Bulletin of Pharmaceutical Research 2013;3(2):44-50
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

STABILITY INDICATING ASSAY OF ORLISTAT AND ITS DEGRADATION PRODUCTS BY HPLC

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Received: April 27, 2013 / Revised: May 11, 2013 / Accepted: May 12, 2013

A simple, selective, rapid, precise and economical RP-HPLC stability-indicating method has been developed and validated for the quantitative estimation of orlistat (API) and their degradation products. Chromatographic separation was accomplished using C18 column with mobile phase consisting of acetonitrile:0.1% formic acid (85:15, *v/v*), flow rate was 1.0 ml/min and the detection wavelength was 215 nm. The method was validated for linearity, accuracy, precision, specificity and robustness. The API was subjected to stress condition of acid decomposition (0.1 N HCl refluxed for 8 h at 80°C), alkali decomposition (0.1 N NaOH refluxed for 8 h at 80°C), neutral hydrolysis (Distilled water refluxed for 12 h at 80°C), oxidative decomposition (3% H_2O_2 for 24 h at RT), thermal decomposition (Drug at 100°C for 24 h), photolytic decomposition (70,000-80,000 lux at 7 days). Percentage of degraded products were 13.37, 9.23, 1.44 and 5.04 for acid, alkali, neutral hydrolysis and oxidative decomposition respectively. No degradation was observed in thermal and photolytic conditions. Forced degradation study showed that orlistat is labile in acid, alkali, neutral and oxidative conditions. It is stable to light and dry heat. No interference of degradation products was found at the retention time of principal peak. The assay can be recommended for analysis of the API and degradation products in stability samples. It may be applied to a routine analysis in industries.

Key words: Orlistat, RP-HPLC, Stability indicating assay, Degradation, Method validation.

INTRODUCTION

The ICH guideline Q1A on stability testing of new drug substances and products (ICH, 1993) emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods. The ICH guideline Q3B entitled 'Impurities in new drug products' emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products (ICH, 1996). The ICH guideline Q6A, which provides note for guidance on specifications (ICH, 1999), also mentions the requirement of stability-indicating assays under universal tests/criteria for both drug substances and drug products. The same is also a requirement in the guideline Q5C on stability testing of biotechnological/biological products (ICH, 1995) since there is no single assay or parameter that profiles the stability characteristics of such products. The standard conditions for photo stability testing are described in ICH Q1A (ICH, 2003). The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics, which need to be evaluated.

International conference on harmonization published its own list of important validation characterization for various procedures (Singh and Garg, 1999; ICH, 2000; Felinger, 1998). In literature, compilation of stability-indicating assays for various drugs is already published (Singh and Bakshi, 2000; Bakshi and Singh, 2002; Xu and Trissel, 1999) and other publications are available which provide general discussion on HPLC method development and validation, with emphasis on stability-indicating assays (Hong and Shah, 2000). Orlistat is (S)-2-Formylamino-4-methyl-pentanoic acid (S)-1-[[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]dodecyl ester (**Figure 1**).

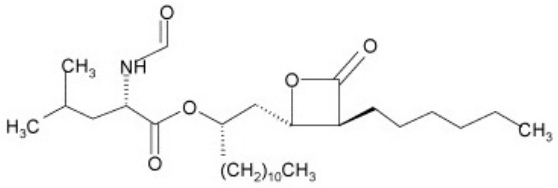

Fig. 1. Chemical structure of orlistat

Orlistat is gastrointestinal lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats but the amount of weight loss achieved with orlistat varies.

A number of methods for the determination of orlistat are reported in the literature (Bennett *et al* 1997; Hemant Kumar *et al* 2011) but there was no method based on selected mobile phase (acetonitrile:0.1% formic acid (85:15, *v/v*). In continuation of establishment of validated stability-indicating assay methods of drugs (Bakshi and Singh, 2004; Dunge *et al* 2005; Giannellini *et al* 2005; Rao *et al* 2009; Kaila *et al* 2010; Bhimavarapu *et al* 2011; Prashanth *et al* 2011), in the present study, an attempt was made to develop a rapid, economical, precise and accurate method for the stability-indicating assay of orlistat (API) and its degradation products by HPLC.

MATERIALS AND METHODS

Chemicals and reagents

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Orlistat was kindly supplied by W.S. Intas Pharmaceutical Ltd (India). Methanol (Merck Ltd, Rankem, RFCL Ltd), Formic acid (Hi Media, India), Sodium hydroxide, Hydrochloric acid, Hydrogen peroxide, Triple distilled water (In-House) was used throughout the experiment. Orlistat tablet formulation (Obelit - 120 mg) was purchased from the local market.

Instrumentation

HPLC analysis were performed on YoungLin system equipped with quaternary SP930D gradient pump, a vacuum degasser and mixer, an UV730D UV/Vis detector and a rheodyne injector holding 20 μl loop. The signals were acquired and analyzed using Windows XP based YoungLin Autochro-3000 software.

Selection of separation variables

Considering the theoretical information and after several trials, separation variables were selected which were constant during whole experiment (**Table 1**).

Table 1. Selection of separation variables

Variable	Condition
Column	Nucleosil
Dimension	250 mm × 4.60 mm
Particle size	5 μ
Bonded phase	Octadecylsilane (C_{18})
Mobile phase	
Acetonitrile:0.1% HCOOH	(85:15, *v/v*)
Flow rate	1.0 ml/min
Temperature	Ambient
Sample size	20 μl
Diluents	Mobile phase
Detection wavelength	215 nm

Mobile phase selection and optimization

Initially several exploratory runs given to estimate orlistat, a number of mobile phase in different ratio were tried and the mobile phase found to be most suitable for analysis was acetonitrile:0.1% formic acid (85:15, *v/v*) taking into consideration the system suitability parameter like resolution, tailing factor, number of theoretical plates. The mobile phase was filtered through a 0.45μ filter to remove particulate matters and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of stock solutions

Accurately weighed 50 mg Orlistat was transferred into 50 ml volumetric flask and dissolved in mobile phase, then volume was made up to 50 ml with mobile phase to get a concentration of 1000 μg/ml (Stock-A). Six

milliliter of stock-A was taken in 25 ml volumetric and diluted up to 25 ml to get concentration of 240 μg/ml (Stock-B). Finally from stock-B solution different of, 12 24, 36, 48, and 60 μg/ml were prepared and used for analysis. Linearity was observed by the linear regression equation (**Figure 2**) and correlation coefficient was found to be 0.9997.

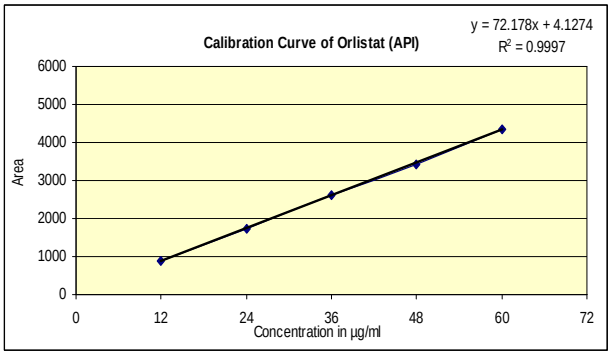

Fig. 2. Calibration curve of orlistat

Chromatographic conditions

Before delivering into the system, mobile phases were filtered through 0.45 μm filter and degassed using vacuum. The chromatographic conditions used for the analysis were given below. The separation of the compound was made on a nucleosil-C18 column (250 mm × 4.6 mm, 5 μm particle size) using isocratic elution. Wavelength: 215 nm, Injection volume: 20 μl, Flow rate: 1.0 ml/min, Column temperature: 25°C, Run time: 10 min (**Figure 3**).

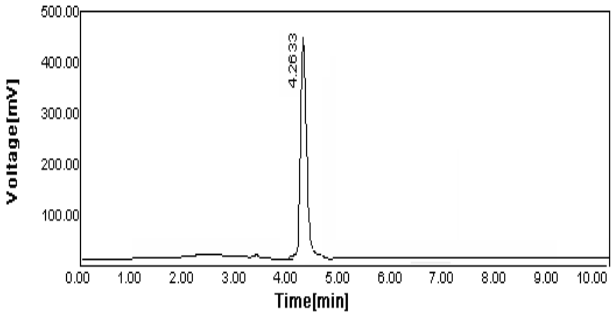

Fig. 3. Chromatogram of orlistat (API)

Procedure for forced degradation study

Stress studies were carried out under the conditions mentioned in ICH Q1A (R2) *viz.* dry heat, hydrolysis, oxidation and photolysis. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 all requires the development and validation of stability indicating assays. Drug at a concentration of 50 μg/ml was used in all degradation studies. Conditions employed for performing stress studies were as follows:

Acid decomposition

Acid decomposition was performed by taking different concentrations (0.01 N, 0.1 N, 1 N, 2 N, 5 N) of HCl with drug (orlistat) at varied temperature (25°C, 40°C, 70°C, 80°C and 100°C) and time period (2, 8, 12, 24 h). The resulting solution was neutralized by base to avoid any interference of acid and suitably diluted with diluent to obtain solution of concentration of 50 μg/ml. All samples were injected into HPLC and the chromatograms were recorded. At the end of these studies, 0.01 N HCl was used and refluxed for 8 h at 80°C (**Figure 4**).

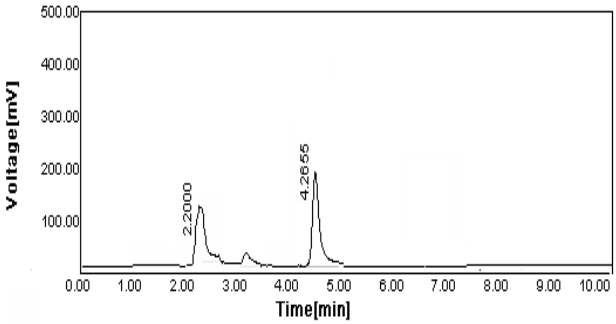

Fig. 4. Chromatogram of orlistat and acid degradation products

Alkali decomposition

Alkali decomposition was performed by taking the different concentrations (0.01 N, 0.1 N, 1 N, 2 N, 5 N) of NaOH with drug (Orlistat) at varied temperature (25°C, 40°C, 70°C, 80°C and 100°C) and time period (2, 8, 12, 24 h). The resulting solution was neutralized by acid to avoid any interference of base and suitably diluted with diluent's to obtain solution of concentration of 50 μg/ml. All samples were injected into HPLC and the chromatograms were recorded. At the end of these studies 0.1 N NaOH was used and refluxed for 8 h at 80°C (**Figure 5**).

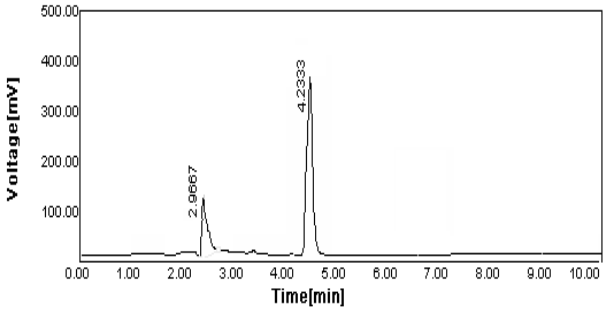

Fig. 5. Chromatogram of orlistat and alkali degradation products

Neutral decomposition

Drug (Orlistat) was subjected to neutral hydrolysis by subjecting to H_2O at varied temperatures (25°C, 40°C, 70°C, 80°C and 100°C) and time period (2, 8, 12, 1 day, 2 days and 5 days). To the resulting solution, diluents were added to obtain concentration of 50 μg/ml. All samples were injected into HPLC and the chromatograms were recorded. At end of these studies, it refluxed for 12 h at 80°C (**Figure 6**).

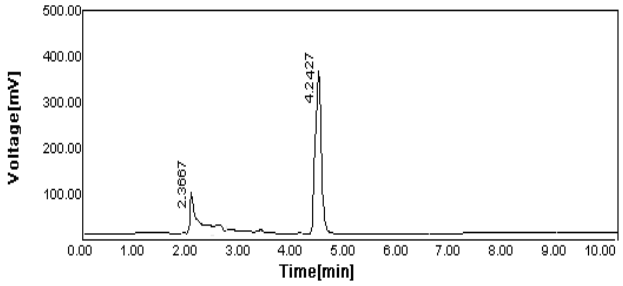

Fig. 6. Chromatogram of orlistat and neutral degradation products in H_2O

Oxidative decomposition

Oxidative decomposition was performed by taking the drug (Orlistat) in 1%, 3%, 10% and 30% of H_2O_2 *v/v* at different temperature and time period (30 min, 3 h, 6 h and 24 h). All samples were injected into HPLC and the chromatograms were recorded. At the end of these studies, 3% of H_2O_2 was used for 24 h at RT (**Figure 7**).

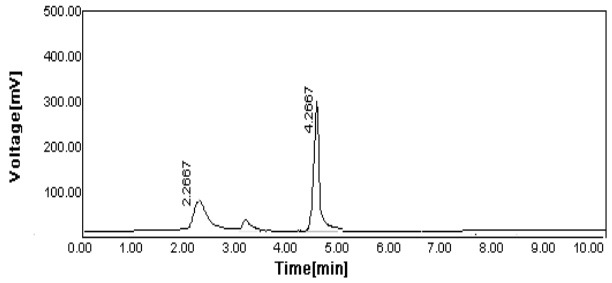

Fig. 7. Chromatogram of orlistat and H_2O_2 degradation products

Thermal decomposition

Thermal studies were also conducted on solid drug (Orlistat), which were heated at varied temperature (40°C, 60°C, 80°C and 100°C) and time period (2, 4, 8, 12 and 24 h) in hot air oven. Samples were withdrawn at appropriate time periods for analysis (**Figure 8**).

Photolytic decomposition

Photo degradation studies were performed by exposing the drug (orlistat) to sunlight for 1day, 2 days, 3 days and 7 days. Samples were withdrawn at appropriate time period for analysis (**Figure 9**).

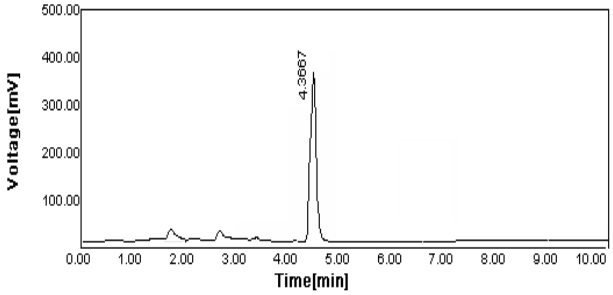

Fig. 8. Chromatogram of orlistat and thermal degradation products

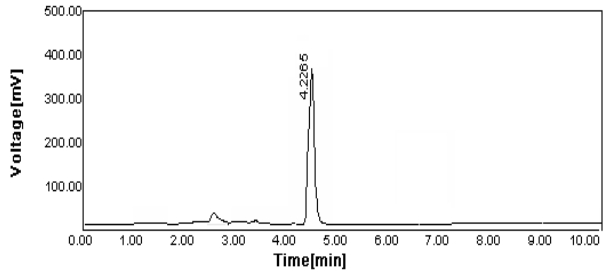

Fig. 9. Chromatogram of orlistat and photolytic degradation products

Preliminary separation studies

The initial analysis of different stressed samples was performed on HPLC system using a C-18 column and mobile phase composed of acetonitrile:0.1% formic acid (85:15, *v/v*). It was filtered through 0.45 μm nylon filter and sonicated before use. The injection volume was 20 μl and the flow rate was set at 1 ml/min. The detection was carried out at 215 nm.

Optimization studies

The separation of drug from its degradation products were optimized by varying the ratio and/or nature of organic modifier. Finally, method was developed using mobile phase composed of acetonitrile:0.1% formic acid (85:15, *v/v*) in which drug and degradation products showed good elution.

RESULTS

Forced degradation study

Degradation of orlistat was observed in acid hydrolysis, alkali hydrolysis, neutral hydrolysis, oxidative conditions. There was no degradation seen in thermal and photolytic conditions. The degradation behaviour of orlistat in various stress conditions is shown in **Figures 4-9** and the results are compiled in **Table 2**.

Table 2. Degradation characteristics of orlistat

S. No.	Stressed parameter			Results of stress degradation			
	Stress condition	Stress temp.	Stress time	AUC of API	% Assay of API	AUC of degraded products	% Assay of degraded products
1	Acid decomposition (0.1 N HCl)	80°C	8 h	3086.33	85.50	482.50	13.37
2	Alkali decomposition (0.1 N NaOH)	80°C	8 h	3235.50	89.64	333.10	9.23
3	Neutral hydrolysis (Distilled water)	80°C	12 h	3527.69	97.75	41.14	1.44
4	Oxidative decomposition (3% H_2O_2)	RT	24 h	3385.86	93.82	181.88	5.04
5	Thermal decomposition	100°C	24 h	3567.75	98.85	–	–
6	Photolytic decomposition	70,000 to 80,000 lux	7 days	3569.56	98.91	–	–

*Each reading is the mean of three replicates.

Method validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, robustness and system suitability in accordance with ICH guidelines Q2A (R1).

Precision

The precision of the method was evaluated by carrying out six independent assays of test samples of orlistat. The precision of the method was also evaluated in same day for repeatability of precision and in different days. The results shown in **Table 3** indicated that the method is reproducible.

Table 3. Results of precision studies

Precision	% Found	SD	%CV
Repeatability	99.50	0.208	2.09
Intermediate precision	99.71	0.295	0.296

Accuracy

Accuracy was calculated as the percentage recovery of the known added amount of orlistat reference substance in the sample solutions using three concentration levels (50%, 100% and 150%) covering the specified range (24, 36 and 48 μg/ml). The accuracy of the method ranged from 99.70-99.94% indicated that this assay is reliable (**Table 4**).

Robustness

To determine the robustness of developed method, experimental condition were purposely altered. The ratio of mobile phase was changed; 85:15 by ±2 to get 87:13 and 83:17 (acetonitrile:0.1% formic acid *v/v*) and changed flow rate by ±0.1 ml/min (flow rate 0.9 ml and 1.1 ml) while the other parameters were held constant in chromatographic condition. The %CV was not more than 2% in both conditions (**Table 5**).

Table 4. Results of recovery studies

Percentage level	% Recovery	SD	%CV
50	99.83	0.3403	0.340
100	99.94	0.3101	0.310
150	99.70	0.4113	0.412

Laboratory samples and tablet formulation

In order to confirm the validity of the method, laboratory samples and tablet samples containing orlistat were prepared in the range of 10 μg/ml-60 μg/ml. The amount of drug present in the standard and test solution was calculated by using the selected linearity equation and the results are tabulated in **Table 6**.

DISCUSSION

Stability-indicating assay method for quantitative estimation of orlistat (API) and its degradation products as well as its validation in tablet dosage form showed good specificity, sensitivity, linearity, precision and accuracy over the entire range.

Table 5. Results of robustness studies

Mobile phase composition change (±2)				Flow rate change (±0.1 ml/min)		
Parameters	**85:15**	**87:13**	**83:17**	**1.0 ml/min**	**0 .9 ml/min**	**1.1 ml/min**
S.D.	1.450	1.301	1.536	1.162	1.34145	1.31426
%CV	0.041	0.036	0.042	0.032	0.037	0.036

Table 6. Results of laboratory samples and tablet formulation

Parameters	Laboratory samples	Tablet formulation
% Mean found	99.86	99.47
S.D.	0.144	0.177
%CV	0.144	0.178

CONCLUSION

Forced degradation study on orlistat was carried out under the conditions of acid, alkali, neutral hydrolysis, oxidation, thermal and photolytic conditions and the study showed that orlistat was labile in acid, neutral, alkali, and oxidative conditions. It was stable to light and dry heat. Based on the information generated by forced degradation, a stability-indicating assay method was developed and validated, which separates all degradation products formed under variety of conditions. The method was found sufficiently linear, precise, accurate, sensitive, robust and specific to drug. No interference of degradation product at retention time of principal peak was found in degradation study. Hence, it can be recommended for analysis of drug/degradation products in stability samples by industry.

REFERENCES

Bakshi M, Singh S. Development of validated stability-indicating assay methods-Critical review. *J. Pharm. Biomed. Anal.* 2002;28(6):1011-40. [DOI: 10.1016/S0731-7085(02)00047-X]

Bakshi M, Singh S. ICH guidance in practice: establishment of inherent stability of secnidazole and development of a validated stability-indicating high-performance liquid chromatographic assay method. *J. Pharm. Biomed. Anal.* 2004;36(4):769-75. [DOI: 10.1016/j.jpba.2004.08.008]

Bennett PK, Li YT, Edom R, Henion J. Quantitative determination of Orlistat (tetrahydrolipostatin, Ro 18-0647) in human plasma by high-performance liquid chromatography coupled with ion spray tandem mass spectrometry. *J. Mass Spectrom.* 1997;32(7):739-49. [DOI: 10.1002/(SICI)1096-9888(199707)32:7<739::AID-JMS526>3.0.CO;2-C]

Bhimavarapu R, Chitra KP, Meda H, Kanikanti D, Anne M, Gowthami N. Forced degradation study of paracetamol in tablet formulation using RP-HPLC. *Bull. Pharm. Res.* 2011; 1(3):13-7.

Dunge A, Sharda N, Singh B, Singh S. Establishment of inherent stability of stavudine and development of a validated stability-indicating HPLC assay method. *J. Pharm. Biomed. Anal.* 2005;37(5):1115-9. [DOI: 10.1016/j.jpba.2004.09.014]

Felinger A. Mathematical Analysis of Multicomponent Chromatograms. *In* Advance in Chromatography. Brown P, Grushka E. (Eds), Vol. 39, Marcel Dekker: New York, 1998; 201-38.

Giannellini V, Salvatore F, Bartolucci G, Coran SA, Bambagiotti-Alberti M. A validated HPLC stability-indicating method for the determination of diacerhein in bulk drug substance. *J. Pharm. Biomed. Anal.* 2005;39(3-4):776-80. [DOI: 10.1016/j.jpba.2005.04.040]

Hemant Kumar T, Manasa Reddy K, Rishika D, Prasanna Kumar R. Estimation of orlistat by UV spectrophotometric method. *Int. J. Pharm. Sci. Res.* 2011;2(9):2469-71.

Hong DD, Shah M, Development and Validation of HPLC Stability-Indicating Assays, Drug Stability: Principles and Practices. 3rd Edition, Marcel Decker, Inc.: New York, 2000; 329-84.

ICH, Stability testing of new drug substances and products. International Conference on Harmonization, IFPMA, Geneva, 1993.

ICH, Quality of biotechnological products: stability testing of biotechnological/biological products. International Conference on Harmonization, IFPMA, Geneva, 1995.

ICH, Impurities in new drug products. International Conference on Harmonization, IFPMA, Geneva, 1996.

ICH, Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances. International Conference on Harmonization, IFPMA, Geneva, 1999.

ICH, Text on validation of analytical procedures. International conference on harmonization of technical requirements for registration of pharmaceutical for human use, Geneva, 2000.

ICH, Stability testing of new drug substances and products Q1A (R2). International Conference on Harmonization, IFPMA, Geneva, 2003.

Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT, Shah AK. A stability-indicating HPLC method for assay of lercanidipine hydrochloride in tablets and for determining content uniformity. *Indian J. Pharm. Sci.* 2010;72(3):381-4. [DOI: 10.4103/0250-474X.70490]

Prashanth S, Anil Kumar A, Madhu B, Vidya Sagar J. LC and LC-MS study on stress decomposition behavior of paclitaxel and establishment of validated stability-

indicating assay method. *Int. J. Pharm. Sci. Drug Res.* 2011;3(3):188-96.

Rao J, Chauhan K, Mahadik KR, Kadam SS. A stability-indicating high performance liquid chromatographic method for the determination of diacerein in capsules. *Indian J. Pharm. Sci.* 2009;71(1):24-9. [DOI: 10.4103/0250-474X.51946]

Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharm. Tech. On-Line* 2000;1-14.

Singh S, Garg S. Understanding analytical method validation. Part 1. Basic validation characteristics. *Pharma Times* 1999;31(8):15-20.

Xu QA, Trissel QA. Stability-indicating HPLC Methods for Drug Analysis, American Pharmaceutical Association: Washington, 1999.

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