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Short communication

HPLC method with UV detection for evaluation of digoxin tablet dissolution in acidic medium after solid-phase extraction

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Abstract

A simple and reliable method for the evaluation of dissolution of digoxin tablets in 0.01 M hydrochloric acid was developed. Digoxin and its degradation products after solid-phase extraction using C18 Sep-Pak cartridges were evaluated. Analyses were performed on C18 column (LiChrospher RP-18e, 5 μm, 125 × 4.0 mm), as mobile phase water and acetonitrile (72:28, v/v) were used. Detection wavelength was 218 nm. Identity of digoxin degradation products was confirmed by HPLC-MS.

Keywords: Digoxin; Degradation products; HPLC; Dissolution; UV; Extraction; Solid-phase

1. Introduction

Hydrolysis of digoxin in acidic medium is the only known degradation route of digoxin. It has been found that in water no apparent hydrolysis of digoxin took place during dissolution testing using BP procedures, but significant hydrolysis occurred during the USP tests, owing to the acidic pH of the medium. 0.1 M HCl is used as dissolution medium in case of USP method and water in case of BP method. Nevertheless, hydrolysis is not a serious problem in digoxin commercial pharmaceutical formulations at room temperature. Digoxin is very stable in most dosage forms when kept in the dark and in well-closed containers [1]. Degradation products of acidic-catalyzed hydrolysis are digoxigenin–bisdigitoxoside, digoxigenin–monodigitoxoside and digoxigenin. Degradation scheme of digoxin is shown in Fig. 1. Due to the low content of digoxin in tablets it was necessary to evaluate tablet dissolution using fluorescence detection. These methods are described in detail in US and British Pharmacopoeias [2,3]. Other works were published about digoxin tablet dissolutions in acidic medium using fluorescence detection [4,5].

In Pharmacopeial Forum [6] a method with UV detection for digoxin tablet dissolution was introduced, but water is the dissolution medium in this case and, therefore, only digoxin is evaluated.

It is appropriate to mention other works, which deal with digoxin determination [7–12] and are

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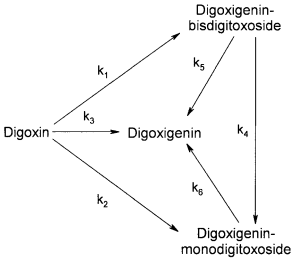

Fig. 1. Degradation scheme of digoxin in acidic medium.

suitable for setting of starting conditions in method development.

The experimentation took place in R&D Analytical Department of Léčiva a.s. Company. Léčiva a.s. is the leader among drug manufacturers in central Europe. Digoxin tables were originally tested in Léčiva a.s. according to USP method, but evaluation by this method was lengthy and laborious. Therefore, it was decided to avoid fluorescence detection from the evaluation of dissolution and to develop a new, quick and robust method.

Authors propose analytical method for the evaluation of dissolution in acidic medium with UV detection in this article.

2. Experimental

2.1. Apparatus

HPLC separations for method development and validation were carried out on a system Hewlett Packard series 1100 equipped with variable wavelength detector (HP GmbH, Waldbronn, Germany). Data acquisition and system control provided Chemstation software revision A.08.03 from the same company. Dissolution tests were carried out using dissolution bath Sotax (Sotax Ltd Basel, Allschwil, Switzerland). HPLC column for the standard method was LiChrospher RP-18e, 5 μm, 125 × 4.0 mm purchased from Merck (Darmstadt, Germany). During HPLC method optimization also following columns were used: LiChrospher CN, 5 μm, 125 × 4.0 mm and LiChrospher RP-select B, 5 μm, 125 × 4.0 mm (Merck) and column Grom-sil 80 amino-1 PR, 5 μm, 100 × 4.6 mm (GromAnalytik + HPLC GmbH, Herrenberg, Germany).

Solid phase extractions were performed with Sep-Pak C18 cartridges obtained from Waters (Milford, MA, USA). Dissolution medium was filtered using glass microfibre filters Whatman 25 mm (Whatman International Ltd, Maidstone, UK).

Other used apparatuses were analytical scale Sartorius BP 211D with precision 0.01 mg (Sartalex, Ústí nad Labem, Czech republic), pH-meter Metrohm 691 (Metrohm, Herisau, Switzerland) and ultrasonic bath with timer (Notus-Powersonic, Vráble, Slovakia).

2.2. Reagents and reference solutions

HPLC-grade acetonitrile and HPLC-grade methanol were from Merck. 37% hydrochloric acid, 85% phosphoric acid and potassium dihydrogenphosphate were from the same manufacturer and were of analytical grade. Potassium hydroxide of analytical grade was purchased from Lachema (Brno, Czech republic). Source of purified water for HPLC separations was Milli-Q water system (Millipore S.A., Molsheim, France). As standard was used in-house reference substance digoxin (assay calculated on dried substance: 99.50%, loss on drying: 0.04%).

Pharmaceutical preparation Digoxin 0.250 tablets (Léčiva a.s., Prague, Czech republic) was used for validation. One tablet weighted 140 mg and contained 0.250 mg of digoxin. Placebo was a mixture of lactose monohydrate, maize starch, gelatin, calcium stearate and polysorbate 80. For method validation were used 1-year-old stability tablet samples. Storage of these tablets was (from the manufacturing date) at conditions corresponding to climatic zone II, i.e. at 25 °C and 60% of relative humidity.

Stock solution of digoxin was prepared as follows: 25.0 mg of reference substance digoxin was dissolved in methanol and diluted to 50.0 ml with the same solvent. 5.0 ml of this solution was diluted to 50.0 ml with 40% methanol (methanol and water, 4:6, v/v).

Reference solution of digoxin was prepared as follows: 3.0 ml of the stock solution of digoxin was diluted to 50.0 ml with 40% methanol (3 μg/ml).

The solutions for verifying linearity (within concentration range from 60 to 3600 ng/ml) were prepared by diluting the stock solution of digoxin (0.05 mg/ml) with 40% methanol.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Different types of columns were tested at first to find the most suitable ones for separation. The separation between digoxin and degradation products, peak shape and selectivity against placebo were set as the most important criteria. Initial mobile phase was a mixture of water and acetonitrile (72:28, v/v) and was based on the work by Fujii, Ikeda and Yamazaki [7]. Different types of commercial columns LiChrospher namely RP-18e, RP-select B, CN and also column Grom-sil 80 amino-1 PR were used for testing.

The column LiChrospher RP-18e appeared as optimal for separation. The separation on column LiChrospher RP-select B was similar, but the efficiency was not as good as in the case mentioned above. Columns LiChrospher CN and Grom-sil 80 amino-1 PR appeared as unsuitable for separation, due to partial, respectively, total disability to separate digoxin degradation products.

After choosing the C18 column as optimal, the influence of other chromatographic conditions on separation was investigated. As mobile phases tested were used different mixtures of water and acetonitrile (starting from the ratio 80:20, v/v to ratio 60:40, v/v). Then usage of phosphate buffers (0.05 M KH_2PO_4 adjusted to pH 4.0 with phosphoric acid and 0.05 M KH_2PO_4 adjusted to pH 6.0 with 1 M potassium hydroxide) instead of water and subsequent influence on separation were investigated. Also influence of flow rate and influence of column temperature within the range 25–60 °C were tested.

There were found following experiences after testing of different chromatographic conditions:

- Increasing temperature of column caused improvement of separation of compounds.
- Increasing amount of acetonitrile in mobile phase caused a shortening of retention times and deterioration of the separation of compounds, especially separation between digoxigenin and digoxigenin-monodigitoxoside.
- Decreasing amount of acetonitrile in mobile phase highly extended time of analysis.
- Change of flow rate had an effect rather on time of analysis than on separation of compounds.
- Usage of buffered mobile phase had little or no effect on separation of compounds.

Final (optimized) chromatographic conditions were followed—column used: LiChrospher RP-18e, 5 μm, 125 × 4.0 mm, mobile phase: water and acetonitrile (72:28, v/v), injection volume: 100 μl, temperature of column: 50 °C, flow rate: 1.1 ml/min, detection wavelength: 218 nm, time of analysis: 8 min.

To ensure the highest possible concentration of digoxin in dissolution medium, the least permitted volume of medium, i.e. 500 ml for the dissolution test, was selected. From source [1] and from our results we decided on 0.01 M hydrochloric acid as dissolution medium. pH of this medium is approximately 2.0.

When dissolution medium is treated immediately after dissolution, digoxin is not completely degraded under chosen conditions. It is not essential to work dissolution medium up immediately, nevertheless authors recommend to work dissolution medium up to 30 min after dissolution. It was proved (see validation), that later sample treatment does not affect accuracy of the results. Half-life of digoxin under chosen conditions is approximately 130 min.

Final (optimized) dissolution conditions were followed—volume of dissolution medium: 500 ml of 0.01 M hydrochloric acid, baskets, rotation speed: 120 rpm, temperature: 37 ± 0.5 °C, dissolution time: 30 min. The dissolution medium was filtered through glass microfibre filters Whatmann and first 10 ml was removed.

For solid phase extraction Sep-Pak C18 cartridges were taken. There was chosen the concentration ratio 30:5 ml (ratio of volume of flushed

dissolution medium through the cartridge: volume of sample solution) for sample preparation. Thus, concentration of digoxin and its degradation products was increased six times, which is sufficient digoxin and its degradation products to be evaluated. The final sample solvent had to be selected with respect to the chosen mobile phase. As it is generally known, methanol has in reversed-phase chromatography lower elution strength than acetonitrile and, therefore, chosen 40% methanol as sample solvent could be used without adverse effect on the peak shape, when mobile phase was 28% acetonitrile. From this reason solid phase extraction was optimized using methanol as elution solvent.

Repeated elution of digoxin from the cartridge by 1.0 ml of methanol appeared to be better than one-step elution by 2.0 ml of methanol. In case of repeated elution, digoxin and its degradation products were eluted quantitatively. 40% methanol as a final sample solvent did not deteriorate the shape of the peaks.

Final (optimized) sample preparation using Sep-Pak C18 cartridges is described in following steps:

- Activation of the cartridge by 5 ml of methanol.
- Washing the cartridge with 5 ml of water.
- Flushing of 30.0 ml of dissolution media through the cartridge.
- Washing the cartridge with 5 ml of water.
- Short drying of the cartridge by vacuum.
- Elution of digoxin from the cartridge twice by 1.0 ml of methanol into 5 ml volumetric flask.
- Flushing of 2.0 ml of water through the cartridge into 5 ml volumetric flask.
- Filling up the flask to the mark with water.

Amount of dissolved digoxin in % was calculated according to the formula stated in precision part of validation (Section 3.2).

3.2. Validation

Information needed for method validation was gathered from sources [13,14]. Following method parameters were validated and are discussed subsequently: selectivity, accuracy, repeatability, linearity and robustness.

3.2.1. Selectivity of the method

The placebo used does not interfere with digoxin and its degradation products. Chromatograms of placebo and sample solutions, see Figs. 2 and 3.

3.2.2. System suitability

Two criteria for system suitability test were set:

- Resolution between digoxigenin-monodigitoxoside and digoxigenin should be at least two.
- Relative standard deviation (R.S.D.) of digoxin peak areas for five replicated injections of digoxin reference solution should not exceed 1.5%.

Usual values of resolution during method validation were about three, usual values of R.S.D. up to 0.1%.

3.2.3. Accuracy of the method

Accuracy was tested as recovery of digoxin at levels 50, 80, 100 and 120% of digoxin declared content, with 100% content of placebo. Placebo (140 mg) and appropriate amount of digoxin, i.e. 0.125 or 0.200 or 0.250 or 0.300 mg, were added into dissolution vessels filled with 500 ml of dissolution medium (three replicates for one concentration level) and dissolution continued for 30 min. Additions of digoxin to dissolution medium were realized as additions of the stock solution of digoxin. Additions were 2.5 or 4.0 or 5.0 or 6.0 ml in dependency on digoxin recovery level tested. After dissolution, dissolution media were filtered and treated as it was written in the text above.

Recovery of digoxin (x) in % was calculated according formula:

$$x = \frac{(A_{DG} + A_{DM} + A_{DB} + A_{DI}) \times c_R \times 100}{A_R \times c_S \times 6} \quad [\%]$$

where, $A_{DG}+A_{DM}+A_{DB}+A_{DI}$ is sum of peak areas of digoxigenin, digoxigenin-monodigitoxoside, digoxigenin-bisdigitoxoside and digoxin in the chromatogram of sample solution. A_R is peak area of digoxin in the chromatogram of reference solution. c_R is concentration of digoxin in the reference solution of digoxin in μg/ml. c_S is concentration of digoxin in the dissolution vessel,

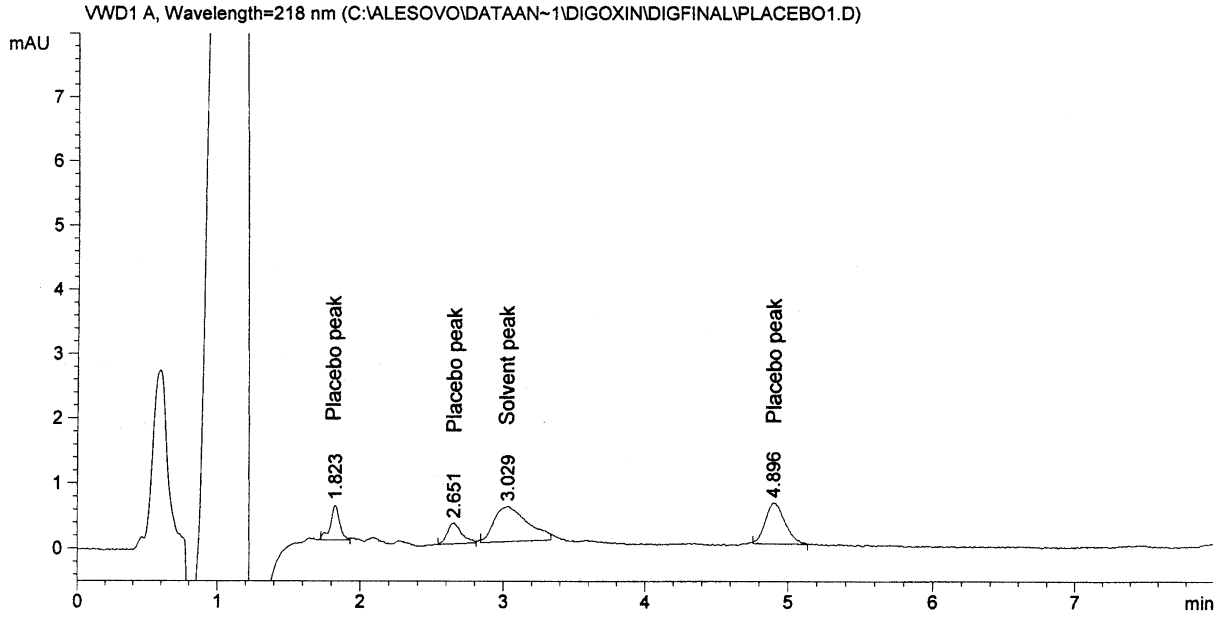

Fig. 2. Chromatogram of placebo solution. Analysis conditions: used column, LiChrospher RP-18e 5 μm (125 × 4.0 mm); mobile phase, water and acetonitrile (72:28, v/v); injection volume, 100 μl; temperature of column, 50 °C; flow rate, 1.1 ml/min; detection wavelength, 218 nm.

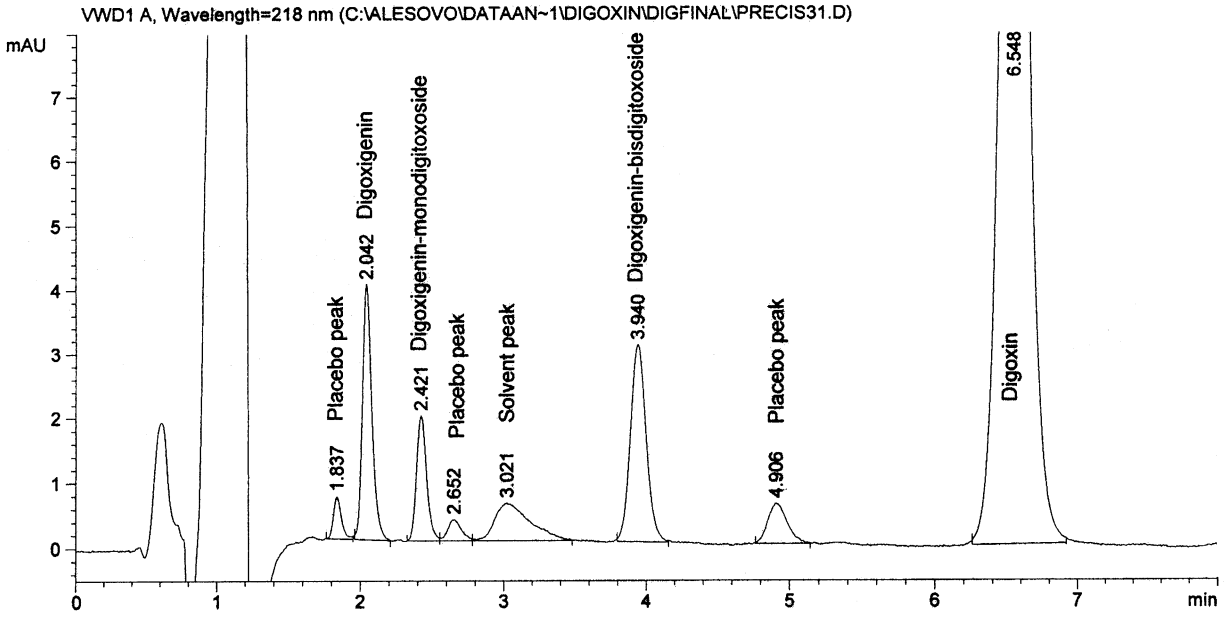

Fig. 3. Chromatogram of sample solution. Analysis conditions: used column, LiChrospher RP-18e 5 μm (125 × 4.0 mm); mobile phase, water and acetonitrile (72:28, v/v); injection volume, 100 μl; temperature of column, 50 °C; flow rate, 1.1 ml/min; detection wavelength, 218 nm.

after addition of the stock solution of digoxin, in μg/ml in time = 0 min.

c_S in μg/ml was calculated according to the formula:

$$c_S = \frac{V_{ST} \times c_{ST}}{(V_{DM} + V_{ST})}$$

where, V_{ST} is volume of the stock solution of digoxin added into the dissolution vessel in ml. c_{ST} is concentration of digoxin in the stock solution of digoxin in μg/ml. V_{DM} is volume of dissolution medium in ml ($V_{DM} = 500$ ml).

Recoveries of digoxin calculated by this formula ranged from 99.5 to 101.4%. 95% confidence intervals for $n = 3$ (each level) were found:

- For 50% recovery level tested: $100.51 \pm 0.71\%$;
- For 80% recovery level tested: $99.98 \pm 0.55\%$;
- For 100% recovery level tested: $100.84 \pm 0.91\%$;
- For 120% recovery level tested: $100.95 \pm 0.15\%$.

It was also confirmed, that for samples prepared from the filtered dissolution medium with different delay, the sum of peak areas of evaluated compounds is always the same. This predication is based on results of measuring described in the following text: dissolution medium was treated immediately after dissolution and then with 30 min intervals up to total time 240 min and analyzed. Amount of degradation products increased at the expense of digoxin, but the sum of peak areas of evaluated compounds was time-unchanging.

Identity of digoxin degradation products was confirmed by HPLC-MS.

3.2.4. Repeatability

Repeatability was carried out as follows: after 30 min of dissolution (one tablet/500 ml of 0.01 M HCl) the dissolution medium was six times taken away from one dissolution vessel, filtered and treated as it was written in the text above. Amounts of dissolved digoxin (x) in % were for repeatability test, as well as for routine dissolutions, calculated according formula:

$$x = \frac{(A_{DG} + A_{DM} + A_{DB} + A_{DI}) \times c_R \times 100}{A_R \times 3} \quad [\%]$$

where, $A_{DG} + A_{DM} + A_{DB} + A_{DI}$ is sum of peak areas of digoxigenin, digoxigenin-monodigitoxoside, digoxigenin-bisdigitoxoside and digoxin in the chromatogram of sample solution. A_R is peak area of digoxin in the chromatogram of reference solution. c_R is concentration of digoxin in the reference solution of digoxin in μg/ml.

The found value of repeatability was 0.44%. 95% confidence interval was found $96.23 \pm 0.34\%$, ($n = 6$). The nominal concentration of digoxin (calculated as sum of concentrations of digoxin and its degradations products) in the sample solution was 3 μg/ml.

Ratio among peak areas of digoxigenin, digoxigenin–monodigitoxoside, digoxigenin–bisdigitoxoside and digoxin after 30 min dissolution (dissolution medium treated immediately) was 6:3:8:83.

3.2.5. Linearity of digoxin

The linearity of digoxin was verified within the range 2–120% of reference solution, which corresponds to concentrations 60–3600 ng/ml. Correlation factor r was found 0.99998 in this range.

3.2.6. Robustness of the chromatographic method and stability of solutions

The change of the ratio of water and acetonitrile in mobile phase has the most important effect on separation and time of analysis. Changes of other chromatographic conditions have little or no effect on the separation. A description about behavior of individual separated compounds, after small changes of chromatographic conditions, expresses Table 1.

The stability of reference and sample solutions was also tested. These solutions appeared stable for 24 h after preparation at laboratory temperature. This information also includes the fact, that not even the ratio among degradation products and digoxin in the sample solution changed.

4. Conclusion

The simple and reliable method was developed for the evaluation of dissolution of digoxin tablets in 0.01 M hydrochloric acid. Due to incorporation of solid-phase extraction step, it is possible, simply

Table 1
Influence of small changes in chromatographic conditions on retention times and resolution between separated compounds (comparison with optimized conditions)

Changed condition	Retention time				Resolution		
	DG	DM	DB	DI	DG/DM	DM/DB	DB/DI
Flow rate							
0.9 ml/min	2.5	3.0	4.9	8.2	3.4	9.9	10.5
Optimized flow rate	2.1	2.4	4.0	6.6	3.2	9.5	10.1
1.3 ml/min	1.7	2.1	3.4	5.6	3.1	9.1	9.7
Temperature							
45 °C	2.1	2.4	3.9	6.4	3.0	9.0	9.5
Optimized temperature	2.1	2.4	4.0	6.6	3.2	9.5	10.1
55 °C	2.0	2.4	4.0	6.8	3.1	9.1	10.4
Amount of ACN in MF							
25%	2.5	3.3	6.4	12.6	4.5	12.0	13.1
Optimized amount of ACN	2.1	2.4	4.0	6.6	3.2	9.5	10.1
31%	1.8	2.0	2.8	4.1	1.9	6.2	7.1
Other type of column							
LiChrospher RP-select B, 5 μm, 125 × 4.0 mm	2.6	3.0	4.5	6.6	2.6	7.0	7.1

Explanatory text: DG, digoxigenin; DM, digoxigenin–monodigitoxoside; DB, digoxigenin–bisdigitoxoside; DI, digoxin; ACN, acetonitrile; MF, mobile phase.

and exactly, to evaluate digoxin in dissolution medium without the need of fluorescence detection. Acquired validation parameters indicate that this method is selective, accurate, precise, linear, robust and hence suitable for routine evaluation of digoxin tablet dissolution.

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