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Novel flow injection spectrophotometric determination of fosinopril using UV-assisted digestion and an orthophosphates calibration graph

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Abstract

This work describes the first flow injection (FI) method for the spectrophotometric determination of the anti-hypertension drug fosinopril (FSP), a phosphorus-containing compound. The method is based on the UV-assisted digestion of the analyte using ammonium peroxodisulfate as the oxidizing reagent. The yielded orthophosphate ions are determined by a rapid and robust FI method employing the molybdenum blue approach. The time needed for complete conversion of a maximum FSP amount concentration of 2.0×10^{-4} mol l^{-1} was 30 min. Based on the capability of the home-made UV digester used in this work to process eight samples simultaneously, an acceptable samples analysis frequency of 16 h^{-1} was achieved. Additionally, a very important advantage of the proposed method is that an orthophosphate ions calibration graph can be used instead of FSP, as 100% conversion of FSP was achieved. Quantitative measurements of FSP were made in the range 1.0×10^{-6} to 2.0×10^{-4} mol l^{-1}. The application of the developed FI method to the analysis of two commercially available pharmaceutical formulations produced accurate results, as the relative errors were <1.5% in both cases, compared to the labeled values.

Keywords: Flow injection; Fosinopril; UV digestion; Spectrophotometry; Pharmaceuticals

1. Introduction

Fosinopril (FSP) (Fig. 1), is a phosphorus-containing compound used for controlling high blood pressure and in the treatment of congestive heart failure. FSP, along with other "-pril" compounds, belongs to angiotensin-converting enzyme (ACE) inhibitors. Its action lies on relaxing blood vessels (causing them to widen) and on reducing sodium retention, lowering in this way blood pressure and decreasing the workload of the heart.

Analytical assays for the determination of FSP include HPLC [1–3], capillary electrophoresis (CE) [4–7] and chemometrics using UV spectrophotometry [2,8,9]. HPLC methods employ UV detection and offer the possibility of determining the drug in the ranges 1.6–30 mg l^{-1} [1], 5–50 mg l^{-1} [2] and 20–200 mg l^{-1} [3]. CE assays are generally less sensitive compared to HPLC, namely 19–380 mg l^{-1} [4] and 20–360 mg l^{-1} [5], except from the method of Lozano et. al. which allows the determination of FSP in the range 1–400 mg l^{-1} [7]. Chemometrics-based procedures employ fourth derivative UV spectrophotometry (determination range 5–45 mg l^{-1} FSP) [2], first-order and ratio spectra derivative UV spectrophotometry (4–50 mg l^{-1} FSP) [8] and multi-wavelength

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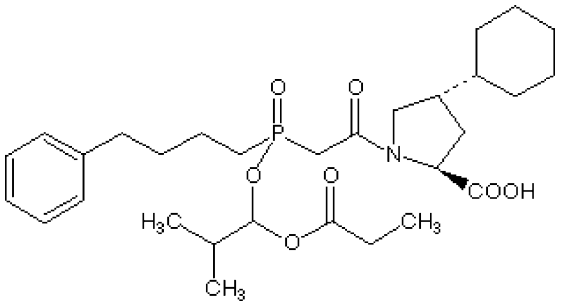

Fig. 1. Chemical structure of fosinopril.

UV spectrophotometry (no analytical data reported) [9].

Separation techniques (HPLC and CE, etc.), generally offer effective procedures in terms of selectivity, when complex biological samples have to be analyzed or multi-compounds determinations are required. However, in the routine quality control of pharmaceuticals usually one analyte, the active ingredient, has to be monitored. In that case, the demands for simplicity, rapidity, automation, easy handling and low operation and instrumentation costs predominate. Compared to separation techniques, flow injection (FI) analysis fulfills the above-mentioned demands, especially in the case of single-compound monitoring. Additionally, routine analytical procedures have to be straightforward, without employing complicated mathematical approaches for data handling. For this reason, chemometrics-based assays are rather unattractive to routine analysis. To the best of our knowledge no FI methods have been reported for the determination of FSP.

The oxidation/conversion methods of phosphorus-containing organic compounds have recently been reviewed [10]. Effective digestion can be achieved by using several techniques, including conventional heating (using a hot plate, sand-bath or aluminum block), UV-induced photo-oxidation or microwave heating. Other techniques such as fusion, ignition and dry-ashing are only recommended for the analysis of solid samples (soils, sediments, etc.). The various oxidizing reagents employed include perchloric acid, hydrogen peroxide and sulfuric acid/peroxodisulfate ions. Among these reagents, peroxodisulfate ions seem to be the most effective. Perchloric acid-based procedures are tedious and dangerous, while excess of hydrogen peroxide in the digestion mixture interferes with the molybdenum blue method and should be completely removed prior to the final measurement.

The reaction of peroxodisulfate ions with various organic and inorganic compounds has been studied extensively [11]. Peroxodisulfate ions decompose under photolysis or heat conditions to generate highly reactive species such as sulfate radicals ($SO_4^{\bullet -}$) and hydroxylradicals ($OH^{\bullet}$) initiating a series of radical chain reactions [11]:

$$S_2O_8^{2-} \overset{\text{heat}/h\nu}{\rightarrow} 2SO_4^{\bullet -}$$

$$S_2O_8^{2-} + M \rightarrow 2SO_4^{\bullet -} + M^{\bullet}$$

(M represents organic compounds)

$$SO_4^{\bullet -} + H_2O \rightarrow OH^{\bullet} + HSO_4^{-}$$

$$SO_4^{\bullet -} + M \rightarrow M^{\bullet} + \text{products}$$

$$OH^{\bullet} + M \rightarrow M^{\bullet} + \text{products}$$

$$S_2O_8^{2-} + M^{\bullet} \rightarrow SO_4^{\bullet -} + \text{products}$$

The novel method described in this paper presents a two-step procedure for the determination of FSP. The first step involves the off-line digestion of the samples under UV irradiation using ammonium peroxodisulfate as the oxidizing reagent. In the second step the generated orthophosphate ion is determined using a well-established FI procedure based on molybdenum blue formation [12]. A very important advantage of the method is that an orthophosphate calibration graph can be used for FSP measurements in the samples, because the conversion of the analyte is quantitative. This is particularly important because FSP pure material is not commercially available. An additional advantage of the proposed method is that it is specific for FSP in the presence of the other ACE inhibitors (captopril, enalapril, lisinopril, quinapril, perindopril, ramipril, benazepril and cilazapril), as FSP is the only phosphorus-containing compound. The developed FI method is simple, robust and cost-effective and could be an advantageous alternative to HPLC and CE methods. Besides, it is more sensitive than all the previously reported assays, allowing the determination of the analyte in the range 1.0×10^{-6} to 2.0×10^{-4} mol l^{-1} FSP (0.6–114 mg l^{-1} FSP). Based on the ability of the home-made UV-digester to process eight samples

at a time, the samples analysis frequency was acceptable ($16\,h^{-1}$). The applicability of the developed method was demonstrated by successfully analyzing two FSP-containing pharmaceutical products.

2. Experimental

2.1. Reagents

All chemicals were of analytical-reagent grade and were provided by Merck (Darmstadt, Germany) unless stated otherwise and all the solutions were made up with doubly de-ionized water.

FSP sodium reference material (batch no. 0C24454, assay 98.6%) was kindly donated by Bristol-Myers Squibb, Inc. (Wallingford, CT, USA). Standard stock solutions of $1.0 \times 10^{-2}\,mol\,l^{-1}$ FSP, as well as of $200\,g\,l^{-1}$ ammonium persulfate and $2.0\,mol\,l^{-1}$ H_2SO_4 were kept in polyethylene (PET) flasks, while working solutions were prepared daily before use.

The reagents for the molybdenum blue method were prepared according to the literature [12]. The molybdate ions reagent ($10\,g\,l^{-1}$ ammonium heptamolybdate in $35\,ml\,l^{-1}$ H_2SO_4 of $\rho = 1.84\,kg\,l^{-1}$) was stable for several months, while the stannous chloride reagent ($0.2\,g\,l^{-1}$ $SnCl_2$ plus $2\,g\,l^{-1}$ hydrazinium sulfate in $28\,ml\,l^{-1}$ H_2SO_4 of $\rho = 1.84\,kg\,l^{-1}$) was stable for at least 1 week if was kept refrigerated and protected from the light. Both solutions were degassed with purified nitrogen daily prior to their use.

Special attention was paid to clean effectively all the glassware to avoid phosphates contamination. For this reason, all glassware were thoroughly washed with a hot 1:1 HCl solution and then rinsed many times with doubly de-ionized water.

2.2. Instrumentation

The FI system used was a Tecator 5010 analyzer with a Type III Tecator chemifold. The detector was a Tecator 5023 FIAstar double-beam spectrophotometer, consisting of a 5032 detector controller and a 5023-011 spectrophotometer optical unit. The absorbance of the colored product was monitored at 690 nm through a 1 cm path length flow cell with an 18 μl internal volume. The flow system used was 0.5 mm i.d. Teflon tubing throughout. Tygon pump tubes were used for delivering the aqueous solutions. A 2 cm long/1.0 mm i.d. piece of an expanded PTFE (e-PTFE, ZEUS, Orangeburg, USA) microporous tube was placed between the UV-digestion unit and the injection valve to ensure the effective de-bubbling of the digestion mixture prior to injection in the FI manifold.

The digestion unit was constructed in-house by placing a 20 W mercury lamp in a cylindrical-shaped metallic box. A ventilator was adjusted at the bottom of the box to avoid overheating of the unit. The design of the unit allowed eight quartz tubes containing the samples to be placed around the lamb, enabling multi-samples process.

2.3. FI procedure for aqueous solutions

The preferred FI setup is depicted in Fig. 2. Two hundred microliters of the orthophosphate standards and the digested samples (FSP in $50\,g\,l^{-1}$ ammonium peroxodisulfate) were injected directly into the carrier stream (C: water) and then merged successively with the molybdate (R_1) and the tin(II)/hydrazinium sulfate (R_2) reagent streams. The molybdenum blue product was formed on passage through a 60 cm long reaction coil (RC_3) and was measured at 690 nm. A 2 cm long/1.0 mm i.d. e-PTFE microporous tube was placed between the digestion unit and the injection valve to remove possible formed O_2 and/or CO_2 bubbles prior to injection. Possible free orthophosphate ions existing in the analyzed samples were measured and subtracted in a first run, prior to digestion. Five replicate injections per sample were made in all instances.

2.4. Determination of FSP in pharmaceuticals by the proposed FI method

Ten tablets of each pharmaceutical formulation were weighed and ground to a fine powder. Accurately weighted amounts were dissolved in $50\,g\,l^{-1}$ ammonium peroxodisulfate. Ten milliliters aliquots of the resulting solutions were transferred in quartz tubes and were UV-digested for 30 min in the digestion unit shown in Fig. 2. Then, the digested samples were analyzed using the above-described FI procedure for aqueous solutions. Based on the capability of the digestion unit to process 8 samples simultaneously, the samples analysis frequency was $16\,h^{-1}$.

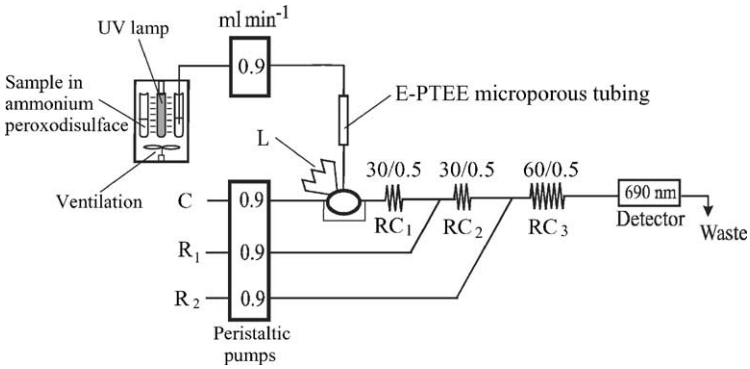

Fig. 2. Preferred FI setup for the determination of FSP. C = carrier stream (water); $R_1 = 10\,g\,l^{-1}$ ammonium heptamolybdate in $35\,ml\,l^{-1}$ of $\rho = 1.84\,kg\,l^{-1}$ H_2SO_4 stream; $R_2 = 0.2\,g\,l^{-1}$ hydrazinium sulfate in $28\,ml\,l^{-1}$ of $\rho = 1.84\,kg\,l^{-1}$ H_2SO_4 stream; L = sample loop (200 μl); RC_1, RC_2, RC_3 = reaction coils; numbers above coils denote the coil length (cm)/i.d. (mm) ratio.

3. Results and discussion

3.1. Preliminary studies

As FSP is a phosphorus-containing compound, it was suspected to be able to liberate orthophosphate ions upon UV-assisted digestion using peroxodisulfate ions as oxidizing reagent [10]. Preliminary studies were carried out at $2.0 \times 10^{-4}\,mol\,l^{-1}$ FSP in $20\,g\,l^{-1}$ ammonium peroxodisulfate. The mixture was UV-digested for 20 min, and then injected in the FI setup shown in Fig. 2. These first experimental results were encouraging as under the above-mentioned conditions, the conversion of the analyte was ca. 20%.

The FI manifold for the determination of orthophosphate ions described by Karlberg and Pacey [12] was simplified as reported in our previous work [13]. A single carrier stream was used instead of two, while all flow rates were fixed at $0.9\,ml\,min^{-1}$.

3.2. Study of digestion variables

The digestion variables studied were the mass concentration of the oxidizing reagent, the effect of the addition of sulfuric acid in the digestion mixture and the digestion time. The starting values of these variables were those mentioned in the previous section.

The effect of the mass concentration of ammonium peroxodisulfate on the digestion of FSP was studied in the range 10–50 $g\,l^{-1}$. The experimental results are shown in Fig. 3. Maximum and constant conversion of FSP was achieved in the range 40–50 $mg\,l^{-1}$. An ammonium peroxodisulfate mass concentration of $50\,g\,l^{-1}$ was, therefore, selected for further experiments. It should also be noted that the addition of sulfuric acid in the digestion mixture in the range 0–0.1 $mol\,l^{-1}$ had no effect in the digestion process.

The effect of the digestion time was studied in the range 0–35 min. As can be seen in Fig. 4, quantitative conversion of the analyte was achieved for digestion times above 30 min. This time was selected as optimal. Based on the capability of the digestion unit to process eight samples simultaneously, the sample analysis frequency was $16\,h^{-1}$. It should be noted that it is not necessary to achieve quantitative conversion of the analyte in order to measure FSP in the pharmaceutical formulations. Even a digestion time of 15 min (30 ± 1% conversion) should be sufficient in terms of

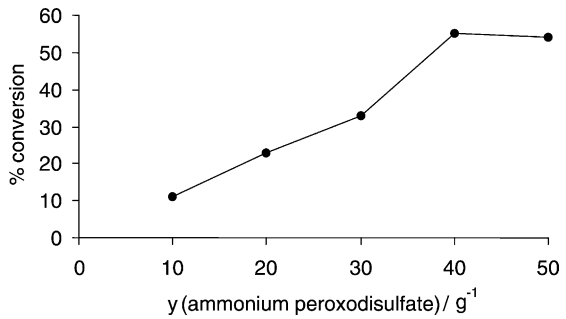

Fig. 3. Effect of the mass concentration of ammonium peroxodisulfate on the UV-digestion of $2.0 \times 10^{-4}\,mol\,l^{-1}$ FSP. $t = 20$ min.

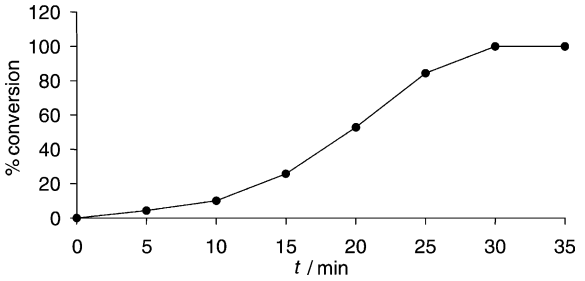

Fig. 4. Effect of time on the UV-digestion of 2.0×10^{-4} mol l^{-1} FSP; γ(ammonium peroxodisulfate) $= 50$ g l^{-1}.

sensitivity. However, the quantitative conversion of the FSP offers the very significant possibility of determining the analyte using an orthophosphates calibration graph. This advantage is particularly important taking into account the fact that FSP reference material is not commercially available.

3.3. Study of interferences

The effect of potential interferences upon the molybdenum blue reaction was studied at 1.0×10^{-4} mol l^{-1} orthophosphate ions. Quantitative recoveries of the analyte were obtained even in the presence of a maximum tested 500-fold excess of Na(I), K(I), Cl^-, SO_4^{2-}, Mg(II), Ca(II), CO_3^{2-}, lactose, glucose, fructose and starch and a 60-fold excess of citrates, tartrates and Cu(II). Regarding the effect of other ACE inhibitors, as it is already mentioned in the introductory section, the developed is specific for FSP, as it is the only phosphorus-containing compound of this group.

3.4. Figures of merit of the proposed FI method

Using the FI setup shown in Fig. 2 and under the chosen conditions described above, a calibration graph was obtained using orthophosphate standards. The calibration graph was linear over the range 1.0×10^{-6} to 2.0×10^{-4} mol l^{-1} PO_4^{3-} (equivalent to 1.0×10^{-6} to 2.0×10^{-4} FSP according to the 1:1 stoichiometry of the digestion reaction) and was described by the equation:

$$A = (0.002 \pm 0.002) + [(0.681 \pm 0.008) \times 10^4]c(\text{FSP})$$

where A is the absorbance as measured by the detector, and c(FSP) is the amount concentration of the analyte, with a correlation coefficient of $r = 0.9999$, a relative standard deviation, s_r, 1.0% (at 1.0×10^{-4} mol l^{-1} FSP, $n = 12$) and 0.6% (at 1.0×10^{-4} mol l^{-1} PO_4^{3-}, $n = 12$), and a 3σ detection limit of 5.0×10^{-7} mol l^{-1} PO_4^{3-} or FSP ($n = 10$).

All the standards were run in five replicate injections ($n = 5$).

3.5. Analysis of real samples

The proposed FI method was applied to the analysis of two commercially available pharmaceutical formulations containing FSP (Monopril and Fozide, Bristol-Myers Squibb). These formulations also contained lactose, microcrystalline cellulose, crospovidone, povidone, and sodium stearyl fumarate as inert excipients, while Fozide also contained hydrochlorothiazide as a co-active ingredient. The results are summarized in Table 1. The calculated average recoveries after spiking the real samples with known amounts of FSP were excellent and ranging between 99.2 and 101.6%. The accuracy of the proposed method was investigated by comparing the results obtained with those claimed by the manufacturer. These comparisons, also shown in Table 1, verified the accuracy of the proposed FI procedure as the relative errors were $e_r = -1.0$ and -1.5% for Monopril and Fozide, respectively.

Table 1
Analysis of pharmaceutical formulations

Sample	FSP added (mg l^{-1})	FSP found[a] (mg l^{-1})	100R[b]	Label[c]	100e_r[d]
Monopril	–	19.8[e]		20.0	−1.0
	25.0	25.4	101.6		
	50.0	49.6	99.2		
Fozide	–	19.7[e]		20.0	−1.5
	25.0	24.8	99.2		
	50.0	49.8	99.6		

[a] Mean of five results.
[b] Percent recovery.
[c] FSP sodium mass, in mg per tablet, according to package label.
[d] Relative error.
[e] FSP sodium mass found, in mg per tablet, mean of the analysis of 10 tablets.

4. Conclusions

The proposed method reports the first FI approach for the determination of FSP. The method offers higher sensitivity over previously reported procedures, robustness and an acceptable samples analysis frequency of $16\,h^{-1}$. It is advantageous over chromatographic and electrophoretic methods in terms of simplicity, cost efficiency and analysis time, while no complicated mathematical approaches are needed for data evaluation compared to chemometrics-based assays. In addition, a very important feature of the proposed method—as FSP pure material is a non-commercially available compound—is that an orthophosphate calibration graph can alternatively be used for the measurement of the analyte. The application of the developed method to two FSP containing pharmaceutical formulations gave very precise and accurate results.

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