

Effect of consumer reconstitution practices on acetaminophen concentration in sachet formulations: Pilot study

Effet des pratiques de reconstitution sur la concentration de paracétamol dans les formulations en sachet: Etude pilote

Yessin Mokni^{1,2}, Nesrine Zitouni^{1,3}, Kaouther Zribi^{1,4}, Sarra Berriri^{1,5}, Fethi Safta¹

1. Laboratory of Chemical, Galenic and Pharmacological Development of Medicines (LR12ES09), University of Monastir, Faculty of Pharmacy of Monastir, Ibn Sina Street, 5000 Monastir, Tunisia
2. Pharmacy Department, Regional Hospital of Djerba, Djerba, Tunisia
3. Pharmacy Department, Regional Hospital of Kef, El Kef, Tunisia
4. Hygiene Laboratory, Hedi Chaker University Hospital, Sfax, Tunisia
5. Pharmacy Department, Farhat Hached University Hospital Center, Sousse, Tunisia

ABSTRACT

Background: Single-dose powder sachets are used to overcome solubility limitations of several actives such as acetaminophen. After reconstitution, residual deposits and sedimentation may reduce the delivered dose.

Objectives: Develop and validate a rapid assay for acetaminophen quantification in sachet preparations and evaluate consumer reconstitution practices to generate provisional, product-specific recommendations.

Methods: Prospective pilot study on four commercial sachet products. Twenty-eight volunteers (7 per product) simulated preparation and administration. Studied preparation variables were: reconstitution volume (mL) and stirring time (s). An aliquot of each preparation was analyzed by the validated chromatography (HPLC) method. Correlation coefficients between measured concentrations and preparation variables were calculated. Product-specific predictive models were generated for variables showing significant association. Optimal reconstitution conditions were estimated from these models.

Results: Inter-individual variability was large: SD(volume) = 27.76 mL; SD(stirring time) = 8.98 s. Recovered concentrations ranged from 59.4% to 99.7% of the predicted value; only 4/28 preparations reached $\geq 97.5\%$ of expected concentration. Product-specific optimal conditions calculated were — Product 1: stir 37 s, volume 90 mL; Product 2: volume 178 mL, stir 7 s; Product 3: stir 11 s, volume 64 mL.

Conclusion: In this exploratory pilot, consumer reconstitution of acetaminophen sachets produced substantial variability and frequent under-delivery. The validated HPLC assay enabled derivation of provisional, product-specific reconstitution parameters.

Keywords: Acetaminophen, Reconstitution, High-performance liquid chromatography, Medication safety

RÉSUMÉ

Contexte : La forme sachet (poudre/granulé) est utilisée pour pallier des problèmes de solubilité de principes actifs tels que le paracétamol. Après reconstitution, des dépôts résiduels et une sédimentation peuvent réduire la dose délivrée.

Objectifs : Développer et valider une méthode rapide pour doser le paracétamol dans des préparations issues de sachets et évaluer les pratiques de reconstitution des usagers afin de proposer des recommandations préliminaires, spécifiques aux produits.

Méthodes : Étude prospective observationnelle pilote portant sur quatre spécialités commerciales. Vingt-huit volontaires (7 par produit) ont simulé la préparation et l'administration. Les variables étudiées sont : volume de reconstitution (mL) et temps d'agitation (s). Chaque préparation a été analysée par la méthode chromatographique HPLC validée. Les coefficients de corrélation entre concentrations mesurées et variables préparatoires ont été calculés ; des modèles prédictifs produits-spécifiques ont été construits pour les corrélations significatives. Conditions optimales ont été estimées à partir des modèles.

Résultats : Grande variabilité interindividuelle : écart-type du volume = 27,76 mL ; écart-type du temps d'agitation = 8,98 s. Les concentrations retrouvées variaient de 59,4 % à 99,7 % de la concentration théorique ; 4/28 préparations atteignaient $\geq 97,5\%$ de la valeur attendue. Les conditions optimales calculées sont : Produit 1 — agitation 37 s pour 90 mL ; Produit 2 — 178 mL et agitation 7 s ; Produit 3 — agitation 11 s pour 64 mL.

Conclusion : Ces données pilotes montrent une variabilité importante des pratiques de reconstitution et une tendance fréquente au sous-dosage. La méthode HPLC validée a permis de proposer des paramètres de reconstitution préliminaires.

Mots-clés : Paracétamol, Reconstitution, Chromatographie liquide haute performance, Sécurité médicamenteuse

Correspondance

Yessin Mokni

Lr12es09 - Faculty of pharmacy Monastir – Monastir University – Tunisia

Email: yassinmokni@live.fr

What is already known

- Sachet formulations of acetaminophen are widely used and can improve bioavailability, but manufacturers often do not provide precise reconstitution instructions (volume, mixing time).
- Reconstitution by caregivers or patients is a known source of dosing error for oral suspensions.
- Analytical quantification of acetaminophen in reconstituted samples is feasible by HPLC, but few data exist describing real-world consumer reconstitution of commercial sachet products.

What this article adds

- Development and validation of a rapid, specific and precise HPLC assay fit-for-purpose to quantify acetaminophen in reconstituted sachet preparations.
- An observational pilot ($n = 28$) showing large inter-individual variability in reconstitution and frequent under-delivery (only 4/28 preparations $\geq 97.5\%$ of expected concentration).
- Product-specific, model-derived provisional reconstitution parameters (Product 1: 90 mL/37 s) that can inform labelling, patient counselling and the design of larger confirmatory studies.

INTRODUCTION

Acetaminophen, alone or in combination with other actives, is marketed in 168 different formulations in Tunisia. It is the most commonly used analgesic for self-medication. Sachet formulations (powder or granulate for oral use) are widely used because they can improve bioavailability, facilitate administration and increase acceptability through flavoring; they also help to mitigate acetaminophen instability in the aqueous phase. (1–3) However, two practical issues are frequently observed after reconstitution: residual powder retained in the sachet and particle sedimentation, both of which may reduce the delivered dose. Moreover, manufacturers do not consistently provide detailed reconstitution instructions (volume, agitation, rest time), leaving substantial room for user-dependent variability. Several studies have assessed reconstitution practices among health professionals—especially for pediatric formulations—but few data describe how lay users actually prepare sachet products. (4–6)

In the present study, we aimed to develop and validate a rapid, specific and linear HPLC method for the quantification of acetaminophen in reconstituted sachet preparations and evaluate consumer reconstitution practices for selected commercial products to generate provisional, product-specific recommendations.

METHODS**Analytical method development and reagents**

All reagents were analytical grade. Methanol 99.8% (LOBA CHEMIE PVT. LTD.[®]), anhydrous sodium dihydrogen phosphate (BIO BASIC INC.[®]), orthophosphoric acid 85% (PANREAC[®]) and laboratory bi-distilled water were used. Pharmaceutical-grade acetaminophen, ascorbic acid and pseudoephedrine served as reference substances.

Chromatographic conditions

Analyses were performed on a YOUNGLIN INSTRUMENTS[®] YL-Inst 9300 HPLC system equipped with a gradient pump, a RHEODYNE[®] manual injector and a UV-Visible detector. Separation used a C18 reverse-phase column (250×4.6 mm, $5 \mu\text{m}$, Discovery HS[®]). Mobile phase: 0.05 M NaH_2PO_4 (solvent A) / methanol (solvent B) 65:35 (v/v); pH adjusted to 3.0 with orthophosphoric acid. The mobile phase was filtered ($0.45 \mu\text{m}$) and degassed. Flow rate: $1.0 \text{ mL} \cdot \text{min}^{-1}$. Detection wavelength: 220 nm. Total run time: 7 min. Before injection, each test solution was filtered through a $0.45 \mu\text{m}$ mixed cellulose ester syringe filter.

Method validation

Validation followed ICH Q2(R1), French Society of Pharmaceutical Sciences and Techniques SFSTP recommendations. (7–9)

- **Specificity:** Assessed by injection of individual standards (acetaminophen, ascorbic acid, and pseudoephedrine) and a mixture to exclude co-elution or interfering peaks at the acetaminophen retention time.
- **Linearity and range:** Linearity was evaluated over $24\text{--}56 \text{ mg} \cdot \text{L}^{-1}$ (five levels in triplicate). The target concentration ($40 \text{ mg} \cdot \text{L}^{-1}$) was chosen as the midpoint of this range, with acceptance defined as $r^2 \geq 0.99$ and evaluation of the regression equation and residuals.
- **Precision** was assessed as repeatability—six replicates at target level ($40 \text{ mg} \cdot \text{L}^{-1}$) analyzed on the same day, results expressed as CVr% (acceptance $\leq 2\%$)—and intermediate precision—six replicates of target level analyzed across three consecutive days, results reported as CVR%.
- **Accuracy** : Determined by preparing homogenized samples from five sachets per product and comparing measured concentrations to the standard calibration; Cochran and Fisher tests used to check variance homogeneity and mean validity ($\alpha = 0.05$). Recovery confidence intervals were evaluated.

Reconstitution procedure and Statistical analysis

For this pilot study, no power calculation was performed. Seven participants per product was deemed the minimum needed for a two-predictor linear regression model. Each participant received: one sachet of the assigned

commercial product, a test tube containing 400 mL of water, a standard drinking glass and a spoon. No instructions were given on how to reconstitute; participants were asked to prepare the product as they would in real use. The following variables were recorded for each preparation:

- Volume of water used (mL)
- Stirring/agitation time (seconds)
- Resting time before ingestion (seconds).

An aliquot, taken from the middle depth of the solution using a micropipette, of each preparation was sampled and diluted with mobile phase to obtain a test solution corresponding to 40 mg·L⁻¹ for HPLC analysis.

Normality was assessed by the Kolmogorov-Smirnov test. Continuous variables are reported as mean ± SD. Pearson correlation coefficients (*r*) were calculated between measured acetaminophen concentration (dependent variable) and each preparation parameter (volume, stirring time, resting time). For variables showing significant associations (*p* < 0.05), simple linear regression models were estimated to generate provisional predictive equations. Model performance was summarized using *R*² and ANOVA. Statistical analyses were performed with GNU PSPP™; $\alpha = 0.05$.

RESULTS

Analytical method validation

- **Specificity:** No interfering peaks were observed at the acetaminophen retention time when ascorbic acid and pseudoephedrine standards were injected (representative chromatogram in figure 1: acetaminophen *t*_R = 4.12 min; ascorbic acid *t*_R = 2.87 min; pseudoephedrine *t*_R = 4.93 min). Chromatograms for all 28 reconstituted preparations are provided in Supplementary Material S1.

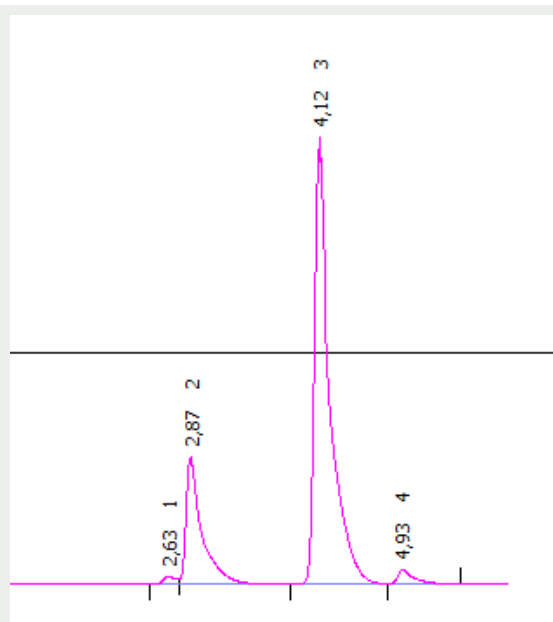


Figure 1. Chromatogram of a mixture of acetaminophen (*t*_R = 4.12 min), ascorbic acid (*t*_R = 2.87 min) and pseudoephedrine (*t*_R = 4.93 min)

- **Linearity:** Calibration across 24–56 mg·L⁻¹ produced equation $Y = 38.592 \cdot X - 57.979$ with $r^2 = 0.9903$.
- **Precision:** For the 40 mg·L⁻¹ level, Cochran *C* = 0.445 (< *C*_{crit} 0.677) indicating homogeneity of variances. Repeatability CV_r = 0.563% and intermediate precision CV_r = 1.248% (both < 2%).
- **Accuracy:** Cochran and Fisher tests satisfied homogeneity and mean comparison criteria (*C* < *C*_{crit}; *F* < *F*_{crit}). Average recoveries and their confidence intervals were acceptable within 24–56 mg·L⁻¹.

Reconstitution procedure and Statistical analysis

The Kolmogorov-Smirnov test was applied to assess normality. The obtained *p*-values were not significant for steering time (*p* = 0.082), volume used (*p* = 0.2), and measured concentration (*p* = 0.2).

Across 28 preparation, mean water volume was 110.14 mL (SD = 27.76 mL), stirring time mean = 14.78 s (SD = 8.98 s), resting time mean = 5.11 s (SD = 3.50 s). Only 4/28 users achieved ≥97.5% of theoretical concentration. Product specific means ranged from 67.56% to 93.04% of the target (Table 1).

Table 1. Comparison of Measured and Theoretical Acetaminophen Concentrations Across the Four Tested Specialties

Specialty	Mean Measured Concentration (mg·L ⁻¹)	Theoretical Concentration (mg·L ⁻¹)	Mean Recovery (%)	Standard Deviation (%)
Specialty 1	37.22	40.00	93.04%	±4.29
Specialty 2	35.50	40.00	88.76%	±9.84
Specialty 3	34.79	40.00	86.96%	±8.52
Specialty 4	27.02	40.00	67.56%	±5.40

Predictive Modeling

Significant correlations were found for three products, No significant correlation was found for product 4, are summarized in Table 2.

Table 2. Pearson Correlation Coefficients between Acetaminophen Concentration and Reconstitution Parameters (Volume, Stirring Time, and Resting Time) across the Four Pharmaceutical Specialties

Specialty	Volume		Stirring Time		Resting Time	
	<i>R</i>	<i>p</i> -value	<i>R</i>	<i>p</i> -value	<i>R</i>	<i>p</i> -value
Specialty 1	0.379	0.201	0.727	0.032	0.132	0.389
Specialty 2	0.823	0.017	-0.197	0.295	0.013	0.488
Specialty 3	-0.809	0.001	0.601	0.033	0.400	0.177
Specialty 4	0.499	0.202	-0.389	0.270	-0.635	0.203

To further quantify the influence of these variables and determine optimal reconstitution conditions, predictive linear models were constructed for each of the three products using the significantly correlated parameters. The statistical significance of each model and its coefficients was evaluated using analysis of variance (ANOVA), are presented in Table 3.

Table 3. Summary of Model Equations and Coefficient Significance (Specialties 1 to 3)

Specialty	Model Equation	X1 Coefficient (p-value)	X2 Coefficient (p-value)	Intercept (p-value)	Statistical Test
Specialty 1	$Y = 0.134 \times X2 + 35.02$	-	0.134 (p = 0.063)	35.02 (p < 0.001)	ANOVA
Specialty 2	$Y = 0.074 \times X1 + 26.79$	-	0.074 (p = 0.035)	26,79 (p < 0.001)	ANOVA
Specialty 3	$Y = -0.137 \times X1 + 0.362 \times X2 + 44.74$	0.137 (p = 0,002)	0.362 (p = 0,016)	44,74 (p < 0.01)	ANOVA

The following optimal conditions were identified:

- Product 1: 90 mL water, 37 seconds stirring
- Product 2: 178 mL water, 7 seconds stirring
- Product 3: 64 mL water, 11 seconds stirring

DISCUSSION

The analytical procedure developed and validated for this work proved fit-for-purpose for the observed concentration range (linearity $r^2 = 0.9903$; repeatability CVr = 0.563%; intermediate precision CVR = 1.248%), supporting the reliability of the measured values and the exploratory models derived from them.

Observed preparation behaviors varied widely: participants used a broad range of water volumes (SD = 27.76 mL) and stirring times (SD = 8.98 s). Such variability materially affected the final concentration (only 4/28 preparations reached $\geq 97.5\%$ of the expected concentration) and product-specific mean recoveries ranged from 67.6% to 93.0%. These findings echo previous reports of frequent reconstitution errors with oral suspensions and highlight that even formulations intended to simplify administration are vulnerable to user-dependent dosing error.(10–12)

The predictive models generated for three of the four tested products suggest that simple, product-specific guidance could substantially reduce under-delivery. For example, model-based provisional parameters (90 mL/37 s for Product 1) provide concrete, testable recommendations that could be integrated into labeling and patient information.

The clinical implications of the observed under-dosing deserve cautious consideration. Systematic under-delivery may reduce symptomatic control (analgesia/antipyresis) and, in some contexts, prompt compensatory behaviors (dose stacking, concomitant medication) that could carry safety risks. Epidemiological data on paracetamol dosing errors indicate that misuse can have severe consequences. However, our pilot did not assess clinical outcomes and thus cannot quantify patient-level risk. Larger, representative studies linking reconstitution behavior to pharmacodynamic or clinical endpoints are therefore needed.

Study strengths include the combined analytical and behavioral approach, the use of validation criteria aligned with ICH/SFSTP/GLP guidance, and the production of actionable, product-specific provisional recommendations. Key limitations are: small sample (n = 28) an observational simulation setting that may not capture real-world distractions (possible Hawthorne effect) and testing of only four commercial products.

Implications are clear and pragmatic: manufacturers

should provide explicit, quantitative reconstitution instructions (specified volume and minimal mixing time) on sachet labeling and consider supplying a simple graduated device or pictograms to reduce user error; pharmacists and other healthcare professionals should systematically counsel patients on correct preparation and, where possible, provide a dosing device or a written instruction leaflet. Packaging innovations may offer a longer-term engineering solution to user variability. (13,14)

CONCLUSION

In this exploratory pilot, consumer reconstitution of acetaminophen sachets produced large inter-individual variability and frequent under-delivery of active substance. The HPLC method validated for this study provided reliable quantification and allowed generation of product-specific provisional reconstitution parameters. Given methodological limitations inherent to a pilot design, these findings should be interpreted as hypothesis-generating: larger, representative and multicentre studies are required to confirm the results and to evaluate clinical impact. Meanwhile, clearer, quantitative reconstitution instructions on product labeling and proactive counseling by pharmacists are pragmatic measures likely to improve dosing accuracy and patient safety.

REFERENCES

1. AMMs par DCI [Internet]. 2025 [cited 2025 Apr 21]. Available from: <http://www.dpm.tn/index.php/medicaments-a-usage-humain/amms-par-dci>
2. De Sutter AI, Eriksson L, van Driel ML. Oral antihistamine-decongestant-analgesic combinations for the common cold. *Cochrane Database Syst Rev*. 2022 Jan 21;1(1):1. doi:10.1002/14651858.CD004976.pub4 PubMed PMID: 35060618; PubMed Central PMCID: PMC8780136.
3. Bouden S, Abid Y, Rouached L, Tekaya AB, Mahmoud I, Tekaya R, et al. Self-medication with analgesics in rheumatology: Frequency and associated factors. *Tunis Med*. 2025;103(12). doi:10.62438/tunismed.v103i12.5918
4. Controlling the Stability of Medicinal Suspensions [Internet]. [cited 2026 May 4]. Available from: <https://www.americanpharmaceuticalreview.com/Featured-Articles/331619-Controlling-the-Stability-of-Medicinal-Suspensions/>
5. Nguyen D, Secretan PH, Auvity S, Vidal F, Postaire M, Cisternino S, et al. Assessment of practices for suspended oral drugs by tablet crushing in pediatric units. *Eur J Pharm Biopharm*. 2020 Dec;157:175–82. doi:10.1016/j.ejpb.2020.10.013 PubMed PMID: 33222769.
6. Ménétré S, Weber M, Socha M, Le Tacon S, May I, Schweitzer C, et al. Préparation et administration des médicaments dans les services

- de pédiatrie. Évaluation des pratiques des soignants. Archives de Pédiatrie. 2018 Apr 1;25(3):3. doi:10.1016/j.arcped.2017.12.002
7. Hubert Ph, Chiap P, Crommen J, Boulanger B, Chapuzet E, Mercier N, et al. The SFSTP guide on the validation of chromatographic methods for drug bioanalysis: from the Washington Conference to the laboratory. *Analytica Chimica Acta*. 1999 May 31;391(2):2. doi:10.1016/S0003-2670(99)00106-3
 8. ICH Q2(R2) Validation of analytical procedures - Scientific guideline | European Medicines Agency (EMA) [Internet]. 2024 [cited 2025 Apr 21]. Available from: <https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures-scientific-guideline>
 9. Hubert P, Nguyen-Huu JJ, Boulanger B, Chapuzet E, Chiap P, Cohen N, et al. Harmonization of strategies for the validation of quantitative analytical procedures. A SFSTP proposal--Part I. *J Pharm Biomed Anal*. 2004 Nov 15;36(3):3. doi:10.1016/j.jpba.2004.07.027 PubMed PMID: 15522533.
 10. Kumarasinghe M, Weerasinghe MC. Reconstitution of oral antibiotic suspensions for paediatric use in households: a cross-sectional study among caregivers of 3-5-year-old children from a selected district, Sri Lanka. *BMC Pediatr*. 2024 Apr 4;24(1):1. doi:10.1186/s12887-024-04725-y PubMed PMID: 38575910; PubMed Central PMCID: PMC10996081.
 11. Berthe-Aucejo A, Girard D, Lorrot M, Bellettre X, Faye A, Mercier JC, et al. Evaluation of frequency of paediatric oral liquid medication dosing errors by caregivers: amoxicillin and josamycin. *Arch Dis Child*. 2016 Apr;101(4):4. doi:10.1136/archdischild-2015-309426 PubMed PMID: 26729746.
 12. Sonji GM, Sonji NM, Boukhary R, Hodeib F, Majzoub MS, Assi M. Caregiver Views and Practices Regarding Children's Antibiotic Suspensions in Lebanon. *Jordan Journal of Pharmaceutical Sciences*. 2025 Mar 25;18(1):90–103. doi:10.35516/jjps.v18i1.2495
 13. World Health Organization. WHO pictograms. Geneva: WHO; 2009.
 14. Idriss-Wheeler D, Ormel I, Assefa M, Rab F, Angelakis C, Yaya S, et al. Engaging Community Health Workers (CHWs) in Africa: Lessons from the Canadian Red Cross supported programs. *PLOS Glob Public Health*. 2024 Jan 18;4(1):1. doi:10.1371/journal.pgph.0002799 PubMed PMID: 38236795; PubMed Central PMCID: PMC10796059.