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Novel strategy for fluorescence determination of glibenclamide in samples with high concentration of caffeine based on a low-pressure flow injection chromatography system

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ABSTRACT

In this work, a new low-pressure flow injection chromatography (FIA-C) system with fluorescence detection was developed for the toxicological control of glibenclamide in beverages with high caffeine content. As caffeine quenched the fluorescence signal of glibenclamide, a separation of the analyte from the sample matrix was proposed as a simple and rapid strategy. The separation was performed in a commercially available monolithic column (RP-18e, 25 mm × 4.6 mm i.d.) inserted in a flow injection system. The mobile phase used for analysis was acetonitrile/acetic acid (50:50 v/v), with a flow rate of 1.03 mL min^{-1}. For each analysis, only 5.2 mL of mobile phase was used.

After the optimization of the variables of the system, a calibration curve with a linear range between 0.50 and 10.0 mg L^{-1} was obtained (R^2 = 0.997). The precision of the proposed method was evaluated in terms of the relative standard deviation obtaining 0.58 and 1.68% for the intra-day precision and inter-day precision, respectively. The detection limit was 0.10 mg L^{-1} and the sample throughput, taking into account the whole procedure, was 12 h^{-1}. The method was applied to fortified real samples with satisfactory recovery values (90.4–103.7%). On the other hand, samples adulterated with commercially GLB pills were also analyzed with very good results (96.5–104.5%).

1. Introduction

Covert administration of incapacitating drugs to commit a crime (sexual assault, robbery) is not a recent phenomenon although reports are still actual and becoming more frequent, especially by teenagers and young people. More recently, covert drugging has become associated with sexually orientated crimes, and reports of women being drugged and sexually assaulted have appeared in media sources around the world [1]. The most common way to introduce some drugs for these purposes is called “drink spiking”, where different kind of pharmaceuticals or abuse drugs are surreptitiously administered together with beverages.

Drugs that are used to facilitate sexual assaults can be difficult to detect (active products at low dosages, chemical instability), and can be rapidly cleared from the body (short half-life). In general, sampling blood or urine has low yields 48–72 h after the offense occurred [2]. Hair segmentation is the more sensitive method, even when several days or months can pass between the occurrence of the crime and the analysis. However, this method has some drawbacks as hair is not a simple matrix and analysis requires an expertise and longer time of analysis [3]. In some countries, drug detection kits to test the presence of some adulterants in drinks before consuming them are available, although the effectiveness of these tests is still doubtful and are not applicable for all kind of substances [4].

Thus, simpler and reliable methods are required to analyze the presence of these drugs in beverages consumed by the victims in order to act immediately and thus, provide a rapid and accurate diagnosis of the situation.

Among the drugs that are used to incapacitate victims, hypoglycemic agents have been recently reported in forensic science [5]. Glibenclamide (GLB) or glyburide is a sulfonylurea indicated for the treatment of non-insulin-dependent diabetes mellitus. This active principle has an acute hypoglycemic effect because it acts on the β cells of the pancreas stimulating the secretion of insulin that causes the cells of the organism to increase their glucose consumption. GLB is rapidly absorbed in the gastrointestinal tract, having 24 h of action, a half-life of 10 h, and a peak response with insulin secretion from 2 or 3 h after oral administration. If GLB is administered to healthy individuals, the

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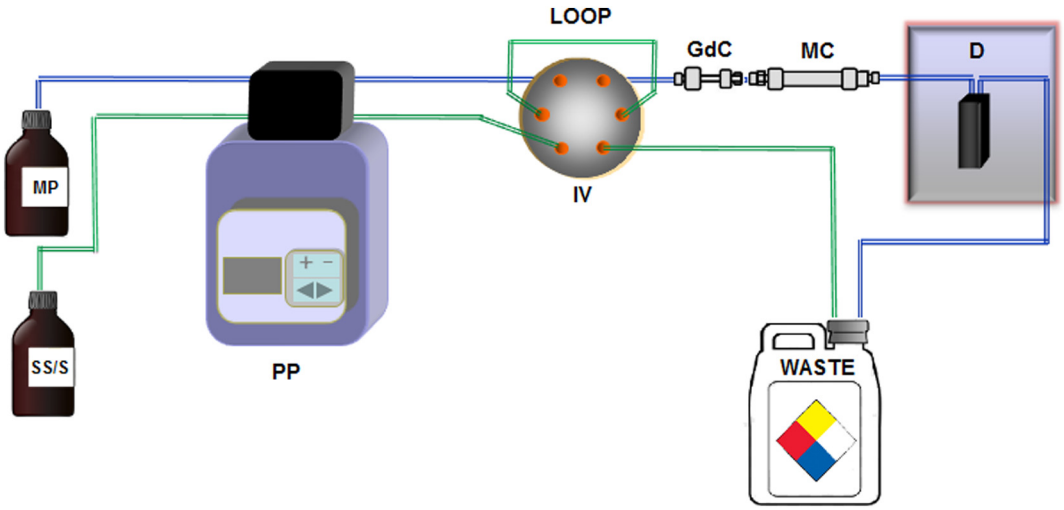

Fig. 1. Scheme of the proposed FIA-C system. MC: monolithic column; GdC: guard column; MP: mobile phase; SS/S: standard solution/sample; PP: peristaltic pump; IV: injection valve, D: detector.

effect is an excess of glucose use, which generates sustained hypoglycemia by sulfonylureas. Usually, the incidence of toxicity caused by hypoglycemic agents is difficult to ensure because there is no adequate report or infrastructure to confirm the poisoning cases, or the number of real exposures is underestimate. In the Annual Report of the American Association of Centers of Poisoning 2015 [6], 3837 cases were mentioned for oral hypoglycemics sulfonylureas agents of which 1413 had unintentional reasons.

Various methods have been proposed for the determination of GLB mainly in pharmaceutical formulations and biological samples using separation techniques. Particularly, liquid chromatography techniques have been extensively used, as part of multi-residue methods, using photodiode array detectors or mass spectrometry [7–10]. Recently, ultra-performance liquid chromatography with photodiode array and fluorescence detector (UHPLC-PDA–FLD) was employed to determine GLB in human plasma samples [11]. Moreover, chemometric techniques have been used with chromatographic techniques, even to optimize the sample pretreatment [12] or to resolve the co-elution of GLB with other sulfonylurea antidiabetic drugs [13]. Additionally, a non-destructive detection of adulterated glibenclamide tablets using near infrared spectroscopy (NIRS) and fluorescence spectroscopy along with chemometric was proposed [14]. The fluorometric determination of GLB has been performed using a multipumping flow system for the toxicological control of GBL in commercially available pharmaceutical formulations and alcoholic beverages [15]. The authors proposed a simple system using sulphuric acid as carrier and sodium dodecylsulphate (SDS) to enhance the fluorescence of GBL. Satisfactory results were obtained for the pharmaceutical formulations, but they informed that some components of soft drinks and red wine interfered in the analytical response and make glibenclamide detection uncertain in these samples. The authors proposed then a new method to separate the drug from the liquid samples through its adsorption into activated charcoal packed within a mini column [16]. This method was applied to tea samples.

In recent years monolithic columns in HPLC have been designed as an alternative to the particulate columns, in order to achieve efficiency of separation at lower pressures, which allows increasing the flow rate, and therefore, significantly reducing the retention times. Monolithic columns consist of a continuous piece of porous silica or an organic polymer with different types of pores (macro-, meso- and micropores, in the range between micrometers and nanometers. The high permeability and porosity of the silica skeleton, and the resulting low back pressure, allow more flexible flow rates compared to the particle columns. As a result, monolithic columns allow high performance analysis without loss of separation efficiency or peak capacity [17].

The use of monolithic columns led to the development of low-pressure chromatographic methods by coupling these columns to flow analysis systems, overcoming one of the limitations assigned to flow-based techniques: the multicomponent analysis. Therefore, chromatographic flow manifolds are presented as an interesting tool to achieve multi-analyte analysis in a flow analysis system in a simple and low-cost manner, which significantly increase its potential. Despite of the simplicity, flow chromatographic methods achieves high performance separations comparable to HPLC. Moreover, the hyphenation of these systems with selected sample pre-treatment processes [18] or post-column derivatization [19] is feasible.

The first chromatographic flow technique was developed by Šatínsky et al., that arises from coupling a monolithic column to a sequential injection analysis (SIA) system: sequential injection chromatography (SIC) [20]. This approach was extended to other flow manifolds such as multi-syringe flow injection approach (multi-syringe chromatography, MSC) [21], and traditional FIA systems (low-pressure flow injection chromatography, FIA-C) [22]. One of the main differences between flow techniques is the diverse propulsion devices that can be used to design the manifold. Unlike SIC manifolds, which are designed with piston pumps that withstand pressures up to 750 psi [23], FIA-C manifolds were designed with milliGAT or peristaltic pumps as propulsion devices, overcoming the limitation of the syringe pump to restrict the volume of the mobile phase available for each analytical cycle. As a drawback, the length of the columns that can be used is limited, between 10 and 50 mm, in order to reduce back-pressure at a level compatible with the propulsion devices that are employed.

In this work, a novel and rapid method with fluorescence detection is proposed for the toxicological control of GLB in energy drink samples, which are highly consumed by adolescents and young adults. As the composition of these beverages contained high concentration of caffeine that affects the fluorescence signal of GLB, a simple FIA-C method was presented as a suitable strategy to perform the separation of GLB from the sample matrix. The analysis of the samples can be performed in a short time (5 min) without extracting the analyte from the sample matrix. To the best of our knowledge, is the first time that a relation between the fluorescence signal of GLB and the content of caffeine in the sample is observed.

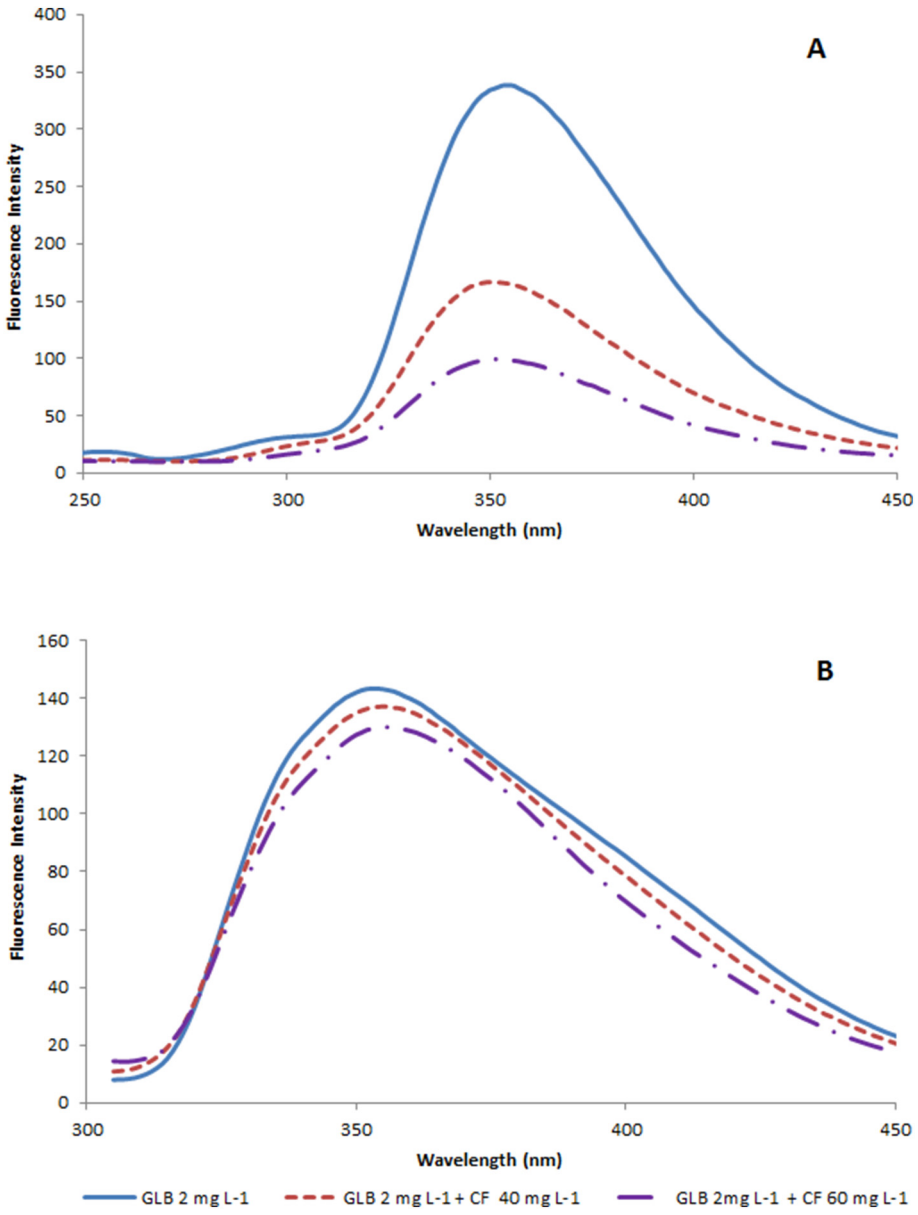

Fig. 2. Effect of CF in the fluorescence intensity of GLB. A: λ_{exc} 234 nm, B: λ_{exc} 298 nm.

2. Experimental

2.1. Reagents

All the solutions were prepared using ultra-pure water (> 18 MΩ cm) and analytical grade reagents. Acetonitrile (ACN) (≥ 99%, HPLC grade, Merck, Germany) and acetic acid (≥ 99%, Merck, Germany) were used for the preparation of both the mobile phase and the sample.

A 200 mg L^{-1} GLB (≥ 99%, Sigma-Aldrich, Germany) stock solution was prepared in ACN and stored in a dark bottle at 4 °C. Standard working solutions of GLB (0.5–10.0 mg L^{-1}) were daily prepared by diluting the appropriate volume of the stock solution with 30% of ACN-acetic acid solution of pH 3.2.

A 200 mg L^{-1} caffeine (ReagentPlus®, Sigma-Aldrich) stock solution prepared in ACN was daily diluted for the preparation of the solutions used to test the effect of caffeine on the GLB fluorescence signals.

2.2. Apparatus and software

As a fluid propulsion system, a Gilson® Minipuls 3 peristaltic pump was used. MHLL Tygon® tubes with an i.d. of 1.14 mm, suitable for organic solvents, were used for all pumping channels. All tubing used for connections were made of PTFE (0.8 mm i.d., Omnifit, England). The injection of the sample was performed using a four-way rotary valve (Rheodyne 5041, Germany). A flow rate of 1.03 mL min^{-1} was selected for the procedure.

A Jasco FP 6500 spectrofluorometer was used to perform the fluorescence measurements (bandwidth of excitation and emission 10 nm; PMT voltage 470 V; data pitch 0.2 s). A Hellma 176.752-QS flow cell with 25 μL internal volume and 1.0 cm optical path was placed in the cuvette holder of the instrument.

The chromatographic separation was performed on a RP-18 silica-based monolithic column (Chromolith® Flash, 25 × 4.6 mm, Merck, Germany) protected with a guard column of the same material (Chromolith®, 10 × 4.6 mm, Merck, Germany).

The pH of the acetic acid solutions and the samples was measured with a pH meter (Isemeter model 710A).

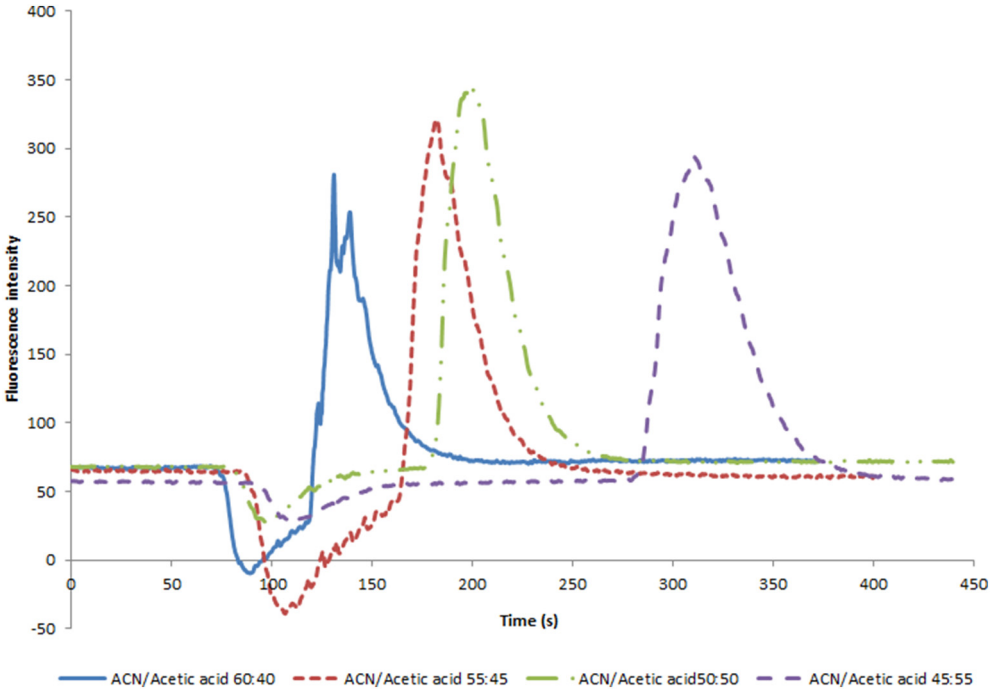

Fig. 3. Optimization of the mobile phase using different percentages of ACN-acetic acid pH 3.2. A solution of GLB of 5 mg L^{-1} was used.

Table 1
Chromatographic characteristics and analytical performance of the proposed FIA-C method.

Parameter	Obtained value
Retention time (s)	120
Repeatability of t_R (RSD %)[1]	0.25
Asymmetry factor	1.20
Calibration range (mg L^{-1})	0.50–10.0
Slope (mg L)$^{-1}$	61.1 ± 0.7
Intercept	80.9 ± 4.1
R^2	0.997
LOD (mg L^{-1})[2]	0.10
Intra-day precision (RSD %)[3]	0.58
Inter-day precision (RSD %)[4]	1.68
Sample throughput (h^{-1})	12

[1,3]n = 9, 5.00 mg L^{-1}; [4]measure by triplicate over 3 days, 5.00 mg L^{-1}; [2]LOD calculated as 3 *s*/A, where, *s*: standard deviation. A: slope of the calibration curve.

2.3. FIA-C system procedure

The proposed FIA-C system is schematically depicted in Fig. 1. The system consisted in a peristaltic pump (PP), an injection valve (IV), the chromatographic monolithic column (MC) and a flow cell (D) placed in the cuvette holder of the spectrofluorometer. The MC was coupled to the guard cartridge (GdC) and was placed between IV and D. Connections lengths between IV and MC, and between the MC and D were reduced as much as possible (25 and 70 mm, respectively) in order to minimize the dispersion. The mobile phase (MP) ACN/acetic acid 50:50 (v/v), which acted as carrier solution, was propelled by PP through the MC for 30 s to achieve stabilization and to condition the column. Then 50 µL of sample were injected into the MP, passed through the MC and afterward by the flow cell, where the FIA-C chromatogram (fluorescence vs. time) for the GLB was obtained. Based on the fluorescence excitation spectrum of the analyte, the measurements were performed at an excitation wavelength of 234 nm and an emission wavelength of 352 nm (470 V, excitation and emission bandwidth 10 nm). The flow rate was kept constant throughout the determination at 1.03 mL min^{-1}.

For the quantification of GLB, the peak height was considered instead of the peak area because the instrument's software (Spectrum Manager, FP-6500/6600) does not allow their precise calculation.

2.4. Sample preparation

Commercial energy drinks and alcoholic drinks (champagne and vodka) samples were acquired from different supermarkets and wine stores of Bahía Blanca, Argentina. GLB could be added directly in the energy drink (aqueous matrix) or in a mixture of energy drink and alcohol. Thus, samples of energy drinks were analyzed immediately after opening the cans without any mixing; and mixed with alcohol in a 50:50 ratio. At first, the pH of the samples was between 3.2 and 3.7. Therefore, there was no need to adjust the pH for the analysis. Then, the samples were degassed by sonication for 10 min in an ultrasound bath. GLB is commercially available in tablets of 5 mg per pill. It was considered that at least one pill of GLB can be mixed with 250 mL of the sample (one can or one glass). To prepare the samples, 10 pills of GLB were weighted (301.8 mg per pill) and blended in a mortar. The quantity of a pill containing approximately 0.02000 mg of GLB was weighted dissolved with 3.0 mL of ACN. Then, 1.0 mL of the sample was added and the mixture was diluted up to 10.00 mL with acetic acid pH 3.2. By this way, the excipients contained in the GLB pill were diluted in the sample and can be tested as possible interferents. The sample was then filtered with a 0.45 µm filter and injected into the FIA-C system.

For the recovery study, the samples were spiked with GLB at two concentration levels within the calibration range: 2.00 and 7.00 mg L^{-1} and the same preparation protocol was followed. The lower concentration level was chosen taking into account the addition of one pill of GLB in 250 mL of sample, a quantity enough to produce the desire effect in a healthy person. The higher level was randomly chosen. Recovery values were calculated according to the AOAC definition [24]. All analyses were performed by triplicate.

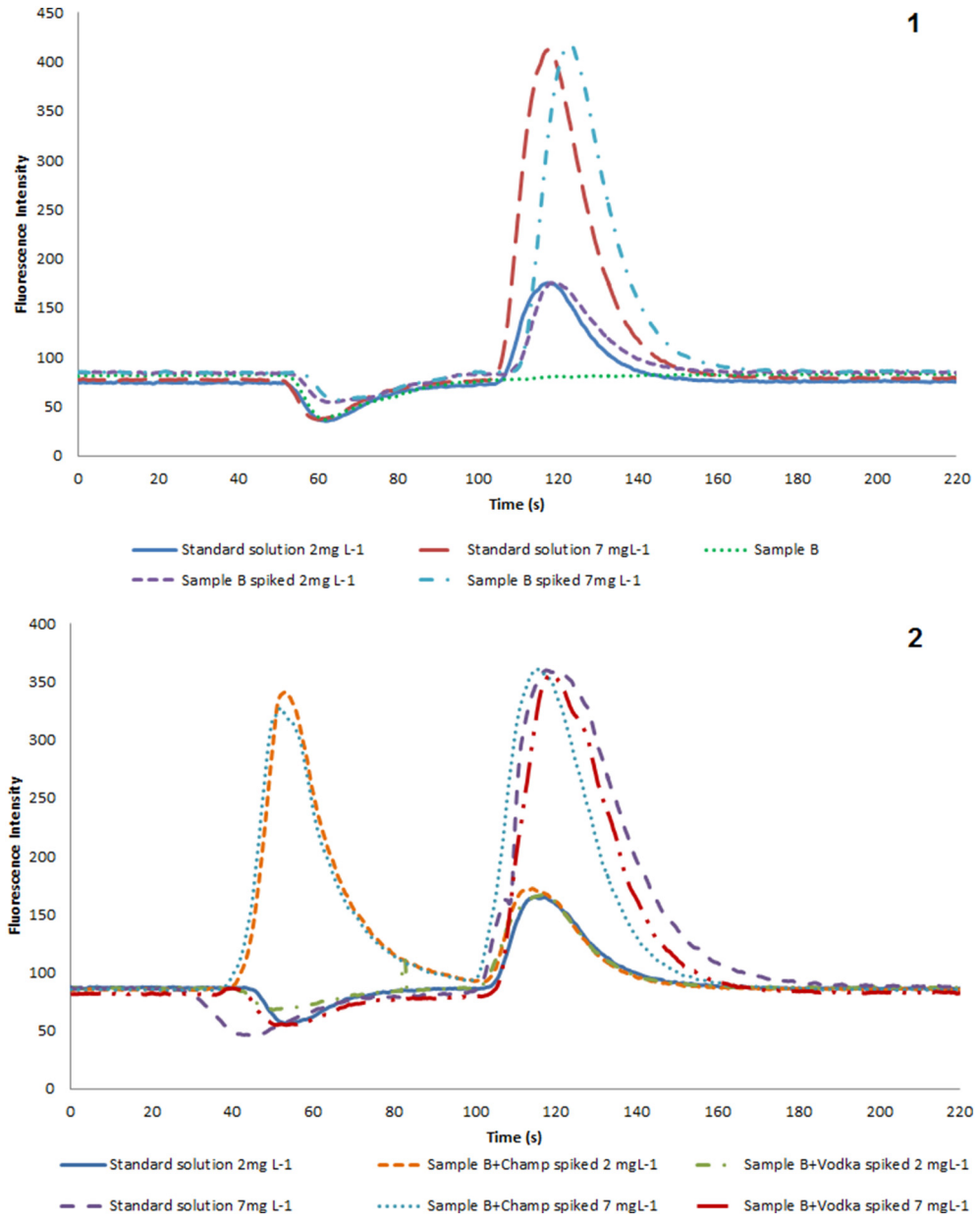

Fig. 4. 1. Comparison between standard solutions of GLB and sample B spiked with GLB at 2 mg L^{-1} and 7 mg L^{-1}. 2. Comparison between standard solutions of GLB and sample B mixed with champagne and vodka in a 50:50 ratio, spiked with GLB at 2 mg L^{-1} and 7 mg L^{-1}. Champ: champagne.

3. Results and discussions

3.1. Fluorescence determination of glibenclamide in presence of caffeine

GLB is a fluorescent compound that presented two excitation maximum at 234 nm and 298 nm, with emission at 352 nm, respectively. On the other hand, CF, which is the major component of the samples to be studied, is not reported as a fluorescent compound [25, 26]. In mixtures of GLB and CF, CF quenched the GLB emission fluorescence signals when the excitation is performed at both excitation wavelengths (Fig. 2A and B). The decrease in the fluorescence intensity is dependent of the CF concentration that is added to the GBA solution. As can be seen in Fig. 2, as the concentration of CF increases the GBA signal decreases for both excitation wavelengths. Thus, in samples that both compounds are presented, the determination of the concentration of GLB cannot be done directly. In general, an extraction step must be performed.

Therefore, to perform the direct determination of GLB in the presence of CF, a FIA-C system was designed in order to separate both compounds, and quantify GLB in samples with high CF content.

3.2. Optimization of the separation conditions

3.2.1. Mobile phase composition

As is well-known, the pH of the mobile phase needs to be carefully considered because it will affect the degree of analyte ionization, and hence its relative hydrophobicity. Considering the pK_a of the GLB (5.3), the pH was studied in a range between 3.0 and 4.5, using acetic acid to adjust the corresponding values. As was expected, the fluorescence intensity was slightly increased when the pH decreased, being higher at pH 3.2. Thus, 3.2 was selected as the optimum pH value.

In order to improve the peak shape, mobile phase composition was studied. Acetonitrile and methanol were tested as organic solvents, and acetic acid and a phosphate buffer, both at pH 3.2, were tested as the aqueous phase. For the mobile phase mixtures containing phosphate buffer, the intensity of the signal was lower than that obtained by using

Table 2
Determination of GLB in energy drink samples adulterated with the standard solution.

Sample[a]	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
A	2.00	1.96 ± 0.02	98.0
	7.00	7.26 ± 0.08	103.7
B	2.00	1.99 ± 0.02	99.5
	7.00	7.11 ± 0.03	101.6
C	2.00	2.04 ± 0.01	102.0
	7.00	7.01 ± 0.05	100.1
D[b]	2.00	1.92 ± 0.04	96.0
	7.00	6.33 ± 0.03	90.4
E[c]	2.00	1.88 ± 0.01	94.5
	7.00	6.41 ± 0.07	91.6
B + champagne[d]	2.00	2.04 ± 0.01	102.0
	7.00	6.93 ± 0.05	99.0
C + champagne[d]	2.00	2.06 ± 0.02	103.2
	7.00	7.14 ± 0.04	102.1
D + champagne[d]	2.00	2.05 ± 0.02	102.5
	7.00	6.95 ± 0.02	99.3
B + vodka[d]	2.00	2.02 ± 0.01	101.0
	7.00	6.90 ± 0.05	98.6
C + vodka[d]	2.00	1.98 ± 0.03	99.0
	7.00	6.91 ± 0.02	98.7
D + vodka[d]	2.00	1.97 ± 0.05	98.5
	7.00	6.99 ± 0.03	99.8

A, B, C, D and E corresponded to energy drink samples of different composition.
[a] The samples were analyzed in triplicate.
[b] Sample D contained Guaraná extract.
[c] Sample E is sugar-free.
[d] Energy drink samples mixed with alcohol in a 50:50 ratio.

Table 3
Determination of GLB in energy drink samples adulterated with GLB pills.

Sample[a]	GLB in pill (mg L^{-1})[b]	GLB found (mg L^{-1})	Recovery (%)
B	2.00	1.93 ± 0.01	96.5
C	2.00	1.96 ± 0.07	98.0
D	2.00	1.98 ± 0.06	99.2
B + champagne	2.00	1.96 ± 0.02	98.0
C + champagne	2.00	1.97 ± 0.03	98.5
D + champagne	2.00	2.09 ± 0.01	104.5
B + vodka	2.00	1.99 ± 0.01	99.5
C + vodka	2.00	2.08 ± 0.02	104.0
D + vodka	2.00	2.02 ± 0.01	101.0

B, C and D corresponded to energy drink samples of different composition. Samples were mixed with champagne and vodka in a 50:50 ratio.
[a] The samples were analyzed in triplicate.
[b] Concentration calculated by adding one pill of GLB in 250 mL of beverage.

the mixtures containing acetic acid. On the other hand, when methanol was used as organic solvent, the baseline was noisy. Based on these results, a mobile phase composition of ACN:acetic acid pH 3.2 was selected for further studies. Afterward, the percentages of ACN were studied, between 45% and 60%. Fig. 3 depicts the chromatograms obtained when different mixtures were tested. Taking into account a proper separation of the GBL peak from the solvent front and the value of the asymmetry factor, 50% of ACN was selected as the optimum percentage.

3.2.2. Flow rate and injection volume

The influence of flow rate regarding retention time was evaluated within the range between 0.40 mL min^{-1} to 1.03 mL min^{-1} using a 5 mg L^{-1} GLB solution. At higher values, backpressure in the chromatographic column was observed giving place to leaking troubles in the FIA-C system. On the other hand, values of flow rate lower than 1.03 mL min^{-1} derived in poor chromatographic parameters. Thus, 1.03 mL min^{-1} was selected as optimum for further studies.

Using the above mentioned experimental conditions of mobile phase and flow rate, three different loop volumes, available in our laboratory, were tested: 10 µL, 50 µL and 100 µL. The sensitivity increased from 10 to 50 µL, but the column was overloaded when 100 µL was injected. Thus, 50 µL was selected as a compromise between sensitivity and column overloading.

3.3. Chromatographic characteristics and analytical performance

The determination of GLB was performed under the above-mentioned conditions for the FIA-C system, namely mobile phase ACN/acetic acid 50:50 (v/v), flow rate 1.03 mL min^{-1}, and injection volume of 50 µL.

As separation is used as a strategy to achieve the determination of GLB in presence of CF, only some parameters were assessed, according to the US FDA guidance [27]. As can be observed in Table 1, the values corresponding to the retention time, peak asymmetry and injection repeatability (calculated with retention times and expressed as RSD value) were satisfactory.

On the other hand, the analytical performance was evaluated in terms of the linearity, limit of detection (LOD), sample throughput, and intra-day and inter-day precision as relative standard deviation (RSD) percentage (Table 1).

In order to test linearity, the calibration curve for the GLB determination was prepared based on the relationship between the concentration of the analytes and the peak height, and was constructed over the range of 0.50–10.0 mg L^{-1} (six points with three replicates). The regression equation was $A = (61.1 \pm 0.7)$ [GBA mg L^{-1}] + (80.9 ± 4.1) with a correlation coefficient of 0.997. The LOD value was 0.10 mg L^{-1} and was calculated from the calibration function.

The intra-day precision was evaluated by RSD (%) values obtained with a 5.00 mol L^{-1} GLB solution and 9-fold repetition while for inter-day precision the same concentration was measured by triplicate on 3 consecutive days. The obtained RSD values of 0.58% and 1.68% proved high repeatability and inter-day precision for the proposed FIA-C method.

The sample throughput of the optimized method was 12 h^{-1} for the complete procedure, meaning that 5 min per sample were needed: 3.5 min for the complete elution of the GLB and to restore the baseline and 1.5 min for column stabilization and loading the new sample in the system.

3.4. Real samples analysis

Hypotonic or energizing drinks that are widely used in the whole world contain high amounts of caffeine, in combination with other presumed energy-enhancing ingredients such as taurine, herbal extracts, and B vitamins [28]. They may or may not be carbonated and they also may contain sugar or other sweeteners. They are most frequently consumed by young people, especially in bars and clubs, and may or may not be mixed with alcohol.

Thus, five energy drinks samples (A to E) with different composition were selected to demonstrate the applicability of the proposed method. The composition of the samples can be found in Table S1 of the Supplementary material. In addition to the components declared in the nutritional information, all samples contained permitted preservatives, colorants, flavorings, regulators of acidity and antioxidants. On the other hand, three energy drink samples (B, C and D) were randomly chosen and mixed in a 50:50 ratio with two different alcoholic beverages commonly used for this purpose: champagne and vodka.

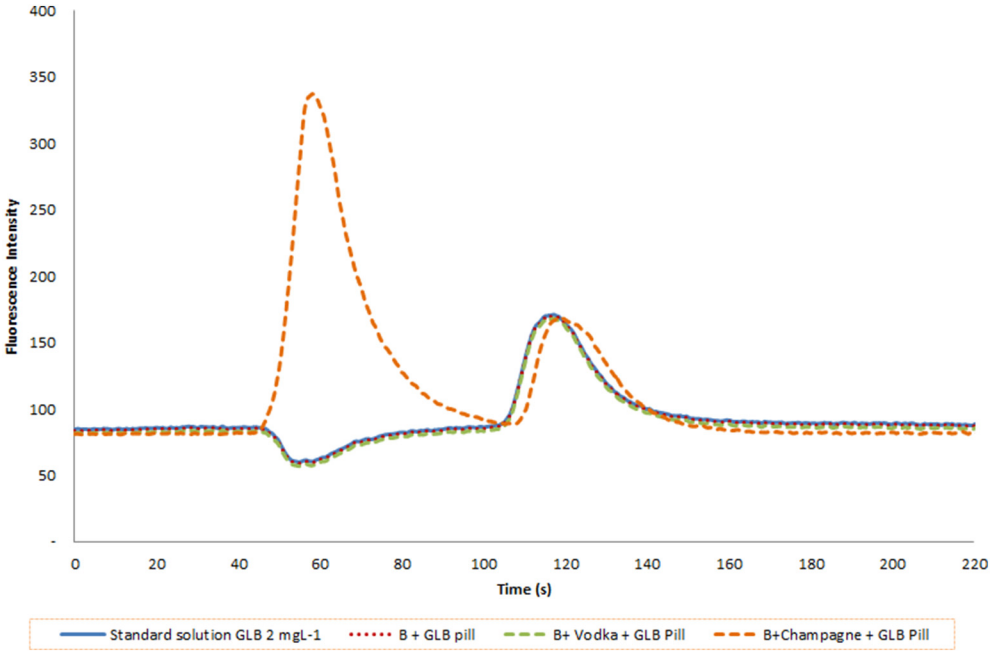

Fig. 5. Energy drink B, unmixed and mixed with alcohol (champagne and vodka), adulterated with commercial GLB pills.

The samples were injected in the FIA-C system for their analysis demonstrating that no other compounds are eluted at the retention time of the GLB (Fig. S1.1 and S1.2, Supplementary material). In addition, the matrix effect was evaluated by comparing the slope of the calibration curve obtained from the standard solutions and the slope from a spiked sample at the same concentration levels by using a t-Student test ($\alpha = 0.05$) [29]. There was no significant difference between the slopes of the regression lines studied for the chosen probability level ($p > 0.1$) meaning that no matrix effect was presented.

For the recovery study, all the analyzed samples were spiked at two concentrations levels within the calibration range: 2.00 and 7.00 mg L^{-1}, treated as described, and introduced into the FIA-C system for their analysis. In Fig. 4, the FIA-C chromatograms of the 2 mg L^{-1} and 7 mg L^{-1} standard solutions, non-spiked B sample and spiked B sample at both levels are shown as model. Fig. 4.1 corresponded to the FIA-C chromatograms of the energy drink sample B without mixing and Fig. 4.2 corresponded to the FIA-C chromatograms of the energy drink sample B mixed with alcohol. The results of the recovery study for the analyzed samples are summarized in Table 2 and they were ranged from 90.4 to 103.7%.

To adulterate the samples with the GLB pills, the procedure described in Section 2.4 was followed. The obtained results were between 96.5 and 104.5% (Table 3) and demonstrated the capacity of the proposed method to perform the analysis under a real situation. Fig. 5 shows the FIA-C chromatogram for sample B, unmixed and mixed with alcohol, with the addition of the GLB pill.

3.5. Comparison with previous methods

Table 4 shows different analytical methods that have been proposed for the determination of the GLB concentration, compared in terms of type of the sample, the necessity of sample preparation and the analytical parameters. An important achievement of the FIA-C method is the possibility to resolve the interference of some components of the sample on the fluorescence signal, such as caffeine, without performing a previous treatment. Compared to the automated methods found in the literature, the proposed method reached lower LODs, and a good sample throughput. It is important to remark that HPLC methods presented LODs values below the value obtained by the FIA-C method, but a step of preconcentration of GLB is required. Nevertheless, the LOD achieved by using the FIA-C method was low enough for determining GLB in beverages. On the other hand, it should be highlighted that excellent values for the intra- and inter- day precision are presented meaning a very good repeatability and reproducibility of the proposed method.

4. Conclusions

Nowadays, the development of analytical methods to determine drugs that can be illegally used in beverages to commit crime is mandatory. This method presents a fast and reliable way to determine one of these drugs, GLB, in energy drink samples that are highly consumed mainly by teenagers and young adults. Due to the interaction of the CF (the major component of energy drinks) in the fluorescence signal of GLB, a FIA-C method was developed as a new strategy to achieve the separation of both compounds. Although other authors have determined GLB by fluorescence, the relation between the high content of caffeine and the decrease of the fluorescent signal had not been previously observed.

The proposed method presented satisfactory values for the calculated analytical parameters. In addition, an important decrease of the consumption of reagents, a very good reproducibility and an excellent sample throughput for the complete procedure were achieved. Moreover, it was performed with a low cost and easy to operate instrumentation is used.

Energy drink samples with different composition were analyzed without mixing and mixed with alcoholic beverages, and were spiked with GLB standard solution and adulterated with GLB pills, obtaining satisfactory results.

Thus, the FIA-C method is proposed as a novel and interesting tool

Table 4
Brief comparison of analytical methods for GLB determination.

Method	Detection technique	Sample	Pre-treatment	Linear range	LOD	Intra-day precision (RSD %)	Inter-day precision (RSD %)	Sample throughput (h^{-1})	Ref
Multipumping flow system	Fluorescence	Tea	Yes	Up to 50 mg L^{-1}	0.81 mg L^{-1}	n.i	n.i.	15	[16]
Multipumping flow system	Fluorescence	Pharmaceutical formulations, alcoholic beverages	No	Up to 75 mg L^{-1}	2.75 mg L^{-1}	n.i	n.i	39	[15]
UHPLC	UV	Human plasma	Yes	0.08–2.50 mg L^{-1}	n.i	n.i.	n.i	10[a]	[11]
HPLC	UV	Human serum	Yes	0.12–2.40 μg L^{-1}	0.047 μg L^{-1}	3.9–9.3	5.5–10.3	10[a]	[12]
FIA-C	Fluorescence	Energy drink	No	0.50–10.0 mg L^{-1}	0.10 mg L^{-1}	0.58	1.68	12	Proposed method

n.i: not informed.
[a] Only the chromatographic run.

to perform a rapid separation and quantification of the selected analyte in samples of high caffeine content, as energy drinks, without performing any previous treatment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2018.07.005.

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