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Analysis of purity in 19 drug product tablets containing clopidogrel: 18 copies versus the original brand

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Abstract

In this study, 18 copies of PLAVIX® tablets containing clopidogrel hydrogensulfate were compared to the innovator drug product for uniformity of mass, impurity profile, content, dissolution properties and stability. In order to be able to separate the R-enantiomer of clopidogrel, an enantiospecific liquid chromatographic method was used to determine the impurities and to perform the assay. The paddle method was used for dissolution testing. Most of the copies were not similar compared to the original drug product: their amount of impurities was higher, the content of clopidogrel lower, the dissolution profiles different and after 3 months under stress conditions in the original packaging, the results for the samples and the reference were significantly different in most of the cases.

Keywords: Clopidogrel; Copies; Liquid chromatography; Dissolution

1. Introduction

The innovator drug product containing clopidogrel hydrogensulfate (named hereafter clopidogrel) was discovered by Sanofi. It is marketed by Sanofi–Synthelabo and BMS worldwide under the brand names PLAVIX® and ISCOVER®.

Clopidogrel is an antiplatelet agent which selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and blocks the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation [1].

The clinical benefits of clopidogrel have been demonstrated in trials involving more than 30,000 patients and it is used worldwide for the long term prevention of atherothrombotic events (myocardial infarction, stroke, peripheral arterial disease, acute coronary syndrome, cardio-vascular death) [2–4]. Several copies of PLAVIX®/ISCOVER® have been brought onto the market in some Asian and South American countries.

As seen in Fig. 1, the molecule is a thienopyridine derivative containing an assymetric carbon leading to the existence of two enantiomers (R and S). Studies from Sanofi–Synthelabo [5] showed that the active compound clopidogrel is the S-enantiomer. This

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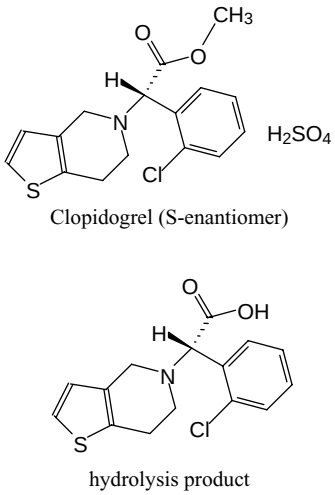

Fig. 1. Chemical structure of clopidogrel and its hydrolysis product.

implies that the content of the R-enantiomer must be carefully controlled in clopidogrel bulk substance and drug products, as required by health authorities. It should be noted that there is currently no official Pharmacopoeia monograph that would set official limits to the impurities and content of clopidogrel bulk samples. According to the ICH guidance (ICH Q3B) entitled "impurities in new drug products", the level of the impurities contained in the drug product should also be carefully controlled and the upper level of the impurities should be determined and supported by adequate data in the dossier filed for registration of a new drug [6].

Few methods for the determination of clopidogrel have been reported in the literature. A non-stereospecific liquid chromatographic (LC) method for the determination of clopidogrel in oral dosage forms has been validated and used for degradation studies under stress conditions [7]. The chiral inversion of clopidogrel in vivo was investigated using a chiral HPLC procedure while a non-stereospecific assay method was applied to monitor the hydrolysis of clopidogrel [8]. A capillary zone electrophoretic method using cyclodextrins for the enantioseparation of drugs also evaluated clopidogrel [9].

Another important part of the quality control is the release of the active from its pharmaceutical formulation, in this case a tablet. In most cases an in vitro dissolution test using paddles is performed to check whether a minimum percentage is dissolved at a predetermined time point. However, dissolution profiles with an equal percentage dissolved at a certain time point can have a different shape before reaching that time point, which, from a pharmacokinetic point of view, can lead to a difference in plasma concentrations. So, for comparison, it is advisable to check multiple time points.

In this paper, the quality of 18 copies is described and compared to the quality of the original drug product (PLAVIX®/ISCOVER®). The mass uniformity, the purity and content using a chiral LC separation method and the dissolution profiles were examined. Samples were analysed at different time points: first at time point zero and then after 3 months in their original packaging at 40 °C and a relative humidity of 75% to check the influence of these stress conditions on the impurity profile.

2. Experimental

2.1. Reagents and samples

Acetonitrile HPLC grade was purchased from Biosolve LTD (Valkenswaard, The Netherlands), methanol from Fisher Chemicals (Loughborough, UK), hydrochloric acid and potassium dihydrogen phosphate analytical reagent grade from Riedel de Haën (Seelze, Germany) and potassium chloride p.a. from Chem-Lab (Lichtervelde, Belgium). Membrane filters 0.45 μm (GHP Acrodisc GF 25 mm syringe filter) were obtained from Gelman Laboratory (Ann Arbor, MI, USA). Demineralised water was distilled from glass apparatus.

Beside three different batches of the reference, a total of 18 copies obtained from five countries, were included in this study. The commercial names, manufacturers and country of origin of each product (expressed as clopidogrel base) are listed in Table 1. Reference substances of clopidogrel, the R-enantiomer and the hydrolysis product (Fig. 1) were kindly provided by Sanofi–Synthelabo (Paris, France).

2.2. Apparatus

The chromatographic analyses were carried out using a L-7100 pump, a L-7200 autosampler and a

Table 1
Overview of the samples used in this study and results for content

Sample	Product	Pharmaceutical company (country of origin)	Mean (percentage of label claim[a])
Ref. batch 1	PLAVIX®[b]	Sanofi–Synthelabo (France)	97.2
Ref. batch 2	PLAVIX®[b]	Sanofi–Synthelabo (France)	99.3
Ref. batch 3	PLAVIX®[b]	Sanofi–Synthelabo (France)	97.3
1	Plagril 75®	Dr. Reddy's Laboratories Ltd. (India)	95.1
2	Clodrel®	Unichem Laboratories Ltd. (India)	88.7
3	Orawis®	Merck (India)	95.6
4	Noklot®	Zydus Medica (India)	91.6
5	Clopigrel®	USV Ltd. (India)	91.6
6	Preva®	Intas Pharmaceuticals Ltd. (India)	93.3
7	Clavix®	Intas Suprima (India)	93.6
8	Clopilet®	Sun Pharmaceutical Ind. Ltd. (India)	91.3
9	Stromix®	Nicholas Piramal (India)	86.5
10	Cloplat-75®	IPCA Laboratories Ltd. (India)	95.9
11	Deplatt®	Torrent Pharmaceutical Ltd. (India)	96.7
12	Ceruvin 75®	Stancare/Reddy (India)	96.1
13	Cloplatic®	Haymann (Uruguay)	90.3
14	Plagrel®	Servimedic (Uruguay)	102.3
15	Nefazan®	Laboratorios Phoenix (Argentina)	96.4
16	Talcom®	Shenzen Salubris (Xi Lin Tai) (China)	94.5
17	Clopifran®	Laboratorios Lufra Farmacos S.A. (Dominican Rep.)	95.3
18	Clopigrel®	Noas Farma Uruguay S.A (Uruguay)	97.7

[a] The dose is 75 mg expressed as clopidogrel base for all products except for sample 16 (Talcom®, China) having a dose of 25 mg.
[b] Marketed as ISCOVER® in some countries.

L-7400 UV-Vis detector from Merck–Hitachi (Darmstadt, Germany). Data were collected and integrated by a PC (Compaq, Houston, TX, USA) by means of a D-7000 interface (Merck–Hitachi).

The column, an ULTRON ES-OVM, 5 μm (4.6 mm × 150 mm i.d.) (Shinwa Chemical Industries, Kyoto, Japan) was immersed in a water bath with a F-10 cooler from Julabo (Seelbach, Germany).

Dissolution tests were performed using a paddle apparatus 72R (Hanson Research Corporation, Northridge, CA, USA). At the sampling times, the assay of the active compound was performed using a Lambda EZ 201 UV-Vis spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) set at 240 nm with 1.0 cm cells and using the dissolution medium as the compensating liquid.

2.3. Mass uniformity

Twenty tablets from each batch were weighed individually and the average mass was calculated. The European Pharmacopoeia (Eur. Ph.) prescribes that not more than two tablets may deviate from the average mass by more than 7.5% for tablets from 80 to 250 mg and 5% for tablets with a mass higher than 250 mg. No tablet can deviate by more than twice the prescribed percentage [10].

2.4. Chromatography

A chiral LC method was used for impurity testing and enantiospecific assay. As stationary phase, an ULTRON ES-OVM column, 5 μm (4.6 mm × 150 mm i.d.) was used. The mobile phase consisted of a mixture of acetonitrile and 0.01 M potassium dihydrogen phosphate solution (25:75 (v/v)). The flow-rate was 1 ml/min, the column temperature 17 °C, the injection volume 10 μl and UV detection was performed at 220 nm.

Sample solutions for chromatography were prepared as follows: the 20 tablets used to check mass uniformity were grinded and mixed in a mortar while a ball mill was used to grind some samples with a very resistant external film. A portion of the ground tablet powder, corresponding to the average weight of one tablet was suspended in 5.0 ml of methanol, shaken

manually during 5 min and further diluted with mobile phase. For the determination of impurities, a final concentration of 0.5 mg/ml and for assay a 0.1 mg/ml solution was used. For sample 16 (Talcom®) having a content of 25 mg of the active compound per tablet, three times the tablet mass was used to prepare the sample solution. All sample solutions were filtered before injection.

2.5. Dissolution

The dissolution medium consisted of 1000 ml of buffer solution pH 2.0, prepared by dissolving 6.57 g of potassium chloride in 119.0 ml of 0.1 M hydrochloric acid and further diluted with water. The reference solution was prepared by dissolving 100.0 mg of clopidogrel working standard in 20.0 ml of methanol; 2.0 ml of this solution were diluted to 100.0 ml with the dissolution medium. In case of sample 16 (Talcom®), containing only 25 mg of clopidogrel per tablet, a reference solution diluted three times was prepared.

The dissolution medium was thermostatically controlled at 37 °C. The rotation speed of the paddles was 50 rpm. A volume of 10 ml was taken at 10, 20, 30 and 40 min and analysed in the spectrophotometer.

Initially the values obtained at 30 min were used to evaluate the differences between the samples. Based on the results of the reference (see Section 3.5) and the acceptance criteria of the USP [11], the *Q*-value was proposed to be 75%. This implied that the percentage of active ingredient dissolved after 30 min for each of the six tablets examined might not be less than 80% ($Q + 5\%$) of the theoretical clopidogrel content. If the tablets did not pass the test, another 6 units were examined. The result was satisfactory if the average of the 12 units was not less than 75% and no unit was less than 60%. If the tablets did not pass the test using these 12 units, another 12 units were tested. The batch was accepted if the average of the 24 units was not less than 75%, not more than 2 units were less than 60% and no unit less than 50%.

3. Results and discussion

3.1. Mass uniformity

The mass uniformity was examined by weighing 20 tablets of each product. Although all products comply with the Eur. Ph. limits [10], the range indicated by the lowest and the highest mass was usually wider for the copies than for the reference product.

3.2. Validation of the chromatographic method

During validation of the chromatographic method, its robustness as well as some quantitative aspects such as repeatability, linearity, limit of detection (LOD) and quantitation (LOQ) were examined.

3.2.1. Robustness

The robustness was studied using the following experimental design. Four chromatographic parameters governing the separation were evaluated: the amount of acetonitrile in the mobile phase (Ac), the buffer concentration in the mobile phase (Bu), the flow rate (Fl) and the column temperature (Te). The set-up and calculation of the central composite design were supported by Modde 4.0 software (Umetrics, Umea, Sweden). The values used at the central (0), high (+1) and low level (−1) are shown in Table 2. A typical chromatogram of the reference sample obtained under the central value conditions is shown in Fig. 2(A). Since the most critical separation is that between peaks 2 and 3, the selectivity between these two peaks was chosen as the response variable. The design required $2^k + 2k + n = 27$ runs, where k is the number of parameters studied ($k = 4$) and n the number of central

Table 2
Chromatographic parameters and range of investigation for the factors of the experimental design

Factor	Abbreviation	Low value (−1)	Central value (0)	High value (+1)
Acetonitrile (%, v/v)	Ac	23	25	27
Temperature (°C)	Te	15	17	19
Buffer concentration (M)	Bu	0.009	0.010	0.011
Flow rate (ml/min)	Fl	0.9	1.0	1.1

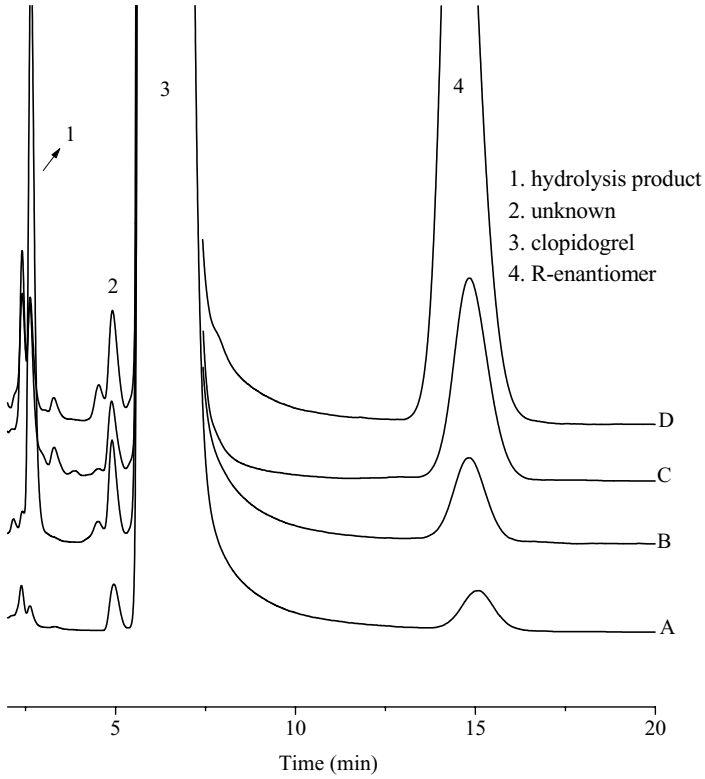

Fig. 2. Superposition of the chromatograms of some samples compared to the reference: (A) reference product; (B) product 3; (C) product 13; and (D) product 5. The chromatographic conditions are mentioned under Section 2.4.

points included ($n = 3$). Multiple linear regression was used to estimate the coefficients of the model representing the relationship between the response variables measured and the chromatographic parameters studied. Single coefficients describe the quantitative effect of a variable on the response, cross products the interaction between variables and squared coefficients the non-linear effects.

The results are shown in Fig. 3. The plot consists of bars that correspond to the regression coefficients with the magnitude of the effects proportional to the regression coefficients. The 95% confidence limits are expressed by using error bars. A regression coefficient smaller than the error bar interval shows that the variation in the response produced by changing that chromatographic parameter is smaller than the experimental error. Therefore the effect of that variable is considered not significant. The results show that the separation under the examined conditions was principally influenced by the amount of acetonitrile in the mobile phase and the temperature of the column. They both have a negative effect on the selectivity, which means that an increase of the percentage of acetonitrile in the mobile phase or an increase of the column

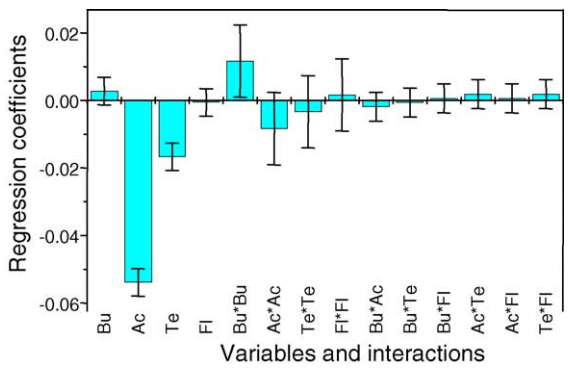

Fig. 3. Regression coefficient plot for the selectivity between peaks 2 and 3 of Fig. 2 (Bu: buffer concentration, Ac: percentage of acetonitrile, Fl: flow rate, Te: column temperature).

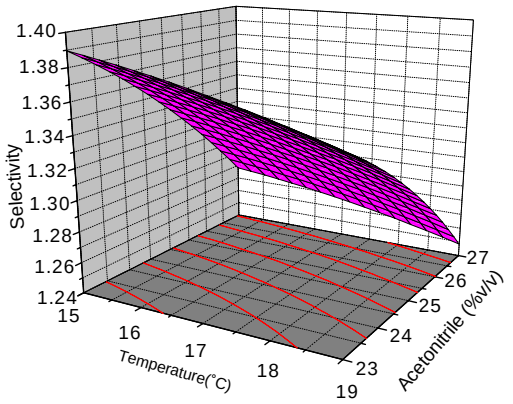

Fig. 4. Response surface plot of the selectivity between peaks 2 and 3 of Fig. 2 as a function of the amount of acetonitrile in the mobile phase and the column temperature.

temperature decreases the selectivity between peaks 2 and 3. The influence of the flow rate of the mobile phase and the concentration of buffer are not significant. No major interactions were found. A better estimation of the effect of the most important parameters can be made by means of a response surface plot. The selectivity for peaks 2 and 3 varies as a function of the amount of acetonitrile in the mobile phase and the temperature of the column and is shown in Fig. 4.

Statistical analysis of the model gave a R^2 value (the fraction of variation of the response that can be explained by the model) of 0.98 and a Q^2 value (the fraction of variation of the response that can be predicted by the model) of 0.93.

3.2.2. Quantitative aspects

For purity testing, 10 μl of a 0.5 mg/ml solution was injected, while for assay a 0.1 mg/ml solution was used. The repeatability of the method was determined by analysing six times a solution containing 0.1 mg/ml. The relative standard deviation (R.S.D.) regarding the area of the main peak was 0.7%. The LOD values were determined at a signal-to-noise ratio of 3 (S/N = 3) and the LOQ values at a S/N = 10. For the hydrolysis product, the LOD–LOQ values amounted to 0.5 and 0.15 ng and for the R-enantiomer to 1.5 and 4.9 ng, respectively. This means that the LOQ is always below 0.1%. The results for the linearity of clopidogrel, its hydrolysis product and the R-enantiomer are shown in Table 3.

3.3. Purity testing

The values obtained at time point zero and after 3 months under stress conditions (40 °C and a relative humidity of 75%) are shown in Table 4. At time point zero, 61% of the copies contained more than four times the amount of hydrolysis product of the innovator product (>0.16%). Three samples (7, 10 and 13) contained even more than 1.0%. A similar result was found for the R-enantiomer at time zero: 67% of the copies showed a fourfold concentration compared to the reference product (>1.0%). Moreover, for three samples (5, 9 and 10) the concentration was above 3%. As could be expected, large differences were also seen for the total amount of impurities. In the LC chromatograms of Fig. 2, the impurities found in some samples can be compared graphically with those found in the reference tablets. After 3 months under stress conditions, the differences were even more pronounced (Table 4). The main degradation pathways are hydrolysis and racemisation. This decline indicates that the excipients, production technology and packaging of the samples are also important and may have a major influence on the global quality of the products. These parameters were best controlled by the originator.

3.4. Content

The mean content expressed as a percentage of the label claim are listed in Table 1. For each sample, two

Table 3
Quantitative aspects of the chromatographic method

Compound	Range (μg/ml)	Equation	r	$S_{y,x}$
Clopidogrel	60–140	$y = 2.04\ x + 0.47$	0.9998	0.96
Hydrolysis product	0.48–9.7	$y = 2.60\ x - 0.02$	0.9999	0.05
R-enantiomer	1.5–29.9	$y = 2.12\ x - 0.41$	0.9999	0.09

y, peak area/100,000; x, concentration (μg/ml); r, coefficient of correlation; and $S_{y,x}$, standard error of estimate.

Table 4
Purity testing of the clopidogrel tablets at time point zero and after 3 months at 40 °C and a relative humidity of 75%

Sample	Product	Hydrolysis product (%)		R-enantiomer (%)		Total impurities (%)	
		Time 0	3 months	Time 0	3 months	Time 0	3 months
Ref. batch 1	PLAVIX®[a]	0.04	0.29	0.24	0.35	0.56	1.71
Ref. batch 2	PLAVIX®[a]	0.04	0.32	0.25	0.31	0.65	1.84
Ref. batch 3	PLAVIX®[a]	0.04	0.33	0.25	0.40	0.61	1.69
1	Plagril 75®	0.45	0.46	1.09	1.14	1.76	3.29
2	Clodrel®	0.85	1.36	2.24	3.61	3.55	5.55
3	Orawis®	0.67	1.36	0.57	3.30	1.52	5.21
4	Noklot®	0.86	1.35	1.71	2.70	2.97	4.85
5	Clopigrel®	0.15	1.40	5.68	6.12	6.65	9.71
6	Preva®	0.57	2.54	1.97	5.30	3.07	10.84
7	Clavix®	1.46	2.06	0.67	6.50	3.13	11.21
8	Clopilet®	<0.01	0.45	0.87	1.26	4.48	5.17
9	Stromix®	0.07	0.70	3.41	3.63	8.87	9.17
10	Cloplat 75®	1.36	2.47	3.20	3.56	5.99	8.53
11	Deplatt®	0.08	0.08	0.95	1.03	1.68	2.54
12	Ceruvin 75®	0.23	0.41	1.50	1.55	2.50	3.46
13	Cloplatic®	1.47	2.20	1.93	3.70	5.88	9.08
14	Plagrel®	0.21	0.26	0.79	0.80	1.78	1.90
15	Nefazan®	0.07	0.07	0.93	0.98	1.28	1.91
16	Talcom®	0.04	0.08	1.11	1.68	2.46	3.43
17	Clopifran®	0.07	0.70	1.13	4.65	3.90	5.60
18	Clopigrel®	0.17	1.78	1.03	3.66	2.59	7.40

[a] Marketed as ISCOVER® in some countries.

extractions were carried out and each solution was analysed three times. All the reference tablets were within the generally accepted 95–105% limits of the clopidogrel label claim. In contrast, half of the copies tested failed to fall within this range.

3.5. Dissolution testing

For the reference product, a total of 94–96% of clopidogrel was dissolved in 30 min. According to the acceptance criteria mentioned in 2.5, only two samples failed to pass the test: product 3 with only 51.3% and product 13 with 60.9%. When the other time points were also taken into account, several copies showed different, much faster or non homogeneous, dissolution profiles.

4. Conclusion

A high level of impurities was found in many copies; over 60% of the copies contained more than four times the amount of hydrolysis product or R-enantiomer compared to the reference drug product. In addition, 50% of the samples did not comply with the 95–105% limits for content. It should be noted that there is no Pharmacopoeia monograph at the moment of writing this document that would set limits to the impurities and content in clopidogrel bulk substance.

Although most copies passed the dissolution specifications at 30 min, no adequate dissolution profiles were obtained for most of them. Differences in excipients led to different tablet masses and they also had an influence, together with the packaging of the tablets, on the stability during 3 months under stress conditions.

Most of the copies are not of equivalent quality compared to the innovator drug product.

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References

[1] B. Jarvis, K. Simpson, Drugs 60 (2000) 347–377.
[2] CAPRIE Steering Committee, Lancet 348 (1996) 1329–1339.
[3] M.E. Bertand, H.J. Rupprecht, P. Urban, A.H. Gershlick, Circulation 102 (2000) 624–629.
[4] S. Yusuf, F. Zhao, S.R. Mehta, S. Chrolavicius, G. Tognoni, K.K. Fox, N. Engl. J. Med. 345 (2001) 494–502.
[5] P. Savi, J. Combalbert, C. Gaich, M.C. Rouchon, J.P. Maffrand, Y. Berger, J.M. Herbert, Thromb. Haemost. 72 (1994) 313–317.
[6] Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Tripartite Guideline Q3B: Impurities in New Drug Products, 1996.
[7] A. Mitakos, I. Panderi, J. Pharm. Biomed. Anal. 28 (2002) 431–438.
[8] M. Reist, M. Roy-de Vos, J.-P. Montseny, J.M. Mayer, P.-A. Carrupt, Y. Berger, B. Testa, Drug Metab. Dispos. 28 (2000) 1405–1410.
[9] K. Zhang, Z. Zhang, Q. Wang, R. Gao, CAN 138 (2002) 95716.
[10] European Pharmacopoeia, fourth ed., European Department for the Quality of Medicines, Strasbourg, France, 2002.
[11] United States Pharmacopeia, 24th ed., United States Pharmacopeial Convention, Rockville, MD, 2000.