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Sensitive fluorimetric method based on sequential injection analysis technique used for dissolution studies and quality control of prazosin hydrochloride in tablets

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Abstract

This report introduces a fully automated flow system for drug-dissolution studies based on the coupling of the sequential injection analysis (SIA) technique with a conventional dissolution apparatus. The methodology described was used for monitoring of dissolution profiles of prazosin hydrochloride (PRH) in pharmaceutical formulation. The very sensitive fluorimetric detection of PRH was performed at $\lambda_{ex} = 244$ nm ($\lambda_{em} \geq 389$ nm). Under the optimal conditions, the calibration curve was linear over the range 0.02–2.43 mg l^{-1} of PRH with R.S.D. 1.89, 1.23, and 1.80% ($n = 10$) when determining 0.02, 1.22, and 2.43 mg l^{-1} of PRH in standard solutions, respectively. Equation of the calibration curve was calculated giving the following values: $F = 4.108c - 3.9$ ($n = 6$), $r = 0.9996$. Detection limit was calculated 0.007 mg l^{-1} of PRH. The dissolution test of Deprazolin® tablets was programmed for 60 min, with a continuous sampling rate of 70 h^{-1} under conditions required by USP 26. Results obtained by SIA technique compared well with HPLC standard method.

Keywords: Sequential injection analysis (SIA); Dissolution test; Prazosin hydrochloride (PRH); Fluorimetric detection

1. Introduction

About three decades ago, problems in biological availability of drugs were brought to attention of regulatory agencies and compendial standards groups. The rate of drug release from dosage form is one of the crucial factors determining the availability of drug. The rate of dissolution of drug from the solid state is defined as the amount of drug substance that goes into solution per unit time under standardised conditions of liquid/solid interface, temperature, and solvent composition.

Scientific evidence has shown that dissolution testing provides the means to evaluate critical parameters of pharmaceuticals such as adequate bioavailability, and information necessary to the formulator in development of more efficacious and therapeutically optimal dosage forms. According to the Food and Drug Administration (USA) bioavailability testing in which humans are used as test subjects should be minimised

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by development and implementation of in vitro dissolution standards that reflect in vivo drug performance [1].

Most drug-dissolution testing procedures involve sampling from the dissolution medium using a rotating basket, paddle or flow-through approach. Samples taken from the dissolution medium are usually analysed "off-line". Automated "on-line" system enables frequent sampling, low sample consumption, possibility of on-line sample pre-treatment, as well as simultaneous determination of agents in multi-components formulations, etc. [2–4].

Flow injection analysis (FIA) and sequential injection analysis (SIA) has played an important role in the automation of dissolution studies over the last period. Compared with the FIA drug-dissolution systems [5–8], the SIA systems [2,9,10] show outstanding advantages of robustness, easy manipulation and simplicity of the instruments. Owing to the use of a reliable syringe pump, full computer control and automated sampling, the SIA system provides higher precision and more reliable and accurate results. Finally, it could be used for fully automated long-term monitoring of not only dissolution profiles, but also for a testing of liberation profiles from the topical formulations [11].

Nowadays hypertension is one of the most frequent civilisation diseases in developing countries. Antihypertensive therapy improves the quality of life of hypertensive patients. A variety of antihypertensive agents contain quinazoline and quinazolidinone ring systems as, e.g. prazosin hydrochloride (PRH), 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl) piperazine monohydrochloride (Fig. 1), which has been proven efficient for a long time in the clinical treatment, acting as α_1-adrenoceptor antagonist [12].

The current USP 26 refers using HPLC method with UV spectrophotometric detection for the determination and monitoring of dissolution profile of PRH [13].

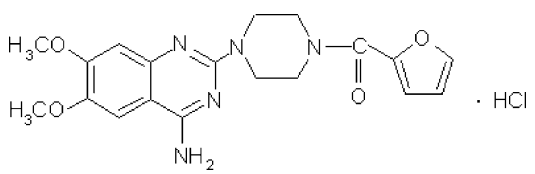

Fig. 1. Chemical structure of prazosin hydrochloride.

Obviously, in case of analysis of only one active compound in pharmaceutical formulation there is no need to use separation techniques, moreover, the UV detection is not very specific and often not sensitive enough in comparison with the fluorimetric detection.

A number of papers dealing with the assay of PRH as pure substance or active component in pharmaceutical formulations or in biological fluids have been published during last decade [14–21]. Besides, the proposed fluorimetric detection [14–16], PRH has been successfully detected by various electrochemical methods, e.g. using voltammetry [17], differential pulse polarography [18], amperometric detection at a glassy carbon electrode [19], further by radio-receptor assay [20] or by densitometric method [21].

The aim of our work was to develop a sensitive, rapid and automated fluorimetric method for the determination and dissolution testing of PRH in tablets using a SIA technique and compare the acquired results with official HPLC method.

2. Experimental

2.1. Apparatus

The proposed SIA manifold used for the determination of PRH (see Fig. 2) consisted of a 2.5 ml volume CAVRO XL 3000 syringe pump, 10-port VICI VALCO selection valve NDU0048 (Valco Instruments Co., Houston, USA) with electrical actuator and holding coil (volume 1.2 ml). All connections and the holding coil were made of a 0.75 mm i.d. PTFE tubing (Upchurch Scientific, USA). A peristaltic pump (Gilson Minipuls 3, France) was employed to achieve a continuous flow of the dissolution medium through a closed circuit during the dissolution test, with the aim to have ready in any time the actual concentration of the dissolved drug close to the sampling port of the selection valve. The communication between the closed circuit and the sampling port was enabled by using a home-made double connector—screw with bore for two tubings reaching the bottom of the screw which was fixed directly to the selection valve. The dead volume of the double connector is less than 10 μl. The total volume from sampling inlet in the dissolution vessel to the selection valve was about 270 μl. A fluorimetric flow detector Schoeffel Instrument (Model

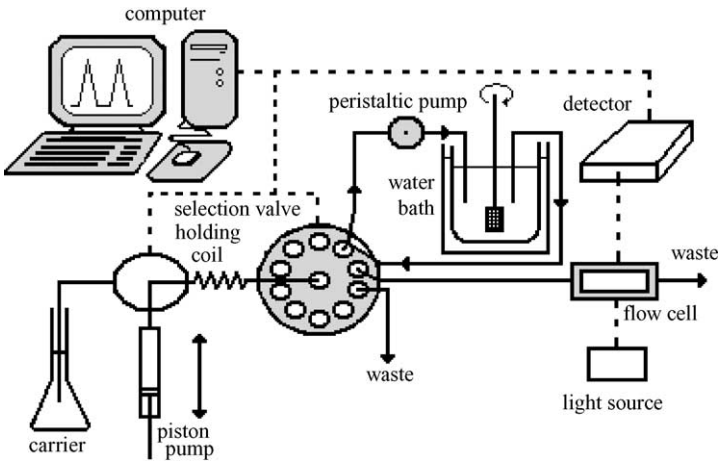

Fig. 2. Scheme of the SIA system.

FS 970, Germany) with 8 μl flow cell was applied. The length of tubing connecting the selection valve and the detector cell was 150 mm. PC equipped with a data acquisitions card AT-MIO-16E10 and an interface card AT-232/4 (National Instruments Corporation, USA) served as a control unit. The operational software FaFSIA version 1.1, written in LabVIEW®, enables the control of the moving of syringe pump, selection valve status and signal processing.

According to USP 26 for the performance of the dissolution study, the following instruments were used—dissolution vessel, rotating basket (Pharmatest, Germany) and digital rotating device (Labortechnik, Germany). The thermostat (Type NBE, Germany) was used for keeping the constant temperature of the dissolution medium throughout the dissolution test. The modified PTFE on-line microfilter (pore size 0.45 μm, ECOM s.r.o., Czech Republic) was used for the filtration of the solution from a dissolution vessel. The filter was fixed halfway between the rotating basket and the surface of the dissolution medium, 10 mm from the sidewall of the vessel.

The HPLC system, used for comparison of methods, consisted of a pump (Ecom LCP 4100, Czech Republic), autosampler (Waters 717), UV detector (Waters 486) and data processing software CSW version 1.7. A Purospher® RP-18 column (Merck, Germany) (125 mm × 4 mm i.d., 5 μm) was used for the HPLC analysis.

2.2. Reagents

All chemicals for the optimisation procedures and final determination of PRH were of analytical grade and they were used without further purification. All solutions were prepared with distilled-deionised water (Millipore, Milli-Q, USA).

An amount of 0.01 mol l^{-1} hydrochloric acid (Lachema a.s., Czech Republic) containing 3% (w/w) sodium lauryl sulphate (Taurus a.s., Czech Republic) was applied as dissolution medium.

Fresh stock solution of PRH (Léèiva a.s., Czech Republic) (2.43 mg l^{-1}) was prepared by dissolving the substance in 0.01 mol l^{-1} hydrochloric acid containing 3% (w/w) sodium lauryl sulphate. Working solutions of PRH were prepared immediately before the experiments by diluting the stock solution with dissolution medium.

Mobile phase for the HPLC measurements was prepared by mixing of 70.0 ml of methanol (Chromasolv®, for HPLC, Sigma–Aldrich, Czech Republic), 1.0 ml of acetic acid 99.8% (Lachema a.s., Czech Republic), 0.02 ml of triethylamine (Sigma–Aldrich, Czech Republic), and 30.0 ml of distilled water, pH 3.83, degassed and used in chromatographic system.

Tablets of Deprazolin® formulation (Léčiva a.s., Czech Republic) contain PRH as active substance, and excipients—microcrystalline cellulose, corn starch,

calcium stearate, sodium lauryl sulfate, calcium biphosphate dihydrate.

2.3. Assays of PRH in pharmaceutical formulation

Following assays were carried out according to the current USP 26 because there is no other pharmacopoeia describing a dissolution assay of PRH.

The uniformity of content for uncoated and film-coated tablets [22] is performed as followed. The content of 10 units is determined individually and the requirement is met if the amount of the active ingredient in each of the 10 dosage units lies within the range of 85.0–115.0% of the label claim and the R.S.D. is less than or equal to 6.0%.

Conditions for *the dissolution studies* of PRH formulations following the USP 26 [2,23] required the rotating basket method with agitation speed 100 rpm, when 900 ml of water, thermostated at $37.0 \pm 0.5\,^{\circ}C$, is used as a dissolution medium. Six tablets are tested, when not less than 75% of the labelled amount of PRH is dissolved in 60 min.

2.4. Procedure

The following procedure was carried out in the proposed SIA system in connection with the dissolution equipment. The SIA system (Fig. 2) employed a peristaltic pump generating continuous flow of the test solution through the closed circuit at a flow rate of $0.5\,ml\,min^{-1}$ during the whole dissolution test. Every 50 s the syringe pump was filled (500 μl) by carrier solution ($0.01\,mol\,l^{-1}$ hydrochloric acid containing 3% (w/w) sodium lauryl sulphate). The pre-defined volume of the test solution (20 μl) was taken from the circuit every 50 s by aspiration through the double connector, transported at a flow rate of $15\,\mu l\,s^{-1}$ to the detection system and the signal was measured by fluorescence detector at $\lambda_{em} \geq 389\,nm$ ($\lambda_{ex} = 244\,nm$). Previously, the aspirated 20 μl of the sample was dispensed directly to the waste whereby the dead volume (10 μl) of the sample in the double connector had been removed. The resulting signal was recorded in the form of peaks; the peak heights were calculated automatically and the data were stored for subsequent processing.

The determination of PRH was not affected by the presence of supplementary compounds (microcrystalline cellulose, corn starch, calcium stearate, sodium lauryl sulphate, calcium biphosphate dihydrate) of Deprazolin® tablet.

The official HPLC method, required for determination of PRH according USP 26, was performed for the comparison of the results obtained by proposed SIA system. The samples and standards were dissolved in $0.01\,mol\,l^{-1}$ hydrochloric acid containing 3% sodium lauryl sulphate. The volume of 10 μl of sample (standards, respectively) was injected onto the column and measured in triplicate. In this case, the method of external standard was applied. PRH dissolved in dissolution medium was determined on the Purospher® RP-18 column (125 mm × 4 mm i.d., 5 μm) using a mobile phase, which composition and preparation are described in the Section 2.2. The HPLC was operated in a constant flow mode and the flow rate was kept at $1.0\,ml\,min^{-1}$. UV detection was performed at 244 nm.

3. Results and discussion

3.1. Optimisation of variables

Sodium lauryl sulphate included in the dissolution medium (composition: see in Section 2.2) was found to exhibit interfering fluorescence signal by PRH determination. Using carrier solution of the same composition as dissolution medium eliminated the problem. The influence of sodium lauryl sulfate contained in tablets as an excipient is inconsiderable in respect with its content per tablet.

Excitation wavelength 244 nm was found in literature and affirmed experimentally as optimal.

The univariant method of optimisation was used in order to achieve optimal SIA variables. The peak height and R.S.D. resulting from the aspiration of $2.43\,mg\,l^{-1}$ of PRH at a constant sample volume of 20 μl was the optimisation criterion.

Flow rate for aspiration of sample into the carrier was optimised. Values in the range 10–80 $\mu l\,s^{-1}$ were tested, $30\,\mu l\,s^{-1}$ was applied as optimum in the final procedure. Flow rate $15\,\mu l\,s^{-1}$ was selected for the passage of flow-through the detector from tested values in the range 10–50 $\mu l\,s^{-1}$, the optimal volume of sample was found to be 20 μl.

With respect to high intensity of PRH fluorescence no pH adjustment was necessary for improving the limit of detection.

3.2. Calibration

The calibration for SIA determination of PRH was carried out under the optimised conditions. The calibration curve relating the intensity of fluorescence to the analyte concentrations (measured in triplicate) involved six calibration points (minimal value = 1% of labelled amount, maximal value = 100% of labelled amount of determined substance in pharmaceutical formulation) Calibration was linear over the range 0.02–2.43 mg l^{-1} of PRH. The R.S.D. was 1.89, 1.23, and 1.80% ($n = 10$) when determining 0.02, 1.22, and 2.43 mg l^{-1} of PRH in standard solutions, respectively. It was described by the following equations: $F = 4.108c - 3.9$ (where F is the intensity of fluorescence and c is the PRH concentration) ($n = 6$); the correlation coefficient was 0.9996. The detection limit (3σ) was 0.007 mg ml^{-1} of PRH for a sample volume 20 μl measured at $\lambda_{ex} \geq 244$ nm ($\lambda_{em} = 389$ nm).

3.3. Determination of PRH in pharmaceutical (tablets)

The above-mentioned procedures (see chapter 2.3) were carried out with Deprazolin® (2.19 mg of PRH per one tablet).

The content of PRH in each of the tablet ($n = 10$) analysed for *the uniformity of content* [22] lied within required range of 90.0–110.0% of the label claim (minimal value = 98.42% and maximal value = 107.06%), with the R.S.D. 2.56% (see Fig. 3).

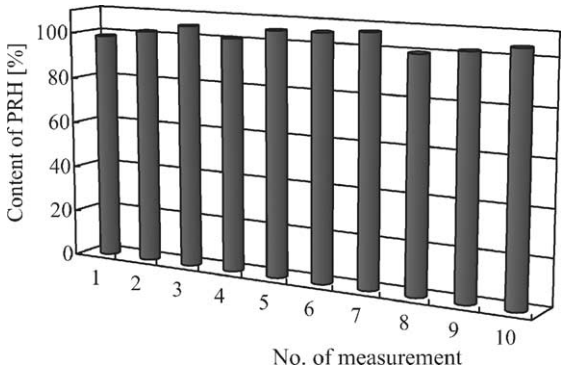

Fig. 3. Experimental values of prazosin in tablets ($n = 10$).

The dissolution studies of the commercial products of Deprazolin® tablets were executed using the mentioned method with the optimised conditions (see Fig. 4). The sampling intervals are initiated from the time of lowering of the basket into the dissolution medium. The resulting signals were recorded in the form of peaks; the peak heights were calculated automatically and the data were stored for subsequent processing. According to the USP 26 [23], not less than 75% of the labelled amount of PRH should be dissolved in 60 min. In the case of Deprazolin® tablets, these criterions has been fulfilled.

Using HPLC method, PRH was determined in the dissolution medium under the described chromatographic conditions and was eluted as a symmetrical peak at retention time (t_R) 3.13 min after the

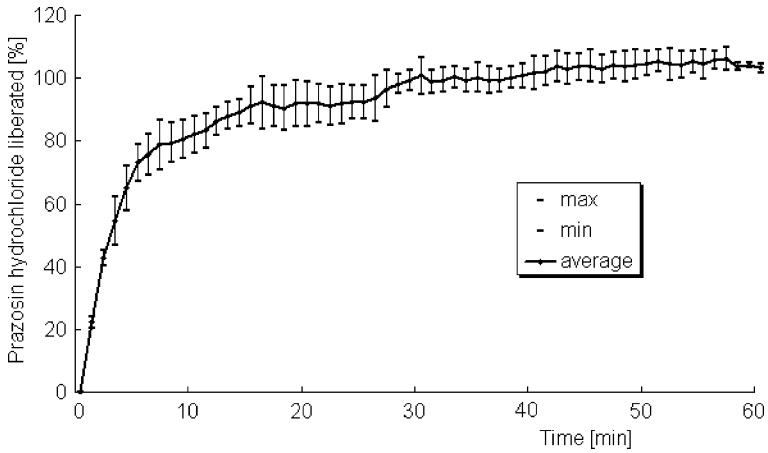

Fig. 4. Dissolution profiles of prazosin in Deprazolin® tablets formulation ($n = 6$).

Table 1
Determination of prazosin hydrochloride in pharmaceutical preparation

Preparation	Nominal content (mg)	Content (%[a] ± S.D.[b]) found		Student's *t*-test[c]
		SIA ($n_A = 3$)	HPLC ($n_B = 3$)	
Prazosin hydrochloride	2.19	99.39 ± 0.02	99.83 ± 0.54	0.576

[a] Percentage value of nominal content of PRH.
[b] Standard deviation of the mean value.
[c] At 95% confidence level, $t_\alpha = 2.776$ (see [24]); n_A and n_B are the number of results.

injection onto the RP-18 column. Results of assay of PRH in preparation obtained by the SIA method compared with the results obtained by a standard pharmacopoeial method are documented in Table 1. The Student's *t*-test [24] did not reveal any statistically significant difference between the results acquired by the proposed and official HPLC methods. This is a positive indication of the fact that any excipients present in the formulation analysed do not cause significant interference.

4. Conclusion

The automatic SIA system proposed in this work for on-line monitoring of dissolution profiles of PRH in tablets utilising sensitive fluorimetric detection was successfully applied and compared well with official method required by the contemporary USP 26. It has exhibited better sensitivity than the officially required method. The other advantages in comparison with HPLC are higher speed (sample throughput $70\,h^{-1}$) of the SIA method, minimal consumption of chemicals and decreased of the waste generation. On-line connection with dissolution equipment enables automation of the whole procedure and generation of a dissolution profile without any human control. The computer control of the SI timing ensures relatively sensitive and reproducible (R.S.D. < 1.80%) determination of PRH as well as in low concentrations.

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