Application of Magnetic Core–Shell Imprinted Nanoconjugates for the Analysis of Hordenine in Human Plasma—Preliminary Data on Pharmacokinetic Study after Oral Administration

Monika Sobiech,* Joanna Giebultowicz, and Piotr Luliński

ABSTRACT: In this paper, we developed and validated a new analytical method to determine the pharmacokinetic profile of hordenine in plasma samples of human volunteers after oral administration of hordenine-rich dietary supplements. For this purpose, a magnetic molecularly imprinted sorbent was fabricated and characterized. The application of a magnetic susceptible material facilitates pretreatment step while working with a highly complex sample, reducing time and costs. An optimized, fast, and reliable separation step was combined with liquid chromatography tandem mass spectrometry, providing an analytical method for analysis of hordenine in human plasma after dietary supplement intake. The method was validated (lower limit of quantification of 0.05 μg/L), enabling the pharmacokinetic profile of hordenine to be determined. The highest concentration of hordenine was noted after 65 ± 14 min, reaching the value of 16.4 ± 7.8 μg/L. The average t1/2 was 54 ± 19 min. The apparent volume of distribution was 6000 ± 2600 L (66 ± 24 L/kg when adjusted for weight).

KEYWORDS: dopamine receptor, hordenine, dietary supplement, pharmacokinetics, magnetic molecularly imprinted polymers

1. INTRODUCTION

The safety and bioactivity efficacy of dietary supplements require extensive investigation since their components may cause unexpected adverse effects or severe intoxication.1,2 Psychoactive compounds are a group of dietary supplement components that could cause complex health risks if an overdose is taken or if administered with other drugs.3 Therefore, pharmacokinetics data are needed to select the efficient dose range and dosing intervals.

The compound that is a common ingredient in numerous dietary supplements is hordenine, which is believed to promote stimulation of the central nervous system and weight loss.3,4 Hordenine (4-(2-dimethylaminoethyl)phenol) is a low-molecular-weight compound, belonging to a group of phenethylamines, a diverse class of bioactive natural and synthetic compounds that includes stimulants, hormones, hallucinogens, neurotransmitters, antidepressants, anorectics, and bronchodilators. The pharmacological action of hordenine is related to its protective role against hyperglycemia (animal study) or with melanogenesis inhibition (in vitro study).5,6 The latter effect attracted attention due to the possible decrease in the overproduction of melanin, a skin pigment that causes a number of skin problems, such as freckles, age spots, and melasma.6 However, the sympathomimetic impact of hordenine could be hazardous for human beings. The positive inotropic effect on the heart, increasing systolic and diastolic blood pressure, and increasing the volume of peripheral blood flow together with increasing respiratory frequencies are attributed to its structural similarity to neurotransmitters. Moreover, the D2R subtype receptor agonist role of hordenine was recently analyzed, revealing its involvement in food reward and addictive behaviors. Thus, hordenine could potentially activate these receptors, affecting rewarding properties of selected food and beverages7,8 or dietary supplements. Therefore, it is necessary to study the impact of hordenine from diet and dietary supplements on dopamine receptor-mediated processes in vivo. Moreover, it should be emphasized that hordenine was placed on the United States Food and Drug Agency Dietary Supplement Ingredient Advisory List.9 Nevertheless, little is known about the efficiency or safety of hordenine and there is little mechanistic evidence of the bioactivity of hordenine and limited pharmacokinetics data.10 Quite recently, hordenine has been analyzed in beverages, such as beer, since this compound is formed in the roots of Hordeum vulgare L. during germination of barley grains.11,12 Moreover, the analysis of hordenine in human plasma and urine samples after beer consumption has been reported.13,14 However, to investigate the psychopharmacological properties of hordenine to understand its possible influence on the reward effects, the need for further in vivo studies was highlighted.8 Recently, Sommer and co-workers10 provided pilot study data related to the biokinetics of hordenine and its metabolites in plasma from four volunteers after beer consumption. To achieve measurable concentrations of the analyte in blood, 1 L of beer with a high hordenine content (6.30 ± 0.19 mg/L) was consumed and the maximum plasma concentrations were...
found to be equal to 1.98–2.85 μg/L. These results seem to be too low to produce an effect by direct interaction with the dopamine D2 receptor (EC_{50} = 610 μg/L).\textsuperscript{8} Thus, the studies presented by Sommer and co-workers inspired us to explore the problem with respect to hordenine-rich dietary supplements could potentially result in sufficient levels of hordenine in the blood to activate the receptors. So far, no data on pharmacokinetics of hordenine after dietary supplement administration exists. Thus, it could be an important scientific goal to assess the probability of hordenine interactions with the dopamine D2 receptor and its pharmacological effects after hordenine-rich dietary supplements.

To realize the above-mentioned goal, the reliable analytical method should be applied. The analytical methods devoted to hordenine determination in plasma/serum with lower limit of quantitation (LLOQ) below 1 μg/L include liquid–liquid extraction with diethyl ether and dichloromethane with LLOQ of 0.2 μg/L.\textsuperscript{11,13} or the protein precipitation method with LLOQ of 0.07 μg/L.\textsuperscript{10} The liquid–liquid extraction process requires the use of harmful solvents, whereas the protein precipitation procedure is characterized by unsatisfactory sample clean-up, resulting in column fouling and faster clogging of capillaries and the ion source. The attractive alternative is solid-phase extraction (SPE), but low selectivity of sorbents caused unsatisfactory limits of quantification (LLOQ = 10 μg/L).\textsuperscript{15} To overcome existing problems, new analytical strategies that allow lower limits of quantification, improve accuracy, enhance selectivity, and minimize matrix effects are urgently needed. Here, molecularly imprinted polymers (MIPs) could be recognized as valuable selective sorbents because of their synthetic process, which proceeds in the presence of the template molecule.\textsuperscript{16–21} Recently, Sobiech and co-workers\textsuperscript{14} designed a MIP sorbent for the extraction of hordenine from human urine samples after beer consumption. However, to elaborate the analytical method available for a more complex matrix as well as to facilitate the process and make it faster, new strategies have been considered. One excellent tool to meet the above-mentioned demands is to merge MIPs with magnetic susceptible materials to obtain advanced core–shell imprinted nanoconjugates.\textsuperscript{22} These materials could easily be employed in the dispersive mode of solid-phase extraction (d-SPE), resulting in the reduction of time and cost of the sample preparation process.\textsuperscript{23}

The aim of this paper was to develop and validate a new analytical method to determine the pharmacokinetic profile of hordenine in plasma samples of human volunteers after oral administration of hordenine-rich dietary supplements. For this purpose, a magnetic molecularly imprinted sorbent was fabricated and characterized, facilitating a pretreatment step while working with a highly complex sample. The magnetic nonimprinted sorbent (NIP) was also fabricated as a control material. It might be expected that in the future, our analytical method will help to provide insight into the probability of hordenine interactions with the dopamine D2 receptor.

2. MATERIALS AND METHODS

2.1. Chemicals. N,N-Dimethylphenethylamine (template), methacrylic acid (functional monomer), ethylene glycol dimethacrylate (cross-linker), tetraethoxysilane, 3-(trimethoxysilyl)propyl methacrylate (MPS), and 2,2′-azobis(2-methylpropionitrile) (initiator) were purchased from Sigma-Aldrich (Steinheim, Germany, http://www.sigmaaldrich.com). Internal standard (IS), hordenine-d_{4} was purchased from Toronto Research Chemicals (Toronto, Canada, https://www.trc-canada.com). Trisodium citrate dehydrate, sodium hydroxide, sodium nitrate, ferrous sulfate heptahydrate, ammonium acetate, ammonium hydroxide, methanol, ethanol, tolune, acetoni-trile, formic acid, and acetone were delivered from POCh (Głogów, Poland, http://www.pocho.com.pl). Ultrapure water was delivered from a Hydrolab HLP 5 system (Straszyn, Poland, http://www.hydrolab.pl). Dietary supplement (Hordenine 98%, 100 mg, 60 capsules) was purchased from Haya Labs (Washington DC, United States, https://www.hayalabs.com).

2.2. Sorbents. The magnetic core–shell polymerization process was used to prepare a magnetite–silane–methacrylate hybrid molecularly imprinted polymer conjugate, Fe_{3}O_{4}@SiO_{3}-MPS@MIP (coded as mag-MIP) as well as a magnetite–silane–methacrylate hybrid non-imprinted polymer conjugate, Fe_{3}O_{4}@SiO_{3}-MPS@NIP (coded as mag-NIP), but during the synthesis of mag-NIP (nonimprinted polymer), the addition of the template was omitted. For the synthesis of mag-MIP, the structural analogue of the target analyte was used as the template.\textsuperscript{14} To 10 mL of toluene, 29.9 mg (0.2 mmol) of N,N-dimethylphenethylamine (a structural analogue of hordenine) and 68.9 mg (0.8 mmol) of methacrylic acid were added and incubated for 24 h. This mixture was then transferred to a round-bottom flask together with 15 mL of toluene, 754 μL (4 mmol) of ethylene glycol dimethacrylate, 20 mg of 2,2′-azobis(2-methylpropionitrile), and 215.4 mg of the magnetite–silane–methacrylate hybrid (Fe_{3}O_{4}@SiO_{3}-MPS). The mixture was sonicated for 5 min, purged with nitrogen for 5 min, and heated to 100 °C on a magnetic stirrer overnight. Next, the polymer was washed (using external magnet) in the following sequence: toluene (2 × 20 mL), methanol (2 × 20 mL), 40 mM aqueous ammonium acetate–methanol 30:70 v/v (2 × 20 mL), and methanol (2 × 20 mL). For mag-MIP, the template removal step was carried out in the Soxhlet apparatus, lasting 36 h (120 mL of methanol) and was monitored by liquid chromatography tandem mass spectrometry (LC–MS/MS).

2.3. Instruments. Quantitative performance was performed using an Agilent 1260 Infinity system (Agilent Technologies, Santa Clara, CA, United States, https://www.agilent.com), equipped with a degasser, an autosampler, and a binary pump coupled to a QTRAP 4000 hybrid triple quadrupole/linear ion trap mass spectrometer (AB Sciex, Framingham, MA, United States, https://sciex.com). The turbo ion spray source was operated in positive mode. The curtain gas, ion source gas 1, ion source gas 2, and collision gas (all high-purity nitrogen) were set at 345, 207, 276 kPa, and “high” instrument units (4.6 × 10^{-5} Torr), respectively. The ion spray voltage and source temperature were 5000 V and 600 °C, respectively. The target compounds were analyzed in multiple reaction monitoring mode. The quantitative multiple reaction monitoring transitions, declustering potential (DP) and collision energy (CE) for hordenine and hordenine-d_{4} were (m/z) 166 > 121 (DP = 61 V, CE = 21 V) and 172 > 121 (DP = 66 V, CE = 19 V), respectively. Chromatographic separation was achieved with a Kinetex EVO C18 column (100 mm × 4.6 mm, 2.6 μm) from Phenomenex (Torrance, CA, United States, https://phenomenex.com). The column was maintained at 40 °C at the flow rate of 0.5 mL/min. The mobile phases consisted of 20 mM aqueous ammonium acetate as eluent A and acetoni-trile with 0.2% formic acid as eluent B. The gradient (% B) was as follows: 0 min 10%, 1 min 10%, 3 min 95%, and 5 min 95%. The reequilibration of the column to the initial conditions lasted 1.5 min.

Quantitative analysis was performed using ultra-high-performance liquid chromatography Dionex Ultimate 3000 with Q-Exactive (Thermo Fisher Scientific, Waltham, MA, United States, https://www.thermofisher.com) hybrid quadrupole-orbitrap mass spectrometer system equipped with heat electrospray ionization. The heat
electrospray ionization was operated in positive mode. Full mass spectrum scans were acquired over m/z in the range of 100–1400 with a resolution of 70,000 (m/z 200). Fragmentation was made at 20, 35, and 50 nominal collision energy units. The mobile phases consisted of high-performance liquid chromatography-grade water with 0.1% formic acid as eluent A and acetonitrile with 0.1% formic acid as eluent B. The gradient (% B) was as follows: 0 min 10%, 1 min 10%, 10 min 90%, and 15 min 90%. The column was maintained at 40 °C at a flow rate of 0.3 mL/min.

The surface morphology analysis using field emission scanning electron microscopy with a Merlin-FE-SEM (Zeiss, Germany) was performed at the Faculty of Chemistry, University of Warsaw, Poland. The samples were Au/Pd sputter-coated before scanning electron microscopy analysis. Transmission electron microscopy was performed with a 120 kV Libra Plus instrument (Zeiss, Germany) at the Faculty of Chemistry, University of Warsaw, Poland. The porosity data were determined using the adsorption isotherm of N2 at 77 K on an ASAP 2420 system (Micromeritics, Inc., United States) at the Faculty of Chemistry, Maria Curie-Skłodowska University, Lublin, Poland. Magnetic measurements were carried out at 300 K in magnetic fields up to 1 T using a Physical Properties Measurement System (PPMS, Quantum Design) equipped with vibrating sample magnetometer at the Institute of Molecular Physics Polish Academy of Sciences, Poznań, Poland. The X-ray diffraction data were collected using a D8 Discover X-ray diffractometer (Bruker, Germany) equipped with a linear position-sensitive VANTEC detector at the Faculty of Chemistry, University of Warsaw, Poland.

2.4. Determination of Adsorption Isotherms and Optimization of d-SPE. For isotherm analysis, polypropylene tubes were filled with 5 mg of mag-MIP or mag-NIP particles and a volume of 1 mL of different methanol–water (85:15 v/v) standard solutions of hordenine (concentrations between 0.5 and 10 μg/L) was added. The tubes were sealed and oscillated by a shaker at room temperature for 45 min. Then, the tubes were centrifuged and the aliquots of supernatant were used to analyze the unbound amount of each compound by LC–MS/MS. For kinetics, the tubes were prepared as above, but different oscillation times were employed (10, 20, 30, 60, 90, 120, and 180 min). Then, the tubes were treated in the same manner as described above. All measurements were carried out in triplicate. The binding capacities (B, μmol/g) of mag-MIP or mag-NIP were calculated according to eq 1

\[ B = \frac{(C_i - C_f)V}{M} \]

where \( C_i \) represents the initial solution concentration (μg/L), \( C_f \) represents the solution concentration after adsorption (μg/L), and \( M \) is the mass of the particles (g).

The adsorption isotherms were characterized using the Freundlich model presented in eq 2

\[ B = aF^n \]

where \( a \) is the measure of the capacity (B_{max}), \( m \) is a heterogeneity index, and \( F \) is the concentration of the analyte in equilibrium state. The kinetics of adsorption of hordenine was calculated according to eq 3

\[ \frac{t}{q_i} = \frac{1}{k_{i}q_{i}^{n-1}} + \frac{1}{q_{i}}t \]

where \( k_i \) is the second-order rate constant at equilibrium, \( q_i \) is the adsorption capacity at equilibrium, and \( q_{i} \) is the adsorption capacity at \( t \) time (in minutes).

For the optimization of the d-SPE process, a mass of 5 mg of mag-MIP was transferred to an Eppendorf tube together with a volume of 300 μL of untreated plasma sample (or in the optimization process: sample diluted with water or sample pretreated with methanol (1:1, v/v)). A volume of plasma pretreated with methanol was centrifuged (10,000g, 5 min) prior to loading to mag-MIP together with 30 μL of IS (c = 50 μg/L). Next, the tube was put on the vortex to provide contact time with the sorbent for 5 min (in the optimization of loading time, the step lasted 5, 15, 30, or 60 min). Then, the supernatant, separated from the sorbent by an external magnetic field, was discarded and the washing step was carried out by applying water, 300 μL of ultrapure water for 0.5 min on the vortex (or in the optimization process, methanol–water 85:15 v/v). The supernatant was removed in the same manner as described above. Finally, the elution took place by adding 300 μL of 5% ammonium hydroxide in methanol. The elution time was set for 5 min (in the optimization process, the step lasted 5, 15, 30, or 60 min). The elution fraction was separated from the sorbent by the application of an external magnetic field. In each optimized step, the recoveries of hordenine were analyzed. The eluate was evaporated to dryness (40 °C, 5 min), reconstituted in a volume of 70 μL of 20% aqueous methanol, and an aliquot of 10 μL was injected into the LC–MS/MS. Each experiment was performed in triplicate. The recovery was calculated by comparing the mean area response of plasma sample spiked before and after extraction.

2.5. Dietary Supplement Analyses. For qualitative analysis of dietary supplements, the contents of three randomly selected capsules were mixed and the equivalent of 10 mg of hordenine was weighed using an analytical balance. Subsequently, the samples were extracted with 1 mL of an acetone–methanol–water (1:1:1 v/v/v) mixture using an ultrasound bath. After centrifugation, the supernatant was diluted to 1 mg/L with water and an aliquot of 10 μL was injected into the LC–MS/MS. The quantitative analysis protocol was performed in a similar way, but the analysis was carried out separately for each of the three capsules, and the final extract was 10 times more diluted to fit the linearity of the calibration line.

2.6. Method Validation. The method was validated according to the EMA guideline.25 The validation covered the entire procedure, i.e., sample preparation and instrumental analysis. The following parameters were analyzed: selectivity, LLOQ, calibration curve performance, precision, accuracy, matrix effect, carryover, and stability of the analyte in the biological matrix and the extract.

All calibration standards and the quality control (QC) samples were prepared by spiking of blank human plasma with hordenine working solution and were stored at −39 °C. Selectivity was evaluated using six blank plasma samples, which were evaluated for interference from the matrix components in the determination of hordenine. The accuracy and precision of the method were determined within runs (n = 5) and between runs (n = 15, five replicate samples on three separate runs) using quality control (QC) samples (0.1, 2.5, and 40 μg/L). Accuracy was expressed as percent relative error of the spiked nominal value, whereas precision was expressed as relative standard deviation (RSD). The linearity range was set between 0.05 and 50 μg/L. Eight point calibration lines (n = 3) were constructed by plotting peak area ratios of each targeted analyte to an area of IS versus the nominal concentration of the analyte. LLOQ was selected to give the signal at least five times higher than the signal of blank samples and satisfactory results of accuracy and precision. LLOQ, QC, and calibration standards were prepared on blank plasma. The matrix effect was evaluated by analyzing low and high QC samples using plasma from six different lots and included hemolyzed and hyperlipidemic plasma samples. Matrix factor (MF) was determined by calculating the ratio of the peak area in the presence of matrix (blank plasma spiked with the analyte post extraction) to the peak area in the absence of matrix (pure solution of the analyte). Additionally, the coefficient of variation (CV) of the IS-normalized MF (expressed as the MF of analyte divided by the MF of IS) was presented. All stability studies were performed at low and high QC samples. The autosampler stability was determined after 48 h of extract storage in an autosampler (4 ± 0.5 °C). The freeze and thaw stabilities were determined in a process of three freeze–thaw cycles at −20 ± 2 °C storage temperature and 25 ± 0.5 °C thawing temperature, at least 12 h after freezing. The short-term stability was determined after 4 h of storage at room temperature, 21 ± 2 °C. Carryover was studied by injecting blank plasma sample after calibration standard at 40 μg/L.

2.7. Pharmacokinetic Study. Three healthy volunteers (men, aged 25–30 years) were enrolled in this study after obtaining written
informed consent. All participants were nonsmokers with body weight no less than 50 kg and body mass index 25.7 ± 3.7 kg/m². Their body weights were 80 kg (two volunteers) and 105 kg (one volunteer). Subjects neither consumed alcohol nor received any medication for 2 weeks prior to the start of the study. Hordenine was administered orally in the morning with a dose of 100 mg (in starch capsules) after overnight fasting. Plasma samples of approximately 2 mL were collected (in vials containing di-potassium ethylene diaminetetraacetic acid as an anticoagulant) before and at 20, 30, 45, 60, 75, 90, 120, 180, and 240 min post-dose. After collection, plasma samples were centrifuged at 2000×g for 15 min at room temperature and plasma samples were analyzed. The pharmacokinetic parameters were calculated using a noncompartmental analysis tool of PKSolver, a freely available menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications.26 The area under the plasma concentration−time curve from 0 h to infinity (AUC₀−∞) was calculated using the linear trapezoidal rule. The maximum time (T_max) refers to the time when plasma concentrations reached a maximum value (C_max). The apparent terminal elimination rate constant, λz, was obtained by linear regression of the log-linear terminal phase of the concentration−time profile using at least three nonzero declining concentrations in the terminal phase with a correlation coefficient of >0.8. The terminal half-life value (t₁/₂) was calculated using the equation (ln 2) × λ/z. The apparent plasma clearance (Cl/F) of

Figure 1. Scanning electron micrographs of magnetite, Fe₃O₄ nanoparticles (a), magnetite–silane functionalized material (b), magnetite–silane–methacrylate hybrid material (c), mag-MIP (d), and mag-NIP (e).
hordenine was calculated using the formula D/AUC_{0→τ}, where D represents the administered dose. The apparent distribution volume (Vd/F) was calculated by multiplying CI/F by mean residence time (MRT).

3. RESULTS AND DISCUSSION

3.1. Characterization of Magnetic Sorbent. To provide information related to the adsorption properties of magnetite–silane–methacrylate hybrid molecularly imprinted polymer conjugate (mag-MIP) and magnetite–silane–methacrylate hybrid nonimprinted polymer conjugate (mag-NIP), the analysis of binding capacities calculated according to eq 1 was carried out for a range of low concentrations of hordenine. The adsorption properties were evaluated using the Freundlich model, eq 2. The straight lines of log B versus log F with the regression coefficient of r² = 0.999 and 0.996 for mag-MIP and mag-NIP, respectively, were evidence that adsorption could be described by the Freundlich equation (Figure S2a). The estimated values of the heterogeneity indices, m, for mag-MIP and mag-NIP were 0.94 and 0.99, respectively. The results indicated that mag-MIP had a more heterogeneous population of adsorption sites with respect to mag-NIP (the heterogeneity increased as the value of m decreased).

Next, the adsorption kinetics of hordenine on mag-MIP and mag-NIP were evaluated since the time of adsorption is an important parameter during the optimization of d-SPE. Kinetics data were analyzed using a pseudo-second-order model, eq 3. The adsorption of hordenine on both materials gave a linear function t/q against t with the correlation coefficient r² = 0.996 and 0.999 for mag-MIP and mag-NIP, respectively. The calculated values k₂ and qₑ were as follows, for mag-MIP: k₂ = 11.17 mg/g/min, qₑ = 0.137 mg/g, and for mag-NIP: k₂ = 4.65 mg/g/min, qₑ = 0.170 mg/g (Figure S2b). These results confirmed differences in the adsorption of hordenine on tested materials.

The evaluation of morphological changes derived from conjugation of subsequent layers of core–shell materials is very important to prove the progress of the process. Thus, the surface morphologies of mag-MIP and mag-NIP at different synthesis steps were analyzed using field emission scanning electron microscopy. The micrographs are presented in Figure 1.

As can be seen, the nanoparticles of magnetite, Fe₃O₄, were characterized by a fairly uniform spherical shape but were agglomerated probably due to their magnetic nature (Figure 1a). In contrast, the submicroparticles obtained after conjugation of the silane layer were characterized by a less uniform structure (Figure 1b). The conjugation of magnetite with the silane layer functionalized by 3-(trimethoxysilyl)-propyl methacrylate resulted in a negligible difference in the morphology of particles (Figure 1c), but the average diameter of particles increased (Table 1). Finally, the external imprinted shell was conjugated and mag-MIP was obtained with the surface characterized by numerous entities uniformly coated by the organic layer (Figure 1d). Similar morphology was observed for mag-NIP (Figure 1e). However, the average diameter of each entity differed significantly (Table 1). This means that the imprinted layer of mag-MIP was thinner. It might be supposed that the presence of the template molecule affects the thickness of the MIP layer. The impact of the template on the thickness of the MIP layer was observed by Sekine and co-workers.²⁷

To reveal the thickness of functionalized silane layers and the imprinted shell, transmission electron microscopy was employed. The diameter of magnetite, Fe₃O₄ ranged between 31 and 53 nm. The thickness of the silane layer conjugated to magnetite particles was between 5 and 8 nm (Figure 2a), and the thickness of the functionalized layer of mag-MIP was between 18 and 31 nm (Figure 2b). Next, the nitrogen adsorption isotherms (Brunauer–Emmett–Teller isotherm) of magnetite, magnetite–silane functionalized material, magnetite–silane–methacrylate hybrid material, mag-MIP, and mag-NIP were analyzed. The isotherms of all tested materials are presented in Figure S3.

As can be seen, all materials revealed physisorption isotherms. All isotherms could be assigned to type IV, possessing characteristic hysteresis loops with the multilayer range of p/p₀ which is associated with capillary condensation that occurs in the mesopore systems.²⁸–³¹ The shape of the hysteresis loops is related to the specific pore structure. Here, the magnetite material revealed the hysteresis loop that could be assigned to H1 type and could refer to the porous materials that form agglomerates. These materials consist of uniform spheres in fairly regular arrays and are characterized by a narrow distribution of pore size. This observation was confirmed by scanning electron microscopy. All other tested materials revealed hysteresis loops that refer to H3- or intermediate H3/H4-type characteristics for slit-shaped pores. The total specific surface area (Brunauer–Emmett–Teller isotherm) was determined together with the cumulative surface area of pores (Barrett–Joyner–Halenda model) and the volume and the area of micropores (Harkins–Jura equation). The data are presented in Table 1. The highest specific surface area was noted for magnetite nanoparticles. After covering a magnetite particle with the silane layer, the specific surface area decreased and the same trend was observed after coating with molecularly imprinted layer. The presence of an organic layer on the surface resulted in an increase of the specific surface area to 11.01 and 19.47 m²/g, for mag-MIP and mag-NIP, respectively.

| Table 1. Characterization Data of Magnetite, Magnetite–Silane Functionalized Material, Magnetite–Silane–Methacrylate Hybrid Material, mag-MIP, and mag-NIP from Porosity Measurements and Vibrating Sample Magnetometer Analysis^a |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| material        | Fe₃O₄           | Fe₃O₄@SiO₂      | Fe₃O₄@SiO₂-MPS  | mag-MIP         | mag-NIP         |
| particle diameter (nm) | 33–96           | 133–258         | 171–308         | 221–336         | 483–778         |
| particle surface area (m²/g) | 34              | 13             | 7.2             | 11              | 19              |
| particle micropore area (m²/g) | 4.5             | 7.4            | 1.1             | 1.7             | 9.2             |
| particle pore diameter (nm) | 53              | 61             | 61              | 56              | 36              |
| particles magnetization (emu/g) | 59.1            | 26.8           | 27.8            | 15.1            | 22.8            |

^aMaterials are coded as: magnetite (Fe₃O₄), magnetite–silane functionalized material (Fe₃O₄@SiO₂), magnetite–silane–methacrylate hybrid material (Fe₃O₄@SiO₂-MPS), magnetite–silane–methacrylate hybrid molecularly imprinted polymer conjugate (mag-MIP), and magnetite–silane–methacrylate hybrid nonimprinted polymer conjugate (mag-NIP).
revealed that mag-MIP was characterized by a very low micropore area (Table 1). The analysis of t-plots for all tested materials revealed that both mag-MIP, and mag-NIP could possess micropore systems. The t-plots from porosity measurements are presented in Figure S4. The vertical drift of measurement points from a straight line could indicate the microporous structure. Finally, one of the parameters considered as related to the imprinting process is the average pore size in the adsorption and desorption processes. Pore size distributions on adsorption and desorption branches of isotherms of all tested materials are presented in Figure 3. The pore diameter data are presented in Table 1. Moreover, the following values of the average pore size were obtained for mag-MIP and mag-NIP: 10.86 and 4.88 nm, respectively. The adsorption and desorption average pore sizes were equal to 17.20 and 14.74 nm for mag-MIP, respectively, and were significantly different from mag-NIP with respective values equal to 12.16 and 16.81 nm. This indicated the mesoporous structure of all tested materials. Smaller average pore diameters in the desorption branch of the isotherm than in the adsorption branch could be proof of the coexistence of bottle-shaped pores. It could indicate the presence of different pore systems in both materials, confirming the impact of the template molecule on the morphology.

Finally, the structural properties of magnetite, magnetite–silane functionalized material, magnetite–silane–methacrylate hybrid material, and mag-MIP were analyzed by X-ray diffraction analysis. Figure 4a shows X-ray diffractometry patterns for the analyzed materials.

As can be seen, for all tested particles, six peaks were observed at 2θ equal to 30.3, 35.7, 43.3, 53.7, 57.4, and 62.6, which can be indexed as (220), (311), (400), (422), (511), and (440), respectively. The results showed that the crystalline structure of magnetite (Fe₃O₄) or maghemite (γ-Fe₂O₃) was well preserved after further covering of silane and molecularly imprinted layers. However, X-ray diffractometry analysis could not discriminate between magnetite or its oxidation product, maghemite, because the patterns overlapped.

The vibrating sample magnetometer analysis was employed to study the magnetic characterization of magnetite, magnetite–silane functionalized material, magnetite–silane–methacrylate hybrid material, and mag-MIP. Figure 4b shows the magnetization curves.

As can be seen, all materials revealed hysteresis loops that demonstrate superparamagnetic properties at room temperature as a response to the external magnetic field (Table 1). The saturation of the magnetization value halved after silanization and functionalization with 3-(trimethoxysilyl)-propyl methacrylate. A further decrease was noted after covering the material with a layer of imprinted or non-imprinted polymers, but the magnetization value for the mag-MIP was the lowest. This fact could be explained by the presence of a polymeric shell on the magnetite nanoparticles.

The Fourier transform infrared analysis was carried out to confirm the structure of magnetic nanoparticles conjugated with an imprinted layer. The spectrum of mag-MIP is presented in Figure S5.

As can be seen, characteristic peaks at 468 and 801 cm⁻¹ are attributed to Si–O–Si stretching vibrations. The bands at 568...
In the next step of optimization, the time for adsorption was analyzed. Different times of contact were applied, viz., 5, 15, 30, or 60 min. As can be seen (Figure S1b), the amount of adsorbed hordenine was very similar after 5, 15, 30, and 60 min of incubation (recoveries equal to 62.0, 67.0, 63.0, and 62.9%, respectively). Thus, the 5 min loading time was selected as optimal.

In the following step, the washing solvent was optimized. The results revealed that almost 60% of adsorbed hordenine was washed out by 85% aqueous methanol (recovery equal to 13.0%) and only 12% of adsorbed hordenine was washed out by water (recovery equal to 58.5%). Here, water was the most appropriate washing solvent due to lower analyte loss.

Finally, the elution was optimized (Figure S1c), revealing high efficacy using 1% ammonium hydroxide in methanol even after 5 min (recovery equal to 60.0%).

To summarize, the optimization of magnetic d-SPE produced a fast, efficient, and easy protocol lasting slightly over 10 min.

In the development of the LC–MS/MS method, the possible interference of phenylalanine should be excluded. The parent ions and some fragments generated in collision cell have undifferentiable \( m/z \) for mass spectrometry, i.e., 91, 95, and 103. The most specific fragment for hordenine is \( m/z \) 121 that is generated by dimethylamine loss; thus, the transition 166 > 121 was selected for quantitation. Next, validation of the analytical method (covered both sample preparation and instrumental analysis) was carried out. The calibration curve was obtained by the weighted (1/x) quadratic regression analysis (n = 3). The values of regression parameters (and their standard deviation) described by the equation \( y = ax^2 + bx + c \) were as follows: \( a = -0.00361 \pm 0.00011 \), \( b = 0.689 \pm 0.015 \), and \( c = -3.2 \pm 4.6 \) with correlation coefficient \( r \).
The range of the calibration line was selected as 0.05–50 μg/L. The precision and accuracy data for the lowest limit of quantification (LLOQ) and quality control (QC) samples within 1 day and between runs is presented in Table 2. No carryover was detected.

The moderate enhancement of the analytical signal was observed because of MF. The MF was 140% and 120% for QC1 and QC3, respectively. The variation of relative matrix factor was 7.3% for QC1 and 5.2% for QC3. Figure 5 presents an extracted chromatogram for hordenine in blank plasma, blank plasma spiked with hordenine, and test plasma collected at 1 h after administration of 100 mg of hordenine.

All stability experiments met the acceptance criteria. Hordenine was stable in plasma samples after freeze–thaw cycles (108% for QC1 and 104% for QC3) and after short-term storage at room temperature (103% for QC1 and 107% for QC3). The analyte was also stable in extract up to 48 h in the autosampler (94% for QC1 and 110% for QC3).

It is known that hordenine levels in plasma after beer consumption are low. Thus, to verify the newly proposed analytical method as well as to reveal its applicability, the evaluation of hordenine pharmacokinetics profiles after beer consumption was also investigated. The results are presented in Supporting Information Section S2. The results proved that the novel method enabled the hordenine level in plasma to be

Figure 5. MRM extracted ion chromatogram for hordenine in blank plasma (a), blank plasma spiked with hordenine at a total concentration of 0.05 μg/L (b), and test plasma (c) collected at 1 h after administration of 100 mg of hordenine (calculated concentration 8.7 μg/L).
reliably quantified from 10 to 240 min after the consumption of 0.5 L of beer with a hordenine content of 2.5 mg/L.

To summarize, all tested validation criteria were fulfilled.

3.3. Pharmacokinetics of Hordenine. Sommer and co-workers\textsuperscript{10} stated that hordenine available as a dietary supplement claims to increase the metabolism and to stimulate cognitive function. Thus, to reveal the applicability of the analytical method, the pharmacokinetic profiles of three healthy volunteers after oral administration of a hordenine-rich dietary supplement were analyzed.

The identity of hordenine was confirmed using high-resolution LC–MS/MS (Figure 6). The calculated mass was 165.1153 g/mol (observed \( m/z \) 166.1223 g/mol) and theoretical 165.1154 g/mol. The fragmentation pattern enabled a match between the compound and the \( m/z \) Cloud library (99.6% of similarity). The analysis confirmed the absence of prohibited contaminants. However, the mean content of hordenine in capsules (and standard deviation) was equal to 101 ± 31 mg. Due to the high variability (RSD = 31%), the contents of the capsules were homogenized in a mortar and the appropriate amount (100 ± 5 mg) of the mass was weighed into the starch capsules. This was also important due to the unknown pharmaceutical availability of the original capsules.

The plasma concentration–time profile for volunteers is presented in Figure 7.

The highest concentration of hordenine in plasma was after 65 ± 14 min and reached the value of 16.4 ± 7.8 μg/L. The average \( t_{1/2} \) was 54 ± 19 min, which is in agreement with the results presented by Sommer and co-workers,\textsuperscript{10} where \( t_{1/2} \) for hordenine after beer consumption varied from 52.7 to 66.4 min. Slower hordenine elimination was noted for oral administration of hordenine in rats (4.6 ± 1.6 h after a dose of 15 mg/kg).\textsuperscript{36} The application of simple allometric scaling relationships: \( t_{1/2}(\text{Human}) \sim 4 t_{1/2}(\text{Rat})^{37} \) estimated \( t_{1/2}(\text{Human}) = 69 \pm 24 \) min, is within the uncertainty of the result obtained experimentally, i.e., 54 ± 19 min. Taking into account the values of \( C_{\text{max}} \) (Table 3), there is a low probability of producing an effect by direct interaction with the dopamine D2 receptor (\( EC_{50} = 610 \mu g/L \)) after oral administration of 100 mg of hordenine from dietary supplements.\textsuperscript{8} However, further studies are required.

In the current study, the apparent volume of distribution (\( V_z/F \)) was 6000 ± 2600 L, and when adjusted for weight, it was equal to 66 ± 24 L/kg. High \( V_z/F \) indicated a low amount of hordenine in plasma with respect to the administered dose. This could be a cause of low bioavailability (low absorption and/or first-pass metabolism) and/or high amounts of tissue distribution. The bioavailability of hordenine determined for rats was 66.2%,\textsuperscript{36} but no data exist for humans. High tissue distribution could be a result of intracellular ion trapping during drug distribution. Hordenine is a weak base (\( pK_a \) of the strongest bases equal 9.19), and when it diffuses into lysosomes, it is converted to an ionized form because of the acidic environment of the lysosomes. The ionization decreases the efficiency of hordenine to diffuse out, resulting in an accumulation of the compound in lysosomes.\textsuperscript{18}

The apparent oral clearance (\( Cl/F \)) determined in the current study was 4700 ± 1700 L/h and 51 ± 11 L/h/kg when adjusted for weight. No data on clearance exist to compare. However, very rapid and extensive metabolism of hordenine was shown.\textsuperscript{10}

![Figure 6. MS2 spectrum of hordenine detected in dietary supplement. The spectrum was obtained with normalized collision energies of 20, 35, and 50 eV using Orbitrap Focus.](image)

![Figure 7. Mean plasma concentration–time profile of hordenine in plasma after administration of a single oral dose of 100 mg hordenine to three healthy volunteers.](image)
Table 3. Main Pharmacokinetic Parameters after Oral Administration of 100 mg of Hordenine to Three Healthy Volunteers

| Parameter       | Unit     | Mean     | SD[^b] |
|-----------------|----------|----------|--------|
| AUC[^a]         | µg min/L | 1440     | 420    |
| AUC\_con        | µg min/L/kg | 17.1    | 6.4    |
| AUC\_con        | µg min/L/kg | 1390   | 420    |
| AUC\_t1/2       | µg min/L/kg | 16.5   | 5.0    |
| C\_max          | µg/L     | 16.4     | 7.8    |
| Cl/F            | L/h      | 4700     | 1700   |
| Cl/F\_norm      | L/h/kg   | 51       | 11     |
| MRT\_t           | min      | 102.0    | 0.9    |
| MRT\_t          | min      | 113.1    | 6.2    |
| T\_max          | min      | 65       | 14     |
| t\_1/2          | min      | 54       | 19     |
| Vz/F            | L        | 6000     | 2600   |
| Vz/F\_norm      | L/kg     | 66       | 24     |

[^a]: Body weight normalized.  
[^b]: Standard deviation.  
[^c]: Parameters: AUC\_con—the area under the plasma concentration–time curve from 0 h to infinity; AUC\_con—the area under the plasma concentration–time curve from 0 h to time t, C\_max—the maximum concentration observed, Cl/F—the apparent plasma clearance, MRT—the mean residence time, T\_max—the maximum time, i.e., time when plasma concentrations reached a maximum value, t\_1/2—the terminal half-life value, Vz/F—the apparent distribution volume.

High variability of pharmacokinetic parameters (CV > 40%) was observed for C\_max (47%), and Vz/F (43%, moderate when adjusted for weight—36%), whereas moderate (20–40%) for t\_1/2 (35%), Cl/F (36, 22% when adjusted for weight), T\_max (22%), and AUC (29%). Similarly, for rats, a high variability was observed for C\_max (47%), moderate for t\_1/2 (35%), and low (<20%) for t\_1/2 (19%). It should be emphasized that no other pharmacokinetic parameters were presented to compare.36

To summarize, small amounts of hordenine related to the administered dose and high variability of pharmacokinetic parameters were observed. As was previously mentioned, taking into account the obtained C\_max (16.4 ± 7.8 µg/L), there is a low probability of an effect by direct interaction with the dopamine D2 receptor (EC\_50 = 610 µg/L)\(^8\) after oral administration of 100 mg of hordenine (ca. 1.1 mg/kg) from dietary supplements. The observed concentrations are lower than expected. In addition, the results could be used to improve knowledge related to the effective and safe dosage of hordenine in dietary supplements. The possibility of hordenine accumulation in the brain exists since it can cross the blood–brain barrier.39

In summary, magnetic core–shell imprinted nanoconjugates allowed for efficient separation of hordenine from human plasma. Morphological and structural characterization methods confirmed that each subsequent preparation step of core–shell magnetic materials resulted in significant modification of the composition of analyzed material. Optimization of magnetic d-SPE provided a fast, easy, and reliable pretreatment step. The proposed analytical protocol was characterized by the lower limit of quantification of hordenine among other presented methods. The method was validated, providing pharmacokinetic studies of hordenine after oral administration to humans. The results are preliminary, and further studies are necessary to assess the probability of hordenine interactions with the dopamine D2 receptor. Here, it could be expected that the analytical method presented in this paper will help to provide more insight into the possibility of hordenine interactions with the dopamine D2 receptor after administration of dietary supplements. Finally, after establishing the relationship between hordenine concentration and its biological effect, the result of our pharmacokinetic study can be used to establish the effective and safe dose of hordenine in dietary supplements.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c05985.

Synthetic process of Fe\(_3\)O\(_4\), Fe\(_3\)O\(_4@\)SiO\(_2\), Fe\(_3\)O\(_4@\)SiO\(_2\)-MPS; determination of pharmacokinetic profile after beer consumption; optimization of MIP d-SPE method regarding sample treatment, loading time, and elution time; Freundlich isotherms and function of \(t/q\) against \(t\) for mag-MIP and mag-NIP; nitrogen adsorption isotherms for Fe\(_3\)O\(_4\), Fe\(_3\)O\(_4@\)SiO\(_2\), Fe\(_3\)O\(_4@\)SiO\(_2\)-MPS, mag-MIP, and mag-NIP; \(t/q\)-plots for Fe\(_3\)O\(_4\), Fe\(_3\)O\(_4@\)SiO\(_2\), Fe\(_3\)O\(_4@\)SiO\(_2\)-MPS, mag-MIP, and mag-NIP; and FT-IR spectrum of mag-MIP (PDF)

**AUTHOR INFORMATION**

Corresponding Author

Monika Sobiech — Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, 02-097, Warsaw, Poland; Phone: +48 22 5720643; Email: monika.sobiech@wum.edu.pl

Authors

Joanna Giebultowicz — Department of Bioanalysis and Drugs Analysis, Faculty of Pharmacy, Medical University of Warsaw, 02-097 Warsaw, Poland; Phone: +48 22 5720643; Email: Joanna.Giebultowicz@wum.edu.pl

Piotr Luśniak — Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, 02-097 Warsaw, Poland; Email: Piotr.Luśniak@wum.edu.pl

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acs.jafc.0c05985

**Funding**

This work was supported by National Science Centre of Poland (Grant No. 2017/01/X/ST5/00270).

**Notes**

The authors declare no competing financial interest.

**REFERENCES**

(1) Dwyer, J. T.; Coates, P. M.; Smith, M. J. Dietary supplements: Regulatory challenges and research resources. *Nutrients* 2018, 10, No. 41.

(2) Crawford, C.; Wang, Y.-H.; Avula, B.; Bae, J.-Y.; Khan, I. A.; Deuster, P. A. The scoop on brain health dietary supplement products containing huperzine A. *Clin. Toxicol. 2020, 58*, 991–996.

(3) Zhao, J.; Wang, M.; Avula, B.; Khan, I. A. Detection and quantification of phenethylamines in sport dietary supplements by NMR approach. *J. Pharm. Biomed. Anal.* 2018, 151, 347–355.

(4) Avula, B.; Bae, J.-Y.; Chittiboyina, A. G.; Wang, Y.-H.; Wang, M.; Khan, I. A. Liquid chromatography-quadrupole time of flight mass spectrometric methods for targeted analysis of 111 nitrogen-based compounds in weight loss and ergogenic supplements. *J. Pharm. Biomed. Anal.* 2019, 174, 305–323.

https://dx.doi.org/10.1021/acs.jafc.0c05985

J. Agric. Food Chem. 2020, 68, 14502–14512
(5) Su, S.; Cao, M.; Wu, G.; Long, Z.; Cheng, X.; Fan, J.; Xu, Z.; Su, H.; Hao, Y.; Li, G.; Peng, J.; Li, S.; Wang, X. Hordenine protects against hyperglycemia-associated renal complications in streptozotocin-induced diabetic mice. *Biomed. Pharmacother.* **2018**, *104*, 315−324.

(6) Kim, S.-C.; Lee, J.-H.; Kim, M.-H.; Lee, J.-A.; Kim, Y. B.; Jung, E.; Kim, Y.-S.; Lee, J.; Park, D. Hordenine, a single compound produced during barley germination, inhibits melanogenesis in human melanocytes. *Food Chem.* **2013**, *141*, 174−181.

(7) Sander, L. C.; Putzbach, K.; Nelson, B. C.; Rimmer, C. A.; Bedner, M.; Brown, T. J.; Porter, B. J.; Wood, L. J.; Schantz, M. M.; Murphy, K. E.; Sharpless, K. E.; Wise, S. A.; Yen, J. H.; Siitonen, P. H.; Evans, R. L.; Nguyen, P. A.; Roman, M. C.; Betz, J. M. Certification of standard reference materials containing bitter orange. *Anal. Bioanal. Chem.* **2008**, *391*, 2023−2034.

(8) Sommer, T.; Hubner, H.; El Kerdawy, A.; Gmeiner, P.; Pischtschieter, M.; Clark, T. Identification of the beer component hordenine as food-derived dopamine D2 receptor agonist by virtual screening a 3D compound database. *Sci. Rep.* **2017**, *7*, No. **44201**.

(9) United States Food and Drug Agency. https://www.fda.gov/food/dietary-supplement-products-ingredients/dietary-supplement-ingredient-advisory-list (accessed May 09, 2020).

(10) Sommer, T.; Göen, T.; Budnik, N.; Pischtschieter, M. Absorption, biokinetics, and metabolism of the dopamine D2 receptor agonist hordenine (N,N-dimethylyleryamine) after beer consumption in humans. *J. Agric. Food Chem.* **2020**, *68*, 1998−2006.

(11) Brauers, G.; Steiner, I.; Daldrup, T. Quantification of the biogenic phenethylamine alkaloid hordenine by LC−MS/MS in beer. *Toxicon. Kronech.* **2013**, *80*, 323−326.

(12) Sommer, T.; Schlüg, G.; Hubner, H.; Gmeiner, P.; Pischtschieter, M. Monitoring of the dopamine D2 receptor agonists hordenine and N-methyltryamine during the brewing process and in commercial beer samples. *Food Chem.* **2019**, *276*, 745−753.

(13) Steiner, I.; Brauers, G.; Temme, O.; Daldrup, T. A sensitive method for the determination of hordenine in human serum by ESIm−UPLC−MS/MS for forensic toxicological applications. *Anal. Bioanal. Chem.* **2016**, *408*, 2285−2292.

(14) Sobiech, M.; Giebultowicz, J.; Lulinski, P. Theoretical and experimental proof for selective response of imprinted sorbent—analysis of hordenine in human urine. *J. Chromatogr. A* **2020**, *1613*, No. 4606777.

(15) Hen, Y.; Meng, J.; Zou, J.; An, J. Selective extraction based on poly(MAA-VB-EGMDA) monolith followed by HPLC for determination of hordenine in plasma and urine samples. *Biomed. Chromatogr.* **2015**, *29*, 869−875.

(16) Lulinski, P.; Giebultowicz, J.; Wroczynski, P.; Maciejewska, D. A highly selective molecularly imprinted sorbent for extraction of 2-aminothiazoline-4-carboxylic acid-Synthesis, characterization and application in post-mortem whole blood analysis. *J. Chromatogr. A* **2015**, *1420*, 16−25.

(17) Lulinski, P.; Dana, M.; Maciejewska, D. Synthesis and characterization of 4-(2-aminoethyl)aniline implanted polymer as a highly effective sorbent of dopamine. *Talanta* **2014**, *119*, 623−631.

(18) Lulinski, P.; Maciejewska, D. Effective separation of dopamine from bananas on 2-(3,4-dimethoxyphenyl)ethylamine implanted polymer. *J. Sep. Sci.** 2012**, *35*, 1050−1057.

(19) Lulinski, P.; Pambucic-Klimkowska, M.; Dana, M.; Szutowski, M.; Maciejewska, D. Efficient strategy for the selective determination of dopamine in human urine by molecularly implanted solid-phase extraction. *J. Sep. Sci.* **2016**, *39*, 895−903.

(20) Lulinski, P.; Sobiech, M.; Zoldek, T.; Maciejewska, D. A separation of tyramine on a 2-(4-methoxyphenyl)ethylamine implanted polymer: An answer from theoretical and experimental studies. *Talanta* **2014**, *129*, 155−164.

(21) Giebultowicz, J.; Sobiech, M.; Ruzyczka, M.; Lulinski, P. Theoretical and experimental approach to hydrophilic interaction dispersive solid-phase extraction of 2-aminothiazoline-4-carboxylic acid from human post-mortem blood. *J. Chromatogr. A* **2019**, *1587*, 61−72.

(22) Ansari, S.; Karimi, M. Recent configurations and progressive uses of magnetic molecularly imprinted polymers for drug analysis. *Talanta* **2017**, *167*, 470−485.

(23) Qiao, F. X.; Wang, M. G. Preparation of selective magnetic dispersive solid-phase sorbent and its application for recognition clenbuterol from bovine urine. *J. Chromatogr. B* **2016**, *1017−1018*, 18−27.

(24) Hu, C.; Deng, J.; Zhao, Y.; Xia, L.; Huang, K.; Ju, S.; Xiao, N. A novel core−shell magnetic nano-sorbent with surface molecularly imprinted polymer coating for the selective solid phase extraction of dimetridazole. *Food Chem.* **2014**, *158*, 366−373.

(25) Smith, G. European Medicines Agency guideline on bioanalytical method validation: what more is there to say? *Bioanalysis* **2012**, *4*, 1−23.

(26) Zhang, Y.; Huo, M.; Zhou, J.; Xie, S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput. Methods Program Biomed.* **2010**, *99*, 306−314.

(27) Sekine, S.; Watanabe, Y.; Yoshimi, Y.; Hattori, K.; Sakai, K. Influence of solvents on chiral discriminative gate effect of molecularly imprinted poly(ethylene glycol dimethacrylate-co-methacrylic acid). *Sens. Actuators, B* **2007**, *127*, S12−S17.

(28) Brunauer, S.; Emmett, P. H.; Teller, E. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **1938**, *60*, 309−319.

(29) Barrett, E. P.; Joyner, L. G.; Halenda, P. P. The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms. *J. Am. Chem. Soc.* **1951**, *73*, 373−380.

(30) Harkins, W. D.; Jura, G. Surfaces of solids. XIII. A vapor adsorption method for the determination of the area of a solid without the assumption of a molecular area, and the areas occupied by nitrogen and other molecules on the surface of a solid. *J. Am. Chem. Soc.* **1944**, *66*, 1366−1373.

(31) Sing, K. S. W. Reporting physiosorption data for gas/solid systems. *Pure Appl. Chem.* **1982**, *54*, 2201−2218.

(32) Marcí, M.; Panuszko, A.; Namięśnik, J.; Wieczorek, P. P. Preparation and characterization of dummy-template molecularly imprinted polymers as potential sorbents for the recognition of selected polybrominated diphenyl ethers. *Anal. Chim. Acta* **2018**, *1030*, 77−95.

(33) Lulinski, P.; Maciejewska, D. Impact of functional monomers, cross-linkers and porogens on morphology and recognition properties of 2-(3,4-dimethoxyphenyl)ethylamine imprinted polymers. *Mater. Sci. Eng., C* **2011**, *31*, 281−289.

(34) Li, L.; He, X.; Chen, L.; Zhang, Y. Preparation of core−shell magnetic molecularly imprinted polymer nanoparticles for recognition of bovine hemoglobin. *Chem. - Asian J.* **2009**, *4*, 286−293.

(35) Advanced Mass Spectral Database. https://www.mzcloud.org/ (accessed August 8, 2020).

(36) Ma, J.; Wang, S.; Huang, X.; Geng, P.; Wen, C.; Zhou, Y.; Yua, L.; Wang, X. Validated UPLC−MS/MS method for determination of hordenine in rat plasma and its application to pharmacokinetic study. *J. Pharm. Biomed. Anal.* **2015**, *111*, 131−137.

(37) Caldwell, G. W.; Masucci, J. A.; Yan, Z.; Hageman, W. Allometric scaling of pharmacokinetic parameters in drug discovery: can human Clss and Vss and t/2 be predicted from in-vivo rat data? *Eur. J. Drug Metab. Pharmacokinet.* **2004**, *29*, 133−143.

(38) Kazmi, F.; Hensley, T.; Pope, C.; Funk, R. S.; Loewen, G. J.; Buckley, D. B.; Parkinson, A. Lysosomal sequestration (trapping) of lipophilic amine (caticonic amphiphile) drugs in immortalized human hepatocytes (Fa2N-4 Cells). *Drug Metab. Dispos.* **2013**, *41*, 897−905.

(39) Konczoł, A.; Rendes, K.; Dekany, M.; Muller, J.; Riethmüller, E.; Balogh, G. T. Blood-brain barrier specific permeability assay reveals N-methylated tyramine derivatives in standardised leaf extracts and herbal products of Ginkgo biloba. *J. Pharm. Biomed. Anal.* **2016**, *131*, 167−174.