# Micro-HPLC-UV Method for Assessing Ibuprofen Content in Pediatric Mini-Tablets and Identification of a Process-Related Impurity

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## Introduction

Age-appropriate pharmaceutical formulations are crucial for ensuring safe and effective drug administration. Mini-tablets (2 mm, 10 mg, **Figure 1a and 1b**) offer a promising solution in pediatrics, as they are easy to swallow, allow flexible dosing, and are well-accepted by patients. Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) consisting of a benzene ring substituted with a 2-propanoic acid group and an isobutyl radical, which enhances lipophilicity and gastrointestinal absorption. It is marketed as a racemic mixture, with the S(+)-enantiomer being the more active form.

The mini-tablets were produced using a new manufacturing process designed through Design of Experiments (DoE, **Table 1**). The formulations contained different ratios of excipients and ibuprofen and were compacted at different pressures. The mini-tablets were characterized by weight, tablet strength, friability, disintegration, and dissolution. The results showed that mini-tablets of acceptable weight uniformity, tablet strength, friability, disintegration time, and dissolution could be obtained for most batches. However, the determination of ibuprofen using a spectrophotometric UV method at 222 nm showed that the API content was lower than the expected nominal level. This observation prompted the development of a modified method for ibuprofen quantification, which was based on the USP compendial method for the assay of ibuprofen in standard-size tablets (Ibuprofen tablets, in USP-NF 2024, Issue 2). The micro-HPLC-UV method confirmed the results obtained by the spectrophotometric assay. This study aimed to compare the two methods applied for determining the ibuprofen content in pediatric mini-tablets and assess their equivalence using the Two One-Sided Test (TOST).


**Figure 1** – Mini-tablet dimensions (a), bag used for storing samples (b), and portable micro-HPLC-UV system used (c).

| Batch # | Manufacturing order | Excipients:ibuprofen, (% w/w) | Compaction pressure, (MPa) |
|---|---|---|---|
| 1 | 1 | 99:1 | 100 |
| 2 | 3 | 95:5 | 100 |
| 3 | 8 | 99:1 | 300 |
| 4 | 7 | 95:5 | 300 |
| 5 | 5 | 99:1 | 200 |
| 6 | 9 | 95:5 | 200 |
| 7 | 2 | 97:3 | 100 |
| 8 | 6 | 97:3 | 300 |
| 9 | 4 | 97:3 | 200 |
| 10 | 10 | 97:3 | 200 |
| 11 | 11 | 97:3 | 200 |

**Table 1.** The factorial $3^2 + 2$ experimental plan used to study mini-tablet manufacturing. Batch#, batch number; Run order, manufacturing order. Batches 9, 10, and 11 are center points. Batches 1 to 9 were used to build the models, whereas batches 10 and 11 served as independent replicates for model validation.

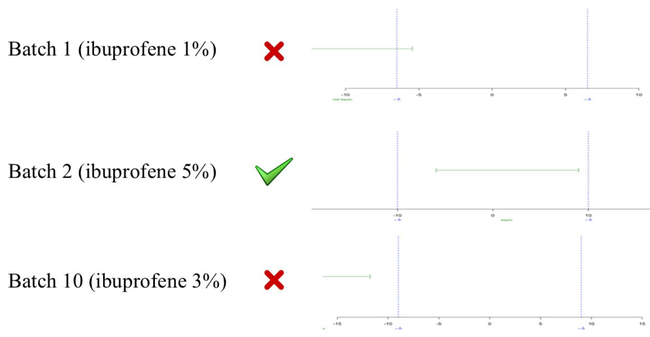


**Figure 2** – An example of the TOST results for three batches

## Chemicals and materials

Methanol and acetonitrile (≥99.9%, gradient grade, LiChrosolv®), Parteck® ODT (mannitol 90-95%, and croscaramellosum-sodium, 3-7%, w/w), magnesium stearate (puriss., meets analytical specification of European Pharmacopoeia), phosphoric acid (85%, for HPLC LiChropur™), ibuprofen (European Pharmacopoeia, Reference Standard), water was produced in-house using a Milli-Q® benchtop EQ 7008/16 Ultrapure and Pure Water Purification System, polyvinylidene fluoride (PVDF)-Millex®-LH 0.45µm membrane filters were used to filter all solutions before analysis. All chemicals and materials were obtained from Merck KGaA (Darmstadt, Germany).

## Spectrophotometry

Apparatus: Perkin-Elmer Lambda 365+ UV-Vis spectrophotometer; 1 cm quartz cuvettes.
Ibuprofen and excipients spectra were acquired in the region from 190 to 400 nm.
Analytical detection wavelength: **222 nm.**

## Chromatography

Apparatus: Axcend Focus LC® micro-HPLC system (**Figure 1c**);

Column: Purospher® STAR RP18-endcapped (2.0 µm), 50x0.3 mm (Merck KGaA, Darmstadt, Germany);

Elution mode, flow rate, column temperature: isocratic, 7 µL/min constant flow, 40°C.

Mobile phase: acetonitrile and water containing 0.05% phosphoric acid 50:50% (v/v),

Analytical detection wavelength: **255 nm**;

Manual injection: 250 nL loop;

**Samples**: Thirteen batches of 500 mini-tablets each were manufactured. The eleven batches listed in Table 1, along with two additional batches, including one placebo batch and one batch optimized for dissolution and physical properties. Each mini-tablet, or group of mini-tablets, was ultrasound-disintegrated in a water-methanol = 1:1 solution for 15 minutes and then left under magnetic stirring for an additional 15 minutes. The suspension was filtered through 0.45 µm (PVDF)-Millex®-LH membrane filters and simultaneously injected into the micro-HPLC system, then submitted to spectrophotometric readings at 222 nm.

## Methods validation

Both methods were validated by the ICH Q2 (R2) guidelines. Briefly, specificity, linearity, precision, and accuracy were assessed and were considered satisfactory for the study (**Table 2**).

| Parameter | Spectrophotometry @222nm | Micro-HPLC-UV @ 255 nm |
|---|---|---|
| Specificity | Specific | Specific (selective) |
| Linearity (5 points, range 10-40 µg/mL) | Absorbance (AU) vs Ibuprofen concentration<br>$y = (0.042 \pm 0.01) \cdot x + (0.03 \pm 0.02)$<br>Residuals ~ N(0, 4E-3), r > 0.999 | Peak area (mAU·s) vs Ibuprofen concentration<br>$y = (0.530 \pm 0.009) \cdot x + (-1.1 \pm 0.2)$<br>Residuals ~ N(0, 0.1), r > 0.998 |
| Quantification limit (µg/mL) | 6 | 6 |
| Repeatability (RSD%, N=6, one day) | 3% | 8% |
| Intermediate precision (RSD%, N=6, two days) | 5% | 13% |
| Accuracy (REC%, N= 3 @ 10, 25, and 40 µg/mL) | 99-104% | 96-98% |

**Table 2** – Methods' validation figures

## Results and discussion

The specificity of either the spectrophotometric or micro-HPLC-UV methods was confirmed by assaying the pure reference compounds and artificial mixtures mimicking the composition of authentic mini-tablets (**Figure 3**). Only ibuprofen exhibited relevant UV absorption at 222 nm in a methanol:water (1:1, v/v) mixture. The analytical wavelength used for the micro-HPLC-UV assay, 255 nm, is recommended by the USP compendial method. Although ibuprofen has a very low extinction coefficient at this wavelength ($\epsilon$ ~0.03 $M^{-1} \cdot cm^{-1}$), it is a suitable choice for detecting ibuprofen and related substances in tablets.

Linearity was satisfactory for both methods (intercept not statistically significant, homoscedastic residuals normally distributed, correlation coefficient r > 0.998), showing proportionality between the method response and ibuprofen nominal concentration. Repeatability and intermediate precision were sufficient for this study. However, the precision figures of the micro-HPLC-UV method did not meet the quality level required for quality control (QC) pharmaceutical analysis (**Table 2**).

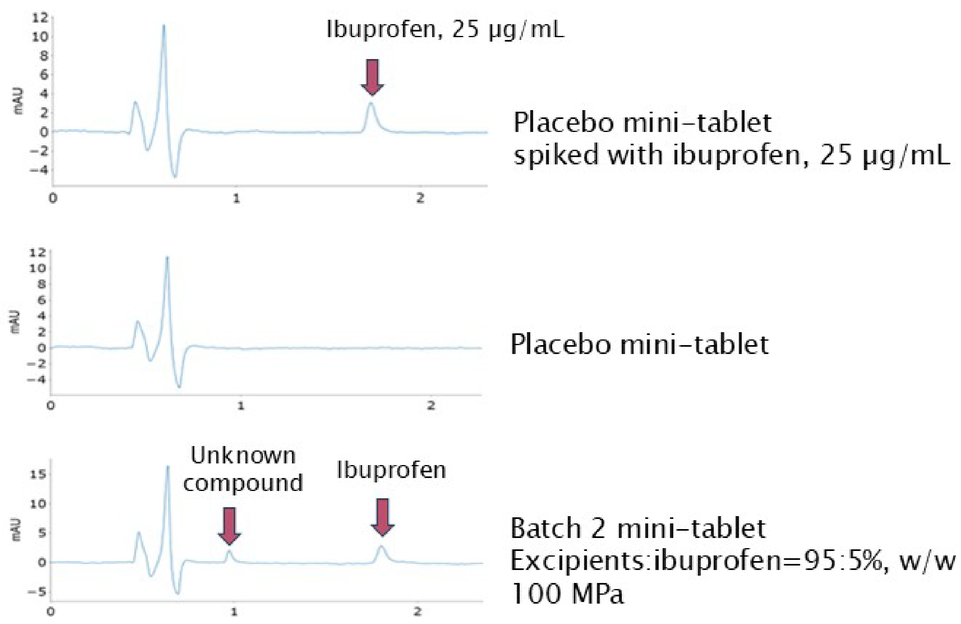


**Figure 3** – Micro-HPLC-UV method selectivity and an example of mini-tablet assay. An unknown compound is found in all batches of mini-tablets manufactured

## Results and discussion *(continued)*

The accuracy study evaluated recovery percentages (REC%) at three concentration levels (low, medium, and high) for each method calibration curve. The REC% values assessed on artificial samples and spiked placebos consistently ranged between 95% and 105%, confirming the quantitative reliability of both methods. However, when authentic samples were assayed, both methods showed recoveries lower than the nominal value expected in most mini-tablet batches (**Table 3**).

| Batch # | Spectrophotometry (222 nm) | micro-HPLC-UV (255 nm) |
|---|---|---|
| 1 | 69.1 | 58.9 |
| 2 | 101.0 | 102.5 |
| 3 | 63.8 | 44.0 |
| 4 | 84.3 | 75.0 |
| 5 | 64.0 | 42.4 |
| 6 | 93.3 | 77.7 |
| 7 | 97.2 | 86.3 |
| 8 | 87.9 | 76. 9 |
| 9 | 107.8 | 93.7 |
| 10 | 95.0 | 78.0 |
| **Optimized** | 86.5 | 87.1 |

**Table 3** – Ibuprofen recoveries of the mini-tablets.

The equivalence test (Two-One-Sided-Test) also failed, as many of the batches studied showed a confidence interval for the difference in the mean results exceeding the boundaries of the equivalence interval set, which was ±10% of the mean recovery for each batch (**Figure 2**). This outcome was attributed to an unknown peak in the chromatograms, likely originating from a compound more polar than ibuprofen. As shown in **Figures 3 and 4**, this compound is less abundant in Batch 2 compared to Batches 1 and 3 and was undetectable by spectrophotometry because it does not absorb at 222 nm.

The presence of this unknown compound in the batches of the formulations manufactured also explains why the recovery of ibuprofen in the mini-tablets was lower than expected. Also, data analysis revealed the amount of the unknown substance is directly proportional to both the compaction pressure and the quantity of mannitol-croscarmellose sodium used (**Figure 4**).

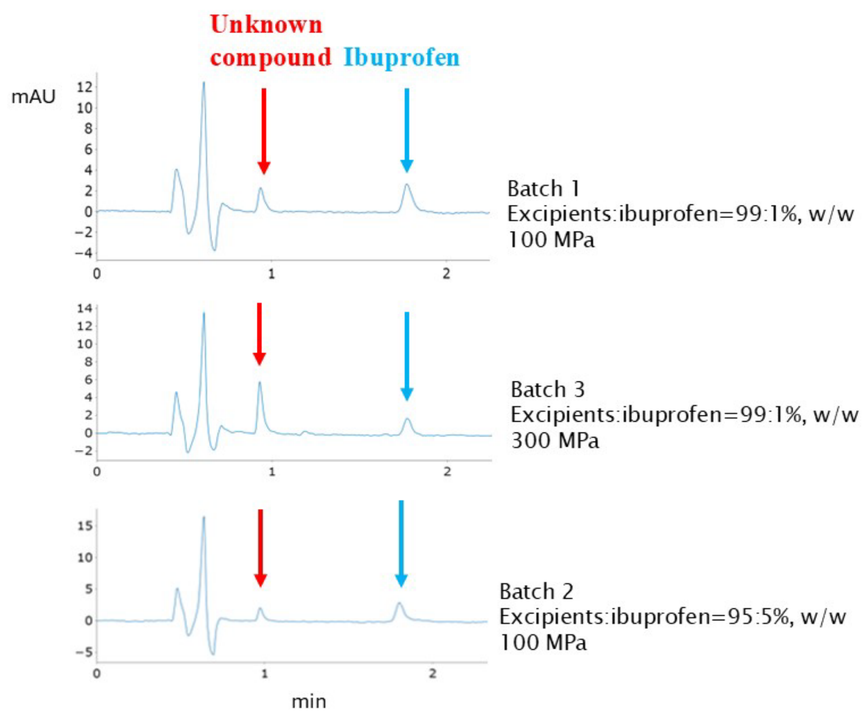


**Figure 4** – Chromatograms of mini-tablets solutions: the abundance of the unknown peak is proportional to the Excipients:ibuprofen ratio and to the compaction pressure.

## Conclusions

Two independent analytical methods were validated for quantifying ibuprofen in pediatric mini-tablets. While the spectrophotometric method was successfully validated as being faster and more cost-effective, it failed to detect an unknown compound that was invisible at the selected analytical wavelength (222 nm). The only indication of its presence came from the non-quantitative recoveries observed in real samples but not in spiked placebo samples.

In contrast, micro-HPLC-UV successfully revealed the unknown peak, corresponding to a more polar compound related to the mini-tablet manufacturing process.

This case study confirms that spectrophotometry alone cannot guarantee sufficient specificity for drug assays when potential interferents cannot be ruled out *a priori*. Thus, chromatographic methods remain essential for reliable quantitative analysis in pharmaceuticals.

An LC-MS/MS study is currently underway to determine the structure of the unknown molecule, with the goals of identifying its origin (e.g., degradation product, excipient interaction, or process-related impurity) and optimizing the manufacturing process of the mini-tablets to prevent its formation.

## References

1. Current Ibuprofen Tablets USP monograph, DOI: https://doi.org/10.31003/USPNF_M39890_01_01. June 10, 2025

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