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DEVELOPMENT OF CHIRAL METHODOLOGIES BY CAPILLARY ELECTROPHORESIS WITH UV AND MASS SPECTROMETRY DETECTION FOR DULOXETINE ANALYSIS IN PHARMACEUTICAL FORMULATIONS

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Abstract

Two chiral methodologies were developed by Capillary Electrophoresis (CE) with UV and Mass Spectrometry (MS) detection to ensure the quality control of the drug duloxetine, commercialized as a pure enantiomer. Both methods were optimized to achieve a high baseline enantioresolution ($Rs > 2$) and an acceptable precision (RSD values < 5 % for instrumental repeatability and < 10 % for intermediate precision). In addition to allow the unequivocal identification of duloxetine enantiomers, the CE-MS method improved the sensitivity with respect to the use of CE-UV (LOD 200 ng/mL by CE-UV and 20 ng/mL by CE-MS) enabling to detect 0.02 % of duloxetine enantiomeric impurity. This is the lowest LOD value ever reported for this drug, being this work the first one enabling to accomplish with the ICH guidelines requirements. The developed methods were validated and applied for the first time to the analysis of four pharmaceutical formulations. The content of R-duloxetine in all these samples was below the detection limit and the amount of S-duloxetine was in good agreement with the labeled content, obtaining results by the two methods that did not differ significantly (p-values > 0.05).

Keywords: capillary electrophoresis / duloxetine / enantioseparation / mass spectrometry / partial filling technique

43 **1. Introduction**

44

45 Chirality acquires special relevance in the pharmaceutical industry since drug
46 properties are strong dependent on the ingested enantiomer as a result of the inherent
47 enantioselectivity of the biological processes. Due to the potentially different bioactivity
48 of the enantiomers of a chiral drug, it is mandatory for the pharmaceutical laboratories to
49 justify the commercialization of a new drug, either as a pure enantiomer or as a racemic
50 mixture. Therefore, a rigorous quality control must be carried out to regulate the drug
51 enantiomeric impurities present in a pharmaceutical formulation, especially if they have
52 adverse effects.

53 As many pharmaceutical compounds, duloxetine ((+)-(S)-N-methyl-γ-(1-
54 naphthalenyloxy)-3-(2-thiopene)-propanamine) is a chiral drug. Both enantiomers of
55 duloxetine are potent norepinephrine and serotonin reuptake inhibitors, although the S-
56 enantiomer was found to be slightly more potent [1]. Since this drug is commercialized
57 as a pure enantiomer in the treatment of major depressive disorder [2], chiral
58 methodologies need to be developed to ensure the quality control of its optical purity.
59 These methodologies must, on the one hand, separate the desired enantiomers of a chiral
60 drug and, on the other hand, detect low amounts of the enantiomeric impurities (the
61 International Conference on Harmonisation (ICH) dictates that a method must be able to
62 detect amounts of a determined impurity lower than 0.1 % [3]).

63 The separation and study of duloxetine enantiomers have been reported by HPLC
64 and CE with UV detection. Regarding HPLC, Rane et al. [4] developed a chiral
65 methodology employing a chiral stationary phase (CSP) based on amylose detecting 250
66 ng/mL of duloxetine impurity, which eluted in first place. Yang et al. [5] described two
67 HPLC methods, one employing a vancomycin CSP and the other using (2-

68 hydroxypropyl)-β-CD (HP-β-CD) as additive in the mobile phase. In both cases, the
69 enantiomeric impurity of duloxetine was the last to elute making it harder to be detected
70 in presence of high amounts of the major enantiomer. CE methods have also been
71 described. Some authors reported methods employing glycogen based selectors [6, 7] and
72 erythromycin lactobionate [8, 9] resulting in analysis times from 15 to 60 min. Rickard et
73 al. reported an acceptable duloxetine enantioseparation by means of HP-β-CD in 25 min
74 detecting up to 0.2 % of enantiomeric impurity as it migrated in first place [10, 11]. This
75 is, so far, the only LOD reported for R-duloxetine in the bibliography; nevertheless, it is
76 not in agreement with the ICH guidelines requirements [3]. This remarks the necessity to
77 develop more sensitive methods to be able to determine duloxetine enantiomers in
78 pharmaceutical formulations.

79 CE is one of the most relevant analytical techniques used in chiral separation since
80 it offers many advantages such as high resolution power, low reagents and sample
81 consumption, and high flexibility given the possibility to easily modify the chiral selector
82 added to the BGE. In addition, CE can be coupled to MS to combine the advantages of
83 CE in chiral analysis with the MS potential to identify unknown chiral compounds with
84 unambiguous assignment and to give structural information [12, 13]. The main problem
85 of the chiral CE-MS coupling is the contamination of the ionization source as a result of
86 the entrance of non-volatile chiral selectors, which produces a loss of the ionization
87 efficiency and a decrease in the detection sensitivity [14, 15]. Although some works
88 demonstrate no significant decrease in the sensitivity when low concentrations of the
89 chiral selector are employed [13, 14, 16], different approaches and strategies have been
90 broadly employed to solve this problem. These strategies include the use of compatible
91 chiral selectors in EKC or CSPs in Capillary Electrochromatography (CEC), or counter
92 migration and partial filling techniques (PFT) [12, 13, 15, 17-19].

93 The aim of this work was the development of two CE methodologies using UV and
94 MS detection for the enantioseparation of duloxetine, along with their validation to
95 establish the content of duloxetine and its enantiomeric impurity in different
96 pharmaceutical formulations.
97

98 **2. Materials and methods**
99

100 *2.1. Reagents and samples*
101 All reagents were of analytical grade. Ortho-phosphoric acid 85 % (v/v), dimethyl
102 sulfoxide (DMSO), and sodium hydroxide were purchased from Merck (Darmstadt,
103 Germany). Methanol was obtained from Scharlau Chemie (Barcelona, Spain) and formic
104 acid, and ammonium hydroxide from Sigma (St. Louis, MO, USA). The employed water
105 was MilliQ quality (Millipore, Bedford, MA, USA). β-CD; methyl-β-CD (M-β-CD) (DS
106 1.7-1.9); HP-β-CD (DS ~4.2); 2,6-di-O-methyl-β-CD and 2,3,6-tri-O-methyl-β-CD were
107 acquired in Fluka (Buchs, Switzerland). Acetyl-β-CD; (2-hydroxybutenyl)-β-CD (HB-β-
108 CD); 2,3,6-tri-O-acetyl-β-CD; γ-CD, acetyl-γ-CD (A-γ-CD); methyl-γ-CD (M-γ-CD)
109 (DS ~12), (2-hydroxy)-butenyl-γ-CD (DS ~4.5), (2-hydroxy)-butenyl-γ-CD (DS ~3.2)
110 and 2,3,6-tri-O-acetyl γ-CD were purchased from Cyclolab (Budapest, Hungary). M-β-
111 CD (DS 10.5-14.7) was bought in Sigma-Aldrich (St. Louis, MO, USA).
112 (R, S)-Duloxetine HCl, (R)-Duloxetine HCl and (S)-Duloxetine HCl were
113 purchased from IS Chemical Technology (Shanghai, China). The commercial
114 pharmaceutical formulations were acquired in pharmacies from Madrid (Spain).
115 According to the labeled data, they contained 30 mg of duloxetine per capsule.
116

117 *2.2. CE-UV conditions*

118 Electrophoretic experiments were carried out on a HP ^{3D}CE system from Agilent
119 Technologies (Palo Alto, CA, USA) with a diode array detector (DAD). The
120 electrophoretic system was controlled by HP ^{3D}CE ChemStation software included the
121 data collection and analysis. BGE employed in the CE-UV experiments consisted on 150
122 mM phosphate buffer (pH 3.0) containing 0.5 % (w/v) of HP-β-CD. Separations were
123 performed in an uncoated fused-silica capillary of 50 µm I.D. and a total length of 64.5
124 cm, acquired from Polymicro Technologies (Phoenix, AZ, USA) at 30 kV (positive
125 polarity) and 20 ºC. Injections were carried out applying 50 mbar for 20 s. Detector
126 parameters were as follows: a response time of 1.0 s and a wavelength of 220 nm
127 (bandwidth 35 nm) including a reference wavelength of 375 nm (bandwidth 100 nm). At
128 the beginning of each working day the capillary was flushed with buffer solution for 10
129 min and at the end of the day it was flushed with MilliQ water for 5 min. In order to
130 ensure the repeatability between injections, the capillary was flushed with DMSO for 2
131 min, buffer solution for 3 min and BGE for 1 min
132
133 *2.3. CE-MS conditions*
134 CE-MS analysis were performed in a HP^{3D}CE instrument (Agilent Technologies,
135 Palo Alto, CA, USA) coupled through an orthogonal coaxial sheath interface (model
136 G1607A from Agilent Technologies, Palo Alto, CA, USA) to the Electrospray Ionization
137 (ESI) source of an Ion Trap (IT) mass spectrometer (model AmaZon SL from Bruker
138 Daltonics, Bremen, Germany) for MS detection. For MS control and data analysis, a
139 TrapControl Software 7.0 for AmaZon was used. BGE employed in the CE-MS
140 experiments consisted of 150 mM ammonium formate buffer (pH 3.0). The separation
141 was achieved in an uncoated fused-silica capillary of 104 cm and 50 µm I.D., at 30 kV
142 (positive polarity) and 15 ºC. Injections were performed applying 50 mbar for 5 s.

143 Between analysis, the capillary was flushed by applying 1 bar with DMSO for 4 min,
144 BGE solution for 5 min and BGE with 0.5 % (w/v) of HP-β-CD during 1 min (38 % of
145 total capillary length).

146 Operating MS conditions consisted on a sheath liquid composition of 80:20 (v/v)
147 methanol/water with 0.1 % (v/v) of formic acid at a flow rate of 3.3 µL/min by a syringe
148 pump (Hamilton, USA). The nebulizer and the drying gas conditions were 3 psi N_2 and 5
149 L/min N_2 at 200 ºC. The mass spectrometer operated with the ESI source in the positive
150 ion mode at -4.5 kV with an end plate of -500 V. The ion optical parameters were tuned
151 in the "expert mode" and the capillary exit value was optimized to 57 V. In MS^2
152 experiments the Ion Charge Control (ICC) was activated with a target up to 100,000 ions
153 using 50 ms of accumulation time and 3 averages. The *m/z* scanned range was from 100
154 to 400 *m/z* in the "UltraScan" mode (32,000 *(m/z)*/s). The isolation width of the precursor
155 ion (298.1 *m/z*) was set to 4.0 *m/z*. Its fragmentation was carried out by collision-induced
156 dissociation with the helium present in the trap for 40 ms with a fragmentation amplitude
157 of 0.5 V (with the "smart-frag" option deactivated) and a fragmentation width of 10 *m/z*.
158 Extracted Ion Electrophoregrams (EIEs) were obtained extracting the product ion 153.8
159 *m/z* with an extraction window of -0.3/+0.7 *m/z* using a smoothed option of the software
160 (Gauss at 4 points).

161

162 *2.4. Preparation of stock and sample solutions*

163 Stock solutions of duloxetine were prepared in DMSO and then diluted with
164 MilliQ water until desired concentration. Commercial pharmaceutical solutions were
165 prepared by homogenizing the content of five capsules of the medicament and grinding
166 it. The required amount for analysis of the grinded powder was dissolved in DMSO and
167 centrifuged for 10 min with a rotational speed of 4000 rpm at 20 ºC. The supernatant was

taken and brought to a known volume. Afterwards, it was diluted to the required concentration employing MilliQ water.

3. Results and discussion

3.1. Development of a chiral methodology for the enantiomeric separation of duloxetine by CE with UV detection

The first step in the method development was to choose a CD offering the best enantioselectivity and enantioresolution (Rs). Since duloxetine is a basic drug (pKa 9.7), an acidic BGE was employed in order to guarantee the presence of its protonated form. A screening test with fifteen neutral CDs was carried out to further investigate the enantiodiscrimination of a high number of CDs towards duloxetine. Initial experimental conditions consisted on the corresponding CDs at 1.0 % (w/v) solved in a 50 mM phosphate buffer at pH 2.0. A capillary of 33.5 cm was employed working at a temperature of 20 ºC and a voltage of 15 kV. Among all the CDs investigated (see Section 2.1), A-γ-CD, HB-β-CD, M-γ-CD, and HP-β-CD were found to be enantioselective towards duloxetine, being M-γ-CD and HP-β-CD those enabling to obtain Rs > 1. Although the optimum concentration was 0.5 % (w/v) for both M-γ-CD and HP-β-CD, HP-β-CD was selected as the best chiral selector as the enantiomeric impurity (R-duloxetine) migrated before the active principle (S-duloxetine) unlike with M-γ-CD. The change in the enantiomer migration order has already been justified by our research group [20]. Once selected the most suitable chiral selector, the effect of the phosphate buffer concentration was studied from 50 to 250 mM, and its pH from 2.0 to 7.0. A 150 mM phosphate buffer (pH 3.0) was chosen as adequate in terms of current intensity and Rs. Temperature was studied from 15 to 35 ºC selecting 20 ºC as the optimum value as it

offered a good Rs and shorter migration times than 15 ºC. Since it was possible to increase the applied voltage from 15 to 30 kV, the total length of the capillary was increased to 64.5 cm in order to improve the Rs. Finally, the effect of the injection volume and detection parameters was investigated to improve the method sensitivity. Injection times between 5 and 80 s were tested, selecting 20 s because it allowed obtaining a greater sensitivity without a significant Rs loss. With regard to the detector parameters optimal values were a response time of 1.0 s with a detection wavelength of 220 nm (bandwidth 35 nm) including the reference wavelength. Under the optimized conditions, the enantioseparation of duloxetine was completed within 20 min with Rs of 2.1, using 0.1 mg/ml as the amount of active principle to be analyzed for purity control (nominal value).

3.2. Validation of the developed CE-UV method

The validation of the CE-UV method was carried out in terms of selectivity, linearity, accuracy, precision, and limits of detection (LOD) and quantitation (LOQ). To study the selectivity of the method, two different pharmaceutical formulations spiked with 1 µg/mL of R-duloxetine and 100 µg/mL of S-duloxetine (nominal value) were analyzed. Interfering peaks were not found under these conditions.

As shown in **Table 1**, linearity was proven to be adequate in all cases as R^2 values were ≥ 99 %, confidence interval for the slope did not include the zero value and confidence interval for the intercept included the zero value (for a 95 % confidence level). The Response Relative Factor (RRF) was between 0.8 and 1.2 as European Pharmacopeia dictates to demonstrate that R- and S-duloxetine responses are equal [21]. The results concluded (for a 95 % confidence level) that no matrix interferences existed~~. Therefore~~, and the external standard calibration method was employed to quantify the content of duloxetine in pharmaceutical formulations.

218 Accuracy was evaluated as the recovery obtained from six pharmaceutical
219 samples replicates containing 100 µg/mL of duloxetine (according to labeled amount)
220 spiked with 1 and 100 µg/mL of R- and S-duloxetine, respectively. Recovery values were
221 acceptable as the 100 % value is included in all cases (see **Table 1**). Moreover, the
222 precision was evaluated and RSD values (%) of migration times and corrected areas were
223 acceptable as shown in **Table 1**.

224 Finally, LOD and LOQ values were 0.2 µg/mL and 0.67 µg/mL, respectively. This
225 LOD was experimentally verified (see **Figure 1**). According to this LOD and the nominal
226 value described above, the method developed allowed the detection of impurities above
227 a 0.2 % of R-duloxetine. Although this value is quite low, following the ICH guideline
228 Q3B [3] this value must be lower than 0.1 % to apply it to determine impurities in
229 pharmaceutical formulations, thus, the adaptation of this methodology to the use of MS
230 detection was carried out.

231

232 *3.3 Modification of the CE-UV method to be compatible with MS detection*

233 Although chiral CE-UV methodologies can be transferred to a CE-MS system,
234 this task is not always easy to achieve, and implies the modification of some parameters
235 of the UV method. These modifications mainly include changes in the employed buffer
236 since the ones employed in UV experiments are non-volatile and generate high currents
237 intensities in the CE system [22]. For this reason, the non-compatible BGE employed in
238 the UV experiments, phosphate buffer, was replaced with an also acidic but volatile
239 medium, ammonium formate buffer. Different buffer pH and concentrations were tested,
240 maintaining in all cases the chiral selector and its optimized concentration. A 150 mM
241 ammonium formate buffer (pH 3.0) with 0.5 % (w/v) HP-β-CD was selected since these
242 conditions originated the best Rs, yielding less than 50 µA of current intensity and making

243 possible the MS coupling. However, under these conditions the obtained Rs was not
244 enough to perform an acceptable enantioseparation. Therefore, different parameters were
245 modified such as the capillary length, the injection time of the sample and the temperature
246 in order to improve the Rs. The total capillary length was enlarged from 64.5 cm to 104
247 cm, sample injection time was reduced from 20 to 5 s and temperature had to be
248 diminished from 20 to 15 ºC. Under these conditions, although migration times increased
249 from 20 to 30 min, it could be possible to obtain a Rs good enough to perform the desired
250 enantioseparation (Rs 2.7).

251 To avoid source contamination and the low sensitivity derived from the use of a
252 non-volatile chiral selector as HP-β-CD, a partial filling strategy was employed. A plug
253 of 0.5 % (w/v) HP-β-CD in 150 mM ammonium formate buffer (pH 3.0) during 1 min
254 (38 % of total capillary length) by applying 1 bar was selected as the best to obtain a
255 satisfactory enantioresolution (Rs 2.5) and an adequate separation from the CD band.
256 Under these conditions, the sensitivity in terms of S/N ratio improved up to 10 times,
257 although the volume injected was about four times lower.

258

259 *3.4 Optimization of the CE-MS method*

260 Several analytical parameters of the MS system related with the ionization source,
261 ionic optics, and analyzer were studied. Firstly, the sheath liquid composition was
262 optimized to establish the electrical contact at the electrospray needle tip. A mixture of
263 methanol:water (80:20, v:v) varying the percentage of formic acid content from 0.1 to 0.5
264 % (v/v) was tested, selecting 0.1 % (v/v) as it offered the best S/N ratio. Sheath liquid
265 flow rate was also optimized (from 1.3 to 5.3 µL/min) and 3.3 µL/min was chosen since
266 it offered the best sensitivity with good stability of the ion current entering the MS system.
267 In CE-MS the nebulizer pressure is an essential parameter to be optimized because the

268 suction effect is produced at high pressures resulting in a loss of Rs. Studied values were
269 2, 3 and 7 psi, selecting 3 psi. With respect to the drying gas, a flow rate of 5 L/min at
270 200 ºC was selected due to the better sensitivity originated. The effect of the applied
271 voltage was evaluated (from 1500 to 6000 V) and better S/N was obtained at 4500 V.
272 Under these conditions, the ion 298.1 *m/z* corresponding to the protonated form of
273 duloxetine as well as its fragment of 153.8 *m/z*, were observed. This indicated that partial
274 fragmentation of duloxetine occurred during the ionization process. Thus, the capillary
275 exit from the ionic optics was optimized (from 0 to 140 V) trying to reduce this
276 fragmentation. 57 V was the selected value since, although fragmentation of the ion m/z
277 298.1 was inevitable, it fragmented the least.

278 Finally, optimization of the MS^2 experiments was performed employing the EIE
279 of the transition from 298.1 to 153.8 *m/z*. The ICC was activated during the analysis to
280 avoid the trap overloading and the target value was set at 100,000 because higher values
281 originated a loss in mass precision. Maximum accumulation time was studied from 25 to
282 300 ms, selecting 50 ms as it ensured the best proportionality between the areas of both
283 enantiomers (when having different levels of concentration), with an average value of 3
284 to obtain enough points to define the peaks. To favor the best entrapment, accumulation
285 and ions fragmentation, more MS^2 parameters had to be optimized. Isolation width (2, 4
286 or 8 *m/z*) was set to 4 *m/z* since it allowed isolating the complete isotopic profile. Different
287 combinations of the fragmentation parameters were tested such as voltage (from 0.2 to
288 1.4 V), time (from 40 to 160 ms), width (from 4 to 40 *m/z*) and the "SmartFrag" option
289 (activated or not) were tested. The best combination was 0.5 V, 40 ms, 10 *m/z*,
290 respectively, with the "SmartFrag" option deactivated. These values allowed obtaining
291 the highest fragmentation of the precursor ion 298.1 *m/z* without 153.8 *m/z* ion

292 fragmentation. **Figure 2** shows the CE-MS^2 EIE at 153.8 *m/z* and the MS^2 spectra
293 obtained for a racemic mixture of duloxetine (25 µg/mL).
294
295 *3.5 Analytical characteristics of the CE-MS^2 method*
296 The analytical characteristics were investigated in terms of linearity, precision and
297 LOD and LOQ. As seen in **Table 2**, once again results show a perfect linearity in the
298 studied range ($R^2 \geq 99$ %), confidence interval for the slope did not include the zero value
299 and confidence interval for the intercept included the zero value in all cases. RRF value
300 was 1.05 meaning that R-duloxetine response could be considered as S-duloxetine. For
301 this CE-MS^2 strategy, only the method repeatability was studied, as it is the most
302 representative parameter of the precision. As **Table 2** shows, precision values were quite
303 better comparing them with those calculated for the CE-UV method (see **Table 1**). **Figure**
304 **3** shows the LOD obtained with the developed CE-MS^2 method being this value 20 ng/mL
305 (20 ppb) for R-duloxetine, reaching the lowest LOD value ever reported for the duloxetine
306 enantiomeric impurity. The sensitivity has been improved 10 times when compared to the
307 CE-UV method, enabling to detect 0.02 % of duloxetine enantiomeric impurity and,
308 according to the ICH Q3B [3], the CE-MS^2 method can be applied to the analysis of
309 impurities. It is important to note that other methods described in the literature do not
310 allow to meet the ICH requirements for the analysis of duloxetine. This highlights the
311 importance of developing CE-MS^2 methodologies in terms of achieving high sensitivity
312 and selectivity, obtaining an unambiguous identification of the studied analytes.
313
314 *3.6 Comparative analysis of pharmaceutical formulations by both methodologies*
315 Four pharmaceutical formulations were analyzed by the developed methods.
316 Enantiomeric impurity percentage, duloxetine content, and established content of

317 duloxetine are shown in **Table 3**. Values obtained by both methods were compared using
318 statistical tests (F- and t-tests). There were not significant differences between the
319 precision achieved by both methods (F-test), nor between the quantities obtained from
320 the analysis of the drugs (t-test), as evidenced by the p-values, in all cases higher than
321 0.05 (confidence level of 95 %). It is worth noted that none of the pharmaceutical
322 formulations analyzed by the two chiral methodologies presented detectable amounts of
323 the enantiomeric impurity and the S-duloxetine content was equivalent to the labeled
324 content (the values of the established duloxetine content included the 100 % value in all
325 cases). **Figure 4** shows the electropherogram corresponding to the analysis of a
326 pharmaceutical formulation by the CE-MS^2 methodology.

327

328 **4. Concluding remarks**

329

330 The developed CE-MS^2 method has been proven to be a valuable tool to enhance
331 the sensitivity obtained for the determination of duloxetine enantiomers by CE-UV. An
332 increase in the sensitivity up to 10 times was obtained, detecting 0.02 % of duloxetine
333 impurity, which is suitable for the analysis of impurities as ICH guidelines dictates. Due
334 to the fact that the Rs decreased when transferring the methodology from the CE-UV to
335 the CE-MS system, some modifications had to be carried out, including an increase of the
336 capillary length, which caused longer migration times (from 20 to 30 min). For the first
337 time, duloxetine pharmaceutical formulations have been analyzed and the results
338 indicated that duloxetine impurity was not detectable and that the duloxetine content was
339 in agreement with the labeled content.

340

341

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343

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348

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408 **Figure legends**

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410 **Figure 1.** CE-UV electropherogram corresponding to the LOD of R-duloxetine (0.2
411 µg/mL) in the presence of 100 µg/mL of S-duloxetine. Experimental conditions: BGE,
412 0.5 % (w/v) of HP-β-CD in 150 mM phosphate buffer (pH 3.0); uncoated fused-silica
413 capillary, 64.5 cm (56 cm effective length) x 50 µm I.D.; temperature, 20 ºC; voltage, 30
414 kV; hydrodynamic injection, 50 mbar x 20 s; UV detection at 220, 4 nm (reference
415 wavelength: 375, 100 nm). * Unknown impurity.

416 **Figure 2.** CE-MS^2 EIE for a 25 µg/mL racemic mixture of duloxetine under the optimized
417 conditions and the corresponding MS^2 spectra for the peaks. Experimental conditions:
418 BGE, 150 mM ammonium formate buffer (pH 3.0); PFT, 0.5 % (w/v) of HP-β-CD in
419 BGE applying 1 bar during 1 min; uncoated fused-silica capillary, 104 cm x 50 µm I.D.;
420 temperature, 15 ºC; voltage, 30 kV; hydrodynamic injection, 50 mbar x 5 s; sheath liquid,
421 3.3 µL/min of 80:20 (v/v) methanol/water with 0.1 % (v/v) of formic acid; nebulizer and
422 drying gas, 3 psi N_2 and 5 L/min N_2 at 200 ºC; ESI+ at -4.5 kV with an end plate of -500
423 V; capillary exit, 57 V; EIE, 153.8 *m/z* (MS^2 transition from 298.1 m/z) fragmentation
424 by collision-induced dissociation with He, 0.5 V for 40 ms to a fragmentation width of
425 10 m/z**.**

426 **Figure 3.** CE-MS^2 EIE corresponding to the LOD of R-duloxetine (0.02 µg/mL) in the
427 presence of 100 µg/mL S-duloxetine. Experimental conditions as in Figure 2. * Unknown
428 impurity.

429 **Figure 4.** CE-MS^2 EIE corresponding to a pharmaceutical formulation with a
430 concentration of 100 µg/mL of S-duloxetine according to the labeled content.
431 Experimental conditions as in Figure 2. * Unknown impurity.

432 **Figure 1**

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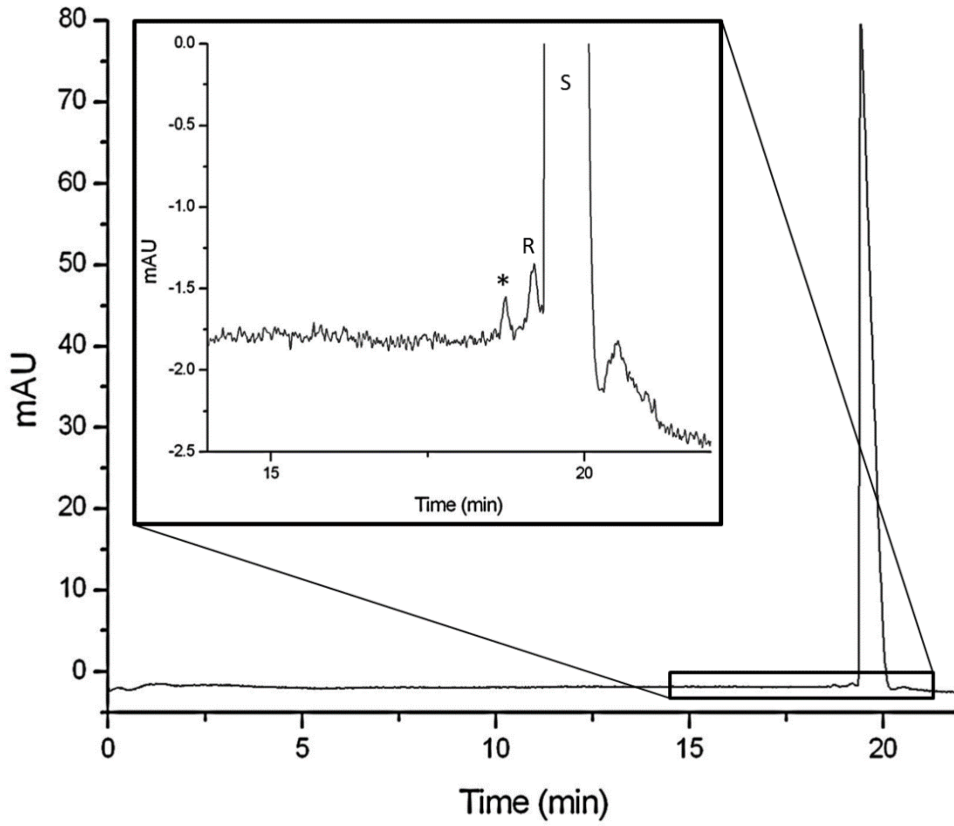

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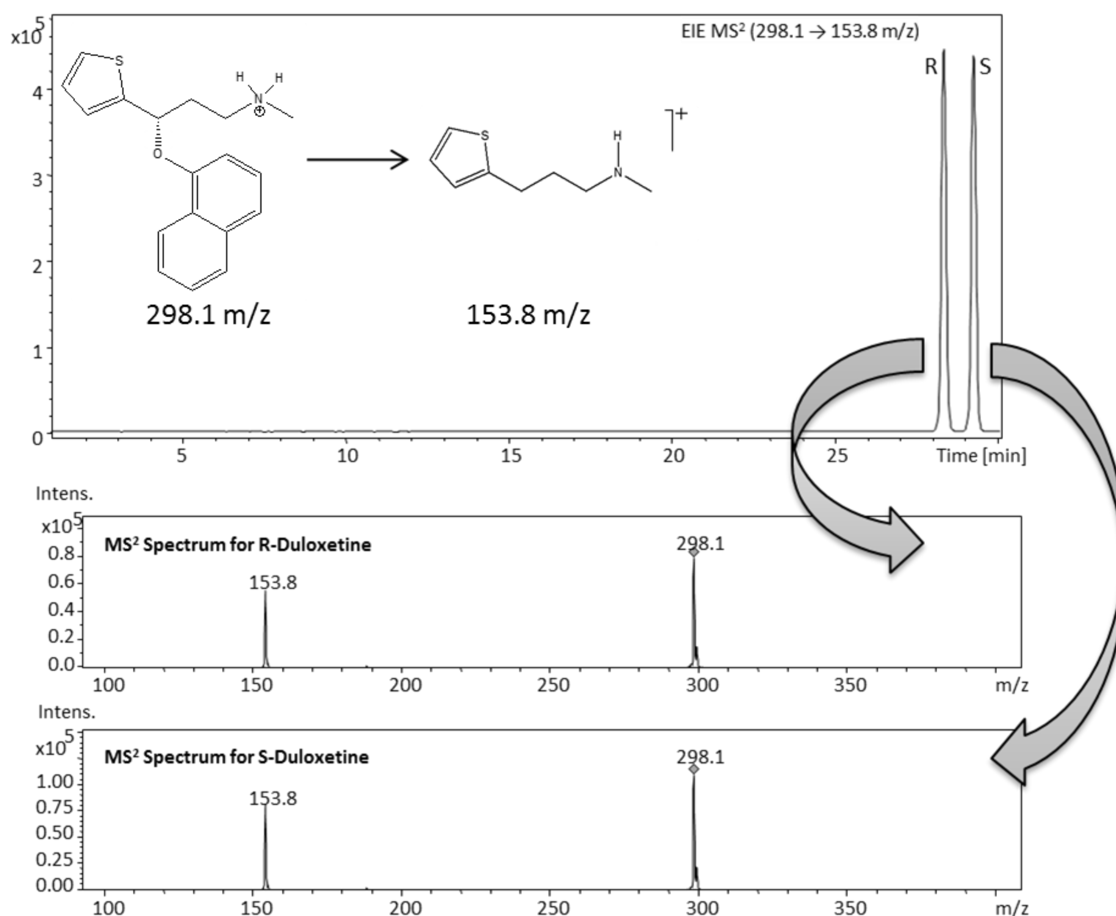
447 **Figure 2**



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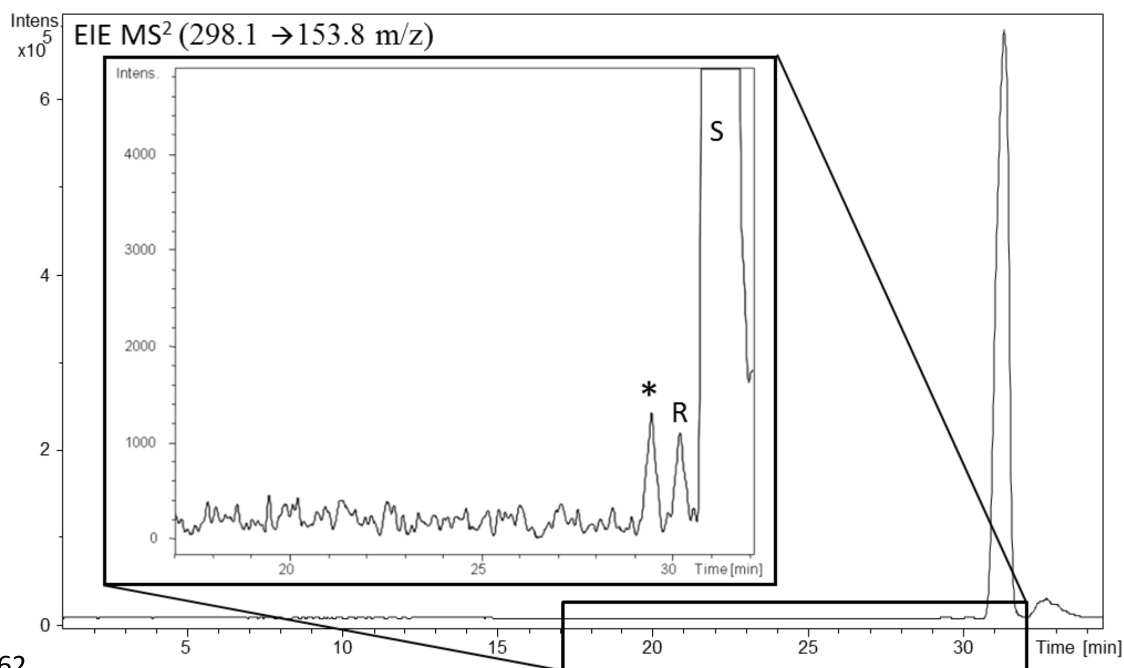
461 **Figure 3**



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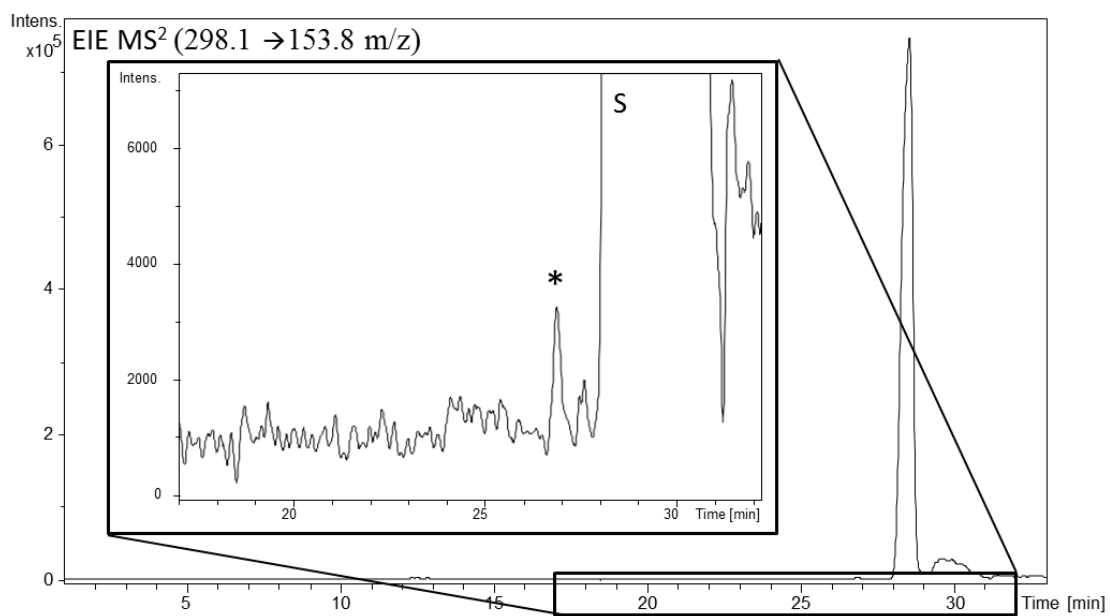
479 **Figure 4**



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