

Tables

Active Ingredient	Mobile Phase (per 1 L)	Flow Rate (mL / min)	Column Details	Injection Volume	Detection Wavelength
Salbutamol Sulphate	600mL - methanol 400mL - deionised water 1g - sodium dodecyl sulphate	1.5	Waters Nova-Pak® C18 5µm 3.9×150 mm,	100 µL	276nm
Fluticasone Propionate / Salmeterol Xinafoate	500mL - 50mM ammonium phosphate pH2.4 1mL - triethylamine 250mL - methanol 250mL - acetonitrile	1.2	Varian Pursuit XRs C18 3µm 4.6 x 150 mm,	200 µL	252nm

Table 1: Details of High Performance Liquid Chromatographic Techniques used for Quantification of Salbutamol Sulphate, Fluticasone Propionate and Salmeterol Xinafoate.

	All	Asthma	COPD	Neuro-muscular Disease	Obesity	Other Respiratory Condition	Miscellaneous/ Healthy
Number	92	27	25	9	7	10	14
Age (years)	53.1±18.0 (18-84)	53.1±16.6 (18-79)	65.8±6.7 (52-80)	39.1±19.0 (17-78)	46.4±14.8 (23-62)	59.2±23.8 (23-84)	38.4±17.4 (21-77)
Gender (M:F%)	42:58	30:70	44:56	78:22	86:14	30:70	29:71
BMI (kg/m²)	27.24±6.35 (16.65-49.20)	27.26±6.03 (16.65-37.80)	26.51±5.24 (19.00-38.02)	26.01±3.80 (21.3-33.6)	39.87±6.80 (30.0-49.2)	24.70±3.00 (20.1-28.7)	24.32±3.96 (18-31.7)
FIVC (L)	2.49±1.11 (0.40-5.42)	2.38±0.74 (1.17-3.78)	2.22±0.81 (0.71-3.59)	2.00±1.84 (0.40-5.42)	3.49±1.18 (1.41-4.74)	2.23±0.93 (0.87-3.85)	3.19±1.25 (1.02-5.25)
PIFR (L/min)	187.3±93.6 (28-456)	205.7±85.4 (59-415)	155.5±66.0 (55-275)	138.1±105.9 (28-323)	233.4±98.0 (104-389)	147.0±73.3 (35-292)	245.8±114.9 (59-456)
FEV₁ (L)	2.17±1.12 (0.24-5.07)	1.82±0.92 (0.82-4.59)	1.75±0.94 (0.24-3.80)	2.65±1.65 (0.33-5.07)	2.98±0.84 (1.58-3.97)	2.07±1.03 (0.84-3.97)	2.93±0.95 (1.02-5.07)
FVC (L)	2.88±1.19 (0.38-5.66)	2.51±0.96 (1.23-5.40)	2.58±0.98 (0.38-4.30)	3.09±1.89 (0.38-5.66)	3.71±1.05 (1.89-4.96)	2.74±1.15 (1.08-4.96)	3.65±1.11 (1.37-5.66)
FEV₁/FVC	0.74±0.15 (0.35-0.99)	0.71±0.16 (0.44-0.94)	0.65±0.18 (0.35-0.89)	0.87±0.10 (0.71-0.99)	0.82±0.06 (0.71-0.89)	0.74±0.11 (0.50-0.87)	0.80±0.08 (0.70-0.92)

Table 2: Demographics and baseline lung function tests for patients by disease category.

^a BMI – Body Mass Index

^b FEV₁ – Forced Expiratory Volume in 1 second

^c FVC – Forced Vital Capacity

^d PEF_R – Peak Expiratory Flow Rate

^e FIVC – Forced Inspiratory Vital Capacity

^f PIF_R – Peak Inspiratory Flow Rate

Reference Method (L/min)	Test Method (L/min)	Sensitivity	Specificity	Correctly Classified
≥30	≥33.55	95.12%	90.00%	94.57%
≥45	≥47.91	91.67%	90.62%	91.30%
≥60	≥66.27	90.48%	96.00%	93.48%
≥90	≥90.57	100.00%	91.86%	92.39%

Table 3: Table showing threshold values of acoustic method for which most inhalations are correctly classified, with corresponding Sensitivity and Specificity. Reference Method represents Spirometric values and Test Method represents Acoustic Method.

Figures

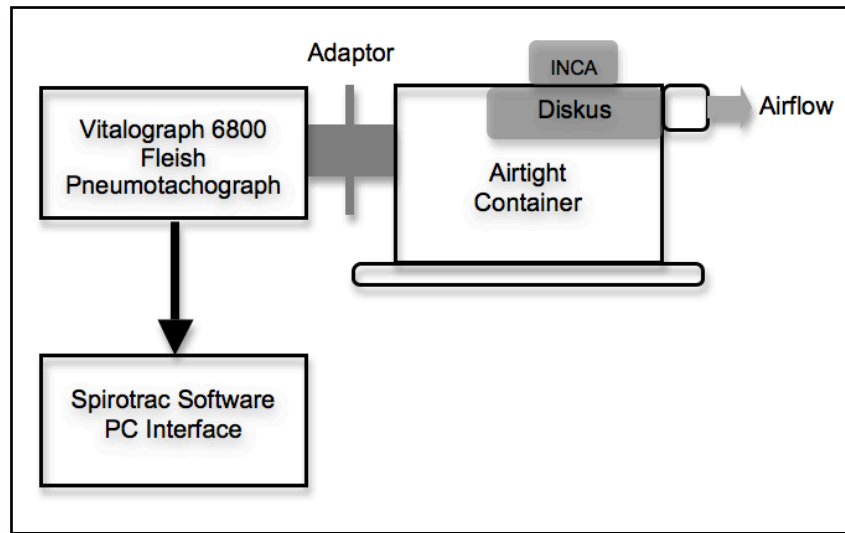


Figure 1: Apparatus setup for Study 1: spirometer with PC connection, airtight container and INCA Device.

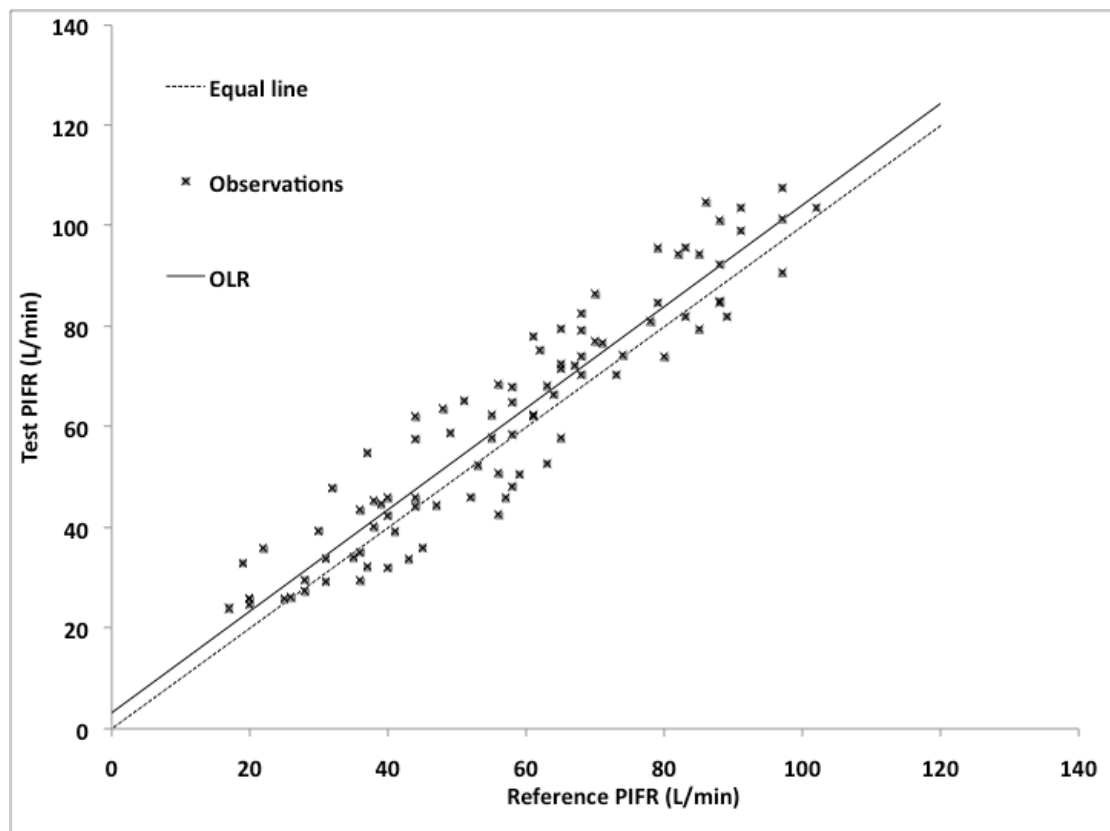


Figure 2: Scatter plot of Test or Acoustically Determined PIFR vs Reference or Spirometrically Determined PIFR. The equal line represents no difference between methods ($y=x$). The Ordinary Least Squares Regression line is also shown ($R^2 = 0.884$, Test PIFR = $1.01 \times \text{Reference PIFR} + 3.18$, Mean bias = 3.78, Mean Relative bias = 6.6%).

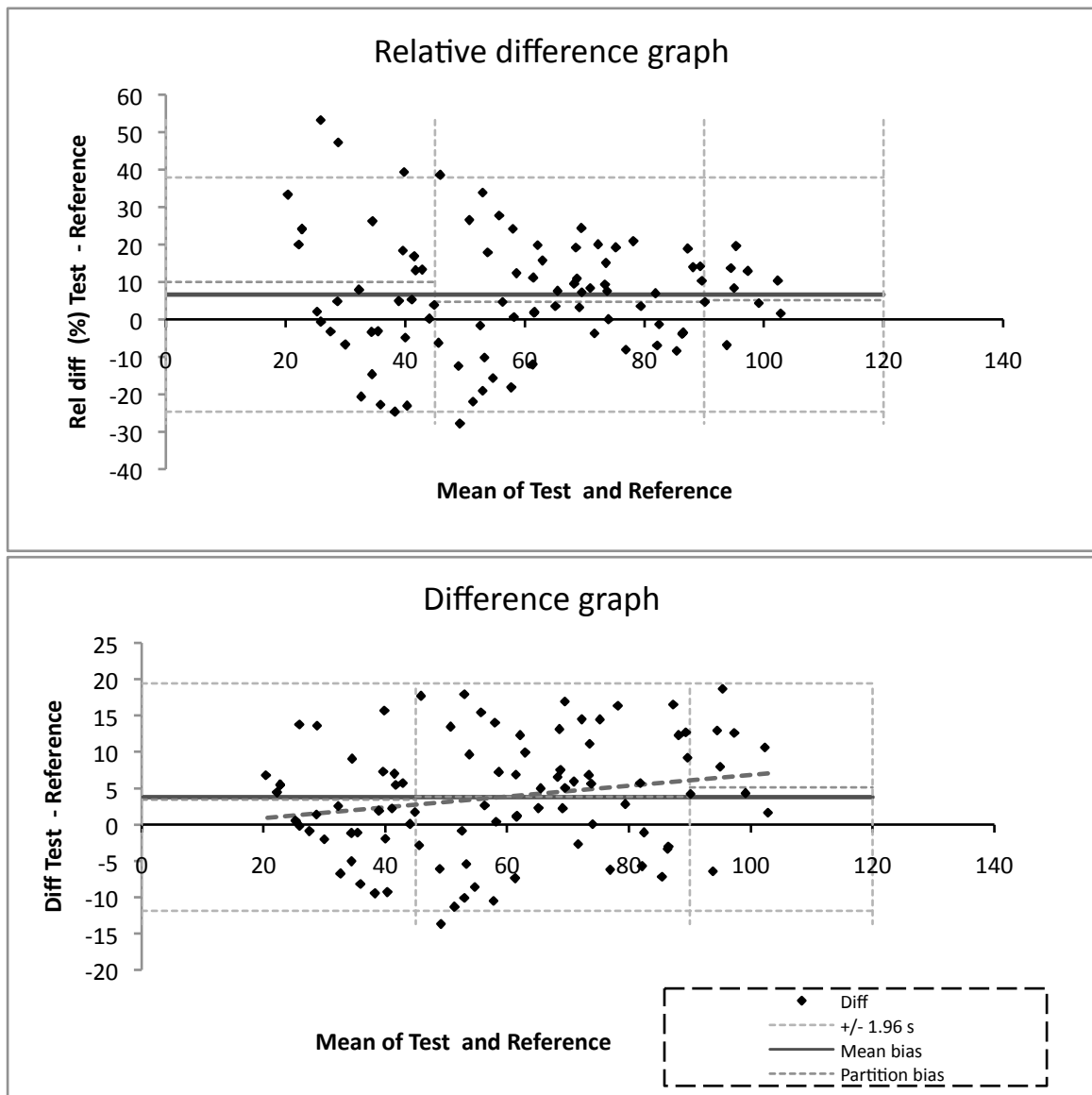


Figure 3: Difference and Relative Difference Plots for Acoustically-determined PIFRc versus Spirometrically-determined PIFRm.

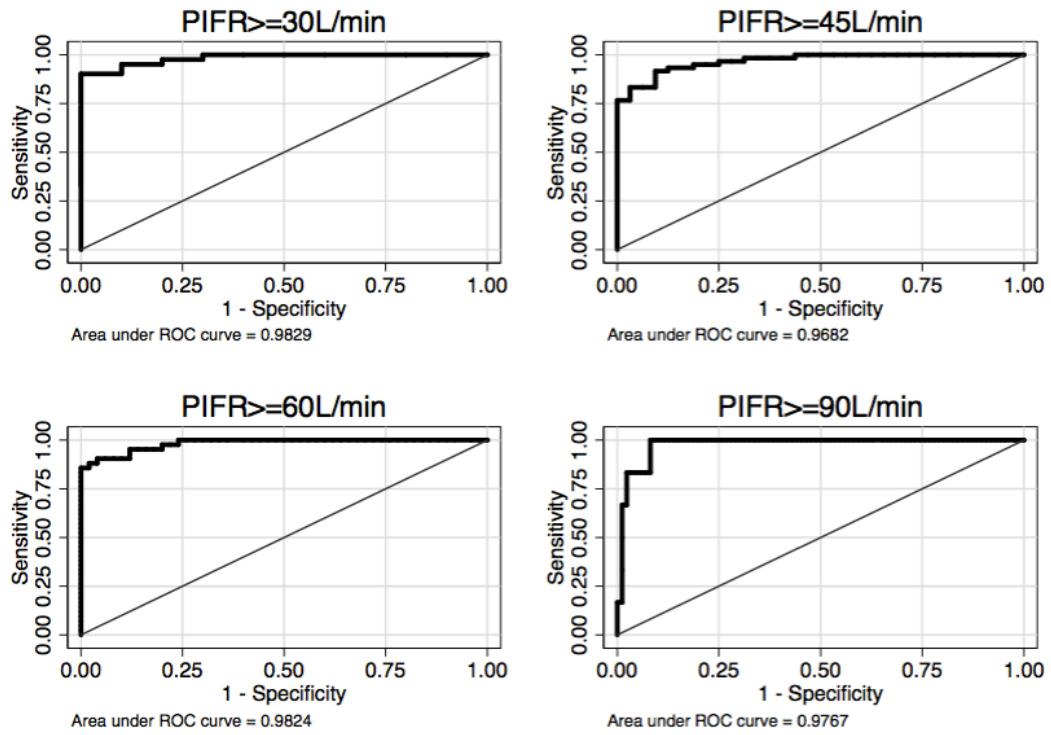


Figure 4: Receiver Operating Characteristic (ROC) Curves for Acoustically Determined PIFR versus thresholds of measured PIFR of 30, 45, 60 and 90 L/min. The equal line represents an AUC of 0.5.

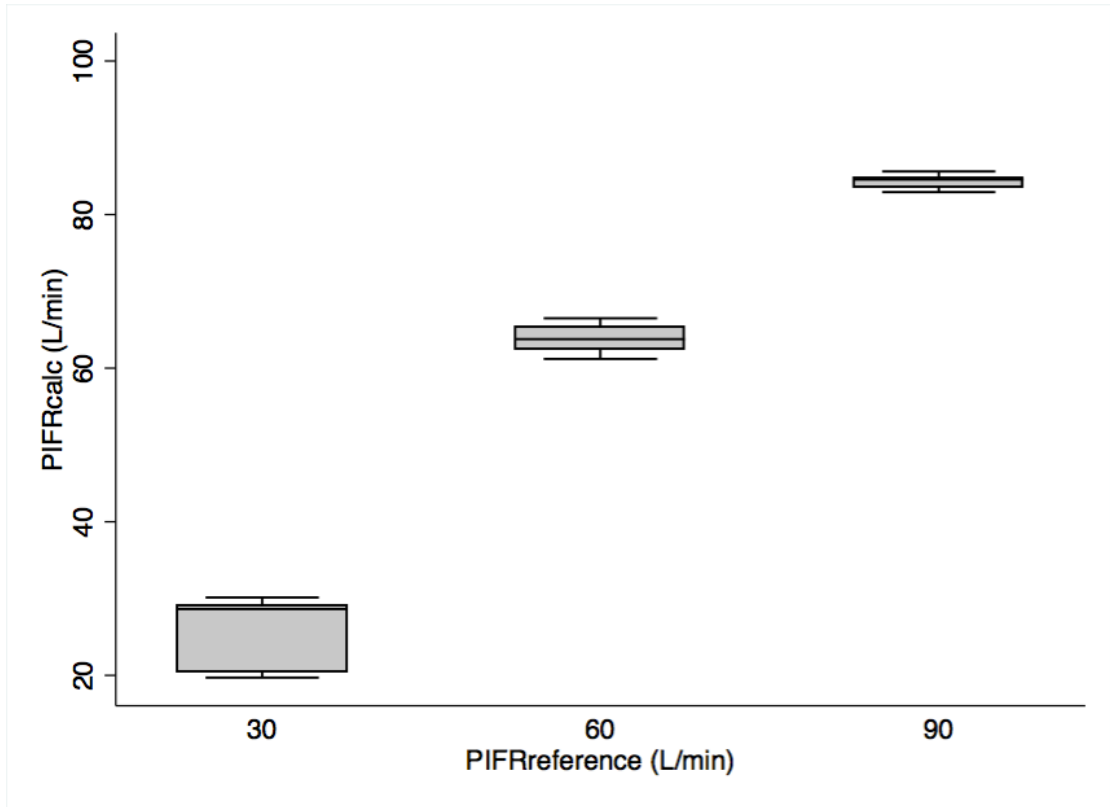


Figure 5: Boxplot of calculated PIFR at each preset PIFR for the NGI Impactor (PIFRm).

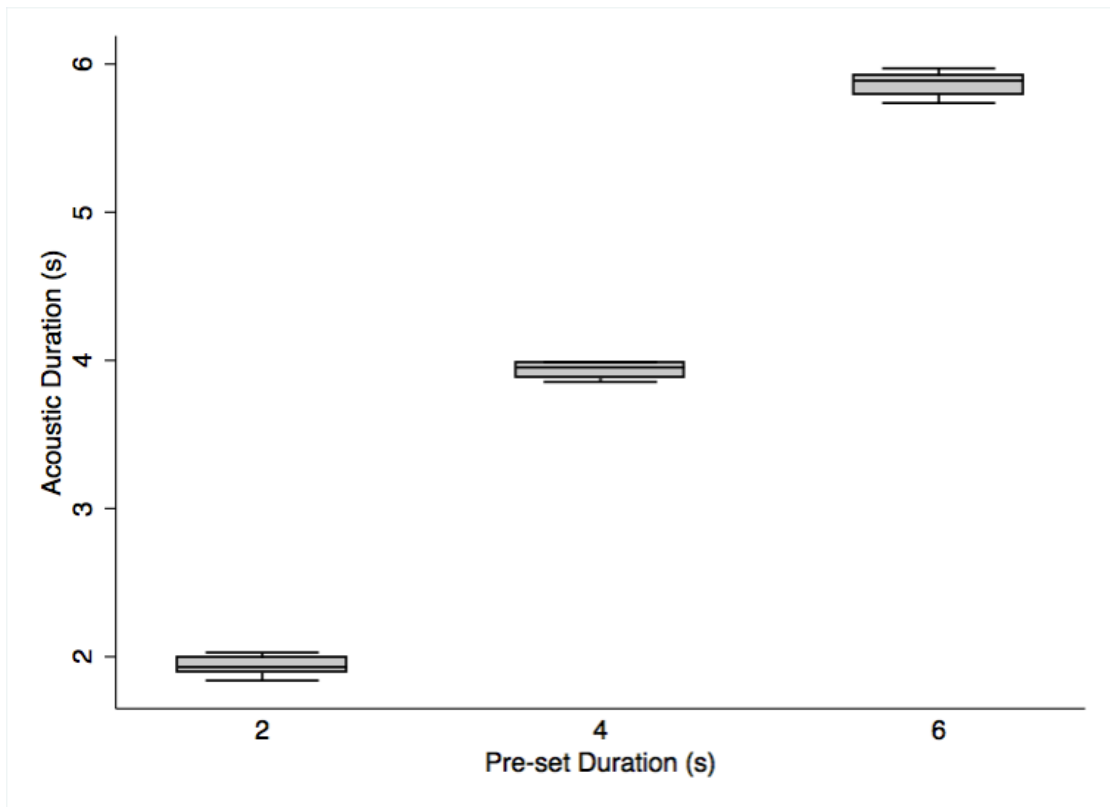


Figure 6: Boxplot of acoustic duration categorized by preset Flow Controller duration for the NGI Impactor.

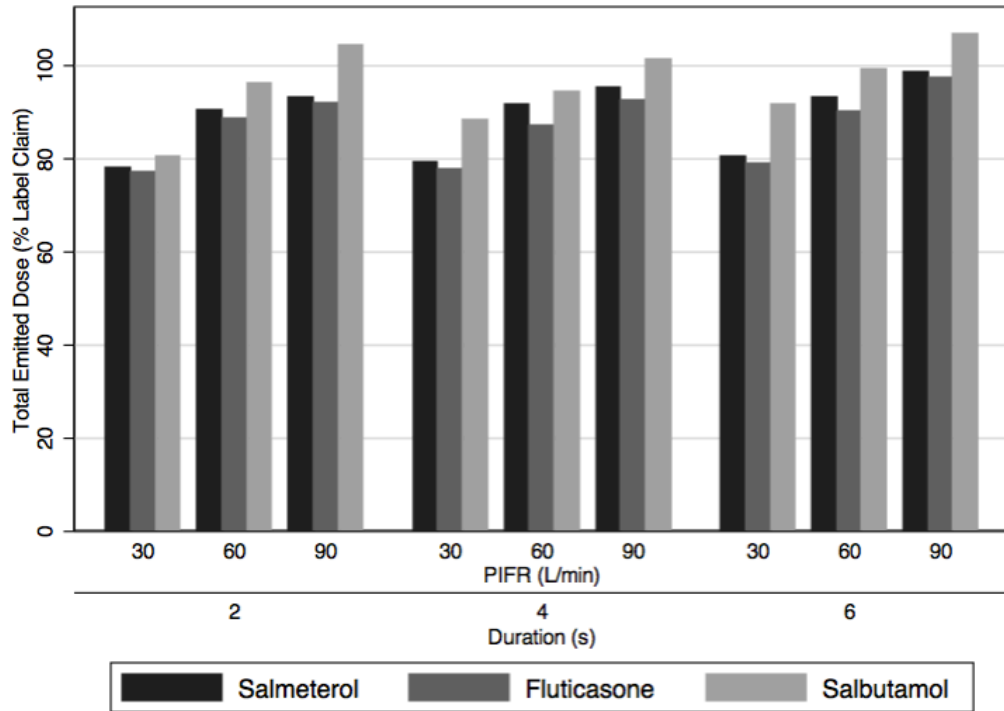


Figure 7: Vertical bar graph of Total Recovey as a % of label claim versus calculated PIFR for Salmeterol and Fluticasone for (a) 2 second inhalation, (b) 4 second inhalation and (c) 6 second inhalation.

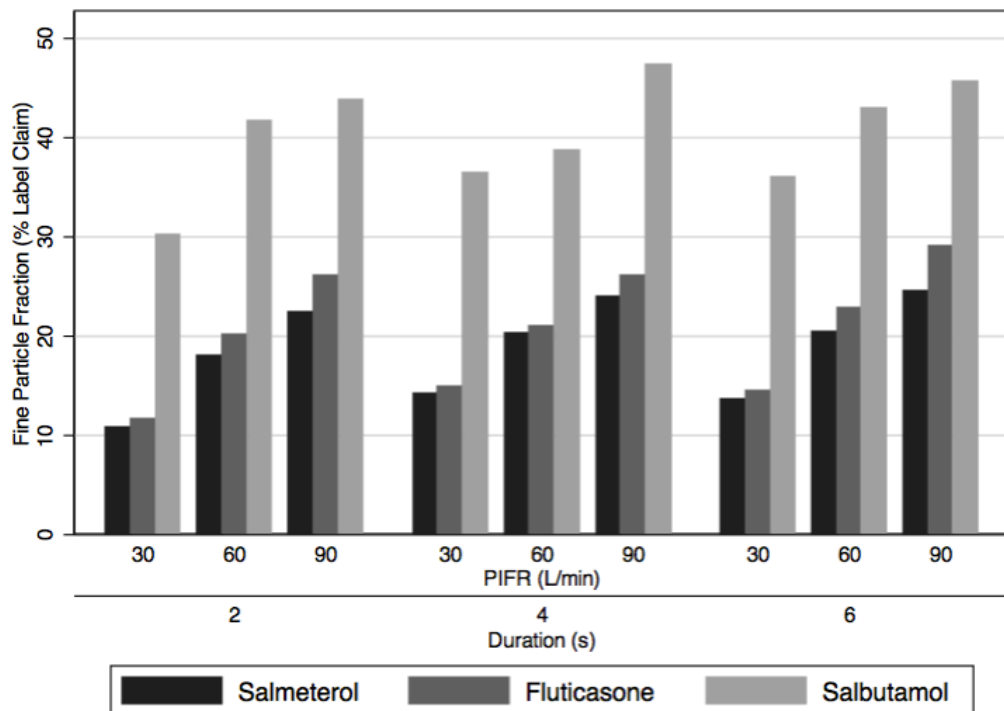


Figure 8: Vertical bar graph of Fine Particle Deposition (less than 5 micron diameter) as a % of label claim versus calculated PIFR for Salmeterol and Fluticasone for (a) 2 second inhalation, (b) 4 second inhalation and (c) 6 second inhalation.

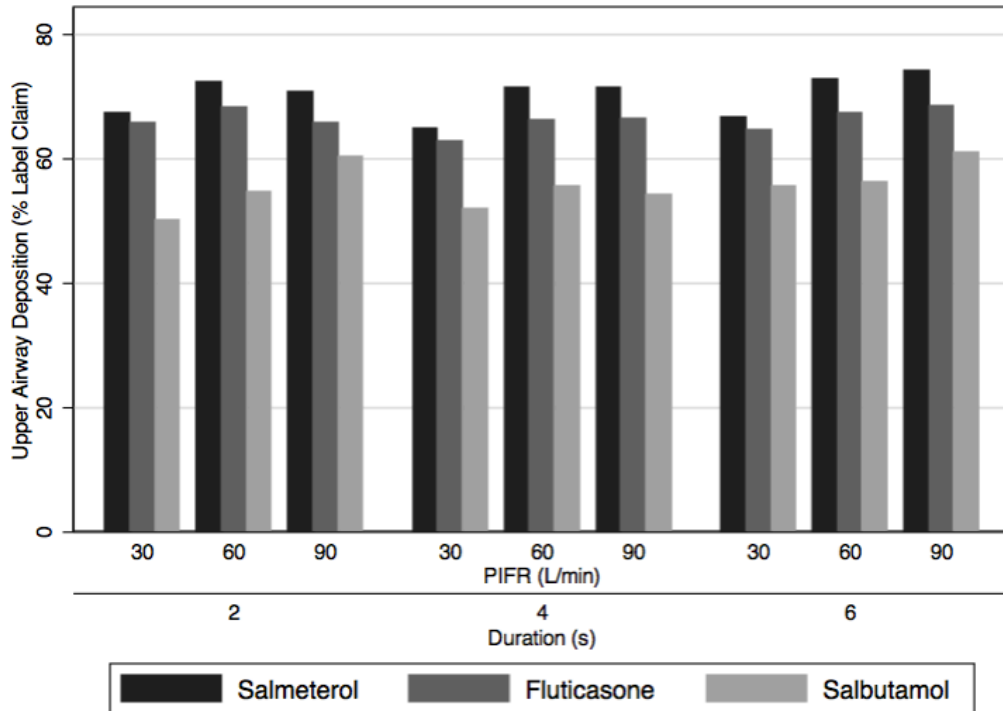


Figure 9: Vertical bar graph of Upper Airway Deposition as a % of label claim versus calculated PIFR for Salmeterol and Fluticasone for (a) 2 second inhalation, (b) 4 second inhalation and (c) 6 second inhalation.

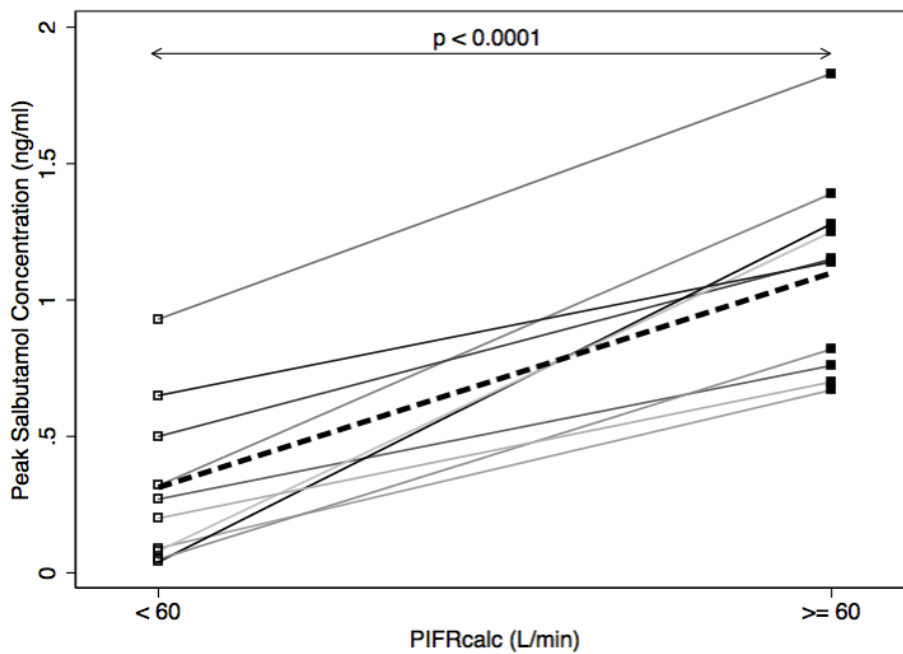


Figure 10: Line and dot plot of peak serum concentration of Salbutamol versus flow rate category (less than or greater than 60 L/min) for ten healthy subjects. Each line represents a separate individual and points represent actual values of concentration and calculated PIFR. The dotted line represents the overall regression line for all the data points. P- value for difference in means between high flow rate and low flow rate groups is less than 0.0001.

Supplementary Appendix

Salmeterol									
Flow Rate (L/min)	30			60			90		
Duration (s)	2	4	6	2	4	6	2	4	6
Throat (mcg)	6.66	5.37	5.81	5.96	6.43	4.99	5.36	5.40	4.51
PS^a (mcg)	24.60	24.05	24.73	27.81	27.05	28.57	27.36	27.36	29.53
S1 (mcg)	1.03	1.10	1.03	2.01	2.03	2.40	3.87	4.25	4.20
S2 (mcg)	1.72	2.42	2.28	3.37	3.35	3.82	3.85	4.07	4.55
S3 (mcg)	2.50	3.15	2.88	3.30	3.42	3.75	3.36	3.61	3.50
S4 (mcg)	2.05	2.76	2.69	2.32	2.68	2.50	2.37	2.48	2.47
S5 (mcg)	BLOQ	0.74	0.65	BLOQ	0.87	BLOQ	BLOQ	BLOQ	BLOQ
S6 (mcg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
S7 (mcg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
MOC^b (mcg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
TED^c (mcg)	39.07	39.60	40.23	45.21	45.82	46.60	46.62	47.65	49.32
FPD^d (mcg)	5.39	7.13	6.63	9.26	10.15	10.48	11.86	12.67	13.00
MMAD^e (mcm)	5.18	5.08	4.95	4.26	4.03	4.32	3.83	3.86	3.91
GSD^f	1.77	1.80	1.88	1.81	1.89	1.87	2.23	2.26	2.12

Table 1: Next Generation Impactor Salmeterol Deposition by Flow Rate and Duration of Inhalation.

^a PS – Pre-separator

^b MOC – Micro-orifice Collector

^c TED – Total Emitted Dose

^d FPD – Fine Particle Dose

^e MMAD – Mass Median Aerodynamic Diameter

^f GSD – Geometric Standard Deviation

BLOQ – Below Limit of Quantification (0.63 micrograms)

Fluticasone									
Flow Rate (L/min)	30			60			90		
Duration (s)	2	4	6	2	4	6	2	4	6
Throat (mcg)	32.72	25.17	28.54	29.77	32.11	23.98	26.64	27.92	23.29
PS^a (mcg)	119.13	116.82	118.02	130.41	123.50	131.70	128.31	128.04	137.15
S1 (mcg)	5.13	5.29	5.04	9.74	9.45	11.10	18.12	19.50	19.65
S2 (mcg)	8.58	12.05	11.58	16.69	13.42	19.45	20.25	20.65	24.74
S3 (mcg)	13.05	16.73	15.23	17.92	17.97	21.16	19.60	18.66	20.88
S4 (mcg)	11.22	14.00	15.26	13.77	13.08	13.86	13.91	13.58	14.94
S5 (mcg)	3.38	4.52	3.66	2.98	8.35	3.82	2.84	2.84	2.96
S6 (mcg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
S7 (mcg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
MOC^b (mcg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
TED^c (mcg)	193.22	194.59	197.54	221.28	217.87	225.07	229.68	231.19	243.62
FPD^d (mcg)	28.83	36.82	35.48	50.35	52.53	56.93	65.42	65.11	72.64
MMAD^e (mcm)	4.98	4.98	4.84	4.03	3.63	4.10	3.47	3.65	3.63
GSD^f	1.79	1.78	1.87	1.82	1.86	1.77	2.22	2.23	2.08

Table 2: Next Generation Impactor Fluticasone Deposition by Flow Rate and Duration of Inhalation.

^a PS – Pre-separator

^b MOC – Micro-orifice Collector

^c TED – Total Emitted Dose

^d FPD – Fine Particle Dose

^e MMAD – Mass Median Aerodynamic Diameter

^f GSD – Geometric Standard Deviation

BLOQ – Below Limit of Quantification (1.50 micrograms)

Salbutamol									
Flow Rate (L/min)	30			60			90		
Duration (s)	2	4	6	2	4	6	2	4	6
Throat (mcg)	14.31	14.77	15.38	15.13	15.18	23.15	19.52	21.88	18.61
PS^a (mcg)	60.89	62.56	67.96	77.88	79.28	67.38	87.57	73.21	90.58
S1 (mcg)	15.00	15.29	17.45	15.32	15.45	18.97	17.56	17.21	16.40
S2 (mcg)	13.42	15.26	14.29	11.27	11.93	17.26	13.38	13.73	13.05
S3 (mcg)	19.48	20.14	22.03	19.12	16.95	21.78	14.98	16.09	16.33
S4 (mcg)	21.90	24.67	23.16	25.90	24.94	29.68	18.77	22.58	21.30
S5 (mcg)	10.42	11.49	11.68	14.14	12.43	13.74	15.27	18.46	16.13
S6 (mcg)	4.12	3.64	3.87	5.59	3.78	2.45	6.77	6.68	6.90
S7 (mcg)	BLOQ	3.66	3.76	3.59	3.81	2.14	6.85	6.30	6.60
MOC^b (mcg)	BLOQ	5.20	3.69	4.62	4.74	2.04	7.72	6.75	7.47
TED^c (mcg)	160.64	176.68	183.26	192.57	188.49	198.59	208.39	202.90	213.37
FPD^d (mcg)	60.52	72.90	72.08	83.29	77.49	85.87	87.66	94.55	91.41
MMAD^e (mcm)	4.51	4.08	4.32	2.60	2.67	3.06	1.92	1.87	1.88
GSD^f	2.24	2.44	2.29	2.17	2.12	2.42	2.94	2.59	2.66

Table 3: Next Generation Impactor Salbutamol Deposition by Flow Rate and Duration of Inhalation.

^a PS – Pre-separator

^b MOC – Micro-orifice Collector

^c TED – Total Emitted Dose

^d FPD – Fine Particle Dose

^e MMAD – Mass Median Aerodynamic Diameter

^f GSD – Geometric Standard Deviation

BLOQ – Below Limit of Quantification (2.00 micrograms)

<i>Demographics</i>	<i>All subjects</i>
Age (years), mean (SD)	31.1 (9.6)
Sex, M:F (%)	70:30
Body mass index (kg/m ²), mean (SD)	23.1 (2.7)
Height (cm), mean (SD)	173.7 (10.0)
Weight (kg), mean (SD)	69.5 (9.8)
Race, n (%)	
White – White/ Caucasian/ European	8 (80)
Asian – Central/ South Asian	2 (20)

Table 4: Demographics of ten healthy volunteers recruited for pharmacokinetic study.



Figure 1: INCA Device and functional position on Diskus™ Inhaler

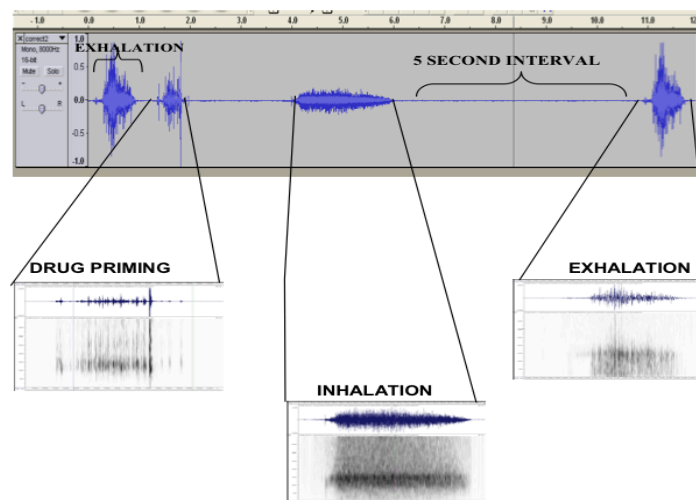


Figure 2: A sample of the acoustic profile obtained from INCA device. The amplitude of the inhalational signal varies proportionately with inhalational flow rate.

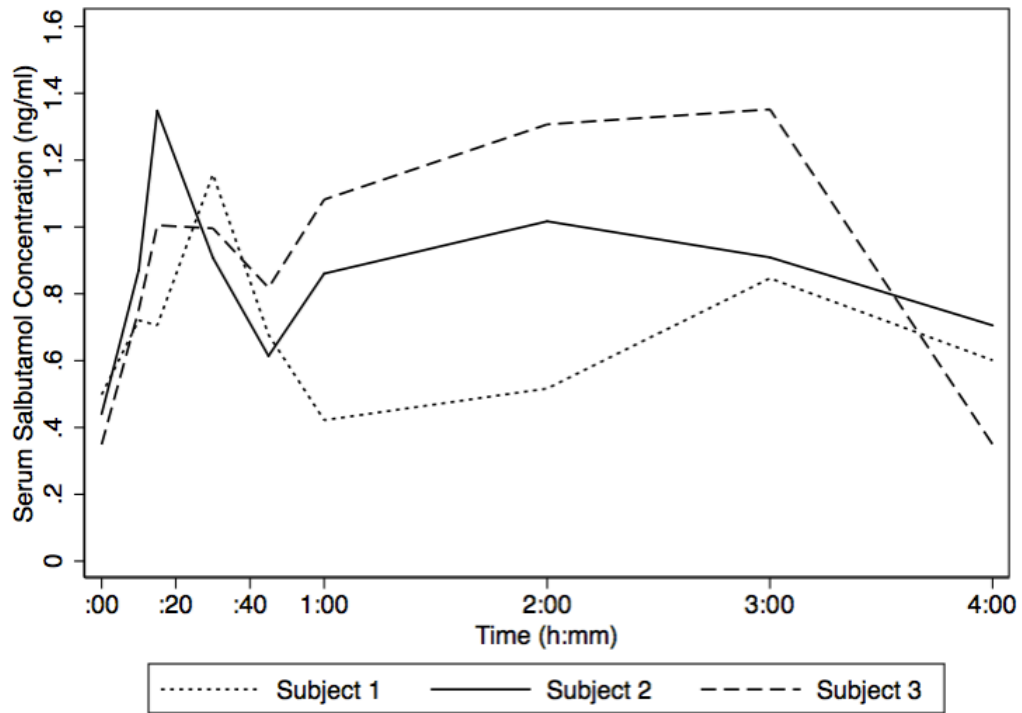


Figure 3: Line graph showing serum drug concentration versus time post-inhalation of a 200 microgram dose of Salbutamol via Diskus inhaler for three healthy individuals. Note the two distinct peaks in drug concentration at 20-25 minutes and at 2-3 hours.