Comparison of the Biotage® RapidTrace® and Gilson® GX-271 ASPEC™ Solid Phase Extraction Automated Liquid Handler Systems for Drug Screening Applications in Forensic Casework

By

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ABSTRACT: Instrument operation and degree of carryover produced by the Biotage® RapidTrace® and the Gilson® GX-271 ASPEC™ solid-phase extraction liquid handler systems was compared. Samples of animal blood, human blood, and human serum were spiked with a high concentration mix of 10 drugs commonly encountered in forensic casework. They were extracted preceding three blank samples of corresponding matrix on both instruments and all samples were subsequently screened on an Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QTOF-MS) instrument to evaluate differences in drug response values. It was found that the GX-271 ASPEC™ instrument produced lower overall drug responses in blank samples and faster time to extinction of drug responses, thereby demonstrating improved control of carryover. This instrument also showed an improvement over the RapidTrace® in overall performance and ease of operation and is therefore recommended as a beneficial replacement.

KEYWORDS: Forensic toxicology, solid-phase extraction, blood, serum, RapidTrace®, GX-271 ASPEC™, UPLC-QTOF-MS, carryover

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Chapter 1: INTRODUCTION AND BACKGROUND

1.1 Introduction

Automated liquid handling systems are commonly used in forensic toxicology laboratories to assist in the extraction of drugs from various biological samples, including whole blood and serum, by Solid-Phase Extraction (SPE). SPE is a commonly used method of sample preparation that assists in the successful identification of substances and can help produce valuable analytical evidence in forensic casework [1,2]. Sample and/or reagent carryover from sample to sample can occur during various steps throughout the procedure, although this is not always taken into consideration by laboratories [3]. Carryover is the low level sample-to-sample contamination that can occur when a sample containing a high concentration of drugs is analyzed prior to other samples that may or may not contain those drugs [4].

The Royal Canadian Mounted Police (RCMP) National Forensic Laboratory Services (NFLS) - Toxicology Services Laboratory currently uses the Biotage® RapidTrace® (Uppsala, Sweden) automated liquid handling system. The laboratory is interested in determining whether it would be beneficial to replace this instrument with the Gilson® GX-274 ASPEC™ (Gilson Inc, Middleton, WI) liquid handler. The Gilson® GX-274 ASPEC™ advertises several features that may represent improvement over the aging Biotage® systems, including intuitive software, liquid sensing capability, less routine maintenance, and reduction of carryover. The comparison of the systems will determine whether it would be beneficial to replace their current instrument with the GX-274 ASPEC™.

1.2 General Background

Preparation of samples, a method of extraction, and instrumental analysis must be validated for mass spectrometry assays. For validation, each laboratory must ensure that the assay used

produces accurate and reliable data based on the evaluation of elementary characteristics including precision, accuracy and carryover potential. To minimize carryover laboratories extract or inject a solvent or reagent blank following high concentration samples. A blank refers to a drug free matrix [4].

General screening in forensic toxicology is necessary for detection of suspected and unsuspected drugs and poisons in biological samples. Liquid chromatography – mass spectrometry methods have proved to exhibit increased specificity and detection sensitivity when compared with gas chromatography – mass spectrometry instruments, which were used as the standard for drug screening over the past years [5]. Specifically, liquid chromatography coupled with time of flight (ToF) mass spectrometry methods of drug screening collect more data about each compound detected, and allows for an expanded compound screen for presence of drugs against a large database. ToF methods may therefore be even more beneficial in drug screening applications than tandem mass spectrometry methods widely used today [6].

Compounds of interest in biological samples received in forensic toxicology casework can be extracted and isolated through liquid-liquid extraction (LLE) or solid-phase extraction (SPE) [4]. SPE proves advantageous in selectivity, reproducibility, throughput and cleanliness of extracts. Mixed-mode columns used in SPE have the ability to extract a wider range of compounds with varied properties at once [7]. For quality control measures, all laboratories should determine the concentration of drug that may result in carryover. This can be assessed by extracting a biological sample containing a concentration of drug that may be encountered in casework prior to the extraction of matrix matched blank samples [4].

As a result of being developed more recently, the GX-274 ASPEC™ system has little current literature outlining advantages and disadvantages of this specific instrument. The

Biotage® RapidTrace® instrument was found to have more studies involving this specific instrument for solid-phase extraction. The RapidTrace® instrument is able to mix reagents, and use one column to collect multiple fractions of the sample. Precision in this instrument comes from reproducible flow rates, and ease in separation of waste liquids for disposal. It also has a common and reproducible method allowing for the extraction procedure to be consistent in all laboratories which demonstrates simplicity and reliability [8]. Current literature also shows consistency between modules of the Biotage® RapidTrace® for majority of drugs of abuse, and relevant metabolites [9].

The experiments conducted to compare the instruments assess the degree of carryover produced in the three blank samples analyzed following the extraction of a sample spiked with high concentrations of ten different drugs. The drugs included in the high concentration sample were chosen because they are analytes commonly encountered in forensic toxicology casework and are known, or suspected to produce, significant carryover on the RapidTrace® SPE robotics system. Highly sensitive Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QToF-MS) instrumentation was chosen for the analysis of these samples through a general screening procedure in order to maximize the detection of any carryover produced. This instrument uses UPLC separation with QToF-MS detection of analytes.

Analytes used in this experiment are: benzoylecgonine (BE), citalopram (CTP), cocaine (COC), diazepam (DIA), diphenhydramine (DPH), ketamine (KET), methadone (MTD), methamphetamine (MPT), methylenedioxymethamphetamine (MDMA), and quetiapine (QTP). These analytes belong to several drug classes and represent a diverse array of chemical structures. Some of these drugs are already known to result in carryover on the RapidTrace® SPE robotics system and the QToF instrument. Central nervous system (CNS) stimulants include

cocaine, methamphetamine, and MDMA. Benzoylecgonine is an inactive metabolite of cocaine. Diazepam belongs to a class of drugs called benzodiazepines, methadone belongs to the opioid class and ketamine is commonly classified as a dissociative anaesthetic. Citalopram, quetiapine and diphenhydramine are various therapeutic drugs [4].

1.3 Purpose and Goals of Study

The purpose of this project is to provide information and data on the degree of carryover produced by each liquid handling system as well as general information on instrument operation in terms of preparation, ease of use, time to complete the extraction, unexpected errors, and amount of consumables and reagents used. The results will allow for the comparison of the RapidTrace® and GX-274 ASPEC™ liquid handlers in order to determine which is more advantageous to the laboratory. To assist in completing these experiments, the RCMP Toxicology Services Laboratory obtained a GX-271 ASPEC™ system on loan from Gilson®. Although the laboratory is interested in purchasing the GX-274 ASPEC™ model which uses a four-probe system to allow for the extraction of multiple samples simultaneously (the GX-271 ASPEC™ uses a single probe), it is not anticipated that this will affect the outcome of the experiment, other than total extraction time.

Chapter 2: MATERIALS AND METHODS

2.1 Sample Preparation

The experiment was conducted three times on three different days in order to control for possible day-to-day differences in extraction and instrument sensitivity. A previously validated procedure [2] for drug extraction and screening using Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UPLC-MS-TOF) was slightly modified for use in this project. The modifications consisted of a reduction in sample volume from 2 mL to 1 mL and the inclusion of a protein precipitation step as opposed to a straight dilution with buffer before loading the samples onto the SPE cartridges for a cleaner extract with fewer problems during extraction.

A high concentration mixture of ten drugs and metabolites commonly encountered in forensic casework was prepared. This was done by pipetting specified amounts (Table 2.1) of Cerilliant 1 mg/mL drug standards into a 5 mL volumetric flask and subsequently filling to volume with methanol. The mix was spiked into three blank matrices: bovine whole blood, human whole blood, and human serum in order to produce a final concentration outlined in Table 2.1. A volume of 400 µL of the high concentration mix was added to three 10 mL volumetric flasks which were then filled to volume with each matrix. Each spiked sample and four matrix-matched blank samples were prepared with 10 µL of an internal standard solution which included doxapram, nalorphine, and nimetzaepam (each with a concentration of 5 µg/mL) prior to extraction. All samples spiked with the high concentration mixture were isolated from the blank samples to eliminate possible cross-contamination during all non-automated steps of the experiment.

Table 2.1 – Spiking volumes for high concentration drug mixture

Analyte	**Amount (µL)**	**Concentration in Matrix (ng/mL)**
Benzoylecgonine	625	5000
Citalopram	315	2520
Cocaine	125	1000
Diazepam	325	2520
Diphenhydramine	750	6000
Ketamine	125	1000
Methadone	315	2520
Methamphetamine	625	5000
MDMA	500	4000
Quetiapine	315	2520

All prepared high concentration and blank samples (of each matrix) were pipetted at a volume of 1 mL into appropriate test tubes for each SPE robotics system. Prior to the extraction procedure, protein precipitation was completed on each sample for a cleaner preparation which began by slowly adding 2 mL of cold acetonitrile:methanol (85:15) to each sample while vortexing. The samples were then centrifuged for 10 minutes at 4400 revolutions per minute (r.p.m.) at a temperature of -5 ºC. The supernatant was transferred to new test tubes and 4 mL of deionized water was added to each sample before being loaded onto the extraction instruments.

2.2 Extraction Procedure

The process of SPE further isolates and purifies the drug containing fractions of the biological samples. Each spiked sample was extracted in parallel on the RapidTrace® and GX-271 ASPEC™ units prior to three blank samples of the same matrix type. A blank also preceded each high concentration sample for quality control purposes and to eliminate robotics carryover as the source of contamination. The order by which each matrix type was extracted on each robotics system was varied for each run of the experiment. Three RapidTrace® modules were used at one time with 5 samples in each module. The three sets of five samples ran on the GX-271 ASPEC™ instrument were also placed in different order for each run of the experiment to keep these locations as consistent as possible with the RapidTrace® sample order. The order of extraction on each day is presented in Tables 2.2.1 and 2.2.2. The abbreviations for the matrices are as follows: AN (animal blood), HB (human blood) and HS (human serum).

Table 2.2.1 - Matrix type ran on each RapidTrace® module

Module	***Day 1***	***Day 2***	***Day 3***
0	AN	-	-
1	HB	AN	HS
2	HS	HB	AN
3	-	HS	HB

Table 2.2.2 - Matrix type ran on the GX-271 ASPEC™

Sample Position	***Day 1***	***Day 2***	***Day 3***
1-5	AN	HB	HS
6-10	HB	HS	AN
11-15	HS	AN	HB

Samples were extracted using Strata™-X-Drug B 33 µm (Phenomenex®, Inc.) cation mixed-mode polymer sorbent SPE cartridges. Parameters for the procedure were consistent between instruments for each step involved. Cartridges were conditioned with 1 mL of methanol and 1 mL of deionized water. Samples were then loaded onto the column at a rate of 2 ml/min, followed by a purge with 5 mL of deionized water and sequential washing with 1 mL of 0.1% formic acid and 1 mL of 10% methanol. Nitrogen gas was then used to dry the resin, and analytes were subsequently eluted out of the column with ethyl acetate:isopropanol:ammonium hydroxide (70:20:10). The final purge used 5 mL of methanol and 5 mL of deionized water to complete the extraction procedure.

Due to the inability to split the nitrogen gas line needed to dry the SPE cartridges during extraction, as well as the length of time required for extraction on the GX-271 ASPEC™, the RapidTrace® samples were extracted during the day following with the GX-271 ASPEC™ extractions which continued overnight. After extraction, the eluates were brought to dryness under nitrogen at ambient temperature using the Pearce ReactiTherm III™ for two hours.

2.3 Screening of Samples (UPLC-QToF-MS)

The resulting residues were reconstituted with 100 µL of UPLC mobile phase and screened for components with Waters® Acquity Ultra Performance Liquid Chromatography (UPLC) Xevo G2-S Quadrupole Time-of-Flight Mass Spectrometry instrumentation (Waters Corp., Milford, MA). The QToF completes a full scan of all parent compounds and fragments detected. During instrumental analysis each blank sample was followed by two acetonitrile solvent blanks to eliminate potential carryover occurring on the instrument. Five acetonitrile blanks were injected between each high concentration sample as well. One-in-twenty (1:20) dilutions of the high concentration eluates were prepared for analysis on the QToF to prevent the

instrumental detection system from being overwhelmed. Samples were analyzed in order of what was predicted to contain the lowest amount of drugs (the blank samples), followed by the high concentration samples at the end of the sequence.

2.4 Assessing Overall Instrument Operation

A review of the operation of both instruments was recorded for each run of the experiment to compare overall differences. The components of each unit considered include ease of set up and user friendliness, time of extraction (total for all 15 samples, and time for one individual sample), any errors that occurred, preparation of consumables, approximate volumes/amount of consumables used and any required maintenance or troubleshooting required during the experiment.

2.5 Positive Identification of Analytes of Interest

The responses of each drug of interest (spiked in the high concentration samples) were compared between liquid handling systems to evaluate the generation of carryover. All positive identifications of drugs present in the high concentration samples and any other drugs or components were recorded and organized. Analytes identified by the UNIFI software as 'Tentative identifications' of the drugs in the high concentration spiking solution were also recorded. The data was flagged for analytes that did not meet the acceptance criteria and analytes that did meet the criteria but were not within 10% of the lowest response detected during instrument validation. The values of these flagged analytes were included in the calculation of the average response of each drug in each matrix over the 3 days.

The criteria used in this experiment for an accepted/positive identification of an analyte are as follows:

1. Mass error – within 5 ppm of library value
2. Retention time – observed values within ±0.35 minutes of expected
3. Fragmentation – fragments found must be ≥50% of fragments expected
4. Isotope match intensity RMS% < 10%

If all four criteria are met within the accepted values then the drug is considered to be positively identified. Figures demonstrating degree of carryover and decay of drug over the three blanks following the high concentration mix were created using a scatterplot. The response values plotted are the mean values of each analyte over the three days in each matrix and results for both instruments were plotted on the same graph to allow direct visual comparison of the degree of carryover produced (see Appendices I-III). Expected analytes that were not present in a blank (i.e., did not carry over) were given a response value of zero, and this number was also included in the calculation of the average.

2.6 Evaluating Degree of Carryover

Evidence of carryover was generated by the UPLC-QToF-MS instrument. Data was compiled in the form of a UPLC-QTOF Drug Screen Report automatically generated by the instrument software (Waters® UNIFI v.1.8.1) after analysis. Identification tables and chromatograms from this report were reviewed, and all identified analytes were recorded with their response values in an Excel spreadsheet as raw data.

The mean response values over the three days for each drug, in each matrix, and on both instruments were calculated. Percent carryover was calculated to assess the amount of each drug

that was carried over in relation to the high concentration sample to blank samples 1, 2, and 3. This was done with the following equation:

$$\% \text{ C/O} = \frac{\text{Blank Mean Response}}{\text{High Concentration Mean Response}} \text{ x } 100\%$$

After percent carryover calculation for each blank on each day, the mean percent carryover over the three was calculated for each blank and was used in a graphical representation of degree of carryover. The statistical significance of percent carryover between instruments in each matrix as well as between matrix types on each instrument was assessed using analysis of variance (ANOVA) tests.

Chapter 3: RESULTS

3.1 Instrument Operation

3.1.1 Set Up and User Friendliness

The set up process for the RapidTrace® instrument has more components involved than the GX-271 ASPEC™. The RapidTrace® requires the manual setup and automated running of 2 purge cycles in each module being used prior to use for sample extraction in order to remove any air bubbles that might affect solvent delivery and subsequently may affect extraction efficiency. Solvent preparation must be completed and lines immersed in solvents before beginning this step. Preparation of these is the same for both instruments and includes deionized water, methanol, 0.1% formic acid, 10% methanol and the elution solvent. However, the solvent bottles used for each instrument are different. The RapidTrace® unit requires manual connection of the lines into solvent bottles and also tightening 4 out of 5 bottles with parafilm due to improperly fitting bottle caps. Once the solvents are connected, the 2 purge cycles can then be run and require 2 ‘dummy’ cartridges and 2 sets of sample and eluate tubes in each module. After this is complete the ‘dummy’ cartridges and tubes must be removed and new cartridges inserted for extraction. In these experiments, 5 prepared sample tubes, 5 empty tubes for eluates and 5 cartridges are placed in each module. After extraction, the cartridges and tubes must be replaced again for the cleanup process which eliminates protein build up in the sample path. Three cartridges, three empty eluate tubes and three sample tubes are required. One sample test tube (per module) must be filled with 8 mL of NaOH base, another filled with 8 mL of HNO_3 acid and the last with 8 mL of deionized water.

The set up for the GX-271 ASPEC™ includes the same solvents required for the extraction procedure, however, the bottles used do not require a cap or manual connection to the

lines. Only one bottle for methanol requires manual immersion of the line and complete covering of the top with Parafilm®. For the other four solvents, the automated system moves the probe to the bottle it needs and inserts it through a small hole in the top. When this set up is applied to the GX-274 ASPEC™ liquid handler that the laboratory would purchase, four bottles of methanol will require manual immersion of the line and complete covering of the top with Parafilm®. The remaining bottles will in this case have four holes in each for each of the four probes. Once the solvents are connected the 15 empty eluate tubes, 15 prepared samples and 15 cartridges can be inserted into the correct positions. One additional step for GX-271 ASPEC™ compared to the RapidTrace® is the placement of caps (with a small hole in the center) on all cartridges before beginning. There is no separate purge or clean up procedure for the Gilson® unit, and the extraction process can begin.

The RapidTrace® instrument requires more time spent manually entering information on the computer to set up the extraction procedure. Solvents used must be confirmed for each line by opening a file listing the reagents required for the analysis being run, and comparing the computer list with the solvents that are physically connected. Procedure steps are confirmed by opening a file corresponding to the extraction procedure required and comparing the computer process with the documented Standard Operating Procedure (SOP) being used in order to ensure changes were not inadvertently made to the program. Three separate portions of the overall process must be entered separately as well. These are the 2 purge cycles, the extraction process for 5 samples on 3 modules, and the 3 wash/clean up cycles. This information must be selected separately for each: what program is being run (after deleting previously selected procedures - purge, extraction, or cleanup), how many samples you have and how many modules are being used. Each module must then be selected separately on the software to start the run. Much less

computer work is involved with the GX-271 ASPEC™ Gilson® instrument. After initially opening the extraction procedure file, the number of cartridges and zone (both inputted as 1-15 for this experiment) are entered and the procedure for all 15 samples can begin by selecting 'run'. A minor problem that was noted is a pop-up that appeared when run was selected, and the process would not begin until this was closed although this only occurred during the first experiment. The prime process begins followed by extraction without separate entries into the software.

3.1.2 Time to Complete Extraction

Providing that no errors occur, the amount of time to extract all fifteen samples is 1.5 hours on the RapidTrace® and 6 hours and 23 minutes on the GX-271 ASPEC™ (on average between the three days). However, if the laboratory purchases the GX-274 ASPEC™ with four probes, it can be expected that extraction will take approximately 1/4th of this time, or approximately 1 hour and 35 minutes for the entire procedure of fifteen samples. Once the 10 minutes to complete the purge cycles and the 38 minutes to complete the wash cycles is added, the RapidTrace® unit takes a total of 2 hours and 18 minutes for the entire procedure. The initial priming/purging step included in the 6 hours and 23 min for the GX-271 ASPEC™ takes just two minutes. When the amount of time it takes to extract one individual sample is compared, the RapidTrace® unit takes approximately 18 minutes and the GX-271 ASPEC™ takes approximately 25.5 minutes to complete. Table 3.1.2 shows a summary of the length of time each instrument requires for the extraction process (providing that no errors occur which need to be addressed). The values in this table do not include time required for equipment set up. The GX-271 ASPEC™ instrument completed the extractions overnight for each run of the experiment therefore potentially saving future time in the lab. The RapidTrace® unit can also be

run overnight, however, as there are five modules with ten sample positions per module there is usually no need.

Table 3.1.2 – Time to Complete Extraction

	RapidTrace®	**GX-271 ASPEC™**	**GX-274 ASPEC™**
Extraction	1 hour 30 min	-	-
Entire process	2 hour 18 minutes	6 hours 23 min	1 hour 35 min
One sample	18 min	25.5 min	25.5 min

3.1.3 Errors and Maintenance

The Biotage® RapidTrace® robotics system was the only instrument to encounter errors throughout this project. On step two of the first run of the experiment (2 minutes in), there was an error on module 0 during the first purge cycle due to a blocked column/line. The issue was resolved after having cut the line at the blockage and reattaching it to the unit. Once the extraction began, there was an error on the first sample at 18 minutes in (animal blood blank 0) during step 11 (methanol purge). Fortunately, the extraction of this sample was complete and able to be saved. It was decided that use of module 0 be discontinued for the rest of the project due to multiple errors encountered within the first run. The remainder of the animal blood samples for this instrument were re-run on module 3 the following day, as the instrument would not start running a new module while extractions were in progress. The remaining four were extracted following a water blank used as 'blank zero'. The unexpected maintenance took a significant amount of time to resolve, and the RapidTrace® instrument was closely observed for the remainder of the extractions this day as well as during the second run of the experiment. No maintenance was required after the extraction began for the GX-271 ASPEC™ for the duration of this project.

3.1.4 Consumables Used

Solvent preparation was the same for both instruments and required the elution solvent of ethyl acetate: isopropanol: ammonium hydroxide (70:20:10) to be prepared fresh each day before use. Table 3.1.4 shows a summary of the approximate volumes of solvent used for each instrument over the course of the project.

Table 3.1.4 – Approximate Volumes of Solvents Used for Each Experiment Run

Solvent	**RapidTrace® (volume in mL)**				**GX-271 ASPEC™ (volume in mL)**			
Day	*1*	*2*	*3*	Avg.	*1*	*2*	*3*	Avg.
Deionized H_2O	687	450	550	500	18	25	20	21
Methanol	138	172	132	152.5	134	286	300	240
0.1% Formic Acid	65	20	53	36.5	13	7	10	10
10% Methanol	61	33	84	58.5	23	11	22	18.7
Elution Solvent	69	58	56	57	37	38	40	38.3

The average volumes of each solvent used for the RapidTrace® unit were calculated from days 2 and 3 only as the errors on day 1 resulted in a fourth module being used and a water blank extraction, using much more solvent than is typically needed.

3.1.5 General Issues with the Extraction

An issue noted for all 3 runs of the experiment is that for both instruments, the five human serum samples for each were not completely drawn into the lines and eluted after extraction. This left some liquid from each sample of this matrix in the test tubes, and is not a result of instrument errors. It also can be noted that the GX-271 ASPEC™ left a very small amount of liquid in each sample tube in all three matrices, not just human serum. However, provided that an internal standard method is used for quantitative analysis, a small residual amount of sample is not expected to affect accuracy of the results.

3.2 Degree of Carryover

3.2.1 Percent Carryover

The percent carryover in each matrix on each instrument were compared to see which SPE robotics system had better control of carryover for all 10 analytes. This was done using multiple bar graphs, and vertical axis scales were kept consistent for each matrix to visually compare values. Figures 3.2.11 to 3.2.16 demonstrate the variation in carryover in each matrix between instruments. Due to such high values of percent carryover compared to the other 9 analytes, diphenhydramine was placed in a graph of its own in order to keep the y-axis scale at a lower value.

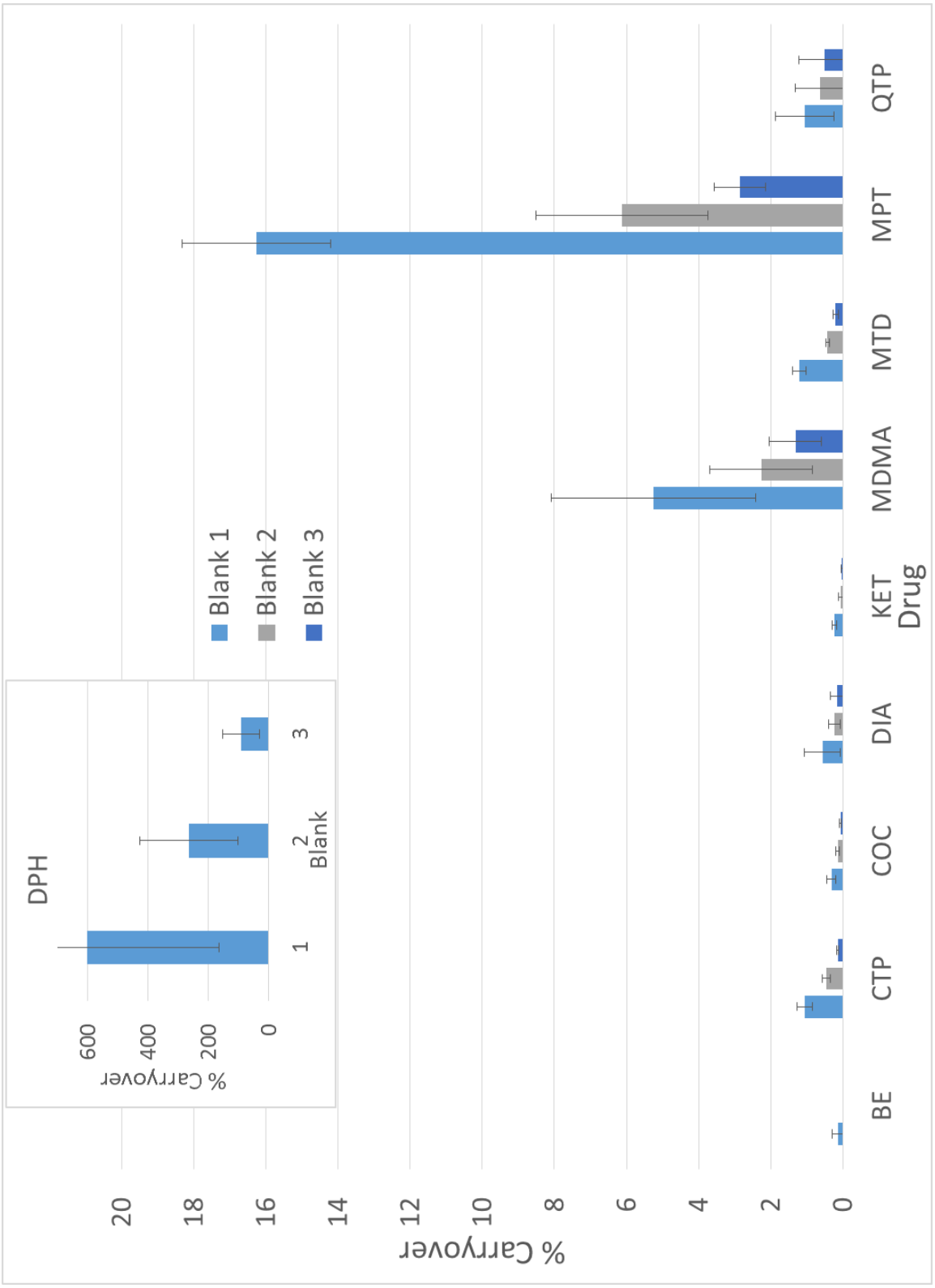

Figure 3.2.11 – Percent carryover in animal blood for BE, CTP, COC, DIA, DPH, KET, MDMA, MTD, MPT, and QTP on the Biotage® RapidTrace® SPE instrument.

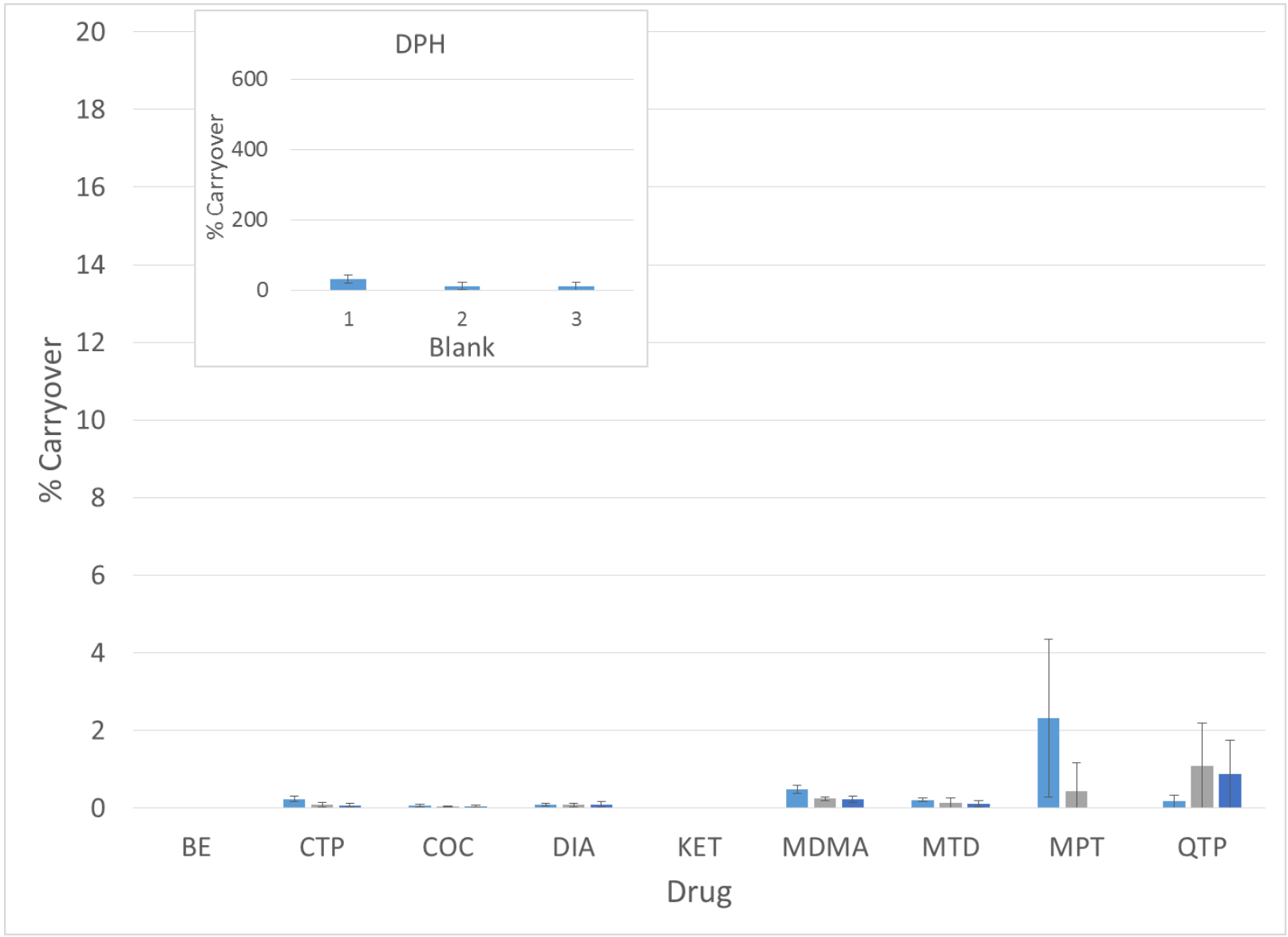

Figure 3.2.12 – Percent carryover in animal blood for BE, CTP, COC, DIA, DPH, KET, MDMA, MTD, MPT, and QTP on the Gilson® GX-271 ASPEC™ SPE instrument.

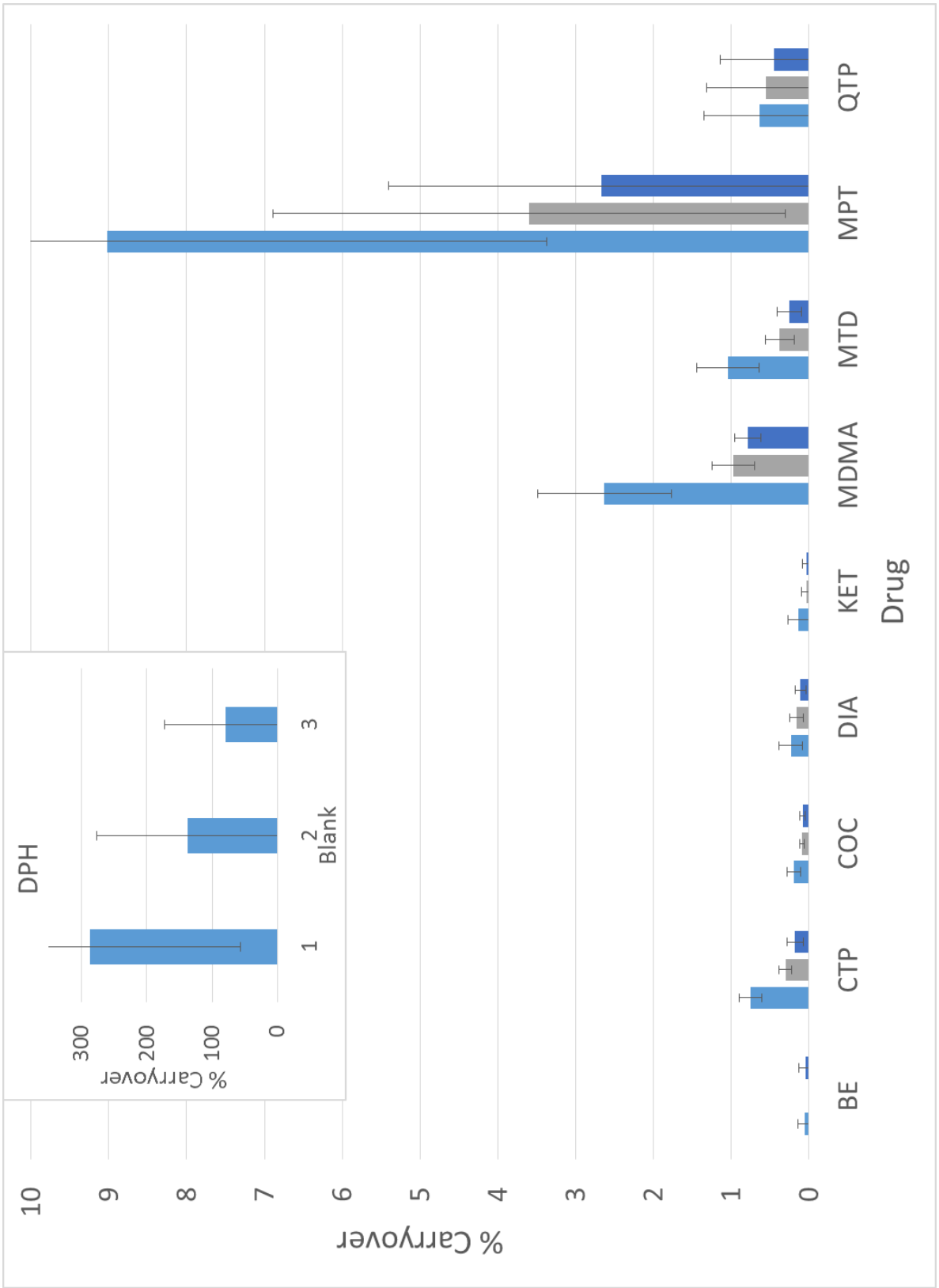

Figure 3.2.13 – Percent carryover in human blood for BE, CTP, COC, DIA, DPH, KET, MDMA, MTD, MPT, and QTP on the Biotage® RapidTrace® SPE instrument.

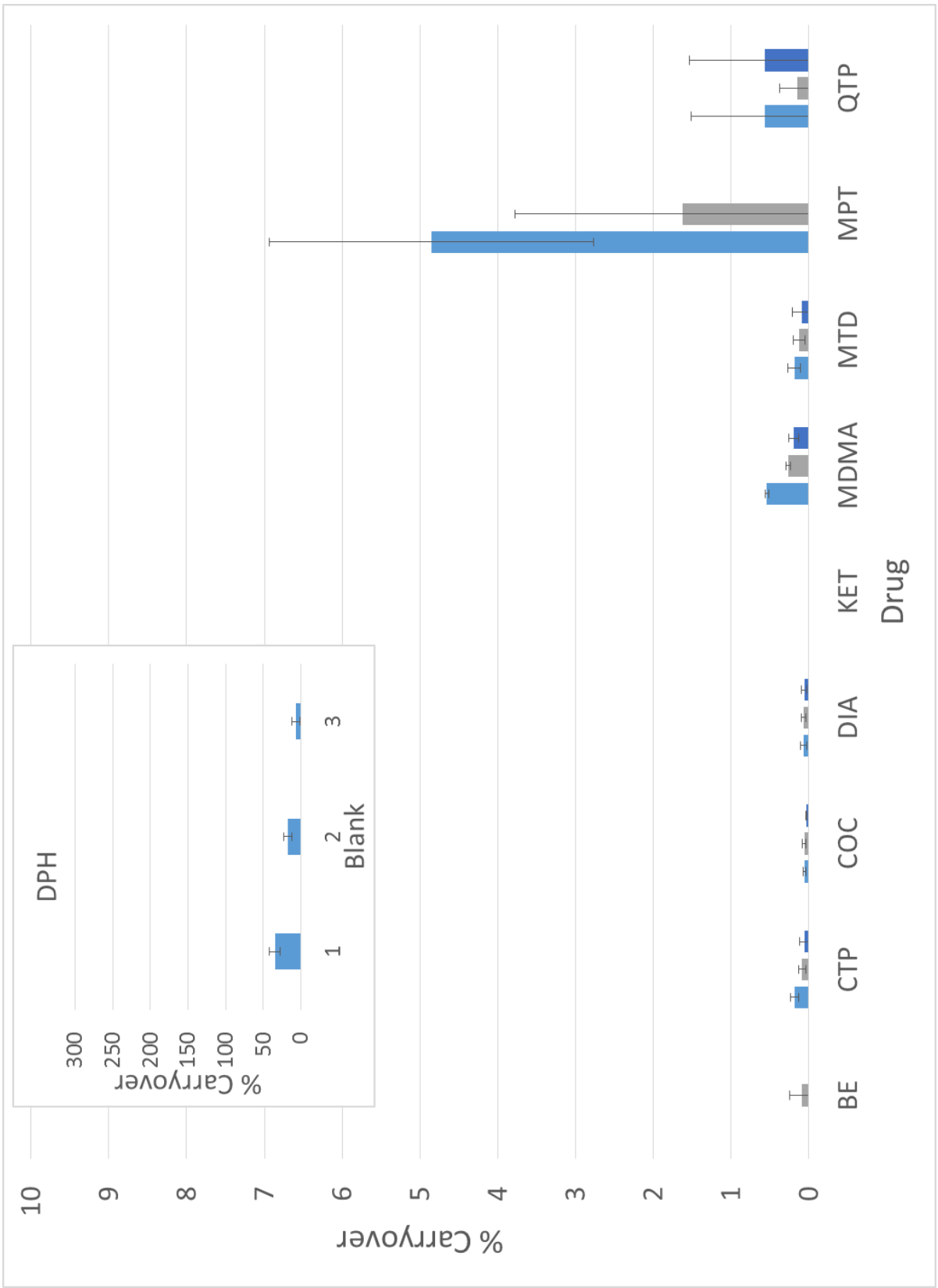

Figure 3.2.14 – Percent carryover in human blood for BE, CTP, COC, DIA, DPH, KET, MDMA, MTD, MPT, and QTP on the Gilson® GX-271 ASPEC™ SPE instrument.

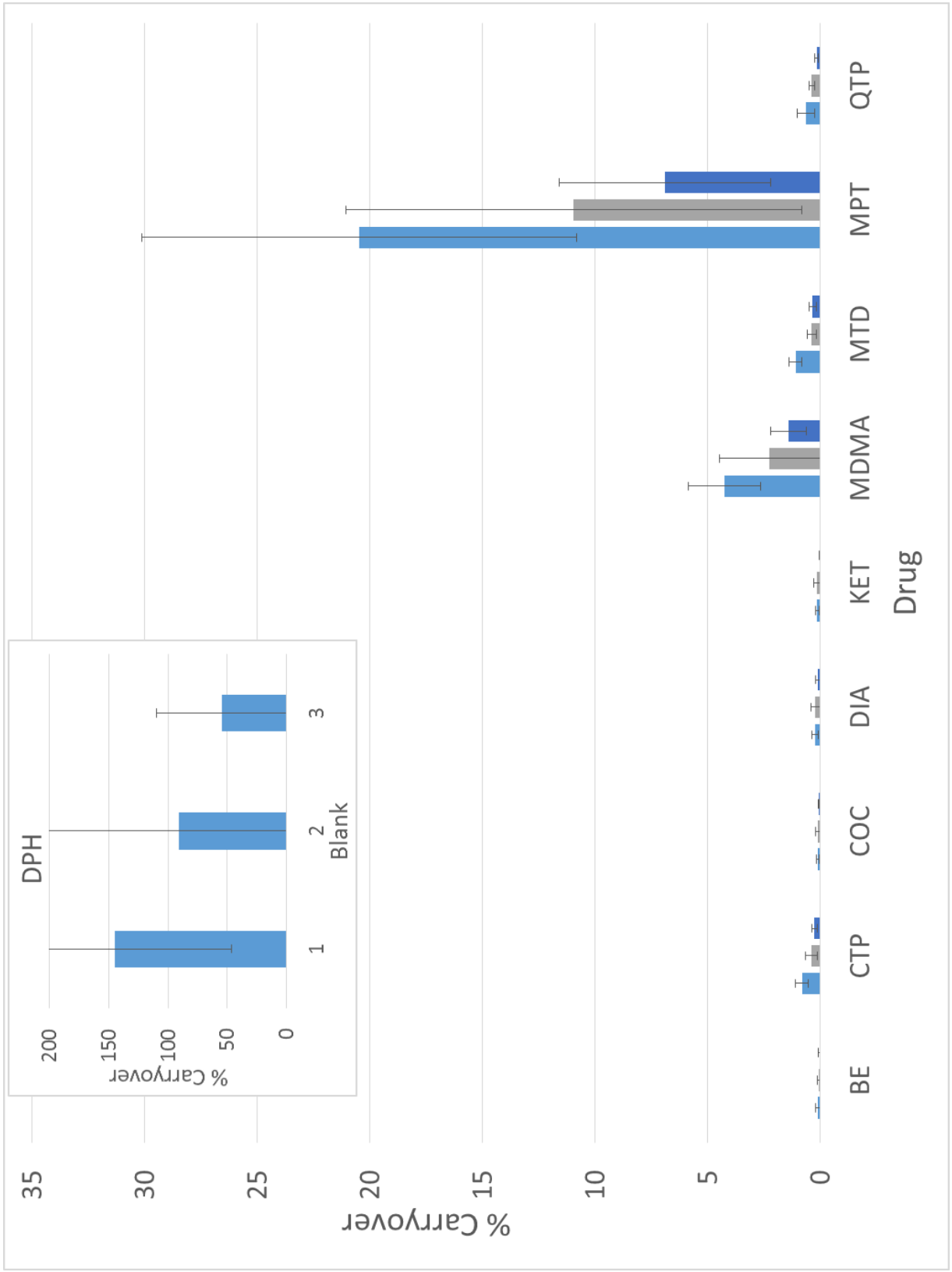

Figure 3.2.15 – Percent carryover in human serum for BE, CTP, COC, DIA, DPH, KET, MDMA, MTD, MPT, and QTP on the Biotage® RapidTrace® SPE instrument.

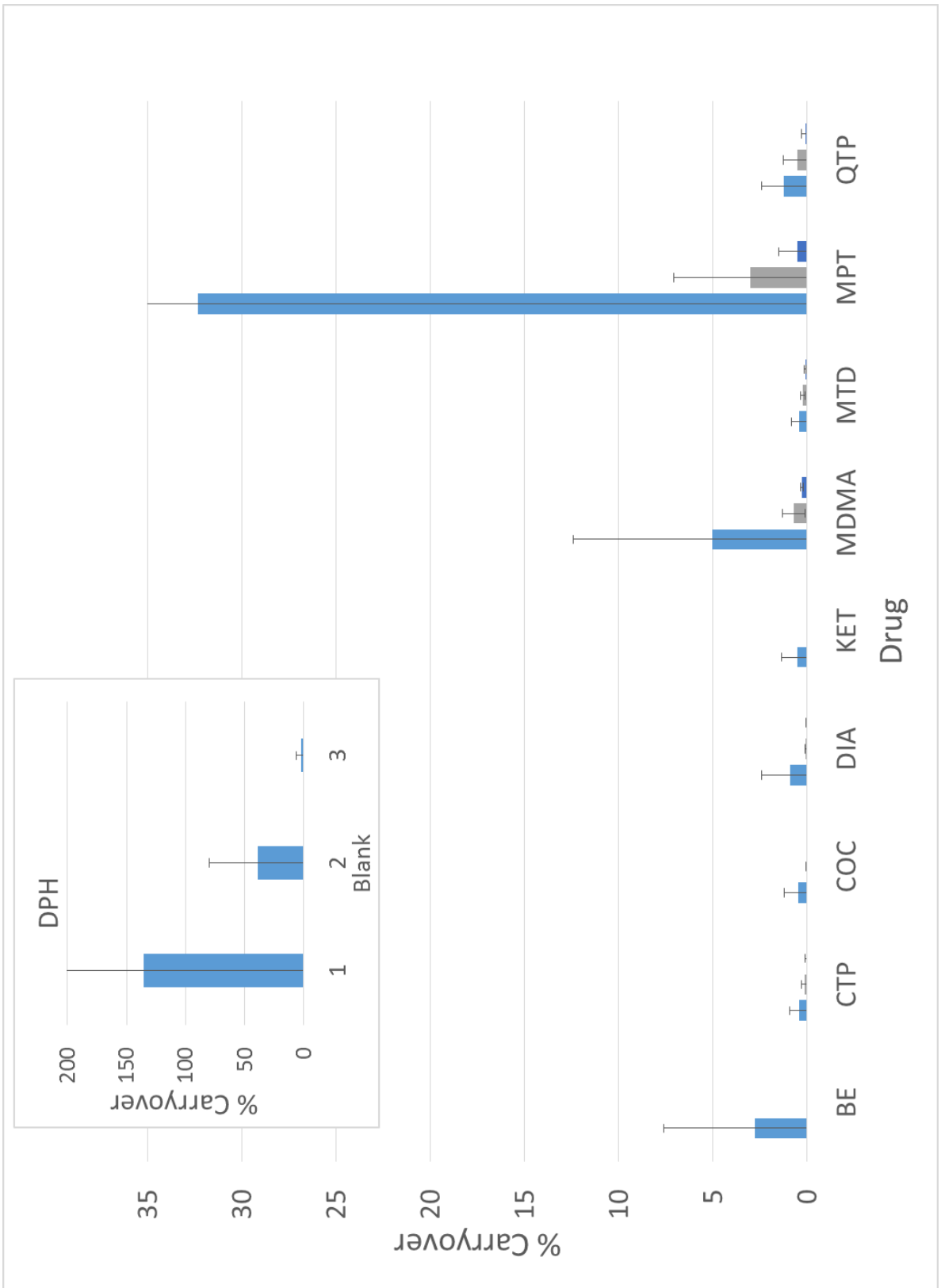

Figure 3.2.16 – Percent carryover in human serum for BE, CTP, COC, DIA, DPH, KET, MDMA, MTD, MPT, and QTP on the Gilson® GX-271 ASPEC™ SPE instrument.

3.2.2 Analysis of Variance (ANOVA)

One-way Analysis of variance (ANOVA) was performed in order to find differences in the means of percent carryover of all 10 analytes. ANOVA was first performed to find differences in carryover in each matrix type between the two instruments in blank 3 (Table 3.2.21). Table 3.2.22 shows this same analysis, however, using blank 1 to see statistical differences in the first sample following the high concentration sample. ANOVA was also performed to find differences of percent carryover of drugs between matrix types on the same instrument. This is demonstrated in tables 3.2.23 to 3.2.26 and was done in blank 1, and blank 3, for each instrument. The critical value for statistical significance for all analyses performed was set to 0.05 (ie. $p < 0.05$).

Table 3.2.21 – ANOVA results for comparing percent carryover means between the two instruments for blank 3 in animal blood, human blood, and human serum

Matrix	F-value	p- value
AN	0.847	0.370
HB	0.932	0.347
HS	1.247	0.279

Table 3.2.22 - ANOVA results for comparing percent carryover means between the two instruments for blank 1 in animal blood, human blood, and human serum

Matrix	F-value	p- value
AN	0.976	0.336
HB	0.823	0.376
HS	0.001	0.973

Table 3.2.23 – ANOVA results for comparing percent carryover means between matrix types on the Biotage® RapidTrace® in blank 1

Matrices	F-value	p- value
AN/HB	0.243	0.628
HB/HS	0.161	0.693
HS/AN	0.545	0.470

Table 3.2.24 – ANOVA results for comparing percent carryover means between matrix types on the Biotage® RapidTrace® in blank 3

Matrices	F-value	p- value
AN/HB	0.014	0.908
HB/HS	0.044	0.836
HS/AN	0.103	0.752

Table 3.2.25 – ANOVA results for comparing percent carryover means between matrix types on the Gilson® GX-271 ASPEC™ in blank 1

Matrices	F-value	p- value
AN/HB	0.014	0.906
HB/HS	1.005	0.329
HS/AN	1.096	0.309

Table 3.2.26 – ANOVA results for comparing percent carryover means between matrix types on the Gilson® GX-271 ASPEC™ in blank 3

Matrices	F-value	p- value
AN/HB	0.154	0.669
HB/HS	0.323	0.577
HS/AN	0.627	0.439

Chapter 4: DISCUSSION

4.1 Instrument Operation

The set up process for the Biotage® RapidTrace® instrument has more components involved than the process for the Gilson® GX-271 ASPEC™ due to the purge and clean cycles that must be run separately from the extraction procedure, and the manual connection of lines to solvent bottles. The set up for the GX-271 ASPEC™ is done altogether; there is no need to connect solvents to the instrument with lines, and there is less computer software work involved. If the laboratory purchases the GX-274 ASPEC™ instrument, it will be able to extract four samples at once, and therefore, will take less time to complete the procedure. Due to absence of any errors during the experiments, it is suggested that the GX-274 ASPEC™ could be run overnight with minimal risk of sample loss due to liquid handling error. The Biotage® RapidTrace® had multiple errors on the first day of the experiment, requiring maintenance and continuous observation for the duration of the project. Overall the GX-271 ASPEC™ also required less volume for each solvent, with the exception of methanol. It is expected that the GX-274 ASPEC™ will use approximately the same volume of each solvent when extracting the same number of samples. The variation in volumes over the three experiments for each instrument is likely due, at least in part, to the glassware used for measurement. Estimation was undertaken according to approximate graduation marks on the glass bottles used to store the solvents. Only the elution solvent was measured using a graduated cylinder, and is therefore, more accurate than other solvents measured using beakers. Amount of consumables used was found to be similar between the instruments. Both used the same amount of test tubes, cartridges, and pipette tips, as they require the same sample preparation. One minor difference is the use of

proprietary cartridge caps on the GX-271 ASPEC™ unit, however it is expected that this will have no significant cost impact.

4.2 Degree of Carryover

The figures produced for the analytes detected in three blank samples, following the high concentration samples, indicate numerous occurrences of carryover for both instruments. Most analytes show a general tendency of having higher percent carryover values in each blank on the RapidTrace® compared to the GX-271 ASPEC™ when observing differences in each matrix type between instruments. The blank samples collected from the GX-271 ASPEC™ extraction also revealed less time to extinction for the ten analytes. There was a significantly faster decay of drugs over time to a very low value or complete extinction by blank 3. The RapidTrace® revealed a significant decay over time as well, however, still showed higher responses in the final blank. Diphenhydramine had more percent carryover in each matrix and on each instrument than any other analyte, however, the values are consistent with the trend of the GX-271 ASPEC™ having lower overall carryover and less time to extinction of the drug.

Positive identifications of any drugs from the high concentration mixture found in quality control blanks (blank 0) could potentially have an impact on the response of an analyte in blanks 1, 2, and 3. They were extracted preceding the high concentration samples for quality control purposes however did result in some positive identifications for drugs on both instruments. This is potentially an indication of pre-existing carryover within the extraction units, or could also be a result of contamination during the following manual steps: dry-down, reconstitution and analysis. The carryover demonstrated in this extraction represents the maximum expected carryover to be encountered when using this analysis, as the concentrations of analytes spiked into these matrices are rarely encountered in criminal forensic casework. In addition, it is

common practice in forensic toxicology to require the detection of a drug in two separate exhibit subsamples, using two separate extraction and detection methods where possible, before a drug finding can be reported. As such, the risk of falsely reporting a drug finding due to carryover in this method is mitigated.

4.2.1 Interpretation of Statistical Analysis

For each ANOVA analysis performed, $p > 0.05$, meaning that there are no statistically significant differences found in any of the six instances. Using percent carryover values from blanks 1 and 3, no significant statistical difference was found between instruments in any of the three matrix types. The ANOVA results when comparing percent carryover between matrix types on each individual instrument also demonstrated no significant differences for blank 1 or blank 3. Although this is the case statistically, it can be seen through visual comparison that the Gilson® GX-271 ASPEC™ demonstrated better overall control of carryover.

4.3 Benefits of Research

To my knowledge, this is the first study to specifically compare the Biotage® RapidTrace®, and the Gilson® GX-271 ASPEC™ automated SPE instruments in terms of instrument operation, and degree of carryover. This comparison is necessary for the RCMP Toxicology Services Laboratory as well as any laboratory considering the replacement of a Biotage® RapidTrace® robotics system. The analytes chosen for this research project are all of forensic relevance, and are known to have existing carryover on the RapidTrace® instrument. Human blood was chosen as a matrix type as it is the most common sample submitted to the lab for casework. Better overall control of carryover by a SPE instrument can increase both extraction and laboratory efficiency when processing high concentration samples.

4.4 Limitations

The main limitation in this research project is the age of the Biotage® RapidTrace® SPE instrument. As a result of the Gilson® GX-271 ASPEC™ SPE instrument never being used prior to these experiments, it is unexpected that errors, maintenance, or issues with the newer system are accurately represented. A newer RapidTrace® instrument also may have the ability to successfully run overnight, impacting time differences. However due to budget considerations, most laboratories would be unable to acquire a new Biotage® RapidTrace® instrument for the purpose of the project. Another limitation is the use of only 3 blank samples following the high concentration sample. Since not all drugs were completely extinct in blank 3, additional blanks should have been implemented to entirely determine the time to extinction of carryover.

Chapter 5: CONCLUSIONS

5.1 Summation

Overall the Gilson® GX-271 ASPEC™ SPE instrument proved to have better overall performance and ease of operation in terms of manual set up process and time, user friendliness, and time to complete extraction (including purge and wash steps). It had no errors, used lower solvent volumes, and used fewer consumables when compared to the Biotage® RapidTrace® instrument. The GX-271 ASPEC™ instrument also produces lower overall drug responses in blank samples following a high concentration sample and faster time to extinction of drugs demonstrating improved control of carryover relative to the RapidTrace® instrument.

5.2 Implementation and Recommendation for Future Work

After assessing the compiled results, the Gilson® GX-274 ASPEC™ is recommended as a viable alternative to the RapidTrace® SPE robotics system for use in forensic casework. The results of this study show that there is no statistical significance to the observed carryover when comparing the two instruments, however, this carryover is still a present issue in laboratories. Future studies should be directed to finding a solution to the carryover problem with automated extraction and analysis instruments, including the QToF-MS instrument. Since the newer instrument advertising a reduction in carryover still in fact does result in some contamination following high concentration samples, the instrument may need a more rigorous wash step after the extraction procedure.

APPENDIX I

Drug Response of the Biotage® RapidTrace® and Gilson® GX-271 ASPEC™ in Animal Blood

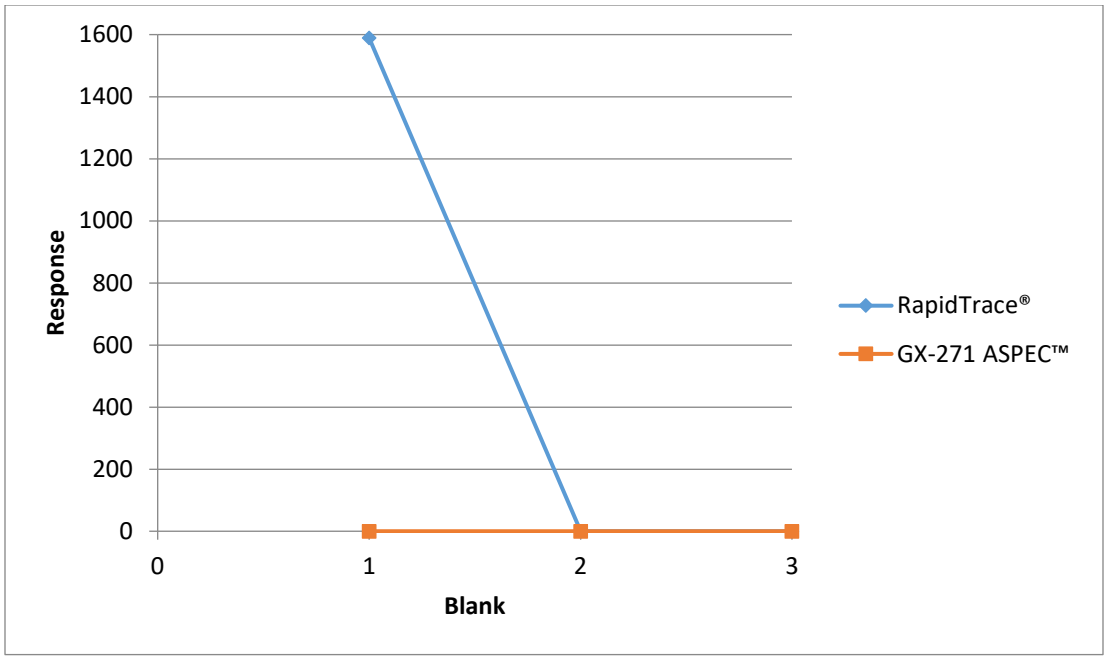

Figure A1 – Benzoylecgonine response in animal blood for the RapidTrace® and GX-271 ASPEC™

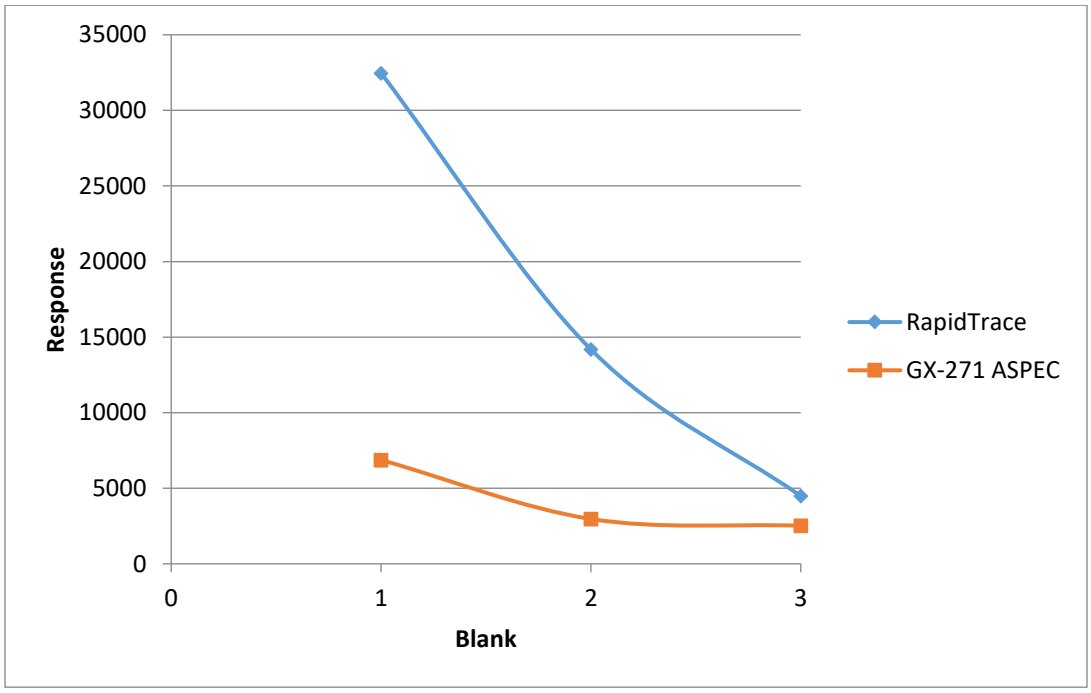

Figure A2 – Citalopram response in animal blood for the RapidTrace® and GX-271 ASPEC™

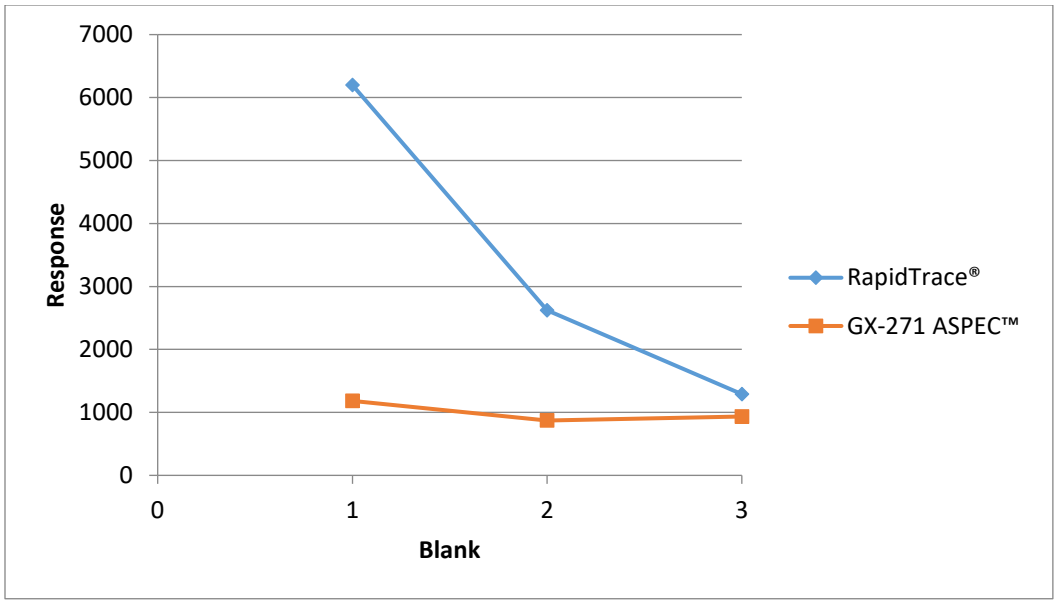

Figure A3 – Cocaine response in animal blood for the RapidTrace® and GX-271 ASPEC™

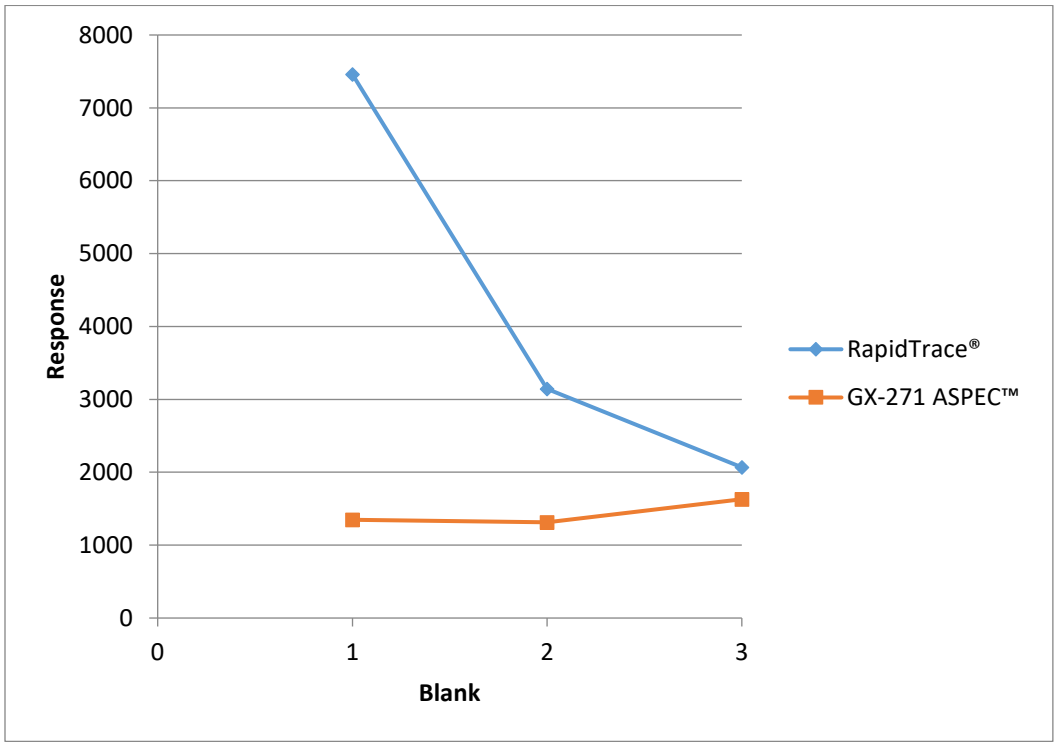

Figure A4 – Diazepam response in animal blood for the RapidTrace® and GX-271 ASPEC™

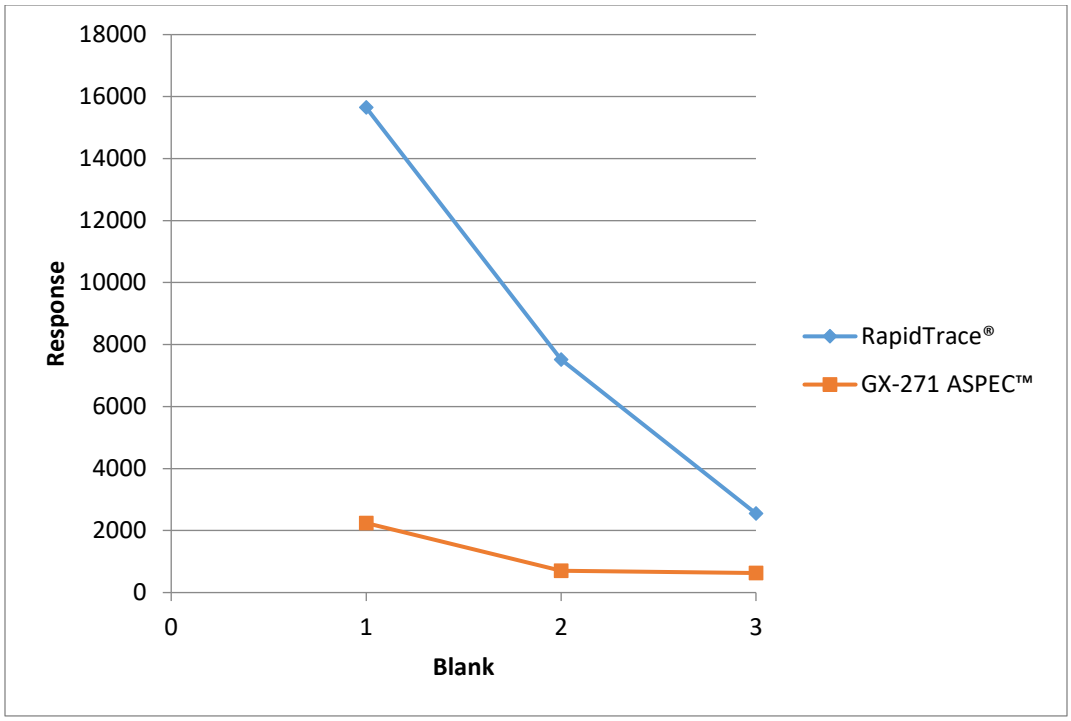

Figure A5 – Diphenhydramine response in animal blood for the RapidTrace® and GX-271 ASPEC™

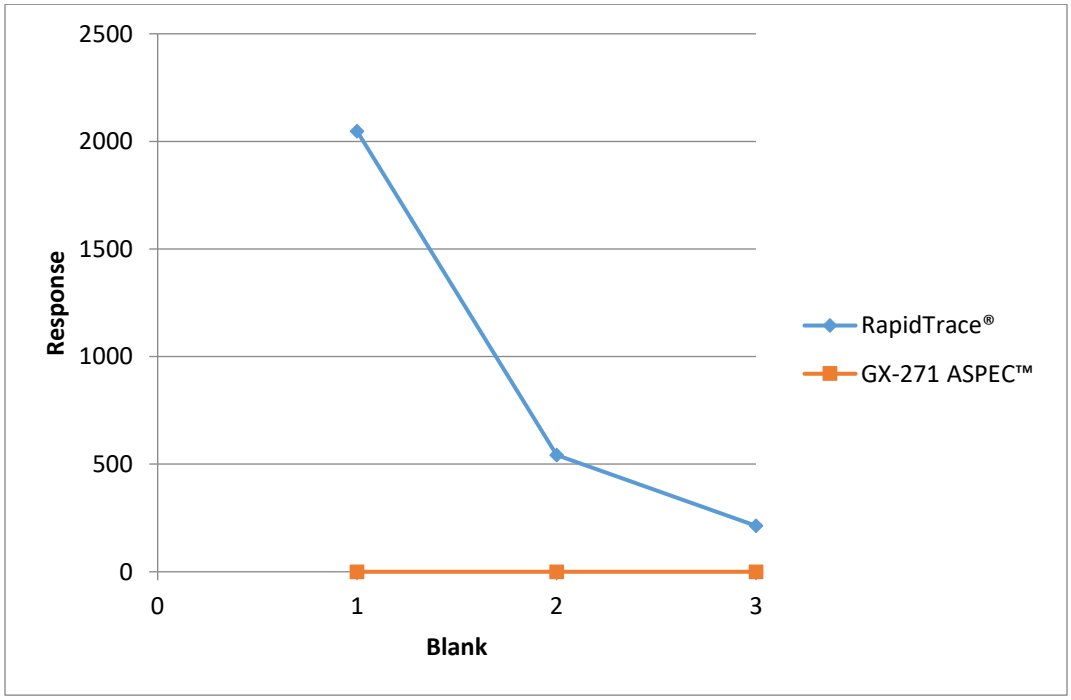

Figure A6 – Ketamine response in animal blood for the RapidTrace® and GX-271 ASPEC™

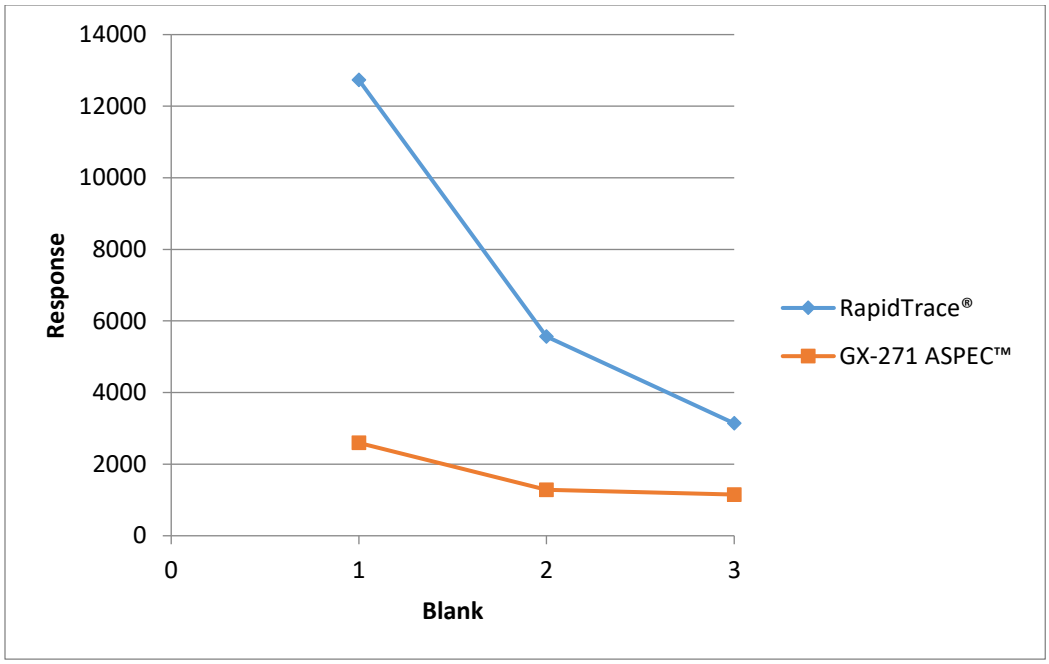

Figure A7 – MDMA response in animal blood for the RapidTrace® and GX-271 ASPEC™

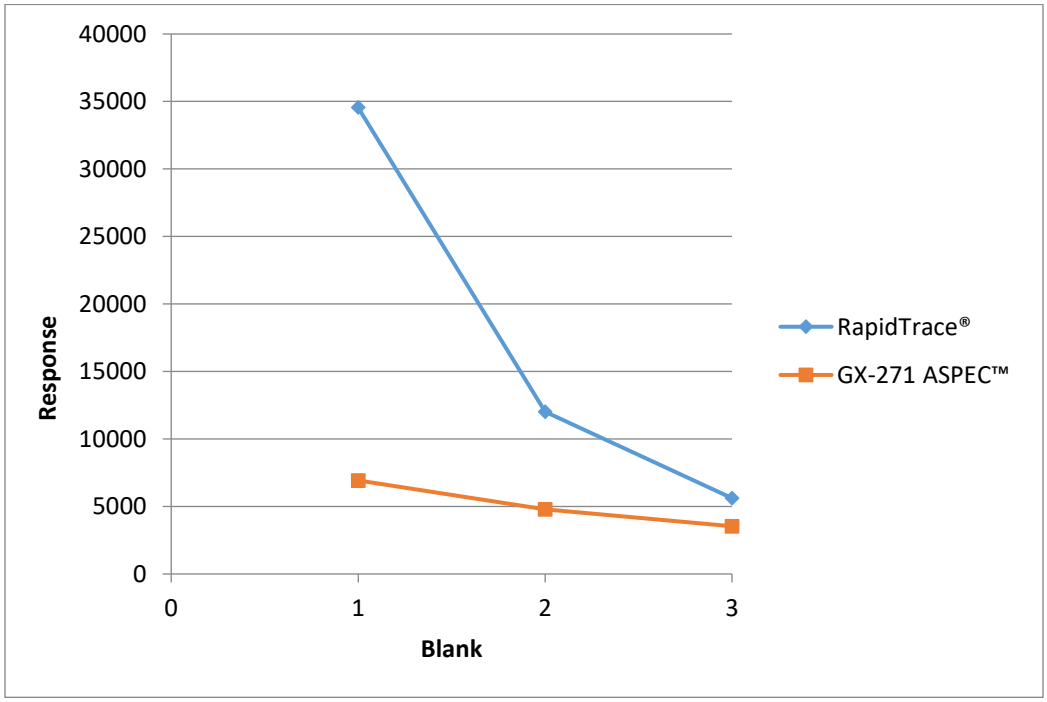

Figure A8 – Methadone response in animal blood for the RapidTrace® and GX-271 ASPEC™

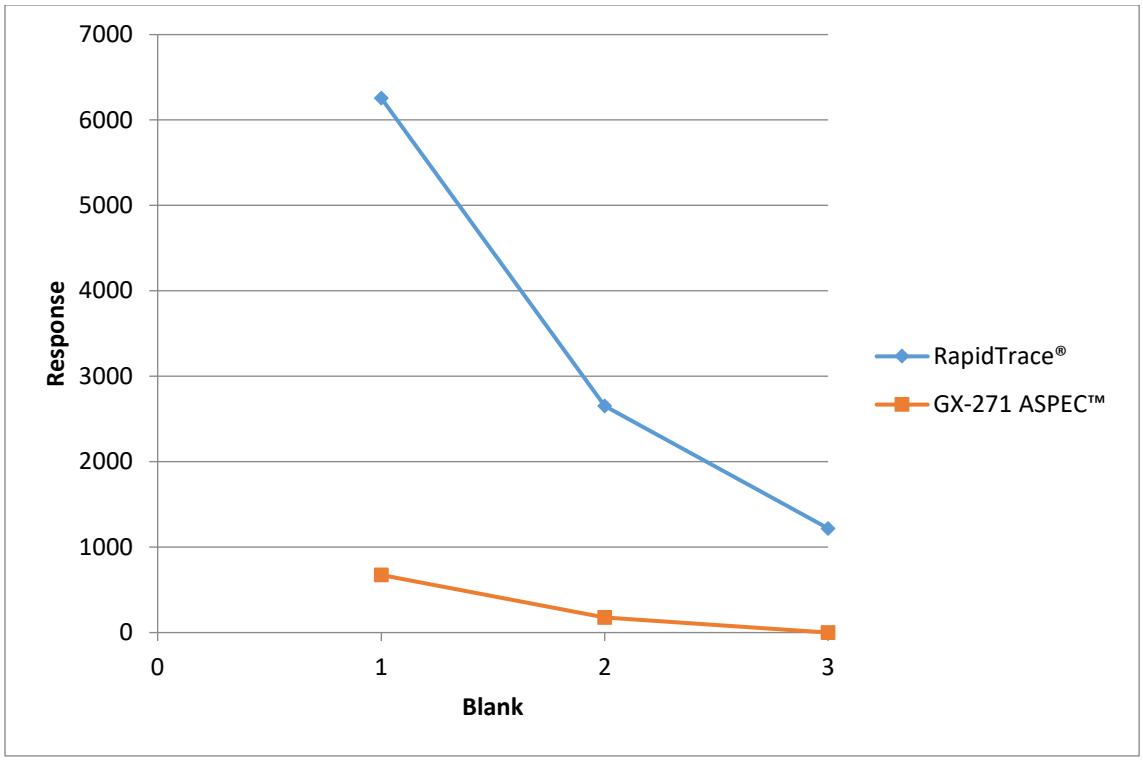

Figure A9 – Methamphetamine response in animal blood for the RapidTrace® and GX-271 ASPEC™

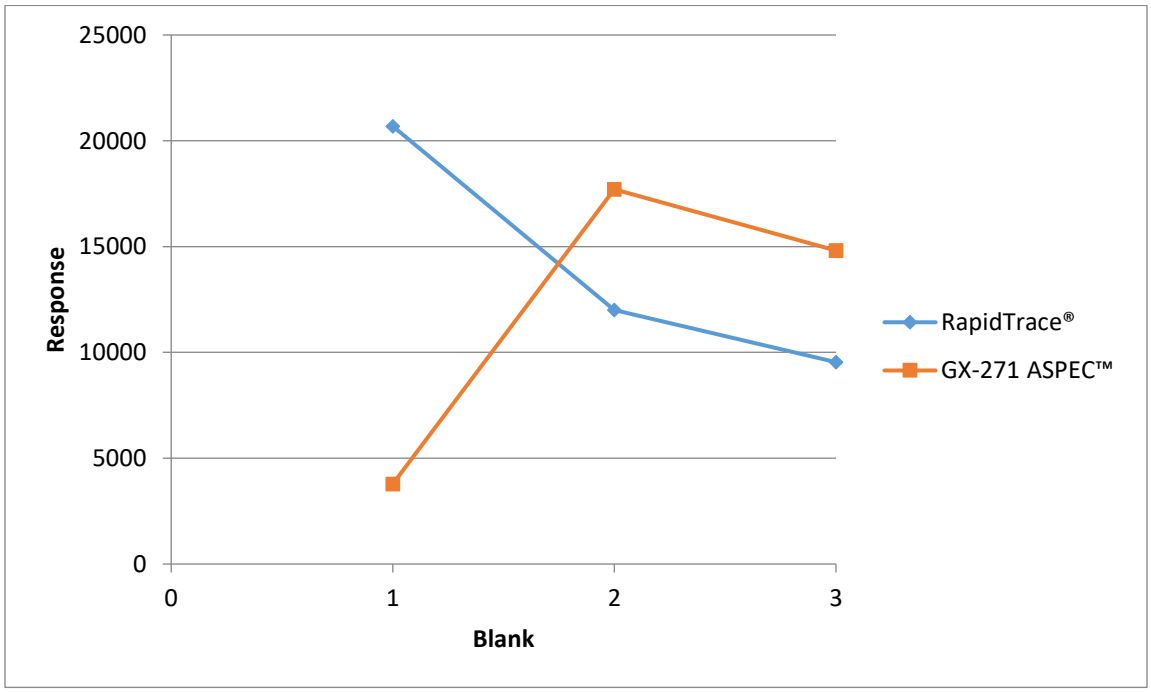

Figure A10 – Quetiapine response in animal blood for the RapidTrace® and GX-271 ASPEC™

APPENDIX II

Drug Response of the Biotage® RapidTrace® and Gilson® GX-271 ASPEC™ in Human Blood

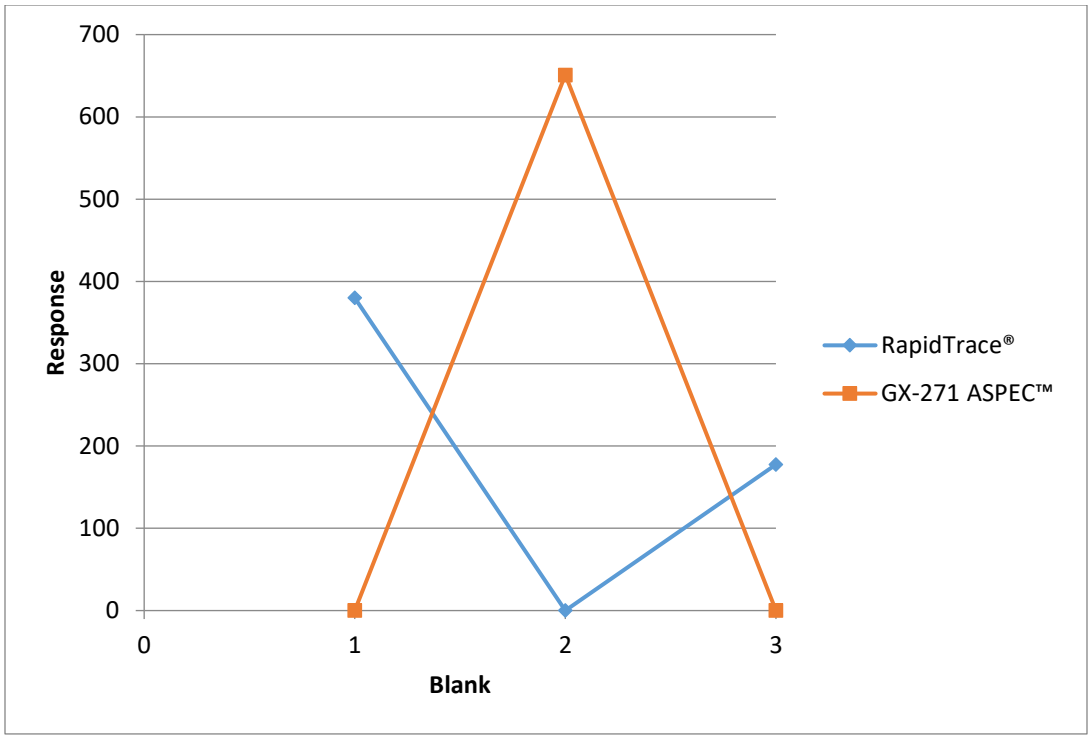

Figure A11 – Benzoylecgonine response in human blood for the RapidTrace® and GX-271 ASPEC™

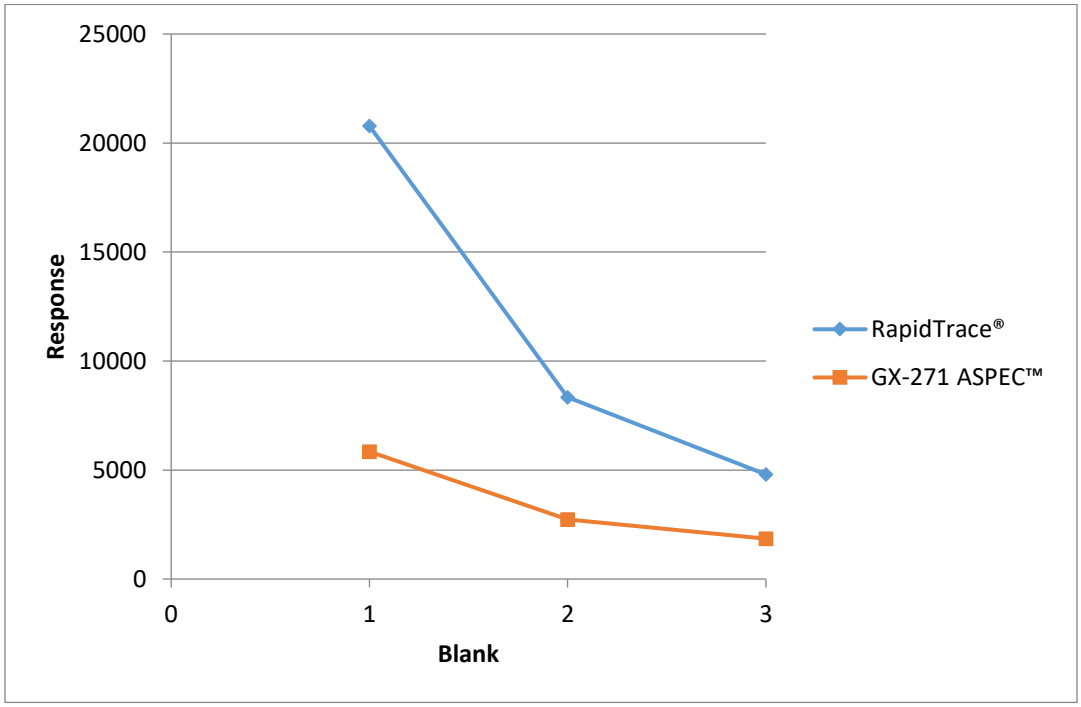

Figure A12 – Citalopram response in human blood for the RapidTrace® and GX-271 ASPEC™

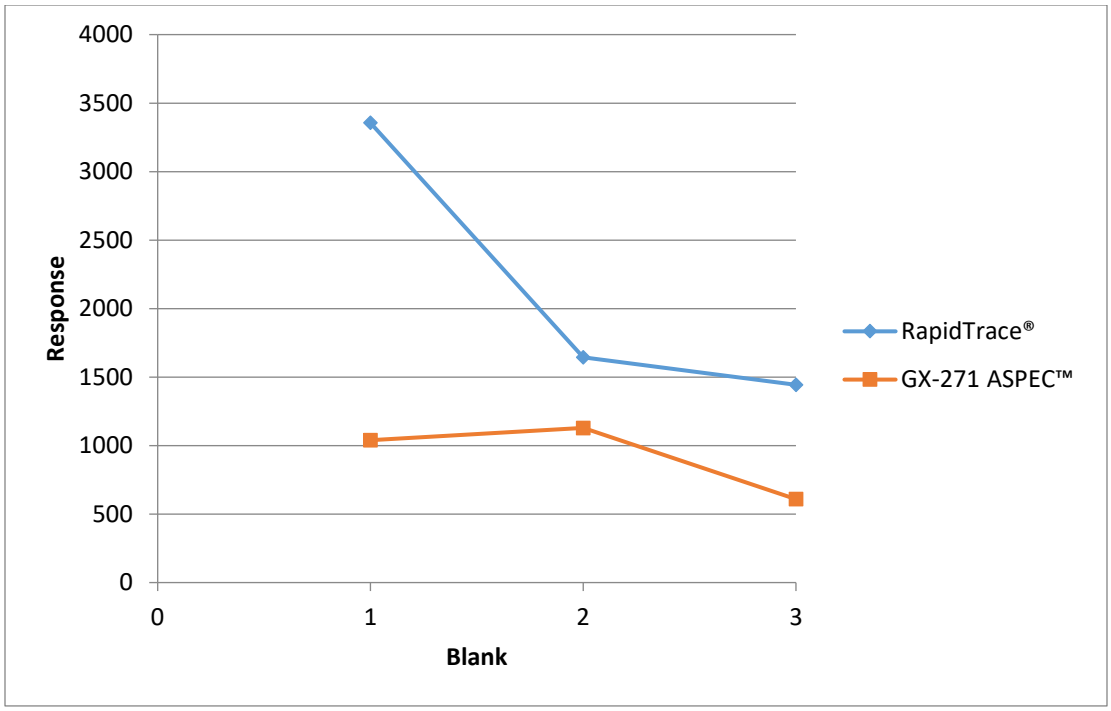

Figure A13 – Cocaine response in human blood for the RapidTrace® and GX-271 ASPEC™

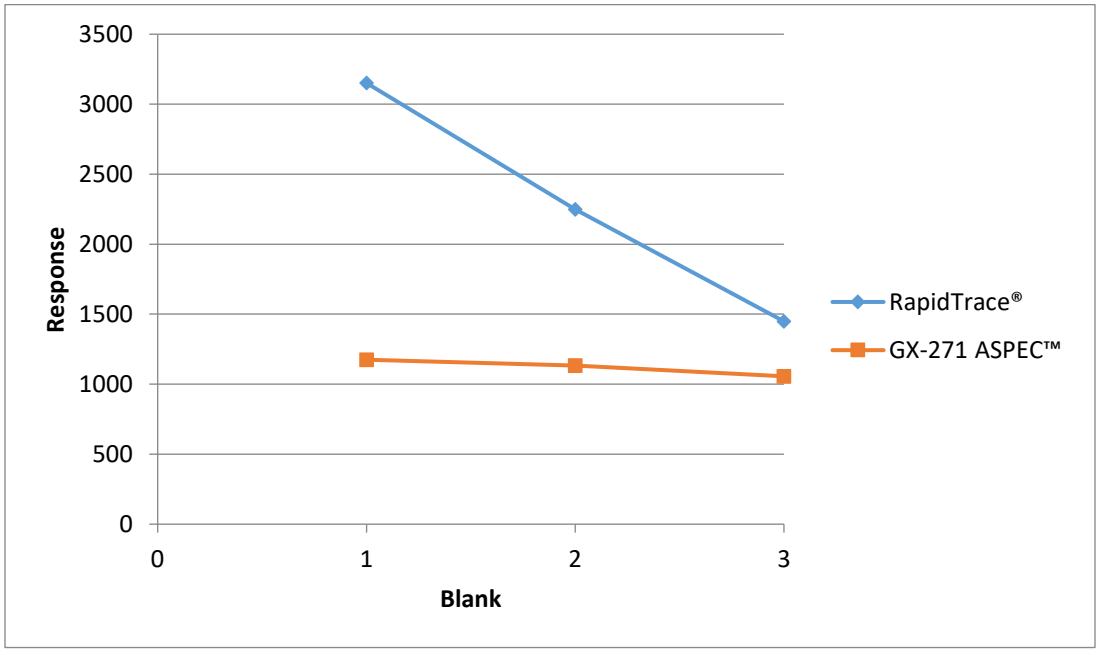

Figure A14 – Diazepam response in human blood for the RapidTrace® and GX-271 ASPEC™

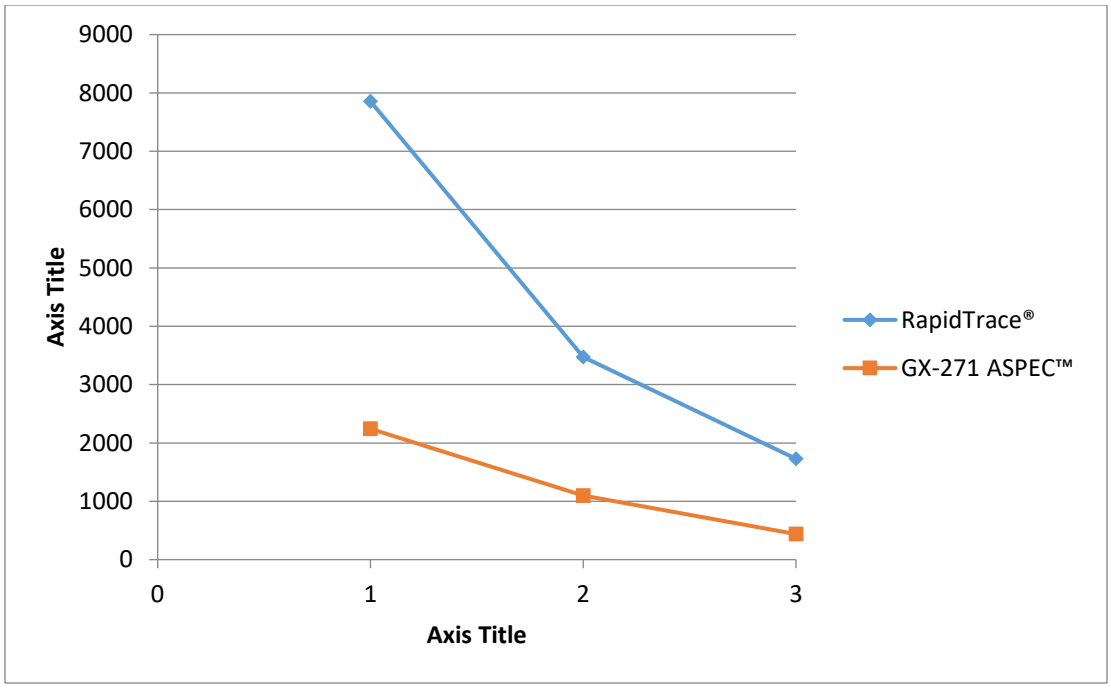

Figure A15 – Diphenhydramine response in human blood for the RapidTrace® and GX-271 ASPEC™

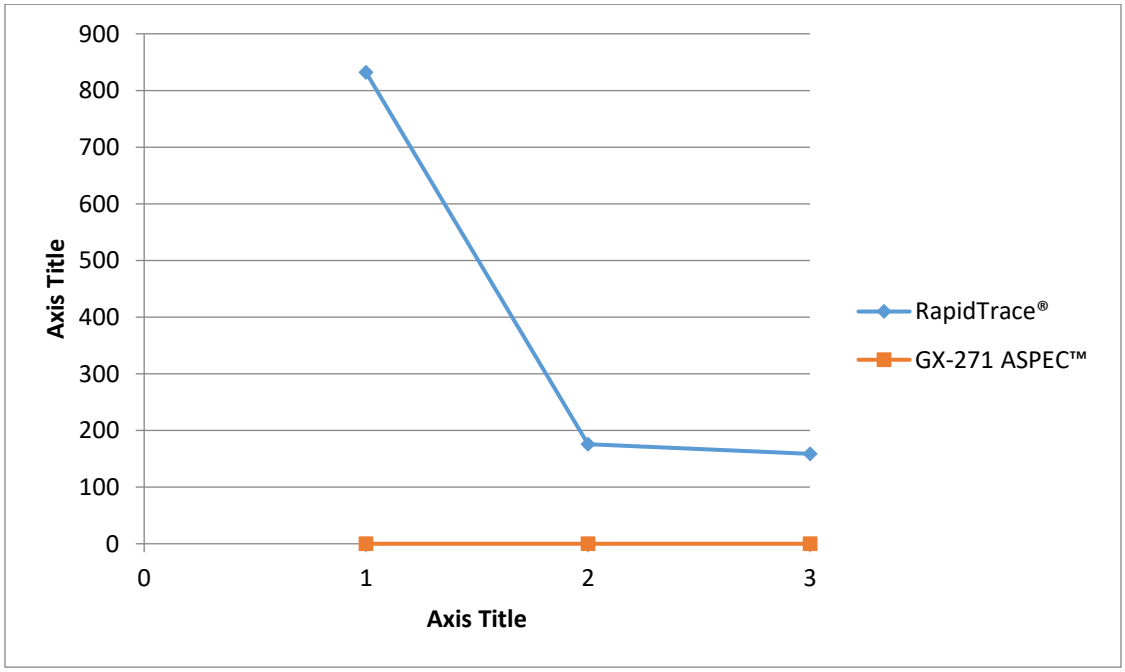

Figure A16 – Ketamine response in human blood for the RapidTrace® and GX-271 ASPEC™

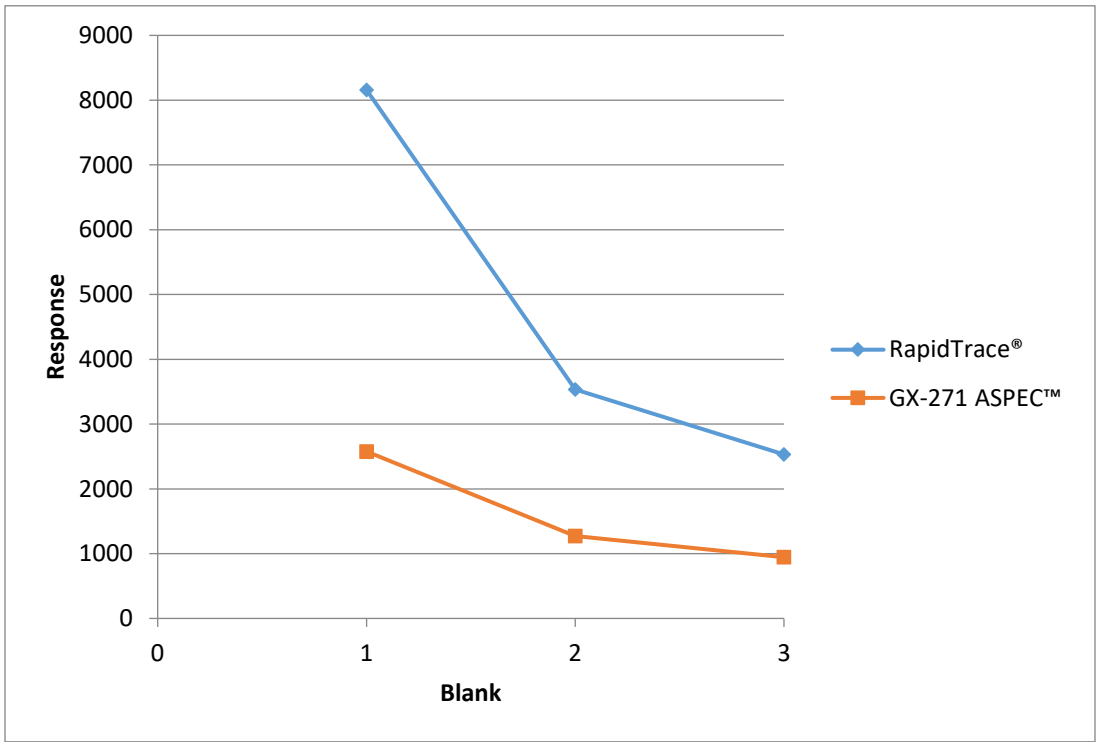

Figure A17 – MDMA response in human blood for the RapidTrace® and GX-271 ASPEC™

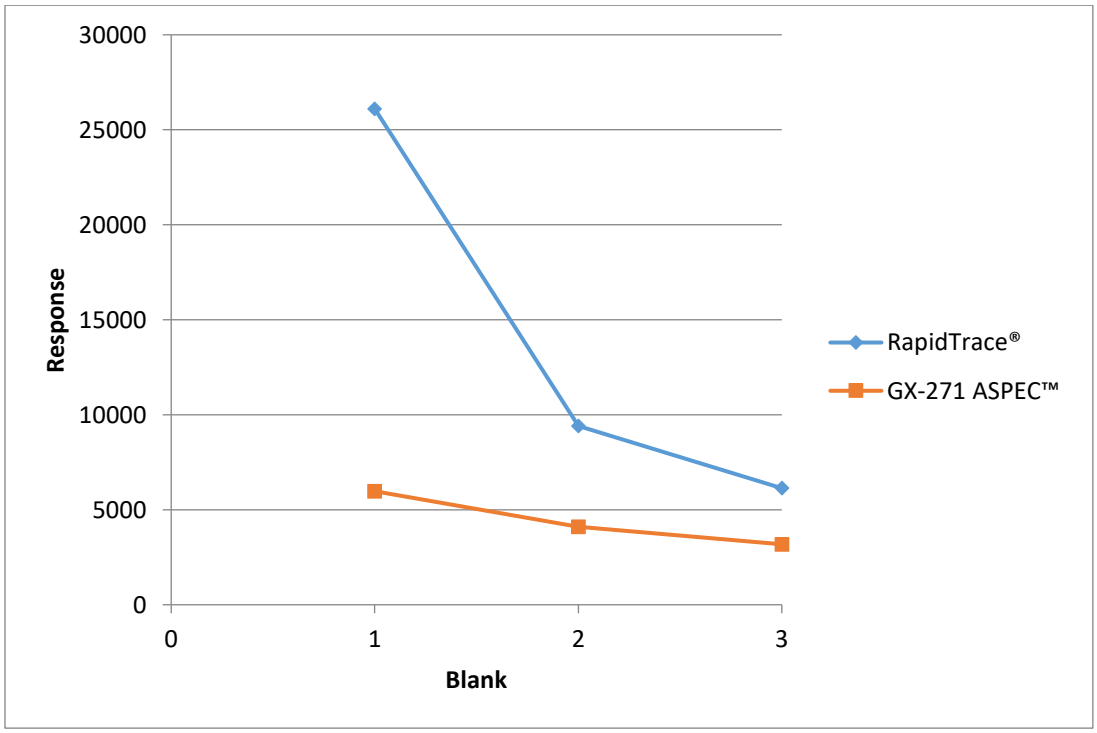

Figure A18 – Methadone response in human blood for the RapidTrace® and GX-271 ASPEC™

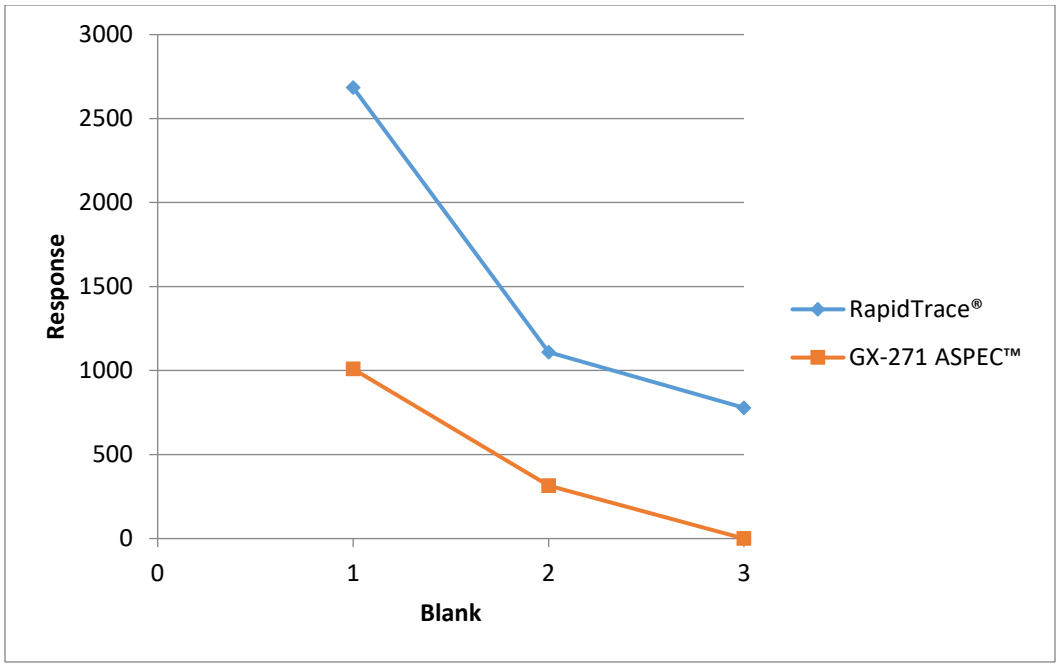

Figure A19 – Methamphetamine response in human blood for the RapidTrace® and GX-271 ASPEC™

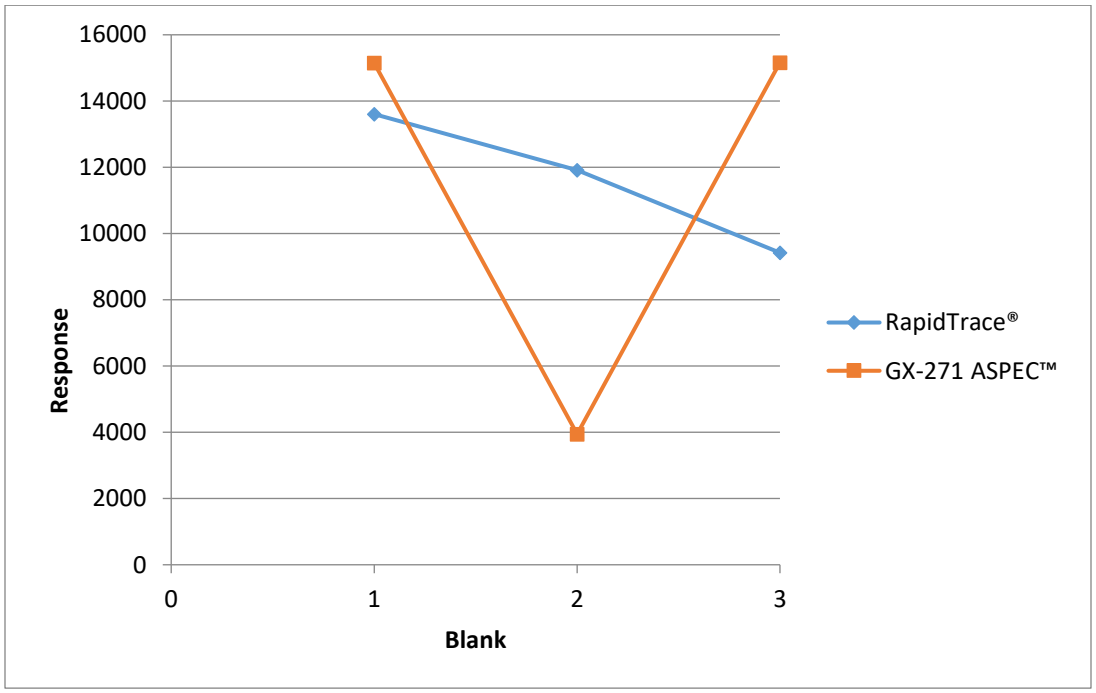

Figure A20 – Quetiapine response in human blood for the RapidTrace® and GX-271 ASPEC™

APPENDIX III

Drug Response of the Biotage® RapidTrace® and Gilson® GX-271 ASPEC™ in Human Serum

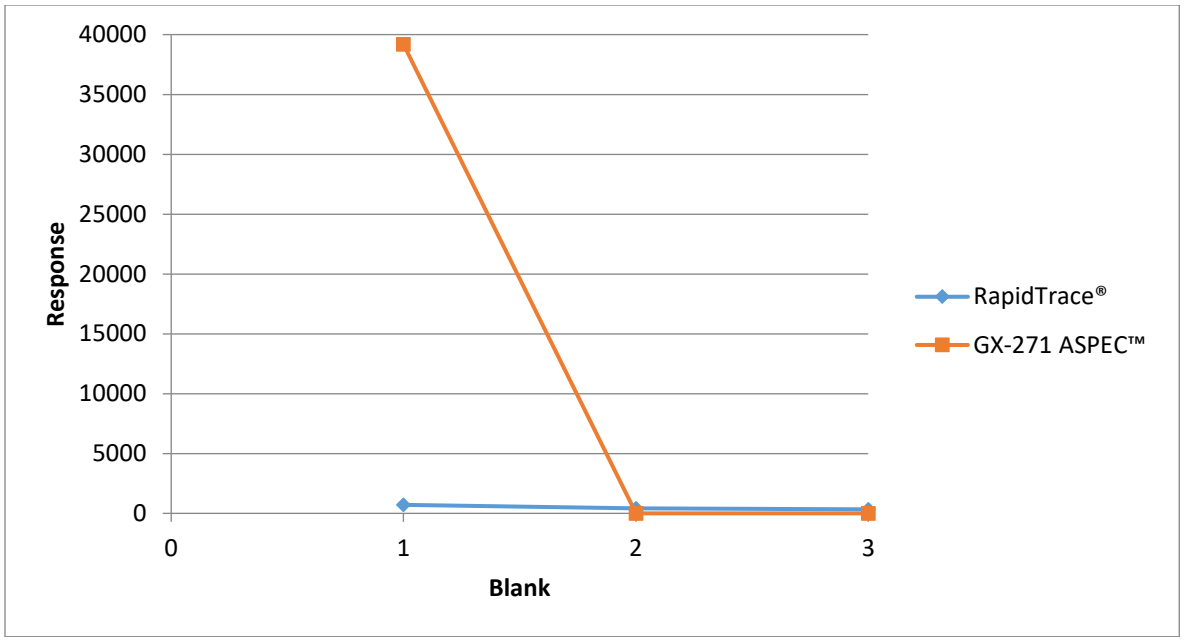

Figure A21 – Benzoylecgonine response in human serum for the RapidTrace® and GX-271 ASPEC™

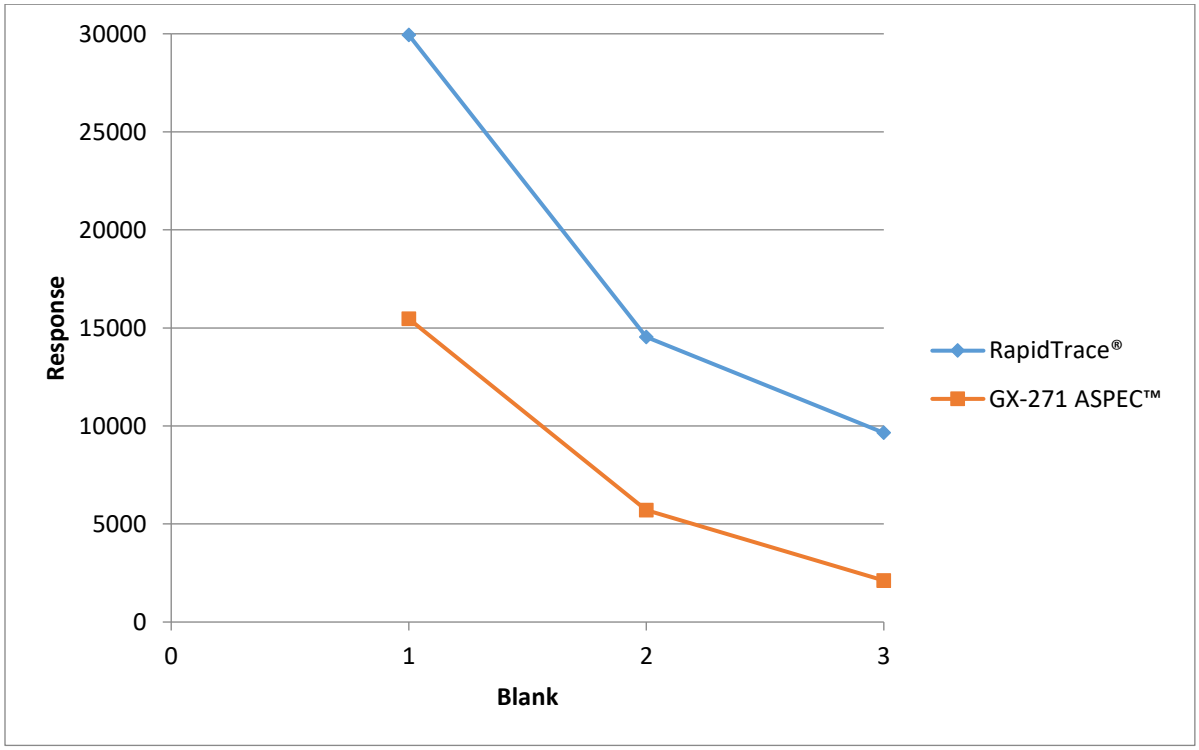

Figure A22 – Citalopram response in human serum for the RapidTrace® and GX-271 ASPEC™

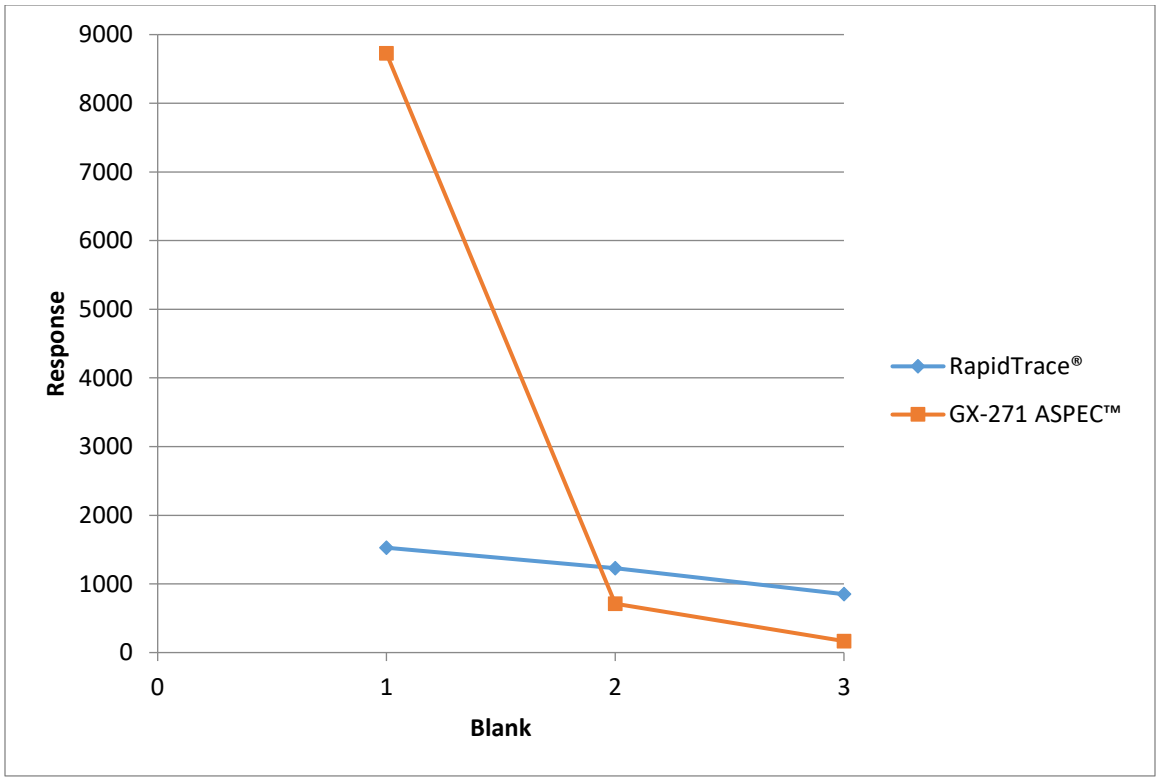

Figure A23 – Cocaine response in human serum for the RapidTrace® and GX-271 ASPEC™

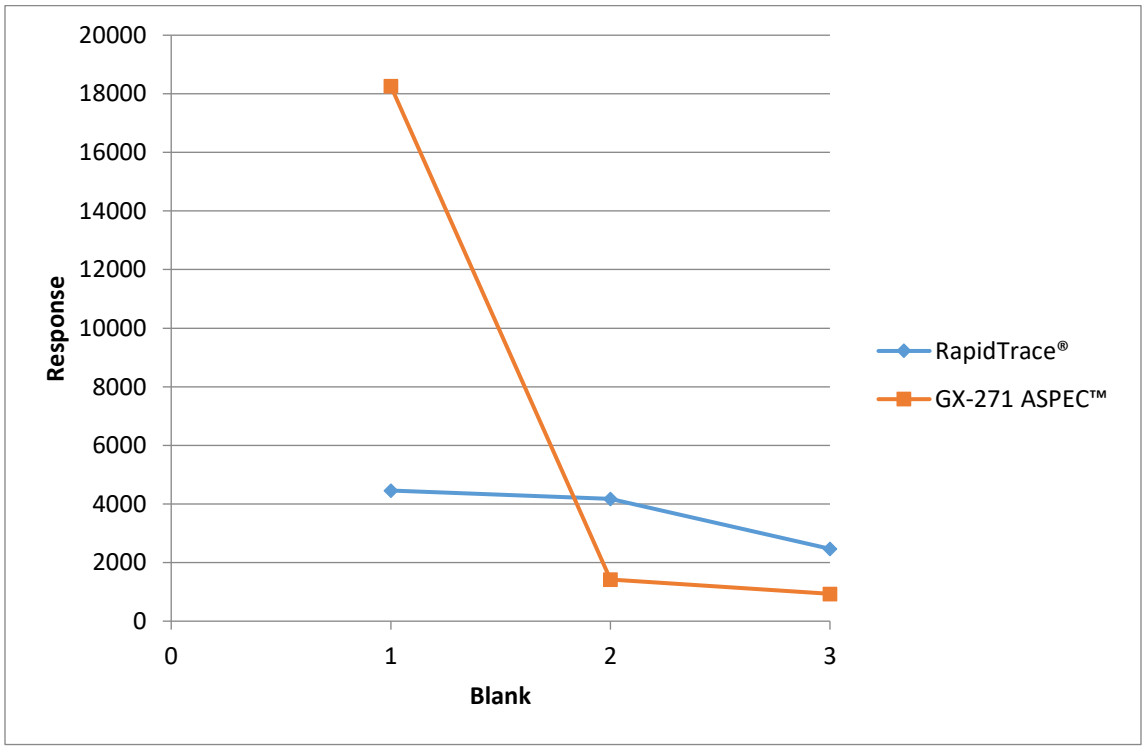

Figure A24 – Diazepam response in human serum for the RapidTrace® and GX-271 ASPEC™

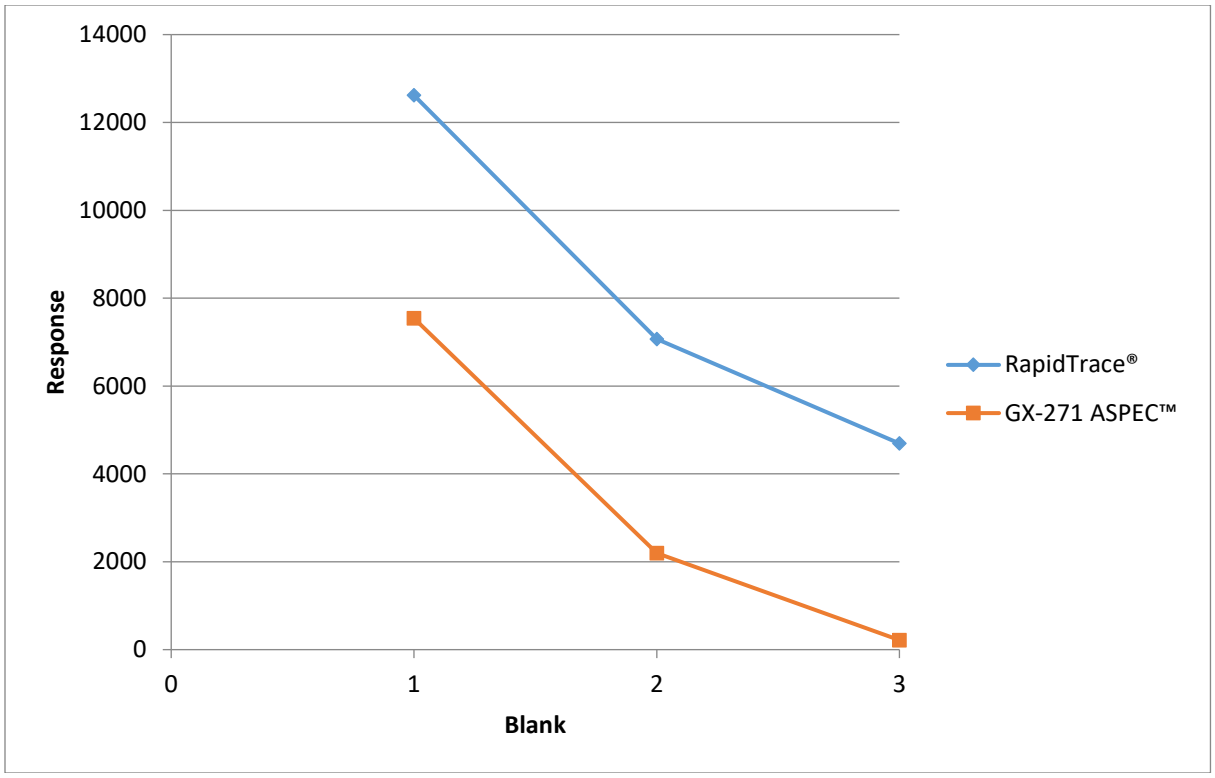

Figure A25 – Diphenhydramine response in human serum for the RapidTrace® and GX-271 ASPEC™

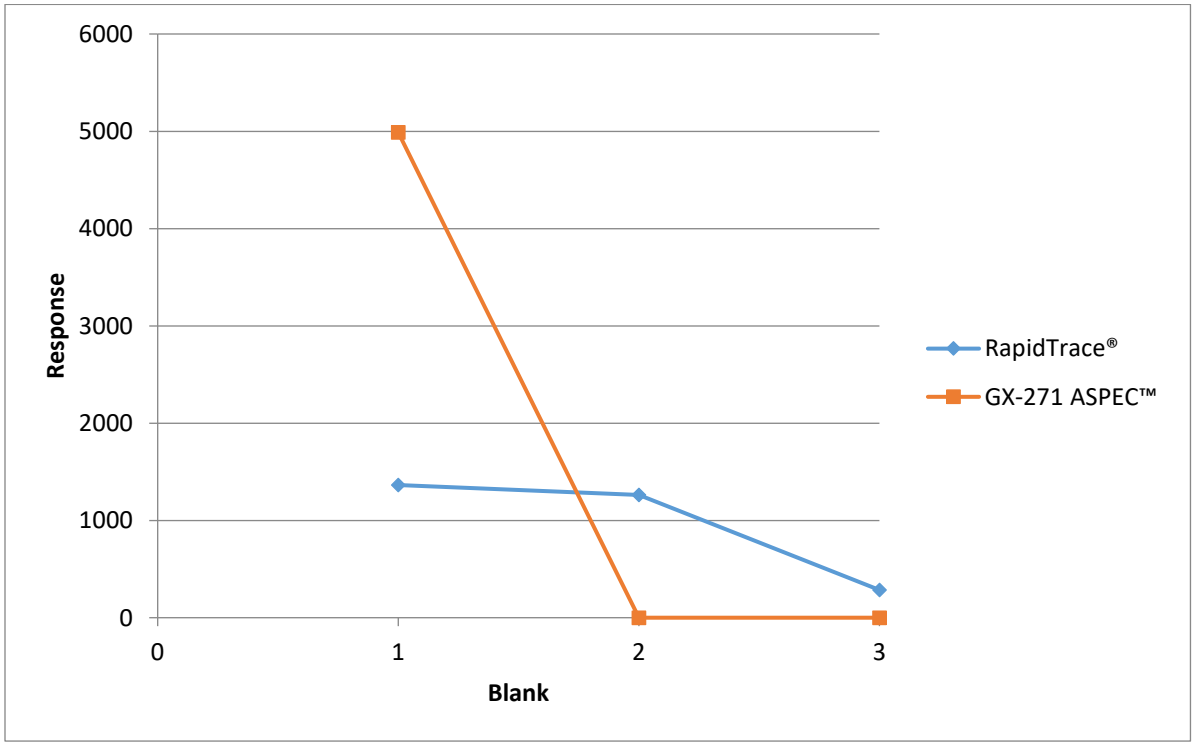

Figure A26 – Ketamine response in human serum for the RapidTrace® and GX-271 ASPEC™

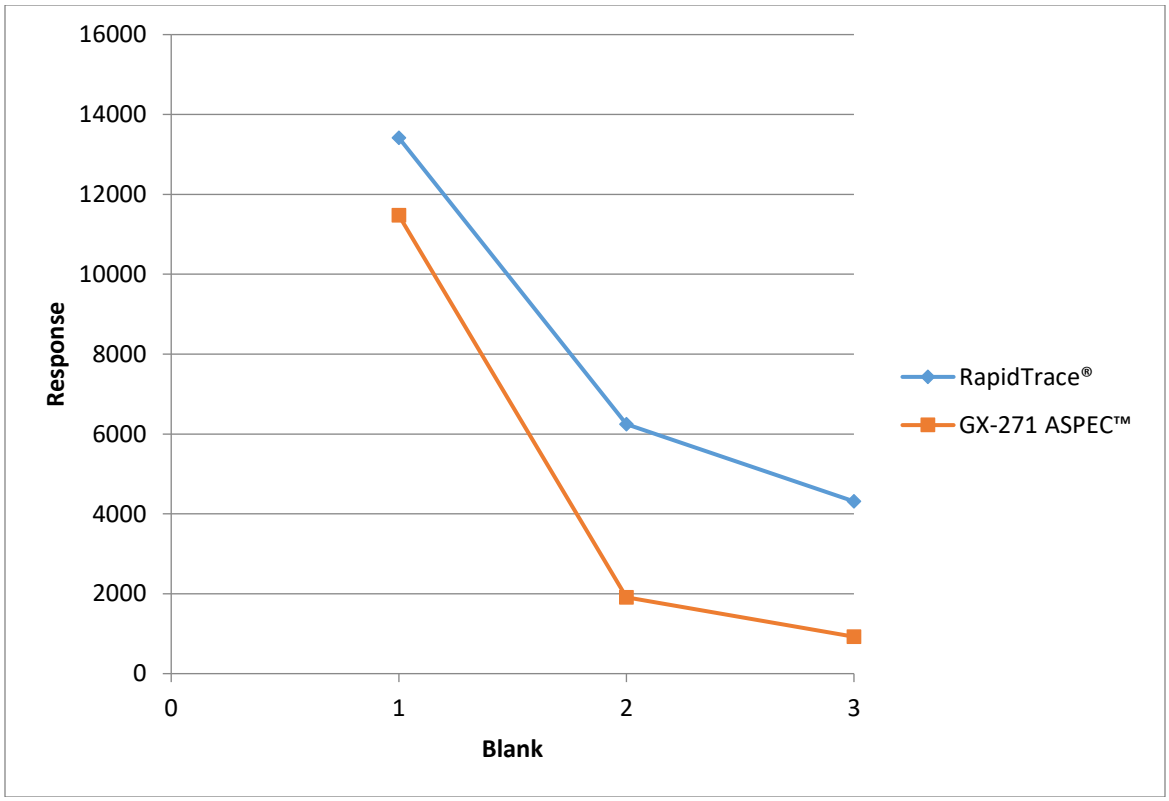

Figure A27 – MDMA response in human serum for the RapidTrace® and GX-271 ASPEC™

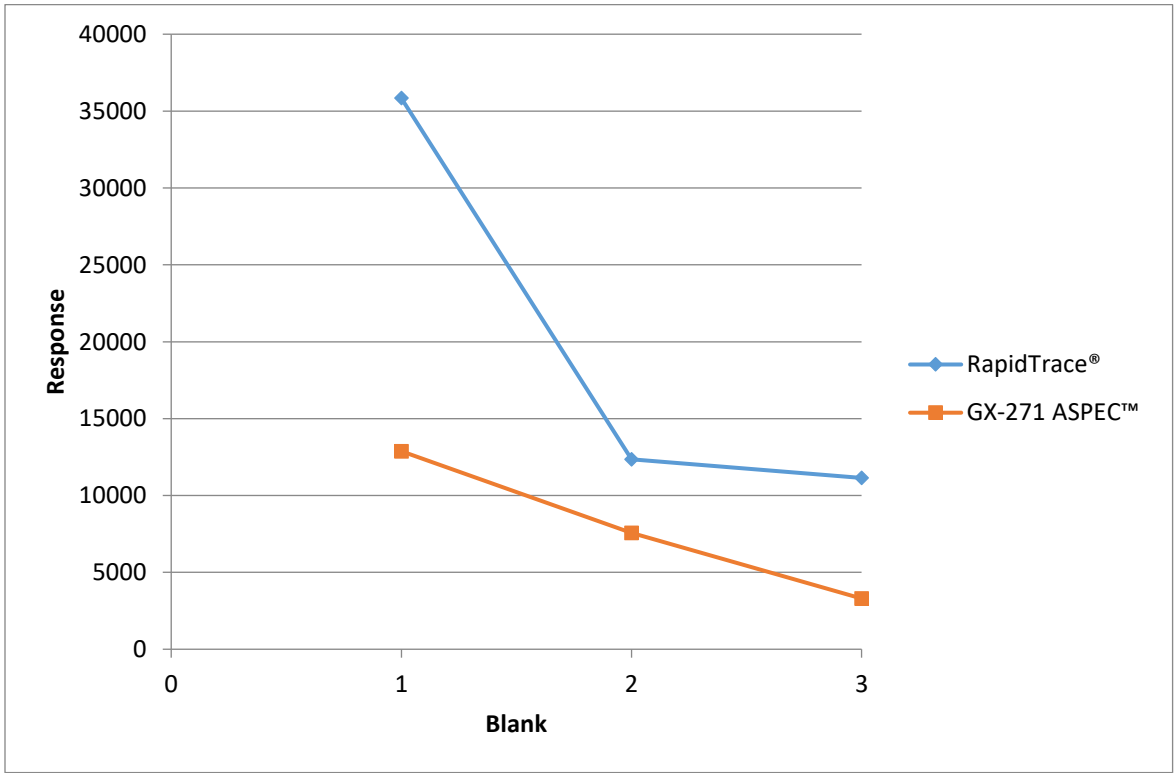

Figure A28 – Methadone response in human serum for the RapidTrace® and GX-271 ASPEC™

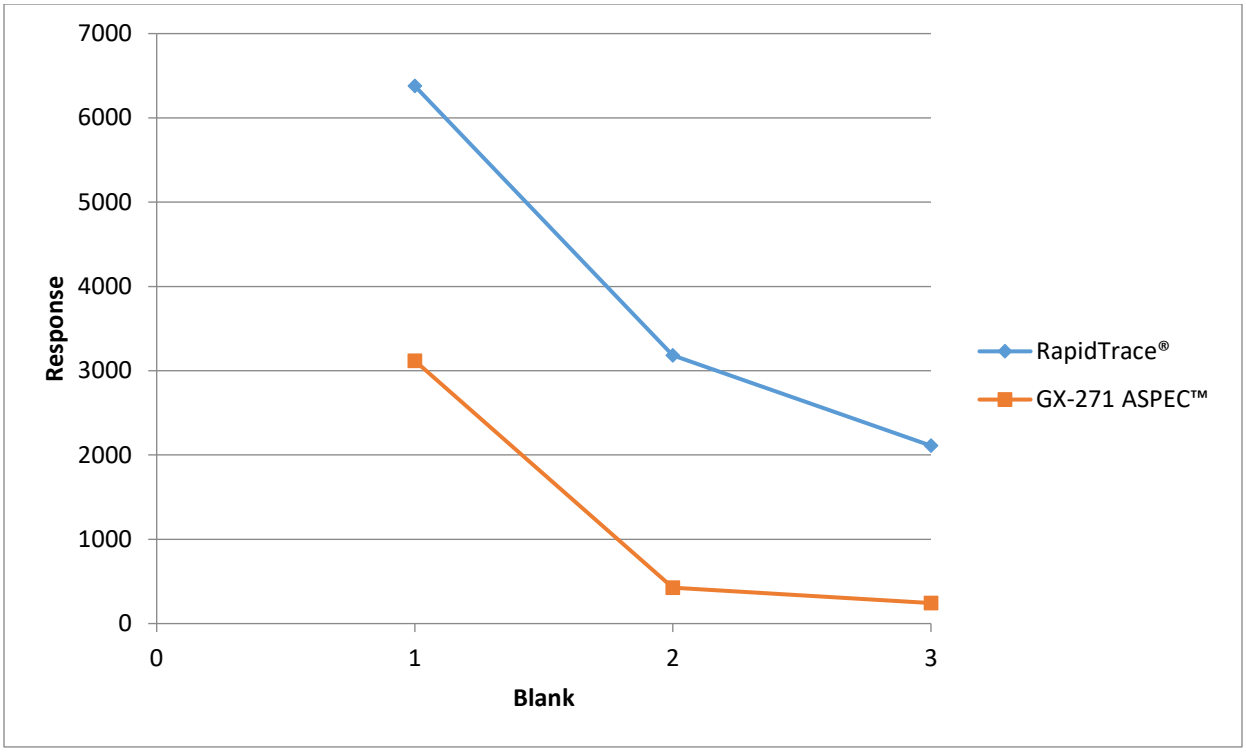

Figure A29 – Methamphetamine response in human serum for the RapidTrace® and GX-271 ASPEC™

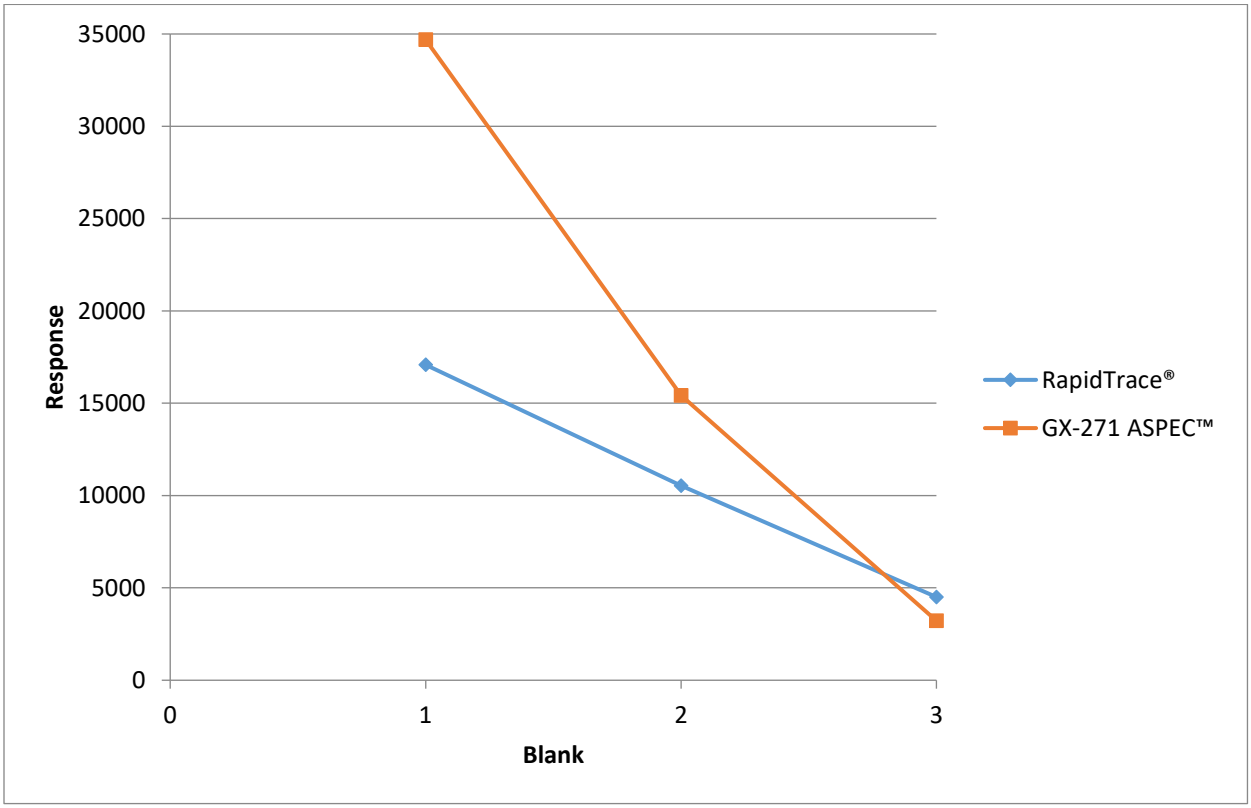

Figure A30 – Quetiapine response in human serum for the RapidTrace® and GX-271 ASPEC™

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