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High Speed Determination of β-Blockers in Human Urine by LC/MS/MS

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Introduction

Since the amount of different β-receptor blocking agents on the international market is enormous and still increases, sensitive and selective methods for the detection and identification of these compounds are required. The gas-chromatographic properties of some β-blockers are unfavourable even after derivatization with MSTFA[12] and/or MBTFA[3] and the EI mass spectra of the underivatized compounds are usually not very informative. Therefore, a fast and sensitive procedure for the analysis of more than 30 β-receptor blocking agents was developed using liquid chromatography coupled to a tandem mass spectrometer.

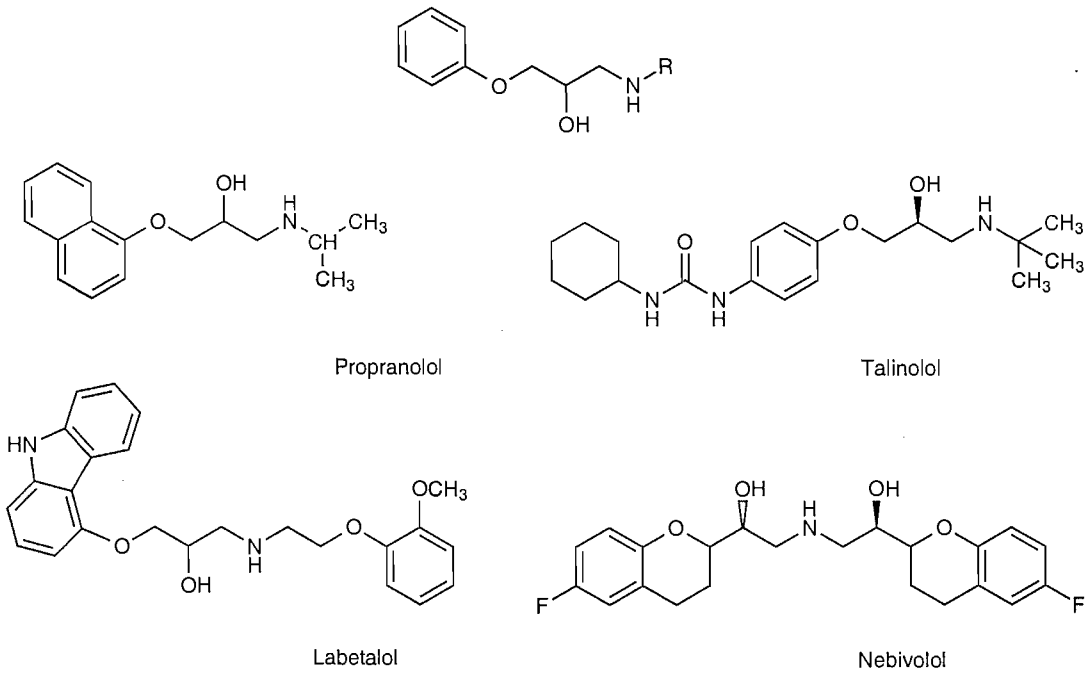

Figure 1: Structure formulas of propranolol, talinolol, nebivolol and labetalol compared to the general structure of group 1

The basic structure of β-blockers can be divided into two groups of which one consists of a phenolic ring structure carrying an oxypropanolamine side chain that terminates either in an isopropyl or tertiary butyl group. Examples for that are propranolol and talinolol and excemptions are nebivolol and labetalol (fig. 1). The second class containing sotalol and nifenalol consists of a substituted phenylethanolamine nucleus (fig. 2) and is considered to be the less potent group of drugs.

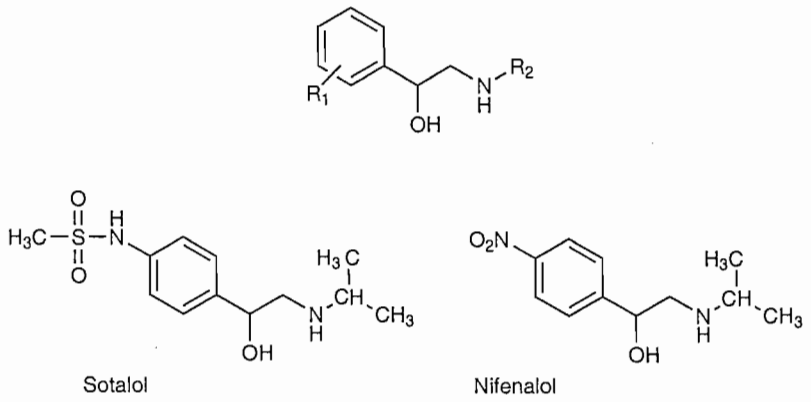

Figure 2: Structure formulas of sotalol and nifenalol compared to the general structure of group 2

The metabolism of β-blockers depends directly on their physiochemical properties as shown by Bourne[4]. The more lipophilic substances are extensively metabolized to produce more water-soluble derivatives by oxidation, hydroxylation or dealkylation following occasional conjugation to glucuronides or sulphates.

Experimental

Chemicals and reference substances

All compounds listed in table 1 were obtained commercially as from manufacturers as reference substances or medicals. Ammonium acetate (p.a.), sodium acetate (p.a.) sodium sulphate (anhydrous, p.a.), potassium carbonate (p.a.), sodium hydrogen carbonate (p.a.), *t*-butanol (p.a.) and *t*-butyl methyl ether were purchased from Merck (Darmstadt, Germany), β-glucuronidase/ arylsulphatase (from *Helix pomatia*) from Boehringer (Mannheim, Germany) and acetonitrile (HPLC grade) from Baker (Deventer, Netherlands).

Analytical parameters

The analyses were performed on a Hewlett Packard HP1100 liquid chromatograph coupled to a PE Sciex API2000 triple quadrupole mass spectrometer. The column used was a Purospher Star RP-18e (55 x 4 mm i.d., 3 µm particle size) from Merck. The LC and MS conditions were: mobile phase, A=ammonium acetate buffer (pH = 3.5, 4 mmol ammonium acetate, 1‰ glacial acetic acid in distilled water) / B = acetonitrile, flow rate: 1.0 ml/min with a post-column split (ratio 10:1), gradient: B: 0% -> 80% in 4 minutes, re-equilibration time: 3 minutes at 0%, injection volume 5 µl, interface and temperature: APCI 400°C, ionisation mode: positive, multiple reaction monitoring of quasi-molecular ions (M^++H) and specific secondary ions after collision induced dissociation of distinct substances (see table 1), dwell time: 50 msec., pause time 5 msec.

Sample preparation

To 5 ml of urine are added 500 ng of bupranolol as internal standard, 0.5 ml of a sodium acetate buffer (1M, pH=5.2) to adjust the pH to 5-5.5 and 50 µl of β-glucuronidase/ arylsulphatase from *Helix pomatia*. The sample is heated for 1 hour at 50 °C and after cooling to ambient temperature 500 mg of a mixture of $NaHCO_3$ and K_2CO_3 (2:1, w:w) are added to adjust the pH to 9.6. Further, 1 ml of tert.-butanol, 5 ml of tert.-butylmethyl ether and 1 g of sodium sulphate were added and the mixture was shaken for 15 minutes. After centrifugation the upper layer was transferred to a fresh test tube and evaporated to dryness. The residue was redissolved in 60 µl of acetonitrile of which 5 µl were injected into the LC/MS/MS system.

Results and discussion

The mass spectrometric behaviour of the selected β-blockers is comparable within the described groups. A common ion for those carrying an oxypropanolamine side chain terminating in an isopropyl group is m/z 116 (e.g. acebutolol, alprenolol, fig. 3, and propranolol) which mainly occurs in high intensities. Therefore, the target ion for multiple reaction monitoring is oftenly m/z 116 as shown in table 1.

Table 1: Compound specific parameters of mass spectrometry and detection limits

			selected common ions								
Compound	mol wt	target ion (m/z)	M^++H - 18	M^++H - 56	M^++H - 56 - 18	M^++H - 77	m/z 116	Individual ions (3 most intensive)			LOD (ng/ml)
Acebutolol	336	116	319	-	-	260	√	218	180	148	10
Alprenolol	249	116	232	-	-	173	√	145	131	91	10
Atenolol	266	145	-	-	-	190	√	145	107	91	50
Befunolol	291	116	274	-	-	215	-	250	203	177	10
Betaxolol	307	56	-	-	-	-	√	121	91	55	10
Bisoprolol	325	116	-	-	-	-	√	133	107	89	10
Bunitrolol	248	193	-	193	175	-	-	120	102	-	10
Bupranolol	271	216	-	216	-	-	-	155	125	91	ISTD
Butofilolol	311	256	-	256	238	-	-	221	209	109	10
Carazolol	298	116	-	-	-	222	√	194	184	139	10
Carteolol	292	237	-	237	-	-	-	202	164	122	10
Carvedilol	406	100	-	-	-	-	-	224	222	100	50
Celiprolol	379	251	-	324	306	-	-	307	251	100	100
Cloranolol	291	236	-	236	218	-	-	175	145	109	10
Esmolol	295	145	278	-	-	219	√	145	133	91	100
Indenolol	247	171	-	-	-	171	√	145	128	98	10
Labetalol	328	91	311	-	-	-	-	294	162	91	50
Levobunolol	291	236	-	236	-	-	-	201	145	91	10
Mepindolol	262	116	-	-	-	186	√	160	148	130	50
Metipranolol	309	116	-	-	-	233	√	191	165	135	10
Metoprolol	267	116	-	-	-	191	√	159	133	77	10
Moprolol	239	116	222	-	-	163	√	121	98	77	10
Nadolol	309	254	-	254	236	-	-	201	145	115	10
Nebivolol	405	151	-	-	-	-	-	151	123	103	10
Nifenalol	224	165	207	-	-	-	-	165	119	118	10
Oxprenolol	265	72	248	-	-	-	√	225	98	-	10
Penbutolol	291	236	-	236	-	-	-	168	133	105	10
Pindolol	248	116	-	-	-	172	√	146	144	134	10
Propranolol	259	116	-	-	-	183	√	157	155	127	10
Sotalol	272	133	255	-	-	-	-	213	133	106	50
Talinolol	363	308	-	308	-	-	-	226	209	100	10
Timolol	316	261	-	261	244	-	-	188	144	113	10
Toliprolol	223	147	-	-	-	147	-	121	119	91	10

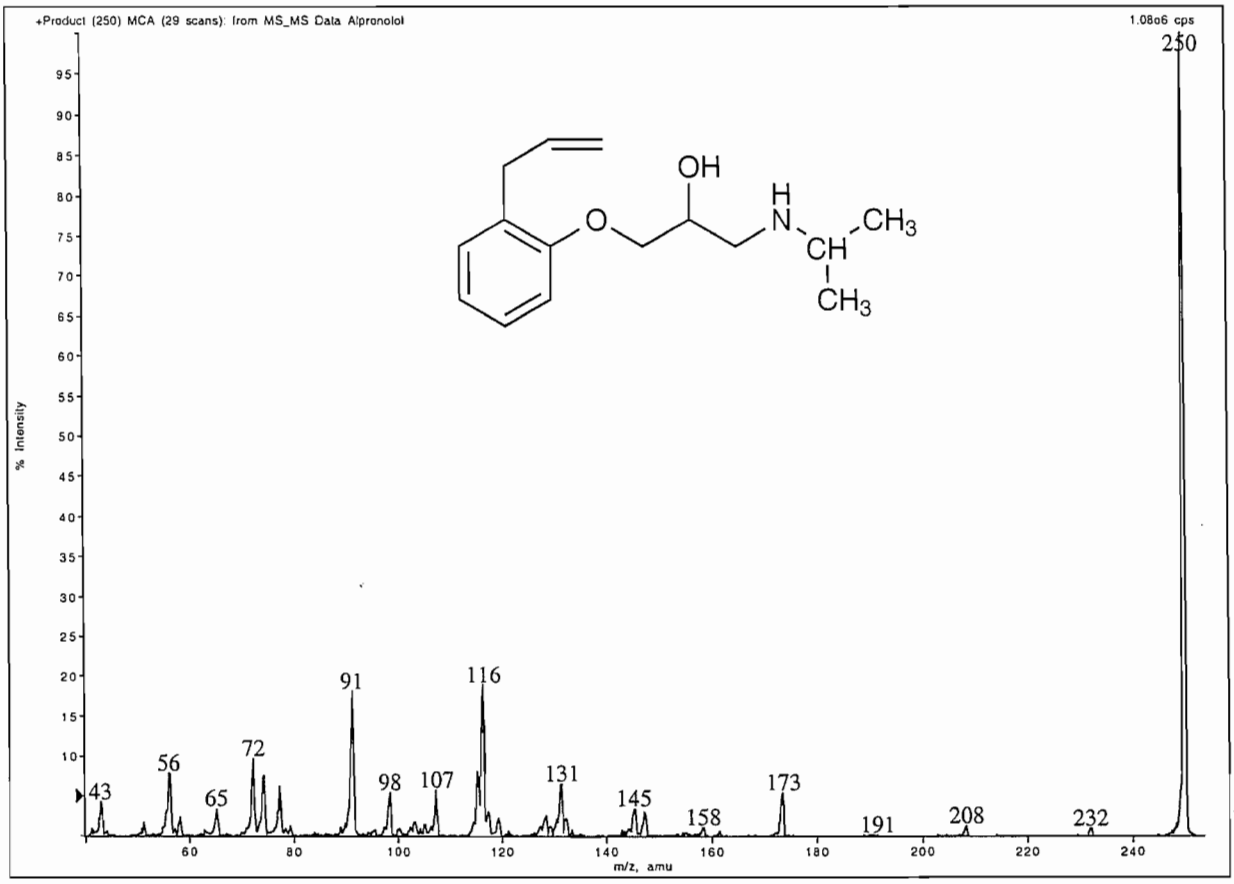

Figure 3: Product ion scan of the quasimolecular ion m/z 250 (M^++H) of alprenolol

The origin of m/z 116 is proposed to be initiated by the protonation of the ether oxygen of the side chain followed by the elimination of the phenolic nucleus. In addition, the fragment $(M+H)^+$ – 77 is found which may be generated by the loss of a water molecule and a subsequent elimination of isopropylamine as demonstrated in figure 4.[5] The more individually structured compounds like nebivolol or carvedilol, for example, showed a different fragmentation pattern and thus different product ions.

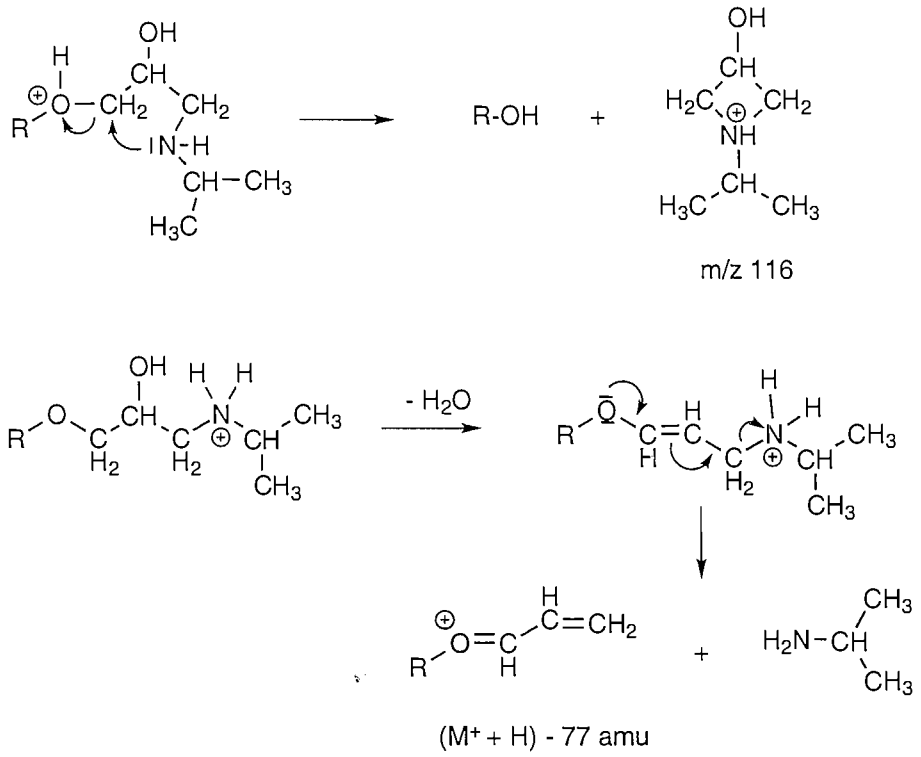

Figure 4: Proposed generation of m/z 116 and $(M+H)^+$ of β-blockers containing an isopropanolamine side chain terminating in an isopropyl group

The selectivity of multiple reaction monitoring and the use of a short and effective HPLC column enable the detection of more than 30 β-receptor blocking agents in a single run with detection limits of less than 100ng/ml each. Only two excemptions were observed with esmolol and celiprolol with were identified at 100ng/ml. In case of a positive screening result the confirmation may be performed with the same LC parameters and a product ion scan of the suspicious quasimolecular ion or by the additional detection of further product ions in a specified MRM experiment.

Acknowledgements

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