Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1254–1260

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Novel automated assay for the quality control of mexiletine hydrochloride formulations using sequential injection and on-line dilution

Paraskevas D. Tzanavaras[a,*], Constantinos K. Zacharis[a], Pantelis Rigas[b]

[a] Laboratory of Analytical Chemistry, Department of Chemistry, Aristotelian University of Thessaloniki, GR-54124 Thessaloniki, Greece
[b] Department of Fisheries and Aquaculture Technology, Technological Educational Institution, GR-63200 Nea Moudania, Greece

ARTICLE INFO

Article history:
Received 5 July 2008
Received in revised form 2 September 2008
Accepted 4 September 2008
Available online 17 September 2008

Keywords:
Mexiletine hydrochloride
Determination
Sequential injection
On-line dilution
o-Phthalaldehyde
Spectrofluorimetry
Pharmaceutical formulations

ABSTRACT

The first automated method for the determination of mexiletine hydrochloride – an antiarrhythmic agent – is reported. The method is based on the reaction of the analyte with *o*-phthalaldehyde (OPA) in the presence of sulfite in basic medium using a sequential injection (SI) manifold. The reaction product was monitored spectrofluorimetrically (λ_{ex} = 350 nm/λ_{em} = 446 nm). A simple and effective on-line dilution approach was adopted in order to expand the linearity and apply the method to assay, dosage uniformity and dissolution tests with minimum sample preparation. Chemical (pH, amount concentrations of OPA and sulfite) and instrumental variables (temperature, flow rate, injection volumes, etc.) that affected the determination were studied. The developed assay was validated in terms of linearity, range, limits of detection (LOD = 3.4 mg L^{-1}) and quantitation (LOQ = 10 mg L^{-1}), accuracy, precision (R.S.D. < 3.4%) and selectivity. The method was applied successfully to the quality control of a mexiletine-containing formulation. The results were in good agreement with the US pharmacopoeia HPLC method.

1. Introduction

Mexiletine·HCl (Fig. 1) is a local anesthetic, antiarrhythmic agent, structurally similar to lidocaine, but orally active. In animal studies, mexiletine·HCl has been shown to be effective in the suppression of induced ventricular arrhythmias, including those induced by glycoside toxicity and coronary artery ligation. It is a white to off-white crystalline powder with slightly bitter taste, freely soluble in water and in alcohol. Mexiletine·HCl is well-absorbed from the gastrointestinal tract, while 50–60% is bound to plasma protein. Approximately 90% is metabolized in the liver into inactive metabolites and 10% is excreted unchanged by the kidney. The most frequent adverse reactions from administration of the drug were upper gastrointestinal distress, lightheadedness, tremor and coordination difficulties [1–3].

From an analytical point of view, active pharmaceutical ingredients (APIs) such as mexiletine·HCl are determined in mainly biological samples (plasma, blood, urine, hair, saliva, etc.) [4–16] and pharmaceutical formulations for quality control purposes [17–22]. The analytical demands differ significantly in each case, based on the complexity of the matrixes and the expected concentration levels of the analytes. Determination of APIs in biological samples is usually carried out by separation techniques coupled to highly sensitive detection systems (e.g. mass spectrometry [4,5] and fluorescence [10–14]), assuring enhanced selectivity and low detection limits.

On the other hand, the quality control of pharmaceutical formulations requires rapidity in analysis, cost effectiveness, high sampling rate, automated procedures prior to detection and simple sample pretreatment protocols, without of course any sacrifice in reliability. Commonly applied separation methods for the quality control of mexiletine·HCl formulations include HPLC-UV [17–19] and capillary electrophoresis (CE) [20]. Potential disadvantages of these assays are the limited determination range at the low mg L^{-1} level demanding serial dilution steps of the samples [17] and the duration of the analysis cycle in the range of 7–16 min [18–20] resulting in low sampling throughput. Ion-pair formation of the analyte with bromothymol blue followed by solvent extraction and photometric detection involves toxic dichloromethane and batch procedures prior to analysis [21]. The reaction system of acetylacetone–formaldehyde coupled to fluorimetric detection [22] also requires extraction with chloroform as sample preparation, offers a very limited determination range (0.4–1.0 mg L^{-1}), while a 30-min reaction time is necessary prior to measurement.

The scope of the present study was to develop, validate and apply a novel, rapid and reliable SI assay for the quality control of several

* Corresponding author. Tel.: +30 2310997721 fax: +30 2310997719.
E-mail address: ptzanava@chem.auth.gr (P.D. Tzanavaras).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.09.017

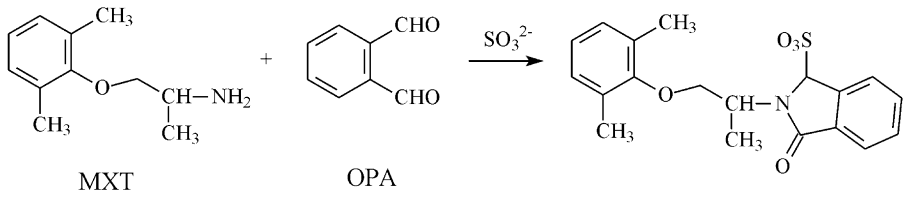

Fig. 1. Scheme of the reaction mechanism between mexiletine (MXT) and *o*-phthalaldehyde (OPA) in the presence of sulfites.

parameters of mexiletine·HCl formulations. Due to their advantages flow and sequential injection have found numerous applications in pharmaceutical analysis [23–25] with reports from pharmaceutical industries being of particular importance [26–29]. To the best of our knowledge so far there are no reports on the determination of mexiletine·HCl using either FI or SI. *o*-Phthalaldehyde reacted rapidly with the analyte in the presence of sulfite ions under SI conditions to form a fluorescent derivative (Fig. 1). An on-line dilution step was incorporated to the sequence in order to expand the linearity of the method and improve its applicability to assay, content uniformity and dissolution tests with the minimum sample preparation. Compared to the previously reported assays, the proposed method offers automation, a higher sampling rate of 25 h^{-1} [18–20,22], simple and environmental friendly sample pretreatment, avoiding organic solvents [21,22] and analytical figures of merit suitable for quality control applications [17,22]. Comparison of the results versus an HPLC assay further confirmed the applicability of the developed SI method.

2. Experimental

2.1. Instrumentation

A schematic diagram of the SI manifold used is shown in Fig. 2. It was comprised of the following parts: a micro-electrically actuated 10-port valve (Valco, Switzerland); an Ultrafluor (Lab Alliance) spectrofluorimetric detector equipped with an 8-μL flow cell; a Gilson (Minipuls3, France) peristaltic pump. The hardware was interfaced to the controlling PC through a multi-function I/O card (6025 E, National Instrument, Austin, TX). The control of the system and the data acquisition from the detector were performed through a special program developed in house using the LabVIEW 5.1.1 instrumentation software package (National Instrument, Austin, TX). Data acquisition was carried out using the EZChrom Elite software (Agilent Technologies).

An HP 1100 HPLC instrument from Agilent Technologies (Palo Alto, CA, USA) was used for HPLC experiments. It comprised a quaternary pump, a vacuum degasser, a column thermostat, an auto-sampler and a DAD spectrophotometric detector. Chromatographic parameters (peak areas, retention times, theoretical plates, etc.) were calculated via the ChemStation® software. A RP-18e monolithic column Chromolith® Performance (100 mm × 4.6 mm i.d.) from Merck (Darmstadt, Germany) was used for separation of the analyte. The mobile phase was filtered using a vacuum filtration system through 0.45-μm membrane filters (RC 55) from Schleicher & Schuell (Dassel, Germany).

Dissolution experiments were carried out using a Distek Premiere 5100 system equipped with a programmable auto-sampler.

2.2. Reagents and materials

All reagents were of analytical grade and were provided by Merck (Darmstadt, Germany), unless otherwise stated. Doubly deionized water produced by a Millipore system was used throughout this study.

Mexiletine·HCl reference material was provided by Sigma–Aldrich. A standard stock solution having a mass concentration of 2000 mg L^{-1} was prepared in water. This solution was stable for 2 weeks if kept refrigerated and protected from the light. Working standard solutions were prepared daily by appropriate dilution of the stock in water.

The standard stock *o*-phthalaldehyde (OPA) solution (c(OPA) = 10 mmol L^{-1}) was prepared by dissolving an accurately weighed amount of the reagent in 500 μL methanol and subsequent dilution with doubly de-ionized water. This solution was stable for 1 week if kept refrigerated and protected from the light. Working OPA solutions (c(OPA) = 0.75 mmol L^{-1}) were prepared daily by appropriate dilution of the stock in water.

Sodium sulfite stock solution (c(sulfite) = 50 mmol L^{-1}) was prepared daily in 0.001 mol L^{-1} NaOH. Working solutions (c(sulfite) = 2 mmol L^{-1}) were also prepared daily in 50 mmol L^{-1} borate buffer (pH 9.7).

The mobile phase of the HPLC reference method was prepared according to USP [30]. A mass of 11.5 g of sodium acetate were dissolved in 500 mL of water, followed by addition of 3.2 mL glacial acetic acid. The pH was adjusted to 4.8 ± 0.1 with HCl and the buffer was finally diluted to 1000 mL with water. The mobile phase was consisted of HPLC grade MeOH:buffer at a ratio of 60:40. Prior to use it was filtered under vacuum through 0.45-μm nylon membrane filters (Whatman) and degassed ultrasonically for 30 min.

Pharmaceutical grade excipients for preparing the placebo mixture used in accuracy and selectivity studies (colloidal silicon dioxide, pre-gelatinized starch, magnesium stearate, titanium dioxide and gelatin) were kindly supplied by Cosmopharm Ltd. (Korinthos, Greece).

Doubly de-ionized water was used as the dissolution media according to USP. It was degassed under vacuum prior to use and stored at 35 °C to minimize re-aeration.

2.3. SI procedure for aqueous solutions

The SI sequence for the determination of mexiletine·HCl is included in details in Table 1. In brief, it consisted of three general parts: (i) on-line dilution (steps 1–6), (ii) aspiration of reagents and sample (steps 7–12) and (iii) detection-washing (steps 13–16). The sampling throughput was 25 h^{-1}.

At the beginning and end of a working day all ports and lines of the SI manifold were flushed with 3 mL of de-ionized water. It should be noted that when changing between samples, an additional washing step was performed in order to avoid carryover effects; 2× 200 μL of the new sample/standard were aspirated to the holding coil (HC), and then flushed through port 6 to waste (W).

2.4. Analysis of pharmaceutical samples

The developed method was applied to the assay, content uniformity and dissolution test of commercially available mexiletine·HCl capsules.

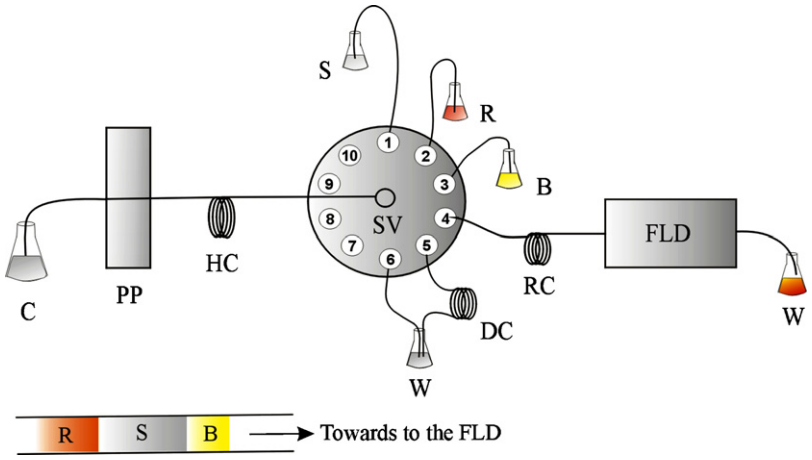

Fig. 2. Schematic representation of the SI setup: C = carrier (water); PP = peristaltic pump; HC = holding coil (300 cm/0.7 mm i.d.); SV = selection valve; DC = dilution coil (300 cm/0.7 mm i.d.); RC = reaction coil (30 cm/0.7 mm i.d.); FLD = fluoerescence detector; S = sample; R = OPA reagent (c = 0.75 mmol L^{-1}); B = sulfite (c = 2 mmol L^{-1})/buffer (pH = 9.7, 50 mmol L^{-1} borate); W = waste.

2.4.1. Assay analysis

The content of 20 capsules was mixed in a mortar and homogenized. Accurately weighed amounts were dispersed in water and ultrasonicated for 15 min. A portion of the resulting solution was filtered through 0.45-μm disposable syringe filters and analyzed according to the SI procedure for aqueous solutions and HPLC.

2.4.2. Content uniformity analysis

Thirty mexiletine·HCl-containing capsules were separately dissolved in 1000 mL of water under shaking and ultrasonication. 5-mL aliquots of the resulting solutions were filtered through 0.45-μm disposable syringe filters and analyzed according to the SI procedure for aqueous solutions and HPLC.

2.4.3. Dissolution test analysis

For each dissolution experiment, 12 mexiletine·HCl-containing capsules were weighed and introduced in the dissolution apparatus in batches of 6. According to USP, apparatus type II was employed. The volume of the dissolution medium was 900 mL and the temperature was set at 37.0 ± 0.5 °C. The rotation speed of the paddles was 50 rpm. Sample aliquots (ca. 7 mL) were withdrawn automatically by the auto-sampler and filtered in-line through 45-μm PTFE disc-filters. No additional pretreatment was required prior to SI or HPLC analysis. Dissolution profiles were constructed at 5, 10, 20, 30, 45 and 60 min in all cases.

2.5. HPLC reference procedure

The HPLC working conditions were fixed to the following values: sample injection volume at 20 μL; flow rate of mobile phase at 1.0 mL min^{-1}; column temperature at 25 °C; UV detection at 254 nm. Aliquots of the samples collected by the auto-sampler of the dissolution apparatus were analyzed by HPLC without any further pretreatment. Peak areas were used for signal evaluation, while each standard or sample was injected in triplicate.

3. Results and discussion

3.1. Preliminary experiments

As can be seen from the chemical structure of Fig. 1, mexiletine comprises a primary amino-group in its molecule and can therefore react with OPA in the presence of sulfites to form a fluorescent derivative according to the reaction scheme shown in Fig. 1. OPA is a "classical" derivatizing reagent for primary amines. It offers high sensitivity and rapid reactions. On the other hand, its main disadvantage is the relatively unstable derivatives that require strict control of the experimental conditions [31]. This drawback can be overcome using sequential injection as the reaction conditions are strictly reproducible due to the fixed geometry of the manifold and the precise computer-controlled operation of the system. Additionally, detection is carried out only a few seconds after mixing of the

Table 1
SI sequence for the determination of mexiletine·HCl.

a/a	Time (s)	Valve position	Pump action	Flow rate (mL min^{-1})	Volume (μL)	Action description
1	1	1	Off	–	–	Selection of sample port
2	5	1	Aspirate	0.6	50	Aspiration of sample in the HC
3	1	5	Off	–	–	Selection of DC port
4	15	5	Deliver	0.6	150	Propulsion of sample to DC
5	1	6	Off	–	–	Selection of waste port
6	15	6	Deliver	1.8	450	Flushing of the HC
7	1	2	Off	–	–	Selection of OPA port
8	5	2	Aspirate	0.6	50	Aspiration of OPA in the HC
9	1	5	Off	–	–	Selection of DC port
10	15	5	Aspirate	0.3	75	Aspiration of sample in the HC
11	1	3	Off	–	–	Selection of sulfite/buffer port
12	5	3	Aspirate	0.3	25	Aspiration of sulfite/buffer in the HC
13	1	4	Off	–	–	Selection of detector port
14	60	4	Deliver	0.9	675	Propulsion of mixture to the detector
15	1	5	Off	–	–	Selection of DC port
16	15	5	Deliver	1.8	450	Flushing of the DC

reagents and therefore potential instability of the product is not generally an issue to worry about.

Preliminary experiments were carried out in order to confirm that the reaction of mexiletine·HCl with OPA can be automated using sequential injection analysis. A three-zones chemistry was adopted for this purpose, namely the sample, the OPA reagent and the buffer that also contained sodium sulfite as coupling reagent. Although pre-mixing OPA and the buffer is a popular approach, we chose to prepare the derivatizing reagent in water as it has been reported to be more stable [32]. The experiments were performed using a similar SI setup to that of Fig. 2, without the dilution coil (DC) and confirmed that the reaction could proceed under SI conditions and that the derivative could be detected fluorimetrically at λ_{ex} = 350 nm/λ_{em} = 446 nm. The starting values of the main variables during these preliminary experiments were: c(OPA) = 1 mmol L^{-1}; c(sulfite) = 2 mmol L^{-1} in 50 mmol L^{-1} borate (pH = 10—as the pK_a of mexiletine·HCl is 9.2 a pH value of 10 was selected initially to ensure de-protonization of the analyte); V(sample) = V(OPA) = V(sulfite) = 50 μL; l(RC) = 60 cm/0.7 mm i.d.; q_V(detector) = 0.6 mL min^{-1}; T = 25 °C.

The order of mixing of the reagents and the sample was examined at three combinations: i) OPA/sulfite-buffer/sample, ii) OPA/sample/sulfite-buffer and iii) sulfite-buffer/OPA/sample. The second aspiration sequence produced the highest signals and derivative/blank ratios and was therefore adopted for all subsequent experiments.

3.2. Study of SI and chemical variables

The most critical instrumental and chemical variables of the method were investigated using the univariate approach. All experiments were carried out at 20 mg L^{-1} mexiletine·HCl, using the starting values of the variables mentioned in the previous section. The derivative/blank signals ratio was used for the evaluation of the results in all cases.

3.2.1. SI variables

The effect of temperature on the reaction was studied in the range of 30–60 °C, by thermostating the reaction coil using a FIAstar 5101 thermostat (Tecator). Higher derivative/blank ratios were obtained at 30 °C, while the blank values increased considerably at higher temperatures. A temperature value of 30 °C was therefore selected for further experiments.

The effect of the flow rate of the reaction mixture towards the flow-through detector was examined in the range of 0.6–1.2 mL min^{-1}. The signal ratios increased in the range of 0.6–0.9 mL min^{-1} and decreased thereafter due to insufficient reaction time. A flow rate of 0.9 mL min^{-1} ensured not only the highest sensitivity, but acceptable sampling rate as well.

The effect of the OPA and buffered sulfite reagents volume was studied in the range of 25–75 μL. Increase of the OPA injection volume resulted in an increase in both the derivative and blank signals. Highest and constant ratios were obtained in the range of 50–75 μL. The value of 50 μL was preferred in terms of reagent consumption. On the other hand, the highest derivative/blank ratios were achieved at lower volumes of the buffered sulfite reagent. 25 μL were selected for subsequent experiments. The sample injection volume had a more profound effect on the sensitivity of the determination. The signals and derivative/blank ratios increased in the range of 25–75 μL and remained practically unaffected up to 100 μL. A mexiletine·HCl injection volume of 75 μL was selected.

The length of the reaction coil (RC) is a potentially important parameter in SI methods, since it determines not only the time that the reaction is allowed to proceed, but the extend of zones overlapping as well. The experiments showed that the signals were constant for reaction coils longer than 60 cm (0.7 mm i.d.). The latter value was selected for further studies.

3.2.2. Chemical variables

The effect of the main chemical variables, namely the pH and the amount concentrations of OPA and sulfites, were examined under the selected values of the SI variables mentioned in the previous section.

The pH had a marking effect on the reaction in the range of 9.25–10.7. Maximum signal ratios were observed at a pH of 9.7, while sharp reduction was observed at higher values. A pH value of 9.7 was selected for further studies. It should be noted that the amount concentration of borate had negligible effect in the range of 30–60 mmol L^{-1}. The starting value of 50 mmol L^{-1} was kept thereafter.

The effect of the amount concentration of OPA was investigated in the range of 0.2–2 mmol L^{-1}. As expected, the blank signals increased almost linearly in the studied range. However, the highest derivative/blank ratio was achieved at an amount concentration of 0.75 mmol L^{-1}, that was selected for subsequent experiments.

Finally, the effect of the amount concentration of sulfites was studied in the range of 0.5–5 mmol L^{-1}. The pH was set at 9.7 and the amount concentration of borate 50 mmol L^{-1}. The experimental results showed maximum signals in the range of 2–5 mmol L^{-1} sulfites. The sulfites amount concentration of 2 mmol L^{-1} was selected for further investigations.

3.3. On-line sample dilution

Under the selected values of the SI and chemical variables, a preliminary evaluation of the linearity of the method was carried out. The experiments showed that the assay was linear up to 30 mg L^{-1} mexiletine·HCl. This range can be satisfactory for routine pharmaceutical assay applications, since the 100% level can be controlled easily by the amount of the formulation and the volume of the solvent. However, content uniformity tests and dissolution studies would require additional dilution steps that would decrease the actual throughput of the assay. For example, according to the USP procedure, the expected theoretical mass concentration of the analyte from dissolution experiments would be ca. 222 mg L^{-1} (200 mg mexiletine·HCl per capsule in 900 mL of dissolution medium).

For this reason an on-line sample dilution protocol was incorporated in the sequence of the assay in order to expand the determination range. Such approaches are quite common in continuous flow injection methods. Typical examples include the use of additional "diluent channels" [33], "zone sampling" [34], "cascade-dilution" [35], dilution-mixing chambers [36], etc. Although these dilution protocols are quite effective, they require re-configuration of the manifolds by addition of extra lines resulting in rather complex systems [33–35], or usage of extra components such as double injection valves [34] or mixing chambers [36].

Our goal was therefore to apply a simple and yet effective on-line dilution protocol that would not require re-configuration of the single-channeled operation of the SI system and would offer the necessary dilution for direct application of the developed method to assay, content uniformity and dissolution quality control tests with minimum manual sample preparation. One of the most effective way to achieve this goal is by controlling the dispersion of the sample zone by applying an extra aspiration–propulsion–aspiration cycle, as described in Section 2.3 and can be seen in Table 1 (steps 1–6) [37,38]. From an instrumentation point of view, only an additional DC is required.

Table 2
On-line dilution experiments.

V_S (μL)	V_{DC} (μL)	V_A (μL)[a]	D_{EF}[b]
50	50	75	2.44
50	100	75	3.77
50	150	75	9.43
50	200	75	30.4
75	50	75	1.98
75	100	75	2.21
75	150	75	4.33
75	200	75	9.65

[a] Fixed at 75 μL according to the experiments of Section 3.2.1.
[b] Mean of five analyses.

In brief, the approach is based on three steps: (i) aspiration of a defined sample volume (V_S) in the HC, (ii) propulsion of an equal or larger volume (V_{DC}) to the dilution coil and (iii) aspiration of a fraction of the zone back in the HC for subsequent analysis (V_A). The analysis volume (V_A) was fixed at 75 μL (see Section 3.2.1). If D_0 is the dispersion coefficient of the sample during the analysis cycle without the application of the on-line dilution steps and D_T is the total dispersion coefficient including the dilution step, the effective dilution factor (D_{EF}) can be derived by the equation:

$$D_T = D_0 \times D_{EF}$$

The dispersion coefficients were determined experimentally according to the procedure described in [38], using quinine sulfate as model fluorescent compound (λ_{ex} = 348 nm/λ_{em} = 446 nm). The results are shown in Table 2. As can be seen from Table 2, by suitable volume combination dilution factors in the range of ca. 2–30 could be achieved. Five injections were made in all instances, while the relative standard deviations were less than 1% in all cases.

The most suitable dilution factor for the proposed method is ca. 10. In this way the calibration curve can be expanded up to 300 mg L^{-1} mexiletine·HCl which is an adequate range for quality control purposes. According to Table 2 this dilution factor can be achieved by two volume combinations (V_S–V_{DC}–V_A), namely 50–150–75 and 75–200–75. The former was selected for subsequent experiments in terms of slightly higher sampling rate.

3.4. Assay validation

The developed FI method was validated for linearity, range, limits of detection (LOD) and quantitation (LOQ), precision (repeatability and reproducibility), selectivity and accuracy.

3.4.1. Linearity and range

The developed assay was found to be linear in the range of 10–300 mg L^{-1} mexiletine·HCl, obeying the following regression equation:

$$FI = 10.036(\pm 1.234) + 0.492(\pm 0.012) \times \gamma(\text{mexiletine} \cdot \text{HCl})$$

where FI is the fluorescence intensity in mV and γ(mexiletine·HCl) is the mass concentration of the analyte in mg L^{-1}. The regression coefficient was higher than 0.999 in all cases.

Two different determination ranges were validated depending on the intended quality control parameter, using the response factor (r.f.) approach [39]. The deviation of the r.f. of each point of the calibration curve from the experimental slope must be within ±3% and is given by the equation:

$$\text{r.f.} = \frac{\text{peak height} - \text{intercept}}{\gamma[\text{mexiletine} \cdot \text{HCl}]}$$

The first range suitable for assay and content uniformity applications varies between 140 and 260 mg L^{-1} (70–130% of the target concentration). Six calibration points were used (140, 150, 180, 200, 230 and 260 mg L^{-1}). The response factors met the ±3% criteria being between −1.6 and +0.9%. The second range is intended for dissolution quality tests and should include lower concentration levels that might appear during dissolution profiles construction. Validation was therefore carried out between 20 and 240 mg L^{-1} (10–120% of the target level) using eight calibration points (20, 50, 75, 100, 150, 180, 200 and 240 mg L^{-1}). In this case, the calculated response factor values were higher ranging between −2.3 and +2.7%.

3.4.2. Limits of detection and quantitation

The detection (LOD) and quantitation limits (LOQ) of the assay were determined based on the criteria proposed by ICH as $3.3 \times s_b/m$ and $10 \times s_b/m$, respectively, where s_b is the standard deviation of the blank measurements (n = 8), and m is the slope of the calibration graph [40]. On this basis, the LOD and LOQ of the proposed method were calculated to be 3.4 and 10 mg L^{-1} mexiletine·HCl, respectively.

3.4.3. Repeatability and intermediate precision

The repeatability (within-day precision) of the proposed SI method was validated by calculation of the relative standard deviations (R.S.D.s) of the peak areas from eight consecutive injections at three mexiletine·HCl standard solutions (140, 200 and 260 mg L^{-1}) at the beginning, middle and end of a working day. The calculated R.S.D.s were less than 3.4% in all cases.

The intermediate precision of the SI method (day-to-day precision) was validated by constructing six consecutive calibration curves (140–260 mg L^{-1} mexiletine·HCl × 6 concentration levels). The experimental results verified the day-to-day precision of the assay, since the R.S.D. of the slopes of the calibration curves was 7.6% (n = 6).

3.4.4. Selectivity

The selectivity of the proposed SI was evaluated against pharmaceutical excipients contained in the mexiletine·HCl capsules (colloidal silicon dioxide, pre-gelatinized starch, magnesium stearate, titanium dioxide and gelatin). The placebo approach was adopted, i.e. all excipients except for the active ingredient. For assay and content uniformity tests, suitable amounts of the placebo mixture were added to mexiletine·HCl standard solutions (at the 100% concentration level). The resulting suspensions were ultrasonicated for 15 min and filtered through 0.45-μm disposable syringe filters prior to SI analyses. Up to 1000 mg L^{-1} of the placebo (maximum concentration tested, 5:1 placebo:analyte ratio) could be tolerated by the proposed method. The criterion for interference was a relative error of >5% at the mexiletine·HCl mass concentration level mentioned above.

For the dissolution test, ca. 1000 mg of the placebo mixture were introduced in six dissolution vessels containing 900 mL aqueous mexiletine·HCl standards (200 mg L^{-1}). The resulting mixtures were subjected to the dissolution test under the conditions described by the USP (see Section 2.4.3). Samples were withdrawn at the maximum tine of 60 min, as worst-case scenario. Again, no interference was observed during SI analyses.

3.4.5. Accuracy

The accuracy of the procedure was validated by analyzing synthetic samples – containing 1000 mg L^{-1} of placebo – spiked with different amounts of mexiletine·HCl in the range of 140–260 mg L^{-1} (namely 140, 180, 200, 220 and 260 mg L^{-1}). The typical ultrasonication–filtration procedure was followed prior to

Table 3
Content uniformity analysis of mexiletine capsules.

Sample	Recovery (%)
MXT1	97.6
MXT2	103.2
MXT3	99.7
MXT4	96.9
MXT5	96.1
MXT6	101.2
MXT7	100.7
MXT8	97.6
MXT9	98.6
MXT10	99.8
MXT11	101.5
MXT12	98.3
MXT13	102.1
MXT14	103.9
MXT15	98.3
MXT16	96.9
MXT17	101.2
MXT18	100.8
MXT19	98.6
MXT20	97.4
MXT21	96.4
MXT22	99.4
MXT23	98.2
MXT24	101.5
MXT25	103.1
MXT26	102.9
MXT27	103.5
MXT28	96.9
MXT29	98.1
MXT30	103.6

each synthetic sample analysis. The percent recoveries were satisfactory in all cases, ranging between 97.1 and 102.3%. It should be noted that the USP assay limits are 90–110% [30].

3.5. Application to pharmaceuticals QC

The developed SI method was applied to the quality control (assay, content uniformity, dissolution) of a pilot batch of mexiletine·HCl capsules (batch size = 10,000 capsules). The results were compared to an in-house validated HPLC method based on the chromatographic conditions proposed by the USP and mentioned in detail in Section 2.5. The results from the assay analysis were satisfactory since the percent recovery was 100.9% (compared to 99.6 from the HPLC assay). The content uniformity results are shown in Table 3. The percent recoveries were in the range of 96.1–103.9%.

Finally, dissolution profiles of mexiletine·HCl capsules were constructed using the proposed SI and the HPLC reference procedure. The profiles were compared by calculating the similarity factors (f_2) derived by the following equation [41,42]:

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right) \times \sum_{t=1}^{n} \times (R_t - T_t)^2\right]^{0.5} \times 100\right\}$$

where n is the number of time points, R_t is the dissolution value using the reference HPLC assay at time t, and T_t is the dissolution value according to the proposed SI method at time t. Generally, f_2 values in the range of 50–100 indicate similarity or equivalence of the compared dissolution profiles. The pilot batch showed good dissolution behavior, since the percent dissolution was higher than 85% after 20 min in all cases, meeting the USP limits (not less than 80% in 30 min). The values of the calculated similarity factors were >85 in all cases confirming the validity and applicability of the developed SI method compared to the HPLC reference method.

4. Conclusions

A new automated SI-fluorimetric method for the quality control of mexiletine·HCl formulations was developed and validated. The reported study is the first FI or SI assay for this analyte. The method is rapid, simple and does not require complicated procedures prior to detection, being advantageous compared to analogous non-separation assays. The analytical figures of merit of the method enable its direct application to assay, content uniformity and dissolution tests of mexiletine·HCl capsules with minimum sample preparation. Validation experiments and comparison of the experimental results with an HPLC reference method confirmed its reliability for the intended quality control applications.

Acknowledgements

The authors would like to thank Mr. A. Kazantzis, Mrs. T. Balloma and Ms. A. Verdoukas (Cosmopharm Ltd., Korinthos, Greece) for providing the dissolution samples and conducting the HPLC experiments.

References

[1] J.P. Monk, R.N. Brogden, Drugs 40 (1990) 374–411.
[2] B. Jarvis, A.J. Coukell, Drugs 56 (1998) 691–707.
[3] N.P.S. Campbell, J.G. Kelly, A.A.J. Adgey, R.G. Shanks, Br. J. Clin. Pharm. 6 (1978) 103–108.
[4] A. Dasgupta, P. Appenzeller, J. Moore, Ther. Drug Monitor. 20 (1998) 313–318.
[5] A. Dasgupta, O. Yousef, J. Chromatogr. B 705 (1998) 283–288.
[6] M.B. Minnigh, J.D. Alvin, M.A. Zemaitis, J. Chromatogr. B 662 (1994) 118–122.
[7] B. Knoche, B. Gehrcke, W.A. Konig, I.W. Wainer, Chirality 8 (1996) 30–34.
[8] S.T. Ulu, Talanta 72 (2007) 1172–1177.
[9] P.K. Kunicki, D. Sitkiewicz, J. Liquid Chromatogr. Relat. Technol. 19 (1996) 1169–1181.
[10] V.L. Lanchote, P.S. Bonato, S.A.C. Dreossi, P.V.B. Goncalves, E.J. Cesarino, C. Bertucci, J. Chromatogr. B 685 (1996) 281–289.
[11] Z. Abolfathi, P.M. Belanger, M. Gilbert, J.R. Rouleau, J. Turgeon, J. Chromatogr. 579 (1992) 366–370.
[12] D.K.W. Kwok, L. Igwemezie, C.R. Kerr, K.M. McErlane, J. Chromatogr. B 661 (1994) 271–280.
[13] N. Shibata, M. Akabane, T. Minouchi, T. Ono, H. Shimakawa, J. Chromatogr. 566 (1991) 187–194.
[14] H. Fieger, I.W. Wainer, J. Pharm. Biomed. Anal. 11 (1993) 1173–1179.
[15] T. Katsu, Y. Tsunamoto, N. Hanioka, K. Komagoe, K. Masuda, S. Narimatsu, Anal. Bioanal. Chem. 387 (2007) 2057–2064.
[16] T. Katsu, Y. Mori, K. Furuno, Y. Gomita, J. Pharm. Biomed. Anal. 19 (1999) 585–593.
[17] R. Pietraś, D. Kowalczuk, H. Hopkata, Chromatographia 60 (2004) 17–23.
[18] S. Kaushik, K.S. Alexander, J. Chromatogr. Relat. Technol. 26 (2003) 1287–1296.
[19] E. Lamparter, J. Chromatogr. 635 (1993) 155–159.
[20] R. Pietraś, D. Kowalczuk, H. Hopkala, J. AOAC Int. 90 (2007) 977–986.
[21] Z. Aydoğmuş, S.M. Çetin, S. Tosunoğlu, Turk. J. Chem. 26 (2002) 839–842.
[22] A.F.M. El Walily, F.A. El-Yazbi, S.F. Belal, O. Abdel-Razak, Anal. Lett. 30 (1997) 2029–2043.
[23] P.D. Tzanavaras, D.G. Themelis, Anal. Chim. Acta 588 (2007) 1–9.
[24] A.M. Pimenta, M.C.B.S.M. Montenegro, A.N. Araujo, J.M. Calatayúd, J. Pharm. Biomed. Anal. 40 (2006) 16–34.
[25] P. Solich, M. Polášek, J. Klimundová, J. Ruzicka, Trends Anal. Chem. 23 (2004) 116–126.
[26] P.D. Tzanavaras, D.G. Themelis, Anal. Lett. 38 (2005) 2165–2173.
[27] P.D. Tzanavaras, A. Verdoukas, D.G. Themelis, Anal. Sci. 21 (2005) 1515–1518.
[28] P.D. Tzanavaras, A. Verdoukas, T. Balloma, J. Pharm. Biomed. Anal. 41 (2006) 437–441.
[29] P.D. Tzanavaras, D.G. Themelis, J. Pharm. Biomed. Anal. 43 (2007) 1820–1824.
[30] U.S. Pharmacopoeia XXIX, 2005, pp. 1430–1431.
[31] Y.V. Tcherkasa, L.A. Kartsova, I.N. Krasnova, J. Chromatogr. A 913 (2001) 303–308.
[32] Z. Genfa, P.K. Dasgupta, Anal. Chem. 61 (1989) 408–412.
[33] D.G. Themelis, P.D. Tzanavaras, F.S. Kika, Talanta 55 (2001) 127–134.
[34] D.G. Themelis, P.D. Tzanavaras, A.V. Trellopoulos, M.C. Sofoniou, J. Agric. Food. Chem. 49 (2001) 5152–5155.
[35] D.G. Themelis, P.D. Tzanavaras, A.N. Anthemidis, J.A. Stratis, Anal. Chim. Acta 402 (1999) 259–266.
[36] F. Albertús, B. Horstkotte, A. Cladera, V. Cerdá, Analyst 124 (1999) 1373–1381.
[37] A. Baron, M. Kuzman, J. Ruzicka, G.D. Christian, Analyst 117 (1992).

[38] D.G. Themelis, A. Economou, A. Tsiomlektsis, P.D. Tzanavaras, Anal. Biochem. 330 (2004) 193–198.
[39] J.M. Green, Anal. Chem. 68 (1996) 305A–309A.
[40] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Q2/R1Validation of analytical procedures. Text and methodology, 1995.
[41] Food and Drug Administration, Guidance for Industry. Dissolution Testing of Immediate Release Solid Oral Dosage Forms, US Department of Health and Human Services/Food and Drug Administration/Center for Drug Evaluation and Research, Rockville, MD, 1997.
[42] P.D. Tzanavaras, D.G. Themelis, A. Zotou, J. Stratis, B. Karlberg, J. Pharm. Biomed. Anal. 46 (2008) 670–675.