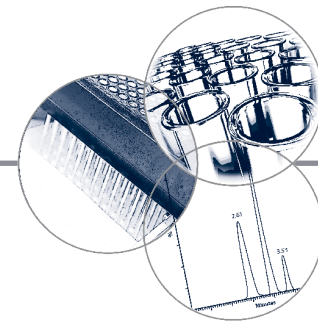
White Paper



Recommendations on bioanalytical method stability implications of co-administered and co-formulated drugs by Global CRO Council for Bioanalysis (GCC)

An open letter written by the Global CRO Council for Bioanalysis (GCC) describing the GCC survey results on stability data from co-administered and co-formulated drugs was sent to multiple regulatory authorities on 14 December 2011. This letter and further discussions at different GCC meetings led to subsequent recommendations on this topic of widespread interest within the bioanalytical community over the past 2 years.

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In 2010, the global bioanalytical community learned of regulatory agency concern about the potential for co-administered compounds present in a bioanalytical matrix to impact the stability of analytes subject to bioanalytical measurement. This pertained to conducting frozen and freeze–thaw stability experiments with spiked matrix samples (stability QCs) that contained only the analytes of bioanalytical interest and not all of the dosed drug compounds. Following several US FDA inspections, laboratories were cited with Form FDA 483 observations proposing that stability experiments should be conducted in the presence of all administered compounds. Additionally, the European Medicines Agency (EMA) guideline on bioanalytical method validation (BMV) suggested that *"In case of a multi-analyte study, and specific for bioequivalence studies, attention should be paid to stability of the analytes in the matrix containing all the analytes"* [1]. Subsequently, the topic has garnered discussion of the scientific merit of conducting the proposed expansion of the cited stability experiments. The topic has been of considerable interest on various discussion forums and at international scientific conferences with relevance to bioanalysis. Discussion was initiated at the 2010 4th Workshop on Recent Issues in Bioanalysis (4th WRIB) [2], and further expanded at the 5th WRIB in April 2011 [3].

At the 2011 5th WRIB in Montreal, Canada, Eric Woolf (Merck Research Laboratories, USA) raised for discussion the need to share experience and data pertaining to the proposed stability experiments. Eric Woolf and Surendra Bansal (Hoffmann-La Roche, USA) then approached the Global CRO Council for Bioanalysis (GCC) to question if CRO laboratories had existing bioanalytical data that could be shared with the bioanalytical community. As reported from the White Paper entitled 'Recommendations on: internal standard criteria; stability; incurred sample reanalysis and recent 483s by the Global CRO Council for Bioanalysis [4], several CRO laboratories suggested that existing data were available and could be shared from non-proprietary bioanalytical method validations. A follow-up request was sent to GCC member companies asking each company to provide details of the combination compound stability experiments and the associated results obtained. In addition to the stability data from combined analytes in spiked matrix, laboratories were requested to provide the corresponding stability results for the primary analyte(s) of interest individually present in the biological matrix.

As a subsequent follow-up to the stability data collected as part of the GCC survey, the GCC sent an open letter addressed to multiple regulatory agencies that contained an assessment of the stability results obtained and the resulting conclusions. The regulatory bodies to whom the letter was addressed were the Canadian Therapeutic Products Directorate (TPD), the US Food and Drug Administration (FDA), the Dutch Medicines Evaluation Board (MEB), the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) and the Brazilian Agência Nacional de Vigilância Sanitária (ANVISA). The survey results and the main conclusions of the open letter to the agencies were presented at the 6th GCC Closed Forum on 27 March, 2012 and at the 6th WRIB on 28–29 March, 2012, both of which took place in San Antonio, TX, USA [5].

Description of the survey data obtained

The data gathered for non-proprietary compounds are shown in Table 1. The data were obtained from three different stability experiments, notably: freezer storage, freeze–thaw and bench-top in matrix stability. All experiments pertained to human plasma or serum bioanalytical methods developed and validated to meet current regulatory guidance. The data provided represent 49 primary compound assessments, of which 41 were unique and six were investigated across more than one laboratory. A total of 56 different combinations of primary compound analyte stability in the presence of one or more co-administered compounds are reported. All methods were LC–MS/MS-based with the exception of one being LC–UV based. Of the LC–MS/MS methods, 36 used a stable-isotope-labeled (SIL) internal standard and nine methods used chemical analog internal standards. Twenty-one methods used SPE, two used solid-supported liquid extraction (SLE), ten used liquid–liquid extraction (LLE) and 11 used protein precipitation (PPT) for sample pretreatment ahead of instrumental analysis.

Stability results reported

Freeze–thaw stability results reported for the primary compounds alone over a range of concentrations ranged from -6.5 to 10.9%. In the presence of co-administered compounds the corresponding stability results ranged from -8.2 to 12.7%.

Stability in matrix at room temperature (bench-top) was not always available but in the cases that it was reported, matrix stability for the primary compounds alone ranged from -6.2 to 10.8%. In the presence of co-administered compounds, the corresponding stability results ranged from -2.7 to 12.8%.

For frozen sample stability (long-term stability) stability results reported for the primary compounds alone over a range of concentrations ranged from -12.0 to 13.6%. In the presence of co-administered compounds, the corresponding stability results ranged from -11.9 to 15.0%.

The reported data represent a good cross section of LC–MS/MS bioanalytical methods developed and validated for small molecules to meet regulatory submission criteria. A broad range of compound chemistries is included both within the primary compounds of bioanalytical measurement interest and the co-administered compounds. When all data are taken into consideration, we conclude that there is no evidence (within this dataset) that stability of the primary compound was impacted by the co-administered compounds. In addition to the observation that all stability values were within ±15% deviation, the difference in stability values without and with the co-administered drug seems to be smaller than the high/low end of the stability range itself. The results seem to be consistent with the scientific basis of LC–MS/MS bioanalytical assays.

Biological matrix is a complex medium containing many known and unknown compounds. Matrix composition is also affected by genetics,

age, metabolism, diet, environment and diurnal variations. Bioanalytical assays are developed to assay a compound in this complex medium without interference from any endogenous and co-administered compounds. As part of the bioanalytical method validation process, analyte stability in this matrix is required to be assessed. Most of the major differences in plasma/serum are currently being evaluated in the form of matrix effect/factor, hemolysis effect and lipemic effect. These evaluations also use commercially available matrix from human donors that are collected under considerably less controlled conditions in regards to food intake and medical screening. Taken in this context, the addition of components in low pico- and nano-molar amounts (i.e., a co-medication), to this already complex matrix would, from a chemical reactivity perspective, be unlikely to impact stability of an analyte that was found to be stable in the co-medication-free matrix.

There are, however, scenarios where the stability of co-administered compounds could be impacted if the following are true:

- The stability of each individual compound is determined in the biological matrix containing enzymes and other components that can potentially destabilize the compound. Stability for the compound in this complex matrix is generally determined individually;
- Once stability is proven individually, if the presence of the co-administered compounds does not change the matrix then the individual stability applies. However, if the matrix is changed (e.g., addition of stabilizers) or if the collection process is changed then the individual stability in matrix cannot be applied. Special attention should also be paid when conducting studies where medications known to affect matrix physiology (e.g., alter matrix pH) are co-administered with the target analyte. If the target analyte stability is known to be altered by a change in matrix physiology, sample collection conditions should be adjusted to compensate for any physiological changes caused by the co-medication.

In these scenarios, stability testing of the target drug in the presence of co-administered compounds may be logically and scientifically justifiable.

The GCC open letter sent to the regulatory agencies concluded by proposing that further practice of conducting co-administered compound stability experiments in routine bioanalytical method validation should be limited to the situation where the co-administered compound may impact stability due to the sample collection process.

Please note that all discussions on this topic (closed discussion at the 6th GCC, and open discussions at the 6th WRIB) were limited to small-molecule xenobiotic drugs. With this in mind, the following recommendations from the GCC only apply to such compounds and do not extend to large-molecule drugs or any endogenously present analytes.

It should also be noted that regulatory authorities have encountered in submitted data the suggestion that failed method validation stability experiments might be attributed to co-administered drugs. While the data collected and presented in this paper does not support such events, it is acknowledged that any such investigation cannot cover all drug combinations. As such, there has been open discussion on whether co-administered drug stability experiments should be conducted as part of bioanalytical method validation.

Recommendations

Following the last GCC closed forum, the GCC came forward with specific recommendations towards conducting stability evaluation experiments in presence of co-administered compounds.

The GCC recommendation for studies with fixed dose drug combination studies is shown in Box 1. This recommendation can be impractical in the case of complex drug combination therapies such as oncology treatments. For such patient studies, other investigative strategies may be appropriate and the GCC encourages open dialogue on best practices. This includes analyte

Box 1. The Global CRO Council for Bioanalysis recommendation for conducting stability validation experiments in presence of co-administered compounds for fixed dose drug combination studies.

- The Global CRO Council for Bioanalysis recommends that matrix stability experiments (i.e., short-term [benchtop] and freeze–thaw stability) in presence of co-administered compounds be performed in support of studies with fixed dose drug combinations. Only if the initial stability experiments fail acceptance criteria is long-term freezer stability evaluation in the presence of co-administered compounds recommended.

Box 2. The Global CRO Council for Bioanalysis consideration for stabilities in presence of co-administered compounds for patient studies.

- The Global CRO Council for Bioanalysis suggests that for patient studies (e.g., oncology studies), viable incurred sample stability experiments including the spiking of predose subject samples with analyte should be considered. However, further discussion within the bioanalytical community and with global regulatory authorities is required before a standardized practice can be recommended or endorsed by the Global CRO Council.

addition to predose study samples and equivalent incurred sample stability experiments.

The GCC consideration for patient studies, such as oncology studies, is shown in Box 2.

Future perspective

The GCC will continue to provide recommendations on hot topics in bioanalysis of global interest and expand its membership by coordinating its activities with the regional and international meetings held by the pharmaceutical industry. Please contact the GCC for the exact date and time of future meetings, and for all membership information.

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Table 1. Stability data in presence of co-administered compounds in human plasma/serum (% deviation).

Primary + co-med compound names	Experimental design information provided	Benchtop stability results (without co-med)	Benchtop stability (with co-med)	Freeze–thaw stability (without co-med)	Freeze–thaw stability (with co-med)	Long-term stability (without co-med)	Long-term stability (with co-med)
Amlodipine + bisoprolol	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	LQC: 4.6 HQC: -0.6 (24.1 h)	N/AV	LQC: 0.3 HQC: -8.2 (3 cycles)	N/AV	LQC: -4.0 HQC: -4.4 (92 days -20°C)
Atorvastatin + free ezetimibe	K_2EDTA plasma, Analog IS, LC–MS/MS, Gradient, SPE	LQC: 2.6 HQC: 2.0 (28.9 h)	LQC: 3.7 HQC: 1.8 (20.8 h)	LQC: 2.6 HQC: 2.0 (6 cycles)	LQC: 3.7 HQC: 1.8 (6 cycles)	LQC: 2.9 HQC: 2.4 (13 days -20°C) LQC: 1.6 HQC: 1.8 (309 days -80°C)	LQC: 5.8 HQC: 0.7 (204 days -80°C)
Atorvastatin + losartan	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, LLE	LQC: 0.2 HQC: -3.1 (4 h)	LQC: 2.7 HQC: -1.4 (4 h)	LQC: -1.0 HQC: -2.4 (4 cycles)	LQC: 3.8 HQC: -5.1 (4 cycles)	LQC: -4.5 HQC: -7.3 (41 days -70°C)	LQC: -2.1 HQC: -1.3 (41 days -70°C)
Atovaquone + proguanil/ cycloguanil	K_2EDTA plasma, SIL IS, LC–MS/MS, Isocratic, SLE/SPE	N/AV	N/AV	N/AV	N/AV	LQC: -0.9 HQC: 4.8 (377 days -20°C)	LQC: -1.3 HQC: -1.3 (107 days -20°C)
Butalbital + codeine/ salicylic acid/caffeine	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	LQC: 5.8 HQC: 5.0 (114 h)	N/AV	LQC: 0.1 HQC: 7.0 (3 cycles)	N/AV	LQC: 0.5 HQC: 1.0 (198 days -80°C) LQC: 2.1 HQC: 5.7 (100 days -20°C)
Bisoprolol + amlodipine	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	LQC: -0.1 HQC: -2.5 (24.1 h)	N/AV	LQC: -0.2 HQC: -6.0 (3 cycles)	N/AV	LQC: -2.6 HQC: 2.1 (92 days -20°C)
Butalbital + codeine	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	LQC: 6.8 HQC: 1.4 (168 h)	N/AV	LQC: 9.9 HQC: 3.0 (3 cycles)	N/AV	LQC: 0.7 HQC: 5.5 (124 days -20°C)
Carbinoxamine + pseudoephedrine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPT	N/AV	LQC: 0.8 HQC: 5.8 (24 h)	N/AV	LQC: 1.5 HQC: 6.1 (3 cycles)	N/AV	LQC: 10.8 HQC: 8.4 (42 days -80°C)
Chlorpheniramine + total phenylephrine /ibuprofen	NaHeparin plasma, Analog IS, LC–MS/MS, Gradient, PPT	N/AV	N/AV	N/AV	N/AV	LQC: 4.8 HQC: 3.1 (312 days -20°C)	LQC: 8.6 HQC: 3.6 (97 days -20°C)
Chlorpheniramine + hydrocodone/ pseudoephedrine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPT	N/AV	LQC: 4.7 HQC: 3.4 (26 h)	N/AV	LQC: 4.3 HQC: 3.1 (3 cycles)	LQC: 8.0 HQC: 1.5 (55 days -80°C)	LQC: 13.0 HQC: 8.3 (91 days -80°C)
Chlorpheniramine + hydrocodone	NaHeparin plasma, Analog IS, LC–MS/MS, Isocratic, SPE	N/AV	LQC: 5.3 HQC: 0.4 (24 h)	N/AV	LQC: 4.0 HQC: 4.9 (3 cycles)	N/AV	LQC: 0.7 HQC: 2.6 (14 days -20°C)

Table 1. Stability data in presence of co-administered compounds in human plasma/serum (% deviation) (cont.).

Primary + co-med compound names	Experimental design information provided	Benchtop stability results (without co-med)	Benchtop stability (with co-med)	Freeze–thaw stability (without co-med)	Freeze–thaw stability (with co-med)	Long-term stability (without co-med)	Long-term stability (with co-med)
Chlorpheniramine + hydrocodone	K_2EDTA plasma, SIL IS, LC–MS/MS, Isocratic, SPE	N/AV	LQC: -2.0 HQC: -1.9 (29.1 h)	N/AV	LQC: -2.7 HQC: -1.5 (3 cycles)	N/AV	LQC: 2.3 HQC: 1.6 (47 days -20°C)
Codeine + butalbital	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	LQC: 8.0 HQC: 3.6 (168 h)	N/AV	LQC: 7.3 HQC: 7.5 (3 cycles)	N/AV	LQC: 0.7 HQC: 6.4 (124 days -20°C)
Codeine + butalbital/salicylic acid/caffeine	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	LQC: 9.3 HQC: 0.6 (114 h)	N/AV	LQC: 4.0 HQC: 0.2 (3 cycles)	N/AV	LQC: 1.3 HQC: 1.1 (198 days -80°C) LQC: 2.0 HQC: 7.1 (198 days -20°C)
Dexchlorpheniramine + pseudoephedrine/ dextromethorphan	NaHeparin plasma, Analog IS, LC–MS/MS, Isocratic, LLE	N/AV	LQC: 8.7 HQC: 8.8 (72 h)	N/AV	LQC: 7.0 HQC: 7.3 (3 cycles)	N/AV	LQC: 13.3 HQC: 12.0 (333 days -80°C)
Dextromethorphan + pseudoephedrine/ dexchlorpheniramine	NaHeparin pasma, Analog IS, LC–MS/MS, Isocratic, LLE	N/AV	LQC: 1.2 HQC: 12.7 (72 h)	N/AV	LQC: 3.6 HQC: 9.1 (3 cycles)	N/AV	LQC: 5.3 HQC: 9.3 (333 days -80°C)
Drospirenone + ethynil estradiol	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, LLE	N/AV	LQC: 5.2 HQC: 0.4 (28.5 h)	N/AV	LQC: 2.0 HQC: -0.1 (3 cycles)	N/AV	LQC: 8.6 HQC: 3.5 (84 days -20°C)
Ethinyl estradiol + norethindrone	K_3EDTA plasma, Analog IS, LC–MS/MS, Isocratic	LQC: 6.7 HQC: 1.0 (18.0 h)	LQC: 5.6 HQC: 2.9 (21.3 h)	LQC: 9.4 HQC: 2.0 (5 cycles -20°C) LQC: 5.7 HQC: 2.5 (3 cycles -80°C)	LQC: 5.6 HQC: 2.9 (5 cycles)	LQC: 6.2 HQC: 2.5 (196 days -20°C) LQC: 5.7 HQC: 2.5 (113 days -80°C)	LQC: 1.8 HQC: 3.8 (111 days -20°C)
Ethinyl estradiol + Drospirenone	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, LLE	LQC: -6.2 HQC: -5.6 (39.9 h)	LQC: 12.2 HQC: 7.4 (21.4 h)	LQC: 2.4 HQC: -1.2 (3 cycles)	LQC: 12.7 HQC: 8.3 (3 cycles)	LQC: 8.2 HQC: 1.6 (126 days -20°C)	LQC: 10.6 HQC: 3.8 (84 days -20°C)
Ethinyl estradiol + norgestrel	K_3EDTA plasma, Analog IS, LC–MS/MS, Isocratic, SPE	LQC: 1.9 HQC: 0.5 (18.7 h)	LQC: 7.1 HQC: 5.2 (19.7 h)	LQC: 8.2 HQC: 2.4 (10 cycles -20°C) LQC: 6.3 HQC: 1.5 (3 cycles -80°C)	LQC: 7.1 HQC: 5.2 (5 cycles)	LQC: 6.2 HQC: 2.5 (196 days -20°C) LQC: 5.7 HQC: 2.5 (113 days -80°C)	LQC: 2.3 HQC: 3.2 (124 days -20°C)
Ezetimibe + atorvastatin	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, PPT	LQC: 2.5 HQC: -4.2 (24 h)	N/AV	LQC: -1.2 HQC: -5.2 (4 cycles)	N/AV	LQC: -9.2 HQC: -3.5 (874 days -20°C)	LQC: -2.4 HQC: -4.4 (343 days -20°C)

Table 1. Stability data in presence of co-administered compounds in human plasma/serum (% deviation) (cont.).

Primary + co-med compound names	Experimental design information provided	Benchtop stability results (without co-med)	Benchtop stability (with co-med)	Freeze–thaw stability (without co-med)	Freeze–thaw stability (with co-med)	Long-term stability (without co-med)	Long-term stability (with co-med)
Ezetimibe + atorvastatin	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, PPT	LQC: 2.5 HQC: -4.2 (24h)	N/AV	LQC: -1.2 HQC: -5.2 (4 cycles)	N/AV	LQC: -9.2 HQC: -3.5 (874 days -20°C)	LQC: -2.4 HQC: -4.4 (343 days -20°C)
Free ezetimibe + atorvastatin	K_2EDTA plasma, Analog IS, LC–MS/MS, Gradient, LLE	LQC: 3.8 HQC: 2.3 (30.3 h)	LQC: 4.2 HQC: 1.3 (25.2 h)	LQC: 3.8 HQC: 2.3 (6 cycles -20°C) LQC: 5.5 HQC: 1.2 (6 cycles -80°C)	LQC: 4.2 HQC: 1.3 (6 cycles)	LQC: 2.3 HQC: 3.0 (243 days -20°C) LQC: 5.5 HQC: 1.2 (12 days -80°C)	LQC: 2.6 HQC: 3.8 (350 days -20°C)
Fentanyl + naltrexone	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, SPE	LQC: 8.3 HQC: 1.0 (141 h)	N/AV	LQC: 6.7 HQC: 1.5 (3 cycles)	N/AV	LQC: 6.3 HQC: 1.1 (97 days -80°C)	LQC: 6.7 HQC: 6.0 (37 days -80°C)
Hydrochlorothiazide + irbesartan	N/AV	LQC: 5 HQC: 2 (24 h)	N/AV	LQC: 2 HQC: 2 (4 cycles -20°C) LQC: 2 HQC: 2 (4 cycles -80°C)	LQC: 1 HQC: 1 (4 cycles -20°C) LQC: 5 HQC: 1 (4 cycles -80°C)	LQC: 3 HQC: 2 (240 days -20°C) LQC: 3 HQC: 2 (328 days -80°C)	LQC: 4 HQC: 4 (52 days -20°C) LQC: 5 HQC: 5 (52 days -80°C)
Hydrocodone + homotropine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPT	N/AV	LQC: 6.3 HQC: 3.7 (24 h)	N/AV	N/AV	N/AV	LQC: 8.3 HQC: 4.5 (173 days -80°C)
Hydrocodone + chlorpheniramine/ pseudoephedrine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, SPE	N/AV	LQC: 7.0 HQC: 7.2 (24 h)	N/AV	LQC: 6.0 HQC: 6.1 (3 cycles)	N/AV	LQC: 13.7 HQC: 8.4 (92 days -80°C)
Hydrocodone + chlorpheniramine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, SPE	N/AV	LQC: 1.7 HQC: 7.4 (24 h)	N/AV	LQC: 4.7 HQC: 4.5 (3 cycles)	N/AV	LQC: 0.0 HQC: 7.9 (14 days -20°C)
Hydrocodone + chlorpheniramine	K_2EDTA plasma, SIL IS, LC–MS/MS, Isocratic, SPE	N/AV	LQC: 0.2 HQC: 2.7 (21.4 h)	N/AV	LQC: -0.3 HQC: -1.5 (3 cycles)	N/AV	LQC: -0.3 HQC: -1.5 (47 days -20°C)
Hydrocodone + ibuprofen	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPT	N/AV	LQC: 7.0 HQC: 2.2 (66.5 h)	N/AV	LQC: 6.7 HQC: 0.8 (3 cycles)	N/AV	LQC: 5.4 HQC: 13.4 (301 days -80°C)
Hydrocodone + ibuprofen	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, SLE	LQC: -3.8 HQC: -6.1 (24h)	N/AV	LQC: -6.5 HQC: -5.6 (4 cycles)	N/AV	LQC: -2.2 HQC: -4.3 (371 days -20°C)	LQC: -1.7 HQC -2.3 (38 days -20°C)
Hydromorphone + ibuprofen	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, SLE	LQC: -0.6 HQC: -3.8 (24h)	N/AV	LQC: -2.0 HQC: -3.4 (4 cycles)	N/AV	LQC: 1.3 HQC: 0.2 (371 days -20°C)	LQC: 1.3 HQC: -0.8 (38 days -20°C)

Table 1. Stability data in presence of co-administered compounds in human plasma/serum (% deviation) (cont.).

Primary + co-med compound names	Experimental design information provided	Benchtop stability results (without co-med)	Benchtop stability (with co-med)	Freeze–thaw stability (without co-med)	Freeze–thaw stability (with co-med)	Long-term stability (without co-med)	Long-term stability (with co-med)
Ibuprofen + chlorpheniramine/ total phenylephrine	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, LLE	N/AV:	N/AV	N/AV	N/AV	LQC: 2.6 HQC: 4.5 (389 days -20°C)	LQC: -2.6 HQC: 1.4 (95 days -20°C)
Ibuprofen† + hydrocodone/ hydromorphone	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPT	LQC: 10.8 HQC: 3.6 (24 h)	LQC: 3.0 HQC: 6.5 (66.5 h)	LQC: 6.8 HQC: 2.6 (3 cycles)	LQC: 1.0 HQC: 5.8 (3 cycles)	LQC: 6.3 HQC: 1.1 (97 days -80°C)	LQC: 8.5 HQC: 11.7 (72 days -80°C)
Irbesartan + hydrochlorothiazide	N/AV	LQC: 5 HQC: 2 (24 h)	N/AV	LQC: 1 HQC: 2 (4 cycles -20°C) LQC: 5 HQC: 6 (4 cycles -80°C)	LQC: 1 HQC: 1 (4 cycles -20°C) LQC: 5 HQC: 1 (4 cycles -80°C)	LQC: 3 HQC: 5 (458 days -20°C) LQC: 4 HQC: 4 (458 days -80°C)	LQC: 12 HQC: 5 (55 days -20°C) LQC: 10 HQC: 7 (55 days -80°C)
Loratadine/ DCL‡ + phenylephrine	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, LLE	N/AV	N/AV	N/AV	N/AV	LQC: 2.8 HQC: 8.8 (311 days -20°C)	pending
Losartan + atorvastatin	K_2EDTA plasma, SIL IS, LC–MS/MS, Gradient, PPT	LQC: -2.9 HQC: 0.0 (6 h)	LQC: -2.7 HQC: -2.5 (6 h)	LQC: 1.1 HQC: 2.8 (5 cycles)	LQC: 4.5 HQC: 2.8 (5 cycles)	LQC: 3.1 HQC: 0.7 (39 days -70°C)	LQC: 2.8 HQC: 2.1 (39 days -70°C)
Metformin + pioglitazone/ hydroxypioglitazone	N/AV	LQC: 1 HQC: 1 (23 h)	N/AV	LQC: 1 HQC: 1 (4 cycles)	LQC: 5 HQC: 2.5 (4 cycles -20°C) LQC: 6 HQC: 2.5 (4 cycles -80°C)	LQC: 5 HQC: 7 (97 days -20°C)	LQC: 7 HQC: 2.0 (14 days -20°C) LQC: 4 HQC: 2.0 (14 days -80°C)
Morphine/M3G§/ M6G¶ + naltrexone	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPE	LQC: 3.0 HQC: 8.3 (24 h)	N/AV	LQC: 6.7 HQC: 9.1 (3 cycles)	N/AV	LQC: 6.7 HQC: 1.9 (73 days -80°C)	LQC: 1.7 HQC: 3.0 (19 days -80°C)
Naproxen + esomeprazole	K_3EDTA plasma, Analog IS, LC–MS/MS, Gradient, PPT	LQC: 5.8 HQC: 5.0 (20.4 h)	LQC: 1.3 HQC: 3.7 (9.4 h)	LQC: 5.8 HQC: 5.0 (5 cycles -20°C) LQC: 4.6 HQC: 2.0 (5 cycles -80°C)	LQC: 1.3 HQC: 3.7 (3 cycles)	LQC: 2.5 HQC: 4.0 (85 days -20°C) LQC: 4.6 HQC: 2.0 (12 days -80°C)	LQC: 3.4 HQC: 1.6 (79 days -20°C)
Niacin + simvastatin/ simvastatin acid	SIL IS, LC–MS/MS, SPE	LQC: 1.0 HQC: 0.2 (26 h)	LQC: 3.7 HQC: 6.7 (24 h)	LQC: 2.1 HQC: 1.3 (4 cycles)	LQC: 1.3 HQC: 1.8 (5 cycles)	LQC: 7.4 HQC: 2.4 (559 days -80°C)	LQC: 0.9 HQC: 1.5 (176 days -80°C)
Nicotinuric acid + simvastatin/ simvastatin acid	SIL IS, LC–MS/MS, SPE	LQC: 8.7 HQC: 1.9 (26 h)	LQC: 1.0 HQC: 0.8 (24 h)	LQC: 1.5 HQC: 1.6 (4 cycles)	LQC: 0.8 HQC: 3.3 (5 cycles)	LQC: 1.3 HQC: 1.0 (559 days -80°C)	LQC: 4.8 HQC: 7.4 (176 days -80°C)

Table 1. Stability data in presence of co-administered compounds in human plasma/serum (% deviation) (cont.).

Primary + co-med compound names	Experimental design information provided	Benchtop stability results (without co-med)	Benchtop stability (with co-med)	Freeze–thaw stability (without co-med)	Freeze–thaw stability (with co-med)	Long-term stability (without co-med)	Long-term stability (with co-med)
Norethindrone + ethinyl estradiol	K_3EDTA plasma, Analog IS, LC–MS/MS, Isocratic	LQC: 1.9 HQC: 0.5 (18.7 h)	LQC: 8.8 HQC: 1.8 (21.3 h)	LQC: 8.2 HQC: 2.4 (10 cycles -20°C) LQC: 6.3 HQC: 1.5 (3 cycles -80°C)	LQC: 8.8 HQC: 1.8 (5 cycles)	LQC: 1.5 HQC: 2.0 (132 days -20°C) LQC: 6.3 HQC: 1.5 (10 days -80°C)	LQC: 8.8 HQC: 1.8 (27 days -20°C)
Norgestrel + ethinyl estradiol	LC–MS/MS	LQC: 2.9 HQC: 4.6 (20 h)	LQC: 3.2 HQC: 1.4 (19.7 h)	LQC: 2.9 HQC: 4.6 (5 cycles -20°C) LQC: 7.8 HQC: 10.9 (5 cycles -80°C)	LQC: 3.2 HQC: 1.4 (5 cycles)	LQC: 6.6 HQC: 5.8 (406 days -20°C) LQC: 10.3 HQC: 10.3 (57 days -80°C)	LQC: 5.8 HQC: 15.0 (112 days -20°C) LQC: 8.4 HQC: 5.0 (100 days -80°C)
Olanzapine/ desmethylolanzapine + valproic acid	NaHeparin plasma, SIL IS, LC–MS/MS, Gradient, SLE	N/AV	N/AV	N/AV	N/AV	LQC: 9.1 HQC: -12.0 (144 days -20°C)	LQC: 2.2 HQC: 0.3 (14 days -20°C)
Oxycodone + naltrexone	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, SPE	LQC: 6.6 HQC: 6.8 (27 h)	N/AV	LQC: 5.5 HQC: 6.1 (3 cycles)	N/AV	LQC: 13.3 HQC: 10.2 (79 days -80°C)	LQC: 14.0 HQC: 11.7 (79 days -80°C)
Paracetamol[#] + codeine	NaHeparin plasma, Analog IS, LC–MS/MS, Isocratic, PPT/SPE	N/AV	N/AV	N/AV	N/AV	LQC: -2.6 HQC: -3.6 (44 days -20°C)	LQC: -11.9 HQC: -4.3 (178 days -20°C)
Total phenylephrine + chlorpheniramine/ ibuprofen	LiHeparin plasma, SIL IS, LC–MS/MS, Gradient, SPE	N/AV	N/AV	N/AV	N/AV	LQC: 13.6 HQC: 6.3 (983 days -70°C)	LQC: 3.6 HQC: 1.7 (106 days -70°C)
Pioglitazone + metformin	N/AV	LQC: 1 HQC: 2 (24 h)	N/AV	LQC: 2 HQC: 2 (4 cycles -20°C) LQC: 2 HQC: 2 (4 cycles -80°C)	LQC: 3 HQC: 2 (4 cycles -20°C) LQC: 3 HQC: 1 (4 cycles -80°C)	LQC: 3 HQC: 2 (95 days -20°C) LQC: 3 HQC: 1 (95 days -80°C)	LQC: 3 HQC: 2 (12 days -20°C) LQC: 2 HQC: 1 (12 days -80°C)
Proguanil + atovaquone/ cycloguanil	K_2EDTA plasma, SIL IS, LC–MS/MS, Isocratic, SLE	N/AV	N/AV	N/AV	N/AV	LQC: 5.9 HQC: 2.8 (197 days -20°C)	LQC: 1.2 HQC: -2.5 (113 days -20°C)
Pseudoephedrine + hydrocodone/ chlorpheniramine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, SPE	N/AV	LQC: 0.3 HQC: 2.4 (25 h)	N/AV	LQC: 0.3 HQC: 2.1 (3 cycles)	LQC: 9.8 HQC: 8.2 (55 days -80°C)	LQC: 8.2 HQC: 12.6 (91 days -80°C)

Table 1. Stability data in presence of co-administered compounds in human plasma/serum (% deviation) (cont.).

Primary + co-med compound names	Experimental design information provided	Benchtop stability results (without co-med)	Benchtop stability (with co-med)	Freeze–thaw stability (without co-med)	Freeze–thaw stability (with co-med)	Long-term stability (without co-med)	Long-term stability (with co-med)
Pseudoephedrine + carbinoxamine	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, PPT	N/AV	LQC: 2.3 HQC: 3.8 (24 h)	N/AV	LQC: 2.7 HQC: 2.9 (3 cycles)	N/AV	LQC: 0.9 HQC: 3.9 (42 days -80°C)
Pseudoephedrine + dexchlorpheniramine/ dextromethorphan	NaHeparin plasma, SIL IS, LC–MS/MS, Isocratic, LLE	N/AV	LQC: 5.3 HQC: 12.4 (72 h)	N/AV	LQC: 1.3 HQC: 12.7 (3 cycles)	N/AV	LQC: 2.0 HQC: 3.2 (333 days -80°C)
Valsartan + hydrochlorothiazide	N/AV	LQC: 1 HQC: 1 (24 h)	N/AV	LQC: 6 HQC: 3 (4 cycles)	LQC: 2 HQC: 1 (4 cycles)	LQC: 1 HQC: 6 (355 days -20°C)	LQC: 5 HQC: 4 (46 days -20°C)

†Ibuprofen alone used human serum and LC–UV.
‡DCL: Descarboethoxyloratadine.
§M3G: Morphine-3-glucuronide.
¶M6G: Morphine-6-glucuronide.
#Paracetamol alone used SPE.
IS: Internal standard; LLE: Liquid–liquid extraction; N/AV: Not available; PPT: Protein precipitation; SIL: Stable-isotope labeled; SLE: Solid-supported liquid extraction.

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