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Compilation of Amino Acids, Drugs, Metabolites and Other Compounds in Masstrak Amino Acid Analysis Solution

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Abstract

The MassTrak Amino Acid Analysis Solution provides a reproducible, robust method with easy identification of the common physiological amino acids. Common compounds detected with the MassTrak AAA Solution include a wide number of pharmaceuticals and metabolite side products found in biological fluids. This compilation provides a guide to more than 100 such compounds, beyond the standard amino acids, that may appear in the analysis of biological fluids with the MassTrak AAA Solution.

Introduction

Physiological amino acid analysis is commonly performed to monitor and study a wide variety of metabolic processes. A wide variety of drugs, foods, and metabolic intermediates that may be present in biological fluids can appear as peaks in amino acid analysis, therefore, it is important to be able to identify unknown compounds.[1,2,3] The reproducibility and robustness of the MassTrak Amino Acid Analysis Solution make this method well suited to such a study as well.[4]

Experimental

Compound sample preparation

A library of compounds was assembled. Each compound was derivatized individually and spiked into the MassTrak AAA Solution Standard prior to chromatographic analysis. The elution position of each tested compound could be related to known amino acids.

1. Each compound was prepared at 500 µM in 0.1 N HCl.
2. Each compound was derivatized according to the standard as described in the MassTrak AAA Solution User's Guide at a concentration of 250 µM.
3. Each compound was also derivatized in conjunction with the MassTrak AAA Standard: 70 µL of borate buffer, 5 µL of MassTrak AAA Standard (250 µM) and 5 µL of compound (500 µM) were combined prior

to derivatization.

Sample preparation utilized the MassTrak AAA derivatization kit and Total Recovery vials. The amino acids were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) (US Patent 5,296,599 and European Patent EP 0533 200 B1). The protocol followed that described in the MassTrak AAA Solution User's Guide.

LC conditions

LC System:	Waters ACQUITY UPLC System with TUV
Column:	MassTrak AAA Column 2.1 x 150 mm, 1.7 µm
Column Temp:	43 ˚C
Flow Rate:	400 µL/min.
Mobile Phase A:	MassTrak AAA Eluent A Concentrate, diluted 1:10
Mobile Phase B:	MassTrak AAA Eluent B
Weak Needle Wash:	5/95 Acetonitrile/Water
Strong Needle Wash:	95/5 Acetonitrile/Water
Gradient:	MassTrak AAA Standard Gradient (as provided in kit)
Detection:	UV @ 260 nm
Injection Volume:	1 µL
Injection Mode:	Partial Loop with Needle Overfill (PLNO)

Results and Discussion

A wide variety of antibiotics, pharmaceutical compounds and metabolite by-products are found in biological fluids. The retention times of a number these compounds have been cataloged for a number of amino acid analysis methods.[1,2,3] A similar study was conducted for the MassTrak AAA Solution.

The compounds listed in Tables, 1, 2 and 3 were analyzed using the MassTrak AAA Solution standard. Retention times were verified for the individual standards, as well as combined with the MassTrak AAA standard. The latter analysis accurately documented coelutions, which include partial resolution. The following tables are compilations of the interference compounds in alphabetical order (Table 1), and elution order (Table 2). Unreactive compounds are listed in both Table 1 and Table 3.

Table 1. Compounds in alphabetical order

Compound	Coelution*	Adjacent Amino Acids	R_T**
Acetyl carnitine			N/D
ε-Acetyl lysine		BAIB, Hyl1	17.30
α-Acetyl lysine		BAIB, Hyl1	17.39
O-Acetyl-serine	GABA(Sh)		15.23
Adenosyl homocysteine	Hyl2		17.71
Adenosyl methionine (peak 2)	His		4.84
Adenosyl methionine (peak 1		HyPro,NH_3	4.36
D-Alanine	Ala		14.60
4-Aminobenzoic acid (peak 1)		HyPro,NH3	4.56
4-Aminobenzoic acid (peak 2)		Nva, Ile	23.92
Aminoethyl cysteine		Cys, Orn	19.54
Aminoethylphosphonic acid	HyPro		4.19
Aminolevulinic acid		Thr, Ala	14.36
Aminopimelic acid		Cys, Orn	18.55
α-Amino-β-guanidinopropionic acid		NH_3, His	4.89
Amoxicllin		Val, Nva	22.21
Argininosuccinic acid	EA		8.48
Argininosuccinic acid anhydride 1		Cit, β-Ala	11.60
Argininosuccinic acid anhydride 2		Ala, GABA	13.20
Argininosuccinic acid anhydride 3		Ala, GABA	14.76
Argininosuccinic acid anhydride 4		GABA, AADA	15.56
Argininosuccinic acid anhydride 5	Deriv Peak (Sh)		20.85
Argininosuccinic acid anhydride 6		Met,Val	21.14
Argininosuccinic acid anhydride 7	Val (Sh)		21.53
Argininosuccinic acid anhydride 8		Nva, Ile	22.81
Azaperone		Nva, Ile	25.33
Azithromycin			N/D
Beclomethasone			N/D
Cadaverine		Nva, Ile	23.10
Caffeine	Ala (Sh)		14.71
Carbamoylphosphate dibasic			N/D
Carnitine			N/D

Compound	Coelution*	Adjacent Amino Acids	R_T**
Choline			N/D
Ciproflaxin		Trp	36.45
Clonazepem Related Compound A		Trp	36.70
Clonazepem Related Compound B			N/D
Creatine			N/D
Cysteic acid		AMQ, HyPro	4.0
cis-Cysteine		AADA, Pro	16.06
Cysteine-Homocysteine dimers (peak 1)		Nva, Ile	22.9
Cysteine- Homocysteine dimers (peak 2)		Nva, Ile	23.1
Cysteine Sulfate	3MH (Sh)		5.25
Diaminopimelic acid (peak 1)		BAIB, Hyl1	17.28
Diaminopimelic acid (peak 2)		Hyl2, AABA	17.92
3, 4- Dihydroxy-D,L-phenylalanine	Cyst, Orn		18.89
N^G, N^G-Dimethyl arginine asymmetrical (ADMA)		Thr, Ala	12.94
N^G, $N^{G'}$-Dimethyl arginine symmetrical (SDMA)		Thr, Ala	13.82
Dopamine	Tyr		20.65
Doxapram			N/D
Ephedrine		Trp	35.48
Epinephrine		Hyl2, AABA	18.06
Erythromycin			N/D
Ethanol (peak 1)		Ala, GABA	15.17
Ethanol (peak 2)	Lys		20.30
Ethionine		Nva, Ile	25.46
Ethosuximide			N/D
Formyl methionine			N/D
Gentamicin (peak 1)	Cyst		18.48
Gentamicin (peak 2)	Orn		19.02
Glucosaminic acid		before PSer	3.16
γ-Glu-ε-Lys	Cys, Lys		20.03
Glutathione, Oxidized		Hyl2, AABA	18.19
Glutathione, Reduced (peak 1)		Thr, Ala	14.44
Glutathioine, Reduced (peak 2)	Orn		19.02
Glycyl-Proline		Pro,BAIB	16.79 (broad Pk)

Compound	Coelution*	Adjacent Amino Acids	R_T**
D-Histamine		Ans, EA	8.13
Histidinol	Ans		5.02
Homoarginine	β-Ala		12.85
Homocitrulline	GABA		15.34
D, L-Homocysteic acid			N/D
Homocysteine	Orn		19.05
Homogentisic acid	Ala (Sh)		14.48
D,L-Homophenylalanine			36.36
Homoserine	Gly,Ans		7.38
Hydroxytryptophan		Cys, Lys	20.20
Hydroxyindollicacetic acid			N/D
cis-Hydroxyproline		Sar, Glu	10.91
Hypotaurine	Ser (Sh)		6.21
Ketorolac		Gln, Carn	7.29
α-keto-γ-(methylthio) butyric acid			N/D
Kynurenine		alle,Leu	28.11
Lanthionine (peak 1)		Pro,BAIB	16.69
Lanthionine (peak 2)	BAIB	Pro,BAIB	16.77
Methanol		Ala, GABA	15.13
2,6-Methionine sulfone		Asp, Sar	9.21
2-Methionine sulfoxide (peak 1)	EA		8.48
Methionine sulfoxide (peak 2)	Asp		8.77
S-Methyl cystine		Hyl2, AABA	18.24
Methyl lysine		Val, Nva	22.31
Se-Methylselenocysteine		Orn, Cys	19.65
Methylxanthine		before PSer	3.52
Momethasone			N/D
Nitrotyrosine	Ile		27.13
Nitrotyrosine methyl ester (peak 1)	Ile		27.13
Nitrotyrosine methyl ester (peak 2)		Trp	35.43
D-Norephedrine		Hcys,Phe	20.94
Norleucine	Phe		29.30

Compound	Coelution*	Adjacent Amino Acids	R_T**
Penicillamin disulfide		After Trp	32.12
Penicillamine		Nva, Ile	22.88
Penicillin g			N/D
Penicllin v			N/D
Phenyl pyruvate			N/D
Phosphocholine			N/D
Pipecolinic acid (peak 1)		Met,Val	21.38
Pipecolinic acid (peak2)		Nva, Ile	24.14
3- Porphobolinogen		Cys, Orn	19.92
Procaine		Sar, Glu	11.39
Pseudoephedrine			35.40
Putrescine		Met,Val	21.27
3-Pyridylethyl cysteine		GABA, AADA	15.70
Pyroglutamic acid			N/D
Saccharopine (peak 1)	EA		8.50
Saccharopine (peak 2)		Nva, Ile	23.58
Salsalate		Trp	35.77
Serotonin		Val, Nva	22.13
Streptomycin			N/D
Tetracycline	Tyr		18.31
3-Thiaproline		Pro,BAIB	16.58
Thioproline (Thiazolidinecarboxylic acid)		Pro,BAIB	26.65
Trimethyl Lysine		Gln, Carn	7.34
Tryptamine		Trp	36.30
Tyramine		Nva, Ile	23.07
Uric acid			N/D
Uridine			N/D
Valproate (Sodium)			N/D
Valproic acid			N/D
VMA			N/D
Xanthine		before PSer	1.56
Zidovudine	Pro (Sh)		16.65

* (Sh)= shoulder, partial resolution

**N/D= Not detected, underivatized

Table 2. Compounds in order of retention time (RT)

Compound	Coelution*	Adjacent Amino Acids	R_T**
Xanthine		before PSer	1.56
Glucosaminic acid		before PSer	3.16
Methylxanthine		before PSer	3.52
Cysteic acid		AMQ, HyPro	4.00
Aminoethylphosphonic acid	HyPro		4.19
Adenosyl methionine (peak 1)		HyPro,NH3	4.36
4-Aminobenzoic acid (peak 1)		HyPro,NH3	4.56
Adenosyl methioine (peak 2)	His		4.84
α-Amino-β-guanidinopropionic acid		NH3, His	4.89
Histidinol	Asn		5.02
Cysteine sulfate	3MH (Sh)		5.25
Hypotaurine	Ser (Sh)		6.21
Ketorolac		Gln, Carn	7.29
Trimethyl lysine		Gln, Carn	7.34
Homoserine	Gly,Ans		7.38
D-Histamine		Ans, EA	8.13
Argininosuccinic acid	EA		8.48
2-Methionine sulfoxide (peak 1)	EA		8.48
Saccharopine (peak 1)	EA		8.50
Methionine sulfoxide (peak 2)	Asp		8.77
2,6-Methionine sulfone		Asp, Sar	9.21
cis-Hydroxyproline		Sar, Glu	10.91
Procaine		Sar, Glu	11.39
Argininosuccinic acid anhydride 1		Cit, β-Ala	11.60
Homoarginine	β-Ala		12.85
N^G, N^G-Dimethyl arginine asymmetrical (ADMA)		Thr, Ala	12.94
Argininosuccinic acid anhydride 2		Ala, GABA	13.20
N^G, N^G-Dimethyl arginine symmetrical (SDMA)		Thr, Ala	13.82
Aminolevulinic acid		Thr, Ala	14.36
Glutathione, Reduced (peak 1)		Thr, Ala	14.44
Homogentisic acid	Ala (Sh)		14.48
D-Alanine	Ala (Sh)		14.60
Caffeine	Ala(Sh)		14.71
Argininosuccinic acid anhydride 3		Ala, GABA	14.76

Compound	Coelution*	Adjacent Amino Acids	R_T**
Methanol		Ala, GABA	15.13
Ethanol (peak 1)		Ala, GABA	15.17
O-Acetyl-serine	GABA(Sh)		15.23
Homocitrulline	GABA		15.34
Argininosuccinic acid anhydride 4		GABA, AADA	15.56
3-Pyridylethyl Cysteine		GABA, AADA	15.70
cis-Cysteine		AADA, Pro	16.06
3-Thiaproline		Pro, BAIB	16.58
Zidovudine	Pro (Sh)		16.65
Lanthionine (peak 1)		Pro,BAIB	16.69
Lanthionine (peak 2)	BAIB(Sh)		16.77
Glycyl-Proline	Pro (Sh)	BAIB(Sh)	16.79 (broad Pk)
Diaminopimelic acid (peak 1)		BAIB, Hyl1	17.28
ε- Acetyl lysine		BAIB, Hyl1	17.30
α- Acetyl lysine		BAIB, Hyl1	17.39
Adenosyl homocysteine	Hyl2		17.71
Diaminopimelic acid (peak 2)		Hyl2, AABA	17.92
Epinephrine		Hyl2, AABA	18.06
Glutathione, Oxidized		Hyl2, AABA	18.19
S-Methyl cystine		Hyl2, AABA	18.24
Teatracycline	Tyr		18.31
Gentamyicin (peak 1)	Cyst		18.48
Aminopimelic acid		Cys, Orn	18.55
3, 4- Dihydroxy-D,L-phenylalanine	Cyst, Orn		18.89
Glutathione, Reduced (peak 2)	Orn		19.02
Gentamyicin (peak 2)	Orn		19.02
Homocysteine	Orn		19.05
Aminoethyl cysteine		Cys, Orn	19.54
Se- Methylselenocysteine		Cys, Orn	19.65
3- Porphobolinogen		Cys, Orn	19.92
γ-Glu-ε-Lys		Cys, Lys	20.03
Hydroxytryptophan		Cys, Lys	20.20
Ethanol (peak 2)	Lys		20.30
Dopamine	Tyr		20.65
Argininosuccinic acid anhydride 5	Deriv Peak (Sh)		20.85
Norephedrine		Hcys,Phe	20.94

Compound	Coelution*	Adjacent Amino Acids	R_T**
Argininosuccinic acid anhydride 6		Met,Val	21.14
Putrescine		Met,Val	21.27
Pipecolinic acid (peak 1)		Met,Val	21.38
Argininosuccinic acid anhydride 7	Val (Sh)		21.53
Serotonin		Val, Nva	22.13
Amoxicllin		Val, Nva	22.21
Methyl lysine		Val, Nva	22.31
Argininosuccinic acid anhydride 8		Nva, Ile	22.81
Cysteine-Homocysteine dimers (peak 1)		Nva, Ile	22.90
Penicillamine		Nva, Ile	22.88
Tyramine		Nva, Ile	23.07
Cadaverine		Nva, Ile	23.10
Cysteine-Homocysteine dimers (peak 2)		Nva, Ile	23.10
Saccharopine (peak 2)		Nva, Ile	23.58
4-Aminobenzoic acid (peak 2)		Nva, Ile	23.92
Pipecolinic acid (peak2)		Nva, Ile	24.14
Azaperone		Nva, Ile	25.33
Ethionine		Nva, Ile	25.46
Thioproline (Thiazolidinecarboxylic acid)		Pro,BAIB	26.65
Nitrotyrosine	Ile		27.13
Nitrotyrosine methyl ester (peak 1)	Ile		27.13
Kynurenine		alle,Leu	28.11
Norleucine	Phe		29.30
D-Penicillamin disulfide		after Trp	32.12
Pseudoephedrine		after Trp	35.40
Nitrotyrosine methyl ester (peak 2)		after Trp	35.43
Ephedrine		after Trp	35.48
Salsalate		after Trp	35.77
Tryptamine		after Trp	36.30
D,L-Homophenylalanine		after Trp	36.36
Ciproflaxin		after Trp	36.45
Clonazepem Related Compound A		after Trp	36.70

TABLE 3. Underivatizable compounds (No Peak Observed)

Compound
Acetyl carnitine
Azithromycin
Beclomethasone
Carbamoylphosphate dibasic
Carnitine
Choline
Clonazepem Related Compound B
Creatine
Doxapram
Erythromycin
Ethosuximide
Formyl methionine
D, L- Homocysteic acid
Hydroxyindollicacetic acid
α-keto-γ-(methylthio) butyric acid
Momethasone
Penicillin g
Penicllin v
Phenyl pyruvate
Phosphocholine
Pyroglutamic acid
Streptomycin
Uric acid
Uridine
Valproate (Sodium)
Valproic acid
VMA

Several compounds produce atypical results upon reaction with the derivatization reagent. These compounds may form isomers or undergo derivatization of an alcohol. Some isomers yield two peaks in the chromatogram (ex. pipecolinic acid). For these compounds, both peaks have been documented and are labeled as Peak 1 and Peak 2. Other compounds, such as argininosuccinic acid, react upon heating to form anhydrides. These secondary products have also been reported.

A number of compounds are directly detectable due to their chemical structure within these chromatographic conditions. These compounds, for example xanthine and caffeine, are not derivatized but appear in the chromatogram.

Conclusion

The MassTrak Amino Acid Analysis Solution provides a reproducible, robust method with easy identification of the common physiological amino acids. These characteristics allow for routine preliminary identification of common interferences and other compounds by relative retention time. This same approach or set of experiments is required for ion exchange methods using protocols, columns and instruments that differ from the historical database. Given the characteristics of ion exchange, this process can be time consuming and variable.

Common compounds detected with the MassTrak AAA Solution include a wide number of pharmaceuticals and metabolite side products found in biological fluids. This compilation provides a guide to more than 100 such compounds, beyond the standard amino acids, that may appear in the analysis of biological fluids with the MassTrak AAA Solution. The listed relative retention times also provide a reliable starting point for identification of such compounds in authentic samples.

References

1. S.A. Cohen, M. Meys, T.L. Tarvin, Pico Tag Method: A Manual for *Advanced Techniques for Amino Acid Analysis*, 1989, Millipore Corp., p 80-90.
2. S. Ian, S. Martin, H. Mick, "Drug Interference in Amino Acid Analysis," SSIEM 2007, National Metabolic Biochemistry Network, Training Documents.
3. S.J. Rehfeld, H. Loken, W.D. Korte, Letters to the Editor: "Interference by Antibiotics in Amino Acid Analysis," *Clin. Chem*., 20 (1974), 11, 1477.
4. P. Hong, T. E. Wheat, D. M. Diehl, Analysis of Physiological Amino Acids with the MassTrak Amino Acid Analysis Solution, 2009, Waters Corp., Application Note, 720002903EN.

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