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Develop and Validate a Stability-Indicating HPLC Assay Method for Paracetamol, and parabens in Pharmaceutical Dosages

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ABSTRACT
The drug's stability, which is a prerequisite for good drug quality, cannot be simply established unless a reliable and trustworthy method for demonstrating stability is applied. Creating such a method is a challenging task. In this work, we developed and validated a reversed-phase stability indicating liquid chromatographic technique with photo diode-array detection to analyze paracetamol (PCM), methylparaben (MP), propylparaben (PP), and their degradants in commercial oral suspension. The compounds and their degradants were separated using a Phenomenex C8 column (250 mm x 4.6 mm, 5 µm) and a mobile phase of pH 4 consisting acetonitrile, potassium di-hydrogen ortho-phosphate, tri-ethylamine, and glacial acetic acid in a 40:58:1.5:0.5 ratio, at 1.2 mL/min. All chemicals separated in within 12 min. The developed method followed the guidelines of the International Conference on Harmonization (ICH) and fulfilled all its requirements in terms of linearity, recovery, reproducibility, detection, and quantitation limits. The dependability of the developed method was shown by recoveries of 98.88% to 101.80% in 102 samples from 34 batches, indicating that it can be used in quality control step for paracetamol, methylparaben, and propylparaben in oral suspension products.

CONTENTS

1. Introduction:

Stability indicating assay method (SIAM) can be defined as a validated analytical method that can reliably be used for the analysis of active ingredients and other substances in pharmaceutical products and produces accurate results without interference from impurities [1]. SIAM is extensively applied during pharmaceutical quality control processes to measure the integrity and stability of pharmaceutical products, which is important for their safety and efficacy [2,3]. Furthermore, each active pharmaceutical ingredient (API) and preservative substance should have a certain

concentration range to be effective [4]. Degradation of these substances to form new chemicals as a result of exposure to undesirable environmental conditions, such as light, humidity, and/or temperature, also has permissible limits; otherwise, toxicity, contamination, or reduced efficacy of the pharmaceutical products may be observed [5]. This indicates that the development of an analytical assay method that is capable of separating and estimating API and preservative substances from their degradants is essential and should meet the SIAM criteria. Paracetamol (PCM) (Acetaminophen, N-acetyl-p-aminophenol) is used as a pain reliever and antipyretic drug [6]. It has a favorable safety profile that is used as a large and important segment of society, especially those requiring special care such as the elderly [6], children [7] and pregnant women [8] compared to other analgesics. PCM is still considered a first-line analgesic for mild to moderate acute pain in patients with liver or kidney disease, cardiovascular disease, gastro-intestinal disorders, and asthma [9].

The paracetamol oral suspension usually contains PCM as an active pharmaceutical ingredient (API) and both methylparaben (MP) and propylparaben (PP) as preservatives in a suitable vehicle that contains flavoring and other aiding substances [10]. On this basis, it is necessary to have a sensitive and accurate analytical SIAM method that can separate and estimate the concentrations of active ingredients, preservatives, and their degradation products that may be formed during manufacturing and storage [11].

HPLC is a necessary technique to be used in stability indicating assay for separating drug substances and degradation impurities simultaneously [12].

A literature survey revealed the availability of several HPLC methods developed as SIAM to determine PCM in combination with other pharmaceutical drugs such as caffeine [13], methylparaben with carmosine [14], and thiamine and pyridoxal phosphate [15]. In addition, simultaneous analysis of paracetamol in combined pharmaceutical formulations was performed using traditional [16] and multivariate HPLC [17].

However, to the best of our knowledge, no method has been reported for the simultaneous separation and determination of a mixture containing PCM, MP, and PP, along with their degradants, in oral suspension pharmaceutical products. Thus, we developed and validated a rapid, accurate, and stability indicating HPLC method for the analysis of oral suspension products containing PCM as an API and both MP and PP as preservatives in the presence of their degradants, flavors, and other aiding substances.

2. Methodology

2.1. Method Development

To develop an analytical method, it must include the steps necessary to conduct each analytical test. This can include the sample, reference materials, reagents, control preparations and use of the device to achieve finally System Suitability Testing (SST) [18,19].

2.1.1. Apparatus

This method was developed and validated using a JASCO high-performance liquid chromatography system with an auto sampler (AS-2055), pump (model PU-2089), and PDA detector (model MD-2018). Analytical balance; Sartorius, QC-CP013 with Sensitivity of 0.0001 mg, water bath; Lab TECH, QC-CP-031, Oven; Lab TECH, QC-CP-075 and Sonicator; POWER SONIC 420 were also used.

2.1.2. Reagents, Chemicals and Standards

HPLC grade KH_2PO_4 (99-103%), acetonitrile, triethylamine, and glacial acetic acid were purchased from Scharlau. ACS grade sodium hydroxide, hydrochloric acid, and hydrogen peroxide were supplied by Scharlau. Deionized water was prepared in-house using a Lab Tech Water Still GS-1007. USP reference standards PCM powder (99.98%), MP (99.96%), PP (99.88%), and HPLC grade 4- aminophenol and

4-hydroxybenzoic acid (> 98%, Fluka) were used for standard preparation.

2.1.3. Preparation of Analytical Solutions

Standards preparation

Stock solutions of PCM, MP, PP, 4-aminophenol and 4-hydroxybenzoicacid were prepared by transferring 55, 55, 25, 15 mg respectively to five separate 50 mL volumetric flasks. Sufficient methanol was added to dissolve the solid, and the volume was brought to the mark. A series dilution steps were carried out with mobile phase for each compound to make working standards as (0.06 mg/mL PCM, 0.00257 mg/mL MP, 0.00125 mg/mL PP, 0.03 mg/mL 4-aminophenol and 0.03 mg/mL 4-hydroxybenzoicacid). A mixed working standard containing 0.06 mg/mL PCM, 0.00257 mg/mL MP, 0.00125 mg/mL PP, 0.03 mg/mL 4-aminophenol and 0.03 mg/mL 4-hydroxybenzoicacid was prepared from the stock solution in a 50-mL volumetric flask. This solution was also called an assay solution.

Sample preparation

The placebo mixture recipe was prepared such that the final concentration of each constituent in a 100 mL solution was as follows: carnosine color (0.06 mg), strawberry flavor liquid (33.75 mg), agar (7.5 mg), avicel (dispersible cellulose (60.0 mg), citric acid anhydrous (0.9 mg), sodium lauryl sulfate (0.05 mg), deionized water (2.5 g), sorbitol solution 70% (1.25 g), sucrose (1.5 g), trisodium citrate:$2H_2O$ (2.5 mg), tween 80 (5 mg), and xanthan gum (20 mg). The placebo mixture was spiked with the PCM, MP, and PP. Sufficient mobile phase was added to bring the volume to the mark. The final concentrations of the PCM, MP, and PP in the placebo solution were 0.06, 0.00275, and 0.00125 mg/mL, respectively.

Preparation of buffer solution (pH = 4)

A buffer solution with pH = 4 was made by dissolving 6.80 g of potassium di-hydrogen phosphate in 1000 mL volumetric flask. Add enough deionized water to dissolve the material and stir well. After the salt dissolved, deionized water was added to reach the desired volume [20].

Preparation of mobile phase

A mixture of acetonitrile, buffer (pH = 4), trimethylamine, and glacial acetic acid in a ratio of 40:58:1.5:0.5 (v/v/v/v) was prepared, and the pH was adjusted to 4.0 ± 0.05 with orthophosphoric acid, filtered through 0.2 µm nylon membrane filter, and degassed for 10 min.

2.2. Stress Degradation Studies

Standards and samples were treated according stability stress testing [1, 21, 22, 23]. A volume of 5 mL of HCl and NaOH solutions with concentrations of 0.5 N and 1 N were used for acid and base degradation. For hydrolysis, 5 mL of deionized water were utilized**.** Thermal degradation was examined at 40 °C for 2–5 days**.** Degradation under sunlight was carried out for 2, 5, and 7 days. The oxidation-stress experiment was performed for 1, 3, and 5 days in 5 mL 3% hydrogen peroxide**.** All stressed solutions were injected into HPLC-PAD system under test procedure conditions. Acid and base stressed solutions were neutralized before HPLC injection. Peak purity and percentage degradation of PCM, MP, and PP were assessed chromatographically under optimum conditions.

2.3. Method Specificity

Method specificity was shown for separating PCM, M.P, and PP from related impurities. The stressed samples and spiked samples were carried out on chromatography condition. The stressed samples after dilution, diluent, placebo solutions (assay and duple ten concentrations), impurity samples for the specification limit (placebo spiked with 4-aminophenol and 4-hydroxybenzoic acid), and spiked solutions (with assay concentrations of PCM, MP, PP, and 4-aminophenol) were prepared. Each solution was analyzed in duplication.

3. Method validation

Under optimal conditions, the proposed HPLC method was validated with respect to LOD, LOQ, linearity, accuracy, and precision, as outlined by ICH Q2 (R^2) [24]and USP [25].

For the LOD and LOQ, serial standard concentrations of PCM, MP, and PP (0.2%,

0.4%, 0.6%, 0.8%, 1.0%, 2.0%, 4.0%, and 6.0%) were prepared, and five injections of each concentration were made. The solutions were injected from the lowest to highest concentrations. Linearity was determined using serial dilutions of a stock solution to prepare seven standard solutions at 40%, 80%, 90%, 100%, 110%, 120%, and 140% of the assay concentration. Six injections of each concentration were administered, starting with the injections of the lowest concentration. Accuracy (as a recovery) and range were determined by preparing nine samples at three concentrations: 80%, 100% hand, and 120%, using three replicate weights for each level, while the placebo remained at 100% of the assay concentration in all samples. Each sample was injected three times and adequately bracketed by a standard. The precision was evaluated in terms of repeatability and intermediate precision. Repeatability of the assay was demonstrated by preparing six replicate sample solutions from the same assay concentration at the 100% level.

Each sample was injected six times. Intermediate Precision was demonstrated by a second analyst and different operating conditions (HPLC; JASCO, Column; Thermo Hypersil ODS,250 mm x 4.6 mm, 5 µm). Six replicate sample solutions with the same assay concentration at the 100% level were injected.

4. Results and discussion

4.1. Method Development

The chromatographic method was refined for stability to separate PCM, MP, and PP from their primary degradants, which may develop under stress. For optimal separation and resolution, different mobile phases and columns were used. The mobile phase was chosen after several trials with methanol, acetonitrile, tetrahydrofuran, glacial acetic acid, triethyl amin, water, and buffer solutions in various compositions and at different pH values. The best separation was obtained using the mobile phase consisting of a mixture of acetonitrile, buffer (KH_2PO_4), triethylamine, and glacial acetic acid (58:40:1.5:0.5 v/v/v/v). A Phenomenex C8 column (150 mm × 4.6 mm, 5 µm) and Phenomenex C8 column (250 mm × 4.6 mm, 5 µm) column were tried in the following order: The C8 column (150 mm × 4.6 mm, 5 µm) gave a poor separation, even when changing the composition of the mobile phase. Phenomenex C8 column (250 mm × 4.6 mm, 5 µm) gave satisfactory result under the same experimental conditions. A flow rate of 1.2 mL/min gave an optimal signal to noise ratio with excellent separation time. The photodiode-array detector was set at 200 to 400 nm and PCM, MP, PP, and (4-aminophenol and 4-hydroxybenzoic acid) components were extracted at maximum absorption at 260 nm and this wavelength was chosen for the assay method.

Figure1 shows the chromatogram-phic separation of PCM, MP, and PP sample spiked with impurities (4-aminophenol and 4-hydroxybenzoic acid), which had retention times (Rt) of 2.73, 5.58, and 11.48 min respectively. Under ideal conditions, all relevant compounds eluted in 12 min. while Figure 2 shows the sample separated without spiking.

Table 1 shows system suitability results for our method. These data showed that the HPLC equipment, including samples, worked as expected and delivered acceptable results. [26].

4.1.1. Degradation behavior of PCM, MP and PP

Table 2 summarizes the stress testing data for PCM, MP, and PP under stress circumstances using HPLC. The data point to the following degradation pattern:

Basic and acidic aqueous hydrolysis

At two distinct HCl and NaOH concentrations (0.5 N and 1.0 N), acid and base hydrolysis was conducted. MP and PP underwent base hydrolysis and produced the breakdown product (4-hydroxybenzoic acid) at a retention time (Rt) of 3.15 min. The stability of the drugs in 0.5 N base was in the order PCM > PP > MP Where the % degradation of MP exceeded 85% for both standard and sample after 4 days of hydrolysis. As expected, similar trend was also observed

when 1.0 N NaOH was used. Both MP and PP were hydrolyzed completely after 4 days according to Table 2. PCM was less labile to base hydrolysis (not surpassing 21.87± 0.29% in 1 N base after 4 days hydrolysis) generating one known degradation product (4-aminophenol) at an Rt of 1.88 min. as shown in (Figures 3-5).

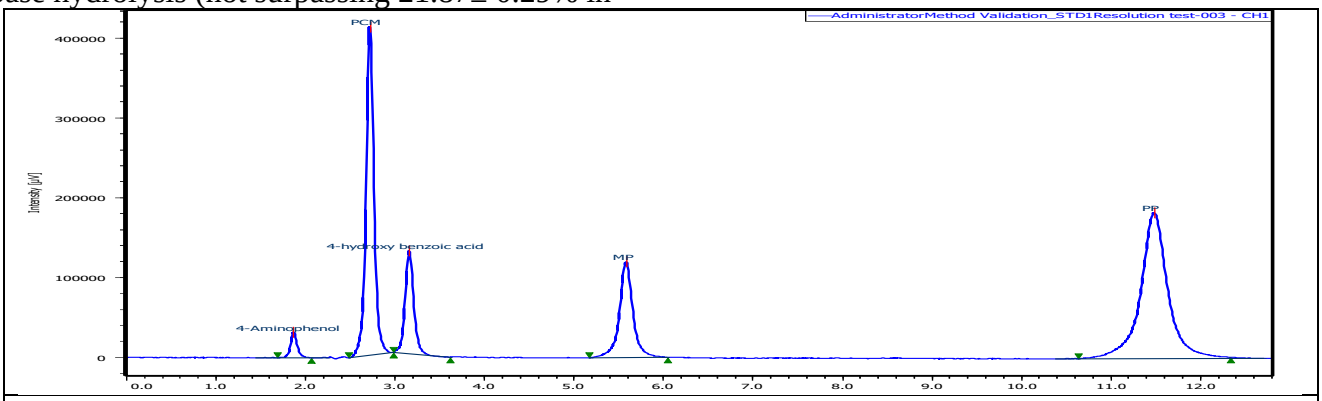

Figure. 1: Chromatogram of Mix of 4-Aminophenol, PCM, 4-Hydroxybenzoic acid, MP, PP and placebo.

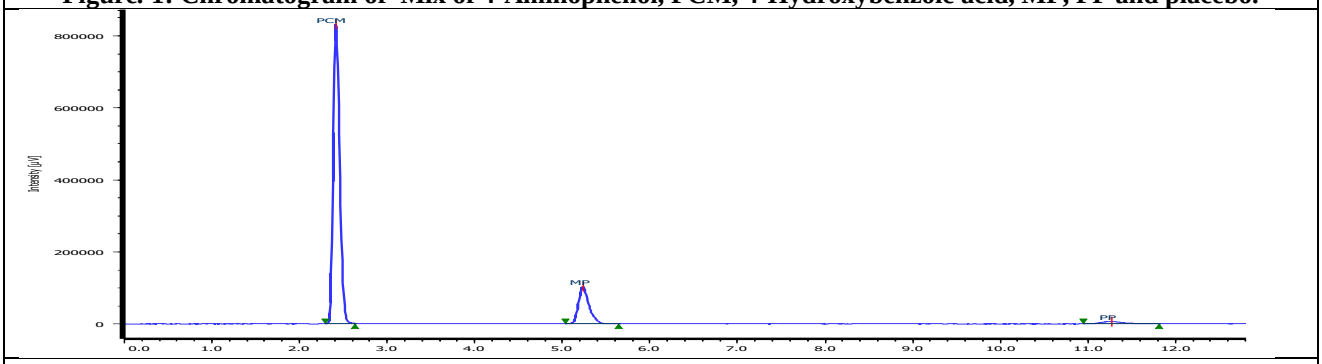

Figure. 2: Chromatogram of a real sample containing PCM, MP, PP and placebo. Separation was caried out under optimum separation condition using compounds' assay concentrations

Table 1: System Suitability Results

Compound	Rt	NTP ≥ 2000	Resolution (R) ≥ 2	%RSD ≤ 2	T ≤ 2
4-aminophenol	1.88	3185	5.96	0.52	1.07
PCM	2.73	5080	2.66	0.54	1.09
4-hydroxybenzoic acid	3.15	5667	11.66	0.43	-
MP	5.58	8122	16.44	0.58	1.01
PP	11.48	9281	>16	0.36	1.00

Where: Rt: retention time, NTP: Number of theoretical plates, RSD: relative standard deviation and T: tailing factor

The three drugs were more stable in acidic medium. Their stability was MP > PP > PCM which was the opposite of that of basic medium. figures 3 and 4 show representative chromograms for the separation of PCM, PP, MP, and their degradation products (4-aminophenol and 4-hydroxybenzoic acid) under acidic and basic stress conditions, respectively. Table 2 shows that PCM degraded faster in a basic medium than an acidic solution. It matches the results of Jawaher et al. [27], Aminu et al. [28] and Jahan et al. [29]. Mohamed MA, however, found that paracetamol degraded faster in acidic than basic media [30].

Table 2 shows no degradation of the three compounds in deionized water for 5 days. Jahan et al. [29] found PCM water degradation of 2.02%.

Stability under thermal, white light and oxidation conditions

PCM, MP and PP were steady in dry heat. Following five days of dry heat at 40 °C, the PCM standard and sample showed just 9.33 ± 0.23% and 10.05 ± 0.56% degradation. Ragab et al. found dry heat did not degrade PCM [31]. According to Mohamed MA [30], exposing the PCM to 80 ^{0}C for 2 h resulted in a negligible deterioration that reached 0.04%.

PCM was more susceptible to white light stress than MP and PP. After seven days of white light exposure, reference and sample PCMs reached 63.81 ± 0.59% and 63.77 ±0.73% degradation. The deterioration rate increased with exposure time.

PCM, MP, and PP levels remained steady after days of 3% H_2O_2 stress.

PP degraded fastest (6.5 ± 0.95%) after 5 days of oxidizing agent contact.

Under the same conditions, the PCM was more consistent at 3.7 ± 1.09%).

Interestingly, PCM stability at oxidizing circumstances (30% H_2O_2) is inconsistent. According to Jawaher et al. [27], PCM degraded just 44.0% after 24 hr.

On the other hand, Aminu et al. [28] report 1.35% PCM degradation in 30% H_2O_2 after 24 hr., while at zero time from addition of 30% H_2O_2, the PCM degradation was 10%.

Figure 5 shows an actual sample was HPLC separated after expiration. The data suggest two known degradants (4-aminophenol and 4-hydroxybezoic acid) and three unknowns that must be identified.

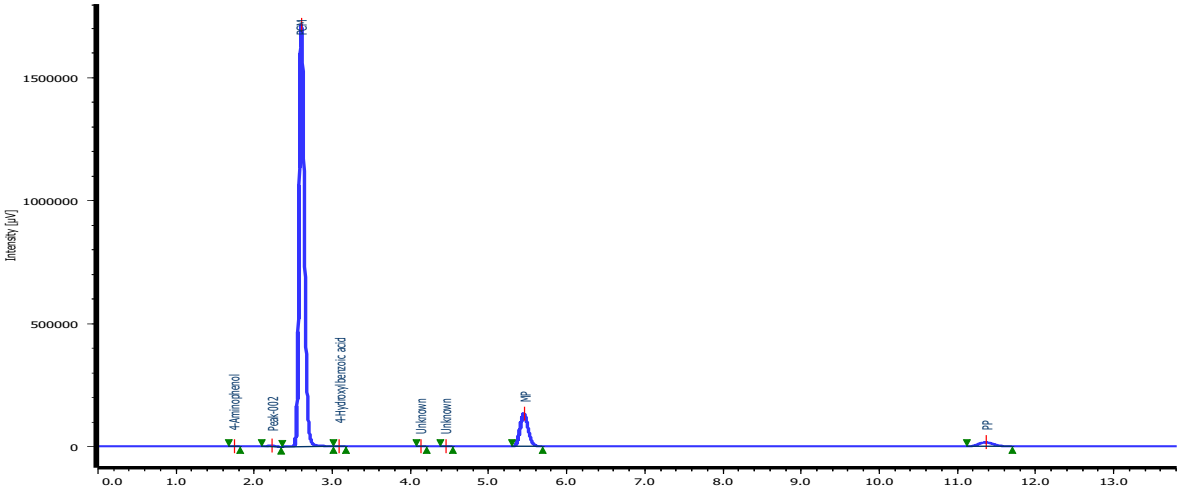

Figure. 3: HPLC chromatogram of a sample underwent a forced degradation in 1.0 N HCl for 5 days.

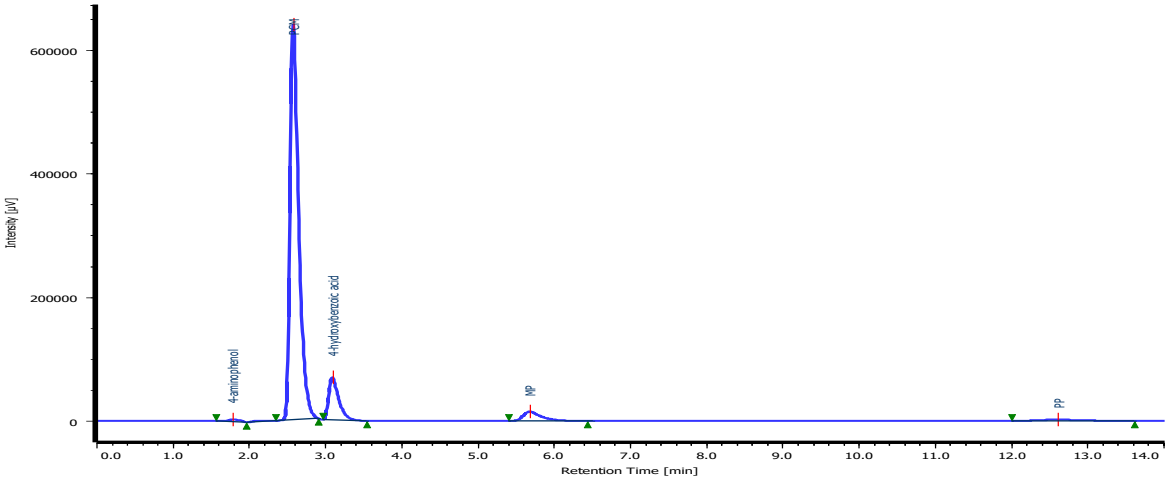

Figure. 4: HPLC chromatogram of a sample underwent a forced degradation in 1.0 N NaOH for 5 days.

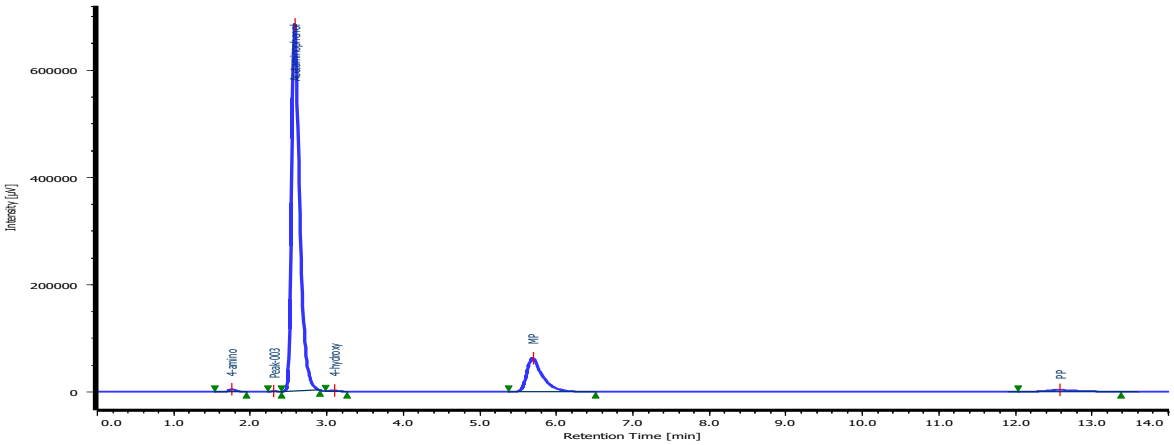

Figure. 5: HPLC Chromatogram of a Real Sample after its Expiration Date.

Table 2: Standard and Sample Stability Results

TREATMENT		N#=2	DEGRADATION %			%RSD		
			PCM	MP	PP	PCM	MP	PP
ACID 0.5 N	1DAY	STD Spike	3.31	1.30	1.38	0.76	0.53	0.77
		SMP Spike	3.02	1.40	1.94	1.09	0.26	0.69
	3DAY	STD Spike	7.20	3.80	3.16	0.54	0.37	0.49
		SMP Spike	6.04	4.26	4.84	0.13	0.43	1.84
	4DAY	STD Spike	9.72	4.9	6.36	0.11	0.46	0.18
		SMP Spike	9.62	4.58	5.81	0.19	0.07	0.01
BASE 0.5 N	1DAY	STD Spike	4.17	38.33	13.58	0.44	0.55	1.22
		SMP Spike	3.09	37.05	14.64	0.07	0.34	0.22
	3DAY	STD Spike	7.78	76.84	30.12	0.27	1.47	1.56
		SMP Spike	6.11	74.65	30.05	0.01	1.55	1.69
	4DAY	STD Spike	10.62	86.31	42.05	0.43	1.24	0.95
		SMP Spike	10.58	85.58	43.45	0.61	0.54	1.08
BASE 1.0 N	2DAY	STD Spike	8.22	100.00	82.97	0.90	N/A	0.45
		SMP Spike	9.05	100.00	83.76	0.25	N/A	0.79
	5DAY	STD Spike	21.74	100.00	100.00	0.38	N/A	N/A
		SMP Spike	21.87	100.00	100.00	0.29	N/A	N/A
ACID 1.0N	2DAY	STD Spike	8.78	2.47	4.27	0.02	0.75	1.20
		SMP Spike	8.68	2.33	3.67	0.03	0.21	0.90
	5DAY	STD Spike	18.92	6.83	7.49	0.85	0.65	0.43
		SMP Spike	18.32	6.32	7.66	0.74	0.43	0.28
WATER	1DAY	STD Spike	<0.10	<0.10	<0.10	0.02	N/A	N/A
		SMP Spike	<0.10	<0.10	<0.10	N/A	N/A	N/A
	5DAY	STD Spike	0.99	<0.10	0.37	0.22	N/A	N/A
		SMP Spike	<0.10	<0.10	-0.73	0.78	N/A	N/A
WIGHT LIGHT	1DAY	STD Spike	11.69	2.26	3.65	0.25	0.74	1.08
		SMP Spike	11.97	2.87	3.17	0.11	0.28	1.12
	5DAY	STD Spike	44.97	4.82	4.20	0.58	0.94	0.01

		SMP Spike	44.97	4.82	4.20	0.77	0.52	0.05
	7DAY	**STD Spike**	63.81	5.20	3.47	0.59	0.78	1.40
		SMP Spike	63.77	5.02	4.13	0.73	0.49	1.62
DRY HEAT 40C	**1DAY**	**STD Spike**	<0.10	<0.10	1.06	N/A	N/A	0.43
		SMP Spike	<0.10	0.73	0.42	N/A	N/A	1.98
	5DAY	**STD Spike**	9.33	2.11	10.00	0.23	1.46	0.66
		SMP Spike	10.05	4.14	9.46	0.56	0.71	0.97
OXIDATION	**1DAY**	**STD Spike**	<0.10	0.64	<0.10	N/A	N/A	N/A
		SMP Spike	<0.10	0.81	0.10	N/A	N/A	N/A
	3DAY	**STD Spike**	0.89	2.14	5.00	N/A	0.08	0.65
		SMP Spike	1.13	1.01	6.20	0.56	1.90	1.75
	5DAY	**STD Spike**	2.40	2.30	6.50	0.75	1.42	0.95
		SMP Spike	3.70	3.70	5.20	1.09	0.71	1.90

Where STD: Standard deviation; SMP = sample, RSD = Relative standard deviation, and N/A: not available.

4.2. VALIDATION of the stability indicating method.

The developed SIAM method was extensively validated for the separation of PCM, MP, PP, and their related impurities using the following parameters and acceptance criteria were met according to [32,33,34]:

4.2.1. Specificity/ Interference from degradation products

Multiple strained samples were injected into the HPLC-PDA and separated under optimal conditions. All degradant peaks were resolved from PCM, MP, and PP peaks, with peak purities of 95.98% to 99.98% for PCM, 98.08% for MP, and 98.16% for PP. These findings suggest that the SIAM technique can be utilized to estimate PCM, MP, and PP stability in pharmaceutical formulations with related compounds and for quality control.

4.2.2. Detection and Quantitation Limits (DL & QL)

Table 3 displays the DL (three times the noise level) for PCM, MP, and PP as well as the QL (ten times the noise level). According to the British National Formulary [35], the SIAM should have a threshold of at least 0.2% (i.e., 2 mg total dose intake TDI) if the maximum daily dose is between 10 mg and 2 g. For newborn patients (6 months to 1 year), the acceptable TDI level of PCM suspension is between 480 and 960 mg [30]. This suggests that 0.2% of the lowest TDI, or 0.96 mg, should be detectable using the SIAM approach. We can conclude that our developed SIAM had DL (3.4 x 10^{-3} mg) significantly below the specified limit.

Table 3: DL and QL

	Conc. (%)	**R^2**	**DL (mg/ml)**	**QL (mg/ml)**
PCM	(0.2%-6.0%)	0.9992	0.00036	0.00102
MP	(0.2%-6.0%)	0.9996	0.000046	0.00014
PP	(0.2%-6.0%)	0.9999	0.0000029	0.0000090

4.2.3. Accuracy (Recovery):

Analyzing the data from spiked placebo solutions with concentrations 80%–120% of PCM drug and MP and PP preservative exhibited high method accuracy. HPLC-based SIAM acceptable recovery is typically 96.0%–104.0%. Table 5 shows that the developed SIAM fits this requirement. A y-intercept analysis showed that the approach had little bias. The average %RSD values of the method recovery for PCM, MP, and PP were 0.89%, 1.14%, and 1.30% respectively.

4.2.4. Precision: Method precision was established by repeatability and intermediate precision. To do so, the preparation of seven samples and one standard was done. Each
Samples and the standards were injected six times to calculate sample peak area/standard peak area averages. Table 6 shows recovery and RSD percentages of our work. High repeatability of the developed SIAM was observed, with %RSD values within the permitted limit (≤ 2%) for analytical repeatability.
Using alternative settings (another analyst, column ODS 250 mm x 4.5 mm, 5µm, HPLC JASCO QC-CP-058, working standard reagent; Global pharma), the procedure showed intermediate precision in preparing five samples and a standard. Each was injected five times to calculate average peak areas. Each sample's percent label claim for chemicals was calculated as presented in Table 7. The SIAM approach is highly precise, as shown in Table 7.

5. Application of the Method

We have verified the developed SIAM through testing the quality of paracetamol oral suspension from Shaphaco pharmaceutical company (a Yemeni company making Amol Suspension with a potency of PCM = 120 mg/5mL, MP = 5.5 mg/5 mL and PP = 2.5 mg/5 mL).
A total of 102 samples from 34 drug batches (three per batch) were evaluated. Table 8 illustrates the results as a percentage of label claims. Average recoveries (n = 3) were 98.00%–102.00.

5.1. Linearity, accuracy (recovery) and precession

Assay linearity was demonstrated by preparing seven standard solutions at 40%, 60% 80%, 90%, 100%, 110%,120 and 140% mg/mL PCM, 0.00275 mg/mL MP and 0.00125 mg/mL PP. Each solution was injected in duplicates. Linear regression analysis was performed, excluding origin as a point. The R^2 which exceeded 0.9999 and the y-intercept value, were shown in Table 4.

The graphs of the concentration versus the area response for PCM, MP and PP were shown in Figures 6 - 8. The data in these graphs do not show a significant departure from zero, indicating that the linearity acceptance criteria were met.

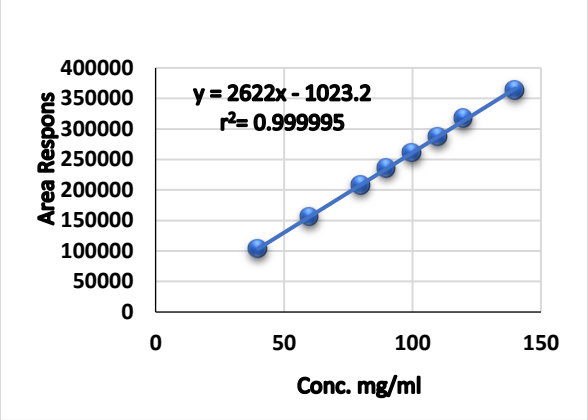

Figure. 6: Calibration Curve of PCM

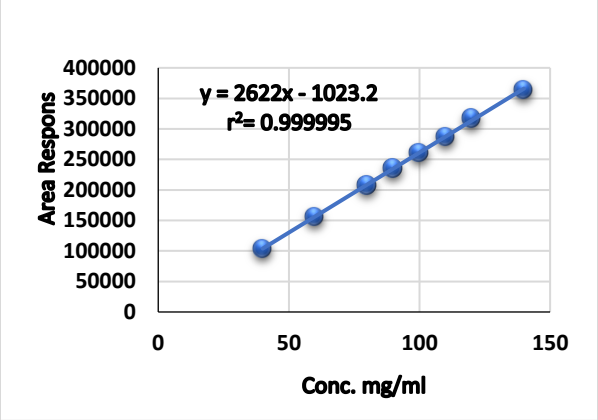

Figure. 7: Calibration Curve of MP

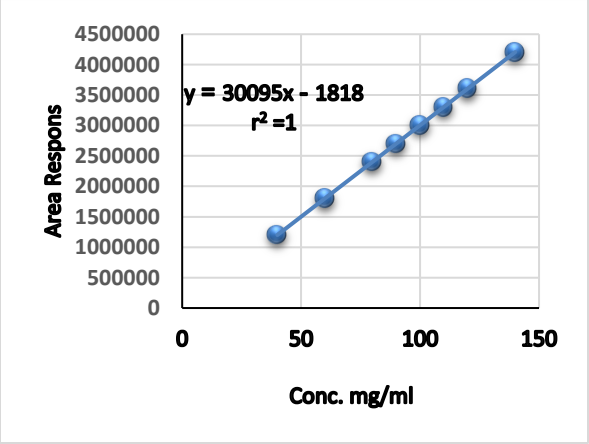

Figure. 8: Calibration Curve of PP

Table 4: Linearity

Level conc.%	Average			% RSD		
	PCM	M.P	P.P	PCM	M.P	P.P
40	1201524	103912	41625	0.6235	0.7984	1. 250
60	1802286	155868	62437	0.4652	0.5231	1.030
80	2403049	207825	84090	0.5002	0.6507	0.8149
90	2699443	235536	94766	0.9251	0.7389	0.7665
100	3005503	260823	104496	0.6377	0.4450	0.6698
110	3300344	287334	116091	0.4771	0.9529	0.8287
120	3611865	317079	127650	0.4610	0.9111	0.8671
140	4205335	363693	145687	0.4214	0.9571	0.8858
SLPOE				**30059.31**	**2622.023**	**1053.509**
INTERCEPT				**-1817.96**	**-1028.21**	**-344.131**
Correlation coefficient (r^2)				**1**	**0.999995**	**0.999988**

Table 5: Accuracy

%	Taken mg/ml(n=6)			Found mg/ml(n=6)			%Recovery Ave.		
	PCM	MP	PP	PCM	MP	PP	PCM	MP	PP
80	0.048	0.0022	0.001	0.04808	0.00222	0.001	99.77-100.81	99.90-100.81	98.85-101.20
100	0.06	0.00275	0.00125	0.05911	0.00272	0.00124	98.14-98.75	98.41-99.38	98.57-99.25
120	0.072	0.0033	0.0015	0.07194	0.00331	0.00151	99.29-100.06	99.36-101.71	99.64-101.70
Overall5 Average							**99.54%**	**100.03%**	**99.87%**
Overall % RSD							**0.89%**	**1.14%**	**1.30%**

Table 6: Method's Repeatability

Name	Average(n=6)								
	Av. Response Area			RSD%			Recovery%		
	PCM	MP	PP	PCM	MP	PP	PCM	MP	PP
STD	2996134	262371	104781	0.536	0.578	0.361	N/A	N/A	N/A
Sample 1	2987293	259210	103703	0.601	0.451	0.540	99.70	98.80	98.97
Sample 2	2987826	259862	104158	1.085	1.292	0.811	99.72	99.04	99.41
Sample 3	3041521	265797	106404	0.465	0.793	0.220	101.51	101.31	101.55
Sample 4	2997480	259870	103973	0.792	0.780	1.174	99.80	100.72	99.38
Sample 5	3021223	263991	106076	0.937	0.842	1.553	100.84	100.62	101.24
Sample 6	3028796	265681	105915	0.443	1.291	1.277	101.09	101.26	101.08
RSD %Overall	0.7200	1.0762	1.0619						
Av. Recovery							100.45	100.29	100.27

Table 7: Method's Intermediate

Name	Average first operator n=5			Average second operator n=5		
	PCM	MP	PP	PCM	MP	PP
STD	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
Sample 1	99.70	98.79	98.97	99.44	99.99	100.47
Sample 2	99.72	99.04	99.40	99.69	100.20	100.68

Sample 3	98.96	98.84	99.15	100.15	99.79	100.21
Sample 4	99.84	99.50	99.37	100.18	100.40	101.10
Sample 5	100.84	100.62	101.24	100.37	100.39	100.22
%Average	**99.81**	**99.36**	**99.63**	**99.97**	**100.15**	**100.54**
%RSD	**1.11**	**1.29**	**1.53**	**0.96**	**0.85**	**1.13**

n = No of replicate

Table 8: Application of the Method

Batch No	**Assay**			**RSD**		
	PCM	**MP**	**PP**	**PCM**	**MP**	**PP**
11121	101.00	100.14	98.45	0.02	0.51	1.02
11321	100.46	102.78	100.10	0.025	0.25	0.92
11421	99.01	98.78	98.04	0.12	0.21	1.60
11521	99.78	99.02	98.42	0.005	0.58	1.26
11621	99.26	98.99	98.00	0.001	0.76	1.07
11721	99.78	99.07	98.70	0.11	0.24	1.32
11821	99.80	98.40	98.12	0.001	0.46	1.01
19520	98.99	98.75	98.05	0.014	0.71	1.06
19620	100.28	99.47	99.40	0.025	0.01	0.96
19720	99.89	98.96	98.78	0.014	0.49	1.11
19820	100.28	99.86	99.01	0.012	0.16	0.25
19920	100.89	100.01	99.83	0.11	0.21	0.87
20020	100.89	99.07	99.90	0.002	0.18	0.62
27520	100.19	100.20	100.45	0.007	0.13	1.02
27620	100.78	98.56	100.02	0.012	0.16	1.54
27720	101.00	99.15	98.70	0.16	0.73	0.96
27920	100.25	99.78	99.96	0.001	0.31	0.76
28121	100.10	99.45	99.00	0.008	0.26	0.92
28220	99.78	98.12	98.02	0.013	0.62	0.05
28321	99.98	99.00	98.05	0.027	0.54	1.60
28421	101.10	100.30	101.45	0.09	0.73	0.98
28521	100.59	100.08	100.79	0.004	0.46	1.28
28621	101.56	99.93	99.90	0.056	0.22	1.39
28721	100.15	99.73	100.45	0.022	0.12	1.07
28821	99.56	99.78	98.78	0.001	0.76	0.55
28921	100.01	101.4	100.78	0.05	0.33	1.31
51520	99.09	100.20	100.78	0.0047	0.51	1.07
51620	100.45	99.70	99.01	0.029	0.92	0.55
51720	101.80	100.19	98.15	0.070	0.22	1.31
51820	101.00	101.59	100.25	0.063	0.83	1.22
51920	101.56	99.01	99.23	0.019	0.45	1.56
52020	100.99	98.41	100.04	0.028	0.13	0.90
52120	101.00	101.20	98.88	0.92	0.76	0.38
27920	100.25	98.13	99.96	0.0084	0.11	1.07

6. Conclusion

The HPLC-DAD method was developed and verified to determine active pharmaceutical components (PCM) and two preservatives (MP and PP) in oral suspension product. Without interference from the blank, placebo, or other degradants, the method was selective, precise, and accurate with high linearity within 80% to 120% of the nominal concentrations of 0.06, 0.00275, and 0.00125 mg/mL of PCM, MP, and MP, respectively. The approach was found to be stable and suitable for quality control of commercial oral solutions including PCM, MP, and PP during routine analysis and stability experiments.

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CONFLICT OF INTEREST:

The authors of this manuscript did not have any conflict of interest to declare.

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