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SIMULTANEOUS ESTIMATION AND STATISTICAL EVALUATION OF DEVELOPED VALIDATED METHODS FOR COMBINED DRUGS IN MARKETED FORMULATION

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ABSTRACT

We have developed rapid derivative methods for simultaneous estimation of paracetamol (PCM), chlorpheniramine maleate (CPM), and phenylephrine hydrochloride (PH) in pure and liquid dosage form. All methods are simple, accurate, rapid, precise, economical and reliable. Methods are third derivative zero crossing spectrophotometry (**I**), single divisor ratio spectra derivative spectrophotometry (**II**), double divisor ratio spectra derivative method (**III**) and Vierdot's method (**IV**). All the method utilizes methanol: 0.1N hydrochloric acid (1:9). For method **I**, **II**, **III** and **IV** Limit of Detection (L.O.D.) values were within 0.59-1.37 µg/mL, 0.60 – 3.72 µg/mL and 0.71- 3.22 µg/mL respectively; and Limit of Quantification (L.O.Q.) values were within 1.83- 4.40 µg/mL, 2.11-11.91 µg/mL and 2.32–10.70 µg/mL for PCM, CPM and PH respectively. The linearity ranges for PCM, CPM and PH were found to be 1.5-15µg/mL, 5-40 µg/mL and 9-64 µg/mL respectively, showing regression coefficient values $0.999 < R^2 < 1$. Precision values were found less than 2% Relative Standard Deviation (R.S.D.) for all methods. The results obtained have been compared statistically via analysis of variance (ANOVA).

Keywords: *Chlorpheniramine maleate; Paracetamol; Phenylephrine hydrochloride; UV spectrophotometry.*

INTRODUCTION

Derivative spectrophotometry is advanced technique used for single and mainly multicomponent analysis by converting the normal spectrum to its first, second and higher derivative spectrum[1]. In derivative spectrophotometry, we have to found the zero crossing points for one drug while other drugs should show substantial absorbance. The derivative spectrophoto - metric technique has many advantages like it enhances resolution and allows identification of analyte with close ëmax, it eliminate baseline shift effect (arises from the instrument) and scattering effects which generally occurs in turbid solutions[2].

Simultaneous estimation involves the estimation of multiple combination drugs at same time period. It is now being used more frequently for the estimation of drugs in multicomponent pharmaceutical formulations due to their inherent advantages like it avoids time consuming extraction and separation, minimize the use of expensive regents and method further being accurate and precise. This work involves the simultaneous estimation of following drugs in pure and marketed formulation.

Paracetamol (PCM), chemically it is *N*-(4-Hydroxy-phenyl) acetamide[3] (PCM; Fig. 1). It is an analgesic, antipyretic and most commonly used over-the-counter remedy for minor aches and pains and relief of headaches and used along with various cold preparations. It may act by inhibiting cyclo-oxygenase (COX-3, a linked variant of COX-1).[4]

Chlorpheniramine maleate (CPM), it is chemically (3*RS*)-3-(4-Chlorophenyl)-*N*, *N*-dimethyl-3-(pyridin-2-yl)propan-1-amine hydrogen (*Z*)-butenedioate[5] (CPM; Fig. 1), is an antihistamine (first-generation alkylamine) used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria, also indicated in food or insect sting allergy. It has low sedative effect than other antihistamines.[6]

Phenylephrine hydrochloride (PH), chemically it is (1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino) ethanol hydrochloride[7] (PH; Fig. 1). PH is a selective $á_1$-adrenergic receptor agonist used primarily in nasal decongestion, as an agent to dilate the pupil, and to increase blood pressure.[8]

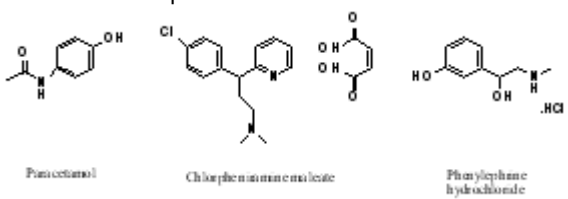

Fig. 1 : *Chemical structure of Paracetamol, Chlorpheniramine maleate and Phenylephrine hydrochloride.*

All three drugs are official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). The physical properties of PCM, CPM and PH were estimated and

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used for active pharmaceutical ingredient (Table 1). The PCM,[9] CPM[10] and PH[11] alone or in combination with other drugs is reported to be estimated by spectrophotometric method,[12-16] derivative spectrophotometric method,[17] chemometric-assisted spectrophotometric,[18] HPLC,[19-20] TLC,[21] LC-MS,[22] FT-IR,[23] amperometric determination,[24] fluorimetry,[25] micellar electrokinetic chromatographic method,[26] electrophoresis,[27] liquid chromatography with two UV absorbance detector[28] and chemometric determination.[29]

Table 1: *Physical properties of the standard drugs.*

Method	Drugs	λ_{max} (nm)	Range (µg/mL)	Slope[a]	Intercept[a]	Correlation coefficient (R^2)	Accuracy ± S.D.[b]	LOD[c] (µg/mL)	LOQ[c] (µg/mL)
I	PCM	253.70	15-35	0.016	0.0010	0.9990	97.00± 1.34	1.37	4.40
	CPM	244.95	5-40	0.036	0.0270	0.9950	97.16± 1.04	0.60	2.11
	PH	291.30	9-64	0.001	0.0003	0.9990	97.02± 0.93	3.22	10.70
II	PCM	218.95	15-35	0.100	0.2000	0.9950	100.9± 2.10	0.80	2.57
	CPM	254.80	5-40	0.020	0.0500	0.9950	96.89± 1.10	2.02	5.77
	PH	261.06	9-64	0.020	0.0100	0.9920	96.74± 1.00	1.60	5.17
III	PCM	232.70	15-35	0.050	0.0200	0.9990	96.76± 0.62	0.70	2.32
	CPM	253.35	5-40	0.050	0.0400	0.9950	97.51± 1.02	0.71	2.34
	PH	237.86	9-64	0.009	0.0200	0.9940	96.27± 0.78	1.08	3.59
IV	PCM	244.00	15-35	0.073	0.0430	0.9991	96.59± 0.76	0.59	1.83
	CPM	264.00	5-40	0.030	0.1000	0.9995	97.23± 0.53	3.72	11.91
	PH	274.00	9-64	0.020	0.0420	0.9995	98.00± 0.51	0.71	2.32

[a]For method II and III slope and intercept were multiplied by 1000.
[b]n=10, [c]n=6

As per literature survey, no analytical method has been developed by using derivative spectrophotometric method for this tertiary combination which is more reliable, simple, accurate and precise method for analysis of multicomponent mixture.

In contrast to other spectrophotometric techniques, the derivative spectrophotometric methods (third derivative zero crossing spectrophotometry, single divisor ratio spectra derivative method, double divisor ratio spectra derivative method) are more rapid, simpler and economical methods and is widely used in the multicomponent analysis of mixtures by UV spectrophotometry. We can resolve tertiary mixture easily with these methods. This method can be applied in pharmaceutical analysis for the determination of individual drugs in multicomponent mixture.

The present study mainly aims at developing a sensitive, simple, rapid, economical, precise and accurate derivative spectrophotometric method for the determination of PCM, CPM and PH in oral suspension and their statistical evaluation by using two- way ANOVA. In present research work 1st, 2nd and 3rd orders of normal spectra were analyzed on trial basis by using different number of data points for slope calculation. All the four methods were developed and satisfactory results were obtained.

EXPERIMENTAL

Selection of analyte wavelength: The wavelength was selected after analyzing the samples in different solvents individually and in combinations like methanol/HCl, and methanol/NaOH at different concentration (in moles). The best results were found in methanol: 0.1N HCl in 1:9 and also satisfactory linearity were obtained at 244 nm, 264 nm and 274 nm for PCM, CPM and PH respectively.

Instruments

UV-Vis double beam spectrophotometer Perkin-Elmer Lambda-35 was used for all spectrophotometric measurements, having slit width of 1 nm, installed with UV Winlab and UV Winlab data processor and viewer software. Ultra bath sonicator (PCI analytics 3.5 L capacity) was used for proper mixing of stock solutions and sonication.

Reagents and materials

All chemicals used were analytical grade (Central Drug House Chemicals, New Delhi, India). Pure PCM, CPM, and PH were obtained from Syncom Health Care Limited, Dehradun, India. Marketed formulation named as COLD-GO which is an oral suspension, Torque Pharmaceutical PVT. Ltd, batch No. HG-278 was procured from local market.

Solutions

Pure samples stock solution 1 mg/mL of PCM, CPM and PH were freshly prepared individually in methanol and further dilutions were made by using 0.1N HCl (for spectrophotometric measurement blank was kept constant in the ratio of 1:9 methanol:0.1N HCl). Marketed formulation which was labeled as each 5 ml of oral suspension contain 125 mg PCM, 2mg CPM and 5mg PH (having coloring agent ponceau4R) was used for the study.

Procedure

All the reagent and analytes were stable up to 24 hour from their preparation at room temperature. All the three drugs were found to be stable during each experimental condition and in the solvent mixture i.e. methanol: 0.1N HCl (1:9) and each spectrophotometric measurement were taken at room temperature.

Extraction procedure

For preparation of marketed dilutions, the equivalent weight were calculated and according to weight, required suspension firstly extracted in methanol and 0.1N HCl (1:1) by using sonication process for 1 hour at room temperature. After this process the resultant solution was filtered through Whatman filter paper number 41. Then further dilutions were made in methanol: 0.1N HCl (1:9) and absorbance were measured and analyzed.

Standard laboratory mixture

Standard laboratory mixture was prepared to obtain precise result by making the concentration same as

marketed formulation i.e. 25 µg/mL, 0.4 µg/mL and 1 µg/mL for PCM, CPM and PH respectively.

Third derivative zero order crossing spectrophotometry (Method I)

All the spectra of the sample were scanned between 320-210 nm using a 1.0 cm quartz cell. The zero order spectra of the three pure drugs in their concentration range (PCM, CPM and PH; 1.5-15µg/mL, 5-40 µg/mL and 9-64 µg/mL respectively) were saved separately. These spectra were derivatized in 1st, 2nd and 3rd order derivative and satisfactory results were found by using third derivative spectra in which number of data points used for slope calculation was set as 49. The third derivative spectra were recorded at 252.77 nm, 244.95 nm and 297.7 nm for PCM, CPM and PH respectively. Standard laboratory mixture and commercial formulation were scanned, derivatized and analyzed at the same wave length as mentioned above.

Single divisor ratio spectra derivative spectrophotometry (Method II)

Pure drug and marketed formulation were scanned between 320 and 210 nm. Pure PH and tertiary mixture spectra were divided by pure spectra of PCM. Pure CPM and their tertiary mixture spectra were divided by pure spectra of PCM. Pure PCM and tertiary mixture spectra were divided by pure spectra of PH (highest concentrations of all three drugs were used as a divisor), all the spectra were stored and derivatized to 3rd order by using 49 data points. PCM was analyzed at 218.97 nm by zero crossing point for CPM and PH as divisor. CPM was analyzed at 254.83 nm through zero crossing point for PH and PCM as divisor, whereas PH was analyzed at 261.06 nm by zero crossing point for CPM and PCM as divisor. Calibration graphs for PCM, CPM and PH were obtained having satisfactory correlation coefficient (R^2) values. Then the standard laboratory mixture and marketed formulation were also determined by above described procedure.

Double divisor ratio spectra derivative spectrophotometry (Method III)

Pure drug and marketed formulation were scanned between 320 and 210 nm. Then the tertiary mixture and pure PCM spectra were divided by a standard spectrum which was obtained by the sum of spectra of CPM and PH. The tertiary mixture and pure CPM spectra were divided by a standard spectrum which was obtained by the sum of spectra of PCM and PH. Similarly for PH, the tertiary mixture and pure PH spectra were divided by a standard spectrum which was obtained by the addition of spectra of PCM and CPM. All the spectra were derivatized into third order by using data points 13. After that the calibration graph were obtained for all the three drugs having satisfactory R^2 values at 232.71 nm, 253.37 nm and 227.86 nm for PCM, CPM and PH respectively and further standard laboratory mixture and marketed formulation were also analyzed according to above procedure.

Vierdot's method[1] (Method IV)

This method is also known as simultaneous equation method.[1] This method utilized simultaneous equations which were obtained from the overlay spectra of three drugs. This method is based on the absorption and absorptivity of PCM, CPM and PH calculated at wavelength maximum of each drug. These parameters were calculated at $\ddot{e}_{max}$ 244 nm, 264 nm and 274 nm for PCM, CPM and PH respectively. The absorptivity values were measured at $\ddot{e}_{max}$ of each other.

Further standard laboratory mixture and marketed formulation were analyzed accordingly as described above. The marketed formulation was analyzed by extracting the suspension and solution was diluted as required and scanned over 320-210 nm region. Finally the equations were solved by using matrix calculations.

Validation

The proposed methods were validated as per International Conference of Harmonization (ICH) guidelines with respects to linearity, range, accuracy, precision, LOD (Limit of detection), LOQ (Limit of quantitation), specificity and robustness[30]

RESULT AND DISCUSSION

Overlay of the absorption spectra of PCM, CPM and PH is shown in the Fig. 2. Concentration ranges for PCM, CPM and PH were found to be 1.5-15 µg/mL, 5-40 µg/mL and 9-64µg/mL respectively. All the proposed methods were applied and satisfactory results were obtained which are shown further.

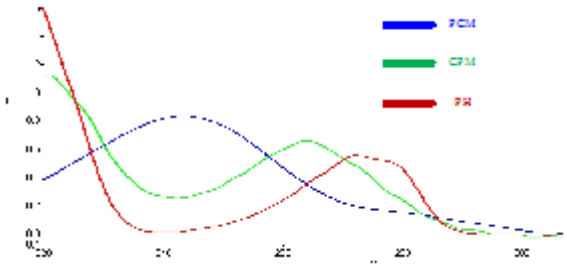

Fig. 2 : *Overlay spectra of PCM, CPM and PH at 244 nm, 264 nm and 274 nm respectively .*

Third order derivative zero crossing spectrophotometry (Method I)

The third order derivative spectra show more resolution in contrast to zero, first and second order spectra [the linearity of the method is shown in Fig. 3(a)] in terms of zero crossing points as shown in Fig. 3b, c &d. The CPM and PH were having zero crossing point at 252.77 nm, PCM and PH at 244.95 nm and PCM and CPM at 297.7 nm that's why the wavelengths were selected for determination of individual as 252.77 nm for PCM (Fig. 3b), 244.95 nm for CPM (Fig. 3c) and 297.7 nm

for PH (Fig. 3d). Method was validated and results of various parameters are shown in Table 2a, 2b and 2c. Further the results of marketed formulation analysis are shown in Table 3a. Results of recovery studies are also shown in Table 3b.

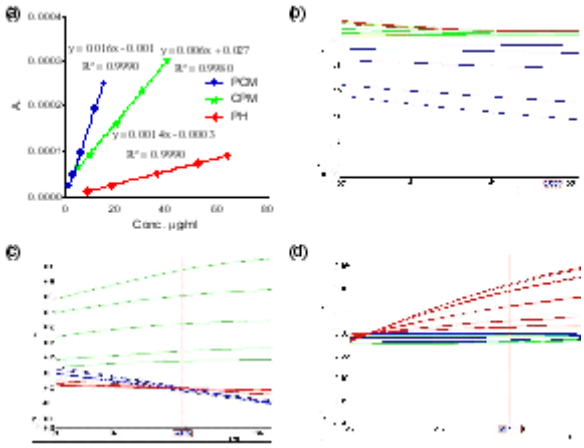

Fig. 3 : *(a) Calibration curve of PCM, CPM and PH. Third order derivative zero crossing spectra; (b) PCM at 252.77 nm, (c) CPM at 244.95 nm (d) PH at 297.7 nm.*

Single divisor ratio spectra derivative spectrophotometry (Method II)

For this method several measurements were made on trial basis to select the accurate standard divisors and different wavelengths. We found changes while changing the divisor concentrations, so accurate pure drug divisor was required to obtain the satisfactory result. For determining working wavelength for PCM, CPM and PH, highest concentration of divisors were selected on trial basis. The linearity of the method is shown in Fig. 4 (a). The derivative wavelengths were selected as 218.97 nm for PCM, 254.83 nm for CPM and 261.06 for PH as shown in Fig. 4 (b), (c) and (d),

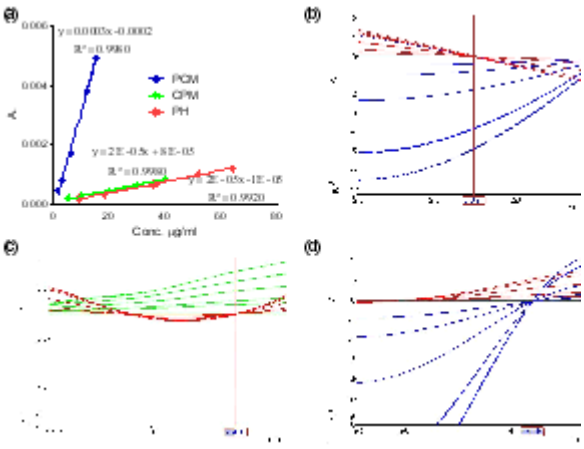

Fig. 4 : *(a) Calibration curve for PCM, CPM and PH. Derivative ratio spectra; (b) PCM at 218.97 nm (c) CPM at 254.83 nm (d) PH at 261.06 nm.*

respectively. Standard laboratory mixture analysis was done according to above described procedure. Method was validated and results of various validation parameters were shown in Table 2a, 2b and 2c. The results of marketed oral suspension analysis are shown in Table 3a. Results of recovery studies are shown in Table 3b.

Table 2a : *Validation parameters obtained by all methods.*

Method	Drugs	λ_{max}(nm)	Range (µg/mL)	Slope[a]	Intercept[a]	Correlation coefficient (R^2)	Accuracy ± S.D.[a]	LOD[a] (µg/mL)	LOQ[a] (µg/mL)
I	PCM	252.77	1.5-15	0.016	0.0010	0.9999	97.00 ± 1.34	1.37	4.40
	CPM	244.95	5-40	0.006	0.0210	0.9980	97.16 ± 1.04	0.60	2.11
	PH	297.70	9-81	0.001	0.0003	0.9990	97.62 ± 0.83	3.22	10.70
II	PCM	218.97	1.5-15	0.100	0.2000	0.9950	100.96 ± 1.30	0.80	2.57
	CPM	254.83	5-40	0.020	0.0500	0.9950	96.99 ± 1.13	2.02	5.77
	PH	261.06	9-81	0.020	0.0100	0.9920	96.74 ± 1.00	1.60	5.17
III	PCM	252.77	1.5-15	0.050	0.0200	0.9990	96.76 ± 0.62	0.70	2.32
	CPM	253.37	5-40	0.050	0.0400	0.9950	97.51 ± 1.02	0.71	2.34
	PH	297.86	9-81	0.009	0.0200	0.9940	96.21 ± 0.75	1.08	3.59
IV	PCM	264.00	1.5-15	0.070	0.0410	0.9991	96.59 ± 0.76	0.59	1.83
	CPM	264.00	5-40	0.030	0.1000	0.9995	97.23 ± 0.53	3.72	11.91
	PH	274.00	9-81	0.020	0.0420	0.9995	95.00 ± 0.53	0.71	2.32

[a]For methods II and III slope and intercept were multiplied by 1000.
[a](n=10), (n=6)

Table 2b : *Robustness study results for all methods.*

Method	Drugs	Variation in wavelength ± 0.2 nm (n=6)		Variation in solvent ± 0.2 (n=6)	
		λ - 0.2 nm (%R.S.D. ± S.D.)	λ + 0.2 nm (% R.S.D. ± S.D.)	Methanol: 0.1M NHCl (1:9) (% R.S.D. ± S.D.)	Methanol: 0.1N HCl (1:9) (%R.S.D. ± S.D.)
I	PCM	0.9857 ± 0.19	0.9899 ± 0.29	0.9258 ± 0.16	0.9211 ± 0.21
	CPM	0.3892 ± 0.34	0.4107 ± 0.12	0.3469 ± 0.02	0.4871 ± 0.31
	PH	1.1913 ± 0.37	1.2121 ± 0.55	1.0069 ± 0.28	0.9885 ± 0.55
II	PCM	0.4565 ± 0.27	0.5195 ± 0.23	0.2455 ± 0.17	0.4654 ± 0.59
	CPM	0.5214 ± 0.37	0.5130 ± 0.42	0.1799 ± 0.02	0.6683 ± 0.67
	PH	0.3770 ± 0.15	0.3037 ± 0.15	0.3872 ± 0.31	0.3525 ± 0.29
III	PCM	0.3855 ± 0.08	1.0380 ± 2.05	1.4047 ± 0.81	0.1917 ± 0.12
	CPM	0.3851 ± 0.15	0.2291 ± 0.15	0.5102 ± 0.37	0.2188 ± 0.27
	PH	0.7068 ± 0.44	0.7914 ± 0.50	0.9317 ± 0.72	0.7506 ± 0.72
IV	PCM	0.4003 ± 0.23	0.4245 ± 0.25	0.2234 ± 0.11	0.5673 ± 0.10
	CPM	1.2826 ± 0.10	0.9473 ± 0.10	0.4837 ± 0.47	1.0110 ± 0.42
	PH	1.0812 ± 0.38	1.1330 ± 0.37	0.7169 ± 0.59	0.7359 ± 0.67

Table 2c : *Precision study (Intra-day and Inter-day) results for all four methods.*

		Method I	Method II	Method III	Method IV
Intra-day (R.S.D. (n=6))	PCM	0.7091 ± 0.29	0.2653 ± 0.17	0.339 ± 0.20	0.385 ± 0.19
	CPM	0.2382 ± 0.04	0.3955 ± 0.22	0.235 ± 0.09	0.678 ± 0.28
	PH	0.8401 ± 0.32	0.5984 ± 0.31	0.837 ± 0.14	1.028 ± 0.10
Inter-day (R.S.D. (n=3))	PCM	0.5140 ± 0.32	0.5038 ± 0.21	0.238 ± 0.11	0.745 ± 0.33
	CPM	0.6111 ± 0.14	0.4690 ± 0.19	0.298 ± 0.05	0.914 ± 0.19
	PH	0.8886 ± 0.63	0.8135 ± 0.34	0.858 ± 0.38	1.088 ± 0.19

Table 3a : *Assay results of PCM, CPM and PH in marketed oral suspension.*

Method	Labeled claimed obtained for PCM[a] (%) (Mean ± S.D.)	Labeled claimed obtained for CPM[a] (Mean ± S.D.)	Labeled claimed obtained for PH[a] (Mean ± S.D.)
I	96 ± 1.34	96.00 ± 1.01	97.21 ± 0.53
II	98.55 ± 2.7	97.22 ± 1.13	96.72 ± 1
III	97.61 ± 0.62	96.57 ± 1.02	96.33 ± 0.782
IV	95.56 ± 0.76	97.5 ± 0.586	97.93 ± 0.523

[a]Paracetamol suspension contains not less than 95.0 per cent and not more than 105.0 per cent w/v solution of the stated amount of paracetamol. Chlorpheniramine suspension contain not less than 95.0 percent and not more than 105.0 per cent of the stated amount of orphenadrine maleate. [a]Pimylephrine suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of phenylephrine hydrochloride.

Table 3b : *Recovery studies results of PCM, CPM and PH by all methods.*

Amount added (µg/mL)				Method I			Method II			Method III			Method IV		
	PCM	CPM	PH	PCM	CPM	PH	PCM	CPM	PH	PCM	CPM	PH	PCM	CPM	PH
80 %	20	3.76	3.8	97.66	96.38	97.87	101.48	98.26	97.21	96.67	97.5	97.36	97.76	97.81	97.38
100 %	25	5.2	7	97.83	97.6	97.38	99.21	96.68	97.83	96.73	98.5	98.66	96.48	97.23	98.93
120 %	30	6.24	7.2	97.81	98.41	96.79	104.83	96.82	96.41	96.76	98.78	96.74	97.3	96.68	98.36
Mean recovery		(n=3)		97.66	97.33	96.83	101.76	96.79	96.76	96.48	98.24	96.26	96.84	97.14	98.33
S.D.		(n=3)		0.333	1.17	1.11	3.703	1.36	1.23	0.31	0.66	0.89	0.40	0.82	0.69

Double divisor ratio spectra derivative method (Method III)

On trial basis, the working derivative wavelengths [the linearity of the method is shown in Fig. 5 (a)] were selected such as 232.71 nm for PCM, 253.37 nm for CPM and 227.86 nm for PH shown in Fig. 5 (b), (c) and (d). The derivative working wavelengths were selected for all drugs having maximum amplitudes and best R^2 value. Standard laboratory mixture analysis was done according to above described procedure. Method was validated and results of various validation parameters are shown in Table 2a, 2b and 2c. Analysis of marketed oral suspension was done and results are shown in Table 3a. Results of recovery studies are shown in Table 3b.

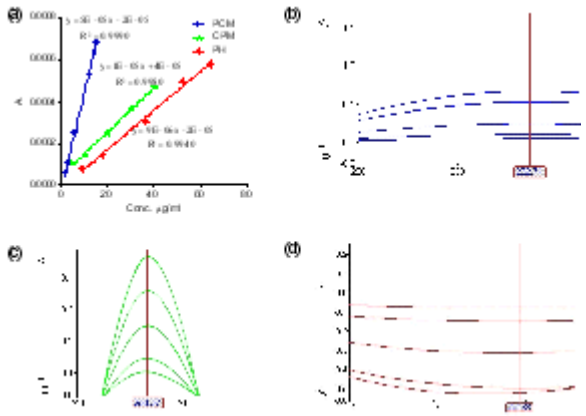

Fig. 5 : *(a) Calibration curve for PCM, CPM and PH. Double divisor ratio derivative spectra; (b) PCM at 232.71 nm (c) CPM at 253.37 nm (d) PH nm at 227.86 nm.*

Vierdot's method (Method IV)

In this method, calibration curve [Fig. 6 (a)] and three equations were obtained from the overlay spectra shown in Fig. 6(b), (c) and (d) of all the three drugs. The absorptivity of PCM, CPM and PH were determined at wavelength maximum of each other after scanning at 320-210 nm. The $\ddot{e}_{max}$ for all three drugs were found at 244 nm, 264 nm and 274 nm for PCM, CPM and PH respectively. The absorptivities were calculated at the maximum wavelength for all drugs. These absorptivity values were found, for PCM; 0.0637, 0.0262and 0.01201, for CPM; 0.00753, 0.02417and 0.01586 and for PH; 0.00175, 0.00781and 0.01127 at 244 nm, 264 nm and 274 nm respectively. Further these absorptivity coefficient values were substituted in equation 1, 2 and 3 to obtain the concentration of the drugs. These equations were solved by matrix calculation by using unscramble software. Equations were listed below:

$A_1 = 0.0637\ b\ C_{PCM} + 0.0075\ b\ C_{CPM} + 0.0018\ b\ C_{PH}$ Eq. (1)

$A_2 = 0.0262\ b\ C_{PCM} + 0.0242\ b\ C_{CPM} + 0.0078\ b\ C_{PH}$ Eq. (2)

$A_3 = 0.0120\ b\ C_{PCM} + 0.0159\ b\ C_{CPM} + 0.0113\ b\ C_{PH}$ Eq. (3)

Where, C_{PCM}, C_{CPM}, and C_{PH} are concentrations of PCM, CPM and PH respectively in µg/mL. A_1 (1.62), A_2 (0.73), and A_3 (0.40) are the absorbance of the marketed

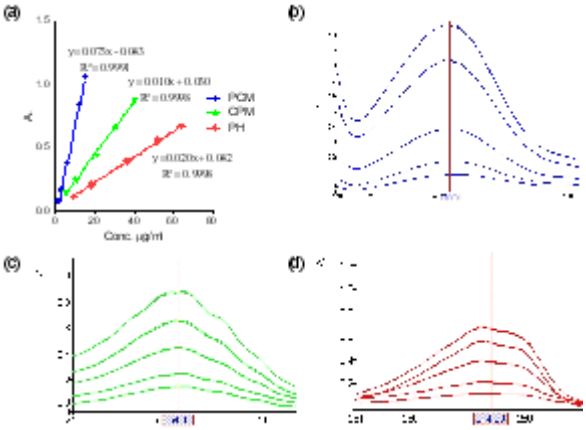

Fig. 6 : *(a) Calibration curve for PCM, CPM and PH. Overlay spectra; (b) PCM at 244 nm (c) CPM at 264 nm (d) PH nm at 274 nm.*

sample for PCM, CPM and PH respectively; b denotes the path length (1cm) These equations were used for simultaneous estimation of PCM, CPM and PH in standard laboratory mixture as well as marketed formulation. Method was validated and results of various validation parameters are shown in Table 2a, 2b and 2c. Analysis of marketed oral suspension was performed and results are shown in Table 3a. Results of recovery studies are shown in Table 3b.

Significant difference of developed four methods statistically

For determination of significant difference between these methods, two-way ANOVA was applied. For this statistics was successfully applied on precision and accuracy data of different methods. Calculated values were less than the theoretical value. Further there is no significant difference between the proposed four methods. Data are shown in Table 4a and results are shown in Table 4b.

Table 4a : *Accuracy and precision data for statistical comparison by all methods.*

Method	Drug	Accuracy		Precision			
		Mean %	S.D. (±)	Intra-day		Inter-day	
				Mean	S.D. (±)	Mean	S.D. (±)
I	PCM	97.00	1.34	0.7091	0.29	0.5140	0.32
	CPM	97.15	1.04	0.2382	0.04	0.6111	0.14
	PH	97.02	0.93	0.8401	0.32	0.8836	0.63
II	PCM	100.96	2.70	0.2953	0.17	0.5336	0.21
	CPM	96.89	1.13	0.3895	0.22	0.4690	0.18
	PH	96.74	1.00	0.5994	0.31	0.6135	0.34
III	PCM	96.76	0.62	0.3337	0.30	0.2360	0.11
	CPM	97.91	1.02	0.2761	0.08	0.2987	0.06
	PH	96.27	0.78	0.8375	0.14	0.8548	0.33
IV	PCM	96.59	0.76	0.3858	0.18	0.7452	0.33
	CPM	97.23	0.53	0.6789	0.24	0.9146	0.19
	PH	98.00	0.53	1.0260	0.10	1.0867	0.19

Table 4b : *Statistically evaluated results for accuracy and precision by two-way ANOVA.*

Parameters	$F_{calculated}$	$F_{theoretical}$
Accuracy (mean ± S.D.)	1.38	19.2
Intra-day (mean ± S.D.)	5.27	19.2
Inter-day (mean ± S.D.)	1.26	19.2

Conclusion

Derivative spectrophotometry analytical technique is frequently used in the contemporary analysis of drugs in multicomponent mixtures in today scenario. The developed spectrophotometric methods which were used for simultaneous estimation of PCM, CPM and PH were selective, specific, simple, accurate, rapid, precise, economical and reliable. All the derivative methods and Vierdot's method was validated accurately and can be used for the simultaneous estimation of PCM, CPM and PH in combined dosage form. By applying ANOVA, it was also concluded that there is no significant difference between all proposed methods, so these methods can be interchangeable for analysis of same drugs. These procedures can be applied for routine quality control analysis of oral suspension as well as solid dosage form.

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