Original Article

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Simultaneous determination of paracetamol, phenylephrine, chlorpheniramine and related compound 4-aminophenol in multi-components pharmaceuticals by high performance liquid chromatography

P.T. Dung[1], K.X. Hai[1]*

[1] Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City 41 Dinh Tien Hoang street, District 1, Ho Chi Minh city, Viet Nam

Abstract

Nowadays, some pharmaceuticals consisted of paracetamol (PAR), phenylephrine hydrochloride (PHE), chlorpheniramine maleate (CPM) which use to reduce several symptoms associated with common cold are used widely. Together with quantitation of these compound, a related compound, 4-aminophenol (PAP) must be controlled. In the previous studies, quantitaion of these compounds were often conducted separately and some researches were confused about identification between chlorpheniramine and maleic acid. This research aims to develop a simple and rapid gradient reversed-phase high performance liquid chromatography method for simultaneous determination of paracetamol, phenylephrine, chlorpheniramine and limit related compound 4-aminophenol in pharmaceutical formulations. These compounds were separated on Eclipse XDB C_{18} column (250 x 4.6 mm i.d., size 5 µm) with a gradient elution using potassium dihydrogen phosphate buffer (pH 2.5) and acetonitrile as mobile phase. The flow rate of mobile phase was adjusted to 1.4 ml/min, temperature 35 ^{o}C and UV detection at wavelenghts of 265 nm for chlorpheniramine and 278 nm for paracetamol, phenylephrine and 4-aminophenol. The method was validated according to ICH guidelines and applied to assay in tablet dosage form without any interference from excipients. The validation characteristics included accuracy, precision, linearity, range, specificity, limit of detection (LOD). The linearity for all the drugs was obtained in the range of 190-455 µg/ml (PAR), 3-7 µg/ml (PHE), 1.2-2.8 µg/ml (CPM) and 0.25-20 µg/ml (PAP) with the correlation coefficients for linearity were in rank 0.9995 – 0.9999. The recovery of PAR, PHE, CPM was found in range 98 – 102% and the RSD of repeatability was found less than 2% . The LOD of 4-aminophenol was 0.0576 µg/ml. Thus, the proposed method can be successfully applicable to the pharmaceutical preparation containing the above mentioned drugs.

Keyword: HPLC; 4-aminophenol; Paracetamol; Pheneylephrine; Chlorpheniramine maleate.

1. INTRODUCTION

Nowadays, some pharmaceuticals consisted of paracetamol, phenylephrine hydrochloride, chlorpheniramine maleate which use to reduce several symptoms associated with common cold are used widely. Chlorpheniramine maleate (CPM), 3-(p-chloro-phenyl)-3-(2-pyridyl)-N,N dimethyl propylamine ($C_{16}H_{19}C_lN_2.C_4H_4O_4$), is a powerful first-generation alkyl amine antihistamine, H1 receptor antagonist, widely used for symptomatic relief of common cold and allergic rhinitis, with weak sedative properties. Phenylephrine hydrochloride (PHE), (*R*)-1-(3-hydroxy-phenyl)-2-methylaminoethanol hydrochloride ($C_9H_{13}NO_2$, HC_l) is useful as a nasal and sinus decongestant. Paracetamol (PAR), acetaminophen, 4-hydroxyacetanilide ($C_8H_9NO_2$), is a popular over-the-counter analgesic and antipyretic drug. Together with quantitation of these compound, a related compound, 4-aminophenol (PAP) must be controlled. In the previous studies (Table 1), quantitaion of these compounds were often conducted separately and some research were

***Corresponding author:** dungpharm@yahoo.com

confused about identification between chlorpheniramine and maleic acid (MAL). Therefore, this research aims to identify cleary chlorpheniamine and maleic acid. Morever, a high performance liquid chromatography procedure to simultaneous assay paracetamol, phenylephrine, chlorpheniramine and limit 4-aminolphenol was prepared.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Chemical reference substance: PAR, batch: QT 009 150414, content: 99.84%; PHE, batch: QT 178 030814, content: 100.02%, CPM, batch: QT 021 070215, content: 99.75%; PAP, batch: QT 216 020914, content: 99.87% were kindly suplied by The Institute of Drug Quality Control – Ho Chi Minh City.

Reagent: Acetonitrile (HPLC grade) was obtained by Prolabo, maleic acid, potassium dihydrogen phosphate, triethylamine were obtained from Merck. Pharmaceutical dosage form contained 325 mg PAR, 5 mg PHE and 2 mg CPM.

2.2. Equipment and chromatographic conditions

The HPLC consisted of a degasser DGU-20 A5, pump LC-20 AT, oven CTO-10 ASVP, SPD M-20A photo diode array detector and a SIL-M 20AC prominence auto sampler with data processor LC solution (Shimadzu, Japan). All pH measurements were performed on a Scott lab 850. Chromatographic separation was carried with gradient elution at 35 ^{o}C with an Eclipse XDB C_{18} column (250 4.6 mm i.d., size 5 μm) from Agilent. Mobile phase was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 1000 ml of double-distilled water, add 3 ml triethylamine. The pH of the dibasic phosphate buffer was adjusted to 2.5 with orthophosphoric acid. Finally, the mobile phase was filtered through a 0.45 μm membrane filter and degassed for 10 minutes. The injection volumes for samples and standards were 20 μl and eluted at a flow rate of 1.4 ml/min at 35 °C. The eluents were monitored at 265 nm and 278 nm. Gradient elution program.

Time (min)	ACN (%)	Buffer (%)
0	0	100
1	3	97
5	5	95
6	10	90
11	10	90
12	15	85
23	15	85
24	0	100
35	0	100

2.3. Preparation of standard solutions

A mixture of standard solution containing CPM 2 μg/ml, PHE 5 μg/ml, PAR 325 μg/ml, maleic acid 10 μg/ml and PAP 10 μg/ml was prepared by dissolving CPM, PHE, PAR and PAP reference standard in diluents (acetonitrile - buffer, 10 : 90, v/v). The mixture was sonicated for 15 min or until the reference standard dissolved completely.

2.4. Preparation of sample solutions

Twenty tablets, each containing 2 mg CPM, 5 mg PHE, 325 mg PAR are weighed accurately and powdered finely. A quantity of powder which is equivalent to 2 mg of CPM, 5 mg of PHE, 325 mg of PAR was weighed accurately and transferred into a 50 ml calibrated volumetric flask. About 50 ml of diluent was added and ultrasonicated for 15 min; finally the volume was adjusted to the mark. The resulting solution obtained was then filtered, through 0.45 μm filter for complete removal of particulate matter. 5 ml of the filtrate was diluted to 100 ml in the volumetric flask with the diluent for analysis.

2.5. Method validation

The proposed method was subjected to validation for various parameters like system suitability, specificity, range and linearity, accuracy, precision and limit of detection in accordance with International Conference on Harmonization guidelines[7]. The replicates of standard solution, which contain (CPM 2 μg/ml, PHE 5 μg/ml, PAR 325 μg/ml), were carried out to assess the system suitability. It was further

evaluated by analyzing the repeatability of retention time, area, tailing factor, theoretical plates of the column. The specificity of the chromatographic method was determined to ensure separation of CPM, PHE, PAR, maleic acid and PAP. Specificity was also determined in the presence of excipients used in formulation, CPM, PHE, PAP, maleic acid and PAR was spiked (at a concentration of 2 µg/ml CPM, 5 µg/ml PHE, 325 µg/ml PAR, 10 µg/ml MAL and 10 µg/ml PAP) in placebo and chromatogram was observed and compared with that of reference standard. A PDA detector was used to check the peak purity.

Table 1. Review of the analytical methods for the determination of paracetamol, phenylephrine and chlorpheniramine

Analytical methods	Compounds	Mobile phase	Column	UV detection	Flow rate	Injection volume
1 [3]	Paracetamol Phenylpropanolamine.HCl Chlorpheniramine maleat Caffein Glycerylguaiacolat	ACN - THF - buffer (7 : 8 : 85) Buffer: Sodium hexasulphonic acid 5mM, dibutylamin 10 mM, glacial acid acetic 0,12% (v/v) pH= 3,3	LiChrospher, 5 µm	310 nm 260 nm 298 nm 284 nm 265 nm	1,0 ml/min	20 *µl*
2 [2]	Paracetamol Chlorpheniramine maleate Phenylephrine HCl	ACN - buffer (78 : 22) Buffer : 1,36 ml acid orthophosphoric in 1000 ml water, add triethylamin to pH= 6,22	µBondapak CN, (5 µm, 3,9 x 150 mm) Temperature: 22 °C	265 nm	1,5 ml/min	15 *µl*
3 [1]	Paracetamol Chlorpheniramine maleate Phenylephrine Dextromethorphan	Methanol - buffer (95 : 5) Buffer: 6 g amoniacetat, 10 ml triethylamine in 1000 ml water, adjusted to pH=5 by acid phosphoric	PerfectSil Target, (250x 4,6 mm, 5µm) Temperature: 30 °C	254 nm 220 nm 227 nm	1,2 ml/min	20 *µl*
4 [4]	Paracetamol 4-aminophenol 4-nitrophenol	Gradient, ACN - buffer Buffer: Solution potassium dihydrophosphat 0,01 M adjusted to pH=3 by acid phosphoric	Symmetry C18, (250 x 4,6 mm, 5 µm)	215 nm	1 ml/min	
5 [6]	Paracetamol Clorpheniramine maleate Phenylpropanolamin.HCl Caffein	ACN- buffer - MeOH (15 : 75 : 10) Buffer: 6,84 ml acid phosphoric in 100 ml water add 0,3 ml triethylamine, 1,05 mg sodium hexasulphonate, adjusted to pH= 2,8 by acid phosphoric.	Inertsil C18 (250 x 4,6 mm, 5 µm)	220 nm	1 ml/min	20 *µl*
6 [5]	Paracetamol Chlorpheniramine maleate Phenylephrine Caffeine	ACN – buffer (7 : 93) Buffer: 6,8 g KH_2PO_4 in 1000 ml water, adjusted to pH 4 by acid phosphoric	Inertsil C18 (250 x 4,6 mm, 5 µm)	215 nm	1,5 ml/min	20 *µl*

The linearity is important to demonstrate that the response of the measurement of detector system is linear over the range of interest of the method. This was determined by means of calibration graph using increasing amounts of a standard solution (60, 80, 100, 120 and 140%) of the standard were tested according to ICH guidelines.

A calibration curve was constructed and the proposed method was evaluated by its correlation coefficient and intercept value (ANOVA, $p < 0.05$). The correlation coefficient was found within limit. The method was validated for accuracy and precision. The accuracy of the method was studied as mean % recovery.

Accuracy was determined by means of recovery experiments, by spiked addition of active drug to placebo formulations. It was shown that the recoveries were independent of the concentration of the active drug over a reasonable concentration range normally 80 to 120% of the nominal concentration. The accuracy of the assay was measured by analyzing samples of CPM, PHE, PAR, by spiking known amounts of said drugs in the placebo, at different concentration levels (80, 100 and 120%).

ICH guidelines recommend that precision must be considered at two levels, i.e., repeatability and intermediate precision. The repeatability of the method was tested by injecting 100% concentration of three analytes of the regular analytical working value consecutively for six samples and examining the effects on the results. The intermediate precision was tested with respect to laboratory variations, by using different equipments, different analysts and days. The relative standard deviation (RSD%) was determined to assess the precision of the assay and it was not more than 2.0%

The limit of detection (LOD) of 4-aminophenol LOD= 3.3×SD/S , where SD is the residual standard deviation of the reponse and S is the slope of the linearity line of 4-aminophenol.

3. RESULT AND DISCUSSION

3.1. Specificity

When chlorpheniramine maleate was dissolved, it was divided into chlorpheniramine and maleic acid. Some research were confused about identification between chlorpheniramine and maleic acid.

During the optimization of the method three columns (Phenyl, C8 and C18 250×4.6 mm, 5 µm), two pH values (4.0 and 2.5) and some gradient elution programs were tested. At low pH and using C18 column had better peak shape, stable baseline and better resolution between fives components (PAR, PHE, CPM, PAP, MAL).

The conditions finally adopted were a C18 column (250 x 4.6mm, 5µm particle size), gradient elution programe (Table 1), 20 µl injection volume at 35 ^{o}C. A typical chromatogram of standard solution contain 5 components and marketed formulation are shown in figure 1 and figure 2, respectively.

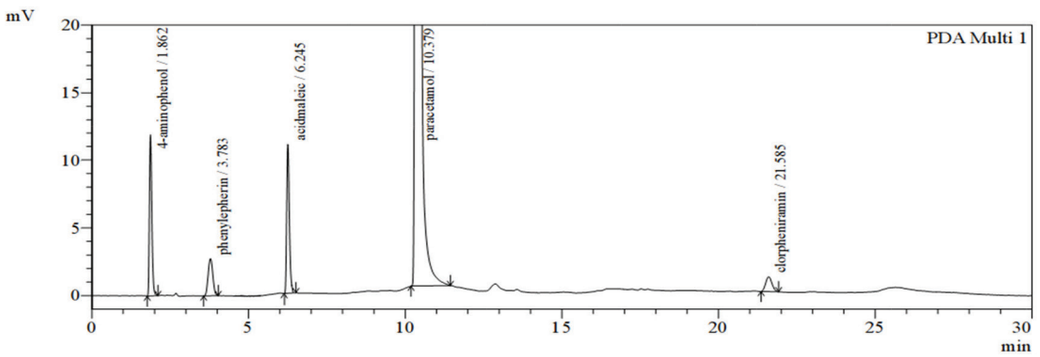

Figure 1. Standard of 5 components with PAP, PHE, MAL, PAR and CPM

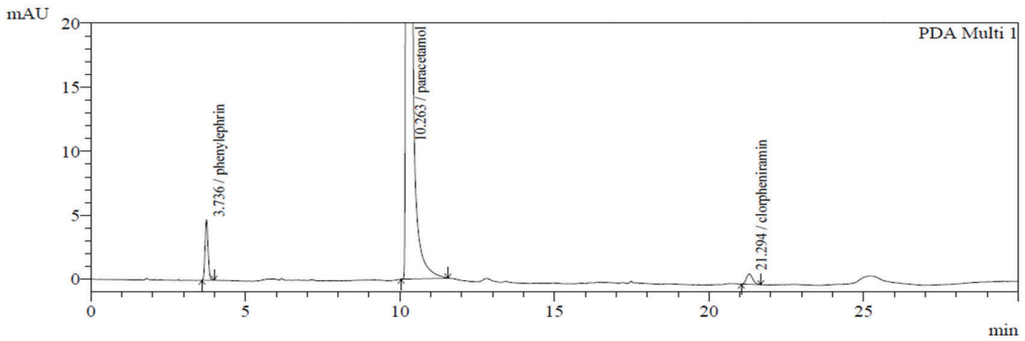

Figure 2. Market sample solution contains PAR 325 µg/ml, PHE 5 µg/ml, CPM 2 µg/ml.

3.2. System suitability testing

The results obtained for system suitability are summarized in table 2. The relative standard deviation of area (S), retention time (tr), theoretical plates (N) and tailing factor (T) were less than 2%.

Table 2. System suitability parameters

		tr	S	T	N
Phenylephrine	Average	3.75	35972	1.1	2717
	RSD	0.67	0.11	0.4	0.65
Paracetamol	Average	10.36	3824512	1.3	46777
	RSD	0.11	0.67	0.4	0.65
Chlorpheniramine	Average	21.6	30922	1.2	52023
	RSD	0.11	0.67	0.4	0.65

3.3. Linearity

Linearity of the method was studied by injecting five concentrations of the drug prepared in the mobile phase into the LC system. The regression equation for phenylephrine HCl, chlorpheniramine, paracetamol were found to be in table 3.

Table 3. The regression equation for PAR, PHE and CPM

No	Name	Range (ppm)	Equation	Correlation coefficient (r^2)
1	PAR	195- 455	$\hat{y} = 11573x$	0.9999
2	PHE	3-7	$\hat{y} = 7161.9x$	0.9999
3	CPM	1.2 – 2.8	$\hat{y} = 15462x$	0.9996

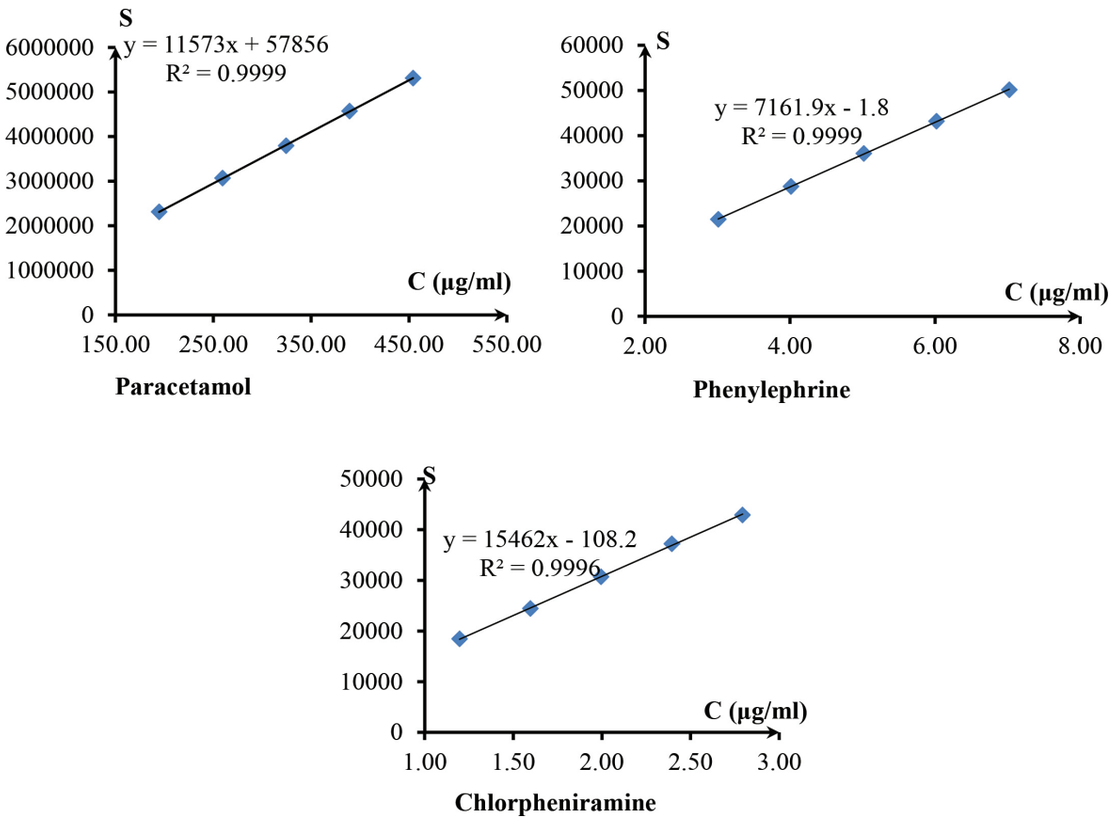

Figure 3. Linearity of PAR, PHE and CPM

3.4. Precision

The precision of this method contain repeatability and intermediate precision. Repeatability was determined on six samples at 100% concentration of PAR, PHE, CPM. It was expressed as RSD% of a series of measurement.

The experimental values obtained for the repeatability of CPM, PE, PCM in samples are presented in table 5. The obtained results show RSD% < 2. The intermediate precision was conducted like the repeatability but it was changed analyst and day. The result have shown in table 4.

Table 4. The parameter of repeatability and intermediate precision

	Different Analyst			
	Analyst 1		Analyst 2	
	Average of area	RSD (%)	Average of area	RSD (%)
Chlorpheniramine	29157	0.24	29298	1.62
Phenyleprine	31766	0.33	31435	0.63
Paracetamol	3473327	1.39	3434586	0.33

3.5. Accuracy

The accuracy of method was confirmed by studying recovery at three different concentrations for all samples, by replicate analysis (n=3). Samples of known concentration (reference standard solutions) were analyzed and the measured values, from the respective area counts, were compared with the true values. The results obtained from the determination of accuracy, expressed as percentage recovery, are summarized in table 5.

Table 5. Results of accuracy of method.

		80%	100%	120%
CPM	Average	99.12	99.60	100.03
	RSD	0.51	1.05	0.88
PHE	Average	100.39	101.81	101.23
	RSD	0.62	0.35	0.15
PAR	Average	100.98	101.04	100.67
	RSD	0.60	0.13	0.30

3.6. Limit of detection of 4-aminophenol

➢ Linearity

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase into the LC. The results have shown in table 6.

➢ Limit of detection

The limit of detection (LOD) of 4-aminophenol LOD= 3.3×SD/S , where SD is the residual standard deviation of the response and S is the slope of the linearity line of 4-aminophenol. The standard deviation (SD) of response (area) was determined by conducting at 8 samples of 0.25 (µg/ml) of 4-aminophenol. The result have shown in table 7.

3.7. Application

To determine the content of CPM, PAR, PHE in commercial drugs. A 20µl volume of sample solution was injected into HPLC, two times, under the conditions described above. The results for CPM, PAR, PHE comparable with their corresponding labeled amounts and % RSD are shown in table 8.

Table 6. Linearity of 4-aminophenol

	C (µg/ml)	Area
PAP	0,25	2632
	0,50	5388
	1,00	11456
	5,00	45900
	10,0	90958
	20,0	182354
Equation	$\hat{y}$ = 9063,3x	
Correlation coefficient (r^2)	0.9999	

Table 7. Standard deviation of response

No.	4-aminophenol (µg/ml)	Area
1	0,25	2632
2	0,25	2491
3	0,25	2455
4	0,25	2710
5	0,25	2602
6	0,25	2435
7	0,25	2606
8	0,25	2918
Average		2606
SD		158

LOD= (3,3 x SD)/S =(3,3 x 158)/9063.3= 0,06 (µg/ml)

Table 8. Application on market drug.

Drug	Label amount (mg)	Amount found (mg)	Content (%)
CPM	2	1.98	99.05
PHE	5	4.82	96.32
PAR	325	314.7	96.83

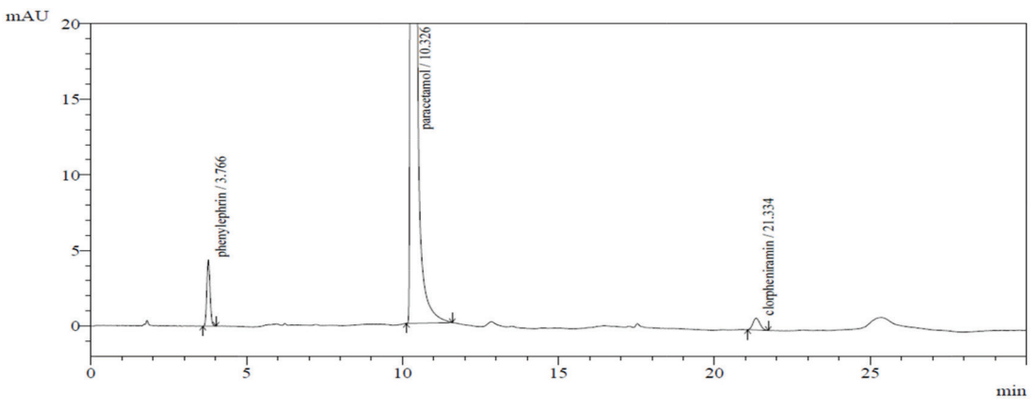

Figure 4. Chromatographic of market drug.

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