Analysis of beta-blocking drugs on Agilent 1100 Series LC systems using UV, fluorescence and mass spectrometry

Application

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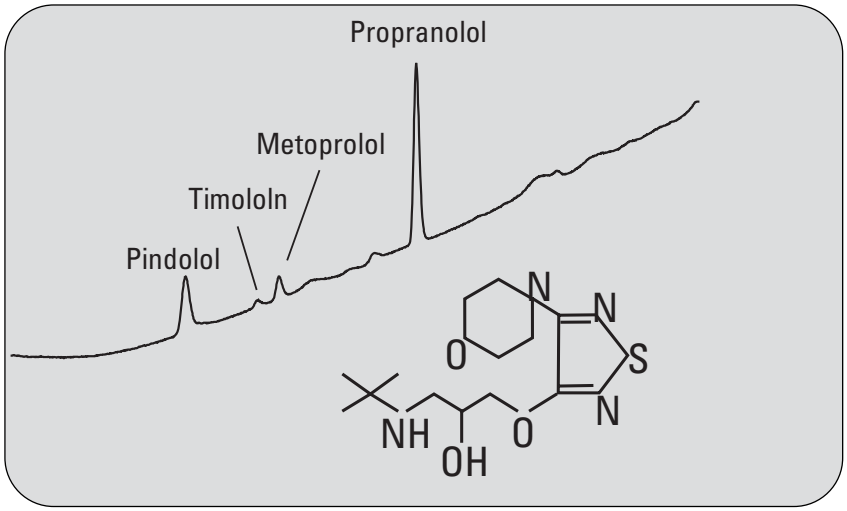

Abstract

This Application Note compares the detection techniques UV, fluorescence and mass spectrometry in combination with standard and capillary LC systems, and columns with different internal diameters. Advantages and disadvantages of the different detector/column/LC system combinations are discussed.

Agilent 1200 Series Verified

This application has been verified using an Agilent 1200 Series LC system, and showed comparable or even better performance.

Application Area
Using different detection systems in drug development

Agilent Technologies

Introduction

Beta-blocking drugs are widely used in the treatment of hypertension and glaucoma. The determination of these drugs in biological fluids is nowadays done by fluorescence or mass spectrometry in combination with standard bore or narrow bore columns[1-3].
With fluorescence detection beta blocking drugs are analyzed in the low ng range. The disadvantage is that not all beta-blocking drugs fluoresce. These compounds can be analyzed using UV detection. The disadvantage of using UV detection is that it is typically less sensitive than fluorescence detection. Recently mass spectrometry is also being used to identify beta-blocking drugs and their metabolites in the low pg range.

The following Agilent 1100 Series LC system combinations were evaluated:

- Agilent 1100 Series standard LC system with standard bore and narrow bore columns using UV, fluorescence and mass spectrometry detection
- Agilent 1100 Series capillary LC system with capillary columns using UV and MS detection

For all system combinations the limit of detection was determined using Pindolol, Timolol, Metoprolol and Propranolol as beta-blocking drugs.

Experimental

A standard Agilent 1100 Series LC system was used in combination with UV detection, fluorescence detection and mass spectrometry. This modular system consists of:

- Agilent 1100 Series binary pump for precise flow rates and a micro degasser with an internal volume of 1 mL
- Agilent 1100 Series well-plate sampler for precise injection in the µL range with cooling option for the samples
- A column thermostat for precise retention times at above or below room temperature
- Agilent 1100 Series diode-array detector equipped with a 8-µL cell for highly precise quantitation
- Agilent 1100 Series LC/MSD Quadrupole SL with an ESI source for identification and highly selective and sensitive analysis. For the Agilent 1100 Series capillary system the ESI source with microspray nebulizer was used.
- Agilent 1100 Series fluorescence detector for multi signal and sensitive analysis of all compounds showing fluorescence activities.

The Agilent 1100 Series capillary LC system was used for experiments using capillary columns with an internal diameter of 0.3 mm. This modular system consists of:

- Agilent 1100 Series capillary pump with electronic flow control for precise flow rates even at changing back-pressure. The capillary pump was also equipped with a micro degasser with an internal volume of 1 mL
- Agilent 1100 Series micro well-plate sampler for precise injection in the nl and µL range with cooling option for the samples
- A column thermostat for precise retention times at room temperature, above or below room temperature
- Agilent 1100 Series diode-array detector equipped with a 500 nl cell for sensitive analysis of small peak volumes
- Agilent 1100 Series LC/MSD Quadrupole with a capillary ESI source for identification and highly selective and sensitive analysis.

Three capillary columns of the same type but of different length were tested:

- 150 x 0.3 mm Zorbax SB C-8, 3.5 µm
- 75 x 0.3 mm Zorbax SB C-8, 3.5 µm
- 50 x 0.3 mm Zorbax SB C-8, 3.5 µm

One narrow bore column was used:

- 30 x 2.1 mm Zorbax SB C-8, 3.5 µm

One standard bore column was used:

- 150 x 4.6 mm Zorbax Eclipse C-8, 5 µm

Beta-blocking drugs Pindolol, Timolol, Metoprolol and Propranolol were purchased from Sigma Aldrich.

Results and discussion

Limit of detection using UV detection

To evaluate optimum performance using UV detection three different combinations were tested:

1. The standard 1100 Series LC system with standard bore column with an internal diameter (id) of 4.6 mm
2. The standard 1100 Series LC system with narrow bore column with an id of 2.1 mm
3. The 1100 Series capillary LC system with a capillary column of 0.3 mm id[4]

Table 1 summarizes the results for all three combinations:
The1100 Series capillary LC system in combination with a 0.3-mm id column delivers the best limits of detection. Figure 1 shows a chromatogram representing the injection of beta-blocking drugs in the low ng range. Typically, UV detection cannot compete with fluorescence detection regarding sensitivity (figure 2). However, using capillary columns in combination with a capillary LC system instead of standard bore or narrow bore columns, comparable limits of detection can be achieved with UV detection (table 5). A significant advantage of the UV LC system is, that all beta-blocking drug can be detected.

Limit of detection (LOD) using fluorescence detection

Fluorescence detection is a very sensitive method for the analysis of beta-blocking drugs compared to UV detection under the same chromatographic conditions, (figure 2). To evaluate this detector the following combination was tested:

	Standard 1100 Series LC sytem			1100 Series capillary LC system	
Compound	**LOD DAD 220 nm**	**LOD DAD 220 nm**	**LOD DAD 290 nm**	**LOD DAD 220 nm**	**LOD DAD 290 nm**
Pindolol	13.49 ng	3.12 ng		0.960 ng	
Timolol	243.99 ng	65.9 ng	4.55 ng	10.16 ng	0.830 ng
Metoprolol	61.99 ng	16.7 ng		2.910 ng	
Propranolol	11.89 ng	3.1 ng		0.12 ng	
Column id	**4.6 mm**	**2.1 mm**	**2.1 mm**	**0.3 mm**	**0.3 mm**

Table 1
Limit of detection for different instrument combinations using UV detection

Chromatographic conditions:
Column: 150 x 0.3 mm Zorbax SB C-8: gradient from 10 % ACN to 80 % ACN in 20min
Flow rate: 5 µL/min
Column oven: 20 ºC
Detection wavelength: 220/10 nm with a reference wavelength at 500/100 nm.
Mobile phase: water + 0.05 % TFA and acetontrile + 0.045 % TFA was used for UV detection.

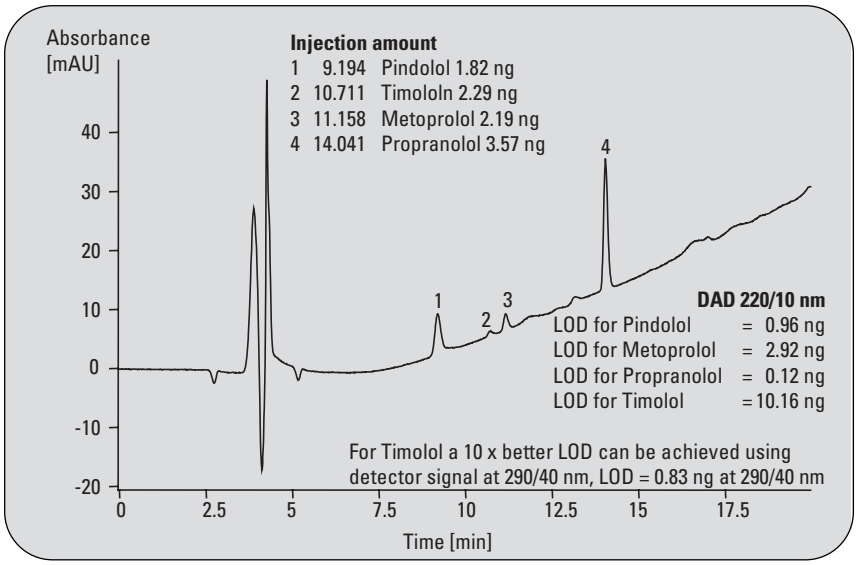

Figure 1
Limit of detection for four beta-blocking drugs, analyzed using a capillary column and diode-array UV detector

- The standard 1100 Series LC system with narrow bore column with an id of 2.1 mm

The advantage is that compounds, which have good fluorescent behavior, can be detected very sensitively and selectively. The disadvantage is that some beta-blocking drugs show only weak fluorescence, for example, Pindolol. Timolol does not fluoresce at all. Both detectors were switched in series and the signal-to-noise ratio were evaluated and compared. Except for Timolol the signal-to-noise ratios for fluorescence detection was significantly better, especially for Propranolol. The limit of detection using the fluorescence detection is in the high pg range for Pindolol and Metoprolol. For Propranolol the limit of detection is about 120 pg. Figure 3 shows a chromatogram with injected amounts in the low ng range. It is quite obvious that Propranolol shows the best limit of detection due to its very good fluorescence.

Chromatographic conditions:
Column: 30 x 2.1 mm Zorbax SB C-8:
Gradient: 10 % ACN to 80 % ACN in 10 min
Flow rate: 0.4 mL/min
Column oven: 20 ºC
FLD wavelength: Multi signal mode Ex/EM = 220/320 nm and 265/320 nm
Mobile phase: water + 0.05 % TFA and acetonitrile + 0.045 % TFA was used for FLD detection.

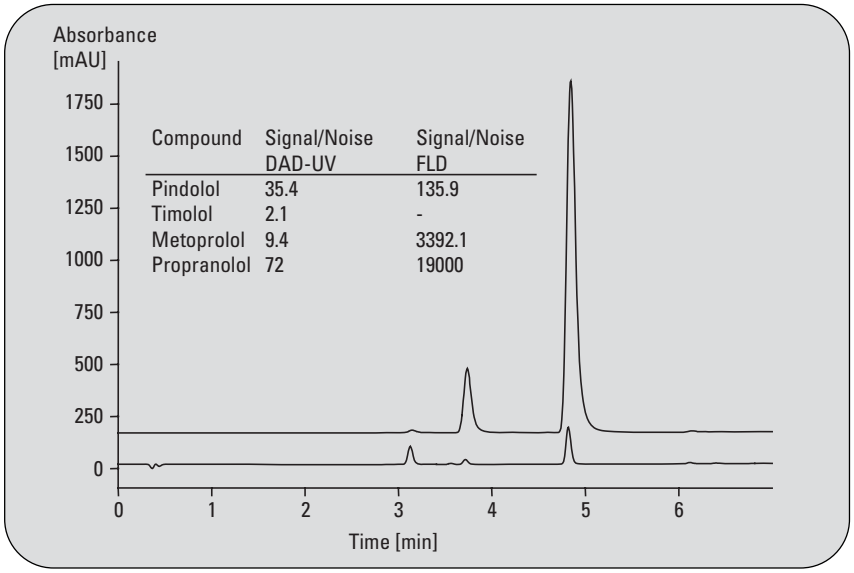

Figure 2
Analysis of beta-blocking drugs with DAD-UV and fluorescence detection using a 2.1-mm id column

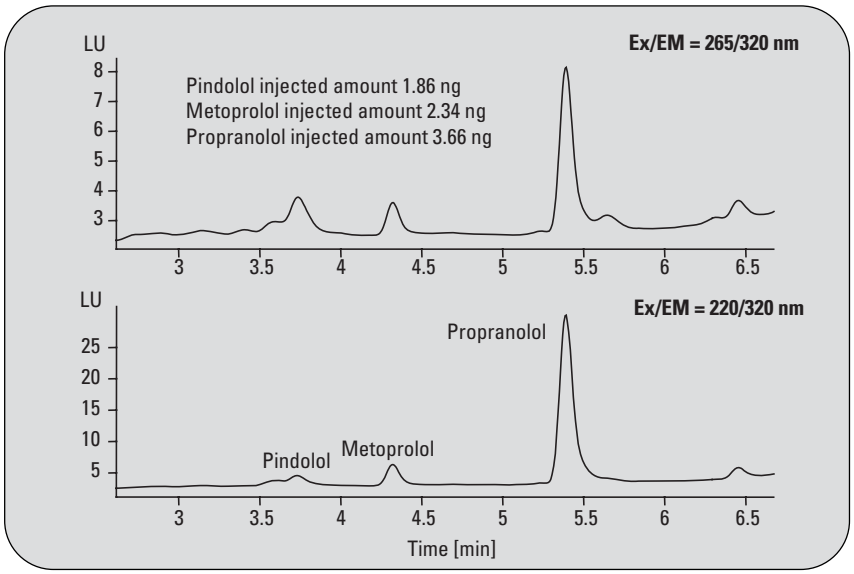

Figure 3
Analysis of beta-blocking drugs with fluorescence detection and 2.1-mm id column in the low ng range

The limit of detection for the three compounds using fluorescence detection is summarized in table 2. The advantage of fluorescence detection is, that strongly fluorescent compounds can be detected with high sensitivity using standard 1100 LC equipment and narrow bore columns.

Compound	FLD EX/Em 220/320	FLD EX/Em 265/320
Pindolol	-	430 pg
Timolol	-	-
Metoprolol	600 pg	-
Propranolol	120 pg	-
Column id/LC system	**2.1 mm / Standard 1100 Series LC system**	

Table 2
Limit of detection for fluorescence detection in combination with a column of 2.1 mm id

Limit of detection using MSD Quadrupole

To evaluate optimum performance using MS detection two different combinations were tested:

- The standard 1100 Series LC system with narrow bore column with an internal diameter of 2.1 mm
- The 1100 Series capillary LC system with a capillary column of 0.3 mm internal diameter

Using the selected ion mode (SIM) beta-blocking drugs can be detected with high sensitivity in the low pg range. (figure 4). A very powerful

Compound	LOD MSD SIM	
Pindolol	9.00 pg	2.04 pg
Timolol	7.70 pg	1.86 pg
Metoprolol	7.40 pg	0.85 pg
Propranolol	16.80 pg	1.64 pg
Column id/ LC system	**2.1 mm standard 1100 Series LC system**	**0.3 mm 1100 Series capillary LC system**

Table 3
Limit of detection for Agilent 1100 Series MSD in selected ion mode using 2.1- and 0.3-mm id columns

Chromatographic conditions:
Column: 50 x 0.3 mm Zorbax SB C-8
Mobile phase: Water+ 0.05 %FA, Acetonirile + 0.045 % FA
Flow rate: 0.01 mL/min
Gradient: at 0 min 10 % ACN, at 10 min 90 % ACN with fast reconditioning
Injection volume: 1 µL with needle wash in flush port

MS conditions:
Source: ESI
Peak width: 0.1 min
Time filter: On
SIM mode: SIM ions 249, 260, 268, 317, Fragmentor 60V, Gain 10, Actual dwell 144
Gas temperature: 350 ºC
Drying gas: 5L/min
Nebulizer pressure: 15 psig
Vcap: 4000V positive
Scan: m/z 120-450

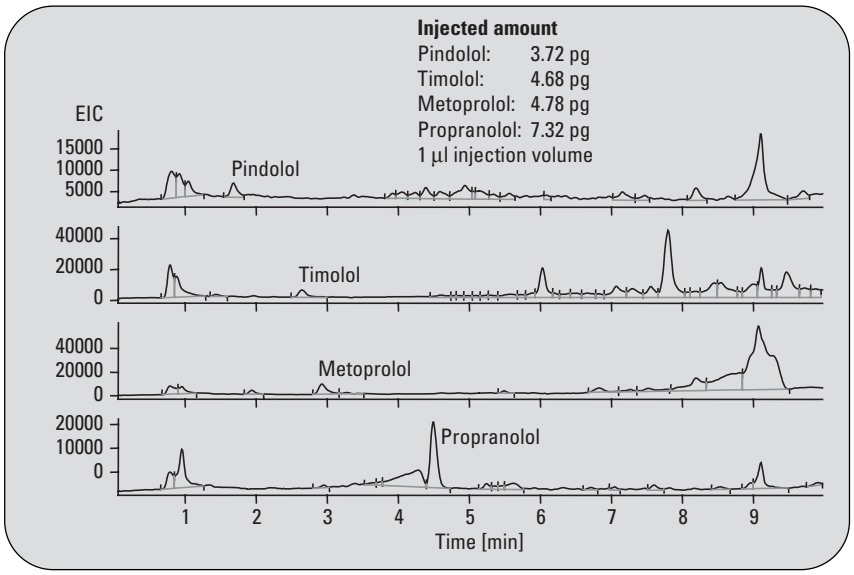

Figure 4
Limit of detection for MS quadrupole using a capillary column with 0.3-mm id

combination regarding speed and sensitivity is the combination of short capillaries with a mass spectrometer. In table 3 the results for the two combinations are summarized. The advantage of MSD quadrupole in combination with capillary columns is that the best limits of detection can be achieved. MS detection is the best choice, if beta-blocking drugs should be identified at trace levels.
The advantage of the other, less sensitive detectors, is that they provide improved quantitative results. In table 4 precisions of areas are compared for all three detectors using 2.1-mm id columns.

Conclusion

The limits of detections were evaluated for different Agilent 1100 Series LC/detector systems using standard bore, narrow bore and capillary columns. For standard and narrow bore columns the standard 1100 Series LC system using DAD-UV, fluorescence and mass spectrometer were used. For capillary columns the 1100 Series capillary LC system was used with DAD-UV and mass spectrometer. The results are summarized in table 5 for a signal-to-noise ratio of 2. From this table it is obvious that the combination 1100 capillary LC/MSD is a very powerful solution, if lowest limits of detection should be achieved.
Best quantitative data are provided if the LC/DAD-UV combination is used. For these combinations precision for areas between 0.16 and 1.02 RSD can be expected.

Compounds	DAD RSD area (%)	FLD RSD area (%)	MSD RSD area (%)	RSD RT (%)
Pindolol (186 ng)	0.16	2.70	2.94	0.11
Timolol (234 ng)	1.02	n.a.	1.59	0.06
Metoprolol (239 ng)	0.20	0.37	2.05	0.06
Propranolol (366 ng)	0.18	0.59	2.00	0.04

Table 4
Precision results for DAD, FLD and MSD

Compound	LOD DAD-UV			LOD FLD	LOD MS Quadrupole SIM mode	
Pindolol	13.49 ng	3.12 ng	0.96 ng	0.43 ng	9 pg	2.04 pg
Timolol	243.90 ng	4.55 ng	0.83 ng	-	7.70 pg	1.86 pg
Metoprolol	61.99 ng	16.70 ng	2.91 ng	0.60 ng	7.40 pg	0.85 pg
Propranolol	11.89 ng	3.10 ng	0.12 ng	0.12 ng	16.80 pg	1.64 pg
Column id	**4.6 mm**	**2.1 mm**	**0.3 mm**	**2.1 mm**	**2.1 mm**	**0.3 mm**

Table 5
LODs for different LC/detector systems using columns with different internal diameter

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