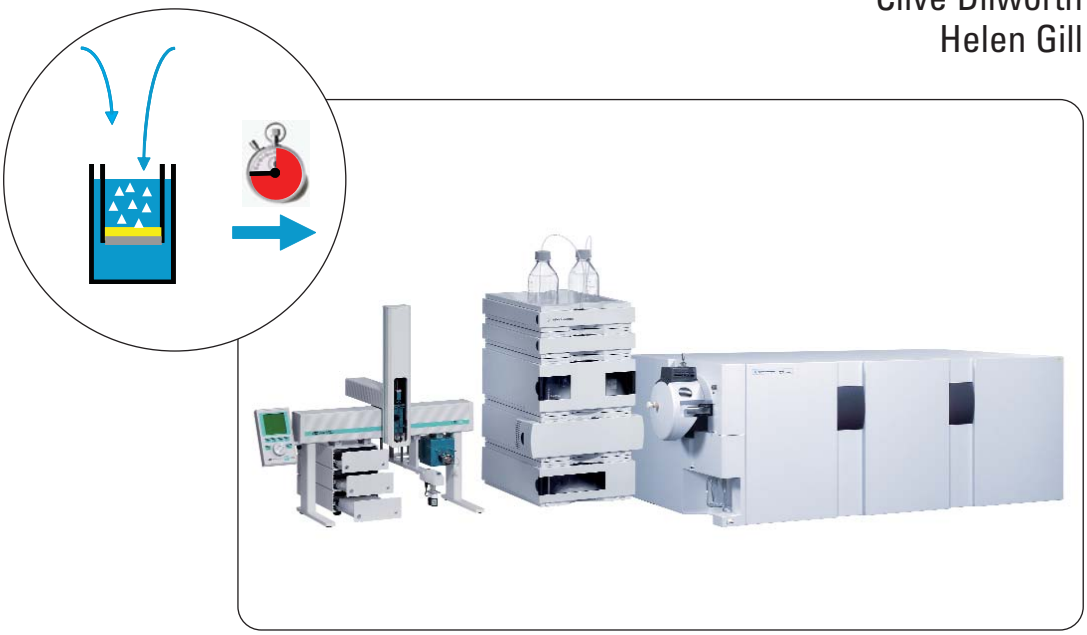
Improving productivity in the determination of parameters for early *in vitro* ADME

Part 3 – Experimental details and results of Caco-2 permeability assay

Application Note

Michael Frank
Clive Dilworth
Helen Gill



Abstract

This Application Note describes the experimental details and results of a Caco-2 permeability (Caco-2) assay. The approach and methodology of rapid resolution liquid chromatography and triple quadrupole mass spectrometry used in this study are described in a separate Application Note[1]. Experimental details and results of other typical assays performed in early ADME profiling of new hits are presented in separate publications[2,3].

Agilent Equipment:
1200 Series RRLC
6410 triple quadrupole LC/MS
CTC HTC-PAL autosampler
ZORBAX RRHT column
MassHunter workstation software

Application Area:
Drug Discovery

cyprotex

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Experimental

Method	Compound	Polarity	Precursor ion [m/z]	Product ion [m/z]	Dwell time [ms]	Fragmentor voltage [V]	Collision energy [V]
Method_6	Tramadol	pos	264.2	58.0	13	100	15
	Propranolol	pos	260.1	183.0	13	120	25
	Atenolol	pos	267.2	145.0	13	140	25
	Metoprolol	pos	268.2	74.1	13	140	25
Method_6neg (time segment 0.5 min)	Flurbiprofen	neg	199.1	199.1	50	100	0
	Metoprolol	pos	268.2	74.1	50	140	25
Method_7	Diclofenac	pos	296.0	215.0	10	90	10
	Digoxin	pos	798.4	651.4	10	120	10
	Nimodipine	pos	343.1	301.0	10	120	20
	Nefazodone	pos	470.2	274.1	10	160	35
	Metoprolol	pos	268.2	74.1	10	140	25
Method_11	Labetolol	pos	329.2	162.0	20	120	25
	Propranolol	pos	260.1	183.0	20	120	25
	Metoprolol	pos	268.2	74.1	20	140	25
Method_8	Buspirone	pos	386.3	122.0	13	180	25
	Atenolol	pos	267.2	145.0	13	140	25
	Talinolol	pos	364.3	100.1	13	140	25

Table 1
Setup of the MRM method for the Caco-2 permeability assay (metoprolol was used as ISTD, atenolol, propranolol and talinolol were used as control substances).

Caco-2 permeability assay[4] – protocol summary

Firstly, Caco-2 cells were allowed to grow for 20 days on special filter plates, after which the cell layers were tested for confluence (figure 1, step 1a). The diluted test compounds were added to the apical side of the cells (step 2). During equilibration time the test compounds either traveled passively or were transported actively to the basolateral side of the cells in the lower compartment (step 3). Samples from both compartments were taken and quantified (step 4) using a calibration curve derived from calibration samples prepared in parallel (step 1b).

Final test compound concentration:	10 µM
Final DMSO concentration:	1.0 %
ISTD:	Metoprolol
Final ISTD concentration:	0.2 µM
Caco-2 cells:	Obtained from the ATCC (passage numbers 40-60), seeded onto Millipore Multiscreen Caco-2 plates at 1 x 105 cells/cm^2, cultured for 20 days in DMEM, media was changed every two or three days. On day 21 the permeability study was performed.
Buffer:	Hanks Balanced Salt Solution (HBSS), pH 7.4 buffer with 25 mM HEPES and 4.45 mM glucose at 37°C
Cell Integrity test:	By Lucifer yellow method
Incubation temperature:	37 °C
Incubation time:	120 min
Sample work-up:	After incubation, samples from the donor and receiver plates were taken and methanol containing internal standard was added to all samples.

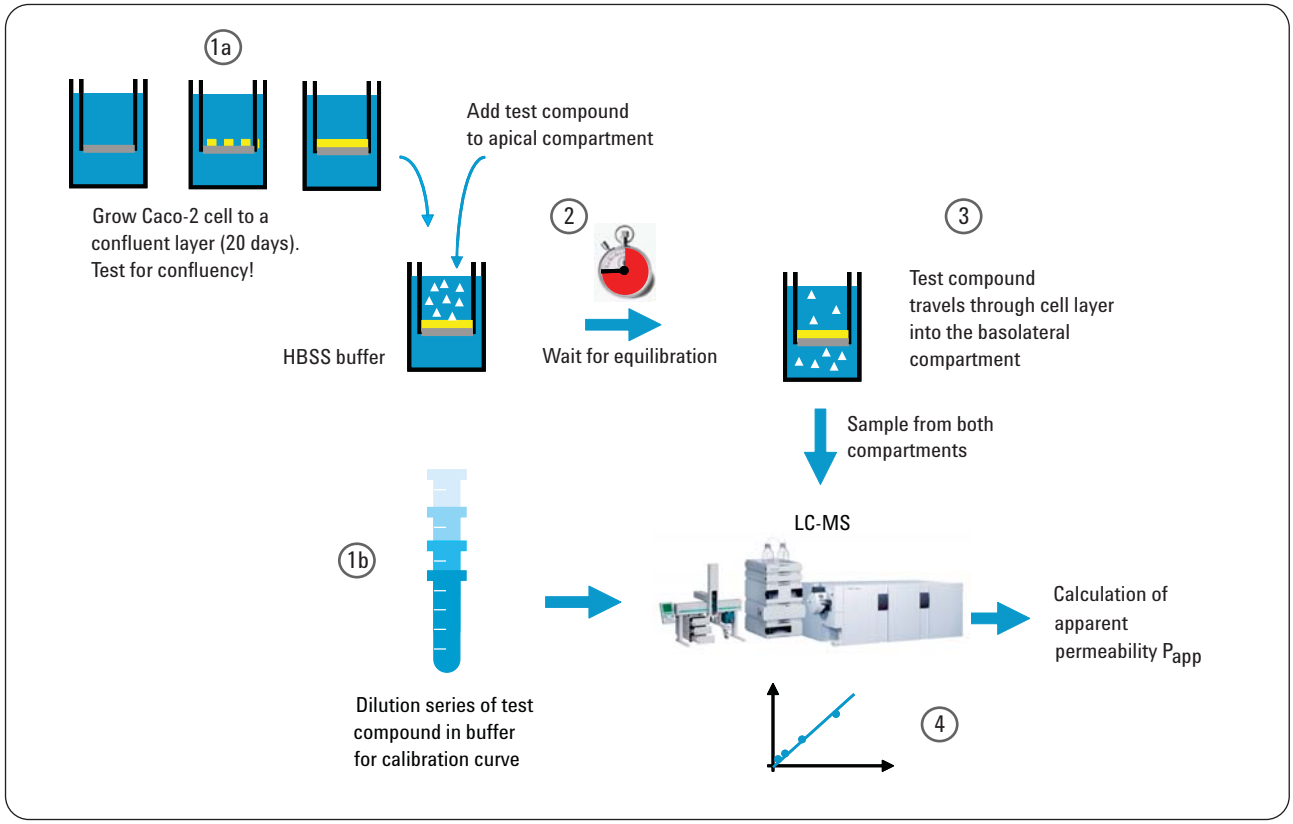

Figure 1
Schematic of the Caco-2 permeation assay.

Results

Compound	Papp [10^{-6} cm/s]	Recovery [%]
Atenolol	0.612	93
Buspirone	52.1	79
Diclofenac	62.7	71
Digoxin	1.82	69
Flurbiprofen	53.0	68
Labetalol	15.5	84
Nefazodone	59.2	32
Nimodipine	60.4	48
Propranolol	53.6	70
Tramadol	49.7	92

Table 2
Summary of the Caco-2 permeability assay results.

References

1.
Improving productivity in the determination of parameters for early in vitro ADME. Part 1 - Study approach and methodology of rapid resolution liquid. *Agilent Technologies publication number 5989-7488EN*, **2007.**

2.
Improving productivity in the determination of parameters for early in vitro ADME.
Part 2 - Experimental details and results of human liver microsome stability assay. *Agilent Technologies publication number 5989-7667EN*, **2007.**

3.
Improving productivity in the determination of parameters for early in vitro ADME. Part 4 - Experimental details and results of plasma protein binding assay. *Agilent Technologies publication number 5989-7669EN*, **2007.**

4.
Per Artusson et al. "Cell Culture Absorption Models – State of the Art", pp. 71–78 in "Pharmaco-kinetic Profiling in Drug Research", Ed. Bernard Testa, Stefanie D. Krämer, Heidi Wunderli-Allenspach, Gerd Folkers, *Verlag Helvetica Chemica Acta*, *Zürich and Wiley-VCH Weinheim*, **2006.**

Clive Dilworth is Senior Principal Scientist at Cyprotex Plc., Macclesfield, UK
Helen Gill is Product Development Manager at Cyprotex Plc., Macclesfield, UK
Michael Frank is Product Manager at Agilent Technologies, Waldbronn, Germany.

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