysteine (NAC) and fluorimetric detection of the formed product ($\lambda_{ex} = 350$ nm; $\lambda_{em} = 450$ nm). The implementation of a SIA flow system allowed for the development of a simple, fast and versatile automated methodology, which exhibits evident advantages regarding the US Pharmacopoeia 24 (USP 24) reference procedure. By combining the SIA time-based sample insertion with a subsequent zone sampling approach, which permitted to select for detection of a well-defined sample zone, it was possible to implement an on-line dilution strategy that enabled the expansion of the analytical working range of the methodology, and thus its application in dissolution studies, without manifold re-configuration.

Linear calibration plots were obtained for aminocaproic acid concentrations up to 6×10^{-5} mol l^{-1}. The developed methodology exhibit a good precision, with a R.S.D. < 2.0% ($n = 15$) and the detection limit was 2.5×10^{-7} mol l^{-1}. The obtained results complied with those furnished by the reference procedure with a relative deviation lower than 1.2%. No interference was found.

Keywords: SIA; Fluorimetric detection; Dissolution test; Aminocaproic acid; On-line dilution; *o*-Phthalaldehyde (OPA)

1. Introduction

Aminocaproic acid is an antifibrinolytic agent used in the treatment and prophylaxis of haemorrhagic states associated to excessive fibrinolysis [1]. With the exception of the reference methodologies proposed by several pharmacopoeias, which are based on non-aqueous titrations, almost all of the available methodologies for aminocaproic acid determination involve either a non-specific potentiometric titration [2] or the utilisation of time-consuming and expensive chromatographic techniques [3–5].

The derivatisation of an amino group with *o*-phthalaldehyde (OPA) [6–8] in the presence of a thiol donator, followed by fluorimetric detection of the formed product, has been used as an expeditious analytical approach for the determination of several species, taking advantage of the sensitivity and selectivity of fluorimetric measurements and extending its range of application to the determination of compounds devoid of native fluorescence. Among the available thiol compounds mercaptoethanol (ME) [6] and *N*-acetylcysteine (NAC) [7,8] are the most commonly used, although thiofluor (TF) has recently emerged as a valuable alternative [9].

Aminocaproic acid is a drug originally non-fluorescent but the presence of an amino group in its molecular structure anticipates the possibility of derivatisation with OPA and, in accordance, the development of a fast and simple analytical procedure for its determination. When implemented with all the automation facilities provided by sequential injection analysis (SIA) technique it combined the advantageous features exhibited by fluorimetric measurements, re-enforced by the reaction simplicity, and the robustness, reliability and easy of operation of SIA [10], guaranteeing a noteworthy

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analytical potential for application in the analysis of pharmaceutical preparations. The effective control of the most relevant analytical parameters at run-time assured a great operational flexibility, which allowed the assessment of distinct analytical strategies without physical reconfiguration of the flow set-up and facilitate system optimisation. In this sense, it was possible to extend the analytical working range of the methodology by implementing an on-line dilution approach based on a time-based selection of defined sample zones of the initially inserted sample plug, that were sent to detection. The developed methodology enabled the analysis of samples within a wide range of concentrations without manifold re-configurations and with the consumption of a low sample volume, which permitted its application in the monitoring of the drug concentration in dissolution studies.

The main goal of this work is the development of an automated methodology for the determination of aminocaproic acid in pharmaceutical formulations, which, supported by its simplicity, versatility, low reagent consumption, robustness and easy of operation could constitute an advantageous alternative to the available procedures.

2. Experimental

2.1. Reagents

All solutions were prepared using analytical grade chemicals and deionised water with specific conductance <0.1 $\mu S\,cm^{-1}$.

A 5×10^{-3} mol l^{-1} aminocaproic acid (minimum 99%) stock solution was prepared in deionised water. Working standard solutions were daily prepared by appropriately diluting the above solution with water.

10 mmol l^{-1} *o*-phthalaldehyde (OPA) and 0.8 mmol l^{-1} *N*-acetylcysteine (NAC) solutions were prepared in deoxygenated borate buffer, pH 9.3, with 4% of methanol. Both reagents were protected from light and kept in ice throughout use. The solutions of thiofluor (TF) and mercaptoethanol (ME) used in the evaluation of the thiol-donator compounds were prepared in the same conditions of NAC.

The solutions of the commercially available aminocaproic acid pharmaceutical formulations were prepared by dissolving the required amounts of each pharmaceutical formulation in water.

2.2. Instruments

Fluorescence measurements (λ_{ex} = 350 nm; λ_{em} = 450 nm) were carried out using a LabAlliance Fluorescence detector LC 305 equipped with an 8 μl flow-cell.

The SIA system (Fig. 1) consisted on a Gilson Minipuls 3, peristaltic pump, equipped with a PVC pumping tube (1.0 mm i.d.) and a 10-port multiposition Vici Valco selection valve.

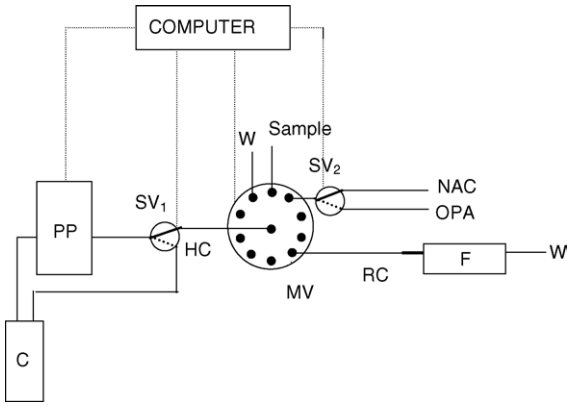

Fig. 1. SIA system used for the determination of aminocaproic acid in pharmaceutical preparations: PP, peristaltic pump; C, carrier (deionised water); SV_1, SV_2—3-way Solenoid valves; HC, holding coil (4 m; 0.8 mm i.d.; straight); MV, multiposition selection valve; RC, reaction coil (1 m; 0.8 mm i.d.; figure eight); F, fluorescence detector; W, waste.

Manifold components were connected by means of PTFE tubing, 0.8 mm i.d., which was also used for the holding and reaction coil (4 and 1 m, respectively).

A 3-way solenoid valve (NResearch 161 T031, W. Caldwell, NJ, USA) (SV_2, Fig. 1), placed at one of the inlets of the selection valve, was used to process the online addition of OPA and NAC. A second solenoid valve (SV_1, Fig. 1) and a contact device (on the peristaltic pump), similar to the one described before by Araújo et al. [11], controlled the pump starting point in order to guarantee reproducibility in the solution aspirated or propelled volumes.

Analytical system control, including the operation of the peristaltic pump, selection valve and solenoid valves, was achieved by means of an Advantech PCL 711B interface card and a Pentium-I based microcomputer. Software was developed in Microsoft Quick-Basic and permitted to control flow rate, flow direction, valve position, sample and reagent volume and data acquisition and processing.

During optimisation the analytical signals were also recorded on a Kipp & Zonen BD 111 strip chart recorder.

Dissolution studies were carried out by coupling the developed SIA system to a stirring basket Erweka DT dissolution apparatus. Sample solutions were aspirated at runtime through an inline filter (Schleicher & Schuell, 1.3 cm diameter).

2.3. SIA manifold

Aminocaproic acid was determined by fluorimetry (λ_{ex} = 350 nm; λ_{em} = 450 nm) upon reaction with OPA and NAC. The analytical cycle started when the reagents were alternately aspirated into the holding coil (HC, Fig. 1), by binary sampling, actuating sequentially solenoid valve SV_2 between position 1 and 0 (on/off). This solenoid valve was placed at position 10 of the multi-port selection valve MV. Each reagents insertion sequence consisted on three cycles of an intercalation time of 1 s (each reagent solution was