EXHIBIT E

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THE INFLUENCE OF PHARMACEUTICAL EXCIPIENTS ON QUINAPRIL HYDROCHLORIDE STABILITY

BEATA STANISZ

Poznań University of Medical Sciences, Department of Pharmaceutical Chemistry,
6 Grunwaldzka Str., 60-780 Poznań, Poland*

Abstract: The objective of this study was to determine the effect of povidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, lactose and magnesium stearate on the stability of quinapril hydrochloride. The applied HPLC method was validated by the following parameters: selectivity, precision, linearity and accuracy. Binary 1: 4 mixtures of quinapril hydrochloride and selected excipients were stable at 318 K/76.4%RH and at 318 K/0%RH. The kinetic parameters of the degradation reaction of quinapril hydrochloride in the presence of excipients were calculated. The degradation of quinapril hydrochloride with acidic excipients was significantly lower than that observed for the basic excipients (magnesium stearate) under the same study conditions. The degradation rate constant was approximately 100 fold greater than the decomposition in the quinapril hydrochloride alone. Thus, the pathway of degradation of quinapril hydrochloride in model drug-excipient mixtures was depended on pH of excipients and humidity.

Keywords: quinapril hydrochloride; pharmaceutical excipients; stability in solid phase

Each of the pharmaceutical formulations both liquid and solid forms (tablets, capsules, ointments) requires at least one of the pharmaceutical excipients to be added. Therefore, pharmaceutical excipients should be carefully examined in respect of incompatibilities and their influence on the active agent stability should be assessed, whether they accelerate or delay its degradation and affect the release profile (1-8). Lactose, stearate magnesium and polymers (povidone, hydroxypropyl methylcellulose, hydroxylpropyl cellulose) are widely used as excipients in the manufacture of pharmaceutical solid dosage forms. Quinapril hydrochloride belongs to a class of dipeptide angiotensin converting enzyme inhibitors (ACE) (9, 10). Like many of these dipeptide angiotensin converting enzyme inhibitors, quinapril hydrochloride in pharmaceutical dosage is unstable, especially in the presence of humidity and temperature (11). The literature does not refer to research into the kinetics of quinapril hydrochloride in the presence of pharmaceutical excipients.

This work aimed at studying the effect of povidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, lactose and magnesium stearate on the stability of quinapril hydrochloride and the pathway to characterize its degradation in the solid phase with respect to temperature and humidity.

EXPERIMENTAL

Material, reagents and apparatus

Quinapril hydrochloride, **QHCl**; quinaprilate, **QAT**; diketopiperazine, **QDKP** were delivered by Biofarm Ltd; benazepril was delivered by Novartis; povidone, **PVD**; hydroxypropyl methylcellulose, **HPMC**; hydroxypropyl cellulose, **HPC**; lactose, **LS**; and magnesium stearate, **SM** were obtained from Sigma-Aldrich. Water was bidistilled, and all other chemicals were of analytical reagent grade.

Instrumentation and Chromatographic Conditions

QHCl, **QAT** and **QDKP** in the presence of excipients were analyzed using a HPLC (Shimadzu Scientific instruments), consisting of a Rheodyne (7125, 100 ml fixed loop injector), a detector (UV--VIS SPO-6AV), a pump (LC-6A) and an integrator (C-RGA chromatopac). An analytical column (Hypersil MOS, 5 mm particle size, 250 mm ´ 4 mm ID Merck) was used as the stationary phase. The mobile phase composed of acetonitrile – phosphate buffer pH = 2.0 (50: 50 v/v); the separations were achieved by isocratic elution with a flow mobile phase rate of 1.0 mL/min; benazepril (internal standard, methanolic solution 0.10 mg/mL) and a UV detector with a wavelength set at 220 nm were used.

* Corresponding author: e-mail – bstanisz@amp.edu.pl; fax – (0-61) 854-66-52

Case 0:12-cv-60704-PAS Document 61-1 Entered on FLSD Docket 12/14/2012 Page 3 of 6

Phosphate buffer solution was prepared by dissolving 0.0680 g of potassium dihydrogen phosphate in 450 mL of water in a 500 mL volumetric flask. The pH value of the solution was adjusted with 80% phosphoric acid to pH = 2.0 and made up the volume with water to a total of 500 mL.

Preparation of stock and working standard solutions

Stock standard solutions of quinapril hydrochloride, **QHCl**, 8.42×10^{-4} mole/L, quinaprilat, **QAT**, 6.71×10^{-4} mole/L and diketopiperazine, **QDKP**, 6.56×10^{-4} mole/L, were prepared by dissolving appropriate amounts of the compounds in methanol. These solutions were stored in the dark under refrigeration at 277 K and were found to be stable for two weeks. Mixed working standard solutions of **QHCl**, **QAT** and **QDKP** in the ratio (1: 1: 1) were prepared by the appropriate dilution of the above mentioned stock standard solutions in methanol.

Calibration Curve

Calibration curves of **QHCl**, **QAT** and **QDKP** were conducted using the series of working standard solutions described previously. The concentration range was: from 8.42×10^{-5} mole/L to 8.42×10^{-4} mole/L for **QHCl**, from 6.71×10^{-5} mole/L to 8.42×10^{-4} mole/L for **QAT** and from 6.56×10^{-5} mole/L to 6.56×10^{-4} mole/L for **QDKP**. For the analysis, 1.0 mL of solutions was mixed with 1.0 mL of the internal standard solution (benazepril). All solutions were analyzed immediately after their preparation. A volume of 100 mL of each sample was injected into the column. All measurements were repeated three times for each concentration. The calibration curves were constructed by plotting the peak area of analyte to I. S. corresponding to the drug concentration.

Drug – excipient mixtures

The drug – excipient mixtures were prepared in a mortar by slowly adding approximately 0.500 g of **QHCl** and approximately 2.0 g of the selected excipient (povidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, lactose and magnesium stearate), mixed using a hand pestle until homogeneous.

Symbols of drug – excipient mixtures: drug with povidone – **QPVD**; drug with hydroxypropyl methylcellulose – **QHPMC**; drug with hydroxypropyl cellulose – **QHPC**; drug with lactose – **QLS** and drug with magnesium stearate – **QSM**.

Kinetic procedure

Accurate weighed samples (0.050 g) of the model drug – excipients mixtures (**QPVD**, **QHMC**, **QHPMC**, **QLS** and **QSM**) and 0.010 g of **QHCl** alone were transferred into 5 mL glass vials.

Samples, **QHCl** alone and in the presence of selected excipients, destined for investigation of effect of temperature at a relative humidity of 0% were placed in a sand bath and kept at a temperature 318 K.

Samples, **QHCl** alone and in the presence of selected excipients, destined for investigation of effect of temperature at a relative humidity of 76.4% were placed in desiccators containing a saturated aqueous solution of sodium chloride and kept at a temperature of 318 K. After definite time intervals, determined by the rate of degradation, the respective vials were withdrawn from the chamber, cooled to room temperature. The contents of vials were transferred into a measuring flask (50 mL), 25.0 mL of methanol was added and the contents were extracted by means of shaking for 15 min. The solutions were then filtered. For the analysis, 1.0 mL of this solution was mixed with 1.0 mL of the internal standard solution and the so obtained solution was analyzed by the HPLC method.

RESULTS AND DISCUSSION

The HPLC method was:

– selective – the applied method was found selective for **QHCl** and for an internal standard in the presence of degradation products of **QHCl** and the excipients (retention times were observed as follows: **QHCl** 10 min, products of its degradation 3 min – **QAT** and 5 min – **QDKP** and internal standard 8 min) (Figure 1a, b, c, d, e).

– linear – all the calibration plots showed excellent linearity with correlation coefficients of better than 0.999; standard deviation ≤ 0.1; *b* – was statistically nonsignificant.

– precise and accurate – the overall percent recovery of **QHCl** in the presence of excipients was 98.95% with a relative standard deviation of 0.91%. The overall percent recoveries of **QAT** and **QDKP** in the presence of excipients were 96.4% and 101.12% with relative standard deviations of 1.31% and 1.86%, respectively.

Drug stability in the presence of excipients

Model drug – excipient of mixtures stored for 6 months at 318 K in absence and presence of humidity. During the experiments, in presence of humidity, the colour of model drug – excipients mixtures **QPVD**, **QHPC** and **QSM** have been changing from white to cream and the mixtures of **QHPMC** and **QLS** have been changing from white to brown; in

Case 0:12-cv-60704-PAS Document 61-1 Entered on FLSD Docket 12/14/2012 Page 4 of 6

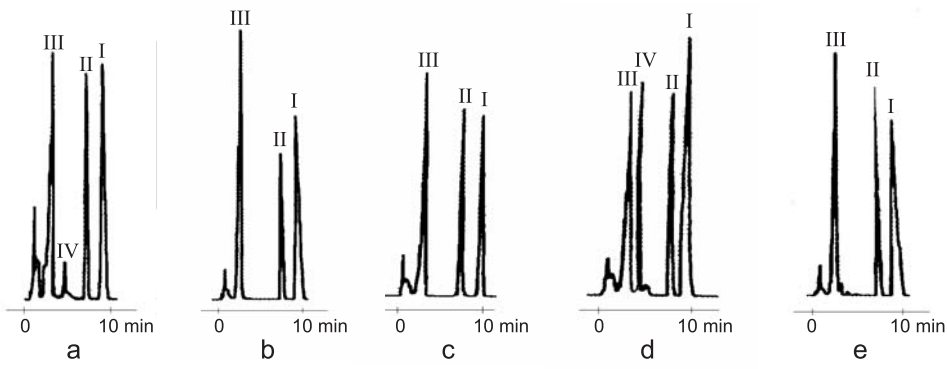


Figure 1. Typical HPLC chromatograms of quinapril hydrochloride, its degradation of products and benazepril (I.S.) in the presence of:
a – povidone
b – hydroxypropylmethyl cellulose
c – hydroxylpropyl cellulose
d – lactose
e – magnesium stearate
I – quinapril hydrochloride; **III** – quinaprilat (product of hydrolysis); **IV** – diketopiperazine (product of intramolecular cyclization); **II** – internal standard.

absence of humidity the colour of all mixtures remained white.

The analysis of concentration changes of **QHCl** as a function of time, in the presence of:

- povidone, hydroxypropyl methylcellulose and hydroxypropyl cellulose, indicates that degradation of **QHCl** proceeded according to the first – order reaction, the plots $ln\ (c_t - c_e) = f\ (t)$ (for 76.4%RH) and $ln\ c_t = f\ (c)$ (for 0%RH) according to the equation: $ln\ (c_t - c_e) = ln\ (c_t - c_0) - k \times t$ (for 76.4%RH) and $ln\ c_t = ln\ c_t - k \times t$ (for 0%RH).
- lactose, indicates that degradation of **QHCl** proceeded according to the following equation: $ln\ c_t = ln\ c_0 - k \times t$ (for 76.4%RH).
- magnesium stearate occurs according to the autocatalytic reaction. The relationship $c = f\ (t)$ was characterized by a sigmoid of curve. For interpretation of this autocatalytic process, the equation of Prout-Tomkins was used: $ln\ c_t/(c_0 - c_t) = C - k \times t$

where: c_t, c_0 and c_e represent the concentration of **QHCl** at time t, 0 and infinite, respectively; C denotes a constant related to the induction period; $k\ (s^{-1})$ is the first order rate constant.

The relationships: $ln\ c_t = f\ (t)$, $ln\ (c_t - c_e) = f\ (t)$ and $ln\ c_t/(c_0 - c_t) = f\ (t)$ were linear (see Figures 2 and 3).

The following statistical parameters of respective equations were estimated: $a \pm \Delta a$, $b \pm \Delta b$, SD and the correlation coefficient. The values of Da and Δb were estimated for f = n-2 degrees of freedom, with $a = 0.05$.

CONCLUSIONS

The degradation of **QHCl** in the presence of typical excipients can be studied by the HPLC stability indicating method. The HPLC method was found to be selective, linear, accurate and precise for **QHCl** assay either in substance or in the presence of povidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, lactose and magnesium stearate. The major degradation kinetics of **QHCl** in the presence of excipients was observed to be the first – order kinetics model, only degradation of **QHCl** in the presence of magnesium stearate in a humid atmosphere proceeds according to the first order autocatalytic kinetic model.

At 318 K/76.4% RH, in the presence of povidone, hydroxypropyl methylcellulose, and magnesium stearate accelerate the decay of **QHCl**. Degradation of **QHCl** in the presence of magnesium stearate was approx. hundredfold faster than the decomposition of **QHCl** alone. The results of the study showed that the drug of **QHCl** has been stabilized by the presence of lactose and hydroxypropyl cellulose does not affect the rate of its degradation. The determination results were given in Table 1. The main degradation product of **QHCl,** in the presence of hydroxypropyl methylcellulose, hydroxypropyl cellulose and magnesium stearate, was quinaprilat **(QAT)** (see Figures 1b, 1c and 1e; Scheme 1).

During degradation of **QHCl** in the presence of povidone and lactose, the following reactions were observed: hydrolysis – product **QAT** and intramole-

Case 0:12-cv-60704-PAS Document 61-1 Entered on FLSD Docket 12/14/2012 Page 5 of 6

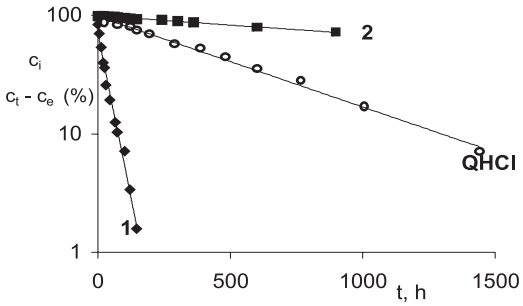


Figure 2. Semilogarithmic plots for decomposition of **QHCl** in the presence of **PVD** in solid phase at 318 K/76.4% RH (1), at 318 K/0% RH (2), and **QHCl** alone at 318 K/76.4% RH **(QHCl).**

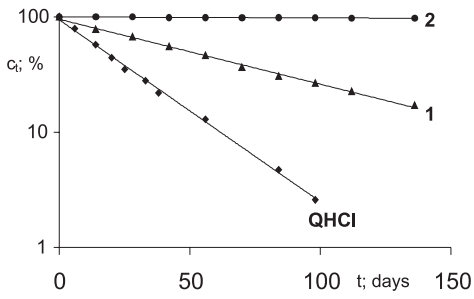
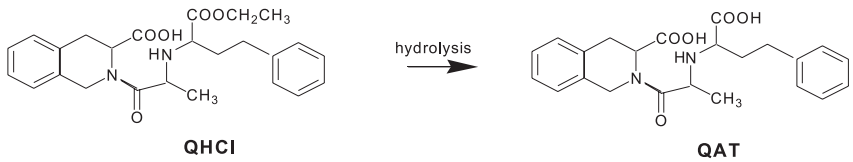


Figure 3. Semilogarithmic plots for decomposition of **QHCl** in the presence of **LS** in solid phase at 318 K/76.4% RH; at 318 K/ 0%RH and **QHCl** alone at 318 K/76.4% RH **(QHCl).**

Table 1. Results of degradation of QHCl : Excipient (1:4) studies at 318 K

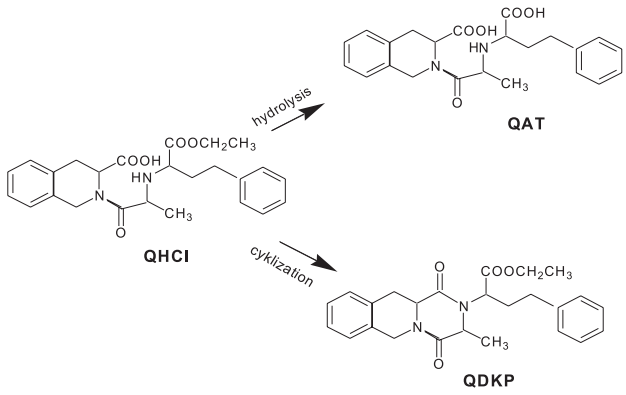
Symbol of model drug-excipients mixtures	Kinetic parameters	
	76.4% RH	0% RH
QPVD	$k = (1.030 \pm 0.032) \times 10^{-6}$ s^{-1} $t_{0.1}$ [1)] = 28.4 h $t_{0.5}$ [2)] = 7.7 days	$k = (1.044 \pm 0.053) \times 10^{-7}$ s^{-1} $t_{0.1}$ [1)] = 11.7 days $t_{0.5}$ [2)] = 76.8 days
QHMPC	$k = (7.153 \pm 0.62) \times 10^{-6}$ s^{-1} $t_{0.1}$ = 4.1 h $t_{0.5}$ = 26.9 h	$k = (2.051 \pm 0.15) \times 10^{-7}$ s^{-1} $t_{0.1}$ = 5.9 days $t_{0.5}$ = 39.1 days
QHPC	$k = (4.45 \pm 0.45) \times 10^{-7}$ s^{-1} $t_{0.1}$ = 2.7 days $t_{0.5}$ = 18.1 days	$k = (4.49 \pm 0.40) \times 10^{-8}$ s^{-1} $t_{0.1}$ = 27.2 days $t_{0.5}$ = 178.7 days
QLS	$k = (1.502 \pm 0.07) \times 10^{-8}$ s^{-1} $t_{0.1}$ = 8.1 days $t_{0.5}$ = 53.4 days	Over the study period (196 days), at 318 K/0%RH degradation, of quinapril hydrochloride in drug-excipients models have not been observed
QSM	$k = (6.835 \pm 1.43) \times 10^{-5}$ s^{-1} $t_{0.1}$ = 21.5 h $t_{0.5}$ = 30.4 h	
Kinetic parameters for QHCl alone		
76.4% RH		0% RH
$k = (4.21 \pm 0.17) \times 10^{-7}$ s^{-1}; $t_{0.1}$ = 2.9 days; $t_{0.5}$ = 19.1 days		$k = (3.56 \pm 0.22) \times 10^{-8}$ s^{-1}; $t_{0.1}$ = 34.1 days; $t_{0.5}$ = 225.3 days

[1)] $t_{0.1}$ – time needed for 10% degradation of QHCl in drug-excipients mixtures
[2)] $t_{0.5}$ – time needed for 50% degradation of QHCl in drug-excipients mixtures



Scheme 1. Degradation of **QHCl** in the presence of hydroxypropyl methylcellulose, hydroxypropyl cellulose and magnesium stearate at 318 K/RH 76.4%

Case 0:12-cv-60704-PAS Document 61-1 Entered on FLSD Docket 12/14/2012 Page 6 of 6



Scheme 2. Degradation of **QHCl** in the presence of povidone, and lactose at 318 K/RH 76.4%

cular cyclization – product **QDKP** (see Figures 1a and 1d; Scheme 2)**.**

The data shown in Table 1 indicate that at 318 K/0% RH, the rate of degradation of **QHCl** in the presence of povidone and hydroxypropyl methylcellulose, hydroxypropyl cellulose (approx. tenfold) was slower than at 318 K/76.4% RH, however (in period of six month, at 318 K/0%RH) the degree of degradation of the **QHCl** in the presence of lactose and magnesium stearate was not observed. **QHCl** in the presence of acidic excipients was more stable than in the presence of basic excipients. The major reasons of the **QHCl** instability in model mixtures are the humidity and pH of excipients. **QHCl** should particularly not be exposed to humidity and temperature in the presence of basic excipients. It is recommended to keep magnesium stearate absent in tablets to ensure proper stability of **QHCl** in pharmaceutical dosage.

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