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DRUG DISCOVERY

Development and validation of a reversed phase HPLC method for the determination of SAHA bulk drug

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ABSTRACT

A rapid sensitive high-performance liquid chromatographic method was developed for the determination of Suberoyl anilide hydroxamic acid (SAHA). The chromatographic system utilized a C_{18} column with the detector wavelength set at 240nm. The mobile phase was composed of A-10mM potassium dihydrogen phosphate (P^H-2.5) and B-Acetonitrile in gradient method (time/%A) of the ratio 0/90, 2/90, 10/30, 12/ 10, 15/90, & 20/90. And it delivered at a flow rate of 1mL/minute. The analytic al run time was less than 10.2 min. The Standard Curves were linear over a range of 0.02-50µ/mL with a correlation coefficient of 0.990. The limit of quantification was assessed to be 0.02µ/mL and the limit of detection 0.01 µ/mL. The RSD values for the intra and inter day precision studies were 1.87-1.88 % and 2.12-1.72 % respectively. The accuracy was ≤5% over the concentration range of 0.02 µ/mL to 40 µ/mL.

Key words: Suberoyl anilide hydroxamic acid; Gradient method; Precision; Accuracy; LOD&LOQ; Acetonitrile; Potassium dihydrogen phosphate.

Abbreviations: SAHA - Suberoyl anilide hydroxamic acid; HDAC- Histone deacetyl transferase; ICH - International Conference on Harmonization; CTCL - Cutaneous T-Cell Lymphoma; LOD - Limit of Detection; LOQ - Limit of Quantitation; USP - United States Pharmacopoeia.

1. INTRODUCTION

Suberoyl anilide hydroxamic acid (SAHA, Vorinostat, Zolinza), (Fig.1) was approved by US FDA for the treatment of advanced cutaneous T-cell lymphoma (CTCL). Its molecular weight is about 264.36. SAHA inhibits the enzyme Histone deacetylase (HDAC), which arrest the cell growth. Mechanism of these inhibitors action is the direct interaction with the active zinc site at the base of the catalytic pocket, which blocks substrate approach active zinc-ion of enzymes. Molecular aspects of HDAC inhibitors effects in tumor cells are complex and not completely elucidated. Therefore, tumor-suppressor genes could be silenced by aberrant histone deacetylation. This epigenetic modification has become an important target for tumor therapy (Baboota et al., 2007). Histone deacetylation of tumor-suppressor genes occur in a variety of human tumors and studies that show HDAC inhibition to result in cell growth arrest, differentiation, apoptosis and alteration in gene expression in cancer cell lines. HDAc inhibitors prevent cell proliferation and survival of tumor cells with very low toxicity towards normal cells.

C_{18} columns were commonly used for the HPLC analysis. Thus separation was achieved on a 4.6 X 250mm C18 Inertsil ODS 3v 5µm analytical column. This column offered adequate separation of compounds and resulted in well-resolved peaks. The mobile phase or eluent interacts with the solute molecules and the stationary phase itself, with the strength of these interactions determining the resolution obtained and hence the efficacy and efficiency of the separation, resulting in the selection of an appropriate mobile phase being imperative. The mobile phase selected must not alter the characteristics of, or be miscible with the stationary phase, as this is potentially detrimental to the life span of the column. The viscosity of the mobile phase is also an important consideration and should not exceed 0.5cps as high viscosity solvents reduce solvent diffusion coefficients, hence decreasing column efficiency. A commonly used mobile phase flow rate is 1-2mL/min.

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MOBILE PHASE SELECTION

The mobile phase or eluent interacts with the solute molecules and the stationary phase itself, with the strength of these interactions determining the resolution obtained and hence the efficacy and efficiency of the separation, resulting in the selection of an appropriate mobile phase being imperative. The mobile phase selected must not alter the characteristics of, or be miscible with the stationary phase, as this is potentially detrimental to the life span of the column.Solutions for use in HPLC systems must be filtered to remove particulate matter that could interfere with the pumping action of the solvent delivery device, cause damage to the seals and valves, or collect on the top of the column causing irregular behavior and subsequent column blockages Solutions must also be degassed to remove dissolved or suspended air bubbles so that they do not collect in the pump or the detector cell and cause erratic behavior of the detector or an irregular pumping action.The viscosity of the mobile phase is also an important consideration and should not exceed 0.5cps as high viscosity solvents reduce solvent diffusion coefficients, hence decreasing column efficiency.

Chromatogram: The electronic result of a chromatographic separation, which is a plot of detectorsignal against elution time. It is represented as a series of peaks.

However, slower flow rates are more advantageous in terms of maximizing the life span of the pump and the column and would thus be more economical. Consequently, a flow rate of 1.0mL/min was selected for the method developed (Atul et al., 1999).

Validation is a process to ensure that the quality of analytical data generated is both reliable and accurate, and it is also capable of identifying potential problems of a particular method. The validation parameters outlined by the United States Pharmacopoeia (USP), the FDA, regulatory bodies in Europe and Canada and International Conference on Harmonization (ICH) may vary slightly (Table 1). The USP and ICH include eight parameters for evaluation, namely accuracy, precision (intermediate precision and repeatability /reproducibility), specificity, limit of detection (LOD)and limit of quantitation (LOQ), linearity, range, Ruggedness and Selectivity (Padadopoulos et al., 2002).

Gradient elution: A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution.

2. METERIALS AND METHODS

The experimental requirements that were necessary for chemicals, reagents, apparatus and instruments with detailed description of HPLC instruments and other chromatographic conditions are mentioned against each method. All the chemicals and solvents used in these experiments were of HPLC and/or analytical grade (Padadopoulos et al., 2002).

2.1. Chemicals

The reference standard of Suberoyl anilide hydroxamic acid was synthesized and purified (99%). HPLC grade acetonitrile was purchased from Alfadan. HPLC grade Ammonium acetate, Acetic acid & Potassium dihydrogen phosphate were obtained from Merck. The Milli Q water was prepared in our Laboratory.

2.2. Apparatus

Limits of detection and Limits of quantitation: The LOD and LOQ are often defined as Concentration which yield a measure peak with S/N of 3 and 10 respectively.

To achieve high accuracy and reliability of the results of research work calibrated glassware was used. All glassware was washed thoroughly with distilled water and then rinsed with methanol and dried before use. Beaker (50 mL, 100 mL, 250 mL, 500 mL and 1000 mL capacity), Standard flasks (10 mL, 50 mL, 100 mL, 250 mL, 500 mL and 1000 mL), Measuring cylinders (50 mL, 100 mL, 250 mL and 500 mL, 1000 mL), HPLC Vials, Micro pipettes (10-100 µL, 20-200 µL and 100-1000 µL) and Membrane Filter.

2.3. HPLC system and chromatographic conditions

The system used for this work consisted of HPLC and other analytical instruments. Analytical balance (Mettler Toledo AX 205), P^H meter (Elinco Li 610), Vacuum pump (Gastdoa-V130-BW), Sonicator (Sonorex super 10 p digital), Shaking Water Bath (Julabo SW 23), HPLC (Waters 2695 separations Module). The HPLC coupled with a UV variable wavelength detector 240nm. The separations were achieved on a reverse phase C18 column (4.6 X 250mm C18 Inertsil ODS 3v 5µm) preceded by guard column (30X4.6mm) packed with the same material. Mobile phase consisted of 10 mM Potassium dihydrogen phosphate (pH 2.5) acetonitrile. Gradient (Time/%A) - 0/90, 2/90, 10/30, 12/10, 15/90 & 20/90. It was filtered and degassed before use and pumped at 1 ml/min flow rate (Ardhani Dwi Lestari et al., 2004).

2.4. Preparation of mobile phase

The mobile phase 'A' was prepared by dissolving 1.36g of Potassium dihydrogen phosphate in 1000 mL of Milli Q Water. And pH was reduced to 2.5 by using ortho-phosporic acid. It was filtered through 0.22 µm membrane filter and degassed by sonication before using in the HPLC system.The mobile phase 'B' was acetonitrile and degassed by sonication before using in the HPLC system (Atul et al., 1999).

2.5. Preparation of stock solution

The stock solution of SAHA (1.0 mg/mL) was prepared by taking 10 mg of SAHA with few mL of DMSO were taken in 10 mL volumetric flask and then completing the volume make up with acetonitrile. The working standard solution was prepared by diluting with mobile phase.

2.6. Analytical Method Validation

2.6.1. Limits of detection and Limits of quantitation (LOD and LOQ)

Limit of detection (LOD) is the lowest concentration of an analyte that can be detected by the proposed method. It is generally referred to as a concentration when the signal to noise ratio is usually 3:1. The limit of quantitation (LOQ) is the lowest concentration of an analyte that can be determined with acceptable accuracy with a signal to noise ratio of 10:1. Two types of solutions i.e. blank and spiked with known progressively

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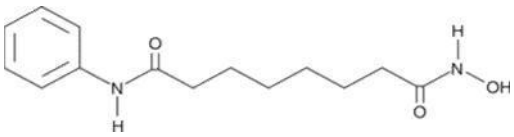

Figure 1
Suberoyl anilide hydroxamic acid (SAHA)

Retention Factor: Retention factor is how long a compound is retained by the stationary phase relative to the time it stays in the mobile phase.

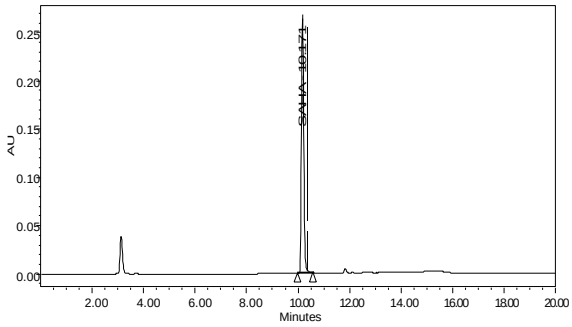

Figure 2
10mM Potassium dihydrogen phosphate (pH 2.5) and acetonitrile resulted in good separation and sharp peaks

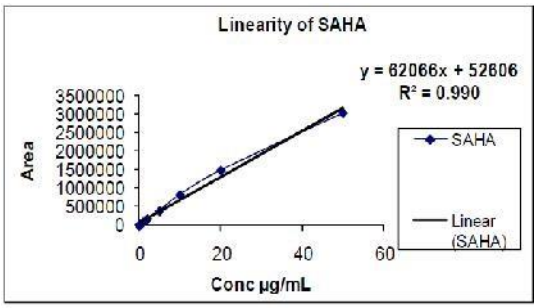

Figure 3
The linear regression equation for SAHA

Precision: The Precision of an analytical method is the degree of arrangement among individual test results when the method is applied respectively to multiple sampling of a homogenous sample.

decreasing concentrations of each analyte were prepared and analyzed (Baboota et al., 2007). The LOD was then calculated by the evaluation of minimum level at which the analyte can be readily detected. The LOQ was calculated by the evaluation of minimum level at which the analyte can be readily quantified with accuracy (Zlata Mrkvickova et al., 2007).

2.6.2. Linearity

The linearity of the methods was confirmed using standard solution at eleven different concentrations (0.02µg/mL, 0.05µg/mL, 0.1µg/mL, 0.2µg/mL, 0.5µg/mL, 1µg/mL, 2µg/mL, 5µg/mL, 10µg/mL, 20µg/mL, 50µg/mL) of analytes within in the range of 0.02-50 µg/mL for SAHA. Mean peak area values were plotted against the corresponding concentrations of analytes. The linearity of this plot was evaluated using least-squares liner regression analysis (Rabert Hartman et al., 2003).

Table 1 The validation parameters

Sl. No	Validation Parameters	Acceptable Limits
1	Linearity	Correlation coefficient should be between 0.98 - 1.00
2	Precision	4% RSD
3	Accuracy	80 to 120% of the theoretical value
4	LOD and LOQ	1:3 and 1: 10 of noise

Tailing: The phenomenon in which the normal Gaussian peak has an asymmetry factorgreater than 1, the peak will have tailing edge.

2.6.3. Accuracy

The accuracy of the method was evaluated by the addition of known amounts of SAHA to the sample solution. The results obtained were compared with the theoretical concentration. Form the Stock Solution to prepared 4 different concentration (0.02µg/mL, 0.05µg/mL, 25µg/mL and 40µg/mL). Each concentration was determined in six times. The accuracy was calculated as a recovery of these analyses (Zlata Mrkvickova et al., 2007).

2.6.4. Precision

Precision was determined as both repeatability and intermediate precision. Precision was expressed in terms of % RSD (Padadopoulos et al., 2002).

2.6.4.1. Repeatability

The intraday precision was based upon the results of six replicate analysis of four different concentrations (0.02µg/mL, 0.05µg/mL, 25µg/mL, 40µg/mL) of analytes on a single day.

2.6.4.2. Intermediate precision

The inter day precision was based upon the results of six replicate analysis of four different concentrations (0.02µg/mL, 0.05µg/mL, 25µg/mL, 40µg/mL) analyzed for three consecutive days (Table 2).

3. RESULTS AND DISCUSSION

3.1. Method Development and Optimization

Initially various mobile phases and stationery phases were tested to obtain the best separation and resolution of SAHA in 10 mM potassium dihydrogen phosphate and acetonitrile in 4.6 X 250mm C18 Inertsil ODS 3v 5µm analytical column were found to be the most appropriate for the separation ofthe components at a the flow rate of 1.0 mL/min. Using the mentioned chromatographic conditions, well resolved sharp peaks can be obtained at retention time of 10.1 minute for SAHA (Ioannis et al., 2004).

The mobile phase of 10mM Potassium dihydrogen phosphate and Acetonitrile was formed tailing in the peak. After the pH was adjusted to 2.5. (toreduce the pH using Ortho-phosphoric acid) to get sharp peak. At the flow rate of 1 mL/min (Table 3). Using the Gradient condition at the different time point at different ratio of mobile phase combinations, well resolved sharp peak can be obtained at retention time of 10.1 minutes for SAHA (Jerome vial et al., 1999).

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Table 2 The inter day precision

Column	4.6 X 250mm C18 Inertsil ODS 3v 5µm
Mobile phase	A= 10 mM Potassium dihydrogen phosphate(pH 2.5)B= Acetonitrile
Flow rate and Detection	1 mL/min and 240 nm
Gradient (Time/%A)	0/90, 2/90, 10/30, 12/10, 15/90 & 20/90.
Retention time	10.1 min
LLOQ	0.02 µg/mL

Method development was started with more polar mobile phase (90 % of 10mM Potassium dihydrogen phosphate) however, no peak could be obtained. The polarity of the mobile phase was then decreased by the addition of acetonitrile (Padadopoulos et al., 2002). A 10mM Potassium dihydrogen phosphate (pH 2.5) and acetonitrile resulted in good separation and sharp peaks (Fig.2).

3.2. Method validation

The developed chromatographic method for the determination of SAHA was validated using ICH guidelines.Validation parameters performed include linearity, limit of detection, limit of quantitation, accuracy, precision and stability of solutions.

3.2.1. Linearity

Table 3 The Linearity measurements for SAHA

Column	4.6 X 250mm C18 Inertsil ODS 3v 5µm
Mobile phase	A= 10 mM Potassium dihydrogen phosphate(pH 2.5) B= Acetonitrile
Flow rate and Detection	1 mL/min and 240 nm
Gradient (Time/%A)	0/90, 2/90, 10/30, 12/10, 15/90 & 20/90.
Retention time	10.1 min;
LLOQ	0.02 µg/mL

Linearity: The Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematically transformation, proportional to the concentration of analytic in sample within a given range.

Samples were prepared by serial dilution of a stock solution to yield concentrations over the range 0.02 – 50 µg/ml concentrations over the. Acceptability of linearity data was judged by examining the correlation coefficient (r^2) on the regression line for the response versus concentration plot. An r^2 value of >0.990 was considered to be sufficient to demonstrate linearity of the method and it falls in the range specified in Table 3.

Linearity of the proposed method was verified by analyzing 11 Concentration in the range of 0.02-50 µg/mL for SAHA. Good linearity was observed over the above range for SAHA. The calibration curve was made using concentration of the analytes versus peak area. The coefficient of determination from the linear regression analysis was calculated and found to be greater than 0.9966 in case of the analytes (Baboota et al., 2007). This indicates that there exists a good linear relationship between concentration of drugs and the peak area. The linear regression equation for SAHA was Y= 62066x + 52606 with value of coefficient of determination equal to 0.9903 (Fig.3).

3.2.2. Limit of detection and Limit of quantitation

Two types of solutions, i.e. blank and spiked with known concentrations of each analyte, were prepared and analyzed. The limit of detection (LOD) and quantification (LOQ) were then established by evaluating the signal to noise ratio of 3:1 and 10:1 respectively. The LOD was found to be 0.01 µg/mL for SAHA. The LOQ was found to be 0.02 µg/mL for SAHA (Atul et al., 1999).

3.2.3. Accuracy

The accuracy of the method was performed by making different concentration of SAHA (0.02µg/mL, 0.05µg/mL,25µg/mL, 40µg/mL) and then analyzed by the proposed method. The percentage of Accuracy and the percentage RSD were calculated from recovery experiments (Table 4).

3.2.4. Precision

Table 4 The results of within-day and between day precision

S.No	Conc (µg/mL)	Mean	Accuracy	% RSD
1	0.02	0.020	100.65	1.895
2	0.05	0.051	102.04	1.615
3	25	25.02	100.06	1.306
4	40	40.39	100.97	1.950

Precision is a measure of the closeness of data values to one another when a number of measurements are taken under the same analytical conditions. The ICH defines precision as having two components, namely: repeatability and intermediate precision. Precision is usually expressed as the percentage relative standard deviation (%RSD) and the tolerance for %RSD was set at ±4% in our laboratory. The precision of the proposed method was determined by the analysis of four different concentrations in terms of % RSD. The intra-day precision was based upon the results of four different concentrations of analytes on a single day. The between-day precision was determined from the same samples analyzed for two consecutive days. The results of within-day and between day precision are given in the Table 4.

3.2.4.1. Repeatability

Repeatability refers to inter-assay precision and is expressed as the degree of variation arising from injections run on the same day. It should be determined using twenty four determinations covering the specified analytical range, for six determinations at four concentration levels (Pallabi Mitra et al., 2004). Consecutive

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Table 5 Inter-day precision was determined using calibrators

			Intra-day Precision		Inter-day	Precision
S. No	Conc (µg/mL)	n	Mean	% RSD	Mean	%RSD
1	0.02	6	0.0205	1.873	0.031	2.121
2	0.05	6	0.051	1.654	0.079	2.204
3	25	6	26.430	1.925	38.961	1.460
4	40	6	42.145	1.885	62.456	1.720

Isocratic: A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic.

Measurements were obtained by repeated injection of the same concentration taken from different vials in succession. Interspersing vials of the same concentration with those of different concentrations was the method used to obtain nonconsecutive measurements. Repeatability of this system was assessed by repeat measurements (n=6) of high, middle, low and very low concentration calibration solutions.

SAHA: Suberoyl anilide hydroxamic acid (SAHA, Vorinostat, Zolinza) recently gained US FDA approval for the treatment of advanced cutaneous T- cell lymphoma (CTCL).

3.2.4.2. Intermediate Precision

Intermediate precision is used to evaluate the reliability of the method in different environments other than that used for the development of the method, and depending on the time, the method may be tested on different days, using different analysts and instruments. Inter-day precision was determined using calibrators of the same concentration as described. Samples were run over a three days (Table 5).

4. CONCLUSION

In this study, an attempt was made to develop a simple, sensitive, robust HPLC method and it was successfully developed and validated. The variables investigated indicate that buffer pH have a significant effect on peak shape and retention time of SAHA in solution. The choice of a suitable wavelength is also important, in terms of method precision, selectivity and sensitivity. The chromatographic conditions were optimized to yield a well-resolved peak with a reasonable retention time. The SAHA was successfully analyzed after developing a simple and accurate HPLC method. The method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation. The method was accurate and precise for the determination of SAHA with good accuracy and precision. As the method can separate the degradation products from the main peak of analyte, so it could be used not only for routine analysis but also for stability studies of SAHA.

SUMMARY OF RESEARCH

1. Suberoyl anilide hydroxamic acid (SAHA, Vorinostat, Zolinza) recently US FDA approval for the treatment of advanced cutaneous T- cell lymphoma (CTCL).
2. SAHA inhibits the enzyme Histone deacetylase (HDAC), which arrest the cell growth.
3. A simple, sensitive HPLC method was developed and validated.
4. After many attempted, that mobile phase 10mM potassium dihydrogen phosphate (pH 2.5) and acetonitrile was chosen. It got sharp peaks and less retention time of SAHA.
5. This method was accurate and precise for the determination of SAHA.

FUTURE ISSUES

1. The method was validated for accuracy, precision, reproducibility, limit of detection and quantification in accordance with ICH guidelines.
2. Statistical analysis proved the method was precise, selective, specific and accurate for analysis of SAHA.
3. The wide linearity range, sensitivity, accuracy, short retention time and simple mobile phase the method is suitable for routine quantification of SAHA with high precision and accuracy.
4. The same method development and validation is used for the stability studies, stress test, physicochemical parameters of the drugs, etc.

DISCLOSURE STATEMENT

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REFERENCES

Atul et al., 1999: Centpropazine is a new anti-depressant compound developed by Central Drug Research Institute, Lucknow (India). This article report the development and validation of a new HPLC assay of a parent drug in the serum of humans, monkeys and rats for pharmacokinetic studies. Centpropazine was eluted on a C18 column with a mobile phase consisting acetonitrile phosphate buffer (60:40) pumped at 1.5 ml min(-1) flow rate and quantitated by UV detector at 270 nm.

1. Ardhani Dwi Lestari, Tini Palupi, Bertha Oktarina, Mochammad Yuwono, Gunawan Indrayanto. HPLC Determination of Cilostazol in Tablets, and Its Validation. *J Liq Chromatogr Relat Technol*. 2004, 27(16), 2603-2612
2. **Atul BV, Paliwal JK, Gupta RC. Development and validation of a new high-performance liquid chromatographic assay of Centpropazine, a new antidepressant compound, in serum. *J Pharm Biomed Anal*. 1999, 20(3), 449-57**
3. Baboota S, Faiyaz S, Ahuja A, Ali J, Shafiq S, Ahmad S. Development and Validation of a stability-indicating HPLC method for analysis of Celecoxib (CXB) IN Bulk drug and microemlusion formulations. *ACTA chromatographica*. 2007, 18, 116-129
4. Ioannis N Papadoyannis, Victoria F. Samanidou. Validation of HPLC Instrumentation. *J Liq Chromatogr Relat Technol*. 2004, 27(5), 753-783
5. Jerome vial and Alain Jardy. Experimental Comparison of the Different Approaches to Estimate LOD and LOQ of an HPLC Method. Anal Chem 1999, 71(14), 2672-2677
6. Padadopoulos P, Parissi-Poulous M, Panderi I. Development and Validation of a Reversed Phase - HPLC method for the determination of Pindolol and clopamide in tablets. *J Liq Chromatogr Relat Technol*. 2002, 25(1), 125-136
7. Pallabi Mitra, Alan T Riga, Alexander KS. A Rapid High-Performance Liquid Chromatographic Determination of Propylthiouracil. *J Liq Chromatogr Relat Technol*. 2004, 27(3), 549-558
8. Rabert Hartman, Ahmed Abrahim, Andrew Claused, Bing Mao, Louis S Crocker, Zhihong Ge. Development and Validation of an HPLC Method for the Impurity and Quantitative Analysis of Etoricoxib. *J Liq Chromatogr Relat Technol*. 2003, 25(26), 2551-2566
9. Zlata Mrkvickova, Petra Kovarikova, Jiri Klimes, Danuta Kalinowski, Des R Richardson. Development and Validation of HPLC-DAD method for the analysis of two novel iron chelators with potent anticancer activity. J Pharm Biomed anal 2007, 43, 1343-1351

RELATED RESOURCE

1. FDA. Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability, *Federal Register (Notices)* 2000, 65(169), 52776-52777
2. International Conference on Harmonization. Q2A: Text on Validation of Analytical Procedures, *Federal Register* 1995, 60(40), 11260-11262
3. International Conference on Harmonization. Q2B: Validation of Analytical Procedures: Methodology; Availability, *Federal Register* 1997, 62(96), 27463-27467
4. Shabir GA. Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities between Validation Requirements of FDA, the US Pharmacopeia and the ICH. *J Chromatogr A* 2003, 987(1-2), 57-66
5. USP 25-NF 20, Validation of Compendial Methods Section (1225) (United States Pharmacopeal Convention, Rockville, Maryland, USA, 2002, p 2256
6. Vichayanrat A, Ploybutr S, Tunlakit M, Watanake-jorn P. Diabetes Res Clin Pract, 2003, 55, 99-103
7. Zhang H, Sun CR, Ishurd O, Pan YP, Ding LS. Analysis of Voglibose by post-column Fluorometric Detection Method using HPLC by Shimadzu application news. *Carbo-hydr Res* 2004, 339, 2027-2030