Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.9 No.6, 2017

Simultaneous Determination of Orphenadrine Citrate and Paracetamol in Tablets by using RP- HPLC Coupled with UV Detection

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ABSTRACT.
A simple, sensitive, specific, and cost effective method for simultaneous determination of Orphenadrine Citrate and Paracetamol was developed and validated in single dosage formulation. The sample solution of PA and ORC was prepared using methanol as a solvent. Separation of PA and ORC was achieved with a mobile phase consisting of 1% Triethylamine aqueous: Methanol: Acetonitrile (35:20:45 v/v) at a flow rate of 2.0 ml/min and the wavelength of 220nm. Separations were performed on Merck Hibar 250-4.6 RP18 (5 µm) column (250 mm X 3.4 mm), using a Lab Alliance HPLC system equipped with a UV-Vis detector, manual injector with 20 µL loop, LC pump, Clarity controller and software. Retention times of PA and ORC were 2.265 and 3.882 minutes respectively. Absolute recovery of PA and ORC was 100.20 and 100.07 % respectively. The lower limit of quantification (LLOQ) of PA and ORC was 0.3097 and 0.1063 ppm and lower limit of detection (LLOD) of PA and ORC was 0.0153 and 0.0135 ppm respectively. Linearity was established for the range of concentrations (20-140) µg/ml and (0.1-50) µg/ml for PA and ORC respectively with the coefficient of determination (R^2) of 0.9991 and 0.9997 for both the compounds. The inter- and intra-day precision in the measurement of PA quality control (QC) sample 450 µg/ml, were in the range 0.1-0.2 % relative standard deviation (R.S.D) and 0.2-0.3 % (R.S.D)., respectively. The inter- and intra-day precision in the measurement of ORC quality control (QC) sample 35 µg/ml, were in the range 0.1-0.2 % (R.S.D)., and 0.0-0.3 % (R.S.D)., respectively. The developed method would be applicable for routine quality control of PA and ORC in bulk as well as in pharmaceutical formulations.
Keywords: Orphenadrine Citrate, Paracetamol, RP-HPLC, Tablets, Validation.

1. INTRODUCTION

Orphenadrine Citrtate (ORC): $C_{18}H_{23}NO$, $C_6H_8O_7$, N, N-dimethyl-2[(2-methylphenyl)-phenylmethoxy] ethanamine;2-hydroxypropane-1,2,3 tricarboxylic acids (Fig. 1a) is an Anticholinergic that treatment acute muscle aches or spasms\pains [1,2]. Paracetamol (PA): $C_8H_9NO_2$, (M. W= 151.20 gr/mole), N-(4-hydroxyphenyl) ethanamide (Fig. 1b) is an Analgesic and Antipyretic that benefit of being completely free of problems with addiction, dependence, tolerance and withdrawal [3,4].

The physico-chemical properties of both PA and ORC are given in Table 1. The numerals of methods are published for the determination of PA and ORC alone or in combination with other drugs in bulk and dosage forms or in biological fluids, including UV Spectroscopy [5-7], Second Kind Electrode detection [8], HPLC [9-14], HPTLC [15-18], Ultra HPLC [19], LC-MS/MS [20-22] and GC [23]. High-performance liquid chromatography coupled with UV-Vis detector (RP-HPLC) [24].

However, the comprehensive literature study revealed that none of the pharmacopoeias or any journals includes these drugs in combination for the simultaneous quantification of PA and ORC. With this regards, the present investigation was carried out to develop a reverse phase high performance liquid chromatography (RP-HPLC) procedure which will serve a reliable, accurate, sensitive and fast method for the simultaneous determination of PA and ORC.

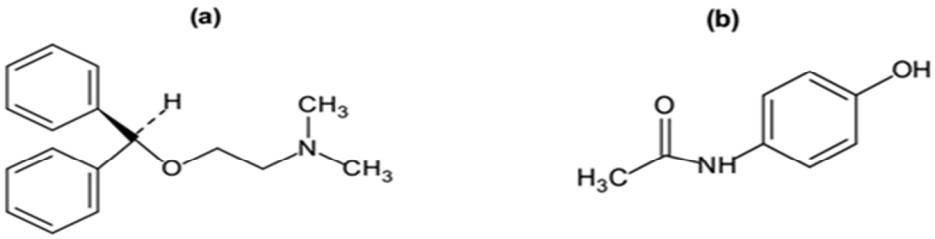

Figure 1. Chemical structure of a) Orphenadrine Citrate b) Paracetamol.

2. MATERIALS AND METHODS

2. 1. Chemicals and Reagents

The reference standards of PA and ORC were gifted from Medico Laboratories for Pharmaceuticals Productions (Homs, Syria). Tablets (Cross Gesic (450+35), Medico Laboratories for Pharmaceuticals

Productions (Homs, Syria). containing PA and ORC of 450 mg and 35 mg respectively were purchased from local market. Water, methanol and Ortho-phosphoric acid of HPLC grade were procured from Merck and TKA, Germany. The other chemicals used in experiments were of HPLC grade and purchased from local market.

2.2. Instrumentation and Chromatographic Conditions

Analytical RP-HPLC separations were performed on Merck Hibar 250-4.6 RP18 (5 µm) column (250 mm X 3.4 mm), using a Lab Alliance HPLC system equipped with a UV-Vis detector, Manual injector with 20 µL loop, LC pump, Clarity LC controller and software. Eluents A (1% Triethylamine aqueous) and B (Methanol) and C (Acetonitrile) in the ratio of 35:20:45 were used as the mobile phases, wavelength set at 220 nm and flow rate was set at 2 mL/min.

2.3. Preparation of stock and standard solutions

Primary stock solutions of PA and ORC for preparation of standard samples were prepared from separate weighing. The primary stock solutions of the analyte were prepared in (50% methanol and 50% Ultra-pure water) as solvent (1.0 mg/ml) and stored at -20 °C, which were found to be stable for one month. Appropriate dilutions were made in methanol for PA and ORC to produce working stock solutions (WSS) of 20, 30, 50, 80, 100, 140 µg/ml and 0.1, 1, 5, 10, 20, 50 µg/ml, respectively, on the day of analysis and these stocks were used to prepare calibration curve (CC).

2.4. Sample preparation

Ten Tablets were weighed each containing 450 mg of Paracetamol and 35 mg of Orphenadrine Citrate powered using mortar and pestle. An amount of pharmaceutical products powder equivalent to 450 mg of PA and 35 mg of ORC were accurately weighed and transferred into a 100 ml volumetric flask and dilute with 50 ml of solvent. Solution was subjected to sonication for 15 minutes for complete extraction of drug and the solution was mark up with the methanol to get final concentration of 4500 µg/ml of PA and 350 µg/ml of ORC respectively. Sample stock solution (SSS) was filtered through 0.45 µm PVDF filter paper (0.45 µm) before further dilution.

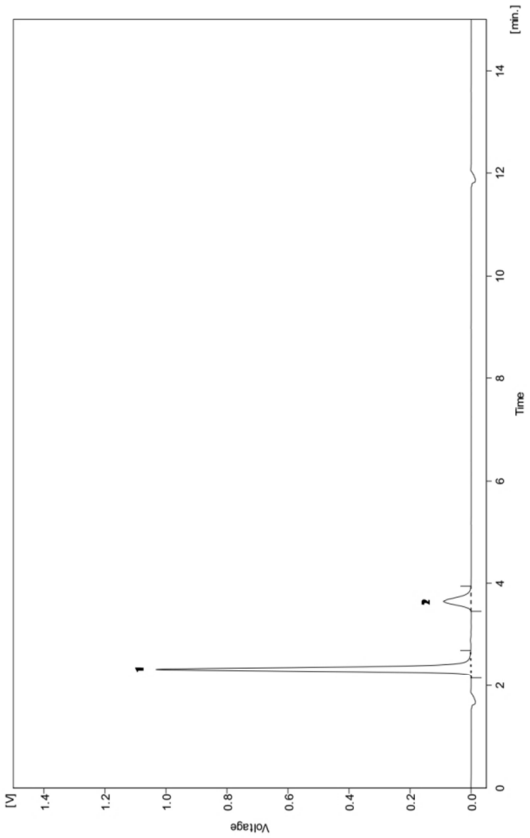

Figure 2. RP-LC chromatogram on Merck Hibar 250-4.6 RP18 (5µm) column representing peaks of Paracetamol (peak 1) and Orphenadrine Citrate (peak 2).

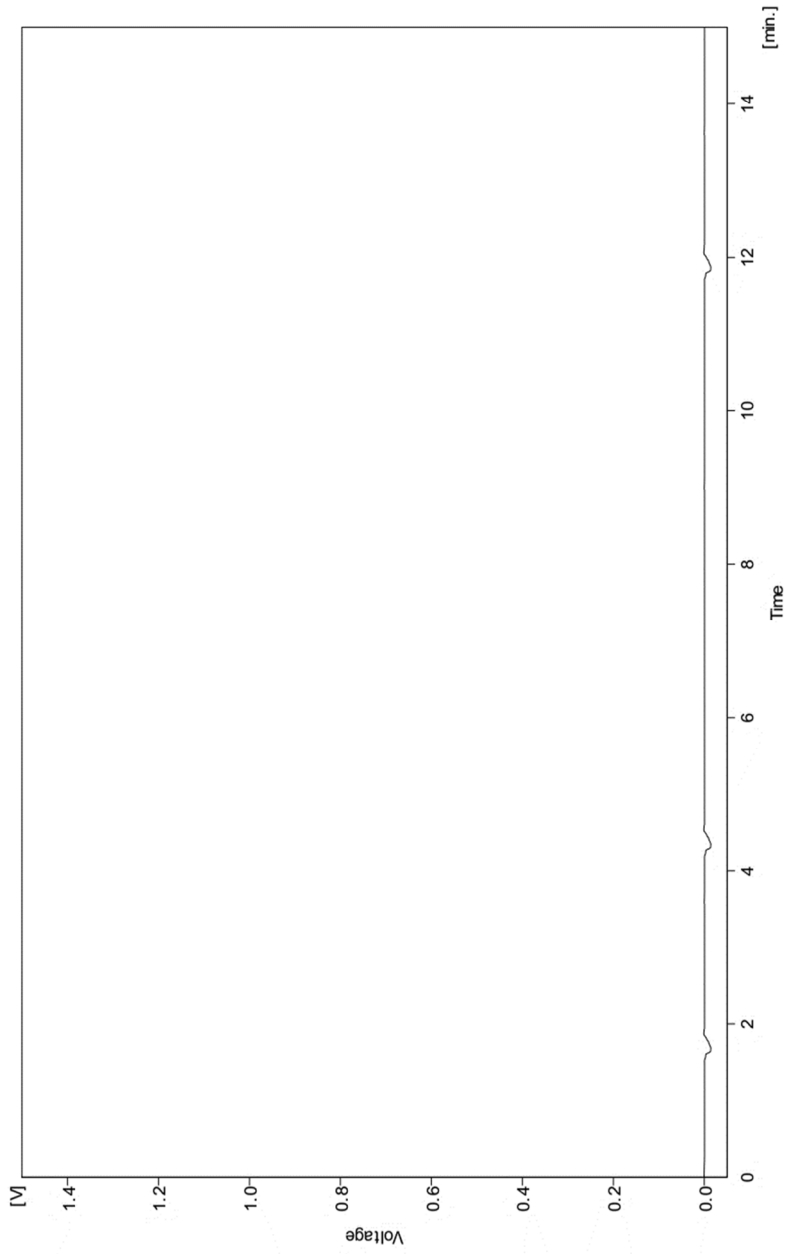

Figure 3. RP-LC chromatogram on Merck Hibar 250-4.6 RP18 (5 µm) column representing blank.

2.5. Validation

Validation studies were performed using the optimized assay conditions following the principles of validation described in the ICH guideline [25]. Key analytical parameters, including, specificity, accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) were evaluated.

2.5.1. Selectivity/selectivity

The specificity/selectivity of the assay method was investigated by verifying the absolute separation and resolution of all the desired peaks of the analytes in mobile phase, and in mixture of excipients and standard. The interference of excipients with drug was measured by recording the retention time and % recovery.

2.5.2. Linearity and Range

For linearity study, six solutions at different concentrations (20, 30, 50, 80, 100, 140 µg/ml of Paracetamol and 0.1, 1, 5, 10, 20, 50 µg/ml of Orphenadrine Citrate) were prepared using six different aliquots of WSS, and the obtained data were used for the linearity calibration plot. Lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for the assay were also calculated using equation 1 and 2 and also determined experimentally by visual evaluation (Table 6).

Lower Limit of Detection (LLOD) = 3.3×σ/s – Eq. 1
Lower Limit of Quantification (LLOQ) = 10×σ/s – Eq. 2

2. 5. 3. Precision

As per the Complementary Guideline on Methodology (dated 6 November 1996 incorporated in November 2005) of ICH. Method precision was performed both in expressions of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days' reproducibility).

2. 5. 4. Repeatability

To determine the repeatability of assay method sample with 4500 µg/ml of PA and 350 µg/ml of ORC were injected 6 times into HPLC system and repeatability of the retention time and peak area was determined and expressed as mean and % RSD calculated from the data obtained.

2.5.5. Intermediate precision

Intermediate precision (intra-day and inter-days' reproducibility), were performed at three different concentration levels (35, 65, 95, µg/ml of Paracetamol and 6, 8, 10, µg/ml of Orphenadrine Citrate) were analyzed three times a day in triplicate injections over three consecutive days and expressed as mean ±SD and % RSD.

2.5.6. Accuracy

The study was performed in triplicates using a mixture of pure drug spiked with its formulation i. e. PA (4500 µg/ml) & ORC (350 µg/ml) solution with three different concentrations of standards at 70 %, 100 % and 120 % (35, 65 and 95 µg/ml for PA and 6, 8 and 10 µg/ml for ORC respectively). Accuracy was determined in terms of percent recovery.

2. 5.7. Robustness

The robustness of the developed assay method was studied by evaluating the manipulating small deliberate variations in procedure variables like column temperature (±1°C), flow rate (±5 %) and pH of the mobile phase (±0.2 units).

3. RESULTS AND DISCUSSION

3.1. Sample preparation

Numerous organic solvents were tried to prepare the stock solution of PA and ORC. Both PA and ORC were having the great solubility in (50% methanol and 50% Ultra-pure water), thus selected as a solvent for this experiment. The corresponding working solutions of PA and ORC were prepared by diluting their stock solutions with methanol.

3.2. Method development and optimization

Feasibility of different solvent system such as water-methanol, buffer-methanol, water- acetonitrile mixture in different strength, pH (2-8), and flow rate (1.0-2.0 ml/min) were experimented. Desired separations were achieved using 1% Triethylamine buffer – methanol–Acetonitrile in the ratio of 35:20:45 v/v (pH adjusted to 3.4 with Ortho phosphoric acid) at a flow rate of 2 ml/min. While optimization of the ratio of different solvents in mobile phase, pH was fixed to 3.4 with Ortho phosphoric acid, at a flow rate of 2 ml/min, the mobile phase composition found better resolution and separation in buffer (1% Triethylamine – methanol–Acetonitrile) (35:20:45 v/v). The pKa values of both PA and ORC were of 9.38 and 8.40 respectively. Resolution and retention of drug component depends upon the pH of the mobile phase, pH range from 2 to 8 were studied to optimize the mobile phase. Buffer: methanol: Acetonitrile ratio (35:20:45 v/v) and flow rate (2 ml/min.) were kept constant, pH 3.4 were found suitable for the current experimentation.

3. 3. Validation of the analytical method

The linear response of PA and ORC was determined by analyzing six independent levels of the calibration curve in the range of 20-140 µg/ml for PA and 0.1-50 µg/ml for ORC. The linearity equations and standard errors for the calibration curves of both ORC and PA are presented in Table 1. Average percent recoveries for PA and ORC were of 100.20 % and 100.07 %, respectively, while % RSD values for both PA and ORC were 0.607 and 0.485, which was less than 1 % and indicates accuracy of the reported method. Method specificity was verified using standard solutions of each drug alone, with excipients, and solvents shows that the resulting peaks on chromatograms at retention times of 2.265 and 3.882 min coincided with PA and ORC respectively with no interference by other substances.

Table 1. Summary of Validation parameters.

Parameters	Paracetamol	Orphenadrine Citrate
Retention time (min)	*2.265*	*3.882*
Theoretical plates	*4423.103*	*4108.956*
Tailing factor	*0.674*	*1.082*
HETP	*99.280*	*61.681*
Linearity range (µg mL^{-1})	*20 - 140 µg/ml*	*0.1 -50 µg/ml*
Linearity Equation	*y = 89456x + 49593*	*y = 68943x + 34013*
Correlation coefficient (r)	*0.9991*	*0.9997*

Table 2. System precision and method precision.

System precision				Method precision			
Paracetamol		Orphenadrine Citrate		Paracetamol		Orphenadrine Citrate	
Injection no.	ea counts (lV s)	Injection no.	Area counts (lV s)	Injection no.	Assay (%claim)	Injectio no.	Assay (%claim)
1	3532926	1	493145	1	80.808	1	9.996064
System precision				Method precision			
Paracetamol		Orphenadrine Citrate		Paracetamol		Orphenadrine Citrate	
Injection no.	ea counts (lV s)	Injection no.	Area counts (lV s)	Injection no.	Assay (%claim)	Injectio no.	Assay (%claim)
2	3537304	2	493299	2	81.078	2	10.10525
3	3533012	3	493012	3	80.985	3	10.15923
4	3530784	4	492812	4	80.688	4	10.09548
5	3536452	5	493437	5	81.022	5	10.07004
6	3529372	6	492948	6	80.781	6	10.04726
Mean	3533308.3	Mean	492812	Mean	80.688	Mean	9.996064
SD	3096.739	SD	231.7407	SD	0.155	SD	0.055431
% RSD	0.087	%RSD	0.047	%RSD	0.192	%RSD	0.554529

The average plate numbers over the concentration range were 2269.153 and 2071.9 for PA and ORC respectively. The chromatograms of blank and the mobile phase do not show any interference at the retention time of Paracetamol and Orphenadrine Citrate as it can be seen from the respective chromatograms (Figure 2, 3).

System precision experiment was performed by preparing the standard solution of PA (4500 µg/ml) and ORC (350 µg/ml) for six times and analyzed as per the ICH guideline (Table 2). Method precision experiment was performed by preparing the test solution of PA (4500 µg/ml) and ORC (350 µg/ml) for six times from different capsule units and analyzed (Table 2). As per the ICH guideline, it expresses within laboratory variations as on different days' analysis or equipment within the laboratory. The Intra-day precision was determined for standard solution of PA (4500 µg/ml) and ORC (350 µg/ml) for different hours in same day, (like 0, 2, 4, 6, 8 and 10 Hours) and results were found 0.1 % and 0.2 % respectively, which were within the limit prescribed by ICH (Table 3). The Inter-day precision was determined for standard solution of PA (4500 µg/ml) and ORC (350 µg/ml) for different days, (like 1, 2, 3, 4, 5 and 6 Days) (Table 3). The accuracy of the method was determined by recovery studies and the percentage recovery was calculated, overall mean percentage recovery was found 99.966 % and 99.347 % for PA and ORC respectively (Table 4). Minor deliberate changes in different experimental parameters such as flow rate (±5 %), pH (±0.2 units) and mobile phase ratio did not significantly affect area under the curve and retention time of both PA and ORC indicating that the proposed method is robust (Table 5). The LOD for PA and ORC standard solution were found to be 0.0445 µg/ml and 0.0046 µg/ml respectively, while LOQ were found to be 0.134697 µg/ml and 0.01386 µg/ml respectively (Table 6).

Table 3. Intra-day precision and Inter-day precision.

Intra-day precision				
Hours	Paracetamol		Orphenadrine Citrate	
	Concentration (ppm)	AUC	Concentration (ppm)	AUC
0	75	3532261	10	493285
2	75	3539762	10	493389
4	75	3531523	10	492912
6	75	3529431	10	492932
8	75	3537213	10	493743
10	75	3530785	10	493678
Mean		3529431	Mean	492912
STDEV		4058.831	STDEV	354.972
% RSD		0.115	% RSD	0.072

Table 3(continue). Intra-day precision and Inter-day precision.

Inter Day				
	Paracetamol		Orphenadrine Citrate	
Day	Concentration (ppm)	AUC	Concentration (ppm)	AUC
1	75	3572364	10	494326
2	75	3586704	10	495284
3	75	3579736	10	490926
4	75	3554987	10	490826
5	75	3563512	10	491295
6	75	3564987	10	492048
Mean		3554987	Mean	490826
STDEV		11585.7	STDEV	1897.71
% RSD		0.3259	% RSD	0.386636

Table 4. Accuracy Study data.

Drugs	Recovery levels	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean % Recovery	SD	%RSD
Paracetamol	Level 1	8	7.999	100	100	0.750555	0.751
		8	8	100			
		8	8.1	101.3			
	Level 2	10	9.998	100	100	0.57735	0.577
		10	10.1	101			
		10	10	100			
	Level 3	12	12	100	99.9	0.493288	0.494
		12	11.99	99.9			
		12	12.1	100.8			
	Overall				99.966	0.607	0.607
Orphenadrine Citrate	Level 1	8	7.99	99.9	99.875	0.832	0.833
		8	8.00	100.0			
		8	8.11	101.4			
	Level 2	10	10.00	100.0	99	1	1.01
		10	9.90	99.0			
		10	10.10	101.0			
	Level 3	12	12.00	100.0	99.166	0.481	0.485
		12	11.90	99.166			
		12	12.00	100.0			
	Overall				99.347	0.481	0.485

Table 5. Robustness study data.

Robustness of Paracetamol				Robustness of Orphenadrine Citrate			
Chromatographic changes							
Factor	Level	Mean AUC	% RSD	Factor	Level	Mean AUC	% RSD
Flow rate (ml/min)				Flow rate (ml/min)			
1.0	-0.1	3350549.7	0.1	1.0	-0.1	486929.7	0.6
1.5	0	3361486.7	0.1	1.5	0	496407.7	0.3
2.0	+0.1	3149075.0	0.4	2.0	+0.1	484091	0.6
Mobile phase ratio (Buffer: Methanol: Acetonitrile)				Mobile phase ratio (Buffer: Methanol: Acetonitrile)			
33:22:47	-2:2	3350549.7	0.1	33:22:47:	-2:2	496929.7	0.3
35:20:45	0:0	3353153.3	0.1	35:20:45	0:0	497421.3	0.3
37:18:43	2:-2	3347486.7	0.1	37:18:43	2:-2	495387.7	0.2
pH				pH			
3.2	-0.2	3472598.7	0.3	3.2	-0.2	493054.3	0.5
3.4	0	3350549.7	0.1	3.4	0	496929.7	0.2
3.6	+0.2	3322326.7	0.4	3.6	+0.2	494721	0.2
Wavelength (nm)				Wavelength (nm)			
220	+2	3345820.0	0.1	220	+2	496487.3	0.2
222	0	3350549.7	0.1	222	0	496929.7	0.1
224	-2	3358686.7	0.2	224	-2	497411.6	0.3

Table 6. LOD and LOQ.

Drug	Based on visual evaluation		on the Standard Deviation of the Response and the Slope	
	LOD	LOQ	LOD	LOQ
Paracetamol	1 ppm	2 ppm	0.01535	0.3097
Orphenadrine Citrate	0.5 ppm	1 ppm	0.01358	0.1063

4. CONCLUSION

Proposed method is simple, rapid and cost effective. Method is validated as per the guideline of ICH and shows satisfactory results. Previously reported methods are either for alone Pa and ORC for with other combination the present validated method allows scientist to perform simultaneous estimation of both PA and ORC at a time. Further this method can be employed for the human clinical pharmacokinetic study of the combination of PA and ORC in future. In summary, the described method provides high throughput for simultaneous quantification of PA and ORC with outstanding accuracy, precision, selectivity and reproducibility.

Acknowledgement

The authors express their thanks to Medico Laboratories for pharmaceutical productions, Homs, Syria, for their assistance during the work.

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