Cypex QC Assays

Substrate:	7-ethoxycoumarin	**Product:**	umbelliferone

Incubation conditions

Assay buffer: 0.05 M potassium phosphate pH 7.4, 5 mM $MgCl_2$
Incubation volume: 200 µl
Stop reagent: 1 M hydrochloric acid (20 µl)
Internal standard: 4-methylumbelliferone (10 µl of 0.1 µg/ml)

HPLC conditions

Column: Hypersil ODS (5 µm) 250 x 4 mm
Temperature: 30ºC
Mobile phase: 0.05% orthophosphoric acid/acetonitrile (gradient separation)

Time (min)	0	3	8	9	9.5
% CH_3CN	25	25	65	65	25

Flow rate: 1.0 ml/min
Run time: 14.5 min
Injection volume: 50 µl
Detection: fluorescence λ_{em} = 324 nm, λ_{ex} = 458 nm (PMT Gain = 13)
Retention times: umbelliferone 5.2 min
4-methylumbelliferone 7.0 min
7-ethoxycoumarin 10.3 min

Substrate:	phenacetin	**Product:**	4-acetamidophenol

Incubation conditions

Assay buffer: 0.05 M potassium phosphate pH 7.4, 5 mM $MgCl_2$
Incubation volume: 200 µl
Stop reagent: 1 M hydrochloric acid (20 µl)
Internal standard: 3-acetamidophenol (10 µl of 10 µg/ml)

HPLC conditions

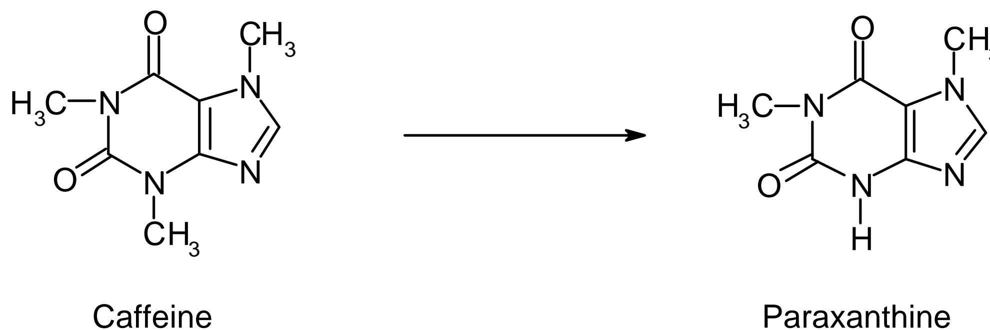
Column: Hypersil ODS (5 µm) 250 x 4 mm
Temperature: 30ºC
Mobile phase: 0.05% orthophosphoric acid/acetonitrile (gradient separation)

Time (min)	0	4	9	12	12.5
% CH_3CN	12	12	37	37	12

Flow rate: 1.0 ml/min
Run time: 18.5 min
Injection volume: 50 µl
Detection: UV, λ = 250 nm
Retention times: 4-acetamidophenol 4.2 min
3-acetamidophenol 6.1 min
phenacetin 12.5 min

Other substrates

Substrate:	Caffeine	**Product:**	Paraxanthine (and Theobromine and Theophylline)



Caffeine Paraxanthine

Reference: Grant, D. *et al* (1987) Biochem. Pharmacol. **36**(8) 1251-1260
Biotransformation of caffeine by microsomes from human liver.

Assayed in 0.12M potassium phosphate buffer pH7.4 / 2mM $MgCl_2$ / 0.23% KCl at 37°C for 15min in 0.5ml final volume.
Reaction stopped by the addition of 10ml chloroform **:** isopropanol 85 **:** 15
0.05ml internal standard solution (0.005mg/ml 7methyl xanthine, 0.005 mg/ml 1,7-dimethyluric acid in H_2O) added, mixture vortexed
Saturating amount ammonium sulphate (~0.5g) added, mixture vortexed and centrifuged.
Organic layer removed and evaporated to dryness under N_2. Residue resuspended in mobile phase.
Analysed by reversed phase HPLC using Beckman Ultrasphere ODS 25cm x 4.6mm column and UV detection at 273nm.
Mobile phase: isocratic, 0.05% acetic acid **:** methanol 88 **:** 12

Substrate:	Ethoxyresorufin	**Product:**	Resorufin

Reference: Grant, M.H. *et al* (1988) Biochem. Pharmacol. **37**(21) 4111-4116
Mixed function oxidase and UDP-glucuronyltransferase activities in the human HEP G2 hepatoma cell line.

Assayed in 0.1M potassium phosphate buffer pH7.6 at 37°C in 0.5ml final volume.
Reaction stopped by addition of 0.75ml ice cold acetone.
Samples centrifuged 12000 *g* for 5min.
Fluorescence of supernatant measured at emission wavelength 600nm and excitation wavelength 580nm

Substrate: Oestradiol	**Product:** 2-hydroxy-oestradiol

Reference: Ball, S. *et al* (1990) Biochem. J. **267** 221-226
Mixed function oxidase and UDP-glucuronyltransferase activities in the human HEP G2 hepatoma cell line.

Assayed in 0.02M Tris / HCl buffer pH7.4 at 37°C for 30min in 3ml final volume.
Reaction stopped by addition of 5ml diethyl ether.
4µg mestranol added as internal standard.
Organic layer removed and evaporated to dryness.
Residue resuspended in mobile phase.
Analysed by reversed phase HPLC using Spherisorb 5-ODS 25cm x 0.46cm column and UV detection at 280nm.
Mobile phase 0.5% w/v ammonium phosphate pH3 **:** methanol (ratio not stated)

Substrate: Imipramine	**Product:** Desipramine

Reference: Koyama, E. *et al* (1997) J. Pharm. Exp. Ther. **281**(3) 1199-1210
Reappraisal of human CYP isoforms involved in imipramine N-demethylation and 2-hydroxylation: a study using poor metabolisers of S-mephenytoin and eleven recombinant human CYPs.

Assayed in 0.1M potassium phosphate buffer pH7.4 / 4mM $MgCl_2$ / 0.1mM EDTA at 37°C for 30min in 0.25ml final volume.
Reaction stopped by the addition of 100µl cold acetonitrile
10µl 25µM mianserin added as internal standard, 50µl 0.1M potassium phosphate pH 3.0 added.
Reaction centrifuged.
Analysed by reversed phase HPLC using CAPCELL PAK phenylSG-120 column and UV detection at 204nm.
Mobile phase: acetonitrile **:** 0.05M potassium phosphate pH3.0 26 **:** 74

Substrate: Clozapine	**Product:** N-demethylclozapine

Reference: Linnet, K. & Olesen, O.V. (1997) Drug Metab. Dispos. **25**(12) 1379-1382
Metabolism of clozapine by cDNA-expressed human cytochrome P450 enzymes.

Assayed in 0.1M potassium phosphate buffer pH7.4 / 4mM $MgCl_2$ / 0.1mM EDTA at 37°C for 15min in 0.3ml final volume.
Reaction stopped by the addition of 1ml 0.5M carbonate/bicarbonate pH 10.5.
50µl 6µM (E)-10-hydroxynortriptylline added as internal standard.
Extracted into 5ml heptane **:** isoamyl alcohol 98.5 **:** 1.5
Evaporated to dryness at 60°C under nitrogen.
Residue dissolved in 75µl mobile phase.

Analysed by reversed phase HPLC using Zorbax 300 SB 3µm C_{18} column and UV detection at 260nm.
Mobile phase: 0.025M K_2HPO_4 / 0.01M triethylamine pH 5.5 **:** acetonitrile 62 **:** 38

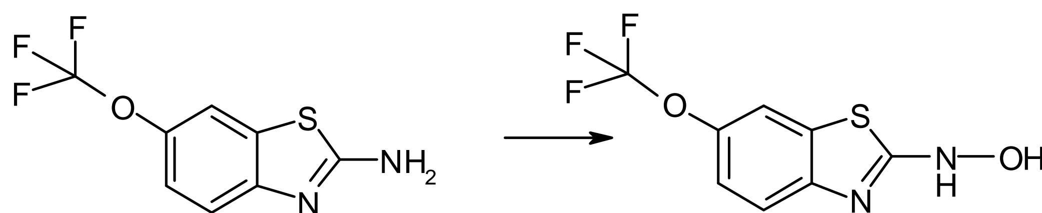
Substrate:	Lisofylline	**Product:**	Pentoxyfylline

Reference: Lee, S.H. & Slattery, J.T. (1997) Drug Metab. Dispos. **25**(12) 1354-1358
Cytochrome P450 isozymes involved in lisofylline metabolism to pentoxifylline in human liver microsomes.

Assayed in 0.1M potassium phosphate buffer pH7.4 at 37°C for 10min in 0.5ml final volume.
Reaction stopped by the addition of 6ml methylene chloride.
Organic layer removed and dried under N_2.
Residue analysed by reversed phase HPLC using a 3µm C_{18} column and UV detection at 273nm.
Mobile phase:
25mM ammonium phosphate containing 0.25% acetic acid (pH 4.5) **:** methanol 65 **:** 35

Substrate:	Riluzole	**Product:**	N-hydroxy riluzole

Reference: Sanderink, G. *et al* (1997) J. Pharm. Exp. Ther. **282**(3) 1465-1472
Involvement of human CYP1A isozymes in the metabolism & drug interactions of riluzole *in vitro*.



Assayed in 0.1M potassium phosphate buffer pH7.4, 10mM $MgCl_2$ at 37°C for up to 20 min.
Reaction stopped by the addition of equal vol. of methanol **:** acetonitrile 3.6 **:** 1.
Reaction centrifuged.
Supernatant analysed by reversed phase HPLC over Lichrosphere 60 RP Select B 5µm column and UV detection at 265nm.
Mobile phase:
10mM K_2HPO_4 **:** methanol **:** acetonitrile **:** glacial acetic acid 108 **:** 72 **:** 20 **:** 1