Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 1197–1200

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Stability of vitamin B complex in multivitamin and multimineral supplement tablets after space flight

Monica C. Chuong[a,*], Dev Prasad[a], Barbara LeDuc[a], Brian Du[b], Lakshmi Putcha[b]

[a] Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, 179 Longwood Ave, Boston, MA 02115, USA
[b] National Aeronautics and Space Administration, Johnson Space Center, Houston, TX, USA

ARTICLE INFO

Article history:
Received 2 February 2011
Received in revised form 19 March 2011
Accepted 21 March 2011
Available online 29 March 2011

Keywords:
Pharmaceutical stability
Vitamin B complex
International Space Station
High performance liquid chromatography

ABSTRACT

The effect of storage in space on the stability of vitamin B complex in two commercial vitamin tablets was examined. Multiple vitamin samples returned after storage on the space shuttle and International Space Station (ISS) along with two ground control and three positive control groups were included in the study. Content of vitamin B_3 in the tablets and *in vitro* dissolution rate were determined using a modified high performance liquid chromatographic assay from USP/NF 2010. Results indicate that vitamin B_3 in one of the brands tested (#2) may be subject to marginal degradation after storage on ISS for 4 months as indicated by the chromatograms for all six tablets showing a split peak appearing as a notch at the peak tip. Chromatograms were not different for ground and flight samples for Brand #1 suggesting that this may be more suitable for use in space.

1. Introduction

Vitamins, including vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), and B_6 (pyridoxine), represent a broad group of organic compounds that are minor, but essential, dietary constituents required for the normal growth, self-maintenance and functioning of the body. Despite good eating habits which incorporate fruits and vegetables in the daily diet, nutrient loss is possible during storage and processing of food. As indicated in the United States Pharmacopeia and the National Formulary (USP/NF) 2010, water and oil soluble vitamin tablets must contain 90.0–150.0% of the labeled amounts [1]. The labels for the tablets in this study indicated that Brand #1 contained 1.5 mg, 1.7 mg, 20 mg and 3 mg of vitamins B_1, B_2, B_3 and B_6, respectively. Similarly, Brand #2 contained 1.5 mg, 1.7 mg, 10 mg and 2 mg, respectively.

Space adaptation involves some very complex changes in the human body, both short-term and long-term [2,3]. Since incidence of illness in space cannot always be predicted in advance, it is extremely important for astronauts in space to have available medications for the treatment and prevention of minor illnesses in space. In a preliminary investigation on the stability of pharmaceuticals in space, some of the formulations were found either degraded prematurely with respect to active pharmaceutical ingredient content [4,5]. Multivitamin and multimineral supplements are included in the space formulary for voluntary consumption by astronauts. For this reason, we examined the stability with respect to content and dissolution of API, B complex, in multiple vitamin nutritional supplement stored in the pharmaceutical kit of a space craft.

2. Materials and methods

2.1. Materials and commercial vitamin products

Reference standards (1 g) of thiamine hydrochloride, riboflavin, nicotinic acid, and pyridoxine hydrochloride (products of Supelco), as well as methylparaben, methanol, acetonitrile, glacial acetic acid, hydrochloric acid (36.5–38%) and sodium 1-hexanesulfonate were purchased from VWR (Bridgeport, NJ). Centrum Silver® Multivitamin/Multimineral Supplements (Wyeth, designated as Brand #1) and OneADay Women's® (Bayer, Brand #2) were purchased from local pharmacies to serve as our laboratory controls (G_L).

2.2. Experimental sample conditions

2.2.1. Study groups

Brand #1 and Brand #2 multivitamin/multimineral supplements retrieved from one space shuttle flight and three ISS expeditions were provided by NASA-JSC. The space shuttle flight group (Payload I) tablets flew on a space shuttle for a short 14–20 day short space journey. The two ISS groups (Payloads II and III) were the tablets taken to the ISS by a space shuttle flight, which remained on the ISS for approximately 12 and 19 months before returning to Earth.

* Corresponding author. Tel.: +1 617 735 1104; fax: +1 617 732 2228.
E-mail addresses: monica.chuong@mcphs.edu, chuongmc@yahoo.com (M.C. Chuong).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.03.030

Table 1
The treatment and absorbed doses of vitamin tablets irradiated in NSRL (only Brand #2 was available in this group).

Kit #	Treatment	Absorbed dose	Irradiation versus control
0001	Heavy iron	10 cGy[a]	Irradiated
0002	Heavy iron	10 Gy	Irradiated
0003	Heavy iron	50 Gy	Irradiated
0004	Proton	10 cGy	Irradiated
0005	Proton	10 Gy	Irradiated
0006	Proton	50 Gy	Irradiated
0007	None	None	NSRL control for heavy metal groups
0012	None	None	NASA control

[a] Gy stands for gray and 1 cGy equals 10^{-2} Gy (refer to text and Ref. [7]).

2.2.2. Ground controls and positive controls

The G_0 groups were the vitamins purchased simultaneously with the space samples (same product lot) by NASA-JSC, but stored in a controlled laboratory on earth for the same time duration as the group flown on a space shuttle or an ISS mission. Our lab also purchased Brand #1 and Brand #2 vitamins from different local pharmacies as another ground control group (G_L). In addition, three positive controls were provided by NASA-JSC. These were vitamin tablets (1) stored in the Orbital Environmental Simulator (OES) for the same time duration under similar temperature and humidity conditions but not the radiations of space flight, (2) retrieved from Kennedy Space Center (KSC), and (3) Brand #2 irradiated in the Space Radiation Laboratory at Brookhaven National Laboratory (NSRL [6]) (Table 1). The samples were shipped to our lab by FedEx without being X-rayed to avoid additional irradiation.

2.3. Preparations of thiamine, riboflavin, niacin, pyridoxine hydrochloride standard curves and HPLC conditions

The liquid chromatographic analyses of thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (nicotinic acid, vitamin B_3 [8]) and pyridoxine (vitamin B_6) content were first conducted according to the Methodology guidelines listed in USP/NF2010 at 280 nm wavelength [1]. However, no significant peaks were seen on the chromatograms. Spectrum scans were using a Hewlett Packard Model 8453 ultraviolet/visual (UV/Vis) spectrophotometer. We selected an optimal absorbance wavelength (268 nm) for simultaneous analysis of these four vitamin Bs (Fig. 1e) in HPLC due to scarcity of samples. Three HPLC columns (X-Terra RP_{18}, μ-Bondapak C_{18} and X-Bridge C_{18} columns) were evaluated. Among the three different HPLC columns studied, X-Terra RP_{18} was found to endure the experiments well in the mobile phase (pH <3.0) by yielding reproducible retention times. The rationale for using such a low pH mobile phase was because one of the analytes, nicotinic acid (vitamin B_3) has pK_a of 2.17. Three separate standard curves for each vitamin B were constructed using water, hydrochloric acid (0.1 M, also cited as 0.1 N HCl in USP/NF 2010) or a "diluting solution" (water–acetonitrile–glacial acetic acid; 94:5:1, v/v/v) as dissolving media.

2.4. Content assays and in vitro release study

For content assays, each vitamin tablet was placed into a beaker with 500 mL of the USP diluting solution for vitamin Bs and stirred until disintegrated. The medium was then filtered through a 0.22 μm syringe filter prior to injecting into the HPLC [1]. The AUC of each vitamin B chromatographic peak was converted into the amount (in mg) through the standard curves and known dissolving medium volume to determine the percentage of the label claim present. For *in vitro* release study, the USP dissolution apparatus was calibrated first using the USP 300 mg salicylic acid and

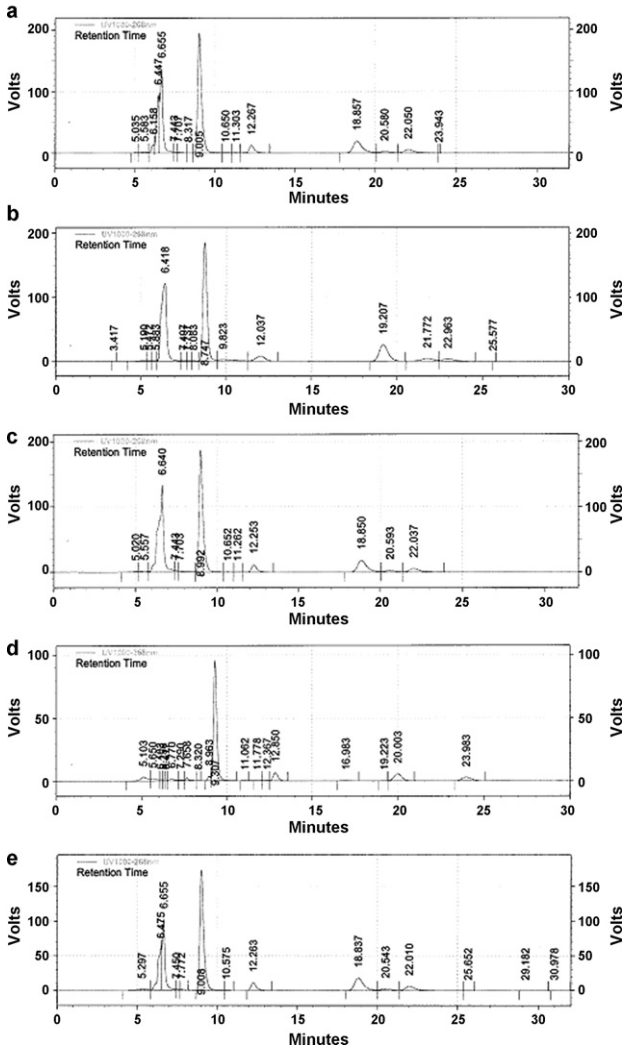

Fig. 1. The representative chromatograms of vitamin B based on study group were a tablet retrieved respectively from a space shuttle flight (a); KSC (b); OES (c); NSRL (d); and our lab control, G_L (e). All tested samples in this figure were product of Brand #1, except NSRL irradiated was only available in Brand #2. Retention times were: vitamin B_3 (niacin) 9 min; vitamin B_6 (pyrodoxin) 12.3 min; vitamin B_2 (riboflavin) 19.2 min; and vitamin B_1 (thiamine) 23.1 min.

10 mg prednisone tablet calibrators. The *in vitro* vitamin release study was then conducted with apparatus 1 (basket method) using two different media (0.1 M HCl versus deionized water), 500 mL each, stirred at 75 rpm to mimic a vitamin tablet administered with a small meal. The USP/NF 2010 suggests using deionized water as the dissolution medium [1]. However, 0.1 M HCl simulates the in vivo gastric fluid better where an immediately release tablet is expected to disintegrate in the stomach [9]. Aliquots of 1 mL were collected every 15 min until all studied vitamin tablets were completely disintegrated. The ChromQuest software generated a chromatographic area under the curve (AUC) value of each vitamin B at each particular sampling point. The AUC was then transformed into a cumulative percent of release versus time plot.

3. Results

3.1. Chromatographic retention time

The four vitamin B retention times are reported in Fig. 1. The peak shape of niacin (vitamin B_3) was asymmetric when its reference standard was dissolved in 0.1 M HCl, but was symmetric

Table 2
Contents of vitamins B_1, B_2, B_3 and B_6 (% label claim) in the vitamin tablets retrieved from a payload containing ISS, OES and NASA ground control samples.

	Sample size	Vitamin B_1 (mean ± SD)	Vitamin B_2 (mean ± SD)	Vitamin B_3 (mean ± SD)	Vitamin B_6 (mean ± SD)
Brand #1					
ISS	3	90.2 ± 34.0	136.0 ± 34.3	103.0 ± 20.3	140.6 ± 21.3
OES	3	93.6 ± 38.6	133.0 ± 33.6	103.4 ± 18.2	174.0 ± 47.2
G_0[a]	3	90.3 ± 30.4	135.9 ± 32.4	102.6 ± 19.8	173.0 ± 47.5
G_L[b]	3	106.2 ± 38.4	133.1 ± 30.2	104.9 ± 18.8	175.3 ± 51.3
Brand #2					
ISS	6	74.9 ± 11.5	120.5 ± 3.0	113.7 ± 5.1	149.3 ± 5.4
OES	6	102.5 ± 11.4	129.0 ± 6.6	113.7 ± 2.9	147.6 ± 5.3
Irradiation					
0001	3	53.5 ± 8.3[c]	104.2 ± 11.8	132.2 ± 28.1	113.7 ± 21.5
0002	3	50.1 ± 6.3[c]	98.0 ± 9.3	123.2 ± 25.0	113.4 ± 21.3
0003	3	47.2 ± 6.7[c]	99.1 ± 6.9	128.1 ± 19.7	106.4 ± 20.9
0004	3	49.9 ± 14.9[c]	96.1 ± 9.6	131.2 ± 31.6	113.0 ± 15.6
0005	3	58.5 ± 14.8[c]	98.1 ± 4.8	130.0 ± 27.1	111.4 ± 19.7
0006	3	56.7 ± 12.3[c]	96.1 ± 4.8	127.2 ± 26.1	107.8 ± 20.4
0007	3	55.7 ± 12.6[c]	94.6 ± 3.9	125.5 ± 22.4	109.7 ± 26.8
0012	3	57.2 ± 11.7[c]	94.6 ± 3.9	130.6 ± 28.0	108.4 ± 22.8
G_0[a]	6	112.4 ± 3.8	136.0 ± 1.1	116.7 ± 3.4	147.5 ± 8.0
G_L[b]	3	104.6 ± 6.4	141.4 ± 0.4	119.5 ± 1.7	152.6 ± 1.1

[a] NASA-JSC ground control.
[b] Our laboratory control.
[c] When irradiated Brand #2 tablets (0001–0007 and 00012) were conducted in deionized water and 0.1 M HCl, vitamin B_1 retrieved was in 90–150% range.

when it was dissolved in diluting solution or deionized water (Fig. 1).

3.2. Content assays for vitamins B_1, B_2, B_3 and B_6

The percent of vitamin B_1, B_2, B_3 and B_6 present in the multivitamin and multimineral supplements retrieved from the spacecrafts (shuttle and ISS), and the positive control groups (OES and KSC) were not significantly different from what was indicated in the USP and on the package labels (90% to 150%), except the vitamin B_1 from the Brand #2 tablets retrieved from one ISS expedition was 74.9 ± 11.5% (Table 2). The percent of vitamins B_1, B_2, B_3 and B_6 present in all groups of Brand #1 were found not significantly different from those of the controls. And all were within but all in 90–150% window.

3.3. In vitro dissolution study

Deionized water was not a good dissolution medium for vitamin B_2 (riboflavin) due to its low solubility, while 0.1 M HCl caused chemical changes in vitamin B_3 (niacin). Brand #1 disintegrated and dissolved 15 min faster than Brand #2 in either medium. There was no significant difference among the averaged release profiles of all experimental groups and control groups, although large variation among individual profiles was observed. Due to complexity in numerous experimental groups with similar results, only one payload of Brand #2 is listed in Table 3 to show the similarity in release performance. As aforementioned, only Brand #2 vitamin tablets were irradiated in the NSRL under eight different source conditions to mimic the potential irradiation exposure and potential dose levels in outer space (Table 1). The *in vitro* released profiles ($n = 2$) using deionized water as dissolution medium and our laboratory tablets as the negative control were found similar among these eight groups (Table 4).

4. Discussion

The ingredient loading allowance for vitamin B complex can be as much as its 150% of the label claims based on USP/NF 2010 [1]. The unused portion of this water soluble vitamin complex may be excreted easily by healthy individuals. Among the four water soluble vitamin B ingredients, vitamin B_2 (riboflavin) is least water soluble (Table 4), but it was released completely when 0.1 M HCl was used as the dissolution medium (Table 4). The dilution solution (water–acetonitrile–glacial acetic acid, 94:5:1, v/v/v) recommended in the USP/NF does not represent a physiological medium. It was used only for retrieving content, but not for *in vitro* dissolution, because acetonitrile may escape into the air by the required agitation. Water is not the ideal *in vitro* dissolution medium for two reasons. First, vitamin B_2 is not soluble in water. Second, water is not a typical disintegrating or dissolution medium when an immediate release tablet is administered by mouth into the stomach. 0.1 M HCl may be used to study vitamin B_1, B_2, and B_6, but preferably not for vitamin B_3 (niacin) due to the appearance of asymmetry observed in the chromatographic peak shape. Therefore, more than one dissolution medium should be used for vitamin B analysis.

The two negative control groups, G_L and G_0 of both Brand #1 and Brand #2 from difference sources were comparable in the study. The chromatographic peak apex of vitamin B_3 (niacin) of all six Brand #2 tablets retrieved from an ISS expedition flight were noted different from those in the ground control group by having a notch

Table 3
In vitro vitamin B_1 (thiamine, % label claim) release from ISS retrieved Brand #2 vitamin tablets in order to compare the difference using deionized water versus 0.1 M HCl as dissolution medium.

Dissolution time (min)	In deionized water ($n = 2$)			In 0.1 M HCl ($n = 1$)		
	ISS	OES	G_0	ISS	OES	G_0
15	69.8	80.1	68.0	116.1	47.0	44.0
30	90.1	96.0	84.8	148.5	76.8	71.6
45	101.4	108.3	99.5	159.2	88.3	78.9
60	113.8	119.1	108.2	140.2	86.8	82.0
90	124.0	129.0	118.9	159.6	97.6	87.1

Table 4
In vitro release of vitamin B_2 (in % label claim) in two different dissolution media (deionized water and 0.1 M HCl).

Dissolution (min)	% of release in deionized water ($n = 2$)								% of release in 0.1 M HCl ($n = 1$)							
	0001	0002	0003	0004	0005	0006	0007[a]	0012[a]	0001	0002	0003	0004	0005	0006	0007[a]	0012[a]
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	16.8	16.0	16.7	15.8	16.4	17.2	16.3	19.3	83.9	89.2	92.4	96.6	87.9	76.8	84.5	81.1
30	19.7	19.1	18.9	18.4	20.2	20.0	18.6	22.8	111.4	110.5	109.9	116.7	93.8	95.3	94.1	96.7
45	22.1	21.8	21.4	20.8	23.3	22.1	21.5	25.8	114.8	113.4	112.2	112.0	95.6	94.4	99.5	97.1
60	25.8	26.4	24.5	24.2	26.4	26.1	25.3	28.6	117.2	116.5	116.1	114.3	96.3	96.2	101.1	100.1
90	30.3	32.6	29.3	28.5	34.4	34.8	31.3	35.0	110.7	118.5	124.0	114.7	96.0	101.1	103.0	103.2

[a] 0007 and 0012 were irradiation controls (see Table 1).

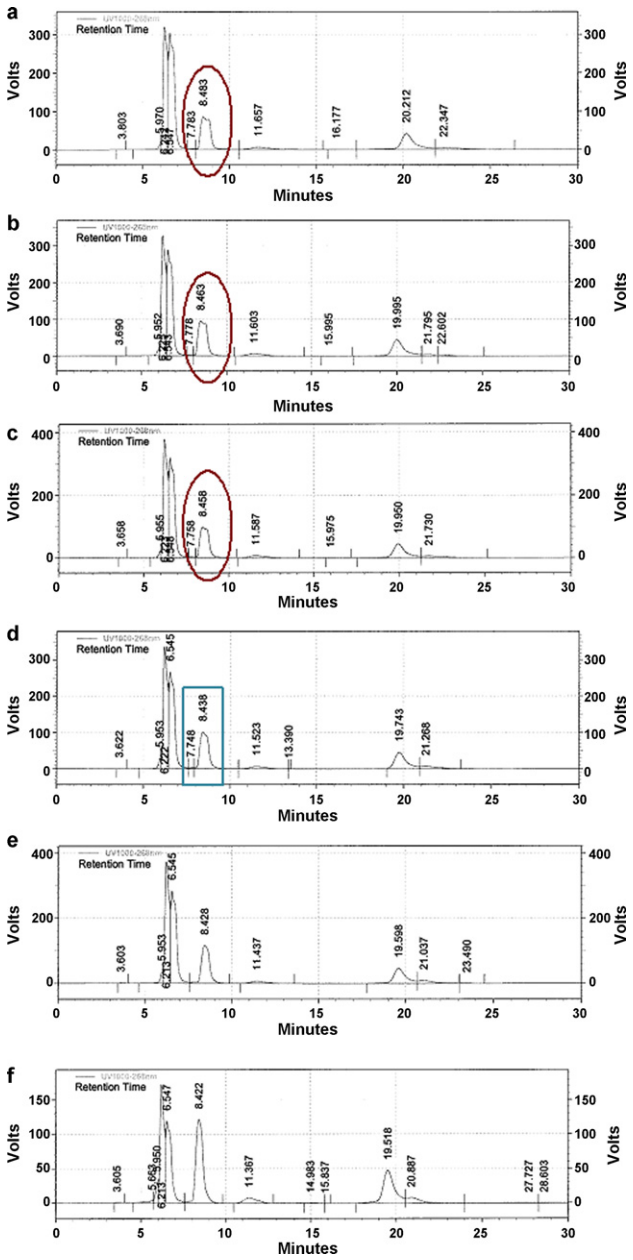

Fig. 2. The chromatograms of vitamin B content assays of Brand #2 product retrieved from International Space Station (a–c); OES (d); NASA control G_0 (e); and our lab control, G_L (f). The vitamin B_3 peak of the ISS samples changed in shape into a notch in the peak apex. This observation was consistent in all six samples in the group. Only the chromatograms of the first three are listed. The vitamin B_3 chromatographic peak of the OES sample also exhibited slightly shape changes in two out of the six tablets of the group.

(Fig. 2a–c). Two out of six Orbital Environmental Simulator tablets in the same payload also started to lose their symmetry (Fig. 2d). A reason for this instability could come from the active pharmaceutical ingredient (API) itself, formulation matrix, API–excipient interaction or the packaging container. Polymer plastic containers are probably better suited for withstanding vibration forces experienced during ascent and decent spacecraft to Earth by being lighter than glass and metal ones and less breakable. However, the polymeric materials used to fabricate the drug and nutrient product containers in space will need to be addressed for long term expedition missions. Formulations intended for use in space should be aimed at having a much longer expiration date than what is labeled on the product.

5. Conclusions

In the tripartite classification of degradation mechanisms, the instability found in this project appears to be more chemical in nature than physical. Although the sample size was understandably small due to the unique nature and limitations of storage and retrieval schedules of space flight experiments, a notch in the chromatographic peak apex was consistent among all six ISS retrieved Brand #2 tablets. Data showed that Brand #1 was stable thus far, but long-term follow up is recommended.

Acknowledgements

This research was supported by National Aeronautics and Space Administration – Johnson Space Center under Contracts T71048 and T71495.

References

[1] Chapter 711: dissolution; oil- and water-soluble vitamins with minerals tablets, nutritional supplements, in: USP/NF, United States Pharmacopeial Convention Inc., Rockville, 2010, pp. 304, 1104, 3076, 3451, 3497, 3718. 3720.
[2] P. Bond, The Continuing Story of the International Space Station, Springer, London, 2002, pp. xvii, 1–11, 343–360.
[3] J.D. Sibonga, H.J. Evans, H.G. Sung, E.R. Spector, T.F. Lang, V.S.A. Oganov, V. Bakulin, L.C. Shackelford, A.D. LeBlanc, Recovery of spaceflight-induced bone loss: bone mineral density after long-duration missions as fitted with an exponential function, Bone 41 (2007) 973–978.
[4] M.C. Chuong, L. Putcha, D.S. Chow, Evaluation of pharmaceutical stability of NitroDur (nitroglycerin) patch and desoximetasone (Topicort) cream during space flights, AAPS PharmSci 4 (S1) (2002) (abstract 795).
[5] M.C. Chuong, L. Putcha, D.S. Chow, Comparing the sensitivity of vertical Franz diffusion cell, VanKel Enhancer Cell™ in detecting changes of in vitro drug release of topical glucocorticoid formulations by using the known degraded quantities of irradiation and thermally degraded samples, AAPS J. 6 (S1) (2004) (abstract 753).
[6] www.bnl.gov/medical/NASA/NSRL_description.asp.
[7] http://en.wikipedia.org/wiki/Gray(unit).
[8] S. Budavari, The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co. Inc., Whitehouse Station, NJ, 2001, pp. 6552–6553, 8075, 8283, 9368.
[9] N. Washington, C. Washington, C.G. Wilson, The stomach, in: Physiological Pharmaceutics, Taylor and Francis Inc., New York, 2001, pp. 83–85.