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Pharmaceutical development and specification of stereoisomers

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Abstract

The pharmaceutical development of chiral drugs requires the activities of many different research and development groups. Guidelines which help to coordinate the activities of these groups and assist in the successful development of compounds with either single or multiple chiral centers are outlined and discussed.

Keywords: Pharmaceutical; Stereoisomers; Enantiomer; Chiral analysis; Specification

1. Introduction

1.1. Development of chiral drugs—a multidisciplinary problem

Although development of chiral drugs as single stereoisomers is the preferred approach, consideration must be given to the unwanted stereoisomers, which may be present as impurities or degradents in the drug substance and in the drug product or generated via metabolism in biological systems. Chiral impurities in pharmaceutical samples may occur as side-products of the synthetic process, as a result of inversion of chiral centers due to chemical degradation of the drug substance or both. Similarly, inversion of the chiral center may occur, in vivo, as a result of metabolism, chemical degradation or both. The issues involved in bringing a chiral compound successfully from discovery to the marketplace are complex and require a coordinated interdisciplinary approach.

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Much has been written on the analytical approaches to the separation and quantitation of chiral compounds [1,2] and on the specific pharmacology, metabolism and pharmacokinetics of chiral compounds [3,4], to the extent that the main scientific issues are well defined. In contrast the regulatory issues surrounding the development of chiral compounds are less well defined and the guidelines published by the various regulatory authorities around the world [5–8] are relatively general and leave much room for interpretation [9]. One notable exception is the recently published step 2 guideline [10] of the international conference on harmonization (ICH) entitled 'Specifications for New Drug Substances and Products: Chemical Substances' (Q6A) which provides guidance for single center chiral compounds with respect to assay, impurities and identification. However, little guidance is given by the ICH or any regulatory authority in regard to the development of chiral compounds with multiple chiral centers. The present contribution describes the multidisciplinary approach being developed at this company for the development of chiral compounds with single or multiple chiral centers.

1.2. Analytical methodology

Although considered outside the specific scope of this paper, consideration of the analytical methodologies required for the determination of chiral compounds in pharmaceutical and biological samples is a key component to the successful development of chiral drugs. Direct methods involving the separation and analysis of the stereoisomers are greatly preferred to indirect methods involving derivatization of the chiral analyte. Direct methods include chromatographic separations, such as chiral HPLC, and physical measurements such as optical rotation [11]. Chiral HPLC is currently the most widely used direct method for analysis of chiral impurities because of sufficient sensitivity, availability of a wide variety of commercial columns, and extensive applications literature [12,13]. Direct methods are preferred to chiral derivatization because of the need to carefully control the chiral purity of the derivatization reagent [11,14]. While not the method of choice, chiral derivatization may be more acceptable in bioanalysis where the need to measure low levels ($< 1\%$) of one enantiomer in the presence of an excess of the other may be less important. Also, chiral derivatization has the potential added advantage of introducing enhanced detection via the use of a fluorescent label in the derivatization reagent [15].

1.3. Nomenclature

Three classes of chiral compounds are discussed in this paper (a) single stereoisomer drugs containing one chiral center, (b) single stereoisomer drugs containing two chiral centers and (c) single stereoisomers containing more than two chiral centers. The development of racemic mixtures of chiral compounds is not currently favored within the pharmaceutical industry [16] and is not considered in any detail within this paper. The discussion of each class of single stereoisomer drug is arranged in terms of the essential activities necessary for the effective development of stereoisomers according to the phase of research and development. Pharmacology and medicinal chemistry are involved in early drug discovery activities. Analytical R&D (AR&D) is responsible for purity, stability testing, and specifications of the drug substance and product. Chemical process research and development (CPR&D) is responsible for development and manufacture of the drug substance. Drug metabolism and pharmacokinetics (ADME) and safety assessment (SA) are responsible for the metabolism and safety testing, respectively, after a drug is nominated for development. Successful and timely development of stereoisomers requires careful coordination of the activities of all of these groups.

Chiral compounds with one chiral center can exist in two enantiomeric forms, both of which have the same chemical and physical properties in an achiral environment but frequently have different biological activities [11]. The unwanted enantiomer is an impurity which usually needs to be quantitated down to levels as low as 0.1% in the drug substance. For multicenter chiral compounds the number of potential stereoisomers that can occur in a given sample increases expo-

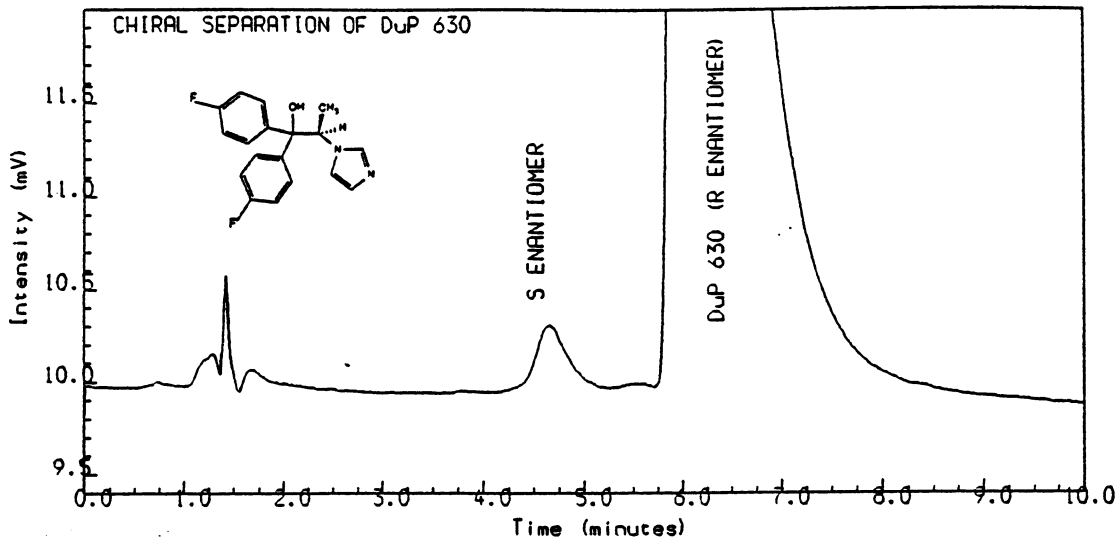

Fig. 1. Separation of drug substance with single chiral center. Chiral separation conditions: chiral AGP column 10 × 0.46 cm; mobile phase of 80% 20 mM NaH_2PO_4 pH 7 and 20% isopropanol (v/v); 0.9 ml min^{-1}; column temp of 30°C; detection in UV at 230 nm; 2 microgram sample of DuP 630.

nentially such that for a compound with n chiral centers 2^n stereoisomers are possible. Each stereoisomer will have one enantiomer with identical chemical properties and (except for meso compounds) 2^n-2 diastereoisomers which can have different chemical properties [17].

2. Compounds with a single chiral center

Guidance on the development of these compounds is relatively general but there is agreement by ICH and regulatory authorities that a chiral method should be used, if possible, to analyze for the amount of enantiomer in drug substance and to establish the chiral stability of the drug substance, the drug product and to investigate for possible in vivo inversion in metabolism samples. An example of a chiral separation of a single center compound is shown in Fig. 1 where a single stereoisomer chiral compound (DuP 630) is separated from its enantiomer which is present as a low level impurity in the drug substance. Chiral methods need to have sufficient sensitivity to analyze for low levels (0.1%) of the enantiomer in the drug substance and sufficient resolution to separate the enantiomer from other impurities. The studies necessary for the discovery and development of single center stereoisomers and the different organizations responsible for these activities are discussed below.

2.1. Discovery phase up to drug candidate nomination

Screening efforts in pharmacology should include identification of the preferred enantiomer for development taking into account the preliminary assessment of efficacy and safety. A single enantiomer should be taken forward unless there are significant technical or financial reasons for developing the racemate. Lead candidates should be screened in aqueous solutions at relevant pH and ionic concentration and in relevant in vitro biological media (enzyme preparations, tissue culture media etc.) to determine the extent of racemization. All aspects of the discovery studies on chiral inversion as well as details of the analytical methods used should be communicated in a timely fashion to the appropriate development groups (AR&D, CPR&D, and ADME).

Once a development candidate has been identified, sufficient quantities (typically 100 mg) of each enantiomer should be prepared by medicinal chemistry for use as analytical standards and, if inversion was seen in vitro, for use by ADME in a single dose pharmacokinetic study using an appropriate animal model. (If synthesis of the unwanted enantiomer is not practical at this stage, a sample of the racemate may be used instead). If the single-dose pharmacokinetic study shows rapid inversion of the drug in vivo, then the decision to develop the drug as a single isomer should be re-evaluated. All aspects of the studies dealing with the issue of candidate selection and chiral inversion, in vitro and in vivo, should be reported by pharmacology and ADME when the discovery compound is taken forward into development.

2.2. Activities between nomination and submission of an investigational new drug application (or equivalent application)

Prior to the filing of an application to conduct phase I clinical investigations, analytical methods will be developed by AR&D for the determination of the chiral purity of the drug substance as well as for the determination of the chiral stability of the drug in the solid state and in dosing forms used in safety assessment. Preliminary information on the chiral identification test, the chiral assay and the stability of the chiral center of the drug should be included in the IND application. Safety and ADME studies should be conducted with the chosen stereoisomer. If inversion of the chiral center was observed in the early pre-nomination studies, then ADME should determine the extent of exposure to the unwanted enantiomer in the safety studies.

2.3. Activities during phase I and phase II clinical trials

During phase I ADME will conduct pharmacokinetic studies in humans to determine if inversion at the chiral center of the selected enantiomer occurs due to metabolism. A special clinical study is not necessary to achieve this objective because representative plasma extracts, selected at appropriate time points from the regular phase I studies, may be used for this purpose.

During phase I and phase II, analytical methods will be developed by AR&D to determine the chiral stability of the chiral center in the drug product by monitoring the levels of unwanted enantiomer. CPR&D will prepare sufficient quantities of each enantiomer for use as analytical standards.

By the end of phase II AR&D will develop chiral specifications for the drug substance involving identity tests for the specified enantiomer and chiral assays/impurity tests capable of quantifying both enantiomers. Optical rotation is acceptable as an identity test for the selected enantiomer provided it has a sufficiently high optical rotation. Alternatively, the chiral assay and the identity test may be combined into a single test method. Specifications for the levels of the unwanted enantiomer should be justified on the basis of the levels qualified in the clinic and appropriate animal models. The ICH guideline entitled 'Specifications for New Drug Substances and Products: Chemical Substances' (Q6A) indicates that if it is technically difficult to develop chiral methodology for the final drug substance then suitable controls should be imposed during the synthesis to assure chiral purity [10]. Furthermore Q6A stipulates that there is no need for a chiral assay/impurity test for the drug product if the chiral purity is controlled in the drug substance and there is no racemization on storage of the drug substance or during manufacture and storage of the drug product [10]. However, there is need for a chiral identity test in the manufacture of the drug product as discussed below in Section 2.4.

2.4. Activities during phase III leading to new drug application (or equivalent) submission

During this period AR&D should finalize and validate the chiral method for drug substance set limits in the final specifications for the level of allowed in the drug substance confirm the chiral stability of the drug substance in the final formulation and finalize and validate a chiral identity test (optical rotation test may be acceptable) for

the drug product. This identity test [6,10] is done at the site of drug product manufacture on the lots of drug substance used to manufacture commercial lots of drug product. This test is necessary if the drug substance is made at a different site than the drug product and even if the drug substance is patented and not available from another source.

3. Compounds with two chiral centers

A single stereoisomeric drug (e.g. *SR* configuration) of this class may contain three chiral impurities; two diastereoisomers (e.g. *SS* and *RR*) and an enantiomer (e.g. *RS*). Some two center chiral compounds (e.g. polypeptide candidates) will require chiral methods to separate both diastereoisomers and enantiomer from the drug and activities in discovery and development will be similar to those for a single center chiral compound. For other compounds achiral methods can resolve the diastereoisomers from the drug (since diastereoisomers can have different physical and chemical properties) and the development process is simplified since achiral methods are preferred for routine testing. However, the diastereoisomeric pair are mirror images and cannot be separated one from the other by achiral methods. An example of this is shown in Fig. 2 where an achiral HPLC method can separate the diastereoisomeric pair (*RR* and *SS*) from the DMP 777 (*SR*) but a chiral method is needed to separate the *RR* from the *SS* as well as the *RS* enantiomer from the drug. For this reason a chiral method will usually be needed early in the development phase of a two chiral center compound in order to separate and measure the individual diastereoisomers and enantiomer.

3.1. Discovery phase up to drug candidate nomination

Screening activities in pharmacology are similar to those outlined in Section 2.1. Once the development candidate is identified Medicinal Chemistry should prepare sufficient quantities (typically 50 mg) of each diastereoisomer and enantiomer for use as analytical standards unless good reason for preparing fewer standards are known and can be justified. If inversion is seen in vitro, further testing should be done by ADME with a single dose pharmacokinetic study as outlined in Section 2.1. All aspects of studies dealing with candidate selection and chiral inversion should be addressed by pharmacology and ADME in reports when the discovery compound is taken forward into development.

3.2. Activities between nomination and investigational new drug (or equivalent) application

AR&D should determine if diastereoisomers can be separated from the stereoisomer drug and analyzed by achiral methods. If so, then development is simplified since achiral methods are usually preferred. However a chiral method may need to be developed to monitor the levels of individual diastereoisomers and enantiomer. The chiral purity, solid state chiral stability and safety assessment dosing form stability should be determined by AR&D as outlined in Section 2.2.

3.3. Activities in phase I and phase II clinical trials

AR&D should determine the chiral stability of the chiral centers in the drug product by testing for increase in concentration of diastereoisomers and enantiomer. ADME should test representative clinical samples to determine if significant inversion to a diastereoisomer occurs in vivo. If inversion occurs further chiral studies may need to be done by ADME. (It should be noted that high speed LC/MS methods now being utilized in metabolism studies probably will not have sufficient resolving power to separate diastereoisomers from the drug substance; slower achiral or chiral methods may be needed to confirm in vivo chiral stability in these studies). AR&D and SA should set specifications for impurity levels of diastereoisomers and enantiomer only if they are significant ($>0.1\%$) im-

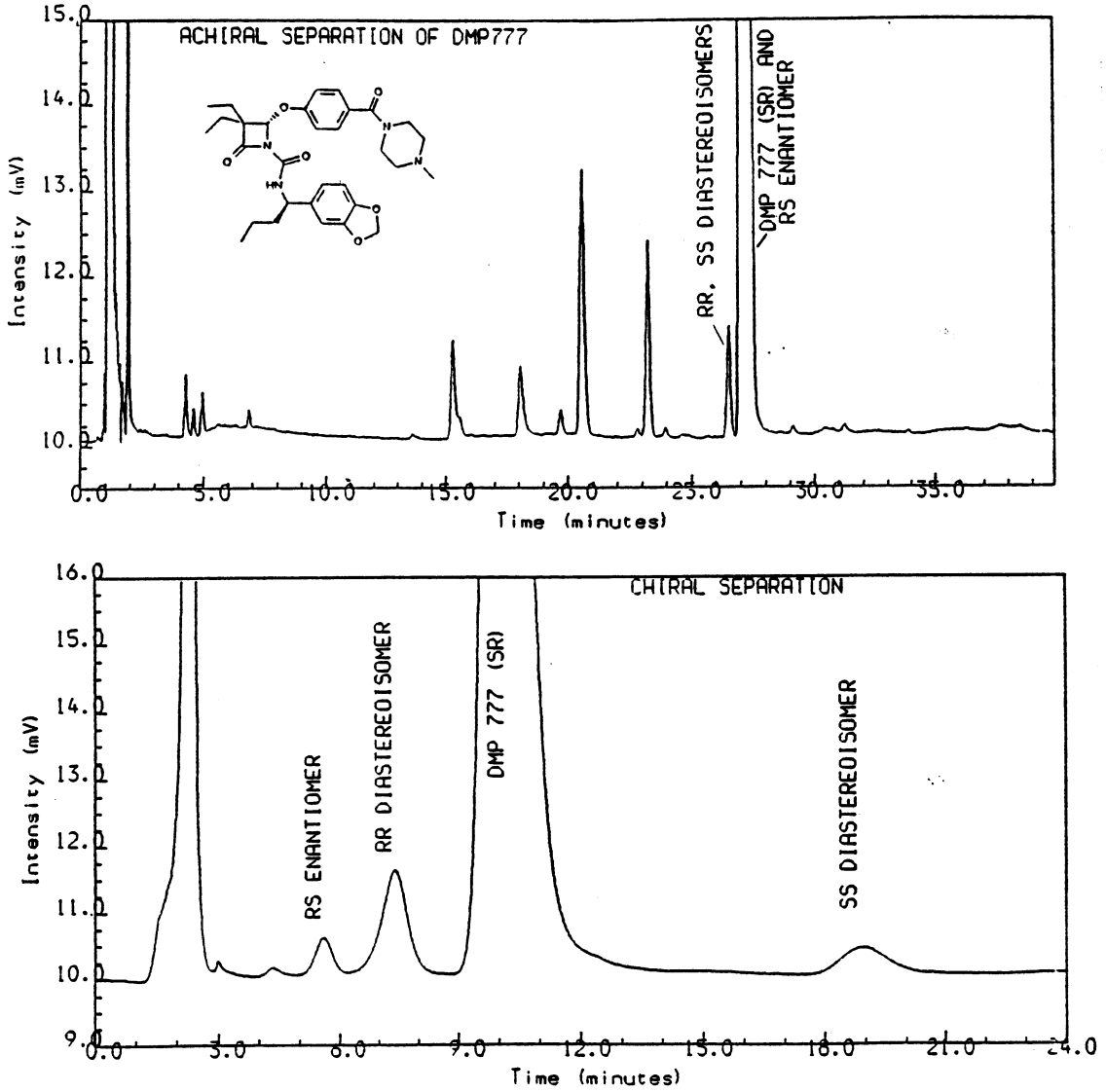

Fig. 2. Separation of drug substance with two chiral centers. Achiral separation conditions: symmetry C18 column 25 × 0.46 cm; mobile phase A of 35% acetonitrile and 65% 10 mM sodium methanesulfonate pH 6.3 with 2 mM sodium octanesulfonate; mobile phase B of 65% acetonitrile and 35% 10 mM sodium methanesulfonate pH 6.3 with 2 mM sodium octanesulfonate; linear gradient from A to B in 35 min; flow rate of 1.5 ml min^{-1}; column temp of 50°C; detection in UV at 250 nm; 2 microgram sample of DMP 777. Chiral separation conditions: Chiracel OD column 25 × 0.46 cm; mobile phase of 70% hexane and 30% isopropanol; flow rate of 1.5 ml min^{-1}; column temp of 30°C; detection in UV at 250 nm; 5 microgram sample of DMP 777.

purities. However, the Q6A ICH guidelines suggest that technical limitations could preclude using the same limits of determination or qualification for chiral impurities as used for achiral impurities [10].

3.4. Activities during phase III and new drug application (or equivalent) submission

The chiral stability of drug substance in the final formulation should be confirmed by AR&D.

If a chiral method is necessary to measure drug substance purity it should be finalized and validated by AR&D. The final limits for the level of diastereosiomer and enantiomer should be set only if they are significant ($>0.1\%$ see Section 3.3) impurities or degradants.

4. Compounds with more than two chiral centers

A single stereoisomer of this class could have 2^n–1 other stereoisomers which could be potential impurities. One of these is the enantiomer (mirror image) and the other diastereoisomers are impurities which can usually be separated from the drug substance by achiral methods. These compounds are developed as achiral candidates. However, some multicenter chiral compounds (e.g. polypeptide drug candidates) may require chiral methods to separate and analyze the diastereoisomer impurities in the drug substance. This should be determined early in the development process. Since there are such a large number of potential stereoisomers (8 with a 3 chiral center compound, 16 with 4 centers, etc), it is realistic to separate and analyze only the most likely impurities. Since the chance of chiral inversion at two chiral centers is small the most likely chiral impurities would be those stereoisomers that have inversion at only one chiral center. As an example, if *RRR* is the selected stereoisomer then the *SRR*, *RSR*, and *RRS* stereoisomers should be considered as potential impurities. However, it may be possible (depending on the synthesis) that other diastereoisomers or even the enantiomer could be the most likely chiral impurities and this should be considered before preparing impurity standards.

4.1. Discovery phase up to drug candidate nomination

Screening activities in pharmacology are similar to those outlined in Section 2.2. Once the candidate is identified, then medicinal chemistry should prepare sufficient quantities (50 mg) of stereoisomers with inversion at one chiral center for use as analytical standards (unless good reason for preparing other chiral impurity standards is known and can be justified). If racemization is observed in the in vitro screens then the stereoisomer should be tested by ADME in a single dose pharmacokinetic study as outlined in Section 2.2. All aspects of studies on candidate selection and chiral inversion should be addressed by pharmacology and ADME in the reports when a discovery compound is nominated for development.

4.2. Activities between nomination and investigational new drug application (or equivalent)

AR&D should determine if diastereoisomers can be separated from the drug substance and analyzed by achiral methods. If so, the drug should be developed as an achiral candidate and development will not be discussed further in these guidelines. If not, chiral methods will need to be developed to monitor purity, stability in solid state, and SA dosing solutions; safety and ADME studies should be conducted with the intended stereoisomer.

4.3. Activities during phase I and phase II

AR&D should determine the chiral stability of the drug substance in the drug product using appropriate methods. Pharmacokinetic studies with representative clinical samples should be done by ADME as outlined in Section 2.3. (As noted in Section 3.3, the high speed LC/MS assays being introduced in metabolism studies may not have sufficient resolving power to separate diastereosiomers from drug substances and slower achiral or chiral methods may be needed to confirm chiral stability during metabolism). Specification for diastereoisomers should be set by AR&D and SA if they are significant impurities (see Section 3.3).

4.4. Activities during phase III and new drug application (or equivalent)

The chiral stability of a drug substance in the final formulation should be confirmed by AR&D. The limits for diastereoisomer and enantiomer

should be set only if they are significant impurities (see Section 3.3).

5. Conclusions

Guidelines on the development of chiral compounds that are published by regulatory authorities around the world are relatively general and leave room for interpretation. At the same time the issues involved in chiral drug development are complex and a coordinated approach among the many research and development groups is necessary. The multidisciplinary approach outlined here will serve as a guide to the development of chiral compounds allowing the coordination of research efforts in the various phases of development.

The recently published step 2 guidelines titled ' Specification on New Drug Substances and Products' (Q6A) provides helpful guidance for the development and specification of compounds with one chiral center. Chiral methods are needed to measure chiral purity, chiral stability of a drug substance and product and chiral stability in metabolism. The specification of chiral purity is necessary. However, little direction is given by any regulatory authority regarding development of compounds with multiple chiral centers. Achiral methods can be used to separate and analyze the diastereoiomeric impurities in many types of multichiral center drug candidates and their development is similar to that of achiral drugs. However, chiral methods may be required for separating and analyzing diastereoisomers in some multicenter chiral compounds and development activities of these candidates have some significant differences from those of single chiral center compounds. Chiral methods are also needed to separate and analyze the individual diastereoisomers in compounds with two chiral centers since these diastereoisomers are enantiotopic. Development activities of chiral compounds are complicated and chiral development guidelines can coordinate the activities that are necessary to successfully bring these different types of chiral compounds from discovery to commercialization.

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References

[1] S.G. Allenmark, Chromatographic Enantioseparation: Methods and Applications, 2, Ellis Horwood, Chichester, 1991.
[2] G. Gubitz, Chromatographia 30 (1990) 555–564.
[3] D.E. Drayer, Clin. Pharmacol. Ther. 40 (1986) 125–133.
[4] F. Jamali, R. Mechvar, F.M. Passuto, J. Pharm. Sci. 78 (1989) 695–715.
[5] FDA Policy Statement for the Development of new Stereoisomer Drugs, Chirality 4, (1992) 3238–340.
[6] W.H. Decamp, J. Pharm. Biomed. Anal. 11 (1993) 1167–1173.
[7] A.G. Rauws, K. Groen, Chirality 6 (1994) 72–75.
[8] M. Gros, A. Cartwright, B. CampbelL, R. Bolton, K. Holmes, K. Kirkland, T. Salmonson, J. Robert, Drug Inf. J. 27 (1993) 453–457.
[9] W.L. Heydorn, Pharm. News 2 (1995) 19–21.
[10] ICH Q6A Step 2 Document, Specification for New Drug Substances and Products: Chemical Substances, Fourth International Conference on Harmonization, Brussels, 1992
[11] A.C. Mebta, J. Chromatogr. 426 (1988) 1–13.
[12] D.R Taylor, K Maher, J. Chromatogr. Sci. 30 (1992) 67–85.
[13] J. Liu, J.T. Stewart, E. Ameyibor, Pharmacopeial Forum 23 (1997) 3908–3916.
[14] C. Pettersson, A. Karlsson, C. Gioela, J. Chromatogr. 426 (1987) 217–229.
[15] S. Einarsson, B. Josefsson, P. Moller, D. Sanchez, Anal. Chem. 59 (1987) 1191–1195.
[16] S. Stinson, Chem. Eng. News 72 (38) (1992) 46–79.
[17] A.L. Ternay, Contemporary Organic Chemistry, Saunders, Philadelphia, PA, 1979.