Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 265–269
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

# Cleaning validation 2: Development and validation of an ion chromatographic method for the detection of traces of CIP-100 detergent

Wilfredo Resto [a], Darimar Hernández [a], Rosamil Rey [b,c], Héctor Colón [b], José Zayas [b,*]

[a] Department of Chemistry, University of Puerto Rico-Cayey, Antonio R. Barceló Ave. Cayey, PR 00736, USA
[b] Research and Development Division, ZAYCOR Industries Corporation, 828 de Diego Ave. Caparra Terrace, San Juan, PR 00921, USA
[c] Interamerican University, Bayamón Campus, Department of Natural Sciences, Bayamon, PR, USA

Received 15 June 2006; received in revised form 17 January 2007; accepted 20 January 2007
Available online 30 January 2007

**Abstract**

A cleaning validation method, ion chromatographic method with conductivity detection was developed and validated for the determination of traces of a clean-in-place (CIP) detergent. It was shown to be linear with a squared correlation coefficient ($r^2$) of 0.9999 and average recoveries of 71.4% (area response factor) from stainless steel surfaces and 101% from cotton. The repeatability was found to be 2.17% and an intermediate precision of 1.88% across the range. The method was also shown to be sensitive with a detection limit (DL) of 0.13 ppm and a quantitation limit (QL) of 0.39 ppm for EDTA, which translates to less than 1 μL of CIP diluted in 100 mL of diluent in both cases. The EDTA signal was well resolved from typical ions encountered in water samples or any other interference presented from swabs and surfaces. The method could be applied to cleaning validation samples. The validated method could be included as a suitable one for rapid and reliable cleaning validation program.
© 2007 Elsevier B.V. All rights reserved.

*Keywords:* Cleaning validation; Ion chromatography; CIP detergent; EDTA analysis

## 1. Introduction

The current good manufacturing practices (c-GMP's) regulates the validation of the cleaning processes in the pharmaceutical industry [1,2]. The Food and Drug Administration (FDA) enforces those cleaning processes and the agency published a guide where they specified that no detergent should remain after the cleaning process [3]. It is common to find reports on cleaning validation of drug residues [4–8]. However, reports on cleaning validation of detergents used for the cleaning process are limited [9,10]. The detection of traces of detergents is a difficult task in cleaning validation programs. The exact amounts of the specific chemical compounds are not revealed, mainly, because the manufacturers of such detergents need to protect their formulations from being copied by competitors. Normally, the amounts of these chemicals are presented in a very wide concentration range. Therefore, a complete disclosure from a detergent manufacturer as to what the components of the detergent are, their ratios and possibly reference materials are not readily available unless secrecy agreements are made between the industry and the manufacturer. A previous report from our research group presented a cleaning validation protocol for the determination of residues of LpHse detergent using HPLC [10]. This article, presents an ion chromatography (IC) approach for the determination of CIP-100 Industrial detergent. In this method, we analyzed the amount of ethylenediamine tetraacetic acid (EDTA) as the measure of the amount of CIP-100 present in the samples under study.

The task of establishing a reliable method falls into the hands of analytical chemists. They must then establish how to determine the traces of the detergent, most often without actual knowledge of the components of the formulation. A further requirement of the analytical method is that its specificity and sensitivity must be established [11,12]. The specific case of detergent CIP-100 presented such an instance. This detergent has been formulated for clean-in-place systems or automated cleaning systems, phosphate-free and claims to be free rinsing. The CIP systems are characterized by the controls and programming required to reproduce the cleaning process over and

* Corresponding author. Tel.: +1 787 999 3016; fax: +1 787 999 8981.
*E-mail address:* jzayas@zaycor.com (J. Zayas).
*URL:* http://www.zaycor.com (J. Zayas).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.01.037

over with the same results in an automated fashion. From the MSDS, it can be seen that the components are water, potassium hydroxide, EDTA and surfactants. The ratio of components are approximated to percentages ranging from 5% to 20% of each component. Therefore, it is very difficult to attain the right amount for each component.

The first step in this case was to establish the amount of water present in the formulation. Typically, detergents range from 75% to 90% of water, the remainder being the "active" ingredients. The process was followed by the assumption that the surfactant would be the component most difficult to remove based on the solubility of the KOH and the EDTA. Thus, detection by means of conductivity would yield a signal proportional to the concentration of EDTA, which is directly proportional to the amount of detergent used and any traces extrapolated to the total detergent concentration present, after correcting by water. The advantage of this method over conventional UV detection is that no modifiers or derivatizing agents are necessary for detection. The method presented here is a direct injection method, not requiring further sample handling. This provides added efficiency and reduction of errors from sample handling.

## 2. Experimental

### 2.1. Equipment

The IC system consisted of a Metrohm-peak 761 compact Ion Chromatography system (Herisau, Switzerland) with conductivity detection and a computer with ICNet 2.1 computer software for data handling.

### 2.2. Materials and reagents

All solvents used were of HPLC and analytical-reagent grade. Water used for mobile phase, sample and standards preparations was obtained from a Barnstead NanoPure (Dubuque, IA, USA) system without further purification. The certified ACS sodium bicarbonate was obtained from Fisher (Fair Lawn, NJ, USA). The sodium carbonate and the EDTA were obtained from J.T. Baker (Philipsburg, NJ, USA). Stainless steel plates were 25 cm × 25 cm dimensions, made out of 304 un-polished material. The CIP-100 detergent was supplied by the Steris Corporation, lot 216811 (St. Louis, MO, USA).

### 2.3. Chromatographic conditions

The column used was a Metrohm-Peak Metrosep A Supp 5-150, polyvinyl alcohol with quaternary ammonium groups, 5 μm, and 4.0 mm × 150 mm with a mobile phase composed of sodium carbonate:sodium hydrogen carbonate (3.2:1.0 mM), flow rate of 0.7 mL/min. The injection volume used was 20 μL. The chromatographic experiments were run at room temperature (20 °C).

### 2.4. Mobile phase preparation

The sodium carbonate:sodium hydrogen carbonate (3.2 mM:1.0 mM) mobile phase was prepared by weighing 0.34 g of sodium carbonate and 0.084 g of sodium hydrogen carbonate dissolved them with deionized water and transferred to a 1.00 L volumetric flask and diluted to volume with deionized water. The mixture was properly filtered and degassed. This solution was used as the mobile phase, diluent for EDTA standards and CIP-100 working samples, and also as the extracting solution.

### 2.5. Preparation of the EDTA standards

The EDTA stock standard solution was prepared by weighing 0.0578 g of EDTA dissolved in deionized water and transferred to a 100.00 mL volumetric flask and diluted to volume with deionized water. The EDTA working solution was prepared by pippeting 5.00 mL of the stock solution to a 50.00 mL volumetric flask and to volume with deionized water. The resulting concentration for the EDTA anion in the stock standard solution and the working solutions were 516 and 51.6 ppm, respectively. From the working solution an aliquot was taken and diluted to volume with the sodium carbonate:sodium hydrogen carbonate (3.2:1.0 mM) mobile phase. Three replicates were prepared for each off the standards solutions. The final concentrations of the standards solutions are presented in Table 1.

### 2.6. Stock CIP-100 detergent sample preparation solutions

A 1.0 mL aliquot of a CIP-100 sample was placed in a 100.00 mL volumetric flask and taken to volume with purified water. Aliquots of this stock solution were further diluted in order to reach the desired concentration for these studies.

### 2.7. Preparation of the CIP-100 sample

The sample for the determination of EDTA concentration in the CIP-100 was prepared by pippeting 0.50 mL of the CIP-100 stock solution to a 10.00 mL volumetric flask and diluted to volume with purified water. From this solution, 2.50 mL were pippeted into a 10.00 mL volumentric flask and diluted to volume with the sodium carbonate:sodium hydrogen carbonate (3.2:1.0 mM) mobile phase. Two replicates were prepared for the CIP sample. The average EDTA concentration determined for this solution was 5.09 ppm by comparing the CIP-100 sample average area with the calibration curve of the EDTA standard solutions (see Table 3).

Table 1
EDTA standard preparation (10.00 mL final volume)

| Aliquot of EDTA working solution (mL) | Theoretical concentration of EDTA (ppm) |
|---|---|
| 0.25 | 1.29 |
| 0.50 | 2.58 |
| 1.00 | 5.16 |
| 2.00 | 10.3 |
| 4.00 | 20.6 |

### 2.8. Preparation for the recovery of CIP-100 from stainless steel surface

The solutions used for recovery from plate were prepared using aliquots from the CIP-100 stock solution. Aliquots of 5.00, 6.00 and 7.00 mL were pippeted to 25.00 mL volumetric flasks and diluted to volume with the sodium carbonate:sodium hydrogen carbonate (3.2:1.0 mM) mobile phase. From the determination of the CIP-100 sample the resulting concentrations for these solutions were 81.5, 97.7 and 114 ppm, respectively. A volume of 100 μL for each of these solutions was spread over a clean and dry 2 in. × 2 in. stainless steel plate. The procedure was repeated for each of the solutions. The metal plates were allowed to dry at room temperature. A TEXWIPE TX761 swab was deposited in a vial that contained 2.00 mL of purified water. For each deposited aliquot, a wet swab was passed over the surface of the plate, one side of the swab was passed horizontally and the other vertically. The swabbing process has been represented schematically elsewhere [10]. The swab was returned to a vial with 2.00 mL of purified water. The vials were shaken mechanically for 120 min and each of them analyzed by IC.

## 3. Results and discussion

### 3.1. System suitability

The ion chromatographic system suitability was evaluated according to the requirements set forth by the United States Pharmacopoeia (USP 27) [11,12]. System precision, theoretical plates ($N$) and tailing factor ($T$) were evaluated. The system precision was obtained from the pooled relative standard deviation (co-variance, percentage of $\text{R.S.D.}_{\text{pooled}}$) of three sets of replicate injections from different days and preparations. Each replicate set consisted of six consecutive injections. This afforded a percentage of $\text{R.S.D.}_{\text{pooled}}$ value of 1.56% by area response factor and 2.50% by height response factor. The average theoretical plates resulted in $N$ of 2900, and the tailing factor, $T$, was calculated at 1.3 on the average. The resolution factor $R$ was calculated against the chlorine peak and set at six based on average determinations. Fig. 1 shows a typical chromatogram for a system suitability run. Fig. 2 shows a typical blank chromatogram.

### 3.2. Repeatability and intermediate precision

The repeatability of the method was determined by using the response factor values obtained for a set of different concentrations. The set consisted of three consecutive injections for each of the three different concentrations. These were averaged and the pooled standard deviation determined ($S_{\text{pooled}}$). These values were used to calculate the pooled percentage of R.S.D. This afforded a percentage of $\text{R.S.D.}_{\text{pooled}}$ value of 2.17% by area response factor and 0.60% by height response factor.

The intermediate precision of the method was determined by using the response factor values for a set of different concentrations prepared by different analyst on the same day and by the same analyst on different days. Each set consisted of three consecutive injections for each of the three different concentrations. These were averaged and the pooled standard deviation determined ($S_{\text{pooled}}$). These values were used to calculate the pooled percentage of R.S.D. This afforded a percentage of $\text{R.S.D.}_{\text{pooled}}$ value of 1.88% by area response factor and 2.41% by height response factor. Table 2 shows the pooled chromatographic data used for the calculations.

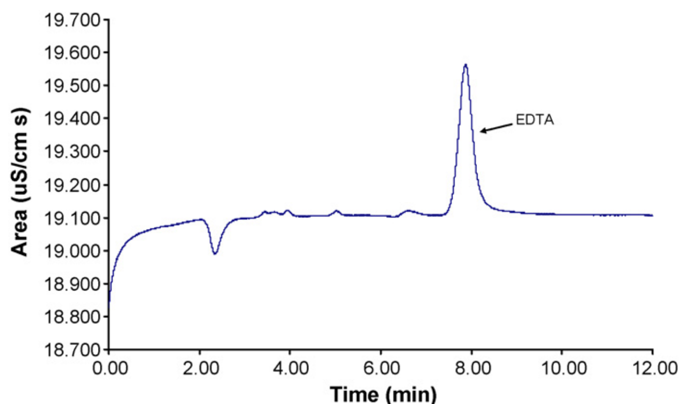


Fig. 1. Typical system suitability chromatogram of the EDTA Standard. Suitability ran at room temperature at 0.7 mL/min. $Na_2CO_3$–$NaHCO_3$: 3.2:1.0 mM. Conductivity detection.

### 3.3. Linearity

The linearity of the method was established by calculating the linear regression of multiple determinations at a concentration range from 1.29 to 20.6 ppm of EDTA standards. The data was combined to determine the linearity of the method. The calibration curve showed a sensitivity of 2.29 (μS/cm s)/ppm with correlation coefficient of 0.99999 for the area response factor and sensitivity of 0.0939 (μS/cm)/ppm with a correlation coefficient of 0.99999 for the height response factor. The method showed outstanding linearity over the concentration range analyzed. The data of the calibration curve of EDTA standards and the CIP-100 EDTA determination is shown in Table 3. Fig. 3 present a stacked arrangement of the typical chromatograms of the EDTA standards.

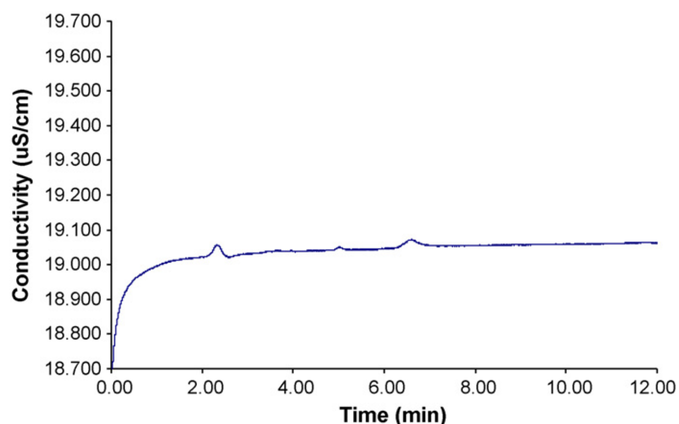


Fig. 2. Typical blank chromatogram. Ran at room temperature at 0.7 mL/min. $Na_2CO_3$–$NaHCO_3$: 3.2:1.0 mM. Conductivity detection.

Table 2
Pooled chromatographic data to asses intermediate precision

| Standard concentration (ppm) | Average area (μS/cm s) | Pooled standard deviation (μS/cm s) | Pooled R.S.D. % | Average height (μS/cm) | Pooled standard deviation (μS/cm) | Pooled R.S.D. % |
|---|---|---|---|---|---|---|
| 2.58 | 5.450 | 0.070 | 1.29 | 0.23 | 0.006 | 2.51 |
| 5.16 | 11.360 | 0.258 | 2.27 | 0.48 | 0.013 | 2.80 |
| 10.3 | 23.002 | 0.420 | 1.83 | 0.95 | 0.022 | 2.35 |

Table 3
Calibration curve and CIP-100 EDTA chromatographic determination data

| Standard concentration (ppm) | Average area (μS/cm s) | Standard deviation (μS/cm s) | R.S.D. % | Average height (μS/cm) | Standard deviation (μS/cm) | R.S.D. % |
|---|---|---|---|---|---|---|
| 1.29 | 2.861 | 0.083 | 2.9 | 0.11 | 0.00 | 0.00 |
| 2.58 | 5.794 | 0.008 | 0.1 | 0.23 | 0.00 | 0.00 |
| 5.16 | 11.706 | 0.067 | 0.6 | 0.47 | 0.00 | 0.00 |
| 10.3 | 23.283 | 0.131 | 0.6 | 0.95 | 0.01 | 0.61 |
| 20.6 | 46.980 | 0.366 | 0.8 | 1.93 | 0.02 | 0.79 |
| CIP-100 sample | 11.503 | 0.069 | 0.6 | 0.47 | 0.01 | 1.2 |

### 3.4. Limit tests

The detection limit (DL) and the quantitation limit (QL) was determined from the calibration curve of five different EDTA standard concentrations. The ICH guide [13] recommends as an alternative for the estimation of the detection (DL) and quantitation (QL) limits the following equation:

$$\frac{S}{N_{\text{Estimate}}} = \frac{S_{xy}}{\text{slope}}$$

where $S/N_{\text{Estimate}}$ is the approximation of the signal-to-noise ratio (semi-empirical) and $S_{xy}$ is the standard error of the intercept and the slope of the linear regression curve from the linearity determination. Multiplying the $S/N_{\text{Estimate}}$ by 3.3 and 10 affords the estimate of the DL and QL, respectively. This calculation yielded an estimated DL of 0.13 ppm, and an estimated QL of 0.39 ppm.

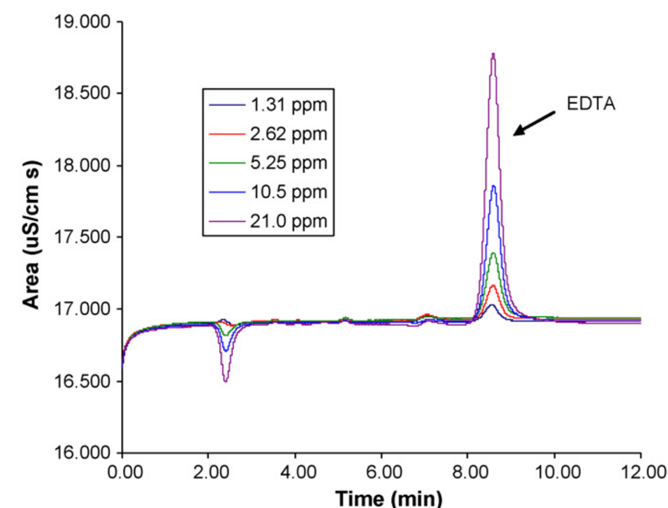


Fig. 3. Typical calibration chromatograms of the EDTA standards. Ran at room temperature at 0.7 mL/min. $Na_2CO_3$–$NaHCO_3$: 3.2:1.0 mM. Conductivity detection.

### 3.5. Determination of EDTA in CIP-100 detergent/recovery experiments

The accuracy of the method was established by means of two separate sets of experiments. The initial set of experiments was carried by extracting the detergent from cotton swabs. The average recovery was calculated to be 101%. This estimation was obtained by dividing the response factor of each concentration recovered and divided by the slope of the linear regression curve of the found versus theoretical concentration for the EDTA. This set of experiments demonstrated that the cotton swab does not interfere with the CIP-100 EDTA analysis.

The second set of experiments was to demonstrate recovery from a complete procedure involving the cotton removal of the detergent from the stainless steel plate, using different recovery solvents. The different solvents used and the respective % recovered are presented in Table 4. After evaluating the solvents included in Table 4, it was determined that water was a suitable solvent to extract the EDTA from the swab. The % recovered of the samples obtained from the stainless steel plates were calculated dividing the area of the samples recovered from the plates by the expected area calculated from a sample directly injected into the system and multiplying by 100.

Once the optimum solvent was selected, a set of recovery experiments was performed to assess the accuracy and precision of the method using CIP-100 samples. A concentration range going from 1.29 to 20.6 ppm of EDTA was used as the calibration curve. The CIP-100 samples were extracted form the

Table 4
Recovery solvents experiments data

| Recovery solvent | Average % recovered |
|---|---|
| 3.2 mM $Na_2CO_3$/1.0 mM $NaHCO_3$ | 53.7 |
| 3.2 mM $Na_2CO_3$/1.0 mM $NaHCO_3$/10% acetone | 53.1 |
| 3.2 mM $Na_2CO_3$/1.0 mM $NaHCO_3$/20% acetone | 70.1 |
| Purified water | 69.2 |

Table 5
Typical recovery experiment data for area response factor

| Deposited concentration (ppm) | Expected concentration (ppm) | Average area (μS/cm s) | Calculated concentration (ppm) | % Recovered |
|---|---|---|---|---|
| 81.5 | 4.07 | 5.987 | 3.45 | 65.6 |
| 97.7 | 4.89 | 7.448 | 4.13 | 67.7 |
| 114 | 5.70 | 8.086 | 5.18 | 63.0 |

Table 6
Typical recovery experiment data for height response factor

| Deposited concentration (ppm) | Expected concentration (ppm) | Average height (μS/cm) | Calculated concentration (ppm) | % Recovered |
|---|---|---|---|---|
| 81.9 | 4.10 | 0.26 | 3.45 | 71.2 |
| 98.3 | 4.91 | 0.32 | 4.13 | 71.3 |
| 114 | 5.73 | 0.36 | 5.18 | 69.5 |

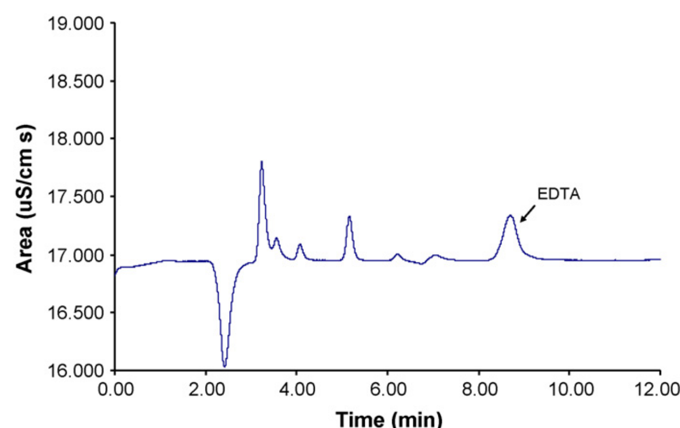


Fig. 4. Typical recovered CIP-100 chromatogram. Chromatogram ran at room temperature at 0.7 mL/min. $Na_2CO_3$–$NaHCO_3$: 3.2:1.0 mM. Conductivity detection.

cotton swab using water as the extracting solvent. One hundred microliters of diluted CIP-100 deposited on the stainless steel plates was diluted further in 2.00 mL of water and after that 20 μL of that was injected into the ion chromatographic system. The average % recovery of the CIP-100 samples was calculated to be 71.4% for the area response factor, and 71.0% for the height response factor. Tables 5 and 6 presents the data obtained from a typical recovery experiment of CIP-100 from the stainless steel plates for area response factor and the height response factor, respectively. Fig. 4 shows a typical chromatogram of the ion chromatography analysis of EDTA contained in a CIP-100 detergent sample.

## 4. Conclusions

The proposed ion chromatographic method has been evaluated over the linearity, precision, accuracy and selectivity and proved to be convenient and effective for the quality control of cleaning validation samples. Other methods for determination of these CIP samples are done by determination of the complexing component in the detergent formulation. The method is fast and reliable affording turn around times convenient for the quality control laboratory. Solvents are mostly aqueous and its consumption is low which makes the method environmentally friendly. The DL and QL of the method are less than 1 ppm, which makes it excellent for determination of traces of CIP-100 in cleaning validation determination.

## Acknowledgment

Darimar Hernández gratefully acknowledges NIH grant number 5R25 GM59429-08 for financial support.

## References

[1] 21 CFR 211.67, Equipment Cleaning and Maintenance.
[2] 21 CFR 211.160 (b), Laboratory Controls.
[3] Guide to Inspections of Cleaning Validation, FDA, 1993.
[4] M. Brindusa Boca, Z. Apostolides, E. Pretorius, J. Pharm. Biomed. Anal. 37 (2005) 461–468.
[5] Z. Katona, L. Vincze, Z. Végh, A. Trompler, K. Ferenczi-Fodor, J. Pharm. Biomed. Anal. 22 (2000) 349–353.
[6] T. Mirza, M.J. Lunn, F.J. Keeley, R.C. George, J.R. Bodenmiller, J. Pharm. Biomed. Anal. 19 (1999) 747–756.
[7] M.J. Nozal, J.L. Bernal, L. Toribio, M.T. Martin, F.J. Diez, J. Pharm. Biomed. Anal. 30 (2002) 285–291.
[8] R. Klinkerberg, B. Streel, A. Ceccato, J. Pharm. Biomed. Anal. 32 (2003) 345–352.
[9] P. Yang, K. Burson, D. Feder, F. Macdonald, Pharm. Technol. 29 (2005) 84–94.
[10] J. Zayas, H. Colón, O. Garced, L.M. Ramos, J. Pharm. Biomed. Anal. 41 (2006) 589–593.
[11] Food and Drug Administration (FDA) Guidance Document for Industry "Analytical Procedures and Methods Validation", August 2000.
[12] The United States Pharmacopoeia, USP 27, 2004 Chapter ⟨1225⟩, Validation of Compendial Methods.
[13] International Conference on Harmonization (ICH-Q2B) Text on "Validation on Analytical Procedures: Methodology Q2B", November 1996.