

Primary Amino Acid Sequence of recombinant Human Arginase 1 (hArg1) expressed in *E. coli*
(SEQ ID NO: 1)

10 20 30 40 50 60
SAKSRITIGII GAPFSKGQPR GGVEEGPTVL RKAGLLEKLK EQECDVKDYG DLPFADIPND
70 80 90 100 110 120
SPFQIVKNPR SVGKASEQLA GKVAEVKKNG RISLVLGGDH SLAIGSISGH ARVHPDLGVI
130 140 150 160 170 180
WVDAHTDINT PLTTSGNLH GQPVSFLLKE LKGKIPDVPG FSWVTPCISA KDIVYIGLRD
190 200 210 220 230 240
VDPGEHYILK TLGIKYFSMT EVDRLGIGKV MEETLSYLLG RKKRPIHLSF DVDGLDPSFT
250 260 270 280 290 300
PATGTPVVGG LTYREGLYIT EEIYKTGLLS GLDIMEVNPS LGKTPEEVTR TVNTAVAITL
310 320
ACFGLAREGN HKPIDYLNPP K

FIG. 1(a)

DNA sequence of human Arginase optimized for expression in *E. coli* (SEQ ID NO: 2).

ATGTCGGAAGAGCCGTACGATCGGCATTATTGGTGCGCCGTTCTCTAAAGGTCAGCCACGCCGGTGGTGTGAAGA
GGTCCGACGGTTCTGCGTAAGGCCGGTTTATTAGAAAAGCTGAAAGACAGGAGTGCCGACGTTAAGGACTACGGTG
ACTTACCATTCCGGACATCCCGAATGATAGCCCGTTCCAAATCGTTAAGAATCCGCGCTCTGTGGTAAAGCAAGC
GAGCAGTTAGCAGTAAGGTGCCGAGGTCAAGAAAACGGTCGTATTAGCCTGGTTTAGCGGTGATCATAGCTT
AGCAATTGGCTCTATCTCTGGTCATGCCCCGTGTGCACCCAGATTTAGGTGCATTTGGGTTGACGCCCCATACGGATA
TCAATACGCCATTAAAGACCAACGCGGCAATCTGCATGCCAGCCGGTTAGCTTCTTACTGAAGGAGCTGAAGGGT
AAAATCCAGATGTTCCGGCTTTAGCTGGTCAAGCCATGTATTTCTGCCAAGGATATCGTGATACATTGGCTTACG
TGACGTCGACCCAGGTGAGCACTACATCTTAAAGACCCCTGGGTATCAAGTATTTTCAGCATGACGGAAGTGGACCCGT
TAGGCATCGGCAAGGTGATGGAGAGACGCTGAGCTATCTGCTGGCCGTAAGAAACGTCCAATCCATCTGAGCTTC
GATGTTGACGGCTTAGACCCGAGCTTTACGCCAGCCACCGCGTCGTTGGTGGTTAACGTATCGCGAAGG
CCTGTATATCACGGAGGAAATCTATAAGACGGGTTTACTGAGCGGCTCTGGACATTATGGAGGTTAATCCAAGCTTAG
GTAAGACGCCGGAAGAGTTACCCGTACCCGTTAACACGGCGGTCCGATCACGTTAGCATGTTTCGGTTTAGCCCCG
GAGGGCAACCATAAACCAATTGATTATCTGAATCCACCGAAGTGA

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FIG. 1(b)

Primary Amino Acid Sequence of Arginase 2 (SEQ ID NO: 3)

SLRGSLSRLQLQTRVHSILKKSVHSAVIGAPFSQGQKRKGVHGPAAIREAGLMKRLSSLGCHLKDFGDLSTFPVPK
DDLNNLIVNPRSVGLANQELAEVVSRAVSDGYSCVTGLGDHSLAIGTISGHARHCPDLCVVWVDAHADINTPLTTS
SGNLHGQPVSFLLRELQDKVPQLPGFSWIKPCISSASIVYIGLRDVPPEHFIKKNYDIQYFSMRDIDRLGIQKVME
RTFDLLIGKRQRPIHLSFDIDAFDPTLAPATGTPVGGTTYREGMYIAEEIHNTGLLSALDLVEVNPQLATSEEEAK
TTANLAVDVIASSFGQTREGGHIVYDQLPTPSSPDESENQARVRI

FIG. 1(c)

Fermentation Process for Arginase I

Step	Process Operation
1	Thaw MCB or WCB vial(s)
↓	
2	Inoculum shake flasks 37°C, shaking
↓	
3	Production Fermentation: 100 liter fermentor Growth medium, 36-38°C pH 7.0-7.4 Agitation and aeration Concentrated growth medium feed
↓	
4	IPTG Induction of Arginase I Concentrated growth medium feed Temperature adjustment 18 hours
↓	
5	Harvest and Separate cells by centrifugation Store in cell slurry polycarbonate containers
↓	
6	Process or freeze cell paste $\leq -60^{\circ}\text{C}$

FIG. 2

Overview of Co-Arginase I Purification Process

Step	Process Operation
1	Cell slurry thaw and re-suspension into 25 mM HEPES
↓	
2	Cell disruption by high pressure homogenization Two passes
↓	
3	Centrifugation and filtration to remove cell debris
↓	
4	Cation exchange capture chromatography High salt elution to provide first protein product
↓	
5	Arginase I cobalt loading 10mM CoCl ₂
↓	
6	Ultrafiltration/diafiltration 1 Buffer exchange 1 into 25mM HEPES, 0.1M NaCl Buffer exchange 2 into 50mM Tris, pH 8.4
↓	
7	Anion Exchange flow-thru chromatography to provide second protein product
↓	
8	Capto MMC Mixed mode capture chromatography High salt elution to provide third protein product
↓	
9	Ultrafiltration/diafiltration 2_3 Buffer exchange 1 into 20mM Sodium Phosphate, 50mM NaCl, 1.5% Glycerol, pH 7.4 and concentrate Buffer exchange 2 into 0.1M sodium phosphate, pH 8.4 and concentrate

FIG. 3(a)

Overview of Co-Arginase I Purification Process

Step	Process Operation
	↓
10	Addition solid methoxy PEG succinimidyl carboxymethyl ester (MW 5000) and incubation pH 8.4
	↓
11	Ultrafiltration/diafiltration 4 Buffer exchange into 20mM Sodium Phosphate, 50mM NaCl, 1.5% Glycerol, pH 7.4 and dilute to target concentration
	↓
12	Filtration of Co-ArgI-PEG drug substance, fill in 5L polycarbonate bottles, and freeze at $\leq -60^{\circ}\text{C}$
	↓
13	Release test drug substance

FIG. 3(b)

Cation Exchange Chromatography – Column 1

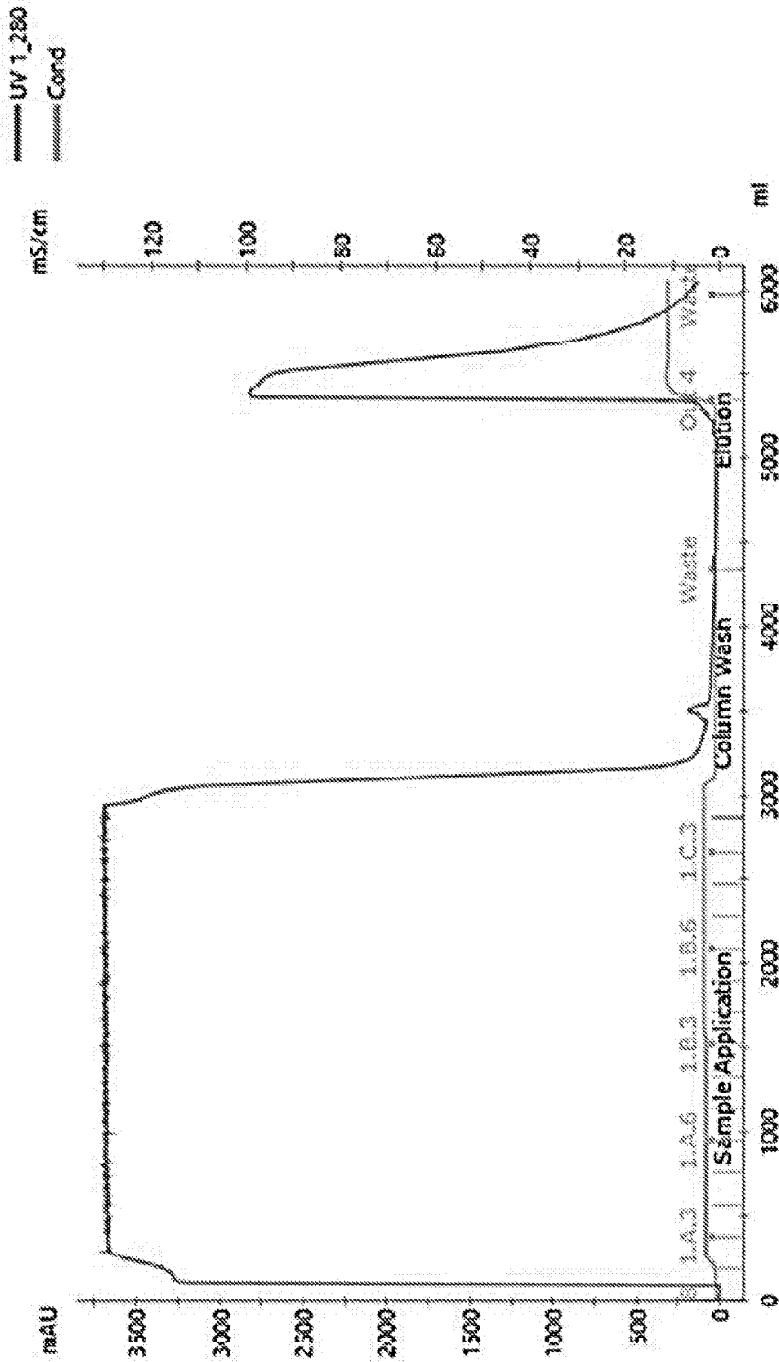


FIG. 4(a)

Anion Exchange Chromatography – Column 2

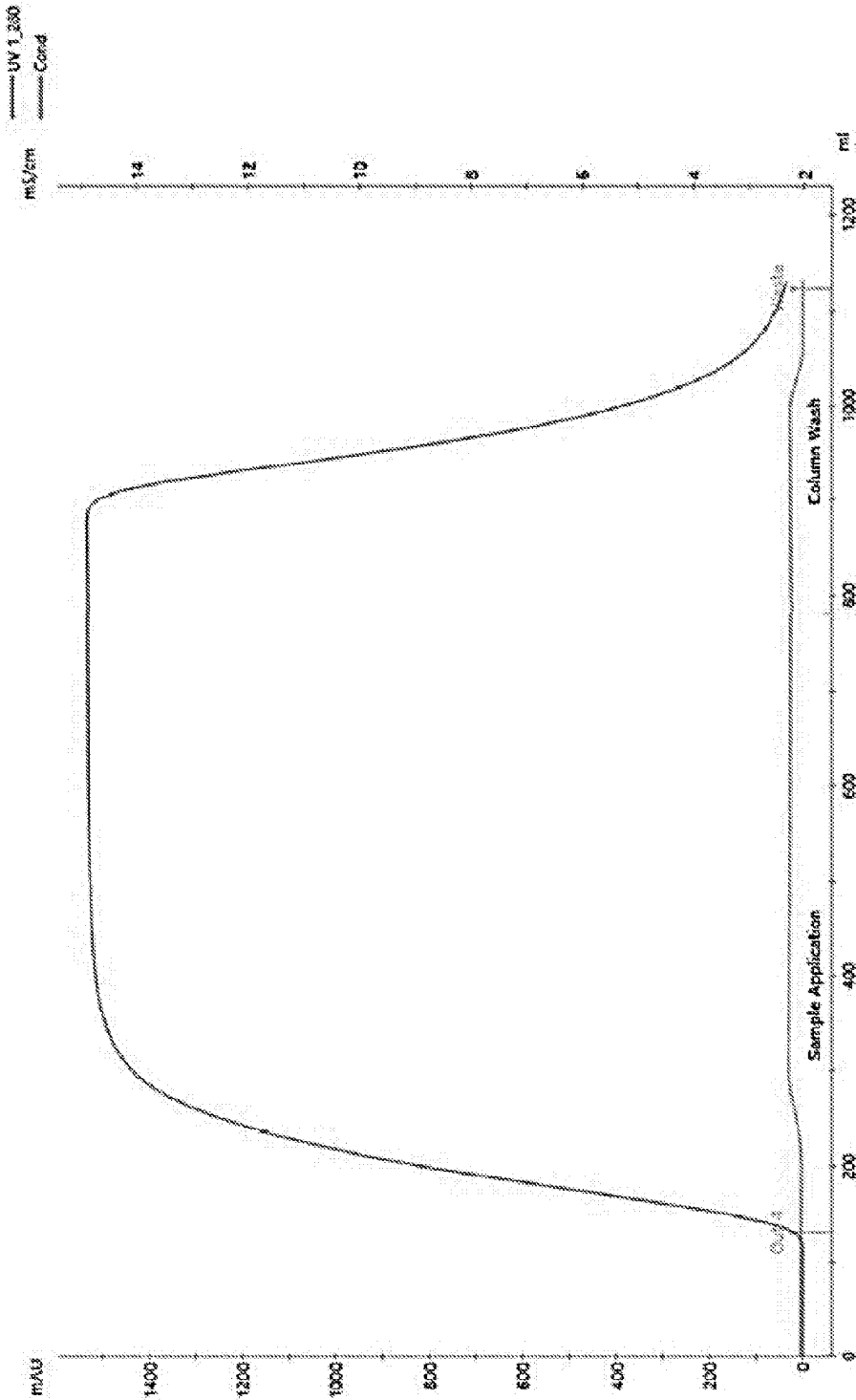


FIG. 4(b)

Capto Multimodal Chromatography – Column 3

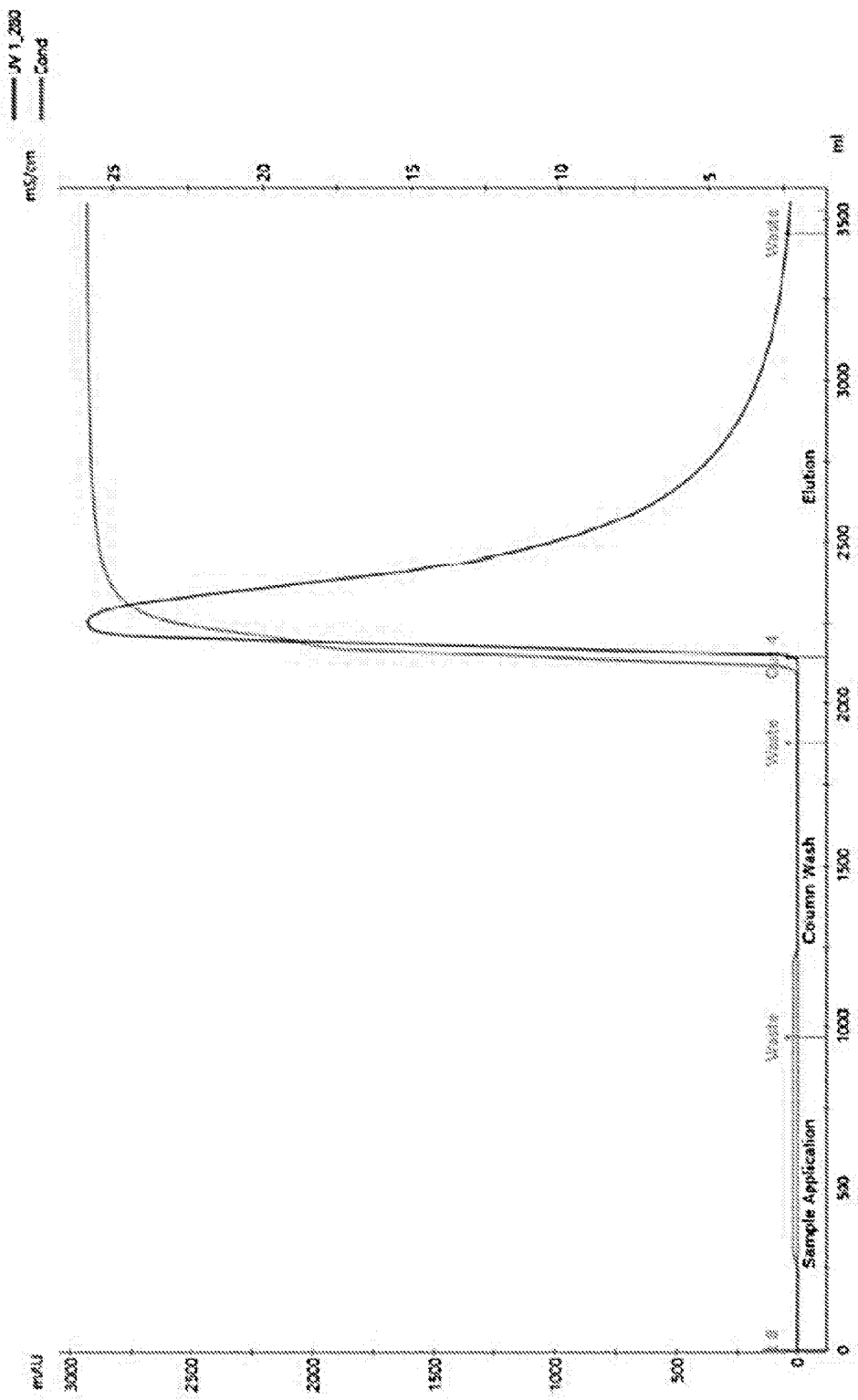


FIG. 4(c)

Charge heterogeneity profile of the Co-Arginase 1 intermediate

Representative Chromatogram of 1 mg/mL Co-Arginase I Intermediate reference standard

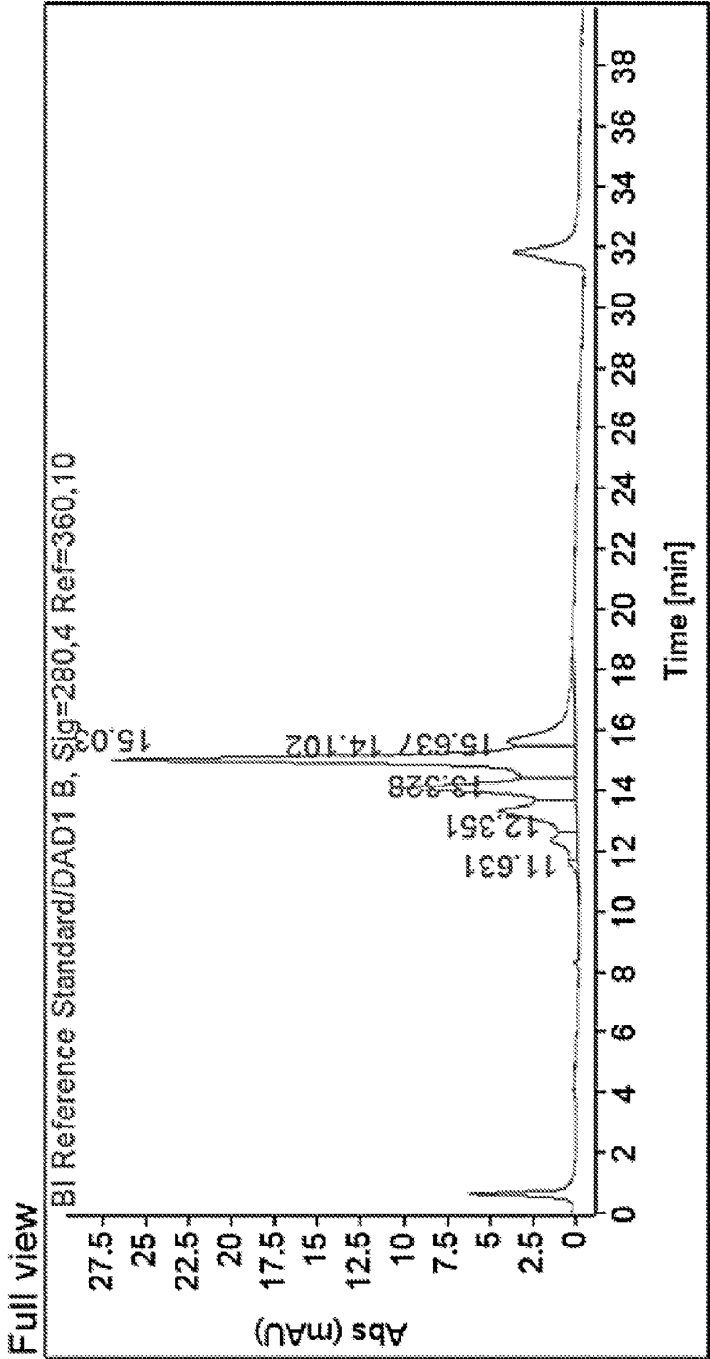


FIG. 5(a)

The same chromatogram as 5(a) with greater magnification of the peaks attributable to Arginase 1.

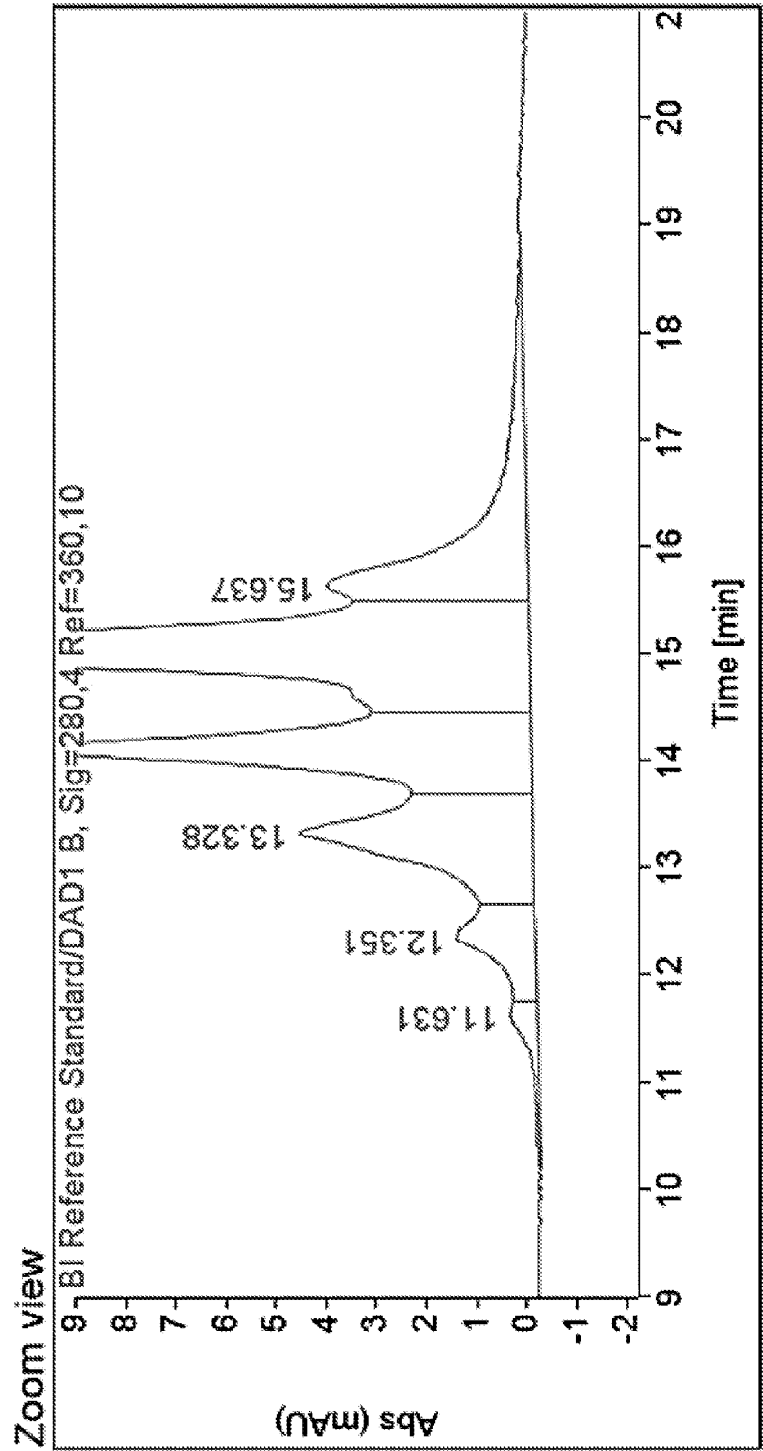


FIG. 5(b)

The assignment of Peak Numbers to the Arginase 1 cation exchange charge variants

Zoom View with Peak Labeling

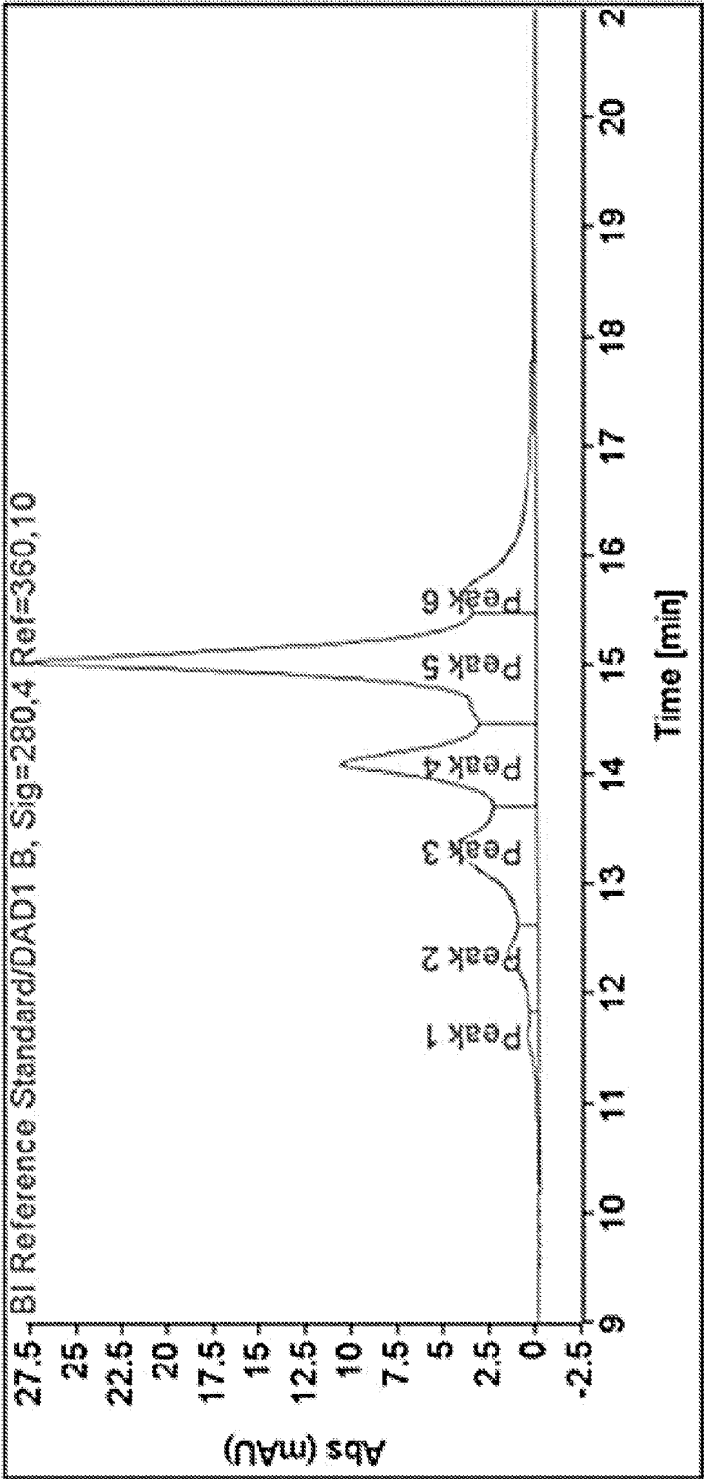


FIG. 5(c)

A typical charge heterogeneity profile of drug substance resolved by an imaging capillary isoelectric focusing (iCIEF) method

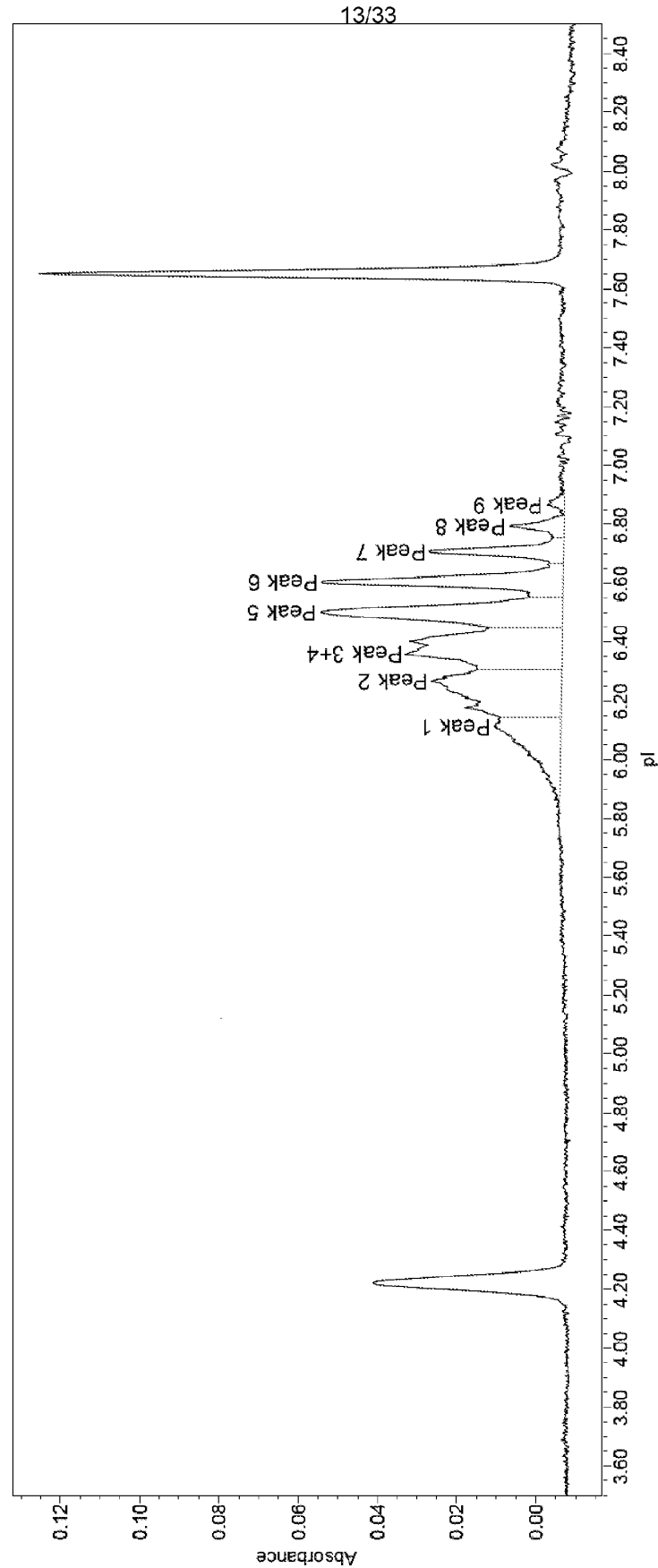


FIG. 5(d)

Analysis of Arginase 1 intermediate gluconylated variants

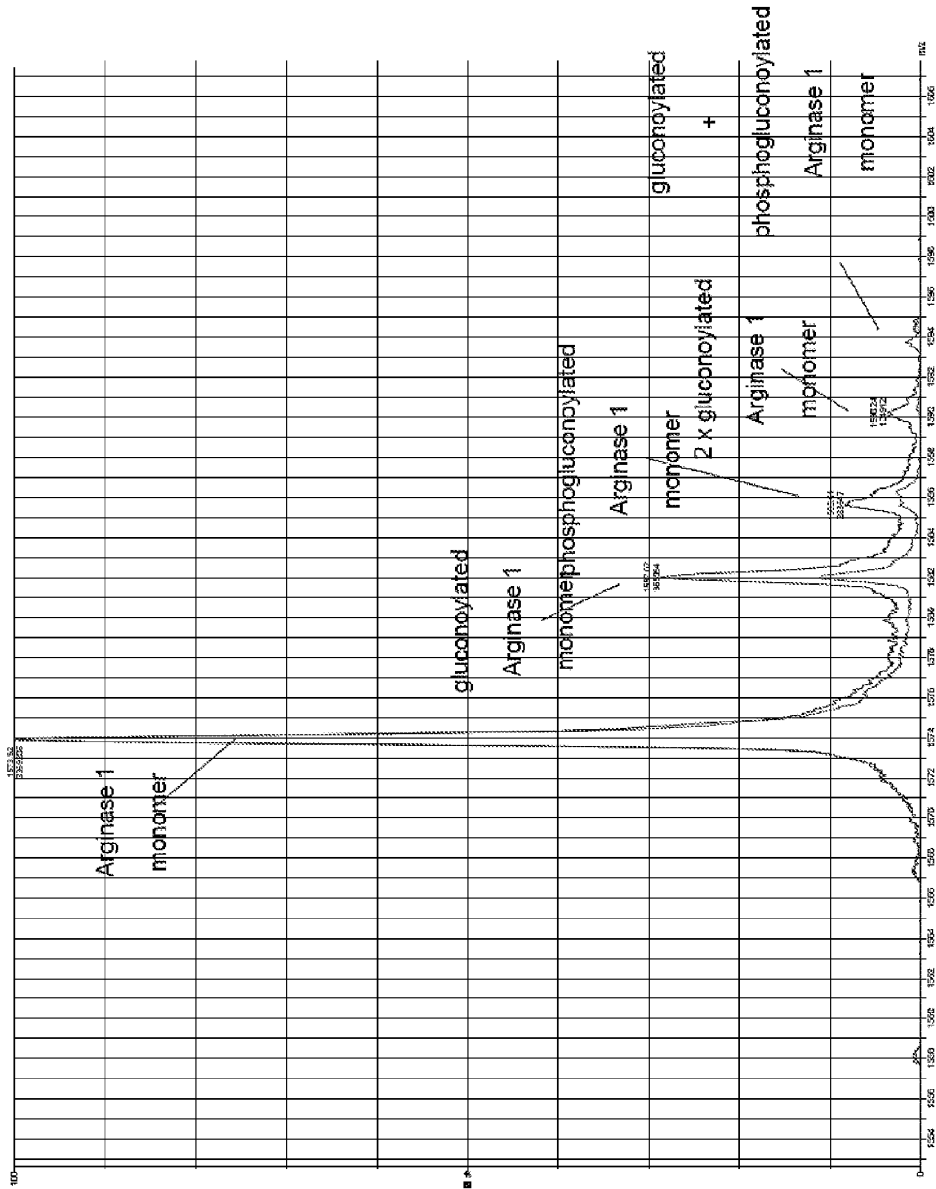


FIG. 6

Gradient of 0.0 - 0.2 M NaCl applied to Column 1

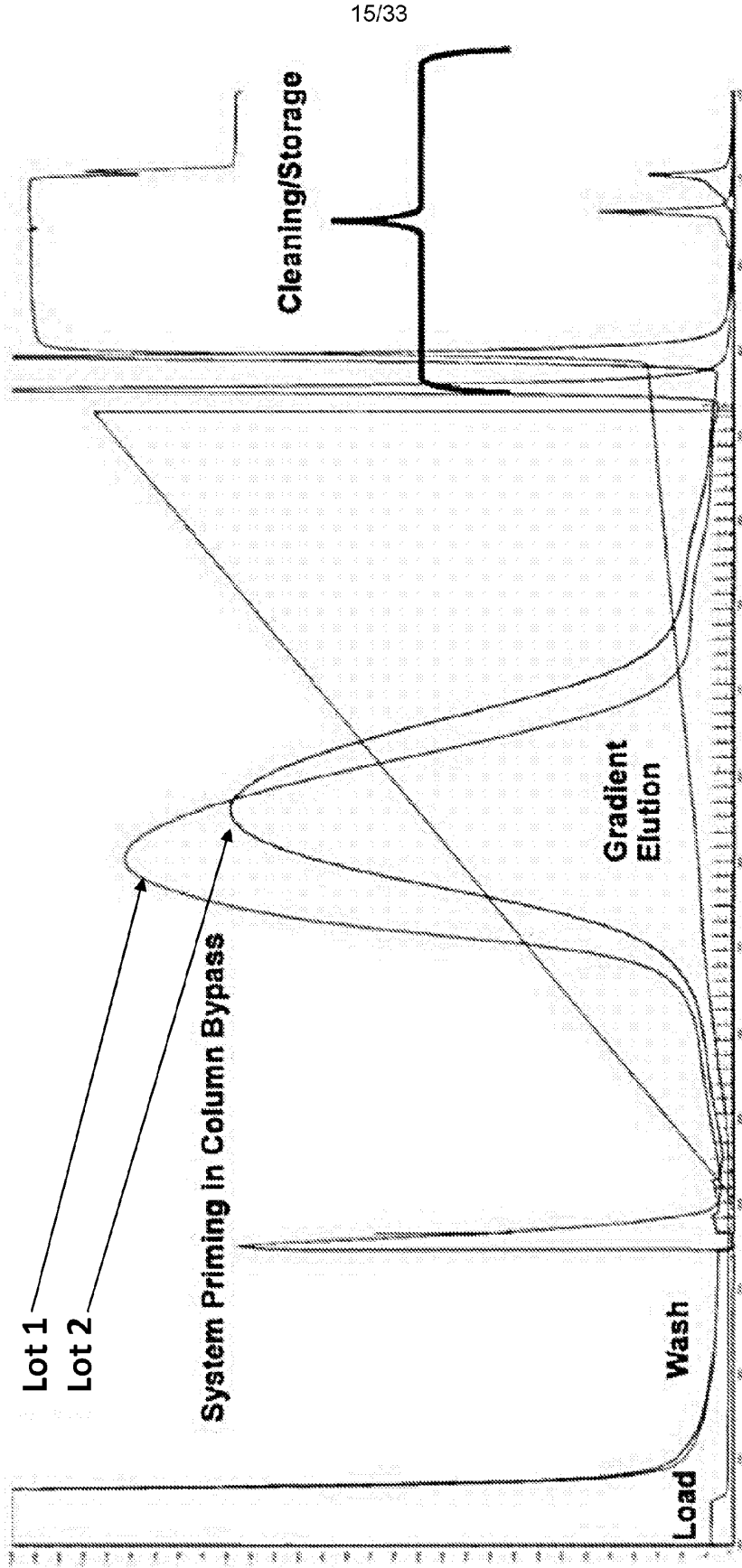
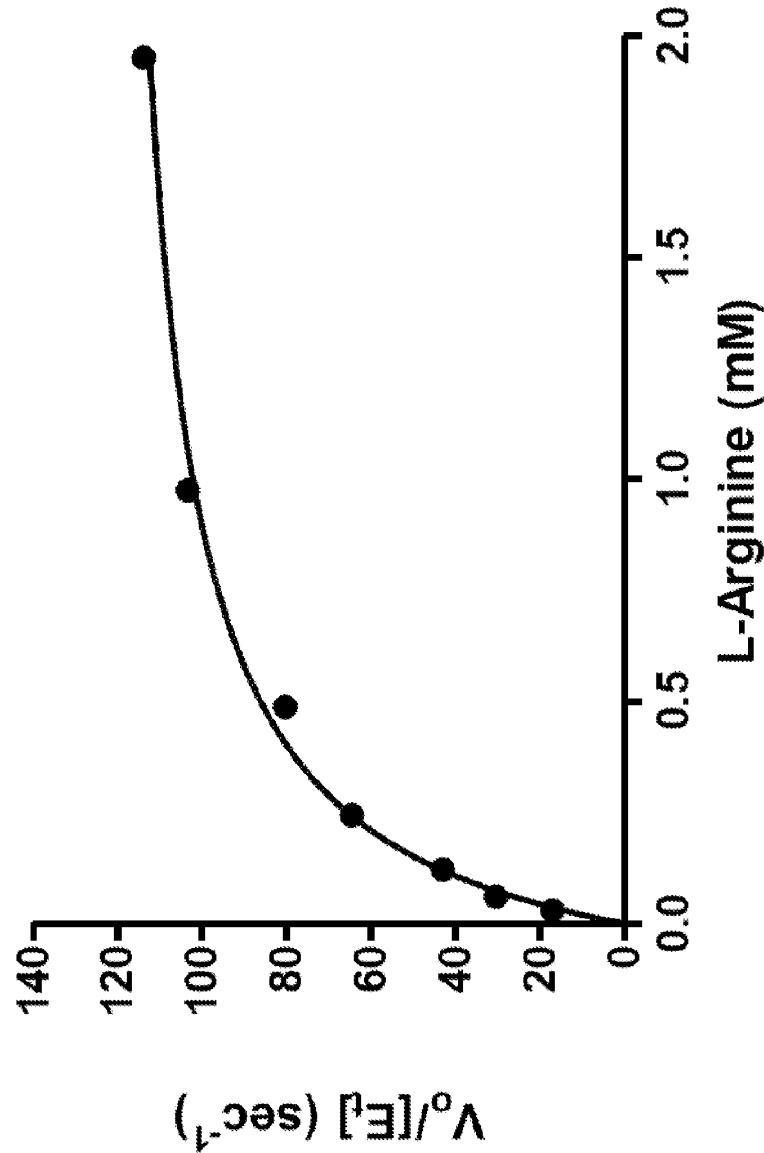


FIG. 7

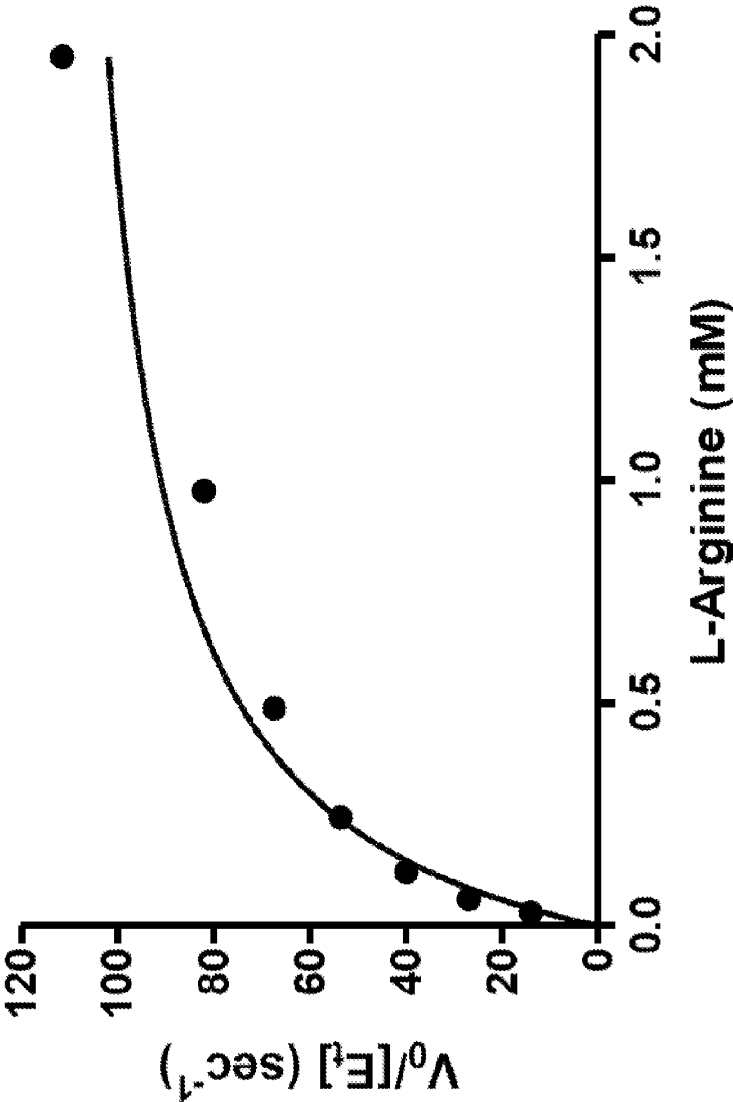
Enzyme Kinetic Analysis of Co-Arginase 1 Intermediate (0-2 mM Arginine)



Lot	k_{cat}	K_m	K_{cat}/K_m
Drug Intermediate	258/sec	0.21 mM	549

FIG. 8(a)

Enzyme Kinetic Analysis of (Co-ArgI-PEG) Drug Substance (0-2 mM Arginine)



Lot	k_{cat}	K_m	K_{cat}/K_m
Drug Substance	307/sec	0.18 mM	415

FIG. 8(b)

Pharmacokinetic Analysis - Linear

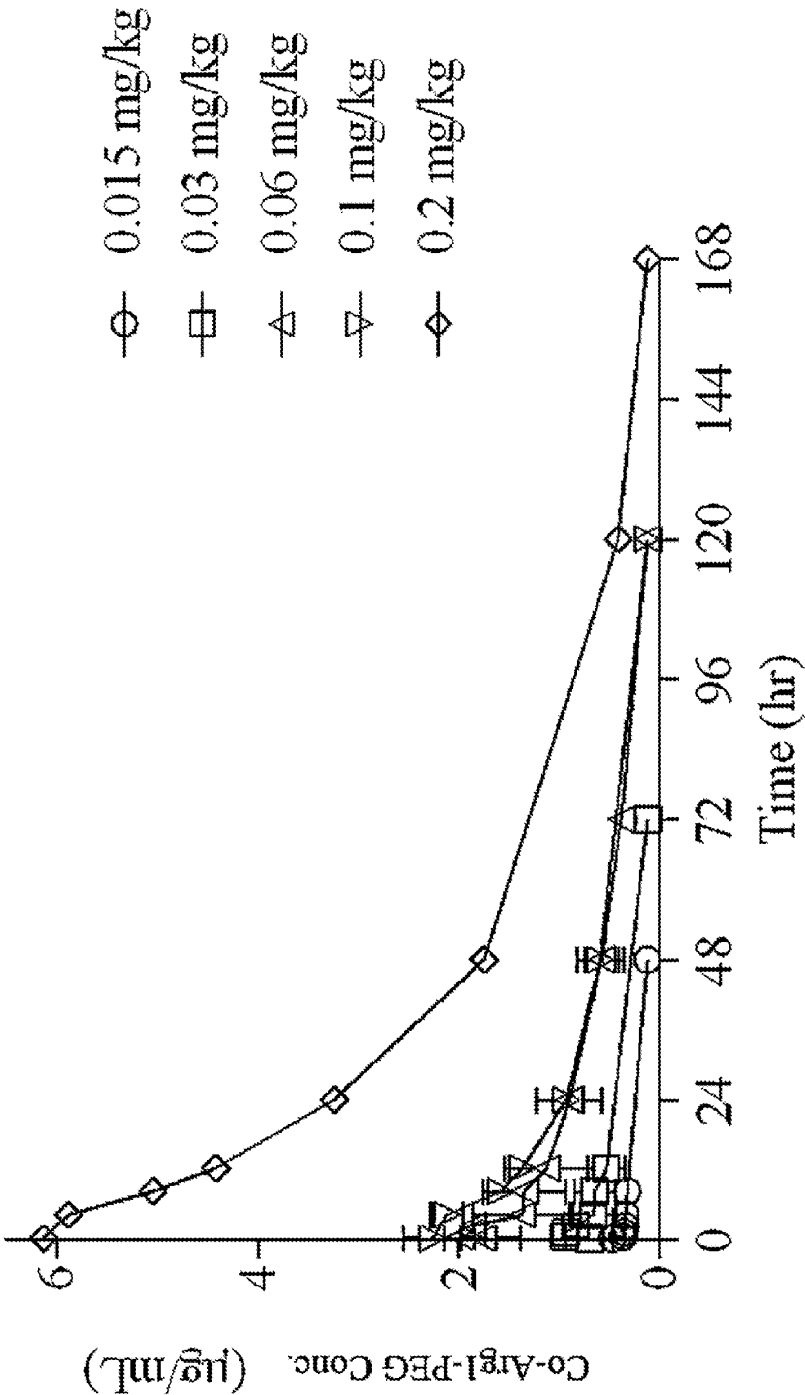


FIG. 9(a)

Pharmacokinetic Analysis – Semi-Log

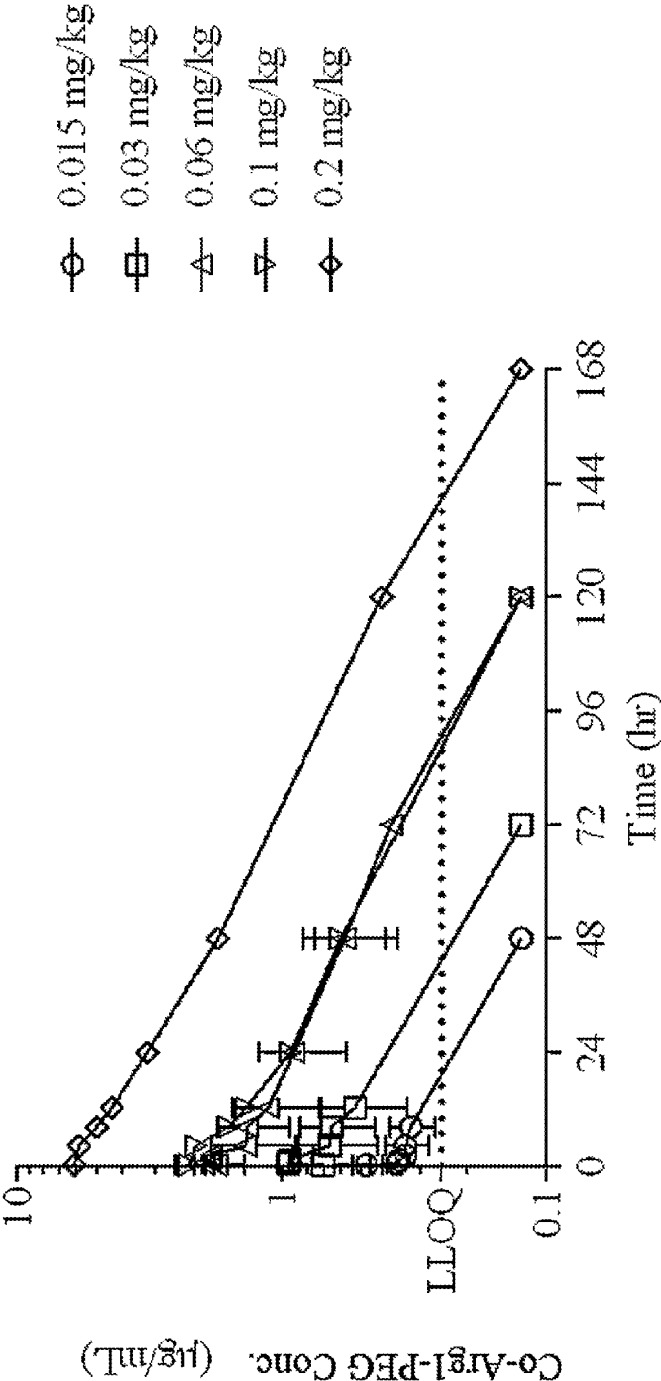
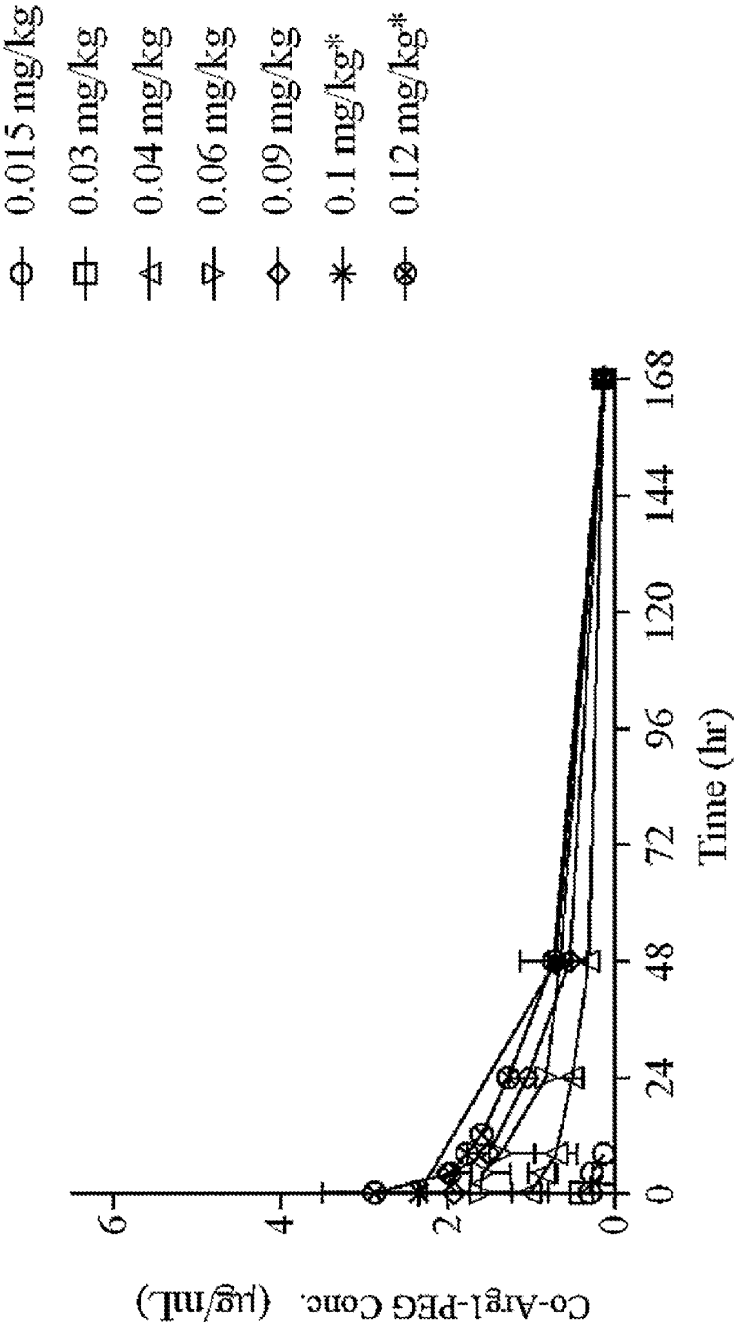


FIG. 9(b)

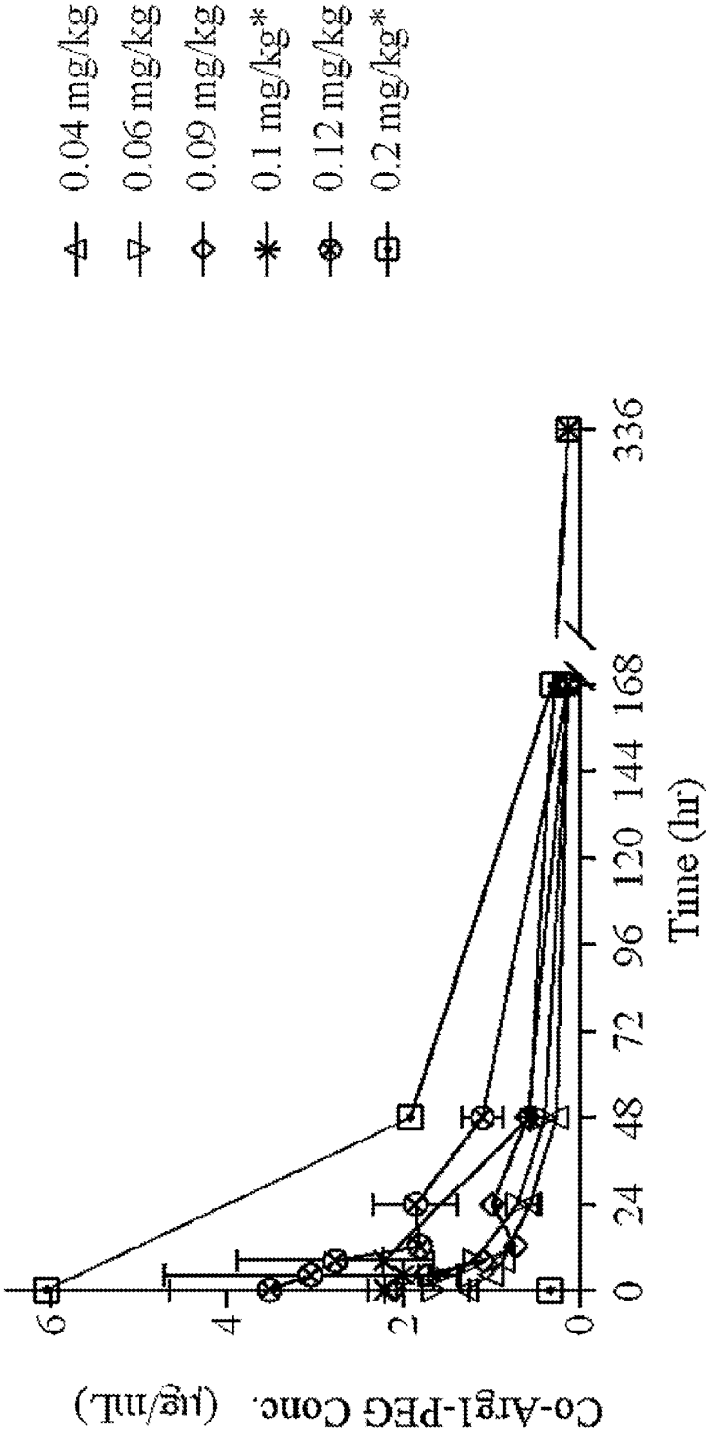
Pharmacokinetic Analysis – Linear, Week 1



* Mean data not available at all time points.

FIG. 9(c)

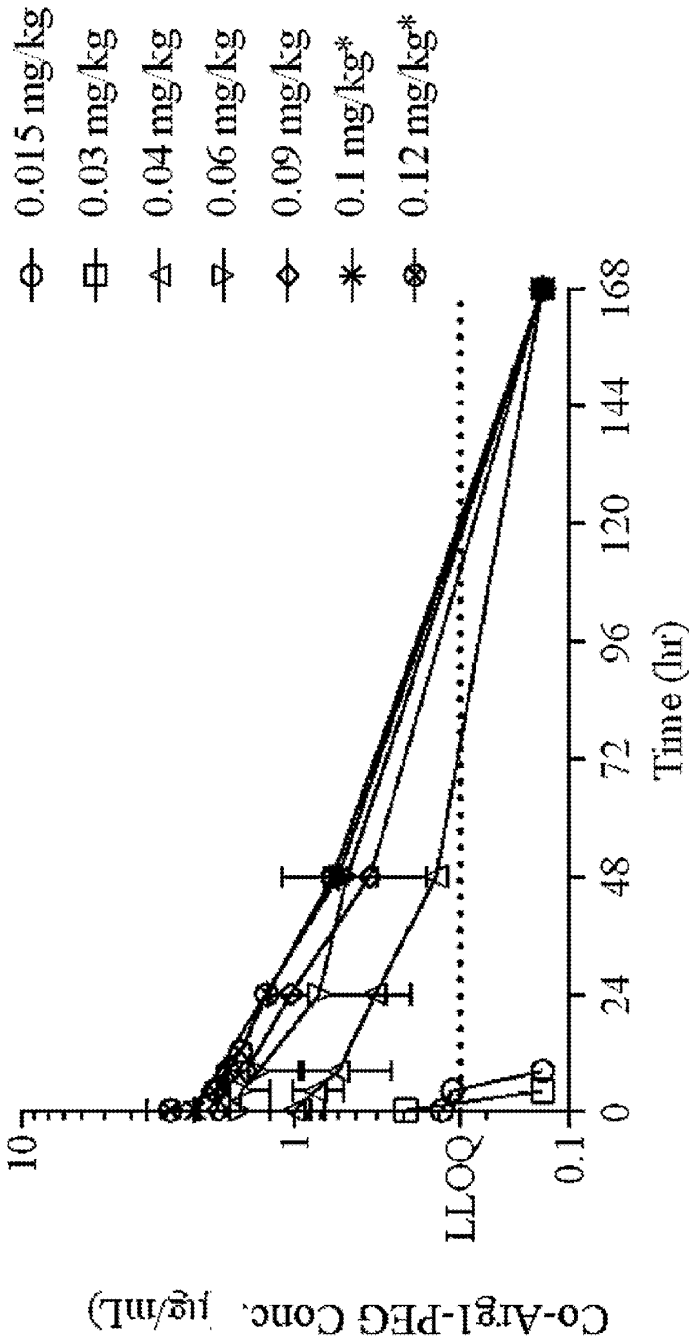
Pharmacokinetic Analysis – Linear, Week 8



* Mean data not available at all time points.

FIG. 9(d)

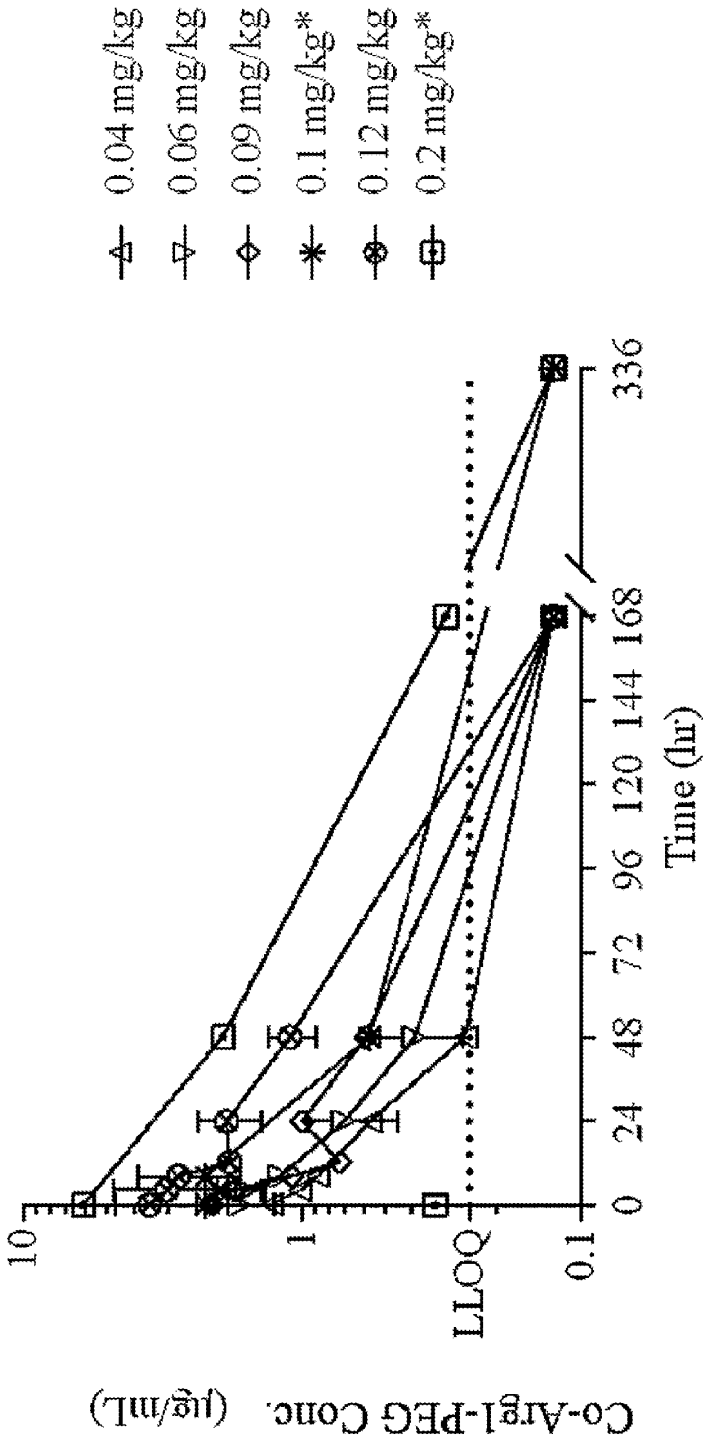
Pharmacokinetic Analysis – Semi-Log, Week 1



* Mean data not available at all time points.

FIG. 9(e)

Pharmacokinetic Analysis – Semi-Log, Week 8



* Mean data not available at all time points.

FIG. 9(f)

Integrated Pharmacokinetics (PK) / Pharmacodynamics (PD) Plot of Co-Arg1-PEG Administration – Patient 1

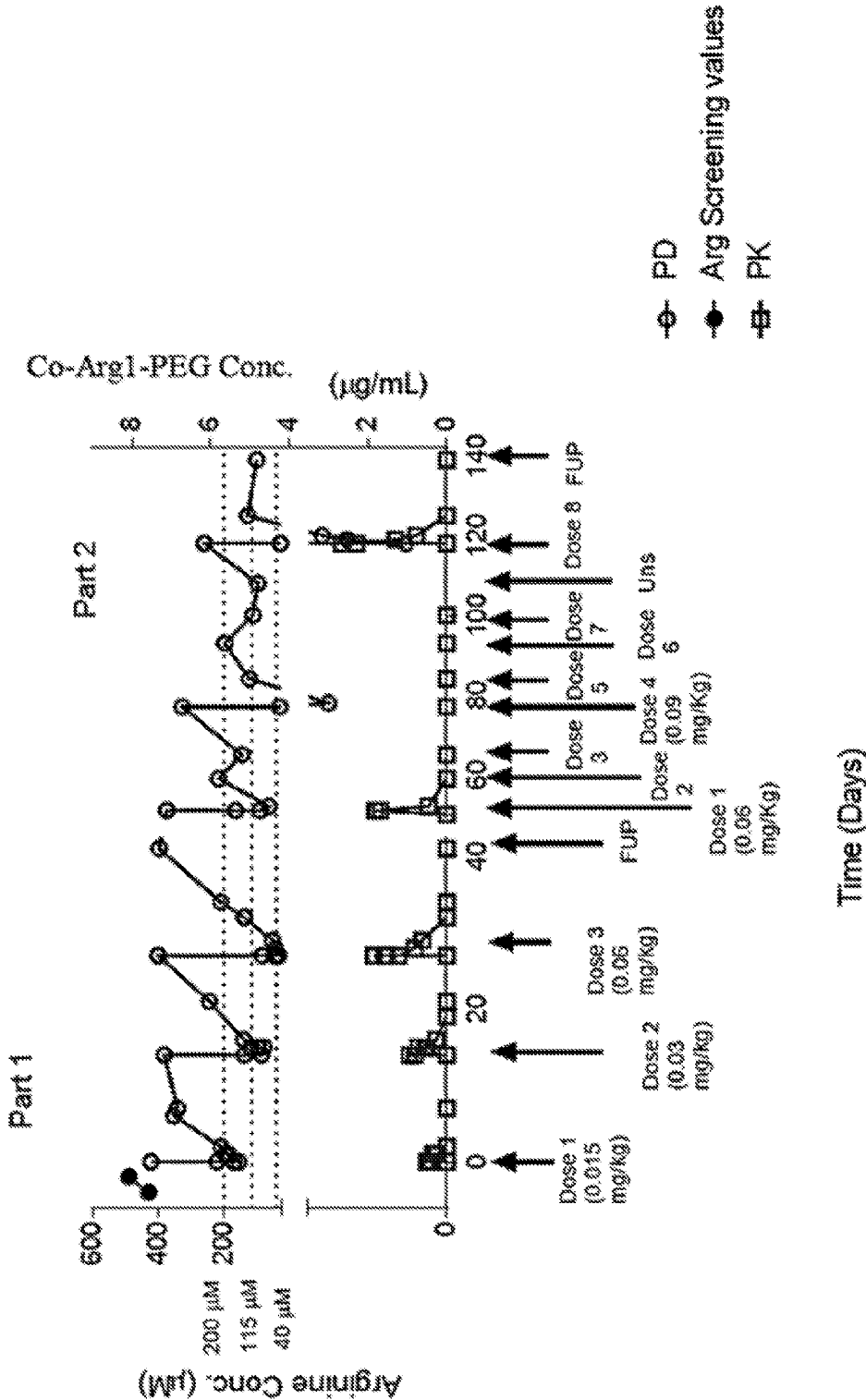


FIG. 10(a)

Integrated Pharmacokinetics (PK) / Pharmacodynamics (PD) Plot of Co-Arg1-PEG Administration – Patient 2

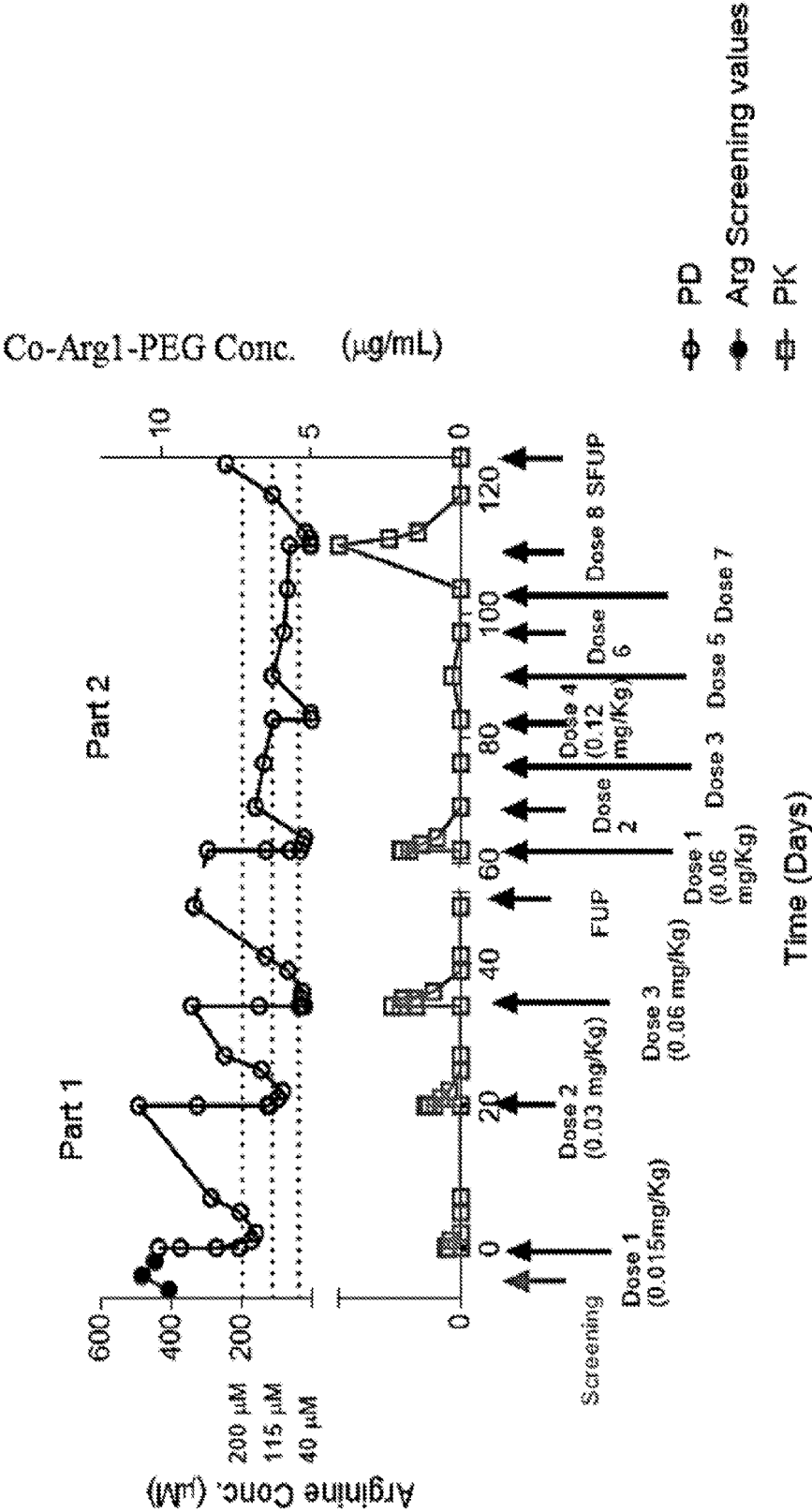


FIG. 10(b)

Integrated Pharmacokinetics (PK)/Pharmacodynamics (PD) Plot of Co-Arg1-PEG Administration – Patient 3

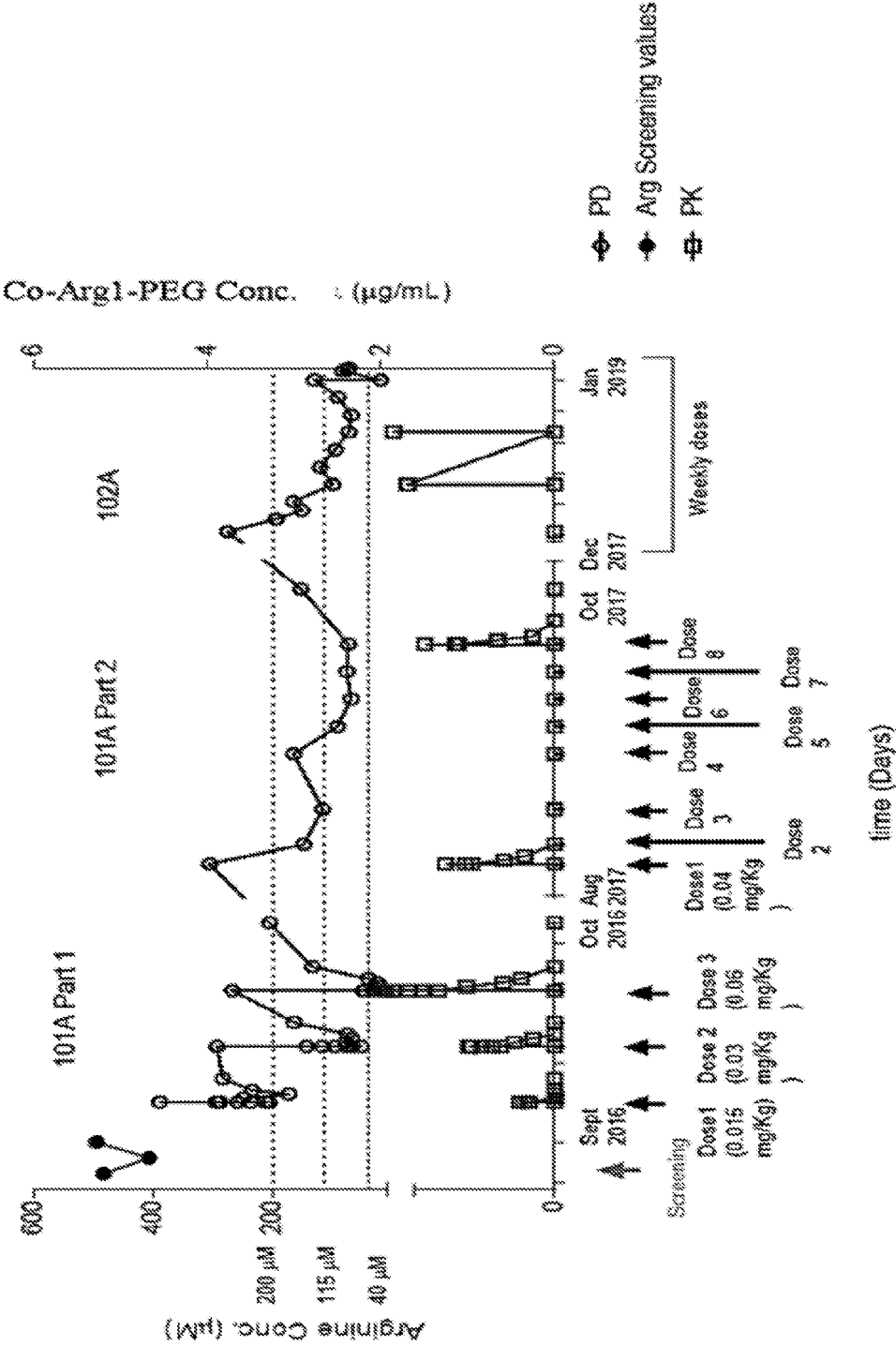


FIG. 10(c)

Comparison of IV and subcutaneous administration of Co-Arg1-PEG

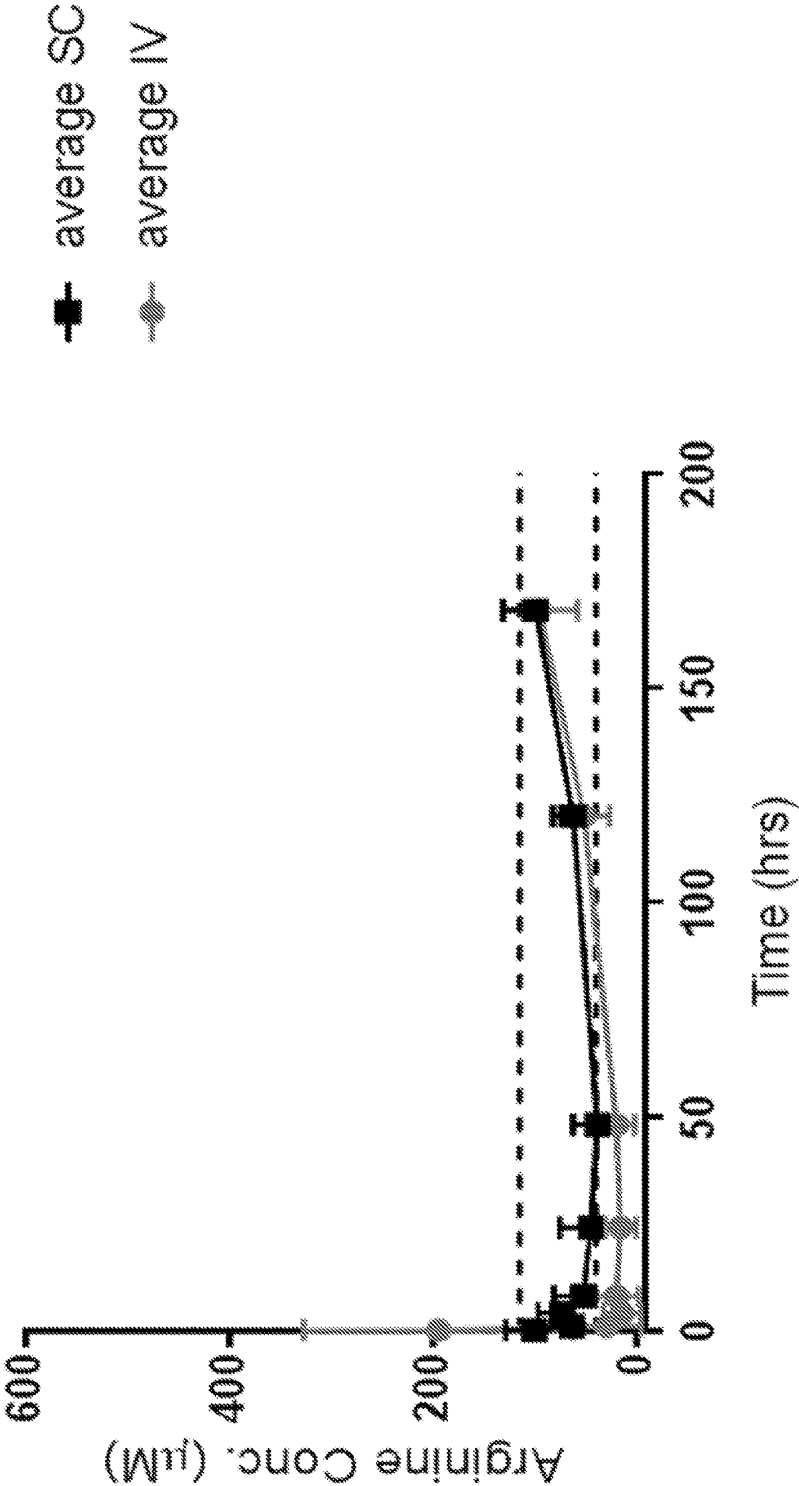


FIG. 11(a)

Comparison of IV and subcutaneous administration of Co-Arg1-PEG

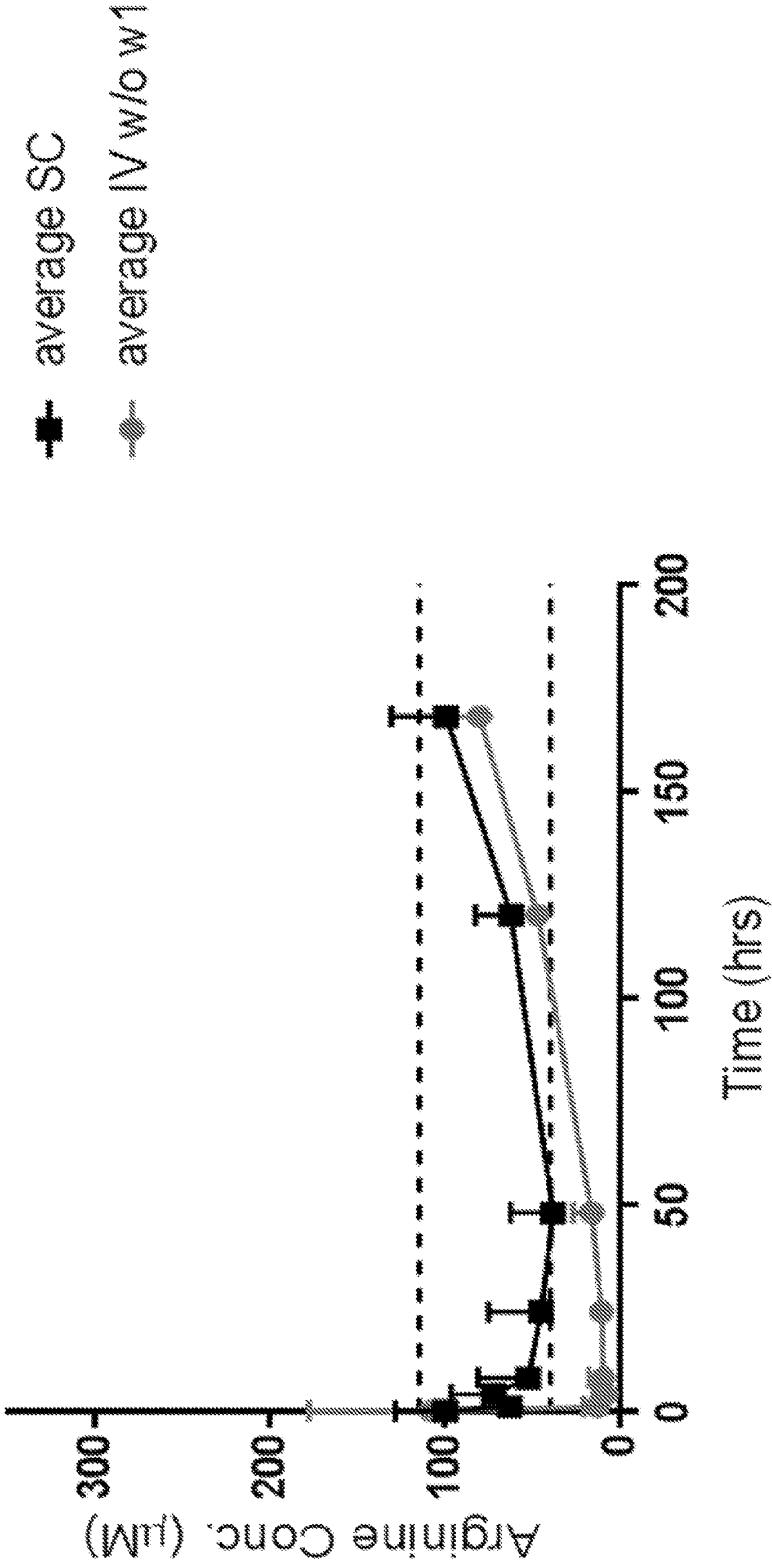


FIG. 11(b)

Plasma Arginine Levels

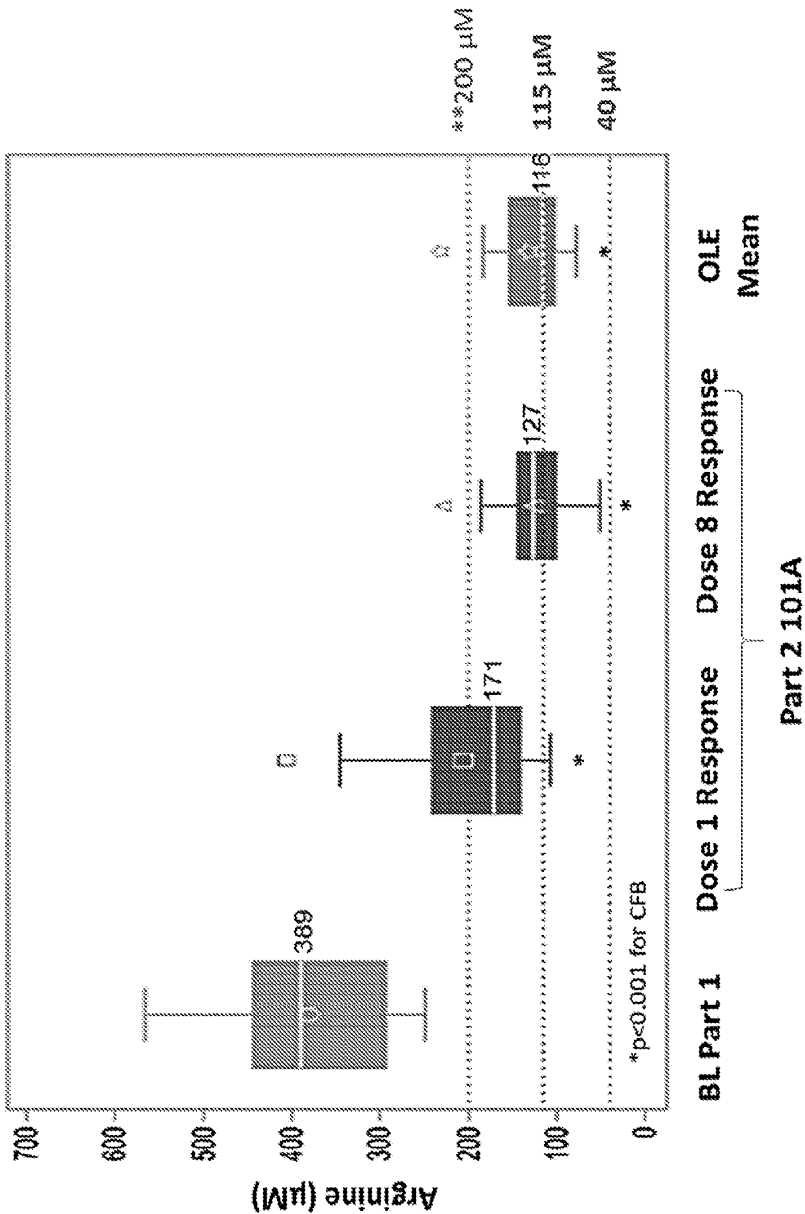


FIG. 12(a)

Plasma Guanidino Compound Levels

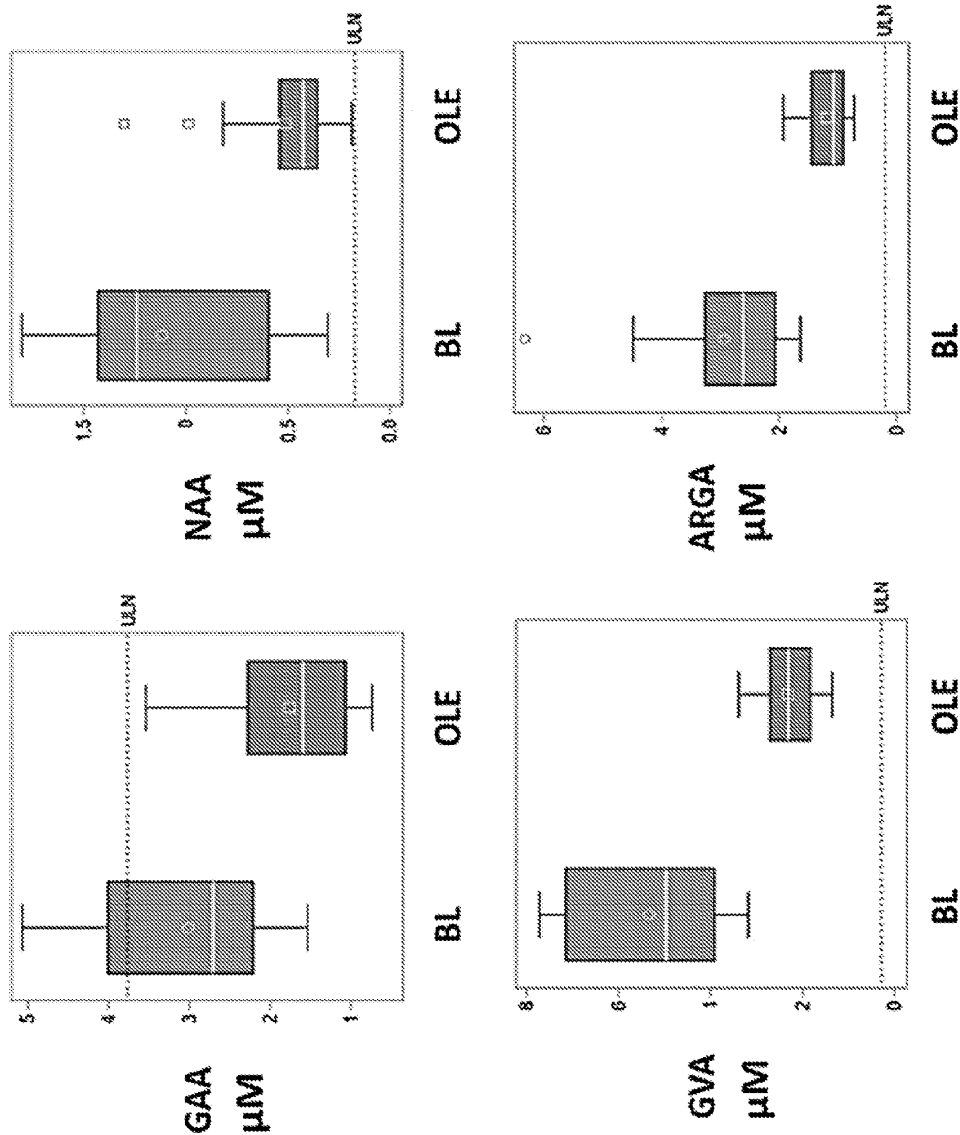


FIG. 12(b)

Baseline Clinical Deficit

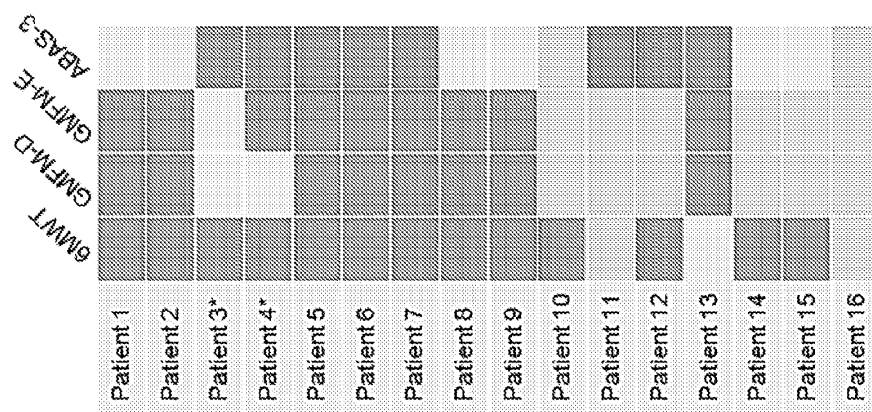


Fig. 13(a)

Clinical Response

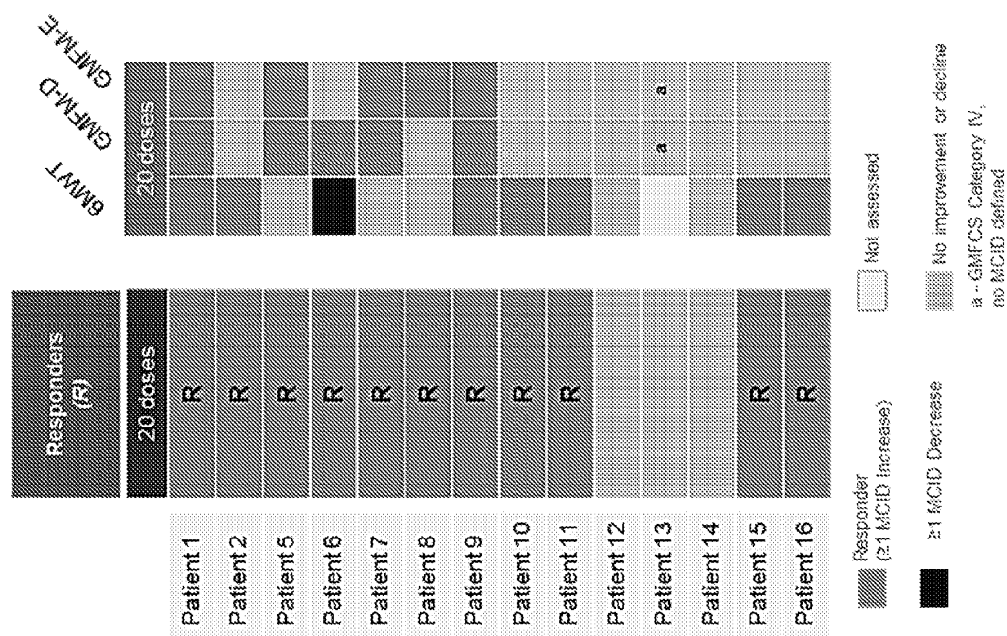
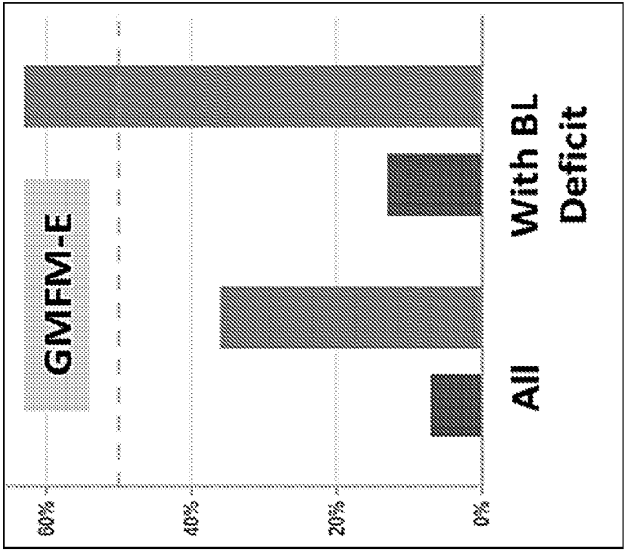


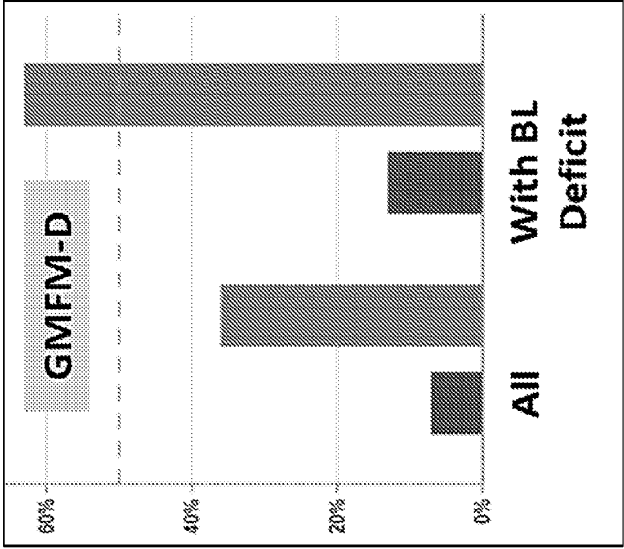
Fig. 13(b)

Time dependent
Improvements in GMFM-E



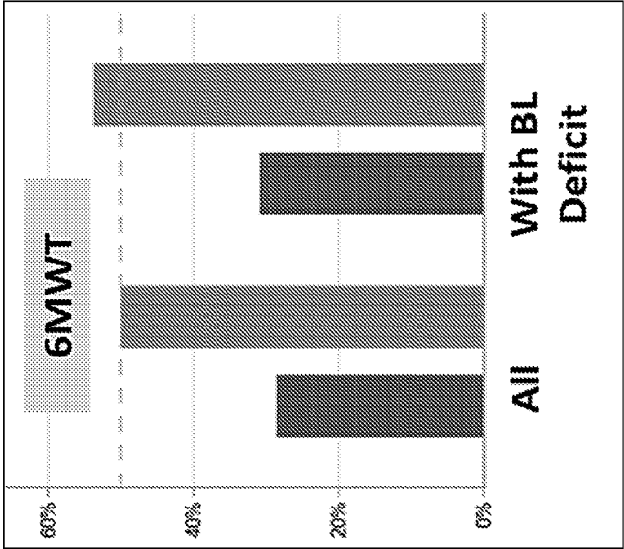
■ Dose 8 ■ Dose 20

Time dependent
Improvements in GMFM-D



■ Dose 8 ■ Dose 20

Time dependent
Improvements in 6MWT



■ Dose 8 ■ Dose 20

Fig. 14(c)

Fig. 14(b)

Fig. 14(a)

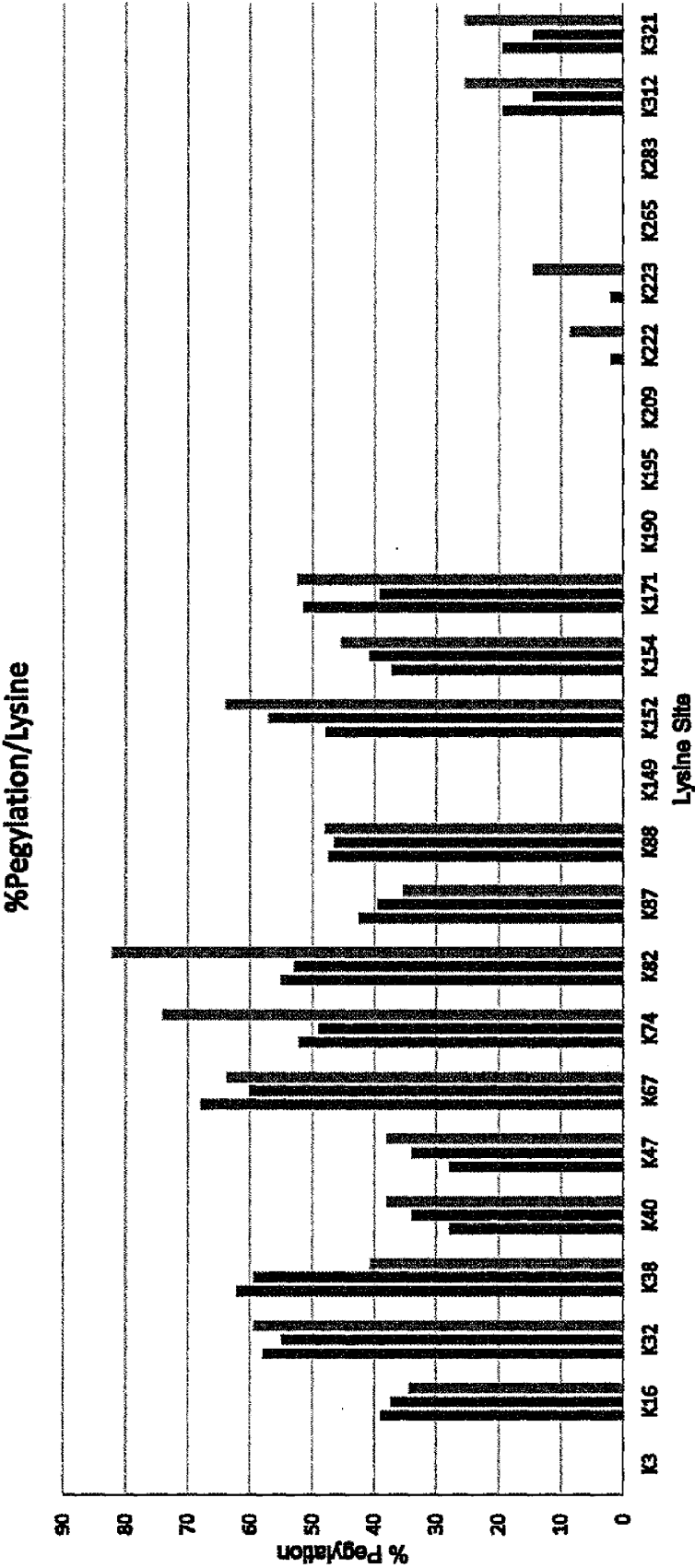


FIG. 15