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The potential of combining solid-phase optosensing and multicommutation principles for routine analyses of pharmaceuticals

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Abstract

In this work, we have explored the analytical potential of combining solid-phase optosensing and multicommutation principles, applied to the field of routine analyses of pharmaceuticals. This marriage benefits from the advantaging features of both concepts: the ability of multicommutation to provide increased repeatability, easier sample handling, reduced sample and reagent consumption as well as minor waste generation, combined with the enhancement of both sensitivity and selectivity obtained when a solid support is used to carry out the spectroscopic measurements directly on it. This approach has been evaluated by developing a method for the simultaneous analysis of two active principles (piroxicam and pyridoxine) in pharmaceutical formulations, using a non-polar sorbent as a solid support to attain the separation and subsequent preconcentration/detection of the targeted analytes. A multicommutated flow-through multisensor based on the direct intrinsic solid-phase UV absorbance measurements of the analytes on a packed C_{18} silica gel bed was then thoroughly developed. The usefulness of this approach was assessed when it was applied to the determination of piroxicam and pyridoxine in different pharmaceutical formulations obtaining remarkable results.

Keywords: Optosensor; Multicommutation; Pharmaceutical analysis; Piroxicam; Pyridoxine

1. Introduction

One of the main research interests of pharmaceutical analysis is the development of fast, single and inexpensive routine methods in order to check whether the commercial preparations meet the specifications regarding the content of the drug. This task has been typically accomplished by HPLC methods with UV and/or vis detection, since the matrix of a pharmaceutical is not complicated enough to require a higher selective detector. Furthermore, the sensitivity is not a factor of paramount importance in this kind of analyses. This has made possible the development of alternative analytical strategies, which can provide benefits in terms of automation, solvent and reagent consumption as well as time and cost-effectiveness. In this sense, the use of flow-injection analysis methodologies (typically FIA and SIA) combined basically with molecular spectroscopic detectors has been thoroughly explored.

Among the advantages concerning flow analysis processes, high sample throughput, automated operation, good accuracy and precision of results and overall robustness have been pointed out. In the basic configuration, a FIA manifold requires a pumping channel for each solution pumped continuously. This disadvantage could be overcome by designing the flow system manifold based on multicommutation [1,3]. In these cases, aliquots of sample and reagents solutions are merged into the reaction coil by controlling the sampling device. The ability of multicommutation to handle samples and reagents using simple manifolds has been exploited to develop several analytical methods [4–7]. Multicommutation enables increased repeatability, easier sample handling, reduced reagent consumption, providing an invaluable tool to develop environmentally friendly methods,

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which provides benefits in terms of reagent waste generation or sample/reagent consumption. Multicommutation provides some valuable advantages versus "classical" FIA, such as the miniaturization of flow analysis, a reduced sample and reagent solution, increased repeatability, facilitates the development of fully automatic analytical methods and improves economy and simplicity [1–7].

Solid-phase spectroscopy (SPS) is a reliable concept to improve both selectivity and sensitivity in non-destructive spectrometric measurements in relation to that carried out in homogeneous solution [8]. This technique is based on the retention/preconcentration of the analyte/target species on an appropriate solid support, implemented with the direct spectrometric measurements on the solid sensing phase. The combination of FIA–SPS (flow-through optosensors [9–15]) combines the advantages of SPS with those inherent to flow injection analysis systems (speed, automation). Separation, preconcentration and detection steps are carried out automatically and simultaneously without sample manipulation during the measurement process.

The combination of flow-through optosensors (solid phase optosensing concept) with multicommutation would benefit from the sum of the advantages provided by each separate concept. In this work, we have evaluated the analytical potential of this combination applied to the field of routine analyses of pharmaceutical formulations. Hence, we have developed a multicommutated UV flow-through multisensor, based on the direct intrinsic absorbance measurements of the analytes on a solid sensing zone with a non-polar sorbent (C_{18} silica gel) packed in the detection flow cell. A temporary sequentiation in the arrival of the analytes to the sensing zone was accomplished by means of an on-line minicolumn filled with C_{18} silica gel placed just before the solid phase UV detection system. The continuous-flow configuration is based on the multicommutation concept, which uses a set of solenoid valves for the insertion of microvolumes of sample and reagents. The flow manifold was designed with computer-controlled three-way solenoid valves for independent handling of sample, carrier and eluting solutions. This approach has been evaluated by developing a multisensor for the simultaneous analysis of two drugs (piroxicam and pyridoxine) in pharmaceutical formulations.

2. Experimental

2.1. Reagents

Pyridoxine chlorhydrate (Vitamin B_6) (Fluka) stock standard solution of 1000 mg l^{-1} was prepared in deionized water. Piroxicam (PX) (Sigma) stock solution of 1000 mg l^{-1} was prepared by diluting the required weigh in the minimum volume of sodium hydroxide 2 M and diluting to 100 ml with deionized water. Both solutions were kept in the refrigerator protected from light. Hydrochloric acid and methanol were purchased from Panreac. All required solutions were prepared in deionized water.

C_{18} bonded phase silica gel (Waters, Milford, USA) with 55–105 μm of average particle size was used as sensing support.

2.2. Apparatus, software and flow diagram

Absorbance measurements were made with a Varian Cary 50 (Madrid, Spain) spectrophotometer that was interfaced to a compatible PC equipped with Varian Cary spectroscopy software (WIN UV). The measurements were recorded at the maximum absorbance wavelengths for both analytes (285 and 334 nm for B_6 and PX, respectively). A Hellma 138-QS quartz flow cell (1 mm light path, 50 μl inner volume) was used to accommodate the solid phase beads. Some glass wool was used in the outlet to retain the C_{18} gel beads.

The manifold is schematized in Fig. 1. A four-channel Gilson Minipuls-3 (Villiers Le Bel, France) peristaltic pump with rate selector and methanol-resistant pump tubes type Solvflex (Elkay Products, Shrewsbury, MA, USA) were used. An electronic interface based on ULN 2803 integrate circuits was employed to generate the electric potential (12 V) and current (100 mA) required to control the four 161T031 NResearch three-way solenoid valves (Neptune Research, MA, USA) valves. The software for system controlling was developed in Java. Flow lines of 0.8 mm internal diameter PTFE tubing and methacrylate connections were also used.

A minicolumn (30 mm length, 1.5 mm i.d.) packed with 30 mg of C_{18} silica gel beads was inserted in the manifold

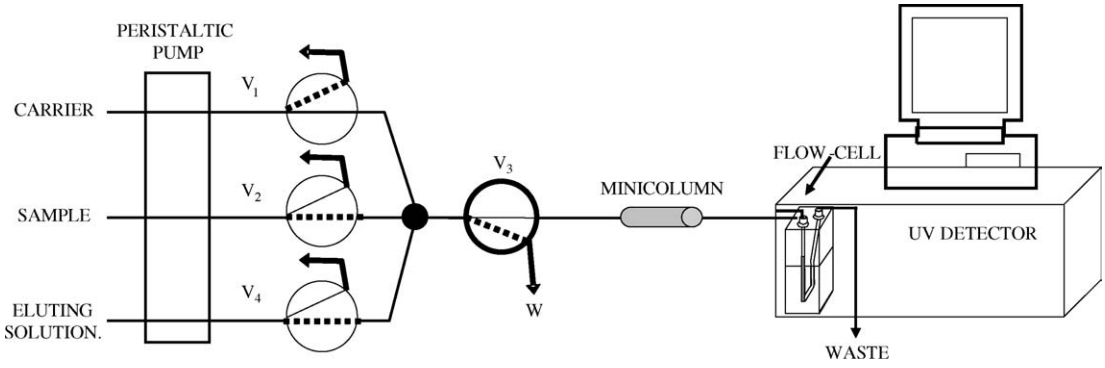


Fig. 1. Multicommutated flow configuration: $V_{1,2,\ldots}$: valves 1, 2, ...

just before the flow cell, to obtain the optimal separation of the analytes.

The HPLC apparatus comprised a Shimadzu SCL-10A vp (USA Manufacturing Inc.) controller with a Rheodyne injector unit Model 7725 (Cotati, CA, USA) equipped with a 20 μl loop. Shimadzu SPD-10AV vp pumps were used and the detection was accomplished with an UV absorbance detector Shimadzu SPD-10AV vp. All the data were recorded by using a LC Solutions 1.02 version software. A Nucleosil 120 5 C_{18} (250 mm × 4.6 mm) column from Scharlau S.L. (Barcelona, Spain) was used for the chromatographic separation. Mobile phases A and B were methanol and water, respectively. The chromatographic method used for comparison purposes held the initial mobile phase composition (5% A) constant for 10 min, followed by a linear gradient to 60% A from 10 to 20 min. The flow-rate used was 0.8 ml min^{-1}.

2.3. Sample treatment

Capsules: Two capsules were completely dissolved in double-distilled water, filtered and diluted to 500 ml in a volumetric flask.

Ampoules: A suitable volume of the ampoule was diluted to 100 ml in a volumetric flask.

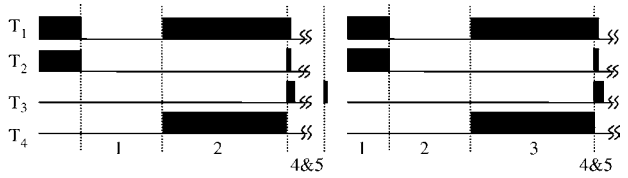
Activated tablets: An accurately portion of two powdered and homogenized activated tablets was weighed and dissolved with double-distilled water, filtered if necessary and diluted to 100 ml in a volumetric flask.

Suitable dilutions were made before measuring with 10^{-2} M HCl. All standard solutions were also prepared in 10^{-2} M HCl.

2.4. General procedure

The flow diagram of the system is shown in Fig. 1. In the initial status, all valves are switched off and the carrier, 10^{-2} M HCl, is flowing through the flow cell while all other solutions are recycling to their vessels. The sample is introduced by simultaneously switching the valves V_1 and V_2 on for 60 s. Vitamin B_6 develops its transitory analytical signal when reaching the flow-through cell and is eluted by the carrier itself, while PX is strongly retained on the solid placed in the minicolumn. After B_6 has developed its signal, by switching valves V_1 and V_4 the 40% MeOH eluting solution is introduced in the flowing system for 180 s; PX is therefore eluted from the solid support in the minicolumn towards the sensing zone, where it develops its signal, being eluted by the methanolic solution. The portion of tubing placed between valve V_1 and V_3 was cleaned between samples in order to avoid any possible contamination. Finally, before a new sample measurement, carrier solution has to be circulated during 20 s in order to conditioning the precolumn. A scheme showing the valve-switching procedure is shown in Fig. 2.

The sample solution is prepared in 10^{-2} M HCl. Calibration standards and samples were analysed by triplicate.



Step	V_1	V_2	V_3	V_4	Time (s)	Description
1°	1	1	0	0	60	Sample introduction
2°	0	0	0	0	120	B_6 signal and elution
3°	1	0	0	1	180	PX signal and elution
4°	1	1	1	0	5	Cleaning step 1
5°	0	0	1	0	5	Cleaning step 2

Fig. 2. Valves scheme: T_1–T_4 refers to the timing courses of solenoid valves V_1–V_4. The shadow surface above the valves timing course line indicates that the corresponding valve was switched on. The steps were the following: (1) sample introduction; (2) B_6 signal and elution; (3) PX signal and elution; (4) and (5) cleaning steps.

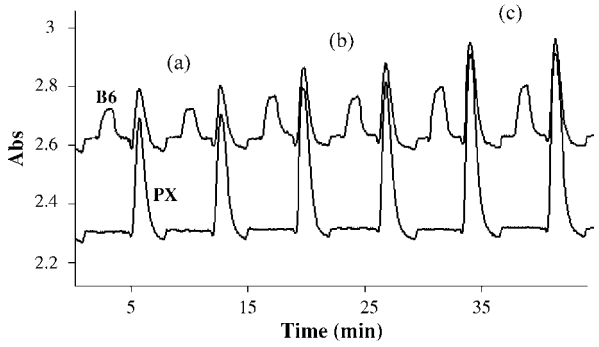


Fig. 3. Profile of the transient signals showing the separation by duplicate of (a) 20 and 8 μg ml^{-1}; (b) 25 and 10 μg ml^{-1}; (c) 30 and 12 μg ml^{-1} of Vitamin B_6 and PX, respectively.

Absorbance measurements were made at 285 and 334 nm for B_6 and PX, respectively. A diagram showing the separation of both analytes is shown in Fig. 3.

3. Results and discussion

3.1. Spectral characteristics

The spectral features of Vitamin B_6 and PX were recorded both in aqueous solution and solid-phase media. An overlapping in the spectra of the analytes was found. For this reason, it was impossible to perform the simultaneous determination without significant errors. The separation achieved by the solid support in the minicolumn just before the detection area makes possible the sequential measurement of the analytes in the proposed method. The maximum absorbance wavelength in the solid phase is 285 and 334 nm for B_6 and PX, respectively, so these were the selected ones for measuring, as the spectrophotometer used allows the measuring at several absorbance wavelengths.

3.2. Selection of the solid support in the cell and in the minicolumn

Various active solid phases were studied for the proposed method: a cation exchanger on dextran (Sephadex SP C-25), a non-polar sorbent (C_{18} silica gel) and a dextran type without exchangeable groups (Sephadex G-15) were tested. C_{18} bonded silica gel beads proved to be the most suitable sensing material, since it was the only solid material that interacted with both analytes in a wide variety of experimental conditions, which allow to separate the two studied compounds.

The solid support in the minicolumn placed before the flow-through cell was also C_{18}, as it could retain PX while B_6 passed through it and developed its transitory analytical signal. Thirty milligrams of solid phase microbeads was shown to be enough for a proper separation of the analytes (a 30 mm minicolumn length, 1.5 mm i.d.). Higher amounts of C_{18} solid support decreased the signal obtained, as the dispersion of the analytes in the solid beads increased [16,17].

3.3. Chemical variables

3.3.1. Carrier solution

The study of the influence from carrier pH value was performed taking into account the structure and pK_a values of the analytes. Due to the pK_a values of B_6, 4.9 and 8.91, it is in the protonated form in acidic pH, while the non-ionic form is predominant for pH range 5–8. PX was completely retained in all the studied pH range, that is, from 2 to 10. The analytical signal obtained for B_6 in all the studied range was practically the same up to 8, but it was not completely eluted by the carrier itself for pH values higher than 4, because B_6 was not completely in the protonated form. 10^{-2} M HCl was appropriate as carrier/selfeluting solution for B_6, without losing sensitivity.

3.3.2. Sample solution

The signal of both analytes was constant when varying the sample pH from 2 up to 8, but a better elution of B_6 was observed when using an acidic medium instead of a neutral one, due to the same reason that the one previously explained. 10^{-2} M HCl was used in order to prepare all the standard and sample solutions.

3.3.3. Eluting solution

After B_6 was eluted from the solid support by the carrier itself, a proper eluting solution had to be used in order to elute PX from the solid support in the minicolumn. Different percentages of MeOH solutions were tested, ranging from 30 to 50% MeOH:water (v:v) solutions. For percentages of MeOH lower than 40% the elution was not complete and the baseline was not restored in this conditions. As the percentage of MeOH was increased, a decrease in the analytical signal was observed due to a faster elution of the analyte from the solid sensing phase. Forty percent MeOH was chosen as the optimum value, obtaining the highest possible analytical signal with an appropriate sample throughput.

3.4. Flow variables

The sample introduction time and the flow-rate of the peristaltic pump were the studied flow variables.

The flow-rate was investigated from 0.8 to 1.4 ml min^{-1}. By increasing it, both the analytical signal and the sample frequency increased. This is explained taking into account that an increase in the flow-rate means a higher volume of sample being introduced into the system in the same time, so sensitivity is increased. The sample frequency is also increased due to the high flow-rate. So the highest possible flow-rate, avoiding overpressures due to the solid phase placed in the minicolumn and in the flow-cell, was selected (1.3 ml min^{-1}).

The sample introduction time was studied ranging from 10 to 80 s, using a flow rate of 1.3 ml min^{-1}. When increasing the sampling time, the signal increased due to a higher amount of analyte being introduced in the flow system, so the amount of analyte concentrated on the solid support is higher. The signal increased linearly up to 60 s for B_6 and 120 s for PX. The study was carried out for 24 and 9 μg ml^{-1} of B_6 and PX, respectively:

$$B_6: \quad A = 0.0245 + 0.0029t(\text{s}), \quad r = 0.9969$$

$$PX: \quad A = -0.0185 + 0.0059t(\text{s}), \quad r = 0.9954$$

For higher sample introduction times, the signal increased only for PX, although not linearly, while for B_6 introduction times higher than 60 s did not provide higher signals. Although sensitivity increased when using high sample times, the sample frequency of the proposed system diminished, so a compromise had to be taken: 60 s was chosen as sampling time.

3.5. Analytical performance

Taking into account the optimised conditions, the analytical parameters of the system were studied. The system responds linearly in the ranges of concentration 5–50 and 1–15 μg ml^{-1} for B_6 and PX, respectively. The calculated R.S.D. ($n = 10$) were 3.0 and 1.2% for 24 and 6 μg ml^{-1} of B_6 and PX, respectively. The detection limits and quantification limits were calculated following the 3σ and 10σ criterion. The detection limits were 1.2 and 0.27 μg ml^{-1} for B_6 and PX. All the analytical parameters are detailed in Table 1. The results obtained compares well with other previously reported spectroscopic methods for these analytes, based mainly on fluorescence spectroscopy [18,19] and chemometric assisted spectrophotometry [20]. On the other hand, the use of multicommutation provides invaluable advantages in terms of degree of automation and sampling frequency in relation to the previous methods that, in most cases, are based on batch measurements. The reagent and sample consumption as well as the waste generation are other key advantaging features of

Table 1
Analytical parameters

Parameter	Pyridoxine	Piroxicam
Dynamic range ($\mu g\,ml^{-1}$)	5–50	1–15
Calibration graph		
Intercept	0.0205	−0.0108
Slope ($ml\,\mu g^{-1}$)	0.0058	0.0418
Correlation coefficient	0.9976	0.9967
Detection limit ($\mu g\,ml^{-1}$)	1.2	0.27
Quantification limit ($\mu g\,ml^{-1}$)	4	0.9
R.S.D. (%) ($n = 10$)	2.97 (10)[a]	1.19 (10)[b]

[a] For a concentration level of 24 $\mu g\,ml^{-1}$.
[b] For a concentration level of 6 $\mu g\,ml^{-1}$.

the proposed approach. For example, the volume of sample, carrier and eluting solutions required (in a batch of analyses) is respectively about 80, 60 and 50% minor than that required in a conventional flow injection-based procedure with continuous delivery of sample and reagents.

3.6. Study of interferences

In order to determine the effect of possible interferences, a tolerance study was carried out with those compounds that are usually found along with B_6 and/or PX in pharmaceuticals.

The study was carried out with 15 and 6 $\mu g\,ml^{-1}$ of B_6 and PX, respectively. Potentially interfering compounds were added to the samples at concentrations higher than those usually found in pharmaceuticals.

A compound was considered to interfere if a variation higher than 5% was observed in the analytical signal regarding the absence of the respective species. If such a variation was observed, the foreign specie concentration was diminished until an error less than 5% was obtained.

The tolerance of potentially interfering compounds is much higher than the amount usually found in pharmaceuticals. The increase of tolerance to foreign species can be observed by comparing the ratios interferent/B_6 in solution and in solid phase for ascorbic acid, thiamine, rivoflavin and cyanocobalamine. They are less than 0.2 in all cases in solution, while they are highly increased (up to 100 times) when using the solid support, as can be observed in Table 2, allowing the determination of pyridoxine in the presence of these compounds. All the obtained results are summarized in Table 2.

Table 2
Interference study

Foreign species	Tolerance ($\mu g\,ml^{-1}$ interferent/ $\mu g\,ml^{-1}$ analyte)	
	Pyridoxine[a]	Piroxicam[b]
Saccharose, glucose, lactose	>20[c]	>20[c]
Nicotinamide, ascorbic acid	>20[c]	>20[c]
Thiamine	1.5	>20[c]
Cyanocobalamine	8	8
Rivoflavin	>2[c]	>4[c]
Saccharin	0.8	>20[c]
Biotin	>2[c]	>2[c]
Carisoprodol	>18[c]	>18[c]

[a] 15 $\mu g\,ml^{-1}$ of pyridoxine.
[b] 6 $\mu g\,ml^{-1}$ of piroxicam.
[c] Maximum ratio tested.

3.7. Analytical applications

Following the general procedure previously described, the system was applied to the determination of B_6 and/or PX in pharmaceutical preparations. Different pharmaceutical preparations of the Spanish Pharmacopoeia were used, such as capsules, activated tablets, and ampoules. As PX and B_6 do not appear together in pharmaceuticals of the Spanish Pharmacopoeia, semi-synthetic preparations were prepared by adding B_6 or PX and carisoprodol (one of the main components of the pharmaceuticals in other countries [18]) to a pharmaceutical containing only one of the active principals. The results of the proposed method, which are in good agree-

Table 3
Applications to pharmaceuticals

Sample[a]	Pyridoxine			Piroxicam		
	Nominal value (mg)	Found[b] (mg)	HPLC method	Nominal value (mg)	Found[b] (mg)	HPLC method
Sasulen[1]	–	–	–	20	20.3 ± 0.9	21.0 ± 0.2
Improntal[2]	–	–	–	20	21.4 ± 0.5	21.2 ± 0.3
Cycladol Dref[3]	–	–	–	20	19.9 ± 0.7	20.1 ± 0.2
Doblexan[4]	–	–	–	20	20.0 ± 0.4	20.2 ± 0.5
Nervobion[5]	100	104 ± 3	101 ± 2	–	–	–
Hidroxil[6]	250	282 ± 10	267 ± 8	–	–	–
Benadon[7]	300	305 ± 12	305 ± 7	–	–	–
Semisynthetic[8]	150	155.0 ± 0.3	156 ± 5	10	9.8 ± 0.2	10.2 ± 0.1
Semisynthetic[9]	100	102 ± 2	102 ± 1	7	7.1 ± 0.2	7.0 ± 0.1

[a] Composition of samples: [1] capsules (Faes): piroxicam, 20 mg; [2] capsules (Fides-Rotttapharm): piroxicam, 20 mg; [3] activated tablets (Chiesi): piroxicam, 20 mg; [4] capsules (Quimifar): piroxicam, 20 mg; [5] ampoules (Merck): thiamine, 100 mg; pyridoxine, 100 mg; cyanocobalamine, 5 mg; [6] activated tablets (Almirall Prodesfarma): hydroxycobalamine, 500 mg; pyridoxine, 250 mg; thiamine, 250 mg; [7] ampoules (Roche): pyridoxine, 300 mg; [8] activated tablets (Cycladol Dref): piroxicam, 10 mg; pyridoxine, 150 mg; carisoprodol, 500 mg; [9] ampoules (Nervobion): pyridoxine, 100 mg; piroxicam, 7 mg; carisoprodol, 165 mg.
[b] $n = 3$.

Table 4
Recovery studies

	Pyridoxine			Piroxicam		
	Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)
Nervobion	30	30 ± 1	100.3	–	–	–
	60	62 ± 2	102.8	–	–	–
	90	87.4 ± 0.6	97.1	–	–	–
Benadon	75	74 ± 3	98.9	–	–	–
	150	149 ± 2	99.5	–	–	–
	300	309 ± 2	103	–	–	–
Cycladol Dref	60	61 ± 2	102	6	6.1 ± 0.1	101.7
	90	88 ± 2	97.3	12	12.2 ± 0.1	101.7
	120	122 ± 2	102	18	17.9 ± 0.4	99.4
Hidroxil	50	53 ± 3	106.8	40	39 ± 2	98.2
	100	99.2 ± 0.6	99.2	50	51.2 ± 0.9	102.4
	150	152 ± 3	101.5	60	62.4 ± 0.4	104
Sasulen	–	–	–	10	9.8 ± 0.1	98
	–	–	–	20	20.6 ± 0.2	103
	–	–	–	30	29.6 ± 0.2	98.7
Improntal	–	–	–	10	9.7 ± 0.1	97
	–	–	–	20	19.8 ± 0.4	99
	–	–	–	30	30.2 ± 0.1	100.7
Doblexan	–	–	–	5	4.9 ± 0.1	98
	–	–	–	10	10.1 ± 0.2	101
	–	–	–	20	20.7 ± 0.1	103.5
Semisynthetic	50	50.7 ± 0.7	101.4	6	5.9 ± 0.1	98.3
	100	101.0 ± 0.3	101	12	12.2 ± 0.2	101.7
	150	154.2 ± 0.3	102.8	20	19.5 ± 0.2	97.5

ment with those obtained with the HPLC reference method, are shown in Table 3.

In addition, a recovery study was performed by adding three different amounts of B_6 and/or PX to each tested preparation. In order to test the recovery of one analyte in the presence of the other, the addition of both analytes was performed in some pharmaceuticals. Recovery results are shown in Table 4.

4. Conclusions

In this work, we have explored the analytical potential of combining multicommutation and solid phase optosensing, applied to routine analysis of pharmaceutical products. The use of a solid support provides remarkable advantages in relation to multicommutated systems with conventional liquid-phase detection. In example, a simplification of the multicommutation scheme of valves is obtained, because the ability of solid-phase optosensing to perform a separation from the matrix and some potential interfering compounds together with the preconcentration of the target analytes. This makes possible the development of UV multicommutation methods for multicomponent determinations, which could not be carried out in liquid phase (except with the use of chemometrics) or with the use of more complicated multi-channel manifolds, implementing different selective reagent-based assays, one for each target analyte, increasing thus the complexity of the multicommutation manifold. Therefore, solid phase optosensing can be used as a tool to simplify the multicommutation configurations whereas its advantaging enhanced sensitivity and selectivity features can be exploited. For example, in this method, the potential interferences of some species which are added to the pharmaceutics are circumvented by the use of the solid support (this is the case of carisopropol). On the other hand, the improvement of the analytical features in terms of reliability, precision, sample handling as well as reagent consumption and minor waste generation in relation to conventional methods based on classical FIA configurations is also remarkable. These advantages are fully complementary with the requirements of a field such as the pharmaceutical analyses in commercials products for routine quality control purposes.

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