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Original scientific paper

Development and application of a validated HPLC method for the analysis of dissolution samples of mexiletine hydrochloride capsules

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Abstract: The aim of this work was to develop and validate a simple, efficient, sensitive and selective method for the analysis of dissolution samples of mexiletine hydrochloride capsules by HPLC without the necessity of any time-consuming extraction, dilution or evaporation steps prior to drug assay. Separation was performed isocratically on a 5 µm LiChrospher 60, RP-Select B column (250×4 mm ID) using the mobile phase buffer–acetonitrile (60:42, v/v) at a flow rate of 1.2 mL min^{-1} and UV detection at 262 nm. The elution occurred in less than 10 min. The assay was linear in the concentration range 50–300 µg mL^{-1} (r^2 = 0.9998). The validation characteristics included accuracy, precision, linearity, specificity, limits of detection and quantification, stability, and robustness. Validation acceptance criteria were met in all cases (the percent recoveries ranged between 100.01 and 101.68 %, *RSD* < 0.44 %). The method could be used for the determination of mexiletine hydrochloride and for monitoring its concentration in *in vitro* dissolution studies.

Keywords: mexiletine hydrochloride; dissolution; method validation; HPLC.

INTRODUCTION

Mexiletine hydrochloride is an orally active class I anti-arrythmic agent. Chemically it is 1-methyl-2-(2,6-xylyloxy)-ethylamine hydrochloride (Fig. 1). Mexiletine hydrochloride 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 available in capsule form.[1]

Mexiletine hydrochloride is a white powder, freely soluble in water and in ethanol, slightly soluble in acetonitrile and practically insoluble in diethyl ether.[2]

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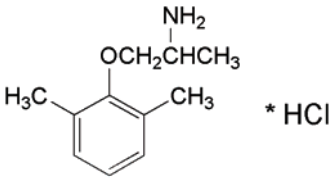

Fig. 1. Chemical structure of mexiletine hydrochloride.

Various analytical methods have been reported in the literature for the quantitative determination of mexiletine hydrochloride in human plasma or serum. These methods are quite limited and typically involve a derivatization step.[3–7] The literature on HPLC analysis of mexiletine hydrochloride in raw materials and pharmaceutical preparations is rather poor.[1,2,8–14] Due to their high sensitivity and selectivity, analytical methods such as liquid chromatography were previously reported to be used for the determination of active substances in dissolution samples.[15–21]

As is known, dissolution testing is a regular quality control procedure in good manufacturing practice, and dissolution data are also a substantial parameter for the evaluation of the bioavailability of drugs. The dissolution test has emerged in the pharmaceutical field as a very important tool for the characterization of the performance of a drug product. It provides measurements of the bioavailability of a drug and can demonstrate bioequivalence from batch-to-batch as well. In addition, dissolution is a requirement for regulatory approval for product marketing. To the best of our knowledge there are only two references for the determination of the amount of mexiletine hydrochloride dissolved from capsules (derivative spectrophotometry and sequential injection method) during dissolution studies.[9,14]

The aim of the present work was to develop and validate a simple, efficient, sensitive and selective method for the analysis of dissolution samples of mexiletine hydrochloride capsules by HPLC. The analytical methods reported were validated in terms of specificity, linearity, accuracy, precision, stability and robustness.[22]

EXPERIMENTAL

Chemicals and reagents

Mexiletine hydrochloride, working certified standard, 100.39 % purity, was purchased from Medichem S.A. (Barcelona, Spain). Acetonitrile (HPLC gradient grade) was purchased from J. T. Baker (Deventer, Holland). Potassium dihydrogen phosphate and ortho-phosphoric acid 85 %, suprapur, were purchased from Merck KgaA (Darmstadt, Germany). Purified water was obtained using an Arium Laboratory Equipment (RO, UV) from Sartorius A.G. (Gottingen, Germany). The mobile phase was filtered through a 0.45 µm Sartorius membrane filter (Gottingen, Germany). Capsules of mexiletine hydrochloride, containing 200 mg of active substances, were obtained in-house and commercially.

Equipment

The HPLC system consisted of a binary pump G1312A, an manual injector G1328A, a thermostated column compartment G1316A and a UV detector G1314A, all 1100 Series, from Agilent Technologies, which was controlled by HP Chemstation software (Waldbroon, Germany). An Erweka DT700LH dissolution apparatus (USP II) (Heusenstamm, Germany) was used. The employed Transsonic 470/H ultrasonic bath was from Elma, (Singen, Germany). The CP224S – OCE analytical balance was from Sartorius A.G. (Gottingen, Germany); the repeatability (standard deviation) of the balance: ±0.0001 g.

Chromatographic conditions

Separation was achieved on a LiChrospher 60, RP-Select B column 250×4 mm, 5 µm (Merck). The elution was isocratic at 1.2 mL min^{-1} using a mobile phase of buffer – acetonitrile (60 : 42, v/v). The column temperature was maintained at 30 °C. The injection volume was 20 µL with UV detection at 262 nm.

The buffer was prepared by dissolve 1.28 g dodecyl sodium sulfate and 4.00 g of anhydrous sodium dihydrogen phosphate in 600 mL of water (pH 4.5).

Dissolution

A calibrated dissolution apparatus (USP II) was used with the paddles turning at 50 rpm and a bath temperature maintained at 37±0.5 °C. Nine hundred milliliter freshly prepared and degassed water was used as the dissolution medium.[9]

Six capsules were evaluated for each tested drug product. Dissolution samples were collected manually at 10, 15, 20, 30 and 45 min. At each time point, a 10 mL sample was removed from each vessel and filtered through a filter (0.45 µm) into glass vials and analyzed by HPLC.

The amount of mexiletine hydrochloride in the test samples was calculated, as percentage dissolved, from the measured peak area for the test samples, which were compared with the peak area for a standard mexiletine hydrochloride solution.

Preparation standard solutions

Mexiletine hydrochloride stock solution I of 1 mg mL^{-1} was prepared in water using a working certified standard, by accurately weighing the mexiletine hydrochloride standard (approximately 25 mg) and transferring it into a 25 mL volumetric flask. Calibration standard solutions were prepared by diluting the stock solution I (50–300 µg mL^{-1}).

A system suitability standard solution, which contained 222 µg mL^{-1}, was prepared by measuring 22.2 mg mexiletine hydrochloride and diluting with purified water to 100 mL.

RESULTS AND DISCUSSION

Selection and optimization of analytical method

UV Spectrophotometry and HPLC are most commonly used for the analysis of dissolution samples. UV is the more preferable choice when compared to HPLC because of the time required for the analysis of the sample. In cases when the interference is higher than 2 %, it is recommended to choose another wavelength, second derivative, baseline subtraction or HPLC. Regarding interference from excipients, sensitivity issues, automated system and regulatory agencies, an HPLC method for the determination of mexiletine hydrochloride after dissolution is recommended, especially if it has a short retention time.

In order to obtain the best chromatographic conditions, the wavelength for detection, the column and the mobile phase composition must be adequately selected.

The starting point for the development of the assay of mexiletine hydrochloride were the chromatographic conditions given in the United States Pharmacopoeia (USP).[9] The USP method utilizes a mobile phase of MeOH and acetate buffer (pH 4.8) (60:40, v/v), UV detection at 254 nm, column Purospher STAR RP18e (250×4.6 mm, 5 µm) and a flow rate of 1 mL min^{-1}. An initial attempt resulted in an acceptable retention time (about 8 min), the USP tailing factor was 1.7, but there was interference from the body of the capsules (Fig. 2).

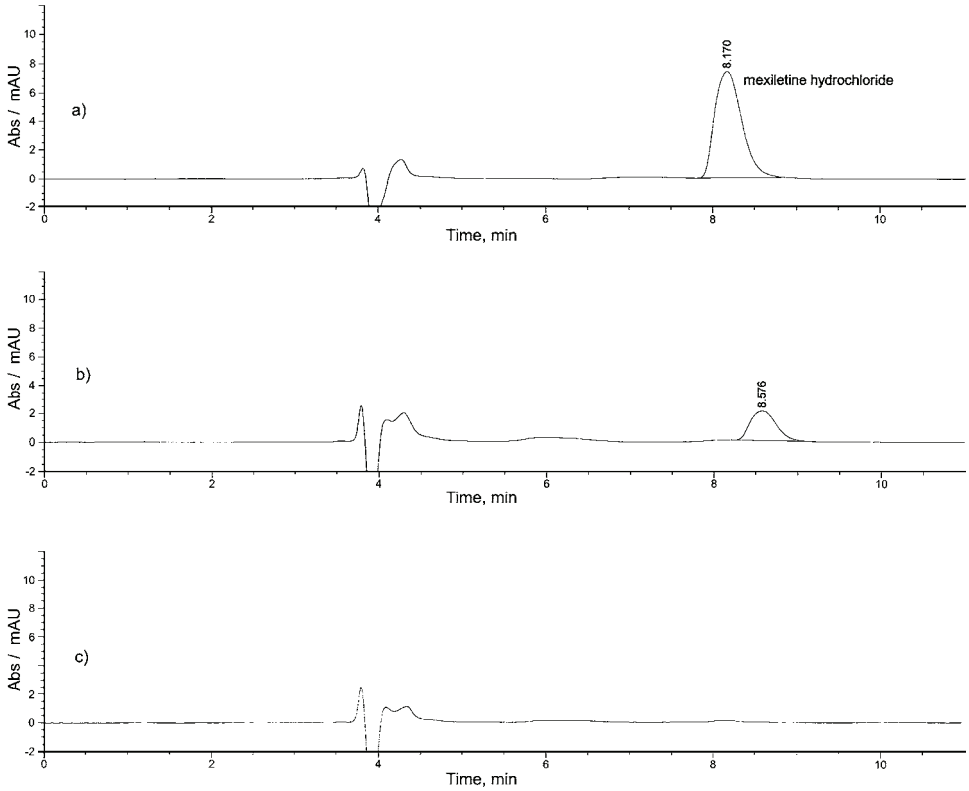

Fig. 2. Chromatograms obtained from: a) the standard solution of mexiletine hydrochloride (200 µg mL^{-1}), b) excipient mixture and c) background sample, using the initial chromatographic conditions.

In order to resolve this problem, the conditions set by the Japanese Pharmacopoeia (UV detection at 210 nm, column 150×4 mm, packed with octyl-silanized silica gel for liquid chromatography, about 7 µm in particle diameter, mobile phase buffer and acetonitrile (60:42, v/v)) were modified in terms of wave-

length, buffer composition and column.[2] A LiChrospher 60, RP Select B, 250×4 mm, 5 μm column was used at 30 °C with a mobile phase flowrate of 1.2 mL min^{-1}. A detection wavelength of 262 nm was selected for the analysis because the drug has sufficient absorption and low quantities of mexiletine hydrochloride may be detected correctly. Furthermore, the calibration curves obtained at 262 nm show good linearity. Regarding the mobile phase, one buffer solution was without an ion-pairing reagent, but every subsequent phase (four in total), contained a different ion-pairing reagent: sodium hexane sulfonate, sodium heptane sulfonate, sodium octane sulfonate and dodecyl sodium sulfate, used in the same molar quantity, respectively (0.004 mol L^{-1}). The obtained results are given in Table I.

TABLE I. Comparative data on the effect of the different ion-pairing reagents (concentration of mexiletine hydrochloride was 200 μg mL^{-1})

Ion-pairing reagent	Retention time, min	Capacity factor	USP Tailing	Number of plates	Width at half height	Symmetry
Without an ion-pairing reagent	2.756	0.826	1.843	1924	0.251	0.410
Sodium hexane sulfonate	2.898	0.845	1.775	3205	0.259	0.475
Sodium heptane sulfonate	3.021	0.923	1.737	3237	0.261	0.477
Sodium octane sulfonate	3.455	1.200	1.598	4153	0.268	0.556
Sodium dodecyl sulfate	13.754	4.789	1.168	12154	0.420	1.104

In order to select the proper reagent, alkyl chain lengths must be taken into consideration. The chain lengths enable selective separation of the analyte. The longer the chain, the greater is the retention (about 14 minutes for dodecyl sodium sulfate), but the features of the peak are better (the USP tailing factor decreased from 1.8 for sodium hexane sulfonate to 1.2 for dodecyl sodium sulfate).

In order to obtain the shortest retention time with the best features of peak and the highest efficiency, the quantity of dodecyl sodium sulfate and sodium dihydrogen phosphate were optimized. Finally, the aforementioned composition of the mobile phase, described in Experimental part was chosen as the mobile phase (buffer–acetonitrile (60:42, v/v)). The retention time was about 9 min (USP tailing factor 1.0, number of plates per column above 12000). When compared to Japanese Pharmacopoeia, using the same flow (1.2 mL min^{-1}) the retention time was obviously shorter.

Taking into account the above-mentioned facts, peak characteristics and retention time that is short enough, for further work, the method given in the Experimental Part was chosen (*i.e.*, the method given in the Japanese Pharmacopoeia was modified concerning the employed wavelength, buffer composition and column).

Validation of the method

The method was validated according to the ICH guidelines (International Conference on Harmonization).[22] The following validation characteristics were determined: specificity, linearity, accuracy, precision, limits of detection and quantification, stability and robustness.

Suitability test. System suitability was determined from six replicate injections of the system suitability standard solution before sample analysis. According to the USP, the acceptance criteria are less than 2 % relative standard deviation (*RSD*) for the peak area, column efficiency (*N*) greater than 2000 column plates and USP tailing factor less than 2.0. The system suitability test ensures the validity of an analytical procedure. All critical parameters tested met the USP acceptance criteria. For this method, the *RSD* of the mean peak area was below 1.8 %, the mean USP tailing factor was 1.0 and *N* (column efficiency) was above 12000 plates per column.

Specificity. The specificity was evaluated by preparing a placebo sample of a commercial formulation of capsules in their usual concentrations. The placebo contained corn starch, silicon dioxide, magnesium stearate and body capsule. This sample was transferred to a vessel with 900 mL of the dissolution medium and stirred at 50 rpm using the paddle for 60 minutes (USP apparatus II) at a temperature of 37 °C. Aliquots of this solution (10 mL) were filtered and analyzed by HPLC.

The specificity analysis revealed that the developed HPLC method did not suffer interference from the formulation excipients, since no other peak was registered at the retention time of mexiletine hydrochloride (Fig. 3).

Linearity. Standard calibration curves were prepared with six calibrators over the mexiletine hydrochloride concentration range of 50–300 μg mL^{-1} (50, 100, 150, 200, 250, 300 μg mL^{-1}), with triplicate determination at each level. The data of peak area *vs*. drug concentration were treated by linear least square regression analysis. Values of the slope, the intercept and the coefficient of determination of the calibration curve for mexiletine hydrochloride are given in Table II. The high value of the coefficient of determination indicated good linearity.

Precision and accuracy. To test the precision and accuracy of the method, solutions containing all excipients in amounts equivalent to one capsule were spiked with a known amount of mexiletine hydrochloride. Taking into consideration the excellent solubility of mexiletine hydrochloride in the dissolution media suggested by the USP, 80, 100 and 120 % were chosen as the three concentration levels for the testing of the accuracy and precision. The determination of mexiletine hydrochloride was realized six times under the same operating conditions over a short period of time (assay working concentrations of 177.78, 222.22 and 266.66 μg mL^{-1} mexiletine hydrochloride, respectively). The precision and accuracy of the chromatographic method, reported as *RSD* (%) and recovery (%),

respectively, were assessed by estimating the repeatability of the results for six replicate injections at three different concentration levels. The recovery, 95 % confidence interval, and *RSD* values obtained per each level illustrated the good precision and accuracy of the method (Table III).

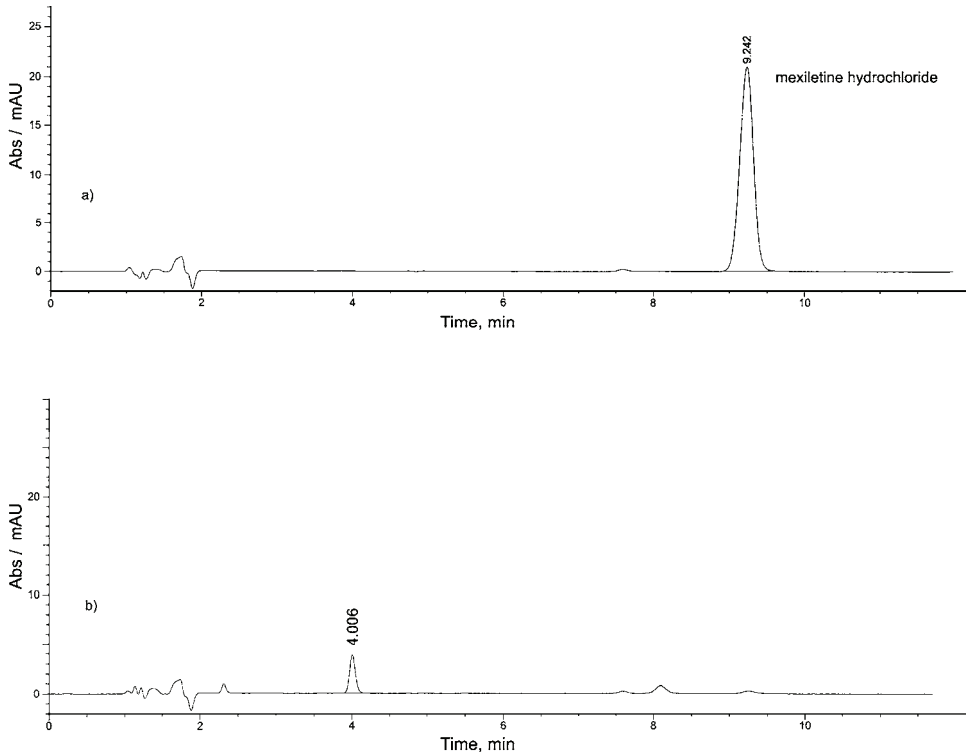

Fig. 3. Chromatograms obtained from: a) the standard solution of mexiletine hydrochloride (222 μg mL^{-1}) and b) excipient mixture, using the selected chromatographic conditions.

TABLE II. Linear regression data in the analysis of mexiletine hydrochloride

Statistical parameters	Obtained values
Concentration range, μg mL^{-1}	50.00–300.00
Regression equation	$y = 1.1772x + 3.497$
Coefficient of determination	$r^2 = 0.9998$
a (*y*-intercept)	1.1772±0.243
b (slope)	3.497±0.472
S(*a*) – error in intercept	0.74
S(*b*) – error in slope	0.02

The intermediate precision of the method was investigated by making ten consecutive injections of a standard solution on two different days by different analysts using two different HPLC instruments. On both days, the *RSD* values

were calculated for the peak area responses obtained for the mexiletine hydrochloride peaks. The data obtained suggested that the method exhibited an acceptable intermediate precision with less than 2.0 % *RSD* for the mexiletine hydrochloride standard solution (Table IV).

TABLE III. Precision and accuracy results obtained for the drug product

Concentration level, %	95 % Confidence interval, %	Recovery, %	*RSD* / % (n = 6)
80	101.47–101.89	101.68	0.26
100	99.66–100.36	100.01	0.44
120	100.29–100.73	100.51	0.27

TABLE IV. Statistical parameters for the intermediate precision (n = 5) at mexiletine hydrochloride concentration of 222 μg mL^{-1}

Statistical parameter	Day 1	Day 2
Mean peak area, mAU	268.78	272.52
SD	0.71	0.88
RSD / %	0.12	0.15

Limits of detection and quantification. The limits of detection (*LOD*) and quantification (*LOQ*) were determined based on the standard deviation of the response (*y*-intercept) and the slope of the calibration plot for low concentrations, in accordance with ICH guidelines.[22] The *LOD* and *LOQ* for mexiletine hydrochloride were 2.07 and 6.21 μg mL^{-1}, respectively.

Sample stability. The stability of mexiletine hydrochloride in aqueous solutions was tested during a 15-day test period. All the samples were kept in the dark at a temperature of +4 °C (in a refrigerator). All the samples were injected into the appropriate HPLC system after 48 h, 7 days and 15 days against fresh standard solutions. No changes in the chromatographic response of the stored samples were found and no additional peaks appeared when compared with chromatograms of the freshly prepared samples.

Robustness. The experimental results of the robustness study are summarized in Table V. Critical chemical and instrumental chromatographic parameters, such as the composition and flow rate of the mobile phase, the column temperature and the injection volume, were deliberately varied in the narrow range compared to their optimal values. The peak areas obtained using mexiletine hydrochloride standard solution of 222 μg mL^{-1} confirmed the robustness of the HPLC assay, since the obtained values were within the acceptance limits (95.0–105.0 %) in all cases.

Analysis of marketed products. The validated method was used for the analysis of two mexiletine hydrochloride drug products. This included drug products from two different manufacturers, as capsules with a dose strength of 200 mg,

using the proposed method. The dissolution profiles of both products are presented in Fig. 4.

TABLE V. Robustness study of the HPLC assay

Chromatographic parameters	Percent recovery[a,b]±*SD*
Optimal conditions[c]	99.32±0.7
Variation of the mobile phase flow rate, mL min^{-1}	
1.0	98.51±0.6
1.4	98.32±1.9
Variation of the buffer : acetonitrile ratio, v/v	
58:44	99.65±0.2
62:40	99.54±1.1
Variation of the injection volume, µL	
18	98.99±1.2
22	100.20±0.4
Variation of column temperature, °C	
28	100.22±0.4
32	100.35±0.8

[a]Percent recovery according to the calibration curve obtained under optimal conditions (mean of three injections ± ± standard deviation); [b]each sample contained 222 µg mL^{-1} (100 % concentration level); [c]for the experimental details, see chromatographic conditions in the Experimental part

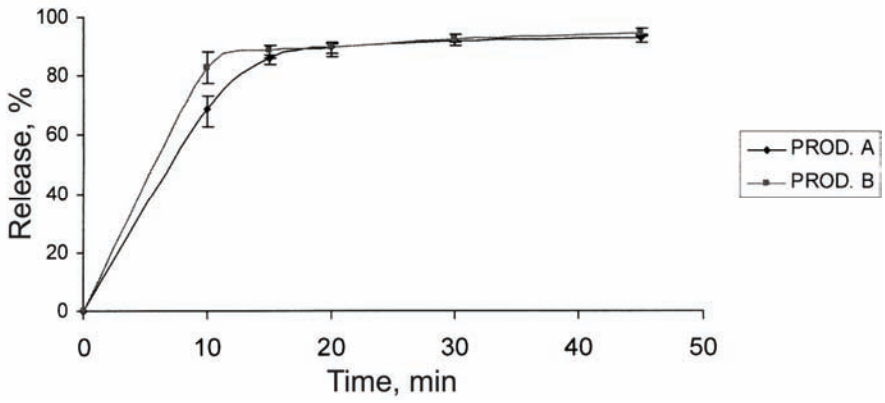

Fig. 4. Dissolution profile of capsule products A and B.

The two capsule products (products A and B) showed a similar dissolution profile with over 85 % dissolution within 15 min (similarity factor f_2 = 85). The dissolution profiles were compared by the calculated similarity factor (f_2) derived using the following equation:[14]

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

where n is the number of time points, R_t the dissolution value of the reference batch at time t and T_t is the dissolution value of the test batch at time t. Generally, f_2 values greater than 50 (50–100) indicate similarity of the compared dissolution profiles. The USP acceptance limits for the dissolution of mexiletine capsules were not less than 80 % of the labeled amount in 30 min.

CONCLUSIONS

A simple and efficient HPLC method was developed and found to be accurate, precise and linear across the analytical range. The method was suitable for the determination of mexiletine hydrochloride in dissolution samples. The methods may be used to assess the quality of commercially available mexiletine hydrochloride drug products. In addition, a significant advantage of the method is that no additional pretreatment of the samples is required prior to the measuring step, thus accelerating the quality control process. An overall duration of the analysis of less than 11 min was achieved (including sample preparation). The method was used successfully to evaluate the dissolution profiles of two marketed drug products.

ИЗВОД

РАЗВОЈ И ПРИМЕНА ВАЛИДИРАНЕ HPLC МЕТОДЕ ЗА АНАЛИЗУ ДИСОЛУЦИОНИХ УЗОРАКА МЕКСИЛЕТИН-ХИДРОХЛОРИДА ИЗ КАПСУЛА

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Циљ овог рада је био развити и валидирати једноставну, ефикасну, осетљиву и селективну методу за анализу узорака мексилетин-хидрохлорида добијених растварањем активне супстанце из капсула помоћу HPLC без претходне посебне припреме узорака (екстракције, разблаживања, упаравања, итд.). Сепарација је изведена са 5 μm LiChrospher 60, RP-Select B колоном (250×4 mm, 5 μm), са мобилном фазом пуфер–ацетонитрил (60:42, в/в) изократским елуирањем за мање од 10 min са протоком од 1,2 mL min^{-1}. Узорци су детектовани на 262 nm. Линеарност је испитана у концентрационом подручју од 50–300 μg mL^{-1} (r^2 = 0,9998). Валидационе карактеристике укључују тачност, прецизност, линеарност, специфичност, лимите детекције и квантификовања, стабилност и робустност. Валидациони критеријуми прихватљивости су постигнути у свим случајевима (рецоверу вредност се креће у опсегу од 100,01 до 101,68 %, *RSD* < 0,44 %). Метода се може користити за одређивање активне супстанце у узорцима након растварања мексилетин-хидрохлорида из капсула.

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REFERENCES

1. S. Kaushik, K. S. Alexander, *J. Liq. Chromatogr. Related Technol.* **26** (2003) 1287
2. *The Japanese Pharmacopoeia XIII*, Official Monographs for Part I, 1996, p. 515
3. Y. Liu, L. Sun, X. Di, Y. Sun, [C. A. 147/2007 249850]
4. H. Zhang, C. Yu, G. Liu, J. Jia, Y. Hong, X. Xu, [C. A. 141/2003 301594]

5. D. K. W. Kwok, L. Igwemezie, C. R. Kerr, K. M. McErlane, *J. Chromatogr. B* **661** (1994) 271
6. Z. Abolfathi, P. M. Belanger, M. Gilbert, J. R. Rouleau, J. Turgeon, *J. Chromatogr.* **579** (1992) 366
7. H. Liu, F. Feng, M. Ma, S. Cui, D. Xie, S. Xu, *J. Chromatogr. B* **858** (2007) 42
8. R. Pietras, D. Kowalczuk, H. Hopkala, *Chromatographia* **60** (2004) 17
9. *The United States Pharmacopoeia*, 31th ed., Official Monographs, USA, 2008, p. 2708
10. E. Lamparter, *J. Chromatogr. A* **635** (1993) 155
11. R. Pietras, D. Kowalczuk, H. Hopkala, *J. AOAC Int.* **90** (2007) 977
12. Z. Aydogmus, S. M. Cetin, S. Tosunoglu, *Turk. J. Chem.* **26** (2002) 839
13. A. M. El Walily, F. A. El Yazbi, S. F. Belal, O. Abdel Razak, *Anal. Lett.* **30** (1997) 2029
14. P. D. Tzanavaras, C. K. Zacharis, P. Rigas, *J. Pharm. Biomed. Anal.* **48** (2008) 1254
15. A. Gupta, A. B. Ciavarella, V. A. Sayeed, M. A. Khan, P. J. Faustino, *J. Pharm. Biomed. Anal.* **46** (2008) 181
16. C. V. Garcia, C. S. Paim, M. Steppe, E. E. S. Schapoval, *J. Pharm. Biomed. Anal.* **41** (2006) 833
17. E. Banoglu, Y. Özkan, O. Atay, *Il Farmaco* **55** (2000) 477
18. A. Savaser, S. Goraler, A. Tasoz, B. Uslu, H. Lingeman, S. A. Özkan, *Chromatographia* **65** (2007) 259
19. P. D. Tzanavaras, A. Verdoukas, D. G. Themelis, *Anal. Sci.* **21** (2005) 1515
20. Y. Özkan, A. Savaser, S. A. Özkan, *J. Liq. Chromatogr. Related Technol.* **28** (2005) 423
21. C. M. B. Rolim, L. Brum, Jr., M. Fronza, M. D. Malesuik, L. Bajerski, S. L. Dalmora, *J. Liq. Chromatogr. Related Technol.* **28** (2005) 477
22. *International Conference on Harmonization Q2 (R1), Validation of Analytical Procedures, Text and Methodology*, http://www.ich.org/LOB/media/MEDIA417.pdf (accessed 20 April, 2009).