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WHITE PAPER

GCC Recommendations on Bioanalytical Method Stability Implications of Co-administered and Co-formulated Drugs

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Note: Except for the first author who provided a major contribution, all the other authors are presented in alphabetical order of company affiliation due to equality principals of Global CRO Council (GCC)

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Abstract

An Open Letter written by the 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 lead to subsequent recommendations on this topic of widespread interest within the bioanalytical community over the past two years.

Introduction

In 2010, the global bioanalytical community learned of regulatory agency concern of 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 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 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 (4^{th} WRIB) [2], and further expanded at the 5th WRIB in April 2011 [3].

At the 2011 5th WRIB in Montreal Canada, Eric Woolf (Senior Director, 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 (Research Director Bioanalytical R&D, Non-Clinical Safety, Hoffmann-La Roche USA) then approached the GCC to question if contract research organization 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 (GCC) for Bioanalysis' [4], several CRO laboratories suggested that existing data was 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 populate an Excel spreadsheet with 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 Canada TPD, the US FDA, the France AFSSAPS, the Brazil ANVISA and the Netherlands MEB. The survey results and the main conclusions of the open letter to the agencies were presented at the 6th WIRB held in San Antonio, Texas, USA on 28 and 29 March 2012 [5].

Description of Survey Data Obtained

The data gathered for non-proprietary compounds is shown in **Table 1**. The data was 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 represents 48 primary compound assessments of which 40 were unique and 5 were investigated across more than one laboratory. A total of 54 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, 34 used a stable-isotope labeled (SIL) internal standard and 9 methods used chemical analog internal standards. 21 methods used solid-phase extraction (SPE), 2 used solid-supported liquid extraction (SLE), 9 used liquid/liquid extraction (LLE), and 10 used protein precipitation (PPT) for sample pre-treatment ahead of instrumental analysis.

Stability Results Reported

Freeze/thaw stability results reported for the primary compounds alone at 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 -2.5% to 12.8%.

For frozen sample stability (long-term stability) stability results reported for the primary compounds alone at 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%.

Table 1. Stability Data in Presence of Co-administered Compounds in Human Plasma/Serum (% deviation)

Primary + Co-med Compound Names	Experimental Design Information Provided	Bench-Top Stability Results (without co-med)	Bench-Top 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	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, SPE	N/AV	LQC: 4.6 HQC: -0.6 (24.1 hrs)	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	K2EDTA plasma, Analog IS, LC-MS/MS, Gradient, SPE	LQC: 2.6 HQC: 2.0 (28.9 hrs)	LQC: 3.7 HQC: 1.8 (20.8 hrs)	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)
Atovaquone + Proguanil/Cycloguanil	K2EDTA plasma, SIL IS, LC-MS/MS, Isocratic, SLE/SPE	N/AV (24 hrs)	N/AV	N/AV (5 cycles)	N/AV (5 cycles)	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 hrs)	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	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, SPE	N/AV	LQC: -0.1 HQC: -2.5 (24.1 hrs)	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 hrs)	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 hrs)	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 (27 hrs)	N/AV	N/AV (5 cycles)	N/AV (5 cycles)	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 hrs)	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 hrs)	N/AV	LQC: 4.0 HQC: 4.9 (3 cycles)	N/AV	LQC: 0.7 HQC: 2.6 (14 days -20°C)
Chlorpheniramine + Hydrocodone	K2EDTA plasma, SIL IS, LC-MS/MS, Isocratic, SPE	N/AV	LQC: -2.0 HQC: -1.9 (29.1 hrs)	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 hrs)	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 hrs)	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 hrs)	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 hrs)	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	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, LLE	N/AV	LQC: 5.2 HQC: 0.4 (28.5 hrs)	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	K3EDTA plasma, Analog IS, LC-MS/MS, Isocratic	LQC: 6.7 HQC: 1.0 (18.0 hrs)	LQC: 5.6 HQC: 2.9 (21.3 hrs)	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	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, LLE	LQC: -6.2 HQC: -5.6 (39.9 hrs)	LQC: 12.2 HQC: 7.4 (21.4 hrs)	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	K3EDTA plasma, Analog IS, LC-MS/MS, Isocratic, SPE	LQC: 1.9 HQC: 0.5 (18.7 hrs)	LQC: 7.1 HQC: 5.2 (19.7 hrs)	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	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, PPT	LQC: 2.5 HQC: -4.2 (24hrs)	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	K2EDTA plasma, Analog IS, LC-MS/MS, Gradient, LLE	LQC: 3.8 HQC: 2.3 (30.3 hrs)	LQC: 4.2 HQC: 1.3 (25.2 hrs)	LQC: 3.8 HQC: 2.3 (6 cycles -20°C) LQC: 5.5 HQC: 1.2	LQC: 4.2 HQC: 1.3 (6 cycles)	LQC: 2.3 HQC: 3.0 (243 days -20°C) LQC: 5.5 HQC: 1.2	LQC: 2.6 HQC: 3.8 (350 days -20°C)

				(6 cycles -80°C)		(12 days -80°C)	
Fentanyl + Naltrexone	NaHeparin plasma, SIL IS, LC-MS/MS, Isocratic, SPE	LQC: 8.3 HQC: 1.0 (141 hrs)	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 hrs)	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 hrs)	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 hrs)	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 hrs)	N/AV	LQC: 4.7 HQC: 4.5 (3 cycles)	N/AV	LQC: 0.0 HQC: 7.9 (14 days -20°C)
Hydrocodone + Chlorpheniramine	K2EDTA plasma, SIL IS, LC-MS/MS, Isocratic, SPE	N/AV	LQC: 0.2 HQC: 2.7 (21.4 hrs)	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 hrs)	N/AV	LQC: 6.7 HQC: 0.8 (3 cycles)	N/AV	LQC: 5.4 HQC: 13.4 (301 days -80°C)
Hydrocodone + Ibuprofen	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, SLE	LQC: -3.8 HQC: -6.1 (24hrs)	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	K2EDTA plasma, SIL IS, LC-MS/MS, Gradient, SLE	LQC: -0.6 HQC: -3.8 (24hrs)	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)
Ibuprofen + Chlorpheniramine/ Total Phenylephrine	NaHeparin plasma, SIL IS, LC-MS/MS, Gradient, LLE	N/AV: (27 hrs)	N/AV	N/AV (4 cycles)	N/AV (4 cycles)	LQC: 2.6 HQC: 4.5 (389 days -20°C)	LQC: -2.6 HQC: 1.4 (95 days -20°C)
Ibuprofen[1] + Hydrocodone/ Hydromorphone	NaHeparin plasma, SIL IS, LC-MS/MS, Isocratic, PPT	LQC: 10.8 HQC: 3.6 (24 hrs)	LQC: 3.0 HQC: 6.5 (66.5 hrs)	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 hrs)	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[2] + Phenylephrine	NaHeparin plasma, SIL IS, LC-MS/MS, Gradient, LLE	N/AV (27 hrs)	N/AV	N/AV (3 cycles)	N/AV (3 cycles)	LQC: 2.8 HQC: 8.8 (311 days -20°C)	pending
Metformin + Pioglitazone/ Hydroxypioglitazone	N/AV	LQC: 1 HQC: 1 (23 hrs)	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 (14days -20°C) LQC: 4 HQC: 2.0 (14 days -80°C)
Morphine/M3G[3]/M6G[4] + Naltrexone	NaHeparin plasma, SIL IS, LC-MS/MS, Isocratic, PPE	LQC: 3.0 HQC: 8.3 (24 hrs)	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	K3EDTA plasma, Analog IS, LC-MS/MS, Gradient, PPT	LQC: 5.8 HQC: 5.0 (20.4 hrs)	LQC: 1.3 HQC: 3.7 (9.4 hrs)	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 hrs)	LQC: 3.7 HQC: 6.7 (24 hrs)	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 hrs)	LQC: 1.0 HQC: 0.8 (24 hrs)	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)
Norethindrone +	K3EDTA plasma, Analog	LQC: 1.9	LQC: 8.8	LQC: 8.2	LQC: 8.8	LQC: 1.5	LQC: 8.8

Ethinyl Estradiol	IS, LC-MS/MS, Isocratic	HQC: 0.5 (18.7 hrs)	HQC: 1.8 (21.3 hrs)	HQC: 2.4 (10 cycles -20°C) LQC: 6.3 HQC: 1.5 (3 cycles -80°C)	HQC: 1.8 (5 cycles)	HQC: 2.0 (132 days -20°C) LQC: 6.3 HQC: 1.5 (10 days -80°C)	HQC: 1.8 (27 days -20°C)
Norgestrel + Ethinyl Estradiol	LC-MS/MS	LQC: 2.9 HQC: 4.6 (20 hrs)	LQC: 3.2 HQC: 1.4 (19.7 hrs)	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 (27 hrs)	N/AV	N/AV (3 cycles)	N/AV (5 cycles)	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 hrs)	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[5] + 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 (14 hrs)	N/AV	N/AV (10 cycles)	N/AV (10 cycles)	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 hrs)	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	K2EDTA plasma, SIL IS, LC-MS/MS, Isocratic, SLE	N/AV (24 hrs)	N/AV	N/AV (5 cycles)	N/AV (5 cycles)	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 hrs)	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)
Pseudoephedrine + Carbinoxamine	NaHeparin plasma, SIL IS, LC-MS/MS, Isocratic, PPT	N/AV)	LQC: 2.3 HQC: 3.8 (24 hrs)	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 hrs)	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 hrs)	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)

[1] Ibuprofen alone used human serum and LC-UV
[3] DCL: Descarboethoxyloratadine
[3] M3G: Morphine-3-Glucuronide
[4] M6G: Morphine-6-Glucuronide
[5] Paracetamol alone used SPE

The reported data represents 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 are included both within the primary compounds of bioanalytical measurement interest and the co-administered compounds. When all data is taken into consideration, we conclude that there is no evidence (within this data set) 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 media 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 media without interference from any endogenous and coadministered 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 into this context, the addition of components in low pico and nanomolar amounts (i.e. a co-med), 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-med free matrix.

There are however scenarios where the stability of co-administered compounds could be impacted if the following is 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 (i.e. alter matrix pH, etc.) are co-administered with the target analyte. If 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 logical 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 sample collection process.

Recommendations

Following the last GCC closed forum held in San Antonio, Texas, USA on March 2012, the GCC came down with specific recommendations towards stabilities in presence of co-administered compounds.

The GCC recommendation for studies with fixed drugs combinations is shown in **Box 1**.

Box 1: The GCC recommendation for stabilities in presence of co-administered compounds for fixed drugs combinations.

The GCC recommends that matrix stabilities (short-term, freeze-thaw and long-term stabilities) in presence of co-administered compounds be performed in support of studies with fixed drug combinations.

The GCC recommendation for patient studies such as oncology studies is shown in **Box 2**.

Box 2: The GCC recommendation for stabilities in presence of co-administered compounds for patient studies.

The GCC recommends that for patient studies (e.g. oncology studies), pre-dose subject samples should be used to evaluate matrix stabilities in presence of co-administered compounds, whenever possible.

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.

Acknowledgments

The GCC would like to thank:

- Steve Lowes (Advion) for leading this initiative within GCC, designing the survey, elaborating the results, and facilitating the discussion during the meeting.
- Surendra Bansal (Hoffmann-La Roche) and Eric Woolf (Merck) for scientific guidance and support.
- Isabelle Dumont and Suzanne Martinez (Algorithme Pharma Inc.) for writing the first draft of this White Paper.
- The GCC member companies who answered the Survey.
- All the member representatives who have sent comments and suggestions to complete this White Paper.

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