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ANALYSIS

COMPARATIVE ANALYSIS OF SELECTED β-BLOCKERS

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Abstract: The suitability and effectiveness of a few spectrophotometric and chromatographic methods (UV, FT-IR, MS,TLC) for differentiating analysis of 6 β-blockers: acebutolol, alprenolol, atenolol, metoprolol, pindolol and propranolol have been tested.

Keywords: comparative analysis, β-blockers

The group of β-adrenolytic drugs was introduced into medical therapy in the 1960s. It is a numerous group of compounds of similar chemical structure (being aryloxyalkylaminopropanol derivatives) and similar physicochemical properties. The small differences in their chemical structure can decide about the pharmacological activity, side effects as well as specific physicochemical properties, e.g., lipophilicity, which is the greatest for the derivatives with no hydrophilic groups in the aryl fragments of the molecule, and which decreases in the following sequence: alprenolol > pindolol > metoprolol > acebutolol > atenolol (1). Differences in activity among the drugs from this group appear in cardioselectivity (greater affinity to β_1 than β_2 receptors), intrinsic sympathomimetic activity and stabilization of cell membranes in the cardiac muscle. Drugs from this group have been mainly applied for the treatment of mild or moderate hypertension, especially that accompanying angina pectoris (2, 3). Because of a wide range of applications, development of effective analytical methods for their fast detection and determination, separation and differential, is of vital importance.

So far, many analytical methods have been proposed for detection and determination of one or a few β-blockers, e.g., the methods based on UV, IR (4-11) or chromatographic methods - TLC, HPLC, LC (12-16). However, it is hard to come across a method that would permit differentiation between particular β-blockers.

The aim of this study was to develop fast and simple methods allowing identification and separation of 6 most popular β-blockers: acebutolol (N-(3-acetyl-4-[2-hydroxy-3-(isopropylamino)propoxy]-phenyl)butanamide hydrochloride), alprenolol (1-(o-allylphenoxy)-3-(isopropylamino)-2-propanol hydrochloride), atenolol (4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide), metoprolol (1-(isopropylamino)-3-[p-(β-methoxyethyl)phenoxy]-2-propanol -tartrate salt, pindolol (1-(1H-indol-4-yloxy)-3-(isopropylamino)-2-propanol) and propranolol (1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride) on the basis of spectroscopic (UV, FT-IR, MS) and chromatographic (TLC) methods. Also other methods such as the Karl Fischer method for determination of water content and Boetius technique for melting point determination were applied.

EXPERIMENTAL

Materials

The compounds studied were 6 β-blockers most often used in medical therapy. Characterisation of the material studied is given in Table 1.

Water content

Portions of about 0.0300 g of the compounds studied were weighted to the accuracy of 0.0001 g, and subjected to determination of water content in an automatic titrator METTLER TOLEDO DL 38 (17).

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Temperature measurement by the Boetius method

The melting point was measured by a heating table with a thermometer and optical microscope ensuring magnification from 60 to 100 times (the Boetius apparatus made by NAGEMA, Germany). The melting point can be determined to an accuracy of 0.50°C. The rate of sample heating was 50°C/min. The substance studied was placed between the microscope slides on the heating table at a spot allowing observations. The substance and the temperature indications were observed simultaneously (17).

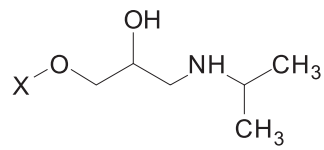
Ultraviolet spectrophotometry (UV)

Portions of 0.0100 g of the β-blockers studied were weighted to the accuracy of 0.0001 g, dissolved in the appropriate solvent (water, methanol, 0.1 mol/cm^3 HCl) in flasks of 10.0 cm^3 in capacity and then the solutions were diluted to get desired concentrations. The absorbance of the solutions was measured by a UV-VIS Perkin-Elmer Lambda 20 spectrometer in the range 200–400 nm against the reference sample which was the solvent used. The method was validated according to the ICH recommendations (18)

Infrared spectroscopy (FT-IR)

Portions of 1 mg of the compounds studied were weighted to the accuracy of 0.05 mg and ground in mortar with 300 mg KBr (preliminary dried at 600°C). After reaching a desired refinement state, tablets were made of the size 1.3 × 0.1 cm with the use of a tabletting machine PYE UNICAM. The

Table 1. Characterisation of examined pharmaceuticals.



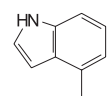
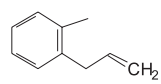
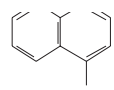
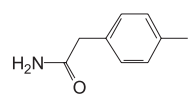
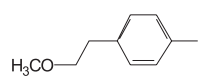
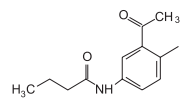
Compound	Molar mass [g/mol]	Symbol	Substituent X =	Purity [%]	Manufacturer Serial number
Pindolol	248.32	**PD**		≥ 98	Sigma-Aldrich Sp. z o.o. LOT: 068K1038
Alprenolol	249.34	**AL**		≥ 98	Sigma-Aldrich Sp. z o.o. LOT: 068H0478
Propranolol	259.34	**PR**		≥ 98	Sigma-Aldrich Sp. z o.o. LOT: S40526-347
Atenolol	266.34	**AT**		≥ 98	Sigma-Aldrich Sp. z o.o. LOT: 126K1463
Metoprolol	267.36	**MT**		≥ 98	Polpharma Starogard Gdański LOT: 1220109/
Acebutolol	336.42	**AC**		≥ 98	Sigma-Aldrich Sp. z o.o. LOT: 065K1622

Table 2. Water content and melting points determined for the β-blockers studied.

Parameter	Compound					
	AL	MT	AC	AT	PR	PD
Melting point [°C]	111-115	121-123	143-146	153-156	159-164	171-176
Color	white	white	white	white	white	white
Water content [%]	0.46	0.28	0.28	0.50	0.49	0.47

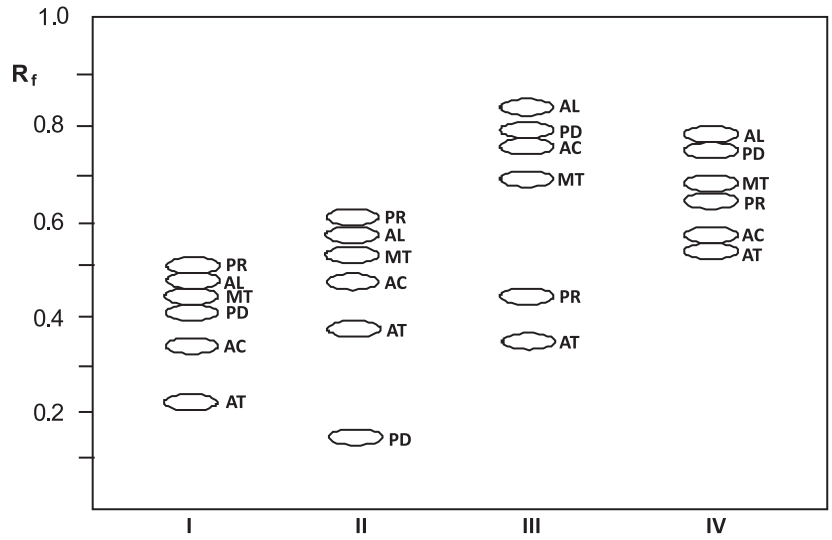


Figure 1. Schemes of chromatograms of β-blockers studied in selected mobile phases: **I**: chloroform - methanol - toluene - 25% ammonia (20 : 25 : 55 : 1, v/v/v/v), **II**: methanol - 25% ammonia (99 : 1, v/v), **III**: chloroform – methanol - 25% ammonia (75 : 35 :1, v/v/v), **IV**: ethyl acetate - methanol - 25% ammonia (6 : 40 : 3, v/v/v)

reference tablet was made of pure KBr. The FT-IR spectra were recorded in the range 4000-400 cm^{-1} on IRAffinity 1-Fourier Spectrophotometer - Shimadzu.

Mass spectrometry (MS)

Mass spectra of the compounds studied were recorded on an Intectra Mass AMD 604 spectrometer in standard conditions, with the electrons of 70 eV (at voltage of 70 V).

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) of the 6 β-blockers studied was performed on plates covered with silica gel Kieselgel F254 at the layer thickness of 0.25 mm and the size 20 × 20 cm. The plates were covered with 25 mL of methanol solutions of the compounds studied at the concentration 10 mg/cm^3, which corresponded to 0.25 mg of the compound studied. The plates were developed over 17 cm for 45 min to 1.5 h, at room temperature. The mobile phases used were: chloroform - methanol - toluene - 25% ammonia (20 : 25 : 55 : 1); methanol - 25% ammonia (99 : 1); chloroform – methanol - 25% ammonia (75 : 35 : 1); ethyl acetate - methanol - 25% ammonia (60 : 40 : 3) (all in volumentric ratios).

After drying the chromatograms, the spots were observed under the UV lamp radiation of 254 nm.

All reagents used were of analytical grade.

Table 3. R_f values determined for the β-blockers studied in selected mobile phases.

Mobile phase ↓ / Compound →		R_f					
		AC	**AL**	**AT**	**MT**	**PD**	**PR**
I	Chloroform - methanol - toluene - 25% ammonia (20 : 25 : 55 : 1, v/v/v/v)	0.35	0.48	0.21	0.46	0.41	0.49
II	Methanol - 25% ammonia (99 : 1, v/v/)	0.48	0.58	0.39	0.54	0.15	0.60
III	Chloroform - methanol - 25% ammonia (75 : 35 : 1, v/v/v)	0.73	0.82	0.33	0.67	0.75	0.41
IV	Ethyl acetate - methanol - 25% ammonia (6 : 40 : 3, v/v/v)	0.59	0.75	0.53	0.68	0.71	0.64

Table 4. Results of UV analysis of the β-blockers studied.

Compound	**CH_3OH**		**H_2O**		**0.1 mol/cm³ HCl**	
	λ_{max} [nm]	**Absorbance**	**λ_{max} [nm]**	**Absorbance**	**λ_{max} [nm]**	**Absorbance**
AC	234.5 320.9	2.3720 0.1502	234.5 320.9	2.3720 0.1502	233.5 320.9	1.4074 0.1715
AL	270.5	0.8058	270.5	0.7577	270.5	0.6471
AT	275.5	0.5610	274.5	0.4910	274.5	0.5053
MT	275.5	0.4752	274.5	0.4183	274.2	0.4410
PD	264.5	1.0911	-	-	264.4	1.0258
PR	291.2	0.6189	290.5	0.7183	289.5	0.6302

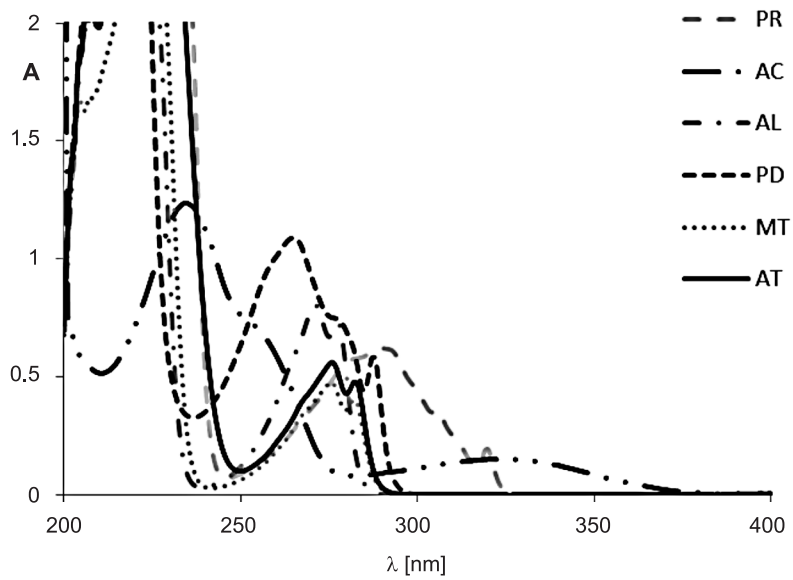


Figure 2. UV spectra of β-blockers studied (solvent: methanol)

Table 5. Validation parameters of the UV spectrophotometric method.

Validation parameter → / **Compound ↓**	**Correlation coefficient (r)**	**Precision W_z [%]**	**Accuracy (content) [%]**	**[%] $\times 10^{-3}$**	**Limit of detection LOD [%] $\times 10^{-4}$**	**Limit of determination LOQ [%] $\times 10^{-4}$**
AC	0.9998	1.16	91.35	1.0 – 13.0	2.45	7.44
AL	0.9998	0.54	98.45	2.0 – 22.0	4.21	0.27
AT	0.9999	0.43	99.74	2.0 – 22.0	3.13	9.45
MT	0.9997	1.05	99.06	1 – 25.0	0.12	0.38
PD	0.9993	1.16	98.45	0.5 – 4.5	1.85	5.61
PR	0.9997	0.44	99.6	0.8 – 6.0	5.49	0.16

Table 6. FT-IR results for the β-blockers studied.

Region of spectrum FT-IR [cm^{-1}]	Compound					
	AC	**AL**	**AT**	**MT**	**PD**	**PR**
600-900	615.87 728.89 802.80 816.02 890.22 903.76	654.15 765.76 844.01	674.12 710.59 796.24 815.14 885.94	704.50 808.30	626.54 721.67 734.71 758.98 820.31 883.70	770.55 797.23
1500-1750	1496.36 1526.13 1590.67 1611.43 1654.72 1663.16 1674.16	1577.64 1598.66 1636.69	1515.55 1583.61 1612.59 1635.35	1512.96 1593.92 1610.89	1508.41 1586.86 1616.95	1510.31 1510.31 1579.27 1596.30 1628.82
2400-3500	2733.55 2796.91 2945.31 2961.51 2973.32 3293.28	2531.80 2584.73 2711.90 2805.67 2831.08 2956.43 2986.48 3387.62	2867.92 2922.77 2964.80 3174.58 3355.64	2825.85 2869.25 2934.54 2979.19 3339.72	2864.17 2872.82 2924.49 2966.36 3129.92 3308.06	2490.60 2710.54 2806.68 2834.73 2964.35 3282.36 3322.22
The most intense bands	1496.36 1663.16 1674.16 3293.28	765.76 1248.66 1493.30 2986.48	1243.02 1516.55 1637.35 3355.64	1111.75 1512.96 1593.92 1610.98	758.98 1246.04 1366.31 2966.36	770.51 797.23 1107.13 1267.77

RESULTS AND DISCUSSION

The preliminary step towards realization of the main aim of the study was to compare the physicochemical properties of selected β-blockers belonging to the same chemical and pharmacological group that could be used for differentiation and identification of particular compounds. This task is very difficult as all compounds from this group have very similar physicochemical parameters as illustrated by the data from Table 2 (white color, similar melting point and water content, similar character of the spectra). It is a consequence of their similar chemical structure and implies that such classical methods

Table 7. MS results obtained for the β-blockers studied.

Compound	Molecular mass [Da]	Molecular ion m/z	Base peak m/z	Fragment ions m/z
AL.	249.34	250.0	72.0	57.0 77.0 90.9 105.0 116.9 130.9 205.1
AT	266.34	267.0	72.0	43.8 56.0 77.0 106.9 222.1
MT	267.36	268.0	72.0	56.1 107.0 223.1
PR	259.34	260.0	72.0	57.3 115.0 143.9 215.3
AC	336.42	337.1	43.0	41.0 56.0 72.0 151.0 221.1
PD	248.32	249.0	133.0	56.0 72.1 104.0 116.0 204.1

Table 8. Suitability of analytical methods in comparative analysis of β-blockers studied.

Compound	Analytical method				
	Temperature measurement by the Boetius method	Ultraviolet spectrophotometry (UV)	Infrared spectroscopy (FT-IR)	Mass spectrometry (MS)	Thin layer chromatography (TLC)
AC	+ -	+	+ -	+	+
AL	+	-	+ -	+ -	+
AT	+ -	-	+	+ -	+
MT	+ -	-	+ -	+ -	+
PD	+	+ -	+ -	+	+
PR	+ -	+ -	+ -	+ -	+

+ suitable method, + - moderately suitable, - unsuitable method

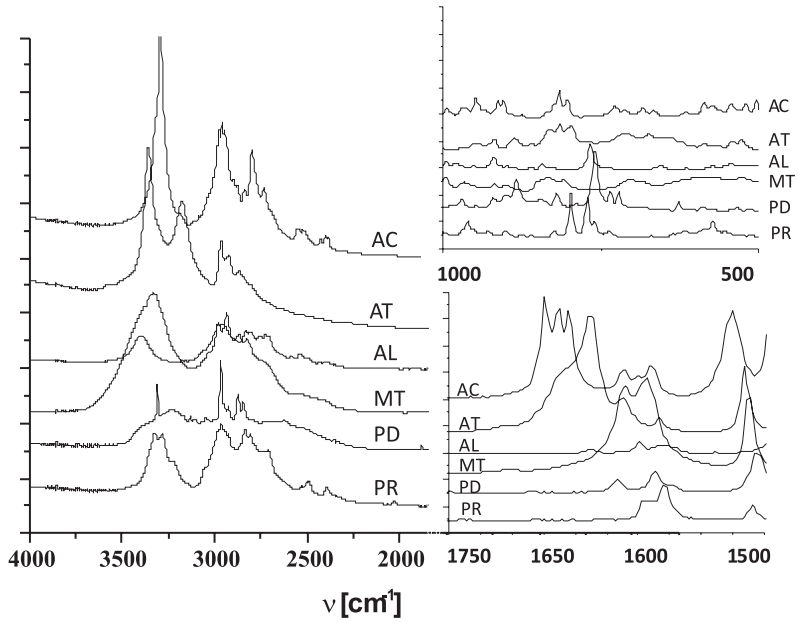


Figure 3. FT-IR spectra of β-blockers studied

of differentiation as chemical reactions will not be effective. From the parameters given in Table 2, only the melting point could be used for identification and sometimes also for differentiation of β-blockers. The lowest melting point (temperature of the beginning of the melting process) has been established for AL (111°C), while the highest PD (171°C), for the other compounds considered the melting point varies from 121 to 159°C. For all 6 compounds the melting process was completed within 4°C.

Literature provides the methods for identification and determination of particular compounds from the group of β-blockers, but to the best of our knowledge no method has been proposed that would allow detection and determination of the compounds from this group occurring together. We subjected to comparative analysis the following 6 most often applied β-blockers: acebutolol, alprenolol, atenolol, metoprolol, pindolol and propranolol. We decided to check if such instrumental methods as UV spectrophotometry, FT-IR spectrophotometry and mass spectrometry MS can be suitable for identification or determination (UV) of the compounds studied, and if the thin layer chromatography (TLC) can be used for their separation. The chromatographic phases applied were chosen on the basis of literature data (19, 20) or our own modifications. Finally, four systems of solvents were chosen, which confirmed the purity of all β-blockers analyzed, satisfied the optimization conditions (sharpness of spots, differences in R_f) and proved the best for the purpose (Table 3, Fig. 1). On the basis of preliminary tests, the best chromatographic system was chloroform - methanol - 25% ammonia (75 : 35 : 1, v/v/v), taking into account the differences in R_f and the distance from the start line. Using this chromatographic system the highest R_f was obtained for AL (0.82) while the lowest for AT (0.33). The results have shown that TLC is effective for differentiating analysis of the compounds studied and it not only allows identification of AC, Al, AT, MT, PD and PR but also permits their separation.

To verify the use of spectral methods (UV, FT-IR) for the above purpose we started from taking UV spectra of all 6 compounds in three solvents (water, methanol and 0.1 mL/cm^3 HCl). The greatest differences between the spectra were noted in methanol so it was selected for further studies. In the range 200–400 nm, the spectra show one or two (AC) absorption maxima (in the range 220-350 nm) of similar shape and similar λ_{max} values (Table 4., Fig. 2). The closest λ_{max} values were found for AT, MT and AL whose differentiation is the most difficult. PR can be easily determined in the presence of AC and PD, while AC is the only compound from the group of 6 β-blockers studied whose λ_{max} permits its differentiation from all other 5 β-blockers. The method was validated and its parameters are given in Table 5. The variation coefficient was low (from 0.43 for AT to 1.16 for AC and PD) and high percent recovery (91.35–99.74%), which means that the UV spectrophotometry is characterized by high precision and high accuracy and can be applied for quantitative analysis of the compounds of interest.

The FT-IR spectra provide much more information than UV ones and permit faster and easier identification and differentiation of particular β-blockers. The greatest number of differences are noted in the range 1500-1700 cm^{-1} and above 2000 cm^{-1} (Fig. 3). For AT, at the wavenumber 1636 cm^{-1} there is a band assigned to the stretching vibrations ν CO, while at 1577 cm^{-1} there is a band assigned to the deformation vibrations δ N-H. In the spectrum of AT at 3350 cm^{-1} there is a well-developed band attributed to the streching vibrations ν N-H, that can also be found in the spectrum of AC at 3330 cm^{-1}. In the same range the spectra of all 6 compounds studied show a broad band corresponding to the stretching vibrations ν O-H. Another range of the FT-IR spectra in which differences are found between the spectra of the 6 β-blockers analyzed is 600-900 cm^{-1}, which contains the bands assigned to the deformation vibrations δ C-H, N-H and O-H. Particular compounds can be identified on the basis of the number and intensities of these bands, reflecting the structural differences between the compounds studied (Table 6). Particular compounds from the group considered can be also identified on the basis of the most intense bands in their entire FT-IR spectra and comparison with the data published in relevant monographs (21).

Much information can also be obtained from comparison of the MS spectra of the 6 β-blockers. In their spectra there is the low-intense molecular ion (electron ionization) and fragmentation ions. Individual compounds can be identified on the basis of the m/z values of the molecular ion and the main ion. On the basis of comparative analysis of the main ion we are able to identify AC (m/z 43.0) and PD (m/z 133.0) and four of the other compounds (AL., AT, MT and PR) which have the main ion at m/z 72.0. Their identification is also possible on the basis of comparison of the molecular ions and fragmentary ions of higher intensities (Table 7). Suitabilities of particular methods for comparative analysis of β-blockers is characterized in Table 8.

CONCLUSIONS

As follows from the above analysis, all four methods applied: UV, FT-IR, MS and TLC permit a relatively fast and easy identification of the β-blockers studied. The chromatographic method (TLC) is most recommended of them because besides differentiation it also permits separation of these 6 compounds.

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