Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 767–772

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Comparison of impurity profiles of Orlistat pharmaceutical products using HPLC tandem mass spectrometry

A. Schneider[a,b,*], L.A. Wessjohann[b]

[a] IDrug GmbH, Berlin, Germany
[b] Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

ARTICLE INFO

Article history:
Received 19 February 2010
Accepted 10 May 2010
Available online 16 May 2010

Keywords:
HPLC-UV
HPLC-MS/MS
Orlistat
Generic

ABSTRACT

HPLC-UV and MS/MS studies of impurity profiles of original (Xenical®, F. Hoffmann-La Roche Ltd., Switzerland) and generic (Cobese™, Ranbaxy Laboratories Limited, India, and Orsoten, KRKA, Russia) products were carried out. The drug and related impurities were extracted by dissolving commercial samples in ethanol. The generic formulations contained higher levels of impurities than the original product. Impurity profiles (HPLC-MS/MS) of the generic samples are similar among themselves, whilst different in comparison to the impurity profile of the original product. The number of detected impurities for generics (14 impurities in Cobese™ and 13 impurities in Orsoten) is higher than for the original product (3 impurities in Xenical®). Based on these analyses the overall analytical quality follows the order Xenical® (best) > Orsoten > Cobese™.

1. Introduction

Orlistat (Fig. 1), also known as (−)-tetrahydrolipstatin, is a patented active pharmaceutical ingredient [1]. There are two different dosage forms of orlistat: 120 mg is marked by F. Hoffmann-La Roche Ltd. worldwide under the brand name Xenical®, and 60 mg is branded as alli® by GlaxoSmithKline. It acts locally in the human gastrointestinal tract, inhibits selectively gastrointestinal lipase and, thus, prevents the absorption of fats [1,2]. The resulting caloric deficit has a positive effect on weight control. In conjunction with a mildly hypocaloric diet, orlistat is used for the treatment of obese patients or overweight patients with associated co-morbidities.

In general there are two different ways to produce the (−)-tetrahydrolipstatin: a fully synthetic route via 5,6-dihydropyran-2-one [3,4], or semi-synthetic one from fermentation products via lipstatin [5]. Original orlistat (Xenical®, alli®) is fully produced in chemical synthesis.

Xenical® received first approval in August 1997 and has subsequently been approved by health authorities in more than 100 countries worldwide. As of July 2009, more than 35 million patients had received Xenical®. The patent of Orlistat expired in most countries in 2009. Several generic formulations of orlistat have been launched recently, mainly by national generic laboratories in emerging markets such as Korea, Russia, India and Brazil. In many cases, the generics do not have the same quality as the original product [6,7]. The presence of certain impurities can lead to a decrease or alteration of biological activity or can impact the safety profile of the drug. The appearance of impurities different from the original innovator dossier, is mostly due to differences in the production processes between originator and generic company.

For example, in the case of tryptophan in 1989 the alteration of the production process from chemical synthesis to fermentation led to the formation of new impurities [8], that were the reason of a severe side effect, eosinophila-myalgia syndrome, causing the death of 27 patients. In 2000 it was reported, that 66 gentamicin patients died, because of different impurities in the active ingredient of various manufacturers [9,10].

There are other cases [6,7,11], in which the quality of the same active ingredient from different producers, was inferior in comparison to the originator quality documented in the product dossier. Therefore, the comparison of impurity profiles of original and generic pharmaceutical products and identification of the chemical structure of impurities (if possible) are relevant for ensuring consistent quality of both originator and generic formulations.

In this paper, two generics of Xenical®, Cobese™ from Ranbaxy Laboratories Limited, India, and Orsoten, KRKA, Russia, are investigated and their quality is compared with the original product. Samples were analyzed using HPLC equipped with UV-detector and tandem mass spectrometer.

2. Experimental

2.1. Reagents and samples

Acetonitrile HPLC grade and acetic acid 100% suprapur® were purchased from Merck (Darmstadt, Germany), phosphoric acid

* Corresponding author at: IDrug GmbH, Prenzlauer Allee 36, 10405 Berlin, Germany. Tel.: +49 30 2886 9966; fax: +49 30 2300 5278.
E-mail address: schneider@idrug.de (A. Schneider).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.05.010

Table 1
Drug product tested.

Product (local name)	Manufacture	Country	Batch number	Expiry date
Xenical®	F. Hoffmann-La Roche Ltd.	Switzerland	B2109/01	04/2011
Cobese™	Ranbaxy Laboratories Limited	India	1941737	06/2010
Orsoten	KRKA	Russia	1070309	03/2011

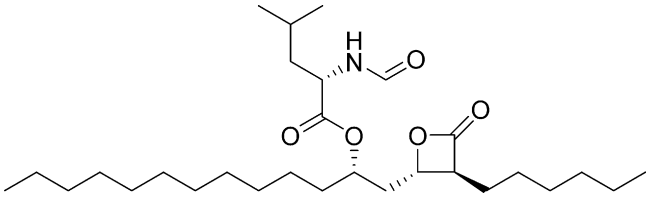


Fig. 1. Structure of orlistat.

85% rotipuran® from Carl Roth GmbH (Karlsruhe, Germany). The generic samples were obtained locally from pharmacies in the respective producers home country and delivered to the investigation laboratory under controlled condition below 25 °C. Product name, manufacture, country and batch numbers are given in Table 1. The reference product, Xenical®, was provided by F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

2.2. Equipment and procedure

2.2.1. HPLC-UV

HPLC-UV investigation of impurity profiles was performed on a Knauer WellChrom K-2800 with integrated photo diode array detector.

Three capsules of the respective sample (active substance content according to leaflet = 120 mg) were opened, put into 10 ml of ethanol and kept in an ultrasonic bath for 20 min. 1 ml of the formed suspension was put into a tube and centrifuged 10 min at 10,000 revolutions/min. 5 μl of this solution was introduced into the HPLC-system. The separation column YMC ODS-A 120 (5 μm, 4.6 mm × 150 mm) at a flow rate of 1 ml/min was used for the analyses. The column was preconditioned for 30 min at room temperature. Substances were eluted with acetonitrile (H_3PO_4 0.005%)–water (H_3PO_4 0.005%) (86/14, v/v) mixture as mobile phase, detection at 210 nm.

2.2.2. HPLC-MS

The positive ion ESI and the collision-induced dissociation (CID) mass spectra were obtained from a Finnigan MAT TSQ Quantum Ultra AM system equipped with a hot ESI source (HESI, electrospray voltage 3.0 kV, sheath gas nitrogen (25 psi); vaporizer temperature: 50 °C; capillary temperature: 250 °C; scan range: 100–1000 a.m.u.; scan rate: 1 scan per 0.6 s). The MS system was coupled with a Surveyor Plus micro-HPLC (Thermo Electron), equipped with an Ultrasep ES RP18E-column (5 μm, 1 mm × 150 mm, HYPERSIL GOLD from Thermo SCIENTIFIC). The column temperature was 25 °C. For the HPLC a gradient system was used starting from H_2O:CH_3CN 30:70 (each of them containing 0.2% acetic acid) to 2:98 within 20 min and then hold on 2:98 for further 35 min; flow rate 50 μl min^{-1}. The collision-induced dissociation (CID) mass spectra were recorded during the HPLC run with a collision energy of 10 or 15 eV (collision gas: argon; collision pressure: 1.5 mTorr). Xcalibur 1.4 was used as software to record and interpret the mass spectra.

Three capsules of the respective sample (active substance content according to leaflet = 120 mg) was opened, put into 10 ml of ethanol and kept in an ultrasonic bath for 20 min. 1 ml of the formed suspension was put into a tube and centrifuged 10 min at 10,000 revolutions/min. 1–2 μl of this solution was introduced into the HPLC/MS system.

3. Results and discussion

The HPLC chromatograms of the analyzed samples are shown in Fig. 2. Retention times, absolute and relative peak areas are shown in Table 2. The relative peak area (%) is an estimation of the content and does not represent the real concentration, as sensitivity coefficients are not known.

As can be seen from chromatograms (Fig. 2), impurity profiles of the generic samples (Cobese™ and Orsoten) are similar between themselves, whilst different in comparison to the impurity profile

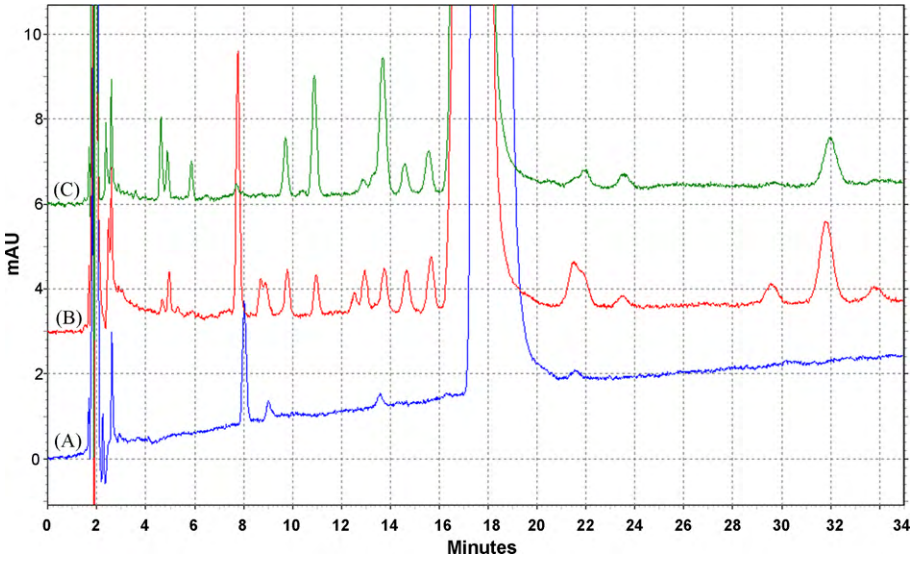


Fig. 2. HPLC-UV chromatograms of samples of Roche A: (Xenical® – blue line), Ranbaxy B: (Cobese™ – red line) and KRKA C: (Orsoten – green line) tetrahydrolipstatin drugs (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Table 2
Relative retention times and relative peak areas of peaks found in the sample of Roche (Xenical®), Ranbaxy (Cobese™) and KRKA (Orsoten) using HPLC-UV.

Retention time, rel.	Area, %		
	Xenical®	Cobese™	Orsoten
0.28		0.02	0.09
0.30		0.05	0.05
0.35			0.04
0.47	0.19	0.39	0.02
0.52	0.04	0.11	
0.53			
0.59		0.09	0.11
0.66		0.08	0.24
0.75		0.05	
0.78	0.03	0.09	0.04
0.82		0.12	0.40
0.88		0.11	0.06
0.94		0.13	0.11
1.00	99.74	97.81	98.46
1.24			
1.29		0.29	
1.32			0.07
1.41		0.04	0.06
1.77		0.08	
1.90		0.45	
1.92			0.25
2.02		0.09	

of the original product (Xenical®). Thus, it follows that the impurities composition for the original pharmaceutical product (Xenical®) and the analyzed generics (Cobese™, Orsoten) are different. The number of registered impurities in the generic formulations (17 impurities in the Ranbaxy sample and 13 impurities in the KRKA sample) is higher than in the original product (3 impurities in the Roche sample). Two impurities are common for all investigated products (relative retention times 0.47 and 0.78), but the concentrations are different. Another impurity (relative retention time 0.52) was found in the Roche and Ranbaxy samples.

Tandem mass spectrometry was used to identify the nature (molecular mass and putative chemical structure) of impurities found in the samples by HPLC. The separation conditions, incl. solvent system containing phosphoric acid, which was used for the HPLC analyses, cannot be applied to the HPLC-MS system. Thus, the method was adjusted for the use of mass spectrometry.

Mass spectra were recorded in positive and in negative mode. The ability of positive ion formation of most substances was better than for negative ions, therefore for the detailed investigation the positive mode was used. Only in the case of two impurities (r.t. 12.43 and 13.59 min, identified molecular mass – 513, see Table 3), the negative mode was considered. The most relevant MS/MS spectra are shown in Fig. 3. Retention times and identified molecular masses, as well as suggested structures are shown in Table 3.

The main fragmentation of the molecular ion of (−)-tetrahydrolipstatin leads to the formation of cations of a substituted β-lactone moiety and an amino acid moiety (Fig. 4). Further, the β-lactone fragment can lose two molecule of water and then decompose to unclarified fragments.

Using HPLC/MS/MS, 3, 14, and 13 impurities were identified in the samples of Roche, Ranbaxy, and KRKA, respectively. One impurity at 12.43 min was common to all samples and can be identified as a degradation product of orlistat [12]. An analogue of this impurity was found at 13.59 min in the samples of the original product Xenical® and the generic Cobese™. Another analogue of the degradation product with the same molecular mass 513 was detected at 18.11 min in both of the generic samples, but not in the original product. Another impurity common to Xenical®, Cobese™ and Orlistat was detected at 19.20 min. The identified molecular mass was 482. It can be assumed that this is one of the valine derivate of the main component.

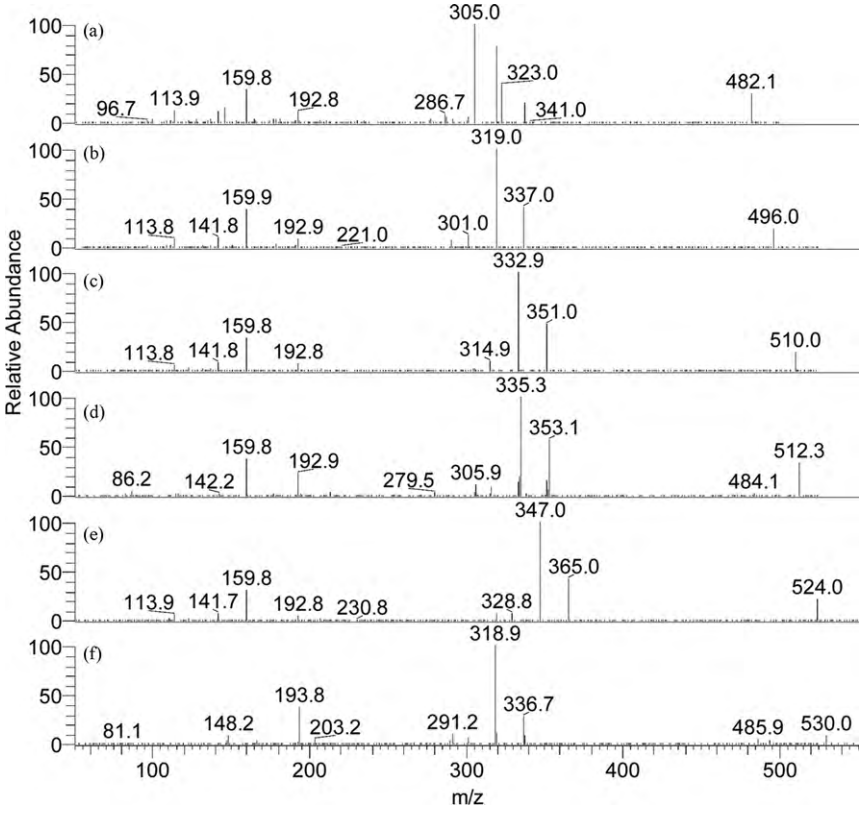


Fig. 3. MS/MS spectra of several impurities found in the investigated samples (a, *m*/*z* 482; b, *m*/*z* 496; c, *m*/*z* 510; d, *m*/*z* 512; e, *m*/*z* 524; f, *m*/*z* 530).

Table 3
Retention times, suggested $[M+H]^+$ and putative structures in the sample of Roche (Xenical®), Ranbaxy (Cobese™) and KRKA (Orsoten) using HPLC-MS.

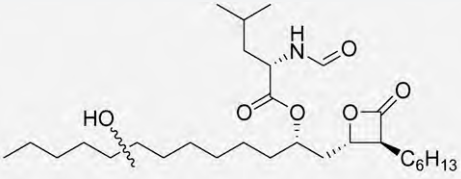
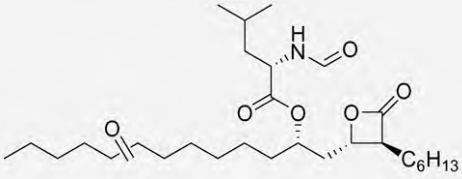
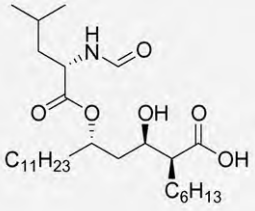
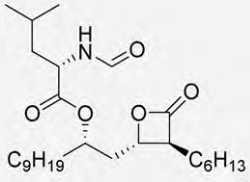
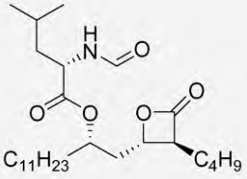
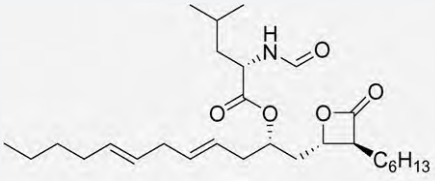
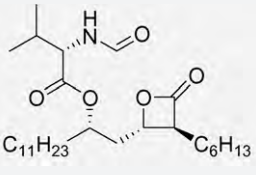
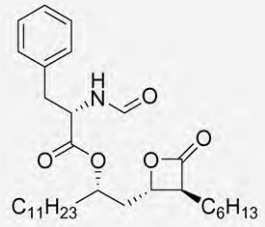
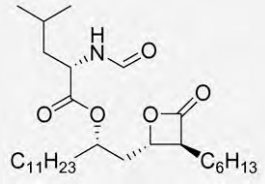
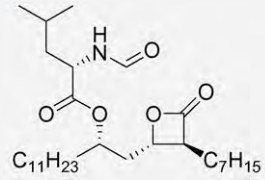
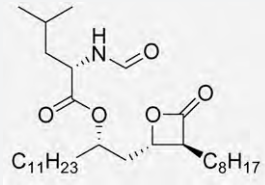
	Retention time (min)	Identified molecular ion $[M+H]^+$	Suggested structure	Presence in samples		
				Xenical®	Cobese™	Orsoten
1	7.85	512				X
2	8.50	510			X	X
3	12.43	514		X	X	X
			or analogue			
4	13.59	514	Analogue to 3	X	X	
5	15.31	468			X	X
			or analogue			
6	16.20	468			X	X
			or analogue			
7	17.52	468	Analogue to 5 and 6		X	
8	18.11	514	Analogue to 3		X	X
9	18.26	478			X	X
			or analogue			
10	18.58	482				X
			or analogue			
11	19.20	482	Analogue to 10	X	X	X
12	19.61	496	Analogue to 14		X	

Table 3 (*Continued*)

	Retention time (min)	Identified molecular ion $[M+H]^+$	Suggested structure	Presence in samples		
				Xenical®	Cobese™	Orsoten
13	20.23	530			X	X
14	21.1	496		X	X	X
			or analogue			
15	23.13	524	Analogue to 18		X (trace)	
16	23.21	510			X	X
			or analogue			
17	23.88	510	Analogue to 16			X
18	26.5	524			X	X
			or analogue			

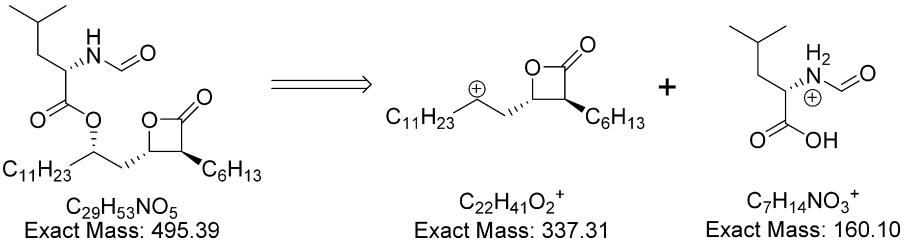


Fig. 4. Putative first fragmentations of orlistat (r.t. ≈ 17 min) and its analogues.

The other impurities were found only in the generic samples Cobese™ and Orsoten. Most of them belong to amino acid analogues (e.g. the phenylalanine derivate of orlistat at 20.23 min) and side-chain derivatives (e.g. impurities eluted at 15.31, 16.20 min, etc.).

In addition, polar impurity 1 (see Table 3) was found in the generic sample Cobese™, and the impurities 1 and 2 in Orsoten. The fragmentation shows that the molecular ion of impurity 1 can lose one more water molecule than the main component. It confirms that the detected impurity is a side-chain oxidation product of orlistat. In the KRKA sample, the hydroxyl- and oxo-derivatives of orlistat were identified, in the Ranbaxy sample only the ketone oxidation product. The exact position of oxidation in the side-chain could not be determined by MS/MS. For a detailed structural clarification of the impurities, it will be necessary to isolate all of them in sizeable amounts for an analysis by NMR. The fatty side-chain oxidation products hint at a fermentative origin of the generic material.

4. Conclusion

The investigation shows quality differences between the samples (Xenical®, Cobese™, and Orsoten). The HPLC(UV) analyses indicate that the number of registered impurities in the generic samples is higher than for the original sample. The same results were obtained using HPLC tandem mass spectrometry. The impurity profiles of generic samples (Cobese™ and Orsoten) are similar, whilst different in comparison to the impurity profile of the original

product (Xenical®). That confirms different production processes of original orlistat drug and the generic formulations. Most identified impurities in the samples Cobese™ and Orsoten were amino acid analogues and side-chain homologues of orlistat. Oxidation products were also found in Cobese™ (around 0.07%) and Orsoten (about 0.14%), which may indicate either a different production process (a fermentation step) or stability problems, e.g. during incorrect storage in the warehouse or pharmacy.

Based on HPLC (UV and MS/MS) data, the relative content of the desired active ingredient can be put into the following sequence: (higher quantity) Xenical® > Orsoten > Cobese™ (lower quantity). The number of detectable impurities accordingly follows the opposite order, with Xenical® product having the fewest, Orsoten most impurities. If the new impurities found in the generics will have toxicological effects was not the focus of this study, but because of the potential problems involved with such extra chemical entities (see Section 1), health authorities might be advised to ask for relevant data with respect to such additional compounds.

Acknowledgement

Part of this work was supported by F. Hoffmann-La Roche Ltd (Basel, Switzerland).

References

[1] P. Hadvary, E. Hochuli, E. Kupfer, H. Lengsfeld, E.K. Weibel, Leucine derivatives, Eur. Pat. Appl. EP 0129748 (A2) (1984).

[2] (a) E.K. Weibel, P. Hadvary, E. Hochuli, E. Kupfer, H. Lengsfeld, Lipstatin, an inhibitor of pancreatic lipase, produced by Streptomyces toxytricini. I. Producing organism, fermentation, isolation and biological activity, J. Antibiot. 40 (1987) 1081–1085;
(b) E. Hochuli, E. Kupfer, R. Mauer, W. Meister, Y. Mercadal, K. Schmidt, Lipstatin, an inhibitor of pancreatic lipase, produced by Streptomyces toxytricini. II. Chemistry and structure elucidation, J. Antibiot. 40 (1987) 1086–1091.

[3] (a) M. Karpf, U. Zutter, Production of oxetanones, Eur. Pat. Appl. EP 0443449 (A2) (1991);
(b) E.A. Broger, M. Karpf, U. Zutter, Process for the preparation of optically active compounds, Eur. Pat. Appl. EP 0643052 (A2) (1994).

[4] M.A. Schwindt, M.P. Fleming, Y.K. Han, L.M. Hodges, D.A. Johnston, R.P. Micheli, C.R. Roberts, R. Snyder, R.J. Topping, K. Puntener, M. Scalone, Enantioselective synthesis of a key intermediate in a new process for orlistat using asymmetric hydrogenation and a Grignard reagent promoted lactone cyclization, Org. Process Res. Dev. 11 (2007) 524–533.

[5] A. Bacher, P. Stohler, Process for the production of lipstatin and tetrahydrolipstatin, Eur. Pat. Appl. EP 0803576 (A2) (1997).

[6] Y. Gomez, E. Adams, J. Hoogmartens, Analysis of purity in 19 drug product tablets containing clopidogrel: 18 copies versus the original brand, J. Pharm. Biomed. Anal. 34 (2004) 341–348.

[7] P.A. Lambert, B.R. Conway, Pharmaceutical quality of ceftriaxone generic drug products compared with Rocephin®, J. Chemother. 15 (2003) 357–368.

[8] A.N. Mayeno, F. Lin, C.S. Foote, D.A. Loegering, M.M. Ames, C.W. Hedberg, G.J. Gleich, Characterization of "peak E", a novel amino acid associated with eosinophilia-myalgia syndrome, Science 250 (1990) 1707–1708.

[9] F Wienen, R. Deubner, U. Holzgrabe, Composition and impurity profile of multisource raw material of gentamicin—a comparison, Pharmeuropa 15 (2003) 273–279.

[10] U. Holzgrabe, R. Deubner, F. Wienen, Quality of APIs—result of a research of different gentamicin products, Am. Pharm. Outsourc. 5 (2004) 24–28.

[11] U. Holzgrabe, Verunreinigung von Arzneistoffen, Deutsche Apotheker Zeitung 143 (2003) 3221–3228.

[12] H. Stalder, P.R. Schneider, G. Oesterhelt, 103. Tetrahydrolipstatin: thermal and hydrolytic degradation, Helv. Chim. Acta 73 (1990) 1022–1036.