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A new validation approach applied to the GC determination of impurities in organic solvents

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Abstract

Organic solvents such as methanol, acetone, dichloromethane or toluene are frequently used in the pharmaceutical industry. The manufacturing of new active pharmaceutical ingredients (APIs) under GMP conditions commands to control adequately the quality of the different ingredients happening in the synthesis. Organic solvents have therefore to be controlled and their purity has to be determined before any GMP synthesis.

A selective gas chromatography (GC) method has been developed to determine the purity of acetone, dichloromethane, methanol and toluene. Using this method, the main contaminants of each organic solvent can be quantified. Moreover, the developed method allows the simultaneous determination of ethanol, isopropanol, chloroform, benzene, acetone, dichloromethane, methanol and toluene. Propionitrile was used as the internal standard.

The separation was obtained on a CP-SIL 8-CB low bleed/MS column (60 m × 0.32 mm i.d. × 1.0 μm coating thickness). The GC method was fully validated using a new approach based on the accuracy profile as a decision tool. The determination of β-expectation tolerance intervals for the estimation of total error – including both bias and precision – is used to better reflect the actual performances of the method, which is definitively the objective of the validation. The different validation criteria such as selectivity, response function, trueness, precision, accuracy, linearity or limits of detection and quantification were considered. The method was found to be able to quantitate with a good accuracy impurities around the 0.1% (v/v) concentration level for the different solvents.

Keywords: Organic solvents; Validation; Gas chromatography; Impurity; Methanol; Acetone; Toluene; Dichloromethane

1. Introduction

Organic solvents such as methanol, acetone, dichloromethane or toluene are frequently used in the pharmaceutical industry. These organic solvents are frequently used in chemistry either as reaction solvent or for extraction or crystallisation processes. The manufacturing of new active pharmaceutical ingredients (APIs) under good manufacturing practices (GMP) conditions commands to control adequately the quality of the different ingredients happening in the synthesis and, as solvents are part of the synthesis, it is of prime importance to control their purity.

Gas chromatography (GC) coupled to flame ionization detection (FID) is obviously the most common technique for analysing organic solvents. There is an abundant literature concerning the analysis of organic solvents, either in GC or in head-space gas chromatography (HS-GC) [1–10] and some methods are described in United States (USP) or European (EP) pharmacopoeias [11,12]. However, the methods mentioned in the USP and EP were methods that are dedicated to the determination of residual solvents in drug substances, excipients or drug products. These methods are selective towards a wide range of organic solvents but are not intended to the determination of purity of one solvent. Indeed, the selectivity of these methods is essentially focused

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on the ability of the method to separate the different solvents used in pharmaceutical industry. However, when the quality of one solvent has to be evaluated, the monographs in pharmacopoeias report some specific method for a given solvent [13,14]. It is therefore very time-consuming to evaluate the purity of different solvents using different methods. Indeed, in a production plant, the quality control of solvents is a repetitive task since the different batches supplied by the manufacturer have to be analysed and since the consumption of these products is often very important.

The first objective of this work was to develop one simple GC method for the evaluation of purity of four main solvents, i.e. methanol, acetone, dichloromethane and toluene. The developed method should ideally be able to determine with a suitable accuracy impurity around the 0.05% (v/v) concentration level. This method allows the simultaneous determination of the considered solvents but also of the main impurities of each solvent. Methanol, ethanol, methyl ethyl ketone (MEK), isopropanol are the main impurities that should be evaluated in acetone; ethanol, acetone, isopropanol and MEK should be researched in methanol; ethanol, methanol and chloroform are the main impurities of dichloromethane and benzene should be limited in toluene. Acetone, methanol, ethanol, dichloromethane, chloroform, toluene, benzene, isopropanol and MEK were therefore considered in this study.

Another objective of the work consists in validating adequately the developed method. Since the control of the solvent needs to quantify low amounts of impurities, the validation of the method was investigated in a low concentration range, from 0.01 to 2.0% (v/v). The validation was performed according to the new strategy proposed by Hubert et al. [15,16]. This validation strategy consists in two steps. The first step, called pre-validation step, consists in selecting the most suitable calibration model using an accuracy profile as a selection tool [15,16]. The second step, corresponding to the validation itself, consists in testing the method selectivity and the assessment of precision, trueness and accuracy [15,17] at different concentration levels and in determining the limits of quantitation and the method linearity [15,18]. The calibration model selected consisted in a linear regression using one concentration level. The relative standard deviation (R.S.D.) values for repeatability and intermediate precision were less than 5% for the different solvents studied, except for MEK, ethanol and isopropanol for which R.S.D. values for intermediate precision were 6.8, 8.8 and 10.3%, respectively. Moreover the method was found to be accurate over the 0.05–2.0% (v/v) calibration range for the nine solvents since the 95% β-expectation tolerance interval of the relative error did not exceed the acceptance limits of −10% and +10%. The LOQ was found to be around 0.05% for all solvents evaluated, except for benzene and toluene for which LOQs were found to be 0.01% (v/v). Finally, the method reported was successfully used to perform the evaluation of acetone, methanol, dichloromethane and toluene.

2. Experimental

2.1. Chemicals

Acetone, benzene, chloroform, dichloromethane, ethanol, methyl ethyl ketone (MEK), isopropanol, methanol, toluene were all for gas chromatography grade from Merck (Darmstadt, Germany). Ethyl acetate was used as solvent and was also for gas chromatography grade from Merck. Propionitrile (99% for gas chromatography grade) was used as internal standard and was supplied by Acros (Geel, Belgium).

2.2. Apparatus

The GC system consisted in a Model 6890N Series gas chromatograph equipped with a an autosampler from Agilent Technologies (Palo Alto, CA, USA). The detection was performed by means of a flame ionization detector (FID).

A PC Compaq Evo GX1 (Round Rock, TX, USA) equipped with Empower Pro 5.0 version software from Waters (Milford, MA, USA) was used to control the GC system and to collect and treat the data. The enoval® software (Arlenda, Liège, Belgium) was used to determine the accuracy profiles and other validation criteria.

2.3. Chromatographic technique

The chromatographic experiments were carried out using a CP-SIL 8-CB Low Bleed/MS column (60 m × 0.32 mm i.d.) coated with 1.0 μm thickness film of 5% phenyl and 95% dimethylpolysiloxane from Varian Inc. (Palo Alto, CA, USA). A second column with the same stationary phase but different dimensions (CP SIL 8-CB, 30 m × 0.32 mmi.d.with 5.0 μm thickness) was also used in the development of the method. The GC was operated under the following conditions: carrier gas was Helium; the inlet pressure was set to 10.0 psi; the injector and detector temperatures were set to 280 and 320 °C, respectively. A 0.2 μl volume was injected using the split mode (ratio 1:50). The column temperature was programmed at 35 °C for 10 min, and then raised to 120 °C at a rate of 40 °C min^{-1}. The 120 °C temperature was kept constant for 8 min and then was raised to 300 °C at a rate of 20 °C min^{-1}.

2.4. Standard solutions

2.4.1. Solutions used for method development

Solutions of each solvent were prepared independently by dissolving the appropriate amount of each compound in ethyl acetate in order to obtain a final concentration of 1.0% (v/v) (1000 μl/100 ml). A solution containing all solvents and propionitrile (IS) was also prepared in ethyl acetate in order to achieve a final concentration of 1.0% (v/v) for each compound to demonstrate the global selectivity of the method.

2.4.2. Solutions used for method validation

A mixed solution containing the nine solvents studied was prepared by diluting stock solutions with ethyl acetate to reach a concentration of 4.0% (v/v) for each compound.

This solution was then diluted adequately and added with suitable amount of IS to obtain solutions ranging from 0.01 to 2.0% (v/v) (10–2000 μl/100 ml) for each solvent. The final concentration of IS is fixed to 0.1% (v/v).

2.4.3. Standard solutions for routine analysis

Dilute 1.0 ml of impurity to 100 ml with ethyl acetate. Two milliliters of this solution is then added to 2.0 ml of internal solution (1 ml/100 ml of IS in ethyl acetate) and diluted to 20 ml with ethyl acetate. This solution corresponds to 0.10% (v/v) concentration for the impurity.

2.5. Sample preparation

The sample solution is prepared by diluting 2.0 ml of internal standard solution (1000 μl/100 ml of IS in the sample to analyze) to 20 ml using the solvent to analyze.

2.6. Routine analysis

The developed GC method was used to control the purity profile of different batches of the four following solvents: methanol, acetone, dichloromethane and toluene.

3. Results and discussion

3.1. Selection of the GC conditions

The GC method for the simultaneous determination of the nine solvents considered was investigated. The first experiments were performed on the 30 m CP SIL 8-CB column using the temperature conditions as described above, a 1.0 μl injection volume and a split ratio of 1:20. In these conditions, the separations obtained on one hand between chloroform and ethyl acetate, and on the other hand, between acetone and isopropanol were not satisfactory. The resolution between chloroform and ethylacetate was found to be 1.2. By increasing the column length, a better separation was obtained between ethyl acetate and chloroform and the resolution between these peaks was increased to 3.5. To improve the separation between isopropanol and acetone, the parameter that was investigated is related to the injection of the sample. Indeed, as isopropanol is a potential impurity of acetone, the difference of concentration of both compounds may cause a problem for quantifying low amounts of isopropanol in acetone. By modifying the split ratio of the injection mode, it was possible to increase the resolution between these two compounds. The injection volume was reduced from 1.0 to 0.2 μl and the split ratio was set to 1:50 instead of 1:20, allowing the resolution between acetone and isopropanol to increase from 0.34 to 1.05. The complete separation of all solvents is illustrated in Fig. 1.

3.2. Validation

3.2.1. Prevalidation step

The response function of an analytical procedure is a very important criteria that must be considered in the validation of the method since it corresponds to the assessment of the relationship between the response (i.e. the chromatographic signal) and the concentration (amount) of the analyte in the sample system [15,18–23]. The approach based on two-sided 95% β-expectation tolerance intervals [15,24] for total measurement error – including bias and precision – was used in order to select the most appropriate response function.

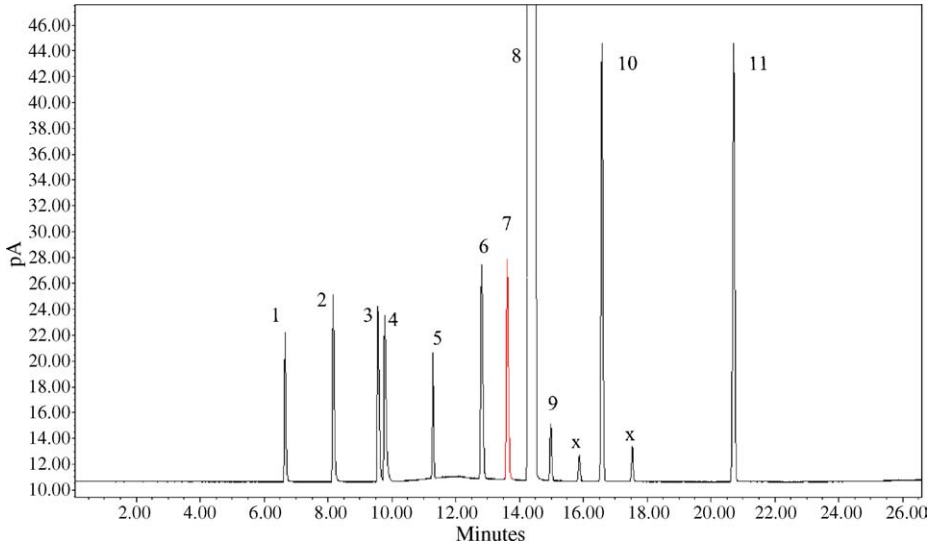

Fig. 1. Chromatographic separation of all compounds investigated: (1) methanol; (2) ethanol; (3) acetone; (4) isopropanol; (5) dichloromethane; (6) propionitrile; (7) MEK; (8) ethyl acetate; (9) chloroform; (10) benzene; (11) toluene; (×) endogenous compound from ethyl acetate.

This approach reflects more truly the performance of future individual assays and reduces the risk of rejecting in-study runs.

The validation of the analytical method was conducted on the different compounds mentioned in the study. However, in order to keep this article as clear as possible, the selection of the suitable calibration model is described only for one compound. Benzene is selected as an example but the same investigations were conducted for acetone, ethanol, methanol, dichloromethane, MEK, isopropanol, toluene and chloroform. The numbers of concentration levels is three for the calibration standards and five for the validation standards. The calibration standards are used to set up the calibration model and the validation standards are used to estimate the precision, trueness and accuracy of the method. Four series ($k = 4$) with three concentrations levels (0.01–0.1 and 2.0%; $n = 3$) and three repetitions per level were performed over the concentration range from 0.01 to 2.0% (or 10–2000 μl/100 ml) to generate the data used for the calibration model while four series with five concentrations levels (ranging from 0.01 to 0.05, 0.1 to 1.0 and 2.0%) and three repetitions per level were performed to estimate the availability of the model proposed. These validation experiments (preparation and analyses) were performed by two different operators.

Fig. 2 illustrates the different accuracy profiles obtained by analysing the validation experiments with different regression models such as linear regression, weighted linear regression, quadratic regression, weighted quadratic regression, linear regression after square root transformation, weighted linear regression after square root transformation, linear regression after logarithm transformation, weighted linear regression after logarithm transformation, linear regression through 0 fitted using the highest level only (2.0%, v/v) and linear regression through 0 fitted using the level 3 only (0.1%, v/v). The selection of the most suitable model is made using the accuracy profile [15]. The accuracy profile is used as a tool to decide the capability of the method to give results inside the acceptance limits. The accuracy profile is obtained by linking on one hand the lower bounds and on the other hand the upper bounds of the β-expectation tolerance limits calculated at each concentration level.

If the β-expectation tolerance interval stays within the predefined 10% acceptance criteria, the corresponding regression model can be used since it guarantees that the method will be able to give a result within the β-expectation tolerance interval 95 times out of 100 experiments. Considering the objective of the present method, i.e. the determination of impurities, it is reasonable to set the acceptance limit to 10%. Regarding the accuracy profiles obtained for benzene, all the calibration models can be used. The analysis of the accuracy profiles of all other solvents was performed using the same approach and among the different possibilities, the linear regression through zero using one concentration level (level 3 = 0.1%, v/v) was selected for all compounds considered in this study since it represents the simplest regression model, even if, in some cases, some other models could also be used. The main advantage of this model consists in its very simple use since it allows the quantification of unknown samples using a single standard solution as reference. It is important to note that the objective of the method was to allow the determination of a 0.1% and ideally a 0.05% (v/v) impurity concentration since the acceptance criterion for the conformity of the solvent is fixed to 99.5% of purity (not more than 0.5%, v/v, for total impurities). This is the reason why the standard concentration level selected corresponds to the 0.1% level. Moreover, even if the concentration ranged investigated is larger than needed, it is still interesting to dispose of a method with a wider concentration range, giving possible the analysis of sample containing higher amounts of impurities than expected. Moreover, in the present case, it was also interesting to illustrate that a relatively wide concentration range (20×) can be covered using a single standard concentration level situated relatively low in the concentration range.

3.3. Stability

The stability of the different solutions was investigated over 24 h at room temperature (22 ± 2 °C). The determination of the different solvents and IS were performed at the beginning and at the end of the storage period. The results obtained were all comprised between 98 and 102% of the initial value. No significant degradation of any solvents studied and internal standard was observed.

Table 1
Response functions

Compound	Series 1	Series 2	Series 3	Series 4	Mean	S.D.
Acetone	7.81	7.74	7.85	7.66	7.76	0.08
Benzene	19.57	19.43	19.63	19.39	19.51	0.11
Chloroform	2.83	2.36	2.38	2.34	2.48	0.24
Dichloromethane	3.76	3.82	3.83	3.83	3.81	0.03
Ethanol	7.43	7.41	7.34	7.20	7.34	0.10
Isopropanol	8.19	8.16	8.13	8.04	8.13	0.07
Methanol	4.91	4.91	4.95	4.77	4.88	0.08
MEK	10.14	10.05	10.00	10.05	10.06	0.06
Toluene	19.05	19.15	19.39	19.11	19.18	0.15

The linear through zero using one single concentration level (0.1%, v/v) calibration model was selected ($Y = bX$).

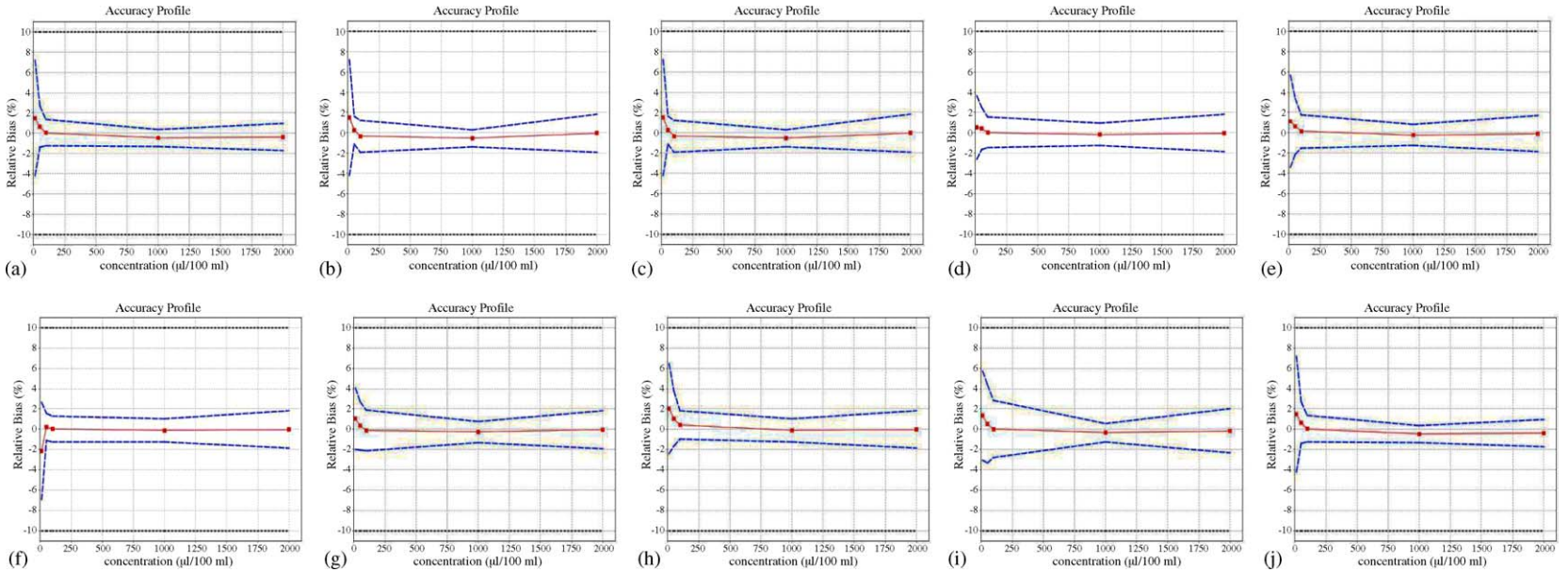

Fig. 2. Accuracy profiles obtained from Enoval® with different calibration models for the benzene compound: (a) weighted linear regression; (b) quadratic regression; (c) weighted quadratic regression; (d) linear regression after square root transformation; (e) weighted linear regression after square root transformation; (f) linear regression; (g) weighted linear regression after logarithm transformation; (h) linear regression through 0 fitted using the highest level only; (i) linear regression after logarithm transformation; (j) linear regression through 0 fitted using the level 3 only.

3.4. Selectivity

The selectivity of the method is clearly demonstrated in Fig. 1 that illustrates the complete separation of the main solvents considered in this work and their corresponding contaminants. All solvents are well separated. It should also be noted that two endogenous peaks coming from ethyl acetate, which was used as a dilution solvent, were observed at about 16 and 17.5 min but were not interfering with compounds of interest.

3.5. Response function

As previously mentioned under the pre-validation step, the linear regression using a single concentration level model was used. Four series ($k = 4$) with the only 0.1% (v/v) concentra-

Table 2
Trueness and precision

Compound	Concentration (μl/100 ml)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
Acetone	10	−7.7	4.3	4.3
	50	−0.4	1.1	1.1
	100	0.0	0.7	0.8
	1000	0.8	0.5	0.7
	2000	1.2	0.5	0.6
Benzene	10	1.2	1.3	1.5
	50	0.2	0.4	0.5
	100	−0.3	0.4	0.6
	1000	−0.9	0.4	0.4
	2000	−0.8	0.4	0.4
Chloroform	10	19.3	1.5	1.5
	50	−1.3	4.7	4.7
	100	0.2	1.8	2.0
	1000	0.6	0.4	0.7
	2000	0.9	0.4	0.5
	10	19.3	1.5	1.5
Dichloromethane	10	11.0	1.5	2.5
	50	−0.4	1.9	2.7
	100	−0.2	1.6	1.9
	1000	0.0	0.5	1.0
	2000	0.2	0.5	1.2
Ethanol	10	10.4	1.5	8.8
	50	1.2	2.0	2.1
	100	0.4	0.8	0.9
	1000	0.7	0.5	1.3
	2000	1.2	0.5	1.1
Isopropanol	10	24.3	4.3	10.3
	50	1.0	1.1	1.7
	100	−0.4	0.9	0.9
	1000	−1.0	0.4	0.8
	2000	−0.8	0.4	0.7
Methanol	10	−13.3	1.1	1.1
	50	0.0	1.2	2.9
	100	0.4	1.3	2.3
	1000	2.7	0.5	1.4
	2000	3.6	0.5	1.3
Methyl ethyl ketone	10	5.1	4.3	6.8
	50	0.6	1.2	1.2
	100	−0.3	0.6	0.7
	1000	−0.6	0.4	0.7
	2000	−0.4	0.4	0.7
Toluene	10	0.5	2.9	3.3
	50	0.1	0.6	0.8
	100	−0.1	0.3	0.4
	1000	−0.9	0.4	0.4
	2000	−0.9	0.4	0.5

tions level and three repetitions at this level were performed. One equation was obtained for each series and the average equation for each compound was calculated (Table 1).

3.6. Trueness

Trueness refers to the closeness of agreement between the mean value obtained from a series of measurements and the conventionally accepted value or reference value [15]. It gives information on systematic error. Trueness is expressed in terms of relative bias (%). It was assessed using validation standards at five concentration levels, ranging from 10 to 2000 μl/100 ml, corresponding to concentration ranging from 0.01 to 2.0% ($k = 4$, $n = 5$). Three independent validation standard solutions were injected for each concentration level. As can be shown in Table 2, the proposed method can be considered as true since the bias did not exceed the values of 10% irrespective to the concentration level for toluene, ben-

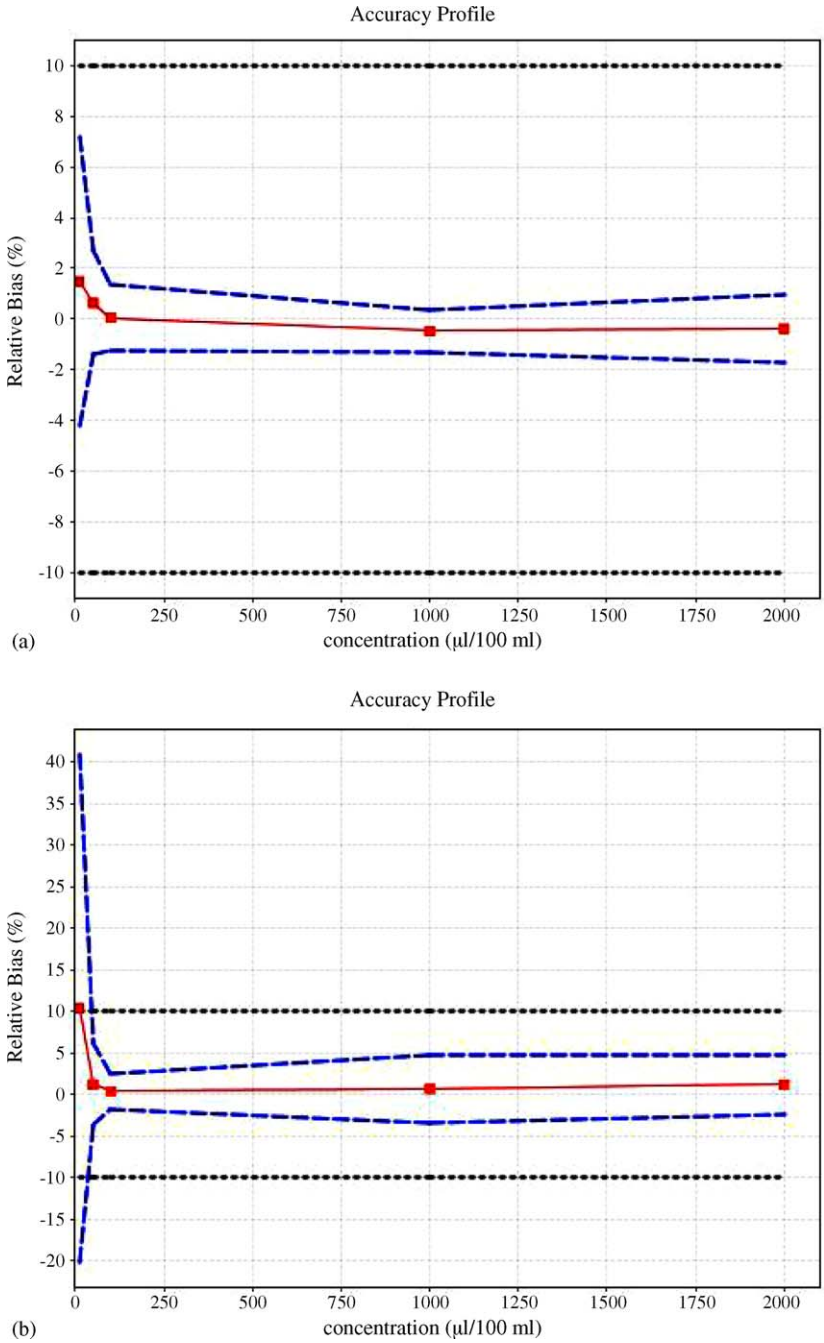

Fig. 3. Accuracy profiles of benzene and ethanol obtained from Enoval® using the linear regression model with one concentration level (level 3 = 0.1%, v/v): (a) accuracy profile of benzene; (b) accuracy profile of ethanol.

zene, acetone and MEK. However, the relative bias observed for chloroform, dichloromethane, isopropanol, methanol and ethanol at the 0.01% (v/v) level are higher than the desired 10% limit, illustrating the importance of the systematic error at the lowest concentration level.

3.7. Precision

The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. It gives information on the random error [15]. The precision was estimated by measuring repeatability and intermediate precision for the different compounds at different concentration levels ranging from 10 to 2000 μl/100 ml (0.01–2.0%, v/v). The variance of repeatability and intermediate precision as well as the corresponding relative standard deviation (R.S.D. (%)) were calculated from the estimated concentrations. The R.S.D. values presented in Table 2 were relatively low, and generally less than 5%,

Table 3
Accuracy

Compound	Concentrated (μl/100 ml)	β-Expectation limit (μl/100 ml)	Relative β-expectation limit (%)	Risk (%)
Acetone	10	8.2–10.2	−17.64 to 2.23	45.2
	50	48.5–51.10	−2.93 to 2.21	2.9×10^{-5}
	100	98.0–102.0	−1.99 to 1.98	2.9×10^{-5}
	1000	989.6–1026	−1.04 to 2.64	2.9×10^{-5}
	2000	1995–2051	−0.23 to 2.57	2.9×10^{-5}
Benzene	10	9.8–10.5	−2.18 to 4.64	1.7×10^{-3}
	50	49.5–50.8	−1.07 to 1.57	2.9×10^{-3}
	100	98.1–101.2	−1.93 to 1.24	2.9×10^{-3}
	1000	982.6–999.7	−1.74 to −0.03	2.9×10^{-3}
	2000	1966–2002	−1.69 to 0.09	2.9×10^{-3}
Chloroform	10	11.6–12.23	15.92 to 22.67	100.0
	50	43.9–54.8	−12.18 to 9.66	12.0
	100	95.3–105.0	−4.65 to 4.95	5.0×10^{-2}
	1000	988.2–1025	−1.18 to 2.45	2.9×10^{-5}
	2000	1994–2041	−0.32 to 2.07	2.9×10^{-5}
Dichloromethane	10	10.4–11.8	4.05 to 18.01	82.8
	50	46.3–53.3	−7.40 to 6.63	1.7
	100	95.2–104.4	−4.76 to 4.38	3.0×10^{-2}
	1000	971.0–1031	−2.90 to 3.09	2.9×10^{-5}
	2000	1932–2076	−3.42 to 3.81	1.2×10^{-3}
Ethanol	10	8.0–14.1	−20.13 to 40.93	75.0
	50	48.2–53.01	−3.69 to 6.13	0.22
	100	98.3–102.5	−1.69 to 2.45	2.9×10^{-5}
	1000	966.2–1048	−3.38 to 4.78	2.0×10^{-2}
	2000	1953–2095	−2.35 to 4.76	5.8×10^{-3}
Isopropanol	10	9.2–15.7	−7.95 to 56.53	98.5
	50	48.3–52.7	−3.44 to 5.38	6.4×10^{-2}
	100	97.5–101.7	−2.47 to 1.72	2.9×10^{-5}
	1000	964.7–1015	−3.53 to 1.46	2.9×10^{-5}
	2000	1944–2024	−2.8 to 1.21	2.9×10^{-5}
Methanol	10	8.4–8.9	−15.78 to −10.90	100.0
	50	45.6–55.5	−8.89 to 8.92	4.8
	100	93.7–107.2	−6.31 to 7.20	1.5
	1000	982.2–1072.4	−1.78 to 7.24	0.7
	2000	1992.0–2152.7	−0.40 to 7.64	0.8
Methyl ethyl ketone	10	8.7–12.4	−13.35 to 23.54	45.2
	50	48.9–51.7	−2.21 to 3.39	2.9×10^{-5}
	100	98.01–101.3	−1.95 to 1.25	2.9×10^{-5}
	1000	974.2–1014	−2.58 to 1.40	2.9×10^{-5}
	2000	1955–2027	−2.23 to 1.35	2.9×10^{-5}
Toluene	10	9.3–10.9	−7.5 to 8.5	3.3
	50	49.1–51.0	−1.8 to 2.0	2.9×10^{-5}
	100	98.7–101.0	−1.3 to 1.0	2.9×10^{-5}
	1000	982.1–999.7	−1.8 to 0.0	2.9×10^{-5}
	2000	1956–2009	−2.2 to 0.5	2.9×10^{-5}

except for ethanol, isopropanol and MEK at the lowest concentration level. The results illustrate the good precision of the proposed method for all considered compounds, especially if it is remembered that two different operators were involved in the realization of the validation.

3.8. Accuracy

Accuracy expresses the closeness of agreement between the calculated value and the accepted reference value, namely the conventionally true value [15]. The accuracy takes into account the total error, i.e. systematic and random errors, related to the test result. It is assessed from the accuracy profile illustrated in Fig. 3. Fig. 3a and b illustrate the accuracy profiles of benzene and ethanol, respectively. They show clearly that the method of determination of benzene is accurate over the whole concentration range while the determination of ethanol is only accurate between 0.05 and 2.0% (50 and 2000 μl/100 ml). The upper and lower β-expectation tolerance limits expressed in μl/100 ml presented in Table 3 as a function of the introduced concentrations demonstrate that the method is accurate for all solvents tested within the range from 0.05 to 2.0% (v/v) since the limits of tolerance of the errors (relative β-expectation tolerance limits) do not exceed the acceptance limits (±10%). However, at the lowest concentration level (0.01% (v/v) or 10 μl/100 ml), the accuracy of the method is clearly not suited to its objective. Indeed, at this concentration level, except for toluene and benzene, the calculated β-expectation tolerance limits are clearly outside the desired limits. Table 3 also indicates the risk of having measurements falling outside of the acceptance limits (10%) and it is very important to note that the risk of giving a concentration value with an error higher than 10% at the lowest concentration level for acetone, chloroform, dichloromethane, ethanol, MEK, isopropanol and methanol is very high, and even sometimes equal to 100%. The determination of chloroform at a 0.05% (v/v) level is also a borderline case since the calculated relative β-expectation tolerance limits are very close to the 10% acceptance limit. The risk of giving a result with an error higher than 10% is evaluated to be 12% that is slightly higher than the acceptance limit.

3.9. Linearity

The ability for an analytical method to give results directly proportional to the concentrations (amounts) of analyte in the sample within a definite concentration range is called linearity [15]. This criterion has to be applied only to results (concentrations or amounts), not to responses (i.e. chromatographic signals). A regression line was therefore fitted between the back-calculated concentrations versus the introduced concentrations applying the linear regression model based on the least squares method. This regression line was calculated for the different considered solvents and the equations are presented in Table 4. A graphic illustration of linearity is presented in Fig. 4, using benzene as an example. It shows clearly the linear relation between the back-calculated concentration and the actual concentration of benzene. The dashed limits correspond to the accuracy profile while the dotted line corresponds to the acceptance limits, set at 10% in the present example. This graph also illustrates the accuracy of the method, expressed in the concentration unit.

Table 4
Linearity

Compound	Slope	Intercept	R^2
Acetone	1.012	−1.362	0.9999
Benzene	0.992	0.269	1.0000
Chloroform	1.008	−0.257	1.0000
Dichloromethane	1.002	0.019	0.9998
Ethanol	1.011	−0.5718	0.9998
Isopropanol	0.991	0.9467	0.9999
Methanol	1.036	−3.218	0.9998
Methyl ethyl ketone	0.995	0.205	0.9999
Toluene	0.991	0.402	1.0000

3.10. Detection and quantitation limits

The limit of detection is defined as the lowest amount of the considered substance that can be detected, but necessarily quantified as an accurate value [15]. The limits of detection of the considered compounds in the present study were estimated using the mean intercept of the calibration model and the residual variance of the regression. The limit of quantitation of an analytical procedure is defined as the smallest quantity of the considered substance in the sample that can be quantitatively determined under the experimental conditions with well defined accuracy [15], i.e. taking into account the systematic and random errors [22,23]. This definition can also be applied to the upper limit of quantitation, which is therefore the highest concentration or quantity that can be determined with a well-defined accuracy. The limits of quantitation can therefore be obtained by calculating the smallest and highest concentration beyond which the accuracy limits or β-expectation tolerance limits go outside the acceptance limits. Limits of detection and quantitation for the considered compounds are mentioned in Table 5. The concentration

Table 5
Limits of detection and quantitation

Compound	LOD (μl/100 ml)	Lower LOQ (μl/100 ml)	Upper LOQ (μl/100 ml)
Acetone	3.039	30.77	2000
Benzene	2.014	10.00	2000
Chloroform	5.55	64.50	2000
Dichloromethane	3.826	38.15	2000
Ethanol	2.783	45.55	2000
Isopropanol	2.158	46.39	2000
Methanol	4.303	43.57	2000
Methyl ethyl ketone	1.248	36.87	2000
Toluene	0.981	10.00	2000

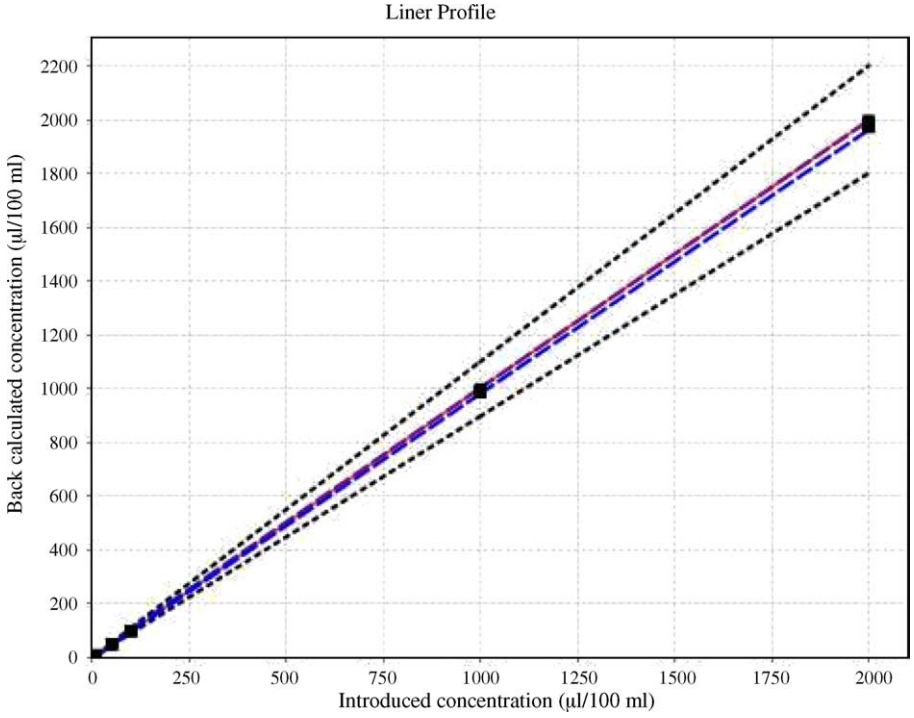

Fig. 4. Linear profile of benzene. The dashed limits correspond to the accuracy profile, i.e. the β-expectation tolerance limits expressed in the concentration unit (µl/100 ml). The dotted curves represent the acceptance limits at 10% expressed in the same concentration unit.

range for which the method is validated is comprised between the lower and the upper limits of quantitation.

3.11. Routine analysis

The GC method was used to analyze different batches of methanol, dichloromethane, acetone and toluene before their utilization in different synthesis of APIs under GMP rules. All batches analyzed were declared to be conformed to the in-house specifications (not less than 99.5%) of purity. The impurities assayed in the four solvents tested were all lower than 0.1% in all batches tested.

4. Conclusions

A sensitive, accurate and precise GC analytical method was developed for the determination of different impurities in methanol, acetone, toluene and dichloromethane. The quality control of these four solvents can be performed with the same method that allows the simultaneous determination of very low concentrations of acetone, benzene, chloroform, dichloromethane, ethanol, isopropanol, methanol, MEK and toluene.

The method was validated using a new approach based on the accuracy profile determination described in previous studies and was found to meet the requirements for a further investigation of traces impurities in solvents. The method was successfully used for the quality control either of methanol, toluene, acetone and dichloromethane.

References

[1] K. Urakami, A. Higashi, K. Umemoto, M. Godo, J. Chromatogr. A 1057 (2004) 203–210.
[2] A.R. Raghani, J. Pharm. Biomed. Anal. 29 (2002) 507–518.
[3] C.C. Camarasu, M. Mezei-Szüts, G.B. Varga, J. Pharm. Biomed. Anal. 18 (1998) 623–638.
[4] A.P. Micheel, C.Y. Ko, C.R. Evans, J. Pharm. Biomed. Anal. 11 (1993) 1233–1238.
[5] K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1040 (2004) 259–272.
[6] Scan View on the Web, Application Search, Application Database from Varian Inc., http://www.varianinc.com.
[7] Agilent's Searchable Chromatogram Database, Agilent Technologies website, http://www.chem.agilent.com/scripts/chromatograms.asp.
[8] Z. Li, Y.-H. Han, G.P. Martin, J. Pharm. Biomed. Anal. 28 (2002) 673–682.
[9] N. Kumar, J.G. Gow, J. Chromatogr. A 667 (1994) 235–240.
[10] R.B. George, P.D. Wright, Anal. Chem. 69 (1997) 2221–2223.
[11] United States Pharmacopoeia, vol. 487, 28th ed., Rockville, MD, USA, 2005, pp. 2322–2332.
[12] European Pharmacopoeia, Directorate for the Quality of Medicines of the Council of Europe, Strasbourg, France, 4th ed., 2002, pp. 96–100.
[13] European Pharmacopoeia, Directorate for the Quality of Medicines of the Council of Europe, Strasbourg, France, 4th ed., Monograph No. 01/2002:0872, 2002.
[14] European Pharmacopoeia, Directorate for the Quality of Medicines of the Council of Europe, Strasbourg, France, 4th ed., Monograph No. 01/2002:0932, 2002.
[15] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, STP Pharma Pratiques 13 (2003) 101–108.
[16] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier,

M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, J. Pharm. Biomed. Anal. 36 (2004) 579–586.
[17] ISO 5725-1, Application of the Statistics—Accuracy (Trueness and Precision) of the Results and Methods of Measurement. Part I. General Principles and definitions, International Organisation for Standardization (ISO), Geneva, Switzerland, 1998.
[18] Ph. Hubert, P. Chiap, J. Crommen, B. Boulanger, E. Chapuzet, N. Mercier, S. Bervoas-Martin, P. Chevalier, D. Grandjean, P. Lagorce, M. Lallier, M.C. Laparra, M. Laurentie, J.C. Nivet, Anal. Chim. Acta 391 (1999) 135–148.
[19] A. Ceccato, R. Klinkenberg, Ph. Hubert, B. Streel, J. Pharm. Biomed. Anal 32 (2003) 619–631.
[20] B. Streel, A. Ceccato, R. Klinkenberg, Ph. Hubert, J. Chromatogr. B 814 (2005) 263–273.
[21] E. Chapuzet, N. Mercier, S. Bervoas-Martin, B. Boulanger, P. Chevalier, P. Chiap, D. Grandjean, Ph. Hubert, P. Lagorce, M. Lallier, M.C. Laparra, M. Laurentie, J.C. Nivet, STP Pharma Pratiques 7 (1997) 169.
[22] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Pharm. Res. 9 (1992) 588.
[23] Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), May 2001.
[24] R.W. Mee, Technometrics 26 (1984) 251–253.