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Evaluation of an International Pharmacopoeia method for the analysis of saquinavir (mesilate) bulk drugs by liquid chromatography

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Abstract

A single gradient LC method for the determination of related substances in both saquinavir (SQV), saquinavir mesilate (SQVM) has been published in a consultation document of the International Pharmacopoeia, WHO Drug Information. The method uses a base deactivated reversed phase C18 column (25 cm × 4.6 mm i.d.), 5 μm kept at a temperature of 30 °C. The mobile phases consist of acetonitrile, methanol, phosphate buffer pH 3.4 and water. The flow rate is 1.0 ml/min. UV detection is performed at 220 nm. A system suitability test (SST) is described to govern the quality of the separation. The separation towards SQV(M) components was investigated on 18 C18 columns and correlation was made with the column classification system developed in our laboratory. The method was evaluated using a Hypersil BDS C18 column (25 cm × 4.6 mm i.d.), 5 μm. A central composite design was applied to examine the robustness of the method. The method shows good precision, linearity, sensitivity and robustness. SQV(M) commercial samples of bulk drugs were examined using this method.

Keywords: Liquid chromatography; Purity control; Saquinavir; Saquinavir mesilate; Validation; Column ranking system

1. Introduction

Saquinavir mesilate (SQVM) and its free base saquinavir (SQV) are selective, competitive inhibitors of the human immunodeficiency virus (HIV) protease enzyme. Both SQVM and SQV are widely used in the treatment against the acquired immune deficiency syndrome (AIDS) and prescribed in combination with other antiretroviral drugs. The chemical structure of SQVM and one of its known related substances (Ro) are shown in Fig. 1.

UV has been described for the determination of SQV(M) [1–4]. Several methods were described to monitor SQV(M) and its metabolites in blood using liquid chromatography (LC) combined with electrochemical detection, with diode array detection (DAD), with UV detection and with mass spectrometric detection (MS) as well as capillary electrophoresis (CE) with MS. So far, LC methods for the assay and purity control of bulk drugs and formulations of SQV(M) have been published in official documents only. Two different LC methods for the assay and purity control of SQV and SQVM bulk drugs have been published in the Indian Pharmacopoeia (IP) [5,6]. An LC method for the purity control of SQV and SQVM bulk drugs has been published in the consultation document of the International Pharmacopoeia (Int. Ph.) [2,3] and the same LC method has been published for the assay and purity control of SQVM capsules [4]. Another LC method for the assay and purity control of SQVM and SQV capsules has been published in the United States Pharmacopeia (USP) [7,8].

The purpose of this study was to evaluate the LC method of the Int. Ph. monographs. For assay, an isocratic method is used, for purity control, a gradient elution is added to that isocratic method. Since no brand names are mentioned in the monograph, the suitability of a set of 18 columns, all complying to the prescriptions of the monographs, was investigated towards the separation of SQV(M) and its impurities. It was checked whether a correlation could be made with the column classification system developed in our laboratory [9–17].

The selectivity, limit of detection, limit of quantitation, linearity, repeatability and intermediate precision were examined on a Hypersil BDS C18 column.

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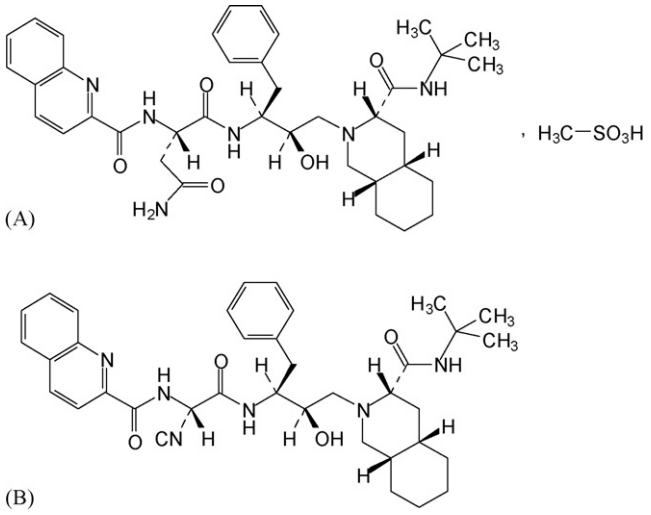

Fig. 1. Chemical structure of SQVM (A) and Ro, one of its known related impurities (B).

2. Experimental

2.1. Reagents and reference substances

HPLC-grade acetonitrile (ACN) was purchased from Acros Organics (Geel, Belgium), methanol (MeOH) and concentrated sulfuric acid from Fisher Scientific (Leicester, UK) and phosphoric acid from Riedel-de Haën (Seelze, Germany). Demineralised water was further purified with a Milli-Q system (Millipore, Milford, MA, USA). Reference substances of SQVM (99.3% on "as is" basis) and of Ro (synthesis by-product) were donated by the WHO (World Health Organization, Geneva, Switzerland). Commercial samples of SQV(M) were obtained from different manufacturers.

2.2. Preparation of standard solutions

For the investigation of the separation of SQV(M) and its impurities on the set of 18 reversed-phase columns and for the robustness study, a spiked sample solution was prepared by dissolving 12.5 mg of SQV, 12.5 mg of SQVM and 0.15 mg of Ro in 50 ml of mobile phase A. For purity control, SQV and SQVM solutions were prepared at a concentration of 0.5 mg/ml (100%) and dilutions were made to obtain 0.5 μg/ml (0.1%) solutions. For assay, 0.5 mg/ml solutions of SQVM reference standard and 0.5 mg/ml test solutions were prepared. The spiked solution was stored in the refrigerator. Fresh solutions were used for quantification experiments.

2.3. Instrumentation and liquid chromatographic conditions

LC equipment I (LaChrom, Merck Hitachi, Darmstadt, Germany) consisted of an L-7100 pump, an L-7200 autosampler, an L-7400 UV detector set at a wavelength of 225 nm and a D-7000 interface. EZChrome Elite 4.0 (Merck Hitachi) software was used for data acquisition. The column was kept in a water bath at 30 °C and the temperature was controlled using an EC Julabo thermostat (Seelbach, Germany). A Hypersil BDS C18 column (25 cm × 4.6 mm i.d.), 5 μm (Thermo Hypersil-Keystone, Cheshire, UK) was used for the validation work. The flow rate was 1.0 ml/min. The injection volume was 20 μl.

For the intermediate precision study, analyses were also carried out by another analyst using a new Hypersil BDS C18 column and LC equipment II (LaChrom Elite, Merck Hitachi) consisting of an L-2130 pump, an L-2200 autosampler and an L-2400 UV detector. Other conditions were identical.

LC equipment I and II were used to for the investigation of the separation of SQV(M) and its impurities on a set of 18 reversed phase columns.

2.4. Mobile phases

Mobile phase A consisted of 50 volumes of a mixture of five parts of ACN and two parts of MeOH, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water. Mobile phase B consisted of 70 volumes of ACN, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water. The phosphate buffer pH 3.4 was prepared by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of purified water, adjusting the pH to 3.4 by adding phosphoric acid (105 g/L) and diluting to 1000 ml with purified water. The gradient applied is shown in Table 1.

2.5. System suitability test

In a LC method, a system suitability test (SST) solution may be proposed to check the quality of the separation. According to the Int. Ph. monographs, a SST solution was prepared by mixing 2.0 ml of a 0.5 mg/ml SQV(M) solution with 5.0 ml of sulphuric acid (475 g/l) and by heating carefully in a boiling water bath for 30 min.

The resolution between the peak due to SQV (retention time about 21 min) (SQV) and the peak of similar size with a retention time of about 0.45 relative to the SQV peak (SSTPK1) should be not less than 14 (SST1) [2–4]. Moreover, the resolution between

Table 1
Gradient program used for assay and purity control of SQV and SQVM

	Time (min)	Mobile phase A (%, v/v)	Mobile phase B (%, v/v)
Isocratic	0–27	100	0
Linear gradient	27–45	100 to 45	0 to 55
Isocratic	45–55	45	55
Return to the initial conditions	55–60	45 to 100	55 to 0
Isocratic	60–75	100	0

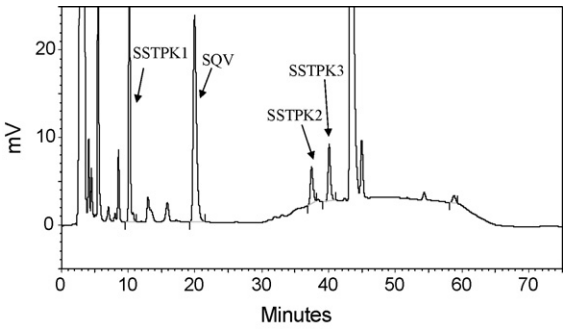

Fig. 2. Typical chromatogram of SST solution prepared by mixing 2.0 ml of a 0.5 mg/ml SQV(M) solution with 5.0 ml of sulphuric acid (475 g/l) and by heating carefully in a boiling water bath for 30 min.

two smaller peaks of similar size, eluted after the SQV speak and which increase during decomposition (SSTPK2 and SSTPK3), should be not less than 4.0 [2,3] or 2 [4] (SST2). The ratio of the retention times of these two peaks relative to the SQV peak is about 1.8 and 1.9 respectively. A typical chromatogram of the SST solution under the chromatographic conditions described in the monographs is shown in Fig. 2.

2.6. Column differentiation based on the CRF

The suitability of a column for a separation can also be examined by calculating the chromatographic response function (CRF), a measure of overall selectivity. Of course, this requires the presence of measurable impurity peaks, which is not always possible in daily routine analysis, unless reference substances are made available. The CRF is defined as

$$\mathrm{CRF} = \prod_{i=1}^{n-1} \frac{f_i}{g_i}$$

where n is the total number of peaks, g the interpolated peak height between two peaks (i.e. the distance between the baseline and a line connecting the two peak apexes, at the location of the valley) and f is the depth of the valley, measured from the line connecting two peak apexes [14–16] (Fig. 3(A)).

In this SQV(M) investigation, UNK1, SQV, UNK2, UNK3 and Ro were used to calculate the CRF values. The baseline separation problems are mainly related to the separation of small peaks eluted before (UNK1) and after (UNK2) the principal peak. Both UNK1 and UNK2 are relatively small compared to SQV and it is difficult to draw a line connecting the peak apexes. For these peak pairs, the calculation of f and g was slightly adapted as follows: g is the height above the baseline of the smallest peak of the pair and f is the distance between the line parallel to the baseline constructed through the highest point of the small peak and the lowest point of the valley between the two peaks (Fig. 3(B)). CRF values are always situated between 0 (two or more peaks are co-eluted) and 1 (all peaks are baseline separated). The CRF is a measure of the selectivity and does not take into account the peak shape (while resolution does).

2.7. Selection of a set of C18 columns

The monographs of SQV and SQVM prescribe the use of a base deactivated reversed phase C18 column (25 cm × 4.6 mm i.d.), 5 μm. This information is not always sufficient to select a column giving the required quality of separation, although the chromatographic conditions given in the monographs may be adjusted to reach the SST. Therefore, it was decided to examine the separation on a set of 18 columns, available in our laboratory and which are at least either base-deactivated or end-capped (the latter were included to check their performance). The columns were chosen based on a column ranking system developed in our laboratory [9–16] and which is also freely accessible on our website [17]. The ranking system is based on the determination of 4 chromatographic parameters. In this system, columns are ranked according to their F-values, calculated versus a reference column (in this case, a Hypersil BDS C18 was taken). The chromatographic parameters of the column with the highest F-value deviate most from these of the reference column. A list of columns examined in this study with their characteristics provided by the manufacturers and ranked by increasing F-values is shown in Table 2.

The SST solution and a spiked sample solution were used to investigate the influence of different stationary phases on the separation. The isocratic re-equilibration time in the gradient program was increased versus the official method by 5 min in order to provide sufficient re-equilibration time for all the different columns examined.

3. Results and discussion

3.1. Column differentiation based on the SST

Some typical chromatograms are shown in Fig. 4. The SST results for all 18 columns are shown in Table 2. Compared to the

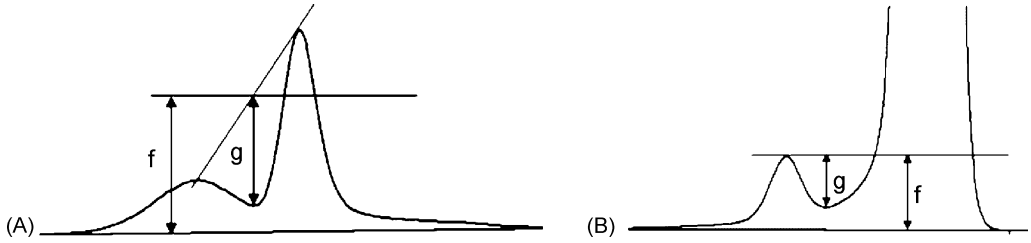

Fig. 3. Illustration of g and f for the calculation of CRF.

Table 2
List of C18 columns (250 mm × 4.6 mm i.d.), 5 μm involved in this study with their characteristics provided by the manufacturer and listed by increasing *F*-value

Column number	*F*-value	Name of the column	Manufacturer/supplier	Pore diameter (Å)	End-capped	Base-deactivated	Opt	SST1 (= or >14)	(= or >2) SST2	CRF
1	0.000	Hypersil BDS C18	ThermoQuest	130	+	+	No	15.69	4.88	1
2	0.168	Brava BDS	Alltech	145	+	+	Yes	14.77	1.67	1
3	0.436	ACE C18	Advanced Chrom. Tech./Achrom	100	+	+	Yes	16.73	1.87	1
4	0.480	Discovery C18	Supelco	180	+	−	Yes	22.27	3.93	1
5	0.667	Supelcosil LC-18 DB	Supelco	120	−	+	Yes	14.08	1.61	0
6	2.135	Nucleosil HD	Macherey-Nagel/Filter Service	100	+	−	Yes	18.85	0.88	1
7	2.303	Validated C18	Perkin-Elmer	100	+	−	Yes	9.89	0.84	1
8	2.813	Platinum C18	Alltech	100	+	+	No	9.42	DI	0
9	3.030	Symmetry	Waters	100	+	−	Yes	11.61	0.00	1
10	3.940	Purospher	Merck	80	+	−	No	15.44	1.54	1
11	4.698	Kromasil EKA	Akzo Nobel/SerCoLab	100	+	−	Yes	23.78	0.00	1
12	4.888	Purospher Star	Merck	80	+	+	Yes	23.82	1.43	1
13	5.456	Alltima C18	Alltech	120	+	+	No	24.20	1.17	1
14	7.162	Platinum EPS C18	Alltech	100	−	+	No	8.49	DI	0
15	9.146	LiChrospher	Merck	100	−	+	Yes	15.68	DI	0
16	10.477	Apex Basic	Jones Chromatography/Sopachem	100	+	+	Yes	DI	DI	0
17	18.397	Hypersil ODS C18	ThermoQuest	120	+	−	Yes	22.19	DI	1
18	26.256	Apex ODS II	Jones Chromatography/Sopachem	100	+	−	Yes	8.36	DI	0

Column 1 (Hypersil BDS C18) is taken as reference column ($F = 0$). The results of SST and CRF values for the separation of SQV(M) and its impurities for a set of 18 columns examined. CRF: chromatographic response function; SST: system suitability test; Opt: organic modifier in mobile phase A optimized; DI: difficult to identify.

Table 3
Correlation between end capping and/or base deactivation, SST1 (= or >14) and quality of separation (CRF = 1)

End-capped	Base-deactivated	Number of columns complying SST1/ number of this type columns examined	Number of columns CRF = 1/ number of this type columns examined
+	+	5/7	5/7
+	−	5/8	7/8
−	+	2/3	0/3

Hypersil BDS C18, faster or slower elution of SQV and its impurities was observed on most of the other columns examined. For the efficient comparison of the separation it was decided to optimize the amount of organic modifier present in mobile phase A so, that SQV was eluted between 18 and 24 min (around 21 min on Hypersil BDS C18 column). The relative retention times (RRTs) of UNK1, UNK2, UNK3 and Ro were calculated with respect to the retention time of SQV for each column and they are shown in Fig. 5. According to the SST1 requirement of the Int. Ph. monograph, columns 7, 8, 9, 14, 16 and 18 have resolutions below 14 (Table 2) and are marked as "not suitable" for this analysis. According to the SST2 requirement of the Int. Ph. monograph, columns 2, 3, 5, 6, 7, 9, 10, 11, 12 and 13 have resolutions below 2. Moreover, identification of SSTPK2 and SSTPK3 is difficult on columns 8, 14, 15, 16, 17 and 18. When the SST and CRF results were closely examined, it was observed that the SST criteria do not always give the required information. The SST2 criterion (> or = 2) was reached by only 2

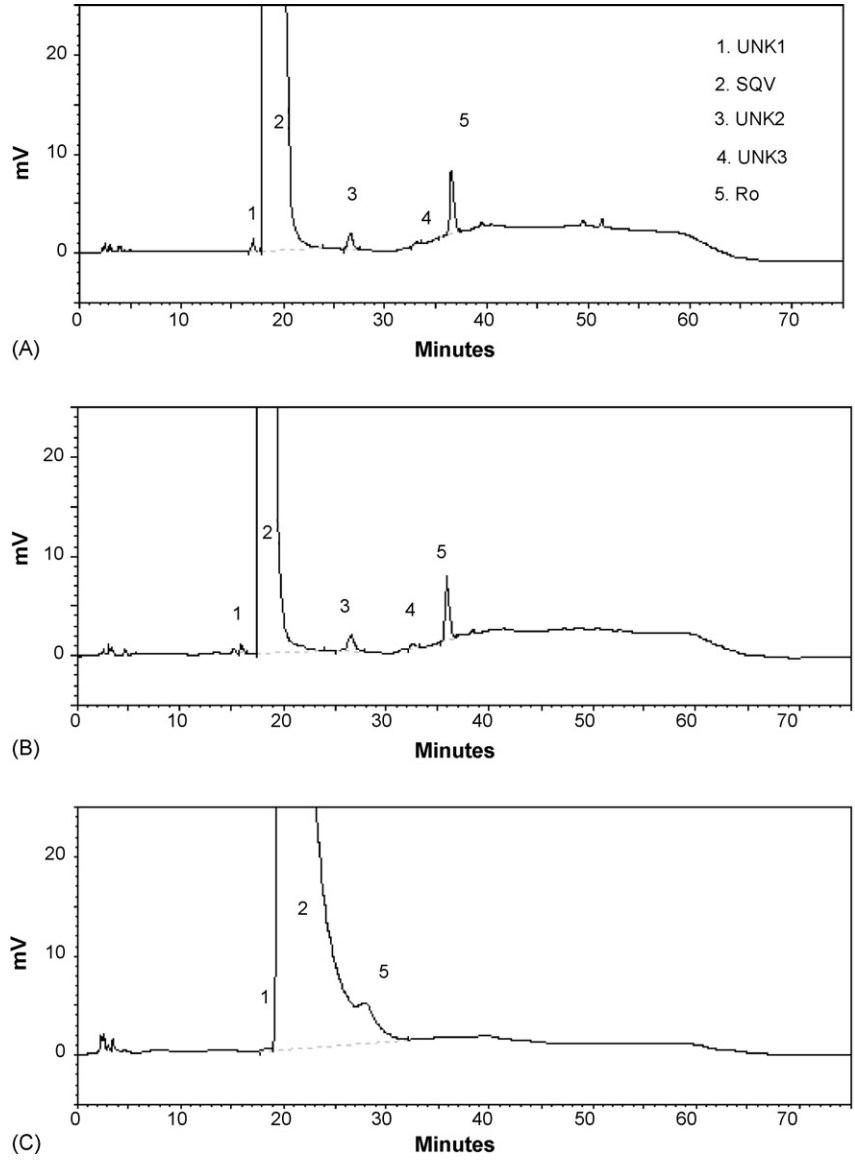

Fig. 4. Chromatograms for purity control obtained on different columns for a spiked SQV(M) sample.(A) Hypersil BDS; $F = 0.000$; CRF = 1.00 (column 1), (B) ACE C18; $F = 0.436$; CRF = 1.00 (column 3) and (C) Apex ODS II; $F = 26.256$; CRF = 0.00 (column 18).

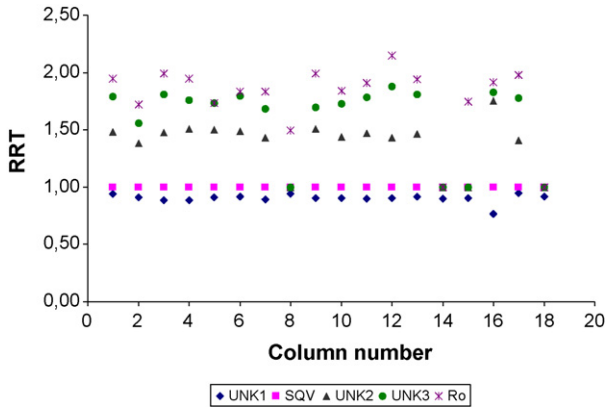

Fig. 5. Relative retention times of UNK1, UNK2, UNK3 and Ro vs. SQV for the 18 C18 columns examined.

columns, although 12 columns gave complete base line separation (CRF = 1). The SST2 criterion (> or = 4.0) was met by only one column. Therefore it is proposed not to consider SST2 to evaluate the quality of the separation. Columns 5 and 15, which comply according to SST1, gave a poor separation (CRF = 0). On the other hand columns 7 and 9, which are not suitable according to the SST1, gave baseline separation for all peaks (CRF = 1). This illustrates that a SST, developed on a single brand of stationary phase, does not allow to adequately distinguish between suitable and non suitable columns.

It is observed that ten out of fifteen columns, which are at least end capped, comply SST1 (= or >14) and also give complete baseline separation for all peaks (CRF = 1). The best separation for the analysis of SQV(M) and its impurities (CRF = 1) was achieved on end capped columns. Correlation between end capped and base deactivated columns with respect to the SST1 and baseline separation of all peaks examined (CRF = 1) is summarized in Table 3.

3.2. Correlation between the column classification and the separation of SQV(M)

It was also examined whether a correlation could be found between the column classification (taking the Hypersil BDS C18 column as reference to calculate *F*-values) and the separation data for SQV(M). The quality of the separations was expressed by the CRF-values. In previous correlation experiments, three arbitrarily chosen ranges of CRF values were distinguished: $F < 2$, $2 < F < 6$, $F > 6$ [14–16].

It is observed in Table 2 that four out of five columns with $F < 2$ give baseline separation for SQV(M) and its impurities (CRF = 1). When *F* is between 2 and 6, still seven out of eight columns give CRF = 1. For columns with $F > 6$ the probability to separate SQV(M) from its impurities clearly decreases: only one of five columns gives complete separation (CRF = 1).

With the increase of the *F*-value, the probability of finding a suitable column (CRF = 1) for the separation of SQV(M) and its impurities clearly decreased. The column classification system indicates to be a helpful tool for choosing a suitable stationary phase. However, it is clear that it is not faultless.

Table 4
Chromatographic parameter setting applied in the robustness investigation, corresponding to low (−), central (0) and high (+) levels

Parameter	Low value (−)	Central value (0)	High value (+)
ACN:MeOH (5:2) (%)	48	50	52
Buffer (%)	14	15	16
Buffer pH	3.3	3.4	3.5
Temperature (°C)	28	30	32

3.3. Method validation

3.3.1. Robustness study

The influence of four (*k*) chromatographic parameters on the separation was investigated using the Hypersil BDS C18 column. The parameters examined were the amount of the mixture CAN–MeOH (5:2) in mobile phase A, the amount and pH of the buffer in the mobile phases and the column temperature (°C). Their effects on the resolution for different pairs of compounds UNK1-SQV, SQV-UNK2, UNK2-UNK3, UNK3-Ro and SSTPK1-SQV were evaluated by means of an experimental design and multivariate data analysis using Modde 5.0 statistical graphic software (Umetrics, Umea, Sweden). The chromatographic parameter settings in the experimental design are shown in Table 4.

A central composite face centered (CCF) design was applied. A central composite design consists of points of a two level full factorial design (2^k), with *n* replicates of the central point, augmented with $2k$ star points to enable this model to estimate the curvature response. So, $2^k + 2k + n = 27$ experiments were performed, where $k = 4$ is the number of parameters and the central point was replicated three times ($n = 3$). The central composite design permits the response surface to be modelled by fitting a second-order polynomial model. The statistical relationship between a response Y and the experimental variables X_i, X_j,... is of the following form:

$$Y = \beta o + \beta_i X_i + \beta_j X_j + \beta_{ij} X_i X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \cdots + E$$

where the β's are the regression coefficients and *E* the overall experimental error. The linear coefficients β_i and β_j describe the quantitative effect of the experimental variables in the model. The cross product coefficient, β_{ij} measures the interaction effect between the variables and the square terms $\beta_{ii} X_i^2$ and $\beta_{jj} X_j^2$ describe non-linear effects on the response [18–21].

The individual and interaction parameter effects on the resolution for pairs UNK1-SQV, SQV-UNK2, UNK2-UNK3, UNK3-Ro and SSTPK1-SQV are summarized in Fig. 6. The effects on other peak pairs are not discussed, as the amount present in commercial samples was less than the disregard limit (0.05%) [2,3]. The plots consist of bars, which correspond to the regression coefficients. The magnitude of the variable effects is proportional to the regression coefficients. The bars denoted by variable *i* * variable *i* reflect the regression coefficients for the non-linear effect of that particular variable *i*, where the bars denoted by variable *i* * variable *j* reflect the interaction

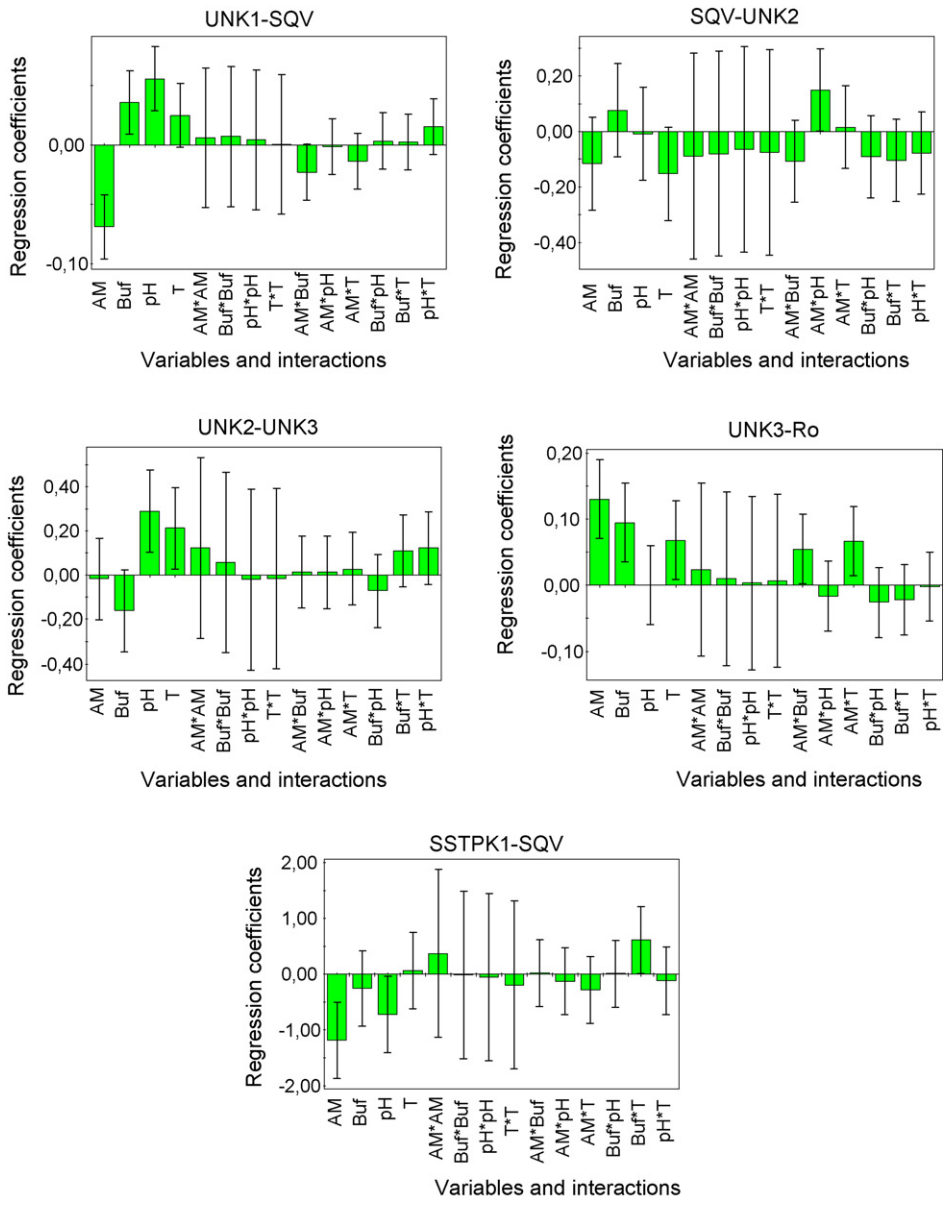

Fig. 6. Regression coefficient plots of the resolution between the peak pairs UNK1-SQV, SQV-UNK2, UNK2-UNK3, UNK3-Ro and SSTPK1-SQV (AM, ACN:MeOH (5:2); Buf, buffer content; pH, pH of the buffer; *T*, column temperature).

between the two variables concerned. The 95% confidence limits are expressed by using error lines. A regression coefficient smaller than the error line shows that the variation of the response caused by changing the variable is smaller than the experimental error. Therefore, the effect of that variable change is considered insignificant when compared to the experimental error. The coefficients of the terms in the model were estimated by the partial least squares (PLS) method. The statistical analysis of the model gave R^2 values above 0.8 for UNK1-SQV and UNK3-Ro, while for the peak pairs SQV-UNK2, UNK2-UNK3 and SSTPK1-SQV R^2 values of 0.7 were found. The R^2 values correspond to the fraction of variation of the response that can be explained by the model.

It is observed that the resolution, under the conditions examined, for peak pairs UNK1-SQV, UNK3-Ro and SSTPK1-SQV is principally influenced by the amount of the mixture of ACN–MeOH (5:2) present in mobile phase A. An increase of organic modifier has a negative effect on the separation of peak pairs UNK1-SQV and SSTPK1-SQV, while it has a positive effect on the peak pair UNK3-Ro. For the peak pair UNK2-UNK3 the pH of the buffer is the most important factor. It has a positive effect on the peak pairs UNK2-UNK3 and UNK1-SQV, while it has a negative effect on the peak pair SSTPK1-SQV. The amount of buffer present in the mobile phase influences positively the separation of the peak pairs UNK1-SQV and UNK3-Ro. For the peak pairs UNK2-UNK3 and UNK3-Ro the temperature of the column has a small positive effect. For peak pair SQV-UNK2 none of the effects is significant within the ranges examined. Neither interactions, nor square terms were found to be important. Fig. 7 shows the variation of the resolutions as a function of the two most influencing parameters for peak pairs UNK1-SQV, UNK2-UNK3, UNK3-Ro and SSTPK1-

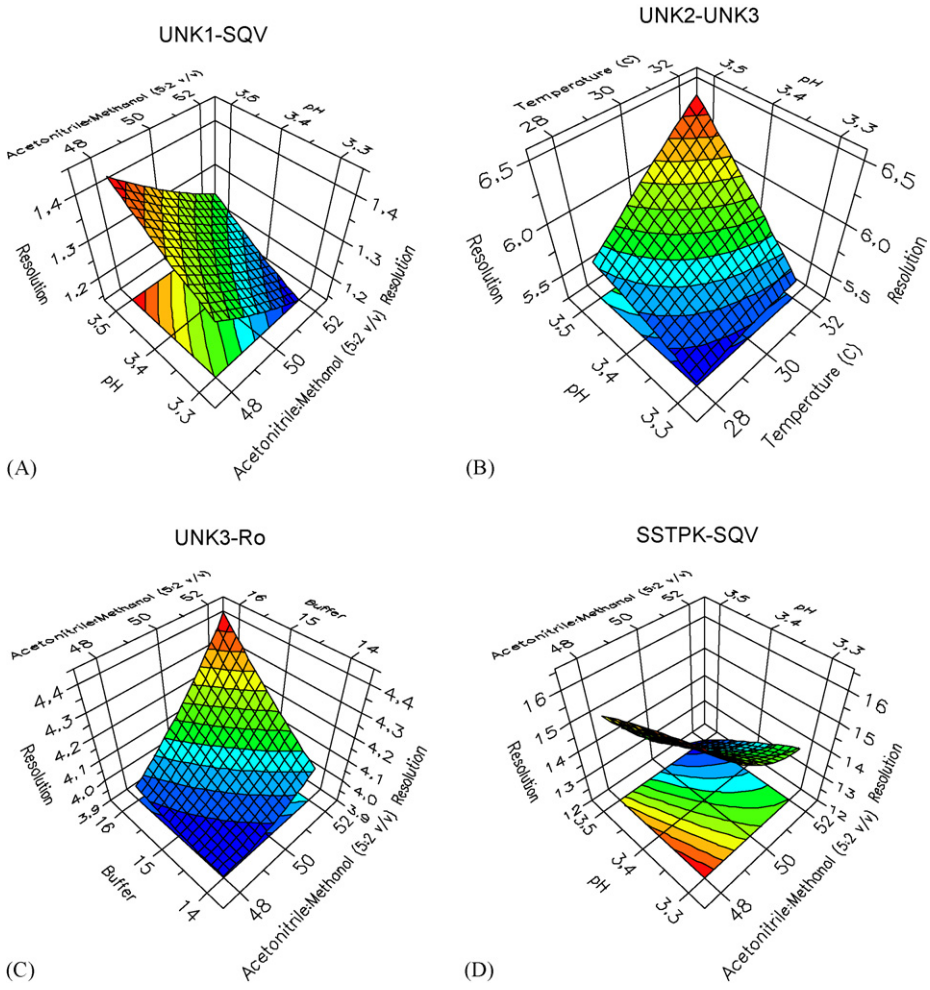

Fig. 7. Response surface plots of the resolution for the pairs: (A) UNK1-SQV as a function of the amount of mixture ACN–MeOH (5:2) and the buffer pH; (B) UNK2-UNK3 as a function of the temperature of the column and the buffer pH; (C) UNK3-Ro as a function of the amount of mixture ACN–MeOH (5:2) and the amount of buffer; (D) SSTPK1-SQV as a function of the amount of mixture ACN–MeOH (5:2) and buffer pH.

SQV. The other parameters were kept constant at the central value.

For SST1 (SSTPK1-SQV), an increase in the mixture of ACN–MeOH content to 52%, in combination with the higher buffer pH 3.5, reduces the resolution to 12 (prescribed limit = 14). However, the reduction in the resolution value (below the prescribed limit) did not affect the quality of the separation.

It can be concluded that any change of the parameter conditions within the examined range will not harmfully affect the quality of the separation since the resolution is always above 1.1.

3.4. Quantitative aspects

3.4.1. Sensitivity and linearity

The limit of detection (LOD) (determined at a signal-to-noise ratio of 3), the limit of quantitation (LOQ) (determined at a signal-to-noise ratio of 10) and the corresponding R.S.D. values are summarized in Table 5. The percentages were calculated with respect to the main component nominal value (0.5 mg/ml = 100%, 20 μl injected).

The linearity was checked by separate analyses of SQVM, SQV and Ro. The concentrations examined were in the range from LOQ to 125% for SQV and SQVM and in the range from LOQ to 5.0% *m/m* for Ro. The linearity data obtained for SQVM and Ro are summarized in Table 6.

3.4.2. Precision

The method was assessed using multiple preparations of a single commercial sample. Three different preparations of the same

Table 5
Limit of detection (LOD), limit of quantitation (LOQ) and corresponding R.S.D. values for SQV(M) and some of its impurities (0.5 mg/ml = 100%, 20 μl injected)

	SQV	SQVM	Ro
LOD			
% *m/m*	0.004	0.005	0.007
Mass on column (ng)	0.4	0.5	0.7
LOQ			
% *m/m*	0.013	0.016	0.020
Mass on column (ng)	1.3	1.6	2.0
R.S.D. (%, *n* = 6)	10	6	3

Table 6
Linearity data for SQVM and one of its impurities (0.5 mg/ml = 100%, 20 μl injected)

	Injected range (μg/ml)	Regression equation, y	R^2	$S_{y,x}$	n_c	n_i
SQVM	0.08–625 (LOQ-125%)	$113{,}386x - 42722$	0.999	117,030	11	3
Ro	0.1–25 (LOQ-5%)	$129{,}455x - 3\,227$	0.999	3,566	4	3

R^2: coefficient of determination; $S_{y,x}$: standard error of estimate; n_c: number of experimental concentrations studied; n_i: number of injections for each concentration; y: peak area; x: concentration injected (μg/ml).

Table 7
Precision data for SQVM and some of its impurities

	UNK1	UNK2	SQV	UNK3	Ro
Level (%)	0.09	0.07	100	0.05	0.15
%R.S.D. ($n = 9$)					
Day 1	2.11	1.81	0.40	0.93	1.00
Day 2	2.37	2.98	0.36	3.09	2.20
Day 3	1.72	3.50	0.68	2.34	3.85
%R.S.D. ($n = 27$)					
Days 1–3	4.40	3.10	0.48	4.68	2.69
%R.S.D. ($n = 9$)					
Day 4	2.85	1.79	0.43	3.13	2.25
%R.S.D. ($n = 18$)					
Days 3–4	2.29	4.68	0.56	5.52	3.09

commercial SQVM sample, each 0.5 mg/ml, were analyzed in triplicate on a single day. New preparations were made and analyzed on each of 3 successive days. An intermediate precision study was performed using another Hypersil BDS C18 column (25 cm × 4.6 mm i.d.) and another LC apparatus. On this system 3 solutions of the same commercial sample were injected in triplicate on a single day (day 4). R.S.D. values obtained for UNK1, UNK2, SQV, UNK3 and Ro on triplicate injections on a single day (day 4) ($n = 9$), three successive days (days 1–3) ($n = 27$) and combining day 3 (LC equipment I) and day 4 (LC equipment II) for the intermediate precision are summarized in Table 7.

3.4.3. Analysis of commercial samples

The Int. Ph. monographs on SQV and SQVM set the limit for any individual impurity not to be more than 0.1% and the sum of impurities not to be more than 0.5% in bulk samples. One SQV sample and two SQVM samples were analyzed for related substances and results obtained are summarized in Table 8. Both SQVM samples do not comply for any individual impurity not to be more than 0.1% and the sum of impurities not to be more than 0.5%. All the impurities are expressed as SQV(M), using a 0.1% dilution (0.5 μg/ml) of the examined sample.

Table 8
Purity control of SQV and SQVM bulk drugs

Bulk drugs	SQV	SQVM	
	Sample 1	Sample 1	Sample 2
Sum of impurities (%)	0.22	0.62	0.67
Total numbers of impurities above disregard limit (0.05%)	3	3	8
Number of impurities above 0.1%	0	3	2
Number of impurities above 0.3%	0	0	0
Complies?	Yes	No	No

4. Conclusions

The gradient LC method proposed in the Int. Ph. monographs shows a good separation of SQV(M) from its impurities in bulk drug substances, depending on the column used. The SST requirements of the Int. Ph. monographs do not always give the required information. The results obtained from SST1 show that a suitability test with only one pair of peaks is insufficient. Ideally to check the suitability of chromatographic conditions in a sufficient way, one should have available reference substances of the impurities. Since it is difficult to realise this in a pharmacopoeial context, the next least is to have available a sample, spiked with the impurities. It is proposed not to consider SST2 to evaluate the quality of the separation because it is difficult to perform on a number of columns and it excludes too many columns showing a good overall separation. The column classification system indicated to be a helpful tool for choosing a suitable column. It was found that, with the increase of the F-values, the probability of finding a column with a suitable separation for the analysis of SQV(M) and its impurities decreased. The method was shown to be robust, sensitive, precise and linear.

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