In vitro dissolution profile study of mucolytic drug ambroxol hydrochloride from solid oral dosage form by UHPLC-MS/MS

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Abstract

In this paper, a simplified dissolution test was performed for the release of ambroxol from tablets according to the European Pharmacopoeia. *In vitro*, three different dissolution media; 0.1 M HCl pH 1.2, acetate buffer (ABS) pH 4.5 and phosphate buffer (PBS) pH 6.8 were used for the simulation of the gastrointestinal conditions at temperature of 37.0±0.5 °C. The drug release was evaluated by a new ultra-high performance liquid chromatography (UHPLC)-tandem mass spectrometry (MS/MS) method. The method was validated to meet requirements as per ICH guidelines which include linearity, specificity, precision, accuracy and robustness. The corresponding dissolution profiles showed more than 80% drug release within 30 min without significant differences. Further, the developed and validated UHPLC-MS/MS method could find a useful application in the process of production, quality control and bioavailability/bioequivalence studies of new pharmaceutical formulations of drugs in order to achieve a safe therapeutic efficacy.

Keywords: ambroxol, dissolution test, UHPLC-MS/MS.

Available online at the Journal website: http://www.ache.org.rs/HI/

SCIENTIFIC PAPER

UDC 615.015:61:543.544

Hem. Ind. **71** (1) 75–83 (2017)

doi: 10.2298/HEMIND160315020V

Ambroxol hydrochloride, *trans*-4-[(2-amino-3,5--dibromobenzyl)amino]cyclohexanol hydrochloride [1], is a semi-synthetic derivative of quinazoline alkaloid vasicine from the Indian shrub *Adhatoda vasica* [2] with the chemical structure shown in Figure 1.

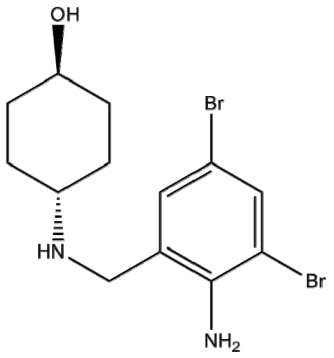

Figure 1. Chemical structure of ambroxol.

Ambroxol is a typical mucolytic drug with secretolytic and secretomotoric activity. Since 1978, ambroxol hydrochloride has been widely used as an expectorant in the treatment of asthma and chronic obstructive pulmonary diseases associated with abnormal mucus secretion and transport [3]. As a liposoluble molecule, it stimulates mucus clearance, decreases mucus adhesion to the bronchial lining and facilitates expectoration which eases a productive cough [4]. Ambroxol hydrochloride has also shown cough-suppressing effects, antioxidant, anti-inflammatory and local anesthetic effects. The local anesthetic effect of ambroxol was explained by its ability to block Na^+-channels observed in the rabbit eye model [5,6]. Recently, the antioxidant activity of ambroxol has been presented in terms of the suppression of lipid peroxidation induced by *t*-butyl hydroperoxide or doxorubicin [7,8]. In addition, anti-inflammatory effects of ambroxol were shown by inhibition of histamine release from mastocytes and the generation of interleukins and cytokines [9]. In addition to its primary pharmacological activity, ambroxol has also been shown to have some analgesic, antibacterial and antiviral effects [10–13].

Ambroxol is contained in several pharmaceutical formulations such as tablets, syrups, lozenges, dry powder and inhalation solution as well as effervescent tablets. The most employed analytical method for the determination of ambroxol in different pharmaceutical forms is high-performance liquid chromatography (HPLC) with UV-visible spectrophotometric [14,15] and electrochemical detection [16]. Pharmaceutical research and development studies of ambroxol formulations have also reported methods such as gas chromatography with an electron capture detector [17], spectrophotometry [18], capillary electrophoresis [19], amperometric method with carbon film resistor electrodes [20] and cyclic voltammetry with a boron-doped diamond electrode [21]. Traditional determination techniques such as HPLC or spectrophotometric detection

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Paper received: 15 March, 2016
Paper accepted: 10 May, 2016

are not suitable for extensive and sensitive validation processes because they are time consuming, have low sensitivity and high input of solvents. Various MS detection procedures, especially MS/MS techniques, are considered to be much more appropriate for this purpose and pharmacokinetic studies. To date, highly sensitive and specific mass spectrometric methods (LC-MS, MS/MS) have been mostly reported for the determination of ambroxol in biological samples [22–25].

In the quality control process, solid oral forms are subjected to appropriate procedures that include a dissolution test under simulated physiological conditions. A dissolution test is primarily a quality control test which represents a standardized method of measuring the rate of drug release from dosage tablets [26]. According to USP and FDA, dissolution test is required for all solid oral dosage forms in which drug absorption is necessary to achieve a desired blood level and therapeutic effect. Dissolution tests are also important in the process of drug development where every product with a solid phase should be developed with drug release characterization [27,28]. All dissolution tests of drugs are conducted according to relevant pharmacopoeia instructions and procedures (Ph. Eur, JP, BP) [29–31].

The present report documents a simple and rapid reversed-phase ultra-high performance liquid chromatographic method using a submicron 1.3 µm Kinetex C-18 column coupled with a tandem-mass spectrometry detector for the quantification of ambroxol. The UHPLC-MS/MS method was completely validated according to ICH guideline with high precision and accuracy [32]. The application of the method was shown in the evaluation of the *in vitro* dissolution test of ambroxol from the tablets as per Euro. Pharm. Dissolution Test Instructions [29].

The developed UHPLC-MS/MS method can find a useful application in the pharmaceutical industry in the study of new pharmaceutical formulations of ambroxol. Thus, the proposed method should enable the *in vitro* preclinical selection of the optimal solid dosage formulation of ambroxol before the *in vivo* bioequivalence study in humans. Furthermore, the method use should contribute to a new pharmaceutical and pharmacokinetic studies of the ambroxol and similar substances.

MATERIAL AND METHODS

Chemicals

Ambroxol hydrochloride (> 99.9%) and verapamil hydrochloride ((> 99.9%, I.S.) were purchased from Fluka (Dorset, UK). Methanol and formic acid (LCMS grade) were obtained from Promochem (Wesel, Germany), whereas, potassium hydrogen phosphate and hydrochloric acid (HCl) (analytical grade) were purchased from Sigma–Aldrich. Ultra-pure LC/MS water was obtained from Fisher Chemical (Waltham, MA, USA). The tested ambroxol tablets (30 mg/tablet, Flavamed®) were provided by Berlin-Chemie AG (Menarini Group).

Instrumental and chromatographic conditions

The data were collected with an ultra high-performance liquid chromatography-tandem mass spectrometer, Shimadzu LCMS-8030. The MS/MS spectrometer was equipped with two atmospheric pressure ionization (API) modes; the electrospray ionization (ESI) and the atmospheric pressure chemical ionization (APCI), binary pumps (LC-30AD, Nexera), column oven (CTO-30A, Nexera), degasser (DGU-20A, Prominence), communication interface (CBM-20A, Prominence) and autosampler (SIL-30AC, Nexera). Chromatographic separation was performed on a Kinetex C_{18} column (2.1 mm×50 mm, 1.3 µm, Phenomenex, USA). The mobile phase consisting of methanol and 0.01% formic acid aqueous solution (90:10 volume ratio) was delivered at a flow rate of 0.3 ml/min. Before use, the mobile phase was filtered through 0.22 µm membrane filter paper. The column temperature was maintained at 40 °C and the cooler of autosampler at 15 °C. The injection volume was 2 µL. Multiple-reaction monitoring (MRM) in a positive (+) ESI mode was used for the quantification at *m/z* 378.9→263.8 for the ambroxol and *m/z* 455.2→165.0 for the internal standard (verapamil). The MRM optimization of the optimum ion optics voltages and collision energies, as well as the highest intensity of the product ions, was performed with an automated wizard. The optimized ionization conditions were the following: interface voltage: 4.5 kV; conversion dynode voltage: 6 kV; detector: –1.92 kV and exit lens: –4.0 V. The collision energy was optimized and applied to –20 and –25 V; Q1 precursor bias/Q3 product bias: –28/–18 V and –11/–10 V for AMB and IS, respectively. The source block and desolvation temperatures were 400 and 270 °C, respectively. Nitrogen was used as the nebulizing and drying gas at flow rates of 3 and 15 L/min, respectively. The UHPLC-MS/MS system was controlled by a Lab Solution software.

Calibration standards (CC) and control samples (QC)

The initial stock solutions of ambroxol and verapamil were prepared separately at the concentration of 1 mg/mL in methanol. Working solutions were obtained by serial dilution of the stock solutions with the methanol or mobile phase and used for the preparation of calibration curves. Calibrations standards (CC) for this method were prepared at the following concentrations 0.5, 10, 20, 100 and 200 ng/mL. Similarly, quality control (QC) samples were run in each assay at lower limit of quantitation (LLOQ): 0.5 ng/mL, low (LQC): 20 ng/mL, medium (MQC): 100 ng/mL and

high level (HQC): 200 ng/mL. The concentration of verapamil used as the internal standard (IS) was 20 ng/mL. All samples were stored at 4 °C and analyzed during the same day. All standard solutions were stored at –20 °C and used up to three weeks.

Method validation

The UHPLC-MS/MS method for the determination of ambroxol was validated according to the ICH Q2A/Q2B guidelines. The validation procedure included the following parameters: linearity, accuracy and precision, sensitivity, specificity, stability tests, and carry-over. The parameters were statistically analyzed by using Microsoft Office Excel 2013. Each value was expressed as the mean ± *SD* and the *RSD* (%).

Dissolution test

The dissolution test was performed according to the Euro. Pharm. with a slight modification due to technical limitations in the laboratory. The tablets were gradually dissolved in three different media, which simulated gastrointestinal conditions. The glass beakers with the solutions and tablets were placed on the magnetic stirrer over a 6-h period. The dissolution media were 1000 mL of HCl pH 1.2, a pH 4.5 acetate buffer solution (ABS) and a pH 6.8 phosphate buffer solution (PBS). The temperature of the medium and the rotation speed of the magnetic stirrer were set at 37.0±0.5 °C and 50 rpm, respectively. At designated time points (1, 3, 5, 10, 15, 30, 60, 120, 240 and 360 min) the samples were withdrawn and filtered through 0.22 µm nylon syringe filter. The aliquot of the filtrate was further diluted with an appropriate volume of fresh medium (with the 20 ng/mL IS added). Two microlitres of the test solution were injected into the instrument, the mass chromatogram was recorded, and then, the amount of the drug was calculated with the dilution factor (DF) of 500. The average percentage of ambroxol at each sampling point was calculated after the correction for the cumulative amount of the removed liquid in the previous sample.

RESULTS AND DISCUSSION

Method development

The aims of the UHPLC-MS/MS validation in this study were the enhancement of the selectivity of the system, the decrease of the limit of detection (increase in sensitivity of detection) and the reduction of the analysis time (increase the results output per time) compared to published methods. To achieve the best instrument performances, the chromatographic and MS/MS parameters optimization were involved in the experimental procedure. The UHPLC conditions, especially the composition of the mobile phase and the type of the analytical column, were optimized through several trials. Three types of analytical columns C_{18} with the same length and diameter (2.1 mm×50 mm), but different particle sizes (1.3, 1.7 and 2.6 µm) were tested. Finally, a Kinetex C_{18} column (2.1 mm×50 mm; 1.3µm, Phenomenex, USA) and security guard C_8 column (Kinetex, Phenomenex, USA) were used. According to literature data, various mixtures of solvents, such as acetonitrile and methanol using different buffers such as ammonium acetate, ammonium formate, formic acid and acetic acid with variable pH (range 4.0–7.0), along with altered flow rates (range 0.2–0.8 mL/min) at the isocratic elution were tested for the chromatographic separation of ambroxol and IS [22––24]. Our studies demonstrated that acidic water and methanol were as efficient as the buffer and other solvents combinations. Furthermore, this procedure avoided the use of the buffer solutions which tend to crystallize the phosphate salts in the column and decrease its separation capacity. Finally, the combination of methanol and 0.01% formic acid solution in water (90:10 volume ratio), was found to be suitable for the method optimization and MS/MS determinations. The retention times (R_t) of ambroxol and IS were 0.79 and 0.91 min, respectively with the total analysis run time of 1.5 min.

MS/MS measurement conditions were selected according to the MS/MS signal response of the target compound, and the results indicated that the positive mode of ionization was much more sensitive than the negative mode. The typical protonated MS ion mass peaks $[M+H]^+$ were observed at *m*/*z* 378.9 for ambroxol and *m*/*z* 455.2 for IS. Furthermore, the quantification of the analytes was performed in the multiple-reaction monitoring (MRM) mode, monitoring the transition pairs within the appropriate run time segments. The *m*/*z* 378.9 precursor ion to the *m*/*z* 263.8 product ion was followed during the 0.4–1.2 min for ambroxol and the *m*/*z* 455.2 precursor ion to the *m*/*z* 165.0 product ion during the 0.5–1.5 min for IS. All applied transitions were in accordance with the literature data [22–24], and the product MS ion mass spectra and the MRM ion chromatograms were previously reported [25]. The detailed optimization of mass spectrometry conditions is provided in the Instrumental and chromatographic condition section. The quadrupole analyzers Q1 and Q3 were set at unit resolution with the dwell/pause time measurement of 200/1 and 100/2 ms for ambroxol and IS, respectively.

Validation of the assay

The standard calibration curves were constructed using five calibration standards (0.5, 10, 20, 100 and 200 ng/mL), without zero points. The calibration standards were injected six times (n = 6) on three different days. The calibration curves were prepared by deter-

mining the best fit of peak ratios *vs*. concentration, and fitted to linear function $y = mx + c$, where y is the peak ratio of ambroxol/IS and x the concentration of ambroxol. The acceptance criteria for each back-calculated standard level was ±15% *RSD* from the nominal value except at *LLOQ*, which was set at ±20% *RSD*. The obtained calibrations curves were: 1STD, $y_1 = 0.0125x_1 - 0.0019$, $r = 0.9995$, 2STD, $y_2 = 0.0125x_2 - 0.0007$, $r = 0.9999$, 3STD, $y_3 = 0.0123x_3 - 0.0037$, $r = 0.9949$ with the average regression line: STD_{av}: $y = (0.0124\pm0.0001)x - (0.0019\pm0.0184)$, $r = 0.9989\pm0.0010$. A least square regression model was used for each standard curve with the mean value of the correlation coefficient (r^2) of 0.9989 and standard deviation of 0.0010. The average regression ($r^2 > 0.99$) for all standard calibration curves indicated very good correlations.

The lower limit of quantification (*LLOQ*) and the limit of detection (*LOD*) were experimentally determined by injecting a series of dilute standard solutions with known concentrations. The lowest concentration levels with the *RSD* ≤ 20% were taken as the *LLOQ* (0.500 ng/mL) and *LOD* (0.250 ng/mL) at signal to noise ratio 10:1 and 3:1, respectively. The accepted *LLOQ* values were consistent with the reported LC-MS/MS limits of quantification for ambroxol [23]. The percentage accuracy observed for the mean of back-calculated concentrations for three different calibration curves was within 102.24–104.14% interval, while the precision *RSD* values ranged from 3.80 to 3.32%.

The intraday assay precision and accuracy were estimated by analyzing six replicates at four QC levels, *i.e*., 0.5, 20, 100 and 200 ng/mL, while interday precision and accuracy were determined by measuring the QC samples on three different days. The acceptance criteria were *RSD* ≤ 20% for *LLOQ* and LQC and ≤ 15% for MQC and HQC. The mean intra- and inter-day precisions were from 0.21 to 14.9% *RSD* and 3.01–16.92% *RSD*, respectively. The mean intraday accuracy was from 96.3 to 119.8% and the corresponding value for inter-day was 97.5–109.7%. The assay values on both occasions, intra- and inter-day, were found to be within the accepted variable limits and the established method could be considered as a highly repeatable and reproducible.

The individual active drug substance and the commercial tablets were used to examine the specificity of the method. Specificity is the ability to assess indubitably the analyte in the presence of interference substances such as excipients, impurities and degradation products. The forced degradation of the tablets was induced under the stress conditions of the base (0.1 M NaOH/1 h), acid (0.1 M HCl/1 h), UV light (254 nm/1 h), high temperature (100 °C/4 h), oxidation (1.0% H_2O_2 at 40 °/1 h) and hydroxylation (60 °C/1 h). The interfering peaks at the retention times of ambroxol and IS were not observed in any of the evaluated samples. Also, to prove the absence of the cross interferences between MS/MS channels used for the ambroxol and IS monitoring, each of the compounds was separately injected at a high concentration (200 ng/ml) and monitored in a different channel. No cross-talk effect was observed.

The carryover effects were studied by the analysis of three replicates of the mobile phase, which was injected immediately after the sample at the concentration 1.5 times higher than HQC (300 ng/mL). The response of the mobile phase at the retention time of ambroxol has not exceeded 20% of the average responses of the analyte at *LLOQ* concentration level.

The stock standard solution stability test was evaluated at one diluted concentration (20 ng/mL, $n = 3$). The average peak area for the stored standard solution (5 h/ambient temperature) compared with the freshly prepared standard showed no significant statistical difference (not higher than 5%). The working standards solutions of ambroxol were stable for at least 20 days stored at the fridge temperature (4 °C).

The robustness of the method was evaluated after minor variations of critical chromatographic parameters, such as organic percentage of the mobile phase (±0.5% of methanol), flow rate (±0.05 mL/min) and column oven temperature (±5 °C). The three replicate injections were performed for each tested parameter, with one parameter changing at a time. The variability between the validated and the modified chromatographic conditions was within ±2%. Obtained results indicated no significant difference between the results and confirmed the robustness of the developed method, with insignificant variability in the retention times.

The ruggedness of the method was examined by comparing standard solutions and QC samples at low and high concentrations with two analysts on different days. The *RSD* values were less than 5%, which indicates the ruggedness of the proposed method with no statistically significant differences in measurement.

Dissolution test

Active substance dissolution rate tests (dissolution tests) are used to test the release rate of a drug from pharmaceutical formulations, according to the procedures regulated by appropriate pharmacopeias. The dissolutions are primarily carried out in quality control and pharmaceutical preparation stability processes, whereas recently they have become significant in determination of the *in-vitro–in-vivo* correlation of tested drugs. Dissolution tests are used to quantitatively connect *in vitro* drug dissolution rate results and pharmacokinetic parameters of drugs, in order to reduce the number of *in vivo* testing. Based on obtained *in vitro* results, mathematical models which describe kinetics and drug dissolution mechanism are set, quantitatively connected with pharmacokinetic parameters and pre-

dict the drug behaviour in the body. Active substance dissolution rate from a pharmaceutical form is the indicator of drug bioavailability in the body, as well as of other pharmacokinetic parameters. Release rate affects the absorption, the amount of drug which gets into the blood and the pharmacological effect.

In order to obtain accurate and reliable dissolution test results, it is necessary to conduct measurements using validated and sensitive analytical methods. This paper presents the application of the validated UHPLC-MS/MS method for the determination of the dissolution rate of ambroxol from tablets. The dissolution rate of ambroxol was tested *in vitro* using three dissolution media at different pH values. Experimental conditions were set in accordance with the guidelines of the Ph. Eur. with slight modifications due to technical limitations. Conditions which enabled the release and determination of ambroxol from tablets included 1000 ml of solution at pH values of 1.2, 4.5, 6.8 (sink conditions) and a temperature of 37.0±0.5 °C, a magnetic stirrer with magnet rotation speed of 50 rpm and sample filtration through 0.22 μm syringe-filter. At specific time intervals (1–360 min), the solutions were sampled, then diluted (1000 x) and injected three times in the UHPLC-MS/MS system. After the measurements and calculations, time dependent curves of the released drug were constructed, percent of the released drug (%) = $f(t)$. After sampling, the volumes of dissolution media were compensated with 0.5 mL of fresh solvent.

Three different media were applied for the simulation of the gastrointestinal tract under sink conditions: 0.1M HCl pH 1.2 for gastric environment, phosphate buffer pH 6.8 for small intestine environment and acetate buffer 4.5 for duodenum environment. The average concentrations of ambroxol and the percentage of released ambroxol from tablets with time intervals at pH 1.2, 4.5 and 6.8 are shown in Tables 1–3, respectively. The curves of ambroxol dissolution rate profiles at pH 1.2, 4.5 i 6.8 and a temperature of 37.0±0.5 °C are shown in Figure 2a–c, respectively.

Table 1. Dissolution data for ambroxol tablets at the pH 1.2, 37.0±0.5 °C; dilution factor: 500

Time, min	C_1[a] / ng mL^{-1}	C[b] / mg mL^{-1}	Released drug, %, at pH 1.2
1	11.87	5.93	20
3	18.67	9.34	31
5	24.69	12.34	41
10	42.70	21.35	71
15	46.89	23.44	78
30	51.10	25.55	85
60	51.78	25.89	86
120	58.15	29.07	97
240	63.48	31.74	106
360	63.66	31.83	106

[a]Concentration calculated from the equation: $Y = 0.0124X - 0.0190$; [b]concentration per a tablet

Table 2. Dissolution data for ambroxol tablets at the pH 4.5, 37.0±0.5 °C; dilution factor: 500

Time, min	C_1[a] / ng mL^{-1}	C[b] / mg mL^{-1}	Released drug, %, at pH 4.5
1	11.97	5.99	20
3	24.07	12.04	40
5	31.07	15.53	52
10	40.76	20.38	68
15	43.07	21.53	72
30	48.36	24.18	81
60	52.71	26.36	88
120	59.04	29.52	98
240	60.40	30.20	101
360	58.62	29.31	98

[a]Concentration calculated from the equation: $Y = 0.0124X - 0.0190$; [b]concentration per a tablet

Table 3. Dissolution data for ambroxol tablets at the pH 6.8, 37.0±0.5 °C; dilution factor: 500

Time, min	C_1[a] / ng mL^{-1}	C[b] / mg mL^{-1}	Released drug, %, at pH 6.8
1	16.20	8.10	27
3	23.03	11.51	38
5	31.37	15.69	52
10	42.37	21.19	71
15	45.99	23.00	77
30	50.51	25.26	84
60	54.88	27.44	91
120	61.30	30.65	102
240	63.10	31.55	105
360	65.32	32.66	109

[a]Concentration calculated from the equation: $Y = 0.0124X - 0.0190$; [b]concentration per a tablet

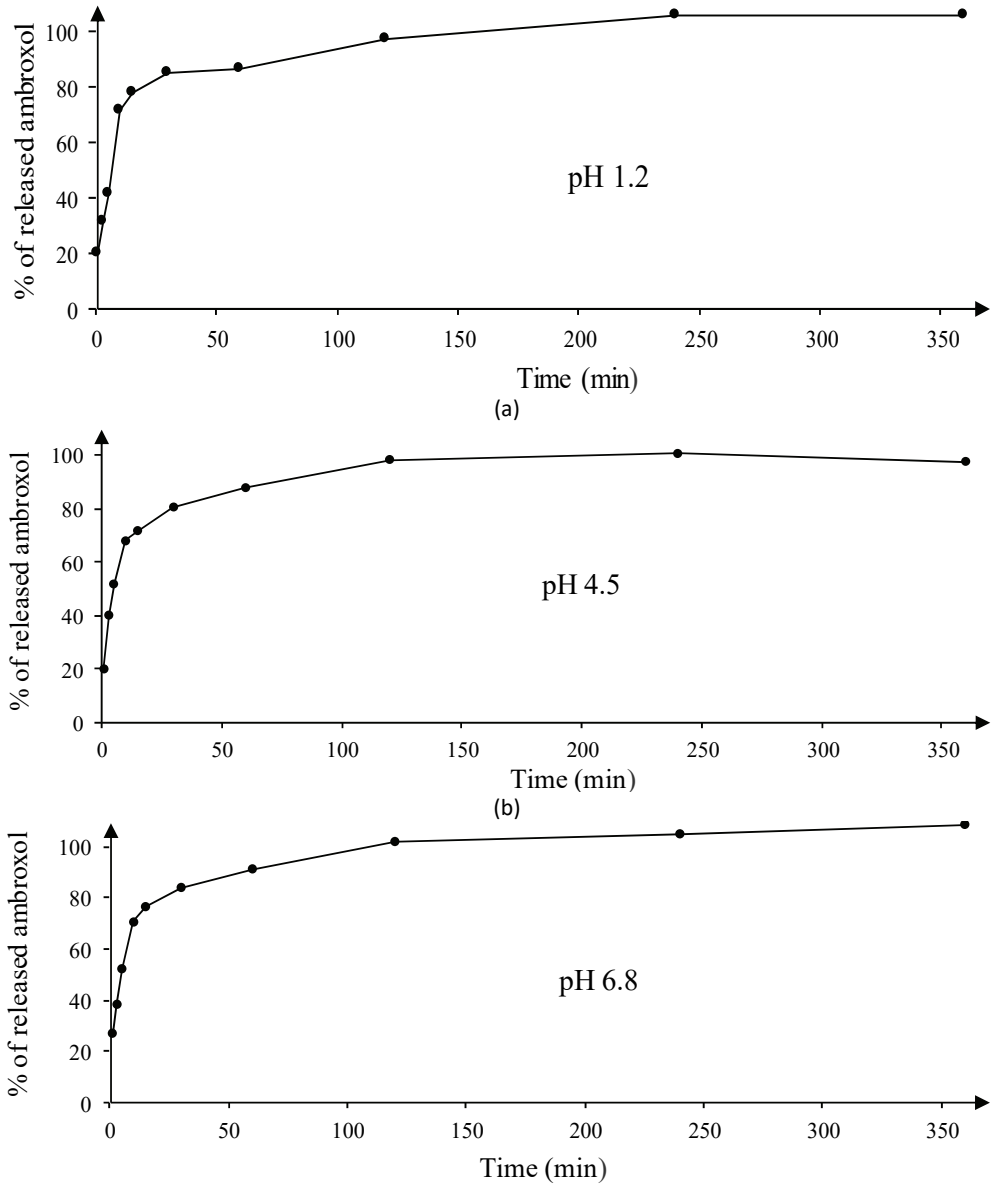

Figure 2. Dissolution profiles of ambroxol at: a) pH 1.2, b) 4.5 and c) pH 6.8 for the temperature of 37.0±0.5 °C.

The results of the dissolution test showed that the ambroxol dissolution rate from tablets is the highest at acidic pH 1.2, then at neutral pH 6.8, and slightly lower in mild acidic environment at pH 4.5. The percentage of released ambroxol compared to the initial amount of the drug in tablets after 30 min was 85% at pH 1.2, 81% at pH 4.5 and 84% at pH 6.8., which indicates that the maximum dissolution rate of ambroxol was in a medium that simulates the stomach, then slightly lower in the medium of the small intestine, and the lowest in the simulated duodenum medium. However, the differences in dissolution profiles at the three pH values were not regarded as significant. The developed and confirmed simple dissolutions test can be considered to be satisfactory with the ambroxol release percentage greater than 80% in 30–60 min from the beginning of dissolution under the simulated biological conditions. Based on the obtained results it can be concluded that the investigated active substance is released from tablets in a high percentage during a period of 30–60 min, under set conditions. Therefore, the formulation can be characterized as a drug with a fast, immediate release of the active pharmaceutical ingredient.

CONCLUSION

The proposed HPLC-MS/MS method for the determination of ambroxol hydrochloride has proved to be a rapid, specific, high-throughput, precise and accurate for analysis. The method was validated according to the ICH Q2A/Q2B guidelines and was found to be acceptable for all validation criterions. Through the validation process the significant improvements in the method were achieved; a chromatographic separation with a shorter run time (1.5 min) in 1.3 µm submicron column without the usage of buffer solutions, and a high sensitivity with a very small amount of injection volume (2 µL). The developed UHPLC-MS/MS method was successfully implemented in the evaluation of the release rate of ambroxol from tablets. Therefore, it is considered that the method can find a useful application in the pharmaceutical industry for the routine quality control of drugs as well as in the development process for product release and stability testing of new pharmaceutical formulations.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project 175045).

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IZVOD

IN VITRO DISOLUCIONI TEST MUKOLITIKA AMBROKSOL HIDROHLORIDA IZ ČVRSTIH FARMACEUTSKIH FORMULACIJA PRIMENOM UHPLC-MS/MS METODE

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(Naučni rad)

Testovi brzine rastvaranja (disolucioni testovi) koriste se za ispitivanje brzine oslobađanja lekovite supstance iz čvrstih farmaceutskih formulacija prema uputstvima relevantnih farmakopeja. Primarno se izvode u procesima kontrole kvaliteta i stabilnosti farmaceutskih preparata, ali poslednjih godina disolucioni testovi dobijaju sve veći značaj zbog mogućnosti uspostavljanja *in vitro-in vivo* korelacije karakteristika ispitivanih lekovitih supstanci. Ovim testiranjima se kvantitativno povezuju *in vitro* rezultati brzine rastvaranja sa farmakokinetičkim parametrima leka kako bi se manjio broj *in vivo* ispitivanja. Na osnovu dobijenih *in vitro* rezultata postavljaju se matematički modeli koji opisuju kinetiku i mehanizam rastvaranja lekovite supstance, kvantitativno povezuju sa farmakokinetičkim parametrima i predviđaju ponašanje leka u organizmu. Brzina oslobađanja aktivne supstance iz farmaceutskog oblika značajan je pokazatelj bioraspoloživosti leka u organizmu kao i drugih farmakokinetičkih parametara. Brzina oslobađanja utiče na resorpciju leka i količinu koja dospeva u krv odnosno na mesto farmakološkog dejstva. Za dobijanje tačnih i pouzdanih rezultata disolucionih testova, merenja je neophodno vršiti validiranim i osetljivim analitičkim metodama. U ovom radu prikazan je jednostavan test brzine rastvaranja ambroksola iz tableta prema uputstvima evropske farmakopeje. U *In vitro* uslovima, tri različita disoluciona medijuma; 0,1 M HCl pH 1,2, acetatni pufer pH 4,5 i fosfatni pufer pH 6,8 na temperaturi od 37,0±0,5 °C su primenjena za simulaciju gastrointestinog trakta. Evaluacija disolucionih profila ambroksola izvršena je primenom metode ultra-brze tečne hromatografije (UHPLC) sa tandem masenim detektorom (MS/MS). Disolucioni profili ambroksola pri različitim pH vrednostima nisu pokazali značajne razlike. Procenat oslobađenog ambrosola iz tableta bio je veći od 80% nakon 30 min u sva tri medijuma. UHPLC-MS/MS metoda je validirana prema ICH smernicama, koje uključuju ispitivanje linearnosti, specifičnosti, preciznosti, tačnosti i robusnosti metode. Hromatografsko razdvajanje analita izvršeno je na reverzno-faznoj 1.3µm Kinetex C-18 (2,1 mm×50 mm) koloni sa mobilnom fazom metanol–0,01% mravlja kiselina u vodi (yapreminski odnos 90:10) pri brzini protoka od 0,3 mL/min. ESI (+) jonizacija u multi-reakcionom monitoring (MRM) režimu je primenjena za MS/MS detekciju. Primenjene jonske tranzicije bile su: *m*/*z* 378,9→263,8 za ambroksol i *m*/*z* 455,2→165,0 za IS. Postavljena UHPLC--MS/MS metoda se pokazala linearnom u opsegu od 0,500–200,0 ng/mL sa limitom detekcije (*LOD*) od 0,25 ng/mL i donjim limitom kvantifikacije (*LLOQ*) 0,5 ng/mL. Intra- i interdnevna preciznost metode bila je <15%, a tačnost u opsegu od 90–119%. Vrednost *carryover* efekta bila je <2% pri koncentraciji 1,5 puta većoj od HQC (300 ng/mL). Nadalje, razvijena i validirana HPLC-MS/MS metoda smatra se da bi mogla naći korisnu primjenu u procesu proizvodnje, kontrole kvalitete gotovih lekova kao i studijama bioraspoloživosti/bioekvivalence drugih farmaceutskih formulacija ambroksola sa ciljem postizanja sigurne terapijske efikasnosti leka.

Ključne reči: Ambroksol • Disolucioni test • UHPLC–MS/MS