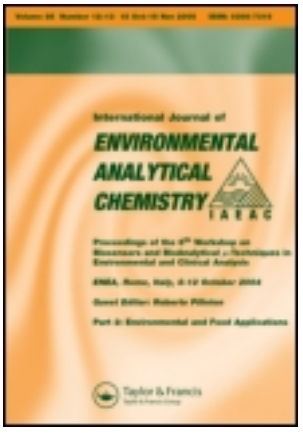

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Determination of phenylglyoxylic acid in urine using a multi-pumping flow system

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In this work a simple, fast and fully automated analytical methodology for the spectrophotometric determination of phenylglyoxylic acid is proposed. Phenylglyoxylic acid is a metabolite of styrene that is excreted in urine, being used as an indicator of styrene occupational exposure. The developed procedure was based on the phenylglyoxylic acid ability to inhibit the formation of the peroxovanadium cation produced by the reaction between vanadate and H_2O_2. The analytical process was implemented in a multi-pumping flow system that employs multiple solenoid actuated micro-pumps as the only active components. This enabled the reproducible insertion and efficient mixing of low volumes of sample and reagents as well as the transportation of the sample zone towards detection. Thus an easily controlled, low cost, compact and reliable analytical system was implemented. A linear working range for phenylglyoxylic acid concentrations up to 700 mg L^{-1} ($r^2 = 0.995$, $n = 7$), was obtained, with a detection limit of 37 mg L^{-1}. The system handles about 43 determinations per hour yielding precise results (relative standard deviation $< 5\%$, $n = 10$). The developed methodology was applied to the determination of phenylglyoxylic acid in urine samples and the obtained results were in agreement with those furnished by the comparison method with relative percentage deviations lower than 6.6%.

Keywords: phenylglyoxylic acid; styrene; vanadate; multi-pumping; spectrophotometry

1. Introduction

Styrene is an important chemical of wide industrial use in the production of polymers, copolymers and glass-reinforced plastics. The highest human exposure to styrene occurs in occupational settings, particularly those involving the production of large fibreglass reinforced polyester products [1]. The two major effects of acute exposure to styrene include irritation of the skin and respiratory tract and central nervous system depression. Other effects, such as reproductive toxicity and neurotoxicity, have been associated with

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chronic exposure in the workplace. Moreover, the International Agency for Research on Cancer has classified styrene as possible carcinogenic to humans (Group 2B).

Occupational exposure to styrene is generally monitored by measuring the concentration in air and by comparing it with the maximum allowable concentrations at the workplace. Ambient monitoring can be complemented by biological monitoring that takes into account other factors such as the uptake by routes additional to inhalation such as skin and oral absorption, and differences in individual metabolic patterns. This way, biological monitoring allows the assessment of the real internal exposure level. Styrene penetrates the organism by inhalation and undergoes hepatic metabolisation producing mandelic acid (MA) and phenylglyoxylic acid (PGA) which are excreted in urine [2]. Urinary concentrations of PGA and MA are quantitatively related to the external exposure levels and the absorbed dose. Thus PGA and MA have been recommended as biological indicators of occupational exposure to styrene. A biological exposure index (BEI) of 240 mg/g creatinine for PGA has been established for samples at the end of a shift [3]. BEIs are reference values intended as guidelines for the evaluation of potential health hazards in the practice of industrial hygiene.

Many analytical methods for the determination of PGA and MA have been reported. The most widely used include gas chromatography with flame ionisation [4] or mass spectrometry [5–7] detection, and liquid chromatography with UV [8–13] or tandem mass spectrometry [14,15] detection. Other methods such as capillary electrophoresis [16,17], fluorimetry [18] and voltammetry [19] were also used to quantify the metabolites of styrene. However most of these methods require enrichment and clean-up steps performed either by solid-phase extraction or liquid-liquid extraction, with an additional derivatisation procedure in the GC methods, which make the determination time-consuming. Moreover, expensive instrumentation is required as well as experienced personnel for operation and maintenance.

Cost and time of analysis are limiting factors that concern both the diagnostic laboratories and the industry that has to monitor its workers. For this reason, a simple, sensitive, inexpensive, fast and automated analytical method is required for routine biological monitoring of workers exposed to styrene.

Flow techniques are powerful and useful tools for developing analytical methodologies, allowing the implementation of precise, reproducible and fast procedures, which make them an excellent choice for routine analysis and screening.

The purpose of this work was to develop a procedure for PGA determination in urine avoiding sample pre-treatments outside the flow system. The developed flow method was based on a multi-pumping flow system (MPFS) that employs, as exclusive active components, multiple solenoid actuated micro-pumps that were simultaneously accountable for solutions insertion, propelling and commutation, resulting on fully automated analytical methodologies characterised by high simplicity and versatility [20]. Moreover, MPFS allow the synchronisation of the reagent additions to a precise sample portion establishing a well-defined reaction zone thereby avoiding superfluous sample and reagent consumption and increasing sampling rate. This aspect makes multi-pumping flow systems suitable for the screening of a large amount of samples in a short period of time which is required for routine biological monitoring.

In this work an automated flow methodology for determination of PGA in urine samples, based on the inhibiting effect of PGA on the reaction of H_2O_2 with ammonium vanadate in acidic medium is proposed.

2. Experimental

2.1 *Reagents*

All chemicals were of analytical reagent grade and doubly deionised water obtained with a Milli-Q system was used throughout (specific conductivity $< 0.1\,\mu S\ cm^{-1}$).

A $0.1\,mol\ L^{-1}$ ammonium vanadate stock solution was prepared by dissolving 292.4 mg in 25 mL of sulphuric acid $1.0\,mol\ L^{-1}$.

A $0.25\,mol\ L^{-1}$ hydrogen peroxide solution was daily prepared by rigorous dilution of a stock solution $10.3\,mol\ L^{-1}$.

A $5.0\,g\ L^{-1}$ phenylglyoxylic acid stock solution was prepared by dissolving the required amount in 25 mL of deionised water and kept in the refrigerator. Working solutions were daily prepared by rigorous dilution of the stock solution.

2.2 *Urine samples*

Urine samples from non-exposed volunteers were collected in polyethylene bottles and refrigerated. If urine samples were not analysed within 48 h after collection, they were kept frozen at $-20^{\circ}C$. Prior to analysis, urine samples were filtered using a cellulose membrane filter of 0.45 μm, and spiked with known amounts of PGA. For the analysis by the proposed multi-pumping flow system the urine samples were diluted two-fold with deionised water, while for analysis by the HPLC comparison procedure [13] the urine samples were diluted 10-fold.

2.3 *Comparison HPLC procedure*

For accuracy assessment of the results obtained by the proposed procedure, urine samples were analysed by HPLC [13]. The HPLC procedure was performed with a Jasco LC-2000 Plus high-pressure liquid chromatograph equipped with a system controller LC-Net II/ADC, a HPLC pump PU-2080 Plus and a PDA detector MD-2015 Plus. Chromatographic separation was performed on a Waters XTerra MS C_8 column (3.9×150-mm). The mobile phase employed was a mixture of phosphate buffer pH 2.3 (50 mM) – methanol (95:5, v/v). The UV-Vis absorbance over the 200–450 nm range was registered and the wavelengths used for the quantification of PGA and creatinine were 254 nm and 225 nm, respectively.

2.4 *Equipment*

The absorbance measurements (443 nm) were carried out in a Jenway 6300 spectrophotometer (Jenway, UK) equipped with an 18 μL inner volume flow-cell of 1 cm optical path length. The flow manifold consisted of a set of four solenoid micro-pumps (Ref. 120SP-BIOCHEM Valve Inc., Boonton, USA) connected by means of flow lines made of 0.8 mm i.d. PTFE tubing. Home-made confluence points, connectors and end-fittings, were also used. Reactor L_1 was thermostatised at $55^{\circ}C$ in a Julabo Variomag EC thermostatised water bath (Julabo Labor Technik GmbH, Germany).

The micro-pumps were of the fixed displacement diaphragm type being solenoid operated and dispensing 10 μL per stroke.

A home-made power drive based on a ULN 2003 integrated circuit or a CoolDrive™ circuit (NResearch Inc., West Caldwell, USA) was used to operate the solenoid pumps.

Data acquisition and control of the analytical system was accomplished through a PC-LABCard model PCL-818L interface card from Advantech (Taipei, Taiwan) and a Pentium I based microcomputer. The software was developed in QuickBASIC 4.5.

2.5 *Flow manifold*

The flow diagram of the system exploiting the multi-pumping approach for PGA determination is shown in Figure 1 and comprised four solenoid micro-pumps (P_1 to P_4) that were responsible for the individual insertion of solutions: P_1 was used to insert vanadate, P_2 was used to insert the sample solution, while P_3 and P_4 were used to insert H_2O_2 and deionised water that was used as carrier, respectively. The pumps acted simultaneously as commuting and propelling devices, controlling at once the sample and reagents volumes, flow rate, timing and sequence of insertion and transportation towards detection.

The analytical cycle started with the intercalated activation of P_1 and P_2, inserting vanadate and sample solutions by resorting to the binary sampling strategy [21]. The insertion of small aliquots of vanadate intercalated with small aliquots of sample, creating multiple reaction interfaces combined with the pulsed flow produced by the micro-pumps' actuation provided a fast reaction zone homogenisation. Then, pump P_4 was activated carrying the reaction zone through L_1, allowing the reaction between vanadate and PGA to take place. When the formed reaction zone reached the confluence point Y, it was mixed with H_2O_2 solution by alternated activation of P_3 and P_4. Once again the binary sampling strategy was implemented and small aliquots of the sample-vanadate reaction zone were intercalated with small aliquots of H_2O_2 resulting on the formation of the coloured

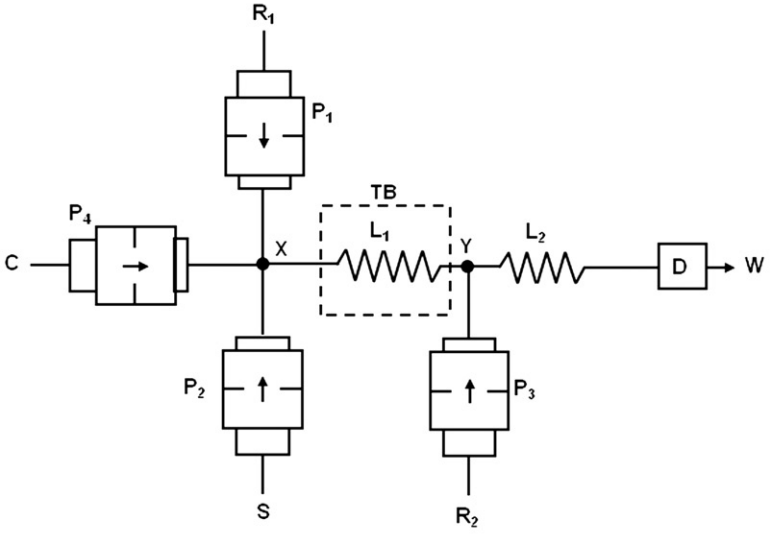

Figure 1. Flow manifold diagram. P_1, P_2, P_3 and P_4, solenoid micro-pumps; X and Y, confluence points; L_1 and L_2, reaction coils (200 cm and 25 cm); TB, thermostatised bath (55°C); D, detector; W, waste; R_1, ammonium vanadate solution; R_2, hydrogen peroxide solution; C, carrier (water); S, sample.

compound, that was carried to the detector through L_2, by actuation of P_4, yielding the analytical signal.

3. Results and discussion

A methodology for PGA determination based on its inhibiting effect on the reaction between H_2O_2 and vanadate was developed for the first time. H_2O_2 reacts with ammonium vanadate in acidic medium leading to the formation of a red-orange colour peroxovanadium cation, with maximum absorption at 443 nm [22]. Vanadate reacts with carboxylic acids to form vanadate esters, therefore, the presence of PGA leads to a decrease on the maximum absorbance by consumption of vanadate. Additionally, α-keto acids such as PGA react with H_2O_2 [23,24], which also contributes to the inhibition of the reaction between H_2O_2 and vanadate.

During system optimisation the effect of several analytical parameters was evaluated in order to achieve the higher inhibiting effect on vanadate–H_2O_2 reaction and aiming the attainment of a detection limit suitable for biomonitoring of workers exposed to styrene.

3.1 *Manifold configuration and optimisation*

An important parameter that influenced the inhibiting effect was the sequence followed for the addition of reagents solutions to PGA. Preliminary studies demonstrated that the inhibiting effect was higher when PGA was first mixed with vanadate. Therefore, the flow system was configured to assure an efficient PGA–vanadate mixing prior to the insertion of the H_2O_2 solution. PGA and vanadate solutions were first put in contact at confluence point X and mixed along L_1 where the reaction took place. The formed reaction zone was subsequently mixed with H_2O_2 that reacted with the remaining vanadate and PGA inside L_2.

The versatility of sample manipulation that characterises multi-pumping flow systems permits the establishment of distinct sampling strategies, this way, single sample volume, merging zones [25] and binary sampling [21] were assayed. Improved results that corresponded to a higher sensitivity and linearity were obtained when the strategy of binary sampling was used to insert PGA and vanadate at confluence point X, as well as to mix the formed reaction zone with H_2O_2 solution at confluence point Y. The multiple interfaces created by binary sampling combined with the pulsed flow resultant from the micro-pumps' actuation provided a fast homogenisation of the reaction zone, thus favouring reaction development. This aspect is particularly relevant for PGA and vanadate mixing since the reaction is relatively slow. The improved mixing conditions presented by MPFS contributed to an enhancement in sensitivity since the reaction zone homogenisation is achieved with low sample dispersion.

In multi-pumping flow systems the sample volume is defined by the stroke volume and by the number of pulses of micro-pump actuation. Since the stroke volume is 10 μL, to evaluate the sample volume the number of pulses was varied from 2 to 12 pulses which corresponded to 20–120 μL (Figure 2). It was verified that the inhibiting effect increased with the number of sample pulses until 10 pulses (100 μl) and then approached stabilisation. The utilisation of a higher number of sample pulses did not significantly increment the inhibiting effect but negatively affected linearity. This way, ten pulses of sample were selected for further work. Since the vanadate solution was inserted by

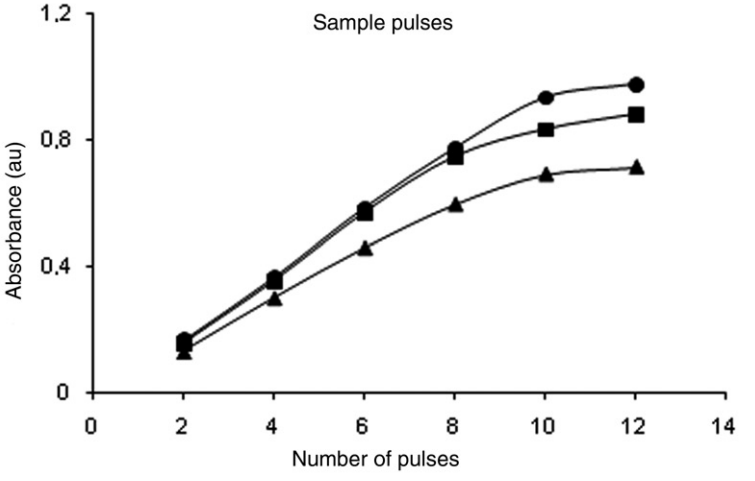

Figure 2. Influence of sample pulses on the analytical signal. The results refer to the analysis of the blank (•) and PGA standards with concentrations of 100 mg L^{-1} (■) and 250 mg L^{-1} (▲).

intercalating small aliquots of vanadate solution with small aliquots of PGA sample solution, the volume of vanadate solution used was also 100 µl.

Flow rate is a relevant parameter affecting the magnitude of the analytical signal once it determined the transport of the reaction zone to detection and thus the reaction time. The influence of flow rate was evaluated by setting pulse frequencies ranging from 43 to 150 pulses min^{-1} that corresponded to flow rates of about 0.43–1.5 mL min^{-1}. An increase in the sensitivity of about 5% was obtained by increasing the flow rate up to 1 mL min^{-1}. When using a flow rate of 1.5 mL min^{-1}, a decrease in sensitivity of about 20% was observed, probably as a consequence of the decreased reaction time. Therefore, a flow rate of 1 mL min^{-1} that also corresponded to the largest linear range of the calibration curve was used for the subsequent assays.

The determination of PGA is based on a two step reaction. In the first step PGA reacted with vanadate and preliminary studies revealed that this reaction was relatively slow. For that reason, a 200 cm reactor (L_1) was used to mix these solutions in order to increase the time for reaction development ensuring adequate sensitivity. The utilisation of a 100 cm reaction coil guaranteed a lower sample dispersion but the corresponding decrease in the reaction time impaired sensitivity. Another parameter that could provide an increase on the PGA–vanadate reaction rate is temperature. Hence, reactor L_1 was thermostatised and the effect of temperature was evaluated for 21, 40 and 55°C. For a temperature higher than 55°C there was an increase on the rate of formation of air bubbles, which affected detection. Since it was observed an increase in sensitivity with the raise of temperature, 55°C were used for further work.

In the second step, the remaining vanadate reacted with H_2O_2 resulting on the formation of the peroxovanadium cation, yielding the analytical signal. The effect of L_2 reactor's length on mixing and on the extent of the reaction between the previously formed reaction zone and H_2O_2 was evaluated by assaying reaction coils of 5, 25, 50 and 100 cm. The reactor of 5 cm corresponded to the minimum length necessary to connect the confluence point Y and the detector. By increasing the reaction coil length there was an

increase of sample dispersion, and consequently, a decrease in sensitivity of about 28% was obtained. For the subsequent work a 25 cm reactor L_2 was used, since it provided a compromise between a good sensitivity and a wider linear range.

3.2 *Reagents concentration*

The formation of the coloured reaction products depended on the concentration of vanadate and H_2O_2. Hydrogen peroxide solutions with concentrations of 0.05, 0.1, 0.25 and 0.5 mol L^{-1} were evaluated and it was verified that the analytical signal increased up to 0.25 mol L^{-1}, which also corresponded to the higher sensitivity, and then approached stabilisation.

The effect of the ammonium vanadate concentration was studied using solutions with concentrations of 0.005, 0.0075, 0.01, 0.015 and 0.02 mol L^{-1}. It was verified that the increase of vanadate concentration resulted in an improvement of the analytical signal. However, for the subsequent work a vanadate concentration of 0.015 mol L^{-1} was selected, since it provided enhanced linearity and sensitivity with lower reagent consumption.

The reaction between vanadate and H_2O_2 occurs in acidic medium, thus the effect of the concentration of sulphuric acid used to prepare the ammonium vanadate solution was studied using solutions with concentrations of 0.05, 0.1, 0.5, 1.0 and 2.0 mol L^{-1}. It was observed that the analytical signal increased up to 1 mol L^{-1} sulphuric acid, remaining stable for higher concentrations.

In flow systems the viscosity of sulphuric acid solutions usually constitutes a drawback since it affects solutions dispersion originating pronounced concentration gradients and unwanted Schlieren effects [26]. In the developed methodology the excellent mixing capability of the pulsed flow produced by micro-pump operation allowed an efficient homogenisation of the viscous sulphuric acid and the aqueous sample zones without the need to resort to complementary strategies to compensate perturbations in the analytical signal.

3.3 *Discrimination between phenylglyoxylic acid and mandelic acid*

Being a metabolite of styrene, PGA is always present in the urine with MA, therefore it is necessary to discriminate between the two metabolites. Due to the similarity of their chemical structure, both acids can react with vanadate inhibiting its reaction with H_2O_2 leading to a decrease in maximum absorbance. Nevertheless, the kinetics of the inhibiting reaction is different for each styrene metabolite, the rate of the PGA–vanadate reaction being markedly higher than the MA–vanadate one.

Using the previously defined conditions PGA produces an inhibition effect that is proportional to its concentration. The optimised L_1 reactor's length and flow rate provided an appropriate residence time for PGA to inhibit the H_2O_2–vanadate reaction, while in these conditions MA does not react with vanadate. The inhibiting effect of a calibration set consisting of 16 standard mixtures with different concentrations of PGA (50, 200, 400, 600 mg L^{-1}) and MA (100, 300, 600 and 900 mg L^{-1}) following a 4^2 factorial design was studied. It was observed that increasing the concentration of PGA there was an increase on the inhibiting effect, while with different concentrations of MA no change on

the analytical signal was verified. Therefore, the developed methodology can be applied to the determination of PGA in urine samples with no interference from MA.

By implementation of a stopped flow-strategy, which consisted in halting the sample-vanadate reaction zone inside the reactor L_1 for a given period of time, it was observed that for the reaction of PGA with vanadate an increase of the stopped flow interval of up to 180 s resulted in an increase on the inhibition. Beyond that time, the inhibition effect remained stable. For the reaction of MA and vanadate no inhibition was observed without stopping the flow, the signal of the standard solutions being similar to the blank. By increasing the stopped flow time there was an increase on the inhibition up to 360 s. The results obtained in these studies indicate that it could be possible to discriminate between the two styrene metabolites on the basis of the different reaction time. In fact, PGA concentration can be related to the analytical signals without stopped flow and MA concentration can be related to the difference between the analytical signals obtained at 180 and 360 s. Based on these results, the possibility of simultaneously determine PGA and MA is being studied.

3.4 *Analysis of urine samples*

After system optimisation a linear working range for PGA concentrations up to 700 mg L^{-1} was obtained. The analytical curve was represented by $A = -4.3 \times 10^{-4}$ ($\pm 0.3 \times 10^{-4}$)C + 1.12($\pm$0.01) where A represented the absorbance at 443 nm and C represented PGA concentration expressed in mg L^{-1}, with a correlation coefficient of 0.995 ($n = 7$). The linearity of the calibration curve was also confirmed by a Student t-test: the estimated *t*-value (32.2) was higher than the tabulated one (2.57) for a confidence level of 95%, indicating that a significant correlation exists. Detection and quantification limits were calculated as the concentrations corresponding to 3 and 10 times the standard deviation of 10 replicates of the blank and values of 37 mg L^{-1} and 129.6 mg L^{-1}, respectively, were estimated [27]. Relative standard deviation (RSD) lower than 5% was obtained in the repetitive analysis of a urine sample (340 mg L^{-1}).

A biological exposure index (BEI) of 240 mg/g creatinine for PGA has been established for samples at the end of a shift [3]. PGA concentration should be corrected for urinary creatinine in order to eliminate the inter-individual variation in urine production rate. Considering that the normal values of creatinine in urine are in the range of 0.8 to 2.0 g/24 h and that the volume of urine produced in 24 h is 0.6 to 2.0 L [28], the obtained detection limit is below BEI for PGA, making the developed method suitable for the quantitative determination of PGA in urine for screening of exposed workers.

Interferences due to different chemical species commonly found in urine were evaluated. Samples containing PGA at a fixed concentration of 250 mg L^{-1} and increasing concentrations of the compound under evaluation were analysed by the developed method. A compound was considered as non-interfering if the analytical signal variation was $\pm$5% when compared to the analytical signal obtained in the absence of the referred compound. The obtained results (Table 1) demonstrated that under the used reaction conditions higher interference was observed for pyruvate, oxalate and ascorbic acid. The presence of these compounds at an interferent/PGA molar ratio of 1 : 1 does not cause interference, however, for a molar ratio five times greater a significant interference was observed. Nevertheless, these levels of interference do not limit the determination of PGA

Table 1. Influence of interferences on the developed methodology.

Added species	Tolerance limit
Glucose, urea, creatinine, acetate	100*
Na^+, Cl^-, NH_4^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}	100
Fe^{3+}	50
CO_3^{2-}	25
K^+, uric acid, hippuric acid	10
Citrate,	5
Oxalate, pyruvate, ascorbic acid	1

*Maximum tested ratio.

Table 2. Results obtained in the determination of PGA in urine samples.

	MPFS[b]		HPLC[b]		
Sample	Concentration found (mg L^{-1})	Concentration found (mg/g creatinine)	Concentration found (mg L^{-1})	Concentration found (mg/g creatinine)	RD[a] (%)
1	198.0 ± 8.3	102.1 ± 4.3	196.3 ± 0.4	101.2 ± 0.2	0.9
2	216.1 ± 8.3	269.0 ± 10.4	217.1 ± 1.8	270.2 ± 2.2	−0.4
3	nd	–	63.0 ± 0.1	57.3 ± 0.1	–
4	162.1 ± 10.2	170.5 ± 10.7	170.9 ± 0.5	179.9 ± 0.5	−5.2
5	297.4 ± 5.6	265.6 ± 5.0	300.0 ± 0.2	267.9 ± 0.2	−0.9
6	368.5 ± 13.9	196.0 ± 7.4	347.2 ± 3.7	184.7 ± 2.0	6.1
7	205.7 ± 15.4	117.7 ± 8.8	220.4 ± 0.1	125.9 ± 0.1	−6.6
8	158.1 ± 6.6	84.1 ± 3.5	150.3 ± 0.5	79.9 ± 0.3	5.2

[a]Relative deviation of the developed methodology with respect to the comparison procedure.
[b]Mean ± SD
nd: Not detectable.

since they correspond to concentration levels higher than the normal values usually found in urine.

The developed methodology was applied to PGA determination in urine samples and the obtained results are showed in Table 2. At present, the real exposure to styrene is very rare (probably due to very strict safety rules obeyed in the production process). To prove the practical application of our procedure and keeping in mind possibility of accident exposure we have analysed urine from healthy volunteers to which known amounts of PGA were added.

The system was stable, no baseline drift was observed and sampling rate was about 43 determinations per hour. The consumption of reagents per determination was about 1.7 mg of H_2O_2, 0.18 mg of ammonium vanadate and about 1.7 mL of liquid waste were generated.

3.5 *Validation of the proposed method*

To evaluate the accuracy of the proposed method, the urine samples were also assayed by the HPLC comparison method and the obtained results (Table 2) were in agreement

Table 3. Accuracy assessment: results of the recovery tests.

	Recovery (%)	
Sample	200 mg L^{-1}	400 mg L^{-1}
1	109	93
2	100	101
3	104	109
4	98	106
5	100	95
6	97	107
7	108	104
8	109	100
9	91	95
10	95	93

as demonstrated by the relative deviation (between −6.6 and 6.1%). Additionally, a linear relationship between the two methods was found $C_{MPFS} = 1.1(\pm 0.2)\ C_{HPLC} - 17.2(\pm 40.0)$, were C_{MPFS} is the concentration (mg L^{-1}) obtained by the proposed method and C_{HPLC} is the concentration (mg L^{-1}) obtained by HPLC, with a correlation coefficient of 0.98. From these figures it is clear that the estimated intercept and slope do not differ significantly from values 0 and 1, respectively. Thus there is no evidence of systematic differences between the two sets of results obtained by the proposed methodology and the comparison procedure. These results were further confirmed by a paired Student's *t*-test: the estimated *t*-value (0.12) was lower than the tabulated one (2.57) for a confidence level of 95% ($n = 10$). The accuracy of the results obtained was also assessed through recovery experiments. Ten urine samples were spiked with two levels of PGA and the obtained recovery data ranged between 91 to 109% (Table 3).

4. Conclusions

A methodology for determination of PGA based on its inhibition effect on the H_2O_2–vanadate reaction using a multi-pumping flow system was proposed. The application of the multi-pumping flow concept allowed the implementation of a precise, reproducible, simple and versatile analytical methodology with high automation level.

The pulsed nature of the flowing stream provided improved mixing conditions resulting in a reduction in reagents consumption as well as in the produced liquid wastes. This led to a reduction of the cost of the analysis, to an increased repeatability and accuracy, to a lessening of the operator intervention and to a high sampling rate.

When compared to the previously reported methods for PGA determination, the developed method presented a higher detection limit, yet was suitable for monitoring styrene exposure; however, it is simpler, it does not require sample pre-treatment, it is faster and it uses less expensive equipment.

All the above-mentioned characteristics demonstrated that the proposed procedure is a valuable method for routine biological monitoring of workers exposed to styrene.

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