A dissolution test for acetaminophen, phenylephrine and carbinoxamine associated in two different combinations in tablets using high performanceliquid chromatography

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Abstract

A dissolution test for analysis of acetaminophen and phenylephrine (CP1) and acetaminophen and carbinoxamine (CP2) in tablets was developed and validated. The dissolution medium employed for CP1 was potassium monobasic phosphate buffer (pH 5.8, 0.15 mol L-1) and for CP2 was purified water. The temperature of dissolution medium (volume 900 and 500 ml in CP1 and CP2 case, respectively) was set at 37.0 ± 0.5oC and the rotation speed of the paddles was set at 100 rpm during 30 minutes. Standards and sample aliquots were diluted in dissolution medium until adequate concentration and filtered before injection in the chromatographic system.

Keywords: Acetaminophen. Phenylephrine. Tablets. Dissolution. HPLC.

1 Introduction

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance. Based on this general consideration, in vitro dissolution tests for immediate release solid oral dosage forms are used to assess the lot-to-lot quality of a drug product, guide development of new formulations, ensure continuing product quality and performance after certain changes in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process (HOTI et al., 2008).

The common cold is normally harmless and generally disappears within one or two weeks unless secondary bacterial infection is diagnosed. Once no specific treatment can be given, the usual procedure consists of alleviating the symptoms. The symptomatic treatment is based on the prescription of drugs that alleviate the nasal congestion, dry the mucous membranes and reduce both temperature and pain. This is achieved through drug combination since no single drugs can have all these effects. The pharmaceutical association is often used as they offer a more convenient treatment than single ones. Some drugs currently available combine three pharmaceuticals: acetaminophen (Ace), an analgesic and antipyretic; phenylephrine hydrochloride (Phe), a nasal decongestant; and carbinoxamine maleate (Car), an antihistamine (BASTOS; OLIVEIRA, 2009).

The three drugs are associated in the pharmaceutical form of oral solution, while in tablet form, is the following associations: Ace/Phe and Car/Ace. Due to the widespread use of these drugs in treating symptoms of colds and flu the development of new or alternatives analytical methodologies able to assist dissolution studies is necessary.

Among the classical analytical methodologies used for analyzing these pharmaceuticals are: titrimetry (BRAZILIAN, 1977), spectrometry UV-Visible (BRAZILIAN, 2010), capillary electrophoresis (CE) (MARCHESINI et al., 2003; MARÍN; BARBAS, 2004; OKAMOTO et al., 2005) and high performance liquid chromatography (HPLC) (BASTOS; OLIVEIRA, 2009; ERK; KARTAL, 1998; GARCÍA et al., 2003; GIL-AGUSTÍ; GARCIA-ALVAREZ-COQUE; ESTEVE-ROMERO, 2000; GUMBHIR;

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MASON, 1996; LAU; MOK, 1995; MARÍN et al., 2005; OLMO et al., 2005; QI; WANG; CHEN, 2004; RAVISANKAR et al., 1998; SHERVINGTON; SAKHNINI, 2000; UNITED states pharmacopeia, 2005).

The Brazilian Pharmacopoeia (2010) describes the dissolution test for acetaminophen tablets, using 900 ml of phosphate buffer pH 5.8 as dissolution medium. The equipment was operated with paddles at 50 rpm during 30 minutes and the assay of samples is done by spectrometry UV-Visible (243 nm). Marin and Barbas (2004) compared CE versus HPLC for the dissolution test in a pharmaceutical formulation containing acetaminophen, phenylephrine and chlorpheniramine. For the dissolution test, one capsule (~610 mg) was added in each one of the six glasses, using 900 ml of water at 37 ± 0.5oC as dissolution medium. The equipment was operated with baskets at 100 rpm. Samples aliquots of 10 ml were taken at 45 min and filtered through 0.45 μm nylon filters to the HPLC or CE vials. The United States Pharmacopeia (2005) describes the dissolution test for carbinoxamine maleate tablets, using 900 ml of water as dissolution medium. The equipment was operated with paddles at 50 rpm during 45 minutes.

Knowing the importance of the dissolution test as a tool in the quality control process in the pharmaceutical industry, the present work has as aim to develop a novel method for monitoring the dissolution of the Ace/Phe and Car/Ace tablets association. Within this context, a simple, rapid, efficient and cheap dissolution test was developed and used to monitoring dissolution of these drugs associated in two different ways (Ace/Phe and Car/Ace) in tablets. After some validation procedures the method was successful applied to evaluate the dissolution of the pharmaceuticals preparations.

2 Material and Methods

The materials and methods used are described below.

2.1 Reagents and chemicals

Methanol was of chromatographic grade and all other chemicals were of analytical grade. Phosphoric acid, potassium phosphate monobasic, methanol, ethanol, triethylamine, sodium lauryl sulfate and sodium hydroxide were purchased from Vetec (Rio de Janeiro, RJ, and Brazil). Water was purified with Milli-Q®, Milipore System. All solvents and solutions were filtered through a 0.45 μm millipore filter (Milipore® millex-HV filter units) (São Paulo, SP, Brazil).

Phenylephrine hydrochloride (Phe) (99.9%) and carbinoxamine maleate (Car) (100.0%) were purchased from American Pharmacopoeia (Rockville, MD, USA). Acetaminophen (Ace) (99.8%) was purchased from Brazilian Pharmacopoeia (Rio de Janeiro, RJ, Brazil).

2.2 Samples

Tablets of commercial product 1 (CP1) (~20 mg Phe and ~400 mg Ace) and commercial product 2 (CP2) (~4 mg Car and ~400 mg Ace) were purchased from local pharmaceutical industry (Juiz de Fora, MG, Brazil).

2.3 Instrumentation

Dissolution experiments were carried out using a Nova Ética 299/8CLA system equipped with a programmable auto-sampler.

HPLC system: the experiments were performed in a high performance liquid chromatography model Waters 1525 (Milford, MA, USA) equipped with a photo diode array detector model 2996, a temperature control device maintained at 27°C and data acquisition and treatment software (Empower Build 1154).

Column: the analytical column was a reversed phase Luna Phenomenex C18 (5 μm, 300 mm x 3.9 mm) (Torrance, CA, USA).

2.4 Dissolution experiments

For each dissolution experiment six tablets of CP1 and CP2 purchased from local pharmaceutical industry were separately weighed and placed in the dissolution apparatus in batches of six. The dissolution medium employed for CP1 was potassium monobasic phosphate buffer (pH 5.8, 0.15 M) and for CP2 was purified water. The temperature of dissolution medium (volume 900 and 500 ml in CP1 and CP2 case, respectively) was set at 37.0 ± 0.5oC and the rotation speed of the paddles was set at 100 rpm. Sample aliquots (ca. 20 ml) were withdrawn automatically by the auto sampler after 30 minutes and filtered in-line through 45 μm millipore filters to the HPLC. Five mL of these CP2 sample aliquots were diluted with dissolution medium in a 50 mL volumetric flask before to filtered through a 0.45 μm millipore filter.

2.5 Chromatographic conditions

The quantitative determination of the sample aliquots was based on a isocratic ion-pair reversed phase high performance liquid chromatography methodology able to perform simultaneous separation of acetaminophen, phenylephrine and carbinoxamine developed, optimized and validated (BASTOS; OLIVEIRA, 2009). All analyses were performed at 27°C under isocratic conditions. A mobile phase consisted of methanol-potassium monobasic phosphate (62.46 mmol L-1) (60:40, v / v) added with 1.0 mL phosphoric acid, 0.50 mL triethylamine and 0.25 g sodium lauryl sulfate (* pH 4.10). Flow rate was 1.0 mL min-1 and volume injection was 50 μL. The UV detection was set at 220 for Car and Phe and at 300 nm for Ace. At the beginning of the day, mobile phase was pumped through the HPLC system

during 30 minutes until achieving baseline stability. The sample analyses were performed within 6 min.

2.6 **Standard solution preparation**

CP1: accurately weighed amount of standard of Phe equivalent to 55.5 mg was transferred to separate volumetric flask containing volume of 50.0 ml (solution 1); 44.4 mg of Ace accurately weighed were transferred to volumetric flask of 100.0 ml containing 2.0 ml of the solution 1, forming the standard solution. Final concentrations were 22.2 and 444.4 mg L-1 for Phe and Ace, respectively.

CP2: accurately weighed amount of standard of Car equivalent to 80.0 mg was transferred to separate volumetric flask containing volume of 100.0 ml (solution 2); 80.0 mg of Ace accurately weighed were transferred to volumetric flask of 100.0 ml containing 1.0 ml of the solution 2. Five ml of this final solution were diluted in a 10 ml volumetric flask, forming the standard solution. Final concentrations were 4.0 and 400.0 mg L-1 for Car and Ace, respectively.

All volumes were completed with dissolution medium and standards solutions were filtered through a 0.45 μm millipore filter before injection.

2.7 **Calibration curves**

The following concentrations levels for CP1 and CP2 were obtained from each standard solution, conveniently diluted with dissolution medium in presence of the excipients:

CP1: Phe (17.8, 20.0, 22.2, 24.4, 26.6 mg L-1) and Ace (355.5, 400.0, 444.4, 488.8, 533.3 mg L-1);

CP2: Car (3.2, 3.6, 4.0, 4.4 and 4.8 mg L-1) and Ace (320.0, 360.0, 400.0, 440.0, 480.0 mg L-1).

Each solution was injected in the chromatographic system (n=3) and mean values of peak areas were plotted against concentration. The curves were fitted by linear regression with least mean square method.

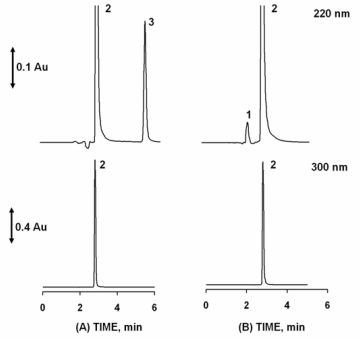
3 Results and Discussion

The development of the dissolution test, validation procedures and dissolution test in samples are described below.

3.1 **Development of the dissolution test**

After checking the solubility of the drugs involved (all featuring some solubility in water), initially was tested for CP1 a methodology for acetaminophen tablets described in the United States Pharmacopeia (2005), which uses 900 ml of phosphate buffer pH 5.8 as dissolution medium, apparatus 2, dissolution time of 30 minutes and rotation of 50 rpm. As initial results were not satisfactory, some experiments were performed and best results were obtained keeping the conditions of the dissolution medium, apparatus and dissolution time, while the rotation was changed for 100 rpm (BRAZIL, 2005). Since the dissolution of the tablets of CP2 was not satisfactory under the conditions developed for CP1, has changed the dissolution medium for purified water, according to a methodology for carbinoxamine maleate tablets described in the same pharmacopoeia. The volume of dissolution medium was changed to 500 mL for the final concentration of carbinoxamine maleate in the dissolution medium to get bigger, since the amount of carbinoxamine maleate in each tablet is only 4 mg. Chromatogram 1 shows the chromatogram for standard mixture.

Chromatogram 1 – Chromatograms for standards mixtures of (1) Car, (2) Ace and (3) Phe using photo diode array detector set at 220 and 300 nm. Operational condtions as indicated in the experimental part.



Source — The authors (2009).

Table 1 shows analytical parameters such as resolution (R), efficiency (N), asymmetry (A/B), and relative retention (α) obtained for the applied conditions.

Table 1 – Analytical parameters such as resolution (R), efficiency (N), asymmetry (A/B), and relative retention (α) obtained for the developed method

	Ace[a]	Phe[a]	Ace[b]	Car[b]
Rc	8.5*		3.2*	
	8.5**		3.1**	
	2.6*		3.6*	
αc	2.6**		3.5**	
A/Bc	1.3*	1.2*	1.5*	1.5*
	1.2**	1.2**	1.5**	1.5**

[a]Commercial product 1, [b]commercial product 2, [c]Mean (n=6), *Standards; **standards + pharmaceutical excipients
Source — The authors (2009).

3.2 **Validation procedures**

Some validation parameters for CP1 and CP2, such as selectivity, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), accuracy and robustness, were determined, as Resolution RE no 899, 29 may 2003 (BRAZIL, 2003) AND ICH (ICH Q2B, 1996).

3.2.1 **Selectivity and linearity**

Method selectivity was assessed by the peak purity test (comparison between analyte peak and auto threshold in the purity plot) using diode array detector. The analyte chromatographic peak was not found to be attributable to more than one component (BRAZIL, 2003; INTERNATIONAL CONFERENCE ON HARMONIZATION Q2B, 1996).

Linearity was evaluated taking into account the correlation coefficient (r). The correlation coefficient equal to or higher than 0.99 is considered evidence of ideal data fitting to line regression performed through least-square treatment (BRAZIL, 2003). In order to evaluate lack of fit of the regression, Shapiro-Wilk Normality Test was performed for the residues (MONTGOMERY, 2005). As the p-value calculated was equal or higher than 0.05 or 0.01, the residue distribution is normal for confidence interval of 95 or 99%, and the model is considered linear within the range evaluated (Table 2).

Table 2 – Statistical results obtained from linearity calculation (standards + pharmaceutical excipients)

	Slope	Intercept	r	p-value[c]
Ace[a]	9 591 (± 132.82)	260497 (± 58987)	0.999	0.088
Phe[a]	75 277 (± 732.44)	-9 950 (± 16775)	0.999	0.010
Ace[b]	11 272 (± 72.55)	-89 084 (± 29568)	0.999	0.316
Car[b]	79 442 (± 1758.46)	23 080 (± 7135)	0.997	0.793

[a]Commercial product 1, [b]commercial product 2, [c]Shapiro-Wilk Normality Test

Source — The authors (2009).

3.2.2 **Precision, limit of detection (LOD) and limit of quantification (LOQ)**

Precision can be determined through the estimate of the relative standard deviation (RSD) (BRAZIL, 2003). The precision in the validation of this developed method was performed at two levels: repeatability and intermediate precision.

Repeatability (n=6) in sample area was carried out for 100.0% of the test concentration. Intermediate precision (n=6) was performed on different days. All results presented acceptable precision values (not exceeding 5.00%) (BRAZIL, 2003) as shown in Table 3.

LOD and LOQ were calculated by means of the standard deviation ratio of the intercept of three calibration curves obtained from linearity by means of the slopes of the respective curves multiplied by 3 and 10, respectively (BRAZIL, 2003). LOD and LOQ obtained presented acceptable values for sample analysis as presented in Table 3.

Table 3 – RSD (%) in concentration found for samples obtained from repeatability and intermediate precision. LOD and LOQ values (mg L-1)

	Ace[a]	Phe[a]	Ace[b]	Car[b]
Repeatability[c]	0.15*	0.06*	0.08*	0.43*
	0.11**	0.28**	0.07**	0.62**
Intermediate precision[c]	1.45*	0.52*	0.12*	3.25*
	0.14**	2.45**	0.89**	0.21**
LOD	6.57**	1.12**	12.51**	0.84**

[a]Commercial product 1, [b]commercial product 2, [c]Mean (n=6), *Standards; **standards + pharmaceutical excipients

Source — The authors (2009).

3.2.3 **Accuracy**

Accuracy, in the present case, was calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample (BRAZIL, 2003; INTERNATIONAL CONFERENCE ON HARMONIZATION Q2B, 1996). Thus, recovery tests were performed by adding known amounts of standard in the sample at five levels of concentrations for each drug, as shown in Table 5. For accuracy test, mean recovery percentage (R%) was 100.0 ± 2.0% and single R% concentration was 100.0 ± 5.0% (Table 4). The results obtained show that the method presents acceptable accuracy.

3.2.4 **Robustness**

The robustness was evaluated by intentional minor modifications in the chromatographic conditions in the proposed methodology (BRAZIL, 2003). Within this context, the parameters selected to evaluate robustness were: mobile phase, flow rate and pH. Table 6 shows the experiments performed for robustness evaluation. It is important to remember that for commercial product 1 the maximum flow rate was set at 1.0 ml min-1 in order to maintain pressure lower than 3000 psi. All parameters were performed in six replicates. For the robustness test, the recovery achieved remained within the interval of 100.0 ± 5.0 % as shown in Table 5. Therefore, little variations in the chromatographic parameters such

as mobile phase, flow rate and pH were found to be acceptable values in relation to the reference value.

Table 4 – Recovery data of standard solutions added to the samples analyzed using the proposed HPLC method

	Added amount (mg)	Found amount [c]	Recovery (%)
	351.79	356.33 ± 0.10	101
	395.77	397.67 ± 0.04	100
Ace[a]	439.74	441.50 ± 0.10	100
	483.71	482.19 ± 0.16	100
	527.69	516.14 ± 0.07	98
			100[d]
	17.78	17.53 ± 0.32	99
	20.01	19.52 ± 0.12	98
Phe[a]	22.23	21.56 ± 0.14	97
	24.45	24.26 ± 0.96	99
	26.68	26.41 ± 0.20	99
			98[d]
	322.82	319.14 ± 0.02	99
	363.17	358.99 ± 0.33	99
Ace[b]	403.52	399.28 ± 0.09	99
	443.87	442.80 ± 0.03	100
	484.22	481.39 ± 0.46	99
			99[d]
	3.21	3.26 ± 4.30	101
	3.62	3.51 ± 1.17	97
Car[b]	4.02	3.88 ± 0.22	97
	4.42	4.34 ± 1.29	98
	4.82	4.70 ± 1.58	97
			98[d]

[a]Commercial product 1, [b]commercial product 2, [c]Mean (n=3); dmean of recovery range

Source — The authors (2009).

Table 5 – Robustness results for commercial product 1 and 2 calculated as the percentage of recovery

Experiments	1	2	3	Ace[a]	Phe[a]	Ace[b]	Car[b]
A[c]	-	0	0	101.0	100.5	101.1	100.7
B[c]	+	0	0	102.0	99.6	100.9	100.7
C[c]	0	-	0	100.2	104.3	100.7	100.5
D[c]	0	+	0	100.7	102.7	100.0	100.2
E[c]	0	0	-	101.1	104.4	100.2	101.8
F[c]	0	0	+	100.8	104.0	101.3	101.2
G[c]	0	0	0	100.3	102.3	101.6	100.6

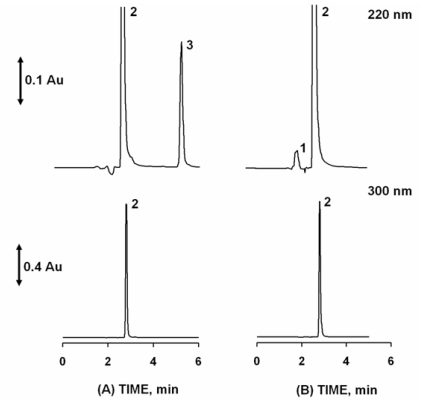
1- Mobile phase (%MeOH): (-) 55; (0) 60; (+) 65; 2- Flow (mL min^{-1}): (-) 0.7; (0) 1.0; (+) 0.4[a]/ 1.3[b] ; 3- pH: (-) 3.1; (0) 4.1; (+) 5.1. [a]Commercial product 1, [b]commercial product 2, [c]Mean (n=6)

Source — The authors (2009).

3.3 **Dissolution test in samples**

After evaluating some validation parameters, the developed method was applied to the sample analysis obtaining 18.5 mg (± 8.0 %) of Phe and 386.6 mg (± 3.3 %) of Ace for CP1 and 4.0 mg (± 1.5 %) of Car and 399.0 mg (± 7.8 x 10-1 %) of Ace for CP2 as results. Chromatogram 2 shows the chromatograms obtained to samples analyzed.

Chromatogram 2 – Chromatograms obtained for samples analysis: (A) - commercial product 1 and (B) commercial product 2. Operational conditions as indicated in the experimental part.



Source — The authors (2009).

4 Conclusions

The present work reports a dissolution test approach for Ace / Phe and Car / Ace tablets was developed and validated. The dissolution approach parameters have been obeyed the variation limits permitted resulting adequate for dissolution quality control routine of these tablets, offering as advantage simplicity, efficiency, speed and low coast.

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Teste de dissolução de paracetamol, fenilefrina e carbinoxamina associados em duas diferentes combinações em comprimidos por cromatografia líquida de alta eficiência

Resumo

Um teste de dissolução para análise de paracetamol e fenilefrina (CP1) e paracetamol e carbinoxamina (CP2) em comprimidos foi desenvolvido e validado. O meio de dissolução empregado foi para CP1 tampão fosfato de potássio monobásico (pH 5,8; 0,15 mol L-1) e para CP2 água purificada. A temperatura do meio de dissolução (volume de 900 e 500 mL no caso de CP1 e de CP2, respectivamente) foi fixada em 37,0 ± 0,5oC e a velocidade de rotação das pás em 100 rpm durante 30 minutos. Padrões e alíquotas das amostras foram diluídos no meio de dissolução até concentração adequada e filtrados antes da injeção no sistema cromatográfico.

Palavras-chave: Paracetamol. Fenilefrina. Comprimidos. Dissolução. HPLC.

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