Analysis of PAMPA samples using the Agilent 1200 Series RRLC system with an Agilent 6460 Triple Quadrupole LC/MS system

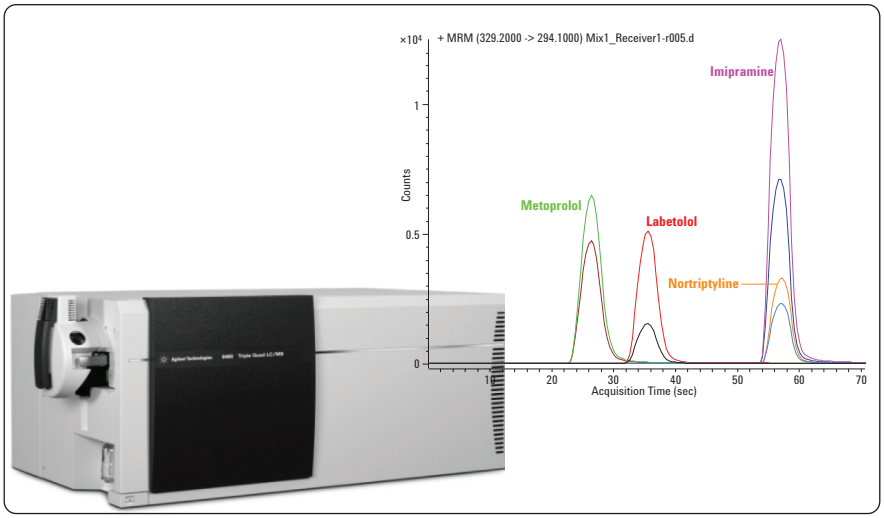
Application Note

Drug Discovery

Authors

Srividya Kailasam
Agilent Technologies
Bangalore
India

Kerry Frost
Cyprotex Discovery Limited



Abstract

In this Application Note an Agilent 1200 Series Rapid Resolution LC system coupled to an Agilent 6460 Triple Quadrupole LC/MS system was used for the analysis of parallel artificial membrane permeability assay (PAMPA) samples, each containing four target compounds. The MassHunter Optimizer software was used to identify the most intense product ions, optimal fragmentor voltages and collision energies. The apparent permeability values of ten pharmaceutical compounds belonging to four different classes of drugs were determined.

Agilent Technologies

Introduction

Parallel artificial membrane permeability assay (PAMPA) is used to determine passive diffusion across a porous filter coated with a lipid-like organic compound or natural lipid extracted from an animal source forming an artificial membrane. Typically, PAMPA experiments are carried out during the drug discovery phase to identify lead candidates with the most promising intestinal absorption potential, and to modify the structure of discovery compounds, improving their in vivo diffusion characteristics. PAMPA in conjunction with Caco-2 assay can explain the observed in vivo permeability behavior of drugs.

In this study the apparent permeability values of four beta blockers (atenolol, labetolol, metoprolol and propranolol), one calcium-channel blocker (verapamil), one serotonin receptor agonist (buspirone) and four antidepressants (nefazodone, nortriptyline, imipramine and trimipramine) along with a control compound (desipramine) were determined.

Experimental

Chemicals

Atenolol, labetolol, metoprolol, propranolol, buspirone, nefazodone, nortriptyline, imipramine, trimiparmine, verapamil and desipramine purchased from Sigma-Aldrich, Bangalore, India. HPLC grade acetonitrile and methanol from Lab-Scan and formic acid from Fluka were used in the study.

Stock solutions and suitable dilutions of target analytes were prepared in methanol for method development.

Sample preparation and PAMPA protocol

A solution of hexadecane in hexane was prepared (5% v/v) and an aliquot added onto the membrane of each well in the filter (donor) plate (multiscreen filter plate for permeability, Millipore). The donor plate was then allowed to dry to ensure evaporation of hexane.

Test compounds were dissolved in DMSO to a final concentration of 10 mM and then diluted to 10 µM with 10 mM phosphate buffer (pH 7) to give a final DMSO concentration of 5%.

A 300-µL amount of 10 mM phosphate buffer (pH 7) containing 5% DMSO was added to each well of the acceptor plate. Another 300-µL amount of 10 mM phosphate buffer solution (pH=7) containing the test compounds and 5% DMSO were added to the wells in the donor plate. Lucifer yellow, a fluorescent marker was added to the test compound solutions to track membrane integrity.

The donor plate was inserted into the acceptor plate and the plates were then incubated at room temperature, in a humid environment for 5 hours. Analytical standards were prepared from test compound solutions. Desipramine, a compound of known permeability, was run on each plate as the control. Methanol was used as the stop solution.

At the end of the incubation period the donor plate was removed from the acceptor plate and the samples pooled to contain four analytes. The concentrations of the test compounds in the donor, acceptor and equilibrium wells were quantified using LC/MS-MS.

Instrumentation

We used an Agilent 1200 Series Rapid Resolution LC system which included a vacuum degasser, binary pump, high-performance autosampler with thermostat and a thermostatted column compartment along with an Agilent 6460 Triple Quadrupole Mass Spectrometer. The mass spectrometer operated in ESI mode with Agilent's Jet Stream Technology. The Agilent MassHunter Optimizer software (B.02.00) was used for identifying the most abundant MRM transitions along with the associated fragmentor voltages and collision energies. The Agilent MassHunter Workstation (version B.02.00) was used for data acquisition and MassHunter Quantitative analysis software (version B.01.04) was used for data analysis.

Method details

The LC conditions are presented in Table 1 and MS conditions in Table 2. The MRM transitions used in the study are presented in Table 3. A 50-ms dwell time used for all transitions provided sufficient data points across all peaks.

Data analyses

Known concentrations of target analytes were loaded onto the 96-well multiscreen filter plate along with donor, receiver and equilibrium samples. After incubation, five replicate injections were performed for all standards and samples and the mean of peak areas were used for all calculations. Calibration curves were constructed by plotting mean of peak areas of target analytes against corresponding concentrations, using a weighting factor of 1/x. The calibration curves were found to be linear with correlation coefficients ranging from 0.95 to 0.99. Concentrations of target analytes in donor, receiver and equilibrium wells were calculated using the mean of peak areas and calibration curves generated for target analytes.

Calculations

The apparent permeability of a compound was calculated using the following equation:

$$P_{app} = C \times -\ln\left(1 - \frac{[drug_{acceptor}]}{[drug_{equilibrium}]}\right)$$

$$\text{wherein} \quad C = \frac{V_D \times V_A}{(V_D + V_A) \times area \times time}$$

Where V_D and V_A are the volume of the donor and acceptor compartments, respectively, area is the surface area of the membrane multiplied by the porosity and the equilibrium drug concentration is the concentration of test compound in the total volume of the donor and acceptor compartments. In the present study, the volumes of both donor and receiver compartments were 0.3 mL and the membrane area was

Parameter	Details
Mobile phase	A: Water (0.1% Formic acid) B: Acetonitrile (0.1% Formic acid)
Flow rate	0.5 mL/min
Gradient conditions	0 min 25% B 0.2 min 60% B 0.3 – 0.5 min 75% B 0.6 – 0.9 min 95% B 1.0 – 1.2 min 100% B 1.25 – 1.5 min 25% B
Run time	1.5 min
Post time	1 min
Injection Volume	1 µl
Needle wash	10 s in wash port with 1:1 methanol:water mix
Column	Agilent Zorbax Eclipse XDB – C18, 2.1 × 50 mm, 1.8 µm particle size (p/n 927700-902)
Column temperature	50 °C

Table 1
LC conditions.

Parameter	Details
Ionization Mode	ESI-positive with Agilent Jet Stream
Drying gas temperature	350 °C
Drying gas flow rate	10 L/min
Nebulization gas pressure	20 Psi
Capillary voltage	2000 V
Sheath gas temperature	400 °C
Sheath gas flow rate	11 L/min
Nozzle voltage	0 V
Q1 resolution	Unit
Q2 resolution	Unit

Table 2
MS/MS conditions.

Compound	Empirical Formula	Precursor Ion	Product Ion(s) Quantifier, Qualifier	Fragmentor voltage (V)	Collision energy (eV) Quantifier, Qualifier
Atenolol	$C_{14}H_{22}N_2O_3$	267.2	145, 190	115	25, 13
Labetolol	$C_{19}H_{24}N_2O_3$	329.2	311.1, 294.1	105	5, 13
Metoprolol	$C_{15}H_{25}NO_3$	268.2	116.1, 74.1	115	13, 17
Propranolol	$C_{16}H_{21}NO_2$	260.2	116.1,183	110	13, 13
Buspirone	$C_{21}H_{31}N_5O_2$	386.3	122, 150	164	29, 29
Verapamil	$C_{27}H_{38}N_2O_4$	455.3	165, 303.1	158	25, 21
Nefazodone	$C_{25}H_{32}ClN_5O_2$	470.2	274.1, 246.1	150	25, 33
Nortriptyline	$C_{19}H_{21}N$	264.2	233.1, 90.9	80	10, 20
Imipramine	$C_{19}H_{24}N_2$	281.2	86.1, 58.1	101	13, 39
Trimipramine	$C_{20}H_{26}N_2$	295.2	100.1, 58.1	105	9, 41
Desipramine	$C_{18}H_{22}N_2$	267.19	72.1	95	11

Table 3
MS/MS parameters used for analyte identification and analysis.

0.0715 cm^2. The permeation time was 5 hours and the logarithm of apparent permeability is reported.

Results and discussion

Rapid resolution chromatography and selective MS determination were used for the analyses of multiple analytes in pooled samples. Figure 1 is a representative chromatogram of a receiver sample. The sample is a mix of four target analytes, metoprolol, labetolol, imipramine, and nortriptyline. A log (P_{app}) value < -5.00 indicates poor permeability, while a log (P_{app}) value > -5.00 indicates high permeability by diffusion [1]. The logarithm of apparent permeability values shown in Table 4 indicates that the chosen compounds are highly permeable.

Conclusion

We have shown the analyses of PAMPA samples using an Agilent 1200 Series RRLC system coupled to an Agilent 6460 Triple Quadrupole MS. The MassHunter Optimizer identified compound specific MS parameters. We determined the apparent permeability values of ten pharmaceutical compounds belonging to four different classes of drugs.

Reference

1. http://www.cyprotex.com/userfiles/file/Cloe_Screen_PAMPA_Permeability(1).pdf, Cyprotex, Cloe Screen PAMPA flyer

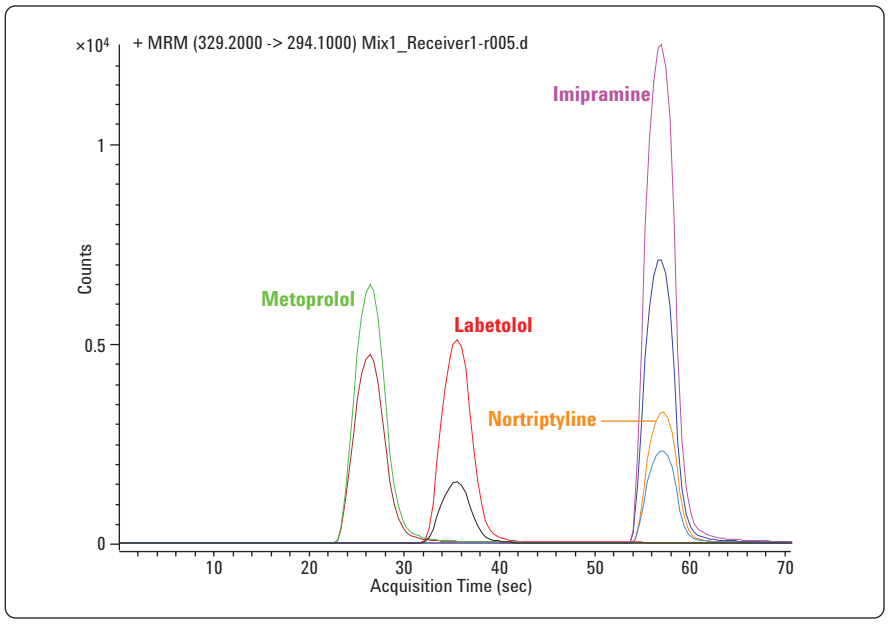


Figure 1
Chromatogram of a sample from a receiver well.

Compound	Log P_{app}	Standard Deviation (n=4)
Atenolol	-3.85	0.08
Labetolol	-4.02	0.09
Metoprolol	-4.07	0.05
Propranolol	-4.08	0.07
Buspirone	-4.31	0.08
Verapamil	-4.12	0.26
Nefazodone	-4.23	0.16
Nortriptyline	-4.24	0.18
Imipramine	-4.24	0.15
Trimipramine	-4.20	0.15
Desipramine	-4.18	0.16

Table 4
Apparent log P values.

www.agilent.com/chem/qqq

October 1, 2009
Publication Number 5990-4878EN

Agilent Technologies