

518 **10 Supplementary Tables**

519 **Table S 1. Assay performance data of the calibration samples for VX-970 in human**  
 520 **plasma.**

Analyte	Conc. (ng/mL)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)
VX-970	3	100.1	3.8	*
	10	99.0	4.0	*
	30	99.7	2.9	2.0
	100	101.9	2.4	0.2
	300	101.0	2.8	2.1
	1000	104.2	3.2	1.7
	3000	99.2	2.3	*
	5000	96.6	3.2	0.5

521 N=15; triplicate results, each in 3 separate runs, for each concentration. \*The mean square of the  
 522 within runs was greater than the mean square of the between runs, indicating that there was no  
 523 significant additional variation due to the performance of the assay in different runs [20].

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527 **Table S 2. Assay performance data for the quantitation of LLOQ, QCL, QCM, and QCH**  
 528 **VX-970 concentrations in human plasma.**

Analyte	Concentration (ng/mL)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)
VX-970	3 (LLOQ)	101.4	6.6	*
	5 (QCL)	98.5	8.4	4.1
	150 (QCM)	98.1	2.6	2.1
	4000 (QCH)	94.6	2.5	*

529 N=18; 6 replicates in 3 separate runs, for each concentration. \*The mean square of the within  
 530 runs was greater than the mean square of the between runs, indicating that there was no  
 531 significant additional variation due to the performance of the assay in different runs [20].  
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534 **Table S 3. Recoveries VX-970 from plasma and respective matrix effect in plasma extract,**  
535 **with coefficients of variation (CV).**

Analyte	Conc. (ng/mL)	Recovery (%)	CV (%)	Matrix Effect (%)	CV (%)
VX-970	5 (QCL)	96.5	7.7	-6.6	8.3
	150 (QCM)	90.4	11.8	-2.3	4.0
	4000 (QCH)	94.2	2.7	-5.5	2.4

536 N=4, for each concentration.

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540 **Table S 4. Assay performance data of QCL, QCM, and QCH VX-970 in EDTA plasma**  
541 **with results based on heparin plasma curves relative to results based on EDTA plasma**  
542 **curves.**

Analyte	Concentration (ng/mL)	Accuracy (%)	Precision (%)
VX-970	5 (QCL)	-8.3	4.3
	150 (QCM)	1.6	3.1
	4000 (QCH)	-0.1	2.0

543 N=4.

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547 **Table S 5. Stability of VX-970 after incubating rat bile containing the glucuronide**  
548 **metabolite at room temperature for 4 h at varying pH.**

Analyte	pH	Stability (%)	CV (%)
VX-970	5	104.3	6.0
	6	96.9	10.3
	7	102.5	4.1
	8	97.1	7.7
	9	101.1	11.1

549 N=4, for each condition

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551 **11 Legends to Supplementary Figures**

552 **Fig. S. 1. Representative calibration curve (N=3 for each concentration) used to quantitate**  
553 **VX-970 human plasma samples (response VX-970 = 0.0773•conc + 0.0339; R<sup>2</sup>=0.9984).**  
554 **Calibration curve is depicted as response ratio versus nominal concentration (A), and as**  
555 **residuals (%) of the back-calculated relative to the nominal concentrations versus the log**  
556 **transformed concentration (B), the log-transformation on x-axis for visual purposes.**

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558 **Fig. S. 2. Representative chromatograms of: (A) VX-970 (*m/z* 464.3>433.5; 1.9 min) added**  
559 **to control plasma at the LLOQ concentration of 3 ng/mL (top trace with an offset of 500**  
560 **counts) and control human plasma (bottom trace with an offset of 100 counts); (B) [d<sub>7</sub>]-VX-**  
561 **970 internal standard (*m/z* 471.3>440.3; 1.9 min) added to control plasma at a**  
562 **concentration of 100 ng/mL (top trace with an offset of 1000 counts) and control human**  
563 **plasma (bottom trace with an offset of 500 counts). (C) Bile of a rat dosed intravenously**  
564 **with 10 mg/kg VX-970 analyzed and monitored with *m/z* 464.3>433.5 (VX-970, top trace**  
565 **with an off-set of 8000 counts) and *m/z* 639.5>433.5 (VX-970 glucuronide, bottom trace**  
566 **with an off-set of 2000 counts).**

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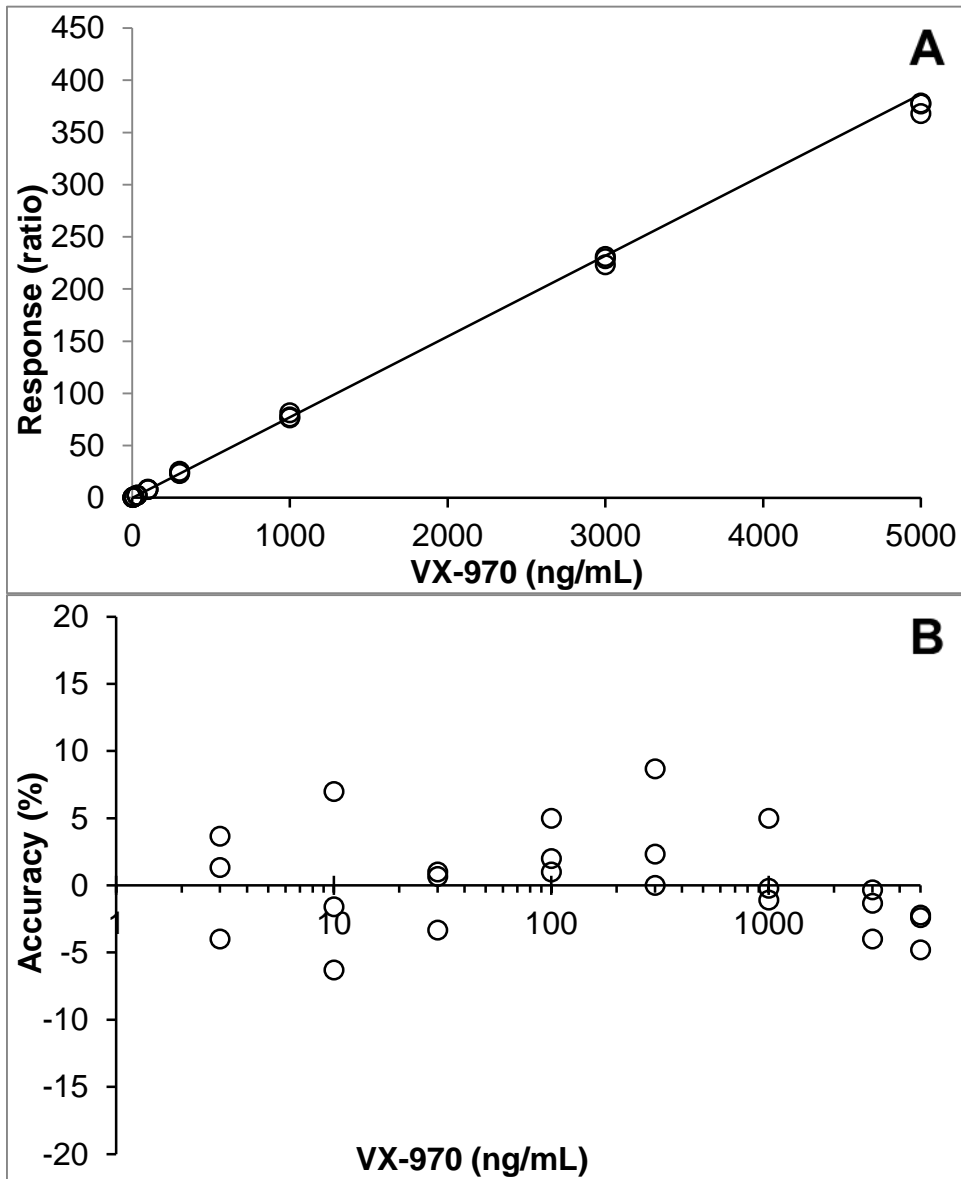
568 **Fig. S. 3. Chromatogram of VX-970 with selected phospholipid MRM channels. For visual**  
569 **purposes phospholipid MRM channels were offset from baseline response in increments of**  
570 **500,000 response counts.**

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573 **12 Supplementary Figures**

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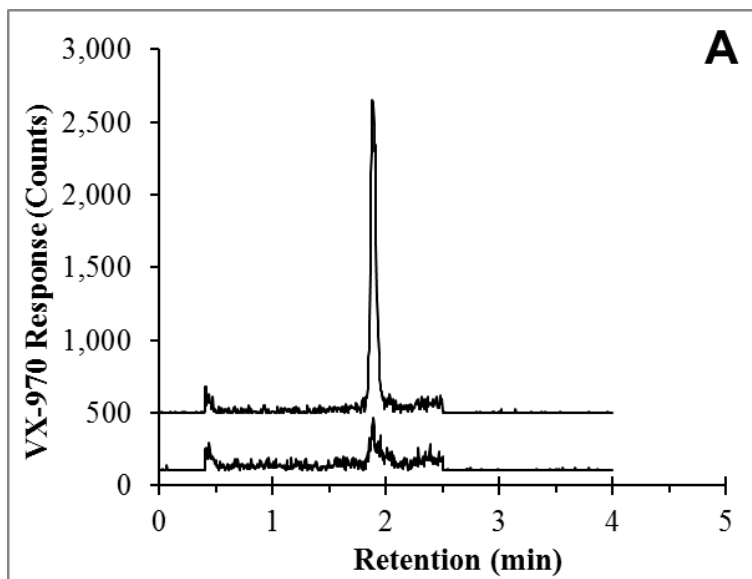
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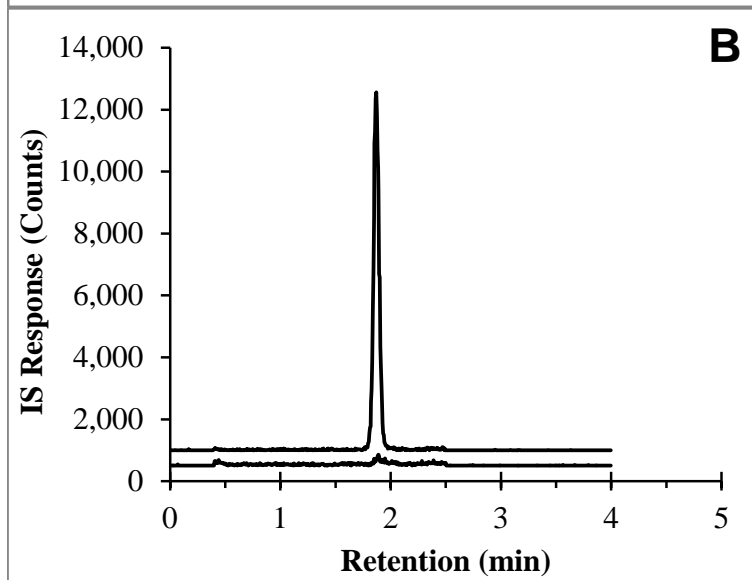
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578 Fig. S. 1. Representative calibration curve (N=3 for each concentration) used to quantitate VX-  
579 970 human plasma samples (response VX-970 = 0.0773•conc + 0.0339; R<sup>2</sup>=0.9984). Calibration  
580 curve is depicted as response ratio *versus* nominal concentration (A), and as residuals (%) of the  
581 back-calculated relative to the nominal concentrations versus the log transformed concentration  
582 (B), the log-transformation on x-axis for visual purposes.  
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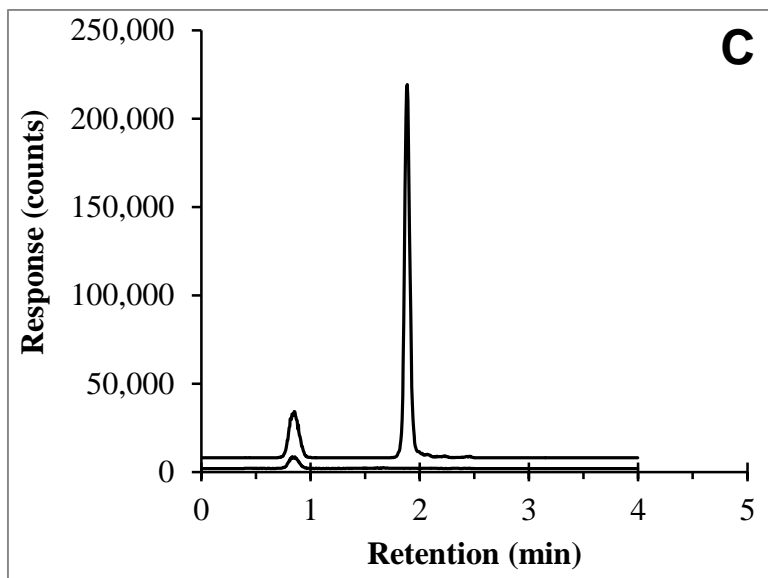


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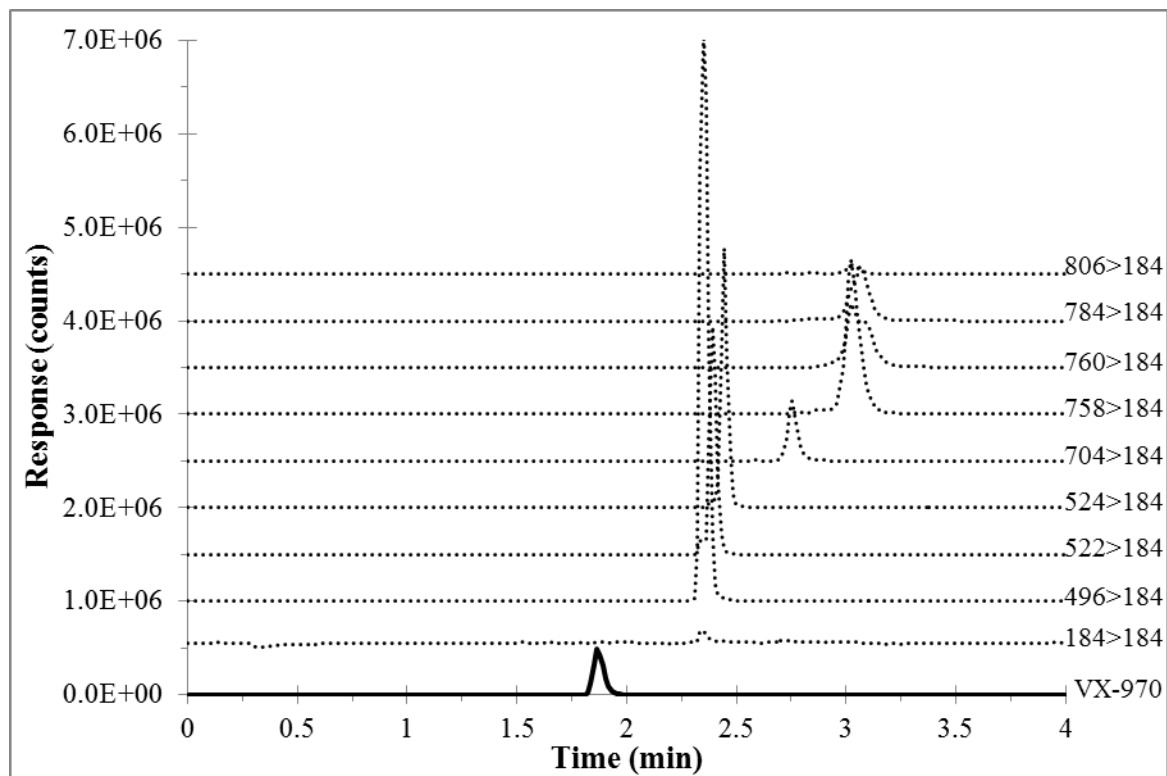
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589 Fig. S. 2. Representative chromatograms of: (A) VX-970 ( $m/z$  464.3>433.5; 1.9 min) added to  
590 control plasma at the LLOQ concentration of 3 ng/mL (top trace with an offset of 500 counts)  
591 and control human plasma (bottom trace with an offset of 100 counts); (B) [d<sub>7</sub>]-VX-970 internal  
592 standard ( $m/z$  471.3>440.3; 1.9 min) added to control plasma at a concentration of 100 ng/mL  
593 (top trace with an offset of 1000 counts) and control human plasma (bottom trace with an offset  
594 of 500 counts). (C) Bile of a rat dosed intravenously with 10 mg/kg VX-970 analyzed and  
595 monitored with  $m/z$  464.3>433.5 (VX-970, top trace with an off-set of 8000 counts) and  $m/z$   
596 639.5>433.5 (VX-970 glucuronide, bottom trace with an off-set of 2000 counts).  
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Fig. S. 3. Chromatogram of VX-970 with selected phospholipid MRM channels. For visual purposes phospholipid MRM channels were offset from baseline response in increments of 500,000 response counts.