gsk do more feel better live longer

Validation of a Clinical assay; LLoQ of 350 fg/mL by Liquid Chromatography + Accelerator Mass Spectrometry in support of Dermal dosing

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Acknowledgements Part I

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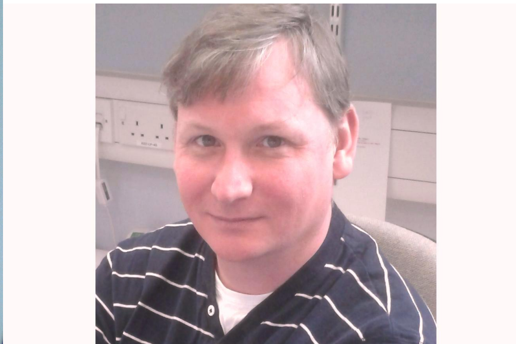

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Validation of an LC+AMS method to 350 fg/mL

Objectives

- Background
- Validation requirements
- Method details
- Data
- Conclusions

Background

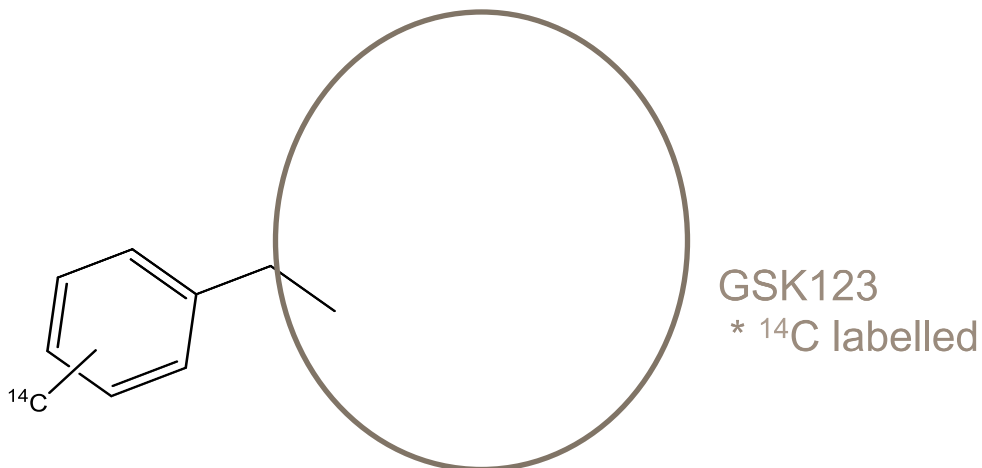
Study Background

- An analytical method was required to support a dermal study in humans
- An LOQ of < 1 pg/mL in plasma was predicted to be required
- The current bioanalytical method (LC-MS/MS) had an LOQ of 10 pg/mL

What LOQ could an AMS based method deliver to the project team?

Background

Compound Background



GSK123
* ^{14}C labelled

- Based on the specific activity of material used for dosing, (218 kBq/mg of free base) the calculated LOQ for the LC+AMS method would be approx. 300-400 fg/mL
- The project team were happy with this potential LOQ
- During method development use of a higher specific activity material produced a potential LOQ of 21 fg/mL

Method Validation

Validation Requirements

Validation based on summarised EBF recommendations for Tiered Approach to Validation[1]

Study data will be used to characterise systemic PK parameters following dermal dosing

Validation data from previous work existed and was reported; LC-MS/MS methodology

Stability in matrix at ambient temperature,
Stock Solution stability at 4°C,
Frozen matrix stability at -20°C,
3 freeze thaw cycles from -20°C to ambient temperature
so **these were not repeated** in this validation

What was done;

Selectivity (for parent, metabolites and co-meds)

In Auto sampler stability

Linearity of [^{14}C]-Parent (AMS) and cold-Parent (UV, ISTD)

Accuracy

Precision

Recovery

1: Higton and Seymour, (2014)

Method Validation

Sample Extraction Procedure

- 1 mL of plasma* sample protein crashed with 3 mL of cold parent (25 µg/mL) in acetonitrile
- Vortex mixed for 30 s and left to stand for at least 30 min.
- Centrifuged at 4200 rpm for 20 min.
- Supernatant transferred and evaporated to dryness
- Reconstituted in 250 µL (75/25 Amm. Form./MeCN (v/v))
- 100 µL injected onto LC system
- LC fraction (2 x 400 µL) collected at parent retention time and dried down
- Dried fraction graphitised and taken for AMS analysis [2]

*The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents”

2: Young *et al*, (2008)

Method Validation

LC Conditions

- Column : Phenomenex Phenyl Luna Hexyl, 250 x 4.6mm, 5µm, 40°C
- MPA: 50 mM Amm. Form. pH 2.5
- MPB: 70% MeOH, 30% MeCN (v/v)
- Flowrate: gradient at 1 ml/min
- Runtime: 22 min
- Wavelength: 254 nm
- Agilent 1100 series HPLC

Gradient Composition

% B

Parent Peak Retention time

Time (mins)

mAU

14.389

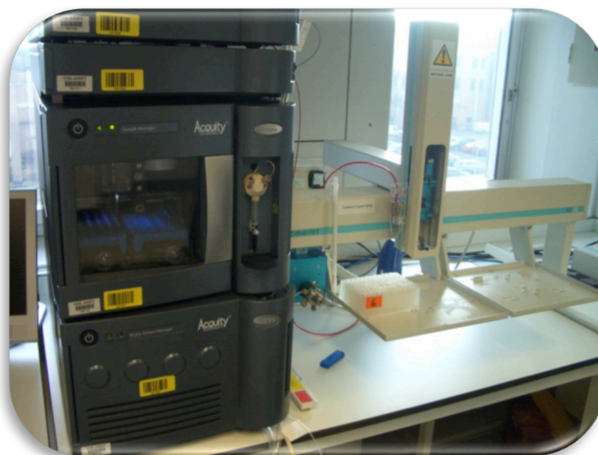
min

Method Details

Sample prep- LC followed by off-line analysis by AMS

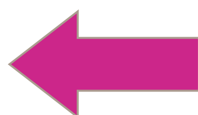
Spiked Human plasma extracted with ACN containing GSK123 as internal standard

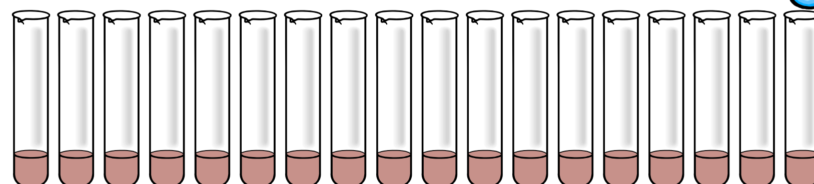
Parent peak isolation by LC-UV, signal of IS measured



Parent fraction collected from each sample

Fractions graphitised prior to analysis by AMS -measure $^{14}C/^{12}C$





Method Validation

Method Validation

- Analytical Range is 0.348 – 94.2 pg/mL (0.0052 to 1.41 dpm/mL)
- Control Plasma was spiked at five concentrations (VCs/QCs)
- Control and spiked plasma was analysed directly by AMS (n=6) to give definitive values
- Lowest two VC levels cannot be analysed directly as <15% above endogenous [^{14}C] in plasma; so calculated by dilution from high level stock (checked by LSC)
- Validation involves generation of a Mean Recovery Constant[3]
- This is calculated at five Recovery Concentration levels (n=3) that are equivalent to the Validation Concentration (VC) levels

3: Lappin G. *et al*, (2008)

Method Validation

Recovery Constant Generation

- Recovery Constant is analogous to single point calibration
- Calculated from the mean of up to 15 measurements
- Analysis of 5 VC levels in triplicate within the validation run
- In the validation run, 2 of these samples failed, no impact on result
- The mean Recovery Value was calculated to be 4.87 E-04 $(mAu*s)^{-1}$
- Currently we are moving away from the Recovery Constant approach towards a calibration standard approach, in order to harmonise our processes with GBC/AMS recommendations

Validation Data

QC values back-calculated for validation

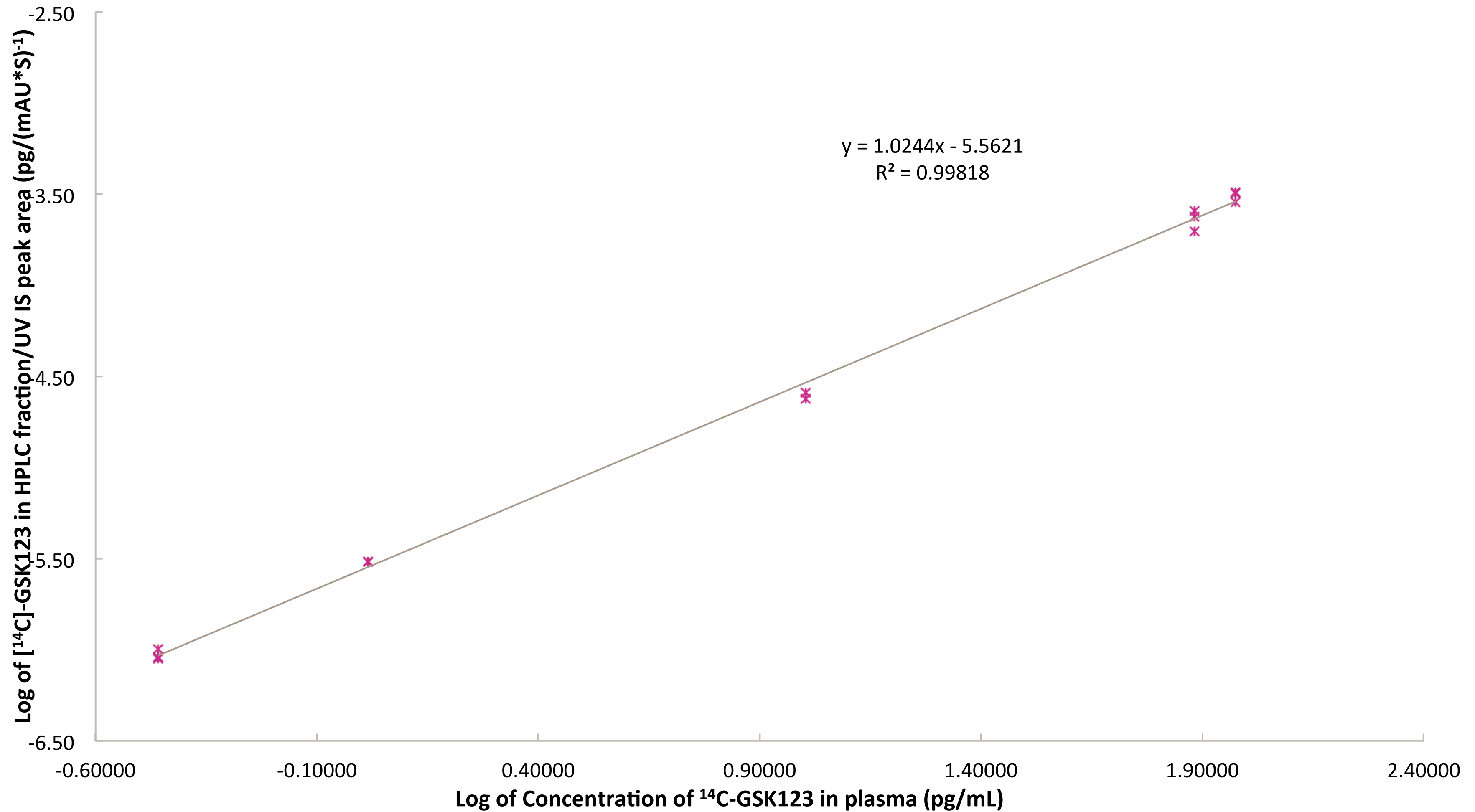
The Recovery Value is then used to back calculate the QCs at five concentrations (n=3, n=5 at LOQ)

QC (dpm/mL)	%Acc	Pass/Fail	GSK123 (pg/mL)	Actual(pg/mL)	Average	SD	%CV	%Bias
QC1, 0.0052	89.1	pass	0.3101					
	109.2	pass	0.3801	0.348	0.340	0.037	10.8	-2.2
	113.2	pass	0.3940					
	91.7	pass	0.3190					
	94.0	pass	0.3270					
	89.6	pass	0.3119					
QC 2, 0.016	110.7	pass	1.1485					
	118.0	fail	1.2246	1.037	1.123	0.062	5.3	11.7
	106.2	pass	1.1019					
QC 3, 0.15	78.8	fail	7.960					
	86.3	pass	8.721	10.107	9.312	0.98	11.1	-12.3
	98.0	pass	9.904					
QC 4, 1.14	87.7	pass	66.94					
	98.2	pass	74.91	76.30	70.23	4.16	5.9	-8.0
	90.2	pass	68.84					
QC 5, 1.40	106.1	pass	100.17					
	94.7	pass	89.33	94.37	94.76	5.42	5.7	0.4
	100.4	pass	94.79					

Acceptance Criteria, ±15% Accuracy and Precision, ±20% at the LOQ

Plot of [^{14}C]-GSK123 conc in HPLC fraction/UV peak area vs [^{14}C]-GSK123 showing linearity from 348 fg/mL to 94 pg/mL

Log-Log Plot of [14C]-GSK123 conc in HPLC fraction/UV peak area vs [14C]-GSK123 showing linearity from 348 fg/mL to 94 pg/mL



Selectivity

Parent, metabolites and Paracetamol co medication

- Parent selectivity was achieved by the analysis of pooled plasma
- Metabolite selectivity was checked by adapting a method previously used for metabolite identification (LC-MS/MS)
- Volunteers on the study were allowed to be administered with Paracetamol should they require it

- UV selectivity was determined by obtaining a plasma sample from a healthy volunteer at post c_{max} who had taken the recommended dose of paracetamol
- The sample was extracted using this method and the UV chromatogram investigated for interferences at the retention time for GSK123
- No interference from Paracetamol or its metabolites was demonstrated in the IS signal

Results

QC Data from Study Runs 1 and 2 (6 runs in total)

QC Data	Actual Conc. (pg/mL)	Result (pg/mL)	pass/fail	%diff.
Study Run 1	Low QC 1.18	1.04	pass	-11.9
		1.09	pass	-7.6
		1.33	pass	12.7
	Mid QC 9.4	8.10	pass	-13.8
		8.31	pass	-11.6
		6.48	fail	-31.1
	High QC 55.9	56.5	pass	1.1
		60.3	pass	7.9
		63.0	pass	12.7
Study Run 2	Low QC 1.18	1.27	pass	7.6
		1.21	pass	2.5
		1.32	pass	11.9
	Mid QC 9.4	9.11	pass	-3.1
		9.50	pass	1.1
		10.8	pass	14.9
	High QC 55.9	59.1	pass	5.7
		66.6	fail	19.1
		66.9	fail	19.7

QCs assayed in triplicate per batch of 63 samples

QC results from two “in study” analytical runs; 15/18 results within 15% acceptance criteria

Conclusions

- Robust bioanalytical method produced
- Sensitive bioanalytical LC+AMS assay with an LOQ of 348 fg/mL
- Used successfully in study support, approx 400 clinical samples
- Data generated was key to C2MD plan for GSK123
- Use of Tiered Validation approach to validate assay

References

- 1: Higton and Seymour, Application of a tiered approach to the validation of accelerator MS assays, Bioanalysis, (2014), 6(5), 665-672

- 2: Young *et al*, Comparison of a 250kV single-stage AMS with a 5MV tandem accelerator mass spectrometer-fitness for purpose in bioanalysis, *Rapid Comms in Mass Spectrometry* (2008), **22**: 4035–4042

- 3: Lappin *et al* ., High-performance liquid chromatography accelerator mass spectrometry: Correcting for losses during analysis by internal standardization., Analytical Biochemistry (2008) 378, 93–95

Acknowledgements

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Thank you