Validation of UV Spectrophotometric and HPLC Methods for Quantitative Determination of Atenolol in Pharmaceutical Preparations

Anelise WEICH, Daniele Carvalho de OLIVEIRA, Janine de MELO,
Karin GOEBEL & Clarice Madalena Bueno ROLIM *

*Department of Industrial Pharmacy, Health Science Center,
Federal University of Santa Maria, 97.105-900 - Santa Maria-RS, Brazil*

SUMMARY. A rapid and sensitive RP-HPLC method with UV detection and UV spectrophotometric method for routine control of atenolol in tablets was developed. Chromatography was performed with mobile phase containing a mixture of 10 mM ammonium acetate buffer (pH 7.0) and acetonitrile (80:20 v/v). The samples were injected onto Purospher RP-18 (250 mm x 4.6 mm, 5 μm) column. The flow rate was 0.8 ml.min^{-1}. The samples were detected at 275 nm. The assay was linear in range from 125 to 375 μg.ml^{-1} with a correlation coefficient (r=0.9999) highly significant for the method. The accuracy was 99.80%. The UV spectrophotometric method was performed at 226 nm, and samples were prepared with a solution of sodium acetate. The linearity demonstrated a correlation coefficient of 0.9986. The proposed methods were simple, rapid, precise, accurate and sensitive, and can be used for the routine of the quality control in pharmaceuticals.

RESUMEN. "Validación de Métodos por Espectrofotometría Utravioleta y HPLC para la Determinación Cuantitativa del Atenolol en Preparaciones Farmacéuticas" . Se ha desarrollado y validado un método de cromatografía líquida y espectrofotometría ultravioleta para la determinación de comprimidos de atenolol. La fase móvil empleada fue buffer acetato de amonio 10 mM (pH 7.0) y acetonitrilo (80:20 v/v). Las muestras se inyectaron en una columna Purospher RP-18 (250 mm x 4,6 mm, 5 mm), con una velocidad de flujo de 0,8 ml.min^{-1}. Las muestras se detectaron a 275 nm. El ensayo resultó lineal en el rango de concentración de 125 a 375 μg.ml^{-1} con un coeficiente de la correlación (r = 0,9999) muy significativo para el método. La exactitud del método fue de 99,8 %. El método por espectrofotometría ultravioleta se realizó a 226 nm y las muestras se preapararon en una solución de acetato de sodio. La linearidad fue adecuada (r = 0,9986). Los métodos propuestos son simples, rápidos, precisos, exactos y sensibles y por lo tanto pueden usarse para la rutina de control de calidad en productos farmacéuticos.

INTRODUCTION

Atenolol, designated chemically as (RS)-4-(2-hidroxy-3-isopropylaminopropoxy) phenylacetamide, is commercially available as a racemic mixture (Fig. 1), it is found in the form of tablets, oral solution, and sterile solution for injectable. Atenolol (ATN) is a β1-selective (cardioselective) β-adrenergic receptor-blocking agent without membrane-stabilizing or intrinsic sympathomimetic (partial agonist) activities [1]. ATN is also used to treat myocardial infarction (heart attack), arrhythmias (rhythm disorders), angina (chest pains), and disorders arising from decreased circulation and vascular constriction, including migraine [2].

In the European Pharmacopoeia [3], the described method for atenolol quantification uses technique of titration with acid percloric. In the British Pharmacopoeia [4] the indicated methods use spectrophotometric in the ultraviolet at 275 nm. Moreover in the American Pharmacopeia [5], it describes the method using high-performance liquid chromatography (HPLC), which uses as mobile phase heptanosulphonate of sodium,
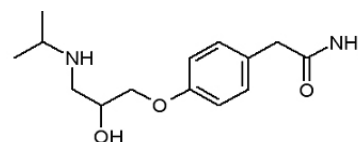

Figure 1. The chemical structure of atenolol.

KEY WORDS: Atenolol, Dosage forms, HPLC, UV Spectrophotometry.
PALABRAS CLAVE: Atenolol, Espectrofotometria ultravioleta, HPLC, Productos farmacéuticos.

* Author to whom correspondence should be addressed. *E-mail:* crolim@smail.ufsm.br

phosphate biphasic of sodium and methanol, with detention in 226 nm.

Other described methods in literature has revealed that several methods such as capillary electrophoresis [6,7], ultra-performance liquid chromatography (UPLC) [8], titrimetric, spectrophotometric and kinetic methods [9] and high-performance liquid chromatography (HPLC) have been reported for the analysis of ATN either in pharmaceutical preparations [5,8-12] or in biological fluids [13-20].

The HPLC method has been highly used for the quality control of drugs due to its sensitivity, reproducibility and specificity. The UV spectrophotometric method is very simple, rapid, economical, and it allows the determination in pharmaceuticals with enough reliability.

Some published works, have reported methods of quantification of atenolol in formulations through Reversed-Phase HPLC method [21] and HPLC with detention in the UV [15,18,22-24], these methods were developed and validated for the determination of atenolol in tablets. Studies with comparative analyses among the methods using high-performance liquid chromatographic and liquid chromatography-mass spectrometry (LC-MS) [15] and ultra-performance liquid chromatography (UPLC) [8] were reported.

There are few articles on HPLC published for the analysis in dosage forms, and these methods adopted a mobile phase containing heptanosulfonate as ion-paring agent, which is very expensive [5,11]. Some studies have used a mobile phase without heptanosulfonate [8-10], which needed internal standard [9], and others do not show the complete method validation.

For the UV spectrophotometric method, the survey of literature revealed very complex methods, using bands of the visible which have used complexometry [25-28], derivative or chemometric assistance [22] and interpolation on the calibration curve [29,30]. In 2006, some studies carried through for Kuban *et al.* [10] compare methods of HPLC of detention for condutivity with the method of detention for UV.

The aim of this work was the development and fully validation of a new HPLC method and a new UV spectrophotometric method, which can be more economical and simpler than the official methods and with other methods published for the quantitative analysis of ATN in dosage forms. The proposed HPLC method needs no ion-paring agent and internal standard, while the other published chromatographic methods use either internal standards or heptanossulfonate as ion-paring agent. The UV spectrophotometric method is simpler than the others studied because it does not need derivative and chemometric assistance. Moreover, this method can be used in dissolution studies because it uses its own dissolution medium as diluent.

MATERIALS AND METHODS
Reagents

The ATN reference standard was obtained from the Brazilian Pharmacopoeia. Pharmaceutical dosage forms (Atenol®) containing 25 mg, 50 mg and 100 mg of ATN were obtained commercially. Acetonitrile, ammonium acetate for chromatography, and sodium acetate p.a. were purchased from Merck or Synth, and heptanosulfonate was purchased from Vetec. The water used in the HPLC experiments was ultrapure obtained from Milli-Q Gradient A10 (Millipore), and distilled water was used to prepare all the solutions for the UV spectrophotometric experiments.

Instrumentation and analytical conditions

The HPLC method was performed on a Shimadzu LC-20AT HPLC system, equipped with a model LC-20AT pump, Diode Array Detector (DAD) SPD-M20A, autosampler SIL-20A, and an oven for the column model CTO-10AS. The method was conducted using a reversed-phase. The samples of 20 µl were injected onto a Purospher Star® RP-18e (250 mm x 4.6 mm i.d., 5 µm), Merck column. ATN was eluted with a flow rate of 0.8 ml.min^{-1} using a mobile phase consisting of 10 mM ammonium acetate buffer (pH 7.0) and acetonitrile (80:20 v/v). The wavelength of the UV-DAD detector was set to 275 nm. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter (Millipore) and sonicated before use. The temperature of the oven was 40 °C. The UV spectrophotometric method was performed on a UV-vis Spectrophotometer SB 1810 (Spectro Vision) at 226 nm using 1.0 cm quartz cells. The samples were prepared with a sodium acetate solution. The UVWin 5.0 Software was used for all absorbance measurements.

Preparation of the standard solutions

For the HPLC method, 50.0 mg of ATN reference standard was accurately weighted, transferred to a 100 ml volumetric flask and dissolved in the mobile phase. 5.0 ml of this solution was diluted to 10.0 ml with mobile phase. Concentrations of 125, 187.5, 250, 312.5 and 375 µg/ml were prepared.

For the UV Spectrophotometric method, 27.76 mg of ATN reference standard was accurately weighted, transferred to a 100 ml volumetric flask and dissolved in a sodium acetate solution. 1.0 ml of this solution was diluted to 20.0 ml with a sodium acetate solution. Concentrations of 8.28, 11.04, 13.88, 15.18 and 16.60 µg.ml^{-1} were prepared.

Preparation of the sample solutions
HPLC method
Twenty tablets were weighted and pulverized by gentle grinding. An accurately weighted amount of powder of ATN tablets, equivalent to 50.0 mg of ATN, was transferred to a volumetric flask of 100 ml and dissolved in the mobile phase. 5.0 ml of this solution was diluted to 10.0 ml with mobile phase (250 µg.ml^{-1}).

UV Spectrophotometric method
An accurately weighted amount of powder of ATN tablets, equivalent to 27.76 mg of ATN, was transferred to a 100 ml volumetric flask and dissolved in a solution of sodium acetate. 1.0 ml of this solution was diluted to 20.0 ml with a sodium acetate solution (13.88 µg.ml^{-1}).

Method validation
The methods were validated according to International Conference on Harmonization (ICH) guidelines [31] for validation of analytical procedures. The System Suitability Test was carried through for both the methods evaluating theoretical plates and asymmetry.

Linearity
The calibration curve was obtained with five concentrations of the standard solution (125-375 µg.ml^{-1} for HPLC method and 8.28 - 16.60 µg.ml^{-1} for UV spectrophotometric method). The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision
The assay precision was carried out by repeatability (within-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples at same concentration and during the same day. The intermediate precision was studied by comparing the assays on three different days. Six sample solutions (250 µg.ml^{-1} for HPLC method and 13.88 µg.ml^{-1} for UV spectrophotometric method) were prepared and assayed in triplicate.

Accuracy
The accuracy of an analytical method is the closeness of the test results obtained by the method to the true value. For the spectrophotometric method, the accuracy was determined by recovery of known amounts of atenolol reference standard added to the samples at the beginning of the process. The accuracy was assessed from five replicate determinations of three concentration levels: 11.04, 13.88 and 16.60 µg.ml^{-1}. The absolute means obtained were 100.61% (within-day) and 100.66% (inter-day) as shown in Table 2, and it is evident that the method is accurate within the desired range. For the HPLC method, the accuracy was determined by the assay of three concentrations of the sample solution (200, 250, and 300 µg.ml-1) in triplicate. The absolute means obtained were 99.80% (within-day) and 99.13% (inter-day) as shown in Table 2.

Specificity
The specificity of the HPLC and UV spectrophotometric methods was determined by using SSE. The specificity of the HPLC method was assessed by spiking the ATN sample solution with known quantities of SSE 25, 62.5 and 125 µg.ml^{-1}. In addition, the sample solutions (250 µg.ml^{-1}) were submitted to accelerated degradation by heat (35 °C) and to the addition of 1.0 N NaOH for 12 h, 0.5 N HCl for 12 h, and H_2O_2 10% for 12 h to verify that none of the degradation products of the ATN interfered with the analysis. For the UV spectrophotometric method, the specificity was obtained by the absorption spectra of the ATN reference standard 13.88 µg/ml and by the use of the simulated sample of excipients.

Robustness
The robustness of the HPLC method was determined by the analysis of the samples under a variety of conditions, such as small changes in the pH (7.0-6.5), percentage of acetonitrile (20-15%) in the mobile phase, flow rate (0.8-0.9 ml.min^{-1}), temperature of the oven (40-35 °C) and column lot. The effects on retention time, quantification of ATN and peak parameters were studied. The robustness of the UV spectrophotometric method was determined by small changes in the pH (4.6- 4.1), temperature of solution (25-20 °C) and solvent lot.

Limit of detection (LOD) and quantification (LOQ)
LOD was determined using the signal-to-

noise ratio, and then comparing the test results from the samples with known concentrations. The analyte concentration that produced a signal-to-noise ratio of 3:1 was accepted as LOD. Limit of quantification (LOQ) is defined as the lowest concentration of the analyte that can be determined with acceptable precision and accuracy under the stated experimental conditions

Official method

The samples were prepared as official monographs in the United States Pharmacopoeia. The mobile phase was prepared dissolving 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 ml of water, and adding 2 ml of dibutylamine. Afterwards, 0.8 M phosphoric acid was used to adjust the pH 3.0, and 300 ml of methanol was added. The chromatographic system was equipped with 226 nm detector and a 3.9 mm x 30 cm column C 18. The flow rate was about 0.6 ml.min^{-1}.

RESULTS AND DISCUSSION
HPLC method

The mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figure 2 shows a typical chromatogram which was obtained from the analysis of a standard and a sample solution of ATN using the proposed method. As shown in figure 2, ATN was eluted forming a symmetrical peak. The retention time observed (2.7 min) allowed a rapid determination of the drug, which is important for routine analyses. The calibration curves for ATN were constructed by plotting peak area *vs.* concentration, and

showed good linearity within 125-375 µg.ml^{-1} range. The representative linear equation was y = 4819658.1x + 42479.9, with a correlation coefficient (r = 0.9999) highly significant for the method (Table 1).

LOD and LOQ were found to be 0.2 and 0.4 µg/ml, respectively, indicating high method sensitivity. The method precision was determined by repeatability (within-day) and intermediate precision (inter-day), and was expressed as R.S.D (%) of a series of measurements. The result obtained showed R.S.D of 0.95% indicating good precision (within-day). Inter-day variability was calculated in three different days and it showed a mean R.S.D of 0.53%. The accuracy of the method was determined and the mean recovery was found to be 99.80 % within day and 99.13% inter-day, indicating an agreement between the true value and the found value (Table 2). The HPLC method is specific to the evaluation of ATN in tablets. No interfering peaks were observed when simulated samples of excipients were added in the sample solutions. When sample solutions were degradated (H$_2$O$_2$ 10% for 12 h), the relative retention time of the peaks of the degradation products were different from the peak for ATN (2.7 min) (Fig. 3). Besides, the samples stayed intact with the addition of 1.0 N NaOH for 12 h and 0.5 N HCl for 12h. The method was found to be robust when the pH, mobile phase, flow rate, temperature of the oven and column lot were varied.

UV Spectrophotometric method

The proposed spectrophotometric method allowed a rapid and accessible quantitation of atenolol in tablets without any time-consuming
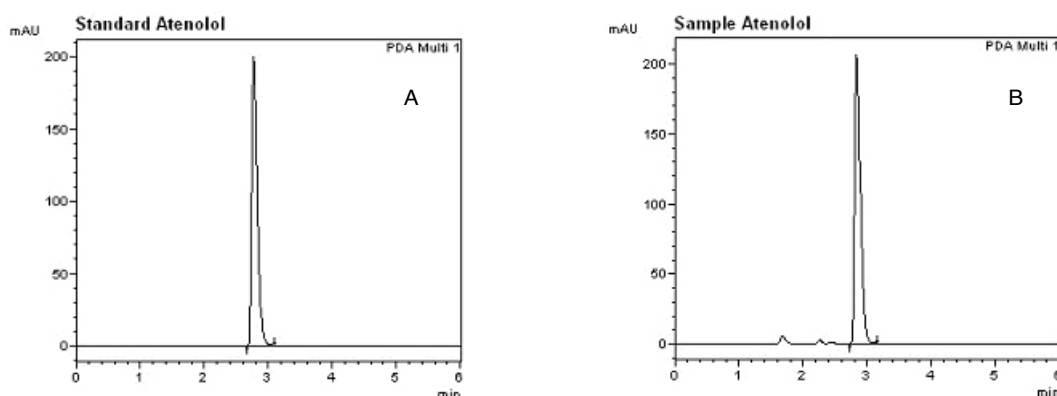
Figure 2. Representative peaks of (A) ATN reference standard 250 µg/ml and (b) ATN tablets 250 µg/ml. Conditions: column Purospher RP-18e (250 mm x 4.6 mm i.d., 5 µm); mobile phase, ammonium acetate buffer-acetonitrile (80:20 v/v); flow rate, 0,8 ml.min^{-1}; UV-DAD detection at 275 nm; temperature of over 40 °C; injection volume, 20 µL.

Statistical parameters	HPLC Method	UV Method
Regression equation*	y = 4819658.1x + 42479.9	y = 31.465x + 0.0141
Correlation coefficient (r)	0.9999	0.9986
Standard error of slope	44925.4	0,6326
Standard error of intercept	5156.6	0.0083
Concentration range (mg/ml)	125-375	0.008-0.016

Table 1. Results of the regression analysis of data for the quantitation of atenolol by the proposed methods.
* Based on three calibration curves.

Method	Precision (R.S.D)		Accuracy (%)		LOD $\mu(g/ml)$	LOQ $\mu(g/ml)$
	Within-day	Inter-day	Within-day	Inter-day		
HPLC	0.95	0.53	99.80	99.13	0.2	0.4
UV	0.81	1,28	100.61	100.66	0.4	0.5

Table 2. Results of the validation of the HPLC and UV spectrophotometric methods for atenolol tablets.
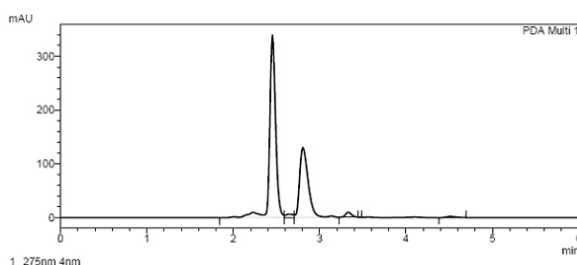
Figure 3. Chromatogram from accelerated degradation of (A) ATN 250 µg/ml and H2O2 10% for 12 h.
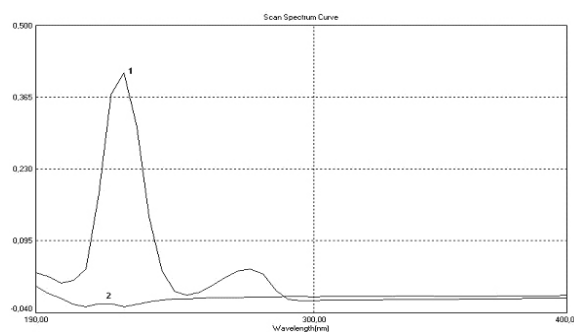
Figure 4. The absorption spectra of the ATN reference standard 13.88 µg/ml (**1**) and of the simulated sample of excipients (**2**).

sample preparation. Moreover, the spectrophotometric method involved simple instrumentation compared with other instrumental techniques. The absorption spectra of atenolol showed λ_{max} was 226 nm, which was the wavelength used. The calibration curves were constructed in the range of expected concentrations (8.28 - 16.60 µg.ml^{-1}). The representative equation analysis was y=31.465x + 0.0141, with a correlation coefficient of 0.9986 (Table 1). LOD and LOQ were found to be 0.4 and 0.5 µg/ml,

respectively, showing that the experimental values obtained for the determination of atenolol in the samples indicated a satisfactory intra-day variability (R.S.D. of 0.81%) and inter-day variability (R.S.D. of 1.28%). A good accuracy of the method was verified with a mean recovery of 100.61% within day and 100.66 % inter-day (Table 2). Finally, the method showed to be specific for the determination of ATN in tablets (Fig. 4).

Comparison between HPLC, UV Spectrophotometric and the official method

The proposed analytical methods were compared using statistical analysis. ANOVA was applied and revealed no significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated F-value (F_{calc} = 3.29) was found to be less than the tabled F-value (F_{tab}= 6.36) at 1% significance level (Table 3).

The HPLC method and the UV method developed and validated for the analysis of atenolol in tablets were found to be reliable, simple, fast, precise, accurate and sensitive. Results of UV spectrophotometric method showed no significant difference from those obtained with the method of HPLC and the official method ($P > 0.01$). The purpose of the new spectrophotometric method is not to replace the available methods for the analysis of atenolol with HPLC, but to serve as an alternative method to be used where advanced instruments e.g. HPLC are not available for routine analysis. Moreover, the HPLC method is suitable for the investigation of the atenolol chemical stability. The methods had presented good eficiencia.

The teoricos plates had been inside of the waited one and asymmetry was minor who 2%. In summary, the proposed method can be used for the routine of quality control in pharmaceuticals.

REFERENCES

1. Physician's Desk Reference-PDR (1999) Medical Economics Company, Inc; Montvale,
2. Wadworth, A.N., D. Murdoch & R.N. Brodgen (1991) *Drugs* **42**: 468-510.
3. European Pharmacopoeia (2005) 5 Ed, France. Conciul of Europe. V 1 and 2.
4. British Pharmacopoeia (2007) London. Her Majesty's Stationary Office. V 1 and 2.
5. U.S. Pharmacopeia (2006) 29th Ed., U. S. Pharmacopoeial Convention, Inc., Rockville, pp. 211-3.
6. Coragem Briguenti, A.C. & P.S. Bonato (2005) *Drug Dev. Ind. Pharm.* **31**: 209-14.
7. Shafaati, A., & B.J. Clarck (1996) *J. Pharm. Biomed. Anal.* **14**: 1547-54.
8. Wren, S.A.C., & P.J. Tchelitcheff (2006) *J. Pharm. Biomed. Anal.* **40**: 571-80.
9. Basavaiah, K., U. Chandrashekar & P. Nagegowda (2006) *J. Serb. Chem. Soc.* **71**: 553-63.
10. Andrisano, V., R. Gotti, A. Leoni, & V.J. Cavrini, (1999) *J. Pharm. Biomed. Anal.* **21**: 851-7.
11. Kuban, P., E.M. Abad-Villar & P.C. Hauser (2006) *J. Chromatogr. A.* **1107**: 159-64.
12. Cuadrado, A., A. Rodriguez Gascon, R.M. Hernandez, A.M. Castilla, A. de la Maza, A.L. Oscariz, A., B.Calvo & J.L. Pedraz (2002) *Arzneimittelforschung* **52**: 371-8.
13. Delamoye, M., C. Duverneuil, F. Paraire, P. Mazancourt, & J.C. Alvarez (2004) *Forensic Sci. Int.* **141**: 23-31.
14. Park, Y., D.W. Lee & W. Lee (2002) *Anal. Chim. Acta* **471**: 51-9.
15. Ranta, V. P., E. Toropainen, A. Talvitie, S. Auriola, & A. Urtti (2002) *J. Chromatogr. B.* **772**: 81-7.
16. Abdel-Hamid, M. E. (2000) *Il Farmaco* **55**: 136-45.
17. Giachetti, C., A. Tenconi, S. Canali & G. Zanolo (1997) *J. Chromatogr B.* **698**: 187-94.
18. Modamio, P., C.F. Lastra, O. Montejo & E.L. Marifio (1996) *Int. J Pharm.* **30**: 137-40.
19. Miller, R.B. (1991) *J. Pharm. Biom. Anal.* **9**: 849-53.
20. Alebié-Kolbah, T., F. Plavsié, & A. Wolf-Coporda (1989) *J. Pharm. Biom. Anal.* **7**: 1777-81.
21. Ceresole, R., M.A. Moyano, M.T. Pizzorno & A.I. Segall (2006) *J. Liquid Chromatogr. Rel. Technol.* **29**: 3009-19.
22. El-Gindy, A., S. Emara & A. Mostafa (2005) *Farmaco* **60**: 269-78.
23. Singh, A.K., E.R.M. Kedor-Hackmann & M.I.R.M. Santoro (2001) *J. Am. Ofic. Anal. Chem. Internat.* **84**: 1724-1729
24. Sivakumar, T., P. Venkatesan, R. Manavalan & K. Valliappan(2007) *Indian J. Pharm. Sci.* **69**: 154-7.
25. Gölcü, A., C. Yücesoy & S. Serin (2004) *Farmaco* **59**: 487-92.
26. Prasad, C.V.N., C. Parihar, & P. Parimoo (1998) *J. Pharm. Biom. Anal.* **17**: 877-84.
27. Al-Ghannam, S.M. (2006) *J. Pharm. Biom. Anal.* **40**: 151-6.
28. Salem, H. (2002) *J. Pharm. Biom. Anal.* **29**: 527-38.
29. Vetuschi, C. & G. Ragno (1990) *Int. J. Pharm.* **65**: 177-81.
30. Kasture, A.V. & M. Ramteke (2006) *Indian J. Pharm. Sci.* **68**: 394-6.
31. International Conference on Harmonisation (2005) *Validation of analytical procedures: Text and Methodology,* Geneva, Switzerland.