Validated Ultra-Performance Liquid Chromatographic and Thin-Layer Chromatographic–Densitometric Methods for the Determination of Paracetamol, Pamabrom, and Pyrilamine Maleate

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Paracetamol
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Ultra-performance liquid chromatography
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Summary

Two chromatographic methods were developed for the simultaneous determination of paracetamol, pamabrom, and pyrilamine maleate in bulk and combined pharmaceutical dosage form. The first method is an ultra-performance liquid chromatographic (UPLC) method, in which separation was carried out by gradient elution using C_{18} column and a mobile phase composed of solution A (acetonitrile) and solution B (phosphate buffer) (pH 3.5). The elution started with 20% (by volume) acetonitrile ramped up linearly to 100% in 2 min, then kept constant till the end of the run at a flow rate of 1.5 mL min^{-1} and ultraviolet (UV) detection at 277 nm. The second method depends on the densitometric determination of thin-layer chromatograms of the three drugs. Separation was carried out at 275 nm using chloroform–acetonitrile (15:35, *v*/*v*) as the mobile phase. The proposed methods were validated according to the International Conference on Harmonisation (ICH) guidelines. Beer's law was obeyed in the range of 5–100 µg mL^{-1} for paracetamol and 0.5–20 µg mL^{-1} for pamabrom and pyrilamine maleate, respectively, with mean recoveries of 98.40–100.32% ± 0.551–0.771 for the UPLC method. Linearity of the thin-layer chromatographic method was achieved in the range of 10–280, 5–45, and 1–20 ng per spot of the three drugs with mean recoveries of 98.75–100.30% ± 0.971–1.061, respectively. The two methods were successfully applied for the simultaneous determination of the cited drugs in their laboratory-prepared mixtures and pharmaceutical dosage form with good accuracy and precision. The results obtained were compared with those of the reported method and found to be in good agreement.

1 Introduction

Paracetamol (PCM) is a non-steroidal anti-inflammatory drug having mild analgesic and antipyretic properties. It is chemically known as *N*-(4-hydroxyphenyl)acetanilide **(Figure 1)** [1]. Pamabrom (PBM), chemically a 1:1 mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate (Figure 1), is a diuretic, works by increasing urination, and is used to treat bloating, swelling, feelings of fullness, and other signs of water weight gain related to menstrual symptoms [2]. Pyrilamine maleate (PAM), 1,2-ethanediamine and *N*-(4-methoxyphenyl)methyl-*N*′,N′-dimethyl-*N*-2-pyridinyl-(*Z*)-2-butenedioate (1:1) (Figure 1), is an antihistaminic drug used to reduce the allergic conditions and symptoms of cold [3]. The combination of the three active ingredients, PCM, PBM, and PAM, is used for the treatment of mild to moderate premenstrual syndrome in addition to its antihistaminic and mild diuretic effects [4].

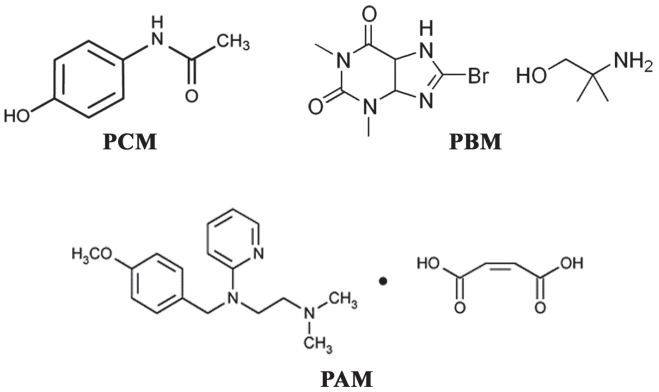

Figure 1

The chemical structures of PCM, PBM, and PAM.

Literature survey revealed that only one high-performance liquid chromatography (HPLC) method [5] concerning the simultaneous estimation of PCM, PBM, and PAM has been reported. Several spectrophotometric [6–11], HPLC [12–18],

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and gas chromatography (GC) [19–21] methods have been reported for the determination of paracetamol either individually or in combination with other drugs. PCM and PBM have been determined simultaneously by different spectrophotometric [22–25], HPLC [26–31], and thin-layer chromatography (TLC) [31–33] methods, whereas few analytical methods [34–36] were reported for the estimation of PAM. The present work provides validated, simple, sensitive, and reproducible chromatographic methods for the determination of the three cited drugs, simultaneously.

2 Experimental

2.1 Instrumentation

- Agilent 1290 Ultra HPLC with a binary pump and an ultraviolet (UV) detector (Agilent Technologies, Santa Clara, CA, USA).
- BDS Hypersil C_{18} column (150 mm × 4.6 mm; particle size: 5 µm). Data were processed using HP ChemStation software (Hewlett Packard, Palo Alto, CA, USA).
- CAMAG TLC Scanner 3, with winCATS computer software (Muttenz, Switzerland).
- Pre-coated TLC plates, silica gel 60 G F_{254} (20 cm × 20 cm) (Fluka Chemie, Buchs, Switzerland).
- Hamilton 50-µL microsyringe (Hamilton Germany GmbH, Martinsried, Germany).
- UV lamp with a short wavelength of 254 nm (Desaga, Wiesloch, Germany).
- Chromatographic tank (25 cm × 25 cm × 9 cm).
- pH meter (Jenway, Radnor, PA, USA).

2.2 Chemicals and Reagents

All reagents used were of analytical grade, and the water used throughout the procedure was freshly distilled.

- Methanol, chloroform, and acetonitrile were of HPLC grade (Sigma-Aldrich, Taufkirchen, Germany).
- Potassium dihydrogen phosphate and *ortho*-phosphoric acid (Merck, Darmstadt, Germany).

2.3 Samples

2.3.1 Pure Samples

PCM (NA106116002) and PBM (NA106116001) were kindly supplied by Haya Pharmacy (Port Said, Egypt). PAM (P 01100211) was obtained from Sigma-Aldrich (Germany). The purities of PCM, PBM, and PAM were found to be 99.16%, 100.47%, and 99.85%, respectively, as stated by the supplier.

2.3.2 Market Sample

Pamprin Multi-Symptom® tablets (lot No. 08F33) were labeled to contain 500 mg PCM, 25 mg PBM, and 15 mg PAM (product of Chattem, Inc., Chattanooga, TN, USA).

2.4 Standard Solutions

Stock solutions of the drugs (1 mg mL^{-1}) were prepared by dissolving 100 mg of PCM, PBM, or PAM in 100 mL methanol. Working solutions of the drugs were prepared by further dilution of their stock solutions with methanol.

2.5 Procedures

2.5.1 Ultra-Performance Liquid Chromatographic (UPLC) Method

2.5.1.1 Chromatographic Conditions

At ambient temperature, gradient separation was carried out on a BDS Hypersil C_{18} column (150 mm × 4.6mm; particle size: 5 µm). The mobile phase consisted of solvent A (acetonitrile) and solvent B (0.05 M phosphate buffer [pH 3.5]) at a flow rate of 1.5 mL min^{-1}. The initial mobile phase composition was maintained at 80% solvent A for 2 min, changed linearly to 100%, and was maintained for 8 min (2–10 min). The injection volume was 20 µL, and the detection was carried out at 277 nm.

2.5.1.2 Calibration Curve

Aliquots of the working PCM solution (0.2 mg mL^{-1}) containing 0.05–1 mg PBM or PAM solutions (0.1 mg mL^{-1} equivalent to 0.005–0.2 mg) were transferred into a series of 10-mL volumetric flasks adjusted to volume with methanol. An amount of 20 µL was injected from each concentration and chromatographed under the conditions described. A calibration graph was constructed by plotting the peak area against the corresponding drug concentration in µg mL^{-1}.

2.5.2 TLC Method

2.5.2.1 Chromatographic Conditions

Analysis was performed on pre-coated 20 cm × 20 cm TLC aluminum silica gel 60G F_{254} plates. The samples were applied to the plates using a Hamilton microsyringe (50 µL). The plates were spotted 1 cm apart from each other and 1 cm apart from the bottom edge. The chromatographic tank was pre-saturated with the mobile phase for 20 min and then developed by ascending chromatography using acetonitrile–chloroform (35:15 *v*/*v*) as the mobile phase. The plates were air-dried, detected under a UV lamp (254 nm) and scanned under the following conditions:

- slit dimension: 6.0 × 0.3 µm,
- wavelength: 275 nm,
- scanning speed: 20 mm s^{-1},
- data resolution: 100 nm per step,
- measurement mode: absorption,
- result output: chromatogram and area.

2.5.2.2 Calibration Curve

In a series of 10-mL volumetric flasks, aliquot of standard solutions PCM (0.1 mg mL^{-1} equivalent to 0.01–0.28 mg), standard solutions of PMB or PAM (0.01 mg mL^{-1} equivalent to 0.005–0.045 mg PMB or 0.001–0.02 mg PAM, respectively) were transferred separately and diluted to volume with methanol. An amount of 10 µL of each solution was applied to a TLC plate following the previously described chromatographic conditions and scanned at 275 nm. The calibration curve was obtained by

plotting the area under the peak *versus* the corresponding drug concentrations in ng per spot, and the regression equation of the linear relation was computed.

2.5.3 Laboratory-Prepared Mixtures

Different aliquots of PCM, PBM, and PAM working solutions were transferred into a series of 10-mL volumetric flasks, and then adjusted to the volume with methanol to prepare synthetic mixtures in different proportion ranges. For UPLC procedure, an amount of 20 µL of each obtained solution was injected into the UPLC column and the obtained chromatograms were detected at 277 nm using the same previous chromatographic conditions. For the TLC procedure, an amount of 10 µL of each solution was applied to TLC plates, and then the spot development was monitored at 275 nm. The concentration of each drug in the prepared mixtures was calculated from the corresponding regression parameters.

2.6 Application to Pharmaceutical Preparation

Ten Pamprin Multi-Symptom® tablets, each labeled to contain 500, 25, and 15 mg of PCM, PBM, and PAM, respectively, were weighed, powdered, and mixed well. An amount equivalent to one tablet was transferred to a 100-mL volumetric flask, dissolved in 50 mL methanol, and sonicated for 10 min. The solution was diluted to volume with methanol and filtered. The clear filtrate labeled to contain 5 mg mL^{-1} of PCM, 0.25 mg mL^{-1} of PBM, and 0.15 mg mL^{-1} of PAM was further diluted to the desired concentration and analyzed by the proposed methods. The drugs concentrations were calculated using appropriate regression equations.

3 Results and Discussion

The aim of this work was to establish sensitive, selective, and accurate UPLC and TLC–densitometric methods for the determination of PCM, PBM, and PAM in bulk powder and pharmaceutical formulations.

3.1 Method Development

For successful method validation, preliminary studies were performed with the objective to select adequate and optimum conditions.

3.1.1 UPLC Method

Different mobile phases were tried such as methanol–phosphate buffer, methanol–acetonitrile–water, and methanol–water, in order to find the optimum conditions for the separation of PCM, PBM, and PAM. Initial experiments showed good separation using acetonitrile, compared with methanol. The effect of different buffers was tested, and it was found that the best resolution with a good peak shape was obtained using phosphate buffer (pH 3.5). Different flow rates (0.5–2 mL min^{-1}) at different wavelengths (200–400 nm) were tried. Isocratic elution with the mobile phase of phosphate buffer (pH 3.5)–acetonitrile cannot be successfully used due to overlapping of PCM and PBM peaks. Trials showed that gradient elution with a mobile phase containing phosphate buffer (pH 3.5)–acetonitrile at a flow rate of 1.5 mL min^{-1} and detection at 277 nm gave sharp, well-defined and resolved peaks with minimum tailing as compared to other mobile phases. Using these conditions, the retention times were observed to be 1.763 min for PCM, 3.888 min for PBM, and 6.024 minutes for PAM **(Figure 2).**

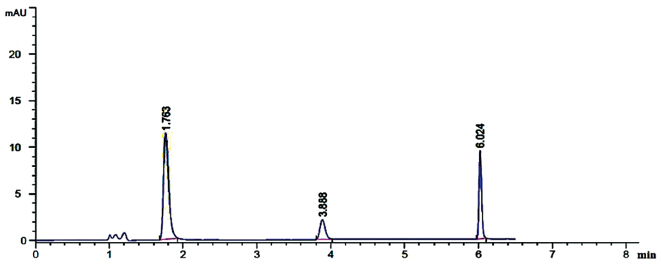

Figure 2

UPLC chromatogram of a mixture of PCM (40 µg mL^{-1}), PBM (5 µg mL^{-1}), and PAM (5 µg mL^{-1}) at 277 nm.

3.1.2 TLC–Densitometric Method

The TLC method used for the simultaneous determination of PCM, PBM, and PAM depends on the difference in R_F values. Initial studies on the cited drugs were carried out to achieve good separation, in which different developing systems with different ratios were tried, such as chloroform–ethyl acetate, methanol–acetone, chloroform–methanol, and acetonitrile–chloroform. The best separation with almost well-defined spots was achieved using acetonitrile–chloroform (7:3, *v/v*) as the mobile phase. The selected mobile phase allows the determination of PCM, PBM, and PAM without tailing of the separated spots or interference, providing better precision.

Different scanning wavelengths were tried such as 242, 275, and 308 nm, and it was found that at 275 nm maximum sensitivity, sharp, symmetrical peaks with minimum noise were obtained, as well as good sensitivity for determination of the three drugs in the presence of each other. Under these conditions, well-defined peaks were obtained with R_F values of 0.76, 0.46, and 0.12 for PCM, PBM, and PAM, respectively **(Figure 3).**

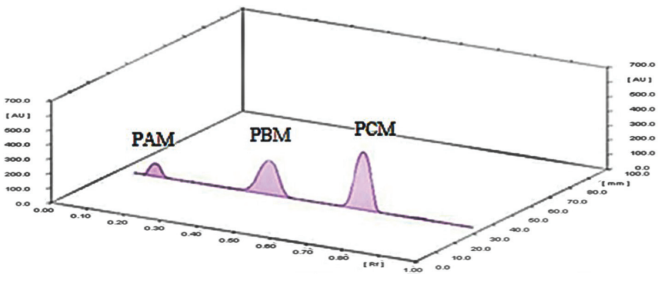

Figure 3

Densitometric chromatograms of the standard of PCM (100 ng per spot), PBM (20 ng per spot), and PAM (20 ng per spot) at 275 nm.

3.2 Method Validation

The proposed methods were validated in accordance to the ICH guidelines [37].

3.2.1 Linearity and Range

Under the previously described experimental conditions, a linear relationship was obtained between peak areas, and the concentrations of the three drugs were over the range of 5–100 µg mL^{-1} for PCM and 0.5–20 µg mL^{-1} for PBM and PAM for the UPLC method. For the TLC method, the linearity was obtained by plotting the peak area of the separated spots, and the corresponding drug concentrations were over the range of 10–280 ng per spot for PCM, 5–45 ng per spot for PBM, and 1–20 ng per spot for PAM **(Table 1).**

3.2.2 Accuracy

Accuracy was checked by applying the proposed methods for the analysis of different samples of pure PCM, PBM, and PAM alkaline phosphatase (ALP). The concentrations were calculated from the corresponding regression equations, and their recoveries were found to be in the range of 99.16–100.44% (Table 1).

3.2.3 Precision

3.2.3.1 Repeatability

Three concentrations of each drug were analyzed three times intra-daily using the proposed methods. Good *%RSD* values ranging from 0.621–1.815% were obtained, confirming the repeatability of the method as shown in Table 1.

3.2.3.2 Intermediate Precision

The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. Acceptable *%RSDs* in the range of 0.814–1.742% were obtained and are given in Table 1.

Table 1

Validation and regression parameters for the determination of PCM, PBM, and PAM by the proposed methods.

Parameter	UPLC			TLC		
	PCM	PBM	PAM	PCM	PBM	PAM
λ Value of detection [nm]	277	277	277	275	275	275
Linearity range	5–100 µg mL^{-1}	0.5–20 µg mL^{-1}	0.5–20 µg mL^{-1}	10–280 ng per spot	5–45 ng per spot	1–20 ng per spot
Regression parameters						
Slope ± SD (S_y)	7.85 ± 0.059	1.97 ± 0.006	6.07± 0.024	436.91 ± 2.241	1245.2 ± 11.212	1747.01 ± 19.813
Intercept ± SD (S_x)	23.59 ± 3.612	0.38 ± 0.082	−1.21 ± 0.301	951.51 ± 38.583	97.00 ± 32.221	516.61 ± 24.313
SD of residual (S_{yx})	4.823	0.114	0.416	48.391	35.471	30.148
Correlation coefficient (r^2)	0.9997	0.9998	0.9996	0.9997	0.9998	0.9998
Accuracy (Mean ± SD%)	99.16 ± 1.014	100.44 ± 0.115	100.37 ± 0.271	100.05 ± 0.511	99.83 ± 1.164	99.64 ± 1.641
Precision *(%RSD)*						
Intra-day	1.484	1.081	0.921	0.831	1.815	0.621
Inter-day	1.742	1.225	1.035	1.134	1.142	0.814

Table 2

Determination of PCM, PBM, and PAM in laboratory-prepared mixtures by the proposed UPLC and TLC methods.

Ratio PCM:PBM:PAM	UPLC method			TLC method		
	Recovery%					
	PCM	PBM	PAM	PCM	PBM	PAM
1:1:1	98.13	98.92	101.33	101.41	99.15	98.27
2:1:2	98.58	99.93	99.36	101.74	98.34	99.86
1:2:2	100.71	97.96	101.66	97.98	100.15	101.64
1:2:1	99.52	100.74	99.21	98.65	101.92	98.33
1:1:2	101.61	99.42	100.18	101.95	98.35	100.19
20:1:0.6[a)]	101.44	101.44	100.35	98.27	98.67	100.83
Mean% ± SD	99.99 ± 1.477	99.74 ± 1.254	100.35 ± 0.998	100.00 ± 1.881	99.34 ± 1.395	99.85 ± 1.348

[a)]Ratio of pharmaceutical formulation

Table 3

Robustness results for the determination of PCM, PBM, and PAM by the proposed UPLC method.

Flow rate		*k*'	R_t [min]	Symmetry	*N*	*α*	*R*
1.4	PCM	1.17	1.75	0.69	4114	6.72	25.43
	PBM	1.59	3.76	0.86	16614	8.74	17.31
	PAM	2.98	5.89	0.79	142986	1.86	22.87
1.5	PCM	0.18	1.76	0.70	4121	6.86	26.47
	PBM	1.6	3.88	0.88	16680	8.91	18.41
	PAM	3.03	6.02	0.81	143008	1.89	23.20
1.6	PCM	0.19	1.84	0.74	4123	6.61	26.93
	PBM	1.33	3.94	0.91	16691	9.12	18.64
	PAM	3.11	6.14	0.87	143112	1.94	23.45

pH		*k*'	R_t [min]	Symmetry	*N*	*α*	*R*
3.4	PCM	0.16	1.68	0.66	4075	6.73	26.37
	PBM	1.42	3.45	0.79	16475	7.39	17.22
	PAM	2.76	5.43	0.73	14284	1.73	22.18
3.5	PCM	0.18	1.76	0.70	4121	6.87	26.51
	PBM	1.6	3.88	0.88	16680	8.91	18.41
	PAM	3.03	6.02	0.81	143008	1.89	23.20
3.6	PCM	0.22	1.94	0.72	4256	6.66	26.48
	PBM	1.73	3.93	0.96	16705	9.34	19.04
	PAM	3.46	7.15	0.88	143248	1.99	24.51

Table 4

Application of a standard addition technique for the determination of PCM, PBM, and PAM by the proposed UPLC and TLC methods.

Recovery% ± SD	Drug Pamprin Multi-Symptom® tablets	UPLC				
		Claimed taken [μg mL^{-1}]	Added [μg mL^{-1}]	Total [μg mL^{-1}]	Recovery%	Mean% ± SD
100.32 ± 0.774	PCM	25	5	30	99.11	100.86 ± 1.632
			15	40	102.36	
			25	50	101.11	
99.68 ± 0.752	PBM	1.25	0.25	1.5	101.31	100.79 ± 1.027
			0.75	2	101.46	
			1.25	2.5	99.61	
98.40 ± 0.553	PAM	0.75	0.15	0.9	100.47	100.91 ± 0.696
			0.45	1.2	100.56	
			0.75	1.5	101.72	
Recovery% ± SD	Pamprin Multi-Symptom® tablets	TLC				
		Claimed taken [ng per spot]	Added [ng per spot]	Total [ng per spot]	Recovery%	Mean% ± SD
99.61 ± 1.672	PCM	100	20	120	100.47	100.07 ± 1.634
			60	160	101.47	
			100	200	98.27	
100.30 ± 0.974	PBM	5	1	6	101.28	100.11 ± 1.615
			3	8	98.27	
			5	10	100.79	
98.75 ± 1.062	PAM	3	0.6	3.6	99.03	100.34 ± 1.137
			1.8	4.8	101.00	
			3	6	101.00	

3.2.4 Specificity

Method specificity was assured by analyzing the laboratory-prepared mixtures of the three studied drugs at different concentrations within the linearity range. Good recoveries (99.43–100.35%) of all drugs indicate high specificity of the two proposed methods **(Table 2).**

3.2.5 Robustness

For the UPLC method, the robustness was assured by studying the influence of deliberate variation in the flow rate and pH of the mobile phase by ±0.1 units. It was found that these variations did not affect the system suitability parameters, where *%RSD* did not exceed 2%, confirming the robustness of the method **(Table 3).** Moreover, the robustness of the proposed TLC method was checked by studying the effect of small variation in the mobile phase ratio. It was observed that there was no significant difference in the R_F value upon changing the acetonitrile volume from 0.05 to 0.1 mL. The *%RSD* did not exceed 1.47%. Also, robustness was tested by studying the effect of different sources of chloroform; it was found that using chloroform (Sigma-Aldrich, Germany and El-Nasr Co., Cairo, Egypt) gave *%RSD* which did not exceed 0.46%, whereas the peak area remained acceptable throughout the assay.

3.3 Application to Pharmaceutical Dosage Form

The developed methods were applied for the quantitative determination of PCM, PBM, and PAM in a Pamprin Multi-Symptom® tablet. The results presented in **Table 4** revealed good mean recoveries, 100.32% ± 0.774 for PCM, 99.68% ± 0.752 for PBM, and 98.4% ± 0.553 for PAM in the UPLC method, while the TLC method showed a recovery range of 99.61 ± 1.672 for PCM, 100.30% ± 0.974 for PBM, and 98.75% ± 1.062 for PAM (Table 4). The selectivity of the developed methods was confirmed by applying a standard addition technique, showing the mean recoveries presented in Table 4. The statistical comparison between the results obtained by applying the proposed procedures and those obtained by applying the reported method showed less calculated *t*- and *F*-values than the tabulated ones revealing no significant difference in accuracy and precision, at 95% confidence limit [38] **(Table 5).**

4 Conclusion

In the present work, sensitive and selective UPLC and TLC–densitometric methods for the determination of PCM, PBM, and PAM in their pure form, laboratory-prepared mixtures, and dosage form have been developed and validated. The developed UPLC method can be recommended as being faster, more sensitive, and more convenient than the reported HPLC method. Moreover, UPLC is cheaper than HPLC, because a higher number of analyses per unit of time can be performed. Regarding the TLC method, it has the advantages of high sensitivity, short run time, large sample capacity, and the use of minimal volume of solvents. All the obtained results were satisfactory, confirming the applicability, accuracy, and precision of these methods. Both methods can be useful in the routine qualitative and quantitative analyses of the studied drugs in their pharmaceutical preparations.

Table 5

Statistical analysis of the proposed methods compared to reported method [5] for the determination of PCM, PBM and PAM in pharmaceutical dosage form.

Parameters	UPLC method			TLC method			Reported method [5][b]		
	PCM	PBM	PAM	PCM	PBM	PAM	PCM	PBM	PAM
Linearity range	5–100 µg mL^{-1}	0.5–20 µg mL^{-1}	0.5–20 µg mL^{-1}	10–280 ng per spot	5–45 ng per spot	1–20 ng per spot	50–150 µg mL^{-1}	2.5–7.5 µg mL^{-1}	1.5–4.5 µg mL^{-1}
N	5	5	5	5	5	5	5	5	5
Mean%	100.32	99.68	98.40	99.61	100.31	98.75	100.14	100.07	99.87
SD	0.771	0.752	0.552	1.673	0.973	1.061	0.512	1.773	0.944
Variance	0.594	0.565	0.304	2.798	0.946	1.125	0.262	3.143	0.891
t-Values[a]	0.343 (2.306)	0.350 (2.306)	2.317 (2.306)	0.521 (2.306)	0.202 (2.306)	1.368 (2.306)	–	–	–
F-values[a]	2.281 (6.39)	5.581 (6.39)	2.865 (6.39)	10.713 (6.39)	3.308 (6.39)	1.261 (6.39)	–	–	–

[a]The values in parenthesis are the theoretical *t*- and *F*-values at $P = 0.05$

[b]The reported method [5] for the determination of PCM, PBM, and PAM is the stability-indicating HPLC assay method using a C_{18} column with methanol and acidified water (pH 1.8) in the ratio of 27:73 *v/v* as the mobile phase at a flow rate of 1.5 mL min^{-1} with detection at 300 nm

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