Review Article

# Design and Development of Stability Indicating Assay Methods as per ICH Guidelines – A Review

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**ABSTRACT**

Stability indicating assay methods helps in detection of degradants in presence of active ingredients formed during storage and thus helps in establishing of storage conditions of the drug. Quantitative determination of these impurities could be used as method for the quality control and validation of drug substances. Regulatory authorities such as US-FDA, COMP, TOA and MCA insists on the impurity profiling of drugs. It is essential to know the structure of impurities in the bulk drug sample to alter the reaction condition and to reduce the quantity of impurity to an acceptable level. Isolation and quantification of impurities helps to obtain pure substance with less toxicity and safe drug therapy. For newly synthesized drug substances the specification should include acceptance criteria for impurities. Stability studies can predict those impurities likely to occur in the commercial product. This review mainly focus on the design and development of stability indicating assay method with emphasis on forced degradation studies which is critical step in stability indicating method development. Forced degradation is essential step for regulatory requirement.

**Keywords:** Stability indicating assay methods, Forced degradation, Storage.

## INTRODUCTION

Stability indicating assay method may be defined as a procedure that affords the selective determination of a drug substance in the presence of its decomposition and reaction products.[1,2] **Specific stability-indicating assay method**:Specific stability-indicating assay method (Specific SIAM)'can be defined as 'a method that is able to measure equivocally the drug(s) in the presence of all degradation products, excipients and additives present in the formulation[3]

**Selective stability-indicating assay method**: 'Selective stability-indicating assay method (Selective SIAM)' was defined as 'a method that is able to measure unequivocally the drug(s) and all degradation products in the presence of excipients and additives, expected to be present in the formulation.[3]

**Objectives**[4]

- To establish shelf life and storage conditions of API.
- To provide evidence on how much quality of API varies with time under influence of various environmental factors such as temperature, humidity and light.
- To study chemical properties of molecules and to establish degradation pathways of drug substances and drug products.
- To generate stable formulations by solving stability relating problems.

**Table 1: GUIDANCE DOCUMENTS FOR STRESS TESTING**

| Standard | Title and reference |
|---|---|
| ICH Q1A(R2) [5] | Stability Testing of New Drug Substances and Products |
| ICH Q1B [6] | Photo stability Testing of New Drug Substances and Products |
| ICH Q2B [7] | Validation of Analytical Procedures: Methodology |
| ICH Q3A(R)[8] | Impurities in New Drug Substances |
| ICH Q3B(R)[9] | Impurities in New Drug Products |
| ICHQ6A[10] | Requirements for stability indication assays under universal tests/criteria for both drug substance sand drug products. |
| FDA Guidance [11] | Submitting Documentation for the Stability of Human Drugs and biological |
| FDA Guideline [12] | Submitting Samples and Analytical Data for Methods Validation |
| FDA Reviewer Guidance [13] | Validation of Chromatographic Methods |
| FDA Guidance [14] | Stability testing of Drug Substances and Drug Products |
| FDA Guidance [15] | Analytical Procedures and Methods Validation. |
| FDA Guidance[16] | INDs for Phase 2 and Phase 3 Studies, Chemistry, Manufacturing, and Control Information. |

**STEPS INVOLDED IN DEVELOPMENT OFVALIDATED STABILITY INDICATING ASSAY METHODS[17]**

**STEP1:** Critical study of the drug structure toassess the likely decomposition routes.
**STEP2:** Collection of information on physico chemical properties.
**STEP3:** Stress (forceddecomposition) studies
**STEP4:** Preliminary separation studies on stressed samples
**STEP5:** Final method development and optimization
**STEP6:** Identification and characterization of degradation products and preparation of standards
**STEP7:** Validation of SIAM s

**1. Critical study of the drug structure to assess the likely decomposition routes[18,19]**

Information on degradation can be easily gained from the structure by the study of functional groups and other key components.

- ✓ Amides, Esters, lactams, lactones – undergo hydrolysis.
- ✓ Thiols, thioethers – undergo oxidation.
- ✓ N-oxides –photodecomposition.

For congener type of drugs the degradation chemistry is based on the reported behavior of other drugs.
Ex: More than 40 penicillin's are in clinical practices which have almost same degradation behavior at beta lactam moiety.
Sometimes congeners might show existence of new degraded products particularly upon influence of the substitutes.
Ex: Amino penicillin show formation of polymers.

**2. Collection of information on physico chemical properties[20]**

Various physico chemical parameters such as $p^{ka,}$ log P, Solubility, absorptivity and absorption maxima of drug are to be studied.
IMPORTANCE OF VARIOUS PARAMETERS:

**$P^{ka}$** - $p^H$ related changes in retention occur at $p^H$ values within 1.5 units of $p^{ka}$ values.

**Ionization values**- helps in selecting $p^H$ of buffer to be used in mobile phase.

**Log P**-identification of log p values for drug and degraded products provides much information about the separation behavior likely to be obtained in particular stationary phase.

**Solubility data**- if solubility profile of drug in aqueous and organic solvents is known it will be useful in selection of sample solvents and mobile phase in HPLC.

**Absorption maxima**- HPLC and UV detector is widely used absorption maxima should be known due to extinction of drug and degradation products in different solvents at different $p^H$.3.**3.**

**Stress (forced decomposition) studies**

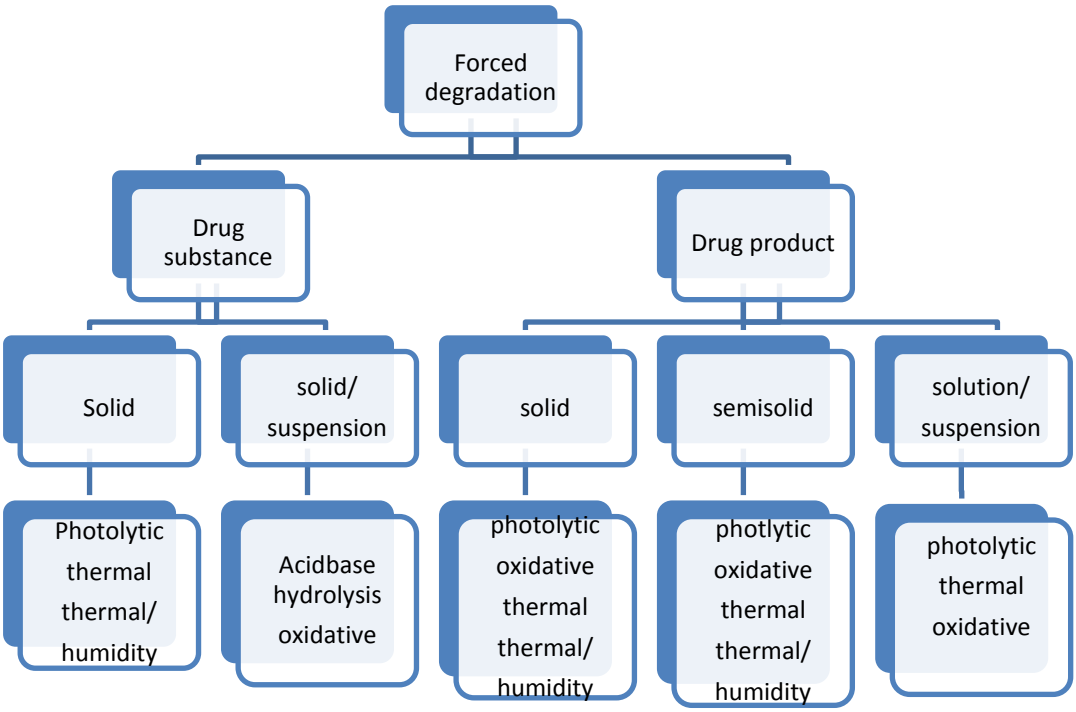


**Fig. 1**

**Objective of forced degradation studies**[20]

Forced degradation studies are carried out to achieve the following purposes:

- To produce the possible degradants by subjecting to various conditions of temperature and humidity.
- By forced degradation studies, degradation mechanisms of drug substance or drug product was revealed.
- By structural elucidation of degradants we can differentiate whether the degradants are formed from API or excipients.

**Table 2: CONDITIONS GENERALLY EMPLOYED FOR FORCED DEGRADATION**[21]

| DEGRADATION TYPE | EXPERIMENTAL CONDITION | STORAGE CONDITION | SAMPLING TIME |
|---|---|---|---|
| | CONTROL API(no acid or base) | $40^0$C ,$60^0$ C | 1,3,5 days |
| HYDROLYSIS | 0.1 N NaOH<br>Acid control(no API)<br>Base control(no API)<br>pH:2,4,6,8<br>3%H2O2 | $40^0$ C,60 $^0$C<br>$40^0$ C,$60^0$ C<br>$40^0$ C,$60^0$ C<br>40 $^0$C,$60^0$ C<br>25 $^0$C | 1,3,5 days<br>1,3,5 days<br>1,3,5 days<br>1,3,5 days<br>1,3,5 days |
| OXIDATION | Peroxide control<br>Azobisisobutyronitrile(AIBN)<br>AIBN control | 25 $^0$C,60 $^0$C<br>$40^0$ C,60 $^0$C<br>$40^0$ C,60 $^0$C | 1,3,5 days<br>1,3,5 days<br>1,3,5 days |
| PHOTOLYTIC | Light, 1X ICH<br>Light,3X ICH<br>Light Control | NA<br>NA<br>NA | 1,3,5 days<br>1,3,5 days<br>1,3,5 days |
| THERMAL | Heat chamber<br>Heat chamber<br>Heat chamber<br>Heat control | $60^0$ C<br>$60^0$ C/75% RH<br>$80^0$ C<br>$80^0$ C/75% RH<br>Room Temp | 1,3,5 days<br>1,3,5 days<br>1,3,5 days |

**Hydrolytic conditions**[20]

- Hydrolysis is a chemical process that includes decomposition of chemical compound by reaction with water.
- Hydrolytic study was done under acidic or basic conditions with which involves catalysis of ionisable functional groups in molecule.
- The drug was exposed to various acidic or basic conditions which generates primary degradants.
- Generally hydrochloric acid or sulphuric acid (0.1-1M) was used for acid hydrolysis and sodium hydroxide and potassium hydroxide (0.1-1M) was used for basic hydrolysis and selection of concentration depends upon stability of drug.
- Solvents used to dissolve HCl or NaOH was based on nature of stress testing sample.
- Hydrolytic stress testing was carried out not more 7 days.

**Oxidation conditions**[20]

- Oxidative degradation involves an electron transfer mechanism to form reactive anions and cations.
- Oxidizing agents such as hydrogen peroxide (widely used), metal ions, oxygen and radical initiators (azobisisobutyronitrile,AIBN) are used.
- 0.1-3% hydrogen peroxide at neutral PH at room temperature for 7days causes 20% degradation.
- Oxidizing agents and its concentrations was based on drug substances.

**Photolytic conditions**[20]

- This type of degradation was done to demonstrate light exposure doesn't result in unacceptable change
- Exposure to uv or fluorescent conditions generates primary degradants
- Wavelength of light in range of 300-800nm causes photolytic degradation.
- Maximum illumination of 6 lxh, light stress conditions can induce photo oxidation by free radical mechanism.

**Thermal conditions**[20]

- Thermal degradation was carried out at strenuous conditions
- Drug products of solid state were subjected to dry and wet heat while liquid drug products are subjected to dry heat.
- Effect of temperature on thermal degradation of a substance is studied by using Arrhenius equation:

$$k = Ae^{-Ea/RT}$$

Where;
k=specific reaction rate,
A = frequency factor,
Ea = energy of activation,
R = gas constant (1.987cal/degmole) and
T = absolute temperature.Thermal degradation study is carried out at 40–80$^0$C.

**Humidity**[20]

- Humidity is the key factor for establishing the potential degradants.
- 90% Humidity for duration of one week causes degradation of samples.

**Table 3: STABILITY TESTING CONDUCTED ACCORDING TO ICH GUIDELINES ARE AS FOLLOWS**[22]

| Type of product | Study | Storage condition | Minimum time period covered |
|---|---|---|---|
| General case | Long term | 25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH | 12 months |
| | Intermediate | 30°C ± 2°C/65% RH ± 5% RH | 6 months |
| | Accelerated | 40°C ± 2°C/75% RH ± 5% RH | 6 months |
| Drug substances storage in refrigerator | Long term | 5°C ± 3°C | 12 months |
| | Accelerated | 25°C ± 2°C/60% RH ± 5% RH | 6 months |
| Drug substances storage in freezer | Long term | - 20°C ± 5°C | 12 months |
| Drug product packed on semipermeable containers | Long term | 25°C ± 2°C/40% RH ± 5% RH or 30°C ± 2°C/35% RH ± 5% RH | 12 months |
| | Intermediate | 30°C ± 2°C/65% RH ± 5% RH | 6 months |
| | Accelerated | 40°C ± 2°C/not more than (NMT) 25% RH | 6 months |
| Drug product intended for storage in refrigerator | Long term | 5°C ± 3°C | 12 months |
| | Accelerated | 25°C ± 2°C/60% RH ± 5% RH | 6 months |
| Drug product intended for storage in freezer | Long term | - 20°C ± 5°C | 12 months |

**APPROPRIATE TIMING**[10,11]

**FDA** guidance states that stress testing should be performed in phase 3 of regulatory submissionprocess. Stress testing in early stages such as preclinical phase or phase 1 of clinical trials is highly encouraged and was conducted on drug substance which helps in identification of degraded products and structural elucidation and optimizing stress conditions and this helps in improving of manufacturing process and proper selection of stability indicating analytical procedures.

4. **Preliminary separation studies on stressed samples**[17]

The stress samples obtained are analyzed to study the number and types of degradation products formed under various conditions.

- Generally RP-HPLC octadecyl column, was generally used. Water- methanol or water-acetonitrile are used as mobile phases in initial stages. Buffer free mobile phases are used in preparative LC or LC-MS studies.
- The solvents are changed if the peak shape or separation problems are found. Degradation products formed are generally polar in nature, pushing the drug peak up to 15 min or more in 25cm column helps in separation of even more degradation products when formed.
- The change in retention time can be observed by changing the mobile phase but should not be pushed very far, due to increase in the resolution but oppositely peak flattens resulting in the decrease in sensitivity. The results should be compared with blank solutions and in this case fall in drug peak is quantitatively followed by a corresponding rise in degradation products peak.
- Sometimes drug fall is clearly seen but no additional rise in peak this is due to the formation of non-chromophoric groups or due to the decomposition into low molecular weight fractions. In such cases detection at multiple wavelengths or LC-MS can be used.
- Sometimes total absence of the simultaneous rise in drug peak is seen which is due to the insolubility of the products in the reaction solution which is confirmed by physical examination of the reaction mixture.in such case product was separated and was injected separately using the solvent in which it is soluble and retention time was determined.
- Sometimes absence of degradation peak can be seen when the product formed is colored and shows no UV-absorption at particular wavelengths at which analysis was conducted. This can be verified by simple observation whether color was developed in the reaction solution. Wavelength of analysis can be adjusted.

**5. Final method development and optimization**[17]

- By preliminary chromatographic analysis RT (retention time) and RRT (relative retention time) was determined.
- The components which has close RT or RRT should be carefully examined. PDA spectra or LC-MS profile of the product should be critically evaluated.
- They give the information that whether the components obtained are same or different .PDA spectra and LC-MS results suggest that any of products which are co eluting then the chromatographic studies should be modified to achieve satisfactory resolution.
- In final study reaction solution mixtures is prepared and subjected for resolution behavior study. Resolution in mixture was closely studied to see whether the resolution is similar to that of obtained from individual samples.
- To separate the co eluting peaks the method was optimized by changing mobile phase ratio, pH, gradient flow rate, temperature, solvent type and column and its types.

**6. Identification and characterization of degradation products and preparation of standards**[17]

The drug degradation products were identified and arranged for their standards. By these specificity /selectivity of the method can be established.

Generally to identify the formed products, they are isolated and then their structure was determined through spectral and elemental analysis but this approach was tedious and time consuming when the degradants are produced.

- Modern approach was using hyphenated LC techniques coupled with MS. This also includes instrumental approach such as analytical HPLC, UV detection, full scan MS and tandem mass spectroscopy (LC-MS-MS) and gives idea about identification of resolving components.
- Integrated approach is becoming popular LC-MS or LC-MS-MS is employed to obtain molecular weight and fragmentation information and detailed information is by LC-NMR analysis. This type of integrated approach proves rapid and unambiguous identification of several degradation products.

✓ For product standards, direct way is to procure from commercial sources. If not available commercially, they should be isolated from degradation reaction solutions or synthesized in the laboratory.

✓ The product is isolated by identifying reaction condition where it is formed selectively and if it crystallizes on completion of reaction it was recovered simply. Otherwise reaction mixture was lyophilized directly. The recovery can also be made by using the organic solvents and solvent was evaporated to recover the product.

✓ When no condition was identified where product is formed quantitatively into single product then the product was isolated from mixture by preparative TLC or preparative HPLC or selective solubility based extraction. Also techniques such as normal column chromatography, medium pressure LC, chromatotron, flash chromatography etc.

✓ If the products identity was already established through sophisticated LC-MS or LC-NMR studies, the molecules can be synthesized, characterized and the presence can be confirmed through spiking in the degraded samples. The synthetic route has advantages that it results in neat product than obtained from isolation.

## 7. Validation of SIAM s[17]

Validation of analytical methods was given under ICH guidelines Q2A, Q2B, FDA guidance and by USP. The validation of SIAM has two stages.

**1st stage**: During early development cycle when drug substances subjected to FDS and SIAM was established based on the drug degradation behavior main focus of validation at this stage is on establishment of specificity or selectivity followed by other parameters like accuracy, precision, range, linearity, robustness. The LOD and LOQ are also determined to establish mass balance. Mass balance is a process of adding the assay value and levels of degradation products to check how closely they add up to 100% of the initial value, with consideration on analytical error. The balance could not be achieved unless all degradation products are separated well. But mass balance is difficult to establish in situations such as

- Formation of multiple degradation products, which involves complex reaction pathways and drug excipient interactions
- Incomplete detection due to loss of UV chromophore or lack of universal detection.
- Loss of drug/ degradation products as volatiles
- Diffusive losses into or through containers
- Elution or resolution problems
- In appropriate or unknown response factors due to lack of standards
- Errors and variability in the drug content assay.[1,39]

**2nd stage**: SIAM developed is extended to formulation, the emphasis gets limited to just prove the pertinence of the established validate parameters in the presence of excipients and other formulation constituents. Here only the critical parameters like specificity, selectivity, accuracy, precision are revalidated.

If SIAM was developed directly for formulation without bulk drug involvement, then all validation parameters should be established.

The specificity or selectivity of SIAM can be established if degradation chemistry of drugs is known and standards of products were available. The main efforts involves development of method that separates the degraded components. Peak purity becomes crucial at this stage. Peak purity can be established by various techniques like PDA detection, dual wavelength ratio chromatography, second order derivative spectroscopy etc generally UV-HPLC detectors allow for simultaneous measurement of multiple

wavelengths which helps in peak purity testing during development of SIAM.

**Accuracy** can be determined by spiking the known amount of drug to either placeboes or the formulations and percent recovery of the drug was determined, better method is spiking the drug in a mixture of degraded solutions.

**Precision** is concerned no special requirements for stability indicating methods and the same procedure for normal assay methods can be applied.

**Linearity** for SIAM s should established initially in the range of 0-100% as drug may fall to very low concentrations during forced decomposition studies. Validation range for the degradation products during stability studies usually should vary from 0 to 20%.

## CONCLUSION

Stability indicating assay method is essential for regulatory requirement and also provides information regarding degradants and degradation pathways that would form during storage. Thus they help in pharmaceutical development in areas such as formulation development, manufacturing and packing in which knowledge of chemical behavior helps to improve drug product.

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## REFERENCES

1. W. F. Head, Jr.,J. Pharm. Sci., 50,1041( 1961).
2. G. J. Papariello, H. Letterman, and R. E. Huetteman,ibid., 53,663( 1964)
3. D.D. Hong, M. Shah, Development and Validation of HPLC stability-indicating assays, In: Drug Stability: Principles and Practices, J.T. Carstensen, C.T. Rhodes (Eds.) Marcel Dekker, New York, 2000, pp. 329–384
4. Sherawat R, Maithani M, Singh R,Regulatory aspects in development of stability indicating method: A review. Chromatographia 2010;72:1-6
5. ICH guidelines, Q1A(R2): Stability Testing of New Drug Substances and Products (revision2), International Conference on Harmonization
6. International Conference on Harmonization. Q1B: Photostability Testing of New Drug Substances and Products. US Department of Health and Human Services; Rockville, MD: 1997;62(95):27115–27122
7. International Conference on Harmonization. Q2(R1): Validation of Analytical Procedures: Text and Methodology. London, UK: European Medicines Agency; 1995;60(11260):27463–27467.
8. International Conference on Harmonization. Q3A (R2): Impurities in New Drug Substances. Published in the Federal Register on Jun 2008.
9. International Conference on Harmonization. Q3B (R2): Impurities in New Drug Products. London, UK: European Medicines Agency; 2003; 68(220):64628–64629.
10. International Conference on Harmonization. Q6A: Requirements for stability indication assays under universal tests/criteria for both drug substance sand drug products London, UK: European Medicines Agency; 1999; 64:44928.
11. Guidance for Industry: Submitting Documentation for the Stability of Human Drugs and Biologics (FDA, Rockville,MD, 1987).
12. Guideline for Submitting Samples and Analytical Data for Methods Validation (FDA, Rockville,MD, 1987).
13. Reviewer Guidance: Validation of Chromatographic Methods (FDA, Rockville,MD,1994).
14. Guidance for Industry (Draft): Stability Testing of Drug Substances and Drug products (FDA, Rockville,MD, 1998).
15. Guidance for Industry (Draft): CMC Content and Format, INDs for Phase 2 and 3 Studies of Drugs, Including Specified Therapeutic Biotechnology-Derived Products(FDA, Rockville,MD, 1999).
16. Draft Guidance for Industry on Analytical Procedures and Methods Validation:Chemistry,Manufacturing, and Controls Documentation (FDA, Rockville,MD, 2000).

17. M. Bakshi, S. Singh / J. Pharm. Biomed. Anal. 28 (2002) 1011–1040
18. K.A. Connors, G.L. Amidon, V.J. Stella (Eds.), Chemical Stability of Pharmaceuticals, Wiley, New York, 1986
19. K. Florey (Ed.), Analytical Profiles of Drug Substances, Academic Press, London L.R. Synder, J.J. Kirkland, J.L. Glajch (Eds.), Practical HPLC Method Development, Wiley, New York, 1997, p. 295.
20. Jitendra Kumar et al / International Journal of Advances in Pharmaceutics 2 (3) 2013
21. H. Brummer, How to approach a forced degradation study, Life Sci. Technol. Bull. 31(2011) 1–4.
22. ICH HARMONISED TRIPARTITE GUIDELINE STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS Q1A(R2)Current Step 4 version dated 6 February 2003
23. A. A.Kondritzer and P. Zvirblis, zbid.,46,531(1957).
24. P.Zvirblis, I.Socholitsky, and A. A.Kondritzer, J. Amer.
25. W. Lund and T. Waaler, Acta Chem. Scand., 22,3085( 1968).
26. E. Bjerkelund, F. Gram, and T. Waaler, Pharm. Acta Helu., 44,745( 1969)
27. I.G. Eisdorfer, J. G. Rosen, and W. C. Ellenbogen, J. PhurmSci., 50, 612(1961).
28. A.J. Khoury and L. J. Cali, ibid., 56,1485(1967)
29. T. Higuchi and C. D. Bias, J. Amer. Pharm. Ass., Sci. Ed.,
30. T.Higuchi, A. D. Marcus, and C. D. Bias, ibid., 43, 129
31. Zbid.,43, 135(1954).
32. T.Higuchi and A. D. Marcus, J. Amer.Pharm. Ass., Sci. Ed., 43, 530(1954).
33. E. R. Garrett, J. Pharm. Sci., 51,1036(1962).
34. Stability-Indicating Assay Methods forDrugs and Their Dosage Forms LESTERCHAFETZ
35. L. Chafetz, J. Phurm. Sci., 53,1162(1964).
36. L. Chafetz and R. E. Daly, ibid., 57, 1977(1968)
37. M. E. Auerbach, Ind. Eng. Chem., Anal. Ed., 15,492(1943).
38. D. 0.Singleton and G. M. Wells, J. Pharm. Pharmacol., 12, 1 7 1 T( 1960)
39. Available from http://www.cmcissues.com/Methods/ HPLC/mass–balance.htm.
40. M. Bakshi, S. Singh / J. Pharm. Biomed. Anal. 28 (2002) 1011–1040.