

# Interference in an Underivatized Assay for Amino Acid and Acylcarnitine Profiling by Tandem Mass Spectrometry

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Table 1

Potential Interferents	
Asparagine	Becomes an interferent for ornithine at 1150 umol/L. The reference levels of asparagine for a neonate are 29-111 umol/L. This compound is unlikely to interfere
Hydroxyproline	Becomes an interferent for leucine at 438 umol/L. Reference levels are 0-44 umol/L for hydroxyproline. It is unlikely to interfere in routine testing.
Methionine sulfone	Becomes an interferent for tyrosine at 317 umol/L. The reference levels for methionine sulfone in neonates are 15-21 umol/L. Because concentrations cannot exceed the reference level of methionine, it is unlikely this compound will interfere. In cases where the specimen's level of methionine are elevated, the presence of methionine sulfone need to be taken into consideration if elevated levels of tyrosine are also present.
Sarcosine	Becomes an interferent for alanine at 561 umol/L. Reference levels for sarcosine are 0-10 umol/L so it is unlikely to interfere.
Flouride	Interferes with C6 at 1219 umol/L. since this compound is rarely used as an anti-coagulant it is unlikely to be found in blood at this level.
Heparin	Becomes an interferent for the internal standards of ornithine and arginine at 400 IU/mL and 2300 IU/mL respectively. The compound could interfere if blood collected with this anti-coagulant is used for screening.
Malic acid	Interferes with aspartic acid at 2157 umol/L. Because aspartic acid has not yet been identified as a marker for any metabolic disorder, this finding has no clinical significance.
Creatine	Becomes an interferent for alanine at 2188 umol/L. Reference levels could not be found. Creatine is used as a performance enhancement dietary supplement. It could possibly interfere. At such high levels no interference is expected.
BSA	Interferes with arginine at 23 g/L. Normal amounts of protein at birth are 40-60 g/L. Total protein can exceed 100 g/L in some monoclonal gammopathies. Such increases are usually due to g-globulin. It is unknown if g-globulin would correlate to the interference observed with BSA.
Amitriptyline	Becomes an interferent for C5DC at 1412 umol/L. Amitriptyline is used as an antidepressant and for chronic pain. Excessive use of the compound by expectant mothers could result in interference for C5DC.
4-aminoantipyrine	Becomes an interferent for C2 at 2583 umol/L. This compound is similar to Felsol, and analgesic. The addition of an amino group in-vivo could result in interference.

## Introduction

In clinical assays other compounds may interfere with the measurement of the intended analyte. These substances may be from endogenous or exogenous sources. An interferent in mass spectrometry may have the same mass or mass transition as the analyte, or alter the analyte by complex formation or precipitation. An underivatized assay utilizing a triple quadrupole mass spectrometer for a full panel of amino acids and acylcarnitines is examined for potential interferents.

## Materials and Methods

Two hundred fifty eight compounds were examined as possible interferents. A final list of thirty-four were included in the study. This study included potential interferential compounds that have obvious molecular mass matches to the analytes or internal standards as well as less obvious substances such as protein (BSA), glucose, and EDTA. The potential interferential compounds were added to whole blood at concentrations suggested by NCCLS guidelines, spotted to filter paper and MS/MS analysis performed to determine the concentration of both amino acids and acylcarnitines in these samples enriched with interferential substances. To be identified as a potential interferent, a compound must have a significant effect on its target analyte concentration when compared to controls. The criteria used are the following: a median value outside +/- 15% of the control, have a median value outside +/- one standard deviation of the control, and have a significant Student's T test. Compounds causing a significant change of target analyte concentrations were then subjected to a Dose Response study. Five levels were made as per NCCLS guidelines. A high and low pool was prepared, then a 25%, mid, and 75% level by appropriate mixing of the high and low pool. Each interferent level of the Dose Response study was evaluated by the same criteria used to identify potential interferents.

## Results and Discussion

Fifteen compounds were found to potentially interfere with either an analyte or internal standard. Eleven, listed in Table 1, interfered during a Dose Response Study. These compounds may interfere by having a similar mass, reducing extraction efficiency, salt effects, precipitation or by complex formation.

Isomers and isobars (non-isomeric compounds with the same mass to charge ratio, m/z) may present a limitation for the analysis of certain analytes by MS/MS. The technology cannot distinguish between two compounds having the same molecular mass and fragmentation patterns. Therefore, isomers and isobars can act as a source of interference for a corresponding analyte of interest.

Table 2 lists potential isomeric and/or isobaric interference for the Underivatized method. The most significant of these is the fact that C5-OH and C4DC share the same m/z (262.1). In this case, both C4DC and C5-OH contribute to the intensity measurement at m/z 262.1. Thus an elevation of either analyte would require confirmation by the Derivatized method. The other isomeric/isobaric combinations listed on Table 2 are less likely to occur and thus are of much lower clinical significance.

Isomeric/isobaric interference is not a result of using a non-derivatized process. This also occurs with the Derivatized method. Many of the dicarboxylated carnitines are of the same mass as longer chain hydroxylated carnitines. Most notably C5DC and C10-OH have the same m/z values, 388.3 amu, by the Derivatized assay.

Table 2. Isomeric Compounds

Internal Standard/Analyte	Protonated Mass	Isomer/Isobar	Protonated Mass
<b>Amino Acids</b>			
Ala	90.0	Sarcosine	90.1
		beta-alanine	90.0
Val <sub>48</sub>	126.1	taurine	126.0
Leu/Ile	132.1	hydroxyproline	132.1
		creatine	132.1
		norleucine	132.1
Om	133.1	asparagine	133.1
<b>Acylcarnitines</b>			
C4DC	262.1	C5-OH	262.2
C8	288.2	C6:1DC	288.1
C14	372.3	C12:1DC	372.2
C18	428.4	C16:1DC	428.3
C4OH	248.1	C3DC	248.1
C6OH	276.1	C5DC	276.1
C7OH	290.1	C6DC	290.2
C8OH	304.1	C7DC	304.2
C9OH	318.1	C8DC	318.2
C10OH	332.2	C9DC	332.2
C11OH	346.2	C10DC	346.2
C12OH	360.3	C11DC	360.2
C13OH	374.3	C12DC	374.2
C14OH	388.3	C13DC	388.2
C12	344.3	C10:1DC	344.2
C8:1DC	316.2	C10	316.2

## Conclusion

Fifteen potential interferents for the NeoGram Non-derivatized amino acids and acylcarnitines MS/MS method were detected. Eleven of these interfered at concentrations unlikely to be found in newborn blood. In a newborn screening program compounds that potentially interfere must be identified and noted. The list provided above serves as a guide that may aid in the troubleshooting of cases with questionable or inconsistent results. It is important to emphasize that because these compounds interfered at concentrations much higher than their reference levels, it is expected that they will not interfere in the routine testing of neonates with this method.