

REVIEWER'S REPORT

Manuscript No.: IJAR-56671

Title: Stability Monitoring of Phytogenic Constituents and Physicochemical Properties of Pulmofarm® T Herbal Respiratory Premix during Feed Pelletization Stress.

Recommendation:

Accept as it is

Accept after minor revision.....

Accept after major revision

Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality	-			
Techn. Quality		-		
Clarity	-	-		
Significance				

Reviewer's ID: JPR- 212

Detailed Reviewer's Report

This manuscript presents a well-designed and executed study evaluating the stability of a commercial herbal feed additive, Pulmofarm® T Premix, under simulated feed pelletization conditions. The research addresses a relevant and practical issue in the animal feed industry, where the efficacy of heat-sensitive phytogenic compounds is a concern. The methodology is robust, employing validated chromatographic techniques (RP-HPLC-PDA and HPTLC) alongside standard physicochemical analyses. The data largely supports the conclusion that the formulation is stable under the tested conditions. The paper is generally well-structured, but several areas require attention to improve clarity, correct inconsistencies, and strengthen the presentation of results before publication.

Major Revisions Required

1. Data Consistency and Presentation:

- **Issue:** There are several inconsistencies between the text, tables, and figures that need to be resolved.
 - **Thymol RT:** The abstract states the retention time (RT) for thymol is 18.30 min (line 19), while Figure 5 shows it as 18.47 min. Please verify and correct for consistency.
 - **Glycyrrhizin RT:** The abstract states the RT for glycyrrhizin is 16.60 min (line 19), while Figure 4 shows it as 16.82 min. Please verify and correct for consistency.
 - **Table 5 vs. Table 6 Control Values:** The control value for thymol in Table 5 is 281.21 ppm, but in the abstract (line 18), it's 280.21 ppm. The abstract's value should be updated to match the data in the results table.
 - **Figure 3 Caption:** The caption for Figure 3 is incomplete. It lists (A), (B), and (D) but omits (C). The text should be revised to correctly identify all panels in the figure (likely A, B, C for the three samples and D for the overlay).

2. Methodology Clarification:

- **Pelletization Stress Conditions (Lines 125-128):** The study exposes the premix to 90°C and 2.0% moisture. The rationale for choosing these specific parameters should be briefly justified. Are these conditions representative of standard industry pelletization processes?

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A short sentence citing industry standards or common practice would strengthen the experimental design's validity.

- **Thymol Assay Development (Lines 152-165):** The manuscript states the thymol protocol underwent new in-house development (line 225). While validation data is presented in Tables 2, 3, and 4, the text should explicitly state that the method was validated according to ICH Q2(R1) guidelines, similar to the statement for glycyrrhizin. The current phrasing ("in-house development and validation") is weaker than a clear statement of adherence to international standards.

3. Discussion of Results:

- **HPTLC Data Interpretation (Table 7):** Table 7 shows a -6.28% difference in total peak area for the chloroform fraction at 480 nm after 10 minutes. While the authors state variations within $\pm 10\%$ are acceptable (line 237), this value is approaching the limit and is notably higher than other measurements. A brief comment in the discussion explaining why this might be (e.g., inherent variability of the chloroform extract, presence of more volatile components) would be a good demonstration of critical data analysis and would preempt potential reader questions. The conclusion that "no significant degradation occurred" is still valid, but acknowledging this point adds nuance.

Minor Revisions / Technical Corrections

1. Abstract:

- Line 18: "280.21 ppm $\pm$ 4.45%" - It is unclear if the $\pm$ value is a standard deviation or relative standard deviation (RSD). Please specify.
- The abstract is clear and provides a good summary of the work.

2. Introduction:

- Line 40: The product name is spelled as "Pulmoform T Premix" here, while it is "Pulmofarm® T Premix" in the title and abstract. Please standardize the spelling throughout the manuscript (Pulmofarm® T appears to be the correct trademarked name).
- Line 54: "Adhatodavascica" should be "Adhatoda vasica" (italicized as per binomial nomenclature).

3. Materials and Methods:

- Line 117: "latest controlled sample" – please clarify what "latest controlled" means. Does it refer to a recently manufactured sample, a quality control sample, or something else?
- Line 192-193: "precooled" – Did the authors mean "pre-coated"? TLC plates are typically pre-coated with silica gel. "Precooled" is an unusual term here and likely a typo.

4. Results and Discussion:

- Line 222: The text says the retention time for glycyrrhizin is 18.30 minutes and for thymol is 16.60 minutes. This appears to be a transposition error based on the figures and abstract. Please correct.
- Table 2: The title "Table 2: Parameters" is too vague. A more descriptive title is needed, such as "Table 2: System Suitability Parameters for the Thymol RP-HPLC-PDA Method."
- Table 4: The table formatting for the "Recovery" data is confusing. The header row for the three concentration levels (0.0, 0.2, 0.4 mg g⁻¹ added) should be clearly separated from the other rows. Consider using a more standard format for presenting recovery data.
- Table 6: The control value in the table body is 2542.91 ppm, but a footnote shows 2450.54 ppm for the 10-minute sample. This is correct, but the footnote in the table seems misplaced, as the main table already contains the data. The footnote format should be cleaned up.

5. Conclusion:

- The conclusion is well-written and accurately summarizes the findings and their implications.

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6. References:

- Reference 5 (Hajare S. et al., 2020) appears incomplete. Please provide full details (journal, volume, issue, pages) if published, or indicate it is a technical report or internal document if that is the case.
- Reference 16 (World Health Organization, 1997) is a report on antimicrobial use and seems out of place for supporting ICH Q2(R1) validation guidelines. The correct reference for ICH Q2(R1) is already provided (Ref 15). Please double-check the citation for Ref 16 in the text (line 226) and remove it if it was cited in error. The current citation seems to be for the WHO report, not the ICH guideline