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Performance qualification of an electronic tongue based on ICH guideline Q2

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ABSTRACT

Recent progress in sensor technology has led to the development and application of electronic taste sensing systems. Especially taste prediction of pharmaceutical formulations is a matter of particular interest and is increasingly performed using electronic tongues. Several studies have dealt with electronic tongues before, but an analytical approach describing successfully conducted qualification has not been reported yet.

Performance qualification of the taste sensing system SA402B (Insent Inc., Atsugi-chi, Japan) equipped with seven lipid membrane sensors was undertaken with special regard to bitter taste assessment. These sensors represent the gustatory stimuli bitterness, umami, saltiness, sourness, and the nociceptive sensation astringency. Specificity, linearity, range, accuracy, precision, detection and quantitation limit as well as robustness were established for each sensor type referring to ICH guideline Q2 (R1). Some items mentioned in the guideline were applicable whereas others had to be modified due to differences of the system to other commonly used analytical techniques. Quinine hydrochloride being one of the bitterest drugs served as model substance. A large range of linearity (0.01–100 mM) with corresponding precision (RSD < 4%) was found for most sensors. One sensor had a lower detection threshold (0.0025 mM) for quinine hydrochloride than humans typically have. Different methods for determination of detection and quantitation limits were implemented and discussed with respect to rationality and feasibility. Therefore the approach based on visual evaluation was found as most adequate. An adapted guidance following ICH guideline Q2 was developed serving qualification of taste sensing systems in the future.

1. Introduction

Today, the use of human taste panels is a commonly applied method for taste assessment of pharmaceutical formulations. However, there are some limitations which led to the development of alternative methods in the past. The human sense of taste is a highly developed mechanism but it also varies between individuals [1]. Even if members of a taste panel are trained and calibrated, taste sensing remains subjective. Other drawbacks of human paneling are ethical and safety concerns coming up due to possible toxicity of the active pharmaceutical ingredients especially for new chemical entities. Furthermore children do have difficulties to make valid statements of differences in taste perception [2]. Particularly in the pediatric population, challenges conducting taste studies can arise and a general approach applicable to the specific characteristics of children has not been established yet [3].

As an alternative electronic taste sensing systems were developed and are increasingly used for taste prediction of pharmaceutical formulations [4–6]. By using electronic tongues possible ethical concerns about taste assessment of medicinal products can be reduced as the absolute number of samples for human taste panels may be reduced. In particular taste assessment at an early stage of formulation development can be conducted without knowing toxicity data of the drug. Furthermore there are numerous fields of application as quality control, stability testing, and screening the taste of ingredients [7,8].

Two electronic tongue systems are commercially available: the taste sensing system SA402B (Insent Inc., Atsugi-chi, Japan) and the α-ASTREE e-tongue (Alpha M.O.S, Toulouse, France) [9]. Both measure changes in electronic potential while investigating liquid samples but the underlying sensor technologies are different. The taste sensing system SA402B is equipped with lipid membrane sensors [10–14] whereas the α-ASTREE uses chemical field effect transistor (ChemFET) technology. In addition other taste sensing systems are under development as for example a voltammetric electronic tongue [9].

To date several studies have been performed using electronic tongues [15–17]. Taste masking by production of sodium benzoate lipid pellets was investigated utilizing electronic tongues, dissolution testing, and a human taste panel [18]. Results of these methods were compared to each other. A correlation between electronic tongue taste prediction and dissolution testing as well as human

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taste sensation was possible. Kayumba et al. [19] developed coated pellets containing quinine sulphate and the α-ASTREE electronic tongue was used for detection of evolution of bitterness intensity as function of time. Therefore the electronic tongue served as tool for selecting the optimal formulation among pellets with different coating thicknesses.

However, there is still the need to learn more about the taste sensing systems. None of the studies has dealt with a method validation before and there is still no systematic approach in order to test the limitations of those systems. Researchers and pharmaceutical industry claim for reliable and reproducible analytical data [8].

Qualification consists of four basic elements. Design qualification (DQ) meaning specification of what exactly the analytical scientist requires the equipment to do, installation qualification (IQ) in which functionality according to the manufacturer's specification is tested, operational qualification (OQ) showing correct working according to the analytical scientist's requirements, and performance qualification (PQ) establishing continuation of operation within the parameters monitored [20]. While IQ and OQ have been investigated and reported in other studies, performance qualification has not been shown for taste sensing systems yet. In this study the taste sensing system SA402B was used for measurements in order to establish a qualified system. Seven lipid membrane sensors representing the different taste stimuli were investigated regarding specificity, linearity, range, accuracy, precision, detection and quantitation limit as well as robustness. Approaches according to International Conference on Harmonization (ICH) guideline Q2 [21] were tested and adapted alternatives were implemented.

As active pharmaceutical ingredients often exhibit a bitter taste, which can lead to non-compliance, quinine hydrochloride was chosen as model drug. Quinine hydrochloride is one of the bitterest drugs coming up with a bitterness value of 200,000 [22]. According to the European Pharmacopoeia the bitterness value is defined as "the reciprocal of the dilution of a compound, a liquid or an extract that still has a bitter taste". For qualification for human taste panel tests, panel members are required to taste certain concentrations of aqueous solutions of quinine hydrochloride. By testing these solution members are calibrated or excluded from the panel, if they are not able to taste the highest concentration (0.015 mM). Nevertheless, the focus was more to find out whether it is possible to establish a qualified system and a valid method than characterizing quinine hydrochloride. The aim was to gain more experience and knowledge about the taste sensing system as well as to determine how and to what extent an electronic tongue can be used as analytical tool.

2. Experimental

2.1. Chemicals and reagents

Quinine hydrochloride was purchased from Caesar & Loretz GmbH (Hilden, Germany). The USP reference standard for quinine hydrochloride was purchased from PHAST Quality standards GmbH (Homburg, Germany). Potassium chloride was acquired from Grüssing GmbH (Filsum, Germany). Tartaric acid was purchased from Sigma–Aldrich Laborchemikalien GmbH (Seelze, Germany). Water was demineralized by reverse osmosis. Distilled water was obtained by in-lab distillation of demineralized water. Absolute ethanol (purity 99.8%) was purchased from VWR International (Leuven, Belgium). Hydrochloric acid (1 mol/l) and potassium hydroxide solution (0.1 mol/l) were acquired from Merck KGaA (Darmstadt, Germany). The inner solution for sensors and reference electrodes consisting of 3.33 mol/l potassium chloride in saturated silver chloride solution was provided by Insent Inc. (Atsugi-chi, Japan). Acetaminophen was obtained from Rhodia Deutschland GmbH (Freiburg, Germany), sodium benzoate from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany), sodium chloride from VWR International (Leuven, Belgium), and quinine sulphate and sodium saccharin were purchased from Caesar & Loretz GmbH (Hilden, Germany). Quinine benzoate was kindly donated by S.C. Microsin S.R.L (Bucharest, Romania).

2.2. Sensors

Sensors and reference electrodes were purchased from TecLabS Europe OHG (Essen, Germany). To enable reproducible electrochemical measurements 0.2 ml saturated AgCl solution called "inner solution" (see Section 2.1) was filled into each sensor prior to the experiments. The reference electrode was completely filled up with inner solution. All sensors were preconditioned in standard solution for one day before the measurement.

2.3. Preparation of standard, washing and sample solutions

Quinine hydrochloride solutions were prepared in demineralized water at different concentrations (0.0005–150 mM). Two washing solutions for negatively and positively charged sensors respectively were made by diluting absolute ethanol to ethanol 30% with distilled water and adding 100 mM hydrochloric acid for the negatively charged sensors or 100 mM potassium chloride and 10 mM potassium hydroxide for the positively charged sensors. A standard solution serving as cleaning and also as "reference solution" was prepared by dissolving 30 mM potassium chloride and 0.3 mM tartaric acid in distilled water.

2.4. Electronic tongue system and measurement setup

All measurements were performed by the taste sensing system SA402B (Insent Inc., Atsugi-chi, Japan). This electronic tongue is equipped with seven lipid membrane sensors providing different taste qualities and three corresponding reference electrodes. The underlying measurement principle is potentiometric and sensor responses are obtained as mV values consequently. According to the Nernst equation the electrode potential depends logarithmically on the activity of the substance [23,24]

$$U = U^0 + \frac{RT}{zF} \ln a_i \quad (1)$$

where U = electrode potential; U^0 = standard electrode potential; R = universal gas constant; T = temperature (K); z = ionic valence of the substance; F = Faraday constant; a_i = activity of the substance.

$$a_i = f_i c_i \quad (2)$$

where c_i = concentration of the substance; f_i = activity coefficient of the substance.

There are three sensors specific for bitterness, bitterness sensor 1 (SB2AC0), bitterness sensor 2 (SB2AN0), and bitterness sensor 3 (SB2C00). The other sensors represent the gustatory stimuli umami (SB2AAE), saltiness (SB2CT0), sourness (SB2CA0), and astringency (SB2AE1). Furthermore an aftertaste can be measured for bitterness, umami, and astringency.

A sensor check was conducted routinely before every measurement in order to assure that sensors were working in the correct mV range. Each sample was measured four times, whereas one measurement cycle (Fig. 1) consisted of measuring the reference solution (V_r), afterwards the sample solution (V_s), a short (2× three seconds) cleaning procedure and measurement of the aftertaste ($V_{r'}$). The aftertaste was measured by determining the change of membrane potential caused by adsorption of the substance to the lipid membrane after the short cleaning procedure. Interpretation

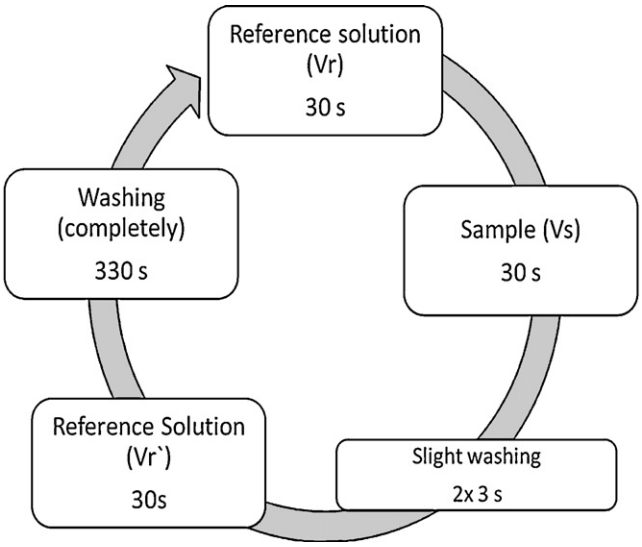

Fig. 1. Measurement routine for one sample.

of aftertaste was not further evaluated as the obtained result is more dependent on the type of substance or formulation investigated than on the sensor. Both, sensor output for taste, also called relative value (R), and sensor output for aftertaste, also called CPA value (change of membrane potential caused by adsorption) were calculated in relation to the preliminary determined sensor response to the reference solution (V_r).

$$R = V_s - V_r \quad (3)$$

$$CPA = V_{r'} - V_r \quad (4)$$

The whole measurement procedure was performed for all samples and repeated afterwards up to four times. For further data treatment the first run was discarded as recommended by the supplier in order to enable conditioning of the sensors. Additionally there was the possibility of calculating so called taste information out of the sensor response in order to ascertain comparability with the human taste. This calculation was skipped here in order to compare raw data without any pretreatment or transformation.

2.5. Evaluation of results

The results were expressed as the raw data in mV of the relative measurement of the sample to the reference. Either sensor signal results alone or combined by multivariate data analysis were evaluated. For the multivariate data analysis raw data was pretreated by mean centering and scaling to unit variance. Data processing, graphical illustration, and statistical interpretation of the results were carried out using Excel 2007 (Microsoft, Redmond, US) and SIMCA-P + v11.5 (Umetrics AB, Umeå, Sweden).

2.6. Performance qualification (PQ)

In order to qualify the electronic tongue quinine hydrochloride was used as a model substance for unpleasant tasting drugs. ICH guideline Q2 [20] served as reference to handle the information necessary for establishing a qualified system as well as a valid method. All items mentioned in the guideline were considered and either transferred or, if not applicable, discussed.

2.6.1. Specificity

Approaches to determine specificity offered in ICH guideline Q2 were not useful as they are aiming on validating the method being specific for the substance under investigation. As verifying of taste masking is the main purpose of the measurement, sensors cannot be specific for only one substance. Every agent present in the solution, which is measured, interacts more or less with the lipid membrane of the sensors and a mV response is obtained. As an alternative method three different drug substances were investigated in demineralized water at 1.0 mM alone and in mixture containing 1.0 mM of each substance. In addition to the cationic quinine hydrochloride, acetaminophen was selected as bitter tasting substance having neutral character. Sodium saccharin known for having bitter taste at higher concentrations was chosen representing anionic bitter substances. The second approach was the investigation of quinine hydrochloride, quinine benzoate, sodium benzoate, and quinine sulphate at 0.1 mM in demineralized water respectively, in order to evaluate the influence of the counterion of a substance. Due to the poor water solubility of quinine benzoate concentrations of 0.1 mM were chosen this time. Furthermore mixtures of quinine hydrochloride and sodium benzoate (0.1 mM each) with and without adding 0.1 mM sodium chloride were measured. Sodium chloride was added in order to imitate the ion pair resulting from mixing quinine hydrochloride and sodium benzoate.

2.6.2. Linearity

Calibration curves were established from 31 different concentrations (0.0005–150 mM of quinine hydrochloride in demineralized water) in order to specify the relationship between concentration and sensor response expressed as difference to the reference (see Eq. (3)). The lowest concentration was 0.0005 mM as there was no difference to demineralized water anymore. The upper end of the concentration series was consistent with the solubility limit of quinine hydrochloride (150 mM). Afterwards linearity was determined according to the guideline by reinvestigation of five concentrations over the observed range of log linear relationship by linear regression. Graphs were generated by plotting the sensor mV response against the logarithm of the concentration. Results were expressed by determination of the y-intercept, slope of the regression line, residual standard deviation, and the coefficient of determination.

2.6.3. Range

The specified range was assessed by confirming an acceptable degree of linearity, accuracy (98–102%) and precision (RSD < 4%) for each sensor. Linearity was considered to be acceptable if the coefficient of determination (R^2) was exceeding 0.98 and residuals were homogeneously distributed.

2.6.4. Accuracy

The accuracy of the sensors was determined at three different drug concentrations (0.2, 0.3 and 1.0 mM of quinine hydrochloride in demineralized water) of a laboratory standard and a USP reference standard. Samples were measured three times and sensor responses of each run were compared to each other for every sample and sensor. The accuracy was evaluated by calculating the difference between laboratory standard and USP reference standard together with the standard deviations and the confidence intervals [95%].

2.6.5. Precision

By measurement of samples in triplicate on the same day the repeatability was determined, whereas intermediate precision was established on three different days with different samples. Precision was investigated at three different concentrations (quinine hydrochloride in demineralized water) within ranges of linearity for each sensor and expressed as relative standard deviation (RSD).

Reproducibility usually shown by inter-laboratory comparison was not investigated.

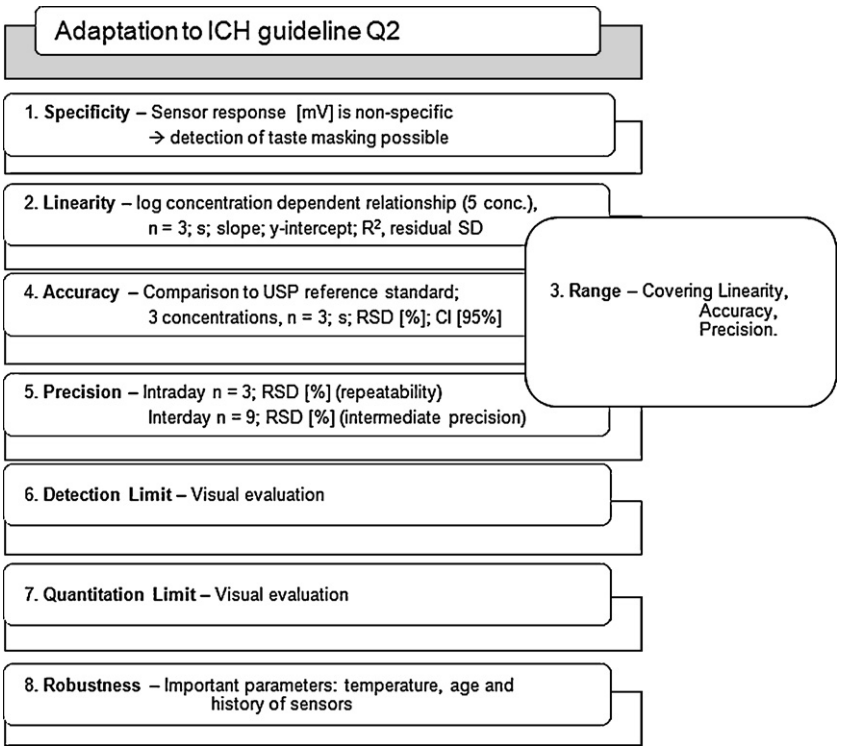

Fig. 2. Guidance on qualification of a taste sensing system.

2.6.6. Detection limit

Different concentrations of quinine hydrochloride (0.0005–100 mM in demineralized water) were measured and every method mentioned in the ICH guideline Q2 was tested in order to asses the best approach identifying the detection limit (Table 1). Demineralized water served as blank. Furthermore concentrations required for human taste testing according to the European Pharmacopoeia (0.009–0.015 mM in demineralized water) were investigated in order to achieve comparability to human taste sensation [22].

2.6.7. Quantitation limit

Different concentrations of quinine hydrochloride (0.0005–100 mM in demineralized water) were measured. Consistent to the evaluation of the detection limit, all methods mentioned in the ICH guideline Q2 were carried out in order to identify the appropriate approach determining the quantitation limit of the sensors.

2.6.8. Robustness

Usually robustness is assessed by doing minor changes to the system to show reliability of the system during typical usage. As it is known that small variations in temperature, pH, and age of sensors may have a huge impact on the sensor responses, robustness testing was not done explicitly as proposed in ICH Q2. Further factors, not mentioned in the guideline, affecting robustness were observed during measurements and will be discussed in the result sections as they might be specific for the taste sensing system.

3. Results and discussion

3.1. Performance qualification (PQ)

After completion of design qualification (DQ), installation qualification (IQ) and operation qualification (OQ), it was possible to qualify the taste sensing system by utilizing ICH guideline Q2. In terms of DQ all sensors commercially available were investigated. IQ and OQ were conducted by routinely performed sensor checks before every measurement and monthly performed maintenance measurements. Determination of specificity and robustness was carried out in a modified way and the best method evaluating the detection limit was identified. In addition a modified guideline following the ICH guideline was developed (Fig. 2).

3.1.1. Specificity

Due to the measurement principle of the taste sensing system neither identification nor purity or content of the analyt could be determined. But interpreting the results of the alternative methods (Tables 2 and 3), which were carried out, some interesting conclusions could be drawn.

3.1.1.1. Influence of ionic structure on sensor responses. Bitterness sensors 1 and 2 could detect quinine hydrochloride in a better

Table 1
Approaches for identification of detection and quantitation limits listed and numbered according to ICH Q2.

Detection limit	Quantitation limit
6.1 Based on visual evaluation	7.1 Based on visual evaluation
6.2 Based on signal-to-noise (2: 1 ratio or 3: 1 ratio)	7.2 Based on signal-to-noise (10: 1 ratio)
6.3 Based on the standard deviation of the response and the slope	7.3 Based on the standard deviation of the response and the slope
6.3.1 Based on the standard deviation of the blank	7.3.1 Based on the standard deviation of the blank
6.3.2 Based on the calibration curve	7.3.2 Based on the calibration curve

Table 2
Specificity – sensor responses [mV] to different bitter tasting drugs (1.0 mM alone and in 1mM equimolar mixture respectively); $\bar{x} \pm s$ ($n = 3$).

1 mM	[mV] ± s						
	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
Quinine hydrochloride	70.98 (±3.05)	29.34 (±0.78)	29.38 (±1.09)	−18.74 (±0.13)	59.40 (±0.30)	−3.55 (±0.23)	69.48 (±0.28)
Acetaminophen	−91.49 (±2.13)	−83.94 (±3.53)	−14.44 (±6.32)	−43.16 (±2.07)	76.80 (±0.27)	−54.15 (±1.65)	111.97 (±1.62)
Sodium saccharin	−76.15 (±0.14)	−65.99 (±1.00)	−121.28 (±0.83)	−43.06 (±0.34)	−31.56 (±1.84)	−54.67 (±0.53)	−103.26 (±0.05)
Quinine hydrochloride + acetaminophen + sodium saccharin	55.94 (±3.30)	29.18 (±0.53)	−99.73 (±0.12)	−26.11 (±0.41)	−23.60 (±1.16)	−9.00 (±0.27)	−100.55 (±0.11)

Table 3
Specificity – sensor responses [mV] to drugs with different counter ions (0.1 mM alone and in 0.1 mM equimolar mixtures respectively); $\bar{x} \pm s$ ($n = 3$).

0.1mM	[mV] ± s						
	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
Quinine hydrochloride	−2.82 (±1.03)	−47.34 (±0.49)	−16.13 (±4.49)	−46.53 (±1.82)	91.03 (±0.42)	−52.45 (±1.71)	107.14 (±3.12)
Quinine benzoate	−16.56 (±1.18)	−70.09 (±1.03)	−20.67 (±3.96)	−49.96 (±2.02)	92.92 (±0.81)	−62.37 (±2.31)	84.06 (±1.32)
Quinine benzoate + NaCl	−17.21 (±1.17)	−70.58 (±0.67)	−19.29 (±3.47)	−45.52 (±0.42)	90.23 (±1.13)	−58.91 (±1.15)	80.37 (±1.20)
Quinine hydrochloride + sodium benzoate	−18.87 (±0.99)	−71.46 (±0.23)	−19.48 (±3.59)	−46.52 (±0.80)	90.10 (±0.97)	−59.26 (±1.68)	85.42 (±1.99)
Sodium benzoate	−113.26 (±0.93)	−118.98 (±1.28)	−56.23 (±6.53)	−53.49 (±1.58)	91.05 (±0.49)	−73.87 (±1.51)	89.70 (±1.37)
Quinine sulphate	22.94 (±1.16)	−25.42 (±0.24)	−7.56 (±2.84)	−39.42 (±0.89)	96.66 (±0.98)	−39.62 (±0.76)	111.91 (±5.12)

way than acetaminophen or sodium saccharin as the sensor signal obtained for the 1 mM equimolar mixture of the three substances was similar to the single quinine hydrochloride sensor response (Table 2). Bitterness sensor 3 detected all three substances but the sensor response to the mixture was most similar to the single sodium saccharin signal. Bitterness sensor 2 gave an almost identical mV response for quinine hydrochloride alone and in presence of acetaminophen and sodium saccharin. In order to visualize the results PCA maps were generated according to these observations (Figs. 3a and b). For both PCA maps the main variance is explained by the first component, the *x*-axis. The smaller part of the variance covered by principal component two is shown on the *y*-axis. Discrimination of the samples can therefore be seen along the *x*-axis whereas variations between one sample due to measurement noise is shown by the *y*-axis. PCA map generated by sensors bitterness 1, bitterness 2, umami, and sourness (Fig. 3a) shows that sensor responses to the mixture were close to the sensor responses to sole quinine hydrochloride whereas the PCA map built up with the remaining sensors discloses that the mixture was found similar to sodium saccharin (Fig. 3b). This can be explained by the differently coated lipid membrane sensors. Bitterness sensors 1 and 2 are more specific for cationic substances as they are coated with anionic lipids, while bitter sensor 3 has cationic lipids and reacts especially to anionic substances. In conclusion, some sensors are more affine to some type of substance but there exists no specificity to only one substance, which is an important condition for assessing whether taste masking was successful or not.

Focusing at acetaminophen, it was not found close to the mixture by any sensor. In addition deviations in sensor outputs of subsequent measurements were obtained leading to the conclusion that detection of non-ionic substances could lead to difficulties as conductivity is necessary for getting a valid sensor signal. This assumption needs to be further analyzed by investigating more non-ionic substances in combination with linearity measurements.

3.1.1.2. Influence of the counterion. By changing the counterion differences between the samples were mainly detected by the three bitter sensors (Table 3). From all bitter sensors the highest mV values were obtained for quinine hydrochloride and quinine sulphate, whereas the lowest value was found for sodium benzoate. The sensor response to quinine benzoate was found in between. Therefore it could be assumed that quinine benzoate was detected as kind of mixture between quinine hydrochloride and sodium benzoate. Focusing at the mixtures of quinine hydrochloride and sodium benzoate with and without sodium chloride this fact could be proved (Fig. 4). Both mixtures lead to the same sensor response as quinine benzoate alone. NaCl is added to the quinine benzoate in order to simulate the ion pair evolving by mixing quinine hydrochloride and sodium benzoate. As a result, the presence of sodium chloride does not have an influence on the sensor response of the bitter sensor whereas the presence of different bitter tasting substances has an influence. From this experiment feasibility for further taste masking measurements can be shown, as the sensors are not specific for only one substance and competition of the different substances at the sensor membrane occurred. This is an important result showing reliability of the sensors. Thinking of taste masking measurements the presence of masking agent and unpleasant tasting API can be detected and comparison to pleasant tasting placebo would be possible. Considering this it becomes obvious how important a preliminary measurement of the single substances and excipients is in order to analyze complex matrices.

3.1.2. Linearity

A log concentration dependent linearity was observed for each sensor. It was found that a logarithmic concentration sensor response relationship is characteristic for the instrument. This can be explained by the Nernst equation which includes the logarithm of the activity of the substance measured (Eq. (1)). Human taste perception also often behaves like that. According to the Weber–Fechner law human response to intensity of taste stim-

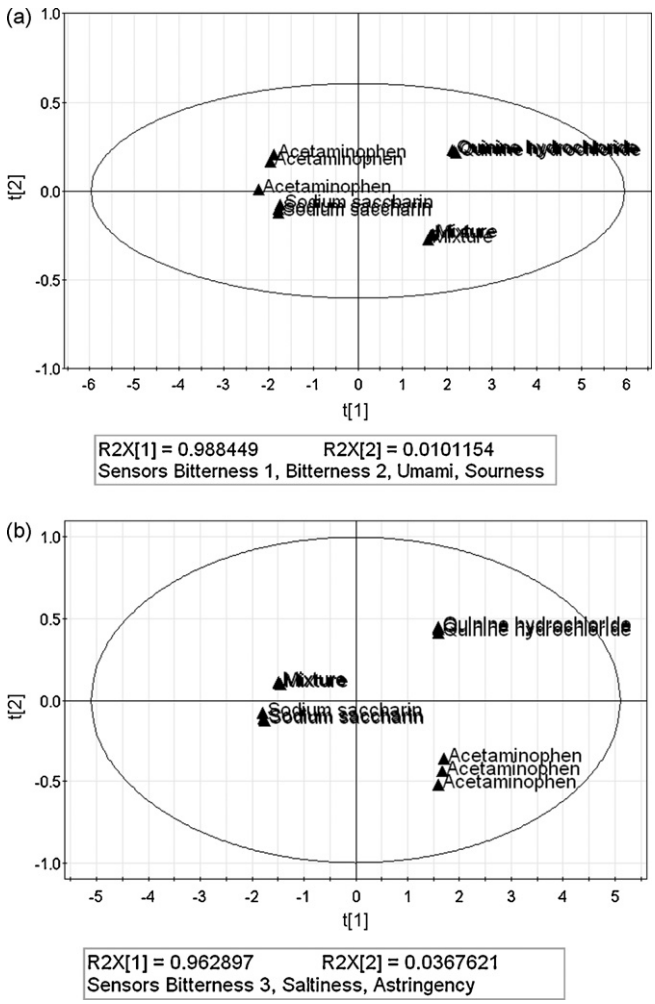

Fig. 3. (a, b) Principal component analysis representing sensor responses to acetaminophen [1 mM], quinine hydrochloride [1 mM], sodium saccharin [1 mM] and a mixture with 1 mM of each substance.

uli depends logarithmically on the concentration of the substance tasted. Once a specific concentration threshold is reached, differentiation between intensity of taste is not possible anymore [25,26].

Table 4 shows ranges of log linearity for the seven sensors with *y*-intercept, slope of the regression line, residual standard deviation, and coefficient of determination.

The slope of calibration curves of sensors for astringency and saltiness was negative whereas a positive slope was obtained for the remaining sensors. This is important to know regarding the interpretation of further (taste masking) measurements. There is no absolute value indicating the taste of a formulation. The results always need to be interpreted in relation to a preliminary performed calibration in order to assess the taste. This can be done by evaluation of one specific sensor as well as by multivariate data analysis. Using a partial least square regression, a model consisting of relevant sensor responses can be build up. Taste masked formulations can be compared to either the univariate calibration or the multivariate model. Regarding detection of bitterness, bitterness sensor 1 had a large range of linearity (0.01–10 mM) with a R^2 of 0.995 whereas bitterness sensor 2 had a likewise large range located at higher concentration ranges (0.02–50 mM) with $R^2 = 0.993$. Bitterness sensor 3 showed log concentration dependent linearity within a smaller concentration interval (0.03–5 mM). This is not surprising as bitterness sensor 3 has a positively charged lipid membrane which detects more specifically negatively charged substances. Conclusively, all sensors show an adequate range of linearity with a good R^2 at the same time, which is relevant for analytical measurements. These ranges of linearity include concentrations of quinine doses which are therapeutically used for the treatment of malaria [27].

3.1.3. Range

The range was established by showing an adequate degree of linearity, precision, and accuracy for every sensor (Tables 4–6). Accuracy was determined at three representing concentrations and considered as valid for higher and lower concentrations.

3.1.4. Accuracy

Determination of accuracy was performed by applying the analytical procedure to the USP reference standard of quinine hydrochloride. Table 5 shows the difference between laboratory standard and reference standard with corresponding standard deviations and confidence intervals. Values (98.00%–100.53%) in an acceptable range were found for all sensors despite bitterness sensor 2, which had a recovery of 109.2% related to the reference standard with a relative standard deviation of 11.2%. The reason for this deviating behavior remains unclear as linearity was given for the whole range and precision was deemed to be satisfying. For the remaining sensors accuracy was considered as established for the specified range.

3.1.5. Precision

The intra- and inter-day precision was investigated within the range of linearity (Table 6). Acceptable values for relative standard deviations were defined as < 4%. Repeatability was found with values of relative standard deviations smaller than 4% for all sensors except bitterness sensor 3 and the sourness sensor. The differences in sensor output for subsequent measurements of the same sample resulted from a drift which was caused by the lower affinity of the cationic sensor to the anionic drug. Fig. 5 shows voltage values [mV] of all sensors for four subsequent measurements of 1.0 mM quinine hydrochloride in aqueous solution. Considering that the first run is discarded, it can be seen that there are drifts for sensors bitterness 1, bitterness 2, bitterness 3, and sourness. However, the coefficient of variation was high for bitterness sensor 3 and the sourness sensor.

The intermediate precision which was investigated over 6 months did not show acceptable values for any sensor. It could be observed that relative standard deviations were generally higher compared to deviations obtained from intra-day precision and sen-

Table 4
Linearity of sensors to quinine hydrochloride (laboratory standard).

	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
Concentration range of linearity [mM]; 5 conc.	0.01–10	0.02–50	0.03–5	0.1–10	0.2–100	0.05–10	0.2–100
y-intercept	73.21	42.01	12.67	−9.91	70.97	10.57	73.02
Slope of the regression line	75.49	81.09	28.43	41.00	−45.32	54.57	−48.61
Residual standard deviation	7.14	10.52	1.94	4.47	3.37	5.59	1.57
Coefficient of determination	$R^2 = 0.995$	$R^2 = 0.993$	$R^2 = 0.995$	$R^2 = 0.985$	$R^2 = 0.996$	$R^2 = 0.991$	$R^2 = 0.999$

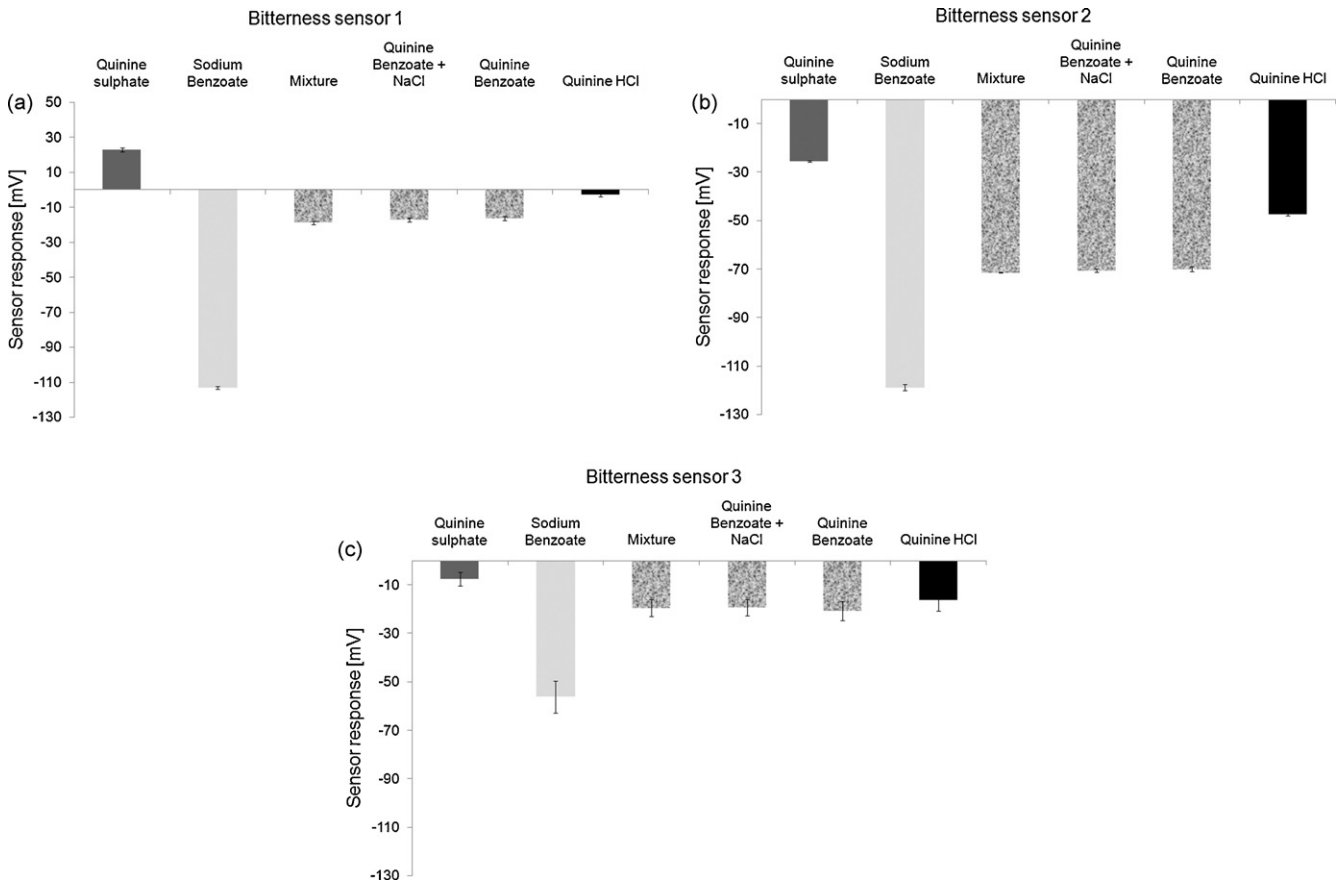

Fig. 4. Sensor responses of bitterness sensors 1–3 to quinine hydrochloride, quinine benzoate, quinine benzoate + sodium chloride, mixture of quinine hydrochloride and sodium benzoate, and quinine sulphate (0.1 mM each); $\bar{x} \pm s$ ($n = 3$).

Table 5
Accuracy – comparison of quinine hydrochloride laboratory standard to USP reference standard.

[mM]	Accuracy between laboratory standard and USP reference standard [%]						
	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
0.2	97.90	108.85	101.39	100.21	99.23	101.41	99.94
0.3	97.21	121.61	98.48	100.57	100.08	103.81	99.35
1.0	98.88	97.12	101.72	100.13	100.64	96.24	101.20
Average value [%]	98.00	109.19	100.53	100.30	99.98	100.49	100.16
Standard deviation	0.84	12.25	1.78	0.23	0.71	3.87	0.95
Relative standard deviation [%]	0.86	11.22	1.77	0.23	0.71	3.85	0.95
95% CI (from)	97.04	95.33	98.52	100.04	99.18	96.11	99.09
95% CI (to)	98.95	123.06	102.55	100.57	100.79	104.87	101.24

Table 6
Precision for quinine hydrochloride at three concentrations within the assessed range of linearity – intra-day $n = 3$; inter-day $n = 9$.

	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
Concentration range of linearity [mM]	0.01–10	0.02–50	0.03–5	0.1–10	0.2–100	0.05–10	0.2–100
Repeatability (intra-day) RSD [%]	**0.01 mM**	**0.02 mM**	**0.03 mM**	**0.1 mM**	**0.2 mM**	**0.05 mM**	**0.2 mM**
	1.92	3.35	10.17	2.63	0.69	4.35	1.86
	1 mM	**2 mM**	**1 mM**	**1 mM**	**2 mM**	**5 mM**	**2 mM**
	1.39	0.82	7.51	0.47	0.03	6.65	0.19
	10 mM	**50 mM**	**5 mM**	**10 mM**	**100 mM**	**10 mM**	**100 mM**
	0.47	1.19	3.02	1.89	1.8	5.03	0.71
Intermediate precision (inter-day) RSD [%]	**0.01 mM**	**0.02 mM**	**0.03 mM**	**0.1 mM**	**0.2 mM**	**0.05 mM**	**0.2 mM**
	32.29	20.07	32.38	0.92	8.52	8.01	3.87
	1 mM	**2 mM**	**1 mM**	**1 mM**	**2 mM**	**5 mM**	**2 mM**
	12.76	21.40	54.75	11.65	7.97	25.63	4.00
	10 mM	**50 mM**	**5 mM**	**10 mM**	**100 mM**	**10 mM**	**100 mM**
	5.55	3.17	6.72	10.96	2.37	22.31	2.47

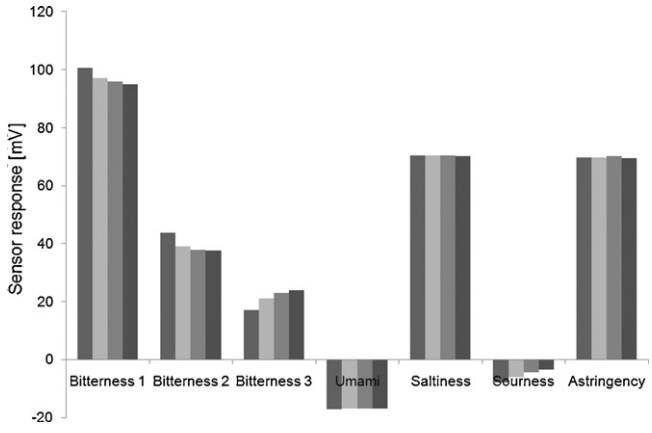

Fig. 5. Drift of mV responses of four subsequent measurements of quinine hydrochloride (1.0 mM in aqueous solution).

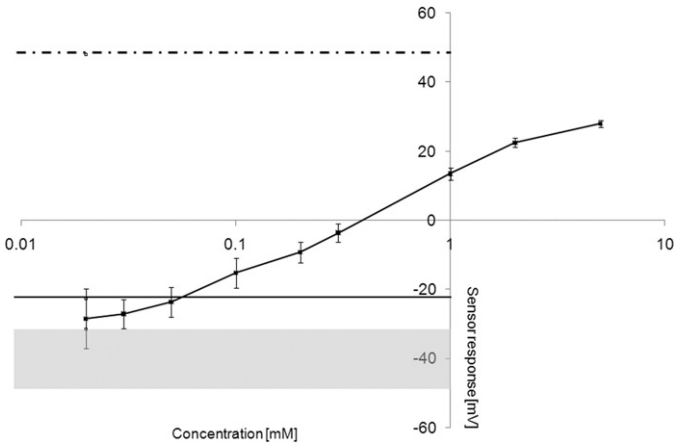

Fig. 6. Identification of detection/quantitation limit with noise; S/N 2:1; S/N 10:1 for bitter sensor 3.

sors with poor response in intra-day measurements showed even higher deviations in inter-day comparison.

This leads to the conclusion that these deviations were caused by general changes affecting the analytical procedure. Further assumptions will be discussed in the robustness part.

3.1.6. Detection limit

The most precise and convenient method for determination of the detection limit was the approach based on visual evaluation. This is due to several reasons. In general it could be seen that the detection limit [mM] varied between the different approaches (Table 7). For every sensor the detection limit for quinine hydrochloride was defined at a higher concentration value using approach ICH Q2 6.3.1. Using the signal-to-noise approach equivalent or slightly higher detection limits would be assessed compared to the visual evaluation. Demineralized water served as blank and the range of three subsequent measurements of demineralized water was considered as noise. Therefore in some cases the detection limit was shifted to higher concentration values due to variation of sensor signals to demineralized water (Fig. 6). Results from ICH Q2 method 6.3.2 were not suitable as the concentration-sensor-response-curve did not cross the origin and too high detection limits resulted compared to visual evaluation. Compared to human taste sensation bitterness sensor 1 was able to pass the calibration which is required by the European Pharmacopeia [22]. According to this calibration humans are only allowed to take part in further taste panels if they are able to taste bitterness of aqueous quinine hydrochloride solutions at concentrations from 0.009 to 0.015 mM. The detection limit for bitterness sensor 1 was found to be 0.0025 mM (Fig. 7).

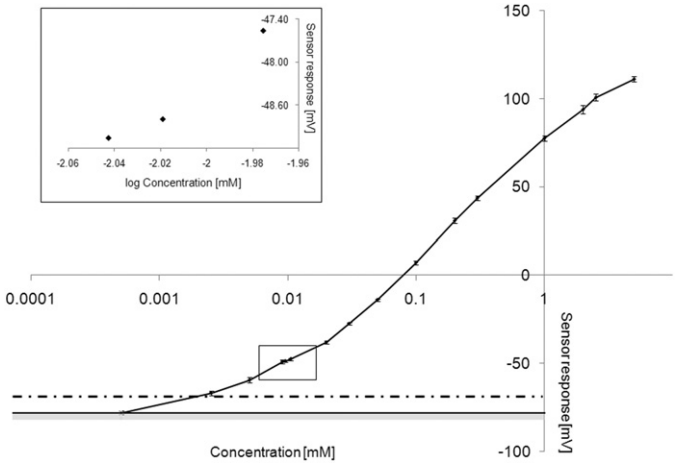

Fig. 7. Identification of detection/quantitation limit with noise; S/N 2:1; S/N 10:1 for bitter sensor 1 and comparison to required sensitivity for human taste panel members according to European Pharmacopeia.

3.1.7. Quantitation limit

As already found out for the detection limit, for the same reasons approaches based on the standard deviation of the response and the slope were not suitable. Results were obtained by the visually performed approach and the signal-to-noise approach (Table 8). Visually determined quantitation limits were congruent with the lower ends of linearity except for bitterness sensor 3 because of missing level of precision. Nevertheless it is to discuss whether determination of content would be the aim of taste measurements and how it could be performed in the best way. Considering the results of intermediate precision and robustness testing a quantitation could only be carried out having a contemporary calibration. Further investigations would be needed to determine precision of quantitation measurements.

3.1.8. Robustness

Results from determination of inter-day precision show that the taste sensing system is susceptible to minor changes in analytical conditions. As an example, measurement sequences of ascending

Table 7
Detection limits for quinine hydrochloride identified by different approaches described in ICH guideline Q2.

Method (ICH Q2)	Detection limit [mM]						
	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
6.1 Visual evaluation	0.0025	0.02	0.03	0.05	0.2	0.05	0.1
6.2 Signal-to-noise ratio	0.0025	0.02	0.1	0.1	0.2	0.1	0.2
6.3.1 Based on the SD of the blank	0.05	0.04	1.00	0.45	0.25	0.28	0.21
6.3.2 Based on the calibration curve	0.06	0.02	0.23	0.05	0.09	0.04	0.04

Table 8
Quantitation limits for quinine hydrochloride identified by different approaches described in ICH guideline Q2.

Method (ICH Q2)	Quantitation limit [mM]						
	SB2AC0 Bitterness 1	SB2AN0 Bitterness 2	SB2C00 Bitterness 3	SB2AAE Umami	SB2CT0 Saltiness	SB2CA0 Sourness	SB2AE1 Astringency
7.1 Visual evaluation	0.01	0.02	–	0.06	0.2	0.05	0.2
7.2 Signal-to-noise ratio	0.005	0.05	–	2.0	5.0	1.0	1.0
7.3.1 Based on the SD of the blank	0.14	0.13	3.04	1.36	0.75	0.85	0.65
7.3.2 Based on the calibration curve	0.18	0.07	0.71	0.15	0.28	0.11	0.14

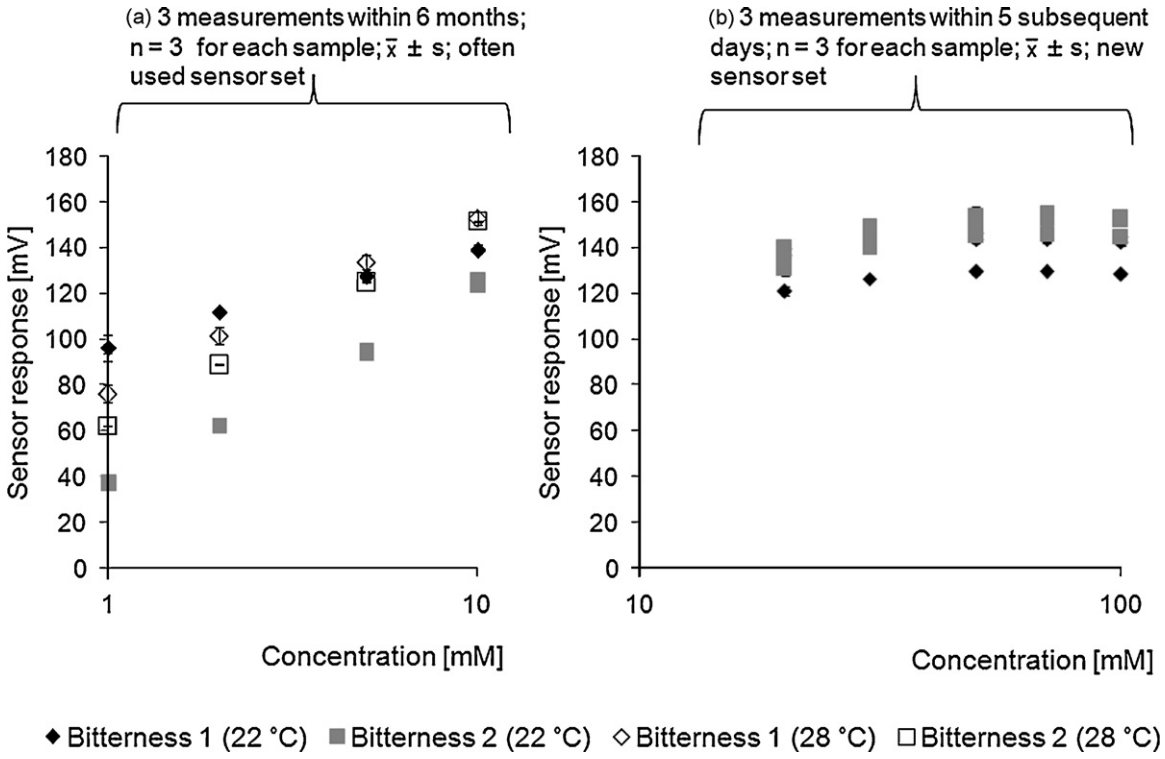

Fig. 8. Sensor responses of bitterness sensors 1 and 2 to quinine hydrochloride under different analytical conditions; (a) = 1–10 mM and (b) = 20–100 mM.

concentrations of quinine hydrochloride performed at different conditions are shown in Fig. 8a and b. Differences in sensor output were found caused by variations in ambient temperature and age and history of sensors. Fig. 8a shows that higher mV values were obtained for lower concentrations compared to higher concentrations shown in Fig. 8b. This can be explained by differences in ambient temperature from 22 °C to 28 °C. Furthermore smaller variations between the sensor responses of measurements performed within five subsequent days at constant ambient temperature of 22 °C (Fig. 8b) can be seen in comparison to measurements conducted within six months (Fig. 8a) showing that age and previously performed measurements have an influence on the mV value. Even if the cleaning procedure is sufficient, irreversible binding of substances to the lipid membrane cannot be prevented and therefore the age of the sensors as well as preliminary performed measurements play an important role. In terms of carryover validity of the cleaning procedure was established during operation qualification by measurement of methylene blue solutions. Furthermore influence of the sample order could be disregarded as the samples were randomly attached to the sample table. Variation of pH was not tested as it is known that sensors react to ions present in the solution. Therefore changes in pH would influence the sensor responses. According to these observations it is recommendable to always have an external standard with known concentration and expected sensor response.

As the external standard should be investigated at the same time within the setup of the samples it should be exposed to the same environment settings as the samples. Therefore it can be assured that those variations can be adjusted for.

Furthermore a contemporary calibration should be conducted by investigation of certain concentrations of the single substances close to the concentration contained in the formulation in order to evaluate the results.

4. Conclusions

This is the first systematic analytical approach reported qualifying a taste sensing system. ICH guideline Q2 on validation of analytical procedures served as background and items that were not feasible were adapted to the measurement principle of an electronic tongue. An approach for performance qualification followed the ICH guideline was developed and can be used for qualification of other taste sensing systems in the future. The taste sensing system SA402B (Insent Inc., Atsugi-chi, Japan) was successfully qualified and offers a new analytical approach for characterization of pharmaceutical formulations. The low detection limit for quinine hydrochloride reveals comparability to human taste sensation. However, it is important to know, that the sensor response can be influenced by temperature and especially by previously performed measurements as well as age of the sensors. This can be handled by

performing a contemporary calibration or using an external standard. A method validation for formulations containing an active pharmaceutical ingredient and a system suitability test would be the next steps to obtain a reliable method for taste assessment.

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