



Synthesis and characterization of a molecularly imprinted polymer (MIP) for solid-phase extraction of the antidiabetic gliclazide from human plasma

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ABSTRACT

Gliclazide is a sulfonylurea frequently prescribed for the management of type 2 diabetes mellitus in elderly patients and for patients with chronic renal or hepatic diseases. Even though it is considered a safer alternative, the drug can provoke side effects in some patients, especially hypoglycemia, due to the high interindividual variability. Therefore, the quantification of gliclazide in biological samples is usually recommended in order to assure efficacy and safety of the pharmacotherapy. However, due to the complexity of biological matrices, therapeutic monitoring can be very challenging, especially in the sample preparation step. For that reason, the synthesis and characterization of a novel and selective molecularly imprinted polymer (MIP) was proposed to be employed as sorbent for the extraction of gliclazide from human plasma samples by a molecularly imprinted solid-phase extraction (MISPE) procedure. Synthesis conditions were optimized (monomer, crosslinker and porogen) and the polymer was characterized for its morphological, physicochemical and stability properties. The influence of drug concentration, solvent composition and pH on the coefficient of distribution (K_d) and imprinting factor (IF) were studied, as well as repeatability between batches and selectivity. A bioanalytical method was developed applying the developed MIP as sorbent in solid phase extraction and liquid chromatography using a Poroshell 120 C18 (100 × 4.6 mm, 4 μm) column, acetonitrile and 10 mM potassium phosphate buffer pH 3.0 (50:50) at a flow-rate of 1.2 mL/min as mobile phase, temperature of 30 °C, injection volume of 40 μL and detection at 230 nm. The best reaction yield, extraction capacity, and selectivity was obtained using 2-hydroxyethyl methacrylate (2-HEMA), ethyleneglycol dimethacrylate (EGDMA) and acetonitrile. The optimized MIP showed coefficient of distribution (K_d) of 59.85 μg/g, imprinting factor (IF) of 1.60, and selectivity for gliclazide and other sulfonylureas compared to possible concurrent drugs. The developed method by MISPE-HPLC-UV showed to be appropriate to determine gliclazide in human plasma samples.

1. Introduction

Diabetes mellitus (DM) is a group of heterogeneous and chronic metabolic disorders that affects approximately 382 million people worldwide, especially in developing countries [1,2]. The disease is characterized by hyperglycemia, due to defects in production, secretion and/or action of insulin. DM can be categorized in type 1 or insulin-

dependent, or type 2 or insulin-independent [3,4]. Type 2 DM is strongly correlated to lifestyle and polygenic factors and its management requires the adoption of healthier life habits, including low carbohydrate diet and regular physical practices [5–7]. DM treatment can also be carried out by pharmacotherapy, based primarily on orally administered antidiabetics, which decrease insulin resistance and improve pancreatic beta cells function. Insulin can also be necessary for

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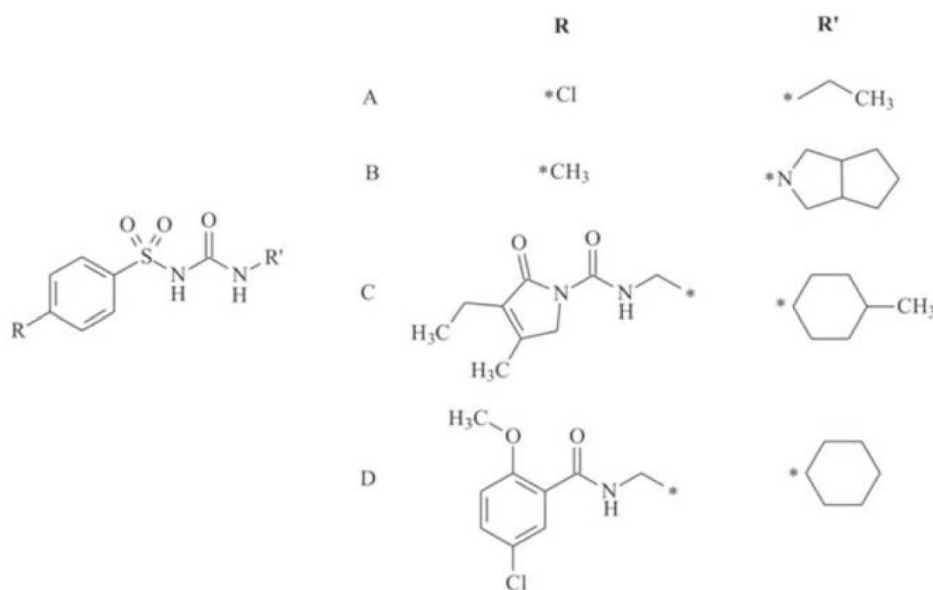


Fig. 1. Chemical structures of sulfonylureas chlorpropamide (A), gliclazide (B), glimepiride (C), and glibenclamide (D).

some patients [1,4,8]. Metformin is the drug of choice and is widely prescribed. However, a sulfonylurea, such as chlorpropamide, glibenclamide, gliclazide, and glimepiride (chemical structures presented in Fig. 1), can be associated in a second step of type 2 DM treatment, when appropriate control of glycemic levels is not reached [9–11].

Gliclazide (GCZ) is a second-generation sulfonylurea and is listed on the Essential Medicines List published by the World Health Organization (WHO). GCZ is the first-choice for the treatment of type 2 DM in elderly people and for patients with kidney or liver chronic diseases, because it is safer, since the incidence of hypoglycemia due to GCZ is lower [9,12]. The drug is available in solid oral dosage forms (controlled-release tablets containing 30, 60 or 80 mg of GCZ) and the usual dose ranges from 40 to 320 mg per day [13]. However, due to the individual variability, a considerable variation in plasma maximum concentrations (C_{max}) following oral administration is frequently reported. For instance, some authors have found C_{max} values ranging from 700 to 4900 ng/mL following a single dose of 80 mg of GCZ [14–16]. Therefore, to assure the efficacy of the treatment and also to minimize side effects, primarily hypoglycemia, an appropriate drug therapeutic monitoring of GCZ after oral administration must be performed [17].

The quantification of drugs in biological matrices, such as whole blood or plasma, is required for therapeutic monitoring. However, it is very challenging, due to the complexity and to the presence of many potential interferences in these matrices, which can influence the quantification [18,19]. Hence, an appropriate sample preparation step is mandatory for bioanalysis of drugs. Different classical and modern approaches have been proposed for sample preparation of biological samples. The determination of GCZ in biological fluids has been commonly performed by conventional techniques, such as liquid-liquid extraction (LLE) [20–22], protein precipitation (PPT) [23–25] or solid-phase extraction (SPE) [26,27]. A few miniaturized approaches, such as dispersive liquid-liquid microextraction (DLLME) [28], microextraction by packed sorbent (MEPS) [29] and magnetic solid-phase extraction (MSPE) have also been described [30]. SPE are in general highly recommended in comparison to LLE or PPT, due to its advantages, such as higher recovery and capacity of preconcentration, lower solvent and reagent consumption, and lower exposure of analysts to toxic solvents [18,31]. However, since these procedures generally employ conventional and commercially available sorbents, selectivity is frequently a major problem [32].

Molecularly imprinted polymers (MIP) are three-dimensional highly cross-linked rigid polymeric network, which contains tailor-made

binding sites, complementary in shape, size and chemical functionality to a target template [33,34]. These biomimetic materials have high selectivity, comparable to immunosorbents. MIP has also great physicochemical robustness and stability, structure predictability and reusability, and their preparation is simple, easy, straightforward and relatively inexpensive [33,35]. Due to these advantages, MIP has emerged in recent years as an alternative in different fields, such as sample preparation, biochemical sensors, separation, purification, drug delivery and catalysis [33,36,37]. For example, Hrichi et al. have proposed a GCZ voltammetric sensor based on an electropolymerized molecularly imprinted polypyrrole film, with acceptable precision, selectivity, and sensitivity for quantification of GCZ in pharmaceutical dosage forms [38].

The most common application of MIP in biological, food, environmental or pharmaceutical analysis is as sorbent for SPE or its miniaturized versions [39–43]. Since the pioneer work of Sellergren and coworkers, in 1994 [44], for the extraction of pentamidine from human urine samples, molecularly imprinted solid-phase extraction (MISPE) has aroused great interest for the determination of several drugs in biological matrices [33]. The procedure is performed as usual in SPE, in 4 different steps (conditioning, loading, washing and elution). However, due to the nature of the material, the enrichment capacity and selectivity of a MISPE procedure are generally higher [45,46]. The growing applicability of MIP as sorbents for MISPE analysis was reviewed in recent years [47,48]. Nonetheless, to date, no study has been presented in the peer-reviewed literature describing the development and application of a MIP for the determination of GCZ in biological samples.

With all this in mind, this study proposes the synthesis and characterization of a MIP for GCZ and the development and optimization of a MISPE procedure for sample preparation of real plasma samples from healthy volunteers, prior to high-performance liquid chromatography with ultraviolet detection.

2. Materials and methods

2.1. Chemicals and reagents

GCZ, glibenclamide (GBC), and chlorpropamide (CPM) chemical reference standards (CRS) were purchased from Instituto Nacional de Controle de Qualidade em Saúde – INCQS (Rio de Janeiro, Brazil). Glimepiride (GMP) CRS was obtained from United States

Pharmacopoeia (Rockville, USA). GCZ and GMP active pharmaceutical ingredients (API) were donated by CIFARMA (Belo Horizonte, Brazil). GBC and metformin (MET) API were donated by Fundação Ezequiel Dias (Belo Horizonte, Brazil). CPM, losartan (LOS), and atorvastatin (ATO) API were bought from Farmácia Artesanal (Belo Horizonte, Brazil), Gemini (Anapólis, Brazil), and DEG (São Paulo, Brazil), respectively. Acetonitrile and methanol HPLC grade were purchased from JT Baker (Ecatepec, Mexico) and Sigma-Aldrich (Steinheim, Germany), respectively. Analytical grade acetic acid, monobasic potassium phosphate, 2-hydroxymethacrylate (2-HEMA), 2-vinylpyridine (2-VP), methacrylic acid (MAA), divinylbenzene (DVB), ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane triacrylate (TRIM) were purchased from Sigma-Aldrich (Steinheim, Germany). Chloroform and potassium bromide analytical grade were obtained from Synth (Diadema, Brazil), whilst phosphoric acid, sodium hydroxide, and 2-2-azobisisobutyronitrile (AIBN) were acquired respectively from Merck (Darmstadt, Germany), Quimex (São Paulo, Brazil), and TCI (Saitama, Japan). Ultrapure water was obtained in a Millipore Direct Q3 system (Billerica, USA) and nitrogen gas was purchased from White Martins (Contagem, Brazil).

Human blood samples were collected from healthy volunteers in heparinized tubes, in fasting or light breakfast conditions, for obtaining blank normal and hemolyzed samples or real samples, following oral administration of Diamicon 30 mg tablets (Servier, Rio de Janeiro, Brazil). For lipemic blank samples, blood collection was performed postprandially. Plasma samples were separated by centrifugation (Jouan, Saint-Herblain, France) at $480 \times g$ for 10 min and stored at -70°C , prior to use. Hemolyzed plasma samples were obtained by freezing-thawing cycles and vortexing. This project was approved by the Committee on Ethics in Research from Federal University of Minas Gerais (protocol number CAAE 68636017.5.0000.5149).

2.2. Chromatographic apparatus and conditions

Analyses were carried out by high-performance liquid chromatography in an Agilent 1200 Infinity Quaternary HPLC system (Santa Clara, USA) equipped with a quaternary pump, an autosampler, a thermostat and a diode-array detector (DAD). Chromatographic separation was achieved in a reversed-phase Poroshell 120 C18 (100×4.6 mm i.d.; particle size of $4 \mu\text{m}$) column from Agilent Technologies (Santa Clara, USA), maintained at 30°C , by isocratic elution of a mobile phase constituted of a mixture of acetonitrile (A) and 10 mM phosphate buffer pH 3.0 (B) (50:50, v/v) at a flow rate of 1.2 mL/min. The detection wavelength and injection volume were set as 230 nm and 40 μL , respectively.

2.3. Synthesis of molecularly imprinted polymers (MIP) for GCZ

Molecularly imprinted polymers (MIP) and non-imprinted polymers (NIP) were synthesized according to a non-covalent approach, by precipitation polymerization using GCZ as template. A comprehensive optimization procedure was performed for determination of synthesis conditions. Eighteen distinctive combinations (Fig. 2) of 3 functional monomers (2-HEMA, 2-VP and MAA), 3 crosslinkers (DVB, EGDMA and TRIM), and 2 porogens (chloroform and acetonitrile) were firstly tested, using a fixed molar ratio of 1:4:20 for template, monomer, and crosslinker, respectively. Free radical polymerization was carried out as follows: 0.1512 mmol (48.9 mg) of GCZ and 0.6048 mmol of monomer (78.7 mg of 2-HEMA, 63.6 mg of 2-VP or 52.1 mg of MAA) were dissolved in 25 mL of porogen in 50 mL threaded glass tubes, and were pre-polymerized at room temperature for 2 h. Then, 3.0240 mmol of the crosslinker (393.8 mg of DVB, 599.6 mg of EGDMA or 896.3 mg of TRIM) and 0.1220 mmol (20 mg) of AIBN were successively added to the solutions. The solutions were purged with nitrogen (N_2) for 10 min for removal of dissolved oxygen. The tubes were then threaded and sealed with parafilm, and placed in a Nova Ética 304D thermostatic

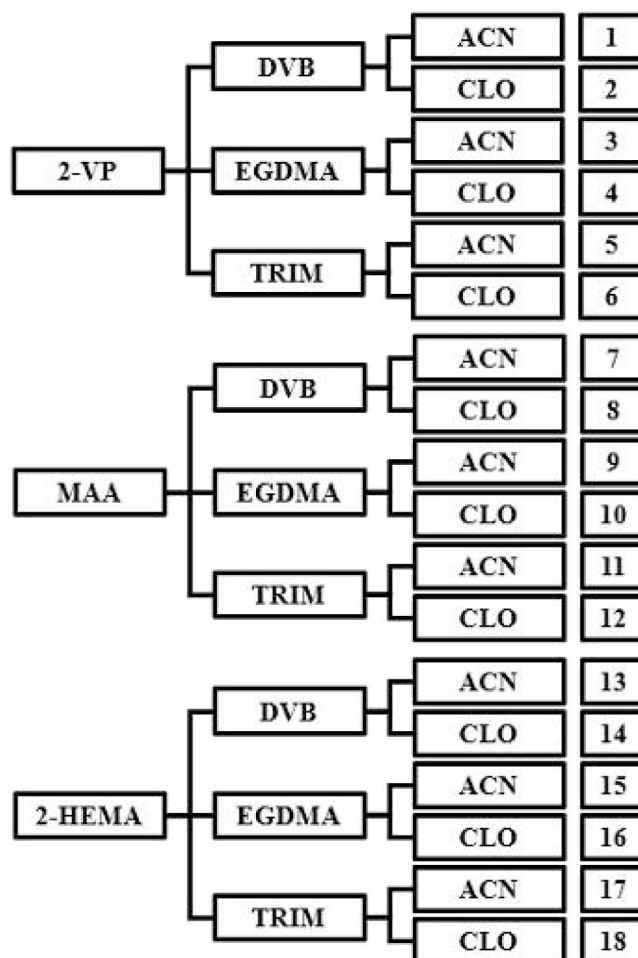


Fig. 2. Eighteen combinations of monomer, crosslinker, and porogen evaluated in the optimization procedure of MIP synthesis.

water bath (Vargem Grande do Sul, Brazil) for the polymerization, under gentle and constant agitation. The temperature was increased from room temperature to 65°C and kept at this temperature for 24 h. Corresponding NIP were synthesized for each condition as proceeded for MIP, in the absence of the template.

After polymerization, the polymers were washed with heated acetonitrile under reflux for 24 h in a Soxhlet apparatus and subsequently with 250 mL of ultrapure water in a vacuum filtration system for removal of the template and residual reagents. The HPLC-UV method developed was used to confirm the complete removal. The leached polymers were dried in a vacuum oven at 65°C for 1 h, the particle size was homogenized in a 24 mesh (0.71 mm) sieve, and the polymers were stored in a desiccator, prior to use.

Additional tests for synthesis optimization were carried out. The influence of increasing the relative proportion of monomer in the template-monomer molar ratio from 1:4 to 1:6 and 1:8, the decrease of porogen volume from 25 mL to 20 mL, and the increase in the amount of the template in the solution from 48.9 mg to 97.8 mg in the adsorption capacity and polymer yield were tested. Ultimately, the pre-polymerization step was optimized, by assessing the benefits of increasing the pre-polymerization time from 2 to 8 h and decreasing the pre-polymerization temperature from room temperature to 4°C .

2.4. Static and kinetic adsorption and binding studies

Static adsorption studies were carried out to investigate the binding properties of the synthesized MIP and NIP following each step of the

optimization procedure. Briefly, 50 mg of the polymers were dispersed in 25 mL sealed conical flasks containing 5 mL of GCZ solution (5 µg/mL) prepared in acetonitrile, 10 mM phosphate buffer pH 3.0 or in a mixture of acetonitrile and 10 mM phosphate buffer pH 3.0 (50:50, v/v). The dispersions were placed in a water bath maintained at room temperature and under gentle shaking for 24 h, and then the supernatants were sampled, filtered through 0.45 µm membranes and analyzed by HPLC-UV for quantification of the unbound GCZ.

Kinetic adsorption studies were performed as proceeded above, but 200 µL aliquots of the supernatants were sampled, with reposi-tion, at 0, 2, 4, 8, 12, 24 and 48 h, prior to chromatographic analysis. The stability of GCZ was also investigated at 0, 2, 4, 8, 12, 24, 48 and 72 h by the same procedure, but in the absence of the polymers.

The adsorption properties of the polymers were also examined in an in-column adapted adsorption study. Firstly, commercial 3 mL polypropylene SPE cartridges were emptied, washed by sonication in methanol and water in a Unique MaxiClean 1400 (Indaiatuba, Brazil) ultrasound bath, and dried in desiccators at room temperature. Then, 50 mg of the polymers were dispersed in methanol, packed into the cartridges in a Phenomenex SPE vacuum manifold (Torrance, USA), using a lower and an upper sieve plate (frit) to keep the sorbent into the cartridge, and stored in desiccators at room temperature prior to use. The cartridges were conditioned with 1 mL of methanol and 1 mL of ultrapure water. Experiments were separately performed to evaluate the effect of three different parameters on the adsorption capacity and selectivity of the polymers in the sample loading step: the proportion of acetonitrile in the diluent (0, 20, 40, 50, 60, 80 and 100%), concentration of GCZ (1, 3, 5, 7 and 9 µg/mL) and pH of the diluent (3.0, 4.0 and 6.0).

Then, the distribution coefficient (K_d) and the imprinting factor (IF) were calculated according to the following equations:

$$K_d = \frac{(C_0 - C_f) \times V}{m} \quad (1)$$

$$IF = \frac{K_{d \text{ MIP}}}{K_{d \text{ NIP}}} \quad (2)$$

where C_0 and C_f are the initial and the equilibrium concentrations of GCZ in the solution (µg/mL), V is the volume of the solution (mL) and m is the mass of the polymers (g).

2.5. Repeatability and selectivity studies

The repeatability of synthesis procedure was evaluated by determining K_d and IF values for 3 independently synthesized batches of the optimized polymer. A solution of GCZ at 2 µg/mL was used for this purpose. The mean values and coefficients of variation (CV%) were calculated. The same procedure was utilized for determining selectivity of MIP and NIP in front of 5 µg/mL aqueous solutions of the sulfonyleureas CPM, GBC and GMP, and commonly concurrent drugs used in senility: MET (antidiabetic), LOS (anti-hypertensive), and ATO (antilipemic).

2.6. Physicochemical, morphological and stability characterization of MIP

Granulometry and particle size distribution analyses were carried out for aqueous suspensions of the optimized polymers by laser diffraction in a Microtrac laser diffractor. The surface morphology, microstructural chemical characterization and elementary mapping of the polymer particles were examined by Field Emission Gun - Scanning Electron Microscopy (FEG-SEM) for the leached and unleached polymers, in a Carl Zeiss Sigma VP (Oberkochen, Germany) microscope coupled to a Bruker GmbH XFlash 410-M (Billerica, USA) detector. Thermal behavior was evaluated by differential scanning calorimetry (DSC), calibrated with Indium (melting: $T_{\text{onset}} = 156.63$ °C, $\Delta H_{\text{fus}} = 28.45$ J g⁻¹), and thermogravimetry (TG) in a Shimadzu

DSC60 cell and in a Shimadzu DTG60 thermobalance (Kyoto, Japan), respectively. Temperature programs ranged from room temperature to 400 °C for DSC analyses and from room temperature to 600 °C for TG analyses, with a heating rate of 10 °C/min, under a dynamic nitrogen atmosphere at a flow rate of 50 mL/min. About 1.5 mg of sample, accurately weighed, were enclosed in partially closed aluminum crucible for DSC analyses; for TG, 2.5 mg of sample, accurately weighed, were transferred to alumina crucible. Fourier transform infrared spectroscopy (FTIR spectroscopy) was performed in a Perkin Elmer Spectrum One operating at attenuated total reflectance (ATR) mode, from 4000 to 650 cm⁻¹, with a resolution of 4 cm, at room temperature. X-ray diffraction (XRD) measures were obtained in parallel geometry in a Shimadzu XRD-7000 diffractometer programed at 40 kV and 30 mA, using CuKα ($\lambda = 1.54056$ Å) radiation, equipped with a polycapillary coupled to a graphite monochromator (at 30 rpm). Experiments were carried out in the angular range from 5 to 35° (2θ), with a step size of 0.01° (2θ) and a time constant of 2 s/step, utilizing Rietveld method for refinement.

2.7. Development and optimization of a molecularly imprinted solid-phase extraction (MISPE) procedure for GCZ extraction from plasma samples

To assure appropriate recovery of GCZ from biological samples, the effect of different variables involved in the MISPE procedure was previously studied in the in-column adapted adsorption study, described in Section 2.4 "Static and kinetic adsorption and binding studies". Moreover, some parameters were optimized: weight of MIP added into cartridges, solvents used for conditioning, sample volume, pH and composition of diluent, solvents used for dilution and clean-up, and volume of solvent for desorption. After optimization, the procedure was as follows. The SPE cartridges were prepared using 50 mg of the optimized polymers (MIP and NIP) and conditioned with 1 mL of methanol and 1 mL of ultrapure water. Then, human plasma blank samples (1 mL) were spiked with GCZ solution to obtain concentration of 5 µg/mL, diluted with 1 mL of 10 mM phosphate buffer pH 3.0 and centrifuged at 480 × g at 4 °C for 10 min, and loaded to the cartridges. The elution of the adsorbed GCZ was carried out with 2 × 500 µL of acetonitrile, diluted with 1 mL of 10 mM phosphate buffer pH 3.0 and centrifuged again at 480 × g and 4 °C for 10 min, prior to chromatographic analysis. The experiments were performed in duplicate.

2.8. Application to real human plasma samples from healthy volunteers

The applicability of the MISPE-HPLC-UV developed method was determined by quantifying the concentration of GCZ in plasma samples from 3 healthy volunteers, obtained from blood samples collected in 6 h following oral administration of 90 mg of GCZ (Diamicon MR, Servier, Rio de Janeiro, Brazil) and chromatographed in duplicate.

3. Results and discussion

3.1. Chromatographic method

The HPLC-UV method was utilized to verify the removal of bound GCZ and residual reagents from the polymers after the washing procedure, to determine the adsorption properties of the polymers and the stability of GCZ in aqueous solutions, and to assess the selectivity and the reproducibility of the polymers. This method was developed by optimizing mobile phase composition, elution mode, temperature and detection wavelength. The best chromatographic conditions for separation of GCZ from other analytes used in this study, especially other sulfonyleureas, were obtained after a comprehensive optimization procedure and the optimized method was described in the Section 2.2 "Chromatographic apparatus and conditions". Even though GCZ possess absorbance maximums at 200 and 230 nm, the latter was set as the detection wavelength because it is slightly more selective. The

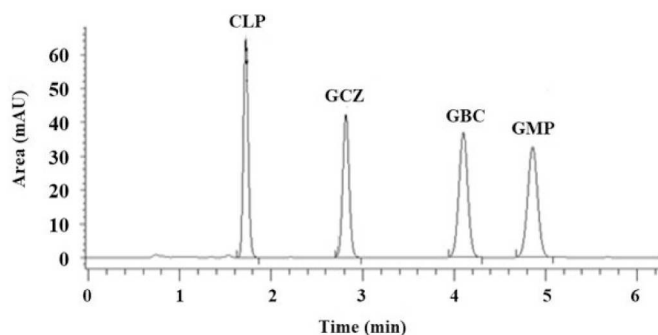


Fig. 3. Chromatogram obtained using the developed liquid chromatographic method for separation of sulfonyleureas chlorpropamide, gliclazide, glibenclamide, and glimepiride at 5 $\mu\text{g/mL}$ (diluted in mobile phase). CLP: chlorpropamide; GCZ: gliclazide; GBC: glibenclamide; GMP: glimepiride.

chromatogram obtained for the simultaneous determination of GCZ and the other three tested sulfonyleureas (CPM, GBC and GMP) selected for the assessment of the selectivity of the polymers is shown in Fig. 3. The retention times of CPM, GCZ, GBC and GMP were 1.72, 2.81, 4.09, and 4.86 min, respectively. As can be seen, an adequate resolution was obtained, since the four chromatographic peaks were sufficiently separated. The analyte was also properly separated from MET, LOS and ATO. The chromatographic peaks also demonstrated appropriated peak shapes and fulfilled system suitability parameters after the definition of the composition of the mobile phase.

Bioanalytical analyses of spiked or real human plasma samples from healthy volunteers were performed after some modifications in the chromatographic method, since interfering peaks were detected in the chromatogram after consecutive analyses. Therefore, an Agilent Security Guard C₁₈ (Torrance, USA) guard column (4.0 \times 3.0 mm i.d.) and a clean-up step by gradient elution, increasing the proportion of acetonitrile in the mobile phase from 50 to 95% for 3 min (4 to 7 min of the chromatographic run), following the 4 initial minutes of isocratic elution, were required. The mobile phase composition was subsequently re-equilibrated for 2 min, leading to a run time of 9 min. The retention time of GCZ did not change, remaining at 2.8 min. These slight changes in the chromatographic conditions have appropriately solved the issue of the separation of the analyte from interfering peaks from the human plasma matrix.

Even though the MISPE approach produced very clean samples, a chromatographic method was required instead of a simpler spectrophotometric approach due to the complexity of the biological matrix (human plasma), which is composed by several potential interferences, such as macromolecules and other endogenous or exogenous substances, including concurrent pharmaceuticals.

3.2. Synthesis of molecularly imprinted polymers (MIP) for gliclazide

The polymers for GCZ were synthesized by a non-covalent approach due to its expanded applicability, simplicity and speed in the processes of binding, rebinding and removal of the template, being more suitable and desirable to be used as SPE sorbent. It is also related to minor occurrence of template leakage, which is usually a major issue in MISPE procedures. The choice of the precipitation polymerization procedure is justified because it leads to spherical and uniform particles and more homogeneous binding cavities, without compromising the simplicity, speed and cost-effectiveness of the procedure.

It is well known that an appropriate choice of the monomer, the crosslinker and the porogen, as well as the polymerization procedure, is mandatory to guarantee a MIP with desirable physicochemical, morphological and binding properties, because polymerization is a complex and sensitive reaction [47]. The selection of a monomer must be a priority, since it is responsible for directly interacts with the template,

forming a stable pre-polymerization complex and is therefore decisive for forming more selective and complementary cavities (binding sites). The crosslinker is crucial to form a rigid and highly crosslinked structure around the template/monomer complex and its properties influence selectivity, binding properties, physicochemical, mechanical stability and rigidity of the polymers, which will determine if the polymer can be successfully applied as a sorbent for solid-phase extraction (SPE). Ultimately, the porogen must be wisely selected, since it substantially influences on the strength and the stability of the template/monomer complex and also on the pore size, surface area, morphology and consequently on its adsorption capacity.

Therefore, 18 distinctive combinations were proposed for the synthesis of a selective and SPE suitable MIP for GCZ. However, an appropriate MIP was obtained only in 5 combinations, in which a precipitate or a solid suspension was observed. Empirically, it is well argued in the literature [34,35,45] that basic monomers, such as 2-vinylpyridine or 4-vinylpyridine, are more recommended for acidic templates, such as sulfonyleureas. The reason is that proton-acceptor functional groups would interact more strongly with proton-donor functional groups in hydrogen-bond formation. Nevertheless, unexpectedly, a MIP for GCZ was only obtained when more acidic monomers (2-HEMA or MAA) were used and no polymerization was observed for 2-VP. This finding, as previously discussed in another study [49] from our research group for lumefantrine (a weak base), firmly suggests that different interactions, such as hydrophobic, dipole-dipole, van der Waals and π - π interactions have prevailed over hydrogen-bonds for GCZ. A possible reason for this result is that the strongest acidic group in GCZ structure is weak and sterically hindered, preventing hydrogen-bonding formation.

The polymerization with 2-HEMA as the monomer has occurred when DVB, EGDMA or TRIM were used as crosslinker, but when MAA was selected as the monomer, polymers were only produced with DVB or EGDMA as the crosslinker. The selection of a porogen also showed to be decisive, since the polymers based on MAA as the monomer were solely synthesized in chloroform, a non-polar and aprotic solvent frequently used in molecularly imprinting. Oppositely, the polymers consisted of 2-HEMA, which is a considerably more polar monomer, required a more polar solvent, such as acetonitrile, to be formed. Both porogens are aprotic, which is preferable for polymerization, but chloroform is usually more recommended since it possess lower dielectric constant and is more favorable for stabilizing monomer-template complex formed during the process, which appears to be critical. Solubility issues or competing interactions between monomer, porogen, and GCZ can possibly explain why certain polymers were formed only in chloroform or acetonitrile, but additional studies must be carried out for more reliable conclusions. The best results in terms of yield and adsorption capacities were achieved for the combination of 2-HEMA as the monomer, EGDMA as the crosslinker and acetonitrile as the porogen.

The template/monomer molar ratio is also known to affect the affinity and imprinting properties of a MIP. The first tested molar ratio (1:4) was too low and fewer binding sites were formed. Furthermore, in the 5 successful combinations, short-chain polymers and fine particles, inappropriate to SPE procedures, with low and unsatisfactory yield, were obtained. Therefore, the molar ratio was increased to 1:6 and to 1:8, which had a progressive beneficial impact on the binding properties of the MIP. The porogen volume was decreased from 25 mL to 20 mL, which favored the precipitation and production of longer-chain polymers, also increasing the yield of the reaction, because more oligomers and nuclei grow into particles under diluted conditions. Posteriorly, to enhance the selectivity, the amount of GCZ was doubled (from 48.9 mg to 97.8 mg) and the pre-polymerization step was modified to 8 h at 4 $^{\circ}\text{C}$, in a refrigerator. The reduced temperature and the longer time of pre-polymerization was positive, since it favored the formation of a more stable complex between template and monomer and consequently more specific binding cavities. The optimal synthesis

Table 1
Optimal synthesis conditions for the molecularly imprinted polymer for gliclazide developed in this study.

Parameter	Optimal condition
Monomer	2-HEMA
Crosslinker	EGDMA
Porogen (volume, mL)	Acetonitrile (20)
Molar ratio	01:08:40
Weight of gliclazide (mg)	97.8
Pre-polymerization step	8 h at 4 °C

condition after an extensive optimization procedure is described in Table 1.

As demonstrated by HPLC-UV analysis, the exhaustive washing procedure by Soxhlet extraction using heated acetonitrile as leaching solvent for 24 h was able to completely remove the bound template from the specific and nonspecific sites from the MIP, since no peak at the retention time of GCZ (2.80 min) was detected in the chromatograms of the washing eluates. However, because of the presence of two chromatographic peaks, at the retention time of 0.75 and 1.44 min, respectively, probably related to more polar residuals or impurities of synthesis, another washing step using ultrapure water in a vacuum filtration system was required and was able to effectively remove the interferences, since no chromatographic peak was further observed. Following washing and vacuum oven drying, heterogeneous particle sizes and aggregates were visible. Thus, a sieving procedure was performed for obtaining more homogenous particles, which allowed an easier packing into SPE cartridges and enhanced reproducibility, making it more suitable for MISPE.

3.3. Static and kinetic adsorption studies

Static and kinetic adsorption studies were carried out for the optimized MIP and NIP in order to investigate their binding properties. The results were however unsatisfactory and unreliable, because some increasingly prominent ghost peaks appeared in the chromatograms. This can be explained because GCZ is very unstable in aqueous and acidic solutions, as already discussed by Doomkaew et al. [50]. Therefore, to confirm it, a stability study for GCZ using the same procedure applied for adsorption studies was replicated in aqueous solution in the absence of the polymers, in a time interval ranging from 0 to 72 h. It was observed that approximately 17% of GCZ degraded in 72 h in aqueous solution. Then, it was not possible to conclude if the unbound GCZ in adsorption studies decreased due to its sorption to the MIP or due to degradation of the analyte. Therefore, in order to avoid exposure of GCZ to unstable conditions for longer periods, binding properties of the polymers were assessed by an adapted in-column adsorption study.

The in-column adapted adsorption study was also used to verify the effect of the proportion of acetonitrile in the diluent, the concentration of GCZ and pH of the diluent in the adsorption capacities of both MIP and NIP. The influence of proportion of organic solvent (acetonitrile) in the diluent and the concentration of GCZ on coefficient of distribution (K_d) and imprinting factor (IF) is presented in Fig. 4. Both MIP and NIP showed higher values of K_d in lower proportions of acetonitrile, up to 40%. Furthermore, it was observed an abrupt decrease in the adsorption capacity when the proportion of acetonitrile is increased to 50% or higher. This can be explained because GCZ is sparingly more soluble in acetonitrile. Thus, higher proportions of acetonitrile in the diluent promote a shift in the equilibrium between bound and unbound GCZ. On the other hand, at higher proportions of aqueous phase (pH of approximately 3.0), GCZ is predominantly found in its non-ionized form, which strongly interacts with the complementary cavities found in the MIP.

Coefficient of distribution of the optimized NIP was slightly more affected, which is indicative that unspecific binding is more remarkably

decreased when higher proportions of acetonitrile is used, since the IF has increased from 1.024 to 1.391 (its highest value), when the percentage of acetonitrile is increased from 0 to 50%. Consecutive increases in the proportion of acetonitrile, higher than 50%, possibly decrease specific binding of GCZ in the MIP and has a prejudicial effect on IF. Therefore, the proportion of acetonitrile was set at 50%, since selectivity, expressed by IF, was prioritized and adsorption capacity of MIP could be posteriorly enhanced.

A progressive and significant increase in the concentration of GCZ from 1 to 9 $\mu\text{g/mL}$ has enhanced the adsorption capacity of both polymers, evidenced by gradually and linearly (r^2 of 0.9884 for the optimized MIP and 0.9733 for the optimized NIP) higher coefficients of distribution, as expected, from 13.44 to 104.62 $\mu\text{g/g}$ for the MIP and from 6.96 to 69.02 $\mu\text{g/g}$ for the NIP. Deviations from linearity can be explained due to the differences on packing of the cartridges or due to particle heterogeneity. No plateau was reached and a maximum value for the adsorption capacity of MIP or NIP could not be achieved. Both specific and unspecific binding have comparably increased at lower concentrations of the analyte. However, the decrease in the IF values at concentrations higher than 5 $\mu\text{g/mL}$ has strongly suggested that the unspecific binding was disproportionately increased, possibly because the specific cavities/binding sites of the MIP have been saturated. The highest IF and selectivity was shown at the concentration of 5 $\mu\text{g/mL}$, which has fortunately been described as the maximum plasma concentration (C_{max}) for GCZ in several pharmacokinetic studies. Therefore, MIP showed to have suitable binding properties for the bioanalysis of GCZ.

The effect of the diluent pH on the selectivity and on the adsorption capacity was assessed at pH 3.0, 4.0, and 6.0. Coefficients of distribution for the MIP and the NIP, as long as the IF, have decreased at higher values of pH. The highest K_d of the optimized MIP was obtained at pH 3.0. At this pH, GCZ is predominantly in its non-ionized form, indicating that the drug-polymer interaction is carried out mainly by hydrophobic interactions or hydrogen bonding. The gradual decrease of the IF at higher pH suggests that a lower pH is also beneficial for specific binding of GCZ in the complementary binding sites. Therefore, to enhance the selectivity and the adsorption capacity of the polymer, the pH of the diluent was set at 3.0.

The presence of selective and complementary cavities or binding sites for GCZ in polymeric network of the MIP was shown, since an IF higher than the unit was obtained for all tested conditions. The results of the optimization procedure for monomer, crosslinker, porogen, pH, and proportion of acetonitrile also suggested that interactions between GCZ and the polymers occurred due to distinctive forces, including hydrophobic, dipole-dipole, van der Waals, and π - π interactions, instead of solely by hydrogen-bond interactions as usual.

3.4. Repeatability and selectivity studies

The repeatability of the synthesis protocol was confirmed since similar K_d and IF values for 3 independently synthesized batches of the polymers were obtained. The mean values were 53.15 $\mu\text{g/g}$ (K_d - MIP), 33.77 $\mu\text{g/g}$ (K_d - NIP), and 1.52 (IF) and the coefficient of variation (CV, %) was lower than 12.0%. However, it is crucial to precisely control the synthesis and MISPE parameters, such as stirring speed, temperature and time of pre-polymerization and polymerization itself, amount of the reagents, nitrogen purging and packing into SPE cartridges.

Table 2 summarizes the IF and the K_d values obtained with the polymers for GCZ, 3 other sulfonylureas (chlorpropamide, glibenclamide, and GMP) and commonly prescribed drugs for diabetic patients, namely MET, LOS, and ATO. The affinity of the MIP for GCZ and the structurally-related sulfonylureas, expressed in terms of K_d values, ranging from 48.81 to 70.80 $\mu\text{g/g}$, remarkably differed from its affinities for the unrelated chemicals, for which the K_d values ranged from 29.10 to 42.65 $\mu\text{g/g}$. These findings showed the selectivity of the

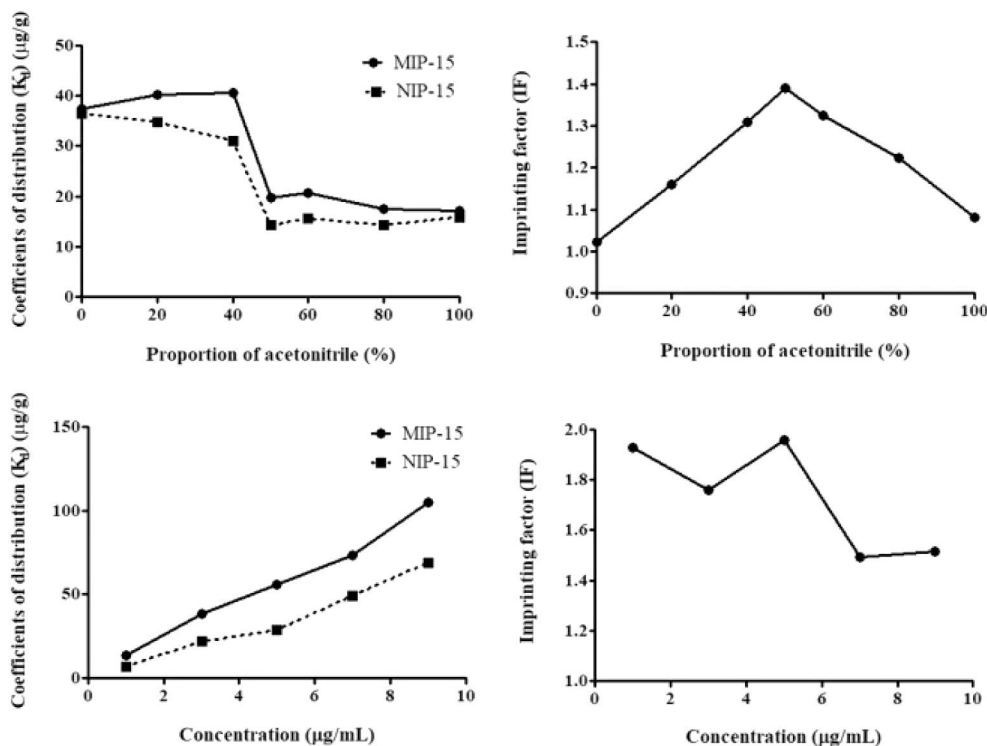


Fig. 4. Influence of proportion of acetonitrile and concentration of gliclazide on coefficient of distribution (K_d) and imprinting factor (IF).

Table 2

Coefficients of distribution (K_d) and imprinting factor (IF) for gliclazide, chlorpropamide, glibenclamide, glimepiride, metformin, losartan, and atorvastatin obtained with optimized MIP and NIP.

Drug	K_d (MIP) ($\mu\text{g/g}$)	K_d (NIP) ($\mu\text{g/g}$)	IF
GCZ	59.85	37.35	1.60
CLP	50.56	39.61	1.28
GBC	48.81	32.68	1.49
GMP	70.80	42.74	1.66
MET	42.65	32.25	1.32
LOS	39.09	28.06	1.39
ATO	29.10	28.17	1.03

ATO: atorvastatin; CLP: chlorpropamide; GBC: glibenclamide; GCZ: gliclazide; GMP: glimepiride; LOS: losartan; MET: metformin.

developed MIP for GCZ, even in the presence of possible interferences, as concurrent drugs. Furthermore, IF for the 4 sulfonylureas tested were considerably higher than 1. Therefore, MIP showed appropriate selectivity and potential applicability for extraction of sulfonylureas in general and not exclusively of GCZ, from pharmaceutical or biological samples. The affinity and adsorption capacity of MIP for sulfonylureas has increased progressively according to increments in hydrophobicity, since GMP, more hydrophobic, was the most retained by the MIP and GBC, more hydrophilic, was the least retained. This is another strong indicative of the importance of hydrophobic forces in the interactions between MIP and sulfonylureas, as discussed previously. On the other hand, GMP, which is a large molecule, was not expected to have strong interaction with the polymer due to steric hindrance. One possible reason for the high K_d observed for this drug is that the group linked in R (Fig. 1) may not be the most important for the interaction with the polymer. Therefore, in this case polarity may have been the parameter that influenced the interaction with the MIP.

The optimized MIP showed an appropriate adsorption capacity, with a K_d of 59.85 $\mu\text{g/g}$ for GCZ, which is approximately 60% higher than the adsorption capacity of the corresponding NIP. It shows that complementary and specific cavities were formed. Considering that the

efficiency of a MIP decreases in aqueous solutions, because water can disturb the equilibrium between the template and the monomer, an IF of 1.6 is appropriate for its purposes.

3.5. Physicochemical, morphological and stability characterization of MIP

Laser diffraction was utilized for determining particle size and particle size distribution of the polymers. The presence of GCZ during polymerization of the MIP contributed for forming specific cavities around GCZ and consequently larger particles and less compacted structures. Therefore, as expected and desirable, particle size of MIP (ranging from 104.7 to 248.9 μm) was considerably higher than particle size of NIP (88.0 μm). Although the particle size obtained for MIP was larger than those generally used in SPE (generally lower than 50 μm), the polymer obtained showed to be suitable for the extraction of GCZ from human plasma samples, as demonstrated in the analysis of real samples.

As shown in the scanning electron microscopy (SEM) micrographs at a magnitude of 1000 \times for the polymers (Fig. 5), both MIP and NIP demonstrated granular aspect, irregular surface morphology and complex structure of internal small pores, dispersed throughout a three-dimensional rigid polymeric network. It was also observed that the MIP structure has larger, more regular and more accessible microporous, which is desirable for a sorbent to be used in MISPE procedures, since it promotes favorable kinetic profile, decreases carryover and enables reusability.

Field Emission Gun (FEG) images (Fig. 6, left) and elementary mappings (Fig. 6, right) are presented for GCZ, NIP, and for the leached and unleached MIP. FEG image for GCZ exhibited a widespread distribution of orange color, because of sulfur (S) exclusively present in its structure. Since monomer 2-HEMA and crosslinker EGDMA are constituted solely by carbon (C), hydrogen (H), and oxygen (O), the absence of orange color in the NIP and in the leached MIP (by Soxhlet extraction with heated acetonitrile and vacuum filtration with ultrapure water), as well as the presence of orange color in unleached MIP, reaffirmed that GCZ (template) was successfully removed from MIP, as

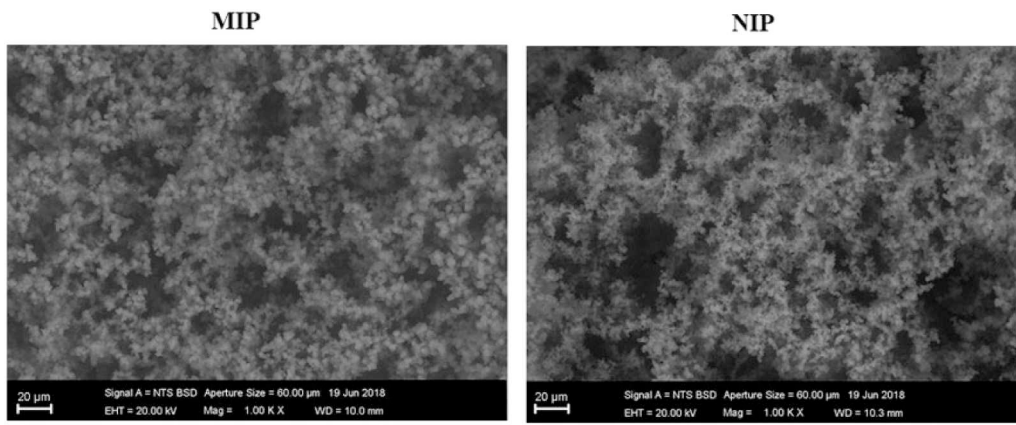


Fig. 5. Scanning electron microscopy (SEM) micrographs at a magnitude of 1000× for the optimized MIP and NIP.

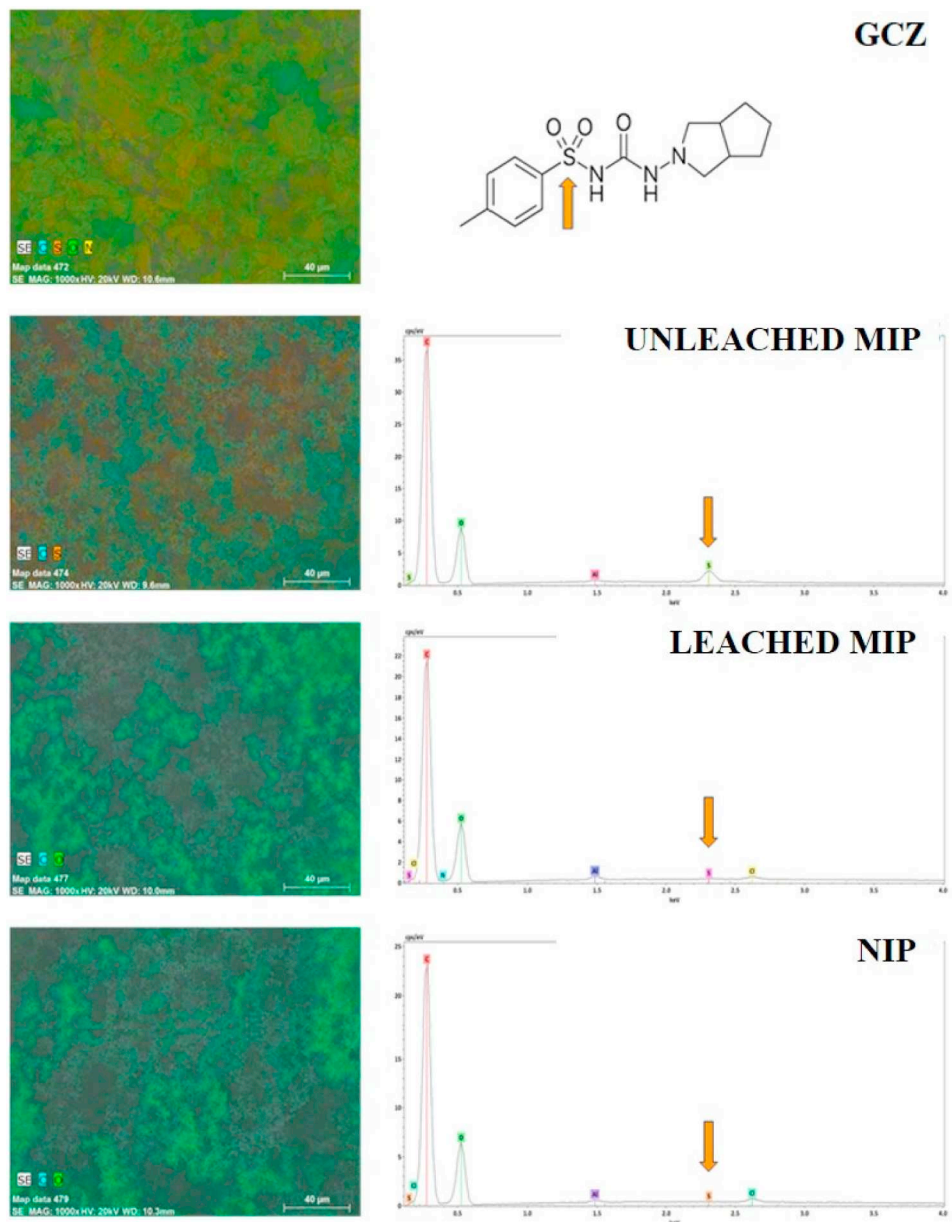


Fig. 6. Field Emission Gun (FEG) images (left) and elementary mapping (right) for gliclazide, unleached MIP, leached MIP, and NIP.

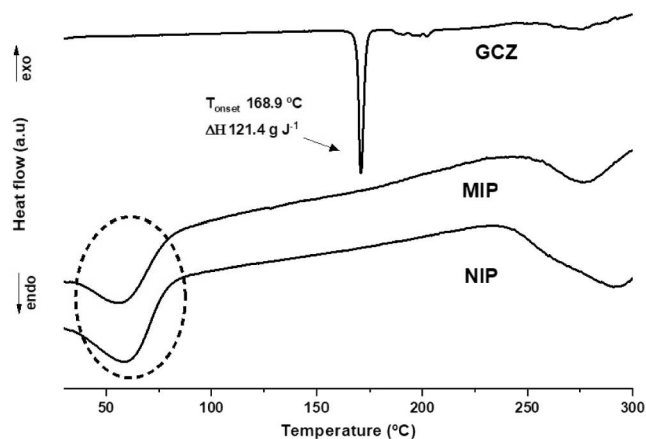


Fig. 7. Differential scanning calorimetry (DSC) curves for gliclazide, MIP, and NIP.

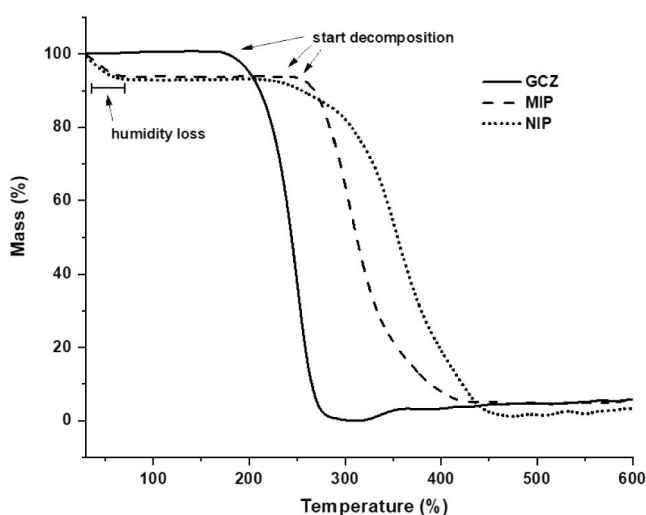


Fig. 8. Thermogravimetry (TG) curves of gliclazide (solid line), MIP (dashed line), and NIP (short dot line).

previously showed by HPLC-UV analysis. Elementary mappings are in consonance with this result, since the peak referring to sulfur is seen only for the un-leached MIP, but not for NIP or leached MIP.

Thermal behavior by DSC and TG is presented in Figs. 7 and 8, respectively. DSC curve for GCZ exhibited a symmetric and sharp endothermic peak, with an onset temperature (T_{onset}) of 168.9 °C and a heat involved of 121.4 g J⁻¹, corresponding to the melting of GCZ. No mass loss was observed, as can be seen in TG curve (Fig. 8). Similar DSC curves, obtained for MIP and NIP, showed a sole, broad, and wide endothermic peak, between 35 and 80 °C (Fig. 7, circled), possibly referred to loss of residual acetonitrile (boiling point at 82 °C). No event related to the glass transition (T_g) was observed in the DSC curves of the polymers.

TG curves for MIP and NIP (Fig. 8) corroborated with DSC curves, since a mass loss of approximately 5% was observed at 80 °C due to the evaporation of residual acetonitrile. Decomposition of GCZ started immediately after melting (~172 °C) in a single ramp, with a total mass loss of 99.3%. MIP and NIP exhibited similar profiles of thermal stability, with decomposition occurring from ~240 °C, in a single ramp and mass loss of ~89% at 600 °C for both. As can be seen, GCZ, MIP and NIP are all thermally stable at the temperature used for polymers synthesis.

FTIR spectra for GCZ, leached and un-leached MIP and NIP are presented in Fig. 9. Both polymers possess comparable spectra, with coincidence of bands at characteristic wavenumbers and similar

relative intensities, which confirmed that MIP and NIP have similar composition, differing only in their structural arrangement. Bands of absorptions at wavenumbers 2947 cm⁻¹ (C–H stretching of aliphatic compound), 1723 cm⁻¹ (C=O stretching of carboxylic acid), 1251 cm⁻¹ (C–O stretching of carboxylic acid), 1140 cm⁻¹ (C–O stretching of ester), and 988 cm⁻¹ (O–H bending of carboxylic acid) were observed. No substantial differences were observed for the un-leached MIP in comparison with the leached MIP, because the template was added in a very low relative proportion compared to monomer and crosslinker and because most of the characteristic bands for GCZ are coincident to the characteristic bands of the polymers, due to the similarity of some functional groups.

Diffraction patterns of GCZ, MIP and NIP are shown in Fig. 10. Following structural refinement by Rietveld method, GCZ X-ray pattern demonstrated high crystallinity and intense reflections at 14.86°, 16.94°, 18.02°, 20.76°, 25.12°, 26.16°, 27.54°, and 28.56°. Comparison of the values experimentally observed in 2θ degrees with predicted values by the Bragg's reflection evidenced that GCZ API used in this study correspond to crystalline form I out of the 3 possible, as described by Rajamma et al. [51]. Graphic presentation of GCZ single crystal from Rietveld refinement is present in Fig. 10 detail. Diffraction patterns obtained for the optimized MIP and NIP exhibited similar profile, without any peaks and no crystallinity, proving that both polymers are amorphous materials, as is usual for polymeric materials.

3.6. Development and optimization of a molecularly imprinted solid-phase extraction (MISPE) procedure for GCZ extraction from human plasma

During the study of the adsorption and binding properties of the developed MIP, some critical parameters for the MISPE procedure were evaluated. For instance, the best results for the spiked and real plasma samples were achieved when the samples were diluted with 10 mM phosphate buffer pH 3.0 (in a proportion of 1:1), following acidification with few microliters of 85% phosphoric acid to pH 3.0, because it was previously shown that GCZ is more strongly and specifically retained in the binding cavities when it is in the non-ionized form, at low pH values. In this condition, the mean recovery of GCZ from the spiked human plasma samples (concentration of 5 µg/mL) was 101.7%. On the other hand, for non-acidified samples, the recovery was significantly decreased to 15.0%. As discussed before, the non-ionized GCZ prevails in acidic conditions and strongly interacts with the complementary cavities of the polymeric network. The desorption step was better carried out with 2 × 0.5 mL of acetonitrile instead of 1 × 1 mL of the same solvent. Acetonitrile was used for desorption because GCZ is very soluble in it and it is more suitable for the subsequent chromatographic analysis, considering the composition of the mobile phase.

The obtained recovery was slightly better when compared to the recovery described by Hrichi et al. [38] for a MIP using polypyrrole as the monomer and electropolymerization for GCZ determination, which ranged from 95.4 to 98.8%. However, this MIP was employed in a voltammetric sensor and although it was shown to be appropriately selective for GCZ against potentially competing sulfonyleureas (GBC, GMP and glipizide), it was not applied to biological samples, but only for pharmaceutical products. Another advantage of the MIP developed in the present study is that the synthesis protocol was simpler and required less instrumentation. Table 3 summarizes the bioanalytical methods for the quantitative determination of GCZ in biological matrices from 2010 up to date. The majority of the 11 published works have used conventional sample preparation techniques, such as PPT or LLE. Although these techniques are very simple and fast, they are also very time and solvent consuming, which is a major problem, due to the high exposure of the analysts and high amount of waste discarded in the environment. Monzon et al. [30] proposed a miniaturized, inexpensive and greener version of a LLE procedure, namely dispersive liquid-liquid microextraction (DLLME), with great sensitivity and outstanding recovery. However, since it is a technique based on differential partition,

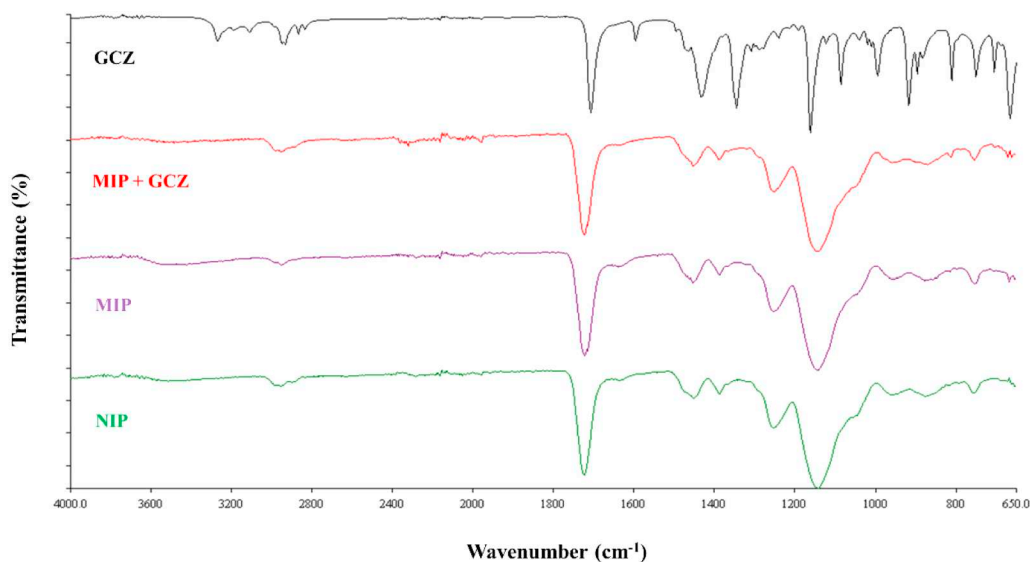


Fig. 9. FTIR spectra from gliclazide, leached and unleached MIP and NIP.

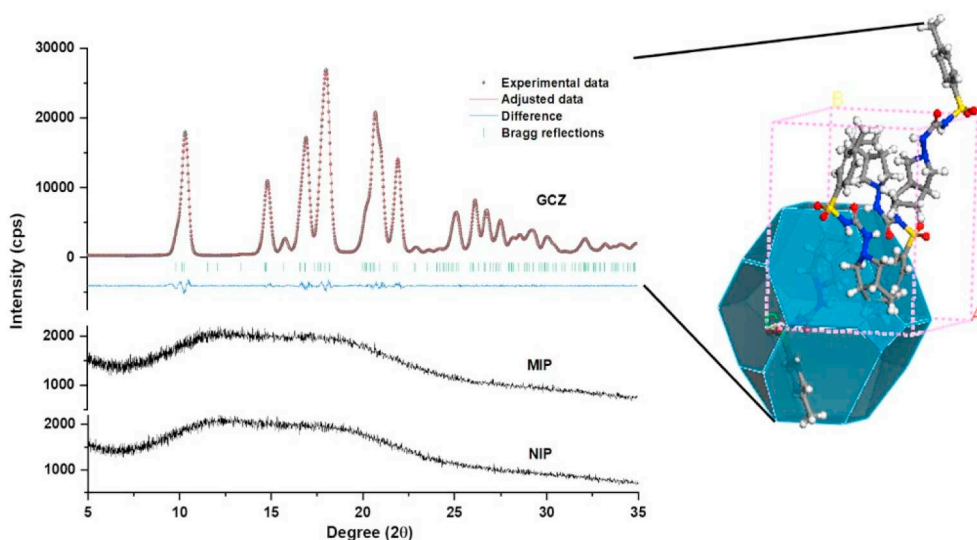


Fig. 10. X-ray pattern of gliclazide, MIP, and NIP. Graphic presentation of GCZ single crystal from Rietveld refinement in detail.

the selectivity is frequently low. Even so, the extraction efficiency of the developed MISPE procedure was comparable to those observed for the LLE and PPT methods. Ali et al. [28] utilized a SPE approach as sample preparation for the extraction of GCZ from human plasma, using a commercially available sorbent (C18). The sensitivity of the procedure was not informed, but low recoveries were obtained – ranging from 10.0 to 22.0%. Aburuz et al. [29] have previously proposed a SPE procedure for the drug in the same matrix, but the copolymer dibutylbenzene-co-vinylpyrrolidinone was selected as sorbent. Even though a higher recovery was obtained (93.2%), it is also inferior to the recovery obtained by the present MISPE procedure. Poor recoveries were also found for miniaturized SPE approaches proposed by Viana et al. and by Souza et al. The MEPS procedure proposed by Viana et al. [29] for sulfonylureas in human plasma, using C18 as sorbent, led to recoveries ranging from 50.2 to 59.4%. Souza et al. [30] poorly extracted GCZ from human plasma (recovery of 3.1%) by MSPE, using a combination of C₁₈ and mesoporous silica-magnetite (Fe₃O₄) nanoparticles.

The optimized MISPE procedure presented in this study has also the advantages of less solvent consumption and higher selectivity when compared to conventional sorbents, even in front of very structurally related molecules. The MIP produced is also simple and inexpensive to

produce and is reusable. These results reaffirm the efficacy and applicability of the developed MIP for bioanalysis of GCZ. In addition to the high recovery presented, the selectivity demonstrated should be highlighted, since it is superior to that demonstrated by the conventional materials previously used for analysis of GCZ.

3.7. Application to real human plasma samples from healthy volunteers

The concentration range of the MISPE-HPLC-UV developed method for determination of GCZ in human plasma was from 1.0 to 10.0 µg/mL and LOQ, calculated as 10 times the signal-to-noise ratio, was 1.0 µg/mL. This method was successfully applied from real samples from 3 healthy volunteers, as demonstrated in the chromatograms presented in Fig. 11. The mean concentrations of GCZ were 4.1, 1.5, and 2.2 µg/mL, respectively, with relative standard deviations (RSD) ranging from 3.7 to 13.6%, inferior to the maximum acceptance criteria determined in guidelines for validation of bioanalytical methods (15.0%).

4. Conclusions

Following a comprehensive and rational optimization procedure, 2-

Table 3
Bioanalytical methods for the determination of glitazide and other sulfonylureas from biological samples, since 2010.

Sample preparation	Solvent/sorbent	Matrix	Analytes	Separation/detection technique	Linear range (ng/mL)	LOQ (ng/mL)	Recovery (%)	Reference
PPT	Acetonitrile	Human plasma	GCZ, MET	SFC-MS/MS	7.5–7500.0	7.5	93.0–96.0	Agrawal et al., 2010 [25]
LLE	Ethyl tert-butyl ether	Human plasma	GCZ, GMP, GBC, GQD, GBM, GSX, GPZ, NAT, REP, ROS, PIO, VIL, SIT, SAX	HPLC-MS/MS	10.0–5000.0	< 1	78.0–105.0	Hess et al., 2011 [20]
PPT	Acetonitrile	Human serum	GCZ, GMP	HPLC-UV	150.0–5000.0	46.0	99.4–100.3	Sultana et al., 2012 [24]
LLE, PPT	Acetonitrile, methanol	Human serum, human hair	GCZ, GPZ, GBC, GDQ, MET	UHPLC-MS/MS	nf	< 1.5/hair, 3.6 pg/mg	56.4	Binz et al., 2012 [52]
MEPS	C ₁₈	Human plasma	GCZ, GMP, CPM	HPLC-UV	1000.0–10,000.0	1000.0	50.2–59.4	Viana et al., 2014 [29]
MSPE	Fe ₃ O ₄ -SBA-C ₁₈	Human plasma	GCZ, GBC, GMP, CPM	HPLC-UV	nf	nf	3.1	Souza et al., 2014 [30]
LLE, PPT	Acetonitrile	Human plasma	GCZ, MON, NIF	HPLC-MS/MS	10.0–5000.0	0.04	nf	Ezzeldin et al., 2014 [53]
PPT	Acetonitrile	Rat plasma	GCZ	HPLC-UV	120.0–2800.0	120.0	99.7–100.0	Adhikari et al., 2014 [23]
Ultrafast AE, UF	HSA	Human serum	GCZ, QUI, DZP, TOL, AHM	Two-dimensional AC	nf	40–190 nM	2.0–2.8	Zheng et al., 2016 [54]
DLLME	Acetonitrile, dichloromethane	Human serum	GCZ, GBC, GMP	HPLC-UV	120.0–2800.0	120.0	99.0	Monzon et al., 2016 [28]
SPE	C ₁₈	Human plasma	GCZ, MET, VIL, LIN, SIT, PIO, GMP, REP	UHPLC-UV	nf	nf	10.0–22.0	Ali et al., 2017 [26]

AC: affinity chromatography; AE: affinity extraction; AHM: acetohexamide; C₁₈: octadecylsilane; CPM: chlorpropamide; DLLME: dispersive liquid-liquid microextraction; DZP: diazepam; Fe₃O₄: magnetite; GBC: glibenclamide; GBM: glibornuride; GCZ: glitazide; GMP: glimepiride; GPZ: glipizide; GQD: gliquidone; GSX: glioxepide; HPLC: high-performance liquid chromatography; HSA: human serum albumin; LIN: linagliptin; LOQ: limit of quantitation; LLE: liquid-liquid extraction; MEPS: microextraction by packed sorbent; MET: metformin; MON: montelukast; MS/MS: tandem mass spectrometry; MSPE: magnetic solid-phase extraction; NAT: nateglinide; nf: not found; ng: nanogram; NIF: nifedipine; nm: nanomolar; PIO: pioglitazone; PPT: protein precipitation; QUI: quinidine; REP: repaglinide; ROS: rosiglitazone; SAX: saxagliptin. SBA: mesoporous silica; SFC: supercritical fluid chromatography; SIT: sitagliptin; SPE: solid-phase extraction; UF: ultrafiltration; UV: ultraviolet detection; VIL: vildagliptin.

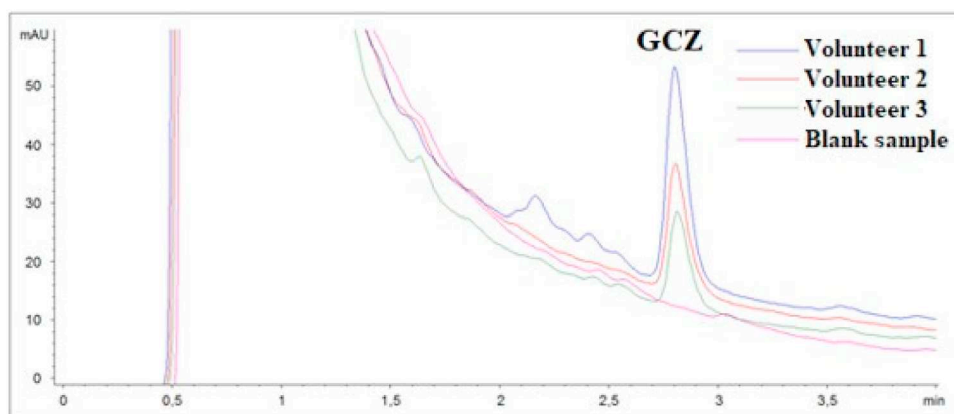


Fig. 11. Chromatogram for real plasma samples from three healthy volunteers collected in 6 h following oral administration of 90 mg of glimepiride using the MISPE-HPLC-UV developed method.

hydroxymethacrylate, ethylene glycol dimethacrylate and acetonitrile were selected as the monomer, the crosslinker and the porogen, respectively, for the synthesis of a molecularly imprinted polymer for the extraction of GCZ from human plasma samples. The proposed protocol of synthesis was reproducible, since the coefficients of variation (for the coefficients of distribution) obtained for 3 different synthesized batches were lower than 15%. The synthesized MIP was characterized by six distinctive techniques, showing morphological, physicochemical and stability characteristics suitable to be used as sorbent in SPE. An adequate molecularly imprinted solid-phase extraction coupled to high-performance liquid chromatography with ultraviolet detection procedure was developed for analysis of GCZ. The polymer showed to be selective for GCZ and other sulfonylureas (CPM, GBC, and GMP) in comparison to possible concurrent drugs. The imprinted polymer showed high affinity for GCZ, with a coefficient of distribution of 59.85 $\mu\text{g/g}$ and an imprinting factor of 1.60. The MISPE approach was applied for real plasma samples, following oral administration of 90 mg of GCZ by healthy volunteers, showing to be a selective, sensitive, reusable, and less expensive alternative for sample preparation for bioanalysis of GCZ.

CRediT authorship contribution statement

Ingrid Vasconcelos: Conceptualization, Formal analysis, Investigation, Methodology, Validation. **Pedro Henrique Reis da Silva:** Investigation, Writing - original draft, Writing - review & editing. **Derick Rodrigues Davila Dias:** Investigation, Methodology, Validation. **Maria Betânia de Freitas Marques:** Investigation, Methodology, Validation, Writing - original draft. **Wagner da Nova Mussel:** Investigation, Methodology. **Tércio Assunção Pedrosa:** Investigation, Methodology. **Maria Elisa Scarpelli Ribeiro e Silva:** Conceptualization, Funding acquisition, Resources. **Roberto Fernando de Souza Freitas:** Conceptualization, Funding acquisition, Resources. **Ricardo Geraldo de Sousa:** Conceptualization, Funding acquisition, Resources. **Christian Fernandes:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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