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# THE DEVELOPMENT AND VALIDATION OF VISIBLE SPECTROPHOTOMETRIC METHODS FOR SIMVASTATIN DETERMINATION IN PURE AND THE TABLET DOSAGE FORMS

*Two simple and sensitive spectrophotometric methods have been developed for the determination of simvastatin (SMT) in pure form and in tablets using insitu generated bromine, and p-phenylenediamine (PPDA) or o-dianisidine (ODA) as reagents. The methods are based on the bromination of SMT by a measured excess of in situ bromine in acid medium followed by the determination of unreacted bromine by reacting with PPDA and measuring the resulting red colour at 510 nm (method A) or reacting with ODA and measuring the absorbance at 470 nm (method B). The conditions for the assay have been optimized. Beer's law is obeyed over the concentration ranges 20-120 and 2--12 μg/ml for method A and method B, respectively. The calculated molar absorbtivities are $2.24\times10^3$ and $1.91\times10^4$ $dm^3$ $mol^{-1}$ $cm^{-1}$ for the method A and the method B, respectively; 0.1868 and 0.0115 μg/$cm^2$ being the corresponding Sandell sensitivities. The LOD and LOQ for method A are found to be 2.96 and 8.97 μg/ml, and the respective values for method B are 0.14 and 0.42 μg/ml. The intra-day and inter-day precision and accuracies were checked. The assay precision was less than 5 % CV and the accuracy was 97.38-103.4 %. The methods were used for the determination of SMT in tablets. No interference from the excipients added to tablets was found. The accuracy and validity of the methods were further ascertained by recovery studies via the standard addition technique.*

*Key words: simvastatin; determination; spectrophotometry; bromate-bromide.*

Simvastatin (SMT), chemically known as (1*S*,2*S*,8*S*,8*aR*)-1,2,6,8,8*a*-hexahydro-1-(2-((2*R*,4*R*)--terahyro-4-hydroxy-6-oxo-2H-pyran-2-yl)-2,6-dimethylnapthalen-8-yl 2,2-dimethylbutanoate (Figure 1), belongs to the group of cholesterol-lowering lactones known as statins which, in 2007, were identified as being the most widely prescribed drugs in the world. Statins lower cholesterol by inhibiting the synthesis of mevalonic acid, which is a key precusor in cholesterol synthesis. SMT, a lipid lowering agent that is derived synthetically from a fermentation product of *Aspergillus terreus* has been found to lessen both normal and elevated LDL-C concentrations. The drug is officially listed in 2004 United States Pharmacopoeia and the official method of its determination is high-performance liquid chromatography [1]. Various other methods such as UV-spectrophotometry [2-7], HPLC [7-12], HPTLC [13], miscellar eletrokinetic chromatography [14] and voltammetry [15] have been reported for the assay of SMT in pharmaceuticals.

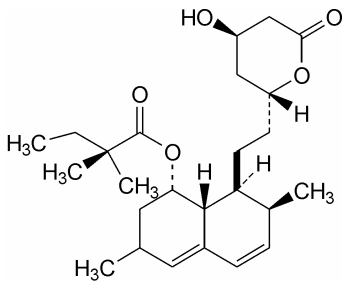


*Figure 1. Structure of simvastatin.*

The present paper describes the development and optimization of two visible spectrophotometric

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methods using bromate-bromide mixture in acid medium as the brominating agent, and *p*-phenylenediamine (PPDA) and *o*-dianisidine (ODA) as chromogenic agents. The methods were found to possess the adequate sensitivity and seletectivity to determine SMT in tablets.

## EXPERIMENTAL

### Apparatus

All absorbance measurements were made on a Systronics model 106 digital spectrophotometer (Ahmedabad, India) provided with 1-cm matched quartz cells.

### Materials and reagents

Reference standard sample of SMT was obtained from Jubiliant Organosis, Nanjangud, Inidia. Tablets containing SMT such as simvofix (Bal Pharm, India), zosta (USV, India) were purchased from local commercial sources. *p*-Phenylenediamine (Loba Chemie, Mumbai, India) solution (1 %) was prepared in 0.5 M HCl. *o*-Dianisidine (Loba Chemie, Mumbai, India) solution (0.1 %) was prepared in 3:2 acetic acid.

Required volumes of concentrated acid (Merck, Mumbai, India) was diluted with water to get 10 M and 1.0 M $H_2SO_4$. Concentrated acetic acid (Merck, mumbai, India) was diluted appropriately with water to get 3:2 acid.

*Bromate-bromide solution*

A stock standard bromate-bromide solution equivalent to 1000 μg/ml $KBrO_3$ was prepared by dissolving accurately weighed 100 mg of pure chemical (Sarabai M. Chemicals, Baroda, India) and 1.0 g of KBr (s.d. fine-chem ltd, India) in water and diluted to the mark in a 100 ml volumetric flask. The stock solution was diluted appropriately with water to get bromate-bromide solutions containing 300 and 50 μg/ml $KBrO_3$ for use in method A and method B, respecttively.

*Simvastatin solution*

A stock standard solution of SMT equivalent to 400 μg/ml was prepared by dissolving accurately weighed about 40 mg of pure drug in 3:2 acetic acid and diluted to the volume in a 100 ml calibrated flask with the same acid. The solution was diluted to get 40 μg/ml SMT for method B.

### Methods

*Method A*

Different aliquots (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml) of 400 μg/ml SMT solution were accurately measured and transferred into a series of 10 ml volumetric flasks and the total volume was brought to 3.0 ml by adding 3:2 acetic acid. To each flask 1.0 ml of 1.0 M $H_2SO_4$ was added, followed by 1.0 ml of bromate-bromide solution (300 μg/ml in $KBrO_3$); the flasks were stoppered and set aside for 10 min. Finally, 1.0 ml of 1.0 % PPDA solution was added, the volume was diluted to the mark with water, mixed well and the absorbance was measured at 510 nm *vs*. the blank after 5 min.

*Method B*

Varying aliquots (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0) of 40 μg/ml SMT solution were buretted out into a set of 10 ml volumetric flaks and the required quantity of 3:2 acetic acid was added to bring the total volume to 3.0 ml in each flask. To each flask 1.0 ml each of 10 M $H_2SO_4$ and bromate-bromide reagent (50 μg/ml in $KBrO_3$) were added, the latter being added by means of a microburette. The flasks were stoppered and set aside for 10 min with occasional shaking. Lastly, 1.0 ml of 0.10 % ODA solution was added to each flask, diluted to the mark with 10 M $H_2SO_4$, mixed well and the absorbance was measured after 5 min at 470 nm against the blank.

In either method, the concentration of the unknown is read from the calibration graph or computed from the regression equation derived using the absorbance-concentration data.

### Procedure for commercial tablets

Twenty tablets were weighed accurately and ground into a fine powder. The powder equivalent to 40 mg of SMT weighed accurately was transferred into a 100 ml volumetric flask and 60 ml of 3:2 acetic acid was added. The content was shaken for 15-20 min and diluted to the volume with the same acid, mixed well and filtered using a Whatman No. 42 filter paper. First, 10 ml portion of filtrate was discarded and a suitable aliquot of the subsequent portion was subjected to the analysis by method A or by method B after the appropriate dilution.

## RESULTS AND DISCUSSION

A number of reagents are reported to oxidize PPDA to a red-coloured product [16] and ODA to an orange-red species [17] in acid medium based on which the micro level determination of such reagents has been accomplished. The work in the authors' laboratory also revealed that bromine brominates SMT in acid medium. These analytical aspects have been successfully utilized to develop two sensitive methods for the determination of SMT. The methods are based

on the bromination of SMT in acid medium by a known excess of *in situ* bromine and the subsequent determination of the unreacted bromine by reacting with either PPDA or ODA and measuring the absorbance either at 510 nm or at 470 nm. The amount of bromine reacted corresponds to the amount of SMT which formed the basis for the assay of SMT and the reaction was found to follow a 3:2 stoichiometry (SMT:$KBrO_3$). The possible reaction scheme is given in Scheme 1.

When a fixed concentration of *in situ* bromine was treated with increasing concentrations of SMT, a concomitant decrease in the bromine concentration occurred which, being reacted with PPDA or ODA, resulted in a corresponding decrease in the absorbance.

### Optimization of the reaction conditions

Separate preliminary experiments were carried out to find the concentrations of $KBrO_3$, in the presence of large excess of KBr, required to produce a convenient maximum absorbance with PPDA and ODA. Under the optimum conditions described, they were found to be 30 and 5 μg/ml with PPDA and ODA, respectively. Hence, 1.0 ml each of 300 and 50 μg/ml $KBrO_3$ (+excess of KBr) was used in method A and method B, respectively, in a total volume of 10 ml.

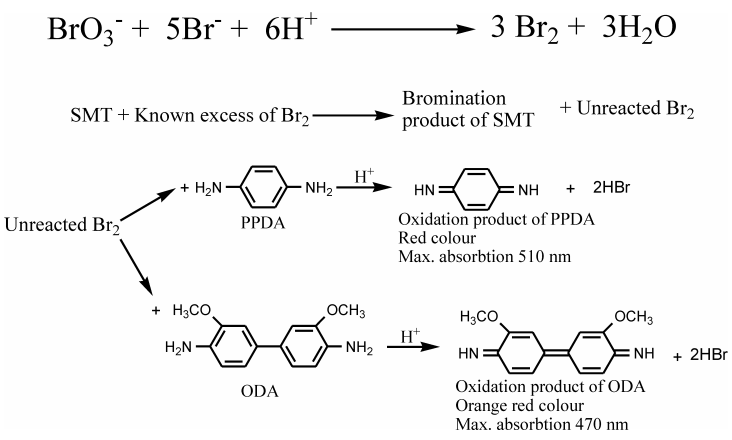
The effects of different medium of acids were studied. The reaction between SMT and liberated $Br_2$ was too slow in HCl medium. Both reaction steps were very slow in $H_3PO_4$ medium. The reaction between SMT and bromine and that between the unused/unreacted bromine and PPDA or ODA were found to proceed quantitatively in $H_2SO_4$ medium. In method A, the first step of the reaction was quantitative with 1.0 ml of 1.0 M $H_2SO_4$ in a total volume of 5.0 ml in addition of 3.0 ml of 3:2 acetic acid and the same quantity of acid was maintained for the second step, *i.e.* the oxidation of PPDA by bromine. However, both steps of the method B required a high concentration; about 0.50 M in $H_2SO_4$ for the first step and an overall 5.0 M in $H_2SO_4$ for the stabilization of the oxidation product of ODA. Lower acid concentrations in method B yielded a less stable coloured oxidation product. In both methods, bromination of SMT was complete in 10 min and any delay up to 30 min did not affect the results whereas the second step was rapid reaching the maximum absorbance in 5 min and the colour remained stable for the next 40 min in method A and 25 min in method B.

The concentration of ODA and PPDA were fixed in order to give the minimum blank absorption and the covenant maximum absorbance in the presence of bromate-bromide mixture. Two blanks were prepared for each system. The first blank which consisted of all reactants except SMT gave maximum absorbance. The absorbance spectrum for both the blanks and products and the tablet excipients are presented (Figure 2). The second blank was prepared in the absence of SMT and bromate-bromide reagent to evaluate the contribution of other reactants to the measured absorbance. Since the second blank had significant absorbance, all absorbance measurements were made against the second blank in both methods.

### Method validation

*Linearity*

A linear correlation was found between the absorbance and the concentration of SMT. Beer's law was obeyed in the inverse manner. The regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (*b*), the intercept (*a*) and the correlation coefficient (*r*) for each system, and the calculated values are given in Table 1. The graphs showed maximum intercept as descrybed by the regression equation, $y = a + bx$, where $y$ is the absorbance and $x$ is the concentration in μg/ml.




*Scheme 1. Probable reaction scheme.*

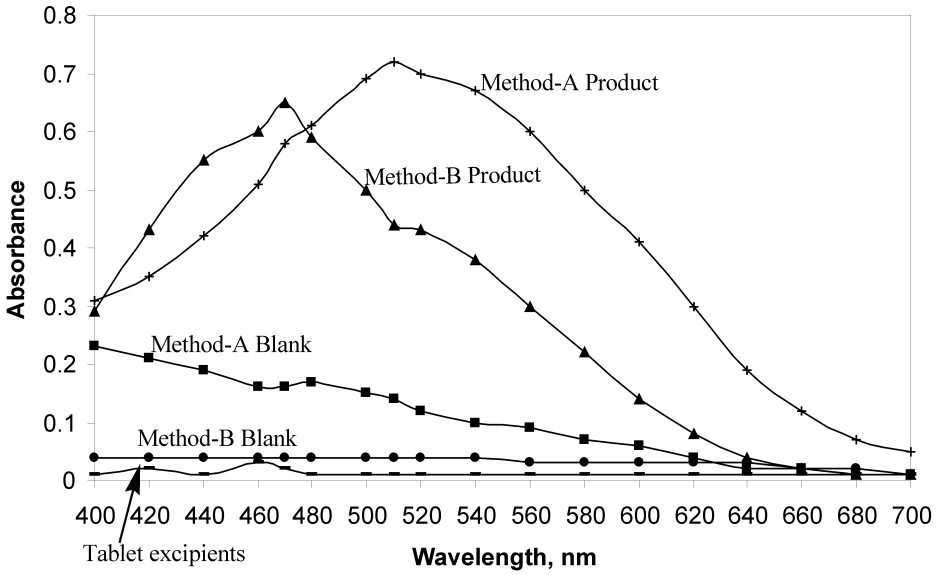



*Fig. 2. Absorption spectra for tablet excipients, both blanks and products.*

*Table 1. Regression and analytical parameters*

| Parameter | Method A | Method B |
|---|---|---|
| $\lambda_{max}$ / nm | 510 | 470 |
| Beer's law limits, μg/ml | 20-120 | 2-12 |
| Molar absorbtivity, $dm^3\ mol^{-1}\ cm^{-1}$ | $2.2\times10^3$ | $1.9\times10^4$ |
| Sandell sensitivity, μg/cm | 0.1868 | 0.0115 |
| Limit of detection, μg/ml | 2.96 | 0.14 |
| Limit of quantification, μg/ml | 8.97 | 0.42 |
| Regression equation, *y** | | |
| Intercept (*a*) | 0.7347 | 0.6373 |
| Slope (*b*) | -0.0058 | -0.0434 |
| Correlation coefficient (*r*) | -0.9987 | -0.9988 |
| $S_a$** | 0.09980 | 0.05716 |
| $S_b$*** | 0.00094 | 0.00540 |

*$y = a+bx$, where $y$ is the absorbance and $x$ the concentration in μg $ml^{-1}$; **standard deviation of the intercept; ***standard deviation of the slope

### *Detection and quantification limits*

The limits of detection (*LOD*) and quantification (*LOQ*) calculated according to the current ICH guidelines [18] are presented in Table 1. The other sensitivity parameters such as molar absorptivity and Sandell sensitivity are also contained in Table 1. These values suggest that method B is nearly 10 times as sensitive as method A which is also fairly sensitive.

### *Selectivity*

The selectivity may be defined as the ability of the method to determine the analyte in the presence of matrix. The selectivity was evaluated by preparing a synthetic mixture and it was confirmed that the change in signal measured (absorbance) was caused only by the analyte. A synthetic mixture with the composition of: SMT (40 mg), talc (20 mg), starch (30 mg), lactose (60 mg), gum arabic (20 mg), magnesium stearate (10 mg) and sodium alginate (15 mg); the extract was prepared according to the procedure described for tablets and analysed. Replicate analyses ($n$ = 7) of the extract by the two methods descrybed earlier at 80 μg/ml (method A) and 8 μg/ml (method B) concentration levels indicated that the inactive ingredients did not interfere with SMT determination. This was supported by the recovery of SMT, in %, which was found to be 98.67±1.3 by method A and 99.12±0.85 by method B. This is amply illustrated in the absorption spectra of the reaction products, respective blanks and the tablet excipients (Figure 2).

### *Precision*

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [19]. Three different concentration of SMT were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The *RSD* (%) values of intra-day and inter-day studies showed that the precision was good (Table 2).

### *Accuracy*

The accuracy of an analytical method expresses the closeness between the reference value and the found value [18-20]. The accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for SMT (Bias %). The results obtained are compiled in Table 2 and show that the accuracy is good for both methods.

*Table 2. Intra-day and inter-day precision and accuracy results*

| Method | SMT taken, μg/ml | Intra-day[a] | | | Inter-day[b] | | |
|---|---|---|---|---|---|---|---|
| | | SMT found[c], μg/ml | Precision[d] | Accuracy[e] | SMT found[c], μg/ml | Precision[d] | Accuracy[e] |
| A | 30 | 30.36±0.16 | 1.38 | 1.2 | 30.8±0.45 | 3.24 | 2.67 |
| | 60 | 59.46±0.25 | 1.12 | -0.59 | 61.4±0.78 | 2.86 | 2.33 |
| | 90 | 89.05±0.55 | 1.64 | -0.05 | 88.16±1.33 | 3.37 | -2.04 |
| B | 3 | 2.97±0.023 | 2.05 | -1.0 | 3.06±0.037 | 2.73 | -2.00 |
| | 6 | 5.55±0.034 | 1.64 | -0.76 | 6.09±0.083 | 3.07 | -1.50 |
| | 9 | 9.11±0.033 | 0.95 | 1.22 | 9.28±0.073 | 1.76 | 3.11 |

[a]$n$ = 7; [b]$n$ = 5; [c]mean±standard error; [d]relative standard deviation, %; [e]bias % : (found – taken/taken)×100

*Application to tablets analysis*

The proposed methods were applied to determine SMT in two brands of tablets with three different doses. The results were compared with those of the literature method [6] which consisted of the measurement of absorbance of the tablet extract at 240 nm in methanolic medium. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95 % confidence level with respect to accuracy and precision (Table 3).

*Recovery study*

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure SMT at three concentration levels (50,100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In all cases, the added SMT recovery percentage values ranged between 96.58 and 104.3 % with relative standard deviation of < 4 %. The results of this study given in Table 4 indicated that the recovery was good, and that the co formulated substances did not interfere in the determination.

*Table 3. The results of the assay of tablets and statistical evaluation. Tabulated t-value at the 95 % confidence level is 2.77; tabulated F-value at the 95 % confidence level is 6.39*

| Tablet | Nominal | Found[a] (% of nominal amount±*SD*) | | |
|---|---|---|---|---|
| | | Literature method | Method A | Method B |
| Simvofix[b] | 10 | 101.6±0.64 | 102.8±0.96<br>$t$ = 2.37<br>$F$ = 2.25 | 100.2±1.45<br>$t$ =2.11<br>$F$ = 5.13 |
| | 20 | 99.74±1.06 | 98.24±1.86<br>$t$ = 1.62<br>$F$ = 3.07 | 100.4±2.2<br>$t$ = 0.64<br>$F$ = 4.30 |
| | 40 | 97.36±0.84 | 96.85±1.33<br>$t$ = 0.74<br>$F$ =2.51 | 98.23±1.72<br>$t$ = 1.07<br>$F$ = 4.19 |
| Zosta[c] | 5 | 102.3±1.24 | 100.6±1.85<br>$t$ = 1.74<br>$F$ =2.22 | 103.6±2.1<br>$t$ = 1.23<br>$F$ = 2.87 |
| | 10 | 98.76±0.92 | 100.1±1.56<br>$t$ = 1.71<br>$F$ = 2.88 | 99.46±1.92<br>$t$ = 0.78<br>$F$ =4.36 |
| | 20 | 103.5±1.04 | 101.7±1.67<br>$t$ = 2.09<br>$F$ =2.58 | 102.3±1.48<br>$t$ = 1.50<br>$F$ = 2.03 |

[a]Mean value of five determinations; [b]Bal Pharma (Servetus); [c]USV (Corvette)

*Table 4. The result of the recovery study via the standard addition technique*

| Tablet studied | Method A | | | | Method B | | | |
|---|---|---|---|---|---|---|---|---|
| | SMT in tablet μg/ml | Pure SMT added, μg/ml | Total found μg/ml | Pure SMT recovered %±*SD** | SMT in tablet μg/ml | Pure SMT added, μg/ml | Total found μg/ml | Pure SMT recovered %±*SD** |
| Zosta 20 mg | 40.68 | 20.0 | 61.54 | 104.3±2.6 | 4.09 | 2.0 | 6.11 | 105.4±3.0 |
| | 40.68 | 40.0 | 81.32 | 101.6±1.9 | 4.09 | 4.0 | 8.15 | 103.7±3.5 |
| | 40.68 | 60.0 | 104.34 | 106.1±2.8 | 4.09 | 6.0 | 10.06 | 101.1±2.7 |
| Simvofix 40 mg | 38.74 | 20.0 | 58.0 | 96.33±3.1 | 3.93 | 2.0 | 5.84 | 95.69±3.2 |
| | 38.74 | 40.0 | 78.2 | 98.56±2.7 | 3.93 | 4.0 | 7.88 | 98.72±2.9 |
| | 38.74 | 60.0 | 97.2 | 97.42±2.4 | 3.93 | 6.0 | 9.71 | 96.34±2.4 |

## CONCLUSIONS

Two simple, rapid, fairly accurate and precise and reasonably sensitive spectrophotometric methods were developed for the determination of simvastatin in bulk drug and in tablets. The methods rely on the use of simple and cheap chemicals and are based on well characterized color reactions. Among the two methods proposed, the method using PPDA has the advantages of employing lower $H_2SO_4$ concentration, measuring at longer wavelength. Though the method using ODA requires higher acid concentration, it offers the advantage in terms of sensitivity. The technique employed is inexpensive but was demonstrated to provide the sensitivity comparable to the expensive technique like HPLC. Thus, the methods can be used for routine analysis laboratories and for quality control purposes.

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## REFERENCES

[1] THE United State Pharmacopoeia 25, the National Formulary 20, United States Pharmocopocial Convention Inc., 2002, 9571

[2] N. Erk, Pharmazie **57** (2002) 817

[3] L. Xu, Y. G. Zazhi, Sci. Finder CAN 135:362691; AN 2001: 606960

[4] L. Zhonghong, T Shurong, Zhongguo Yaoxue Zazhi **35** (2000) 554

[5] L. Wang, M. Asgharnejad, J.Pharm.Boimed.Anal. **21** (2000) 1243

[6] M. S. Arayne, N. Sultana, F. Hussain, S. A. Ali, J. Anal. Chem. **62** (2007) 536

[7] G. Carlcucci, P. Mazzeo, Farmaco **47** (1992) 817

[8] X. Jianwei, L. Ying, Yaowu Fenxi Zazhi **25** (2005) 523

[9] A. Ali, E. S. M. Nameh, R. A. Shawabkeh. J. Anal. Chem. **61** (2006) 63

[10] G. Carolina, G. C. Gloria, D. D. Marta, G. Ricardo, J.Chilean Chem. Soc. **49** (2004) 289

[11] X. Xan, G. Cao, X. He, X. Hu, D. Gu, Huaxi Yaoxue Zazhi **15** (2000) 205

[12] J. Wang, Thongguo Yiyao Gongye ZaZhi **31** (2000) 121; Sci Finder, CAN 132:284323; AN 2000:293986

[13] B. G. Chaudhari, N. M. Patel, P. B. Shah, Indian J. Pharm. Sci. **69b** (2007) 130

[14] M. K. Srinivasu, A. N. Raju, G. O. Reddy. J. Pharm Biomed. Anal. **29** (2002) 715

[15] O. Coruh, S. A. Ozkan. Die Pharmazie **61** (2006) 285

[16] Z. Marczenko, Separation and spectrophotometric determination of elements, Mary Masson, Ed., ELLIS HORWOOD Limited Publishers, pp. 213, 252, 304

[17] Z. Marczenko, Separation and spectrophotometric determination of elements, Mary Masson, Ed., ELLIS HORWOOD Limited Publishers, pp. 216, 222, 240, 319, 435, 631

[18] ICH topic Q2B, Validation of analytical procedures: methodology (CPMP/ICH/281/95)

[19] G. A. Shabir, J. Chromatogr A **987** (2003) 57

[20] J. Ermer. J. Pharm. Biomed. Anal. **24** (2001) 755.