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Short communication

A stability-indicating HPLC assay method for docetaxel

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Abstract

A novel stability-indicating high-performance liquid chromatographic assay method was developed and validated for docetaxel in the presence of degradation products generated from forced decomposition studies. A gradient HPLC method was developed to separate the drug from the degradation products, using a Hichrom RPB HPLC column. Mixture of water and acetonitrile was used as mobile phase. The flow rate was 1.0 ml/min and the detection was done at 230 nm. Using the above method one can carry out the quantitative estimation of impurity namely DCT-1 and docetaxel. The developed gradient LC method was subsequently validated.
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Keywords: RP-LC; Forced degradation; Validation; Solution stability; Diode array detector (DAD)

1. Introduction

Docetaxel is an antineoplastic agent and belongs to the taxoid family. It is prepared by a semi-synthetic process, beginning with a precursor extracted from the renewable needle biomass of yew plants. Docetaxel is available as both anhydrous and trihydrate in the market. Docetaxel has a trade name of Taxotere and was developed by Aventis Pharmaceuticals for the treatment of certain type of cancer. The drug is now approved in 90 countries to treat advanced breast cancer and 70 countries to treat patients with advanced non-small lung cancer.

A sensitive high-performance liquid chromatographic method for the determination of docetaxel in human plasma or urine is available in the literature [1]. Rapid analysis of docetaxel in human plasma by tandem mass spectrometry with on-line sample extraction is also published [2]. According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. So far, to our present knowledge, no stability-indicating assay method for docetaxel is available in the literature. Keeping into the view of susceptibility of docetaxel under variety of conditions, it was felt that a HPLC method of analysis that separates the drug from the degradation products formed under ICH suggested conditions (hydrolysis, oxidation, photolysis and thermal stress) [3] would be of general interest. These studies provide valuable information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies [4]. Therefore, the aim of present study was to develop a stability-indicating HPLC assay method for docetaxel. The developed HPLC assay method was validated as per ICH guidelines [5].

2. Experimental

2.1. Materials

A sample of docetaxel, its potential impurity DCT-1 and possible degradant 7-Epimer (Fig. 1) were received from Pro-

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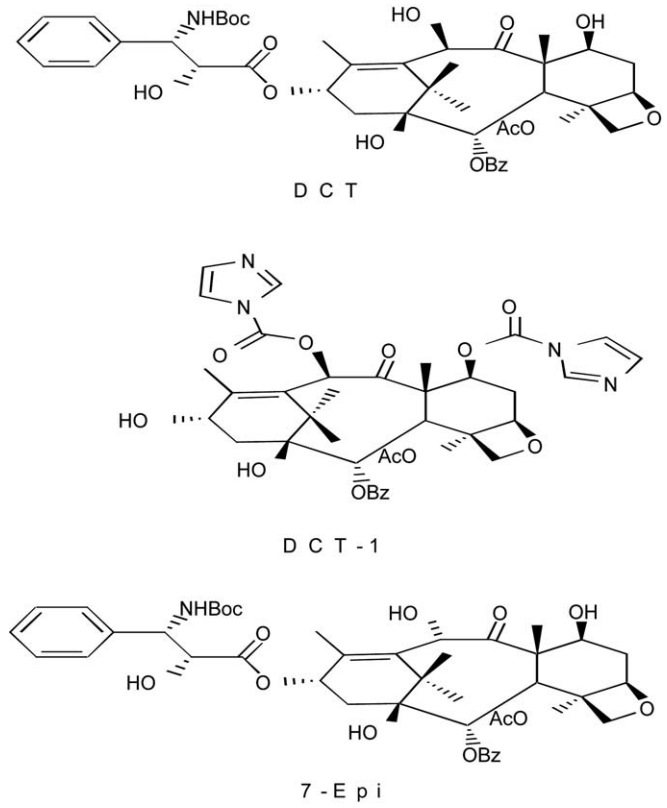

Fig. 1. Chemical structures of docetaxel (DCT), DCT-1 and 7-Epimer (7-Epi).

cess Research Department of Custom Pharmaceutical Services of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile and water were purchased from Qualigens Fine Chemicals, Mumbai, India.

2.2. HPLC

The HPLC instrument employed was Agilent 1100 series (manufactured by Agilent Technologies, Waldbronn, Germany) LC system with variable wavelength detector (VWD) and also a diode array detector (DAD). The out put signal was monitored and processed using Chemstation Software (designed by Agilent Technologies, Waldbronn, Germany).

2.3. Chromatographic conditions

The chromatographic column used was a Hichrom (manufactured by Hichrom Ltd., Berkshire, UK) RPB 250 mm × 4.6 mm column with 5 μm particles. Solvent A consisted of HPLC grade water and solvent B contains HPLC grade acetonitrile. The flow rate of the mobile phase was 1.0 ml/min. The HPLC gradient was kept as *T*/%*B*: 0/35, 15/65, 25/75, 30/95, 35/100, 39/100 and 40/35 with a post run time of 5 min. The column was maintained at 25 °C. The detection wavelength was 230 nm. The injection volume was 10 μl. The diluent used was a 1:1 mixture of water and acetonitrile.

2.4. Preparation of standard solutions

A stock solution of 5.0 mg/ml was prepared by dissolving appropriate amount of substance in the diluent. Working solution of 500 μg/ml was prepared from stock solution for related substances and assay determination. A stock solution of impurity (DCT-1) at 0.5 mg/ml was also prepared in the diluent.

2.5. Forced decomposition studies

Forced degradation studies were performed on docetaxel bulk drug. Intentional degradation was attempted to stress conditions of UV light (254 nm), heat (60 °C), acid (0.5N HCl), base (0.005N NaOH) and oxidation (3% H_2O_2) to determine the ability of the proposed method to separate docetaxel from its impurity DCT-1 and degradation products generated during forced decomposition studies. For heat and light studies, study period was 10 days whereas for acid, base and oxidation it was 48 h. Peak purity test was carried out on the stressed samples by using DAD. Assay studies were carried out for stress samples against qualified reference standard and the mass balance (% assay+ % impurities + % degradation products) was calculated. Assay was also calculated for bulk sample by spiking with DCT-1 at the specification level (0.5%).

2.6. Method validation

The analytical method validation was carried out as per ICH method validation guidelines [5]. The following validation parameters were addressed: specificity, precision, linearity, accuracy, limit of detection, limit of quantitation, robustness and stability of docetaxel in diluent.

3. Results and discussion

3.1. HPLC method development

DCT-1 was the only potential impurity present in bulk samples produced by Dr. Reddy's Laboratories. From the chemistry point of view, the formation of 7-Epimer is suspected when docetaxel was treated with alkali. The key objective of 'chromatographic method' was to get the separation of DCT-1, 7-Epimer from docetaxel peak. Attempts were made by using different stationary phases like C18 and C8 and using a combination of water and organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. Selection of suitable HPLC column was also of major concern. Hichrom RPB is high purity base deactivated silica, which consists of a unique C8/C18 multi-alkyl bonding and exhaustive end capping. This offers a unique selectivity not achieved by a C8 or C18 column and combines the robustness of a C18 phase with the high coverage of a C8 phase. The chromatographic separation was achieved using a mobile phase containing a mixture of water and acetonitrile using a Hichrom RPB column. Very good peak shape of docetaxel noticed when Hichrom RPB column employed. In the optimized conditions (see Section 2) docetaxel and its impurity DCT-1 and the possible degradant 7-Epimer were well separated with

Table 1
System suitability report

Compound ($n = 3$)	USP resolution	USP tailing factor (R_S)	No. of theoretical plates (N) USP tangent method
DCT-1	–	1.2	74352
Docetaxel	12.0	1.1	104784

n, number of determinations.

a resolution of greater than 2.5. The typical retention times of DCT-1, docetaxel and 7-Epimer were about 12.1 min, 14.4 min and 17.3 min, respectively. The system suitability results are given in Table 1 and the developed LC method was found to be specific for docetaxel and its impurity, namely DCT-1 and its possible degradant, namely 7-Epimer.

3.2. Results of forced degradation studies

Degradation was not observed in docetaxel samples under stress conditions like acid hydrolysis, thermal exposure and oxidation. The drug degradation was observed when docetaxel was exposed in solid state to UV light for 10 days and when treated with mild alkali (0.005N NaOH for 2 h at RT). The major impurity formed under UV stress was observed as DCT-1, which was again confirmed by co-injection with the standard. Under alkali hydrolysis (in 0.005N NaOH for 2 h stress at RT), one major degradant and some unknown degradation products were formed (Fig. 2). The major degradant formed under alkali hydrolysis was observed as 7-Epimer as suspected, which was again confirmed by co-injection with the standard. Peak purity test results confirmed that the docetaxel peak is homogeneous and pure in all the stress samples, analyzed under DAD. The mass balance of stressed samples was close to 99.5% (Table 2). The assay of docetaxel was unaffected by the presence of DCT-1 and 7-Epimer which confirms the stability-indicating power of the method.

4. Validation

4.1. Precision

The injection (system) precision was evaluated by performing six replicate injections of the standard docetaxel solution (nominally, 0.5 mg/ml), spiked with 0.5% of DCT-1. The procedure precision (intra-day repeatability) was established by performing five replicate assays of independently prepared samples of docetaxel. The R.S.D. values were 1.0 and 0.6%, respectively. The assay results of docetaxel solutions (nominally, 0.5 mg/ml), prepared and assayed on each of 3 consecutive days averaged 99.7% and the precision (R.S.D.) was 0.9%.

4.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) of DCT-1 was checked and obtained at 0.04% (of 500 μg/ml) for 10 μl injection volume. The limit of quantification (LOQ) of DCT-1 was about 0.12% under the same conditions.

4.3. Linearity

Linear calibration plot for above method was obtained over the calibration range 250 μg/ml to 750 μg/ml (50–150% of docetaxel, nominal concentration 500 μg/ml) and the correlation coefficient obtained was greater than 0.999. Linearity was checked for DCT-1 between 60 μg/ml and 600 μg/ml (LOQ to 120% of impurity limit, i.e. 0.5%). The correlation coefficient obtained for DCT-1 was greater than 0.990. The results show that an excellent correlation existed between the peak area and concentration of the analyte and impurity DCT-1.

4.4. Accuracy

The recovery of docetaxel in bulk drug samples ranged from 99.2 to 101.5%. The recovery of DCT-1 in bulk drugs samples ranged from 94.4 to 104.5%. HPLC chromatograms of blank, pure and spiked samples at 0.5% level of DCT-1 and 7-Epimer in docetaxel bulk drug sample are presented in Fig. 3.

4.5. Robustness

In all the deliberate varied chromatographic conditions (flow rate and column temperature), the resolution between docetaxel and its impurity DCT-1 was greater than 4.0, illustrating the robustness of the method.

Table 2
Summary of forced degradation results

Stress condition	Time	% Assay of active substance	Mass balance (% assay + % impurities + % degradation products)	Remarks
Acid hydrolysis (0.5N HCl at RT)	48 h	99.0	99.4	No degradation products formed
Base hydrolysis (0.005N NaOH at RT)	2 h	75.5	99.6	Major degradant is observed as 7-Epimer. Also some unknown degradation products were formed
Oxidation (3% H_2O_2 at RT)	48 h	99.0	99.4	No degradation products formed
Thermal (60 °C)	10 days	99.2	99.5	No degradation products formed
UV (254 nm)	10 days	88.0	99.3	Major degradant is observed as DCT-1. Also some unknown degradation products were formed

RT, room temperature.

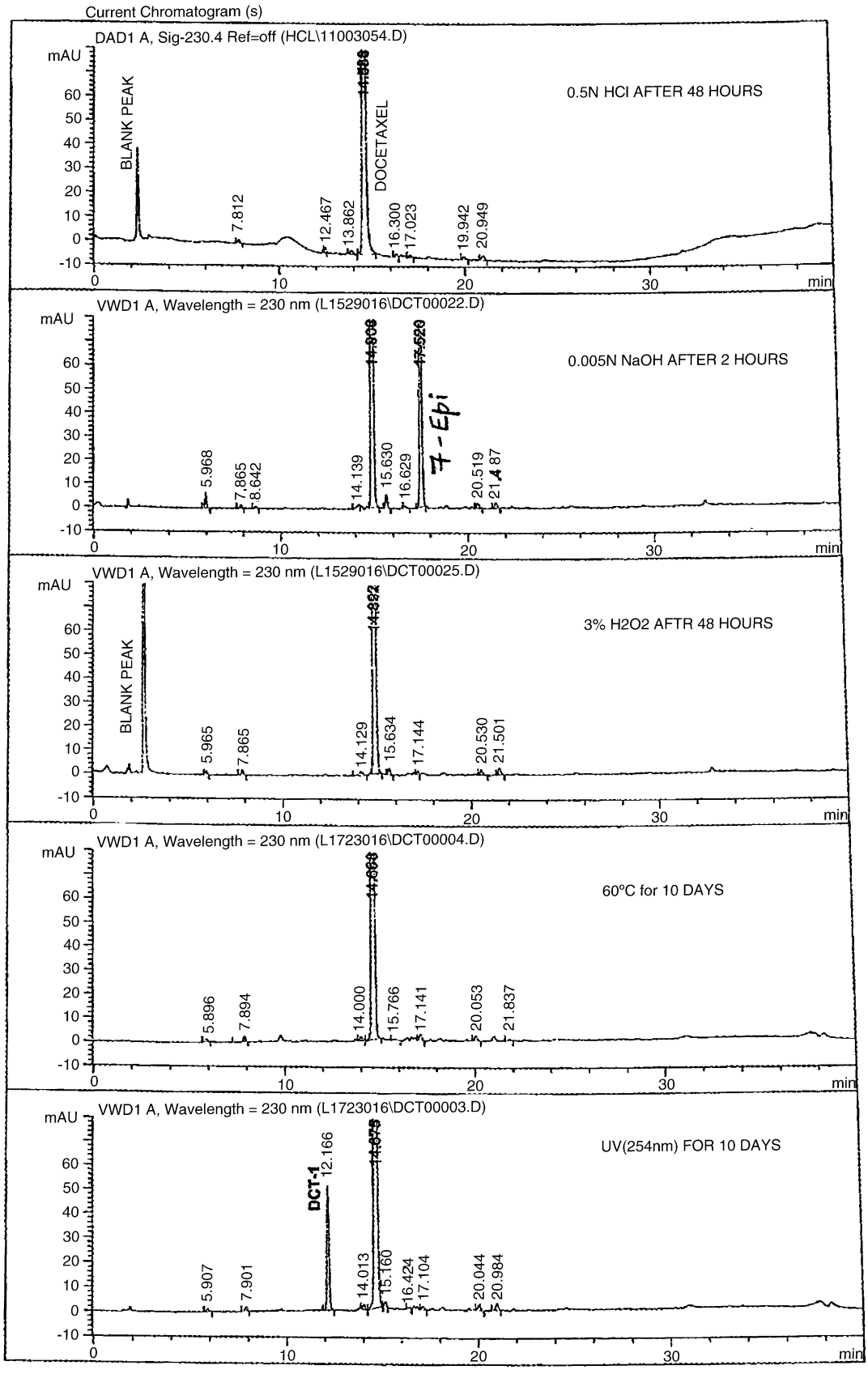

Fig. 2. Typical HPLC chromatograms recorded during forced degradation studies.

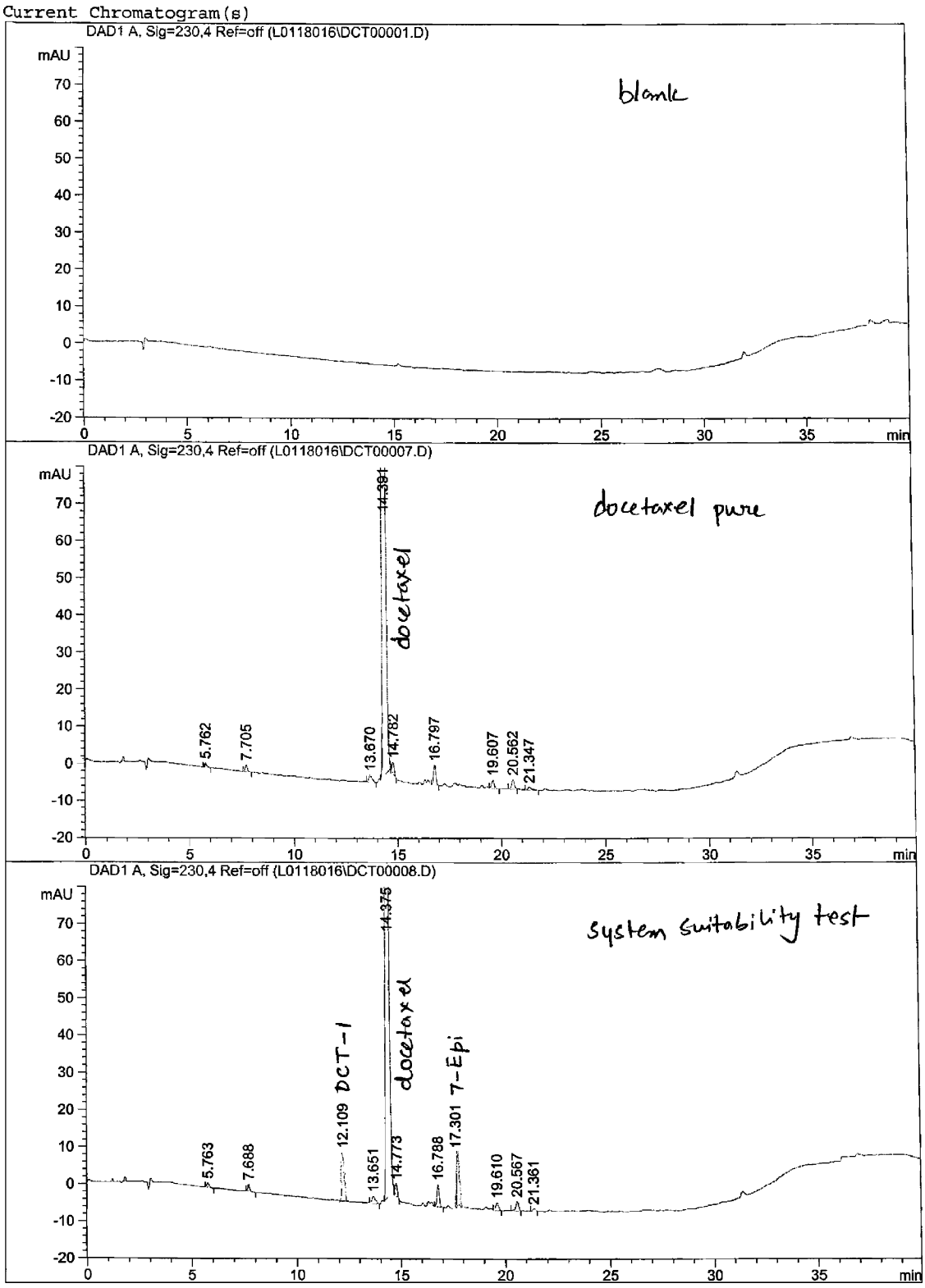

Fig. 3. Typical HPLC chromatograms of blank (top), pure bulk (docetaxel pure, middle) and spiked (DCT-1 spiked in docetaxel at 0.5% level) (system suitability test, SST; bottom) samples.

4.6. Solution stability

The R.S.D. of assay of docetaxel during solution stability experiments was within 1.0%. No significant change was observed in the content of DCT-1 during solution stability experiments. The experimental data confirmed that sample solutions used during assay and related substance determinations were stable up to 48 h.

5. Conclusions

A stability-indicating HPLC assay method was developed for the quantitation of docetaxel, its potential impurity, i.e. DCT-1. The developed method is specific, accurate, precise and robust. The procedure permitted an accurate and quantitative determination of docetaxel and its impurity DCT-1. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrates that the developed method was specific and stability-indicating. This method can be used to carry out the analysis of docetaxel in stability samples.

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References

[1] M.B. Garg, S.P. Ackland, J. Chromatogr. B Biomed. Sci. Appl. 748 (2000) 383–388.
[2] A.G. Grozav, T.E. Hutson, X. Zhou, R.M. Bukowski, R. Ganapathi, Y. Xu, J. Pharm. Biomed. Anal. 36 (2004) 125–131.
[3] M. Bakshi, B. Singh, A. Singh, S. Singh, J. Pharm. Biomed. Anal. 26 (2001) 1011–1040.
[4] Stability Testing of New Drug Substances and Products (Q1AR2), ICH Harmonised Tripartite Guideline.
[5] Validation of Analytical Procedures: Methodology (Q2B), ICH Harmonised Tripartite Guideline.