Development and Validation of Method for Determination of Clobetasol Propionate and Salicylic Acid from Pharmaceutical Dosage Form by HPLC

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors MHUB and AFMAI designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors HAR and MIT managed the literature searches. Author AFMAI analyses of the study performed the chromatographic analysis. All authors read and approved the final manuscript.

ABSTRACT

**Aim:** A new reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of Clobetasol Propionate and Salicylic Acid in ointment was developed and validated as per ICH (International Conference on Harmonization) guidelines.

**Methodology:** Separation of Clobetasol Propionate and Salicylic Acid was achieved on Zorbax Eclipse XDB-C18 (250 mm x 4.6 mm, 5µ) stationary phase by using mobile phase solution A, solution B and solution C (Solution A, B and C was composed of potassium dihydrogen phosphate, methanol [HPLC grade] and Acetonitrile [HPLC Grade]) with gradient flow rate. The injection volume and temperature of the column oven were 20µl and 30°C± 2°C respectively.
Results: The developed method shows high specificity for Clobetasol Propionate and Salicylic Acid. The calibration curve for Clobetasol Propionate and Salicylic Acid was linear and correlation coefficient (r²) for Clobetasol Propionate and Salicylic Acid was 1.0000 & 0.9999 respectively. The proposed method was adequate selective, reproducible and specific for the determination of Clobetasol Propionate and Salicylic Acid from ointment. Average percent of recovery for the drugs Clobetasol Propionate and Salicylic Acid were found 100.33% and 100.62% respectively. All of the results are within the acceptance criteria.

Conclusion: The proposed method was accurate and precise for the quantification of Clobetasol Propionate and Salicylic Acid in the ointment, also be used for routine analysis in quality control. The method parameters like selectivity, precision, intermediate precision, accuracy, linearity, recovery and stability was validated.

Keywords: Gluco-corticosteroid; HPLC column; NSAID; ICH; voltametry.

1. INTRODUCTION

Clobetasol Propionate is the most potent Gluco-corticosteroids for topical use. It is used for short term relief of anti-inflammatory, Pruritic manifestations of moderate to severe corticosteroid responsive dermatoses and in Psoriasis. It is available in dosage forms such as cream, gel, ointment etc. [1-3]. The non steroid anti-inflammatory drugs (NSAID) are among the most prescribed because of their analgesic and anti-inflammatory properties [4]. In the literature, several analytical methods primarily based on separation were described. Therefore, quantitative methods to assay, for instance a drug substance or its impurities, have needed to be fully validated after development. Depending on the type of analytical method and its intended use, a check for linearity, precision, accuracy, specificity, range, limits of detection and quantification, and/or robustness, is required [5-9], gas chromatography coupled with the mass spectrometry were the most usually used methods for analytes separation [10].

From Literature survey it has been concluded that many methods have been developed for SAL and CLO in combination of drugs by using HPLC, UV Spectrophotometry, Colorimetry, GC, Voltammetry, HPCE [11-23].

Only few methods are available for determination of Clobetasol Propionate and Salicylic Acid from pharmaceutical dosage form, so present work was undertaken with the aim to develop and validate a rapid and consistent reversed phase high performance liquid chromatographic method for determination for Clobetasol Propionate and Salicylic Acid according to International Conference on Harmonization (ICH) guideline [24].

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

The following chemicals were used for the process: Water [HPLC grade] Acetonitrile [HPLC grade] Methanol [HPLC grade], Potassium dihydrogen phosphate. All these chemicals were from Merck Germany. Clobetasol Propionate [Working Standard] and Salicylic acid [Working standard] were from Crystal Pharma S. A., Spain and Alta Laboratories Ltd., India.

2.2 Apparatus and Chromatographic Conditions

The equipment used for the method was Analytical balance Sartorious (model: TE214S). HPLC were Dionex Ultimate 3000(equipped with Auto sampler and UV-visible detector), HPLC Shimadzu Prominence (equipped with Auto sampler and PDA detector). The Column selected for the method was 250 mm x 4.6 mm,
5 µm (Zorbax Eclipse XDB-C18). The flow rate was monitored to separate the peak of methyl paraben and salicylic acid and minimize retention time of clobetasol propionate as per Table 1. The wavelength selected for the method was 240 nm and the injection volume was 20 µl. The temperature of the column oven was 30ºC±2ºC.

2.3 Method Development

2.3.1 Preparation of mobile phase

Solution A, Solution B and Solution C run at gradient elution program.

2.3.2 Preparation of solution A

Solution A was prepared 6.80 mg per ml of Potassium dihydrogen phosphate in water.

2.3.3 Solution B

Solution B was filtered and degassed Methanol.

2.3.4 Solution C

Solution C was mixture of Methanol and Acetonitrile in the ratio of 1:1.

All these solution was filtered through 0.45 µ filter under vacuum filtration and degassing in ultrasonic water bath for 5 minutes.

2.3.5 Preparation of diluent

The diluent consists of Solution A and Methanol in the ratio of 46:54.

2.3.6 Preparation of the clobetasol propionate and salicylic acid standard and sample solution

2.3.6.1 Standard solution preparation

The Standard solution was prepared by accurately weighing and transferring about 20 mg Clobetasol propionate working standard and about 60 mg of Salicylic Acid into a 100 ml clean and dry volumetric flask. About 70 ml of HPLC grade methanol was added and sonicated for 10 minutes with intermittent shaking. The sample was kept for few minutes to cool at room temperature and the volume was made up to mark with same solvent. 5 ml of the resulting solution was transferred into a 50 ml clean and dry volumetric flask and the volume was made up to the mark with diluting solvent. The content was filtered through 0.45 µm membrane filter before injection.

2.3.6.2 Sample solution preparation

The Sample solution was prepared by accurately weighing and transferring about 4.0 g ointment that contained about 2.0 mg of clobetasol propionate and 120 mg Salicylic Acid into a 50 ml clean and dry volumetric flask. About 20 ml of HPLC grade methanol was added and content was heated in a water bath at about 65ºC for 15 minutes with intermittent shaking. The sample was kept for few minutes to cool at room temperature and the volume was made up to mark with the same solvent. The content was shaking vigorously. The content was filtered through whatman filter paper No. 42 and filtrate was collected by discarding first few ml. From the filtrate 10 ml was pipette out into 20 ml clean and dry volumetric flask and diluted up to mark with diluting solvent (Sample for clobetasol Propionate). Further 5 ml was pipette out into 100 ml clean and dry volumetric flask and diluted up to mark with diluting solvent (Sample for Salicylic Acid). Then both Clobetasol propionate and Salicylic Acid solutions was filtered through 0.45 µm membrane filter before injection.

2.3.7 System suitability solution

The final standard solution is used as system suitability solution and inject 20 µl of six replicate injections of standard solution were injected. The chromatogram was recorded and the system suitability parameters for each of the injection were checked for % RSD of area within 1%. Tailing factor must not exceed 2.0 and resolution must be less than 15.

Table 1. Gradient program

| Time (minutes) | Flow rate (ml/ minute) | Solution A (%) | Solution B (%) | Solution C (%) |
|---------------|------------------------|----------------|----------------|----------------|
| 0             | 1.0                    | 46             | 54             | 0              |
| 3             | 1.0                    | 46             | 54             | 0              |
| 5             | 1.5                    | 37             | 0              | 63             |
| 24            | 1.5                    | 37             | 0              | 63             |
| 25            | 1.0                    | 46             | 54             | 0              |
| 30            | 1.0                    | 46             | 54             | 0              |
3. METHOD VALIDATION

3.1 System Suitability

The system was deemed suitable if the following acceptance criteria were satisfied. The relative standard deviation (% RSD) of the peak area responses Clobetasol Propionate and Salicylic Acid from six replicate injections of standard solution is not more than 2.0%. Tailing factor must not exceed 2.0 and resolution must be less than 15.

3.2 Specificity

Specificity and selectivity is defined as the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The standard solution was prepared and injected to the column and the retention time was checked. There were no interferences found. The method was found to be precised and specific.

3.3 Linearity

It is the relationship between instrument response and known concentrations of the analyte. The linearity was carried out by observing the correlation coefficient (r) of standard solution.

3.4 System Precision

System precision was carried out by performing six replicate injections of standard at 100% of the test concentration and calculating the % RSD of the measured area.

3.5 Method Precision

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of sample. To demonstrate method precision, six replicate of sample against standard at 100% of test concentration was carried out and the precision of method was calculated by computing % RSD of six measurements.

3.6 Intermediate Precision (Ruggedness)

Intermediate precision or ruggedness study of an analytical method is the degree of reproducibility of the test results obtained by the analysis of the same samples under a variety of normal test conditions. Test sample of representing single batch was analyzed by two different analysts on two different equipments on two different days. The ruggedness of the test method was calculated by measuring % RSD of six results and % RSD of results of two analysts.

3.7 Accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Study was carried out over a range of 80% - 120% (3 replicate each) of the test concentration. The % recovery and RSD of % recovery for each concentration was also measured.

3.8 Range

Data generated in linearity, precision and accuracy were considered for establishing the range of the analytical method.

3.9 Robustness

Robustness of the method was investigated by changing flow rate 0.80 ml/min and 1.30 and 1.20 ml/min and 1.70 ml/min, changing column temperature (±5°C) and ratio of components of mobile phase.

3.10 Stability Study

The solution stability experiments were carried out under room temperature at intervals of 0 h 6 h 12 h 18 h 24 h 30 h and 48 h.

4. RESULTS AND DISCUSSION

4.1 System Suitability

System suitability is an integral part of analytical procedures. In optimized chromatographic conditions Relative standard Deviation (%RSD) of area of Clobetasol propionate and Salicylic Acid 0.223% (NMT 1.0%) and 0.074% (NMT 1.0%) respectively. Average tailing factor Clobetasol Propionate and Salicylic Acid were 1.14 and 1.10 respectively. Resolution was found 39 (Table 2).

4.2 Specificity

Specificity of an analytical method is its ability to assess unequivocally the analyte in the presence
of components that may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. From the specificity study it is observed that the chromatogram for Clobetasol Propionate and Salicylic Acid sample with reference standard showed positive response and Placebo had no response, so the method was specific.

4.3 System Precision

System precision was carried out by performing six replicate injections at 100% of the test concentration and calculating the % RSD, Tailing factor, resolution and Theoretical plate count. From the data it is observed that the % Relative standard Deviation (% RSD) of area of Clobetasol propionate and Salicylic Acid 0.223% (NMT 1.0%) and 0.074% (NMT 1.0%) respectively. Average tailing factor Clobetasol Propionate and Salicylic Acid were found 1.14 and 1.10 respectively. Theoretical plate count for Clobetasol Propionate and Salicylic Acid were 12800 and 5114 respectively. Resolution was found 39 (Table 2).

4.4 Intermediate Precision or Ruggedness

Assay results by two different analysts at different days have been found very much close to each other. The % RSD of two analysts (12 samples) was 0.22% and 0.15% for clobetasol propionate and salicylic acid respectively. This was within acceptance criteria. So the method can be considered to be rugged enough (Table 3).

4.5 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The result shows that Average % recovery at different accuracy level is 100.33% and 100.62% for Salicylic Acid and Clobetasol propionate respectively (Table 4, Figs. 1 and 2).

4.6 Linearity

The linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte in samples within given range. Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from mean peak area of Clobetasol Propionate and Salicylic Acid at different concentrations level (80%, 90%, 100%, 110% and 120%). Correlation coefficient of Clobetasol Propionate and Salicylic Acid was 1.0000 and 0.9999 respectively (Table 5, Figs. 5 and 6).

4.7 Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of the analyte within the extremes of the specified range of the analytical procedure. Based on the linearity, precision and accuracy results, the Range of the method was determined as 80% to 120% of the target concentration.

Fig. 1. Representation of chromatogram of clobetasol propionate and salicylate acid obtained from the standard solution
4.8 Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of this method was determined by analyzing the same batch of sample by deliberately changing the method parameters like machine, ratio of mobile phase and column temperature. From the results presented on table it is clear that the system suitability criteria meet with the acceptance limit. Hence the method is robust (Table 6).

Fig. 2. Representation of chromatogram of clobetasol propionate obtained from the test solution

Fig. 3. Representation of chromatogram of salicycle acid obtained from the test solution

Fig. 4. Representation of chromatogram of placebo matrix obtained from the placebo solution
Table 2. Data for system precision

| Sl. no. | Retention time | Peak area | Theoretical plate | Resolution | Asymmetry factor |
|---------|----------------|-----------|-------------------|------------|-----------------|
|         | Clobetasol propionate | Salicylic acid | Clobetasol propionate | Salicylic acid | Clobetasol propionate | Salicylic acid |
| 01      | 18.750          | 2.800     | 873365           | 3502679    | 12851           | 5234         | 39        | 1.13 | 1.12 |
| 02      | 18.793          | 2.800     | 873495           | 3503150    | 12590           | 5192         | 39        | 1.13 | 1.09 |
| 03      | 18.707          | 2.797     | 873870           | 3498655    | 12974           | 5146         | 39        | 1.13 | 1.10 |
| 04      | 18.690          | 2.797     | 873232           | 3496389    | 13261           | 5113         | 40        | 1.15 | 1.10 |
| 05      | 18.720          | 2.800     | 871649           | 3501734    | 12694           | 5030         | 39        | 1.15 | 1.10 |
| 06      | 18.673          | 2.800     | 868736           | 3500553    | 12430           | 4970         | 38        | 1.15 | 1.11 |
| Average | 18.722          | 2.799     | 872391           | 3500527    | 12800           | 5114         | 39        | 1.14 | 1.10 |
| Relative standard deviation | 0.233% | 0.062% | 0.223% | 0.074% | ----- | -- | --- |

Table 3. Data for intermediate precision

| Sample no. | % of label claim |
|------------|------------------|
|            | Day-1            | Day-2            |
|            | Clobetasol propionate | Salicylic acid | Clobetasol propionate | Salicylic acid |
| 01         | 100.20%           | 99.95%           | 99.88%           | 100.02% |
| 02         | 99.85%            | 99.76%           | 99.78%           | 100.25% |
| 03         | 100.15%           | 99.85%           | 100.30%          | 100.08% |
| 04         | 100.12%           | 100.17%          | 99.95%           | 100.25% |
| 05         | 99.93%            | 100.08%          | 99.81%           | 99.78% |
| 06         | 99.87%            | 99.87%           | 100.25%          | 100.04% |

% RSD (12 samples), Clobetasol propionate, 0.22% salicylic acid 0.14%
Table 4. Data for accuracy

| Concentration level | Sample no. | Amount added in (µg/ml) | Amount recovered in (µg/ml) | % recovery |
|---------------------|------------|-------------------------|-----------------------------|------------|
|                     |            | Salicylic acid | Clobetasol propionate | Salicylic acid | Clobetasol propionate | Salicylic acid | Clobetasol propionate |
| 80%                 | Sample-1   | 48.8           | 16.3                     | 49.09       | 16.40                   | 100.59         | 100.61                  |
|                     | Sample-2   | 48.9           | 16.3                     | 49.08       | 16.46                   | 100.57         | 100.98                  |
|                     | Sample-3   | 48.6           | 16.4                     | 48.95       | 16.40                   | 100.72         | 100.61                  |
| 100%                | Sample-1   | 60.2           | 20.4                     | 60.36       | 20.56                   | 100.27         | 100.78                  |
|                     | Sample-2   | 60.2           | 20.5                     | 60.38       | 20.60                   | 100.30         | 100.49                  |
|                     | Sample-3   | 60.6           | 20.5                     | 60.76       | 20.67                   | 100.26         | 100.83                  |
| 120%                | Sample-1   | 72.2           | 24.3                     | 72.20       | 24.41                   | 100.00         | 100.45                  |
|                     | Sample-2   | 72.1           | 24.3                     | 72.11       | 24.35                   | 100.00         | 100.21                  |
|                     | Sample-3   | 72.9           | 24.6                     | 73.11       | 24.76                   | 100.29         | 100.65                  |
| Average             |            |               |                          |             |                         | 100.33         | 100.62                  |

Table 5. Data for linearity

| Concentration level | Concentration (µg/ml) | Peak area | Correlation co-efficient |
|---------------------|-----------------------|-----------|--------------------------|
|                     | Salicylic acid | Clobetasol propionate | Salicylic acid | Clobetasol propionate | Salicylic acid | Clobetasol propionate |
| 80%                 | 48.16         | 16.16        | 2829054                 | 701140               |                     |                       |
| 90%                 | 54.18         | 18.18        | 3165420                 | 786188               |                     |                       |
| 100%                | 60.20         | 20.20        | 3500414                 | 874565               | 0.9999              | 1.0000                 |
| 110%                | 66.22         | 22.22        | 3866651                 | 960699               |                     |                       |
| 120%                | 72.24         | 24.24        | 4200993                 | 1049951              |                     |                       |
From the stability study data, it was observed that the test sample solution is found to be stable up to 48 h at ambient condition.

4.10 Discussion

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple,
convenient but also better in terms of efficiency, stability and reproducibility. C18 column was selected because less retentive and it is less polar compare to C4 and C8 columns. C18 column allows eluting non-polar compounds more quickly compare to polar compounds. In addition to this, UV detector is used, to optimize response of the components. At 240 nm Clobetasol Propionate gives maximum response where 270 nm for Salicylic Acid. At 240 nm peak height of Clobetasol Propionate shows maximum peak height and partially decline peak height of Salicylic Acid which allows easy detection of the compounds. A 250 x 4.6 mm column of 5 μm particles packing was preferred as a starting point for method development. Flow rate was changed to separate the peak of methyl paraben and salicylic acid and minimize retention time of clobetasol propionate. Gradient mode was chosen due to simplicity in application and robustness with respect to longer column stability. This configuration provides a large number of theoretical plate’s values for most separation. Various solvent ratios of solution A, solution B and solution C were tried as mobile phase for proper separation, good peak shape and sharpness, more Theoretical plates, good resolution & less tailing of Clobetasol propionate and Salicylic Acid. All the results were found within acceptance criteria.

5. CONCLUSION

A simple RP-HPLC Method is developed to quantify Clobetasol Propionate and Salicylic Acid in pharmaceutical ointment. The validated method was covers the wide range of linearity. The method adopted for estimation of by RP-HPLC is precise, linear, accurate, rugged and robust enough. The sample solution is found to be stable up to 48 h at ambient condition. Hence this method can be considered validated for its intended purpose to establish the quality of the drug substance during routine analysis with consistent and reproducible results.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Stanislaw Dyderski, Edmund Grzeskowiak, Edyta Szalek, Agnieszka Mrzyglod. Pharmaceutical availability of clobetasol-17-propionate from cream and ointment. Acta Polonae Pharmaceutica – Drug Research. 2001;58(6):435-438.
2. JM Carrascosa, F Vanaclocha, et al. Update of the topical treatment of psoriasis, Actas Dermosifiliogr. 2009;100:190-200.
3. Elise A, Olsen MD, Roger C, Cornell MD. Topical clobetasol-17-propionate: Review of its clinical efficacy and safety. J. American Academy of Dermatolog. 1986;15(2):246-255.
4. Wolfe MM, Lichtenstein DR, Singh GN. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. Engl J Med. 1999;340:1888–99.
5. Defilippi A, Piancone G, Costa Laia R, Tibaldi GP. An HPLC screening method for the detection of isonicotinic acid hydrazide in cattle milk. Chromatographia. 1995;40:170.
6. Defilippi A, Piancone G, Costa Laia R, Balla S, Tibaldi GP. High-performance liquid chromatography with UV detection and diode-array UV confirmation of isonicotinic acid hydrazide in cattle milk. J. Chromatogr. 1994;656:466.
7. Sadeg N, Pertat N, Dutertre H, Dumontet MJ. Rapid, specific and sensitive method for isoniazid determination in serum. Chromatogr. 1996;675:113.
8. Seifart HI, Kruger PB, Parkin DP, Van Jaarsveld PP, Donald PR. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system. J. Chromatogr. 1993;619,285.
9. Hansen Jr. EB, Dooley KL, Thompson Jr. HCJ. High-performance liquid chromatographic analysis of the antituberculosis drugs aconiazide and isoniazid. Chromatogr. 1995;670:259.
10. Karlaganis G, Peretti E, Lauterburg BH. J. Chromatogr. 1987;137.
11. Tian J, Chen XS, Wang RD. Determination of free salicylic acid in chewing aspirin tablets by HPLC. Di Yi Jun Yi Da Xue Xue Bao. 2003;23(7):744-747.
12. Fogel J, Epstein P, Chen P. Simultaneous high-performance liquid chromatography
assay of acetylsalicylic acid and salicylic acid in film-coated aspirin tablets. J. Chromatogr. 1984;317:507-511.

13. Sawyer M, Kumar V. A rapid HPLC method for simultaneous quantification of aspirin, salicylic acid and caffeine in Effervescent tablets. J. Chromatogr. Sci. 2003;41:393.

14. Kedor Hackmann ER, Gianotto EA, Santoro MI. Determination of betamethasone dipropionate and salicylic acid in pharmaceutical preparations by HPLC. Drug Dev. Ind. Pharm. 1998;24(6):553-555.

15. McLaughlin JR, Sherma J. Quantitative HPTLC determination of salicylic acid in topical acne medications. J. Liq. Chromatogr. & Related Techn. 1996;19(1):17-21.

16. Tinker RB, McBay AJ. Spectrophotometric determination of acetylsalicylic and salicylic acids. J. Amer. Pharmaceu. Asso. 1954;43(5):315–317.

17. Ahmad I, Vaid FH. Determination of benzoic acid and salicylic acid in commercial benzoic and salicylic acids ointments by spectrophotometric method. Pak. J. Pharm. Sci. 2009;22(1):18-22.

18. Rance MJ, Jordan BJ, Nichols JD. A simultaneous determination of acetylsalicylic acid, salicylic acid and salicylamide in plasma by gas liquid chromatography. J. Pharm. Pharmacol. 1975;27(6):425-429.

19. Yongchun Zhu, Xinyan Guan, Hongguo Ji. Electrochemical solid phase micro extraction and determination of salicylic acid from blood samples by voltammetry and differential pulse voltametry. J. Solid State Electrochem. 2009;13:1417-1423.

20. Blake MI. The determination of benzoic acid and salicylic acid mixtures by differential nonaqueous titration. J. Amer. Pharma. Asso. 1957;46(5):287-290.

21. Konstantinos DG, Ioannou PC. Simultaneous determination of diflunisal and salicylic acid in human serum by second-derivative synchronous fluorescence spectroscopy. Eur. J. Pharma. Sci. 1994;1(4):209–217.

22. Fontana MC, Bastos MO, Beck RC. Development and validation of a fast RPHPLC method for the determination of clobetasol propionate in topical nanocapsule suspensions. J. Chro. Sci. 2010;48(8):637-640.

23. Yang Qingxiong, Wang Zhaizhong and Xiao Yiling. Determination of clobetasol propionate by the method of linear sweep adsorptive stripping voltammetry. Chem. Resea. And App. 1997;2:21-27.

24. International conference on harmonization. Draft revised guidance on validation of analytical procedure: Text and methodology. Q2A (R1). Federal Register, step 4 version; 2005. Available: www.ich.org/products/guidelines/quality-guidelines 20/02/13.