Spectrometric Smartphone-Based System for Ibuprofen Quantification in Commercial Dosage Tablets

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ABSTRACT

A rapid and portable analytical methodology has been developed for ibuprofen (IBU) quantification in commercial dosage tablets using a spectrometric smartphone-based system. The analytical methodology employs point-of-use approaches both for sample preparation and detection, demonstrating its potential utility for portable quality control of pharmaceutical products. In this work, IBU is dissolved in methanol and then treated with a Co(II) aqueous solution, forming a blue complex which is extractable by dispersive liquid-liquid microextraction. Then, the sample’s absorption spectrum is directly measured by a spectrometric smartphone-based system using cartridge made of polyoxymethylene for solvent compatibility. The main experimental factors affecting the dispersive liquid-liquid microextraction of Co-IBU complex were optimized using a multivariate analysis. Under optimized conditions, a working range between 20 and 80 µg mL \(^{-1}\) was obtained with a correlation coefficient of 0.996 for 5 calibration points. The limit of detection and limit of quantification obtained were 4 and 12 µg mL \(^{-1}\), respectively. The performance of the proposed methodology was evaluated in commercial tablet dosage forms, and the results demonstrate the ability of the method to determine IBU in samples representative of those used in real-world quality control applications. Recovery values between 97% and 105% were obtained, which are comparable to those obtained via standard titrimetric methodology.

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Introduction

Nonsteroidal anti-inflammatory drugs are among the most frequently prescribed drugs worldwide, and they are used for relief of inflammatory pain conditions in both acute (e.g., headache, postoperative pain, and orthopedic fractures) and chronic (e.g., rheumatoid arthritis, osteoarthritis, and gout) disease.¹

Ibuprofen (IBU), \((R,S)-2-(4-isobutylphenyl)\) propionic acid, is a white crystalline powder that is partially insoluble in water but easily dissolved in acetone, methanol, and chloroform, while it is partially soluble in dilute hydroxide and carbonate solutions. It has become one of the most common nonsteroidal anti-inflammatory drugs, and the general acceptance of its safety led to its approval by the US Food and Drug Administration in 1984 for nonprescription, over-the-counter sale to consumers (<1200 mg/d).² Since then, its utilization as a general analgesic has led it to become the third most popular pharmaceutical in the world, with an annual global production reported as \(10^6\) kg.³

As with any pharmaceutical product, achieving stringent quality controls over both the quantity of the active pharmaceutical ingredient (API) and quality of that product is essential to ensuring that end-user dosage is in agreement with national standards. Several factors can result in a loss of potency and quality. For instance, IBU may be synthesized via different chemical pathways, resulting in different process impurities remaining in the API and final drug products.⁴ Furthermore, during shipping and storage,
different degradation impurities may be formed due to environment- 
ment variations in temperature and humidity. Quality control of 
both API and final consumer products is an essential component of 
all pharmaceutical manufacturing.

Quality control of raw materials and consecutive monitoring of 
potency and safety of pharmaceutical products constitutes an im-
portant current subject of investigation in the pharmaceutical 
sector. The decomposition process can result in a loss of potency 
and quality or in a loss of safety of drugs and drug formulations due 
to creation of minor ineffective or toxic degradation products.

The pharmacopeias for different countries report conflicting 
methods for IBU determination in pharmaceutical product quality 
control. For instance, the method used throughout the United 
Kingdom and the European Union is based on an acid-base titra-
tion in nonaqueous media (a solution of methanol and sodium hy-
droxide) and is measured by observing the color change of 
phenolphthalein as a visually observable indicator. This classic 
method of titration is quite time consuming and agent intensive.
In the US pharmacopeia, standard procedures dictate the use of HPLC 
with UV detection, and capillary electrophoresis. These 
methods are attractive choices for the analysis of IBU in terms of their 
limit of quantification (LoQ), which can extend to concentrations less 
than 0.05 μg L⁻¹. These improvements in detection sensitivity involve 
high-performance liquid chromatography, including the dependence on sophisticated, costly, 
and bulky instruments compounded by time-consuming analysis, and the need for expensive reagents in high volumes.

After extensive analysis and low-cost, high-sensi-
tivity detection is necessary, researchers have looked to take 
avantage of smartphone-based systems to measure a variety of 
analytes including biomarkers, target DNA, viruses, drugs, allergens, 
ascorbic acid, molecular beacon, proteins, among others. Modern smartphones make use of miniaturized high-quality cameras and more powerful microprocessors than the desktop computers of 
even a decade ago while maintaining reasonable affordability and 
ease of use compared with standard laboratory equipment. In fact, 
smartphone-based systems have recently been demonstrated for 
several chemical analysis for quality control applications that include methanol and ethanol determinations in sugar cane spirits, sul-
fadiazine and sulfasalazine determinations in pharmaceutical and veterinary formulations, ascorbic acid determination in commer-
cial vitamin C tablets, iodine determination in biodiesel, and 
even furfural quantification for beer freshness assessment. These 
smartphone-based systems offer several advantages including (1) 
their ability to use a low volume of reagents and samples, (2) cost-effectiveness, (3) shortened analysis time, (4) high portability for 
on-site and in-field analysis, (5) simple operational steps and familiar user experience, and (6) high-throughput capabilities. Nevertheless, the use of a spectrometric smartphone-based system for 
IBU quantification has not been previously reported.

In this article, we demonstrate the first use of a spectroscopic 
smartphone-based system as a simple, fast, portable, and low-cost analytical procedure of IBU in commercial dosage tables. The 
demonstrated methodology uses strategies compatible with a 
point-of-use approach for both sample preparation and portable, 
high-sensitivity readout: centrifuge-less dispersive liquid-liquid 
microextraction (DLLME) and a smartphone-based spectrometric 
device. The DLLME offers a simple, easy-to-use, low-reagent vol-
ume technique that allows for both the reduction in byproducts 
generated and a much higher enrichment factor when compared 
with traditional liquid—liquid extraction techniques. In this work, 
we have optimized the chemical assay via a multivariate optimi-
ization and then used the developed assay to accurately measure 
IBU concentrations in commercial IBU tablets, successfully 
demonstrating comparable results to those obtained by a conven-
tional titrimetric method.

Analytical Methodology

Spectrometric Smartphone-Based System

The transmission, reflection, intensity (TRI) analyzer used in this 
work is the result of the evolution of diverse prototypes already 
described elsewhere, and it has been previously introduced for multimodal analysis of absorptive, fluorescent, and photonic 
crystal-based measurements. Briefly, the TRI-analyzer instru-
ment comprised a 3D-printed cradle for a commercially available 
smartphone that enables the rear-facing camera to function as a 
spectrophotometer. The system gathers light from the white light-
emitting diode that is ordinarily used for flash illumination into an 
optical fiber. The light emerging from the opposite end of the fiber 
is directed through a cartridge, and after passing through it, the 
light is reflected back by a mirror, so the white light makes 2 passes 
through a liquid test sample. When the liquid sample contains 
material that absorbs some of the light-emitting diode wave-
lenghts, the intensity of the reflected spectrum will be reduced at 
those specific wavelengths. The back-reflected light is gathered into 
a second optical fiber that directs the light through a transmission 
grating that is placed in front of the phone’s rear-facing camera, 
thus dispersing the wavelength components in one direction to 
generate a spectrum on the image sensor’s pixels. The cartridge 
contains multiple liquid compartments in one direction to 
generate a spectrum on the image sensor’s pixels. The cartridge 
contains multiple liquid compartments in a serial configuration 
that can be passed through the measurement head in a linear 
sequence, and when at least one of the liquid compartments is 
filled with colorless material (such as pure water), the spectrum 
from a test sample can be directly compared with a negative control 
spectrum to generate an absorption spectrum. Although the TRI-
analyzer can perform 3 different classes of spectrometric mea-
surements, we will use the system solely to measure the absorption 
spectrum of the test sample via optical transmission.

Reagents and Samples

A stock solution (2000 mg L⁻¹) of S-ibuprofen (Sigma-Aldrich, 
St. Louis, MO) was prepared in methanol (Fisher Scientific, Fair 
Lawn, NY) and stored at 4°C. Working solutions were prepared by 
dilution of the stock standard solution. Chlorofluorine (Sigma-Aldrich) 
was used as an extractant solvent, and methanol was used as a 
dispersant. A stock cobalt (Co(II)) solution (10% w/v) was pre-
pared by dissolving CoCl₂·6H₂O (Sigma-Aldrich) in water and 
stored at 4°C. Working solutions was obtained by diluting the stock 
cobalt solution. Diluted sodium hydroxide solution, prepared from 
solid NaOH pellets (Fisher Scientific), was used for pH adjustment. 
Sodium chloride was purchased from Sigma-Aldrich. D-glucose 
was purchased from Merck (Darmstadt, Germany), while 
MgCl₂·6H₂O was purchased from Sigma-Aldrich.

Pharmaceutical Sample Analysis

Two different commercial IBU products were analyzed: 200 mg 
ibuprofen tablets from Major Pharmaceuticals, Inc. (tablet 1) and 
Supervalu, Inc. (tablet 2). For each sample, 5 tablets were weighed, 
finely powdered, and then homogenized. From these powders, 3 
samples each of 200.0 mg IBU (the weight of one tablet) were dis-
solved in 50 mL of methanol for both the standard (titrimetric) and
proposed methodologies. As the proposed methodology was designed to use smaller volumes, aliquots of 100 µL were measured. Sodium hydroxide solution (0.100 M) was prepared and standardized using potassium hydrogen phthalate (Sigma-Aldrich). Phenolphthalein solution 0.5 wt. % in ethanol:water (1:1) (Sigma-Aldrich) was used throughout as a colorimetric indicator. In addition, for the method described here, spiked samples with 15 µg mL\(^{-1}\) of IBU were analyzed to characterize the limit of quantification (LOQ).

Distilled, deionized water (18.3 MΩ cm) from a Millipore water purification system (Millipore Corporation, Bedford, MA) was used for producing all aqueous solutions. All stock solutions were stored at 4 °C, and all working solutions were prepared immediately before each experiment.

**Multivariate Optimization**

Cobalt(II) forms a complex with IBU. Physical studies of Co(II) ibuprofenate (Co\(_2\)(Ibu)\(_4\)(H\(_2\)O)\(_2\)) show that 2 cobalt atoms bridge 4 deprotonated carboxylate groups and 2 water molecules in the coordination sphere.\(^\text{31}\) Thus, Co-IBU complex extraction can be influenced by several modifiable experimental factors that were optimized by multivariate analysis. The main experimental factors affecting the extraction include extractant solvent volume, dispersant volume, sample pH, salt concentration (NaCl), and cobalt concentration. We employed a two-step multivariate technique: (1) a Plackett-Burman design for screening followed by (2) a circumscribed central composite design (CCCD) for optimization. This study was carried out using the TRI-analyzer platform (Spectrometric Smartphone-Based System), and a model 10 mL sample containing 80 µg L\(^{-1}\) of IBU was used to optimize the assay procedure. For both steps, 12 experiments were randomly performed to nullify the effect of extraneous factors. The peak measurement intensity at a wavelength of λ = 585 nm, where Co-IBU complex exhibits a strong absorbance band, was used as the response function in designing both the Plackett-Burman and CCCD studies.

Extractant volume of chloroform was studied in the range of 75-100 µL as microextraction procedure must use an extractant volume equal or below 100 µL.\(^\text{32}\) In addition, the study was carried out by varying the dispersant volume in the range of 200-400 µL due to the fact that those amounts of methanol showed enough dispersive effect to form a cloudy solution. Furthermore, the effect of the pH was studied in the range 5-7. The basic media was not evaluated to avoid the precipitation of the cobalt(II) hydroxide. The vast excess of sodium chloride added was to improve the extraction of the analyte and promoted a fast phase separation. Finally, a huge excess of cobalt salt was used to guarantee the formation of the Co-IBU complex.

**Data Processing**

A multivariate optimization strategy was performed to determine the optimum conditions for the microextraction method. Statgraphics statistical computer package “Statgraphics Centurion XVI” (Warrenton, VA) was used to construct the experimental design matrices and evaluate the results. Image analysis software is developed with computational software (Matlab, MathWorks, Natick, MA) to process spectral data acquired by the smartphone. Details about spectrum processing has been previously explained by Long et al.\(^\text{19}\)

**DLLME Procedure**

Using the results of our multivariate analysis, our final DLLME protocol calls for a mixture of 80 µg mL\(^{-1}\) of IBU, 5% (w v\(^{-1}\)) of cobalt salt, and 5% (w v\(^{-1}\)) of NaCl solutions mixed well in a 15-mL test tube, with a pH corrected to 7 and a final volume adjusted to 10 mL. pH measurements were performed with a pH meter (model Orion 3 Star; Thermo Scientific, Waltham, MA). Then, a mixture of 99 µL of extractant solvent (i.e., chloroform) and 319 µL of dispersant volume (methanol) are added using a syringe. A cloudy solution immediately forms, and the phase separation is allowed to proceed for 1 min. Chloroform was chosen over nontoxic solutions (e.g., undecanol) as it allows phase separation to occur without centrifugation, allowing for a truly portable sample preparation. Afterward, the aqueous phase is removed, and the organic phase is retrieved with a pipette and analyzed by the smartphone-based system (Fig. 1). A novel component of this work is the direct measurement of the organic phase using a custom cartridge that integrates a linear series of fluid compartments made of polyoxymethylene, which has excellent chemical resistance to most organic solvents (Figs. 1b and 1c). From the beginning to end, the overall procedure lasts less than 5 min.

**Results and Discussion**

**Optimization of the Experimental Factors**

Table 1 shows the experimental factors and levels used in both Plackett-Burman and circumscribed central composite

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental Factors and Levels of the Plackett—Burman and Circumscribed Central Composite Designs</th>
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<tbody>
<tr>
<td>Plackett—Burman Design</td>
<td>Level</td>
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<tr>
<td>Factors</td>
<td>Low (−1)</td>
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<td>Extractant volume (µL)</td>
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<tr>
<td>Dispersant volume (µL)</td>
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<tr>
<td>Sample pH</td>
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<tr>
<td>[NaCl] (%,w v(^{-1}))</td>
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</tr>
<tr>
<td>[CoCl(_2)] (%,w v(^{-1}))</td>
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<tr>
<td>Circumscribed Central Composite Design</td>
<td>Level</td>
</tr>
<tr>
<td>Factors</td>
<td>Low (−1)</td>
</tr>
<tr>
<td>Extractant volume (µL)</td>
<td>80</td>
</tr>
<tr>
<td>Dispersant volume (µL)</td>
<td>200</td>
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</tbody>
</table>

Figure 1. (a) Scheme of the analytical procedure for IBU quantification. (b) Photography of the cartridge used throughout the work. (c) Scheme of the top view of the cartridge made of glass substrates with double-sided adhesive (DSA) and polyoxymethylene (POM) which is completely resistant to chloroform.

optimizations. The results obtained from the screening study (Plackett-Burman design) are shown in the Pareto chart in Figure 2a. In this chart, gray bars indicate a positive influence in the response function for the DLLME procedure when increasing the value of the experimental factor, whereas blue bars indicate a negative influence. The vertical line indicates the 95% confidence interval and the factors with a statistically significant influence with respect to the selected response function extend past this threshold. As observed, the extractant solvent and the dispersant volumes have a significant influence in DLLME. On the other hand, the sample pH, CoCl2 concentration, and NaCl addition are not statistically significant factors in the procedure optimization and as such can be fixed at reasonable values. Sample pH was fixed at its highest level and the other 2 nonsignificant factors (CoCl2 and NaCl concentration) were fixed at their lower limits for subsequent extractions.

The extractant solvent and the dispersant were each assessed at 5 different volumes using a CCD. Table 1 shows the experimental factors and volumes selected for the CCD. Results of this study are illustrated in Figure 2b as a response surface, showing the variation in the absorption of the Co-IBU complex as a function of the extractant solvent and the dispersant volumes.

Increasing the extractant solvent volume in DLLME leads to an increase in the quantity of tiny droplets dispersed throughout the aqueous solution, thereby increasing the interfacial contact area and consequently the extraction efficiency. On the other hand, increasing the extractant solvent volume also leads to an increase in the analyte-enriched phase (i.e., dilution) and therefore to a decrease in the analyte concentration in the organic phase. The observed optimum value of 99 μL for extractant solvent volume is a direct consequence of these competitive effects.

The dispersant volume should be controlled to ensure adequate extractant solvent dispersion, thus leading to the formation of fine droplets that are responsible for maximizing extraction efficiency for the DLLME. However, an excess of dispersant may increase the dilution of the organic phase, thus resulting in a lower extraction efficiency.

In summary, the optimum experimental conditions for our procedure were selected as the following parameters for subsequent experiments: extractant solvent volume, 99 μL; dispersant volume, 319 μL; sample pH, 7; CoCl2 concentration, 5% w v⁻¹ and NaCl concentration, 5% w v⁻¹.

Analytical Figures of Merit

Analytical figures of merit of the combination of DLLME and the spectrometric smartphone-based system were evaluated to assess the analytical capability of this procedure for the quantification of IBU in pharmaceutical products. Under optimized conditions, the working range was established between 20 and 80 μg mL⁻¹, and the absorption spectra were measured (Figs. 3a-3c). The calibration curve was constructed using 5 concentration levels, evaluated in
The proposed method is significantly faster than aggregation-based, solid-phase microextraction, or kinetic spectrophotometry technique.28–40

Comparison With Previously Published Spectrophotometric Methods and a Smartphone-Based System

Several analytical methods for IBU quantification using spectrophotometric systems have been published, though none of them are portable smartphone systems (Table 2). It should be noted that, as a result of the combination of DLLME and spectrometric capability of the smartphone-based system, our observed LOQ values of IBU in this work are significantly better than those obtained using benchtop spectrophotometer instruments.33–36 One research work obtained comparable LOQ values using a complex and nonportable flow injection system.37 Few works using nonportable systems reported significantly lower LOQ than what was obtained using the proposed method, and we believe that in each case our demonstrated methodology provides significant improvements. First, the proposed method does not require gold nanoparticles that makes cheaper the analysis, and the methodology does not depend on the low colloidal stability of gold nanoparticle solution.39 Second, our proposed method is significantly faster than aggregation-based, solid-phase microextraction, or kinetic spectrophotometry techniques.38–40

Interference Study

Typical excipients used in pharmaceutical dosage include binders, fillers, glucose, and some salts of sodium and magnesium.

Table 3

<table>
<thead>
<tr>
<th>Interference Species</th>
<th>RE (%)</th>
<th>Tolerance Level</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>–5</td>
<td>750</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>5</td>
<td>750</td>
</tr>
<tr>
<td>NaCl</td>
<td>–7</td>
<td>500</td>
</tr>
</tbody>
</table>

* Relative error defined as RE (%) = (C – C0)/C0*100.

Table 4

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (μg per Tablet)</th>
<th>Titrimetric Method</th>
<th>Proposed Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found Value (μg per Tablet)²</td>
<td>Recovery (%)</td>
<td>Found Value (μg per Tablet)²</td>
</tr>
<tr>
<td>Tablet 1</td>
<td>200</td>
<td>107 ± 2</td>
<td>204 ± 10</td>
</tr>
<tr>
<td>Tablet 2</td>
<td>200</td>
<td>103 ± 2</td>
<td>210 ± 19</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± SD, n = 3.

Binders, such as gelatin, and fillers, such as talc and silicon dioxide, are insoluble in methanol, which we used for dissolving the pharmaceutical preparations. For this reason, only glucose and salts of magnesium and sodium were evaluated for potential interference in this study.

To assess the selectivity of our method, the interference of these excipients were studied. Four different concentration ratios were studied (Cexcipient/CIBU = 250:1, 500:1, 750:1 and 1000:1) in the quantification of 40 μg mL⁻¹ IBU, and the resultant tolerance limits are shown in Table 3. The tolerance limit is defined as concentration ratio causing a relative error of ±10% from pure IBU. Both glucose and MgCl₂ were found to have the same upper tolerance limit ratio (i.e., 750:1), while NaCl has an upper tolerance limit of 500:1. The anticipated Cexcipient/CIBU ratios for pharmaceutical dosages are much lower than the ratios investigated in this interference study, typically on the magnitude of 0.05:1–0.1:1.

Real Sample Analysis

Table 4 shows the results obtained for the determination of IBU in 2 commercial tablets. The results were compared with those obtained by standard reference methods of the British and European pharmacopoeias, and a high level of agreement was found (recovery values ranged from 103% to 107%). In addition, both tablets were spiked at concentrations near that of the determined LOQ, 15 μg mL⁻¹ of IBU (Table 5). No significant difference between the concentrations added and those measured via our proposed methodology were found with either commercial tablet, with recovery values ranging from 97% to 105%.

Conclusion

In this work, a spectrometric smartphone-based system has been successfully combined with a novel, optimized DLLME procedure for the quantification of IBU in pharmaceutical tablets. These results demonstrate that the combination of DLLME and a spectrometric smartphone-based system can improve the figures of merit even beyond those of recent conventional benchtop laboratory spectrophotometers. This promising analytical methodology would be suitable for a routine and rapid IBU quality control and can be performed in virtually any location or field environment.
Acknowledgments

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References

2. Rainsford KD. Fifty years since the discovery of ibuprofen. Inflammopharmacology. 2011;19:293-297.
35. Ziafati HE, Elzafarly ES, Soudi AT, Salem MY. Application of the ratio difference spectrophotometry to the determination of ibuprofen and famotidine in their combined dosage form; Comparison with previously published spectrophotometric methods. Spectrochim Acta A Mol Biomol Spectrosc. 2015;143:251-255.