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A quality by design approach to impurity method development for atomoxetine hydrochloride (LY139603)

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Abstract

The development of an ion-pairing HPLC method and associated system suitability parameters for the analysis of atomoxetine hydrochloride (LY139603 HCl) using a quality by design approach is described. Potential method conditions were evaluated for their ability to meet design requirements and statistically designed experiments were used to optimize conditions and demonstrate method robustness for the separation of atomoxetine and impurities. The separation of two early eluting impurities, phenyl methylaminopropanol (PMAP (±)3-methylamino-1-phenylpropanol) and mandelic acid is correlated to the separation of other impurities that elute near the main sample component and the resolution of this peak pair is used as a system suitability test without the need for impurity reference standards.

Keywords: Liquid chromatography; Ion-pairing; Quality by design; System suitability; Column equivalence; *In situ* degradation; Atomoxetine

1. Introduction

Quality by design (QbD) is a key principle that has gained much discussion since its initiation as part of the U.S. Food and Drug Administration's vision for the 21st century cGMPs and the International Conference on Harmonisation (ICH) guidance on pharmaceutical development [1,2]. The fundamental principle of the initiative is to demonstrate both understanding and control of pharmaceutical processes to deliver high quality pharmaceutical products while affording opportunities for continuous improvement. While it is clear that the initiative is primarily intended for pharmaceutical product development, its use in the development of an integrated control strategy that involves analytical technology and methods should not be underestimated. In fact, many of the terms used in the QbD initiative are very familiar to analytical chemists when put into the context of method development activities for new pharmaceutical ingredients.

Analytical methods used for the analysis of active pharmaceutical ingredients (API) and drug products are an integral part of the quality by design concept that is outlined in ICH Guideline Q8 for pharmaceutical development [2]. It is important that methods used for analysis meet their intended purpose similar to the product requirements for a clinical dosage form. It is also clear that in order to develop robust, stability indicating analytical methods, a solid set of design requirements must be established to ensure that the method meets its intended use. Methods used for impurity analysis need to be capable of detecting both process and degradation related impurities. Impurities arising from starting materials and/or reaction by-products, whether they carry through the synthetic process unaltered or participate in chemical reactions, must be part of the design requirements for the appropriate impurity method. This type of holistic consideration of impurity nature and fate becomes a key piece of the overall analytical control strategy. Intentional application of quality by design principles to the control strategy can result in a paradigm shift from quality through analytical testing to one where the analytical method verifies that the API or drug product process has been executed as designed.

Design requirements, however, are just one piece of analytical method development activities that mirror the 21st Century GMP initiatives. Analytical chemists are quite familiar with design space or a combination of parameters, within which, the process

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(or method) delivers the desired outcome. The deliberate evaluation of the range around a specified set of conditions where the desired property is intended to be measured is often referred to as the evaluation of the robustness of the method. Robust methods, given a defined region, need very little intervention to remain suitable for the intended use, whereas sensitive methods require stringent controls (due to limited design space) on method parameters in order to operate as intended. The compendia have viewed design space as the acceptability of changes in method conditions within outlined guidances [3]. Method performance within this variation is confirmed with system suitability requirements. In a way, system suitability can be viewed as another element of quality by design for analytical chemists, when applied appropriately, as it helps to identify failure modes and can prevent the generation of erroneous results.

In this paper, the development of the impurity method for atomoxetine hydrochloride is described in terms of quality by design concepts. Considerations for method development or design are discussed in terms of potential impurities, actual impurities and the linkage between the analytical method and the overall process control strategy. Statistically designed experiments were used to identify the optimal operating conditions as well as evaluate the range of several important method parameters. Knowledge from method development and validation experiments proved quite beneficial in the establishment of a correlated peak system suitability approach that affords control and demonstration of the acceptability of the method each time it is run without the need for impurity standards.

2. Experimental

2.1. Equipment

Chromatographic analyses were performed on Agilent Technologies G1100 systems (Waldbronn, Germany) equipped with a vacuum degasser, quaternary pump, refrigerated autosampler, thermostatted oven device and a variable wavelength UV detector. The chromatographic data were acquired and analyzed using Millenium^{32} software, version 3.2 (Waters Corporation, Milford, MA), Empower (version 5.00) or on an in-house-modified HP1000 data acquisition system. The voltage units plotted in the chromatograms are proportional to absorbance. Statistically designed experiments were designed and analyzed using JMP 5.1.1 (SAS Institute, Cary, NC).

2.2. Chromatographic mobile phases and sample preparation

2.2.1. Ion-pairing

Isocratic separations were carried out on a 15 cm × 4.6 mm i.d. Zorbax Eclipse XDB-C8, 3.5 μm particle size column using a mixed aqueous/organic mobile phase consisting of 73% 25 mM *o*-phosphoric acid, pH 2.5, 25 mM octanesulfonic acid; and 27% *n*-propanol, with a column temperature of 40 °C unless otherwise indicated. The flow rate was 1.0 ml/min with UV detection at 215 nm. The mobile phase mixtures used in the robustness study were prepared as outlined in Table 1. Samples for the

Table 1
Fractional factorial design (FFD) to assess method robustness

Code	Buffer concentration (mM)	pH	Ion-pairing agent (mM)	*n*-Propanol (%)	Column temperature (°C)	Pressure (bar)	R_s (1–2)	R_s (3–4)	R_s (4–5)	Run time (min)	Tailing
+−−++	30	2.3	20	28	45	181	4.45	7.64	4.50	12.68	1.23
00000	25	2.5	25	27	40	192	6.49	8.88	5.17	16.80	1.21
+−−++	30	2.3	20	28	45	179	4.54	7.81	4.65	12.96	1.25
00000	25	2.5	25	27	40	199	6.95	8.56	4.97	19.43	1.21
−+−+−	20	2.7	20	28	35	218	5.88	8.22	4.89	15.42	1.23
++++	30	2.7	30	28	45	179	6.13	8.10	4.61	14.21	1.23
+−+−−	30	2.3	30	26	35	211	8.83	10.14	5.84	26.13	1.18
00000	25	2.5	25	27	40	200	6.83	8.48	4.92	19.04	1.21
00000	25	2.5	25	27	40	189	6.84	9.15	5.35	18.43	1.23
+−+−−	30	2.3	30	26	35	211	9.11	10.35	5.97	27.50	1.19
−+−+−	20	2.7	20	28	35	221	6.22	8.18	4.85	17.29	1.24
−+−−+	20	2.7	30	26	45	177	8.57	9.52	5.43	21.15	1.21
−−−−+	20	2.3	20	26	45	181	6.94	9.37	3.00	20.12	1.25
++−−−	30	2.7	20	26	35	214	7.36	9.62	5.78	24.81	1.21
++−−−	30	2.7	20	26	35	200	7.58	10.42	6.35	23.24	1.22
++++	30	2.7	30	28	45	187	5.85	7.72	4.37	13.57	1.21
−−++−	20	2.3	30	28	35	223	7.30	8.38	4.77	18.24	1.20
−−−−+	20	2.3	20	26	45	182	7.47	8.71	5.15	22.82	1.23
−++−+	20	2.7	30	26	45	181	8.74	8.87	5.03	23.35	1.21
−−++−	20	2.3	30	28	35	212	7.17	8.72	5.00	17.46	1.22

robustness studied were prepared at 0.02–0.3 mg/ml and authentic atomoxetine samples were prepared at 2.5 mg/ml for impurity analysis in the mobile phase and a 10 μl injection was used for all analyses unless otherwise noted. System suitability preparations were made by degradation of atomoxetine hydrochloride (4 mg/ml) in 0.5 M sulfuric acid for 3 h at 85 °C. Final solutions were prepared by dilution of the degraded solution and addition of mandelic acid to achieve approximate degradant and mandelic acid concentrations of 0.3 and 0.09 mg/ml, respectively.

2.2.2. Gradient conditions

Gradient elution separations were performed using aqueous organic mixtures of trifluoroacetic acid (0.07%, v/v) and acetonitrile at a flow rate of 1.0 ml/min unless otherwise noted. The gradient profile consisted of an initial hold at 20% acetonitrile for 3 min, followed by a linear ramp to 85% acetonitrile over 15 min (4.33% per minute) with a 5 min hold at 85% acetonitrile before re-equilibration to initial conditions.

2.3. Materials

Aqueous portions of the mobile phases were prepared in deionized water (18.2 MΩ) from a Millipore Milli-Q Plus water purification system (Millipore, Billerica, MA). Aqueous phosphate systems were prepared from *o*-phosphoric acid (85%) unless otherwise specified. Potassium phosphate monobasic (EM Science, Darmstadt, Germany) and *ortho*-phosphoric acid (85%, w/w, HPLC grade) were purchased from Fisher Chemicals (Fair Lawn, NJ). Adjustments to the pH of the aqueous phase were achieved by addition of 5 M potassium hydroxide (reagent grade, Sigma–Aldrich). HPLC grade (Omnisolv) solvents *n*-propanol (*n*-propyl alcohol), acetonitrile, and methanol were obtained from EM Science (Gibbstown, NJ). Octanesulfonic acid sodium salt monohydrate (>98%) was purchased from Fluka. Authetic atomoxetine hydrochloride and related impurity samples that were not commercially available were supplied by the Chemical Process Research and Development Laboratories of Eli Lilly and Company. Trifluoroacetic acid, D(−)mandelic acid was purchased from Sigma–Aldrich (St. Louis, MO) and used as received. The Zorbax Eclipse, XDB C-8 (15 cm × 4.6 mm i.d., 3.5 μm), Zorbax RX-C8 (25 cm × 4.6 mm i.d., 5 μm) and Zorbax Bonus RP (15 cm × 4.6 mm i.d., 3.5 μm) columns were purchased from Agilent (Waldbronn, Germany).

3. Results and discussion

Quality by design for analytical methods can be envisioned to occur in many different ways, often guided by the experience of the individuals who are performing it. One approach to describe how analytical chemists embrace quality by design in the pharmaceutical industry, as applied to analytical method development is pictorially outlined in Fig. 1. Method development is initiated after the appropriate patient and product requirements are identified, and by necessity, after some initial process or product development work has been performed to generate samples to enable method development. The pharmaceutical analytical chemist works in concert with process or formulation colleagues to identify an integrated control strategy, where the method is only one element of the control strategy. The method's purpose is to assess the quality of the product, not impart quality into the product. In developing this analytical control strategy, a cycle of design requirements (design space), followed by development efforts that lead to initial control and investigative methods, ensues. As experience and knowledge are gained with the methods, this leads to better definition of the analytical design space, more refined methods and ultimately methods that are suitable for control laboratories. The control lab methods are further refined and validated with additional experience and knowledge, and transferred to quality control laboratories with appropriate method controls (system suitability) for long-term commercial use. The development of an API impurity method for atomoxetine hydrochloride is an ideal example to illustrate how these concepts have been practiced to develop a control method for an API.

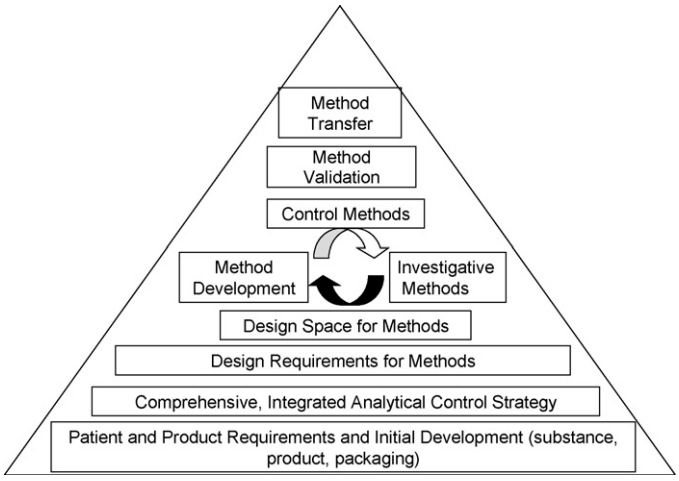

Fig. 1. A quality by design approach for analytical method development.

An important consideration in developing impurity profiling methods is to appropriately define the requirements of the method. In a quality by design approach, this involves establishing what impurities need to be separated and eluted from the chromatographic column followed by detection. Examination of the route of synthesis for the compound of interest, and structurally similar compounds is often a good starting point to define the impurities that may be considered in method development. Fig. 2 shows potential process impurities and degradation products that might be present in atomoxetine hydrochloride (IV). The structure of fluoxetine (VI), a structurally similar compound, is also shown. However, for atomoxetine hydrochloride, the impurity method represents only one element of the overall impurity control strategy. Additional analytical or process chemistry elements such as methods for the atomoxetine positional isomers and undesired enantiomer [4], starting material quality control strategy [5] and manufacturing process understanding (participation, carry-through and rejection) contribute to an encompassing impurity control strategy [6–8]. The use of multiple elements to provide control simplifies the requirements of the analytical method and greatly enhances the efficiency with which methods are developed through better scope def-

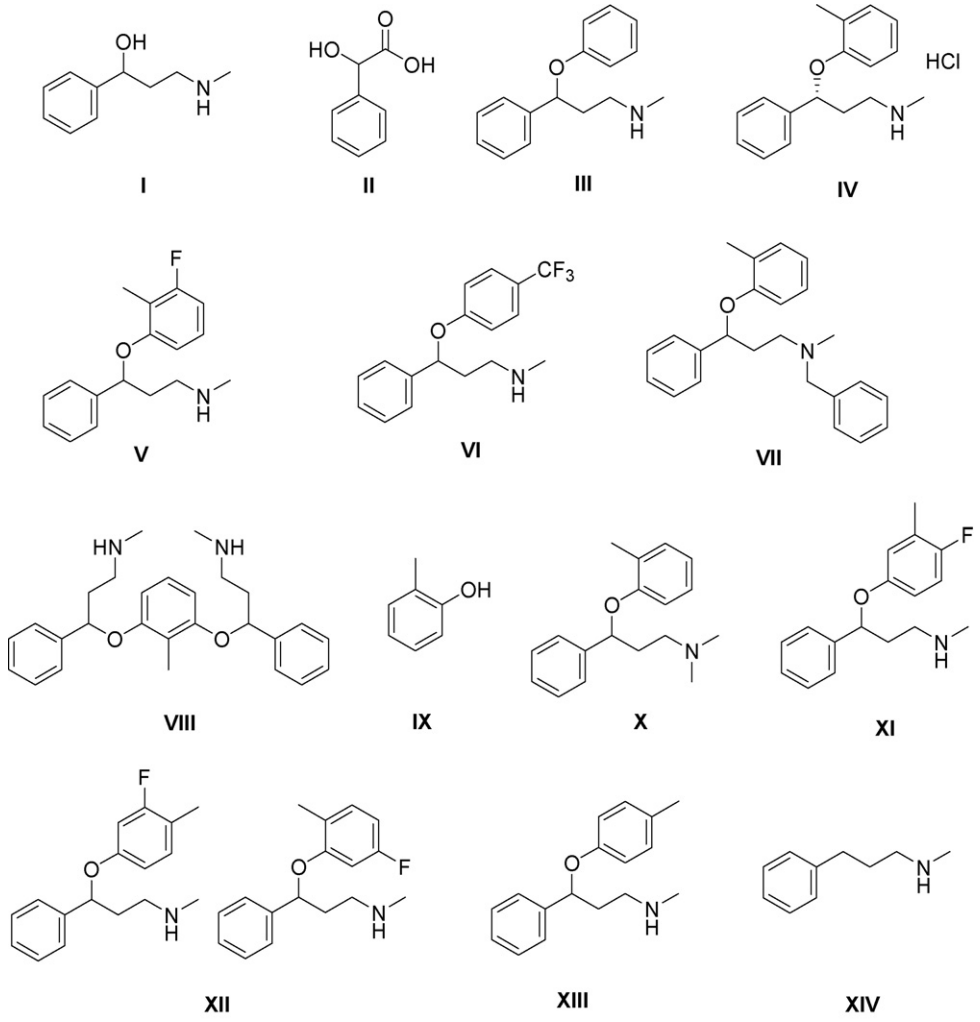

Fig. 2. Impurities considered in atomoxetine hydrochloride (IV) method development. Fluoxetine (VI), while not an impurity of atomoxetine, is included due to its similarity in structure and synthetic route to atomoxetine [5]. *Note*: chirality is not indicated for impurities.

inition. This approach limits the critical design requirements from Fig. 1 to five key objectives. Specifically, (1) the need to retain and selectively separate PMAP (compound I in Fig. 2), a highly polar starting material and degradation product, (2) resolve known and potential process related impurities from the API, (3) elute several potential non-polar reaction by-products such as *N*-benzyl atomoxetine (VII), (4) achieve separation in a reasonable analysis time and (5) user-friendly methodology for quality control laboratories.

3.1. Selection of method conditions

Several approaches for impurity method development can readily be envisioned given the five design requirements. The impurity method must provide retention of polar species such as (I), yet have the capability to detect impurities such as the *N*-benzyl derivative of atomoxetine (VII). Impurities very similar in structure to atomoxetine such as *N*-desmethyl atomoxetine (III) or 3-fluoroatomoxetine (V) must also be separated from the API. Thus, the overall separation goal represents the classical elution window problem in chromatographic science and gradient HPLC readily comes to mind as a reasonable solution. Consideration of the structural similarity of atomoxetine and fluoxetine (VI) suggests that existing gradient HPLC methods may already be suitable to meet the design requirements. Thus, existing in-house approaches, methods available in the literature or subtle modifications of either might provide adequate retention of PMAP and greatly reduce method development efforts [9].

Fig. 3 demonstrates that modified conditions from the literature using a trifluoroacetic acid/acetonitrile mobile phase gradient can certainly provide some retention of PMAP and separation of several impurities of interest from Fig. 2. However, these conditions do not meet all of the design requirements. Notably, PMAP (I) displays poor peak shape (peak splitting) and does not meet a key design requirement for the method. This poor peak shape may be due to the high sample concentration needed to meet detection sensitivity requirements for an impurity control method as has been observed for fluoxetine [9]. The gradient HPLC approach using a mobile phase compatible with mass spectrometric detection is an attractive option for the elution window problem and has potential benefit for impurity profiling but does not meet the requirements for an atomoxetine

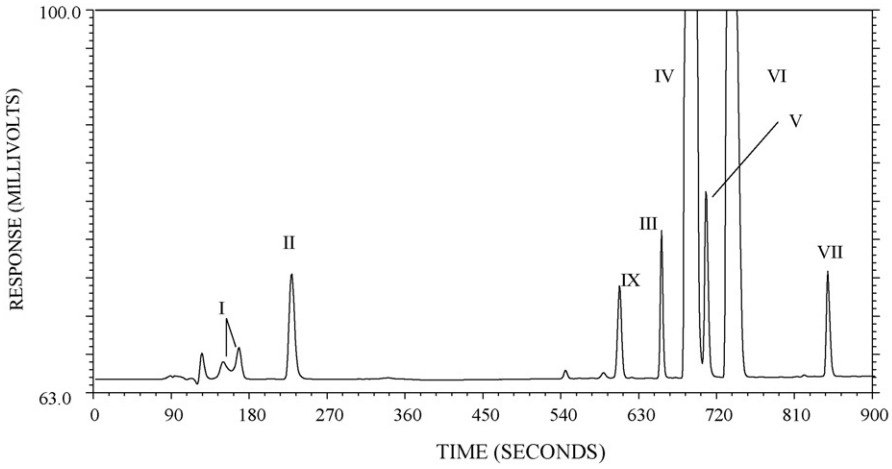

Fig. 3. Separation of a crude mixture of fluoxetine, atomoxetine and related impurities using a MS-compatible mobile phase system. The TFA/acetonitrile gradient elution profile was modified from Ref. [9] for use on a 4.6 mm × 150 mm, 3.5 μm particle size Zorbax SB-C8 column with UV detection at 260 nm.

impurity method. Further refinement might have resulted in an optimal balance between peak shape, retention and detection sensitivity, however, additional investigation into alternative stationary phases with the gradient separation did not readily meet the design requirements. To avoid some of the potential drawbacks with routine use of gradient method HPLC methods [10], alternative methods were investigated to determine if mobile phase modifiers (solvent strength, type and ion-pair reagents) could meet the design requirements under isocratic separation conditions.

The pharmacopeial assay and impurity methods for fluoxetine hydrochloride are run under isocratic separation conditions using an aqueous organic mixture of triethylamine, tetrahydrofuran and methanol [11,12]. The structural similarity of fluoxetine to atomoxetine makes this mobile phase system an attractive choice to investigate however, the retention of PMAP (I) was low. To improve this situation, the use of ion-pairing reagents to afford retention of PMAP under acidic conditions (pH 2.5) and the organic modifier type and relative composition in the mobile phase were investigated using a solution containing several of the compounds of interest from Fig. 2. An ion-pairing system was known to have provided retention and good selectivity for PMAP and its associated impurities [5]. Both PMAP and atomoxetine had significant increases in retention upon addition of an alkyl sulfonate ion-pairing agent and the retention could be tailored based upon the concentration of ion-pair reagent and alkyl chain length. As expected, acidic compounds such as II in Fig. 2 were relatively unaffected by the addition of the ion-pairing reagent. Two organic modifiers were used in the mobile phase for fluoxetine, however it was not clear whether this additional complexity was necessary for the separation of atomoxetine impurities under ion-pairing conditions.

3.2. Choice of organic modifier

Single organic modifiers *n*-propanol, methanol, acetonitrile and tetrahydrofuran were evaluated for the separation of mixtures of compounds I, II and IV. Methanol was a weaker solvent than *n*-propanol in that 60% methanol provided a similar retention factor (*k*) to 29% *n*-propanol. For atomoxetine, *n*-propanol mobile phases showed similar selectivity yet afforded better peak shape than methanol, and thus methanol was not evaluated further. Interestingly, tetrahydrofuran exhibited different selectivity than either acetonitrile or *n*-propanol, with PMAP eluting earlier than mandelic acid. The retention order difference in some cases can be used to the enhance selectivity, however for this peak pair, the differences in selectivity and acceptable results with a single modifier (*n*-propanol) suggested that further optimization would not add significant benefit [13]. Indeed, 29% *n*-propanol (with octanesulfonic acid) provided the most suitable solvent composition for separating a simple mixture of impurities. Fig. 4a shows the performance of the *n*-propanol/ion-pair conditions for a mixture of impurities and atomoxetine (IV). The impurities of interest are resolved from atomoxetine (IV) in the separation, however many of the potential impurities in the complex mixture in Fig. 4a are not observed when an authentic sample of atomoxetine is analyzed under similar conditions (Fig. 4b). The comparison of the chromatograms highlights a relevant attribute of the role of analytical methods in the overall impurity control strategy. It would be ideal if all actual and potential impurities could be well-resolved from atomoxetine and each other and indeed it would be a necessity if this was the only point of control in the overall impurity control strategy. However, the API impurity method is only one element of the control strategy and it works in concert with the other elements to provide the desired quality of the API. For example, it is not of concern that impurities XI and XII are not fully resolved in Fig. 4a chromatogram since the absence of these impurities in authentic samples was by design of an integrated, overall control strategy (see Fig. 1). The knowledge of these potential impurities led to their elimination in the API by application of control specifications on the quality of starting materials used in the synthesis. Thus, the burden on the API impurity method is lessened by designing quality into the control strategy for the API rather than relying on testing alone to assure the quality of the API. Fig. 4a and b thus demonstrate the ability of the conditions used to meet design requirements 1–4, showing the retention, selectivity and elution of impurities of interest in a

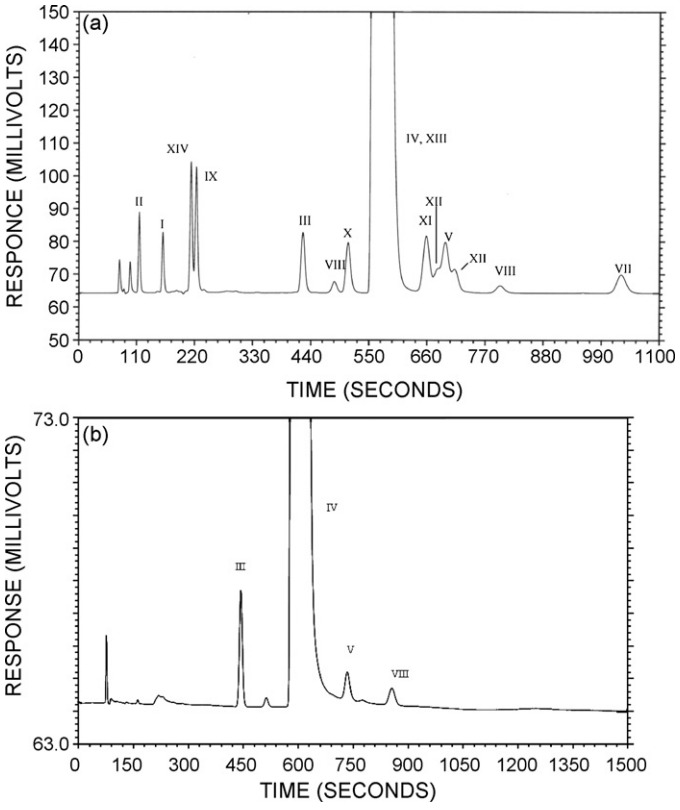

Fig. 4. Ion-pairing separation of atomoxetine impurities demonstrating method performance. (a) Separation of a complex mixture of potential impurities as outlined in Fig. 1 that may be present in the absence of additional impurity controls or process interventions. (b) Impurity profile of an authentic sample of atomoxetine hydrochloride demonstrating the role of the impurity method as an element of the overall impurity control strategy. Isocratic separation on a Zorbax Eclipse XDB-C8 stationary phase at 40 °C with a flow rate of 1 ml/min and UV detection at 215 nm. The mobile phase was an aqueous–organic mixture consisting of *o*-phosphoric acid buffer–octanesulfonate (25 mM, 25 mM, pH 2.5)–*n*-propanol (73:27) (%, v/v).

reasonable analysis time. However, the requirement for a robust user-friendly method must also be addressed.

Robust analytical methods are required in control laboratories for ease of implementation and routine use. The effects of stationary phase chemistry in columns from single or multiple manufacturers and slight changes in mobile phase composition are primary factors in achieving robustness. One approach to the investigation of stationary phase robustness during development is to examine the separations obtained on different manufacturers' columns with respect to the similarity that would be predicted for the columns. Several approaches to the evaluation of similarity between columns as well as selectivity classification of columns according to their hydrophobicity, steric effects, hydrogen bonding acidity or basicity characteristics as well as cation exchange properties have recently been described [14–19]. These approaches enable the selection of a column that is likely to be equivalent or quite different compared to the initial column. One approach utilizes a quantitative descriptor, F_s, to indicate column similarity in terms of selectivity, with F_s values less than 3 indicating a high likelihood of similar chromatographic performance [14]. Separations that are not sensitive to

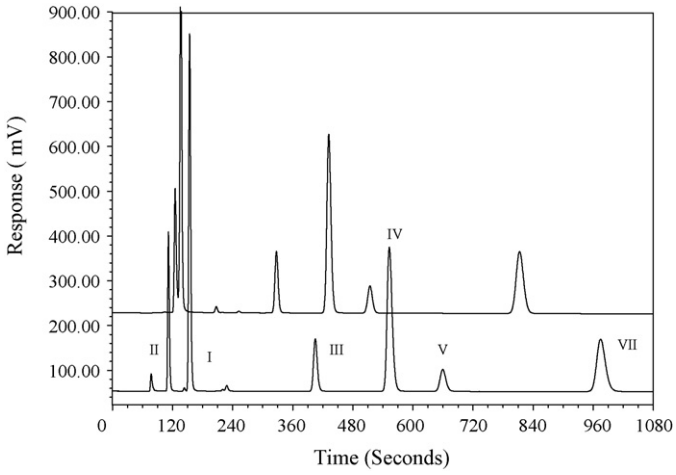

Fig. 5. A comparison of an ion-pairing separation of an atomoxetine sample on (top) Zorbax Bonus RP and (bottom) Zorbax XDB-C8 stationary phases that should provide different stationary phase selectivity ($F_s \geq 3$) by column classification approaches. The separation conditions were the same as those in Fig. 4.

stationary phase characteristics, i.e., those where selectivity is primarily driven by the mobile phase composition, may provide equivalent results on columns that are not predicted to be similar. This would suggest that the separation will be robust with respect to changes in column characteristics (e.g. lot to lot differences and column age).

The influence of stationary phase characteristics using the ion-pair/*n*-propanol mobile phase conditions for atomoxetine was investigated by comparing the separation of an impurity-containing atomoxetine sample performed on Bonus RP and Zorbax XDB-C8 columns (Fig. 5). The column comparison value of $F_s > 175$ would suggest that the Bonus RP column, with its polar embedded phase, should have quite different selectivity than a Zorbax XDB-C8. In addition, it would be anticipated that the Bonus RP would afford less retention than the Zorbax XDB-C8 for a similar series of analytes due to its decreased hydrophobicity. The decrease in retention between the columns is apparent in Fig. 5 and matches well with the expected behavior. However, the selectivity of the separation is largely unaffected as the elution order remains unchanged. This supports the conclusion that for the ion-pairing conditions employed, the selectivity is driven by the mobile phase composition. The column choice may not necessarily impact the selectivity in ion-pairing separations, but may be critical in providing efficiency and peak shape adequate for separating impurities eluting near the main component.

3.3. Statistically designed robustness experiments

Statistically designed experimentation can be an effective tool for screening the robustness of an analytical method once the initial mobile phase composition and column chemistry are defined. The use of design of experiments (DOE) in robustness evaluation of method conditions can result in significant knowledge to establish optimal robust operating conditions as well as identify potential failure modes. For the atomoxetine impurity

method, where the mobile phase composition drives selectivity, statistically designed experiments were used to understand the importance of mobile phase composition (pH, buffer concentration, organic solvent concentration and ion-pair concentration) and column temperature as well as guide method optimization and selection of appropriate system suitability parameters. A five-factor, two-level fractional factorial design with four centerpoints was performed (see Table 1 for design). Specifically, the five factors were phosphate buffer concentration, pH, sodium octanesulfonate concentration, column operating temperature and *n*-propanol concentration in the mobile phase. The centerpoints of the design were the midpoints of the range for each of the five factors. In addition to selecting the experimental design, the choice of what sample(s) to use in the evaluation and what parameters or responses to measure in the evaluation of method robustness can enhance the information gained and often are as important as the design of the experiment itself. Sample selection was based on design requirements 1–4 and the resulting chromatography for two design points in the study is shown in Fig. 6. The chromatograms show that the sample contained peak pairs to assess retention of PMAP (I) and mandelic acid (II) (R_s 1–2 in Fig. 6) and to check the separation of potential critical impurities (III and V) around the main peak (R_s 3–4 and R_s 4–5). These are

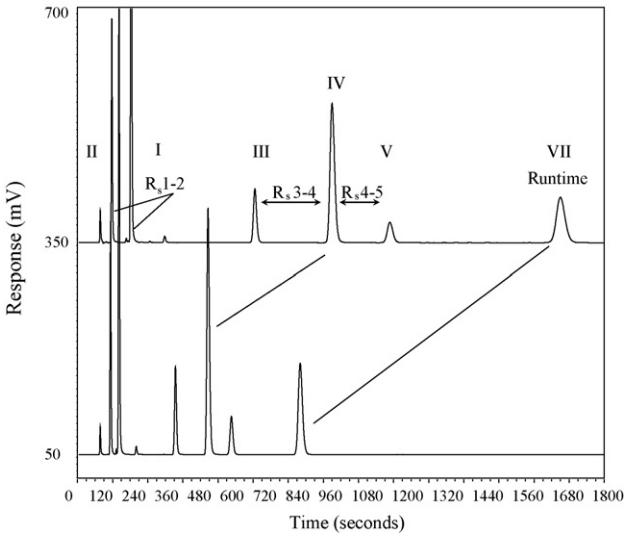

Fig. 6. Chromatograms of crude impurity sample from the design experiment. (Top) *o*-phosphoric acid buffer–octanesulfonate (25 mM, 30 mM, pH 2.5)–*n*-propanol (74:26) (%, v/v) at 35 °C; (Bottom) *o*-phosphoric acid buffer–octanesulfonate (25 mM, 20 mM, pH 2.5)–*n*-propanol (72:28) (%, v/v) at 45 °C.

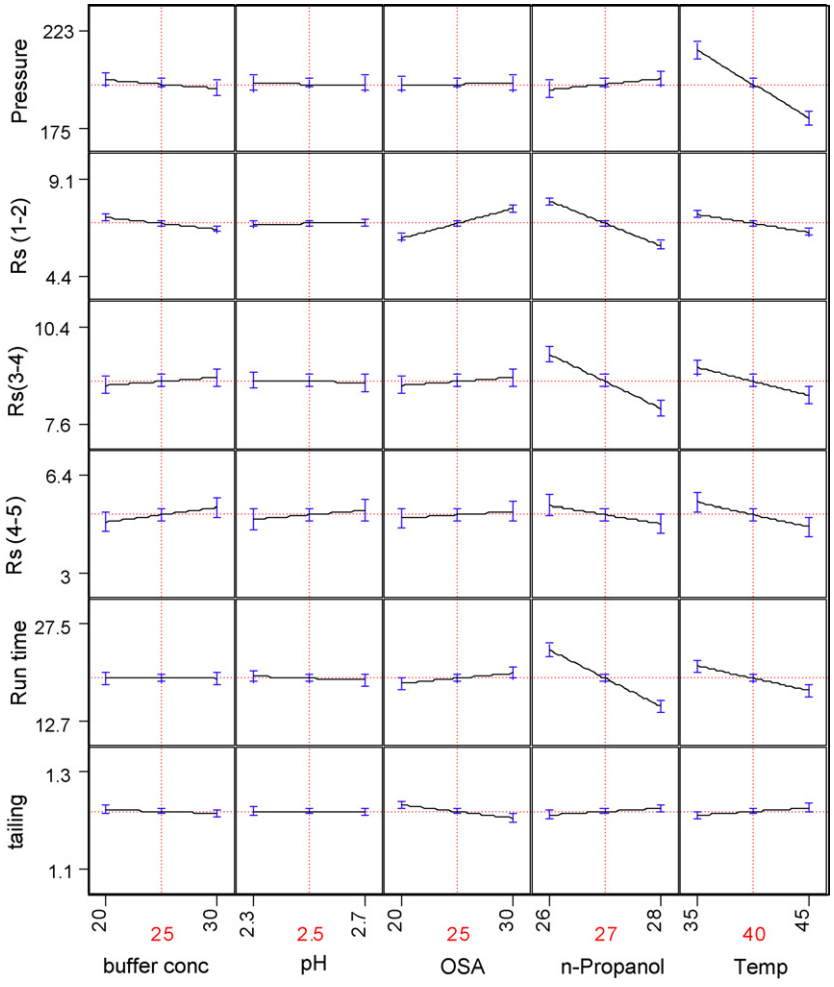

Fig. 7. Prediction profiles for the mobile phase robustness study.

the key separations of interest for the method, as other would be critical impurities are managed by process understanding and additional analytical methods as part of an integrated impurity control strategy for atomoxetine hydrochloride [4,5]. In addition, the sample also contained VII, a late eluting impurity which can be a marker for an assessment of method run time. The responses from the design included not only resolution and run time, but also tailing and backpressure to monitor effects on peak shape and operational characteristics due to the viscous mobile phase. Fig. 7 shows the results of the designed experiment as prediction traces from a least squares fit of the data. The plot shows the prediction of how the responses for each of the five measured parameters of interest (*y*-axis), change as the input parameters (*x*-axis) are varied. Thus, in viewing Fig. 7, horizontal responses indicate that the output is relatively unaffected by changes to the input and can be interpreted as being robust over the range studied. Sloping lines indicate an impact of the *x*-variable on the response as the parameter is varied. Specifically, the data indicate that changes to the buffer concentration, pH and sodium octanesulfonate concentration have minor impact relative to the organic modifier percentage and temperature. Interestingly, it appears that a 2% change in *n*-propanol, can result in a change in *k* by a factor of 1.6. This dramatic decrease in retention is also observed in the chromatography in Fig. 6. Thus, the method conditions are quite sensitive to the percentage of organic modifier in the mobile phase. This may suggest a lack of robustness for routine operation and the need to assess suitability of mobile phase preparation with appropriate system suitability criteria. Fig. 7 also shows that temperature control of the column is important as it can impact the run time and observed backpressure. The use of the predicted responses from the design experiment enables the method developer to optimize the conditions quite readily and observe predicted impact of the changes being made. The final method conditions were selected based upon a balance of resolution and retention, and incorporated a column temperature chosen to balance run time and backpressure considerations. In Fig. 7, the final method conditions are shown as the midpoints of the variables along the *x*-axis, and the figure can be used to predict typical values of backpressure, resolution and retention times. The predictive ability obtained through the statistical analysis (in Fig. 7) also provides starting points to establish meaningful controls that insure long-term method performance within the desired design space.

3.4. Correlated peak system suitability development

System suitability is intended to demonstrate that the method is performing as it was intended or designed in order to insure that the method provides precise, accurate and reliable results each time it is used. Information from the designed experiments may be used to identify key parameters that must be controlled to insure acceptable method performance. In Fig. 7, it is clear that resolution of the impurities studied can be impacted by the percentage *n*-propanol and the column temperature. Therefore, the development of the system suitability test and criteria was focused on being able to demonstrate that these parameters were adequately controlled. System suitability requirements for peak resolution often are defined with a peak pair that includes the main component and a closely eluted impurity with the intent of demonstrating that closely eluting peaks are resolved [20]. Often a reference standard of the impurity is needed for system suitability tests used in pharmacopeial monographs. There are, however, examples where peak pairs other than the main component and a closely eluting impurity are used to demonstrate that the system is operating as intended [21,22]. In these cases, maintenance and supply of impurity standards may still be required. In some cases, the parent drug under study could be used to generate the suitability mixture by degradation thus eliminating the need to supply a separate impurity standard. This latter approach also provides the capability to perform direct retention time identification of key components that may be degradation products of the API and confidence in a relative retention approach to identify other impurities.

The resolution system suitability approach for atomoxetine considered the information gained from the robustness study to provide a convenient approach for demonstrating control of the method. Specifically, the data in Fig. 7 show that the resolution responses for the impurities of interest all follow the same trend when method parameters (*n*-propanol and temperature) are varied. Thus, resolution of two early eluting impurities (R_s 1–2 for PMAP (I) and mandelic acid (II)) is correlated to resolution of impurities that elute closer to the main component (R_s 3–4 and R_s 4–5), suggesting that control of any one of the peak pairs affords control of the chromatographic performance of the method. The early eluting acid/base impurity pair was chosen for system suitability as the compounds are easily generated or commercially available. PMAP (I) can be generated by *in situ* degradation of atomoxetine and mandelic acid (II) is commercially available. Fig. 8 shows the chromatographic profile for the acid decomposition of atomoxetine that results in formation of PMAP (I). A chromatogram of the resulting solution after mandelic acid has been added to the reaction mixture is also shown in Fig. 8. The peak pair in the solution is more than baseline resolved ($R_s \gg 1.5$) however, establishment of criteria based on a minimum resolution of 1.5 would not result in adequate control of the method.

Methods that provide separation of impurities with resolution values greater than 2 are often desired as they afford some level of operational variation (mobile phase preparation, column-to-column variation or column aging effects) without detrimental impact on the method performance or results. In the case of atomoxetine impurity analysis, a resolution of not less than 5.0 was proposed for the early eluting peak pair. This criterion was established based upon a combination of the results from the robustness DOE (see R_s 1–2 in Fig. 7) and additional investigation using authentic samples as opposed to an artificial impurity mixture. This approach highlights the utility of using a DOE, not only for robustness, but to support the selection of the system suitability criterion and to demonstrate the meaningfulness (or lack thereof) of the proposed criterion. The chromatograms in Fig. 9 show the analysis of authentic samples of atomoxetine where the system suitability criterion are met as well as conditions designed to demonstrate system suitability failure. The system suitability chromatograms under these conditions are not

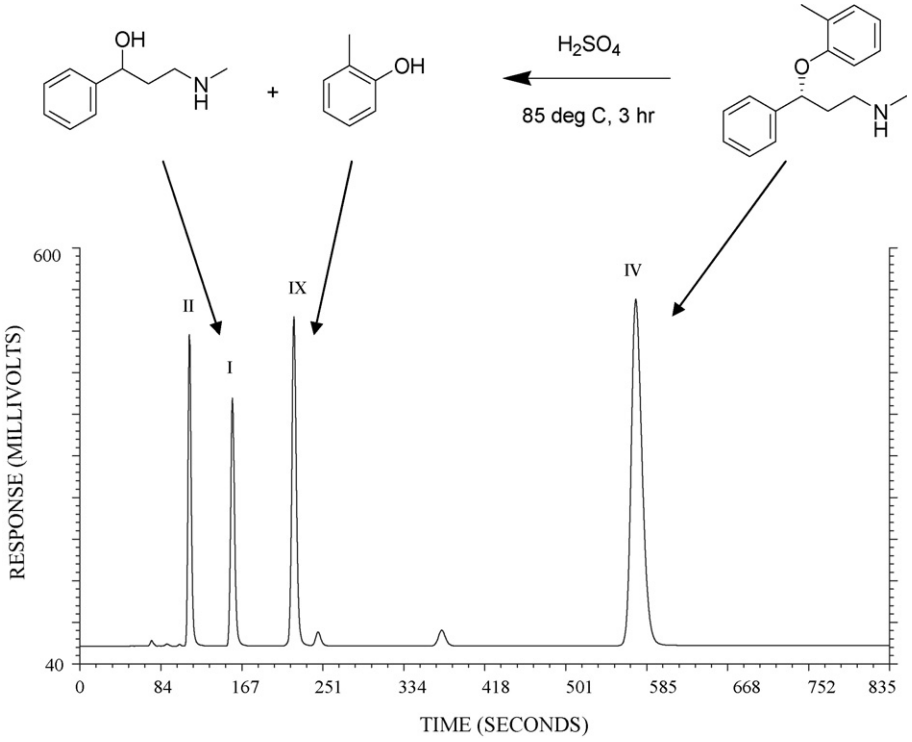

Fig. 8. Resolution system suitability solution generated by *in situ* degradation of atomoxetine (IV) and addition of commercially available mandelic acid. The chromatographic conditions used to generate the separation are provided in Fig. 4. *Note*: chirality is not indicated for degradation products or impurities in this *in situ* preparation.

shown; however the resolution values from their analysis are identified in each chromatogram. The chromatograms show that under the defined operating conditions, when the system suitability criteria are met, acceptable resolution of all impurities in authentic samples is obtained. As the system suitability limit (middle chromatogram in Fig. 9) is approached, the separation is still acceptable as all impurities can be distinguished, yet the two later eluting impurities are not as well resolved. Also, the separation of impurity V from atomoxetine is reduced, compromising the accuracy of quantitation. Clearly, when the chromatographic system is tested by increasing the level of *n*-propanol by 4%, resulting in a resolution value of 4.3 for the system suitability solution, the separation degrades and results in unacceptable performance. Thus, a resolution criterion of greater than 5 provides sufficient control to demonstrate that the system is performing as intended and is in agreement with results from the robustness study where this peak pair (R_s 1–2) had resolution values greater than 5 under the centerpoint conditions for the method. Furthermore, this also shows that setting system suitability limits based upon historical practice may often place overly stringent demands on method performance that while typical, may not be required.

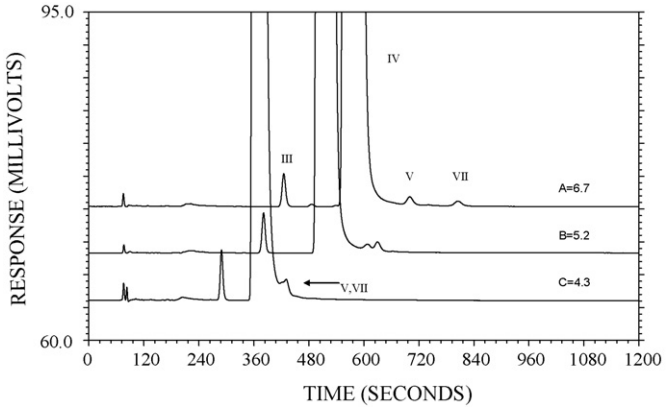

Fig. 9. Chromatograms of an atomoxetine sample evaluated under (a) *o*-phosphoric acid buffer–octanesulfonate (25 mM, 25 mM, pH 2.5)–*n*-propanol (73:27) (%, v/v) at 40 °C, (b) *o*-phosphoric acid buffer–octanesulfonate (25 mM, 25 mM, pH 2.5)–*n*-propanol (73:27) (%, v/v) at 42 °C and (c) *o*-phosphoric acid buffer–octanesulfonate (25 mM, 25 mM, pH 2.5)–*n*-propanol (69:31) (%, v/v) at 40 °C. System suitability results for the resolution of PMAP (I) and mandelic acid (II) are given for each chromatogram.

3.5. Validation studies

The final method conditions were assessed against ICH validation characteristics, specifically examining linearity, precision, accuracy (recovery) and limit of quantitation (LOQ) and demonstrated acceptable validation results. Quantitation of impurity levels for the method was performed against an external standard of atomoxetine hydrochloride. Linearity and accuracy of selected impurities was assessed across the range listed in Table 2 by spiking authentic impurity samples into the atomoxetine hydrochloride matrix. Intermediate precision was assessed using a common sample analyzed across 16 independent method executions. Variance analysis of the data was used to generate an overall method relative standard deviation (method R.S.D.)

Table 2
Summary of validation studies for atomoxetine impurity method

Validation characteristic	Validation performed	Validation result
Accuracy (recovery at 0.05%)	Compound I	100.1
	Compound III	101.3
	Compound V	94.1
Limit of quantitation (LOQ) for compound IV	Signal to noise measurement	S/N = 10 at 0.008%
Precision: repeatability	Number of preparations	6
	Average result (wt%)	0.08%
	R.S.D.	4.9%
Precision: intermediate precision	Number of independent method executions	15
	Average result (wt%)	0.07%
	Method R.S.D.	13.2%
Linearity of external standard Compound IV	Number of points	9
	Target (mg/ml)	0.0025
	Correlation coefficient	0.9999
	Range[a]	0.013–1.35%
Linearity of impurities		
Compound I	Number of points	8
	Correlation coefficient	0.9999
	Relative response factor[b]	0.55
	Range[c]	0.01–0.5%
Compound III	Number of points	9
	Correlation coefficient	0.9999
	Relative response factor	0.99
	Range	0.01–1.15%
Compound V	Number of points	8
	Correlation coefficient	0.9999
	Relative response factor[b]	0.93
	Range	0.01–0.5%

Accuracy was assessed at all levels in the linearity study, however for comparison, only the results for the 0.05% level are provided.
[a] Range expressed as percentage of nominal standard (0.0025 mg/ml) concentration.
[b] Reported as the ratio of response per unit concentration for impurity divided by response per unit concentration of compound IV.
[c] Ranges expressed as percentage of nominal sample concentration (2.5 mg/ml).

listed in Table 2. A summary of the validation characteristics for the atomoxetine impurity method is included in Table 2.

4. Conclusions

The development of the impurity method for atomoxetine hydrochloride has been described in terms of several key concepts of the quality by design paradigm. Design space considerations involving process knowledge of likely impurities and end-user requirements have been discussed along with approaches to guide method development. The use of statistical tools to design robustness experiments and optimize method parameters has led to a sensitive yet well-controlled, validated analytical method for impurity analysis. Understanding gained from the method development and robustness experiments enabled a choice of a peak pair and resolution criterion for system suitability that provided assurance of separation of all significant impurities. The system suitability approach minimizes the need for establishing and maintaining impurity reference standards, yet ensures consistency of method performance.

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