DRUG FORMULATIONS AND CLINICAL METHODS

Simultaneous Assay of Olanzapine and Fluoxetine in Tablets by Column High-Performance Liquid Chromatography and High-Performance Thin-Layer Chromatography

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This paper describes validated high-performance liquid chromatographic (LC) and high-performance thin-layer chromatographic (TLC) methods for the simultaneous estimation of olanzapine and fluoxetine in pure powder and tablet formulations. The LC separation was achieved on a Lichrospher 100 RP-180, C18 column (250 mm, 4.0 mm id, 5 μm) using 0.05 M potassium dihydrogen phosphate buffer (pH 5.6 adjusted with *o*-phosphoric acid)–acetonitrile (50 + 50, v/v) as the mobile phase at a flow rate of 1 mL/min and ambient temperature. The TLC separation was achieved on aluminum sheets coated with silica gel 60F_{254} using methanol–toluene (40 + 20, v/v) as the mobile phase. Quantitation was achieved by measuring ultraviolet absorption at 233 nm over the concentration range of 10–70 and 40–280 μg/mL with mean recovery of 99.54 ± 0.89 and 99.73 ± 0.58% for olanzapine and fluoxetine, respectively, by the LC method. Quantitation was achieved by measuring ultraviolet absorption at 233 nm over the concentration range of 100–800 and 400–3200 ng/spot with mean recovery of 101.53 ± 0.06 and 101.45 ± 0.35% for olanzapine and fluoxetine, respectively, by the TLC method with densitometry. These methods are simple, precise, and sensitive, and they are applicable for simultaneous determination of olanzapine and fluoxetine in tablet formulations.

Olanzapine is an antipsychotic agent, chemically a thienobenzodiazepine described as a 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*] [1,5] benzodiazepine (1). Fluoxetine is an antidepressant agent, acting as a selective serotonin re-uptake inhibitor (SSRI), chemically described as a (±)-*N*-methyl-3-phenyl-3-[(α,α,α,-trifluoro-*p*-tolyl)oxy] propylamine (2). Olanzapine in combination with fluoxetine is used in treatment-resistant depression (TRD). The combination of olanzapine and fluoxetine produced robust, sustained increases of extracellular levels of dopamine and norepinephrine, which were significantly greater than with either drug alone. This combination produced a slightly smaller increase of serotonin than fluoxetine alone (3).

A literature survey found different analytical methods involving column high-performance liquid chromatography (LC) for determination of olanzapine in human plasma (4–6), rat plasma (7), rat brain tissue (8), rat brain using coulometric detection (9), and human blood by LC/tandem mass spectrometry (LC/MS/MS; 10). Determination of olanzapine in suspension by LC for study of stability (11) is also reported. Reports concerning determination by LC, capillary zone electrophoresis (CZE), derivative spectrometry, and linear voltammetry for the quantitation of olanzapine in tablets have been published (12). Recently, olanzapine has been determined by spectrophotometric procedures: one direct method was based on oxidation of the drug with excess of *N*-bromosuccinimide in acidic medium, and 2 indirect methods were based on the oxidation of the drug with excess of *N*-bromosuccinimide and cerium (IV) sulfate, followed by the reaction of the unconsumed oxidants with celestine blue (13).

Reports are available for determination of fluoxetine hydrochloride using LC with chiral stationary phases (14), gas chromatography (GC; 15), and LC both in direct (16) and reverse (17, 18) modes and also with precolumn derivation (19); in human plasma by LC with UV detection (20); and using a rapid LC diode array detection (DAD) method (21), LC/MS/MS detection (22), LC with fluorimetric detection (23), for plasma analysis (24), and spectrophotometry (25, 26).

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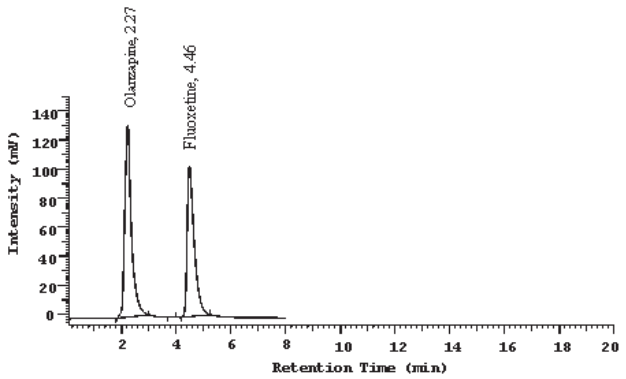

Figure 1. Column liquid chromatogram of olanzapine and fluoxetine and corresponding retention times (2.27 min for olanzapine and 4.46 min for fluoxetine) with detection at 233 nm.

So far, no LC or TLC method has been reported for the estimation of olanzapine and fluoxetine in combined dosage forms. This paper describes precise, specific, accurate, and sensitive LC and TLC methods for simultaneous estimation of olanzapine and fluoxetine in tablets.

Experimental

Apparatus

A Merck-Hitachi isocratic LC instrument equipped with a Hitachi L-7420 UV-visible (UV-Vis) detector, Rheodyne universal injector 77251 with an injection volume of 20 μL, and a Lichrospher 100 RP-180, C18 column (250 mm, 4.0 mm id, 5 μm particle size) was used. For TLC, a Camag (Muttens, Switzerland) Linomat V automatic sample applicator, Camag Scanner III, Camag WinCATS software, Hamilton 100 μL syringe (Anchrom Enterprises Pvt. Ltd, Mumbai, India), Camag twin trough developing chamber (for 10 × 10 cm plates), and viewing cabinet with dual wavelength UV lamps was used. TLC plates used were 10 × 10 cm silica gel $60F_{254}$, layer thickness 0.2 mm, with aluminum backing (E. Merck, Mumbai, India).

Reagents and Materials

Olanzapine and fluoxetine standards were procured as a gift sample from Sun Pharmaceuticals Ltd (Baroda, India) with 99.96 and 99.95% purity, respectively. Tablets containing 5 mg olanzapine and fluoxetine hydrochloride USP equivalent to 20 mg fluoxetine were procured from the local market (Olanex-F, Ranbaxy Laboratories Ltd, Secunderabad, India). LC grade acetonitrile and water were purchased from Ranbaxy Fine Chemicals Ltd (New Delhi, India). Potassium dihydrogen phosphate and *o*-phosphoric acid were procured from SD Fine Chemicals Ltd (Mumbai, India) and were of analytical grade.

Chromatographic Conditions

(**a**) *LC method.*—A Lichrospher 100 RP-180 C18 column was used at ambient temperature. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer (pH 5.6 adjusted with *o*-phosphoric acid)–acetonitrile (50 + 50, v/v) and was pumped at a flow rate of 1 mL/min. The mobile phase was filtered through a nylon 0.45 μm, 47 mm membrane filter and degassed before use. The eluent was monitored at 233 nm, and the injection volume was 20 μL.

(**b**) *TLC method.*—Solutions of olanzapine and fluoxetine were applied to silica gel $60F_{254}$ TLC plates (10 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 μL syringe and operated with a settings of band length 6 mm, distance between bands 8 mm, distance from the plate edge 12 mm, and distance from the bottom of the plate 15 mm. The plate was developed for a distance of 7 cm in a twin trough chamber previously saturated for 30 min with the mobile phase methanol–toluene (40 + 20, v/v). The spots on the air-dried plates were scanned with a Scanner III at 233 nm in the absorption mode.

Preparation of Olanzapine and Fluoxetine Standard Stock Solutions

Olanzapine (5 mg) and fluoxetine (20 mg) were transferred to 50 mL volumetric flask and dissolved in and diluted to mark with methanol to obtain a mixed standard solution of olanzapine (100 μg/mL) and fluoxetine (400 μg/mL) for the LC and TLC method.

Preparation of Sample Solutions

Powder of 20 tablets was weighed and analyzed as follows: A mass of powder equivalent to 1 tablet was weighed and transferred into a 50 mL volumetric flask, and methanol (40 mL) was added. The suspension was sonicated for 15 min, and the final volume was made up to the mark with methanol to obtain solution of olanzapine (100 μg/mL) and fluoxetine (400 μg/mL). The mixture was then filtered through a nylon 0.20 μm, 47 mm membrane filter.

Validation of the Method

(**a**) *Calibration curve (linearity of the LC method).*—Calibration curves were constructed by plotting

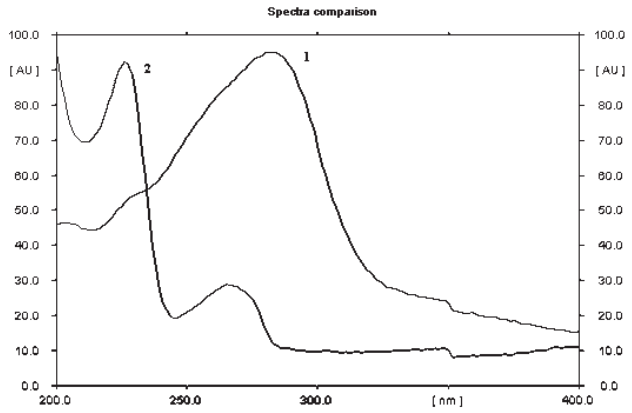

Figure 2. Absorbance spectra of olanzapine (1) and fluoxetine (2).

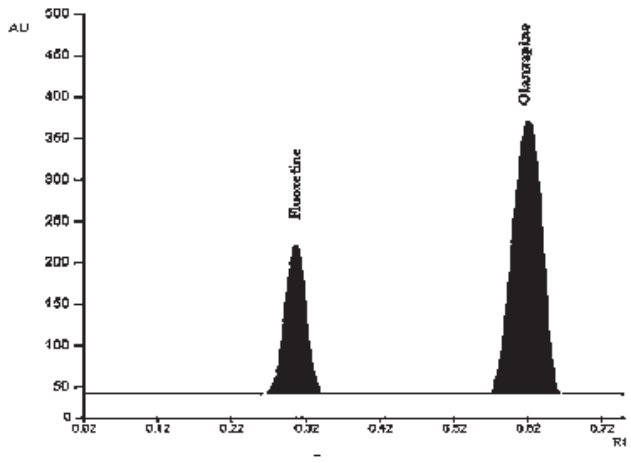

Figure 3. TLC densitogram of olanzapine and fluoxetine with scanning at 233 nm.

peak areas vs concentrations of olanzapine and fluoxetine, and the regression equations were calculated. The calibration curves were plotted over the concentration ranges of 10–70 and 40–280 μg/mL for olanzapine and fluoxetine, respectively. Aliquots of the standard working solution of olanzapine and fluoxetine (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mL) were transferred to a series of 10 mL volumetric flasks and completed to mark with mobile phase. Aliquots (20 μL) of each solution were injected under the operating chromatographic conditions as described above.

(**b**) *Calibration curve (linearity of the TLC method).*—Calibration curves were constructed by plotting peak areas vs concentrations of olanzapine and fluoxetine, and the regression equations were calculated. The calibration curves were plotted over the concentration ranges of 100–800 and 400–3200 ng/spot for olanzapine and fluoxetine, respectively. Aliquots of the standard working solution of olanzapine and fluoxetine (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 μL) were applied to the plate. The calibration curves were constructed by plotting peak areas vs concentrations with the help of the WinCATS software. Each reading was the average of 3 determinations.

(**c**) *Accuracy (% recovery).*—The accuracy of the methods was determined by calculating recoveries of olanzapine and fluoxetine by the standard addition method. Known amounts of standard solutions of olanzapine (8.0, 10, and 12 μg/mL) and fluoxetine (32, 40, and 48 μg/mL) for the LC method, and olanzapine (100, 200, and 300 ng/spot) and fluoxetine (400, 800 and 1200 ng/spot) for the TLC method, were added to prequantified sample solutions of tablet dosage forms. The amounts of olanzapine and fluoxetine were estimated by applying these values to the regression equations of the calibration curves.

(**d**) *Method precision (repeatability).*—The instrumental precision was checked by repeatedly injecting ($n = 6$) standard solutions of olanzapine (30 μg/mL) and fluoxetine (120 μg/mL) for the LC method and by repeated scanning of the same spot ($n = 6$) of olanzapine (300 ng/spot) and fluoxetine (1200 ng/spot) without changing the position of the plate for the TLC method.

(**e**) *Intermediate precision (reproducibility).*—The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of olanzapine (20, 30, and 40 μg/mL) and fluoxetine (80, 120, and 160 μg/mL) for the LC method, and olanzapine (200, 300, and 400 ng/spot) and fluoxetine (800, 1200, and 1600 ng/spot) for the TLC method. The results are reported in terms of relative standard deviation (RSD).

(**f**) *Limit of detection (LOD) and limit of quantitation (LOQ).*—LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines (27):

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Table 1. Regression analysis of the calibration curves for olanzapine and fluoxetine in the proposed LC and TLC methods

	LC method		TLC method	
Parameter	Olanzapine	Fluoxetine	Olanzapine	Fluoxetine
Concentration range	10–70 μg/mL	40–280 μg/mL	100–800 ng/spot	400–3200 ng/spot
Slope	50645	23212	10.019	5.0287
Standard deviation of the slope	1.52	0.93	0.056	0.16
Intercept	168722	73661	8.467	189.58
Standard deviation of the intercept	0.512	0.157	0.0189	0.0154
Correlation coefficient	0.9995	0.9998	0.9995	0.9991

Table 2. Summary of the validation parameters for the proposed LC and TLC methods

	LC method		TLC method	
Parameter	Olanzapine	Fluoxetine	Olanzapine	Fluoxetine
LOD[a]	3.429 μg/mL	13.37 μg/mL	33.13 ng/spot	132.08 ng/spot
LOQ[b]	10.392 μg/mL	40.53 μg/mL	100.42 ng/spot	400.25 ng/spot
Accuracy, %	98.05–100.6	98.9–100.26	99.66–100.42	101.33–101.370
Repeatabilty (RSD[c], %; *n* = 6)	0.53	0.64	0.19	0.62
Precision (RSD, %)				
Interday, *n* = 3	0.42–0.68	0.3—0.76	0.21–0.85	0.16–0.77
Intraday, *n* = 3	0.25–0.48	0.31–0.58	0.13–0.3	0.23–0.71

[a] LOD = Limit of detection.
[b] LOQ = Limit of quantitation.
[c] RSD = Relative standard deviation.

where σ = the standard deviation of the response and S = the slope of the regression equation.

Analysis of Olanzapine and Fluoxetine in Combined Tablet Dosage Forms

Tablets containing olanzapine (5 mg) and fluoxetine (20 mg) of the brand from Ranbaxy Laboratories Ltd were purchased from the local market. The responses of the tablet dosage forms were measured at 233 nm for quantification of olanzapine and fluoxetine by using LC and TLC as described above. The amounts of olanzapine and fluoxetine present in sample solutions were determined by fitting the responses into the regression equations for olanzapine and fluoxetine.

Results and Discussion

LC Method

To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for olanzapine and fluoxetine were obtained with a mobile phase consisting of 0.05 M, 5.6 pH potassium dihydrogen phosphate buffer–acetonitrile (50 + 50, v/v). Quantitation of the drug was performed at 233 nm. Resolution of the components with clear baseline separation was obtained (Figure 1). Absorption spectra of the 2 active components are shown in Figure 2.

Table 3. System suitability test parameters for olanzapine and fluoxetine for the proposed LC method

Parameter	Olanzapine ± RSD[a], %	Fluoxetine ± RSD[a], %
Retention time, min	2.27 ± 0.01	4.46 ± 0.01
Tailing factor	1.18 ± 0.03	1.25 ± 0.02
Capacity factor, k′	2.02	4.94
Selectivity factor, α	—	2.44
Resolution, Rs	—	1.96
Theoretical plates	6875 ± 0.08	5462 ± 0.09

[a] RSD = Relative standard deviation.

TLC Method

Several mobile phases were tried to accomplish good separation of olanzapine and fluoxetine. Using the mobile phase methanol–toluene (40 + 20, v/v) and 10 × 10 cm TLC silica gel $60F_{254}$ aluminum-backed plates with fluorescent indicator, good separation was attained with R_f values of 0.63 for olanzapine and 0.31 for fluoxetine. Quantitation of the drug was performed at 233 nm. Resolution of the components with clear baseline separation was obtained (Figure 3).

Validation of the Proposed Method

Linearity.—Linear correlation was obtained between peak areas and concentrations of olanzapine and fluoxetine in the ranges of 10–70 and 40–280 μg/mL, respectively, for LC and 100–800 and 400–3200 ng/spot, respectively, for TLC. The linearity of calibration curves was validated, and correlation coefficients of regression were found near to 1 (Table 1).

Accuracy.—The recovery experiments were performed by the standard addition method. The recoveries obtained were 99.54 ± 0.89 and 99.73 ± 0.58% for olanzapine and fluoxetine, respectively, by LC and 100.02 ± 0.06 and 101.35 ± 0.35% for olanzapine and fluoxetine, respectively, by TLC (Table 2). The high values indicate that both methods are accurate.

Method precision.—The RSD values for olanzapine and fluoxetine in combined formulations were found to be 0.53

Table 4. System suitability test parameters for olanzapine and fluoxetine for the proposed TLC method

Parameter	Olanzapine ± RSD[a], %	Fluoxetine ± RSD[a], %
R_f value	0.63 ± 0.04	0.31 ± 0.03
Area (average)	3104.2 ± 0.19	1713.2 ± 0.09

[a] RSD = Relative standard deviation.

Table 5. Assay results for the combined dosage form using the proposed LC and TLC methods

	Olanzapine ± SD[a]		Fluoxetine ± SD[a]	
Formulation	LC	TLC	LC	TLC
A	99.2% ± 0.85	101.53% ± 1.06	100.4% ± 0.78	101.45% ± 0.35

[a] SD = Standard deviation, 5 determinations.

and 0.64%, respectively, using LC and 0.19 and 0.62%, respectively, for TLC (Table 2). The low RSD values indicate that the proposed methods are repeatable.

Intermediate precision.—The intraday and interday RSD values for olanzapine and fluoxetine were 0.25–0.48 and 0.31–0.58% and 0.42–0.68 and 0.34–0.76%, respectively, using LC and 0.13–0.3 and 0.23–0.71%, and 0.21–0.85 and 0.16–0.77%, respectively, using TLC. These low values reveal that the proposed methods are reproducible (Table 2).

LOD and LOQ.—LOD values for olanzapine and fluoxetine were found to be 3.429 and 13.37 μg/mL, respectively, for LC and 33.13 and 132.08 ng/spot, respectively, for TLC. LOQ values for olanzapine and fluoxetine were found to be 10.392 and 40.53 μg/mL, respectively, for LC and 100.42 and 400.25 ng/spot, respectively, for TLC (Table 2). These data show that both of the methods are sensitive for the determination of olanzapine and fluoxetine.

System suitability parameters.—Resolution (Rs), %RSD, N, k', α, and T were measured as the criteria for system suitbability testing according to ICH guidelines (27) as shown in Tables 3 and 4.

Assay of Tablet Dosage Form (Olanzapine 5 mg and Fluoxetine 20 mg/Tablet)

The proposed validated method was successfully applied to determine olanzapine and fluoxetine in their combined tablet dosage form. The results obtained were in good agreement with the corresponding labeled amounts (Table 5).

Comparison of the Proposed Methods

The assay results for olanzapine and fluoxetine in their combined dosage form obtained using the LC and TLC methods were compared by applying the paired t-test. The calculated t-values of 0.14 for olanzapine and 0.31 for fluoxetine are less than the tabulated t-value 1.85 at the 95% confidence level. Therefore, there was no significant difference in a determined content of olanzapine and fluoxetine by the LC and TLC methods.

Conclusions

The results of analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives in the pharmaceutical formulations of the assayed samples did not interfere with determination of olanzapine and fluoxetine. The methods can be used for the routine simultaneous analysis of the olanzapine and fluoxetine in pharmaceutical preparations. We propose the use of the TLC method based on statistical analysis as well as its advantages such as better sensitivity, speed, and economy.

References

(1) Seeman, P. (1995) *Int. Clin. Psychopharmacol.* **10**, 5–13

(2) Labat, L., Deveaux, M., Dallet, P., & Dubost, J.P. (2002) *J. Chromatogr. B* **773**, 17–23

(3) Zhang, M.D., Kenneth, W.P., David, T.W., Brian, D.P., Jingqi, B., Gary, D.T., & Frank, P.B. (2000) *Neuropsychopharmacology* **23**, 250–262

(4) Manickam, A., Donna, A., William, C.W., & Stephen, R.M. (1997) *Ther. Drug Monit.* **19**, 307–313

(5) John, T.C., Richard, D.B., Matt, C., Todd, A.G., Michael, G., & Steven, P.S. (1995) *J. Chromatogr. B* **668**, 85–90

(6) Dasandi, B., Shah, P., Gandhi, C., Bhat, K.M., & Shivprakash, (2003) *Indian Drugs* **40**, 350–354

(7) Chiu, J.A., & Franklin, R.B. (1996) *J. Pharm. Biomed. Anal*. **14**, 609–615

(8) Bao, J., & Potts, B.D. (2001) *J. Chromatogr. B* **752**, 61–67

(9) Saracino, M.A., Gandolfi, O., Dallolio, R., Albers, L., Kenndler, E., & Raggi, M.A.J. (2006) *J. Chromatogr. A* **1122**, 21–27

(10) Berna, M., Ackermann, B., Ruterbories, K., & Glass, S. (2002) *J. Chromatogr. B* **767**, 161–163

(11) Harvey, E.J., Flanagan, R.J., & Taylor, D.M. (2000) *Pharm. J.* **265**, 275–276

(12) Raggi, M.A., Casamenti, G., Mandrioli, R., Izzo, G., & Kenndler, E. (2000) *J. Pharm. Biomed. Anal.* **23**, 973–981

(13) Anna, K., Barbara, S., Helena, P., & Joanna, S. (2006) *Anal. Sci.* **22**, 829–836

(14) Olsen, B.A., Wirth, D.D., & Larew, J.S. (1998) *J. Pharm. Biomed. Anal.* **17**, 623–630

(15) Ulrich, S. (2005) *Ther. Drug Monit.* **27**, 463–468

(16) Lantz, R.J., Farid, K.J., Koons, J., Tenbarg, J.B., & Boop, R.J. (1993) *J. Chromatogr.* **614**, 175–179

(17) Potts, B.D., & Paril, C.J. (1992) *J. Liq. Chromatogr.* **15**, 665–681

(18) Wong, S.H.Y., Dellafera, S.S., Fernandes, R., & Kranzler, H. (1990) *J. Chromatogr*. **499**, 601–608

(19) Nichols, J.H., Chrison, J.R., & Lawson, G.M. (1994) *Clin. Chem.* **40**, 1312–1316

(20) Suckow, R.F., Zhang, M.F., & Cooper, T.B. (1992) *Clin. Chem*. **38**, 1756–1761

(21) Maya, M.T., Domingos, C.R., Guerreiro, M.T., & Morasis, J.A. (2000) *J. Pharm. Biomed. Anal*. **18**, 989–996

(22) Koves, E.M. (1995) *J. Liq. Chromatogr.* **692**, 103–119
(23) Sutherland, F.C.W., Badenhorst, D., & Hundt, A.F. (2001) *J. Chromatogr. A* **914**, 45–51
(24) Raggi, M.A., Andrioli, R.M., Casamenthi, G., Bugamelli, F., & Volterra, V. (1998) *J. Pharm. Biomed. Anal.* **18**, 193–199
(25) Khan, G.J.G.V., Trivedi, C., Soni, K., Khan, I.J., Namjoshi, D.R., & Saraf, M.N. (2005) *Indian. Drugs* **42**, 580–584
(26) Khan, I.U., Aman, T., Muhammad, A.I., & Asrar, A.K. (2000) *Microchim. Acta* **134**, 27–31
(27) International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996) *Guideline on Validation of Analytical Procedures-Methodology*, Geneva, Switzerland